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SYNTHESIS AND BIOLOGICAL EVALUATION OF NOVEL PYRAZOLINE AND ISOXAZOLINE DERIVATIVES.

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ABSTRACT

A series of novel Pyrazoline **3a-l** and Isoxazoline **4a-l** derivatives have been synthesized as potential antibacterial agents. The Pyrazoline derivatives **3a-l** have been synthesized by reaction of various Chalcones **2a-l** with hydrazine hydrate in ethanol. The Isoxazolines **4a-l** were prepared by the reaction of various Chalcones **2a-l** with hydroxyl amine hydrochloride in presence of sodium acetate using ethanol as a solvent. The structures of the new synthesized compounds were established on the basis of ^{1}H-NMR, Mass spectra, IR and elemental analysis data. All the newly synthesized compounds were screened for their antibacterial activity against *E. coli*, *S. thyphi* (Gram-negative bacteria), *S. aureus*, *M. luteus* (Gram-positive bacteria) and antifungal activity against *Candida albicans (Fungi)*.

KEYWORDS: Pyrazoline, Isoxazoline, Antimicrobial activity.

INTRODUCTION

Compounds incorporating heterocyclic ring systems continue to attract considerable interest due to their wide range of biological activities.[1,3] Pyrazolines and Isoxazoline represents an important class of nitrogen containing heterocyclic compounds.[4,7] A classical pathway for the synthesis of pyrazoline and isoxazoline are based on the reaction of compounds having α,?-unsaturated group in conjugation with carbonyl system with hydrazine hydrate and hydroxylamine hydrochloride respectively. Some Pyrazolines have played a crucial role in the development of heterocyclic chemistry and were also extensively used as key synthons in organic synthesis.[8,9] As a consequence, a large number of different substituted pyrazoline derivatives were prepared.[10,14] Numerous pyrazolines have been reported to possess

important bioactivities, viz. antimicrobial and antimycotic,[15,16] immunosuppressive,[17] etc. activities.

In addition, Synthesis of novel isoxazoline derivative remains a main focus of medicinal chemist, due to their diverse pharmacological activity. Acivicin, a known Isoxazoline derivative is used for treatment of tumor. Isoxazoline derivatives have been reported to possess antinociceptive,[18] antibacterial,[19] anti-tuberculosis,[20] etc. activities.

Vanilla is a natural aromatic compound that can be used as an antimicrobial, antioxidant and masking agent. However, the antimicrobial inherent qualities of vanillin are very intriguing. Vanillin exhibit inhibitory activity against bacteria, fungi and molds. Vanillin is a food-grade ingredient and due to this reason it can be incorporated into the product of interest. *Aspergillus niger*, *Rhizopus stolonifer*, *Penicillium notatum* and *Saccharomycopsis fibulgera* are sensitive to vanillin. The antimicrobial activity of vanillin was investigated against *E. coli*, *Lact. plantarum* and *L. innocua* in laboratory media. MIC levels of vanillin indicated that the inhibitory action of vanillin was bacteriostatic rather than bactericidal.[21]

