

Polymorphism of an $N\alpha$ -Aroyl- N -Aryl-Phenylalanine Amide: An X-ray and Electron Diffraction Study

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Abstract: In view of the rise of drug-resistant tuberculosis and difficult-to-treat related diseases caused by non-tuberculous mycobacteria, there is an urgent need for antimycobacterial drug discovery. $N\alpha$ -aroyl- N -aryl-phenylalanine amides (AAPs) have been identified as antimycobacterial agents and are subject to lead optimization. The aim of the present study is to evaluate the impact of N -aryl *ortho* cyano substitution in a lead compound on the crystal and molecular structure and its in vitro activity against *Mycobacterium abscessus*. The title AAP can be conveniently synthesized from N -Boc-protected D-phenylalanine in two amide coupling steps using a previously established racemization-free method. Two polymorphic forms in the solid-state are described, as discovered by X-ray and electron diffraction. The introduction of a cyano group in the *ortho* position of the AAP N -aryl ring, however, leads to loss of in vitro activity against *M. abscessus* subsp. *abscessus*.

Keywords: amino acids; amides; drug discovery; antibiotics; mycobacteria; tuberculosis; NTM infections; crystal structure; polymorphism; electron diffraction



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1. Introduction

Mycobacterial infections are a serious public health threat of global concern. They can manifest as pulmonary or extrapulmonary tuberculosis (TB) [1,2], localized or disseminated non-tuberculous mycobacterial (NTM) diseases [3], leprosy and chronic ulcers (Buruli ulcer) [4]. The rise of drug-resistant TB [5,6] and hard-to-treat NTM pulmonary disease [7] necessitate drug discovery and development efforts in this field [8]. $N\alpha$ -Aroyl- N -aryl-phenylalanine amides (AAPs) have been identified as antimycobacterial agents that specifically and stereoselectively inhibit the mycobacterial RNA polymerase with a low probability of cross-resistance to rifamycins [9]. Their mechanism of action was unveiled in a seminal work by Lin et al. [10]. In vitro activity of compound **1** against *Mycobacterium tuberculosis*, the pathogen causing TB, and the clinically relevant NTM species *Mycobacterium avium* and *Mycobacterium abscessus* has been discovered in several phenotypic compound screenings [11–15]. Hit-to-lead optimization driven by whole-cell activity against *M. abscessus* subsp. *abscessus* resulted in lead compound **2** (Figure 1) [16]. While retaining the phenylalanine side chain proved beneficial, replacement of the 2-thiophenoyl moiety in **1** by a 2-fluorobenzoyl moiety and the morpholine ring by thiomorpholine 1,1-dioxide lowered the minimum inhibitory concentration (MIC) against *M. abscessus* subsp. *abscessus*

by about an order of magnitude to the submicromolar level. The most recent work on AAPs focused on improving their metabolic stability [17]. In the present contribution, we disclose the influence of a cyano group in *ortho* position on the AAP *N*-aryl ring instead of the morpholino group, while retaining the phenylalanine side chain the 2-fluorobenzoyl moiety as in the lead compound 2.

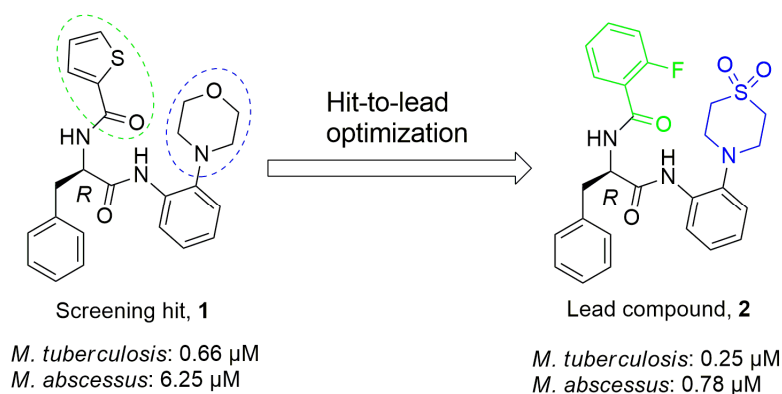
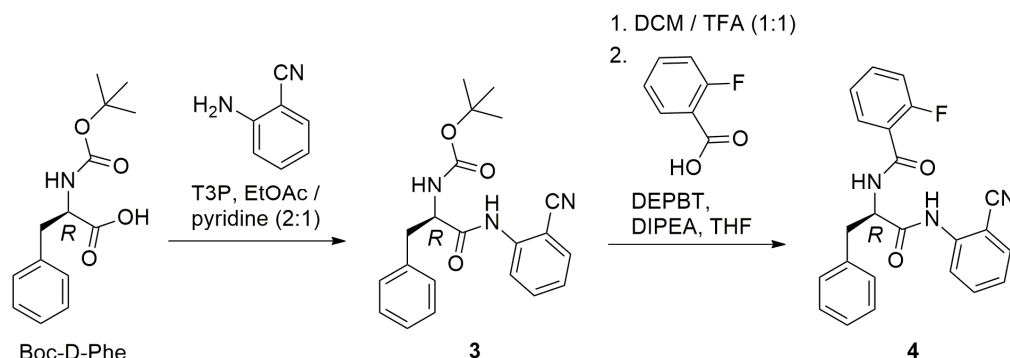


Figure 1. Screening hit 1 and derived lead compound 2 and the corresponding MICs against *M. tuberculosis* H37Rv and *M. abscessus* ATCC 19977 [17].

