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Synthesis of some Novel 2, 5- Disubstituted 1, 3, 4-Oxadiazole and its Analgesic, Anti-Inflammatory, Anti-Bacterial and Anti-Tubercular Activity

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Abstract: Research on 1, 3, 4-oxadiazole and their synthetic analogs have revealed a variety of pharmacological activities including anti-microbial, anti-tubercular and insecticidal agents. Some of these compounds have also analgesic ,anti-inflammatory, anti-cancer,anti-HIV agent,anti-parkinsonian and anti-priliferative agent,It was our interested to make novel derivatives of the titled compounds and evaluate the anti-bacterial, analgesic,anti-inflammatory and anti-tubercular activities.1,3,4-oxadiazole and its derivatives **(3a-3e)** were obtained from the intermediate pyridine-4-carbohydrazide **(I)** from which schiffs base were obtained on treatment with various aromatic aldehyde ,further on condensation with acetic anhydride produced the title compounds.The remaining derivatives **(4f-4j,5,6)** were also obtained from the same intermediate pyridine-4-carbohydrazide **(I)** by condensation with different cyclizing reagent like phosphoryl chloride. The structures of the compounds were confirmed by IR, ^{1}H NMR, MASS spectral data.All the synthesized compounds shown to significant analgesic, anti-inflammatory,anti-bacterial and anti-tubercular activities.But compound **4g** and **4j** was found to possess better activity then others.Structure activity relationship and mass fregmentation has also been studied.

Key word: 1, 3, 4-Oxadiazole, Schiffs base, Analgesic, Anti-Inflammatory,Anti-Bacterial and Anti-Tubercular Activity.

Introduction

Due to the interesting activity of 2, 5-disubstituted 1, 3, 4-oxadiazole as biological agent's considerable attention has been focused on this class. The pharmaceutical importance of these compounds lies in the fact that they can be effectively utilizing as antibacterial, antitubercular and insecticidal agents [1, 2, 3, 4]. Some of these compounds have also analgesic, antiinflammatory, anticancer,anti-HIV agent, anti parkinsonian and antipriliferative agent [5,6,7,8,9]. In addition, 1,3,4-oxadiazole have played a crucial part in the development of theory in heterocyclic chemistry and also used extensively in organic synthesis [10,11].

Among the methods employed in synthesis of 1,3,4-oxadiazole, condensation of hydrazide and its derivatives with variety of substituted acids and bases are commonly used [12,13]. 2,5-disubstituted 1,3,4-oxadiazole can be conveniently synthesized by the treatment of pyridine-4-carbohydrazide with different acids and bases and carbon disulfide in basic and acidic media [14,15]. In this method, pyridine-4-carbohydrazide are formed as intermediates, which can be subsequently cyclized to 2,5-disubstituted 1,3,4-oxadiazole in the presence of a suitable cyclizing reagent like phosphoryl chloride [16,17,18].

As evident from the literature, in recent years a significant portion of research work in heterocyclic chemistry has been devoted to 1,3,4-oxadiazole containing different aryl groups as substituents. Reddy, *et al*.,Cyclization by dehydrosulphurization of 1-(-aminobenzoyl)-4-aryl-3-thiosemicarbazide, formation of 1,3,4-benzotriazepinones, 1,3,4-thiadiazolea & 1,3,4-oxadiazoles [19]. Madhukar S.chande, *et al*., Carbon oxysulfide: A novel reagent for the synthesis of 4-amino/aryl//aryloxymethyl/thiophenoxymethyl, 1,2,4-thriazolin-5-ones-5-arylamino-2-mercapto-1,3,4-oxadiazole[20]. Xing-Ping Hui, *et al*.,Synthesis and antibacterial activity of 1,3,4 thiadiazole, 1,3,4-oxadiazole, 1,2,4-triazole derivative of 5-methylisoxazole[21]. Mogilaiah, *et al*.,Hypervalent iodine mediated solid state synthesis of 1,8-naphthyridinyl-1,3,4-oxadiazoles. Synthesized compounds were screened for their antimicrobial activity group**.** [22].

According to a literature survey, it was noted that very little research has been carried out regarding 1,3,4-oxadiazole carrying pyridine/substituted pyridine groups as substituents on 1,3,4-oxadiazole ring. In this area Liszkiewicz have recently reported the synthesis and anti-proliferative activity in vitro of new 5-(-2-amino-3-pyridyl)-2-thixo-3H-1,3,4-oxadiazole derivative.[28]

Therefore, as a part of our program focused on 1,3,4-oxadiazole with biological activity, and in connection with our interest in the chemistry of 2,5-disubstituted 1,3,4-oxadiazole.In this paper we report the synthesis of some novel 2, 5- disubstituted 1,3,4-oxadiazole and its analgesic, anti-inflammatory,anti-bacterial and anti-tubercular activity. A study of the effects of certain substituent groups attached both 2 and 5 position of 1,3,4-oxadiazole rings on the anti-tubercular activity of these compounds was also planned.

Experimental

Material and Method

General procedure for the synthesis of pyridine-4-carbohydrazide (1):

To a solution of isonicotinamide (3.8gm in 20ml methanol), 3ml of hydrazine hydrate was added and refluxed for 4 hr at 110 °C. The reaction mixture was cooled, filtered, and the separated product was purified by recrystallization from ethanol.

General procedure for the synthesis of Schiff Base (2):

Compound 1 (0.01mole) was dissolved in 30 ml of ethanol containing few drop of GAA. The appropriate aromatic aldehyde (0.01mole) was added and reaction mixture was refluxed for 5 hr at 70 °C. The reaction mixture was cooled, poured in crushed ice, filtered, and the separated product was purified by recrystallization from ethanol.

General procedure for the synthesis of 2-aryl-5-pyridine-1, 3, 4-oxadiazole by Schiff base (3):

A mixture of Schiff base (0.002 moles) and acetic anhydride (10ml) was refluxed for 4 hr. The excess of acetic anhydride was distilled off and residue was poured into ice cold water, filtered, and the separated product was purified by recrystallization from ethanol.

General procedure for the synthesis of 2-aryl-5-pyridine-1, 3, 4-oxadiazole (4):

A mixture of 1 (0.01mole) and appropriate aromatic acid (0.01mole) in 50 ml ethanol containing phosphoryl chloride as a catalyst was refluxed for 4-5 hr at 120 °C. The mixture was cooled and poured into crushed ice and made basic by 20 % NaOH. The resulting solid was, filtered, and the separated product was purified by recrystallization from ethanol.

General procedure for the synthesis of 2-thiol-5-pyridine-1, 3, 4-oxadiazole (5):

A mixture of 1 (0.01mole), carbon disulfide (0.02mole) and KOH (30%, 5ml) in 50 ml ethanol was refluxed on water bath for 4 hr. The mixture was cooled and poured into crushed ice and made acidic by HCl. The resulting solid was, filtered, and the separated product was purified by recrystallization from ethanol.

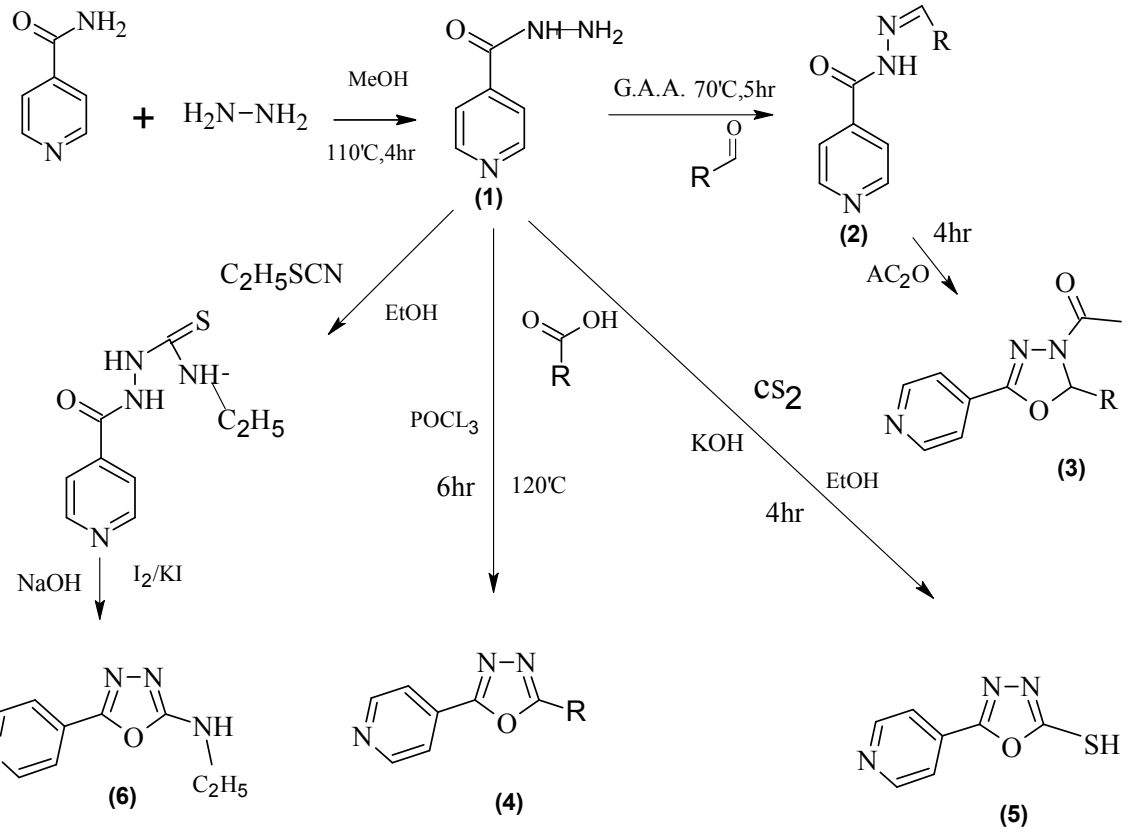
General procedure for the synthesis of N-ethyl-2-(pyridine-4-ylcarbonyl) hydrazinecarbothioamide :

