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Short communication

Synthesis and antimicrobial activities of Schiff bases derived from 5-chloro-salicylaldehyde

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Abstract

A series of Schiff bases (compounds **1**–**26**) were synthesized by reacting 5-chloro-salicylaldehyde and primary amines, 15 (compounds **2**–**4**, **6**, **7**, **10**, **12**–**17**, **23**, **25** and **26**) of which were first reported. The chemical structures of these compounds were confirmed by means of ^{1}H NMR, ^{13}C NMR, ESI-MS and elemental analyses. The compounds were assayed for antibacterial (*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescence* and *Staphylococcus aureus*) and antifungal (*Aspergillus niger*, *Candida albicans* and *Trichophyton rubrum*) activities by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl trtrazolium bromide) method. Among the compounds tested, (*E*)-4-chloro-2-((4-fluorobenzylimino)-methyl)phenol (**2**) showed the most favorable antimicrobial activity with MICs of 45.2, 1.6, 2.8, 3.4, and 47.5 μg/mL against *B. subtilis*, *E. coli*, *P. fluorescence*, *S. aureus* and *A. niger*, respectively.

Keywords: Aldehyde; Primary amine; Schiff base; Antibacterial activity; Antifungal activity; Structure–activity relationship

1. Introduction

Compounds with the structure of AC = NB are known as Schiff bases, which are usually synthesized from the condensation of primary amines and active carbonyl groups. Some Schiff bases were reported to possess antibacterial [1–6], antifungal [3–6] and antitumor activities [7,8]. Lots of researchers studied the synthesis, characterization and structure–activity relationship (SAR) of Schiff bases [9–12]. It is also reported that Salicylaldehyde derivatives, with one or more halo-atoms in the aromatic ring, showed variety of biological activities like antibacterial and antifungal activities [13]. These investigations led to the conception that Schiff bases of 5-chloro-salicylaldehyde would possess potential antimicrobial properties. In this paper, the synthesis of some Schiff bases of 5-chloro-salicylaldehyde and their antimicrobial properties were reported. The results of this study may be useful to researchers attempting to gain more understanding of the antimicrobial activity of Schiff base compounds.

2. Chemistry

In the present study, 26 primary amines were subjected to reaction with 5-chloro-salicylaldehyde to prepare the corresponding Schiff bases (Scheme 1). All the compounds gave satisfactory chemical analyses (±0.4%). ^{1}H NMR, ^{13}C NMR and ESI-MS spectra were consistent with the assigned structures.

3. Results and discussion

The ways in which different Schiff base compounds react with bacteria and fungi vary due to the difference in their

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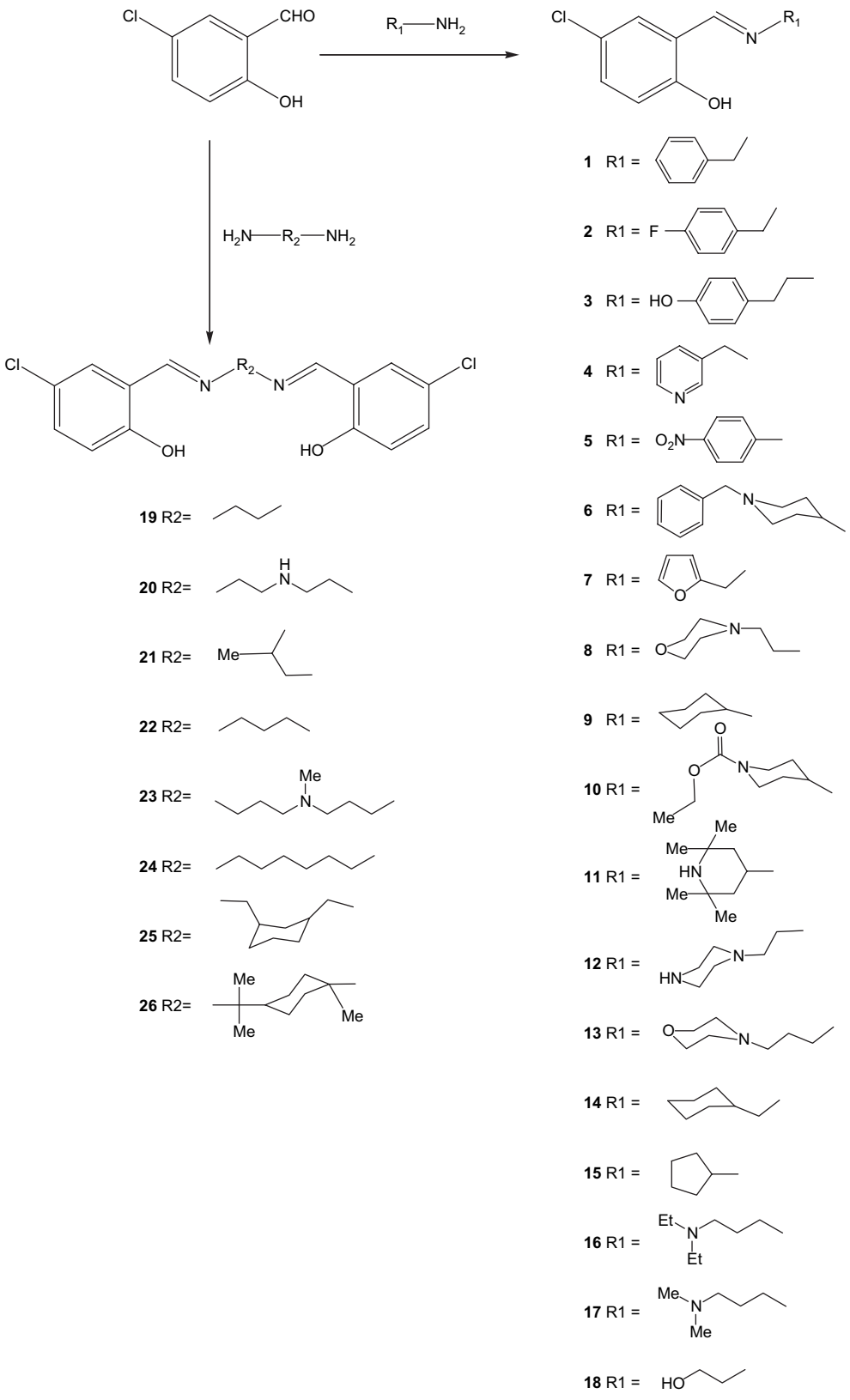

