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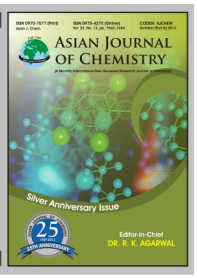

Synthesis and Spectral Studies of Acetophenone Schiff Bases and Evaluation of their Antimicrobial Activities

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Seven acetophenone derived Schiff bases with different amines were synthesized including, (Z)-2-[(1-phenylethylidene)amino]benzoic acid (**HL**[1]), (Z)-4-[(1-phenylethylidene)amino]benzoic acid (**HL**[2]), (E)-N-(1-phenylethylidene)naphthalen-2-amine (**HL**[3]), (E)-1-phenyl-2-(1-phenylethylidene)hydrazine (**HL**[4]), N^1,N^2-*bis*(1-phenylethylidene)ethane-1,2-diamine (**HL**[5]), N^1(Z),N^2(Z)-N^1,N^2-*bis*(1-phenylethylidene)benzene-1,2-diamine (**HL**[6]) and N^1,N^4-*bis*(1-phenylethylidene)benzene-1,4-diamine (**HL**[7]) and characterized by spectral studies (IR, MS and NMR) and elemental analysis. Their antimicrobial activity was extensively investigated against 20 Gram-negative and 10 Gram-positive bacterial strains. Schiff bases **HL**[1-5] showed good antibacterial activity against almost all bacterial strains. **HL**[3] showed remarkable activity against *Enterococcus* sp., *Citrobacter freundii, Salmonella typhi* and *Pseudomonas aurantiaca.* **HL**[4] gave incredible inhibition against *Enterobacter aerogenes*. **HL**[7] demonstrated notable activity against *Staphylococcus aureus.* (E)-N-(1-phenylethylidene)naphthalen-2-amine (**HL**[3]) is reported for the first time.

Key Words: Acetophenone, Schiff bases, Spectroscopic, Antimicrobial activity.

INTRODUCTION

Growing pathogenic resistance to existing antibiotic drugs is one of the most baffling challenges to human health in the modern times and necessitates constant efforts to discover more effective and safer therapeutic agents[1-3]. The infectious diseases are, in fact, one of the main causes of deaths the world over[4]. The screening of both the natural products and the synthetic compounds against different pathogenic microorganisms has thus emerged to be one of the most active fields of research today.

The Schiff bases[5-8], or substances with azomethine functionality, comprise a major class of organic compounds being investigated for multifarious applications including their possible development into therapeutic agents. They have been found to possess antibacterial[9,10], antifungal[11], antiviral[12], anti-inflammatory[13], analgesic[14], anti- tumour[15], anticonvulsant[16], anti HIV[17] and antileishmanial[18] activities. They are also good ligands[19] and can coordinate with metals *in vitro* as well as *in vivo*.

Acetophenone, a ketone having both an aliphatic and an aromatic moiety, has been shown to form Schiff bases exhibiting considerable antibacterial[20,21] and antifungal[22,23] activities along with other useful applications[24-28].

As part of our ongoing search for new therapeutic agents, seven different Schiff bases were synthesized from unsubstituted acetophenone and different primary amines including mono- and diamines of both aromatic and aliphatic nature. According to our knowledge, acetophenone derived Schiff base with β-naphthylamine is being reported for the first time. Spectral studies including ^{1}H NMR, ^{13}C NMR, IR and MS were carried out to characterize the compounds. Synthesised Schiff bases were then screened for the first time ever against thirty different bacterial strains including gram positive and gram negative bacteria, pathogenic as well as non-pathogenic.

EXPERIMENTAL

All the reagents and solvents were of analytical-grade quality purchased from Aldrich Co and were used without further purification. Melting points were found on Gallenkamp (Electronic) melting point apparatus and were uncorrected. The reactions were monitored by thin-layer chromatography (TLC) on silica gel plates (Sigma-Aldrich). A mixture of ethanol and ethyl acetate (1:1) was employed as mobile phase and UV lamp, Model UVLS-225 D, was used for detection.

The IR spectra of the compounds were taken using KBr disks on Varian 640-IR spectrometer (cm^{-1}). UV-VIS absorption spectra were recorded with Spectro UV-VIS Double PC 8 Auto Cell with variable bandwidth of 0.5, 1.0, 2.0 and 5.0 nm, Model UVD-3200 spectrometer. Both ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz NMR spectrometer

(H.E.J. Research Institute of Chemistry, Karachi, Pakistan), with chemical shift in ppm downfield from TMS as an internal reference. High resolution mass spectra were captured on Varian MAT 312 mass spectrometer (H.E.J. Research Institute of Chemistry, Karachi, Pakistan).

