Published in final edited form as:

Lett Drug Des Discov. 2010 March ; 7(3): 165–170. doi:10.2174/157018010790596678.

Synthesis and Antimicrobial Activity of N,N'-Bis(2-hydroxybenzyl)-1,2-ethanediamine Derivatives

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Abstract

A series of N,N'-Bis(2-hydroxybenzyl)-1,2-ethanediamine derivatives and its schiff bases were synthesized, characterized and screened for *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella enterica*. Result indicated that the ethylenediamine derivatives, N,N'-Bis(2-hydroxy-5-bromobenzyl)-1,2-ethanediamine (**21**), and N,N'-Bis(2-hydroxy-5-chlorobenzyl)-1,2-ethanediamine (**22**) showed the most favorable antimicrobial activity exhibiting LC₅₀ of 11.6 and 8.79 μ M against *S. enterica*, 86 and 138 μ M against *P. aeruginosa*, and 140 and 287 μ M against *S. aureus*, respectively. These compounds displayed highest level of resistance with *S. aureus*. Thus, the high level of activity seen with the compounds (**21**, **22**) suggests that these compounds could serve as the leads for development of novel synthetic compounds with enhanced antimicrobial activity.

Keywords

Schiff's base; N; N-ethylenediamine and antimicrobial activity

Introduction

The emergence and spread of multidrug-resistant bacteria have made treatment of infectious diseases difficult and have, over the last few decades, become a serious medical problem [1-2]. For example, strains of *Salmonella enterica*, a leading cause of bacterial gastroenteritis, are no longer susceptible to front line antibiotics [3] and the distribution of methicillin-resistant *S. aureus* (MRSA) strains has increased [4]. Moreover, the high level of inherent antibiotic resistance in *P. aeruginosa* makes treatment of these infections problematic [5]. Several approaches to negating antibiotic resistance are currently being investigated, including inactivation of enzymes in essential metabolic pathways and inhibiting signal transduction systems [6,7]. These approaches involve the development of new antimicrobial drugs with modes of action that circumvent current resistance mechanisms [8,9].

Schiff's bases (or azomethine), functional group generally represented as $-\text{CH}=\text{N}-$, possessed antibacterial [10-12], antifungal [11,12], antitumor [13,14], analgesic [15,16], anti-inflammatory [17,18], antibiotic and antimicrobial activities [19]. Schiff's bases of salicylaldehyde derivative with one or more halo-atom in the aromatic ring had been shown to exhibit variety of biological activities including antibacterial and antifungal activities [20]. For example, recent investigation of the Schiff's base derivatives of 5-chloro-salicylaldehyde, (*E*)-4-chloro-2-((4-fluoro-benzylimino)-methyl) phenol (**1**, Fig. 1), displayed favorable antimicrobial activity against *B. subtilis*, *E. coli*, *P. fluorescence*, *S. aureus* and *A. niger* [19]. Furthermore, the development of a second-generation diamine antibiotic by incorporating 1,2-ethylenediamine pharmacophore to ethambutol (EMB), a useful anti-tuberculosis chemotherapeutic agent, led to the identification of a novel drug candidate, SQ109 (*N*-geranyl-*N'*-(2-adamantyl)ethane-1,2-diamine) (**2**, Fig. 1), with potent antimicrobial activity against *M. tuberculosis* [21-23]. Also, investigation involving antibacterial and antifungal activity of *N,N'*-Bis(1,2,3,4-tetrahydrocarbazol-1-ylidene)ethane-1,2-diamines derivatives (**3**, Fig. 1) revealed that the Schiff's base derivatives having the methyl group and chloro group at the C-6 position displayed antibacterial and antifungal activities; with the chloro derivative exhibiting better activity than the methyl counterpart [24]. Structure activity relationship (SAR) study involving the size and the nature of the alkyl group on the ethylenediamine nitrogen indicated that modification either by introducing heteroatoms into the chain or lengthening of the ethylene unit diminishes activity [25,26].

In the present study focusing on further exploring the antimicrobial activity of Schiff's base derivatives and its reduced forms, the ethylenediamine derivatives; we herein report the synthesis of compounds (**11-24**) as outlined in Scheme 1 and their *in vitro* antimicrobial activity against gram-positive (*S. aureus*) and gram-negative (*P. aeruginosa* and *S. enterica*) bacteria.

