Synthesis and Cytotoxic Evaluation of 3-(4-Fluorophenyl)-4,5-dihydro-5-(3,4,5-trimethoxy/4-nitrophenyl)-*N*-(substituted-phenyl)pyrazole-1-carboxamide Analogues

Mohamed J. Ahsan^{*a}

Bhawani S. Kumawat^a

Sonu Kumawat^a

Piush Sharma^a

Mohammad A. Bakht^b

Mohd Z. Hassan^c

Afzal Hussain^d

Pankaj Saraswat^e

Habibullah Khalilullah^f

^a Department of Pharmaceutical Chemistry, Maharishi Arvind College of Pharmacy, Ambabari Circle, Jaipur, Rajasthan 302 039, India

jawedpharma@gmail.com

^b Department of Chemistry, College of Science & Humanities, Prince Sattam Bin Abdulaziz University, P.O. Box 11323, Saudi Arabia

^c Department of Pharmaceutical Chemistry, College of Pharmacy, King Khalid University, Abha 62529, Saudi Arabia

^d Department of Pharmaceutical Science & Technology, Birla Institute of Science & Technology, Mesra, Ranchi, Jharkhand 835 215, India

^e Department of Pharmaceutical Chemistry, Alwar Pharmacy College, Alwar, Rajasthan 301 030, India

^f Department of Pharmaceutical Chemistry, Unaizah College of Pharmacy, Qassim University, Al-Qassim 51911, Kingdom of Saudi Arabia

Received: 24.01.2018

Accepted after revision: 14.03.2018

Published online: 23.04.2018

DOI: 10.1055/s-0036-1591980; Art ID: so-2018-d0007-op

License terms:

Abstract A novel series of 3-(4-fluorophenyl)-4,5-dihydro-5-(3,4,5-trimethoxy/4-nitrophenyl)-*N*-(substituted-phenyl)pyrazole-1-carboxamide analogues **4a–n** was synthesized in two steps from 4-fluoroacetophenone. The pyrazoline analogues were evaluated for cytotoxicity against two breast cancer cell lines (MCF-7 and MBA-MD-231) by the sulforhodamine B (SRB) assay. *N*-(4-Chlorophenyl)-3-(4-fluorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (**4b**) showed the most promising cytotoxicity among the series, with GI_{50} < 0.1 and 45.8 μ M against the cancer cell lines, MCF-7 and MDA-MB-231, respectively. The anticancer activity of **4b** was found to be comparable to that of the standard drug adriamycin (GI_{50} < 0.1) against the MCF-7 cancer cell line. Structure activity relationships (SAR) are also considered.

Key words anticancer agents, breast cancer cell lines, MCF-7, MDA-MB-231, SRB assay, pyrazolines

In 2015, nearly 8.8 million cancer related deaths were reported. In India, every year over 0.7 million new cancer patients are registered and 0.5 million cancer related death are reported,^{1,2} and it is expected that new cases of cancer will amount to 19.3 million per annum worldwide by

2025.³ Chemotherapy is an important approach to cancer treatment, but it has drawbacks of toxicity, resistance, and genotoxicity.⁴ Today, we need to focus on drug discovery programmes to develop effective and safer anticancer agents for cancer treatment.

Five-membered pyrazoline rings have received much attention because of their diverse biological potential. Some of the pyrazoline incorporated compounds that have promising biological activities are shown in Figure 1. Pyrazoloacridine (**I**) is a new anticancer agent in Phase II clinical trial.^{5–7} 3-(5'-Hydroxymethyl-2'-furyl)-1-benzyl indazole (YC-1) (**II**) is a hypoxia-inducible factor (HIF)-1 inhibitor.^{8,9} Axitinib (AG013736) (**III**) is an endothelial growth factor receptor (VEGFR) inhibitor in clinical practice.^{10,11} 4-(4-Chlorophenyl)-2-(3-(3,4-dimethylphenyl)-5-(4-fluorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)thiazole (**IV**) is an EGFR TK inhibitor (IC_{50} = 0.06 μ M).¹² 5-Bromo-3-{2-[5-(4-methoxyphenyl)-3-naphthalen-2-yl-4,5-dihydropyrazol-1-yl]-4-oxo-4,5-dihydro-1,3-thiazol-5-ylidene}-2,3-dihydro-1*H*-indol-2-one (**V**) is a potent anticancer agent with promising activity against HOP-92 (GI_{50} < 0.01 μ M), HCT-116 (GI_{50} = 0.018 μ M), SNB-75 (GI_{50} = 0.0159 μ M), RXF 393 (GI_{50} = 0.0197 μ M), and NCI/ADR-RES (GI_{50} = 0.0169 μ M).¹³ 3-Benzofuran-2-yl-5-(4-dimethylaminonaphthalen-1-yl)-4,5-dihydropyrazole-1-carbothioic acid amide (**VI**) was found to

