

## Synthesis and preliminary evaluation of brominated 5-methyl-2,4-dihydropyrazol-3-one and its derivatives as cytotoxic agents

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### Abstract

Derivatives of 5-methyl-2,4-dihydro-pyrazol-3-one (**1a**), 5-methyl-2-phenyl-2,4-dihydro-pyrazol-3-one (**1b**) and 2-(2,4-dinitro-phenyl)-5-methyl-2,4-dihydro-pyrazol-3-one (**1c**) have been brominated in various ways. The compounds (**1a**, **1b**, **1c**) were synthesized by condensation reaction between the ethylacetoacetate and hydrazine derivatives. All the products have been characterized by extensive use of IR, <sup>1</sup>H-NMR, <sup>13</sup>C-NMR and Mass spectral analysis. Investigation of the cytotoxicity of these compounds was carried out against brine shrimp by lethality bioassay. The cytotoxicity studies of the synthesized compounds revealed that compound 4,4-dibromo-2-(2,4-dinitro-phenyl)-5-methyl-2,4-dihydro-pyrazol-3-one (**2c**) had shown tremendous bioactivity against brine shrimp. However, the compounds 4,4-dibromo-5-methyl-2,4-dihydro-pyrazol-3-one (**2a**), 4,4-dibromo-2-(2,4-dibromo-phenyl)-5-methyl-2,4-dihydro-pyrazol-3-one (**2b**) and 4,4-dibromo-2-(dinitro-phenyl)-5-methyl-2,4-dihydro-pyrazol-3-one (**2c**) showed higher activity leaving the other compounds almost inactive.

### Introduction

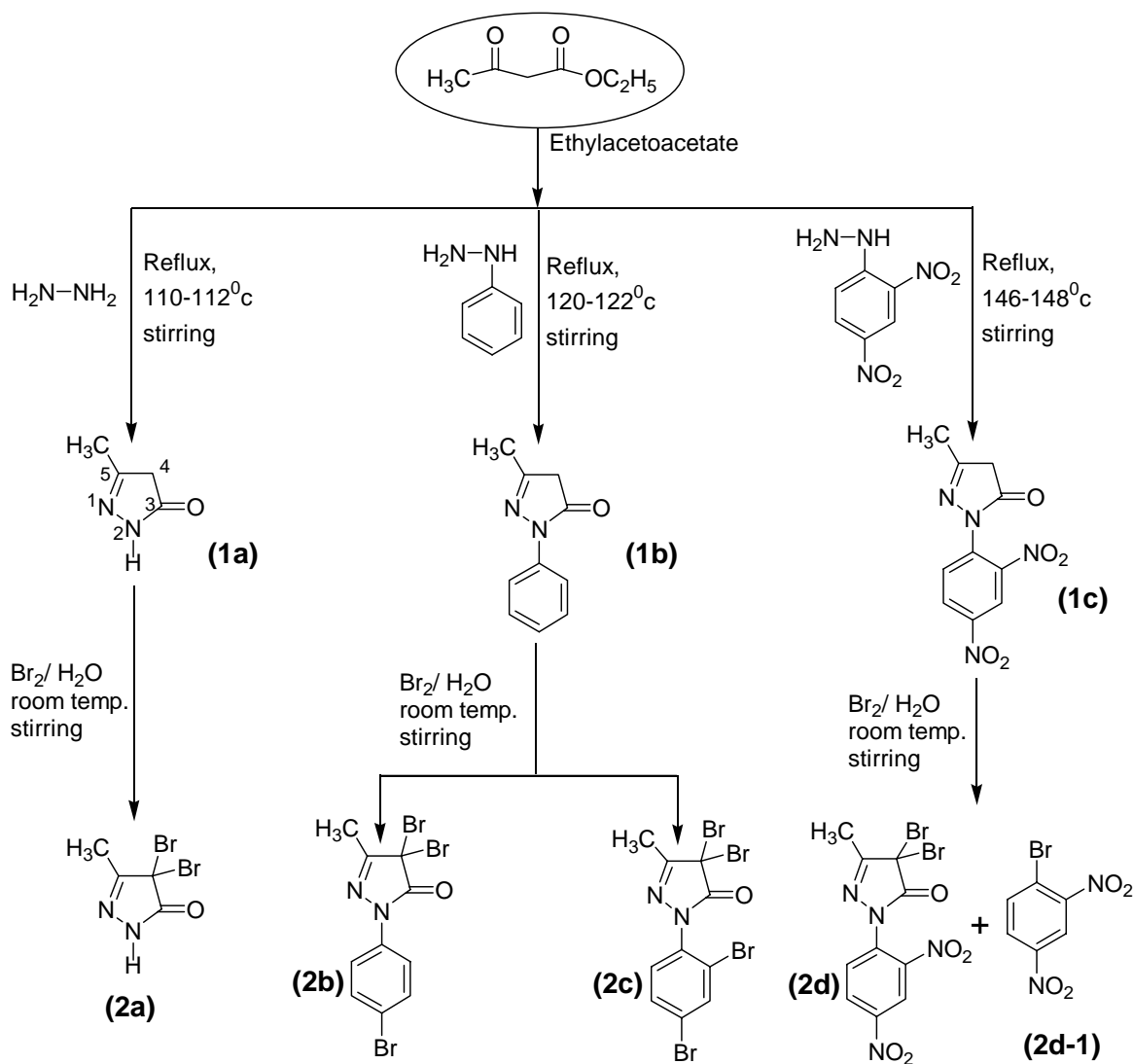
Pyrazole chemically known as 1,2-diazole has become a popular topic due to its manifold uses. The chemistry of pyrazolone and its derivatives is particularly interesting because of their potential application in medicinal chemistry as analgesic (Gürsoy et al., 2000; Amir et al., 2008), anti-inflammatory (Satyanarayana and Rao, 1995), antipyretic (Manna et al., 1992), antiparasitic (Kuettel et al., 2007), antimalarial (Kativar et al., 2005), antifungal (Bekhit and Fahmy, 2003) and enzyme inhibitory agents (Regan et al., 2003; Li et al., 2004 and Dolle et al., 1994). As an example, edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one,