Scheme 1. Syntheses of the Schiff bases.

structures. Structural analysis of these compounds may provide some explanation for the structure–activity relationships. Such an analysis might be helpful in the design of better inhibitors. The biological activity of a particular substance depends on a complex sum of individual properties including compound structure, affinity for the target site, survival in the medium of application, survival within the biological system, transport properties, and state of the target organism [14]. In this study,

we focused our attention on the structure–activity relationships.

All the synthesized compounds were screened for antibacterial activity against two Gram (+) bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram (−) bacterial strains (*Escherichia coli* and *Pseudomonas fluorescence*) by MTT method. The MICs (minimum inhibitory concentrations) of the compounds against four bacteria are presented in Table 1. The activity of reference compounds kanamycin (Nanjing Zhuyan Biotechnology Co. Ltd, Amresco 060D0504, Nanjing 210002, China) and penicillin (North China Pharmaceutical Co. Ltd, D0211107, Hebei 050015, China) was included. Compounds **5**, **10**, **14**, **16**, **17** and **23**–**26** were found to be inactive against all the bacterial strains. Compound **21** showed highest activity against *B. subtilis* (1.8 μg/mL) while compounds **2**, **3** and **7** exhibited moderate activity (45.2–49.8 μg/mL). Compounds **1**, **2**, **7**, **8**, **15**, **21** and **22** showed significant activity against *E. coli* (1.6–5.7 μg/mL) while compound **9** exhibited moderate activity (48.6 μg/mL). Compounds **1**–**3**, **7**, **8**, **15**, **19**, **21** and **22** showed significant activity against *P. fluorescence* (2.5–5.2 μg/mL) while compounds **4**, **9**, **11** and **20** exhibited mild to moderate activity (12.5–40.5 μg/mL). Compounds **2** and **3** showed significant activity against *S. aureus* (1.8–3.4 μg/mL) while compounds **4**, **5**, **9**, **12**, **13** and **18** exhibited mild activity (15.5–25 μg/mL).

Compounds **1**–**5** and **7** showed higher antibacterial activity than lots of other compounds. This result disclosed that compounds with aromatic rings were more active than compounds with aliphatic chains.

Compounds **6**, **8**–**14**, **25** and **26** had a slight difference in the structure. All of them contained a subset similar to cyclohexyl, but their antibacterial activity was very different. Compound **8** showed the highest activity, compounds **9**, **12** and **13** exhibited moderate activity while compounds **6**, **10**, **14**, **25** and **26** almost had no activity. The order of activity indicated that the activity of compounds against bacterial strains was decreased when the complexity of the cyclohexyl-like subset was increased. The results also implied that heteroatoms such as oxygen and nitrogen were helpful in the activity of the compounds. Compound **5** with a cyclopentyl ring was more active than the compounds above. This observation indicated that simpler and smaller the alicyclic ring higher the activity of the compounds.

The antifungal activity of the compounds was studied with three fungal strains (*Aspergillus niger*, *Candida albicans* and *Trichophyton rubrum*) by MTT method. The results are summarized in Table 1. Ketoconazole (Sigma-Aldrich Inc, 3050 Spruce Street, St. Louis) was used as reference for inhibitory activity against fungi. Almost none of the compounds showed antifungal activity except compound **18** which showed mild activity (12.5 μg/mL) against *C. albicans* and compound **2**

