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Synthesis and Biological Evaluation of Mannich Bases of Isoxazoline Derivatives as Novel Anti-Microbial Agents

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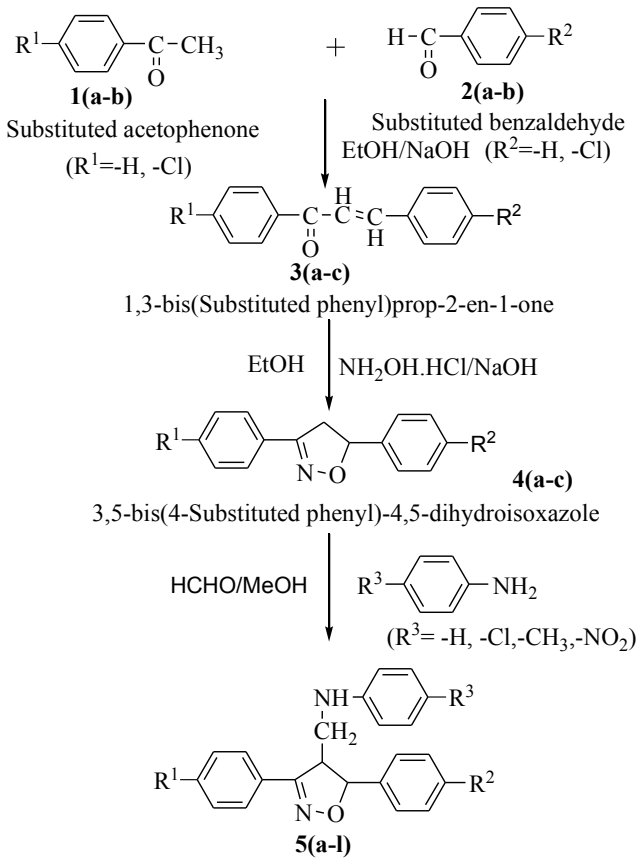
Abstract: A novel series of compounds were synthesized by condensation reaction of substituted acetophenone **(1a-b)** with substituted aldehyde **(2a-b)** in presence of alcoholic sodium hydroxide to get intermediate chalcones **(3a-c)**, which were further treated with hydroxylamine hydrochloride in presence of sodium hydroxide to get isoxazolines derivatives **(4a-c)**. The latter were refluxed with substituted primary amines and formaldehyde for 6-10 h to afford Mannich bases. The synthesized compounds were characterized on the basis of their spectral (IR, ^{1}HNMR) data and evaluated for the antimicrobial activity by using Zone of Inhibition by cup plate method and Minimum Inhibitory Concentration by broth dilution method.

Keywords- Chalcone, Isoxazoline, Mannich bases, Antimicrobial activity.

Introduction

Heterocyclic compounds have so far been synthesized mainly due to the wide range of biological activities[1]. Isoxazoline derivatives have been reported in the literature to possess antifungal[2], antibacterial[3], anticonvulsant[4], anti-inflammatory[5], antiviral[6] and analgesic activity[7]. Mannich bases of heterocyclic molecules have been grabbing the attention of the synthetic chemists for their wide range of biological activities ranging from antibacterial, antifungal, anticancer, antiparkinson to anticonvulsant and anti–HIV[8-10]. In the present work, chalcones (**3a-c**) were synthesized by the reacting of substituted acetophenones (**1a-b**) with substituted aldehydes **(2a-b)** in the presence of aqueous solution of sodium hydroxide and ethanol at room temperature by Claisen-Schmidt condensation method. The synthesized chalcones were reacted with hydroxylamine hydrochloride in presence of sodium hydroxide to obtain isoxazolines derivatives (**4a-c**) [11]. The synthesized isoxazolines were reacted with formaldehyde and primary amines to afford Mannich bases **(5a-l).**



N-((3,5-bis(4-Substituted phenyl)-4,5-dihydroisoxazol-4-yl)substituted)-4-substituted benzenamine

R^1= -H,-Cl R^3= -H, -Cl, -NO_2, -CH_3 R^2= -H, -Cl

Figure 1. Scheme of synthesis.

Experimental

All the chemicals used in the synthesis were of laboratory grade. Melting points were determined in open capillary on Veego (model: VMP-D) electronic apparatus and are uncorrected. The IR spectra of the synthesized compounds were recorded on Shimadzu 8400-S FT-IR Spectrophotometer using potassium bromide or chloroform. The ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO using NMR Varian-Mercury 300 MHz spectrometer and chemical shifts are given in units as per million, downfield from Tetramethylsilane (TMS) as an internal standard. To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on microscopic slides (2 x 7.5 cm) coated with silica gel-G, using appropriate solvent systems and the spots were visualized under ultra-violet light or by exposure to iodine vapors.