**1a**) has been recently shown to produce marked attenuation of brain damage caused by ischemia-reperfusion (Anzai et al., 2004) and its pharmacological actions were attributed to its antioxidant activity, as a potent hydroxyl radical scavenger (Parmar et al., 1999). The useful properties of pyrazole derivatives as insecticides, fungicides and sedatives drew attention of many investigators. Earlier experiments showed that pyrazole derivatives can be used as blood platelet aggregation inhibitor. That is pyrazole and its derivatives are clearly important in the field of enzyme inhibition. Selvam et al. (2006) showed that brominated derivatives are strikingly cytotoxic and possess more bioactivity. So the



present work is the continuation of bromination of 5-Methyl-2,4-dihydro-pyrazol-3-one at its active methylene group (Islam et al., 2001). The bromination of 5-Methyl-2-phenyl-2,4-dihydro-pyrazol-3-one (**1b**) led to the expected bromination at active methylene group along with bromination at *ortho*- and *para*- position of the phenyl ring. This result is not consistent with the reported work of

Elguero et al., 1984. So the bromination on some of the pyrazoline-5-one derivatives (Scheme 1) was taken for investigation. Hence the results of bromination on the active methylene group of ethyl acetoacetate followed by the ring-closure reaction with hydrazine and hydrazine derivatives leading to the pyrazolone ring are reported along with its cytotoxicity studies.



**Scheme - 1**

### Materials and Methods

The melting points of the synthesized compounds were recorded by thin disc method on a Fischer Johns electro-thermal melting point apparatus. Infrared spectra were recorded on DR-8001,

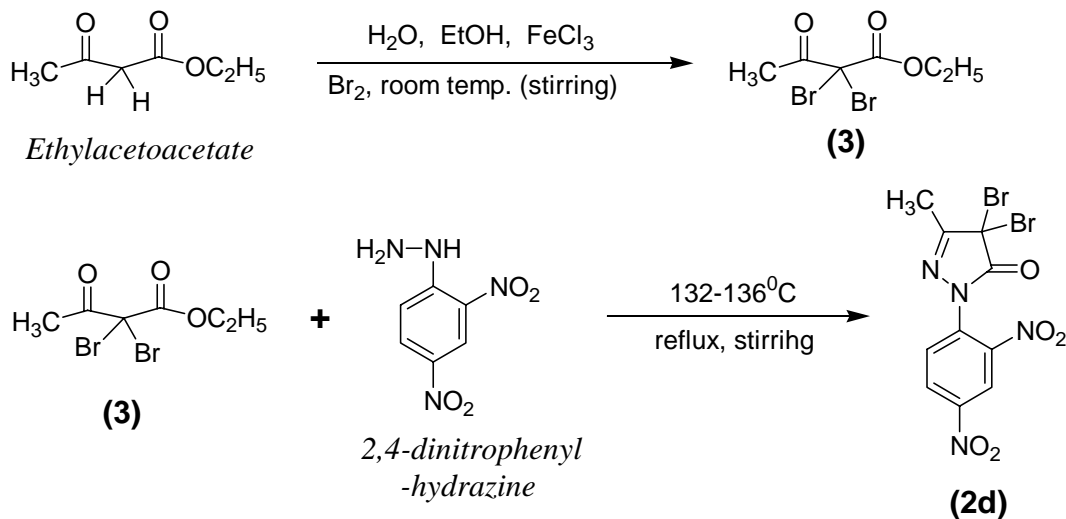
Shimadzu FT-IR spectrophotometer, <sup>1</sup>H-NMR spectra on a WP -400 NMR spectrophotometer using tetramethylsilane as internal standard. Mass spectra were obtained on a Kratos MS-25 using DH-88 data system.

