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Antibacterial activity of Schiff base ligands containing pyridine and disulphide moieties against some chosen human bacterial pathogens

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The objective of the study was to evaluate the antibacterial activities of N,N'[1,1'-dithiobis(phenylene)]bis(benzyldeneimine), referred to as L1 and *o,o'*-(N,N-dipicolinyldene) diazadiphenyldisulfide, referred to as L2, containing disulfide moieties against some ophthalmic pathogens (*Klebsiella* species, *Escherichia coli*, *Streptococcus* species, *Proteus morganii, Pseudomonas* species, *Streptococcus pneumoniae, Acinetobacter* species, *Streptococcus pyogenes* and *Streptococcus viridans*), urinary tract infectious pathogens (*P. morganii, E. coli, Pseudomonas* spp., *Enterobacter* species, and *Klebsiella* spp.) and antibiotic resistant pathogens (*Staphylococcus* species, *Streptococcus*, *Pseudomonas* spp., and *Klebsiella* spp.) for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The MIC for the ophthalmic pathogens and antibiotic resistant pathogens were found to be 400 to 500 µg/ml, while for the urinary tract infectious pathogens a lower MIC value (200 µg/ml) was obtained. The MBC for the compounds against all the pathogens tested was 400 to 500 µg/ml. The synthesized Schiff L1 and L2 showed the MIC values for all the tested ophthalmic and antibiotic resistant bacterial pathogens more or less similar. Further studies are needed to prove the safe and efficacy needed for these compounds to develop as a drug after completing successful preclinical and clinical tests.

Key words: Antibacterial activity, minimum bactericidal concentration (MBC), minimum inhibitory concentration (MIC), N, N'[1,1'-dithiobis(phenylene)]bis(benzyldeneimine), ophthalmic pathogens, Urinary tract infectious pathogens (UTI), antibiotic resistant pathogens.

INTRODUCTION

Soon after each antibacterial agent entered into clinical practice, resistance was reported in at least one bacterial pathogen (Bell et al., 1998). Resistance determinants had already accumulated in the environment from which these tagents originated. The environmental resistance determinants were established shortly before the selection pressures that were being treated with the new antibiotics. The clinical utility of the antibiotic was severely

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diminished within a 5-year time span (Schmitz et al., 1999). The first documented example of the rapid selection of a resistant population was the increase in penicillin resistance from ≤8% to almost 60% in *Staphylococcus aureus* from 1945 to 1949 (Schmitz et al., 1999). That was why, the pharmaceutical industry has responded by designing new classes of drugs. Examples of these were the development of the 'penicillinase-stable penicillins' to counteract Gram-positive β-lactamases, aminoglycosides to avoid streptomycin resistance, cephalosporins to provide improved activity against Gram-negative pathogens, the ketolide telithromycin to avoid macrolide resistance in the pneumococci or linezolid in a novel class of synthetic oxazolidinones with no cross-resistance to any known antibiotic class. However, resistance has emerged to the new agents and thus repeating the cycle (Bush, 2004).

In the history of the antibiotic world, one might imagine that all resistance mechanisms could be overcome by some agents. Indeed, some have stated that virtually all infections can be treated by combination of effective drugs. Therefore, it is not necessary to develop new agents. Unfortunately, that is not the case, as evidenced by the multidrug-resistant enteric bacteria and the pan-resistant pseudomonas that are currently being treated with the toxic membrane-disruptive polymyxins (Evans et al., 1999). With the approval of the three most recent antibacterial agents, namely, linezolid in 2000, daptomycin in 2003 and telithromycin in 2002 to 2004, three new classes of agents have been introduced into the market place. However, resistance has already been reported for all the three agents (Evans et al., 1999), thus providing an opportunity for additional agents in these classes to overcome the new resistance.

However, there will be also unpredictable factors such as the appearance of new diseases, or the newly recognized association of established diseases with infectious agents (Paul, 2010). Finally, this will lead to the threat of bioterrorism due to epidemic outbreaks of resistant organisms. Although, the mail attacks of anthrax in the USA was fortunately conducted with a highly susceptible *Bacillus anthracis* strain in 2001 (Dalton, 2001). It is now well established that antibiotic resistant strains can be readily selected *in vitro* (Brook et al., 2001), thus leading to the possibility of more resistant strains appearing in the future. Opportunities are available to approach this disease more creatively, with the development of new drugs to attack the toxin and the use of classical antibacterial agents to eradicate the bacteria, because inhalation anthrax is the most frequently causing fatal disease due to rapid toxin production (Shen et al., 2004). Development of safe and effective medicines is still an option that is frequently ignored. Hence, this study has been focused on the development of safe and effective medicines. Derivatives of pyridine (Mei-Ying et al., 2005; Li-Xia et al., 2006; Tarafder et al., 2001) and diphenyl-disulphides (Bhowonet al., 2005; Jiang et al., 2003) are known to possess significant antibacterial, antifungal, anticancer and anti-human immunodeficiency virus (HIV) properties. This gave a great insight to search for potential pharmacologically active Schiff bases containing pyridine and disulphide moieties.

