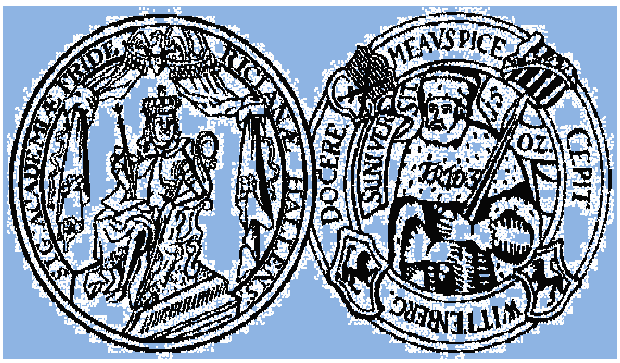

Development of Novel Indolyl-derived Biologically Active Compounds.

Dissertation

zur Erlangung des akademischen Grades
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von

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ToMyFamily

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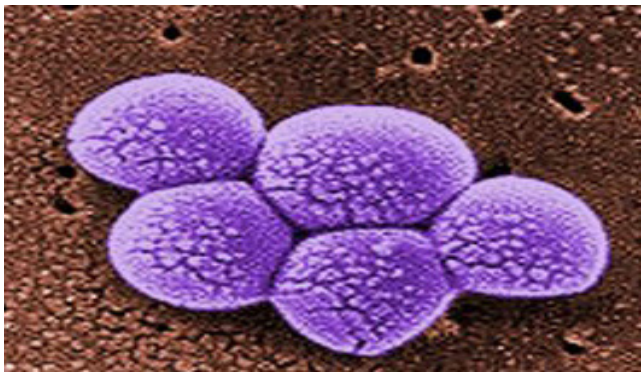
A-498	renal cancer cell
A-549	human lung cancer cell lines
Ac	Acetyl
AcOH	acetic acid
AKT	serine/threonine protein kinase
aliph.	Aliphatic
AML	Acute myeloid leukaemia
aq	Aqueous
arom	aromatic
ASC	ascorbigen
B16	Mouse melanoma cell line
Bcl-2	B-cell lymphoma 2
Bcl-X_L	B-cell leukemia XL
BID	BH3 interacting-domain death agonist
BEL-7402	human hepatocellular carcinoma cells
BIM	bisindolylmethane
BIMs	bisindolylmethanes
Bn	benzyl
br	broad
B. Subtilis	Bacillus subtilis
CAN	cerium ammonium nitrate
C. Albicans	candida albicans
CDC	centers for Disease Control and Prevention
CML	chronic myeloid leukemia
conc.	concentrated
COX-2	cyclooxygenase-2
d	Doublet
DDQ	dichlorodicyanoquinone
DIM	3,3'-diindolylmethane
DMAP	4-(dimethylamino)pyridine
DMF	N, N-dimethylformamide
DMSO	dimethyl sulfoxide
DMT	dimethyltryptamine
DNA	deoxyribonucleic acid, deoxyribonucleic acid
DTP	Developmental Therapeutics Program
DU-145	prostatic cancer

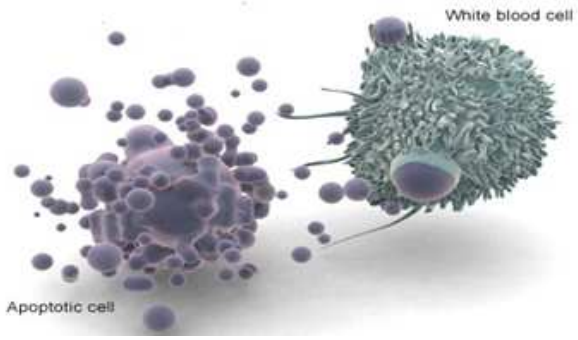
EA	elemental analysis
E.Coli	Escherichia coli
EE	ethyl acetate
eq	equivalent
ErbB2	erythroblastic leukemia viral oncogene homolog 2
ESI	electrospray ionization
Et	ethyl
FDA	Food and Drug Administration
FGFR	fibroblast growth factor receptor
Fig	Figure
FLIP	Fas-associated death domain protein-like interleukin-1-beta-converting enzyme inhibitory protein
FOXO3a	Forkhead box O3
GB	glucobrassicin
GI_{50}	50 % growth inhibition
GIST	gastrointestinal stromal tumor
GSK-3β	glycogen synthase kinase-3β
HCC	hepatocellular carcinoma, hepatocellular carcinoma
HCT-8	human colon cancer cell lines
HEC1A	Human Endometrial Cancer Cells
HeLa	an immortal cell line
HepG2	human liver hepatocellular carcinoma cell line
HER	human epidermal growth factor receptor
HIF-1	hypoxia-inducible factor in breast cancer
HIV	human immunodeficiency virus
HL-60	Human promyelocytic leukemia cells
HOME-1	human nasopharyngeal carcinoma
HOP-92	lung cancer cell
HT29	Human colon adenocarcinoma cell line
$HUVEC_S$	human umbilical vein endothelial cells
Hz	Hertz
IAP	inhibitor of apoptosis proteins
Ic_{50}	inhibition concentration 50%
I3C	indole-3-carbinol
IGF-1-R	insulin-like growth factor 1 receptor
IR	infrared
J	Coupling constant

253J-BV	bladder cancer cells
kcal/mol	kalio calorie ber mole
KB	human epidermoid carcinoma cells
KU7	bladder cancer cells
L-1210	murine leukaemia cells
LC_{50}	lethal concentration 50%
M	molar
m	multiplet
MCF-7	breast cancer cell line
MDAMB	human mammary cancer cell lines.
MDA-MB-231	breast cancer cell line
MDA-MB-453	breast cancer cell line
MDAMB-231/1TCC	breast cancer cell
MDR	multidrug resistance
Me	methyl
MEL-28	Human Skin Melanoma cell line
MeOH	methanol
mg	milligram
MG-MID	mean graph midpoint
MIC	minimum inhibitory concentration
min	minute (s)
ml	Milliliter
mmol	millimole
MONO-MAC-6	human monocytic cell lines
Mp	Melting point
MRSA	methicillin-resistance Staphylococcus aureus
MS	mass spectrometry
NBS	bromosuccinimide
NCI	national cancer institute
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NMR	nuclear magnetic resonance
NSCLC	non small cell lung cancer
NSCLC-N6	human bronocopuemonary cancer cells
NUGC-3	gastric adeno carcinoma cell
Nurr 1	Nuclear receptor related 1 protein
Nurr 77	Nuclear receptor related 77 protein
p	Primary

P-388	lymphocytic leukemia
PAGE	polyacrylamide gel electrophoresis
PAR	parental cell line
PDGFR	platelet-derived growth factor receptor
PK	pyruvate kinase
p38 MAPK	Mitogen-activated protein kinase
PMM	pentamethylmelamine
p75(NTR)	p75 neurotrophin receptor
Ph	phenyl
Phe	phenylalan
PPA	polyphosphoric acid
PPARγ	peroxisome proliferator-activated receptors gamma
ppm	parts per million
Pr	propyl
RCC	renal cell carcinoma, renal cell carcinoma
R_f	ratio of fronts
RNA	ribonucleic acid, ribonucleic acid
ROS	reactive oxygen species
RT	room temperature
s	Singlet
S. aureus	Staphylococcus aureus
SKOV3	ovarian cancer cells
SRB	Sulforhodamine B colorimetric assay for cytotoxicity screening
SSA	silica sulfuric acid
t	triplet
tab	table
TCA	trichloroacetic acid
TCQ	tetrachloroquinone
TGI	Total growth inhibition
THF	tetrahydrofuran
TIMs	trisindolylmethanes
TLC	Thin layer chromatography
Ts	toluenesulfonic acid
U.S	United State
UV	ultraviolet
VEGFR	vascular endothelial growth factor receptor
VRE	Vancomycin Resistant Enterococcus

XIAP	X-chromosome-linked Inhibitor Apoptose-Protein
q	Quartet





Introduction

Introduction

Indole is an aromatic heterocyclic compound that has a bicyclic structure, consisting of a six-membered ring fused to a five-membered nitrogen-containing pyrrole ring. All compounds that contain an indole ring system are indoles. Indole itself is obtained from coal tar or various plants and produced by the bacterial decomposition of tryptophan in the intestine. It has been synthesized by one of the oldest and most reliable methods known as *Fischer indole synthesis*[1]. Indole functions are popular components of fragrances, indicator of some diseases and function as signal molecule in plant, animal and microorganism, respectively. It also serves as precursor, core building block and functional group of many important biochemical molecules and compounds, such as plant hormones, alkaloids, indigoids, certain proteins and enzymes. Most of these important molecules and compounds if not all, are originated, fully or partly, from bio oxidation of indole.

1.1. Indoles as natural products

Indoles are natural compounds that are found in many plants but particularly associated with cruciferous vegetables[2,3]. Cruciferous vegetables include cauliflower, cabbage, turnip, broccoli and Brussels sprouts, figure (1). The specific compounds in these vegetables that are thought to be of value are indoles. Indoles belong to a class of phytonutrients compounds (plant compounds which are thought to have health-protecting qualities) which have been scientifically shown to benefit the body in a number of important ways. Consuming cruciferous vegetables has been associated with a decreased risk of colon, breast and prostate cancers. Cruciferous vegetables are a rich source of many phytochemicals, including indole derivatives, dithiolthiones and isothiocyanates. Cruciferous vegetables contain glucobrassicin (GB) which, during metabolism, yields indole-3-carbinol (I3C), 3,3'-diindolylmethane (DIM) and ascorbigen (ASC), figure (2). The ant carcinogenic effects of I3C and DIM were exhibited in human cancer cells. It appears that these indolic compounds may offer effective means against several cancer cell lines[4].

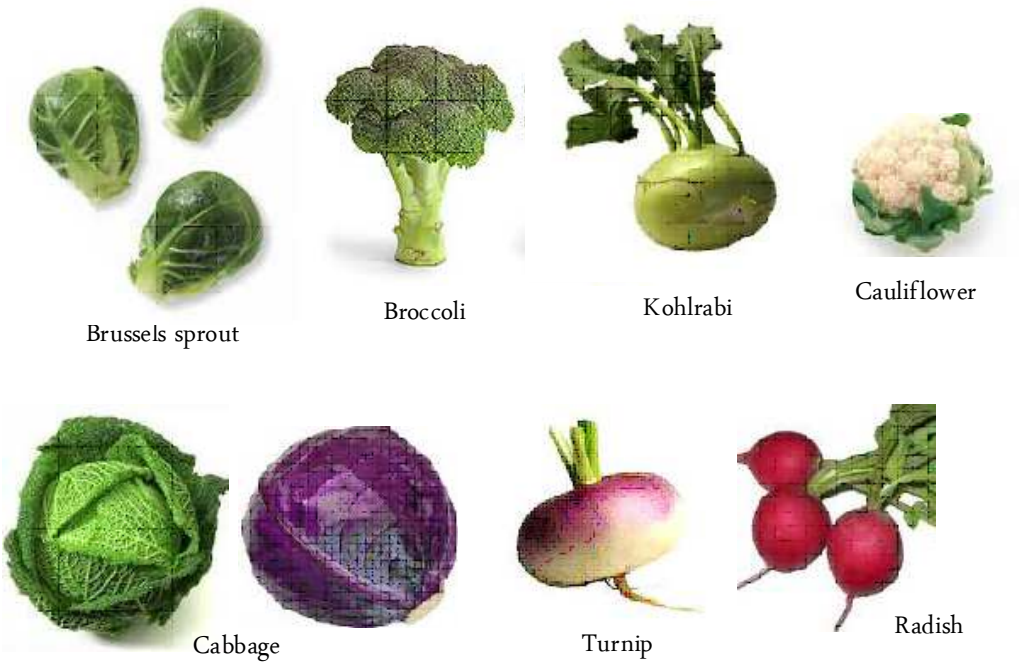


Figure (1): Cruciferous Vegetables.
http://www.fotosearch.com/photos-images/cruciferous-vegetables.html

A number of natural products found in fruits and vegetables are known to possess anti-mutagenic and anti-carcinogenic properties. A beneficial effect of high dietary intake of fruits and vegetables against carcinogenesis is known and an inhibitory effect of indoles and cruciferous vegetables against tumorgenesis and risk of cancers has also been demonstrated[5]. Epidemiological data suggest that populations that consume higher amounts of cruciferous vegetables have lower incidence of cancer or improved biochemical parameters, such as decreased oxidative stress compared to controls. Cruciferous vegetables protect more effectively against cancer than the total intake of fruits and other vegetables. The National Research Council, Committee on Diet, Nutrition, and Cancer has recommended increased consumption of cruciferous vegetables as a measure to decrease the incidence of cancer[6,7,8,9].

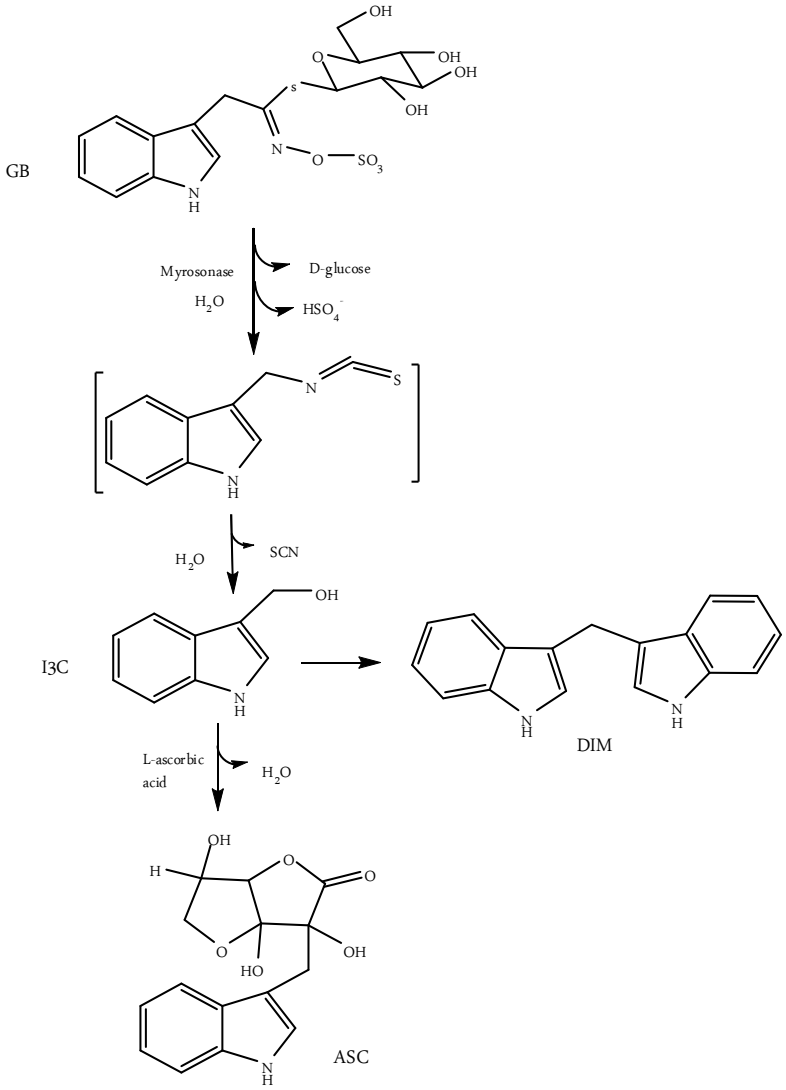


Figure (2): The derivation and chemical structure of the anticarcinogenic indole compounds I3C, DIM and ASC from GB.

Many indole alkaloid derivatives were found in nature such as the plant growth hormone (Auxin) which contains indole-3-acetic acid[10]. Indoles are precursors of many pharmaceuticals. Indoles are present in many important biological compounds such as in tryptophan which is a significant indole derivative while serotonin and melatonin are biochemically active indole molecules[10], figure (3).

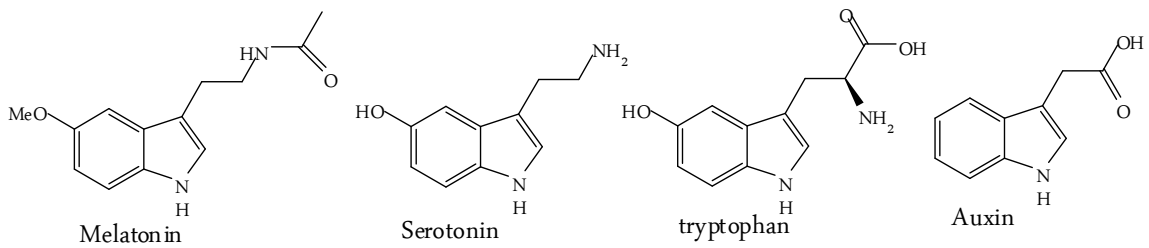


Figure (3): Structure of biochemically some active indole molecules

1.2. Indoles in Medicinal Chemistry

Indole derivatives are certainly very important heterocycles in the drug-discovery studies. They are a very important class of compounds that play a major role in cell physiology and are potential intermediates for many biological reactions. Indole derivatives represent many important classes of therapeutically agents in medicinal chemistry such as anti-cancer[11], antioxidant[12], antirheumatoidal[13], and anti-HIV[14,15], antimicrobial[16,17,18], antiinflamatory[19], analgesic[20], antipyretic[21], anticonvulsant[22,23], anthelmintic cardiovascular[24], and selective COX-2 (cyclooxygenase-2) inhibitory activities[25,26,27,28] (which is an enzyme responsible for inflammation and pain) and DNA binding ability[29]. Furthermore, many important indole derivatives are used in diseases treatment, for example, the non-steroidal anti-inflammatory drug indomethacin (Indocin®), the beta blocker pindolol (Viskin®) for treatment of high blood pressure (hypertension), the naturally occurring hallucinogen dimethyltryptamine (DMT)[10] and Bio Response DIM for healthy estrogens for men and women, (http://www.bioresponse.com/Home.asp), figure (4).

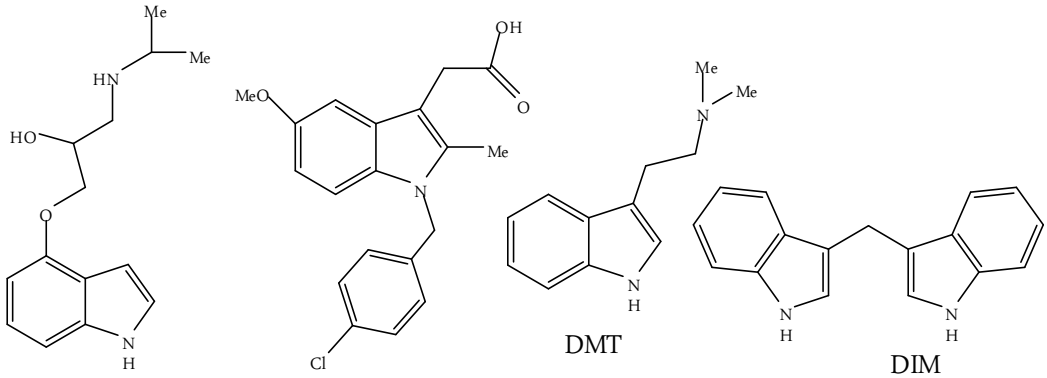


Figure (4): Marketed indole drugs.

1.3- Indoles as antimicrobial active agents

Several indole derivatives either naturally isolated or synthesized have been reported in the literature as active antimicrobial agents. For example from the *frish marin sponge*

Hyrtios altum a new antibiotic indole trimer called trisindoline was isolated which showed antibiotic activities against *E.coli*, *Bacillus subtilis* and *staphylococcus aureus*[30]. Also numerous bis- and tris-indole derivatives were isolated from *a North Sea bacterium* that was closely related to vibrio parahaemolyticus (98 % homology). 1,1,3-Tris(3-indolyl)butane, 3,3/-bis(3-indolyl)butane-2-one, arundine (DIM) and 1,1,1-tris(3-indolyl)methane, figure (5), were isolated from a microorganisms. These compounds were showed to have a broad spectrum as active antibacterial and antifungi[31].

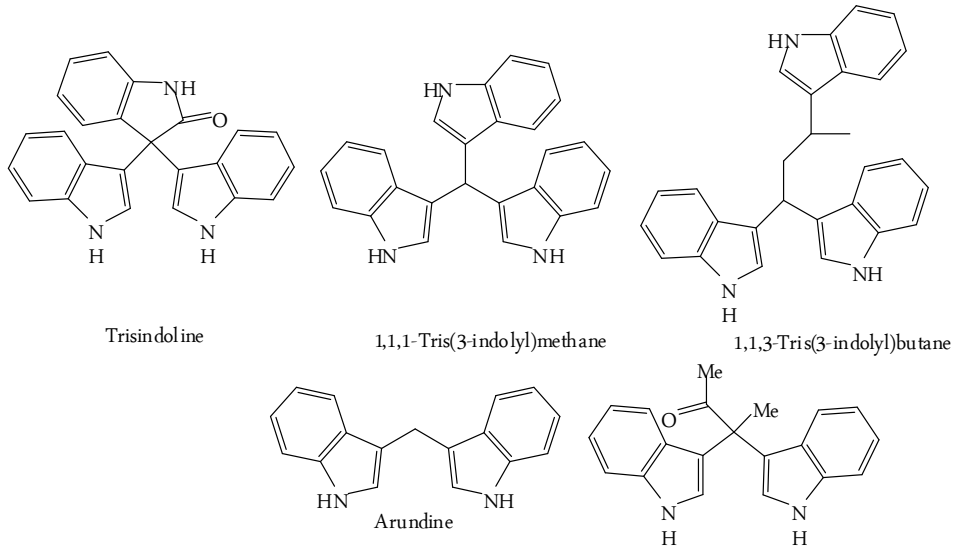


Figure (5): Naturally antimicrobial indole derivatives.

The antibiotics turbomycin A and B are natural products which were derived from a metagenomic library of soil microbial DNA, figure (6) [32].

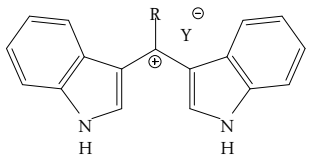


Figure (6): Structure of turbomycine A and B

1.3.1. Indoles as anti-MRSA agents

MRSA[33] is a methicillin-resistance Staphylococcus aureus shown under microscope figure (7a) and a strain of Staphylococcus aureus that developed resistance to the killing effect of the β-lactam antibiotics, which include the penicillins (methicillin, dicloxacillin, nafcillin, oxacillin, etc.) This strain of common "staph" bacteria causes infections in different parts of the body including the skin, lung and other areas. MRSA is sometimes

called a "superbug" because it is very difficult to treat and it causes a huge number of infections every year in hospitals all over the world due to the resistance to many antibiotics. Although most MRSA infections are not serious, some can be life-threatening. In addition, these organisms have been termed "flesh-eating bacteria" because of their occasional rapid spread and destruction of human skin. Statistical data suggest that as many as 19,000 people per year have died from MRSA in the U.S., data supplied by the CDC in (Centers for Disease Control and Prevention). In 2010 this number has declined by about 28 % from 2005 to 2008, in part because of prevention practices in hospitals and home care[34].

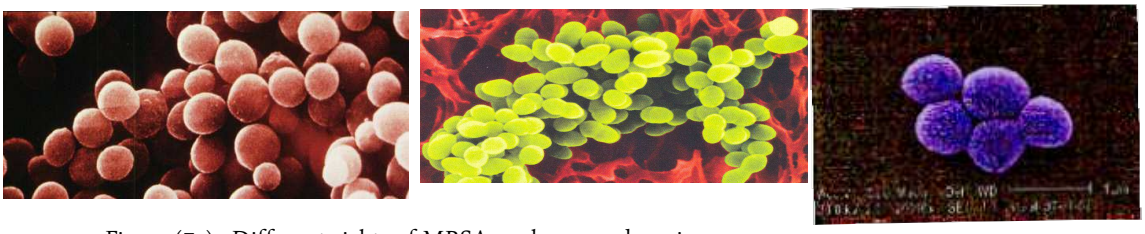


Figure (7a) : Different sights of MRSA as shown under microscope.
(http://www.medicinenet.com/mrsa_infection/article.htm#)

1.3.2- Signs and symptoms of MRSA infection

Most MRSA infections are skin infections that produce the following signs and symptoms[35]:

(1) **Cellulitis**: Infection of the skin or the fat and tissues that lie immediately under the skin, usually starting as small red bumps in the skin with some areas like a bruise, figures (7b).

(2) **Boils**: Pus-filled infections of hair follicles.

(3) **Abscesses**: Collections of pus in or under the skin.

(4) **Sty**: Is an acute infection of the secretory oil glands of the eyelids.

(5) **Rash**: Skin appears to be reddish or

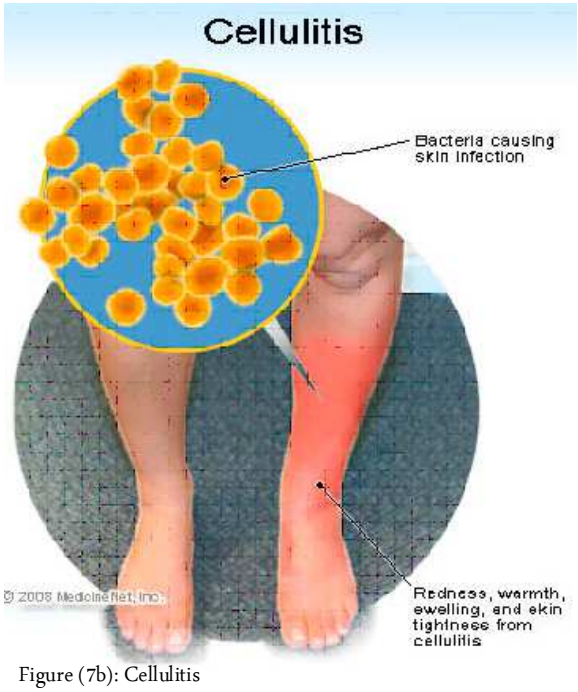
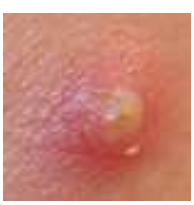


Figure (7b): Cellulitis
http://www.medicinenet.com/mrsa_infection/article.htm#

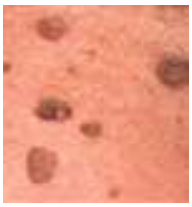
have red-colored areas.

(6) **Impetigo**: Skin infection with pus-filled blisters.

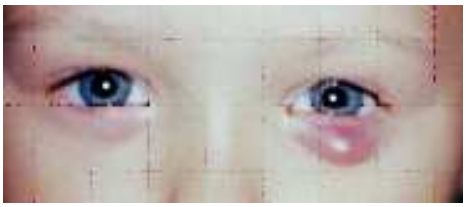
(7) **Carbuncles**: Infections larger than an abscess, usually with several openings to the skin.



Boils

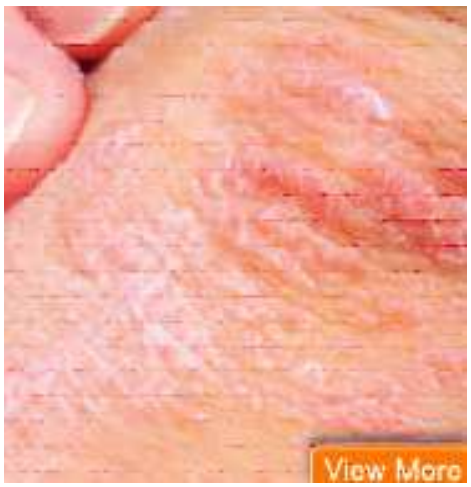


Abscesses

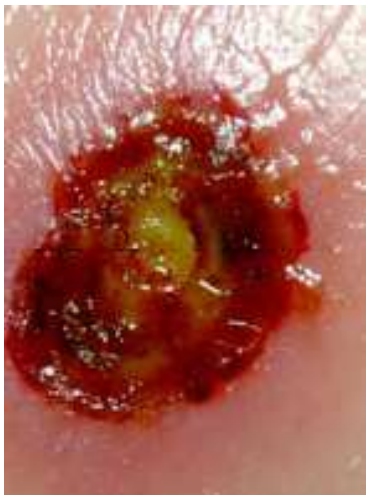


Sty

Impetigo



Rash



Carbuncles

Figure (7c): Signs and symptoms of MRSA.
http://www.medicinenet.com/mrsa_infection/article.htm#

Most of the above signs and symptoms, figure (7c), represent the early stages of MRSA infections. One major problem with MRSA and occasionally with other Staphylococcus infections is that in some times the skin infection can spread to almost any other organ in the body. When this happens, more severe symptoms develop. MRSA that spreads to internal organs can become life threatening. Fever, chills, low blood pressure, joint pains, severe headaches, shortness of breath, and "rash over most of the body" are symptoms that need immediate medical attention, especially when associated with skin infections. Some MRSA infections become severe and complications such as endocarditic, necrotizing fasciitis, osteomyelitis, sepsis, and death may occur. There are two major ways people become infected with MRSA. The first is physical contact with someone who is either infected or is a carrier (people who are not infected but are colonized with the bacteria on their body) of MRSA. The second way is for

people who have physically contact to MRSA on any objects such as door handles, floors, sinks or towels that have been touched by a MRSA-infected person or carrier. People with higher risk of MRSA infection are those with obvious skin breaks (for example, patients with surgical or traumatic wounds or hospital patients with intravenous lines, burns, or skin ulcers) and people with a depressed immune systems (infants, the elderly or HIV-infected individuals) or those with chronic diseases (diabetes or cancer). People with pneumonia (lung infection) due to MRSA can transmit MRSA by airborne droplets.

1.3.3. Treatment of MRSA

The following antibiotics[36], figure (8), are currently in clinical use for treatment of the MRSA

[1] **Oxazolidinones antibiotics**: It is a group of synthetic antibiotics which work by stopping the growth of bacteria, such as linezolid.

[2] **Lipopeptides antibiotics**: A molecules consists of lipids connected to peptide such as daptomycin.

[3] **Glycylcycline antibiotics**: It is a new class of antibiotic derivatives from the tetracycline type, such as tigecycline.

[4] **Glycopeptides antibiotics**: They are composed of glycosylated cyclic or polycyclic non-ribosomal peptides, such as vancomycin.

[5] **Lip glycopeptides antibiotics**: It is a class of antibiotics that has lipophilic side chains liked to glycopeptides, such as oritavancin.

[6] **Cephalosporin's antibiotics**: They are β-lactam antibiotics, such as ceftobiprole.

[7] **Enzyme inhibitors**: These are molecules that bind to enzymes and decrease their activities, such as iclaprim.

MRSA infections cause appreciable mortality and morbidity. Vancomycin, figure (9), has been the mainstay of therapy for serious MRSA infections. However, new data show that vancomycin may not be suitable for therapy of so called VRE (Vancomycin Resistant Enterococcus) infections. Moreover the advent of several new antibiotics in the last few years has provided the clinicians with reasonable alter-natives for therapy of MRSA infections.

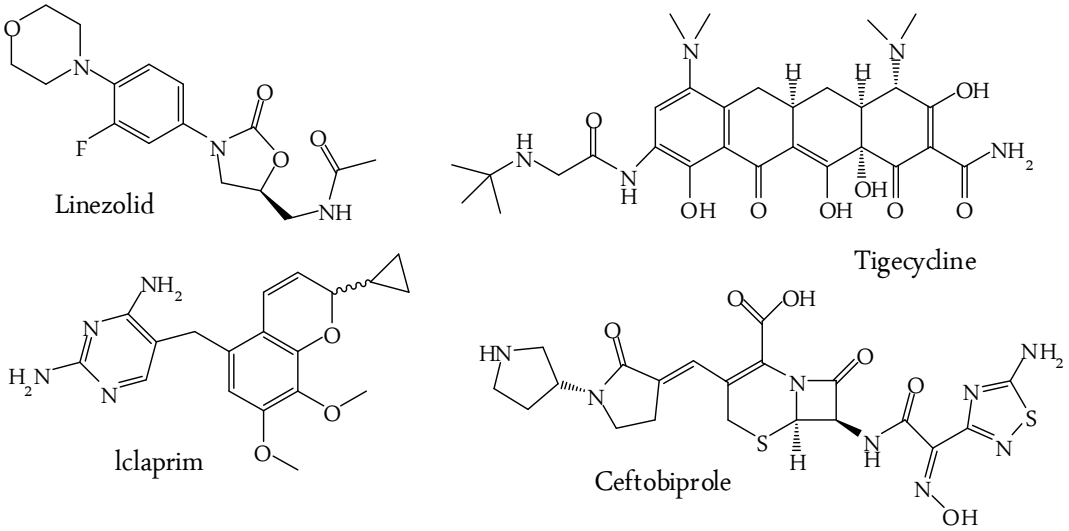


Figure (8) : Chemical structures of some antibiotics used for MRSA treatment.

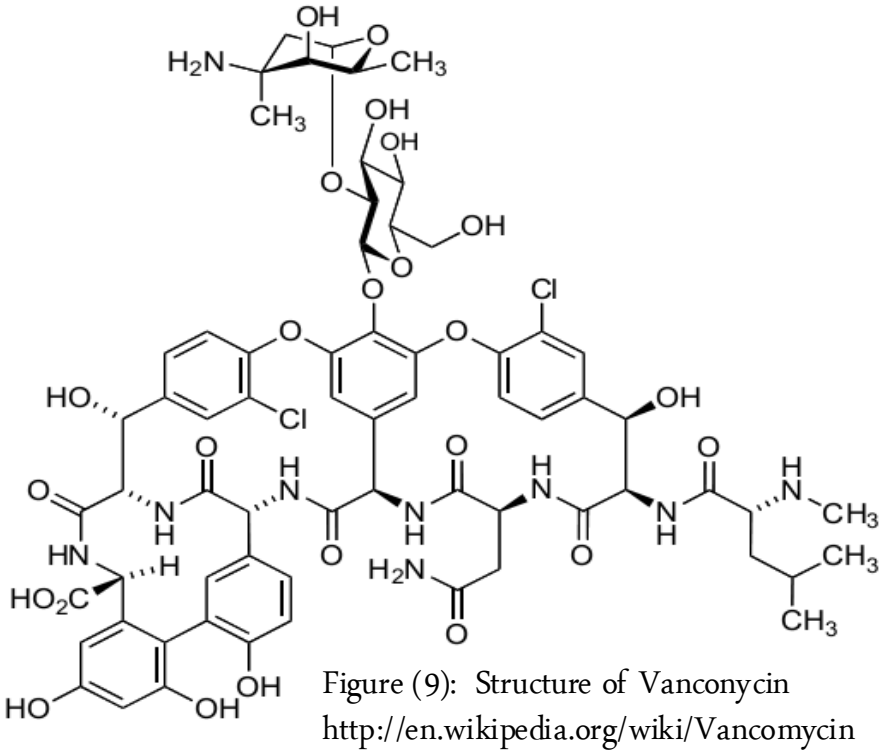
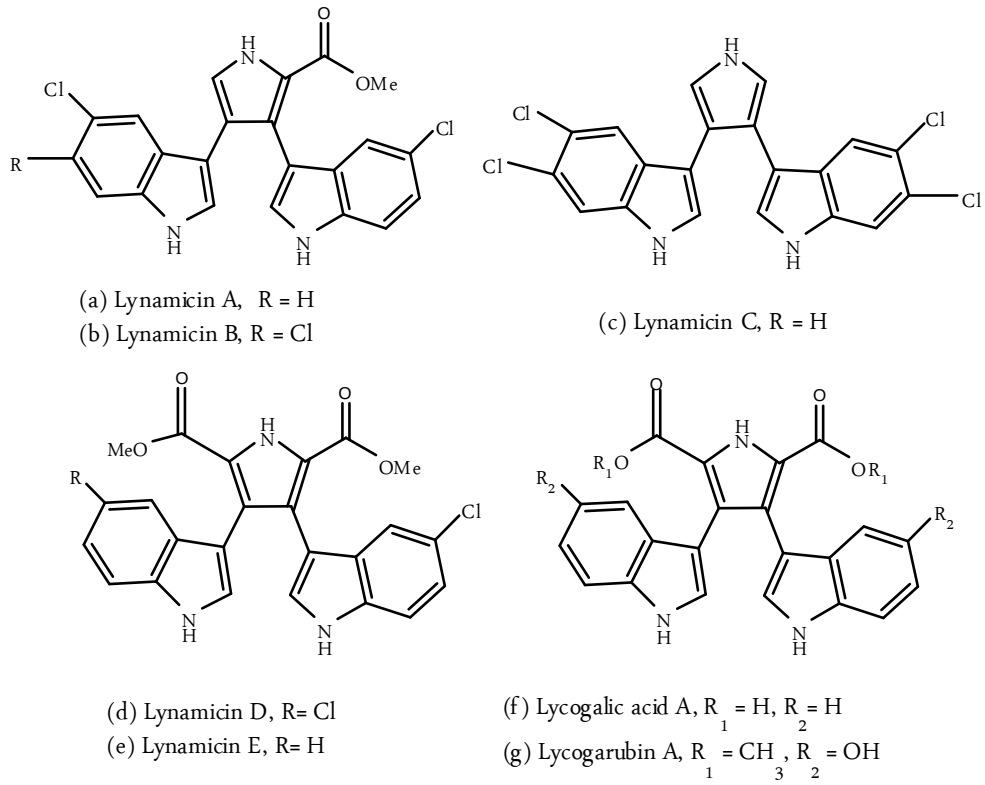


Figure (9): Structure of Vanconycin
http://en.wikipedia.org/wiki/Vancomycin

1.3.4- Indoles as active agents against MRSA

A vast number of indole compounds either synthesized or naturally occurring have been reported in the literature as active components against MRSA. In the present part we will describe some of the most important and recent discovered indoles that have been examined as anti-MRSA agents. The bioassay-guided fractionation of the extracts from the culture broth of another *Marinispora* species led to the five halogenated

bisindole pyrroles, lynamicins A to E, figure (10), which showed activity against MRSA and VRE[37]. MIC values in the range 1–3 μg/ml and 2–8 μg/ml were recorded for lynamicins A-D against MRSA and VRE, respectively. Lynamicin E was somewhat less active (MIC 12 μg/ml and > 24 μg/ml against MRSA and VRE)[37, 38, 39]. Lycogalic acid A and the lycogarubins A bisindoles were isolated from *Chromobacterium violaceum* and *Lycogala epidendrum*[40, 41, 42]. Antibacterial activity for these latter bisindolyl compounds has opened windows for using these indole derivatives in a clinical treatment of MRSA and VRE, which are largely responsible for the increase in numbers of hospital-acquired, such as nosocomial infections.



(a) Lynamicin A, R = H
(b) Lynamicin B, R = Cl

(c) Lynamicin C, R = H

(d) Lynamicin D, R= Cl
(e) Lynamicin E, R= H

(f) Lycogalic acid A, R_1 = H, R_2 = H
(g) Lycogarubin A, R_1 = CH_3, R_2 = OH

Figure (10): Structure of bisindole pyrroles and bisindolylmaleimide derivatives.

The four bis-(imidazo-linylindole) compounds, MBX 1113, MBX 1090, MBX 1066 and MBX 1128, figure (11), were shown to have potent antibacterial activity as measured by the inhibition of bacterial growth in vitro. These compounds were effective against a broad range of gram-positive and gram-negative bacteria species, including several antibiotic resistant strains[43].

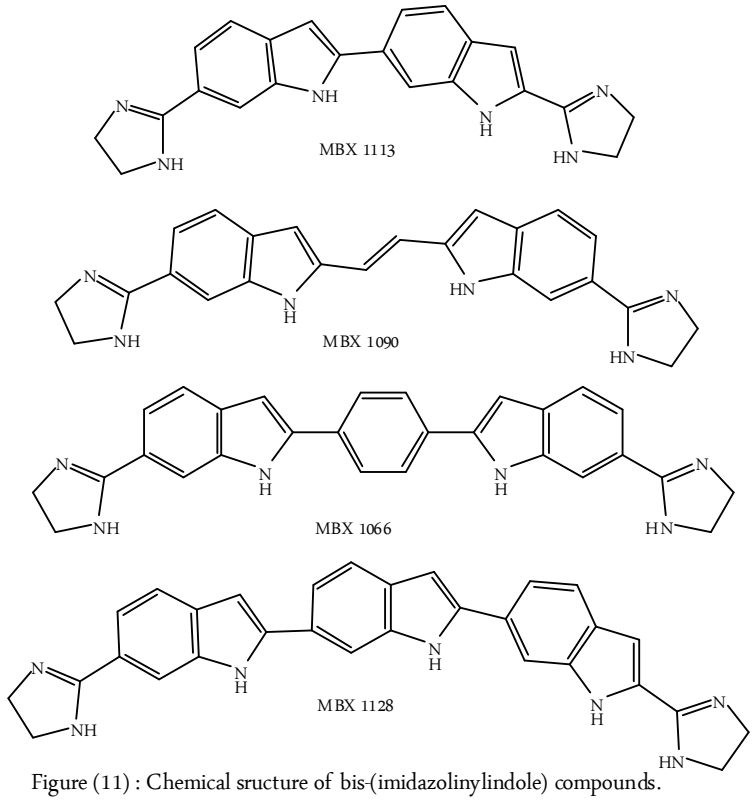


Figure (11) : Chemical sructure of bis-(imidazolinylindole) compounds.

Reserpine, strychnine and harmaline, figure (12) are plant-based alkaloids indoles which have been isolated and tested against MRSA and compared with vancomycin and oxacillin antibiotics. The results demonstrated that harmaline exhibited a notable inhibitory potential against MRSA and this suggests that interesting phytocompounds have yet to be discovered as resistance modifying agents[44].

A new indole-containing compound, figure (12) has been successfully synthesized by one-pot reaction and several of its analogous exhibited good to excellent *in vitro* activities against S. aureus and E. faecium including MRSA and VRE[45].

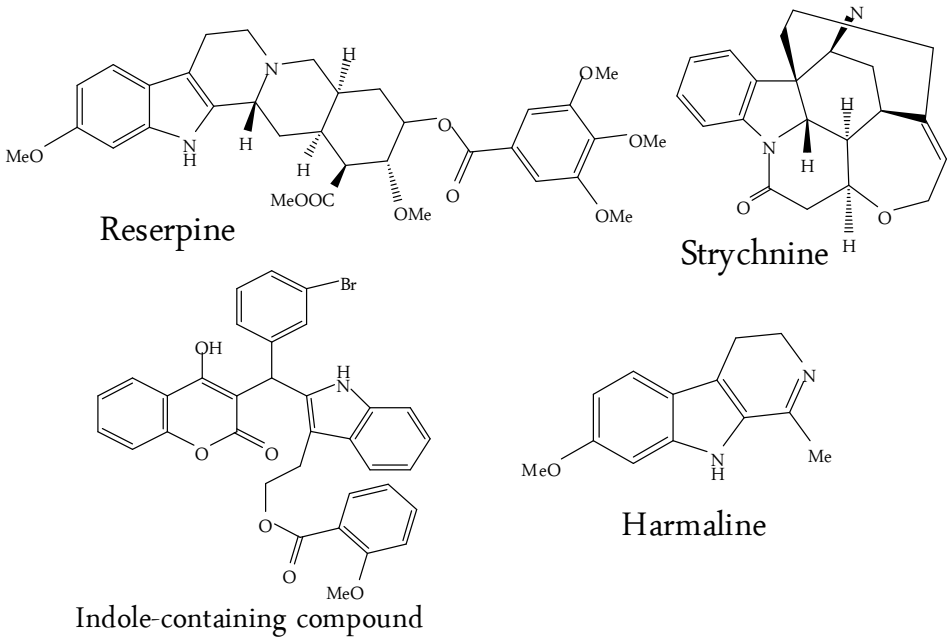


Figure (12): Structure of some plant-based alkaloids and Indole-containing compounds.

Screening of a marine extract library led to the identification of several bis-indole alkaloids (spongotine A, bromotopsentin, bromodeoxytopsentin and *cis*-3,4-dihydrohamacanthin B), figure (13) which was reported as novel potent and selective MRSA PK (Pyruvate Kinase) inhibitors. These results help to understand the mechanism of the antibacterial activities of marine bis-indole alkaloids and provide the basis for the development of potential novel antimicrobial drugs[46].

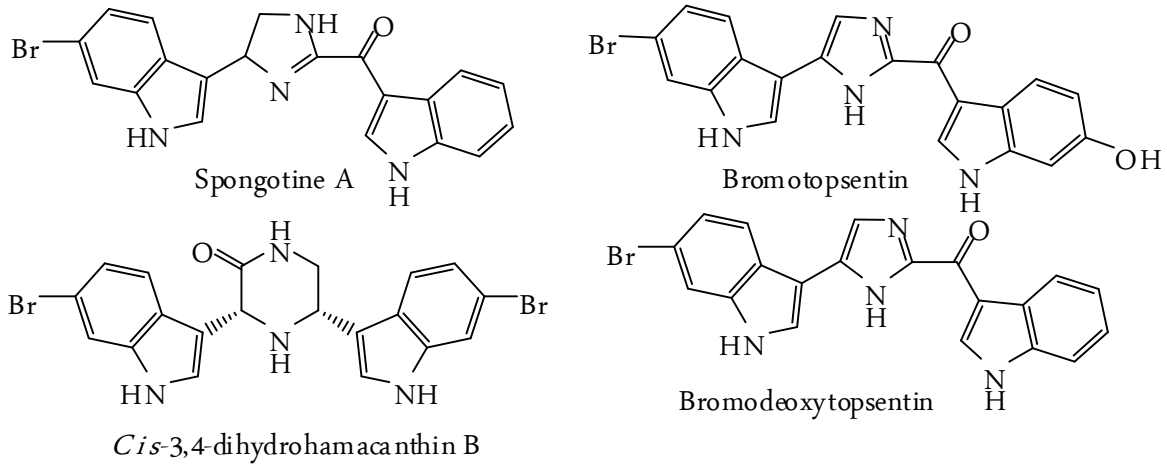


Figure (13): Molecular structure of marine bisindole alkaloids.

1.4. Indoles as anticancer active agents

Carcinogenesis is an uncontrolled growth of the cells in the human body and the ability of these cells to migrate from the original site and spread to distant sites. If the spread is not controlled, cancer can result in death[48]. The burden of cancer is growing and it is the leading cause of death worldwide. Global cancer population is more than 6.75 billion and it is still increasing predominantly in developing countries. About 12.7 million new cancer cases and 7.6 million cancer deaths occurred across 182 countries in 2008. An increase of new cancer cases (56 %) and cancer deaths (63 %) occurred in the less developed nations[47]. Efforts are being made with the aim to prevent, control and cure the cancer through various research activities across the globe supporting hands from various funding bodies. Indole compounds are well-known for their anticancer properties. In particular indole-3-carbinol (I3C), its dimeric product 3,3′-diindolylmethane (DIM) and other derivatives of DIM have been widely investigated for their effectiveness against a number of human cancers *in vitro* as well as *in vivo*. These compounds are effective inducers of apoptosis (programmed cell death) and the accumulating evidence documenting the ability of indoles to modulate multiple cellular signalling pathways that are considered as a testimony to their pleiotropic behaviour, (http://www.dimfaq.com/site/cancer.htm).

1.4.1. Induction of cell death by indoles

Anti-cancer agents have been traditionally evaluated for their apoptosis-inducing action and this is true for indole compounds as well, where they have been demonstrated to inhibit the proliferation, growth and invasion of human cancer cells[49,50,51,52]. As a mechanism of apoptosis induction, indole derivatives, I3C and DIM, as summerized in figure (14), have been shown to (a): Down-regulate anti-apoptotic gene products such as Bcl-2 (B-cell lymphoma 2) and Bcl-X_L (B-cell leukaemia XL), (b): Down-regulate the inhibitor of apoptosis proteins e.g. CIAPs, X-chromosome linked Inhibitor of apoptose-protein (XIAP) and surviving, (c): Up-regulate pro-apoptotic factors such as Bax gene, (d): Release mitochondrial cytochrome C as well as activate

caspase-9 and caspase-3[53], (e): Inhibition of the NF-$_k$B signalling pathway[54-60]. A vast number of different mechanisms of apoptosis induction by indoles have also been reported[60-67].

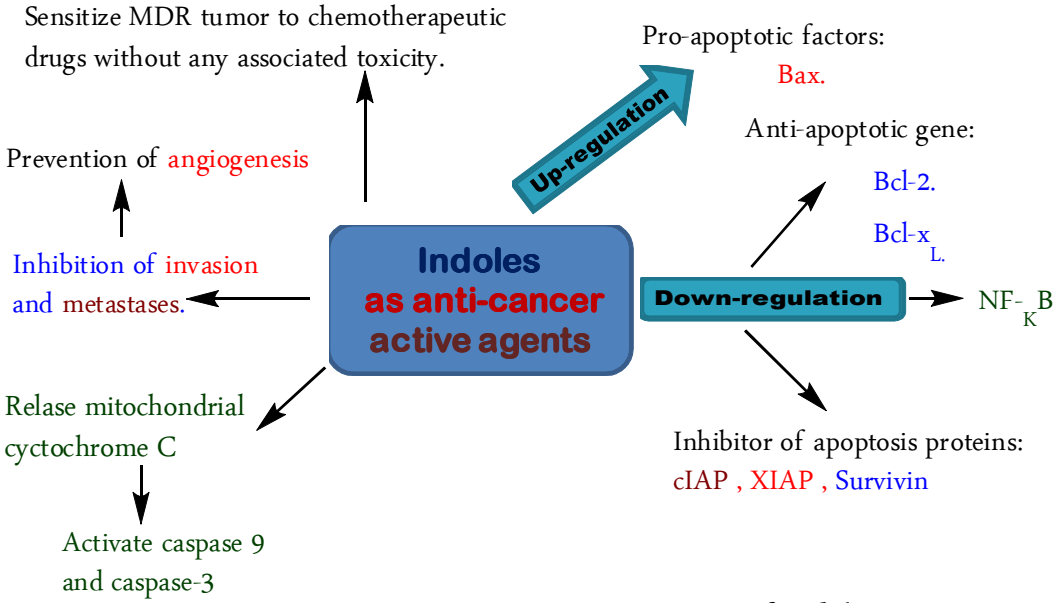


Figure (14) : Some anticancer activity of indoles.

Figure (15) illustrated the extrinsic and the intrinsic pathways of apoptosis (programmed cell death). *The Extrinsic Pathway*: In the extrinsic pathway, signal molecules known as ligands, which are released by the immune system's natural killer cells possess the Fas ligand (FasL) on their surface to bind to a transmembrane death receptors on the target cell. After the binding of the death ligand to the death receptor the target cell triggers multiple receptor to aggregate together on the surface of the target cell. The aggregation of these receptors recruits an adaptor protein known as Fas-associated death domain protein (FADD) on the cytoplasmic side of the receptors. FADD, in turn, recruits caspase-8. Then caspase-8 will be activated and it is now able to directly activate caspase-3, and caspase-7. The activation of caspase-3 will initiate degradation of the cell[68]. *The Intrinsic Pathway*: The intrinsic pathway is triggered by cellular stress specifically mitochondrial stress caused by factors such as DNA damage from the chemotherapy or UV exposure. Upon receiving the stress signal the proapoptotic proteins in the cytoplasm (BAX and BID) bind to the outer membrane of the mitochondria to signal the release of the internal content. The interaction between

the proapoptotic (BAX and BID) and the antiapoptotic proteins (Bcl-2) at the surface of the mitochondria is thought to be important in the formation of the PT pores in the mitochondria and hence the release of cytochrome c and the intramembrane content from the mitochondria. Following the release, cytochrome c forms a multi protein complex known as apoptosome which consists of cytochrome c, Apaf-1, procaspase-9 and ATP. Following its formation, the complex will activate caspase-9 and then the activated caspase-9 will turn the pro-caspase-3 and pro-caspase-7 into active caspase-3 and active caspase-7. These activated proteins initiate cell degradation or cell death. Besides the release of cytochrome c from the intramembrane space, the intramembrane also releases Smac/Diablo proteins to inhibit the inhibitor of apoptosis (IAP). These IAP as protein family consists of 8-human derivatives and their function is to stop apoptotic cell death by binding to caspase-3, caspase-7 and caspase-9 and inhibit them[68].

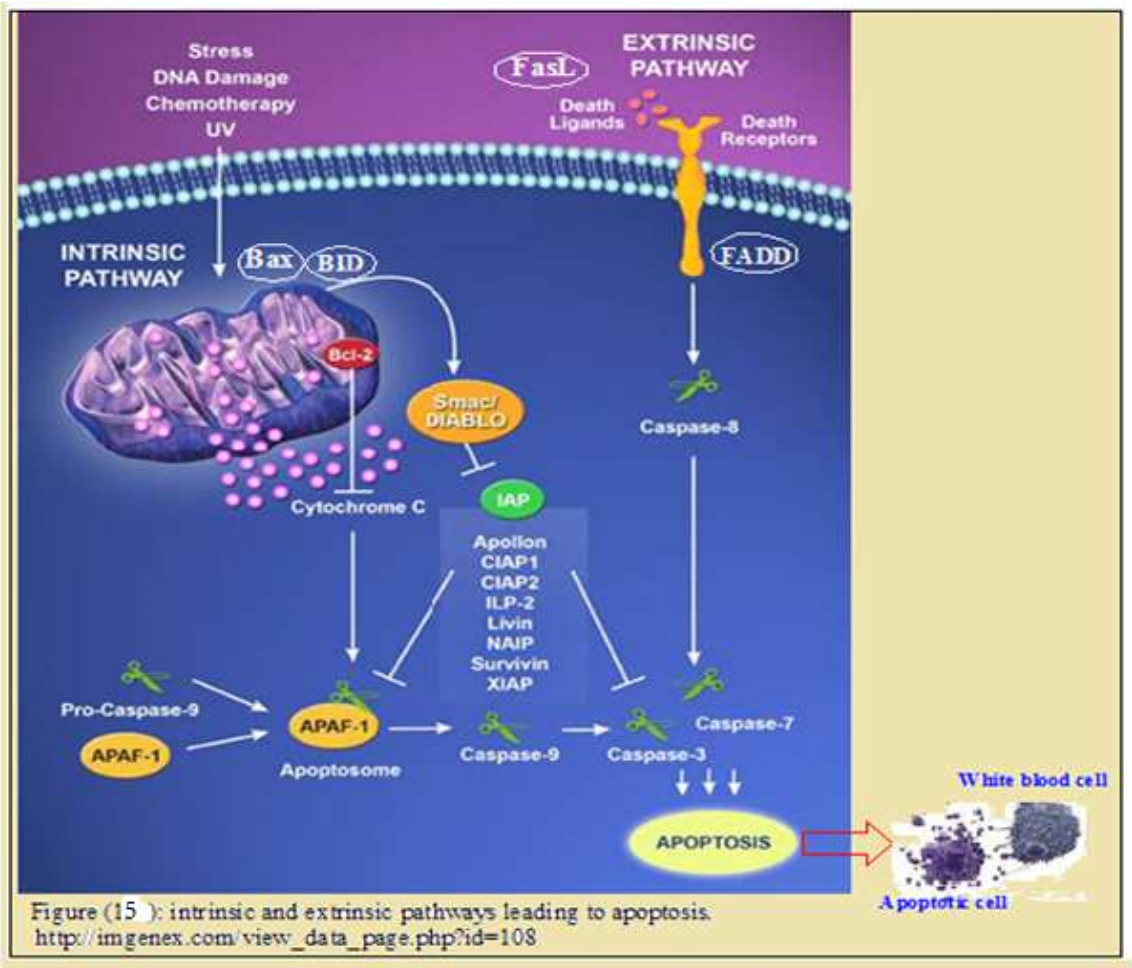


Figure (15): intrinsic and extrinsic pathways leading to apoptosis.
http://imgenex.com/view_data_page.php?id=108

1.4.2. Inhibition of invasion and metastasis by indoles

The ability of cancer cells to penetrate into lymphatic and blood vessels, circulate through the bloodstream, and then invade and grow in normal tissues elsewhere. This ability to spread to other tissues and organs makes cancer to a potentially life threatening disease. Tumour angiogenesis is the proliferation of a network of blood vessels that penetrates into cancerous growths, supplying blood and oxygen and removing waste products. Tumour angiogenesis actually starts with cancerous tumour cells releasing molecules that send signals to surrounding normal host tissue. This signalling activates certain genes in the host tissue that, in turn, make proteins to encourage growth of new blood vessels. Figure (16) shows the concept of angiogenesis and its relation to the tumour growth. Indole derivatives, I3C and DIMs have been reported to inhibit the invasion of cancer cells[69,70,71] and the development of new blood vessels (angiogenesis)[58,72].

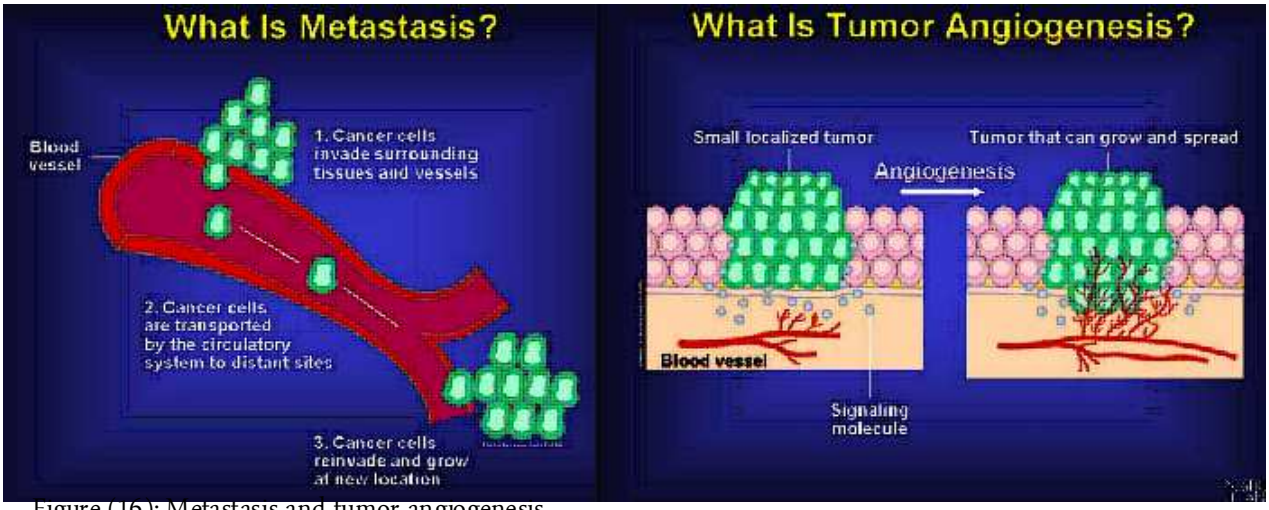


Figure (16): Metastasis and tumor angiogenesis. (http://www.cancer.gov/search/results)

1.4.3. Chemosensitization by Indole Compounds

Chemosensitization is the process by which compounds eg. indole compounds, I3C and DIM modulate the cellular signalling pathways leading to apoptosis and thus overcome the chemo- as well as immune-resistance of established chemotherapeutic drugs[73]. I3C has been reported to sensitize multidrug resistant tumours to chemotherapeutic drugs without any associated toxicity[73].

1.4.4. Reported indole derivatives as anticancer agents

In human cancer cell models, indoles (I3C and DIM or its derivatives) have been shown to induce apoptosis in breast[74-80], squamous cell carcinoma[81], cholangiocarcinoma[82], colon[83-86], cervical[87], ovarian[88], pancreatic[89,90] and prostate[91-94] cancer cells. There are many other indole derivatives were reported as active anticancer agent we will list some of them. The potential prodrug (1,2-dimethyl-3-(*N*-(4,6-bis(dimethylamino)-1,3,5-triazin-2-yl)-*N*-trideuteromethylaminomethyl)-5-methoxyindole-4,7-dione),pentamethylmelamine (PMM), figure (17) in which the labelled pentamethylmelamine is attached to an indole-4,7-dione moiety has attracted much interest as antitumor agent over the past 35 years. In particular, it entered the clinic in the **1970s** for the treatment of ovarian carcinoma but difficulties were encountered, as it was insoluble in water and thus is difficult to formulate. However, it has recently been recognised as a second-line treatment for ovarian carcinoma[95-97].

Schoentjes and et. al[98] introduced a patent of indole derivatives of the general formula (I), figure (17) in 2011 with reported their use for the treatment of cancers.

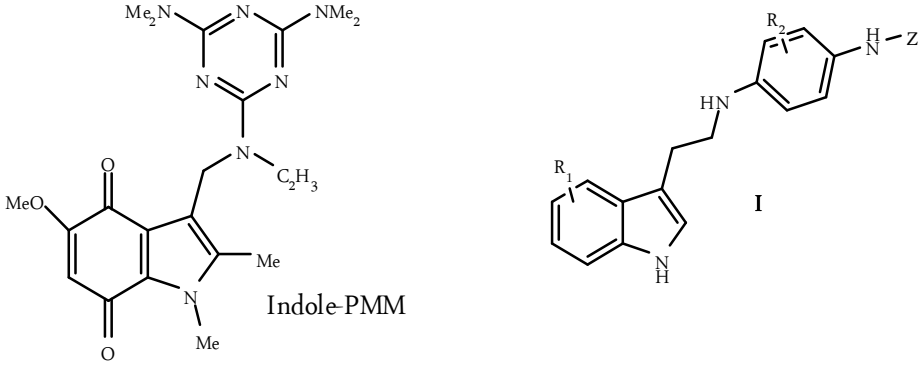


Figure (17): Structure of prodrug indole-PMM derivative and tryptamine derivative I.

Several aroylamide indole analogues, figure (18) have been synthesized and preliminarily evaluated for their *in vitro* cytotoxic activity in A431 and H460 cell lines. All the compounds examined conferred unusual potency in a tumour cell cytotoxicity assay. The findings showed that the indole derivatives would be promising candidates for the development of new anticancer agents[99]. 3-Aroylindole is a potential anticancer

drug candidate designed and proposed from *in vitro* human microsome studies with better pharmaco kinetics and improved potency in the tumour xenograft model[100].

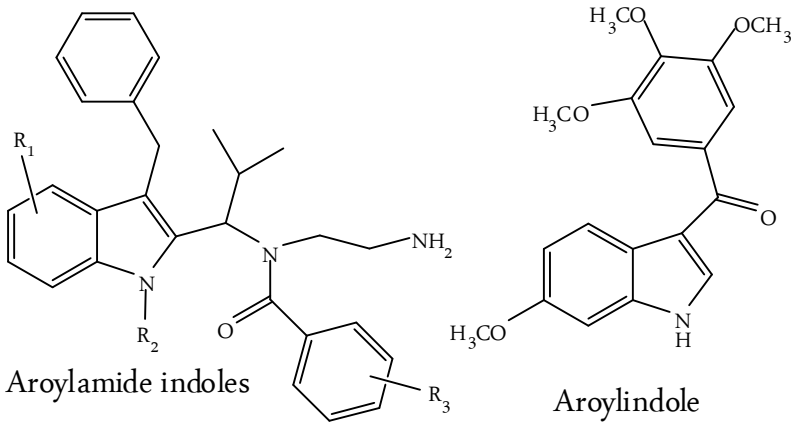


Figure (18): Molecular structure of aroyl- and aroylamide-indoles.

Dragmacidin is an isolated bisindole alkaloid, figure (19), from a deep water marine sponge[101]. Dragmacidin was found to contain two indole groups joined by a piperazine ring system. Dragmacidin exhibited *in vitro* cytotoxicity with IC_{50} values of 15 μg/ml against P-388 cell lines and 1-10 μg/ml against A-549 (human lung), HCT-8 (human colon) and MDAMB (human mammary) cancer cell lines.

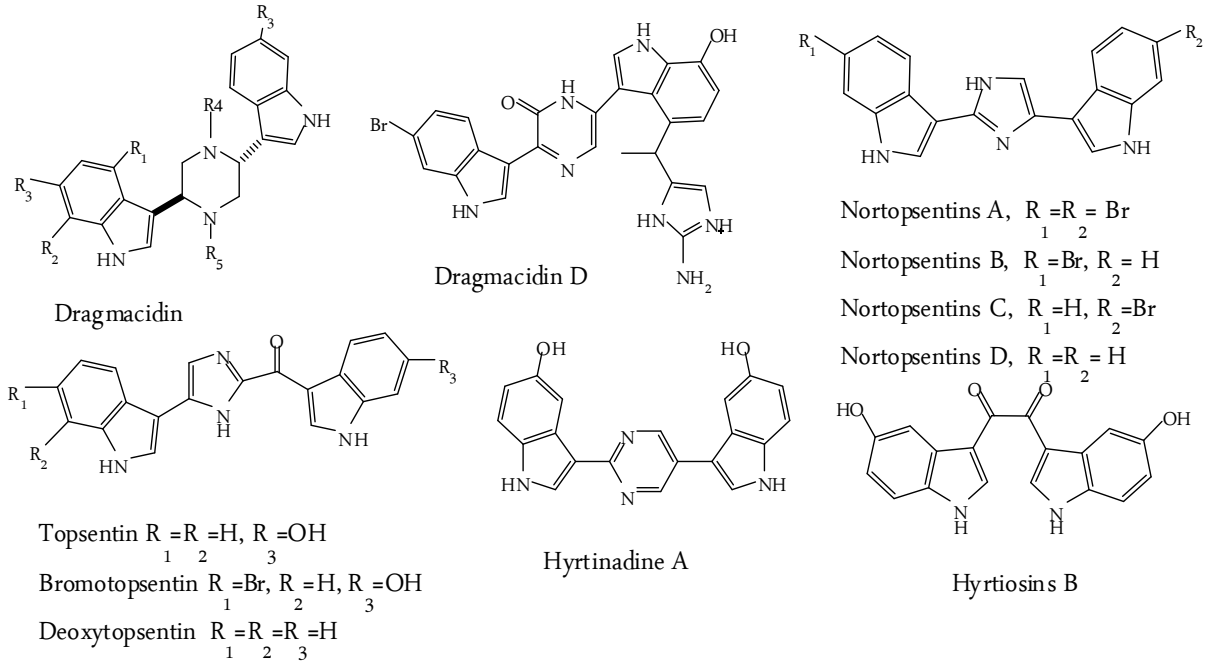
In 1995, *Capon* and et al. reported the isolation of dragmacidin D, figure (19), from a deep water marine sponge Spongosorites collected from the southern Australian coast[102]. Dragmacidin D was found to be active against human lung tumour cell lines and inhibited in *vitro* growth of the P-388 murine and A-549 with IC_{50} values of 1.4 and 4.5 μg/ml respectively[101].

Four new bisindole alkaloids nortopsentins A-D, figure (19), were isolated from the *Caribbean deep sea sponge Spongosorites ruetzleri*[103]. These derivatives of nortopsentins A-D exhibited cytostatic activity against P-388 cells with IC_{50} values of 7.6, 7.8, 1.7 and 0.9 μg/ml, respectively.

Topsentin inhibited proliferation of cultured human and murine tumour cells. It exhibited *in vitro* activity against P-388 with IC_{50} value of 3 μg/ml, human tumour cell

(HCT-8, A-549, T47D) with IC_{50} value of 20 μg/ml and *in-vivo* activity against P-388 (T/C 137 %, 150 mg/kg) and B16 melanoma (T/C 144 %, 37.5 mg/kg)[104]. Bromotopsentin showed ant proliferative activity against human bronocopuemonary cancer cells (NSCLC-N6) with an IC_{50} = 12 μg/ml[105]. Deoxytopsentin[106] showed antiproliferative activity against human bronocopulmanary cancer cells (NSCLC-N6) with an IC_{50} value of 6.3 μg/ml. It also displayed moderate activity against breast cancer and hepatoma (HepG2) with an IC_{50} of 10.7 and 3.3 μg/ml, respectively.

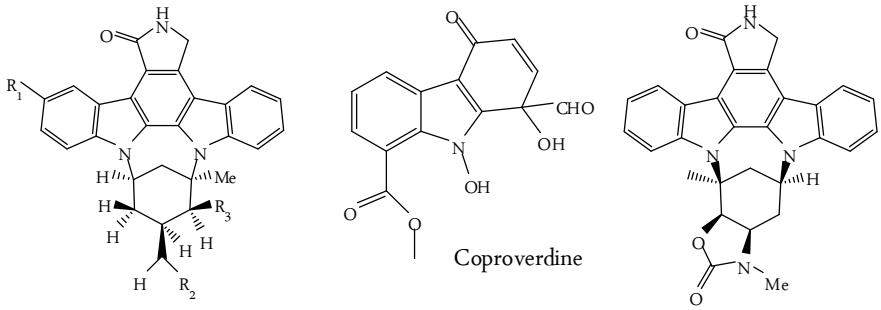
Recently, *Kobayashi* et al. isolated a new cytotoxic bis-indole alkaloid hyrtinadine A, figure (19) from an Okinawan marine sponge Hyrtios sp[107]. Hyrtinadine A exhibited *in-vitro* cytotoxicity against marine leukaemia L-1210 and human epidermis carcinoma KB cells with IC_{50} values of 1.0 and 3 μg/ml, respectively.



Figure(19): Marine natural bis-indole alkaloids as anticancer agents

Schupp et al. isolated two new indolocarbazole alkaloids staurosporines, figure (20) from the marine ascidians Eudistoma toealensis and its predator[108]. *Schupp* et al. evaluated the potential of these staurosporine derivatives as inhibitors of cell

proliferation and macromolecule synthesis[109]. Staurosporine D was found to be the most active staurosporine derivative as MONO-MAC-6 (human monocytic cell lines) inhibitor and inhibitor of RNA and DNA synthesis. The IC_{50} values of staurosporine A, D, E, F for inhibiting MONO-MAC-6 cells were 24.4, 13.3, 33.3 and 29.7 ng/ml, respectively, while those of staurosporine B and C were > 100 μg/ml. The percentage inhibition of RNA and DNA synthesis of compounds staurosporine A and D were 93 and > 98, 98 and > 98, respectively. Staurosporine H inhibited the proliferation of human cancer A-549, BEL-7402, HL-60 cells and mouse leukaemia P-388 cells with the percentage inhibition of 82.6 %, 57.3 %, 76.1 % and 62.2 % in the SRB assay[109]. It also inhibited the proliferation of mouse cancer tsFT210 cells with the inhibition rates of 28.3 % at 21 μM and 20.5 % at 2.1 μM in the SRB assay. Analysis of structure activity relationship demonstrated that hydroxylation of staurosporine at position 3 of the indolocarbazole moiety causes an increase in antiproliferative activity. The position of OH group is crucial to determine the antiproliferative properties of the various staurosporine analogues. A novel carbazole alkaloid, coproverdine, figure (20), was isolated from an unidentified ascidians Anchorina sp. collected from the north Island of New Zealand[113]. Coproverdine was evaluated against a variety of murine and human tumour cell lines such as P-388, A-549, HT-29, MEL-28 and DU-145 exhibiting IC_{50} values of 1.6, 0.3, 0.3, 0.3 and 0.3 μM, respectively.

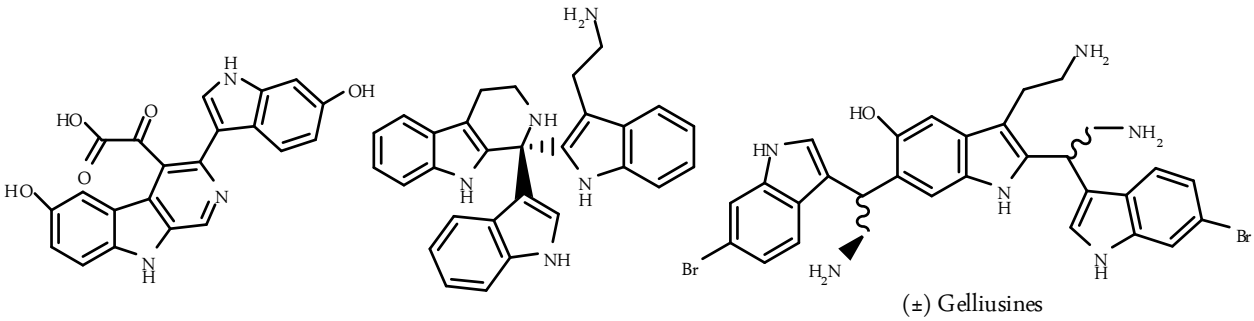


Staurosporine A: R_1=H, R_2=CH_3, R_3=OCH_3, R_4=H
Staurosporine B: R_1=OH, R_2=CH_3, R_3=OH, R_4=H
Staurosporine C: R_1=H, R_2=H, R_3=OCH_3, R_4=OH
Staurosporine D: R_1=OH, R_2=CH_3, R_3=OCH_3, R_4=H
Staurosporine E: R_1=H, R_2=CH_3, R_3=OH, R_4=H
Staurosporine F: R_1=H, R_2=H, R_3=OH, R_4=H

Figure (20):Chemical structures of marine natural products, staurosporines and coproverdine.

The hyrtioerectine alkaloid A, figure (21), was isolated from a red coloured marine sponge Hyrtios erectus[111]. Hyrtioerectines A was evaluated for its cytotoxicity against HeLa cells and showed moderate cytotoxic activity with IC_{50} value of 10 μg/ml. *Foderaro* et al. reported the isolation of a new tetrahydro-β-carboline alkaloid, figure (21) bengacarboline from t*he Fijian ascidians Didemnum sp*[112]. Bengacarboline was found to be cytotoxic towards a 26 cell line human tumour panel in vitro with a mean IC_{50} value of 0.9 μg/ml and also inhibited the catalytic activity of topoisomerase II at 32 μM.

In 1994, *Bifulco* et al. reported the isolation of two tris-indole alkaloids, Gelliusines A and B, figure (21) from a deep water new Caledonian sponge Gellius or Orina sp[113]. Gelliusin A and B were found to be diastereomeric compounds made up by the coupling of three indole units in which two 6-bromo tryptamine units are linked through their aliphatic chains to the C-2 and C-6 position of a central serotonin moiety. The coupling of the indole unit appears to be nonstereoselective giving two enantiomeric pairs, having different relative configuration at C-8 and C-8 named (±) Gelliusines A and B. Gelliusines A and B showed cytotoxicity with an IC_{50} value of between 10 and 20 μg/ml against KB, P-388, P-388/dox, HT-29 and NSCLCN-6 cell lines.



Figure(21): Molecular structures of Hyrtioerectine A, Bengacarboline and (±) Gelliusines.

Dendridine A, figure (22), a unique C_2-symmetrical 4,4′-bis(7-hydroxy)indole alkaloid was isolated from an Okinawan marine sponge Dictyodendrilla[114]. It exhibited moderate cytotoxicity against murine leukaemia L-1210 cells with IC_{50} value of 32.5 μg/ml Chetomin; figure (22) was identified as natural product antitumor compound which inhibited the formation of the HIF-1, P300 complex. Systemic administration of chetomin inhibited hypoxia-inducible transcription within tumours and inhibited tumour growth[115].

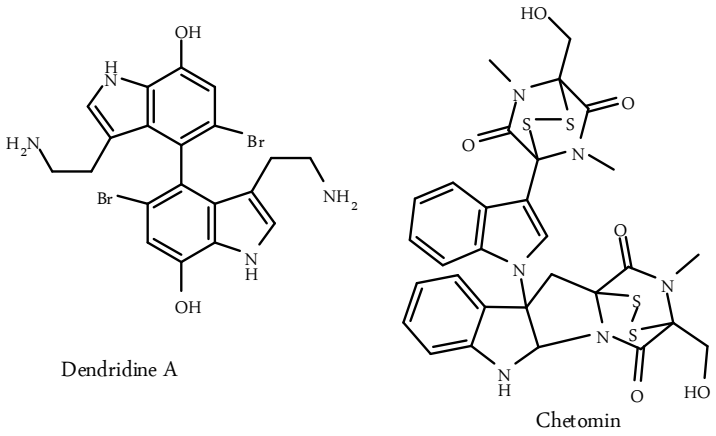
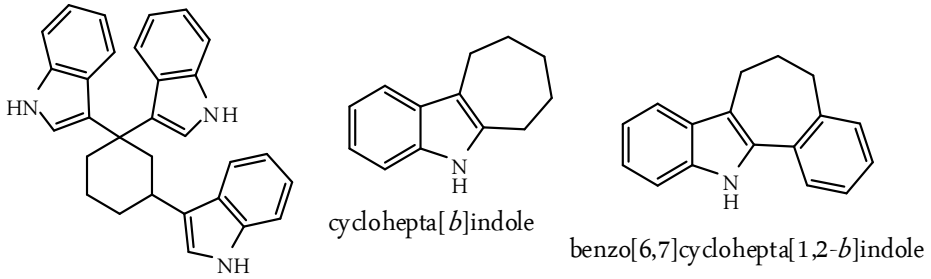


Figure (22): Chemical structure of Dendridine A and Chetomin

Recently *Lee* and co-workers have found that 1,1,3-tri(3-indolyl)cyclohexane, figure (23), inhibits cancer cell growth in lung cancer cell of xenograft models[116]. Thus it is a potential anticancer compound based on its strong tumour growth inhibition with favourable pharmacologic properties. In addition, it increases the production of reactive oxygen species (ROS) and triggers DNA damage[116]. Cyclohepta[*b*]indole and benzo[6,7]cyclohepta[1,2-*b*]indole, figure (23) were subsequently screened for cytotoxic

activity against human nasopharyngeal carcinoma (HOME-1) and gastric adeno carcinoma (NUGC-3) cell lines, where the result show significant cytotoxic activity at a concentration of 4 μg[117].



3,3',3''-(cyclohexane-1,1,3-triyl)tris(1*H*-indole)

cyclohepta[*b*]indole

benzo[6,7]cyclohepta[1,2-*b*]indole

Figure (23): Chemical structure of some cycloalkano indoles have anticancer activity.

2. Objectives of this work

Synthetic objectives:

The first aim of this work was the using of an aliphatic dialdehydes and indoles for the synthesis and elucidation of a novel highly substituted diastereromeric tetrahydrocyclopenta indoles, tetrahydrocarbazoles, hexahydrocyclohepta and hexahydrocycloocta indoles with triindole substituents in the form of *cis* or *trans* compounds $\mathbf{2}_{\mathbf{a-j}}$ and a tetraindole of propane, pentane and hexane compounds $\mathbf{3}_{\mathbf{a-j}}$, figure (24). In addition to investigate some chemical reactivity of these compounds for example the acetylation and the oxidation reactions.

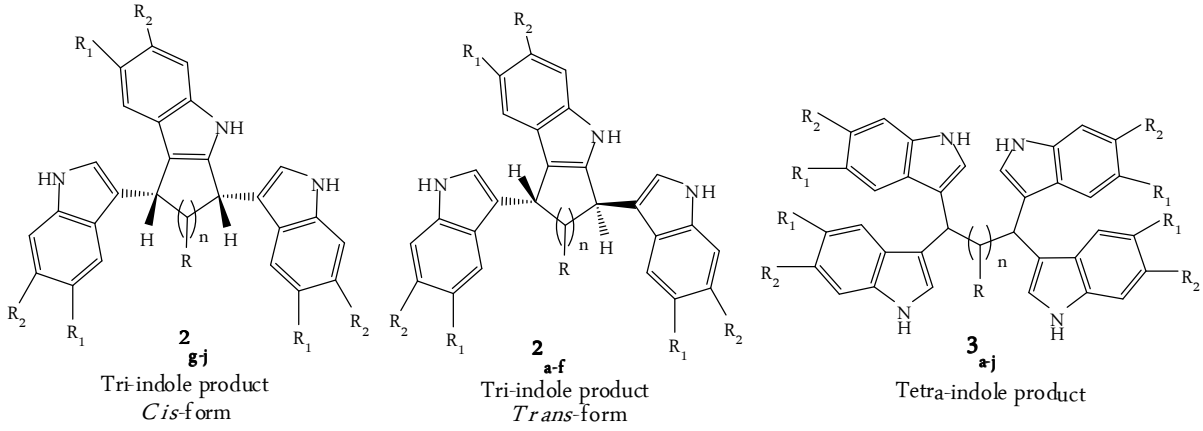


Figure (24): Structure of compounds 2 and 3.

The second aim of this work was to determine the reaction products (compounds $\mathbf{7}_{\mathbf{a,b}}$, $\mathbf{8}_{\mathbf{a,b}}$, **10**, **11** and **13**), figure (25), which have been formed from the application of the aromatic dialdehydes e.g. *o*-phthalaldehyde, homophthalaldehyde and tarphthalaldehyde with indoles under the same reaction conditions. And further condensation, acetylation and oxidation of some of these products have been applied.

The third objective of this work was a series of substituted aryl or heteroaryl aldehydes were efficiently converted to the corresponding BIMs **17**$_{a-p}$, table (1). And as an extending study of the work, the prepared BIMs **17**$_{a-p}$ were used as a starting materials for the synthesis of new biologically active tetrahydroindolo[2,3-*b*]carbazoles of type **18**$_{a-m}$, novel of 4-(8-(3-(benzyloxy)-4-methoxyphenyl)1,1a,2,2a,3,7b,8,8a-octahydroindolo[2,3-*b*]carbazol-2-yl)-*N*,*N*-dimethylaniline (**19**) and the novel spirocyclic biscarbazoles **20**. Some BIMs were oxidized affording bisindolylmethenes **21**$_{a-k}$ and its salts **22**$_{a,b}$, figure (26).

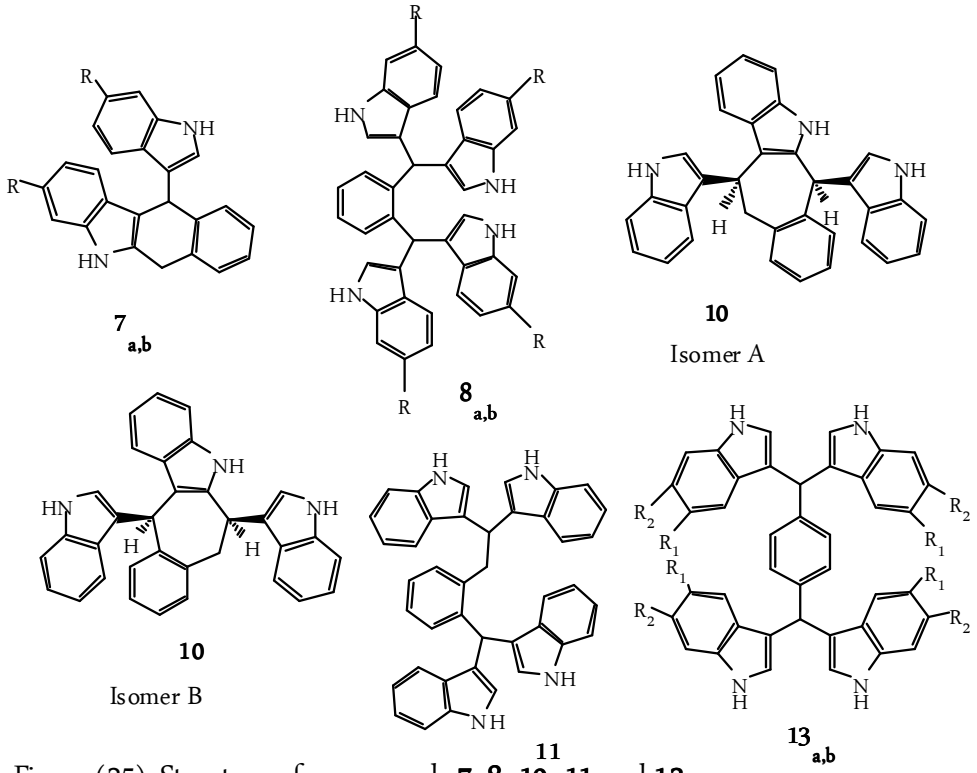


Figure (25): Structure of compounds **7**, **8, 10**, **11** and **13**.

The fourth aim of this work was extended a similar electrophilic condensation of indoles with other carbonyl compounds included different types of ketones e.g. heteroacetyl ketones (3-acetylindole and 3-acetylpyridine), cyclohexanone, isatin, cyclohexane-1,4-dione and ninhydrin as a possible way for the synthesis a novel spirocyclic structures **26**, **27**, **30**, **32**, **33** and **34**, figure (27).

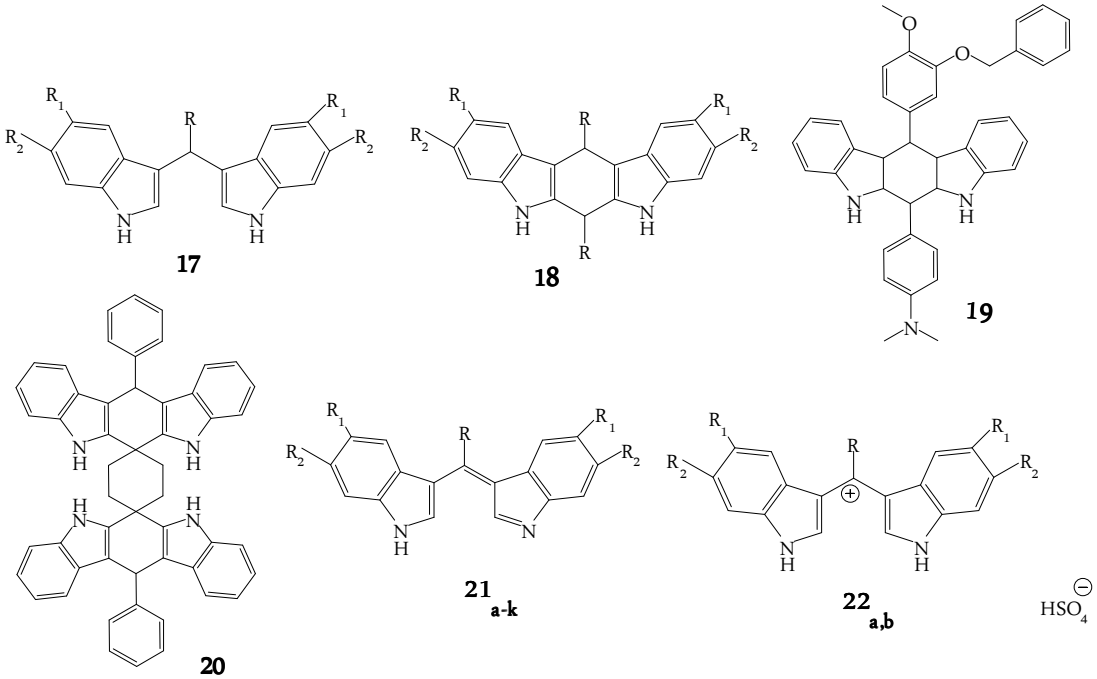


Figure (26): Structure of compounds **17, 18, 19, 20, 21** and **22.**

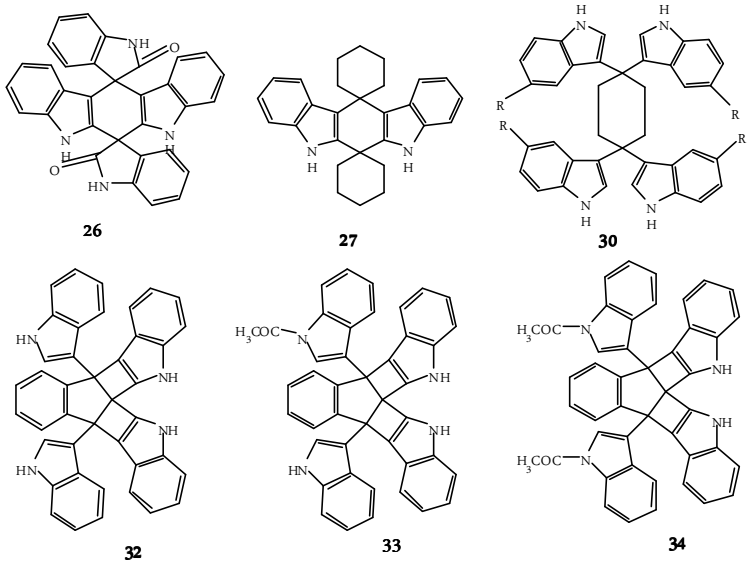


Figure (27): Structure of compounds **26, 27, 30, 32, 33** and **34.**

Pharmacological objectives:

Antimicrobial assays:

Some selected indole compounds, figure (28), were tested for their *in vitro* growth inhibitory activity against Candida albicans ATCC 10145 as fungus, S. aureus ATCC

25923, Bacillus subtilis ATCC 6633, MRSA standard ATCC 43300 and MRSA isolate as Gram-positive bacteria and E. coli ATCC 23556 as Gram-negative bacteria.

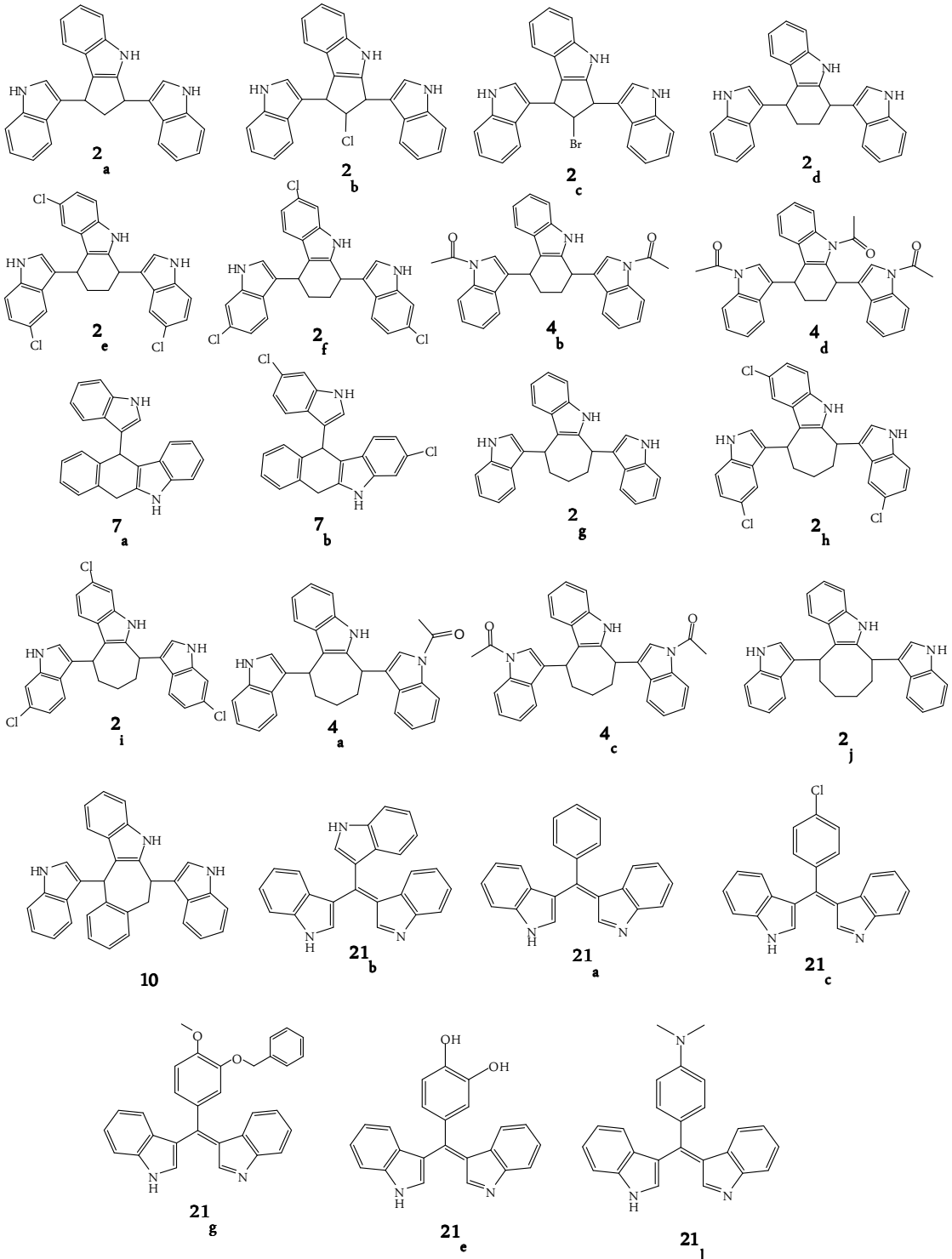


Figure (28): Selected compounds for antimicrobial tests.

Anticancer screen

Ten substances have been selected by the NCI for one dose screening which were ($\mathbf{17}_{e,g,i,j,l}$) and ($\mathbf{18}_{d,f,h,i,l}$), figure (29) and figure (30).Compounds $\mathbf{17}_{j}$ and $\mathbf{18}_{d}$ were further selected for the five screening to further characterize the ant proliferative activities.

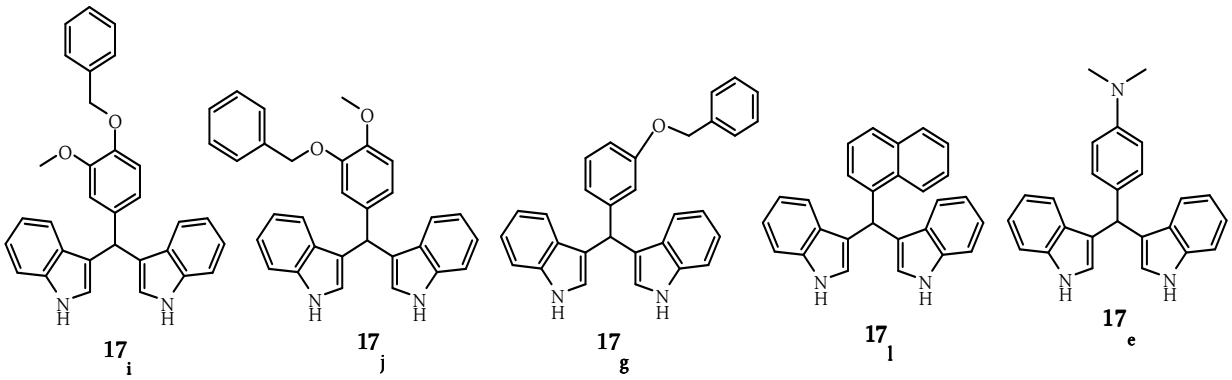


Figure (29): Selected BIMs (**17e,g,i,j,l**) for NCI screenings.

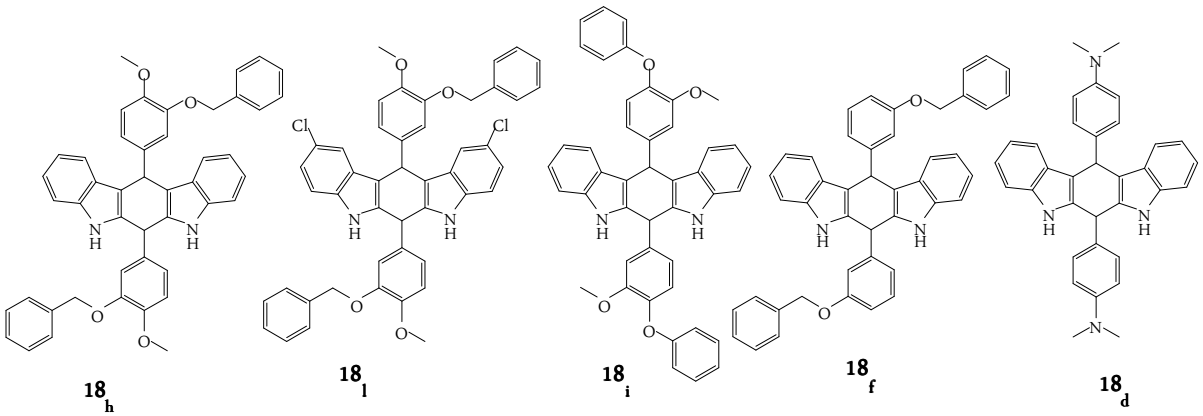
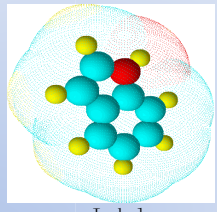
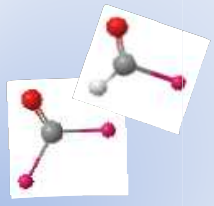


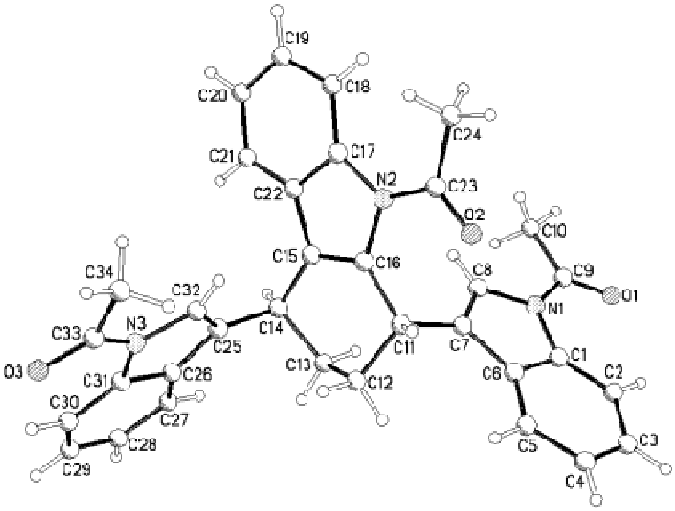
Figure (30): Selected indolocarbazoles ($\mathbf{18}_{d,f,h,i,l}$) for NCI screening.

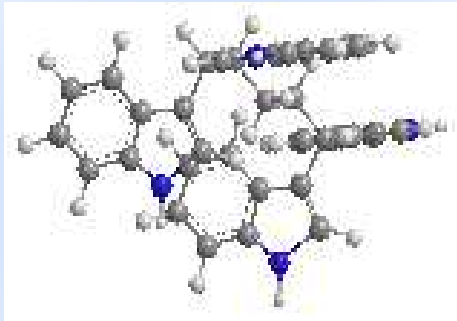


Indole

Synthesis Part







3. Results and Discussion

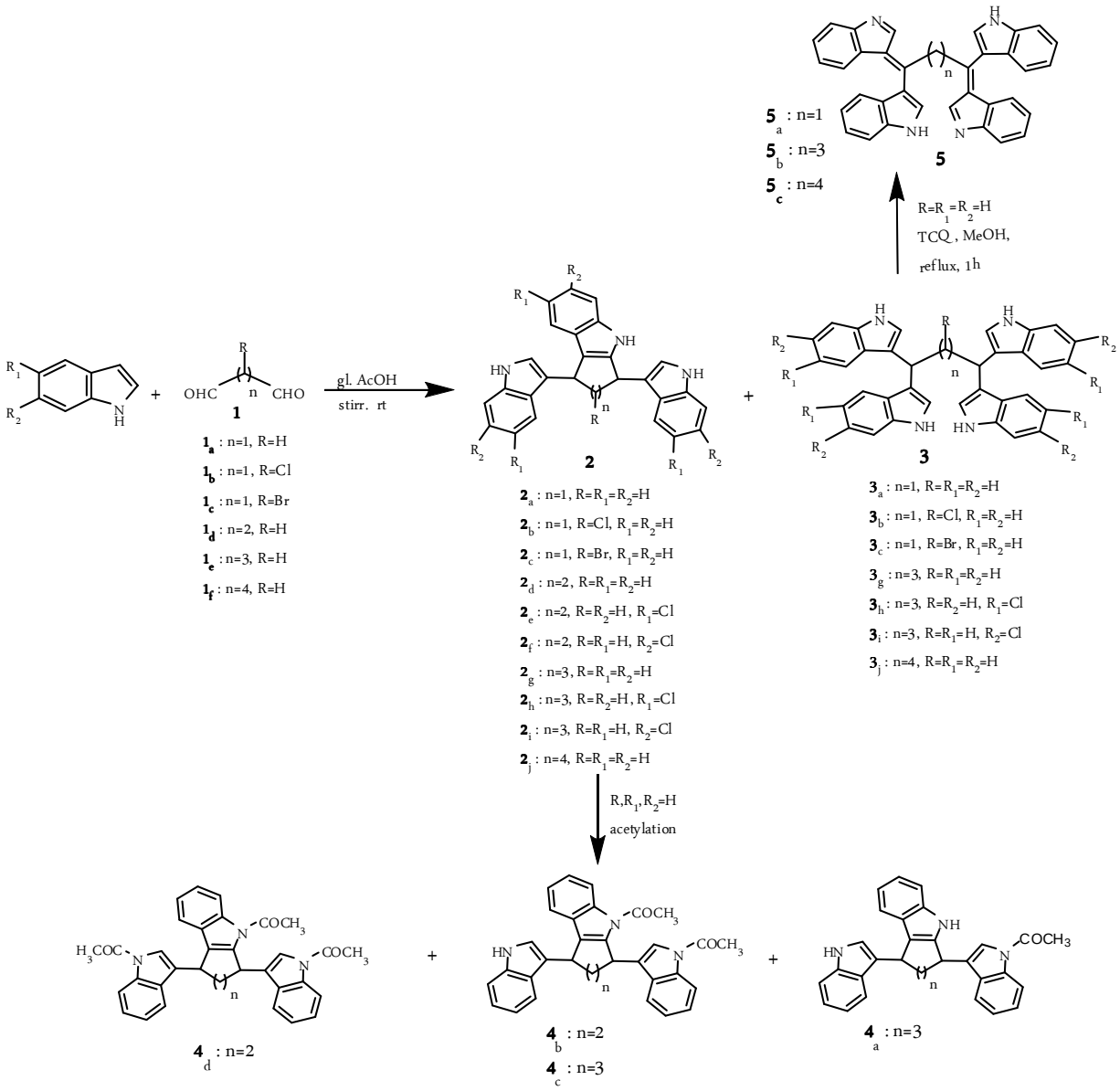
3.1. Synthetic Results

The electron rich indole nucleus shows an enhanced reactivity towards carbon electrophiles that generally results in the formation of three substituted indole derivatives[118]. The 3-position of the indole is the preferred site for the electrophilic substitution reactions. 3-Alkyl or 3-acyl indoles are versatile intermediates for the synthesis of a wide range of indole derivatives[119]. A simple and direct method for the synthesis of 3-alkylated indoles involves the condensation with aliphatic or aromatic aldehydes. Normally these reactions occur in presence of several types of catalysts for example protic or Lewis acids. Protic acids used to catalyze the reaction for example silica sulphuric acid (SSA)[120], oxalic acid[121], zeolites HY[122,123] and ZnY[124], amberlyst[125,126], HBr[127,128], HCl[129,130], HCOOH[131], CH_3COOH[132,133], *p*-TsOH[134], $NaHSO_3$[135], $KHSO_4$[136], H_3PO_4-SiO_2[137] etc. Lewis acids are lanthanide resins[138], zeolite (ZnY)[139], bentonic clay/IR[140], montmorillonite clay K-10[141,142], cerium ammonium nitrate (CAN)[143], $ZrCl_4$[144], $IndF_3$[145], $Bi(OTf)_3$[146], $TiCl_4$[147], $Al(OTf)_3$[148] etc. As seen from these reported literatures numerous catalysts can efficiently promote the reaction of aldehydes or ketones and indoles afforded 3-alkylated indole compounds in good to high yield in a reasonable time.

In the present work we wished to introduce AcOH as a mild and efficient catalyst for the synthesis of novel highly substituted diastereromerics tetrahydrocyclopentaindoles, tetrahydrocarbazoles, hexahydrocyclohepta indoles and hexahydrocycloocta indoles with triindole substituents as a minor product and a tetraindole of propane, pentane and hexane as the major product. Our reaction meant the introduction of a novel and simple chemical reaction method that has not been reported in literature before. We have divided the whole work of this thesis into four different schemes depend on the type of the carbonyl compounds that reacted with indole or its derivatives. The first scheme will be for the reactions of indoles with different aliphatic dialdehydes and some further reactions on the products. And the

second was for the same reactions with aromatic dialdehydes whereas the third was for the reactions with aryl and heteroaryl aldehydes and the last one involved the reactions with ketones as a new method for the synthesis of novel indolospirocyclic compounds.

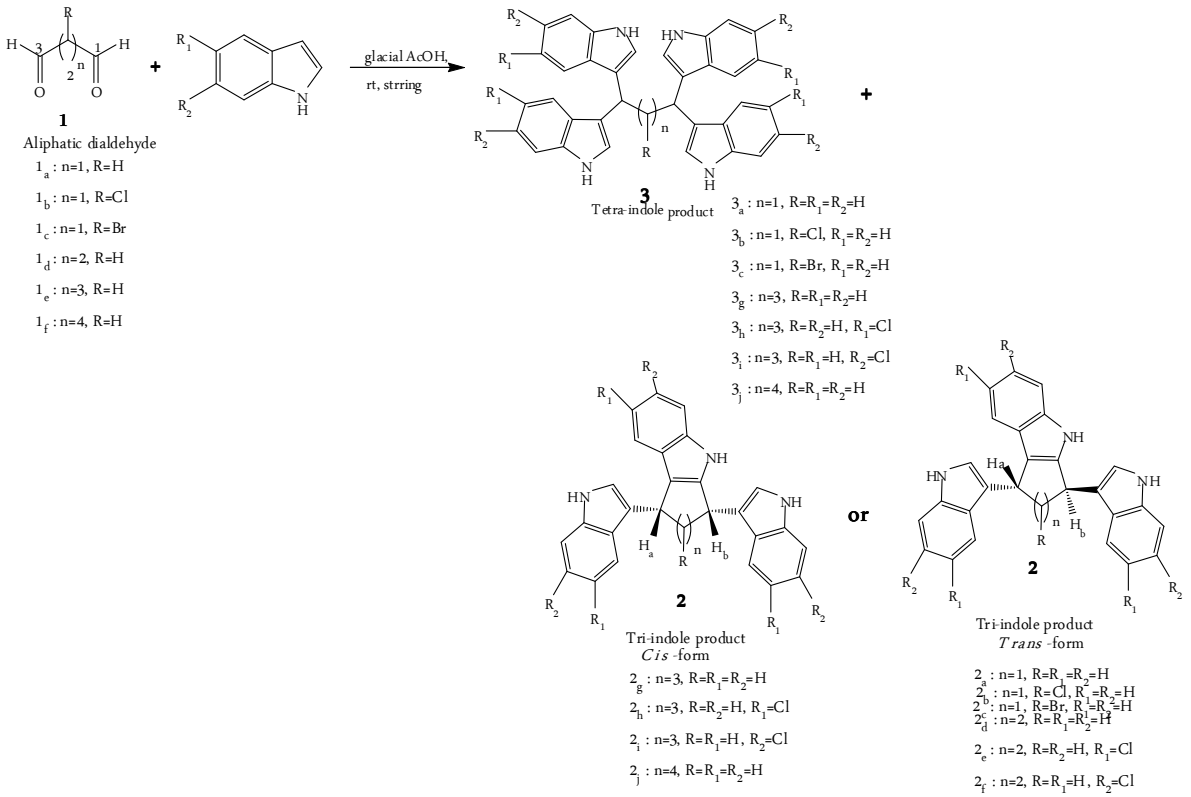
3.1.1. Electrophilic substitution reactions of indoles with aliphatic dialdehydes



Schme (1): The whole scheme of electrophilic substitution reactions of indoles with aliphatic dialdehydes.