2. Results and Discussion

2.1. Synthesis

Compound 4 was obtained in good yield by applying a previously published racemization-free method [16], shown in Scheme 1. In brief, commercially available *N*-tert-butyloxycarbonyl (Boc) D-phenylalanine (Boc-D-Phe) was reacted with 2-aminobenzonitrile (2-cyanoaniline) in ethyl acetate to form anilide 3, using T3P® (propanephosphonic acid anhydride) [18] as coupling reagent in the presence of pyridine [19]. T3P® is known to cause a low degree of racemization of racemization-prone carboxylic acids [20], and the presence of the *N*-bound carbamate group further helps prevent racemization in the first amide coupling step [21]. After removal of the Boc protecting group, the 2-fluorobenzoyl moiety was introduced in the second amide coupling step using 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one (DEPBT) [22,23] as a coupling reagent in the presence of Hünig's base (*N,N*-diisopropylethylamine, DIPEA). For a brief discussion of advantages and disadvantages of DEPBT in the synthesis of AAPs, we refer the interested reader to a recent review [9]. Compound 4 was identified by ^1H and ^{13}C -APT NMR spectroscopy, FT-IR spectroscopy and high-resolution mass spectrometry (see Supplementary Materials).



Scheme 1. Two-step synthesis of compound 4 from Boc-D-Phe.

2.2. Structural Elucidation

Crystallization of 4 from ethanol afforded fine needle-shaped crystals, hereafter referred to as 4-I. X-ray crystallography at 100 K unambiguously confirmed the molecular

structure (Figure 2a). Both amide groups adopt the *Z* conformation. The molecular conformation of the solid form **4-I** is nonetheless distinctly different from that of **2** (CSD refcode: KODROO) [17] and related thiomorpholinoanilide AAP derivatives [16], whose crystal structures feature an intramolecular N–H···O hydrogen bond between the amide groups and intermolecular N–H···O hydrogen bonds to form *pseudo* centrosymmetric $R_2^2(10)$ hydrogen-bonded dimers [9]. In contrast, the solid-state structure of **4-I** lacks a comparable intramolecular N–H···O hydrogen bond. Instead of forming dimers, the molecules in the crystal structure of **4-I** form polymeric stacks through two N–H···O classical hydrogen bonds with an $R_2^2(12)$ motif [24], assisted by C–H···N weak hydrogen bonds (Figure 2b). These stacks extend by translational symmetry in the crystallographic *a*-axis direction (Figure 2b). Table 1 lists the corresponding hydrogen bond parameters. The packing index of **4-I** is 72.0% [25]. Determination of the unit cell parameters of **4-I** from the single-crystal at room temperature indicated that there was no phase change upon cooling to 100 K (Section 3.3).

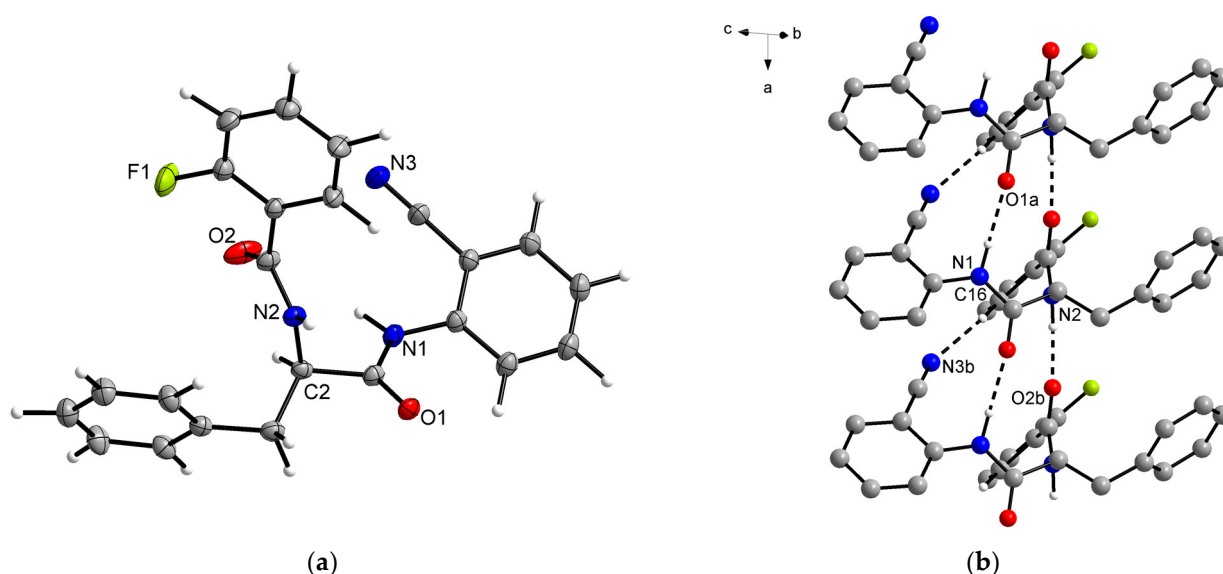


Figure 2. (a) Molecular structure in crystal form **4-I**. Displacement ellipsoids are drawn at the 50% probability level. Hydrogen atoms are represented by small spheres of arbitrary radius. (b) Part of the crystal structure of **4-I**, viewed along the [011] direction. Dashed lines represent hydrogen bonds. Hydrogen atoms not involved in hydrogen bonds are omitted for clarity. Symmetry codes: (a) $x - 1, y, z$; (b) $x + 1, y, z$. Color scheme: C, gray; H, white; N, blue; O, red; F, lime.

