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Research Article

STUDY OF INFLUENCE OF LINKERS AND SUBSTITUTIONS ON ANTIMICROBIAL ACTIVITY OF SOME SCHIFF BASES

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ABSTRACT

A series of twelve different Schiff bases in four sets each with different linkers and substitutions was synthesized by condensing appropriate aromatic amines with different aromatic aldehydes in the presence of glacial acetic acid at pH 4-6. The structures of all the synthesized compounds have been characterized on the basis of analytical and spectral data. The antimicrobial activities of all these compounds 3(a-l) were evaluated by measuring zone of inhibition using agar diffusion method. The results of the antimicrobial activity showed that the compounds 3e (amide liker and p-chloro phenyl substitution) and 3h (acetoxy amide linker and p-chloro phenyl substitution) had LogP values of 3.77 and 3.74, respectively, and these were equivalent to the LogP values of standard Ketoconazole that showed significant antifungal activities. None of the compounds showed good antibacterial activity except 3f (amide linker with o-hydroxy phenyl substitution), 3g (with acetoxy amide linker) and 3j (with succinamide linker) that had LogP values of 3.03, 3.06 and 2.7, respectively compared to the standard ciprofloxacin with LogP value of -0.77. The results showed the significance of linkers and different substitution on Schiff bases over antimicrobial activities that should be considered for the design of new antimicrobial agents.

Keywords: Synthesis, Schiff bases, Linkers, Antimicrobial activity.

INTRODUCTION

The growing incidence of bacterial resistance to existing antibiotics poses a serious medical problem in treating pathogenic infections[1, 2]. Hence, there is an urgent need for novel molecules which are more potent and less sensitive to developing resistance properties than current clinically used antibacterials [3]. Many novel approaches have been employed in this regard, these include exploitation of new targets [2], structural modifications to the existing molecules, combining two pharmacophores in one molecule[14], and exploitation of structural variants like spacers or linkers[6-9]. Among these, use of structural variants like spacers or linkers is getting popular nowadays in the process of discovery of novel compounds as antibacterials through the reactions involving Schiff bases [9, 16, 17].

Imines/Schiff bases were first discovered by Hugo (ugo) Schiff more than a century ago. Since then Schiff bases constitute one of the most widely used families of organic compounds[4]. Compounds which posses R-CH=N-R' as a general formula are called Imines or Schiff bases and can be efficiently prepared by condensation of an aromatic aldehyde or ketone with an appropriate aromatic amine at an optimum pH of 4-6 using dry alcohol as a solvent[4, 10]. Schiff bases were reported to possess antifungal, antibacterial, anti-*T. cruzi*, estrogenic and cytotoxic activities [11-14,16]. Hearn et al. (2004) demonstrated structural variant of Isoniazid (INH) i.e., INH Schiff base that displayed strong activity, low toxicity and excellent bioavailability[15]. Shi et al. (2007) studied Structural Activity Relationship (SAR) of some Schiff bases derived form 5-chloro-salicyladehyde and concluded that the hydrophilicity and aromaticity are important parameters for antimicrobial activity[16]. Paula et al. (2009) verified SAR considering the lipophilicity potential maps and calculated logP values for the set of novel 5-nitro-heterocyclic Schiff bases and concluded that chlorine substitution on furfuryliden indicated optimum lipophilicity value and hence had better biological effect[13]. Linkers can be used for the selection of desirable properties associated with structural dynamics[5, 6].

The present study is oriented towards dual conceptualization that comprised the synthesis of various Schiff bases with different linkers and varying lipophilicity value (LogP value) between imine functionality and a planar phenyl ring. This study also involves effect of substitutions with different electronic influences on the phenyl ring directly attached to imine carbon. In addition, an attempt was made to justify SAR with lipophilicity values (LogP) of synthesized Schiff bases, since hydrophobic property is important for the drugs to diffuse through the pathogenic biological system[13]. Hence, this study may be helpful for the medicinal chemists in understanding antimicrobial activity of Schiff bases, one with different inter atomic distances (linkers) and with different electronic environments.

