SYNTHESIS, MOLECULAR STRUCTURES, AND ANTIMICROBIAL ACTIVITIES OF *N'*-(3,5-DIBROMO-2-HYDROXYBENZYLIDENE)-2-FLUOROBENZOHYDRAZIDE AND *N'*-(4-DIETHYLAMINO-2-HYDROXYBENZYLIDENE)-2-FLUOROBENZOHYDRAZIDE

YUN-XIAO FENG[1], LING-WEI XUE[1,], CAI-XIA ZHANG[2]*

[1] *College of Chemistry and Chemical Engineering, Pingdingshan University, Pingdingshan Henan 467000, P.R. China*
[2] *Coal Chemical Industry Branch of Shenhua Ningxia Coal Group, Yinchuan Ningxia 750411, P.R. China*

ABSTRACT

Two new carbohydrazone derivatives, *N'*-(3,5-dibromo-2-hydroxybenzylidene)-2-fluorobenzohydrazide (**1**), and *N'*-(4-diethylamino-2-hydroxybenzylidene)-2-fluorobenzohydrazide (**2**), were prepared by the reaction of 2-fluorobenzohydrazide with 3,5-dibromo-2-hydroxybenzaldehyde and 4-diethylamino-2-hydroxybenzaldehyde, respectively. The compounds were characterized by elemental analyses, infrared spectra, and single crystal X-ray diffraction. The crystal of **1** crystallizes in monoclinic space group *C*2/*c*, with unit cell dimensions $a = 36.0853(5)$ Å, $b = 10.1711(2)$ Å, $c = 17.3627(5)$ Å, $\beta = 109.973(2)°$, $V = 5989.3(2)$ Å^3, $Z = 16$, $R_1 = 0.0544$, $wR_2 = 0.1300$. The crystal of **2** crystallizes in monoclinic space group $P2_1/c$, with unit cell dimensions $a = 10.6827(2)$ Å, $b = 16.5256(3)$ Å, $c = 9.8174(1)$ Å, $\beta = 102.564(2)°$, $V = 1691.64(5)$ Å^3, $Z = 4$, $R_1 = 0.0772$, $wR_2 = 0.2156$. The compounds were assayed for antibacterial (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescence* and *Staphylococcus aureus*) and antifungal (*Aspergillus niger* and *Candida albicans*) activities by MTT method.

Keywords: Synthesis; crystal structure; carbohydrazone; X-ray diffraction; antibacterial activity; antifungal activiy.

INTRODUCTION

Various carbohydrazides and their derivatives have been reported to possess interesting biological activities. For instance, some of the compounds are found useful for the treatment of autoimmune and inflammatory diseases, tumors, osteoarthritis and hemorrhage,[1] and some of the compounds have antibacterial, antifungal, antiviral, antiparasitic, antituberculous, and many other activities.[2–5] The emphasis on structural studies of carbohydrazone derivatives is a consequence of our interests in compounds having potential biological activity. In addition, the carbohydrazides and their derivatives have also been used as preferred ligands in construction of versatile structures of complexes with various metal salts.[6–9] As an extensive of the work on the structures and antimicrobial activities of such compounds, in the present paper, two new carbohydrazone derivatives, *N'*-(3,5-dibromo-2-hydroxybenzylidene)-2-fluorobenzohydrazide (**1**), and *N'*-(4-diethylamino-2-hydroxybenzylidene)-2-fluorobenzohydrazide (**2**) (Chart 1), were reported.

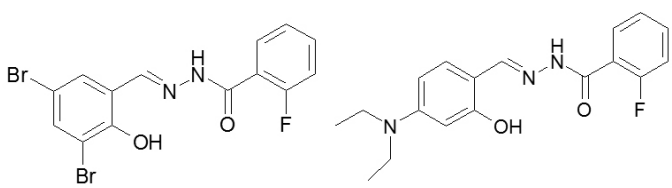

Chart 1: The compounds **1** and **2**.

EXPERIMENTAL

Materials and Methods: 3,5-Dibromo-2-hydroxybenzaldehyde, 4-diethylamino-2-hydroxybenzaldehyde and 2-fluorobenzohydrazide with AR grade were purchased from Fluka and used as received. All other chemicals with AR grade were obtained commercial and used without purification. Elemental analyses were carried out on a Perkin-Elmer model 240 analyzer. NMR spectra were determined in deuterated chloroform containing *ca.* 1% tetramethylsilane as an internal standard, using a Bruker AVANCE DRX-500 NMR instrument. FT-IR spectra were recorded on a Nicolet 55XC spectrometer.

