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ORIGINAL PAPER

Synthesis and the Crystal Structure of (*E*)-2-(7-(3-(Thiophen-2-yl) acrylamido)-2,3-dihydro-5-oxobenzo[e][1,4]oxazepin-1(5H)-yl) ethyl acetate

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Abstract Details of the synthesis and crystal structure determination of (*E*)-2-(7-(3-(thiophen-2-yl)acrylamido)-2,3-dihydro-5-oxobenzo[e][1,4]oxazepin-1(5H)-yl)ethyl acetate are presented. The compound crystallizes in the triclinic $P-1$ space group ($a = 8.3377(17)$, $b = 9.792(2)$, $c = 12.469(3)$ Å, $\alpha = 96.39(3)°$, $\beta = 108.50(3)°$, $\gamma = 97.68(3)°$, $V = 943.9(3)$ Å^3, $Z = 2$). Interesting features of the structure include intermolecular hydrogen bonding between the amide proton on one molecule and the carbonyl oxygen of the dihydrooxazepinone ring on an adjacent molecule, the boat conformation of the dihydrooxazepinone ring, and π-π stacking between thiophene and phenyl rings on adjacent molecules with a distance between centroids of 3.79(2) Å.

Keywords *E* isomer · Arylacrylamide · Oxazepinone · X-ray · SrtA inhibitor · Hydrogen bonding

Introduction

We have identified inhibitors of the bacterial surface enzyme Sortase A by conducting in silico virtual screening of commercial compound libraries against the Sortase A active site using FlexX software package. These small molecules have inhibited the catalytic activity of the enzyme with IC_{50} values ranging from low to high micromolar values [1]. The most active inhibitor (**1**) identified from these studies has an IC_{50} value of 75 μM [1] (Fig. 1).

Inhibitor **1** has a three-ring structure consisting of a thiophene ring, phenyl ring and a morpholine ring. Thiophene and phenyl rings are connected by an acrylamido linkage in which the double bond has a trans stereochemistry. Middle phenyl ring bears a carboxylic acid group. The morpholine ring is connected to the middle phenyl ring at the position ortho to the carboxylic acid group through the morpholine N atom. The FlexX docking model of inhibitor **1** revealed several critical interactions of the inhibitor within the active site residues. The model indicated that the morpholine ring O has a H bonding interaction with a backbone NH in $SrtA_{\Delta 59}$ active site along with several other critical interactions. In order to improve the H bonding interaction of the ring O it is proposed to synthesize the analog **2** which has a bis(2-hydroxyethyl)amino group in the place of morpholine ring. The FlexX docking model of the proposed analog **2** has revealed possible additional H bonding interactions with NH of Trp194, CO of the Ala92 backbone and CO of Gly192 backbone in the enzyme active site. We have synthesized this new analog **2** using a six step synthesis starting from 2-fluoro-5-nitrobenzoic acid (**3**). The synthesis involves a 7 membered dihydrooxazepinone as a key intermediate (**10**) in the penultimate step. The structure of this intermediate has been confirmed by a high resolution X-ray crystallographic structure. This manuscript describes the synthesis of inhibitor **2** and the X-ray structure determination of the 7-membered ring intermediate dihydrooxazepinone, **10**.

Experimental Section

Synthesis: 5-[(*E*)-3-(thiophen-2-yl)acrylamido)-2-(*bis* (2-hydroxyethyl)amino]benzoic acid (**2**) is prepared in six

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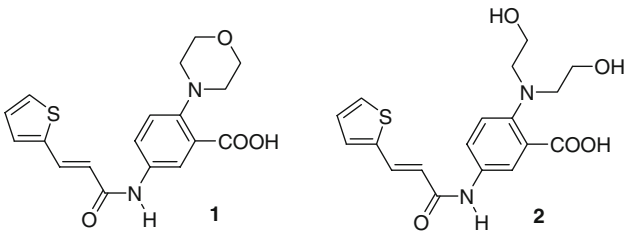


Fig. 1 Sortase A inhibitors

steps from commercially available methyl 2-fluoro-5-nitrobenzoate (**3**) as outlined in Scheme 1. The experimental details follow.

The melting point was determined using a Mel-Temp II melting point instrument apparatus and is uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz spectrometer using TMS as internal standard. The values of chemical shifts (δ) are given in ppm and coupling constants (J) in Hz. Reactions were monitored by TLC (Whatmann, Si gel, UV 254, 25 μM plates). The solvents used for reactions were purchased as anhydrous in Sure-Seal^{TM} bottles from Aldrich chemical company.