Table 1
Antimicrobial activity of the synthesized compounds

Compound	Minimum inhibitory concentrations (μg/mL)						
	Bacillus subtilis	*Escherichia coli*	*Pseudomonas fluorescence*	*Staphylococcus aureus*	*Aspergillus niger*	*Candida albicans*	*Trichophyton rubrum*
1	>50	3.1	3.2	>50	>50	>50	>50
2	45.2	1.6	2.8	3.4	47.5	>50	>50
3	48.5	>50	3.1	1.8	>50	>50	>50
4	>50	>50	12.5	19.5	>50	>50	>50
5	>50	>50	>50	18.5	>50	>50	>50
6	>50	>50	>50	>50	>50	>50	>50
7	49.8	5.7	5.2	>50	>50	>50	>50
8	>50	3.5	2.5	>50	>50	>50	>50
9	>50	48.6	12.5	25.0	>50	>50	>50
10	>50	>50	>50	>50	>50	>50	>50
11	>50	>50	40.5	>50	>50	>50	>50
12	>50	>50	>50	22.5	>50	>50	>50
13	>50	>50	>50	18.5	>50	>50	>50
14	>50	>50	>50	>50	>50	>50	>50
15	>50	4.1	3.4	>50	>50	>50	>50
16	>50	>50	>50	>50	>50	>50	>50
17	>50	>50	>50	>50	>50	>50	>50
18	>50	>50	>50	15.5	>50	12.5	>50
19	>50	>50	2.8	>50	>50	>50	>50
20	>50	>50	32.5	>50	>50	>50	>50
21	1.8	4.9	3.6	>50	>50	>50	>50
22	>50	5.0	4.5	>50	>50	>50	>50
23	>50	>50	>50	>50	>50	>50	>50
24	>50	>50	>50	>50	>50	>50	>50
25	>50	>50	>50	>50	>50	>50	>50
26	>50	>50	>50	>50	>50	>50	>50
Ketoconazole	>50	>50	>50	>50	7.8	3.9	3.9
Kanamycin	0.39	3.9	3.9	1	>50	>50	>50
Penicillin	0.78	>50	>50	2	>50	>50	>50

exhibited moderate activity (47.5 μg/mL) against *A. niger*. These results revealed that most of the synthesized compounds exhibited significant antibacterial activity but showed low antifungal activity.

The study on structure–activity relationships of these Schiff base derivatives indicated that the hydrophilicity and aromaticity seemed to be important for the antimicrobial activity. Generally speaking, the activity of compounds was increased with the increase of hydrophilicity and aromaticity of the compounds. Heteroatoms were helpful in the activity of the compounds. Compound **2** was found to be the most potent antimicrobial agent indicated that fluorine atom played an important role in the antimicrobial activity.

4. Experimental protocols

4.1. Chemistry

All chemicals (reagent grade) used were commercially available. 5-chloro-salicylaldehyde (>95%) was purchased from Zhangjiagang Feihang Industry Co., Ltd, Jiangsu, China and was used without further purification. All the ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX 500 or DPX 300 model Spectrometer in DMSO-d_6. Chemical shifts (δ) for ^{1}H NMR spectra were reported in parts per million to residual solvent protons. ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values. Melting points were measured on a Boetius micro melting point apparatus.

*4.1.1. General method for the synthesis of compounds **1–18***

Equimolar quantities (0.6 mmol) of 5-chloro-salicylaldehyde and the primary amine with one amino group were dissolved in methanol (10 mL) and stirred at room temperature for 10 min to give a clear solution. After standing for approximately 3 d, the precipitates were separated by filtration, recrystallized from methanol, washed with methanol for three times, and dried in a vacuum desiccator containing anhydrous $CaCl_2$.

*4.1.1.1. (E)-2-((Benzylimino)methyl)-4-chlorophenol (**1**).* Yellow crystals, yield 92%, mp: 83–84 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 4.82 (s, 2H); 6.91 (d, $J = 8.9$ Hz, 1H); 7.30 (t, $J = 7.2$ Hz, 1H); 7.34 (d, $J = 7.2$ Hz, 2H); 7.35 (m, 1H); 7.39 (d, $J = 7.2$ Hz, 2H); 7.60 (d, $J = 2.5$ Hz, 1H); 8.71 (s, 1H); 13.53 (s, 1H). ESI-MS: 246.1 ($C_{14}H_{13}ClNO^+$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{12}ClNO$: C, 68.44%; H, 4.92%; N, 5.70%. Found: C, 68.68%; H, 4.96%; N, 5.68%.

*4.1.1.2. (E)-4-Chloro-2-((4-fluorobenzylimino)methyl)phenol (**2**).* Yellow crystals, yield 92%, mp: 97–99 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 4.80 (s, 2H); 6.91 (d, $J = 8.8$ Hz, 1H); 7.20 (t, $J = 8.9$ Hz, 2H); 7.36 (m, 1H); 7.40 (d, $J = 8.9$ Hz, 2H); 7.59 (d, $J = 2.5$ Hz, 1H); 8.69 (s, 1H); 13.42(s, 1H). ^{13}C NMR (DMSO-d_6, δ ppm): 166.5, 163.7, 161.8, 135.9, 133.4, 131.9, 131.2, 123.4, 121.2, 119.8, 116.6, 62.5. ESI-MS: 264.0 ($C_{14}H_{12}ClFNO^+$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{11}ClFNO$: C, 63.77%; H, 4.20%; N, 5.31%. Found: C, 63.96%; H, 4.22%; N, 5.34%.