Table 1. Hydrogen bond parameters in **4-I** (Å, °).

	$d(D-H)$	$d(H\cdots A)$	$d(D\cdots A)$	$\angle(DHA)$
N1–H1···O1a	0.99 (3)	2.05 (3)	2.935 (3)	146 (2)
N2–H2A···O2b	0.98 (3)	1.80 (3)	2.777 (3)	169 (2)
C16–H16···N3b	1.10 (3)	2.42 (3)	3.491 (3)	165 (2)

Symmetry codes: (a) $x - 1, y, z$; (b) $x + 1, y, z$.

To shed light on the crystal and molecular structure of **4** in the powder as synthesized, hereafter **4-II**, we performed electron diffraction on a crystallite. Figure 3a shows the molecular structure in **4-II**. As in **4-I**, both amide groups exhibit the *Z* conformation. In contrast to **4-I**, the anilide group forms an intramolecular N–H···O hydrogen bond to the benzoyl oxygen atom (O2) with an $S(7)$ motif, as previously observed in the crystal structures of **2** and related AAPs [9]. Nonetheless, hydrogen-bonded dimers, as in **2** and related AAPs, are not encountered in **4-II**. Instead, **4-II** features N–H···O hydrogen-bonded chains formed by a single N–H···O hydrogen bond formed between the $N\alpha$ N2–H2A moiety and the benzoyl oxygen atom (O2). The hydrogen-bonded chains so formed extend

by translational symmetry in the *a*-axis direction (Figure 3b). Table 2 lists the corresponding hydrogen bond parameters. Notably, the carbonyl oxygen atom of the anilide group (O1) and the nitrile nitrogen atom (N3) do not act as hydrogen bond acceptors. The packing index calculated for 4-II is 70.2%, which suggests that the crystal packing in 4-II is slightly less dense than in 4-I. The calculated densities of 4-I and 4-II at room temperature (see Sections 3.3 and 3.4) are similar. A powder X-ray diffraction (PXRD) analysis of the bulk material of 4 as synthesized suggests that the sample did not exclusively consist of 4-II (see Supplementary Materials).

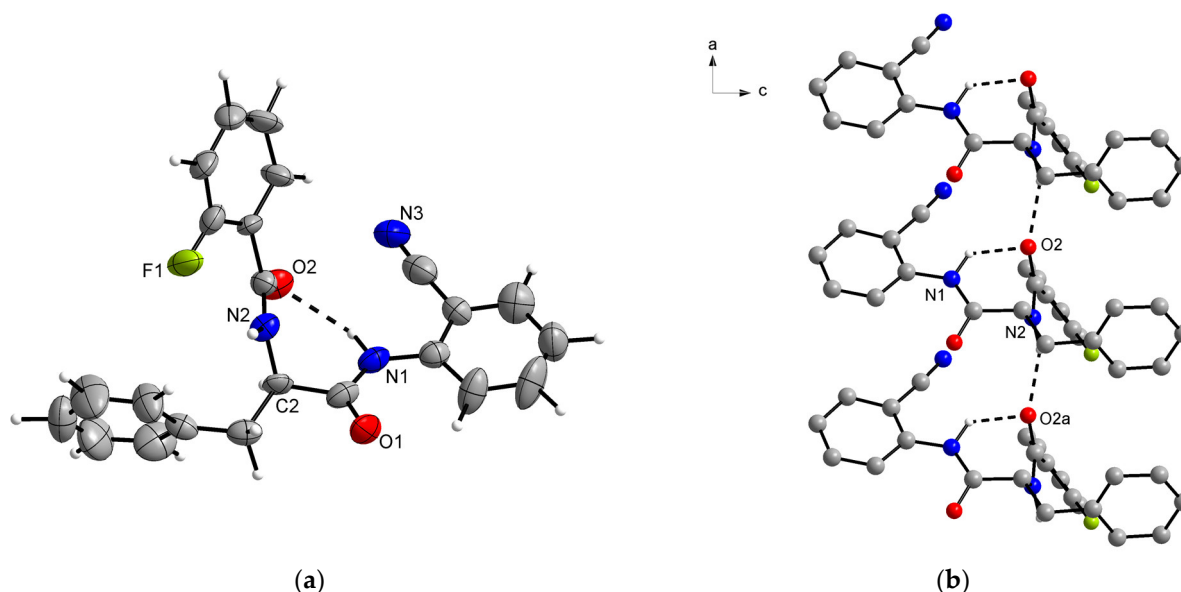


Figure 3. (a) Molecular structure in crystal form 4-II. Displacement ellipsoids are drawn at the 50% probability level. Hydrogen atoms are represented by small spheres of arbitrary radius. (b) Part of the crystal structure of 4-II, approximately viewed along the *b*-axis direction. Dashed lines represent hydrogen bonds. Hydrogen atoms not involved in hydrogen bonds are omitted for clarity. Symmetry code: (a) $x - 1, y, z$. Color scheme: C, gray; H, white; N, blue; O, red; F, lime.

Table 2. Hydrogen bond parameters in 4-II (Å, °).

