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Rapid Synthesis of 3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives and their Characterization, anticancer and antifungal activities

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Abstract: A series of 3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives were synthesized through a facile and efficient synthetic methodology using pyrazole acid **4a**, amine **5a-5v** with HOBt/EDC.HCl under very mild reaction condition. The synthesized compounds were further confirmed by IR, ¹H NMR and mass spectroscopy. All these synthesized compounds were tested for their biological potency against different strains of fungi. Anticancer screening were also done against MCF-7 cell line.

Keywords: Pyrazole Carboxamide, HOBt/EDC.HCl, amines, peptide coupling, antifungal activity, anti-cancer activity.

1. Introduction

Pyrazole derivatives have boosted the attention of synthetic medicinal chemists in last few years due to their usefulness as intermediates in the preparation of new biological potent molecules.¹ As it hold immense potency as inhibitors of protein glycation, antibacterial, antifungal, anticancer, antidepressant, anti-inflammatory, anti-tuberculosis, antioxidant as well as antiviral agents.²⁻³ Literature reveals that, amide functionality is found in up to 25% of all pharmaceuticals present in the market.⁴⁻⁵ It is also estimated that 16% of reactions involved

in the synthesis of modern pharmaceuticals are the acylation of an amine. These facts make it the most valuable asset in the field of organic synthesis.⁶⁻⁷ There are well known facts present in literature for pyrazole derivatives and the acylation of amine as both are the integrated part of biologically active natural products, agrochemicals and pharmaceuticals.⁸⁻⁹

There are many reports from industries on pyrazole-4-carboxamide derivatives, as they are widely and effectively introduced into the market as agricultural fungicides¹⁰⁻¹¹ (**Fig. 1**) in recent years along with their therapeutic nature

as also narrated by Yong Xu and co-workers in 2014. They reported that these moieties have excellent properties as activators of the pyruvate kinase M2 (PKM2) and potential in anticancer therapies (Fig. 2).¹²

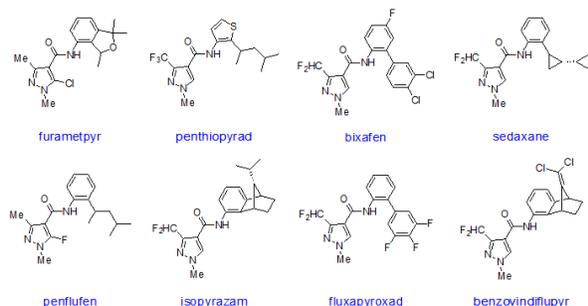


Fig. 1. Pyrazole carboxamides SQR inhibitors.

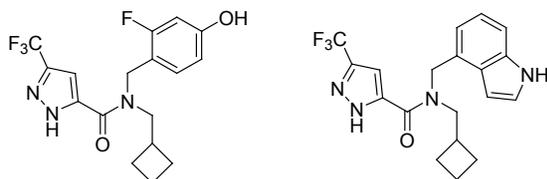


Fig. 2. Structures of biologically active pyrazole carboxamides.

In the series of investigations of potential use of pyrazole scaffold we also reported a series of biologically active pyrazole carboxamides¹³ as 3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives showed significant role in the field of agrochemicals.

2. Materials and Methods

General

¹H NMR (400 MHz) spectra were recorded in CDCl₃/DMSO-d₆. Chemical shifts for protons 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 F₂₅₄ silica gel plates and visualized by either UV irradiation or by staining with I₂ 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 comparison of their physical and spectral data (m.p, ¹H NMR and mass spectroscopy) with those reported in literature.

To a solution of (0.4 g, 2.27 mmol) **3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid 4a** in 10 mL of DMF was treated sequentially with (0.382 g, 2.499 mmol) of HOBT, (0.388 g, 2.499 mmol) of EDC.HCl and (0.243 g, 2.27 mmol) of *p*-Toluidine. The suspension was stirred for 15 min at 5 °C, (0.93 mL, 6.609 mmol) of TEA was added, and the mixture stirred for 10–12 h at r.t.. The reaction was quenched by pouring into 50 mL of ethyl acetate and extracting with 50 mL of 1N aq. HCl, 100 mL of water, 50 mL of brine, 50 mL of saturated aqueous NaHCO₃, and finally 50 mL of brine. The solution was then dried over anhydrous Na₂SO₄, filters and concentrated under reduced pressure. The crude material was purified by column chromatography on silicagel (15 g, 15:85 ethyl acetate/hexanes) to provide 0.532 g with 88.20% yield of product as a off-white solid.