Starting with the first part which involved the reactions of indoles with aliphatic dialdehydes, scheme (1). The reactions were accomplished by mixing an aliphatic

dialdehyde ($\mathbf{1}_{a\text{-}f}$) for example malonaldehyde and its derivatives, succinaldehyde, glutaraldehyde and adipaldehyde with indoles in glacial acetic acid to react smoothly at room temperature under stirring overnight, until the reaction solution became dark. The formation of our two products was detected by TLC. The reaction gave two products compound $\mathbf{2}_{a\text{-}c}$ as a minor product appeared at higher R_f values and compound $\mathbf{3}_{a\text{-}c}$ at lower R_f values than indole or its derivatives. The TLC in CH_2Cl_2 (100 %) showed the higher spot for indole, the middle spot for compound $\mathbf{2}_{a\text{-}c}$ and the lower spot for compound $\mathbf{3}_{a\text{-}c}$ as a major product,. Cycloalkano indoles of type **2** have been isolated in either *cis* or *trans* form. In the case of succindialdehyde the reaction did not afford the tetraindole product and the only product was the triindole with a high yield. The overall reaction equation can be summarized in scheme (2).

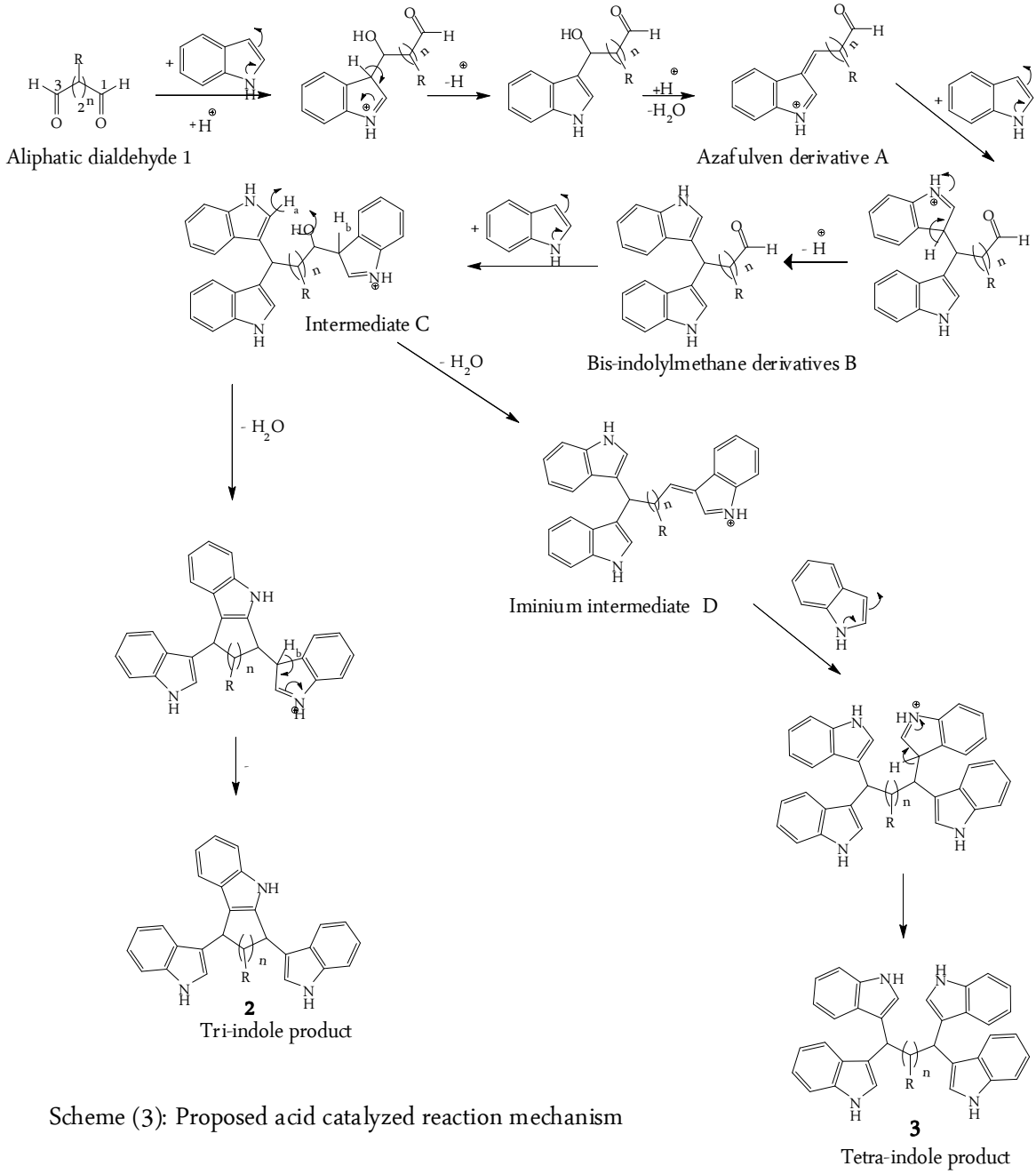


Scheme (2): General equation for the reaction of indoles with aliphatic dildehydes.

Although, there are several types of bis-indolylmethanes known, which were made in presence of several types of catalysts our tetraindole products considered as types of

bis-indolylmethane derivatives are novel compounds have not reported yet which were identified on the basis of their analytical and spectral data.

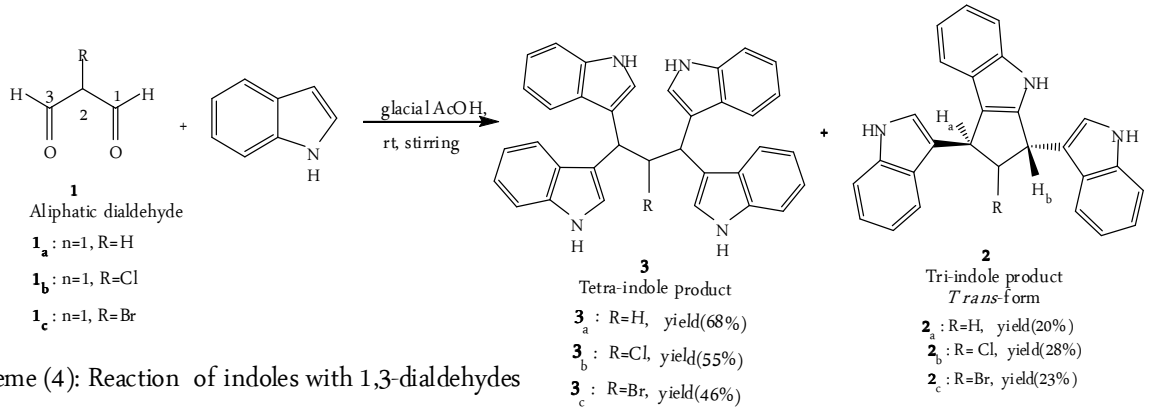
A plausible mechanistic pathway for the formation of compounds **2** and **3** is shown in scheme (3). The acid catalyzed reaction of electron rich indoles with aliphatic dialdehydes (**1**) produces *azafulven* derivative A, according to the *Ehrlich test* mechanism[149]. The azafulven A undergoes a further addition with a second indole molecule to give bis-indolylmethane derivative B[150,151,152]. A condensation step with the third indole molecule leads to the formation of the intermediate C. Intermediate C, passed through two different expected pathways to form compounds **2** and **3**. In the first pathway intermediate C undergo as a successful intermolecular condensation and cyclization with α-indole H_a giving the cycloalkanotriindole product (**2**)[153-158]. In the second pathway intermediate C follows condensation with 3-indole proton H_b to form the unsaturated iminium intermediate D which provides the tetraindole product (**3**) *via* a successful addition of the fourth indole molecule. The α-electrophilic attacks in the indole units leading to cyclisation have been reported in several indole reactions yielding indole alkaloids[159,160].



Scheme (3): Proposed acid catalyzed reaction mechanism

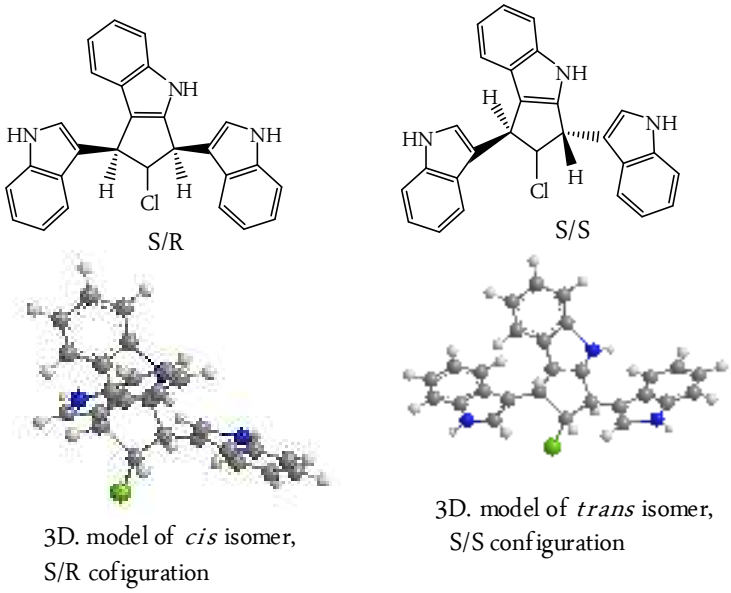
3.1.1.1. Reaction with malonaldehyde and its derivatives

Malonaldehyde itself is not commercially available It is available in the form of malonaldehydebisdimethylacetal which is converted into malonaldehyde in an acidic solution. However 2-chloromalonaldehyde and 2-bromomalonaldehyde are commercially available. The reaction of malonaldehydes ($\mathbf{1_{a-c}}$) with indole, scheme (4) gives the diastereomers (*trans*-form) of the tetrahydrocyclopenta indolo compound $\mathbf{2_{a-c}}$ with the formation of tetraindole $\mathbf{3_{a-c}}$.



Scheme (4): Reaction of indoles with 1,3-dialdehydes

The ^{1}H-NMR spectra of $\mathbf{2_{a-c}}$ showed the presence of one signal for both H_a and H_b which indicated that the isolated form of $\mathbf{2_{a-c}}$ is the *trans*-form this by referring to the X-ray crystallography of compound $\mathbf{4_d}$ that will later show in figure (36). Which confirmed that both the C-atoms with H_a and H_b have the same configuration *S/S* as well as compound $\mathbf{2_{a-c}}$ are expected to be *S/S* configuration. This is the reason for the two hydrogen protons H_a and H_b have one signal for both in the ^{1}H-NMR spectrum of these compounds because they are identically protons have the same configuration. Thus we can conclude that the substituted cyclopenta indolo structures $\mathbf{2_{a-c}}$ prefer to give the *trans* isomer in which the two bulky residues of indole units have a less satirical hindrance because they are far apart from each other compared to *cis*. This may be noticed from the 3 dimensional model of *cis* and *trans* compound $\mathbf{2_b}$ as an example of these derivatives, figure (31).



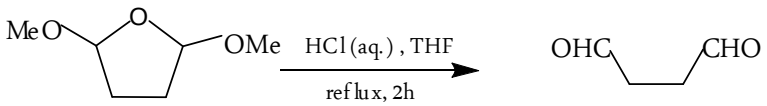
3D. model of *cis* isomer, S/R cofiguration

3D. model of *trans* isomer, S/S configuration

Figure (31): Three dimentional models of *cis* and *trans* of compound $\mathbf{2_b}$.

3.1.1.2. Reaction with succindialdehyde

Succinaldehyde ($\mathbf{1_d}$) has been prepared from 2,5-dimethoxytetrahydrofuran according to *Alan Arnstrong*[161], scheme (5).

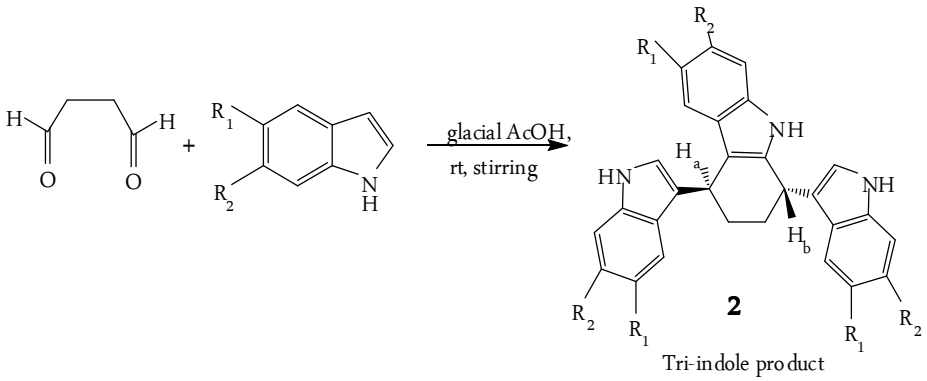


2,5-dimethoxytetrahydrofuran $\mathbf{1_d}$

Scheme (5): Synthesis of succinaldehyde.

Succinaldehyde condensed with indoles to form highly substituted tetrahydrocarbazoles of type $\mathbf{2_{d\text{-}f}}$ in a high yield. *Ming-Zhong*[162] and et al. reported that acetylenic aldehydes react with indoles to form diastereomeric tetrahydrocarbazoles (*trans* and *cis*) in presence of AgOTf as a catalyst. These tetrahydrocarbazoles were prepared also by the reaction of indoles with hexan-2,5-dione in ethanol containing toluene-*p*-sulphuric acid[163]. The new strategy of our novel chemical reaction to synthesize a highly substituted tetrahydrocarbazoles by the reaction of aliphatic dialdehyde (succinaldehyde) with indoles in acetic acid at room temperature has not been reported yet. The reaction yielded only the triindole products $\mathbf{2_{d\text{-}f}}$ in high yields (77

– 85 %) and the isolated form was only the *trans* isomer without the formation of a possible tetraindole product similar to malonaldehydes and its derivatives, scheme (6). This behaviour can be explained in the scheme (3) by cyclization step to form the triindole product of a sixmembered ring which expected to be faster and easier than the condensation step to form the iminium intermediate D which leads to the formation of the tetraindole product. The Sixmembered rings are more stable than 5-,7- and 8-membered rings. This stability may be confirmed by the higher yields of the formed compounds **2**$_{\mathbf{d\text{-}f}}$ (77 % - 85 %) compared with the yields of the 5-membered rings (20 % - 32 %).



Scheme (6): Reaction of indoles with succinaldehyde.

^{1}H-NMR spectrums of these tri-indoles indicated the presence of one signal multiplite for both H_a and H_b and this in agreement with the ^{1}H-NMR spectrum of its triacetylated derivative **4**$_{\mathbf{d}}$, figure (36) (will mention latter), which confirmed by X-ray single crystal as *S/S* configuration. Refering to the X-ray of compound **4**$_{\mathbf{d}}$ The two protons H_a and H_b in the tri indoles **2**$_{\mathbf{d,e,f}}$ should configurated as pseudoaxial/pseudoaxial and the two indole units should configurated as pseudoequatorial/pseudoequatorial. Acorrding to the rules of cyclohexene conformation the *trans* form of 3,6-disubstituted cyclohexene the *trans* form consists of (pseudoaxial/pseudoaxial) or (pseudoequatorial/ pseudoequatorial). Figure (32) illustrate the pseudoaxial/ pseudoequatorial orientations in the most stable conformation (half-chair) of cyclohexene[164(a)]. Where the smaller

groups (H_a and H_b) tend to be pseudoaxial and the bulky groups (the indole units) tend to be pseudoequatorial in order to decrease the interactions and the stiric hindrance. And this in agreement with the conformation of the compound **4_d** as shown from its X-ray single crystal. Thus *trans* isomer of **2_{d-f}** is stable and logically to be formed rather than the *cis* isomer.

^{1}H-^{1}H COSY spectrum of compound **2_d** showed ^{1}H-^{1}H correlation between the two NH indole proton signals at δ = 10.67 ppm and 10.88 ppm values and the two adjucent aromatic CH protons (CH pyrroles) at δ = 7.05 ppm and 7.18 ppm. Whereas the NH indole signal at 10.42 ppm has not any ^{1}H-^{1}H correlation cross peaks with adjucent protons. These data confirmed that the NH indole proton signals at 10.42 ppm is belonging to the NH indole of the fused indole unit fused with sixmemmberd ring "NH of the tetrahydrocarbazole" and othe two NH indole proton signals is related to the free indole units.

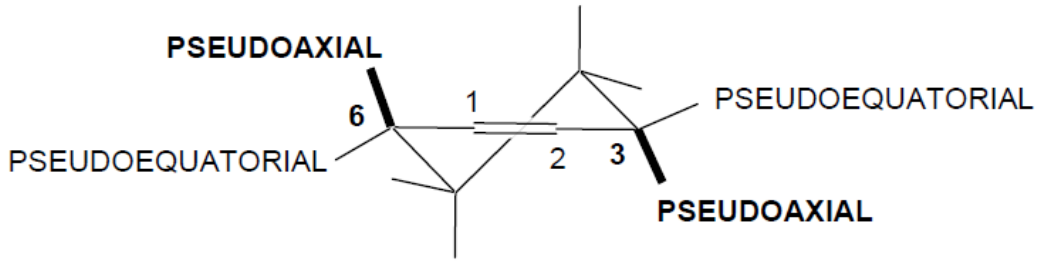
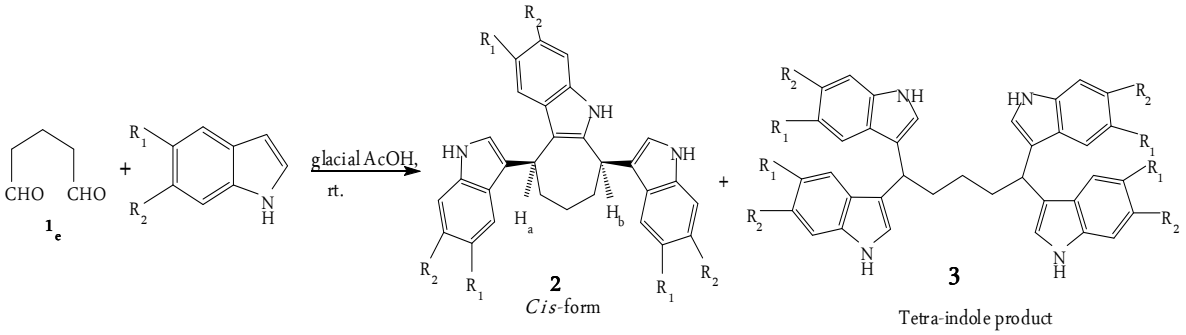


Figure (32): Pseudoaxial/Pseudoequatorial orientations in cyclohexene half-chair form.

3.1.1.3. Reaction with glutaraldehyde

Similar to malonaldehyde or its derivatives, glutaraldehyde in a 40 % solution reacts with indoles to form hexahydrocycloheptaindole derivatives of the type **2_{g-i}** in low yields of (18 % - 25 %) and the tetraindole product of the type **3_{g-i}** in a high yield of (47 % - 65 %) as shown in scheme (7). The only isolated form of compounds **2_{g-i}** is the *cis*-isomer which could be derived from the ^{1}H-NMR of compounds **2_{g-i}** with one signal for H_a and one signal for H_b this confirmed that the two chairal carbon atoms with C-H_a

and C-H_b have two different configurations of *R/S* or *S/R*. So that compound **2_g** can defined as *(6R,10S)-6,10-di(1H-indol-3-yl)-5,6,7,8,9,10-hexahydrocyclohepta[b]indole* or (6S,10R)-6,10-di(1H-indol-3-yl)-5,6,7,8,9,10-hexahydrocyclohepta[b]indole. Figure (33) showed the two possible configurations of compound **2_g** and the expected three dimensional pictures. In this case of using glutaraldehyde *cis*-isomer product is more stable than *trans*-isomer product this can be referred to the fact that the *cis*-cycloheptene is always assumed due to the double bond in the *trans* isomer is very strained because of the resulting ring strain and angle strain (http://en.wikipedia.org/wiki/Cycloheptene). These compounds were characterized and identified based on their analytical and spectral data. Figures (34) and (35) illustrated the difference between ^{1}H-NMR spectra of compound **2_e** as an example of *trans*-isomer and **2_g** as example of *cis*-isomer, for more spectra see the appendix.



Scheme (7): Reaction of indoles with glutaraldehyde.

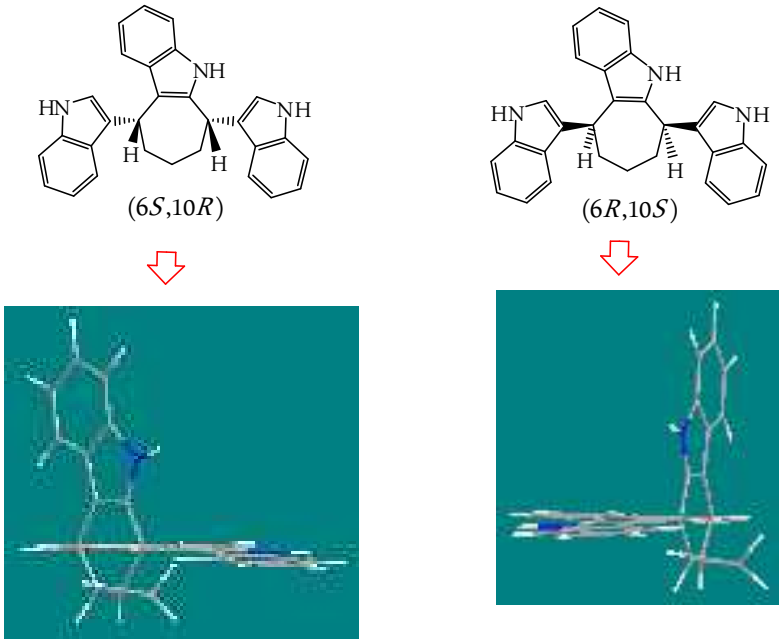


Figure (33): Expected two possible configurations of **2g**.

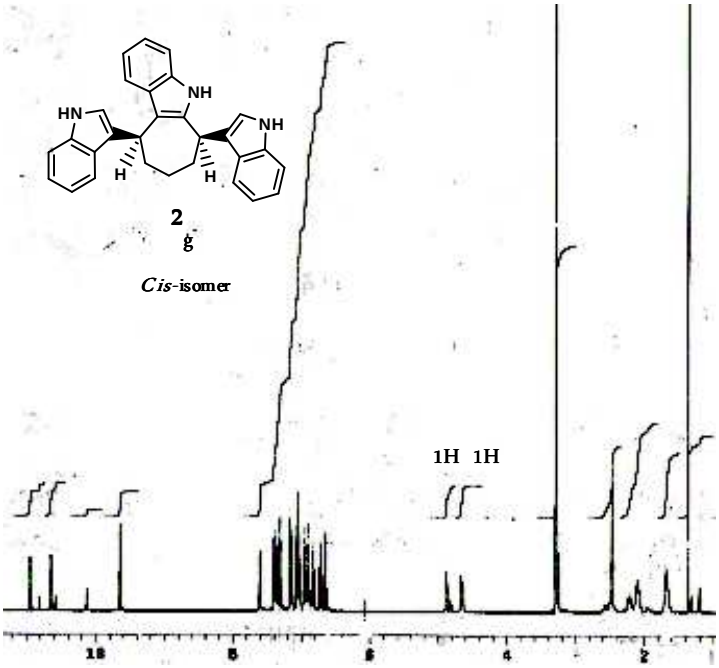


Figure (34): ^{1}H-NMR spectra of compounds **2g** in DMSO-*d6*.

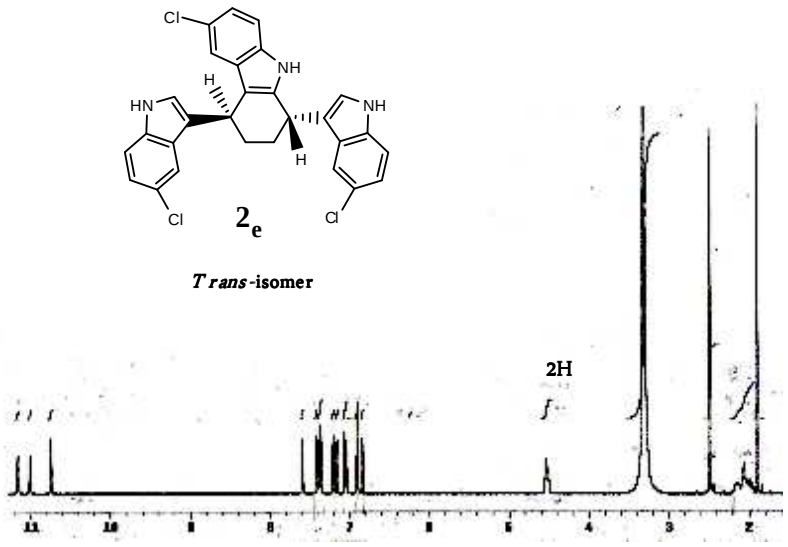
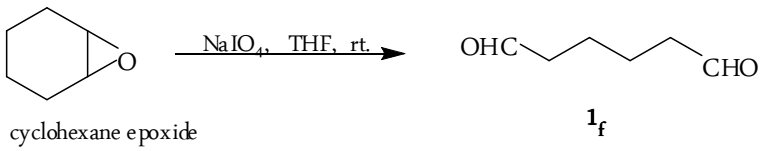


Figure (35): ^{1}H-NMR spectra of compounds **2**$_e$ in DMSO-*d6*.

3.1.1.4. Reaction with adipaldehyde

Adipaldehyde (**1**$_f$) was synthesized by sodium meta preiodate oxidation of cyclohexane epoxide according to the reported case[165], scheme (8).

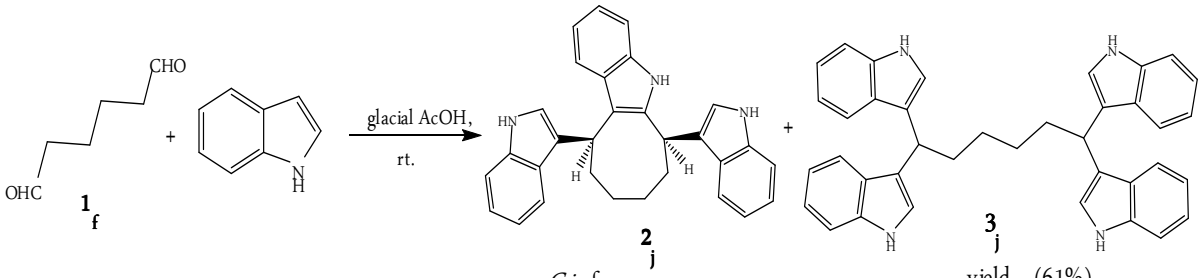


cyclohexane epoxide

1$_f$

Scheme (8): Synthesis of adipaldehyde

Adipaldehyde (**1**$_f$) reacts as well as glutaraldehyde and a malonaldehyde affording the *cis*-isomer (in either *S/R* or *R/S* configuration) of hexahydrocycloocta[*b*]indole derivative **2**$_j$ in 27 % yield and 3-(1,6,6-tri(1*H*-indol-3-yl)hexyl)-1*H*-indole (**3**$_j$) in a yield of 61 % see scheme (9). The isolation of the *cis*-octene dreivative rather than trans-octene may be referred to the fact of the commercially available stereoisomer is *cis*-cyclooctene which is more stable than the *trans*-isomer. Because of the *trans*-cyclooctene has a high ring-strain where the energy of the *trans*-form is significantly higher than that of the *cis*-form[164(b)].



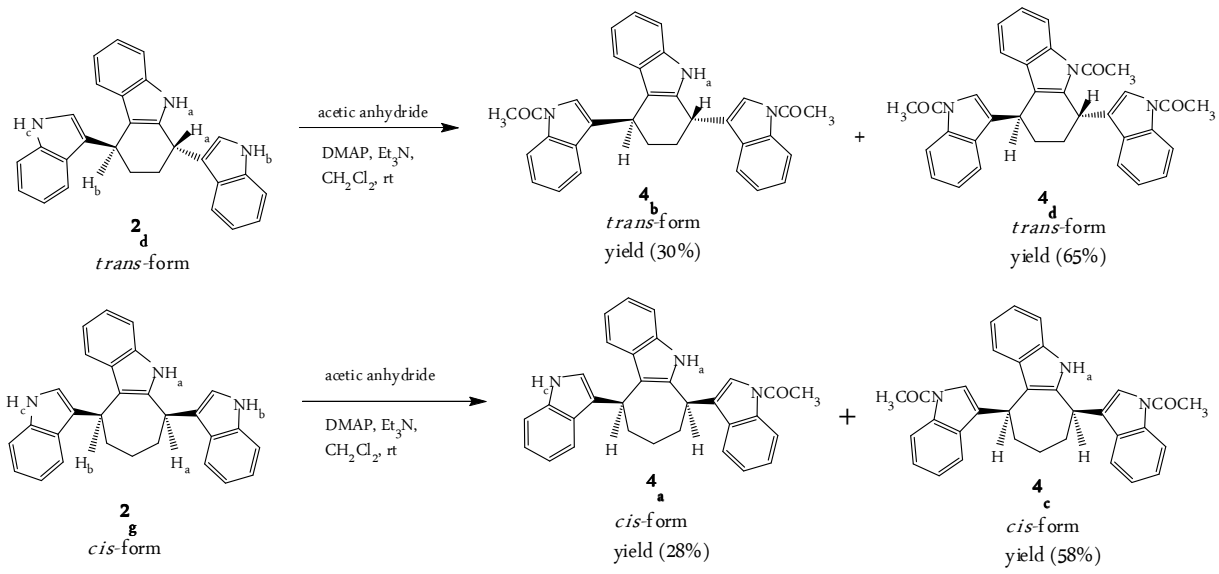
Scheme (9): Reaction of indole with adipaldehyde

From the overall reactions of aliphatic dialdehydes with indoles we summarize that, the diastereomer *trans* isomer is preferable for the smaller ring size (n = 1, 2), in case of 5-membered, and 6-membered rings. The *trans*-form (in *S/S* configuration) is more stable to avoid the steric hindrance from the two bulky indole residues. While in 7- and 8-membered rings the *cis*-isomer (in *S/R* or *R/S* configuration) is more stable because of the two indole units are far from each other.

3.1.1.5. Acetylation reaction of triindole product

The acetylation reaction of the triindole products **2$_d$** and **2$_g$** have been accomplished with acetic anhydride in dichloromethane in presence of 4-(dimethylamino)pyridine (DMAP) as catalyst [166]. The reaction mixture was left stirring at room temperature for several weeks, while the product formation was monitored by TLC. After about eight weeks the reaction mixtures were worked up and the products were isolated, purified by column chromatography and identified by their analytical and spectral data. The acetylation reaction of the *trans* isomer of compound **2$_d$** leads to the formation of the diacetylated product **4$_b$** and the triacetylated derivative **4$_d$**. Based on the spectral data, 1D- and 2D-NMR spectrums, the two NH indole that acetylated were NH_b and NH_c. ^{1}H-NMR spectrum of **4$_b$** which showed only one NH proton at 10.72 ppm, which was supported to be the NH_a with some shift to a higher ppm value. In case of the *cis* form of the compound **2$_g$** the reaction afforded two products as *cis* forms, the mono-acetylated compounds **4$_a$** and the diacetylated one **4$_c$**. The results that were observed from 2D-NMR spectra of the mono-acetylated product **4$_a$** which showed two signals for

two NH indole protons, one at δ = 9.79 ppm for NH_a and the other at 10.65 ppm value for NH_c and NH_b disappeared. Thus the only acetylated NH indole proton were NH_b based on the ^1H-^1H zTOCSY, ^1H-^1H ROESY and ^1H-^1H gDQCOSY spectrums and ^1H-^{13}C gHMBCAD spectrum. Whereas in the diacetylated product the remaining signal at δ = 9.94 ppm is attributed to NH_a. We can conclude that, the acetylation reactions of these triindole products takes place step by step, where the first reaction lads to the monoacetylated derivative which then reacts to give the diacetylated and finally the triacetylated derivative. The first acetylated NH protons should be NH_b and NH_c and the last one is NH_a, scheme (10). The structure of **4_d** was further confirmed by single crystal X-ray crystallography, figure (36) which indicates that the **4_d** is in the *trans*-isomer and (*S/S*) configuration. Compound **4_d** was determined as 1,1'-(3,3'-((1*S*,4*S*)-9-acetyl-2,3,4,9-tetrahydro-1*H*-carbazole-1,4-diyl)bis(1*H*-indole-3,1-diyl))diethanone. the two hydrogen atoms are one pseudoaxial vertical up (H_b) and one pseudoaxial vertical down (H_a) and the two bulky groups of acetylated indole are in pseudoequatorial/pseudoequatorial orientations which is the most stable conformation based on stereochemistry rules of 3,6-disubstituted cyclohexene. This rule state that the 3,6-disubstituted cyclohexene a *trans* configuration leads to either both groups pseudoaxial or both pseudoequatorial and the di pseudoaxial conformation is effectively prevented by its high steric strain more than the di pseudoequatorial, figure (33).



Scheme (10): Acetylation reactions of triindole products **2**$_{d}$ and **2**$_{g}$.

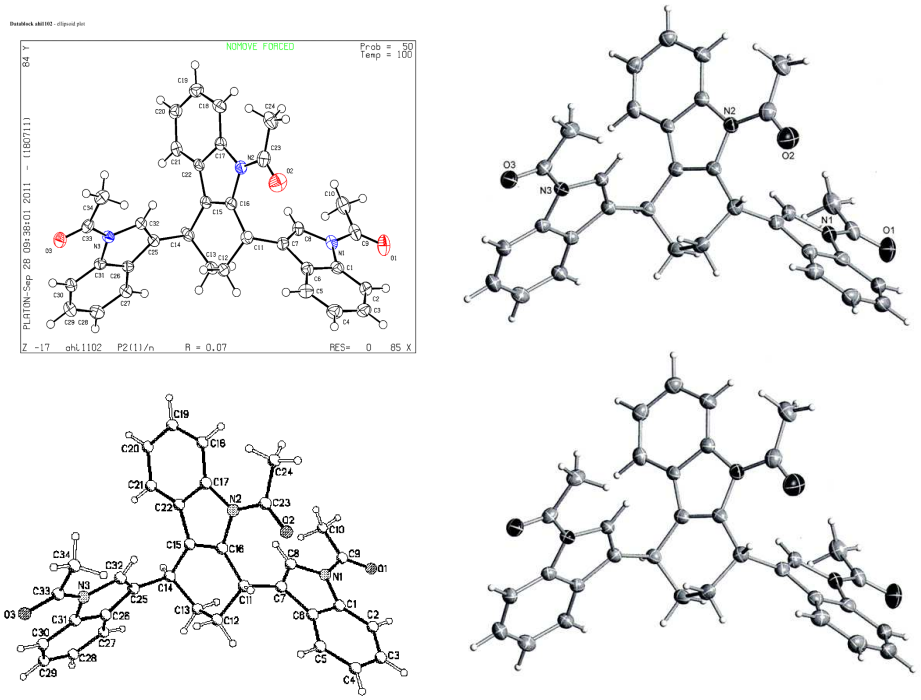
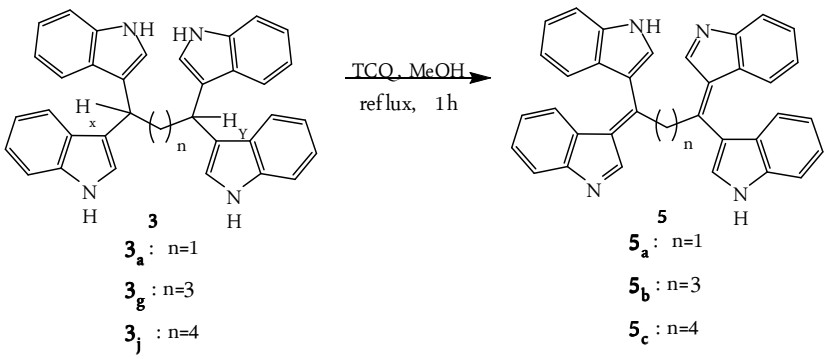


Figure (36): X-ray crystal structure of compound **4**$_{d}$.

3.1.1.6. Oxidation reaction of tetraindole products

Our novel tetraindole products are very similar as two molecules of bis-indolylmethane (BIM) were connected by an alkyl chain $(CH_2)_n$ with n = 1, 2, 3, 4. These tetraindoles are novel structures and have not been reported in the literature. The

novel tetraindoles can act as a good precursor for several organic reactions e.g. further condensation reactions and oxidation or dehydration reactions. The stereochemistry of these molecules which illustrated from the Three-dimensional models (3D) for example compounds $\mathbf{3_a}$ and $\mathbf{3_g}$, figure (37a), leads to expect the highly reactivity of these compounds towards the oxidation chemical reaction where the molecule does not show any stric hindrance of the configuration of the four indole units around the aliphatic hydrocarbon chain. This new compounds expect to be highly biologically active as well as the reported BIMs which exhibit a range of biological activities. Cancer chemotherapy with BIMs was recently reviewed and numerous activities are reported[167] where they inhibit bladder cancer growth, renal cell carcinoma growth, have growth inhibitory activity on lung cancer cells, are active against colon cancer, induce apoptosis in prostate cancer and exhibit antimicrobial, antifungal[30,31] and antibiotic activities[32]. In view of all these reported references we hope that these new tetraindole compounds are promising to show biological activities. The oxidized form of bis-and tris-indolylmethanes are utilized as dyes[168,169,170,171,172,173], as well as colorimetric sensors[174,1175,176,177]. The oxidation reaction of tetraindoles ($\mathbf{3_{a,g,j}}$) takes place as well as that of BIMs. It has been found that these tetraindoles are highly sensitive towards oxidation reagents under similar conditions used for the oxidation of BIMs [174,178]. The oxidation reaction was done by using TCQ (tetrachloroquinone) or DDQ (dichlorodicyanoquinone) either in methanol or acetonitrile at room temperature or by reflux for a short time to yield the oxidized form of $\mathbf{5_{a-c}}$ in good yields (73 % - 85 %), scheme (11).



3

$\mathbf{3_a}$: n=1

$\mathbf{3_g}$: n=3

$\mathbf{3_j}$: n=4

5

$\mathbf{5_a}$: n=1

$\mathbf{5_b}$: n=3

$\mathbf{5_c}$: n=4

Scheme(11): Oxidation reactions of tetraindoles $\mathbf{3_{a,g,j}}$.

The products were purified by column chromatography and identified on the bases of their spectral data. The ^{1}H-NMR spectra of the resulting oxidized forms **5$_{a-c}$** showed the disappearance of the signal related to the protons H_x and H_y in a tetraindoles **3$_{a,g,j}$**. The remaining two acidic protons (NH indole) appear as broad signal at high ppm values. For example the ^{1}H-NMR spectrum of compound **5$_a$**, showed a broad signal at δ = 13.17 ppm for the remaining two NH indole protons, figure (37b). The broad signal is due to the conjugation within the indoles, where every side of compound **5$_a$** is considered as monoprotonated form of BIMs[171,172,178,179]. These types of compounds showed broad peaks in their IR spectra at 3064 - 3350 cm^{-1}, 3105 - 3340 cm^{-1} and 3250 - 3348 cm^{-1} for the NH indole in compounds **5$_a$**, **5$_b$** and **5$_c$** respectively. Figure (37a) shows the acidic conjugated form of compound **5$_a$** as an example. The conjugated oxidized forms of type **5$_{a-c}$** are expected to be excellent receptors for the colorimetric detection of anions similar to the oxidized forms of BIMs[179]. Compounds **5$_{a-c}$** are colored compounds so that they can be used as dyes like BIMs[168-173].

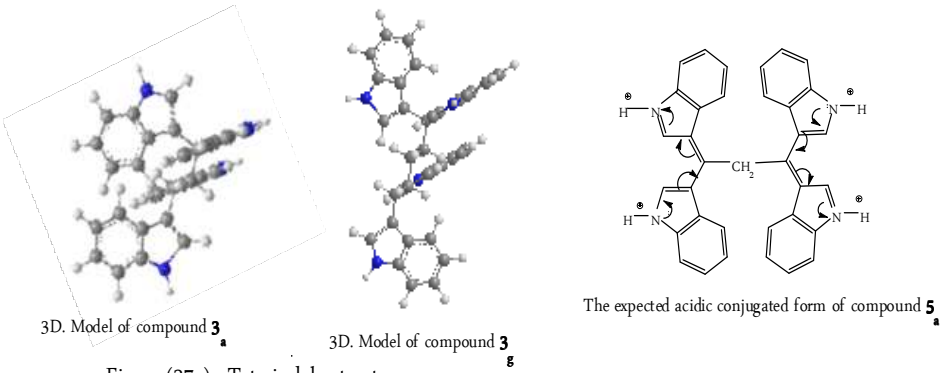


Figure (37a): Tetraindole structures

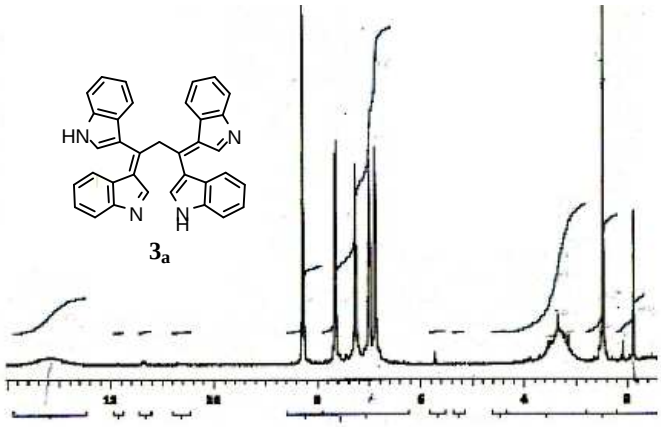
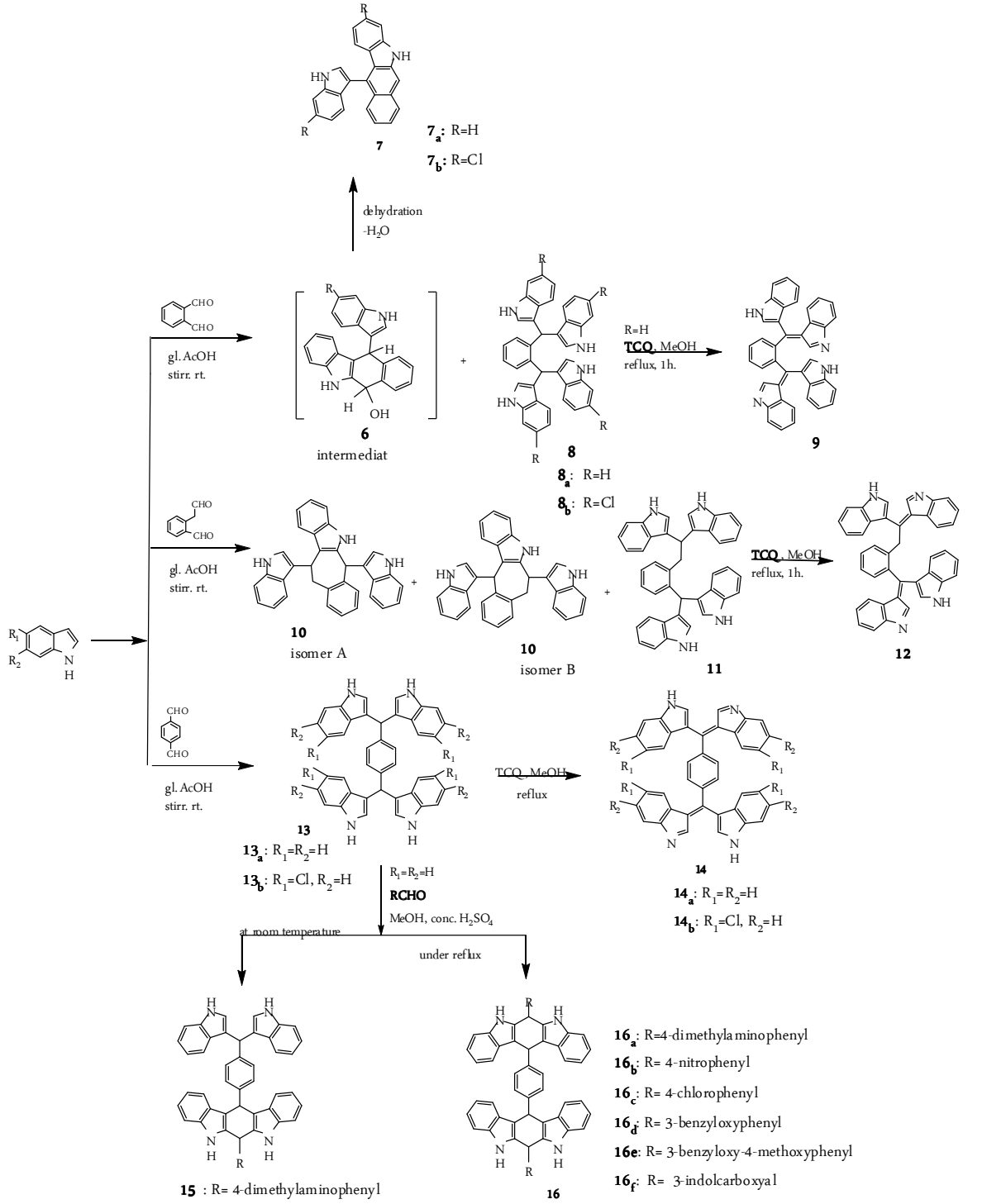


Figure (37$_b$): ^{1}H-NMR spectra of compound **3$_a$** in DMSO-*d6*.

3.1.2. Electrophilic substitution reactions of indoles with aromatic dialdehydes



Scheme (12): The whole scheme of electrophilic substitution reactions of indoles with aromatic dialdehydes.

3.1.2.1. Domino reaction in organic synthesis

The usual procedure for the synthesis of organic compounds is the stepwise formation of the individual bonds in the target molecule. However, it would be much more efficient if one could form several bonds in one sequence without isolating the intermediates, changing the reaction conditions or adding reagents. It is obvious that this type of reaction would allow the minimization of waste and thus making the waste management unnecessary since compared to stepwise reactions the amount of solvents, reagents, adsorbents and energy would be dramatically decreased, in addition the amount of labour would go down. Thus, these reactions would allow an ecologically and economically favourable production. We call this type of transformation a *domino reaction*. The name was chosen from the game where one puts up several domino pieces in one row and in agreement with the time-resolved succession of reactions, if one knocks over the first domino, all the others follow without changing the conditions, figure (38). Domino reactions, on the other hand, allow access to a myriad of complex molecules with high a stereo-control in an efficient, atom-economical manner. Nikolaou noted that the descriptors *domino*, *cascade*, *tandem* and *sequential* are often used indistinguishably from one another in the literature[180,181].

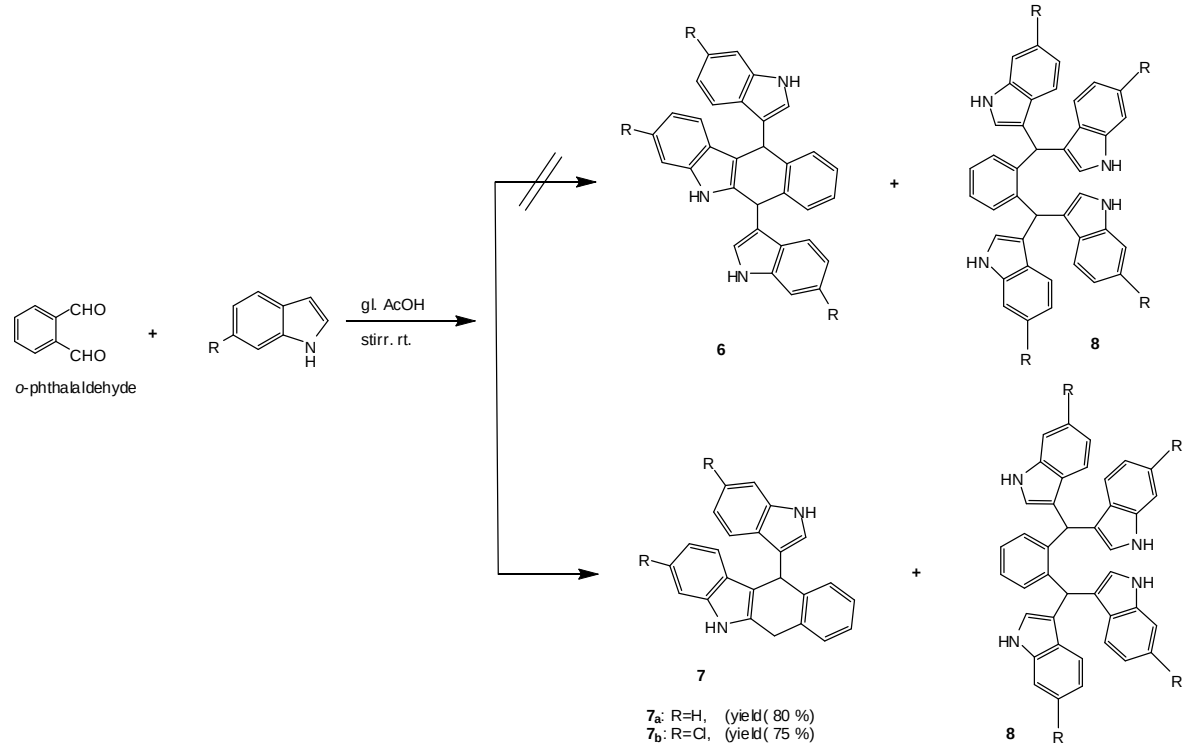
Figure (38): Domino game.
http://www.123rf.com/stock-photo/domino.html

According to *Tietze*, a *domino* (or *cascade*) reaction is defined as a process in which two or more bond-forming transformations occur based on functionalities formed in the previous step. Furthermore, no additional reagents, catalysts or additives can be added to the reaction vessel, nor can reaction conditions be changed[182,183]. The usefulness of

this reaction is correlated *firstly* to the number of bonds which are formed in one sequences we call this the bond-forming efficiency (or bond-forming economy), *secondly*, to the increase in the structural complexity (structure economy), and, *thirdly*, to its suitability for a general application[180].

3.1.2.2. Domino reactions of *o*-phthalaldehyde with indoles

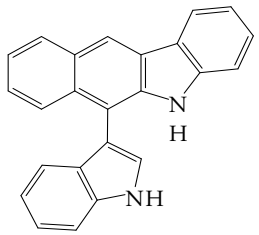
The reaction of *o*-phthalaldehyde with electron rich arenes or heteroarenes such as indole, in presence of AcOH at room temperature was found to be successfully affording the product **7$_{a-b}$** in 75 to 80 % yields, in addition to the formation of the expected tetraindole products **8$_{a,b}$** in a poor yield of 10 to 15 %, scheme (13).



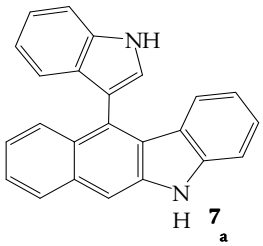
Scheme (13): Domino reaction of indoles with *o*-phthalaldehyde.

The formation of the heterocyclic compounds **7$_{a,b}$** took place instead of the formation of our expected compound **6**. The expectation for the reaction to form compound **6** was in view of our pervious results of the reactions of an aliphatic dialdehyde specially succinaldehyde with compound **6** identified and confirmed by X-ray crystallography, see scheme (6) and figure (36). However, the reaction of indoles with *o*-

phthalaldehyde took different courses and afforded the heterocyclic compounds **7a,b**, and this behavior has been reported in the literature[184,185]. It has been known as *domino reaction* of *o*-phthalaldehyde with arenes, which has been formed in presence of strong acid catalyst such as $POCl_3$ or a mild acid catalyst such as *p*-toluene sulphuric acid. The combination of *o*-phthalaldehyde in the presence of phosphoryl chloride in chloroform gave the isomer 11-(1*H*-indol-3-yl)-5*H*-benzo[*b*]carbazole, figure (39), whereas the use of *p*-toluene sulphuric acid in methanol yielded the isomeric, 6-(1*H*-indol-3-yl)-5*H*-benzo[*b*]carbazole, figure (39).



6-(1*H*-indol-3-yl)-5*H*-benzo[*b*]carbazole



7a

11-(1*H*-indol-3-yl)-5*H*-benzo[*b*]carbazole

Figure (39): The two isomers of indolylbenzo[*b*]carbazoles.

The result of our procedure, by using similar reactants in glacial acetic acid at room temperature, is identical with the procedure using $POCl_3$ in the formation of the isomers 11-(1*H*-indol-3-yl)-5*H*-benzo[*b*]carbazoles (**7a**) in good yields. The ^{1}HNMR of compound **7a** explained the presence of eleven signals in an aromatic region (6.76 - 8.03) ppm values related to the fourteen aromatic protons in the structure **7a**. In addition the ^{13}C NMR spectra illustrated the presence of ten signals for ten aromatic quaternary carbon C atoms and fourteen signals for fourteen aromatic carbons CH atoms. These spectral data confirmed that our new reaction method afforded only isomer A (**7a**) in a purely case while isomer B has not been formed. This result was confirmed by single crystal X-ray crystallography of compound **7a**, figure (40).

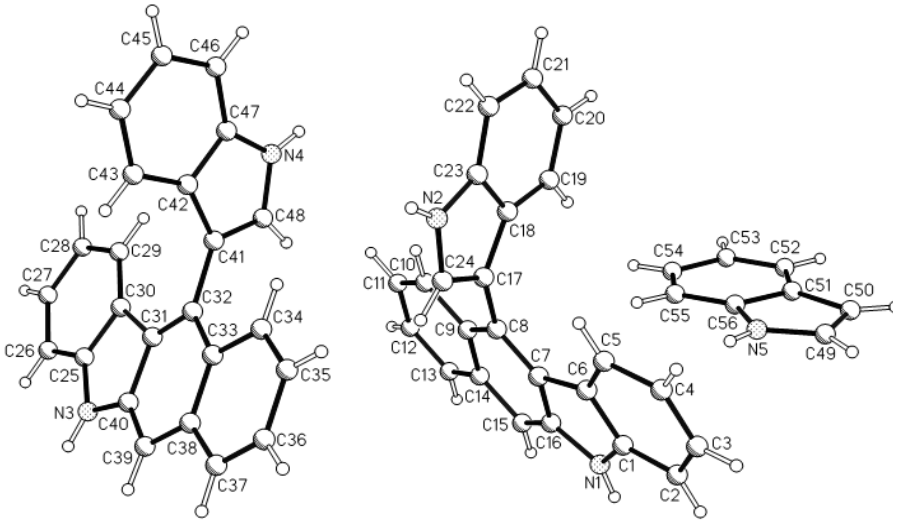
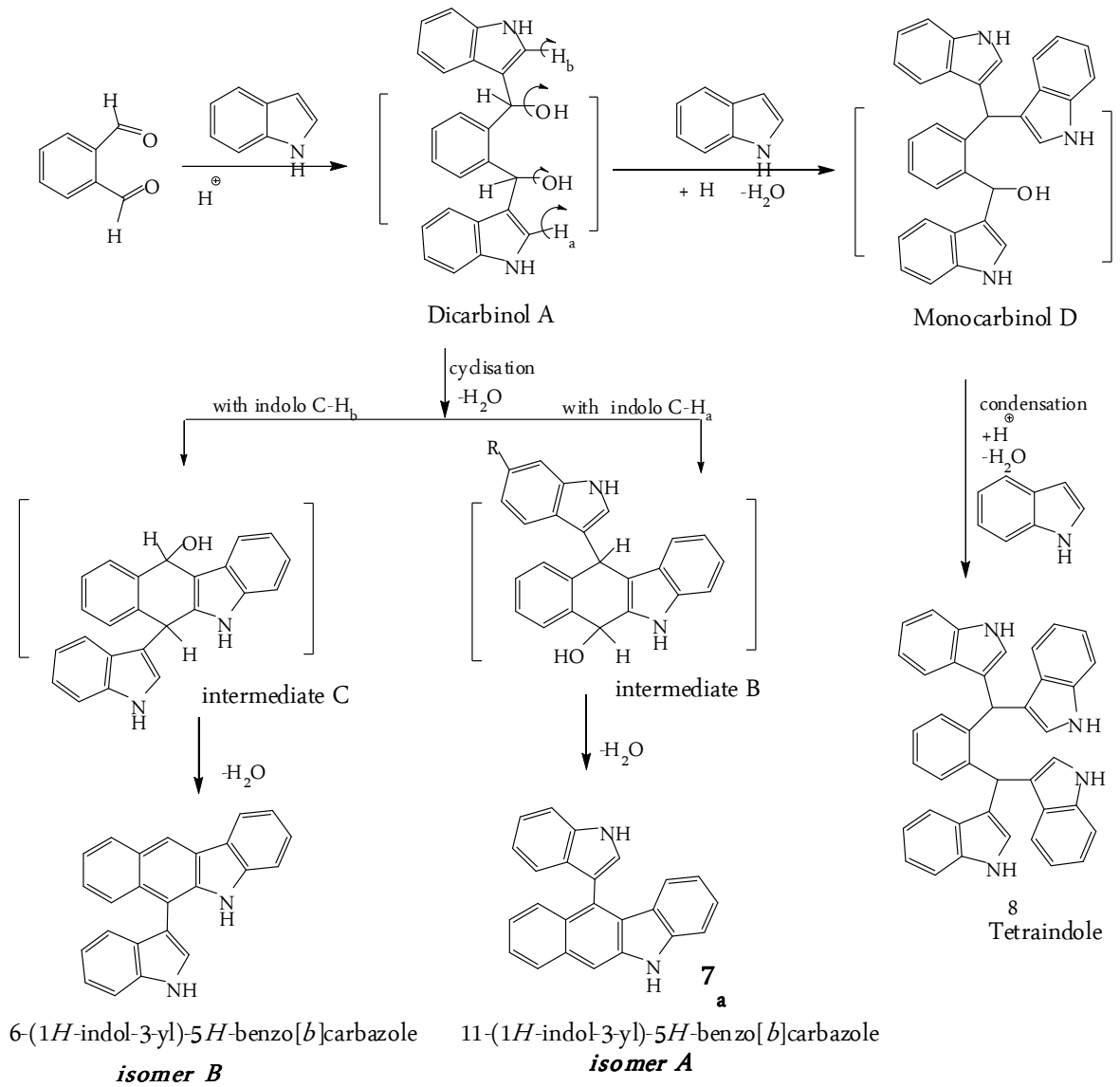


Figure (40): X-ray structure of compound 7a.

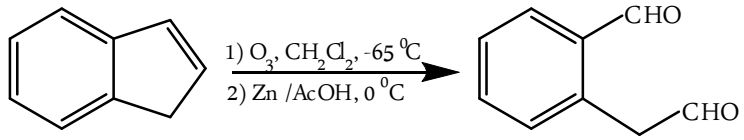
The suggested mechanism of this *domino reaction* is shown in scheme (14). It involves the formation of the dicarbinol A, which undergoes dehydration and cyclization with the α-indolo C-H_a to form the intermediate B. Intermediate B afforded our product **7** in the form of isomer A *via* aromatization. Whereas the cyclization with α-indolo C-H_b leads to the formation of intermediate C which undergoes a dehydration or an aromatization to form isomer B[184]. Dicarbinol A follows the other pathway to yield the tetraindole product **8** by condensation of the third indole molecule forming the monocarbinol D, which undergoes a successful fourth condensation step with the fourth indole molecule[149-152]. Benzo[*b*]carbazoles (**$7_{a,b}$**) principally are of interest because of their relationship to the antitumor agent *ellipticene*[162] and are considered as desirable synthetic target structures as potentially DNA intercalating agents[186-188]. So that, our simple reaction enhances the simplicity of the synthetic access to new benzo[*b*]carbazoles.



Scheme (14): Reaction mechanism of indoles with *o*-phthalaldehyde.

3.1.2.3. Domino reaction of indole with homophthalaldehyde

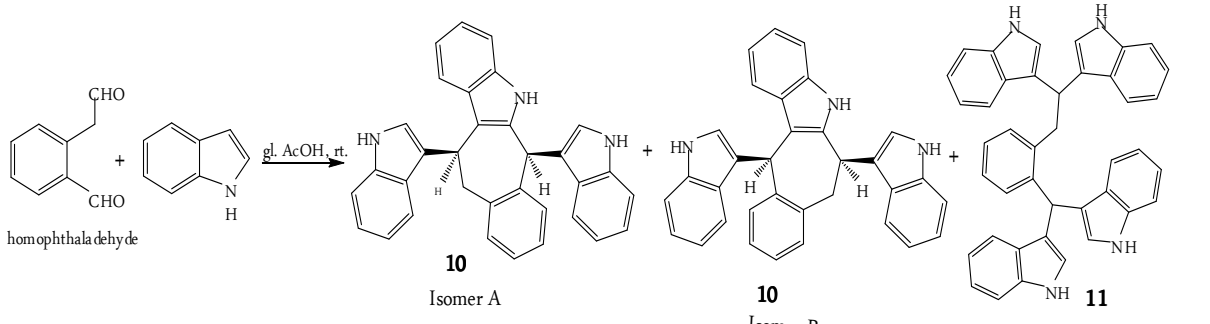
Homophthalaldehyde is of considerable interest as a precursor of isoquinoline and its derivatives[189-193]. Homophthalaldehyde is not commercially available so that we were synthesized it *via* an ozonolysis of indene in dry dichloromethane at - 65 ^{0}C followed by reduction with zinc in acetic acid at 0 ^{0}C affording the homophthalaldehyde after an azeotropic distillation to remove the water[194], scheme (15).



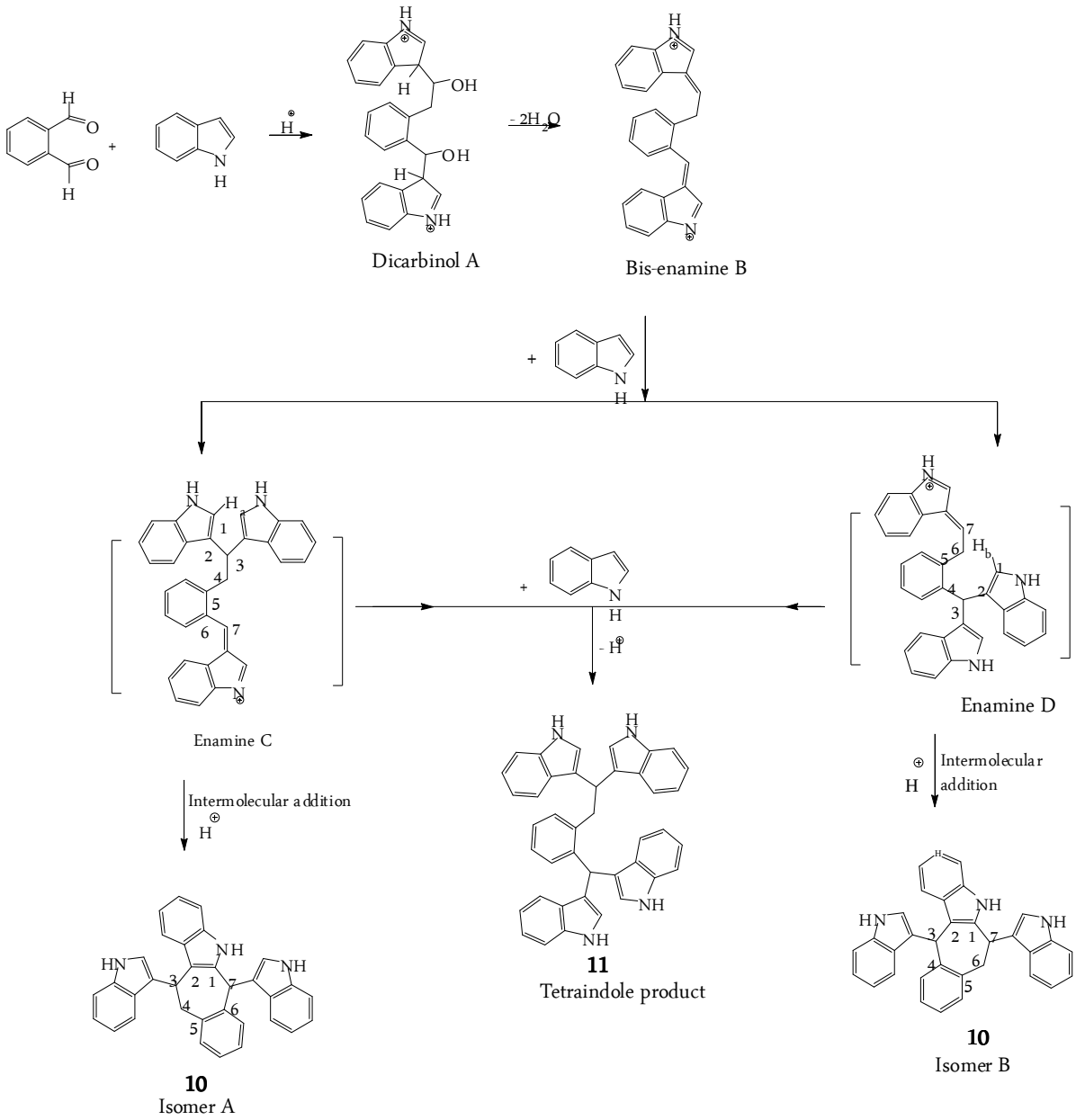
indene homophthalaldehyde

Scheme (15): Synthesis of homophthalaldehyde.

After an azeotropic distillation, the dialdehyde was used directly in the condensation reaction with indole in acetic acid at room temperature yielding a novel benzo[7]annulene derivatives of type **10** in a moderate yield (46 %) and the tetraindole product 11 in 38 % yield. Compound 10 was found to be a mixture consists of two isomers A and B, where its ^{1}H-NMR spectrum showed two signals multiplets for the two protons at C_3 of both isomer A and B at δ = 4.98 ppm and 5.71 ppm values, and two signal multiplets for the two protons at C_7 of both isomers A and B at δ = 5.86 and 6.03 ppm. Also the CH_2 group at position 4 in isomer A and at position 6 in *isomer* B gives each one multiplet signal at 2.85 - 2.89 ppm and 3.94 - 4.03 ppm values, see figure (94) in the appendix. These ^{1}H-NMR data give strong indication that we have a mixture of both isomers A and B. From the ^{1}H-NMR spectra and referring to the X-ray crystallography of compound $\mathbf{4_d}$, the presence of two different signals for the two protons of C_3 and C_7 in every *isomer* of compound 10, pointed to that we have cis-forms for both *isomers* A and B, scheme (16).



Scheme (16): Domino reaction of indole with homophthaladehyde.



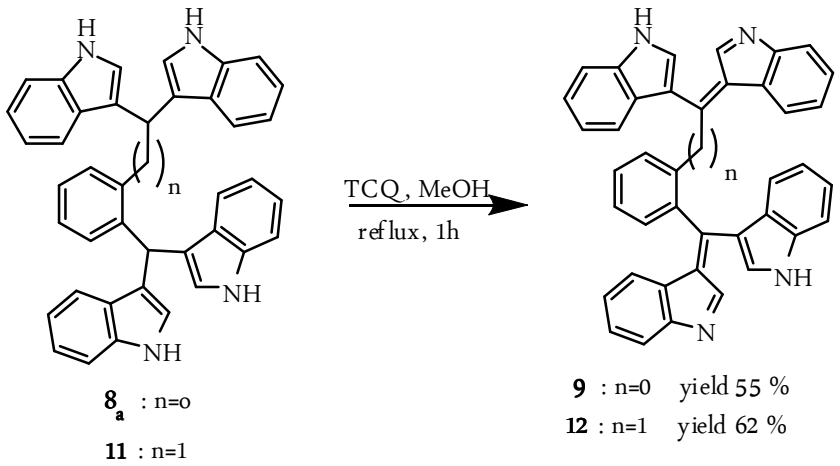
Scheme (17): Expected reaction mechanism for the condensation of indole with homophthaladehyde.

The formation of both *isomers* A and B of triindole (**10**) accompanied with tetraindole (**11**) formation can be explained by the mechanism showed in scheme (17). Indole condensed with homophthalaldehyde in acid medium formed the dicarbinol A which underwent dehydration *via* an elimination of two molecules of water to give the bisenamine B. This bisenamine B reacted with the third indole to give the mono enamine C or D. If the intermolecular addition takes place via α-indole C-H_a in enamine C, the formed triindole (10) was *isomer* A. The intermolecular addition with

α-indole C-H_b in enamine D yielded the triindole *isomer* B. A successful addition of the fourth indole to either enamine C or enamine D tends to the formation of the tetraindole product 11. Both tri- and tetraindole products are novel compounds which have not reported in the literature.

3.1.2.4. Oxidation reaction of tetraindoles 8_a and 11

From the previous oxidation reaction, scheme (11), which involve the oxidation reaction of the tetraindoles $\mathbf{3_{a,g,j}}$ yielding bisdehydrated forms $\mathbf{5_{a\text{-}c}}$, we found that the tetraindoles which result from the condensation of aromatic dialdehydes (*o*-phthalaldehyde and homophthalaldehyde) compounds $\mathbf{8_a}$ and **11** can also undergo dehydration reaction using TCQ affording the dehydrated forms **9** and **12** in good yields, scheme (18).



$\mathbf{8_a}$: n=o

11 : n=1

9 : n=0 yield 55 %

12 : n=1 yield 62 %

Scheme (18): Oxidation reaction of compound $\mathbf{8_a}$ and **11**

The reaction occurred by similar chemical procedure using TCQ in methanol and reflux under stirring. The colour of the reaction solution turned to deep dark after few minutes. The products were purified by column chromatography eluted with methanol/dichloromethane (5 – 10 %). The ^{1}H-NMR spectra showed no detection for both the aliphatic methylene protons, and detected a broad signal for the remaining two acidic NH indole protons due to the delocalization resulting from the conjugation of the remaining acidic NH indole. Every side of these oxidized forms appears to be as a

monoprotonated form of oxidized BIMs[171,172,178,179]. The suggested mono protonated forms of **9** and **12** are shown in figure (41). Concerning to the importance of these compounds, we expect them to be favourable receptors for a colorimetric detection[179], and as a dyes due to their colours as well as the oxidized BIMs[168-173].

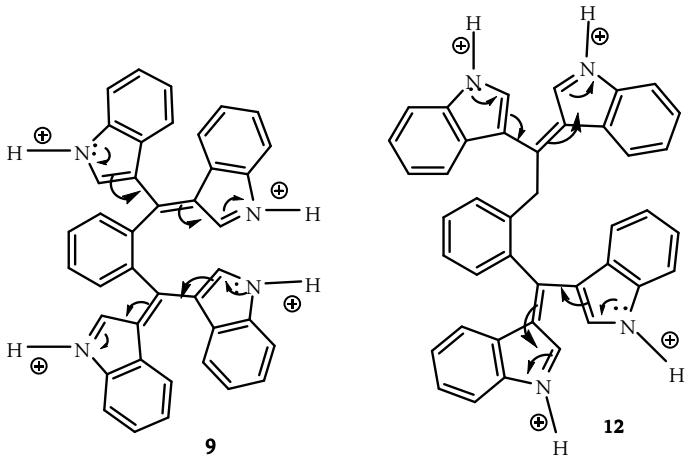
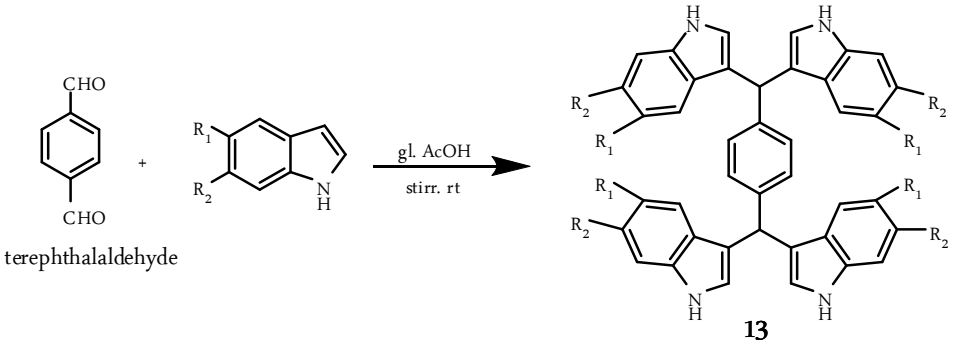


Figure (41): NH indole resonance of compounds **9** and **12**

3.1.2.5. Domino reaction of indoles with terephthaladehyde

The electrophilic substitution reactions of indoles with terephthaladehyde have been reported in the literature as a possible way for the synthesis of supramolecular compounds containing BIMs, namely 3,3′,3″,3‴-tetraindolyl(terphthalayl)dimethane (**13$_a$**)[195]. This reaction has been done in presence of catalyst such as iodine and *N*-bromosuccinimide (NBS)[196,197] affording the tetrasubstituted product in good yields. In the present work, terephthaladehyde condensed with indoles in glacial acetic acid in a molar ratio (1:4) affording compounds **13$_{a,b}$** in a high yield of 93 - 95 % respectively after a short time of stirring at room temperature (2 - 4 h), scheme (19).

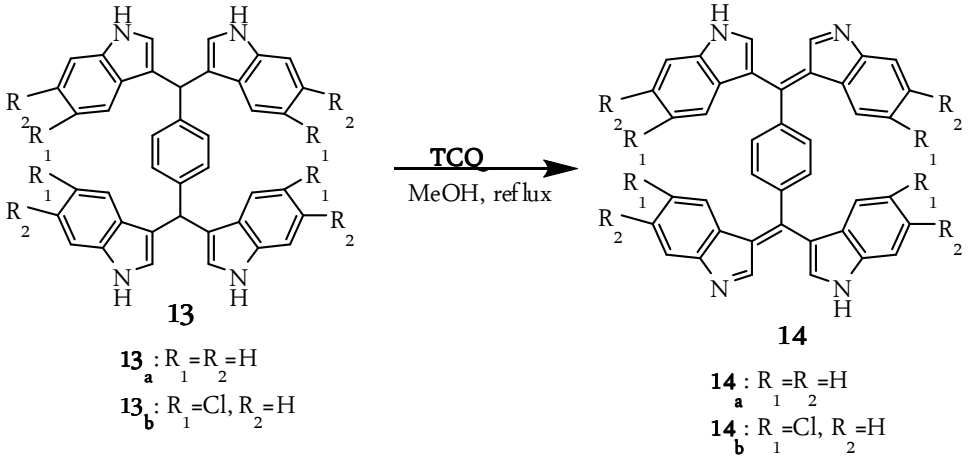


Scheme (19): Reaction of indoles with terephthaladehyde.

The analytical and spectral data of compound **13a** was found to be identical to the data of the published tetrasubstituted indole product [196,197], another novel derivative using 5-chloroindole has been prepared accordingly and identified.

3.1.2.6. Oxidation reaction of tetrasubstituted indoles 13a,b

The oxidation reaction using TCQ as oxidizing agent in methanol solution has been extended for the synthesis of the expected novel bisdehydrated forms of type **14a,b** scheme (20).



Scheme (20): Oxidation reaction of compound **13a,b**.

The reaction occurs as well as the oxidation reaction of all pervious tetrasubstituted indoles which were produced from the condensation of indoles either with aliphatic dialdehydes (compound **3**) or aromatic dialdehydes (compounds **8** and **11**). Compound $\mathbf{14_{a,b}}$ was formed with a high yields, 86 - 90 % with respect to the other oxidized compounds $\mathbf{5_{a-c}}$ or **9** or **12.** The structure of compound $\mathbf{14_{a,b}}$ has not reported yet and was identified on the bases of their analytical and spectroscopic data. As we discussed previously, the figure (42) showed the expected NH indole resonance structure of $\mathbf{14_a}$.

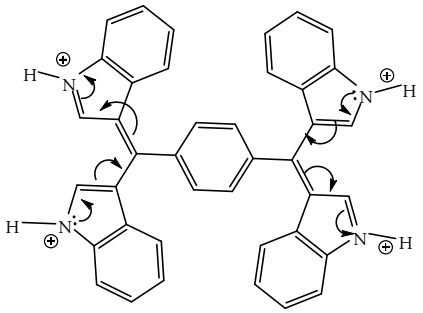
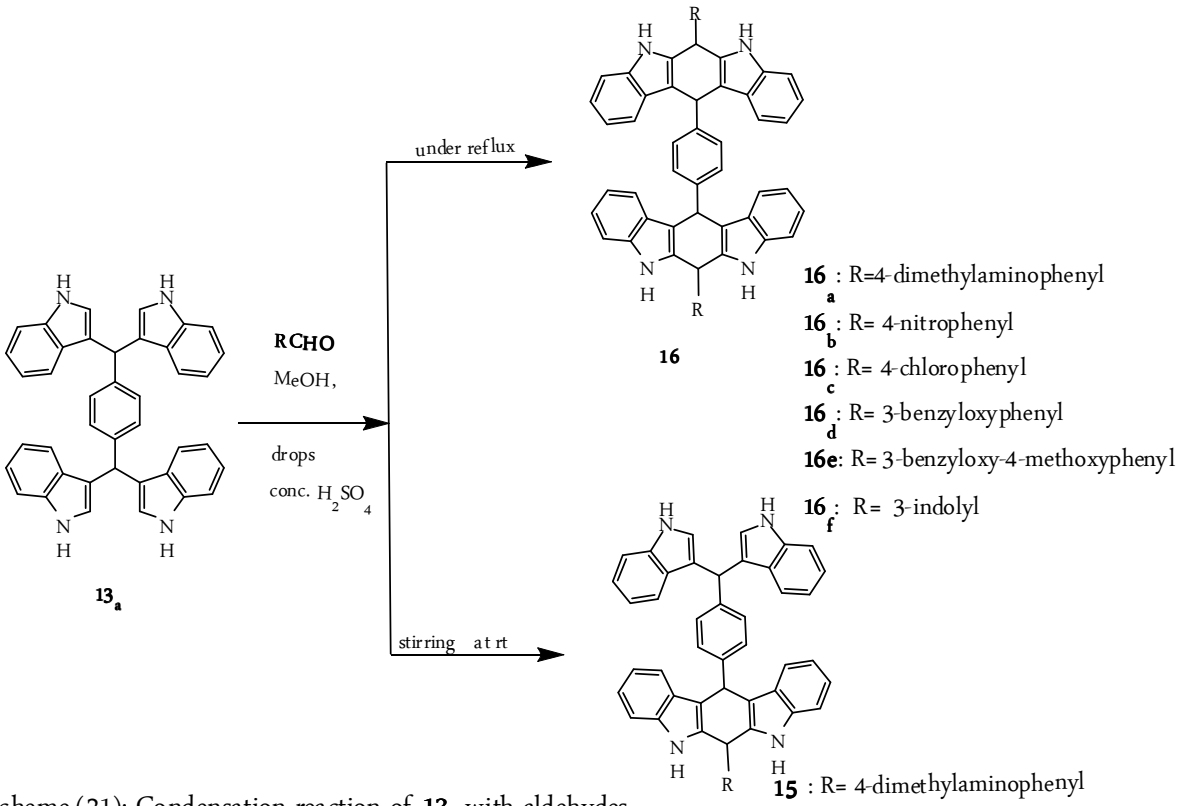


Figure (42): NH indole resonance structure of $\mathbf{14_a}$.

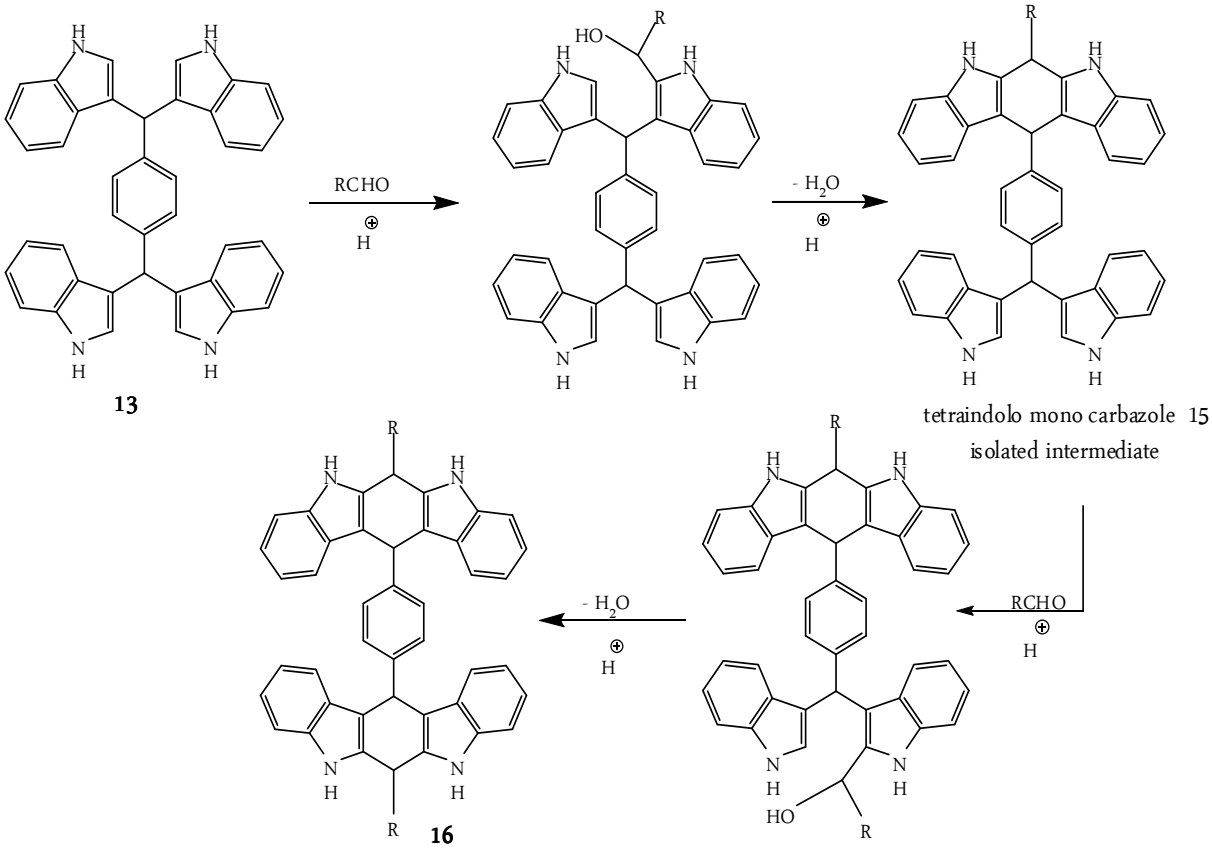
3.1.2.7. Condensation reaction of the tetraindole (13_a) with aryl and heteroaryl aldehydes

Compounds $\mathbf{13_{a,b}}$ can acts as nucleophile due to the unoccupied two positions of the four indole rings, hence we now present a convenient method for the synthesis of the novel extended ring systems ($\mathbf{16_{a,f}}$) *via* the condensation reaction of compound $\mathbf{13_a}$ with aryl or heteroaryl substituted aldehydes in a molar ratio (1:2), scheme (21). The reaction was accomplished by let the compounds reacting in methanolic solution containing drops of conc. H_2SO_4 under reflux. The product was detected by TLC and isolated easily column chromatography using dichloromethane as an eluent. However, when the reaction has carried out at room temperature under stirring for long time, the main product which was separated and identified was compound **15** by using *p*-dimethylaminobenzaldehyde. Compound **15** can be considered as an intermediate product for the formation of the compound **16**. A modified synthesis for the novel extended ring system indolo-carbazoles ($\mathbf{16_{a-f}}$) has been done by one step procedure by an acid-catalyzed intermolecular reaction as in the reported cases of condensation of BIMs and aldehydes to afford the corresponding substituted indolo[3,2-*b*]carbazoles[198-203].



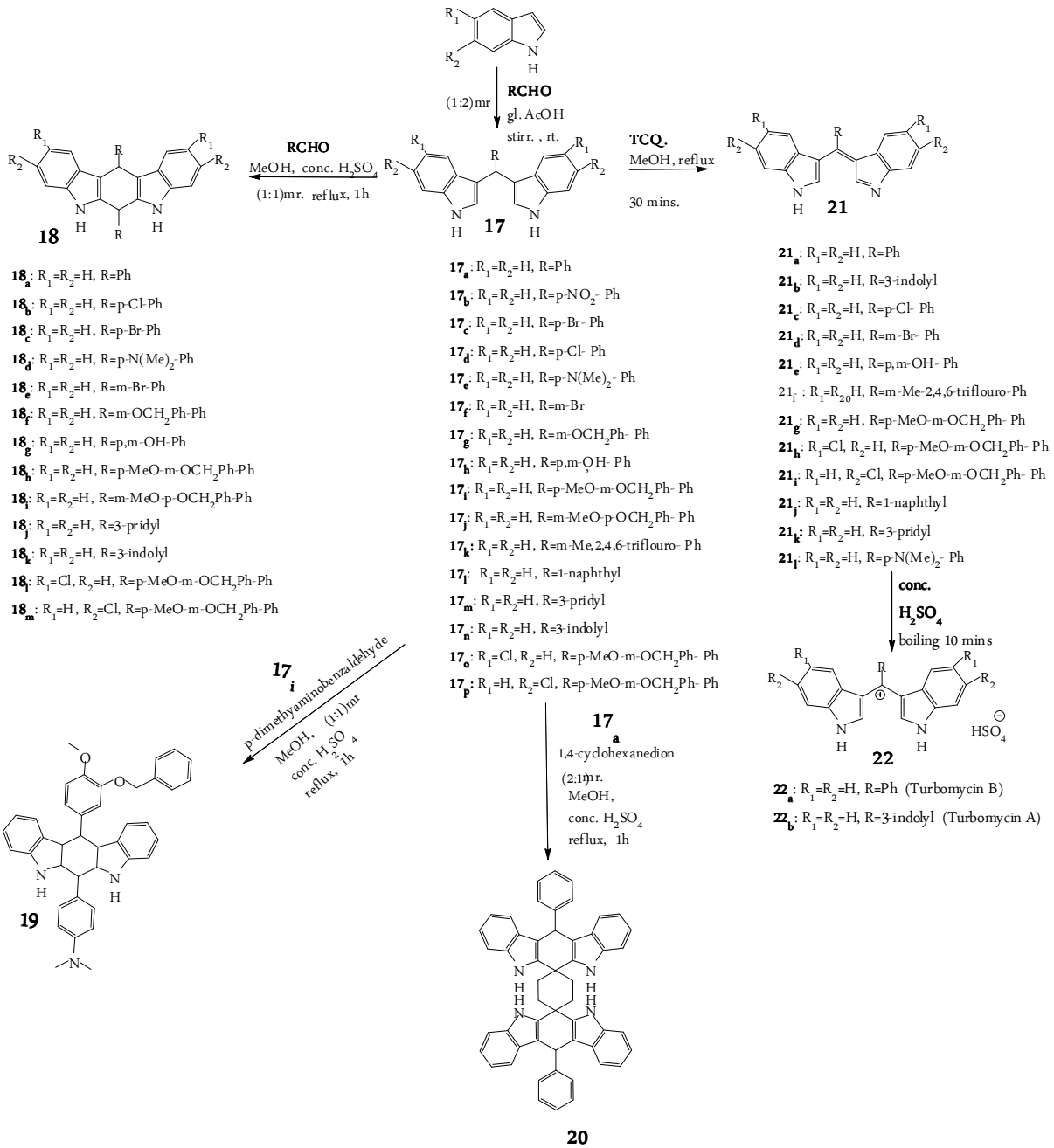
Scheme (21): Condensation reaction of **13**$_a$ with aldehydes.

The proposed mechanism is illustrated as shown in the scheme (22), where the successful condensation of the tetra substituted indole **13** with the first mole of aldehyde afforded the bisindole monocarbazole as an intermediate which can be isolated from the reaction as in case of compound **15**. This intermediate gave the novel extended ring system indolocarbazoles (**16**) as a final product as a result of the condensation with the second mole of aldehyde. This suggested mechanism is supported by an isolation of the intermediate **15**, so we can say that the second condensation step with the second mole of aldehyde may need heat energy with the acid catalyst for promotion of the reaction to take place. The ^{1}HNMR spectrum of compound **16** detected the aliphatic hydrogenated protons of dihydroindolo[3,2-*b*]carbazoles as a singlet signal at a δ value of about 4.5 to 5.5 ppm with an integral for 4 protons and one for 3 protons in the case of compound **15**.



Scheme (22): Proposed reaction mechanism of compound **16**

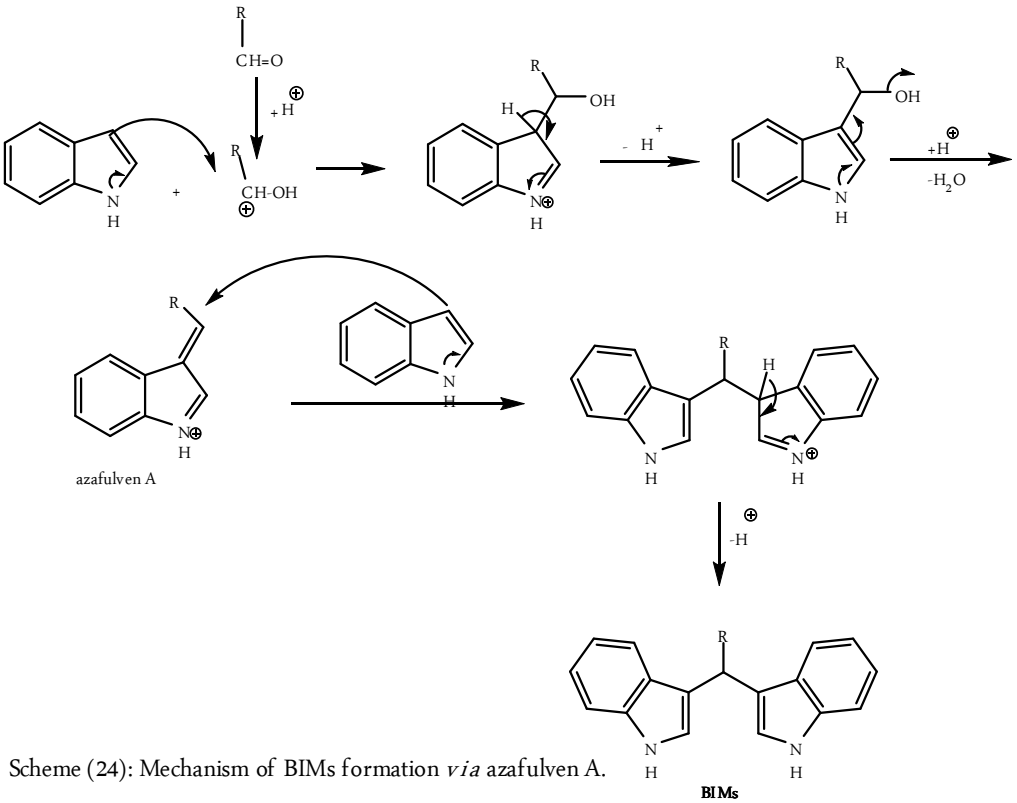
3.1.3. Electrophilic substitution reactions of indoles with aryl and heteroaryl aldehydes



Scheme (23): The whole scheme of electrophilic substitution reactions of indoles with aryl and heteroaryl substituted aldehydes.

In recent years a considerable attention has been paid on the synthetic methods leading to indole derivatives because of their biological activities. Various indole derivatives, such as 3-substituted indoles, are common components of drugs and are generally found to be of pharmaceutical interest in a variety of therapeutic areas[204]. In

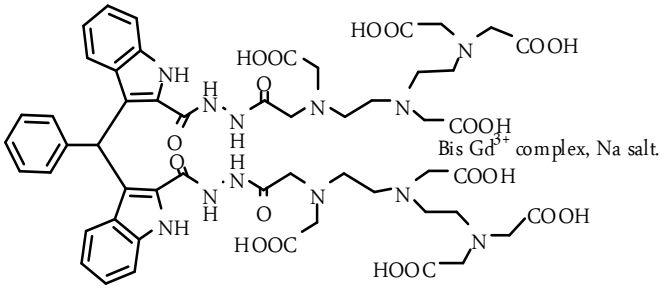
addition, 3-substituted indole derivatives are also versatile intermediates in organic synthesis[205], due to the feasibility of their 3-position for an electrophilic substitution. The electrophilic substitution reactions of indoles with aromatic aldehydes afford corresponding BIMs. Several catalysts such as protic acids[120-137], Lewis acids[138-148], ionic liquids[206] and others are known to promote these reactions. The 3-position of indole is the preferred site for the electrophilic substitution reactions. A simple and direct method for the synthesis of 3-alkylated indole derivatives involves the condensation of indoles or its substituted derivatives with electrophiles (aldehydes or ketones or imines). Aldehydes either aliphatic or aromatic are the most important and widely used as electrophiles in such reactions. The acid catalyzed reactions of electron rich heterocyclic compounds such as indoles or pyrroles with *p*-dimethylaminobenzaldehyde have been known as the *Ehrlich test* [151,207-210]. Generally, BIMs are synthesized by an analogous reaction to the *Ehrlich test*, where indoles react with aliphatic or aromatic aldehydes or ketones in presence of an acid catalyst to give *azafulven* A[150-152], which undergoes further addition with the second indole molecule to afford BIMs, scheme (24)[207-210].



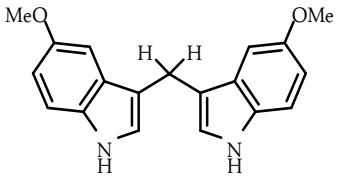
Scheme (24): Mechanism of BIMs formation *via* azafulven A.

3.1.3.1. Synthesis of BIMs

BIMs are molecules containing two indolyl moieties connected to the same carbon. Many advances in the strategy of BIMs synthesis were published as result of the variation of the catalyst. Other factors that prompted new research include the price of catalysts, yield of products, reaction rates, simplicity of the work up procedure, green chemistry etc. Bisindolylalkanes and their derivatives are found in bioactive metabolites of terrestrial and marine origin. A recent patent describes the synthesis of BIMs forming complex A, figure (43), with radioactive metal ions (Gd^{3+}), which are found to be useful contrast agents for radio-imaging and visualization of various tissues and organs[211]. Recently, *Maciejewska* et al.[212] used DNA-based electrochemical biosensors to demonstrate that bis(5-methoxyindol-3-yl)methane[213], figure (43), considerably reduces the growth of the cancer cell lines such as HOP-92 (lung), A498 (renal) and MDAMB-231/1TCC (breast) Their results also indicate that BIMs could potentially be applied as chemotherapeutic agents against tumors[212,214]. BIMs and trisindolylmethanes (TIMs), have been used as legands for the synthesis of many complex molecules and different properties of these complexes molecules have been investigated[215-220].



BIM complex A

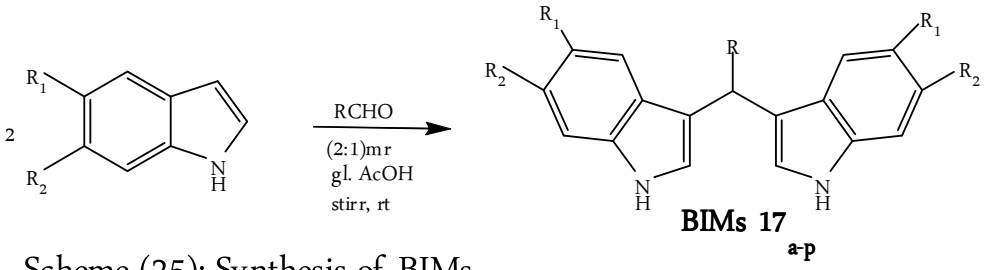


bis(5-methoxy-1*H*-indol-3-yl)methane

Figure (43): Structure of BIM complex A and bis(5-methoxy-1*H*-indol-3-yl)methane.

In view of our previous work using glacial acetic acid as a protic acid for promotion of the condensation reaction of indoles with aldehydes, we will try to estimate the yield formation of BIMs *via* using glacial acetic acid with indoles and aromatic aldehydes. Some of these BIMs have been reported by using different types of catalysts[120-148,206]. It has been found that, glacial acetic acid acts as a protic acid without solvent to catalyze

the reaction of indoles (two equivalent moles) and aryl or heteroaryl aldehydes (one equivalent mole). Acetic acid has only been reported as a catalyst in the preparation of BIM derived from 4-cyanoindole and formaldehyde solution. The reaction was done by using drops of acetic acid and finished after about 60 h[221]. In our present work *via* glacial acetic acid as a solvent (5 ml) the corresponding BIMs were given in a high yields (86 - 98 %) and after few hours (4 - 6) of stirring at room temperature. In comparison to the reported methods glacial acetic acid under a solvent free condition was found to be an efficient catalyst in terms of handling, temperature, yields and reaction times, scheme (25) and table (1). A series of substituted aryl or heteroaryl aldehydes were efficiently converted to the corresponding BIMs $\mathbf{17}_{a\text{-}p}$, as shown in table (1), which give the reaction times and the formed yields. Concerning to the substituent on the carbonyl compounds we can summarize that the presence of either electron-donating group (such as dimethylamino, methoxy, benzyloxy or hydroxy) or electron-withdrawing group (e.g. nitro, chloro, bromo or trifluoro) has not noticeable effect on the reaction time or the percent of the yield. So we can conclude that glacial acetic acid promotes the electrophilic substitution reaction of indoles with aromatic aldehydes whatever the substituent on the aromatic aldehyde and this makes it different from all the catalysts used in these reactions. In addition to the substituent on the indole phenyl ring (5-chloro and 6-chloro indole) play a role in the reaction which enhances the product formation as indicated by a shorter reaction time and higher yield (entry 15 and 16). BIMs ($\mathbf{17}_{a,b,c,d,e,f,l}$) are known[222-230], their identities were proven by means of MS, NMR, IR spectra, and the other BIMs, ($\mathbf{17}_{g,h,i,j,k,o,p}$), are novel could not be found in the literature. The short reaction time coupled with the simplicity of the reaction procedure makes this method one of the most efficient methods for the synthesis of this class of compounds.



Scheme (25): Synthesis of BIMs.

entry	Aryl or heteroarylaldehydes	Indoles	product	Reaction time (h)	Yield (%)
1	R = Ph	Indole	**17_a**	5	90
2	R = *p*-NO2-Ph	"	**17_b**	4	98
3	R = *p*-Br- Ph	"	**17_c**	6	99
4	R = *p*-Cl- Ph	"	**17_d**	5	76
5	R = *p*-N(Me)2- Ph	"	**17_e**	5	91
6	R = *m*-Br-Ph	"	**17_f**	4	88
7	R= *m*-OCH_2Ph- Ph	"	**17_g**	5	87
8	R = *p,m*-OH- Ph	"	**17_h**	6	73
9	R=*p*-MeO-*m*-OCH_2Ph-Ph	"	**17_i**	4	89
10	R=*m*-MeO-*p*-OCH_2Ph-Ph	"	**17_j**	5	92
11	R = *m*-Me,2,4,6-tri-F-Ph	"	**17_k**	6	77
12	R = 1-naphthyl	"	**17_l**	4	97
13	R = 3-pridyl	"	**17_m**	6	95
14	R = 3-indolyl	"	**17_n**	6	98
15	R=*p*-MeO-*m*-OCH_2Ph-Ph	5-Cl-indole	**17_o**	4	91
16	R=*p*-MeO-*m*-OCH_2Ph-Ph	6-Cl-indole	**17_p**	4	93

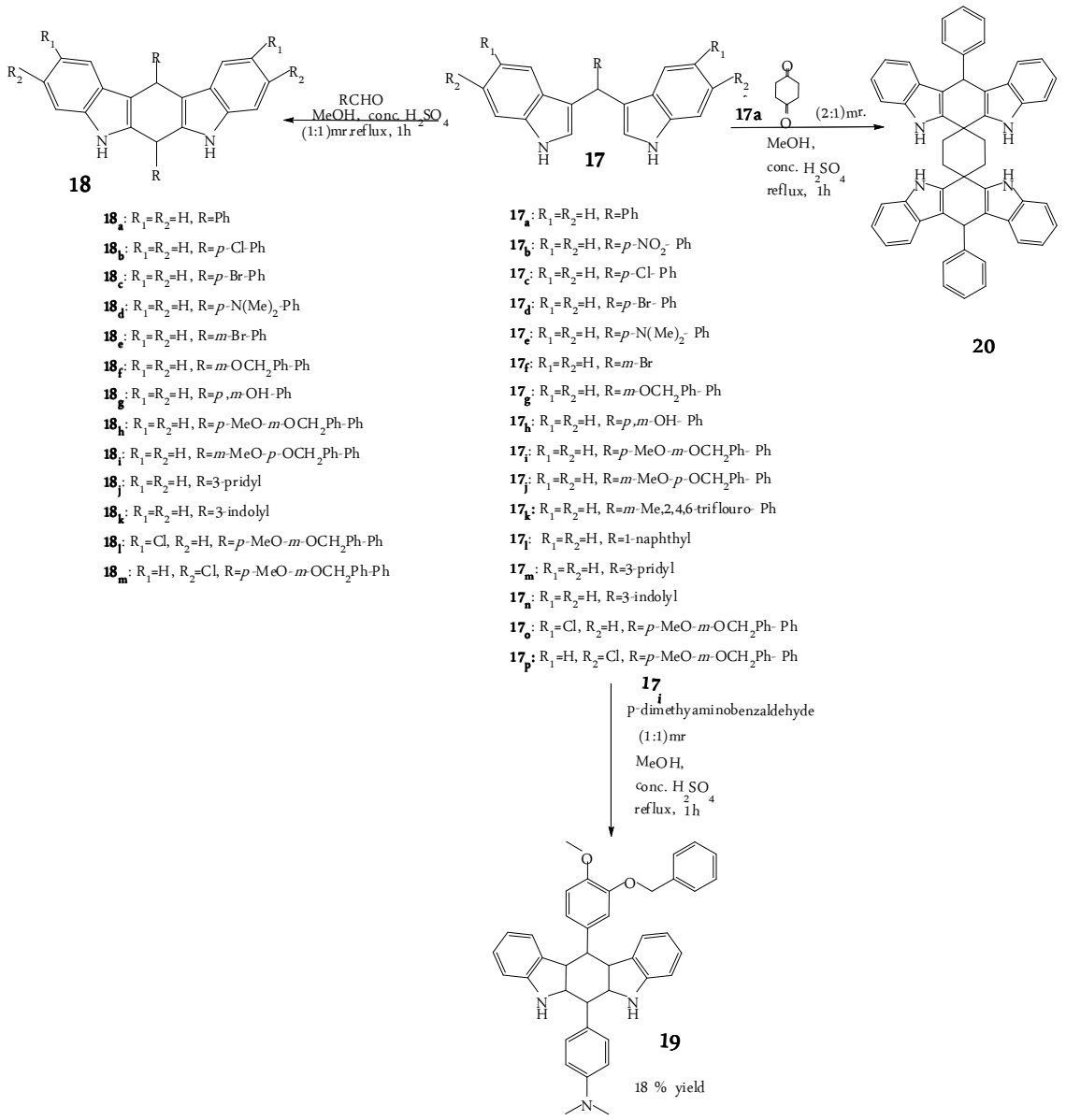
Table (1): Synthesized BIMs (**17_{a-p}**).

3.1.3.2. Synthesis of tetrahydroindolo[2,3-*b*]carbazoles

Indolocarbazoles have been reported as a primary compound for the synthesis of various drugs and possesses important biological, pharmacological, and medicinal activities[231-240]. Indolocarbazoles are associated with anticancer, antimicrobial and antifungal activities. In most of these cases biological activity is correlated with indolocarbazoles containing heteroatom. The biological activity depends on the interaction potential with DNA[241-242]. Furthermore, many experimental studies have indicated that the size, shape and planarity of this structure are important criteria in such DNA interaction[243]. As an extending study of our present work, we used the prepared BIMs (**17$_{a-p}$**) as a starting materials for the synthesis of biologically active tetrahydroindolo[2,3-*b*]carbazoles of type **18$_{a-m}$**, **19** and the extended spirocyclic biscarbazoles **20.** The reaction has been done according to the reported cases of condensation of BIMs with aldehydes or ketones[244] in which the BIM and the aromatic aldehyde (the same aldehyde which condensed with indoles in the synthesis of the used BIM) in molar ratios (1:1) were dissolved in methanol and few drops (0.5 - 1 ml) of conc. H_2SO_4 were added dropwisly. The mixture was refluxed under stirring for about 1 h. The product precipitated and was isolated from the reaction mixture while the solution is hot yielding about the half amount of the pure tetrahydroindolo[2,3-*b*]carbazoles of type **18$_{a-m}$**. The rest of the compounds **18$_{a-m}$** could be extracted and purified from the reaction mixture affording the second corp in good to better yields, scheme (26).