*Synthesis of chalcones **(3a-c)*** [11]-

Substituted acetophenone (0.01mol) was taken in 100ml conical flask and dissolved in 25 mL of absolute ethanol. Ethanolic NaOH (0.01mol) was then added and stirred for 30 min.

To this solution appropriately substituted benzaldehyde (0.01mol) was added and stirred for about 12 h. The precipitated product was filtered, dried and recrystalized from ethanol.

Table 1. Physical data of chalcones **(3a-c)**

Code No.	**-R^1**	**-R^2**	***M.P. ^{O}C**	****R_f**	**IR**	**% yield**
3a	-H	-Cl	156-158	0.42	3327.5 (Ar-H), 1604.92 (C=O), 1559.10(C=C), 892.42(C-Cl).	66
3b	-H	-H	170-172	0.46	3066.21(Ar -H), 1656.51 (C=O), 1333.83(C=C),	56
3c	-Cl	-Cl	170-172	0.42	3030.27(Ar- H), 1682.95 (C=O), 1385.81 (C=C), 762.87 (C-Cl)	78
*All melting points are uncorrected. **Mobile phase- Chloroform: Methanol (8:2) Recrystallization solvent: Ethanol (80%).						

Synthesis of 3, 5-bis (4-substituted phenyl)-4, 5-dihydroisoxazole ***(4)*** [11]-

The isoxazolines were prepared by reacting a mixture of purified chalcones (0.01mol), hydroxylamine hydrochloride (0.03 mol) and a solution of NaOH (0.01 mol) in dry distilled ethanol by refluxing for 14-16 h. After completion of the reaction, an excess of the solvent was removed by distillation and the resultant mass was poured into ice water with vigorous stirring. The solution was acidified with dilute HCl. It was kept overnight in cool condition. The resultant solid product was filtered, washed with sufficient cold water, dried and purified by recrystallization from ethanol.

Table 2. Physical data of isoxazolines **(4a-c).**

Compound code	-R^1	-R^2	*M.P. ^{O}C	**R_f	% yield
4a	-H	-Cl	185-188	0.52	62
4b	-H	-H	142-145	0.50	75
4c	-Cl	-Cl	135-138	0.45	58
*All melting points are uncorrected. **Mobile phase- Chloroform: Methanol (8:2). Recrystallization solvent: Ethanol (80%).					

Table 3. Spectral data of isoxazolines **(4a-c)**

Compound	-R^1	-R^2	I.R. (KBr, cm^{-1})	^{1}H NMR (δ ppm)
4a	-H	-Cl	3319.60 (Ar -H), 1653.05(C=N), 1506.46 (C=C), 1352.14(C-O-N), 844.85 (C-Cl)	7.796-7.807(5H, m, Ar-H), 6.742-6.755(5H, m, Ar-H), 5.623-5.670(1H, dd, Hc), 4.807-4.914(1H, d, -CH), 3.723-3.960 (1H, dd, Ha), 3.205-3.266(1H, dd, Hb)
4b	-H	-H	3309.98 (Ar -H), 1610.22 (C=N), 1513.12(C=C), 1339.61 (C-O-N)	8.341-8.363 (4H, m, Ar-H), 7.657-7.767 (4H, m, Ar-H), 5.623-5.670 (1H, dd, Hc), 4.807-4.914 (1H, d, -CH), 3.723-3.960 (1H, dd, Ha), 3.205-3.266 (1H, dd, Hb),
4c	-Cl	-Cl	32288.95 (Ar -H), 1523.82 (C=N), 1344.43 (C-O-N), 795.32 (C-Cl)	8.341-8.363 (4H, m, Ar-H), 7.657-7.767 (4H, m, Ar-H), 5.623-5.670 (1H, dd, Hc), 4.807-4.914 (1H, d, -CH), 3.723-3.960 (1H, dd, Ha), 3.205-3.266 (1H, dd, Hb),

Synthesis of N-{[3, 5-bis (4-substituted phenyl)-4, 5-dihydroisoxazole-4-yl] substituted}-4-substituted benzamine ***(5a-l)*** [12]

To a solution of compounds (4a-c) (0.01mol) in methanol (30ml), formaldehyde (0.02mol) and corresponding substituted aniline (0.02mol) were added. The reaction-mixture was refluxed for 6 -10 h. The solvent was distilled off and the residue was poured into ice water. The precipitate solid was filtered off, dried and recrystallised from ethanol. Following the same procedure, all the derivatives were synthesized.

