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Synthesis, Characterization, anticancer and antifungal studies of Pyrazole carboxamide derivatives

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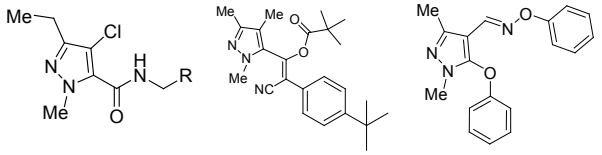
Abstract: We have developed a highly efficient approach for the preparation of pyrazole carboxamide derivatives **7a–7x** and **7a1–7n1** by using pyrazole acid **5a**, amine with HOBt under mild reaction condition. The synthesized compounds were further confirmed by IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. All the synthesized compounds were screened for their biological activity against different strains of fungi and anticancer activity against MCF–7 cell line.

Keywords: Carboxamide, HOBt, amines, peptide coupling, antifungal activity, anticancer activity.

1. Introduction

Pyrazole carboxamides attracted the attention of all chemists in the past few decades because they serve extensively as the key intermediate for the preparation of new biological materials. The pyrazole ring is present in area of medicines[1] and agrichemicals[2] along with it the amide functionality is also one of the most fundamental chemical building blocks found in nature, having wide range of applications.[3] It constitutes the backbone of the biologically crucial proteins, and it is present in a vast number of synthetic structures. Peptides and proteins paved a wide spectrum in living organisms and display a range of properties starting from the potent hormonal activity of small peptides to the structural support and protection for the organisms shown by insoluble proteins. In an industrial context, formation of the amide bonds represents the largest application in reactions in medicinal chemistry. A key step is the formation of the peptide bond, which involves amide bond formation in which the activation of a carboxylic acid group is required, which is usually carried out by using peptide coupling reagent. Amide bonds are generally synthesized from the reaction of carboxylic acids and amines. Amide bond is essential to sustain life, making up the peptide bonds in proteins, such as enzymes.

It is also one of the most prolific moieties in pharmaceutical molecules, agrochemicals and natural products. Usefulness of this amide bond in the preparation of insecticides and acaricides i.e. Tebufenpyrad, [4] Tolfenpyrad, [5] Cyanopyrafen, [6] and Fenpyroximate[7] (Figure 1). The introduction of substituted aliphatic, aromatic and heterocycle rings into pyrazole carboxmide link made possible the discovery of new bioactive products.[8] In particular, highly substituted pyrazoles carboxamides are considerable interest due to their agrochemical and pharmaceutical properties.[9] Because Tebufenpyrad is a commercially available *N*-methylpyrazole derivative that displays important acaricidal activity, we decided to pursue the preparation of substituted *N*-methylpyrazole carboxamides derived from Tebufenpyrad with several different substitution patterns (RF) to replace the tert-butylbenzyl group by aliphatic, aromatic and heterocycles on the carboxamide linkage (**Table** 1 and 2).



R = *p*-t-BuC_6H_4 (Tubefenpyrad)
R = 4-C6H4-O-(4-Me)C6H4 (Tolfenpyrad)

Cyenopyrafen

Fenpyroximate

Figure 1. Biologically active pyrazole carboxamide analogs

Despite of various literature processes available for the synthesis of pyrazole carboxamides, there is an urgent need for efficient methodology which starts from inexpensive and readily available starting materials for their synthetic interest ot synthetic chemists. In past few decades, construction of molecular libraries or heterocyclic via peptide coupling reactions has been attracted growing interest in organic synthesis due to their degree of atom economy, convergence, productivity and importance of their activity in routine life.

Pyrazoles and its derivatives has been attracted much attention due to their presence in biologically active natural products, agrochemicals and pharmaceuticals. Among these substituted pyrazole carboxamides are plays significant role in the development of biologically active molecules.

In 2007, Tobias Persson and co-worker has been reported that MCF-7 breast cancer cells after incubation with carboxamide (**Figure** 2), [10] exposing a lipophilic appendage was the most promising demonstration of inhibitory activity.

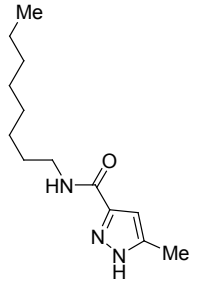


Figure 2

In 2007, Ding et al has been reported that series of novel 3-aryl-1-arylmethyl-1Hpyrazole-5-carboxamide derivatives which could suppress A549 lung cancer cell growth (**Figure** 3).[11]

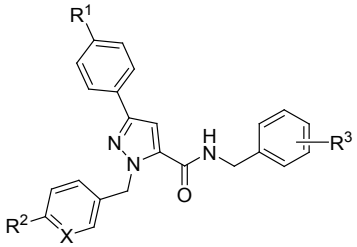


Figure 3

Pyrazoles and its derivatives has been attracted much attention due to their presence in biologically active natural products, agrochemicals and pharmaceuticals. Among these substituted pyrazole carboxamides are

plays significant role in the development of biologically active molecules.

2. Materials and Methods

General

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$/DMSO-d_6. Chemical shifts for carbons are reported in ppm from TMS and are referenced to the carbon resonance of the solvent. Data are reported as follows: chemical shift, multiplicity (s = singlet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet), coupling constants in Hertz, and integration. Mass spectra were recorded with electro spray mass spectrometer. FTIR spectra were recorded with a Perkin Elmer spectrum 65 FTIR spectrometer using KBr plates, and characteristic wave numbers are given in cm^{-1}. TLC analysis was performed on commercially prepared 60 F_{254} silica gel plates and visualized by either UV irradiation or by staining with I_2 and ninhydrin stain. All purchased chemicals were used as received. All melting points are done by using M-565, Buchi melting point apparatuses and are uncorrected.

2.1 General procedure for the synthesis of Carboxamides

The starting materials were prepared by reported procedure. The structure and purity of known material and were confirmed by their physical and spectral data (^{1}H NMR and ^{13}C NMR) with those reported in literature.

To a solution of (400 mg, 2.127mmol) **4-Chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid (5a)** in 10 mL of DMF was treated sequentially with (358.18 mg, 2.339 mmol) of HOBT, (363.23 mg, 2.339 mmol) of EDC.HCl and (188 mg, 2.02 mmol) of aniline. The suspension was stirred for 15 min at 5 ºC, (0.89 mL, 6.381 mmol) of TEA was added, and the mixture stirred at r.t. under N_2 atmosphere for 8 -10 h. The reaction was quenched by pouring into 100 mL of Water and extracted with 50x2 mL of ethyl acetate, wash the organic layer wash with 1% HCl Solution and 100x3 mL of saturated aqueous $NaHCO_3$, 50 mL of brine wash to the organic layer, the final organic layer was dried over anhydrous Na_2SO_4, filters and concentrated under reduced pressure. The crude material was purified by column chromatography on silica gel (15 g, 20:80 ethyl acetate/hexanes) to provide 430 mg (76.8%) of product as a off-white needles.

Characterization of Representative 4-Chloro-3-ethyl-1-methyl-*N*-o-tolyl-1*H*-pyrazole-5-carboxamide (7j):

Compound **7j** was synthesized by **4-Chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid** with o–Toludine inpresence of base and peptide coupling reagents. The product was obtained as a colorless needles in 70.30% yield with m.p. 122–123 °C. The structure of compound **7j** was established on the basis of spectral data analysis. In the ^{1}H NMR spectrum in $CDCl_3$ at 400 MHz, the characteristic peak of amide linkage appeared at δ 8.32 ppm, a singlet appeared at δ 4.19 ppm corresponds to pyrazole –NMe, aromatic –Me group proton appeared at δ 2.37 ppm and Ethyl group of –Me proton of pyrazole ring appeared at δ 1.29 ppm respectively. Similarly in the ^{13}C NMR spectrum (DMSO-d_6, 100 MHz), the characteristic peak corresponds to Carbonyl attched to –NH group of toluene appeared at δ 157.01 ppm, another carbon corresponds to corbonyl attched pyrazole ring appeared at δ 132.51 ppm and -NMe of pyrazole carbon appears at δ 38.65 ppm, pyrazole ring carbon appeared at δ 148.41 ppm, aromatic –Me group of toluene appeared at δ 18.03 ppm and pyrazole ethyl of –Me carbon C_1appeared at δ 12.73 ppm respectively confirmed the coupling of pyrazole acid with amine of o-Toluidine. The peaks of all

other protons and carbons of the molecules were presented in ^{1}H NMR and ^{13}C NMR spectra of the molecule. Its mass specturm showed $[M+H]^+$ peak at 278.1132, which confirmed its molecular formula to be $C_{14}H_{16}ClN_3O$.