The formation of the tetrahydroindolo[2,3-*b*]carbazoles (**18$_{a-m}$**) were due to the fact that a cyclizative condensation can occur by an acid catalyzed nucleophilic attack of indole nucleus at the two positions, when the two positions are free. The unsubstituted two positions of the two indole nucleus in BIMs can react with a carbonyl group of either aldehydes or ketones, affording the corresponding tetrahydroindolo[2,3-*b*]carbazoles[244]. The ^{1}H-NMR of compounds **18$_{a-m}$** showed the two aliphatic CH protons as a singlet signal at δ between 5.50 to 5.90 ppm values. The acid catalyzed

condensation of indoles with aldehydes has been reported as a method for the preparation of a mixture of substituted isomers of tetrahydroindolo[3,2-*b*]carbazole (*trans* isomer) and tetrahydroindolo[2,3-*b*]carbazoles (*cis*-isomer), in the presence of phosphoryl chloride as acid catalyst directly with indole itself [244(d)]. However the products are not stable under this reaction condition where they readily converted *via* oxidation with air to the dihydroindolocarbazoles, figure (44). The formation of the *trans* isomer has also recently confirmed and published by *Rong Gu* and et al[245(a)] however the reaction has been done under a completely different reaction conditions of using indoles with aromatic aldehydes in (1:1) molar ratio in presence of 2 mol % of iodine as a catalyst in acetonitrile under refluxing for 14 hours (long time reaction) afforded 6,12-trans–isomer, tetrahydroindolo[3,2-*b*]carbazole derivative, which was confirmed by X-ray crystallography.



Scheme (26a): Synthesis of tetrahydroindolo[2,3-*b*]carbazoles.

This behaviour is due to that the iodine catalyze the transformation or the isomerisation of 3,3/-BIMs into 2,3-BIMs under a long time reaction conditions (***The Plancher Rearrangement***)[245(b)]. Then the 2,3-BIMs can undergo an electrophilic attack at a carbonyl group of the second molecule of aldehyde leading to the formation of tetrahydroindolo[3,2-*b*]carbazoles[246]. The reaction of indoles with aromatic aldehydes using iodine as a catalyst is a selective reaction for the preparation of tetrahydroindolo[3,2-*b*]carbazoles and none of the other isomer tetrahydroindolo[2,3-*b*]carbazoles were observed in the reaction mixture. In our reaction using BIMs and

aldehydes in methanolic sulphuric acid solution under refluxing for one hour (short time reaction) afforded the *cis*-isomer, tetrahydroindolo[2,3-*b*]carbazoles (**18$_{a-m}$**) in good yields which were given without isolation of other *trans* isomer, tetrahydroindolo[3,2-*b*]carbazoles. Scheme (26b) showed the mechanism for the formation of our [2,3-*b*] carbazoles (*cis* form) and the other [3,2-*b*] carbazoles (*trans* form). It has been reported that the short reaction time is important to promote the reaction in direction of forming our *cis*-isomer (**18$_{a-m}$**)[246]. The products **18$_{a-m}$** which were prepared by this method were found to be more stable than the same products using $POCl_3$ as a catalyst which were quickly oxidized to form dihydroindolo[2,3-*b*]carbazoles.

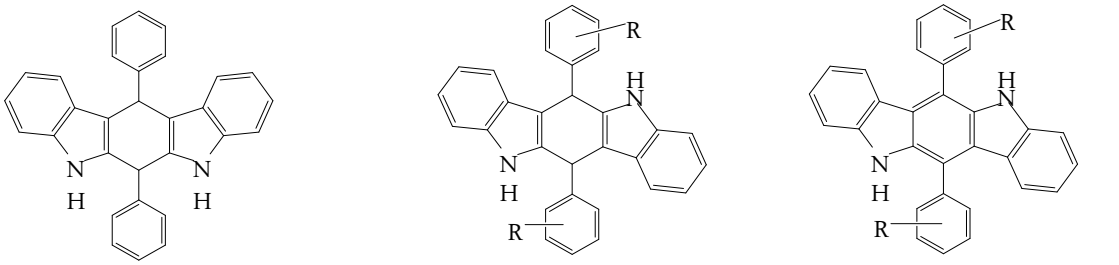
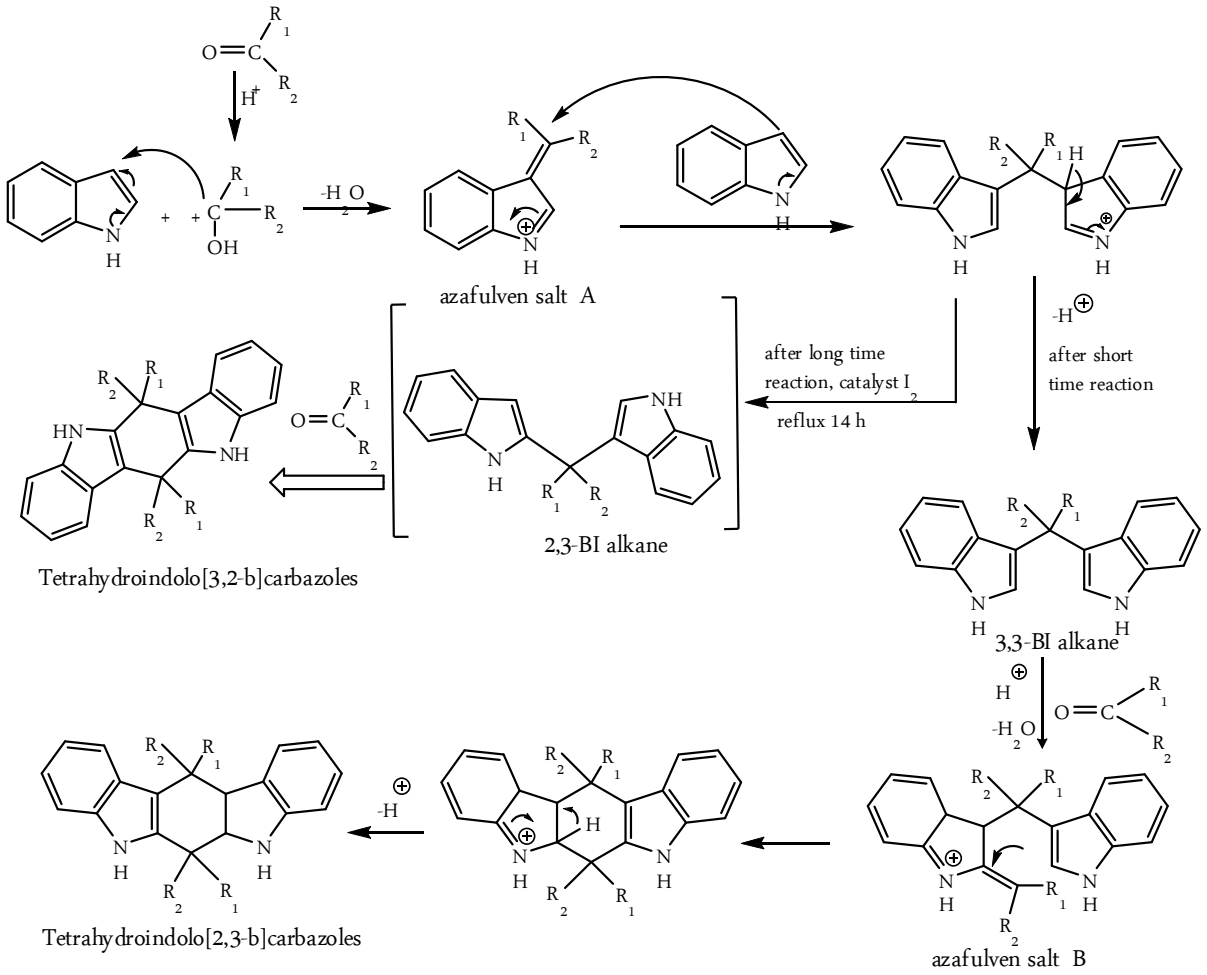


Figure (44): Cis and Trans isomers of indolocarbazoles.

In this context and as a continuation of our work concerning the synthesis of tetrahydroindolo[2,3-*b*]carbazoles with an attempt to prepare the mixed indolocarbazoles (with two different aldehydes). The reaction of BIMs (**17$_i$**) (1 mole equivalent) and *p*-dimethylaminobenzaldehyde (1 mole equivalent) has been done by the method of methanol sulphuric acid solution as a possible route for the synthesis of 4-(8-(3-(benzyloxy)-4-methoxyphenyl)1,1a,2,2a,3,7b,8,8a-octahydroindolo[2,3-*b*]carbazol -2-yl)-*N*,*N*-dimethylanilin (**19**). The desired compound **19** was formed with a low yield of 18 % and confirmed by the means of ^{1}H-NMR, ESI-MS and IR spectra, where the ^{1}H-NMR spectra of **19**, indicated the single signal for 2-protons at 5.79 ppm for two aliphatic CH protons.



scheme (26 b): Mechanism for the formation of tetrahydroindolo carbazoles.

The extended spirocyclic structure (**20**) was synthesized in a better yield of 52 %, by the way of MeOH and conc.H_2SO_4 using BIM (**17$_a$**) (2 moles equivalent) and 1,4-cyclohexanedione (1 mole equivalent). The reaction solution turned from pink colour to dark violet by leaving it stirring for one hour under reflux. The product was detected, purified and confirmed by means of ESI-MS (m/z): 719.29 [M^+-H] and EI-MS (m/z): 720 [M^+] 32 %. Its ^{1}H-NMR spectrum showed a single signal at δ = 5.91 ppm value for 2 protons (2 CH), and two triplet signals every one for 4 protons (2 CH_2) at δ = 2.03 ppm and 2.27 ppm. The four NH indole protons appeared at 9.94 ppm as a broad signal. The structure was confirmed additionally by its APT ^{13}C-NMR spectrum that showed the presence of only twenty six carbon signals as two signals for 2CH_2 carbon, eleven signals for quaternary carbons, one carbon signal for one CH aliphatic carbon

and thirteen carbon signals for 13 CH aromatic carbons. These data proved that the novel spirocyclic compound **20** is a symmetrical structure.

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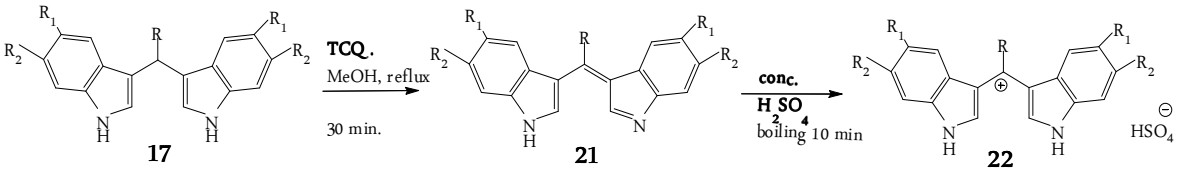
3.1.3.3. Oxidation reactions of BIMs

The antibiotic agent turbomycin A has been isolated as a product of *saccharomyces cerevisiae* fermentation and identified as the salt of tris(indol-3-yl)methylium[171]. Turbomycin A and Turbomycin B were obtained from soil microorganisms by a metagenomic approach using a 24.546-member DNA library expressed in *Escherichia coli*[32], in which turbomycin A was designated as tris(indol-3-yl)methylium and *turbomycin B* as bis(indol-3-yl)phenyl)methylium, figure (7). Both of these agents were capable of killing gram-negative and gram-positive microorganisms[32]. These results provided strong evidence that turbomycins comprise a perspective class of biologically active compounds.

In view of this, we will introduce a novel class of biologically active turbomycines *via* oxidation reaction of some previously prepared BIMs ($\mathbf{17_{a-p}}$). The diindolylmethenes were first named "*Rosindoles*" by *Fisher*[247]. The substituted di- or tri- indolylmethenes have been synthesized in literature by the oxidation of the analogous BIMs using oxidizing agents such as DDQ, TCQ[171, 33], tritylperchlorate[248] or $FeCl_3$[249], where if the reaction was accomplished in presence of acid (H^+X^-) as a source of the anion it will afford the corresponding methylium salts e.g. turbomycin A and turbomycin B, compounds $\mathbf{22_a}$ and $\mathbf{22_b}$ in presence of conc. sulphuric acid. In the absence of the acid the reaction afforded only the free bases compounds ($\mathbf{21_{a-k}}$). BIMs are found to be very sensitive compounds against the oxidizing agents. Our prepared BIMs ($\mathbf{17_{a-p}}$) were dissolved in methanol and 1.5 mole equivalent of TCQ or DDQ (as oxidized agents) were added. The reaction solution turned from light yellow to dark red and allowed to reflux for 30 to 60 minutes yielding our bisindolylmethenes of type $\mathbf{21_{a-k}}$ as a free base due to the absence of the anion source (the acid), scheme (27).



17

21

22

17_a: $R_1=R_2=H$, R=Ph
17_b: $R_1=R_2=H$, R=*p*-NO_2- Ph
17_c: $R_1=R_2=H$, R=*p*-Cl- Ph
17_d: $R_1=R_2=H$, R=*p*-Br- Ph
17_e: $R_1=R_2=H$, R=*p*-$N(Me)_2$- Ph
17_f: $R_1=R_2=H$, R=*m*-Br
17_g: $R_1=R_2=H$, R=*m*-OCH_2Ph- Ph
17_h: $R_1=R_2=H$, R=*p,m*-OH- Ph
17_i: $R_1=R_2=H$, R=*p*-MeO-*m*-OCH_2Ph- Ph
17_j: $R_1=R_2=H$, R=*m*-MeO-*p*-OCH_2Ph- Ph
17_k: $R_1=R_2=H$, R=*m*-Me,2,4,6-triflouro- Ph
17_l: $R_1=R_2=H$, R=1-naphthyl
17_m: $R_1=R_2=H$, R=3-pridyl
17_n: $R_1=R_2=H$, R=3-indolyl
17_o: $R_1=Cl$, $R_2=H$, R=*p*-MeO-*m*-OCH_2Ph- Ph
17_p: $R_1=H$, $R_2=Cl$, R=*p*-MeO-*m*-OCH_2Ph- Ph

21_a: $R_1=R_2=H$, R=Ph
21_b: $R_1=R_2=H$, R=3-indolyl
21_c: $R_1=R_2=H$, R=*p*-Cl- Ph
21_d: $R_1=R_2=H$, R=*m*-Br- Ph
21_e: $R_1=R_2=H$, R=*p,m*-OH- Ph
21_f: $R_1=R_2=H$, R=2,4,6-trifluro-3-methylphenyl
21_g: $R_1=R_2=H$, R=*p*-MeO-*m*-OCH_2Ph- Ph
21_h: $R_1=Cl$, $R_2=H$, R=*p*-MeO-*m*-OCH_2Ph- Ph
21_i: $R_1=H$, $R_2=Cl$, R=*p*-MeO-*m*-OCH_2Ph- Ph
21_j: $R_1=R_2=H$, R=1-naphthyl
21_k: $R_1=R_2=H$, R=3-pridyl
21_l: $R_1=R_2=H$, R=*p*-$N(Me)_2$-Ph

22_a: $R_1=R_2=H$, R=Ph (Turbomycin B)
22_b: $R_1=R_2=H$, R=3-indolyl (Turbomycin A)

Scheme (27): Synthesis of bisindolylmethenes and its salt formation

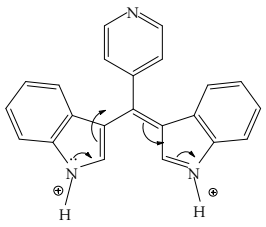


Figure (45): Reported monoprotonated form of diindolylpyridylmethene

The structural features of the free base **21_{a-l}** were determined by its analytical and spectral data ESI-MS, ^{1}H-NMR, ^{13}C-NMR and IR, where the ^{1}H-NMR gives strong indication for the disappearance of the protons related to the aliphatic CH function and no detection for the NH indole protons. The disappearance of the remaining indolic NH has been explained as in the previous reported cases of these bisindolylmethenes, due to the high delocalization which resulted from the conjugation of the remaining strong acidic NH indole. This fact was well documented by the reported monoprotonated form of diindolylpyridylmethene, figure (45)[172]. This indolic NH proton has not also been found in the IR spectra. Figure (46), shows the two IR spectra of the known compound **17_a** (BIM) and compound **21_a** (oxidized form), in which **17_a** has a sharp peak at 3410 for the NH indole, however compound **21_a** has no detection for the remaining NH indole.

The ^{1}H-NMR and ^{13}C-NMR spectrums illustrated that these structures (**21$_{a-l}$**) show not any isomerisation as *E/Z* or *cis/trans* forms because the pure spectra did not show any repetition for the signals of the protons related to the presence of *E/Z* forms as it is in the published examples of these compounds. The spectral data of our prepared compounds **21$_a$**, **21$_b$** and **21$_k$** were found to be identically to those of the published compounds[171, 32]. The synthesized BIMs and bisindolylmethenes were recently reported as new host molecules for anion recognition and sensing by hydrogen bonding interaction[250]. Indole based receptors have attracted considerable attention due to the acidity of the indole NH group which was expected to be enhanced by the conjugation with benzene which would lead to higher binding affinity for anions[251]. These anions have a wide range of importance in medicinal, environmental and biological process[252]. The large conjugated bis(3-indolyl)methene skeleton has been demonstrated to be an efficient chromogenic and fluorescent moiety for metal ions sensing by *Kim* and coworkers[253]. In view of this recent documentation the mono sulphate salts **22$_{a;\ b}$** can easily be synthesized just by the addition of the anion as an acid or in its tetrabutylammonium salt in methanol or acetonitrile solution and boiling the mixture for 10 minutes. The crude product can be crystallized or purified by column chromatography. There were a lot of acids which have been used for this reactions[174-177], that have been known in the branch of physical chemistry by colorimetric chemosensors. Bisindolylmethenes (**21$_{a-l}$**) and its monosulfate salts (**22$_{a,\ b}$**) were formed with a high yield over 76 %. The ^{1}H-NMR spectra of the sulphate salt **22$_{a,\ b}$** appear to be the same that of the free bases **21$_a$** and **21$_b$** respectively upon addition of 0.5 to 2.5 equivalents of the anion, and it showed broading of the signals after the addition of 5 to 10 equivalents[174, 254]. This has been illustrated by the reported ^{1}H-NMR spectra in DMSO-d_6 of bisindolylmethene **21$_a$** before and after the addition of various quantities of the fluoride anion F^- in zero, 0.4, 2.5, 5 and 10 equivalents as shown in figure (47)[174]. The ^{1}H-NMR spectrum of our synthesized monosulfate salts **22$_{a,b}$** appeared to be identical to the case of the addition of 5 to 10 equivalents of the anion which indicates that we have used an excess of conc. H_2SO_4.

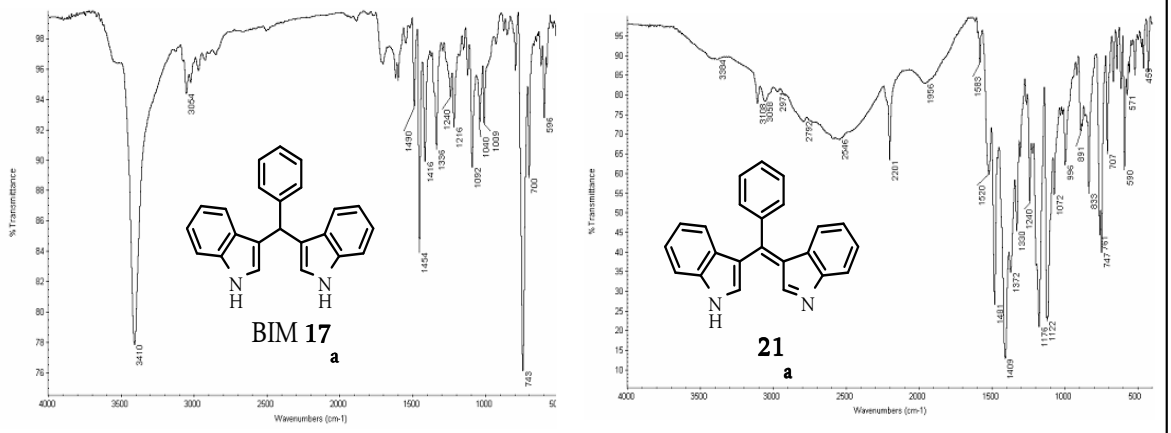


Figure (46): IR spectra of BIM **17** and its oxidized form **21**
a a

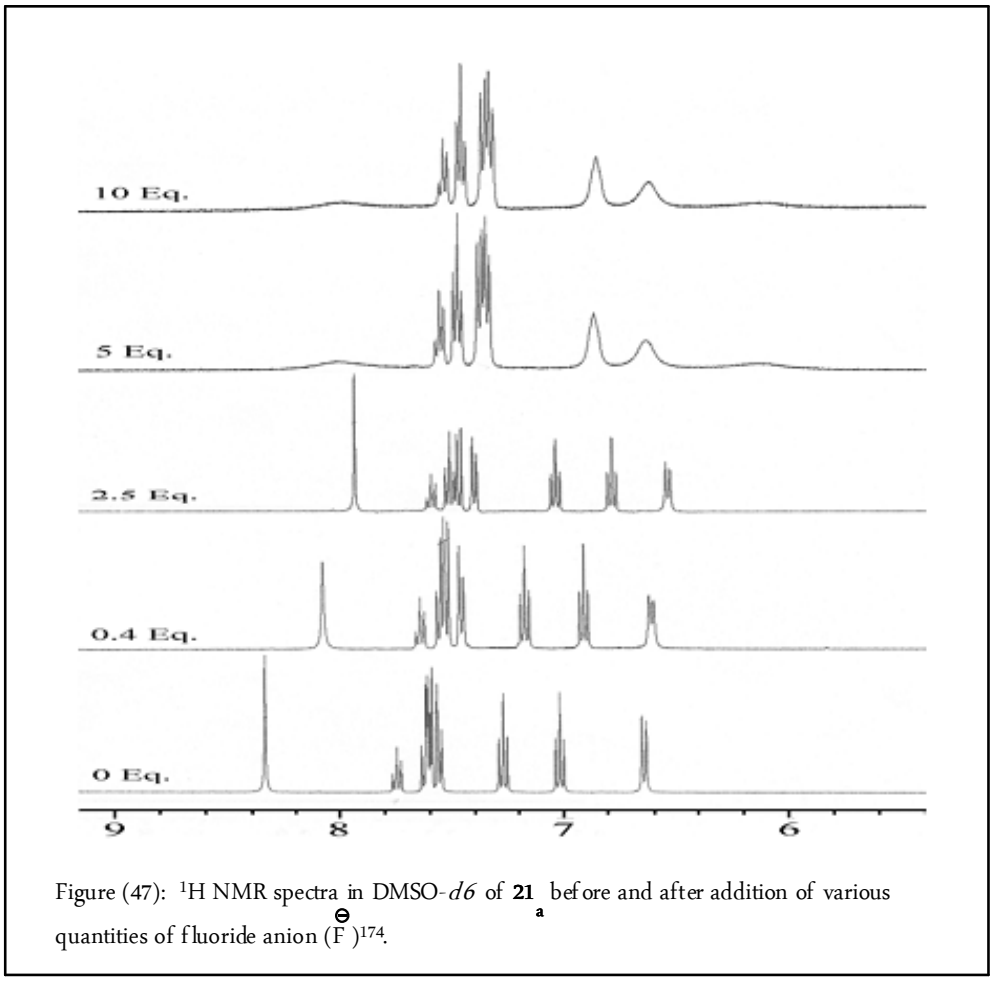
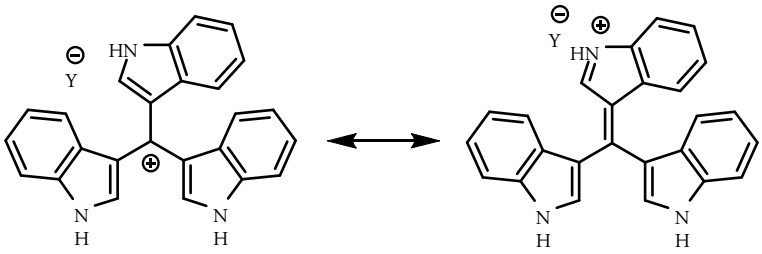


Figure (47): ^{1}H NMR spectra in DMSO-*d6* of **21** before and after addition of various quantities of fluoride anion ($F^{\ominus}$)[174].
a

The salts $\mathbf{22_{a,b}}$ recorded lower R_f values than the free bases $\mathbf{21_{a,b}}$ and different melting points. It has been recently reported that, the salts of type $\mathbf{22_{a,b}}$ have two possible structures the indolyl methylium cation form and the indolenine form as illustrated in

figure (48). However the quantum chemical calculations of the two structures showed that the delocalized form of the inolylmethylium cation is more stable than the indolenine form by 1.4 kcal/mol[254].

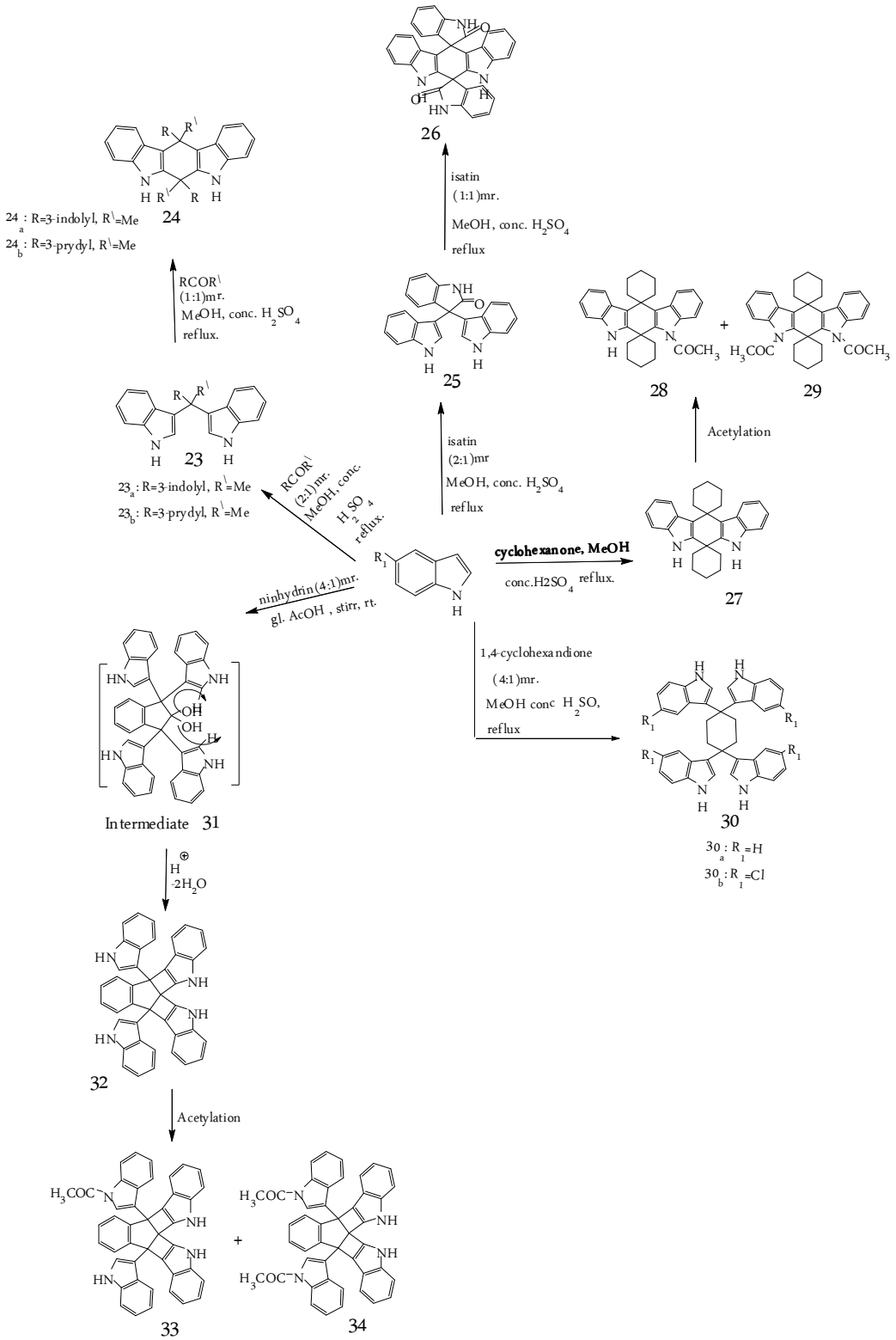


Indolylmethylium cation form

Indolenine form

Figure (48): Resonance stabilization of turbomycin A

3.1.4. Condensation reactions of indoles with different types of ketones:



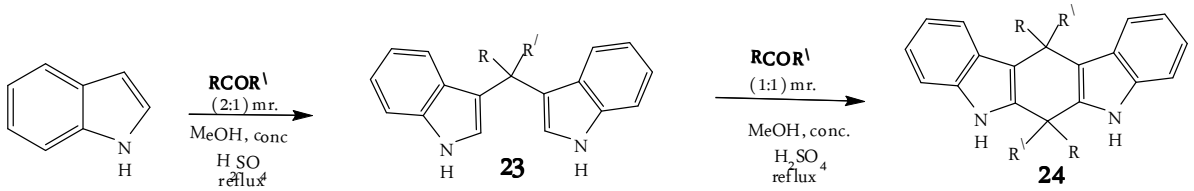
Scheme (28) : The whole scheme of Condensation reactions of indoles with different types of ketones.

In this part we extended our investigation to similar electrophilic condensation reactions of indoles with carbonyl compounds including different types of ketones, e.g. heteroacetyl ketones (3-acetylindole and 3-acetylpyridine), cyclohexanone, isatin, cyclohexane-1,4-dione and ninhydrine, scheme (28). This condensation of ketones was carried out in a molar ratio of 1:2, one mole of ketones with two moles of indoles, and afforded the corresponding BIMs. These BIMs were isolated from the reaction mixture as in the case of compounds **23**$_{a,b}$ or directly used without an isolation from the reaction mixture to condense with another equivalent mole of ketones for the formation of tetrahydroindolo[2,3-*b*]carbazole (**24**$_{a,b}$).

The main propose of this part of our work was an attempt to synthesis novel spirocyclic structures. The formation of BIMs derived from ketones follow the similar reaction mechanism of the formation of BIMs derived from aldehydes, scheme (26 b).
The acids have been utilized for the reaction of indoles with ketones as well as with aldehydes. The formation of our BIMs derived from aldehydes was carried out smoothly at room temperature in a solution of glacial acetic acid. However the BIMs derived from ketones did not give products by the method of using glacial acetic acid. Numerous procedures for the synthesis of BIMs derived from ketones using different types of catalysis have been reported in literature[120-148,244,245]. Glacial acetic acid has been catalyzed this reaction but under reflux and in presence of 2 N H_3PO_4[255]. We were used the known method of methanol sulphuric acid solution as a suitable way for the synthesis of these BIMs and also the corresponding tetrahydroindolo[2,3-*b*]carbazoles. This method was useful in the condensation reactions of indole with cyclohexanone, cyclohexan-1,4-dione and isatin, however, the reaction of indoles with ninhydrin was carried out in glacial acetic acid.

3.1.4.1. Condensation reactions of indoles with acetylketones

Indole (two equivalents) was condensed with acetyl indole or acetyl pyridine (one equivalent) in methanol solution containing drops of conc. H_2SO_4 under reflux. The reaction afforded BIMs of type **23$_{a,b}$** in yields of 52 % and 55 % respectively. The formed moderate yield is due to the lower reactivity of ketones if compared to aldehydes, thus the reaction was accomplished under vigorous conditions and produced moderate yields. Several arylacetylketones have been reported in these condensation reactions. However only a limited numbers of heteroaryl acetyl ketones have been reported.



23$_a$: R=3-indolyl, R\=Me
23$_b$: R=3-pyridyl, R\=Me

24$_a$: R=3-indolyl, R\=Me
24$_b$: R=3-pyridyl, R\=Me

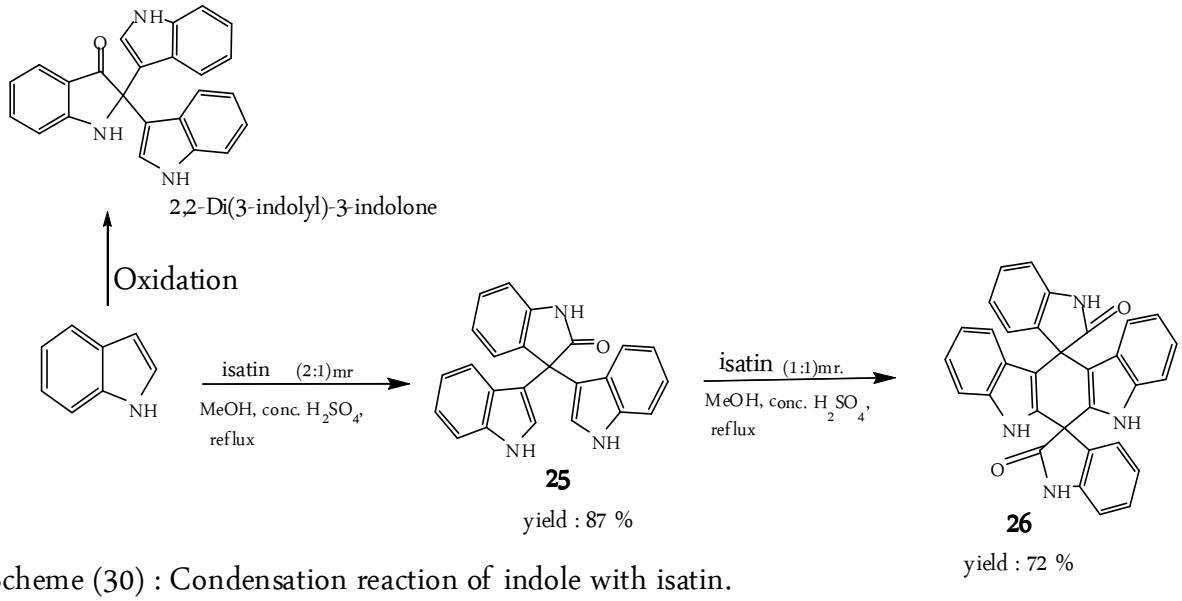
Scheme (29): Condensation of indole with acetylketones.

Under the similar reaction conditions we had the primary intention of using the ketones as precursors for the preparation of the corresponding tetrahydroindolo[2,3-*b*]carbazoles of type **24$_{a,b}$**, scheme (29). BIMs **23$_{a,b}$** were isolated from the reaction mixture and were used in a new reaction flask with an equivalent amount of the desired ketone in methanol sulphuric acid solution under reflux affording compounds **24$_{a,b}$**. Compounds **23$_{a,b}$** and **24$_{a,b}$** have been identified by means of their spectroscopic data. The mechanism of the reaction follow the same previous mechanism of condensation reactions of indoles with aldehydes in scheme (26 b).

3.1.4.2. Condensation reaction of indole with isatin

Isatin as an example of a 1,2-diketone was condensed with indole for the preparation of the indole trimer by following the similar reaction conditions of the methanol-sulphuric acid solution. The indole trimer **25** was a known natural product which has been isolated from the frish marine sponge *Hyrtios altum*, and was named as trisindole shown to have antibiotic activities against bacteria (*E.Coli*, *Bacillus Subtilis*, *Staphylococcus aureus* and *Streptomyces*) and (*Candida albicans* and *Mucor miehei*)[31,257].

An attempt to prepare trisindole **25** in glacial acetic acid at room temperature was failed. The use of methanol sulphuric acid solution method in a molar ratio of two moles of indole and of one mole of isatin under reflux for two hours yielded the trisindole **25** in an 87 % yield. This method is considered to be simple and efficient if compared to the reported chemical procedures for the preparation of **25**, scheme (30). Where trisindoline (**25**) was synthesized in the following manner using oxindole which was treated with $CuBr_2$, in ethylactate under reflux for 3 hours to give 3,3-dibromooxindole which was then treated with indole and silver carbonate in THF at 25 ^{0}C for 1.5 h to furnish trisindoline (**25**) in 47 % yield. The spectral data of our synthesized trisindoline (**25**) were identical with those of the natural product isolated from the marine sponge *Hyrtios alum*. The trinuclear indole derivative 2,2-di(3-indolyl)-3-indolone, was reported recently as an oxidized product of indole [258]. This compound was unstable and decomposed gradually.

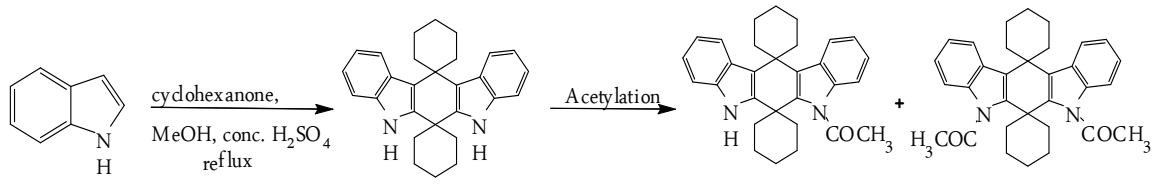


Scheme (30) : Condensation reaction of indole with isatin.

The trisindoline (**25**) was used as a precursor for a condensation with an equimolecular amount of isatin as a possible way for the synthesis of the expected novel spirocyclic structure **26** which was confirmed on the basis of its spectroscopic data, where the ^{1}H-NMR spectrum demonstrated the presence of four multiplet signals each one for four aromatic protons and two broad signals each one for two NH protons.

3.1.4.3. Condensation reaction of indole with cyclohexanone

Cyclohexanone was condensed with indole using different types of catalysts as well as aldehydes[120-148]. Using the method of MeOH/conc. H_2SO_4 in the reaction of indole with cyclohexanone in a molar ratio of 2:1 the known (3,3′-(cyclohexane-1,1-diyl)bis(1-*H*-indole)) was isolated. It was detected by TLC and ESI-MS of the reaction mixture and not isolated from the reaction mixture but directly used into the second condensation step with the second mole of cyclohexanone under the same conditions of MeOH/conc. H_2SO_4 leading to our second novel spirocyclic structure **27** in a 97 % yield. Compound **27** was determined to be the 2,8,2′,8′-bis(cyclohexane-1,1-diyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (**27**), scheme (31).

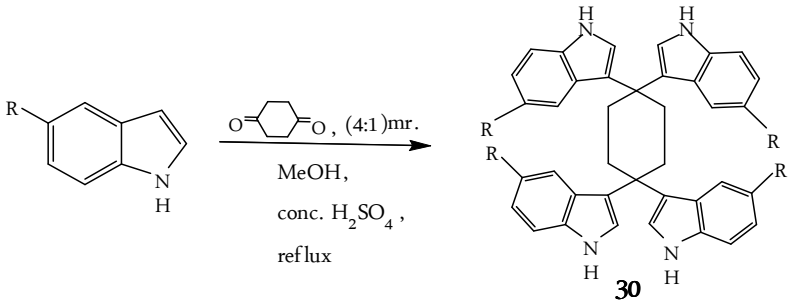


Scheme (31): Reaction of indole with cyclohexanone.

The spirocyclic structure **27** exhibited an EI-MS with m/z 395 [M^+] 20 % and its ^{1}H-NMR spectra showed five multiplet signals for the twenty aliphatic protons (10 CH_2) of the cyclohexyl groups and one singlet signal at 10.66 ppm for two NH indole protons. The ^{13}C-NMR (*Apt*) spectra of compound **27** showed the presence of five carbon signals for the five CH_2 groups and one signal for the spirocyclic atom. Eight signals were observed for the aromatic indole carbons. Based on these data it was presumed that compound **27** possesses an asymmetrical structure. In order to verify the structure of compound **27** it was acetylated using acetic anhydride and triethylamine in presence of 4-(dimethylamino)pyridine (DMAP) as a catalyst. The reaction afforded two products one was determined as monoacetylated product (**28**) in a 59 % yield and was confirmed by its ESI-MS, ^{1}H-NMR, ^{13}C-NMR and IR spectra. These data showed the presence of the carbonyl group in the ^{13}C-NMR spectrum at δ = 169.66 ppm and a carbon signal at δ = 14.54 ppm for the methyl group. The IR spectrum of compound **28** exhibited a strong peak at 1670 cm^{-1} for the C=O group. The ^{1}H-NMR indicated the disappearance of one NH indole proton and the presence of singlet signal at δ = 2.69 ppm integrated for three protons for the acetyl methyl group. ESI-MS showed the molecular weight of compound **27** plus only one acetyl group. The second reaction product had a lower R_f value and was identified based on its spectroscopic data as the diacetylated product (**29**) in a lower yield of 32 % than that of the monoacetylated product **28**. Thus the acetylation reaction takes place stepwisly and the formation of the diacetylated products needs more time. This may be due to striric hindrance of the two NH indole which more expected to be in the same direction.

3.1.4.4. Condensation reactions of indoles with 1,4-cyclohexandione

The electrophilic substitution reactions of indoles with cyclohexan-1,4-dione has been reported in the literature as a possible way for the synthesis of the extended supramolecular compounds **30$_{a,b}$** named as 1,1,4,4-tetrakis(1*H*-indol-3-yl)cyclohexane (**30$_a$**)[189]. The reaction takes place in presence of catalyst such as iodine and *N*-bromosuccinimide (NBS)[190,191,196,197] affording the tetra substituted product in good yield. In the course of this study cyclohexan-1,4-dione was condensed with indole in MeOH/conc. H_2SO_4 solution in a molar ratio of 1:4 yielding compounds **30$_{a,b}$** in a 82 and 87 % yield, respectively after refluxing of 2 hours, scheme (32).



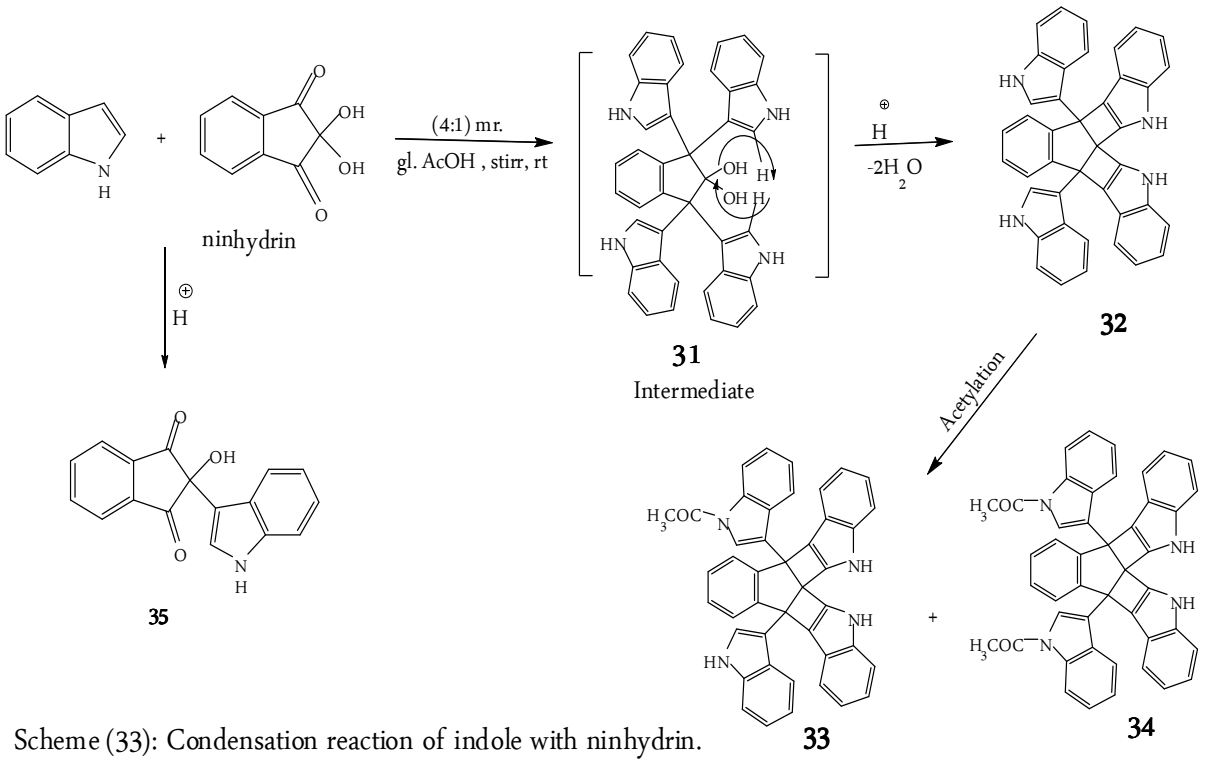
Scheme (32): Condensation of indoles with cyclohexane-1,4-dione

The spectroscopic data of compound **30$_a$** were identical to the data of the reported tetra-substituted indole product[196,197]. The other novel derivative **30$_b$** using 5-chloroindole was synthesized and elucidated in the same manner.

3.1.4.5. Condensation reaction of indole with ninhydrin

Indole based macrocyclic systems attract increasing interest in a relation to their conformational and self-association properties, stacking interactions, spectroscopic features, cavity shape, and performance as ligands or ion sensing scaffolds[259]. A synthetic trial for the synthesis of the novel indole based macrocyclic systems of type **31** was made *via* a condensation reaction of indole with 2,2-dihydroxy-1*H*-indene-1,3-(2*H*)-dione (ninhydrin) in glacial acetic acid as solvent. The reaction was done in a molar ratio of

four mole of indole to one mole of ninhydrin by refluxing for 10 - 15 minutes. Then the mixture was left overnight under stirring at room temperature. The reaction was worked up *via* neutralization with NaOH solution (10 %), extracted with dichloromethane, concentrated and the product had R_f value of 6.6 in 100 % of CH_2Cl_2 was purified by column chromatography eluted with dichloromethane. This attempt was made with an intention to prepare the expected tetraindole **31**. However the spectroscopic data confirmed the fantastic and novel carbon skeltol structure of compound **32** that has not reported yet, scheme (33). The mechanism of the formation of this compound **32** was attributed to that the four indole molecules were condensed with the two carbonyl groups of the ninhydrin under acid catalyzed conditions result in the tetraindole **31** as an intermediate which was not isolated from the reaction mixture. Then the tetraindole **31** underwent a fast and successful acid dehydration with an elimination of two moles of water from the two hydroxyl groups and the two protons at the α-positions of the nearest two indole units affording compound **32** with a 91 % yield, scheme (33).



Scheme (33): Condensation reaction of indole with ninhydrin.

The ^{1}H-NMR indicated the presence of thirteen signals in the area of 6.68 ppm to 8.59 ppm values related to the twenty two aromatic and indolic protons, and one signal

doublet at 10.85 ppm for two protons belonging to the two NH indole and the other two NH indole protons appeared as one signal doublet at 11.80 ppm. There were two signals each one for two protons belonging to the aromatic indene protons and five signals each one for two protons related to the ten indolic protons of the two free indole units. The other eight indolic protons related to the two fused indole units appeared separately one signal for each proton. These data confirmed that in comopound **32** the two free indole units are symmetrically whereas the two fused ones are non symmetrical. This was also proved by a ^{1}H-^{1}H zTOCSY spectrum which confirmed that compound **32** consists of only four different spin systems instead of five spin systems. The ^{13}C-NMR / DEPT spectrum indicated that the compound contains twenty two aromatic CH carbon atoms and nineteen quaternary carbon atoms. For differentiating between the two signals of the NH indole protons the ^{1}H-^{1}H COSY spectrum of compound **32** showed that the signal at 10.85 ppm correlated through the bond with the signal at 7.34 ppm which is related to the two CH pyrrole of the two free indole units. Whereas the two signals at 11.80 ppm value has not any correlation through one bond due to the absence of the adjacent αCH pyrrole protons that disappeared by the cyclisation. In addition the gHMBCAD spectrum of compound **32** indicated the presence of ^{1}H-^{13}C correlation through space between the two NH indole protons of the fused indole units and the C_3. These data confirm that the 10.85 ppm is related to the two NH indole protons of the free indole units whereas the signal at 11.80 ppm is related to the two NH indole protons of the fused indole rings. The structure of compound **32** was additionally confirmed by ^{1}H-^{1}H ROESY and ^{1}H-^{13}C gHSQCAD spectrums, see all the spectrums of compounds **32**, **33** and **34** in the appendix figures (68 to 73). Concerning the structure configuration of compound 32 as a non planar molecule and a three dimentional model of compound **32** showed that it it may have two different possible structure configurations as in figure (49). To confirm the suggested structure configurations of compound **32** we need other 3D-NMR experiments which will be one of our future objectives.

Compound **32** was submitted for acetylation reaction which afforded two products after long time of stirring at room temperature. The products were determined to be the monoactylated form **33** and the diacetylated form **34**, scheme (33). The ^{1}H-NMR spectrum of the monoacetylated compound **33** demonstrated the disappearance of one NH indole proton which appeared in compound **32** at a ppm value of 10.85. This confirmed that the acetylated NH indole is one of the free rotable indole units, whereas the ^{1}H-NMR spectrum of the diacetylated **34** indicated the disappearance of the second NH proton at 10.85 ppm. From this data we can summarize that the two free rotatable indole moieties were acetylated at first. Moreover, their acetylation was found to be easier than that of the two NH indole protons of the fixed or fused indole units. Because of the two acetylated products **33** and **34** were formed after few hours and although the reaction was left standing over one month we did not found any tri or tetra acetylated compounds. This can be attributed to the steric hindrance which has been expected from the three dimensional models of the two possible structures of compound **32**, figure (49).

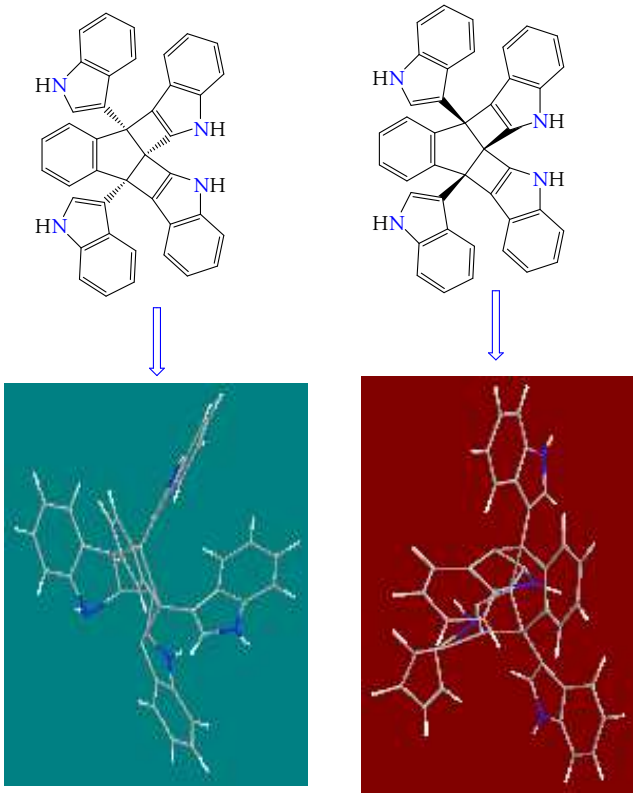
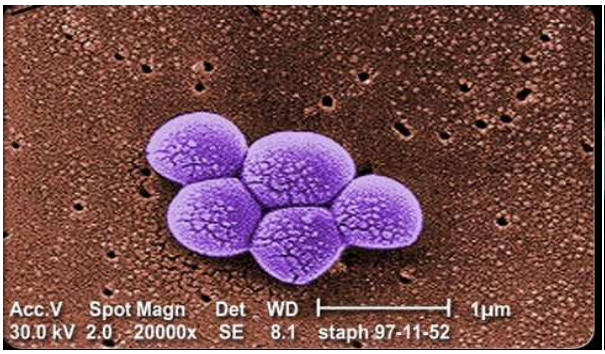
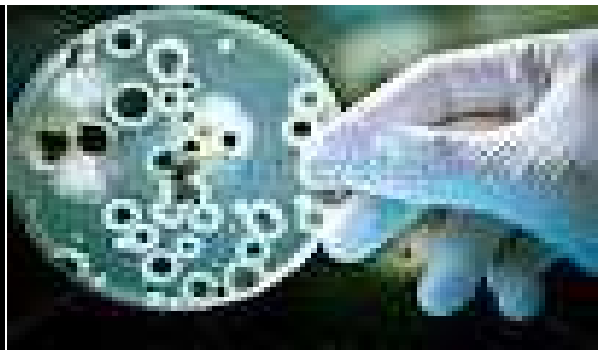
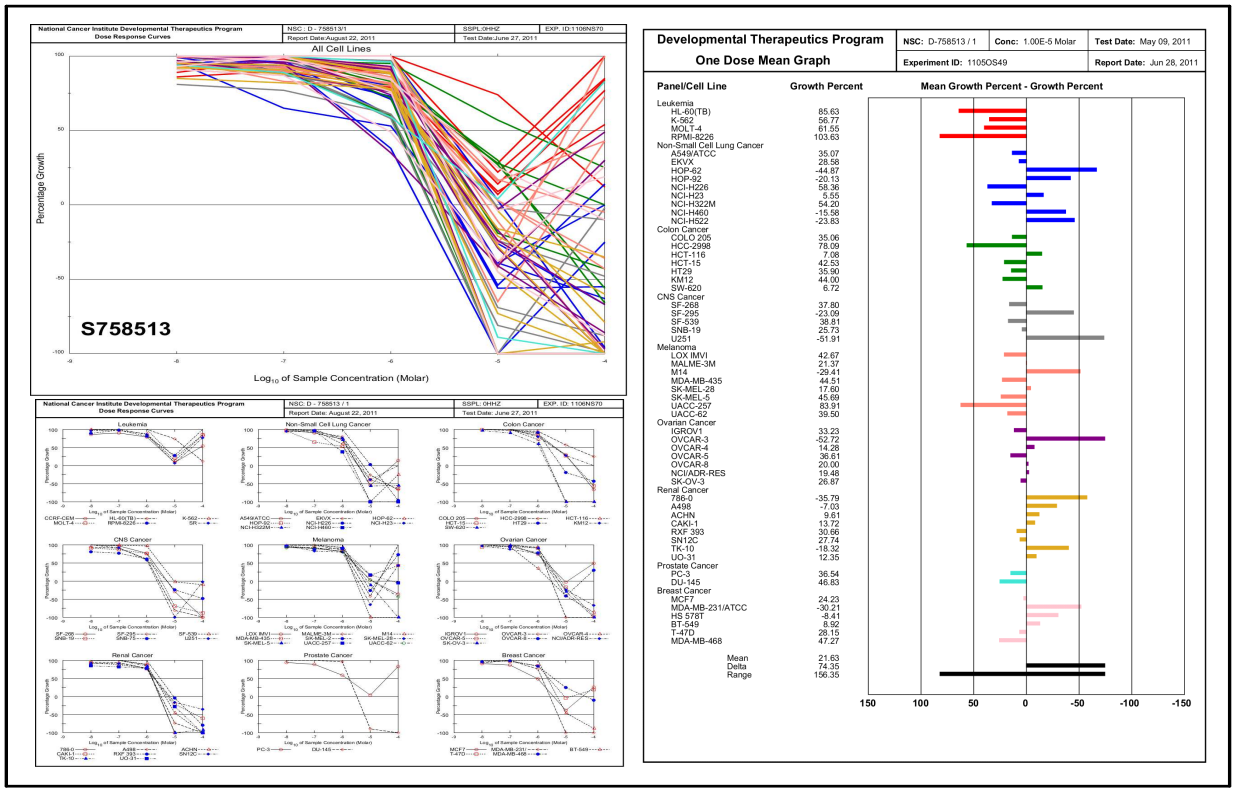


Figure (49): 3D models of the two possible structure configurations of compound **32**.





Pharmacology



3.2- Results of Pharmacological Studies

3.2.1- Results of Antimicrobial assays

As a result of the rising number of multidrug resistant bacteria, the recent years have witnessed an increased demand for novel antibiotic compounds. Indeed examples of multiple resistances have been reported for strains of streptococcus pneumonia and staphylococcus aureus across Asia, South America, Australia and Europe[262-267]. Methicillin resistant Staphylococcus aureus (MRSA) continues to be a leading cause of nosocomial infections[268]. Over the past several years, Community-associated MRSA strains have been involved in an increasing number of serious infections not originated in the hospital setting[269]. Despite the availability of several classes of antibiotics including the relatively recently introduced oxazolidinones (linezolid) and lipopeptides (daptomycin), these infections cause significant morbidity and mortality. Resistance to a number of antibacterial agents has slowly, but steadily, increased over time including reports of clinical isolates resistant to vancomycin, a widely used antistaphylococcal drug[270]. The current clinical landscape suggests that both the prudent use of existing drugs coupled with new antibacterial discovery is required to combat these serious medical issues[271]. A valuable new addition to our antibacterial arsenal for MRSA infections would be new bactericidal agents effective against resistant isolates or standard.

3.2.1.1- Biological evaluation and discussion

ATCC strains of the microorganisms used in this study were obtained from the culture collection of the Refik Saydam Health Institution of Health Ministry, Ankara, and maintained at the Microbiology Department of the Faculty of Pharmacy of the Ankara University. All the compounds were tested for their in vitro growth inhibitory activity against Candida albicans ATCC 10145 as fungus, S. aureus ATCC 25923, Bacillus subtilis ATCC 6633, MRSA standard ATCC 43300 and MRSA isolate as Gram-positive bacteria and E. coli ATCC 23556 as Gram-negative bacteria. The antimicrobial

activity test for the selected new indolyl compounds was performed using the Agar Cup-diffusion technique as described by *Arret* et al[272a] and code of Fedral Regulations[272b] and by two fold serial dilution methods (http://www.fao.org/docrep/005/ac802e/ac802e0q.htm). Our selected novel bis and trisindolyl compounds for these tests that were illustrated in figure (30), can be classified according to the structure and derivatives into six groups:

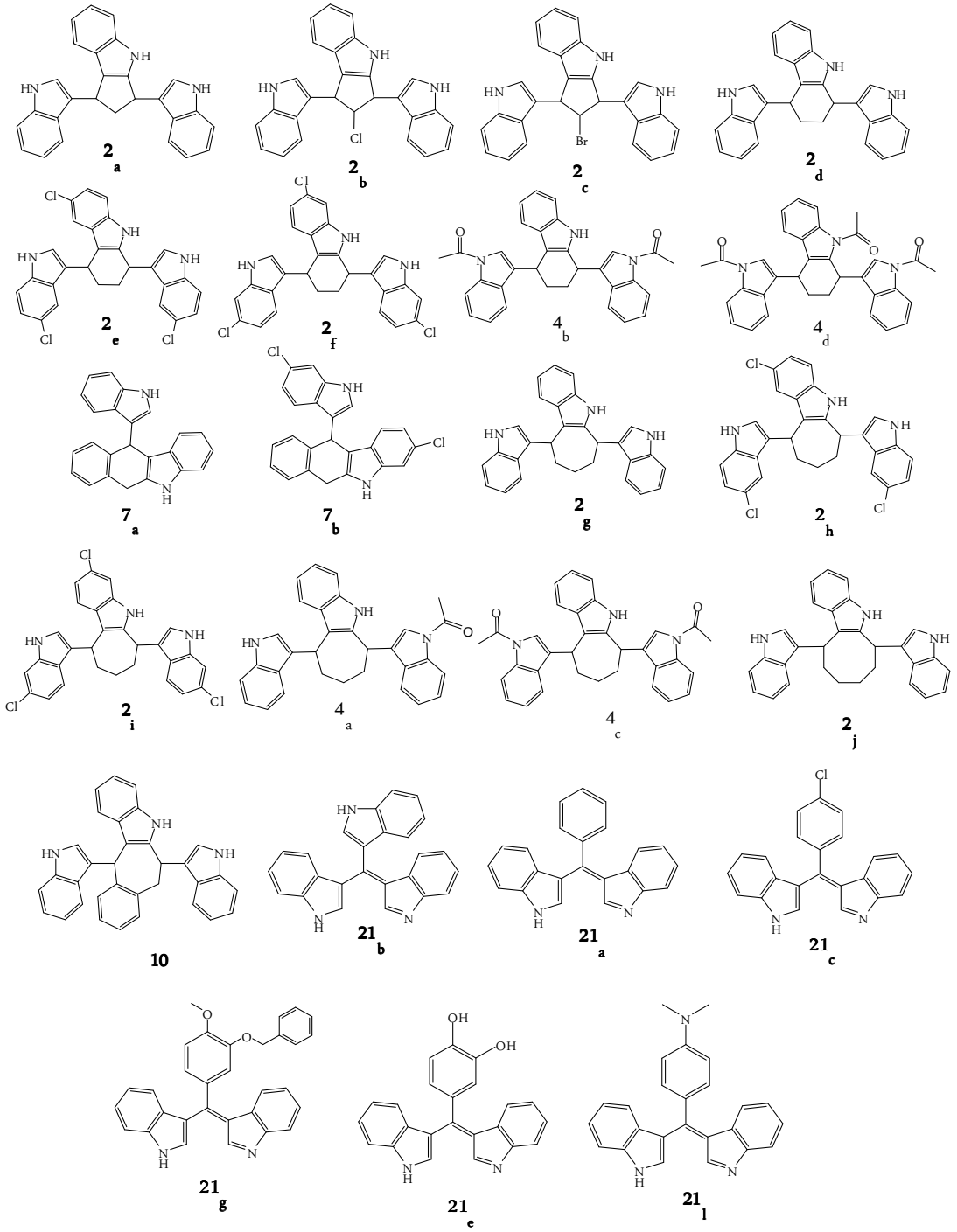
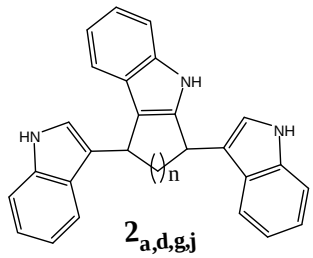


Figure (30): Selected compounds for antimicrobial tests.

3.2.1.2- Group (A), according to ring size compounds ($2_{a,d,g,j}$)



$2_{a,d,g,j}$

Table (2): Variety ring size of compound **2**

Compound No.	n
2_a	1
2_d	2
2_g	3
2_j	4

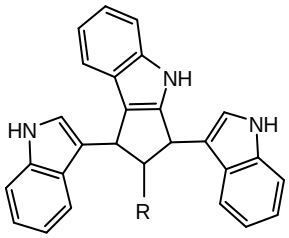
Table (3): MIC values µg/ml of compounds **$2_{a,d,g,j}$**.

compounds	S. aureus	$MRSA_{standard}$	$MRSA_{isolate}$	E. coli	B.subtilies	C.albicans
2_a	50	12.5	12.5	50	50	12.5
2_d	3.125	3.125	6.25	50	6.25	12.5
2_g	3.125	6.25	12.5	50	6.25	6.25
2_j	3.125	6.25	12.5	25	6.25	12.5
Sultamicillin	0.39	25	25	25	0.78	-
Ampicillin	0.78	50	50	50	50	-
Fluconazole	-	-	-	-	-	0.78
Ciprofloxacin	0.78	6.25	12.5	0.19	0.09	-

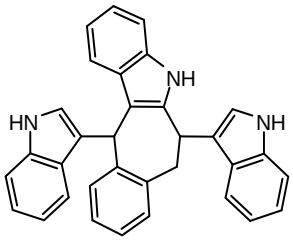
Increasing the ring size is favourable where the six memberd ring compound **2_d** is the best one, with good activity against S. aureus and MRSA standard and less activity against MRSA isolate and B. subtilis. Seven memberd ring compound **2_g** indicates good activity against S. aureus, less activity against MRSA standard, B. subtilis and C. albicans. Compound **2_j** has good activity against S.aureus, and unchanged activity towards others as in case of compound **2_g**.

3.2.1.3. Group (B), according to ring substitutions compounds ($2_{b,c}$ and 10)

The halogen ring substitution in case of five memberd rings is favourable both for chloro and for bromo in compounds **2_b** and **2_c** whereas a benzoannelation as ring substitution is not favourable.



2_b: R=Cl
2_c: R=Br



10

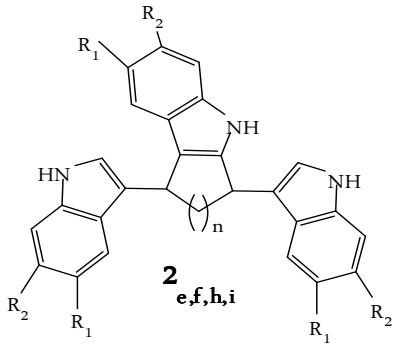
Table (4): MIC values μg/ml of compounds **$2_{b,c}$** and **10.**

compounds	S. aureus	$MRSA_{standard}$	$MRSA_{isolate}$	E. coli	B.subtilies	C.albicans
2_b	3.125	3.125	6.25	50	50	25
2_c	3.125	3.125	6.25	50	6.25	50
10	25	12.5	25	25	25	12.5
Sultamicillin	0.39	25	25	25	0.78	-
Ampicillin	0.78	50	50	50	50	-
Fluconazole	-	-	-	-	-	0.78
Ciprofloxacin	0.78	6.25	12.5	0.19	0.09	-

3.2.1.4. Group (C) according to indole phenyl ring substitution compounds ($2_{e,f,h,i}$)

Table (5): Indole phenyl ring substitutions of compound **2**

Compound No.	n	R_1	R_2
2_e	2	Cl	H
2_f	2	H	Cl
2_h	3	Cl	H
2_i	3	H	Cl



$2_{e,f,h,i}$

Table (6): MIC values μg/ml of compounds **$2_{e,f,h,i}$** .

compounds	S. aureus	$MRSA_{standard}$	$MRSA_{isolate}$	E. coli	B.subtilies	C.albicans
2_e	3.125	6.25	12.5	25	6.25	12.5
2_f	12.5	12.5	12.5	100	50	50
2_h	3.125	6.25	6.25	50	25	6.25
2_i	3.125	6.25	12.5	25	6.25	12.5
Sultamicillin	0.39	25	25	25	0.78	-
Ampicillin	0.78	50	50	50	50	-
Fluconazole	-	-	-	-	-	0.78
Ciprofloxacin	0.78	6.25	12.5	0.19	0.09	-

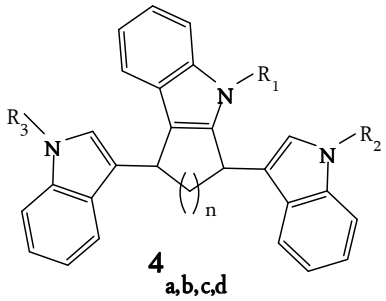
Indole phenyl ring substitution in a six-memberd ring with a 5-chloro substituent is less favourable for both MRSA standard and isolate and favourable for S. aureus,

whereas a 6-chloro substituent is less favourable for all. Thus, a chloro substituent is important for activity either at a 5- or 6- position of the indole phenyl ring. The indole phenyl ring substitution in the 7-membered ring proves that a 5-chloro substituent to be more favourable for MRSA isolated while a 6-chloro substituent gave unchanged activity. As similar to the 6-membered ring in the 7-membered ring chloro substituents are important for activity for MRSA isolate and unchanged activity for S. aureus and MRSA standard. The target structure of our compounds is unknown so far, referring to the different activity of chloro indole pyrroles[37]. The antibiotic activity of these bisindole pyrroles was associated with a family of related compounds with one to four chlorine atoms per molecule and evaluated against a panel of pathogenic bacteria Gram positive and Gram negative demonstrating a broad spectrum antibiotic activity[37].

3.2.1.5. Group (D) according to indole *N*-acetylated compounds ($4_{a,b,c,d}$)

Table (7): Indole *N*-acetylated compounds

Compound No.	n	R_1	R_2	R_3
4_a	3	H	$COCH_3$	H
4_b	2	H	$COCH_3$	$COCH_3$
4_c	3	H	$COCH_3$	$COCH_3$
4_d	2	$COCH_3$	$COCH_3$	$COCH_3$



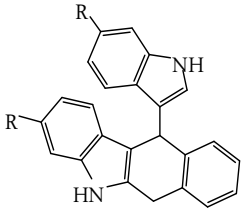
4 a,b,c,d

Table (8): MIC values µg/ml of compounds **$4_{a,b,c,d}$**.

Compounds	S. aureus	$MRSA_{standard}$	$MRSA_{isolate}$	E. coli	B.subtilies	C.albicans
4_a	25	12.5	12,5	50	50	3.125
4_b	100	100	100	50	50	50
4_c	100	50	50	100	50	12.5
4_d	50	50	50	50	25	25
Sultamicillin	0.39	25	25	25	0.78	-
Ampicillin	0.78	50	50	50	50	-
Fluconazole	-	-	-	-	-	0.78
Ciprofloxacin	0.78	6.25	12.5	0.19	0.09	-

A potential *N*-acetylation is very unfavourable. So it can be concluded that the NH indoles are involved in binding to a potential target structure. Monoacetylation is tolerated by MRSA standard and MRSA isolate and less favourable for S. aureus.

3.2.1.6. Group (E) indolobenzocarbazoles compounds ($7_{a,b}$)



7_a: R=H

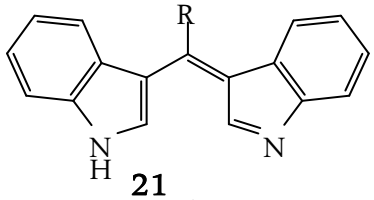
7_b: R=Cl

Table (9): MIC values µg/ml of compounds **$7_{a,b}$**.

Compounds	*S. aureus*	*MRSA$_{standard}$*	*MRSA$_{isolate}$*	*E. coli*	*B.subtilies*	*C.albicans*
7_a	3.125	3.125	6.25	50	50	50
7_b	12.5	12.5	25	100	50	50
Sultamicillin	0.39	25	25	25	0.78	-
Ampicillin	0.78	50	50	50	50	-
Fluconazole	-	-	-	-	-	0.78
Ciprofloxacin	0.78	6.25	12.5	0.19	0.09	-

In case of our bis-indoles, compound **7_a** shows good activity similar to the 6-membered ring derivative with three indoles **2_d**. With a 6-chloro substituent indole compound **7_b** shows a loss of activity similar to the 6-memberd ring derivative **2_f** with three indoles. So it may be supported with a similar binding to a potential target structure with one indole probably not necessary for activity.

3.2.1.7. Group (f) oxidized bis(indolyl)arylmethanes compounds ($21_{a,b,c,e,g,m}$)



21
a,b,c,e,g,m

Table (10): Selected bisindolylmethenes **$21_{a,b,c,e,g,m}$**.

Compound No.	R
21_a	Ph
21_b	3-indolyl
21_c	*p*-Cl-Ph
21_e	*p,m*-dihydroxy. Ph
21_g	*p*-MeO-*m*-OCH_2Ph.Ph
21_l	*p*-$N(CH_3)_2$.Ph

Table (11): MIC values μg/ml of compounds **$21_{a,b,c,e,g,m}$**.

Compounds	S.aureus	$MRSA_{standard}$	$MRSA_{isolate}$	E.coli	B.subtilies	C.albicans
21_a	3.125	3.125	6.25	100	3.125	25
21_b	100	50	50	50	50	12.5
21_c	3.125	3.125	6.25	100	50	12.5
21_e	100	100	100	50	50	25
21_g	50	25	25	100	50	50
21_m	6.25	6.25	6.25	50	12.5	50
Sultamicillin	0.39	25	25	25	0.78	-
Ampicillin	0.78	50	50	50	50	-
Fluconazole	-	-	-	-	-	0.78
Ciprofloxacin	0.78	6.25	12.5	0.19	0.09	-

The salts of this oxidized bis-indolylmethenes have been reported in the literature as active agents capable to kill Gram-positive and Gram-negative microorganisms[32]. The salt of compound (**21_b**) is known as Turbomycin A, and the salt of compound **21_a** as known as Turbomycin B. The antibiotics Turbomycin A and Turbomycin B are natural products were isolated from a metagenomic library of soil microbial DNA. The reported MIC of synthetic Turbomycin A was 6.2 μg/ml for E. herbicola, B. subtilis, S. aureus and S. pyogenes and 12.5 μg/ml for S. enterica serovar Typhimurium[32]. Our bis-indolylmethenes were tested for antibacterial activity as free bases instead of their salts. The free base **21_b** shows no activity compared to its salt Turbomycin A as in the reported case. Compound **21_a** indicates good activity data like its salt Turbomycin B and it has also show good activity against MRSAs (standard and isolate) and B. Subtilis. These compounds have a potential broad spectrum as antibiotics against Gram-positive bacteria. Structure activity relationship of the tested bisindolylmethenes demonstrated that the presence of $N(Me)_2$ substitution in the phenyl ring of the derivative **21_c** is tolerable with some decreases in activity whereas the high hydrophilic hydroxy substitution is unfavourable with almost a complete loss of activity. The lipophilic methoxy and benzyloxy substituent of compounds **21_c** and **21_g** is unfavourable with decreases of activity. The chloro substitution leads to similar activity against S. aureus and MRSA while a loss of activity against B. subtilis and a potential for antifungal

activity against C.albicans was observed due to the chloro substitution as a more lipophilic hydrogen bond acceptor function. In conclusion, we have shown for the first time that the cycloalkanoindoles (**2**$_{a,b,c,d,e,h,i,g,j}$), bis-indolobenzocarbazoles (**7**$_a$) and the oxidized bis-indolylmethenes (**21**$_a$, **21**$_c$, **21**$_m$) inhibited growth of drug resistant MRSA either standard or isolate and other Gram-positive bacteria at low concentrations. These novel bis- and tris-indolyl inhibitors described here can be synthesized easily and cost-effectively and structural modifications to improve the inhibitory activity in vitro can be achieved in a time efficient manner. The results are expected to be of significance in terms of discovering new molecules that can be developed into drugs to combat MRSA and Gram- positive pathogens.

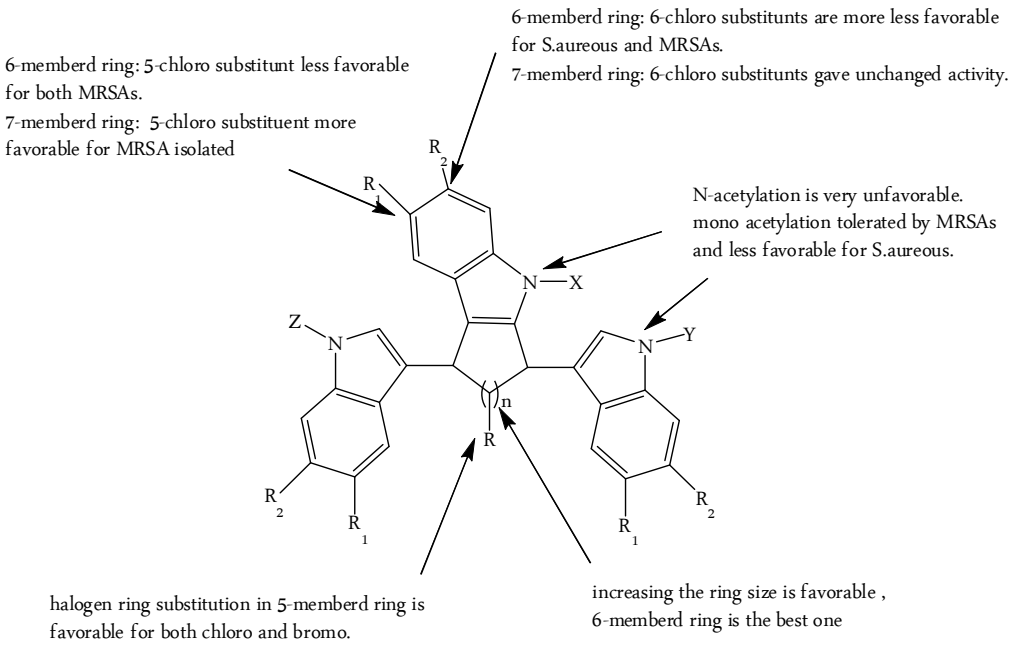


Figure (50): Summary of structure-activity of tris-cycloalkanoindoles **2**$_{a-j}$

3.2.2. Results of the in vitro cancer screen

3.2.2.1. Activity of BIMs and indolocarbazoles as antitumor agents

Bisindolylalkanes and their derivatives are found in bioactive metabolites of terrestrial and marine origin. Recently, *Maciejewska* et al.[212] used DNA-based electrochemical biosensors to demonstrate that bis(5-methoxyindol-3-yl)methane[213] considerably reduces the growth of the cancer cell lines such as HOP-92 (lung), A498 (renal) and MDAMB-231/1TCC (breast) Their results also indicate that BIMs could potentially be applied as chemotherapeutic agents against tumors[212,214]. It has been reported that, DIM-C-*p*-PhC_6H_5 substituted in the phenyl ring with a para-*t*-butyl, trifluoromethyl (DIM-C-*p*-$PhCF_3$) substituent and indole ring-substituted analogs are selective PPARγ modulators[273] in several cancer cell lines such as HEC1A endometrial cancer cells with antiproliferative activity[274], renal adenocarcinoma[275], basal-like breast cancer lines[276], estrogens receptor α-negative MDA-MB-231 and MDA-MB-453 breast cancer cells[277], SKOV3 ovarian cancer cells[278], KU7 and 253J-BV bladder cancer cells[279] and MCF-7 breast cancer cells[280]. 1,1-bis(3-indolyl)-1-(*p*-chlorophenyl)-methane (DIM-C-*p*-PhCl) activated the ligand-binding domain of Nurr1. Treatment of bladder cancer cells with Nurr1-active C-DIM resulted in decreased cell survival and induction of cell death pathways, resulting in poly(ADP-ribose)polymerase cleavage and DNA fragmentation[281]. 1,1-Bis(3'-indolyl)-1-(*p*-bromophenyl)methane (DIM-C-*p*-PhBr) and the 2,2'-dimethyl analog (2,2'-diMeDIM-C-*p*-PhBr) inhibited proliferation and induced apoptosis in SW480 (human colon adenocarcinoma cell line)[282]. The RNA interference studies with small inhibitory RNA for Nur77 demonstrated that DIM-C-*p*-PhOCH3 induces Nur77-dependent and independent apoptosis in colon cancer cell growth (5–10 mM) and induces cell death[283]. Other study investigated the antileukaemic activity and molecular mechanisms of action of a newly synthesized ring-substituted diindolylmethane derivative, 1,1-bis[3′-(5-methoxyindolyl)]-1-(*p*-t-butylphenyl) methane in acute myeloge leukaemia (AML) cells[284].

Indolocarbazoles have been reported as a primary compound for the synthesis of various drugs and possesses important biological, pharmacological, and medicinal activities[231-240]. Indolocarbazoles are associated with anticancer, antimicrobial, and antifungal activities. In most cases biological activity is correlated with indolocarbazoles containing heteroatom. The biological activity depends on the interaction potential with DNA[241-242]. Furthermore, many experimental studies have indicated that the size, shape and planarity of this structure are important criteria in such DNA interaction[243].

All the submitted compounds [BIMs (**17**$_{e,g,j,i,l}$) and the indolocarbazoles (**2**$_{e,f,h,i,j}$)] to National Cancer Institute (NCI) in USA have been selected by the NCI for anticancer screening. The tumour growth inhibition properties of the ten compounds **17**$_e$, **17**$_g$, **17**$_j$, **17**$_i$, **17**$_l$, **18**$_d$, **18**$_f$, **18**$_h$, **18**$_i$ and **18**$_l$ with the NCI codes **NSC D-755521/1**, **D-755518/1**, **D-755517/1**, **D-755519/1**, **D-755520/1**, **D-758513/1**, **D-758511/1**, **D-758510/1**, **D-758512/1** and **D-758514/1**. The selected compounds were screened on human tumour cell lines at 10^{-5} M at the 60-Cell-Line Screenings of the Developmental Therapeutics Program (DTP) of the National Cancer Institute (NCI, Bethesda, Maryland, USA) under the drug discovery program of the NCI. Among the selected 10 compounds the two compounds **17**$_j$ (**NSC D-755517/1**) and **18**$_d$ (**D-758513/1**) were further screened for five-log dose molar range as they have shown prominent cell growth inhibition at 10^{-5} M concentration against variety of cancer cell lines. The 60-cell-line-screening of the NCI includes 60 different tumour cell lines, the nine various organs and tumour types derived (leukaemia, non-small-cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer).

3.2.2.2. Results of 60-Cell-Line-Screening for BIMs (17$_{e,g,i,j,l}$)

All the five selected BIMs (**17**$_{e,g,i,j,l}$), figure (31), by the NCI for *in vitro* anticancer assay were evaluated for their anticancer activity. Primary *in vitro* One dose anticancer assay was performed in full NCI 60 cell panel representing leukaemia, melanoma and cancers of lung, colon, brain breast, ovary, kidney and prostate in accordance with the

protocol of the NCI, USA. The compounds were added at a single concentration (10^{-5} M) and the culture was incubated for 48 h. End point determinations were made with a protein binding dye, Sulforhodamine B. Results for each compound were reported as a mean graph of the percent growth of the treated cells when compared to the untreated control cells. After obtaining the results for one dose assay, analysis of historical Development Therapeutics Programme (DTP) was performed and compound **17_j** (**NSC D-755517/1**) which satisfied predetermined as effective inhibition criteria was selected for NCI full panel 5 dose assays. The tested BIMs showed a distinctive pattern of selectivity with regard to sensitivity against individual cell lines all the percent growth inhibition and the mean growth percent has been collected in table (12). Compound **17_j** (**NSC D-755517/1**) exhibited broad spectrum cell growth inhibition against leukaemia cancer cell MOLT-4 (growth inhibition 20.53 %), non small lung cancer cell NCI-H460 (growth inhibition 9.25 %), colon cancer cells HCT-116 and HT29 with recorded growth inhibition values 19.91 % and 20.89% respectively, melanoma cancer cell M14 (growth inhibition 19.50 %), ovarian cancer cell IGROV1 (growth inhibition 23.79 %), and renal cancer cells (CAKI-1 and UO-31) with growth inhibition 15.65 % and 18.10 % respectively. This data confirmed that as a result of a Single dose assay concentration of 10^{-5} M the average highest cytostatic effects were recorded for the compound **17_j** (**NSC D-755517/1**) that showed the lowest over all mean value (47.39 %), figure (51). The two substituted derivatives **17_g** and **17_i** were observed as moderate cytostatic properties with over all mean values 75.51 % and 86.38 % respectively, especially for the cancer cell lines "leukaemia MOLT-4, non small lung cancer NCI-H460, ovarian cancer cell lines IGROVI and OVCAR-3 and renal cancer cell lines CAKI-1 and UO-31" with growth percent values in a range from 58.65 % to 34.07 %. Compounds **17_e** and **17_l** were shown as inactive cytostatics against all selected cell lines with a mean values 101.60 % and 92.63 % respectively. Table (12) showed the sixty human tumour cell line anticancer screening data at single dose assay (10^{-5} M) as percent growth inhibition of BIMs **$17_{e,g,i,j,l}$**, figure (51) represent the one dose mean graph of compound **17_j**, see all the

figures of the one dose mean graph for all the tested compounds in the appendix, figures (55) to (68).

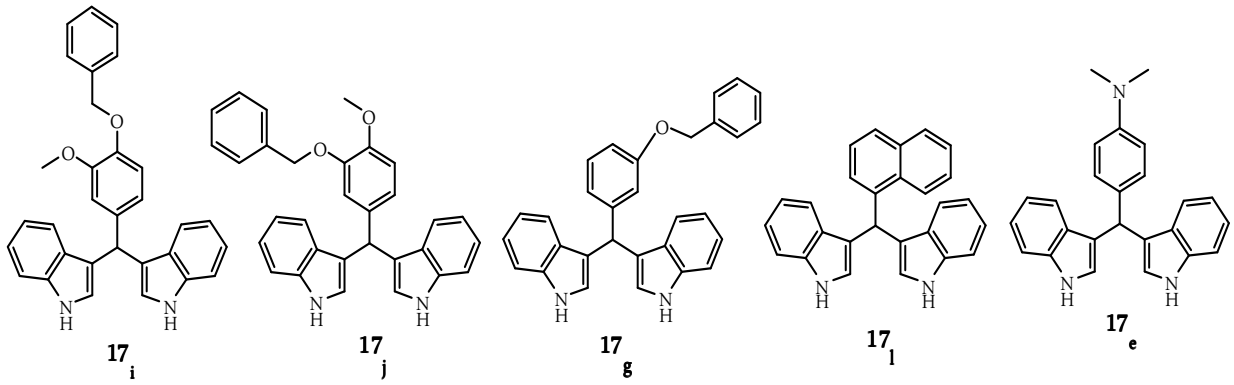


Figure (31): Selected BIMs (**17e,g,i,j,l**) for NCI screenings.

All the cell lines (NCI 60), representing nine tumour subpanels, were incubated at five different concentrations (0.01, 0.1, 1, 10 & 100 μM). The outcomes were used to create log concentration Vs % growth inhibition curves and three response parameters (GI_{50}, TGI and LC_{50}) were calculated for each cell line. The GI_{50} value (growth inhibitory activity) corresponds to the concentration of the compound causing 50 % decrease in net cell growth, the TGI value (cytostatic activity) is the concentration of the compound resulting in total growth inhibition and LC_{50} value (cytotoxic activity) is the concentration of the compound causing net 50 % loss of initial cells at the end of the incubation period of 48 h. Compound under investigation **17_j** (**NSC D-755517/1**) exhibited remarkable anticancer activity against most of the tested cell lines representing nine different subpanels with GI_{50} values between "1.20 – 9.56 μM" as shown in table (13). Whereas three cell lines of non small lung cancer cell subpanel namely HOP-62, melanoma cancer cell line MALME-3M and breast cancer cell line HS 578T were found to be insensitive at the highest tested concentration 100 μM therefore a sign of ">" is used as prefix to the concentration. With regard to the sensitivity against some individual cell lines, compound **17_j** (**NSC D-755517/1**) showed obvious activity toward CNS Cancer cell lines SNB-7 and U251, Melanoma cell lines MDA-MB-43 and UACC-62, Renal Cancer cell lines A498 and RXF 393 and breast cancer cell line MDA-MB-468, (GI_{50} value ranging from 1.20 to 1.87 μM). The criterion for selectivity of a compound depends upon the ratio obtained by dividing the full panel MID (the average sensitivity of all cell lines toward the test agent) by their individual subpanel MID (the average

sensitivity of all cell lines of a particular subpanel toward the test agent). Ratios between 3 and 6 refer to moderate selectivity, ratios greater than 6 indicate high selectivity toward the corresponding cell line, while compounds not meeting either of these criteria rated non-selective. As per this criterion, compound under investigation was found to be non selective toward all the cell panels, table (13). The five-in-box screening for **17$_j$** gave the parameters log GI_{50}, log TGI and log LC_{50} , figures (52) and (53), which are summarized in table (13). In the full NCI screening data report, three additional numbers are printed at the base of each of the three respective mean-graphs provided. These numbers are the *MG-MID* (Average), the *Delta* and the *Range*. The *MG-MID* or the average is the calculated logarithmic of a mean panel of GI_{50}, TGI or LC_{50}. The *Delta* is the differences of concentrations with parameters between the most sensitive cell line and the mean. Similarly, the *Range* is the number of log_{10} units by which the delta of the most sensitive line(s) of the panel differs from the delta of the least sensitive line(s). On the other hand the given Delta and Range values quite accurately reflect a true range of differential sensitivity among the full panel of cell lines to the compound under investigation. Likewise, the given MG-MID (Average) value quite accurately reflects a true overall panel-average sensitivity of the cell lines to this agent, and therefore is a useful basis for comparison of overall potency of the given agent with related or unrelated compounds. Compound **17$_j$** has average GI_{50} responses at sub-micromolar concentrations (11 μM), cytostatic effects at micromolar concentrations (95.5 μM) and the average cytotoxic effects on cancer cell lines at micromolar concentrations (100 μM) which is 10 fold higher than GI_{50}. Based on all these data compound **17$_j$** showed a high degree of variability in its response. Compound **17$_j$** with GI_{50} values in the micromolar range is more effective than the cytostatic drugs Etoposid, Melphalan and Irinotecan (GI_{50} values of 38.9 μM, 14.5 μM and 14.1 μM respectively)[290h].

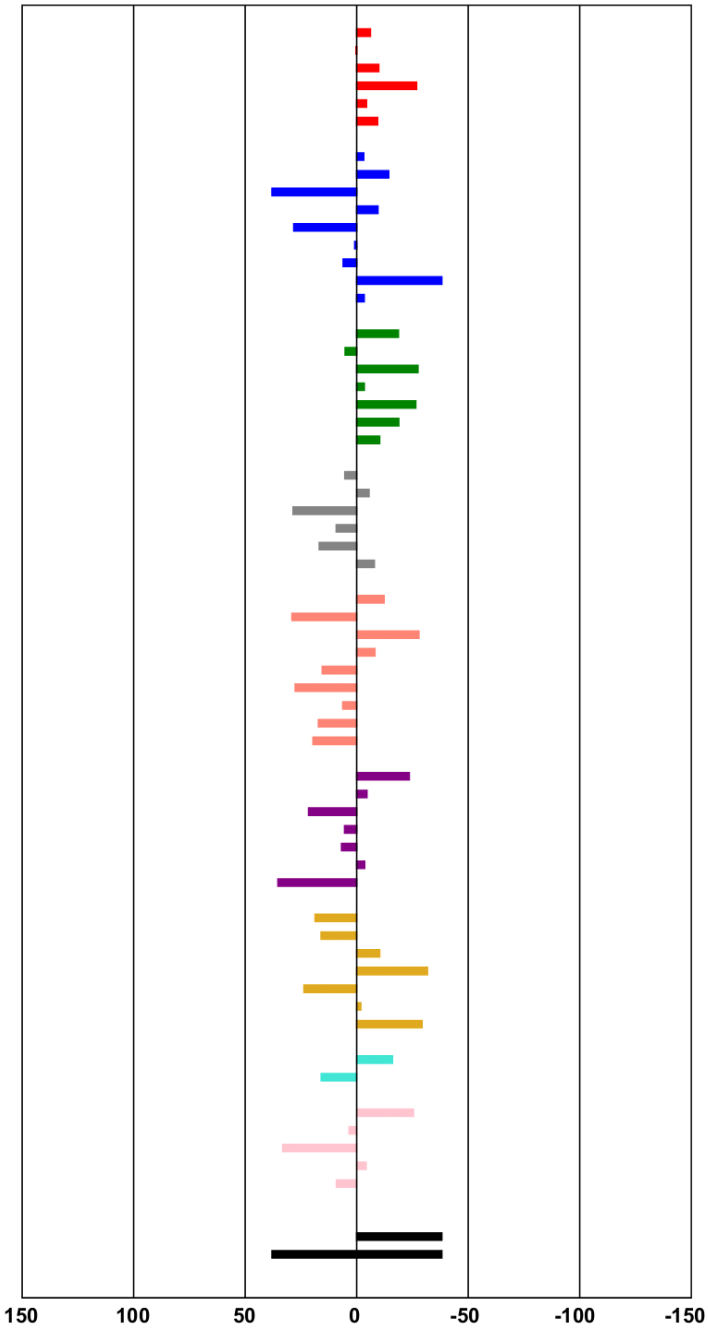
Table (12): 60 human tumour cell line anticancer screening data at single dose assay (10^{-5} M) as percent growth inhibition of BIMs **$17_{e,g,i,j,l}$**.

Panel/Cell Line	Growth Percent				
	BIM (**17_j**)	BIM (**17_e**)	BIM (**17_g**)	BIM (**17_i**)	BIM (**17_l**)
Leukaemia					
CCRF-CEM	41.24	94.29	65.84	82.70	90.92
HL-60(TB)	47.74	128.59	85.73	97.61	100.22
K-562	37.52	91.47	63.92	71.05	76.69
MOLT-4	20.53	96.36	52.47	58.65	75.09
RPMI-8226	42.97	94.00	75.01	94.76	93.10
SR	38.00	100.85	67.99	77.82	89.79
Non-Small Cell Lung Cancer					
A549/ATCC	44.24	98.14	70.37	80.66	78.08
EKVX	33.06	94.88	70.13	75.34	92.09
HOP-62	85.39	103.12	117.07	89.05	96.56
HOP-92	37.86	97.98	77.26	74.21	65.77
NCI-H226	75.60	103.98	96.26	86.74	95.12
NCI-H23	48.28	105.48	83.58	83.17	90.04
NCI-H322M	53.46	89.81	76.91	72.67	94.19
NCI-H460	9.25	111.93	40.92	87.36	90.80
NCI-H522	44.03	103.14	81.51	90.05	88.29
Colon Cancer					
COLO 205	28.69	104.13	98.17	102.68	103.48
HCC-2998	52.61	107.97	89.58	102.35	101.88
HCT-116	19.91	89.91	49.68	63.15	78.21
HCT-15	43.97	100.56	71.42	80.96	93.30
HT29	20.89	100.05	67.05	80.75	92.07
KM12	28.51	102.97	61.54	88.20	83.31
SW-620	37.08	93.36	66.72	84.23	87.23
CNS Cancer					
SF-268	52.76	116.79	67.22	89.60	99.12
SF-295	41.84	98.27	90.26	86.27	75.79
SF-539	75.91	91.54	90.70	97.53	104.56
SNB-19	56.58	109.25	88.91	101.55	113.06
SNB-75	64.21	92.69	69.63	80.78	76.20
U251	39.48	111.50	71.87	94.01	91.64
Melanoma					
LOX IMVI	35.11	96.58	70.34	84.68	91.70
MALME-3M	76.44	108.26	86.23	95.57	104.86
M14	19.50	102.85	60.84	94.58	91.67
MDA-MB-435	39.23	100.64	66.55	97.07	104.96
SK-MEL-2	62.89	108.23	87.10	106.14	117.60
SK-MEL-28	75.02	115.84	94.00	107.74	110.32
SK-MEL-5	53.71	102.49	69.90	89.22	104.38
UACC-257	64.60	113.36	88.90	97.97	97.73
UACC-62	67.03	92.49	65.78	75.87	90.79
Ovarian Cancer					
IGROV1	23.79	99.14	51.22	53.76	92.57
OVCAR-3	42.79	108.57	57.39	95.78	89.75
OVCAR-4	68.99	108.38	85.86	93.36	105.91
OVCAR-5	52.85	102.74	84.10	77.30	92.79
OVCAR-8	54.18	100.41	84.14	99.85	98.97
NCI/ADR-RES	43.84	105.48	83.51	95.00	95.63
SK-OV-3	82.74	105.24	99.10	92.84	100.06
Renal Cancer					
786-0	66.10	101.34	93.63	96.46	105.42
A498	63.36	106.51	104.66	89.10	95.52
ACHN	37.06	93.95	63.05	74.33	86.38
CAKI-1	15.65	74.45	34.27	57.43	53.07
RXF 393	71.08	115.46	90.57	110.45	94.67
SN12C	45.51	103.75	71.34	87.36	103.59
UO-31	18.10	64.28	42.30	51.55	66.86
Prostate Cancer					
PC-3	31.31	88.06	58.42	67.79	69.02
DU-145	63.31	120.00	74.41	109.45	105.82
Breast Cancer					
MCF7	21.93	105.91	52.85	76.47	104.09
MDA-MB-231/ATCC	50.81	104.98	86.20	68.39	79.28
BT-549	80.58	109.82	102.59	99.13	102.05
T-47D	43.17	89.79	65.05	72.49	99.38
MDA-MB-468	56.56	110.54	97.31	119.11	101.32
Mean	47.39 % Selected for 5-dose	101.60 % Non selected	75.51 % Non selected	86.38 % Non selected	92.63 % Non selected

Developmental Therapeutics Program
One Dose Mean Graph

NSC: D-755517 / 1	Conc: 1.00E-5 Molar	Test Date: Jan 10, 2011
Experiment ID: 1101OS87		Report Date: Feb 03, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	41.24
HL-60(TB)	47.74
K-562	37.52
MOLT-4	20.53
RPMI-8226	42.97
SR	38.00
Non-Small Cell Lung Cancer	
A549/ATCC	44.24
EKVX	33.06
HOP-62	85.39
HOP-92	37.86
NCI-H226	75.60
NCI-H23	48.28
NCI-H322M	53.46
NCI-H460	9.25
NCI-H522	44.03
Colon Cancer	
COLO 205	28.69
HCC-2998	52.61
HCT-116	19.91
HCT-15	43.97
HT29	20.89
KM12	28.51
SW-620	37.08
CNS Cancer	
SF-268	52.76
SF-295	41.84
SF-539	75.91
SNB-19	56.58
SNB-75	64.21
U251	39.48
Melanoma	
LOX IMVI	35.11
MALME-3M	76.44
M14	19.50
MDA-MB-435	39.23
SK-MEL-2	62.89
SK-MEL-28	75.02
SK-MEL-5	53.71
UACC-257	64.60
UACC-62	67.03
Ovarian Cancer	
IGROV1	23.79
OVCAR-3	42.79
OVCAR-4	68.99
OVCAR-5	52.85
OVCAR-8	54.18
NCI/ADR-RES	43.84
SK-OV-3	82.74
Renal Cancer	
786-0	66.10
A498	63.36
ACHN	37.06
CAKI-1	15.65
RXF 393	71.08
SN12C	45.51
UO-31	18.10
Prostate Cancer	
PC-3	31.31
DU-145	63.31
Breast Cancer	
MCF7	21.93
MDA-MB-231/ATCC	50.81
BT-549	80.58
T-47D	43.17
MDA-MB-468	56.56
Mean	47.39
Delta	38.14
Range	76.14



Figure(51): Results of the one-dose screening of $\mathbf{17_j}$.

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 755517 / 1	Experiment ID : 1102NS97	Test Type : 08	Units : Molar
Report Date : April 08, 2011	Test Date : February 07, 2011	QNS :	MC :
COMI : Elm 168a (101196)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0HHZ	

			Log10 Concentration											
	Time		Mean Optical Densities					Percent Growth						
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.313	1.220	1.147	1.169	1.148	0.694	0.456	92	94	92	42	16	6.91E-6	> 1.00E-4	> 1.00E-4
HL-60(TB)	0.835	2.419	2.537	2.518	2.480	1.571	0.865	107	106	104	46	2	8.67E-6	> 1.00E-4	> 1.00E-4
K-562	0.221	1.713	1.724	1.758	1.672	0.804	0.594	101	103	97	39	25	6.49E-6	> 1.00E-4	> 1.00E-4
MOLT-4	0.527	1.800	1.874	1.938	1.866	0.919	0.452	106	111	105	31	-14	5.51E-6	4.83E-5	> 1.00E-4
RPMI-8226	0.750	1.982	1.961	1.844	1.815	1.046	0.915	98	89	86	24	13	3.83E-6	> 1.00E-4	> 1.00E-4
SR	0.351	1.070	1.007	1.108	1.072	0.651	0.557	91	105	100	42	29	7.20E-6	> 1.00E-4	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.380	1.540	1.560	1.595	1.519	0.866	0.761	102	105	98	42	33	7.18E-6	> 1.00E-4	> 1.00E-4
EKVX	0.709	1.863	1.805	1.772	1.677	1.167	1.007	95	92	84	40	26	5.83E-6	> 1.00E-4	> 1.00E-4
HOP-62	0.346	0.976	0.936	0.952	0.918	0.815	0.745	94	96	91	74	63	> 1.00E-4	> 1.00E-4	> 1.00E-4
HOP-92	1.088	1.583	1.464	1.429	1.438	1.225	1.164	76	69	71	28	15	3.03E-6	> 1.00E-4	> 1.00E-4
NCI-H226	0.896	1.877	1.812	1.721	1.649	1.422	1.357	93	84	77	54	47	3.49E-5	> 1.00E-4	> 1.00E-4
NCI-H23	0.696	1.953	1.854	1.820	1.759	1.281	1.196	92	89	85	47	40	8.10E-6	> 1.00E-4	> 1.00E-4
NCI-H322M	0.515	1.214	1.203	1.180	1.150	0.940	0.854	98	95	91	61	48	7.51E-5	> 1.00E-4	> 1.00E-4
NCI-H460	0.201	1.575	1.625	1.616	1.538	0.549	0.438	104	103	97	25	17	4.54E-6	> 1.00E-4	> 1.00E-4
NCI-H522	0.538	1.262	1.136	1.114	1.067	0.733	0.650	82	79	73	27	15	3.15E-6	> 1.00E-4	> 1.00E-4
Colon Cancer															
COLO 205	0.277	1.275	1.329	1.261	1.209	0.766	0.410	105	99	93	49	13	9.47E-6	> 1.00E-4	> 1.00E-4
HCC-2998	0.490	1.731	1.627	1.584	1.630	1.094	0.918	92	88	92	49	34	9.31E-6	> 1.00E-4	> 1.00E-4
HCT-116	0.251	1.594	1.645	1.667	1.614	0.631	0.504	104	105	101	28	19	5.05E-6	> 1.00E-4	> 1.00E-4
HCT-15	0.315	1.662	1.577	1.513	1.419	0.763	0.613	94	89	82	33	22	4.53E-6	> 1.00E-4	> 1.00E-4
HT29	0.225	1.096	1.093	1.061	1.047	0.475	0.364	100	96	94	29	16	4.74E-6	> 1.00E-4	> 1.00E-4
KM12	0.405	1.671	1.701	1.682	1.631	0.796	0.575	102	101	97	31	13	5.12E-6	> 1.00E-4	> 1.00E-4
SW-620	0.226	1.502	1.491	1.394	1.398	0.796	0.611	99	92	92	45	30	7.71E-6	> 1.00E-4	> 1.00E-4
CNS Cancer															
SF-268	0.544	1.747	1.708	1.743	1.714	1.194	1.085	97	100	97	54	45	2.79E-5	> 1.00E-4	> 1.00E-4
SF-295	0.706	2.433	2.454	2.418	2.426	1.532	1.487	101	99	100	48	45	9.07E-6	> 1.00E-4	> 1.00E-4
SF-539	0.818	2.032	1.972	1.862	1.907	1.463	1.401	95	86	90	53	48	4.12E-5	> 1.00E-4	> 1.00E-4
SNB-19	0.425	1.187	1.174	1.209	1.166	0.849	0.733	98	103	97	56	40	2.35E-5	> 1.00E-4	> 1.00E-4
SNB-75	0.713	1.161	1.072	1.072	1.048	0.965	0.861	80	80	75	56	33	1.87E-5	> 1.00E-4	> 1.00E-4
U251	0.237	1.113	1.112	1.110	1.075	0.690	0.556	100	100	96	52	36	1.28E-5	> 1.00E-4	> 1.00E-4
Melanoma															
LOX IMVI	0.259	1.776	1.655	1.610	1.568	0.845	0.623	92	89	86	39	24	5.76E-6	> 1.00E-4	> 1.00E-4
MALME-3M	0.698	1.499	1.436	1.468	1.470	1.292	1.107	92	96	96	74	51	> 1.00E-4	> 1.00E-4	> 1.00E-4
M14	0.403	1.198	1.177	1.169	1.150	0.560	0.373	97	96	94	20	-8	3.91E-6	5.28E-5	> 1.00E-4
MDA-MB-435	0.537	2.111	2.109	2.200	2.153	1.358	1.165	100	106	103	52	40	1.50E-5	> 1.00E-4	> 1.00E-4
SK-MEL-2	0.899	1.312	1.316	1.264	1.229	1.016	0.870	101	88	80	28	-3	3.78E-6	7.87E-5	> 1.00E-4
SK-MEL-28	0.542	1.335	1.298	1.288	1.296	1.082	0.900	95	94	95	68	45	6.12E-5	> 1.00E-4	> 1.00E-4
SK-MEL-5	0.534	2.357	2.227	2.303	2.222	1.679	1.277	93	97	93	63	41	3.81E-5	> 1.00E-4	> 1.00E-4
UACC-257	0.613	1.100	1.120	1.142	1.142	0.950	0.834	104	109	109	69	45	6.40E-5	> 1.00E-4	> 1.00E-4
UACC-62	0.735	1.842	1.797	1.807	1.642	1.335	1.029	96	97	82	54	27	1.42E-5	> 1.00E-4	> 1.00E-4
Ovarian Cancer															
IGROV1	0.518	1.792	1.851	1.783	1.706	1.248	1.138	105	99	93	57	49	6.98E-5	> 1.00E-4	> 1.00E-4
OVCAR-3	0.405	1.215	1.215	1.214	1.138	0.749	0.627	100	100	90	42	27	6.95E-6	> 1.00E-4	> 1.00E-4
OVCAR-4	0.453	1.309	1.291	1.245	1.200	0.798	0.687	98	92	87	40	27	6.22E-6	> 1.00E-4	> 1.00E-4
OVCAR-5	0.575	1.425	1.330	1.291	1.186	0.922	0.735	89	84	72	41	19	5.04E-6	> 1.00E-4	> 1.00E-4
OVCAR-8	0.313	1.208	1.229	1.224	1.218	0.855	0.742	102	102	101	61	48	6.81E-5	> 1.00E-4	> 1.00E-4
NCI/ADR-RES	0.581	1.885	1.798	1.816	1.697	1.224	1.112	93	95	86	49	41	9.56E-6	> 1.00E-4	> 1.00E-4
Renal Cancer															
A498	0.939	1.720	1.645	1.667	1.521	1.355	1.201	90	93	75	53	34	1.46E-5	> 1.00E-4	> 1.00E-4
ACHN	0.424	1.224	1.187	1.093	1.019	0.612	0.551	95	84	74	23	16	3.01E-6	> 1.00E-4	> 1.00E-4
CAKI-1	0.844	2.467	2.340	2.292	2.013	1.487	1.373	92	89	72	40	33	4.78E-6	> 1.00E-4	> 1.00E-4
RXF 393	0.711	1.373	1.362	1.317	1.324	1.044	1.021	98	92	93	50	47	1.20E-5	> 1.00E-4	> 1.00E-4
SN12C	0.650	1.718	1.657	1.681	1.565	1.035	0.836	94	97	86	36	17	5.22E-6	> 1.00E-4	> 1.00E-4
TK-10	0.640	1.124	1.106	1.078	1.087	0.809	0.715	96	91	92	35	16	5.47E-6	> 1.00E-4	> 1.00E-4
UO-31	0.562	1.821	1.503	1.431	1.396	1.023	0.921	75	69	66	37	29	3.52E-6	> 1.00E-4	> 1.00E-4
Prostate Cancer															
PC-3	0.450	1.300	1.199	1.142	1.118	0.745	0.690	88	81	79	35	28	4.47E-6	> 1.00E-4	> 1.00E-4
DU-145	0.392	1.368	1.407	1.401	1.428	1.015	0.783	104	103	106	64	40	3.81E-5	> 1.00E-4	> 1.00E-4
Breast Cancer															
MCF7	0.480	2.257	2.088	2.044	2.015	1.157	1.012	90	88	86	38	30	5.67E-6	> 1.00E-4	> 1.00E-4
MDA-MB-231/ATCC	0.594	1.170	1.229	1.083	0.965	0.782	0.528	110	85	64	33	-11	2.84E-6	5.57E-5	> 1.00E-4
HS 578T	0.509	1.289	1.235	1.220	1.186	1.073	1.013	93	91	87	72	65	> 1.00E-4	> 1.00E-4	> 1.00E-4
T-47D	0.580	1.280	1.276	1.305	1.299	0.919	0.893	99	104	103	48	45	9.32E-6	> 1.00E-4	> 1.00E-4
MDA-MB-468	0.606	1.367	1.317	1.319	1.307	1.004	0.835	93	94	92	52	30	1.27E-5	> 1.00E-4	> 1.00E-4

Figure (52): Five dose testing results of compound **17$_j$**.