	<i>d</i> (D–H)	<i>d</i> (H···A)	<i>d</i> (D···A)	∠(DHA)
N1–H1···O2	1.03 (3)	2.14 (5)	2.98 (2)	137 (4)
N2–H2A···O2a	1.02 (3)	2.10 (3)	3.06 (2)	156 (4)

Symmetry code: (a) $x - 1, y, z$.

2.3. Hirshfeld Surface Analysis and Density Functional Theory (DFT) Study

In order to compare the crystal structures of 4-I and 4-II and to gain insight into the environments of the molecules in the two polymorphs, we generated and visualized the Hirshfeld surfaces, which represent the shortest distances between molecules in a crystal and their neighbors [26]. Figure 4 shows the Hirshfeld surface mapped with the normalized contact distance (d_{norm}) for the molecules in 4-I and 4-II and their fingerprint plots, which are a summary of the corresponding interatomic distances. A red color in the d_{norm} plot indicates contacts shorter than, white equal to and blue longer than the sum of the van der Waals radii of the nearest atoms to a point on the Hirshfeld surface.

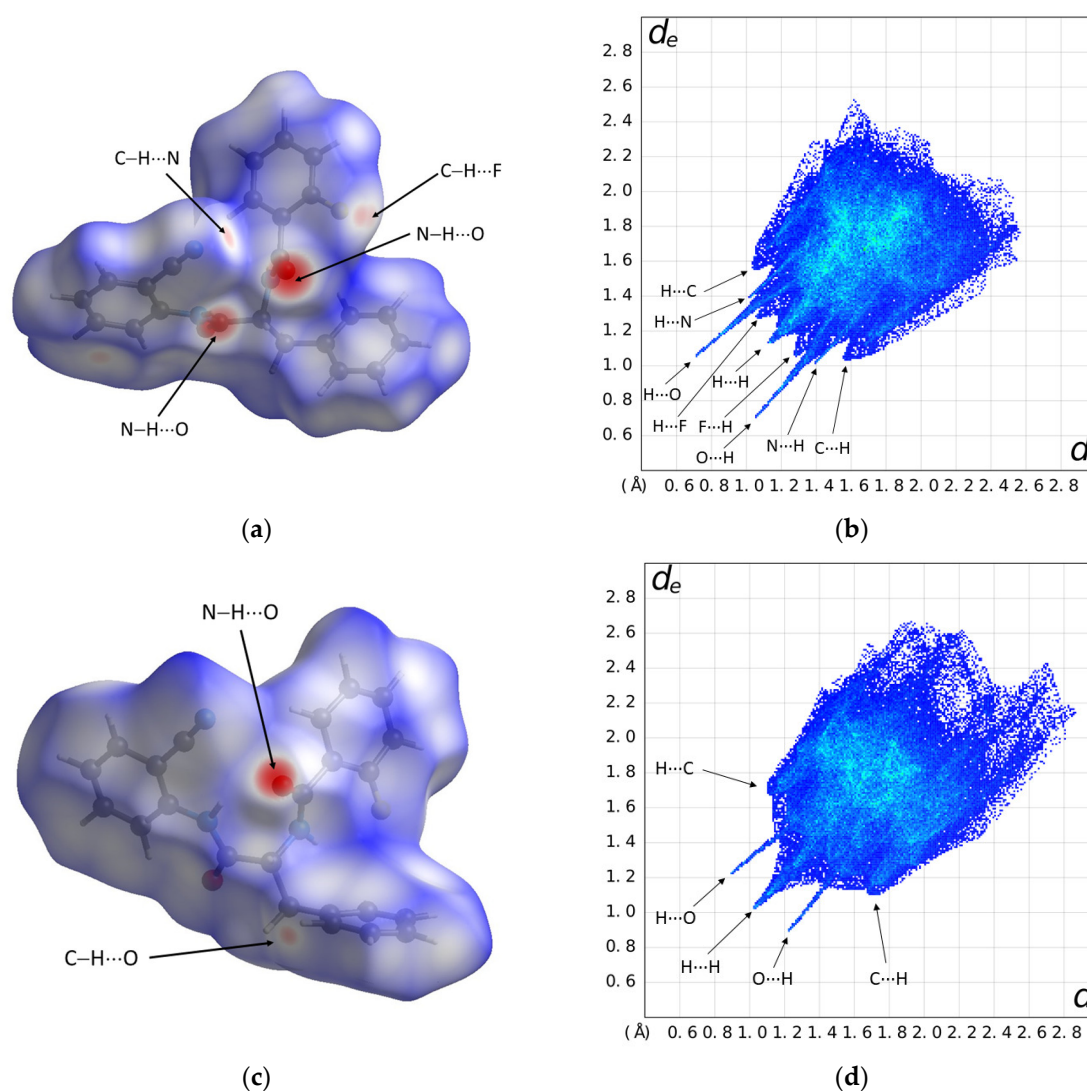


Figure 4. (a) Hirshfeld surface mapped with d_{norm} and (b) the corresponding fingerprint plot for 4-I. (c) Hirshfeld surface mapped with d_{norm} and (d) the corresponding fingerprint plot for 4-II. d_i and d_e are the distances from a point on the Hirshfeld surface to the nearest internal and external atom, respectively.

The N-H...O and C-H...N intermolecular hydrogen bond donor and acceptor sites, as described in Section 2.2, show up as red areas. Minor red areas resulting from a C-H...F contact in 4-I and a C-H...O contact in 4-II are also visible. Inspection of the Hirshfeld surface mapped with d_{norm} also confirmed that the carbonyl oxygen atom O1 in 4-II remains without a short contact resulting from hydrogen bonding. This may be regarded as an exception to Etter's first general hydrogen bond rule for organic compounds, which states that all good proton donors and acceptors are used in hydrogen bonding [27].

