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Synthesis of disulphide-Schiff base derivatives and investigations of *in vitro* antimicrobial activities against some human pathogens

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Abstract

Thio-Schiff bases are becoming increasingly widespread in various branches such as the preparation of certain medicines, cosmetic products, and polymer production. In particular, the presence of antibacterial, antifungal, antiviral, antitumor and antimalarial properties of Schiff bases containing sulfur in the structure has made these compounds attractive in different disciplines. In this study, different derivatives of dimeric disulfide-Schiff bases have been synthesized.

The antibacterial and antifungal activities of the synthesized these compounds were investigated *in vitro* against some human pathogens (*Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Candida albicans*, *C. tropicalis*, *C. guilliermondii* and *C. glabrata)*. Test microorganisms were isolated from the patients appyling to Medical Faculty Hospital of Duzce University were used. Diffusion method was used to determine the antimicrobial activities of the compounds.standard antibacterial (Cefotaxime, Amoxicillin/clavulanicacid) and antifungal (Posaconazole) antibiotics were used as the control group and the results were compared.

The result indicated that antimicrobial activity of Disulphide-Schiff Base Derivatives exhibited less activity against bacteria as compared to AMC30 (Amoxicillin/clavulanicacid), but highly effective against bacteria as compared to CTX30 (Cefotaxime). In addition, the compounds exhibited less activity against yeast.

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Introduction

Schiff bases are compounds with the structure consisting of a -C=N- bond, generally formed by condensation of an amine and active carbonyl group, initially developed by Hugo Schiff (1, 2). Despite many years of work on Schiff bases, research on compounds containing ONS (oxygen-nitrogen-sulphur) donor atoms has been limited. However, thio-Schiff bases and their derivatives play an important role in the development of modern inorganic chemistry. Thio-Schiff bases constitute one of the broadest classes of Schiff bases obtained by condensation of active carbonyl groups and amine compounds which generally form azomethine -C=N- bonds (3). The Super Bug (multidrug resistant organisms) poses a significant threat to global health. Schiff bases bearing sulphur, aryl or heterocyclic groups having nitrogen are known to possess biological activities (4).Recent studies have focused on the biological, especially antitumor (5-7), antibacterial (8, 9), antifungal, anti- HIV (10) properties of disulphide-Schiff bases. *In vitro* biological screenings of the synthesized ligands were carried out against the human pathogens. We have been observed that the ligands have significant affect against test microorganisims.

Materials and Methods

Reagents

Fourier transform infrared-attenuated total reflection spectroscopy (FTIR–ATR) results were recorded with using Perkin Elmer spectrometer, and wave numbers were averaged across the spectral range of 550–4000 cm^{-1}. Elemental analyses were carried out a Thermo