Table (13): NCI in vitro testing result of compound **17_j (NSC D-755517/1)** at five dose level in µM.

Panel/Cell Line	GI_{50} Concentration per cell line	MID[b]	selectivity ratio (MID[a]:MID[b])	TGI	LC_{50}	$LogGI_{50}$	Log TGI	Log LC_{50}
Leukemia	.....	6.44	0.79	.....	.....	.....	....	.....
CCRF-CEM	6.91			> 1.00	> 1.00	-5.16	> -4.00	> -4.00
HL-60(TB)	8.67			> 1.00	> 1.00	-5.06	> -4.00	> -4.00
K-562	6.49			> 1.00	> 1.00	-5.19	> -4.00	> -4.00
MOLT-4	5.51			4.83	> 1.00	-5.26	-4.32	> -4.00
RPMI-8226	3.83			> 1.00	> 1.00	-5.42	> -4.00	> -4.00
SR	7.20			> 1.00	> 1.00	-5.14	> -4.00	> -4.00
Non-Small Cell Lung Cancer	.....	5.35	0.96	.....	.....	.....	.....	.....
A549/ATCC	7.18			> 1.00	> 1.00	-5.14	> -4.00	> -4.00
EKVX	5.83			> 1.00	> 1.00	-5.23	> -4.00	> -4.00
HOP-62	> 1.00			> 1.00	> 1.00	> -4.00	> -4.00	> -4.00
HOP-92	3.03			> 1.00	> 1.00	-5.52	> -4.00	> -4.00
NCI-H226	3.49			> 1.00	> 1.00	-4.46	> -4.00	> -4.00
NCI-H23	8.10			> 1.00	> 1.00	-5.09	> -4.00	> -4.00
NCI-H322M	7.51			> 1.00	> 1.00	-4.12	> -4.00	> -4.00
NCI-H460	4.54			> 1.00	> 1.00	-5.34	> -4.00	> -4.00
NCI-H522	3.15			> 1.00	> 1.00	-5.50	> -4.00	> -4.00
Colon Cancer	.....	6.56	0.78	.....	.....	.....	.....	.....
COLO 20	9.47			> 1.00	> 1.00	-5.02	> -4.00	> -4.00
HCC-2998	9.31			> 1.00	> 1.00	-5.03	> -4.00	> -4.00
HCT-116	5.05			> 1.00	> 1.00	-5.30	> -4.00	> -4.00
HCT-1	4.53			> 1.00	> 1.00	-5.34	> -4.00	> -4.00
HT29	4.74			> 1.00	> 1.00	-5.32	> -4.00	> -4.00
KM12	5.12			> 1.00	> 1.00	-5.29	> -4.00	> -4.00
SW-620	7.71			> 1.00	> 1.00	-5.11	> -4.00	> -4.00
CNS Cancer	.....	3.58	1.42	.....	.....	.....	.....	.....
SF-268	2.79			> 1.00	> 1.00	-4.55	> -4.00	> -4.00
SF-29	9.07			> 1.00	> 1.00	-5.04	> -4.00	> -4.00
SF-539	4.12			> 1.00	> 1.00	-4.39	> -4.00	> -4.00
SNB-19	2.35			> 1.00	> 1.00	-4.63	> -4.00	> -4.00
SNB-7	1.87			> 1.00	> 1.00	-4.73	> -4.00	> -4.00
U251	1.28			> 1.00	> 1.00	-4.89	> -4.00	> -4.00
Melanoma.....	.....	4.09	1.24	.....	.....	.....	.....	.....
LOX IMVI	5.76			> 1.00	> 1.00	-5.24	> -4.00	> -4.00
MALME-3M	> 1.00			> 1.00	> 1.00	>-4.00	> -4.00	> -4.00
M14	3.91			5.28	> 1.00	-5.41	-4.28	> -4.00
MDA-MB-43	1.50			> 1.00	> 1.00	-4.82	> -4.00	> -4.00
SK-MEL-2	3.78			7.87	> 1.00	-5.42	-4.10	> -4.00
SK-MEL-28	6.12			> 1.00	> 1.00	-4.21	> -4.00	> -4.00
SK-MEL-	3.81			> 1.00	> 1.00	-4.42	> -4.00	> -4.00
UACC-257	6.40			> 1.00	> 1.00	-4.19	> -4.00	> -4.00
UACC-62	1.42			> 1.00	> 1.00	-4.85	> -4.00	> -4.00
Ovarian Cancer	.....	6.93	0.73	.....	.....	.....	.....	.....
IGROV1	6.98			> 1.00	> 1.00	-4.16	> -4.00	> -4.00
OVCAR-3	6.95			> 1.00	> 1.00	-5.16	> -4.00	> -4.00
OVCAR-4	6.22			> 1.00	> 1.00	-5.21	> -4.00	> -4.00
OVCAR-	5.04			> 1.00	> 1.00	-5.30	> -4.00	> -4.00
OVCAR-8	6.81			> 1.00	> 1.00	-4.17	> -4.00	> -4.00
NCI/ADR-RES	9.56			> 1.00	> 1.00	-5.02	> -4.00	> -4.00
Renal Cancer	.....	3.5	1.45	.....	.....	.....	.....	.....
A498	1.46			> 1.00	> 1.00	-4.83	> -4.00	> -4.00
ACHN	3.01			> 1.00	> 1.00	-5.52	> -4.00	> -4.00
CAKI-1	4.78			> 1.00	> 1.00	-5.32	> -4.00	> -4.00
RXF 393	1.20			> 1.00	> 1.00	-4.92	> -4.00	> -4.00
SN12C	5.22			> 1.00	> 1.00	-5.28	> -4.00	> -4.00
TK-10	5.47			> 1.00	> 1.00	-5.26	> -4.00	> -4.00
UO-31	3.52			> 1.00	> 1.00	-5.45	> -4.00	> -4.00
Prostate Cancer	.....	2.1	2.42	.....	.....	.....	.....	.....
PC-3	4.47			> 1.00	> 1.00	-5.35	> -4.00	> -4.00
DU-14	3.81			> 1.00	> 1.00	-4.42	> -4.00	> -4.00
Breast Cancer	.....	4.78	1.07	.....	.....	.....	.....	.....
MCF7	5.67			> 1.00	> 1.00	-5.25	> -4.00	> -4.00
MDA-MB-231/ATCC 0.594	2.84			5.57	> 1.00	-5.55	-4.25	> -4.00
HS 578T	>1.00			> 1.00	> 1.00	> - 4.00	> -4.00	> -4.00
T-47D	9.32			> 1.00	> 1.00	-5.03	> -4.00	> -4.00
MDA-MB-468	1.27			> 1.00	> 1.00	-4.90	> -4.00	> -4.00
MID[a]	5.09							
Average:						-4.96 (11 µM)	-4.02 (9.6 µM)	-4.0 (>100µM)
Delta:						0.59	0.30	0.00
Range:						1.55	0.32	0.00

MID[a]: Average sensitivity of all cell line in µM. MID[b]: Average sensitivity of all cell line of a particular subpanel in µM.

3.2.2.3. Structure Activity Relationship (SAR) of BIMs

Structure-activity correlation of the synthesized BIMs revealed that, by comparison of **17_i** and **17_j** being the position of the functional groups is of great importance of either *meta* or *para*. Where the *para*-methoxy is much more favourable than *meta* substituent, moreover the comparison of **17_j** with **17_g** indicated that the methoxy function in addition to benzyloxy group ensures mainly increased activity. If the methoxy function is positioned in *meta* position the effect is similar concerning no favour of a *meta* methoxy function as indicated by a comparison **17_i** and **17_g**. The lipophilic fixed substituent in the naphthyl derivative **17_l** is not favourable compared to the routable benzyloxy substituent compound **17_g**. BIM **17_e** containing the basic substituent (NMe_2) was unfavourable concerning the all over anticancer activities. Whereas, its activities in a renal cancer cells indicated different anticancer activities comparable to compound **17_g**.

In conclusion, all BIMs (**$17_{e,g,i,j,l}$**) showed best activities in the same cell lines MOLT-4 in leukaemia cell line, IGROV1 in ovarian cell line and the cell lines CKAI-1 and UO-31 renal cancer cell line. Also the basically substituted derivative demonstrates good Activity for leukaemia cell line MOLT-4, non small cell lung cancer NCI-H460, colon cancer cell lines HCT-116 and HT29, melanoma cell line M14, ovarian cancer cell line IGROV1, the renal cancer cell lines CAKI-1 and UO-31, and the breast cancer cell line MCF7 moreover compound **17_j** showed no cytotoxic properties.

3.2.2.4. Results of 60 cell line screening for aryl substituted tetrahydroindolo[2,3-*b*]carbazoles (**$18_{d,f,h,i,l}$**)

We further developed the series of the substituted bis(indolyl)substituted phenylmethanes with the synthesis of new structures as aryl substituted tetrahydroindolo[2,3-*b*]carbazoles to constrain the flexibility of the molecule. The NCI selected five derivatives of these substituted indolocarbazoles for the one-dose screening program at a concentration of 10^{-5} μM. The selected substances (**$18_{d,f,h,i,j}$**) showed a distinctive pattern of selectivity with regard to sensitivity against individual cell lines all the percent growth inhibition and the mean growth percent has been collected in table (14). Compound **18_d** exhibited broad spectrum cell growth inhibition against non small

lung cancer cell NCI-H23 (growth inhibition 5.55 %), colon cancer cell lines HCT-116 and SW-620 (growth inhibition 7.08 % and 6.72%), renal cancer cell line ACHN, CAKI-1 and UO-31 (growth inhibition 9.61 %, 13.72% and 12.35% respectively) and breast cancer cell line BT-549 (growth inhibition 8.92%) at single dose assay concentration of 10^{-5} M. The average highest anti cancer activity were Scored for the compound **18_d** that showed the lowest mean value (21.63 %), figure (53). The two substituted derivatives **18_h** and **18_i** were observed as moderate cytotoxic properties with mean values 84.67 % and 76.84 % respectively and the other two derivatives **18_f** and **18_l** were shown as inactive cytostatics.

All the cell lines (NCI 60), representing nine tumour subpanels, were incubated at five different concentrations (0.01, 0.1, 1, 10 & 100 μM). Compound under investigation **18_d** (**D-758513/1**) exhibited remarkable anticancer activity against most of the tested cell lines representing nine different subpanels with GI_{50} values between ''1.07 – 5.65 μM" except the two cancer cell lines non small cell lung cancer NCI-H460 and breast cancer cell line MCF7 with GI_{50} values of 6.22 and 9.29 μM respectively, table (15). From the five-in-box screening for **18_d** and similar to compound **17_j** the criterion for selectivity of a compound **18_d** indicated that it also non selective toward the cancer subpanels with selectivity ratio in range of "0.62 - 1.46", table (15) and figures (53) and (54). Compound **18_d** has average GI_{50} responses at sub-micromolar concentrations (2 μM), cytostatic effects at micromolar concentrations (48.9 μM) and the average cytotoxic effects on cancer cell lines at micromolar concentrations (34.6 μM) which is 17 fold higher than GI_{50}. Furthermore, the basically substituted derivative **18_d** gave the highest antipoliferative activity "nanomolar active" in a selected cancer cell lines which is non small lung cancer cell line NCI-H460 with GI_{50} = 616 nM and ovarian cancer cell line OVCAR-4 with GI_{50} = 562 nM with non critical cytotoxic properties. Based on these data we observed that compound **18_d** is more effective than the cytostatic drugs Etoposid, Melphalan and Irinotecan (GI_{50} values of 38.9 μM, 14.5 μM and 14.1 μM respectively).

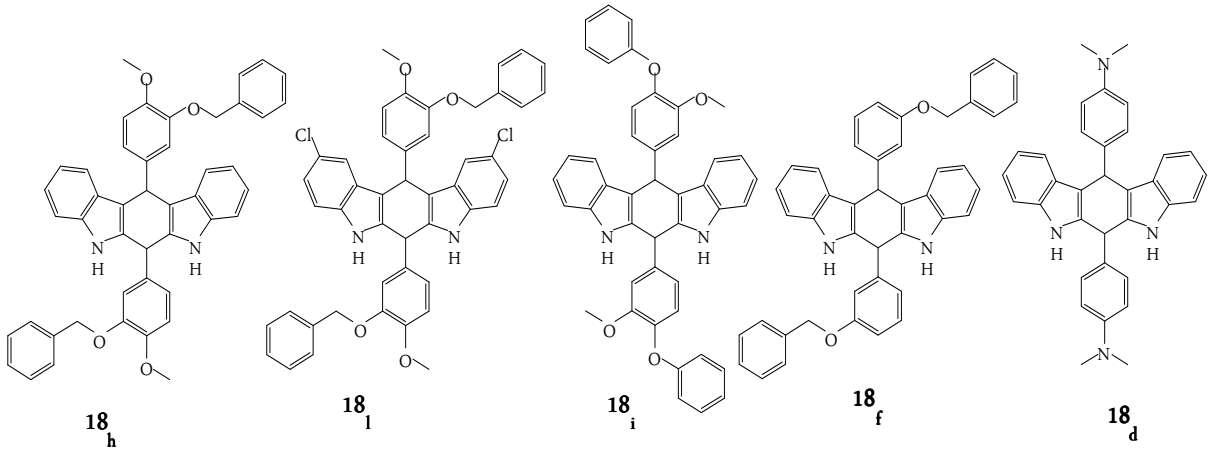


Figure (32): Selected indolocarbazoles ($\mathbf{18}_{d,f,h,i,l}$) for NCI screening.

3.2.2.5. Structure activity relationship (SAR) of indolocarbazoles

The structure activity relationship our synthesized indolocarbazoles indicated that, the basic substituted derivative have the highest activity which recorded the very potent and broad spectrum of activity against several cancers cell lines indicated with the negative values of percent growth (-7.03 % to -52.72 %) promoted at one dose with GI_{50} value of (1.07 μM to 5.65 μM) against almost all the selected cell lines at five dose assay. The chloro-substitution on the indole phenyl ring is unfavourable with a main loss of activity in the selected cancer cell lines. Comparing compound $\mathbf{18_h}$ with $\mathbf{18_i}$ a *para*-benzyloxy substituent increases the activity in some novel sensitive cell lines (NCI-H522 as non small lung cancer cell lines and CAKI-1 and UO-31 as renal cancer cell. By comparison of compound $\mathbf{18_h}$ and $\mathbf{18_f}$ a *para*-methoxy substituent ensures the activity especially in selected cell lines. The *Para*-benzyloxy compound $\mathbf{18_i}$ more active than the *meta*-benzyloxy compound $\mathbf{18_f}$. The basically substituted derivative $\mathbf{18_d}$ gave the highest antipoliferative activity (nanomolar active) in selected cell lines (non small lung cancer cell NCI-H460 and ovarian cancer cell OVCAR-4) with non critical cytotoxic properties (ten-to hundred full higher LC_{50} than TGI_{50} values) however this compound was found to be non selective toward the cancer subpanels.

Table (14): 60 cell line anticancer screening data at single dose assay (10^{-5} M) as percent growth inhibition of indolocarbazoles **$18_{d,f,h,i,l}$**.

Panel/Cell Line..... Growth Percent (%).....	**18_d**	**18_f**	**18_h**	**18_i**	18_l
Leukaemia		.....	.....	.....	.....
HL-60(TB)	.....	93.01	93.83	92.43	71.69
K-562	85.63	93.77	90.31	69.13	91.85
MOLT-4	56.77	87.83	89.45	57.59	100.00
RPMI-8226	61.55	93.28	101.84	85.35	98.07
Non-Small Cell Lung	103.63	.....	.....	.....	.....
Cancer.....	.....	101.29	87.05	70.47	102.55
A549/ATCC	35.07	85.39	100.47	60.37	100.02
EKVX	28.58	103.65	75.37	87.43	82.44
HOP-62	-44.87	117.34	98.69	56.36	89.60
HOP-92	-20.13	113.20	121.56	95.18	113.72
NCI-H226	58.36	99.57	85.95	72.69	87.64
NCI-H23	5.55	92.60	94.27	74.83	106.18
NCI-H322M	54.20	101.36	11.95	68.71	74.67
NCI-H460	-15.58	76.28	69.76	40.64	67.55
NCI-H522	-23.83	.....	.....	.....	.....
Colon Cancer	.....	102.90	46.32	89.45	104.17
COLO 205	35.06	94.79	102.40	89.55	nd
HCC-2998	78.09	93.94	72.65	58.49	92.35
HCT-116	7.08	91.90	97.68	67.40	101.24
HCT-15	42.53	90.98	97.62	57.82	103.79
HT29	35.90	105.92	70.71	73.25	104.52
KM12	44.00	91.96	11.44	80.96	75.56
SW-620	6.72	.....	.....	.....	.....
CNS Cancer	.....	104.78	95.54	93.10	111.10
SF-268	37.80	108.54	97.81	67.24	99.02
SF-295	-23.09	89.59	87.95	95.85	100.18
SF-539	38.81	104.31	101.34	89.22	109.29
SNB-19	25.73	97.54	44.79	75.14	91.47
U251	-51.91	.....	.....	.....	.....
Melanoma	.....	91.90	91.85	76.41	95.39
LOX IMVI	42.67	89.19	70.52	95.88	101.60
MALME-3M	21.37	87.83	86.36	78.68	102.10
M14	-29.41	102.34	90.59	79.49	100.85
MDA-MB-435	44.51	96.34	87.37	95.93	102.29
SK-MEL-28	17.60	113.79	100.59	71.78	106.20
SK-MEL-5	45.69	104.58	109.35	97.58	106.52
UACC-257	83.91	93.61	90.58	77.85	100.27
UACC-62	39.50	.....	.....	.....	.....
Ovarian Cancer	.....	94.67	94.15	62.03	105.11
IGROV1	33.23	124.29	122.18	96.52	108.60
OVCAR-3	-52.72	92.04	78.48	73.12	101.27
OVCAR-4	14.28	88.96	94.14	85.87	104.54
OVCAR-5	36.61	107.28	59.24	89.33	96.21
OVCAR-8	20.00	106.83	75.38	79.55	108.71
NCI/ADR-RES	19.48	105.46	94.19	94.58	91.66
SK-OV-3	26.87	.....	.....	.....	.....
Renal Cancer.....	.....	97.88	84.04	76.33	92.18
786-0	-35.79	113.64	86.53	78.86	118.27
A498	-7.03	98.38	94.06	70.22	104.77
ACHN	9.61	80.17	84.65	41.36	95.32
CAKI-1	13.72	112.55	105.50	85.59	104.21
RXF 393	30.66	96.88	97.15	81.56	99.00
SN12C	27.74	106.78	95.31	94.84	139.73
TK-10	-18.32	57.58	60.84	39.78	76.77
UO-31	12.35	.....	.....	.....	.....
Prostate Cancer.....	.....	107.22	58.82	48.49	76.27
PC-3	36.54	115.99	39.16	98.24	97.18
DU-145	46.83	.....	.....	.....	.....
Breast Cancer.....	.....	89.39	77.15	59.07	94.71
MCF7	24.23	89.23	86.89	63.58	84.64
MDA-MB-231/ATCC	-30.21	109.72	91.24	88.18	97.29
HS 578T	-8.41	113.56	87.17	81.59	94.79
BT-549	8.92	85.57	92.62	59.13	96.62
T-47D	28.15	115.17	108.96	112.86	121.33
MDA-MB-468	47.27				
Mean	21.63 % Selected	98.65 % Non selected	84.67% Non selected	76.84% Non selected	98.24% Non selected

Table (15): NCI in vitro testing result of compound **18_d (D-758513/1)** at five dose level in µM.

Panel/Cell Line	GI_{50} Concentration per cell line	MID[b]	selectivity ratio (MID[a]:MID[b])	TGI	LC_{50}	Log GI_{50}	Log TGI	Log LC_{50}
Leukemia	.....	2.41	1.0	.....	.....	.....	.....	.....
CCRF-CEM	nd			> 1.00	> 1.00	nd	> -4.00	> -4.00
HL-60(TB)	2.41			> 1.00	> 1.00	-4.62	> -4.00	> -4.00
K-562	nd			> 1.00	> 1.00	nd	> -4.00	> -4.00
MOLT-4	nd			> 1.00	> 1.00	nd	> -4.00	> -4.00
RPMI-8226	nd			> 1.00	> 1.00	nd	> -4.00	> -4.00
SR	nd			> 1.00	> 1.00	nd	> -4.00	> -4.00
Non-Small Cell Lung Cancer	.....	2.29	1.06	.....	.....	.....	.....	.....
A549/ATCC	1.51			nd	nd	-5.82	nd	nd
EKVX	1.92			5.72	3.90	-5.72	-5.24	-4.41
HOP-62	1.16			2.38	nd	-5.94	-5.62	nd
HOP-92	1.07			3.73	2.83	-5.97	-5.43	-4.55
NCI-H226	3.28			1.08	3.42	-5.48	-4.97	-4.47
NCI-H23	1.69			4.59	> 1.00	-5.77	-5.34	> -4.00
NCI-H322M	1.46			3.63	9.01	-5.83	-5.44	-5.05
NCI-H460	6.22			1.88	4.34	-6.21	-5.72	-5.36
Colon Cancer	.....	2.68	0.9	.....	.....	.....	.....	.....
COLO 20	4.22			2.05	6.77	-5.38	-4.69	-4.17
HCC-2998	1.69			> 1.00	> 1.00	-4.77	>-4.00	> -4.00
HCT-116	1.34			2.61	5.11	-5.87	-5.58	-5.29
HCT-1	4.24			2.12	8.44	-5.37	-4.67	-4.07
HT29	2.36			6.72	> 1.00	-4.63	-5.17	> -4.00
KM12	3.77			> 1.00	> 1.00	-5.42	>-4.00	> -4.00
SW-620	1.15			2.36	4.86	-4.94	-5.63	-5.31
CNS Cancer	.....	1.66	1.46	.....	.....	.....	.....	.....
SF-268	1.79			5.35	1.99	-5.75	-5.27	-4.70
SF-29	1.19			2.69	6.06	-5.92	-5.57	-5.22
SF-539	3.00			9.53	> 1.00	-5.52	-5.02	> -4.00
SNB-19	1.52			3.35	7.40	-5.82	-5.47	-5.13
SNB-7	1.34			5.24	> 1.00	-5.87	-5.28	> -4.00
U251	1.13			2.33	nd	-5.95	-5.63	nd
Melanoma.....	.....	2.51	0.96	.....	.....	.....	.....	.....
LOX IMVI	2.56			1.21	> 1.00	-5.59	-4.92	> -4.00
MALME-3M	nd			nd	> 1.00	nd	nd	> -4.00
M14	1.67			3.03	5.51	-5.78	-5.52	-5.26
MDA-MB-43	3.10			> 1.00	> 1.00	-5.51	>-4.00	> -4.00
SK-MEL-2	nd			nd	> 1.00	nd	nd	> -4.00
SK-MEL-28	1.62			nd	nd	-5.79	nd	nd
SK-MEL-	2.57			7.81	2.74	-5.59	-5.11	-4.56
UACC-257	3.54			5.93	> 1.00	-5.45	-4.23	>-4.00
UACC-62	2.48			1.15	> 1.00	-5.61	-4.94	> -4.00
Ovarian Cancer	.....	2.5	0.97	.....	.....	.....	.....	.....
IGROV1	2.06			nd	> 1.00	-5.69	nd	> -4.00
OVCAR-3	1.67			3.03	5.51	-5.78	-5.52	-5.26
OVCAR-4	5.65			2.89	1.58	-6.25	-5.54	-4.80
OVCAR-	2.39			6.97	2.60	-5.62	-5.16	-4.58
OVCAR-8	1.72			nd	> 1.00	-5.76	Nd	> -4.00
NCI/ADR-RES	1.82			5.77	3.99	-5.74	-5.24	-4.40
SK-OV-3	2.19			5.72	1.97	-5.66	-5.24	-4.70
Renal Cancer	.....	1.94	1.25	.....	.....	.....	.....	.....
786-0	1.60			2.95	5.43	-5.80	-5.53	-5.27
A498	1.53			3.28	7.05	-5.82	-5.48	-5.15
ACHN	1.96			4.61	1.22	-5.71	-5.34	-4.91
CAKI-1	1.90			6.11	5.51	-5.72	-5.21	-4.26
RXF 393	3.19			9.24	4.14	-5.50	-5.03	-4.38
SN12C	2.00			6.81	> 1.00	-5.70	-5.17	> -4.00
TK-10	1.52			2.85	5.34	-5.82	-5.54	-5.27
UO-31	1.82			5.41	2.01	-5.74	-5.27	-4.70
Prostate Cancer	.....	1.77	1.37	.....	.....	.....	.....	.....
PC-3	nd.			> 1.00	> 1.00	nd	>-4.00	> -4.00
DU-14	1.77			3.30	6.13	-5.75	-5.48	-5.21
Breast Cancer	.....	3.86	0.62	.....	.....	.....	.....	.....
MCF7	9.29			nd	> 1.00	-6.03	nd	> -4.00
MDA-MB-231/ATCC 0.594	1.41			2.70	5.20	-5.85	-5.57	-5.28
HS 578T	2.40			5.08	1.29	-5.62	-5.29	-4.89
T-47D	2.46			nd	> 1.00	-5.61	Nd	> -4.00
MDA-MB-468	3.74			5.23	> 1.00	-5.43	-4.28	> -4.00
MID[a]	2.42							
Avarege:						-5.69 (2 µM)	-5.01 (10 µM)	-4.46 (34.6 µM)
Delta:						0.56	0.71	0.9
Range:						1.63	1.72	1.36

MID[a]: Average sensitivity of all cell line in µM. MID[b]: Average sensitivity of all cell line of a particular subpanel in µM.

Developmental Therapeutics Program One Dose Mean Graph	NSC: D-758513 / 1	Conc: 1.00E-5 Molar	Test Date: May 09, 2011
	Experiment ID: 1105OS49		Report Date: Jun 28, 2011

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	85.63
K-562	56.77
MOLT-4	61.55
RPMI-8226	103.63
Non-Small Cell Lung Cancer	
A549/ATCC	35.07
EKVX	28.58
HOP-62	-44.87
HOP-92	-20.13
NCI-H226	58.36
NCI-H23	5.55
NCI-H322M	54.20
NCI-H460	-15.58
NCI-H522	-23.83
Colon Cancer	
COLO 205	35.06
HCC-2998	78.09
HCT-116	7.08
HCT-15	42.53
HT29	35.90
KM12	44.00
SW-620	6.72
CNS Cancer	
SF-268	37.80
SF-295	-23.09
SF-539	38.81
SNB-19	25.73
U251	-51.91
Melanoma	
LOX IMVI	42.67
MALME-3M	21.37
M14	-29.41
MDA-MB-435	44.51
SK-MEL-28	17.60
SK-MEL-5	45.69
UACC-257	83.91
UACC-62	39.50
Ovarian Cancer	
IGROV1	33.23
OVCAR-3	-52.72
OVCAR-4	14.28
OVCAR-5	36.61
OVCAR-8	20.00
NCI/ADR-RES	19.48
SK-OV-3	26.87
Renal Cancer	
786-0	-35.79
A498	-7.03
ACHN	9.61
CAKI-1	13.72
RXF 393	30.66
SN12C	27.74
TK-10	-18.32
UO-31	12.35
Prostate Cancer	
PC-3	36.54
DU-145	46.83
Breast Cancer	
MCF7	24.23
MDA-MB-231/ATCC	-30.21
HS 578T	-8.41
BT-549	8.92
T-47D	28.15
MDA-MB-468	47.27
Mean	21.63
Delta	74.35
Range	156.35

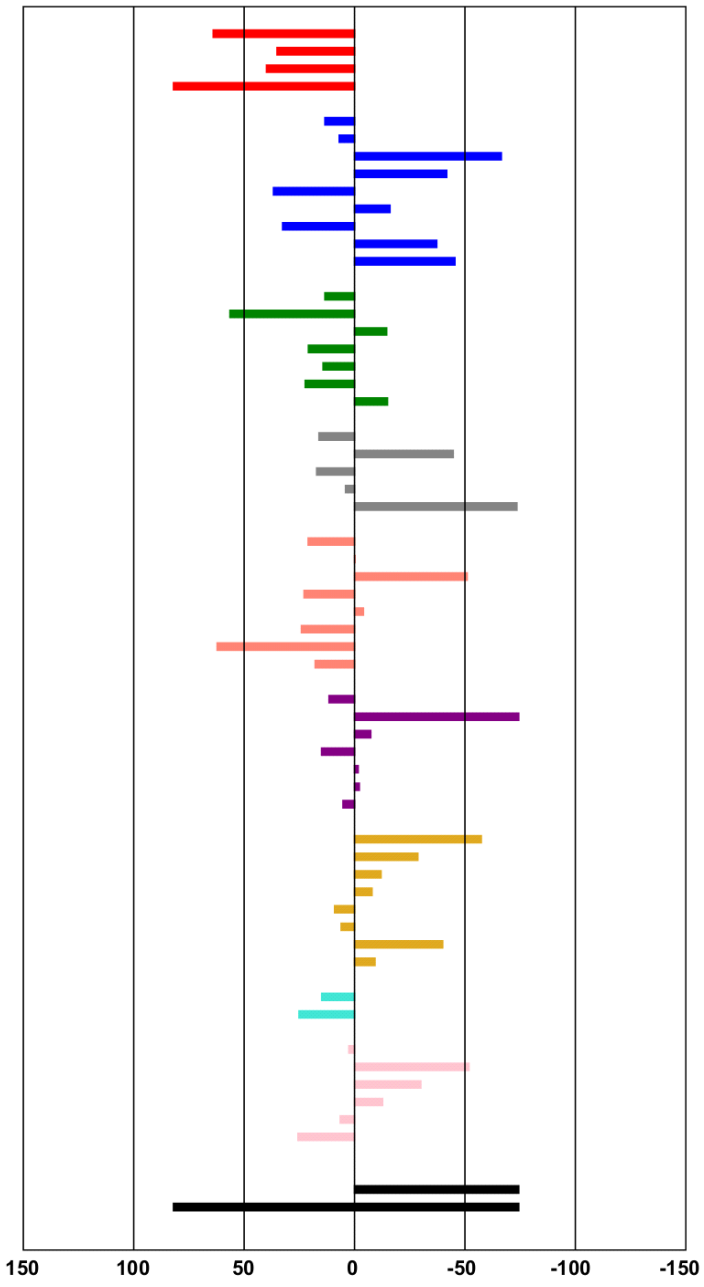


Figure (53): Results of the one dose screening of **18d**

National Cancer Institute Developmental Therapeutics Program
In-Vitro Testing Results

NSC : D - 758513 / 1	Experiment ID : 1106NS70	Test Type : 08	Units : Molar
Report Date : August 22, 2011	Test Date : June 27, 2011	QNS :	MC :
COMI : Elm-213a (105821)	Stain Reagent : SRB Dual-Pass Related	SSPL : 0HHZ	

		Log10 Concentration												
	Time	Mean Optical Densities					Percent Growth							
Panel/Cell Line	Zero	Ctrl	-8.0	-7.0	-6.0	-5.0	-4.0	-8.0	-7.0	-6.0	-5.0	-4.0	GI50	TGI	LC50
Leukemia															
CCRF-CEM	0.557	1.688	1.525	1.571	1.455	0.663	1.164	86	90	79	9	54	.	> 1.00E-4	> 1.00E-4
HL-60(TB)	0.977	2.710	2.699	2.783	2.718	2.252	1.182	99	104	100	74	12	2.41E-5	> 1.00E-4	> 1.00E-4
K-562	0.168	1.073	1.064	1.049	0.965	0.299	0.930	99	97	88	14	84	.	> 1.00E-4	> 1.00E-4
MOLT-4	0.522	1.548	1.592	1.584	1.574	0.748	1.393	104	104	103	22	85	.	> 1.00E-4	> 1.00E-4
RPMI-8226	0.384	1.035	0.960	1.024	0.914	0.564	1.193	89	98	81	28	124	.	> 1.00E-4	> 1.00E-4
SR	0.374	1.380	1.341	1.387	1.236	0.440	1.145	96	101	86	7	77	.	> 1.00E-4	> 1.00E-4
Non-Small Cell Lung Cancer															
A549/ATCC	0.333	1.611	1.593	1.488	1.263	0.153	0.509	99	90	73	-54	14	1.51E-6	.	.
EKVX	0.707	1.743	1.692	1.674	1.537	0.526	0.235	95	93	80	-26	-67	1.92E-6	5.72E-6	3.90E-5
HOP-62	0.364	1.094	1.111	1.075	0.804	-0.031	0.274	102	97	60	-100	-25	1.16E-6	2.38E-6	.
HOP-92	1.098	1.382	1.370	1.282	1.247	0.666	0.407	96	65	53	-39	-63	1.07E-6	3.73E-6	2.83E-5
NCI-H226	0.752	1.456	1.470	1.453	1.455	0.775	0.027	102	100	100	3	-96	3.28E-6	1.08E-5	3.42E-5
NCI-H23	0.627	1.698	1.645	1.662	1.446	0.383	0.626	95	97	76	-39	.	1.69E-6	4.59E-6	> 1.00E-4
NCI-H322M	0.347	0.757	0.770	0.776	0.638	0.154	0.155	103	105	71	-56	-55	1.46E-6	3.63E-6	9.01E-6
NCI-H460	0.224	1.797	1.843	1.739	0.821	-0.071	-0.173	103	96	38	-100	-100	6.22E-7	1.88E-6	4.34E-6
Colon Cancer															
COLO 205	0.246	1.305	1.395	1.343	1.130	0.563	0.083	109	104	83	30	-66	4.22E-6	2.05E-5	6.77E-5
HCC-2998	0.650	2.205	2.233	2.159	2.133	1.541	1.045	102	97	95	57	25	1.69E-5	> 1.00E-4	> 1.00E-4
HCT-116	0.170	1.128	1.170	1.138	0.856	-0.016	-0.350	104	101	72	-100	-100	1.34E-6	2.61E-6	5.11E-6
HCT-15	0.310	1.842	1.822	1.864	1.664	0.727	0.136	99	101	88	27	-56	4.24E-6	2.12E-5	8.44E-5
HT29	0.223	1.076	1.199	1.134	1.001	0.181	0.126	114	107	91	-19	-43	2.36E-6	6.72E-6	> 1.00E-4
KM12	0.398	1.756	1.753	1.777	1.482	0.779	0.405	100	102	80	28	.	3.77E-6	> 1.00E-4	> 1.00E-4
SW-620	0.189	1.094	1.067	1.004	0.729	-0.029	-0.316	97	90	60	-100	-100	1.15E-6	2.36E-6	4.86E-6
CNS Cancer															
SF-268	0.327	1.134	1.133	1.114	0.946	0.234	-0.437	100	97	77	-29	-100	1.79E-6	5.35E-6	1.99E-5
SF-295	0.783	2.216	2.125	2.085	1.655	0.151	0.005	94	91	61	-81	-99	1.19E-6	2.69E-6	6.06E-6
SF-539	0.824	2.025	2.073	2.019	1.995	0.807	0.740	104	99	97	-2	-10	3.00E-6	9.53E-6	> 1.00E-4
SNB-19	0.436	1.503	1.403	1.363	1.252	0.135	0.052	91	87	76	-69	-88	1.52E-6	3.35E-6	7.40E-6
SNB-75	0.617	1.459	1.295	1.261	1.128	0.471	0.319	81	77	61	-24	-48	1.34E-6	5.24E-6	> 1.00E-4
U251	0.291	1.233	1.237	1.153	0.839	-0.039	0.285	100	92	58	-100	-2	1.13E-6	2.33E-6	.
Melanoma															
LOX IMVI	0.314	1.879	1.783	1.716	1.604	0.364	0.202	94	90	82	3	-36	2.56E-6	1.21E-5	> 1.00E-4
MALME-3M	0.544	0.860	0.858	0.857	0.816	0.313	1.138	99	99	86	-42	188	.	.	> 1.00E-4
M14	0.369	1.251	1.278	1.305	1.189	-0.034	-0.418	103	106	93	-100	-100	1.67E-6	3.03E-6	5.51E-6
MDA-MB-435	0.384	1.689	1.634	1.591	1.461	0.598	0.945	96	92	82	16	43	3.10E-6	> 1.00E-4	> 1.00E-4
SK-MEL-2	0.846	1.603	1.700	1.730	1.611	0.624	1.396	113	117	101	-26	73	.	.	> 1.00E-4
SK-MEL-28	0.321	1.063	1.042	0.939	0.917	0.112	0.641	97	83	80	-65	43	1.62E-6	.	.
SK-MEL-5	0.644	1.929	1.991	1.940	1.831	0.573	-0.221	105	101	92	-11	-100	2.57E-6	7.81E-6	2.74E-5
UACC-257	0.691	1.412	1.386	1.332	1.343	0.812	0.657	96	89	90	17	-5	3.54E-6	5.93E-5	> 1.00E-4
UACC-62	0.566	2.336	2.193	2.204	1.996	0.616	0.322	92	93	81	3	-43	2.48E-6	1.15E-5	> 1.00E-4
Ovarian Cancer															
IGROV1	0.420	1.215	1.279	1.268	1.011	0.408	0.814	108	107	74	-3	49	2.06E-6	.	> 1.00E-4
OVCAR-3	0.433	1.091	1.111	1.103	1.045	-0.017	-0.409	103	102	93	-100	-100	1.67E-6	3.03E-6	5.51E-6
OVCAR-4	0.396	1.499	1.497	1.440	0.785	0.233	0.057	100	95	35	-41	-86	5.65E-7	2.89E-6	1.58E-5
OVCAR-5	0.467	1.181	1.137	1.154	1.114	0.389	0.016	94	96	91	-17	-97	2.39E-6	6.97E-6	2.60E-5
OVCAR-8	0.311	1.275	1.290	1.231	1.065	0.183	0.601	102	95	78	-41	30	1.72E-6	.	> 1.00E-4
NCI/ADR-RES	0.532	1.620	1.585	1.487	1.360	0.405	0.174	97	88	76	-24	-67	1.82E-6	5.77E-6	3.99E-5
SK-OV-3	0.566	1.510	1.546	1.499	1.423	0.402	-0.003	104	99	91	-29	-100	2.19E-6	5.72E-6	1.97E-5
Renal Cancer															
786-0	0.607	1.874	1.890	1.892	1.729	-0.113	-0.606	101	101	88	-100	-100	1.60E-6	2.95E-6	5.43E-6
A498	0.970	1.418	1.397	1.392	1.318	0.264	-0.462	95	94	78	-73	-100	1.53E-6	3.28E-6	7.05E-6
ACHN	0.438	1.657	1.672	1.675	1.526	0.240	-0.192	101	101	89	-45	-100	1.96E-6	4.61E-6	1.22E-5
CAKI-1	0.599	2.093	1.993	1.917	1.756	0.473	0.239	93	88	77	-21	-60	1.90E-6	6.11E-6	5.51E-5
RXF 393	0.605	0.934	0.950	0.947	0.949	0.583	0.129	105	104	104	-4	-79	3.19E-6	9.24E-6	4.14E-5
SN12C	0.469	1.740	1.639	1.627	1.462	0.396	0.304	92	91	78	-16	-35	2.00E-6	6.81E-6	> 1.00E-4
TK-10	0.590	1.158	1.177	1.185	1.065	-0.044	0.045	103	105	84	-100	-92	1.52E-6	2.85E-6	5.34E-6
UO-31	0.674	1.480	1.361	1.336	1.299	0.484	-0.280	85	82	78	-28	-100	1.82E-6	5.41E-6	2.01E-5
Prostate Cancer															
PC-3	0.524	1.626	1.563	1.504	1.176	0.572	1.439	94	89	59	4	83	.	> 1.00E-4	> 1.00E-4
DU-145	0.342	1.031	1.107	1.057	1.004	0.036	-0.553	111	104	96	-89	-100	1.77E-6	3.30E-6	6.13E-6
Breast Cancer															
MCF7	0.212	1.153	1.073	1.026	0.671	0.133	0.458	91	87	49	-38	26	9.29E-7	.	> 1.00E-4
MDA-MB-231/ATCC	0.437	1.064	1.018	1.065	0.914	-0.136	-0.537	93	100	76	-100	-100	1.41E-6	2.70E-6	5.20E-6
BT-549	0.905	1.618	1.667	1.692	1.680	0.495	0.111	107	110	109	-45	-88	2.40E-6	5.08E-6	1.29E-5
T-47D	0.448	1.150	1.179	1.131	1.043	0.430	0.580	104	97	85	-4	19	2.46E-6	.	> 1.00E-4
MDA-MB-468	0.682	1.377	1.352	1.370	1.263	0.856	0.615	96	99	84	25	-10	3.74E-6	5.23E-5	> 1.00E-4

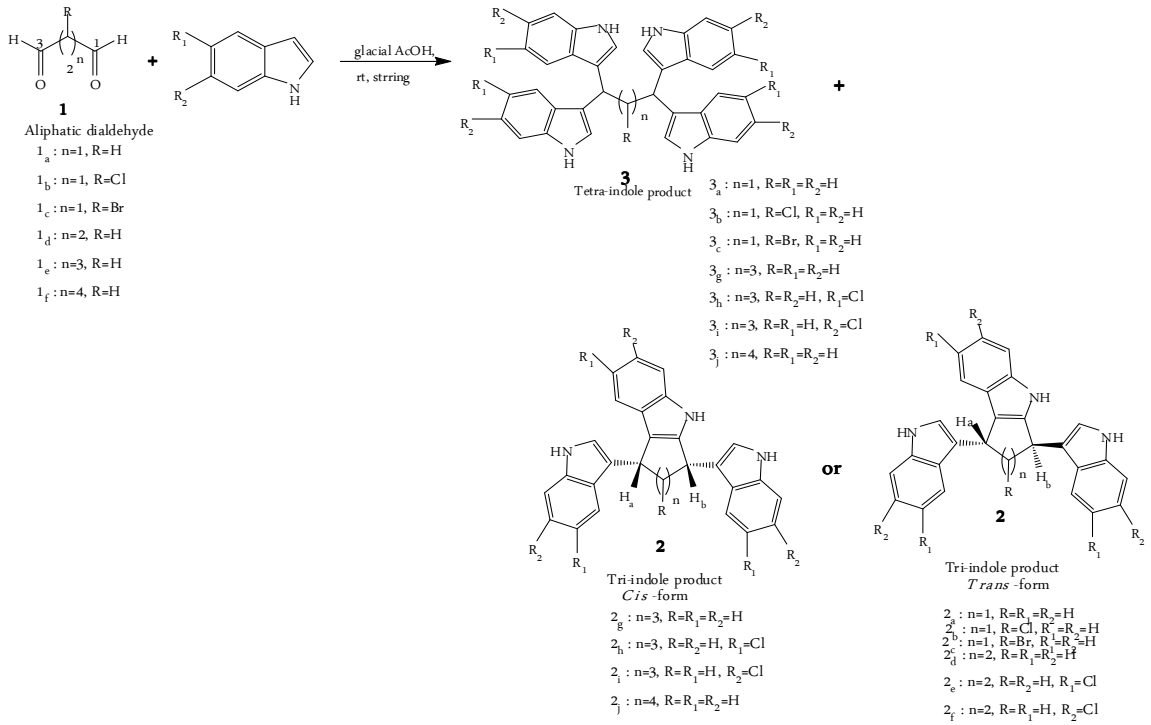
Figure (54): Five dose testing results of compound **18$_d$**.

4. Summary and Future work

4.1. Summary

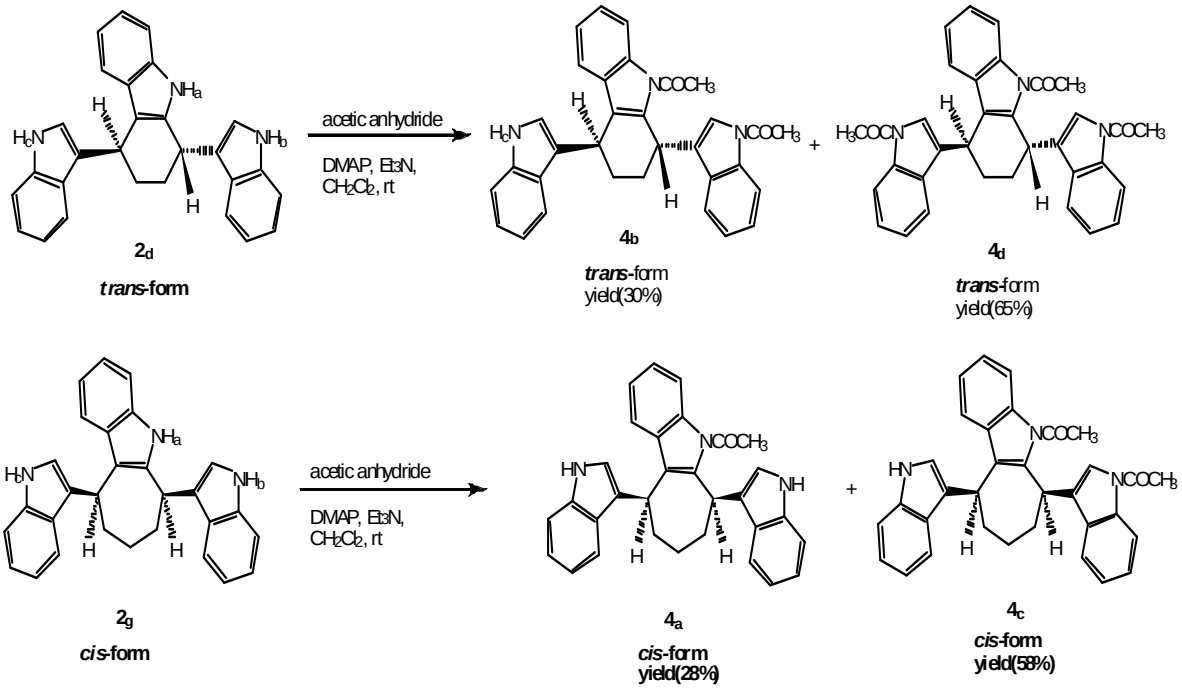
Synthetic part

In the present work we introduce AcOH as a mild and efficient catalyst for the synthesis of novel highly substituted diastereromeric tetrahydrocyclopenta indoles, tetrahydrocarbazoles, hexahydrocyclohepta and hexahydrocycloocta indoles with triindole substituents in the form of *cis* or *trans* as a minor product and a tetraindole of propane, pentane and hexane as the major product. Our reaction means the introduction of a novel and simple chemical reaction method that has not been reported in literature before. We left indoles react smoothly with aliphatic dialdehydes (**1_{a-f}**) for example malonaldehyde and its derivatives, succinaldehyde, glutaraldehyde and adipaldehyde to afford substituted diastereromeric tetrahydrocyclopenta indoles, tetrahydrocarbazoles, hexahydrocyclohepta and hexahydrocycloocta indoles of type **2** as a minor product and propane, pentane or hexane substituted with four indole units of type (**3**) as the major product, scheme (2).



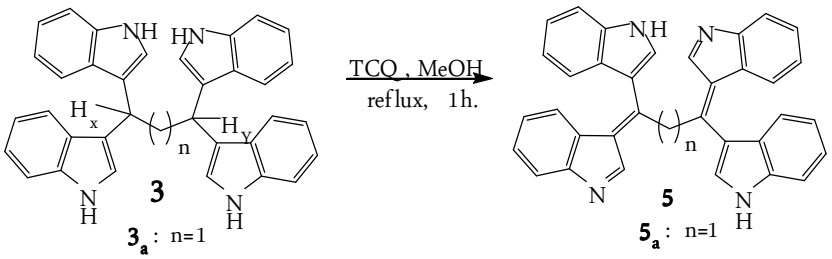
Scheme (2): General equation for the reaction of indoles with aliphatic dildehydes.

The acetylation reaction of the *trans* isomer of compound $\mathbf{2_d}$, led to the identified diacetylated product $\mathbf{4_b}$ and the triacetylated derivative $\mathbf{4_d}$. In case of the *cis* form of the compound $\mathbf{2_g}$ the reaction afforded two products as *cis* forms, the mono-acetylated compounds $\mathbf{4_a}$ and the diacetylated one $\mathbf{4_c}$, scheme (10).



Scheme (10): Acetylation reactions of triindole products. 2_d and 2_g.

The oxidation reaction of tetraindoles ($\mathbf{3_{a,g,j}}$) took place under a mild condition as reported for the oxidation of BIMs by using TCQ or DDQ, scheme (11).

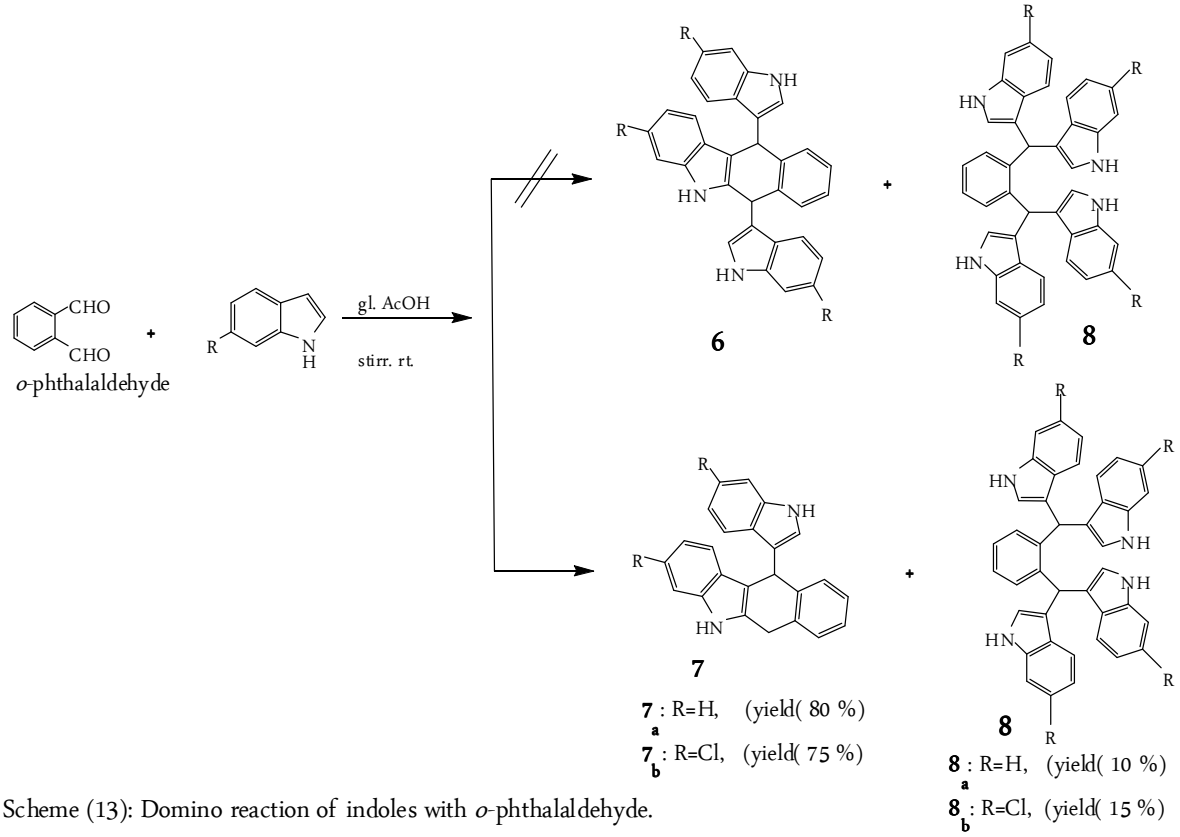


$\mathbf{3_a}$: n=1
$\mathbf{3_g}$: n=3
$\mathbf{3_j}$: n=4

$\mathbf{5_a}$: n=1
$\mathbf{5_b}$: n=3
$\mathbf{5_c}$: n=4

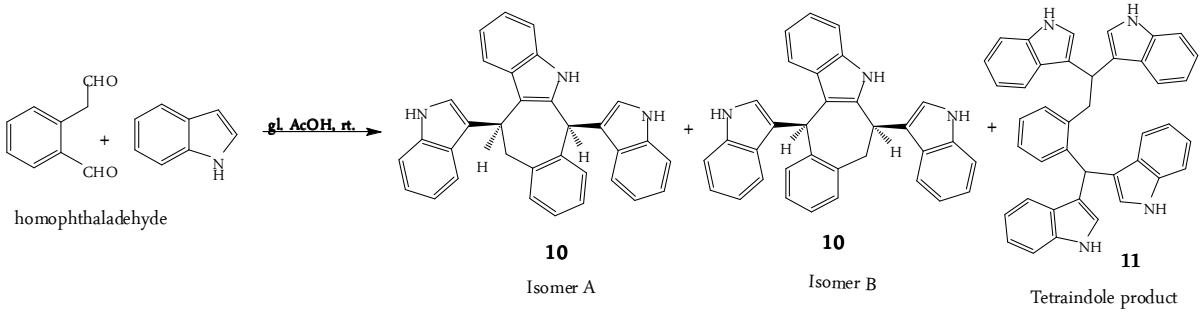
Scheme(11): Oxidation reactions of tetraindoles $\mathbf{3_{a,g,j}}$.

The reaction of *o*-phthalaldehyde with indole, in the presence of AcOH at room temperature was found to be successful affording the product $\mathbf{7_{a-b}}$ in 75 to 80 % yields, in addition to the formation of the expected tetraindole products $\mathbf{8_{a,b}}$ in a very low yield of 10 to 15 %, scheme (13).



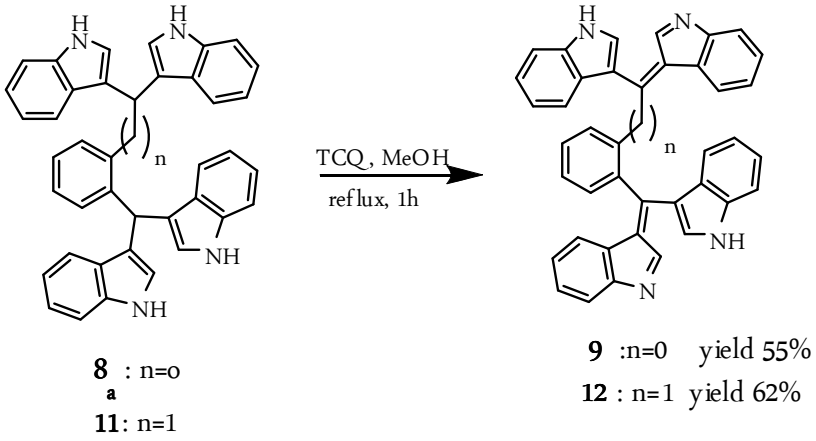
Scheme (13): Domino reaction of indoles with *o*-phthalaldehyde.

The dialdehyde homophthalaldehyde was used directly in the condensation reaction with indole in acetic acid at room temperature yielding a novel benzo[7]annulene derivative of type **10** in a moderate yield (46 %) and the tetraindole product **11** in a 38 % yield. Compound **10** was isolated in two *isomers* **A** and **B**, scheme (16).



Scheme (16): Domino reaction of indole with homophthaladehyde.

Compounds **8**$_a$ and **11** could also undergo dehydration reaction using TCQ affording the dehydrated forms **9** and **12** in good yields, scheme (18).

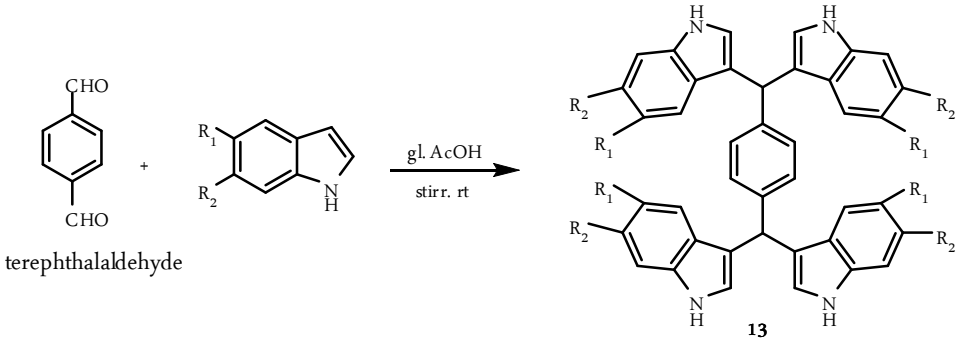


8$_a$: n=o
11: n=1

9 :n=0 yield 55%
12 : n=1 yield 62%

Scheme (18): Oxidation reaction of compound **8**$_a$ and **11**

The electrophilic substitution reactions of indoles with terephthaladehyde have been reported in the literature as a possible way for the synthesis of supramolecular compounds containing BIMs, namely 3,3$^{/}$,3$^{//}$,3$^{///}$-tetraindolyl(terphthalayl)dimethane (**13**$_a$) in good yields. In the present work, terephthaladehyde condensed with indoles in glacial acetic acid in a molar ratio (1:4) affording compounds **13**$_{a,b}$ in a high yield of 93-95 % respectively after a short time of stirring at room temperature (2-4 h), scheme (19).



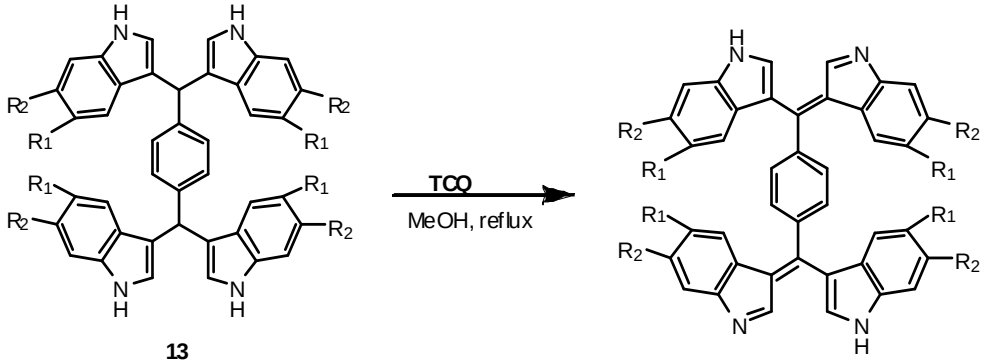
terephthalaldehyde

13

13$_a$: R$_1$=R$_2$=H
13$_b$: R$_1$=Cl, R$_2$=H

Scheme (19): Reaction of indoles with terephthaladehyde.

The oxidation reaction using TCQ as oxidizing agent in methanol solution had been extended for the synthesis of the expected novel bishydrated forms of type **$14_{a,b,}$** scheme (20).



13

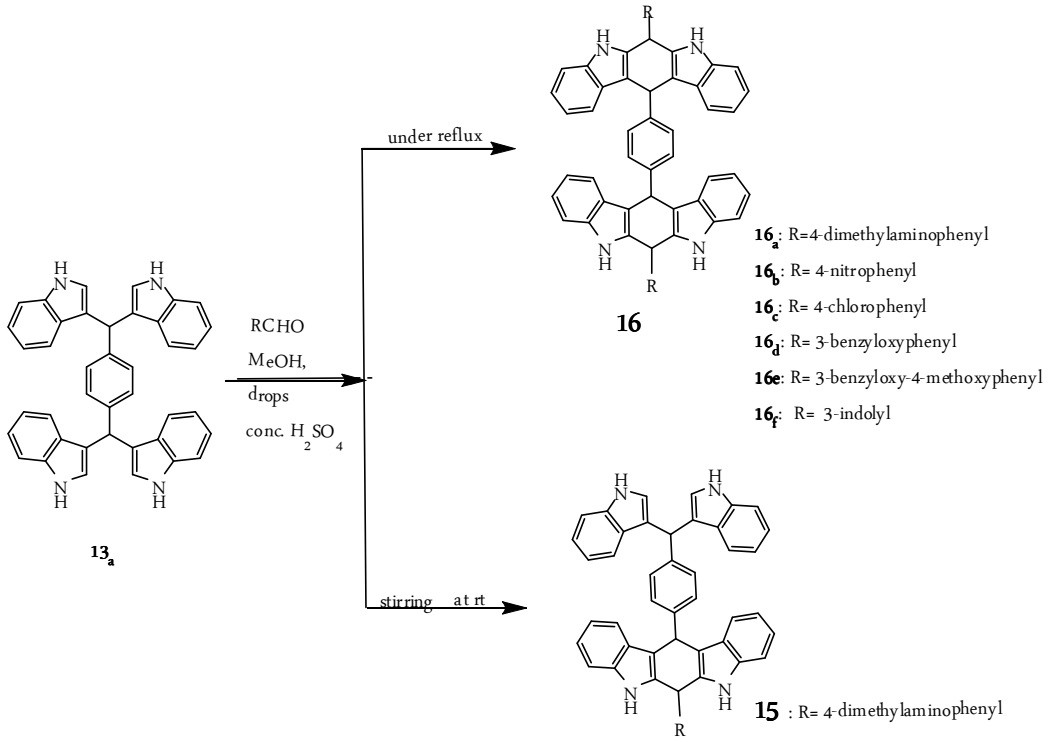
13_a: R_1=R_2=H
13_b: R_1=Cl, R_2=H

14

14_a: R_1=R_2=H
14_b: R_1=Cl, R_2=H

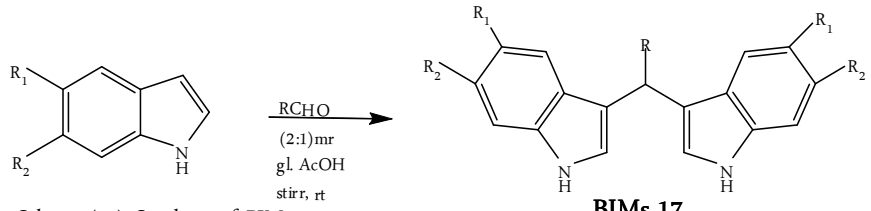
Scheme (20): Oxidation reaction of compound **$13_{a,b}$.**

Compounds **$13_{a,b}$** can act as nucleophile due to the unoccupied two positions of the four indole rings, hence we now present a convenient method for the synthesis of the novel extended ring systems (**$16_{a,f}$**) *via* the condensation reaction of compound **13_a** with aryl or heteroaryl substituted aldehyde in a molar ratio (1:2), scheme (21). However, when the reaction was carried out at room temperature under stirring for long time, the main product which was separated and identified was compound **15** by using *p*-dimethylaminobenzaldehyde. Compound **15** can be considered as an intermediate product for the formation of the compound **16_a**.



Scheme (21): Condensation reaction of **13**$_{a}$ with aldehydes.

A series of substituted aryl or heteroaryl aldehydes were efficiently converted to the corresponding BIMs **17**$_{a\text{-}p}$ as shown in table (1) and scheme (25), which gives the reaction times and the formed yields. The short reaction time coupled with the simplicity of the reaction procedure makes this method one of the most efficient methods for the synthesis of this class of compounds.

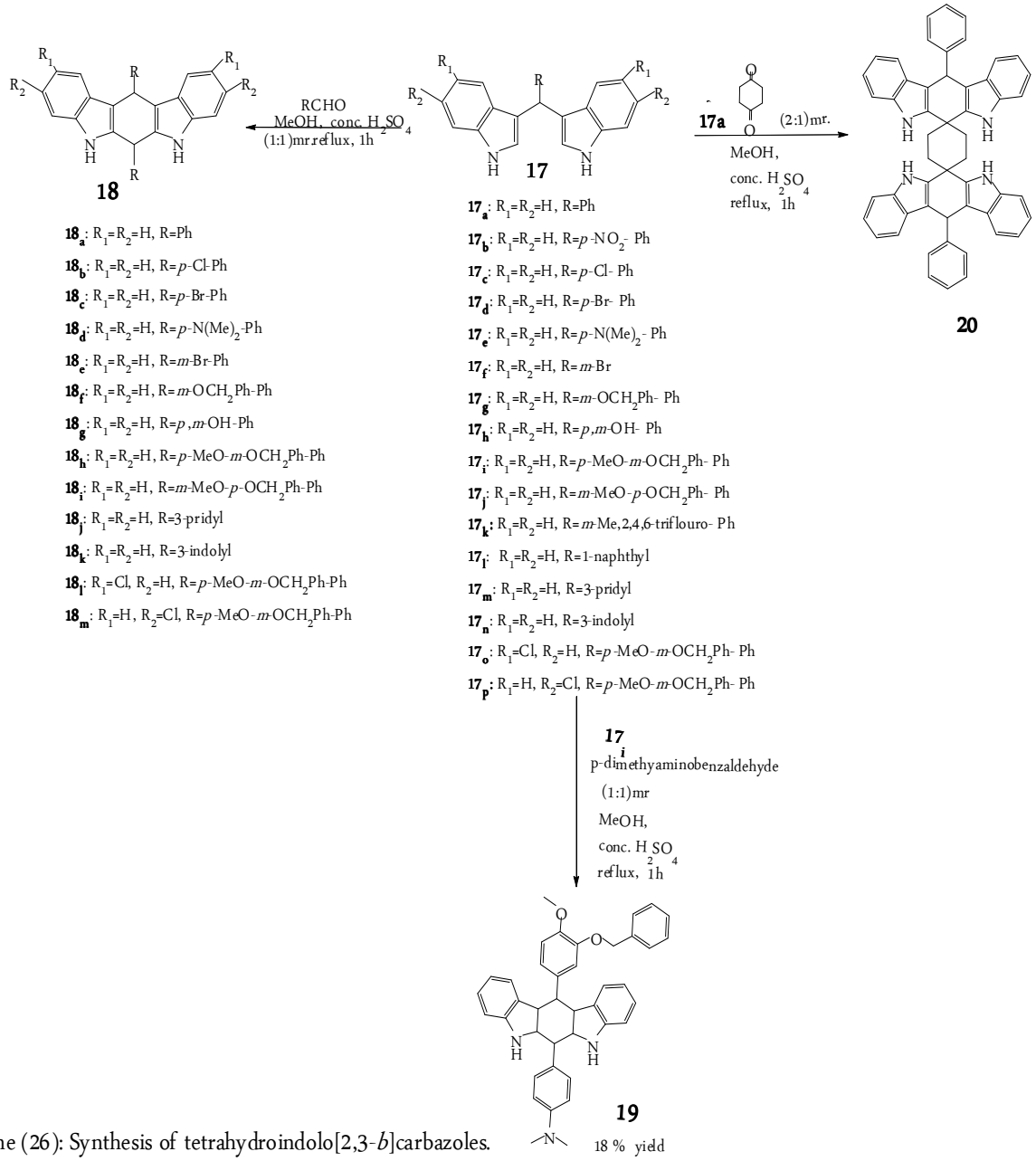


Scheme (25): Synthesis of BIMs.

entry	Aryl or heteroarylaldehydes	Indoles	Product	Reaction time (h)	Yield (%)
1	**R** = Ph	Indole	**17$_a$**	5	90
2	**R** = *p*-NO_2-Ph	"	**17$_b$**	4	98
3	**R** = *p*-Cl- Ph	"	**17$_c$**	6	99
4	**R** = *p*-Br- Ph	"	**17$_d$**	5	76
5	**R** = *p*-N(Me)2- Ph	"	**17$_e$**	5	91
6	**R** = *m*-Br-Ph	"	**17$_f$**	4	88
7	**R**= *m*-OCH_2Ph- Ph	"	**17$_g$**	5	87
8	**R** = *p,m*-OH- Ph	"	**17$_h$**	6	73
9	**R**=*p*-MeO-*m*-OCH_2Ph-Ph	"	**17$_i$**	4	89
10	**R**=*m*-MeO-*p*-OCH_2Ph-Ph	"	**17$_j$**	5	92
11	**R** = *m*-Me,2,4,6-tri-F-Ph	"	**17$_k$**	6	77
12	**R** = 1-naphthyl	"	**17$_l$**	4	97
13	**R** = 3-pridyl	"	**17$_m$**	6	95
14	**R** = 3-indolyl	"	**17$_n$**	6	98
15	**R**=*p*-MeO-*m*-OCH_2Ph-Ph	5-Cl-indole	**17$_o$**	4	91
16	**R**=*p*-MeO-*m*-OCH_2Ph-Ph	6-Cl-indole	**17$_p$**	4	93

Table (1): Synthesized BIMs (**17$_{a-p}$**).

As an extending study of our present work, we used the prepared BIMs **17$_{a-p}$** as a starting materials for the synthesis of biologically active tetrahydroindolo[2,3-*b*]carbazoles of type **18$_{a-m}$, 19** and the extended spirocyclic biscarbazoles **20**. The BIM and the aromatic aldehyde (the same aldehyde which condensed with indoles in the synthesis of the used BIM) were used in a molar ratio (1:1) for the synthesis of the pure tetrahydroindolo[2,3-*b*]carbazoles of type **18$_{a-m}$** in good to better yields, scheme (26).



18

18a: R_1=R_2=H, R=Ph
18b: R_1=R_2=H, R=*p*-Cl-Ph
18c: R_1=R_2=H, R=*p*-Br-Ph
18d: R_1=R_2=H, R=*p*-N(Me)$_2$-Ph
18e: R_1=R_2=H, R=*m*-Br-Ph
18f: R_1=R_2=H, R=*m*-OCH$_2$Ph-Ph
18g: R_1=R_2=H, R=*p*,*m*-OH-Ph
18h: R_1=R_2=H, R=*p*-MeO-*m*-OCH$_2$Ph-Ph
18i: R_1=R_2=H, R=*m*-MeO-*p*-OCH$_2$Ph-Ph
18j: R_1=R_2=H, R=3-pridyl
18k: R_1=R_2=H, R=3-indolyl
18l: R_1=Cl, R_2=H, R=*p*-MeO-*m*-OCH$_2$Ph-Ph
18m: R_1=H, R_2=Cl, R=*p*-MeO-*m*-OCH$_2$Ph-Ph

17

17a: R_1=R_2=H, R=Ph
17b: R_1=R_2=H, R=*p*-NO$_2$- Ph
17c: R_1=R_2=H, R=*p*-Cl- Ph
17d: R_1=R_2=H, R=*p*-Br- Ph
17e: R_1=R_2=H, R=*p*-N(Me)$_2$- Ph
17f: R_1=R_2=H, R=*m*-Br
17g: R_1=R_2=H, R=*m*-OCH$_2$Ph- Ph
17h: R_1=R_2=H, R=*p*,*m*-OH- Ph
17i: R_1=R_2=H, R=*p*-MeO-*m*-OCH$_2$Ph- Ph
17j: R_1=R_2=H, R=*m*-MeO-*p*-OCH$_2$Ph- Ph
17k: R_1=R_2=H, R=*m*-Me,2,4,6-triflouro- Ph
17l: R_1=R_2=H, R=1-naphthyl
17m: R_1=R_2=H, R=3-pridyl
17n: R_1=R_2=H, R=3-indolyl
17o: R_1=Cl, R_2=H, R=*p*-MeO-*m*-OCH$_2$Ph- Ph
17p: R_1=H, R_2=Cl, R=*p*-MeO-*m*-OCH$_2$Ph- Ph

19
18 % yield

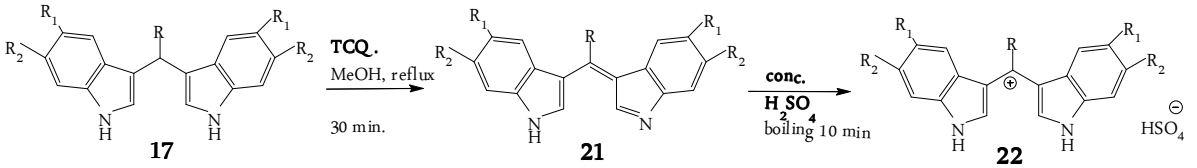
20

Scheme (26): Synthesis of tetrahydroindolo[2,3-*b*]carbazoles.

In this context and as a continuation of our work concerning the synthesis of tetrahydroindolo[2,3-*b*]carbazoles with an attempt to prepare the mixed indolocarbazole (two different aldehydes). The reaction of BIMs (**17$_i$**) (1 mole equivalent) and *p*-dimethylaminobenzaldehyde (1 mole equivalent) has been done by the method of using methanol sulphuric acid solution as a possible route for the synthesis of 4-(8-(3-(benzyloxy)-4-methoxyphenyl)1,1a,2,2a,3,7b,8,8a-octahydroindolo-[2,3-*b*]carbazol-2-yl)-*N*,*N*-dimethylaniline (**19**), scheme (26). The extended spirocyclic structure (**20**) was

synthesized in a better yield of 52 %, by the way of MeOH and conc.H_2SO_4 using BIM ($\mathbf{17_a}$) (2 moles equivalent) and 1,4-cyclohexanedione (1 mole equivalent) scheme (26).

BIMs were found to be sensitive compounds towards oxidizing agents. Our prepared BIMs, $\mathbf{17_{a-p}}$ was oxidized with (1.5) mole equivalent of TCQ or DDQ yielding bisindolylmethenes of type $\mathbf{21_{a-k}}$ as a free base. The monosulfate salts $\mathbf{22_{a,b}}$ could easily be synthesized by the addition of the anion in the form of an acid or in its tetrabutylammonium salt in methanol or acetonitrile solution and boiling the mixture for few minutes, scheme (27).



17

21

22

$\mathbf{17_a}$: R_1=R_2=H, R=Ph
$\mathbf{17_b}$: R_1=R_2=H, R=*p*-NO_2- Ph
$\mathbf{17_c}$: R_1=R_2=H, R=*p*-Cl- Ph
$\mathbf{17_d}$: R_1=R_2=H, R=*p*-Br- Ph
$\mathbf{17_e}$: R_1=R_2=H, R=*p*-$N(Me)_2$- Ph
$\mathbf{17_f}$: R_1=R_2=H, R=*m*-Br
$\mathbf{17_g}$: R_1=R_2=H, R=*m*-OCH_2Ph- Ph
$\mathbf{17_h}$: R_1=R_2=H, R=*p,m*-OH- Ph
$\mathbf{17_i}$: R_1=R_2=H, R=*p*-MeO-*m*-OCH_2Ph- Ph
$\mathbf{17_j}$: R_1=R_2=H, R=*m*-MeO-*p*-OCH_2Ph- Ph
$\mathbf{17_k}$: R_1=R_2=H, R=*m*-Me,2,4,6-triflouro- Ph
$\mathbf{17_l}$ R_1=R_2=H, R=1-naphthyl
$\mathbf{17_m}$: R_1=R_2=H, R=3-pridyl
$\mathbf{17_n}$: R_1=R_2=H, R=3-indolyl
$\mathbf{17_o}$: R_1=Cl, R_2=H, R=*p*-MeO-*m*-OCH_2Ph- Ph
$\mathbf{17_p}$: R_1=H, R_2=Cl, R=*p*-MeO-*m*-OCH_2Ph- Ph

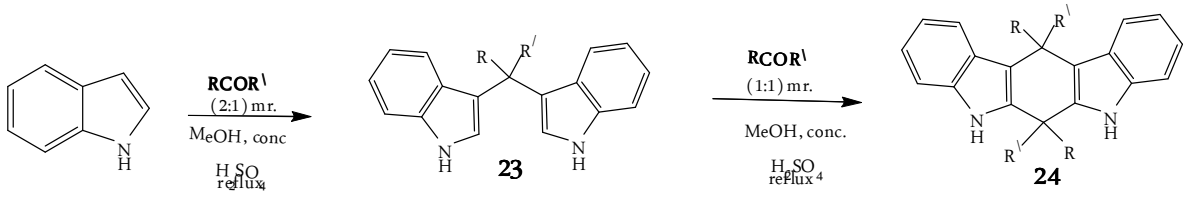
$\mathbf{21_a}$: R_1=R_2=H, R=Ph
$\mathbf{21_b}$: R_1=R_2=H, R=3-indolyl
$\mathbf{21_c}$: R_1=R_2=H, R=*p*-Cl- Ph
$\mathbf{21_d}$: R_1=R_2=H, R=*m*-Br- Ph
$\mathbf{21_e}$: R_1=R_2=H, R=*p,m*-OH- Ph
$\mathbf{21_f}$: R_1=R_2=H, R=2,4,6-trifluro-3-methylphenyl
$\mathbf{21_g}$: R_1=R_2=H, R=*p*-MeO-*m*-OCH_2Ph- Ph
$\mathbf{21_h}$: R_1=Cl, R_2=H, R=*p*-MeO-*m*-OCH_2Ph- Ph
$\mathbf{21_i}$: R_1=H, R_2=Cl, R=*p*-MeO-*m*-OCH_2Ph- Ph
$\mathbf{21_j}$: R_1=R_2=H, R=1-naphthyl
$\mathbf{21_k}$: R_1=R_2=H, R=3-pridyl
$\mathbf{21_l}$: R_1=R_2=H, R=*p*-$N(Me)_2$-Ph

$\mathbf{22_a}$: R_1=R_2=H, R=Ph (Turbomycin B)
$\mathbf{22_b}$: R_1=R_2=H, R=3-indolyl (Turbomycin A)

Scheme (27): Synthesis of bisindolylmethenes and its salt formation

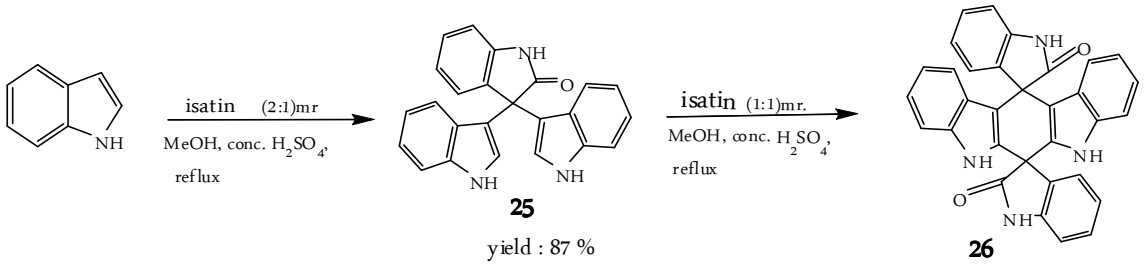
This condensation of acetyl ketones with indole was carried out in a molar ratio of 1:2 to afford the corresponding BIMs. These BIMs isolated from the reaction mixture as in the case of compounds $\mathbf{23_{a,b}}$ or directly used without an isolation from the reaction mixture to condense with the other equivalent mole of ketones for the formation of tetrahydroindolo[2,3-*b*]carbazole ($\mathbf{24_{a,b}}$), scheme (29). Isatin as an example of a 1,2-diketone was condensed with indole for the preparation of the indole trimer by

following the similar reaction conditions of the methanol-sulphuric acid solution. The indole trimer **25** is a known natural product which has been isolated from the fresh marine sponge *Hyrtios altum*, and was named as trisindole.



Scheme (29b): Condensation of indole with acetylketones.

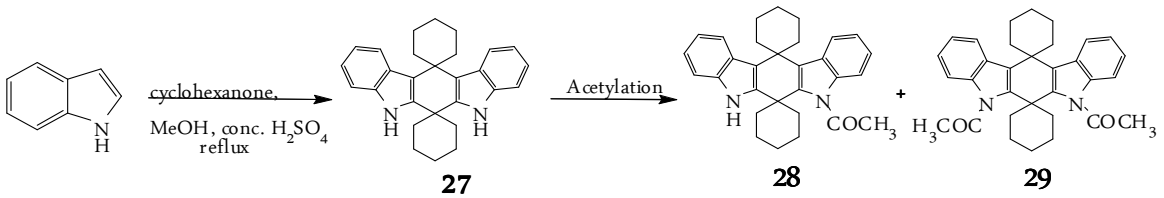
An attempt to prepare trisindole **25** in glacial acetic acid at room temperature failed. The use of methanol sulphuric acid solution in a molar ratio of two moles of indole and of one mole of isatin under reflux for two hour yielded trisindole **25** in an 87 % yield. This method is considered to be simple and efficient if compared to the reported chemical procedures for the preparation of **25**. The trisindoline (**25**) was further used as a precursor for a condensation with an equimolecular amount of isatin as a possible way for the synthesis of the expected novel spirocyclic structure **26,** scheme (30).



Scheme (30) : Condensation reaction of indole with isatin.

Cyclohexanone was condensed with indole using different types of catalysts as well as aldehydes. Using the method of MeOH/conc. H_2SO_4 in the reaction of indole with cyclohexanone in a molar ratio of 2:1 the known (3,3′-(cyclohexane-1,1-diyl)bis(1-*H*-indole)) was isolated. It was detected by TLC and ESI-MS of the reaction mixture and not isolated from the reaction mixture but directly used into the second condensation step with the second mole of cyclohexanone under the same conditions of MeOH/conc.

H_2SO_4 leading to our second novel spirocyclic structure **27** in a 97 % yield. Compound **27** was determined to be the 2,8,2′,8′-bis(cyclohexane-1,1-diyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (**27**), scheme (31).

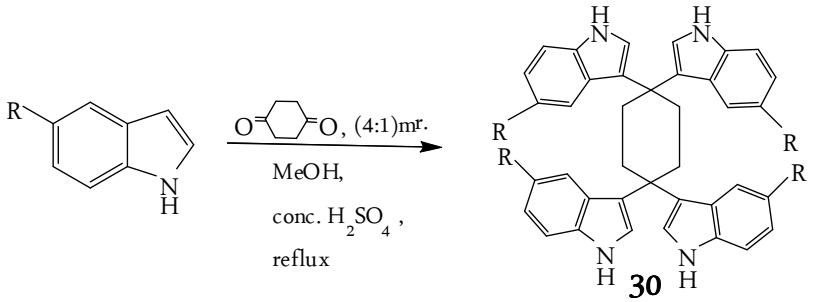


27 **28** **29**

Scheme (31): Reaction of indole with cyclohexanone.

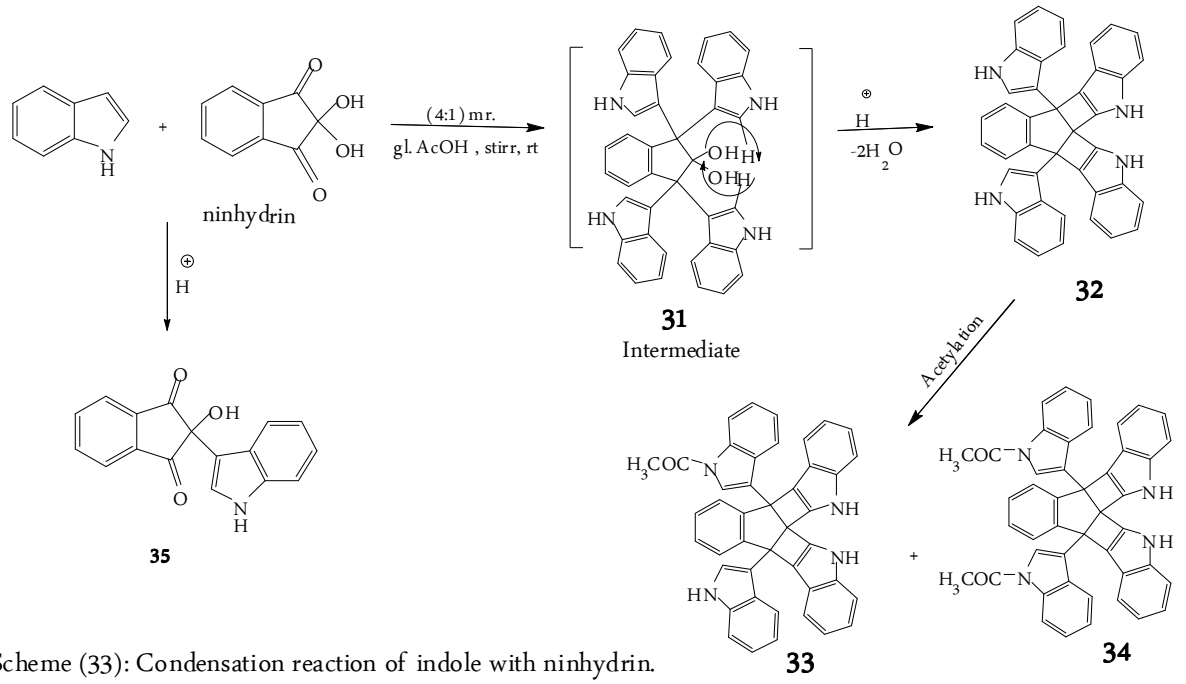
In order to verify the structure of compound **27** it was acetylated using acetic anhydride and triethylamine in presence of 4-(dimethylamino)pyridine (DMAP) as catalyst. The reaction afforded two products one was determined as monoacetylated product (**28**) in a 59 % yield. The second reaction product was identified as the diacetylated product (**29**) in a lower yield of 32 % than that of the monoacetylated product **28**. Thus the acetylation reaction took place stepwisly and the formation of the diacetylated products needed more time, scheme (31).

The electrophilic substitution reactions of indoles with cyclohexane-1,4-dione has been reported in the literature as a possible way for the synthesis of the extended supramolecular compounds **30$_{a,b}$** named as 1,1,4,4-tetrakis(1*H*-indol-3-yl)cyclohexane (**30$_a$**). The reaction took place in the presence of catalyst such as iodine and *N*-bromosuccinimide (NBS) affording the tetrasubstituted product in good yield. In the course of this study cyclohexan-1,4-dione was condensed with indole in MeOH/conc. H_2SO_4 solution in a molar ratio of 1:4 yielding compounds **30$_{a,b}$** in a 82 and 87 % yield, respectively after refluxing for 2 hours, scheme (32).



Scheme (32): Condensation of indoles with cyclohexane-1,4-dione

Condensation reaction of indole with ninhydrin was made with the intention to prepare the expected tetraindole **31**, however the spectroscopic data confirmed the structure of compound **32.** Compound **32** was submitted for the acetylation reaction in which afforded two products after long time of stirring at room temperature. The products were determined to be the monoactylated form **33** and the diacetylated form **34**, scheme (33).



Scheme (33): Condensation reaction of indole with ninhydrin.

Pharmacological Studies

Results of antimicrobial assays

ATCC strains of the microorganisms used in this study were obtained from the culture collection of the Refik Saydam Health Institution of Health Ministry, Ankara, and maintained at the Microbiology Department of the Faculty of Pharmacy of the Ankara University. All the compounds were tested for their in vitro growth inhibitory activity against Candida albicans ATCC 10145 as fungus, S. aureus ATCC 25923, Bacillus subtilis ATCC 6633, MRSA standard ATCC 43300 and MRSA isolate as Gram-positive bacteria and E. coli ATCC 23556 as Gram-negative bacteria. The selected compounds for antimicrobial tests are shown in the figure (30). We have shown for the first time that the cycloalkanoindoles (**2**$_{a,b,c,d,e,h,i,g,j}$), bis-indolobenzocarbazoles (**7**$_a$) and the oxidized bis-indolylmethenes (**21**$_a$, **21**$_c$, **21**$_m$) inhibited growth of drug resistant MRSA either standard or isolated and other Gram-positive bacteria at low concentrations. These novel bis- and trisindolyl inhibitors described here can be synthesized easily and cost-effectively and structural modifications to improve the inhibitory activity in vitro can be achieved in a time efficient manner. The results are expected to be of significance in terms of discovering new molecules that can be developed into drugs to combat MRSA and gram-positive pathogens.

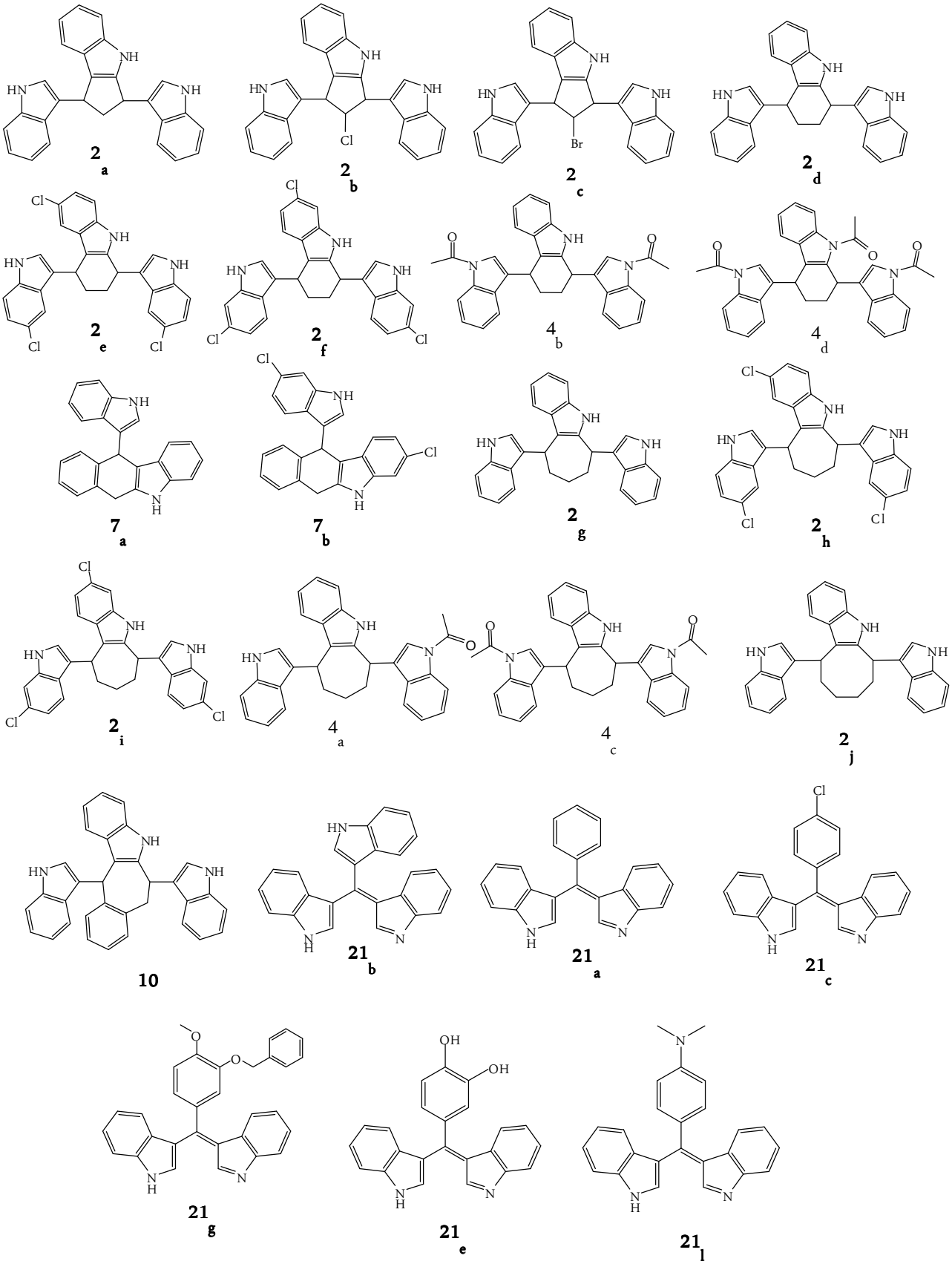


Figure (30): Selected compounds for antimicrobial tests.

Results of the in vitro cancer screen

Activity of BIMs as antitumor agents

The selected substances for the One dose screening ($\mathbf{17_{e,g,i,j,l}}$) are shown in figure (31) and $\mathbf{17_j}$ was further selected for the five dose mean graph determination.

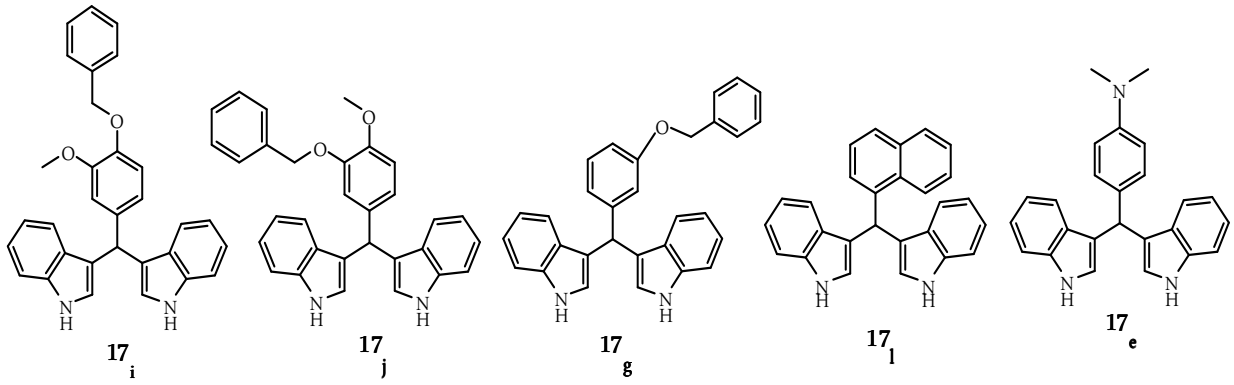


Figure (31): Selected BIMs (**17e,g,i,j,l**) for NCI screenings.

Structure Activity Relationship (SAR) of BIMs

The comparison of $\mathbf{17_i}$ and $\mathbf{17_j}$ showed that the position of the functional groups is of great importance of being either *meta* or *para*, where the *para*-methoxy is much more favourable than *meta*. Moreover and by comparison of $\mathbf{17_j}$ with $\mathbf{17_g}$ the methoxy function in addition to the benzyloxy group ensured mainly increased activity. If the methoxy function is positioned in *meta* position the effect is similar concerning no favour of a *meta* methoxy function as indicated in comparison of $\mathbf{17_i}$ and $\mathbf{17_g}$. The lipophilic fixed substituent (naphthyl derivative, compound $\mathbf{17_l}$) is not favourable compared to the routable benzyloxy substituent compound $\mathbf{17_g}$. BIM ($\mathbf{17_e}$) containing a basic substituent (NMe_2) was unfavourable concerning the all over anticancer activities. Whereas, its activities in a renal cancer cell lines indicated different anticancer activities comparable to compound $\mathbf{17_g}$.

On conclusion, all BIMs ($\mathbf{17_{e,g,i,j,l}}$) show good activities in the same cell lines (MOLT-4 as leukaemia cancer cell line and IGROV1 as ovarian cancer cell line. Also the

basically substituted derivatives demonstrate good activity for the cell line CAKI-1 and UO-31 as a renal cancer cell lines.

Results of 60 Cell Line Screening for Aryl substituted tetrahydroindolo[2,3-*b*]carbazoles ($18_{d,f,h,i,l}$)

We further developed the series of the substituted bis(indolyl)substituted phenylmethanes to yield new structures known as aryl substituted tetrahydroindolo[2,3-*b*]carbazoles with a constrained flexibility of the molecule. The NCI selected five derivatives of these substituted indolocarbazoles for the One dose screening program at concentration 10 μM. These selected substances (**$18_{d,f,h,i,l}$**) are illustrated in figure (32) and according to the data from the one dose screening compound **18_d** showed the lowest mean value (21.63). Thus compound **18_d** was further selected for the five dose screening program which showed the highest activity.

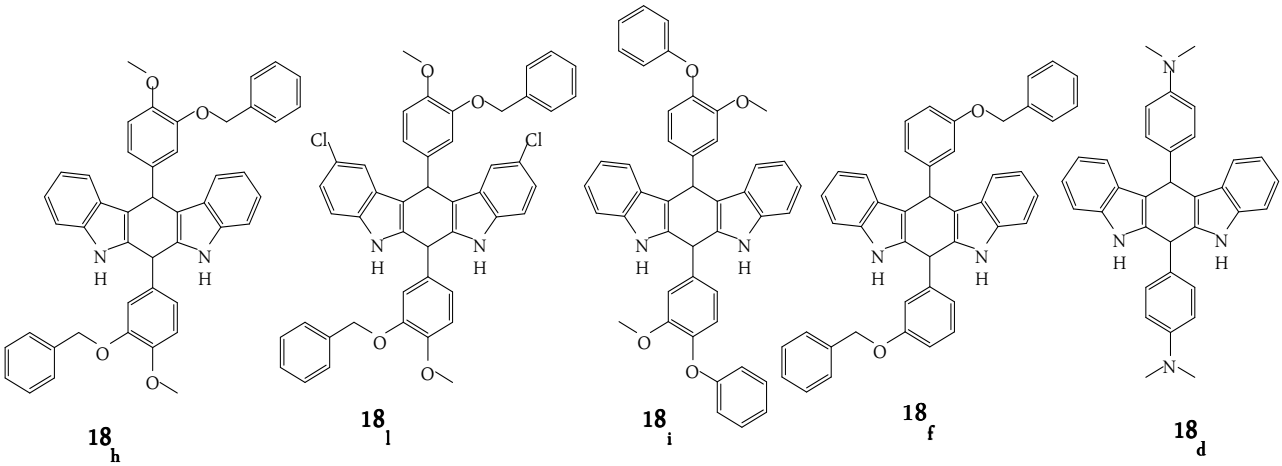


Figure (32): Selected indolocarbazoles (**$18_{d,f,h,i,l}$**) for NCI screening.

Structure Activity Relationship (SAR) of indolocarbazoles

The basically substituted derivative showed the highest activity. Chloro-substitution on the indole phenyl ring was unfavourable (main loss of activity in all these cancer cell lines. Comparing compound **18_h** with **18_i** showed that *para*-benzyloxy increased the activity in some novel sensitive cancer cell line (NCI-H522 as non small lung cancer cell line and CAKI-1 and UO-31 as renal cancer cell.

By comparison of compound **18$_h$** and **18$_f$** a *para*-methoxy function ensures the activity especially in selected cell lines. The *para*-benzyloxy compound **18$_i$** was more active than the *meta*-benzyloxy compound **18$_f$**. The basically substituted derivative showed the highest antipoliferative activity (nanomolar active) in a selected cell lines with noncritical cytotoxic property (ten-to hundredful highes LC_{50} than GI_{50} values).

4.2. Future work

Our novel electrophilic substitution reaction of indole or its derivatives with aliphatic or aromatic dialdehydes will open new prospects of using different substituents of the indole ring and dialdehydes. Especially for the bis- and tris-indole products that showed in in vitro antimicrobial assays strong anti-MRSA activity (compounds **2$_{b,c,d}$**, **7$_a$**, and **21$_{a,c,e}$**), it would be important to modify the structure of these compounds. Furthermore we currently make further antibacterial tests to identify the target structure. It is of interest also to involve the substitution possibilities of such derivatized indole compounds that can be synthesized by solid phase support methods. Figure (A)[286] point to the recent published solid phase pathways that can be used to synthesize different indole ring systems.

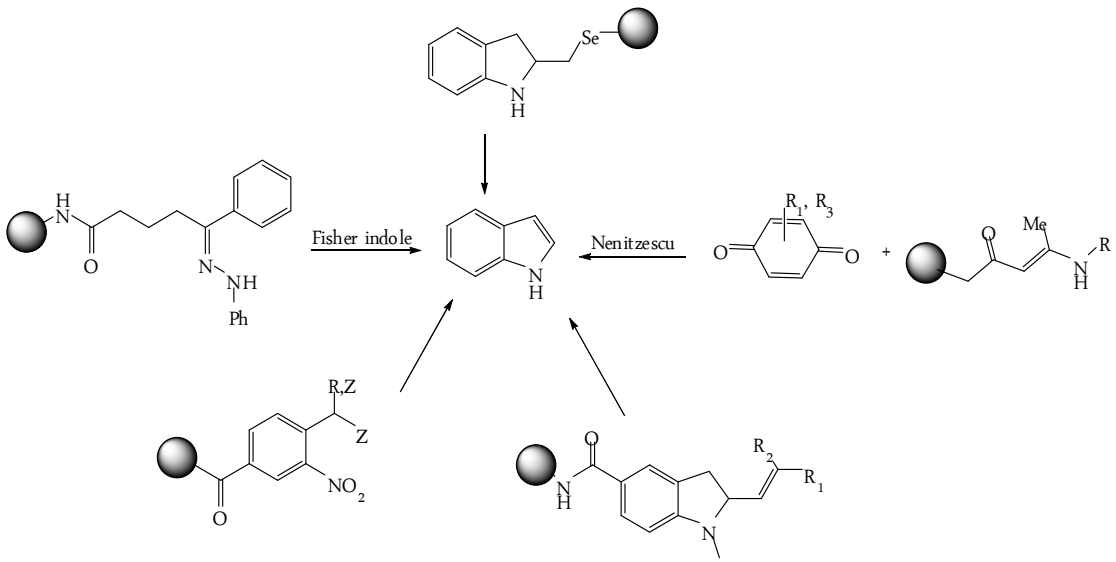


Figure (A): Solid phase pathways towards the indole core structure.

For the synthetic evolution of our novel substances and the modification of the bis-, tri- and tetra-indoles it would be logically and interestingly to introduce various derivatives of substituted aliphatic and aromatic dialdehydes. For example the vast number of the substituted aliphatic dialdehydes (A, B, C, D, E, F and G), figure (A), that can be prepared according to the literature the references[287] may be used.

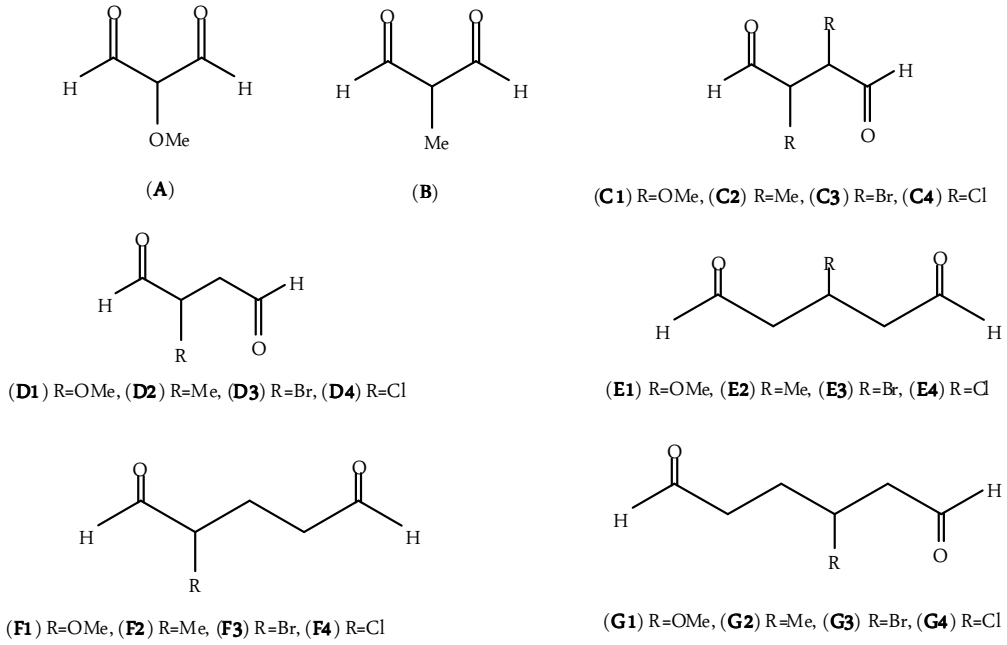
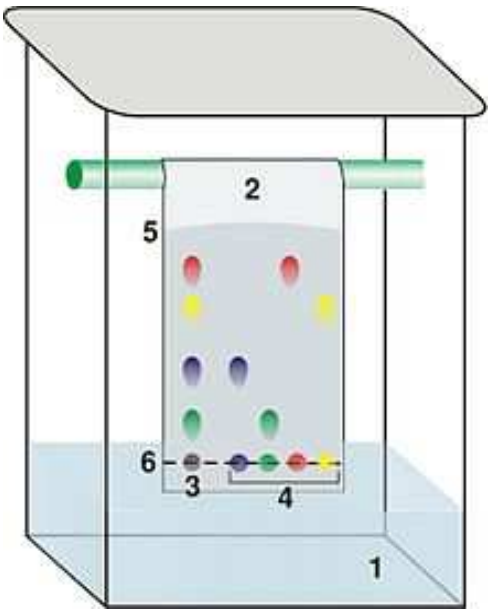
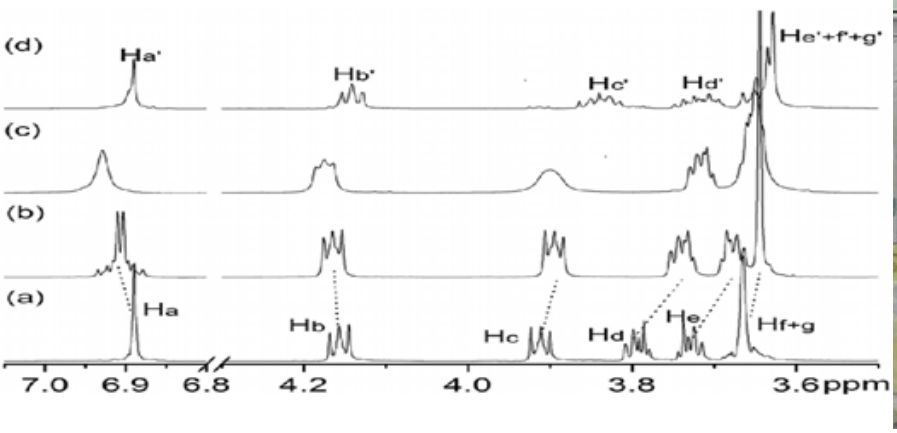


Figure (B): Varied starting substituted aliphatic dialdehydes.

