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Synthesis, Characterization and Antimicrobial Evaluationof New Di-Schiff Bases Derived From 2,2′-[Butane-1,4-Diylbis (Oxy)] Dibenzaldehyde

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ABSTRACT:
Two novel di-Schiff bases were synthesized through the condensation of the prepared dialdehyde intermediate, *2,2'-[butane-1,4-diylbis(oxy)]dibenzaldehyde*, with amine moieties, namely semithiocarbazide and 4-aminoantipyrine, in a 2:1 molar ratio using DMF as the reaction medium under controlled conditions. The progress of the reactions and the purity of the synthesized compounds were monitored by thin-layer chromatography (TLC). The obtained Schiff bases were characterized by solubility tests, melting point determination, FT-IR spectroscopy, and elemental analysis. The compounds were found to be partially soluble in most organic solvents while remaining insoluble in water.The antimicrobial potential of the synthesized Schiff bases was evaluated by the disc diffusion method on agar medium against selected Gram-positive and Gram-negative bacterial strains, *Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris,* and *Escherichia coli*, along with the fungal strain *Candida albicans*, at three different concentrations. The results revealed that the Schiff bases exhibited variable inhibitory activity against the tested microorganisms, indicating their promising antimicrobial properties.

KEYWORDS: Semithiocarbazide, 4-amino antipyrine, Schiff bases, Antimicrobial Activities

INTRODUCTION:

Hugo Schiff first described Schiff bases, a well-known class of imines, in 1864 [1]. They have the generic formula R–CH=N–R′, where R and R′ can be alkyl, aryl, or heteroaryl groups with various substituents that alter their steric and electronic characteristics. Usually, a reversible, acid-catalyzed condensation of primary amines with carbonyl compounds-most frequently aldehydes-forms them. Because of the unique reactivity and coordination ability that the azomethine (C=N) bond offers, these molecules are extremely versatile in both organic and inorganic chemistry.Because of their easy one-pot synthesis from cheap precursors, structural tunability, and potent donor properties, Schiff bases are among of the most studied ligands in coordination chemistry. In multidentate complexes, additional donor atoms such phenolic oxygen, pyridine nitrogen, or sulfur frequently aid in coordination, which typically takes place through the imine nitrogen. The production of stable chelate complexes with a broad range of metal ions, such as heavier transition elements, lanthanides, main-group metals, and first-

row transition metals, is made possible by its polydentate nature. As a result, a variety of mono-, bi-, and polynuclear as well as supramolecular structures with tunable physicochemical characteristics like luminescence, magnetic behavior, redox activity, and nonlinear optical responses can be produced. Because the electrophilic azomethine group can interact with biomolecular targets, Schiff bases are of great biological interest. Through processes like DNA binding, reactive oxygen species production, apoptosis induction, and cell-cycle arrest, both free ligands and, more successfully, their metal complexes exhibit significant cytotoxic effects against a variety of cancer cell lines in addition to wide antibacterial and antifungal activities. Research on transition metal complexes made from salicylaldehyde has shown improved bioactivity when compared to the parent ligands, sometimes reaching levels on par with conventional medications [2-10].

Schiff base coordination molecules are significant in a variety of fields. They are useful chiral auxiliaries, protective groups, and intermediates in organic synthesis. They serve as metal ion and anion extractants and selective sensors in analytical chemistry [11-13]. Their π-conjugated frameworks serve as the foundation for commercial dyes and pigments [14], and in materials science, they are employed in processes including electroplating, rare-earth separation, corrosion inhibition, and photography. In addition, metal–Schiff base complexes frequently compete with porphyrin-based systems as effective and recyclable catalysts for processes such olefin epoxidation, aldol condensation, hydrogenation, and C–C bond formation [15-18]. Their bioactivity and lipophilicity enable their use as plant growth regulators, herbicides, and fungicides in agriculture [19, 20].Beyond their use in medicine, their versatility in synthesis and coordination aids in the creation of spin-crossover systems, luminous probes, and molecular magnets. These varied characteristics highlight Schiff bases' ongoing importance as multipurpose platforms that connect organic, inorganic, and bioinorganic chemistry.

