Journal of Pharmaceutical Research Vol. 12, No. 1, January-March 2013 : 5-14.

SYNTHESIS OF PYRAZOLE DERIVATIVES: A NEW THERAPEUTIC APPROACH FOR ANTIUBERCULAR AND ANTICANCER ACTIVITY

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Received on : 02.11.2012 Revised : 16.01.2013 Accepted : 21.01.2013

ABSTRACT

In present study (5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(substitutedphenyl)-1*H*-pyrazol-1-yl)(pyridine-4-yl)methanone **(4a-4o)**were synthesized by starting with acetophenone and phenylhydrazine resulting in the formation of acetophenonephenylhydrazone**(1).** Compound **(1)** on Vilsmeier-Haack reaction yielded 5-chloro-1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde **(2)**, which on condensation with substituted acetophenone gave substituted arylidenepyrazoles**(3a-3o)** followed by the reaction with isoniazid yielded the titled compounds **(4a-4o)**. All the newly synthesized pyrazolederivatives were characterized by UV, FTIR, ^{1}H NMR, MASS spectrometry and elemental analysis. All the newly synthesized derivatives were also evaluated for their antitubercular activity against *Mycobacterium tuberclosis* H_{37}Rv using agar dilution method as well as for anticancer activity against MCF7 (Human breast cancer) cells by SRB assay.

Keywords: *Pyrazole derivatives; Antitubercular activity; Anticancer activity.*

INTRODUCTION

Pyrazole scaffold represent a common motif demonstrating a wide range of pharmacological activities like anticancer[1-2], anti-inflammatory[3], adenosine receptor antagonists[4-5], antibacterial[6], anti-HIV[7],antiulcer[8], antitubercular[9] and anti-oxidantactivity[10]. Tuberclosis and cancer are diseases which becomes a cause of highest mortality rate worldwide[11]. About one-third world population is suffering from these two diseases.

In search of better anticancer and antitubercular drug, it was thought worthwhile to synthesize a series of pyrazole derivatives. Keeping in view the diverse therapeutic activities of pyrazole containing compounds and as a part of our ongoing development of efficient protocols for preparation of bioactive heterocycles, the present studies describes a simple and novel synthesis of the titled compounds in hope of getting potent biodynamic agents and evaluate their potential for antitubercular and anticancer activities.

EXPERIMENTAL

The purity of all the newly synthesized compounds were checked by TLC on silica gel-coated aluminum sheets (Type 60 F_{254}, Merck) and the spots were detected by exposure to iodine vapors and UV-lamp at $ë_{max}$ 254 nm. The melting points were determined in open capillary tube and are uncorrected. The infrared (FTIR) spectra were recorded on 470-Shimadzu Infrared Spectrophotometer using the KBr disc prepared by pressed pellet technique and expressed in cm^{-1}. ^{1}H NMR spectra were recorded on Bruker DRX-300 using DMSO-d_6 as solvent. The chemical shift was given in ä (ppm) in a downfield manner using tetramethylsilane (TMS) as an internal standard. Elemental analysis was carried on Elemental Vario EL III Carlo Erba 1108 and the values were within permissible limit of ±0.04% of the theoretical values. Mass spectrum was obtained using LC-MS (Shimadzu-2010AT) under Electrospray Ionization (ESI) technique.

SYNTHETIC PROCEDURE

Synthesis of Acetophenone phenyl hydrazone (1)

A mixture of acetophenone (0.167 moles, 20.04 gm) and Phenyl hydrazine (0.167 moles, 18.00 gm) in 60 ml of ethanol with few drops of glacial acetic acid was heated for 9 hrs between 50-60°C. Then it was cooled, filtered and washed with dilHCl and rectified spirit, the dried solid was recrystallized from ethanol to get off-white acetophenone phenyl hydrazone **(1)** (70.6%, m.p. 105-106°C).

Synthesis of 5-chloro-1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde (2)

Acetophenone phenyl hydrazone**(1)** (0.004 mole, 0.84gm) was dissolved in 10ml of DMF at 0-5°C with continuous stirring. To this solution 1.1 ml of $POCl_3$was added drop wise with stirring. Then the reaction mixture was heated on water bath at 50-60°C for 15 hrs and cooled at room temperature.The mixture was poured into crushed ice and neutralized by adding 5% $NaHCO_3$ solution. The resulting solid was filtered, washed with water and dried. The dried solid was recrystallized with aqueous methanol to get yellow coloured compound **(2) (**52.5%, m.p.118-119°C).

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Synthesis of 3-(5-chloro-1,3-diphenyl-1*H*-pyrazol-4-yl)-1-(substituted phenyl)prop-2-en-1-one (3a-3o)

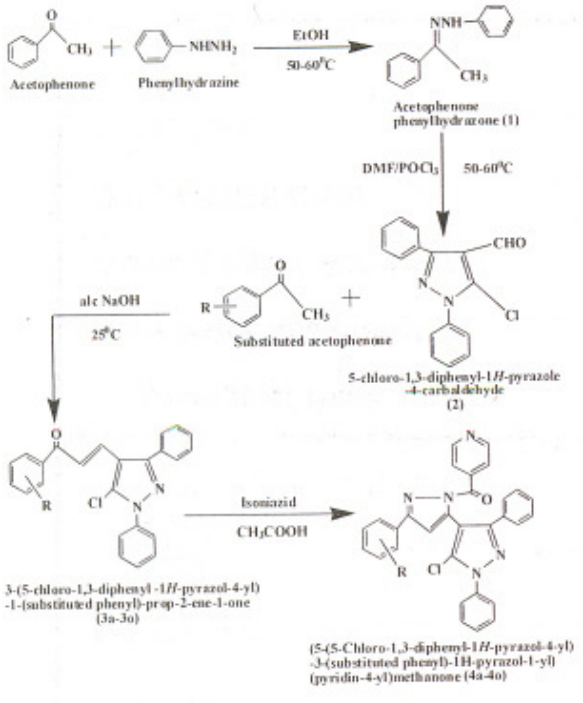
To a solution of 5-chloro-1,3-diphenyl-1*H*-pyrazole-4-carbaldehyde**(2)** (0.01 mol, 2.82gm) in 10 ml of 10% ethanolic sodium hydroxide solution, substituted acetophenone (0.01mol) was added slowly with vigorous stirring at 25ºC. The completion of reaction was checked by TLC.After completion of reaction the product was filtered, dried and recrystallized from ethanol to obtained 3-(5-chloro-1,3-diphenyl-1*H*-pyrazol-4-yl)-1-(substituted phenyl)prop-2-en-1-one**(3a-3o)**.

Synthesis of(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(substituted phenyl)-1*H*-pyrazol-1-yl) (pyridine-4-yl) methanone (4a-4o)

A mixture of compound **(3a-3o)**(0.01 moles), Isoniazid (0.02 moles, 2.44 gm) and 2 ml of glacial acetic acid was refluxed for 22-24 hrs. The resulting solution was poured into ice cold water with constant stirring, solid was separated, filtered, dried and recrystallized from suitable solvent to obtained titled compounds **(4a-4o)**.