Characterization of Representative 3-(difluoromethyl)-1-methyl-N-*p*-tolyl-1H-

pyrazole-4-carboxamide (6v):

Compound **6v** was synthesized by **3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid 4a** with *p*-Toluidine in presence of base and peptide coupling reagents. The product was obtained as a colorless needles in 70.30% yield with m.p. 99–100 °C. The structure of compound **6v** was established on the basis of spectral data analysis. In the ¹H NMR spectrum in CDCl₃ at 400 MHz, the characteristic peak of amide linkage appeared at δ 8.10 ppm, a singlet appeared at δ 3.91 ppm corresponds to pyrazole –NMe, aromatic –Me group proton appeared at δ 2.32 ppm respectively confirmed the coupling of pyrazole acid with amine of *p*-toluidine. The peaks of all other protons of the molecules are presented in ¹H NMR spectra of the molecule. Its mass spectrum showed [M+H]⁺ peak at 266.1027, which confirmed its molecular formula to be C₁₃H₁₃F₂N₃O.

N-(4-methoxybenzyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (6a):

The product was obtained as yellow needles (680 mg, 89.83% yield); m.p. 139–140 °C. ¹H NMR (400 MHz, CDCl₃): δ 7.88 (br s, 1H), 8.08 (s, 2H), 7.32 (m, 2H), 6.86 (s, 1H), 3.95 (s, 3H), 2.36 (s, 3H) ppm. HRMS (ESI): calcd. for C₁₄H₁₅F₂N₃O₂ [M+H]⁺ 296.1133; found 296.1130 IR (KBr, cm⁻¹): ν_{max} = 3665, 2974, 2383, 1615, 1396, 1158.

3-(difluoromethyl)-1-methyl-N-(2,5-dimethylphenyl)-1H-pyrazole-4-carboxamide (6b):

The product was obtained as Off-white needles (540 mg, 85.14% yield); m.p. 115–116 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.02 (br s, 1H), 7.88 (s, 1H), 7.79 (d, *J* = 7.6 Hz, 1H), 7.04 (m, 2H), 6.87 (s, 1H), 3.93 (s, 3H), 2.30 (s, 3H), 2.26 (s, 1H) ppm. HRMS (ESI): calcd. for C₁₄H₁₅F₂N₃O [M+H]⁺ 280.1183; found 280.1192. IR (KBr, cm⁻¹): ν_{max} = 3661, 2975, 2373, 1625, 1390, 1157.

(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-yl)(piperidin-1-yl) methanone (6c): The product was obtained as brown oil (480 mg, 86.89% yield); ¹H NMR (400 MHz, CDCl₃): δ 7.46 (s, 3H), 6.81 (m, 1H), 3.93 (s, 3H), 3.54 (m, 4H), 1.66-1.56 (m, 6H) ppm. HRMS (ESI): calcd. for C₁₁H₁₅F₂N₃O [M+H]⁺ 244.1183; found 244.1183. IR (KBr, cm⁻¹): ν_{max} = 2974, 2383, 1635, 1394, 1157.

N-(5-chloro-2-methylphenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (6d):

The product was obtained as White needles (452 mg, 66.51% yield); m.p. 142–143 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.15 (d, *J* = 2, 1H), 7.98 (br s, 1H), 7.23–7.06 (m, 2H), 6.85 (s, 1H), 3.96 (s, 3H), 2.26 (s, 3H) ppm. HRMS (ESI): calcd. for C₁₃H₁₂ClF₂N₃O [M+H]⁺ 300.0637; found 300.0802. IR (KBr, cm⁻¹): ν_{max} = 3660, 2975, 2371, 1624, 1392, 1159, 856.

3-(difluoromethyl)-1-methyl-N-(2-(trifluoromethoxy)phenyl)-1H-pyrazole-4-carboxamide (6e):

The product was obtained as White needles (250 mg, 32.83% yield); m.p. 62–63 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.33 (d, *J* = 8.4 Hz, 1H), 8.11 (br s, 1H), 8.09 (m, 1H), 7.88 (m, 1H), 7.48 (d, *J* = 9.2 Hz, 1H), 7.42 (s, 1H), 6.98 (s, 1H), 3.96 (s, 3H) ppm. HRMS (ESI): calcd. for C₁₃H₁₀F₅N₃O [M+H]⁺ 336.0693; found 336.0694. IR (KBr, cm⁻¹): ν_{max} = 3659, 2975, 2373, 1621, 1396, 1156, 1071.

(3-(difluoromethyl)-1-methyl-1H-pyrazole-4-yl)(pyrrolidin-1-yl)methanone (6f):

The product was obtained as brown oil (460 mg, 88.37% yield); ¹H NMR (400 MHz, CDCl₃): δ 7.58 (s, 3H), 7.17 (m, 1H), 3.95 (s, 3H), 3.61 (m, 2H), 3.55 (m, 2H), 1.91 (m, 4H) ppm. HRMS (ESI): calcd. for C₁₀H₁₃F₂N₃O [M+H]⁺ 230.1027; found 230.1027. IR (KBr, cm⁻¹): ν_{max} = 2972, 2384, 1635, 1392, 1154.

