CHAPTER–IV

PART–I

Synthesis of Pyrazolyl-1,3,4-thiadiazole Analogues
4.1.1 Introduction

1,3,4-Thiadiazoles constitute a class of important heterocycles with “N–C–S” linkage which can work as the active center, chelate with certain metal ions in vivo and show good tissue permeability. The lower toxicity and in vivo stability of thiadiazole nucleus is attributed to its aromaticity.\(^1\) Amide bonds are neutral, stable and have both hydrogen-bond accepting and donating properties. Hence, they play a crucial role in the composition of biological systems and are present in more than 25% of the known drugs\(^2\) including major marketed drug Atorvastatin which blocks the production of cholesterol.\(^3\)

In an attempt to the quest and design of more promising and economical drugs in the clinical arena, hybrid molecules were designed through the combination of different pharmacophores in one frame. Thus pyrazole incorporated thiazole,\(^4\) 1,2,4-oxadiazole,\(^5\) 1,3,4-oxadiazole,\(^6\) 1,2,4-triazoles and benzoxazoles\(^7\) were synthesized and observed in the enhancement of pharmacological effect. Also several biologically active pyrazolyl-1,3,4-thiadiazole analogues have been reported.\(^8,9,10\) Therefore, interest in the synthesis of pyrazole integrated thiadiazole derivatives is significant.
4.1.2 Plan of the Synthesis

5-phenyl-1,3,4-thiadiazol-2-amines\textsuperscript{11} 53(a-e) were synthesized by heating a mixture aryl carboxylic acids I(a-e) and thiosemicarbazides (TSC) in presence of POCl\textsubscript{3} over a period of 3 to 4h as shown in Scheme 4.1.

\[
\begin{align*}
\text{I(a-e)} & \quad \text{TSC} & \quad \text{POCl}_3 & \quad \text{80-90°C} & \quad \text{53(a-e)} \\
\text{Ar}^2\text{COOH} & \quad \text{H}_2\text{N} & \quad \text{H} & \quad \text{N} & \quad \text{S} & \quad \text{Ar}^2 \\
a & \quad \text{C}_6\text{H}_5 & \quad \text{b} & \quad \text{4-Cl-C}_6\text{H}_4 & \quad \text{c} & \quad \text{4-NO}_2\text{-C}_6\text{H}_4 & \quad \text{d} & \quad \text{4-CH}_3\text{-C}_6\text{H}_4 & \quad \text{e} & \quad \text{4-CH}_3\text{-C}_6\text{H}_4
\end{align*}
\]

**Scheme 4.1.** Synthesis of 5-phenyl-1,3,4-thiadiazol-2-amines 53(a-e)

The two new series of pyrazole integrated 1,3,4-thiadiazole derivatives; 2-(5-methyl-1,3-diphenyl-1H-pyrazol-4-yl)-5-phenyl-1,3,4-thiadiazole 52(a-e) and 5-methyl-1,3-diphenyl-N-(5-phenyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-4-carboxamide 54(a-e) were synthesized. Compounds 52(a-e) were synthesized by the thionation followed by cyclization using Lawesson’s reagent (LR) and compounds 54(a-e) via coupling reaction between pyrazole-4-carboxylic acids 45(a-f) and 5-phenyl-1,3,4-thiadiazol-2-amines 53(a-e) utilizing a peptide coupling reagent EDC.HCl and an additive HOEt in DCM (Scheme 4.2).
Scheme 4.2: Synthesis of pyrazole integrated thiadiazole analogues
4.1.3 Discussion on the synthesis of 2-(5-methyl-1,3-diphenyl-1H-pyrazol-4-yl)-5-phenyl-1,3,4-thiadiazoles 52(a-e) and 5-methyl-1,3-diphenyl-N-(5-phenyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-4-carboxamides 54(a-e)

The synthesis of compounds 52(a-e) and 54(a-e) was began with pyrazole-4-carboxylates 44(a-e) as starting material and it was converted into the corresponding pyrazole-4-carboxylic acid 45(a-f) by refluxing with 10% NaOH solution in methanol for 4h followed by acidification with dilute hydrochloric acid. The pyrazole-4-carboxylic acid 45(a-f) was then treated with hydrazide compound 46(a-e) in the presence of coupling reagents EDC.HCl and HOBT in DCM to afford N’-benzoyl-5-methyl-1,3-diphenyl-1H-pyrazole-4-carbohydrazide 47(a-e). This was converted into the corresponding 2-(5-methyl-1,3-diphenyl-1H-pyrazol-4-yl)-5-phenyl-1,3,4-thiadiazole 52(a-e) by thionation followed by cyclization using LR. The 5-methyl-1,3-diphenyl-N-(5-phenyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-4-carboxamides 54(a-e) were achieved in one step by coupling reaction between the pyrazole-4-carboxylic acids 45(a-f) and 5-phenyl-1,3,4-thiadiazol-2-amins 53(a-e) utilizing a peptide coupling reagents EDC.HCl and HOBT in DCM.

To explore the influence of solvent, temperature, time and number of equivalents of LR on the conversion rate of the compound (47a) to (52a), various conditions were tested on a model compound and the results are summarized in Table 4.1.
Table 4.1: Reaction condition for step wise synthesis of 52a

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>Lawesson’s Reagent (equiv)</th>
<th>Yield(^a) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>THF</td>
<td>60</td>
<td>0.5</td>
<td>0.5</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>THF</td>
<td>60</td>
<td>1</td>
<td>1.0</td>
<td>52</td>
</tr>
<tr>
<td>3</td>
<td>THF</td>
<td>70</td>
<td>1</td>
<td>1.0</td>
<td>58</td>
</tr>
<tr>
<td>4</td>
<td>THF</td>
<td>70</td>
<td>2</td>
<td>1.0</td>
<td>86</td>
</tr>
<tr>
<td>5</td>
<td>THF</td>
<td>80</td>
<td>2</td>
<td>2.0</td>
<td>70</td>
</tr>
<tr>
<td>6</td>
<td>THF</td>
<td>80</td>
<td>2</td>
<td>2.5</td>
<td>72</td>
</tr>
<tr>
<td>7</td>
<td>THF</td>
<td>80</td>
<td>2</td>
<td>3.0</td>
<td>68</td>
</tr>
<tr>
<td>8</td>
<td>THF</td>
<td>80</td>
<td>3</td>
<td>2.0</td>
<td>74</td>
</tr>
<tr>
<td>9</td>
<td>Toluene</td>
<td>80</td>
<td>2</td>
<td>1.0</td>
<td>82</td>
</tr>
<tr>
<td>10</td>
<td>Toluene</td>
<td>80</td>
<td>2</td>
<td>2.0</td>
<td>72</td>
</tr>
<tr>
<td>11</td>
<td>Toluene</td>
<td>80</td>
<td>3</td>
<td>2.0</td>
<td>70</td>
</tr>
<tr>
<td>12</td>
<td>CH(_3)CN</td>
<td>80</td>
<td>2</td>
<td>2.0</td>
<td>62</td>
</tr>
<tr>
<td>13</td>
<td>CH(_3)CN</td>
<td>80</td>
<td>3</td>
<td>2.5</td>
<td>56</td>
</tr>
<tr>
<td>14</td>
<td>Xylene</td>
<td>120</td>
<td>2</td>
<td>2.0</td>
<td>72</td>
</tr>
<tr>
<td>15</td>
<td>Xylene</td>
<td>120</td>
<td>3</td>
<td>2.5</td>
<td>70</td>
</tr>
</tbody>
</table>

