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SYNTHESIS AND SCREENING OF ANALGESIC ACTIVITY OF SOME NOVEL PYRAZOLE

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ABSTRACT: The present study was aimed to study the analgesic potency of some new pyrazole compounds. Various Quinazolinone clubbed Pyrazole derivative compounds (**7a₁**, **7a₂**, **7b₁**, **7b₂**) were synthesized by using anthranilic acid as starting material as per standard chemical procedure. The synthesized compounds were characterized by physical and spectral analysis by using UV-visible spectrophotometer, Fourier transform infra red (FTIR) Spectrophotometer, ¹H Nuclear Magnetic Radiation spectrometer and Gas Chromatography Mass Spectrophotometer.-QC-2010. Moreover Pyrazole derivatives have found their clinical application as analgesic agent. In this study the synthesized novel Pyrazole derivatives were screened for in-vivo analgesic properties by tail-flick method. The result obtained is compare with standard diclofenac. It has been observed that the maximum analgesic activity was recorded by the compound **7a₁** having 359 % analgesic activity followed by compound **7b₁** (268 %), **7b₂** (204 %) & **7a₂** (159%) analgesic potential. The compounds **7a₁** showed nearly similar percentage analgesic activity compared to standard drug Diclofenac sodium, whereas **7b₁**, **7b₂** & **7a₂** registered percentage analgesic activity relatively less than standard drug. The compound **7a₁** possess maximum analgesic potency.

INTRODUCTION: International Association for the Study of Pain (IASP) has defined pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It can be defined as characteristic neuro-physiological sensation which arises from noxious stimuli. Analgesics are the agents which relieve or decrease pain sensation by increasing threshold to painful stimuli without causing loss of consciousness.

The cause of the pain may be physiologic, inflammation and neuropathic. Pain can be classified into two types: Integumental pain which is superficial and related to skin muscle and joints and visceral pain which is deep seated and related to heart, kidney, stomach, gall bladder etc.

Analgesics are the drugs which decrease the pain sensation. There are 2 types of analgesic agents: Opioid analgesics and Non steroidal anti-inflammatory agents (NSAIDs). Opioid analgesics are mainly used to treat the visceral pain. They cause respiratory depression, CNS depression, drug dependence. But opioid analgesics lack anti-inflammatory, antipyretic or uricosuric action. NSAIDs on the other hand are mainly used to treat integumental pain.

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The main physiological peripheral receptors are sensitized by pro-inflammatory autocooids like prostaglandin, 5-HT, histamine, bradykinin, interleukin etc. These drugs are most effective against pain associated with inflammation.

When a tissue is injured, prostaglandin synthesis increases in that tissue. The prostaglandins have 2 major actions: They are the mediators of inflammation as well as they sensitize the pain receptors at the nerve endings by lowering the threshold of response to painful stimuli. Moreover allows the other mediators (histamine, bradykinin, 5-HT etc.) which cause inflammation to intensify activation of the sensory neurons. Thus, a drug that prevents synthesis of prostaglandins will be effective in treating pain due to inflammation. The mechanism of action involves the inhibition of cyclooxygenases enzymes in the arachidonic acid cascade for synthesis of prostaglandins.

Pyrazoles are five member ring heterocyclic compounds, have some structural features with two nitrogen atoms in adjacent position and are also called as Azoles¹. Recently Pyrazole derivatives have been found in nature¹, β - [1-pyrazolyl] alanine was isolated from the seeds of water melons [Citrullus lanatus]. The best described property of almost every group of pyrazoles is in the treatment of inflammation and inflammation associated disorders, such as arthritis². Pyrazole derivatives are the subject of many research studies due to their widespread potential biological activities such as antimicrobial³, antiviral⁴, antitumor^{5, 6}, antihistaminic⁷, antidepressant⁸, insecticides⁹ and fungicides⁹.

Several pyrazole derivatives have been found to possess significant activities such as 5- α -red-uctase inhibitor¹⁰, antiproliferative¹¹, antiparasitic¹², herbicides¹³. A good number of pyrazoles have also been reported to have interesting biological activities like anti-inflammatory¹⁴ and antiprotozoal¹⁵⁻¹⁶ which render them valuable active ingredients of medicine and plant protecting agents. Further, current literature indicates 1, 2-pyrazole derivatives to possess various biological activities¹⁷. Quinazolinone are classes of fused heterocycles that are of considerable interest because of the diverse range of their biological

properties, for example, anticancer, diuretic, anti-inflammatory, anticonvulsant and antihypertensive activities¹⁸.

The survey of literature reveals the analgesic activity possessed by most of the Pyrazole and Quinazolinone derivatives. Therefore, we think it is worthful to evaluate the analgesic activity of all the synthesized compounds. The analgesic activity of the synthesized compounds was evaluated by tail-flick model.

MATERIALS AND METHODS: Melting points were determined on an electro thermal apparatus using open capillaries and are uncorrected. Thin-layer chromatography was accomplished on 0.2-mm precoated plates of silica gel G60 F254 (Merck). Visualization was made with UV light (254 and 365nm) or with an iodine vapor. IR spectra were recorded on a Shimadzu-Fourier transform infra-red (FTIR)-8400 Spectrophotometer using KBr disc. ¹H NMR spectra were recorded on a Bruker DPX-400 MHz spectrometer. Chemical shifts are expressed in δ ppm downfield from TMS as an internal standard. The mass spectral data were obtained with a SHIMADZU-GCMS-QC-2010.