Spectral data of the synthesized derivatives (4a-4o)

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-phenyl-1*H*-pyrazol-1-yl)(pyridine-4-yl) methanone (4a) (MS fragmentation chart in figure 1)



Scheme 1: Synthesis of Pyrazole Derivatives**(4a-4o)**

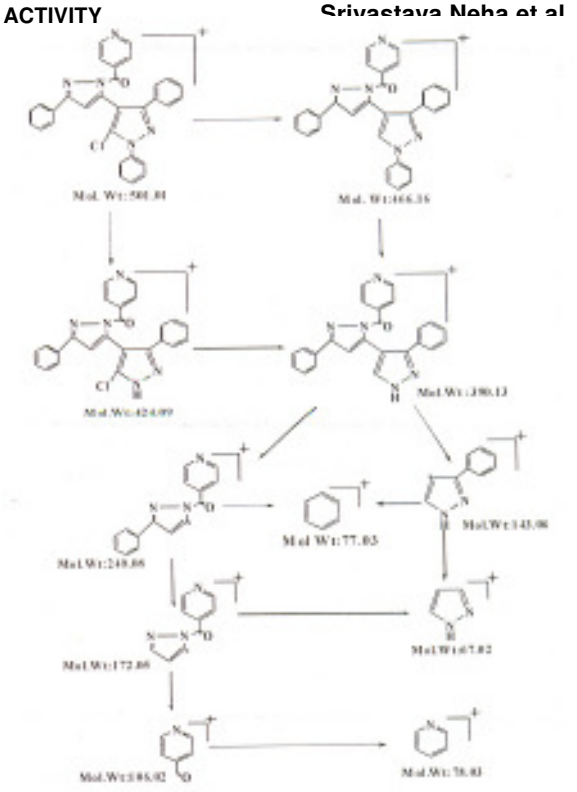


Fig.1: *Mass fragmentation pattern of compound 4a*

UV I_{max} (Chloroform): 293 nm;**FTIR (KBr, cm^{-1}):***v* 3048.53 (Ar C-H, str.), 1666.18 (C=O, str.), 1510.01 (C=N, str.), 1415.65 (Ar C=C, str.), 1346.06 (C-N, str.), 1089.64 (Ar C-Cl, str.), 753.35 (MonosubstitutedC-H def.).**^{1}H NMR (**DMSO-d_6**):** *d* 5.63 (s,1H,CH-pyrazole), 6.78-6.94 (m,5H,Ar-H), 7.00-7.18 (d,2H,Ar-H pyridine), 7.26-7.48 (m,5H,Ar-H), 7.58-7.82 (m,5H,Ar-H), 8.56-8.53 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 501.0189 (10) $[M]^+$, 502.1437 (3.4) $[M+1]^+$, 503.1332 (3.8) $[M+2]^+$,248.0885 (100). Fragments: 466.1636 (14), 424.0906 (28), 390.1336 (47), 172.0553 (8), 143.0614 (20), 106.0296 (7), 78.0321 (33), 67.0631 (19). **Elemental analysis:**Calcd. for $C_{30}H_{20}ClN_5O$: C,71.78; H, 4.02; N, 13.95. Found: C, 71.77; H, 4.01; N, 13.93%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(4-methoxyphenyl)-1*H*-pyrazol-1-yl)(pyridine-4-yl) methanone (4b) (MS fragmentation chart in figure 2)

UV I_{max} (Chloroform): 306 nm;**FTIR (KBr, cm^{-1}):***v* 3048.17 (Ar C-H, str.), 1674.31 (C=O, str.), 1520.21 (C=N, str.), 1480.26 (Ar C=C, str.), 1326.21 (C-N, str.), 1226.64 (C-O-C asym, str.), 1091.90 (Ar C-Cl, str.), 1029.92 (C-O-C sym, str.), 835.12 (Disubstitued C-H def.), 778.35 (Monosubstituted C-H def.).**^{1}H NMR (**DMSO-d_6**):** *d*3.42 (s,3H, OCH_3), 5.66 (s,1H,CH-pyrazole), 6.38-6.44 (d, 2H, Ar-H), 6.92-7.08 (m,5H,Ar-H), 7.09-7.30 (d, 2H, Ar-H pyridine), 7.23-7.32 (d,2H,Ar-H), 7.60-7.81 (m,5H,Ar-H), 8.32-8.53 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 531.0135 (11) $[M]^+$, 532.1533

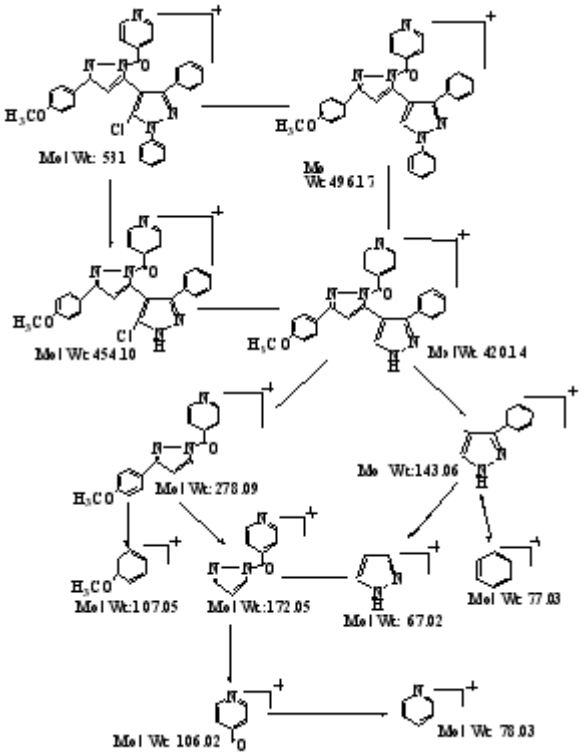


Fig. 2 : Mass fragmentation pattern of compound 4b

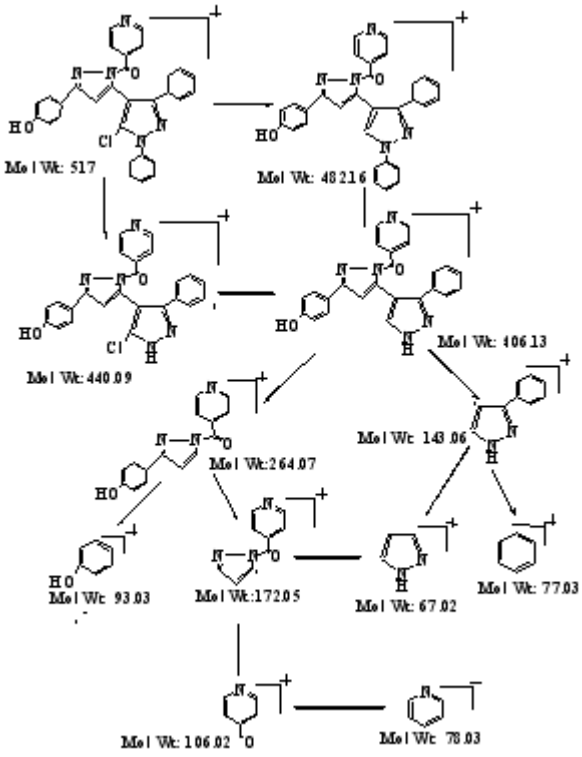


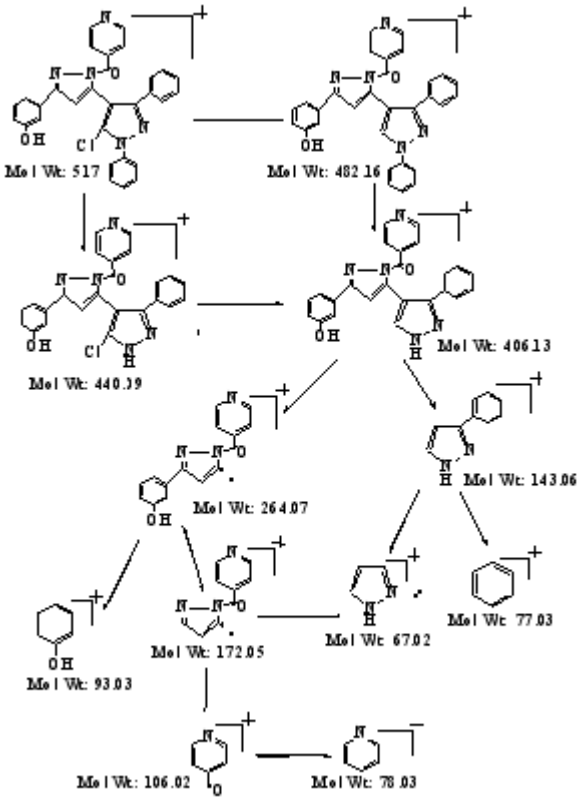
Fig. 3 : Mass fragmentation pattern of compound 4c