Three active methylene compounds (**1a-1c**) were prepared by condensation of ethylacetoacetate and hydrazine and its derivatives in moderate yields (Scheme 1). Bromination was carried out on these three products (**1a-1c**) by following the standard procedure of Elguero et al., 1984 and dibromo (**2a** and **2d**), tribromo (**2d**), tetrabromo (**2c**) along with a side product (**2d-1**) were obtained. Compound **2d** was also prepared by an alternative method as shown in Scheme 2. Here bromination was carried out first on ethylacetoacetate to get compound **3**. This compound on condensation with 2,4-dinitrophenyl hydrazine at 132-136°C afforded **2d** in high yield. Compound (**2e**) was obtained from (**1c**) by bromination with NBS.

*General procedure for the Preparation of 5-methyl-2,4-dihydro-pyrazol-3-ones, (1a-1c):* Ethylacetoacetate was taken in a round bottomed flask and hydrazine hydrate and its derivatives were added to the flask dropwise with constant stirring at room temperature whereupon a vigorous reaction set in. A precipitate was formed quickly. As the reaction was exothermic, the reaction mixture was

allowed to cool at room temperature. The resulting precipitate was collected by filtration. The crude product (1.5 g) was purified by recrystallization. The products were identified by spectroscopic techniques.

*General procedure for the Preparation of 4,4-dibromo-5-methyl-2,4-dihydro-pyrazol-3-ones, (2a-2d):* Compound **1a-1c** were taken in a round-bottomed flask and dissolved in water (300 cm<sup>3</sup>). Bromine (15 cm<sup>3</sup>) was added to the solution from dropping funnel whereupon a vigorous reaction set in. After hydrogen bromide had ceased to evolve, the solid product was isolated by suction, washed with water several times and recrystallized from petroleum ether (60-80°C) to give crystals of **2a-2d**. During the Bromination of **1c** an unexpected byproduct was found from column chromatographic separation as the second fraction which lowers the % of yield of our main product, **2d**. The products were identified by spectroscopic techniques.



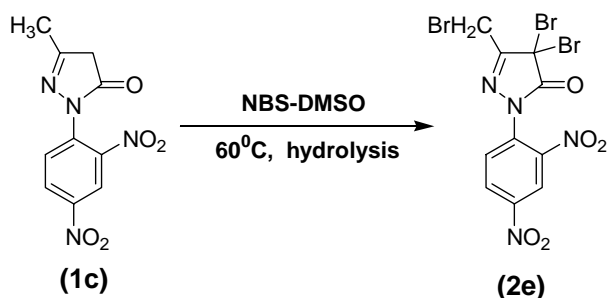
**Scheme-2**

*Preparation of 2,2-dibromo-3-oxo-butyric acid ethyl ester, 3:* Ethylacetoacetate (14.5123 g, 0.1209 mmol) was taken in a two-necked round bottomed flask and dissolved in ethanol (15 ml). 2-3 drops of aqueous solution of FeCl<sub>3</sub> was added to the stirred solution which produced deep violet-red coloration. 100ml of water was added to it with constant stirring. To this solution Br<sub>2</sub>/H<sub>2</sub>O was

added from a dropping funnel until the deep violet-red color was just disappeared. Upon standing the deep violet-red color was reappeared. This process was continued until the deep red-violet coloration was disappeared permanently. Some excess amount of Br<sub>2</sub>/H<sub>2</sub>O was added with constant stirring for 0.5 hour. Then the excess amount of Br<sub>2</sub> was removed by adding 5% aqueous

solution of  $\text{NaHSO}_3$ . Compound-3 was extracted by chloroform and was pale yellow colored liquid (Scheme 2).

*Preparation of 4,4-dibromo-2-(dinitro-phenyl)-5-methyl-2,4-dihydro-pyrazol-3-one, 2d:* A mixture of compound 3 (0.0500 g, 0.1737 mmol) and an equivalent amount of 2,4-dinitrophenyl hydrazine (0.0344 g, 0.1737 mmol) was taken in a 100 ml two necked quick-fit round-bottomed flask and was refluxed in an oil-bath for 3.5 hours. The color of the reaction mixture was changed to deep red during the reflux period. The reaction mixture was allowed to cool at room temperature and was kept overnight to be precipitated out. Then the reaction mixture was treated with water, immediately brown solid came out. The crude product was

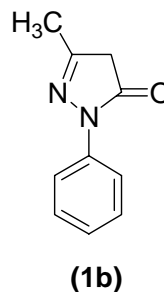
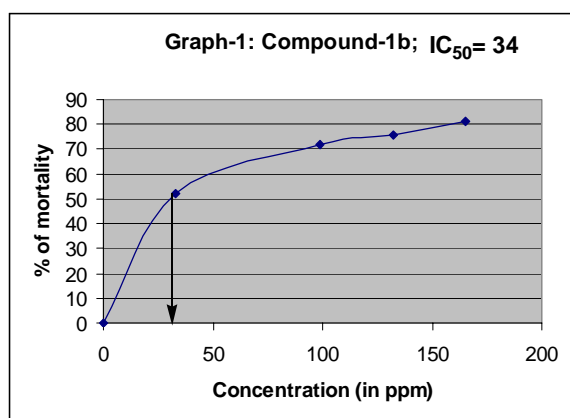


**Scheme-3**

purified by recrystallization from chloroform that gave brown crystalline solid of 2c; m.p. 120-121°C; yield 0.0641g (88%),  $R_f$  0.65 (PE:EA, 4:1). The product was characterized spectroscopically (Scheme 2).