Synthesis of Schiff bases

Schiff bases HL[1] to HL[4]: Acetophenone (1.16 mL, 10 mmol) with *o*-aminobenzoic acid (1.37 g, 10 mmol) (for **HL[1]**), *p*-aminobenzoic acid (1.37 g, 10 mmol) (for **HL[2]**), β-naphthylamine (1.43 g, 10 mmol) (for **HL[3]**) and phenylhydrazine (0.99 mL, 10 mmol) (for **HL[4]**) in different round bottom flasks were refluxed in 20 mL methanol at 40 ºC for 3 h in the presence of 1 mL glacial acetic acid. In each case, the precipitated base was filtered off, recrystallized from absolute ethanol and dried in vacuum desiccator. The structure of the synthesized schiff bases are given in Fig. 1.

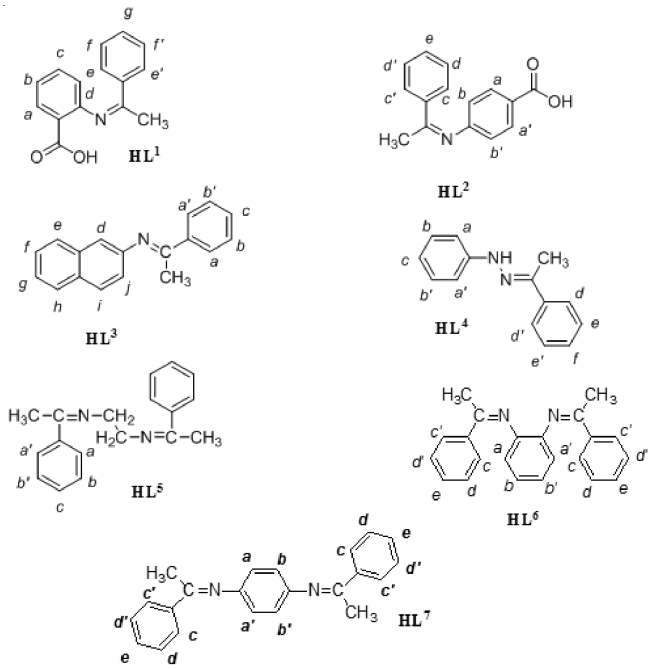

Fig. 1. Structures of synthesized Schiff bases

Schiff bases HL[5] to HL[7]: In different experiments, acetophenone (2.33 mL, 20 mmol) with ethylene diamine (0.66 mL, 10 mmol) (for **HL[5]**), *o*-amino aniline (1.08 g, 10 mmol) (for **HL[6]**) and *p*-amino aniline (1.08 g, 10 mmol) (for **HL[7]**) was refluxed at 40 ºC for 3 h in 20 mL of methanol in the presence of 1 mL glacial acetic acid. The resultant ligands were filtered, recrystallized from absolute ethanol and dried in vacuum desiccator. The structures of proposed ligands are shown in Fig. 1.

Antimicrobial analysis

Test microbes: Seventeen different bacterial cultures were acquired from The Children Hospital, Lahore, Pakistan including two strains of *E. coli* [*Escherichia coli* (1) and *Escherichia coli* (2)], two strains of *S. aureus* [*Staphylococcus aureus* (1) and *Staphylococcus aureus* (2)], two strains of *P. aeruginosa* [*Pseudomonas aeruginosa* (1) and *Pseudomonas aeruginosa* (2)], two unidentified *Bacillus* species [*Bacillus* sp.(1) and *Bacillus* sp.(2)] and one strain each of *Pseudomonas aurantiaca* , *Salmonella typhi* (1), *Stenotroph maltophilia*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Bacillus subtilis* and *Bacillus megaterium.* Thirteen different microbial colonies were attained from biotechnology laboratory, Forman Christian College, Lahore, Pakistan including two strains of *E. coli* [*Escherichia coli* (3) and *Escherichia coli* (4)] and single strain each of *Pseudomonas aeruginosa* (3), *Pseudomonas* sp., *Salmonella typhi* (2), *Salmonella* sp., *Achromobacter xylosoxidans, Azospirillum lipoferum, Rhizobium* sp., *Citrobacter freundii, Staphylococcus aureus* (3), *Bacillus* sp.(3) and *Enterococcus* sp. All strains were stored at -20 ºC until utilized.

Determination of zones of inhibition (ZI): Agar well diffusion method[29,30] was employed for antimicrobial screening in terms of the zones of inhibition.

Preparation of standard dilutions: In each case, 2 mg of a synthesized compound was dissolved per mL of DMSO to yield stock solution, which was serially diluted to 0.5-1 mg/mL. Three antibiotic drugs levofloxacin, cefixime and amoxylin were used as standard with final concentration of 2 mg/mL in DMSO.