A mixture of 1(0.01mole) and ethyl isothiocyanate (0.01 moles) in conc. HCl and methanol (50ml) ethanol was refluxed on water bath for 4 hr. The mixture was cooled and poured into crushed ice. The resulting solid was, filtered, and the separated product was purified by recrystallization from ethanol.

General procedure for the synthesis of N-ethyl-5-(pyridine-4-yl)-1, 3, 4-oxadiazole-2-amine (6):

A mixture of 2 in ethanol (30ml) was dissolved in cold aq. NaOH (5ml,4N) at 4°C.To this clear solution, iodine in aq, KI (5%) was added gradually with stirring till the colour of iodine persisted at room temp. The mixture was heated under refluxed for 2hr. The mixture was cooled and poured into crushed ice. The resulting solid was, filtered, and the separated product was purified by recrystallization from ethanol.

Scheme:



(3a): R = 2-OH-C_6H_4	**(4f): R** = C_8H_8
(3b): R = 4-OCH_3-C_6H_4	**(4g): R** = C_5H_4N
(3c): R = 3-OCH_3-4-OH-C_6H_3	**(4h): R** = 2-NH_2-C_6H_4
(3d): R = 2-C_4H_3O	**(4i): R** = 4-NH2-C_6H_4
(3e): R = -C_6H_5	**(4j): R** = 2-COOH-C_6H_4

Melting points were determined in open capillaries and were uncorrected. Purity of the compounds was checked by TLC. IR spectra (KBr, cm^{-1}) were recorded on Perkins Elmer Infrared-283 FTIR. ^1H NMR (CDCl3) on a Bruker 300MHz spectrometer using TMS as an internal reference.The mass spectra were recorded on a API 3000 LC-MS.

1-[2-(2-hydroxyphenyl)-5-(pyridin-4-yl)-1, 3, 4-oxadiazol-3(2*H*)-yl]ethanone(3a):
Mol.formula: $C_{15}H_{13}N_3O_3$, M.P.(ºC):188-199, Mol.wt.: 282, App.: Yellow White, Rf: 0.57, Solibility: Freely soluble in Ethanol, soluble in Acetone and Water, %yield(w/w): 66.666 , λmax : 404(nm), IR(HBr cm^{-1}) - 2820.99(Aromatic-C-H str), 274.03(C-O str of 1,3,4-oxadiazole), 1624.12(C=N str of 1,3,4 oxadiazole), 1160.22(aromatic C-C str), 1067.64 (C-N str), 568.18(N=C str), 820.99(aliphatic C-H str) , 613.51(C=O str) , 3200.98 (N-H str), 3003.27(O-H str).

1-[2-(4-methoxyphenyl)-5-(pyridin-4-yl)-1, 3, 4-oxadiazol-3(2*H*)-yl]ethanone(3b):
Mol.formula:$C_{16}H_{15}N_3O_3$, ,M.P.(ºC):158-162, Mol.wt.: 297, App.: white solid,
Rf: 0.53, Solibility:Freely soluble in Ethanol, soluble in Water,Acetone, % yield (w/w): 48.898, λ max : 354(nm), IR(HBr cm^{-1}) - 2919.36(Aromatic-C-H str) 1285.60(C-O str of 1,3,4-oxadiazole), 1683.91(C=N str of 1,3,4-oxadiazole) 1149.61(aromatic C-C str), 1064.74(C-N str), 1520.91(N=C str),3027.38(aliphatic C-H str),

1542.14(C=O str), 3198.08(N-H str) , 1229.66(C-O str of OCH3)

1-[2-(4-hydroxy-3-methoxyphenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2*H*)-yl]ethanone(3c):Mol.formula:$C_{16}H_{15}N_3O_4$, M.P.(ºC): 155-160, Mol wt:313, App.: white soild, Rf: 0.65, Solibility:Freely soluble in Ethanol, soluble in Methanol,% yield(w/w): 67.171, λ max: 355(nm), IR(HBr cm^{-1}) - 3203.87(Aromatic-C-Hstr) , 1655.94(C-Ostr of 1,3,4-oxadiazole),1597.11(C=Nstr of 1,3,4-oxadiazole) , 1166.97(aromatic C-Cstr) 1371.43(C-Nstr),1544.07(N=Cstr) , 3036.06(aliphatic C-Hstr), 1733.10(C=O str), 3446.91(N-Hstr) , 3203.87(O-H str) ,1256.67(C-Ostr of OCH3), ^{1}HNMR(δ ppm) - 8.246-7.849(4H of 4-pyridine) , 7.592-7.544(4H of 1-benzene), 4.246(1H of OH), 3.253(1H of OCH_3),1.317(1H of O=C-CH_3), MS(m/z+)- 7.96(M+), 316.94, 314.96, 312.94.

1-[2-(furan-2-yl)-5-(pyridin-4-yl)-1, 3, 4-oxadiazol-3(2*H*)-yl]ethanone(3d):
Mol.formula: $C_{13}H_{11}N_3O_3$, M.P.(ºC): 208-214, Mol.wt.: 257, App.: grey solid, Rf: 0 .56, Solibility: Freely soluble in Ethanol, soluble in Water and Methanol, % yield (w/w): 16.177, λ max : 378(nm) , IR(HBr cm^{-1}) -3051.49(Aromatic-C-H str), 651.12(C-O str of 1,3,4-oxadiazole) , 1619.29(C=N str of 1,3,4-oxadiazole), 1296.21(aromatic C-C str) , 1161.19(C-N str) , 1522.85(N=C str), 2847.03(aliphatic C-H str) , 1619.29(C=O str), 3255.95(N-H str), ^{1}HNMR(δ ppm) - 7.392-7.315(4H of 4-pyridine), 8.352-7996 (3H of 2-furan) 2.097(1H of O=C-CH_3), MS(m/z+)- 277.98(M+), 274.16, 270.01,265.00

1-[2-phenyl-5-(pyridin-4-yl)-1,3, 4-oxadiazol-3(2*H*)-yl]ethanone(3e):
Mol.formula: $C_{15}H_{13}N_3O_2$, M.P.(ºC): 230-240, App.:yellow solid, Rf: 0.57, Solibility: Freely soluble in Ethanol, soluble in Water and Acetone, % yield (w/w): 54.75, Mol.wt.: 267, λmax: 367(nm), IR(HBr cm^{-1}) - 3076.56(Aromatic-C-H str), 666.55(C-O str of 1,3,4 -oxadiazole), 1559.50(C=N str of 1,3,4-oxadiazole), 1286.56(aromaticC-C str),1460.16(C-N str), 1652.09(N=C str), 3013.86(aliphatic C-H str) , 1700.31(C=O str), 3220.27(N-Hstr).

