CRYSTAL STRUCTURES AND ANTIMICROBIAL ACTIVITIES OF *N'*-[2,5-DIHYDROXYBENZYLIDENE]-3,4,5-TRIMETHOXYBENZOHYDRAZIDE, *N'*-[2,4-DICHLOROBENZYLIDENE]-3,4,5-TRIMETHOXYBENZOHYDRAZIDE, AND 2,4-DICHLORO-*N'*-(2-HYDROXY-3-METHOXY-5-NITROBENZYLIDENE)BENZOHYDRAZIDE ETHANOL SOLVATE

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ABSTRACT

Three benzohydrazone compounds, *N'*-[2,5-dihydroxybenzylidene]-3,4,5-trimethoxybenzohydrazide (1), *N'*-[2,4-dichlorobenzylidene]-3,4,5-trimethoxybenzohydrazide (2), and 2,4-dichloro-*N'*-(2-hydroxy-3-methoxy-5-nitrobenzylidene)benzohydrazide ethanol solvate (3), have been synthesized and structurally characterized by elemental analysis and X-ray single crystal structure determination. Compound (1) crystallizes in the orthorhombic space group *Pbca*, with unit cell dimensions $a = 15.193(2)$, $b = 11.086(2)$, $c = 20.203(3)$ Å, $V = 3402.8(8)$ Å³, $Z = 8$. Compound (2) crystallizes in the monoclinic space group *P2₁/c*, with unit cell dimensions $a = 17.127(3)$, $b = 13.326(3)$, $c = 7.984(2)$ Å, $\beta = 99.689(2)^\circ$, $V = 1796.2(6)$ Å³, $Z = 4$. Compound (3) crystallizes in the monoclinic space group *P2₁/n*, with unit cell dimensions $a = 12.919(3)$, $b = 10.068(2)$, $c = 15.205(4)$ Å, $\beta = 98.288(2)^\circ$, $V = 1957.0(7)$ Å³, $Z = 4$. X-ray crystallography reveals that each compound has an *E* configuration with respect to the C=N double bond and C–N single bond. The molecular structures of the compounds are stabilized by hydrogen bonds and weak $\pi \cdots \pi$ stacking interactions. The antimicrobial activities against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus magaterium* were studied. The electron-withdrawing groups, such as Cl and NO₂, are preferred groups for the antimicrobial material. Among the compounds, (3) showed the most effective activity against *Escherichia coli*, which is even stronger than the controlled drug Tetracycline.

Keywords: Synthesis; crystal structure; benzohydrazone; hydrogen bonds; antimicrobial activity

INTRODUCTION

Benzohydrazone compounds are readily synthesized by the reaction of aldehydes with benzohydrazides.¹⁻³ The chemistry of these compounds has been intensively investigated in recent years. The reasons are manifold: the versatile biological activities, such as antibacterial and antitubercular,⁴⁻⁶ the coordination abilities to most metal ions,^{7,8} and promising properties for analytical applications.^{9,10} As is well known that properties of any material are based on their structures, so, in recent years, the crystal structures of benzohydrazone compounds have attracted much attention.^{1,11,12} Recently, Zhang and co-workers have reported the structures and antimicrobial activity of a series of halo-substituted aroylhydrazones.¹³ As a continuation of the structural and antimicrobial investigation of such compounds, in this paper, three new benzohydrazone compounds, *N'*-[2,5-dihydroxybenzylidene]-3,4,5-trimethoxybenzohydrazide (1), *N'*-[2,4-dichlorobenzylidene]-3,4,5-trimethoxybenzohydrazide (2), and 2,4-dichloro-*N'*-(2-hydroxy-3-methoxy-5-nitrobenzylidene)benzohydrazide ethanol solvate (3), have been synthesized and structural characterized. The antimicrobial activities of the compounds against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus magaterium* have been studied. Compound (3) shows the most effective activity against *Escherichia coli*.

EXPERIMENTAL

Materials and Methods: 3,4,5-Trimethoxybenzohydrazide, 2,4-dichlorobenzohydrazide, 2,5-dihydroxybenzaldehyde, 2,4-dichlorobenzaldehyde, and 2-hydroxy-3-methoxy-5-nitrobenzaldehyde were analytical pure grade from Aldrich Chemical Co., Milwaukee, Wisconsin, and used without further purification. Other reagents were also analytical pure grade and obtained from Beijing Reagent Factory, used without further purification. C, H, N analyses were carried out using a Perkin-Elmer model 240 analyzer.