Table 4. Physical data of target compounds **(5a-l)**

Compound code	-R^1	-R^2	-R^3	Molecular formula	Molecular weight	Melting Point(^{0}C)	R_f	Yield (%)
5a	-H	-Cl	-H	$C_{22}H_{19}ClN_2O$	362.5	55-57	0.62	68
5b	-H	-Cl	-Cl	$C_{22}H_{18}Cl_2N_2O$	396	85-87	0.62	73
5c	-H	-Cl	$-CH_3$	$C_{23}H_{21}ClN_2O$	376.5	60-63	0.69	73
5d	-H	-Cl	$-NO_2$	$C_{22}H_{18}ClN_3O_3$	407.85	75-78	0.57	75
5e	-H	-H	-H	$C_{22}H_{20}N_2O$	328	45-47	0.69	83
5f	-H	-H	-Cl	$C_{22}H_{19}ClN_2O$	362.5	62-65	0.65	68

5g	-H	-H	$-CH_3$	$C_{23}H_{22}N_2O$	340	55-57	0.68	68
5h	-H	-H	$-NO_2$	$C_{22}H_{19}N_3O_3$	373	58-60	0.63	75
5i	-Cl	-Cl	-H	$C_{22}H_{18}Cl_2N_2O$	396	76-79	0.70	67
5j	-Cl	-Cl	-Cl	$C_{22}H_{17}Cl_3N_2O$	430.5	82-85	0.70	77
5k	-Cl	-Cl	$-CH_3$	$C_{23}H_{20}Cl_2N_2O$	410	77-80	0.67	82
5l	-Cl	-Cl	$-NO_2$	$C_{22}H_{17}Cl_2N_3O_3$	441	52-55	0.65	76

*All melting points are uncorrected.
**Mobile phase- Benzene: Methanol (7:3).
Recrystallization solvent: Ethanol (80%).
Spectral data of target compounds **(5a-l)**

N [{5-(4-chlorophenyl)-3-phenyl-4, 5-dihydroisoxazole-4-yl} methyl] benzenamine (5a)

IR (KBr, cm-1): N-H (3336.00), Ar Stretch C-H (3130.34), Aliphatic C-H (2834.23), C=N (1608.69), C=C (1344.43), C-O-N (1181.44), C-Cl (827.49).

^{1}*H NMR (δ ppm):* 7.677-7.687(5H, m, Ar-H), 7.194-7.203(4H, m, Ar-H), 6.933-6.987(5H, m, Ar-H), 6.879(2H, d, CH2), 5.283(1H, s, HN-Ar), 5.203-5.286(1H, d, -CH), 3.357-3.347(1H, m, -CH).

4-chloro-N-[{5-(4-chlorophenyl)-3-phenyl-4, 5-dihydroisoxazole-4-yl} methyl] benzenamine (5b)

IR (KBr, cm-1): N-H (3302.29), Ar Stretch C-H (3006.29), Aliphatic C-H (2903.83), C=N (1526.15), C=C (1318.38), C-O-N (1178.98), C-Cl (821.21).

^{1}*H NMR (δ ppm):* 7.688-7.776(4H, m, Ar-H), 7.146-7.174(4H, m, Ar-H), 6.877-6.904(5H, m, Ar-H), 6.483-6.512(2H, d, CH2), 5.175(1H, s, HN-Ar), 4.807-4.914(1H, d, -CH), 3.304-3.357(1H, m, -CH).

N [{5-(4-chlorophenyl)-3-phenyl-4, 5-dihydroisoxazole-4-yl} methyl]-4-methylbenzenamine (5c)
IR (KBr, cm-1): N-H (3319.50), Ar Stretch C-H (3267.89), Aliphatic C-H (2834.23), C=N (1623.82), C=C (1344.43), C-O-N (1157.43), C-Cl (739.45).

^{1}*H NMR (δ ppm):* 7.677-7.687(4H, m, Ar-H), 7.194-7.203(4H, m, Ar-H), 6.933-6.987(5H, m, Ar-H), 6.879(2H, d, CH2), 5.283(1H, s, HN-Ar), 5.203-5.286(1H, d, -CH), 3.357-3.347(1H, m, -CH), 1.600(3H, s, CH3).

N-[{5-(4-chlorophenyl)-3-phenyl-4, 5-dihydroisoxazole-4-yl} methyl]-4-nitrobenzenamine (5d)

IR (KBr, cm-1): N-H (3429.28), Ar Stretch C-H (3084.03), C=N (1614.07), C=C (1422.02), C-O-N (1247.50), C-Cl (754.13).

^{1}*H NMR (δ ppm):* 7.688-7.776(4H, m, Ar-H), 7.146-7.174(4H, m, Ar-H), 6.877-6.904(5H, m, Ar-H), 6.483-6.512(2H, d, CH2), 5.175(1H, s, HN-Ar), 4.807-4.914(1H, d, -CH), 3.304-3.357(1H, m, -CH).