Therefore, two novel Schiff bases with N, O, and/or S, donor atoms were synthesized in this work. FT-IR spectroscopy, CHN elemental analysis, and melting point determination were used to characterize these synthesized Schiff base ligands.In the view of these observations, we planned to synthesize new Schiff bases derived from semithiocarbazide and 4-amino antipyrine.

EXPERIMENTAL

Material and Methods

All the required chemicals were purchased from Sigma-Aldrich, E. Merck (India), SD Fine, and Hi-Media Chemicals and used as supplied. The apparatus is SMP30 melting points equipment, melting points were measured in open capillary tubes and were not adjusted. The infrared spectra were recorded by the Shimadzu FTIR model 4800S spectrophotometer in the range of 4,000-400 cm^{-1} using KBr pellets. The progress of all reactions and purity of prepared compounds was checked by thin layer chromatography (TLC) on aluminum plates coated with layer of silica gel, supplied by Merck. The spots were visualized utilizing an iodine chamber. A CHNS microanalyzer was used to record microanalyses for the elements C, H, and N.

METHODOLOGY

Synthesis of dialdehyde intermediate- *2, 2'-[butane-1, 4-diylbis (oxy)] dibenzaldehyde(A)*

A solution of 1,4-dibromobutane (0.1 mol, 21.592 g) in about 40-50 mL of DMF was mixed dropwise with salicylaldehyde (0.2 mol, 24.4 g) and anhydrous K_2CO_3 (0.1 mol, 13.8 g) diluted in a suitable amount of DMF. After four hours of refluxing, the reaction mixture was stirred at room temperature for

4h and then, water was added once the reaction was finished, and the liquid cooled to promote precipitation. The resulting solid was subjected to vacuum drying, recrystallization from 1-butanol, various distilled water treatments, and filtering [21].

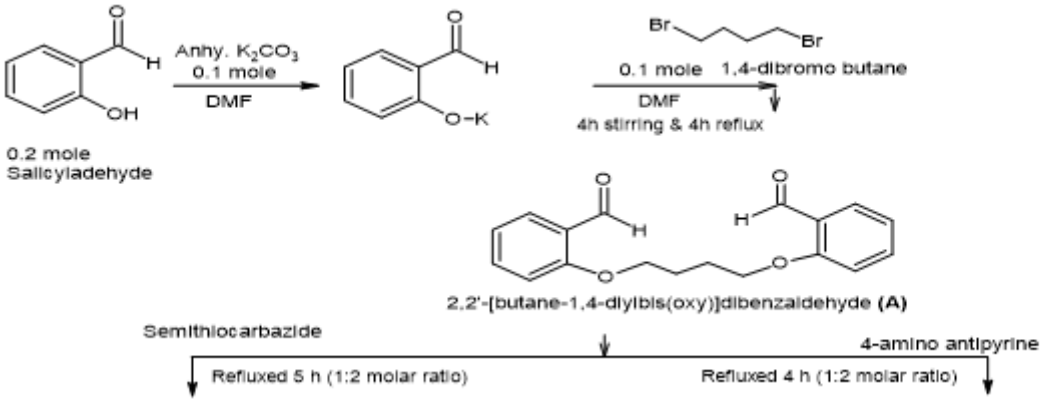
Synthesis ofsemithiocarbazide- and 4-amino antipyrine- basedSchiff bases-(A1 and A2)

