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RESEARCH ARTICLE

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF SPIROQUINAZOLINONES AS ANTI-INFLAMMATORY AND ANALGESIC AGENTS

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Abstract

A series of ten new 6',8'-Disubstituted-5-methyl-3'-substitutedphenyl-2,4-dihydro-1'H-spiro[pyrazole-3,2'-quinazolin]-4'(3'H)-one derivatives have been synthesized by cyclization of substituted anthranilic acid and 5-Methyl-2,4-dihydro-3H-pyrazol-3-one giving corresponding 5'-methyl-2',4'-dihydrospiro [3,1-benzoxazine-2,3'-pyrazol]-4(1*H*)-one followed by amination. The structure of synthesized compounds were established by spectral (FTIR, NMR, and MS) and elemental (C, H, N) analysis. These synthesized compounds were screened for analgesic and anti-inflammatory activities by hot plate method and carrageenan induced rat paw method respectively. The spectral analysis of Spiro compound was found to be IR (KBr, $cm^{-}1$): 3337, 3218 (2NH), 3061 (CH, aromatic), 2936 (CH, alicyclic), and, 1670 (CO). Compound 1, 3, 5, 6, 8 and 9 showed good anti-inflammatory whereas compound 2, 3, 7, and 10 exhibits promising analgesic activity when compared with Indomethacin and Tramadol respectively as a standard.

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Introduction

It is well known that non steroidal anti-inflammatory drugs (NSAIDs) are associated with several side effects such as gastrointestinal mucosal damage, bleeding, intolerance and renal toxicity.[1,2] Production of safer and more active NSAIDs and analgesic drugs is still needed. Quinazoline is one of the most frequently reported heterocyclic compound in medicinal chemistry, which possess diverse biological activities like analgesic and anti-inflammatory,[3] antihypertensive,[4] antimicrobial,[5] antibacterial,[6] anticonvulsant,[7] anticancer,[8] antimalarial[9] and antidepressant[10] activities etc. Additionally, different known anti-inflammatory drugs such as: Proquazone, Fluoroquazone and Diproquazone are bearing quinazolinone nucleus. It has been showed that *2nd* and *4th* position of quinazoline plays a very crucial role in exhibiting anti-inflammatory activity. On the other hand, sulphonamides,[11] imidazoles,[12] pyrazoles[13] are other important pharmacodynamic heterocyclic nuclei which when incorporated into different heterocyclic templates, have been reported to possess potent anti-inflammatory activity. In present investigation, we planned to target these positions for substitution of certain bulkier groups. Based on the importance of these molecules, our attention was attracted towards synthesis of novel quinazoline derivatives with structure modifications involving incorporation of the spiro pyrazole moiety at 2^{nd} position of quinazolinone moiety as a trial to obtain safer and potent anti-inflammatory and analgesic agents.

Material and Methods

The chemicals used in the present work were AR grade and LR grade, purchased from Loba, Merck and Fisher scientific fine chemicals. The synthesized compounds were scaled for yield and purified by recrystallization with suitable solvent system. The purified compounds were assigned for physical constant determination was carried out by open capillary method using LABHOSP melting point apparatus and recorded without correction. The

structures were further confirmed by elemental (CHN) and spectral analysis like IR, NMR and MS.

EXPERIMENTAL

Synthesis of 5-methyl-2, 4-dihydro-3H-pyrazol-3-one.

Ethyl acetoacetate (0.5 mol) was taken in conical flask and hydrazine hydrate (1 mol) in ethanol (40 ml) was added dropwise to it with stirring. The temperature raised during this addition and it was maintained at 60°C when a crystalline solid separated. The reaction-mixture was further stirred for 80 min at room temperature then cooled in an ice bath to complete the crystallization. Separated solid was washed with ice cold ethanol.