N-[{3, 5-diphenyl-4, 5-dihydroisoxazole-4-yl} methyl] benzenamine (5e)

IR (KBr, cm-1): N-H (3336.17), Ar Stretch C-H (3120.34), Aliphatic C-H (2467.43), C=N (1637.73), C=C (1368.33), C-O-N (1104.64).

^{1}*H NMR (δ ppm):* 7.460-7.688(5H, m, Ar-H), 7.174-7.260(5H, m, Ar-H), 6.744-6.904(5H, m, Ar-H), 6.483(2H, d, CH2), 5.175(1H, s, HN-Ar), 4.914(1H, d, -CH), 4.868-4.950(1H, d,-CH).

4-chloro-N-[{3, 5-diphenyl-4, 5-dihydroisoxazole-4-yl} methyl] benzenamine (5f)

IR (KBr, cm-1): N-H (3250.58), Ar Stretch C-H (2922.25), Aliphatic C-H (2258.98), C=N (1735.42), C=C (1490.06), C-O-N (1234.54), C-Cl (809.17).

^{1}*H NMR (δ ppm):* 7.694-7.730(4H, m, Ar-H), 7.169-7.180(5H, m, Ar-H), 6.879-6.915(5H, m, Ar-H), 6.660-6.879(2H, d, -CH2), 4.927 (1H, s, HN-Ar), 4.868-4.950(1H, d,-CH), 3.323-3.340(1H, d, -CH).

N-[{3, 5-diphenyl-4, 5-dihydroisoxazole-4-yl} methyl]-4-methylbenzenamine (5g)

IR (KBr, cm-1): N-H (3318.60), Ar Stretch C-H (3105.50), C=N (1683.91), C=C (1352.14), C-O-N (1217.42), C-Cl (844.85).

^{1}*H NMR (δ ppm):* 7.698-7.731(4H, m, Ar-H), 7.500-7.664(5H, m, Ar-H), 6.908-7.010(5H, m, Ar-H), 6.879(2H, d, CH2), 5.043(1H, s, HN-Ar), 4.806-4.845(1H, d, -CH), 2.811-2.924(1H, m, -CH), 2.640(3H, s, CH3).

N-[{3, 5-diphenyl-4, 5-dihydroisoxazole-4-yl} methyl]-4-nitrobenzenamine (5h)

IR (KBr, cm-1): N-H (3329.56), Ar Stretch C-H (3120.56), Aliphatic C-H (2361.91), C=N (1605.19), C=C (1345.61), C-O-N (1217.12).

^{1}*H NMR (δ ppm):* 7.688-7.776(4H, m, Ar-H), 7.146-7.174(5H, m, Ar-H), 6.877-6.904(5H, m, Ar-H), 5.175(1H, s, HN-Ar), 6.483-6.512(2H, d, CH2), 4.807-4.914(1H, d, -CH), 3.304-3.357(1H, m, -CH).

N-[{3, 5-bis (4-chlorophenyl)-4, 5-dihydroisoxazole-4-yl} methyl] benzenamine (5i)

IR (KBr, cm-1): N-H (3350.45), Ar Stretch C-H (3088.85), Aliphatic C-H (2756.23), C=N (1693.95), C=C (1456.30), C-O-N (1135.34), C-Cl (837.98).

^{1}*H NMR (δ ppm):* 7.664-7.715(5H, m, Ar-H), 7.180-7.185(4H, m, Ar-H), 6.883-6.920(4H, m, Ar-H), 6.651-6.658(2H, d, CH2), 5.174 (1H, s, HN-Ar), 3.323-3.340(1H, d, -CH), 4.868-4.950(1H, d, -CH).

N-[{3, 5-bis (4-chlorophenyl)-4, 5-dihydroisoxazole-4-yl} methyl]-4-chlorobenzenamine (5j)

IR (KBr, cm-1): N-H (3437.35), Ar Stretch C-H (3126.94), Aliphatic C-H (2362.88), C=N (1518.03), C=C (1318.38), C-O-N (1346.36), C-Cl (850.64).

^{1} H NMR (δ ppm): 7.677-7.687(4H, m, Ar-H), 7.194-7.203(4H, m, Ar-H), 6.933-6.987(4H, m, Ar-H), 6.879(2H, d, CH2), 5.283(1H, s, HN-Ar), 5.203-5.286(1H, d, -CH), 3.357-3.347(1H, m, -CH).

N-[{3, 5-bis (4-chlorophenyl)-4, 5-dihydroisoxazole-4-yl} methyl]-4-methylbenzenamine ***(5k)***

IR (KBr, cm-1): N-H (3437.35), Ar Stretch C-H (3126.94), Aliphatic C-H (2362.88), C=N (1518.03), C=C (1318.38), C-O-N (1346.36), C-Cl (850.64).

