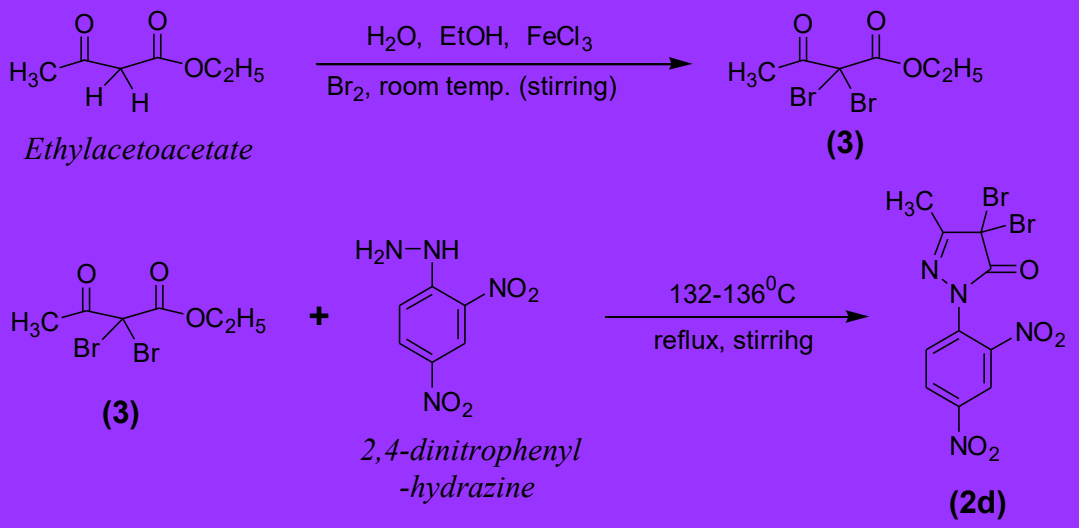


BJP

Bangladesh Journal of Pharmacology

Research Article

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

A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index;
Bangladesh J Pharmacol 2008; 3: 27-35
ISSN: 1991-0088

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

Rahat Khan, Md. Imam Uddin, Md. Sultan Alam, Mohammad Mamun Hossain and Md. Rabiul Islam

Department of Chemistry, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh.

Article Info

Received: 11 November 2007
Accepted: 23 April 2008
Available Online: 1 May 2008

DOI: 10.3329/bjp.v3i1.834

Cite this article:
Khan R, Uddin MI, Alam MS, Hossain MM, Islam MR. Synthesis and preliminary evaluation of brominated 5-methyl-2,4-dihydropyrazol-3-one and its derivatives as cytotoxic agents. Bangladesh J Pharmacol. 2008; 3: 27-35.

