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

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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-1H-pyrazole-1-carboxamide (4b) showed the most promising cytotoxicity among the series, with GI50 <0.1 and 45.8 μM 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 (GI50 <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 deaths 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-1H-pyrazol-1-yl)thiazole (IV) is an EGFR TK inhibitor (IC50 = 0.06 μM).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-1H-indol-2-one (V) is a potent anticancer agent with promising activity against HOP-92 (GI50 < 0.01 μM), HCT-116 (GI50 = 0.018 μM), SNB-75 (GI50 = 0.0159 μM), RXF 393 (GI50 = 0.0197 μM), and NCI/ADR-RES (GI50 = 0.0169 μM).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 (IC50 = 1.3±0.4), PC-3 (IC50 = 0.9±0.5), MCF-7 (IC50 = 1.3±0.4), H-460 (IC50 = 1.4±0.8), A-549 (IC50 = 1.10±0.5), PaCa-2 (IC50 = 0.9±0.8), and Panc-1 (IC50 = 1.2±0.2) targeting tyrosinase. All compounds including HT-29 (IC50 = 1.3±0.4), PC-3 (IC50 = 0.9±0.5), MCF-7 (IC50 = 1.3±0.4), H-460 (IC50 = 1.4±0.8), A-549 (IC50 = 1.10±0.5), PaCa-2 (IC50 = 0.9±0.8), and Panc-1 (IC50 = 1.2±0.2) possessed anticancer activity against various cancer cell lines, including HT-29 (IC50 = 1.3±0.4), PC-3 (IC50 = 0.9±0.5), MCF-7 (IC50 = 1.3±0.4), H-460 (IC50 = 1.4±0.8), A-549 (IC50 = 1.10±0.5), PaCa-2 (IC50 = 0.9±0.8), and Panc-1 (IC50 = 1.2±0.2) targeting tyrosinase.

The 4-fluorophenyl pyrazoline pharmacophore was chosen because the same pharmacophore is present in several anticancer agents, such as Pyrazoloacridine and 4-(4-Chlorophenyl)-2-(3-(3,4-dimethoxyphenyl)-3-(4-fluorophenyl)-1H-pyrazol-1-yl)thiazole. The synthetic protocol is summarized in Scheme 1.

Analogue 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 δ = 3.16 and 3.78 ppm corresponding to the two protons of the pyrazoline ring (Ha and Hb); a multiplet δ = 7.01–7.21 ppm was observed for the corresponding four aromatic protons (4-fluorophenyl) was added dropwise with continuous stirring at room temperature for 4 h. The reaction mixture was kept standing overnight and further poured into the crushed ice to obtain a pale-yellow precipitate of (2E)-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. The reaction was monitored throughout by thin-layer chromatography (TLC silica gel 60 F254; mobile phase benzene/aceton, 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.

In the initial step, (2E)-1-(4-fluorophenyl)-3-(substituted-phenyl)prop-2-en-1-one derivatives 3a and 3b were synthesized from 4-fluorocacetophenone by Claisen-Schmidt condensation. 4-Fluorocacetophenone 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 solution was added dropwise with continuous stirring at room temperature for 4 h. The reaction mixture was kept standing overnight and further poured into the crushed ice to obtain a pale-yellow precipitate of (2E)-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. The reaction was monitored throughout by thin-layer chromatography (TLC silica gel 60 F254; mobile phase benzene/aceton, 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.

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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 $^1$H 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 $\delta = 3.94$ ppm corresponding to the other proton of pyrazoline ring (H$_c$); a singlet peak at $\delta = 3.84$ ppm corresponding to the six methoxy protons (OCH$_3$); a singlet peak at $\delta = 3.93$ ppm corresponding to the three methoxy protons (OCH$_3$); a singlet peak at $\delta = 7.23$ ppm corresponding to the two aromatic protons (3,4,5-trimethoxyphenyl); a multiplet $\delta = 7.32–7.42$ 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 $\delta = 8.26$ 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 [M+H]$^+$ signal at m/z 484, corresponding to the molecular formula C$_{25}$H$_{23}$ClFN$_3$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 μM 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 μM against MCF-7 cancer cell lines and >100 μM against MDA-MB-231 cancer cell line. The pyrazoline analogues 4a–n showed promising cytotoxicity against the MCF-7 (GI$_{50}$ = <0.1 to 42.9 μM), whereas the cytotoxicity was found to be moderate or low against MDA-MB-231 (GI$_{50}$ = 45.8 to >100 μM). Eight compounds, 4d, 4j, 4m, 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 μM. Five compounds, 4i (GI$_{50}$ = 6.6 μM), 4k (GI$_{50}$ = 12.4 μM), 4a (GI$_{50}$ = 14.2 μM), 4h (GI$_{50}$ = 14.9 μM) and 4m (GI$_{50}$ = 16.0 μM) showed moderate cytotoxicity against MCF-7. N-(4-Chlorophenyl)-3-(4-fluorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide (4b) showed the highest cytotoxicity among the series, with GI$_{50}$ of <0.1 μM against the cancer cell line MCF-7. The cytotoxicity of compound 4b and the standard drug adriamycin (GI$_{50}$ = <0.1 μM) 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 μM. Images of growth for some of the pyrazoline with significant anticaner 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