4-Chloro-3-ethyl-1-methyl-*N*-phenyl-1*H*-pyrazole-5-carboxamide (7a): The product was obtained as a Off-white needles (430 mg, 76.84% yield); m.p. 112–113 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (br s, 1H), 7.62 (d, *J* = 7.6 Hz, 2H,), 7.40 (d, J = 8.4 Hz, 2H), 7.20 (dd, *J* = 7.2 Hz, 2H), 4. 17 (s, 3H), 2.70 (q, *J* = 7.6 Hz, 2H), 1.29 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.75, 18.61, 38.65, 106.78, 125.38, 126.47, 130.51, 132.78, 134.73, 148.42, 157.12 ppm. HRMS (ESI): calcd. for $C_{13}H_{14}ClN_3O$ $[M+H]^+$ 263.0825; found 264.1169. IR (KBr, cm^{-1}): ν_{max}= 3671, 2974, 2383, 1615, 1396, 1059, 882.

4-Chloro-3-ethyl-1-methyl-*N*-(2-methyl-4-(perfluoropropan-2-yl)phenyl)-1*H*-pyrazole-5-carboxamide(7b): The product was obtained as a light yellow needles (600 mg, 63.33% yield); m.p. 101–102 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (br s, 1H), 8.34 (d, *J* = 8.8 Hz, 1H,), 7.51 (d, J = 9.2 Hz, 1H), 7.46 (s, 1H), 4. 19 (s, 3H), 2.71 (q, *J* = 7.6 Hz, 2H), 2.44 (s, 3H), 1.30 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.78, 18.13, 18.76, 38.72, 103.59, 106.75, 125.34, 126.73, 130.39, 132.72, 134.67, 135.72, 136.41, 136. 53, 148.42, 157.79 ppm. HRMS (ESI): calcd. for C17H15ClF7N3O $[M+H]^+$ 445.0792; found 446.1416. IR (KBr, cm^{-1}): ν_{max}= 3674, 2976, 2382, 1613, 1398, 1372, 1058, 882.

4-Chloro-3-ethyl-1-methyl-*N*-(4-nitrophenyl)-1*H*-pyrazole-5-carboxamide(7c):The product was obtained as a yellow needles (470 mg, 71.72% yield); m.p. 193–194 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (br s, 1H), 8.28 (d, *J* = 5.2 Hz, 2H,), 7.83 (d, J = 5.2 Hz, 2H), 4.19 (s, 3H), 2.71 (q, *J* = 7.6 Hz, 2H), 1.29 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.98, 18.23, 39.13, 107.52, 119.04, 123.81, 132.51, 142.56, 148.59, 156.70 ppm. HRMS (ESI): calcd. for $C_{13}H_{13}ClN_4O_3$ $[M+H]^+$ 308.0676; found 309.1623. IR (KBr, cm^{-1}): ν_{max}= 3676, 2975, 2381, 1649, 1613, 1397, 1059, 886.

***N*-(4-bromophenyl)-4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxamide(7d)**:The product was obtained as a colorless needles (510 mg, 70.30% yield); m.p. 175–176 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (br s, 1H), 7.53 (m, 4H,), 4.16 (s, 3H), 2.69 (q, *J* = 7.6 Hz, 2H), 1.28 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.73, 18.63, 38.50, 107.01, 116.12, 121.79, 131.72, 134.57, 137.1, 148.51, 156.94 ppm. HRMS (ESI): calcd. for $C_{13}H_{13}BrClN_3O$ $[M+H]^+$ 340.9931; found 343.9873. IR (KBr, cm^{-1}): ν_{max}= 3673, 2974, 2380, 1613, 1649, 1397, 1059, 886, 659.

4-Chloro-3-ethyl-1-methyl-*N*-(2-(trifluoromethoxy)phenyl)-1*H*-pyrazole-5-carboxamide(7e): The product was obtained as a light yellow needles (430 mg, 76.84% yield); m.p. 83–84 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.01 (br s, 1H), 8.53 (d, *J* = 8.4 Hz, 1H,), 7.36 (d, J = 8.4 Hz, 2H), 7.19 (d, *J* = 8.4 Hz, 1H), 4. 20 (s, 3H), 2.71 (q, *J* = 7.6 Hz, 2H), 1.29 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.72, 18.65, 38.56, 107.15, 118.12, 122.79, 123.43, 131.12, 134.56, 135.21, 136.32, 137.23, 148.56, 156.74 ppm. HRMS (ESI): calcd. for $C_{14}H_{13}ClN_3O_2$ $[M+H]^+$ 347.0648; found 348.1624. IR (KBr, cm^{-1}): ν_{max}= 3671, 2973, 2310, 1643, 1650, 1397, 1249, 1069, 856.

4-Chloro-3-ethyl-1-methyl-*N*-(pyridine-3-yl)-1*H*-pyrazole-5-carboxamide(7f):The product was obtained as a Brown colour needles (325 mg, 75.98% yield); m.p. 148–149 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (br s, 1H), 7.92 (s, 1H), 7.40 (d, *J* = 7.6 Hz, 1H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.20 (dd, *J* = 7.2 Hz, 2H), 4. 17

(s, 3H), 2.70 (q, *J* = 7.6 Hz, 2H), 1.29 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.76, 18.21, 38.63, 107.91, 125.24, 126.57, 130.56, 132.88, 134.73, 148.42, 157.72 ppm. HRMS (ESI): calcd. for $C_{12}H_{13}ClN_4O$ $[M+H]^+$ 264.0778; found 265.0781. IR (KBr, cm^{-1}): ν_{max}= 3675, 2971, 2384, 1617, 1643, 1396, 1059, 881.

4-Chloro-3-ethyl-1-methyl-*N*-(2,4-dimethylphenyl)-1*H*-pyrazole-5-carboxamide(7g): The product was obtained as a Off-white needles (525 mg, 84.77% yield); m.p. 116–117 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (br s, 1H), 7.84 (d, *J* = 8.8 Hz, 1H,), 7.07 (m, 2H), 4.18 (s, 3H), 2.70 (q, *J* = 7.6 Hz, 2H), 2.32 (s, 6H), 1.29 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.78, 18.31, 18.68, 38.42, 106.47, 127.17, 127.88, 128.32, 132.17, 133.01, 135.1, 148.43, 157.21 ppm. HRMS (ESI): calcd. for $C_{15}H_{18}ClN_3O$ $[M+H]^+$ 291.1138; found 292.1232. IR (KBr, cm^{-1}): ν_{max}= 3670, 2973, 2379, 1614, 1392, 1058, 884.

4-Chloro-*N*-(2-chloro-5-methylphenyl)-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxamide(7h): The product was obtained as a Off-white needles (410 mg, 61.96% yield); m.p. 149–150 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (br s, 1H), 8.21 (d, *J* = 2.0 Hz, 1H,), 7.14 (m, 1H), 7.10 (m, 1H), 4.19 (s, 3H), 2.71 (q, *J* = 7.6 Hz, 2H), 2.33 (s, 3H), 1.29 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.79, 17.82, 19.28, 41.12, 107.62, 122.17, 125.14, 126.46, 130.96, 131.40, 132.25, 136.28, 149.67, 156.46 ppm. HRMS (ESI): calcd. for $C_{14}H_{15}Cl_2N_3O$ $[M+H]^+$ 311.0592; found 312.1329. IR (KBr, cm^{-1}): ν_{max}= 3673, 2984, 2373, 1613, 1396, 1057, 798, 880.

4-Chloro-*N*-(2-chloro-4-nitrophenyl)-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxamide(7k): The product was obtained as a yellow needles (310 mg, 42.60% yield); m.p. 106–107 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.76 (br s, 1H), 8.21 (s, 1H,), 7.86 (d, *J* = 7.6 Hz, 1H), 7.37 (d, *J* = 7.6 Hz, 1H), 4.09 (s, 3H), 2.72 (q, *J* = 7.6 Hz, 2H), 1.28 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.78, 17.89, 41.12, 108.61, 124.18, 127.19, 129.46, 130.92, 131.47, 135.28, 136.29, 149.67, 157.46 ppm. HRMS (ESI): calcd. for $C_{13}H_{12}Cl_2N_4O_3$ $[M+H]^+$ 342.0286; found 343.0287. IR (KBr, cm^{-1}): ν_{max}= 3671, 2982, 2363, 1623, 1593, 1386, 1058, 791, 882.