(3.9) $[M+1]^+$, 533.1478 (4.2) $[M+2]^+$, 278.0981 (100) Fragments: 496.1736 (17), 454.1006 (26), 420.1371 (51), 172.0529 (7), 143.0634 (22), 107.0502 (13), 78.0318 (29), 67.0296 (17). **Elemental analysis:**Calcd. for $C_{31}H_{22}ClN_5O_2$: C, 69.99; H, 4.17; N, 13.16. Found: C, 69.96; H, 4.15; N, 13.15%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(4-hydroxyphenyl)-1*H*-pyrazol-1-yl)(pyridine-4-yl) methanone (4c) (MS fragmentation chart in figure 3)

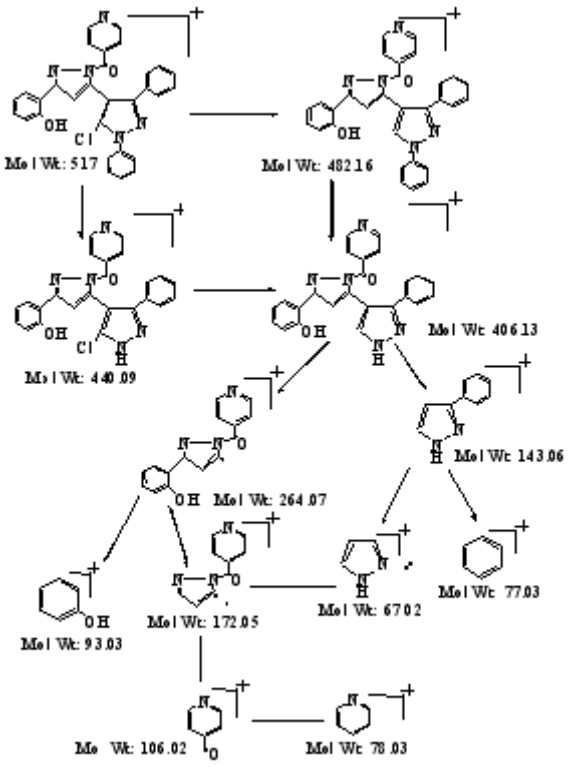
UV I_{max} (Chloroform): 298 nm;**FTIR (KBr, cm^{-1}):***v* 3427.11 (O-H, str.), 3006.82 (Ar C-H, str.), 1683.74 (C=O, str.), 1527.52 (C=N, str.), 1442.66 (Ar C=C, str.), 1338.51 (C-N, str.), 1186.14 (C-O, str.), 1091.78 (Ar C-Cl, str.), 810.05 (Disubstituted C-H def.), 692.40 (Monosubstituted C-H def.).**^{1}H NMR (**DMSO-d_6**):** *d*4.01 (s,1H, OH, D_2O exchangeable), 5.43 (s,1H,CH-pyrazole), 7.08-7.13 (d,2H,Ar-H pyridine), 7.24-7.47 (m,5H, Ar-H), 7.50-7.56(d,2H,Ar-H), 7.66-7.83 (m, 5H, Ar-H), 7.88-7.90 (d,2H,Ar-H), 8.57-8.61 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 517.0111 (13) $[M]^+$, 518.1327 (4.5) $[M+1]^+$, 519.1390 (4.9) $[M+2]^+$, 264.0769 (100) Fragments: 482.1632 (19), 440.0930 (24), 406.1308 (46), 172.0530 (6), 143.0629 (21), 106.0219 (11), 93.0341 (12), 78.0367 (31), 67.0211 (15). **Elemental analysis:**Calcd. for $C_{30}H_{20}ClN_5O_2$: C, 69.56; H, 3.89; N, 13.52. Found: C, 69.54; H, 3.87; N, 13.50%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(3-hydroxyphenyl)-1*H*-pyrazol-1-yl)(pyridine-4-yl) methanone (4d) (MS fragmentation chart in figure 4)



UV l_{max} (Chloroform): 295 nm; **FTIR (KBr, cm^{-1}):***v* 3463.62 (O-H, str.), 3055.03 (Ar C-H, str.), 1622.74 (C=O, str.), 1525.59 (C=N, str.), 1444.58 (Ar C=C, str.), 1365.51 (C-N, str.), 1164.92 (C-O, str.), 1062.70 (Ar C-Cl, str.), 815.83 (Disubstituted C-H def.), 750.26 (Monosubstituted C-H def.).**^{1}H NMR (**DMSO-d_6**):** *d*4.08 (s,1H, OH, D_2O exchangeable), 5.79 (s,1H,CH-pyrazole), 6.90-7.09 (d,2H,Ar-H pyridine), 7.21 (s,1H,Ar-H), 7.31-7.41 (dd, 1H,Ar-H), 7.51-7.60 (d,2H,Ar-H), 7.71-7.99 (m,5H, Ar-H), 8.02-8.59 (m,5H,Ar-H), 8.61-8.71 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 517.0171 (16) [M]$^+$, 518.1267 (5.5) [M+1]$^+$, 519.1321 (6.1) [M+2]$^+$, 264.0721 (100) Fragments: 482.1637 (18), 440.0911 (21), 406.1393 (48), 172.0539 (12), 143.0676 (22), 106.0214 (10), 93.0329 (13), 78.0341 (27), 77.0367 (23), 67.0241 (14). **Elemental analysis:**Calcd. for $C_{30}H_{20}ClN_5O_2$: C, 69.56; H, 3.89; N, 13.52. Found: C, 69.55; H, 3.88; N, 13.51%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(2-hydroxyphenyl)-1*H*-pyrazol-1-yl)(pyridine-4-yl) methanone (4e) (MS fragmentation chart in figure 5)



UV l_{max} (Chloroform): 307 nm; **FTIR (KBr, cm^{-1}):***v* 3540.19 (O-H, str.), 3064.68 (Ar C-H, str.), 1644.95 (C=O, str.), 1575.73 (C=N, str.), 1492.20 (Ar C=C, str.), 1333.22 (C-N, str.), 1216.64 (C-O, str.), 1081.99 (Ar C-Cl, str.), 835.12 (Disubstituted C-H def.), 707.83 (Monosubstituted C-H def.).**^{1}H NMR (**DMSO-d_6**):** *d*4.09 (s,1H, OH, D_2O exchangeable), 5.68 (s,1H,CH-pyrazole), 6.41-6.52 (d,2H,Ar-H pyridine), 6.73-6.91 (m,5H, Ar-H), 7.02-7.39 (m,5H,Ar-H), 7.47-7.50 (d,1H,Ar-H), 7.50-7.90 (t,2H,Ar-H), 8.21-8.32 (d,1H,Ar-H), 8.57-8.61 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 517.0132 (12) [M]$^+$, 518.1176 (4.1) [M+1]$^+$, 519.1345 (4.6) [M+2]$^+$, 264.0714 (100) Fragments: 482.1676 (19), 440.0986 (20), 406.1327 (47), 172.0513 (11), 143.0634 (23), 106.0286 (11.5), 93.0332 (14), 78.0390 (26), 77.0322 (22), 67.0213 (13). **Elemental analysis:**Calcd. for $C_{30}H_{20}ClN_5O_2$: C, 69.56; H, 3.89; N, 13.52. Found: C, 69.53; H, 3.86; N, 13.50%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(4-chlorophenyl)-1*H*-pyrazol-1-yl)(pyridine-4-yl) methanone (4f) (MS fragmentation chart in figure 6)