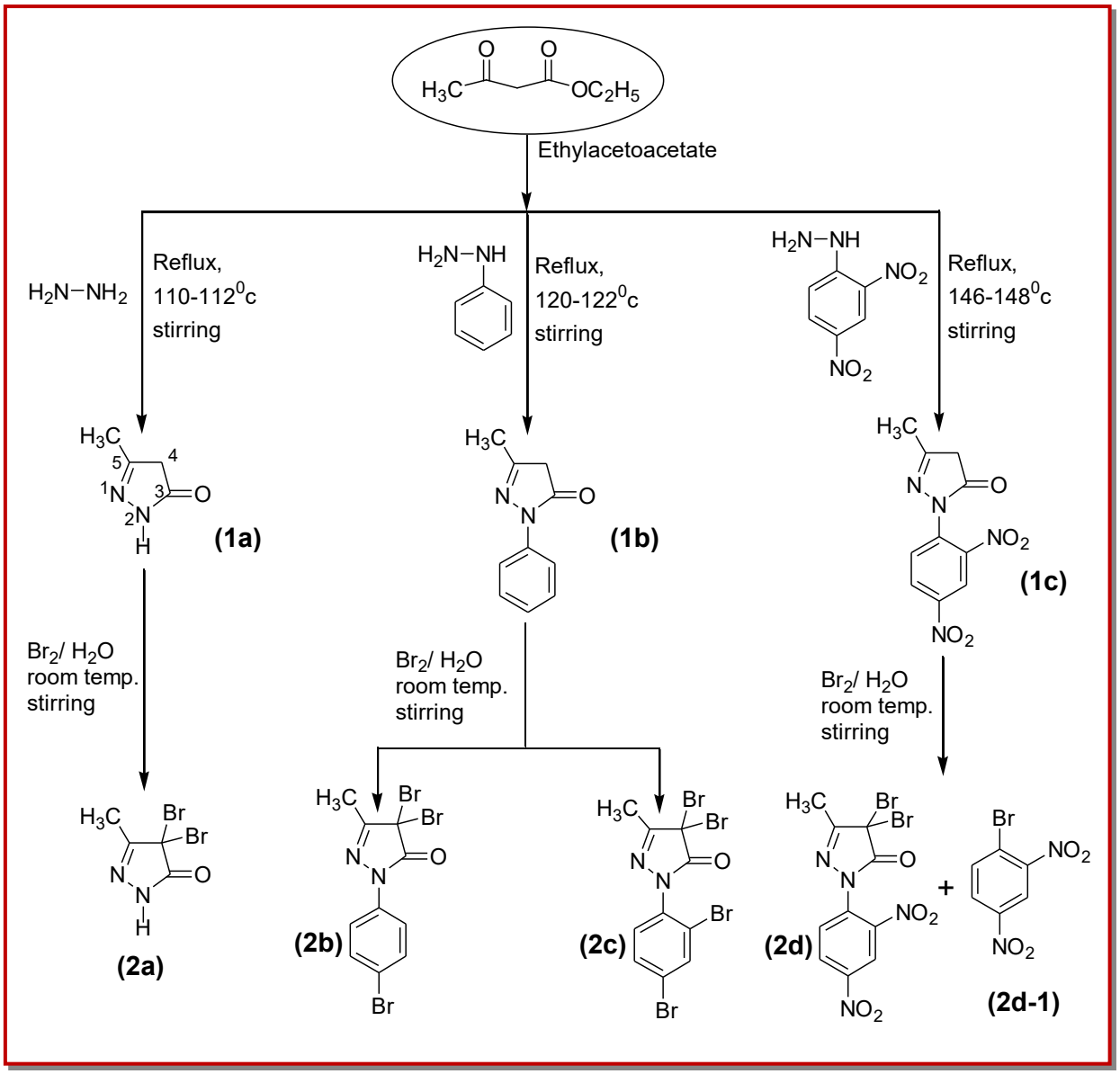
Abstract

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, ^{1}H-NMR, ^{13}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 anti-oxidant 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*-



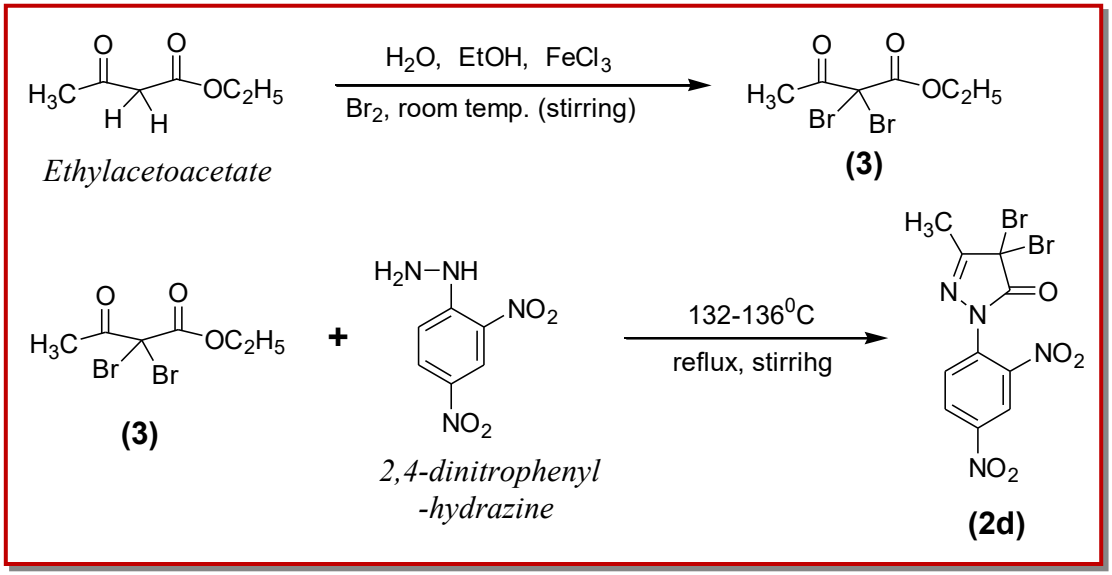
Scheme 1

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.