Experimental Procedure:

Synthesis of 3, 5 dibromo Anthranilic Acid (1): 20gm of anthranilic acid dissolved in 25 c.c. bromine in glacial acetic acid(9 cc bromine in 25 cc glacial acetic acid) was added drop by drop from separating funnel till the reddish color of the liquid persist. Then, content was converted to a thick mass. So it will form dibromoanthranilic acid. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under *vacuo* when the reaction was completed. The solid was crystallized from methanol to give pure product (2). Their melting points, yields and molecular formula are given in **Table 1**.

Yield: 79%; mp 202-204 °C; IR (cm⁻¹): 3312 (N-H stretching of primary amine), 3066 (C-H stretching of aromatic ring), 1726, 1675 (C=O stretching of carboxylic acid), 1604 (N-H deformation of NH₂ group), 1560 and 1432 (C=C stretching of aromatic ring), 1004 (C-H in plane bending for aromatic

ring); 695(C-Br); ¹H NMR (CDCl₃) δ ppm: 7.03-7.58 (m, 2H, ArH), 5.38 (s, 1H, NH), 9.94 (s, 1H, COOH); MS: *m/z* 293,275,213, 189, 174, 134; Anal. Calcd. For C₇H₅Br₂NO₂: C, 28.51; H, 1.71; N, 4.75. Found: C, 28.80; H, 1.64; N, 4.77%.

Synthesis of 6, 8-dibromo-2-methyl-4H-3, 1-benzoxazin-4-one (2): A mixture of 3, 5 di Bromo Anthranilic Acid (1,0.01 mol) and acetic anhydride (10.2 ml(0.1 mol) was refluxed on gentle flame for 1 hr. The excess of acetic anhydride was distilled off under reduce pressure and the residue was dissolve in petroleum ether and kept a side for 1 hr. The reaction was monitored by TLC. After completion of reaction, the solvent was removed under *vacuo* when the reaction was completed. The solid was crystallized from ethanol to give pure product (2). Their melting points, yields and molecular formula are given in Table 1.

Yield: 71%; mp 179-181 °C; IR (cm⁻¹): 3076,3000 (C-H stretching of aromatic ring), 1689 (C=O stretching of *benzoxazin-4-one* ring), 1639 (C=N stretching of pyridine ring), 1495 (C=C stretching of aromatic ring), 1127 (COC stretching of *benzoxazin-4-one* ring), 933 (C-H in plane bending for aromatic ring); 684(C-Br stretching of aromatic ring); ¹H NMR (CDCl₃) δ ppm: 1.87 (s, 3H, CH₃), 7.397.89(m,ArH); MS: *m/z*317, 289, 276, 237, 213, 158, 105; Anal. Calcd. for C₉H₅Br₂NO₂: C, 33.89; H, 1.58; N, 4.39. Found: C, 33.91; H, 1.54; N, 4.42%.

Synthesis of 3-amino-6, 8-dibromo-2-methylquinazolin-4(3H)-one (3): A 100mL round-bottom flask equipped with condenser and septum was charged with a solution of 6,8-dibromo-2-methyl-4H-3,1-benzoxazin-4-one (2, 0.01 mmol) in ethyl alcohol (30 mL), followed by the hydrazine hydrate (0.03 mmol) was added and the mixture was reflux for 2 hr. The reaction was monitored by TLC, after complete the reaction mixture was then cooled down to room temperature, poured into crushed ice. When precipitated, the product is filtered, washed with water, and purified by recrystallization from ethanol to give pure product. Their melting points, yields and molecular formula are given in Table 1.

Yield: 69%; mp 138-140 °C; IR (cm⁻¹):3361(N-H stretching of primary amine), 3063(C-H stretching of aromatic ring),2914(CH₃ Str.),1682 (C=O stretching of ring), 1599(C=N stretching of pyridine ring), 1451 (C=C stretching of aromatic ring), 880(C-H in plane bending for aromatic ring); 688(C-Br stretching of aromatic ring); ¹H NMR (CDCl₃) δ ppm: 1.84 (s, 3H, CH₃), 4.73 (s,2H,NH₂),7.408.07(m,ArH);MS:*m/z*331,314,303, 290,251,227,211,172,103; Anal. Calcd. for C₉H₇Br₂N₃O: C, 32.46; H, 2.12; N, 12.62. Found: C, 32.49; H, 2.09; N, 12.58%.

Synthesis of N-(6, 8-dibromo-2-methyl-4-oxoquinazolin-3(4H)-yl) acetamide (4): A 100mL round-bottom flask equipped with condenser and septum was charged with a solution of 3-amino-6, 8-dibromo-2-methylquinazolin-4(3H)-one (3, 0.01 mmol) in Glacial acetic acid (30 mL), followed by the acetic anhydride (0.03 mmol) was added and the mixture was reflux for 1 hr. The reaction was monitored by TLC, after complete the reaction mixture was then cooled down to room temperature, poured into crushed ice. When precipitated, the product is filtered, washed with water, and purified by recrystallization from ethanol to give pure product. Their melting points, yields and molecular formula are given in Table 1.