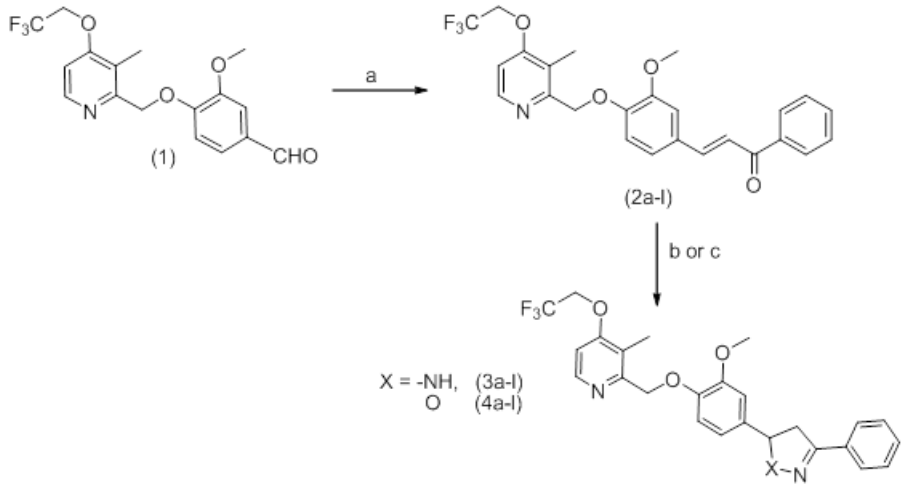
The incorporation of fluorine into a chemical compound allows simultaneous modulation of electronic, lipophilic and steric parameters, all of which can critically influence both the pharmacodynamic and pharmacokinetic properties of drugs.[22] Fluorine occupies a van der Waals radius (1.47 A°) positioned between oxygen (1.52 A°) and hydrogen (1.20 A°) allowing it to mimic a hydroxyl group, and to participate in hydrogen bonding interactions.[23] So, by this idea in view synthesis of fluorine containing heterocyclic compounds is the interesting area of research.

Recently, vanillin containing aryl substitution reported as anticancer,[24] antimitotic and apoptotic,[25] and antimalarial,[26] activities. Considering importance of vanillin and fluorine in medicinal chemistry, we prompted to incorporate these two moieties in a single molecule. In continuation with our ongoing research program on synthesis of heterocyclic compounds.[27] We now report on the synthesis of 2-((2-Methoxy-4-(3-aryl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)methyl)-3-methyl-4-(2,2,2-trifluoroethoxy)pyridine and 5-(3-Methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy) pyridin-2-yl)methoxy)phenyl)-3-aryl-4,5-dihydroisoxazole.

RESULTS AND DISCUSSION

Chemistry

The synthetic route adopted to synthesize the Pyrazoline derivatives **3a-l** and Isoxazoline derivatives **4a-l** is shown in Scheme 1. The required key chalcone intermediate 2a-l,[27] was synthesized by reacting aldehyde **1** with substituted aetophenone using catalytic 40% aq. NaOH in EtOH at room temperature. After recrystallization from ethanol all corresponding Chalcones were obtained varied in 68-90% yield. The pyrazoline derivatives **3a-l** were prepared from Chalcones **2a-l** by reacting with hydrazine hydrate in ethanol at 76-78^{O}C. The isolated product was washed with diethyl ether to get pyrazolines in 71-82% yield. The isoxzoline derivatives **4a-l** were prepared from Chalcones **2a-l** by reacting with hydroxyl amine hydrochloride in ethanol at 76-78^{O}C in presence of sodium acetate. The isolated product was washed with diethyl ether to get pyrazolines in 71-82% yield. The structures of all newly synthesized compounds were assigned on the basis of spectral data such as IR, ^{1}H-NMR, ^{13}C-NMR, Mass and elemental analysis.



Reagents and conditions: (a) $ArCOCH_3$, 40% NaOH, Ethanol, RT, 5-6 hrs; (b) hydrazine hydrate, 78 ^{O}C, 4-6 hrs, EtOH. (c) NH_2OH-HCl, NaOAc, EtOH

Scheme 1: The Synthetic scheme for the preparation of compounds 3a-l and 4a-l.

