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Synthesis and Biological Evaluation of Some Novel Mannich Bases of Isoxazoline Derivatives as Possible Antimicrobial Agents

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Novel isoxazoline derivatives were synthesized by condensation of substituted acetophenones with aldehyde in presence of alcoholic NaOH to get intermediate chalcones, which were further treated with hydroxylamine hydrochloride in presence of sodium hydroxide to get isoxazoline derivatives. The latter were refluxed separately with isonicotinic acid hydrazide and sulphanilamide in presence of formaldehyde for 6-10 h to afford corresponding Mannich bases. The structures of synthesized compounds were established on the basis of melting point, TLC, IR, ^{1}H NMR and HRMS. Antimycobacterial activity of compounds (**3a-j**) were assessed against *M. tuberculosis* (vaccine strain, H37 Rv strain) ATCC27294 using microplate Alamar Blue assay (MABA). Further the derivatives were evaluated for the antibacterial activity against Gram positive bacteria *S. aureus* (ATCC 9144), *S. epidemidis* (ATCC12228) and Gram negative bacteria *E. coli* (ATCC 25922), *Klebsiella* (ATCC 4352), while antifungal activity against *A. flavus* (ATCC 9643) and *A. niger* (ATCC 16404) by using agar well diffusion method using ciprofloxacin and fluconazole as standards, respectively. The results of antimicrobial studies showed that some of the derivatives posses mild to moderate biological activity as compared to standard.

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

INTRODUCTION

The isoxazoles are five membered rings containing a nitrogen and an oxygen atom adjacent to each other. Dihydro derivatives of isoxazoles are called as isoxazolines. Isoxazolines are generally synthesized from chalcones represents a class of compounds of great biological importance. Isoxazoline possess a broad spectrum of biological activity [1,2]. Isoxazoline derivatives have been reported in the literature to possess antifungal [2,3], antibacterial, anticonvulsant [4], anti-inflammatory [5], antiviral [6] analgesic activity [7] and antitubercular [8]. It serves as an important building block for the synthesis of biologically active molecules [3].

Mannich bases of heterocyclic molecules have been attracting the attention of the synthetic chemists for their wide range of biological activities ranging from antibacterial, antitubercular, antifungal, anticancer, anti-Parkinson, anticonvulsant and anti-HIV [9-12]. In the present work, chalcones were synthesized by the reaction of various substituted acetophenones with aldehydes in the presence of alcoholic NaOH at room temperature by Claisen-Schmidt condensation method. The synthesized chalcones were further reacted with hydroxylamine hydrochloride in presence of NaOH to obtain isoxazolines derivatives [13]. The synthesized isoxazolines were reacted with formaldehyde and isonicotinic acid hydrazide and sulphanilamide to give Mannich bases. The purpose of this study was to develop new Mannich bases of isoxazoline derivatives as potent antibacterial, antifungal and antitubercular agents.

EXPERIMENTAL

All the chemicals were purchased from S.D. Fine Chemicals Ltd., Mumbai, India. Melting points were determined by open capillary method 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 ATR sampling tech-nique. ^{1}H NMR spectra was obtained on Bruker AV III 500 MHz spectrometer

spectra in $CDCl_3$ and chemical shifts are given in parts per million, downfield from tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained from Bruker Impact HD 3050 system instrument. 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 × 7.5 cm) coated with silica gel G F_{254}, using benzene: methanol (7:3) solvent systems and the spots were visualized under ultra-violet light (254 nm) or by exposure to iodine vapours.

Synthesis of chalcones (1a-f): In an Erlenmeyer flask, appropriate acetophenone (0.01 mol) and aromatic aldehyde (0.01 mol) in ethanol were separately dissolved in ethanol. The two were mixed and 10 mL of 40 % NaOH solution was added with stirring. The resulting solution was kept overnight at room temperature. The completion of reaction was monitored by TLC. The contents of mixture was then poured over crushed ice and acidified with dil. HCl. The solid obtained was filtered, dried and recrystallized from ethanol.

