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STRUCTURE AND BIOLOGICAL EVALUATION OF (*E*)-5-BROMO-2-METHOXY-4-((PHENYLIMINO)METHYL)PHENOL DERIVATIVES AS ANTIBACTERIAL AGENTS

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Three (*E*)-5-bromo-2-methoxy-4-((phenylimino)methyl)phenol derivatives (**1—3**) are synthesized and characterized by elemental analysis and single-crystal X-ray diffraction. The antibacterial activities of compounds **1—3** against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *and Staphylococcus aureus* are evaluated by the MTT method.

K e y w o r d s: vanillin derivatives, Schiff base, crystal structure, antibacterial activity.

INTRODUCTION

Although several classes of antibacterial agents are presently available, resistance to these drugs constantly emerges in most of the pathogenic bacteria. In order to prevent this serious medical problem, the elaboration of new types of antibacterial agents is a very important task [1]. Apart of its flavor qualities, vanillin exhibits the antimicrobial potential and has been used as a natural food preservative [2]. Schiff bases, named after Hugo Schiff [3], have also been shown to exhibit a broad range of biological activities, including antifungal, antibacterial, anti-malarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic properties [4—12].

The molecular hybridization approach is one of the most valuable structural modification tools useful for the discovery of ligands and prototypes presenting either optimized affinity for one bioreceptor or the ability to modulate more than one bioreceptor associated with the target disease [13]. The growing efforts to discover hybrid drugs resulting from a combination of pharmacophoric moieties of different known lead. Hence, the impressive results of vanillin derivatives and various phenylamine fueled our interest in combining two scaffolds and exploring their possibilities as antibacterial agents. The crystal structures of compounds (**1—3**) were determined.

EXPERIMENTAL

All chemicals and reagents used in the current study were of analytical grade. The reactions were monitored by thin layer chromatography (TLC) on Merck pre-coated silica GF254 plates. Melting points (uncorrected) were determined on a XT4MP apparatus (Taike Corp., Beijing, China). ESI mass spectra were obtained on a Mariner System 5304 mass spectrometer, and ^{1}H NMR spectra were collected on a Bruker DPX300 spectrometer at room temperature with TMS and solvent signals allotted as the internal standards. Chemical shifts are reported in ppm (δ). Elemental analyses were performed on a CHN-O-Rapid instrument, and were within ±0.4 % of the theoretical values.

General synthesis method of (*E*)-5-bromo-2-methoxy-4-((phenylimino)methyl)phenol derivatives (1—3). Vanillin was suspended in methylene chloride, and then acetic anhydride and pyridine were added. The resultant solution was stirred at room temperature for 18 h. Water was added to

the reaction mixture, and ethyl acetate was then added. The organic layer was washed with 1 N HCl, a saturated sodium hydrogen carbonate aqueous solution, and saturated brine, and was dried with anhydrous sodium sulfate. The organic layer was filtered, and concentrated under reduced pressure to give the pure compound **a**.

The compound **a** and potassium bromide were suspended in water, and then bromine was added at 0℃. The solution was stirred at room temperature for 15 h. The resultant solid was filtered, washed with water, and dried to give the pure compound **b**.

HCL was added to the compound **b**, and stirred at 90 °C for 4 h. After cooling in the air, the resultant solid was filtered, washed with water, and dried to give the compound **c**.

The equivocal compound **c** and substituted aromatic amine in methanol or acetonitrile was stirred for 4—6 h at room temperature, and then the reaction mixture was concentrated under reduced pressure, recrystallized from ethanol to give vanillin derivatives **1—3**.

(*E*)-5-Bromo-2-methoxy-4-((*p*-tolylimino)methyl)phenol (1). Mp: 134—136 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 1.54 (s, 6H), 6.47 (m, 1H), 6.96—6.97 (d, J = 3.0 Hz, 1H), 7.11—7.13 (m, 3H), 7.23—7.24 (d, J = 3.0 Hz, 1H), 8.34 (s, 1H), 9.74 (s, 1H). MS (ESI): 320 ($C_{15}H_{15}BrNO_2$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{14}BrNO_2$: C, 56.27; H, 4.41; N, 4.37; Found: C, 56.31; H, 4.23; N, 4.19.