\(^a\)Isolated yields; Reaction were carried out in 1mmol scale of reactants.

From the Table 4.1 it is clear that 1.0 equivalents of LR is required for the better yield. In some cases, more equivalents do not improve the reaction rate (see entries 5-7). Best conversion rates were obtained with tetrahydrofuran (THF) and toluene as a solvent performing the reaction at 70-80°C for 2h (entry 4 and entry 9 with, 86% and 82% yield respectively).
The structures assigned to the compounds were substantiated by their analytical and other spectral data. For the compounds 52(a-e) the IR spectra showed the appearance of (C=S) peak at 1290-1250 cm\(^{-1}\) and absence of carbonyl stretching band at 1720-1710 cm\(^{-1}\) of the carboxylic acid function and also showed the characteristic signals at 1660-1610 cm\(^{-1}\) for (C=N). The formation of the compounds 54(a-e) was supported by the appearance of peak at 1690-1660 cm\(^{-1}\) (C=O amide) and 3335-3310 cm\(^{-1}\) for (−NH− amide).

In the \(^1\)H NMR spectra the signals of the newly synthesized compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The disappearance of peak at \(\delta\) 12.44 for (−OH, 45a) and singlet due to (−NH−, 47a) at \(\delta\) 8.90-8.95 confirms the formation of cyclised biheterocycle (52a). Similarly the appearance of singlet at \(\delta\) 8.60-8.65 for −CONH− bond confirms the formation of compound (54a). The aryl moiety exhibited characteristic signals in the aromatic region of the spectrum. In \(^{13}\)C NMR spectra, absence of peak at \(\delta\) 166.2 due to carbonyl group (C=O) of compound (47a) substantiated the formation of product (52a).

The mass spectrum of all the compounds showed molecular ion peak at M+1 corresponding to its molecular formula, which confirmed its chemical structure. The single crystal X-ray structure analysis\(^{12}\) of compounds (47a)\(^{13}\) and (54a)\(^{14}\) confirmed the structure assignment based on the \(^1\)H NMR spectra.
The possible mechanism for the formation of pyrazolyl-1,3,4-thiadiazoles 52(a-e).

Although we have not established the mechanism for the reaction between hydrazides 47(a-e) and LR to form compounds 52(a-e) in an experimental manner, a tentative explanation is proposed based on the literature.\textsuperscript{15} Initially LR (51) forms penta-coordinated phosphorus intermediate (a). As in Wittig reaction, the addition of mercapto function to carbonyl group of 47(a-e) to yield thiocarboxamides (b). These thiocarboxamides cyclized with the elimination of H$_2$S to get 52(a-e) as shown in Scheme 4.3.

\begin{center}
\begin{tikzpicture}
  % Draw the mechanism here
\end{tikzpicture}
\end{center}

\textbf{Scheme 4.3}: Possible mechanism for the conversion of 47(a-e) to 52(a-e)
4.1.4 Experimental results for the synthesis of 2-(5-methyl-1,3-diphenyl-1H-pyrazol-4-yl)-5-phenyl-1,3,4-thiadiazoles 52(a-e) and 5-methyl-1,3-diphenyl-N-(5-phenyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-4-carboxamides 54(a-e)

General

Benzohydrazides 46(a-e) were purchased from Merck India Pvt. Ltd. All other chemicals were obtained from commercial suppliers and used without further purification. Melting points were determined in open capillaries on a Buchi oil melting point apparatus and are uncorrected. Reactions were monitored by using TLC on aluminum sheets precoated with silica gel 60 F$_{254}$ (0.2 mm, Merck). Chromatographic spots were visualized by UV light and/or with iodine. For column chromatography, silica gel of 100-200 mesh size was used. $^1$H NMR spectra were acquired on a Bruker Avance 400 MHz instrument in DMSO-$_d$6 or CDCl$_3$. $^{13}$C NMR spectra were recorded on a Bruker AMX-400 (100 MHz) with complete proton decoupling. Chemical shifts are reported in ppm from tetramethylsilane (TMS) with the solvent as the internal reference. Mass spectrometry was performed with a Bruker-Franzen Esquire LC mass spectrometer unless otherwise stated.

**General procedure for the synthesis of 5-phenyl-1,3,4-thiadiazol-2-amine (53a):**

A mixture of the benzoic acid (Ia, 0.12g, 1.00mmol) and thiosemicarbazide (TSC, 1.00mmol) was added in portions over 0.5h to
POCl₃ (5mL) at 80-90°C and the mixture was stirred at this temperature for 3 to 4h. Then the reaction mixture was cooled, water/ice was added and it was finally basified with NH₃ (0.88g/mL). The solids isolated by filtration was washed with water, dried and recrystallized in ethyl alcohol to give pure 5-phenyl-1,3,4-thiadiazol-2-amine **53a** as a white solid in 70% (0.12g) yield.

**5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (53b):**

Obtained from heating 4-chlorobenzoic acid (Ib, 0.15g, 1.00mmol) and thiosemicarbazide (TSC, 1.00mmol) in presence of POCl₃ at 80-90°C for about 3 to 4h as white solid in 88% yield (0.18g).

**5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine (53c):**

Obtained from heating 4-methoxybenzoic acid (Ic, 0.15g, 1.00mmol) and thiosemicarbazide (TSC, 1.00mmol) in presence of POCl₃ at 80-90°C for about 3 to 4h as white amorphous solid in 88% (0.18g) yield.