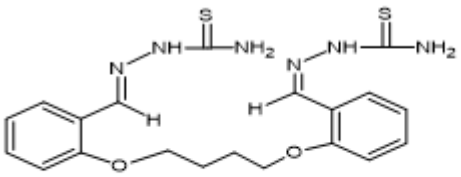
The new Schiff bases were synthesized by the portion-wise addition of 0.2 mol of semithiocarbazideor 4-amino antipyrine in DMF solvent to a dimethyl formamide solution of the 0.1 mol of dialdehyde compound (A) and mixture enriched by a few drops of concentrated hydrochloric acid. The reaction mixture was refluxed for 4-5 h, after which it was allowed to cool to room temperature, leading to the formation of a precipitate. The resulting solid was filtered and washed several times with cold water to remove unreacted materialsand acidic contaminants. The crude product was recrystallized from a DMF: ethyl alcohol (9:1) solvent system to afford yellow crystalline material. The purified precipitate was further washed successively with water followed by ethanol and then dried in a desiccator for 24 h to obtain the corresponding Schiff base in pure form.

Antimicrobial Screening

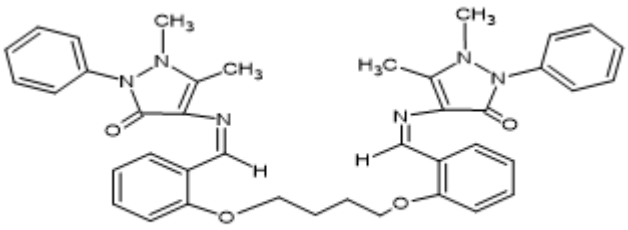
A modified Kirby–Bauer disc diffusion method was used to evaluate the synthesized Schiff bases' in vitro antibacterial and antifungal properties against the fungal strain Candida albicans, Gram-positive bacteria (Bacillus subtilis and Staphylococcus aureus), and Gram-negative bacteria (Proteus vulgaris and Escherichia coli). Mueller-Hinton agar and Sabouraud dextrose agar were used to maintain bacterial and fungal cultures, respectively. The usual instructions provided by the manufacturer were followed in the preparation of the culture media. Aseptically, sterile molten agar was transferred onto sterile Petri dishes and let to set. After that, 100 µL of freshly made microbial culture was added to each plate, evenly distributed across the agar surface, and aseptically dried.Final concentrations of 500, 1000 and 2000 µg per disc were achieved by impregnating sterile paper discs with dimethyl sulphoxide(DMSO) solutions of the test compounds. The discs were aseptically positioned on pre-inoculated agar plates at equal intervals and then gently pressed. For 24 hours for the bacterial strains and 48 hours for the fungal strain, the plates were incubated at 37 °C. DMSO-loaded discs were employed as negative controls, whereas conventional antibacterial and antifungal drugs were employed as positive controls. Following incubation, each disc's zone of inhibition's diameter (in mm) was measured [22, 23].

Reaction Scheme





semithiocarbazide-based Schiff base **(A1)**



4-amino antipyrine -based Schiff base **(A2)**

RESULT AND DISCUSSION

The prepared Schiff bases (A1,and A2) were synthesized by condensation of dialdehyde intermediate compounds with both, semithiocarbazide and 4-amino antipyrine in molar ratio (1:2) in dimethyl formamide (DMF). The reactions proceeded smoothly, producing corresponding Schiff bases ligands in good yield. All prepared compounds are sparingly soluble in most of the common organic solvents but completely insoluble in water. All prepared compounds are non-hygroscopic solids and stable in air. The structures of the prepared compounds were supported by melting points, IR spectral studies and elemental analyses. Obtained physical and analytical data agreed well with the proposed composition of synthesized dialdehyde compound and Schiff bases, summarized in Table 1.

Compounds [Mol. Formula]	**Mol. Wt. gmol^{-1}**	**Color**	**Crystallization Solvent**	**M. P. (^{0}C)**	**Yield %**	**Elemental Composition in % Found /(Calc.)**		
						C	**H**	**N**
Dialdehyde (**A**) [$C_{18}H_{18}O_4$]	298.34	White	n-Butanol	72-74	85	72.49 (72.47)	6.11 (6.08)	---
Schiff base (**A1**) [$C_{20}H_{24}O_2N_6S_2$]	444.63	Pale yellow	DMF:Ethanol (9:1)	196-198	78	54.11 (54.03)	5.51 (5.44)	18.87 (18.90)
Schiff base (**A2**) [$C_{40}H_{40}O_4N_6$]	668.8	Yellow	DMF:Ethanol (9:1)	216-218	75	71.90 (71.84)	6.09 (6.03)	12.51 (12.57)