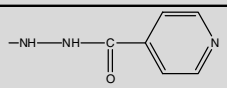
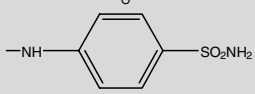
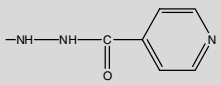
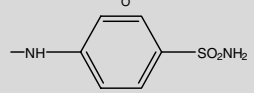
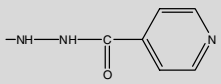
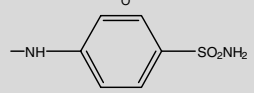
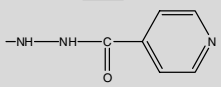
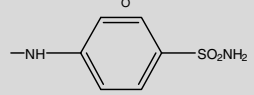
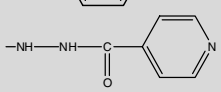
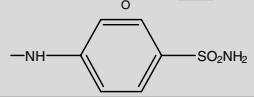
Synthesis of isoxazolines (2a-f): The isoxazolines were prepared by refluxing a mixture of purified chalcones (0.01 mol) and hydroxylamine hydrochloride (0.03 mol) in ethanolic NaOH (0.01mol) for 6 h. The progress of reaction was monitored by TLC. After completion of the reaction, excess solvent was removed by distillation and the resultant mass was poured into ice-water with vigorous stirring. The solution was acidified with dil. 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.

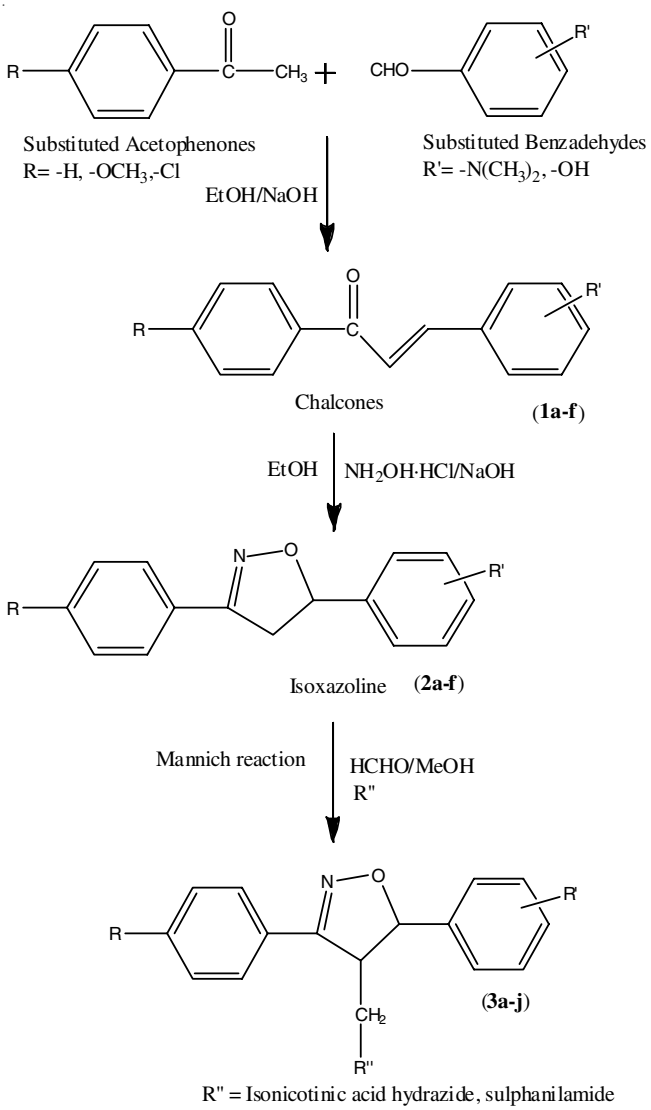
General procedure for the synthesis of substituted isoxazolines (Mannich reaction) (3a-j): To a solution of isoxazoline (0.01 mol, **2**) in methanol (30 mL), formaldehyde (0.02 mol) and corresponding isonicotinic acid hydrazide/sulphanilamide (0.02 mol) were added. The reaction-mixture was refluxed for 6-10 h. The progress of reaction was monitored by TLC. The solvent was distilled off and the residue was poured into ice-water. The precipitate was filtered off, dried and recrystallized from ethanol (**Scheme-I**). The results of physical characteristics of synthesized derivatives are presented in Table-1.