Results and Discussion

Synthesis

The synthesis of Schiff's bases, reaction involving amine and carbonyl compound, and their metal complexes are well known [27-32]. In general, 1,2-ethylenediamine were reacted with various substituted benzaldehydes to generate the Schiff's bases (**11-17**), which were further reduced using sodium borohydride to afford the ethylenediamine derivatives (**18-24**) in moderate to good yields (82-91%; Scheme 1, Table 1). Mass spectroscopic (FAB) analysis of compound (**11**) revealed molecular ion at m/z 269 (MH^+) (schiff's base) and m/z 273 (MH^+) (reduced derivative, ethylenediamine, **18**). The ^1H NMR spectrum of compound (**11**) shows a singlet at δ 3.92ppm corresponding to $=\text{NCH}_2\text{CH}_2\text{N}=\text{}$ linkage of the schiff's base, while its reduced form (**18**), displays two characteristic peaks (singlet) at δ 3.98 and 2.83ppm corresponding to the CH_2 -linkages represented as C-1 and C-2, respectively (Scheme 1). Other characteristic peak of compound **11** is δ 13.2ppm, which corresponds to the aromatic hydroxyl group of the Schiff's base. Compound **18-22** did not show the presence of the aromatic hydroxyl group (ArOH) and / or amine NH- group when the spectra were recorded in CDCl_3 as NMR solvent has indicated in the experimental section. However, the ^1H NMR spectrum of Compound (**23**) in $\text{DMSO}-d_6$ showed a chemical shift (broad peak) at δ 4.53ppm and Compound (**24**) at δ 5.32ppm corresponding to the presence of the aromatic hydroxyl peak (ArOH), which disappeared upon the addition of D_2O .

These compounds (**11-24**) were characterized using ^1H NMR, ^{13}C NMR, and MS analyses and then tested for antimicrobial activity against three bacterial strains: *Staphylococcus aureus*, *Salmonella enterica* and *Pseudomonas aeruginosa*.

Antimicrobial activity

Results from antimicrobial screening (Table 2) of compounds (**11-24**) against some common infectious agents i.e. *S. aureus*, *S. enterica*, and *P. aeruginosa* indicate that the reduced ethylenediamine derivatives containing the chlorine (Cl) or bromine (Br) groups at C-6 position of the aromatic ring (**21, 22**), exhibited antimicrobial activity. They are highly effective against *S. enterica* with the lethal concentration 50 (LC₅₀) of 11.6 and 8.79 μ M compared to *P. aeruginosa* and *S. aureus*, LC₅₀ of 86 and 138 μ M, and 140 and 287 μ M, respectively (Table 2 and Fig 2). The LC₅₀, indicated by the vertical arrow, is the concentration that killed 50% of the cells within 15 minutes. Furthermore, the poor solubility of many of these compounds, especially those with the dibromo- or dichloro- substitution, apparently rendered them ineffective under the test conditions. It is to be noted that only the diamine derivatives, which were readily soluble in 0.1M HCl, showed significant activity. None of the Schiff's bases has shown any observable activity against *S. enterica* or *E. coli* at concentrations >500 μ M.

Conclusions

The synthesis and antimicrobial activities of N,N'-Bis(2-hydroxybenzyl)-1,2-ethanediamine derivatives and their Schiff bases have been described. The *in vitro* results indicated that for the type of assays performed, water solubility is essential for antimicrobial activity. Only N,N'-Bis(2-hydroxy-5-bromobenzyl)-1,2-ethanediamine (**21**), and N,N'-Bis(2-hydroxy-5-chlorobenzyl)-1,2-ethanediamine (**22**), containing bromine and chlorine, respectively showed antimicrobial activity against aerobic gram-positive and gram-negative bacteria while other analogs tested were all inactive. *S. enterica* showed the highest level of susceptibility after 15 minutes exposure. On the basis of these results, N,N'-Bis(2-hydroxy-5-bromobenzyl)-1,2-ethanediamine (**21**), and N,N'-Bis(2-hydroxy-5-chlorobenzyl)-1,2-ethanediamine (**22**) could be considered as attractive leads for the future development of antimicrobial agents. Further research is needed to establish the molecular mechanism and/or the chemical reasons for the activity of the diamines and inactivity of the Schiff's bases.