4-Chloro-3-ethyl-1-methyl-*N*-(2-nitrophenyl)-1*H*-pyrazole-5-carboxamide(7l):The product was obtained as a yellow needles (4350 mg, 53.40% yield); m.p. 120–121 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 8.74 (d, *J* = 7.6 Hz, 1H,), 7.24 (d, *J* = 6.8 Hz, 1H), 7.69 (m, 1H), 7.25 (m, 1H), 4.14 (s, 3H), 2.71 (q, *J* = 7.6 Hz, 2H), 1.29 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.97, 18.22, 39.15, 107.56, 119.13, 125.82, 132.53, 143.57, 147.58, 157.79 ppm. HRMS (ESI): calcd. for $C_{13}H_{13}ClN_4O_3$ $[M+H]^+$ 308.0676; found 309.1623. IR (KBr, cm^{-1}): ν_{max}= 3677, 2975, 2382, 1612, 1647, 1396, 1059, 887.

4-Chloro-3-ethyl-1-methyl-*N*,*N*-diphenyl-1*H*-pyrazole-5-carboxamide(7m): The product was obtained as a Off-white needles (450 mg, 62.38% yield); m.p. 172–173 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (br s, 1H), 7.61 (d, *J* = 7.6 Hz, 4H,), 7.42 (d, *J* = 7.6 Hz, 4H), 7.20 (dd, *J* = 7.2 Hz, 2H), 4. 27 (s, 3H), 2.71 (q, *J* = 7.6 Hz, 2H), 1.28 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.75, 18.61, 38.65, 107.68, 124.98, 126.32, 131.52, 133.68, 134.43, 148.72, 156.92 ppm. HRMS (ESI): calcd. for $C_{19}H_{18}ClN_3O$ $[M+H]^+$ 339.1138; found 340.1142. IR (KBr, cm^{-1}): ν_{max}= 3672, 2974, 2378, 1611, 1393, 1057, 881.

4-Chloro-3-ethyl-*N*-(2-ethyl-6-methylphenyl)-1-methyl-1*H*-pyrazole-5-carboxamide(7n):The product was obtained as a white needles (425 mg, 65.48% yield); m.p.

97–98 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (br s, 1H), 7.22 (m, 1H,), 7.14 (m, 2H), 4.15 (s, 3H), 2.72 (q, *J* = 7.6 Hz, 2H), 2.29 (s, 3H), 1.31 (t, *J* = 7.6 Hz, 3H), 1.23 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.78, 14.511, 18.32, 21.01, 24.43, 38.42, 106.49, 128.17, 127.86, 128.42, 131.27, 133.41, 135.32, 147.43, 158.21 ppm. HRMS (ESI): calcd. for C16H20ClN3O $[M+H]^+$ 305.1295; found 306.1301. IR (KBr, cm^{-1}): ν_{max}= 3671, 2972, 2381, 1612, 1393, 1059, 887.

4-(4-Chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxamido)phenyl4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylate(7o):The product was obtained as a Brown colour needles (415 mg, 43.44% yield); m.p. 200–201 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.48 (br s, 1H), 7.71 (d, *J* = 8.8 Hz, 2H,), 7.25 (d, *J* = 8.4 Hz, 2H), 4. 18 (s, 6H), 2.72 (q, *J* = 7.6 Hz, 4H), 1.29 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.74, 18.03, 18.48, 38.07, 38.65, 107.32, 106.72, 121.12, 125.47, 127.93, 132.51, 133.79, 135.53, 136.32, 147.19, 148.41, 149.32, 157.23 ppm. HRMS (ESI): calcd. for $C_{20}H_{21}Cl_2N_5O_3$ $[M+H]^+$ 449.1021; found 450.1023. IR (KBr, cm^{-1}): ν_{max}= 3672, 2972, 2383, 1735, 1613, 1397, 1145, 1058, 887.

4-Chloro-3-ethyl-1-methyl-*N*-*p*-tolyl-1*H*-pyrazole-5-carboxamide (7p): The product was obtained as a white needles (420 mg, 71.25% yield); m.p. 117–118 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (br s, 1H), 7.50 (d, *J* = 8.4 Hz, 2H,), 7.19 (d, *J* = 8.0 Hz, 2H), 4. 17 (s, 3H), 2.69 (q, *J* = 7.6 Hz, 2H), 2.34 (s, 3H), 1.28 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.72, 18.01, 18.67, 38.66, 106.32, 125.34, 126.41, 130.48, 132.52, 134.57, 148.46, 157.15 ppm. HRMS (ESI): calcd. for $C_{14}H_{16}ClN_3O$ $[M+H]^+$ 277.0982; found 278.0983. IR (KBr, cm^{-1}): ν_{max}= 3671, 2973, 2379, 1616, 1398, 1058, 878.

4-Chloro-3-ethyl-*N*,1-dimethyl-*N*-phenyl-1*H*-pyrazole-5-carboxamide(7q): The product was obtained as a brown oil (430 mg, 72.94% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.40 (br s, 1H), 7.53 (d, *J* = 7.6 Hz, 2H,), 7.32 (d, J = 8.4 Hz, 2H), 7.05 (dd, *J* = 7.2 Hz, 1H), 4. 18 (s, 3H), 2.81 (s, 3H), 2.70 (q, *J* = 7.6 Hz, 2H), 1.29 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.74, 18.65, 28.16, 38.25, 106.38, 125.36, 126.23, 130.59, 132.18, 134.23, 148.52, 157.02 ppm. HRMS (ESI): calcd. for $C_{14}H_{16}ClN_3O$ $[M+H]^+$ 277.0982; found 278.0991. IR (KBr, cm^{-1}): ν_{max}= 3672, 2964, 2382, 1613, 1394, 1052, 881.

4-Chloro-3-ethyl-*N*-(5-(trifluoromethyl)-2-methylphenyl)-1-methyl-1*H*-pyrazole-5-carboxamide (7r): The product was obtained as a white needles (372 mg, 50.67% yield); m.p. 137–138 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.44 (s, 1H), 8.42 (br s, 1H), 7.37 (m, 2H), 4.20 (s, 3H), 2.71 (q, *J* = 7.6 Hz, 2H), 2.42 (s, 3H), 1.30 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.71, 18.07, 18.65, 38.87, 107.10, 121.19, 121.22, 122.33, 122.37, 131.56, 134.02, 136.10, 136.95, 148.50, 157.22 ppm. HRMS (ESI): calcd. for $C_{15}H_{15}ClF_3N_3O$ $[M+H]^+$ 345.0856; found 346.0859. IR (KBr, cm^{-1}): ν_{max}= 3671, 2971, 2381, 1612, 1393, 1145, 1059, 886.

4-Chloro-3-ethyl-*N*-(4-(trifluoromethyl)-2-methylphenyl)-1-methyl-1*H*-pyrazole-5-carboxamide (7s): The product was obtained as a white needles (360 mg, 49.03% yield); m.p. 118–119 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (br s, 1H), 8.33 (d, *J* = 8.4 Hz, 1H), 7.53 (d, *J* = 8.8 Hz, 1H), 7.49 (s, 1H), 4.19 (s, 3H), 2.71 (q, *J* = 7.6 Hz, 2H), 2.43 (s, 3H), 1.30 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.72, 18.07, 18.66, 38.84, 107.11, 121.18, 121.23, 122.21, 122.41, 131.42, 134.10, 136.09, 136.94, 148.48, 157.19 ppm. HRMS (ESI): calcd. for $C_{15}H_{15}ClF_3N_3O$ $[M+H]^+$ 345.0856; found 346.0859. IR (KBr, cm^{-1}): ν_{max}= 3671, 2969, 2382, 1614, 1393, 1145, 1058, 887.