Antibacterial activity: All the synthesized compounds (**3a-j**) have been screened *in vitro* for the antibacterial activity by the agar well diffusion method using DMSO as solvent, against two strains of Gram positive and Gram negative bacteria at five concentrations in a Brain Heart Infusion agar medium. Antibacterial activity of test compound was evaluated against gram positive bacteria *S. aureus* (ATCC 9144) and *S. epidermis* (ATCC 12228) and Gram negative bacteria *E. coli* (ATCC 25922) and *Klebsiella* (ATCC 9027) using ciprofloxacin as standard. Stock solution was prepared by dissolving accurately weighed 10 mg of compound in 1 mL DMSO. With the help of micropipette a volume of 75, 50, 25, 10 and 5 µL were transferred in each well. The plates were incubated for 18-24 h at 37 ºC in incubator. Plates were read only if the lawn of growth is confluent or nearly confluent and diameter of inhibition zone was measured to nearest whole millimeter by holding the measuring device [14-17].

Antifungal activity: Fungicidal activity of the synthesized compounds was evaluated against *A. niger* (ATCC 16404) and

TABLE-1
PHYSICAL CHARACTERISTICS OF SYNTHESIZED DERIVATIVES (**3a-j**)

Compd.	R	R'	R"	m.f.	m.w.	m.p. (°C)	R_f value	Yield (%)
3a	-H	*p*-$N(CH_3)_2$		$C_{24}H_{25}N_5O_2$	415.49	88-90	0.54	55
3b	-H	*p*-$N(CH_3)_2$		$C_{24}H_{26}N_4O_3S$	450.55	161-163	0.72	65
3c	*p*-OCH_3	*p*-OH		$C_{23}H_{22}N_4O_4$	418.45	270-275	0.62	60
3d	*p*-OCH_3	*p*-OH		$C_{23}H_{23}N_3O_5S$	453.51	175-178	0.82	56
3e	*p*-OCH_3	*p*-$N(CH_3)_2$		$C_{25}H_{27}N_5O_3$	445.51	155-159	0.71	53
3f	*p*-OCH_3	*p*-$N(CH_3)_2$		$C_{25}H_{28}N_4O_4S$	480.58	156-158	0.80	58
3g	*p*-Cl	*p*-OH		$C_{22}H_{19}N_4O_3Cl$	422.86	85-87	0.63	63
3h	*p*-Cl	*p*-OH		$C_{22}H_{20}N_3O_4SCl$	457.93	75-78	0.62	67
3i	*p*-Cl	*p*-$N(CH_3)_2$		$C_{24}H_{24}N_5O_2Cl$	449.93	125-130	0.81	59
3j	*p*-Cl	*p*-$N(CH_3)_2$		$C_{24}H_{25}N_4O_3SCl$	485.00	132-135	0.72	65



Scheme-I: Synthesis of substituted isoxazole derivatives

A. flavas (ATCC 9643) by agar well diffusion method, Sabouraud agar medium is used instead of brain heart infusion agar. The method of testing the antifungal activity is same as that adopted for evaluating antibacterial activity [18-22]. Fluconazole was used as the standard and DMSO was used as solvent.

