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Antibacterial Activities of 4-Substituted-2-[(*E*)-{(*1S,2R*)/(*1R,2S*)-1-Hydroxy-1-Phenylpropan-2-Ylimino}Methyl]Phenol

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A series of norephedrine-based Schiff bases (1a–6a and 1b–6b) were synthesized by reacting substituted salicylaldehydes with *d*-norephedrine or *l*-norephedrine. The structure of these compounds was confirmed by elemental analyses and spectroscopic techniques. The molecular structures of 5a and 6a have been determined by X-ray crystallography, which revealed that the compounds are in the oxoamino form, with bent intramolecular N-H···O (N···O ≈ 2.58 Å) hydrogen bonds and that they are associated in dimers bridged by linear intermolecular O-H···O (O···O ≈ 2.69 Å) hydrogen bonds. The density functional theory calculations on 5a confirmed that the oxoamino form is more stable than the phenolimino form by 12.2 kcal/mol. All the compounds were evaluated for their antibacterial activity using resazurin dye as indicator by twofold dilution method against four bacteria namely, *Bacillus subtilis* (NCIM2718), *Staphylococcus aureus* (NCIM5021), *Escherichia coli* (NCIM2931), and *Proteus vulgaris* (NCIM2813).

Key words: antibacterial studies, norephedrine, Schiff base, spectral studies

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Schiff bases are known therapeutical agents. They are intermediates in various therapeutically active chemical entities. The antitubercular activity of D-mannitol-based Schiff bases against *Mycobacterium tuberculosis* $H_{37}Rv$ using alamar blue susceptibility test has been reported (1). Schiff and Mannich bases of isatin derivatives have been synthesized and their antibacterial (against 26 of pathogenic bacteria), antifungal (against eight pathogenic fungi), and anti-HIV activity (replication of HIV-1 (IIIB) in MT-4 cells) have been reported (2). Schiff bases also exhibit antiinflammatory, antiproliferative, and antipyretic activities (3–5). Various references explain the importance of imine group in bioactive molecules (6–14). In the present study, Schiff bases have been synthesized from norephedrine and substituted salicylaldehydes. Earlier *l*-norephedrine was used in the synthesis of oxazaborolidines, which exhibited antibacterial activity against *Streptococcus mutans*, an organism associated with the tooth decay (8). Virtual screening for quorum sensing inhibitors which included ephedrine to eradicate, the biofilm formed by *Pseudomonas aeruginosa* has been performed (15). The 5-chloro salicylaldehyde-based Schiff bases were also evaluated for their antibacterial activity against four bacteria and three of the fungi (16). We herein report the synthesis of 4-substituted-2-[(*E*)-{[(*1S,2R*)/(*1R,2S*)-1-hydroxy-1-phenylpropan-2-ylimino}methyl]phenols (Schiff bases) (Table 1) from *d*-norephedrine/*l*-norephedrine and substituted salicylaldehyde, and their antibacterial activity against two Gram-positive and two Gram-negative bacteria.

Materials and Methods

All the chemicals used were of analytical grade. Solvents were purified and dried according to standard procedure (17). Salicylaldehyde was purchased from Loba Chemie and was used after double distillation. The 2-hydroxy-5-methylbenzaldehyde, 2-hydroxy-5-methoxybenzaldehyde, 5-chlorosalicylaldehyde, 2-hydroxy-5-nitrobenzaldehyde, 5-bromo-2-hydroxybenzaldehyde, *d*-norephedrine·HCl, and *l*-norephedrine·HCl were purchased from Sigma-Aldrich and were used without further purification. Microbial strains *Bacillus subtilis* (NCIM2718), *Staphylococcus aureus* (NCIM5021), *Escherichia coli* (NCIM2931), and *Proteus vulgaris* (NCIM2813) were obtained from National Chemical Laboratory, Pune, India. Media components, including broth and agar, and control drugs (ampicillin and tetracycline) were purchased from Himedia, Mumbai, India.

Physical measurements

The FT-IR spectra were recorded on a PerkinElmer Spectrum One FT–IR spectrometer as KBr pellets in the frequency range of 400–4000 per cm. The C, H, and N contents were determined by Thermoflash EA1112 series elemental analyzer. Specific rotation was recorded with a Rudolph Autopol IV polarimeter (589 nm and $CHCl_3$). The

Table 1: Crystal Data, data collection, and structure refinement parameters for **5a** and **6a**

Parameters	**5a**	**6a**
Empirical formula	C_{16} H_{16} Br N O_2	C_{16} H_{16} N_2 O_4
Formula weight	334.21	300.31
Color	Yellowish orange	Orange
Habit	Block	Block
Crystal dimension	0.28 × 0.16 × 0.11 mm	0.60 × 0.50 × 0.40 mm
Crystal system	Triclinic	Triclinic
Space group	*P*1	*P*1
a (Å)	7.1016 (14)	6.0305 (5)
b (Å)	9.2799 (18)	10.2143 (9)
c (Å)	12.299 (2)	12.2730 (11)
α (°)	69.075 (2)	92.325 (1)
β (°)	80.006 (2)	100.757 (1)
γ (°)	75.075 (2)	92.917 (1)
Volume (Å^3)	728.5 (2)	740.77 (11)
Z	2	2
Temperature (K)	100 (2)	100 (2)
D_C (Mg/m^3)	1.523	1.346
Absorption coefficient (mm^{-1})	2.822	0.098
F (000)	340	316
λ (Å)	0.71073	0.71073 Å
θ range (°)	2.98–30.13	2.68–30.00
Scan type	*ω*, *φ*	*ω*, *φ*
Index ranges	−9 ≤ h ≤ 10, −13 ≤ k ≤ 12, −17 ≤ l ≤ 17	−8 ≤ h ≤ 8, −14 ≤ k ≤ 14, −16 ≤ l ≤ 17
Reflns collected/ unique/obs.	11045/8163/8019	10928/8245/8089
R_{int}	0.0144	0.0174
Refinement method	Full-matrix least squares on F^2	Full-matrix least squares on F^2
Diffractometer	Bruker Kappa APEX-II CCD	Bruker Kappa APEX-II CCD
Absorption correction	Multiscan, program SADABS	Multiscan, program SADABS
Final *R* indices $R1/wR_2$ [$I > 2\sigma$ (I)]	0.0292/0.0744	0.0321/0.0908
$R1/wR_2$ (all data)	0.0297/0.0748	0.0327/0.0916
Goodness-of-fit on F^2	1.057	1.056
$\Delta\rho_{max/min}$, e Å^{-3}	0.54 and −0.26	0.37 and −0.18
Data/restraints/ parameters	8163/4/371	8245/58/409