The corresponding fingerprint plots (Figure 4b,d) reveal distinct differences between the two polymorphs. Both plots show the spikes indicative of the N-H...O hydrogen bonds. The shortest hydrogen bond distance (d_i plus d_e from the tips of the spikes) is shorter in 4-I than in 4-II (cf. Tables 1 and 2). Wings characteristic of C...H contacts, resulting from C-H... π interactions, are observed for both structures. A triangular feature on the diagonal, typical of C...C contacts from π ... π stacking, is not pronounced in either 4-I or 4-II. Both structures have in common a feature characteristic of H...H contacts from close packing. In contrast to 4-II, the fingerprint plot for 4-I (Figure 4b) exhibits small spikes resulting from a weak C-H...N hydrogen bond associated with the nitrile nitrogen atom (see Section 2.2)

and a feature resulting from short C–F···H contacts. The asymmetry in the fingerprint plot for 4-II suggests packing inefficiency [28], which appears to be consistent with a lower packing index and the lower calculated density of 4-II at room temperature compared with 4-I at 100 K (see Section 2.2).

To shed light on the preferred molecular conformation of the free molecule of 4, we performed DFT calculations. Figure 5 shows a structure overlay plot of the molecular structures in the polymorphs 4-I and 4-II and the minimum energy structure from the DFT structure optimization. The results show that the conformation of the DFT-optimized free molecule is similar to the molecular structure encountered in 4-II and features a similar intramolecular N–H···O hydrogen bond. The absence of a comparable intramolecular N–H···O hydrogen bond in form 4-I is presumably a result of the favorable formation of additional N–H···O and C–H···N intermolecular hydrogen bonds and close packing in this polymorph.

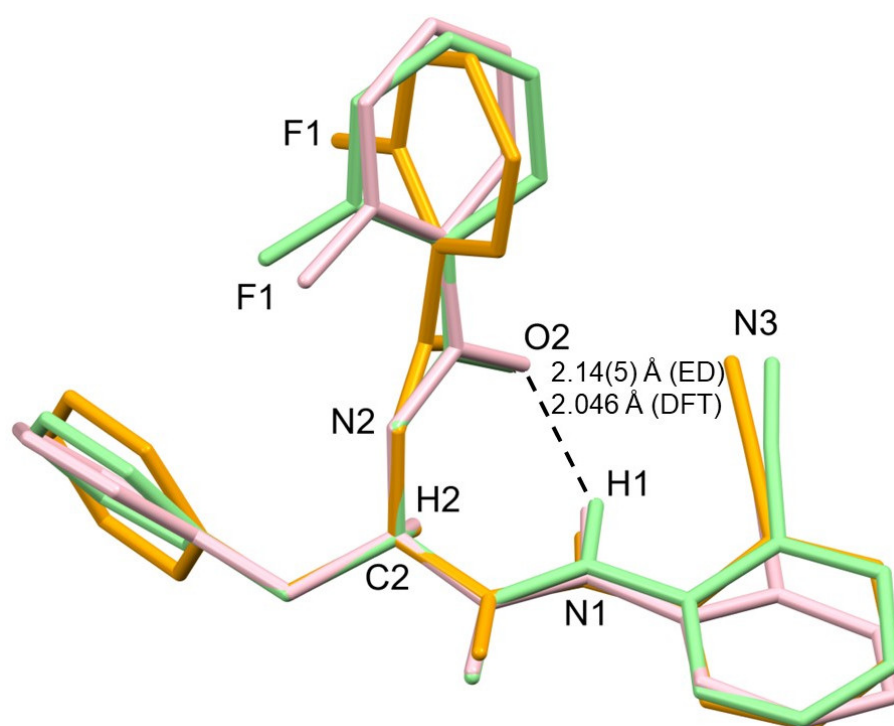


Figure 5. Overlay plot of the molecular structures of 4-I (orange), 4-II (green), and the DFT-optimized structure of the free molecule (pink). The dashed line represents the intramolecular N–H···O hydrogen bond. The structures are each superimposed at C2 and its three adjacent non-hydrogen atoms. Hydrogen atoms are omitted for clarity, except for H1 and H2.

2.4. Antimycobacterial Evaluation

We subjected compound 4 to antimicrobial susceptibility testing against *M. abscessus* subsp. *abscessus* using the broth microdilution method (Middlebrook 7H9 medium supplemented with 10% albumin-dextrose-saline). Up to a compound concentration of 100 μ M, we did not observe growth inhibition of the reference strain *M. abscessus* ATCC 19977. Likewise, the compound proved inactive (MIC > 50 μ M) against *M. abscessus* Bamboo, a clinical isolate [29].

