Research Article

Microwave Assisted Facile Synthesis of some pyrimidine and isoxazole Derivatives and their Antimicrobial Activity

Kumudini Bhanat*[1], Bharat Parashar [2] and V. K. Sharma[1]

[1]Microwave Chemistry Laboratory, Department of Chemistry, Mohanlal Sukhadia University, Udaipur - 313 001 (Raj.), India
[2]Department of Pharmacy, M. B. University, Solan (H.P.), India.

Abstract
Synthesis of some pyrimidine and isoxazole derivatives has been carried out under microwave irradiation. The synthesis of various pyrimidine and isoxazole derivatives can be achieved from α, β- unsaturated ketones using microwave irradiation. The structures of the products were supported by IR, ^{1}HNMR, ^{13}CNMR and Mass spectral data. All these compounds have also been screened for their antimicrobial activity against bacteria *E.coli, S. aureus, P. aeruginosa* and *S. pyogenes* and fungi such as *C. albicans* and *A. niger*. Introduction of Cl, F, NO_2, and OH groups in the heterocyclic framework enhanced antibacterial and antifungal activities.

***Correspondence**
Kumudini Bhanat,
Department of Chemistry,
Mohanlal Sukhadia University
Email: kumudini23@gmail.com

Keywords: Microwave irradiation, Pyrimidine, Isoxazole, Chalcones, Antimicrobial activity.

Introduction

Organic synthesis, especially diversity-oriented synthesis, may likely to play a vital role in drug discovery in the future. However, the manufacture of fine chemicals and pharmaceuticals through traditional processing generates a lot of waste, the bulk of which consists of byproducts and inorganic salts. Hence, for cleaner production, waste minimization is essential which can be achieved using newer techniques. The use of microwave irradiation in organic synthesis has become increasingly popular within the pharmaceutical and academic arenas, because it is a new enabling technology for drug discovery and development. [1] Microwave assisted organic synthesis offers a cleaner and greener route, since higher yields are obtained in few minutes, leading to minimization of wastes.
Isonicotinohydrazide is reported as a well known drug.[2,3]Isonicotinohydrazide is one of the primary drugs used in combination with ethanbutol, rifampin, streptomycin and pyrazinamide to treat tuberculosis.[4] Despite the large number of compounds containing the isoniazid moiety which have already been synthesized and tested, there is still a need for new compounds of this kind, due to the increasing resistance of bacterial strains of certain type of antibiotics.[5]

The efficiency of pyrimidine and isoxazole as chemotherapeutic agent is well established and their chemistry has been extensively studied. Pyrimidines have been paid increasing attention, due to their various therapeutic and pharmacological properties, such as antiviral, antibacterial, antihypertensive, and antitumor effects. Isoxazoline derivatives have also been reported to possess antidiabetic, diuretic, analgesic, anthelimintic and hypolipaemic activity.[6] Generally, these compounds are prepared using α, β - unsaturated ketones as starting materials.
The α, β - unsaturated ketones have been attracting much more attention, particularly the α, β-unsaturated derivatives of cyclohexanone, not only due to their intriguing biological activities such as antiangiogenic [7,8], cytotoxicity [9,10], cholesterol - lowering activity [11], use in agrochemicals, pharmaceuticals and perfumes [12], and as liquid crystalline polymer units [13], but also as important precursors for the synthesis of many heterocyclic compounds.

Looking to the great-diversified role of pyrazole moieties as drugs, synthesis of some pyrazole derivatives have been carried out under microwave irradiation.

Experimental Section

Materials and Method: All reactions were carried out in a modified microwave oven (KENSTAR- OM-20DSP, 2450 Hz). Melting points are uncorrected and determined in open capillaries. Reactions were monitored by thin layer chromatography using silica gel-G as adsorbent using ethyl acetate: n-hexane (7:3) as eluent and products were detected by iodine vapors. IR spectra (KBr pellets) were recorded on Perkin-Elmer 1800 (FTIR) spectrometer. ^{1}H NMR spectra (DMSO-d6) were taken on a Bruker AVANCE II 400 NMR spectrometer using TMS as internal standard and chemical shift were expressed in δppm. Mass spectra were taken on a Jeol SX- 102/PA-6000 (EI) spectrometer. Elemental analysis was carried out on C, H, N analyzer (Elemental Vario Carlo Alba 1108), Anti-microbial activity has been carried out in Microcare Laboratory, Surat (Gujarat).

