IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 11, May 2026
Impact Factor: 8.2

Pharmacological Activities of DI-IMINE Bases and their Antimicrobial Study

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Abstract: *This paper is normally focused on the pharmacology and design of Vaniline containing di-imines. They can be prepared by the condensation of the primary amines with carbonyl compounds. In this paper, we are going to synthesized new derivatives of di-imines SB1 and SB2 by condensing semithiocarbazide and 4-amino antipyrine with 4,4'-[butane-1,4-diylbis(oxy)] bis(3-methoxybenzaldehyde) in DMF solvent. They are potent intermediates for the synthesis of pharmaceutical and bioactive materials and thus, they are used extensively in the field of pharmaceutical chemistry. di-imines of semithiocarbazide and 4-amino antipyrine are often having promising biological activities like anti-inflammatory, antidepressant, antiglycation, antibacterial, etc. The structures of the compounds have been characterized on the basis of their IR data. Studies have been conducted on compounds bearing -N-C=S, and-CH=N- as pharmacophore. Accordingly, by considering this biological potential herein, this paper also provides a brief overview on biological applications, namely potent candidates with antibacterial and antifungal activities. This work is aim to organize the different biological activities of some synthesized semithiocarbazide and 4-amino antipyrine derivatives. They were tested against two gram positive (bacillus subtilis, Staphylococcus aureus) and two gram negative bacteria (P. vulgaris, E. coli) bacterial strains and the antifungal activity of di-imine ligand was carried out using C. albicans by using agar well diffusion method.*

Keywords: Semithiocarbazide, 4-amino antipyrine, Vaniline, Schiff bases, Antimicrobial Activities

I. INTRODUCTION

The term Schiff's base derives from the name of the German chemist Hugo Schiff, who reported in 1864, to describe the products resulting from the reaction of primary amines with carbonyl compounds [1]. Schiff bases (imines) are defined as chemical compounds Consist of a hydrocarbyl group on the nitrogen atom $R_2C = NR$. They are the products of condensation between aldehydes or ketones (active carbonyl compound) with specifically primary amines called aldimines or ketimines. They are also considered as synonymous with azomethines [2]. The different applications of di-imine and their complexes are because of having coordination sites containing donor atoms, so that they can be used as organometallic reagents, Diels Alder reactions, various reactions liquefaction reaction taking place between different aldehyde and amines. [3] For the last decades, a lot of research groups have been investigating Schiff bases and reporting on the huge scope of bioactivities these compounds possess–antiviral [4,5], antifungal [6], antibacterial [6,7], antioxidant [7,8], anti-inflammatory [8], antitumor [9,10], anticancer [11] and antimicrobial properties [10,12]. Additionally, their potential use as urease inhibitors has also been suggested [4-12]. The Understanding the biological gap and magnifying the potency of these Schiff bases may benefits from antibacterial and antifungal resistance [13]. SBs were showed antibacterial activity against some bacterial strains like *Acinetobacter baumannii*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli*, *M. tuberculosis*, *Micrococcus luteus*, *Micrococcus flavus*, *Proteus vulgaris*, *Salmonella enteric*, *Staphylococcus aureus*, *Streptococcus epidermidis* and *S. pyogenes*, SBs were reported to exhibit antifungal activity against fungal strains including *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Candida tropicalis*, *Candida guilliermondii*, *Candida glabrata*, *Cryptococcus neoformans*, *Epidermophyton floccosum*, *etc*. [14-20]. Large number of Schiff bases containing N, O, S as donor atoms are obtained by huge variety of applications in field like food industry, dye industry, luminescence chemosensors, intermediates in many organic reactions as well as in photoactive solar energy and in agrochemicals [21-23]. In recent decades, numerous researchers focused on forming new framework Schiff base resulting an interest in kinetic, thermodynamic, chelating, magnetic properties [24]. Some of Schiff bases are utilized for their unpleasant fragrance or taste so that they can be used as healthy beverages derived from raw material like protein, starch, and carbohydrates. Now days, Schiff bases are extensively playing integrative role in different field including metal ion detectors, ion selective detectors in colorimetric and fluorescent probes, in material science they have been used as nanocomposites, in biomedical field used as biosensors, drug delivery systems and platforms for tissue related applications [25-26].

