SYNTHESIS, STRUCTURES, AND ANTIBACTERIAL ACTIVITIES OF 3-CHLORO-N'-(2-HYDROXY-5-METHOXYBENZYLIDENE)BENZOHYDRAZIDE AND N'-(2-HYDROXY-5-METHOXYBENZYLIDENE)-3-METHYLBENZOHYDRAZIDE

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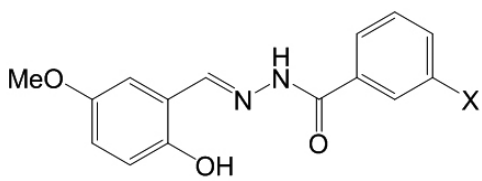
ABSTRACT

By reaction of 5-methoxysalicylaldehyde with 3-chlorobenzohydrazide and 3-methylbenzohydrazide, respectively, two structurally similar hydrazone compounds, 3-chloro-N'-(2-hydroxy-5-methoxybenzylidene)benzohydrazide (**1**) and N'-(2-hydroxy-5-methoxybenzylidene)-3-methylbenzohydrazide (**2**), were synthesized. The compounds were characterized by elemental analyses, infrared and UV-vis spectra, and single crystal X-ray diffraction. The crystal of **1** crystallizes in monoclinic space group $P2_1/n$, with unit cell dimensions a = 5.8872(8) Å, b = 31.649(2) Å, c = 7.6309(9) Å, β = 94.427(2)°, V = 1417.6(3) Å^3, Z = 4, R_1 = 0.0462, wR_2 = 0.1053. The crystal of **2** crystallizes in orthorhombic space group *Pbca*, with unit cell dimensions a = 10.415(1) Å, b = 9.781(1) Å, c = 29.548(2) Å, V = 3010.2(5) Å^3, Z = 8, R_1 = 0.0519, wR_2 = 0.1577. 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: Hydrazone; crystal structure; hydrogen bonds; X-ray crystallography; antibacterial activity; antifungal activity.

INTRODUCTION

Hydrazides and their hydrazone 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 hydrazone derivatives is a consequence of our interests in compounds having potential biological activity. In addition, hydrazone 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 hydrazone compounds, 3-chloro-N'-(2-hydroxy-5-methoxybenzylidene)benzohydrazide (**1**) and N'-(2-hydroxy-5-methoxybenzylidene)-3-methylbenzohydrazide (**2**) (Scheme 1), are reported.



Scheme 1. The hydrazone compounds. **1**: X = Cl; **2**: X = CH_3.

EXPERIMENTAL

Materials and Methods: 2-Hydroxy-5-methoxybenzaldehyde, 3-chlorobenzohydrazide and 3-methylbenzohydrazide 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. ^{1}H NMR spectra were measured with a Bruker AVANCE 300 MHz spectrometer. ^{13}C NMR spectra were measured with an Oxford NMR spectrometer. FT-IR spectra were recorded on a Nicolet 55XC spectrometer. UV-vis spectra were recorded on a Lambda 900 spectrophotometer in methanol. HRMS data was obtained with ESI (electrospray ionization) mode.

*Synthesis of **1**:* To a solution of 2-hydroxy-5-methoxybenzaldehyde (0.152 g, 1.0 mmol) in absolute MeOH (20 mL), 3-chlorobenzohydrazide (0.171 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, 76%. IR (cm^{-1}, KBr pellets): 3445 (br, w), 3215 (sh, m), 3054 (w), 2925 (w), 2843 (w), 1648 (vs), 1561 (s), 1487 (s), 1332 (w), 1264 (s), 1202 (w), 1158 (m), 1078 (w), 1034 (m), 960 (w), 898 (w), 836 (w), 787 (m), 743 (w), 681 (w), 632 (w), 477 (w). UV-vis data (λ, nm; ε, L mol^{-1} cm^{-1}): 293, 12700; 365, 6440. ^{1}H NMR data (300 MHz, $CDCl_3$, δ, ppm): 10.35 (s, 1H), 8.53 (s, 1H), 7.63 (m, 2H), 7.67 (d, 1H), 7.45 (m, 1H), 7.18 (s, 1H), 6.82 (dd, J = 8.5, 2.6 Hz, 1H), 6.62 (d, 1H), 3.74 (s, 3H). ^{13}C NMR data (75 MHz, d_6-DMSO, δ, ppm): 163.0, 152.1, 151.5, 147.7, 136.5, 133.8, 132.3, 131.6, 128.1, 126.2, 119.1, 118.1, 117.2, 112.6, 55.5. Anal. Calcd. (%) for $C_{15}H_{13}ClN_2O_3$: C, 59.1; H, 4.3; N, 9.2. Found (%): C, 59.3; H, 4.4; N, 9.0. HRMS (ESI): m/z calcd for $C_{15}H_{13}ClN_2O_3$ $[M + H]^+$ 305.6631; found: 305.6635.