General Procedure for Microwave induced Synthesis of 3-amino-1-isonicotinoyl-1H-pyrazol-5(4H)-one (1): A mixture of Isonicotinohydrazide (0.01 mol) and Ethyl-2-cyanoacetate (0.05 mol), Acetic Acid (4-5drops) in ethanol (20 ml.) were taken in an Erlenmeyer flask and mixed thoroughly. The mixture was subjected to microwave irradiation at 600W for 7 min (Succesive irradiation of 30-40 sec. with cooling interval of time). On completion of reaction, indicated by TLC, The solvent was removed by evaporating the compound at the room temperature, solid thus obtained was dried and recrystallised from ethanol to afford pure product (1) in good yield.
Spectral Data

3-amino-1-isonicotinoyl-1H-pyrazol-5(4H)-one (1): M.P. 116-118°C (Found): C, 53.25; H, 4.24; N, 27.77; (calculated): C, 52.92; H, 3.95; N, 27.44; Mol. Formula: $C_9H_8N_4O_2$;

IR (KBr cm^{-1}): 1290 (N-N), 1574, 1556(C=N), 2200(pyridine ring), 1698, 1672(C=O), 1412(C=C str.), 3334, 3218(-NH2).

^{1}HNMR (400MHz, DMSO) δ ppm: 7.20-7.77 (m, 4H, pyridine); 5.12 (s, 2H, NH_2), 3.33 (s, 2H, CH_2).

^{13}CNMR ($CDCl_3$) δ: 149.6-121.7(CH-pyridine), 141.3(C-pyridine), 167.7(C=O), 162.7(C=O), 158.5(C-NH_2), 162.4(N=CH), 32.5(-CH_2).
Mass(m/z): $[M]^+$ 204

Microwave induced Synthesis of the Schiff bases 3-(substituted benzylideneamino)-1-isonicotinoyl-1H-pyrazol-5 (4H)-one (2): Mixture of 3-amino-1-isonicotinoyl-1H-pyrazol-5(4H)-one (0.01 mol) and different aromatic aldehydes (0.01 mol) and conc. HCl (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 solid thus obtained was dried and recrystallised from alcohol to yield compound The purity of compounds was analyzed by TLC using ethyl acetate: n-hexane (7: 3) as mobile phase.

M.P. 134-137°C (Found) C, 66.05; H, 4.44; N, 19.37; (calculated) C, 65.75; H, 4.14; N, 19.17; Mol. Formula: $C_{16}H_{12}N_4O_3$;
IR (KBr cm^{-1}): 1315 (N-N), 1638(C=O), 3310(N-H str), 2210(pyridine ring), 1483 (aromatic ring str.), 3082(C-H str. Ar-H), 3410 (OH).

^{1}HNMR (400MHz, DMSO) δ ppm: 7.55-8.01 (m, 4H, pyridine), 6.79-7.21 (m, 4H, Ar-H), 8.89 (s, 1H, N=CH-Ar), 10.35 (s, H, OH), 2.72 (s, 2H, CH_2, pyrazolidine); (s, 2H, CH_2).

^{13}CNMR ($CDCl_3$) δ: 148.0-121.7(CH-pyridine), 141.3(C-pyridine), 171.7(C=O), 165.7(C=O), 154.5(C-N), 162.4(N=CH), 32.5(-CH_2), 133.7-133.9(C-Ar), 115.5-129.4(CH-Ar).

Mass (m/z): $[M]^+$ 308

Microwave induced Synthesis of the Chalcones 4-(substituted benzylidene)-3-(4-hydroxybenzylideneamino)-1-isonicotinoyl-1H-pyrazol-5(4H)-one (3a-d)

The chalcones 3a-d were prepared as starting material to obtain the desired derivatives. To a Mixture of chalcone (3a-d) (0.01 mol), 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 reaction flask was irradiated in microwave oven for 5-6 min at 600 W (i.e. 50 % microwave power). Completion of the reaction was established by TLC using silica gel-G. After completion of the reaction, the reaction mixture was cooled at room temperatures and poured into crushed ice with constant stirring. The solid obtained 3a-d was filtered, washed with water and recrystallized from ethanol.

(4-(4-hydroxybenzylidene)-3-(4-hydroxybenzylideneamino)-1-isonicotinoyl-1H-pyrazol-5(4H)-one (3a):
M.P. 135-137°C (Found): C, 66.66; H, 4.38; N,13.52; (calculated): C, 66.25; H, 4.78; N, 13.34; Mol. Formula: $C_{23}H_{16}N_4O_4$
IR (KBr cm^{-1}): 1180(N-N), 1690(C=O str.), 3076 (C-H str., Ar-H), 1485 (aromatic ring str.), 3292(OH), 3052(=C-H, SP^2).

^{1}HNMR (400MHz, DMSO) δ ppm): 7.50-7.83 (m, 4H, pyridine), 6.90-7.30 (m,4H, Ar-H), 8.63(s, 1H, N=CH-Ar), 7.3(=C-H), 5.4 (s, H, OH).