ESI-MS was recorded by a Bruker Daltonics HCT-Ultra ETDII ion trap mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). The ^{1}H and ^{13}C{^{1}H} NMR spectra were recorded in $CDCl_3$ solution on a Bruker AVANCE III 500 MHz spectrometer (Bruker BioSpin AG, Fällanden, Switzerland) using TMS as internal standard. The ^{1}H-^{13}C HSQC spectrum was obtained using the standard Bruker pulse programs. Melting points were determined with Khera digital melting point apparatus (Khera Instuments Pvt. Ltd., New Delhi, India).

X-ray crystallography

Single crystals of **5a** and **6a** suitable for X-ray diffraction studies were grown at room temperature from ethanol/hexane solution by slow evaporation. Diffraction data were collected on a Bruker Kappa APEX-II CCD diffractometer (Bruker AXS GmbH, Karlsruhe, Germany) using graphite-monochromated Mo K_α radiation (λ = 0.71073 Å). After data integration with program SAINT, absorption corrections with the multiscan method and program SADABS were applied.[a] The structures were solved by direct methods (SHELXS-97) and refined by full-matrix least squares fitting based on F^2 using the program SHELXL-97 (18). All non-hydrogen atoms were refined with anisotropic displacement parameters. All H atoms were located by different Fourier syntheses and were then included in the refinement mostly with idealized geometry riding on the atoms to which they were bonded. Only the N-bonded hydrogen atoms were refined in *x*, *y*, *z* using a SADI distance restraint for the N-H distance. In addition, the crystal structure of compound **4a**, the Cl analogue of the Br compound **5a**, was determined and found to be isostructural with **5a**. Due to crystal quality problems and larger *R*-values, this structure is not reported in the present work, but structural data can be obtained on request.

Computational details

Calculations were performed using the GAUSSIAN 03 software package,[b] and the B3LYP functional (19–21) without symmetry constraints. The functional includes a mixture of Hartree–Fock (22) exchange with DFT (23) exchange-correlation, given by Becke's three parameter functional with the Lee, Yang, and Parr correlation functional, which includes both local and non-local terms. For all atoms, the 6-31g** basis set was employed. Frequency calculations were performed to confirm the nature of the stationary points yielding no imaginary frequency for the minima (24–30). A scaling factor of 0.9614 was applied for the IR frequencies. The NMR chemical shift calculations were obtained with the GIAO method at the B3LYP/6-31g** level (31). The TMS, calculated at the same level of theory, was used as reference to scale the absolute shielding value.

Synthesis of chiral Schiff bases

The *d*-norephedrine.HCl or *l*-norephedrine.HCl (0.5 g, 2.66 mmol) and potassium carbonate (0.368 g, 2.66 mmol) were added to 20 mL of absolute ethanol and stirred for 0.5 h at 27 °C. To the resulting solution, 5-substituted salicylaldehyde (2.66 mmol) was added and stirred for 4 h at 27 °C. The reaction was monitored by TLC. After completion of reaction, the resulting solution was evaporated to dryness, then dissolved in dichloromethane, and filtered through Whatman filter paper (no. 1). The filtrate was evaporated to dryness. An oily residue was obtained for **1a**, **1b**, **2a**, **2b**, **3a**, and **3b** and for others solid was obtained.

Antibacterial evaluation

In vitro antibacterial inhibitory activities of the Schiff bases were determined by twofold dilution assay (32) with modifications from the reported method (33) using resazurin as an indicator dye. Muller Hinton broth was used to grow the bacterial strains to a final inoculum size of 5×10^5 CFU/mL. The Schiff bases were dissolved in absolute ethanol to a concentration of 10 mg/mL (34). Schiff base solutions were added to successive wells in a 96-well microtiter plate and incubated with the organisms for 18 h at 37 °C. Growth and sterility controls were maintained during the experiments. Compounds without the microorganism were also kept in the 96-well

microtiter plates to check the precipitation of compounds during the course of the experiments. Ten microliters of 0.01% resazurin solution was added to each well and incubated for 2 h. A blank array with ethanol alone and its effect on the growth of the microorganisms was also studied. The color change was assessed visually, with the highest dilution remaining blue (inhibition of growth) indicating minimum inhibitory concentration (MIC). Ampicillin and tetracycline were used as control drugs.

Results and Discussions

The reaction between equimolar ratio of 5-substituted salicylaldehyde and *d*-norephedrine or *l*-norephedrine in the presence of base (K_2CO_3) in ethanol yielded the new 4-substituted-2-[(*E*)-{[(*1S,2R*)/(*1R,2S*)-1-hydroxy-1-phenylpropan-2-ylimino}methyl]phenols in good yield. They were found to be soluble in CH_2Cl_2, CH_3CN, C_6H_6, DMSO, DMF, and C_2H_5OH. The analytical and ESI-MS data for these compounds are in good agreement with the proposed molecular formula.