possess anticancer activity against various cancer cell lines, including HT-29 ($IC_{50} = 1.3 \pm 0.4$), PC-3 ($IC_{50} = 0.9 \pm 0.5$), MCF-7 ($IC_{50} = 1.3 \pm 0.4$), H-460 ($IC_{50} = 1.4 \pm 0.8$), A-549 ($IC_{50} = 1.10 \pm 0.5$), PaCa-2 ($IC_{50} = 0.9 \pm 0.8$), and Panc-1 ($IC_{50} = 1.2 \pm 0.2$) targeting tyrosinase.¹⁴ All compounds **I–VI** contain the pyrazoline nucleus (Figure 1). Furthermore, reports of other biological activities of pyrazoline analogues include antitubercular,¹⁵ anticonvulsant,¹⁶ antimicrobial,^{17,18} selective HER inhibition,¹⁹ anti-inflammation,²⁰ anti-HIV,¹⁷ carbonic anhydrase inhibition,²¹ antiproliferatin^{22,23} and tyrosinase inhibition.¹⁴ Hence, we have synthesized some novel pyrazoline analogues and report herein their cytotoxicity evaluation. The 4-fluorophenyl pyrazoline pharmacophore was chosen because the same pharmacophore is present in **IV**, which shows excellent anti-EGFR TK inhibition.

Figure 1 Pyrazoline incorporated compounds and their biological activities

In the initial step, (2*E*)-1-(4-fluorophenyl)-3-(substituted-phenyl)prop-2-en-1-one derivatives **3a** and **3b** were synthesized from 4-fluoroacetophenone by Claisen-Schmidt condensation. 4-Fluoroacetophenone **1** (0.05 mol, 6.07 mL) and aromatic aldehyde **2a** or **2b** (0.05 mol) were dissolved in absolute ethanol (50 mL) and 30% NaOH solu-

tion was added dropwise with continuous stirring at room temperature for 4 h.^{15,19} The reaction mixture was kept standing overnight and further poured into the crushed ice to obtain a pale-yellow precipitate of (2*E*)-1-(4-fluorophenyl)-3-(substituted-phenyl)prop-2-en-1-one derivatives **3a** and **3b**. In the subsequent step an equimolar mixture of **3a** or **3b** and substituted phenyl semicarbazide was heated at reflux in glacial acetic acid for 12 h to obtain the 3-(4-fluorophenyl)-4,5-dihydro-5-(3,4,5-trimethoxy/4-nitrophenyl)-*N*-(substituted phenyl)pyrazole-1-carboxamide analogues **4a–n**. The substituted phenyl semicarbazides were synthesized as per the reported method.²¹ The reaction was monitored throughout by thin-layer chromatography (TLC silica gel 60 F₂₅₄; mobile phase benzene/acetone, 8:2). The title compounds **4a–n** were further recrystallized from ethanol to give the pure compounds in yields of 65–81%. The synthetic protocol is summarized in Scheme 1.

Analogues **4a–n** were further characterized based on their IR, NMR (¹H and ¹³C) and MS data. The prototype compound **4b** showed amide stretching band at 3302 cm^{−1} for NH, and 1682 cm^{−1} for the carbonyl function (C=O) in the IR spectra. Other prominent absorption peaks were found at 1522, 810, and 690 cm^{−1} for C=N (pyrazoline), C–F and C–Cl, respectively. In the ¹H NMR spectrum, two doublet peaks at $\delta = 3.16$ and 3.78 ppm corresponding to the two protons of pyrazoline ring (H_a and H_b), a triplet at $\delta = 5.19$ ppm corresponding to the other proton of the pyrazoline ring (H_c); a multiplet $\delta = 7.01$ –7.21 ppm was observed for the corresponding four aromatic protons (4-fluorophenyl); four doublet $\delta = 7.25$, 7.68, 7.74, and 8.11 ppm for eight aromatic protons. The CONH peak was obtained as a singlet at $\delta = 8.36$ ppm. The ¹³C NMR spectrum showed peaks at $\delta = 166.01$, 165.19, 151.83, 149.63, 146.29, 134.02, 130.82, 129.61, 129.11, 127.92, 123.09, 120.91, 115.61, 61.31, and 39.43 ppm. The mass spectral data showed the $[M+H]^+$ signal at m/z 439, corresponding to the molecular formula C₂₂H₁₆ClFN₄O₃.

Similarly, the prototype compound **4i** showed amide stretching band at 3312 cm^{-1} for NH, and 1685 cm^{-1} for the carbonyl function (C=O) in the IR spectra. Other prominent absorption peaks were found at 1523 , 812 and 694 cm^{-1} for C=N (pyrazoline), C-F and C-Cl, respectively. In the ^1H NMR spectrum, two doublet peaks at $\delta = 3.42$ and 3.58 ppm corresponding to the two of pyrazoline ring (H_a and H_b), a triplet at $\delta = 5.41\text{ ppm}$ corresponding to the another proton of pyrazoline ring (H_c); a singlet peak at $\delta = 3.84\text{ ppm}$ corresponding to the six methoxy protons (OCH_3); a singlet peak at $\delta = 3.93\text{ ppm}$ corresponding to the three methoxy protons (OCH_3); a singlet peak at $\delta = 7.23\text{ ppm}$ corresponding to the two aromatic protons (3,4,5-trimethoxyphenyl); a multiplet $\delta = 7.32\text{--}7.42\text{ ppm}$ was observed for the corresponding four aromatic proton (4-fluorophenyl); two doublet $\delta = 7.70$ and 7.89 ppm for four aromatic protons (4-chlorophenyl). The CONH peak was obtained as singlet $\delta = 8.26\text{ ppm}$. The ^{13}C NMR showed peaks at $\delta = 166.71$, 165.02 , 151.83 , 150.63 , 137.89 , 137.23 , 134.02 , 130.81 , 129.99 , 129.63 , 129.11 , 123.01 , 115.66 , 104.33 , 61.95 , 56.56 , 56.24 , and 39.41 ppm . The mass spectral data showed the $[\text{M}+\text{H}]^+$ signal at m/z 484, corresponding to the molecular formula $\text{C}_{25}\text{H}_{23}\text{ClFN}_3\text{O}_4$. The purity was checked by microanalysis (C, H and N analysis).

