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Fungal biofilm inhibition by piperazine-sulphonamide linked Schiff bases: Design, synthesis, and biological evaluation

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Abstract

We report the synthesis of some new piperazine-sulphonamide linked Schiff bases as fungal biofilm inhibitors with antibacterial and antifungal potential. The biofilm inhibition result of *Candida albicans* proposed that the compounds **6b** (IC_{50} = 32.1 μM) and **6j** (IC_{50} = 31.4 μM) showed higher inhibitory activity than the standard fluconazole (IC_{50} = 40 μM). Compound **6d** (MIC = 26.1 μg/mL) with a chloro group at the *para* position was found to be the most active antibacterial agent of the series against *Bacillus subtilis* when compared with the standard ciprofloxacin (MIC = 50 μg/mL). Compound **6j** (MIC = 39.6 μg/mL) with an OH— group at the *ortho* position showed more potent antifungal activity as compared to that of the standard fluconazole (IC_{50} = 50 μM) against *C. albicans*. Thus, the synthesized compounds **6a–k** were found to be potent biofilm inhibitors as well as active antibacterial and antifungal agents. The molecular docking study of the synthesized compounds against the secreted aspartyl protease (SAP5) enzyme of *C. albicans* exhibited good binding properties. The *in silico* ADME properties of the synthesized compounds were also analyzed and showed their potential to be developed as potential oral drug candidates.

KEYWORDS
antibacterial, fungal biofilm inhibition, piperazine, Schiff bases, sulphonamide

1 | INTRODUCTION

Discovery of potent and effective antimicrobial drugs indicates most important developments in therapeutics, not only in the management of serious infections, but also in the control and treatment of some infectious complications of other therapeutic modalities such as cancer chemotherapy and surgery. In last decade, microbial infection becomes an important complication and a main cause of morbidity and mortality in immuno-compromised patients such as those suffering from cancer, AIDS, and tuberculosis and in organ transplantation cases. In spite of the availability of large antimicrobial drugs for the treatment, the emergence of antimicrobial resistant microbial strains in the last decades constitutes a substantial need for the development of new classes of antimicrobial agents.[1–4]

The therapeutic resistance phenomena, which are very often associated with the biofilm formation is one of the major problem related to the treatment of *Candida albicans* infections.[5] Biofilms are defined as complex microbial communities that are encapsulated in a matrix of extracellular polymeric substances. They develop when such community of microorganisms irreversibly adheres to an inert or living surface. Contact with a solid surface triggers the expression of a panel of enzymes, which catalyze the formation of sticky polysaccharides that

promote the surface colonization and the microbial cells protection. This adherent community is considered an important virulence factor because it is difficult to eradicate and often responsible for treatment failures.[6] Indeed, the biofilm represents a physical barrier that prevents drugs from entering and expressing their activity.

Schiff bases are considered as an important antimicrobial scaffold. Our group reported the synthesis and screening of linezolid-like Schiff bases as inhibitors of biofilm formation and antibacterial agents.[7] Yuan et al.[8] investigated the *in vitro* activity of taurine-5-bromosalicylaldehyde Schiff base (TBSSB) against methicillin-resistant *Staphylococcus aureus* (MRSA); as the therapy for MRSA infections is becoming more difficult because of multidrug resistance and strong biofilm forming properties and Schiff bases have attracted attention as promising antibacterial agents. Also piperazine and sulphonamide containing compounds have been reported as important scaffolds for antibacterial and antifungal agents. Hatnapure et al.[9] prepared and screened a series of novel piperazine derivatives of biological interest for their antibacterial and antifungal activity and many compounds were shown to have potent antibacterial and antifungal activities when compared with standard ciprofloxacin and miconazole. Zoumpoulakis et al.[10] designed the synthesis of a series of novel sulfonamide compounds and tested *in vitro* for antibacterial and antifungal activity and some analogues exhibited very promising results especially as antifungal agents. Better antifungal activity than commercial ketoconazole and bifonazole were observed. Thus, we decided to synthesize a series of some new Schiff bases with piperazine and sulphonamide-coupled scaffolds as antimicrobial agents.