^{1}*H NMR (δ ppm):* 7.664-7.686(4H, m, Ar-H), 7.421-7.457(4H, m, Ar-H), 6.949-7.034(4H, m, Ar-H), 6.652-6.659(2H, d, CH2), 5.043(1H, s, HN-Ar), 4.845-4.926(1H, d, -CH), 2.904-2.924(1H, m, -CH), 2.641(3H, s, -CH3).

N-[{3, 5-bis (4-chlorophenyl)-4, 5-dihydroisoxazole-4-yl} methyl]-4-nitrobenzenamine ***(5l)***

IR (KBr, cm^{-1}): N-H (3105.21), Ar Stretch C-H (2784.31), Aliphatic C-H (2661.81), C=N (1721.23), C=C (1333.43), C-O-N (1034.84), C-Cl (821.51).

^{1}*H NMR (δ ppm):* 7.688-7.776(4H, m, Ar-H), 7.146-7.174(4H, m, Ar-H), 6.877-6.904(4H, m, Ar-H), 6.483-6.512(2H, d, CH2), 5.286(1H, s, HN-Ar), 4.807-4.914(1H, d, -CH), 3.304-3.357(1H, m, -CH).

Antibacterial Activity

Various Mannich bases derivatives were synthesized and evaluated for the antimicrobial activity by the cup plate method, against three strains of gram positive bacteria and gram negative bacteria and at three concentrations using DMSO as solvent in a nutrient broth as nutrient medium. Antibacterial activity of test compound was evaluated against gram positive bacteria *S.aureus* (ATCC 9144), *B. subtilis* (ATCC 6633) and *S.epidermis* (ATCC12228) and gram negative bacteria *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 9027), *S. typhi* (ATCC 13044), using Amoxicillin as standard by cup plate method. The bacteria were subcultured in the nutrient agar medium containing peptone (0.6%), yeast extract (0.3%), beef extract (0.13%) and agar (2.1%) in distilled water. The solution was sterilized for 20 min at 15 psi pressure in an autoclave at 121^{0}C. The basal medium 15-20 ml was poured in the sterile petridishes. After the solidification of medium, holes of 6 mm diameter were bored to form cups with the help of a sterile cork borer. To this cup 50 µg/mL, 100 µg/mL, 150 µg/mL of the test compounds were added by the micropipette. The petridishes were kept in a cold room to facilitate the diffusion of the solvent for about 2 h. The extent of inhibition was measured by the width of the inhibition zone in mm.

Antifungal Activity

Fungicidal activity of the test compound was evaluated against *A. Niger* (ATCC 16404) and *A.flavas* (ATCC 9643) by cup plate method. The fungi were subcultured in the medium containing potato infusion from 200 gm (4 gm), dextrose (20 gm) and agar (15 gm) in distilled water. The pH of the medium is adjusted to 5.6 ± 0.2 and sterilized for 20 min at 121 ^{0}C. The method of testing the antifungal activity is same as that adopted for evaluating antibacterial activity. Fluconazole was used as the standard; DMSO was used as solvent control and potao-dextose-agar as nutrient medium.

Minimum Inhibitory Concentration

The Minimum inhibitory concentration study is done by broth dilution method as follows.

Broth Dilution Method

Prepare the nutrient broth test tubes. In the first tube, inoculum is not added which is used for checking the sterility of medium and as a negative control. In other all test tubes inoculum is added. In all test tubes, test antimicrobial compound is added ranging from 15 to 50µg/10µL except uninoculated (negative control) and control (positive tube). The positive control tube is used to check the suitability of the medium for the growth of test microorganism and the viability of the inoculum. Adjust the final volume in all the test tube by using sterile water. All test tubes are properly shaken and then inoculated at 37 ^{0}C for 24 h.

Table 5. Data of mean of Zone of inhibition of Gram positive Bacteria.

Compound code	150 µg/mL	Gram positive bacteria		
		S.aureus	*S. epidermis*	*B.subtilis*
Amoxycillin	Mean	26	27	29
	SEM	±1.36	±1.45	±1.54
5a	Mean	12	15	15
	SEM	±1.36	±1.21	±1.15
5b	Mean	18	20	22
	SEM	±1.06	±1.08	±1.19
5c	Mean	14	14	15
	SEM	±1.46	±1.51	±1.65
5d	Mean	9	11	12
	SEM	±0.67	±0.60	±0.67
5e	Mean	10	14	14
	SEM	±1.10	±1.01	±1.04
5f	Mean	12	15	14
	SEM	±1.06	±1.12	±1.14
5g	Mean	12	14	12
	SEM	±1.16	±1.12	±1.27
5h	Mean	12	15	15
	SEM	±1.16	±1.12	±1.13
5i	Mean	17	19	20
	SEM	±1.59	±1.45	±1.55
5j	Mean	17	18	20
	SEM	±1.59	±1.57	±1.55
5k	Mean	17	15	17
	SEM	±1.59	±1.52	±1.53
5l	Mean	10	14	14
	SEM	±0.61	±1.51	±1.64

N=6, * - P < 0.05, ** - P < 0.01 compared with control treated group,
Data expressed as Mean ± SEM.
Data was analysed by one way ANOVA followed by Dunnett's test.