4-Chloro-3-ethyl-*N*-(4-(trifluoromethyl) phenyl)-1-methyl-1*H*-pyrazole-5-carboxamide(7t): The product was obtained as a white needles (215 mg, 30.52% yield); m.p. 168–169 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.73 (br s, 1H), 8.21 (d, *J* = 7.2 Hz, 2H,), 7.85 (d, *J* = 7.2 Hz, 2H), 4.19 (s, 3H), 2.72 (q, *J* = 7.6 Hz, 2H), 1.28 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.97, 18.24, 39.15, 107.52, 121.04, 123.81, 125.15, 126.37, 132.52, 142.57, 148.58, 156.71 ppm. HRMS (ESI): calcd. for $C_{14}H_{13}ClF_3N_3O$ $[M+H]^+$ 331.0699; found 332.0702. IR (KBr, cm^{-1}): ν_{max}= 3672, 2971, 2378, 1616, 13955, 1146, 1057, 887.

4-Chloro-*N*-(3-chloro-2-methylphenyl)-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxamide(7u): The product was obtained as a Off-white needles (500 mg, 75.56% yield); m.p. 167–168 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (br s, 1H), 7.85 (d, *J* = 7.6 Hz, 1H,), 7.27 (m, 1H), 7.21 (m, 1H), 4.18 (s, 3H), 2.71 (q, *J* = 7.6 Hz, 2H), 2.42 (s, 3H), 1.30 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.78, 17.83, 19.29, 41.32, 107.63, 122.27, 125.04, 126.45, 130.97, 131.41, 132.26, 136.30, 149.68, 156.47 ppm. HRMS (ESI): calcd. for $C_{14}H_{15}Cl_2N_3O$ $[M+H]^+$ 311.0592; found 312.1329. IR (KBr, cm^{-1}): ν_{max}= 3672, 2983, 2372, 1614, 1397, 1058, 796, 882.

4-Chloro-3-ethyl-1-methyl-*N*-(2,6-dimethylphenyl)-1*H*-pyrazole-5-carboxamide(7v): The product was obtained as a Off-white needles (517 mg, 83.48% yield); m.p. 118–119 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (br s, 1H), 7.16 (m, 3H,), 4. 15 (s, 3H), 2.70 (q, *J* = 7.6 Hz, 2H), 2.30 (s, 6H), 1.30 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.78, 18.30, 18.67, 38.42, 106.41, 127.17, 127.88, 133.99, 135.0, 135.34, 148.45, 157.12 ppm. HRMS (ESI): calcd. for $C_{15}H_{18}ClN_3O$ $[M+H]^+$ 291.1138; found 292.1232. IR (KBr, cm^{-1}): ν_{max}= 3672, 2974, 2378, 1615, 1394, 1056, 887.

4-Chloro-3-ethyl-1-methyl-*N*-(2,5-dimethylphenyl)-1*H*-pyrazole-5-carboxamide(7w):The product was obtained as a Off-white needles (471 mg, 76.1% yield); m.p. 120–121 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (br s, 1H), 7.16 (m, 3H,), 4. 15 (s, 3H), 2.70 (q, *J* = 7.6 Hz, 2H), 2.30 (s, 6H), 1.30 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.79, 18.33, 18.78, 38.32, 106.51, 121.79, 127.18, 127.76, 128.31, 132.20, 133.11, 135.21, 148.41, 157.23 ppm. HRMS (ESI): calcd. for $C_{15}H_{18}ClN_3O$ $[M+H]^+$ 291.1138; found 292.1232. IR (KBr, cm^{-1}): ν_{max}= 3671, 2973, 2378, 1612, 1393, 1058, 883.

4-benzyl-4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxamide (7a1): The product was obtained as a white needles (480 mg, 81.44% yield); m.p. 90–91 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (m, 5H), 7.03 (br s, 1H,), 4.65 (d, J = 6.0 Hz, 2H), 4. 14 (s, 3H), 2.65 (q, *J* = 7.6 Hz, 2H), 1.25 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): 12.72, 18.63, 38.64, 42.51, 106.31, 126.91, 127.16, 128.35, 134.17, 138.68, 148.34, 158.42 ppm. HRMS (ESI): calcd. for $C_{14}H_{16}ClN_3O$ $[M+H]^+$ 277.0982; found 278.1316. IR (KBr, cm^{-1}): ν_{max}= 3372, 2923, 2249, 1645, 1350, 1058, 883.

4-Chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-yl(pyrrolidine-1-yl)methanone(7b1):The product was obtained as a light brown oil (420 mg, 81.90% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.82 (s, 3H), 3.65 (t, *J* = 6.8 Hz, 2H,), 3.49 (t, J = 6.8 Hz, 2H), 2.63 (q, J = 7.6 Hz, 2H), 2.00 (m, 4H), 1.24 (t, *J* = 7.6 Hz, 3H) ppm. HRMS (ESI): calcd. for $C_{11}H_{16}ClN_3O$ $[M+H]^+$ 241.0982; found 242.1732. IR (KBr, cm^{-1}): ν_{max}= 3371, 2924, 2245, 1681, 1351, 1059, 885.

4-Chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-yl(piperidin-1-yl)methanone(7c1): The product was obtained as a light yellow oil (450 mg, 82.92% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.82 (s, 3H), 3.71 (br s, 2H,), 3.37

(br s, 2H), 2.63 (q, J = 7.6 Hz, 2H), 1.67 (br s, 6H), 1.25 (t, *J* = 7.6 Hz, 3H) ppm. HRMS (ESI): calcd. for $C_{12}H_{18}ClN_3O$ $[M+H]^+$ 255.1138; found 256.2103. IR (KBr, cm^{-1}): ν_{max}= 3373, 2921, 2247, 1679, 1353, 1057, 884.

4-Chloro-3-ethyl-1-methyl-*N*-propyl-1*H*-pyrazole-5-carboxamide (7d1): The product was obtained as a light yellow needles (415 mg, 85.15% yield); m.p. 62–63 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.69 (br s, 1H), 4.12 (s, 3H,), 3.43 (q, *J* = 6.8 Hz, 2H), 2.66 (q, *J* = 7.6 Hz, 2H), 1.69 (q, *J* = 10.4 Hz, 2H), 1.25 (t, *J* = 7.6 Hz, 3H), 1.02 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 11.33, 12.72, 18.62, 22.11, 38.44, 40.67, 105.98, 134.66, 148.23, 158.22 ppm. HRMS (ESI): calcd. for $C_{10}H_{16}ClN_3O$ $[M+H]^+$ 229.0982; found 230.1523. IR (KBr, cm^{-1}): ν_{max}= 3671, 2973, 2384, 1650, 1615, 1398, 1058, 887.

***N,N*-dibutyl-4-Chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxamide(7e1)**: The product was obtained as a brown oil (540 mg, 84.85% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.10 (s, 3H,), 3.01 (q, *J* = 6.8 Hz, 4H), 2.65 (q, *J* = 6.8 Hz, 2H), 1.63 (m, 4H), 1.42 (m, 4H), 1.23 (t, *J* = 7.6 Hz, 3H), 0.97 (t, 6H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 11.34, 12.72, 18.64, 20.21, 38.47, 41.63, 106.95, 134.69, 148.25, 159.23 ppm.. HRMS (ESI): calcd. for $C_{15}H_{26}ClN_3O$ $[M+H]^+$ 299.1764; found 300.2132. IR (KBr, cm^{-1}): ν_{max}= 3668, 2972, 2376, 1649, 1613, 1389, 1052, 882.

2-(4-Chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxamido)ethyl4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylate (7g1):The product was obtained as a white needles (385 mg, 45.12% yield); m.p. 99–100 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.11 (br s, 1H), 4.50 (t, *J* = 4.8 Hz, 2H), 4.11 (s, 6H,), 3.88 (q, *J* = 5.6 Hz, 2H), 2.66 (m, 4H), 1.24 (t, *J* = 7.6 Hz, 6H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 12.76, 18.03, 18.498, 36.67, 38.07, 38.65, 62.89, 107.32, 109.72, 132.15, 136.18, 147.18, 148.43, 149.31, 157.28 ppm. HRMS (ESI): calcd. for $C_{16}H_{21}Cl_2N_5O_3$ $[M+H]^+$ 401.1021; found 402.1023. IR (KBr, cm^{-1}): ν_{max}= 3676, 2972, 2381, 1648, 1613, 1398, 1059, 886.