Preparation of inoculums: All microbial cultures were re-isolated thrice successively on Mueller-Hinton Agar, MHA (Merck) with incubation period of 24 h at 37 ºC and identity confirmed by standard bacteriological methods. A loop full of bacterial suspension was then diluted with sterile physiological solution to standardize inoculum density up to 10^8 CFU/mL of bacterial cells (equivalent to turbidity of McFarland, barium sulphate standard 0.5) followed by 24 h incubation at 37 ºC.

Antimicrobial screening (zones of inhibition): The bacterial inoculums were uniformly swabbed on prepared Mueller-Hinton Agar plate followed by a session of drying, after which three wells of 7 mm diameter each were dug in the agar gel 33 mm apart from one another using a sterile cork borer. A 100 µL volume of test compound dilutions were pipetted into the triplicate wells and allowed to stand for an hour for diffusion to take place. Finally, plates were incubated at 37 ºC for 24 h after which zones of inhibition were recorded to the nearest mm.

Determination of minimum inhibitory concentration (MIC): Minimum inhibitory concentration (MIC) is defined as the lowest concentration of an anti-microbial agent requisite to inhibit bacterial growth. The agar dilution method[31,32] was executed to evaluate MICs of synthesized compounds and antibiotics. Briefly, 0.004 g/mL stock solution of each of the test compounds was prepared in DMSO, from which graded concentrations were made to achieve final concentrations (mg/mL) of 0.02, 0.04, 0.08, 0.12, 0.16, 0.20, 0.24, 0.28, 0.32, 0.36 and 0.40 in total 20 mL of Mueller-Hinton Agar plates by varying the volume of agar and test stock solutions. The plates were spotted with 0.1 mL overnight activated cultures of the microbes and incubated at 37 ºC for 24 h.

RESULTS AND DISCUSSION

In the present study, seven different Schiff bases of the ketone acetophenone were synthesized with different amines using the conventional reflux method. The structures (Fig. 1)

of the bases were elucidated on the bases of elemental analysis and spectroscopic data. The bases were then subjected to antimicrobial screening against different bacterial strains.

(Z)-2-((1-Phenylethylidene)amino)benzoic acid (HL^1): Orange yellow; yield (%): 86; m.p. 233 ºC; anal. calcd. (%) for $C_{15}H_{13}NO_2$: 75.31, C; 5.43, H; 5.85, N; 13.38, O. Found (%): 75.30, C; 5.42, H; 5.83, N; 13.35, O. Selected IR (KBr, ν_{max}, cm^{-1}) 1627 (-C=N-), 2900-3100 (O-H), 1721 (C=O), 1290 (C-O). ^{1}H NMR (CD_3OD, 300 MHz) δ 2.28 (s, 3H, CH_3), 6.82 (m, 3H, $ArH_{f=f',g}$), 7.25(d, 1H, ArH_d, ^3J = 7.2 Hz), 7.52 (t, 1H, ArH_c, ^3J = 7.2Hz), 7.74(d, 2H, $ArH_{e=e'}$, ^3J = 7.5 Hz), 7.85(t, 1H, ArH_b, ^3J = 7.2Hz), 8.12(d, 1H, ArH_a, ^3J = 7.2 Hz), 11.9 (s, 1H, COOH); ^{13}C NMR (CD_3OD, 75 MHz) δ 18.3 (1C, CH_3), 111.2 (1C), 122.3 (1C), 125.0 (1C), 126.7 (3C), 127.8 (1C), 133.1 (1C), 135.2 (1C), 137.8 (1C), 150.2 (1C, C-N), 165.3 (1C, C=N), 171.0 (1C, C=O); MS: m/z 239 [M^+].

(Z)-4-((1-Phenylethylidene)amino)benzoic acid (HL^2): Lime yellow; yield (%): 82; m.p. 227 ºC; anal. calcd. (%) for $C_{15}H_{13}NO_2$: 75.31, C; 5.43, H; 5.85, N; 13.38, O. Found (%): 75.31, C; 5.42, H; 5.84, N; 13.36, O. Selected IR (KBr, ν_{max}, cm^{-1}): 1629 (-C=N-), 2900-3100 (O–H), 1720 (C=O), 1293 (C–O). ^{1}H NMR (CD_3OD, 300MHz) δ 2.27 (s, 3H, CH_3), 6.86 (m, 3H, $ArH_{d=d',e}$), 7.34(d, 2H, $ArH_{b=b'}$, ^3J = 7.5 Hz), 7.52(d, 2H, $ArH_{c=c'}$, ^3J = 7.5Hz), 8.0(d, 2H, $ArH_{a=a'}$, ^3J = 7.5Hz), 11.8 (s, 1H, COOH); ^{13}C NMR (CD_3OD, 75 MHz) δ 24.2(1C, CH_3), 112.2 (2C), 120.3(2C), 126.0(1C), 130.8(2C), 133.1(2C), 135.4(4C), 137.8(1C), 152.1(1C, C-N), 163.4(1C, C=N), 171.1(1C, C=O); MS: m/z 239 [M^+].