Taking into account all of the aforementioned, and as a part of our ongoing work toward identifying biologically active molecules[11,12] we report the synthesis of a series of novel piperazine-sulphonamide linked Schiff bases, and the study of their effects on inhibition of *Candida albicans* biofilm. Also antifungal activity was evaluated against *Candida albicans*. Antibacterial activity was screened for two Gram-negative bacteria namely, *Escherichia coli* and *Pseudomonas aeruginosa*, and two Gram-positive bacteria, namely *Staphylococcus aureus* and *Bacillus subtilis*. We have also evaluated the synthesized compounds for *in silico* ADMET prediction and results showed that compounds could be exploited as oral drug candidates.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

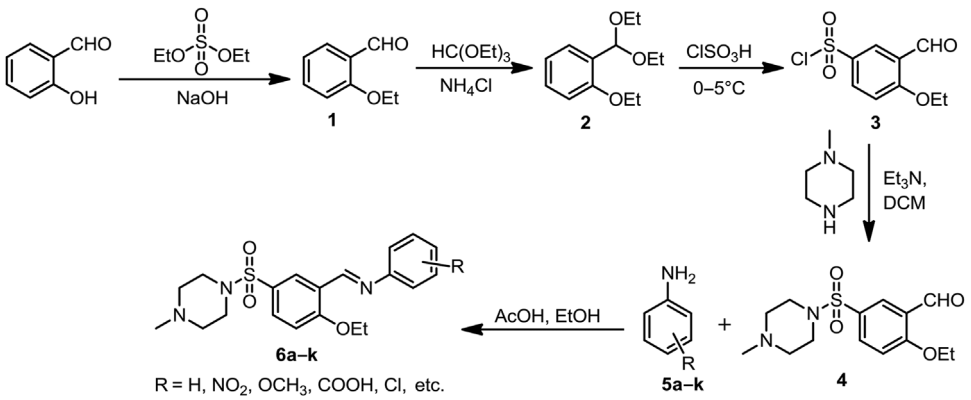
2.1.1 | Synthesis of the title compounds

The Schiff bases **6a–k** were synthesized by refluxing a mixture of 2-ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)benzaldehyde **4** (1.0 mmol) and of various substituted aromatic amines **5a–k** (1.0 mmol) in 15 mL absolute ethanol using glacial acetic acid (3.0 mmol) as catalyst (Scheme 1). After completion of reaction as indicated by TLC, the reaction mixture was poured in petri plate and allowed to stand overnight. The solid substance obtained was collected and recrystallized from ethanol. All the derivatives were obtained by similar method by treating with corresponding amines. The physical data of synthesized compounds are presented in Table 1. The synthesized compounds were obtained in good yield (82–90%) within 10–12 h. Purity of the synthesized compounds was checked by thin layer chromatography (TLC) and melting points were determined in open capillaries on melting point apparatus and were uncorrected. ^{1}H NMR, ^{13}C NMR, and mass spectral analysis confirmed the formation of the synthesized compounds and the data also suggested the proposed structures (Supporting Information).

2.2 | Biological assays

2.2.1 | *In vitro* biofilm inhibition assay

The synthesized compounds **6a–k** were evaluated for anti-biofilm activity to explore a possible role of piperazine-sulphonamide linked Schiff bases in inhibiting/impeding the formation of biofilm in *C. albicans* using MTT assay method. This method is based on the fact that the higher the biofilm formation, the greater is the extent of absorption of crystal violet and, thus, the less is the effectiveness of the compounds. Fluconazole was used as standard for the comparison of biofilm inhibition activity. The IC_{50} value (concentration that decreased biofilms by 50%) of synthesized compounds is presented in Table 2.