4-benzyl-4-chloro-3-ethyl-*N*,1-dimethyl-1*H*-pyrazole-5-carboxamide(7h1): The product was obtained as a yellow needles (415 mg, 67.01% yield); m.p. 96–97 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (m, 5H), 4.21 (s, 3H), 4. 14 (s, 3H), 2.63 (q, *J* = 7.6 Hz, 2H), 1.25 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): 12.74, 18.65, 38.62, 42.53, 45.32, 106.34, 126.97, 127.18, 128.34, 134.27, 138.72, 148.35, 158.43 ppm. HRMS (ESI): calcd. for $C_{15}H_{18}ClN_3O$ $[M+H]^+$ 291.1138; found 292.1141. IR (KBr, cm^{-1}): ν_{max}= 3372, 2923, 2248, 1649, 1351, 1058, 885.

4-Chloro-3-ethyl-*N,N*,1-trimethyl-1*H*-pyrazole-5-carboxamide(7k1):The product was obtained as a brown oil (315 mg, 68.84% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.82 (s, 3H,), 3.12 (s, 3H), 3.05 (s, 3H), 2.63 (q, *J* = 7.6 Hz, 2H), 1.25 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 11.39, 12.71, 36.44, 40.68, 105.98, 134.69, 148.24, 158.23 ppm. HRMS (ESI): calcd. for $C_9H_{14}ClN_3O$ $[M+H]^+$ 215.0825; found 216.0825.

N-butyl-4-Chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxamide (7n1): The product was obtained as a yellow needles (350 mg, 67.76% yield); m.p. 47–48 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.67 (br s, 1H), 4.10 (s, 3H,), 3.45 (q, *J* = 6.8 Hz, 2H), 2.64 (q, *J* = 7.6 Hz, 2H), 1.62 (m, 2H), 1.43 (m, 2H), 1.23 (t, *J* = 7.6 Hz, 3H), 0.98 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 11.32, 12.71, 18.64, 22.21, 32.63, 38.47, 40.63, 105.97, 134.69, 148.22, 158.25 ppm. HRMS (ESI): calcd. for $C_{11}H_{18}ClN_3O$ $[M+H]^+$ 243.1138; found 244.1139. IR (KBr, cm^{-1}): ν_{max}= 3671, 2973, 2382, 1649, 1616, 1396, 1059, 882.

4-Chloro-3-ethyl-1-methyl-*N*-(2-methylthio)propan-2-yl)-1*H*-pyrazole-5-carboxamide(7o1): The product was obtained as a light yellow oil (435 mg, 70.73% yield); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.69 (br s, 1H), 4.08 (s, 3H,), 3.03 (s, 2H), 2.65 (q, *J* = 7.6 Hz, 2H), 2.15 (s, 2H), 1.50 (s, 6H), 1.25 (t, *J* = 7.6 Hz, 3H) ppm. ^{13}C NMR (100 MHz, DMSO–d_6): δ 11.31, 12.74, 17.14, 27.21, 38.47, 40.63, 42.76, 105.96, 131.68, 147.27, 158.29 ppm. HRMS (ESI): calcd. for $C_{12}H_{20}ClN_3OS$ $[M+H]^+$ 289.1016; found 290.1023. IR (KBr, cm^{-1}): ν_{max} = 3659, 2963, 2381, 1651, 1615, 1397, 1058, 880.

Antifungal activity

The *in vitro* antifungal activities of synthesized pyrazole carboxamides were carried out at Department of Microbiology, R.C. Patel Arts commerce and Science College Shirpur, Dhule (Maharashtra). Antifungal activity of all the synthesized pyrazole carboxamides were evaluated against *F. moniliformis (*NFCCI 2949), *C.lunata (*MTCC 2033*)* and *G.cingulata* fungi strains at 100 µg/mL concentration by agar disc diffusion method, [12] and the zone of inhibition reported in mm. Cultures used were obtained from National collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune (India). Potato Dextrose Agar microbiological media used for fungi (*F. moniliformis*, *C.lunata* and *G.cingulata*) was obtained from Hi-media and its compostion (grams per litre) has Potato infusion form, 200.0; Glucose, 10.0; 0 (pH 5.6). Stock solution [1000 microgram per ml] of each compound was prepared in DMSO. Assay carried out by taking concentration 100 µg/mL disk. Hi-media antibiotics disk: Amphotericin-B (100 µg/mL) moistened with water are used as standard. The zone of inhibition was measured in millimeter (mm) for **7a–7x** and **7a1–7n1**.

Anticancer activity

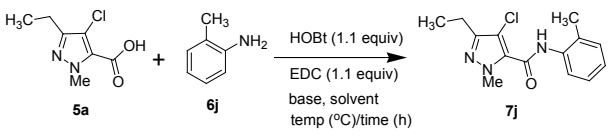
In *vitro* anticancer activities of synthesized compounds were investigated at Anticancer Drug Screening Facility, Tata Memorial Advanced centre for Treatment, Research and Education in Cancer (ACTREC) Kharghar, Navi Mumbai, India.

The *in vitro* anticancer activity of compounds **7b, 7c, 7e, 7x** and **7d1** were performed on the breast cancer cell line MCF-7 at four dose levels 10,20, 40 and 80 µg/L in DMSO, the test consisted of 48 h continuous drug exposure protocol using sulforhodamine B (SRB) assay to estimate cell growth. [13] This assay relies on the uptake of the negatively charged pink aminoxanthine dye, sulforhodamine B (SRB) by basic amino acids in the cells. The greater the number of cells, the greater amount of dye is taken up and, after fixing, when the cells are analyzed, the released dye gives a greater absorbance. The SRB assay was found to be more reliable, sensitive, simple, reproducible and more rapid than the formazan-based assay and gives better results.[14]

3. Results and Discussion

In order to find the optimal reaction for proposed peptide coupling reaction, we analyzed various factors affecting reaction by using 4-Chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid **5a** with *o*–Toludine inpresence of 1.1 mol eq of HOBt and 1.1 mole eq of EDC as the couling reagents and Et_3N as base at rt under N_2 atmophere in DMF for 8–10 hrs. This set of condition afforded desired product **7j** in good yield (**Table** 1, entry 1).

Table 1. Optimization of the reaction conditions[a]



entry	Catalyst/reagent	base (equiv)	solvent	(°C)/(h)	Yield (%)[b]
1	HOBt/EDC.HCl	Et3N (3)	THF	rt/6	51%
2	HOBt/EDC.HCl	Et3N (3)	MDC	rt/6	45%
3	HOBt/EDC.HCl	DIPEA(3)	DMF	rt/8	65%
4	EDC.HCl	Et3N(3)	DMF	rt/8	24%
5	HOBt/EDC.HCl	Et3N (3)	Dioxane	rt/8	54%
6	**HOBt/EDC.HCl**	**Et3N (3)**	**DMF**	**rt/8**	**77%**
7	HATU [c]	Et3N (3)	MDC	rt/8	35%
8	HOBt/EDC.HCl	Et3N (3)	THF	rt/10	52%
9	HATU	Et3N (3)	DMF	rt/8	50%
10	HOBt/EDC.HCl	Na_2CO3(2.5)	Dioxane	rt/8	00
11	HOBt/EDC.HCl	K3PO4.3H2O (2)	Dioxane	rt/8	00
12	HOBt/EDC.HCl	Et3N (3)	EDC	rt/8	52%

[a] Reaction were performed using 1.0 equiv of acid **5**, 0.95 equiv of amine **6j**, base (3.0 equiv) in 10 mL of solvent under N_2 atmosphere. [b] Isolated yields. [c] HATU as peptide reagent.

Various bases like K_3PO_4. $3H_2O$ and Na_2CO_3 were failed to afford the desired product **7j** in good yields (**Table** 1, entry **10-11**). Whereas changes of solvents from THF, MDC, dioxane, to DMF was found effective (**Table** 1, entry **1-2,4-6**) where as only EDC.HCl used a peptide reagent with DMF to get poor yield with recimized products observed .when changed the peptide reagent to get a lower yields (**Table** 1, entries **7,8,9**).