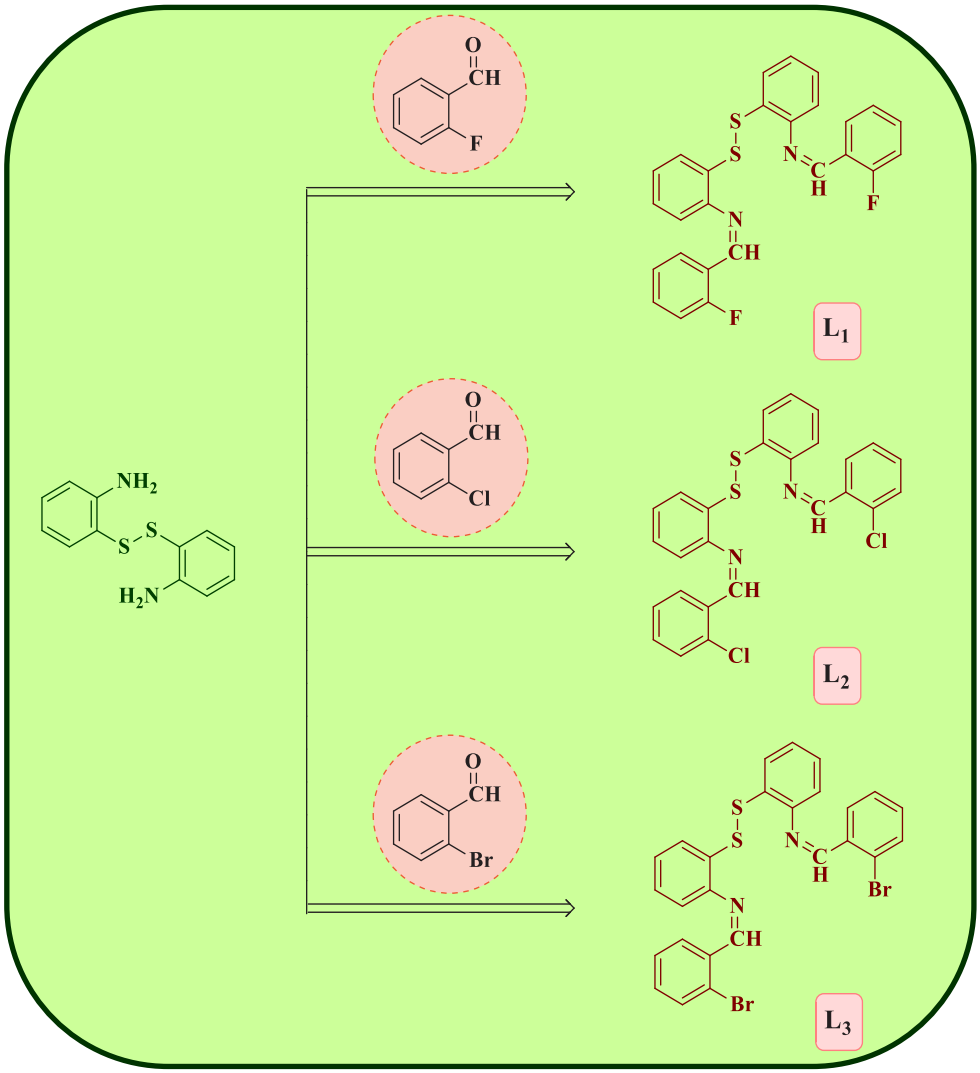

Figure 1. Synthesis of dimeric disulphide-Schiff base derivatives.

Scientific Flash 2000.All reagents were purchased in commercially. The compounds 2-floro benzaldehyde, 2-chloro benzaldehyde, 2-bromo benzaldehyde and solvents were purchased from Merck.

Synthesis of dimeric disulphide-Schiff bases

Synthesis of 2,2'-diaminodiphenyl disulphide: 2,2'-diaminodiphenyl disulphide was synthesized by oxidation of 2-aminothiophenol (11). Compounds were synthesized according to previous studies (11, 12). In this study, different derivatives of Schiff bases were synthesized as a result of condensation of dimeric sulphurous aromatic a amine and various aldehydes (NZ,N'Z) - 2,2' - disulfanediylbis (N-(2-fluorobenzylidene) aniline), (NZ,N'Z)-2,2'-disulfanediylbis (N - (2-chlorobenzylidene)aniline), (NZ,N'Z)-2,2'-disulfanediylbis (N-(2-bromobenzylidene)aniline). The synthesized dimeric disulphide-Schiff base compounds are given in **Fig. 1**.

Test organisms

The bacteria and fungi species in this study were isolated from the patients appyling to Medical Faculty Hospital of Duzce University. Microorganisms samples were incubated at 30-35°C for 24h in Brain Heart Infusion Broth for identification. Afterwords, the counts of bacterial and yeast cultures were adjusted to yield 10^7 -10^8 /mL and 10^5 -10^6 /mL, respectively, using the standard McFarland method. The isolates were identified with VITEK 2 microbial identification system (BioMeriéux, France).

Disc diffusion method

The antibacterial and antifungal activities of the compounds have been screened *in vitro* against the organisms *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae* (Gram negative bacteria), *Staphylococcus aureus* (Gram positive bacteria) and *Candida albicans*, *C. tropicalis*, *C. guilliermondii*, *C. glabrata*. The antimicrobial activity of the compounds was evaluated using the disc diffusion method (13). For this purpose, stock solutions of the compounds were prepared in DMSO to a final concentration of 30µg/mL. All the bacteria were incubated at 30-35°C for 24h in Nutrient Broth. The yeasts were incubated in Sabouraud Dextrose Agar (SDA) for 48h. Inoculums containing 1.5x10^8 cfu/ml bacterial cells or yeast cells were spread on Mueller Hinton Agar plates (1mL inoculums for each plate).

