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Microwave assisted synthesis of Some Isoxazole and Pyrazole Compound as possible Antimicrobial Agents

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ABSTRACT

3-substitutedphenyl-4-methylhexahydro-6H-pyrazolo[3,4-c]isoxazol-6-yl](pyridine-4-yl) methanone-ethane (3a-d) and (3-methyl-4-(substitutedphenyl)-3,3a,4,5-teterahydro pyrazolo [3,4-c]pyrazol-1(2H)-yl)(pyridine-4-yl)methanone(4a-d) were synthesized from 4-(substituted benzylidene)-isonicotinoylpyrazolidine 3, 5-dione (2a-d) using microwave-assisted route. The structures of all the compounds have been established on the basis of analytical and spectral data. The newly synthesized compounds were evaluated for their anti-microbial activity and have shown significant anti-microbial activity.

Keywords: Isoxazole, Pyrazole, Microwave, Antibacterial, Antifungal.

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INTRODUCTION

Industrialization has led to immense environmental deterioration. The increasing environmental consciousness all over the world has put a pressing need to develop an alternative synthetic approach for synthetically and biologically important compounds. This requires a new approach, which will reduce the material and energy intensity of chemical processes and products, to minimize or eliminate the dispersion of harmful chemicals in the environment in a way that enhances industrially benign approach and meets the challenges of green chemistry [1]. Organic synthesis through microwave irradiation is a new and interesting technique and is becoming popular now. Microwave-assisted organic synthesis (MAOS) continues to affect synthetic chemistry significantly by enabling rapid, reproducible and scalable chemistry development [2]. Numerous reactions including condensations, cycloadditions, heterocycle formations, and metal catalyzed cross coupling have been explored under microwave conditions [3]. MAOS can facilitate the discovery of new reactions and reduce cycle time in optimization of reactions. In addition, it serves to expand chemical space in compound library synthesis [4].

Recently, microwave reactions have attracted considerable attention in organic chemistry for their ability to accelerate slow thermal reactions [5]. Microwave irradiation provides advantages over conventional heating in chemical transformations; these advantages include accelerated reaction rates, significant energy savings, high chemical yields, and cleaner reactions [6] and several eco-friendly advantages in the context of green chemistry [7].

The isoxazole nucleus is well known for its medicinal importance [8] and a number of related compounds are known to exhibit antitumor [9], anti-HIV [10] and cestoidal [11] agents. The marketed drugs of isoxazole, such as, Acetylsulfisoxazole, Cycloserine, Drazoxol on, Sulfisoxazole and Zonisamide have a great medicinal value. Compounds containing an isoxazole scaffold are known to possess antimicrobial [12-13], anti-inflammatory [14], antiviral [15], anticonvulsant [16], antileukemic [17] and antagonist ionotropic glutamate receptor [18] activities, and are also modulators of the Multidrug Resistance Protein (MRP1) [19] and these activities are also observed in their derivatives, led to the search for newer bioactive compounds of this class.

The pyrazoles constitutes an interesting class of organic compounds with diverse chemical and biological application. They are known to possess variety of biological activities such as analgesic, anti-inflammatory, protein kinase C inhibitor [20]. Many pyrazole derivatives possess remarkable antiepileptic and antimicrobial, [21] potent and selective inhibitors of tissue-nonspecific alkaline phosphatase, [22] antiamoebic, [23] and antiandrogenic activities [24]. The pyrazole nucleus is a ubiquitous feature of pharmacological interest and has been proven to be a fertile source of medicinal agents. The isoxazole and pyrazole nucleus is a ubiquitous feature of pharmacological interest and has been proven to be a fertile source of medicinal agents.

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EXPERIMENTAL

Materials and equipments

All reactions were carried out in a microwave oven (KENSTAR- OM-26EGO). Melting points were determined in open capillaries and are uncorrected. Reaction was monitored by thin layer chromatography using silica gel-G as adsorbent using ethyl acetate: benzene (7:3) as eluent and products were detected by iodine vapour. IR spectra (KBr pellets) were recorded on Perkin-Elmer 1800 (FTIR) spectrometer. ^{1}H NMR spectra (DMSO-d_6) were taken on a Bruker DRX spectrometer (300 MHz, FT NMR) using TMS as internal standard and chemical shift were expressed in δ. Mass spectra were taken on a Jeol SX-102/PA-6000 (EI) spectrometer. The starting compounds were prepared according to reported method.