Cytotoxicity

The cytotoxicity of analogues **4a–n** was tested against two breast cancer cell lines (MCF-7 and MDA-MB-231) according to the sulforhodamine B (SRB) assay.²⁴ The percent growth control was recorded for each drug at four different drug molar concentrations (10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M). Most of the pyrazoline analogues showed significant cytotoxicity at higher dose of 10^{-4} M . The cytotoxicity of compounds **4d** (growth percent (GP) = -55.1%) and **4m** (GP = -54.6%) was found to be comparable to the standard drug adriamycin (GP = -57.8%) at the higher dose concentration (10^{-4} M). The results of percent growth control at different molar concentrations are summarized in Table 1. The plotted growth curve is shown in Figure 2a (MCF-7) and Figure 2b (MDA-MB-231).

Three further dose-related parameters, LC_{50} , GI_{50} , and TGI, were also recorded for each compound; these are given in Table 2. The LC_{50} was found to be $>100\text{ }\mu\text{M}$ for each of the pyrazoline analogues **4a–n** against both the breast cancer cell lines (MCF-7 and MDA-MB-231). The TGI was found to be between <0.1 and $97.3\text{ }\mu\text{M}$ against MCF-7 cancer cell lines and $>100\text{ }\mu\text{M}$ against MDA-MB-231 cancer cell line. The pyrazoline analogues **4a–n** showed promising cytotox-

Figure 2 (a) Growth curve of analogues **4a–n** against (a) MCF-7 and (b) MDA-MB-231

icity against the MCF-7 ($\text{GI}_{50} = <0.1$ to $42.9\text{ }\mu\text{M}$), whereas the cytotoxicity was found to be moderate or low against MDA-MB-231 ($\text{GI}_{50} = 45.8$ to $>100\text{ }\mu\text{M}$). Eight compounds, **4d**, **4j**, **4n**, **4l**, **4f**, **4c**, **4e**, and **4g** showed less cytotoxicity against MCF-7 cancer cell line, with GI_{50} ranging between 21.3 and $42.9\text{ }\mu\text{M}$. Five compounds, **4i** ($\text{GI}_{50} = 6.6\text{ }\mu\text{M}$), **4k** ($\text{GI}_{50} = 12.4\text{ }\mu\text{M}$), **4a** ($\text{GI}_{50} = 14.2\text{ }\mu\text{M}$), **4h** ($\text{GI}_{50} = 14.9\text{ }\mu\text{M}$) and **4m** ($\text{GI}_{50} = 16.0\text{ }\mu\text{M}$) showed moderate cytotoxicity against MCF-7. *N*-(4-Chlorophenyl)-3-(4-fluorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (**4b**) showed the highest cytotoxicity among the series, with GI_{50} of $<0.1\text{ }\mu\text{M}$ against the cancer cell line MCF-7. The cytotoxicity of compound **4b** and the standard drug adriamycin ($\text{GI}_{50} = <0.1\text{ }\mu\text{M}$) was found to be equal against the MCF-7 cancer cell line. The images of growth for some of the pyrazoline having significant cytotoxicity against MCF-7 cancer cell line are shown in Figure 3.

All the pyrazoline analogues showed moderate or low cytotoxicity against the MDA-MB-231 cancer cell line, and compound **4b** showed the highest cytotoxicity among the series of pyrazoles **4a–n**, with GI_{50} of $45.8\text{ }\mu\text{M}$. Images of growth for some of the pyrazoline with significant anticancer activity against MDA-MB-231 cancer cell line are shown in Figure 4.

Table 1 Anticancer Activity (% Control Growth) of **4a–n** against Breast Cancer Cell Lines (MCF-7 and MDA-MB-231) at Four Molar Concentrations

Comp.	Molar Drug Concentrations (M)							
	MCF-7				MDA-MB-231			
	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻⁷	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴
4a	78.9	78.2	13.4	–12.4	103.5	84.9	75.6	33.3
4b	–8.4	–12.5	–15.9	–34.9	92.4	62.0	63.4	21.3
4c	88.3	101.9	74.3	–31.0	112.8	104.3	98.9	31.9
4d	81.7	88.4	50.1	–55.1	107.2	100.0	82.3	30.7
4e	100.8	106.7	42.7	1.3	100.7	106.4	78.6	35.2
4f	89.8	103.3	52.3	–18.7	115.1	98.1	99.1	39.1
4g	115.9	105.0	32.6	2.1	108.9	106.6	81.9	37.1
4h	74.3	77.4	18.0	–5.3	118.8	127.7	113.6	38.5
4i	53.4	77.9	–8.2	–24.1	102.4	101.4	86.8	41.9
4j	86.5	95.1	7.8	–4.0	125.8	125.3	106.7	94.2
4k	79.1	74.1	13.8	–16.0	107.7	104.0	89.2	173.3
4l	92.4	88.0	16.6	–6.7	133.0	145.3	124.0	68.4
4m	77.2	89.6	27.7	–54.6	102.5	95.7	72.1	41.1
4n	86.1	91.8	18.8	–8.1	105.7	99.0	94.0	32.0
ADR ^a	5.5	–24.1	–61.3	–57.8	31.8	39.8	–19.7	–15.7