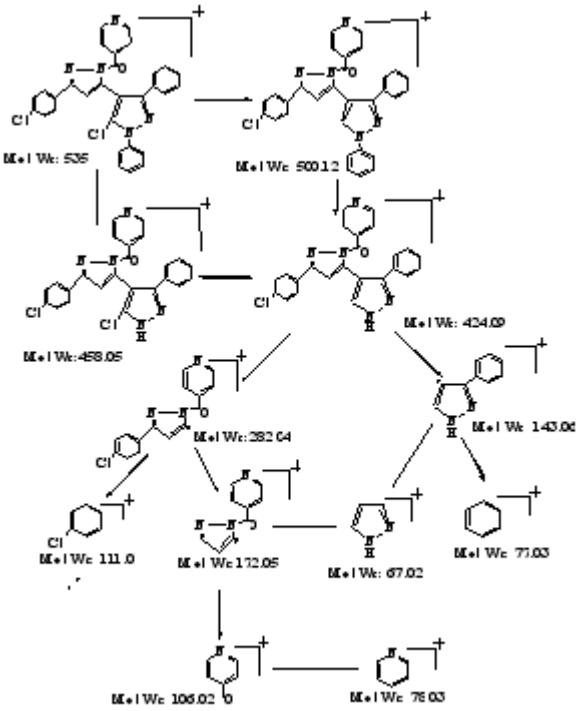


Fig. 6 : Mass fragmentation pattern of compound 4f

UV l_{max} (Chloroform): 278 nm; **FTIR (KBr, cm^{-1}):***v* 3010.67 (Ar C-H, str.), 1668.15 (C=O, str.), 1566.09 (C=N, str.), 1488.60 (Ar C=C, str.), 1319.75 (C-N, str.), 1095.22 (Ar C-Cl, str.), 820.83 (Disubstituted C-H def.), 681.54 (Monosubstituted C-H def.).**^{1}H NMR (**DMSO-d_6**):** *d*5.32 (s,1H,CH-pyrazole), 6.68-6.93 (m,5H,Ar-H), 7.01-7.21 (d,2H,Ar-H pyridine), 7.28-7.43 (d, 2H, Ar-H), 7.57-7.89 (m,5H, Ar-H), 7.99-8.09 (d,2H,Ar-H), 8.62-8.68 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 535.0134 (18) [M]$^+$, 536.1002 (6.2) [M+1]$^+$, 537.0933 (12.7) [M+2]$^+$, 539.0112 (1.9) [M+4]$^+$, 282.0466 (100) Fragments: 500.1221 (21), 458.0539 (23), 424.0933 (53), 172.0513 (13), 143.0622 (25), 111.0013 (16), 106.0296 (18), 78.0321 (28), 77.0325 (24), 67.0239 (15). **Elemental analysis:**Calcd. for $C_{30}H_{19}Cl_2N_5O$: C, 67.17; H, 3.57; N, 13.06. Found: C, 67.13; H, 3.55; N, 13.04%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(4-bromophenyl)-1*H*-pyrazol-1-yl) (pyridine-4-yl)methanone (4g) (MS fragmentation chart in figure 7)

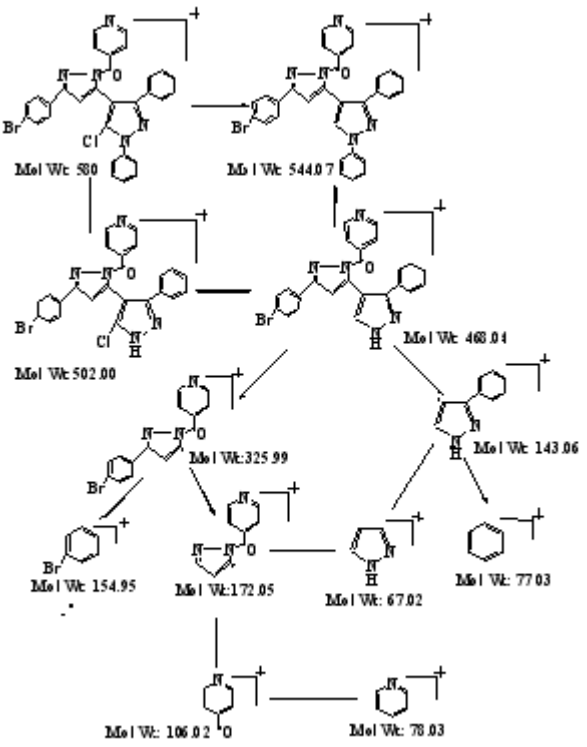
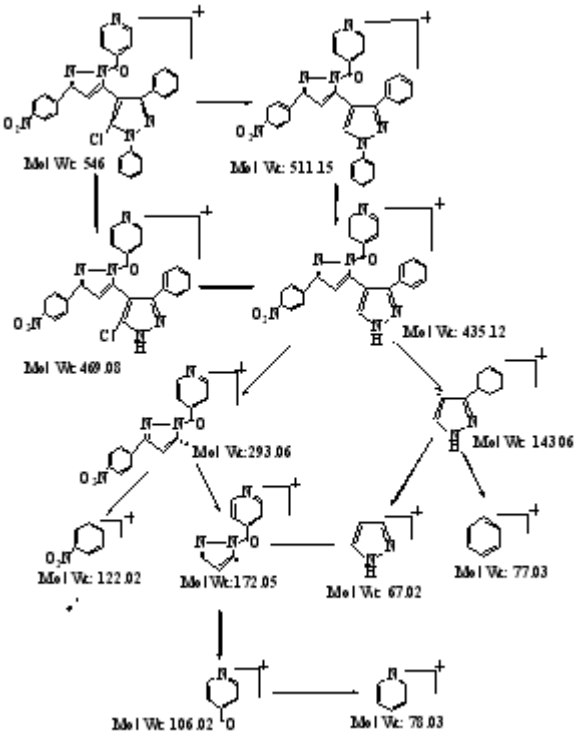


Fig. 7 : Mass fragmentation pattern of compound 4g

UV l_{max} (Chloroform): 304 nm;**FTIR (KBr, cm^{-1}):***v* 3060.82 (Ar C-H, str.), 1722.31 (C=O, str.), 1569.95 (C=N, str.), 1525.59 (Ar C=C, str.), 1350.08 (C-N, str.), 1016.42 (Ar C-Cl, str.), 814.41 (Disubstituted C-H def.), 750.26 (Monosubstituted C-H def.), 661.54 (C-Br, str.).**^{1}H NMR (**DMSO-d_6**):***d*5.61 (s,1H,CH-pyrazole), 6.72-6.90 (m,5H,Ar-H), 6.90-7.18 (d,2H,Ar-H pyridine), 7.24-7.72 (m, 5H, Ar-H), 7.82-7.98 (d, 2H, Ar-H), 8.01-8.17 (d,2H,Ar-H), 8.65-8.66 (d,2H, Ar-H pyridine).**MS (ESI) m/z [% rel. abundance] :** 580.0189 (12) [M]$^+$, 581.0478 (4.1) [M+1]$^+$, 582.0523 (16.25) [M+2]$^+$, 584.0176 (3.8) [M+4]$^+$, 325.9910 (100) Fragments: 544.0840 (19), 502.0092 (22), 468.0436 (46), 172.0586 (11), 154.9534 (15), 143.0617 (24), 106.0286 (17), 78.0346 (27), 77.0323 (23), 67.0201 (14). **Elemental analysis:**Calcd. for $C_{30}H_{19}BrClN_5O$: C, 62.03; H, 3.30; N, 12.06 Found: C, 62.00; H, 3.28; N, 12.04%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(4-nitrophenyl)-1*H*-pyrazol-1-yl) (pyridine-4-yl)methanone (4h) (MS fragmentation chart in figure 8)