Materials and Methods

The melting points of the synthesized compounds were recorded by thin disc method on a *Fischer Jonhns* electro -thermal melting point apparatus. Infrared spectra were recorded on DR-8001, Shimadzu FT-IR spectrophotometer, ^{1}H-NMR spectra on a WP-400 NMR spectrophotometer using tetramethylsilane as internal standard. Mass spectra were obtained on a Kratas MS-25 using DH-88 data system.

Three active methylene compounds (**1a-c**) 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-c**) 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



Scheme 2

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-c)

Ethylacetoacetate was taken in a around 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 recrystalization. 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)

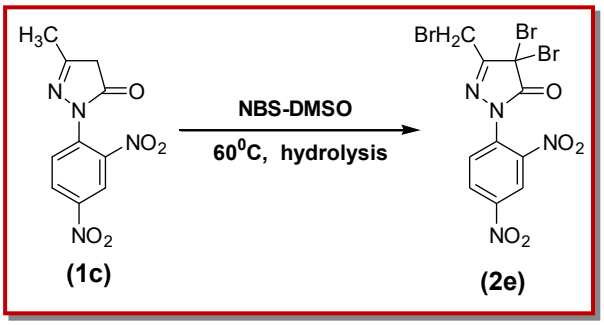
Compound **1a-1c** were taken in a round-bottomed flask and dissolved in water (300 cm^3). Bromine (15 cm^3) was added to the solution from dropping funnel whereupon a vigorous reaction set in. After hydrogen bromide had ceased to evolve, the solid product was isolated by suction, washed with water several 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.

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 $FeCl_3$ was added to the stirred solution which produced deep violet-red coloration. 100 mL of water was added to it with constant stirring. To this solution Br_2/H_2O 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_2/H_2O was added with constant stirring for 0.5 hour. Then the excess amount of Br_2 was removed by adding 5% aqueous solution of $NaHSO_3$. 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 purified by recrystalization 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



Scheme 3

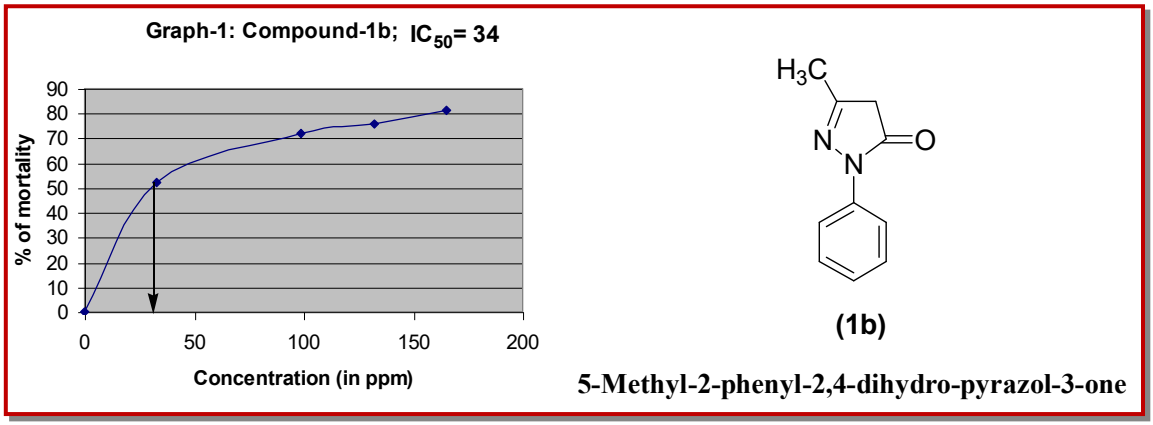


Figure 1: Graph that was used for determining the IC_{50} of compound 1b

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 recrystalization 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 1 mL 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 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_{50} (inhibitory concentration-50) values of the corresponding compounds (Figure 1). IC_{50} 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_f 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 I).

