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Design, synthesis, characterization and antimicrobial evaluation of isoxazole derivatives

Renu Beniwal and Archana Kapoor*

Drug Discovery and Research Laboratory, Department of Pharmaceutical Sciences, Guru Jambeshwar University of Science and Technology, Hisar (Haryana), India

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ABSTRACT

*The present study comprises of design and synthesis of some newer derivatives by incorporating isoxazole nucleus in the pharmacophore and characterizing them physicochemically and by spectral means. In vitro tube dilution method was followed for their antimicrobial screening against Gram positive bacteria: Staphylococcus aureus, Bacillus subtilis, Gram negative bacteria: Escherichia coli, and two fungal strain: Candida albicans and Aspergillus niger respectively. The results indicated that compound **TPI-2, TPI-5** and **TPI-14** were found to be the most active antibacterial and antifungal agents respectively.*

Keywords: Isoxazole, chalcone antibacterial activity, antifungal activity, minimum inhibitory concentration (MIC)

INTRODUCTION

Isoxazoles are unsaturated aromatic heterocyclic compounds that contains a ring with three carbon atoms and one oxygen atom. The isoxazole behavior can be modified by the effects of substituent at position 1 and one nitrogen atom at position 2 [1]. Isoxazoles exhibits broad spectrum of pharmacological and biological activities which include anti-HIV [2], GABA antagonist [3], anticancer [4], antinociceptive [5], antithrombotic [6], antifungal [7], antibacterial [8], dopamine D4 receptors antagonist [9], immunomodulatory [10]. The chalcones are reactive intermediate in the synthesis of isoxazole that exhibit broad range of biological activities.

The chalcone compounds are more biologically active due to the presence of α, β-unsaturated carbonyl. Chalcones and their derivatives have been reported to possess many useful biological activities such as antibacterial, antifungal, antiviral, antioxidant, anti-tubercular, insecticidal, antiprotozoal, nitric oxide inhibition, ulcerogenic, anti-inflammatory, anticancer and antihyperglycemic etc [11, 12].

Keeping in view the above facts, we have designed some more isoxazole derivatives and evaluated them for the antimicrobial activity.

MATERIALS AND METHODS

Chemistry

All the chemicals and reagents were of analytical grade and were used without further purification. Melting points were determined by capillary tube method and are uncorrected. All the reaction and purity of synthesized

compounds was deduced by thin layer chromatography (TLC) using silica-G plate. The plates were developed by exposing to the iodine vapours. Infrared spectra were recorded by Perkin Elmer spectrophotometer using KBr pellets. Proton nuclear magnetic resonance spectra were recorded on BRUKER AVANCE ÌÌ 400 MHz NMR spectrophotometer.

Synthesis of 2, 4, 5-trisubstituted-1*H*-imidazole derivatives (4)

The synthesis of 2,4,5-trisubstituted-1*H*-imidazoles **(4)** was carried out by refluxing benzil (**1)** (10mmol) with different aromatic aldehydes (**2)** (12mmol**)** in the presence of ammonium acetate (**3)** (40mol) and sulphanilic acid (10mol%, 1.7gm) catalyst in ethanol (20mL) in round bottom flask at 80°C for 2hours. The completion of reaction was checked by thin layer chromatography (TLC) using solvent system having toluene, ethyl acetate and formaldehyde in ratio 4:4:2. Then reaction mixture was cooled to room temperature and poured on ice-cold water (50mL) to get the solid precipitated out. It was collected by filtration, washed with cold water and recrystallized with ethanol [13].

Synthesis of acetylated 2,4,5-trisubstituted-1*H* imidazole derivatives (5)

The synthesis of acetylated 2,4,5-trisubstituted-1*H*-imidazoles **(5)** was carried out by refluxing mixture of 0.0M of **(4)** and 0.01M of chloroacetone into a 250mL round bottom flask. Then 150mL of dry acetone and 30g of anhydrous potassium carbonate were added and the reaction mixture was refluxed for 6h at 75°C. Filtrate obtained was concentrated under vacuum. The product **(5)** was dried and recrystallized from acetone. The purity of the compound was checked by TLC and melting point [14].