(*E*)-5-Bromo-2-methoxy-4-((*o*-tolylimino)methyl)phenol (2). Mp: 119—120 °C. 1H NMR (300 MHz, $CDCl_3$): 1.56 (s, 6H), 6.95—6.96 (d, J = 3.0 Hz, 1H), 7.17—7.20 (m, 3H), 7.22—7.23 (m, 1H), 7.33—7.35 (m, 1H), 9.97 (s, 1H), 10.06 (s, 1H). MS (ESI): 320 ($C_{15}H_{15}BrNO_2$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{14}BrNO_2$: C, 56.27; H, 4.41; N, 4.37; Found: C, 56.11; H, 4.24; N, 4.52.

(*E*)-5-Bromo-4-((3,4-dimethylphenylimino)methyl)-2-methoxyphenol (3). Mp: 139—141 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 1.23 (s, 6H), 2.08 (m, 3H), 7.01—7.03 (m, 1H), 7.11—7.12 (m, 3H), 7.19—7.21 (d, J = 9.0 Hz, 1H), 8.11 (s, 1H), 8.78 (s, 1H). MS (ESI): 334 ($C_{16}H_{17}BrNO_2$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{16}BrNO_2$: C, 57.50; H, 4.83; N, 4.19; Found: C, 57.71; H, 4.65; N, 3.98.

Crystallographic data collection and structure determination. Single crystals of **1** (0.32× ×0.27×0.25 mm), **2** (0.30×0.22×0.28 mm), and **3** (0.38×0.26×0.25 mm) were mounted on a *D*-8 venture diffractometer with graphite-monochromated MoK_α (λ = 0.71073 Å) radiation. The SAINT program was used for the integration of the diffraction profiles and the SADABS program was used for semi-empirical absorption corrections. The structure was solved by direct methods using the SHELXS-97 program [14] and refined by full-matrix least-squares techniques on F^2 with SHELXL-97 [15].

For **1**, a total of 15248 reflections were collected, out of which 5238 were unique with R_{int} = 0.024, and 4218 observed reflections with $I > 2\sigma(I)$ were used in the succeeding structure calculations. The final cycle of the refinement of full matrix least-squares was converged to R = 0.0355 and wR = 0.0970. The highest and lowest residual peaks in the final difference Fourier map are 0.75 e/Å^3 and –0.42 e/Å^3, respectively.

For **2**, a total of 13871 reflections were collected, out of which 5183 were unique with R_{int} = 0.033, and 4157 observed reflections with $I > 2\sigma(I)$ were used in the succeeding structure calculations. The final cycle of the refinement of full matrix least-squares was converged to R = 0.0366 and wR = 0.0994. The highest and lowest residual peaks in the final difference Fourier map are 0.45 e/Å^3 and –0.54 e/Å^3, respectively.

For **3**, a total of 30996 reflections were collected, out of which 5787 were unique with R_{int} = 0.048, and 4331 observed reflections with $I > 2\sigma(I)$ were used in the succeeding structure calculations. The final cycle of the refinement of full matrix least-squares was converged to R = 0.0492 and wR = 0.1338. The highest and lowest residual peaks in the final difference Fourier map are 0.56 e/Å^3 and –0.51 e/Å^3, respectively.

Antibacterial studies. The antibacterial activity of the synthesized compounds was tested against *B. subtilis, E. coli, P. aeruginosa,* and *S. aureus* using the MTT method [16]. The optical density (OD) was measured with a microplate reader at 550 nm.