NMR spectra

In ^1H NMR spectrum of all the compounds, a singlet observed in the region 8.07–8.20 ppm has been assigned to azomethine proton (-CH=N-). A quintet observed in the region 3.0–3.5 ppm in all the Schiff bases is assigned to hydrogen present in C9 (for atom numbering see Table 1). Two doublets observed in the region 1.28–1.37 and 4.75–4.83 ppm with the proton count of three and one, respectively, in all the Schiff bases are assigned to proton(s) on C8 and C10. Schiff bases **2a** and **2b** exhibit a singlet at 2.26 ppm, and **3a** and **3b** show a singlet at 3.75 ppm correspond to methyl and methoxy protons, respectively. Remaining aromatic protons in norephedrine moiety appeared as a multiplet in the region 7.20–7.39 ppm. In the ^{13}C NMR spectra of all the compounds, azomethine carbon resonance is observed in the 163.35–164.38 ppm range. The methyl carbon present in all the Schiff bases appeared in the region 17.97–18.01 ppm. The resonance for phenolic C-O carbon appeared in the region 158.18–160.36 ppm for **1a**–**5a** and **1b**–**5b**, and for **6a** and **6b**, it appeared at 170.28 and 169.97 ppm respectively. Peaks due to aliphatic C-O and C-N carbons are observed in the region 77.09–77.66 and 69.73–70.21 ppm, respectively. Methyl (**2a** and **2b**) and methoxy carbons (**3a** and **3b**) of Schiff bases exhibit a resonance at 20.33 and 55.95 ppm, respectively. In **5a** and **5b**, signals due to protons present in C3 and C5 are merged in the region 7.21–7.37 ppm, where signals of aromatic protons of norephedrine moiety are also present. To confirm the presence of protons in C3 and C5, ^1H-^{13}C HSQC was recorded for **5a** which establishes the connectivity of carbons, namely C3 (134.97) and C5 (133.50) with its protons (Figure 1).

IR spectra

The IR spectra of all the compounds exhibit a strong C=N stretching vibration around 1630–1653 per cm. A band appeared in the region 1565–1612 per cm in all the compounds may be assigned to aromatic C=C stretching vibration (35). The bands in the region 1322–1327 and 1033–1052 per cm have been assigned to phenolic ν(C-O)

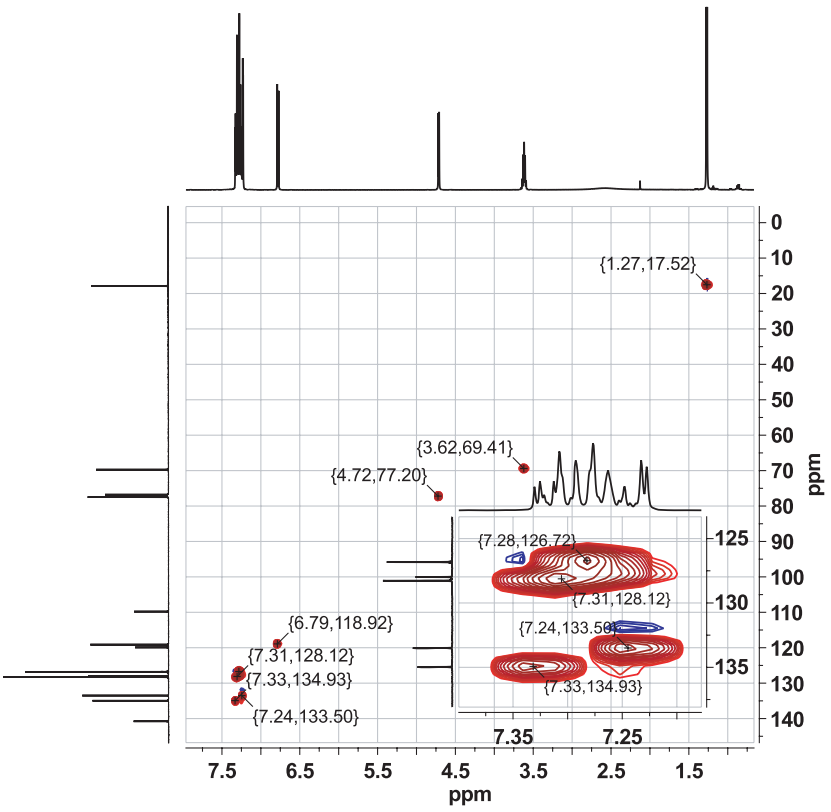


Figure 1: ^1H-^{13}C HSQC NMR spectrum of **5a**.

and aliphatic *ν*(C-O), respectively (35,36). The *ν*(C-N) stretching frequency was appeared in the region 1226–1277 per cm. A broad band appeared around 3400 per cm was assigned to merged aliphatic as well as phenolic *ν*(O-H). For compounds **6a** and **6b**, antisymmetric (out-of-phase) stretching vibration of the aromatic nitro group gives rise to a strong IR band at 1545 and 1547 per cm, respectively. The symmetric (in-phase) stretching vibration of the nitro group present in **6a** and **6b** exhibits a strong band at 1323 and 1324 per cm, respectively.

X-ray crystallography

The compounds **5a** and **6a** were structurally determined by X-ray crystallography. The crystallographic and measurement data are shown in Table 1 and representative bond lengths and bond angles are listed in Table 2. The molecular structures and the adopted atom numbering are presented in Figures 2 and 3, respectively. Both compounds crystallize in triclinic lattices of space group symmetry *P*1 (no. 1) with unit cell contents of each two independent molecules linked to dimers by a pair of O-H···O hydrogen bonds. Schiff bases of salicylaldehydes are known for their intramolecular short hydrogen bonds between O and N and the associated tautomerism between hydroxyimino (O-H···N) and oxoamino (O···H-N) form, this last being also interpretable as an *ortho*-quinoid or a zwitter ionic form (O^{1-}···H-N^{1+}) (37,38). In the solid state, **5a** and **6a** adopt the oxoamino form with a short C(1)-O(1) bond of ∼1.29 Å and an alternating long–short–long bond length pattern in the phenyl ring C(1) through C(6) related to an *ortho*-quinoid structure. These features become clearly evident when the ring bond lengths of **5a** and **6a** (Table 2) are compared with those of a Schiff base in the

Table 2: Selected bond lengths (Å) and angles (°) for compounds **5a** and **6a**