IR Spectral Analysis

The FTIR spectra provide valuable information regarding the nature of the functional groups present in the compounds. The FT-IR spectra of the dicarbonyl precursor was initially captured and carefully studied to verify the successful synthesis of the Schiff base. The initial dicarbonyl compound's spectral data and those of the produced Schiff bases were then compared. The formation of the imine linkage and the successful condensation reaction were clearly demonstrated by the appearance and disappearance of specific distinctive peaks in the Schiff base spectra. The characteristic IR bands of the dicarbonyl compound and its former ligand (KBr pellets, cm^{-1}) are given in Table 2. The IR spectrum of the semithiocarbazide-based Schiff base (A1) showed peak at 1599 cm^{-1}was assigned totheazomethine group ν(CH=N) vibrationindicated that the free-NH_2 group of semicarbazide was converted to azomethine group [22].The presence three absorption bands at 3331, 3389 and 3234 cm^{-1} is due to N-H stretching vibrations of the semithiocarbazide part of the Schiff base. The presence of band at 1361 cm^{-1} indicating presence of ν(C=S) groups in the Schiff base [22-23]. The absorption bands at 1292 cm^{-1} are due to ν(C-N) stretching. In the IR spectra of compound (A), the band appeared at 1681 cm^{-1} assigned to carbonyl group (C=O) has disappeared in the Schiff base (A1) suggesting the consumption of carbonyl

group in the formation of imine group. The characteristic absorptions at 1597, 1487, 1456 cm^{-1} and 1286, 1246, 1035 cm^{-1} can be attributed to the aromaticν(C=C), and ν(C-O), respectively.The presence of bands at 825 and 763 cm^{-1} indicate presence of ortho disubstitution. The band appears at 2943 and 3151 cm^{-1} are due to aromatic ν(C-H)stretching vibrations [21, 24-27].

The IR spectrum of 4-amino antipyrine-based Schiff base (A2) showed peak at 1651 $cm^{-1}$was assigned to theazomethine group ν(CH=N) vibration indicated that the free -NH_2group of 4-amino antipyrine was consumed in the formation of azomethine group of the Schiff base[25].The three absorption band at 1389, 1363 and 1298 cm^{-1} due to ν(C-N) stretching vibrations. The presence of bands at 759 and 705 cm^{-1} are indicating presence of ortho disubstitution[26]. In the IR spectra of compound (A), the band appeared at 1681 cm-1 assigned to carbonyl group (C=O) has disappeared in the Schiff base (A2) suggesting the involvement of carbonyl oxygen atom in the formation of imine group. The characteristic absorptions at 1570, 1487, 1454 cm^{-1} and 1242, 1022 cm^{-1} can be attributed to the aromaticν(C=C), and ν(C-O), respectively[27].The peak at 1593 cm^{-1} in the Schiff base (A2) indicating presence of free cyclic carbonyl group in 4-amino antipyrine part of the Schiff base. The band appears at 2947 and 2862 cm^{-1} are due to the aliphatic and aromatic ν(C-H)stretching vibrations [21, 24-29].

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

Comp. Code	υ C=O cm^{-1}	υald. C-H cm^{-1}	υ C=N cm^{-1}	υ (C-N) cm^{-1}	(C=C) cm^{-1}	orthodisub. cm^{-1}	υ (C-H) cm^{-1}	υ (N-H) cm^{-1}	C=S cm^{-1}
A	1681	1392			1597, 1487, 1456	831, 760	2949, 2929, 2850	-	-
A1			1599	1292	1508, 1489, 1452	825,763	2943, 3151	3331, 3389, 3234	1361
A2	1593		1651	1389, 1363, 1298	1570, 1487, 1454	759,705	2947, 2862	-	-