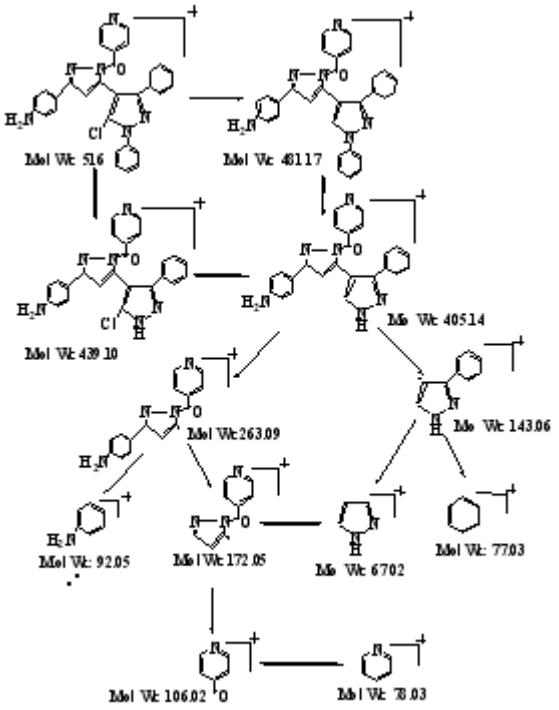
UV l_{max} (Chloroform): 276 nm;**FTIR (KBr, cm^{-1}):***v* 3058.89 (Ar C-H, str.), 1674.10 (C=O, str.), 1595.02 (C=N, str.), 1541.02 (Ar.NO_2 , asym str.), 1500.52 (Ar C=C, str.), 1332.72 (C-N, str.), 1294.50 (Ar.NO_2 , sym str.), 1064.63 (Ar C-Cl, str.), 837.05 (Disubstituted C-H def.), 736.76 (Monosubstituted C-H def.).**^{1}H NMR**



(DMSO-d_6**):** *d*5.72 (s,1H,CH-pyrazole), 6.57-6.79 (m,5H,Ar-H), 7.12-7.22 (d,2H,Ar-H pyridine), 7.32-7.56 (m,5H, Ar-H), 7.60-7.72 (d, 2H, Ar-H), 8.01-8.12 (d,2H, Ar-H), 8.53-8.68 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 546.0029 (11) [M]$^+$, 547.1289 (3.8) [M+1]$^+$, 548.1289 (4.2) [M+2]$^+$, 293.0609 (100) Fragments: 511.1536 (17), 469.0906 (20), 435.1240 (48), 172.0553 (13), 143.0613 (25), 122.0240 (21), 106.0204 (16), 78.0332 (28), 77.0321 (25), 67.0211 (15). **Elemental analysis:**Calcd. for $C_{30}H_{19}ClN_6O_3$: C, 65.88; H, 3.50; N, 15.36. Found: C, 65.89; H, 3.48; N, 15.33%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(4-aminophenyl)-1*H*-pyrazol-1-yl)(pyridine-4-yl) methanone (4i) (MS fragmentation chart in figure 9)

UV l_{max} (Chloroform): 285 nm;**FTIR (KBr, cm^{-1}):***v* 3291.97 (Ar.N-H primary, str.) 3055.03 (Ar C-H, str.), 1650.95 (C=O, str.), 1598.88 (C=N, str.), 1506.30 (Ar C=C, str.), 1342.36 (C-N, str.), 1064.63 (Ar C-Cl, str.), 827.41 (Disubstituted C-H def.), 761.83 (Monosubstituted C-H def.).**^{1}H NMR (**DMSO-d_6**):** *d*5.76 (s,1H,CH-pyrazole), 6.24 (s, 2H, NH_2,D_2O exchangeable), 6.52-6.75 (m,5H,Ar-H), 6.90-6.99 (d,2H,Ar-H pyridine), 7.19-7.41 (m,5H, Ar-H), 7.55-7.66 (d,2H,Ar-H), 7.81-7.93 (d,2H, Ar-H), 8.23-8.39 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 516.0069 (14) [M]$^+$, 517.1512 (4.9) [M+1]$^+$, 518.1433 (5.3) [M+2]$^+$, 263.0952 (100) Fragments: 481.1726 (15), 439.0910 (19), 405.1436 (51), 172.0538 (10), 143.0625 (23), 92.0521 (21), 106.0272 (17), 78.0352 (28), 77.0345 (26), 67.0251 (16). **Elemental analysis:**Calcd. for



$C_{30}H_{21}ClN_6O$: C, 69.70; H, 4.09; N, 16.26. Found: C, 69.69; H, 4.08; N, 16.23%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(2,5-dihydroxyphenyl)-1*H*-pyrazol-1-yl)(pyridine-4-yl) methanone (4j) (MS fragmentation chart in figure 10)

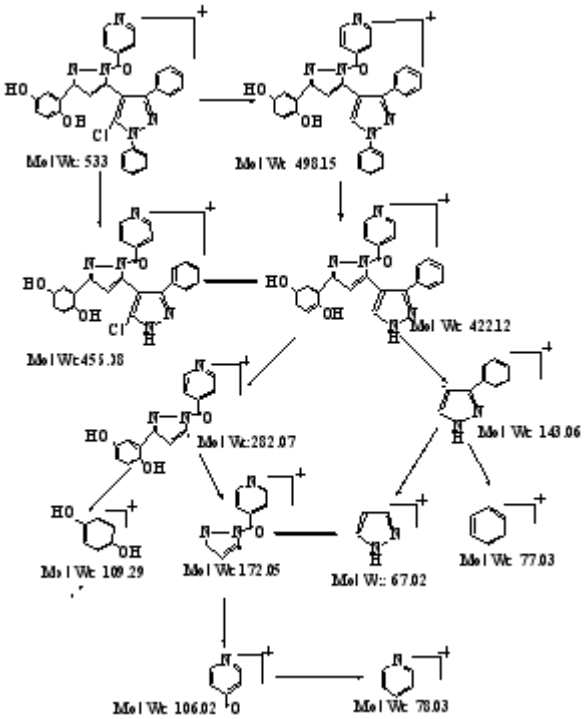
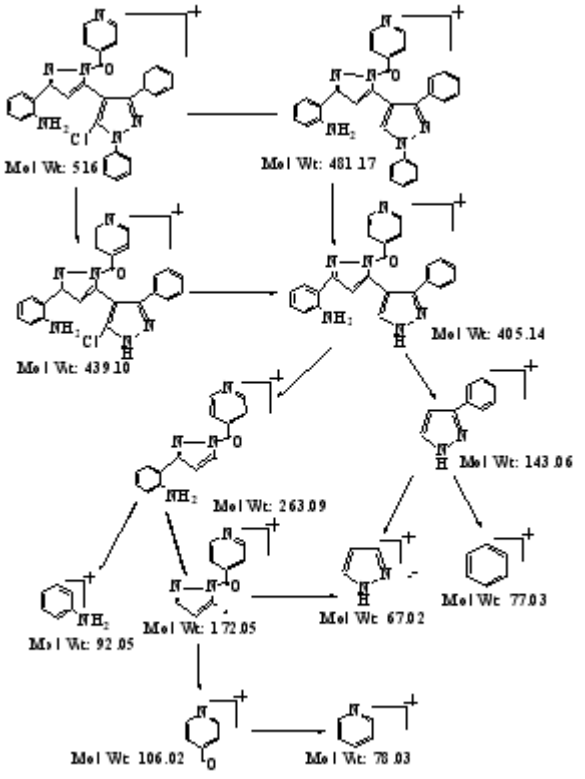