*Synthesis of **2**:* To a solution of 2-hydroxy-5-methoxybenzaldehyde (0.152 g, 1.0 mmol) in absolute MeOH (20 mL), 3-methylbenzohydrazide (0.150 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, 55%. IR (cm^{-1}, KBr pellets): 3450 (br, w), 3203 (sh, m), 3042 (w), 2955 (w), 2837 (w), 1643 (vs), 1585 (w), 1541 (s), 1493 (m), 1326 (m), 1282 (s), 1208 (m), 1165 (w), 1034 (w), 963 (w), 812 (w), 743 (w), 693 (w), 656 (w), 477 (w). UV-vis data (λ, nm; ε, L mol^{-1} cm^{-1}): 292, 14400; 355, 6590. ^{1}H NMR data (300 MHz, d_6-DMSO, δ, ppm): 10.11 (s, 1H), 8.52 (s, 1H), 7.59 (m, 2H), 7.26 (m, 2H), 7.18 (s, 1H), 6.82 (dd, J = 8.7, 2.7 Hz, 1H), 6.62 (d, 1H), 3.74 (s, 3H), 2.33 (s, 3H). ^{13}C NMR data (75 MHz, d_6-DMSO, δ, ppm): 162.9, 152.1, 151.5, 147.5, 137.8, 132.9, 132.5, 128.4, 128.1, 124.8, 119.0, 118.1, 117.2, 112.3, 55.5, 20.9. Anal. Calcd. (%) for $C_{16}H_{16}N_2O_3$: C, 67.6; H, 5.7; N, 9.9. Found (%): C, 67.4; H, 5.7; N, 9.8. HRMS (ESI): m/z calcd for $C_{16}H_{16}N_2O_3$ $[M + H]^+$ 285.1234; found: 285.1229.

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 Mo *Kα* radiation (λ = 0.71073 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 coordinates refined freely with N–H distances restrained to 0.893(2) Å. The remaining hydrogen atoms were located at their calculated positions. 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.

Table 1 Crystal data for the compounds.

Compound	**1**	**2**
Empirical formula	$C_{15}H_{13}ClN_2O_3$	$C_{16}H_{16}N_2O_3$
Formula weight	304.72	284.3
Crystal shape, color	Block, colorless	Block, colorless
Temperature, K	298(2)	298(2)
Wavelength, Å	0.71073	0.71073
Crystal system	Monoclinic	Orthorhombic
Space group	$P2_1/n$	*Pbca*
Unit cell dimensions		
a, Å	5.8872(8)	10.415(1)
b, Å	31.649(2)	9.781(1)
c, Å	7.6309(9)	29.548(2)
β, °	94.427(2)	
Volume, Å^3	1417.6(3)	3010.2(5)
Z	4	8
Calculated density, g/cm^3	1.428	1.255
Absorption coefficient, mm^{-1}	0.281	0.088
F(000)	632	1200
Crystal size, mm	0.17 × 0.17 × 0.13	0.17 × 0.15 × 0.13
Limiting indices	$-7 \le h \le 7$ $-36 \le k \le 38$ $-9 \le l \le 8$	$-13 \le h \le 13$ $-12 \le k \le 12$ $-35 \le l \le 37$
Reflections collected	12345	21403
Independent reflections [R_{int}]	2626[0.0345]	3161[0.0372]
Observed reflections [$I \ge 2\sigma(I)$]	2026	1984
Data/restraints/parameters	2626/1/195	3161/1/196
Goodness-of-fit on F^2	1.097	1.114
T_{min}	0.9538	0.9852
T_{max}	0.9644	0.9887
Final *R* indices [$I \ge 2\sigma(I)$]	0.0462, 0.1053	0.0519, 0.1577
R indices (all data)	0.0654, 0.1136	0.0962, 0.2096
Largest difference peak and hole, e Å^{-3}	0.186 and -0.214	0.273 and –0.345