**5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine (53d):**

Obtained from heating 4-nitrobenzoic acid (Id, 0.16g, 1.00mmol) and thiosemicarbazide (TSC, 1.00mmol) in presence of POCl₃ at 80-90°C for about 3 to 4h as yellow solid in 67% (0.15g) yield.
5-(p-tolyl)-1,3,4-thiadiazol-2-amine (53e): Obtained from heating 4-methylbenzoic acid (Ie, 0.13g, 1.00mmol) and thiosemicarbazide (TSC, 1.00mmol) in presence of POCl₃ at 80-90°C for about 3 to 4h as brown solid in 72% (0.13g) yield.

**General procedure for the synthesis of 5-methyl-1,3-diphenyl-1H-pyrazole-4-carboxylic acid (45a)**

Ethyl 5-methyl-1,3-diphenyl-1H-pyrazole-4-carboxylate (44a, 0.30g, 1.00mmol) with 10% NaOH (10mL) solution was taken in methanol (10mL) and refluxed for 4h. After completion of the reaction (monitored through TLC), the reaction mixture was evaporated to half of its volume, cooled and acidified with ice-cold dilute hydrochloric acid (pH ≈ 2). The obtained solid was filtered, washed with water, dried and recrystallised from ethyl alcohol to obtain the corresponding pyrazole-4-carboxylic acid (45a) as a white solid in 95% yield (0.26g), m.p. 198-200°C; IR (KBr): 3390-3044 cm⁻¹ (OH acid), 1724 cm⁻¹ (C=O), 1610 cm⁻¹ (C=N); ¹H NMR (400 MHz, DMSO-ｄ₆): δ 2.48 (s, 3H), 7.36-7.41 (m, 3H), 7.48-7.52 (m, 1H), 7.54-7.63 (m, 6H), 12.44 (s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 13.1, 109.5, 125.9, 127.8, 128.4, 128.8, 129.3, 129.5, 132.7, 138.6, 146.3, 154.3, 169.6; MS: m/z = 280.0 [M+2], 279.0 [M+H]+ (100%), 235.0; Anal. %
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Calculated for C\textsubscript{17}H\textsubscript{13}N\textsubscript{2}O\textsubscript{2}: C 73.37, H 5.07, N 10.07; Found: C 73.33, H 5.02, N 10.11.

3-(4-chlorophenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid (45b):

![Structure of 45b]

Obtained from ethyl 3-(4-chlorophenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxylate (44b), 0.34g, 1.00mmol and 10% NaOH (10mL) in methanol (10mL) as white solid in 92% yield (0.28g), IR (KBr):3195 cm\textsuperscript{-1} (OH acid), 1715 cm\textsuperscript{-1} (C═O acid), 1625 (C═N); \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6): \textit{δ} 2.44 (s, 3H), 7.37-7.40 (m, 2H), 7.42-7.44 (m, 3H), 7.49 (dd, 2H), 7.65 (dd, 2H), 12.36 (s, 1H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}): \textit{δ} 13.2, 109.5, 125.8, 127.7, 128.8, 129.6, 129.7, 131.6, 135.7, 138.6, 146.3, 154.3, 169.6; MS: m/z = 315.1 [M+3], 313.2 [M+H]\textsuperscript{+} (100%), 269.2, 255.3, 229.4; Anal. % Calculated for C\textsubscript{17}H\textsubscript{13}ClN\textsubscript{2}O\textsubscript{2}: C 65.29, H 4.19, N 8.96; Found: C 65.35, H 4.17, N 8.92.

3-(4-methoxyphenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid (45c):

![Structure of 45c]

Obtained from ethyl 3-(4-methoxyphenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxylate (44c), 0.34g, 1.00mmol and 10% NaOH (10mL) in methanol (10mL) as white amorphous solid in 87% yield (0.27g).

IR (KBr): 3200 cm\textsuperscript{-1} (OH acid), 1710 cm\textsuperscript{-1} (C═O acid), 1642 cm\textsuperscript{-1} (C═N); \textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6): \textit{δ} 2.43 (s, 3H), 3.75 (s, 3H), 7.32-7.40 (m,
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5-methyl-3-(4-nitrophenyl)-1-phenyl-1H-pyrazole-4-carboxylic acid (45d):

 Obtained from ethyl 5-methyl-3-(4-nitrophenyl)-1-phenyl-1H-pyrazole-4-carboxylate (44d, 0.35g, 1.00mmol) and 10% NaOH (10mL) in methanol (10mL) as brown amorphous solid in 67% yield (0.22g). IR (KBr): 3200 cm⁻¹ (OH acid), 1710 cm⁻¹ (C=O acid), 1628 cm⁻¹ (C=N); ¹H NMR (400 MHz, DMSO-ᵈ): δ 2.46 (s, 3H), 7.50-7.57 (m, 5H), 7.92-7.94 (d, 2H, J = 8.8 Hz), 8.18-8.20 (d, 2H, J = 8.8 Hz), 12.32 (s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 13.0, 109.5, 124.5, 125.8, 127.7, 128.8, 129.3, 138.5, 138.6, 146.3, 149.2, 154.3, 169.5; MS: m/z = 324.2 [M+H]⁺ (100%), 280.1, 260.3, 240.2; Anal. % Calculated for C₁₇H₁₃N₃O₄: C 63.16, H 4.05, N 13.00; Found: C 63.12, H 4.08, N 13.06.

5-methyl-1-phenyl-3-(p-tolyl)-1H-pyrazole-4-carboxylic acid (45e):

 Obtained from ethyl 5-methyl-1-phenyl-3-(p-tolyl)-1H-pyrazole-4-carboxylate (44g, 0.32g, 1.00mmol) and 10% NaOH (10mL) in methanol (10mL) as off white
amorphous solid in 77% yield (0.22 g). IR (KBr): 3192 cm$^{-1}$ (OH acid), 1725 cm$^{-1}$ (C=O acid), 1635 cm$^{-1}$ (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.40 (s, 3H), 2.43 (s, 3H), 7.02-7.10 (m, 2H), 7.25-7.40 (m, 1H), 7.45-7.60 (m, 4H), 7.66-7.79 (m, 2H), 12.33 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 12.6, 13.0, 110.1, 125.6, 127.6, 128.3, 128.7, 129.8, 130.2, 138.1, 138.6, 146.1, 154.0, 169.2; MS: m/z = 293.3 [M+H]$^+$ (100%), 247.4, 234.2; Anal. % Calculated for C$_{18}$H$_{16}$N$_2$O$_2$: C 73.95, H 5.52, N 9.58; Found: C 73.90, H 5.60, N 9.62.