Binding model of compounds into the FtsZ structure. FtsZ, considered to be the most critical component of the division machinery, is an essential protein for bacterial viability [17—19] and it is a

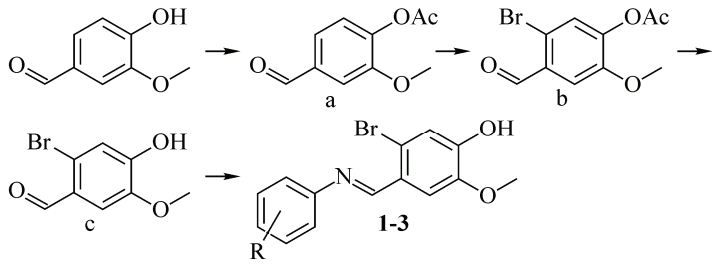
highly conserved and potentially broad-spectrum antibacterial target; it does have structural and functional homology suggesting that FtsZ may also be amenable to inhibitor development. Therefore, we displayed the binding model of compound **1** and FtsZ. Automated docking studies were carried out using Discovery Studio (version 3.1) as implemented through the graphical user interface DS-CDocker protocol.

The three-dimensional structures of the aforementioned compounds were constructed using the Chem. 3D ultra 11.0 software (Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2009)). Then they were energetically minimized using MOPAC with 100 iterations and a minimum RMS gradient of 0.10. The Gasteiger-Hückel charges of ligands were assigned. The crystal structures of the FtsZ protein (PDB code: 2VAM) complex were retrieved from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/home/home.do). All bound water molecules and ligands were eliminated from the protein and the polar hydrogen atoms and the Kollman-united charges were added to the proteins.

RESULTS AND DISCUSSION

Crystal structures of compounds 1—3. Crystals of three compounds were obtained from a methanol solution. Figures show a perspective view of the monomeric unit with the atomic numbering scheme, and the spatial arrangement that is affected by intermolecular hydrogen bonds. The important bond distances and bond angles are showed in Table 1. The hydrogen bond lengths and bond angles are given in Table 2.

All three structures have two benzene rings, but they have a great difference in the dihedral angles between the two phenyl groups. The dihedral angle of compound **3** (Fig. 1) is 6.6(3)°, while the



Scheme 1. Synthesis route of title compounds **1—3**

T a b l e 1

Important bond lengths (Å) *and bond angles* (deg.) *for compounds* **1—3**

Compound **1**		Compound **2**		Compound **3**	
Bond	Dist	Bond	Dist	Bond	Dist
Br(2)—C(20)	1.899(3)	Br(2)—C(20)	1.903(3)	Br(1)—C(1)	1.908(6)
O(1)—C(16)	1.422(4)	O(4)—C(17)	1.358(3)	O(3)—C(5)	1.364(6)
O(1)—C(17)	1.358(3)	O(4)—C(22)	1.424(4)	O(3)—C(27)	1.401(7)
O(2)—C(22)	1.353(4)	O(3)—C(16)	1.348(3)	O(4)—C(11)	1.344(7)
N(1)—C(23)	1.270(4)	N(1)—C(23)	1.264(4)	N(2)—C(8)	1.270(6)
N(1)—C(24)	1.423(4)	N(1)—C(24)	1.421(4)	N(2)—C(16)	1.418(7)
Angle	(deg.)	Angle	(deg.)	Angle	(deg.)
C(23)—N(1)—C(24)	118.9(3)	C(23)—N(1)—C(24)	120.2(3)	C(8)—N(2)—C(16)	119.7(5)
Br(2)—C(20)—C(19)	120.9(2)	Br(2)—C(20)—C(19)	120.6(2)	Br(1)—C(1)—C(10)	117.1(5)
C(17)—O(1)—C(16)	117.2(3)	C(17)—O(4)—C(22)	117.7(2)	C(5)—O(3)—C(27)	118.5(5)