MATERIAL AND METHODS

Experimental

Melting points were determined by open capillary and are uncorrected. The purity of the compounds was checked using precoated TLC plates (MERCK, 60F) using n-Hexane: Ethyl acetate solvent system with a gradient of polarity from 5:5 to 8:2. The plates were visualized under UV light (254 nm). IR spectra were recorded using KBr on Shimadzu FTIR model 8400 Spectrophotometer, ^{1}H NMR spectra was performed in DMSO (D_6) on a BRUKER FT-NMR instrument using TMS as internal standard. LogP value of synthesized compounds and standard drugs was obtained from Molinformation Cheminformatics 2009 software[18, 19].

General procedure for the synthesis of Schiff bases 3(a-c) without linker.

A mixture of aniline **1a** (0.1 mol), different aldehydes **2(a-c)** (0.1 mol) and few drops of glacial acetic acid in dry alcohol (50 ml) was refluxed for 3-4 h or until a distinct spot on TLC was obtained. The reaction mixture was cooled and poured into ice cold water. The solid obtained was filtered, washed with ice cold water, dried and recrystallized from absolute alcohol to give compounds **3(a-c)**. The solvent system for TLC used was *n*-Hexane: Ethyl acetate (8:2).

General procedure for the synthesis of Schiff bases 3(d-f) having amide linker.

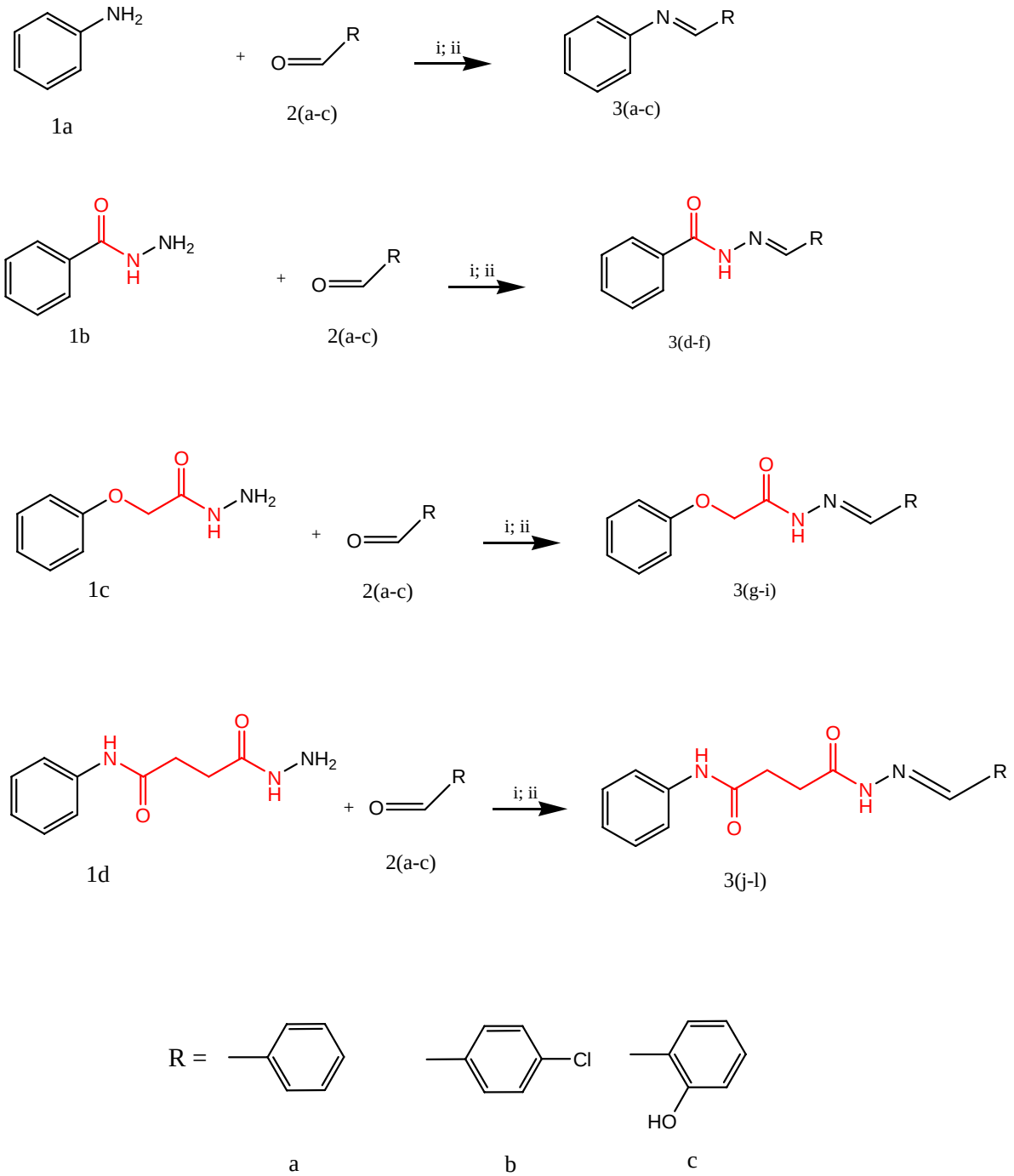
A mixture of benzohyrazide **1b** (0.1 mol), different aldehydes **2(a-c)** (0.1 mol) and few drops of glacial acetic acid in dry alcohol (50 ml) was refluxed for 5-6 h or until a distinct spot on TLC was obtained. The reaction mixture was cooled and poured into ice cold water. The solid obtained was filtered, washed with ice cold water, dried and recrystallized from absolute alcohol to give compounds **3(d-f)**. The solvent system for TLC used was *n*-Hexane: Ethyl acetate(6:4).