The structural assignment of the title compounds **3a-l** and **4a-l** have been made on the basis of ^{1}H-NMR, Mass spectra, elemental analysis and IR spectral studies which were in full agreement with the proposed structures. The structure of **3a** is interpreted from spectroscopic data. The IR spectrum of **3a** showed a characteristic absorption band in the region 1120 cm-1 corresponding to C-N stretching and 1624 cm-1 due to C=N of Pyrazoline ring. In ^{1}H-NMR spectra of **3a** the one CH_3 protons absorbed as a singlet at δ 2.35 and methoxy group at δ

3.82 and the doublet of doublet at δ 3.10, 3.65 and 5.48 due to pyrazoline ring for 1H proton and rest of the aromatic proton appear at their respective position. Mass spectrum of 2-((2-Methoxy-4-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl) phenoxy)methyl)-3-methyl-4-(2,2,2-trifluoroethoxy)pyridine showed (M+) peak at 472.64 which support the formation of desired product. The structure of Isoxazoline **4a** is interpreted from spectroscopic data. The IR spectrum of **6a** showed a characteristic absorption band in the region 815 cm-1 corresponding to N-O stretching and 1641 cm^{-1} due to C=N of Isoxazoline ring. In ^{1}H-NMR spectra of **4a** the one methyl protons absorbed as a singlet at δ 2.32 and methoxy group at δ 3.81 and the doublet of doublet at δ 3.10, 3.65 and 5.78 due to pyrazoline ring for 1H proton and rest of the aromatic proton appear at their respective position. Mass spectrum of 5-(3-methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy) pyridin-2-yl)methoxy) phenyl)-3-phenyl-4,5-dihydroisoxazole showed (M+) peak at 473.3 which support the formation of product.

Experimental

All the melting points were determined on electro-thermal apparatus using open capillaries and are uncorrected. Formation of the compounds was routinely checked by TLC on silica gel-G plates of 0.5mm thickness and spots were located by iodine and UV (254nm). The IR spectra were recorded on a Shimadzu FT-IR-8400 instrument using KBr pellet method. Mass spectra were recorded on Shimadzu GCMS-QP2010 model using Direct Injection Probe technique. ^{1}H-NMR was determined in $CDCl_3$/DMSO-d_6 solution on a Bruker AC 400MHz spectrometer using TMS as internal standard and coupling constants (J) are expressed in Hertz (Hz). Elemental analysis of the all the synthesized compounds were carried out on Elementar Vario EL III Carlo Erba 1108 model and the results are in agreements with the structures assigned. All the reagents were purchased from Rankem (New Delhi, India) and Sigma-Aldrich (New Delhi, India) and are used without further purification.

General procedure for synthesis of 3-(3-Methoxy-4-((3-methyl-4-(2,2,2-trifluoro ethoxy)pyridin-2-yl)methoxy)phenyl)-1-arylprop-2-en-1-one (2a-l). To a solution of 3-Methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy) benzaldehyde 1 (2.81 mmol) in ethanol was added appropriate Acetophenone (3.09 mmol) followed by catalytic amount of 40% aqueous NaOH solution and the reaction mixture was stirred for 5-6 hrs at room temperature. After completion of reaction on TLC, the reaction mixture was filtered. The product was crystallized in ethanol to afford title compounds **2a-l**.

General procedure for synthesis of 2-((2-Methoxy-4-(3-Aryl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)methyl)-3-methyl-4-(2,2,2-trifluoroethoxy)pyridine (3a-l). To a solution of Chalcone **2a-l** (1.62 mmol) in Ethanol (5 ml) was added hydrazine hydrate (0.4 ml, 8.12mmol) and heated at 70-75^{O}C for 4-6 hrs. After completion of the reaction, the reaction mixture was poured in ice water and extracted with ethyl acetate (2 x 20 ml). The organic layer was washed with brine, dry over sodium sulphate and evaporated under reduced pressure. The crude residue was washed with diethyl ether to give pure product as solid.