^{13}CNMR ($CDCl_3$) δ: 148.5-121.7(CH-pyridine), 141.6(C-pyridine), 171.9(C=O), 165.7(C=O), 154.2(C-N), 162.4(N=CH), 40.2(CH), 127.2(C=CH), 144.8(C=CH), 133.7-133.9(C-Ar), 115.5-129.4(CH-Ar).

Mass (m/z): $[M]^+$ 412

(4-(4-cholrobenzylidene)-3-(4-hydroxybenzylideneamino)-1-isonicotinoyl-1H-pyrazol-5(4H)-one (3b):
M.P. 92-94 (Found): C, 62.82; H, 3.96; N,12.94; (calculated): C, 62.76; H, 3.56; N, 12.23; Mol. Formula: $C_{23}H_{15}ClN_4O_3$
IR (KBr cm^{-1}): 1176(N-N), 1672(C=O str.), 3055 (C-H str., Ar-H), 1453(aromatic ring str.), 3291(OH), 765 (C-Cl), 1152(C-N str.), 3155(=C-H, SP^2).

^{1}HNMR (400MHz, DMSO) δ ppm: 7.70-8.40 (m, 4H, pyridine), 6.90-7.30 (m, 4H, Ar-H), 8.5(s, 1H, N=CH-Ar), 7.1(=C-H), 5.9(s, H, OH).

^{13}CNMR ($CDCl_3$) δ: 148.9-121.7(CH-pyridine), 141.6(C-pyridine), 171.4(C=O), 165.6(C=O), 154.9(C-N), 162.7(N=CH), 40.8(CH), 127.5(C=CH), 144.6(C=CH), 133.7-133.9(C-Ar), 115.5-129.4(CH-Ar).

Mass (m/z): 432[M+2], $[M]^+$ 430

(4-(3-nitrobenzylidene)-3-(4-hydroxybenzylideneamino)-1-isonicotinoyl-1H-pyrazol-5(4H)-one (3c):
M.P. 90-92°C (Found): C, 62.30; H, 3.86; N, 15.79; (calculated): C, 62.80; H, 3.46;N, 15.73; Mol. Formula: $C_{23}H_{15}N_5O_5$
IR (KBr cm^{-1}): 1173(N-N), 1675(C=O str.), 3091 (C-H str., Ar-H), 3045 (C-H str., Ar-H), 1540 (NO_2), 3296(OH), 1145(C-N str.), 3177(=C-H, SP^2).

^{1}HNMR (400MHz, DMSO) δ ppm: 7.88-8.83 (m, 4H, pyridine), 6.80-7.50 (m,4H,Ar-H), 8.4(s, 1H, N=CH-Ar), 7.4(=C-H), 5.7 (s, H, OH).

^{13}CNMR ($CDCl_3$) δ: 148.4-121.2(CH-pyridine), 141.3(C-pyridine), 171.5(C=O), 165.8(C=O), 154.7(C-N), 162.5(N=CH), 40.6(CH), 127.8(C=CH), 144.4(C=CH), 133.7-133.9(C-Ar), 115.5-129.4(CH-Ar).

Mass (m/z): $[M]^+$ 441

(4-(4-flourobenzylidene)-3-(4-hydroxybenzylideneamino)-1-isonicotinoyl-1H-pyrazol-5(4H)-one (3d):
M.P. 123-125°C (Found): C, 66.34; H, 4.11; N, 13.45; (calculated): C, 66.55; H, 4.29; N, 13.12; Mol. Formula: $C_{23}H_{15}FN_4O_3$

IR (KBr cm^{-1}): 1160 (N-N), 1680(C=O str.), 3073(C-H str., Ar-H), 3176(Ar-CH), 3297(OH), 1180(C-F), 1145(C-N str.), 3089(=C-H, SP^2).

^{1}HNMR (400MHz, DMSO)δ ppm: 7.67-7.83 (m, 4H, pyridine), 6.95-7.67 (m, 4H, Ar-H), 8.9(s, 1H, N=CH-Ar), 7.9(=C-H), 5.4(s, 1H, OH).

^{13}CNMR ($CDCl_3$) δ: 148.2-121.5(CH-pyridine), 141.7(C-pyridine), 171.3(C=O), 165.4(C=O), 154.6(C-N), 162.8(N=CH), 40.7(CH), 127.8(C=CH), 144.5(C=CH), 133.7-133.9(C-Ar), 115.5-129.4(CH-Ar).

Mass (m/z): $[M]^+$ 414

Microwave induced Synthesis of (3-(substituted benzylideneamino)-4-(4-hydroxyphenyl)-6-thioxo-3a,4,5,6-tetrahydropyrazolo[3,4-d]pyrimidine-1-yl)(pyridine-4-yl)methanone (4a-d): Mixture of compound **3a-d**(0.01 mol) and thiourea (0.01 mol) with KOH (2-3 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 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 ethyl acetate: n-hexane (7: 3) as mobile phase.