3-(difluoromethyl)-N-(5-(trifluoromethyl)-

2-methylphenyl)-1-methyl-1H-pyrazole-4-carboxamide (6g): The product was obtained as yellow needles (350 mg, 46.24% yield); m.p. 139–140 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.42 (br s, 1H), 8.08 (s, 2H), 7.32 (m, 2H), 6.86 (s, 1H), 3.95 (s, 3H), 2.36 (s, 3H) ppm. HRMS (ESI): calcd. for C₁₄H₁₂F₅N₃O [M+H]⁺ 334.0901; found 334.0902. IR (KBr, cm⁻¹): ν_{max} = 3658, 2974, 2375, 1620, 1396, 1159.

N-(2-chloro-4-nitrophenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide(6h): The product was obtained as yellow needles (290 mg, 38.67% yield); m.p. 62–63 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.10 (br s, 1H), 7.97 (s, 1H), 7.48 (d, *J* = 8.4 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 6.91 (m, 1H), 3.91 (s, 3H), 2.32 (s, 3H) ppm. HRMS (ESI): calcd. for C₁₂H₉ClF₂N₄O₃ [M+H]⁺ 331.0331; found 331.0330. IR (KBr, cm⁻¹): ν_{max} = 3658, 2977, 2372, 1621, 1396, 1345, 1156, 587.

3-(difluoromethyl)-1-methyl-N-(2-methyl-5-(perfluoropropan-2-yl)phenyl)-1H-pyrazole-4-carboxamide (6i): The product was obtained as Off-white needles (520 mg, 52.84% yield); m.p. 123–124 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.33 (d, *J* = 8.4 Hz, 1H), 8.11 (d, *J* = 8.4 Hz, 2H), 7.48 (m, 1H), 7.42 (br s, 1H), 6.98 (m, 1H), 3.96 (s, 3H), 2.37 (s, 3H) ppm. HRMS (ESI): calcd. for C₁₆H₁₂F₉N₃O [M+H]⁺ 434.0837; found 434.0836. IR (KBr, cm⁻¹): ν_{max} = 3657, 2972, 2371, 1622, 1394, 1155, 1075.

N-(4-bromophenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide(6j): The product was obtained as Off-white needles (650 mg, 86.95% yield); m.p. 145–146 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.10 (br s, 1H), 7.97 (s, 1H), 7.51 (d, *J* = 9.2 Hz, 2H), 7.44 (d, *J* = 9.2 Hz, 2H), 6.87 (s, 1H), 3.95 (s, 3H) ppm. HRMS (ESI): calcd. for C₁₂H₁₀BrF₂N₃O [M+H]⁺ 329.9975; found 329.9971. IR (KBr, cm⁻¹): ν_{max} = 3654, 2971, 2371, 1623, 1395, 1158, 1071, 587.

N-(2-ethyl-6-methylphenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (6k): The product was obtained as White needles (480 mg, 72.07% yield); m.p. 104–105 °C. ¹H NMR (400 MHz, CDCl₃): δ 7.97 (s, 1H), 7.74 (br s, 1H), 7.23 (m, 1H), 7.14 (m, 2H), 6.89 (m, 1H), 3.90 (s, 3H), 2.63 (q, *J* = 7.6 Hz, 2H), 2.30 (s, 3H), 1.19 (t, *J* = 7.2 Hz, 3H) ppm. HRMS (ESI): calcd. for C₁₅H₁₇F₂N₃O [M+H]⁺ 294.1340; found 294.1340. IR (KBr, cm⁻¹): ν_{max} = 3655, 2972, 2850, 2378, 1621, 1394, 1156, 1061.

N,N-dimethyl-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (6l): The product was obtained as brown oil (450 mg, 85.69% yield); ¹H NMR (400 MHz, CDCl₃): δ 7.45 (s, 1H), 6.86 (m, 1H), 3.93 (s, 3H), 3.46 (q, *J* = 7.2 Hz, 4H), 1.12 (t, *J* = 7.2 Hz, 6H) ppm. HRMS (ESI): calcd. for C₁₀H₁₅F₂N₃O [M+H]⁺ 232.1183; found 232.1182. IR (KBr, cm⁻¹): ν_{max} = 3654, 2972, 2851, 2374, 1620, 1395, 1158, 1059.

3-(difluoromethyl)-1-methyl-N-o-tolyl-1H-pyrazole-4-carboxamide (6m): The product was obtained as White needles (430 mg, 71.39% yield); m.p. 118–119 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.01 (br s, 1H), 7.96 (d, *J* = 8.0 Hz, 2H), 7.23 (m, 2H), 7.11 (m, 1H), 6.96 (m, 1H), 3.92 (s, 3H), 2.30 (s, 3H) ppm. HRMS (ESI): calcd. for C₁₃H₁₃F₂N₃O [M+H]⁺ 266.1027; found 266.1027. IR (KBr, cm⁻¹): ν_{max} = 3656, 2972, 2859, 2372, 1623, 1393, 1159, 1058.

