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SYNTHESIS, SPECTRAL STUDY AND ANTIMICROBIAL ACTIVITY OF ISOXAZOLE MOLECULES BEARING IN PYRIDINES NUCLEUS

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ABSTRACT

A new series of compounds, namely 3-{3'-(methoxy)-4'-[3"-(methyl)-4"-(2"',2"',2"'-trifluoroethoxy)-pyridin-2"-yl]methoxy phenyl}-5-aryl-isoxazoles (4a-4j), were synthesized. The chemical structures of these compounds were confirmed by ^{1}H-NMR, IR, Mass spectral analysis. The compounds (4a-4j) have been evaluated their antimicrobial activity.

***KEYWORDS*:** Isoxazole, Anti-bacterial & Anti-fungal activity (Heterocyclic compounds).

INTRODUCTION

Existing data shows that nitrogen-containing heterocyclic compounds such as isoxazole, pyrazoline,pyrimidine have medicinal importance because of their biological activities[1]. Isoxazole are another class of important heterocyclic compounds containing a five-membered ring with one nitrogen atoms, one oxygen atom and three carbon atoms.[2,3] Isoxazole are synthesized by the chalcone condensation with hydroxylamine hydrochloride.[4] The combination of chalcone and isoxazole moieties allows medicinal chemists to create hybrid molecules with a broad range of biological activities.[5-6-7]

Isoxazole have been found to exhibit various biological properties, such as Antibacterial[8-9], Antifungal[10-11], Antitumor[12], Anti-inflammatory[13], Anthelmintic[14], Anticancer[15], Anti-

HIV[16], Antihypertensive[17], Antihistaminic[18], Anti-oxidant[19], Anti-convulsant[20] and Analgesic[21] activities.

Our research work focuses on synthesized heterocyclic compounds derived from chalcones, which exhibit many biological activities and find various applications in industries. In our study, we have dedicated our efforts to synthesis isoxazole derivatives (4a-4j) derived from chalcones (3a-3j). Subsequently, we evaluated the antimicrobial properties of these newly synthesized chalcones and isoxazole derivatives and compared them with known standard drugs using the cup-plate method.[22]

The cup-plate method is commonly used to assess the antimicrobial activity of compounds. This method depends on the diffusion of an antibiotic from a vertical cavity, through the agar layer containing microorganisms in a petri plate. In this method, prepare an agar plate, and a swap of pure bacterial culture is evenly spread on the agar plate. Then, the synthesized compounds are added to the agar plate containing microorganisms, now kept this petri plate for incubation for 24 hours to allow them to diffuse and come into contact with the microorganisms. After an incubation period, a clear area (Zone of inhibition) around the tested sample is observed and measured. This measured zone indicates the antimicrobial activity of the compounds.

MATERIALS AND METHOD

The synthesis process involved the use of analytical grade (AR) chemicals sourced from SRL and Phenar companies, which were employed without any additional purification. All reactions took place under specified conditions. The purity of the synthesized compounds was assessed using TLC with silica gel G (Merk) and the solvent system ethyl acetate: hexane (2:3). The TLC plates were visualized under UV at 260nm. Characterization of the compounds was performed through spectral analysis, including MS (Mass Spectrometry), IR, and ^{1}H-NMR. Mass spectra were recorded using a water Mass spectrometer. Infrared spectroscopy was conducted using KBr on a Shimadzu IR Affinity FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker 400MHz spectrometer in DMSO solvent with TMS as an internal standard. Melting points of the synthesized compounds were determined in open glass capillary tubes and are uncorrected. The antimicrobial activities, of the synthesized compounds (3a-3j), (4a-4j) have been taken by the Cup plate method, with known standard drugs utilized for comparison.