(3-(4-hydroxybenzylideneamino)-4-(4-hydroxyphenyl)-6-thioxo-3a,4,5,6-tetrahydropyrazolo[3,4-d]pyrimidine-1-yl)(pyridine-4-yl)methanone) (4a):
M.P. 203-205°C (Found): C, 61.00; H, 4.27; N, 17.79; (calculated): C, 60.86; H, 4.59;N, 17.50; Mol. Formula: $C_{24}H_{19}N_6O_3S$
IR (KBr cm^{-1}): 1162 (N-N), 1687(C=O str.), 3079(C-H str., Ar-H), 3175(Ar-CH), 3297(OH), 1636(C=N str.) 1556 (C=C ring skeleton Ar. moiety), 1270(C=S).

^{1}HNMR (400MHz, DMSO) δ ppm: 9.18-7.80(m, 4H, Ar-H, pyridine), 7.5(1H, s, N=CH), 6.5(1H, s, NH-pyrimidine), 2.3-3.5(CH) 4.80(CH), 8.08-6.93(m, 4H, Ar-H), 5.8(1H, s, OH).

^{13}CNMR ($CDCl_3$) δ: 148.2-121.5(CH-pyridine), 141.7(C-pyridine), 171.3(C=O), 163.9(C=N) 154.6(C-N), 162.8(N=CH), 186.3(C=S) 40.7(CH), 133.7-133.9(C-Ar), 115.5-129.4(CH-Ar).

Mass (m/z): $[M]^+$ 470

(3-(4-chlorobenzylideneamino)-4-(4-hydroxyphenyl)-6-thioxo-3a,4,5,6 tetrahydropyrazolo[3,4-d]pyrimidine-1-yl)(pyridine-4-yl) methanone (4b):
M.P. 107-109°C (Found): C, 58.71; H, 3.90; N, 17.12; (calculated): C, 58.15; H, 3.33; N, 17.30; Mol. Formula: $C_{24}H_{17}ClN_6O_2S$

IR (KBr cm^{-1}): 1168 (N-N), 1680(C=O str.), 3074(C-H str., Ar-H), 3170(Ar-CH), 3310(OH), 1638(C=N str.), 1560(C=C), 747 (C-Cl), 1275(C=S).

^{1}HNMR (400MHz, DMSO) δ ppm: 9.17-7.84(m, 4H, Ar-H, pyridine), 7.45(1H, s, N=CH), 6.4(1H, s, NH-pyrimidine), 2.6-3.5(CH) 4.80(CH), 8.18-6.94(m, 4H, Ar-H), 5.6(1H, s, OH).

^{13}CNMR ($CDCl_3$) δ: 148.6-121.8(CH-pyridine), 141.7(C-pyridine), 171.3(C=O), 163.5(C=N) 154.8(C-N), 162.3(N=CH), 186.9(C=S) 40.5(CH), 133.6-133.8(C-Ar), 115.0-129.2(CH-Ar).

Mass (m/z): 490[M+2], $[M]^+$ 488

(3-(3-nitrobenzylideneamino)-4-(4-hydroxyphenyl)-6-thioxo-3a,4,5,6-tetrahydropyrazolo[3,4-d]pyrimidine-1-yl)(pyridine-4-yl) methanone (4c):
M.P. 178-180°C (Found): C, 57.48; H, 3.82/; N, 19.55/; (calculated): C, 57.05; H, 3.10; N, 19.20; Mol. Formula: $C_{24}H_{17}N_7O_4S$
IR (KBr cm^{-1}): 1160 (N-N), 1682(C=O str.), 3078(C-H str., Ar-H), 3178(Ar-CH), 3250(OH), 1149(C-N str.), 1565 (C=C ring skeleton Ar. moiety), 1135(C=N str.), 1386 (NO_2), 1272(C=S).

^{1}HNMR (400MHz, DMSO) δ ppm: 9.11-7.80(m, 4H, Ar-H, pyridine), 7.5(1H, N=CH), 6.2(1H, s, NH-pyrimidine), 2.2-3.5(CH) 4.80(CH), 8.38-6.93(m, 4H, Ar-H), 5.6(1H, s, OH).

^{13}CNMR ($CDCl_3$) δ: 148.0-121.0(CH-pyridine), 141.5(C-pyridine), 171.9(C=O), 163.4(C=N) 154.1(C-N), 162.7(N=CH), 186.2(C=S) 40.4(CH), 133.6-133.8(C-Ar), 115.6-129.2(CH-Ar).