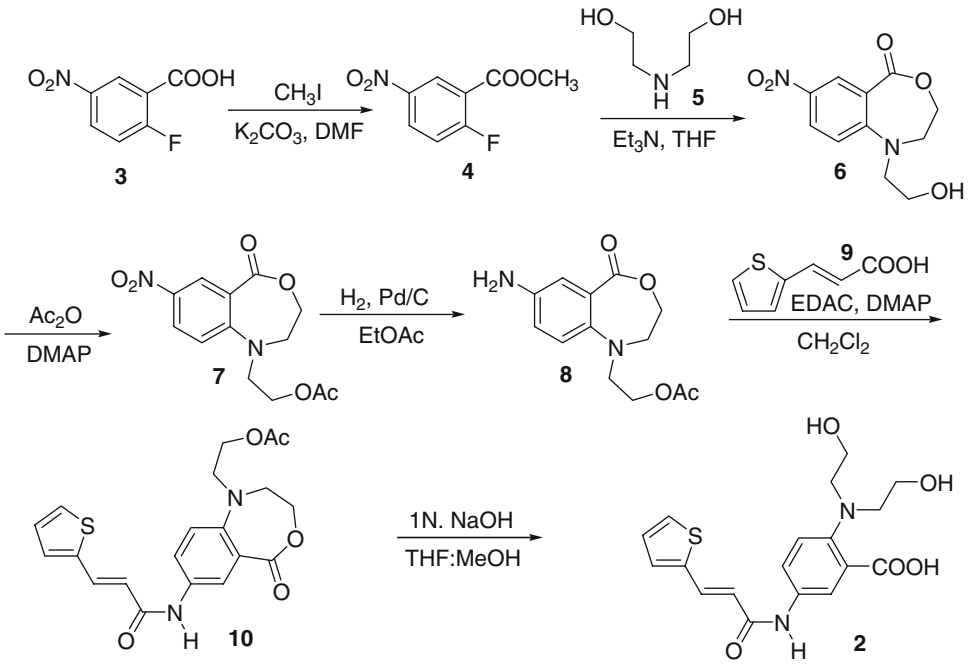
Methyl 2-fluoro-5-nitrobenzoate (**4**)

To a 2-fluoro-5-nitrobenzoic acid (0.5 g, 2.7 mmol) in anhydrous DMF (8 mL), K_2CO_3 (0.86 g, 6.23 mmol) was added and stirred for 20 min at room temperature under N_2 atmosphere. CH_3I (0.5 mL, 8.0 mmol) was added to the mixture and stirred for additional 30 min. TLC examination (50% EtOAc in hexanes) showed that the reaction is complete. Solvent was completely removed and the residue obtained was diluted wit EtOAc (50 mL). The EtOAc extract was washed with water (3 × 25 mL), brine (25 mL) and dried over Na_2SO_4. The drying agent was filtered off, and the solvent was completely removed in vacuo to obtain the crude product. This was purified by chromatography over Si gel (20 × 2 cm) using 10% EtOAc in hexanes as eluent to afford the pure product **4** (0.53 g, 99%); ^{1}H NMR ($CDCl_3$) δ 3.95 (s, 3H), 7.34(t, 1H, $J = 9$ Hz), 8.38–8.47 (m, 1H) and 8.85 (dd, 1H, $J_1 = 6.3$ Hz, $J_2 = 3.0$ Hz); ^{13}C NMR ($CDCl_3$) δ 53.0, 118.3 and 118.6 (C–F coupling), 119.7 and 119.9 (C–F coupling), 128.2 and 128.3, 129.4 and 129.6 (C–F coupling), 143.9, 162.6 and 163.3 (C–F coupling) and 166.9; MS (ES+) m/z 169 (M-OCH_3).

2,3-Dihydro-1-(2-hydroxyethyl)-7-nitrobenzo[e][1,4]oxazepin-5(1H)-one (**6**)

To a solution of compound **4** (0.59 g, 2.9 mmol) in anhydrous THF (12 mL), bis(2-hydroxyethyl)amine (0.4 g, 3.8 mmol) and triethylamine (0.67 mL, 4.8 mmol) were added and stirred at room temperature for 17 h. TLC analysis (25% EtOAc in hexane) revealed that the reaction was complete. The solvent was removed, and the residue was diluted with EtOAc (100 mL). The EtOAc extract was washed with water (3 × 35 mL) and brine (35 mL) and dried over Na_2SO_4. The drying agent was filtered off, and the solvent was removed in vacuo to obtain the crude product. The crude product was purified by crystallization from $CHCl_3$/EtOAc/MeOH (70:25:5) to afford the pure product **6** (0.67 g, 90%); ^{1}H NMR (acetone-d_6) δ 3.75