^a ADR = Adriamycin **Figure 3** Images of growth control against MCF-7 cancer cell line**Table 2** LC₅₀, TGI, and GI₅₀ of **4a–n** against MCF-7 and MDA-MB-231 cancer cell lines

Comp.	Drug concentrations calculated from graph (μM)					
	MCF-7			MDA-MB-231		
	LC ₅₀	TGI	GI ₅₀	LC ₅₀	TGI	GI ₅₀
4a	>100	79.0	14.2	>100	>100	69.6
4b	>100	<0.1	<0.1	>100	>100	45.8
4c	>100	74.7	34.5	>100	>100	76.1
4d	>100	58.3	21.3	>100	>100	70.9
4e	>100	97.7	42.1	>100	>100	75.0
4f	>100	80.6	34.0	>100	>100	83.7
4g	>100	97.3	42.9	>100	>100	78.5
4h	>100	87.5	14.9	>100	>100	>100
4i	>100	61.5	6.6	>100	>100	85.0
4j	>100	88.4	23.0	>100	>100	>100
4k	>100	75.3	12.4	>100	>100	NE ^a
4l	>100	86.4	24.8	>100	>100	>100
4m	>100	55.1	16.0	>100	>100	80.4
4n	>100	85.2	24.3	>100	>100	>100
ADR ^b	82.9	2.7	<0.1	>100	50.9	<0.1

^a NE = Not evaluated^b ADR = Adriamycin.

Figure 4 Images of growth control against MDA-MB-231 cancer cell line

Structure activity relationship studies (SAR) were established with the anticancer data (GI_{50}) and the results are summarized in Figure 5.

Pyrazolines with a 4-nitrophenyl substituent at position 5 of the pyrazoline showed higher anticancer activity than those with 3,4,5-trimethoxyphenyl substitution. Furthermore, substrates with a 4-chloro substitution at the *N*-phenyl group showed the maximum anticancer activity. The order of anticancer activity was found to be 4-Cl>4-F>2-Cl>4-Br>4-OCH₃>4-CH₃>2,6-(CH₃)₂. The anticancer results showed that electron-withdrawing substituents at the *N*-phenyl group were found to be more significant than electron-releasing substitutions.

In conclusion, novel pyrazolines have been synthesized in satisfactory yield. All the pyrazoline analogues were evaluated for cytotoxicity against two breast cancer cell lines (MCF-7 and MDA-MB-231). *N*-(4-Chlorophenyl)-3-(4-fluorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (**4b**) showed the most promising anticancer activity among the series. Further structure activity relationships are also presented. Pyrazolines with 4-nitrophenyl substitution at position 5 of the pyrazoline showed higher anticancer activity and 4-chloro-substitution on the *N*-phenyl group led to maximum anticancer activity.

Preparation of 3-(4-Fluorophenyl)-4,5-dihydro-5-(3,4,5-trimethoxy/4-nitro phenyl)-*N*-(substituted-phenyl)pyrazole-1-carboxamide Analogues **4a–n**; General Procedure

An equimolar mixture of (2*E*)-1-(4-fluorophenyl)-3-(substituted phenyl)prop-2-en-1-one **3a** or **3b** (0.0015 mol) and substituted phenyl semicarbazides (0.0015 mol) was heated at reflux in glacial acetic acid for 12 h. The reaction mixture was further concentrated and excess of solvents was removed under vacuum and the reaction mixture was poured onto crushed ice, filtered, dried and washed with water to obtain analogues **4a–n**. The title compounds were further recrystallized from ethanol. Thin-layer chromatography (benzene/acetone, 8:2) was used to monitor the reaction.

N,3-Bis(4-fluorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (**4a**)

Yield: 0.513 g (81%); brown solid; m.p. 180–182 °C.

IR (KBr): 3322 (NH), 1685 (C=O), 1522 (C=N), 810 (C-F) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.29 (dd, *J* = 4.1, 13.1 Hz, 1 H, H_a), 3.63 (dd, *J* = 4.1, 13.1 Hz, 1 H, H_b), 5.19 (t, 1 H, H_c), 7.01–7.62 (m, 8 H, ArH), 7.64 (d, *J* = 8.1 Hz, 2 H, ArH), 8.12 (d, *J* = 8.1 Hz, 2 H, ArH), 8.26 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.01, 165.21, 158.41, 151.81, 149.61, 146.21, 131.52, 130.81, 129.63, 127.91, 123.21, 120.92, 115.79, 115.61, 61.13, 39.41.

LC-MS: *m/z* = 423 [M + H]⁺.

Anal. calcd.: C, 62.56; H, 3.82; N, 13.26; found: C, 62.54; H, 3.85; N, 13.22.

N-(4-Chlorophenyl)-3-(4-fluorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (**4b**)

Yield: 0.494 g (75%); pale-brown solid; m.p. 178–180 °C.