Mass (m/z): $[M]^+$ 499

(3-(4-flourobenzylideneamino)-4-(4-hydroxyphenyl)-6-thioxo-3a,4,5,6-tetrahydropyrazolo[3,4-d]pyrimidine-1-yl)(pyridine-4-yl)methanone (4d):
M.P. 193-195°C (Found): C, 60.75; H, 4.04; N, 17.71; (calculated): C, 60.2; H, 4.80;N, 17.23; Mol. Formula: $C_{24}H_{17}FN_6O_2S$
IR (KBr cm^{-1}): 1166 (N-N), 1684(C=O str.), 3074(C-H str., Ar-H), 3178(Ar-CH), 3290(OH), 1139(C=N str.), 1568 (C=C ring skeleton Ar. moiety), 1140(C-N str.), 1180 (F), 1270(C=S).

^{1}HNMR (400MHz, DMSO) δ ppm: 79.15-7.70(m, 4H, Ar-H, pyridine), 7.8(1H, N=CH), 6.8(1H, s, NH-pyrimidine), 2.7-3.9(CH) 4.80(CH), 8.87-6.90(m, 4H, Ar-H), 5.6(1H, s, OH).

^{13}CNMR ($CDCl_3$) δ: 148.3-121.8(CH-pyridine), 141.5(C-pyridine), 171.2(C=O), 163.9(C=N) 154.7(C-N), 162.8(N=CH), 186.8(C=S) 40.1(CH), 133.2-133.4(C-Ar), 115.5-129.5(CH-Ar).

Mass (m/z): $[M]^+$ 472

Microwave induced Synthesis of (3-(substituted benzylideneamino)-4-(4-hydroxyphenyl)-3,3a-dihydropyazolo[3,4-c]isoxazole-6-yl)(pyridine-4-yl)methanone (5a-d): Mixture of compound **3a-d**(0.01 mol) and hydroxylamine hydrochloride (0.05 mol) with KOH (2-3 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 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 ethyl acetate: n-hexane (7: 3) as mobile phase.

(3-(4-hydroxybenzylideneamino)-4-(4-hydroxyphenyl)-3,3a-dihydropyazolo[3,4-c]isoxazole-6-yl)(pyridine-4-yl)methanone (5a):
M.P. 205-207°C (Found): C, 64.33; H, 4.46; N, 16.31; (calculated): C, 64.87; H, 4.86; N, 16.82; Mol. Formula: $C_{23}H_{17}N_5O_4$
IR (KBr cm^{-1}): 1171 (N-N), 1680(C=O str.), 3082(C-H str., Ar-H), 3168(Ar-CH), 3415(OH), 1142(C-N str.) 1550 (C=C ring skeleton Ar. moiety),1568 (C=C ring skeleton Ar. moiety), 1648(C=N str.), 1091 (C-O str.), 943 (N-O str.).

^{1}HNMR (400MHz, DMSO) δ ppm: 9.15-7.70(m, 4H, Ar-H, pyridine), 7.8(1H, N=CH), 2.7-3.9(CH) 4.80(CH), 8.87-6.90(m, 4H, Ar-H), 5.3(1H, s, OH), 4.72 (1H, d, Ar-CH isoxazoline ring)

^{13}CNMR ($CDCl_3$) δ: 150.3-120.8(CH-pyridine), 139.8(C-pyridine), 169.3(C=O), 163.4(C=N) 154.5(C-N), 162.3(N=CH), 40.3(CH), 133.7-133.5(C-Ar), 115.5-129.3(CH-Ar).

Mass (m/z): $[M]^+$ 427

(3-(4-chlorobenzylideneamino)-4-(4-hydroxyphenyl)-3,3a-dihydropyazolo[3,4-c]isoxazole-6-yl)(pyridine-4-yl) methanone (5b):
M.P. 105-107°C (Found): C, 61.68; H, 4.05; N, 15.64; (calculated): C, 61.21; H, 3.98; N, 14.08; Mol. Formula: $C_{23}H_{16}ClN_5O_3$
IR (KBr cm^{-1}): 1158 (N-N), 1669(C=O str.), 3088(C-H str., Ar-H), 3164(Ar-CH), 3410(OH), 1157(C-N str.), 1563(C=C ring skeleton Ar. moiety), 1645(C=N str.), 750(C-Cl), 1083 (C-O str.), 936 (N-O str.).

^{1}HNMR (400MHz, DMSO) δ ppm: 9.11-7.72(m, 4H, Ar-H, pyridine), 7.8(1H, N=CH), 2.7-3.9(CH) 4.82(CH), 8.83-6.90(m, 4H, Ar-H), 5.4(1H, s, OH), 4.78 (1H, d, Ar-CH isoxazoline ring),

^{13}CNMR ($CDCl_3$) δ: 150.1-120.8(CH-pyridine), 139.8(C-pyridine), 169.3(C=O), 163.5(C=N) 154.3(C-N), 162.5(N=CH), 40.2(CH), 133.7-133.4(C-Ar), 115.5-129.3(CH-Ar).