*4.1.1.3. (E)-4-Chloro-2-((4-hydroxyphenethylimino)methyl)-phenol (**3**).* Yellow crystals, yield 90%, mp: 116–118 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2.83 (t, $J = 6.6$ Hz, 2H); 3.78 (t, $J = 6.6$ Hz, 2H); 6.66 (d, $J = 7.4$ Hz, 2H); 6.87 (d, $J = 9.0$ Hz, 1H); 7.02 (d, $J = 7.4$ Hz, 2H); 7.33 (dd, $J = 9.0$ and 2.5 Hz, 1H); 7.48 (d, $J = 2.5$ Hz, 1H); 8.44 (s, 1H); 9.16 (s, 1H); 13.64 (s, 1H). ^{13}C NMR (DMSO-d_6, δ ppm): 165.8, 160.9, 156.8, 133.2, 131.8, 131.0, 130.6, 123.0, 120.9, 119.9, 116.3, 61.3, 37.0. ESI-MS: 276.2 ($C_{15}H_{15}ClNO_2^+$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{14}ClNO_2$: C, 65.34%; H, 5.12%; N, 5.08%. Found: C, 65.49%; H, 5.10%; N, 5.05%.

*4.1.1.4. (E)-4-Chloro-2-((pyridin-3-ylmethylimino)methyl)phenol (**4**).* Yellow crystals, yield 86%, mp: 92–94 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 4.85 (s, 2H); 6.91 (d, $J = 8.8$ Hz, 1H); 7.37 (m, 1H); 7.40 (m, 1H); 7.60 (d, $J = 2.0$ Hz, 1H); 7.76 (d, $J = 7.5$ Hz, 1H); 8.51 (d, $J = 4.8$ Hz, 1H); 8.59 (s, 1H); 8.72 (s, 1H); 13.10 (s, 1H). ^{13}C NMR (DMSO-d_6, δ ppm): 166.9, 160.2, 150.4, 149.9, 137.0, 135.4, 133.5, 131.9, 125.1, 123.5, 121.2, 119.8, 60.8. ESI-MS: 247.1 ($C_{13}H_{12}ClN_2O^+$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{11}ClN_2O$: C, 63.29%; H, 4.49%; N, 11.36%. Found: C, 63.41%; H, 4.53%; N, 11.42%.

*4.1.1.5. (E)-4-Chloro-2-((4-nitrophenylimino)methyl)phenol (**5**).* Yellow crystals, yield 88%, mp: 190–192 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 7.04 (d, $J = 8.9$ Hz, 1H); 7.50 (dd, $J = 8.9$ and 2.7 Hz, 1H); 7.58 (d, $J = 8.9$ Hz, 2H); 7.82 (d, $J = 2.7$ Hz, 1H); 8.32 (d, $J = 8.9$ Hz, 2H); 8.95 (s, 1H); 12.14 (s, 1H). ESI-MS: 277.0 ($C_{13}H_{10}ClN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{13}H_9ClN_2O_3$: C, 56.43%; H, 3.28%; N, 10.13%. Found: C, 56.49%; H, 3.31%; N, 10.11%.

*4.1.1.6. (E)-2-((1-Benzylpiperidin-4-ylimino)methyl)-4-chlorophenol (**6**).* Yellow crystals, yield 83%, mp: 99–101 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 1.65 (m, 2H); 1.79 (m, 2H); 2.14 (m, 2H); 2.76 (m, 2H); 3.36 (m, 1H); 3.49 (s, 2H); 6.89 (d, $J = 8.9$ Hz, 1H); 7.25 (m, 1H); 7.31–7.35 (m, 5H); 7.53 (d, $J = 2.1$ Hz, 1H); 8.59 (s, 1H); 13.30 (s, 1H). ESI-MS: 329.2 ($C_{19}H_{22}ClN_2O^+$, $[M+H]^+$). ^{13}C NMR (DMSO-d_6, δ ppm): 164.2, 160.7, 139.8, 133.2, 131.8, 130.0, 129.4, 128.1, 123.1, 121.0, 119.8, 63.5, 53.4, 52.3, 34.2. Anal. Calcd for $C_{19}H_{21}ClN_2O$: C, 69.40%; H, 6.44%; N, 8.52%. Found: C, 69.56%; H, 6.47%; N, 8.48%.

*4.1.1.7. (E)-4-Chloro-2-((furan-2-ylmethylimino)methyl)phenol (**7**).* Yellow crystals, yield 92%, mp: 77–78 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 4.81 (s, 2H); 6.40 (d, $J = 2.9$ Hz, 1H); 6.45 (m, 1H); 6.92 (d, $J = 8.9$ Hz, 1H); 7.37 (dd, $J = 8.9$ and 2.7 Hz, 1H); 7.61 (d, $J = 2.7$ Hz, 1H);

7.65 (d, $J = 1.8$ Hz, 1H); 8.66 (s, 1H); 13.21 (s, 1H). ^{13}C NMR (DMSO-d_6, δ ppm): 167.1, 160.0, 152.6, 144.2, 133.5, 131.9, 123.6, 121.2, 119.8, 112.0, 109.2, 55.8. ESI-MS: 234.2 ($C_{12}H_9ClNO_2^-$, $[M-H]^-$). Anal. Calcd for $C_{12}H_{10}ClNO_2$: C, 61.16%; H, 4.28%; N, 5.94%. Found: C, 61.04%; H, 4.24%; N, 5.93%.