MATERIALS AND METHODS

Chemistry

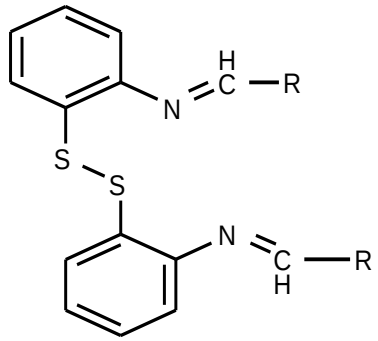
Melting points were uncorrected and were determined on a Stuart Scientific Electric Melting Point Apparatus. Infrared (IR) spectra (KBr) were recorded on a Mattson 1000 Fourier transform Infrared (FTIR) spectrometer in the range of 400 to 4000 cm^{-1}. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker spectrometer at 250 MHz.

Synthesis of phenylenediimines N,N'[1,1'-dithiobis(phenylene)]bis(benzyldeneimine) (L1) and *o,o'*-(N,N-dipicolinyldene) diazadiphenyldisulfide (L2)

To a vigorously stirred solution of bis (2-aminophenyl) disulphide (0.9 mmol) in EtOH (40 ml), benzaldehyde or 2-pyridine carboxaldehyde (1.9 mmol) was added. The yellow mixture was allowed to stir at room temperature. The precipitated product was collected by filtration, washed thoroughly with EtOH (10 ml) and dried *in vacuo*. **L1**: mp: 140°C (Krebs, 1995); ^{1}H NMR: δ 8.74 (s, 2H, 2-CH=N), 8.02 (m, 4H, Ar-H), 7.59-7.55 (m, 8H, Ar-H), 7.34-7.24 (m, 6H, Ar-H); ^{13}C NMR: δ 118.4, 125.7, 127.7, 128.0, 129.5, 131.5, 132.5, 136.3, 148.8, (benzene carbons), and 161.5 ppm (2 imine carbons); UV-Visible: 258 (4507), 324 (11,646). **L2: m**p: 139 to 140°C (lit 139 to 140°C); ^{1}H NMR**:** δ 8.71 ppm (d, J=5, 2H, 2-pyridyl-H), 8.66 (s, 2H, 2-CH=N), 8.33 (d, J=8, 2H, 2-Ar-H); 7.81 (dt, J=16, 8, 1, 2H, 2-pyridyl-H), 7.61 (dd, J=7, 2, 2H, 2-pyridyl H), 7.32 ppm (dt, J=12, 5, 1Hz, 2H, 2-pyridyl-H), 7.14 (m, 6H, 6-Ar-CH); ^{13}C NMR**:** δ 122.2, 126.0, 136.8, 147.8, 154.5 (pyridyl –carbons) δ 117.3, 125.5, 127.1, 127.9, 132.7, 149.7 (benzene carbons), and 160.4 ppm (2 imine carbons); UV-Visible: 260 (31,382), 285 (25,947), 349 (11,295).

Minimum Inhibitory Concentration (MIC)

Different concentrations of stock solutions were prepared by dilution with sterile distilled water so as to obtain 200, 300, 400, 500 and 600 µg/ml of L1 and L2. MIC determination was carried out by mixing 5 ml of varied concentration of L1 and L2 and mixed with 0.5 ml of nutrient broth. 50 µl of bacterial inoculums of ophthalmic pathogens, namely, *Klebsiella* species*, Escherichia coli*, *Streptococcus* species, *Proteus morganii, Pseudomonas* species, *Streptococcus pneumoniae, Acinetobacter* species, *Streptococcus pyogenes* and *Streptococcus viridans,* obtained from Aravind Eye Hospital, Madurai, India; Urinary tract infectious pathogens (UTI), namely, *P. morganii, E. coli, Pseudomonas* spp., *Enterobacter* species, and *Klebsiella* spp. obtained from Vivek Laboratories, Nagercoil, India and antibiotic resistant pathogens, namely, *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Streptococcus pneumoniae* and *Klebsiella* spp. resistant to co-trimoxazole, gentamycin, ampicillin, tetracyclin, chloramphenicol, ciprofloxacin, and kanamycin, obtained from Vinayaga Mission Hospital, Salem India. Nutrient broth alone served as negative control. Whole setup in duplicate was incubated at 37°C for 24 h. The MIC was the lowest concentration of the synthetic compounds that did not permit any visible growth of bacteria during 24 h of incubation after inoculation examined on the



L 1 = C_6H_5
L 2 = C_5H_5N

Figure 1. Structure of L1 and L2. L1: R=Ph; L2: R= 2-pyridyl.

basis of turbidity (Hammond and Lambert, 1978).

Minimum bactericidal concentration (MBC)

To avoid the possibility of misinterpretations due to the turbidity of insoluble compounds if any, the MBC was determined by sub-culturing the MIC serial dilutions after 24 h in Nutrient agar plates using 0.01 ml loop and incubated at 37°C for 24 h. MBC was regarded as the lowest concentration that prevents the growth of bacterial colony on this solid media (Hammond and Lambert, 1978).