Mass (m/z): 447[M+2], $[M]^+$ 445

(3-(4-nitrobenzylideneamino)-4-(4-hydroxyphenyl)-3,3a-dihydropyazolo[3,4-c]isoxazole-6-yl)(pyridine-4-yl)methanone (5c):
M.P. 195-197°C (Found): C, 60.26; H, 3.96; N, 18.33; (calculated): C, 59.32; H, 3.03; N, 18.96; Mol. Formula: $C_{23}H_{16}N_6O_5$
IR (KBr cm^{-1}): 1153 (N-N), 1668(C=O str.), 3083(C-H str., Ar-H), 3163(Ar-CH), 3415(OH), 1155(C-N str.), 1560 (C=C ring skeleton Ar. moiety), 1640(C=N str.), 1388 (NO_2), 1080 (C-O str.), 930 (N-O str.)

^{1}HNMR (400MHz, DMSO) δ ppm: 9.15-7.77(m, 4H, Ar-H, pyridine), 7.8(1H, N=CH), 2.2-3.9(CH) 4.80(CH), 8.87-6.90(m, 4H, Ar-H), 5.6(1H, s, OH), 4.81 (1H, d, Ar-CH isoxazoline ring),

^{13}CNMR ($CDCl_3$) δ: 150.4-120.5(CH-pyridine), 139.2(C-pyridine), 169.4(C=O), 163.7(C=N) 154.8(C-N), 162.5(N=CH), 40.1(CH), 133.7-133.6(C-Ar), 115.5-129.5(CH-Ar).

Mass (m/z): $[M]^+$ 456

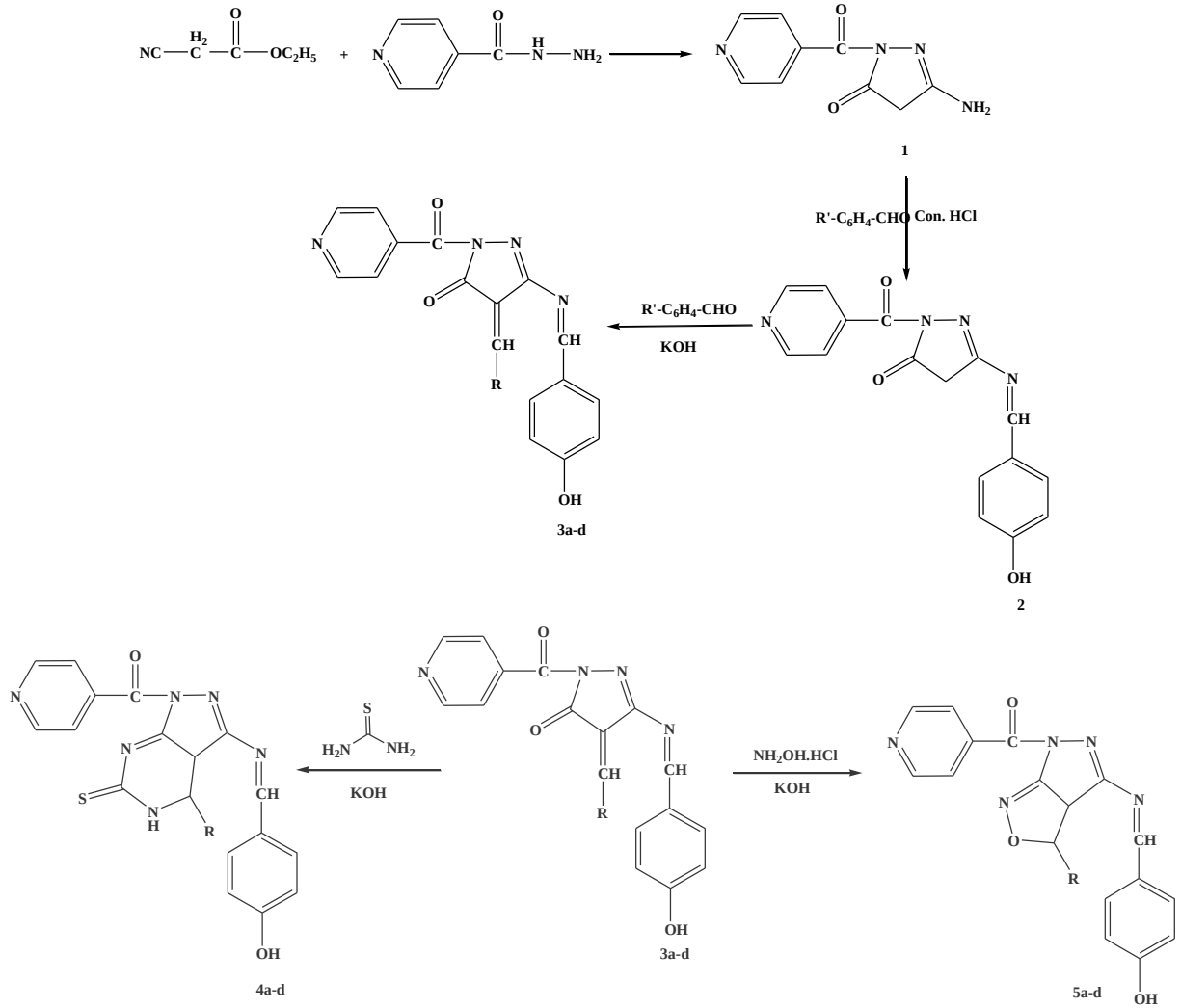
(3-(4-flourobenzylideneamino)-4-(4-hydroxyphenyl)-3,3a-dihydropyazolo[3,4-c]isoxazole-6-yl)(pyridine-4-yl)methanone (5d):
M.P. 178-179°C (Found): C, 64.03; H, 4.21; N, 16.23; (calculated): C, 66.97; H, 4.85; N, 16.57; Mol. Formula: $C_{23}H_{16}FN_5O_3$