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

1			
C(7)–N(1)	1.272(3)	N(1)–N(2)	1.379(2)
N(2)–C(8)	1.339(3)	O(2)–C(8)	1.224(2)
C(7)–N(1)–N(2)	117.07(17)	N(1)–N(2)–C(8)	118.64(16)
O(2)–C(8)–N(2)	122.52(19)		
2			
C(7)–N(1)	1.272(3)	N(1)–N(2)	1.377(3)
N(2)–C(8)	1.351(3)	O(2)–C(8)	1.231(3)
C(7)–N(1)–N(2)	118.81(17)	N(1)–N(2)–C(8)	118.13(18)
O(2)–C(8)–N(2)	120.3(2)		

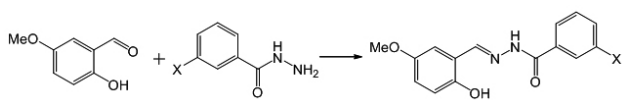
Table 3 Distances (Å) and angles (°) involving hydrogen bonding of the compounds

D–H···*A*	d(*D*–H)	d(H··*A*)	d(*D*···*A*)	Angle(*D*–H···*A*)
1				
O(1)–H(1)···N(1)	0.82	1.91	2.619(2)	144.6
N(2)–H(2)···O(2)$^{\#1}$	0.893(10)	2.013(13)	2.871(2)	161(3)
2				
O(1)–H(1)···N(1)	0.82	1.87	2.593(3)	145.6
N(2)–H(2)···O(2)$^{\#2}$	0.893(10)	1.948(12)	2.820(2)	165(2)

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

RESULTS AND DISCUSSION

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



Scheme 2: The synthesis of the compounds. X = Cl for **1**, X = CH_3 for **2**.

Structure Description of the Compounds: The solid state structures of compounds **1** and **2** determined by X-ray diffraction are shown in Figures 1 and 2, respectively. Selected bond lengths and angles are listed in Table 2. The hydrogen bond geometry is given in Table 3. Compound **1** is isostructural with 3-bromo-*N'*-(2-hydroxy-5-methoxybenzylidene)benzohydrazide.[21] The bonds C8–O2 of 1.224(2) Å in **1** and 1.231(3) Å in **2** have double bond character, whereas the bonds C8–N2 of 1.339(3) Å in **1** and 1.351(3) Å in **2** are typical for single bonds. It is obvious that both C8–O2 and C8–N2 bonds in **1** are shorter than those in **2**. This is caused by the existence of electro-withdrawing group (Cl) in **1**, while electro-donoting group (CH_3) in **2**. The remaining bond lengths in both structures are similar to each other. With the normal deviations, all the bond lengths are in agreement with the values found in analogues compounds.[22,23] The molecules of the compounds are not to be planar. The dihedral angles between the phenyl rings are 8.26(11)º for **1** and 10.80(3)º for **2**. This conformation is stabilized by intramolecular hydrogen bonds between the amide hydrogen atom and the oxygen atom of the phenoxy groups.

In the crystal packing of the compounds, molecules are linked through intermolecular N–H···O hydrogen bonds, to form 1D chains (Figure 3 for **1** and Figure 4 for **2**). Using graph-set analysis to describe patterns in the hydrogen-bond network, we find C_4 chains.[24] The infinite chains are subjected to π···π interactions acting between the aromatic rings in **2**. Here, *Cg*···*Cg*(–*x*, –*y*, 1–*z*) is 3.9074(14) Å, the dihedral angle is 0.00(10)°, the interplanar distance is –3.3344(9) Å and the offset is 2.037 Å.

Infrared and UV-vis Spectra: The broad bands with the maximum at about 3450 cm^{-1} in the spectra of both compounds are due to stretching vibrations of hydroxyl groups. The sharp and medium stretching vibrations at about 3210

cm^{-1} in both compounds indicate the presence of amino groups. Compounds **1** and **2** exhibit strong vibrations of imino bonds at 1648 cm^{-1} and 1643 cm^{-1}, respectively.[25,26] The Ar-O stretching vibration frequencies of hydroxyl groups substituted on the benzene rings are observed at 1264 cm^{-1} for **1** and 1282 cm^{-1} for **2**.