General procedure for the synthesis of Schiff bases 3(g-i) having acetoxy amide linker.

A mixture of phenoxy acetohydrazide **1c** (0.1 mol), different aldehydes **2(a-c)** (0.1mol) and few drops of glacial acetic acid in dry alcohol (50 ml) was refluxed for 30min-1h or until a distinct spot on TLC was obtained. The reaction mixture was cooled and poured into ice cold water. The solid obtained was filtered, washed with ice cold water, dried and recrystallized from absolute alcohol to give compounds **3(g-i).** The solvent system for TLC used was *n*-Hexane: Ethyl acetate (5:5).

General procedure for the synthesis of Schiff bases 3(j-l) having succinamide linker.

A mixture of succinanilic acid hydrazide **1d** (0.1 mol), different aldehydes **2(a-c)** (0.1mol) and few drops of glacial acetic acid in dry alcohol (50 ml) was refluxed for 3-4 h or until a distinct spot on TLC was obtained. The reaction mixture was cooled and poured into ice cold water. The solid obtained was filtered, washed with ice cold water, dried and recrystallized from absolute alcohol to give compounds **3(j-l).** The solvent system for TLC used was *n*-Hexane: Ethyl acetate (7:3).



Scheme 1: Synthesis of Schiff bases

i = Glacial acetic acid.

ii = Dry ethyl alcohol.

N-Benzylidene benzenamine (3a):

IR (KBr cm^{-1}): 1583(HC=N).

^{1}H-NMR (DMSO δ ppm): 8.63 (1H, s, =CH-Ar), 7.10-7.82(10H, m, Ar-H).

N-(4-Cholrobenzylidene)-bezenamine (3b):

IR (KBr cm^{-1}): 1585(HC=N), 827(Ar-Cl).

^{1}H-NMR (DMSO δ ppm): 8.62 (1H, s, =CH-Ar), 7.23-7.96(9H, m, Ar-H).

2-[(Phenylimino)-methyl]-phenol (3c):

IR (KBr cm^{-1}): 1581(HC=N), 1389(Ar-OH).