Synthesis of the compounds: Equimolar quantities (0.1 mmol each) of aldehydes and benzohydrazide were dissolved in a sufficient volume of ethanol and the mixture was refluxed for 2 h. The mixture was then allowed to cool, poured into a beaker and kept aside for evaporation. The resulting crude sample was recrystallized twice from ethanol. X-ray quality single crystals were formed by slow evaporation of the solution containing the compound in air.

For (1): 3,4,5-Trimethoxybenzohydrazide and 2,5-dihydroxybenzaldehyde. Yield: 27.0 mg (78%). Analysis: Calcd. (%) for C₁₇H₁₈N₂O₆: C 59.0, H 5.2, N 8.1. Found: C 58.8, H 5.3, N 8.0. For (2): 3,4,5-Trimethoxybenzohydrazide and 2,4-dichlorobenzaldehyde. Yield: 25.0 mg (65%). Analysis: Calcd. (%) for C₁₇H₁₆Cl₂N₂O₄: C 53.3, H 4.2, N 7.3. Found: C 53.4, H 4.2, N 7.4. For (3): 2,4-Dichlorobenzohydrazide and 2-hydroxy-3-methoxy-5-nitrobenzaldehyde. Yield: 32.0 mg (74%). Analysis: Calcd. (%) for C₁₇H₁₇Cl₂N₃O₆: C 47.5, H 4.0, N 9.8. Found: C 47.3, H 3.9, N 9.6.

X-ray Crystallography: X-ray single crystal structure determination was carried out at 298(2) K on a Bruker Smart 1000 CCD area diffractometer equipped with a graphite-monochromatic Mo *K* α radiation ($\lambda = 0.71073$ Å) for data collection. The unit cell dimensions were obtained with the least-squares refinements and the structures were solved by direct methods with SHELXTL-97 package.¹⁴ The final refinements were performed by full-matrix least-squares methods with anisotropic thermal parameters for the non-hydrogen atoms on *F*². The amino H atoms in the compounds were located in difference Fourier maps and refined isotropically, with the U_{iso} (H) values fixed at 0.08 Å², and with N–H distance restrained to 0.90(1) Å. Other H atoms were placed in the calculated positions and constrained to ride on their parent atoms. Multi-scan absorption correction was applied by using the SADABS program.¹⁵

RESULTS AND DISCUSSION

The compounds were crystallized as well-shaped single crystals, soluble in the common polar organic solvents, such as methanol, ethanol, acetonitrile, and DMF, insoluble in water. The crystallographic data for the compounds are summarized in Table 1.

Table 1: Crystallographic and experimental data for the compounds.

Compound	(1)	(2)	(3)
Gross formula	C ₁₇ H ₁₈ N ₂ O ₆	C ₁₇ H ₁₆ Cl ₂ N ₂ O ₄	C ₁₇ H ₁₇ Cl ₂ N ₃ O ₆
<i>M</i>	346.3	383.2	430.2
Crystal system	orthorhombic	monoclinic	monoclinic
Space group	<i>Pbca</i>	<i>P2₁/c</i>	<i>P2₁/n</i>
<i>T</i> , K	298(2)	298(2)	298(2)
<i>a</i> , Å	15.193(2)	17.127(3)	12.919(3)
<i>b</i> , Å	11.086(2)	13.326(3)	10.068(2)
<i>c</i> , Å	20.203(3)	7.984(2)	15.205(4)
β , °		99.689(2)	98.288(2)
<i>V</i> , Å ³	3402.8(8)	1796.2(6)	1957.0(7)
<i>Z</i>	8	4	4
<i>D_c</i> , g cm ⁻³	1.352	1.417	1.460
Crystal dimensions, mm ³	0.40×0.31×0.07	0.13×0.10×0.10	0.38×0.19×0.19
μ , mm ⁻¹	0.104	0.385	0.371
Radiation λ , Å	0.71073	0.71073	0.71073
<i>T_{min}</i> / <i>T_{max}</i>	0.960/0.993	0.952/0.962	0.871/0.933
Reflections measured	19344	10640	11509
Total no. of unique data	3709	3894	4248
No. of observed data, <i>I</i> > 2σ(<i>I</i>)	2201	2055	2656
No. of variables	234	233	260
No. of restraints	1	1	1
<i>R_{int}</i>	0.0514	0.0451	0.0289
<i>R₁</i> , <i>wR₂</i> [<i>I</i> ³ 2σ(<i>I</i>)] ^a	0.0453, 0.1047	0.0489, 0.1063	0.0431, 0.1056
<i>R₁</i> , <i>wR₂</i> (all data) ^a	0.0893, 0.1240	0.1093, 0.1299	0.0762, 0.1228

$$^a R_1 = \frac{\sum ||F_o| - |F_c||}{\sum |F_o|}, wR_2 = [\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2]^{1/2}$$