RESULTS

Chemistry

The Schiff bases L1 and L2 (Figure 1) were synthesized by the reaction of bis (2-aminophenyl) disulphide with benzaldehyde (Bhowon et al., 2007) and 2-pyridine carboxaldehyde (Uma and Palaniandavar, 1993) in ethanol. The IR spectrum of L1 and L2 showed a sharp peak at 1623 to 1624 cm^{-1} (Bhowon et al., 2005) indicating the presence of imines group, whereas the peak at 1583 cm^{-1} was due to C=N of the pyridine in L2. The other spectral data of L1 and L2 as described in the experimental section were concordant with the proposed structure. The mass spectrum of L1 showed peaks at m/z 425 and 214 corresponding to the molecular formula $C_{26}H_{20}N_2S_2$ (100%) and a fragment ion $C_{13}H_{10}NS$ (12%). The mass spectrum of L2 showed peaks at m/z 427 and 428 accounting for the molecular ion $[C_{24}H_{18}N_4S_2]^+$ (M^+, 100% base peak) and for $[C_{24}H_{19}N_4S_2]^+$ (M+1, 37.2% base peak) ion peaks. Other peaks at m/z 245 were assigned to the ion $[C_{12}H_9N_2S_2]^+$ (5.7% base peak).

Determination of MIC of synthesized compounds L1 and L2 were carried out against 9 ophthalmic bacterial pathogens, 5 urinary tract infectious bacterial pathogens and 5 antibiotic resistant bacterial pathogens. In addition, multidrug resistant *S. aureus* strains are often isolated from human clinical specimens (Oplachenova and Obreshkova, 2003). The results reported in Table 1 show differential sensitivity of the investigated bacterial pathogens. The concentration of L1 and L2 required to inhibit bacterial growth were higher for the chosen bacterial pathogens. Moreover, the MIC values for all the tested ophthalmic and antibiotic resistant bacterial pathogens were quite similar (400 to 500 µg/ml) for L1 and L2 compounds. Lower MIC concentration was reported against urinary tract infectious bacterial pathogens (200 µg/ml). MIC values for *S. aureus* were similar for all tested fractions, while MIC for *Bacillus subtilis* varied between 0.15 and 2.50 µg/ml, depending on the fraction applied (Dragana et al., 2005). Lower sensitivity of *S. aureus* compared to *B. subtilis* to sage essential oil was also reported by other authors (Carvalho et al., 1999). MBC results for all the tested ophthalmic pathogens, UTI pathogens, and antibiotic resistant pathogens were more or less similar (400 to 500 µg/ml) for L1 and L2 compounds.

Table 1. MIC and MBC determinations of 2 synthesized compounds (L1 and L2) against chosen ophthalmic, UTI and antibiotic resistant pathogens.

Pathogen	MIC (µg)		MBC (µg)	
	L_1	L_2	L_1	L_2
Ophthalmic pathogens				
Klebsiella species	500	400	500	500
Escherichia coli	400	400	400	500
Streptococcus species	500	500	500	500
Proteus morganii	400	500	400	400
Pseudomonas species	500	400	500	500
Streptococcus pneumoniae	500	400	500	500
Acinetobacter species	500	500	500	400
Streptococcus pyogenes	500	500	500	500
Streptococcus viridans	400	500	400	500
UTI Pathogens				
Proteus morganii	400	300	500	500
Escherichia coli	300	400	500	500
Pseudomonas species	200	400	500	500
Enterobacter species	400	300	500	500
Klebsiella species	300	400	400	400
Antibiotic resistant pathogens				
Staphylococcus aureus	500	500	500	500
Streptococcus species	400	400	400	400
Pseudomonas aeruginosa	500	500	500	500
Streptococcus pneumoniae	500	500	500	500
Klebsiella species	400	400	400	500

Moreover, the differential MIC value is due to the differences in their physiology as well as in the variations in their sensitivity. Although, the MIC test is considered more accurate for quantitative evaluation of antimicrobial activity, it does not represent an absolute value either. MIC is somewhere between the lowest test concentration which inhibits the bacterial growth and the next lower test concentration.

DISCUSSION

Bactericidal activity of antimicrobial agents can also be assessed by performing a MBC test. Table 1 presents data on the MBC values of tested compounds L1 and L2 against chosen bacterial pathogens. Both of the investigated compounds exhibited a strong bactericidal effect against the tested bacteria. However MBC is slightly higher compared with the MIC values due to the varying in the incubation period. Moreover, the survival of the tested pathogens at higher concentrations of MBC is probably due to the presence of endospores, which are resistant to conditions to which vegetative cells are intolerant. This study was designed to study the MIC and MBC against some ophthalmic pathogens, UTI and anti-biotic resistant pathogens. The synthesized Schiff bases L1 and L2, showed the MIC values for all the tested ophthalmic and antibiotic resistant bacterial pathogens quite similar. Therefore, both compounds could score as putative drugs.

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