Materials and Methods

Commercial grade solvents and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Alfa Aesar (Ward Hill, MA, USA) and used without further purification. NMR spectra were recorded on Varian 300 MHz spectrometer. The appropriate deuterated solvents are indicated in the procedure, and line positions recorded in ppm from the reference signal. ESI-TOF Mass Spectrometer was recorded on Agilent 6210 TOF with 1200 HPLC using fast atom bombardment (FAB), (TOF H⁺). Melting points were determined on a Gallenkamp (UK) apparatus and are uncorrected.

Synthesis of the Schiff's Bases (11-17)

To a solution of the benzaldehyde (10mmol) in ethanol (5mL) was added ethylenediamine (5 mmol, 0.33mL) and the solution stirred at rt for 20mins to afford a yellow precipitate that was collected using vacuum filtration.

Compound 11—(1.14g, 86%), m.p. 126-128 °C; ¹H-NMR (300 MHz, CDCl₃) 3.92 (s, 4H, =NCH₂CH₂N=) 6.82-6.95 (4H, ArH), 7.20-7.31 (4H, ArH), 8.35 (s, 2H, Ar-CH=N-), 13.2 (s, 2H, ArOH); ¹³C-NMR (75MHz, CDCl₃) 60.13 (CH₂), 117.34, 119.03, 131.85, 132.75, 161.40 (Ar-C) and 166.87 (CH=N-); MS (FAB): *m/z* = 269 (MH⁺, 100%); HRMS (*m/z*): calcd for C₁₆H₁₆O₂N₂, 268.121178; found, 268.121196.

Compound 12—(1.60, 98%), m.p. 160-163 °C; ¹H-NMR (300 MHz, CDCl₃) 3.88 (s, 6H, OCH₃), 3.95 (s, 4H, =NCH₂CH₂N=), 6.74-6.91 (6H, ArH), 8.33 (s, 2H, ArH), 13.53 (s, 2H,

ArOH); ^{13}C -NMR (75MHz, CDCl_3) 56.47 (OCH_3), 59.87 (CH_2), 114.61, 118.41, 118.86, 123.56, 148.70, 151.84 (Ar-C) and 167.03 ($\text{CH}=\text{N}$ -); MS (FAB): m/z = 329 (MH^+ , 100%); HRMS (m/z): $[\text{M}]^+$ calcd for $\text{C}_{18}\text{H}_{20}\text{O}_4\text{N}_2^+$, 328.142307: found, 328.142358.

Compound 13—(1.70g, 96%), m.p. 135-137 °C; ^1H -NMR (300 MHz, CDCl_3) 1.46 (t, 6H, J 6.9 and 7.2 Hz, $2 \times \text{OCH}_2\text{CH}_3$), 3.94 (s, 4H, $=\text{NCH}_2\text{CH}_2\text{N}=\text{}$), 4.10 (q, 4H, J 7.2 Hz, $2 \times \text{OCH}_2\text{CH}_3$), 6.72-6.91 (6H, ArH), 8.32 (s, 2H, ArH), 13.55 (s, 2H, ArOH); ^{13}C -NMR (75MHz, CDCl_3) 15.21 (OCH_2CH_3), 59.89 and 64.91 (CH_2), 116.17, 118.37, 118.98, 123.62, 147.90, 152.06 (Ar-C) and 167.04 ($\text{CH}=\text{N}$ -).

Compound 14—(2.01g, 94%), m.p. 193-195 °C; ^1H -NMR (300 MHz, CDCl_3) 3.95 (s, 4H, $=\text{NCH}_2\text{CH}_2\text{N}=\text{}$), 6.84 (d, 2H, J 8.7Hz, ArH), 7.33-7.38 (4H, ArH), 8.28 (s, 2H, ArH), 13.2 (s, 2H, ArOH); ^{13}C -NMR (75MHz, CDCl_3) 59.96 (CH_2), 110.54, 119.41, 120.29, 133.91, 135.53, 160.42 (Ar-C) and 165.68 ($\text{CH}=\text{N}$ -); HRMS (m/z): $[\text{M}+1]^+$ calcd for $\text{C}_{16}\text{H}_{15}\text{O}_2\text{N}_2\text{Br}_2^+$, 426.9402: found, 426.9475.