3.1 Scope and Limitation of the Reaction

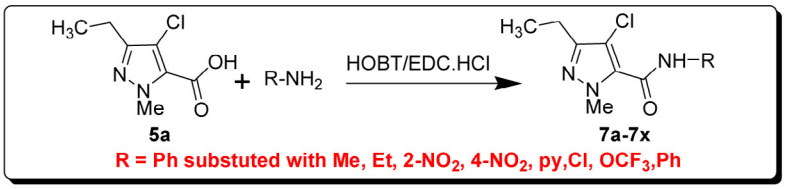
With this standard protocol in hand (**Table** 2, Entry **1–24**), we examined the scope and generality of the reaction with a different substituted aliphatic, heterocycle amines with **4-Chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid (Table** 2, entries **1-29**). The Aromatic amines bearing electron–donating substituents such as –Me, –OMe, –Et aromatic amines with pyrazole acid **5a** and afforded the desired products **7a, 7g, 7j, 7n, 7p, 7v, 7x** in yield 72–79% (**Table** 2). Where as aromatic ring bearing with strong electron withdrawing groups like $–CF_3$, $–NO_2$ are ortho,meta, para to amine provided the desired products **7c, 7l, 7t** comparatively in lower yield 50–62 % (**Table** 2), this may be due to the reduced necluophility at the proximal end of the amine moiety thereby reducing the efficiency of the desired coupling and also amine having both electron donating aswell as strong electron withdrawing group these coupling products **7b, 7r, 7s** gave yield 49–63 % (**Table** 2). Whereas aromatic amines having $–OCF_3$ group gave yield 58.51% (**Table** 2) due to may increase the nucleophility of the amine at the same way aromatic amines having moderating elctron with drawing group like –Br coupling product **7d, 7w** gave excellent yield 65–70.3% (**Table** 2) and also amine containing both electron releasing aswell as moderating EWG compounds **7h, 7u** gave yield 52–70%, amines having strong EWG amines coupling with acid **1a** products **7l, 7t** gave yield between 50–52 % (**Table** 2), whereas amines having both EWG compound **7k** gave very less yield 42.60% (Table 1) due to loss of nucleophility of amines. Chemoselective coupling between hydroxy and amine with acid to gave compound **7o** with yield 43.44%.Whereas heterocyclic amines couple with acid compounds **7f, 7i** gave yield 62–65 % (**Table** 2), whereas secondry amine couple with acid to compounds **7m, 7q** gave yield 62–73% (**Table** 2). Alipahtic amines coupling with acid products **8a1, 8m1, 8n1** gave yield 77–83 % (**Table** 3), whereas aliphatic 2° amine coupling with acid to products **8e1, 8i1, 8k1, 8l1** gave yield 65–81.85% (**Table** 3). Benzylic amine coupling with acid products **8a1, 8h1** and **8j1** gave yields are 81.44%, 67.01% and 77% respectively (**Table** 3), cyclic amines couple with acid products **8b1, 8c1** gave yields are 79.41%, 76.97 % (**Table** 3) respectively. Whereas sulfur aliphatic containing amine couple with acid product **8o1** gave yield 70.73 % (**Table** 3).

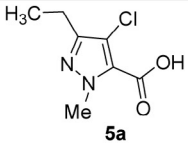
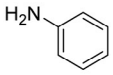
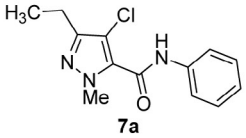
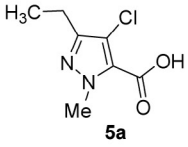
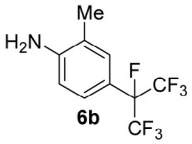
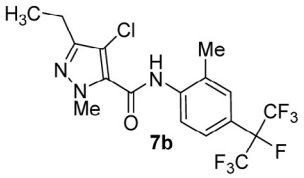
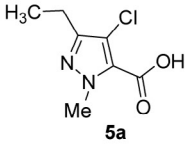
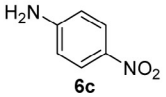
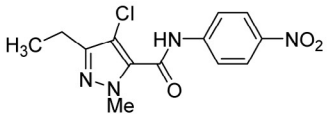
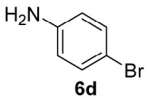
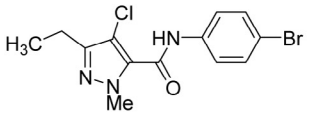
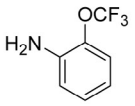
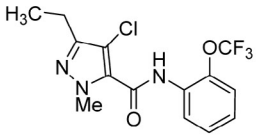
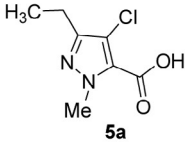
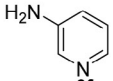
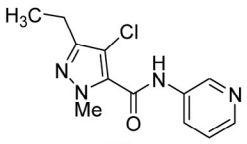
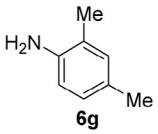
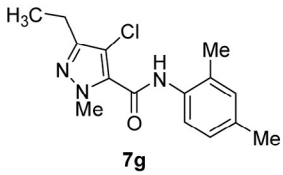
4. Biological activities

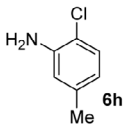
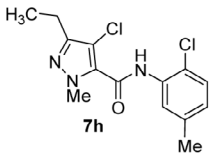
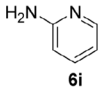
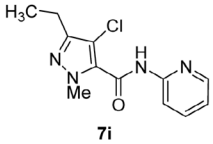
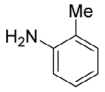
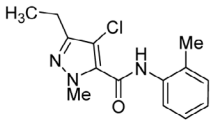
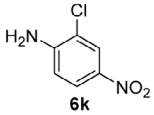
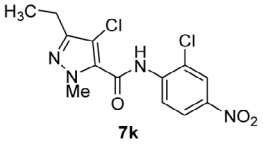
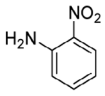
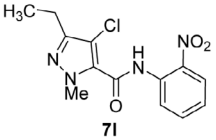
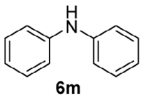
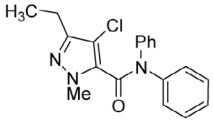
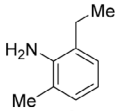
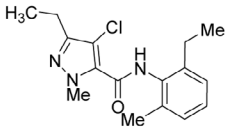
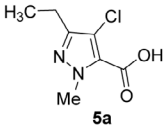
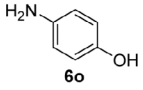
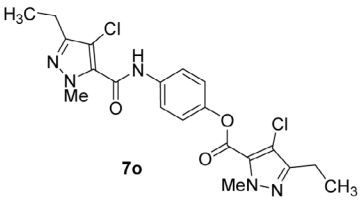
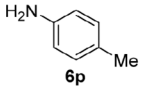
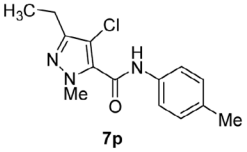
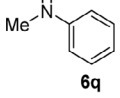
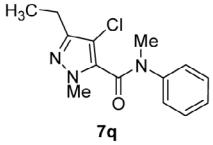
Antifungal activity

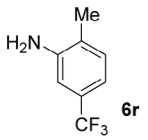
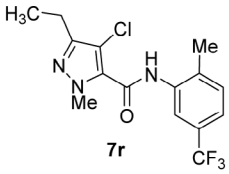
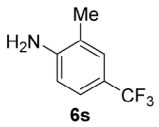
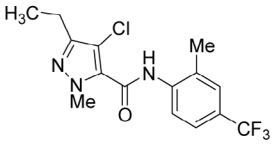
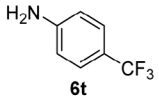
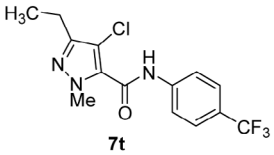
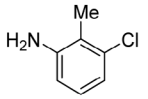
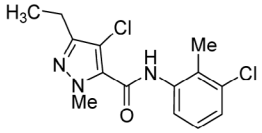
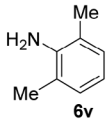
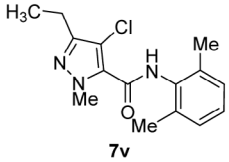
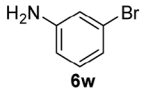
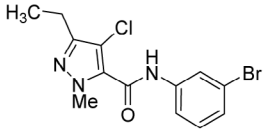
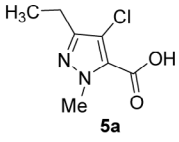
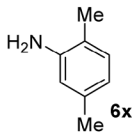
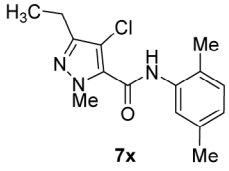
The antifungal activities of pyrazole