Antitubercular activity: Antimycobacterial activity of the synthesized compounds were assessed against *M. tuberculosis* (Vaccine strain, H37 Rv strain) ATCC27294 using microplate Alamar Blue assay (MABA). This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation. The 96 wells plate received 100 µL of Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/mL. Plates were covered and sealed with parafilm and incubated at 37 ºC for five days. After this time, 25µL of freshly prepared 1:1 mixture of Almar Blue reagent and 10 % Tween 80 was added to the plate and incubated for 24 h. A blue colour in the well was interpreted as no bacterial growth and pink colour was scored as growth. The MIC was defined as lowest drug concentration which prevented the colour change from blue to pink [23-25].

N′-((5-(4-(Dimethylamino)phenyl)-3-phenyl-4,5-dihydroisoxazol-4-yl)methyl)isonicotinohydrazide (3a): IR (ATR, cm^{-1}): 3051.49 (Ar-H *str.*), 1664.62 (-CO *str.* amide), 1529.60 (-C=C-), 1614.47 (-C=N), 1323.21 (-C-O-N), 3429.55 (NH *str.*), 3408.33 (-NH *str.* hydrazide), 2889.46 & 2908.75 (aliph. -CH *str.*), 1664.62 (-C=O *str.* amide), 1359.86 (Ar-CN *str.* amine), 947.08 (-N-O *str.* isoxazole), 1230.63 (-C-N *str.* amine), 1166.97 (N-N *str.* hydrazide). ^{1}H NMR (δ ppm): 2.977 (6H, s, aliph. -CH_3), 3.68-3.75 (1H, d, isoxazole ring -O-CH-CH-C=), 3.34-3.4 (1H, q, isoxazole ring CH-CH), 6.73-6.77 (2H, d, aliph. -CH_2), 5.64-5.7(2H,NH, d, amine nitrogen), 7.28-7.31 (4H, d, arom. H), 7.42-7.46, (5H, d, arom. H), 7.71-7.75, (2H, d, arom. H), 7.63-7.64, (2H, d, arom. H), HRMS *m/z* (%): $[M]^+$: 415.

4-((5-(4-(Dimethylamino)phenyl-4,5-dihydroisoxazole-4-yl)methyl)amino)benzene sulfonamide (3b): IR (ATR, cm^{-1}): 3373.71 (Ar-H *str.*), 1381.79 (-C-O-N), 1230.63 (-C-N *str.* amide), 1159.26 (-S=O symm. *str.*), 1361.79 (-S=O asymm. *str.*), 3242.45 & 3373.61 (-NH *str.* sulphonamide), 1546.60 (NH bend of sulphonamide), 952.87 (-N-O *str.* isoxazole), 1309.71 (C-N *str.* tertiary amine). ^{1}H NMR (δ ppm): 2.97-2.99 (6H, s, aliph. -CH_3), 3.67-3.74 (1H, d, isoxazole ring -O-CH-CH-C=), 3.35-3.4 (1H, q, isoxazole ring CH-CH), 6.73-6.76 (2H, d, aliph. -CH_2), 5.65-5.70 (1H, t, amine nitrogen), 9.76 (2H, s, -SO_2NH_2), 7.28-7.32 (4H, d, arom. H), 7.42-7.49 (5H, m, arom. H), 7.71-7.75 (2H, d, arom. H), 7.46-7.48 (2H, d, arom. H), HRMS *m/z* (%): $[M]^+$: 450.55.

N′-((5-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazol-4-yl)methyl)isonico tinohydrazide (3c): IR (ATR, cm^{-1}): 3373.71 (Ar-H *str.*), 1668.48 (-CO *str.* amide), 1508.38 (-C=C-), 1668.48 (-C=N), 1371.43 (-C-O-N), 3201.94 (-NH *str.* hydrazide), 1259.56 (-CN *str.*), 857.49 (-CH bend), 1668.48 (-C=O *str.* amide), 1062.81 & 1259.56 (-C-O of phenyl alkyl ether), 970.23 (-N-O *str.* isoxazole), 1259.56 (-CN *str.* amine), 1165.04 (N-N *str.* hydrazide).