Fig. 10 : *Mass fragmentation pattern of compound 4j*

UV I_{max} (Chloroform): 287 nm;**FTIR (KBr, cm^{-1}):** *v* 3505.47 (O-H, str.), 3056.96 (Ar C-H, str.), 1662.52 (C=O, str.), 1548.73 (C=N, str.), 1504.37 (Ar C=C, str.), 1353.94 (C-N, str.), 1226.64 (C-O, str.), 1062.70 (Ar C-Cl, str.), 958.56 (Trisubstituted C-H def.), 761.83 (Monosubstituted C-H def.).**^{1}H NMR (**DMSO-d_6**):** *d* 4.12 (s,2H, OH, D_2O exchangeable), 5.68 (s,1H,CH-pyrazole), 6.60-6.82 (m,5H,Ar-H), 6.98-7.09 (d,2H,Ar-H pyridine), 7.21-7.50 (m,5H, Ar-H), 7.60 (s,1H,Ar-H), 7.93-8.08 (d,2H, Ar-H), 8.54-8.64 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 533.0046 (13) $[M]^+$, 534.1232 (4.5) $[M+1]^+$, 535.1267 (5.0) $[M+2]^+$, 282.0765 (100) Fragments: 498.0802 (17), 456.0843 (22), 422.1236 (49), 172.0521 (11), 143.0676 (21), 109.2625 (23), 106.0225 (19), 78.0321 (26), 77.0331 (25), 67.0291 (14). **Elemental analysis:**Calcd. for $C_{30}H_{20}ClN_5O_3$: C, 67.48; H, 3.78; N, 13.12. Found: C, 67.46; H, 3.77; N, 13.10%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(2-aminophenyl)-1*H*-pyrazol-1-yl)(pyridine-4-yl) methanone (4k) (MS fragmentation chart in figure 11)



UV I_{max} (Chloroform): 298 nm; **FTIR (KBr, cm^{-1}):** *v* 3252.34 (Ar.N-H primary, str.), 3041.53 (Ar C-H, str.), 1598.88 (C=O, str.), 1496.66 (Ar C=C, str.), 1527.26 (C=N, str.), 1319.22 (C-N, str.), 1070.42 (Ar C-Cl, str.), 748.33 (Disubstituted C-H def.), 692.40 (Monosubstituted C-H def.).**^{1}H NMR (**DMSO-d_6**):** *d* 5.74 (s,1H,CH-pyrazole), 6.31 (s,2H, NH_2, D_2O exchangeable), 6.51-6.75 (m,5H,Ar-H), 6.97-7.01

(d,2H,Ar-H pyridine), 7.13-7.32 (m,5H, Ar-H), 7.46-7.51 (dd,1H,Ar-H), 7.71-7.85 (t,2H, Ar-H), 8.03-8.13 (dd,1H,Ar-H), 8.63-8.70 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 516.0020 (12) $[M]^+$, 517.1234 (4.2) $[M+1]^+$, 518.1289 (4.5) $[M+2]^+$, 263.0975 (100) Fragments: 481.1712 (16), 439.0931 (23), 405.1491 (45), 172.0525 (10), 143.0632 (19), 106.0272 (14), 92.0569 (17), 78.0321 (24), 77.0322 (21), 67.0243 (13). **Elemental analysis:**Calcd. for $C_{30}H_{21}ClN_6O$: C, 69.70; H, 4.09; N, 16.26. Found: C, 69.68; H, 4.07; N, 16.24%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(2-chlorophenyl)-1*H*-pyrazol-1-yl) (pyridine-4-yl) methanone (4l) (MS fragmentation chart in figure 12)

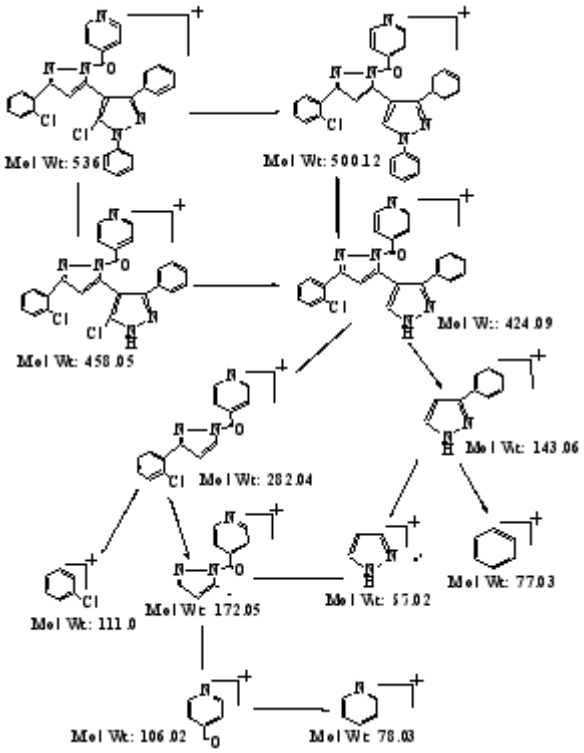
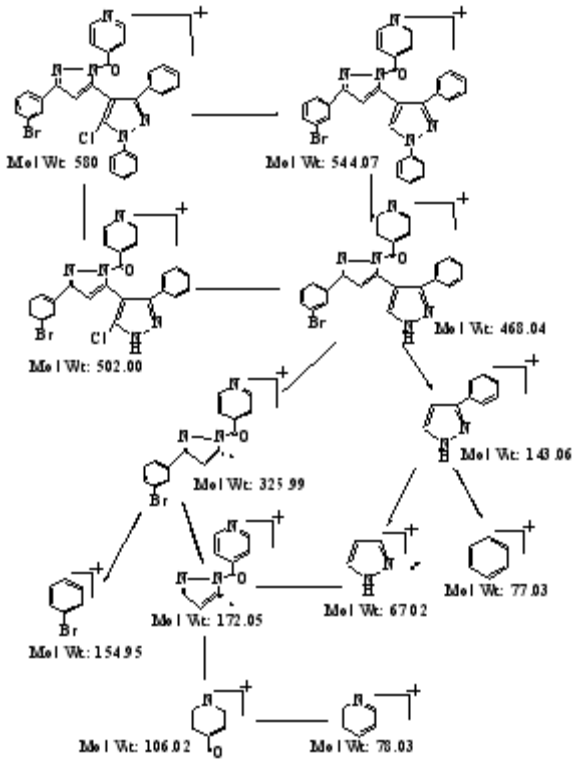