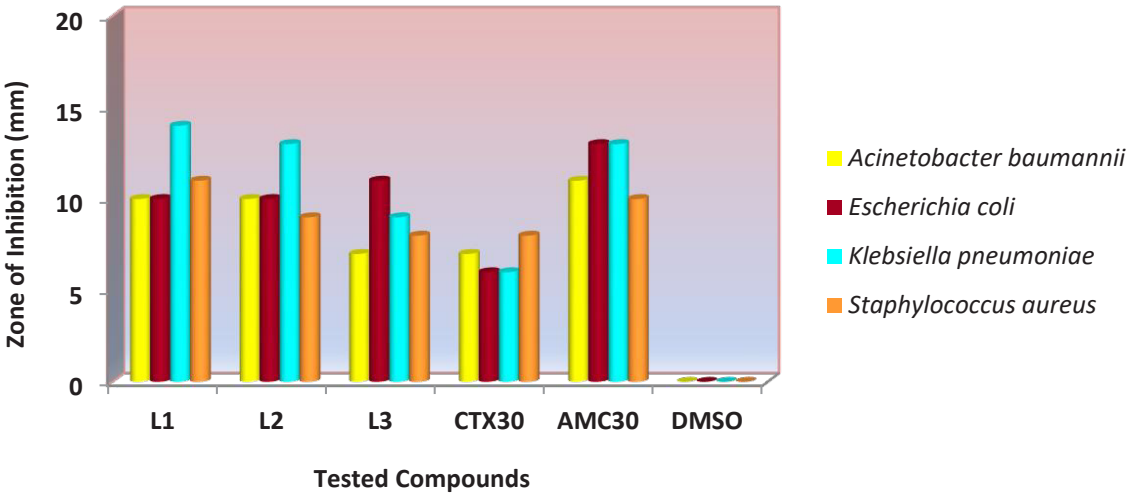

Figure 2. The in vitro antibacterial activity of compounds (**L1-L3**).

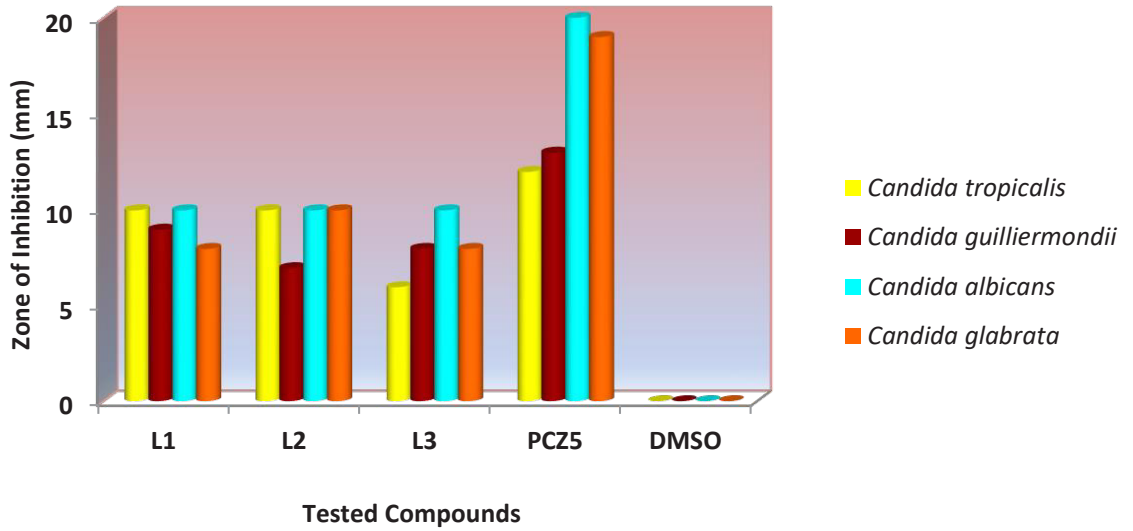

Figure 3. The in vitro antifungal activity of compounds (**L1-L3**).

After 50µL of these compounds were impregnated into standard sterile discs (6mm, Bioanalyse) and the discs were placed on the agar and incubated at 35 °C (24h) and at 25 °C (72h) for bacteria and yeast, respectively. As the control group standard antibacterial antibiotics (CTX30: Cefotaxime30µg; AMC30: Amoxicillin/clavulanicacid 30µg) and antifungal antibiotic (PCZ5: Posaconazole 5µg) were used and the results were compared.