^{1}H-NMR (DMSO δ ppm): 8.95 (1H, s, =CH-Ar), 6.95-7.66(9H, m, Ar-H), 4.99(1H, m, Ar-OH).

N-Benzylidene benzohydrazide (3d):

IR (KBr cm^{-1}): 1548(HC=N), 1645(C=O).

^{1}H-NMR (DMSO δ ppm): 8.55 (1H, s, =CH-Ar), 8.86 (1H, s, CO-NH), 6.90-7.94(10H, m, Ar-H).

N-(4-ChloroBenzylidene)-benzohydrazide (3e):

IR (KBr cm^{-1}): 1541(HC=N), 1664(C=O), 825(Ar-Cl).

^{1}H-NMR (DMSO δ ppm): 8.62 (1H, s, =CH-Ar), 8.82 (1H, s, CO-NH), 6.90-7.86 (9H, m, Ar-H).

N-(2-Hydroxy Benzylidene)-benzohydrazide (3f):

IR (KBr cm^{-1}): 1562(HC=N), 1681(C=O), 1365(Ar-OH).

^{1}H-NMR (DMSO δ ppm): 8.64 (1H, s, =CH-Ar), 8.61 (1H, s, CO-NH), 6.90-7.94 (9H, m, Ar-H), 5.01 (1H, m, Ar-OH).

N-Benzylidene-2-Phenoxy-acetohydrazide (3g):

IR (KBr cm^{-1}): 1593(HC=N), 1681(C=O), 3370(-NH).

^{1}H-NMR (DMSO δ ppm): 8.55 (1H, s, =CH-Ar), 8.20 (1H, s, CO-NH), 6.82-7.70 (10H, m, Ar-H).

N-(4-Chloro Benzylidene)-2-Phenoxy-acetohydrazide (3h):

IR (KBr cm^{-1}): 1531(HC=N), 1676(C=O), 3390(-NH), 825(Ar-Cl).

^{1}H-NMR (DMSO δ ppm): 8.32 (1H, s, =CH-Ar), 8.46 (1H, s, CO-NH), 6.92-7.62 (9H, m, Ar-H).

N-(2-Hydroxy Benzylidene)-2-Phenoxy-acetohydrazide (3i):

IR (KBr cm^{-1}): 1592(HC=N), 1681(C=O), 3429(-NH) 1345(Ar-OH).

^{1}H-NMR (DMSO δ ppm): 8.55 (1H, s, =CH-Ar), 8.29 (1H, s, CO-NH), 6.82-7.70 (9H, m, Ar-H), 5.09 (1H, m, Ar-OH).

N-Benzylidene-4-oxo-4-(Phenylamino)-butanehydrazide(3j):

IR (KBr cm^{-1}): 1554(HC=N), 1654(C=O), 3194(-NH), 2962-2970(CH_2).

^{1}H-NMR (DMSO δ ppm): 8.35 (1H, s, =CH-Ar), 8.28 (1H, s, CO-NH), 2.54-2.90(4H, m, CH_2), 6.88-7.63 (10H, m, Ar-H).

N-(4-Chloro Benzylidene)-4-oxo-4-(Phenylamino)-butanehydrazide(3k):

IR (KBr cm^{-1}): 1550(HC=N), 1656(C=O), 3201(-NH), 3057 (-CH_2), 825(Ar-Cl).

^{1}H-NMR (DMSO δ ppm): 8.32 (1H, s, =CH-Ar), 8.76 (1H, s, CO-NH), 2.52-2.85(4H, m, CH_2), 6.72-7.68 (9H, m, Ar-H).

N-(2-Hydroxy Benzylidene)-4-oxo-4-(Phenylamino)-butanehydrazide(3l):

IR (KBr cm^{-1}): 1572(HC=N), 1652(C=O), 3201(-NH), 3049 (-CH_2), 1388(Ar-OH).

^{1}H-NMR (DMSO δ ppm): 8.34 (1H, s, =CH-Ar), 8.99 (1H, s, CO-NH), 2.52-2.90(4H, m, CH_2), 7.69-6.85 (9H, m, Ar-H), 5.00 (1H, m, Ar-OH).