(E)-N-(1-Phenylethylidene)naphthalen-2-amine (HL^3): Faded pink; yield (%): 84; m.p. 239 ºC; anal. calcd. (%) for $C_{18}H_{15}N$: 88.16, C; 6.12, H; 5.72, N. Found (%): 88.08, C; 6.11, H; 5.71, N. Selected IR (KBr, ν_{max}, cm^{-1}): 1631 (-C=N-). ^{1}H NMR (CD_3OD, 300 MHz) δ 2.28 (s, 3H, CH_3), 7.23(d, 1H, ArH_j, ^3J = 7.2 Hz), 7.32 (m, 3H, $ArH_{b=b',c}$), 7.42(m, 2H, $ArH_{g,f,}$), 7.72(d, 2H, $ArH_{a=a'}$), 7.88(m, 4H, $ArH_{d,e,h,i}$); ^{13}C NMR (CD_3OD, 75MHz) δ 18.3(1C, CH_3), 110.2(1C), 115.3(1C), 120.0(2C), 123.3(1C), 125.1(2C), 125.3(2C), 125.6(2C), 126.2(1C), 127.4(1C), 128.2(1C), 134.2(1C), 135.2(1C, C-N), 146.3(1C, C=N); MS: m/z 245 [M^+].

(E)-1-Phenyl-2-(1-phenylethylidene)hydrazine (HL^4): Chocolate brown; yield (%): 90; m.p. 64 ºC; anal. calcd. (%) for $C_{14}H_{14}N_2$: 80.00, C; 6.66, H; 13.37, N. Found (%): 79.92, C; 6.64, H; 13.34, N. Selected IR (KBr, ν_{max}, cm^{-1}): 1637 (-C=N-), 3350 (NH). ^{1}H NMR(CD_3OD, 300 MHz) δ 2.28 (s, 3H, CH_3), 6.68 (t, 1H, Ar_c), 7.27(m, 3H, $ArH_{b=b',c}$), 7.02 (t, 2H, $ArH_{b=b'}$, ^3J = 7.2 Hz), 7.25 (d, 2H, $ArH_{a=a'}$, ^3J = 7.5Hz), 7.52 (t, 3H, $ArH_{e=e',f}$), 12.96 (s, 1H, NH); ^{13}C NMR (CD_3OD, 75 MHz) δ 18.3 (1C, CH_3), 115.2(2C), 115.4(1C) 116.5.2 (2C), 116.9 (2C), 118.6 (2C), 136.6 (1C), 138.7 (1C), 168.2 (1C, C=N); MS: m/z 210 [M^+].

N^1,N^2-*Bis*(1-phenylethylidene)ethane-1,2-diamine (HL^5): Pale yellow; yield (%): 80; m.p. 120 ºC; anal. calcd. (%) for $C_{18}H_{20}N_2$: 81.20, C; 7.15, H; 10.17, N. Found (%): 81.81, C; 7.57, H; 10.60, N. Selected IR (KBr, ν_{max}, cm^{-1}): 1635 (-C=N-). ^{1}H NMR (CD_3OD, 300 MHz) δ 2.26 (s, 6H, CH_3), 4.24 (s, 2H, $-CH_2-$), 7.27(m, 6H, $ArH_{b=b',c}$), 7.54 (d, 4H, $ArH_{a=a'}$, ^3J = 7.2Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ 18.0 (2C, CH_3), 54.1 (2C, $-CH_2-$), 127.2 (4C), 128.1 (6C), 128.6 (2C), 142.7 (2C), 168.3 (2C, C=N); MS: m/z 264 [M^+].