Table 6. Data of mean of Zone of inhibition of Gram negative Bacteria.

Compound code	150 µg/ml	Gram negative bacteria		
		E.coli	*P.aeroginosa*	*S.typhi*
Amoxycillin	Mean	24	25	28
	SEM	±1.35	±1.33	±1.30
5a	Mean	11	15	15
	SEM	±1.55	±1.18	±1.16
5b	Mean	18	20	23
	SEM	±1.51	±1.45	±1.35
5c	Mean	14	13	15
	SEM	±1.52	±1.16	±1.16
5d	Mean	12	15	15
	SEM	±1.50	±1.48	±1.28
5e	Mean	7	12	12
	SEM	±0.72	±1.47	±0.45
5f	Mean	10	14	13
	SEM	±1.18	±1.17	±1.13
5g	Mean	12	15	14
	SEM	±1.50	±1.48	±1.41
5h	Mean	12	15	15
	SEM	±0.50	±1.48	±1.48
5i	Mean	17	15	22
	SEM	±1.49	±1.48	±1.34
5j	Mean	15	18	22
	SEM	±1.53	±1.45	±1.34
5k	Mean	17	15	17
	SEM	±1.49	±1.48	±1.39
5l	Mean	10	14	14
	SEM	±0.68	±1.47	±1.11
N=6, * - P < 0.05, ** - P < 0.01 compared with control treated group, Data expressed as Mean ± SEM. Data was analysed by one way ANOVA followed by Dunnett's test.				

Results and Discussion

Substituted chalcones **3(a-c)** were synthesized by condensation reaction using equimolar quantities of substituted acetophenones, ethanolic NaOH and substituted benzaldehydes. 3, 5-bis (4-substituted phenyl)-4, 5-dihydroisoxazole **4(a-c)** were synthesized by refluxing corresponding chalcones and hydroxylamine hydrochloride in presence of sodium hydroxide using ethanol as solvent. The target compound Mannich bases of isoxazolines **5(a-l)** are synthesized from 3, 5-bis (4-substituted phenyl)-4, 5-dihydroisoxazole **4(a-c)** by using methanol as solvent and using substituted primary anilines and formaldehyde. The solid state IR (KBr,cm^{-1}) spectra of these compounds reveal a characteristic N-H Stretch

(Secondary amine) between 3300-3400 $cm^{-1}$and aromatic Stretch between 3000-3200 cm^{-1}. The cyanide (C=N) group present in the isoxazoline ring reveal peaks at 1600-1700 cm^{-1} .The C=C group of Aromatic ring showed stretching vibrations at around 1300-1400 cm^{-1}. C-O-N group reveal peaks at 1150-1250 cm^{-1}. The ^{1}H NMR spectra of all target derivatives **(5a-l)** were recorded in $CDCl_3$ Synthesized target compounds were screened for anti-microbial activity by Zone of Inhibition and Minimum Inhibitory Concentration methods. Amongst the twelve synthesized derivatives, compounds **5b, 5h, 5i, 5j and 5l** exhibited promising activity against gram positive bacteria. The compounds **5b, 5h, 5i, 5j and 5l** exhibited promising activity against gram negative bacteria. The compounds **5b, 5h, 5i, 5j and 5l** exhibited promising activity against fungi.

Table 7. Data of mean of Zone of inhibition of fungi.

Compound code	150 µg/ml	Fungi	
		A.niger	*A.flavus*
Fluconazole	Mean	25	27
	SEM	±1.35	±1.33
5a	Mean	11	15
	SEM	±1.15	±1.18
5b	Mean	18	20
	SEM	±1.11	±1.15
5c	Mean	14	13
	SEM	±1.12	±1.16
5d	Mean	12	15
	SEM	±1.10	±1.18
5e	Mean	7	12
	SEM	±0.72	±1.17
5f	Mean	10	14
	SEM	±1.08	±1.17
5g	Mean	12	15
	SEM	±1.10	±1.18
5h	Mean	12	15
	SEM	±1.10	±1.28
5i	Mean	17	15
	SEM	±1.09	±1.28
5j	Mean	15	18
	SEM	±1.03	±1.15
5k	Mean	17	15
	SEM	±1.19	±1.08
5l	Mean	15	14
	SEM	±1.18	±1.07

N=6, * - $P < 0.05$, ** - $P < 0.01$ compared with control treated group,
Data expressed as Mean ± SEM.
Data was analysed by one way ANOVA followed by Dunnett's test.