Fig. 12 : *Mass fragmentation pattern of compound 4l*

UV I_{max} (Chloroform): 292 nm;**FTIR (KBr, cm^{-1}):***v* 3018.39 (Ar C-H, str.), 1695.31 (C=O, str.), 1573.81 (C=N, str.), 1471.59 (Ar.C=C, str.), 1315.36 (C-N, str.), 1010.63 (Ar C-Cl, str.), 765.69 (Disubstituted C-H def.), 673.11 (Monosubstituted C-H def.).**^{1}H NMR (**DMSO-d_6**):** *d*5.66 (s,1H,CH-pyrazole), 6.59-6.83 (m,5H,Ar-H), 7.02-7.19 (d,2H,Ar-H pyridine), 7.30-7.61 (m,5H, Ar-H), 7.81-7.90 (dd,1H,Ar-H), 7.98-8.13 (t,2H, Ar-H), 8.15-8.22 (dd,1H,Ar-H), 8.58-8.63 (d,2H, Ar-H pyridine).**MS (ESI) m/z :** 536.0197 (13) $[M]^+$, 536.0956 (4.5) $[M+1]^+$, 537.0276 (9.2) $[M+2]^+$, 539.0812 (1.3) $[M+4]^+$, 282.0464 (100) Fragments: 500.1225 (17), 458.0543 (23), 424.0933 (50), 172.0517 (10), 143.0604 (15), 111.0032 (19), 106.0225 (12), 78.0391 (22), 77.0322 (21), 67.0214 (11). **Elemental analysis:**Calcd. for $C_{30}H_{19}Cl_2N_5O$: C, 67.17; H, 3.57; N, 13.06 Found: C, 67.16; H, 3.57; N, 13.04%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(3-bromophenyl)-1*H*-pyrazol-1-yl)(pyridine-4-yl) methanone (4m) (MS fragmentation chart in figure 13)



UV I_{max} (Chloroform): 309 nm;**FTIR (KBr, cm^{-1}):***v* 3051.18 (Ar C-H, str.), 1606.59 (C=O, str.), 1521.73 (C=N, str.), 1456.16 (Ar C=C, str.), 1338.51 (C-N, str.), 1045.64 (Ar C-Cl, str.), 806.19 (Disubstituted C-H def.), 740.61 (Monosubstituted C-H def.), 640.32 (C-Br, str.).**^{1}H NMR (**DMSO-d_6**):** *d*5.69 (s,1H,CH-pyrazole), 6.60-6.82 (m,5H,Ar-H), 7.09-7.16 (d,2H,Ar-H pyridine), 7.30-7.52 (m, 5H, Ar-H), 7.81-8.00 (t,1H, Ar-H), 8.16-8.21 (dd,2H,Ar-H), 8.29 (s,1H,Ar-H), 8.59-8.67 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 580.0133 (11) $[M]^+$, 581.0402 (3.8) $[M+1]^+$, 582.0564 (14.9) $[M+2]^+$, 584.0612 (3.5) $[M+4]^+$, 325.9959 (100) Fragments: 544.0634 (16), 502.0022 (25), 468.0423 (47), 172.0521 (15), 154.9528 (21), 143.0615 (21), 106.0282 (14), 78.0326 (23), 77.0365 (19), 67.0201 (11). **Elemental analysis:**Calcd. for $C_{30}H_{19}BrClN_5O$: C, 62.03; H, 3.30; N, 12.06. Found: C, 62.01; H, 3.28; N, 12.03%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(3-fluorophenyl)-1*H*-pyrazol-1-yl)(pyridine-4-yl) methanone (4n) (MS fragmentation chart in figure 14)

UV I_{max} (Chloroform): 295nm;**FTIR (KBr, cm^{-1}):***v* 3056.96 (Ar C-H, str.), 1740.12 (C=O, str.), 1579.59 (C=N, str.), 1458.08 (Ar C=C, str.), 1326.93 (C-N, str.), 1238.21 (C-F, str.), 1046.77 (Ar C-Cl, str.), 820.05 (Disubstituted C-H def.), 752.19 (Monosubstituted

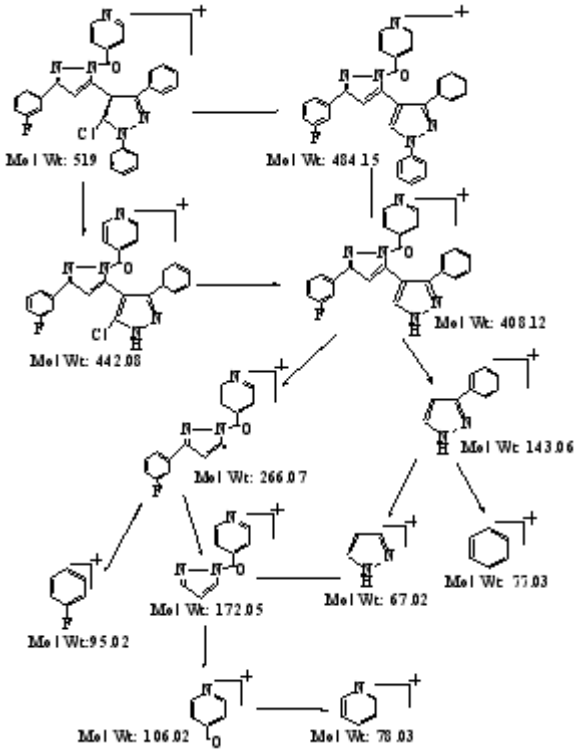


Fig. 14 : *Mass fragmentation pattern of compound 4n*

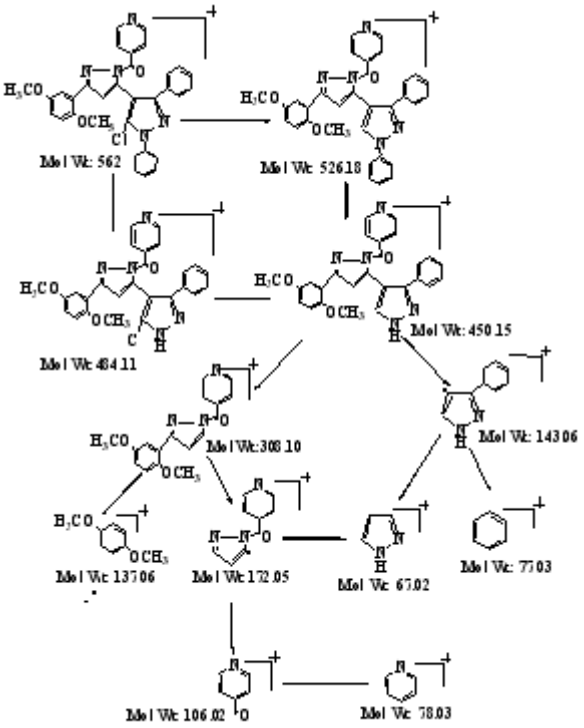


Fig. 15 : *Mass fragmentation pattern of compound 4o*

C-H def.).**^{1}H NMR (**DMSO-d_6**):** *d*5.70 (s,1H,CH-pyrazole), 6.56-6.89 (m,5H,Ar-H), 7.10-7.18 (d,2H,Ar-H pyridine), 7.33-7.55 (m,5H, Ar-H), 7.92-8.12 (t,1H, Ar-H), 8.24-8.32 (dd,2H,Ar-H), 8.39 (s,1H,Ar-H), 8.64-8.78 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 519.0116 (15) $[M]^+$, 520.1367 (5.2) $[M+1]^+$, 521.1298 (5.7) $[M+2]^+$, 266.0721 (100) Fragments: 484.1516 (19), 442.0896 (24), 408.1225 (49), 172.0553 (17), 143.0614 (11), 106.0296 (12), 95.0259 (16), 78.0318 (22), 77.0321 (20), 67.0231 (13). **Elemental analysis:**Calcd. for $C_{30}H_{19}ClFN_5O$: C, 69.30; H, 3.68; N, 13.47. Found: C, 69.28; H, 3.66; N, 13.49%

(5-(5-chloro-1,3-diphenyl-1*H*-pyrazole-4-yl)-3-(2,5-dimethoxyphenyl)-1*H*-pyrazol-1-yl)(pyridine-4-yl) methanone (4o) (MS fragmentation chart in figure 15)