SCHEME 1 Synthesis of the title compounds **6a–k**

TABLE 1 Physical data for the piperazine-sulphonamide linked Schiff bases **6a–k**

Entry	R	Mol. formula	Yield (%)	Rf value	Mp (°C)
6a	*p*-OCH_3	$C_{21}H_{27}N_3O_4S$	87	0.71	110
6b	H	$C_{20}H_{25}N_3O_3S$	82	0.68	90–92
6c	*p*-COOH	$C_{21}H_{25}N_3O_5S$	90	0.65	220
6d	*p*-Cl	$C_{20}H_{24}ClN_3O_3S$	85	0.60	110–112
6e	*o*-NO_2	$C_{20}H_{24}N_4O_5S$	83	0.70	80
6f	*m*-NO_2	$C_{20}H_{24}N_4O_5S$	84	0.76	140–144
6g	*p*-NO_2	$C_{20}H_{24}N_4O_5S$	84	0.65	138–140
6h	*o*-CF_3	$C_{21}H_{24}F_3N_3O_3S$	84	0.68	110–114
6i	*m*-CF_3	$C_{21}H_{24}F_3N_3O_3S$	84	0.72	130–134
6j	*o*-OH	$C_{20}H_{25}N_3O_4S$	85	0.70	140–142
6k	*p*-OH	$C_{20}H_{25}N_3O_4S$	86	0.62	98–100

The synthesized compounds **6a–k** had shown good biofilm inhibition activity (IC_{50} range = 31.4–169.1 μM) against *C. albicans* strain. Compounds **6b** (IC_{50} = 32.1μM), **6i** (IC_{50} = 37.2 μM), **6j** (IC_{50} = 31.4 μM), and **6k** (IC_{50} = 39.5 μM) were found to show potent biofilm inhibition activity against *C. albicans* when compared with standard fluconazole (IC_{50} = 40.0 μM). The compounds **6a** (IC_{50} = 46.2 μM) and **6d** (IC_{50} = 47.5 μM) had shown significant biofilm inhibition activity when compared with standard fluconazole. The compound **6j** having —OH at the *ortho* position was found to be more potent than unsubstituted phenyl analogue **6b**, while compounds **6i** and **6k** having —CF_3 at the *meta* position and —OH at *para* position, respectively, were found to be less potent compared to unsubstituted phenyl analogue **6b**. Compounds **6a**, **6c**, and **6d** with —OCH_3, —COOH, and —Cl at the *para* position, respectively, and compound **6h** with —CF_3 at the *ortho* position showed somewhat decreased in activity, while —NO_2 at *meta* and *para* position in compounds **6f** and **6g** showed great decrease in activity. Among all the synthesized compounds, compound **6j** was found to be the most active biofilm inhibitory compound. As observed from activity data, compounds **6a**, **6j**, and **6k** with electron donating groups like —OH and —OCH_3 were more active than compounds **6c–h** with electron withdrawing groups (except **6i**) like —Cl, —NO_2, —COOH, and —CF_3 on the phenyl group.

2.2.2 | Field emission scanning electron microscopy analysis

After identifying the lead with potent anti-biofilm activity and to better understand the biofilm inhibition by compounds, we carried out the field emission scanning electron microscopy (FESEM) analysis of most active anti-biofilm compound **6j** (IC_{50} = 31.4 μM). Result revealed that

TABLE 2 *In vitro* biofilm inhibition, antibacterial and antifungal activities of piperazine-sulphonamide linked Schiff bases **6a–k**

	Antibiofilm activity, IC_{50} (μM)	Antibacterial activity, MIC (μg/mL)				Antifungal activity, MIC (μg/mL)
Entry	*C. albicans* (NCIM-3471)	*P. aeruginosa* (NCIM-2036)	*E. coli* (NCIM-2256)	*B. subtilis* (NCIM-2063)	*S. aureus* (NCIM-2901)	*C. albicans* (NCIM-3471)
6a	46.2	39.0	158.2	36.6	195.0	82.2
6b	32.1	40.0	228.1	34.7	125.5	51.1
6c	53.6	115.0	225.1	47.2	112.2	56.0
6d	47.5	175.0	197.0	26.1	147.3	75.0
6e	76.2	122.0	111.2	40.9	98.5	84.1
6f	169.1	119.6	92.6	62.3	118.6	247.8
6g	121.1	188.4	95.5	94.6	174.8	244.3
6h	66.3	49.2	190.1	33.6	35.7	185.2
6i	37.2	181.6	84.0	29.2	85.4	45.0
6j	31.4	74.5	131.4	37.66	129.2	39.6
6k	39.5	68.5	99.16	41.1	111.9	47.2
CP	–	50.0	50.0	50.0	25.0	–
FA	40.0	–	–	–	–	50.0