*Preparation of 4,4-dibromo-5-bromomethyl-2-(2,4-dinitrophenyl)-2,4-dihydro-pyrazol-3-one, 2e:* N-bromosuccinimide, NBS (3.57 g, 0.0201 mol) in anhydrous DMSO (15 ml) was added slowly to the stirred solution of the compound, 1c (7.3495 g, 0.0278 mol) in anhydrous DMSO (5 ml) under an inert atmosphere. The solution was stirred at 60°C for 6 hours under an ambient pressure to give a red solution. After hydrogen bromide had ceased to evolve, the red solution was hydrolyzed with water, whereupon the solid product isolated. After hydrolysis the compound, 2e was isolated by filtration and washed several times with water and dried by suction (Scheme 3). The crude product was purified by recrystallization from dichloromethane that gave red powdered solid of 2e. The product was characterized spectroscopically.

*Demonstration of cytotoxicity:* The cytotoxicity study of the synthesized compounds 1a, 1b, 1c, 2a, 2b, 2c, 2d, 2d-1, 2e and 3 was investigated on brine shrimp as a test organism for convenience (Solis et al., 1993). 1.6 mg of each of the compounds was taken in the corresponding sample vials with 1.6 ml of dimethyl sulfoxide to prepare stock solution. From this stock solution 33, 99, 132 and 165 ppm of each compounds were placed in separate test tubes by micro syringe 1ml of extra dimethyl sulfoxide was given in each test tube with 10-12 brine shrimp. After 1, 2, 3 and 4 hours the test tubes were observed and the number of survived naupli in each test tube was counted and results



**5-Methyl-2-phenyl-2,4-dihydro-pyrazol-3-one**

**Figure 1:** Graph that was used for determining the  $\text{IC}_{50}$  of compound 1b.

were noted. From this the percentage of lethality of brine shrimp naupli was calculated at each concentration for each sample. Then graphs are drawn by plotting percentage of lethality of brine shrimp versus doses (in ppm) of the synthesized compounds which gave rise to the IC<sub>50</sub> (inhibitory concentration-50) values of the corresponding compounds (Figure 1). IC<sub>50</sub> of an agent is the dose which will inactive 50% of the test animal.

## Results

The formations of the new compounds were ensured by its several physical constants, such as

melting temperature (°C), R<sub>f</sub> values and color. However, the introduction of bromine atoms into the pyrazolone ring was identified by chemical method. Finally, the structures of the compounds were determined by the spectroscopic methods.

Physical constants gave the preliminary idea about the formation of new compounds. These constants are given here as a tabular form (Table 1).

Lassaigene test was performed on each of the synthesized compounds to detect the upcoming Bromine atoms. The results of the chemical tests are given bellow (Table II):

**Table I: Some physical data of the synthesized compounds**

| Compounds | Melting points (°C) | % of yield | R <sub>f</sub> values (PE:EA=1:2) | Color        |
|-----------|---------------------|------------|-----------------------------------|--------------|
| 1a        | 223                 | 30         | 0.32                              | White        |
| 1b        | 127                 | 78         | 0.52                              | Pale yellow  |
| 1c        | 81                  | 98         | 0.74                              | Yellow       |
| 2a        | 131                 | 64         | 0.41                              | White        |
| 2b        | 78                  | 76         | 0.29                              | Pale yellow  |
| 2c        | 134                 | 72         | 0.35                              | Pale yellow  |
| 2d        | 120                 | 49         | 0.65                              | Dark red     |
| 2d-1      | 92                  | 48         | 0.62                              | Light yellow |
| 2e        | 123                 | 87         | 0.57                              | Yellow       |
| 3         | 113 (b.p)           | 97         | 0.68                              | Yellow       |

**Table II: Results of the chemical tests**

| Compounds | Nitrogen Present | Bromine Present |
|-----------|------------------|-----------------|
| 1a        | +ve              | -ve             |
| 1b        | +ve              | -ve             |
| 1c        | +ve              | -ve             |
| 2a        | +ve              | +ve             |
| 2b        | +ve              | +ve             |
| 2c        | +ve              | +ve             |
| 2d        | +ve              | +ve             |
| 2d-1      | +ve              | +ve             |
| 2e        | +ve              | +ve             |
| 3         | -ve              | +ve             |

The resulting spectroscopic datas are given bellow:

**Compound-1a:** IR (Nujol): 3480 (ν<sub>N-H</sub>); 2980, 2880 (ν<sub>C-H</sub> aliphatic); 2700 (ν<sub>O-H</sub> enolic); 1680 (ν<sub>C=O</sub> lactam); 1615 (ν<sub>C=N</sub>); 1580 (ν<sub>C=C</sub> aromatic); 1515 (δ<sub>N-H</sub>) cm<sup>-1</sup>; <sup>1</sup>H-NMR (DMSO-d<sub>6</sub>): δ 5.2 (s, 1H, =CH); 2.1 (s, 3H, CH<sub>3</sub>); 10.0 (s, 1H, NH); 10.5 (s, 1H, OH); <sup>13</sup>C-NMR (DMSO-d<sub>6</sub>): δ 11.4 (CH<sub>3</sub>), 89.23 (C-4); 139.82 (C-3); 161.42 (=C-OH); **MS:** m/z (% of rel. intensities): 98 (M<sup>+</sup>, 100), 97(17), 81(1.0), 73(1.0), 67(18).