Crystal Structure Description of the Compounds: The molecular structures of the compounds (1), (2), and (3) are shown in Figs. 1, 2, and 3, respectively. Selected bond lengths and bond angles are listed in Table 2. There is only one benzohydrazone molecule for (1) and (2). Compound (3) contains a benzohydrazone molecule and an ethanol molecule of crystallization. The benzohydrazone molecules in the compounds have *E* configurations with respect to the C=N double bonds and C–N single bonds. In each compound, the benzohydrazone molecule is twisted, with the dihedral angle between the two benzene rings of 28.5(3)° for (1), 40.9(3)° for (2), and 48.8(3)° for (3). All the bond lengths in the compounds are within normal ranges,¹⁶ and comparable to each other. The C8–N2 bond length of 1.345(2) Å in (1), C8–N2 bond length of 1.355(3) Å in (2), and C9–N2 bond length of 1.349(3) Å in (3) are relatively short, suggesting delocalization in the acetohydrazide systems. In

the benzohydrazone molecules of (1) and (3), the intramolecular O1–H1⋯N1 hydrogen bonds make *S*(6) ring motifs.¹⁷

Hydrogen bonding interactions of the compounds are listed in Table 3. In the crystal structure of (1), the benzohydrazone molecules are linked through intermolecular O–H⋯O and N–H⋯O hydrogen bonds, to form layers parallel to the *ab* plane (Fig. 4). In the crystal structure of (2), the benzohydrazone molecules are linked through intermolecular N–H⋯N and N–H⋯O hydrogen bonds, to form chains running along the *c* axis (Fig. 5). In the crystal structure of (3), the benzohydrazone molecules and ethanol molecules are linked through intermolecular O–H⋯O hydrogen bonds, to form a 3D network (Fig. 6). In addition, weak $\pi \cdots \pi$ stacking interactions among the aromatic rings are also observed in the compounds (Table 4).

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

(1)		(2)		(3)	
Bond lengths					
O1–C2	1.363(2)	C7–N1	1.272(3)	O1–C2	1.342(2)
C7–N1	1.279(2)	N1–N2	1.376(3)	C8–N1	1.275(3)
N1–N2	1.383(2)	N2–C8	1.355(3)	N1–N2	1.374(2)
N2–C8	1.345(2)	C8–O1	1.226(3)	N2–C9	1.349(3)
C8–O3	1.232(2)			C9–O5	1.220(2)
Bond angles					
C7–N1–N2	115.6(2)	C7–N1–N2	115.0(2)	C8–N1–N2	118.3(2)
N1–N2–C8	119.2(2)	N1–N2–C8	118.7(2)	N1–N2–C9	117.9(2)
N2–C8–O3	122.6(2)	N2–C8–O1	123.0(2)	N2–C9–O5	123.4(2)
N2–C8–C9	115.8(2)	N2–C8–C9	113.8(2)	N2–C9–C10	113.5(2)

Table 3: Hydrogen-bond geometry of the compounds.

$D-H\cdots A$	$d(D-H)$ (Å)	$d(H\cdots A)$ (Å)	$d(D\cdots A)$ (Å)	Angle($D-H\cdots A$) (°)
(1)				
O1-H1 $\cdots$ N1	0.82	1.96	2.677(2)	145
O2-H2A $\cdots$ O3 ⁱ	0.82	1.88	2.663(2)	160
N2-H2 $\cdots$ O2 ⁱⁱ	0.90(1)	2.23(1)	3.127(2)	172(2)
(2)				
N2-H2 $\cdots$ N1 ⁱⁱⁱ	0.90(1)	2.68(2)	3.340(3)	131(2)
N2-H2 $\cdots$ O1 ⁱⁱⁱ	0.90(1)	2.05(2)	2.906(3)	160(3)
(3)				
O1-H1 $\cdots$ N1	0.82	1.86	2.577(2)	145
O6-H6 $\cdots$ O5 ^{iv}	0.82	2.41	2.980(3)	127
O6-H6 $\cdots$ O3 ^v	0.82	2.53	3.127(3)	131
N2-H2 $\cdots$ O6	0.90(1)	1.99(1)	2.872(2)	170(2)