Result and Discussion

Dimeric disulphide-Schiff base derivatives have been showed biological activities against different types of microorganisms (14).The analytical and physical data of obtained the compounds are shown in (**Table 1**). According to the results, the *in vitro* antimicrobial activities of compounds L_1, L_2 were investigated and L_3, the inhibition zones measured against various clinical isolates are summarized in (**Table 2, Fig. 2** and **Fig. 3**). Especially, the compound L_1(fluorine contained compound) showed more effect against bacteria as compared to other compounds L_2, L_3. When the results of the antimicrobial activity are examined, it has been found that the test compounds are more effective against the bacteria than the yeast. *Klebsiella pneumaniae* is the most sensitive bacterium that is affected by L_1, having the diameter zones of 14mm. *Acinetobacter baumannii* have been the most resistant bacterium with a diameter of 7mm which is least affected by L_3, and this compound has similarly effect with the standard antibiotic CTX30. The compounds L_1 and L_2 show a similar antibacterial effect on *Acinetobacter baumannii* and *Escherichia coli*. Besides, the compound L_2 is equivalent to the standart antibiotic AMC30 against the *Klebsiella pneumaniae*. Compound L_1 was showed good antibacterial activity against *Staphylococcus aureus* as compared to standard antibiotics CTX30 and AMC30. L_1 and L_2 compounds were similar affected against *Candida tropicalis* and *C. albi-*

Table 1. Analytical and physical data of the obtained compounds

Compounds	Color	Yield (%)	MP (°C)	IR (cm^{-1}) $\nu_{C=N}$	Analytical Calculated / Found Calculated (%) C	H	N	S
L_1	Yellow	85	175	1610	67.80	3.94	6.08	13.92
					67.86	3.85	6.11	13.86
L_2	Yellow	90	173	1614	63.28	3.68	5.68	13.00
					63.39	3.74	5.73	13.12
L_3	Pale yellow	93	156	1613	53.62	3.12	4.81	11.01
					53.73	3.07	4.75	11.19

Table 2. The *in vitro* antibacterial and anti-Candidal activity of compounds (L_1-L_3)

Inhibition zones (mm)*						
	Compounds			Antibiotics		
Microorganisms (Gram positive bacteria)	L_1	L_2	L_3	CTX30	AMC30	PCZ5
Acinetobacter baumannii	10.0	10.0	7.0	7.0	11.0	-
Escherichia coli	10.0	10.0	11.0	6.0	13.0	-
Klebsiella pneumaniae	14.0	13.0	9.0	6.0	13.0	-
Microorganisms (Gram negative bacteria)						
Staphylococcus aureus	11.0	9.0	8.0	8.0	10.0	-
***Candida* species**						
Candida tropicalis	10.0	10.0	6.0	-	-	12.0
Candida guilliermondii	9.0	7.0	8.0	-	-	13.0
Candida albicans	10.0	10.0	10.0	-	-	20.0
Candida glabrata	8.0	10.0	8.0	-	-	19.0

CTX30: Cefotaxime 30 µg; AMC30: Amoxicillin/clavulanicacid 30 µg; PCZ5: Posacanazole 5 µg
(*): The figures on the scale show the inhibition diameters.

cans. However, L_3 did not show any activity against *C. tropicalis*. Generally, L_1, L_2 and L_3 compounds showed a low activity against *Candida* species when compared to the standard antibiotic PCZ5. According to previously, a series of Schiff bases were assayed for antibacterial and antifungal activities using disc diffusion method by Narain *et.al.* Researchers have found-

ed that the [bis(p-methoxybenzaldimino)phenyldisulfide] has the most favourable antibacterial and [o,o'-(N,N'-dipicolinyldene)diazadiphenyldisulfide] antifungal activities with MICs of 1mg/mL and 0.025 mg/mL against *S. aureus* and *C. albicans* respectively (4). A similar study, *in vitro* biological screening of the synthesized Schiff-bases has been carried out against the

phyto pathogenic bacteria (*Azotobacter* and *Rhizobium*) and fungi (*Aspergillus niger* and *Fusarium oxysporium*). It has been observed that the antimicrobial activities of metal complexes are higher than the free ligands (15).

Conclusion

In this study, three dimeric disulphide-Schiff base derivatives were synthesized and the in vitro antimicrobial activities of these synthesized compounds were examined against human pathogens. The aromatic substituents present in the *ortho* position in these synthesized disulphide-Schiff base compounds showed different antimicrobial activity with their different electronic properties. Furthermore, the presence of the disulfide bond in the compounds is very important for antimicrobial activity. In particularly, L_1 had a greater effect on bacterial resistance than other compounds L_2, L_3. The fluorine containing L_1 compound has been exhibited antimicrobial activity higher than bromine and chlorine containing L_2 and L_3 compounds. As a result of, our study indicate that test compounds have similarly affected against test microorganisms as compared to antibiotics. The results of our study may help to obtain new antibiotics in advanced pharmacological research.

Acknowledgements

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Conflict of interest statement

The authors declare no commercial or financial conflict of interest.

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