Table 2. synthesis of pyrazole carboxamides[a]



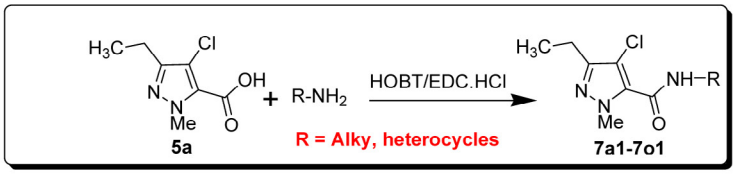
Entry	substrate	amine	product	yield[b]
1	 **5a**	 **6a**	 **7a**	76.84%
2	 **5a**	 **6b**	 **7b**	63.33%
3	 **5a**	 **6c**	 **7c**	61.72%
4	5a	 **6d**	 **7d**	70.30%
5	5a	 **6e**	 **7e**	58.51%
6	 **5a**	 **6f**	 **7f**	65.98%
6	5a	 **6g**	 **7g**	74.77%

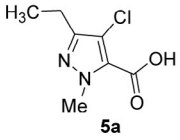
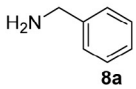
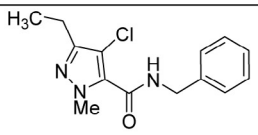
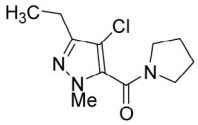
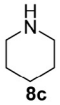
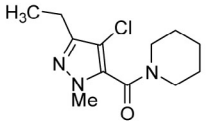
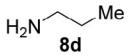
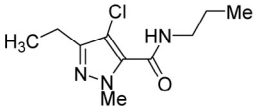
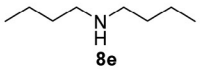
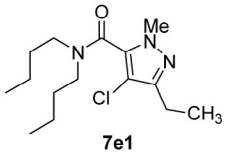
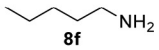
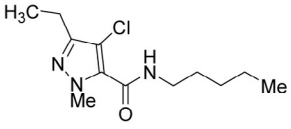
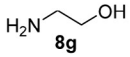
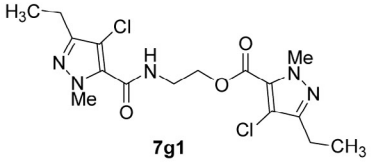
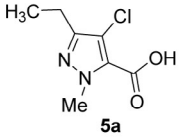
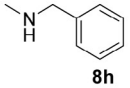
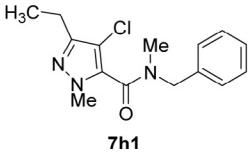
7	5a	 **6h**	 **7h**	51.96%
8	5a	 **6i**	 **7i**	62.30%
10	5a	 **6j**	 **7j**	81.43%
11	5a	 **6k**	 **7k**	42.60%
12	5a	 **6l**	 **7l**	53.40%
13	5a	 **6m**	 **7m**	62.38%
14	5a	 **6n**	 **7n**	75.48%
15	 **5a**	 **6o**	 **7o**	43.44%
16	5a	 **6p**	 **7p**	75.25%
17	5a	 **6q**	 **7q**	72.94%

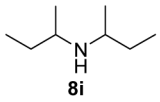
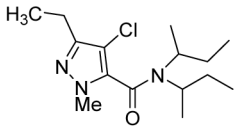
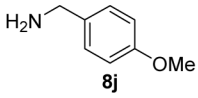
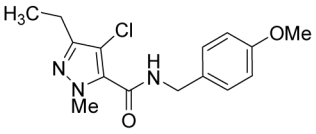
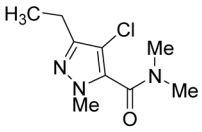
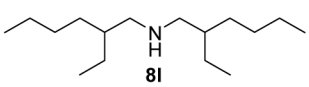
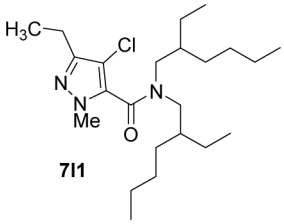
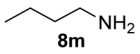
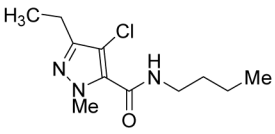
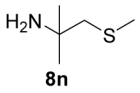
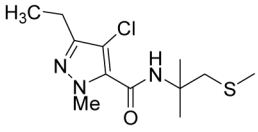
18	5a	 **6r**	 **7r**	50.67%
19	5a	 **6s**	 **7s**	49.03%
20	5a	 **6t**	 **7t**	50.52%
21	5a	 **6u**	 **7u**	69.56%
22	5a	 **6v**	 **7v**	79.48%
23	5a	 **6w**	 **7w**	65.93%
24	 **5a**	 **6x**	 **7x**	76.1%

[a] Reaction were performed using 1.0 equiv of acid **5a**, 0.95 equiv of amine **(6a–6x)**, TEA (3.0 equiv) in DMF as solvent under N_2 atmosphere. [b] Isolated yields.

Table 3. synthesis of pyrazole carboxamides[a]



Entry	substrate	amine	Product	yield[b]
1	 5a	 8a	 7a1	81.44%
2	5a	 8b	 7b1	79.41%
3	5a	 8c	 7c1	76.97%
4	5a	 8d	 7d1	83.15%
5	5a	 8e	 7e1	81.85%
6	5a	 8f	 7f1	76.2%
7	5a	 8g	 7g1	45.12%
8	 5a	 8h	 7h1	67.01%

9	5a	 **8i**	 **7i1**	70.49%
10	5a	 **8j**	 **7j1**	76.8%
11	5a	 **8k**	 **7k1**	68.84%
12	5a	 **8l**	 **7l1**	65.14%
13	5a	 **8m**	 **7m1**	77.86%
14	5a	 **8n**	 **7n1**	70.73%

[a] Reaction were performed using 1.0 equiv of acid **5a**, 0.95 equiv of amine **(6a–6x)**, TEA (3.0 equiv) in DMF as solvent under N_2 atmosphere. [b] Isolated yields.

carboxamides were determined using the Agar disc diffusion method against *F. moniliformis, C.lunata, G. cingulata* at Stock solution [1000 microgram per ml] of each compound was prepared in DMSO. Assay carried out by taking concentration 100 microgram per disk. Hi-media antibiotics disk: Amphotericin-B (100 µg/mL) moistened with water are used as standard, and the zones of inhibition are summarized in **Table** 4. It is evident from the zone of inhibition data that pyrazole carboxamides **7o, 7a1, 7d1, 7f1, 7h1** and **7j1** showed excellent activity against *F. moniliformis* at 100 µg/mL concentration but these compounds showed no zone of inhibition against *C.lunata, G. cingulata* strains where as remaining **7e1, 7i1** and **7m1** showed good activity against *F. moniliformis* at 100 µg/mL. Pyrazole carboxamides **7a, 7b, 7c, 7d, 7e, 7f, 7g, 7h, 7j, 7k, 7l, 7m, 7n, 7r, 7s** and **7u** showed poor activity against *F. moniliformis* at 100 µg/mL and also some of these compounds showed poor zone of inhibition activity against *C.lunata* at 100 µg/mL concentration. Thus it's clear that benzyl group and different alky groups attached chain compounds to be responsible for the excellent to good antifungal activity because rest of the pyrazole ring is same for the all compounds. Whereas as aromatic and heterocycle attached compounds showed no significant antifungal activity.

whereas compounds **7a, 7e, 7g, 7h, 7j, 7m, 7n** and **7s** showed very poor zone of inhibition against *C.lunata* at 100 µg/mL concentration Rest of the compounds showed no significant activity thus it's clear that aromatic group attached compounds showed poor activity because rest of pyrazole ring is same. Whereas **7k** compound showed excellent activity against *G. cingulata* at 100 µg/mL other compound **7b, 7c, 7l** and **7r** showed poor activity against G. *cingulata* 100 µg/mL, rest of the pyrazole compound showed no significant activity thus it's clear that aromatic group attached compounds showed poor activity.

Table 4 *In vitro* testing expressed as zone of inhibition for antifungal activities.