3. Materials and Methods

3.1. General

All chemicals were purchased and used as received. Solvents were distilled before use. HPLC analysis was conducted on a Shimadzu instrument equipped with two LC-10AD pumps, an SPD-M10A VP photodiode array detector, and a Waters XTerra[®] RP18 column (3.5 μ m, 3.9 mm $\times$ 100 mm), using gradient elution with methanol/water

containing 0.05% trifluoroacetic acid. NMR spectra were recorded on an Agilent Technologies VNMRs 400 MHz NMR spectrometer. Chemical shifts are reported relative to the residual solvent signal of chloroform-*d* ($\delta_{\text{H}} = 7.26$ ppm, $\delta_{\text{C}} = 77.23$ ppm). Abbreviations: s = singlet, d = doublet, dd = doublet of doublets, m = multiplet, td = triplet of doublets, and qd = quartet of doublets. The IR spectrum was recorded on a Bruker Alpha FT-IR spectrometer with a resolution of 4 cm^{-1} , using the ATR technique; a total of 16 scans were accumulated. The APCI mass spectrum was measured on an Advion Expression compact mass spectrometer. The HRMS analysis was performed on a Thermo Scientific Q ExactiveTM Plus Orbitrap mass spectrometer using methanol as a solvent.

3.2. Synthesis

(R)-Boc-2-amino-*N*-(2-cyanophenyl)-3-phenylpropanamide (**3**): The synthesis of **3** was carried out essentially following “General Procedure A” in ref. [16] Boc-D-Phe (1.24 g, 4.66 mmol) and 2-aminobenzonitrile (0.50 g, 4.23 mmol) were dissolved in a mixture of ethyl acetate (10 mL) and pyridine (5 mL). After cooling to $-20\text{ }^{\circ}\text{C}$, T3P[®] (2.69 g, 8.46 mmol) was added with stirring. The reaction mixture was then allowed to slowly warm up to room temperature and stirred for 48 h. Subsequently, ca. 25 mL of ethylacetate was added, and the mixture was washed three times each with an equal volume of a 0.25 M aqueous KH_2PO_4 solution. The organic phase was then dried over anhydrous Na_2SO_4 , and the solvent was removed under reduced pressure. The product was purified by flash chromatography (silica gel, heptane/ethyl acetate gradient). Yield: 1.32 g (3.61 mmol, 85%). ^1H NMR (400 MHz, chloroform-*d*) δ 8.59–8.31 (m, 2H), 7.63–7.51 (m, 2H), 7.36–7.21 (m, 6H), 7.17 (td, $J = 7.6, 1.1$ Hz, 1H), 4.94 (d, $J = 7.2$ Hz, 1H), 4.62–4.46 (m, 1H), 3.25 (dd, $J = 14.0, 6.2$ Hz, 1H), 3.16 (dd, $J = 14.1, 7.6$ Hz, 1H), 1.43 (s, 9H). MS (APCI⁺) m/z 366.2 $[\text{M} + \text{H}]^+$, 310.1 $[\text{M} - \text{C}_4\text{H}_8 + \text{H}]^+$, and 266.0 $[\text{M} - \text{Boc} + \text{H}]^+$ 266.0.

(R)-*N*-(1-((2-cyanophenyl)amino)-1-oxo-3-phenylpropan-2-yl)-2-fluorobenzamide (**4**): The synthesis of **4** was performed essentially following “General Procedure B” in ref. [16] Compound **3** (1.32 g, 3.61 mmol) was dissolved in 20 mL dichloromethane, and trifluoroacetic acid (20 mL) was added with stirring. The reaction mixture was stirred for 1 h at room temperature, and subsequently, dichloromethane/trifluoroacetic was removed under reduced pressure. The residue was taken up with ca. 30 mL of ethyl acetate, and the solution was washed three times with an equal volume of saturated aqueous NaHCO_3 solution. The organic phase was dried over anhydrous Na_2SO_4 , and the solvent was removed with a rotary evaporator to obtain unprotected *(R)*-2-amino-*N*-(2-cyanophenyl)-3-phenylpropanamide in virtually quantitative yield (0.96 g, 3.60 mmol), which was used without further purification.

An amount of 88 mg (0.33 mmol) of the residue was dissolved in 5 mL of THF, and 2-fluorobenzoic acid (51 mg, 0.36 mmol) and DEPBT (298 mg, 1.00 mmol) were added with stirring. After the addition of DIPEA (0.17 mL, 1.00 mmol), the mixture was stirred for 4 h at room temperature. Subsequently, ca. 25 mL of brine was added, and the mixture was extracted thrice with an equal volume of ethyl acetate. The combined organic phases were washed each once with 0.25 M aqueous KH_2PO_4 solution, water, saturated aqueous NaHCO_3 , water and, finally, brine. After drying over anhydrous Na_2SO_4 , the solvent was removed under reduced pressure. The product was purified by flash chromatography twice (silica gel, dichloromethane containing 1% of a 4% methanolic ammonia solution, followed by silica gel, heptane/ethyl acetate gradient) to yield **4** as an off-white solid (98 mg, 0.25 mmol, 76%). Purity: >98% (HPLC analysis at 254 nm). ^1H NMR (400 MHz, chloroform-*d*) δ 8.53 (s, 1H), 8.34 (dd, $J = 8.5, 1.0$ Hz, 1H), 8.12 (td, $J = 7.9, 1.9$ Hz, 1H), 7.61–7.44 (m, 3H), 7.37–7.22 (m, 7H), 7.20–6.97 (m, 2H), 5.08 (qd, $J = 6.9, 1.9$ Hz, 1H), and 3.40–3.27 (m, 2H) ppm. ^{13}C APT NMR (101 MHz, chloroform-*d*) δ 169.5, 164.0 (d, $^4J_{\text{C},\text{F}} = 3$ Hz), 160.8 (d, $J = 248$ Hz), 139.8, 135.8, 134.0 (d, $^3J_{\text{C},\text{F}} = 10$ Hz), 133.9, 132.4, 132.3 (d, $^4J_{\text{C},\text{F}} = 2$ Hz), 129.2, 129.0, 127.4, 124.9 (d, $^4J_{\text{C},\text{F}} = 3$ Hz), 124.5, 121.5, 119.7 (d, $^3J_{\text{C},\text{F}} = 11$ Hz), 116.1 (d, $^2J_{\text{C},\text{F}} = 25$ Hz), 115.8, 103.0, 56.1, and 37.3 ppm. FT-IR (ATR): ν 3326 (C–H), 2218 (C $\equiv$ N),