	5a			**6a**	
Bond lengths	Molecule 1	Molecule 2	Bond lengths	Molecule 1	Molecule 2
O(1)-C(1)	1.288 (3)	1.290 (3)	O(1)-C(1)	1.279 (1)	1.288 (1)
O(2)-C(10)	1.410 (2)	1.409 (2)	O(2)-C(10)	1.422 (1)	1.415 (1)
N(1)-C(7)	1.294 (3)	1.292 (3)	N(1)-C(7)	1.292 (1)	1.295 (1)
N(1)-C(9)	1.469 (2)	1.473 (3)	N(1)-C(9)	1.462 (1)	1.473 (1)
C(1)-C(2)	1.437 (3)	1.436 (3)	C(1)-C(2)	1.429 (1)	1.430 (1)
C(1)-C(6)	1.440 (3)	1.441 (3)	C(1)-C(6)	1.449 (1)	1.443 (1)
C(2)-C(3)	1.379 (3)	1.376 (3)	C(2)-C(3)	1.367 (1)	1.368 (1)
C(3)-C(4)	1.411 (3)	1.408 (3)	C(3)-C(4)	1.407 (1)	1.408 (1)
C(4)-C(5)	1.374 (3)	1.373 (3)	C(4)-C(5)	1.380 (1)	1.378 (1)
C(5)-C(6)	1.403 (3)	1.405 (3)	C(5)-C(6)	1.401 (1)	1.402 (1)
C(6)-C(7)	1.429 (3)	1.435 (3)	C(6)-C(7)	1.433 (1)	1.437 (1)
C(8)-C(9)	1.526 (3)	1.519 (4)	C(8)-C(9)	1.524 (1)	1.521 (1)
C(9)-C(10)	1.553 (3)	1.542 (3)	C(9)-C(10)	1.545 (1)	1.544 (1)
C(10)-C(11)	1.520 (3)	1.520 (3)	C(10)-C(11)	1.515 (1)	1.515 (1)
C(4)-Br(1)	1.894 (2)	1.897 (2)	C(4)-N(2)	1.444 (1)	1.446 (1)
			N(2)-O(3)	1.236 (1)	1.232 (1)
			N(2)-O(4)	1.234 (1)	1.235 (1)
Bond angles			Bond angles		
C(2)-C(1)-C(6)	115.8 (2)	115.8 (2)	C(2)-C(1)-C(6)	116.5 (1)	117.1 (1)
C(1)-C(6)-C(5)	121.6 (2)	121.8 (2)	C(1)-C(6)-C(5)	121.0 (1)	120.6 (1)
C(1)-C(6)-C(7)	120.4 (2)	120.0 (2)	C(1)-C(6)-C(7)	120.5 (1)	120.5 (1)
C(5)-C(6)-C(7)	118.1 (2)	118.3 (2)	C(5)-C(6)-C(7)	118.4 (1)	118.9 (1)
C(6)-C(7)-N(1)	122.2 (2)	121.8 (2)	C(6)-C(7)-N(1)	121.4 (1)	120.7 (1)
C(7)-N(1)-C(9)	127.8 (2)	127.6 (2)	C(7)-N(1)-C(9)	127.3 (1)	128.5 (1)
N(1)-C(9)-C(8)	113.6 (2)	112.6 (2)	N(1)-C(9)-C(8)	108.1 (1)	112.5 (1)
N(1)-C(9)-C(10)	106.1 (2)	107.4 (2)	N(1)-C(9)-C(10)	106.2 (1)	105.4 (1)
C(8)-C(9)-C(10)	112.5 (2)	113.2 (2)	C(8)-C(9)-C(10)	112.9 (1)	113.7 (1)
O(2)-C(10)-C(9)	109.8 (2)	110.6 (2)	O(2)-C(10)-C(9)	105.2 (1)	107.4 (1)
O(2)-C(10)-C(11)	111.0 (1)	110.4 (2)	O(2)-C(10)-C(11)	112.0 (1)	112.9 (1)
C(9)-C(10)-C(11)	112.0 (2)	111.4 (2)	C(9)-C(10)-C(11)	111.9 (1)	113.1 (1)
			O(3)-N(2)-O(4)	122.2 (1)	123.0 (1)
			O(3)-N(2)-C(4)	118.5 (1)	118.1 (1)
			O(4)-N(2)-C(4)	119.3 (1)	118.9 (1)
Dihedral angles			Dihedral angles		
C7-N1-C9-C10	−118.1 (2)	−126.2 (3)	C7-N1-C9-C10	129.3 (1)	−137.9 (1)
N1-C9-C10-C11	−160.5 (2)	−161.9 (2)	N1-C9-C10-C11	173.8 (1)	−166.7 (1)
C9-C10-C11-C12	−126.6 (2)	−126.3 (2)	C9-C10-C11-C12	−90.5 (1)	−91.4 (1)

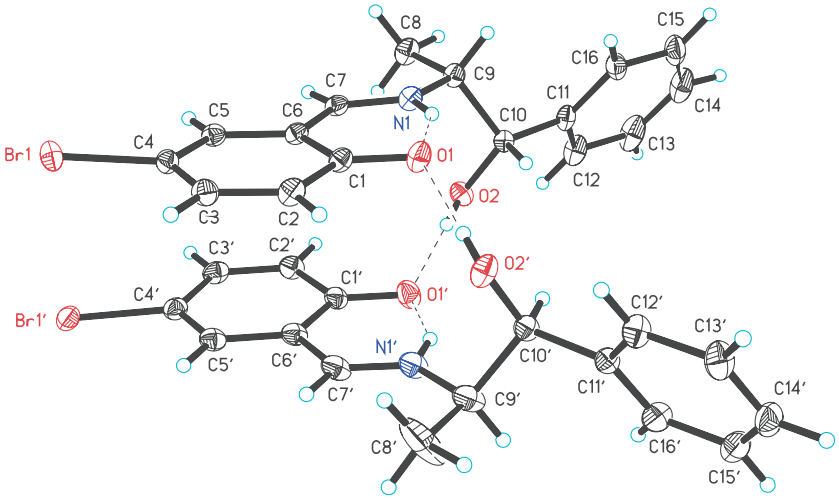


Figure 2: Molecular structure of compound **5a** showing the two crystallographically independent molecules with hydrogen bonds and 50% displacement ellipsoids.