General Procedure for Microwave induced Synthesis of 1-isonicotinoylpyrazolidine 3, 5-dione (1)

A mixture of Isonicotinohydrazide (0.01 mol) and Diethyl malonate (0.05 mol), Acetic Acid (4-5drops) in ethanol (20 ml.) were taken in an Erlenmeyer flask and mixed thoroughly. The mixture was irradiated under microwave for 6 min at 600 W (i.e, 50 % microwave power) with constant shaking and intermittent radiation of 30 sec interval. The progress of the reaction was monitored by TLC. The solid thus obtained was dried and recrystallised from alcohol to yield compound (1)

Microwave induced Synthesis of 4-(substituted benzylidene)-isonicotinoylpyrazolidine 3, 5-dione (2a-d)

Mixture of 1-isonicotinoylpyrazolidine-3, 5-dione (0.01 mol) and different aromatic aldehydes (0.01 mol) and KOH (2 to 3 drops) in 30 ml of DMF were taken in an Erlenmeyer flask and mixed thoroughly. The well-stirred mixture was irradiated in microwave oven for 5-6 min at 600 W (i.e. 50 % microwave power). The completion of the reaction was monitored by TLC. The reaction mixture was then cooled at room temperatures and poured into crushed ice. The solid obtained 2a-e was filtered, washed with water and recrystallized from ethanol. The purity of compounds was analyzed by TLC using hexane: ethyl acetate (7:3) as mobile phase.

Microwave induced Synthesis of 3-substituted phenyl-4-methylhexahydro-6H-pyrazolo [3,4-c]isoxazol-6-yl](pyridine-4-yl)methanone-ethane (3a-d)

Mixture of compound **2** (0.01 mol) and hydroxylamine hydrochloride (0.05 mol) with few drops of acetic acid in 30 ml of DMF were taken in an Erlenmeyer flask and mixed thoroughly. The well-stirred mixture was irradiated in microwave oven for 7-8 min at 600 W (i.e. 50 % microwave power). The completion of the reaction was monitored by TLC. The reaction mixture was then cooled at room temperatures and poured into crushed ice. The obtained solid was filtered, washed with water and recrystallized from ethanol. The purity of compounds was analyzed by TLC using hexane: ethyl acetate (7:3) as mobile phase.