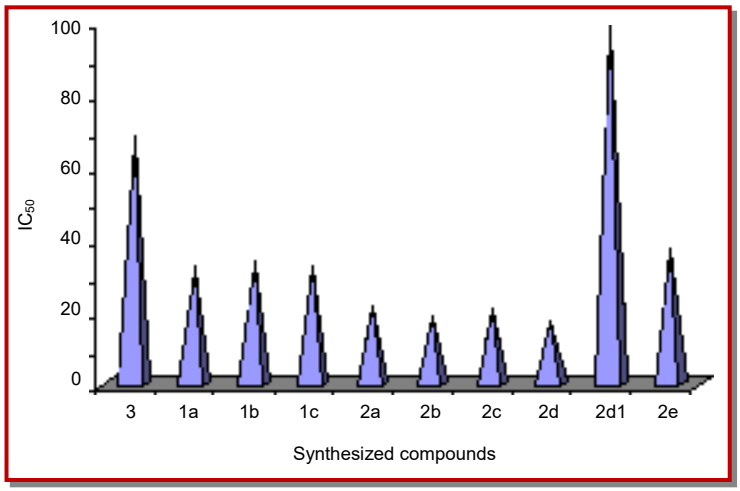


Figure 2: Graphical representation of IC_{50} of the synthesized compounds

Table I

Some physical data of the synthesized compounds

Compounds	Melting points (°C)	% of yield	R_f 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

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): The resulting spectroscopic datas are given bellow:

Compound 1a: IR (Nujol): 3480 (ν_{N-H}); 2980, 2880 (ν_{C-H} aliphatic); 2700 (ν_{O-H} enolic); 1680 ($\nu_{C=O}$ lactam); 1615 ($\nu_{C=N}$); 1580 ($\nu_{C=C}$ aromatic); 1515 (δ_{N-H}) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 5.2 (s, 1H, =CH); 2.1 (s, 3H, CH_3); 10.0 (s, 1H, NH); 10.5 (s, 1H, OH); ^{13}C-NMR (DMSO-d_6): δ 11.4 (CH_3), 89.23 (C-4); 139.82 (C-3); 161.42 (=C-OH); MS: *m/z* (% of rel. intensities): 98 (M^+, 100), 97(17), 81(1.0), 73 (1.0), 67(18).

Compound 1b: IR (Nujol): 2980, 2880 (ν_{C-H} aliphatic); 2700 (ν_{O-H} enolic); 1697.7 ($\nu_{C=O}$ lactam); 1489.2, 1541.3 ($\nu_{C=N}$); 1616.5, 1558.8 ($\nu_{C=C}$ aromatic); 1311.5 (ν_{C-N}); 773.5 (δ_{C-H}) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 11.45 (br, s, 1H, OH); 5.36 (s, 1H, =CH); 2.1 (s, 3H, CH_3); 7.43 (dd, 2H, J_m=1.1Hz, J_o=8.0Hz); 7.71 (dd, 2H, J_o=7.4Hz, J_m= 1.1Hz); 7.52 (dd, 1H, J_m=2.0Hz, J_o=7.2Hz); ^{13}C-NMR ($CDCl_3$): δ 14.60 (CH_3), 178.3 (=C-OH); 45.10 (CH_2); 132.1 (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 (ν_{C-H} aromatic); 2925 (ν_{C-H} saturated); 1728 ($\nu_{C=O}$ lactam); 1620 ($\nu_{C=N}$); 1597, 1473 (($\nu_{C=C}$ aromatic); 1410, 1518 (($\nu_{N=O}$ nitro group); 1340 (ν_{C-N}); 1026 (ν_{C-O}); 1455, 1378 (δ_{C-H}); 1357 (ν_{N-N}) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ10.85 (br, s, 1H, OH); 3.61 (s, 2H, CH_2); 2.19 (s, 3H, CH_3); 8.92 (d, H, J_m=3.2Hz); 8.43 (dd, 1H, J_o=5.3Hz, J_m=2.5Hz); 7.86 (dd, 1H, J_o=7.6Hz); **^{13}C-NMR** ($CDCl_3$): δ 16.525 (CH_3), 169.45 (=C-OH); 61.03 (CH_2); 153.54 (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 (M^+), 137 (18%), 219 (28%), 173 (6%), 145 (8%), 131 (6%), 104 (14%), 77 (32%), 51