4-[5-{(E)-2-phenylethyl}-1, 3, 4-oxadiaziole-2-yl] pyridine (4f):
Mol.formula: $C_{15}H_{11}N_3O$, M.P.(ºC): 0-130, Mol.wt.: 249, App.: white solid, Rf: 0.63, Solibility: Freely soluble in Ethanol and soluble in Water, % yield (w/w): 42.033, λ max:243(nm), IR(HBr cm^{-1}) - 3025.45(Aromatic-C-H str), 1313.57(C-O str of 1,3,4-oxadiazole), 1795.79(C=N str of 1,3,4- oxadiazole), 1227.73(aromatic C-C str), 1062.81(C-N str),630.87(N=C str), 2885.07(aliphatic C-H str), 1684.98(C=O str) , 3445.94(N-H str) ,1619.34(aliphatiC=C str), ^{1}HNMR(δ ppm) - 7.995-7878(4H of 4-pyridine),7.593-7.262(4H of 1-benzene),6.729-6.577(2H of 1-ethylene), MS(m/z+)- 285.01(M+), 280.04,260.99,248.99

3-[5-pyridine-5-yl] 1, 3, 4-oxadiazole-2-yl] pyridine (4g):
Mol.formula: $C_{12}H_8N_4O$, M.P.(ºC): 225-232, Mol.wt.: 224, App.: yellow solid, Rf: 0.66, Solibility: Freely soluble in Ethanol and soluble inWater, % yield (w/w): 7.738 ,λ max(nm) : 216, IR(HBr cm^{-1})- 2981.08 (Aromatic-C-H str), 1228.70(C-O str of 1,3,4-oxadiazole), 1683.91(C=N str of 1,3,4 -oxadiazole), 1301.99(aromatic C-C str) , 1127.43(C-N str) ,1555.64(N=C str), 999.16(aliphatic C-Hstr),1683.91(C=O str), 3425.69(N-H str), ^{1}HNMR(δ ppm) - 7.955-7.878(4H of 4-pyridine), 7..593-7.262(4H of 3-pyridine), MS(m/z+)- 286.09(M+), 285.06,278.47,267.50

2-[5-(pyridine-4-yl)-1,3,4-oxadiazole-2-yl]aniline(4h):
Mol.formula: $C_{13}H_{10}N_4O$, M.P.(ºC): 230-236, Mol.wt.: 238
App.:brownish yellow, Rf: 0.55, Solibility: Freely soluble in Ethanol and soluble in Water, % yield (w/w): 21.72, λ max : 281(nm) , IR(HBr cm^{-1})- 3197.12(Aromatic-C-H str), 1301.99(C-O str of 1,3,4 -oxadiazole) ,1683.91(C=N str of 1,3,4 oxadiazole), 1227.73(aromatic C-C str), 1126.47(C-N str),1651.12(N=C str) , 2977.23(aliphatic C-H str) , 1746.60(C=O str), 3442.09(N-Hstr) , ^{1}HNMR(δ ppm) -7.615-7.556(4H of 4-pyridine), 6.964-6.867(4H of 1-benzene), 4.618 (2H of aromatic C-NH), MS(m/z+)- 318.92(M+),315.01,303.02,301.00

4-[5-(pyridine-4-yl)-1,3,4-oxadiazole-2-yl]aniline(4i):
Mol.formula: $C_{13}H_{10}N_4O$, M.P.(ºC): <240, Mol.wt.: 238, App.: brownish white, Rf: 0.61, Solibility: Freely soluble in Ethanol and soluble in Acetone, % yield (w/w): 3.55, λ max : 281(nm), IR(HBr cm^{-1})- 2983.98(Aromatic-C-H str) , 1228.70(C-O str of 1,3,4 -oxadiazole), 1684.88(C=N str of 1,3,4- oxadiazole) , 1301.99(aromatic C-C str), 1127.43(C-N str),1540.21 (N=C str), 999.16(aliphatic C-H str), 1773.61(C=Ostr), 3443.05(N-H str),

2-[5-(pyridine-4-yl)-1,3,4-oxadiazole-2-yl] benzoic acid(4j):
Mol.formula: $C_{14}H_9N_3O_3$, M.P.(ºC): 215-233, Mol.wt.: 267, App.: brownish white, Rf: 0.67, Solibility: Freely soluble in Ethanol and soluble in Methanol, % yield (w/w): 20.00, λ max : 246(nm),

IR(HBr cm^{-1})- 3025.45(Aromatic-C-H str), 1226.77(C-O str of 1,3,4-oxadiazolest), 1540.21(C=N str of 1,3,4- oxadiazole), 983.73(aromatic C-Cstr), 931.65(C-N str),1540.21(N=Cstr), 2884.64(aliphatic C-H str), 1700.31(C=O str) , 3547.21(N-Hstr), ^{1}HNMR(δ ppm)- 7.643-7.474(4H of 4-pyridine) ,7.123-6.064(4H of 1-benzene), 10(1H of aromatic COOH), MS(m/z+)- 294.96(M+),293.98,293.02,289.98..

5-(pyridine-4-yl)-1.3.4-oxadiazole-2-thiol (5):

Mol.formula:$C_7H_5N_3O$, M.P.(ºC): <240, Mol.wt.: 179, App.: yellow solid

Rf: 0.57, Solibility: Freely soluble in Ethanol and soluble in Acetone, Water

% yield (w/w): 38.666, λ max :363(nm), IR(HBr cm^{-1})- 2880.78(Aromatic-C-H str), 1233.52(C-O str of 1,3,4-oxadiazole), 1716.70(C=N str of 1,34-oxadiazole), 1007.84(aromatic C-Cstr),1052.20(C-N str),1540.21(N=Cstr) , 2360.95(aliphatic C-H str), 1734.06 (C=O str) ,3032.20(N-Hstr), ^{1}HNMR(δ ppm)- 8.009-7.936(4H of 4-pyridine),3.111(1HofaromaticC-SH),MS(m/z+)- 677.27(M+),634.27,585.24,583.28

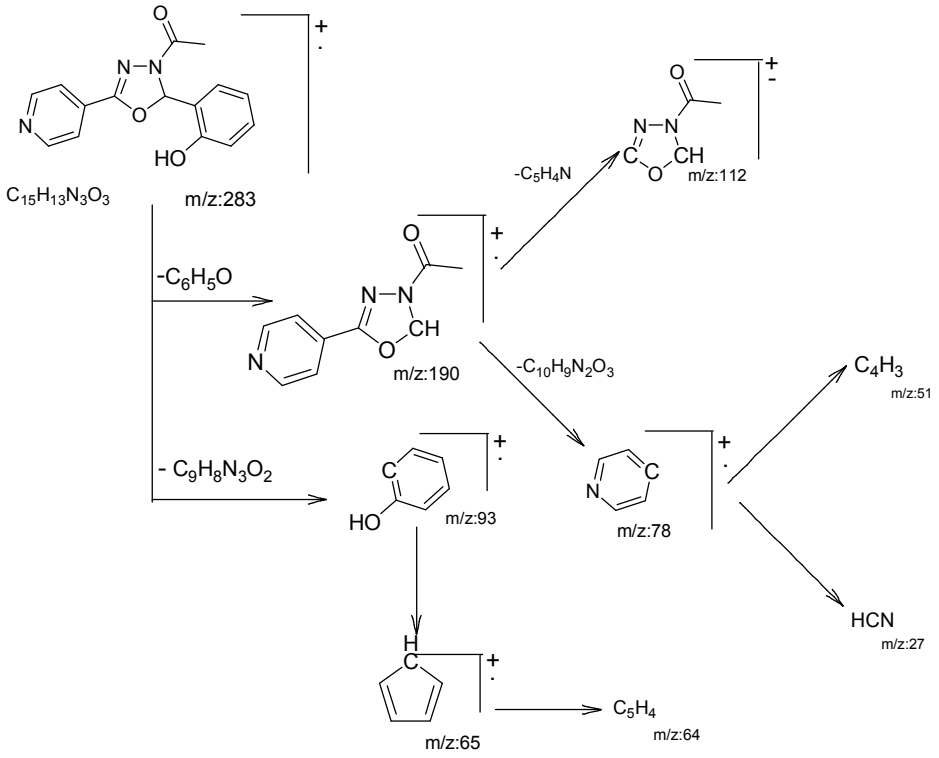
N-ethyl-5-(pyridine-4-yl)-1.3.4-oxadiazole-2-amine(6):

Mol.formula: $C_9H_{10}N_4O$, M.P.(ºC): 245-256, Mol.wt.: 190, App.: brown solid, Rf: 0.67, Solibility: Freely soluble in Ethanol and soluble in Water, % yield (w/w): 42.50