**Compound-1b:** IR (Nujol): 2980, 2880 (ν<sub>C-H</sub> aliphatic); 2700 (ν<sub>O-H</sub> enolic); 1697.7 (ν<sub>C=O</sub> lactam); 1489.2, 1541.3 (ν<sub>C=N</sub>); 1616.5, 1558.8 (ν<sub>C=C</sub> aromatic); 1311.5 (ν<sub>C-N</sub>); 773.5 (δ<sub>C-H</sub>) cm<sup>-1</sup>; <sup>1</sup>H-NMR (CDCl<sub>3</sub>): δ 11.45 (br, s, 1H, OH); 5.36 (s, 1H, =CH); 2.1 (s, 3H, CH<sub>3</sub>); 7.43 (dd, 2H, J<sub>m</sub>=1.1Hz, J<sub>o</sub>=8.0Hz); 7.71 (dd,

2H,  $J_o=7.4\text{Hz}$ ,  $J_m=1.1\text{Hz}$ ); 7.52 (dd, 1H,  $J_m=2.0\text{Hz}$ ,  $J_o=7.2\text{Hz}$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  14.60 ( $\text{CH}_3$ ), 178.3 ( $=\text{C-OH}$ ); 45.10 ( $\text{CH}_2$ ); 132.1 ( $\text{N=C}$ ); 147.0 (aromatic C-1); 132.01 (aromatic C-2/6); 142.30 (aromatic C-3/5); 130 (aromatic C-4).

**Compound-1c: IR** (KBr): 3116 ( $\nu_{\text{C-H}}$  aromatic); 2925 ( $\nu_{\text{C-H}}$  saturated); 1728 ( $\nu_{\text{C=O}}$  lactam); 1620 ( $\nu_{\text{C=N}}$ ); 1597, 1473 ( $\nu_{\text{C=C}}$  aromatic); 1410, 1518 ( $\nu_{\text{N=O}}$  nitro group); 1340 ( $\nu_{\text{C-N}}$ ); 1026 ( $\nu_{\text{C-O}}$ ); 1455, 1378 ( $\delta_{\text{C-H}}$ ); 1357 ( $\nu_{\text{N-N}}$ )  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ):  $\delta$  10.85 (br, s, 1H, OH); 3.61 (s, 2H,  $\text{CH}_2$ ); 2.19 (s, 3H,  $\text{CH}_3$ ); 8.92 (d, H,  $J_m=3.2\text{Hz}$ ); 8.43 (dd, 1H,  $J_o=5.3\text{Hz}$ ,  $J_m=2.5\text{Hz}$ ); 7.86 (dd, 1H,  $J_o=7.6\text{Hz}$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  16.525 ( $\text{CH}_3$ ), 169.45 ( $=\text{C-OH}$ ); 61.03 ( $\text{CH}_2$ ); 153.54 ( $\text{N=C}$ ); 116.30 (aromatic C-6); 123.31 (aromatic C-1); 129.81 (aromatic C-2); 130.54 (aromatic C-4); 137.52 (aromatic C-5); 144.88 (aromatic C-3). **MS:**  $m/z$  (% of rel. intensities): 264 ( $\text{M}^+$ ), 137 (18%), 219 (28%), 173 (6%), 145 (8%), 131 (6%), 104 (14%), 77 (32%), 51 (8%); *Elemental analysis:* Calcd. for  $[\text{C}_{10}\text{H}_8\text{N}_4\text{O}_5]$ : C (46.48%), H (4.276%), N (18.43%). Found: C (46.41%), H (4.241%), N (18.36%).

**Compound-2a: IR** (Nujol): 3353 ( $\nu_{\text{N-H}}$ ); 2880 ( $\nu_{\text{C-H}}$  aliphatic); 1723 ( $\nu_{\text{C=O}}$  lactam); 1620 ( $\nu_{\text{C=N}}$ ); 1300 ( $\nu_{\text{C-N}}$ ); 1515 ( $\delta_{\text{N-H}}$ ); 1357 ( $\nu_{\text{N-N}}$ )  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ):  $\delta$  12.0 (s, 1H, NH); 2.1 (s, 3H,  $\text{CH}_3$ ); **MS:**  $m/z$  (% of rel. intensities): 254, 256, 258 (M, M+2, M+4, 10:20:10); 177/175 (100); 98 (3.0); 94 (2.0).