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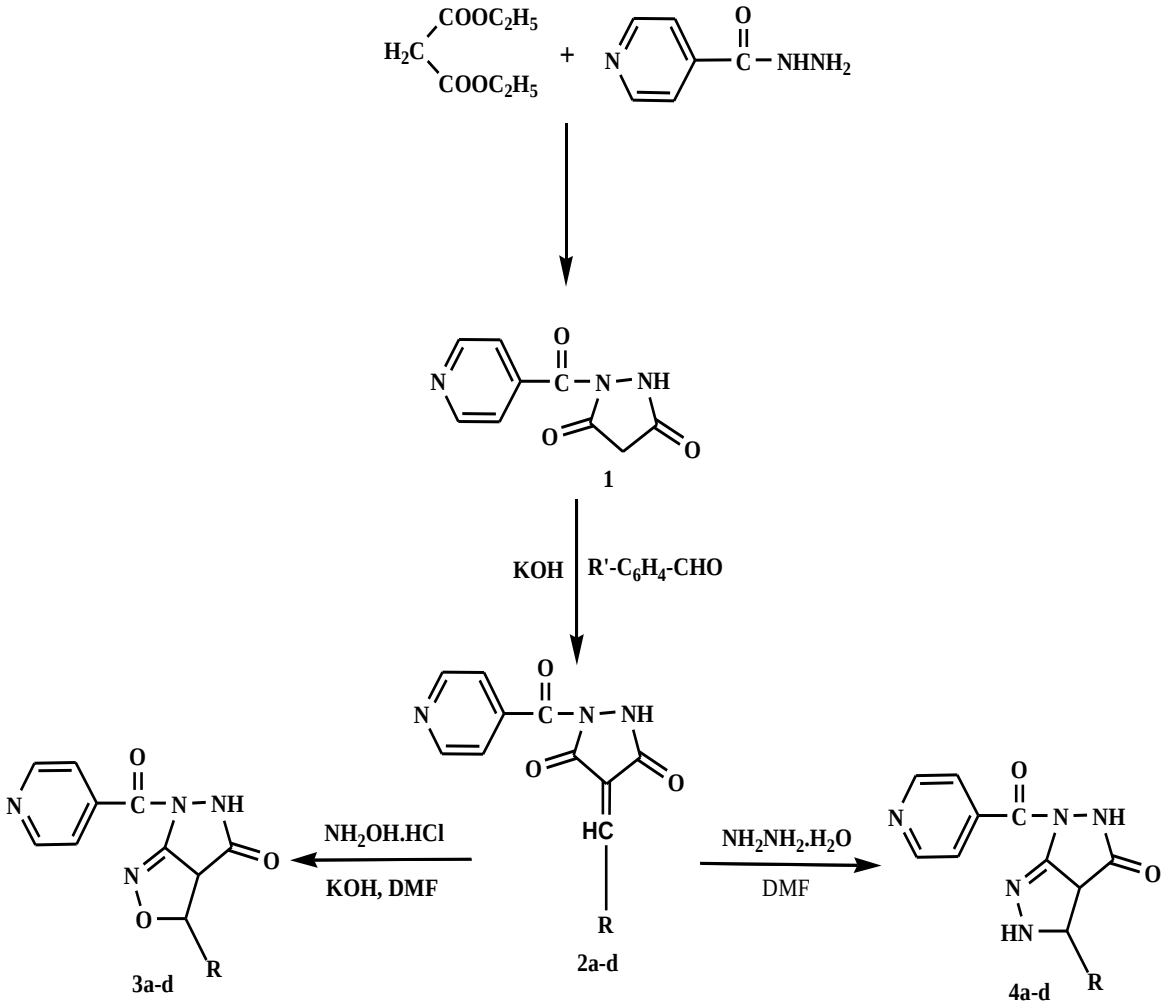
Microwave induced Synthesis of (3-methyl-4-(substituted phenyl)-3,3a,4,5-teterahydropyrazolo[3,4-c]pyrazol-1(2H)-yl)(pyridine-4-yl)methanone (4a-d)

Mixture of compound **2**(0.01 mol) and hydrazine hydrate (0.05 mol) in 30 ml of DMF were taken in an Erlenmeyer flask and mixed thoroughly. The well-stirred mixture was irradiated in microwave oven for 6-8 min at 600 W (i.e. 50 % microwave power). The completion of the reaction was monitored by TLC. The reaction mixture was then cooled at room temperatures and poured into crushed ice. The obtained solid was filtered, washed with water and recrystallized from ethanol. The purity of compounds was analyzed by TLC using hexane: ethyl acetate (7:3) as mobile phase.



R'= 4-OH, 4-Cl, 3-NO_2, 4-F.
R= 4-OH-C_6H_4, 4-Cl-C_6H_4, 3-NO_2-C_6H_4, 4-F-C_6H_4.

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Reaction Scheme: Synthesis of isoxazole and Pyrazole Compounds