CP, ciprofloxacin; FA, fluconazole. Experiments were performed in triplicates and compared to DMSO-treated control. Standard errors were all within 10% of the mean.

in absence of compound, cells of *C. albicans* were seen as enmeshed-covered structures, and cells were enclosed, a typical of biofilm structures. When the cells of *C. albicans* were subjected to inhibitory concentration of compound **6j**, there was a prominent decrease in the biofilm formation and cells were seen as spatially distributed. More importantly, the numbers of the planktonic cells (cells in suspension) were not affected, suggesting that the inhibition of the biofilm in *C. albicans* is quorum sensing (QS) mediated (Figure 1).[13]

2.2.3 | *In vitro* antifungal activity

Minimum inhibitory concentration (MIC) values for antifungal activity against *C. albicans* were determined using standard agar method using fluconazole as standard. Dimethyl sulfoxide was used as solvent control. The results of *in vitro* antifungal activity (Table 2) showed that synthesized compounds **6a–k** have moderate to good activity. Also our results demonstrated that most potent biofilm inhibitors **6a**, **6i–k** showed a significantly potent antifungal activity against *C. albicans* when compared with standard fluconazole. Comparison of antifungal activity of compounds with that of antifungal drug fluconazole (MIC = 50.0 μg/mL) showed that compound **6b** (MIC = 51.1 μg/mL) had same antifungal profile against *C. albicans*. Substituted phenyl analogue —CF_3 at the *meta* position in compound **6i** (MIC = 45.0 μg/mL) and —OH at the *ortho* and *meta* position in compound **6j** (MIC = 39.6 μg/mL) and **6k** (MIC = 47.2 μg/mL) showed increased activity when compared with standard drug fluconazole. All the other synthesized compounds like **6a**, **6c–h** were found less active than fluconazole. Among all synthesized, compound **6j** (MIC = 39.6 μg/mL) was found to be the most active compound against *C. albicans*. Structure–activity relationship (SAR) revealed that compounds **6a**, **6j**, and **6k** with electron donating groups like —OH and —OCH_3 were more active than compounds **6c–h** with electron withdrawing groups (except **6i**) like —Cl, —NO_2, —COOH, and —CF_3 on the phenyl group.

2.2.4 | *In vitro* antibacterial activity

MIC values for antibacterial activity were determined using standard agar method using ciprofloxacin as standard. Dimethyl sulfoxide was used as solvent control. From activity data (Table 2), the synthesized compounds **6a–k** had exhibited moderate to good antibacterial activity. Unsubstituted phenyl analogue **6b** showed significant activity against *P. aeruginosa* and *B. subtilis* than activity against *E. coli* and *S. aureus*. Introduction of —OCH_3 at the *para* position of phenyl **6a** led to increase in the antibacterial activity against *P. aeruginosa*, while other substitutions showed decreased activity for the same organism. The substituted phenyl analogue had shown increased activity against *E. coli*. Compound **6d** with —Cl at the *para* position, compound **6h** with —CF_3 at the *ortho* position and compound **6i** with —CF_3 at the *meta* position gave potent compounds against *B. subtilis* and showed the increased antibacterial activity, at the same time other substitutions decreased the activity for the same organism. Compound **6c** having —COOH at the *para* position, compounds **6e** and **6f** having —NO_2 at the *ortho* and *meta* position, respectively, compounds **6h** and **6i** having —CF_3 at the *ortho* and *meta* position, respectively, and —OH at the *para* position in compound **6k** showed increased activity against *S. aureus* while other substitutions showed decrease in activity for the same organism. Among all the synthesized analogues, compound **6d** was found to be the most active compound against *B. subtilis*. Compound **6h** had shown broad spectrum of antibacterial activities. As observed from activity data (Table 2), compounds **6a**, **6j**, and **6k** with electron donating groups like —OH and —OCH_3 were more active than compounds **6c–h** with electron withdrawing groups (except **6h** and **6i**) like —Cl, —NO_2, and —COOH on the phenyl group.