Increasing knowledge of cancer chemotherapy, it is needful to design new compounds to overcome the resistance of available anticancer drugs amino phosphonates are an important class of compounds that have received wide attention due to various pharmaceutical applications such as anticancer, antiviral, antibacterial drugs They showed a low inhibition of cell vitality of mammalian cells which defines them as promising drug candidates and necessitates the extensive study of their pharmacological potential [27-30].

II. EXPERIMENTAL PROTOCOLS

2.1 REAGENTS AND INSTRUMENTS

For synthesizing the di-imine ligands, we were used analytical grade chemicals and were used without further purification. All reagents, 4-aminoantipyrine, Vaniline, Semithiocarbazide were of Aldrich and Merck products. Commercial solvents were distilled and then used for the preparation of Schiff base ligands. Microanalyses (C, H and N) were performed using the Perkin Elmer elemental analyzer. Infrared spectra were measured using KBr discs on a Jasco FT-IR 410 spectrometer in the range 4000-400cm^{-1}. Purity of Schiff base ligands were monitored by using Thin Layer Chromatography (TLC) and iodine chamber was used to detect the spot of Schiff bases.

2.2 METHODOLOGY

Protocol-I: Synthesis of 4,4'-[butane-1,4-diylbis(oxy)] bis(3-methoxybenzaldehyde)

0.2 millimoles of Vaniline and 0.1 millimoles of potassium carbonate was dissolved in 100 ml DMF, to this solution portion wise addition of 0.1 millimoles of 1,4-dibromobutane in DMF for about 1 hrs. at room temperature. The reaction mixture then heated for 4 hours at 170-180 ^{0}C. The progress of reaction was monitored using TLC. Precipitate obtained was cooled at room temperature, filtered and wash with plenty of distilled water. Precipitate was then filtered in vacuum and dried and recrystallized from n-butanol.

Yield: - 88%, M.P. 152-154^{0}C, empirical formula- ($C_{20}H_{22}O_6$), Mol.Wt. 358.39 gm.

Protocol-II: Synthesis of di-imine ligands using semithiocarbazide (SB1) and 4-amino antipyrine (SB2) with Dialdehyde Compound

The di-imine ligands were prepared by reacting dialdehyde compound with drop-wise addition of 0.2 millimoles equimolar mixture of semithiocarbazide or 4-amino antipyrine in DMF solvent under constant stirring at elevated temperature, little drop of HCl was introduced to aid the condensation process. Mixture was then refluxed for 4-5 h, TLC was used to track and record the reaction progress. The resulting product was then subjected to cooled at room temperature, wash with distilled water plenty of times to eliminate unreacted matter. The resulting yellow coloured product was subjected to recrystallization using DMF: Ethanol (9:1) system. The purified compound was filtered, rinse with ethanol and dried in oven.

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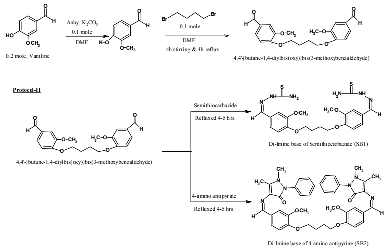

Fig. 1: Reaction Scheme

Table 1: Indicating physical and Analytical data of newly synthesized Schiff bases.

Compounds [Mol. Formula]	Mol. Wt. gmol^{-1}	Color	Crystallization Solvent	M. P. (^{0}C)	Yield %	Elemental Composition in % Found /(Calc.)		
						C	H	N
Dialdehyde [$C_{20}H_{22}O_6$]	358.39	White	n-Butanol	152-154	88	67.02 (67.09)	6.19 (6.12)	---
Di-Imine base (**SB1**) [$C_{22}H_{28}O_4N_6S_2$]	504.64	Pale yellow	DMF: Ethanol (9:1)	220-222	74	52.36 (52.40)	5.59 (5.52)	16.65 (16.61)
Di-Imine base (**SB2**) [$C_{42}H_{44}N_6O_6$]	728.85	Pale yellow	DMF: Ethanol (9:1)	202-204	81	69.21 (69.25)	6.09 (6.02)	11.53 (11.59)