Yield: 74%; mp 126-128 °C; IR (cm⁻¹):3366(N-H stretching of primary amine), 3082(C-H stretching of aromatic ring), 2870(CH₃ Str.), 1703(C=O stretching of *Acetyl group*), 1673(C=O stretching of ring), 1609(C=N stretching of pyridine ring), 1462 (C=C stretching of aromatic ring), 900(C-H in plane bending for aromatic ring); 689(C-Br stretching of aromatic ring); ¹H NMR (CDCl₃) δ ppm:1.83(s,3H,CH₃),2.50(s,3H,CH₃),8.94(s,1H,NH),7.408.17(m,ArH);MS:*m/z*373,345,331,314,293,278,269,190,103; Anal. Calcd. for C₁₁H₉Br₂N₃O₂: C35.23; H, 2.42; N, 11.20. Found: C 35.19; H, 2.44; N, 11.24%.

Synthesis of N-(6, 8-dibromo-4-oxoquinazolin-3(4H)-yl)-3-(2-hydroxyphenyl) prop-2-enamide (5a): A mixture of N-(6, 8-dibromo-2-methyl-4-oxoquinazolin-3(4H)-yl) acetamide (4,0.01 mol) was dissolved in ethanol. Then sodium hydroxide(5 ml,30%) solution and salisaldehyde(0.01 mol) were added to the resulting solution with continuous

stirring for 10 hr then solution so obtained refluxed on gentle flame for 6 hr.

The reaction was monitored by TLC. After complete the reaction mixture was then cooled down to room temperature, poured into crushed ice. When precipitated, the product is filtered, washed with water. The solid was crystallized from ethanol to give pure product (5a). 5b were prepared similarly. Their melting points, yields and molecular formula are given in Table 1.

Yield: 70%; mp 152-154 °C; IR (cm⁻¹): 3471(O-H stretching of Hydroxy group), 3356(N-H stretching of primary amine), 3076(C-H stretching of aromatic ring), 2915(CH₃ Str.), 1730(C=O stretching of keto group), 1673 (C=O stretching of ring), 1607(C=N stretching of pyridine ring), 1449 (C=C stretching of aromatic ring), 876(C-H in plane bending for aromatic ring); 687(C-Br stretching of aromatic ring); ¹H NMR (CDCl₃) δ ppm: 1.84 (s, 3H, CH₃), 10.83 (s, 1H, OH), 9.01 (s, 1H, NH), 6.210-6.216(d, 1H, CH), 7.19-7.25 (d, 1H, CH), 7.418.10(m, ArH); MS: *m/z* 477, 459, 449, 397, 331, 316, 314, 163, 161, 103; Anal. Calcd. for C₁₈H₁₃Br₂N₃O₃: C, 45.12; H, 2.73; N, 8.77. Found: C, 45.16; H, 2.69; N, 8.81%.

Synthesis of N- (6, 8-dibromo-4-oxoquinazolin-3(4H)-yl)-3-(2-hydroxyphenyl) prop-2-enamide (5b): A mixture of N-(6, 8-dibromo-2-methyl-4-oxoquinazolin-3(4H)-yl) acetamide (4,0.01 mol) was dissolved in ethanol. Then sodium hydroxide(5 ml, 30%) solution and benzaldehyde (0.01 mol) were added to the resulting solution with continuous stirring for 10 hr then solution so obtained refluxed on gentle flame for 6 hr. The reaction was monitored by TLC. After complete the reaction mixture was then cooled down to room temperature, poured into crushed ice. When precipitated, the product is filtered, washed with water. The solid was crystallized from ethanol to give pure product (5a). Their melting points, yields and molecular formula are given in Table 1.

Yield: 73%; mp 151-153 °C; IR (cm⁻¹): 3377(N-H stretching of primary amine), 3076(C-H stretching of aromatic ring), 2932(CH₃ Str.), 1727(C=O stretching of keto group), 1670 (C=O stretching of ring), 1616(C=N stretching of pyridine ring),

1485, 1460 (C=C stretching of aromatic ring), 924(C-H in plane bending for aromatic ring); 659(C-Br stretching of aromatic ring); ¹H NMR (CDCl₃) δ ppm: 1.80(s, 3H, CH₃), 9.73(s, 1H, NH), 6.2106.216(d, 1H, CH), 7.197.27(d, 1H, CH), 7.408.57(m, ArH); MS: *m/z* 461, 433, 381, 331, 314, 235, 147, 103; Anal. Calcd. for C₁₈H₁₃Br₂N₃O₂: C, 46.68; H, 2.83; N, 9.07. Found: C, 46.66; H, 2.81; N, 9.10%.

Synthesis of 6, 8-dibromo-3-[[5-(2-hydroxyphenyl)-4, 5-dihydro-1H-pyrazol-3-yl] amino] quinazolin-4(3H)-one (6a): A 100mL round-bottom flask equipped with condenser and septum was charged with a solution of N- (6, 8-dibromo-4-oxoquinazolin-3(4H)-yl)-3-(2-hydroxyphenyl) prop-2-enamide (5a, 0.01 mol) in Glacial acetic acid (30 mL), followed by the Hydrazine hydrate (0.01 mol) was added and the mixture was reflux for 4 hr. The reaction was monitored by TLC, after complete the reaction mixture was then cooled down to room temperature, poured into crushed ice. When precipitated, the product is filtered, washed with water, and purified by recrystallization from ethanol to give pure product. Their melting points, yields and molecular formula are given in Table 1.