2.3 | Molecular docking analysis

The synthesized compounds **6a–k** showed very good binding interactions with the active site of SAP5 enzyme. The docking interactions have been studied into substrate binding site pocket, namely, S4, S3, S2, S1, S1′, S2′, and S3′ of SAP5. The docking interactions indicated that compounds were held deep into these pockets by combination of various hydrophobic, van der Waal's interactions and charge interactions. The most active (fungal biofilm inhibitors) compounds **6b**, **6i–k** were held into substrate binding pockets by forming a most number of interactions namely van der Waal's interactions with active site amino acid residues such as Ile12, Trp51, Asp86, Gly220, Thr221, Asn249, Phe281, Thr283, Glu295, Arg297, and Ile305. 4-Methylpiperazine ring buried deep into active site and mostly had formed the hydrophobic interactions with active site residues such as Lys50, Trp51, Arg52, Gly85, Asp86, Thr221,

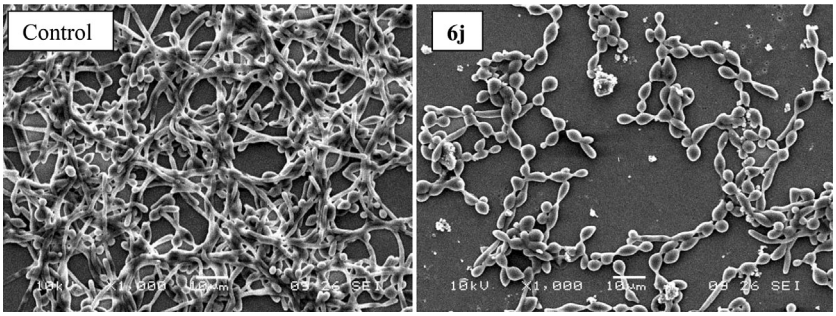


FIGURE 1 Inhibition of *C. albicans* biofilm (FESEM images). FESEM analysis of *C. albicans* biofilm (control) shows bunches of cells surrounded by biofilm. However, in presence of compound **6j** individual cells were observed, indicating an inhibition of biofilm formation

Thr222, Ile223, Asn249, Thr283, Arg229, Ser301, and Ile305. The nitrogen atom of imine group (—C═N—) had formed strong charge interactions with amino acid residues such as Trp51, Arg52, Gly85, Asp86, Thr221, Thr222, Tyr225, Phe281, Thr283, Glu295, Arg297, and Ile305. The most active compound **6j** (Figure 2) had shown very good binding affinity, that is −52.81 kcal/mol. The compound **6j** had shown very strong non-covalent interactions with amino acids such as Ile12, Trp51, Arg52, Asp86, Thr222, Ile223, Tyr225, Asn249, Phe281, Thr283, Glu295, Arg297, and Ile305.

Native inhibitor of SAP5 that is PepA was also docked into binding pockets of SAP5 and had shown number of non-covalent interactions such as van der Waal's and hydrophobic interactions but no charge interactions (Figure 2). The binding affinity of PepA was least, that is −25.78 kcal/mol when compared with all synthetic compounds. The amino acid residues such as Glu10, Lys83, Asp86, Gly131, Phe281, Glu300, and Asp303 had shown van der Waals interactions with two —C═O functional groups and aliphatic nitrogen atoms. The amino acids like Ala11, Ile12, Trp51, Lys193, and Leu216 had formed hydrophobic interactions with terminal —CH_3 groups of PepA. The mode of interactions of active synthetic compounds when compared with co-crystallized complex (PepA) of SAP5 found that important amino acid residues such as Ile12, Lys83, Gly85, Asp86, Gly220, Thr221, Thr222, Thr222, Ile223, Tyr225, and Ile305 had interacted with active compounds and PepA by forming van der Waal's, hydrophobic and charge interactions.