III. RESULT AND DISCUSSION

The former di-imine ligands have been synthesized from reaction between semithiocarbazide and 4-aminoantipyrine with dicarbonyl moiety to form di-imines in DMF solvent by producing excellent yield of products. The synthesised di-imine ligands are insoluble in water, but sparingly soluble in ethanol, chloroform, acetone, ethyl acetate, ether solvents but freely soluble in dimethyl formamide (DMF) and Dimethyl Sulfoxide (DMSO). Their structures were determined by using IR technique and elemental analysis. Below data indicating the physical and analytical data of prepared di-imine ligands and dicarbonyl compound outlined in Table 1.

DOI: 10.48175/IJARSCT-35833

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3.1 IR SPECTRAL ANALYSIS

IR spectroscopy provides valuable insites to examines different vibrational insites of molecules. It allows details about functional groups. It is an instrumental technique to identifying peaks associated with C=N vibrations. In FTIR Spectra of former Schiff bases were examined in Nujol or KBr medium in 450-4000 cm^{-1} range to determine the formation of di-imine ligand, Strength of bond and to investigate the various vibrational bonding modes in Schiff bases. In solid state the signal obtained at 1681 cm^{-1} for SB1 and 1664 cm^{-1} for SB2 conforms the presence of v (C=N) i.e. azomethine group indicating establishment of linkage between di-imine [31-33]. Moreover, absence of aldehyde and amine C=O and NH_2 vibrations, serves as a fundamental mechanism of production of di-imine.

The FTIR spectra of dialdehyde shown that peak appears at 1675 cm^{-1} is indicating presence of carbonyl group (C=O) [38]. The lower frequency observed for carbonyl group is because of presence of conjugation. As well as signals appears at 1584, 1506, 1464 indicates presence of aromatic ring (C=C) [35]. The signals appear at 1261, 1150, 1031 indicating (C-O) stretching vibrations [37]. The peak obtained in the range of 2936 and 2921 was because of stretching (C-H) vibrations [38].

The compound SB1 is characterized by the appearance of absorption bands obtained at 1591, 1539,1503 cm^{-1} is due to presence of aromatic (C=C) stretching frequency. Another band appears due to stretching frequency at 1333, 1398 $cm^{-1}$of (C-N) [35]. FTIR spectra indicate presence of N-H stretching vibrations of semithiocarbazide at 3161, 3120, 3080 cm^{-1}[33, 34]. Other signal belongs to (C-O) vibrations at 1235, 1133, 1033 cm^{-1} [36]. The spectrum of SB1 showed a signal at 1265 cm^{-1} indicating presence of (C=S) vibrations [42].

The FTIR spectra of SB2 exhibit intense band at 1664 cm^{-1} indicating formation of imine bond (C=N) between carbonyl group and NH_2 group [31]. The band assigned at 1595 cm^{-1} confirms exocyclic ketonic carbonyl group of anti-pyrine [37-39]. Presence of 1230, 1160, 1035 cm^{-1} was indicating (C-O) [36, 37]. [26-Anti]. Additionally, bands displayed at 2930 cm^{-1} and 2860 cm^{-1} indicating presence of (C-H) stretching [38]. The absorption peak obtained at 1574, 1508, 1463 cm^{-1}assigned as aromatic (C=C) band [42]. The band obtained in the range of 1384, 1306 cm^{-1} was due to vibrations involving interaction between (C-N) stretching [40, 41].