Table 1: Physical data of synthesized compounds

Compds.	R	Mol formula	Mol Wt.	M.P. (°C)	CHN Calculated/Found	Yield (%)	Reaction Time (min)
1	---	$C_9H_7N_3O_3$	205	115-118	C:52.69/52.50 H:3.44/3.89 N:20.48/20.32	88%	6 min
2a	4-OH-C_6H_4	$C_{16}H_{11}N_3O_4$	309	190-192	C:62.14/65.78 H:3.58/3.34 N:13.59/13.53	82%	5 min
2b	4-Cl-C_6H_4	$C_{16}H_{10}ClN_3O_3$	327	18-183	C:58.64/58.7 H:3.01/3.11 N:12.82/12.76	74%	5 min
2c	3-NO_2-C_6H_4	$C_{16}H_{10}N_4O_5$	338	152-154	C:56.81/56.90 H:2.98/2.13 N:16.56/16.9	75%	5 min
2d	4-F-C_6H_4	$C_{17}H_{10}FN_3O_3$	311	165-167	C:61.74/61.46 H:3.24/3.05 N:13.50/13.8	82%	5 min
3a	4-OH-C_6H_4	$C_{16}H_{12}N_4O_4$	324	205-207	C:59.26/59.14 H:3.73/3.57 N:17.28/17.30	72%	7 min
3b	4-Cl-C_6H_4	$C_{16}H_{11}ClN_4O_3$	342	162-164	C:56.07/56.76 H:3.23/ 3.89 N:16.35/16.63	78%	8 min
3c	3-NO_2-C_6H_4	$C_{16}H_{11}N_5O_5$	353	194-196	C:54.39/54.83 H:3.14/3.30 N:19.82/19.76	69%	7 min
3d	4-F-C_6H_4	$C_{16}H_{11}FN_4O_3$	326	180-182	C:58.12/58.42 H:3.73/3.59 N:19.93/19.20	76%	7 min
4a	4-OH-C_6H_4	$C_{16}H_{13}N_5O_3$	323	203-205	C:59.44/59.42 H:4.05/4.78 N:21.66/21.88	80%	7 min
4b	4-Cl-C_6H_4	$C_{16}H_{12}ClN_5O_2$	341	187-189	C:56.23/56.92 H:3.54/3.22 N:20.49/20.98	85%	8 min
4c	3-NO_2-C_6H_4	$C_{16}H_{12}N_6O_4$	352	198-200	C:54.55/54.2 H:3.42/3.30 N:23.85/23.02	83%	7 min
4d	4-F-C_6H_4	$C_{16}H_{12}FN_5O_2$	325	182-185	C:59.08/59.39 H:3.72/3.53 N:21.53/21.76	76%	6 min

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Table 2: Spectral data of synthesized compounds