T a b l e 2

Hydrogen bond lengths (Å) *and bond angles* (deg.) *for compounds* **1—3**

Compounds		D—H…A	*d*(D—H)	*d*(H…A)	*d*(D…A)	∠DHA
1	Intra	O(2)—H(2)…O(1)	0.82	2.22	2.670(3)	115
		O(4)—H(4)…O(3)	0.82	2.27	2.683(3)	111
		C(7)—H(7A)…Br(1)	0.93	2.74	3.190(3)	111
		C(23)—H(23A)…Br(2)	0.93	2.81	3.189(3)	106
	Inter	C(5)—H(5A)…O(2)	0.93	2.44	3.358(4)	170
		C(18)—H(18A)…O(4)	0.93	2.54	3.161(3)	124
		O(4)—H(4)…N(1)	0.82	2.23	2.973(3)	152
		O(2)—H(2)…N(2)	0.82	2.21	2.943(3)	148
2	Intra	O(3)—H(3)…O(4)	0.82	2.23	2.675(3)	114
		O(2)—H(2)…O(1)	0.82	2.28	2.686(3)	111
		C(7)—H(7A)…Br(1)	0.93	2.75	3.206(3)	111
		C(23)—H(23A)…Br(2)	0.93	2.87	3.194(3)	102
		C(30)—H(30A)…N(1)	0.96	2.40	2.857(5)	109
	Inter	O(2)—H(2)…N(1)	0.82	2.14	2.897(3)	154
		C(18)—H(18A)…O(2)	0.93	2.57	3.039(3)	112
		O(3)—H(3)…N(2)	0.82	2.16	2.907(3)	152
		C(6)—H(6A)…O(3)	0.93	2.33	3.251(4)	170
3	Intra	O(2)—H(2)…O(1)	0.82	2.25	2.674(5)	112
		O(4)—H(4)…O(3)	0.82	2.24	2.680(6)	114
		C(8)—H(8)…Br(1)	0.93	2.80	3.154(5)	104
		C(15)—H(15)…Br(2)	0.93	2.82	3.185(6)	104
	Inter	O(4)—H(4)…N(1)	0.82	2.12	2.846(6)	147
		O(2)—H(2)…N(2)	0.82	2.06	2.803(6)	151
		C(10)—H(10)…O(2)	0.93	2.42	3.342(7)	169
		C(6)—H(6)…O(4)	0.93	2.43	3.358(7)	178