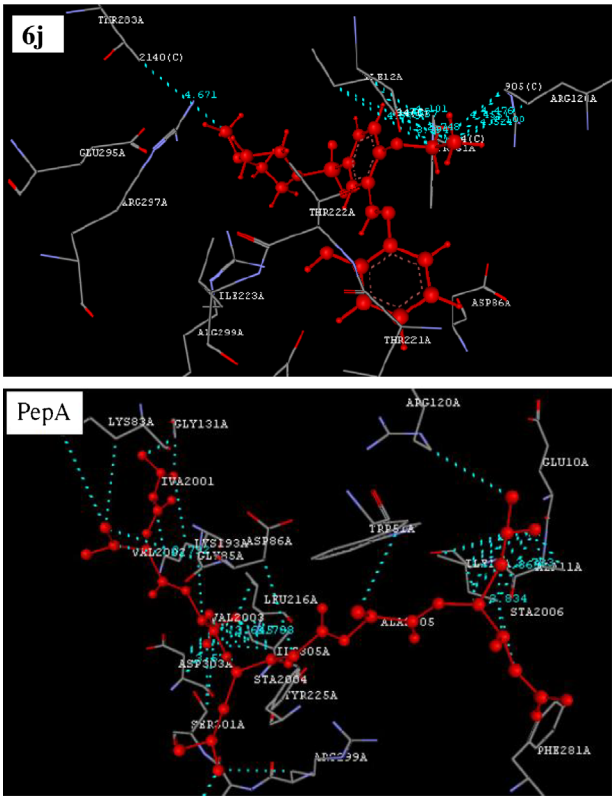


FIGURE 2 Molecular docking study of compound **6j** and PepA with SAP5 enzyme of *C. albicans* (PDB ID: 2QZX). Ligands are shown in red color ball and sticks

2.4 | ADMET prediction

Due to unfavorable absorption, distribution, metabolism, elimination, and toxicity (ADMET) properties, many potential therapeutic agents may fail to reach the clinical stage. Therefore, *in silico* study was carried out for assessment of ADMET parameters and result obtained is presented in Table 3. The data obtained for all the synthesized compounds **6a–k** were within the range of accepted values. None of the synthesized compounds had violated the Lipinski's rule of five for its variants. The value of polar surface area (PSA), logP, and H/C ratio for synthesized compounds **6a–k** were within the accepted range thus indicating for good oral bioavailability. The parameters like number of rotatable bonds and number of rigid bonds are linked with intestinal absorption result, and the result showed that all synthesized

TABLE 3 Physicochemical pharmacokinetic parameters important for agents to have good oral bioavailability of synthesized compounds **6a–k**

Entry	MW	LogP	PSA	n-Rot bond	n-Rig bond	HBD	HBA	Rings	Ratio H/C	Toxicity
6a	417.18	3.73	79.8	7	21	0	5	3	0.38	NT
6b	387.49	3.72	70.5	6	21	0	4	3	0.35	NT
6c	431.50	3.42	107	7	22	1	6	3	0.428	NT
6d	421.94	4.38	70.5	6	21	0	4	3	0.4	NT
6e	433.50	4.05	115	7	22	1	6	3	0.5	NT
6f	433.50	4.05	115	7	22	1	6	3	0.5	NT
6g	433.50	4.05	115	7	22	1	6	3	0.5	NT
6h	411.44	4.34	61.3	5	21	0	3	3	0.473	NT
6i	455.49	4.74	70.5	7	21	0	4	3	0.476	NT
6j	403.49	3.43	90.8	6	21	1	5	3	0.4	NT
6k	403.49	3.43	90.8	6	21	1	5	3	0.4	NT

MW, molecular weight; LogP, logarithm of partition coefficient of compound between *n*-octanol and water; PSA, polar surface area; n-Rot bond, number of rotatable bonds; n-Rig bond, number of rigid bonds; HBA, hydrogen bond acceptors; HBD, hydrogen bond donor; NT, non-toxic.

compounds **6a–k** had good absorption. Also, all the synthesized compounds **6a–k** were found to be non-toxic.