Scheme 1 Synthesis of compound **2**



(t, 2H, $J = 5.4$ Hz), 3.89 (t, 2H, $J = 5.4$ Hz), 4.03(t, 2H, $J = 3.9$ Hz), 4.60 (t, 2H, $J = 3.9$ Hz), 7.20 (d, 1H, $J = 9.6$ Hz), 8.13 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 3.0$ Hz) and 8.61(d, 1H, $J = 3.0$ Hz); ^{13}C NMR (acetone-d_6) δ 56.4(2C), 59.4, 66.1, 116.8, 117.5, 128.7, 132.7, 138.10, 152.0 and 170.2; MS (ES+) *m/z* 253 (M + H).

2-(2,3-Dihydro-7-nitro-5-oxobenzo[e][1,4]oxazepin-1(5H)-yl)ethyl Acetate (**7**)

A solution of compound **6** (0.103 g, 0.41 mmol) and DMAP (0.011 g, 0.09 mmol) in acetic anhydride (2 mL) was stirred at room temperature for 12 h. TLC (50% EtOAc in $CHCl_3$) examination indicated the completion of the reaction. Acetic anhydride was completely removed under high vacuum, and the residue was diluted with EtOAc (25 mL). The EtOAc extract was washed with water (3 × 15 mL) and brine (15 mL) and then dried over Na_2SO_4. The drying agent was filtered off, and the filtrate was concentrated in vacuo to yield the pure product **7** (0.091 g, 75%); ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 3H), 3.72 (t, 2H, $J = 6.0$ Hz), 3.82–3.91(m, 2H), 4.35 (t, 2H, $J = 6.0$ Hz), 4.49–4.59 (m, 2H), 6.92 (d, 1H, $J = 9.6$ Hz), 8.19 (dd, 1H, $J_1 = 9.6$ Hz, $J_2 = 3.0$ Hz) and 8.72 (d, 1H, $J = 3.0$ Hz); ^{13}C NMR ($CDCl_3$) δ 21.0, 52.0, 55.1, 60.3, 65.1, 116.4, 117.3, 129.0, 132.3, 138.9, 150.5, 169.6 and 170.9; MS (ES+) *m/z* 295 (M + H).

(*E*)-2-(7-Amino-2,3-dihydro-5-oxobenzo[e][1,4]oxazepin-1(5H)-yl)ethyl acetate (**8**)

To a solution of compound **7** (0.165 g, 0.56 mmol) in EtOAc (40 mL), 10% Pd/C (0.034 g) was added and then this mixture was stirred at room temperature for 1 h under H_2 gas. TLC examination (25% EtOAc in $CHCl_3$) showed that the reaction was complete. The catalyst was removed by filtration through Celite®. The filtrate was concentrated in vacuo to obtain the product **8** (0.148 g, 100%); ^{1}H NMR ($CDCl_3$) δ 2.05 (s, 3H), 3.33–3.39 (m, 4H), 4.21 (t, 2H, $J = 6.0$ Hz), 4.29 (t, 2H, $J = 5.6$ Hz), 6.77–6.84 (m, 2H) and 6.93–6.98 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 20.7, 50.5, 54.6, 61.1, 65.2, 117.2, 120.0, 120.3, 126.2, 137.3, 141.8, 170.9 and 171.9; MS (ES+) *m/z* 265 (M + H).

(*E*)-2-(7-(3-(Thiophen-2-yl)acrylamido)-2,3-dihydro-5-oxobenzo[e][1,4]oxazepin-1(5H)-yl)ethyl acetate (**10**)