4-(((5-(4-Hydroxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazol-4-yl)methyl)amino)benzene sulfonamide (3d): IR (ATR, cm^{-1}): 3063.06 (Ar-H *str.*), 2864.39 (aliph. -CH *str.*), 1650 (-CONH *str.*), 1600.97 (-C=C-), 1311.64 (Ar-CN *str.*), 1157.33 (-S=O symm. *str.*), 1361.79 (-S=O asymm. *str.*), 3244.38 & 3335.03 (-NH *str.* sulphonamide), 1514.17 (-NH bend of sulphonamide), 1323.21 (-C-O-N), 3500 (Ar-OH).

N′-((5-(4-(Dimethylamino)phenyl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole-4-yl)methyl)isonicotinohydrazide (3e): IR (ATR, cm^{-1}): 3068.85 (Ar-H *str.*), 3408.33 (-NH *str.* hydrazide), 2845.10 (aliph. -CH *str.*), 1664.62 (-CONH *str.*), 1597.11 (-C=N *str.*), 1519.96 (-C=C-), 750.33 (-CH bend), 1058.96 and 1249.91 (-C-O of phenyl alkyl ether), 1180.47 (N-N *str.* hydrazide), 1311.64 (-C-O-N), 1365.65 (C-N *str. tert.* arom. amine), 1232.55 (-CN *str.* amine). ^{1}H NMR (δ ppm): 2.93-2.97 (6H, s, aliph. -CH_3), 3.65-3.68 (1H, d, isoxazole ring -O-CH-CH-C=), 3.06-3.67 (1H, q, isoxazole ring CH-CH), 3.88 (3H, s, -OCH_3), 6.73-6.75 (2H, d, aliph. -CH_2), 5.62-5.66 (2H NH, d, amine-N), 6.60-6.75 (4H, d, arom. H), 6.91-6.94 (4H, d, arom. H), 7.28-7.32 (2H, d, arom. H), 7.60-7.67 (2H, d, arom. H), HRMS *m/z* (%): $[M]^+$: 445.5.

4-(((5-(4-(Dimethylamino)phenyl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazol-4-yl)methyl)amino)benzene sulfonamide (3f): IR (ATR cm^{-1}): 3066.92 (Ar-H *str*.), 2839.31 (aliph. -CH *str*.), 3375.54 (-NH *str*.), 1244.13 (-C-O-N), -S=O sym. Str. (1155.40), 1350.22 (-S=O asymm. *str*.), 1301.99 (C-N *str*. *tert*. amine), 1030.02 & 1244.13 (-C-O of phenyl alkyl ether). ^{1}H NMR (δ ppm): 2.93-2.96 (6H, s, aliph. $-CH_3$), 3.62-3.69 (1H, d, isoxazole ring -O-CH-CH-C=), 3.28-3.35 (1H, q, isoxazole ring CH-CH), 3.842 (3H, s, $-OCH_3$), 6.71-6.74 (2H, d, Aliph. $-CH_2$), 5.59-5.63 (1H, s, amine -N), 6.69-6.73 (4H, d, arom. H), 6.9-6.94 (4H, d, arom. H), 7.226-7.27(2H, d, arom. H), 7.63-7.64 (2H, d, arom. H). HRMS *m/z* (%): $[M]^+$: 480.

N′-((3-(4-Chlorophenyl)-5-(4-hydroxyphenyl)-4,5-dihydroisoxazol-4-yl)methyl)isonico tinohydrazide (3g): IR (ATR, cm^{-1}): 3063.06 (Ar-H *str*.), 821.70 (-C-Cl), 3240.52 (NH *str*.), 1670.41 (-CONH *str*.), 1492.95 (-C=C), 3550 (Ar-OH), 1396.44 (-C-O-N), 746.48 (-CH bend), 1182.40 (N-N *str*. hydrazide), 1307.78 (CN *str*. amine), 1369.50 (Ar-CN *str*. pyridine), ^{1}H NMR (δ ppm): 8.89 (1H, s, arom.-OH), 3.30-3.36 (1H, d, isoxazole ring -O-CH-CH-C=), 3.29-3.34 (1H, q, isoxazole ring CH-CH), 6.70-6.74 (2H, d, aliph. $-CH_2$), 5.65-5.69 (1H, s, amine-N), 6.53 (1H, s, amide-N) 7.25-7.29 (4H, d, arom. H), 7.34-7.45 (4H, d, arom. H), 7.54-7.56 (2H, d, arom. H), 7.63-7.64 (2H, d, arom. H). HRMS *m/z* (%): $[M]^+$: 422.