Synthesis of chalcone derivatives (6)

In a flat bottom flask, placed a solution of sodium hydroxide (0.013mol) in 5ml of water and 3ml of ethanol and then placed the flask on the stirrer. Immersed the flask in a bath of crushed ice and acetylated 2, 4, 5-trisubstituted-1*H*-imidazolyl (0.01mol) and substituted benzaldehyde (0.01mol) were added. The mixture was stirred for 12-15 hr. until the entire mixture becomes very cloudy or thick. The mixture was kept in refrigerator overnight. Then the mixture was poured slowly into the water with the constant stirring, and acidified with dil HCl. Then precipitate was obtained, filtered, washed and recrystallized from ethanol. The formation of the chalcone derivative was then confirmed by performing TLC using Hexane: Ethyl acetate (8:2 v/v) as mobile phase [15].

Synthesis of isoxazole derivatives by cyclization (7)

A mixture of chalcone **6** (0.02mol) and hydroxylamine hydrochloride (0.02mol) were dissolved in 25ml of ethanol, then 0.01mol of sodium acetate was added and the mixture was heated under reflux for 6hr. The mixture was concentrated by distilling out the solvent under reduced pressure and poured into ice water. Then, precipitate obtained was filtered, washed and recrystallized from ethanol. The completion of reaction was confirmed by TLC using chloroform: methanol (9:1 v/v) as mobile phase [16].

Table 1 Physicochemical characterization of synthesized compounds

Sr. No.	Product Code	Mol. Formula	Mol. Wt.	M.P(˚C)	Rf Value	% Yield
1	**TPI-1**	$C_{33}H_{27}N_3O_2$	497.59	220-223	0.62	55.41
2	**TPI-2**	$C_{33}H_{26}N_4O_5$	558.58	217-219	0.53	59.62
3	**TPI-3**	$C_{33}H_{27}N_3O_3$	513.59	225-228	0.48	65.72
4	**TPI-4**	$C_{34}H_{30}N_4O$	510.63	205-207	0.59	62.34
5	**TPI-5**	$C_{33}H_{27}N_5O_3$	541.6	227-229	0.64	66.41
6	**TPI-6**	$C_{32}H_{24}BrN_3O$	546.46	224-226	0.45	58.05
7	**TPI-7**	$C_{34}H_{29}N_3O_5$	559.61	213-216	0.51	73.22
8	**TPI-8**	$C_{32}H_{25}N_3O_2$	483.56	219-221	0.43	63.64
9	**TPI-9**	$C_{32}H_{24}ClN_3O$	502.01	210-214	0.47	61.30
10	**TPI-10**	$C_{32}H_{24}ClN_3O_3$	534	221-223	0.67	65.09
11	**TPI-11**	$C_{34}H_{30}N_4O_2$	526.63	225-227	0.57	57.87
12	**TPI-12**	$C_{32}H_{24}ClN_3O_2$	518	216-219	0.52	54.98
13	**TPI-13**	$C_{36}H_{33}N_3O_6$	603.66	213-215	0.65	64.32
14	**TPI-14**	$C_{34}H_{28}ClN_3O_4$	578.06	207-210	0.44	68.26
15	**TPI-15**	$C_{35}H_{31}N_3O_4$	557.64	226-228	0.58	71.39
16	**TPI-16**	$C_{31}H_{21}N_5O_5$	543.53	221-224	0.52	67.85
17	**TPI-17**	$C_{32}H_{24}N_4O_5$	544.56	215-218	0.63	66.83
18	**TPI-18**	$C_{31}H_{22}FN_3O_2$	487.52	222-225	0.49	60.94
19	**TPI-19**	$C_{32}H_{24}FN_3O_2$	501.55	209-212	0.50	59.77
20	**TPI-20**	$C_{33}H_{27}BrN_4O$	575.5	214-216	0.68	60.68

Spectral data of synthesized isoxazole compounds

2-(4-methoxyphenyl)-4, 5-diphenyl-1-((5-p-tolylisoxazol-3-yl) methyl)-1*H*-imidazole (TPI-1): IR (KBr Pellets) cm^{-1}: 3006(-C-H), 3115(=C-H aromatic), 1605(C=N *str*.), 1253(N-O *str*.), 1146(C-O *str*.). **^{1}H-NMR (DMSO)δ ppm:** 7.24-7.59 (14H, m, rest of aromatic proton on benzene ring), 6.85-7.12 (4H, m, H-3", H-5", H-3"', H-5"'), 6.74 (1H, s, isoxazole), 4.82 (2H, s, imidazole-N-CH_2-isoxazole ring), 3.76 (3H, s, -OCH_3) and 2.34 (3H, s, -CH_3)