2-((2-Methoxy-4-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)phenoxy)methyl)-3-methyl-4-(2,2,2-trifluoroethoxy)pyridine (3a): Yield 87% (off white solid); m.p 138 ^{O}C; IR (KBr, cm^{-1}): 1120 (C-N), 1624 (C=N); ^{1}H-NMR ($CDCl_3$, □ ppm): 8.33-8.35 (d, 1H, *J* = 5.6 Hz, aromatic), 7.61-7.63 (m, 2H, aromatic), 7.21-7.23 (m, 3H, aromatic), 6.96-6.98 (d, 1H, *J* = 8 Hz, aromatic), 6.65-6.75 (m, 3H, aromatic), 5.48-5.51 (dd, 1H, Pyrazoline), 5.21 (s, 2H, -O-CH_2-), 4.35-4.41 (q, 2H, -O-CH_2-CF_3), 3.82 (s, 3H, -OCH_3), 3.65-3.69 (dd, 1H, Pyrazoline), 3.10-3.16 (dd, 1H, Pyrazoline), 2.35 (s, 3H, -CH_3); MS : (m/z) 472.64 (M^+); Anal. Calcd. for $C_{25}H_{24}F_3N_3O_3$: C: 63.69%; H: 5.13%; N: 8.91%; Found: C: 63.60%, H: 5.19%, N: 8.75%.

General procedure for synthesis of 5-(3-Methoxy-4-((3-methyl-4-(2,2,2-trifluoro ethoxy)pyridin-2-yl)methoxy)phenyl)-3-aryl-4,5-dihydroisoxazole (4a-l). To a solution of Chalcone **2a-i** (1.09 mmol) in Ethanol (5 ml) was added Sodium acetate (4.37mmol) and Hydroxylamine hydrochloride. Reaction mixture was heated at 70-75^{O}C for 12 hrs. After completion of the reaction, the reaction mixture was poured in ice water and extracted with ethyl acetate (2 x 20 ml). The organic layer was washed with brine, dry over sodium sulphate and evaporated under reduced pressure. The crude residue was washed with diethyl ether to give pure product as solid.

5-(3-Methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy)phenyl)-3-phenyl-4,5-dihydroisoxazole (4a): Yield 52% (off white solid); m.p 128^{O}C; IR (KBr, cm^{-1}): 815 (N-O), 1641 (C=N); ^{1}H-NMR ($CDCl_3$, □ ppm): 8.34-8.36 (d, 1H, *J* = 5.6 Hz, aromatic), 7.62-7.64 (m, 2H, aromatic), 7.20-7.22 (m, 3H, aromatic), 6.95-6.97 (d, 1H, *J* = 8 Hz, aromatic), 6.64-6.74 (m, 3H, aromatic), 5.78-5.81 (dd,1H, Isoxazoline), 5.20 (s,2H,-O-CH_2-), 4.34-4.40 (q, 2H, -O-CH_2-CF_3), 3.82 (s, 3H, -OCH_3), 3.64-3.68 (dd, 1H, Isoxazoline), 3.09-3.14 (dd, 1H, Isoxazoline), 2.32 (s, 3H, -CH_3); MS: (m/z) 473.3 (M^+); Anal. Calcd. for $C_{25}H_{23}F_3N_2O_4$: C: 63.55%; H: 4.91%; N: 5.93%; Found: C: 63.42%, H: 4.82%, N: 5.86%.

Similarly other Pyrazoline and Isoxazoline derivative has been synthesized and all data have been recorded in **Table 1.**