To a solution of compound, **8** (0.166 g, 0.63 mmol) and (E)-3-(thiophen-2-yl)acrylic acid **9** (0.116 g, 0.75 mmol) in CH_2Cl_2 (10 mL), EDAC (0.246 g, 1.28 mmol) and DMAP (0.01 g, 0.082 mmol) were added and the mixture was stirred under N_2 atmosphere for 12 h. TLC examination (25% EtOAc in $CHCl_3$) indicated the completion of the reaction. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with 1 M $NaHCO_3$ (3 × 20 mL), water (2 × 20 mL), brine (20 mL) and then dried over Na_2SO_4. The drying agent was filtered off, and solvent was completely removed to yield the crude product. The crude product was purified by column chromatography over Si gel (20 × 2 cm) using $CHCl_3$ as the eluent to afford the pure product **10** (0.162 g, 64%); ^{1}H NMR (DMSO-d_6) δ 1.96 (s, 3H), 3.35–3.58 (m, 4H), 4.18 (t, 2H, $J = 6.0$ Hz), 4.36 (t, 2H, $J = 4.5$ Hz), 6.51(d, 1H, $J = 15.3$ Hz), 7.03(d, 1H, $J = 9.0$ Hz), 7.14 (dd, 1H, $J_1 = 9.0$ Hz, $J_2 = 4.6$ Hz), 7.44 (d, 1H, $J = 3.3$ Hz), 7.65 (d, 1H, $J = 5.1$ Hz), 7.71(d, 1H, $J = 15.6$ Hz), 7.80 (dd, 1H, $J_1 = 8.7$ Hz, $J_2 = 2.7$ Hz) and 7.86 (d, 1H, $J = 2.4$ Hz); ^{13}C NMR (DMSO-d_6) δ 20.6, 50.1, 54.2, 60.6, 65.1, 118.6, 120.6, 122.3, 122.5, 124.7, 128.4(2C), 131.2, 132.2, 132.9, 139.7, 141.8, 162.9, 170.3 and 170.8; MS (ES−) *m/z* 399 (M − H); Anal Calcd for $C_{20}H_{20}N_2O_5S$: C, 59.99; H, 5.03; N, 7.00. Found: C, 59.33; H, 4.98; N, 6.87.

5-((*E*)-3-(Thiophen-2-yl)acrylamido)-2-(bis(2-hydroxyethyl)amino)benzoic acid (**2**)

To a solution of compound **10** (0.087 g, 0.22 mmol) in MeOH/THF (6 mL, 1:1), 1 N NaOH (0.5 mL) was added, and the mixture was stirred at room temperature for 4 h. TLC examination (25% EtOAc/hexanes) showed the completion of the reaction. Solvents were completely evaporated in vacuo, and the residue was dissolved in water (20 mL). The aqueous solution was extracted with $CHCl_3$ (2 × 10 mL) and acidified with 6 N HCl to pH 2 to precipitate the crude product. The crude product was filtered off and purified by column chromatography over Si gel (10 × 1 cm) using 20% MeOH/$CHCl_3$ as the eluent to furnish the pure product **2** (0.21 g, 95%) as a white solid; mp: 116 °C; ^{1}H-NMR (DMSO-d_6): δ 3.21–3.53 (m, 8H), 4.91(bs, 2H), 6.69 (d, 1H, $J = 15.3$ Hz), 7.13 (s, 1H), 7.46 (s, 1H), 7.58–7.90 (m, 3H), 8.07 (d, 1H, $J = 7.8$ Hz), 8.43 (s, 1H) and 10.88 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 57.1, 58.7, 120.9, 121.0, 121.1, 121.2, 124.6, 124.7, 125.2, 129.0, 129.2, 132.0, 134.1, 140.1, 164.1 and 167.4; MS (ES−) *m/z* 375 (M − H).

X-ray Data Collection and Structure Solution

Compound **10** was crystallized by slow diffusion of hexanes into a THF solution of **10** to give yellow plate-like crystals. A suitable single crystal of compound **10** was glued on a glass fiber with epoxy and aligned upon an Enraf Nonius CAD4 single crystal diffractometer under aerobic conditions. Standard peak search and automatic indexing routines followed by least squares fits of 25 accurately centered reflections resulted in accurate unit cell

parameters. The space group was assigned on the basis of systematic absences and intensity statistics. All data collection was carried out using the CAD4-PC software [2], and details of the data collection are given in Table 1. The analytical scattering factors of the complex were corrected for both $\Delta f'$ and $i\Delta f''$components of anomalous dispersion. All data were corrected for the effects of absorption using a psi scan with four reflections with $\chi \geq 80°$ and for Lorentz and polarization effects.

All crystallographic calculations were performed with the Siemens SHELXTL-PC program package [3]. All heavy atom positions were located using Direct Methods while the amide hydrogen atom was located in difference Fourier maps. Full matrix refinement of the positional and anisotropic thermal parameters for all these atoms versus F^2 was carried out. All other hydrogen atoms were placed in calculated positions with the appropriate molecular geometry and the δ(C–H) = 0.96Å. The isotropic thermal parameter associated with each hydrogen atom was fixed equal to 1.2 times the U_{eq} of the atom to which it was bound.