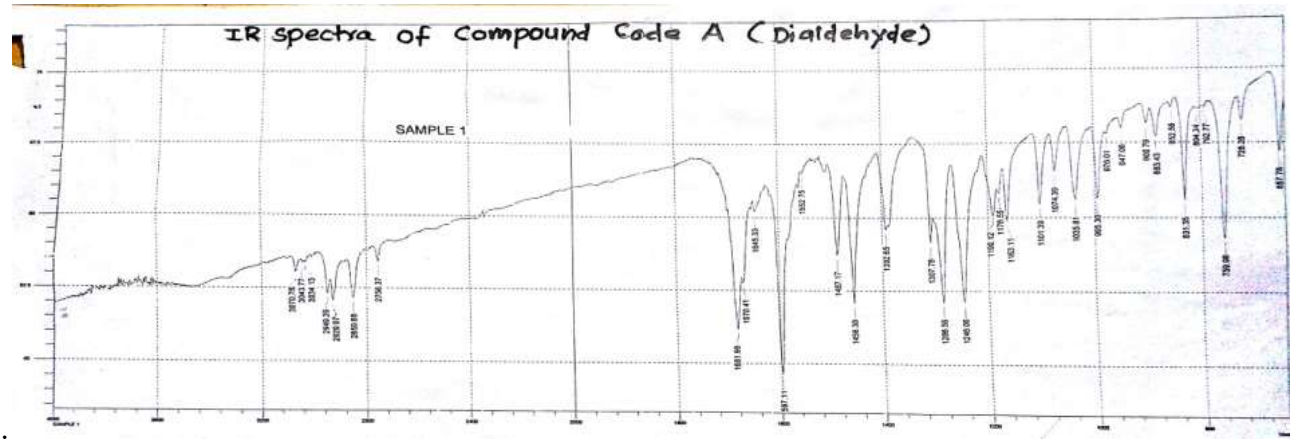


Figure 1.FT-IR spectrum of 2, 2'-[butane-1, 4-diylbis (oxy)] dibenzaldehyde(A)

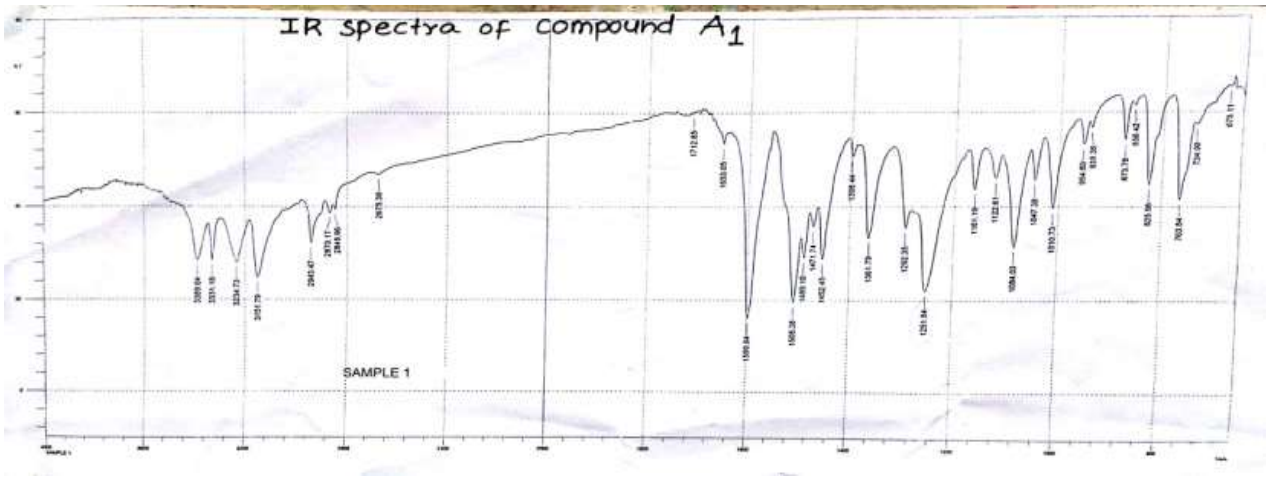


Figure 2.FT-IR spectrum of Schiff base(A1)