IR (KBr): 3322 (NH), 1680 (C=O), 1522 (C=N), 810 (C-F), 690 (C-Cl) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.16 (dd, *J* = 4.1, 13.1 Hz, 1 H, H_a), 3.78 (dd, *J* = 4.1, 13.1 Hz, 1 H, H_b), 5.19 (t, 1 H, H_c), 7.01–7.21 (m, 4 H, ArH), 7.25 (d, *J* = 8.0 Hz, 2 H, ArH), 7.68 (d, *J* = 8.1 Hz, 2 H, ArH), 7.74 (d, *J* = 8.1 Hz, 2 H, ArH), 8.11 (d, *J* = 8.0 Hz, 2 H, ArH), 8.14 (d, *J* = 8.1 Hz, 2 H, ArH), 8.36 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.01, 165.19, 151.83, 149.63, 146.29, 134.02, 130.82, 129.61, 129.11, 127.92, 123.09, 120.91, 115.61, 61.31, 39.43.

LC-MS: *m/z* = 439 [M + H]⁺.

Anal. calcd.: C, 60.21; H, 3.67; N, 12.77; found: C, 60.24; H, 3.69; N, 12.74.

Figure 5 Structure activity relationship studies on analogues **4a–n**

***N*-(4-Bromophenyl)-3-(4-fluorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (4c)**

Yield: 0.536 g (74%); creamy solid; m.p. 150–152 °C.

 IR (KBr): 3302 (NH), 1682 (C=O), 1519 (C=N), 811 (C-F), 598 (C-Br) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.19 (dd, *J* = 4.1, 13.1 Hz, 1 H, H_a), 3.63 (dd, *J* = 4.1, 13.1 Hz, 1 H, H_b), 5.21 (t, 1 H, H_c), 7.01–7.19 (m, 4 H, ArH), 7.35 (d, *J* = 8.0 Hz, 2 H, ArH), 7.41 (d, *J* = 7.9 Hz, 2 H, ArH), 7.54 (d, *J* = 7.9 Hz, 2 H, ArH), 8.14 (d, *J* = 8.0 Hz, 2 H, ArH), 8.29 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.91, 165.09, 151.89, 149.61, 146.49, 134.91, 131.91, 130.81, 129.61, 127.93, 123.87, 120.95, 115.69, 61.37, 39.49.

 LC-MS: *m/z* = 484 [M + H]⁺.

Anal. calcd.: C, 54.67; H, 3.34; N, 11.59; found: C, 54.64; H, 3.37; N, 11.57.

***N*-(2-Chlorophenyl)-3-(4-fluorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (4d)**

Yield: 0.526 g (80%); pale-brown solid; m.p. 112–114 °C.

 IR (KBr): 3321 (NH), 1678 (C=O), 1523 (C=N), 812 (C-F), 694 (C-Cl) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.21 (dd, *J* = 4.3, 11.1 Hz, 1 H, H_a), 3.53 (dd, *J* = 3.1, 12.1 Hz, 1 H, H_b), 5.23 (t, 1 H, H_c), 6.94–7.52 (m, 8 H, ArH), 7.55 (d, *J* = 7.9 Hz, 2 H, ArH), 8.14 (d, *J* = 7.9 Hz, 2 H, ArH), 8.26 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.92, 165.06, 151.83, 149.65, 146.51, 134.95, 130.83, 130.51, 129.64, 129.10, 127.91, 127.11, 125.85, 123.01, 120.91, 115.61, 61.36, 39.43.

 LC-MS: *m/z* = 439 [M + H]⁺.

Anal. calcd.: C, 60.21; H, 3.67; N, 12.77; found: C, 60.25; H, 3.65; N, 12.75.

***3*-(4-Fluorophenyl)-*N*-(4-methylphenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (4e)**

Yield: 452 g (72%); pale-brown solid; m.p. 184–186 °C.

 IR (KBr): 3319 (NH), 1681 (C=O), 1516 (C=N), 812 (C-F) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 2.34 (s, 3 H, CH₃), 3.19 (dd, *J* = 3.1, 10.1 Hz, 1 H, H_a), 3.51 (dd, *J* = 3.2, 11.1 Hz, 1 H, H_b), 5.23 (t, 1 H, H_c), 7.01 (d, *J* = 7.8 Hz, 2 H, ArH), 7.04–7.22 (m, 4 H, ArH), 7.38 (d, *J* = 7.9 Hz, 2 H, ArH), 7.52 (d, *J* = 7.8 Hz, 2 H, ArH), 8.14 (d, *J* = 7.9 Hz, 2 H, ArH), 8.29 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.90, 165.11, 151.85, 149.61, 146.41, 134.05, 132.93, 130.81, 129.63, 129.39, 127.93, 121.55, 120.95, 115.61, 61.39, 39.45, 21.35.

 LC-MS: *m/z* = 419 [M + H]⁺.

Anal. calcd.: C, 66.02; H, 4.58; N, 13.39; found: C, 66.05; H, 4.55; N, 13.37.

***3*-(4-Fluorophenyl)-*N*-(4-methoxyphenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (4f)**

Yield: 0.424 g (65%); pale-brown solid; m.p. 136–138 °C.