*4.1.1.8. (E)-4-Chloro-2-((2-morpholinoethylimino)methyl)phenol (**8**).* Yellow powder, yield 84%, mp: 51–53 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2.42 (m, 4H); 2.59 (t, $J = 6.4$ Hz, 2H); 3.55 (m, 4H); 3.72 (t, $J = 6.4$ Hz, 2H); 6.88 (d, $J = 8.9$ Hz, 1H); 7.33 (dd, $J = 8.9$ and 2.4 Hz, 1H); 7.53 (d, $J = 2.4$ Hz, 1H); 8.54 (s, 1H); 13.70 (s, 1H). ESI-MS: 267.2 ($C_{13}H_{16}ClN_2O_2^-$, $[M-H]^-$). Anal. Calcd for $C_{13}H_{17}ClN_2O_2$: C, 58.10%; H, 6.38%; N, 10.42%. Found: C, 58.24%; H, 6.36%; N, 10.43%.

*4.1.1.9. (E)-4-Chloro-2-((cyclohexylimino)methyl)phenol (**9**).* Yellow crystals, yield 85%, mp: 41–42 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 1.38–1.77 (m, 10H); 3.36 (m, 1H); 6.88 (d, $J = 8.8$ Hz, 1H); 7.34 (dd, $J = 8.8$ and 1.8 Hz, 1H); 7.52 (d, $J = 1.8$ Hz, 1H); 8.57 (s, 1H); 13.93 (s, 1H). ESI-MS: 238.1 ($C_{13}H_{17}ClNO^+$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{16}ClNO$: C, 65.68%; H, 6.78%; N, 5.89%. Found: C, 65.46%; H, 6.82%; N, 5.92%.

*4.1.1.10. (E)-Ethyl 4-(5-chloro-2-hydroxybenzylideneamino)-piperidine-1-carboxylate (**10**).* Yellow powder, yield 82%, mp: 144–146 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 1.19 (t, $J = 7.2$ Hz, 3H); 1.54 (m, 2H); 1.80 (m, 2H); 3.39 (m, 1H); 3.55 (m, 2H); 3.89 (m, 2H); 4.04 (q, $J = 7.2$ Hz, 2H); 6.90 (d, $J = 8.7$ Hz, 1H); 7.35 (dd, $J = 8.7$ and 2.4 Hz, 1H); 7.55 (d, $J = 2.4$ Hz, 1H); 8.61 (s, 1H); 13.85 (s, 1H). ^{13}C NMR (DMSO-d_6, δ ppm): 164.6, 160.4, 156.0, 133.3, 131.9, 123.3, 121.2, 119.8, 65.0, 49.0, 42.9, 33.8, 15.9. ESI-MS: 311.1 ($C_{15}H_{20}ClN_2O_3^+$, $[M+H]^+$). Anal. Calcd for $C_{15}H_{19}ClN_2O_3$: C, 57.97%; H, 6.16%; N, 9.01%. Found: C, 58.12%; H, 6.12%; N, 8.98%.

*4.1.1.11. (E)-4-Chloro-2-((2,2,6,6-tetramethylpiperidin-4-ylimino)methyl)phenol (**11**).* Yellow crystals, yield 80%, mp: 125–126 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 1.07 (s, 6H); 1.18 (s, 6H); 1.25 (m, 2H); 1.67 (m, 2H); 3.80 (m, 1H); 6.88 (d, $J = 8.8$ Hz, 1H); 7.33 (dd, $J = 8.8$ and 2.1 Hz, 1H); 7.52 (d, $J = 2.1$ Hz, 1H); 8.65 (s, 1H); 13.92 (s, 1H). ESI-MS: 295.2 ($C_{16}H_{24}ClN_2O^+$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{23}ClN_2O$: C, 65.18%; H, 7.86%; N, 9.50%. Found: C, 65.33%; H, 7.89%; N, 9.43%.

*4.1.1.12. (E)-4-Chloro-2-((2-(piperazin-1-yl)ethylimino)methyl)phenol (**12**).* Yellow oil, yield 74%, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.36–2.69 (m, 10H); 3.69 (t, $J = 6.4$ Hz, 2H); 6.87 (d, $J = 8.9$ Hz, 1H); 7.33 (dd, $J = 8.9$ and 2.6 Hz, 1H); 7.51 (d, $J = 2.6$ Hz, 1H); 8.51 (s, 1H); 13.75 (s, 1H). ^{13}C NMR (DMSO-d_6, δ ppm): 166.4, 161.7, 133.4, 131.8, 122.7, 120.8, 120.2, 59.7, 56.3, 54.7, 46.4. ESI-MS: 268.2 ($C_{13}H_{19}ClN_3O^+$, $[M+H]^+$). Anal. Calcd for $C_{13}H_{18}ClN_3O$: C, 58.31%; H, 6.78%; N, 15.69%. Found: C, 58.46%; H, 6.84%; N, 15.76%.