Table 1: **Physical constants data of synthesized compounds**

Compound	R	Mol. formula	Yield (%)	M.P.(ºC)	R_f value
3a	C_6H_5	$C_{13}H_{11}N$	90	58	0.173
3b	4-Cl.C_6H_4	$C_{13}H_{10}NCl$	88	63	0.162
3c	2-OH.C_6H_4	$C_{13}H_{11}NO$	96	52	0.180
3d	C_6H_5	$C_{14}H_{12}N_2O$	76	204	0.383
3e	4-Cl.C_6H_4	$C_{14}H_{11}N_2OCl$	80	184	0.218
3f	2-OH.C_6H_4	$C_{14}H_{12}N_2O_2$	86	188	0.200
3g	C_6H_5	$C_{15}H_{14}N_2O_2$	79	238	0.176
3h	4-Cl.C_6H_4	$C_{15}H_{13}N_2O_2Cl$	82	215	0.191
3i	2-OH.C_6H_4	$C_{15}H_{14}N_2O_3$	88	192	0.183
3j	C_6H_5	$C_{17}H_{17}N_3O_2$	96	190	0.450
3k	4-Cl.C_6H_4	$C_{17}H_{16}N_3O_2Cl$	94	242	0.442
3l	2-OH.C_6H_4	$C_{17}H_{17}N_3O_3$	86	225	0.483

Table 2: Calculated Log P values for synthesized compounds[18, 19]

Compound	Calculated LogP value	Compound	Calculated LogP value	Compound	Calculated LogP value	Compound	Calculated LogP value
3a	3.47	**3d**	3.09	**3g**	3.06	**3j**	2.73
3b	4.14	**3e**	3.77	**3h**	3.74	**3k**	3.40
3c	3.41.	**3f**	3.03	**3i**	3.00	**3l**	2.67

Table 3: Log P values for Standard compounds[18, 19]

Standard compounds	Calculated LogP value
Ketoconazole	3.77
Ciprofloxacin	-0.77

Antibacterial activity[7, 16]

The antibacterial activities of all the synthesized compounds 3(a-l) were screened against different Gram-positive (*B. subtilis*) and Gram-negative (*E. coil* and *P. aeruginosa*) organisms by measuring zone of inhibition[7, 16]. The antibacterial activity was performed by Agar diffusion method at concentration level of 250µg/ml. Ciprofloxacin was used as standard drug at a concentration of 250µg/ml. Nutrient agar was used as culture media and DMSO was used as solvent control. The results of the antibacterial activity are shown in Table 4.

Antifungal activity[16, 20]

The antifungal activities of all the synthesized compounds 3(a-l) were screened against *A. niger* and *C. albicans* by measuring zone of inhibition[16, 20]. The antifungal activity was performed by Agar diffusion method at a concentration level of 250µg/ml. Ketoconazole was used as standard drug at a concentration of 250µg/ml. Sabouraud dextrose agar was used as culture media and DMF was used as solvent control. The results of the antifungal activity are shown in Table 4.

Table 4: Antimicrobial activity (Zone of inhibition in mm) data of synthesized compounds

Compound	Antibacterial activity			Antifungal activity	
	P. aeruginosa	*B. subtilis*	*E. coli*	*A. niger*	*C. albicans*
3a	4	-	-	15	21
3_b	-	8	-	21	16
3c	-	14	12	18	18
3d	6	-	12	16	15
3e	-	-	-	22	22
3f	14	12	14	18	16
3g	13	14	12	18	18
3h	-	-	-	23	24
3i	12	-	-	19	12
3j	15	14	13	16	11
3k	4	-	-	18	15
3l	6	-	10	17	16
Control	-	-	-	-	-
Ciprofloxacin	24	26	25	-	-
Ketoconazole	-	-	-	24	26