 IR (KBr): 3309 (NH), 1684 (C=O), 1514 (C=N), 814 (C-F) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.21 (dd, *J* = 5.6, 12.1 Hz, 1 H, H_a), 3.53 (dd, *J* = 5.3, 12.1 Hz, 1 H, H_b), 3.73 (s, 3 H, OCH₃), 5.26 (t, 1 H, H_c), 6.77 (d, *J* = 7.9 Hz, 2 H, ArH), 7.02–7.19 (m, 4 H, ArH), 7.39 (d, *J* = 8.1 Hz, 2 H, ArH), 7.53 (d, *J* = 7.9 Hz, 2 H, ArH), 8.14 (d, *J* = 8.1 Hz, 2 H, ArH), 8.26 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.90, 165.11, 156.33, 151.81, 149.63, 146.40, 130.88, 129.63, 128.21, 127.93, 122.66, 120.91, 115.55, 114.51, 61.31, 55.93, 39.43.

 LC-MS: *m/z* = 435 [M + H]⁺.

Anal. calcd.: C, 63.59; H, 4.41; N, 12.90; found: C, 63.56; H, 4.45; N, 12.88.

***3*-(4-Fluorophenyl)-*N*-(2,6-methylphenyl)-5-(4-nitrophenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (4g)**

Yield: 0.486 g (75%); pale-brown solid; m.p. 190–192 °C.

 IR (KBr): 3309 (NH), 1684 (C=O), 1514 (C=N), 814 (C-F) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 2.21 (s, 6 H, CH₃), 3.14 (dd, *J* = 6.1, 7.8 Hz, 1 H, H_a), 3.75 (dd, *J* = 6.1, 11.1 Hz, 1 H, H_b), 5.18 (t, 1 H, H_c), 6.84 (s, 1 H, ArH), 6.77–7.19 (m, 7 H, ArH), 7.39 (d, *J* = 8.0 Hz, 2 H, ArH), 8.14 (d, *J* = 8.0 Hz, 2 H, ArH), 8.16 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.29, 165.14, 151.81, 149.63, 146.41, 134.69, 134.20, 130.81, 129.61, 127.91, 126.31, 124.21, 120.93, 115.61, 61.39, 39.46, 15.56.

 LC-MS: *m/z* = 433 [M + H]⁺.

Anal. calcd.: C, 66.66; H, 4.89; N, 12.96; found: C, 66.63; H, 4.87; N, 12.99.

***N*,3-Bis(4-Fluorophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (4h)**

Yield: 0.546 g (78%); creamy solid; m.p. 170–172 °C.

 IR (KBr): 3319 (NH), 1682 (C=O), 1553 (C=N), 810 (C-F) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.41 (dd, *J* = 6.04, 11.2 Hz, 1 H, H_a), 3.58 (dd, *J* = 6.4, 10.1 Hz, 1 H, H_b), 3.83 (s, 6 H, OCH₃), 3.91 (s, 3 H, OCH₃), 5.42 (t, 1 H, H_c), 7.23 (s, 2 H, ArH), 7.32–7.62 (m, 8 H, ArH), 8.26 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.91, 165.12, 158.66, 151.83, 150.66, 137.89, 137.21, 131.51, 130.83, 129.63, 123.21, 115.71, 115.60, 104.93, 61.93, 56.56, 56.25, 39.46.

 LC-MS: *m/z* = 468 [M + H]⁺.

Anal. calcd.: C, 64.23; H, 4.96; N, 8.99; found: C, 64.25; H, 4.99; N, 8.96.

***N*-(4-Chlorophenyl)-3-(4-fluorophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (4i)**

Yield: 0.537 g (74%); yellow solid; m.p. 164–166 °C.

 IR (KBr): 3312 (NH), 1685 (C=O), 1523 (C=N), 812 (C-F), 694 (C-Cl) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.42 (dd, *J* = 4.0, 11.2 Hz, 1 H, H_a), 3.58 (dd, *J* = 13.2, 10.0 Hz, 1 H, H_b), 3.85 (s, 6 H, OCH₃), 3.93 (s, 3 H, OCH₃), 5.41 (t, 1 H, H_c), 7.23 (s, 2 H, ArH), 7.32–7.42 (m, 4 H, ArH), 7.70 (d, *J* = 15.2 Hz, 2 H, ArH), 7.89 (d, *J* = 15.6 Hz, 2 H, ArH), 8.26 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.71, 165.02, 151.83, 150.63, 137.89, 137.23, 134.02, 130.81, 129.99, 129.63, 129.11, 123.01, 115.66, 104.33, 61.95, 56.56, 56.24, 39.41.

 LC-MS: *m/z* = 484 [M + H]⁺.

Anal. calcd.: C, 62.05; H, 4.79; N, 8.68; found: C, 62.05; H, 4.81; N, 8.65.

***N*-(4-Bromophenyl)-3-(4-fluorophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (4j)**

Yield: 0.515 g (65%); pale-brown solid; m.p. 170–172 °C.

IR (KBr): 3318 (NH), 1680 (C=O), 1543 (C=N), 810 (C-F), 598 (C-Br) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.41 (dd, *J* = 4.1, 13.1 Hz, 1 H, H_a), 3.61 (dd, *J* = 4.2, 12.1 Hz, 1 H, H_b), 3.85 (s, 6 H, OCH₃), 3.91 (s, 3 H, OCH₃), 5.39 (t, 1 H, H_c), 7.22 (s, 2 H, ArH), 7.24–7.39 (m, 4 H, ArH), 7.41 (d, *J* = 8.0 Hz, 2 H, ArH), 7.53 (d, *J* = 8.0 Hz, 2 H, ArH), 8.24 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.92, 165.12, 151.89, 150.61, 137.81, 137.21, 134.92, 131.89, 130.89, 129.61, 123.83, 118.71, 115.61, 104.36, 61.92, 56.56, 56.21, 39.43.