Selected torsion angles for compound **10** are given in Table 2. Crystallographic data for the complex has been deposited with the Cambridge Crystallographic Database (**10**: CCDC 712737).

Table 1 Crystal and structure data for **10**

Empirical formula	$C_{20}H_{20}N_2O_5S$
CCDC deposit no.	712737
Formula weight	400.44
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	$P-1$
Unit cell dimensions	$a = 8.3377(17)$ Å
	$b = 9.792(2)$ Å
	$c = 12.469(3)$ Å
	$\alpha = 96.39(3)°$
	$\beta = 108.50(3)°$
	$\gamma = 97.68(3)°$
Volume	943.9(3) Å^3
Z	2
Density (calculated)	1.409 mg/m^3
Absorption coefficient	0.207 mm^{-1}
$F_{(000)}$	420
Crystal size	0.4 × 0.5 × 0.8 mm^3
θ range for data collection	2.13°–22.47°
Index ranges	$-8 \leq h \leq 8$
	$-10 \leq k \leq 1$
	$-13 \leq l \leq 13$
Reflections collected	2969
Independent reflections	2450 [R(int) = 0.0575]
Completeness to $\theta = 22.47°$	100.0%
Absorption correction	Empirical
Maximum transmission	0.6643
Minimum transmission	0.4442
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2450/0/258
Goodness-of-fit on F^2	1.113
Final R indices [I > 2σ (I)]	$R_1 = 0.0694$, $wR_2 = 0.1720$
R indices (all data)	$R_1 = 0.0982$, $wR_2 = 0.1935$
Largest diff. peak and hole	0.670 and −0.441 e Å^{-3}

Table 2 Selected torsion angles (°) for compound **10**

C(2)–C(3)–C(4)–S	0.2(6)
C(1)–C(2)–C(3)–C(4)	−177.0(4)
N(1)–C(1)–C(2)–C(3)	178.7(4)
C(2)–C(1)–N(1)–C(8)	−174.5(4)
C(9)–C(8)–N(1)–C(1)	161.4(4)

Results and Discussion

X-ray Crystal Structure

The crystal structure of compound **10** shows that the molecule has a rigid backbone with a conformationally flexible dihydrooxazepinone ring and side chain as seen in Fig. 2. Table 2 gives selected torsion angles of the backbone portion of the molecule. The data show that the thiophene, alkene and amide are essentially coplanar as indicated by the small deviation (±5°) in torsion angles from 180°. This conformation of the backbone suggests

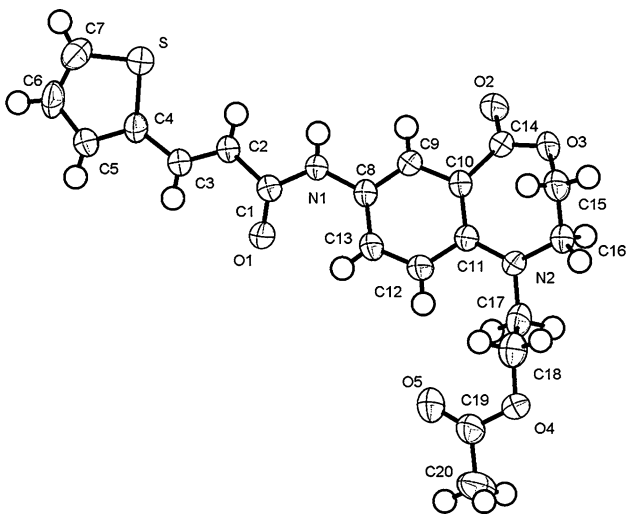


Fig. 2 ORTEP [6] drawing of the molecular structure of compound **10**. Thermal ellipsoids are drawn at 50% and hydrogen labels are omitted for clarity

that conjugation is preserved throughout the backbone. In contrast, the amide and phenyl ring are twisted by approximately 18.6(4)° relative to each other, which breaks the conjugation of the π-system.

Another important feature of the structure of compound **10** is the conformation of the dihydrooxazepinone ring. Besides the side chain, the dihydrooxazepinone ring is the most conformationally flexible portion of the molecule. The dihydrooxazepinone ring adopts a boat conformation with C(15) occupying the bow and C(10)–C(11) occupying the stern. A least squares plane through C(14), O(3), N(2), and C(16) shows that these four atoms are only slightly out of plane. This creates a mirror plane of symmetry through the C(10)–C(11) bond and bisecting C(15), which is indicative of the boat conformation. Fillers et al. also found that the dihydrooxazepinone ring adopts a boat conformation as opposed to the half-chair conformation seen in other dihydrooxazepinone rings [4, 5]. This conformation is further supported by the lack of axial-axial interactions between H(15) and C(10) or C(11) due their lack of hydrogens.