Compound 15—(1.60g, 95%), m.p. 175-178 °C; ^1H -NMR (300 MHz, CDCl_3) 3.95 (s, 4H, $=\text{NCH}_2\text{CH}_2\text{N}=\text{}$), 6.89 (d, 2H, J 8.4 Hz, ArH), 7.20-7.26 (4H, ArH), 8.29 (s, 2H, ArH), 13.06 (s, 2H, ArOH); ^{13}C -NMR (75MHz, CDCl_3) 59.97 (CH_2), 118.96, 119.68, 123.71, 130.93, 132.71, 159.95 (Ar-C) and 165.78 ($\text{CH}=\text{N}$ -); MS (FAB): m/z = 336 (M^+ , 98.17%), 338 (75.13%) and 141 (100%); HRMS (m/z): $[\text{M}]^+$ calcd for $\text{C}_{16}\text{H}_{14}\text{O}_2\text{N}_2\text{Cl}_2$, 336.043233: found, 336.043288.

Compound 16—(2.10g, 72%), m.p. 241-243 °C; ^1H -NMR (300 MHz, DMSO) 4.0 (s, 4H, $=\text{NCH}_2\text{CH}_2\text{N}=\text{}$), 7.62 (d, 2H, J 2.7 Hz, ArH), 7.81 (d, 2H, J 2.4 Hz, ArH), 8.58 (s, 2H, ArH), ^{13}C -NMR (75MHz, DMSO) 55.91 (CH_2), 106.93, 114.52, 119.23, 134.56, 138.46, 162.17 (Ar-C) and 167.09 ($\text{CH}=\text{H}$ -).

Compound 17—(1.91g, 94%), m.p. 207-209 °C; ^1H -NMR (300 MHz, CDCl_3) 4.00 (s, 4H, $=\text{NCH}_2\text{CH}_2\text{N}=\text{}$), 7.15 (s, 2H, ArH), 7.40 (s, 2H, ArH), 8.28 (s, 2H, ArH), 13.91 (s, 2H, ArOH); ^{13}C -NMR (75MHz, CDCl_3) 59.36 (CH_2), 119.76, 123.10, 123.49, 129.60, 132.85, 156.46 (Ar-C) and 165.63 ($\text{CH}=\text{NH}$ -).

Synthesis of N,N'-Bis(2-hydroxybenzyl)-1,2-ethanediamine (18-24)

To a solution of the Schiff's bases (3.24mmol) in ethanol (30mL) was added sodium borohydride (4.50mmol, 170.19mg). The mixture was heated for 1h, cool to rt and further stirred for another 2-3h to afford precipitate that was collected using vacuum filtration.

Compound 18—(800mg, 91%), m.p. 113-116 °C; ^1H -NMR (300 MHz, CDCl_3) 2.83 (s, 4H, $2 \times \text{NHCH}_2$), 3.98 (s, 4H, $2 \times \text{Ar-CH}_2\text{NH}$ -), 6.75-6.84 (4H, ArH), 6.97 (d, J 7.5 Hz, 2H, ArH), 7.14-7.19 (2H, ArH); ^{13}C -NMR (75MHz, CDCl_3) 48.08 and 52.84 (CH_2), 116.39, 116.64, 119.44, 122.37, 128.54, 128.67, 129.12, 129.18, 158.20 (Ar-C); HRMS (m/z): $[\text{M}+1]^+$ calcd for $\text{C}_{16}\text{H}_{20}\text{O}_2\text{N}_2^+$, 272.1525: found, 272.1519.

Compound 19—(930mg, 86%), m.p. 167-170 °C; ^1H -NMR (300 MHz, CDCl_3) 2.81 (s, 4H, $2 \times \text{NHCH}_2$), 3.87 (s, 6H, $2 \times \text{OCH}_3$), 3.98 (s, 4H, $2 \times \text{Ar-CH}_2\text{NH}$), 6.63 (d, J 7.2 Hz, 2H, ArH), 6.71-6.82 (4H, ArH); ^{13}C -NMR (75MHz, CDCl_3) 47.97 and 52.04 (CH_2), 56.16 (OCH_3), 11.23, 119.08, 120.98, 123.15, 147.10, and 148.15 (Ar-C).