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Molar Drug Concentrations (M)</th>
<th>MCF-7</th>
<th>MDA-MB-231</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10^{-3}</td>
<td>10^{-6}</td>
<td>10^{-5}</td>
</tr>
<tr>
<td>4a</td>
<td>78.9</td>
<td>78.2</td>
<td>13.4</td>
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<tr>
<td>4b</td>
<td>-8.4</td>
<td>-12.5</td>
<td>-15.9</td>
</tr>
<tr>
<td>4c</td>
<td>88.3</td>
<td>101.9</td>
<td>74.3</td>
</tr>
<tr>
<td>4d</td>
<td>81.7</td>
<td>88.4</td>
<td>50.1</td>
</tr>
<tr>
<td>4e</td>
<td>100.8</td>
<td>106.7</td>
<td>42.7</td>
</tr>
<tr>
<td>4f</td>
<td>89.8</td>
<td>103.3</td>
<td>52.3</td>
</tr>
<tr>
<td>4g</td>
<td>115.9</td>
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<td>32.6</td>
</tr>
<tr>
<td>4h</td>
<td>74.3</td>
<td>77.4</td>
<td>18.0</td>
</tr>
<tr>
<td>4i</td>
<td>53.4</td>
<td>77.9</td>
<td>-8.2</td>
</tr>
<tr>
<td>4j</td>
<td>86.5</td>
<td>95.1</td>
<td>7.8</td>
</tr>
<tr>
<td>4k</td>
<td>79.1</td>
<td>74.1</td>
<td>13.8</td>
</tr>
<tr>
<td>4l</td>
<td>92.4</td>
<td>88.0</td>
<td>16.6</td>
</tr>
<tr>
<td>4m</td>
<td>77.2</td>
<td>89.6</td>
<td>27.7</td>
</tr>
<tr>
<td>4n</td>
<td>86.1</td>
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</tr>
<tr>
<td>ADRx</td>
<td>5.5</td>
<td>-24.1</td>
<td>-61.3</td>
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</table>

*a ADR = Adriamycin

### Table 2  LC_{50}, TGI, and GI_{50} of 4a–n against MCF-7 and MDA-MB-231 cancer cell lines

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Drug concentrations calculated from graph (μM)</th>
<th>MCF-7</th>
<th>MDA-MB-231</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC_{50}</td>
<td>TGI</td>
<td>GI_{50}</td>
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<tr>
<td>4a</td>
<td>&gt;100</td>
<td>79.0</td>
<td>14.2</td>
</tr>
<tr>
<td>4b</td>
<td>&gt;100</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>4c</td>
<td>&gt;100</td>
<td>74.7</td>
<td>34.5</td>
</tr>
<tr>
<td>4d</td>
<td>&gt;100</td>
<td>58.3</td>
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<tr>
<td>4e</td>
<td>&gt;100</td>
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<tr>
<td>4n</td>
<td>&gt;100</td>
<td>85.2</td>
<td>24.3</td>
</tr>
<tr>
<td>ADRx</td>
<td>82.9</td>
<td>2.7</td>
<td>&lt;0.1</td>
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</table>

*a NE = Not evaluated

b ADR = Adriamycin.
Structure activity relationship studies (SAR) were established with the anticancer data (GI\textsubscript{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-OCH\textsubscript{3} > 4-CH\textsubscript{3} > 2,6-(CH\textsubscript{3})\textsubscript{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-1H-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 (2E)-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/acetonitrile, 8:2) was used to monitor the reaction.

### N,3-Bis(4-fluorophenyl)-5-(4-nitrophenyl)-4,5-dihydro-1H-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\textsuperscript{-1}.

\[ \text{1H NMR} (400 MHz, DMSO-\text{d}_6): \delta = 3.29 (dd, J = 4.1, 13.1 Hz, 1 H, H\textsubscript{a}), 3.63 (dd, J = 4.1, 13.1 Hz, 1 H, H\textsubscript{b}), 5.19 (t, 1 H, H\textsubscript{c}), 7.01–7.62 (m, 8 H, ArH), 7.64 (d, J = 8.1 Hz, 2 H, ArH), 8.12 (d, J = 8.1 Hz, 2 H, ArH), 8.26 (s, 1 H, CONH). \]

\[ \text{13C NMR} (100 MHz, DMSO-\text{d}_6): \delta = 166.01, 165.21, 158.41, 151.81, 149.61, 146.21, 141.52, 132.91, 129.63, 127.91, 126.13, 115.79, 115.61, 61.13, 39.41. \]

\[ \text{LC-MS: } m/z = 423 [M + H]^+. \]

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-1H-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\textsuperscript{-1}.