4-(((3-(4-Chlorophenyl)-5-(4-hydroxyphenyl)-4,5-dihydroisoxazol-4-yl)methyl)amino)benzene sulfonamide (3h): IR (ATR, cm^{-1}): 3242.45 (Ar-H *str*.), 1641.48 (C=N), 1307.78 (-C-O-N), 3242.45 & 3296.42 (-NH *str*. sulphon-amide), 1597.11 (-NH bend sulphonamide), 1149.61 (-S=O symm. *str*.), 1367.58 (-S=O asymm. *str*.), 1220.98 (-CN *str*. amine), 1492.95 (-C=C-), 821.70 (C-Cl). ^{1}H NMR (δ ppm): 8.9 (1H, s, arom.-OH), 3.67-3.74 (1H, d, isoxazole ring -O-CH-CH-C=), 3.35-3.4 (1H, q, isoxazole ring CH-CH), 6.73-6.76 (2H, d, aliph. $-CH_2$), 5.65-5.70 (1H, d, amine-N), 9.7-10 (2H, s, $-SO_2NH_2$), 7.28-7.32 (4H, d, arom. H), 7.43-7.46 (5H, m, arom. H), 7.65-7.68 (2H, d, arom. H), 7.63-7.64 (2H, d, arom. H). HRMS m/z (%): $[M]^+$: 457.93.

N′-((3-(4-Chlorophenyl)-5-(4-(dimethylamino)phenyl)-4,5-dihydroisoxazol-4-yl)methyl)isonicotinohydrazide (3i): IR (ATR, cm^{-1}): 3039.91 (Ar-H *str*.), 2974.33 (aliph. -CH *str*.), 1670.41 (-CONH *str*. amide), 1523.82 (-C=N *str*.), 1491.02 (-C=C-), 1296. 21 (-C-O-N), 1228.70 (-CN *str*. amine), 1350.22 (-CN *str*. amine), 1165.04 (N-N *str*. hydrazide), 3201.94 (-NH *str*. hydrazide), 1402.30 (Ar-CN *str*. pyridine), 827.49 (-C-Cl), ^{1}H NMR (δ ppm): 2.975 (6H, s, aliph. $-CH_3$), 3.31-3.36 (1H, d, isoxazole ring -O-CH-CH-C=), 3.64-3.69 (1H, q, isoxazole ring CH-CH), 6.61-6.73(2H, d, aliph. $-CH_2$), 5.66-5.70 (1H, s, amine-N), 6.52 (1H, s, -NH-NH-), 7.25-7.28 (4H, d, arom. H), 7.38-7.45 (4H, d, arom. H), 7.51-7.52 (2H, d, arom. H), 7.63-7.64 (2H, d, arom. H). HRMS *m/z* (%): $[M]^+$: 449.93.