Compounds	IR(cm^{-1})	^{1}HNMR(400MHz,DMSO)δ ppm	^{13}CNMR δ ppm	MS (m/z)
1	1301(N-N),1606(C=O), 1510(C=N), 3250(Ar-CH str.), 2200(pyridine ring), 3300(N-H str), 1530(N-H bending), 1410(C=C str).	8.77-7.84 (m, 4H, pyridine), 8.34(1H, s, NH of Pyrazolidine), 2.40(CH_2), 3.12(CH).	148.7 (CH-pyridine), 121.3(CH pyridine), 141.4(pyridine), 172.9(C=O), 170.2(C=O), 32.8(CH_2),C_7- 40.9(CH).	205 (M^+), $[C_4H_3N_2O_3]^+$127, $[C_4H_3N_2O_2]^+$99 $[C_6H_4NO]^+$106 $[C_5H_5N]^+$79
2a	1209 (N-N), 1638(C=O), 1695 (C=N str.), 3082(C-H str., Ar-H), 2150(pyridine ring), 3310(N-H str), 1520(N-H bending), 3050(=C-H, SP^2), 1487 (aromatic ring str.), 3412 (OH).	8.76-7.83 (m, 4H, pyridine), 8.54(1H, s, NH of Pyrazolidine), 7.50(CH=), 3.45(CH-pyrazolidine), 6.45-6.83(m, 4H,Ar-H), 5.3(OH).	148.5(CH-pyridine), 121.7(CH-pyridine), 141.6(C-pyridine), 171.9(C=O), 131.7(C=O), 40.2(CH), 164.2(C=CH), 137.8(CH), 133.7-133.9(C-Ar), 126.5-129.4(CH-Ar).	309(M^+) $[C_{16}H_{15}N_3O_3]^+$293 $[C_{10}H_6N_3O_3]^+$216 $[C_{11}H_7N_2O_4]^+$231 $[C_{10}H_7N_2O_3]^+$203 $[C_5H_5N]^+$79 $[C_6H_4NO]^+$106
2b	1310 (N-N), 1720(C=O), 1685 (C=N str.), 3062(C-H str., Ar-H), 2210(pyridine ring), 3400(N-H str), 1620(N-H bending), 3150(=C-H, SP^2), 1470(aromatic ring str.), 662(Cl)	9.26-8.83(m, 4H, pyridine), 8.14(1H, s, NH of Pyrazolidine), 8.10(CH=), 3.45(CH-pyrazolidine), 6.85-7.23(m, 4H,Ar-H).	148.2-122.2(CH-pyridine), 140.2(C-pyridine), 172.9(C=O), 130.3(C=O), 39.2(CH), 163.2(C=CH), 137.9(CH), 133.8-129.4(CH-Ar).	329(M+2), 327(M^+) $[C_{16}H_{11}N_3O_3]^+$293 $[C_{10}H_6N_3O_3]^+$216 $[C_{11}H_6ClN_2O_3]^+$249 $[C_{10}H_6ClN_2O_2]^+$221 $[C_6H_4NO]^+$106 $[C_5H_5N]^+$79
2c	1220 (N-N), 3018 (C-H str., Ar-H), 1648 (C=O str.), 1615 (C=N str.), 1612(N-H bending), 3170(=C-H, SP^2),1484 (aromatic ring str.), 1553(C-NO_2).	8.57-7.77(m, 4H, pyridine), 8.15(1H, s, NH of Pyrazolidine), 7.56(CH=), 3.59(CH-pyrazolidine), 6.55-6.83(m, 4H,Ar-H).	149.5-121.9(CH-pyridine), 139.9(C-pyridine), 171.5(C=O), 130.2(C=O), 39.2(CH), 164.8(C=CH), 138.9(CH), 133.9-127.4(CH-Ar).	338(M^+) $[C_{16}H_{11}N_2O_3]^+$293 $[C_{10}H_6N_3O_3]^+$216 $[C_{11}H_6N_3O_5]^+$260 $[C_{10}H_6N_3O_4]^+$232 $[C_5H_5N]^+$79 $[C_6H_4NO]^+$106
2d	1232 (N-N), 3085 (C-H str., Ar-H), 1692 (C=O str.), 1622 (C=N str.), 1575(N-H bending), 3085(=C-H, SP^2), 1472(aromatic ring str.) 810 (C-F str.).	9.76- 6.85 (m, 4H, pyridine), 8.14(1H, s, NH of Pyrazolidine), 7.30(CH=), 3.45(CH-pyrazolidine), 7.38-6.93(m, 4H, Ar-H).	148.5-122.0(CH-pyridine), 141.2(C-pyridine), 173.2(C=O), 130.2(C=O), 38.0(CH), 164.0(C=CH), 138.8(CH), 133.7-128.4(CH-Ar).	311(M^+) $[C_{16}H_{11}N_3O_3]^+$293 $[C_{10}H_6FN_2O_2]^+$205 $[C_{10}H_6N_3O_3]^+$216 $[C_{11}H_6FN_2O_3]^+$233 $[C_5H_5N]^+$79 $[C_6H_4NO]^+$106
3a	1565 (C=C ring skeleton Ar. moiety), 1510 (N-H bending), 1685(C=O), 3215 (N-H str.), 3410 (OH), 1140(C-N str.), 1080 (C-O str.), 949 (N-O str.).	8.80-6.84(m, 4H, pyridine), 7.5(1H, s, NH of Pyrazolidine), 3.4(CH-pyrazolidine), 2.8(CH), 4.84(CH), 7.05-6.80(m, 4H, Ar-H), 5.2(OH), 4.73 (1H, d, Ar-CH isoxazoline ring).	148.0-123.9(CH-pyridine), 142.4(C-pyridine), 174.4(C=O), 162.5(C=N, pyrazole), 49.8(CH), 87.2(CH), 132.7(C-Ar), 129.3-116.2(CH-Ar), 156.5(C-Ar).	324(M^+), $[C_{16}H_{11}N_4O_3]^{+\cdot}$307 $[C_{10}H_7N_4O_3]^{+\cdot}$231, $[C_{10}H_8N_3O_3]^{+\cdot}$218 $[C_{11}H_8N_3O_4]^{+\cdot}$246′ $[C_5H_4N]^{+\cdot}$78 $[C_6H_4NO]^{+\cdot}$106