Compound 20—(1.02g, 87%), m.p. 142-145 °C; ^1H -NMR (300 MHz, CDCl_3) ^1H -NMR (300 MHz, CDCl_3) 1.45 (t, 6H, J 7.2 and 6.9 Hz, $2 \times \text{OCH}_2\text{CH}_3$), 2.81 (s, 4H, $2 \times \text{NHCH}_2$), 3.95 (s, 4H, $2 \times \text{Ar-CH}_2\text{NH}$), 4.10-4.12 (q, 4H, $2 \times \text{OCH}_2\text{CH}_3$), 6.63 (d, J 7.5 Hz, 2H, ArH),

6.71 (t, 2H, J 8.1 and 7.5 Hz, ArH), 6.78-6.81 (dd, 2H, J 1.2 Hz, ArH); ^{13}C -NMR (75MHz, CDCl_3) 15.15 (CH_3), 48.03, 51.93 and 64.58 (CH_2), 112.55, 119.04, 121.01, 123.48, 147.20, 147.31 (Ar-C).

Compound 21—(1.2g, 87%), m.p. 159-162 °C; ^1H -NMR (300 MHz, CDCl_3) ^1H -NMR (300 MHz, CDCl_3) 2.82 (s, 4H, $2 \times \text{NHCH}_2$), 3.96 (s, 4H, $2 \times \text{Ar-CH}_2\text{NH}$), 6.71 (d, J 9.0 Hz, 2H, ArH), 7.09 (d, 2H, J 1.8 Hz, ArH), 7.25 (dd, 2H, J 2.4 and 1.8 Hz, ArH); ^{13}C -NMR (75MHz, CDCl_3) 47.96 and 52.31 (CH_2), 111.18, 118.49, 124.21, 131.27, 131.88 and 157.33 (Ar-C); HRMS (m/z): $[\text{M}]^+$ calcd for $\text{C}_{16}\text{H}_{18}\text{Br}_2\text{O}_2\text{N}_2^+$, 429.97146; found, 429.9710.

Compound 22—(980mg, 89%), m.p. 152-155 °C; ^1H -NMR (300 MHz, CDCl_3) ^1H -NMR (300 MHz, CDCl_3) 2.82 (s, 4H, NHCH_2), 3.95 (s, 4H, $2 \times \text{Ar-CH}_2\text{NH}$), 6.76 (d, J 8.4 Hz, 2H, ArH), 6.95 (d, 2H, J 2.7 Hz, ArH), 7.12 (dd, 2H, J 2.7 and 2.4 Hz, ArH); ^{13}C -NMR (75MHz, CDCl_3) 47.98 and 52.41 (CH_2), 117.97, 123.62, 124.04, 128.38, 128.94 and 156.80 (Ar-C); HRMS (m/z): $[\text{M}+1]^+$ calcd for $\text{C}_{16}\text{H}_{18}\text{Cl}_2\text{O}_2\text{N}_2^+$, 340.07453; found, 341.0818.

Compound 23—(1.56g, 82%), m.p. 197-199 °C; ^1H -NMR (300 MHz, DMSO) ^1H -NMR (300 MHz, CDCl_3) 2.71 (s, 4H, NHCH_2), 3.91 (s, 4H, $2 \times \text{Ar-CH}_2\text{NH}$), 7.25 (d, J 2.4 Hz, 2H, ArH), 7.54 (d, 2H, J 2.7 Hz, ArH); ^{13}C -NMR (75MHz, DMSO) 46.06 and 50.17 (CH_2), 108.05, 110.88, 126.52, 130.29, 132.61 and 155.51 (Ar-C).

Compound 24—(1.14g, 86%), m.p. 165-168 °C; ^1H -NMR (300 MHz, DMSO) ^1H -NMR (300 MHz, CDCl_3) 2.70 (s, 4H, $2 \times \text{NHCH}_2$), 3.90 (s, 4H, $2 \times \text{CH}_2\text{NH}$), 7.11 (d, J 2.4 Hz, 2H, ArH), 7.32 (d, 2H, J 2.7 Hz, ArH); ^{13}C -NMR (75MHz, DMSO) 47.09 and 50.81 (CH_2), 121.43, 127.17, 127.69, 128.02 and 154.61 (Ar-C).

Antimicrobial Screening

Preparation of stock compound solutions

Stock solutions of the antimicrobial compounds (**11-24**) were prepared in a 0.1 M HCl solution to a final concentration of 7.5 mg/mL (eq. to 17.4 mM for **21**), which was diluted to 1.5 mg/mL (eq. to 3.5 mM for **21**) for assays involving *S. enterica*. Stock solutions were placed under refrigeration when not in use, whereas solution that would precipitate under refrigeration was kept at room temperature. Typically, each compound was tested within one week of solubilization.