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*

Table III

Remarks of the synthesized compounds according to their bioactivity

Compounds	IC_{50}	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

(8%); *Elemental analysis:* Clalc. for [$C_{10}H_8N_4O_5$]: C (46.48%), H (4.276%), N (18.43%). Found: C (46.41%), H (4.241%), N (18.36%).

Compound 2a: IR (Nujol): 3353 (ν_{N-H}); 2880 (ν_{C-H} aliphatic); 1723 ($\nu_{C=O}$ lactam); 1620 ($\nu_{C=N}$); 1300 (ν_{C-N}); 1515 (δ_{N-H}); 1357 (ν_{N-N}) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 12.0 (s, 1H, NH); 2.1 (s, 3H, 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).

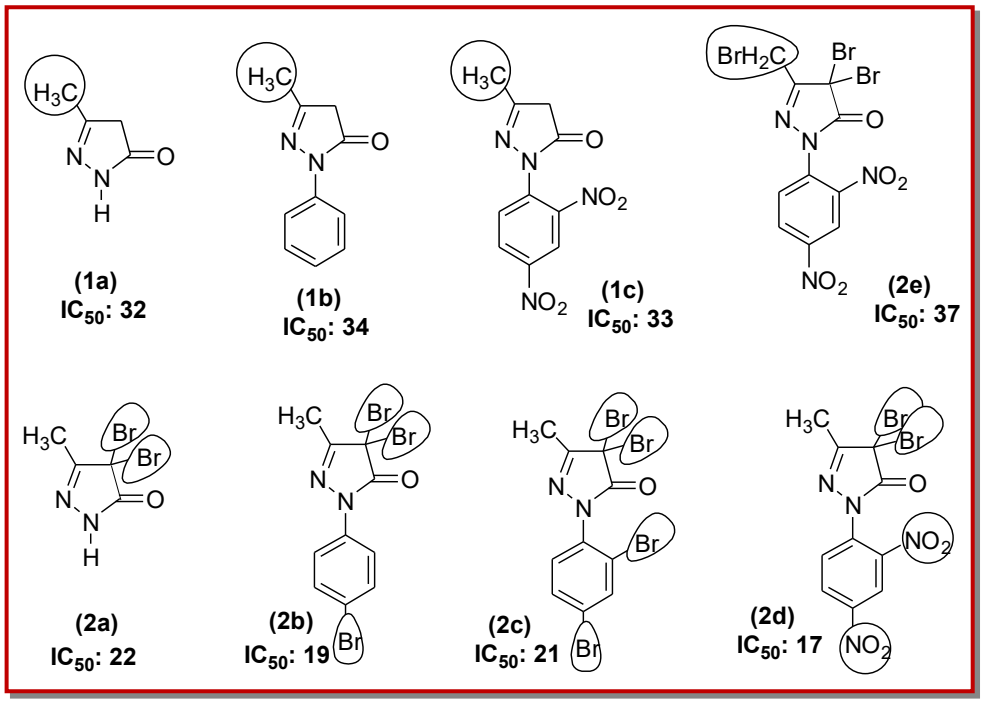
Compound 2b: IR (Nujol): 3094 (ν_{C-H} aromatic); 2916 (ν_{C-H} aliphatic); 1738 ($\nu_{C=O}$ lactam); 1574 ($\nu_{C=N}$); 1616.5, 1558.8 ($\nu_{C=C}$ aromatic); 1279 (ν_{C-N}); 773.5 (δ_{C-H} *p*-substituted benzene ring); 650 (ν_{C-Br}); cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3); 8.15 (d, 1H, J_m=2.26Hz); 7.77 (dd, 1H, J_o=8.54Hz, J_m=2.26Hz); 7.52 (d, 1H, J_o=8.54Hz). MS: *m/z* (% of rel. intensities): 486, 488, 490, 492, 494 (M^+, M+2, M+4, M+6, M+8, 1:4:6:4:1); 408, 410, 412, 414 (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 (ν_{C-H} aromatic); 2927 (ν_{C-H} saturated); 1714 ($\nu_{C=O}$ lactam); 1612 ($\nu_{C=N}$); 1593, 1498 (($\nu_{C=C}$ aromatic); 1427, 1548 (($\nu_{N=O}$ nitro group); 1338 (ν_{C-N}); 1026 (ν_{C-O}); 1455, 1315 (δ_{C-H}); 1357 (ν_{N-N}); 742 (ν_{C-Br}); cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.55 (s, 3H, CH_3); 8.71 (d, H, J_m=2.5Hz); 8.30 (dd, 1H, J_o=8.7Hz, J_m=2.5Hz); 8.01 (d, 1H, J_o=8.07Hz); ^{13}C-NMR ($CDCl_3$): δ 14.28 (CH_3), 190.20 (C=O); 107.20 (CBr_2); 187.11 (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 (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:* Clalc. for [$C_{10}H_6Br_2N_4O_5$]: C (28346%), H (1.43%), N (13.28%). Found: C (28.45%), H (1.46%), N (13.26%).