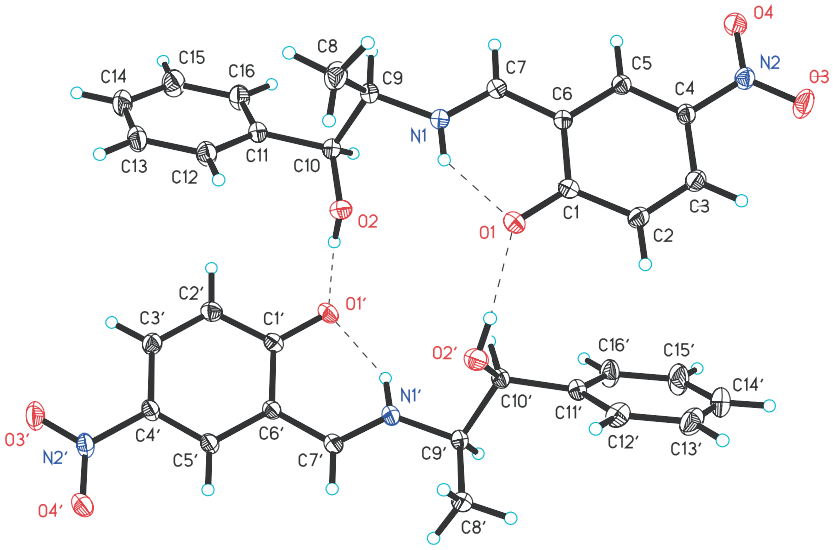


Figure 3: Molecular structure of compound **6a** showing the two crystallographically independent molecules with hydrogen bonds and 50% displacement ellipsoids.

hydroxyimino form, for example, 2-(N-salicylidene)amino-2-methyl-propan-1,3-diol (39), where following bond lengths (Å) were found [values in brackets are corresponding mean values of **5a** and **6a**, e.s.d.s 0.002 Å]: O(1)-C(1) = 1.356(2) [1.286], C(1)-C(2) = 1.392(2) [1.433], C(2)-C(3) = 1.383(2) [1.372], C(3)-C(4) = 1.388(3) [1.409], C(4)-C(5) = 1.385(2) [1.376], C(5)-C(6) = 1.399(2) [1.403], C(6)-C(1) = 1.409(2) [1.443], C(6)-C(7) = 1.456(2) [1.434], C(7)-N(1) = 1.274(2) [1.293], N(1)-C(8) = 1.477(2) [1.469]. The bond angles in both compounds are unremarkable except for the ring bond angles C(6)-C(1)-C(9), which are with values of about 115°, distinctly smaller than all other ring bond angles. The bond angles C(7)-N(1)-C(9) are about 128° and thus, by about 10° larger than for corresponding compounds in hydroxyimine form. The hydrogen bonds are compiled in Table 3. The intramolecular N-H···O bonds are very short (N···O ≈ 2.58 Å) and distinctly bent (140–150°). The intermolecular O-H···O are also relatively short (O···O ≈ 2.69 Å) but essentially linear (164–179°). It is interesting to note that the O-H···O bonds in both **5a** and **6a** link pairs of symmetry independent molecules to dimers in a pseudosymmetric fashion (Figures 2 and 3). In **5a,** the two independent molecules are related by a non-crystallographic C_2-axis that extends at $x \approx 1/2$, $z \approx 1/2$ infinitely parallel to the *b*-axis to form columns. As the crystallographic *a*- and *c*-axes are not perpendicular to the *b*-axis, in three-dimensional space instead of a monoclinic lattice of space group *P*2, a truly triclinic lattice of space group symmetry *P*1 is formed with different neighborhoods for the two independent kinds of molecules. Interestingly, also **6a** shows pseudosymmetry – here, however, C_i with a pseudoinversion at *x*, *y*, *z* ≈ 1/2, 1/2, 1/2 – which relates approximately the two independent molecules except for their groups C(8)H_3 and O(9)H (cf. Figure 3). These latter groups define the handedness of the two

Table 3: Hydrogen bonds in **5a** and **6a** (Å, °). Both crystal structures contain each two pseudosymmetry-related independent molecules (unprimed and primed atom labels).

D-H···A	D-A	D-H	H···A	D-H···A
Compound **5a**				
N(1)-H(1n) ···O(1)	2.593 (2)	0.92 (2)	1.76 (3)	149 (3)
O(2)-H(2o) ···O(1′)	2.670 (2)	0.84	1.83	178
N(1′)-H(1′n) ···O(1′)	2.576 (2)	0.92 (2)	1.74 (3)	150 (3)
O(2′)-H(2′o) ···O(1)	2.671 (2)	0.84	1.83	179
Compound **6a**				
N(1)-H(1n) ···O(1)	2.580 (1)	0.86 (1)	1.859 (14)	140 (2)
O(2)-H(2o) ···O(1′)	2.701 (1)	0.84	1.86	174
N(1′)-H(1n′) ···O(1′)	2.570 (1)	0.86 (1)	1.819 (15)	145 (2)
O(2′)-H(2o′) ···O(1)	2.714 (1)	0.84	1.90	164

independent but homochiral molecules and consequently, violate the C_i pseudosymmetry most (a true inversion would invert the chirality of the symmetry-related molecule). However, different from **5a**, the C_i pseudosymmetry in **6a** is not limited to one dimension but covers the triclinic lattice in three dimensions (Appendix S1).

DFT/B3LYP calculations

The DFT calculations reveal that molecules of the type (**I**) (Figure 4) undergo an intra-molecular proton transfer from the phenolic OH to the azomethine nitrogen resulting in a quinoid structure (**II**) with the latter being more stable by a free energy of 12.2 kcal/mol. However, **II** is able to form dimeric structures **A** and **B** which are connected via strong intra- and inter-molecular hydrogen bonds with another molecule as depicted in Figure 5. These are more stable than the monomeric form by 26.2 kcal/mol. Structure **A**, where the two 'benzoquinone' rings show π-π-stacking, is slightly disfavored over **B** by 3.2 kcal/mol. The gas phase structures agree very well with solid-state structures of **5a** and **6a**, as determined by X-ray crystallography, where it was found that structure **A** is formed in the case of **5a**, whereas for **6a,** the structural type **B** is observed. The calculated IR spectra of monomeric **I** and **II** display strong peaks of the C=N stretching vibration at 1669 and 1633 per cm, respectively, whereas the dimeric compounds **A** and **B** give rise to signals at 1640 and 1635 per cm, respectively. The scaled calculated frequencies $\nu_{C=N}$ show a reasonably good agreement with the experimentally observed value for **5a** being 1633 per cm. In the ^{1}H NMR spectrum of **A** and **B**, the C=NH proton exhibits a signal at about 13.7 ppm (cf. 13.32 in **5a**).