Sr No	R	Molecular Formula	M.W	M.P ^{o}C	Yield %	% of Nitrogen	
						Calcd.	Found.
3a	C_6H_5-	$C_{25}H_{24}F_3N_3O_3$	471.3	138	87	8.91	8.75
3b	4-CH_3-C_6H_4-	$C_{26}H_{26}F_3N_3O_3$	485.3	101	84	8.66	8.78
3c	4-OCH_3-C_6H_4-	$C_{26}H_{26}F_3N_3O_4$	501.2	144	83	8.38	8.51
3d	4-OH-C_6H_4-	$C_{25}H_{24}F_3N_3O_4$	487.4	238	65	8.62	8.72
3e	3-Br-C_6H_4-	$C_{25}H_{23}BrF_3N_3O_3$	550.3	179	73	7.63	7.55
3f	4-Br-C_6H_4-	$C_{25}H_{23}BrF_3N_3O_3$	550.3	173	87	7.63	7.69
3g	3-Cl-C_6H_4-	$C_{25}H_{23}ClF_3N_3O_3$	506.1	161	80	8.31	8.29
3h	4-Cl-C_6H_4-	$C_{25}H_{23}ClF_3N_3O_3$	506.1	170	81	8.31	8.42
3i	3-NO_2-C_6H_4-	$C_{25}H_{23}F_3N_4O_5$	516.3	185	75	10.85	10.71
3j	2-Thiophenyl-	$C_{23}H_{22}F_3N_3O_3S$	477.5	143	71	8.80	8.93
3k	2-Furanyl-	$C_{23}H_{22}F_3N_3O_4$	461.3	156	68	9.11	9.23
3l	2-Pyridinyl	$C_{24}H_{23}F_3N_4O_3$	472.3	170	72	11.86	11.95
4a	C_6H_5-	$C_{25}H_{23}F_3N_2O_4$	472.3	128	52	5.93	5.86
4b	4-CH_3-C_6H_4-	$C_{26}H_{25}F_3N_2O_4$	486.3	118	58	5.76	5.81
4c	4-OCH_3-C_6H_4-	$C_{26}H_{25}F_3N_2O_5$	502.2	134	55	5.58	5.67
4d	4-OH-C_6H_4-	$C_{25}H_{23}F_3N_2O_5$	488.4	205	49	5.74	5.82
4e	3-Br-C_6H_4-	$C_{25}H_{22}BrF_3N_2O_4$	551.3	174	58	5.08	5.14
4f	4-Br-C_6H_4-	$C_{25}H_{22}BrF_3N_2O_4$	551.3	153	63	5.08	5.15
4g	3-Cl-C_6H_4-	$C_{25}H_{22}ClF_3N_2O_4$	507.1	156	53	5.53	5.60
4h	4-Cl-C_6H_4-	$C_{25}H_{22}ClF_3N_2O_4$	507.1	169	71	5.53	5.58
4i	3-NO_2-C_6H_4-	$C_{25}H_{22}F_3N_3O_6$	517.3	189	48	8.12	8.24
4j	2-Thiophenyl-	$C_{23}H_{21}F_3N_2O_4S$	478.4	123	65	5.85	5.92
4k	2-Furanyl-	$C_{23}H_{21}F_3N_2O_5$	462.3	135	61	6.06	6.19
4l	2-Pyridinyl	$C_{24}H_{22}F_3N_3O_4$	473.3	160	51	8.88	8.79

Antibacterial and antifungal activity

The newly synthesized compounds were screened for their antibacterial activity against Gram-positive (*S. aureus* ATCC 6538, *M. luteus* ATCC 9345), Gram negative (*E. coli* ATCC 4230, *S.thyphi* ATCC 14028) bacteria, as described by the guidelines in NCCLS-approved standard document M7-A4, using the micro dilution broth procedure (Clause, G.W. 1989).[28] Ampicillin trihydrate was used as the reference antibacterial agent. The antifungal activities of the newly synthesized chemical compounds were tested against yeast strain (*C. albicans* ATCC 14053) according to the guidelines in NCCLS-approved standard document M27-A2, using the micro dilution broth procedure (NCCLS 1997).[29] Fluconazole was used as the reference antifungal agent. The solutions of test compounds and reference drug were prepared by dissolving in DMSO at a concentration of 2560 µg/mL. The 2-fold dilutions of the compounds and the reference drug were prepared (1280, 640, 320, 160, 80, 40, 20, 10 µg/mL). Antibacterial activities of the newly synthesized chemical compounds were

performed in Mueller-Hinton broth medium at a pH of 7.2 with an inoculum of (1-2) × 10^3 cells/mL by the spectrophotometric method and an aliquot of 100µL solution was added to each tube of serial dilution. The chemical compounds-broth medium serial tube dilutions inoculated with each bacterium were incubated on a rotary shaker at 37°C for 18 hr at 150 rpm. The minimum inhibitory concentration (MIC) of each chemical compound was recorded as the lowest concentration of each chemical compound in the tubes with no growth (i.e., no turbidity) of inoculated bacteria. Minimum inhibitory concentration (MIC, µg/mL) was measured and compared with control; the MIC values of the compound screened are given in **Table 2.**