Table 2. The selected IR Spectral data of the prepared compounds

Comp. Code	υ(C=O) cm^{-1}	υ ald (C-H) cm^{-1}	υ(C=N) cm^{-1}	υ (C-N) cm^{-1}	Ar (c=c) cm^{-1}	(C-O) cm^{-1}	υ (C-H) cm^{-1}	υ (N-H) cm^{-1}	(C=S) cm^{-1}
B	1675	1275	-	-	1584, 1506, 1464	1261, 1150, 1031	2936, 2921	-	-
SB1	-	-	1681	1333, 1398	1591, 1539, 1503	1235, 1133, 1033	2980, 2990	3161, 3120, 3080	1265
SB2	1595	-	1664	1384, 1306	1574, 1508, 1463	1230, 1160, 1035	2930, 2860	-	-

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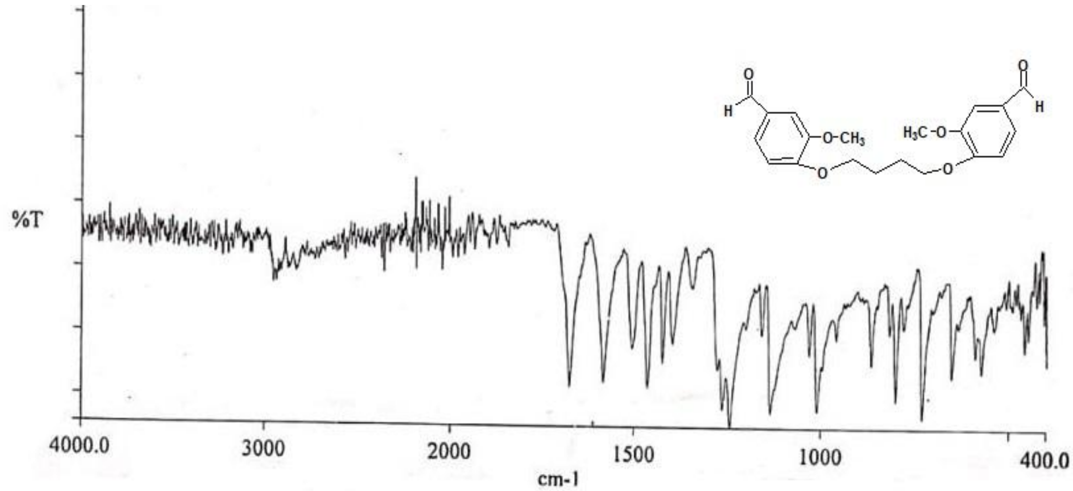

Figure 2. FT-IR spectrum of 4,4'-[butane-1,4-diylbis(oxy)] bis(3-methoxybenzaldehyde

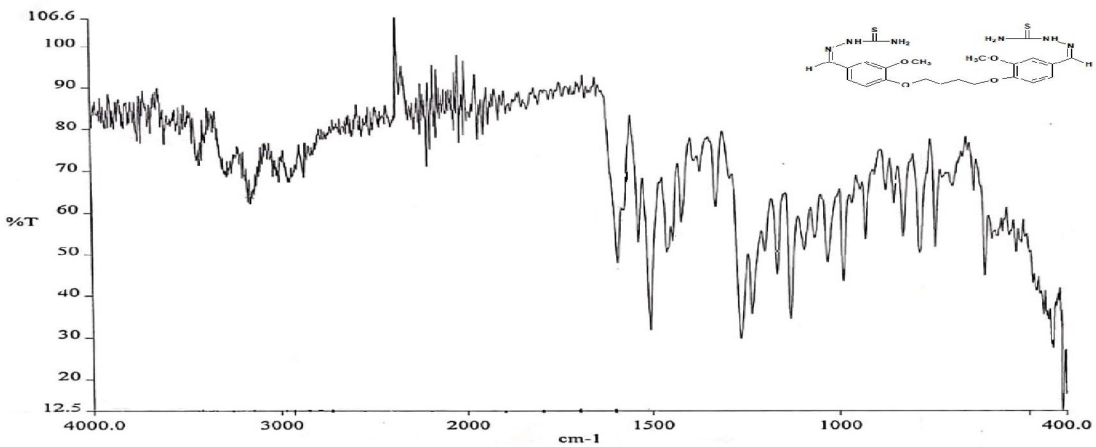

Figure 3. FT-IR spectrum of Di-Imine base (SB1)