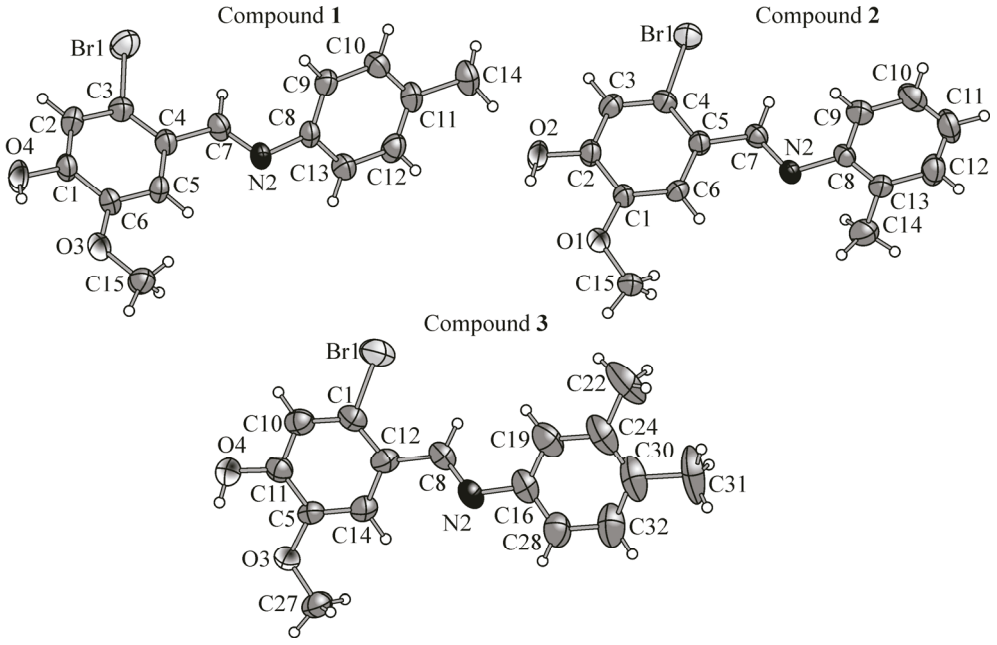


Fig. 1. Molecular structures of the title compounds with atomic numbering scheme

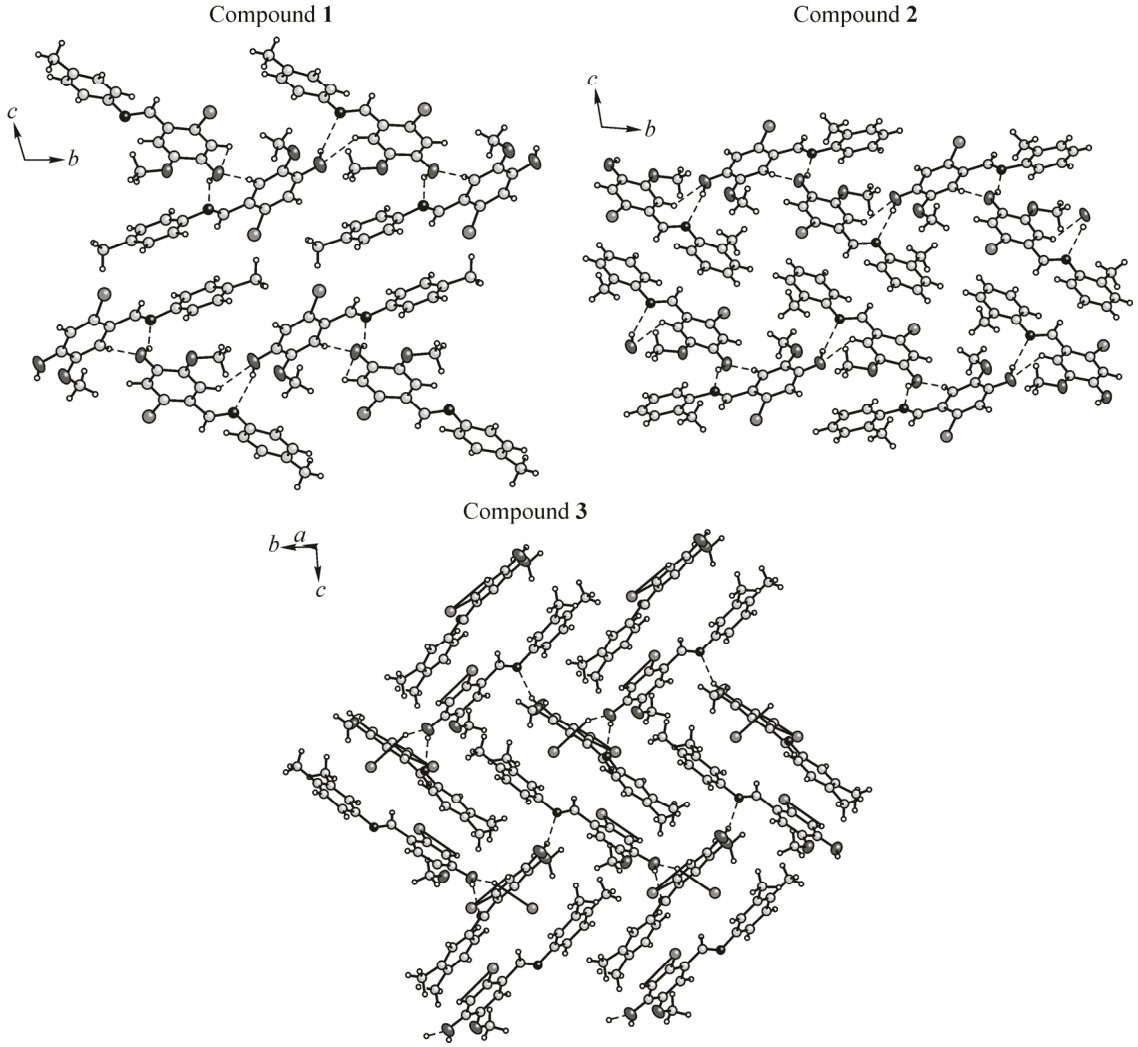


Fig. 2. Crystal packing of the title compounds

other two are close to 50°. The difference between the three structures is that they have different substituents at the second phenyl ring. The ranges of the Br—C bond length are 1.899(3) ~ 1.913(6)Å, which is consistent with the reported lengths, and the O—C bond length is also consistent with the normal lengths. With respect to the regularity of these values, the N—C bond lengths may be slightly longer or shorter than the reported lengths. Moreover, all the structures around the nitrogen atom are almost planar, thus, the differences between the dihedral angles are due to the torsion of the phenyl rings. Furthermore, we also showed the crystal packing of the title compounds in the Fig. 2. We can clearly observe that many molecules are regularly combined into the spatial structure due to the presence of several hydrogen bonds. All the intermolecular hydrogen bonds happened to be H…O and H…N, with O or N atoms being the acceptors. The spatial structure of compounds **1** and **2** are similar, however, the spatial chains of compound **3** are connected to each other by H-bonds and arranged particularity in a helix.