3b	3415 (N-H stretching), 1657 (C=O stretching), 749 (C-Cl), 1592(N-H bending), 1565(C=C), 1145(C-N str.), 1088 (C-O str.), 943 (N-O str.).	8.8-6.7 (m, 4H, pyridine), 7.8(1H, s, NH of Pyrazolidine), 3.6(CH-pyrazolidine), 2.6(CH), 4.5(CH), 7.8-6.4(m, 4H, Ar-H), 4.71 (1H, d, Ar-CH isoxazoline ring).	148.0-123.9(CH-pyridine), 142.4(C-pyridine), 174.4(C=O), 162.7(C=N, pyrazole), 49.4(CH), 87.4(CH), 132.7(C-Ar), 129.4-116.6(CH-Ar), 156.5(C-Ar).	344(M+2), 342(M^+), $[C_{16}H_{11}N_4O_3]^{+\cdot}$307 $[C_{10}H_7N_4O_3]^{+\cdot}$231, $[C_{10}H_9ClN_3O_2]^{+\cdot}$236 $[C_{11}H_7ClN_3O_3]^{+}$264, $[C_6H_4NO]^{+\cdot}$106 $[C_5H_5N]^{+\cdot}$78
3c	1560(C=C ring skeleton Ar. moiety), 1380 (NO_2), 1692 (C=O), 3213 (N-H str.), 1620(N-H bending), 1154(C-N str.), 1093 (C-O str.), 948 (N-O str.).	8.55-6.83 (m, 4H, pyridine), 7.9(1H, s, NH of Pyrazolidine), 3.95(CH-pyrazolidine), 2.74(CH), 4.71(CH), 8.4-6.9(m, 4H, Ar-H), 4.74 (1H, d, Ar-CH isoxazoline ring),	148.0-123.9(CH-pyridine), 142.9(C-pyridine), 174.7(C=O), 162.3(C=N, pyrazole), 49.4(CH), 87.2(CH), 132.6(C-Ar), 129.6-116.2(CH-Ar), 156.2(C-Ar).	353(M^+), $[C_{10}H_7N_4O_4]^{+\cdot}$247, $[C_{11}H_7N_4O_5]^{+\cdot}$275, $[C_{16}H_{11}N_4O_3]^{+\cdot}$307 $[C_{10}H_7N_4O_3]^{+\cdot}23[C_6H_4NO]^{+\cdot}$106 $[C_5H_5N]^{+\cdot}$78
3d	1560 (C=C), 1180 (F), 1585(N-H bending),1693 (C=O) , 3312 (N-H str.), 1144(C-N str.), 1091 (C-O str.), 940 (N-O str.).	1560 (C=C), 1180 (F), 1585(N-H bending),1693 (C=O) , 3312 (N-H str.), 1144(C-N str.), 4.70 (1H, d, Ar-CH isoxazoline ring).	148.0-123.9(CH-pyridine), 142.4(C-pyridine), 174.7(C=O), 162.7(C=N, pyrazole), 49.6(CH), 87.2(CH), 132.7(C-Ar), 129.3-116.2(CH-Ar), 156.5(C-Ar).	326(M^+), $[C_{16}H_{11}N_4O_3]^{+\cdot}$307 $[C_{10}H_7N_4O_3]^{+\cdot}$231, $[C_6H_4NO]^{+\cdot}$106 $[C_5H_5N]^{+\cdot}$78, $[C_{10}H_7FN_3O_2]^{+\cdot}$220 $[C_{11}H_7FN_3O_3]^{+\cdot}$248
4a	1564 (C=C ring skeleton Ar. moiety), 1512 (N-H bending), 1684(C=O), 3214 (N-H str.), 3412 (OH), 1149(C-N str.).	8.8-6.3m, 4H, pyridine), 7.8(1H, s, NH of Pyrazolidine), 3.73(CH-pyrazolidine), 2.5(CH), 4.76(CH), 8.8-6.9(m, 4H, Ar-H),	148.2-123.3(CH-pyridine), 142.6(C-pyridine), 174.8(C=O), 157.7(C=N, pyrazole), 42.3-50.2(CH), 132.7(C-Ar), 129.3-116.2(CH-Ar), 156.5(C-Ar).	323(M^+), $[C_{16}H_{13}N_5O_2]^{+\cdot}$307 $[C_{10}H_8N_5O_2]^{+\cdot}$230, $[C_{10}H_9N_4O_2]^{+\cdot}$217 $[C_{11}H_9N_4O_3]^{+\cdot}$245, $[C_6H_4NO]^{+\cdot}$106 $[C_5H_4N]^{+\cdot}$78
4b	3413 (N-H stretching), 1654 (C=O stretching), 746 (C-Cl), 1593(N-H bending), 1565(C=C), 1145(C-N str.).	8.8-6.8(m, 4H, pyridine), 7.5(1H, s, NH of Pyrazolidine), 3.74(CH-pyrazolidine), 2.6(CH), 4.76(CH), 8.8-6.9(m, 4H, Ar-H),	148.6-123.3(CH-pyridine), 142.5(C-pyridine), 174.8(C=O), 157.7(C=N, pyrazole), 42.5-50.2(CH),132.7(C-Ar), 129.6-116.2(CH-Ar), 156.7(C-Ar).	343(M+2), 341(M^+), $[C_{16}H_{13}N_5O_2]^{+\cdot}$307 $[C_{10}H_8N_5O_2]^{+\cdot}$230, $[C_{10}H_8ClN_4O]^{+\cdot}$235 $[C_{11}H_8ClN_4O_2]^{+\cdot}$263, $[C_6H_4NO]^{+\cdot}$106 $[C_5H_4N]^{+\cdot}$78
4c	1560(C=C ring skeleton Ar. moiety), 1380 (NO_2), 1692 (C=O), 3215 (N-H str.), 1618(N-H bending), 1154(C-N str.).	8.8-6.8(m, 4H, pyridine), 8.8(1H, s, NH of Pyrazolidine), 3.76(CH-pyrazolidine), 2.7(CH), 4.76(CH), 8.8-6.9(m, 4H, Ar-H),	148.3-123.9(CH-pyridine), 142.7(C-pyridine), 174.7(C=O), 157.5(C=N, pyrazole), 42.6-50.4(CH),132.6(C-Ar), 129.3-116.2(CH-Ar), 156.8(C-Ar).	352(M^+), $[C_{16}H_{13}N_5O_2]^{+\cdot}$307 $[C_{10}H_8N_5O_2]^{+\cdot}$230, $[C_{10}H_8N_5O_3]^{+\cdot}$246 $[C_{10}H_8N_5O_4]^{+\cdot}$274, $[C_6H_4NO]^{+\cdot}$106 $[C_5H_4N]^{+\cdot}$78