**Compound-2b: IR** (Nujol): 3094 ( $\nu_{\text{C-H}}$  aromatic); 2916 ( $\nu_{\text{C-H}}$  aliphatic); 1738 ( $\nu_{\text{C=O}}$  lactam); 1574 ( $\nu_{\text{C=N}}$ ); 1616.5, 1558.8 ( $\nu_{\text{C=C}}$  aromatic); 1279 ( $\nu_{\text{C-N}}$ ); 773.5 ( $\delta_{\text{C-H}}$  *p*-substituted benzene ring); 650 ( $\nu_{\text{C-Br}}$ )  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\text{DMSO-d}_6$ ):  $\delta$  2.3 (s, 3H,  $\text{CH}_3$ ); 8.15 (d, 1H,  $J_m=2.26\text{Hz}$ ); 7.77 (dd, 1H,  $J_o=8.54\text{Hz}$ ,  $J_m=2.26\text{Hz}$ ); 7.52 (d, 1H,  $J_o=8.54\text{Hz}$ ). **MS:**  $m/z$  (% of rel. intensities): 486, 488, 490, 492, 494 ( $\text{M}^+$ , M+2, M+4, M+6, M+8, 1:4:6:4:1); 408, 410, 412, 414 ( $\text{M}^+$ -Br, 1:3:3:1); 488(100%); 329(20%); 289(5%); 263(50%); 235(65%); 182(5%); 154(18%); 117(5%); 75(20%).

**Compound-2c: IR** (KBr): 3118 ( $\nu_{\text{C-H}}$  aromatic); 2927 ( $\nu_{\text{C-H}}$  saturated); 1714 ( $\nu_{\text{C=O}}$  lactam); 1612 ( $\nu_{\text{C=N}}$ ); 1593, 1498 ( $\nu_{\text{C=C}}$  aromatic); 1427, 1548 ( $\nu_{\text{N=O}}$  nitro group); 1338 ( $\nu_{\text{C-N}}$ ); 1026 ( $\nu_{\text{C-O}}$ ); 1455, 1315 ( $\delta_{\text{C-H}}$ ); 1357 ( $\nu_{\text{N-N}}$ ); 742 ( $\nu_{\text{C-Br}}$ )  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  1.55 (s, 3H,  $\text{CH}_3$ ); 8.71 (d, H,  $J_m=2.5\text{Hz}$ ); 8.30 (dd, 1H,  $J_o=8.7\text{Hz}$ ,  $J_m=2.5\text{Hz}$ ); 8.01 (d, 1H,  $J_o=8.07\text{Hz}$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  14.28 ( $\text{CH}_3$ ), 190.20 ( $\text{C=O}$ ); 107.20 ( $\text{CBr}_2$ ); 187.11 ( $\text{N=C}$ ); 120.97 (aromatic C-6); 121.02 (aromatic C-1); 128.08 (aromatic C-2); 136.56

(aromatic C-4); 147.31 (aromatic C-5); 150.08 (aromatic C-3). **MS:**  $m/z$  (% of rel. intensities): 420, 422, 424 ( $\text{M}^+$ , M+2, M+4, 1:2:1); 281,283 (1:1, 1.5%); 246, 248 (1:1, 100%); 167 (3.99%), 156 (53.8%), 154 (23.7%), 75 (93.41%); *Elemental analysis:* Calcd. for  $[\text{C}_{10}\text{H}_6\text{Br}_2\text{N}_4\text{O}_5]$ : C (28.346%), H (1.43%), N (13.28%). Found: C (28.45%), H (1.46%), N (13.26%).

**Compound-2d: IR** (KBr): 3118 ( $\nu_{\text{C-H}}$  aromatic); 2927 ( $\nu_{\text{C-H}}$  saturated); 1714 ( $\nu_{\text{C=O}}$  lactam); 1612 ( $\nu_{\text{C=N}}$ ); 1593, 1498 ( $\nu_{\text{C=C}}$  aromatic); 1427, 1548 ( $\nu_{\text{N=O}}$  nitro group); 1338 ( $\nu_{\text{C-N}}$ ); 1026 ( $\nu_{\text{C-O}}$ ); 1455, 1315 ( $\delta_{\text{C-H}}$ ); 1357 ( $\nu_{\text{N-N}}$ ); 742 ( $\nu_{\text{C-Br}}$ )  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  1.55 (s, 3H,  $\text{CH}_3$ ); 8.71 (d, H,  $J_m=2.5\text{Hz}$ ); 8.30 (dd, 1H,  $J_o=8.7\text{Hz}$ ,  $J_m=2.5\text{Hz}$ ); 8.01 (d, 1H,  $J_o=8.07\text{Hz}$ );  $^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  14.28 ( $\text{CH}_3$ ), 190.20 ( $\text{C=O}$ ); 107.20 ( $\text{CBr}_2$ ); 187.11 ( $\text{N=C}$ ); 120.97 (aromatic C-6); 121.02 (aromatic C-1); 128.08 (aromatic C-2); 136.56 (aromatic C-4); 147.31 (aromatic C-5); 150.08 (aromatic C-3). **MS:**  $m/z$  (% of rel. intensities): 420, 422, 424 ( $\text{M}^+$ , M+2, M+4, 1:2:1); 281,283 (1:1, 1.5%); 246, 248 (1:1, 100%); 167 (3.99%), 156 (53.8%), 154 (23.7%), 75 (93.41%); *Elemental analysis:* Calcd. for  $[\text{C}_{10}\text{H}_6\text{Br}_2\text{N}_4\text{O}_5]$ : C (28.346%), H (1.43%), N (13.28%). Found: C (28.45%), H (1.46%), N (13.26%).