3 | CONCLUSION

In conclusion, we have synthesized a series of new piperazine-sulphonamide linked Schiff bases **6a–k** in good yield. All the synthesized compounds were tested for fungal biofilm inhibition activity, antibacterial and antifungal activities. Based on the activity data, SAR for the series has been developed. Interestingly, compounds **6b**, **6i–k** had shown good results for fungal biofilm inhibition activity. Also, compounds **6i–k** had shown very good potential for the development of novel antifungal agents. Compounds **6a**, **6b**, **6d**, **6h**, and **6i** were shown to have potent antibacterial activity and can serve as important pharmacophores for the design and development of new leads as antibacterial agent. The mechanism for fungal biofilm inhibition is demonstrated by molecular docking study and result had shown good binding interactions with SAP5. *In silico* ADME study of synthesized compounds indicated that compounds had potential to develop as good oral drug-like candidate. Thus, suggesting that the compounds from the present series can serve as important gateway for the design and development of new good oral drug-like antimicrobial agents.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All the starting material used in synthesis of title compounds and solvents were purchased from Sigma or Avra synthesis and used without further purification. The purity of the synthesized compounds was checked by ascending thin layer chromatography (TLC) on silica gel-G (Merck) coated aluminum plates, visualized by iodine vapor. The melting points of synthesized compounds were measured in open capillary tubes. ^{13}C NMR and ^{1}H NMR spectra were recorded on 400 MHz Bruker spectrometer. Chemical shifts for NMR studies are reported in parts per million (ppm), using TMS as an internal standard. Agilent technology 1200 series HPLC paired to a 6130 mass spectrometer with electron spray ionization (ESI) was used for mass spectra.

The InChI codes of the investigated compounds together with some biological activity data are also provided as Supporting Information.

4.1.2 | Synthesis of the title compounds

The synthetic approach applied is outlined in Scheme 1. Initially to a suspension of salicylaldehyde and aqueous sodium hydroxide solution, diethyl sulfate was slowly added dropwise to obtain 2-ethoxybenzaldehyde **1**. Ethyl orthoformate and ammonium chloride were added to 2-ethoxybenzaldehyde **1** in presence of ethanol to produce diethylacetal of 2-ethoxybenzoic aldehyde **2** which on chlorosulphonation in presence of chlorosulphonic acid at 0°C yielded 2-ethoxy-5-chlorosulfonylbenzaldehyde **3**. The compound **3** on reaction with 1-methylpiperazine in presence of triethylamine as a base in methylene dichloride as solvent reacted to give 2-ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)benzaldehyde **4** in good yield (90%). The Schiff bases **6a–k** were synthesized by refluxing a mixture of compound **4** (1.0 mmol) and of various substituted aromatic amines **5a–k** (1.0 mmol) in 15 mL absolute ethanol using glacial acetic acid (3.0 mmol) as catalyst.[14] After completion of reaction as indicated by TLC, the reaction mixture was poured in petri plate and allowed to stand overnight. The solid substance obtained was collected and recrystallized from ethanol. All the derivatives were obtained by similar method by treating with corresponding amines.

4.2 | Biological assays

4.2.1 | *In vitro* biofilm inhibition assay

The piperazine-sulphonamide linked Schiff bases **6a–k** were evaluated for anti-biofilm via evaluating the metabolic activity of cells by dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In this assay, 10^5 cells/well of *C. albicans* (yeast form) were inoculated in RPMI in presence of various concentrations (0–100 μM) of compounds **6a–k** and standard drug fluconazole, and incubated for 24 h at 30°C. After incubations, the media was removed, and biofilm was washed with phosphate buffer. A 100 μL of MTT (1 μg/μL) was added to each well, and incubated in dark for 3 h. After 3 h, the clear solution (without violet granules) was removed and 100 μL of dimethylsulfoxide was added to each well, and optical density recorded at 575 nm. The concentration that decreased biofilms by 50% (IC_{50}) was computed from growth inhibition curve.[15,16]