Experimental





5. Experimental Part

5.1. Synthesis of the compounds

5.1.1. General Information

All moisture and / or air-sensitive reactions were carried out under argon atmosphere in dried apparatus under vacuum.

Solvent:

The solvents were distilled as needed and according to literature Method dried.

TLC:

For the analyzes were TLC with aluminium foil fluorescent indicator from Merck KGaA (silica gel 60 F254, layer thickness 0.2 mm) used. Detection was with UV light at 254 nm and 366 nm ratios are provided. R_f -values (run level relative to the solvent front) were used as the eluent in the test requirements specified mixtures used.

Column chromatography:

The separations were with column chromatography at atmospheric pressure on silica gel 60 (Grain size from 0.063 to 0.200 mm) from Merck KGaA. The eluent was the used in the test requirements specified mixtures.

5.1.2. Instruments used

NMR spectra:

The NMR spectra were recorded on a "Gemini 2000" (400/100 MHz) or on an "INOVA 500" (500 MHz) of the firm "Varian measured" served as the internal standard residual resonance signal of the respective deuterated solvent. The interpretation of the NMR Spectra was carried out using the spectral simulation tools, the programs "ACD/Labs7.00" (Advanced Chemistry Development Inc.) and "ChemDraw Ultra 9.0" (CambridgeSoft). The Assignment of the signals has been done by the inclusion of

appropriate 2-D NMR Spectra (^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, ^{1}H-^{1}H ROESY, gHSQCAD, gHMBCAD and zTOCSY) at 600 MHz.

^{1}H-NMR:

It is the transmitter frequency and the used deuterated solvents indicated. The Chemical shifts are δ in parts per million (ppm). Follows in parentheses then the multiplicity of the signal. Where: s = singlet, d = doublet, t = triplet, q = Quartet, qu = quintet, sep = septet, m = multiplet and b = broad signal. Combinations of multiplicities, eg dd = doublet of doublets, are optionally listed. Furthermore, the integrated proton number, the coupling constant J and The chemical interpretation of the signal indicated.

^{13}C-NMR:

The ^{13}C-NMR spectra were recorded broad-band decoupled. It is the Transmitter frequency, the used deuterated solvents and the chemical Shifts δ in parts per million (ppm). In parentheses following the chemical interpretation of the signals. The abbreviations are: p = primary carbon atoms s = secondary carbon atom, t = tertiary C-atom and q = quaternary C atom.

IR spectra:

The ATR spectra were recorded on a FT-IR spectrometer "IFS 28" by "Bruker", the KBr spectra on a FT-IR Spectrometer "Spectrum BX" "the Company "Perkin-Elmer" measured. For each signal is the wave number ν in cm^{-1}, the intensity and the chemical Interpretation given. The abbreviations are: s = strong (strong), m = medium (medium) and w = weak (weak), br = broad is a broad peak.

Mass spectra:

The ESI mass spectra were recorded on a "Finnigan LCQ Classic" by "thermal Electron measured" the sample was injected directly. The EI mass spectra were recorded on an "Intel 402" the Company "AMD Intectra GmbH" measured. The ionization was 70 eV. The interpretation of EI mass spectra was carried out using the Spectra

simulation tools "ACD/MS Fragmenter" (Advanced Chemistry Development Inc.).

Melting points:

The melting points were measured on a Boetius-Mikroheiztisch the company "VEB weighing. Rapido Radebeul / VEB NAGEMA "measured and are uncorrected.

Elemental analysis:

The carbon, hydrogen and nitrogen content of the substances were performed on a "CHNS-932" automatic analyzer of the company "LECO Corporation" in the automatic Microchemical. The halogen content was determined by titration in semimicro method.

5.1.3. Reagents:

For the synthesis of the compounds prepared in this work we were used the following solvents and reagents:

Acetone (Roth)
Acetyl chloride (Lancaster)
Ammonia, conc. (Roth)
Acetic anhydride (Roth)
3-Acetylindole (Sigma-Aldrich)
3-Acetylpyridine (Sigma-Aldrich)
2-Bromomalonaldehyde (Across Organics)
N-Bromosuccinimide (Merck)
p-Bromobenzaldehyde (Merck)
m-Bromobenzaldehyde (Merck)
m-Benzyloxybenzaldehyde (Sigma-Aldrich)
Benzaldehyde (Merck)
p-Chlorobenzaldehyde (Merck)
Chloroacetyl chloride (Merck)
Chloroform (Roth)
Cyclohexane (Roth)
5-Chloroindole (Across Organics)
6-Chloroindole (Across Organics)
2-Chloromalonaldehyde (Across Organics)

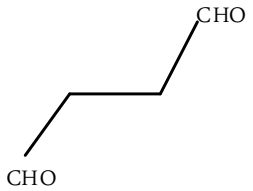
Cyclohexan epoxide (Across Organics)
2,4-Cyclohexanedion (Across Organics)
Cyclohexanone (Merck)
Diphenyl ether (Across Organics)
Dimethyl sulfoxide (Roth)
N, *N*-dimethylformamide (laboratory chemistry Apolda)
Diethyl ether (Kraemer and Martin)
Dichloromethane (Roth)
4-(Dimethylamino)pyridine (Sigma-Aldrich)
2,5-Dimethoxy tetrahydrofuran (Across Organics)
Dichlorodicyanoquinone (Across Organics)
p-Dimethylaminobenzaldehyde (Merck)
p,*m*-Dihydroxybenzaldehyde (Merck)
Ethanol (Roth)
Ethyl acetate (Roth)
Glacial acetic acid (Roth)
Glutaraldehyde (Across Organics)
Hydrochloric acid (Roth)
3-Indolcarboxyaldehyde (Merck)
Isatin (Merck)
Indole (Merck)
Indene (Across Organics)
Methanol (Sigma-Aldrich)
Malonaldehydebisdimethylacetal (Across Organics)
p-Methoxy-m-benzyloxybenzaldehyde (Sigma-Aldrich)
m-Methoxy-p-benzyloxybenzaldehyde (Sigma-Aldrich)
m-Methyl-2,4,6-triflourobenzaldehyde (Sigma-Aldrich)
p-Nitrobenzaldehyde (Merck)
1-Naphthaldehyde (Across Organics)
Ninhydrin (Merck)
Potassium hydroxide (Roth)
Phosphorus oxychloride (Sigma-Aldrich)
O-phthalaldehyde (Across Organics)

3-Pridincarboxyaldehyde (Sigma-Aldrich)
Sodiummetaperiodate (Fluka)
Tetrahydrofuran (Sigma-Aldrich)
Triethylamine (Fluka)
Tetrachloroquinone (Sigma-Aldrich)
Terphthaladehyde (Across Organics)

5.1.4. Synthesis and analytical data

5.1.4.1. Procedure for the preparation of succinaldeyde (1_d):

In a flask containing a magnetic stirrer 10 mmol, 13.22 ml of 2,5-dimethoxytetrahydrofuran was mixed with 20 ml of 70 % HCl/H_2O. The mixture was allowed to stirr under reflux for about 2 h at 100 ^{0}C. After that the mixture was extracted with ether for three times (200 ml), washed with water and brine and dried over anhydrous sodium sulphate. The ether solution was distilled at 62 ^{0}C to give succinaldeyde (**1_d**) as colorless light oil, bp. 62 ^{0}C.



Chemical Formula: $C_4H_6O_2$

Molecular Weight: 86.09 g/mol

Boiling point: 62 ^{0}C

ESI-MS: (m/z) = 108.94 [M^++Na]

IR-Spectrum: (ATR, cm^{-1}): 1711 (CHO), 2956 (CH_2)

^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 2.62 - 2.65 (m, 4H, $2CH_2$), 9.58 (t, 2H, J=2.7 Hz, 2CHO)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 35.47 (CH_2), 200.82 (CHO).

5.1.4.2. Procedure for the preparation of adipaldehyde (1_f):

Tinely powdered sodium periodate (20 mmol, 4.3 mg) was stirred in 40 ml THF/H_2O [2:1] for five minutes. The epoxide 7-oxabicyclo[4.1.0]heptane (10 mmol, 1 ml) was added and the reaction mixture was stirred at room temperature. Upon the reaction completion, as monitored by TLC (30 % ethylacetate/hexane), the white precipitate that was formed was filtered away and the water layer was washed with 30 ml Et_2O creating two distinct layers. The aqueous layer was extracted with two 30 ml portions of Et_2O, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuum. The crude reaction mixtures was purified *via* column chromatography on silica gel

eluted with 45 % (EtOAc/hexane) affording a slightly viscous light yellow oil in a yield of 65 %.

Chemical Formula: $C_6H_{10}O_2$

Molecular Weight: 114.14 g/mol

ESI-MS: (m/z) = 137.05 [M^++Na]

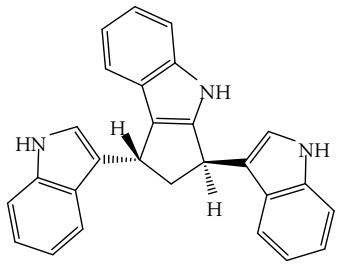
^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 1.48 - 1.54 (m, 4H, 2CH_2), 2.39 - 2.45 (m, 4H, 2CH_2), 9.65 (s, 2H, 2CHO)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 21.01 (CH_2), 42.70 (CH_2), 203.16 (CHO)

5.1.4.3. General procedure for the preparation of compounds 2 and 3:

To 15 ml glacial acetic acid 2 mmol of aliphatic dialdehyde was added under stirring at room temperature. Then 5mmol of indole or its derivatives (5-chloroindole or 6-chloroindole) was added to the reaction mixture. The clear light yellow solution was left stirring overnight until the solution became dark brown. The product was detected by TLC (100 % CH_2Cl_2). The TLC showed the formation of the two products, compound 2 with a high R_f value and 3 with a law R_f value, where the indole or its derivatives have not been finished from the reaction. At this point the reaction was worked up by neutralization with a cold solution of 10 % NaOH affording a brown precipitate. Then the mixture was extracted with CH_2Cl_2 for three times, washed with water for two times and brine for two times then dried over anhydrous sodium sulphate, and concentrated in vacuum. The crude reaction mixture was purified *via* column chromatography on silica gel eluted with CH_2Cl_2 to remove the unreacted indoles and then compound $\mathbf{2_{a-j}}$ was collected. Finally compound $\mathbf{3_{a-c}}$ and $\mathbf{3_{g-j}}$ were collected.

Trans-diastereomer:1,3-Di(1*H*-indol-3-yl)-1,2,3,4-trahydrocyclopenta[*b*]indole (2_a):



Molecular Formula: $C_{27}H_{21}N_3$

Molecular Weight: 387.48 g/mol

Melting point: 110 - 115 ^{0}C

Colour and shape: light brown powder

EI-MS:(m/z) = 387 [M^+] 100 %, 386 [M^+-H] 25 %, 373 [M^+-CH_2] 10 %, 269 [M^+-indolyl] 30 %, 257 [M^+-CH_2-indolyl] 77 %, 130 [CH_2.indolyl] 22 %, 117 [indolyl] 10 %, 7 [Ph] 8 %

IR/Spectrum: (ATR, cm^{-1}) = 2923 (CH_2), 3404 (NH) .

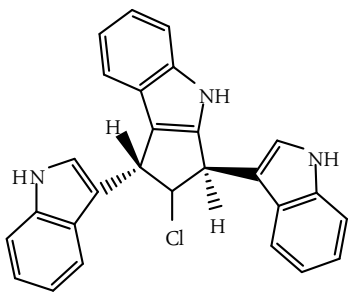
^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 1.89 (d, J=7.9 Hz, 2H, CH_2), 5.01 - 5.19 (m, 2H, 2CH), 6.45 - 6.47 (m, 1H, ArH), 6.82 - 6.83 (m, 1H, ArH), 6.90 - 6.92 (m, 2H, ArH), 7.01 - 7.03 (m, 2H, ArH), 7.06 - 7.09 (m, 1H, ArH), 7.26 - 7.28 (m, 4H, ArH), 2.37 - 7.39 (m, 2H, ArH), 7.56 - 7.58 (m, 1H, ArH), 9.97 (s, 1H, NH), 9.99 (s, 1H, NH), 10.19 (s, 1H, NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 30.57 (CH), 41.13 (CH), 70.27 (CH_2), 102.26, 111.97, 112.05, 114.62, 116.45, 118.59, 119.29, 119.63, 119.83, 120.24, 120.99, 121.43, 121.51, 122.02, 124.50, 127.37, 128.39, 128.06, 129.06, 129.14, 138.02, 138.46, 147.62, 159.70

R_f - value: 0.51 (CH_2Cl_2).

Yield: (200 mg), 20 %

Trans-diastereomer: 2-Chloro-1,3-di(1*H*-indol-3-yl)-1,2,3,4-tetrahydrocyclopenta[*b*]indol (2_b):



Chemical Formula: $C_{27}H_{20}N_3Cl$

Molecular Weight: 421.92 g/mol

Melting point: 168 - 171 ^{0}C

Colour and shape: light brown powder

EI-MS: (m/z) = 421 [M^+] 75 %, 385 [M^+-Cl] 3 %, 269 [M^+-indolyl-Cl] 4 %, 130 [CH_2-indolyl] 9 %, 117 [indolyl] 100 %

ESI-MS: (m/z) = 423.20 [M^++H]

IR-Spectrum : (ATR, cm^{-1}) = 1455 (CHCl), 3403 (NH)

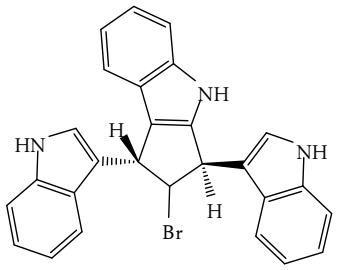
^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 3.30 (s,br., 2H, 2CH), 4.85 - 5.01 (m, 1H, CHCl), 6.92 - 6.97 (m, 4H, ArH), 7.07 - 7.11 (m, 4H, ArH), 7.19 (d, J=7.7 Hz, 2H, ArH), 7.35 - 7.43 (m, 2H, ArH), 7.63 (d, J=8.2 Hz, 2H, ArH), 9.88 (s, 1H, NH), 10.10 (s, 1H, NH), 10.46 (s, 1H, NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 28.97 (CH), 29.16 (CH), 44.81 (CHCl), 110.71, 111.17, 111.36, 111.39, 116.19, 116.92, 117.84, 118.19, 118.64, 119.29, 119.34, 119.54, 120.91, 121.25, 121.84, 123.84, 123.99, 124.68, 127.22, 127.64, 132.49, 135.67, 137.09, 137.21, 145.30

R_f-value: 0.57 (CH_2Cl_2)

Yield: (236 mg), 28 %

Trans-diastereomer: 2-Bromo-1,3-di(1*H*-indol-3-yl)-1,2,3,4-tetrahydrocyclopenta[*b*]indole (2_c):



Chemical Formula: $C_{27}H_{20}BrN_3$

Molecular Weight: 466.37 g/mol

Melting point : 130 - 132^{0}C

Colour and shape: light brown powder

EI-MS: (m/z) = 466 [M^+] 20 %, 386 [M^+-Br] 15 %, 269 [M^+-Br-indolyl] 7%, 245 [indolyl-CH_2-indolyl] 35 %, 130 [CH_2-indolyl] 10 %, 117 [indolyl] 100 %

IR-Spectrum: (ATR, cm^{-1}) = 1454 (CHBr), 3401 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 3.58 (d, 2H, J=7.63 Hz, 2CH), 4.85 (t, 1H, J=7.8 Hz, CHBr), 6.96 - 6.98 (m, 2H), 7.03 (d, J=7.2 Hz, 1H), 7.20 (t, J=8.4 Hz, 3H),

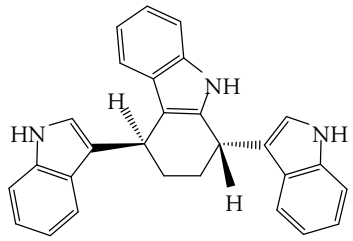
7.28 (d, J=7.5 Hz, 2H), 7.35 - 7.42 (m, 2H), 7.44 (d, J=8 Hz, 3H), 7.56 (d, J=7.7 Hz, 1H), 10.67 (s, 1H, NH), 10.91 (s, 1H, NH), 11.35 (s, 1H, NH)

^{13}C-NMR: (100 MHz, DMSO-*d6*) δ (ppm) = 38.98 (CH), 39.14 (CH), 40.01 (CHBr), 109.66, 111.48, 111.60, 111.55, 115.09, 116.46, 117.69, 118.23, 118.40, 119.00, 119.22, 120.89, 121.67, 123.86, 126.62, 127.00, 127.55, 128.32, 131.52, 132.12, 132.12, 135.12, 136.35, 136.52

R_f-value: 0.65 (CH_2Cl_2)

Yield: (215 mg), 23 %

Trans-diastereomer: 1,4-Di(1*H*-indol-3-yl)-2,3,4,9-tetrahydro-1*H*-carbazole (2_d):



Chemical Formula: $C_{28}H_{23}N_3$

Molecular Weight: 401.50 g/mol

Melting point: 139 - 145 ^{0}C

Colour and shape: light brown powder

EI-MS: (m/z) = 401 [M^+] 85 %, 373 [M^+-2CH_2] 38 %, 284 [M^+-indolyl] 100 %, 269 [M^+-indolyl-CH_2] 12 %, 258 [M^+-indolyl-2CH_2] 52 %, 167 [M^+-2indolyl] 19 %, 130 [CH_2-indolyl] 12 %, 117 [indolyl] 55 %, 90 [Ph-CH] 23 %

ESI-MS: (m/z) = 402.28 [M^++H], 400.23 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 2923 (CH_2), 3398 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 2.05-2.23 (m, 4H, 2CH_2), 4.49-4.58 (m, 2H, 2CH), 6.60-6.67 (m, 2H), 6.81-6.94 (m, 4H), 7.05 (t, J=7 Hz, 2H), 7.18 (t, J=6.5 Hz, 1H), 7.28-7.39 (m, 3H), 7.45 (d, J=7.9 Hz, 1H), 7.59 (d, J=7.9 Hz, 1H), 10.42 (s, 1H, NH), 10.67 (s, 1H, NH), 10.88 (d, J=9.13 Hz, 1H, NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 29.75 (CH_2), 30.52 (CH_2), 32.20 (CH), 32.51 (CH), 111.03, 111.62, 111.80, 117.53, 117.92, 118.19, 118.29, 118.51, 118.88, 119.03, 119.39, 119.94, 120.11, 120.92, 121.09, 123.05, 126.42, 126.69, 127.07, 136.34, 136.37, 136.84, 137.33, 137.56

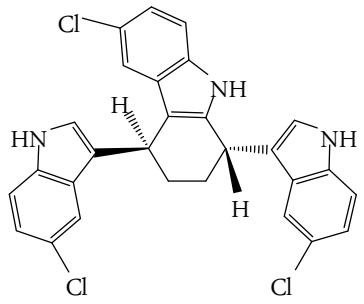
Elemental Analysis: Calcd. C, 83.76, H, 5.77, N, 10.47

. Found C, 83.80, H, 5.73, N, 10.51

R_f-value: 0.65 (CH_2Cl_2)

Yield: (683 mg), 85 %

Trans-diastereomer: 6-Chloro-1,4-bis(5-chloro-1*H*-indol-3-yl)-2,3,4,9-tetrahydro-1*H*-carbazole (2_e):



Chemical Formula: $C_{28}H_{20}C_{l3}N_3$

Molecular Weight: 504.84 g/mol

Melting point: 210 - 214 ^{0}C

Colour and shape: white powder

EI-MS: (m/z) = 504 [M^+] 100 %, 475 [M^+-$2CH_2$] 62 %, 439 [M^+-Cl-$2CH_2$] 22 %, 405 [M^+-2Cl-$2CH_2$] 11 %, 369 [M^+-3Cl-$2CH_2$] 10 %, 352 [M^+-chloroindoly] 55 %, 326 [M^+-$2CH_2$-chloroindolyl] 100 %, 316 [M^+-chloroindolyl-Cl] 30 %, 280 [M^+-chloroindolyl-2Cl] 15 %, 202 [M^+-2chloroindolyl] 24 %, 151 [chloroindolyl] 28 %

IR-Spectrum: (ATR,cm^{-1}) = 1689 (CCl), 3009 (CH_2), 3411 (NH)

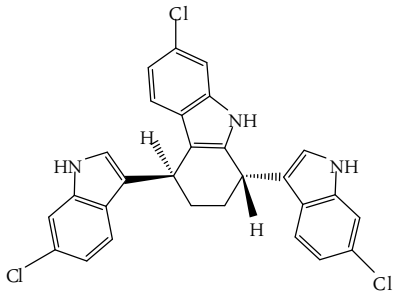
^{1}H-NMR: (400 MHz, DMSO-*d6*) δ (ppm) = 1.99-2.17 (m, 4H, $2CH_2$), 4.50-4.54 (m, 2H, 2CH), 6.84 (s, 1H), 6.92 (dd, J=1.9,8.72 Hz, 2H), 7.07 (d, J=8.72 Hz, 2H), 7.15-7.16 (m, 1H), 7.22 (d, J=8.5 Hz, 1H), 7.36-7.42 (m, 3H), 7.59-7.60 (m, 1H), 10.75 (s, 1H, NH), 11.01 (s, 1H, NH), 11.16 (s, 1H, NH)

^{13}C-NMR: (100 MHz, DMSO-*d6*) δ (ppm) = 29.66 (CH_2), 30.23 (CH_2), 40.39 (CH), 40.56 (CH), 111.52, 112.89, 113.57, 117.29, 117.70, 118.26, 118.39, 119.08, 120.38, 121.23, 121.33, 122.86, 123.30, 123.45, 125.32, 125.57, 127.71, 127.82, 127.95, 128.30, 135.07, 135.47, 135.57, 139.6

R_f-value: 0.72 (CH_2Cl_2)

Yield: (777 mg), 77 %

Trans-diastereomer: 7-Chloro-1,4-bis(6-chloro-1*H*-indol-3-yl)-2,3,4,9-tetrahydro-1*H*-carbazole (2_f):



Chemical Formula: $C_{28}H_{20}Cl_3N_3$

Molecular Weight: 504.84 g/mol

Melting point: 154 - 158 ^{0}C

Colour and shape: light green powder

ESI-MS: (m/z) = 503 [M^+-H]

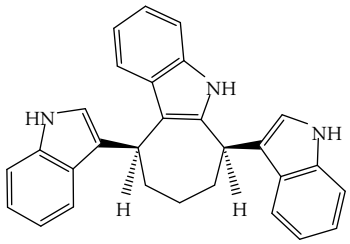
IR-Spectrum : (ATR, cm^{-1}) = 1613 (CCl), 2923 (CH_2), 3418 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 0.91-1.11 (m, 2H, CH_2), 1.38 - 1.41 (m, 2H, CH_2), 2.43 - 2.60 (m, 2H, 2CH), 6.87 - 6.97 (m, 1H), 6.98 - 700 (m, 2H), 7.20 (t, J=7.6 Hz, 1H), 7.31 - 7.34 (m, 2H), 7.43 - 7.47 (m, 3H), 7.51 (d, J=8.6Hz, 1H), 7.96 - 8.01 (m, 1H), 9.70 (s, 1H, NH), 10.19 (s, 1H, NH), 10.62 (s, 1H, NH)

R_f-value: 0.77 (CH_2Cl_2)

Yield: (788 mg), 78 %

Cis-diastereomer: 6,10-Di(1*H*-indol-3-yl)-5,6,7,8,9,10-hexahydrocyclohepta[*b*]indole (2_g)



Chemical Formula: $C_{29}H_{25}N_3$

Molecular Weight: 415.53 g/mol

Melting point: 152 - 155 ^{0}C

Colour and shape: brown powder

ESI-MS: 414.02 [M^+-H]

EI-MS:(m/z) = 415 [M^+] 35 %, 372 [M^+-3CH_2] 5 %, 298 [M^+-indolyl] 100 %, 283 [M^+-indolyl-CH_2] 18 %, 269 [M^+-indolyl-2CH_2] 25 %, 257 [M^+-indolyl-3CH_2] 25 %, 245 [indolyl.CH.indolyl] 15 %, 156 [indolyl.CH.CH_2.CH_2] 40 %, 130 [indolyl.CH_2] 32 %, 117 [indolyl] 75 %, 90 [Ph.CH] 33 %.

IR-Spectrum: (ATR,cm^{-1}) = 2852, 2925 (CH_2), 3416 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 1.62 - 1.74 (m, 2H, CH_2), 2.09 - 2.19 (m, 4H, $2CH_2$), 4.66 (dd, J=3,10 Hz, 1H, CH), 4.91 (t, J=8.5 Hz, 1H, CH), 6.66 (dd, J=1.2, 7.5 Hz, 1H), 6.74 (t, J=6.88 Hz, 1H), 6.85 (t, J=6.88 Hz, 1H), 6.69 - 6.99 (m, 2H), 7.05-7.10 (m, 3H), 7.19 (dd, J=2.3, 11.7 Hz, 1H), 7.33 - 7.43 (m, 4H), 7.62 (d, J=7.7 Hz, 1H), 9.66 (s, 1H, NH), 10.66 (s, 1H, NH), 10.97 (s, 1H, NH)

^{13}C-NMR: (100MHz, DMSO-d_6) δ (ppm) = 25.66 (CH_2), 33.14 (CH_2), 33.71 (CH_2), 36.13 (CH), 36.23 (CH), 110.33, 111.03, 111.45, 114.95, 117.88, 118.17, 118.95, 119.11, 119.93, 120.73, 121.37, 121.70, 122.54, 123.59, 126.61, 126.87, 127.17, 129.37, 133.84, 134.31, 136.55, 137.18, 138.67, 139.65

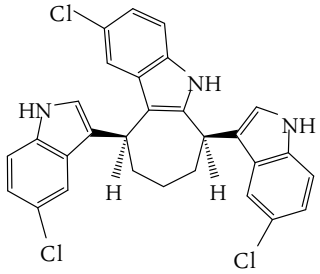
Elemental analysis: Calcd. C, 83.82; H, 6.06; N, 10.11

Found. C, 83.89, H, 6.05, N, 10.24

R_f-value: 0.67 (CH_2Cl_2)

Yield: (208 mg), 25%

Cis-diastereomer: 2-Chloro-6,10-bis(5-chloro-1*H*-indol-3-yl)-5,6,7,8,9,10-hexahydrocyclohepta[*b*]indole (2_h):



Chemical Formula: $C_{29}H_{22}Cl_3N_3$

Molecular Weight: 518.86 g/mol

Melting point: 130 - 133 ^{0}C

Colour and shape: brown powder

ESI-MS: (m/z) = 517.18 [M^+-H]

EI-MS: (m/z) = 518 [M^+] 5 %, 517 [M^+-H] 11 %, 411 [M^+-3Cl] 4 %, 366 [M^+-indolyl-Cl] 85 %, 352 [M^+-chloroindolyl-Cl-CH_2] 9 %, 339 [M^+-chloroindolyl-Cl-$2CH_2$] 12 %, 303 [M^+-chloroindolyl-2Cl-$2CH_2$] 6 %, 290 [M^+-chloroindolyl-2Cl-$3CH_2$] 12 %, 215 [chloroindolyl.CH.CH.CH_2.CH_2.CH_2] 38 %, 190 [chloroindolyl.CH_2.CH_2.CH] 35 %, 164 [chloroindolyl.CH_2] 20 %, 151 [chloroindolyl] 100 %, 116 [indolyl] 25 %, 89 [Ph.CH] 35 %

IR-Spectrum: (ATR, cm^{-1}) = 1460 (CCl), 2921 (CH_2), 3423 (NH)

^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 1.72 - 1.96 (m, 2H, CH_2), 2.11 - 2.21 (m, 2H, CH_2), 2.61 - 2.70 (m, 2H, CH_2), 4.51 - 4.53 (m, 1H, CH), 4.88 (t, J=7.9 Hz, 1H, CH), 6.57 (s, 1H), 6.93 - 6.99 (m, 2H), 7.04 - 7.14 (m, 4H), 7.17 - 7.25 (m, 1H), 7.34 - 7.41 (m, 1H), 7.44 - 7.45 (m, 1H), 7.52 (s, 1H), 7.72 - 7.73 (m, 1H, NH), 7.92 (s, 1H, NH), 8.27 (s, 1H, NH)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 33.07 (CH), 33.51 (CH_2), 34.28 (CH_2), 35.93 (CH_2), 37.91 (CH), 111.41, 112.25, 112.61, 114.56, 117.24, 117.65, 118.18, 118.63, 118.97, 119.06, 121.23, 122.19, 123.13, 123.79, 124.72, 124.98, 125.06, 125.85, 127.73, 130.36, 132.19, 134.84, 135.51, 139.76 .

Elemental analysis:

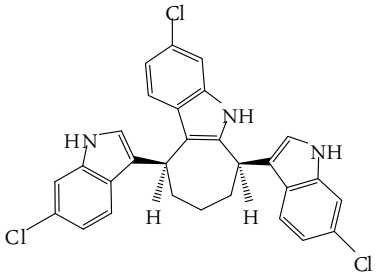
Calcd. C, 67.13; H, 4.27; Cl, 20.50; N, 8.10

Found. C, 67.18, H, 4,30, Cl, 20.49, N, 8.13

R_f-value: 0.69 (CH_2Cl_2)

Yield: (208 mg), 20 %

Cis-diastereomer: 3-Chloro-6,10-bis(6-chloro-1*H*-indol-3-yl)-5,6,7,8,9,10-hexahydrocyclohepta[*b*]indole (2_i):



Chemical Formula: $C_{29}H_{22}C_{l3}N_3$

Molecular Weight: 518.86 g/mol

Melting point: 107 - 110 ^{0}C

Colour and shape: brown powder

ESI-MS: (m/z) = 557 [M^++K], 516.05 [M^+H]

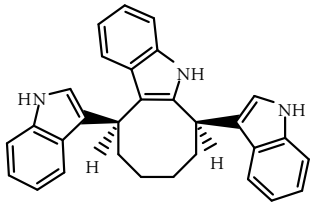
IR-Spectrum: (ATR, cm^{-1}) = 1464 (CCl), 2919 (CH_2), 3432 (NH)

^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 1.59 - 1.78 (m, 2H, CH_2), 1.78 - 1.99 (m, 2H, CH_2), 2.61 - 2.70 (m, 2H, CH_2), 4.53 (dd, J=7.3, 11.3 Hz, 1H, CH), 4.91 - 4.93 (m, 1H, CH), 6.52 - 6.53 (m, 1H), 6.89 (dd, J=1.89, 8.51 Hz, 1H), 6.95 - 7.04 (m, 2H), 7.11 - 7.16 (m, 1H), 7.23 (m, 7.5 Hz, 1H), 7.27 - 7.32 (m, 1H), 7.34 - 7.37 (m, 2H), 7.37 - 7.44 (m, 1H), 7.50 (s, 1H, NH), 7.62 (d, J=8.5 Hz, 1H), 7.89 (s, 1H, NH), 8.25 (s, 1H, NH)

R_f-value: 0.74 (CH_2Cl_2)

Yield: (187 mg), 18 %.

Cis-diastereomer: 6,11-Di(1*H*-indol-3-yl)-6,7,8,9,10,11-hexahydro-5*H*-cycloocta[*b*]indole (2_j):



Chemical Formula: $C_{30}H_{27}N_3$

Molecular Weight: 429.56 g/mol

Melting point: 105 - 108 ^{0}C

Colour and shape: light brown powder

ESI-MS: (m/z) = 428.43 [M^+-H]

EI-MS: (m/z) = 429 [M^+] 15 %, 312 [M^+-indolyl] 73 %, 297 [M^+-indolyl-CH_2] 5 %, 283 [M^+-indolyl-2CH_2] 37 %, 269 [M^+-indolyl-3CH_2] 20 %, 256 [M^+-indolyl-3CH_2-CH] 9 %, 245 [indolyl.CH.indolyl] 100 %, 194 [M^+-2indolyl] 12 %, 130 [indolyl-CH_2] 35 %, 117 [indolyl] 100 %, 90 [Ph.CH_2] 43 %, 77 [Ph] 8 %

IR-Spectrum: (ATR, cm^{-1}): 2936(CH_2), 3375(NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ(ppm) = 1.86 - 1.95 (m, 2H, CH_2), 1.98 - 2.07 (m, 6H, 3CH_2), 4.61 (d, J=10.2 Hz, 1H, CH), 5.57 - 5.61 (m, 1H, CH), 6.95 (t, J=7.1 Hz, 1H), 7.00 - 7.07 (m, 2H), 7.15 - 7.18 (m, 1H), 7.18 - 7.32 (m, 4H), 7.37 (d, J=8 Hz, 1H), 7.49 - 7.59 (m, 4H), 7.65 (d, J=7.8 Hz, 1H), 9.05 (s, 1H, NH), 10.85 (s, 1H, NH), 11.86 (s, 1H, NH)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 24.91 (CH_2), 30.37 (CH_2), 34.58 (CH_2), 36.96 (CH), 41.23 (CH_2), 49.78 (CH), 110.75, 110.85, 110.98, 118.79, 118.80, 118.99, 119.58, 119.76, 119.91, 120.47, 120.64, 121.47, 121.54, 121.62, 122.46, 127.02, 127.75, 128.16, 136.13, 136.23, 136.65, 139.65, 143.61, 147.09

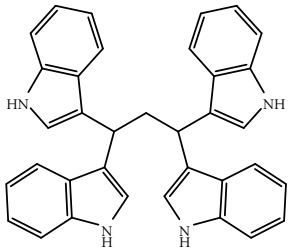
Elemental analysis: Calcd. C, 83.88; H, 6.34; N, 9.78

Found. C, 83.89, H, 6.36, N, 9.81

R_f-value: 0.63 (CH_2Cl_2)

Yield: (232 mg), 27 %

1,1,3,3-Tetra(1*H*-indol-3-yl)propane (3_a):



Chemical Formula: $C_{35}H_{28}N_4$

Molecular Weight: 504.62 g/mol

Melting point: 240 - 244 ^{0}C

Colour and shape: yellow powder

ESI-MS: (m/z) = 505.22 [M^++H], 503.40 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 3052 (CH_2), 3438 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 3.11 (t, J=7.2 Hz, 2H, CH_2), 4.32 (t, J=7.2 Hz, 2H, 2CH), 6.75 (t, J=7.5 Hz, 4H), 6.96 (t, J=7.5 Hz, 4H), 7.23 - 7.25 (m, 8H), 7.31 (d, J=8.1 Hz, 4H), 10.79 (s, 4H, 4NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 31.88 (CH), 40.36 (CH_2), 111.34, 117.75, 118.63, 119.11, 120.56, 122.17, 126.62, 136.62

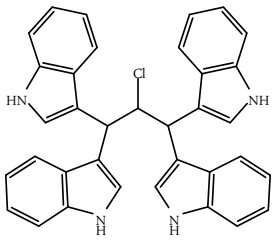
Elemental analysis: Calcd. C, 83.30; H, 5.59; N, 11.10

Found. C, 83.32, H, 5.54, N, 11.14

R_f-value: 0.56 (CH_2Cl_2)

Yield: (686 mg), 68 %

3,3$^{/}$,3$^{//}$,3$^{///}$-(2-Chloropropane-1,1,3,3-tetrayl)tetrakis(1*H*-indole (3_b):



Chemical Formula: $C_{35}H_{27}N_4Cl$

Molecular Weight: 539.07 g/mol.

Melting point: 220 - 226 ^{0}C

Colour and shape: brown powder

ESI-MS: (m/z) = 538.46 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}): 2848, 2922 (CH), 34401 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 3.09 (t, J=7 Hz, 2H, 2CH), 4.29 (t, J=7.3 Hz, 1H, CHCl), 6.73 (t, J=7.8 Hz, 4H), 6.95 (t, J=7 Hz, 4H), 7.23 (d, J=7.4 Hz, 2H),

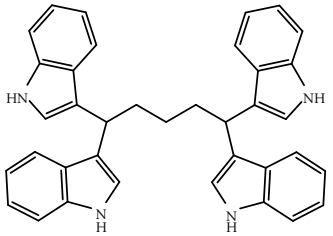
7.27 (d, J=8.2 Hz, 2H), 7.29 - 7.38 (m, 4H), 7.51 (d, J=6.8 Hz, 2H), 7.57 (d, J=6.8 Hz, 2H), 10.73 (s, 4H, 4NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 26.79 (CH), 50.00 (CHCl), 111.74, 118.17, 119.07, 119.55, 120.98, 122.62, 127.06, 137.05

R_f-value: 0.55 (CH_2Cl_2)

Yield: (496 mg), 46 %

3,3′,3″,3‴-(2-Bromopropane-1,1,3,3-tetrayl)tetrakis(1*H*-indole) (3_c):



Molecular Formula: $C_{35}H_{27}BrN_4$

Molecular Weight: 583.52 g/mol

Melting point: 180 - 185 ^{0}C

Colour and shape: light brown powder

ESI-MS: (m/z) = 622.16 [M^++K]

IR-Spectrum: (ATR, cm^{-1}) = 3455 (NH)

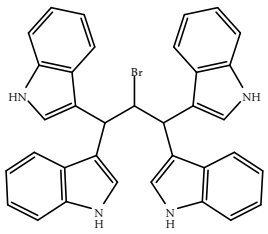
^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 3.13 (t, J=7.2 Hz, 2H, 2CH), 4.25 (t, J=7.3 Hz, 1H, CHBr), 6.76 (t, J=7.8 Hz, 4H), 6.88 (t, J=7.2 Hz, 4H), 7.23 (d, J=7.2 Hz, 2H), 7.27 (d, J=7.6 Hz, 2H), 7.30 - 7.38 (m, 4H), 7.52 (d, J=6.8 Hz, 2H), 7.58 (d, J=6.8 Hz, 2H), 10.72 (s, 4H, 4NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 25.88 (CH), 52.30 (CHBr), 111.75, 118.12, 119.53, 119.70, 120.98, 122.62, 127.07, 137.52

R_f-value: 0.5 (CH_2Cl_2)

Yield: (642 mg), 55 %

1,1,5,5-Tetra(1*H*-indol-3-yl)pentane (3_g):



Chemical Formula: $C_{37}H_{32}N_4$

Molecular Weight: 532.68 g/mol

Melting point: 219 - 221 ^{0}C

Colour and shape: light brown powder

ESI-MS: (m/z) = 555.51 [M^{+}+Na], 531.30 [M^{+}-H]

IR-Spectrum: (ATR, cm^{-1}) = 2931 (CH_2), 3409 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 1.21 - 1.48 (m, 2H, CH_2), 2.06 - 2.24 (m, 4H, $2CH_2$), 4.29 (t, J=7.4 Hz, 2H, 2CH), 6.80 (t, J=7.4 Hz, 4H), 6.94 (t, J=7.6 Hz, 4H), 7.10 (s, 4H), 7.24 (dd, J=3.42,8 Hz, 4H), 7.41 (t, J=7.9 Hz, 4H), 10.62 (s, 4H, 4NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 26.47 (CH_2), 33.41 (CH_2), 34.96 (CH), 111.22, 117.76, 118.86, 118.97, 120.46, 121.79, 126.62, 136.42

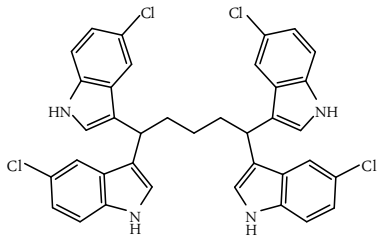
Elemental analysis: Calcd. C, 83.43; H, 6.06; N, 10.52

Found. C, 83.39, H, 6.09, N, 10.61

R_f-value: 0.63 (CH_2Cl_2)

Yield: (693 mg), 65 %

1,1,5,5-Tetrakis(5-chloro-1*H*-indol-3-yl)pentane (3_h):



Chemical Formula: $C_{37}H_{28}C_{l4}N_4$

Molecular Weight: 670.46 g/mol

Melting point: 210 - 213 ^{0}C

Colour and shape: brown powder

ESI-MS: (m/z) = 669.02 [M^{+}-H]

IR-Spectrum: (ATR, cm^{-1}) = 2858, 2931 (CH_2), 3409 (NH)

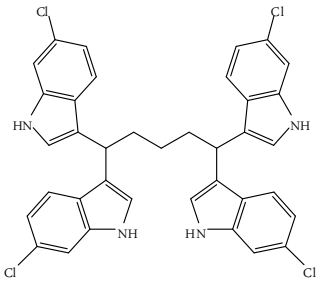
^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 0.94 - 1.22 (m, 2H, CH_2), 1.53 - 1.56 (m, 2H, CH_2), 2.19 - 2.36 (m, 2H, CH_2), 4.42 (t, j=7.47 Hz, 2H, 2CH), 6.97 (d, j=6.4 Hz, 4H), 7.08 - 7.10 (m, 4H), 7.32 (d, j=8.9 Hz, 4H), 7.3 - 7.47 (m, 4H), 10.09 (s, 4H, 4NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 22.50 (CH_2), 32.25 (CH_2), 38.90 (CH), 109.00, 111.05, 116.87, 118.02, 119.87, 121.02, 127.02, 137.42

R_f-value: 0.51 (CH_2Cl_2)

Yield: (670 mg), 50 %

1,1,5,5-Tetrakis(6-chloro-1*H*-indol-3-yl)pentane (3_i):



Chemical Formula: $C_{37}H_{28}C_{l4}N_4$

Molecular Weight: 670.46 g/mol

Melting point: 215 - 218 ^{0}C

Colour and shape: brown powder

ESI-MS: (m/z) = 669.02 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 2842, 2933 (CH_2), 3440 (NH)

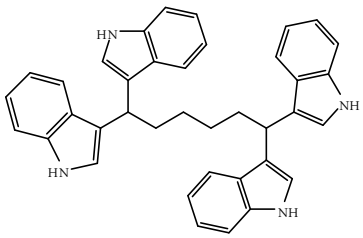
^{1}H-NMR: (400 MHz, CD_3OD) δ (ppm) = 2.25 - 2.45 (m, 2H, CH_2), 2.65 - 2.79 (m, 2H, CH_2), 3.32 - 3.6 (m, 2H, CH_2), 5.63 (t, J=8.6 Hz, 2H, 2CH), 6.85 (s, 4H), 8.05 (s, 1H), 8.22 (d, J=7.9 Hz, 2H), 8.33 - 8.42 (m, 1H), 8.55 (d, J=8.72 Hz, 4H), 8.66 - 8.72 (m, 4H), 11.35 (s, 4H, 4NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 23.36 (CH_2), 33.26 (CH_2), 38.92 (CH), 110.05, 111.53, 116.90, 118.03, 119.99 121.34, 127.05, 138.00

R_f-value: 0.53 (CH_2Cl_2)

Yield: (630 mg), 47 %

1,1,6,6-Tetra(1*H*-indol-3-yl)hexane (3_j):



Chemical Formula: $C_{38}H_{34}N_4$

Molecular Weight: 546.70 g/mol

Melting point: 164 - 168 ^{0}C

Colour and shape: light brown powder

ESI-MS: (m/z) = 545.26 [M^+-H]

IR-Spectrum: (ATR,cm^{-1}) = 2986 (CH_2), 3456 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 1.21 - 1.34 (m, 4H, $2CH_2$), 2.29 - 2.10 (m, 4H, $2CH_2$), 4.28 (t, J=7.5 Hz, 2H, 2CH), 6.79 (t, J=7.5 Hz, 4H), 6.95 (t, J=7.6 Hz, 4H),

7.11 (d, J=7.9 HZ, 4H), 7.25 (d, J=8.1 Hz, 4H), 7.41 (d, J=7.9 Hz, 4H), 10.63 (s, 4H, 4NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 27.79 (CH_2), 33.30 (CH_2), 35.11 (CH), 111.12, 117.69, 118.82, 120.37, 121.00, 121.66, 126.58, 136.29

Elemental analysis: Calcd. C, 83.48; H, 6.27; N, 10.25

Found. C, 83.46, H, 6.30, N, 10.30

R_f-value: 0.62 (CH_2Cl_2)

Yield: (645 mg), 59 %

5.1.4.4. General procedure for the acetylation reaction of triindoles $2_{d,g}$:

The triindole product $\mathbf{2_d}$ or $\mathbf{2_g}$, (1 mmol) was filled into a flask containing 5 ml CH_2Cl_2, 0.1 mmol of 4-(dimethylamino)pyridine (DMAP), 1.2 mmol triethylamine and 1.2 mmol acetic anhydride. The reaction mixture was left to stirring at room temperature for several days. The products were detected by TLC (100 % CH_2Cl_2) for mono and diacetylated products and TLC (2 % MeOH/CH_2Cl_2) for the triacetylated product. After about 2 months, the reaction mixture was neutralized with NH_4OH solution and extracted with CH_2Cl_2, washed with water and brine, dried over anhydrous sodium sulphate. The product was purified by using 100 % CH_2Cl_2 to collect the monoacetylated product first and then the diacetylated one. After that the tri-acetylated compound was collected by using eluent (2 % MeOH/CH_2Cl_2)

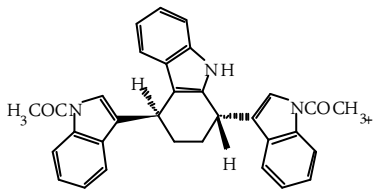
Trans-diastereomer: 1-(1-(1-acetyl-1*H*-indol-3-yl)-4-(1*H*-indol-3-yl)-3,4-dihydro-1*H*-carbazol-9(2*H*)-yl)ethanone (4_b):

Chemical Formula: $C_{32}H_{27}N_3O_2$

Molecular Weight: 485.58 g/mol

Melting point: 142 - 148 ^{0}C

Colour and shape: white powder



ESI-MS: (m/z) = 508.11 [M^++Na], 484.20 [M^+-H]

EI-MS (m/z): 485 [M^+] 100 %, 457 [M^+-$2CH_2$] 18 %, 443 [M^+-$COCH_3$] 21 %, 415 [M^+-$2CH_2$-$COCH_3$] 35 %, 399 [M^+-$2COCH_3$] 23 %, 373 [M^+-indolyl] 20 %, 326 [M^+-indolyl-$COCH_3$] 31 %, 283 [M^+-indolyl-$2COCH_3$] 45 %, 258 [indolyl.CH_2.CH_2.indolyl] 32 %, 68 [CH.CH_2.CH_2.CH.indolyl] 9 %, 130 [CH_2.indolyl] 5 %

IR/Spectrum: (ATR, cm^{-1}) = 1693 (C=O), 2923 (CH_2), 3335 (NH)

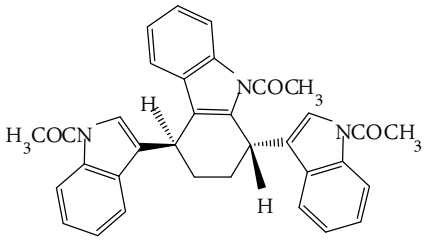
^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 2.07-2.46 (m, 4H, $2CH_2$), 2.51 (s, 3H, $COCH_3$), 2.61 (s, 3H, $COCH_3$), 4.55-4.60 (m, 2H, 2CH), 6.56 (t, J=10.2 Hz, 1H), 6.78 (t, J=7.8 Hz, 1H), 7.03 (d, J=7.8 Hz, 1H), 7.19-7.25 (m, 3H), 7.30-7.49 (m, 3H), 7.52 (d, J=8.2 Hz, 1H), 7.63 (s, 1H), 7.69 (d, J=7.9 Hz, 1H), 8.30-8.36 (m, 2H), 10.72 (s, 1H, NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 23.56 (CH_3), 24.37 (CH_3), 26.79 (CH_2), 27.19 (CH_2), 29.95 (CH), 32.19 (CH), 100.01, 101.56, 108.02, 109.11, 110.02, 116.05, 117.23, 118.63, 119.00, 120.15, 121.32, 122.00, 123.01, 123.55, 124.02, 124.34, 125.04, 127.36, 128.44, 129.18, 130.90, 134.00, 135.00, 136.50, 168.00, 169.02

R_f-value: 0.52 (CH_2Cl_2)

Yield: (146 mg), 30 %

Trans-diastereomer: 1,1'-(3,3'-(9- acetyl-2,3,4,9-tetrahydro-1*H*-carbazole-1,4-diyl)bis(1*H*-indole-3,1-diyl))diethanone (4_d):



Chemical Formula: $C_{34}H_{29}N_3O_3$

Molecular Weight: 527.61 g/mol

Melting point: 264 - 268 ^{0}C

Colour and shape: light yellow crystal

EI-MS: (m/z) = 527 [M^+] 100 %, 485 [M^+-$COCH_3$] 80 %, 442 [M^+-$2COCH_3$] 24 %, 415 [M^+-$2COCH_3$-$2CH_2$] 25 %, 398 [M^+-$3COCH_3$] 8 %, 325 [M^+-indolyl-$2COCH_3$] 98 %, 300 [M^+-indolyl-$2COCH_3$-CH_2-CH] 31 %, 283 [M^+-indolyl-$3COCH_3$] 90 %, 268 [M^+-

indolyl-3$COCH_3$-CH_2] 10 %, 256 [M^+-indolyl-3$COCH_3$-CH_2] 22 %, 243 [indolyl.CH.indolyl] 9 %

IR/Spectrum: (ATR, cm^{-1}) = 1689 (C=O), 2923 (CH_2), 3266 (NH)

^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 1.41 - 2.07 (m, 2H, CH_2), 2.15 - 2.27 (m, 2H, CH_2), 2.42 (s, 3H, CH_3), 2.48 (s, 3H, CH_3), 2.61 (s, 3H, CH_3), 4.57 - 4.65 (m, 2H, 2CH), 6.76 (s, 2H), 6.91 - 6.97 (m, 2H), 7.09 - 7.19 (m, 2H), 7.31 - 7.43 (m,3H), 7.58 - 7.69 (m, 2H), 8.03 (d, J=8.3 Hz, 1H), 8.46 - 8.48 (m, 2H)

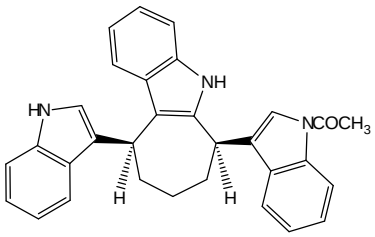
X-ray diffraction analysis:[287] a colourless plate shaped crystal $C_{34}H_{29}N_3O_3$ (from DMSO), crystal size 0.12 x 0.08 x 0.02 mm^3 was measured at 100 ^{0}K temperature by using a Bruker kappa APEX21μ SDuo Dffractometer with MOK$_\alpha$ (QUAZAR focussing Montel optics) radiation (λ = 0.71073 Å) and a graphitemonochromator. 3642 reflexions were collected in w/2ϑ scanning mode in the range $4.1^0 \leq 2\vartheta \leq 52.8^0$, h,k,l range from -11, -13, -14 to 11, 13, 14. Crystal system: monoclinic, space group p2_1/n (Nr. 14), Z = 4, a = 5.6167(5)Å, b = 39.258(3), c = 11.931(2) Å, $\alpha = 90^0$, $\beta = 95.079(2)^0$, $\gamma = 90^0$, v = 2620.4(4)$Å^3$, D_{ber} = 1.337 gcm^{-3}, μ = 0.086mm^{-1}. The structure was solved by direct methods (SHELXS-86)[288] using 21426 independent reflexions. Structure refinement: Full-matrix least-squares methods on F^2 using SHELXL-93[289], all the non-hydrogen atoms with isotropic displacement parameters. All hydrogen atoms are in calculated positions. The refinement converged to a final WR^2=0.1600 for 5316 unique reflections and R^1 = 0.0714 for 4129 observed reflections [$I_o \geq 4.06\sigma(I_o)$] and 364 refined parameters (p)

R_f-value: 0.27 (CH_2Cl_2)

Yield: (343 mg), 65 %

Cis- diastereomer-1-(6,10-Di(1*H*-indol-3-yl)-7,8,9,10-tetrahydrocyclohepta[*b*]indol-5(6*H*)-yl)ethanone (4_a):

Chemical Formula: $C_{31}H_{27}N_3O$



Molecular Weight: 457.57g/mol

Melting point: 265 - 270 ^{0}C

Colour and shape: White powder

ESI-MS: (m/z) = 456.24 [M^{+}-H]

IR-Spectrum: (ATR, cm^{-1}) = 1689 (C=O), 2843, 2922 (CH_2), 3406 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 1.69 - 1.99 (m, 2H, CH_2), 2.04 - 2.08 (m, 2H, CH_2), 2.22 - 2.28 (m, 2H, CH_2), 2.62 (s, 3H, $COCH_3$), 4.63 (dd, J=2.9,11 Hz, 1H, CH), 4.91 - 4.93 (m, 1H, CH), 6.57 - 6.61 (m, 1H), 6.74 (t, J=7.4 Hz, 1H), 6.84 (t, J=7.5 Hz, 1H), 6.96 (t, J=7.5 Hz, 1H), 7.04 (t, J=7.5 Hz, 1H), 7.11 (d, J=7.7 Hz, 1H), 7.13 - 7.29 (m, 2H), 7.31 - 7.49 (m, 3H), 7.62 (d, J=7.9 Hz, 1H), 7.79 (s, 1H), 8.37 (d, J=8.3 Hz, 1H), 9.79 (s, 1H, NH), 10.65 (s, 1H, NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 23.73 (CH), 24.39 (CH_2), 32.18 (CH), 33.36 (CH_2), 34.59 (CH_2), 36.74 (CH_3), 110.64, 11.25, 114.14, 115.93, 117.29, 117.45, 117.72, 117.95, 118.58, 119.48, 119.71, 120.49, 122.79, 122.88, 123.36, 124.39, 124.79, 126.19, 128.37, 29.59, 134.36, 135.56, 136.58, 137.38, 169.15 (C=O)

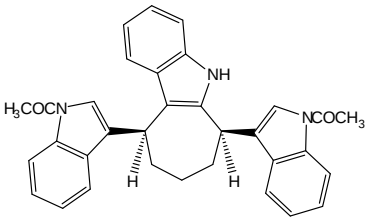
Elemental analysis: Calcd. C, 81.37; H, 5.95; N, 9.18

Found. C, 81.40, H, 5.90, N, 9.19

R_f-value: 0.62 (CH_2Cl_2)

Yield: (128 mg), 28 %

Cis- diastereomer-1-(3-(5-acetyl-10-(1*H*-indol-3-yl)-5,6,7,8,9,10-hexahydrocyclohepta[*b*]indol-6-yl)-1*H*-indol-1-yl)ethanone (4_c):



Chemical Formula: $C_{33}H_{29}N_3O_2$

Molecular Weight: 499.6 g/mol

Melting point: > 360 ^{0}C

Colour and shape: white powder

ESI-MS: (m/z) = 498.36 [M^{+}-H]

EI-MS: (m/z) = 499 [M^+] 40 %, 457 [M^+-$COCH_3$] 10 %, 415 [M^+-$2COCH_3$] 3 %, 384 [M^+-indolyl] 5 %, 339 [M^+-indolyl-$COCH_3$] 100 %, 297 [M^+-indolyl-$2COCH_3$] 46 %, 283 [M^+-indolyl-$2COCH_3$-CH_2] 8 %, 269 [M^+-indolyl-$2COCH_3$-$2CH_2$] 20 %, 259 [M-indolyl-$2COCH_3$-$3CH_2$] 30 %, 245 [indolyl.CH.indolyl] 9 %, 156 [indolyl.CH.CH_2.CH_2] 20 %, 130 [indolyl.CH_2] 32 %, 117 [indolyl] 8 %

IR-Spectrum: (ATR,cm^{-1}) = 1694 (C=O), 2942 (CH_2), 3374 (NH)

^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 1.78 - 1.84 (m, 2H, CH_2), 2.14 - 2.08 (m, 2H, CH_2), 2.00 - 2.22 (m, 2H, CH_2), 2.45 (s, 3H, $COCH_3$), 2.61 (s, 3H, $COCH_3$), 4.56 (dd, J=4.1, 10.8 Hz, 1H, CH), 4.92 (br., 1H, CH), 6.79 - 689 (m, 2H), 7.10 - 7.21 (m, 4H), 7.23 - 7.34 (m, 3H), 7.36 - 7.42 (m, 1H), 7.75 (d, J=7.5 Hz, 1H), 7.83 (s, 1H), 8.33 (d, J=7.7 Hz, 1H), 8.43 (d, J=8.2 Hz, 1H), 9.94 (s, 1H, NH)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 24.97 (CH_2), 31.12 (CH), 32.56 (CH), 33.34 (CH_2), 35.05 (CH_2), 37.04 (CH_3), 40.07 (CH_3), 108.20, 110.00, 11.48, 112.93, 114.53, 115.00, 116.44, 116.59, 117.87, 118.61, 119.05, 120.12, 120.54, 123.31, 123.56, 123.59, 125.09, 125.22, 128.82, 130.19, 130.29, 135.03, 136.18, 138.58, 169.47 (C=O), 169.85 (C=O)

Elemental analysis: Calcd. C, 79.33; H, 5.85; N, 8.41

Found. C, 79.34, H, 5.89, N, 8.42

R_f-value: 0.14 (CH_2Cl_2)

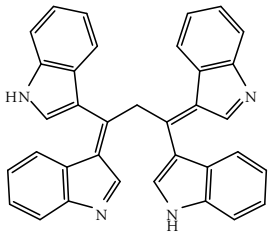
Yield: (290 mg), 58 %

5.1.4.5. General procedure for the preparation of compounds (5_{a-c}):

Compound 3_a or 3_g or 3_j (1 mmol) was dissolved in MeOH (25 ml). TCQ (tetrachloroquinone) (0.34 mg, 1.5 mmol) was added to the reaction mixture. The reaction was allowed to stirring under reflux for 1-1.5 h until the start was finished, when the colour of the reaction became dark red. The product was detected by TLC (5 % MeOH/CH_2Cl_2). Then methanol was evaporated and the product was purified by

column chromatography by eluent at first 0.5 L of 100 % CH_2Cl_2, then 0.5 L of 2 % MeOH/CH_2Cl_2 and finally 5 % MeOH/CH_2Cl_2 to afford the pure colord compounds **5_a** or **5_b** or **5_c** respectively

1,3-Di(1*H*-indol-3-yl)-1,3-di(3*H*-indol-3-yl)propane (5_a):



Chemical Formula: $C_{35}H_{24}N_4$

Molecular Weight: 500.59 g/mol

Melting point: 210 - 214 ^{0}C

Colour and shape: dark yellow powder

ESI-MS: (m/z) = 501.20 [M^++H]

IR-Spectrum: (ATR, cm^{-1}) = 1455 (CH=N), 2920 (CH_2), 3064 - 3350 (br. NH)

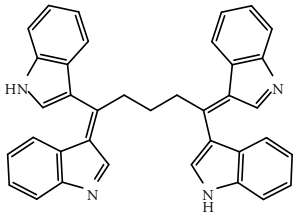
^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 1.88 (s, 2H, CH_2), 6.88 (d, J=7.8 Hz, 4H), 6.99 (t, J=7.4, 4H), 7.27 (t, J=7.4 Hz, 4H), 7.65 (d, J=7.8 Hz, 4H), 8.27 (s, 4H), 13.17 (br., 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 29.56 (CH_2), 113.95, 119.05, 120.60, 122.74, 124.30, 126.67, 139.61, 140.00, 142.50, 157.74, 171.93(C=N)

R_f-value: 0.03 (10 % MeOH/CH_2Cl_2)

Yield: (365 mg), 73 %

1,5-Di(1*H*-indol-3-yl)-1,5-di(3*H*-indol-3-ylidene)pentane (5_b):



Chemical Formula: $C_{37}H_{28}N_4$

Molecular Weight: 528.65 g/mol

Melting point: > 350 ^{0}C

Colour and shape: dark violet powder

ESI-MS: (m/z) = 528.47 [M^+], 527.10 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1452 (CH=N), 2919 (CH_2), 3105 - 3340 (br, NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 1.90 - 1.22 (m, 2H, CH_2), 3.41 - 3.44 (m, 4H, $2CH_2$), 6.92 (d, J=7.4 Hz, 4H), 7.06 (t, J=7.7 Hz, 2H), 7.09 - 7.17 (m, 2H), 7.24 (t,

J=7.5 Hz, 1H), 7.33 (t, J=6.7 Hz, 2H), 7.54 (d, J=8.2 Hz, 1H), 7.65 - 7.69 (m, 2H), 7.82 (s, 1H), 7.92 (s, 2H), 8.06 - 8.12 (m, 1H), 8.40 (s, 2H), 11.99 (br., 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ(ppm) = 35.00 (CH_2), 40.56 (CH_2), 110.01, 114.24, 121.20, 123.45, 125.50, 127.05, 136.05, 139.55, 140.02, 157.05, 172.42 (C=N)

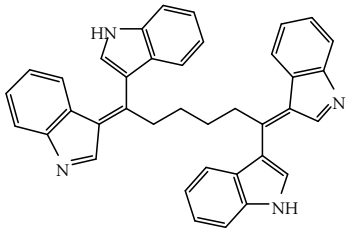
Elemental analysis: Calcd. C, 84.06; H, 5.34; N, 10.60

Found. C, 84.08, H, 5.39, N, 10.62

R_f-value: 0.05 (7 % MeOH/ CH_2Cl_2)

Yield: (449 mg), 85 %

1,6-Di(1*H*-indol-3-yl)-1,6-di(3*H*-indol-3-ylidene)hexane (5_c):



Chemical Formula: $C_{38}H_{30}N_4$

Molecular Weight: 542.67 g/mol

Melting point: 175 - 178 ^{0}C

Colour and shape: dark brown powder

ESI-MS: (m/z) = 582.40 [M^++K], 541.12 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1459 (CH=N), 2852, 2924 (CH_2), 3250 - 3348 (br, NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 1.42-1.57 (m, 4H, $2CH_2$), 3.23-3.24 (m, 4H, CH_2), 3.41 - 3.57 (m, 2H, CH_2), 6.68 (d, J=8.4 Hz, 1H), 6.78 (t, J=7.6 Hz, 1H), 6.85 - 6.88 (m, 1H), 6.91 - 7.10 (m, 2H), 7.15 - 7.22 (m, 4H), 7.25 - 7.38 (m, 4H), 7.45 - 7.60 (m, 4H), 7.64 - 7.78 (m, 2H), 7.81 (s, 1H), 9.93 (br., 1H, NH), 10.95 (br., 1H, NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 23.69 (CH_2), 43.27 (CH_2), 111.99, 112.99, 118.74, 119.89, 121.71, 123.19, 125.87, 128.68, 137.94, 158.20, 166.50 (C=N)

Elemental analysis: Calcd C, 84.10; H, 5.57; N, 10.32

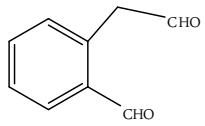
Found. C, 84.12, H, 5.61, N, 10.35

R_f-value: 0.58 (7 % MeOH / CH_2Cl_2)

Yield: (429 mg), 79 %

5.1.4.6. Procedure for the preparation of Homophthalaldehyde

Ozonolysis of indene: freshly distilled indene (10 ml, 9.8 mg, 85 mmol) was added to dry dichloromethane (200 ml, distilled from P_2O_5). The solution was cooled dawn to -65 ^{0}C, and ozone (about 3 % in oxygen, flow rate 11/min.) was bubbled through the solution for 10 mines. The resulting blue solution was flushed with nitrogen until the blue colour disappeared for about 10 mines, and then zinc (4.0 mg) and acetic acid (20 ml) were added. The solution was allowed to warm to 0 ^{0}C under stirring. Four similar portions of zinc in acetic acid were added over the next 2 and 1/2 h. The resulting mixture was then filtered, the filtrate washed with aqueous 2 N sodium carbonate solution (50 ml), water (3 x 50 ml), and dried over sodium sulphate. Evaporation of the solvent under reduced pressure gave pale yellow oil. Dry benzene (100 ml) was added and the mixture was heated to distil off the benzene. This isotropic distillation was repeated, and the residue was then distilled under reduced pressure with a yield of 65 % b.p. 90 / 0.1 mm. The distillation temperature must be kept below 100 ^{0}C otherwise extensive decomposition occurs.



Chemical Formula: $C_9H_8O_2$

Molecular Weight: 148.16 g/mol

EI-MS: (m/z) = 148 [M^+] 6 %, 147 [M^+-H] 4 %, 134 [M^+-CH_2] 6 %, 120 [M^+-CO] 97 %, 119 [M^+-CHO] 100 %

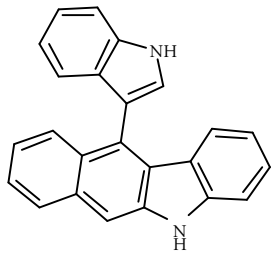
^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 4.75 (d, 2H, J=12.5 Hz, CH_2), 7.01 - 7.22 (m, 2H), 7.30 - 7.52 (m, 2H), 9.72 (t, 1H, J=0.7 Hz, CH_2CHO), 10.00 (s, 1H, CHO)

5.1.4.7. General procedure for the preparation of compound 7_{a-b} and 8_{a-b}:

The aromatic dialdehyde (*o*-phthalaldehyde) 2 mmol was added to 15 ml glacial acetic acid in a round bottom flask equipped with a magnetic stir-bar at room temperature. Then 5 mmol of indole or its derivative (6-chloroindole) was added to the

reaction mixture. The clear light yellow solution was left stirring overnight, and the solution became dark brown. The product was detected by TLC (20 % EtAc/Hexane). The TLC showed the formation of the two products, compound **7_{a-b}**, at high R_f value and **8_{a-b}** at low R_f value, where the indole or its derivative was not finished from the reaction. At this point the reaction mixture was worked up by neutralization with a cold solution of 10 % NaOH affording a brown precipitate. Then the mixture was extracted with CH_2Cl_2 for three times (200 ml), washed with water for two times and brine for two times. Then it was dried over anhydrous sodium sulphate, filtered, and concentrated in vacuum. The crude reaction mixture was purified *via* column chromatography on silica gel eluted with (20 % EtAc/hexane) to remove at first the unreacted indole or its derivative. Then compound **7_{a-b}** was collected followed by compound **8_{a-b}**

11-(1H-indol-3-yl)-5H-benzo[b]carbazole (7_a):



Chemical Formula: $C_{24}H_{16}N_2$

Molecular Weight: 332.40 g/mol

Melting point: 250 - 254 ^{0}C

Colour and shape: light green crystal

ESI-MS: (m/z) = 331.57 [M^+-H]

EI-MS: (m/z) = 332 [M^+] 100 %, 215 [M^+-inolyl] 0.1 %, 117 [indolyl] 31 %, 90 [Ph.CH] 15 %

IR-Spectrum: (ATR, cm^{-1}) = 3435 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 6.76 (t, 1H, J=7.5 Hz), 6.94 (t, 1H, J=7.4 Hz), 7.03 (t, 2H, J=7.14 Hz), 7.20 - 7.29 (m, 2H), 7.31 (t, 1H, J=7.3 Hz), 7.41 - 7.46 (m, 2H), 7.58 (d, 1H, J= 7.9 Hz), 7.68 (d, 1H, J=8.2 Hz), 7.89 (d, 1H, J=8.6 Hz), 7.94 (s, 1H), 8.03 (d, 1H, J=8.3 Hz), 10.27 (s, 1H, NH), 10.74 (s, 1H, NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 104.74, 109.95, 111.73, 111.78, 112.73, 118.16, 119.39, 119.55, 121.81, 121.98, 123.05, 123.31, 124.44, 124.47, 124.61, 126.51, 126.64, 126.96, 127.98, 128.82, 133.01, 136.89, 139.86, 142.98

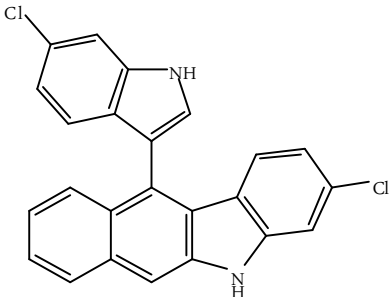
Elemental analysis: Calcd. C, 85.50; H, 5.16; N, 9.35

Found C, 85.52, H, 5.18, N, 9.36

R_f-Value: 0.34 (20 % EtAc/Hexane)

Yield: (1.33 g), 80 %

3-chloro-11-(6-chloro-1H-indol-3-yl)-5H-benzo[b]carbazole (7_b):



Chemical Formula: $C_{24}H_{14}Cl_2N_2$

Molecular Weight: 401.29 g/mol

Melting point: 235 - 238 ^{0}C

Colour and shape: light green crystal

ESI-MS: (m/z) = 402.30 [M^++H]

EI-MS: (m/z) = 401 [M^+] 42 %, 400 [M^+-H] 100 %, 364 [M^+-H-Cl] 25 %, 330 [M^+-2Cl] 11 %, 165 [chloroindolyl.CH] 32 %, 151 [chloroindolyl] 11 %

IR-Spectrum: (ATR, cm^{-1}) = 1605 (CCl), 3426 (NH)

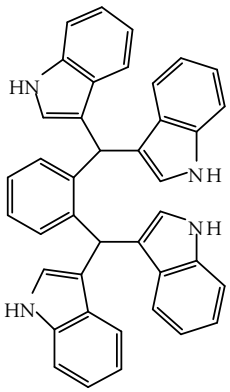
^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 6.74 (d, 1H, J=8.3 Hz), 6.81 - 6.84 (m, 1H), 6.89 (d, 1H, J=6.8 Hz), 7.22 - 7.24 (m, 1H), 7.37 - 7.39 (m, 1H), 7.45 (t, 2H, J=8 Hz), 7.65 - 7.68 (m, 1H), 7.72 - 7.76 (m, 2H), 7.95 (s, 1H), 8.07 (d, 1H, J=8.2 Hz), 11.42 (s, 1H, NH), 11.72 - 11.73 (s, br., 1H, NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 109.00, 110.50, 111.37, 111.64, 118.08, 119.62, 120.32, 121.27, 122.47, 123.31, 123.58, 124.85, 125.52, 125.89, 126.02, 126.24, 127.10, 128.01, 131.21, 132.45, 136.73, 136.87, 139.48, 143.36

R_f-Value: 0.87 (CH_2Cl_2)

Yield: (601 mg), 75 %

1.2-Bis(di(1*H*-indol-3-yl)methyl)benzene (8_a):



Chemical Formula: $C_{40}H_{30}N_4$

Molecular Weight: 566.69 g/mol

Melting point: 230 – 233 ^{0}C

Colour and shape: light brown powder

ESI-MS: (m/z) 565.20 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 3408 (NH)

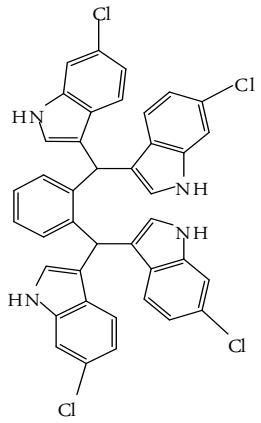
^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 5.65 (s, 2H, 2CH), 6.06 (s, 4H), 6.93 (t, 4H, J=8 Hz), 7.05 (t, 4H, J=7.5 Hz), 7.13 (d, 8H, J=7.8 Hz), 7.29 (d, 4H, J=7.8 Hz), 9.96 (s, 4H, 4NH)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 40.84 (CH), 111.07, 118.84, 119.42, 119.83, 121.62, 123.59, 124.18, 126.93, 128.43, 129.88, 136.49, 139.30, 141.45. 143.51

R_f-Value: 0.29 (CH_2Cl_2)

Yield: (56 mg), 10 %

1.2-Bis(di(6-chloro-1*H*-indol-3-yl)methyl)benzene (8_b):



Chemical Formula: $C_{40}H_{26}C_{l4}N_4$

Molecular Weight: 704.4 g/mol

Melting point : 175 - 180 ^{0}C

Colour and shape: brown powder

ESI-MS: (m/z) = 703.27 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1703 (CCl), 2954 (CH_2), 3312 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 5.89 (s, 2H, 2CH), 6.39 -6.42 (m, 2H), 6.49-6.69 (m, 2H), 6.79 - 6.83 (m, 4H), 7.17 (t, 4H, J=8.2 Hz), 7.41 - 7.51 (m, 4H), 7.58 -7.59 (m, 2H), 7.77 - 7.81 (m, 2H). 10.82 (s,br., 4H, 4NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 48.55 (CH), 109.22, 110.00, 111.82, 117.50, 120.62, 121.73, 123.34, 124.12, 125.77, 126.00, 128.55, 129.73, 130.00, 134.15, 137.32, 144.00

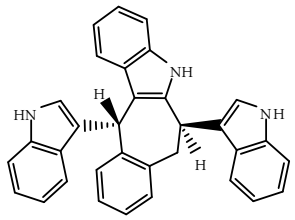
R_f-Value: 0.34 (CH_2Cl_2)

Yield: (99 mg), 14 %

5.1.4.8. Procedure for the preparation of compounds 10 and 11:

2 Mmol of the aromatic dialdehyde homophthalaldehyde were added in a round bottom flask containing 5 ml of glacial acetic acid at room temperature. Then 5 mmol of indole were added to the reaction mixture. The clear light yellow solution was left to stirring overnight, when the solution became dark brown. The product was detected by TLC (100 % CH_2Cl_2). The TLC showed the formation of the two products, compound 10 at high R_f value and compound 11 at low R_f value where the indole was not finished from the reaction mixture. At this point the reaction was worked up by neutralization with a cold solution of 10 % NaOH affording a brown precipitate. Then the mixture was extracted with CH_2Cl_2 three times 200 ml, washed with water for two times and brine for two times then dried over anhydrous sodium sulphate, filtered and finally concentrated in vacuum. The crude reaction mixture was purified *via* column chromatography on silica gel eluting with dichloromethane, to remove at first the unreacted indole, and then compound **10** was collected, followed by compound **11**.

Trans- distereomer: 6,10-Di(1*H*-indol-3yl)-5,10,11,12-tetrahydrodibenzo[*a*,*g*]azulene[*b*]indole (10):



Chemical Formula: $C_{33}H_{25}N_3$

Molecular Weight: 463.57 g/mol

Melting point : 255 – 257 ^{0}C

Colour and shape: puff powder

ESI-MS: (m/z) = 462.14 [M^+-H]

EI-MS: (m/z) = 463 [M^+] 4 %, 347 [M^+-indolyl] 100 %, 333.9 [M^+-indolyl-CH_2] 13 %, 256.6 [M^+-indolyl-CH_2-Ph] 3 %, 245 [indolyl.CH.indolyl] 9 %, 230 [indolyl.indolyl] 15 %, 217 [indolyl.CH_2.CH.Ph] 12 %, 130 [indolyl.CH_2] 10 %, 117 [indolyl] 20 %, 90 [Ph.CH_2] 8 %

IR-Spectrum: (ATR,cm^{-1}) = 2922 (CH_2), 3401 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 2.85 - 2.89 (m, 2H, CH_2), 3.94 - 4.03 (m, 2H, CH_2), 4.98 (s,br., 1H, CH), 5.71 (s,br., 1H, CH), 5.85 - 5.89 (m, 1H, CH), 6.03 (s, br., 1H, CH), 6.25 - 6.32 (m, 2H,), 6.22 (s, 1H), 6.68 (s, 1H), 6.85 (t, 2H, J=9.1 Hz), 6.88 - 7.02 (m, 4H), 7.09 - 7.34 (m, 5H), 7.44 - 7.51 (m, 1H), 7.67 (d, 1H, J=7.1 Hz), 7.78 (s, 1H), 10.33 (s, 1H, NH), 10.57 (d, 1H, NH), 10.92 (s, 1H, NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 26.32 (CH), 26.34 (CH), 31.47 (CH_2), 32.06 (CH_2), 110.43, 111.17, 111.31, 111.46, 112.73, 116.37, 116.59, 117.34, 117.72, 117.89, 117.97, 118.03, 118.26, 118.49, 119.02, 119.32, 120.23, 120.32, 120.48, 120.69, 123.02, 123.34, 124.77, 125.39, 125.64, 125.90, 126.00, 126.28, 126.33, 126.44, 126.89, 127.73, 128.31, 128.42, 128.89, 131.44, 131.64, 131.89, 134.71, 134.87, 135.13, 135.98, 136.57, 136.73, 138.10, 138.84, 141.51

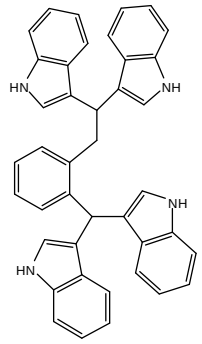
Elemental analysis: Calcd. C, 85.50; H, 5.44; N, 9.06

Found C, 85,53, H, 5.42, N.8.99

R_f-Value: 0.72 (CH_2Cl_2)

Yield: (427 mg), 46 %

3,3'-((2-(2,2-di(1*H*-indol-3-yl)ethyl)phenyl)methylene)bis(1*H*-indole) (11):



Chemical Formula: $C_{41}H_{32}N_4$

Molecular Weight: 580.72 g/mol

Melting point: 168 - 172^{0}C

Colour and shape: light brown powder

ESI-MS: (m/z) = 579.08 [M^{+}-H]

IR-Spectrum: (ATR, cm^{-1}) = 2922 (CH_2), 3407 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 3.59 (d, 2H, J=6.9 Hz, CH_2), 4.80 - 4.83 (m, 1H, CH-CH_2), 5.71 (s, 1H, CH), 6.13 (s, 2H), 6.47 (s, 2H), 6.72 - 6.77 (m, 4H), 6.89 - 7.19 (m, 8H), 7.22 (d, 4H, J=7.9 Hz), 7.32 (t, 4H, J=7.7 Hz), 10.63 (s, 2H, 2NH), 10.71 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 41.52 (CH_2), 46.00 (CH), 59.01 (CH), 102.00, 105.22, 111.33, 115.02, 116.55, 117.80, 118.03, 118.23, 118.93, 120.44, 122.00, 124.00, 125.50, 126.02, 129.00, 130.00, 132.23, 134.61, 135.02, 138.52, 139.00, 142.00

Elemental analysis: Calcd. C, 84.80; H, 5.55; N, 9.65

Found C, 84.87, H, 5.56, N, 9.56

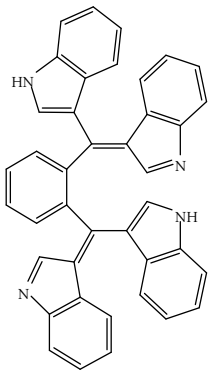
R_f-Value: 0.54 (CH_2Cl_2)

Yield: (441 mg), 38 %

5.1.4.9. General procedure for the preparation of compound 9, 12:

Compounds **8$_a$** or **11** (1 mml, 0.6 gm) respectively, was dissolved in MeOH (25 ml). TCQ (tetrachloroquinone) (0.34 gm, 1.5 mmol) was added to the reaction mixture. The reaction was allowed to stir for 2 h under reflux until the reaction was finished, where the colour of the reaction became dark red. The product was monitored by TLC (7 % MeOH/CH_2Cl_2). Then methanol was evaporated and the product was purified by column chromatography by using first 0.5 L of 100 % CH_2Cl_2 then 0.5 L of 2 % MeOH/CH_2Cl_2 and finally 5 % MeOH/CH_2Cl_2 as eluent to afford the pure colored compounds **9** or **12** respectively.

1,2-Bis-(1*H*-indol-3-yl)(3*H*-indol-3-ylidene)methyl)benzene (9):



Chemical Formula: $C_{40}H_{26}N_4$

Molecular Weight: 562.66 g/mol

Melting point: 220 - 223 ^{0}C

Colour and shape: dark green powder

ESI-MS: (m/z) = 561.44 [M^+-H]

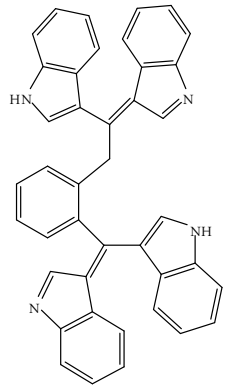
IR-Spectrum: (ATR, cm^{-1}) = 1151 (CH=N), 2922 (CH_2), 3253 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 6.91 - 6.99 (m, 4H), 7.05 (d, 2H, J=7.8 Hz), 7.11 (t, 2H, J=7.6 Hz), 7.20 (t, 2H, J=11 Hz), 7.27 - 7.39 (m, 4H), 7.40 (s, 2H), 7.60 - 7.80 (m, 4H), 8.10 (m, 2H), 8.19 (s, 2H), 11.14 (s, br., 2H, 2NH)

R_f-Value: 0.1 (7 % MeOH/CH_2Cl_2)

Yield: (309 mg), 55 %

3-(2-(2-(1*H*-indol-3-yl)(3*H*-indol-3-ylidene)methyl)phenyl)-1-(1*H*-indol-3-yl)ethylidene)-3*H*-indole (12):



Chemical Formula: $C_{41}H_{28}N_4$

Molecular Weight: 576.69 g/mol

Melting point: 150 - 155 ^{0}C

Colour and shape: dark violet powder

ESI-MS: (m/z) = 576.50 [M^+], 575.49 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1067 (CH=N), 2922 (CH_2), 3303 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 3.60 (s, 2H, CH_2), 6.96 - 6.99 (m, 4H), 7.03 (d, 2H, J=9.8 Hz), 7.05 (d, 2H, J=7.8 Hz), 7.21 (t, 2H, J=7.4 Hz), 7.26 - 7.39 (m, 4H), 7.45 (s, 2H), 7.47 - 7.48 (m, 2H), 7.60 - 7.79 (m, 2H), 8.09 (d, 2H, J=8.2 Hz), 8.19 (s, 2H), 11.19 (s, br., 2H, 2NH)

^{13}C-NMR: (100 MHz, Acetone-d_6) δ (ppm) = 36.49 (CH_2), 102.45, 112.09, 112.25, 114.34, 119.52, 119.71, 120.09, 120.39, 120.53, 120.84, 121.79, 122.04, 122.32, 122.79, 123.07, 123.27, 123.56, 124.04, 125.39, 125.94, 126.14, 126.35, 126.50, 126.98, 127.19, 127.73, 127.99, 128.47, 128.59, 128.81, 129.79, 131.92, 132.29, 132.39, 137.16, 142.27, 143.95, 148.18, 161.00 (C=N), 162.04 (C=N)

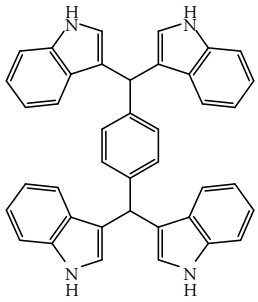
R_f-Value: 0.83 (7 % MeOH/CH_2Cl_2)

Yield: (558 mg), 62 %

5.1.4.10. General procedure for the preparation of compound $13_{a,b}$:

In a flask containing 20 ml glacial acetic acid, (1 mmol, 0.134 gm) of terphthalaldehyde was added under stirring at room temperature. And after all the amounts of the dialdehyde were dissolved, indole (4 mmol, 0.47 gm) or 5-chloroindole (4 mmol, 0.61 gm) was added. Then the reaction mixture was allowed to stirring overnight at room temperature. The reaction solution turned from light yellow to dark pink colour. The product was detected by TLC (100 % CH_2Cl_2) until the reaction was finished. In the case of indole the product was precipitated from the reaction mixture, filtered off, washed with AcOH, and washed with water, dried over P_2O5 to give a pure light pink powder of compound **13_a**, in 98 % yield. The case of 5-chloroindole the product precipitated by the addition of 10 ml water and filtered off, washed with water under suction and dried to afford compound **13_b**.

1,4-Bis(di(1*H*-indol-3-yl)methyl)benzene (13_a):



Chemical Formula: $C_{40}H_{30}N_4$

Molecular Weight: 566.69 g/mol

Melting point: 137 - 139 ^{0}C

Colour and shape: light pink, powder

ESI-MS: (m/z) = 566.31 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 3404 (NH)

^{1}HNMR: (400 MHz, acetone-d_6) δ (ppm) = 5.91 (s, 2H, 2CH), 6.82 - 6.83 (m, 4H), 6.89 (t, 4H, J=7.2 Hz), 7.05 (t, 4H, J=8.1 Hz), 7.35 - 7.38 (m, 12H), 9.96 (s, 4H, 4NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 40.84 (CH), 112.08, 119.22, 120.06, 120.32, 121.95, 124.46, 128.13, 129.19, 138.03, 143.42

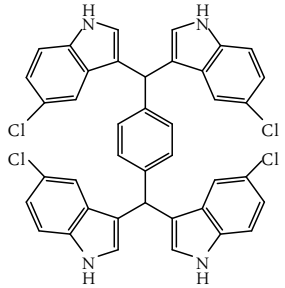
Elemental Analysis: Calcd. C, 84.78, H, 5.34, N, 9.89

Found C, 84.69, H, 5.29, N, 10.00

R_f-Value: 0.54 (CH_2Cl_2)

Yield: (527 mg), 93 %

1,4-Bis(bis(5-chloro-1*H*-indol-3-yl)methyl)benzene (13_b):



Chemical Formula: $C_{40}H_{26}C_{l4}N_4$

Molecular Weight: 704.47 g/mol

Melting point : 160 - 163^{0}C

Colour and shape: pink powder

ESI-MS: (m/z) = 705.35 [M^++H]

IR-Spectrum: (ATR, cm^{-1}) = 1458 (CCl), 3424 (NH)

^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 5.63 (s, 2H, 2CH), 6.49 - 6.50 (m, 4H), 7.05 (dd, 4H, J=1.9, 8.6 Hz), 7.11 (s, 4H), 7.17 (d, 4H, J=7.4 Hz), 7.20 - 7.29 (m, 4H), 7.79 (s, 4H, 4NH)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 39.67 (2CH), 112.21, 119.15, 119.26, 122.31, 124.97, 125.09, 128.06, 128.71, 135.06, 141.35

Elemental Analysis: Calcd. C, 68.20; H, 3.72; Cl, 20.13; N, 7.95

Found C, 68.24, H, 3.75, Cl, 20.7, N, 8.00

R_f-Value: 0.55 (CH_2Cl_2)

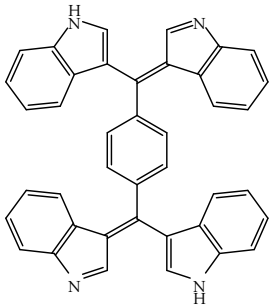
Yield: (669 mg), 95 %

5.1.4.11. General procedure for the preparation of compound $14_{a,b}$:

Compounds 13_a or 13_b (1mmol) was dissolved in MeOH 25 ml, TCQ (tetrachloroquinone) (0.34 gm, 1.5 mmol) was added to the reaction mixture. The reaction was allowed to stir for 2 h under reflux until it was finished. The colour of the reaction mixture became dark red. The product was detected by TLC 5 % MeOH/CH_2Cl_2. Methanol was evaporated and the product was purified by column

chromatography by eluting with 1 L of 2 % MeOH/CH_2Cl_2 and then with 5 % MeOH/CH_2Cl_2 to afford the pure colord compounds **14_a** or **14_b** respectively.

1,4-(1*H*-indol-3-yl)(3*H*-indol-3-ylidene)methyl)benzene (14_a):



Chemical Formula: $C_{40}H_{26}N_4$

Molecular Weight: 562.66 g/mol

Melting point: > 350 ^{0}C

Colour and shape: dark red powder

ESI-MS: (m/z) = 563.26 [M^++H], 561.35 [M^+-H]

IR-Spectrum:(ATR, cm^{-1}) = 1459 (CH=N), 3035 – 3450 (br. NH)

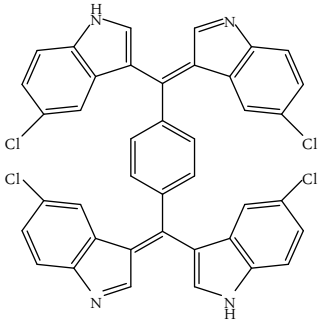
^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 6.95 (d, 4H, J=7.9 Hz), 7.05 (t, 4H, J=7.5 Hz), 7.27 (t, 4H, J=7.6 Hz), 7.59 (d, 4H, J=7.8 Hz), 7.69 (s, 4H), 8.23 (s, 4H), 10.91 (s,br., 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 110.92, 111.60, 115.00, 115.94, 116.60, 117.32, 120.68, 120.87, 121.50, 122.96, 125.21, 127.63, 131.82, 134.33, 137.03, 139.34, 140.00, 141.33, 144.04, 161.05 (C=N)

R_f-Value: 0.52 (10 % MeOH/CH_2Cl_2)

Yield: (484 mg), 86 %

1,4-Bis((5-chloro-1*H*-indol-3-yl)(5-chloro-3*H*-indol-3-ylidene)methyl)benzene (14_b):



Chemical Formula: $C_{40}H_{22}C_{l4}N_4$

Molecular Weight: 700.44 g/mol

Melting point: 220 - 225 ^{0}C

Colour and shape: dark red powder

ESI-MS: (m/z) = 701.10 [M^++H], 699.27 [M^+-H]

IR-Spectrum:(ATR, cm^{-1}) = 1130 (CH=N), 3106 – 3450 (br. NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 6.64 (s, 2H), 6.87 (s, 2H), 6.97 (dd, 2H, J=2.3, 8.6 Hz), 7.12 (d, 2H, J=7.9 Hz), 7.29 - 7.34 (m, 4H), 7.42 (d, 2H, J=7.8 Hz), 7.47 - 7.53 (m, 4H), 8.09 (s, 2H), 10.26 (s,br, 2H, 2NH)

Elemental Analysis: Calcd. C, 68.59; H, 3.17; Cl, 20.25; N, 8.00

Found C, 68.61, H, 3.20, Cl, 20.19, N, 7.98

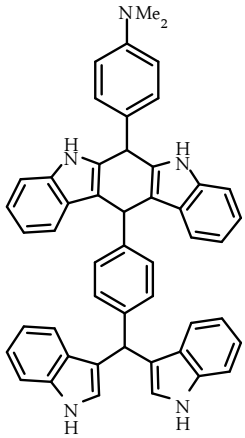
R_f-Value: 0.33 (7 % MeOH/CH_2Cl_2)

Yield: (630 mg), 90 %

5.1.4.12. General procedure for the preparation of compounds 15 and 16_{a-f}:

To a flask containing 50 ml MeOH 1mmol, 0.567 mg of compound **10_a** was added under stirring and heating until it completely dissolved. Then 2 mmol of the appropriate aromatic or heterocyclic aldehyde was added to the reaction mixture, and after the aldehyde was dissolved, a few drops of conc. H_2SO_4 were added drop wisely, where the reaction solution became pink colour. Then the reaction mixture was left stirring for about 1h under reflux. The reaction afforded a precipitate and when the reaction was finished, the precipitate was filtered off while the solution was still hot, dried to afford compound **16_{a-e}**, which was purified by passing over a column and eluted with 30 % Et.Ac/hexane. Whereas the monocondensed product (**15**) was formed by leaving the same reactants in a ratio of 1 mmol, 0.567 mg of compound **10_a** and 2 mmol, 0.3 gm of 4-*N*,*N*-dimethylaminobenzaldehyde under stirring at room temperature for a long time.

4-(8-(4-(Di(1*H*-indol-3-yl)methyl)phenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazol-2-yl)-*N*,*N*-dimethylaniline (15):



Chemical Formula: $C_{49}H_{39}N_5$

Molecular Weight: 697.87 g/mol

Melting point: 218 - 220 ^{0}C

Colour and shape: light brown powder

ESI-MS: (m/z) = 699.12 [M^{+}+H], 697.09 [M^{+}-H]

IR-Spectrum: (ATR, cm^{-1}) = 1590 ($N(Me)_2$), 2850, 2922 (CH), 3407 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 2.80 (s, 3H, CH_3), 2.84 (s, 3H, CH_3), 5.93 (s, 2H, 2CH), 6.18 (s, 1H, CH), 6.85 (s, 4H), 6.91 (t, 4H, J=7.3 Hz), 7.10 (t, 6H, J=7.6 Hz), 7.12 - 7.34 (m, 4H), 7.39 (t, 8H, J=8.6 Hz), 9.96 (s, 2H, 2NH)

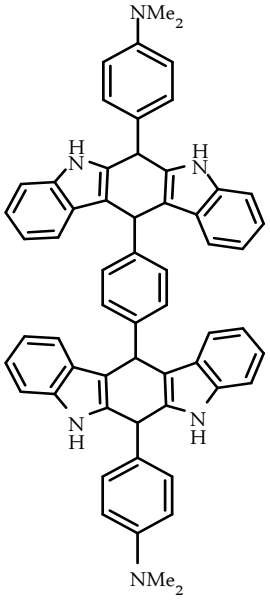
^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 29.95 (CH_3), 30.05 (CH_3), 30.26 (CH), 40.86 (CH), 40.93 (CH), 111.90, 112.08, 112.24, 114.01, 118.32, 119.24, 120.10, 120.35, 121.96, 123.15, 123.92, 124.48, 125.18, 128.17, 129.21, 131.82, 133.07, 134.17, 137.33, 143.48

Elemental Analysis: Calcd. C, 84.33; H, 5.63; N, 10.04

Found C, 84.24, H, 5.70, N, 10.00

R_f-Value: 0.6 (CH_2Cl_2)

Yield: (426 mg), 61 %



4,4'-(8,8'-(1,4-Phenylene)bis(1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole-8,2-diyl))bis(*N*,*N*-dimethylaniline (16_a):

Chemical Formula: $C_{58}H_{48}N_6$

Molecular Weight: 829.04 g/mol

Melting point: 140 - 144 ^{0}C

Colour and shape: pink powder

ESI-MS: (m/z) = 828.26 [M^{+}-H]

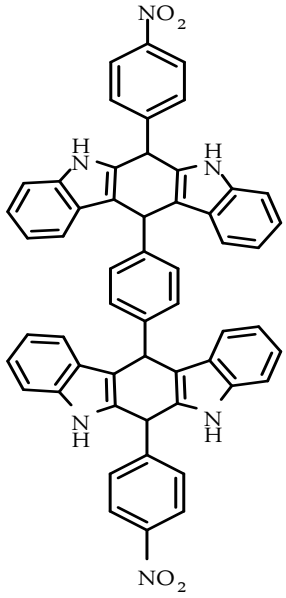
IR-Spectrum: (ATR, cm^{-1}) = 1540 ($N(Me)_2$), 3377 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 2.79 (s, 6H, $2CH_3$), 2.98 (s, 6H, $2CH_3$), 5.79 (s, 4H, 4CH), 6.69 (d, 8H, J=8.8 Hz), 6.78 (t, 4H, J=7 Hz), 6.95 (t, 4H, J=6.7 Hz), 7.26 (t, 8H, J=7.3 Hz), 7.62 (d, 4H, J=9 Hz), 9.84 (s, br., 4H, 4NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 26.64 (Me), 39.23 (CH), 40.01 (CH), 111.07, 111.24, 118.39, 118.64, 119.25, 119.50, 121.11, 121.38, 123.48, 123.63, 125.47, 127.31, 128.36,129.36, 129.41, 131.38, 137.06, 137.21, 142.59

R_f-Value: 0.44 (100 % CH_2Cl_2)

Yield: (580 mg), 70 %



1,4-Bis(2-(4-Nitrophenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazol-8-yl)benzene (16_b):

Chemical Formula: $C_{54}H_{36}N_6O_4$

Molecular Weight: 832.90 g/mol

Melting point: 260 – 262 ^{0}C

Colour and shape: yellow powder

ESI-MS: (m/z) = 832.43 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1310, 1516 (NO_2), 2852, 2921 (CH), 3406 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 6.02 (s, 4H, 4CH), 6.86 -6.97 (m, 8H), 7.04 (t, 4H, J=7.5 Hz), 7.27 (d, 4H, J=8 Hz), 7.35 (d, 4H, J=8.3 Hz), 7.59 (d, 4H, J=8.6 Hz, 8.15 (d, 4H, J=8.7 Hz), 10.91 (s, br., 4H, 4NH)

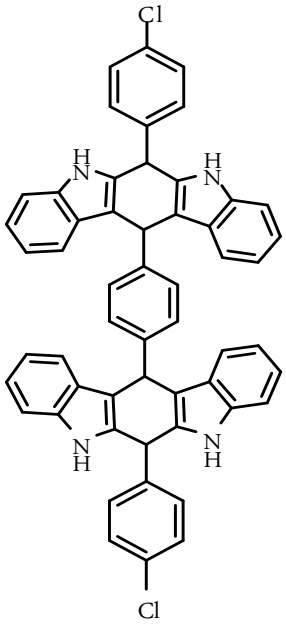
^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 29.46 (CH), 30.87 (CH), 108.32, 110.33, 111.05, 111.92, 112.06, 112.50, 115.20, 116.14, 117.15, 117.82, 118.34, 118.89, 119.38, 121.02, 121.57, 122.00, 123.89, 124.33, 126.84, 128.50, 129.93, 137.06, 144.22, 146.00, 147.90

Elemental Analysis: Calcd. C, 77.87; H, 4.36; N, 10.09

Found C, 78.00, H, 4.39, N, 10.12

R_f-Value: 0.77 (CH_2Cl_2)

Yield: (433 mg), 52 %



1,4-Bis(2-(4-Chlorophenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazol-8-yl)benzene (16_c):

Chemical Formula: $C_{54}H_{36}Cl_2N_4$

Molecular Weight: 811.80 g/mol

Melting point: >350 ^{0}C

Colour and shape: yellow powder

ESI-MS: (m/z) = 811.06 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 2863, 2922 (CH), 3410 (NH)

^{1}H-NMR: (100 MHz, acetone-d_6) δ (ppm) = 5.68 (s, 4H, 4CH), 6.77 (s, 4H), 6.82 (t, 4H, J= 4.1 Hz), 6.86 - 6.91 (m, 4H), 6.95 - 7.11 (m, 4H), 7.14 - 7.25 (m, 2H), 7.27 - 7.30 (m, 4H), 7.39 - 7.46 (m, 2H), 7.62 - 7.73 (m, 4H), 9.92 (s, br., 4H, 4NH)

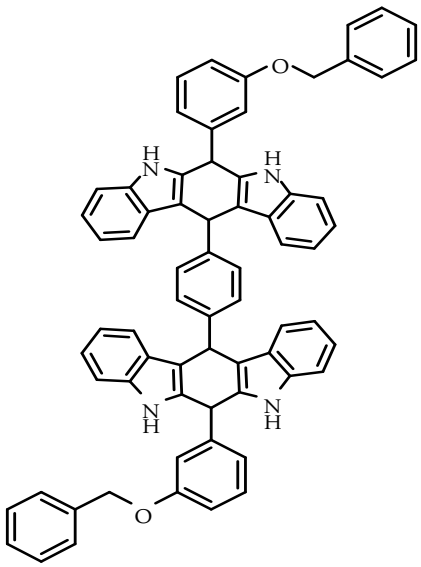
^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 29.71 (CH), 31.77 (CH), 111.24, 111.99, 114.50, 115.40, 117.34, 118.62, 119.37, 121.11, 121.87, 122.40, 124.39, 125.33, 126.14, 127.55, 128.37, 129.12, 129.92, 130.01, 131.13, 132.50, 134.00, 136.62, 137.21, 137.97, 144.15

Elemental Analysis: Calcd. C, 79.89, H, 4.47, Cl, 8.73, N, 6.90

Found C, 80.02, H, 4.51, Cl, 8.75, N, 6.93

R_f-Value: 0.74 (CH_2Cl_2)

Yield: (666 mg), 82 %



1,4-Bis(2-(3-(benzyloxy)phenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazol-8-yl)benzene (16_d):

Chemical Formula: $C_{68}H_{50}N_4O_2$

Molecular Weight: 955.15 g/mol

Melting point : >350 ^{0}C

Colour and shape: yellow powder

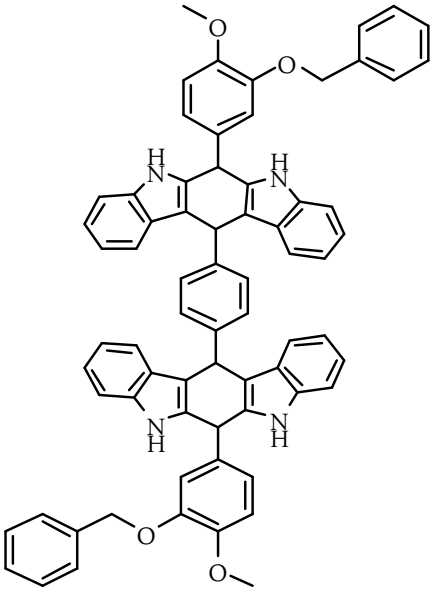
ESI-MS: (m/z) = 954.23 [M^{+}-H]

IR-Spectrum: (ATR, cm^{-1}) = 1456 (C-O), 2922 (CH_2), 3414 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 5.05 (s, 4H, 2CH_2), 5.77 (s, 4H, 4CH), 5.95 - 6.08 (m, 4H), 6.43 - 6.77 (m, 8H), 6.82 - 6.85 (m, 4H), 6.92 - 6.95 (m, 6H), 7.03 - 7.09 (m, 4H), 7.11 - 7.24 (m, 8H), 7.34 - 7.41 (m, 4H), 7.51 (s, 1H), 10.68 (s, br., 4H, 4NH)

R_f-Vlaue: 0.75 (CH_2Cl_2)

Yield: (544 mg), 57 %



1,4-Bis(2-(3-(benzyloxy)-4-methoxyphenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazol-8-yl)benzene (16_e):

Chemical Formula: $C_{70}H_{54}N_4O_4$

Molecular Weight: 1015.20 g/mol

Melting point: 152 - 158 ^{0}C

Colour and shape: yellow powder

ESI-MS: 1014.25 [M^{+}-H]

IR-Spectrum: (ATR, cm^{-1}) = 1453 (C-O), 2922 (CH_2), 3391 (NH)

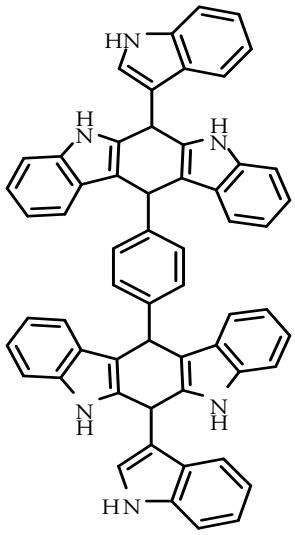
^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 3.69 (s, 3H, OMe), 3.94 (s, 3H, OMe), 5.01 (s, 4H, 2CH_2), 5.58 (s, 4H, 4CH), 6.66 (d, 4H, J=8.51 Hz), 6.76 - 6.71 (m, 6H), 6.86 - 6.93 (m, 6H), 6.97 - 7.18 (m, 4H), 7.21 (t, 6H, J=7.2 Hz), 7.26 - 7.38 (m, 4H), 7.41 (d, 4H, J=7.9 Hz), 7.52 (s, 2H), 10.55 (s, 4H, 4NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 48.40 (CH), 55.38 (OMe), 55.53 (OMe), 69.83 (CH_2-O), 109.32, 109.58, 110.79, 111.17, 112.04, 112.57, 114.55, 117.92, 118.38, 119.16, 120.26, 120.64, 122.13, 123.23, 123.89, 124.76, 125.66, 126.44, 127.53, 127.75, 128.07, 128.18, 129.12, 136.42, 136.87, 137.02, 141.81, 147.19, 147.61, 148.14, 148.52

R_f-Value: 0.69 (CH_2Cl_2)

Yield: (731 mg), 72 %

1,4-Bis(2-(1*H*-indol-3-yl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazol-8-yl)benzene (16_f):



Chemical Formula: $C_{58}H_{40}N_6$

Molecular Weight: 820.98 g/mol

Melting point : 115 - 118 ^{0}C

Colour and shape: light brown powder

ESI-MS: (m/z) = 820.25 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 2853, 2923 (CH), 3391 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 4.21 (s, 4H, 4CH), 6.94 (t, 8H, J=7.4 Hz), 6.99 (s, 2H), 7.05 (d, 4H, J=7.7 Hz), 7.08 (d, 6H, J=6.9 Hz), 7.35 (d, 4H, J=8.1 Hz), 7.55 (d, 6H, J=8.1 Hz), 9.86 (s,br., 4H, 4NH), 9.96 (s,br., 2H, 2NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 30.26 (CH), 111.27, 111.97, 112.02 (3C), 115.83 (2C), 119.16 (3C), 119.36, 119,67 (3C), 121.89 (3C), 123.28, 123.44 (3C), 128.64, 137.87

Elemental Analysis: Calcd: C, 84.85; H, 4.91; N, 10.24

Found C, 84.88, H, 5.01, N, 10.22

R_f-Value: 0.76 (CH_2Cl_2)

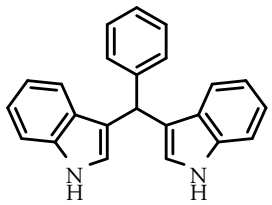
Yield: (402 mg), 49 %

5.1.4.13. General procedure for the preparation of compounds 17_{a-p} :

In a flask containing 5 ml of glacial acetic acid and 2 mmol of indole (0.234 gm) or 5-chloroindole 0.303 mg or 6-chloroindole 0.303 mg was added under stirring until all

the indole was dissolved. Then 1 mmol of the appropriate aromatic or heterocyclic aldehyde was added under vigorous stirring. The reaction mixture was allowed to stir over 4 to 6 h, where the reaction solution turned from light yellow to light pink to dark red colour. The product was detected by TLC (100 % CH_2Cl_2), and when the reaction was finished 10 ml of water were added and the solution was extracted with ethyl acetate, washed with water and brine, dried over anhydrous sodium sulphate and concentrated in vacuum. The product was purified by passing over a column and eluted with dichloromethane.