Table 8. Data of minimum inhibitory concentration (μg/mL) of Gram positive bacteria

Sr.No.	Compound Name	Gram positive bacteria		
		S.aureus	*S. epidermis*	*B.subtilis*
1	**5a**	25	30	30
2	**5b**	20	25	25
3	**5c**	25	25	35
4	**5d**	30	30	35
5	**5e**	25	25	30
6	**5f**	25	30	30
7	**5g**	30	35	35
8	**5h**	30	35	30
9	**5i**	20	25	20
10	**5j**	20	25	25
11	**5k**	25	35	30
12	**5l**	30	35	35

Table 9. Data of minimum inhibitory concentration (μg/ml) of Gram negative bacteria.

Sr.No.	Compound Name	Gram negative bacteria		
		E.coli	*p.aeruginosa*	*s.typhi*
1	**5a**	30	30	25
2	**5b**	25	25	25
3	**5c**	30	30	30
4	**5d**	35	25	30
5	**5e**	25	30	35
6	**5f**	35	30	30
7	**5g**	25	25	25
8	**5h**	30	30	35
9	**5i**	25	25	25
10	**5j**	20	25	30
11	**5k**	25	30	35
12	**5l**	30	30	30

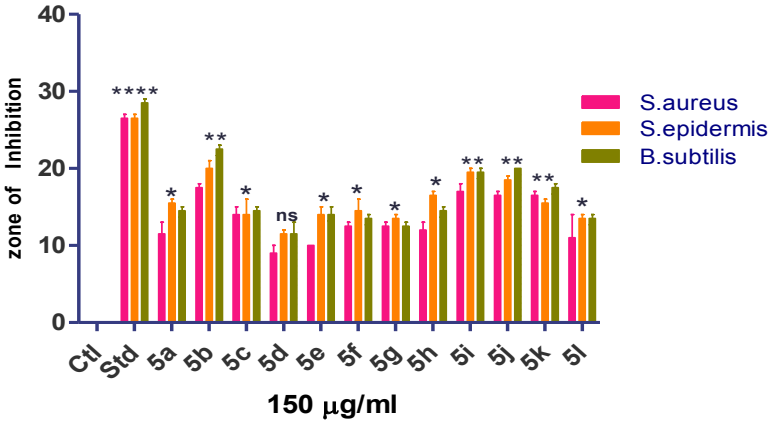


Figure 2. Graph of Zone of inhibition against Gram positive bacteria.

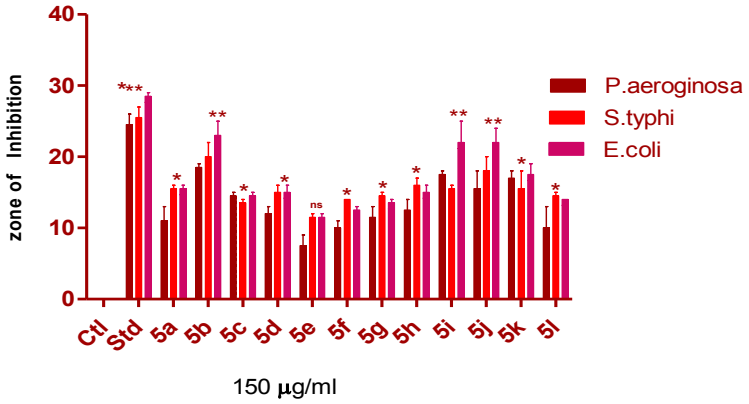


Figure 3. Graph of zone of inhibition against Gram negative bacteria.

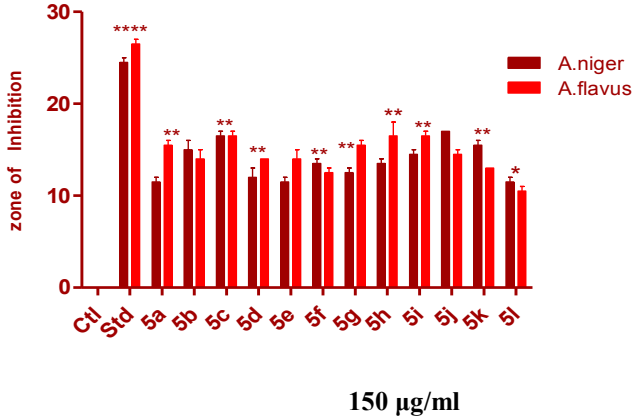


Figure 4. Graph of zone of inhibition against Fungi.