LC-MS: *m/z* = 528 [M + H]⁺.

Anal. calcd.: C, 56.83; H, 4.39; N, 7.95; found: C, 56.85; H, 4.41; N, 7.92.

***N*-(2-Chlorophenyl)-3-(4-fluorophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (4k)**

Yield: 0.522 g (72%); pale-yellow solid; m.p. 174–176 °C.

IR (KBr): 3321 (NH), 1680 (C=O), 1551 (C=N), 812 (C-F), 698 (C-Cl) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.34 (dd, *J* = 3.1, 10.1 Hz, 1 H, H_a), 3.59 (dd, *J* = 3.1, 10.2 Hz, 1 H, H_b), 3.83 (s, 6 H, OCH₃), 3.91 (s, 3 H, OCH₃), 5.38 (t, 1 H, H_c), 7.12 (s, 2 H, ArH), 7.14–7.42 (m, 8 H, ArH), 8.22 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.80, 165.09, 151.81, 150.69, 137.85, 137.21, 134.92, 130.81, 130.51, 129.61, 129.10, 127.11, 125.86, 123.02, 115.61, 104.37, 61.92, 56.55, 56.26, 39.49.

LC-MS: *m/z* = 484 [M + H]⁺.

Anal. calcd.: C, 62.05; H, 4.79; N, 8.68; found: C, 62.05; H, 4.81; N, 8.65.

***N*-(4-Methylphenyl)-3-(4-fluorophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (4l)**

Yield: 0.486 g (70%); pale-yellow solid; m.p. 176–178 °C.

IR (KBr): 3319 (NH), 1688 (C=O), 1549 (C=N), 811 (C-F) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 2.34 (s, 3 H, CH₃), 3.35 (dd, *J* = 4.3, 12.1 Hz, 1 H, H_a), 3.61 (dd, *J* = 3.1, 11.1 Hz, 1 H, H_b), 3.85 (s, 6 H, OCH₃), 3.92 (s, 3 H, OCH₃), 5.33 (t, 1 H, H_c), 6.98 (d, *J* = 7.9 Hz, 2 H, ArH), 7.13 (s, 2 H, ArH), 7.14–7.25 (m, 4 H, ArH), 7.53 (d, *J* = 7.9 Hz, 2 H, ArH), 8.23 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.81, 165.09, 151.83, 150.63, 137.82, 134.05, 132.91, 130.81, 130.21, 129.61, 129.30, 121.56, 115.62, 104.32, 61.91, 56.56, 56.22, 39.49, 23.31.

LC-MS: *m/z* = 463 [M⁺], 464 [M + H]⁺.

Anal. calcd.: C, 67.37; H, 5.65; N, 9.07; found: C, 67.35; H, 5.67; N, 9.05.

***N*-(4-Methoxyphenyl)-3-(4-fluorophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (4m)**

Yield: 0.503 g (70%); pale-yellow solid; m.p. 176–178 °C.

IR (KBr): 3312 (NH), 1682 (C=O), 1523 (C=N), 812 (C-F) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 3.41 (dd, *J* = 3.6, 12.8 Hz, 1 H, H_a), 3.62 (dd, *J* = 2.0, 16.8 Hz, 1 H, H_b), 3.70 (s, 6 H, OCH₃), 3.72 (s, 6 H, OCH₃), 5.22 (t, 1 H, H_c), 7.23 (s, 2 H, ArH), 7.24–7.42 (m, 4 H, ArH), 7.69 (d, *J* = 8.0 Hz, 2 H, ArH), 7.88 (d, *J* = 8.0 Hz, 2 H, ArH), 8.25 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.91, 165.19, 156.31, 151.81, 150.61, 137.81, 130.81, 130.23, 129.61, 128.21, 122.66, 115.61, 114.05, 104.38, 61.97, 56.56, 56.26, 39.41.

LC-MS: *m/z* = 480 [M + H]⁺.

Anal. calcd.: C, 65.13; H, 5.47; N, 8.76; found: C, 65.15; H, 5.49; N, 8.73.

***N*-(2,6-Dimethylphenyl)-3-(4-fluorophenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carboxamide (4n)**

Yield: 0.537 g (75%); creamy solid; m.p. 182–184 °C.

IR (KBr): 3288 (NH), 1678 (C=O), 1541 (C=N), 812 (C-F) cm⁻¹.

¹H NMR (400 MHz, DMSO-*d*₆): δ = 2.49 (s, 3 H, CH₃), 3.56 (dd, *J* = 11.6, 13.2 Hz, 1 H, H_a), 3.63 (dd, *J* = 7.2, 7.2 Hz, 1 H, H_b), 3.85 (s, 6 H, OCH₃), 3.93 (s, 3 H, OCH₃), 5.19 (t, 1 H, H_c), 7.23 (s, 2 H, ArH), 7.32–7.42 (m, 4 H, ArH), 7.43–7.71 (m, 4 H, ArH), 8.25 (s, 1 H, CONH).

¹³C NMR (100 MHz, DMSO-*d*₆): δ = 166.91, 165.19, 151.81, 150.61, 137.81, 137.24, 134.61, 134.23, 130.81, 129.61, 126.31, 124.21, 115.63, 104.31, 61.91, 56.56, 56.24, 39.41, 15.51.