4.2.2 | Field emission scanning electron microscopy (FESEM) analysis

To further confirm the impedance of biofilm, surface topography of the biofilm was observed in presence of title compounds by FESEM (SEI NOVA, NANO-SEM, 450, USA). For this purpose, 10^5 cells/well of *C. albicans* were inoculated in RPMI media in presence of IC_{50} value of **6j** compound, and incubated for 24 h at 30°C. After incubations, the media was removed, and biofilm was washed with phosphate buffer followed by series of ethanol concentrations (10–100%, each for 15 min), dried, mounted on aluminum stubs conductive carbon cement, and finally coated with a gold film. The biofilm with planktonic cells were observed under FESEM at 1000× magnifications.

4.2.3 | *In vitro* antifungal activity

Antifungal activity was determined by standard agar dilution method as per CLSI (formerly, NCCLS) guidelines.[17] The synthesized compounds and standard fluconazole were dissolved in DMSO solvent. The medium yeast nitrogen base was dissolved in phosphate buffer pH 7 and it was autoclaved at 110°C for 10 min. With each set a growth control without the antifungal agent and solvent control

DMSO were included. The fungal strains were freshly subcultured on to Sabouraud dextrose agar (SDA) and incubated at 25°C for 72 h. The fungal cells were suspended in sterile distilled water and diluted to get 10^5cells/mL. Ten microliters of standardized suspension was inoculated onto the control plates and the media incorporated with the antifungal agents. The inoculated plates were incubated at 25°C for 48 h. The readings were taken at the end of 48 and 72 h. The MIC was the lowest concentration of drug preventing growth of macroscopically visible colonies on drug containing plates when there was visible growth on the drug free control plates.

4.2.4 | *In vitro* antibacterial activity

All the synthesized compounds were screened for *in vitro* antibacterial activity. Minimum inhibitory concentration (MIC) values were determined using method recommended by National Committee for Clinical Laboratory Standards (NCCLS). *In vitro* antibacterial activities of the synthesized compounds **6a–k** were tested in nutrient broth (NB) for bacteria by the twofold serial dilution method. Seeded broth (broth containing microbial spores) was prepared in NB from 24 h old bacterial cultures on nutrient agar (Hi-media) at 37 ± 1°C. The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^{-4}–10^{-5}colony forming units (CFU). The synthesized compounds and standard drug ciprofloxacin were prepared by twofold serial dilutions to obtain the required concentrations of 400, 200, 100, 50, 25, 12.5, 6.25, and 3.13 µg/mL. The tubes were incubated in BOD incubators at 37 ± 1°C for bacteria. The MICs were recorded by visual observations after 24 h of incubation.[17]

4.3 | Molecular docking study

The molecular docking study was carried out in order to understand basis of biofilm inhibition in fungal species and importance of structural features of synthesized series of compounds mentioned in SAR. Fungal organism secretes various integral proteins mainly include proteases responsible for the formation of biofilm which inherently provides pathogenesis to fungal organism. The role of secreted aspartyl protease (SAP5) in *Candida* spp. has been reported to provide key characteristics such as gives tissue adhesion, invasions degrading cell surface structures and intercellular substances host system.[18,19] Molecular docking study of synthesized compounds and classical inhibitor of SAP5, pepA enzyme of *C. albicans* (PDB ID: 2QZX)[20] was performed using VLife MDS 4.3 package following standard procedure.[21]

4.4 | ADMET prediction

A computational study of synthesized compounds **6a–k** was performed for prediction of ADMET properties. In this study, we assessed ADMET properties using ADMET predictor FAFDrugs2 which runs on Linux OS. This tool is freely available and used for *in silico* ADMET filtering.[22] In this study, we calculated the compliance of synthesized compounds to the Lipinski's rule of five.[23] This approach has been widely used as a filter for substances that would likely be further developed for drug design programs. We have also assessed parameters like number of rotatable bonds (>10) and the number of rigid bonds which signify that the compound may have good oral bioavailability and good intestinal absorption.[24]

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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SUPPORTING INFORMATION

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