N-butyl-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (6n): The product was obtained as Brown oil (460 mg, 87.59% yield). ¹H NMR (400 MHz, CDCl₃): δ 7.97 (s, 1H), 7.89 (s, 1H), 7.23 (m, 1H), 6.83 (m, 1H), 6.38 (br s, H), 3.89 (s, 3H), 3.38 (q, 2H), 1.56 (m, 2H), 1.38 (m, 2H), 0.92 (q, 3H) ppm. HRMS (ESI): calcd. for C₁₀H₁₅F₂N₃O [M+H]⁺ 232.1183; found 232.1184. IR (KBr, cm⁻¹): ν_{max} = 3564, 2967, 2376, 1620, 1395, 1158, 1057.

3-(difluoromethyl)-1-methyl-N-(2,5-dimethylphenyl)-1H-pyrazole-4-carboxamide (6o): The product was obtained as Off-white needles (547 mg, 86.24% yield); m.p. 107–108 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.01 (br s, 1H), 7.88 (s, 1H), 7.78 (d, *J* = 7.6 Hz, 1H), 7.01 (m, 2H), 6.87 (s, 1H), 3.93 (s, 3H), 2.30 (s, 3H), 2.26 (s, 1H) ppm. HRMS (ESI): calcd. for C₁₄H₁₅F₂N₃O [M+H]⁺ 280.1183; found 280.1192. IR (KBr, cm⁻¹): ν_{max} = 3654, 2972, 2851, 2374, 1620, 1395, 1158, 1059.

3-(difluoromethyl)-1-methyl-N-phenyl-1H-pyrazole-4-carboxamide (6p): The product was obtained as White needles (490 mg, 85.88% yield); m.p. 116–117 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.19 (br s, 1H), 7.99 (s, 1H), 7.60 (d, *J* = 7.6 Hz, 2H), 7.34 (d, *J* = 7.6 Hz, 2H), 7.15 (m, 1H), 6.92 (m, 1H), 3.91 (s, 3H) ppm. HRMS (ESI): calcd. for C₁₂H₁₁F₂N₃O [M+H]⁺ 252.087; found 252.088. (KBr, cm⁻¹): ν_{max} = 3654, 2972, 2858, 2371, 1643, 1389, 1158, 1057.

N-(5-chloro-2-methylphenyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (6q): The product was obtained as yellow needles (410 mg, 60.33% yield); m.p. 142–143°C. ¹H NMR (400 MHz, CDCl₃): δ 8.15 (d, *J* = 2, 1H), 8.05 (s, 1H), 7.98 (br s, 1H), 7.12 (d, *J* = 7.6 Hz, 1H), 7.04 (m, 1H), 6.85 (m, 1H), 3.95 (s, 3H), 2.36 (s, 3H) ppm. HRMS (ESI): calcd. for C₁₃H₁₂ClF₂N₃O [M+H]⁺ 300.0637; found 300.0639. IR (KBr, cm⁻¹): ν_{max} = 3658, 2976, 2371, 1627, 1394, 1156, 586.

3-(difluoromethyl)-1-methyl-N-(2-methyl-1-(methylthio)propan-2-yl)-1H-pyrazole-4-carboxamide (6r): The product was obtained as Brown oil (520 mg, 82.58% yield). ¹H NMR (400 MHz, CDCl₃): δ 7.88 (s, 1H), 6.84 (m, 1H), 6.32 (br s, H), 3.89 (s, 3H), 3.02 (s, 2H), 2.12 (s, 3H), 1.45 (s, 6H) ppm. HRMS (ESI): calcd. for C₁₁H₁₇F₂N₃OS [M+H]⁺ 278.106; found 278.108. IR (KBr, cm⁻¹): ν_{max} = 3561, 2972, 2371, 1624, 1394, 1342, 1156, 1275, 585.

N,N-bis(2-ethylhexyl)-3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxamide (6s): The product was obtained as Brown oil (640 mg, 70.53% yield). ¹H NMR (400 MHz, CDCl₃): δ 7.42 (s, 1H), 6.96 (m, 1H), 3.93 (s, 3H), 3.42 (m, 2H), 3.27 (m, 2H), 1.50 (m, 4H), 1.25 (m, 20H), 0.87 (m, 6H) ppm. HRMS (ESI): calcd. for C₂₂H₃₉F₂N₃O [M+H]⁺ 400.3061; found 400.3063. IR (KBr, cm⁻¹): ν_{max} = 2967, 2376, 1620, 1360, 1158, 1057.