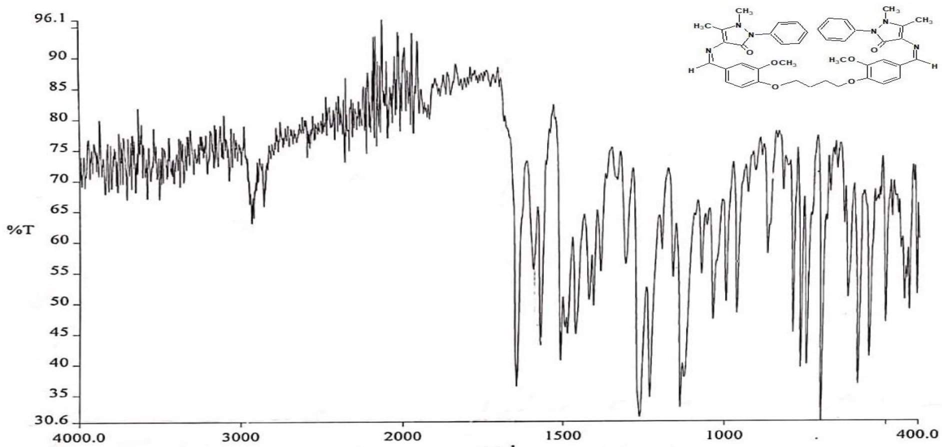

Figure 4. FT-IR spectrum of Di-Imine base (SB2)

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IV. ANTI-MICROBIAL STUDIES

The bacteriological activity of the di-imine ligand was carried out by using two gram positive (*bacillus subtilis*, *Staphylococcus aureus*) and two gram negative bacteria (*P. vulgaris, E. coli*) under different concentration. The antifungal activity of di-imine ligand was carried out using C. albicans. The antimicrobial activity of ligands was shown in **Figure 4.** The agar well diffusion method was employed for the bacteria with respect to standard drug as Ciprofloxacin and fluconazole as a standard drug for antifungal activity. The antimicrobial studies suggested that the di-imine ligands were found to be biologically active against microbial strains [43].

The solution of di-imine ligands was prepared containing 100 mg/ml concentration using DMSO as solvent. 100 µL volume of test solution having concentration 500, 1000, and 2000 µg per disc were put into four wells plates (6 mm in diameter). Two out of four wells have one Schiff base (SB1) and two have another Schiff base (SB2). DMSO solvent was used as negative control and anti-bacterial drug Ciprofloxacin was used as standard drug and Fluconazole drug was used as standard antifungal drug. The results of the antibacterial and antifungal activity showed that the activities of the synthesized di-imines were screened against different types of minimum inhibition concentrations (MIC). The result indicated that, SB2 ligand shown grater potency at concentration of 500 µg against all stain in comparison to SB1. The antibacterial and antifungal efficacy of di-imine ligand prepared from 4-amino antipyrine against *S. aureus, P. vulgaris, E. coli* and *C. albicans* at concentrations 500 µg showed considerable action against standard antibacterial drug Ciprofloxacin and antifungal drug Fluconazole with inhibition zone 16-18 mm range. Therefore, the inhibition zone results were indicated that SB2 ligand showed moderate antimicrobial activity against the given strains, the inhibitory zone values of each di-imine ligand have been shown in the table below. [44, 45]

Table 3. Antibacterial and antifungal activities of the di-imine ligand

Compo-unds	Conc. (µg/disc)	Zone Inhibition Diameter (mm)				
		Gram +ve Bacteria		Gram –ve Bacteria		Fungus
		B. subtillis	S. aureus	P. vulgaris	E. coli	C. albicans
SB1	500	10	15	11	14	12
	1000	12	11	13	12	13
	2000	15	14	13	14	11
SB2	500	16	18	17	18	19
	1000	13	15	16	14	15
	2000	17	13	14	15	14
Ciprofloxacin		24	23	22	20	Fluconazole 22