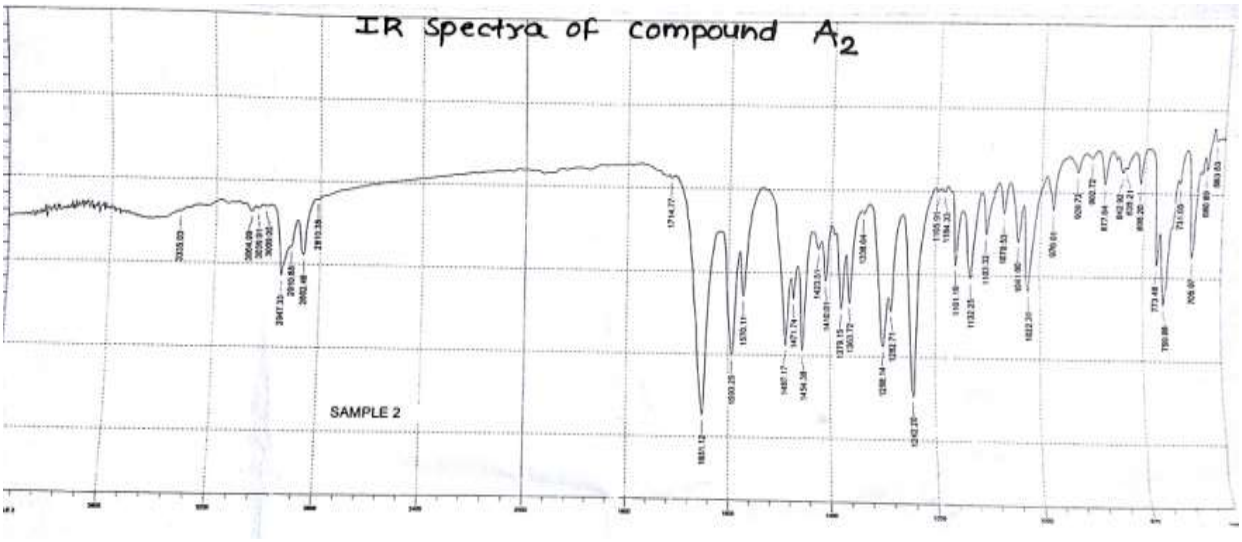


Figure 3.FT-IR spectrum of Schiff base(A2)