3-(difluoromethyl)-N-(4-(trifluoromethyl)-2-methylphenyl)-1-methyl-1H-pyrazole-4-carboxamide (6t): The product was obtained as Off-white needles (367 mg, 48.49% yield); m.p. 118–119 °C. ¹H NMR (400 MHz, CDCl₃): δ 8.30 (d, *J* = 8.4 Hz, 1H), 8.11 (br s, 1H), 8.07 (s, 1H), 7.50 (d, *J* = 8.8 Hz), 7.45 (s, 3H), 6.85 (m, 1H), 3.95 (s, 3H), 2.35 (s, 3H) ppm. HRMS (ESI): calcd. for C₁₄H₁₂F₅N₃O [M+H]⁺ 334.0901; found 334.0902. IR (KBr, cm⁻¹): ν_{max} = 3656, 2973, 2371, 1621, 1395, 1158, 1059.

Antifungal activity

The *in vitro* antifungal activities of synthesized 3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives were carried out at Department of Microbiology, R.C. Patel Arts commerce and Science College Shirpur, Dhule (Maharashtra). Antifungal activity of all the synthesized 3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives 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,¹⁴ 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 composition (grams per liter) 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 millimetre (mm) for compounds **6a–6v**.

Anticancer activity

In *in vitro* anticancer activities of synthesized 3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives were investigated at Anticancer Drug Screening Facility, Tata Memorial Advanced centre for Treatment, Research and Education in Cancer (ACTREC) Kharghar, Navi Mumbai.

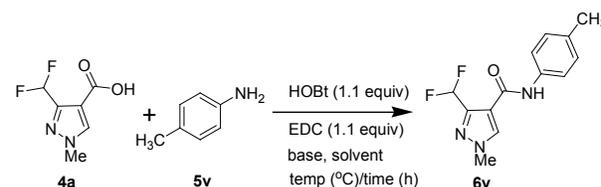
The *in vitro* anticancer activity of compounds **6d**, **6e**, **6g** and **6i** were performed on the breast cancer cell line MCF-7 at four dose levels 10, 20, 40 and 80 µg/L in DMSO, the test consisted of 48 h continuous drug exposure protocol using sulforhodamine B (SRB) assay to estimate cell growth.¹⁵ 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.¹⁵

3. Results and Discussion

In order to search the optimal reaction condition for proposed peptide coupling reaction, we analyzed various factors affecting reaction by using **3-(difluoromethyl)-1-methyl-1H-pyrazole-4-carboxylic acid 4a** with *p*-Toluidine **5v** in presence of 1.1 mol eq of HOBT and 1.1 mole eq of EDC.HCl as the coupling reagents and

Et₃N as base at rt under N₂ atmosphere in DMF for 10–12 hrs. This set of condition afforded desired product **6v** in good yield (**Table 1**, entry 6).

Table 1. Optimization of the reaction conditions^a



Entry	Reagent	Base	Solvent	T(h) / T(°C)	Yield (%) ^b
1	HOBT/EDC.HCl	Et ₃ N	DMF	6 / 25	51
2	HOBT/EDC.HCl	Et ₃ N	DMF	12 / 70	50
3	HATU	Et ₃ N	DMF	12 / 25	54
4	PyBOP	Et ₃ N	DMF	12 / 25	48
5	HBTU	Et ₃ N	DMF	12 / 25	50
6	TBTU	Et ₃ N	DMF	12 / 25	40
7	HOBT/EDC.HCl	DIPEA	DMF	12 / 25	48
8	HOBT/EDC.HCl	DIPA	DMF	12 / 25	40
9	HOBT/EDC.HCl	K ₂ CO ₃	DMF	12 / 25	15
10	HOBT/EDC.HCl	Et ₃ N	DMSO	12 / 25	27
11	HOBT/EDC.HCl	Et ₃ N	MeCN	12 / 25	41
12	HOBT/EDC.HCl	Et ₃ N	Dioxan	12 / 25	67
13	HOBT/EDC.HCl	Et₃N	DMF	12 / 25	88.2
14	HOBT/EDC.HCl	Et ₃ N	THF	24 / 25	65
15	HOBT/EDC.HCl	Et ₃ N	THF	8 / 25	56
16	HOBT/EDC.HCl	Et ₃ N	THF	6 / 25	60
17	HOBT/EDC.HCl	Et ₃ N	THF	6 / 60	65
18	HOBT	Et ₃ N	THF	12 / 25	-
19	EDC.HCl	Et ₃ N	THF	12 / 25	10
20	HOBT/EDC.HCl	Et ₃ N	THF	8 / 25	55 ^c

^a Reaction were performed using 1.0 mol eq of pyrazole acid **4a**, 1.0 mol. equiv. of *p*-Toluidine, base (3.0 equiv.) in 10 mL of solvent under N₂ atmosphere. ^bIsolated yields. ^c 1.5 equiv. of amine was used.