Antibacterial activity

The synthesized compounds were tested for antibacterial activity against *E. coli, P. vulgaris, S. aureus*, and *B. subtilis* by twofold dilution method. Antibacterial activity was expressed in terms of MIC in μM concentration (Table 4). Compounds **1a**, **5a**, **2b**, **3b**, and **5b** were the most active and **2a** and **3a** were the least active against *E. coli*. A strong correlation [correlation coefficient (r) = 0.96] exists between the antibacterial activity of the most active comounds (**1a**, **5a**, **2b**, **3b**, and **5b**) and ClogP (measure of lipophilicity or hydrophobicity of a compound). Compounds **1a**, **2a**, **5a**, **2b**, **5b**, and **6b** were the most active and compounds **1b** and **4b** were the least active against *P. vulgaris*. Once again, a strong positive correlation exists between the antibacterial activity and ClogP of the most active compounds (**1a**, **2a**, **5a**, **2b**, **5b**, and **6b**) (r = 0.99). Compounds **1a**, **2b**, **3b**, and **6b** were the most active and

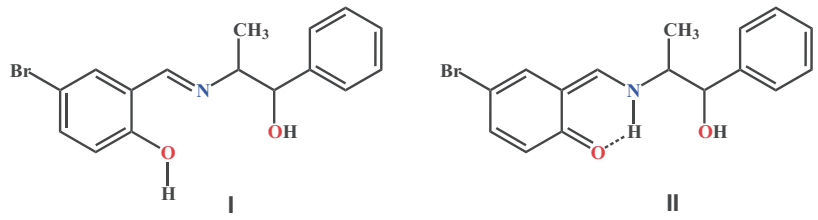


Figure 4: Possible configurations of **5**.

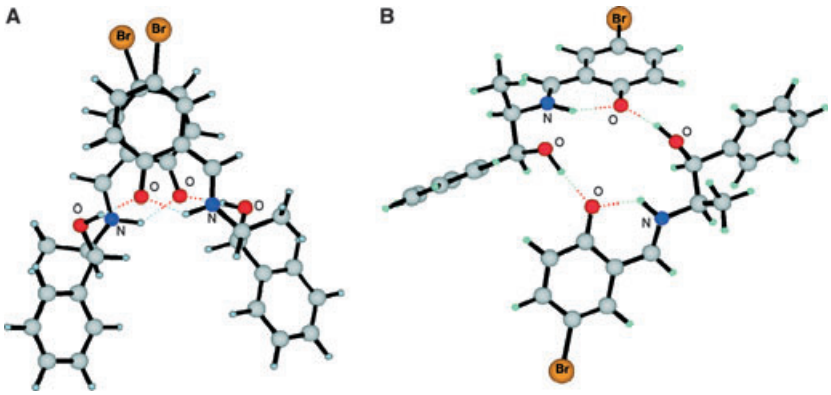
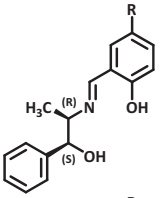
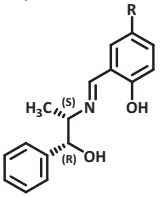


Figure 5: DFT/B3LYP optimized structures of two molecules of compound **5a** connected *via* strong NH and OH hydrogen bonds.

Table 4: *In vitro* antimicrobial activity (minimum inhibitory concentration, μM) of the compounds and standard reagents

Structure	Compound	Substitution at *R*	ClogP	Minimum inhibitory concentration (μM)			
				Gram-negative		Gram-positive	
				E. coli NCIM 2931	*P. vulgaris* NCIM2813	*B. subtilis* NCIM2718	*S. aureus* NCIM5021
	**1a**	H	2.374	0.031	0.015	0.031	0.031
	2a	CH_3	2.873	0.232	0.058	0.232	0.232
	3a	OCH_3	2.483	0.219	0.110	0.219	0.438
	4a	Cl	3.191	0.216	0.108	0.216	0.216
	5a	Br	3.341	0.094	0.094	0.187	0.094
	6a	NO_2	2.339	0.208	0.104	0.416	0.416
	**1b**	H	2.374	0.122	0.245	0.061	0.979
	2b	CH_3	2.873	0.058	0.058	0.029	0.464
	3b	OCH_3	2.483	0.055	0.110	0.027	0.876
	4b	Cl	3.191	0.108	0.216	0.108	0.863
	5b	Br	3.341	0.094	0.094	0.187	0.748
	6b	NO_2	2.339	0.104	0.013	0.026	0.204
–	Ampicillin	–		0.005	0.168	0.168	0.084
–	Tetracycline	–		0.001	0.001	0.001	0.002

compounds **2a**, **3a** and **6a** were the least active against *B. subtilis*. A negative correlation exists between the antibacterial activity of the least active compounds (**2a**, **3a**, and **6a**) and ClogP ($r = -0.66$). Compounds **1a**, **4a**, **5a**, and **6b** were the most active and **1b**, **3b**, and **4b** were the least active against *S. aureus*. A negative correlation exists between the antibacterial activity and ClogP and the activity of least active compounds (**1b**, **3b**, and **4b**) ($r = -0.68$). Enantiomers may have either similar or different pharmacodynamic profiles. In our case, active compounds **1a** and **1b** (enantiomers) have similar pharmacodynamic profiles whereas in the cases of **3a**, **5a**, **2b**, **4b**, and **6b**, antibacterial activity is higher in a single stereoisomer and other exert lower activity. Similar observation has already been made (40,41). In general, compounds **1a**, **5a**, **2b**, and **6b** were the most active and compounds **1b**, **4b**, and **3a** were the least active in this series against all strains. Most active compounds showed a positive correlation with ClogP especially against Gram-negative organisms (*E. coli* and *P. vulgaris*). The least active compounds showed a negative correlation with ClogP, especially against Gram-positive organisms (*B. subtilis* and *S. aureus*). This study clearly shows that increasing the hydrophobicity increases the antibacterial activity against Gram-negative organisms and decreases the antibacterial activity against Gram-positive organisms. Earlier reports related to acetophenones (42), chalcones (43), and 1,3,5-triphenyl-2-pyrazolines (44) as antibacterial agents showed that hydrophilic and lipophilic balance of the molecules influence their antibacterial activity. The mode of action of the Schiff bases may involve the formation of the hydrogen bond through the azomethine group (C=N) with the active centers of the cell constituents resulting in the interference with normal cell process (45). Interestingly, the activity of some of the compounds reaches the effectiveness of conventional bactericide ampicillin, particularly against *P. vulgaris* and *B. subtilis*. However, activity did not reach the effectiveness of tetracycline, against all bacteria tested.