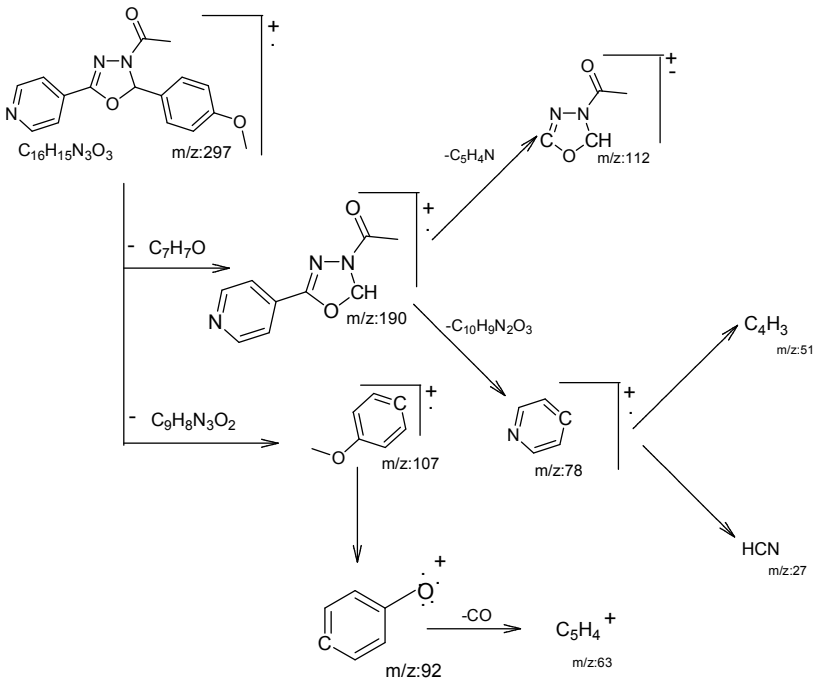
λ max :320(nm), IR(HBr cm^{-1})- 2837.38(Aromatic-C-H str), 1273.06(C-O str of 1,3,4 oxadiazole), 1699.34(C=N str of 1,3,4 -oxadiazole), 1091.75(aromaticC-Cstr),1033.88(C-Nstr) ,1540.21 (N=C str), 2945.40(aliphatic C-H str) , 1772.64 (C=O str) , 3270.42(N-Hstr),

Mass Fragmentation of 1, 3, 4-oxadiazole derivative:

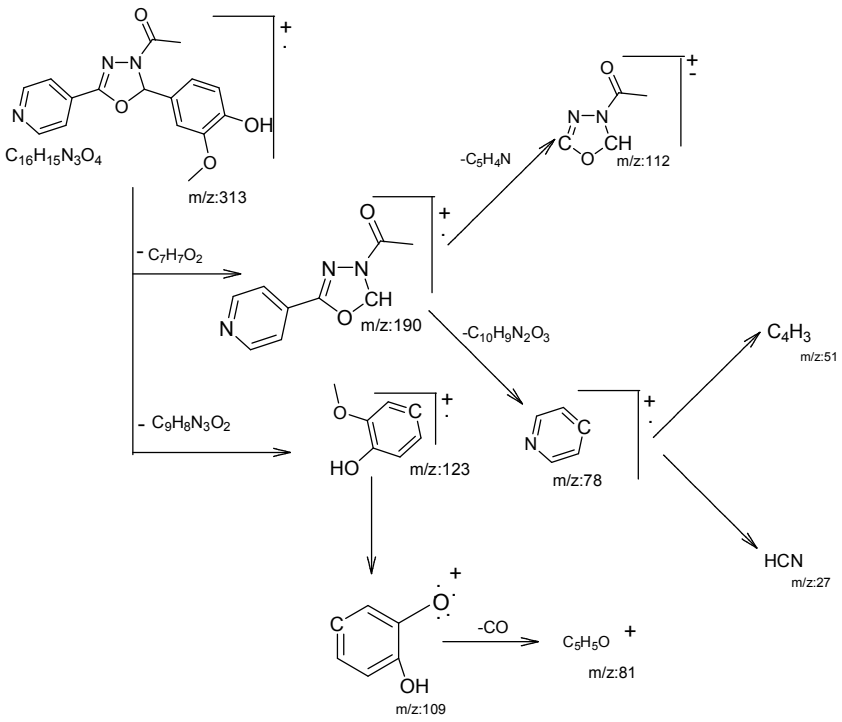
1-[2-(2-hydroxyphenyl)-5-(pyridin-4-yl)-1, 3, 4-oxadiazol-3(2*H*)-yl]ethanone(3a):



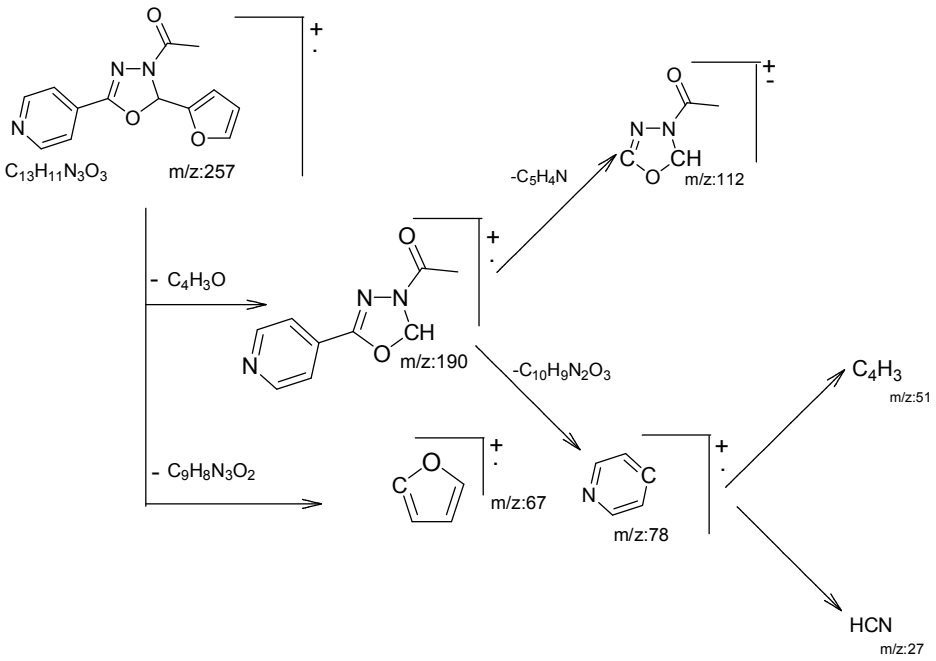
1-[2-(4-methoxyphenyl)-5-(pyridin-4-yl)-1, 3, 4-oxadiazol-3(2*H*)-yl]ethanone(3b):



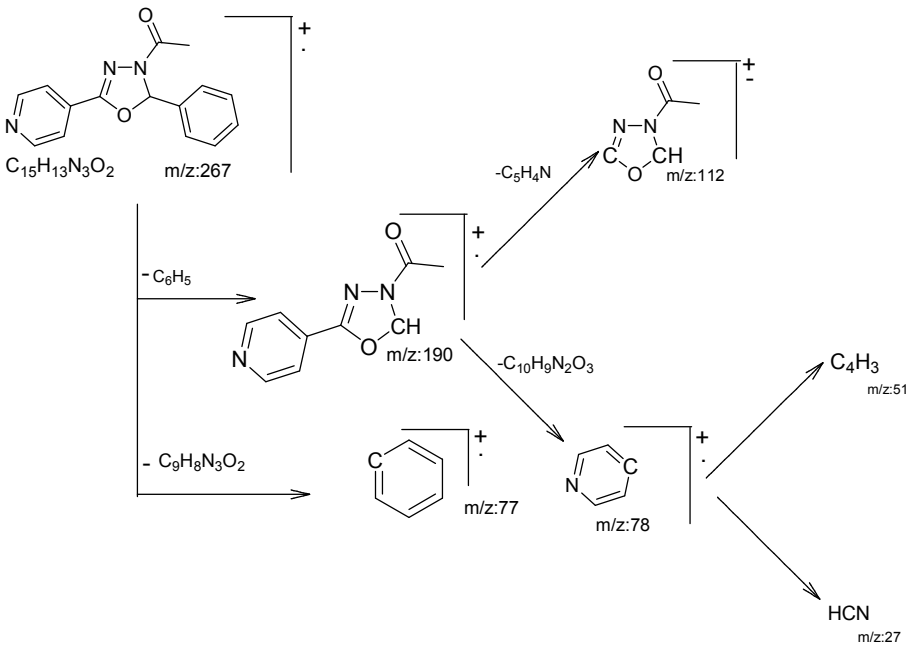
1-[2-(4-hydroxy-3-methoxyphenyl)-5-(pyridin-4-yl)-1, 3, 4-oxadiazol-3(2*H*)-yl]ethanone(3c):