*4.1.1.13. (E)-4-Chloro-2-((3-morpholinopropylimino)methyl)-phenol (**13**).* Yellow oil, yield 72%, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.78 (m, 2H); 2.32 (m, 6H); 3.56 (m, 4H); 3.62 (m, 2H); 6.88 (d, $J = 8.8$ Hz, 1H); 7.33 (dd, $J = 8.8$ and 2.4 Hz, 1H); 7.52 (d, $J = 2.4$ Hz, 1H); 8.53 (s, 1H); 13.72 (s, 1H). ^{13}C NMR (DMSO-d_6, δ ppm): 165.9, 161.4, 133.2, 131.7, 122.9, 121.0, 120.0, 67.5, 57.6, 57.1, 54.7, 28.4. ESI-MS: 281.1 ($C_{14}H_{18}ClN_2O_2^-$, $[M-H]^-$). Anal. Calcd for $C_{14}H_{19}ClN_2O_2$: C, 59.47%; H, 6.77%; N, 9.91%. Found: C, 59.58%; H, 6.72%; N, 9.97%.

*4.1.1.14. (E)-4-Chloro-2-((cyclohexylmethylimino)methyl)phenol (**14**).* Yellow crystals, yield 82%, mp: 49–51 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 0.98 (m, 2H); 1.13 (m, 1H); 1.20 (m, 2H); 1.59 (m, 2H); 1.68 (m, 4H); 3.45 (d, $J = 6.2$ Hz, 2H); 6.88 (d, $J = 8.8$ Hz, 1H); 7.33 (dd, $J = 8.8$ and 2.4 Hz, 1H); 7.52 (d, $J = 2.4$ Hz, 1H); 8.49 (s, 1H); 13.88 (s, 1H). ^{13}C NMR (DMSO-d_6, δ ppm): 166.0, 160.5, 133.3, 131.9, 123.0, 120.8, 120.0, 66.1, 39.7, 31.9, 27.4, 26.8. ESI-MS: 252.2 ($C_{14}H_{19}ClNO^+$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{18}ClNO$: C, 66.79%; H, 7.21%; N, 5.56%. Found: C, 66.83%; H, 7.23%; N, 5.52%.

*4.1.1.15. (E)-4-Chloro-2-((cyclopentylimino)methyl)phenol (**15**).* Yellow crystals, yield 94%, mp: 45–47 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.62 (m, 4H); 1.74 (m, 2H); 1.91 (m, 2H); 3.86 (m, 1H); 6.88 (d, $J = 8.8$ Hz, 1H); 7.32 (dd, $J = 8.8$ and 2.6 Hz, 1H); 7.53 (d, $J = 2.6$ Hz, 1H); 8.55 (s, 1H); 13.76 (s, 1H). ^{13}C NMR (DMSO-d_6, δ ppm): 163.7, 161.0, 133.0, 131.7, 123.0, 120.9, 119.8, 69.8, 35.4, 25.1. ESI-MS: 224.1 ($C_{12}H_{15}ClNO$, $[M+H]^+$). Anal. Calcd for $C_{12}H_{14}ClNO$: C, 64.43%; H, 6.31%; N, 6.26%. Found: C, 64.35%; H, 6.37%; N, 6.31%.

*4.1.1.16. (E)-4-Chloro-2-((3-(diethylamino)propylimino)methyl)phenol (**16**).* Yellow crystals, yield 86%, mp: 75–77 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 0.91 (t, $J = 7.1$ Hz, 6H); 1.72 (m, 2H); 2.40 (m, 6H); 3.59 (t, $J = 6.6$ Hz, 2H); 6.87 (d, $J = 8.9$ Hz, 1H); 7.31 (dd, $J = 8.9$ and 2.5 Hz, 1H); 7.49 (d, $J = 2.5$ Hz, 1H); 8.51 (s, 1H); 13.78 (s, 1H). ^{13}C NMR (DMSO-d_6, δ ppm): 166.1, 161.0, 133.2, 131.8, 124.9, 123.7, 121.4, 57.2, 50.5, 47.6, 28.0, 12.0. ESI-MS: 269.2 ($C_{14}H_{22}ClN_2O^+$, $[M+H]^+$). Anal. Calcd for $C_{14}H_{21}ClN_2O$: C, 62.56%; H, 7.88%; N, 10.42%. Found: C, 62.73%; H, 7.78%; N, 10.48%.

*4.1.1.17. (E)-4-Chloro-2-((3-(dimethylamino)propylimino)methyl)phenol (**17**).* Yellow crystals, yield 84%, mp: 76–78 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 1.74 (m, 2H); 2.11 (s, 6H); 2.54 (t, $J = 7.0$ Hz, 2H); 3.59 (t, $J = 6.7$ Hz, 2H); 6.87 (d, $J = 8.9$ Hz, 1H); 7.32 (dd, $J = 8.9$ and 2.4 Hz, 1H); 7.50 (d, $J = 2.4$ Hz, 1H); 8.51 (s, 1H); 13.81 (s, 1H). ^{13}C NMR (DMSO-d_6, δ ppm): 166.0, 161.1, 133.3, 131.8, 124.9, 123.0, 121.3, 57.4, 57.2, 45.8, 28.8. ESI-MS: 241.2 ($C_{12}H_{18}ClN_2O^+$,

$[M+H]^+$). Anal. Calcd for $C_{12}H_{17}ClN_2O$: C, 59.87%; H, 7.12%; N, 11.64%. Found: C, 59.76%; H, 7.09%; N, 11.68%.