2-(3, 5-dimethoxyphenyl)-1-((5-(4–nitrophenyl) isoxazol-3-yl)methyl)-4, 5-diphenyl-1*H*-imidazole (TPI-2): IR (KBr Pellets) cm^{-1}: 3120(=C-H aromatic), 3010(-C-H), 1611(C=N *str*.), 1358, 1541(NO_2), 1341(C-O *str*.), 1267(N-O *str*.). **^{1}H-NMR (DMSO)δ ppm:** 7.22-7.52(14H, m, rest of aromatic proton on benzene ring), 6.89-7.09(2H, H-3"', H-5"'), 5.04(2H, s, imidazole-N-CH_2-isoxazole ring), 6.76(1H, s, isoxazole), 3.88(3H, s, -OCH_3)

4-(4, 5-diphenyl-1-((5-p-tolylisoxazol-3-yl)methyl)-1*H*-imidazole-2-yl)-2-methoxyphenol (TPI-3): IR (KBr Pellets) cm^{-1}: 3438-3311(OH str., br), 3106(=C-H aromatic), 3015(-C-H), 1602(C=N *str*.), 1245(N-O *str*.), 1237(C-O *str*.).**^{1}H-NMR (DMSO)δ ppm:** 7.24-7.59(14H, m, rest of aromatic proton on benzene ring), 6.95-7.17(3H, H-5", H-3"', H-5"'), 6.74-6.77(1H, s, isoxazole), 4.82-5.19(2H, s, imidazole-N-CH_2-isoxazole ring), 3.71(3H, s, -$0CH_3$),5.78(-OH) and 2.39(3H, s, -CH_3)

4-(4, 5-diphenyl-1-((5-p-tolylisoxazol-3-yl) methyl)-1*H*-imidazole-2-yl)-N, N-dimethylbenzenamine (TPI-4): IR (KBr Pellets) cm^{-1}: 3124(=C-H aromatic), 3013(-C-H), 1601(C=N *str*.), 1260(N-O *str*.), 1251(C-O *str*.). **^{1}H-NMR (DMSO)δ ppm:** 7.24-7.59(14H, m, rest of aromatic proton on benzene ring), 6.95-7.17(4H, H-3", H-5",H-3"',H-5"'), 6.74-6.77(1H, s, isoxazole), 4.82-5.19(2H, s, imidazole-N-CH_2-isoxazole ring), 2.98(6H, s, -$N(CH_3)_2$ and 2.45(3H, s, -CH_3).

N, N-dimethyl-4-(1-((5-(4-nitrophenyl)isoxazol-3-yl)methyl)-4, 5-diphenyl-1*H*-imidazole-2-yl)benzenamine (TPI-5): IR (KBr Pellets) cm^{-1}: 1350, 1543(OH *str*., br.), 3118(=C-H aromatic), 3014(-C-H), 1615(C=N *str*.), 1255(N-O *str*.), 1252(C-O *str*.).

1-((5-(3-bromophenyl) isoxazol-3-yl)methyl)-4, 5-diphenyl-2-*p*-tolyl-1*H*-imidazole (TPI-6): IR (KBr Pellets) cm^{-1}: 3123(=C-H aromatic), 3015(-C-H), 1597(C=N *str*.), 1278(N-O *str*.), 1238(C-O *str*.), 677(C-Br).

3-(4, 5-diphenyl-1-((5-(3, 4, 5-trimethoxyphenyl) isoxazole-3-yl) methyl)-1*H*-imidazole-2-yl)phenol (TPI-7): IR (KBr Pellets) cm^{-1}: 3447-3292(OH *str*., Br), 3120(=C-H aromatic), 3013(-C-H), 1615(C=N *str*.), 1271(N-O *str*.), 1256(C-O *str*.).

2-(4, 5-diphenyl-1-((5-*p*-tolylisoxazole-3-yl)methyl)-1*H*-imidazole-2-yl) phenol (TPI-8): IR (KBr Pellets) cm^{-1}: 3449-3298(OH *str*., br.), 3128(=C-H aromatic), 3008(-C-H), 1612(C=N *str*.), 1261(C-O *str*.), 1239(N-O *str*.).