4-(((3-(4-Chlorophenyl)-5-(4-(dimethylamino)phenyl)-4,5-dihydroisoxazol-4-yl)methyl)amino)benzenesulfonamide (3j): IR (ATR, cm^{-1}): -C-Cl(827.49), 3084.28 (Ar-H *str*.), 2850.88 (aliph. -CH *str*.), 1612.54 (-C=C-), 1595.18 (-C=N *str*.), 1230.63 (-CN *str*. amine), 1525.74 (-NH bend sulphonamide), 1315.50 (C-O-N), 1350.22 (-S=O asymm. *str*.), 1193.98 (-CN *str*. amine), 1149.61 (-S=O symm. *str*.). ^{1}H NMR (δ ppm): 2.977(6H, s, aliph. $-CH_3$), 3.64-3.69 (1H, d, isoxazole ring -O-CH-CH-C=), 3.31-3.36 (1H, q, isoxazole ring CH-CH), 6.75-6.76 (2H, d, aliph. $-CH_2$), 5.66-5.70 (1H, s, amine-N), 9.977 (2H, s, -NH-NH-), 7.27-7.28 (4H, d, arom. H), 7.39-7.47(4H, d, arom. H), 7.64-7.72(2H, d, arom. H), 7.81-7.82(2H, d, arom. H). HRMS *m/z* (%): $[M]^+$: 485.

RESULTS AND DISCUSSION

Initially, chalcones were synthesized by using substituted acetophenones and aromatic aldehyde following Claisen-Schmidt′s condensation reaction. Further chalcones were treated with nucleophilic reagent hydroxylamine hydrochloride under reflux to get isoxazoline compounds. The target compounds, Mannich bases of isoxazolines (**3a-j**) were synthesized from isoxazoles (**2a-f**) by using methanol as solvent and using isonicotinic acid hydrazide and sulphanilamide with formaldehyde. Structures of all the derivaties have been elucided by ^{1}H NMR, HRMS and IR spectral measurements. The results confirmed that the product has formed. The solid state IR (ATR) spectra of these compounds revealed a characteristic N-H *str*. 3408-3200 cm^{-1} of hydrazide and aromatic *str*. between 3200-3000 cm^{-1}. The imine group of isoxazole ring (C=N) group present in isoxazoline ring revealed a peak at 1600-1550 cm^{-1} .The C=C group of aromatic ring showed stretching vibrations at around 1500-1400 cm^{-1}. The C-O-N group revealed peaks at 1396-1250 cm^{-1}.

The ^{1}H NMR spectra of all the synthesized derivatives (**3a-j**) were recorded in $CDCl_3$. ^{1}H NMR has revealed signal around at δ 3.62-3.74 accounting for isoxazole nucleus. Signal for the aromatic protons were present in between δ 8 and 7. Thus, all the protons were accounted for the respective structures.

Antibacterial study of the titled compounds revealed that amongst the ten synthesized derivatives, **3h**, **3g**, **3j**, **3a**, **3c**, and **3g**, **3h**, **3c**, **3j**, **3a** exhibited promising activity against Gram positive and negative bacteria, respectively. The compounds **3a**, **3b**, **3g** and **3h** exhibited promising activity against fungi. Remaining isoxazolines have shown considerable activity against most of the tested bacteria and fungi. The results are presented in Tables 2 and 3.

The synthesized derivatives were also evaluated for their *in vitro* antitubercular activity against *M. tuberculosis*. They showed equivalent antitubercular activity as the parent drug (Table-4). Derivatives **3i** and **3j** showed equivalent antitubercular activity as ciprofloxacin and pyrazinamide at 3.125 μg/mL conc. and were the potent antitubercular derivatives. Derivatives **3f**, **3i** and **3j** showed equivalent antitubercular activity as streptomycin at 6.25 μg/mL, respectively but showed intermediate anti-tubercular activity at other concentrations. MIC values of compounds **3i** and **3j** have been found to be 3.125 μg/mL and compounds **3f**, **3i** and **3j** were found to be 6.25 μg/mL. Other derivatives showed intermediate antitubercular activity at all concentrations (Table-4).