Conclusion

The approach of the present study was to synthesize various mannich bases and evaluate the antibacterial and antifungal activities. From result generated it can be concluded that test compounds **5b, 5h, 5i, 5j and 5l** were found to possess moderate antibacterial activity against gram positive bacteria and gram negative bacteria compared with Amoxicillin. The observed antimicrobial and antifungal activities are attributed to the substitution of 4-chloro, 4- methyl group at 4 position of benzenamine ring of synthesized compounds. These results suggest that the Mannich bases of appropriately substituted isoxazolines have good potential for further development as antimicrobial agents. Further study is required to predict mechanism of action. The data reported in this article may be helpful guide for the medicinal chemist who is working in this area.

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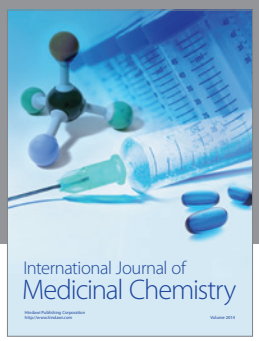

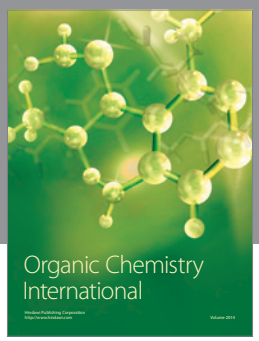

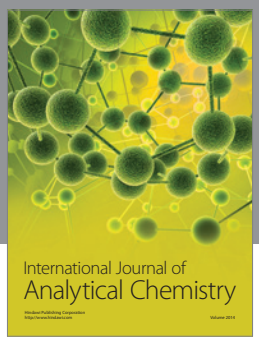

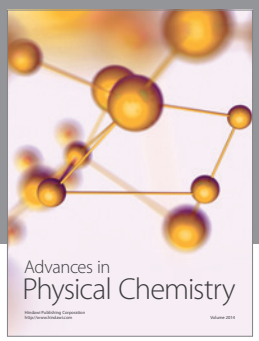

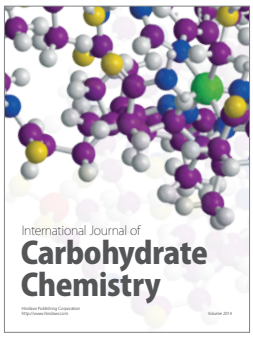

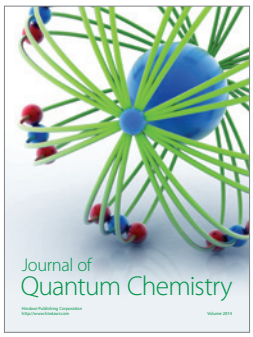

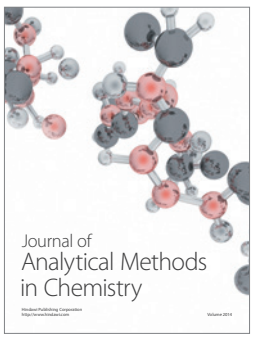

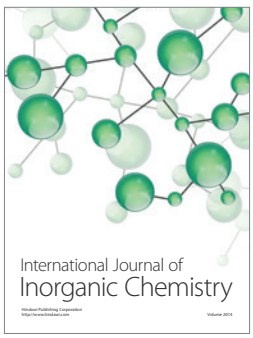

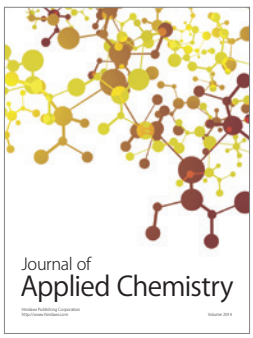

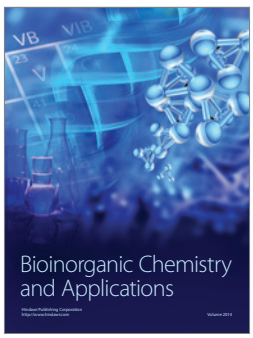

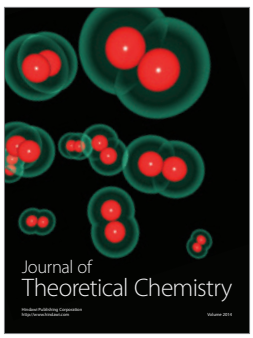

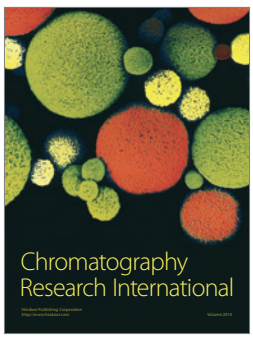

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