One final point of interest is the manner in which the molecules stack in the crystal lattice. Figure 3 shows that a portion of the molecules stack about a center of symmetry, which allows for intermolecular hydrogen bonding between the amide hydrogen and the carbonyl of the dihydrooxazepinone ring. The O(2)–H_{N1} distance is 2.08(4) Å and the N(1)–$H_{N(1)}$ distance is 0.87(4) Å. The angle between N(1)–$H_{N(1)}$–O(2) is almost linear with a value of 174(4)°. The orientation of the rings relative to each other in the crystal lattice shows that π-π stacking is present. The thiophene

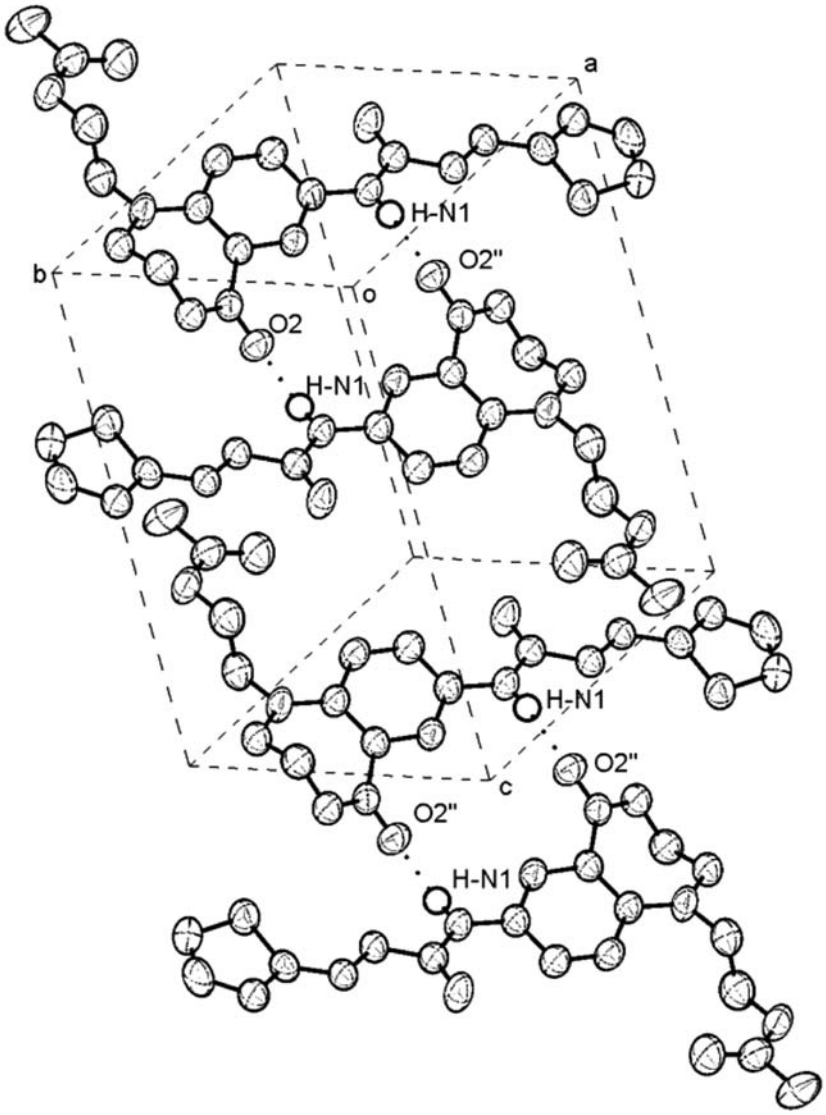


Fig. 3 ORTEP [6] Packing Diagram of the molecular structure of compound **10** showing centrosymmetric intermolecular hydrogen bonding. Thermal ellipsoids are drawn at 50% and hydrogens are omitted for clarity

ring stacks directly over an adjacent phenyl ring with a distance between centroids of 3.79(2) Å. However, the rings are not coplanar due to the free rotation about the N(1)–C(8) bond causing the phenyl group to twist relative to the rigid backbone.

Supplementary Material

CCDC 712737 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via (please use the link below) by e-mailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK, Fax: + 44(0)1223-336033.

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