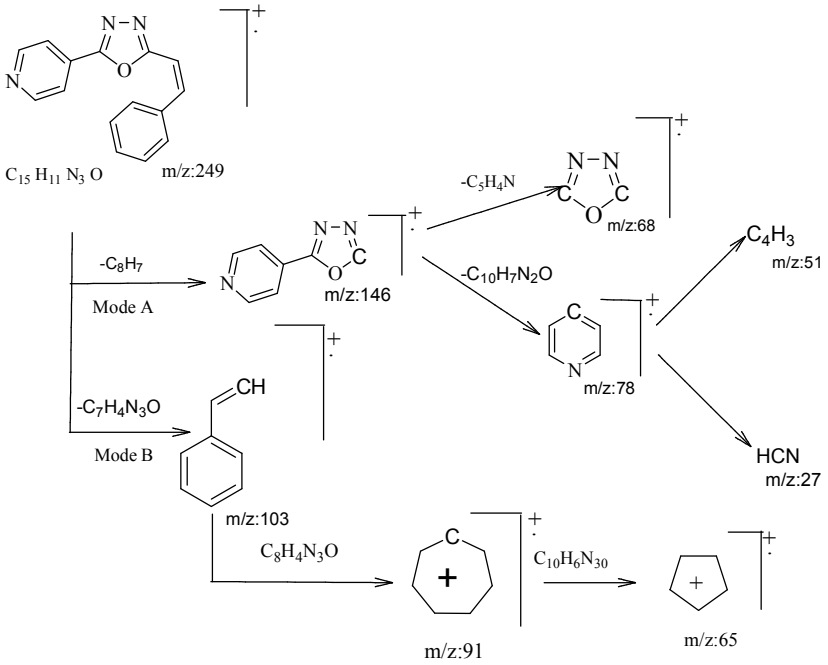
1-[2-(furan-2-yl)-5-(pyridin-4-yl)-1, 3, 4-oxadiazol-3(2*H*)-yl]ethanone(3d):



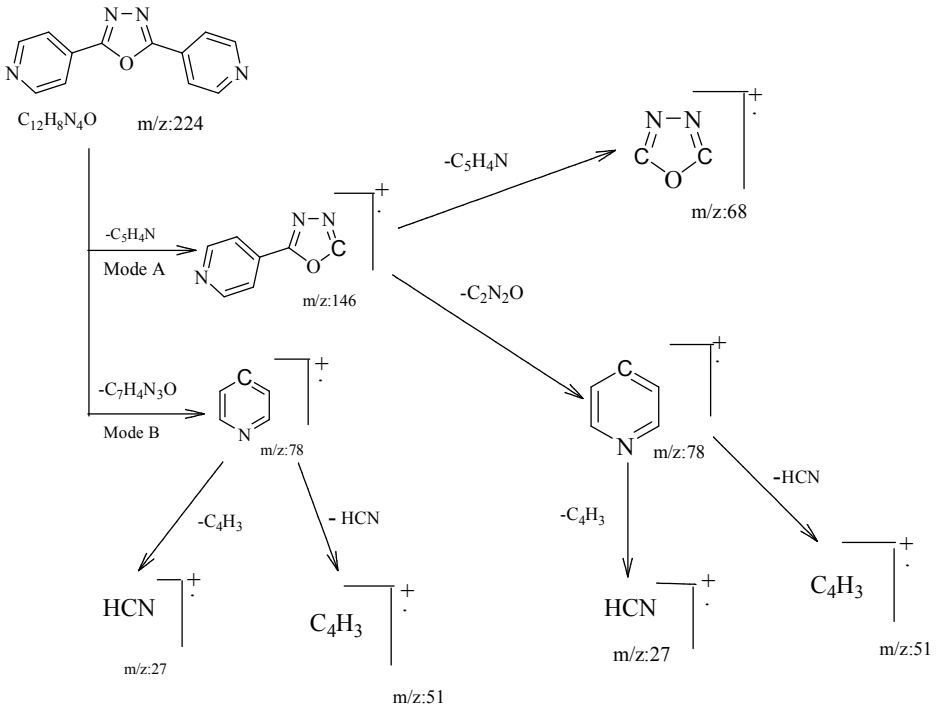
1-[2-phenyl-5-(pyridin-4-yl)-1, 3, 4-oxadiazol-3(2*H*)-yl]ethanone(3e):



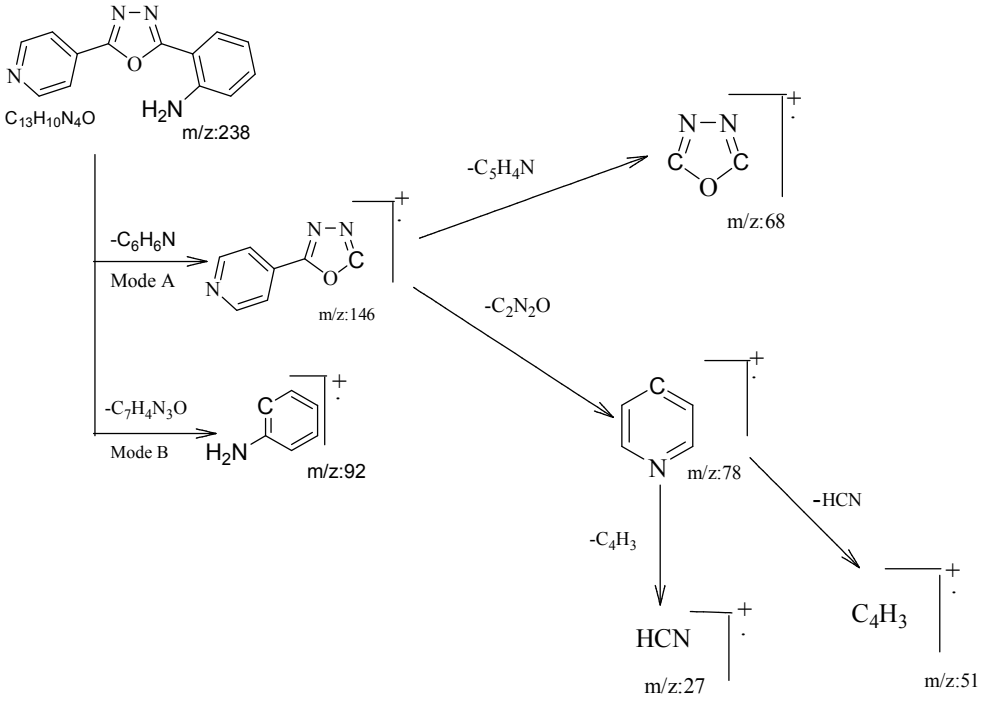
4-[5-{(E)-2-phenylethyl}-1, 3, 4-oxadiaziole-2-yl] pyridine (4f):



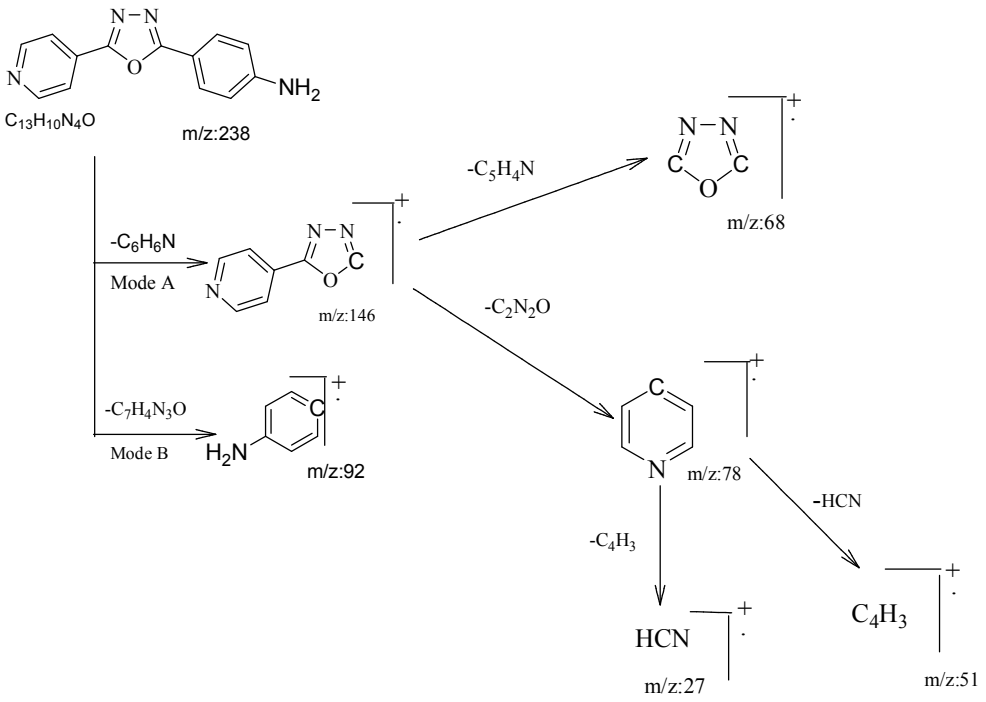
3-[5-pyridine-5-yl] 1,3,4-oxadiazole-2-yl]pyridine(4g):