3-(4-hydroxyphenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid (45f):

Obtained from ethyl 3-(4-hydroxyphenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxylate (44h, 0.32 g, 1.00 mmol) and 10% NaOH (10 mL) in methanol (10 mL) as amorphous solid in 80% yield (0.23 g). IR (KBr): 3260 cm$^{-1}$ (OH-phenolic), 3190 cm$^{-1}$ (OH acid), 1727 cm$^{-1}$ (C=O acid), 1644 cm$^{-1}$ (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.43 (s, 3H), 7.38-7.45 (m, 5H), 7.88 (dd, 2H), 7.91 (dd, 2H), 9.35 (s, 1H), 12.40 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 12.7, 110.2, 115.1, 125.4, 125.8, 127.7, 128.6, 129.4, 138.2, 146.8, 154.1, 156.2, 169.2; MS: m/z = 295.2 [M+H]$^+$ (100%); Anal. % Calculated for C$_{17}$H$_{14}$N$_2$O$_3$: C 69.38, H 4.79, N 9.52; Found: C 69.30, H 4.85, N 9.58.
**General procedure for the synthesis of N'-benzoyl-5-methyl-1,3-diphenyl-1H-pyrazole-4-carbohydrazide (47a):**

5-methyl-1,3-diphenyl-1H-pyrazole-4-carboxylic acid (45a, 0.28g, 1.00mmol) in dry DCM (5mL) was cooled to 0°C. Then EDC.HCl (0.23g, 1.20mmol) and HOBt (0.18g, 1.20mmol) were added under nitrogen atmosphere and stirred the reaction mixture at the same temperature for 0.5h. To this reaction mixture benzohydrazide (46a, 0.13g, 1.00mmol) was added and stirred at 0°C for another 0.5h. The reaction mixture was slowly brought to room temperature and stirring was continued for 8-12h. The progress of the reaction was monitored by TLC. After completion of the reaction the reaction mixture was extracted with ethyl acetate (2x25mL) and the combined organic phase was washed with brine solution (3x20mL) and dried over anhydrous sodium sulfate. Ethyl acetate was distilled off and the obtained residue was purified by column chromatography using hexane:ethyl acetate (8:2) as eluent to afford pure pyrazole-4-carbohydrazide (47a) as white solids with 72% yield (0.28g); IR (KBr): 3220 cm\(^{-1}\) (NH), 1640 cm\(^{-1}\) (C=O), 1625 cm\(^{-1}\) (C=N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.47 (s, 3H), 7.33–7.41 (m, 1H), 7.49–7.55 (m, 8H), 7.76–7.82 (m, 2H), 7.89–7.93 (m, 4H), 8.01 (s, 1H), 8.03 (s, 1H); \(^1^3\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 12.9, 109.8, 125.6, 126.2, 127.4, 128.2, 128.9, 129.5, 129.8, 130.1, 133.4, 138.7, 145.1, 147.2, 153.2, 166.2; MS: m/z...
= 397.2 [M+H]+ (100%), 306.2, 235.2, 194.1; Anal. % Calculated for C_{24}H_{20}N_4O_2: C 72.71, H 5.08, N 14.13; Found: C 72.68, H 5.10, N 14.17.

**N’-(4-chlorobenzoyl)-3-(4-chlorophenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carbohydrazide (47b):**

Obtained from 3-(4-chlorophenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid (45b, 0.31g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 4-chlorobenzohydrazide (46b, 0.17g, 1.00mmol) as pale yellow amorphous solid in 70% yield (0.32g); IR (KBr): 3215 cm⁻¹ (NH), 1647cm⁻¹ (C=O), 1623 cm⁻¹ (C=N); ^1H NMR (400 MHz, DMSO-d₆): δ 2.50(s, 3H), 7.23-7.31 (m, 5H), 7.37-7.39 (dd, 4H, J = 8.2 Hz), 7.52-7.55 (dd, 4H, J = 8.0 Hz), 8.95 (s, 2H); ^13C NMR (100 MHz, CDCl₃): δ 13.0, 109.8, 125.6, 127.4, 127.5, 128.8, 129.5, 129.7, 130.1, 130.2, 136.3, 137.0, 142.3, 145.1, 147.2, 153.1, 166.2; MS: m/z = 469.7 [M+5], 467.6 [M+3], 465.1 [M+H]+ (100%), 270.5, 230.6; Anal. % Calculated for C_{24}H_{18}Cl_{2}N_4O_2: C 61.95, H 3.90, N 15.24; Found: C 61.99, H 3.88, N 15.28.

**N’-(4-methoxybenzoyl)-3-(4-methoxyphenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carbohydrazide (47c):**

Obtained from 3-(4-methoxyphenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid (45c, 0.31g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 4-methoxybenzohydrazide (46c, 0.17g, 1.00mmol) as gum in 68% yield (0.31g); IR (KBr): 3215 cm⁻¹ (NH), 1640 cm⁻¹ (C=O),
1633 cm$^{-1}$ (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.54 (s, 3H), 3.82 (s, 6H), 7.30-7.35 (m, 5H), 7.43-7.59 (m, 8H), 8.95 (s, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 13.0, 55.8, 109.8, 115.6, 115.7, 123.2, 125.6, 127.2, 127.4, 129.2, 129.7, 131.4, 145.1, 147.2, 153.1, 159.8, 165.9, 166.2.; MS: m/z = 457.3 [M+H]$^+$, 266.3, 226.1; Anal. % Calculated for C$_{26}$H$_{24}$N$_4$O$_4$: C 68.41, H 5.30, N 12.27; Found: C 68.38, H 5.35, N 12.30.