*Synthesis of **1**:* To a solution of 3,5-dibromo-2-hydroxybenzaldehyde (0.280 g, 1.0 mmol) in absolute MeOH (20 mL), 2-fluorobenzohydrazide (0.154 g, 1.0 mmol) was added. The mixture was stirred at room temperature for 30 min and the solvent was partially concentrated at reduced pressure. The colorless precipitate of the compound was isolated by filtration, and washed with methanol. Single crystals suitable for X-ray diffraction were obtained by recrystallization of the product in methanol. Yield, 67%. Characteristic IR (cm^{-1}, KBr pellets): 3353 (m, O-H), 3231 (m, N-H), 1657 (s, C=O), 1626 (s, C=N). ^{1}H NMR data in $CDCl_3$: 12.57 (s, 1H, -N*H*), 10.33 (s, 1H, -O*H*), 8.71 (s, 1H, C*H*=N), 8.00 (dd, $J = 7.3$, 1H), 8.04 (d, $J = 7.3$, 1H), 7.85 (s, 1H), 7.72 (s, 1H), 7.37 (dd, $J = 7.3$, 1H), 7.45 (d, $J = 7.3$, 1H). ^{13}C NMR data in $CDCl_3$: 163.7, 159.8, 158.5, 145.7, 138.2, 134.3, 131.7, 129.6, 127.1, 123.8, 123.1, 115.8, 115.2, 114.0. Anal. Calcd. (%) for $C_{14}H_9Br_2FN_2O_2$: C, 40.4; H, 2.2; N, 6.7. Found (%): C, 40.3; H, 2.2; N, 6.8.

*Synthesis of **2**:* To a solution of 4-diethylamino-2-hydroxybenzaldehyde (0.193 g, 1.0 mmol) in absolute MeOH (20 mL), 2-fluorobenzohydrazide (0.154 g, 1.0 mmol) was added. The mixture was stirred at room temperature for 30 min and the solvent was partially concentrated at reduced pressure. The colorless precipitate of the compound was isolated by filtration, and washed with methanol. Single crystals suitable for X-ray diffraction were obtained by recrystallization of the product in methanol. Yield, 71%. Characteristic IR (cm^{-1}, KBr pellets): 3387 (m, O-H), 3212 (m, N-H), 1651 (s, C=O), 1623 (s, C=N). ^{1}H NMR data in $CDCl_3$: 12.02 (s, 1H, -N*H*), 9.87 (s, 1H, -O*H*), 8.72 (s, 1H, C*H*=N), 8.00 (dd, $J = 7.3$, 1H), 8.04 (d, $J = 7.3$, 1H), 7.37 (dd, $J = 7.3$, 1H), 7.45 (d, $J = 7.3$, 1H), 7.52 (d, $J = 7.6$, 1H), 6.35 (d, $J = 7.6$, 1H), 6.28 (s, 1H), 3.39 (q, 4H, $N(CH_2CH_3)_2$), 1.13 (t, 6H, $N(CH_2CH_3)_2$). ^{13}C NMR data in $CDCl_3$: 12.7, 46.6, 99.3, 103.8, 108.2, 115.5, 124.7, 126.2, 130.9, 133.0, 134.3, 146.5, 155.0, 159.1, 162.7, 163.5. Anal. Calcd. (%) for $C_{18}H_{20}FN_3O_2$: C, 65.6; H, 6.1; N, 12.8. Found (%): C, 65.4; H, 6.2; N, 12.8.