**Compound-2d-1: IR** (KBr): 3118 ( $\nu_{\text{C-H}}$  aromatic); 1593, 1498 ( $\nu_{\text{C=C}}$  aromatic); 1427, 1548 ( $\nu_{\text{N=O}}$  nitro group); 1338 ( $\nu_{\text{C-N}}$ ); 742 ( $\nu_{\text{C-Br}}$ )  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  8.84 (d, H,  $J_m=2.39\text{Hz}$ ); 8.38 (dd, 1H,  $J_o=8.7\text{Hz}$ ,  $J_m=2.39\text{Hz}$ ); 8.21 (d, 1H,  $J_o=8.79\text{Hz}$ ). **MS:**  $m/z$  (% of rel. intensities): 246/248 ( $\text{M}^+$ / M+2, 11%/10%); 216/218 (5%); 200/202 (3.60%, 3.27%); 170/172 (4.64%, 3.80%); 167 (3.02%); 154/156 (6.2%, 7.32%); 134 (100%); 120(2.95%); 91(6.76%); 90 (3.29%); 75 (15.02%); 74 (3.84%).

**Compound-2e: IR** (KBr): 3030 ( $\nu_{\text{C-H}}$  aromatic); 2926 ( $\nu_{\text{C-H}}$  saturated); 1684 ( $\nu_{\text{C=O}}$  lactam); 1620 ( $\nu_{\text{C=N}}$ ); 1549, 1498 ( $\nu_{\text{C=C}}$  aromatic); 1376, 1538 ( $\nu_{\text{N=O}}$  nitro group); 1318 ( $\nu_{\text{C-N}}$ ); 1445, 1315 ( $\delta_{\text{C-H}}$ ); 744 ( $\nu_{\text{C-Br}}$ )  $\text{cm}^{-1}$ ;  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ):  $\delta$  2.44 (s, 2H,  $\text{CH}_2$ ); 8.84 (d, 1H,  $J_m=2.79\text{Hz}$ ); 8.379 (dd, 1H,  $J_o=8.79\text{Hz}$ ,  $J_m=2.79\text{Hz}$ ); 8.20 (d, 1H,  $J_o=8.79\text{Hz}$ ). **MS:**  $m/z$  (% of rel. intensities): 498/500/502/504 ( $\text{M}^+$ , M+2, M+4, M+6, 29.24%); 458/460/462/464 (59.76%); 418/420/422 (8.92%); 338/340 (8.53%); 292/298/296/298 (100%); 277/279 (64.88%); 264/266/268/270 (11.81%); 251/253/255 (30.84%); 184/186/188 (12.63%); 183 (52.0%); 105 (17.23%); 93 (17.07%).

**Compound-3: IR** (Nujol): 2960, 2873 ( $\nu_{\text{C-H}}$  aliphatic); 1708.5 ( $\nu_{\text{C=O}}$  ketone); 1756.02 ( $\nu_{\text{C=O}}$  ester);

1455, 1358 ( $\delta_{C-H}$ ); 1015 ( $\nu_{C=O}$ ); 742 ( $\nu_{C-Br}$ );  $cm^{-1}$ ;  $^1H$ -NMR ( $CDCl_3$ ):  $\delta$  2.85 (s, 3H); 1.36 (t, 3H,  $J=6.89Hz$ ); 4.82 (q, 2H,  $J=6.82Hz$ ).

| Table III: Remarks of the synthesized compounds according to their bioactivity |                  |                    |
|--|------------------|--------------------|
| Compounds  | IC <sub>50</sub> | Remarks            |
| 1a   | 32               | Weakly active      |
| 1b   | 34               | Weakly active      |
| 1c   | 33               | Weakly active      |
| 2a   | 22               | Highly active      |
| 2b   | 19               | Highly active      |
| 2c   | 21               | Highly active      |
| 2d   | 17               | Very Highly active |
| 2d-1   | 99               | Inactive           |
| 2e   | 37               | Weakly active      |
| 3  | 68               | Less active        |

The comparative IC<sub>50</sub> values of the synthesized compounds are shown bellow.