Antibacterial activities. MICs of compounds **1—3** against these bacterial strains are tested by the MTT method. Based on the data obtained, we found that compound **1** exhibits better inhibitory activities (MICs: 1.56—6.25 μg/ml) than **2** (MICs: 6.25—12.5 μg/mL) and **3** (MICs > 25 μg/ml), and it can be a potential antibacterial agent.

Experimental protocol of the docking study. Molecular docking of the synthesized compounds and FtsZ was performed on the binding model based on the FtsZ protein complex structure (2VAM.

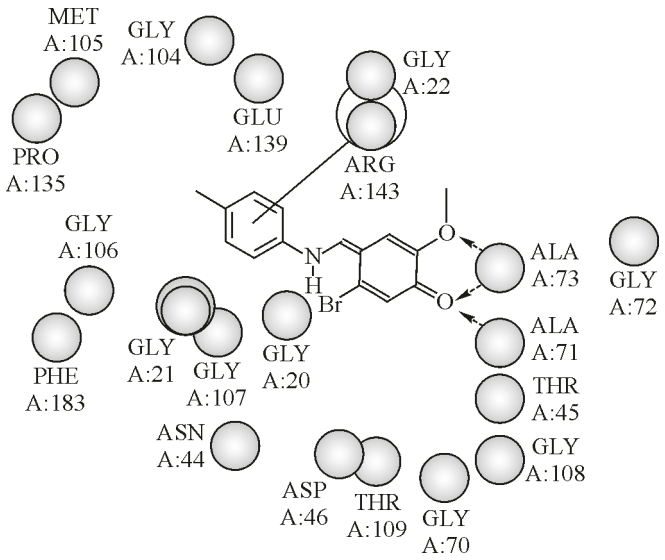


Fig. 3. 2D molecular docking modeling of compound **1** with 2VAM (*a*),
3D molecular docking modeling of compound **1** with 2VAM (*b*)

pdb). The binding model of compound **1** and FtsZ was depicted in Fig. 3. In the binding model, compound **1** was nicely bound to FtsZ by three interaction bonds. Moreover, the π cation interactions existed between the benzene ring and Arg 143 amino acids.

CONCLUSIONS

The present paper reports the synthesis and crystal structures of three new (*E*)-5-bromo-2-methoxy-4-((phenylimino)methyl)phenol derivatives and the study of their antibacterial activities. Compound **1** exhibited the best inhibitory activity and can be a potential antibacterial agent.

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