LC-MS: *m/z* = 478 [M + H]⁺.

Anal. calcd.: C, 67.91; H, 5.91; N, 8.80; found: C, 67.95; H, 5.93; N, 8.78.

Acknowledgment

The Management of Maharishi Arvind College of Pharmacy is acknowledged for providing the necessary research facilities. The authors are also grateful to Dr. Reddy Lifescience, Hyderabad and Anticancer Drug Screening Facility (ACTREC), Navi Mumbai, India.

References

- (1) Siegel, R. L.; Miller, K. D.; Jemal, A. CA: *Cancer J. Clin.* **2017**, 67, 7.
- (2) Nandakumar, A. *National Cancer Registry Programme. Indian Council for Medical Research, Consolidated Report of the Population Based Cancer Registries 1990-96. New Delhi: Indian Council of Medical Research; 2009.*
- (3) Cancer fact sheet February 2017: <http://www.who.int/media-centre/factsheets/fs297/en/>
- (4) Aydemir, N.; Bilaloglu, R. *Mutat. Res., Genet. Toxicol. Environ. Mutagen.* **2003**, 537, 43.
- (5) Dees, E. C.; Rowinsky, E. K.; Noe, D. A.; O'Reilly, S.; Adjei, A. A.; Elza-Brown, K.; Donehower, R. C. *Invest. New Drugs* **2003**, 21, 75.
- (6) Berg, S. L.; Blaney, S. M.; Sullivan, J.; Bernstein, M.; Dubowy, R.; Harris, M. B. *J. Pediatr. Hematol./Oncol.* **2000**, 22, 506.
- (7) Ramaswamy, B.; Mrozek, E.; Kuebler, J. P.; Bekaii-Saab, T.; Kraut, E. H. *Invest. New Drugs* **2011**, 29, 347.
- (8) Na, J. I.; Na, J. Y.; Choi, W. Y.; Lee, M. C.; Park, M. S.; Choi, K. H.; Lee, J. K.; Kim, K. T.; Park, J. T.; Kim, H. S. *Am. J. Transl. Res.* **2015**, 7, 751.
- (9) Chang, L. C.; Lin, H. Y.; Tsai, M. T.; Chou, R. H.; Lee, F. Y.; Teng, C. M.; Hsieh, M. T.; Hung, H. Y.; Huang, L. J.; Yu, Y. L.; Kyo, H. C. *Br. J. Pharmacol.* **2014**, 171, 4010.
- (10) George, D. J. *Clin. Cancer Res.* **2007**, 13, 753.

- (11) Rini, B.; Wilding, G.; Hudes, G.; Stadler, W. M.; Kim, S.; Tarazi, J.; Bycott, P.; Liao, K.; Dutcher, J. *EJC Suppl.* **2007**, 5, 300.
- (12) Lv, P. C.; Li, D. D.; Li, Q. S.; Lu, X.; Xiao, Z. P.; Zhu, H. L. *Bioorg. Med. Chem. Lett.* **2011**, 21, 5374.
- (13) Havrylyuk, D.; Zimenkovsky, B.; Vasylenko, O.; Gzella, A.; Lesyk, R. *J. Med. Chem.* **2012**, 55, 8630.
- (14) Qin, H.; Shang, Z.; Jantan, I.; Tan, O. U.; Hussain, M. A.; Sher, M.; Bukhari, S. N. A. *RSC Adv.* **2015**, 5, 463330.
- (15) Ahsan, M. J.; Samy, G. J.; Habibullah, K.; Bakht, M. A.; Hassan, M. Z. *Eur. J. Med. Chem.* **2011**, 46, 5694.
- (16) Ahsan, M. J.; Samy, G. J.; Khalilullah, H.; Moham, G.; Stables, J. P. *Bioorg. Med. Chem. Lett.* **2012**, 22, 7029.
- (17) Shamsuzzaman, ; Khanam, H.; Dar, A. M.; Siddiqui, N.; Rehman, S. *J. Saudi Chem. Soc.* **2016**, 20, 7.
- (18) Yang, B.; Yang, Y. S.; Yang, N.; Li, G.; Zhu, H. L. *Sci. Rep.* **2016**, 6, 27571.
- (19) Elbayaa, R. Y.; Badr, M. H.; Khalil, A. A.; Abdelhadi, M. *Drug Res. (Stuttgart, Ger.)* **2013**, 63, 271.
- (20) Ahsan, M. J.; Samy, G. J.; Stables, J. P. *Med. Chem. Res.* **2013**, 22, 2746.
- (21) Gul, H. I.; Yamali, C.; Sakagami, H.; Angeli, A.; Leitans, J.; Kazaks, A.; Tars, K.; Ozgun, D. O.; Supuran, C. T. *Bioorg. Chem.* **2018**, 77, 411.
- (22) Yakaiah, S.; Kumar, P. S. V.; Rani, P. B.; Prasad, K. D.; Aparna, P. *Bioorg. Med. Chem. Lett.* **2018**, 28, 630.
- (23) Ahsan, M. J. *Lett. Drug Des. Discovery* **2012**, 9, 823.
- (24) Vichai, V.; Kirtikara, K. *Nat. Protoc.* **2006**, 1, 1112.