Yield: 69%; mp 234-236 °C; IR (cm⁻¹): 3472(O-H stretching of Hydroxy group), 3354(N-H stretching of primary amine), 3074(C-H stretching of aromatic ring), 2901(CH₃ Str.), 1733(C=O stretching of keto group), 1671 (C=O stretching of ring), 1608(C=N stretching of pyridine ring), 1449 (C=C stretching of aromatic ring), 875(C-H in plane bending for aromatic ring); 688(C-Br stretching of aromatic ring); ¹H NMR (CDCl₃) δ ppm: 1.84 (s, 3H, CH₃), 10.95 (s, 1H, OH), 9.19(s, 1H, NH), 4.50(s, 1H, NH), 6.216.91(t, 1H, CH), 7.197.29(d, 1H, CH), 7.408.27(m, ArH); MS: *m/z* 491, 473, 463, 411, 397, 331, 178, 160, 135, 120, 103; Anal. Calc d. for C₁₈H₁₅Br₂N₅O₂: C, 43.84; H, 3.07; N, 14.20. Found: C, 43.88; H, 3.10; N, 14.22%.

Synthesis of 6, 8-dibromo-3-[[5-(phenyl)-4, 5-dihydro-1H-pyrazol-3-yl] amino] quinazolin-4(3H)-one (6b): A 100mL round-bottom flask equipped with condenser and septum was charged with a solution of N- (6, 8-dibromo-4-oxoquinazolin-3(4H)-yl)-3-(phenyl) prop-2-enamide (5a, 0.01 mol) in Glacial acetic acid (30 mL), followed by the Hydrazine hydrate (0.01 mol)

was added and the mixture was reflux for 4 hr. The reaction was monitored by TLC, after complete the reaction mixture was then cooled down to room temperature, poured into crushed ice. When precipitated, the product is filtered, washed with water, and purified by recrystallization from ethanol to give pure product. Their melting points, yields and molecular formula are given in Table 1.

Yield: 78%; mp 230-231 °C; IR (cm⁻¹): 3322(N-H stretching of primary amine), 3079(C-H stretching of aromatic ring), 2932(CH₃ Str.), 1727(C=O stretching of keto group), 1672 (C=O stretching of ring), 1616(C=N stretching of pyridine ring), 1485,1460 (C=C stretching of aromatic ring), 924(C-H in plane bending for aromatic ring); 650(C-Br stretching of aromatic ring); 1H NMR (CDCl₃)δppm: 1.84(s,3H,CH₃), 9.00(s,1H,NH), 6.20-6.22(d,1H,CH), 7.167.25(d,1H,CH), 7.428.07(m,Ar H); MS:m/z 475,447,395,371,331,316,161,159,144,119,104; Anal.Calcd. for C₁₈H₁₅Br₂N₅O: C, 45.31; H, 3.17; N, 14.68. Found: C, 45.37; H, 3.20; N, 14.71%.

Synthesis of 6, 8-dibromo-3-([5-(2-hydroxyphenyl) - 1- [(4-nitro Phenylamino) methyl]-4, 5-dihydro-1H-pyrazol-3-yl] amino) quinazolin-4(3H)-one (7a₁): To a mixture of 6,8-dibromo-3-[[5-(2-hydroxyphenyl) 4,5dihydro 1 Hpyrazol3yl] amino}quinazolin-4(3H)-one (6a) (0.005 mol) , 37% formalin (1 mL) and few drop of Acetic acid in ethanol (20 mL) was added drop wise appropriate p-nitro aniline (0.005 mol) with stirring over 15 min. The stirring was continued for 1h at room temperature and the reaction mixture then warmed for 15 min on a water bath. The mixture was poured into ice-cold water and stored in a refrigerator for 24 hr. The solid thus separated was filtered, washed with water, dried and recrystallized from appropriate solvent. Their melting points, yields and molecular formula are given in Table 1.

Yield: 81%; mp 170-172 °C; IR (cm⁻¹): 3476(O-H stretching of Hydroxy group) ,3350(N-H stretching of primary amine), 3076(C-H stretching of aromatic ring), 2918(CH₃ Str.), 1732(C=O stretching of keto group), 1670 (C=O stretching ring), 1609(C=N stretching of pyridine ring), 1449 (C=C stretching of aromatic ring), 879(C-H in

plane bending for aromatic ring); 685(C-Br stretching of aromatic ring); 1H NMR (CDCl₃) δ ppm: 1.84(s,3H,CH₃), 9.72(s,1H,NH), 4.47(s,1H,NH) 6.576.91(t,1H,CH), 7.167.26(d,1H,CH), 7.408.11(m, ArH); ¹³CNMR(400MHz,CDCl₃): 19.10,32.40,33.29 ,36.24,37.45,40.32,41.43,43.05,46.54,48.15,50.28,51.44,70.12,101.12,101.15,104.76,105.25,123.25,129.23,144.12,154.66. MS:m/z 641,623,613,561,520,327,285,150,121,104; Anal.Calcd. for C₂₅H₂₁Br₂N₇O₄: C, 46.48; H, 3.29; N, 15.24. Found: C, 46.46; H, 3.32; N, 15.29%.