4d	1564(C=C), 1182 (F), 1584(N-H bending), 1695 (C=O), 3315 (N-H str.), 1146(C-N str.).	8.8-6.8(m, 4H, pyridine), 8.7(1H, s, NH of Pyrazolidine), 3.73(CH-pyrazolidine), 2.8(CH), 4.75(CH), 8.8-6.9(m, 4H, Ar-H),	148.5-123.5(CH-pyridine), 142.7(C-pyridine), 174.8(C=O), 157.3(C=N, pyrazole), 42.8-50.6(CH), 132.3(C-Ar), 129.3-116.7(CH-Ar), 156.5(C-Ar).	325(M^+), $[C_{16}H_{13}N_5O_2]^{+\cdot}$307 $[C_{10}H_8N_5O_2]^{+\cdot}$230, $[C_{10}H_8FN_4O]^{+\cdot}$219 $[C_{11}H_8FN_4O_2]^{+\cdot}$247, $[C_6H_4NO]^{+\cdot}$106 $[C_5H_4N]^{+\cdot}$78

Antimicrobial Activity

The compounds (4,5a-d) were tested for their antimicrobial activities against gram-positive and gram-negative bacterial and fungal strain. The resulting MIC (µg/ml) values are indicated in **Table 3**. It was observed that more than half compounds exhibited excellent activity in comparison to standards used, while the remaining were good and one or two of them poor in comparison to the standards. The standard used for antifungal activity was Greseofulvin and Amphicilin was used as a standard for antibacterial assay.

The newly synthesized compounds **(3,4a-d)** were screened for their antibacterial activity against gram-negative bacteria *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 441) and gram-positive bacteria *Staphylococcus aureus* (MTCC 96) and *Streptococcus pyogenes* (MTCC 442) and antifungal activity against *A. nigar* and *C. albicans.* The samples were tested by broth dilution method. The screening for antimicrobial activity was carried out by diluting the solution and preparing the sets consecutively from 1000, 500, 250, 200, 100, 50, 25, 12.5, 6.25 micro/ml. The highest dilution showing at least 99 % inhibition zone is taken as MIC. The result of this is much affected by the size of the inoculum. The test mixture should contain 10^8 organism/ml. Among all the synthesized derivatives **3c, 4c** and **4d** were exhibited the best MIC values. **3a, 4a** and **4c** showed equivalent activity to standard and rest of compounds were showed moderate to poor activity.