X-Ray Crystallography: Single-crystal X-ray diffraction measurements for the compounds were carried out on a CrysAlis CCD diffractometer equipped with a graphite crystal monochromator for data collection at 298(2) K. The determinations of unit cell parameters and data collections were performed with Cu *Kα* radiation ($\lambda = 1.5418$ Å) and unit cell dimensions were obtained with least-squares refinements. Both structures of the compounds were solved by direct methods using SHELXS-97 and refined with SHELXL-97,[10] non-hydrogen atoms were located in successive difference Fourier syntheses. The final refinement was performed by full-matrix least-squares methods with anisotropic thermal parameters for non-hydrogen atoms on F^2. The hydrogen atoms were treated by a mixture of independent and constrained refinement. The amino H atoms in the two compounds were located from difference Fourier maps and refined isotropically, with N–H distances restrained to 0.90(1) Å. The remaining hydrogen atoms were located at their calculated positions. The F atoms in **1** are over two sites with occupancies of 0.723(2) and 0.277(2) for F1, and 0.625(2) and 0.375(2) for F2. Crystallographic data and experimental details for structural analyses are summarized in Table 1. Selected bond values are summarized in Table 2. Hydrogen bonds are listed in Table 3.

Antimicrobial Test: The antibacterial activity of the compounds was tested against *B. subtilis*, *E. coli*, *P. fluorescence* and *S. aureus* using MH medium (Mueller–Hinton medium: casein hydrolysate 17.5 g, soluble starch 1.5 g,

beef extract 1000 mL), the antifungal activity of the compounds was tested against *A. niger* and *C. albicans* using RPMI-1640 medium (RPMI-1640 (GIBCO BRL) 10 g, $NaHCO_3$ 2.0 g, 0.165 mol/L morpholinepropanesulfonic acid (MOPS) (Sigma) 34.5 g, triple distilled water 900 mL, buffered to pH 7.0 with 1 mol/L NaOH (25 °C), metered volume to 1000 mL, filtered sterilization, conservation in 4 °C). The MICs of the test compounds were determined by a colorimetric method using the dye MTT.[11] A stock solution of the synthesized compound (50 μg/mL) in DMSO was prepared and graded quantities of the test compounds were incorporated in specified quantity of sterilized liquid medium (MH medium for antibacterial activity and RPMI-1640 medium for antifungal activity). A specified quantity of the medium containing the compound was poured into microtitration plates. Suspension of the microorganism was prepared to contain approximately 10^5 cfu/mL and applied to microtitration plates with serially diluted compounds in DMSO to be tested and incubated at 37 °C for 24 h and 48 h for bacterial and fungi, respectively. After the MICs were visually determined on each of the microtitration plates, 50 μL of PBS (phosphate buffered saline 0.01 mol/L, pH 7.4, $Na_2HPO_4 \cdot 12H_2O$ 2.9 g, KH_2PO_4 0.2 g, NaCl 8.0 g, KCl 0.2 g, distilled water 1000 mL) containing 2 mg of MTT/mL was added to each well. Incubation was continued at room temperature for 4–5 h. The content of each well was removed, and 100 μL of isopropanol containing 5% 1 mol/L HCl was added to extract the dye. After 12 h of incubation at room temperature, the optical density (OD) was measured with a microplate reader at 550 nm.

RESULTS AND DISCUSSION

The synthesis of the compounds was carried out as outlined in Chart 2. The single crystals were obtained by slow evaporation of the methanolic solutions of the compounds.

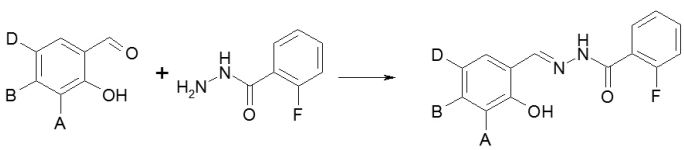

Chart 2: The synthesis of the compounds. A = D = Br, B = H for **1**, A = D = H, B = $N(CH_3CH_2)_2$ for **2**.

Structure Description of the Compounds: The structures of the compounds **1** and **2** together with the atom numbering scheme are shown in Figs. 1 and 2, respectively. The asymmetric unit of **1** contains two independent carbohydrazone molecules. In both compounds, the carbohydrazone molecules adopt *E* configurations about the C=N bonds. The dihedral angles between the benzene rings C(1)-C(6) and C(9)-C(14) in **1**, C(15)-C(20) and C(23)-C(28) in **1**, and C(1)-C(6) and C(9)-C(14) in **2** are 32.5(3), 28.6(3), and 40.5(3)°, respectively. The bond distances C(7)–N(1) and C(21)–N(3) (1.273(5) Å) in **1** and C(7)–N(1) (1.283(3) Å) in **2** correspond to C=N double bonds, and are comparable to the previously reported analogous of carbohydrazones.[12–14] The bond distances C(8)–N(2) (1.359(5) Å) and C(22)–N(4) (1.340(5) Å) in *(1)* and C(8)–N(3) (1.348(3) Å) in *(2)* are shorter than the typical values for C–N single bonds, suggesting the existence of conjugation in the carbohydrazone molecules.