Synthesis of 6, 8-dibromo-3-([5-(2-hydroxyphenyl)-1-[(4-chloroPhenylamino)methyl]-4, 5-dihydro-1H-pyrazol-3-yl] amino) quinazolin-4(3H)-one (7a₂): To a mixture of 6,8-dibromo-3-[[5-(2-hydroxyphenyl) 4,5dihydro 1Hpyrazol 3yl]amino} quinazolin-4(3H)-one (6a) (0.005 mol) , 37% formalin (1 mL) and few drop of Acetic acid in ethanol (20 mL) was added drop wise appropriate p-chloro aniline (0.005 mol) with stirring over 15 min. The stirring was continued for 1h at room temperature and the reaction mixture then warmed for 15 min on a water bath. The mixture was poured into ice-cold water and stored in a refrigerator for 24 hr. The solid thus separated was filtered, washed with water, dried and recrystallized from appropriate solvent. Their melting points, yields and molecular formula are given in Table 1.

Yield: 79%; mp 168-169 °C; IR (cm⁻¹): 3475(O-H stretching of Hydroxy group) ,3354(N-H stretching of primary amine), 3078(C-H stretching of aromatic ring), 2815(CH₃ Str.), 1734(C=O stretching of keto group), 1672 (C=O stretching ring), 1617(C=N stretching of pyridine ring), 1449 (C=C stretching of aromatic ring), 877(C-H in plane bending for aromatic ring); 686(C-Br stretching of aromatic ring); 1H NMR (CDCl₃) δ ppm: 1.88(s,3H,CH₃), 9.84(s,1H,NH), 5.11(s,1H,NH), 3.123.16(d,1H,CH), 3.803.91(t,1H,CH), 7.408.12 (ArH); ¹³CNMR(400MHz,CDCl₃): 19.40,32.40,33.99 ,36.24,37.78,39.86,40.41,41.43,42.50,43.05,46.51, 47.55,48.43,50.28,51.84,72.12,101.11,101.26,103.20,105.25,123.25,129.23,144.12,156.99 MS:m/z 630, 612, 604, 550, 520, 491, 316, 299, 274, 139,127,104; Anal.Calcd. for C₂₅H₂₁Br₂N₆O₂: C, 47.46; H, 3.35; N, 13.28. Found: C, 47.51; H, 3.38; N, 13.31%.

Synthesis of 6,8-dibromo-3-({5-(phenyl)-1-[(4-nitro Phenylamino)methyl]-4,5-dihydro-1H-pyrazol-3-yl}amino)quinazolin-4(3H)-one (7b₁):

To a mixture of 6,8-dibromo-3-[[5-(2-hydroxy phenyl)4,5dihydro1Hpyrazol3yl]amino}quinazolin-4(3H)-one (6a) (0.005 mol), 37% formalin (1 mL) and few drop of Acetic acid in ethanol (20 mL) was added drop wise appropriate p-nitro aniline (0.005 mol) with stirring over 15 min. The stirring was continued for 1h at room temperature and the reaction mixture then warmed for 15 min on a water bath. The mixture was poured into ice-cold water and stored in a refrigerator for 24 hr. The solid thus separated was filtered, washed with water, dried and recrystallized from appropriate solvent. Their melting points, yields and molecular formula are given in Table 1.

Yield: 76%; mp 164-166 °C; IR (cm⁻¹):3323(N-H stretching of primary amine), 3076(C-H stretching of aromatic ring), 2811(CH₃ Str.), 1724(C=O stretching of keto group),1672 (C=O stretching), 1611(C=N stretching of pyridine ring), 1471(C=C stretching of aromatic ring), 917(C-H in plane bending for aromatic ring); 687(C-Br stretching of aromatic ring); 1H NMR (CDCl₃) δ ppm: 1.89 (s, 3H,CH₃),9.81(s,1H,NH),5.10(s,1H,NH)3.863.96(t, 1H,CH),3.113.16(d,1H,CH),7.418.12(m,ArH);¹³CNMR(400MHz, CDCl₃): 18.18, 32.40, 33.99, 36.04, 37.05, 39.66,40.41,41.05, 42.12, 46.51, 47.51, 48.72, 49.51, 72.03, 100.01-100.07, 103.70, 104.76, 105.75, 123.95, 129.23, 144.02, 145.02. MS: m/z 625, 608, 597, 545, 331, 309, 294, 150, 138, 121, 104; Anal.Calcd. For C₂₅H₂₁Br₂C₁N₇O₃: C, 47.87; H, 3.37; N, 15.63. Found: C, 47.84; H, 3.41; N, 15.83%.

Synthesis of 6,8-dibromo-3-({5-(phenyl)-1-[(4-chloro-Phenylamino)methyl]-4,5-dihydro-1 H-pyrazol-3-yl}amino)quinazolin-4(3H)-one (7b₂):

To a mixture of 6,8-dibromo-3-[[5-(phenyl)-4,5-dihydro-1H-pyrazol-3-yl]amino}quinazolin-4(3H)-one (6b₁) (0.005 mol) , 37% formalin (1 mL) and few drop of Acetic acid in ethanol (20 mL) was added drop wise appropriate p-chloro aniline (0.005 mol) with stirring over 15 min. The stirring was continued for 1h at room temperature and the reaction mixture then warmed for 15 min on a

water bath. The mixture was poured into ice-cold water and stored in a refrigerator for 24 hr.