IR (KBr cm^{-1}): 1155 (N-N), 1663(C=O str.), 3082(C-H str., Ar-H), 3169(Ar-CH), 3412(OH), 1155(C-N str.), 1568(C=C ring skeleton Ar. moiety), 1650(C=N str.), 1184 (F), 1082 (C-O str.), 934(N-O str.).

^{1}HNMR (400MHz, DMSO) δ ppm: 9.11-7.73(m, 4H, Ar-H, pyridine), 7.5(1H, N=C<u>H</u>), 2.5-3.9(CH) 4.84(CH), 8.87-6.90(m, 4H, Ar-H), 5.7(OH), 4.84 (1H, d, Ar-CH isoxazoline ring)

^{13}CNMR ($CDCl_3$) δ: 150.7-120.1(<u>C</u>H-pyridine), 139.1(<u>C</u>-pyridine), 169.3(<u>C</u>=O), 163.4(<u>C</u>=N) 154.5(<u>C</u>-N), 162.2(N=<u>C</u>H), 40.3(<u>C</u>H), 133.7-133.4(<u>C</u>-Ar), 115.5-129.3(<u>C</u>H-Ar).

Mass (m/z): $[M]^{+}$ 429



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.

Reaction Scheme: Synthesis of pyrimidine and isoxazole derivatives

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 1**. 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.

Table1 Result of antibacterial and antifungal screening for compounds (4,5a-d)

Compounds	R	MIC(µg/ml) Bacteria				Fungi	
		E. coli	*P. euroginosa*	*S. aureus*	*S.pyogenus*	*A. nigar*	*C.albicans*
4a	4-OH-C_6H_4	125	250	250	125	250	125
4b	4-Cl-C_6H_4	250	125	125	125	250	125
4c	3-NO_2-C_6H_4	62.5	125	62.5	125	500	250
4d	4-F-C_6H_4	125	125	100	62.5	500	500
5a	4-OH-C_6H_4	250	125	250	250	250	125
5b	4-Cl-C_6H_4	200	100	100	125	250	500
5c	3-NO_2-C_6H_4	100	62.5	200	200	125	100
5d	4-F-C_6H_4	62.5	100	125	100	500	100
Amphicilin		100	----	250	100	----	----
Greseofulvin		----	----	----	----	500	100

The newly synthesized compounds **(4,5a-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 **4c, 5c** and **5d** were exhibited the best MIC values. **4a, 5a** and **5c** showed equivalent activity to standard and rest of compounds were showed moderate to poor activity.

In fungal activity only **5c** was showed excellent activity against *A. nigar* and **4c, 4d** and **5d** showed equivalent activity to standard and rest of compounds showed moderate to poor activity against fungal standard.

Result and Discussion

3-amino-1-isonicotinoyl-1H-pyrazol-5(4H)-one was synthesized by condensation of Isonicotinohydrazide and Ethyl-2-cyanoacetate in acetic acid and microwave exposure. The IR spectrum of **(1)** showed absorptions at 3218 cm-1 (NH_2 group) and 1698, 1672 (C=O), 1574, 1556 (C=N), 1H NMR of **(1)** showed broad singlet at 5.12 δ due to NH_2 protons whereas signals of pyridine protons are in the region 7.20-7.77 δ (4 protons), a singlet at 3.33 δ due to two protons of -CH2- group supported the formation of the desired product. Mass spectrum showed molecular ion peak at m/z 204.