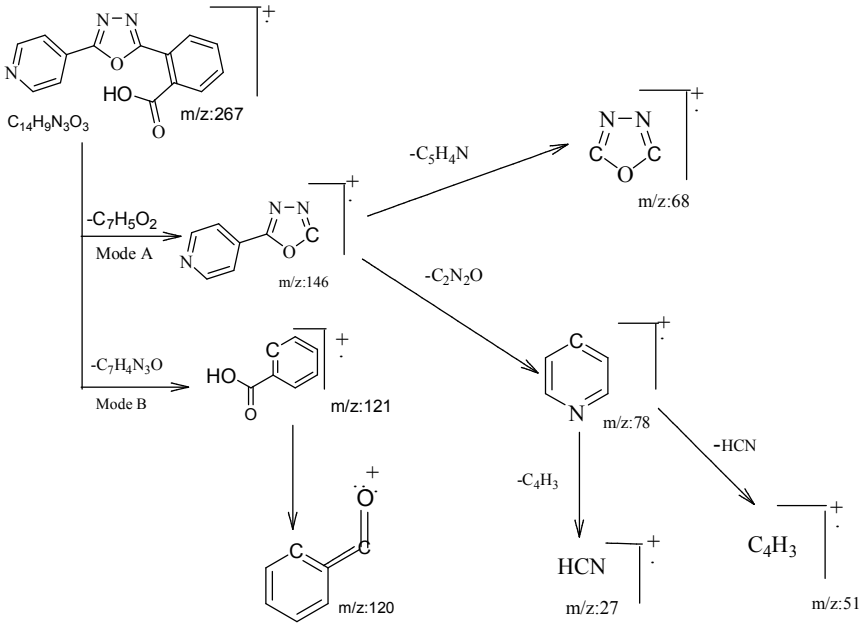
2-[5-(pyridine-4-yl)-1.3.4-oxadiazole-2-yl]aniline(4h):



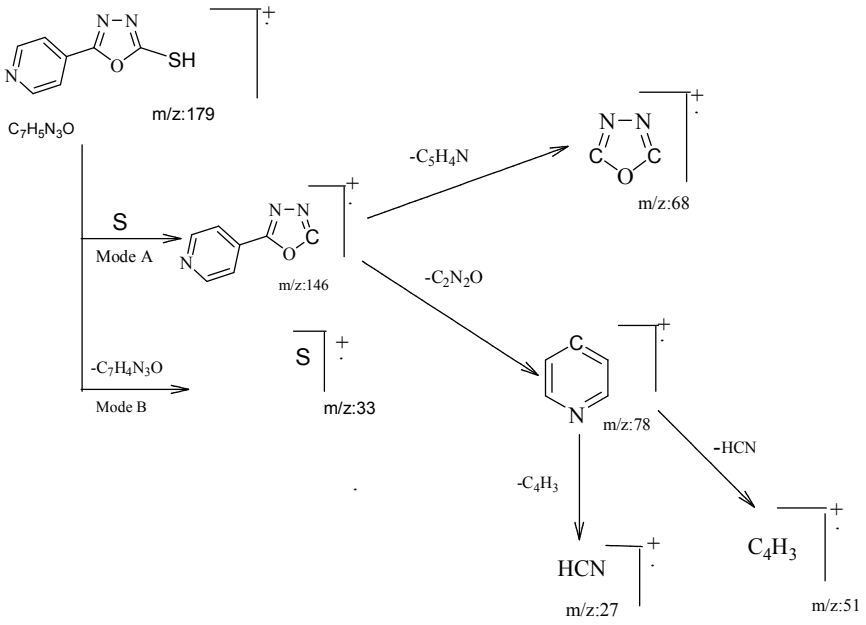
4-[5-(pyridine-4-yl)-1,3,4-oxadiazole-2-yl]aniline(4i):



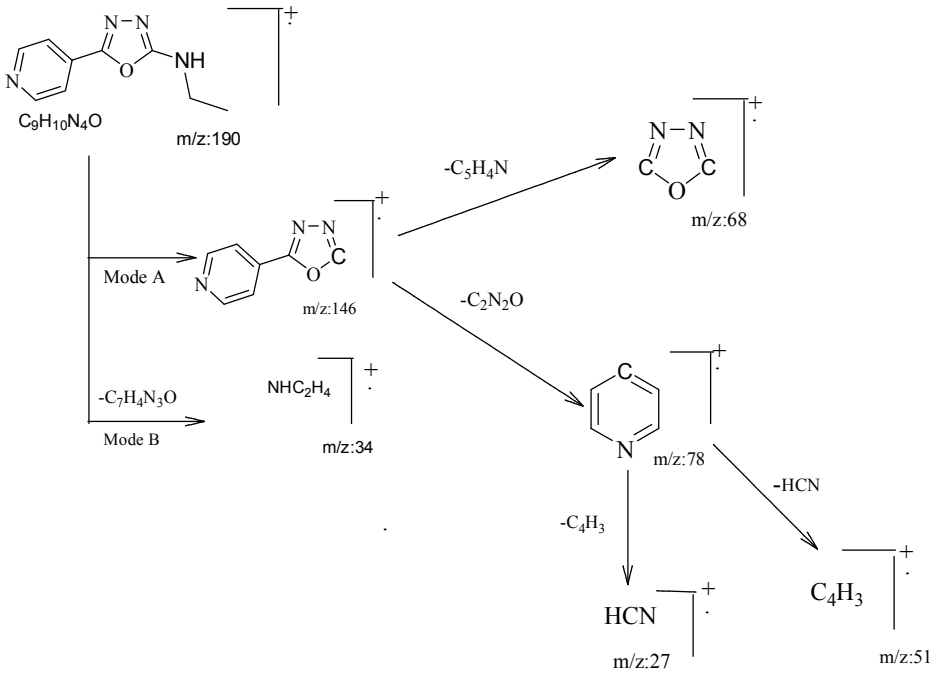
2-[5-(pyridine-4-yl)-1,3,4-oxadiazole-2-yl]benzoic acid(4j):



5-(pyridine-4-yl)-1,3,4-oxadiazole-2-thiol(5):

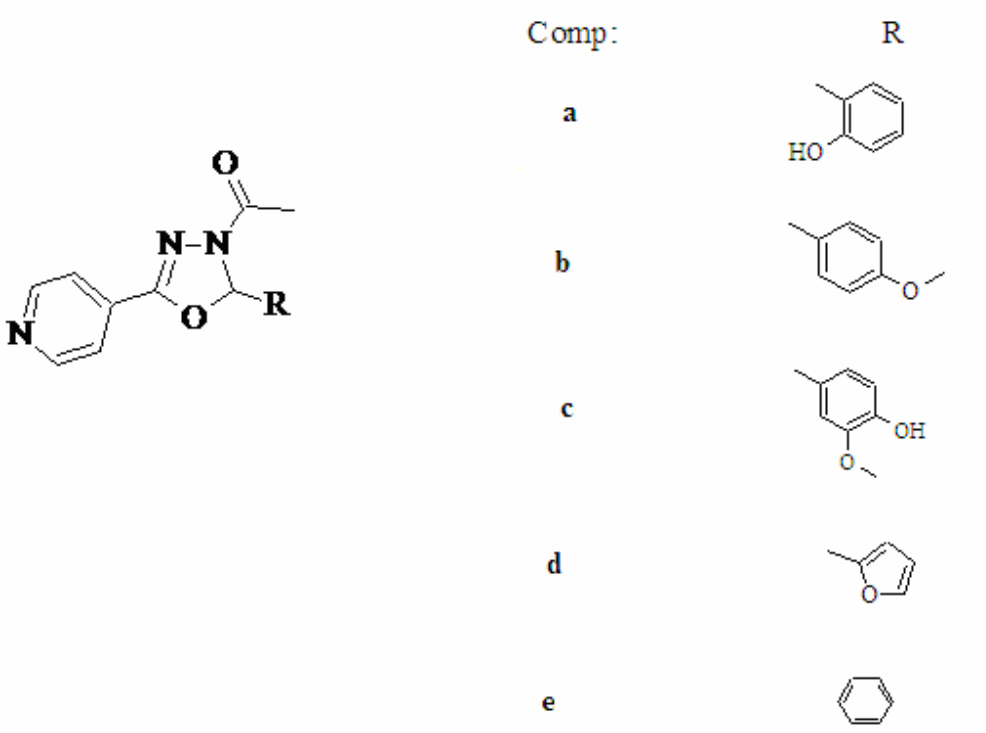


N-ethyl-5-(pyridine-4-yl)-1,3,4-oxadiazole-2-amine(6):