\[ \text{1H NMR} (400 MHz, DMSO-\text{d}_6): \delta = 3.16 (dd, J = 4.1, 13.1 Hz, 1 H, H\textsubscript{a}), 3.78 (dd, J = 4.1, 13.1 Hz, 1 H, H\textsubscript{b}), 5.19 (t, 1 H, H\textsubscript{c}), 7.01–7.21 (m, 4 H, ArH), 7.25 (d, J = 8.0 Hz, 2 H, ArH), 7.68 (d, J = 8.1 Hz, 2 H, ArH), 7.74 (d, J = 8.1 Hz, 2 H, ArH), 8.11 (d, J = 8.0 Hz, 2 H, ArH), 8.14 (d, J = 8.1 Hz, 2 H, ArH), 8.36 (s, 1 H, CONH). \]

\[ \text{13C NMR} (100 MHz, DMSO-\text{d}_6): \delta = 166.01, 165.19, 151.83, 149.63, 146.29, 134.02, 132.91, 129.11, 129.63, 127.91, 121.21, 120.92, 115.79, 115.61, 61.31, 39.43. \]

\[ \text{LC-MS: } m/z = 439 [M + H]^+. \]

Anal. calcd.: C, 60.21; H, 3.67; N, 12.77; found: C, 60.24; H, 3.69; N, 12.74.
N-(4-Bromophenyl)-3-(4-fluorophenyl)-5-(4-nitrophenyl)-4,5-di-
hydro-1H-pyrazole-1-carboxamide (4c)
Yield: 0.536 g (74%); creamy solid; m.p. 150–152 °C.
IR (KBr): 3302 (NH), 1682 (C=0), 1519 (C=N), 811 (C-F), 598 (C-
Br) cm⁻¹.
¹H NMR (400 MHz, DMSO-d₆): δ = 3.19 (dd, J = 4.1, 13.1 Hz, 1 H, H₈),
3.63 (dd, J = 4.1, 13.1 Hz, 1 H, H₈), 5.21 (t, 1 H, H₂), 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-d₆): 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 = 435 [M + H]⁺.
Anal. calcld.: 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-di-
hydro-1H-pyrazole-1-carboxamide (4d)
Yield: 0.526 g (80%); pale-brown solid; m.p. 112–114 °C.
IR (KBr): 3321 (NH), 1678 (C=0), 1523 (C=N), 812 (C-F), 694 (C-
Cl) cm⁻¹.
¹H NMR (400 MHz, DMSO-d₆): δ = 3.21 (dd, J = 4.3, 11.1 Hz, 1 H, H₈),
3.53 (dd, J = 3.1, 12.1 Hz, 1 H, H₈), 5.23 (t, 1 H, H₂), 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-d₆): 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. calcld.: 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-1H-pyrazole-1-carboxamide (4e)
Yield: 452 g (72%); pale-brown solid; m.p. 184–186 °C.
IR (KBr): 3319 (NH), 1681 (C=0), 1516 (C=N), 812 (C-F) cm⁻¹.
¹H NMR (400 MHz, DMSO-d₆): δ = 2.34 (s, 3 H, CH₃), 3.19 (dd, J = 3.1,
10.1 Hz, 1 H, H₈), 3.51 (dd, J = 3.2, 11.1 Hz, 1 H, H₈), 5.23 (t, 1 H, H₂),
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-d₆): 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. calcld.: 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-1H-pyrazole-1-carboxamide (4f)
Yield: 0.424 g (65%); pale-brown solid; m.p. 136–138 °C.
IR (KBr): 3309 (NH), 1684 (C=0), 1514 (C=N), 814 (C-F) cm⁻¹.
¹H NMR (400 MHz, DMSO-d₆): δ = 3.21 (dd, J = 5.6, 12.1 Hz, 1 H, H₈),
3.53 (dd, J = 5.3, 12.1 Hz, 1 H, H₈), 3.73 (s, 3 H, OCH₃), 5.26 (t, 1 H, H₂),
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-d₆): δ = 166.91, 165.11, 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]⁺.
LC-MS: 115.61, 104.36, 61.92, 56.56, 56.21, 39.43.
137.81, 137.21, 134.92, 131.89, 130.89, 129.61, 123.83, 118.71,

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-d6): δ = 3.41 (dd, J = 4.1, 13.1 Hz, 1 H, H₂),
3.61 (dd, J = 4.2, 12.1 Hz, 1 H, H₂), 3.85 (s, 6 H, OCH₃), 3.91 (s, 3 H, OCH₃),
5.39 (t, 1 H, Hc), 7.22 (s, 2 H, ArH), 7.24–7.39 (m, 4 H, ArH),
7.41 (d, J = 8.0 Hz, 2 H, ArH), 7.88 (d, J = 8.0 Hz, 2 H, ArH).

13C NMR (100 MHz, DMSO-d6): δ = 137.82, 134.05, 132.91, 130.81,
127.21, 123.83, 118.71. 1H, CONH).

Anal. calcd.: C, 62.05; H, 4.79; N, 8.68; found: C, 62.05; H, 4.81; N, 8.73.

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References

(24) Vichai, V.; Kirtikara, K. *Nat. Protoc.* **2006,** *1,* 1112.