The solid thus separated was filtered, washed with water, dried and recrystallized from appropriate solvent. Their melting points, yields and molecular formula are given in Table 1.

Yield: 78%; mp 162-163 °C; IR (cm⁻¹): 3364(N-H stretching of primary amine), 3028(C-H stretching of aromatic ring), 2928(CH₃ Str.), 1665(C=O stretching of keto group), 1586(C=N stretching of pyridine ring), 1466,1451 (C=C stretching of aromatic ring), 909(C-H in plane bending for aromatic ring); 751(C-Cl stretching of aromatic ring), 654(C-Br stretching of aromatic ring); 1H NMR (CDCl₃) δ ppm: 1.91 (s, 3H, CH₃), 9.89(s,1H,NH), 5.09(s,1H, NH) 3.813.90(t,1H,CH), 3.103.14(d,1H,CH),7.438.19(m,ArH);¹³CNMR(400 MHz,CDCl₃):18.44,32.40,33.99,36.04,37.78,39.66, 40.41,41.03,42.12,46.05,47.55,48.43,49.47,74.44, 100.00,100.17,101.11,103.07,104.76,105.25,129.13 ,144.12. MS: m/z 614,586,534,283,139,127,104; Anal. Calcd. for C₂₅H₂₁Br₂C₁N₆O: C, 48.69; H, 3.43; N, 13.63. Found: C, 48.37; H, 3.49; N, 13.59%.

General Procedures for Synthesis: Given in scheme 1.

Pharmacological evaluation:

Acute toxicity study: Healthy and adult Swiss albino mice weighing between 20- 25 gms were used in the present investigation. The animals were fasted for 24 hrs with *water ad libitum* and divided into groups of 4 animals each. The test compounds i.e. **7a1**, **7a2**, **7b1**, **7b2** suspended in tween-40 and normal saline were administered intraperitoneally in doses of 25mg/kg to 500 mg/kg b.w. The control group of animals received only the vehicle (Tween-40 + normal saline). The animals were observed for 48hrs from the time of administration of test compounds to record the mortality¹⁹.

As no mortality was observed with the administered dose, a dose 50 mg/Kg b.w. was selected for the further pharmacological evaluation.

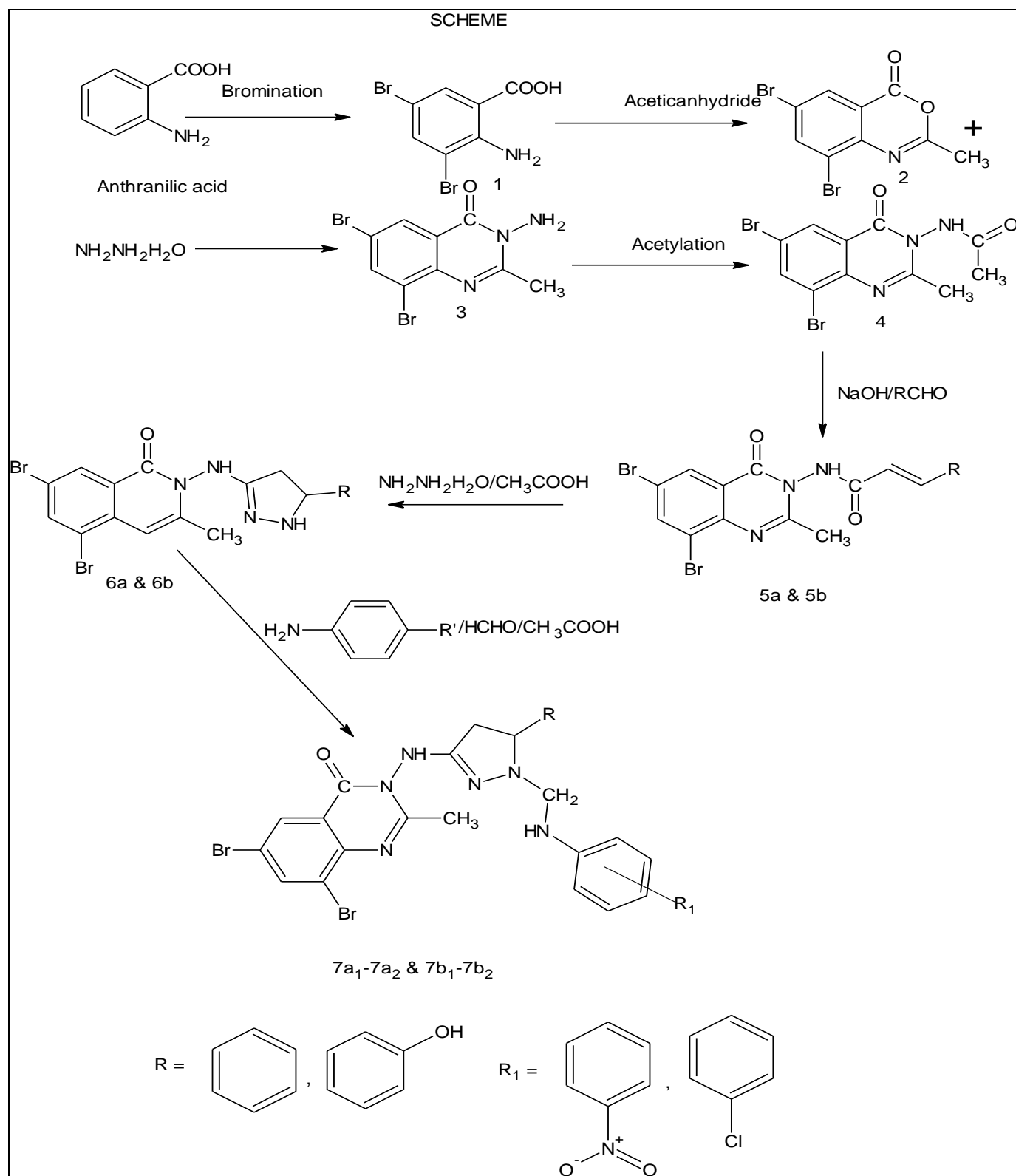
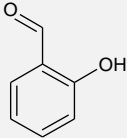
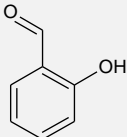
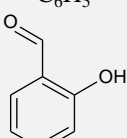
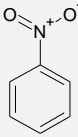
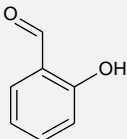
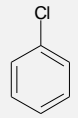
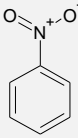
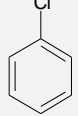
**SCHEME 1: SYNTHESIS OF 7A₁, 7A₂, 7B₁ AND 7B₂**