Different bases like DIPEA, DIPA and K₂CO₃ were used to afford the desired product **6v** in poor yields (**Table 1**, entry 7-9). Whereas

changes of solvents from THF, MeCN, DMSO, dioxane to DMF was found effective (Table 1, entry 13) where as only EDC.HCl used a peptide reagent with THF to get poor yield with recimized products observed .when changed the peptide reagent like HATU, PyBOP, HBTU and TBTU to get a moderate yields (Table 1, entries 3–4).

3.1 Scope and Limitation of the Reaction

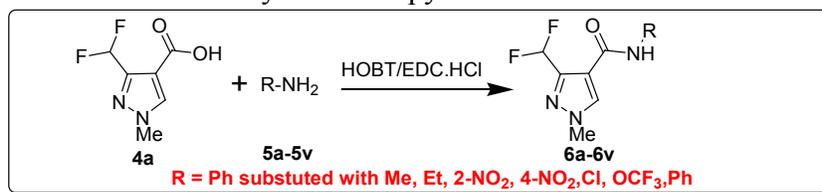
With this standard protocol in hand (Table 2, Entry 1–21), we examined the scope and generality of the reaction with a different substituted aliphatic, aromatic amines with 3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxylic acid **4a** (Table 2, entries 1-21). The Aromatic amines bearing electron–donating substituents such as –Me, H substiuted aromatic amines with pyrazole acid **4a** and afforded the desired products **6m**, **6p** in excellent yield 71–85% (Table 2). Whereas the aromatic amine having strong electron withdrawing group like –CF₃ desired product **6g** gave poor yiled 46.24% (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 **6g**, **6i**, **6t** gave yield 46–53 % (Table 2). Whereas aromatic amines having –OCF₃ group gave yield 32.83% (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 **6j** gave excellent yield 86.95% (Table 2) and also amine containing both electron releasing aswell as moderating EWG compounds **6d**, **6q** gave yield 60–66.5%, whereas amines having both EWG compound **6h** gave very less yield 38.67% (Table 2) due to loss of nucleophility of amines. In the same way aromatic amines having both electron donating group amines coupling with pyrazole acid **4a** gave desired compounds

6b, **6k** and **6o** gave good yield 72–73%(Table 2).Benzylic amine having –OMe coupling with acid products **6a** gave excellent yield 89.93% (Table 2), alipahtic cyclic amines couple with pyrazoleacid products **6c**, **6f** gave yields are 86.89%, 88.37 % (Table 2) respectively. Whereas sulfur aliphatic containing amine couple with acid product **6r** gave yield 82.58 % (Table 2). Whereas aliphatic primary amine coupling with pyrazole acid product **6n** gave yield 87.59% (Table 2). In the same way secondary amine couple with pyrazole acid products **6k** and **6s** gave yield are 85.69% and 70.53% respectively (Table 2).

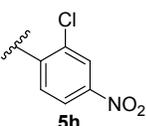
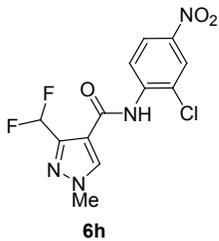
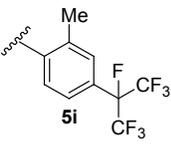
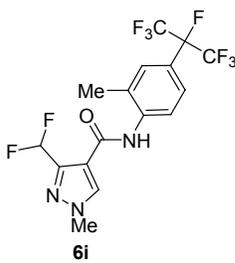
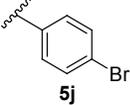
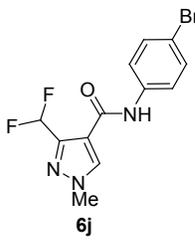
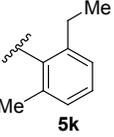
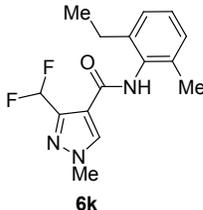
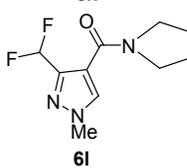
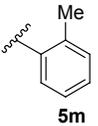
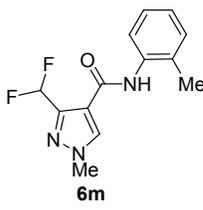
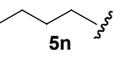
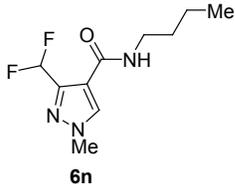
4. Biological activities