5-methyl-$N'$(4-nitrobenzoyl)-3-(4-nitrophenyl)-1-phenyl-1H-pyrazole-4-carboxylic acid (45d, 0.32g, 1.00mmol), EDC.HCl (1.20mmol), HOBr (1.20mmol) and 4-nitrobenzohydrazide (46d, 0.18g, 1.00mmol) as brown solid in 61% yield (0.29g); IR (KBr): 3244 cm$^{-1}$ (NH), 1655 cm$^{-1}$ (C=O), 1620 cm$^{-1}$ (C=N); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.53 (s, 3H), 7.33-7.41 (m, 5H), 7.57-7.59 (dd, 4H, $J = 8.2$ Hz), 7.61-7.63 (dd, 4H, $J = 8.2$ Hz), 8.93 (s, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 13.0, 109.8, 124.7, 125.1, 125.6, 126.9, 127.4, 129.1, 129.7, 135.3, 144.3, 145.1, 147.2, 150.1, 153.1, 155.8, 166.2; MS: m/z = 487.1 [M+H]$^+$ (100%), 486.3, 281.2, 241.1; Anal. % Calculated for C$_{24}$H$_{18}$N$_6$O$_5$: C 59.26, H 3.73, N 17.28; Found: C 59.22, H 3.74, N 17.33.
5-methyl-N'(4-methylbenzoyl)-1-phenyl-3-(p-tolyl)-1H-pyrazole-4-carbohydrazide (47e):

Obtained from 5-methyl-1-phenyl-3-(p-tolyl)-1H-pyrazole-4-carboxylic acid (45e, 0.29g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 4-methylbenzohydrazide (46c, 0.15g, 1.00 mmol) as white amorphous solid in 69% yield (0.29g); IR (KBr): 3222 cm\(^{-1}\) (NH), 1645 cm\(^{-1}\) (C=O), 1610 cm\(^{-1}\) (C=N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.39 (s, 6H), 2.53 (s, 3H), 7.21-7.30 (m, 5H), 7.37-7.52 (m, 8H), 8.95 (s, 2H); \(^13\)C NMR (100 MHz, CDCl\(_3\)):\(\delta\) 13.0, 17.9, 20.1, 109.8, 125.6, 126.1, 127.1, 127.4, 128.0, 129.7, 130.4, 135.8, 139.0, 145.1, 147.2, 153.1, 166.2; MS: m/z = 425.4 [M+H]\(^+\), 249.2, 209.1; Anal. % Calculated for C\(_{26}\)H\(_{24}\)N\(_4\)O\(_2\): C 73.56, H 5.70, N 13.20; Found: C 73.63, H 5.68, N 13.29.

General procedure for the synthesis of 2-(5-methyl-1,3-diphenyl-1H-pyrazol-4-yl)-5-phenyl-1,3,4-thiadiazole (52a):

The compound N'-benzoyl-5-methyl-1,3-diphenyl-1H-pyrazole-4-carbohydrazide (47a, 0.39g, 1.00mmol) dissolved in THF (10mL) was taken in a two neck round bottomed flask and LR (0.40g, 1.00mmol) was added slowly and refluxed for 2h at 70°C. During the course of the reaction, solution turns yellow or orange color indicates the
progress of the reaction. After completion of the reaction (monitored through TLC, hexane:ethyl acetate in 7:3 ratio), the solvent was evaporated to half of its volume and it was then allowed to cool and the resulting viscous liquid was dissolved in DCM and evaporated on silica gel. Flash column chromatography on silica (200-425 mesh) provided the corresponding pyrazolyl-1,3,4-thiadiazole (52a) as white amorphous solid in 86% yield (0.33g); IR (KBr): 1645 cm\(^{-1}\) (C═N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.54 (s, 3H), 7.34-7.43 (m, 3H), 7.49-7.54 (m, 3H), 7.58-7.63 (m, 5H), 7.86-7.88 (d, 2H, \(J = 7.24\) Hz), 7.94-7.95 (d, 2H, \(J = 7.32\) Hz) (Figure 4.2); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 13.3, 105.1, 108.3, 120.4, 124.7, 125.8, 128.1, 128.6, 129.0, 129.4, 129.5, 130.0, 131.9, 138.1, 143.4, 147.5, 155.0, 160.0 (Figure 4.3); MS: m/z = 396.2 [M+2], 395.2 [M+H]\(^+\) (100%) (Figure 4.4); Anal. % Calculated for C\(_{24}\)H\(_{18}\)N\(_4\)S: C 73.07, H 4.60, N 14.20; Found: C 73.02, H 4.63, N 14.18.

2-(4-chlorophenyl)-5-(3-(4-chlorophenyl)-5-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-thiadiazole (52b):

Obtained from \(N'\)-(4-chlorobenzoyl)-3-(4-chlorophenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carbohydrazide (47b, 0.46g, 1.00mmol) and LR (1.00mmol) in THF (10mL) as pale yellow amorphous solid in 80% yield (0.36g); IR (KBr): 1623 cm\(^{-1}\) (C═N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.50 (s, 3H), 7.13-7.18 (m, 5H), 7.48 (dd, 4H), 7.54 (dd, 4H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 13.3, 108.3,
125.8, 127.6, 128.2, 129.4, 129.6, 129.8, 133.5, 135.4, 136.0, 136.5, 143.4, 147.4, 155.0, 159.1; MS: m/z = 467.2 [M+5], 465.1 [M+3], 463.2 [M+H]+ (100%), 269.2, 235.1, 229.2, 196.4; Anal. % Calculated for C_{24}H_{16}Cl_{2}N_{4}S: C 62.21, H 3.48, N 12.09; Found: C 62.28, H 3.45, N 12.17.

**2-(4-methoxyphenyl)-5-(3-(4-methoxyphenyl)-5-methyl-1-phenyl-1H-pyrazol-4-yl)-1,3,4-thiadiazole (52c):**

\[
\text{ Obtained from } \quad \text{N'-}(4\text{-methoxybenzoyl})-3\text{-}(4\text{-methoxyphenyl})-5\text{-methyl-1-phenyl-1H-pyrazole-4-carbohydrazide (47c, 0.45g, 1.00mmol) and LR (1.00mmol) in THF (10mL) as thick oil in 78% yield (0.35g); IR (KBr): 1640 cm}^{-1}\text{ (C=N); } \quad ^1\text{H NMR (400 MHz, DMSO-}d_6\text{)}: } \delta \quad 2.50 \text{ (s, 3H), 3.84 (s, 6H), 7.23-7.40 (m, 5H), 7.49-7.53 (m, 8H); } \quad ^{13}\text{C NMR (100 MHz, CDCl}_3\text{): } \delta \quad 13.3, 108.3, 125.8, 127.6, 128.2, 129.4, 129.6, 129.8, 133.5, 135.4, 136.0, 136.5, 143.4, 147.4, 155.0, 159.1; \quad \text{MS: m/z = 455.2 [M+H]+ (100%), 264.1, 251.4, 225.1, 192.1; Anal. % Calculated for C}_{26}\text{H}_{22}\text{N}_4\text{O}_2\text{S: C 68.70, H 4.88, N 12.33; Found: C 68.67, H 4.94, N 12.30.}
\]