When 3-amino-1-isonicotinoyl-1H-pyrazol-5 (4H)-one **(1)** was condensed with substituted benzaldehydes and a catalytic amount of acetic acid in DMF under microwave irradiation, product 3-(substituted benzylidene amino)-1-isonicotinoyl-1H-pyrazol-5 (4H)-one **(2)** were obtained in good yields. IR, 1H NMR and mass spectral data have characterized these compounds. ^{1}H NMR showed broad singlet at 8.89 due to proton N=CH-Ar and at 2.72 for CH_2 protons. Multiplets for aromatic protons in the range 6.79-7.21 for benzene and 7.55-8.01 for pyridine were observed. The compound **(2)** possesses an active methylene group, which undergoes condensation with various aromatic aldehydes in the presence of KOH to give 4-(substituted benzylidene)-3-(4-hydroxybenzylideneamino)-1-isonicotinoyl-1H-pyrazol-5(4H)-one **(3a-d)**. The IR spectra of compounds **(3a-d)** show the presence of band of arylidine proton (=CH-Ar) of chalcone moiety also appeared at 3072-3177 cm^{-1}. ^{1}H NMR spectra of compounds **(3a-d)** were characterized by appearance of a new singlet at 7.1-7.9 ppm due to arylidine proton (=CH-Ar) of chalcone moiety.

Formation of pyrimidine derivatives **(4a-d)** by the reaction of chalcones and thiourea takes place in basic medium using DMF as solvent. Structures of compounds **(4a-d)** were elucidated by IR, 1H NMR. Their spectra showed a strong band for the C=S (1270-1275 cm^{-1}) and a band at (1635 - 1639 cm - 1) for C=N str. in IR spectra. It is supported by the presence of 1 NH protons in the region of 6.2-6.8 ppm in ^{1}H NMR spectrum.

Chalcone derivatives **(3a-d)** were allowed to react with hydroxylamine hydrochloride in the presence of KOH as a catalyst to afford isoxazole derivatives (**5a-d)** with excellent yields.The structures of (**5a-d)** were confirmed by appearance of absorption band at 1080-1091 cm^{-1} due to C-O stretch and at 930-943 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 4.72-4.84 ppm in ^{1}H NMR.

Conclusion

Synthesis of pyrimidine and isoxazole derivatives using microwave irradiation technique found efficient and time saving. In the present research work, we have synthesized some pyrimidine and isoxazole derivatives as microorganism growth inhibitor. The antibacterial and antifungal data displayed significant activity of synthesized compounds. Some of the derivatives show higher bacterial and fungal growth inhibition. It can be concluded that there is wide scope in developing this compounds as potent lead molecule.

References

[1] N. Singh, S. S. Kshirsagar, H. M. Nimje, P. S. Chaudhari, J. P. Bayas , R. J. Oswal, *Int. J. Pharm. Pharm. Sci.*, **2011,** 3(1), 109.
[2] L. Mc Neill, M. Allen, C. Estrada, P. Cook, *Chest,* **2003**, 123(1), 102.
[3] R. M. Jasmer, J. J. Sankonnen, H. M. Blumberg, C. L. Daley, J. Bemardo, E. Vittinaghoff, *Ann. Intern. Med.*, **2002,** 137, 640.

[4] World Health Organization: Geneva; WHO Global Tuberculosis Programme, 1997.
[5] S. Sakkarapandi, V. Revathi, R. Kalaiselvi, J. Balamurugan, M. Sevukarajan, *Ind. J. Heterocycl. Chem.*, **2002**, 11, 327.
[6] A. Wasi, B. K. Sharma, A. K. Gupta, K. Intodia, *J. Chem. Biol. Phy. Sci.*, **2013,** 3(4), 2505.
[7] T. P. Robinson, T. Ehlers, R. B. Hubbard, X. Bai, J. L. Arbiser, D. J. Goldsmith, J. P. Bowena, *Bioorg. Med. Chem. Lett.*, **2003,** 13, 115.
[8] T. P. Robinson, R. B. Hubbard, T. J. Ehlers, J. L. Arbiser, D. J. Goldsmith, J. P. Bowena, *Bioorg. Med. Chem.*, **2005**, 13, 4007.
[9] J. R. Dimmock, M. P. Padmanilayam, G. A. Zello, K. H. Nienaber, T. M. Allen, C. L. Santos, *Eur. J. Med. Chem.*, **2003**, 38, 169 - 177.
[10] Modzelewska, C. Pettit, G. Achanta, N. E. Davidson, P. Huang, S. R. Khan, *Bioorg. Med. Chem.*, **2006**, 14, 3491.
[11] I. H. Piantadosi, J. L. Hall, Irvine, G. L. Carlson, *J. Med. Chem.*, **1973**, 16, 770.
[12] M. Ogawa, Y. Ishii, T. Nakano, S. Irifune, Jpn. Kohai Tokyo JP., 63238034 A2 1988
[13] K. K.Gangadhara, *Polymer*, **1995**, 36, 1903.

Publication History
Received 27th Feb 2014
Revised 10th Mar 2014
Accepted 18th Mar 2014
Online 03rd Apr 2014