3,3'-(Phenylmethylene)bis(1*H*-indole) (17_a):



Chemical Formula: $C_{23}H_{18}N_2$

Molecular Weight: 322.40 g/mol

Melting point : 126 - 127 ^{0}C

Colour and shape: pink powder

ESI-MS: (m/z) = 321.32 [M^+-H]

IR-Spectrum: (ATR,cm^{-1}) = 3141 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 5.90 (s, 1H, CH), 6.79 (d, 2H, J=1.5 Hz), 6.87 (t, 2H, J=7.2 Hz), 7.04 (t, 2H, J=7.6 Hz), 7.16 (d, 1H, J=7.3 Hz), 7.25 (t, 2H, J=7.5 Hz), 7.32 - 7.39 (m, 6H), 9.99 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 40.26 (CH), 110.94, 119.68, 120.59, 121.79, 121.85, 123.49, 123.99, 125.99, 126.98, 128.08, 128.59, 136.55, 143.88

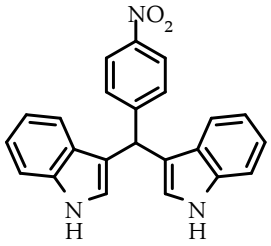
Elemental analysis: Calcd. C, 85.68, H, 5.63, N, 8.69

Found C, 85.72, H, 5.58, N, 8.66

R_f-Value: 0.76 (CH_2Cl_2)

Yield: (580 mg), 90 %

3,3/(4-Nitrophenyl)methylene)bis(1*H*-indole (17_b):



Chemical Formula: $C_{23}H_{17}N_3O_2$

Molecular Weight: 367.40 g/mol

Melting point : 219 - 221 ^{0}C

Colour and shape: yellow powder

ESI-MS: (m/z) = 366.29 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1456, 1507 (C-NO_2), 3052 (CH), 3455 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 5.98 (s, 1H, CH), 6.83 - 6.86 (m, 4H), 7.02 (d, 2H, J=8 Hz), 7.26 (d, 2H, J=7.9 Hz), 7.35 (d, 2H, J=8.1 Hz), 7.56 (d, 2H, J=8.72 Hz), 8.09 (d, 2H, J=8.92 Hz), 10.88 (s, br, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 54.82 (CH), 111.51, 116.62, 118.36, 118.79, 121.04, 123.27, 123.74, 126.26, 129.32, 136.48, 145.65, 152.94

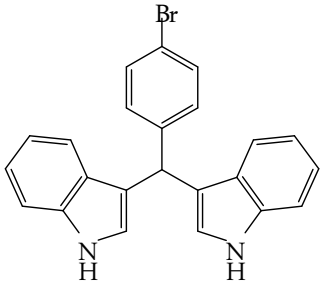
Elemental analysis: Calcd. C, 75.19, H, 4.66, N, 11.44

Found C, 75.28, H, 4.51, N, 11.60

R_f-Value: 0.29 (CH_2Cl_2)

Yield: (720 mg), 98 %

3,3'-((4-bromophenyl)methylene)bis(1H-indole) (17_c):



Chemical Formula: $C_{23}H_{17}BrN_2$

Molecular Weight: 401.30 g/mol

Melting point : 100 - 103 ^{0}C

Colour and shape: yellow crystals

ESI-MS: (m/z) = 402 [M^++H]

IR-Spectrum: (ATR,cm^{-1}) = 4356 (NH)

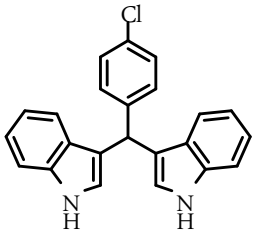
^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 5.91 (s, 1H, CH), 6.79 (d, 2H, J=7.2 Hz), 6.87 (t, 2H, J=7.5 Hz), 7.07 (t, 2H, J=7.4 Hz), 7.28 (d, 2H, J=8 Hz), 7.36 -7.40 (m, 6H), 10.93 (s, 2H, 2NH)

^{13}C-NMR: (400 MHz, acetone-d_6) = 57.50 (CH), 111.40, 117.48, 118.14, 118.99, 119.89, 120.80, 120.99, 123.48, 124.99, 127.89, 129.99, 136.50, 144.02

R_f-Value: 0.65 (CH_2Cl_2)

Yield: (700 mg), 76 %

3,3'-((4-Chlorophenyl)methylene)bis(1H-indole) (17_d):



Chemical Formula: $C_{23}H_{17}N_2Cl$

Molecular Weight: 356.85 g/mol

Melting point: 104 - 106 ^{0}C

Colour and shape: pink powder

ESI-MS: (m/z) = 355.11 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 3410 (NH)

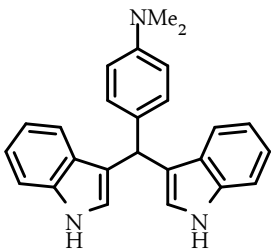
^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 5.85 (s, 1H, CH), 6.83 (d, 2H, J=7.2 Hz), 6.86 (t, 2H, J=7.4 Hz), 7.04 (t, 2H, J=7.6 Hz), 7.28 (d, 2H, J=7.9 Hz), 7.29 - 7.36 (m, 6H), 10.83 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) = 59.65 (CH), 111.38, 117.48, 118.14, 118.89, 119.85, 123.48, 124.99, 127.84, 129.97, 130.16, 136.49, 143.87

R_f-Value: 0.87 (CH_2Cl_2)

Yield: (649 mg), 99 %

4-Di(1*H*-indol-3-yl)methyl)-*N*,*N*-dimethylaniline (17_e):



Chemical Formula: $C_{25}H_{23}N_3$

Molecular Weight: 365.47 g/mol

Melting point : 225 - 226 ^{0}C

Colour and shape: pink powder

ESI-MS: (m/z) = 366.25 [M^++H], 364.38 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 3314 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 4.60 (s,br., 6H, 2CH_3), 5.89 (s, 1H, CH), 6.84 - 6.88 (m, 4H), 7.03 (t, 2H, J=7.99 Hz), 7.28 (d, 2H, J=7.9 Hz), 7.34 (d, 2H, J=8.1 Hz), 7.49 (t, 4H, J=10.6 Hz), 10.84 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 40.13 (CH_3), 43.62 (CH_3), 45.07 (CH), 111.39, 114.52, 117.43, 118.13, 118.85, 119.08, 120.83, 121.40, 123.47, 124.23, 126.37, 129.47, 136.46, 141.84

Elemental analysis: Calcd. C, 82.16; H, 6.34; N, 11.50

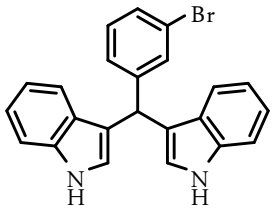
Found C, 82.20, H, 6.37, N, 11.53

R_f-Value: 0.29 (CH_2Cl_2)

Yield: (665 mg), 91 %

3,3'-((3-Bromophenyl)methylene)bis(1H-indole) (17_f):

Chemical Formula: $C_{23}H_{17}BrN_2$



Molecular Weight: 401.30 g/mol

Melting point : 93 - 95 ^{0}C

Colour and shape: red crystals

ESI-MS: (m/z) = 401.26 [M^++H], 399.31 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 3405 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 5.86 (s, 1H, CH), 6.85 - 6.86 (m, 3H), 7.03 (t, 2H, J=7.6 Hz), 7.22 (t, 1H, J=7.8 Hz), 7.28 (d, 2H, J=7.9 Hz), 7.34 - 7.37 (m, 5H), 7.49 (s, 1H), 10.84 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 39.16 (CH), 111.38, 117.20, 118.16, 118.80, 120.84, 121.25, 123.51, 126.32, 127.23, 128.54, 130.08, 130.69, 136.42, 147.78

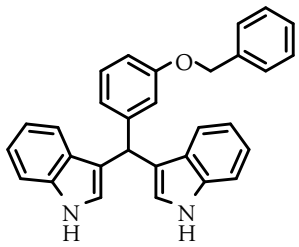
Elemental analysis: Calcd. C, 68.84, H, 4.27, Br, 19.91, N, 6.98

Found C, 68.90, H, 4.30, Br, 19.95, N, 7.00

R_f-Value: 0.74 (CH_2Cl_2)

Yield: (787 mg), 98 %

3,3′-((3-Benzyloxy)phenyl)methylene)bis(1*H*-indole (17$_g$):



Chemical Formula: $C_{30}H_{24}N_2O$

Molecular Weight: 428.52 g/mol

Melting point : 190 - 192 ^{0}C

Colour and shape: white powder

ESI-MS: (m/z) = 428.24 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1262 (C-O), 2852, 3034 (CH), 3425 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 5.01 (s, 2H, CH_2), 5.90 (s, 1H, CH), 6.82 (d, 2H, J=7.5 Hz), 6.85 (d, 2H, J=7.2 Hz), 6.90 (t, 2H, J=7.5 Hz), 7.00 - 7.11 (m, 4H), 7.18 (t, 1H, J=7.9 Hz), 7.26 - 7.33 (m, 2H), 7.37 - 7.39 (m, 6H), 9.95 (s, br., 2H, 2NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 41.18 (CH), 70.31 (CH_2-O), 112.06, 112.91, 116.41, 119.26, 119.63, 120.21, 121.98, 122.13, 123.51, 124.45, 128.04, 128.32, 128.37, 129.07, 129.23, 129.69, 137.98, 138.38, 147.55, 159.66

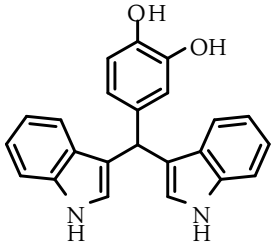
Elemental analysis: Calcd. C, 84.08; H, 5.65; N, 6.54

Found C, 84.12, H, 5.55, N, 6.58

R_f-Value: 0.79 (CH_2Cl_2)

Yield: (746 mg), 87 %

4-(Di(1*H*-indol-3-yl)methyl)benzene-1,2-diol (17$_h$):



Chemical Formula: $C_{23}H_{18}N_2O_2$

Molecular Weight: 354.40 g/mol

Melting point: 105 – 107 ^{0}C

Colour and shape: light brown powder

ESI-MS: (m/z) = 392.89 [M^++K], 354.25 [M^+], 353.24 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1215 (C-O), 2922, 3051 (CH), 3400 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 5.77 (s, 1H, CH), 6.45 (s, 1H), 6.76 (d, 2H, J=8.9 Hz), 6.86 - 6.89 (m, 2H), 7.04 (s, 2H), 7.29 (s, 1H), 7.35 (s, 4H), 7.55 (s, 1H), 9.89 (s, 2H, 2NH)

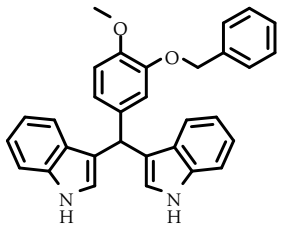
Elemental analysis: Calcd. C, 77.95; H, 5.12; N, 7.90

Found C, 78.01, H, 5.20, N, 7.96

R_f-Value: 0.62 (CH_2Cl_2)

Yield: (517 mg), 73 %

3,3/-(3-Benzyloxy)-4-methoxyphenyl)methylene)bis(1*H*-indole (17$_i$):



Chemical Formula: $C_{31}H_{26}N_2O_2$

Molecular Weight: 458.55 g/mol

Melting point : 75 - 78 ^{0}C

Colour and shape: orange crystals

ESI-MS: (m/z) = 481.16 [M^++Na], 457.24 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1262 (C-O), 2850, 2925 (CH), 3398 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 3.71 (s, 3H, OMe), 4.95 (s, 2H, CH_2), 5.71 (s, 1H, CH), 6.74 - 6.76 (m, 2H), 6.81 - 6.86 (m, 4H), 7.02 (t, 2H, J=7.5 Hz), 7.06 (s, 1H), 7.23 (d, 2H, J=7.9 Hz), 7.29 - 7.31 (m, 6H), 7.34 (s, 1H), 10.73 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 55.59 (CH), 59.70 (OMe), 70.08 (OCH_2), 111.29, 111.98, 114.94, 118.00, 118.24, 119.03, 120.71, 123.29, 126.24, 126.56, 127.63, 127.75, 127.86, 128.18, 128.35, 136.49, 137.09, 137.39, 147.14, 147.38

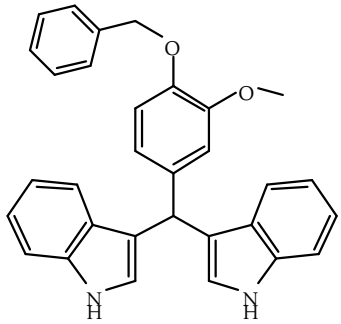
Elemental analysis: Calcd. C, 81.20; H, 5.72; N, 6.11

Found C, 81.22, H, 5.75, N, 6.14

R_f-Value: 0.79 (CH_2Cl_2)

Yield: (816 mg), 89 %

3,3′-((4-Benzyloxy)-3-methoxyphenyl)methylene)bis(1*H*-indole (17$_j$):



Chemical Formula: $C_{31}H_{26}N_2O_2$

Molecular Weight: 458.55 g/mol

Melting point : 215 – 219 ^{0}C

Colour and shape: light orange crystals

ESI-MS: (m/z) = 457.20 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1245 (C-O), 2961, 3036 (CH), 3416 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 3.70 (s, 3H, OMe), 5.04 (s, 2H, CH_2), 5.85 (s, 1H, CH), 6.81 (s, 2H), 6.85 - 6.92 (m, 4H), 7.04 (t, 2H, J=7.6 Hz), 7.09 (s, 1H), 7.29 (d, 1H, J=7.5 Hz), 7.33 - 7.37 (m, 6H), 7.47 (d, 2H, J=7.7 Hz), 9.95 (s, 2H, 2NH)

^{13}C-NMR: (400 MHz, acetone-d_6) δ (ppm) = 40.75 (CH), 56.19 (OMe), 71.61 (OCH_2), 112.05, 112.10, 114.33, 114.93, 119.23, 120.09, 120.32, 121.47, 121.98, 124.32, 124.47, 128.12, 128.45, 128.49, 129.12, 138.08, 138.83, 139.31, 147.72, 150.64

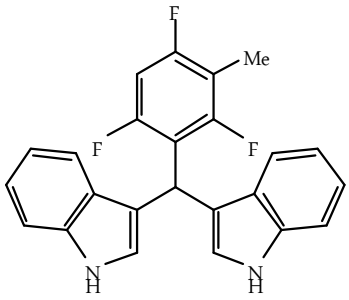
Elemental analysis: Calcd. C, 81.20; H, 5.72; N, 6.11

Found C, 81.02, H, 5.90, N, 6.22

R_f-Value: 0.71 (CH_2Cl_2)

Yield: (844 mg), 92 %

2,4,6-(3,3′-(Trifluoro-3-methylphenyl)methylene)bis(1*H*-indole) (17$_k$):



Chemical Formula: $C_{24}H_{17}F_3N_2$

Molecular Weight: 390.40 g/mol

Melting point : >350 ^{0}C

Colour and shape: white powder

ESI-MS: (m/z) = 391.90 [M^++H], 389.31 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 2960, 3055 (CH), 3443 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 3.15 (s, 3H, Me), 5.73 (s, 1H, CH), 6.86 (t, 1H, J=10.9 Hz), 6.99 - 7.14 (m, 2H), 7.19 (d, 1H, J=8.2 Hz), 7.21 - 7.29 (m, 2H), 7.35 (t, 1H, J=7.7 Hz), 7.44 (s, 1H), 7.66 (d, 1H, J=8.2 Hz), 7.74 (t, 2H, J=10.4 Hz), 8.37 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 38.87 (Me), 52.77 (CH), 109.00, 110.02, 117.32, 119.88, 120.17, 122.73, 126.12, 126.21, 127.37, 128.25, 128.78, 129.21, 134.22, 142.00

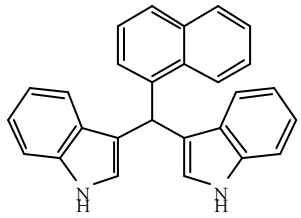
Elemental analysis: Calcd. C, 73.84; H, 4.39; F, 14.60; N, 7.18

Found C, 74.01, H, 4.52, F, 14.52, N, 7.23

R_f-Value: 0.71 (CH_2Cl_2)

Yield: (390 mg), 77 %

3,3/-(Naphthalen-1-ylmethylene)bis(1*H*-indole (17$_l$):



Chemical Formula: $C_{27}H_{20}N_2$

Molecular Weight: 372.46 g/mol

Melting point: 252 - 255 ^{0}C

Colour and shape: White powder

ESI-MS: (m/z) = 371.30 [M^{+}-H]

IR-Spectrum: (ATR, cm^{-1}) = 2834, 3048 (CH), 3407 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 5.71 (s, 1H, CH), 6.59 (s, 1H), 6.68 (d, 2H, J=7 Hz), 6.81 (t, 2H, J=7.5 Hz), 6.99 (t, 2H, J=7.6 Hz), 7.23 (d, 4H, J=8.1 Hz), 7.32 (t, 2H, J=9 Hz), 7.41 (t, 2H, J=7.7 Hz), 7.73 (d, 1H, J=8 Hz), 7.88 (d, 1H, J=7.5 Hz), 8.22 (d, 1H, J=8 Hz), 10.74 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 35.33 (CH), 111.41, 117.62, 118.15, 118.84, 120.77, 123.84, 124.13, 125.15, 125.19, 125.42, 125.68, 126.43, 126.54, 128.42, 131.23, 133.49, 136.56, 140.18

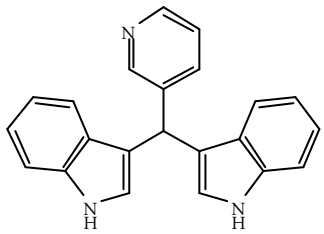
Elemental analysis: Calcd. C, 87.07; H, 5.41; N, 7.52

Found C, 87.00, H, 5.51, N, 7.55

R_f-Value: 0.87 (CH_2Cl_2)

Yield: (722 mg), 97 %

3,3′-(Pyridin-3-ylmethylene)bis(1*H*-indole (17$_m$):



Chemical Formula: $C_{22}H_{17}N_3$

Molecular Weight: 323.39 g/mol

Melting point : 98 - 101 ^{0}C

Colour and shape: light pink powder

ESI-MS: (m/z) = 324.16 [M^++H]

IR-Spectrum: (ATR, cm^{-1}) = 2917, 3055 (CH), 3403 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 5.70 (s, 1H, CH), 5.88 (s, 1H, CH), 6.84 (t, 4H, J=7.1 Hz), 7.01 (t, 2H, J=7.6 Hz), 7.22 - 7.29 (m, 3H), 7.32 (d, 2H, J=8.1 Hz), 7.65 (d, 1H, J=7.9 Hz), 8.34 - 8.37 (m, 1H), 8.58 (d, 1H, J=7.9 Hz), 10.84 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 54.79 (CH), 54.78 (CH), 111.45, 117.07, 118.24, 118.83, 120.94, 123.15, 123.56, 126.29, 135.47, 136.51, 140.15, 146.99, 149.50

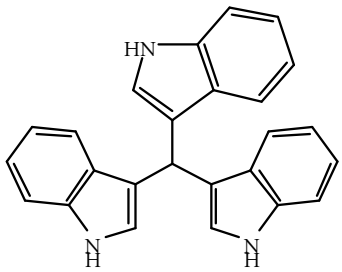
Elemental analysis: Calcd. C, 81.71; H, 5.30; N, 2.99

Found C, 81.90, H, 5.35, N, 13.02

R_f-Value: 0.46 (7 % MeOH/CH_2Cl_2)

Yield: (614 mg), 95 %

Tri(1*H*-indol-3-yl)methane (17$_n$):



Chemical Formula: $C_{25}H_{19}N_3$

Molecular Weight: 361.44 g/mol

Melting point: 235 - 240 ^{0}C

Colour and shape: light yellow powder

ESI-MS: (m/z) = 360.32 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 2882, 3054 (CH), 3424 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 6.19 (s, 1H, CH), 6.85 - 6.93 (m, 6H), 7.03 (t, 4H, J=7.6 Hz), 7.37 (t, 3H, J=7.8 Hz), 7.48 (t, 2H, J=7.4 Hz), 9.88 (s, 3H, 3NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 31.33 (CH), 111.13, 118.95, 119.08, 120.12, 121.09, 123.17, 124.60, 127.35, 128.17, 137.19

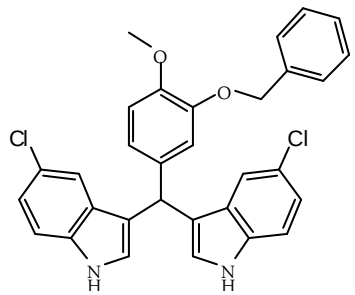
Elemental analysis: Calcd. C, 83.08; H, 5.30; N, 11.63

Found C, 83.09, H, 5.33, N,11.71

R_f-Value: 0.73 (CH_2Cl_2)

Yield: (708 mg), 98 %

3,3′-((3-Benzyloxy)-4-methoxyphenyl)methylene)bis(5-chloro-1*H*-indole (17_o):



Chemical Formula: $C_{31}H_{24}Cl_2N_2O_2$

Molecular Weight: 527.44 g/mol

Melting point: 82 - 85 ^{0}C

ESI-MS: (m/z) = 528.18 [M^++H]

IR-Spectrum: (ATR, cm^{-1}) = 1259 (C-O), 2850, 2924 (CH), 3369 (NH)

^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 3.77 (s, 3H, OMe), 4.93 (s, 2H, CH_2), 5.52 (s, 2H, CH), 6.41 (d, 2H, J=7.6 Hz), 6.73 (t, 4H, J=7.3 Hz), 7.02 (d, 2H, J=7 Hz), 7.13 (d, 2H, J=8.6 Hz), 7.18 (dd, 6H, J=3.1, 7.1 Hz), 7.88 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 39.39 (CH), 55.99 (OMe), 71.03 (OCH_2), 111.51, 111.76, 112.12, 115.37, 119.15, 121.20, 122.31, 124.77, 124.99, 126.91, 127.46, 127.50, 127.66, 127.96, 128.64, 135.04, 135.74, 137.10, 147.63, 148.36

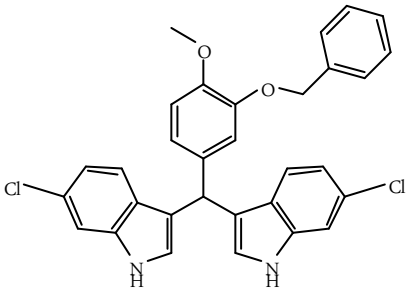
Elemental analysis: Calcd. C, 70.59; H, 4.59; Cl, 13.44; N, 5.31

Found C, 70.62, H, 4.55, Cl, 13.55, N, 5.51

R_f-Value: 0.68 (CH_2Cl_2)

Yield: (960 mg), 91 %

3,3/-((3-(Benzyloxy)-4-methoxyphenyl)methylene)bis(6-chloro-1*H*-indole (17_p):



Chemical Formula: $C_{31}H_{24}Cl_2N_2O_2$

Molecular Weight: 527.44 g/mol

Melting point : 85 – 87 ^{0}C

Colour and shape: light orange crystals

ESI-MS: (m/z) = 526.14 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1253 (C-O), 2866, 2928 (CH), 3420 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 3.70 (s, 3H, OMe), 4.94 (s, 2H, OCH_2), 5.69 (s, 1H, CH), 6.77 (d, 2H, J=2 Hz), 6.79 (d, 1H, J=1.9 Hz), 6.84 (t, 2H, J=7.9 Hz), 7.00 (d, 1H, J=2 Hz), 7.17 (d, 2H, J=8.6 Hz), 7.30 (t, H, J=5.7 Hz), 7.37 (d, 2H, J=1.6 Hz), 10.91 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 26.78 (CH), 55.99 (OMe), 70.41 (OCH_2), 111.47, 112.41, 115.11, 118.79, 118.95, 120.83, 121.08, 125.02, 125.79, 126.10, 127.02, 128.17, 128.29, 128.71, 128.89, 137.22, 137.40, 137.59, 147.70, 147.99

Elemental analysis: Calcd. C, 70.59; H, 4.59; Cl, 13.44; N, 5.31

Found C, 70.63, H, 4.72, Cl, 13.53, N, 5.34

R_f-Value: 0.68 (CH_2Cl_2)

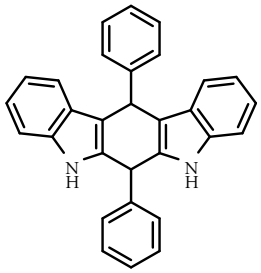
Yield: (960 mg), 91 %

5.1.4.14. General procedure for the preparation of compounds 18_{a-m}:

In a round bottom flask containing 1 mmol of BIMs derivatives $\mathbf{17_{a-p}}$ was stirred with 50 ml MeOH under heating until it completely dissolved. The aromatic or heterocyclic aldehyde 1 mmol which has been used for the synthesis of the BIMs was added and the reaction mixture was stirred under heating until the reaction solution became clear. Then a few drops of conc. H_2SO_4 were added. The reaction solution became pink turned to dark red by refluxing for about 1 h. Upon the reaction

completion, as monitored by TLC (100 % CH_2Cl_2) the reaction was worked up by adding 50 ml water, which was neutralized by NH_4OH addition, extracted with ethylacetate 100ml for two times, washed with water and then brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The crude reaction mixture was purified *via* column chromatography on silica gel using (30 % EtAc/hexane) as a solvent to afford the alternative carbazole derivatives **18_{a-m}**.

2,8-Diphenyl-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (18_a)



Chemical Formula: $C_{30}H_{22}N_2$

Molecular Weight: 410.51 g/mol

Melting point: 352 – 355 ^{0}C

Colour and shape: light brown powder

ESI-MS: (m/z) = 409.35 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 2864, 3018 (CH), 3389 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 5.66 (s, 2H, 2CH), 6.74 (t, 2H, J=7.5 Hz), 6.74 (t, 2H, J=7.5 Hz), 6.91 (t, 2H, J=7.6 Hz), 7.05 (d, 2H, J=7.9 Hz), 7.15 - 7.26 (m, 8H), 7.30 (d, 4H, J=7.1 Hz), 10.63 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 30.57 (CH), 39.40 (CH), 109.67, 110.85, 117.93, 118.24, 120.31, 125.46, 126.14, 128.01, 128.23, 128.98, 129.79, 136.38, 136.88, 143.84 .

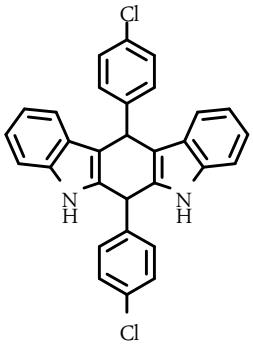
Elemental analysis: Calcd. C, 87.77; H, 5.40; N, 6.82

Found C, 87.79, H, 5.36, N, 6.86

R_f-Value: 0.89 (CH_2Cl_2)

Yield: (333 mg), 81 %

2,8-Bis(4-Chlorophenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (18_b):



Chemical Formula: $C_{30}H_{20}Cl_2N_2$

Molecular Weight: 479.40 g/mol

Melting point: 339 - 342 ^{0}C

Colour and shape: light green powder

ESI-MS: (m/z) = 478.27 [M^+-H]

IR-Spectrum: (ATR,cm^{-1}) = 2922, 3059 (CH), 3414 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 5.69 (s, 2H, 2CH), 6.78 (t, 2H, J=7.5 Hz), 6.84 (t, 2H, J=7.6 Hz), 6.93 (t, 2H, J=7.4 Hz), 7.06 (dd, 2H, J=8, 15.2 Hz), 7.21 (d, 2H, J=8 Hz), 7.24 - 7.32 (m, 3H), 7.40 (d, 1H, J=8 Hz), 7.67 (d, 1H, J=8.3 Hz), 7.75 (d, 1H, J=8.3 Hz), 10.57 (s, 1H, NH), 10.72 (s, 1H, NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 38.66 (CH), 40.17 (CH), 109.54, 111.10, 118.32, 120.73, 125.40, 128.18, 129.26, 130.23, 130.88, 131.93, 132.68, 136.19, 137.09, 142.89

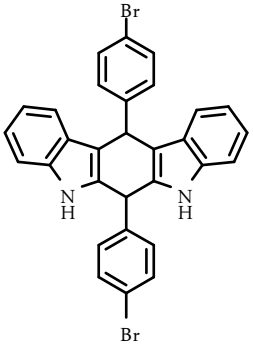
Elemental analysis: Calcd. C, 75.16; H, 4.21; Cl, 14.79; N, 5.84

Found C, 75.18, H, 4.24, Cl, 14.82, N, 5.79

R_f-Value: 0.96 (CH_2Cl_2)

Yield: (268 mg), 56 %

2,8-Bis(4-bromophenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (18_c):



Chemical Formula: $C_{30}H_{20}Br_2N_2$

Molecular Weight: 568.30 g/mol

Melting point : >350 ^{0}C

Colour and shape: yellow powder

ESI-MS: (m/z) = 569.19 [M^++H], 567.12 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 2847, 3054 (CH), 3436 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 5.66 (s, 2H, 2CH), 6.78 (t, 2H, J=7.5 Hz), 6.94 (t, 2H, J=7.5 Hz), 7.05 (d, 2H, J=7.9 Hz), 7.12 - 7.29 (m, 6H), 7.43 (d, 4H, J=8 Hz), 10.71 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 39.02 (CH), 39.99 (CH), 110.22, 112.34, 119.05, 120.22, 126.40, 128.80, 130.55, 136.40, 137.62, 139.00, 143.01

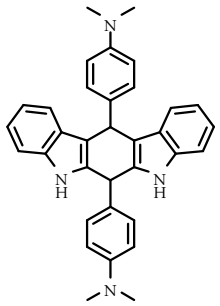
Elemental analysis: Calcd. C, 63.40; H, 3.55; Br, 28.12; N, 4.93

Found C, 63.42, H, 3.58, Br, 28.16, N, 4.98

R_f-Value: 0.87 (CH_2Cl_2)

Yield: (494 mg), 87 %

4,4′-(8,3,2,1-Tetrahydroindolo[2,3-*b*]carbazole-2,8-diyl)bis(*N*,*N*-dimethylaniline) (18_d):



Chemical Formula: $C_{34}H_{32}N_4$

Molecular Weight: 496.64g/mol

Melting point : 324 - 325^{0}C

Colour and shape: dark gray powder

ESI-MS: (m/z) = 497.21 [M^{+}+H]

IR-Spectrum: (ATR, cm^{-1}) = 2980, 3039 (CH), 3304 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 3.05 (s, 12H, 4Me), 5.73 (s, 2H, 2CH), 6.78 (t, 2H, J=7.5 Hz), 6.94 (t, 2H, J=7.5 Hz), 7.09 (d, 2H, J=7.9 Hz), 7.23 (d, 2H, J=8.1 Hz), 7.35 - 7.41 (m, 8H), 10.73 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 38.59 (Me), 44.58 (Me), 52.81 (CH), 109.59, 111.06, 118.27, 118.34, 120.66, 125.39, 127.80, 129.61, 133.32, 136.24, 137.01

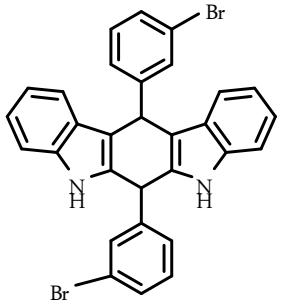
Elemental analysis: Calcd. C, 82.22, H, 6.49, N, 11.28

Found C, 82.25, H, 6.51, N, 11.38

R_f-Value: 0.66 (CH_2Cl_2)

Yield: (452 mg), 91 %

2,8-Bis(3-bromophenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (18_e):



Chemical Formula: $C_{30}H_{20}Br_2N_2$

Molecular Weight: 568.30 g/mol

Melting point : 255 - 257 ^{0}C

Colour and shape: light green powder

ESI-MS: (m/z) = 569.16 [M^++H], 567.01 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 2986, 3058 (CH), 3390 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 5.75 (s, 2H, 2CH), 6.82 (t, 4H, J=7.4 Hz), 6.97 (t, 2H, J=7.3 Hz), 7.09 (d, 2H, J=7.9 Hz), 7.26 (d, 2H, J=7.5 Hz), 7.27 - 7.34 (m, 4H), 7.47 (s, 2H), 10.81 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 40.12 (CH), 109.39, 111.15, 118.31, 120.82, 121.53, 125.32, 127.55, 128.07, 129.32, 130.88, 136.03, 137.06, 146.41, 146.68

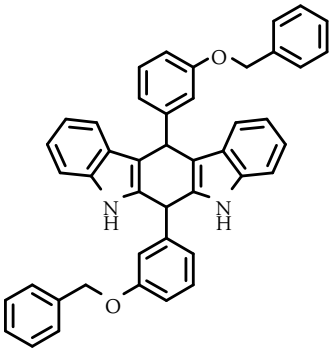
Elemental analysis: Calcd. C, 63.40; H, 3.55; Br, 28.12; N, 4.93

Found C, 63.40, H, 3.58, Br, 28.18, N, 5.00

R_f-Value: 0.92 (CH_2Cl_2)

Yield: (307 mg), 54 %

2,8-Bis(3-(benzyloxy)phenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (18_f):



Chemical Formula: $C_{44}H_{34}N_2O_2$

Molecular Weight: 622.75 g/mol

Melting point : 275 - 279 ^{0}c

Colour and shape: white powder

ESI-MS: (m/z) = 623.26 [M^++H], 621.31 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 2986, 3058 (CH), 3390 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 5.00 (s, 4H, 2CH_2), 5.62 (s, 2H, 2CH), 6.76 (t, 4H, J=7.3 Hz), 6.82 (d, 2H, J=7.9 Hz), 6.93 (t, 2H, J=7.2 Hz), 7.03 (d, 2H, J=7.7 Hz),

7.14 (t, 2H, J=8 Hz), 7.23 (d, 2H, J=7.9 Hz), 7.24-7.32 (m, 6H), 7.37 (d, 4H, J=6.7 Hz), 10.62 (s, 2H, 2NH)

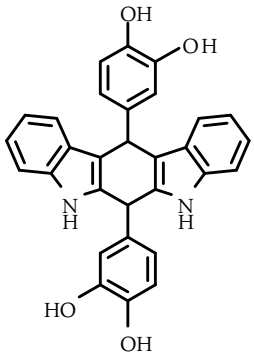
Elemental analysis: Calcd. C, 84.86; H, 5.50; N, 4.50

Found C, 84.89, H, 5.54, N, 4.53

R_f-Value: 0.85 (CH_2Cl_2)

Yield: (448 mg), 72 %

4,4-(1,2,3,8-Tetrahydroindolo[2,3-*b*]carbazole-2,8-diyl)dibenzene-1,2-diol (18_g):



Chemical Formula: $C_{30}H_{22}N_2O_4$

Molecular Weight: 474.51 g/mol

Melting point : 273 - 275 ^{0}C

Colour and shape: dark brown powder

ESI-MS: (m/z) = 475.10 [M^++H], 473.09 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1266 (C-O), 3250 (OH), 3430 (NH)

^{1}H-NMR: (400MHz, acetone-d_6) δ (ppm) = 5.53 (s, 2H, 2CH), 6.65 (d, 2H, J=2 Hz), 6.74 (d, 7H, J=7.9 Hz), 6.78 - 6.92 (m, 4H), 6.94 (t, 2H, J=7 Hz), 7.18 (d, 2H, J=7.9 Hz), 7.25 (d, 2H, J=8 Hz), 7.59 (s,br., 4H, 4OH), 9.75 (s, 2H, 2NH)

^{13}C-NMR: (100MHz, acetone-d_6) δ (ppm) = 40.67 (CH), 111.42, 111.79, 115.93, 116.39, 119.32, 119.97, 121.05, 121.69, 127.63, 136.81, 138.28, 138.55, 144.82, 145.94

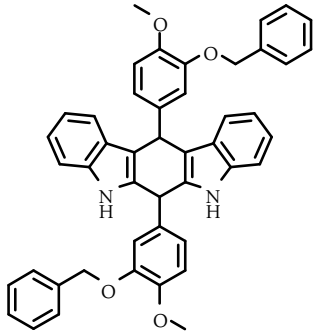
Elemental analysis: Calcd. C, 75.94; H, 4.67; N, 5.90

Found C, 75.99, H, 4.69, N, 5.93

R_f-Value: 0.54 (10 % MeOH/CH_2Cl_2)

Yield: (214 mg), 45 %

2,8-Bis(3-(benzyloxy)-4-methoxyphenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (18$_h$):



Chemical Formula: $C_{46}H_{38}N_2O_4$

Molecular Weight: 682.80 g/mol

Melting point : 310 - 313 ^{0}C

Colour and shape: white powder

ESI-MS: (m/z) = 705.19 [M^++Na], 681.41 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1253 (C-O), 2838, 3045 (CH), 3389 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 3.66 (s, 6H, 2OMe), 5.53 (s, 4H, 2CH_2), 5.69 (s, 2H, 2CH), 6.69 - 6.78 (m, 4H), 6.82 (d, 2H, J=8.3 Hz), 6.92 (t, 2H, J=7.3 Hz), 6.98 (d, 2H, J=7.9 Hz), 7.06 (d, 2H, J=7.7 Hz), 7.19 (d, 2H, J=10.4 Hz), 7.22 - 7.26 (m, 4H), 7.32 (dd, 4H, J=3, 6.6 Hz), 7.41 (d, 4H, J=7.9 Hz), 10.51 (s, 2H, 2NH)

^{13}C-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 38.97 (CH), 55.63 (OMe), 70.09 (OCH_2), 109.68, 110.00, 111.55, 112.44, 114.04, 118.35, 119.21, 119.44, 120.55, 121.00, 123.50, 127.20, 127.55, 127.61, 128.24, 137.19, 137.96, 138.40, 146.83, 149.75

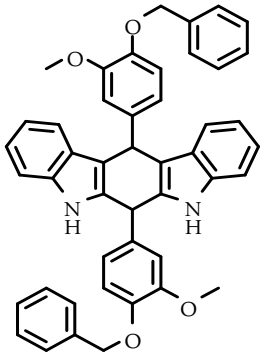
Elemental analysis: Calcd. C, 80.92; H, 5.61; N, 4.10

Found C, 80.95, H, 5.62, N, 4.16

R_f-Value: 0.71 (CH_2Cl_2)

Yield: (574 mg), 84 %

2,8-Bis(4-(benzyloxy)-3-methoxyphenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (18$_i$):



Chemical Formula: $C_{46}H_{38}N_2O_4$

Molecular Weight: 682.80 g/mol

Melting point : 289 - 291 ^{0}c

Colour and shape: dark green powder

ESI-MS: (m/z) = 683.20 [M^++H]

IR-Spectrum: (ATR, cm^{-1}) = 1264 (C-O), 2853, 2922 (CH), 3301 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 3.70 (s, 6H, 2OMe), 5.04 (s, 4H, 2CH_2), 5.84 (s, 2H, 2CH), 6.81 (d, 4H, J=1.7 Hz), 6.84 - 6.92 (m, 4H), 7.04 (t, 4H, J=8 Hz), 7.09 (d, 2H, J=1.9 Hz), 7.28 (d, 2H, J=7.3 Hz), 7.33 - 7.37 (m, 4H), 7.46 (d, 4H, J=7 Hz), 9.95 (s, 2H, 2NH)

^{13}C-NMR: (400MHz, acetone-d_6) δ (ppm) = 39.87 (CH), 55.31 (OMe), 70.72 (OCH_2), 110.98, 111.22, 113.44, 113.98, 114.04, 118.35, 119.20, 119.44, 120.58, 121.10, 123.59, 127.23, 127.56, 127.61, 128.24, 137.19, 137.94, 138.42, 146.83, 149.75

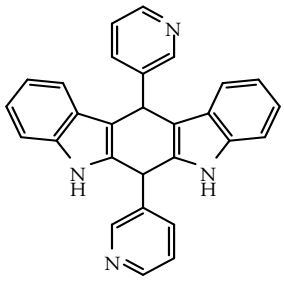
Elemental analysis: Calcd. C, 80.92; H, 5.61; N, 4.10

Found C, 80.95, H, 5.64, N, 4.17

R_f-Value: 0.65 (CH_2Cl_2)

Yield: (600 mg), 88 %

2,8-Di(pyridin-3-yl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (18$_j$):



Chemical Formula: $C_{28}H_{20}N_4$

Molecular Weight: 412.49 g/mol

Melting point : 129 - 132 ^{0}C

Colour and shape: light pink powder

ESI-MS: (m/z) = 413 [M^++H]

EI-MS: (m/z) = 412 [M^+] 10 %, 334 [M^+-pyridine] 5 %, 323 [M^+-pyridine.CH] 100 %, 245 [indolyl.CH.indolyl] 80 %

IR-Spectrum: (ATR, cm^{-1}) = 1338 (C=N), 2853, 2908 (CH), 3398 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 5.43 (s, 1H, CH), 5.89 (s, 1H, CH), 6.86 (d, 4H, J=7.3 Hz), 7.02 (t, 2H, J=7.5 Hz), 7.22 - 7.25 (m, 1H), 7.28 (d, 2H, J=8 Hz), 7.35 (d, 2H, J=7.5 Hz), 7.66 - 7.69 (m, 2H), 8.35 (d, 1H, J=7.6 Hz), 8.51 (d, 1H, J=7.6 Hz), 8.57 (dd, 1H, J=1.6, 11.6 Hz), 10.86 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 37.72 (CH), 53.34 (CH), 101.78, 112.04, 117.67, 118.85, 119.42, 121.55, 123.85, 124.15, 126.89, 134.13, 134.75, 136.09, 137.12, 140.75, 147.56, 148.39, 150.04, 150.06

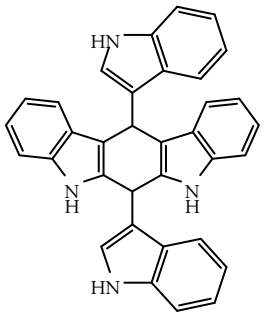
Elemental analysis: Calcd. C, 81.53; H, 4.89; N, 13.58

Found C, 81.50, H, 4.95, N, 13.62

R_f-Value: 0.49 (7 % MeOH/CH_2Cl_2)

Yield: (600 mg), 58 %

2,8-Di(1*H*-indol-3-yl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (18$_k$):



Chemical Formula: $C_{34}H_{24}N_4$

Molecular Weight: 488.58 g/mol

Melting point : 190 - 193 ^{0}C

Colour and shape: light yellow powder

ESI-MS: (m/z) = 489.18 [M^++H]

IR-Spectrum: (ATR, cm^{-1}) = 2852, 2921 (CH), 3406 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 5.85 (s, 2H, 2CH), 6.81 - 6.89 (m, 2H), 7.00 - 7.07 (m, 2H), 7.14 (t, 2H, J=7 Hz), 7.31 - 7.37 (m, 4H), 7.46 (d, 2H, J=7.2 Hz), 7.48 - 7.55 (m, 4H), 7.74 (s, 1H), 8.16 (s, 1H), 9.95 (s, 2H, 2NH), 10.13 (s, 2H, 2NH)

^{13}C-NMR: (100MHz, acetone-d_6) δ (ppm) = 27.42 (CH), 111.22, 112.00, 115.23, 118.55, 119.38, 120.28, 120.55, 121.89, 122.10, 124.52, 124.61, 129.00, 130.32, 138.00, 138.26, 142.55 .

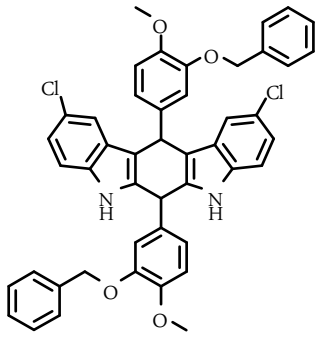
Elemental analysis: Calcd. C, 83.58; H, 4.95; N, 11.47

Found C, 83.55, H, 5.01, N, 11.49

R_f-Value: 0.65 (CH_2Cl_2)

Yield: (229 mg), 47 %

2,8-Bis(3-(benzyloxy)-4-methoxyphenyl)-6,10-dichloro-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (18_l):



Chemical Formula: $C_{46}H_{36}Cl_2N_2O_4$

Molecular Weight: 751.70 g/mol

Melting point : 320 - 322 ^{0}C

Colour and shape: brown powder

ESI-MS: (m/z) = 752.10 [M^++H], 749.17 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1239 (C-O), 2839, 2933 (CH), 3304 (NH)

^{1}H-NMR: (400MHz, DMSO-d_6) δ (ppm) = 3.70 (s, 6H, 2OMe), 4.97 (s, 4H, 2OCH_2), 5.60 (s, 2H, 2CH), 6.82 (dd, 2H, J=1.9, 8.2 Hz), 6.90 (d, 2H, J=8.2 Hz), 6.97 (dd, 2H, J=2, 8.6 Hz), 7.05 (dd, 4H, J=1.9, 8.6 Hz), 7.23 - 7.27 (m, 8H), 7.33 (dd, 4H, J=2.6, 6.7 Hz), 10.81 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 26.29 (CH), 55.53 (OMe), 70.13 (OCH_2), 109.45, 112.19, 112.54, 114.48, 117.67, 120.44, 120.85, 122.64, 126.86, 127.72, 127.89, 128.19, 128.34, 133.00, 135.56, 135.81, 136.89, 138.57, 147.56, 148.00

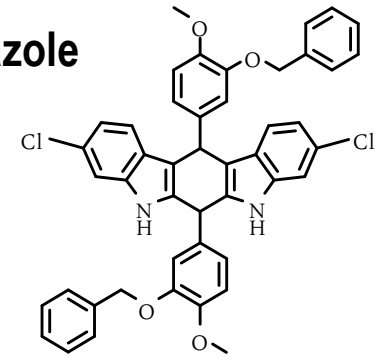
Elemental analysis: Calcd. C, 73.50; H, 4.83; Cl, 9.43; N, 3.73

Found C, 73.52, H, 4.85, Cl, 9.45, N, 3.75

R_f-Value: 0.85 (CH_2Cl_2)

Yield: (669 mg), 89 %

2,8-Bis(3-(benzyloxy)-4-methoxyphenyl)-5,11-dichloro-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (18_m):



Chemical Formula: $C_{46}H_{36}C_{l2}N_2O_4$

Molecular Weight: 751.70 g/mol

Melting point : 322 – 324 ^{0}C

Colour and shape: white powder

ESI-MS: (m/z) = 751.27 [M^+], 752.30 [M^++H], 750.26 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1260 (C-O), 2836, 2927 (CH), 3348 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 3.69 (s, 6H, 2OMe), 4.94 (s, 4H, 2OCH_2), 5.52 (s, 2H, 2CH), 6.71 (dd, 2H, J=1.9, 8.6 Hz), 6.81 (s, 4H), 6.96 (d, 4H, J=7.2 Hz), 7.17 - 7.21 (m, 8H), 7.30 (dd, 4H, J=2, 7.5 Hz), 10.31 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 30.55 (CH), 56.00 (OMe), 74.05 (OCH_2), 108.99, 112.89, 112.99, 114.50, 115.20, 117.68, 120.00, 120.95, 122.90, 126.58, 127.72, 127.89, 128.18, 128.34, 135.56, 135.81, 136.89, 138.55, 147.56, 148.05

Elemental analysis: Calcd. C,73.50; H, 4.83; Cl, 9.43; N, 3.73

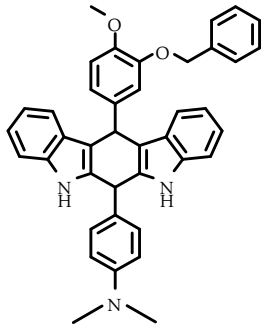
Found C,73.49, H, 4.88, Cl, 9.39, N, 3.80

R_f Value: 0.79 (CH_2Cl_2)

Yield: (676 mg), 90 %

5.1.4.15. Procedure for the preparation of 4-(8-(3-(Benzyloxy)-4-methoxyphenyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazol-2-yl)-*N,N*-dimethylaniline (19):

BIM (**17$_i$**) 1 mmol, 0.5 gm was dissolved in 25 ml of MeOH, and 1 mmol 0.149 mg of *p-N,N*-dimethylaminobenzaldehyde was added to the reaction mixture. The reaction was allowed to stir under reflux until all the reactants had dissolved. After that few drops of conc. H_2SO_4 were dropwisly added. Then the reaction was allowed to stirring under reflux for about one hour. The reaction was worked up by adding 10 ml of water, neutralization with a solution of NH_4OH, extracted by CH_2Cl_2, dried over anhydrous Na_2SO_4, evaporated and purified by column chromatography eluted with CH_2Cl_2.



Chemical Formula: $C_{40}H_{35}N_3O_2$

Molecular Weight: 589.72 g/mol

Melting point: 299 - 301 ^{0}C

Colour and shape: light pink powder

ESI-MS: (m/z) = 590.26 [M^++H]

IR-Spectrum: (ATR, cm^{-1}) = 1214 (C-O), 2833, 2960 (CH), 3416 (NH)

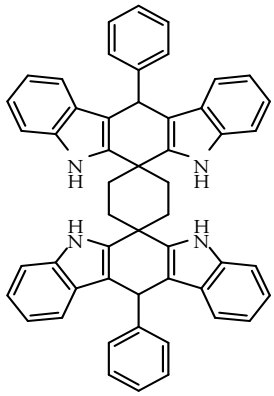
^{1}H-NMR: (100 MHz, acetone-d_6) δ (ppm) = 2.03 (s, 6H, 2Me), 3.78 (s, 3H, OMe), 4.98 (s, 2H, CH_2), 5.79 (s, 2H, 2CH), 6.73 (s, 2H), 6.85 - 6.91 (m, 4H), 7.03 (t, 2H, J=7.2 Hz), 7.09 (d, 2H, J=7.9 Hz), 7.27 - 7.37 (m, 8H), 9.93 (s, br., 2H, 2NH)

R_f-Value: 0.55 (CH_2Cl_2)

Yield: (106 mg), 18 %

5.1.4.16. Procedure of the preparation of the Spirocyclic structure (20):

In a round bottom flask containing 50 ml of MeOH 2 mmol (0.65 mg) of BIMs derivatives **17$_a$** was added under stirring until it completely dissolved. Cyclohexane-1,4-dione (1 mmol, 0.112 mg) was added to the reaction mixture. When the reaction solution became clear, few drops of conc. H_2SO_4 were added slowly. The reaction solution became pink and the colour turned to dark violet by leaving it stirring under reflux for one hour. Upon the reaction completion as monitored by TLC (100 % CH_2Cl_2) the reaction was worked up by added of 50 ml of water, neutralized by NH_4OH, extracted with ethylacetate 200 ml two times washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The crude reaction mixture was purified *via* column chromatography on silica gel eluted with (30 % EtAc/hexane) to afford compound **20** in a moderate yield.



Chemical Formula: $C_{52}H_{40}N_4$

Molecular Weight: 720.90 g/mol

Melting point: 149-152 ^{0}C

Colour and shape: light pink powder

ESI-MS: (m/z) = 719.29 [M^+-H]

EI-MS: (m/z) = 720 [M^+] 32 %, 322 [3,3'-(phenylmethylene)bis(1H-indole)] 100 %, 245 [indolyl.CH.indolyl] 75 %, 117 [indolyl] 75 %, 90 [Ph.CH] 31 %

IR-Spectrum: (ATR, cm^{-1}) = 2852, 2921 (CH), 3409 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 2.03 (t, 4H, 2CH_2, J=7 Hz), 2.27 (t, 4H, 2CH_2, J=11.4 Hz), 5.91 (s, 2H, 2CH), 6.79 (s, 2H), 6.88 (t, 2H, J=7.5 Hz), 7.04 (t, 4H, J=7.6 Hz), 7.11 - 7.20 (m, 4H), 7.25 (t, 4H, J=7.5 Hz), 7.35 (dd, 4H, J=7.9, 15.8 Hz), 7.38 (d, 4H, J=8 Hz), 7.47 (t, 2H, J=8.8 Hz), 9.94 (s, br., 2H, 2NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 26.69 (CH_2), 26.96 (CH_2), 29.66 (CH), 29.69 (C), 110.22, 111.73, 117.21, 118.48, 120.39, 122.24, 123.27, 125.58, 125.65, 126.72, 127.82, 128.22, 128.50, 128.82, 128.84, 129.46, 130.09, 130.86, 130.89, 134.11, 137.03, 140.96

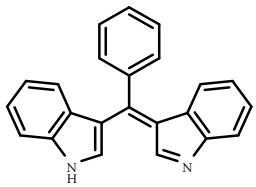
R_f-Value: 0.97 (CH_2Cl_2)

Yield: (375 mg), 52 %

5.1.4.17. General procedure for the preparation of compounds 21_{a-l}:

1 Mml of the alternative BIMs from the list of compounds $\mathbf{17_{a-p}}$ was dissolved in 25 ml of MeOH and TCQ (tetrachloroquinone) 1.5 mmol, 0.37 mg was added to the reaction mixture. Then the reaction was allowed to stir under reflux for 1-2 h until the reaction was finished as monitored by TLC (5 % MeOH/CH_2Cl). when the reaction was finished the dark red solution was concentrated in vacuum, and the product was purified by column chromatography eluted at first with 1 L of (2.5 % MeOH/CH_2Cl_2) then with (5 % MeOH/CH_2Cl_2) to afford the pure colord compound $\mathbf{21_{a-l}}$.

3-((1*H*-indol-3-yl)(phenyl)methylene)-3*H*-indole (21_a):



Chemical Formula: $C_{23}H_{16}N_2$

Molecular Weight: 320.39 g/mol

Melting point : 210 - 215 ^{0}C

Colour and shape: dark red powder

ESI-MS: (m/z) = 321.36 [M^++H], 319.26 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1404 (C=N), 1475 (C=C)

^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 6.75 (d, 3H, J=7.8 Hz), 6.96 (t, 3H, J=7.9 Hz), 7.19 - 7.23 (m, 3H), 7.45 - 7.51 (m, 3H), 7.64 (d, 3H, J=7.5 Hz)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm): 98.38, 114.97, 116.28, 120.94, 122.02, 123.64, 125.38, 127.07, 129.13, 130.47, 131.93, 132.53, 138.36, 141.83, 147.28, 151.87

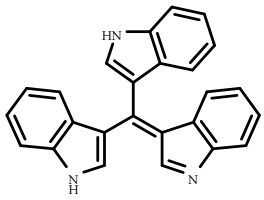
Elemental analysis: Calcd. C, 86.22; H, 5.03; N, 8.74

Found C, 86.19, H, 5.00, N, 8.74

R_f-Value: 0.23 (10 % MeOH/CH_2Cl_2)

Yield: (263 mg), 82 %

3,3′-(3*H*-indol-3-ylidene)methylene)bis(1*H*-indole) (21_b):



Chemical Formula: $C_{25}H_{17}N_3$

Molecular Weight: 359.14g/mol

Melting point : > 350 ^{0}C

Colour and shape: dark red powder

ESI-MS: (m/z) = 360.31 [M^++H], 358.26 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}): 1411 (CH=N)

^{1}H-NMR: (400MHz, DMSO-d_6) δ (ppm) = 6.89 (d, 3H, J=7.8 Hz), 7.02 (t, 3H, J=7.6 Hz), 7.27 (dd, 3H, J=3.9, 11.4 Hz), 7.69 (d, 3H, J=7.8 Hz), 8.34 (s, 3H)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 113.86, 113.99, 115.38, 118.09, 120.62, 120.80, 122.95, 123.52, 124.45, 126.52, 130.02, 139.06, 142.53, 160.36

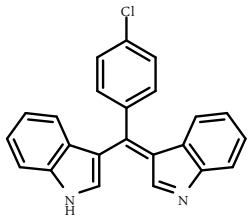
Elemental analysis: Calcd. C, 83.54; H, 4.77; N, 11.69

Found C, 83.57, H, 5.01, N, 11.99

R_f-Value: 0.29 (7 % MeOH/CH_2Cl_2)

Yield: (305 mg), 85 %

3-((4-chlorophenyl)(1*H*-indol-3-yl)methylene)-3*H*-indole (21$_c$):



Chemical Formula: $C_{23}H_{15}ClN_2$

Molecular Weight: 354.83 g/mol

Melting point : 136 - 140 ^{0}C

Colour and shape: dark red powder

ESI-MS: (m/z) = 355.22 [M^++H], 353.39 [M^+-H]

IR-Spectrum:(ATR, cm^{-1}) = 1435 (C=N), 1502 (C=C)

^{1}H-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 6.84 (d, 2H, J=7.9 Hz), 7.03 (t, 2H, J=7.6 Hz), 7.28 (d, 2H, J=7.4 Hz), 7.49 (d, 4H, J=7.5 Hz), 7.66 (d, 2H, J=8.2 Hz), 7.93 (s, 2H)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 110.91, 112.31, 115.73, 118.20, 119.32, 121.31, 123.55, 123.81, 125.83, 127.54, 129.33, 133.28, 137.53, 139.02, 144.23, 146.00, 160(C=N)

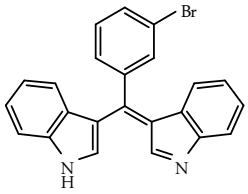
Elemental analysis: Calcd. C, 77.85; H, 4.26; Cl, 9.99; N, 7.89

Found C, 77.91, H, 4.31, Cl, 10.03, N, 8.00

R_f-Value: 0.62 (10 % MeOH/CH_2Cl_2)

Yield: (305 mg), 86 %

3-((3-Bromophenyl)(1*H*-indol-3-yl)methylene)-3*H*-indole (21$_d$):



Chemical Formula: $C_{23}H_{15}BrN_2$

Molecular Weight: 399.28 g/mol

Melting point : 150 - 152 ^{0}C

Colour and shape: dark red powder

ESI-MS: 400.18 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1413 (C=N), 1505 (C=C)

^{1}H-NMR: (100 MHz, acetone-d_6) δ (ppm) = 6.80(d, 2H, J=8 Hz), 7.00 (d, 2H, J=7.4 Hz), 7,25 (t, 2H, J=7.4 Hz), 7.52 (d, 4H, J=6.8 Hz), 7.60 (d, 2H, J=8 Hz), 7.99 (s, 2H)

^{13}C-NMR: (400 MHz, CD_3OD) δ (ppm) = 110.90, 112.13, 116.46, 117.81, 119.63, 120.44, 122.18, 122.35, 124.34, 126.40, 126.63, 129.91, 131.46, 135.02, 136.00, 138.25, 164.65

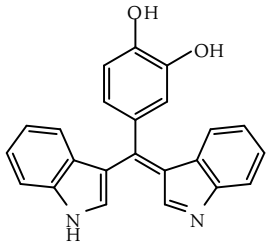
Elemental analysis: Calcd. C, 69.19; H, 3.79; Br, 20.01; N, 7.02

Found C, 69,25, H, 3,95, Br, 20.21, N, 7.30

R_f-Value: 0.26 (7 % MeOH/CH_2Cl_2)

Yield: (327 mg), 82 %

4-((1*H*-indol-3-yl)(3*H*-indol-3-ylidene)methyl)benzene-1,2-diol (21$_e$):



Chemical Formula: $C_{23}H_{16}N_2O_2$

Molecular Weight: 352.39 g/mol

Melting point: 247 – 250 ^{0}C

Colour and shape: dark red powder

ESI-MS: (m/z) = 353.24 [M^++H], 351.27 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1375 (C=N), 1479 (C=C), 3105 (OH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 6.65 (d, 1H, J=8.6 Hz), 6.88 (d, 2H, J=7.8 Hz), 6.96 (t, 4H, J=7.7 Hz), 7.18 (d, 3H, J=7.9 Hz), 7.56 (d, 2H, J=7.9 Hz), 7.88 (s, 1H)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 111.22, 113.64, 115.33, 116.50, 118.74, 119.81, 120.32, 121.69, 123.41, 125.23, 127.39, 130.01, 134.00, 146.03, 148.03, 151.02, 171.96 (C=N)

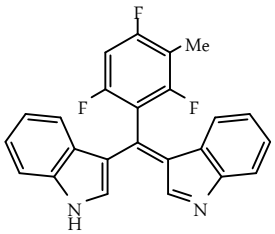
Elemental analysis: Calcd. C, 78.39; H, 4.58; N, 7.95

Found C, 78.43, H, 4.82, N, 7.98

R_f-Value: 0.23 (10 % MeOH/CH_2Cl_2)

Yield: (278 mg), 79 %

3-((1*H*-indol-3-yl)(2,4,6-trifluoro-3-methylphenyl)methylene)-3*H*-indole (21_f):



Chemical Formula: $C_{24}H_{15}F_3N_2$

Molecular Weight: 388.38 g/mol

Melting point : 155 - 158 ^{0}C

Colour and shape: dark red powder

ESI-MS: (m/z) = 389.31 [M^++H]

IR-Spectrum: (ATR, cm^{-1}) = 1329 (C=N), 1455 (C=C), 2852, 2921 (CH_3)

^{1}H-NMR: (400 MHz, CD_3OD) δ (ppm) = 3.30 (s, 3H, Me), 6.32 - 6.40 (m, 1H), 6.83 - 6.95 (m, 2H), 6.96 - 7.00 (m, 3H), 7.19 (d, 1H, J=7.6 Hz), 7.34 (d, 1H, J=7 Hz), 7.36 (d, 1H, J=7.5 Hz), 7.50 (d, 1H, J=7.2 Hz), 7.50 (d, 1H, J=6.9 Hz)

^{13}C-NMR: (100 MHz, CD_3OD) δ (ppm) = 38.11 (Me), 110.22, 112.21, 116.23 119.92, 120.99, 121.96, 122.11, 123.25, 125.31, 129.50, 132.02, 138.00, 145.03, 158.00, 162.32, 163.21, 163.51

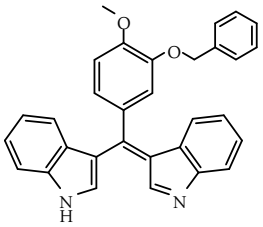
Elemental analysis: Calcd. C, 74.22; H, 3.89; F, 14.67; N, 7.21

Found C, 74.30, H, 4.00, F, 14.69, N, 7.23

R_f-Value: 0.35 (7 % MeOH/CH_2Cl_2)

Yield: (590 mg), 76 %

3-((3-(benzyloxy)-4-methoxyphenyl)(1*H*-indol-3-yl)methylene)-3*H*-indole (21_g):



Chemical Formula: $C_{31}H_{24}N_2O_2$

Molecular Weight: 456.53 g/mol

Melting point : > 350 ^{0}C

Colour and shape: red powder

ESI-MS: (m/z) = 457.11 [M^++H], 455.17 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1140 (C-O), 1414 (C=N), 1484 (C=C), 2847, 2926 (CH_2)

^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 4.03 (s, 3H, OMe), 4.94 (s, 2H, OCH_2), 6.89 (s, 2H), 7.01 (s, 3H), 7.24 (s, 10H), 7.82 (d, 2H, J=6.7 Hz), 7.92 (s, 1H)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 56.38 (OMe), 71.29 (OCH_2), 107.55, 111.62, 115.01, 121.02, 122.50, 124.29, 125.87, 126.32, 127.27, 128.26, 128.62, 129.30, 130.14, 132.65, 135.87, 136.40, 139.70, 140.75, 144.42, 147.33, 149.00, 150.43, 169.52 (C=N)

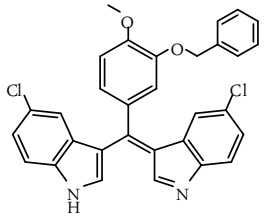
Elemental analysis: Calcd. C, 81.56; H, 5.30; N, 6.14

Found C, 81.49, H, 5.32, N, 6.10

R_f-Value: 0.54 (10 % MeOH/CH_2Cl_2)

Yield: (803 mg), 88 %

3-((3-(Bnzyloxy)-4-methoxyphenyl)(5-chloro-1*H*-indol-3-yl)methylene)-5-chloro-3*H*-indole (21_i):



Chemical Formula: $C_{31}H_{22}Cl_2N_2O_2$

Molecular Weight: 525.42 g/mol

Melting point : 149 - 151 ^{0}C

Colour and shape: red powder

ESI-MS: (m/z) = 526.15 [M^++H], 524.08 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1199 (C-O), 1377 (C=N), 1454 (C=C), 2851, 2921 (CH_3)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 3.89 (s, 3H, OMe), 4.98 (s, 2H, OCH_2), 6.78 (d, 2H, J=7.5 Hz), 7.15 - 7.18 (m, 6H), 7.22 (d, 2H, J=8.2 Hz), 7.27 (d, 2H, J=7.5 Hz), 7.62 (dd, 2H, J=4.6, 11.2 Hz), 8.03 (s, 2H)

^{13}C-NMR: (100 MHz, acetone-d_6) = 55.62 (OMe), 70.94 (OCH_2), 100.00, 111.89, 112.20, 114.50, 117.31, 118.00, 120.76, 121.06, 124.77, 126.52, 127.55, 127.81, 127.97, 128.32, 129.30, 129.68, 130.11, 135.84, 137.09, 139.35, 142.33, 153.00, 165.50 (C=N)

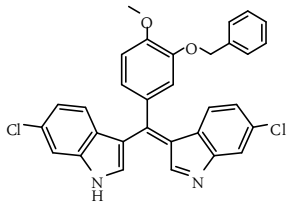
Elemental analysis: Calcd. C, 70.86; H, 4.22; Cl, 13.49; N, 5.33

Found C, 70.89, H, 4.25, Cl, 14.00, N, 5.34

R_f-Value: 0.46 (7 % MeOH/CH_2Cl_2)

Yield: (820 mg), 78 %

3-((3-(Benzyloxy)-4-methoxyphenyl)(6-chloro-1*H*-indol-3-yl)methylene)-6-chloro-3*H*-indole (21_j):



Chemical Formula: $C_{31}H_{22}Cl_2N_2O_2$

Molecular Weight: 525.42 g/mol

Melting point : 125 - 127 ^{0}C

Colour and shape: red powder

ESI-MS: (m/z) = 526.13 [M^++H], 524.18 [M^+-H].

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 3.84 (s, 3H, OMe), 4.94 (s, 2H, OCH_2), 6.69 (d, 2H, J=8.6 Hz), 6.83 (d, 2H, J=1.9 Hz), 6.99 - 7.04 (m, 2H), 7.09 (d, 2H, J=1.9 Hz), 7.12 - 7.19 (m, 2H), 7,24 (d, 2H, J=6.2 Hz), 7.48 (d, 2H, 7.5 Hz), 7.87 (s, 2H)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 55.49 (OMe), 70.64 (OCH_2), 106.00, 111.73, 115.95, 117.83, 122.32, 122.65, 123.61, 125.48, 126.49, 126.69, 127.10, 127.51, 127.74, 127.76, 128.29, 129.76, 131.66, 137.14, 147.73, 148.26, 153.01, 153.21, 162.63 (C=N) .

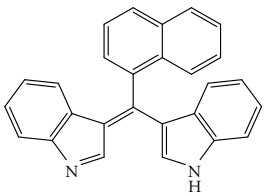
Elemental analysis: Calcd. C, 70.86; H, 4.22; Cl, 13.49; N, 5.33

Found C, 70.89, H, 426, Cl, 13.52, N, 5.35

R_f-Value: 0.36 (7 % MeOH/CH_2Cl_2)

Yield: (872 mg), 83 %

3-((1*H*-indol-3-yl)(naphthalen-1-yl)methylene)-3*H*-indole (21_k):



Chemical Formula: $C_{27}H_{18}N_2$

Molecular Weight: 370.45 g/mol

Melting point : >350 ^{0}C

Colour and shape: red powder

ESI-MS: (m/z) = 371.24 [M^++H]

IR-Spectrum:(ATR, cm^{-1}) = 1406 (C=N), 1476 (C=C)

^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 6.23 (s, 1H), 6.84 (t, 1H, J=7.7 Hz), 7.19 (t, 2H, J=7.8 Hz), 7.24 (s, 6H), 7.49 (t, 1H, J=6.3 Hz), 7.62 (d, 2H, J=7.9 Hz), 7.78 (d, 1H, J=7.8 Hz), 7.99 (d, 1H, J=8.2 Hz), 8.19 - 8.21 (m, 1H), 8.45 (s, 1H).

Elemental analysis: Calcd. C, 87.54; H, 4.90; N, 7.56

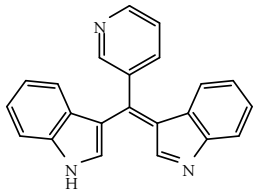
Found C, 87.58, H, 4.95, N, 7.59

R_f-Value: 0.15 (7 % MeOH/CH_2Cl_2)

Yield: (630 mg), 85 %

3-((1*H*-indol-3-yl)(pyridin-3-yl)methylene)-3*H*-indole (21_l):

Chemical Formula: $C_{22}H_{15}N_3$



Molecular Weight: 321.37 g/mol

Melting point : >350 ^{0}C

Colour and shape: dark red powder

ESI-MS: (m/z) = 322.23 [M^++H], 320.49 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1410 (C=N), 1535 (C=C)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 6.77 (d, 1H, J=7.8 Hz), 6.99 (t, 1H, J=7.7 Hz), 7.24 (t, 2H, J=7.5 Hz), 7.46 - 7.49 (m, 1H), 7.59 (d, 1H, J=8.2 Hz), 7.86 (d, 1H, J=7.8 Hz), 8.01 (s, 1H), 8.73 (d, 1H, J=7.5 Hz), 8.86 (dd, 1H, J=1.6, 7.7 Hz), 8.97 (s, 4H)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 110.44, 115.83, 119.92, 121.19, 123.54, 123.77, 123.96, 125.95, 127.25, 134.74, 139.46, 143.79, 146.50, 151.33, 151.74, 176.51 (C=N)

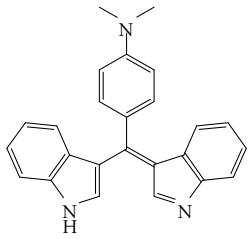
Elemental analysis: Calcd. C, 82.22; H, 4.70; N, 13.08

Found C, 82.15, H,4.55, N, 12.98

R_f-Value: 0.62 (10 % MeOH/CH_2Cl_2)

Yield: (578 mg), 90 %

4-((1*H*-indol-3-yl)(3*H*-indol-3-ylidene)methyl)-*N*,*N*-dimethylaniline (21_m):



Chemical Formula: $C_{25}H_{21}N_3$

Molecular Weight: 363.45 g/mol

Melting point : 232 - 233 ^{0}C

Colour and shape: dark red powder

ESI-MS: (m/z) = 364.30 [M^++H], 362.35 [M^+-H]

^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 3.15 (s, 6H, 2Me), 6.62 (d, 2H, J=9 Hz), 6.95 - 7.01 (m, 4H), 7.18 (t, 2H, J=7.5 Hz), 7.40 (d, 2H, J=9 Hz), 7.68 (d, 4H, J=8.6 Hz)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 40.23 (2C, 2Me), 111.59, 114.0, 120.29, 120.78, 123.18, 124.81, 127.43, 137.4, 139.88, 143.24, 154.85, 175.01

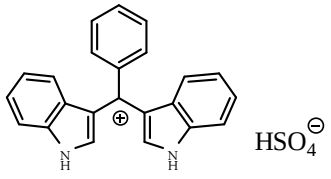
R_f-Value: 0.57 (10 % MeOH/CH_2Cl_2)

Yield: (422 mg), 58 %

5.1.4.18. Procedure for the preparation of the salts $22_{a,b}$:

To a solution of (1 mmol, 0.32 g) of $\mathbf{21_a}$ or (1 mmol, 0.359 g) of $\mathbf{21_b}$ in 10 ml MeOH or CH_3CN an equimolecular amount or excess of conc. H_2SO_4 was added drop by drop under stirring. The mixture was boiled for 10 mints, then the reaction mixture was left to cool at room temperature and the solvent concentrated in a vacuum and the product was purified by column chromatography eluted with (10 % MeOH/CH_2Cl_2) to afford the pure red powder of the monosulfate salt $\mathbf{22_a}$ and $\mathbf{22_b}$ respectively in good yields.

Di(1*H*-indol-3-yl)(phenyl)methylium hydrogenmonosulfate (22_a):



Chemical Formula: $C_{23}H_{18}N_2O$

Molecular Weight: 418.47 g/mol

Melting point : 139 – 142 ^{0}C

Colour and shape: dark red powder

ESI-MS: (m/z) = 322.14 [M^++H], 321.27 [M^+]

^{1}H-NMR: (400 MHz, CD_3Cl_3) δ (ppm) = 6.73 (d, 1H, J=7.8 Hz), 6.96 (t, 1H, J=7.9 Hz), 7.19 - 7.23 (m, 4H), 7.45 - 7.51 (m, 4H), 7.64 (d, 4H, J=8.5 Hz), 7.88 (s, br., 1H).

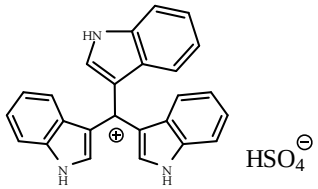
^{13}C-NMR: (100 MHz, CD_3OD) δ (ppm) = 110.72, 111.94, 118.19, 119.59, 119.88, 120.55, 121.77, 121.98, 123.49, 123.99, 125.99, 126.99, 128.38, 129.99, 136.58, 142.99

R_f-Value: 0.1 (10 % MeOH/CH_2Cl_2)

Yield: (511 mg), 61 %

Tri(1*H*-indol-3-yl)methylium hydrogenmonosulfate (22_b):

Chemical Formula: $C_{25}H_{19}N_3O_4S$



Molecular Weight: 457.50g/mol

Melting point : 130 - 135^0 C

Colour and shape: red powder

ESI-MS: (m/z) = 361.32 [M^++H], 360.32 [M^+]

^{1}H-NMR: (400 MHz, CD_3OD) δ (ppm) = 7.03 (s, br., 5H), 7.32 (t, 4H, J=8.2 Hz), 7.68 (d, 4H, J=8.2 Hz), 8.07 (s, br., 3H)

^{13}C-NMR: (100 MHz, CD_3OD) δ (ppm) = 114.56, 122.02, 124.47, 126.17, 128.59, 140.34, 143.32, 163.11

R_f–Value 0.07 (10 % MeOH/CH_2Cl_2)

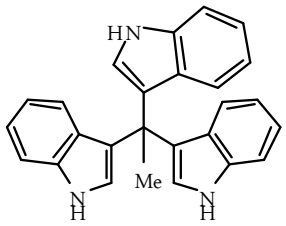
Yield: (714 mg), 78 %

5.1.4.19. Procedure for preparation of compound $23_{a,b}$:

1 mmol (0.159 mg) of acetylindole or 1 mmol (0.121 mg) of acetylpyridine and 2 mmol (0.234 mg) of indole were added to a flask which contained 50 ml MeOH under heating until it completely dissolved. The reaction mixture was stirred under heating until the reaction solution became clear. Then a few drops of conc. H_2SO_4 were added. The reaction solution became pink. The colour turned to dark red by leaving it to stir

under reflux for 1h. Upon the reaction completion as monitored by TLC (5 % $MeOH/CH_2Cl_2$) the reaction was worked up by adding 50 ml water, and neutralized by NH_4OH. The water phase was extracted with ethylacetate 100 ml for two times washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The crude reaction mixture was purified *via* column chromatography on silica gel eluted with (5 % $MeOH/CH_2Cl_2$) to afford compound **23_a** and **23_b**, respectively.

3,3/,3//-(Ethane-1,1,1-triyl)tris(1*H*-indole (23a):



Chemical Formula: $C_{26}H_{21}N_3$

Molecular Weight: 375.47 g/mol

Melting point : 110 - 115 ^{0}C

Colour and shape: light brown powder

ESI-MS: (m/z) = 376.14 [M^++H], 374 [M^+-H]

IR-Spectrum:(ATR, cm^{-1}) = 2923 (CH_3), 3403 (NH)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 2.44 (s, 3H, Me), 6.76 (t, 3H, J=7.5 Hz), 6.92 (d, 3H, J=2 Hz), 6.97 (t, 3H, J=7.6 Hz), 7.34 (d, 2H, J=8.1 Hz), 7.38 (d, 4H, J=8.1 Hz), 9.89 (s, 3H, 3NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 8.60 (Me), 39.86 (CMe), 111.87, 111.92, 118.50, 121.21, 122.28, 123.75, 123.91, 124.12, 124.16, 127.45, 127.48, 138.15, 138.31

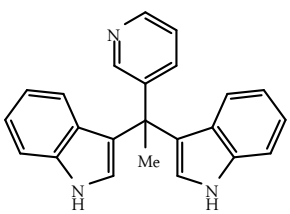
Elemental analysis: Calcd. C, 83.17; H, 5.64; N, 11.19

Found C, 83.18, H, 5.68, N, 11.22

R_f-Value: 0.7 (CH_2Cl_2)

Yield: (391 mg), 52 %

3,3/-(Pyridin-3-yl)ethane-1,1-diyl)bis(1*H*-indole (23b):



Chemical Formula: $C_{23}H_{19}N_3$

Molecular Weight: 337.42 g/mol

Melting point: 180 - 182 ^{0}C

Colour and shape: light yellow crystals

ESI-MS: (m/z) = 338.17 [M^++H]

EI-MS: (m/z) = 337 [M^+] 45 %, 322 [M^+-Me] 100 %, 259 [M^+-pyridyl] 15 %, 220 [M^+-indolyl] 99 %, 205 [M^+-Me-indolyl] 55 %, 117 [indolyl] 98 %, 90 [Ph.CH] 90 %

IR-Spectrum: (ATR, cm^{-1}) = 2920 (CH_3), 3411 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 2.33 (s, 3H, Me), 6.81 (t, 2H, J=7.6Hz), 6.86 (d, 2H, J=7.3 Hz), 7.01 - 7.07 (m, 4H), 7.39 (d, 2H, J=8.1 Hz), 7.95 (dd, 1H, J=7.6, 8.1 Hz), 8.46 (d, 1H, J=8.3 Hz), 8.65 (s, 1H), 8.77 (d, 1H, J=7.4 Hz), 11.13 (d, 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 28.27 (Me), 42.19 (C-Me), 111.88, 118.47, 119.98, 120.13, 120.89, 124.02, 125.15, 126.40, 137.09, 139.58, 140.19, 144.54, 147.79

Elemental analysis: Calcd. C, 81.87; H, 5.68; N, 12.45

Found C, 81.89, H, 5.75, N, 12.48

R_f -Value: 0.48 (7 % MeOH/CH_2Cl_2)

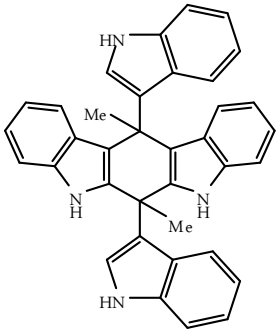
Yield: (371 mg), 55 %

5.1.4.20. Procedure for the preparation of compounds $24_{a,b}$:

Compound $\mathbf{23_a}$ 1 mmol (0.375 mg) or 1 mmol (0.337 mg) of compound $\mathbf{23_b}$ and 1 mmol (0.159 mg) of acetylindole or 1 mmol (0.121 mg) of acetylpyridine, respectively, were added to a flask containing 50 ml MeOH under heating until it completely dissolved. When the reaction solution became clear a few drops of conc. H_2SO_4 were added. The reaction solution became pink then the colour turned to dark red by leaving it stirring under reflux for one hour. Upon the reaction completion, as monitored by TLC (7.5 % MeOH/CH_2Cl_2) the reaction was worked up by adding 50 ml of water, and neutralized by NH_4OH the water phase was extracted with ethylacetate 100 ml for two times washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The crude reaction mixture was purified *via* column

chromatography on silica gel eluted with (7.5 % MeOH/CH_2Cl_2) affording compounds **24_a** and **24_b** respectively.

2,8-Di(1*H*-indol-3-yl)-2,8-dimethyl-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (24_a):



Chemical Formula: $C_{36}H_{28}N_4$

Molecular Weight: 516.63 g/mol

Melting point: 190 - 193 ^{0}C

Colour and shape: dark violet powder

ESI-MS: (m/z) = 517.45 [M^++H]

EI-MS: (m/z) = 516 [M^+] 25 %, 501 [M^+-Me] 100 %, 117 [indolyl] 30 %

IR-Spectrum: (ATR, cm^{-1}) = 2851, 2923 (CH_3), 3255 (NH)

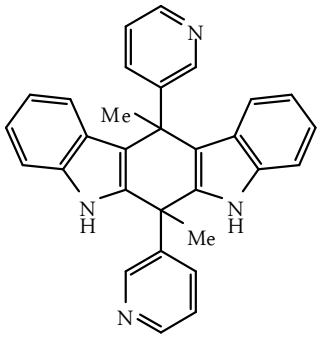
^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 2.31 (s, 3H, Me), 3.29 (s, 3H, Me), 6.99 (t, 2H, J=7.4 Hz), 7.11 (t, 1H, J=7.4 Hz), 7.26 (t, 1H, J=7.5 Hz), 7.34 (d, 3H, J=7.8 Hz), 7.39 (t, 1H, J=7.8 Hz), 7.44 (d, 1H, J=8.4 Hz), 7.64 (d, 1H, J=8 Hz), 7.69 (d, 1H, J=8.4 Hz), 7.79 - 7.84 (m, 3H), 8.19 (d, 1H, J=8 Hz), 8.29 (s, 1H), 8.35 (s, 1H), 8.40 (s, 1H), 11.88 (s, 2H, 2NH), 12.72 (s, 2H, 2NH)

^{13}C-NMR: (400 MHz, acetone-d_6) δ (ppm) = 26.05 (Me), 26.39 (Me), 48.75 (C-Me), 53.27 (C-Me), 113.46, 114.12, 119.36, 120.92, 121.38, 121.62, 122.79, 123.71, 123.79, 123.98, 125.06, 125.37, 126.87, 134.37, 139.17, 141.72

R_f-Value: 0.15 (10 % MeOH/CH_2Cl_2)

Yield: (434 mg), 42 %

2,8-Dimethyl-2,8-di(pyridin-3-yl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (24_b):



Chemical Formula: $C_{30}H_{24}N_4$

Molecular Weight: 440.54 g/mol

Melting point: 155 - 60^0 C

Colour and shape: dark yellow powder

ESI-MS: (m/z) = 442.18 [M^++H], 440.28 [M^+-H]

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 1.43 (s, 3H, Me), 1.85 (s, 3H, Me), 6.74 (s, 1H), 6.80 (t, 1H, J=7.8 Hz), 6.99 - 7.05 (m, 2H), 7.09 - 7.17 (m, 2H), 7.28 - 7.33 (m, 2H), 7.40 (t, 2H, J=9 Hz), 7.73 (dd, 1H, J=1.6, 7.4 Hz), 7.78 (dd, 1H, J=1.6, 7.4 Hz), 8.31 (dd, 1H, J=1.53, 7.73 Hz), 8.43 (dd, 1H, J=1.53, 7.73 Hz), 8.67 (t, 2H, J=7.8 Hz), 10.12 (s, 1H, 1NH), 10.48 (s, 1H, 1NH)

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 18.99 (Me), 28.16 (C), 112.37, 113.19, 119.31, 119.54, 120.25, 120.69, 121.62, 121.85, 122.12, 123.56, 123.61, 123.71, 124.32, 124.43, 126.41, 134.28, 135.54, 138.33, 142.68, 143.18, 145.81, 147.11, 147.61, 148.15, 148.91, 149.89

Elemental analysis: Calcd. C, 81.79, H, 5.49, N, 12.72

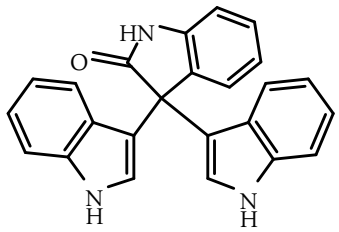
Found C, 81.78, H, 5.52, N, 12.59

R_f-Value: 0.7 (10 % MeOH/CH_2Cl_2)

Yield: (414 mg), 47 %

5.1.4.21. Preparation of 3,3-Di(3-indolyl)-2-indoline (25):

1 Mmol (0.147 mg) of isatin and (2 mmol, 0.234 gm) of indole were added to a flask which contained 50ml of MeOH under stirring and heating until it completely dissolved. When the reaction solution became clear a few drops of conc. H_2SO_4 were added. The reaction solution became pink the colour was turned to dark red by leaving it to about 2 h under stirring and reflux. Upon the reaction completion, as monitored by TLC (5 % MeOH/CH_2Cl_2) the reaction was worked up by adding 50 ml of water, and neutralized by NH_4OH, extracted with ethylacetate 100 ml two times washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The crude reaction mixture was purified *via* column chromatography on silica gel eluted with (5 % MeOH/CH_2Cl_2) to afford compound **25**.



Chemical Formula: $C_{24}H_{17}N_3O$

Molecular Weight: 363.41 g/mol

Melting point : 290 - 293 ^{0}C

Colour and shape: light pink powder

ESI-MS: (m/z) = 363.20 [M^+], 362.28 [M^+-H]

IR-Spectrum: (ATR,cm^{-1}) = 1687 (C=O), 3439 (NH)

^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 6.69 (d, 2H, J=1.9 Hz), 6.78 - 6.82 (m, 2H), 6.97 - 7.05 (m(t,d), 4H, J=7, 7.4 Hz), 7.13 - 7.23 (m, 6H), 7.94 (s, br., 2H, 2NH), 8.45 (s, br., 1H, 1NH)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 53.25, 110.02, 111.36, 114.87, 119.41, 119.66, 120.00, 121.19, 121.61, 121.67, 122.61, 124.05, 125.51, 125.44, 125.82, 126.01, 126.39, 127.96, 134.52, 136.02, 136.92, 139.94, 141.00, 180.03 (C=O)

Elemental analysis: Calcd. C, 79.32; H, 4.72; N, 11.56

Found C, 79.40, H, 4.75, N, 11.61

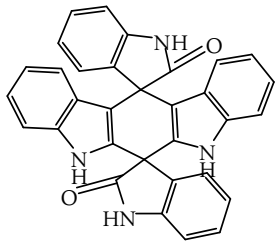
R_f-Value: 0.43 (7 % MeOH/CH_2Cl_2)

Yield: (316 mg), 87 %

5.1.4.22. Preparation of 2,8,2′,8′-Bis(1*H*-indolonyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (26):

1 mmol (0.147 mg) of isatin and 1 mmol (0.363 mg) of compound **25** were added to the flask which contained 50 ml of MeOH under stirring and heating until it completely dissolved. Upon the reaction solution became clear a few drops of conc. H_2SO_4 were added dropwsily. The reaction solution became pink and the colour turned to dark red by leaving it stirring under reflux for about one hour. Upon the reaction completion, as monitored by TLC (5 % MeOH/CH_2Cl_2) the reaction was worked up by added 50 ml of water, neutralized by NH_4OH, extracted with ethylacetate 100ml two times washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in

vacuum. The crude reaction mixture was purified *via* column chromatography on silica gel eluted with (7 % MeOH/CH_2Cl_2) to afford compound **26**.



Chemical Formula: $C_{32}H_{20}N_4O_2$

Molecular Weight: 492.53 g/mol

Melting point : >350 ^{0}C

Colour and shape: light green powder

ESI-MS: (m/z) = 493.16 [M^++H]

IR-Spectrum: (ATR, cm^{-1}) = 1699 (C=O), 3273 – 3450 (br., NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 6.80 - 6.90 (m, 4H), 6.92 - 7.01 (m, 4H), 7.12 - 7.24 (m, 4H), 7.26 - 7.42 (m, 4H), 10.66 (s, br., 2H, 2NH), 10.93 (s, br., 2H, 2NH)

Elemental analysis: Calcd. C, 78.03; H, 4.09; N, 11.38

Found C, 78.00, H, 4.06, N, 11.39

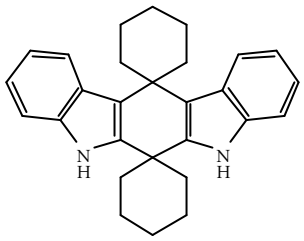
R_f-Value: 0.28 (7 % MeOH/CH_2Cl_2)

Yield: (355 mg), 72 %

5.1.4.23. Preparation of 2,8,2′,8′-Bis(cyclohexyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (27):

2.5 Mmol (0.25 mg) of cyclohexanone and 2 mmol (0.234 mg) of indole were added to a flask which contained 50 ml MeOH under stirring and heating until it completely dissolved. When the reaction solution became clear a few drops of conc. H_2SO_4 were added. The reaction solution became pink then the colour turned to dark red by leaving it to stir under reflux for about one hour. Upon the reaction completion, as monitored by TLC (100 % CH_2Cl_2) the reaction was worked up by adding 50 ml of water. The reaction mixture was neutralized with NH_4OH, extracted with ethylacetate 100 ml for two times washed with water and brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuum. The crude reaction mixture was purified via

column chromatography on silica gel eluted with (100 % CH_2Cl_2) to afford compound **27**.



Chemical Formula: $C_{28}H_{30}N_2$

Molecular Weight: 394.55 g/mol

Melting point : 90 – 92 ^{0}C

Colour and shape: light yellow crystals

EI-MS: (m/z) = 395 [M^+] 20 %, 394 [M^+-H] 65 %, 393 [M^+-2H] 40 %, 314 [M^+-cyclohexanone-2H] 100 %, 285 [314-2CH_2] 20 %, 271 [314-3CH_2] 99 %, 257 [314-4CH_2] 65 %, 245 [indolyl.CH.indolyl] 20 %, 130 [indolyl.CH] 98 %, 117 [indolyl] 95 %, 90 [PhCH] 97 %

IR-Spectrum: (ATR, cm^{-1}) = 2925 (CH_2), 3404 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 1.48 - 1.49 (m, 4H), 1.50 - 1.60 (m, 4H), 1.70 - 1.74 (m, 4H), 2.19 - 2.29 (m(t,t), 4H, J=6.64, 6.5 Hz), 2.39 - 2.46 (m, 4H), 6.65 (t, 2H, J=7.6 Hz), 6.85 (t, 2H, J=7.5 Hz), 7.22 (d, 1H, J=8 Hz), 7.26 (d, 1H, J=7.5 Hz), 7.32 (d, 2H, J=7.9 Hz), 10.66 (s, br., 2H, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 22.55 (CH_2), 26.30 (CH_2), 26.35 (CH_2), 36.63 (CH_2), 38.87 (CH_2), 40.13 (C), 111.21, 117.35, 120.01, 120.49, 121.94, 122.03, 125.85, 136.90

Elemental analysis: Calcd. C, 85.24; H, 7.66; N, 7.10

Found C, 85.18, H, 7.69, N, 7.15

R_f-Value: 0.75 (100 % CH_2Cl_2)

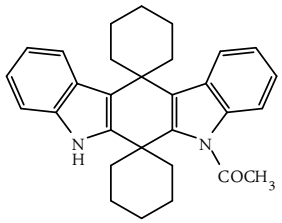
Yield: (383 mg), 97 %

6.1.4.24. General procedure for acetylation reaction (compounds 28 and 29):

Compound **27** (1 mmol, 0.395 mg) was added to a flask containing 5 ml CH_2Cl_2, 0.1 mmol of 4-(dimethylamino)pyridine (DMAP), 1.2 mmol of triethylamine and 2.2 mmol of acetic anhydride. The reaction mixture was left to stirring at room temperature

for several days. The products formation was detected by TLC (100 % CH_2Cl_2). After several days the reaction was worked up and the solution was neutralized with NH_4OH solution, extracted with CH_2Cl_2, washes with water and brine, dried over anhydrous sodium sulphate. The product was purified by using 100 % CH_2Cl_2 to collect the monoacetylated spirocyclic product **28** first and then the diacetylated spirocyclic product **29**.

2,8,2′,8′-Bis(cyclohexyl)-1-acetylindolyl-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (28):



Chemical Formula: $C_{30}H_{32}N_2O$

Molecular Weight: 436.59 g/mol

Melting point : 290 - 293 ^{0}C

Colour and shape: white powder

ESI-MS: (m/z) = 435.34 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1677 (C=O), 2949 (CH_2), 3287 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 1.52 - 1.59 (m, 4H, 2CH_2), 1.67 - 1.73 (m, 6H, 3CH_2), 2.07 (d, 4H, J=7.9 Hz, 2 CH_2), 2.69 (s, 3H, COMe), 6.94 - 6.96 (m, 4H), 7.15 - 7.21 (m, 2H), 7.69 (t, 1H, J=10 Hz), 8.33 (d, 1H, J=9.95 Hz), 10.66 (s, 1H, 1NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 14.54 (Me), 23.23 (CH_2), 23.33 (CH_2), 23.89 (CH_2), 24.47 (CH_2), 24.82 (CH_2), 25.71 (CH_2), 31.13 (CH_2), 35.48 (CH_2), 43.39 (CH_2), 48.82, 55.35, 112.47, 116.45, 118.98, 119.84, 120.63, 120.72, 122.29, 123.37, 124.64, 124.69, 124.91, 126.00, 129.68, 136.21, 140.83, 149.74, 169.66 (C=O).

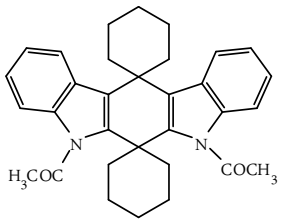
Elemental analysis: Calcd. C, 82.53; H, 7.39; N, 6.42

Found C, 82.56, H, 7.42, N, 6.50

R_f-Value: 0.66 (100 % CH_2Cl_2)

Yield: (258 mg), 59 %

2,8,2/,8/-Bis(cyclohexyl)-bis(1-acetylindolyl)-1,2,3,8-tetrahydroindolo[2,3-*b*]carbazole (29):



Chemical Formula: $C_{32}H_{34}N_2O_2$

Molecular Weight: 478.62 g/mol

Melting point: >350 ^{0}C

Colour and shape: white powder

ESI-MS: (m/z) = 477.52 [M^+-H]

IR-Spectrum:(ATR, cm^{-1}) = 1680 (C=O), 2949 (CH_2)

^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 1.41 - 1.43 (m,4H, 2CH_2), 1.46 - 1.59 (m,4 H, 2CH_2), 1.90 - 1.92 (m, 4H, 2CH_2), 2.14 (d, 4H, J=7.8 Hz), 2.42 (t, 4H, CH_2, J=7.9 Hz), 2.63 (s, 6H, 2COMe), 6.79 - 6.83 (m, 2H), 6.99 (t, 2H, J=7 Hz), 7.42 (d, 2H, J=7.8 Hz), 7.84 (s, 1H), 8.24 (d, 1H, J=8.3 Hz)

^{13}C-NMR: (100 MHz, acetone-d_6) = δ (ppm) = 19.67 (Me), 22.51 (CH_2), 23.37 (Me), 26.44 (CH_2), 26.64 (CH_2), 28.49 (CH_2), 28.65 (CH_2), 28.79 (CH_2), 29.11 (CH_2), 29.20 (CH_2), 29.27 (CH_2), 29.42 (CH_2), 35.99, 38.89, 100.81, 116.14, 121.09, 122.55, 123.63, 124.12, 127.21, 129.39, 136.59, 162.02 (C=O), 168.90 (C=O)

R_f-Value: 0.58 (CH_2Cl_2)

Yield: (153 mg), 32 %

6.1.4.25. Procedure for the preparation of compound 30$_{a,b}$:

1 Mmol (0.112 mg) of cyclohexane-1,4-dione and 4 mmol (0.468 mg) of indole or 4 mmol (0.606 mg) of 5-chloroindole were added to a flask without solvent and 22 mmol (0.39 gm) of N-bromosuccinimide was slowly added to the mixture and the reaction mixture was left to stirring at room temperature overnight. Upon the reaction completion, as monitored by TLC (100 % CH_2Cl_2) the reaction was worked up by adding 50 ml of water, the solution was extracted with ethylacetate 100 ml for two times washed with water and brine, dried over Na_2SO_4 anhydrous, filtered and concentrated in vacuum. The crude reaction mixture was purified *via* column chromatography on silica

gel eluted with (100 % CH_2Cl_2) to afford compound **$30_{a,b}$** respectively.

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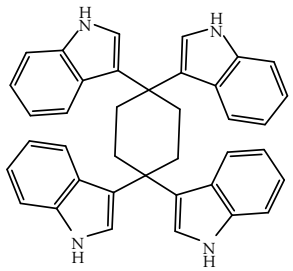
1,1,4,4-Tetrakis(1*H*-indol-3-yl)cyclohexane (30_a):



Chemical Formula: $C_{38}H_{32}N_4$

Molecular Weight: 544.69 g/mol

Melting point: 122 - 125 ^{0}C

Colour and shape: light green crystals

ESI-MS: (m/z) = 543.19 [M^+-H]

EI-MS: (m/z) = 544 [M^+] 50 %, 427 [M^+-indolyl] 58 %, 399 [M^+-indolyl-2CH_2] 100 %, 310 [M^+-2indolyl] 25 %, 258 [indolyl.C.CH_2.indolyl] 55 %, 117 [indolyl] 60 %, 90 [Ph.CH] 30 %

IR-Spectrum: (ATR,cm^{-1}) = 2923 (CH_2), 3399 (NH)

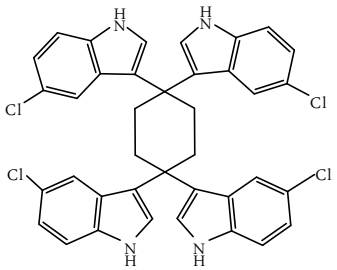
^{1}H-NMR: (400 MHz, $CDCl_3$) δ (ppm) = 2.06 (s, 4H), 2.09 - 2.14 (m, 2H), 2.18 - 2.22 (m, 8H), 2.27 - 2.31 (m, 4H), 2.49 - 2.62 (m, 2H), 2.65 - 2.69 (m, 2H), 2.72 - 2.76 (m, 2H), 6.49 (d, H, J=8 Hz), 6.60 (d, H, J=7.5 Hz), 6.84 (t, H, J=7.6 Hz), 6.98 (t, H, J=7.2 Hz), 7.05 - 7.07 (m, H), 7.09 - 7.21 (m, H), 7.26 (t, H, J=6.9 Hz), 7.30 (t, H, J=7.6 Hz), 7.45 (d, H, J=7.9 Hz), 7.51 (d, H, J=8.23 Hz), 7.62 (d, H, J=7.9 Hz), 7.71 (d, H, J=7.6 Hz), 7.77 (s, H), 7.92 (s, H), 8.23 (s, 2H, 2NH), 8.46 (s, 2H, 2NH)

^{13}C-NMR: (100 MHz, $CDCl_3$) δ (ppm) = 33.61 (CH_2), 33.92 (CH_2), 37.05 (CH_2), 38.09 (CH_2), 51.85 (C), 60.40 (C), 110.78, 111.20, 111.39, 111.73, 115.48, 117.98, 118.13, 119.42, 119.59, 119.66, 119.73, 119.98, 120.28, 120.38, 120.81, 120.93, 121.02, 121.28, 121.38, 121.99, 122.33, 124.28, 125.99, 127.78, 128.65, 134.62, 135.66, 136.02, 136.94, 136.99, 137.08, 137.18, 141.35, 143.62

R_f-Value: 0.66 (CH_2Cl_2)

Yield: (447 mg), 82 %

1,1,4,4-Tetrakis(5-chloro-1*H*-indol-3-yl)cyclohexane (30_b):



Chemical Formula: $C_{38}H_{28}Cl_4N_4$

Molecular Weight: 682.47 g/mol

Melting point : 320 - 323 ^{0}C

Colour and shape: white powder

ESI-MS: (m/z) = 681.11 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1194 (CCl), 2993 (CH_2), 3373 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 2.48 - 2.53 (m, 8H, 4CH_2), 6.89 (dd, 4H, J=1.9, 8.6 Hz), 7.22 (d, 4H, J=7.9 Hz), 7.28 (d, 4H, J=8.6 Hz), 7.37 - 7.49 (m, 4H), 10.98 (s, 4H, 4NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 28.39 (CH_2), 30.54 (CH_2), 47.62 (C), 48.57 (C), 99.88, 112.92, 119.37, 120.20, 122.15, 126.71, 135.49, 147.08

Elemental analysis: Calcd. C, 66.88; H, 4.14; Cl, 20.78; N, 8.21

Found C, 66.79, H, 4.20, Cl, 20.82, N, 8.25

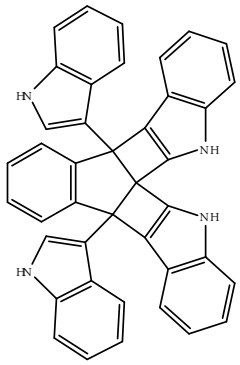
R_f-Value: 0.72 (CH_2Cl_2)

Yield: (594 mg), 87 %

5.1.4.26. Procedure for the preparation of compound 32:

To 15 ml glacial acetic acid 1mmol (0.178 mg) of 2,2-dihydroxy-1*H*-indene-1,3(2*H*)-dione was added under stirring at room temperature with 4 mmol (0.468 mg) of indole. The clear light yellow solution was left stirring overnight. The solution became dark brown after a few hours. The product was detected by TLC (100 % CH_2Cl_2). And upon the reaction was finished, it was worked up by adding 50ml of water then neutralization with a cold solution of 10 % NaOH, extracted with CH_2Cl_2 for three times, washed with water for two times and brine for two times and then dried over anhydrous sodium sulphate and concentrated in vacuum. The crude reaction mixture was purified *via* column chromatography on silica gel eluted with (100 % CH_2Cl_2) to afford compound **32**.

7*c*,11*b*-Di(1*H*-indol-3-yl)-di(7*c*,11*b*-dihydro-3*H*-biphenyleno[2,1-*b*]indole) (32):



Chemical Formula: $C_{41}H_{26}N_4$

Molecular Weight: 574.67 g/mol

Melting point : > 350 ^{0}C

Colour and shape: light gray powder

ESI-MS: (m/z) = 573.38 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 3396 (NH)

^{1}H-NMR: (600 MHz, acetone-d_6) δ(ppm) = 6.68 (t, 2H, J=7.6 Hz), 6.75 (t, 1H, J=7.7 Hz), 6.91 (t, 2H, J=7.6 Hz), 7.03 (d, 2H, J=7.5 Hz), 7.08 (t, 1H, J=7.6 Hz), 7.27 (t, 1H, J=9.3 Hz), 7.34 (d, 2H, J=7.5 Hz), 7.38 - 7.42 (m, 2H), 7.50-7.54 (m, 2H), 7.60 (d, 1H, J= 8 Hz), 7.68(d, 1H, J=7.8 Hz), 7.85 (d, 1H, J=8 Hz), 8.50 (d, 1H, J=7.5 Hz), 8.77 (d, 1H, J=7.7 Hz), 10.85 (s, 2H, 2NH), 11.80 (d, 2H, 2NH).

^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = (Quaternary C: 58.16 (2C), 106.82, 112.77, 114.35 (2C), 116.57, 121.34, 122.25, 125.83 (2C), 133.18, 134.46, 136.93 (2C), 138.74, 139.73, 145.93, 153.22), (CH: 110.53, 111.54 (2CH), 111.57, 118.00, 118.04 (2CH), 119.35, 120.04, 120.47 (2CH), 120.75 (2CH), 120.92, 122.91, 124.30, 124.35, 124.82, 124.84, 125.60 (2CH), 126.75).

Elemental analysis: Calcd. C, 85.69; H, 4.56; N, 9.75

Found C, 85.62, H, 4.60, N, 9.71

R_f-Value: 0.66 (100 % CH_2Cl_2)

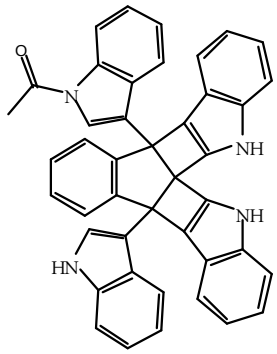
Yield: (523 mg), 91 %

5.1.4.27. Procedure for the acetylation reaction of compound 32:

Compound **32** 1mmol (0.575 mg) was added to a flask containing, 5 ml CH_2Cl_2, 0.1mmol of 4-(dimethylamino)pyridine (DMAP), 1.2mmol triethylamine and 4.2 mmol acetic anhydride. The reaction mixture was left stirring at room temperature for several

days. The products were detected by TLC (100 % CH_2Cl_2). After several days and the reaction was neutralized with NH_4OH solution and extracted with CH_2Cl_2, washed with water and brine, dried over anhydrous sodium sulphate. The crude product was purified by using (100 % CH_2Cl_2) to collect the monoacetylated product 33 firstly and then the diacetylated product compound **34**.