**2-(5-methyl-3-(4-nitrophenyl)-1-phenyl-1H-pyrazol-4-yl)-5-(4-nitrophenyl)-1,3,4-thiadiazole (52d):**

\[
\text{ Obtained from 5-methyl-}\text{N'-}(4\text{-nitrobenzoyl})\text{-3-(4-nitrophenyl)-1-phenyl-1H-pyrazole-4-carbohydrazide (47d, 0.48g 1.00mmol) and LR (1.00mmol) in THF (10mL) as thick paste in 67% yield (0.32g); IR (KBr):}
\]
1630 cm\(^{-1}\) (C=\(\equiv\)N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.54 (s, 3H), 7.38-7.42 (m, 5H), 7.51 (dd, 4H), 7.61 (d, 4H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 13.2, 108.3, 123.1, 123.5, 125.8, 127.6, 128.8, 129.0, 129.4, 138.8, 143.4, 144.2, 147.3, 150.2, 155.0, 159.2; MS: \(m/z = 485.3\) [M+H]\(^+\) (100%), 279.5, 266.2, 240.4, 207.0; Anal. % Calculated for C\(_{24}\)H\(_{16}\)N\(_6\)O\(_4\): C 59.50, H 3.33, N 17.35; Found: C 59.47, H 3.30, N 17.39.

2-(5-methyl-1-phenyl-3-(p-tolyl)-1H-pyrazol-4-yl)-5-(p-tolyl)-1,3,4-thiadiazole (52e):

Obtained from 5-methyl-\(N^+\)-(4-methylbenzoyl)-1-phenyl-3-(p-tolyl)-1H-pyrazole-4-carbohydrazide (47e, 0.42g, 1.00mmol) and LR (1.00mmol) in THF (10mL) as white amorphous solid in 74% yield (0.31g); IR (KBr): 1635 cm\(^{-1}\) (C=\(\equiv\)N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.40 (s, 3H), 2.42 (s, 3H), 2.54 (s, 3H), 7.27-7.62 (m, 13H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 13.3, 14.1, 20.1, 108.3, 125.8, 126.8, 127.6, 128.1, 129.4, 130.0, 130.1, 130.3, 137.8, 138.8, 143.4, 147.3, 155.1, 160.0; MS: \(m/z = 423.1\) [M+H]\(^+\) (100%), 248.1, 235.3, 209.5, 176.4; Anal. % Calculated for C\(_{26}\)H\(_{22}\)N\(_4\)O\(_2\): C 73.90, H 5.25, N 13.26; Found: C 73.95, H 5.21, N 13.35.
General procedure for the synthesis of 5-methyl-1,3-diphenyl-N-(5-phenyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-4-carboxamide (54a):

A solution of 5-methyl-1,3-diphenyl-1H-pyrazole-4-carboxylic acid (45a, 0.28g, 1.00mmol) in dry DCM (5mL) was cooled to 0°C and added EDC.HCl (1.20mmol) and HOBt (1.20mmol) under nitrogen atmosphere and stirred the reaction mixture at the same temperature for 0.5h. To this reaction mixture compound 5-phenyl-1,3,4-thiadiazol-2-amine (53a, 0.18, 1.00mmol) was added and stirred at 0°C for 0.5h. The reaction mixture was slowly brought to room temperature and stirring continued for 6-8h. After completion of the reaction, the reaction mixture was extracted with ethyl acetate (2x25mL) and the combined organic phase was washed with brine solution and dried over anhydrous sodium sulfate. Ethyl acetate was distilled off and the residue thus obtained was purified by recrystallization in ethyl alcohol afforded the corresponding (54a) as white powder with 82% yield (0.36g). (Single crystal suitable for X-ray analysis was obtained with ethyl alcohol/water (9.5:0.5) solvent system). IR (KBr): 3310 cm\(^{-1}\) (NH), 1665 cm\(^{-1}\) (amide C═O), 1635 cm\(^{-1}\) (C═N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.50 (s, 3H), 7.38-7.45 (m, 3H), 7.52-7.55 (m, 3H), 7.59-7.69 (m, 5H), 7.77-7.80 (d, 2H, \(J = 8.4\) Hz), 7.92-7.94 (d, 2H, \(J = 8.3\) Hz), 8.62 (s, 1H) (Figure 4.5); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 13.0, 108.3, 125.6, 126.4, 127.5, 128.2, 129.6, 129.8, 131.9,
139.0, 143.4, 145.1, 147.5, 155.0, 160.0, 165.4, 166.2 (Figure 4.6); MS: 

\text{m/z = 438.2 [M+H]⁺ (100%), 234.2, 221.3, 195.3, 176.1 (Figure 4.7);}

Anal. % Calculated for C_{25}H_{19}N_{5}OS: C 68.63, H 4.38, N 16.01; Found: C 68.69, H 4.34, N 16.08.

\textbf{3-(4-chlorophenyl)-N-(5-(4-chlorophenyl)-1,3,4-thiadiazol-2-yl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxamide (54b):}

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{54b}
\caption{54b}
\end{figure}

Obtained from 3-(4-chlorophenyl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid (45b, 0.31g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 5-(4-chlorophenyl)-1,3,4-thiadiazol-2-amine (53b, 0.21g, 1.00mmol) in dry DCM (5mL) as off white amorphous solid in 78% yield (0.39g); IR (KBr): 3330 cm\(^{-1}\) (NH), 1670 cm\(^{-1}\) (amide C═O), 1625 cm\(^{-1}\) (C═N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.52 (s, 3H), 7.20-7.31 (m, 5H), 7.38 (dd, 4H), 7.40 (dd, 4H), 8.60 (s, 1H); \(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 13.2, 108.3, 125.5, 127.4, 127.5, 129.5, 129.7, 129.9, 130.1, 130.2, 136.2, 136.9, 142.6, 143.4, 147.5, 155.0, 160.0, 165.4, 166.2 ; 

MS: m/z = 510.2 [M+5], 508.1 [M+3], 506.2 [M+H]⁺ (100%), 268.2, 255.4, 229.5, 211.2; Anal. % Calculated for C\(_{25}\)H\(_{17}\)Cl\(_2\)N\(_5\)OS: C 59.29, H 3.38, N 13.83; Found: C 59.25, H 3.33, N 13.80.