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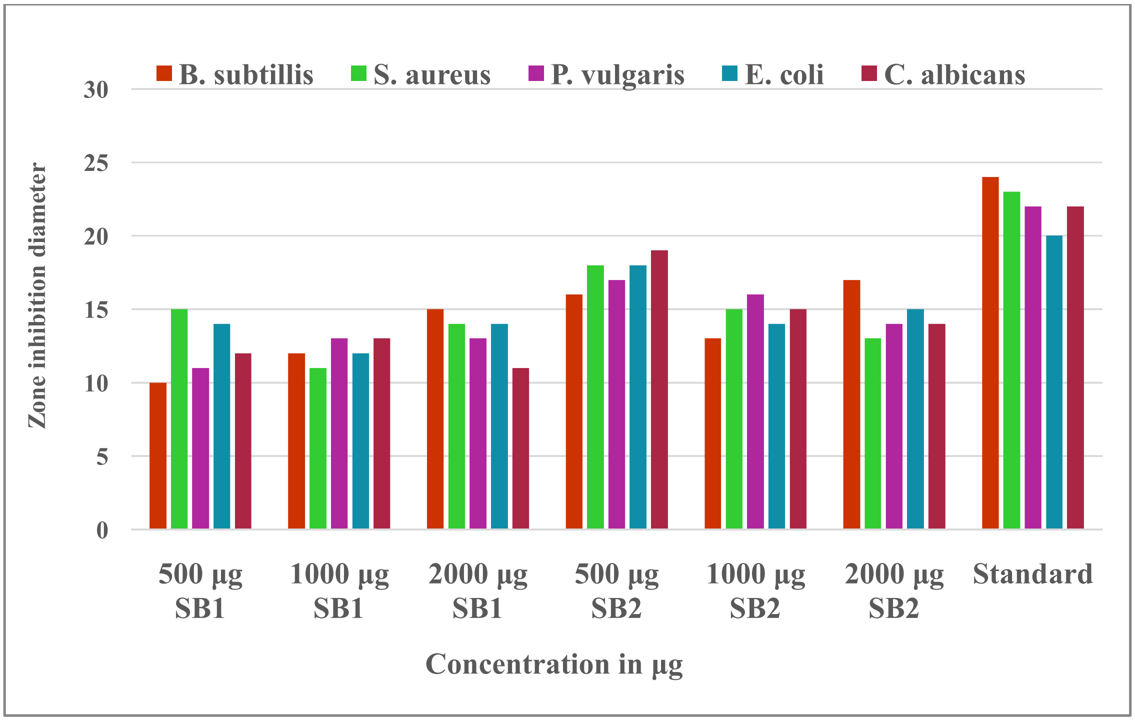

Figure 5. Graphical representation of antibacterial and antifungal activities of the di-imine ligand

V. CONCLUSION

In spite of the developments in the production of new and modern chemical compounds for several and variety of applications, still the interest toward the production of di-imine ligand is highly considerable due to their importance in several fields and applications, such as biological and pharmaceutical activity, inorganic and coordination chemistry, biochemistry, etc. Therefore, we have synthesized an di-imine ligands (SB1 and SB2) derived from 4-aminoantipyrine and semithiocarbazide by the condensation reaction with dicarbonyl compound at elevated temperature under DMF solvent, in which the products were synthesized with excellent yield and simple workout. Furthermore, the presence of imine bond in former ligands was identified by IR and CHN analysis. Antimicrobial activity of di-imine ligand was conducted against two gram positive (*bacillus subtilis*, *Staphylococcus aureus*) and two gram negative bacteria (*P. vulgaris, E. coli*) under different concentration using agar well diffusion method. The inhibition results indicated that di-imine ligand prepared from 4-amino antipyrine (SB2) shown significant antibacterial and antifungal potency (inhibition zone 16-18 mm) than di-imine ligand prepared from semithiocarbazide. The di-imine ligand containing nitrogen and oxygen as donor atoms making them suitable for formation of metal complexes and deeper understanding of variety of applications including clinical, analytical, industrial and as catalyst.

ACKNOWLEDGMENTS

The authors express their sincere gratitude to the Department of Chemistry at Vasantrao Naik College of Arts, Commerce and Barrister A. R. Antulay Science College, Mhasala-Raigad, for the provision of laboratory facilities and technical support.

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