1-(3-(7c-(1*H*-Indol-3-yl)-di(7*c*,11*b*-dihydro-3*H*-biphenyleno[2,1-*b*]indole)-7*c*,11*b*-(1*H*-indol-1-yl)ethanone (33):



Chemical Formula: $C_{43}H_{28}N_4O$

Molecular Weight: 616.71 g/mol

Melting point : 265 - 268 ^{0}C

Colour and shape: yellow powder

ESI-MS: (m/z) = 616.36 [M^++H]

IR-Spectrum: (ATR, cm^{-1}) = 1684 (C=O), 2922 (CH_3), 3409 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 2.48 (s, 3H, CH_3CO), 6.35 (d, 1H, J= 8.2 Hz), 6.63 (t, 1H, J= 8.2 Hz), 6.74 - 6.88 (m, 2H), 7.00 (t, 2H, J= 7.9 Hz), 7.04 - 7.13 (m, 3H), 7.14 - 7.15 (m, 2H), 7.16 - 7.54 (m, 3H), 7.58 (d, 1H, J= 8.2 Hz), 7.71 - 7.85 (m, 2H), 8.13 - 8.23 (m, 2H), 8.53 (d, 1H, J= 7.4 Hz), 8.77 (d, 1H, J= 7.8 Hz), 10.88 (d, 1H, J= 2.4 Hz, 1NH), 11.83 (d, 2H, J= 2.4 Hz, 2NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 23.96 (Me), 40.12, 40.21, 56.08, 107.14, 110.71, 111.59, 111.96, 112.69, 113.60, 115.52, 116.89, 118.42, 119.41, 120.05, 120.25, 120.73, 120.97, 121.21, 121.50, 121.88, 122.71, 123.16, 124.11, 124.39, 124.51, 125.05, 125.66, 127.29, 128.71, 133.09, 134.50, 135.49, 137.18, 138.98, 139.76, 139.78, 143.94, 151.37, 168.82 (C=O)

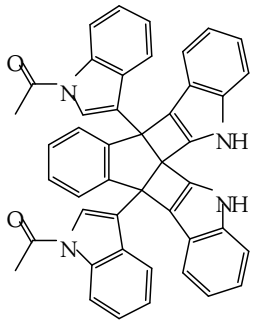
Elemental analysis: Calcd. C, 83.74, H, 4.58, N, 9.08

Found C, 83.70, H, 4.62, N, 9.00

R_f-Value: 0.55 (CH_2Cl_2)

Yield: (265 mg), 43 %

Bis(7*c*,11*b*-dihydro-3*H*-biphenyleno[2,1-*b*]indole)-di(7*c*,11*b*-(1*H*-indol-1-yl)ethanone (34):



Chemical Formula: $C_{45}H_{30}N_4O_2$

Molecular Weight: 658.75 g/mol

Melting point: 230 - 233 ^{0}C

Colour and shape: yellow powder

ESI-MS: (m/z) = 681.15 [M^++Na], 657.32 [M^+-H]

IR-Spectrum: (ATR, cm^{-1}) = 1703 (C=O), 2922 (CH_3), 3392 (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 2.58 (s, 6H, 2COMe), 6.61 (d, 2H, J= 7.83 Hz), 6.74 (t, 2H, J= 7.24 Hz), 6.93 (t, 1H, J= 7Hz), 7.03 - 7.08 (m, 2H), 7.14 (t, 2H, J= 8.23 Hz), 7.34 - 7.41 (m, 2H), 7.42 - 7.55 (m, 2H), 7.77 (d, 1H, J= 7.44 Hz), 7.86 (t, 2H, J= 7.24 Hz), 8.06 - 8.08 (m, 1H), 8.23 (d, 1H, J= 8.22 Hz), 8.27 (s, 2H), 8.58 (d, 1H, J= 7.83 Hz), 8.78 (d, 1H, J= 7.83 Hz), 11.86 (s, 1H, 1NH), 11.89 (s, 1H, 1NH)

^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 24.57 (Me) (2C), 40.62, 40.67, 56.12, 107.94, 110.74, 110.74, 111.31, 112.15, 112.27, 113.23, 116.15, 117.65, 119.05, 119.24, 119.99, 120.54, 120.77, 121.07, 121.59, 121.62, 121.75, 122.04, 123.30, 123.92, 124.33, 124.46, 124.75, 125.20, 125.53, 125.93, 127.63, 128.39, 128.97, 133.49, 135.06, 136.03, 137.18, 140.0, 140.24, 140.33, 142.79, 150.31, 169.78 (2 (C=O))

Elemental analysis: Calcd. C, 82.05, H, 4.59, N, 8.51

Found C, 82.08, H, 4.65, N, 8.61

R_f-Value: 0.47 (CH_2Cl_2)

Yield: (210 mg), 32 %

5.2. Biological part

5.2.1. Antimicrobial assay

The antimicrobial activity of the synthesized compounds was tested in the Microbiology Department of the Faculty of Pharmacy of the *Ankara University*. ATCC strains of the microorganisms used in this study were obtained from the culture collection of the *Refik Saydam Health Institution of Health Ministry*, *Ankara*, and maintained at the Microbiology Department of the Faculty of Pharmacy of the *Ankara University*.

The Agar cup diffusion technique[272a,b] and two fold serial dilution methods, (http://www.fao.org/docrep/005/ac802e/ac802e0q.htm), were used to determine the antimicrobial activity against against Candida albicans ATCC 10145 as fungus, S. aureus ATCC 25923, Bacillus subtilis ATCC 6633, MRSA standard ATCC 43300 and MRSA isolate as Gram-positive bacteria and E. coli ATCC 23556 as Gram-negative bacteria.

5.2.1.1. *In-vitro* assay with agar Cup-diffusion Technique

The *in-vitro* antimicrobial screening is done by Agar Cup-diffusion method[272a], In brief, 200 µl of microbial suspension were uniformly spread over solidified Sabouraud Dextrose Agar (SDA) plates with the help of a sterilized spreader. Wells of 6 mm diameter were made in the centre of these agar plates with the help of a sterile cork borer. The wells were then filled with 200 µl of the respective test extract at different concentrations. The compounds and the standards were dissolved in 12.5 % DMSO at concentrations of 200 µg/ml. Further dilutions of the compounds and standard drugs in the test medium were prepared using two fold serial dilution methods at the required quantities of 400, 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 µg/ml concentrations with Mueller-Hinton broth and Sabouraud dextrose broth. Then all the plates were allowed to diffuse at room temperature for an hour followed by incubation at 28 ± 2 °C for 72 - 96 hours to 2 weeks depending on the growth rate of the test pathogen. The antimicrobial activities of the compounds were determined by measuring the diameter of

the inhibition zone around the well that was filled with the substrate. The minimum inhibitory concentrations (MIC) were regarded as "the lowest concentration of an antibiotic that did not permit any visible growth after 72 - 96 hours of inoculation".

5.2.2. *In vitro* cancer screen

The screening is a two-stage process, beginning with the evaluation of all compounds against the 60 cell lines at a single dose of 10^{-5} M. The output from the single dose screen is reported as a mean graph and is available for analysis by the COMPARE program. Compounds which exhibit significant growth inhibition are evaluated against the 60 cell panel at five concentration levels. The human tumour cell lines of the cancer-screening panel are grown in RPMI 1640 medium containing 5 % fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells are inoculated into 96 well microtiter plates in 100 ml at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates are incubated at 37 C, 5 % CO, 95 % air and 100% relative humidity for 24 h prior to addition of experimental drugs. After 24 h, two plates of each cell line are fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs are solubilised in dimethylsulfoxide at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate is dissolved and diluted to twice the desired final maximum test concentration with complete medium containing 50 mg/ml gentamicin. Additional four, 10-fold or ½ log serial dilutions are made to provide a total of five drug concentrations plus control. Aliquots of 100 ml of these different drug dilutions are added to the appropriate microtiter wells already containing 100 ml of medium, resulting in the required final drug concentrations. Following drug addition, the plates are incubated for an additional 48 h at 37 ^{0}C, 5 % CO, 95 % air, and 100% relative humidity. For adherent cells, the assay is terminated by the addition of cold TCA. Cells are fixed in situ by the gentle addition of 50 ml of cold 50 % (w/v) TCA (final concentration, 10 % TCA) and

incubated for 60 min at 4 C. The supernatant is discarded, and the plates are washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 ml) at 0.4 % (w/v) in 1 % acetic acid is added to each well, and plates are incubated for 10 min at room temperature. After staining, unbound dye is removed by washing five times with 1 % acetic acid and the plates are air dried. Bound stain is subsequently solubilised with 10 μM trizma base, and the absorbance is read on an automated plate reader at a wavelength of 515 nm. For suspension cells, the methodology is the same except that the assay is terminated by fixing settled cells at the bottom of the wells by gently adding 50 ml of 80 % TCA (final concentration, 16 % TCA). Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the five concentration levels (Ti)], the percentage growth is calculated at each of the drug concentrations levels. Percentage growth inhibition is calculated as:

$[(Ti-Tz)/(C-Tz)] \times 100$ for concentrations for which $Ti > / = Tz$

$[(Ti-Tz)/Tz] \times 100$ for concentrations for which $Ti < Tz$

Three dose response parameters are calculated for each experimental agent. Growth inhibition of 50 % (GI_{50}) is calculated from $[(Ti-Tz)/(C-Tz)] \times 100 = 50$, which is the drug concentration resulting in a 50 % reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. The drug concentration resulting in total growth inhibition (TGI) is calculated from (Ti = Tz). The LC_{50} (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) indicating a net loss of cells following treatment is calculated from $[(Ti-Tz)/Tz] \times 100 = -50$. Values are calculated for each of these three parameters if the level of activity is reached; however, if the effect is not reached or is exceeded, the value for that parameter is expressed as greater or less than the maximum or minimum concentration tested[290].

6. Appendix

6.1. Mean graphs of One and Five dose anticancer screening

Developmental Therapeutics Program One Dose Mean Graph	NSC: D-755518 / 1	Conc: 1.00E-5 Molar	Test Date: Jan 10, 2011
	Experiment ID: 1101OS87		Report Date: Feb 03, 2011

Panel/Cell Line	Growth Percent
Leukemia	
CCRF-CEM	65.84
HL-60(TB)	85.73
K-562	63.92
MOLT-4	52.47
RPMI-8226	75.01
SR	67.99
Non-Small Cell Lung Cancer	
A549/ATCC	70.37
EKVX	70.13
HOP-62	117.07
HOP-92	77.26
NCI-H226	96.26
NCI-H23	83.58
NCI-H322M	76.91
NCI-H460	40.92
NCI-H522	81.51
Colon Cancer	
COLO 205	98.17
HCC-2998	89.58
HCT-116	49.68
HCT-15	71.42
HT29	67.05
KM12	61.54
SW-620	66.72
CNS Cancer	
SF-268	67.22
SF-295	90.26
SF-539	90.70
SNB-19	88.91
SNB-75	69.63
U251	71.87
Melanoma	
LOX IMVI	70.34
MALME-3M	86.23
M14	60.84
MDA-MB-435	66.55
SK-MEL-2	87.10
SK-MEL-28	94.00
SK-MEL-5	69.90
UACC-257	88.90
UACC-62	65.78
Ovarian Cancer	
IGROV1	51.22
OVCAR-3	57.39
OVCAR-4	85.86
OVCAR-5	84.10
OVCAR-8	84.14
NCI/ADR-RES	83.51
SK-OV-3	99.10
Renal Cancer	
786-0	93.63
A498	104.66
ACHN	63.05
CAKI-1	34.27
RXF 393	90.57
SN12C	71.34
UO-31	42.30
Prostate Cancer	
PC-3	58.42
DU-145	74.41
Breast Cancer	
MCF7	52.85
MDA-MB-231/ATCC	86.20
BT-549	102.59
T-47D	65.05
MDA-MB-468	97.31
Mean	75.51
Delta	41.24
Range	82.80

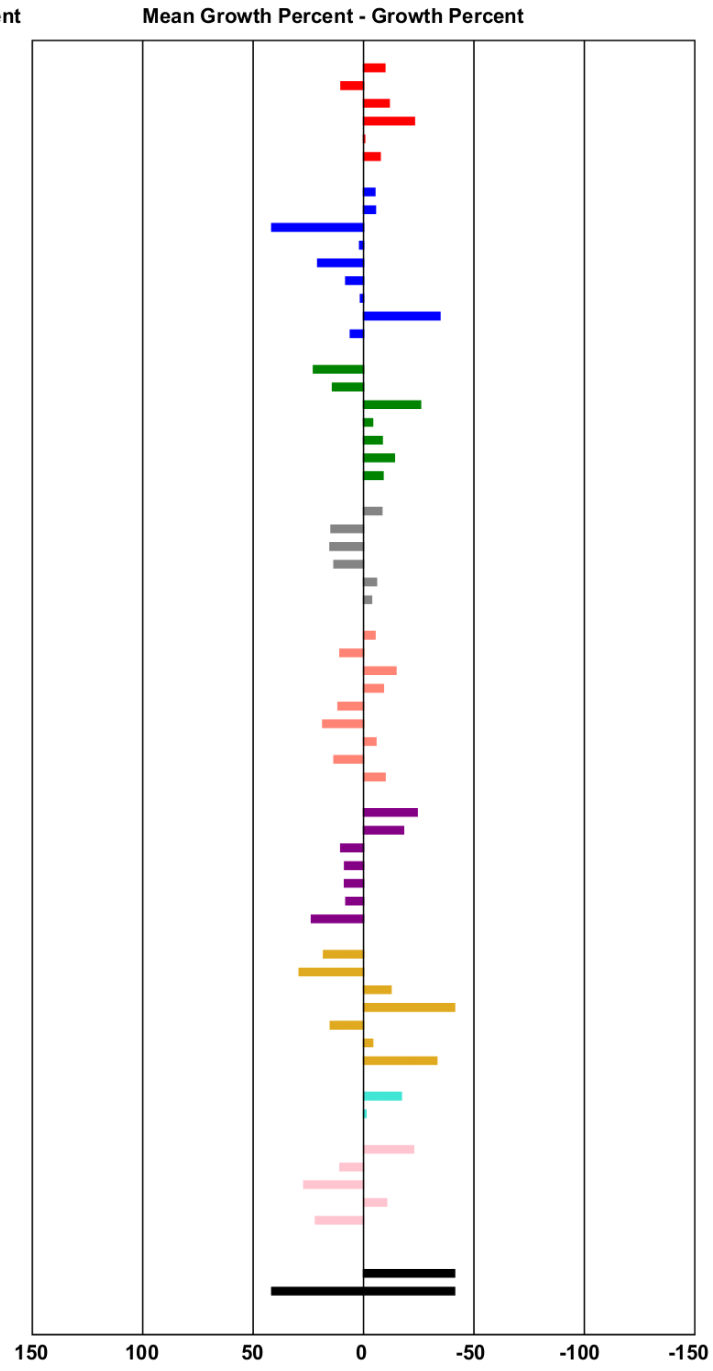


Figure (55): Maen graph one dose screening of **17g**

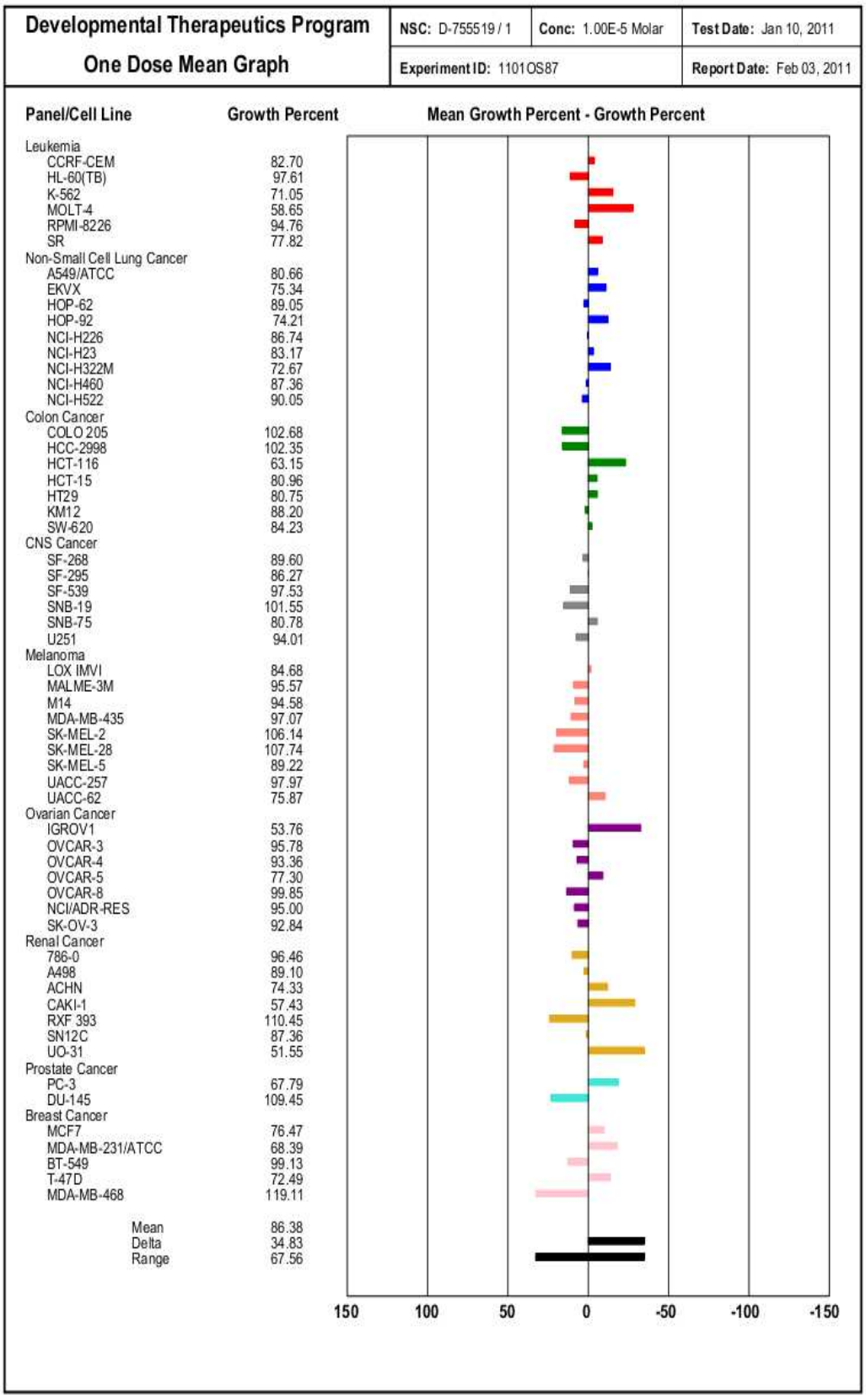


Figure (56): Maen graph one dose screening of **17**$_{i}$.

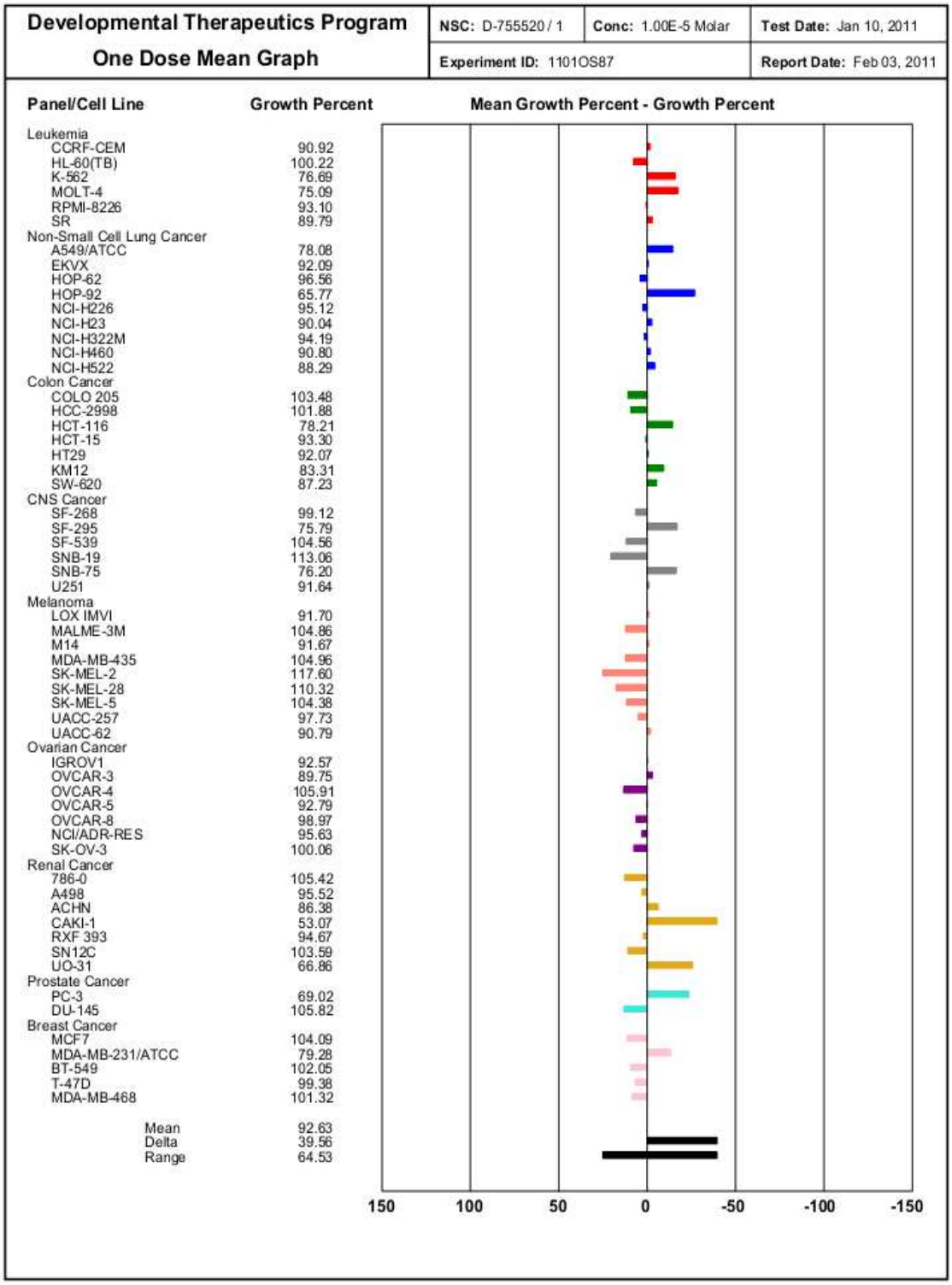


Figure (57): Maen graph one dose screening of **17**$_{l}$.

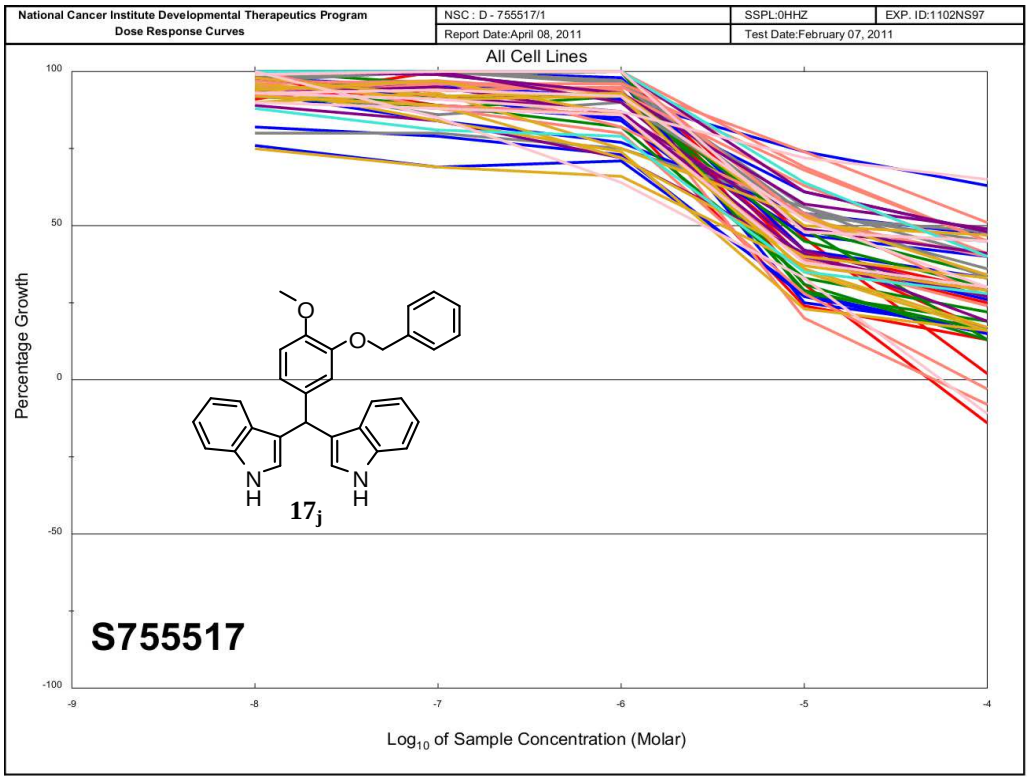


Figure (58): Superposition of all the growth curves of compound $\mathbf{17_j}$

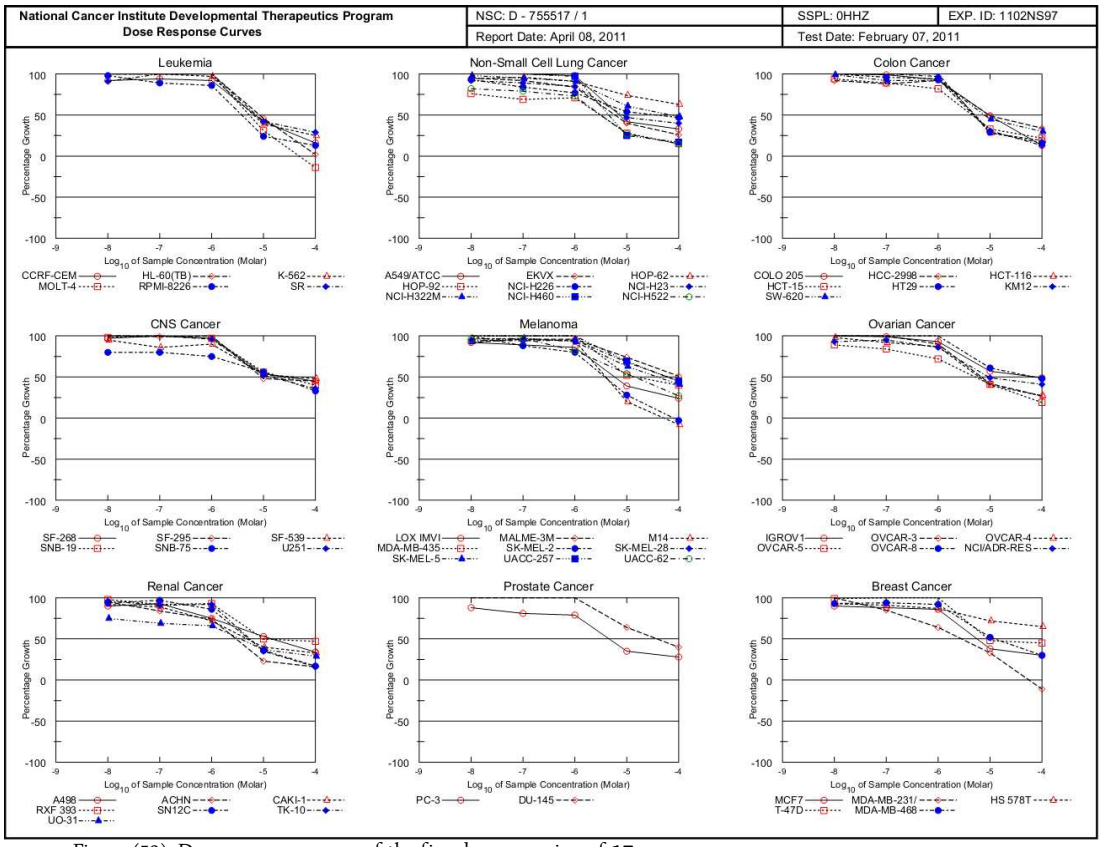


Figure (59): Dose-response curves of the five-dose screening of $\mathbf{17_j}$

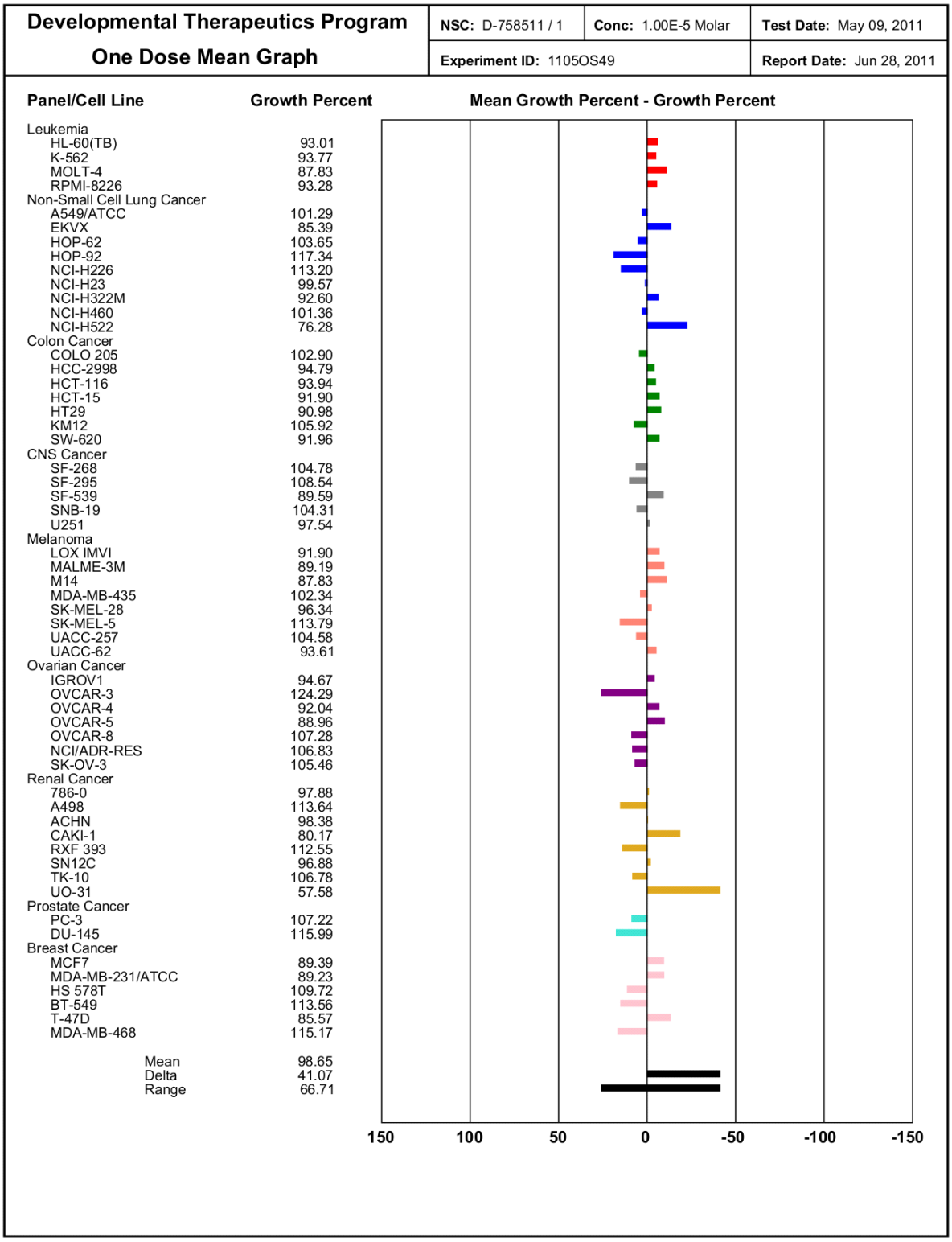


Figure (60): Maen graph one dose screening of **18**$_{f}$

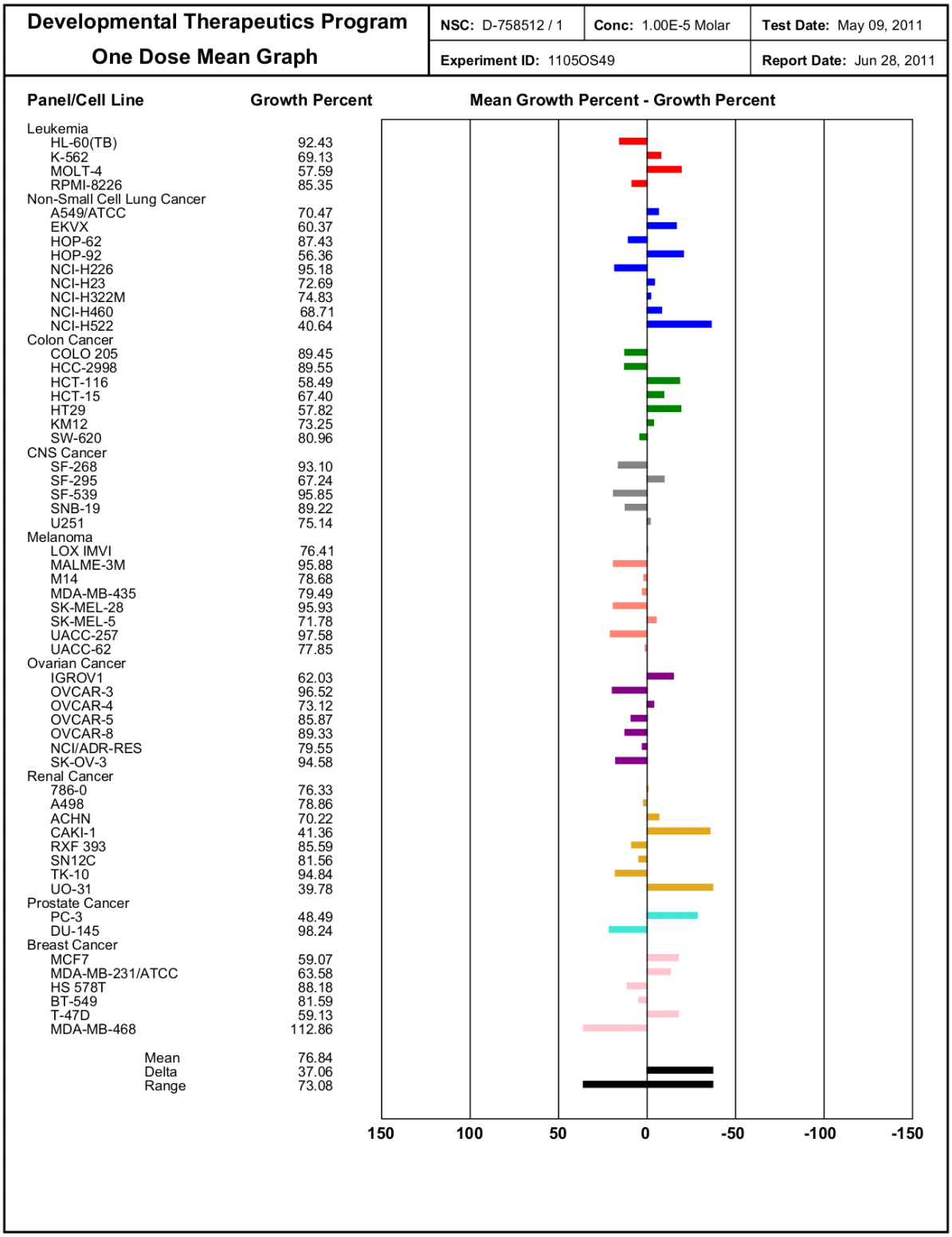


Figure (61): Maen graph one dose screening of **18**$_{h}$

Developmental Therapeutics Program

One Dose Mean Graph

NSC: D-758512 / 1	**Conc:** 1.00E-5 Molar	**Test Date:** May 09, 2011
Experiment ID: 1105OS49		**Report Date:** Jun 28, 2011

Panel/Cell Line	Growth Percent
Leukemia	
HL-60(TB)	92.43
K-562	69.13
MOLT-4	57.59
RPMI-8226	85.35
Non-Small Cell Lung Cancer	
A549/ATCC	70.47
EKVX	60.37
HOP-62	87.43
HOP-92	56.36
NCI-H226	95.18
NCI-H23	72.69
NCI-H322M	74.83
NCI-H460	68.71
NCI-H522	40.64
Colon Cancer	
COLO 205	89.45
HCC-2998	89.55
HCT-116	58.49
HCT-15	67.40
HT29	57.82
KM12	73.25
SW-620	80.96
CNS Cancer	
SF-268	93.10
SF-295	67.24
SF-539	95.85
SNB-19	89.22
U251	75.14
Melanoma	
LOX IMVI	76.41
MALME-3M	95.88
M14	78.68
MDA-MB-435	79.49
SK-MEL-28	95.93
SK-MEL-5	71.78
UACC-257	97.58
UACC-62	77.85
Ovarian Cancer	
IGROV1	62.03
OVCAR-3	96.52
OVCAR-4	73.12
OVCAR-5	85.87
OVCAR-8	89.33
NCI/ADR-RES	79.55
SK-OV-3	94.58
Renal Cancer	
786-0	76.33
A498	78.86
ACHN	70.22
CAKI-1	41.36
RXF 393	85.59
SN12C	81.56
TK-10	94.84
UO-31	39.78
Prostate Cancer	
PC-3	48.49
DU-145	98.24
Breast Cancer	
MCF7	59.07
MDA-MB-231/ATCC	63.58
HS 578T	88.18
BT-549	81.59
T-47D	59.13
MDA-MB-468	112.86
Mean	76.84
Delta	37.06
Range	73.08

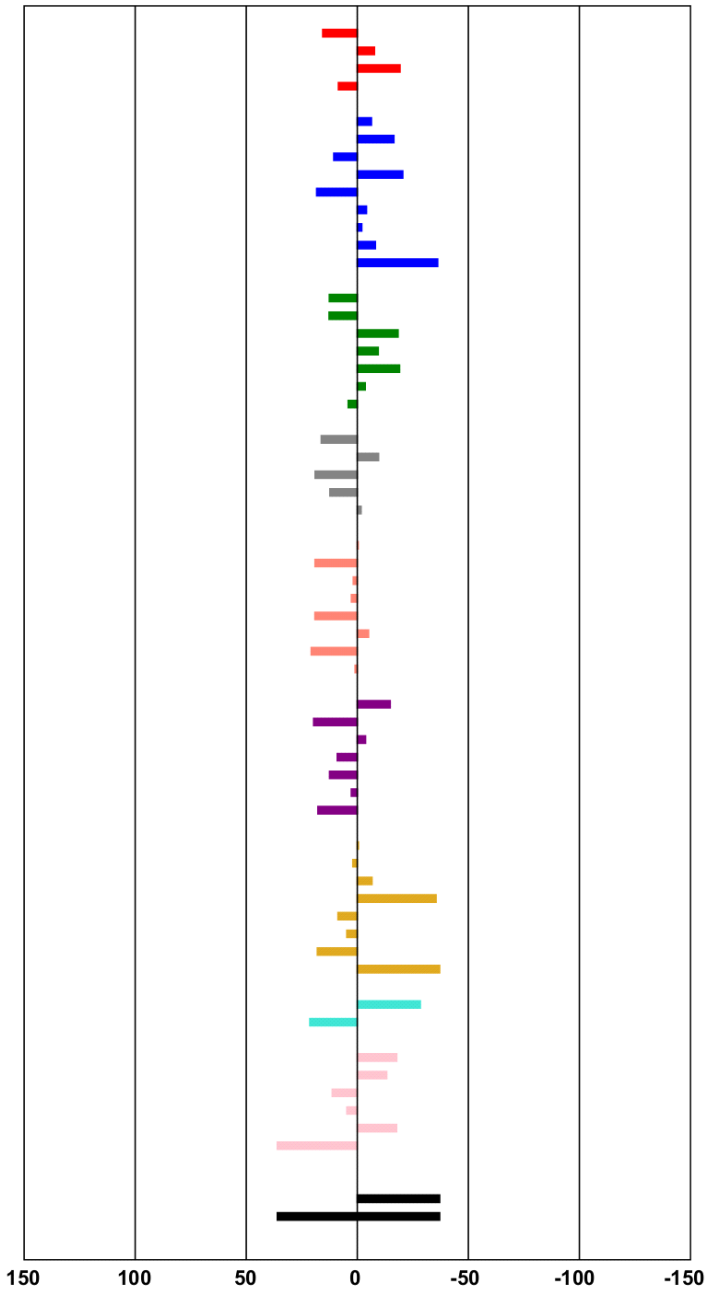


Figure (62): Maen graph one dose screening of **18**$_i$

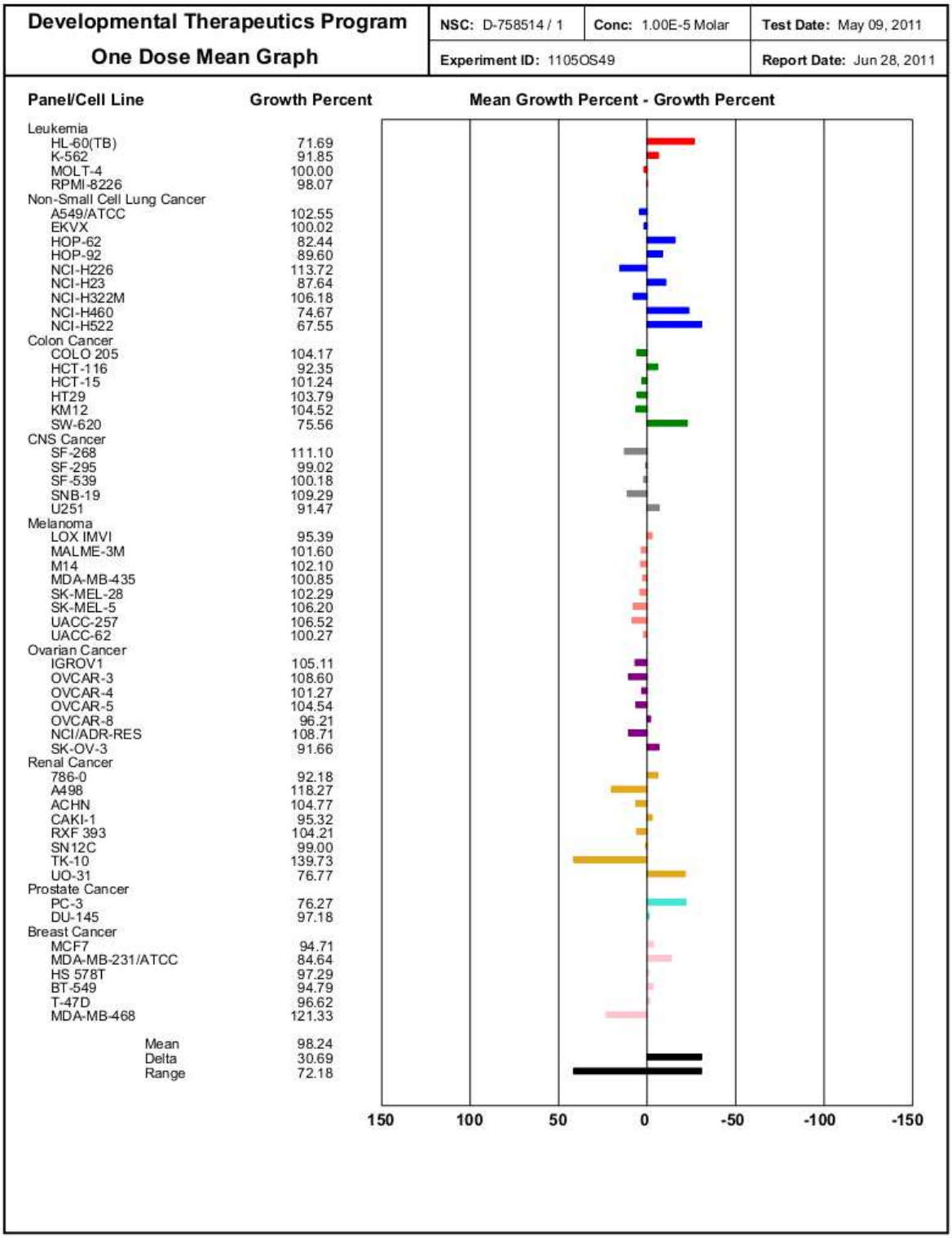


Figure (63): Maen graph one dose screening of **18**$_1$

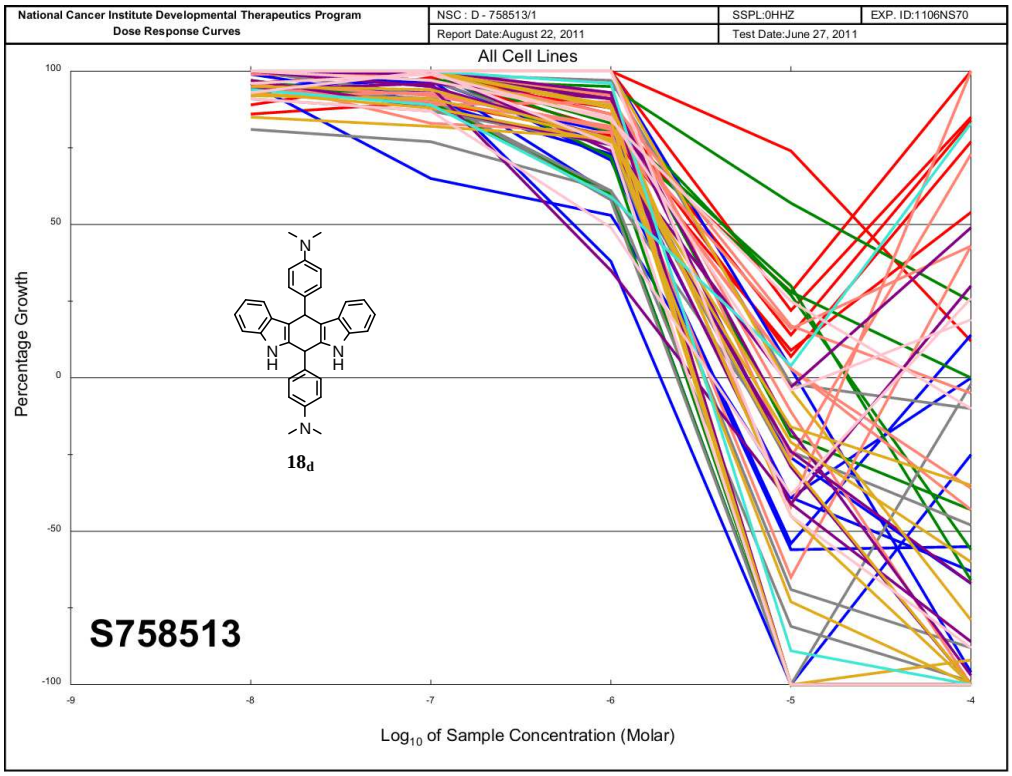


Figure (64): Superposition of all the growth curves for compound **18**$_{d}$

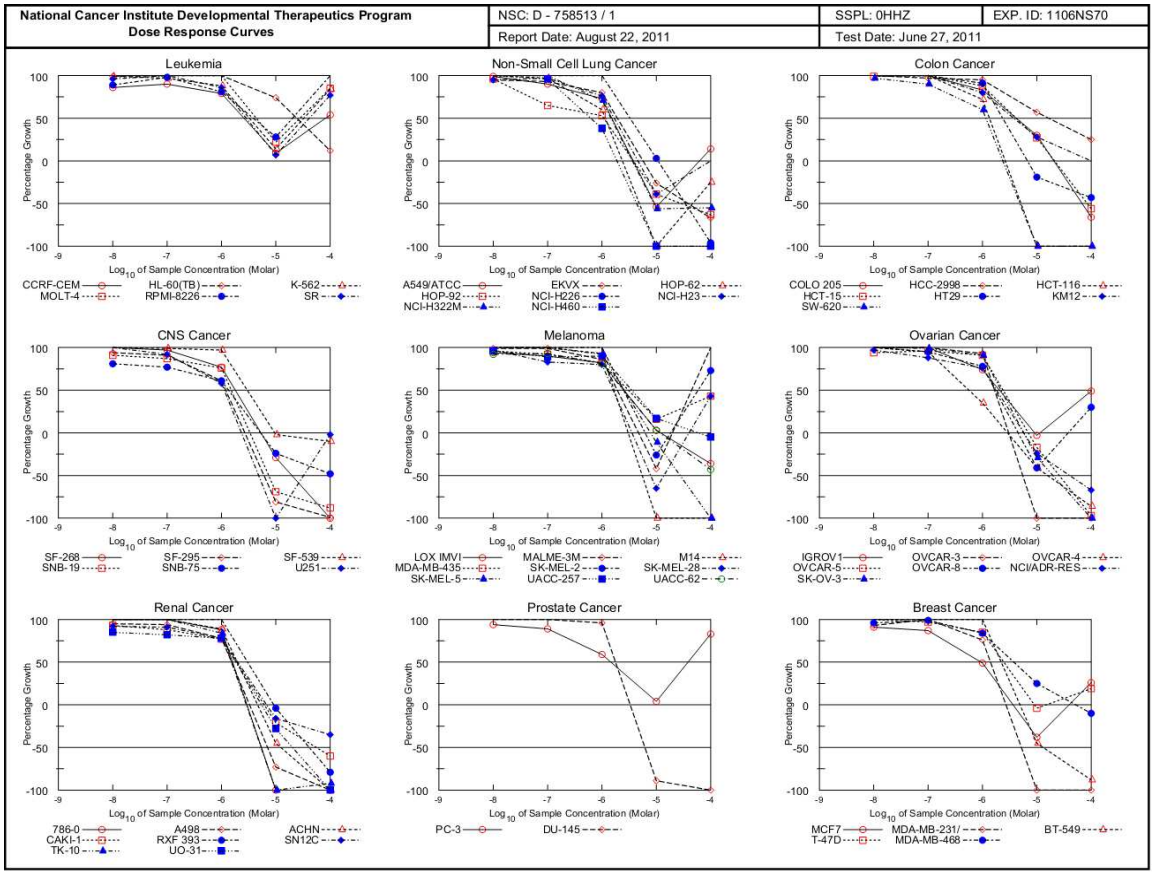


Figure (65): Dose response curves of compound **18**$_{d}$

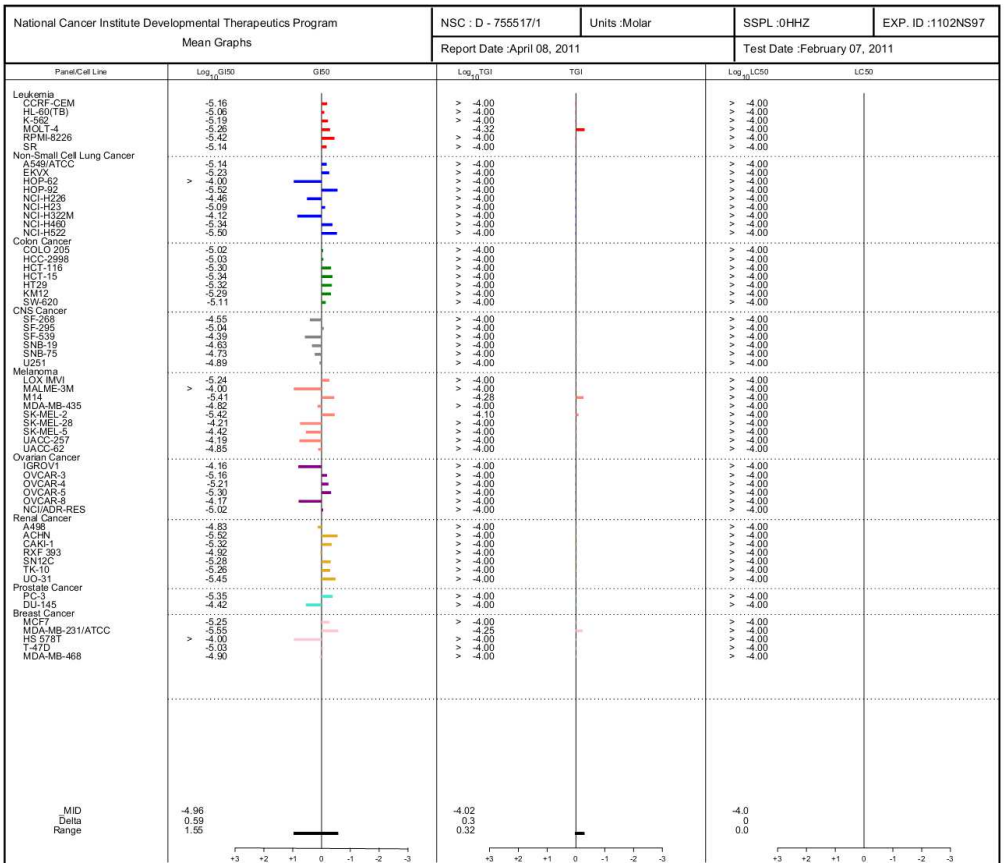


Figure (66): Five dose test results of compound **17**$_{j}$

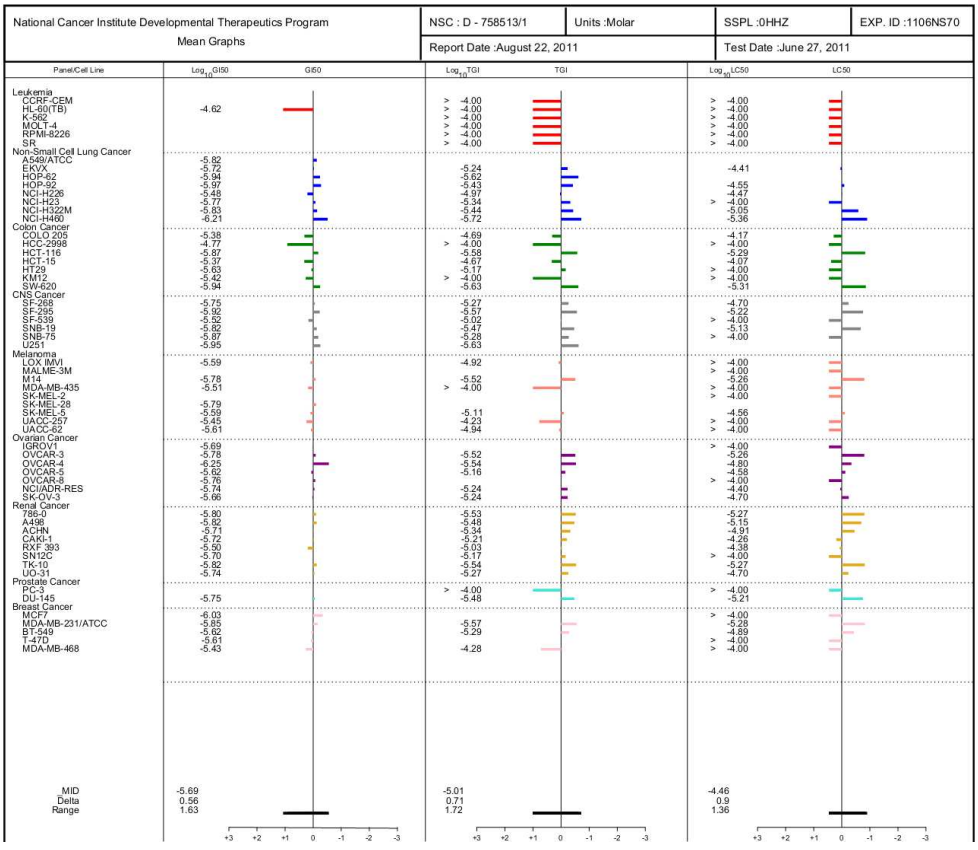
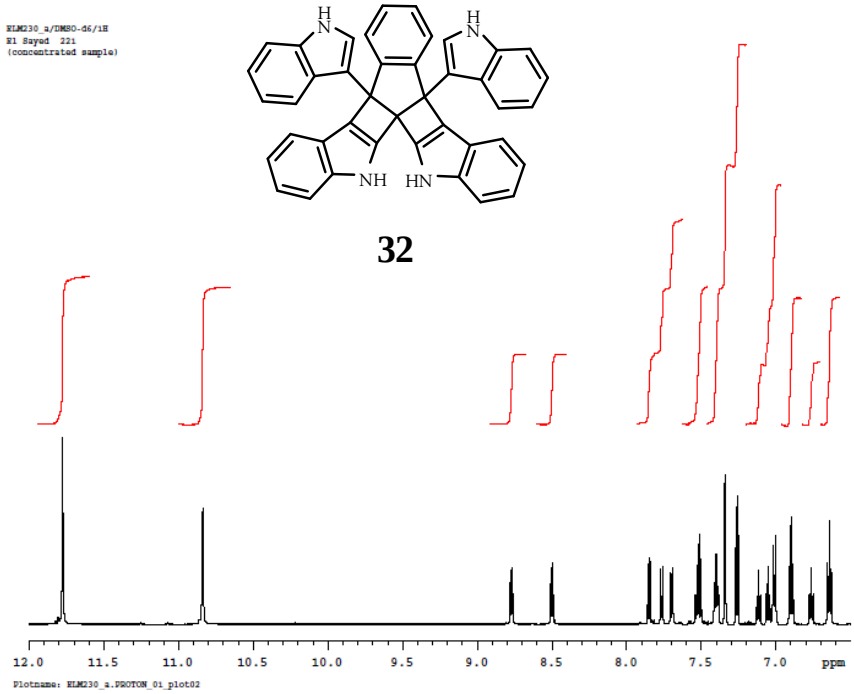


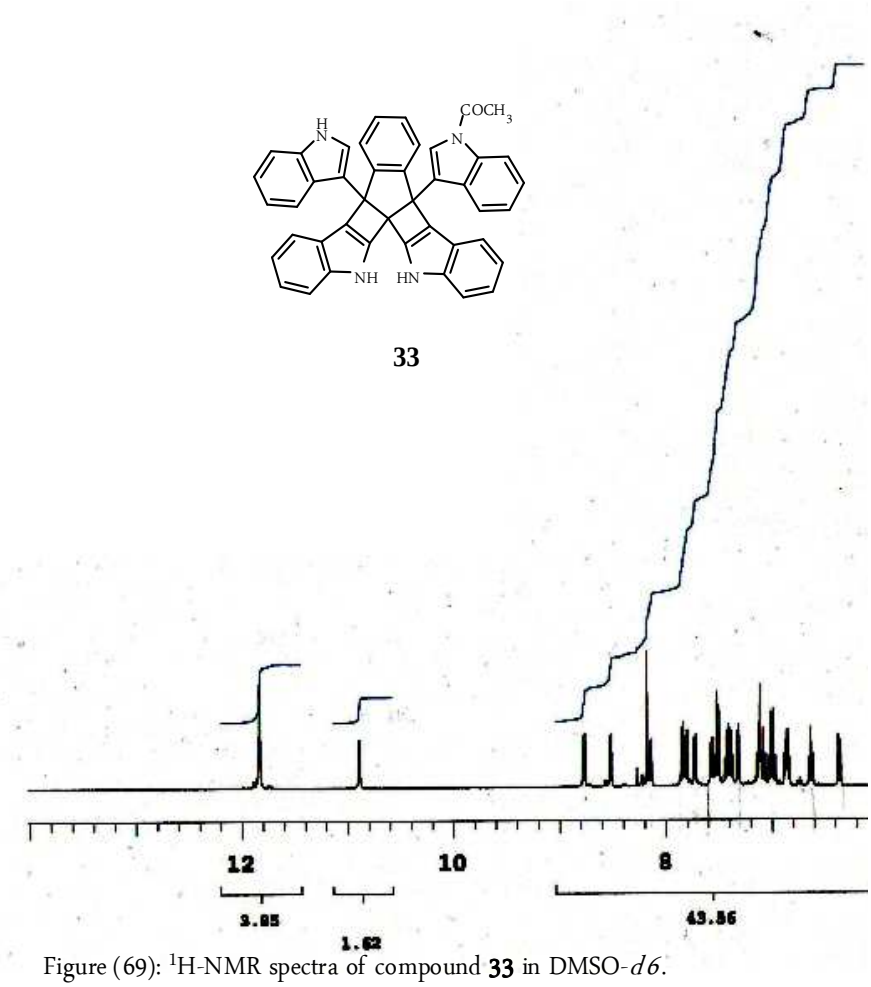
Figure (67): Five dose test results of compound **18**$_{d}$

6.2. Some 1D- and 2D- NMR spectrum of selected compounds



32

Figure (68): ^{1}H-NMR spectra of compound 32 in DMSO-*d6*.



33

Figure (69): ^{1}H-NMR spectra of compound **33** in DMSO-*d6*.

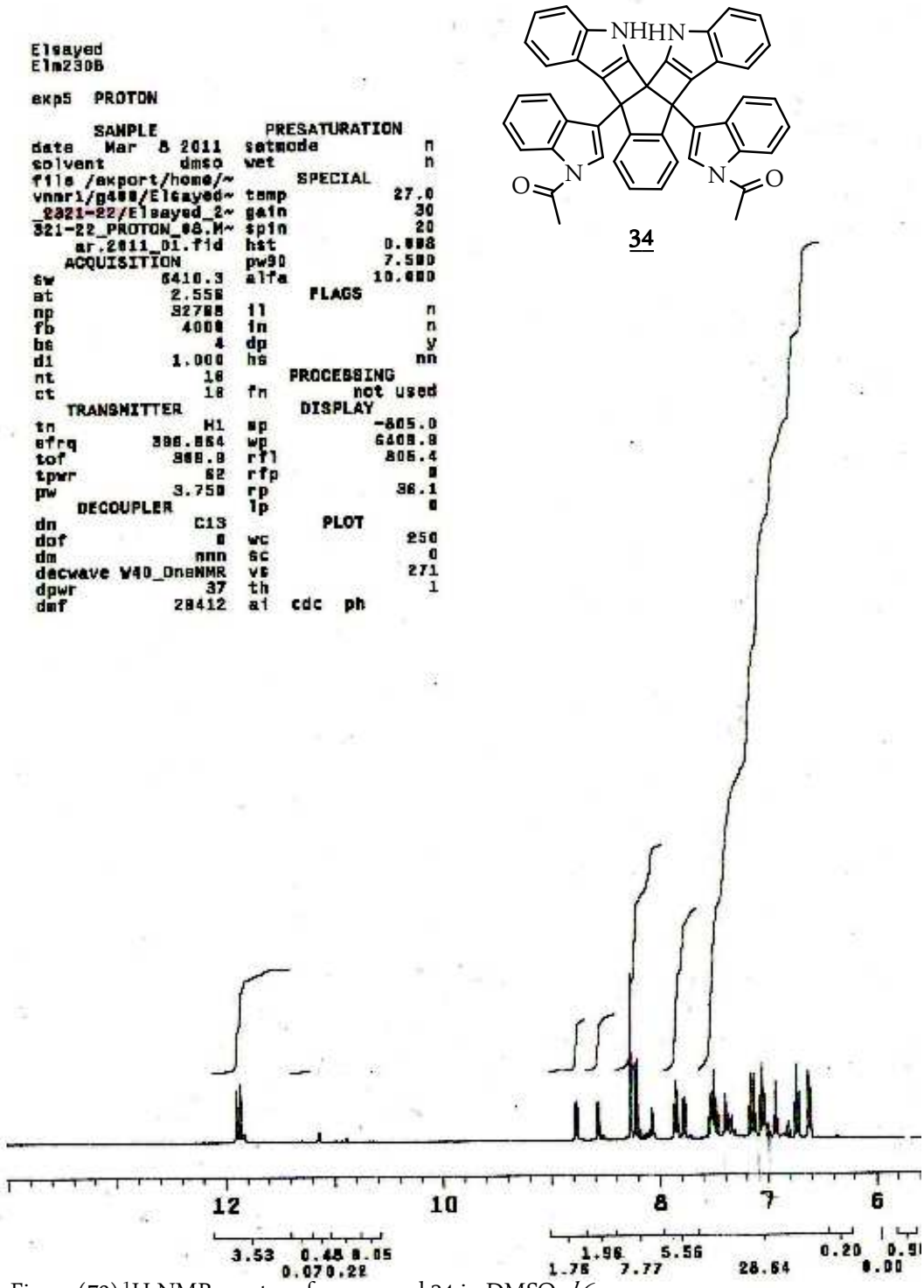


Figure (70):^{1}H-NMR spectra of compound 34 in DMSO-*d6*.

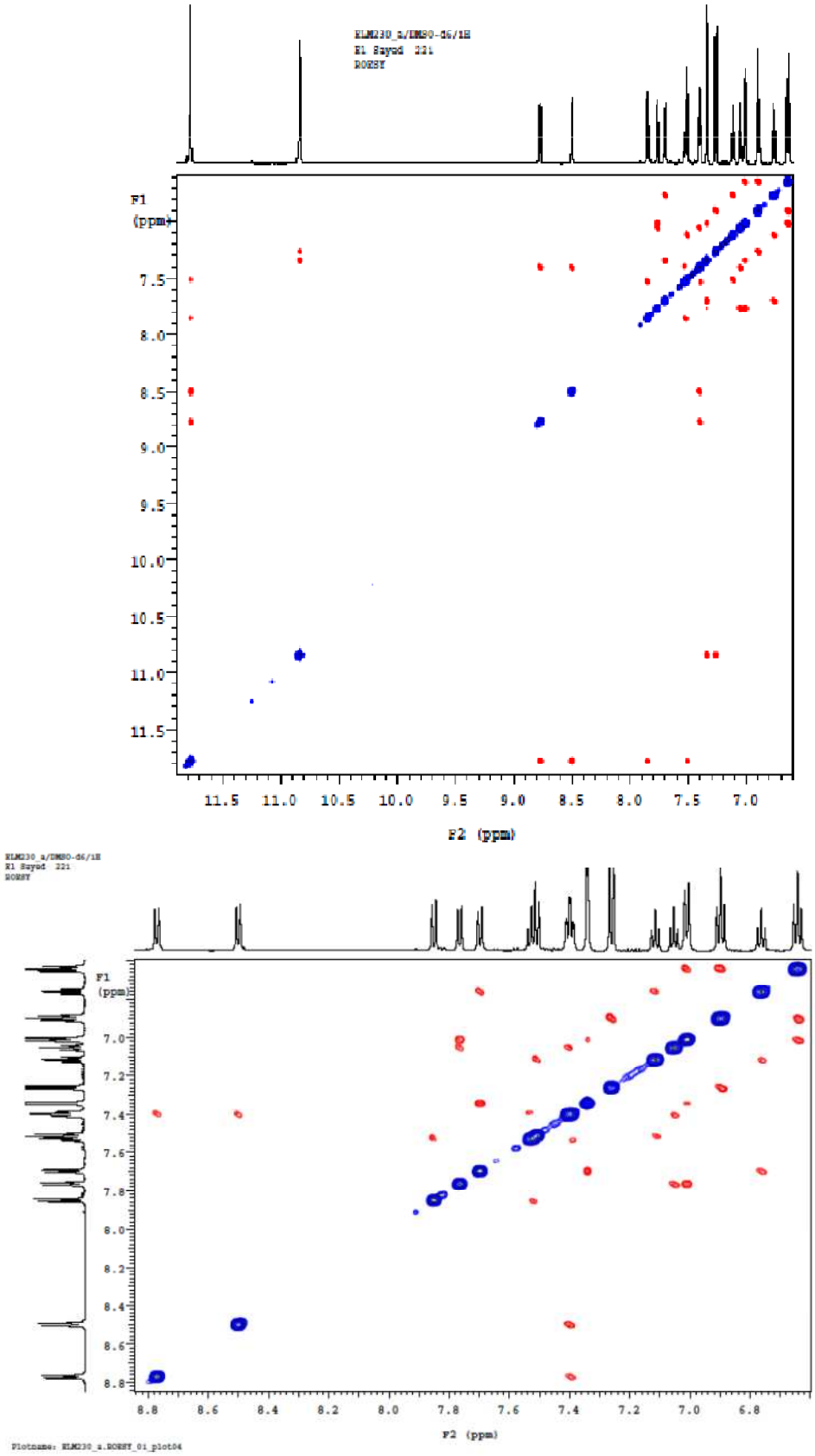


Figure (71): ROESY spectrum of compound **32** in DMSO-*d6*.

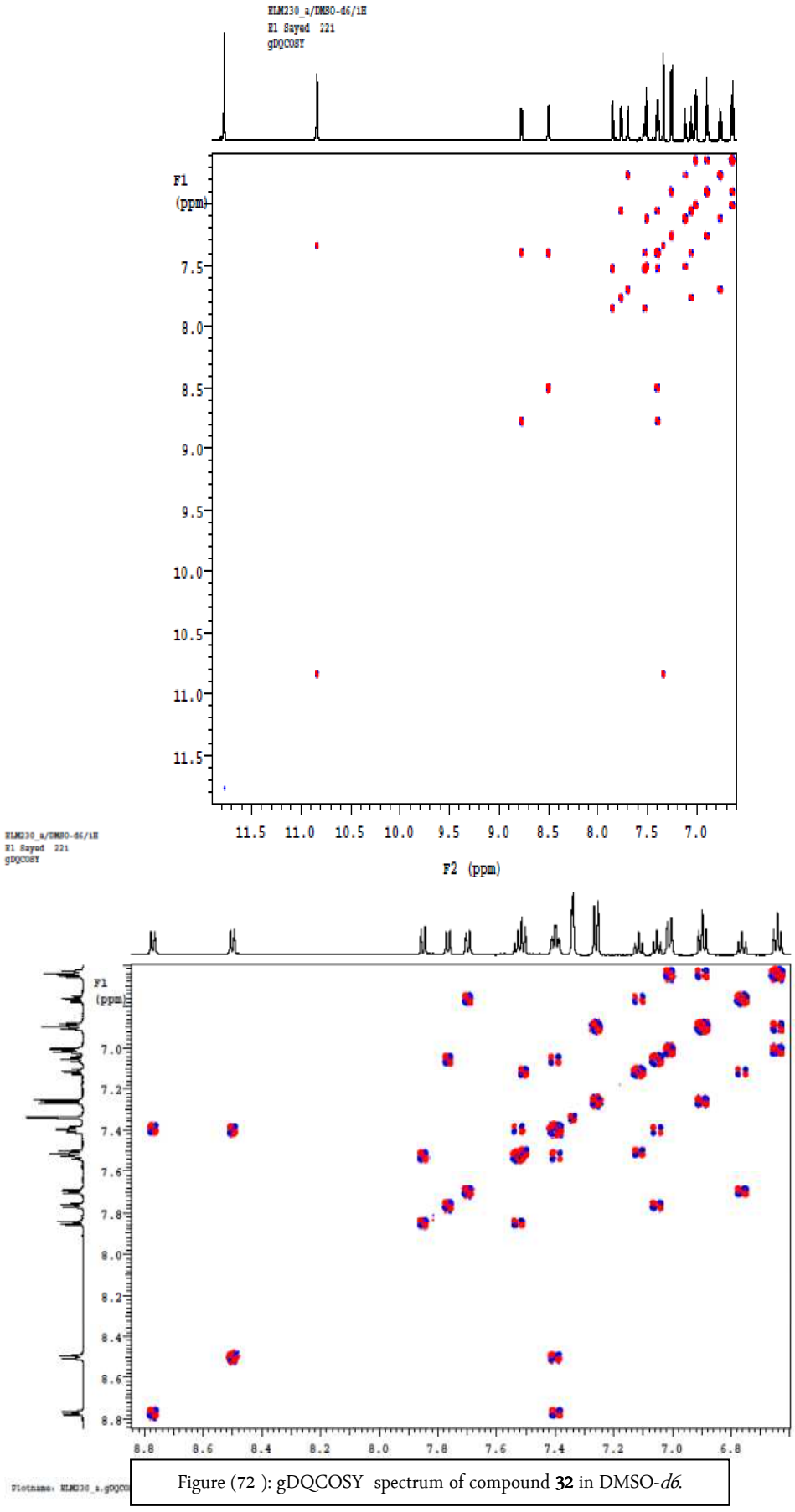


Figure (72): gDQCOSY spectrum of compound **32** in DMSO-*d6*.

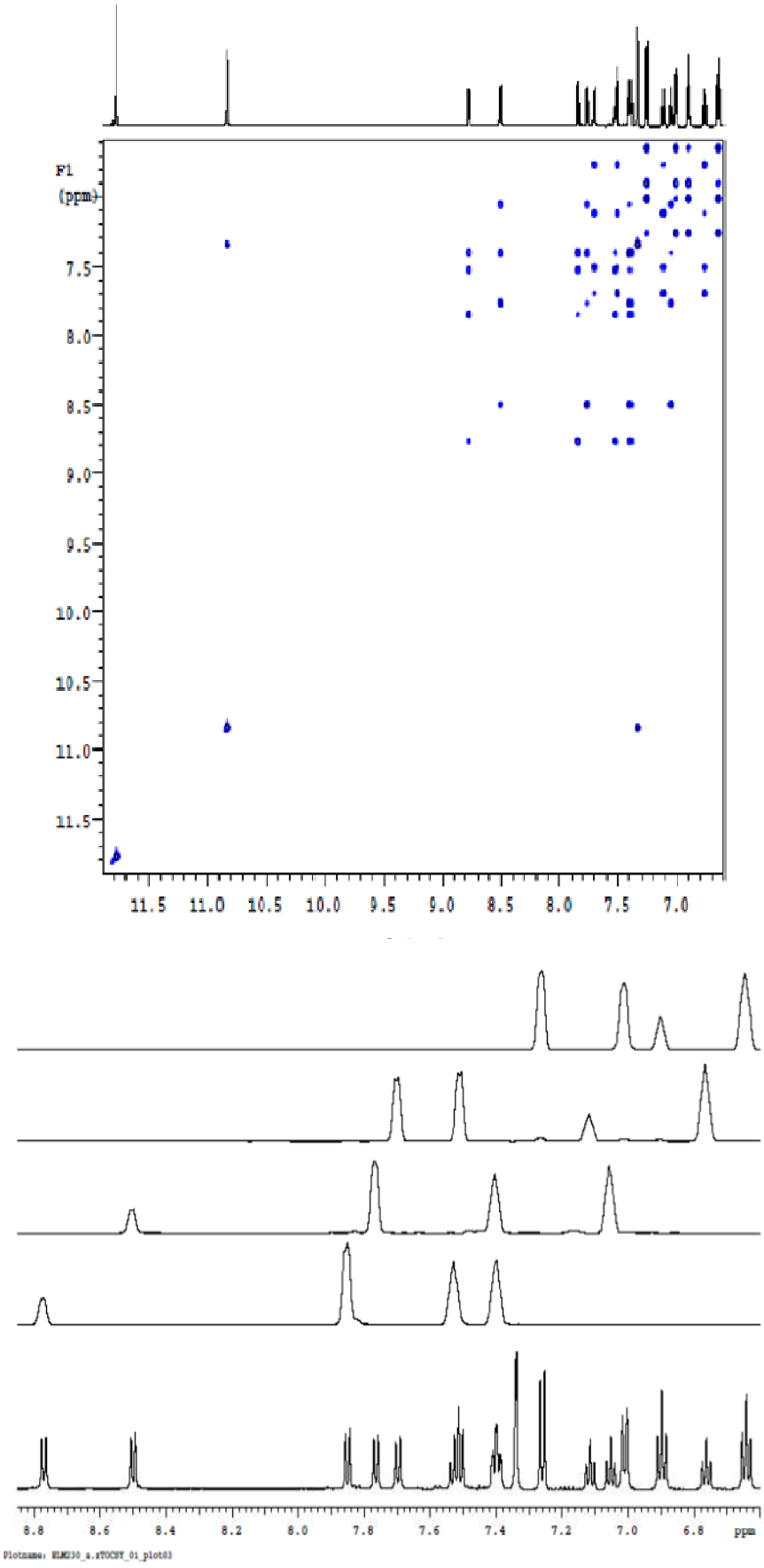


Figure (73): zTOCSY spectra of compound **32** in DMSO-*d6*.

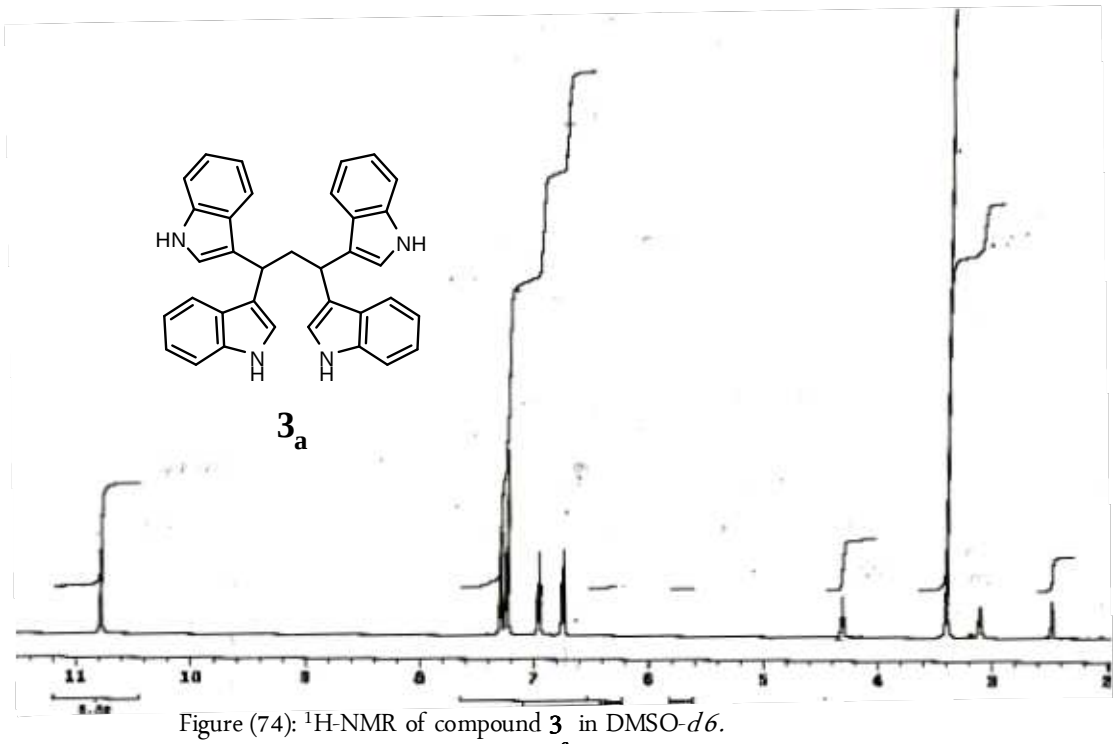


Figure (74): ^{1}H-NMR of compound **3$_a$** in DMSO-*d6*.

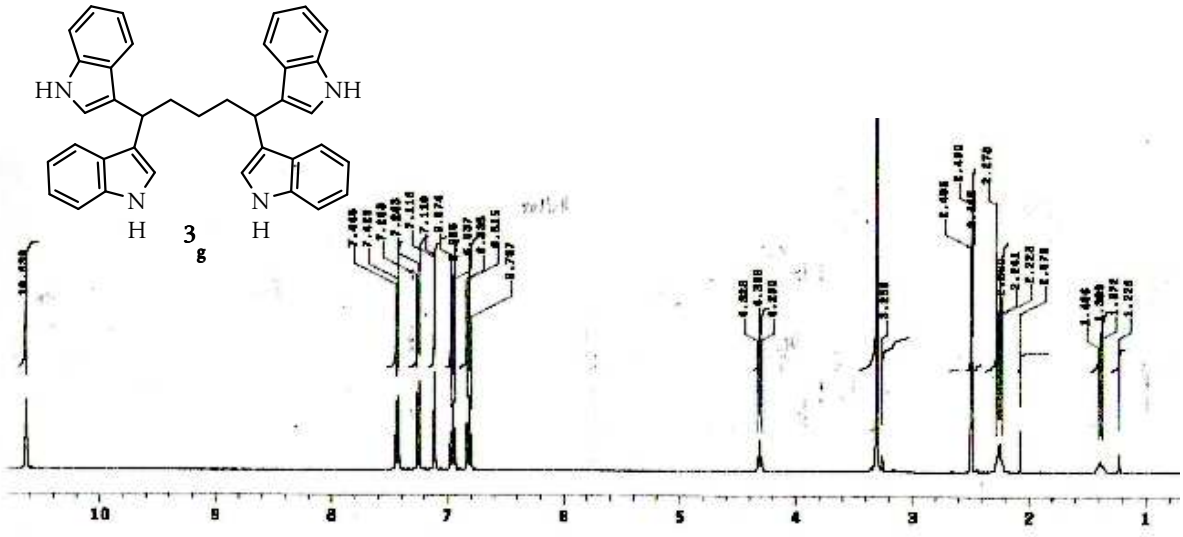


Figure (75): ^{1}H-NMR of compound **3$_g$** in DMSO-*d6*

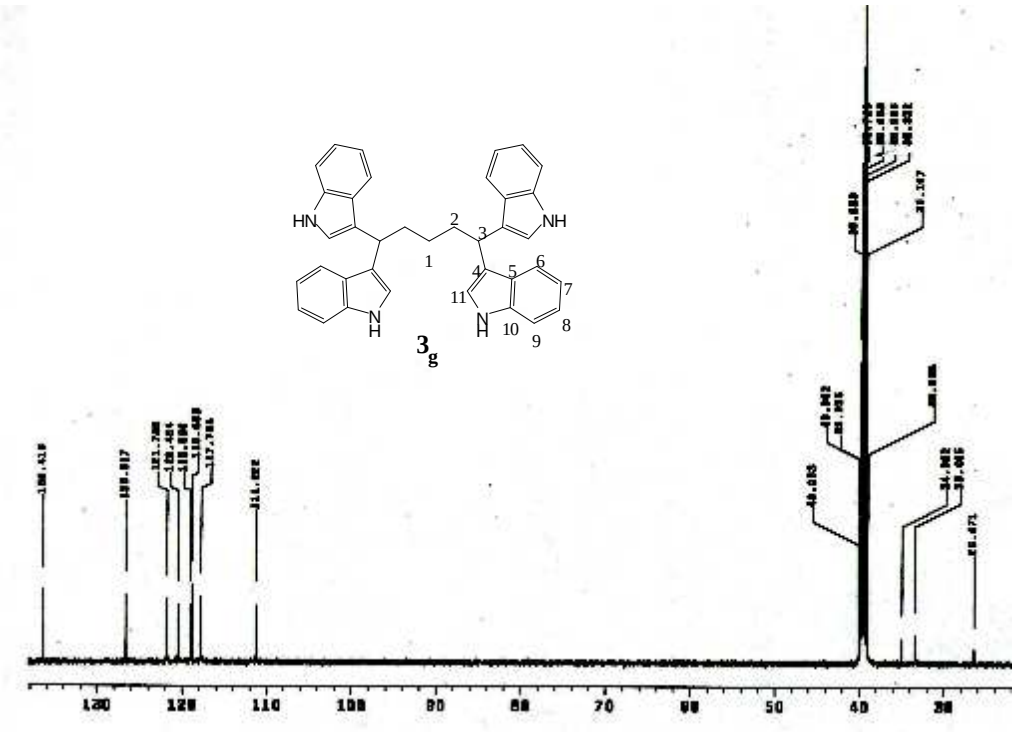


Figure (76): ^{13}C-NMR spectra of compound **3**$_g$ in DMSO-*d6*.

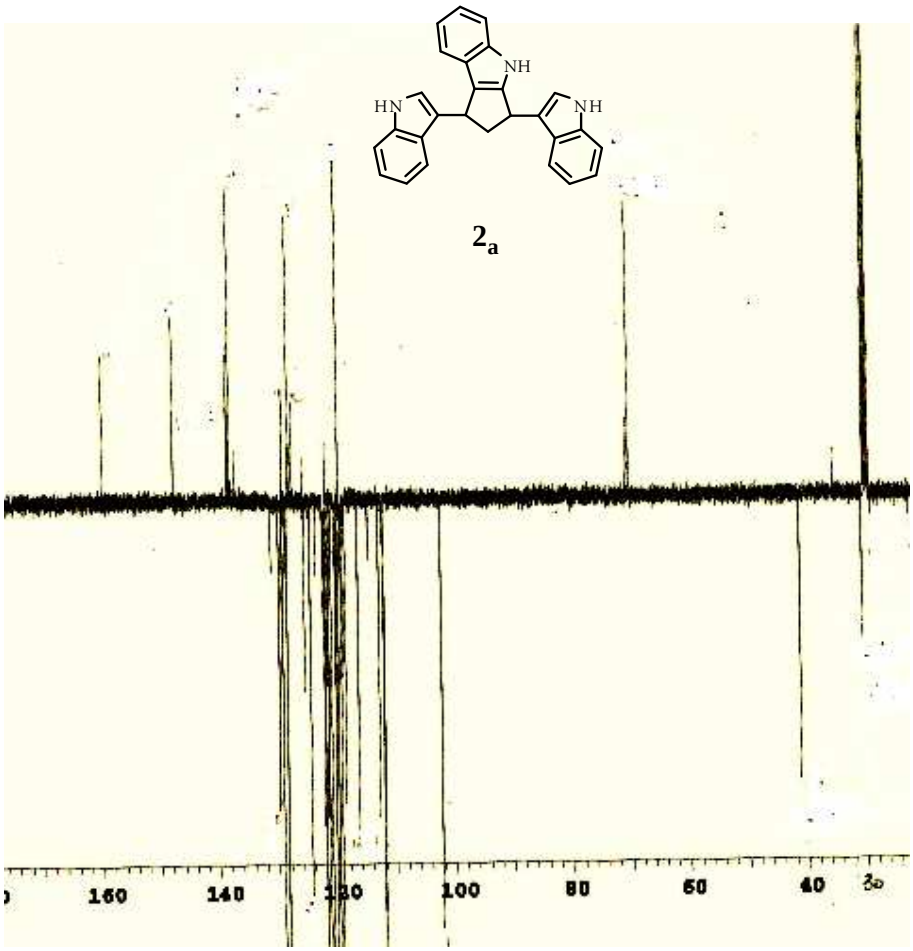


Figure (77): ^{13}C- APT spectrum of compound **2**$_a$ in DMSO-*d6*.

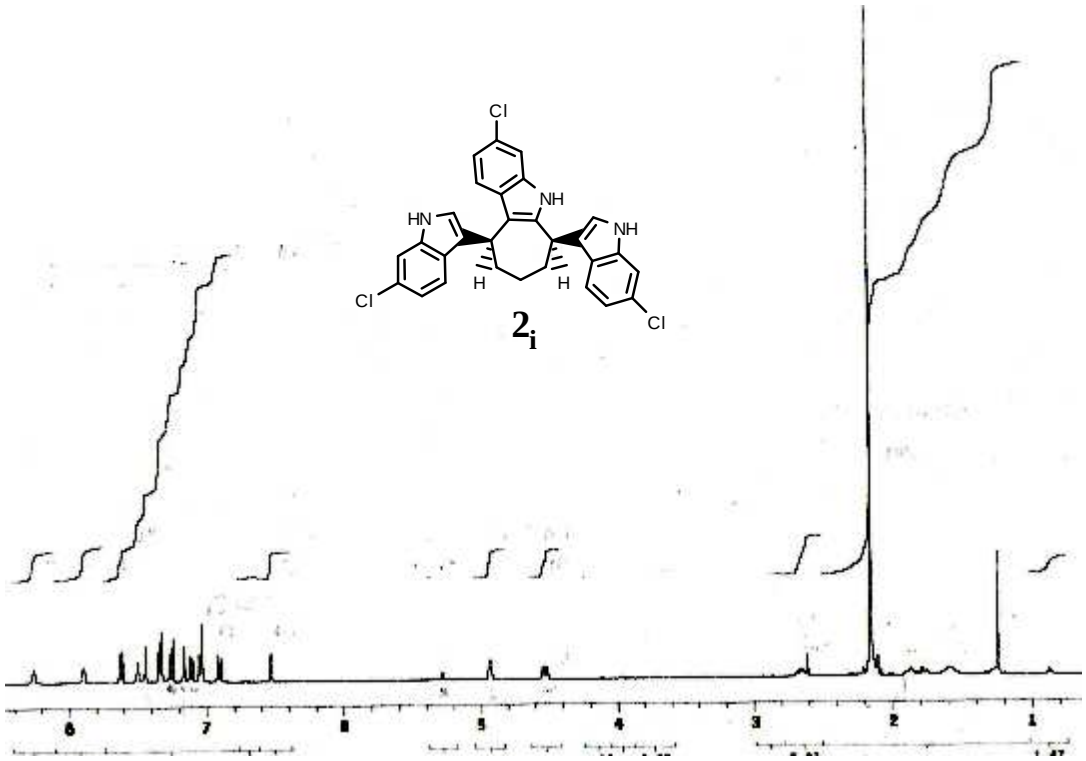


Figure (78): ^{1}H-NMR spectra of compound **2$_i$** in $CDCl_3$

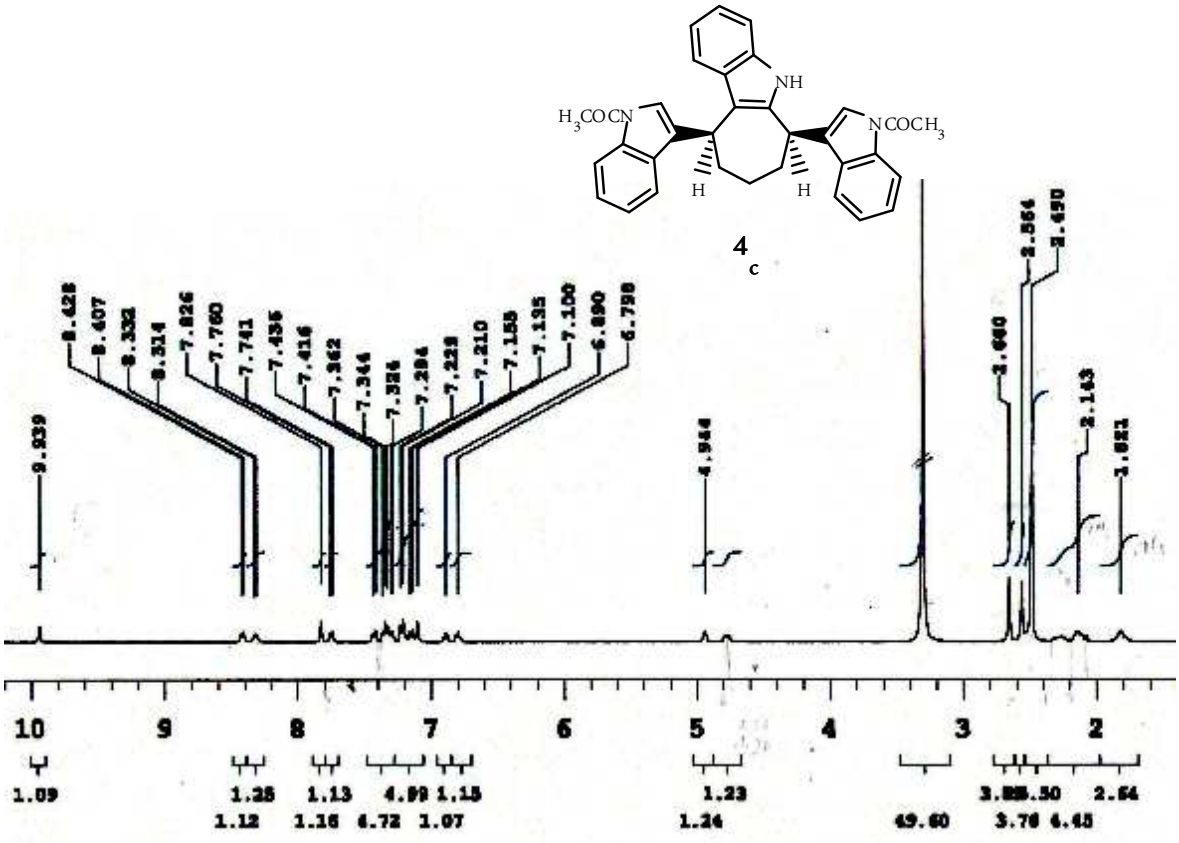


Figure (79): ^{1}H-NMR spectra of compound **4$_c$** in DMSO-*d6*.

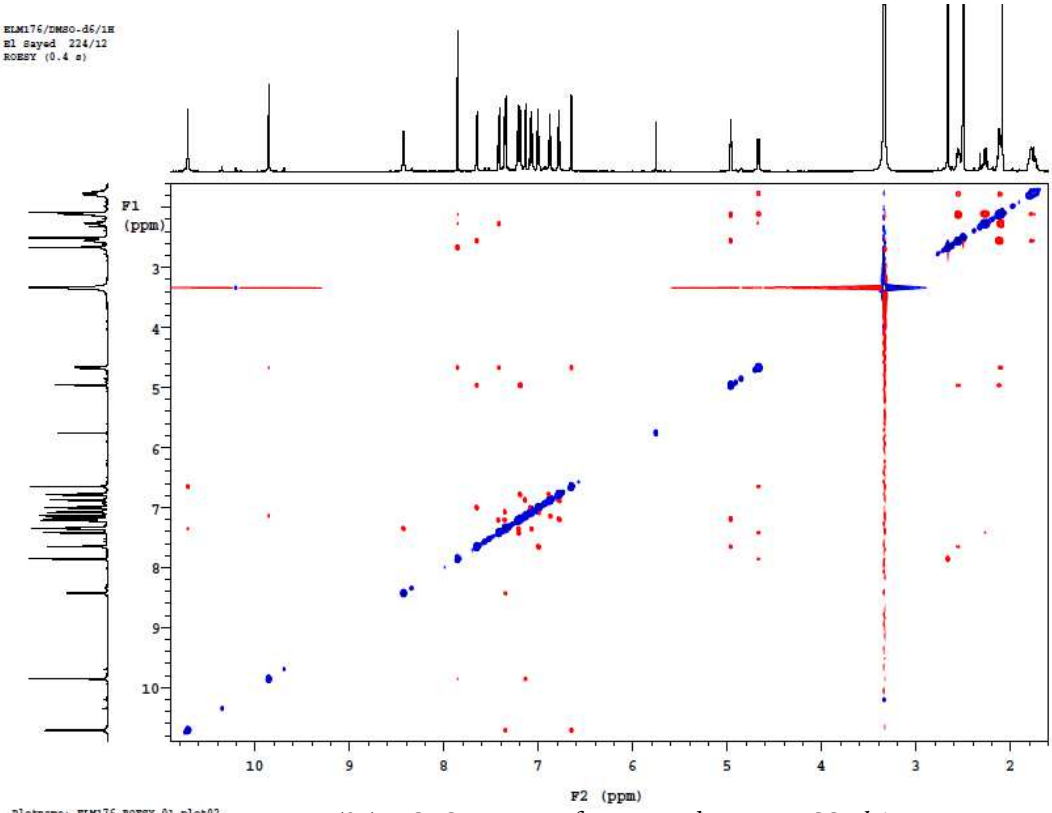


Figure (80): ROESY spectra of compound **4**$_{a}$ in DMSO-*d6*.

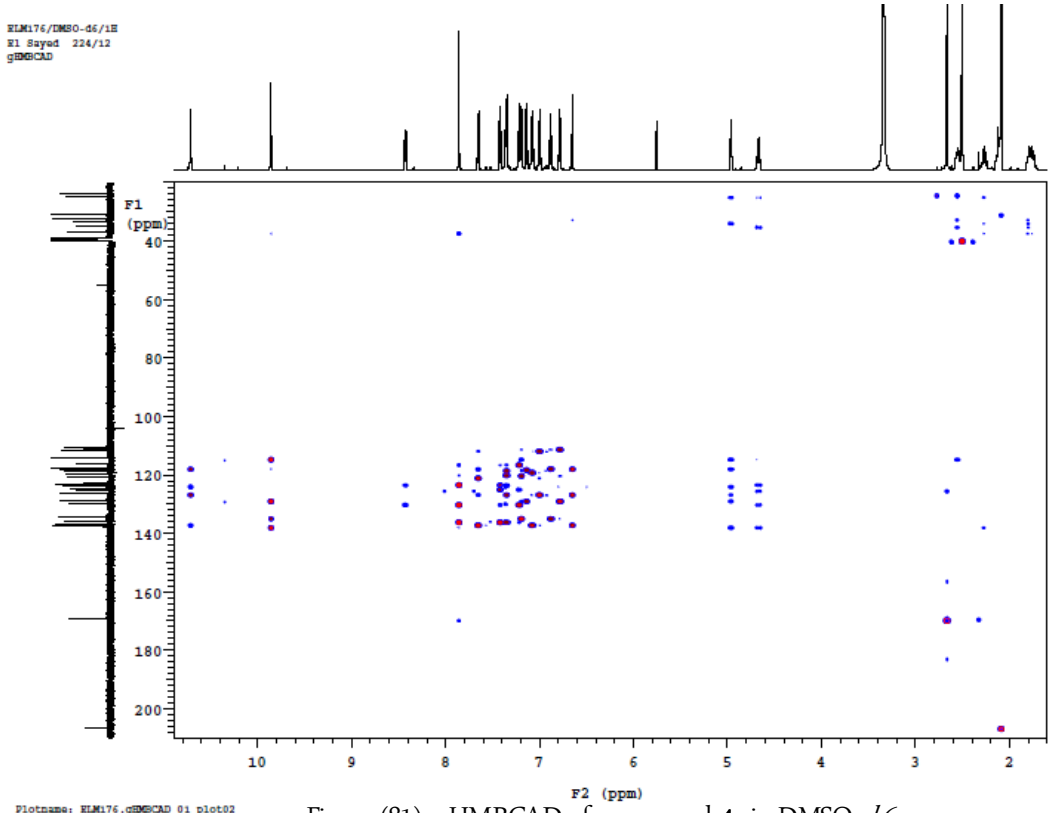


Figure (81): gHMBCAD of compound **4**$_{a}$ in DMSO-*d6*.

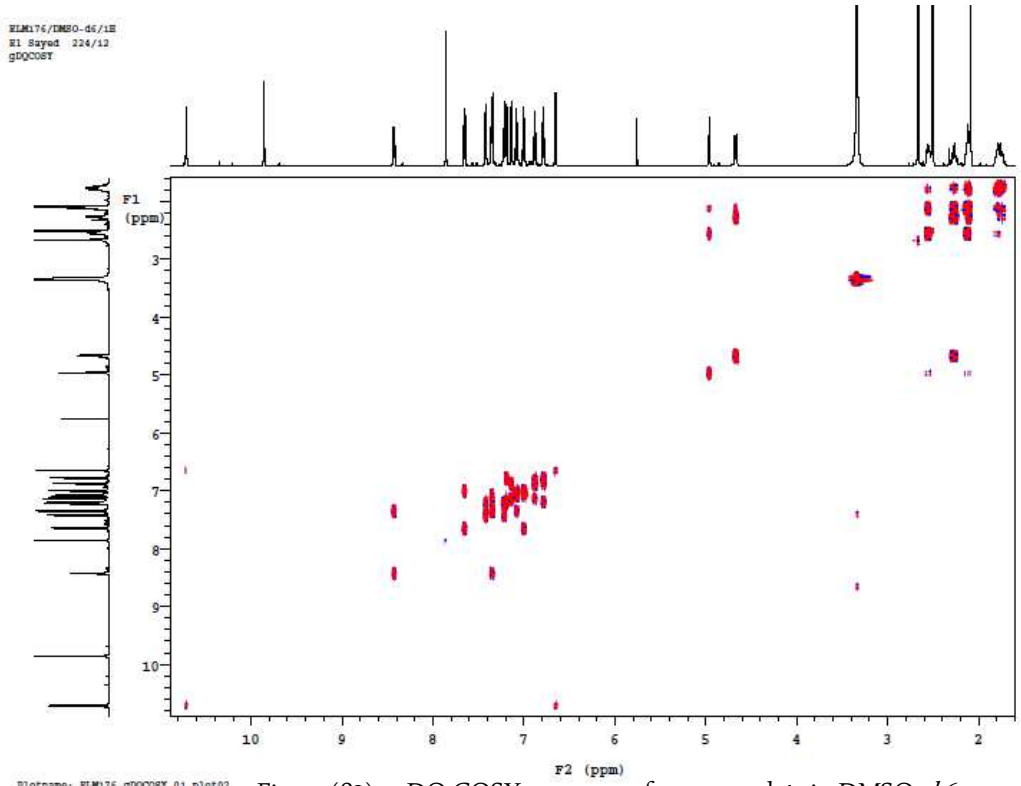


Figure (82): g DQ COSY spectrum of compound **4**$_{a}$ in DMSO-*d6*.

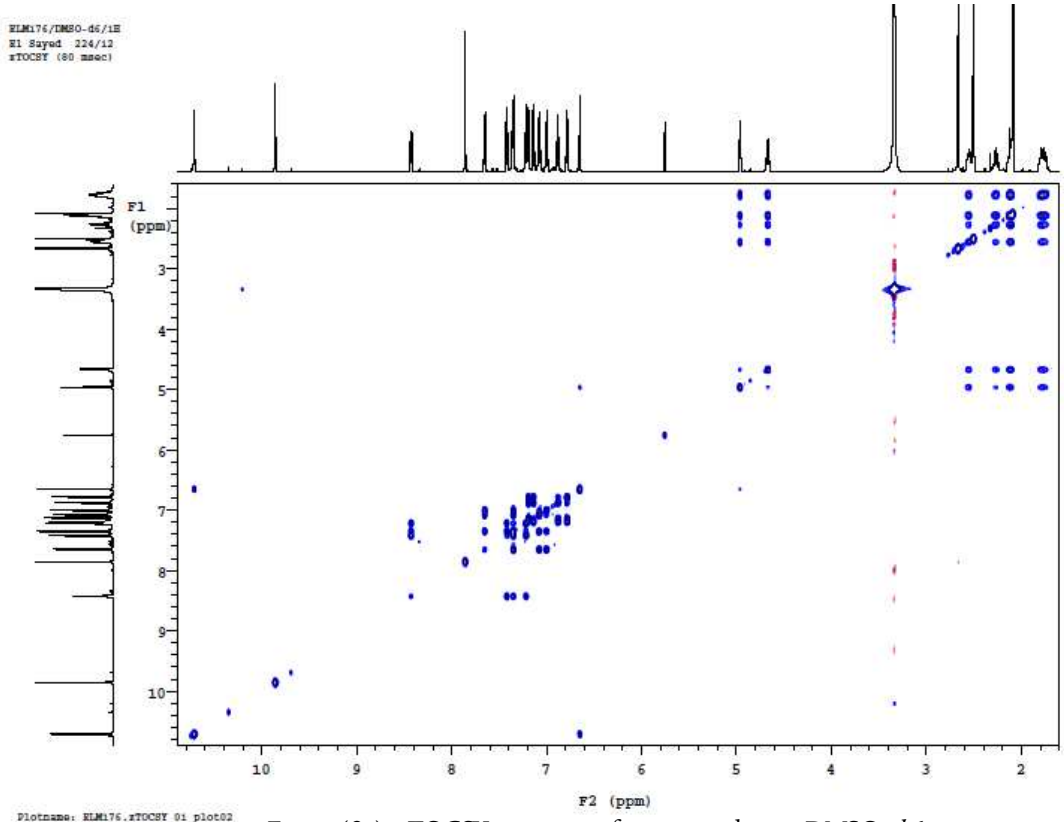
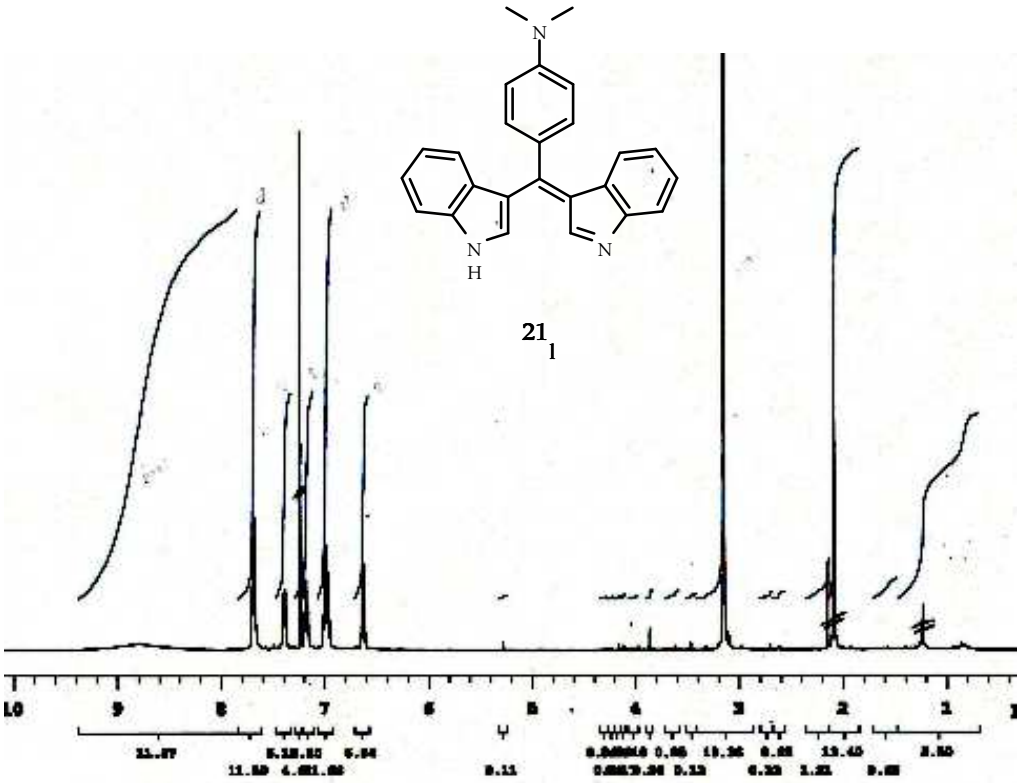
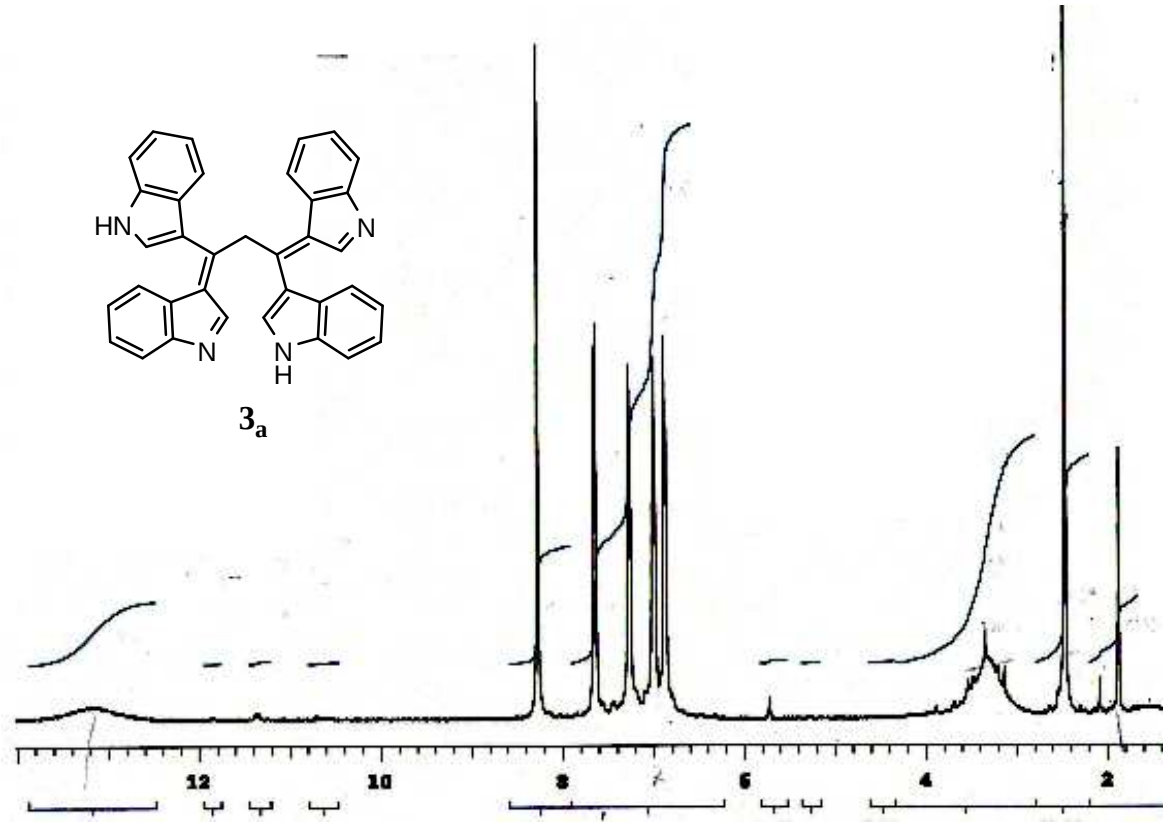


Figure (83): zTOCSY spectrum of compound **4**$_{a}$ in DMSO-*d6*



21$_1$

Figure (84): ^{1}H-NMR of compound 21$_1$ in $CDCl_3$



3$_a$

Figure (85): ^{1}H-NMR spectrum of compound **3**$_a$ in DMSO-*d6*.