Compound 2d: IR (KBr): 3118 (ν_{C-H} aromatic); 2927 (ν_{C-H} saturated); 1714 ($\nu_{C=O}$ lactam); 1612 ($\nu_{C=N}$); 1593, 1498 (($\nu_{C=C}$ aromatic); 1427, 1548 (($\nu_{N=O}$ nitro group); 1338 (ν_{C-N}); 1026 (ν_{C-O}); 1455, 1315 (δ_{C-H}); 1357 (ν_{N-N}); 742 (ν_{C-Br}); cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.55 (s, 3H, CH_3); 8.71 (d, H, J_m=2.5Hz); 8.30 (dd, 1H, J_o=8.7Hz, J_m=2.5Hz); 8.01 (d, 1H, J_o=8.07Hz); ^{13}C-NMR ($CDCl_3$): δ 14.28 (CH_3), 190.20 (C=O); 107.20 (CBr_2); 187.11 (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 (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:* Clalc. for [$C_{10}H_6Br_2N_4O_5$]: C (28346%), H (1.43%), N (13.28%). Found: C (28.45%), H (1.46%), N (13.26%).

Compound 2d-1: IR (KBr): 3118 (ν_{C-H} aromatic); 1593, 1498 (($\nu_{C=C}$ aromatic); 1427, 1548 (($\nu_{N=O}$ nitro group); 1338 (ν_{C-N}); 742 (ν_{C-Br}); cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 8.84 (d, H, J_m=2.39Hz); 8.38 (dd, 1H, J_o=8.7Hz, J_m=2.39Hz); 8.21 (d, 1H, J_o=8.79Hz). MS: *m/z* (% of rel. intensities): 246/248 (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 (ν_{C-H} aromatic); 2926 (ν_{C-H} saturated); 1684 ($\nu_{C=O}$ lactam); 1620 ($\nu_{C=N}$); 1549, 1498 (($\nu_{C=C}$ aromatic); 1376, 1538 (($\nu_{N=O}$ nitro group); 1318 (ν_{C-N}); 1445, 1315 (δ_{C-H}); 744 (ν_{C-Br}); cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.44 (s, 2H, CH_2); 8.84 (d, 1H, J_m=2.79Hz); 8.379 (dd, 1H, J_o=8.79Hz, J_m=2.79Hz); 8.20 (d, 1H, J_o=8.79Hz). **MS:** *m/z* (% of rel. intensities): 498/500/502/504 (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 (ν_{C-H} aliphatic); 1708.5 ($\nu_{C=O}$ ketone); 1756.02 ($\nu_{C=O}$ ester); 1455, 1358 (δ_{C-H}); 1015 (ν_{C-O}); 742 (ν_{C-Br}); cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.85 (s, 3H); 1.36 (t, 3H, *J*=6.89Hz); 4.82 (q, 2H, *J*=6.82Hz).