'-' Indicates no inhibition

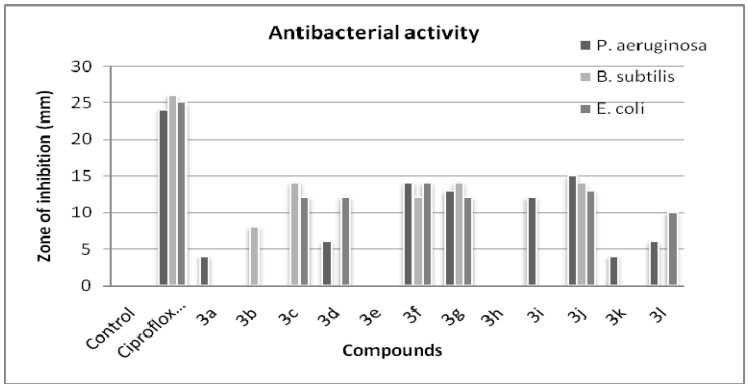


Fig. 1: Antibacterial activity of synthesized compounds

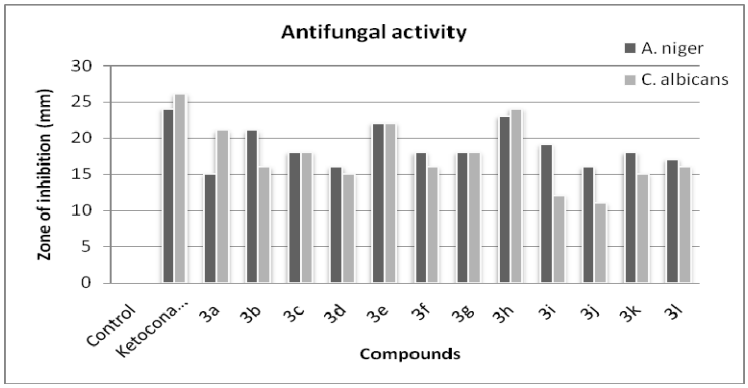


Fig. 2: Antifungal activity of synthesized compounds

RESULTS AND DISCUSSION

In this study, a series of substituted Schiff base compounds were synthesized. All the set of synthesized compounds 3(a-l) possess their own characteristic crystalline structure and were purified by successive recrystallization using absolute alcohol. The purity of the synthesized compounds was confirmed by performing TLC using different ratios of *n*-Hexane: ethyl acetate as developing solvent with gradient of polarity as described in methods. The structures of the synthesized compounds were determined on the basis of their FTIR and ^{1}H NMR data.

The IR spectra of the synthesized compounds showed the presence of $\nu_{C=N}$ stretching bands at 1531-1585 cm^{-1} which corresponded to imine or Schiff bases and $\nu_{C=O}$ stretching bands at 1645-1681 cm^{-1}. In

^{1}H NMR spectra of the synthesized compounds, singlet peak was obtained in the range of 8.32 – 8.94 δ ppm for imine protons**.**

Various substituted Schiff bases with three different linkers between planar phenyl ring and imine functionality were prepared in an attempt to vary LogP value (Lipophilicity parameter) which is important factor for the drug to diffuse through the parasite biological membrane. It is found that none of the compounds showed good antibacterial activity except the compound 3f (amide linker with o-hydroxy phenyl substitution), 3g (with acetoxy amide linker) and 3j (with succinamide linker) (Table 4; Fig 1) that had LogP values of 3.03, 3.06 and 2.7, respectively (Table 2 and 3). This may be due to the large variation in the LogP values of the synthesized compounds compared to the value of -0.77 for the standard ciprofloxacin (Table 2 and 3). Further, compounds 3e (acetoxy amide linker) and 3h (acetoxy amide linker and p-chlorophenyl substitution) showed significant antifungal activity (Table 4; Fig 2) and this may be due to the fact that their LogP value was found to be 3.77 and 3.74, which are equivalent to standard ketoconazole (Table 2 and 3). All these data correlated that there is a strong relationship between different linkers used and substitutions occurred with the Schiff bases upon antimicrobial activity, which must be taken into consideration during design of new antimicrobials.

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