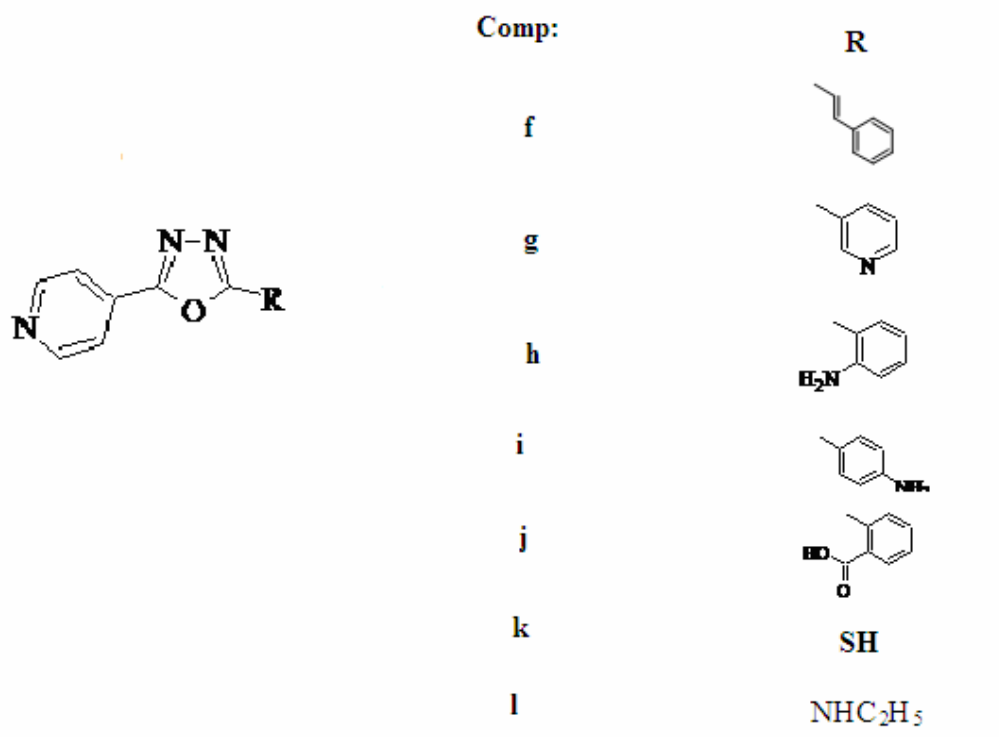


Structure Activity Relationship of 1, 3, 4-Oxadiazole Derivatives (SAR):

Compound a-Compound e:



Compound f-Compound l:



Exhaustive SAR studies have been conducted with the 1, 3, 4-oxadiazole derivative.The 2-position and 5-position is an extremely important site of molecular modification,which play a dominant role in determining the pharmacological activites of 1,3,4-oxadiazole derivatives

Direct substitution of the 2-position with an 2-methoxy phenol,pyridine and benzoic acid ,with pyridine in 5-position enhance the antimicrobial activity of 1,3,4-oxadiazole derivative.

Substitution of the 2-position with an methoxybenzene,ethenylbenzene and benzoic acid , with pyridine in 5-position enhance the analgesic activity of 1,3,4-oxadiazole derivative.

Direct substitution of the 2-position with an aminobenzene and benzoic acid, with pyridine in 5-position enhance the anti-inflammatory activity of 1, 3, 4-oxadiazole derivative.

Direct substitution of the 2-position with an 2-methoxy phenol,pyridine and benzoic acid, with pyridine in 5-position enhance the Tuberculostatic Activity of 1,3,4-oxadiazole derivative.

So the best substitutents include –COOH and pyridine groups and compound G and J gives the bettar result as compare to other derivatives.

Pharmacological activity:

Acute Toxicity**:** Animals: Swiss albino mice weighing 20 – 25 gms were used for the study. Animals were fed a standard pellet (Pranav Agro Industries Ltd., Sangli) and water ad libitum and maintained at 24 – 28 C temperature, 60 – 70% relative humidity and 12 hr day and nigh cycle. Animals described as fasted were deprived of food for 4 days, but had free access to water.

In-vitro Antibacterial Screening of Synthesized Compounds:

Plate hole diffusion method**:**

The synthesized compounds were tested for their *in vitro* antbacterial activity against the gram-negative bacteria *E.Coli* ,by cup-plate method.Oxytetracycline were used as standard drug for antibacterial studies. Nutrient Agar(Beef extract 10 gm,Peptone 10 gm, Sodium Chloride 5 gm ,Agar 20 gm, urfied water 1000ml) was employed as culture media for antibacterial studies. The ingredients were dissolved in water, and adjust the PH to 7.2 to 7.4 by using dilute alkali/dilute acid and autoclave at 120 ºC for 20 min.30-35 ml of nutrient agar was transferred to the Petri dish. 1000µg/disc,500µg/disc,250µg/dish concentration of the test compounds are prepared &

Dimethyl Foramide (DMF) was used as vehicle and Oxytetracycline(1000µg/disc) was used as standard. Nutrient agar plates were prepared aseptically to get a thickness of 5-6 mm.The plates were allowed to solidify and inverted to prevent condensate falling on the agar surface. The plates were dried at 37 ºC just before inoculation. The standard inoculums is inoculated in the plates prepared earlier aseptically by dipping a sterile swab in the inoculums,removing the excess of inoculums by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking the swab all over the surface of 60 after each application.finally press the swab round the edge of the agar surface.The strrilized discs for the test drugs were placed in the Petri dishes aseptically.Incubate the Petri dish at 37 ºC ± 0.2 ºC for about 18-24 hrs, after placing them in the refrigerator for one hour to facilitate uniform diffusion.The average zone diameter of the plates were measured and recorded.All compounds synthesized were tested for antibacterial activity against five gram + ve & five gram (–) ve bacteria.

Analgesic activity:

Test for analgesic activity was performed by Acetic acid induced writhing method using Swiss albino mice (25-35g) of male sex selected by random sampling technique. Diclofenac (5mg/kg) was used as standard drug for comparison. The test compound (1,3,4-Oxadiazole Derivatives) were administered at dose of 5 mg/kg. The inhibition of writhing in mice by synthesized compound will be compared against to the inhibition of writhing by a standard analgesic agent the reaction time was recorded after 10 min of the administration of standard / test compounds. The percent analgesic activity (PAA) was calculated by the following formula:

PAA = 1-Treated/Control ×100

Anti-inflammatory activity:

The inhibitory activity of synthesized compound on carrageenean induced rat paw edema will be determined according to mercury displacement method by using plethismograph. 8 groups of adult male albino rates (150-180gm) four animals in each will be orally dosed with synthesized compound one hour before carrageenan challenge, foot paw edema will be induced by sub planter injection of 0.05ml of 1% suspension of carrageenan in saline in to the planter tissue of one hind paw. The equal vol. of saline will be injected serve as a control group. The standard group will receive indomethacin (10mg/kg) s.c. The mercury displacement will be compared with standard for evaluation of anti-inflammatory activity of synthesized compound.

Anti-Tubercular Activity of Synthesized Compounds

The REMA plate method was performed to determine the MICs of test compounds for all the mycobacterial isolates briefly, a 100 mL volume of Middlebrook 7H9 broth (Difco, USA) was dispensed in each well of a 96-well cell culture plate (Nunc, Denmark). Test compound concentrations prepared directly in the medium were 1.25, 2.5, 3.75, 5.0, 6.25, 7.5, 8.75 and 10.0 mg/L. Perimeter wells of the plate were filled with sterile water to avoid dehydration of the medium during incubation. A standard bacterial suspension equivalent in turbidity to that of a no. 1 McFarland standard was prepared and diluted 1:20 in 7H9 broth; a 100 mL inoculum was used to inoculate each well of the plate. A growth control containing no test compound and a sterile control without inoculum were also included e. Plates were sealed and incubated at 37 ºC for 1 week.

Twenty-five microlitres (25µL of 0.02% resazurin (Sigma Chem. Co.) Solution was added to each well; plates were re-incubated for an additional 2 days.

A change in colour from blue to pink indicated the growth of bacteria, and the MIC was read as the minimum test compound concentration that prevented the colour change in resazurin solution.