*4.1.1.18. (E)-4-Chloro-2-((2-hydroxyethylimino)methyl)phenol (**18**).* Yellow crystals, yield 83%, mp: 44–46 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 3.65 (m, 4H); 4.75 (t, $J = 3.4$ Hz, 1H); 6.87 (d, $J = 8.8$ Hz, 1H); 7.32 (dd, $J = 8.8$ and 2.4 Hz, 1H); 7.53 (d, $J = 2.4$ Hz, 1H); 8.49 (s, 1H); 13.76 (s, 1H). ESI-MS: 200.1 ($C_9H_{11}ClNO_2^+$, $[M+H]^+$). Anal. Calcd for $C_9H_{10}ClNO_2$: C, 54.15%; H, 5.05%; N, 7.02%. Found: C, 54.02%; H, 5.08%; N, 7.09%.

*4.1.2. General method for the synthesis of compounds **19–26***

They were prepared by a similar procedure to that described above, with 5-chloro-salicylaldehyde (0.6 mmol) and the primary amine (0.3 mmol), which have two amino groups.

*4.1.2.1. 2,2′-(1E,1′E)-(Ethane-1,2-diylbis(azan-1-yl-1-ylidene)) bis(methan-1-yl-1-ylidene)bis(4-chlorophenol) (**19**).* Yellow powder, yield 80%, mp: 163–165 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 3.93 (s, 4H); 6.89 (d, $J = 8.8$ Hz, 2H); 7.34 (dd, $J = 8.8$ and 2.6 Hz, 2H); 7.54 (d, $J = 2.6$ Hz, 2H); 8.58 (s, 2H); 13.42 (s, 2H). ESI-MS: 337.1 ($C_{16}H_{15}Cl_2N_2O_2^+$, $[M+H]^+$). Anal. Calcd for $C_{16}H_{14}Cl_2N_2O_2$: C, 56.99%; H, 4.18%; N, 8.31%. Found: C, 57.13%; H, 4.14%; N, 8.38%.

*4.1.2.2. 2,2′-(1E,1′E)-(2,2′-Azanediylbis(ethane-2,1-diyl)bis(azan-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene)bis(4-chlorophenol) (**20**).* Yellow powder, yield 82%, mp: 54–56 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 2.83 (m, 4H); 3.60 (m, 4H); 6.83 (d, $J = 8.8$ Hz, 2H); 7.22 (dd, $J = 8.8$ and 2.5 Hz, 2H); 7.43 (d, $J = 2.5$ Hz, 2H); 8.50 (s, 2H); 13.63 (s, 2H). ESI-MS: 380.1 ($C_{18}H_{20}Cl_2N_3O_2^+$, $[M+H]^+$). Anal. Calcd for $C_{18}H_{19}Cl_2N_3O_2$: C, 56.85%; H, 5.04%; N, 11.05%. Found: C, 56.98%; H, 5.02%; N, 11.09%.

*4.1.2.3. 2,2′-(1E,1′E)-(Propane-1,2-diylbis(azan-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene)bis(4-chlorophenol) (**21**).* Yellow powder, yield 86%, mp: 126–128 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 1.31 (d, $J = 5.6$ Hz, 3H); 3.78–3.84 (m, 3H); 6.88 (d, $J = 8.4$ Hz, 1H); 6.90 (d, $J = 8.5$ Hz, 1H); 7.33–7.35 (m, 2H); 7.54 (d, $J = 2.4$ Hz, 2H); 8.55 (s, 1H); 8.58 (s, 1H); 13.45 (s, 1H); 13.46 (s, 1H). ESI-MS: 351.0 ($C_{17}H_{17}Cl_2N_2O_2$, $[M+H]^+$). Anal. Calcd for $C_{17}H_{16}Cl_2N_2O_2$: C, 58.13%; H, 4.59%; N, 7.98%. Found: C, 58.18%; H, 4.56%; N, 7.82%.

*4.1.2.4. 2,2′-(1E,1′E)-(Propane-1,3-diylbis(azan-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene)bis(4-chlorophenol) (**22**).* Yellow crystals, yield 88%, mp: 85–86 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 2.03 (m, 2H); 3.68 (t, $J = 6.7$ Hz, 4H); 6.89 (d, $J = 8.8$ Hz, 2H); 7.35 (dd, $J = 8.8$ and 2.6 Hz, 2H); 7.54 (d, $J = 2.6$ Hz, 2H); 8.57 (s, 2H); 13.57 (s, 2H). ESI-MS: 349.3 ($C_{17}H_{15}Cl_2N_2O_2$, $[M-H]^-$). Anal. Calcd for $C_{17}H_{16}Cl_2N_2O_2$: C, 58.13%; H, 4.59%; N, 7.98%. Found: C, 57.96%; H, 4.52%; N, 7.89%.