	Ar	Antibacterial Activity				Antifungal activity
		S.aureus	***M.luteus***	***E.coli***	***S.typhi***	***C.albicans***
3a	C6H5-	160	80	160	160	160
3b	4-CH_3-C_6H_4-	160	80	160	160	160
3c	4-OCH_3-C_6H_4-	80	160	160	80	160
3d	4-OH-C_6H_4-	40	20	80	40	80
3e	4-Br-C_6H_4-	40	40	40	20	80
3f	4-Cl-C_6H_4-	20	20	40	40	160
3g	3-Cl-C_6H_4-	80	40	80	40	80
3h	3-Br-C_6H_4-	80	40	160	80	160
3i	3-NO_2-C_6H_4-	40	40	80	40	80
3j	2-Thiophenyl	80	40	40	40	160
3k	2-Furanyl	40	20	80	20	80
3l	2-Pyridinyl	20	20	40	40	80
4a	C_6H_5-	160	160	80	160	320
4b	4-CH_3-C_6H_4-	80	160	160	80	80
4c	4-OCH_3-C_6H_4-	80	80	80	20	160
4d	4-OH-C_6H_4-	40	40	80	160	80
4e	4-Br-C_6H_4-	20	20	80	40	160
4f	4-Cl-C_6H_4-	40	80	40	40	160
4g	3-Cl-C_6H_4-	20	40	40	20	80
4h	3-Br-C_6H_4-	80	80	40	80	160
4i	3-NO_2-C_6H_4-	40	40	80	40	160
4j	2-Thiophenyl	40	20	160	80	80
4k	2-Furanyl	40	40	80	80	80
4l	2-Pyridinyl	80	40	80	40	80
	Ampiciline	20	20	40	20	-
	Fluconazole	-	-	-	-	10

From the result of biological evaluation, it has been observed that the compounds exhibited interesting biological activity, however with a degree of variation. Most of the compounds tested were found to have comparable antibacterial and exhibit low antifungal activity. From the Table 1, it can be observed that compounds 3d, 3g, 3i, 3k, 4h, 4i and 4k were moderate

active against *S. aureus* A, *M. luteus*, *E. coli*, *S. thyphi*. Compounds 3h, 3l, 4d and 4k were moderate active against *S. aureus* A, *M. luteus* and 3f, 4g, 4l were moderate active against *E. coli, S. thyphi*, while all the synthesized compounds shows low antifungal activity against *C. albicans*. So result of all preliminary study indicated that the substituted 2-((2-Methoxy-4-(3-Aryl-4,5-dihydro-1H-pyrazol-5-yl) phenoxy)methyl)-3-methyl-4-(2,2,2-trifluoroethoxy) pyridine and 5-(3-methoxy-4-((3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl)methoxy) phenyl)-3-Aryl-4,5-dihydroisoxazole moiety represent a new class of pharmacophore for broad spectrum antibacterial activity.

CONCLUSION

In summary, we have synthesized a series of vanillin incorporated novel Pyrazoline and Isoxazoline derivatives. All the newly synthesized compounds were confirmed with spectroscopic data like ^{1}H-NMR, Mass, IR Spectra, elemental analysis and evaluated antibacterial and antifungul activity. The antibacterial study shows that some of the pyrazoline and isoxazoline derivatives showed moderate in activity with MICs between 20 and 40 µg/mL. The Pyrazolines and Isoxazoline showed low antifungal activity. The importance of such work lies in the possibility that the new compounds might be more efficacious drugs against bacteria, which could be helpful in designing more potent antibacterial agent for therapeutic use.

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