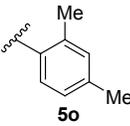
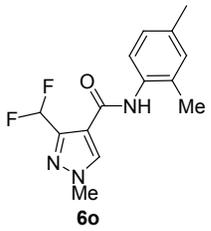
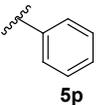
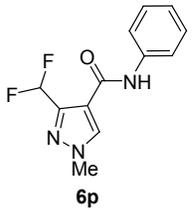
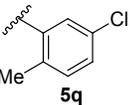
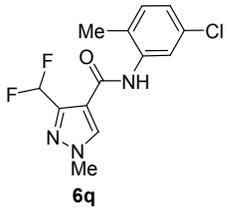
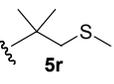
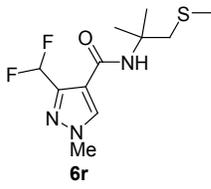
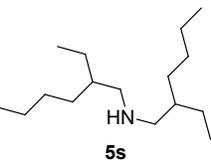
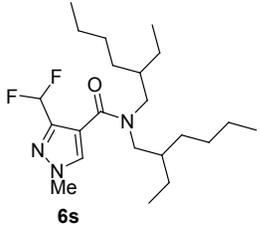
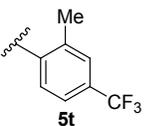
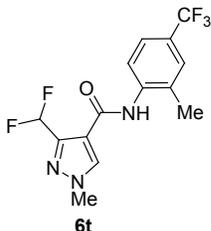
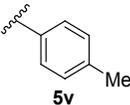
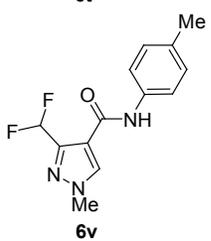
Antifungal activity

The antifungal activities of 3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives 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 units/disk) moistened with water are used as standard, and the zones of inhibition are summarized in Table 3. It is evident from the zone of inhibition data that 3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives **6a**, **6r** 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 **6c**, **6f**, **6l** and **6n** compound showed good activity against *F. moniliformis* and *C.lunata* strain at 100 µg/mL. None of compound showed activity against *G.cingulata* strain at 100 µg/mL. As per this observation none of aromatic ring attached compound is showed activity against all these strains at 100 µg/mL. Thus it's clear that benzyl group and different alky groups attached chain

Table 2. synthesis of pyrazole carboxamides^a

Entry	substrate	amine	product	yield ^b
1	4a			89.93%
2	4a			85.14%
3	4a			86.89%
4	4a			66.51%
5	4a			32.83%
6	4a			88.37%
7	4a			46.24%

8	4a	 5h	 6h	38.67%
9	4a	 5i	 6i	52.84%
10	4a	 5j	 6j	86.95%
11	4a	 5k	 6k	72.07%
12	4a	 5l	 6l	85.69%
13	4a	 5m	 6m	71.39%
14	4a	 5n	 6n	87.59%

15	4a	 <p>5o</p>	 <p>6o</p>	86.24%
16	4a	 <p>5p</p>	 <p>6p</p>	85.88%
17	4a	 <p>5q</p>	 <p>6q</p>	60.33%
18	4a	 <p>5r</p>	 <p>6r</p>	82.58%
19	4a	 <p>5s</p>	 <p>6s</p>	70.53%
20	4a	 <p>5t</p>	 <p>6t</p>	48.49%
21	4a	 <p>5v</p>	 <p>6v</p>	88.20%

^a Reaction were performed using 1.0 mol. equiv of acid **4a**, 1.0 mol. equiv of amine (**5a–5v**), TEA (3.0 mol. equiv) in DMF as solvent under N₂ atmosphere. ^b Isolated yields.

compounds to be responsible for the excellent to good antifungal activity because rest of the pyrazole ring is same for the all compounds. Whereas aromatic compounds showed no significant antifungal activity.

Table 3 *In vitro* testing expressed as zone of inhibition for antifungal activities.

Compound	Zone of inhibition in mm [1000 microgram per ml]		
	<i>F. moniliformis</i> ^a	<i>C. lunata</i> ^b	<i>G. cingulata</i> ^c
6a	11.59	-	-
6b	-	-	-
6c	17.32	15.32	-
6d	-	-	-
6e	-	-	-
6f	17.8	16.31	-
6g	-	-	-
6h	-	-	-
6i	-	-	-
6j	-	-	-
6k	-	-	-
6l	15.38	12.16	-
6m	-	-	-
6n	16.39	13.07	-
6o	-	-	-
6p	-	-	-
6q	-	-	-
6r	10.06	-	-
6s	11.75	13.98	-
6t	-	-	-
6v	-	-	-
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.

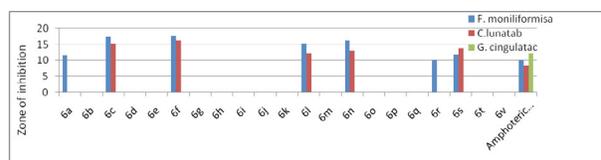


Fig. 3. Zone of inhibition in mm versus 3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives and Amphotericin B concentration.