2-(4-chlorophenyl)-4, 5-diphenyl-1-((5-*p*-tolylisoxazole-3-yl)methyl)-1*H*-imidazole (TPI-9): IR (KBr Pellets) cm^{-1}: 3130(=C-H aromatic), 3018(-C-H), 1618(C=N *str*.), 1254(C-O *str*.), 1248(N-O *str*.), 779(C-Cl).

4-(3-((2-(3-chlorophenyl)-4, 5-diphenyl-1*H*-imidazole-1-yl)methyl) isoxazole-5-yl)-2-methoxyphenol (TPI-10): IR (KBr Pellets) cm^{-1}: 3429-3320(OH *str*., br.), 3134(=C-H aromatic), 3009(-C-H), 1601(C=N *str*.), 1236(C-O *str*.), 1232(N-O *str*.), 782(C-Cl).

4-(3-((2-(4-methoxy)-4, 5-diphenyl-1*H*-imidazol -1-yl)methoxy) isoxazole-5-yl)-*N*, *N*-dimethylbenzenamine (TPI-11): IR (KBr Pellets) cm^{-1}: 3124(=C-H aromatic), 3012(-C-H), 1256(C-O *str*.), 1240(N-O *str*.), 1611(C=N *str*.).

1-((5-(4-chlorophenyl) isoxazol-3-yl) methyl)-2-(4-methoxyphenyl)-4, 5-diphenyl-1*H*-imidazole(TPI-12): IR (KBr Pellets) cm^{-1}: 3132(=C-H aromatic), 3001(-C-H), 1657(C=N *str*.), 1267(N-O *str*.), 1236(C-O *str*.), 782 (C-Cl).

2-(3, 5-dimethoxyphenyl)-4, 5-diphenyl-1-((5-(3,4,5-trimethoxyphenyl)isoxazol-3-yl)methyl)-1*H*-imidazole (TPI-13): IR (KBr Pellets) cm^{-1}: 3116(=C-H aromatic), 3018(-C-H), 1619(C=N *str*.), 1257(C-O *str*.), 1254(N-O *str*.).

1-((5-(4-chlorophenyl) isoxazol-3-yl)methyl)-4, 5-diphenyl-2-(3, 4, 5-trimehoxyphenyl)-1*H*-imidazole (TPI-14): IR (KBr Pellets) cm^{-1}: 3127(=C-H aromatic), 3019(-C-H), 1601(C=N *str*.), 1255(C-O *str*.), 1226(N-O *str*.), 782(C-Cl).

4, 5-diphenyl-1-((5-*p*-tolylisoxazol-3-yl)methyl)-2-(3, 4, 5-trimethoxyphenyl)-1*H*-imidazole (TPI-15): IR (KBr Pellets) cm^{-1}: 3134(=C-H aromatic), 3021(-C-H), 1589(C=N *str*.), 1268(N-O *str*.), 1237(C-O *str*.).

2-(2,4-dinitrophenyl)-4,5-diphenyl-1-((5-phenylisoxazol-3-yl)methyl)-1*H*-imidazole (TPI-16): IR (KBr Pellets) cm^{-1}: 3123(=C-H aromatic), 3022(-C-H), 1592(C=N *str*.), 1362, 1553(NO_2)1239(C-O *str*.), 1236(N-O *str*.).

2-methoxy-4-(3-((2-(4-nitrophenyl)-4, 5-diphenyl-1*H*-imidazol-1-yl) methyl) isoxazol-5-yl) phenol (TPI-17): IR (KBr Pellets) cm^{-1}: 3427-3291(OH *str*., br.), 3108(=C-H aromatic), 3010(-C-H), 1612(C=N *str*.), 1345, 1558(NO_2), 1259(C-O *str*.), 1221(N-O *str*.).

2-(3-((2-(4-fluorophenyl)-4, 5-diphenyl-1*H*-imidazol-1-yl)methyl)isoxazol-5-yl)phenol (TPI-18): IR (KBr Pellets) cm^{-1}:. 3445-3311(OH *str*., br.), 3136(=C-H aromatic), 2989(-C-H), 1616(C=N *str*.), 1253(C-O *str*.), 1249(N-O *str*), 976 (C-F).