In the crystal structure of **1**, the carbohydrazone molecules are linked *via* N–H···O intermolecular hydrogen bonds, to form 1D chains running along the *b* axis (Fig. 3). In the crystal structure of **2**, the carbohydrazone molecules are linked *via* N–H···O intermolecular hydrogen bonds, to form 1D chains running along the *c* axis (Fig. 4). In addition, the weak π···π interactions are observed in the compounds, with centroid to centroid distances from 4.549 to 4.773 Å for **1**, and 4.431 Å for **2**.

Infrared Spectra: The broad and middle bands centered at 3372 cm^{-1} in **1** and 3403 cm^{-1} in **2** are due to the O-H streching vibrations of the hydroxyl groups. The sharp bands at 3237 cm^{-1} in **1** and 3219 cm^{-1} in **2** are due to the N-H stretching vibrations. The compounds exhibit strong absorptions at 1623 cm^{-1} for **1** and 1620 cm^{-1} for **2**, which can be attributed to the C=N vibrations. The bands originating from the stretching vibrations of the C=O groups are observed at 1655 cm^{-1} for **1** and 1651 cm^{-1} for **2**.

Antimicrobial Activities: The MICs (minimum inhibitory concentrations) of the compounds against four bacteria strains are presented in Table 4. The activities of reference compounds Kanamycin and Penicillin were included. The two compounds were found to be inactive against *Bacillus subtilis*. Compound **1** showed the highest activity against *Escherichia coli*, and effective activities against *Pseudomonas fluorescence* and *Staphylococcus aureus*. Compound **2** showed significant activity against *Staphylococcus aureus* and *Pseudomonas fluorescence*, and weak activity against *Escherichia coli*. It is notable that compound **1** showed much more stronger activity against *Escherichia coli* than the reference drug Kanamycin. When detailed comparison with the structures and activities of the compounds, we found that the existence of the bromo and fluoro groups in the compounds can increase the antibacterial activities.

The antifungal activity of the compounds was studied with two fungal strains by MTT method. The results are summarized in Table 4. Ketoconazole was used as a reference. The results indicate that both compounds showed no activity against *Aspergillus niger* and *Candida albicans*.

Table 1 Crystal data for the compounds

Compound	**1**	**2**
Empirical formula	$C_{14}H_8Br_2FN_2O_2$	$C_{18}H_{20}FN_3O_2$
Formula weight	415.0	329.4
Crystal shape, color	Block, colorless	Block, colorless
Temperature, K	298(2)	298(2)
Wavelength, Å	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic
Space group	*C*2/*c*	$P2_1/c$
Unit cell dimensions		
a, Å	36.0853(5)	10.6827(2)
b, Å	10.1711(2)	16.5256(3)
c, Å	17.3627(5)	9.8174(1)
β, °	109.973(2)	102.564(2)
Volume, $Å^3$	5989.3(2)	1691.64(5)
Z	16	4
Calculated density, g/cm^3	1.841	1.293
Absorption coefficient, mm^{-1}	7.030	0.770
F(000)	3216	696
Crystal size, mm	0.20 ´ 0.20 ´ 0.19	0.18 ´ 0.18 ´ 0.17
Limiting indices	$-32 \le h \le 44$ $-10 \le k \le 12$ $-21 \le l \le 18$	$-13 \le h \le 12$ $-19 \le k \le 20$ $-6 \le l \le 12$
Reflections collected	20876	11296
Independent reflections [R_{int}]	5864 [0.0224]	3307 [0.0126]
Observed reflections [$I \ge 2\sigma(I)$]	4859	3092
Data/restraints/parameters	5864/8/411	3307/1/223
Goodness-of-fit on F^2	1.078	1.037
T_{min}	0.3337	0.8738
T_{max}	0.3485	0.8802
Final *R* indices [$I \ge 2\sigma(I)$]	0.0544, 0.1300	0.0772, 0.2156
R indices (all data)	0.0646, 0.1370	0.0801, 0.2185
Largest difference peak and hole, e $Å^{-3}$	0.613 and -0.578	0.744 and –0.414