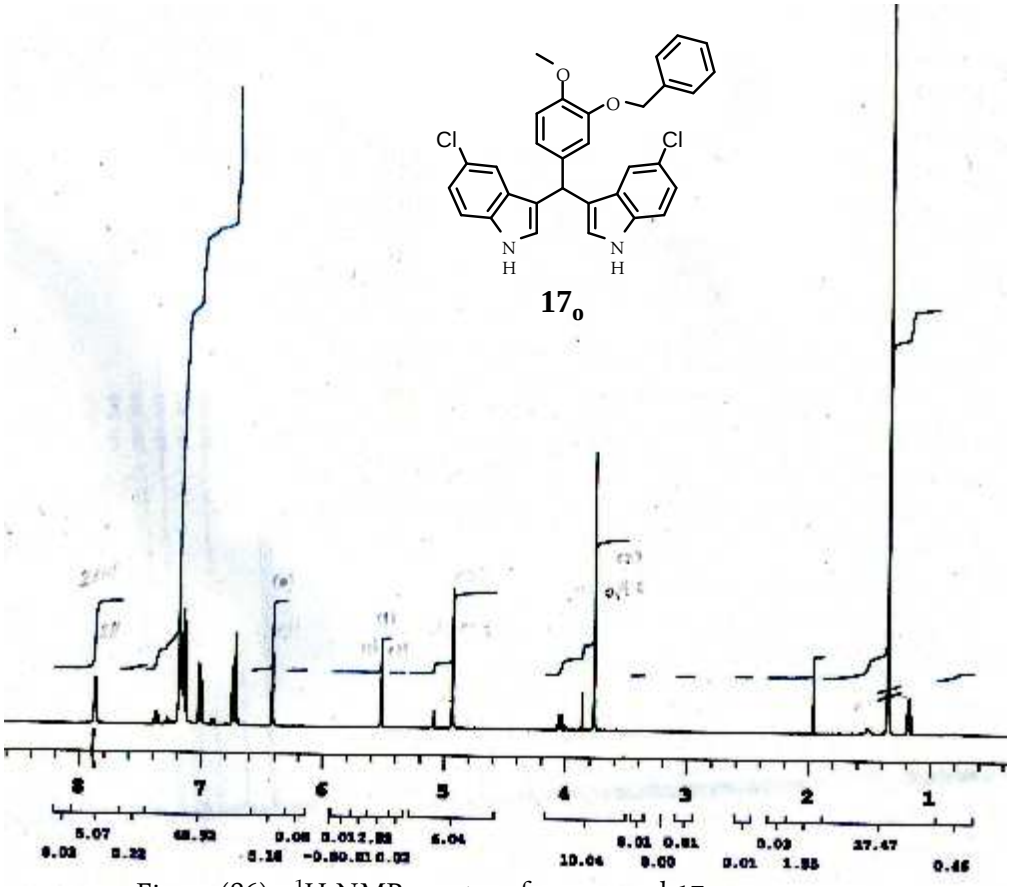


Figure (86): ^{1}H-NMR spectra of compound 17_o

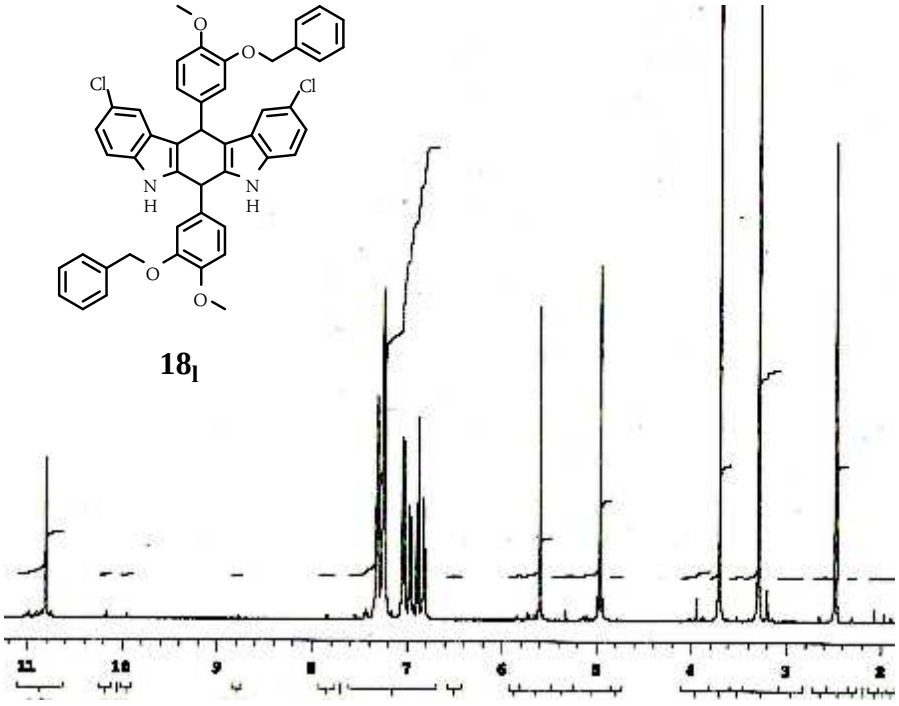
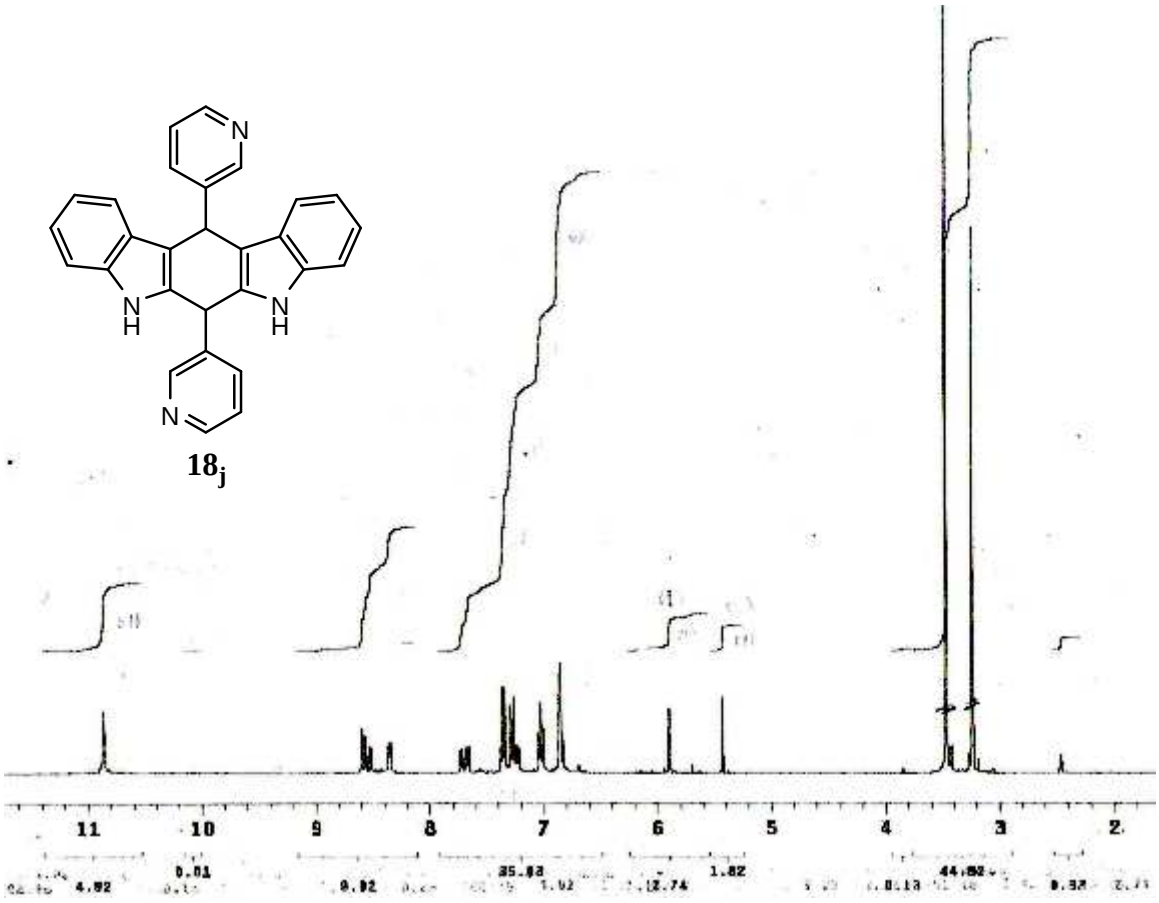
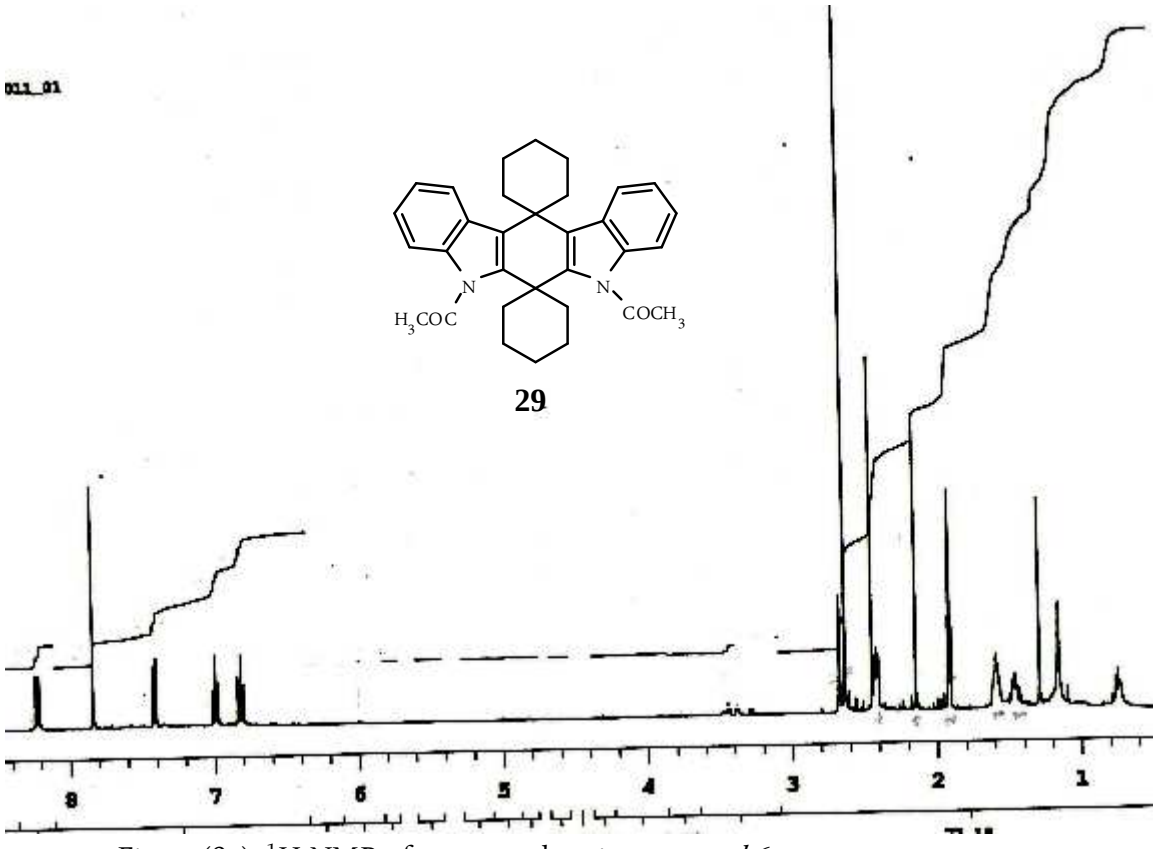


Figure (87): ^{1}H-NMR of compound 18_l DMSO-*d6*.



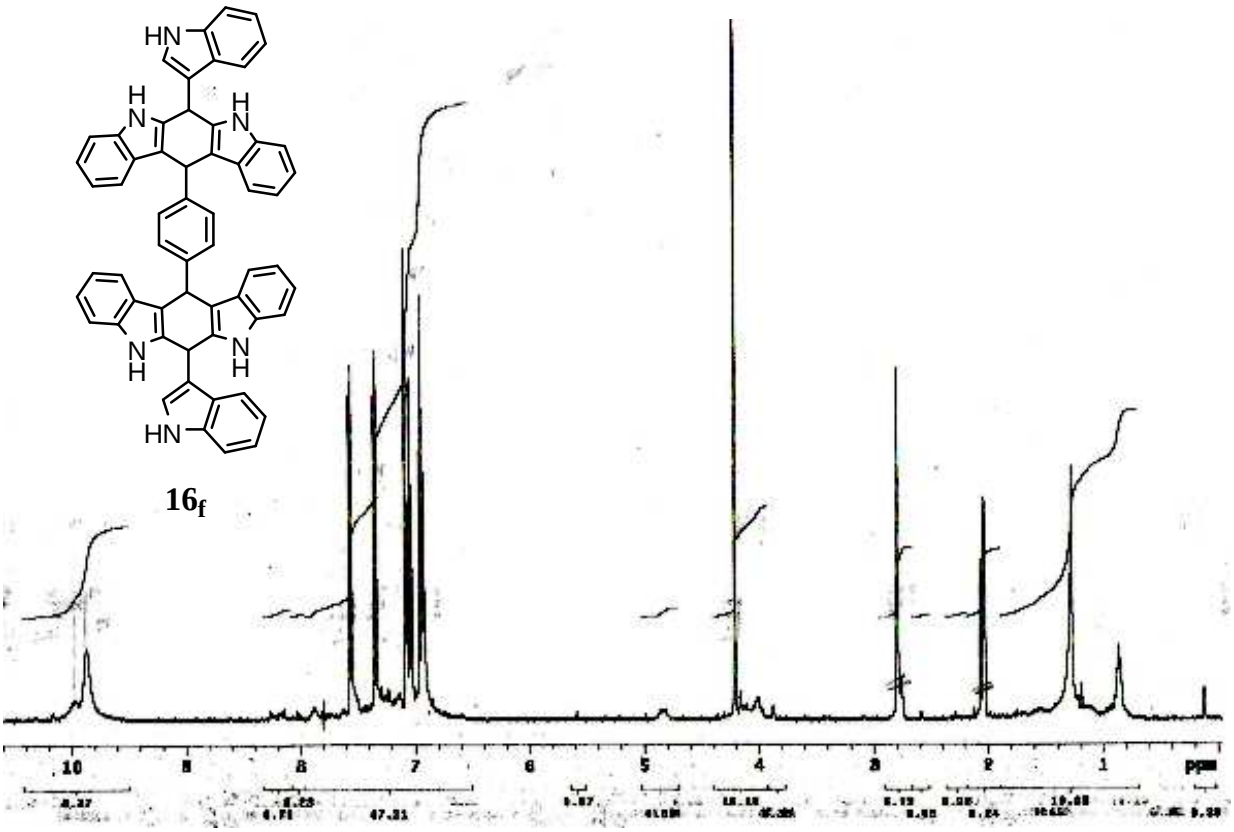
18_j

Figure (88): ^{1}H-NMR of compound **18_j** in DMSO-*d6*.



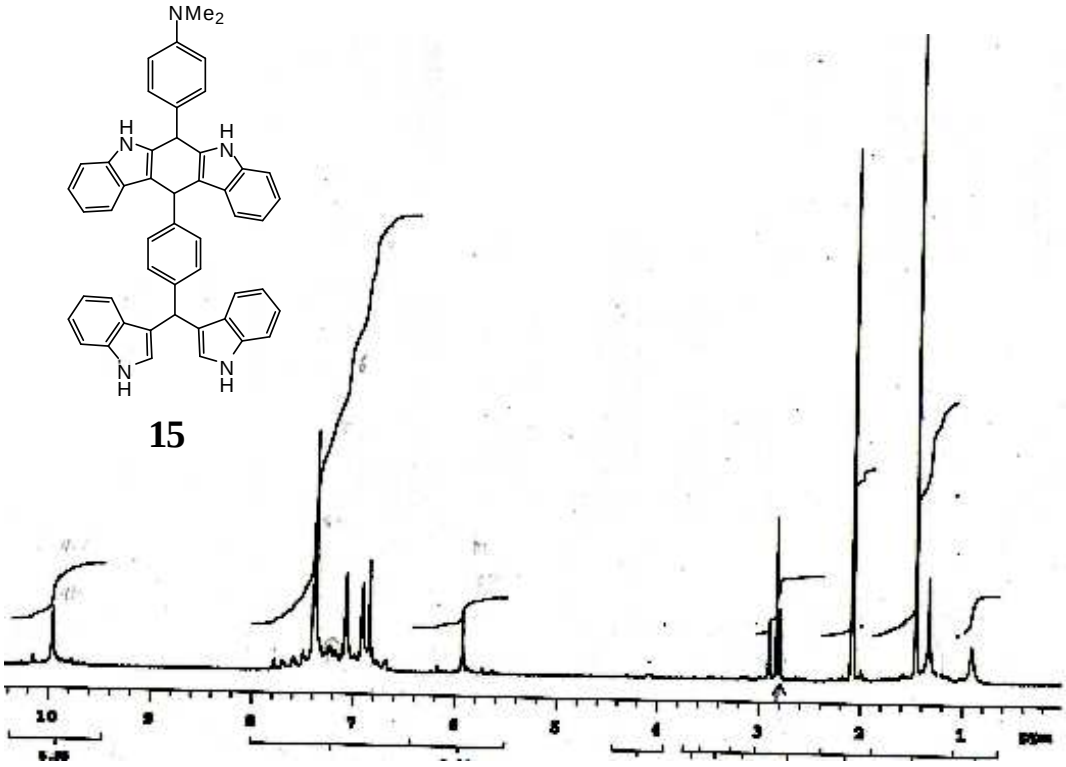
29

Figure (89): ^{1}H-NMR of compound **29** in acetone-*d6*.



16$_f$

Figure (90): ^{1}H-NMR of compound **16$_f$** in acetone-*d6*.



15

Figure (91): ^{1}H-NMR spectra of the intermediate 15 in acetone-d6

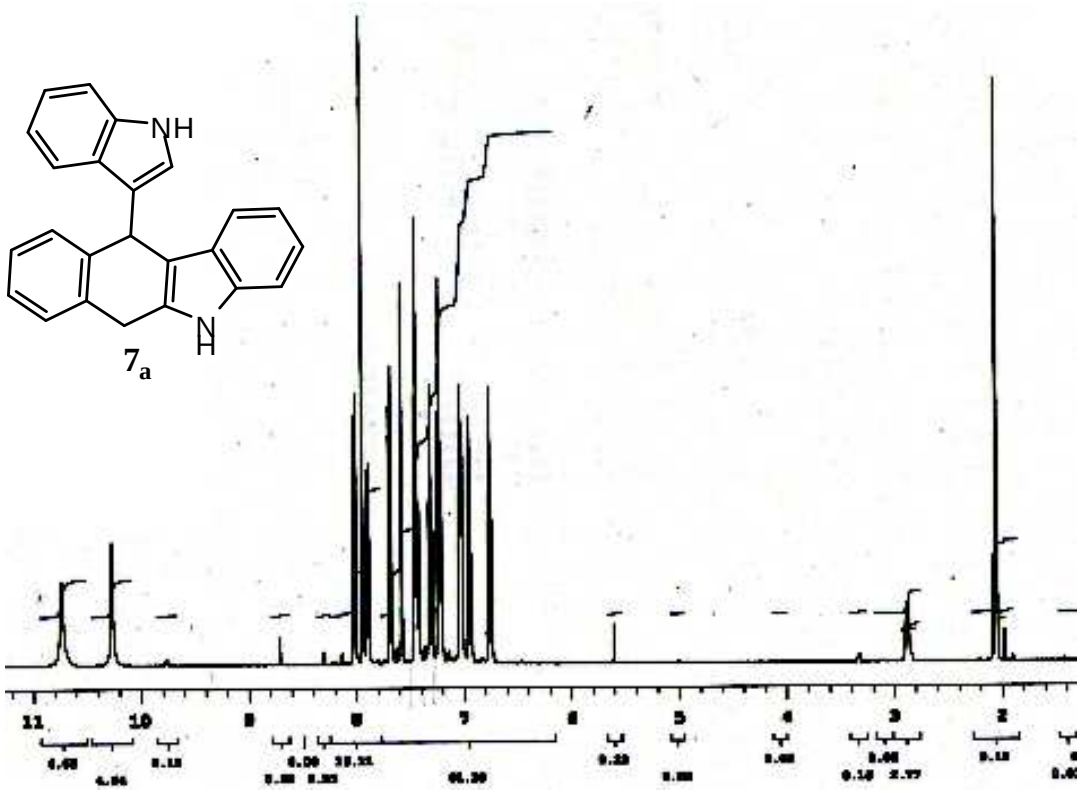


Figure (92): ^{1}H-NMR spectra of compound **7**$_a$ in DMSO-*d6*.

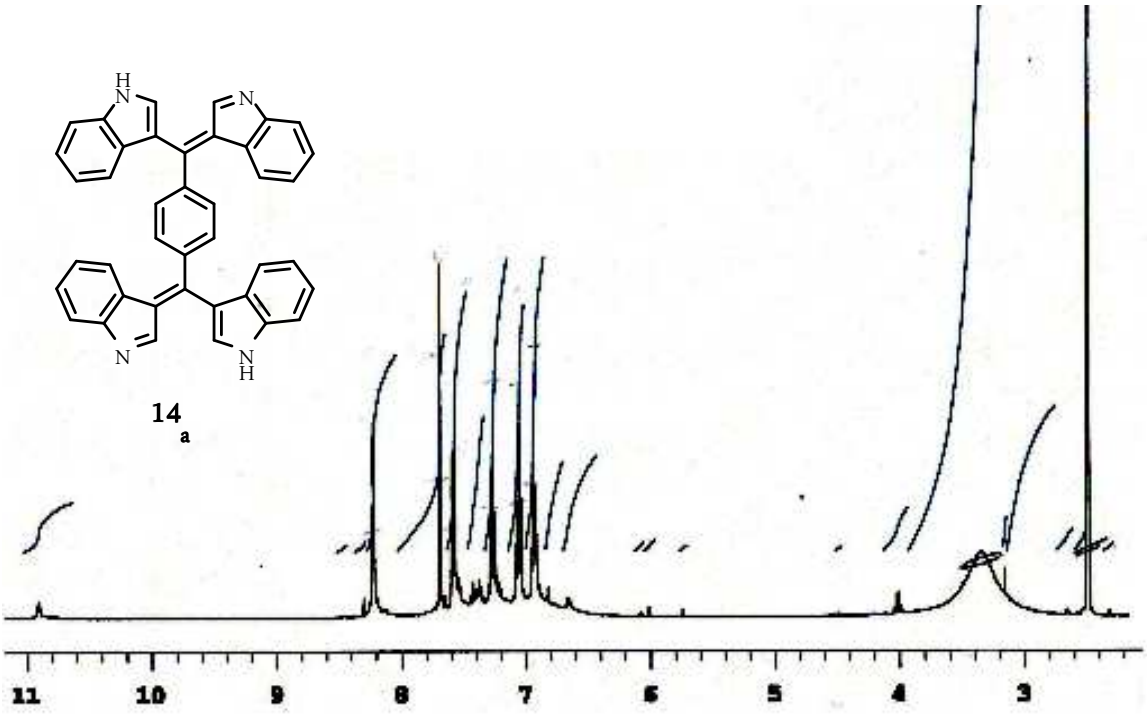


Figure (93): ^{1}H-NMR of compound **14**$_a$ in DMSO-*d6*.

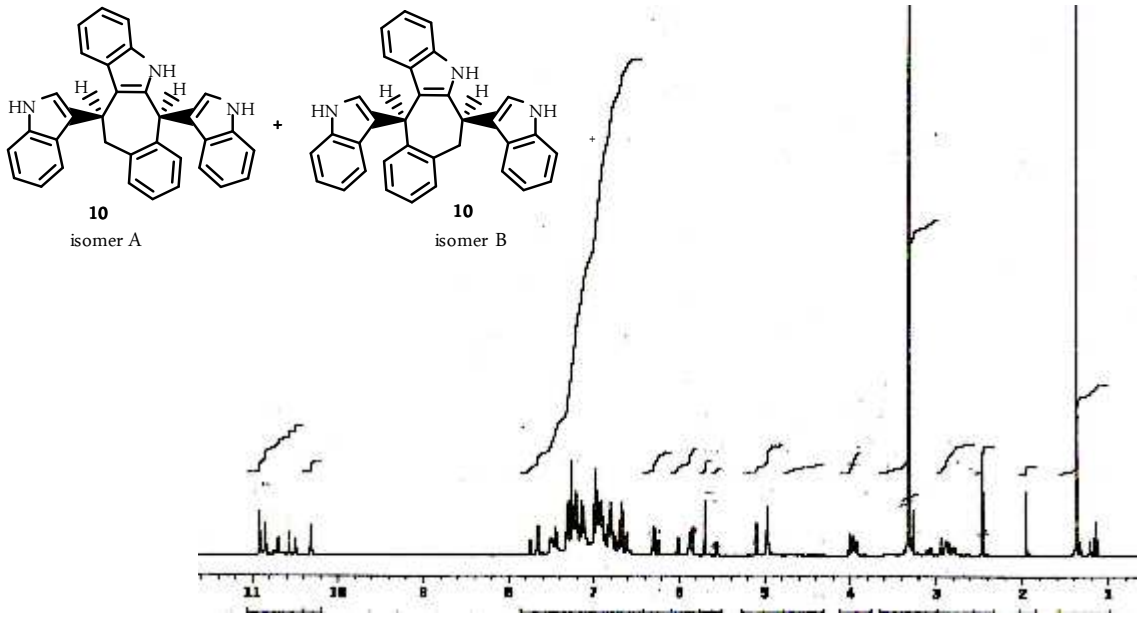


Figure (94): ^{1}H-NMR spectra of the mixture of two isomer of compound **10** in DMSO-*d6*.

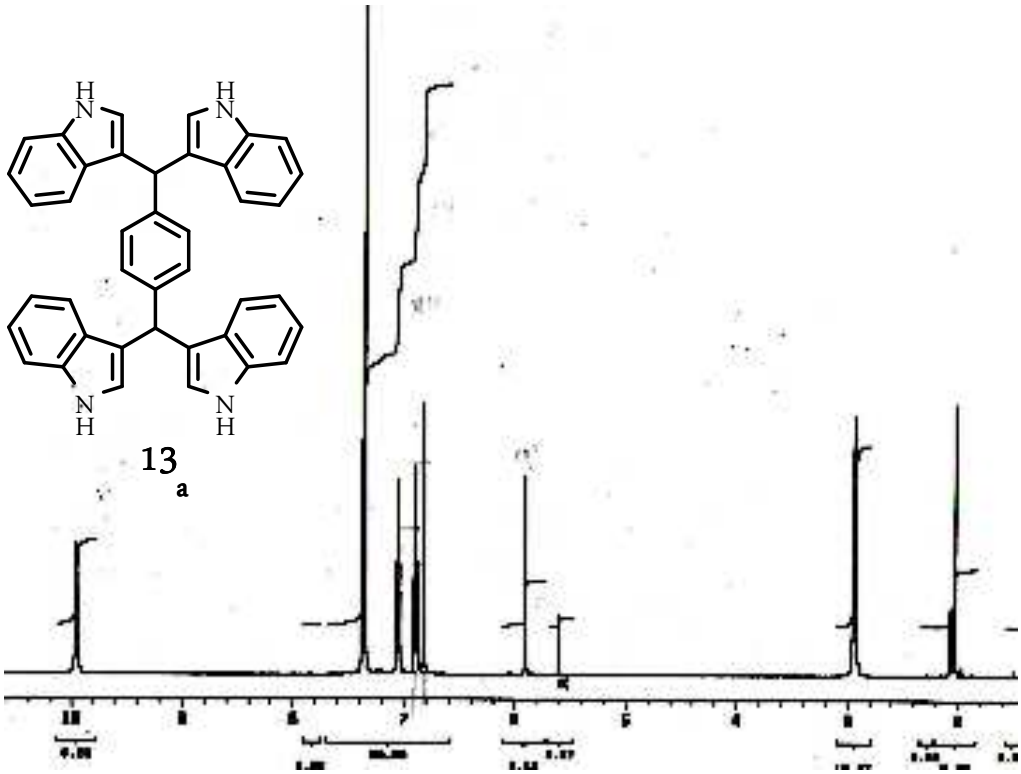


Figure (95): ^{1}H-NMR spectra of compound **13**$_{a}$ in DMSO-*d6*.

6.3. Summary details of X-ray crystallography of compound 4_d and 7_a

Compound 4_d:

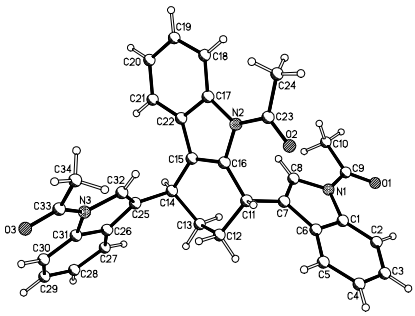
Dr. Frank W. Heinemann, Institute für Anorganische Chemie II, Egerlandstrasse 1, D91058 Erlangen, Tel.: +49 (9131) 8527383, Fax: +49 (9131) 8527367, E-mail: frank.heinemann@chemie.uni-erlangen.de

Summary of details for X-ray crystallography	28. 09. 2011

AHI1102 (ELM215c)

1. Compound name:

2. Formula: $C_{34}H_{29}N_3O_3$



3. Crystal data (e.s.d.'s in parentheses):

a(Å)	=	5.6167(5)	α(°)	=	90
b(Å)	=	39.258(3)	β(°)	=	95.079(2)
c(Å)	=	11.931(2)	γ(°)	=	90
V(Å^3)	=	2620.4(4)	Z	=	4

Number of reflections used for cell refinement: 3642

Range (°): $4.6 \leq 2\theta \leq 52.0$

4. Crystal system, space group (number in „International Tables") monoclinic, $P2_1/n$ (Nr. 14)

5. Experimental conditions:

Radiation: MoK_α (QUAZAR focussing Montel optics), λ = 0.71073 Å

2θ-range (°): $4.1 \leq 2\theta\theta \leq 52.8$

Diffractometer: Bruker Kappa APEX 2 *IµS* Duo

Scan: ω- and φ-rotations with 0.50° and 60 s per frame

Temperature (K): 100

Crystal shape and colour: plate, colourless

Crystal size (mm): 0.12×0.08×0.02

D_{ber} (g·cm^{-3}): 1.337

μ(mm^{-1}): 0.086

Recrystallized from:

6. Absorption correction: SADABS (semiempirical from multiple measurements of equivalent reflections) T_{min} = 0.644, T_{max} = 0.746

7. Extinction correction: none

8. Number of reflections (N)

collected:	21426
unique:	5316
observed ($F_o \geq 4.0\sigma(F)$):	4129

Structure solution: direct methods

Number of refined parameters (p): 364

9. Fractional atomic coordinates and equivalent isotropic displacement parameters (Tab. 2 containing x, y, z with e.s.d.'s, U_{eq} included)

10. Source of atomic scattering factors and anomal dispersion correction terms (f' and f''): International Tables for Crystallography, Vol. C (1992), Ed. A. J. C. Wilson, Kluwer Academic Publishers, Dordrecht: Tables 6.1.1.4 (pp. 500-502), 4.2.6.8 (pp.219-222) und 4.2.4.2 (pp.193-199).

11. Table of anisotropic displacement parameters included (Tab. 4)

(anisotropic according to: $U_{eq} = \frac{1}{3} \sum_i \sum_j U_{ij} a^*_i a^*_j \mathbf{a}_i \mathbf{a}_j$)

12. Table of bond distances and angles (Tab. 3) with e.s.d.'s in parentheses included.

13. Table of hydrogen coordinates and isotropic displacement parameters (Tab. 5) included. Geometrically positioned H-atoms are given without e.s.d.'s (see also Remarks)

14. Final R indices:

$$wR_2 = \left[\frac{\Sigma[w(F_o^2 - F_c^2)^2]}{\Sigma[w(F_o^2)^2]} \right]^{\frac{1}{2}} = \quad 0.1600$$

$$R_1 = \left[\frac{\Sigma \| F_o | - | F_c \|}{\Sigma | F_o |} \right] = \quad 0.0714 \text{ (for observed reflections)}$$

$$\text{GooF} = S = \left[\frac{\Sigma[w(F_o^2 - F_c^2)^2]}{(n-p)} \right]^{\frac{1}{2}} = \quad 1.148$$

15. Weighting scheme: $w = 1 / [\sigma^2(F_o^2) + (0.0323 \cdot P)^2 + 4.3934 P]$

$P = (F_o^2) + 2 \cdot F_c^2) / 3$

16. Residual electron density (largest peak and hole, e·Å^{-3}) in the final difference fourier synthesis: max.: 0.523, min.: -0.397

17. Remarks

Representation of the molecular structure with the atomic numbering scheme is included. All non-hydrogen atoms were refined anisotropically. Treatment of hydrogen atoms: All hydrogen atoms were placed in positions of optimized geometry, their isotropic displacement parameters are tied to those of their corresponding carrier atoms by a factor of 1.2 or 1.5.

18. Software:

Measurement: APEX 2 (Bruker AXS, 2009)

Data reduction: SAINT (Bruker AXS, 2009)

Absorption correction: SADABS (Bruker AXS, 2009)

Structure solution: SHELXTL NT 6.12 (Bruker AXS, 2002)

SHELXTL NT 6.12 (Bruker AXS, 2002) Refinement: SHELXTL NT 6.12 (Bruker AXS, 2002) Graphical representation:

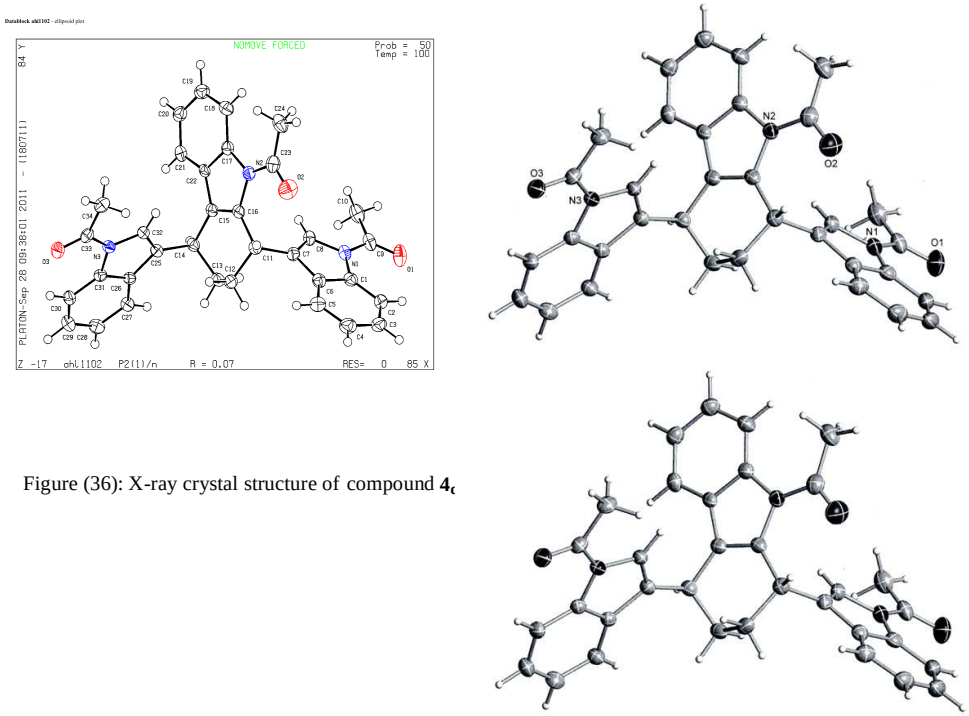


Figure (36): X-ray crystal structure of compound **4**

Compound 7_a:

The data quality of 7_a (**ELM258**) was poor so that it was just a preliminary structure determination and we received the following photo:

AHI1101 (ELM258):

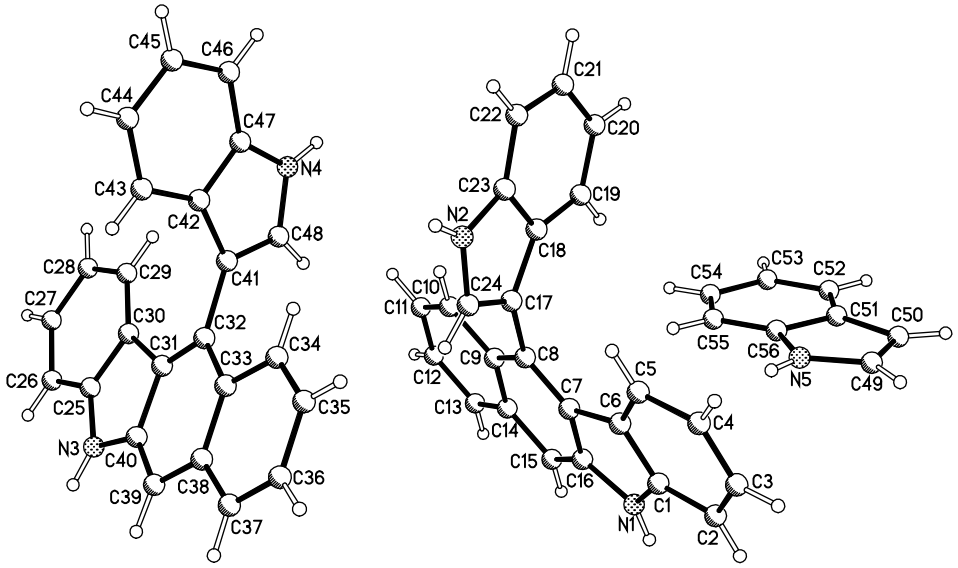


Figure (40): X-ray structure of compound 7_a.

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charge on application to CCDC, 12 Union Road, Cam bridge CB2 1EZ, UK [Fax: (internat.) 44 (1223) 336 - 033; E-mail: deposit@ccdc.cam.ac.uk].

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Publications

Papers:

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Reviews:

[2] Mardia Telep El-Sayed, Kazem Mahmoud, Andreas Hilgeroth; Synthesis of β-Nitro amines *via* Classical Mannich and Aza-Henry Reactions; *Curr. Org. Chem.* (Accepted).

Patent:

[1] Mardia Telep El-Sayed, Andreas Hilgeroth, *Novel Bis- and Tris-Indolyl as Anti-MRSA*, (under registeration).

Presentations in conferences :

[1] Poster Presentation, titled (*β*-Nitrostyrene as a precursor to Nitro Mannich Bases: Synthesis and Biological Evaluation of Some New Nitro Mannich Bases), in International conference on chemistry "***Chem 05***" under the theme "Green and Sustainable Chemistry in Developing Countries " from 3rd to 6th March **2008** in Faculty of Science, Cairo University, **Egypt**.

[2] Poster Presentation, titled (*Bis-Indolyl as Cytostatics*), in "**6th Summer School Medicinal Chemistry**" at the University of Regensburg, in 26th to 29th September **2012**, Regensburg, **Germany**.

[2] Oral Presentation, titled "*Synthesis of Novel Indolo Spirocyclic Compounds*" in International Congress of Young Chemists "**YoungChem2012**" 10th to 14th October **2012** in Gdansk, **Poland**.

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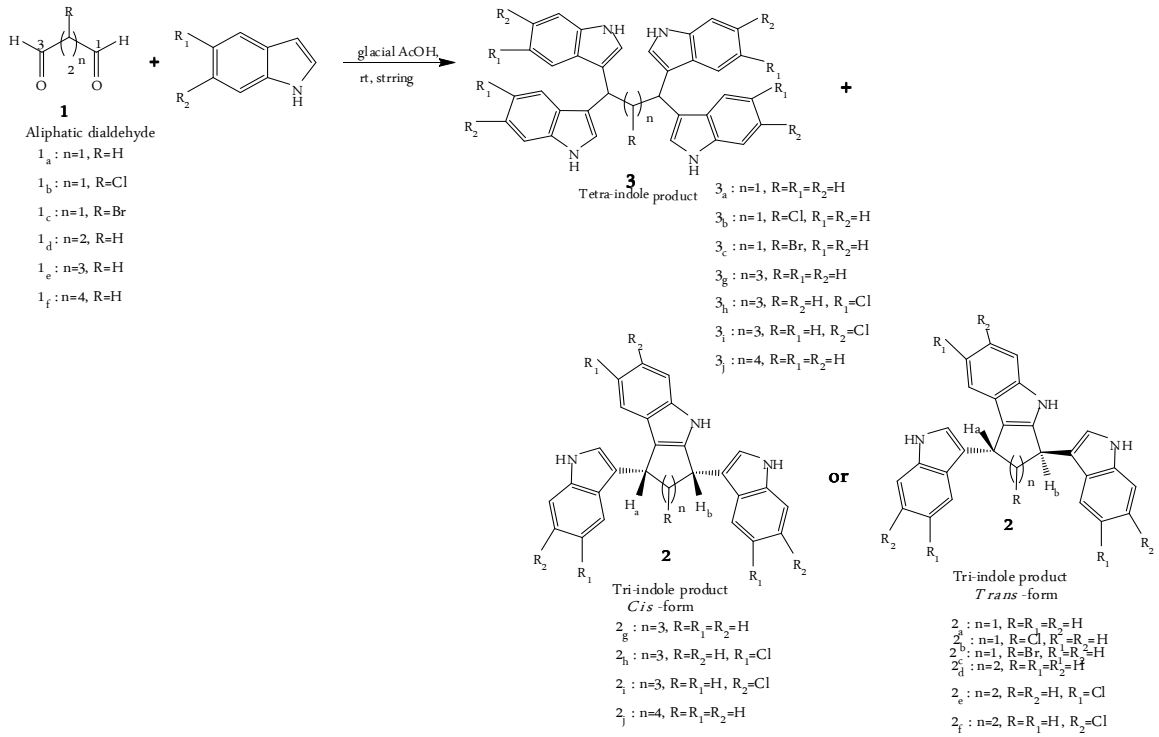
Zusammenfassung

Kunststoffteil

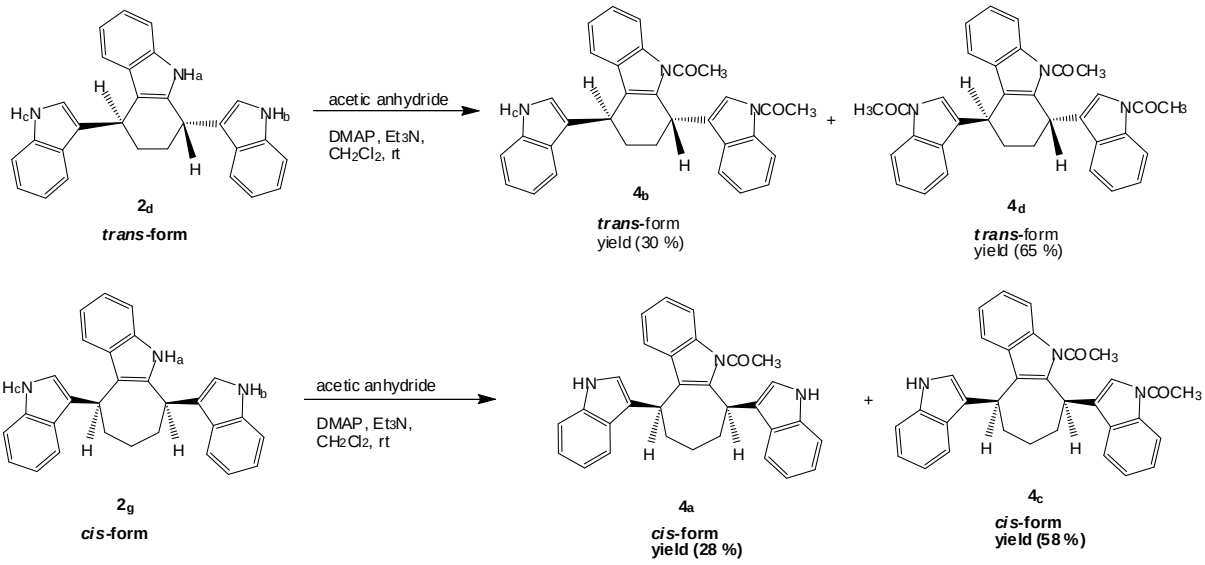
In der vorliegenden Arbeit stellen wir AcOH als mild und effizienter Katalysator für die Synthese von neuartigen hochsubstituierten diastereromeric tetrahydrocyclopenta Indole, Tetrahydrocarbazolen, hexahydrocyclohepta und hexahydrocycloocta Indolen mit triindole Substituenten in Form von *cis*-oder trans-als Nebenprodukt und tetraindole von Propan, Pentan und Hexan als Hauptprodukt. Unsere Reaktion bedeutet die Einführung eines neuen und einfachen chemischen Reaktion Methode, die nicht in der Literatur wurde bereits berichtet. Wir verließen Indole reagieren leicht mit aliphatischen Dialdehyden ($\mathbf{1_{a-f}}$) zum Beispiel Malonaldehyd und seinen Derivaten, Succinaldehyd, Adipaldehyd und Glutaraldehyd zu substituierten Indolen diastereromeric tetrahydrocyclopenta leisten, Tetrahydrocarbazole, hexahydrocyclohepta und hexahydrocycloocta Indolen vom Typ **2** als Nebenprodukt und Propan, Pentan oder Hexan substituiert mit vier Einheiten von Indol-Typ (**3**) als Hauptprodukt, Schema (2).



Scheme (2): General equation for the reaction of indoles with aliphatic dildehydes.

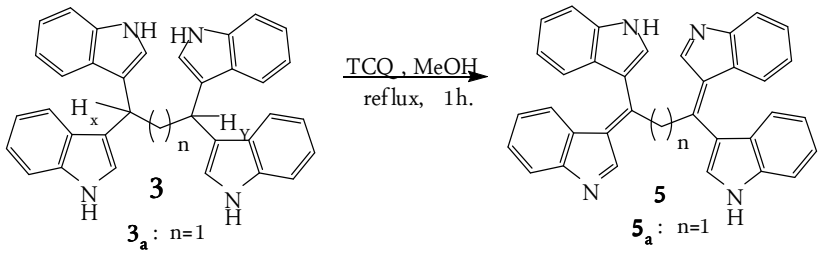
Die Acetylierungsreaktion des trans-Isomers der Verbindung $\mathbf{2_d}$, führte zu dem identifizierten Produkt diacetylierten $\mathbf{4_b}$ und $\mathbf{4_d}$ der triacetylierte Derivat. Im Falle der

cis-Form der Verbindung **2g** die Reaktion ergab zwei Produkte als *cis*-Formen, die Mono-acetylierten Verbindungen **4a** und die diacetylierten ein **4c**, Schema (10).



Scheme (10): Acetylation reactions of triindole products.**2d** and **2g** .

Die Oxidationsreaktion von tetraindoles (**3a, g, j**) fanden unter milden Bedingungen, wie für die Oxidation von BIMs mithilfe TCQ oder DDQ, Schema (11) gemeldet.

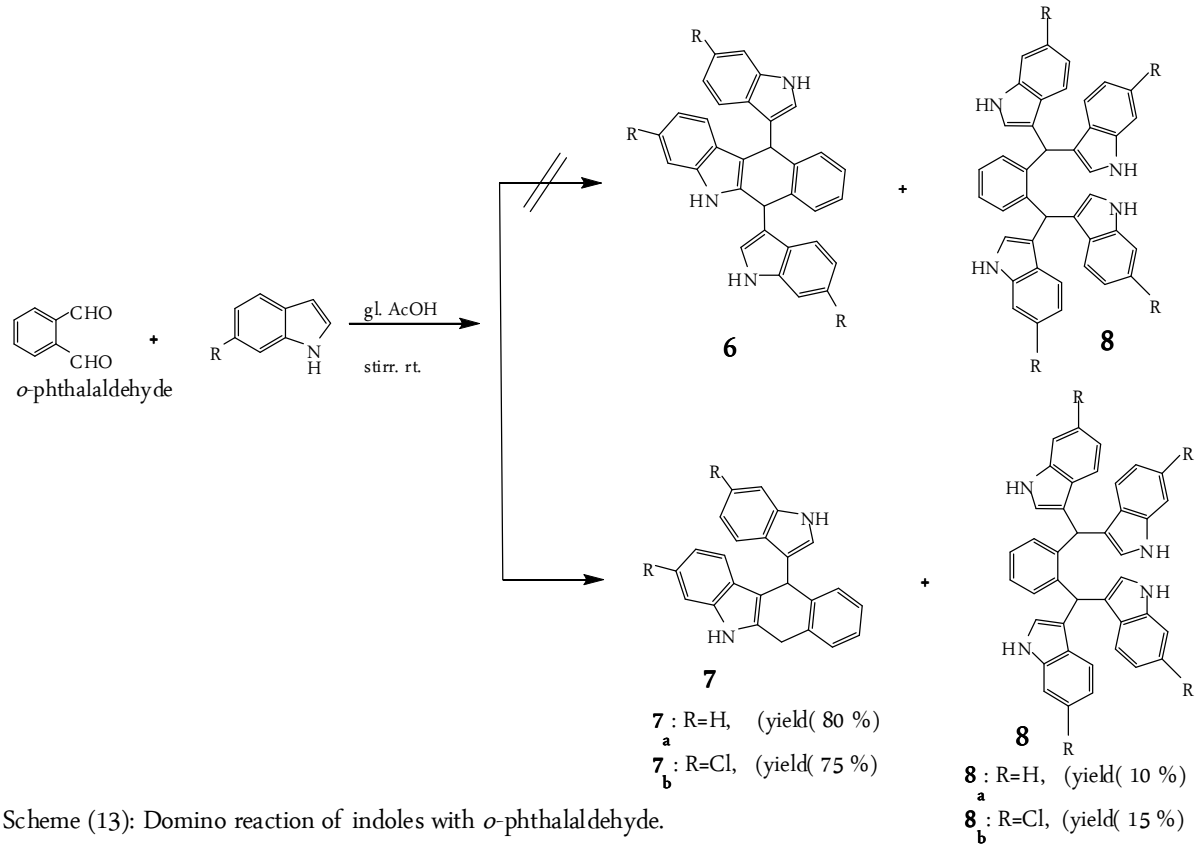


3a : n=1
3g : n=3
3j : n=4

5a : n=1
5b : n=3
5c : n=4

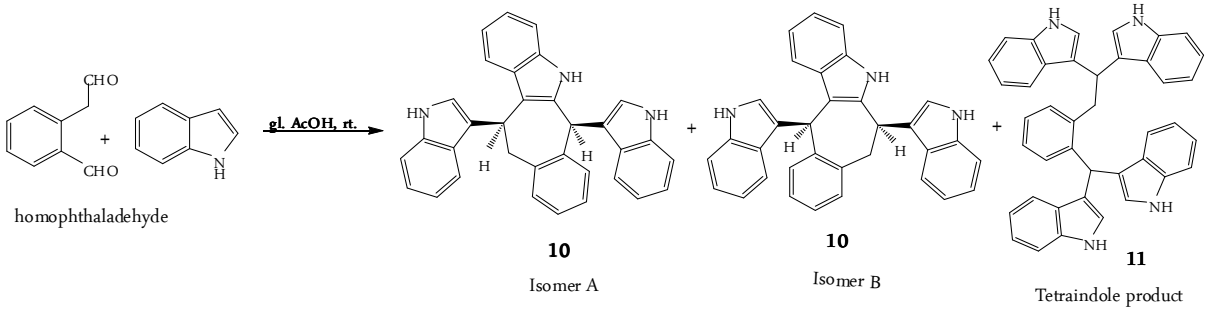
Scheme(11): Oxidation reactions of tetraindoles **3a,g,j**.

Die Umsetzung von o-Phthalaldehyd mit Indol, in Gegenwart von AcOH bei Raumtemperatur wurde gefunden, dass erfolgreiche Man erhält das Produkt **7a-b** in 75 bis 80 % ergibt, zusätzlich zu der Ausbildung der zu erwartenden Produkte tetraindole **8a,b** in eine sehr geringer Ausbeute von 10 bis 15 %, Schema (13).



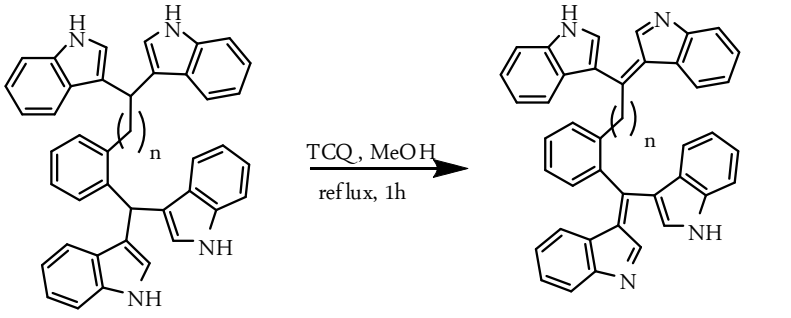
Scheme (13): Domino reaction of indoles with *o*-phthalaldehyde.

Das Dialdehyd homophthalaldehyde wurde direkt in der Kondensationsreaktion mit Indol in Essigsäure bei Raumtemperatur verwendet was eine neuartige Benzo[7] annulen-Derivat vom Typ **10** in mäßiger Ausbeute (46 %) und dem tetraindole Produkt 11 in einer 38 % Ausbeute. Verbindung **10** wurde in zwei Isomeren A und B, Schema (16) isoliert.



Scheme (16): Domino reaction of indole with homophthaladehyde.

Verbindungen $\mathbf{8_a}$ und **11** könnte auch unterziehen Dehydratisierungsreaktion mit TCQ liefern, das die dehydratisierten Formen **9** und **12** in guten Ausbeuten, Schema (18).

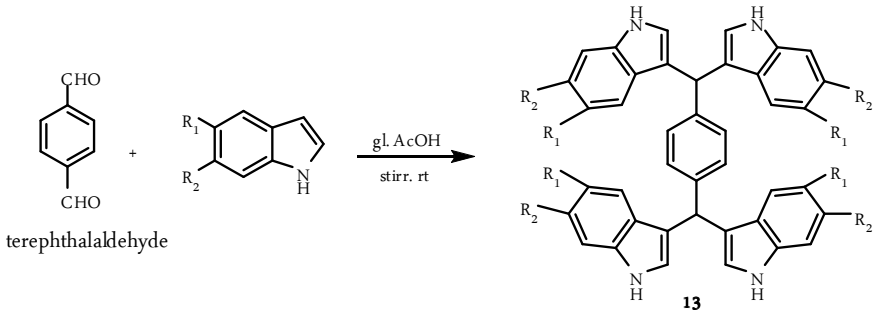


8 : n=o
a
11: n=1

9 :n=0 yield 55%
12 : n=1 yield 62%

Scheme (18): Oxidation reaction of compound **8** and **11**
a

Die elektrophilen Substitutionsreaktionen von Indolen mit terephthaladehyde wurden in der Literatur bereits als möglicher Weg zur Synthese von Verbindungen, die supramolekularen BIMs, nämlich 3,3′,3″,3‴-tetraindolyl(terphthalayl)methan (**13**$_a$) gemeldet in guten Ausbeuten. In der vorliegenden Arbeit terephthaladehyde mit Indolen in Eisessig in einem Molverhältnis (1:4) Man erhält Verbindungen **13**$_{a,b}$ in einer hohen Ausbeute von 93 – 95 % nach einer kurzen Zeit von Rühren bei Raumtemperatur (2 - 4 kondensiert h), Schema (19).

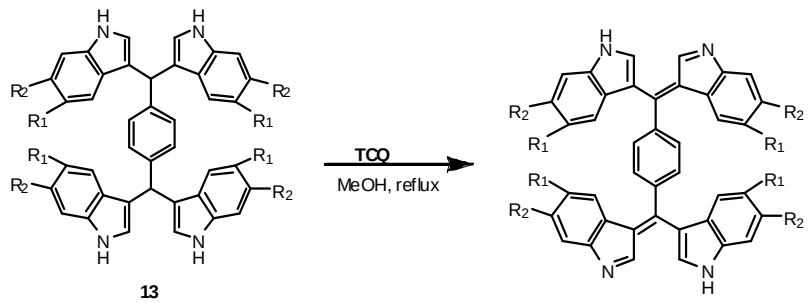


13

13$_a$: R_1=R_2=H
13$_b$: R_1=Cl, R_2=H

Scheme (19): Reaction of indoles with terephthaladehyde.

Die Oxidationsreaktion mithilfe TCQ als Oxidationsmittel in Methanollösung hatte für die Synthese der zu erwartenden neuen bishydrated Formen vom Typ **14**$_{a,b}$, Schema (20) verlängert.



13

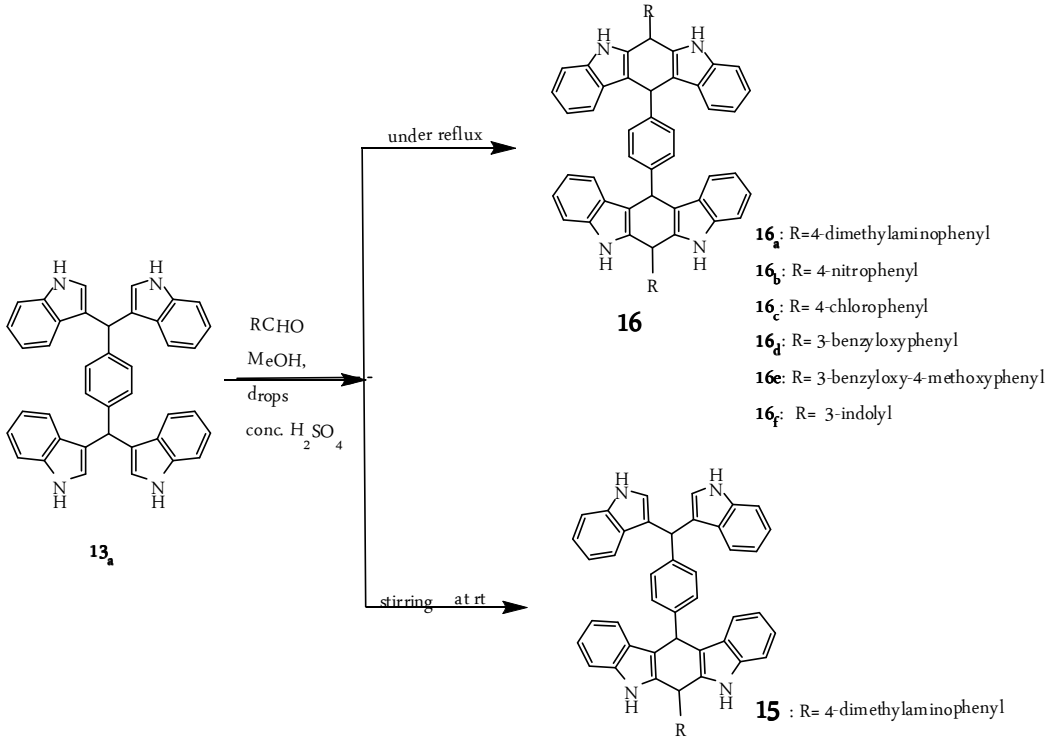
13$_a$: R_1=R_2=H
13$_b$: R_1=Cl, R_2=H

14

14$_a$: R_1=R_2=H
14$_b$: R_1=Cl, R_2=H

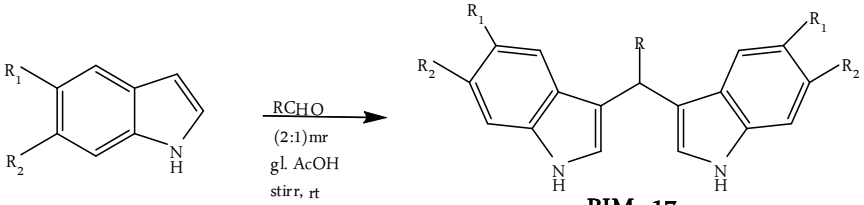
Scheme (20): Oxidation reaction of compound **13**$_{a,b}$.

Verbindungen **13**$_{a,b}$ kann als Nukleophil aufgrund der unbesetzten zwei Positionen der vier Indolringe wirken, damit wir nun ein praktisches Verfahren für die Synthese der neuen erweiterten Ringsysteme (**16**$_{a,f}$) über die Kondensationsreaktion der Verbindung **13**$_a$ mit Aryl oder Heteroaryl substituierten Aldehyd in einem Molverhältnis (1:2), Schema (21). Wenn jedoch die Reaktion wurde bei Raumtemperatur unter Rühren für lange Zeit durchgeführt wird, war das Hauptprodukt, das getrennt und identifiziert wurde Verbindung **15** unter Verwendung von *p*-Dimethylaminobenzaldehyd. Verbindung **15** kann als Zwischenprodukt für die Bildung der Verbindung **16**$_a$ zu berücksichtigen.



Scheme (21): Condensation reaction of **13**$_a$ with aldehydes.

Eine Reihe von substituierten Aryl-oder Heteroaryl Aldehyde wurden effizient zu den entsprechenden **17**$_{a-p}$ BIMs wie in Tabelle (1) und Regelung (25), die die Reaktionszeit und die gebildeten Ausbeuten liefert umgewandelt. Die kurze Reaktionszeit mit der Einfachheit der Reaktionsführung gekoppelt macht diese Methode eine der effizientesten Methoden zur Synthese dieser Klasse von Verbindungen.

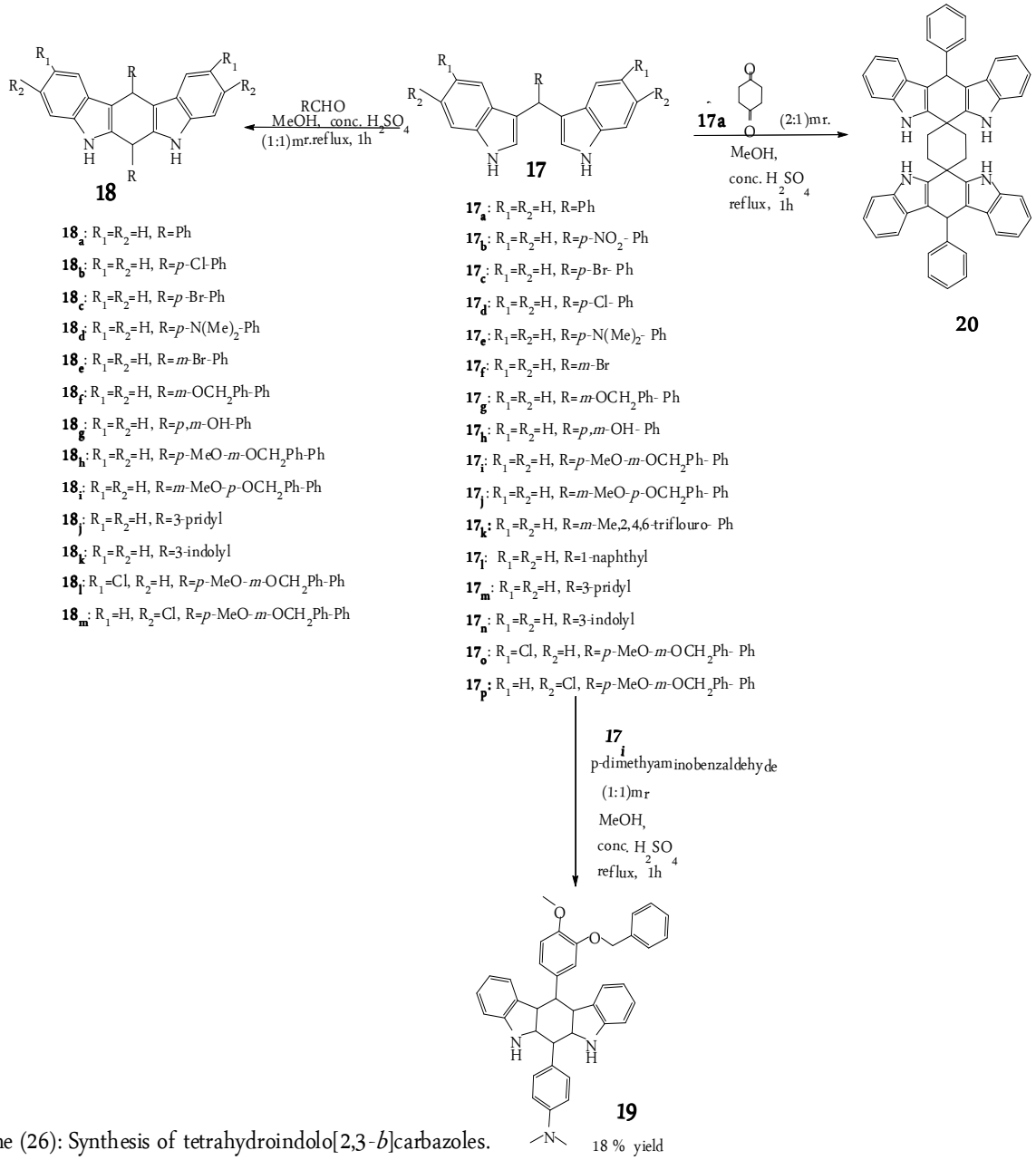


Scheme (25): Synthesis of BIMs.

entry	Aryl or heteroarylaldehydes	Indoles	Product	Reaction time (h)	Yield (%)
1	**R** = Ph	Indole	**17$_a$**	5	90
2	**R** = *p*-NO_2-Ph	"	**17$_b$**	4	98
3	**R** = *p*-Br- Ph	"	**17$_c$**	6	99
4	**R** = *p*-Cl- Ph	"	**17$_d$**	5	76
5	**R** = *p*-N(Me)2- Ph	"	**17$_e$**	5	91
6	**R** = *m*-Br-Ph	"	**17$_f$**	4	88
7	**R**= *m*-OCH_2Ph- Ph	"	**17$_g$**	5	87
8	**R** = *p,m*-OH- Ph	"	**17$_h$**	6	73
9	**R**=*p*-MeO-*m*-OCH_2Ph-Ph	"	**17$_i$**	4	89
10	**R**=*m*-MeO-*p*-OCH_2Ph-Ph	"	**17$_j$**	5	92
11	**R** = *m*-Me,2,4,6-tri-F-Ph	"	**17$_k$**	6	77
12	**R** = 1-naphthyl	"	**17$_l$**	4	97
13	**R** = 3-pridyl	"	**17$_m$**	6	95
14	**R** = 3-indolyl	"	**17$_n$**	6	98
15	**R**=*p*-MeO-*m*-OCH_2Ph-Ph	5-Cl-indole	**17$_o$**	4	91
16	**R**=*p*-MeO-*m*-OCH_2Ph-Ph	6-Cl-indole	**17$_p$**	4	93

Table (1): Synthesized BIMs (**17$_{a-p}$**).

Als erstreckenden Studie unserer vorliegenden Arbeit verwendeten wir die vorbereitete BIMs **17$_{a-p}$** als Ausgangsstoffe zur Synthese von biologisch aktiven tetrahydroindolo [2,3-*b*] Carbazolen vom Typ **18$_{a-l}$**, **19** und die erweiterten spirocyclischen biscarbazoles **20**. Das BIM und der aromatische Aldehyd (dasselbe Aldehyd, mit Indolen in der Synthese des eingesetzten BIM kondensiert) wurden in einem molaren Verhältnis (1:1) für die Synthese der reinen tetrahydroindolo [2,3-*b*] Carbazolen des Typs verwendet **18$_{a-l}$** in guten bis besseren Ausbeuten, Schema (26).



18

18$_a$: R_1=R_2=H, R=Ph
18$_b$: R_1=R_2=H, R=*p*-Cl-Ph
18$_c$: R_1=R_2=H, R=*p*-Br-Ph
18$_d$: R_1=R_2=H, R=*p*-N(Me)$_2$-Ph
18$_e$: R_1=R_2=H, R=*m*-Br-Ph
18$_f$: R_1=R_2=H, R=*m*-OCH_2Ph-Ph
18$_g$: R_1=R_2=H, R=*p*,*m*-OH-Ph
18$_h$: R_1=R_2=H, R=*p*-MeO-*m*-OCH_2Ph-Ph
18$_i$: R_1=R_2=H, R=*m*-MeO-*p*-OCH_2Ph-Ph
18$_j$: R_1=R_2=H, R=3-pridyl
18$_k$: R_1=R_2=H, R=3-indolyl
18$_l$: R_1=Cl, R_2=H, R=*p*-MeO-*m*-OCH_2Ph-Ph
18$_m$: R_1=H, R_2=Cl, R=*p*-MeO-*m*-OCH_2Ph-Ph

17

17$_a$: R_1=R_2=H, R=Ph
17$_b$: R_1=R_2=H, R=*p*-NO_2- Ph
17$_c$: R_1=R_2=H, R=*p*-Br- Ph
17$_d$: R_1=R_2=H, R=*p*-Cl- Ph
17$_e$: R_1=R_2=H, R=*p*-N(Me)$_2$- Ph
17$_f$: R_1=R_2=H, R=*m*-Br
17$_g$: R_1=R_2=H, R=*m*-OCH_2Ph- Ph
17$_h$: R_1=R_2=H, R=*p*,*m*-OH- Ph
17$_i$: R_1=R_2=H, R=*p*-MeO-*m*-OCH_2Ph- Ph
17$_j$: R_1=R_2=H, R=*m*-MeO-*p*-OCH_2Ph- Ph
17$_k$: R_1=R_2=H, R=*m*-Me,2,4,6-triflouro- Ph
17$_l$: R_1=R_2=H, R=1-naphthyl
17$_m$: R_1=R_2=H, R=3-pridyl
17$_n$: R_1=R_2=H, R=3-indolyl
17$_o$: R_1=Cl, R_2=H, R=*p*-MeO-*m*-OCH_2Ph- Ph
17$_p$: R_1=H, R_2=Cl, R=*p*-MeO-*m*-OCH_2Ph- Ph

19

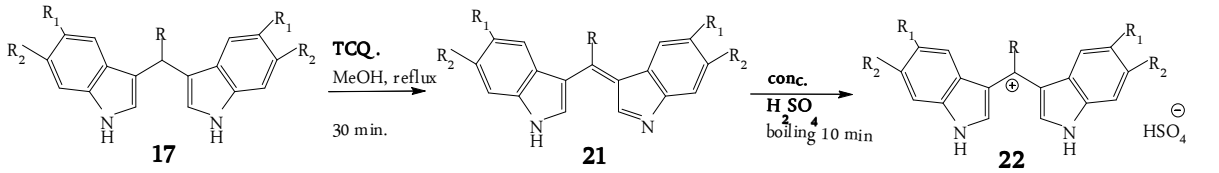
18 % yield

20

Scheme (26): Synthesis of tetrahydroindolo[2,3-*b*]carbazoles.

In diesem Zusammenhang und als Fortsetzung unserer Arbeit über die Synthese von tetrahydroindolo [2,3-*b*] Carbazolen mit einem Versuch, die gemischte Indolocarbazol (zwei verschiedene Aldehyde) vorzubereiten. Die Reaktion von BIMs (**17$_i$**) (1 Moläquivalent) und *p*-Dimethylaminobenzaldehyd (1 Moläquivalent) durch das Verfahren der Verwendung von Methanol Schwefelsäurelösung als mögliche Route zur Synthese geschehen (**19**), Schema (26). Der erweiterte spirocyclischen Struktur (**20**) wurde in einer besseren Ausbeute von 52 % synthetisiert übrigens aus MeOH und

conc.H2SO4 Verwendung BIM ($\mathbf{17_a}$) (2 Mol-Äquivalent) und 1,4-Cyclohexandion (1 Moläquivalent) Schema (26). BIMs erwiesen sich Verbindungen empfindlich gegenüber Oxidationsmitteln sein. Unsere vorbereiteten BIMs, $\mathbf{17_{a-p}}$ wurde mit (1,5) Moläquivalent TCQ oder DDQ Nachgeben bisindolylmethenes vom Typ $\mathbf{21_{a-k}}$ als freie Base oxidiert. Die Salze Monosulfat $\mathbf{22_{a,b}}$ konnte leicht durch die Addition des Anions in Form einer Säure oder in ihrer Tetrabutylammoniumsalz in Methanol oder Acetonitril und Sieden der Mischung für einige Minuten, Schema (27) synthetisiert werden.



17

21

22

$\mathbf{17_a}$: R_1=R_2=H, R=Ph
$\mathbf{17_b}$: R_1=R_2=H, R=*p*-NO_2- Ph
$\mathbf{17_c}$: R_1=R_2=H, R=*p*-Br- Ph
$\mathbf{17_d}$: R_1=R_2=H, R=*p*-Cl- Ph
$\mathbf{17_e}$: R_1=R_2=H, R=*p*-$N(Me)_2$- Ph
$\mathbf{17_f}$: R_1=R_2=H, R=*m*-Br
$\mathbf{17_g}$: R_1=R_2=H, R=*m*-OCH_2Ph- Ph
$\mathbf{17_h}$: R_1=R_2=H, R=*p*,*m*-OH- Ph
$\mathbf{17_i}$: R_1=R_2=H, R=*p*-MeO-*m*-OCH_2Ph- Ph
$\mathbf{17_j}$: R_1=R_2=H, R=*m*-MeO-*p*-OCH_2Ph- Ph
$\mathbf{17_k}$: R_1=R_2=H, R=*m*-Me,2,4,6-triflouro- Ph
$\mathbf{17_l}$: R_1=R_2=H, R=1-naphthyl
$\mathbf{17_m}$: R_1=R_2=H, R=3-pridyl
$\mathbf{17_n}$: R_1=R_2=H, R=3-indolyl
$\mathbf{17_o}$: R_1=Cl, R_2=H, R=*p*-MeO-*m*-OCH_2Ph- Ph
$\mathbf{17_p}$: R_1=H, R_2=Cl, R=*p*-MeO-*m*-OCH_2Ph- Ph

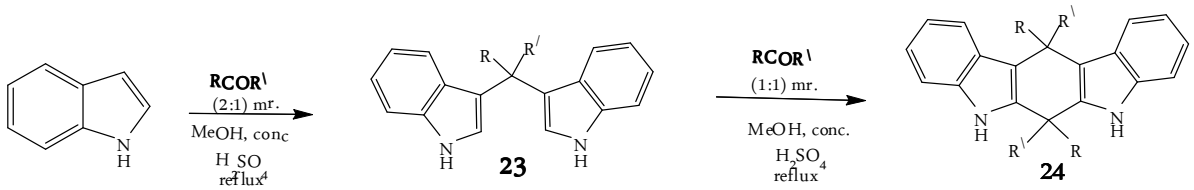
$\mathbf{21_a}$: R_1=R_2=H, R=Ph
$\mathbf{21_b}$: R_1=R_2=H, R=3-indolyl
$\mathbf{21_c}$: R_1=R_2=H, R=*p*-Cl- Ph
$\mathbf{21_d}$: R_1=R_2=H, R=*m*-Br- Ph
$\mathbf{21_e}$: R_1=R_2=H, R=*p*,*m*-OH- Ph
$\mathbf{21_f}$: R_1=R_2=H, R=2,4,6-trifluro-3-methylphenyl
$\mathbf{21_g}$: R_1=R_2=H, R=*p*-MeO-*m*-OCH_2Ph- Ph
$\mathbf{21_h}$: R_1=Cl, R_2=H, R=*p*-MeO-*m*-OCH_2Ph- Ph
$\mathbf{21_i}$: R_1=H, R_2=Cl, R=*p*-MeO-*m*-OCH_2Ph- Ph
$\mathbf{21_j}$: R_1=R_2=H, R=1-naphthyl
$\mathbf{21_k}$: R_1=R_2=H, R=3-pridyl
$\mathbf{21_l}$: R_1=R_2=H, R=*p*-$N(Me)_2$-Ph

$\mathbf{22_a}$: R_1=R_2=H, R=Ph (Turbomycin B)
$\mathbf{22_b}$: R_1=R_2=H, R=3-indolyl (Turbomycin A)

Scheme (27): Synthesis of bisindolylmethenes and its salt formation

Diese Kondensation von Ketonen mit Acetyl indol wurde in einem molaren Verhältnis von 1:2 durchgeführt, um die entsprechenden BIMs leisten. Diese BIMs aus dem Reaktionsgemisch wie im Fall der Verbindungen $\mathbf{23_{a,b}}$ oder direkt, ohne Isolation aus dem Reaktionsgemisch verwendet werden, um mit dem anderen äquivalenten Mol Ketone für die Bildung von tetrahydroindolo[2,3-*b*]carbazol ($\mathbf{24_{a,b}}$ kondensieren isoliert , b), Schema (29). Isatin als Beispiel eines 1,2-Diketons mit Indol zur Herstellung des Indols Trimer, indem Sie die ähnlichen Reaktionsbedingungen des Methanol-Schwefelsäure-Lösung kondensiert. Die Indol-Trimer **25** ist ein bekanntes natürliches

Produkt, das aus der frischen marinen Schwamm Hyrtios altum isoliert wurde, und wurde als trisindole benannt.



23

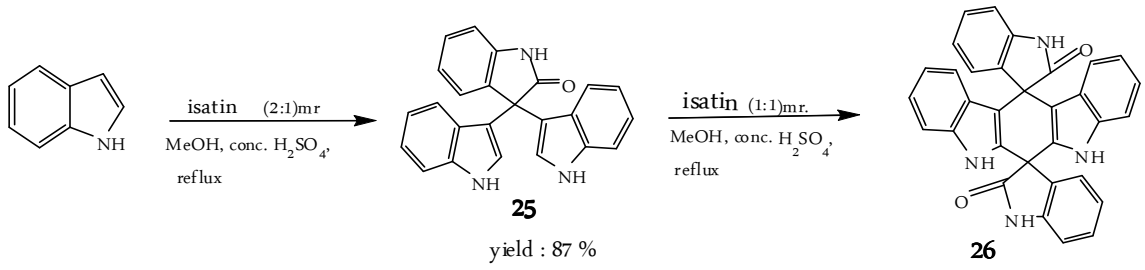
23$_a$: R=3-indolyl, R\=Me
23$_b$: R=3-pyridyl, R\=Me

24

24$_a$: R=3-indolyl, R\=Me
24$_b$: R=3-pyridyl, R\=Me

Scheme (29): Condensation of indole with acetylketones.

Ein Versuch, trisindole **25** in Eisessig bei Raumtemperatur herzustellen fehlgeschlagen. Die Verwendung von Methanol Schwefelsäurelösung in einem Molverhältnis von zwei Mol Indol und von einem Mol Isatin unter Rückfluss 2 Stunden ergab trisindole **25** in einer Ausbeute von 87 %. Diese Methode wird als einfach und effizient, wenn an den gemeldeten chemische Verfahren zur Herstellung von **25** verglichen. Die trisindoline (**25**) wurde weiter als Vorstufe für eine Kondensationsreaktion mit einer äquimolaren Menge von Isatin als möglichen Weg für die Synthese von dem erwarteten neuen spirocyclischen Struktur **26**, Schema (30) verwendet.



25

yield : 87 %

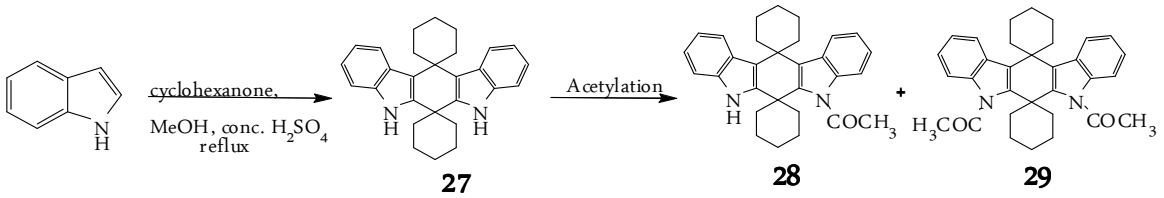
26

yield : 72 %

Scheme (30) : Condensation reaction of indole with isatin.

Cyclohexanon wurde mit Indol Verwendung unterschiedlicher Arten von Katalysatoren sowie Aldehyde kondensiert. Verwendung des Verfahrens nach MeOH / konz. H2SO4 bei der Reaktion von Indol mit Cyclohexanon im molaren Verhältnis von 2:1 die bekannten (3,3′-(Cyclohexan-1,1-diyl)-bis-(1-*H*-indol)) wurde isoliert. Es wurde durch TLC und ESI-MS der Reaktionsmischung detektiert und nicht isoliert aus dem Reaktionsgemisch aber direkt in die zweite Kondensationsstufe mit der zweiten Mol Cyclohexanon unter den gleichen Bedingungen von MeOH / konz verwendet. H2SO4, was zu unserem zweiten Roman spirocyclische Struktur **27** in einer Ausbeute von 97 %.

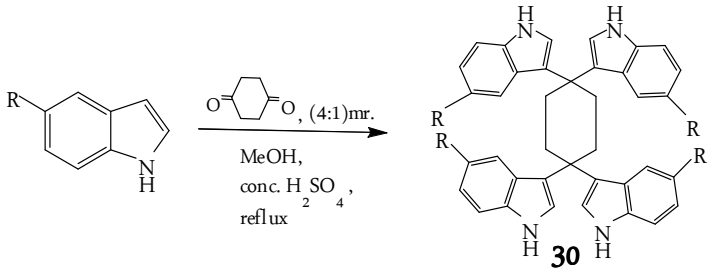
Verbindung **27** wurde als die 2,8,2′,8′-bis(Cyclohexan-1,1-diyl)-1,2,3,8-tetrahydroindolo [2,3-*b*]carbazol (**27**), Schema werden (31).



27 **28** **29**

Scheme (31): Reaction of indole with cyclohexanone.

Um die Struktur der Verbindung **27** verifizieren wurde acetyliert Verwendung von Essigsäureanhydrid und Triethylamin in Gegenwart von 4-(Dimethylamino)pyridin (DMAP) als Katalysator. Die Reaktion ergab zwei Produkte eine als monoacetylierten Produkt (**28**) in einer 59 % Ausbeute wurde bestimmt. Das zweite Reaktionsprodukt wurde als diacetylierten Produkt (**29**) in einer geringeren Ausbeute von 32 % als die der monoacetylierten Produkt **28** identifiziert. So ist die Acetylierung erfolgte stepwisly und die Bildung der diacetylierten Produkte benötigt mehr Zeit, Schema (31). Die elektrophilen Substitutionsreaktionen von Indolen mit Cyclohexan-1,4-dion wurde in der Literatur bereits als möglicher Weg zur Synthese der verlängerten supramolekularen Verbindungen $\mathbf{30_{a,b}}$, wie 1,1,4,4-Tetrakis benannt berichtet (1*H*-indol -3-yl) cyclohexan ($\mathbf{30_a}$). Die Umsetzung erfolgte in Gegenwart von Katalysatoren wie Jod und N-Bromsuccinimid (NBS) unter Bildung des tetrasubstituierten Produkt in guter Ausbeute. Im Zuge dieser Untersuchung cyclohexan-1,4-dion wurde mit Indol in MeOH / konz kondensiert. H_2SO_4 Lösung in einem molaren Verhältnis von 1: 4 so Verbindungen $\mathbf{30_{a,b}}$ in einem 82 bis 87 % Ausbeute, jeweils nach 2 Stunden Refluxieren, Schema (32).

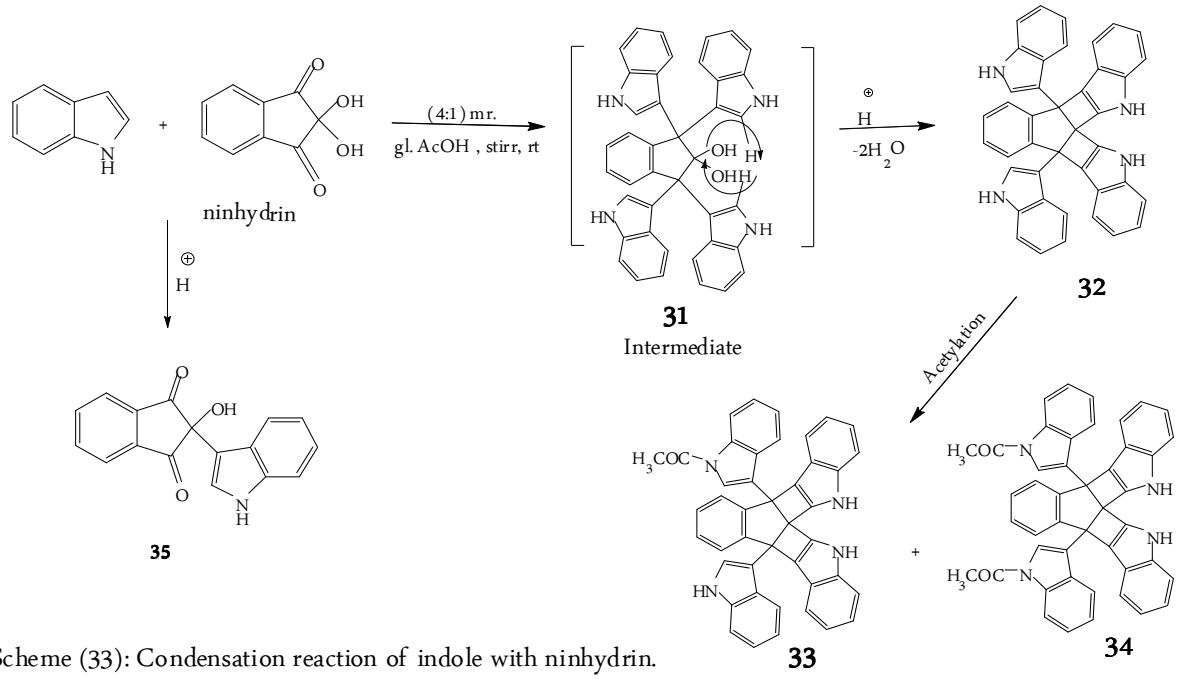


30

$\mathbf{30_a}$: R=H

Scheme (32): Condensation of indoles with cyclohexane-1,4-dione $\mathbf{30_b}$: R=Cl

Kondensationsreaktion von Indol mit Ninhydrin in der Absicht, um die erwartete tetraindole **31** vorbereitet wurde jedoch die spektroskopischen Daten bestätigen die Struktur von Verbindung **32**. Verbindung **32** wurde für die Acetylierungsreaktion in der zwei Produkte, die nach langem Rühren bei Raumtemperatur ergab vorgelegt. Die Produkte wurden bestimmt, um die monoactylated Form **33** und die diacetylierten Form **34**, Schema (33) sein.



Scheme (33): Condensation reaction of indole with ninhydrin.

Pharmakologische Untersuchungen
Ergebnisse der antimikrobiellen Tests

ATCC-Stämme der Mikroorganismen in dieser Studie verwendet wurden aus der Stammsammlung des Refik Saydam Health Institution of Gesundheitsministerium, Ankara, erhalten und gepflegt an der Abteilung Mikrobiologie der Fakultät für Pharmazie der Universität Ankara. Alle Verbindungen für ihre In-vitro-Wachstum hemmende Aktivität gegen Candida albicans ATCC 10145 als Pilz, S. aureus ATCC 25923, Bacillus subtilis ATCC 6633 getestet, Isolieren MRSA Standard ATCC 43300 und MRSA als Gram-positive Bakterien und E.coli ATCC 23.556, wie gram-negative

Bakterien. Die ausgewählten Verbindungen zur antimikrobiellen Tests sind in der Figur (30) dargestellt. Wir haben zum ersten Mal gezeigt, dass die cycloalkanoindoles (**2**$_{a,b,c,d,e,h,i,g,j}$), Bis-indolobenzocarbazoles (**7**$_a$) und die oxidierte Bis-indolylmethenes (**21**$_a$, **21**$_c$, **21**$_l$) hemmte Wachstum von resistenten MRSA entweder Standard-oder isolierten und andere Gram-positive Bakterien bei niedrigen Konzentrationen. Diese neuartigen Bis-und trisindolyl hier beschriebenen Inhibitoren kann leicht und kostengünstig hergestellt werden und strukturelle Modifikationen, um die inhibitorische Aktivität in vitro zu verbessern kann in zeitsparender Weise erfolgen. Die Ergebnisse werden voraussichtlich von Bedeutung im Hinblick auf die Entdeckung neuer Moleküle, die in Medikamente entwickelt werden, um MRSA und gram-positive Erreger bekämpfen kann.

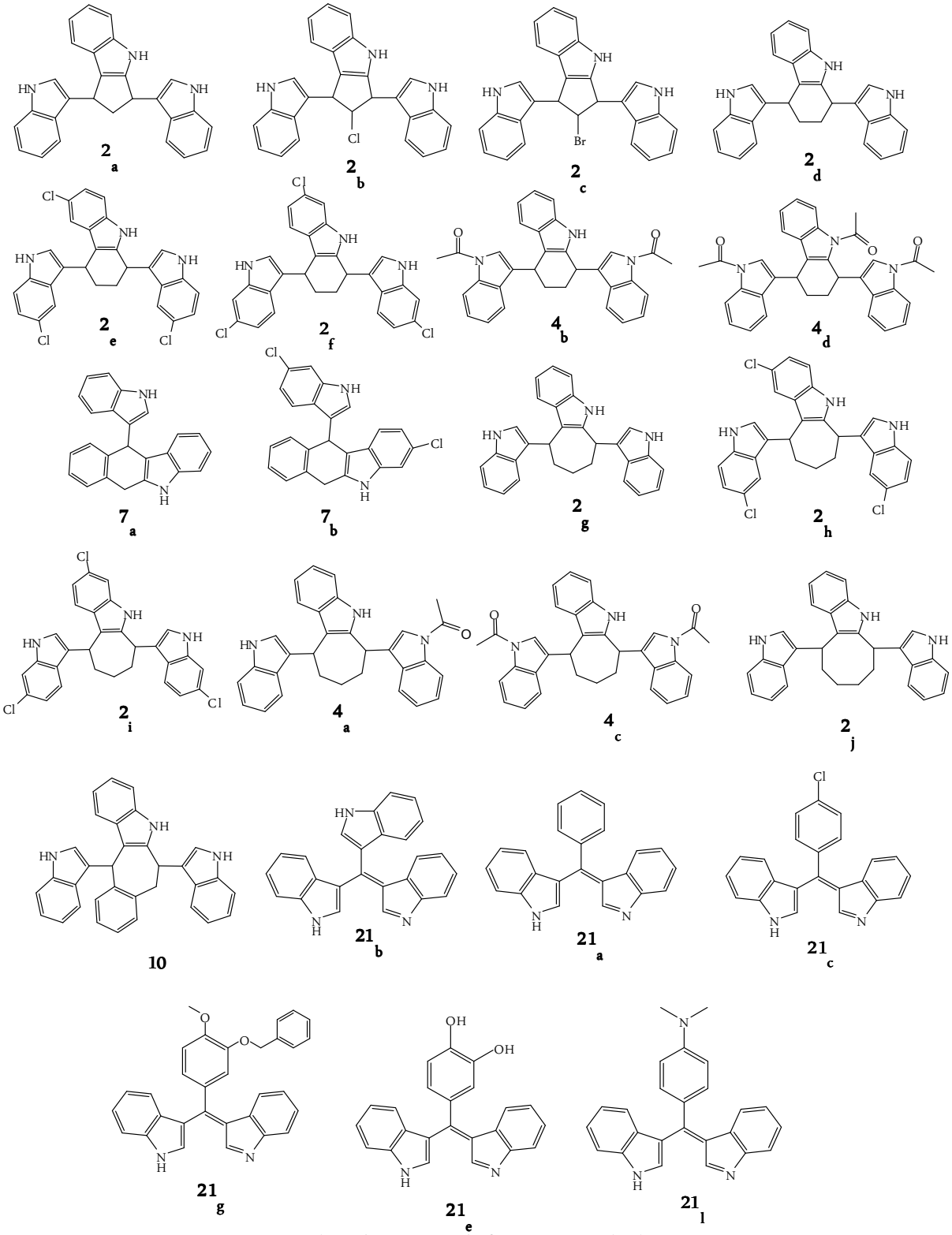


Figure (30): Selected compounds for antimicrobial tests.

Ergebnisse der in-vitro-Krebs-Bildschirm Aktivität der BIM als Antitumormittel

Die ausgewählten Substanzen für die Eine Dosis-Screening ($\mathbf{17}_{e, g, i, j, l}$) sind in Abbildung (31) und $\mathbf{17}_j$ wurde für weitere fünf Dosis ausgewählt meine Grafik Entschlossenheit.

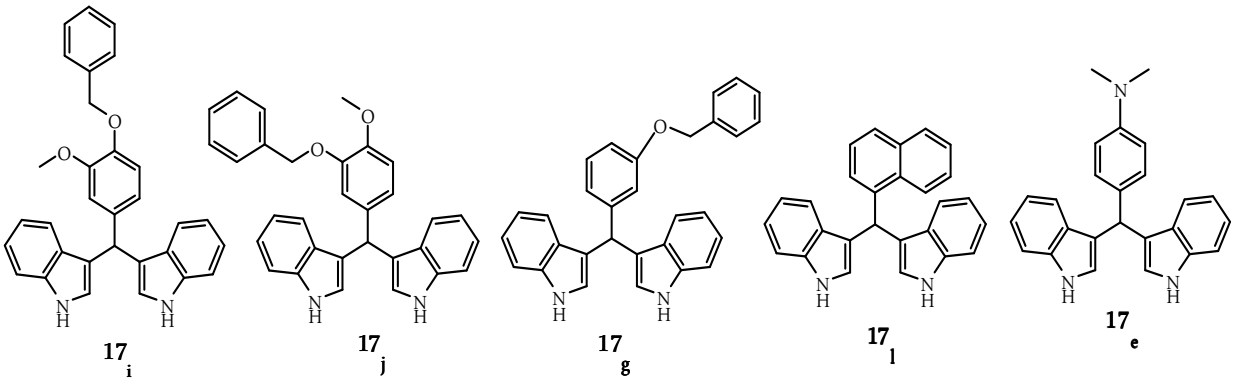
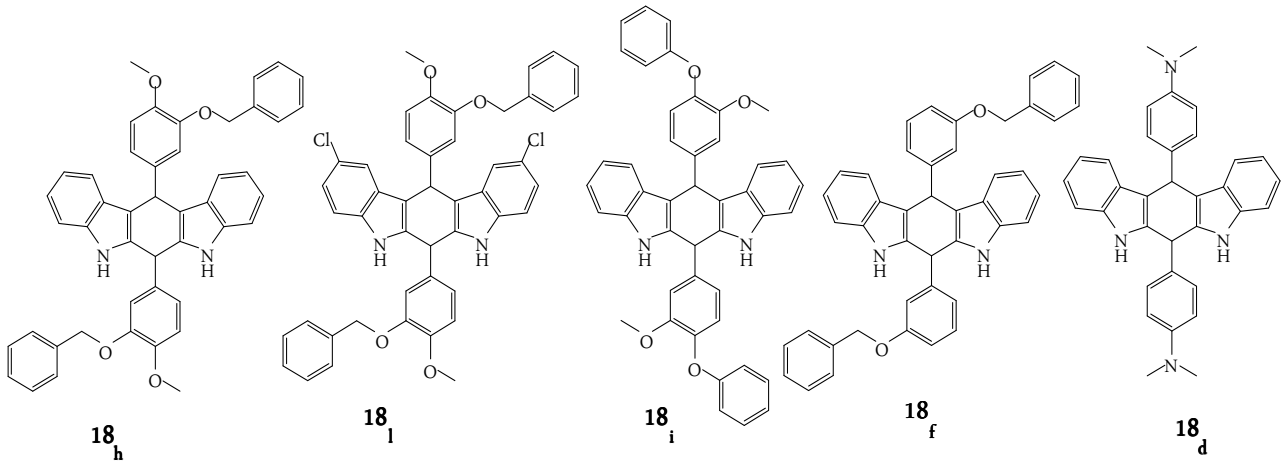


Figure (29): Selected BIMs (**17e,g,i,j,l**) for NCI screenings.

Structure Activity Relationship (SAR) der BIM

Der Vergleich $\mathbf{17}_i$ und $\mathbf{17}_j$ zeigten, daß die Position der funktionellen Gruppen von großer Bedeutung für die entweder meta oder para, wo der *p*-Methoxy ist viel günstiger als meta ist. Darüber hinaus und durch Vergleich mit **17g 17j** die Methoxy-Funktion zusätzlich zu der Benzyloxygruppe sichergestellt hauptsächlich erhöhte Aktivität. Wenn die Funktion Methoxy in meta-Stellung angeordnet ist, die Wirkung ist ähnlich über keinem zugunsten eines meta Methoxy Funktion wie im Vergleich von **17i** und **17g** angedeutet. Der lipophile feste Substituent (Naphthylderivat, Verbindung $\mathbf{17}_l$) ist nicht günstig im Vergleich zu den routbare benzyloxy Substituent Verbindung $\mathbf{17}_g$. BIM ($\mathbf{17}_e$), die eine basische Substituenten (NMe_2) ungünstig war über die ganzen Anti-Krebs-Aktivitäten. Während angegeben, ihre Tätigkeit in einem Nierenkrebs Zelllinien verschiedenen Krebs Tätigkeiten vergleichbar $\mathbf{17}_g$ verschlimmern. Nach Abschluss zeigen alle BIMs ($\mathbf{17}_{e,g,i,j,l}$) gute Aktivitäten in den gleichen Zelllinien (MOLT-4) als krebs-Zelllinie und IGROV1 als Ovarialkarzinom-Zelllinie. Auch die basisch substituierte Derivate zeigen eine gute Aktivität bei der Zelllinie CAKI-1 und UO-31 als renale Krebszelllinien. Ergebnisse von 60 Zelllinien-Screening für Aryl substituiert tetrahydroindolo [2,3-*b*]Carbazolen ($\mathbf{18}_{d, f, h, i, l}$) Wir weiterentwickelt die Reihe der substituierten Bis (indolyl) substituiert phenylmethanes, neue Strukturen wie Aryl substituiert tetrahydroindolo [2,3-*b*] Carbazolen mit einer eingeschränkten Flexibilität des Moleküls bekannten ergeben. Das NCI gewählt fünf Derivate dieser substituierten Indolocarbazole für die Eine Dosis-Screening-Programm in einer Konzentration 10 μM. Diese ausgewählten Substanzen ($\mathbf{18}_{d,f, h,i,l}$) sind in der Abbildung (32) dargestellt und entsprechend den Daten aus den eine Dosis Screening

Verbindung $\mathbf{18_d}$ zeigten die niedrigsten Mittelwert (21.63). So Verbindung $\mathbf{18_d}$ wurde für weitere fünf Dosis-Screening-Programm, die höchste Aktivität zeigte ausgewählt.



$\mathbf{18_h}$ $\mathbf{18_l}$ $\mathbf{18_i}$ $\mathbf{18_f}$ $\mathbf{18_d}$

Figure (30): Selected indolocarbazoles ($\mathbf{18_{d,f,h,i,l}}$) for NCI screening.

Structure Activity Relationship (SAR) der BIM

Der Vergleich **17i** und **17j** zeigten, daß die Position der funktionellen Gruppen von großer Bedeutung für die entweder meta oder para, wo der p-Methoxy ist viel günstiger als meta ist. Darüber hinaus und durch Vergleich mit **17g** **17j** die Methoxy-Funktion zusätzlich zu der Benzyloxygruppe sichergestellt hauptsächlich erhöhte Aktivität. Wenn die Funktion Methoxy in meta-Stellung angeordnet ist, die Wirkung ist ähnlich über keinem zugunsten eines meta Methoxy Funktion wie im Vergleich von **17i** und **17g** angedeutet. Der lipophile feste Substituent (Naphthylderivat, Verbindung $\mathbf{17_l}$) ist nicht günstig im Vergleich zu den routbare benzyloxy Substituent Verbindung $\mathbf{17_g}$. BIM ($\mathbf{17_e}$), die eine basische Substituenten (NMe2) ungünstig war über die ganzen Anti-Krebs-Aktivitäten. Während angegeben, ihre Tätigkeit in einem Nierenkrebs Zelllinien verschiedenen Krebs Tätigkeiten vergleichbar $\mathbf{17_g}$ verschlimmern. Nach Abschluss zeigen alle BIMs (**17e,g,i,j,l**) gute Aktivitäten in den gleichen Zelllinien (MOLT-4) als krebs-Zelllinie und IGROV1 als Ovarialkarzinom-Zelllinie. Auch die basisch substituierte Derivate zeigen eine gute Aktivität bei der Zelllinie CAKI-1 und UO-31 als renale Krebszelllinien. Ergebnisse von 60 Zelllinien-Screening für Aryl substituiert tetrahydroindolo [2,3-*b*] Carbazolen ($\mathbf{18_{d,f,h,i,l}}$) Wir weiterentwickelt die Reihe der substituierten Bis (indolyl) substituiert phenylmethanes, neue Strukturen wie Aryl substituiert tetrahydroindolo [2,3-*b*] Carbazolen mit einer eingeschränkten Flexibilität des Moleküls bekannten ergeben. Das NCI gewählt fünf Derivate

dieser substituierten Indolocarbazole für die Eine Dosis-Screening-Programm in einer Konzentration 10 μM. Diese ausgewählten Substanzen (**18**$_{d, f, h, i, l}$) sind in der Abbildung (32) dargestellt und entsprechend den Daten aus den eine Dosis Screening Verbindung 18d zeigten die niedrigsten Mittelwert (21.63). So Verbindung **18**$_{d}$ wurde für weitere fünf Dosis-Screening-Programm, die die höchste Aktivität zeigte ausgewählt

Selbststandigkeitserklarung

Hiermit erklare ich gemas § 5(2)b der Promotionsordnung der Naturwissenschaftlichen Fakultat I – Biowissenschaften der Martin-Luther-Universitat Halle-Wittenberg, dass ich die Vorliegende Arbeit selbststandig und ohne Benutzung anderer als der angegebenen Hilfsmittel und Quellen angefertigt habe. Alle Stellen, die wortlich oder sinngemas aus Veroffentlichungen entnommen sind, habe ich als solche kenntlich gemacht. Ich erklare ferner, dass diese Arbeit in gleicher oder ahnlicher Form bisher keiner anderen Prufbehorde zur Erlangung des Doktorgrades vorgelegt wurde.

Halle, Saale, Germany, November 2012.

Mardia El-Dessoky Telep El-Sayed