TABLE 1: PHYSICAL DATA OF SYNTHESIZED COMPOUNDS

Comp. No.	R	R ¹	Mol. Formula	Colour	MP (°C)	Yield (%)
1	-----	-----	C ₇ H ₅ Br ₂ NO ₂	Reddish brown	202-204	73
2	-----	-----	C ₉ H ₅ Br ₂ NO ₂	Orange	179-181	71
3	-----	-----	C ₉ H ₇ Br ₂ N ₃ O	Dirty Yellow	138-140	69
4	-----	-----	C ₉ H ₉ Br ₂ N ₃ O ₂	Pale Yellow	126-128	74
5a		--	C ₁₇ H ₁₁ Br ₂ N ₃ O ₃	Grey	152-154	70
5b	C ₆ H ₅	--	C ₁₇ H ₁₁ Br ₂ N ₃ O ₂	Pale Yellow	151-153	73
6a		--	C ₁₇ H ₁₃ Br ₂ N ₃ O ₂	Brick red	234-236	69
6b	C ₆ H ₅	--	C ₁₇ H ₁₃ Br ₂ N ₃ O	Yellowish brown	230-231	78
7a1			C ₂₄ H ₁₉ Br ₂ N ₇ O ₄	Dirty Yellow	170-172	81
7a2			C ₂₄ H ₁₉ Br ₂ N ₆ O ₂ Cl	Brick red	168-169	79
7b1	C ₆ H ₅		C ₂₄ H ₁₉ Br ₂ N ₇ O ₃	Reddish Yellow	164-166	76
7b2	C ₆ H ₅		C ₂₄ H ₁₉ Br ₂ N ₆ OCl	Brownish Yellow	162-163	78

Analgesic Activity: Test compounds were evaluated for their analgesic activity using male albino rats weighing between 125-150g as the experimental animals. The test compounds were suspended in Tween-40 & normal saline solution and their analgesic activity was determined by Tail-flick method²⁰.

Each of the test compounds (all synthesized compounds (**7a1**, **7a2**, **7b1**, **7b2**) were administrated at a dose of 50mg/kg (b.w.) intraperitoneally. Diclofenac sodium at a dose of 10mg/kg (b.w.) was taken as a standard drug for comparison.

The basal reaction time as noted at 1, 2 & 3 hrs after administration of the synthesized compound by placing the tip of the rats 1-2cm on the radiant heat of analgesimeter, heated at 55°C ± 0.5°C. A cut off time period of 10sec was taken into account to prevent the tissue damage. The percentage analgesic activity was calculated by observing the following formula:

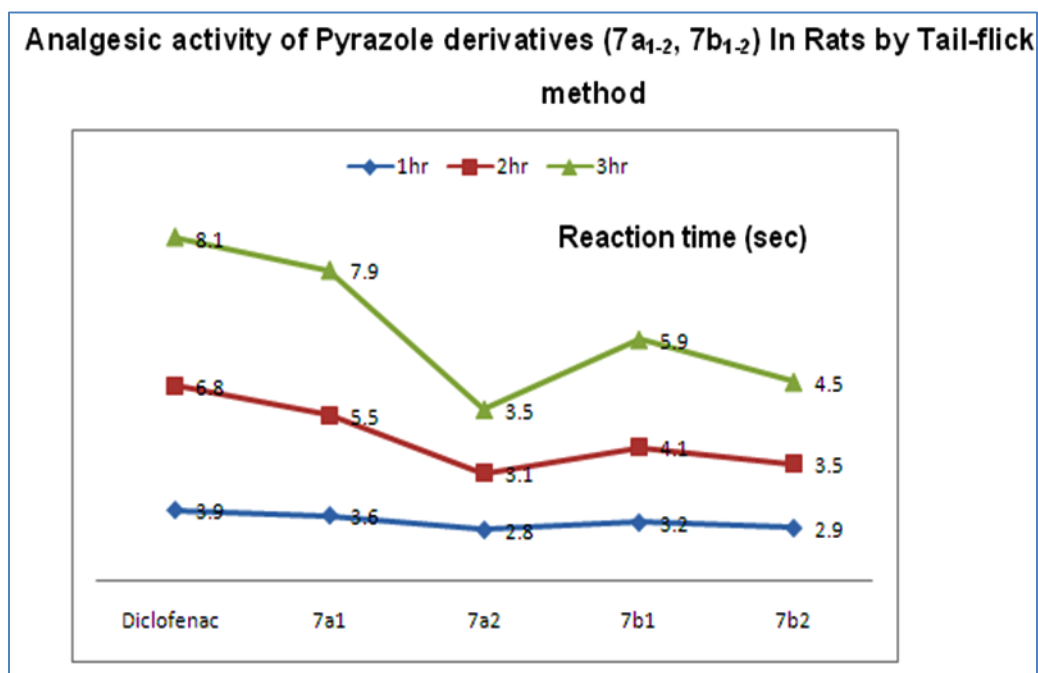
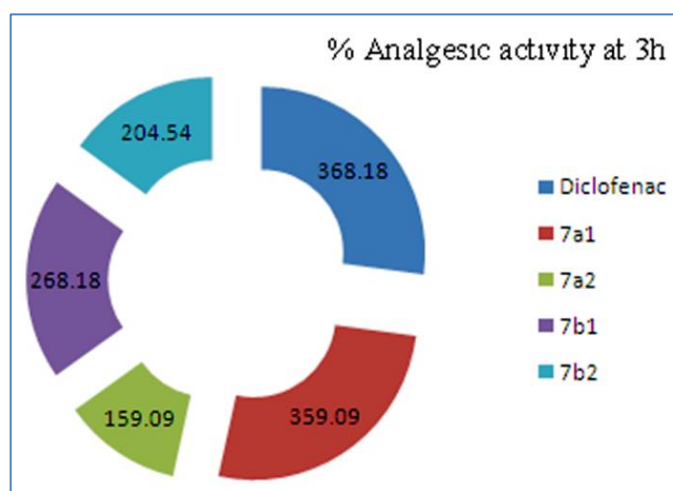
$$\% \text{ analgesic activity} = \frac{\text{Reaction time of test} \times 100}{\text{Reaction time of the solvent}}$$

The results of the analgesic activities are depicted in **table 2**.

TABLE 2: ANALGESIC ACTIVITY OF PYRAZOLE DERIVATIVES (7A₁₋₂, 7B₁₋₂) IN RATS BY TAIL-FLICK METHOD

Sl. No.	Compds	Reaction time (min)			%Analgesic activity at 3h
		1h	2h	3h	
1	Control	2.3±0.11	2.5±0.08	2.2±0.06	-----
2	Diclofenac	3.9±0.17***	6.8±0.12***	8.1±0.08***	368.18
3	7a ₁	3.6±0.04***	5.5±0.06***	7.9±0.15***	359.09
4	7a ₂	2.8±0.08**	3.1±0.13***	3.5±0.11***	159.09
5	7b ₁	3.2±0.11***	4.1±0.14***	5.9±0.09***	268.18
6	7b ₂	2.9±0.13**	3.5±0.23**	4.5±0.08***	204.54

Values expressed as Mean ± SEM, n=6, *p<0.05, **p<0.01, ***p<0.001, Dose of the test compound: 50 mg/kg. (ANOVA followed by Dunnet's t-test).

**FIGURE 2: ANALGESIC ACTIVITY OF COMPOUND 7A₁₋₂ AND 7B₁₋₂****FIGURE 3: % ANALGESIC ACTIVITY OF COMPOUND 7A₁₋₂ AND 7B₁₋₂**

RESULT AND DISCUSSION: The study of analgesic activity by tail flick method showed that the percentage analgesic activity ranges between 159 and 359 at the end of 3 hrs, where as the standard drug Diclofenac sodium registered 368 (Table 2). It has been observed that the maximum analgesic activity was recorded by the compound **7a₁** having 359 % analgesic activity followed by compound **7b₁** (268 %), **7b₂** (204 %) & **7a₂** (159%) analgesic potential.

It has been found that the reaction time increases significantly from p<0.05 to p<0.001 when compared with solvent control. The compounds **7a₁** showed nearly similar percentage analgesic activity compared to standard drug Diclofenac sodium,

where as **7b₁**, **7b₂** & **7a₂** registered percentage analgesic activity relatively less than standard drug. The compound **7a₁** that possess maximum potency are 6, 8-dibromo-3-({5-(2-hydroxyphenyl)-1-[(4-nitroPhenylamino) methyl] - 4, 5-dihydro-1H pyrazol-3yl} amino) quinazolin-4(3H)-one (**7a₁**).

The compounds **7b₁**, **7b₂** & **7a₂** which showed 2nd, 3rd & 4th most potent analgesic activity are 6,8-dibromo3({5(phenyl)1[(4nitroPhenylamino)methyl]-4,5dihydro1Hpyrazol3yl}amino)quinazolin4(3H)-one (**7b₁**) & 6,8-dibromo-3-({5-(phenyl)-1-[(4-chloro-Phenylamino)methyl]4,5dihydro1Hpyrazol-3-yl}amino)quinazolin-4(3H)-one(**7b₂**) respectively.

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