CONCLUSION

The reaction of 2-fluorobenzohydrazide with 3,5-dibromo-2-hydroxybenzaldehyde and 4-diethylamino-2-hydroxybenzaldehyde,

respectively, products two new carbohydrazone compounds *N'*-(3,5-dibromo-2-hydroxybenzylidene)-2-fluorobenzohydrazide and *N'*-(4-diethylamino-2-hydroxybenzylidene)-2-fluorobenzohydrazide. The compounds were characterized by elemental analyses, infrared spectra, and single crystal X-ray diffraction. The structure-activity relationship indicates that the existence of the bromo and fluoro groups in the compounds can increase the antibacterial activities. The compounds may be used as potential antibacterial drugs, which deserve further study.

Table 2 Selected bond lengths (Å) and angles (°) for the compounds

1			
C(7)–N(1)	1.273(5)	N(1)–N(2)	1.373(5)
N(2)–C(8)	1.359(5)	O(2)–C(8)	1.218(5)
C(21)–N(3)	1.273(5)	N(3)–N(4)	1.376(4)
N(4)–C(22)	1.340(5)	O(4)–C(22)	1.213(5)
C(7)–N(1)–N(2)	120.2(4)	N(1)–N(2)–C(8)	115.9(3)
O(2)–C(8)–N(2)	120.7(4)	C(21)–N(3)–N(4)	118.8(3)
N(3)–N(4)–C(22)	118.3(3)	O(4)–C(22)–N(4)	122.3(4)
2			
C(7)–N(2)	1.283(3)	N(2)–N(3)	1.384(3)
N(3)–C(8)	1.348(3)	O(1)–C(8)	1.224(3)
C(7)–N(2)–N(3)	116.3(2)	N(2)–N(3)–C(8)	118.6(2)
N(3)–C(8)–O(1)	122.7(2)		

Table 3 Distances (Å) and angles (°) involving hydrogen bonding of the compounds

D–H···*A*	d(*D*–H)	d(H··*A*)	d(*D*···*A*)	Angle(*D*–H···*A*)
1				
O(1)–H(1)···N(1)	0.85(1)	1.80(3)	2.537(5)	145(5)
O(3)–H(3)···N(3)	0.85(1)	1.82(3)	2.576(4)	148(5)
N(2)–H(2)···O(4)$^{\#1}$	0.90(1)	2.10(2)	2.940(4)	156(4)
N(4)–H(4)···F(1)	0.90(1)	2.60(4)	3.203(5)	125(4)
N(4)–H(4)···F(2)	0.90(1)	2.32(5)	2.792(5)	113(4)
N(4)–H(4)···O(2)	0.90(1)	2.06(2)	2.921(4)	162(4)
2				
N(3)–H(3)···O(1)$^{\#2}$	0.90(1)	2.09(2)	2.947(3)	161(3)
O(2)–H(2)···N(2)	0.82	1.93	2.646(3)	146

Symmetry codes: #1: x, $1 + y$, z; #2: x, $1/2 - y$, $-1/2 + z$.

SUPPLEMENTARY MATERIAL

CCDC –869274 for **1** and 869275 for **2** contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44(0)1223-336033; e-mail: deposit@ccdc.cam.ac.uk].

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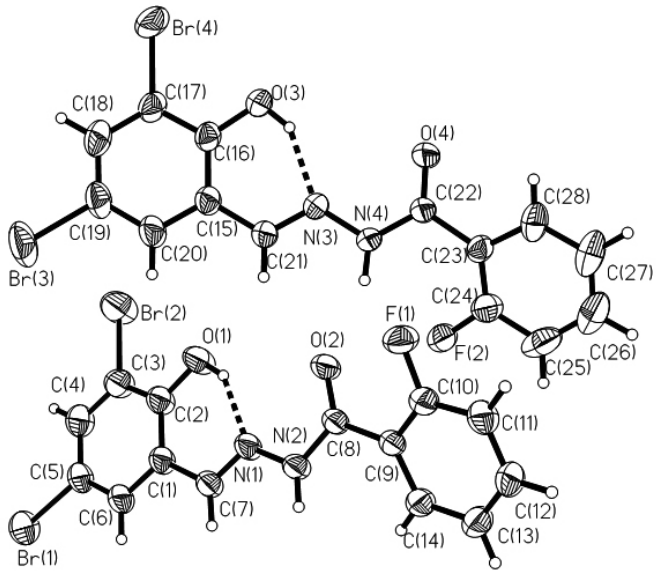

Figure 1 The molecular structure of **1**. The ellipsoids are shown with 30% probability. Intramolecular O–H···N hydrogen bonds are shown as dashed lines.