2-(4-fluorophenyl)-1-((5-(4-methoxyphenyl)isoxazol-3-yl)methyl)-4,5-diphenyl-1*H*-imidazole (TPI-19): IR (KBr Pellets) cm^{-1}: 3115(=C-H aromatic), 2990(-C-H), 1614(C=N *str*.), 1256(C-O *str*.), 1224(N-O *str*.), 960 (C-F).

4-(3-((2-(3-bromophenyl)-4, 5-diphenyl-1*H*-imidazol-1-yl)methyl) isoxazol-5-yl)-N, N-dimethylbenzenamine (TPI-20): IR (KBr Pellets) cm^{-1}: 3127(=C-H aromatic), 3021(-C-H), 1611(C=N *str*.), 1259(C-O *str*.), 1251(N-O *str*.), 682(C-Br).

ANTIMICROBIAL ACTIVITY

The antimicrobial activity of synthesized derivatives was evaluated by the tube dilution method. Ciprofloxacin and Fluconazole were used as the standard drugs for evaluation of antibacterial and antifungal activity respectively. In this method minimum inhibitory concentration (MIC) of the antimicrobial agent was determined. The MIC is the (lowest concentration of the antimicrobial agent which is essential to inhibit the growth of micro-organism *in vitro***)** [17].

ANTIBACTERIAL ACTIVITY

In vitro antibacterial evaluation of synthesized derivatives was carried out against the gram positive bacteria: *Staphylococcus aureus*, *Bacillus subtilis* and gram negative bacteria: *Escherichia coli* by serial dilution method using nutrient broth media. Inoculated tubes were incubated at 37±1 °C for 24 hours (*S. aureus* and *B. subtilis* and *E.coli*) and was observed. From the observed MIC values, the exact MIC values were determined by making dilution of stock solution.

Preparation of suspension of microorganisms

The suspension of microorganism was made by transferring the microorganism from culture to 10 mL of sterile normal saline solution.

Preparation of standard and test solutions

Standard drug and the test compounds were dissolved in DMSO to obtain the concentration of 100µg/mL. Suspended 13.0 g of the nutrient broth in 1000 mL of distilled water, boiled to dissolve and the media was sterilized by autoclaving at 15 lbs pressure (121˚C) for 15 minutes.

Determination of minimum inhibitory concentration

1 mL of sterilized media was poured into sterilized test tubes. 1 mL of 100 stock solution of test compound was transferred in one test tube and serially diluted to give a concentration of 50µg/ml, 25µg/ml, 12.5µg/ml, 6.25µg/ml and 3.12µg/ml. In the same way, five different concentrations from stock solution of standard drug (Ciprofloxacin) were prepared. To all the test tubes 0.1 mL of suspension of bacteria in saline was added and test tubes were incubated at 37 °C for 24 hours for *S.aureus* and *B.subtilis* and 48 hours for *E.coli*. The growth in the test tube was observed visually for turbidity and inhibition was determined by the absence of growth. [18]. (Table 2 and Table 3)

ANTIFUNGAL ACTIVITY

Antifungal activity of the synthesized compounds was evaluated against *Aspergillus niger* and *Candida albicans* similarly to antibacterial assay. The nutrient media was used sabouraud's dextrose broth. To all the test tubes 0.1mL of suspension of fungal strain in saline was added and the test tubes were incubated at 25±1 °C for *A. niger* for 7 days and at 37±1°C for *C. albicans* for 48 hrs. Fluconazole was used as the standard drug and the activity of the synthesized compounds was compared with the standard drug [17,1 8, 19]. (Table 2 and Table 3)

Table 2 Antimicrobial Activity (MIC, µg/ml) of isoxazole derivatives

Comp. Code	(*B. subtilis*)	(*S. aureus*)	(*E. coli*)	(*C. albicans*)	(*A. niger*)
TPI-1	12.50	12.50	6.25	12.50	6.25
TPI-2	6.25	6.25	6.25	6.25	12.50
TPI-3	6.25	6.25	12.50	12.50	12.50
TPI-4	6.25	6.25	6.25	6.25	3.12
TPI-5	6.25	6.25	6.25	6.25	6.25
TPI-6	6.25	6.25	6.25	12.50	12.50
TPI-7	12.50	12.50	6.25	6.25	6.25
TPI-8	6.25	6.25	12.50	6.25	12.50
TPI-9	6.25	12.50	6.25	6.25	6.25
TPI-10	12.50	6.25	12.50	6.25	6.25
TPI-11	6.25	6.25	6.25	12.50	6.25
TPI-12	6.25	12.50	12.50	12.50	12.50
TPI-13	12.50	12.50	6.25	12.50	12.50
TPI-14	6.25	6.25	6.25	6.25	6.25
TPI-15	12.50	12.50	6.25	12.50	12.50
TPI-16	6.25	6.25	6.25	3.12	3.12
TPI-17	6.25	6.25	6.25	12.50	6.25
TPI-18	12.50	6.25	6.25	6.25	12.50
TPI-19	12.50	6.25	6.25	6.25	12.50
TPI-20	6.25	6.25	12.50	6.25	12.50
Ciprofloxcin	1.56	1.56	1.56	-	-
Fluconazole	-	-	-	3.12	3.12