In vitro Antimicrobial studies

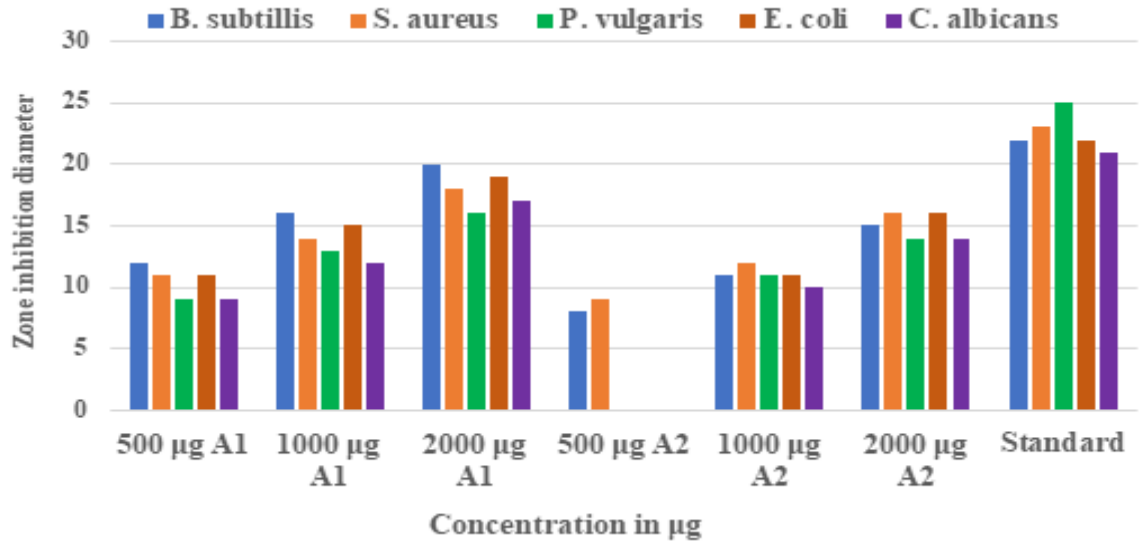
The disc diffusion method was used to assess the synthesized Schiff bases' antibacterial and antifungal properties against Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris, Escherichia coli, and Candida albicans on agar medium. Table 3 and Figure 4 show the antimicrobial screening results of tests conducted on the compounds at 500, 1000, and 2000 µg per disc. The inhibitory effects of each tested Schiff base against the chosen bacterial and fungal species varied.The Schiff base (A1), which is derived from semithiocarbazide, exhibited poor inhibition against P. vulgaris but substantially stronger antibacterial activity against the majority of the examined organisms among the compounds under investigation. The Schiff base (A1) showed modest inhibition against B. subtilis, S. aureus, and E. coli at lower concentrations, but at higher concentrations (2000 µg), it showed substantial action with inhibition zones in the 18–20 mm range. On the other hand, the antibacterial effectiveness of the Schiff base (A2) derived from 4-aminoantipyrine was comparatively lower. The Schiff base (A2) was ineffective against P. vulgaris and E. coli and very slightly inhibited B. subtilis and S. aureus at 500 µg per disc. However, when concentration increased, a considerable level of antibacterial activity was noted.In the antifungal assay, Schiff base (A1) had superior antifungal action against *C. albicans*, whereas Schiff base (A2)

remained inactive at lowest concentration. Both compounds showed moderate antifungal activity at the highest tested concentration. Overall, the synthesized Schiff bases showed significant antibacterial potential, with Schiff base (A1) being the most promising option. These findings imply that these substances may serve as promising scaffolds for further biological and pharmacological investigations.

Table 3. Antibacterial and antifungal activities of the Schiff bases (where NA= non-active)

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
A1	500	12	11	09	11	09
	1000	16	14	13	15	12
	2000	20	18	16	19	17
A2	500	08	09	NA	NA	NA
	1000	11	12	11	11	10
	2000	15	16	14	16	14
Streptomycin		22	23	25	22	Miconazole 21

Figure 4.Graphical representation of antibacterial and antifungal activities of the Schiff bases



CONCLUSION

The current study describes the successful synthesis of new multidentate Schiff base ligands (A1 and A2) through the condensation of the dialdehyde intermediate, 2,2′-[butane-1,4-iylbis(oxy)]dibenzaldehyde, with semithiocarbazide and 4-aminoantipyrine, respectively. The formation of the azomethine (–C=N–) linkage in the synthesized compounds was confirmed based on existed

literature data and supported by FT-IR spectral analysis and CHN elemental analysis, which verified the proposed structures. The in vitro antimicrobial activity of the prepared Schiff bases was evaluated against four bacterial strains and one fungal strain using the disc diffusion method. The antimicrobial screening results revealed that both compounds exhibited inhibitory effects against the selected microorganisms, with the semithiocarbazide-derived Schiff base (A1) showing comparatively higher antimicrobial activity (inhibition zone 18-20 mm) than the 4-aminoantipyrine-derived Schiff base (A2).Owing to the presence of multiple donor atoms, these Schiff bases can act as multidentate ligands with strong coordination ability and can be utilized for the synthesis of metal–ligand (M–L) complexes to explore their improved biological and physicochemical properties. Furthermore, they may also be useful analytical reagents for quantifying metal ions from alloys, biological samples, and synthetic mixtures.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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