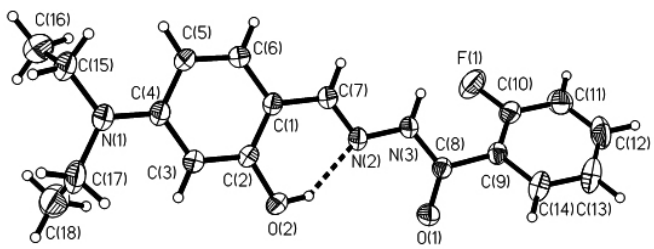

Figure 2 The molecular structure of **2**. The ellipsoids are shown with 30% probability. Intramolecular O–H···N hydrogen bond is shown as a dashed line.

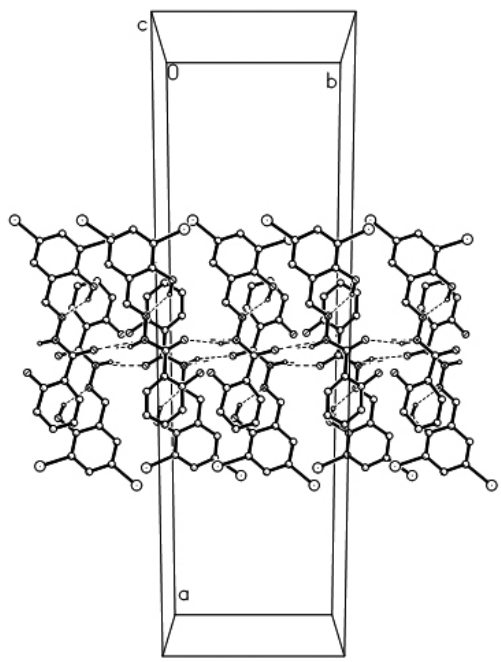

Figure 3 The packing diagram of **1**. Hydrogen bonding interactions are shown as dashed lines.

Table 4. MIC values of the compounds (μg/mL)

Compound	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas fluorescence*	*Staphylococcus aureus*	*Aspergillus niger*	*Candida albicans*
1	> 50	1.6	3.1	3.1	> 50	> 50
2	> 50	12.5	6.2	3.1	> 50	> 50
Ketoconazole	> 50	> 50	> 50	> 50	7.8	3.9
Kanamycin	0.39	3.9	3.9	1	> 50	> 50
Penicillin	0.78	> 50	> 50	2	> 50	> 50

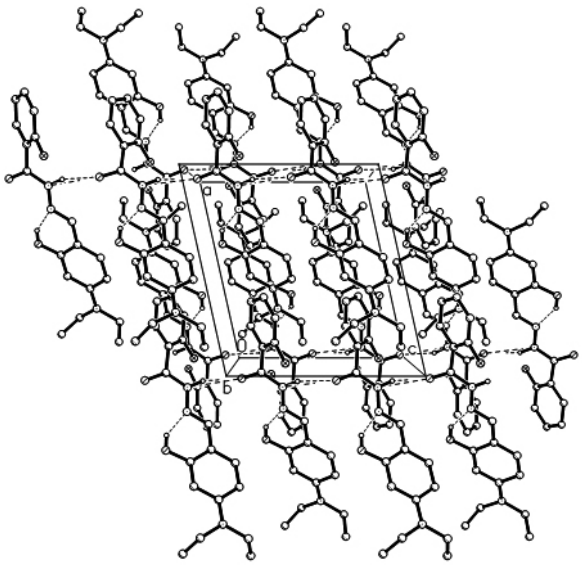

Figure 4 The packing diagram of **2**. Hydrogen bonding interactions are shown as dashed lines.

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