and 1699 (C=O) cm^{-1} . HRMS (ESI): m/z calcd. for $\text{C}_{23}\text{H}_{18}\text{O}_2\text{N}_3\text{NaF}^+$, 410.127524, found 410.127360 $[\text{M} + \text{Na}]^+$.

3.3. X-ray Crystallography

Very fine needles of **4-I** suitable for single-crystal X-ray diffraction were grown from a solution in ethanol. The X-ray diffraction data were measured on a Bruker AXS D8 Venture diffractometer, equipped with an Incoatec I μ S Diamond microfocus X-ray source, Incoatec multilayer optics and a Photon III detector. The data collection was controlled with APEX4 (Bruker AXS, Karlsruhe, Germany), and the raw diffraction data were processed with SAINT (Bruker AXS, Karlsruhe, Germany). An absorption correction, using the Gaussian method based on indexed crystal faces, was carried out with SADABS-2016/2 (Bruker AXS, Karlsruhe, Germany). The crystal structure was solved with XM 2013/2 (Bruker AXS, Karlsruhe, Germany, 2013) and initially refined using the independent atom model with SHELXL-2019/3 [30]. The crystal structure was subsequently refined using NoSpherA2 [31,32] in Olex2 [33], with the Hirshfeld partitioning of the electron density calculated using ORCA 5.0 [34] (B3LYP [35,36]/def2-TZVPP [37]). The absolute structure was inferred from the known absolute configuration of the starting material Boc-D-Phe in the synthesis. Anisotropic atomic displacement parameters were introduced for all non-hydrogen atoms. Hydrogen atoms were located in a difference Fourier map, and their positions were refined with $U_{\text{iso}}(\text{H})$ values constrained to 1.2 $U_{\text{eq}}(\text{C,N})$. For the calculation of the packing index with Platon [38], the X–H distances were neutron-normalized with Mercury [39]. Structure pictures were drawn with Diamond (Crystal Impact GbR, Bonn, Germany).

Crystal data for 4-I: $\text{C}_{23}\text{H}_{18}\text{FN}_3\text{O}_2$, $M_r = 387.41$, $T = 100(2)$ K, $\lambda = 0.71073$ Å, monoclinic, space group $P2_1$, $a = 4.9144(4)$, $b = 16.6111(15)$, $c = 11.6035(11)$ Å, $\beta = 95.808(4)^\circ$, $V = 942.37(15)$ Å³, $Z = 2$, $\rho_{\text{calc}} = 1.365$ g cm^{−3}, $\mu_{\text{calc}} = 0.096$ mm^{−1}, $F(000) = 404.280$, crystal size = $0.165 \times 0.023 \times 0.022$ mm, θ range = $2.15\text{--}30.50^\circ$, 31,125 reflections collected, 5661 reflections unique, $R_{\text{int}} = 0.1263$, observed reflections [$I > 2\sigma(I)$] 3372, 1 restraint, 18 constraints, 316 parameters, $S = 1.0494$, $R1$ [$I > 2\sigma(I)$] = 0.0520, $wR2 = 0.1134$, $\Delta\rho_{\text{max}} = 0.5708$ eÅ^{−3}, and $\Delta\rho_{\text{min}} = -0.5288$ eÅ^{−3}. *Crystal data at 300(2) K:* $\text{C}_{23}\text{H}_{18}\text{FN}_3\text{O}_2$, $M_r = 387.41$, $a = 4.949(4)$, $b = 16.80(1)$, $c = 11.85(1)$ Å, $\beta = 96.67(2)^\circ$, $V = 978(1)$ Å³, $Z = 2$, $\rho_{\text{calc}} = 1.315$ g cm^{−3}.

3.4. Electron Crystallography

The crystallites taken from the material of **4-II** as synthesized were topically applied to a carbon-coated 200 mesh copper grid. The electron diffraction data were measured at ambient temperature on an Eldico ED-1 electron diffractometer equipped with an Excillum LaB₆ electron source, a 6-axis automatic centering goniometer, and a Dectris Quadro hybrid pixel detector. The electrons were accelerated with 160 kV potential ($\lambda = 0.02851$ Å), and the emission current was maintained at 10 μA during the data collection. The Eldix (Eldico Scientific, Alschwill, Switzerland, 2024) software was used to control the diffractometer [30]. The beam size at the crystallite was ca. 600 nm. The crystal was continuously rotated by 120° with a rotation speed of 1.044° per second. The frames were recorded at 0.522° intervals (0.5 s per frame) in the crystallographic binary file (CBF) format [40]. The raw data were processed with APEX5 and SAINT (Bruker AXS, Karlsruhe, Germany). The intensity data were not corrected for absorption. The crystal structure was solved with SHELXT [33], and an independent atom model refinement was carried out with SHELXL-2018/3 [34]. Carbon-bound hydrogen atoms were placed in geometrically calculated positions with neutron-normalized C–H distances [41] and subsequently refined with an appropriate riding model. N–H bond lengths were restrained to a target value of 1.027 Å, as derived from neutron diffraction data [41], with an estimated standard deviation of 0.02 Å. $U_{\text{iso}}(\text{H})$ values were constrained to 1.2 $U_{\text{eq}}(\text{C,N})$. The absolute configuration was verified using dynamical scattering effects [42]. Further details are given in the supplementary

crystallographic data (CIF). The packing index was calculated with Platon [38]. Structure pictures were drawn with Diamond (Crystal Impact GbR, Bonn, Germany).