Table 3 Antimicrobial Activity (pMIC, µmol/ml) of isoxazole derivatives

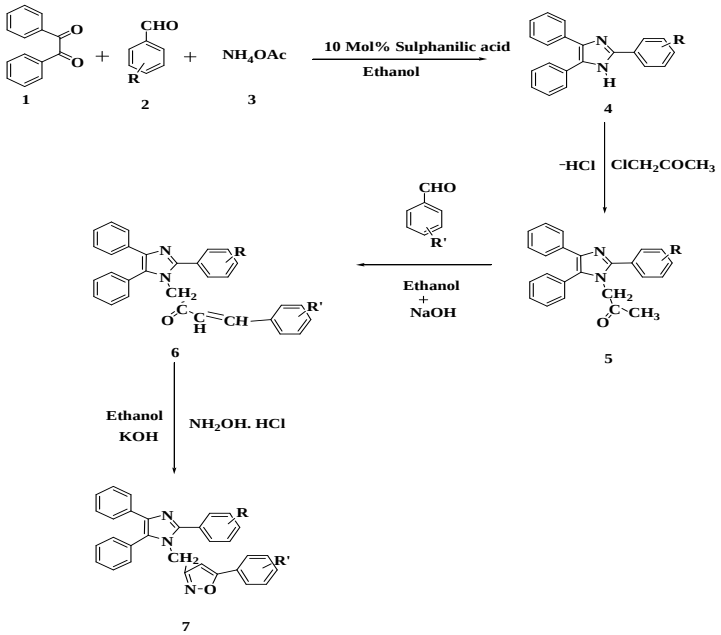
Comp. Code	(*B. subtilis*)	(*S. aureus*)	(*E. coli*)	(*C. albicans*)	(*A. niger*)
TPI-1	1.60	1.60	1.90	1.60	1.90
TPI-2	1.95	1.95	1.95	1.95	1.65
TPI-3	1.92	1.92	1.61	1.61	1.61
TPI-4	1.91	1.91	1.91	1.91	2.22
TPI-5	1.95	1.95	1.95	1.95	1.95
TPI-6	1.95	1.95	1.95	1.63	1.63
TPI-7	1.65	1.65	1.95	1.95	1.95
TPI-8	1.88	1.88	1.60	1.88	1.60
TPI-9	1.90	1.61	1.90	1.90	1.90
TPI-10	1.63	1.95	1.63	1.95	1.95
TPI-11	1.95	1.95	1.95	1.63	1.95
TPI-12	1.92	1.61	1.61	1.61	1.61
TPI-13	1.69	1.69	1.98	1.69	1.69
TPI-14	2.00	2.00	2.00	2.00	2.00
TPI-15	1.65	1.65	1.95	1.65	1.65
TPI-16	1.95	1.95	1.95	2.30	2.30
TPI-17	1.95	1.95	1.95	1.65	1.95
TPI-18	1.60	1.92	1.92	1.92	1.60
TPI-19	1.61	1.92	1.92	1.92	1.61
TPI-20	1.96	1.96	1.67	1.96	1.67
Ciprofloxcin	2.32	2.32	2.32	-	-
Fluconazole				1.99	1.99

RESULTS AND DISCUSSION

Chemistry

Scheme 1 was followed for synthesis of isoxazole derivatives. Reflucation of benzil with aromatic aldehyde gave compounds 1 which further acetylation with chloroacetone to yielded compound 2. The heating of compound 2 with

NaOH in the presence of ethanol yielded chalcone compound which further reacted with hydoxyalamine for cyclization yielded isoxazole derivatives. All the derivatives were characterized by FTIR and ^{1}H-NMR spectra. All the derivatives were synthesized in good yields and physicochemical parameters are summarized in table 1.