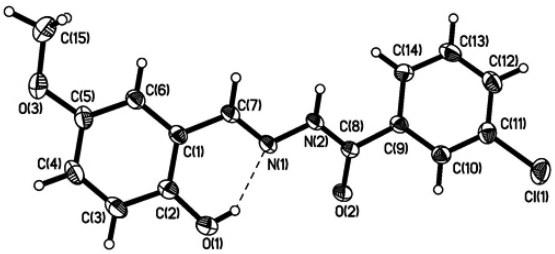


Figure 1 The molecular structure of **1**. The ellipsoids are shown with 30% probability. Intramolecular O–H···N hydrogen bond is shown as a dashed line.

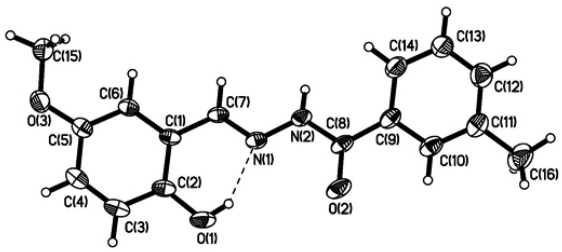


Figure 2 The molecular structure of **2**. The ellipsoids are shown with 30% probability. Intramolecular O–H···N hydrogen bond is shown as a dashed line.

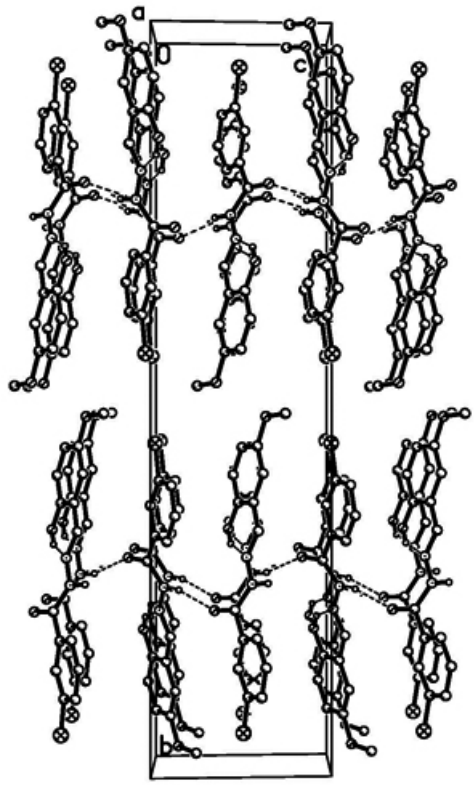


Figure 3 The packing diagram of **1**. Hydrogen bonding interactions are shown as dashed lines.

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

Compound	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas fluorescence*	*Staphylococcus aureus*	*Aspergillus niger*	*Candida albicans*
1	> 50	3.1	3.1	12.5	> 50	> 50
2	> 50	25	12.5	> 50	> 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

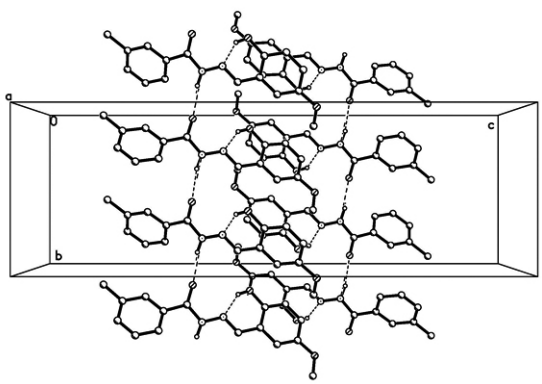


Figure 4 The packing diagram of **2**. Hydrogen bonding interactions are shown as dashed lines.

The compounds have two sets of bands in the UV region. The first at about 290 nm may be assigned to the π→π* transitions. The second set at about 360 nm may be assigned to the *n*→π* transitions.

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 *Pseudomonas fluorescence*, and effective activities against *Staphylococcus aureus*. Compound **2** showed medium activities against *Escherichia coli* and *Pseudomonas fluorescence*, and weak or no activity against *Staphylococcus aureus*. Compound **1** showed stronger activities against *Escherichia coli* and *Pseudomonas fluorescence* than the reference drug Kanamycin. When detailed comparison with the structures and activities of the compounds, we found that the existence of the chloro group in the compound may 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*.

SUPPLEMENTARY MATERIAL

CCDC –1021369 for **1** and 1021370 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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