Symmetry codes: (i) $1/2 + x, y, 1/2 - z$; (ii) $1 - x, -1/2 + y, 1/2 - z$; (iii) $x, 3/2 - y, -1/2 + z$; (iv) $3/2 - x, 1/2 + y, 1/2 - z$; (v) $1 - x, 1 - y, -z$.

Table 4: $\pi\cdots\pi$ interactions (Å).

(1)			
$Cg1\cdots Cg2^{\#1}$	4.6868		
(2)			
$Cg3\cdots Cg3^{\#2}$	3.6305	$Cg3\cdots Cg4^{\#3}$	4.7407
$Cg4\cdots Cg4^{\#4}$	4.9040		
(3)			
$Cg5\cdots Cg5^{\#5}$	4.7016	$Cg5\cdots Cg6^{\#6}$	4.4537

$Cg1$, $Cg2$, $Cg3$, $Cg4$, $Cg5$, and $Cg6$ are the centroids of the C1–C6 (1), C9–C14 (1), C1–C6 (2), C9–C14 (2), C1–C6 (3), C10–C15 (3), respectively. Symmetry codes: #1) $1/2 - x, 1/2 + y, z$; #2) $-x, -y, -z$; #3) $x, 1/2 - y, -1/2 + z$; #4) $1 - x, 1 - y, 1 - z$; #5) $1 - x, -y, -z$.

Fig. 1 Molecular structure of (1) with 30% probability thermal ellipsoids. An intramolecular hydrogen bond is shown as a dashed line. **Fig. 2** Molecular structure of (2) with 30% probability thermal ellipsoids. **Fig. 3** Molecular structure of (3) with 30% probability thermal ellipsoids. Hydrogen bonds are shown as dashed lines. **Fig. 4** Molecular packing of (1), viewed along the a axis. Hydrogen bonds are shown as dashed lines.

Fig. 5 Molecular packing of (2), viewed along the *b* axis. Hydrogen bonds are shown as dashed lines.

Fig. 6 Molecular packing of (3), viewed along the *a* axis. Hydrogen bonds are shown as dashed lines.

Antimicrobial activity: Qualitative determination of antimicrobial activities of the compounds was done using the disk diffusion method.^[18,19] The results are summarized in Table 5. Compound (1) showed weak activities against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus magaterium*, and no activity against *Bacillus subtilis*. Compound (2) showed weak activities against *Bacillus subtilis* and *Staphylococcus aureus*, and moderate activities against *Escherichia coli* and *Bacillus magaterium*. Compound (3) showed moderate activities against *Bacillus subtilis* and *Staphylococcus aureus*, and strong activities against *Escherichia coli* and *Bacillus magaterium*. It can be seen that the activities agree well with the general order (3) > (2) > (1). Detailed investigation of the structures and activities reveals that the electron-withdrawing groups, such as Cl and NO₂, are preferred groups for the antimicrobial material, which is in accordance with that reported in the literature^[13]. Among the compounds, (3) showed the most effective activity against *Escherichia coli*, which is even stronger than the controlled drug Tetracycline. The strong antimicrobial activities of (3) may be caused by the existence of Cl substitute groups, which is considered to be a functional groups for antimicrobial processes.^[20] The remaining results are lower than Tetracycline, which may not deserve further investigation.

Table 5. Antimicrobial activities of the compounds (inhibitory ratio).

Compound	<i>Bacillus subtilis</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus magaterium</i>
(1)	0	7%	10%	13%
(2)	15%	45%	16%	54%
(3)	28%	73%	21%	82%
Tetracycline	57%	62%	33%	95%

Supplementary material

Crystallographic data for the compounds have been deposited with the Cambridge Crystallographic Data Centre, CCDC reference numbers 879671 for (1), 879672 for (2), and 879673 for (3). This information may be obtained free of charge from: the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk; website: <http://www.ccdc.cam.ac.uk>).

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