Anticancer activity. The *in vitro* anticancer activities of compounds **6d**, **6e**, **6g** and **6i** were determined against the human breast cancer MCF-7 cell lines at four dosage levels of 10, 20, 40 and 80 $\mu\text{g/mL}$ in DMSO. A test consisted for 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. 4** and **5**) to calculate numerous parameters.

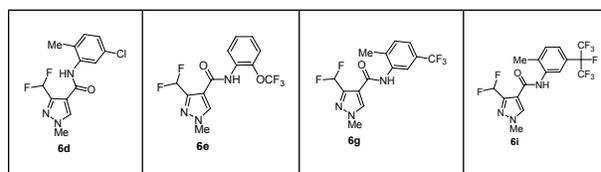


Fig. 4. Screening molecules for anticancer activity

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 LC_{50} (concentration of the drug that kills 50% of the cells) values calculated from the mean graphs (**Fig. 4**) and are given in **Table 4**. 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 4**. The compounds which have GI_{50} values of $<10 \mu\text{g}/\text{mL}$ were considered to demonstrate anticancer activity against MCF-7 cell line. The GI_{50} of each 3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives against the MCF-7 cell line exceeded $80 \mu\text{g}/\text{mL}$, hence these pyrazole amides were found to inactive.

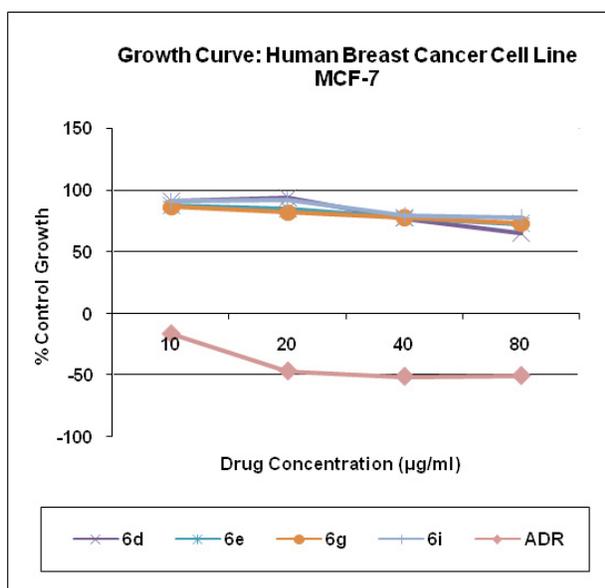


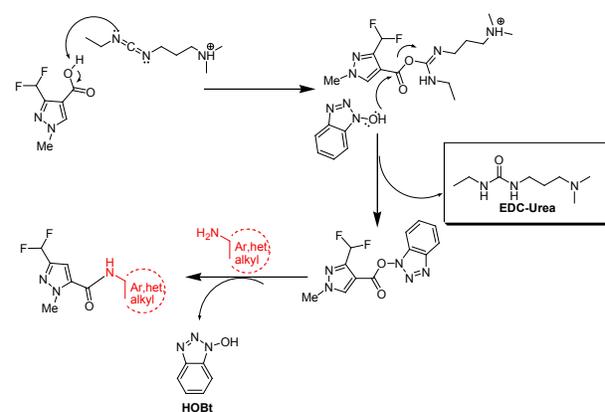
Fig. 5. MCF-7 cell line growth as a percentage as a percentage of the control versus drug (3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives and ADR) concentration.

Table 4 *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} *
6d	>80	>80	>80
6e	>80	>80	>80
6g	>80	>80	>80
6i	>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 $\leq 10 \mu\text{g}/\text{mL}$ is considered to demonstrate anticancer against MCF-7 cell line.

Plausible Mechanistic pathways



4. Conclusion

We developed excellent protocol for the synthesis of 3-(difluoromethyl)-1-methyl-pyrazole carboxamide derivatives with high molecular complexity with excellent yield. Their characterization were done by using ^1H NMR, HRMS and IR techniques to confirm the desired structure. These synthesized compounds were also screened for antifungal and anticancer activity. *In vitro* studies pyrazole carboxamide derivatives **6r** showed excellent activity against *F. moniliformis* compare to Amphotericin-B, where as **6a**, **6c**, **6f**, **6l**, **6n** and **6s** showed very good activity against *F. moniliformis* and **6l**, **6n** and **6s** showed very good activity against *G. cingulata* other compound **6c** and **6f** showed moderate activity against *G. cingulata*. None of the pyrazole carboxamide derivatives showing significant anticancer activity against MCF-7 cell line compare to standard ADR. The

developed synthetic protocol may become an efficient alternative for the synthetic chemist for diverse biological active pyrazole carboxamide compounds.

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