UV l_{max} (Chloroform): 278nm**; FTIR (KBr, cm^{-1}):***v* 3058.89 (Ar C-H, str.), 1643.24 (C=O, str.), 1504.37 (C=N, str.), 1444.58 (Ar C=C, str.), 1344.29 (C-N, str.), 1260.08 (C-O-C asym, str.), 1064.63 (Ar C-Cl, str.), 1026.06 (C-O-C asym, str.), 914.20 (Trisubstituted C-H def.), 761.83 (Monosubstituted C-H def.).**^{1}H NMR (**DMSO-d_6**):** *d* 3.56 (s,6H, OCH_3), 5.74 (s,1H,CH-pyrazole), 6.52-6.78 (m,5H,Ar-H), 6.92-6.94 (d,2H,Ar-H pyridine), 7.09-7.56 (m,5H, Ar-H), 7.73 (s,1H,Ar-H), 8.00-8.13 (d,2H,Ar-H), 8.54-8.63 (d,2H, Ar-H pyridine).**MS (ESI) m/z:** 562.0190 (11.5) $[M]^+$, 563.1533 (4.2) $[M+1]^+$, 564.1564 (4.5) $[M+2]^+$, 308.1042 (100) Fragments: 526.1843 (17), 484.1120 (23.5), 405.1536 (47), 172.0560 (17.5), 143.0629 (11), 137.0610 (17), 106.0243 (14), 78.0304 (21), 77.0321 (19), 67.0256 (12). **Elemental analysis:**Calcd. For $C_{32}H_{24}ClN_5O_3$: C, 68.39; H, 4.30; N, 12.46**.** Found: C, 68.37; H, 4.28; N, 12.43%

Pharmacological Screening

Antitubercular Activity

The newly synthesized compounds were tested for their antitubercular activity against *M. tuberculosis* $H_{37}Rv$ using agar dilution method[12].It is a semi-quantitative method for determination of *in vitro* activity of antitubercular agents, which provides a specific Minimum Inhibitory Concentration (MIC). In dilutions tests, microorganisms were tested for their ability to produce visible growth on series of Middlebrook and Cohn 7H11 agar[13] in quadrant plates containing dilutions of antitubercular agents. DMSO was used as a vehicle and isoniazid was used as standard. All the plates were incubated at 37°C in 5% CO_2 for 4 weeks, after which viable counting was performed. The antitubercular activity exhibited by the compounds is shown in Table-1.

Anticancer Activity

All the newly synthesized pyrazole derivatives were screened for their anticancer activity against MCF7 (Human breast cancer) cell line by SRB (Sulphorodamine B) assay[14-15]. Under mild conditions, SRB binds to protein basic amino acid residue in

Table 1: *Antitubercular Activity of the Synthesized compounds (4a-4o)*

CompoundCode	Percent inhibition of M. tuberculosis (*in vitro*)			
	1.5µg l^{-1}	3.12 µg ml^{-1}	6.25µg ml^{-1}	12.5 µg ml^{-1}
4a	33	40	43	54
4b	47	47	52	59
4c	38	42	45	61
4d	38	47	46	53
4e	41	43	43	52
4f	49	52	65	75
4g	41	46	57	69
4h	46	52	62	73
4i	31	33	41	61
4j	29	34	39	41
4k	31	43	53	65
4l	45	48	61	69
4m	44	44	59	69
4n	47	51	76	88
4o	29	33	45	58
Isoniazid(standard)	87	89	95	99

trichloroacetic acid (TCA) fixed cells to provide sensitive index of cellular protein content that is linear over a cell density. Colour development in SRB assay is rapid, stable and visible. The developed colour can be measured over a broad range of visible wavelength in either a spectrophotometer or a 96 well plate reader. The cell lines were grown in RPMI1640 medium containing 10% foetal bovine serum and 2mM L-glutamine. After cell inoculation, the microtiter plates were incubated at 37° C, 5 % CO_2, 95 % air and 100 % relative humidity for 24 h prior to addition of experimental drugs. Vehicle used was DMSO and adriamycin was used as standard drug. Drug concentration resulting in 50 % reduction in the net protein in control cells during the drug incubation was calculated. Anticancer activity exhibited by the synthesized compounds is shown in Table-2.

Table 2: *Anticancer Activity of Synthesized compounds (4a-4o)*

Human Breast Cancer Cell Line (MCF7) % Control Growth Drug Concentration (µg/ml)				
Compound no.	10	20	40	80
4f	12.7	-3.0	-5.3	-27.9
4g	92.8	85.9	74.6	39.4
4h	43.7	19.1	10.2	2.8
4l	92.7	89.1	81.7	60.2
4n	40.6	21.4	10.3	2.1
Adriamycin	0.2	-10.7	-33.5	-59.9

RESULTS AND DISCUSSION

A series of substituted pyrazole derivatives**(4a-4o)**was prepared by following the **scheme-1**. The physical properties like melting point, R_f value are shown in Table-3. All the newly synthesized derivatives were characterized by FTIR, ^{1}H NMR, Mass spectral studies and Elemental analysis. The synthesized compounds were screened for antitubercular (by Agar dilution test) and anticancer (by Sulphorodamine B assay) activity.

Table 3: *Physical data of the synthesized compounds (4a-4o)*

Compound code	R	Mol. Weight	Melting Point (°C)	% Yield	R_f*
4a	H	501.5	215-216°C	77.0	0.71
4b	*p*-Methoxy	531.5	231-232°C	51.7	0.74
4c	*p*- Hydroxy	517.5	226-227°C	59.6	0.58
4d	*m*- Hydroxy	517.5	235-236°C	63.8	0.71
4e	o- Hydroxy	517.5	216-217°C	71.7	0.63
4f	*p*- Chloro	536.3	234-235°C	76.9	0.67
4g	*p*- Bromo	580.5	246-247°C	66.6	0.69
4h	*p*- Nitro	546.5	218-219°C	73.4	0.67
4i	*p*- Amino	516.5	210-211°C	65.5	0.66
4j	2,5-dihydroxy	533.2	232-233°C	56.2	0.70
4k	o- Amino	516.5	239-240°C	63.2	0.68
4l	o-Chloro	536.1	243-244°C	59.3	0.63
4m	*m*-Bromo	580.5	266-267°C	51.4	0.65
4n	*m*-Fluoro	519.3	259-260°C	67.0	0.71
4o	2,5-dimethoxy	562.5	287-288°C	61.2	0.66

**Mobile phase: n-Butanol: Acetic acid: Water (4:1:5)*

*In vitro*antitubercular screening was done against *M. tuberculosis* H_{37}Rv using agar dilution method. Results (as shown in Table-1) revealed that compound **4n** exhibited promising antitubercular activity with 88% inhibition and found to be most potent. Compounds **4f and 4h** exhibited good activity with 75% and 73% inhibition respectively. The other derivatives showed marked antitubercular activity. The electron withdrawing groups on benzene ring at C_3 of the pyrazole moiety showed promising activity compared to others. The selected derivatives evaluated for anticancer activities were **4f, 4g, 4h, 4l and 4n** (results as shown in Table-2). Compounds **4f**, **4h** and **4n** were active on Human Breast Cancer cell line. Compound **4f** has minimum GI50value among all the tested compounds and was found to be most potent.Other compounds possessed good activity in compare to the standard drug adriamycin.

ACKNOWLEDGEMENT

Authors are thankful to Central Drug Research Institute, Lucknow for performing antitubercular activity and Tata Memorial Centre, Navi Mumbai for performing anticancer activity. Authors are also thankful to the management of Rajiv Academy for Pharmacy, Mathura for providing necessary facilities for synthetic work.

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