\textbf{3-(4-methoxyphenyl)-N-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)-5-methyl-1-phenyl-1H-pyrazole-4-carboxamide (54c):}

Obtained from 3-(4-methoxyphenyl)-5-methyl-1-phenyl-1H-pyrazole-4-
carboxylic acid (45c, 0.31g, 1.00mmol), EDC.HCl (1.20mmol), HOBt (1.20mmol) and 5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-amine (53c, 0.21g, 1.00mmol) in dry DCM (5mL) as white amorphous solid in 78% yield (0.39g); IR (KBr): 3320 cm\(^{-1}\) (NH), 1690 cm\(^{-1}\) (amide C=O), 1625 cm\(^{-1}\) (C=N); \(^1\)H NMR (400 MHz, DMSO-\(d_6\)): \(\delta\) 2.53 (s, 3H), 3.80 (s, 3H), 3.81 (s, 3H), 7.32-7.41 (m, 5H), 7.50-7.58 (m, 8H), 8.63 (s, 1H); \(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 13.3, 108.3, 115.6, 115.8, 124.7, 125.5, 127.2, 127.4, 129.2, 129.7, 131.6, 143.4, 147.5, 155.0, 159.8, 160.0, 165.4, 165.8, 166.2; MS: \(m/z = 498.1\) [M+H]\(^+\) (100%), 264.1, 231.4, 225.0, 206.3; Anal. % Calculated for C\(_{27}\)H\(_{23}\)N\(_5\)O\(_3\)S: C 65.17, H 4.66, N 14.08; Found: C 65.22, H 4.62, N 14.15.

**5-methyl-3-(4-nitrophenyl)-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)-1-phenyl-1H-pyrazole-4-carboxamide (54d):**

Obtained from 5-methyl-3-(4-nitrophenyl)-1-phenyl-1H-pyrazole-4-carboxylic acid (45d, 0.32g, 1.00mmol), EDC.HCl (1.20mmol), HOBt(1.20mmol) and 5-(4-nitrophenyl)-1,3,4-thiadiazol-2-amine (53d, 0.22g, 1.00mmol) in dry DCM (5mL) as buff colored amorphous solid in 68% yield (0.35g); IR (KBr): 3295 cm\(^{-1}\) (NH), 1695 cm\(^{-1}\).
(amide C═O), 1644 cm⁻¹ (C═N); ¹H NMR (400 MHz, DMSO-d₆): δ 2.50 (s, 3H), 7.28-7.39 (m, 5H), 7.49 (dd, 4H), 7.58 (d, 4H), 8.65 (s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 13.2, 108.3, 124.8, 125.3, 125.5, 127.1, 127.4, 129.1, 129.7, 137.8, 143.4, 144.7, 147.5, 149.8, 155.0, 155.8, 160.0, 165.4, 166.2; MS: m/z = 528.2 [M+H]⁺ (100%), 266.1, 240.4, 238.1, 221.4; Anal. % Calculated for C₂₅H₁₇N₇O₅S: C 56.92, H 3.25, N 18.59; Found: C 56.99, H 3.23, N 18.67.

**5-methyl-1-phenyl-3-(p-tolyl)-N-(5-(p-tolyl)-1,3,4-thiadiazol-2-yl)-1H-pyrazole-4-carboxamide (54e):**

Obtained from 5-methyl-1-phenyl-3-(p-tolyl)-1H-pyrazole-4-carboxylic acid (45e, 0.29g, 1.00mmol), EDC.HCl (1.20mmol), HOBT (1.20mmol) and 5-(p-tolyl)-1,3,4-thiadiazol-2-amine (53e, 0.19g, 1.00mmol) in dry DCM (5mL) as off white solid in 76% yield (0.35g); IR (KBr): 3310 cm⁻¹ (NH), 1690 cm⁻¹ (amide C═O), 1629 cm⁻¹ (C═N); ¹H NMR (400 MHz, DMSO-d₆): δ 2.40 (s, 3H), 2.41 (s, 3H), 2.54 (s, 3H), 7.30-7.61 (m, 13H), 8.61 (s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 13.2, 108.3, 125.5, 126.1, 127.4, 128.1, 129.0, 129.7, 130.2, 130.5, 135.9, 138.9, 143.4, 144.9, 147.4, 154.9, 160.0, 165.3, 166.1; MS: m/z = 466.2 [M+H]⁺ (100%), 248.1, 238.4, 209.1, 190.1; Anal. % Calculated for C₂₇H₂₃N₅O₂S: C 69.65, H 4.98, N 15.04; Found: C 69.69, H 4.95, N 15.10.
Figure 4.2: $^1$H NMR spectrum of compound 52a
Figure 4.3: $^{13}$C NMR spectrum of compound 52a
Figure 4.4: Mass spectrum of compound 52a
Figure 4.5: $^1$H NMR spectrum of compound 54a
Figure 4.6: $^{13}$C NMR spectrum of compound 54a
Figure 4.7: Mass spectrum of compound 54a
CHAPTER–IV

PART–II

Biological Activity of
Pyrazolyl-1,3,4-thiadiazole
Analogues
4.2.1 Anticancer activity

4.2.1.1 Evaluation of anticancer activity of compounds 52(a-e) and 54(a-e).

Materials and methods:

MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] assay.\textsuperscript{16-19}

The assay detects the reduction of MTT by mitochondrial dehydrogenase to blue formazan product, which reflects the normal functioning of mitochondria and hence the cell viability. Human breast adenocarcinoma cells (MCF-7) (2x10\textsuperscript{3}) were seeded in each well of a 96-well plate and were allowed to adhere and spread for 24h. Subsequently, cells were treated with different concentrations (0.1, 1.0, 10.0 and 100 \mu g/mL) of test compounds prepared in 10\% dimethyl sulphoxide (DMSO) and incubated at 37°C for 24h. Doxorubicin was used as a positive control and the first column of the micro plate was used as negative control (containing no drug). After 24h, MTT solution (10\mu l of 10 mg/mL) was added to each well, and the cultures were incubated for an additional 4h to allow the formation of formazan crystals. A further 100\mu l of MTT solution was added and incubation continued overnight. The optical density at 570 nm was determined in each well with an ELISA plate reader (ELx800, BioTek, VT, USA) and results were compared with untreated control. The cell viability was determined using the formula:
CHAPTER-IV

Viability % = (optical density of sample/optical density of control) × 100

IC\textsubscript{50} values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell line.