(N^1(Z),N^2(Z)-N^1,N^2-*Bis*(1-phenylethylidene)benzene-1,2-diamine(HL^6): Earth brown; yield (%): 88; m.p. 238 ºC; anal. calcd. (%) for $C_{22}H_{20}N_2$: 84.61, C; 6.41, H; 8.98, N. Found (%): 84.58, C; 6.40, H; 8.97, N. Selected IR (KBr, ν_{max}, cm^{-1}): 1632 (-C=N-). ^{1}H NMR (CD_3OD, 300 MHz) δ 1.98 (s, 6H, CH_3), 6.92-7.11 (m, 4H, $Ar_{b=b',e}$), 7.15-7.30 (m, 4H, $ArH_{d=d'}$), 7.43 (d, 4H, $ArH_{c=c'}$, ^3J = 7.5 Hz), 7.59 (d, 4H, $ArH_{a=a'}$, ^3J = 7.5Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ 22.2 (2C, CH_3), 121.9 (2C), 122.7 (2C), 126.9 (4C), 127.7 (4C), 128.4 (2C), 129.0 (2C), 129.1 (2C), 169.7 (2C, C=N); MS: m/z 312 [M^+].

N^1,N^4-*Bis*(1-phenylethylidene)benzene-1,4-diamine (HL^7): Earth brown; yield (%): 88; m.p. 238 ºC; anal. calcd. (%) for $C_{22}H_{20}N_2$: 84.61, C; 6.41, H; 8.98, N. Found (%): 84.60, C; 6.39, H; 8.95, N. Selected IR (KBr, ν_{max}, cm^{-1}): 1631 (-C=N-). ^{1}H NMR (CD_3OD, 300 MHz) δ 2.28 (s, 6H, CH_3), 6.88 (s, 4H, $Ar_{a=a'=b=b'}$), 7.47(m, 6H, $ArH_{d=d',e}$), 7.92 (dd, 4H, $ArH_{c=c'}$, ^3J = 7.2 Hz, ^4J = 2.5 Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ 18.2 (2C, CH_3), 121.7(4C), 128.4(4C), 129.2(4C), 131.7(2C), 141.0(2C), 148.5(2C), 169.8(2C, C=N); MS: m/z 312 [M^+].

The IR spectra of the synthesized Schiff bases showed a strong band in the region of 1610-1640 cm^{-1}, which is characteristic of the azomethine group (C=N)[33]. Moreover, O-H stretch in 3300-2500 cm^{-1} , C=O stretch in 1760-1690 cm^{-1} and C-O stretch in 1320-1210 cm^{-1} was noticed in **HL^1** and **HL^2**, together with ^{1}H NMR signal between 11.8-12.5 ppm confirming the presence of a carboxylic group in them. ^{1}H NMR signal in 12-14 ppm and an IR band in 3400-3200 cm^{-1} indicating presence of (NH) group was observed in **HL^4**.

Antimicrobial activities: The Schiff bases in general showed moderate activity against most of the tested microbes. In some cases, however, the activity was very good while some compounds exhibited no appreciable activity against different microorganisms. Notably, bases with single azomethine group were more active than those having two azomethine functionalities (Table-1). **HL^4** and **HL^3** proved to be most versatile antimicrobial agents combating all 30 strains with medium to high lethality, while **HL^5** was least active. As the Table-1 displays, **HL^3** showed remarkable activity against *Enterococcus* sp., *Citrobacter freundii, Salmonella typhi* and *Pseudomonas aurantiaca.* **HL^4** exhibited remarkable activity against *Enterobacter aerogenes*, while **HL^7** was notably potent against *Staphylococcus aureus.*

HL^1 was weakly active against *Escherichia coli* (2), *Salmonella typhi* (1), *Staphylococcus aureus* (3) and *Enterococcus* sp. with zones of inhibition between 10-11 mm and MIC between 200-240 µg/mL. It was moderately active against *Escherichia coli* (4), *Pseudomonas aeruginosa (*1) and (2), *Staphylococcus aureus* (2), *Bacillus* sp. (3), *Staphylococcus epidermidis* and *Salmonella* sp. with zones of inhibition in the range of 16-19 mm and MIC value between 120-160 µg/mL (Table-2).

HL^2 was moderately active against *Escherichia coli* (3) and (4), *Pseudomonas aeruginosa (*2) and (3), *Pseudomonas aurantiaca*, *Salmonella typhi* (1), *Salmonella* sp., *Rhizobium* sp., *Citrobacter freundii*, *Staphylococcus aureus* (2), *Bacillus subtilis*, *Bacillus* sp. (3) and *Enterococcus* sp. with zones of inhibition between 17-24 mm and MIC between 80-160 µg/mL (Table-2).