Scheme 1. General synthetic pathway for the synthesis of the derivatives

Compounds	R	R'
TPI-1	4-OCH_3	4-CH_3
TPI-2	3,5-OCH_3	4-NO_2
TPI-3	4-OH, 3-OCH_3	H
TPI-4	4-$N(CH_3)_2$	4-CH_3
TPI-5	4-$N(CH_3)_2$	4-NO_2
TPI-6	4-CH_3	3-Br
TPI-7	3-OH	3,4,5-OCH_3
TPI-8	2-OH	4-CH_3
TPI-9	4-Cl	4-CH_3
TPI-10	3-Cl	4-OH, 3-OCH_3
TPI-11	4-OCH_3	4-$N(CH_3)_2$
TPI-12	4-OCH_3	4-Cl
TPI-13	3,5-OCH_3	3,4,5-OCH_3
TPI-14	3,4,5-OCH_3	4-Cl
TPI-15	3,4,5-OCH_3	4-CH_3
TPI-16	2-NO_2	H
TPI-17	4-NO_2	4-OH, 3-OCH_3
TPI-18	4-F	2-OH
TPI-19	2-F	4-OCH_3
TPI-20	3-Br	4-$N(CH_3)_2$

ANTIMICROBIAL ACTIVITY

The synthesized derivatives were evaluated for antibacterial and antifungal studies by serial dilution method using ciprofloxacin and fluconazole as standard drugs respectively. Double strength nutrient broth and sabouraud dextrose broth have been employed as media for growth of bacterial and fungal cells respectively. Different concentrations of synthesized and standard derivatives were made and then inoculated with the microorganisms and incubated for a specified period at specified temperature. The minimum inhibitory concentration (MIC) was calculated by visual examination of turbidity.

The synthesized isoxazole derivatives **(TPI-1 to TPI-20)** exhibited good antimicrobial activity as compared to the standard drugs. It was observed from the results that the derivatives **TPI-2**, **TPI-5** and **TPI-14** showed maximum

antibacterial and antifungal activities (1.95µM/ml and 2.00µM/ml). The derivatives **TPI-6**, **TPI-11**, **TPI-16 and TPI-17** showed maximum antibacterial activity against *B. subtilis*, *S. aureus* and *E. coli* with pMIC 1.95µM/ml. The derivative **TPI-16** showed highest antifungal activity against *A. niger* and *C. albicans* with pMIC 2.30µM/ml respectively. The **TPI-4** derivative exhibited remarkable antifungal activity against *A.niger*. The derivatives **TPI-11**, **TPI-14**, **TPI-17** and **TP1-20** were found to have good antibacterial activity against gram-positive bacteria and antifungal activity against *A.niger*. The derivative **TPI-13** showed potent antibacterial activity against gram-negative *E. coli* bacteria. The compounds **TPI-6**, **TPI-7** and **TPI-10** displayed good antifungal activity. The derivatives **TPI-1**, **TPI-9** and **TPI-8** showed lowest antibacterial activity in comparison to other derivatives.

From the above discussion made, following SAR can be derived
1. Substitution of ring with methoxy group has a prominent effect on antimicrobial activity. Compounds **TPI-14** was found to display best activity with methoxy substitution. Compound with methoxy and nitro has also showed comparable results.
2. Nitro group at ortho position has shown good activity against *Aspergillus niger* and *Candida albicans.*
3. Substitution with hydroxyl (*p*-position) and methoxy (*m*-position) groups showed the moderate activity. Compounds TPI-10 and TPI-17 showed moderate activity against all the strains.
4. Substitution with methyl group has resulted in decrease in activity compounds.

CONCLUSION

Present study described the synthesis of some new isoxazole derivatives. All the synthesized compounds were characterized by FTIR and ^{1}H-NMR spectra and evaluated for antimicrobial activity. The results indicated that compound **TPI-2, TPI-5** and **TPI-14** were found to be the most active antibacterial and antifungal agents respectively. Compound **TPI-6**, **TPI-11**, **TPI-16** and **TPI-17** showed potent antibacterial effect against Gram positive and Gram- negative bacteria. Compound **TPI-4** was found to be the most active antifungal agent. The remaining compounds also possessed antimicrobial activity.

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