General preparation: 1-{3'-(methoxy)-4'-[3''-(methyl)-4''-(2''', 2''', 2'''-trifluoroethoxy) pyridin-2''-yl] methoxy phenyl}-3-(3'''',4''''-dimethoxyphenyl)prop-2-en-1-one. (3a)

To a solution containing 1-{3'-(methoxy)-4'-[3"-(methyl)-4"-(2"',2"',2"'-trifluoroethoxy) pyridin-2"-yl]methoxy phenyl}ethan-1-one (0.01 m) in methanol, an appropriate 3, 4-dimethoxy benzaldehyde (0.01 m) was added. A catalytic amount of 40% NaOH solution was then introduced, and the resulting reaction mixture was stirred at room temperature for 24 hrs. The reaction is monitored by (TLC). After completion of the reaction, the reaction mixture was poured into ice and filtered, dry it. The obtained product was crystalized in methanol, leading to the formation of the desired compound (3a). M.P. :175^{0}C ; % of Yield :90%. ^{1}H NMR (400 MHz, DMSO) δ 8.37 (d, *J* = 5.7 Hz, 1H), 7.90 (d, *J* = 6.7 Hz, 1H), 7.82 (d, *J* = 7.8Hz, 1H), 7.64 (d, 1H), 7.52 (d, 1H), 7.40 (d, *J* = 7.3 Hz, 1H), 7.26 (d, 1H), 7.16 (d, *J* = 5.6 Hz, 1H), 7.08 – 6.97 (m, *J* = 8.3, 4.7 Hz, 1H), 5.32 (s, 1H), 4.92 (q, 1H), 3.84 (s, 9H), 2.23 (s, 3H). IR (cm-) 3527 (C-H Str. Aromatic), 2848 (C-H Str. Alkane), 1452-1361(C-H def. Alkane), 1581(C=C Str. Aromatic), 1107(C-H Def. Aromatic), 1649(C=O Str.), 1510(CH=CH Str. Vinyl), 1240(C-O-C Str.), 1022(C-F Str.). MS: at M/Z = 517, 419, 354, 313, 204, 191, 163, 151, 136 Anal. Calc. for $C_{27}H_{26}F_3NO_6$: C:62.66%,H:5.02%,O:20.88%,N:2.70% Found C:62.61%,H:5.00%,O:20.78%,N:2.66%.

Similarly other (3a-3j) compounds have been synthesized.

The synthesised chalcone (3a-3j) are reported in following paper.

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General preparation : 3-{3'-(methoxy)-4'-[3''-(methyl)-4''-(2''', 2''', 2'''-trifluoroethoxy)-pyridin-2''-yl] methoxy phenyl}-5-(3'''',4''''-dimethoxyphenyl)-isoxazole: (4a)

To a solution of 1-{3'-(methoxy)-4'-[3"-(methyl)-4"-(2"',2"',2"'-trifluoroethoxy)pyridin-2"-yl] methoxy phenyl}-3-(3"",4""-dimethoxyphenyl)prop-2-en-1-one (0.01 m) and hydroxyl amine hydrochloride (0.02 m) in methanol add small amount of alcoholic NaOH and reflux it for 8hrs at 80^{0}C. The reaction is monitored by (TLC). After completion of the reaction, the reaction mixture was poured into maltreated ice and filtered, dry it. The obtained product was crystalized in methanol, leading to the formation of the desired compound (4a). Yield :80%; M.P. :180^{0}C, ^{1}H NMR (400 MHz, DMSO) δ 8.36 (d, 1H), 7.47 (d, 1H), 7.27 (d, 2H), 7.17 (d,

1H), 7.00 – 6.87 (m, 3H), 6.78 (s, 1H), 5.22 (s, 2H), 4.94 (qr, 2H), 3.78 (d, *J* = 7.9 Hz, 9H), 2.26 (s, 3H). IR (cm-) 3064 (C-H Str. Aromatic), 2920 (C-H Str. Alkane), 1462-1350(C-H def. Alkane), 1583(C=C Str. Aromatic), 1136(C-H Def. Aromatic), 808(N-O Str.), 1583(CH=CH Str. Vinyl), 1255(C-O-C Str.), 1311(C-F Str.), 1514(C=N Str.). MS: at M/Z = 530. Anal. Calc. for $C_{27}H_{25}F_3N_2O_6$; C:61.13%,H:4.75%,N:5.28%,O:18.09% Found C:61.11%,H:4.70%,N:5.16%,O:18.00%.