Crystal data for 4-II: $C_{23}H_{18}FN_3O_2$, $M_r = 387.41$, $T = 298(2)$ K, $\lambda = 0.02851$ Å, orthorhombic, space group $P2_12_12_1$, $a = 5.21(4)$, $b = 16.73(11)$, $c = 22.21(15)$ Å, $V = 1935(23)$ Å³, $Z = 4$, $\rho_{\text{calc}} = 1.330$ g cm^{−3}, $F(000) = 318$, crystal size = $0.6 \times 0.5 \times 0.5$ µm, θ range = 0.088 – 1.020° , 8676 reflections collected, 3833 reflections unique, $R_{\text{int}} = 0.1277$, observed reflections [$I > 2\sigma(I)$] 1905, 269 parameters, 2 restraints, extinction factor $x = 1617(22)$ whereby $F_c^* = kF_c [1 + 0.001 \times F_c^2 \lambda^3 / \sin(2\theta)]^{-1/4}$ and k is the overall scale factor, $S = 0.996$, $R1 [I > 2\sigma(I)] = 0.138$, $wR2 = 0.369$, $\Delta\rho_{\text{max}} = 0.148$ eÅ^{−3}, and $\Delta\rho_{\text{min}} = -0.153$ eÅ^{−3}.

3.5. Powder X-ray Diffraction

The PXRD pattern of **4** as synthesized was measured on a Stoe STADI P powder diffractometer (Debye–Scherrer geometry) at room temperature using a 0.7 mm borosilicate capillary in a moving PSD fixed-omega scan mode (0.005° , 30 s/step). Eight scans were collected and summed after data collection. The diffractometer was equipped with a Cu sealed-tube X-ray source ($\lambda = 1.54060$ Å) running at 40 kV, 40 mA, a curved germanium (111) monochromator, and a linear position-sensitive detector. The theoretical PXRD patterns of **4-I** and **4-II** were calculated from the single-crystal data using Mercury [39].

3.6. Computational Methods

Hirshfeld surface analysis was carried out with CrystalExplorer [43]. DFT calculations on the free molecule of **4** were performed starting from the molecular structure in **4-II** using ORCA (version 5.0) [34] with a B3LYP/G (VWN1) hybrid functional (20% HF exchange) and a def2-TZVPP basis set with an auxiliary def2/J basis. Optimization of the structure used the BFGS method from an initial Hessian according to Almlöf's model with a very tight self-consistent field convergence threshold. The optimized local minimum-energy structures exhibited only positive modes. The structure overlay in Figure 5 was generated with Mercury [39]. Cartesian coordinates of the DFT-optimized structure of **4** can be found in the Supplementary Materials.

3.7. Microbiological Assays

Susceptibility testing against *M. abscessus* ATCC 19977 transformed with plasmid pTEC27) to express the tomato red fluorescent protein (RFP) using a dual readout (optical density and fluorescence) [14], and *M. abscessus* Bamboo was performed as described in detail in a recent publication [16]. The clinical isolate *M. abscessus* Bamboo was provided by the Taichung Veterans General Hospital (Taichung, Taiwan).

4. Conclusions

The title compound, **4**, can be readily synthesized from commercially available Boc-protected D-phenylalanine in a two-step synthesis. Two polymorphs, **4-I** and **4-II**, were discovered. Single-crystal X-ray diffraction revealed the crystal structure of form **4-I**, whereas electron diffraction on a crystallite taken from **4** as synthesized unveiled the solid-state structure of form **4-II**. Replacement of the thiomorpholine 1,1-dioxide moiety on the *N*-aryl ring in the lead compound **2** by an *ortho* cyano group, however, leads to complete loss of whole-cell activity against *M. abscessus* subsp. *abscessus*.

Supplementary Materials: Figure S1: ¹H NMR spectrum of **3** in chloroform-*d*; Figure S2: APCI⁺ mass spectrum of **3**; Figure S3: HPLC analysis of **4**; Figure S4: ¹H NMR spectrum of **4** in chloroform-*d*; Figure S5: ¹³C APT NMR spectrum of **4** in chloroform-*d*; Figure S6: ATR-FT-IR spectrum of **4**; Figure S7: HRMS(ESI⁺) spectrum of **4**; Figure S8: PXRD analysis of **4**. DFT-calculated structure of **4** in MOL file format.

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T.D.; data curation, M.L., R.G., M.P., U.S.G. and A.R.; writing—original draft preparation, R.W.S.; writing—review and editing, M.L., R.G., M.P., U.S.G., T.D. and A.R.; visualization, R.G. and R.W.S.; supervision, A.R. and T.D.; project administration, A.R. and R.W.S.; funding acquisition, A.R. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: CCDC 2352084-2352085 contains the supplementary crystallographic data for this paper. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/structures. The Cartesian coordinates for the DFT-calculated structure of **4** can be found in the Supplementary Materials.

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