The comparative IC_{50} values of the synthesized compounds are shown bellow.

According to the IC_{50} values the compounds are remarked as very highly active (IC_{50}<19), highly active (19< IC_{50}<29), weakly active (29< IC_{50}<39), less active (39< IC_{50}<69) and inactive (69< IC_{50}).

Figure 2 represents the comparative IC_{50} 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_{50}) 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_2 group to show better activity.

Financial Support

Self-funded

Conflict of Interest

Authors declare no conflict of interest

Acknowledgement

We express our gratitude and thanks to Prof. Helmut Duddeck, Institute of Organic Chemistry, Hannover University, Germany for supplying the MS and NMR spectra of the synthesized compounds. We also express our gratitude to Mrs. Rabeya Khatun, Quality Control Manager, Supreme Pharmaceuticals Ltd. for supplying the IR spectra of the synthesized compounds.

References

Amir M, Kumar H, Khan SA. Synthesis and pharmacological evaluation of pyrazoline derivatives as new anti-inflammatory and analgesic agents. Bioorg Med Chem Lett. 2008; 18: 918-22.

Anzai K, Furuse M, Yoshida A, Matsuyama A, Moritake T, Tsuboi K, Ikota N. *In vivo* radioprotection of mice by 3-methyl-1-phenyl-2pyrazolin-5-one (edaravone; Radicut), a clinical drug. J Radiat Res (Tokyo). 2004; 45: 319-23. http://dx.doi.org/10.1269/jrr.45.319

Bekhit AA, Fahmy HT. Design and synthesis of some substituted 1H-pyrazolyl-oxazolidines or 1H-pyrazolyl-thiazolidines as anti-inflammatory-antimicrobial agents. Arch Pharm (Weinheim). 2003; 336: 111-18. http://dx.doi.org/10.1002/ardp.200390007

Dolle RE, Singh J, Rinker J, Hoyer D, Prasad CV, Graybill TL, Salvino JM, Helaszek CT, Miller RE, Ator MA. Aspartyl alpha-[1-phenyl-3-(trifluoromethyl)-pyrazol-5-yl]methyl ketons as interleukin-1 beta converting enzyme inhibitors. Significance of the P1 and P3 amido nitrtogens for enzyme-peptide inhibitor binding. J Med Chem. 1994; 37: 3863-66.

http://dx.doi.org/10.1021/jm00049a001 http://dx.doi.org/10.1021/jm00031a003 http://dx.doi.org/10.1021/jm00043a002

Elguero PJ, Guiraud G, Jacquier R. Nouvelles Réactions de bromination en séric pyrazole. Obtention de bromo-4 pyrazolones. Bulletin de la Socété Chimique de France. 1967; 62: 328-32.

Elzein E, Palle V, Wu Y, Maa T, Zeng D, Zablocki J. 2-Pyrazolyl -N(6)-sustituted adenosine derivatives as high affinity and selective adenosine A(3) receptor agonists. J Med Chem. 2004; 47: 4766-73. http://dx.doi.org/10.1021/jm049682h

Friebolin H. Basic one and two-dimensional NMR Spectroscopy. 3rd ed. Germany, Wiley-VCH, 1998, p 47.

GÜrsoy A, Demiravak S, Capan G, Erol K, Vural K. Synthesis and preliminary evaluation of new 5-pyrazolione derivatives as analgesic agents. Eur J Med Chem. 2000; 35: 359-64. http://dx.doi.org/10.1016/S0223-5234(00)00117-3

Huang LJ, Hour MJ, Teng CM, Kuo SC. Synthesis and antiplatelat activities of N-arylmethyl-3,4-dimethylpyrano [2,3-c]pyrazole-6-one derivatives. Chem Pharm Bull (Tokyo). 1992; 40: 2547-51.

Ikeda M, Maruyama K, Nobuhara Y, Yamada T, Okabe S. Synthesis and cytoprotective antiulcer activity of 2- or 4-(1H-pyrazol-1-yl)pyrimidine derivatives related to mepirizole and dulcerozine. Chem Pharm Bull (Tokyo). 1996; 44: 1700-06.