Conclusion

Novel 4-substituted 2-[(*E*)-{[(*1S,2R*)/(*1R,2S*)-1-hydroxy-1-phenylpropan-2-ylimino}methyl] phenols have been prepared from *d*-norephedrine or *l*-norephedrine and substituted salicylaldehydes, and characterized by analytical, spectral, and crystallographic techniques. Single crystal X-ray crystallography and DFT studies showed that compounds are in the quinoid form and exist as dimers at least in solid state. The antibacterial activity of the synthesized compounds was evaluated using twofold dilution technique against four bacteria using resazurin as indicator. Compounds **1a**, **5a**, **2b**, and **6b** were the most active and compounds **3a**, **1b**, and **4b** were the least active against all the four tested organisms. This study clearly indicates that hydrophobicity of the Schiff bases influences their antibacterial activity.

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References

1. Ferreira M.L., Vasconcelos T.R.A., de Carvalho E.M., Lourenço M.C.S., Wardell S.M.S.V., Wardell J.L., Ferreira V.F., de Souza M.V.N. (2009) Synthesis and antitubercular activity of novel Schiff bases derived from D-mannitol. Carbohydr Res;344:2042–2047.
2. Pandeya S.N., Sriram D., Nath G., De Clercq E. (1999) Synthesis, antibacterial, antifungal and anti HIV evaluation of Schiff and