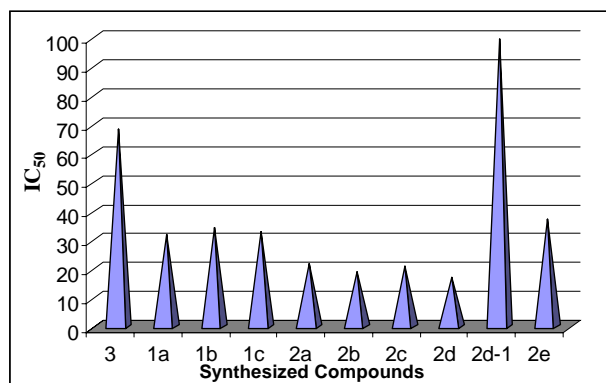
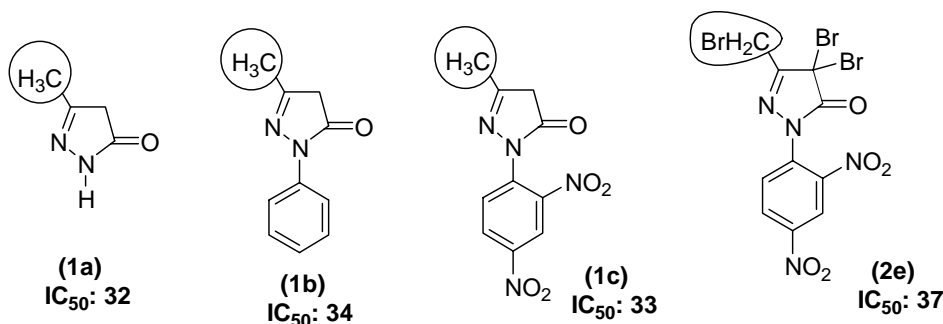


Figure 2: Graphical representation of IC<sub>50</sub> of the synthesized compounds



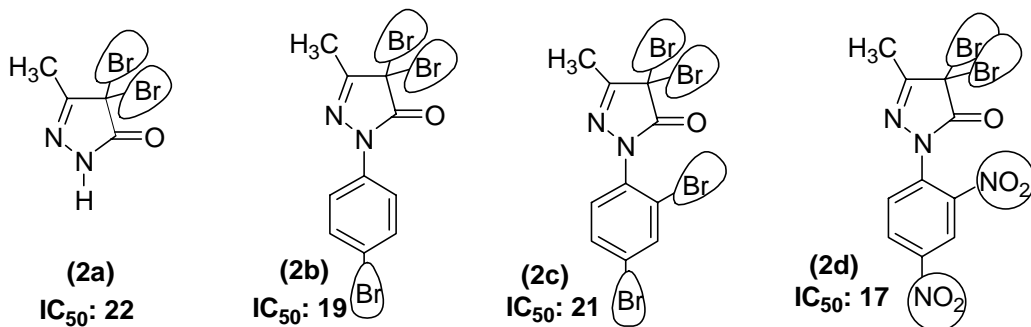
According to the IC<sub>50</sub> values the Compounds are remarked as very highly active (IC<sub>50</sub><19), highly active (19< IC<sub>50</sub><29), weakly active (29< IC<sub>50</sub><39), less active (39< IC<sub>50</sub><69) and inactive (69< IC<sub>50</sub>).

Figure 2 represents the comparative IC<sub>50</sub> values of the synthesized compounds (**1a**, **1b**, **1c**, **2a**, **2b**, **2c**, **2d**, **2d-1**, **2e** and **3**) and Table III indicates the remarks about the cytotoxicity.

## Discussion

After performing the bioassay of the synthesized molecules, it was found that the terminal methyl group is necessary for its activity. When the compounds **1a**, **1b** and **1c** were brominated the activity of the molecule was increased. The activity was further increased when two nitro-groups were introduced in the benzene moiety in *ortho*- and *para*- position.

Hence the terminal methyl chain should be undisturbed to show fruit-full bioactivity. According to Regan et al. 2003 the terminal small alkyl chains are sterically fit into the lipophilic active pocket of the enzyme. It is also proved by the present work. When the terminal methyl group is disturbed by introducing a bulky group -Br, the activity (hence IC<sub>50</sub>) reduces by 3 to 5. Thus when the methyl side chain at position 5 was mono-brominated along with the bromination (in **2c-2**) at the active methylene group (position-4), the activity was decreased remarkably. Thus the methyl side chain, brominated active methylene group (position-4) and the two nitro-groups containing phenyl ring in the pyrazolone ring are very important to show biological activity.



The present studies agree with the earlier experiments. Jiang et al. (1990) showed that 2-styrylquinazolin-4(3H)-ones have potential antimitotic anticancer activity. Their extensive structure activity relationship (SAR) studies suggested that the entire quinazolinone was required but activity was further enhanced by introducing halide group into the 4 position of pyrazole ring. The present findings are also consistent with this literature. But in the case of compound-2e in which the methyl side chain is also brominated showed lower activity. On the other hand, Elzein et al. 2004 showed that alkyl group on pyrazole ring substantially increase its activity as selective adenosine A(3) receptor. Furthermore, Ikeda et. al. 1996 and Huang et al. 1992 showed that methyl group is necessary for the binding of drug through the lipophilic domain of the enzymes. Thus the methyl group on 5 position of pyrazolone ring should be unsubstantiated for showing bioactivity.

Besides, extensive SAR studies showed that the benzene ring of the 5-methyl-2-phenyl-2,4-dihydro-pyrazol-3-one should be substituted by -Br or -NO<sub>2</sub> group to show better activity.

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