HL^3 was weakly active against *Klebsiella pneumonia* and *Bacillus* sp. (1) with zones of inhibition of 10 and 11 mm,

TABLE-1
ZONES OF INHIBITION (MM) OF ACETOPHENONE DERIVED SCHIFF BASES IN THREE DIFFERENT CONCENTRATIONS AGAINST THIRTY DIFFERENT BACTERIAL STRAINS AND THEIR COMPARISON WITH THREE STANDARD ANTIBIOTICS

S. No.	Bacterial strains	Zones of inhibition (mm)											
		Antibiotics (2 mg/mL)			**HL[1]** (mg/mL)			**HL[2]** (mg/mL)			**HL[3]** (mg/mL)		
		L	C	A	2	1	0.5	2	1	0.5	2	1	0.5
1	*Escherichia coli* (1)	38	20	16	–	–	–	12	10	7	10	9	7
2	*Escherichia coli* (2)	40	23	20	12	9	–	11	7	–	24	20	17
3	*Escherichia coli* (3)	40	25	20	–	–	–	19	15	11	10	9	8
4	*Escherichia coli* (4)	40	21	20	18	15	11	17	13	10	21	16	11
5	*Pseudomonas aeruginosa* (1)	40	20	15	16	11	8	11	7	–	24	19	15
6	*Pseudomonas aeruginosa* (2)	38	19	22	18	14	11	17	11	7	28	24	20
7	*Pseudomonas aeruginosa* (3)	38	16	10	9	7	–	17	13	10	27	21	18
8	*Pseudomonas* sp.	24	10	13	–	–	–	15	10	8	22	17	13
9	*Pseudomonas aurantiaca*	32	20	12	7	–	–	20	17	14	33	28	23
10	*Salmonella typhi* (1)	28	16	10	11	7	–	16	11	8	32	26	22
11	*Salmonella typhi* (2)	32	15	10	–	–	–	15	12	8	33	27	21
12	*Salmonella* sp.	33	16	17	18	13	9	20	17	13	29	24	19
13	*Stenotroph maltophilia*	38	27	24	–	–	–	14	10	8	22	17	11
14	*Enterobacter cloacae*	32	21	10	–	–	–	15	9	7	27	24	20
15	*Enterobacter aerogenes*	33	16	14	8	–	–	13	11	8	24	19	15
16	*Klebsiella pneumonia*	38	30	15	–	–	–	12	10	7	10	8	–
17	*Achromobacter xylosoxidans*	38	22	15	–	–	–	13	9	–	21	17	12
18	*Azospirillum lipoferum*	35	28	20	7	–	–	12	8	–	23	19	16
19	*Rhizobium* sp.	30	16	15	–	–	–	16	12	9	24	20	17
20	*Citrobacter freundii*	32	16	11	7	–	–	22	16	12	32	26	21
21	*Staphylococcus aureus* (1)	35	22	16	–	–	–	8	–	–	27	24	19
22	*Staphylococcus aureus* (2)	37	11	9	19	11	8	19	16	12	32	24	20
23	*Staphylococcus aureus* (3)	35	28	10	11	7	–	14	11	7	9	7	–
24	*Bacillus subtilis*	35	20	18	–	–	–	16	12	9	18	15	10
25	*Bacillus megaterium*	37	22	26	9	–	–	14	10	7	20	18	14
26	*Bacillus* sp.(1)	38	17	20	–	–	–	15	13	9	11	9	7
27	*Bacillus* sp.(2)	42	28	18	–	–	–	15	11	7	20	16	13
28	*Bacillus* sp.(3)	35	25	14	19	14	10	24	20	14	30	27	21
29	*Staphylococcus epidermidis*	36	20	16	17	14	9	17	13	10	31	27	22
30	*Enterococcus* sp.	28	21	17	11	8	–	24	19	14	30	25	20
		HL[4] (mg/mL)			**HL[5]** (mg/mL)			**HL[6]** (mg/mL)			**HL[7]** (mg/mL)		
1	*Escherichia coli* (1)	21	18	16	–	–	–	–	–	–	7	–	–
2	*Escherichia coli* (2)	13	10	7	–	–	–	–	–	–	17	14	9
3	*Escherichia coli* (3)	10	8	7	–	–	–	12	10	7	–	–	–
4	*Escherichia coli* (4)	25	21	18	–	–	–	–	–	–	16	14	10
5	*Pseudomonas aeruginosa* (1)	17	14	11	–	–	–	–	–	–	18	15	9
6	*Pseudomonas aeruginosa* (2)	15	12	9	–	–	–	–	–	–	–	–	–
7	*Pseudomonas aeruginosa* (3)	21	19	16	22	19	16	16	12	7	11	7	–
8	*Pseudomonas* sp.	14	11	8	–	–	–	–	–	–	–	–	–
9	*Pseudomonas aurantiaca*	23	20	18	20	17	15	15	10	8	15	11	8
10	*Salmonella typhi* (1)	13	9	7	–	–	–	–	–	–	9	–	–
11	*Salmonella typhi* (2)	22	19	16	–	–	–	–	–	–	–	–	–
12	*Salmonella* sp.	19	18	16	–	–	–	–	–	–	–	–	–
13	*Stenotroph maltophilia*	26	23	19	–	–	–	–	–	–	–	–	–
14	*Enterobacter cloacae*	19	15	11	–	–	–				–	–	–
15	*Enterobacter aerogenes*	28	24	21	–	–	–	–	–	–	–	–	–
16	*Klebsiella pneumonia*	15	10	7	–	–	–	–	–	–	–	–	–
17	*Achromobacter xylosoxidans*	22	18	16	–	–	–	–	–	–	–	–	–
18	*Azospirillum lipoferum*	14	12	9	–	–	–	–	–	–	–	–	–
19	*Rhizobium* sp.	15	12	8	–	–	–	–	–	–	–	–	–
20	*Citrobacter freundii*	20	16	13	–	–	–	–	–	–	–	–	–
21	*Staphylococcus aureus* (1)	20	18	15	–	–	–	–	–	–	35	30	24
22	*Staphylococcus aureus* (2)	19	17	13	16	11	–	–	–	–	–	–	–
23	*Staphylococcus aureus* (3)	18	16	15	–	–	–	–	–	–	22	18	13
24	*Bacillus subtilis*	14	11	8	–	–	–	–	–	–	9	–	–
25	*Bacillus megaterium*	16	11	7	–	–	–	–	–	–	11	9	–
26	*Bacillus* sp.(1)	16	13	9	–	–	–	–	–	–	10	8	–
27	*Bacillus* sp.(2)	13	10	8	–	–	–	–	–	–	–	–	–
28	*Bacillus* sp.(3)	35	30	27	26	21	18	17	13	10	31	26	20
29	*Staphylococcus epidermidis*	20	18	14	–	–	–	–	–	–	–	–	–
30	*Enterococcus* sp.	21	17	15	–	–	–	21	17	13	20	16	11