Screening for antimicrobial activity

Bacterial growth and antimicrobial assays of compounds (**11-24**) against *S. enterica*, *P. aeruginosa*, and *S. aureus* were done with Luria-Bertani (LB) broth. Early-log phase cells were prepared by inoculating twenty ml of LB broth 1:100 with cells from an overnight culture. After ~ 90 minutes of incubation (to OD_{600} 0.2), 0.1 mL of culture was removed and diluted in LB broth to $\sim 3 \times 10^3$ cfu/mL. 0.25 mL aliquots of diluted cell suspension were transferred to four 1.5 mL Eppendorf tubes. The antimicrobial compound, in increasing concentration, was added to three of the tubes, leaving the fourth tube as a control. 0.1 mL from the control tube was plated immediately (the T0 count). After 15 minutes incubation in a 37°C water bath, 0.1 mL of cell suspension from each of the tubes was spread onto tryptic soy agar (TSA) plates and incubated overnight. Percent survival was determined by dividing colony counts from plates spread with treated cells (the T15 counts) by the colony counts on the T0 control plates. For *S. enterica* and *S. aureus*, the T15 control counts were always greater than the T0 control counts, so the T0 counts were used for survival determinations. However, the *P. aeruginosa* T15 control counts were consistently lower than the T0 counts, in which case the T15 counts were used for survival determinations. Importantly, in the three bacterial strains used in these

assays, a 5 μ L volume of 0.1 M HCl solution (the largest volume of antimicrobial compound solution tested) had no effect on cell viability. KaleidaGraph[®] was used to generate a percent survival versus concentration of the compound plot, from which the lethal concentration 50 (LC₅₀) was determined by interpolation as shown in Fig. (2). Overall, the method is similar to that is described by Parra-Lopez *et al.* [33].

Acknowledgments

Florida A & M University TITLE III PROGRAM and The Faculty Research Development Funds (NIH/NCRR grant G12 RR0 3020, NIH/NCRR grant 1 C06 RR12512-01 and NIH/DHHS grant 1 S11 ES011182 01) are gratefully acknowledged for financial support. The authors also gratefully acknowledge Dr. David H. Powell of the Mass Spectrometry Unit, Department of Chemistry at University of Florida for assistance with Mass Spectra analysis.

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Fig. (1).

Chemical structure of (*E*)-4-chloro-2-((4-fluorobenzylimino)methyl) phenol (**1**), *N*-geranyl-*N'*-(2-adamantyl)ethane-1,2-diamine (**2**, SQ109) and *N,N'*-Bis(1,2,3,4-tetrahydro-carbazol-1-ylidene)ethane-1,2-diamines derivatives (**3**)

Fig. (2). Cell viability in the presence of compounds (**21**, **22**) against *Salmonella enterica*, closed circles (●); *Staphylococcus aureus*, open squares (◊); and *Pseudomonas aeruginosa*, open circles (○). Values are the average of three trials.

Scheme 1. Reagents and condition

(i) 1,2-diaminoethane, ethanol, stirred at rt; (ii) NaBH_4 , ethanol, reflux for 1hr and then stirred for 2-3hrs.

Table 1
Structures of synthesized compounds (**11-24**) screened for antimicrobial activities.

Schiff's Bases	R ₁	R ₂	% Yield	Reduced forms	R ₁	R ₂	% Yield
11	H	H	86	18	H	H	91
12	H	OCH ₃	98	19	H	OCH ₃	86
13	H	OCH ₂ CH ₃	96	20	H	OCH ₂ CH ₃	87
14	Br	H	94	21	Br	H	87
15	Cl	H	95	22	Cl	H	89
16	Br	Br	72	23	Br	Br	82
17	Cl	Cl	94	24	Cl	Cl	86

Table 2Antimicrobial activity of compounds (**11-24**).

Compounds	Lethal concentration 50 (LC ₅₀) in μ M		
	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. enterica</i>
11	>500	>500	>500
12	>500	>500	>500
13	>500	>500	>500
14	>500	>500	>500
15	>500	>500	>500
16	>500	>500	>500
17	>500	>500	>500
18	>500 ^a	>500	>500
19	ND	ND	>500
20	ND	ND	>500
21	140	86	11.6
22	287	138	8.79
23	>500	>500	>500
24	>500	>500	>500

^a No antimicrobial effect with 500 μ M after 30 minutes exposure at 37°C. ND, not determined as was inactive against *S. enterica*. Values for the calculated LC₅₀ are the average from three independent trials.