Results and Discussion

IR data of compounds clearly shows a strong C=N stretching band around 1624.12 cm^{-1} and C-O absorption band around 1274.03 cm^{-1} which indicates ring closure of 1,3,4-oxadiazole ring.All final compounds have strong absorption around 3200 cm^{-1} and around 1590 cm^{-1} which are evidence for aromatic C-H and aromatic C-C bonds respectively.IR data also confirms the presence of specific functional groups present in final synthesized compounds.^{1}HNMR data also also confirms the presence of specific functional groups present in final synthesized compounds.The presence of shift value 3.253, 4.246, 4.618, 10 shown the presence of methoxy, hydroxyl,amino, carboxylic group respectively in synthesized compounds.Exhaustive pharmacological studies have been conducted with the 1,3,4-oxadiazole derivative.The 2-position and 5-position is an extremely important site of molecular modification,which play a dominant role in determining the pharmacological activites of 1,3,4-oxadiazole derivatives.. The synthesized compounds were screened *in-vitro* anti-bacterial with *E.coli*, which is cause for common cold and cough. Few compounds like compound **3c, 4g** and **4j** were shows good anti-bacterial activity against standard. So the compound contain 2-position with an -3-OCH_3-4-OH-C_6H_3, C_5H_4N, and -2-COOH-C_6H_4, with pyridine in 5-position enhance the antimicrobial activity of 1,3,4-

oxadiazole derivative Substitution of the 2-position with an -4-OCH_3-C_6H_4, -C_8H_8 and -2-COOH-C_6H_4, with pyridine in 5-position enhance the analgesic activity of 1,3,4-oxadiazole derivative. So compound 3**b, 4f** and **4j** were shown significant analgesic activity. The compound it also shown anti-inflammatory activity Direct substitution of the 2-position with an -C_5H_4N and -2-COOH-C_6H_4, with pyridine in 5-position enhance the anti-inflammatory activity of 1, 3, 4-oxadiazole derivative. Few compounds like compound **3c, 4g** and **4j** were shows good anti- inflammatory activity against standard. Further explored the synthesized compounds for anti-tubular activity against *Mycobacterium tuberculosi*s (organism). Direct substitution of the 2-position with an C_5H_4N and 2-COOH-C_6H_4, with pyridine in 5-position enhance the Tuberculostatic Activity of 1, 3, 4-oxadiazole derivative. The **MIC** of synthesized compound **4g** and **4j** were shown positive response as compaired to other compounds.

Of the all the compounds **4g** and **4j** were shown comparatively significant activity. How ever, still need some more novel approach towards the functional group at the SAR to explore the pharmacological activity.

Table No 1: Anti –Bacterial Activity of 1, 3, 4-oxadiazole derivatives using plate hole diffusion method

S.No.	Comp.	*E.coli* (-) Zone of inhibition (mm)			
		1000µg/ml	500µg/ml	250µg/ml	Oxytetracycline (Std. drug) 1000µg/ml
1	Comp.A	18	14	11	22
2	Comp.B	16	13	10	20
3	Comp.C	19	16	9	22
4	Comp.D	16	12	7	22
5	Comp.E	17	12	11	22
6	Comp.F	17	15	12	21
7	Comp.G	19	15	11	22
8	Comp.H	16	13	7	22
9	Comp.I	18	15	9	22
10	Comp.J	19	15	9	21
11	Comp.K	16	12	8	21
12.	Comp.L	17	14	10	20

From the above Table No.:1.The synthesis compound shows moderate activity against the gram (-) *E.coli*. The **Comp (3E), Comp. (4G), Comp.(4J)** possess good activity when compared to other compounds.

Table No 2:Analgesic effect of 1,3,4-Oxadiazole derivatives (5 mg/ kg) and standard drug (5 mg/kg) on Acetic acid induced writhing test in Swiss albino male mice.

Treatment group	Treatment group	%Inhibition
Control (Vehicle)	70.0 ± 0.3	--
Standard drug	23.67 ± 0.	66.67
Comp.A	46.3 ± 0.5	34.78
Comp.B	37.3 ± 0.4	47.46
Comp.C	43.33 ± 0.88	38.11
Comp.D	42.16 ± 0.94	39.77
Comp.E	42.50 ± 0.76	39.28
Comp.F	40.33 ± 0.80	42.38
Comp.G	38.16 ± 0.54	45.48
Comp.H	42.50 ± 0.92	39.28
Comp.I	41.33 ± 0.71	40.95
Comp.J	38.16 ± 0.30	45.48
Comp.K	32.70 ± 0.58	38.76
Comp.L	36.25± 0..69	42.76

(n=3,p<0.1) The experimental groups compared with control

From the above Table No:2. The synthesis compound shows moderate activity in the Swiss albino male mice. The **Comp (3B), Comp. (4F), Comp. (4J)** possess good activity when compared to other compounds.However,further some more models of analgesic activity has to screen for explore the pharmacological activity of synthesized compounds.

Table No 3:Anti-inflammatory effect of 1, 3, 4 Oxadiazole derivative on Carrageenan -induced paw edema in rats.(10mg/kg)

Treatment group	Edema induced by Carrageenan (mm.)	%Inhibition
Control (Vehicle)	6.41 ± 0.02	--
Standard drug	3.81 ± 0.03	40.56
Comp.A	2.11 ± 0.03	23.292
Comp.B	1.82 ± 0.03	18.293
Comp.D	2.57 ± 0.03	25.449
Comp.G	3.67 ± 0.03	33.073
Comp.H	2.92 ± 0.02	28.825
Comp.J	2.27 ± 0.02	30.605

(n=4,p<0.1) The experimental groups compared with control.

From the above Table No:3. The synthesis compound shows significant activity. The **Comp (4G), Comp. (4H), Comp. (4J)**, possess significant activity when compared to other compounds.The result were compared with standard drugs Indomethacin(10mg/kg).However,the compound B not shows significant activity,it may be due to the other mediator pathway.

Table No 4:Anti -tubularcular activity of 1,3,4 – Oxadiazole and its derivatives :

Compound	Antitubular Activity data in MIC (mg/l) (Mycobacterium tuberculosis)
Comp.A	7.3
Comp.B	7.3
Comp.C	7.5
Comp.D	7.2
Comp.E	7.4
Comp.F	7.4
Comp.G	7.5
Comp.H	7.4
Comp.I	7.3
Comp.J	7.5
Comp.K	7.1
Comp.L	7.3

From the above table no:4 , the entire compound shows good activity against Mycobacterium tuberculosis. The **Comp (4g)** and **Comp. (4h)** possess good activity when compared to other compounds.

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References

1. G.Grover, S.G.Kini. Indian Journal of Heterocyclic Chemistry., 2003, 12, 289.
2. J.Hazarika , J.C.S Kataky. Indian Journal of Heterocyclic Chemistry. 1997; 7: 47.
3. P.R.Kagthara, N.S.Shah, R.K.Doshi, H.H.Parekh,.Indian. Journal of Chemistry.1999; 38B: 572.
4. B.Shivarama Holla, C.S.Prasanna, Boja Poojary, K.S. Rao, K.Shridhara, U.Ganesha Bhat. Indian Journal of Chemistry. 2004; 43B: 864.
5. Zheng Li & Xicum Wang. Indian Journal of Chemistry.2003; 42B: 941.
6. M.Amir, S. Shahani. Indian Journal of Heterocyclic Chemistry.1998; 8:107.
7. H.P.Shah, B.R.Shah, J.J.Bhatt, N.C.Desai, P.B.Trivadi, N.K.Undavia. Indian Journal of Chemistry.1996; 37B: 180.
8. J.Hazarika, J.C.S.Kataky.Indian Journal of Heterocyclic Chemistry.1998; 7: 83.
9. H.Liszkiewicz, M.W.Kowalska, J.Wietrzyk A.Opolski. Indian Journal of Chemistry.2003; 42B:2846.
10. K.Mogilaiah, N.Vasudeva Reddy,.Indian Journal of Chemistry.2003; 42B:2124.
11. M.S.Chande, A.A.Godbole, E.Coutinho, P.Desai Indian Journal of Chemistry 2003;42B: 397.
12. K.Subrahmanya Bhat, M.S.Karthikeyan, B.Shivarama Holla, N. Suchetha Shetty. Indian Journal of Chemistry2004; 42B: 1765.
13. M.S.Y. Khan, M.Akhtar. Indian Journal of Chemistry.2003; 42B: 900.
14. P.Mishra, H.Rajak, A.Mehta. Joural of General Applied Microbial.2005; 15: 133.
15. H.Rajak, M.Kharya, P.Mishra. International Journal of Chemical Science. 2007;5: 365.
16. P.V Frank, B.Kalluraya. Indian Journal of Chemistry. 2005; 44B:1456.
17. Xio-Wen Sun, Xin-Ping Hui, Chang-Hu Chu, , Zi-Xi Zhang. Indian Journal of Chemistry.2002; 41B: 2176.
18. CH.K.Reddy, P.C.S.Reddy, P.S.N.Reddy, C.V, Ratnam. Indian Journal of Heterocyclic Chemistry.1997; 7:17.
19. M.S.chande, Ms K.Singh. Indian Journal of Chemistry.1998; 37B:352.
20. Xing-Ping Hui, Lin-Mei Zhang & Zi-Yi Zhang. Indian Journal of Chemistry.1999; 38B:1066.
21. K.Mogilaiah, P.Raghotham Reddy. Indian Journal of Chemistry. 2001; 40B: 619.

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