Where L = Levofloxacin, C = Cefixime and A = Amoxylin; the blank boxes show no appreciable activity.

respectively. It was strongly active against *Pseudomonas aeruginosa* (2) and (3), *Pseudomonas aurantiaca*, *Pseudomonas aurantiaca*, *Salmonella typhi* (1) and (2), *Salmonella* sp., *Enterobacter cloacae*, *Citrobacter freundii*, *Staphylococcus aureus* (1) and (2), *Bacillus* sp. (3), *Staphylococcus epidermidis* and *Enterococcus* sp. with zones of inhibition between 27-33 mm and MIC values between 20-40 µg/mL. It was moderately active against rest of microbes with MIC values in the range 80-120 µg/mL (Table-2).

HL[4] was weakly active against *Escherichia coli* (3) with zone of inhibition of 10 mm and MIC value of 200 µg/mL. It was strongly active against *Stenotroph maltophilia*, *Enterobacter aerogenes* and *Bacillus* sp. (3) with zones of inhibition between 26-33 mm and MIC values between 20-40 µg/mL. It was moderately active against the rest with MIC values 80-160 µg/mL (Table-2).

HL[5] was moderately active against *Pseudomonas aeruginosa (*3), *Pseudomonas aurantiaca, Staphylococcus aureus* (2) and *Bacillus* sp. (3) with zones of inhibition between 16-25 mm and MIC values between 80-160 µg/mL. It was totally inactive against rest of microbes (Table-2).

HL[6] was weakly active against *Escherichia coli* (3) with zone of inhibition of 12 mm and MIC value of 200 µg/mL. It showed moderate activity against *Pseudomonas aeruginosa* (3), *Pseudomonas aurantiaca*, *Bacillus* sp. (3) and *Enterococcus sp.* with zones of inhibition in between 15-21 mm and MIC values between 120-160 µg/mL. It was inactive against rest microbes (Table-2).

HL[7] was weakly active against *Pseudomonas aeruginosa (*3), *Bacillus megaterium* and *Bacillus sp.* (1) with zones of inhibition between 10-11 mm and MIC value of 200 g/mL each. It was moderately active against *Escherichia coli* (2) and (4), *Pseudomonas aeruginosa* (1), *Staphylococcus aureus* (3) and *Enterococcus* sp. with zones of inhibition in between 17-22 mm and MIC range between 80-160 µg/mL. It showed strong activity against *Staphylococcus aureus* (1) and *Bacillus* sp. (3) with zones of inhibition 35 and 31, respectively and MIC value of 20 µg/mL each (Table-2).

Comparing the screening results of **HL**[1] and **HL**[2] and **HL**[6] and **HL**[7] gave a trend proving that *p*-substitution of benzene with azomethine group is more effective than *o*-substitution and hence are better antimicrobial agents.