Similarly other (4a-4j) compounds have been synthesized.

Reaction scheme: 1

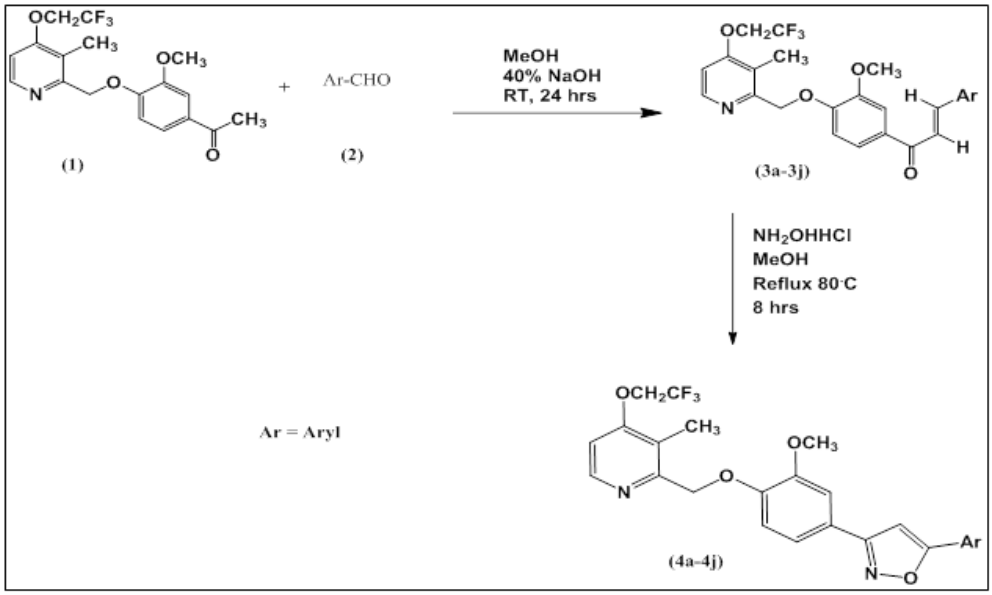

Table 1: physical and analytical data of 3-{3'-(methoxy)-4'-[3''-(methyl)-4''-(2''', 2''', 2'''-trifluoroethoxy)-pyridin-2''-yl]methoxy phenyl}-5-aryl-isoxazoles. (4a-4j)

Sr. No	Ar-	M.F	M. W	M.P	%Yield	% of Nitrogen	
						Calc.	Found
4a	3,4-$(OCH_3)_2$-C_6H_3-	$C_{27}H_{25}F_3N_2O_6$	530	180	80	5.28	5.16
4b	4-OH,3-OCH_3-C_6H_3-	$C_{26}H_{23}F_3N_2O_6$	516	195	60	5.42	5.40
4c	C_6H_5-	$C_{25}H_{21}F_3N_2O_4$	470	130	77	5.95	5.90
4d	4-OCH_3-C_6H_4-	$C_{26}H_{23}F_3N_2O_5$	500	140	67	5.60	5.55
4e	4-OH-C_6H_4-	$C_{25}H_{21}F_3N_2O_5$	486	220	68	5.76	5.70
4f	2-NO_2-C_6H_4-	$C_{25}H_{20}F_3N_3O_6$	515	190	73	8.15	8.00
4g	3-NO_2-C_6H_4-	$C_{25}H_{20}F_3N_3O_6$	515	185	78	8.15	8.10
4h	4-Cl-C_6H_4-	$C_{25}H_{20}ClF_3N_2O_4$	504	150	74	5.55	5.50
4i	4-Br-C_6H_4-	$C_{25}H_{20}BrF_3N_2O_4$	549	160	73	5.10	5.00
4j	3,4,5-$(OCH_3)_3$-C_6H_2-	$C_{28}H_{27}F_3N_2O_7$	560	210	66	5.00	5.00