Table 3: Result of antibacterial and antifungal screening for compounds 3,4a-d)

Comp.	R	MIC(µg/ml)					
		Bacteria				Fungi	
		E. coli	*P. euroginosa*	*S. aureus*	*S.pyogenus*	*A. nigar*	*C. albicans*
3a	4-OH-C_6H_4	125	250	250	125	250	125
3b	4-Cl-C_6H_4	250	125	125	125	250	125
3c	3-NO_2-C_6H_4	62.5	125	62.5	125	500	250
3d	4-F-C_6H_4	125	125	100	62.5	500	500
4a	4-OH-C_6H_4	250	125	250	250	250	125
4b	4-Cl-C_6H_4	200	100	100	125	250	500
4c	3-NO_2-C_6H_4	100	62.5	200	200	125	100
4d	4-F-C_6H_4	62.5	100	125	100	500	
Amphicilin		100	----	250	100	----	----
Greseofulvin		----	----	----	----	500	100

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In fungal activity only **4c** was showed excellent activity against *A. nigar* and **3c, 3d** and **4d** showed equivalent activity to standard and rest of compounds showed moderate to poor activity against fungal standard.

RESULTS AND DISCUSSION

In the present investigation, Isonicotinohydrazide treated with diethyl malonate with acetic acid(2-3 drops) in DMF yielded 2-isonicotinoyl-5-methylpyrazolidine-3-one **(1)**, which is confirmed by the presence of a band at 1301 cm^{-1} due to (N-N), 3300 cm^{-1} due to (N-H str.) and 1530 cm^{-1} due to N-H bending of pyrazolidine in IR spectrum. 1H NMR of **(1)** showed broad singlet at 8.70 δ due to NH proton whereas signals of pyridine protons are in the region 8.77-7.84 δ (4 protons), a singlet at 2.40 δ due to two protons of $-CH_2-$ group supported the formation of the desired product. Mass spectrum show molecular ion peak at m/z 205. The compound **(1)** was irradiated with different aromatic Aldehydes to give Chalcones (4-(substituted benzylidene)-2-isonicotinoyl-5-methylpyrazolidine-3-one **(2a-d)**. Formation of compound **(2a-d)** is confirmed by the new singlet at 7.30-8.10 δ due arylidine proton (=CH-Ar) and disappearance of active $-CH_2-$ 2.40δ (s, 2H, CH_2), Multiplets for aromatic protons in the range 6.45-7.38δ for benzene and 9.76-6.85δ for pyridine were observed in ^{1}HNMR and appearance of bands at 3050-3170 of (=C-H) and 1470-1487 cm^{-1} of aromatic ring str. in IR region.

Compound **(2a-d)** was treated with hydroxylamine hydrochloride in the presence of KOH to give 3-substituted phenyl-4-methylhexahydro-6H-pyrazolo[3,4-c]isoxazol-6-yl](pyridine-4-yl)methanone-ethane **(3a-d)**. The structure of compound **(3a-d)** was confirmed by the appearance of a band due to C-N str. at 1140-1154 cm^{-1} and disappearance of band at 3050-3170cm^{-1} due to (=C-H) in IR spectrum. The structures of (**3a-d)** were confirmed by appearance of absorption band at 1080-1093 cm^{-1} due to C-O stretch and at 940-949 cm^{-1} due to N-O stretch in IR spectra. It is supported by the presence of two doublets of two methine protons of isoxazolino moiety at about 4.70-4.74 ppm in ^{1}H NMR.

In second pathway, Compound **(2a-d)** was reacted with hydrazine hydrate in presence to obtain (3-methyl-4-(substitutedphenyl)-3,3a,4,5-teterahydropyrazolo[3,4-c]pyrazol-1(2H)-yl)(pyridine-4-yl)methanone **(4a-d).** The formation of compounds **(4a-d)** was confirmed by the appearance of a band of C=N str. at 1145-1154 cm^{-1} in IR spectrum.

CONCLUSION

In conclusion, this work demonstrates a rapid, efficient and environment friendly method for the synthesis of excellent potentially bioactive isoxazole and pyrazole compounds in excellent yield under microwave irradiation. The results obtained confirm that the microwave assisted leads to considerable saving in reaction time and energetically profitable over the conventional method. All the synthesized compounds have shown excellent activity in bacterial and fungal studies. The biological activity of these compounds will trigger more interest in the

synthesis of such compounds from the easily available starting materials in eco-friendly manner.

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