HL[4] was strongly active against *Escherichia coli* (1) and (4), *Pseudomonas aeruginosa (*3), *Pseudomonas aurantiaca*, *Stenotroph maltophilia*, *Enterobacter aerogenes*, *Achromobacter xylosoxidans* and *Bacillus* sp. (3) even in 0.5 mg/mL concentration with zones of inhibition between 16-27 mm.

HL[5] was strongly active against *Bacillus sp.* (3) and *Pseudomonas aeruginosa* (3) in its lowest concentration with zones of inhibition 18 mm and 16 mm, respectively whereas **HL**[7] was strongly active against *Staphylococcus aureus* (1)

TABLE-2
MIC (MINIMUM INHIBITORY CONCENTRATION) VALUES OF ACETOPHENONE DERIVED SCHIFF BASES AGAINST GRAM NEGATIVE AND GRAM POSITIVE BACTERIAL STRAINS

S. No.	Bacterial strains	MIC (µg/mL)						
		HL[1]	**HL**[2]	**HL**[3]	**HL**[4]	**HL**[5]	**HL**[6]	**HL**[7]
1	*Escherichia coli* (1)	240	200	200	120	240	320	200
2	*Escherichia coli* (2)	200	240	80	160	280	240	120
3	*Escherichia coli* (3)	240	120	200	200	240	200	240
4	*Escherichia coli* (4)	120	160	80	80	240	320	160
5	*Pseudomonas aeruginosa* (1)	160	200	80	160	240	280	120
6	*Pseudomonas aeruginosa* (2)	120	160	40	160	240	200	240
7	*Pseudomonas aeruginosa* (3)	200	120	40	120	120	160	200
8	*Pseudomonas sp.*	240	240	80	160	160	280	240
9	*Pseudomonas aurantiaca*	240	120	20	120	120	160	160
10	*Salmonella typhi* (1)	200	120	40	160	240	320	200
11	*Salmonella typhi* (2)	240	240	20	120	280	280	240
12	*Salmonella sp.*	160	120	40	120	280	360	320
13	*Stenotroph maltophilia*	240	240	80	40	240	280	400
14	*Enterobacter cloacae*	240	200	40	120	240	320	240
15	*Enterobacter aerogenes*	240	200	80	40	280	320	280
16	*Klebsiella pneumoniae*	240	200	200	160	280	320	200
17	*Achromobacter xylosoxidans*	240	200	80	120	240	320	360
18	*Azospirillum lipoferum*	200	200	80	160	280	360	240
19	*Rhizobium sp.*	240	160	80	160	240	280	240
20	*Citrobacter freundii*	200	120	20	120	240	280	320
21	*Staphylococcus aureus* (1)	240	240	40	120	240	280	20
22	*Staphylococcus aureus* (2)	120	120	20	120	160	360	320
23	*Staphylococcus aureus* (3)	200	240	200	120	240	360	80
24	*Bacillus subtilis*	240	160	120	160	280	280	200
25	*Bacillus megaterium*	200	200	120	160	280	360	200
26	*Bacillus sp.*(1)	240	240	200	160	240	280	200
27	*Bacillus sp.*(2)	240	200	120	160	240	320	240
28	*Bacillus sp.*(3)	120	80	40	20	80	160	20
29	*Staphylococcus epidermidis*	160	160	20	120	280	360	280
30	*Enterococcus sp.*	200	80	40	120	280	120	120

and *Bacillus* sp. (3) with zones of inhibition of 21 mm and 20 mm.

Conclusion

Seven different acetophenone derived Schiff bases were prepared with different amines. The base (E)-N-(1-phenylethylidene)naphthalen-2-amine is being reported for the first time. The synthesized compounds were characterized by spectral study and screened against 30 different bacterial strains. N^1,N^4-*bis*(1-phenylethylidene)benzene-1,4-diamine had outstanding activity against *Staphylococcus aureus*, thus it could be used as a hit to cure human skin and nasal passage nosocomial diseases. The new compound (E)-N-(1-phenylethylidene)naphthalen-2-amine had a remarkable versatility to combat *Enterococcus* sp., *Citrobacter freundii, Salmonella typhi* and *Pseudomonas aurantiaca* much better than any of reported antibiotics and hence, could be a lead molecule to prevent wide range of nosocomial infections including respiratory, urinary and blood infections and typhoid. (*E*)-1-phenyl-2-(1-phenylethylidene)hydrazine had extraordinary activity against *Enterobacter aerogenes* equal to that of Levofloxacin and can be formulated into drug fighting against septic arthritis, endocarditis and osteomyelitis among others, after *in vivo* testing. Our results confirmed the widely reported antimicrobial efficacy of the Schiff bases.

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