4.2.1.2 Discussion on the Anticancer activity of compounds 52(a-e) and 54(a-e).

The MTT cell proliferation assay has been widely accepted as a reliable way to measure the cell proliferation rate and conversely when metabolic events lead to apoptosis or necrosis. Although the number of tested compounds in this study is limited, some structural features that are important for explanation of their cytotoxic effects can be referred. In general, the pyrazole integrated 1,3,4-thiadiazole derivatives 5-methyl-1,3-diphenyl-N-(5-phenyl-1,3,4-thiadiazol-2-yl)-1H-pyrazole-4-carboxamide 54(a-e) were more potent than the 2-(5-methyl-1,3-diphenyl-1H-pyrazol-4-yl)-5-phenyl-1,3,4-thiadiazoles 52(a-e). Moreover, it was envisioned that the incorporation of amide (–CONH–) functionality between pyrazole and 1,3,4-thiadiazole scaffold as in 54(a-e) may increase the anticancer activity of compounds. The results were reported as percentage survival of the cells when compared to that of the untreated control cells ± standard deviation in Table 4.2. A comparison of the para-substituents on the phenyl rings on both pyrazole and
thiadiazole moiety demonstrated that an electron-donating group 54b, 54c, 52b and 52c have better activity. Comparing with -OCH$_3$ and –Cl, –CH$_3$ substituents mostly had minimal and –NO$_2$ substituents had least effects. Among the ten compounds analyzed for cytotoxicity, compound 54b emerged as a potent anticancer agent with IC$_{50}$ value 15µg/mL. The maximum cytotoxicity of compounds was observed in the following order 54c>52b>52c with IC$_{50}$ values 24, 26, 29µg/mL respectively. All other compounds showed low to moderate cytotoxicity. The IC$_{50}$ for the standard drug Doxorubicin (DOX) was found to be 18µg/mL.
Table 4.2: MTT assay of the compounds 52(a-e) and 54(a-e)

<table>
<thead>
<tr>
<th>Products</th>
<th>Ar₁</th>
<th>Ar₂</th>
<th>Vehicle Control</th>
<th>0.1 µg/mL</th>
<th>1.0 µg/mL</th>
<th>10 µg/mL</th>
<th>100 µg/mL</th>
<th>IC₅₀ µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>52a</td>
<td>C₆H₅</td>
<td>C₆H₅</td>
<td>100 ± 3.90</td>
<td>99.7 ± 8.02</td>
<td>100 ± 7.70</td>
<td>82.4 ± 3.08</td>
<td>58.7 ± 2.30</td>
<td>100</td>
</tr>
<tr>
<td>52b</td>
<td>4–Cl–C₆H₄</td>
<td>4–Cl–C₆H₄</td>
<td>100 ± 7.03</td>
<td>64.9 ± 4.04</td>
<td>69.4 ± 2.47</td>
<td>72.4 ± 4.65</td>
<td>26.8 ± 7.77</td>
<td>26</td>
</tr>
<tr>
<td>52c</td>
<td>4–OCH₃–C₆H₄</td>
<td>4–OCH₃–C₆H₄</td>
<td>100 ± 4.20</td>
<td>100 ± 7.50</td>
<td>100 ± 4.60</td>
<td>99.0 ± 2.50</td>
<td>86.5 ± 5.10</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>52d</td>
<td>4–NO₂–C₆H₄</td>
<td>4–NO₂–C₆H₄</td>
<td>100 ± 3.29</td>
<td>83.0 ± 5.07</td>
<td>88.0 ± 4.55</td>
<td>76.7 ± 2.10</td>
<td>28.4 ± 2.20</td>
<td>29</td>
</tr>
<tr>
<td>52e</td>
<td>4–CH₃–C₆H₄</td>
<td>4–CH₃–C₆H₄</td>
<td>100 ± 5.00</td>
<td>100 ± 5.01</td>
<td>100 ± 2.70</td>
<td>96.0 ± 4.83</td>
<td>46.5 ± 2.56</td>
<td>89</td>
</tr>
<tr>
<td>54a</td>
<td>C₆H₅</td>
<td>C₆H₅</td>
<td>100 ± 4.90</td>
<td>100 ± 2.17</td>
<td>70.5 ± 3.74</td>
<td>61.3 ± 4.90</td>
<td>54.8 ± 7.30</td>
<td>100</td>
</tr>
<tr>
<td>54b</td>
<td>4–Cl–C₆H₄</td>
<td>4–Cl–C₆H₄</td>
<td>100 ± 7.00</td>
<td>74.5 ± 4.60</td>
<td>80.1 ± 5.02</td>
<td>64.8 ± 2.40</td>
<td>12.6 ± 7.41</td>
<td>15</td>
</tr>
<tr>
<td>54c</td>
<td>4–OCH₃–C₆H₄</td>
<td>4–OCH₃–C₆H₄</td>
<td>100 ± 2.16</td>
<td>93.2 ± 4.80</td>
<td>90.3 ± 9.45</td>
<td>88.6 ± 4.20</td>
<td>43.6 ± 2.52</td>
<td>77</td>
</tr>
<tr>
<td>54d</td>
<td>4–NO₂–C₆H₄</td>
<td>4–NO₂–C₆H₄</td>
<td>100 ± 7.50</td>
<td>80.3 ± 2.30</td>
<td>86.8 ± 4.44</td>
<td>84.3 ± 2.77</td>
<td>22.5 ± 1.22</td>
<td>24</td>
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<tr>
<td>54e</td>
<td>4–CH₃–C₆H₄</td>
<td>4–CH₃–C₆H₄</td>
<td>100 ± 5.19</td>
<td>94.7 ± 4.87</td>
<td>95.8 ± 2.46</td>
<td>91.2 ± 5.20</td>
<td>45.6 ± 5.53</td>
<td>80</td>
</tr>
<tr>
<td>DOX</td>
<td>---</td>
<td>---</td>
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<td>---</td>
<td>---</td>
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</tr>
</tbody>
</table>

The values are expressed as mean ± SD of six separate experiments, DOX = Doxorubicin.
4.3.1 References


12. CCDC 962806 (47a) and CCDC 962807 (54a) contain the supplementary crystallographic data for this paper. They can be obtained free of charge from The Cambridge Crystallographic Data Centere via www.ccdc.cam.ac.uk/data_request/cif.