RESULT AND DISCUSSION

Antimicrobial activity

The antibacterial and antifungal activity is done by the cup plate method through zone of inhibition in mm. The concentration of the sample & standard drug is 50 µg/ml using DMSO as solvent. The anti-bacterial activity was taken by Gram-positive bacteria *Bascillis Substilis, Staphylococcus aureus* and Gram-negative bacteria *proteus vulgaris, Escherichia coli*. The anti-fungal activity was taken by *Aspergillus niger* fungus. The antimicrobial activity compared with known standard drugs Streptomycin, Ampicillin, Tetracyclin and Nystatin. The Zone of inhibition was measured in mm. Were Zone of inhibition of compounds (4a-4j) is shown in the Table No. 2. Comparable antimicrobial activity represent in Table No. 3.

Table 2: Antimicrobial activity data of 3-{3'-(methoxy)-4'-[3''-(methyl)-4''-(2''', 2''', 2'''-trifluoroethoxy)-pyridin-2''-yl]methoxy phenyl}-5-aryl-isoxazoles. (4a-4j)

Sr. No.	Ar-	Antibacterial activity, zone of inhibition in m.m.				Antifungal activity, Zone of inhibition m.m.
		Gram-positive bacteria		Gram-negative bacteria		
		B. subtilis	*S. aureus*	*E. coli*	*P. vulgaris*	*A. niger*
4a	3,4-$(OCH_3)_2$-C_6H_3-	8	18	6	10	12
4b	4-OH,3-OCH_3-C_6H_3-	10	22	7	11	12
4c	C_6H_5-	20	22	10	9	10
4d	4-OCH_3-C_6H_4-	20	22	11	12	12
4e	4-OH-C_6H_4-	21	23	6	13	13
4f	2-NO_2-C_6H_4-	20	23	13	13	11
4g	3-NO_2-C_6H_4-	18	20	20	12	11
4h	4-Cl-C_6H_4-	19	20	19	10	8
4i	4-Br-C_6H_4-	22	18	21	11	9
4j	3,4,5-$(OCH_3)_3$-C_6H_2-	20	21	22	10	10

Table: 3 Compounds (4a-4j) showing antibacterial and antifungal activity compared with known standard drugs.

Compound No.	Antibacterial activity, zone of inhibition in mm.				Antifungal activity, zone of inhibition in mm.
	Gram-positive bacteria		Gram-negative bacteria		
	B. Subtilis	***S. aureus***	***E. Coli***	***P. vulgaris***	***A. Niger***
(4a-4j)	4c,4d,4e,4f,4i,4j	4b,4c,4d,4e,4f,4j	4g,4i,4j	4e,4f	4e
Activity of known standard drugs					
Drugs	***B. Subtilis***	***S. aureus***	***E. Coli***	***P. vulgaris***	***A. Niger***

Streptomycin	26	27	28	20	0
Ampicillin	25	26	26	19	0
Tetracycline	25	26	27	19	0
Nystatin	0	-	-	-	22

CONCLUSION

We have synthesized The Isoxazole derivatives (4a-4j) 3-{3'-(methoxy)-4'-[3"-(methyl)-4"-(2"',2"',2"'-trifluoroethoxy)-pyridin-2"-yl]methoxy phenyl}-5-aryl-isoxazoles**.** The structure of the compounds confirmed by ^{1}H-NMR, IR, Mass spectra. the synthesized compounds were subjected to antibacterial & antifungal activities. Compounds **4b,4c,4d,4e,4f,4i,4j** exhibited significant antibacterial activity against Gram-positive bacteria as compared to known standard drugs and Compounds **4e,4f,4g,4i,4j** give remarkable activity against Gram-negative bacteria as compared to known standard drugs and Compounds **4e,4f** displayed good antifungal activity as compared to known standard drugs with the same concentration 50 µg/ml.

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