Compound	Zone of inhibition in mm [1000 microgram per ml]		
	F. moniliformis[a]	*C.lunata*[b]	*G. cingulata*[c]
7a	25.07	17.39	-
7b	15.11	19.45	16.39
7c	18.39	-	16
7d	13.5	-	-
7e	20.92	15.23	-
7f	17.5	19.1	
7g	21.6	14.58	-
7h	23.98	18.71	-
7i	-	-	-
7j	22.75	15.9	-
7k	18.02	-	**10.83**
7l	19.72	-	15.67
7m	23.54	17.98	-
7n	24.89	16.72	-
7o	**10.78**	-	-
7r	17.26	19.23	16.96
7s	18.29	16.24	-
7u	19.86	-	-
7a1	**9.21**	-	-
7d1	**10.79**	-	-
7e1	11.21	-	-
7f1	**10.35**	-	-
7g1	**9.56**	-	-
7h1	**8.23**	-	-
7i1	11.98	-	-
7j1	**8.89**	-	-
7m1	11.07	-	-
Amphotericin-B	10.11	8.33	12.22

Data represent is mean of three replicates for each concentration.

The compounds were dissolved in DMSO.[a] Compounds with zone ≥10.11 mm are sensitive against *F. moniliformis;* [b] Compounds with zone ≥8.33 mm are sensitive against *C.lunata;* [c] Compounds with zone ≥12.22 mm are sensitive against *G. cingulata;*'-' Means no zone of inhibition; Diameter in mm calculated by Vernier Caliper.

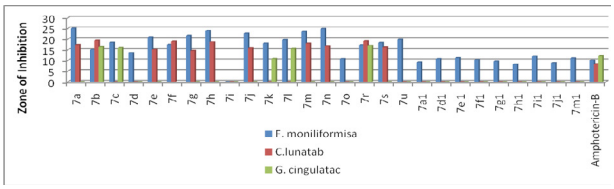


Figure.4 Zone of inhibition in mm *versus* pyrazole carboxamides derivatives and Amphotericin B concentration.

Anticancer activity. The *in vitro* anticancer activities of compounds **7b, 7c, 7e, 7x** and **7d1** were determined against the human breast cancer MCF-7 cell lines at four dosage levels of 10, 20, 40 and 80 µg/mL in DMSO. A test consisted of a 48 h continuous drug exposure protocol using SRB assay to estimate cell growth. Suitable positive controls were run in every experiment. Each experiment was repeated in triplicate, and growth relative to the control was plotted as a function of drug concentration (**Fig.** 6) to calculate numerous parameters.

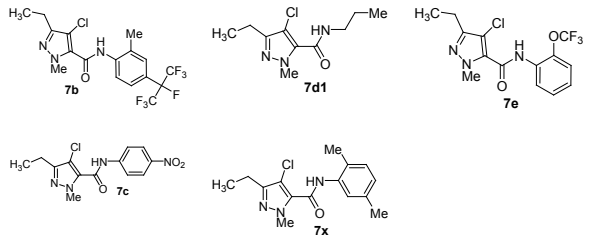
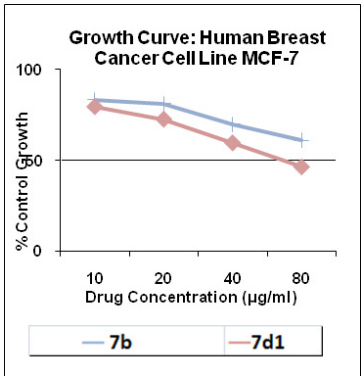


Figure 5. Selected molecules for anticancer activity

Results are given in terms of GI_{50} (concentration of drug that produces 50% inhibition of the cell), TGI (concentration of the drug that produces total inhibition of the cells) and LC50 (concentration of the drug that kills 50% of the cells) values calculated from the mean graphs (Fig. 3 and 4) and are given in Table Xx. Adriamycin (ADR), which is chemotherapy drug often used to kill cancerous cells, was used as the standard anticancer drug. Reported parameters are given in **Table** 5. The compounds which have GI_{50} values of <10 µg/mL were considered to demonstrate anticancer activity against MCF-7 cell line. The GI_{50} of each pyrazole carboxamide against the MCF-7 cell line exceeded 80 µg/mL, hence these pyrazole amides were found to inactive; in contrast, ADR showed a better result (GI_{50} ~**68.7** µg/mL).

In general the LC_{50}, which is a parameter of cytotoxicity and reflects the molar concentration needed to kill 50% of the cells, was found to exceed 80 µg/mL for the pyrazole carboxamides as well as ADR for the MCF-7 cell line. Thus, it can be concluded that pyrazole carboxamides **7d1**compound showing activity of GI 50 value ~ **68.7** µg/mL against standard ADR (<10 µg/mL) due to alkyl group attached carboxamide. Rest of other pyrazole carboxamides **7b, 7c, 7e** and **7x** showed no significant anticancer activity against MCF-7 cell line.



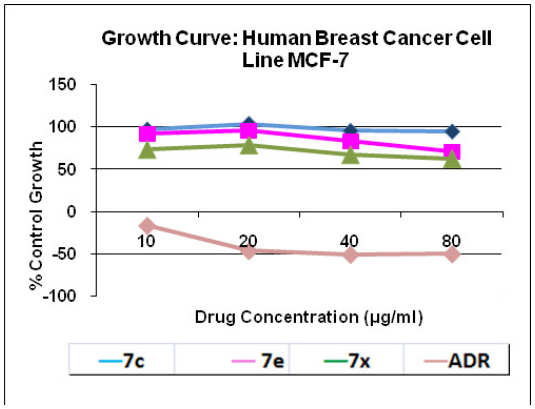


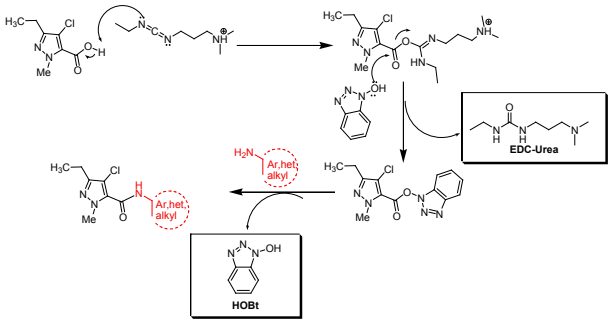
Figure.6 MCF-7 cell line growth as a percentage as a percentage of the control *versus* drug (pyrazole carboxamides and ADR) concentration.

Table 5 *In vitro* testing expressed as growth inhibition of human breast cancer cell line MCF-7 by Pyrazole carboxamides [a]

MCF-7			
Pyrazole carboxamides	LC_{50}*	TGI*	GI_{50}*
7b	>80	>80	>80
7d1	>80	>80	**68.7**
7c	>80	>80	>80
7e	>80	>80	>80
7x	>80	>80	>80
ADR	62.5	<10	<10

[a] Data represents means of three replicates for each concentration DMSO was used as solvent. *LC_{50} = Concentration of drug causing 50% cell kill. *GI_{50} = Concentration of drug causing 50% inhibition of cell growth. TGI = Concentration of drug causing total inhibition of cell growth. *ADR = Adriamycin, Positive control compound. GI_{50} value of ≤ 10 µg/mL is considered to demonstrate anticancer against MCF-7 cell line.

Plausible Mechanistic pathways



4. Conclusion

In Conclusion, we have developed reaction methodology for the synthesis of substituted pyrazole carboxamides with high molecular complexity in good to excellent yield. Their characterization was done by using various spectral techniques.These synthesized compounds were screened for antifungal and anticancer activity. *In vitro* studies pyrazole carboxamides **7o, 7a1, 7d1, 7f1, 7h1** and **7j1** showed excellent activity against *F. moniliformis* whereas remaining **7e1, 7i1** and **7m1** showed good activity against *F. moniliformis*. Pyrazole carboxamide **7k** c showed excellent activity against *G. cingulata* other compound **7b, 7c, 7l** and **7r** showed poor activity against G. *cingulata.* Pyrazole carboxamides **7d1**compound showing moderate anticancer activity against MCF-7 cell line compare to standard ADR. This protocol is efficiently developed 1-hydroxy benzotriazole mediated peptide coupling of pyrazole acid with amine under normal condition. Owing to the great skeletal diversity of substitution pattern, this developed chemistry is potentially attractive for the synthesis of libraries of bioactive pyrazole carboxamide compounds by using peptide chemistry.

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