*4.1.2.5. 2,2′-(1E,1′E)-(3,3′-(Methylazanediyl)bis(propane-3,1-diyl)bis(azan-1-yl-1-ylidene))bis(methan-1-yl-1-ylidene)bis(4-chlorophenol) (**23**).* Yellow oil, yield 74%, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.76 (m, 4H); 2.15 (s, 3H); 2.36 (t, $J = 6.7$ Hz, 4H); 3.61 (t, $J = 6.5$ Hz, 4H); 6.88 (d, $J = 8.8$ Hz, 2H); 7.32 (dd, $J = 8.8$ and 2.4 Hz, 2H); 7.49 (d, $J = 2.4$ Hz, 2H); 8.51 (s, 2H); 13.75 (s, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 165.7, 161.5, 133.1, 131.6, 122.8, 120.9, 120.1, 57.4, 55.9, 43.0, 29.2. ESI-MS: 422.2 ($C_{21}H_{26}Cl_2N_3O_2^+$, $[M+H]^+$). Anal. Calcd for $C_{21}H_{25}Cl_2N_3O_2$: C, 59.72%; H, 5.97%; N, 9.95%. Found: C, 59.91%; H, 5.92%; N, 9.98%.

*4.1.2.6. 2,2′-(1E,1′E)-(Hexane-1,6-diylbis(azan-1-yl-1-ylidene)) bis(methan-1-yl-1-ylidene)bis(4-chlorophenol) (**24**).* Yellow powder, yield 82%, mp: 117–118 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 1.38 (m, 4H); 1.64 (m, 4H); 3.59 (t, $J = 6.5$ Hz, 4H); 6.88 (d, $J = 8.9$ Hz, 2H); 7.33 (dd, $J = 8.9$ and 2.4 Hz, 2H); 7.52 (d, $J = 2.4$ Hz, 2H); 8.53 (s, 2H); 13.59 (s, 2H). ESI-MS: 393.2 ($C_{20}H_{23}Cl_2N_2O_2^+$, $[M+H]^+$). Anal. Calcd for $C_{20}H_{22}Cl_2N_2O_2$: C, 61.08%; H, 5.64% N, 7.12%. Found: C, 61.24%; H, 5.55%; N, 7.16%.

*4.1.2.7. 2,2′-(1E,1′E)-(Cyclohexane-1,3-diylbis(methylene))bis (azan-1-yl-1-ylidene)bis(methan-1-yl-1-ylidene)bis(4-chlorophenol) (**25**).* Yellow powder, yield 84%, mp: 120–122 °C, ^{1}H NMR (500 MHz, DMSO-d_6, δ ppm): 0.93 (m, 2H); 1.32 (m, 2H); 1.67 (m, 2H); 1.74 (m, 4H); 3.47 (d, $J = 2.8$ Hz, 4H); 6.88 (d, $J = 8.8$ Hz, 2H); 7.33 (dd, $J = 8.8$ and 2.5 Hz, 2H); 7.52 (d, $J = 2.5$ Hz, 2H); 8.50 (s, 2H); 13.63 (s, 2H). ^{13}C NMR (DMSO-d_6, δ ppm): 166.0, 160.3, 133.2, 131.8, 122.9, 120.8, 120.0, 66.0, 34.6, 31.7, 30.4, 26.2. ESI-MS: 419.2 ($C_{22}H_{25}Cl_2N_2O_2^+$, $[M+H]^+$). Anal. Calcd for $C_{22}H_{24}Cl_2N_2O_2$: C, 63.01%; H, 5.77%; N, 6.68%. Found: C, 62.85%; H, 5.81%; N, 6.60%.

*4.1.2.8. 4-Chloro-2-((E)-(2-(4-((E)-5-chloro-2-hydroxybenzylideneamino)-4-methylcyclohexyl)propan-2-ylimino)methyl)-phenol (**26**).* Yellow powder, yield 78%, mp: 123–124 °C, ^{1}H NMR (300 MHz, DMSO-d_6, δ ppm): 1.22–1.28 (m, 11H); 1.55 (m, 5H); 1.83 (m, 2H); 6.78 (d, $J = 8.8$ Hz, 1H); 6.83 (d, $J = 8.4$ Hz, 1H); 7.29 (dd, $J = 8.8$ and 2.7 Hz, 1H); 7.32 (dd, $J = 8.4$ and 2.8 Hz, 1H); 7.56 (d, $J = 2.7$ Hz, 1H); 7.59 (d, $J = 2.8$ Hz, 1H); 8.52 (s, 1H); 8.61 (s, 1H); 13.60 (s, 1H); 13.68 (s, 1H). ^{13}C NMR (DMSO-d_6, δ ppm): 164.1, 163.0, 161.8, 160.9, 133.3, 133.2, 132.1, 131.8, 122.3, 122.0, 120.7, 120.5, 119.7, 118.9, 63.1, 59.2, 48.2, 39.3, 29.4, 25.4, 23.5. ESI-MS: 447.2 ($C_{24}H_{29}Cl_2N_2O_2^+$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{28}Cl_2N_2O_2$: C, 64.43%; H, 6.31%; N, 6.26%. Found: C, 64.56%; H, 6.25%; N, 6.37%.

4.2. Antimicrobial activity

The antibacterial activity of the synthesized 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*, *C. albicans* and *T. rubrum* 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 [15]. 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. The observed MICs are presented in Table 1.

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