Mannich bases of isatin derivatives with 3-amino-methylmercapto quinazolin-4(3H) one. Pharm Acta Helv;74:11–17.
3. Dhar D.N., Taploo C.L. (1982) Schiff bases and their applications. J Sci Ind Res;41:501–506.
4. Przybylski P., Huczynski A., Pyta K., Brzezinski B., Bartl F. (2009) Biological properties of Schiff bases and azo derivatives of phenols. Curr Org Chem;13:124–148.
5. da Silva C.M., da Silva D.L., Modolo L.V., Alves R.B., de Resendec M.A., Martins C.V.B., Fátimaa A. (2010) Schiff bases: a short review of their antimicrobial activities. J Adv Res;2:1–8.
6. Satyanarayana V.S.V., Sreevani P., Sivakumar A., Vijayakumar V. (2008) Synthesis and antimicrobial activity of new Schiff bases containing coumarin moiety and their spectral characterization. ARKIVOC; xvii:221–233.
7. Karthikeyan M., Prasad D.J., Poojary B., Subramanya Bhat K., Holla B.S., Kumari N.S. (2006) Synthesis and biological activity of Schiff and Mannich bases bearing 2,4-dichloro-5-fluorophenyl moiety. Bioorg Med Chem;14:7482–7489.
8. Singh K., Barwa M.S., Tyagi P. (2006) Synthesis, characterization and biological studies of Co(II), Ni(II), Cu(II) and Zn(II) complexes with bidentate Schiff bases derived by heterocyclic ketone. Eur J Med Chem;41:147–153.
9. Pannerselvam P., Nair R.R., Vijayalakshmi G., Subramanian E.H., Sridhar S.K. (2005) Synthesis of Schiff bases of 4-(4-aminophenyl)-morpholine as potential antimicrobial agents. Eur J Med Chem;40:225–229.
10. Sridhar S.K., Saravan M., Ramesh A. (2001) Synthesis and antibacterial screening of hydrazones, Schiff and Mannich bases of isatin derivatives. Eur J Med Chem;36:615–625.
11. de Souza A.O., Galetti F.C.S., Silva C.L., Bicalho B., Parma M.M., Fonseca S.F. (2007) Antimycobacterial and cytotoxicity activity of synthetic and natural compounds. Quim Nova;30:1563–1566.
12. Bringmann G., Dreyer M., Faber J.H., Dalsgaard P.W., Staerk D., Jaroszewski J.W., Ndangalasi H., Mbago F., Brun R., Christensen S.B. (2004) Ancistrotanzanine C and related 5,1′- and 7,3′-coupled naphthylisoquinoline alkaloids from Ancistrocladus tanzaniensis. J Nat Prod;67:743–748.
13. Guo Z.Y., Xing R.G., Liu S., Zhong Z.M., Ji X., Wang L., Li P.C. (2007) Antifungal properties of Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan. Carbohydr Res;342:1329–1332.
14. Jabbour A., Steinberg D., Dembitsky V.M., Moussaieff A., Zaks B., Srebnik M. (2004) Synthesis and evaluation of oxazaborolidines for antibacterial activity against streptococcus mutans. J Med Chem;47:2409–2410.
15. Zeng Z., Qian L., Cao L., Tan H., Huang Y., Xue X., Shen Y., Zhou S. (2008) Virtual screening for novel quorum sensing inhibitors to eradicate biofilm formation of Pseudomonas aeruginosa. Appl Microbiol Biotechnol;79:119–126.
16. Shi L., Ge H., Tan S., Li H., Song Y., Zhu H., Tan R. (2007) Synthesis and antimicrobial activities of Schiff bases derived from 5-chloro-salicylaldehyde. Eur J Med Chem;42:558–564.
17. Vogel A.I. (1989) Textbook of Practical Organic Chemistry, 5th edn. London: Longman.
18. Sheldrick G.M. (2008) A short history of SHELX. Acta Crystallogr;A64:112–122.
19. Becke A.D. (1993) Density-functional thermochemistry III. The role of exact exchange. J Chem Phys;98:5648–5653.
20. Miehlich B., Savin A., Stoll H., Preuss H. (1989) Results obtained with the correlation energy density functionals of Becke and Lee, Yang and Parr. Chem Phys Lett;157:200–206.
21. Lee C., Yang W., Parr R.G. (1988) Development of the Colle-Salvetti correlation energy formula into a functional of the electron density. Phys Rev B;37:785–789.
22. Hehre W.J., Radom L., Schleyer P.V., Pople J.A. (1986) Ab Initio Molecular Orbital Theory. New York: John Wiley & Sons.
23. Parr R.G., Yang W. (1989) Density Functional Theory of Atoms and Molecules. New York: Oxford University Press.
24. McLean A.D., Chandler G.S. (1980) Contracted Gaussian basis sets for molecular calculations. I. Second row atoms, Z = 11–18. J Chem Phys;72:5639–5648.
25. Krishnan R., Binkley J.S., Seeger R., Pople J.A. (1980) Self-consistent orbital methods. XX. A basis set for correlated wave functions. J Chem Phys;72:650–654.
26. Wachters A.J.H. (1970) Gaussian basis set for molecular wave functions containing third row atoms. J Chem Phys;52:1033–1036.
27. Hay P.J. (1977) Gaussian basis sets for molecular calculations – representation of 3D orbitals in transition-metal atoms. J Chem Phys;66:4377–4385.
28. Raghavachari K., Trucks G.W. (1989) Highly correlated systems. Excitation energies of first row transition metals Sc-Cu. J Chem Phys;91:1062–1066.
29. Curtiss L.A., Mc Grath M.P., Blaudeau J.-P., Davis N.E., Binning R.C., Radom L. (1995) Extension of Gaussian-2 theory to molecules containing third row atoms Ga-Kr. J Chem Phys;103:6104–6113.
30. McGrath M.P., Radom L. (1991) Extension of Gaussian-1 (G1) theory to bromine containing molecules. J Chem Phys;94:511–516.
31. Scott A.P., Radom L. (1996) Harmonic vibrational frequencies: an evaluation of Hartree–Fock, Moller–Plesset, quadratic configuration interaction, density functional theory and semiempirical scale factors. J Phys Chem;100:16502–16513.
32. Candan F., Unlu M., Tepe B., Daferera D., Polissiou M., Sokmen A., Akpulat A. (2003) Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. millefolium Afan. (Asteraceae). J Ethnopharmacol;87:215–220.
33. Sarker S.D., Nahar L., Kumarasamy Y. (2007) Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods;42:321–324.
34. Charles E.S., Agrawal V.K., Sharma S., Iyer R.N. (1979) Synthesis of 2,5-disubstituted benzimidazoles as potential antihookworm and antimicrobial agents. Eur J Med Chem Chim Ther;14:435–438.
35. Florey K. (ed.) (1983) Analytical Profiles of Drug Substances, Vol. 12. New York: Academic Press.
36. Bellamy L.J. (1975) The Infrared Spectra of Complex Molecules, 3rd edn. London, UK: Chapman and Hall Ltd.
37. Sharif S., Denisov G.S., Toney M.D., Limbach H.-H. (2006) NMR studies of solvent-assisted proton transfer in a biologically relevant Schiff base: toward a distinction of geometric and equilibrium H-bond isotope effects. J Am Chem Soc;128:3375–3387.
38. Bilge S., Kilic Z., Hayvali Z., Hökelek T., Safran S. (2009) Intramolecular hydrogen bonding and tautomerism in Schiff bases: Part VI. Synthesis and structural investigation of salicylaldimine and naphthaldimine derivatives. J Chem Sci;121:989–1001.

39. Dey M., Rao C.P., Saarenketo P.K., Rissanen K., Kolehmainen E., Guionneau P. (2003) Mn(IV) and Co(III)-complexes of OH-rich ligands possessing O_2N, O_3N and O_4N cores: syntheses, characterization and crystal structures. Polyhedron;22:3515–3521.
40. Nichols A.J., Sulpizio A.C., Ashton D.J., Hieble J.P., Ruffolo R.R. (1989) The interaction of the enantiomers of carvedilol with a1- and b1-adrenoceptors. Chirality;1:265–270.
41. Patel B.K., Hutt A.J. (2004) Stereoselectivity in drug action and disposition. In: Reddy I.K., Mehvar, R, editor. Chirality in Drug Design and Development. New York, USA: Marcel Dekker; p. 127–174.
42. Sivakumar P.M., Sheshayan G., Doble M. (2008) Experimental and QSAR of acetophenones as antibacterial agents. Chem Biol Drug Des;72:303–313.
43. Sivakumar P.M., Sobana P., Doble M. (2009) Synthesis, biological evaluation, mechanism of action and quantitative structure-activity relationship studies of chalcones as antibacterial. Chem Biol Drug Des;73:403–415.
44. Sivakumar P.M., Ganesan S., Veluchamy P., Doble M. (2010) Novel chalcones and 1,3,5-triphenyl-2-pyrazoline derivatives as antibacterial agents. Chem Biol Drug Des;73:403–415.
45. Prabhakaran R., Renukadevi S.V., Karvembu R., Huang R., Mautz J., Huttner G., Subashkumar R., Natarajan K. (2008) Structural and biological studies of mononuclear palladium(II) complexes containing N-substituted thiosemicarbazones. Eur J Med Chem;43:268–273.

Notes

[a]APEX2, version 2009.9-0; SAINT, versions 7.68A; SADABS, version 2008/1; SHELXTL, version 2008/4. Madison, WI: Bruker AXS Inc.
[b]Frisch M.J. *et al.* Gaussian 03, Revision C.02. Wallingford, CT: Gaussian, Inc., 2004.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix. S1. CCDC 805343 & CCDC 727008 contains the supplementary crystallographic data for **5a** and **6a**. Chacterization data of **1a** to **6a** and **1b** to **6b** are provided. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

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