Islam MR, Khayer KK, Ahmad MM. Synthesis of 3-methylpyrazolin-5-one and its 3,3-dimethyl-5,5-ethene-bis-2-pyrazolin-4,4-dione, substituted cyclic tri-azathione and N-acetylated derivatives. J Bangladesh Chem Soc. 2001; 14: 33-39.

Jiang JB, Hesson DP, Dusak BA, Dexter DL, Kang GI, Hamel E. Synthesis and biological evaluation of 2-styrylquinazolin-4 (3H)-ones, a new class of antimitotic anti-cancer agents which inhibit tubulin polymerization. J Med Chem. 1990; 33: 1721-28. http://dx.doi.org/10.1021/jm00168a029

Kativar SB, Srivastava K, Puri SK, Chauhan PM. Synthesis of 2-[3,5-substituted pyrazole-1-yl]-4,6-trisubstituted triazine derivatives as antimalarial agents. Bioorg Med Chem Lett. 2005; 15: 4957-60. http://dx.doi.org/10.1016/j.bmcl.2005.08.023

Kuettel S, Zambon A, Kaiser M, Brun R, Scapozza L, Perozzo R. Synthesis and evaluation of antiparasitic activities of new 4-[5-(4-phenoxyphenyl)-2H-pyrazol-3-yl]morpholine derivatives. J Med Chem. 2007; 50: 5833-39. http://dx.doi.org/10.1021/jm700938n

Li J, DeMello KM, Cheng H, Sakya SM, Bronk BS, Rafka RJ, Jaynes BH, Ziegler CB, Kilroy C, Mann DW, Nimz EL, Lynch MP, Haven ML, Kolosko NL, Minich ML, Li C, Dutra JK, Rast B, Crosson RM, Morton BJ, Kirk GW, Callaghan KM, Koss DA, Shavnya A, Lund LA, Seibel SB, Silvia A. Discovery of a potent, selective and orally active canine COX -2 inhibitor, 2-(3-difluoromethyl-5-phenyl-pyrazol-1-yl)-5-methane-sulfonyl-pyridine. Bioorg Med Chem Lett. 2004; 14: 95-98. http://dx.doi.org/10.1016/j.bmcl.2003.10.004

Manna F, Chimenti F, Bolasco A, Amico MD, Parrillo C, Rossi F, Marmo E. Anti-inflammatory, analgesic and antipyretic N -acetyl-Δ2-pyrazolines and dihydro-thienocoumarines. Eur J

Med Chem. 1992; 27: 633-39. http://dx.doi.org/10.1016/0223-5234(92)90142-N

Parmar VS, Kumar A, Prasad AK, Singh SK, Kumar N, Mukherjee S, Raj GH, Goel S, Errington W, Puar MS. Synthesis of E- and Z-pyrazolylacrylonitriles and their evaluation as novel antioxidants. Bioorg Med Chem. 1999; 7: 1425-36. http://dx.doi.org/10.1016/S0968-0896(99)00056-5

Regan J, Capolino A, Cirillo PF, Gilmore T, Graham AG, Hickey E, Kroe RR, Madwed J, Moriak M, Nelson R, Pargellis CA, Swinamer A, Torcellini C, Tsang M, Moss N. Structure activity relationships of the p38alpha MAP kinase inhibitor 1-(5-tert-butyl-2-p-tolyl-2H-pyrazol-3yl)-3-[4-(2-morpholin-4-yl-ethoxy) maphthalen-1-yl]urea (BIRB 796). J Med Chem. 2003; 46: 4676-86. http://dx.doi.org/10.1021/jm030121k

Satyanarayana K, Rao MNA. Synthesis of 4-[5-(substituted aryl)-4,5-dihydro-1H-pyrazol-3-yl]-3-phenyl-sydnones as anti-inflammatory, antiarthritic and analgesic agents. Eur J Med Chem. 1995; 30: 641-45. http://dx.doi.org/10.1016/0223-5234(96)88280-8

Solis PN, Wright CW, Anderson MM, Gupta MP, Philipson JD. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). Planta Med. 1993; 59: 250-52. http://dx.doi.org/10.1055/s-2006-959661

Author Info
Md. Rabiul Islam (Principal contact)
e-mail: rabiulju@gmail.com