Conclusion

The present study reported the synthesis and antimicrobial evaluation of some novel Mannich bases of isoxazoline in which some compounds are good and some are moderately active like standard and can be employed for comparison. From the structure of potent antimicrobial compounds amongst the

TABLE-2
ANTIBACTERIAL ACTIVITY RESULTS OF SYNTHESIZED MANNICH BASES OF ISOXAZOLINE DERIVATIVES (**3a-j**)

Compound	Mean zone of inhibition (mm)																			
	Gram-positive bacteria										Gram-negative bacteria									
	S. aureus (conc., µg/mL)					*S. spidermis* (conc., µg/mL)					*Klebsiella* (conc., µg/mL)					*E. coli* (conc., µg/mL)				
	75	50	25	10	5	75	50	25	10	5	75	50	25	10	5	75	50	25	10	5
3a	17	14	11	9	R	28	25	23	15	13	18	15	R	R	R	14	12	10	8	R
3b	19	15	13	10	8	25	23	18	13	10	15	12	R	R	R	15	12	–	R	R
3c	16	13	10	8	R	25	23	20	19	16	15	13	10	R	R	16	14	12	R	R
3d	18	15	13	10	8	23	20	17	15	13	12	10	R	R	R	15	10	–	R	R
3e	10	08	R	R	R	18	15	13	–	–	18	15	R	R	R	15	10	–	R	R
3f	15	10	R	R	R	21	16	12	10	8	21	13	10	R	R	16	13	–	R	R
3g	20	18	15	10	R	40	38	35	18	15	42	40	34	20	18	23	21	10	R	R
3h	22	20	18	R	R	38	35	28	25	23	36	32	30	18	10	25	22	20	10	R
3i	18	15	R	R	R	15	13	12	R	R	15	13	R	R	R	13	10	R	R	R
3j	19	16	14	10	8	18	15	13	R	R	18	15	13	R	R	12	R	R	R	R
Ciprofloxacin (10 µg)			26					26					30					32		

R = Resistant

TABLE-3
ANTIFUNGAL ACTIVITY RESULTS OF SYNTHESIZED MANNICH BASES OF ISOXAZOLINE DERIVATIVES (**3a-j**)

Compound	Mean zone of inhibition (mm) (conc.)									
	A. flavus (conc., µg/mL)					*A. niger* (conc., µg/mL)				
	75	50	25	10	5	75	50	25	10	5
3a	30	28	25	12	10	30	20	18	-	R
3b	32	28	24	12	10	32	28	25	20	R
3c	18	15	14	R	R	28	20	R	R	R
3d	15	10	R	R	R	26	20	R	R	R
3e	30	28	25	12	10	30	20	18	R	R
3f	15	13	12	R	R	18	15	10	R	R
3g	30	28	25	15	R	40	38	27	15	12
3h	13	12	10	R	R	28	25	20	R	R
3i	15	13	10	R	R	12	8	R	R	R
3j	18	15	13	R	R	19	13	R	R	R
Fluconazole (30 µg)			26					26		

R = Resistant

TABLE-4
ANTIMYCOBACTERIAL ACTIVITY RESULTS OF SYNTHESIZED MANNICH BASES OF ISOXAZOLINE DERIVATIVES (**3a-j**)

Samples	Concentrations (µg/mL)							
	100	50	25	12.5	6.25	3.12	1.6	0.8
3a	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
3b	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
3c	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant
3d	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
3e	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant	Resistant
3f	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant
3g	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant
3h	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant	Resistant	Resistant
3i	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant
3j	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant
Pyrazinamide	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant
Ciprofloxacin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant
Streptomycin	Sensitive	Sensitive	Sensitive	Sensitive	Sensitive	Resistant	Resistant	Resistant

synthesized series, it can be concluded that the groups like -Cl, -OH and -OCH_3 at substituent on phenyl ring as well as isonicotinic acid hydrazide/sulphanilamide on isoxazoline positively contributes for antimicrobial potential. All the synthesized derivatives were tested for antimycobacterial activity against *M. tuberculosis* using microplate Alamar Blue assay (MABA). Compounds **3i** and **3j** showed equivalent antitubercular activity as standard. Hence in the present study, the aromatic substituted ketone, aromatic substituted aldehydes and isonicotinic acid hydrazide/sulphanilamide when linked with isoxazole moiety showed good potential for further development as antimycobacterial and antimicrobial agents.

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CONFLICT OF INTEREST

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

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