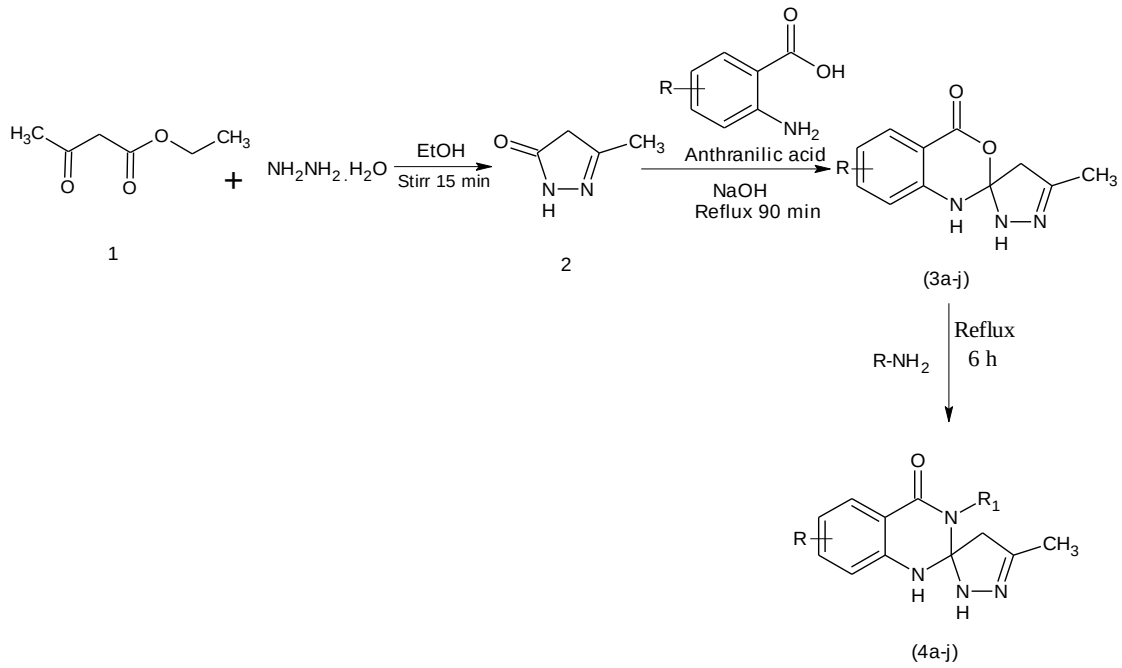
Preparation of mono and disubstituted anthranilic acid.

Anthranilic acid (20g, 0.17mol) was dissolved in acetic acid 250mL. The solution was cooled below 16°C, the bromine (6.5, 0.1mol) in glacial acetic acid (500ml) was run in above solution. The reddish colour persists with thick mass which was filtered, washed with benzene and dried. The mass was boiled with water 500ml containing conc. Hydrochloric acid 25mL. The undissolved precipitate as 3, 5-dibromoanthranilic acid was filtered and recrystallized from methanol. The filtrate was cooled gradually. The precipitate obtained as 5-bromoanthranilic acid was filtered and recrystallized with methanol.

Synthesis of 6',8'-Disubstituted-5-methyl-3'-substitutedphenyl-2,4-dihydro-1'H-spiro[pyrazole-3,2'-quinazolin]-4'(3'H)-one.

An equimolar mixture of benzoxizanone derivatives and substituted anilines was refluxed for 6 h with glacial acetic acid and progress of reaction was monitored by TLC. After completion of reaction, contents were poured onto crushed ice to form solid mass which was collected and recrystallized from ethanol.

Schematic representation of 6',8'-Disubstituted-5-methyl-3'-substitutedphenyl-2,4-dihydro-1'H-spiro[pyrazole-3,2'-quinazolin]-4'(3'H)-one derivatives.



1 2 (3a-j) (4a-j)

SPECTRAL DATA

Synthesis of 6'-Bromo-5-methyl-3'-phenyl-2,4-dihydro-1'H-spiro[pyrazole-3,2'-quinazolin]-4'(3'H)-one (4a).
IR (KBr, cm^{-1}): 3477 (NH), 3066 (CH-aromatic), 2890 (CH-aliphatic), 1680 (CO,quinazolinone), 1320(CN str),534 (Br), **^{1}HNMR (CDCl3) δppm;** 9.77(s, 1H, NH), 2.15(d, 1H, ,N–NH), 8.17(s, 1H, –N–NH), 3.05(d, 1Ha), 3.56(d, 1Hb), 6.47(t, 1Hx),6.47–7.96(m, 7H, Ar–H), **MS (m/z):** 374 (M^{+}+2), 373 (M^{+}).

Synthesis of 6',8'-Dibromo-5-methyl-3'-phenyl-2,4-dihydro-1'H-spiro[pyrazole-3,2'-quinazolin]-4'(3'H)-one (4b)

IR (KBr, cm^{-1}): 3474 (NH), 3059 (CH-aromatic), 2880 (CH-aliphatic), 1675 (CO,quinazolinone), 1330(CN str),549 (Br), **^{1}HNMR (CDCl3) δppm;** 9.78(s, 1H, –NH), 2.19(d, 1H, ,N–NH), 8.21(s, 1H, –N–NH), 3.06(d, 1Ha), 3.46(d, 1Hb), 6.49(t, 1Hx), 6.45–7.91(m, 6H, Ar–H), **MS (m/z):** 454 (M^++2), 453 (M^+).

Synthesis of 6'-Bromo-3'-(4-chlorophenyl)-5-methyl-2,4-dihydro-1'H-spiro[pyrazole-3,2'-quinazolin]-4'(3'H)-one (4c).

IR (KBr, cm^{-1}): 3486 (NH), 3071 (CH-aromatic), 2889 (CH-aliphatic), 1675 (CO,quinazolinone), 1346(CN str),542 (Br), **^{1}HNMR (CDCl3) δppm;** 9.76(s, 1H, –NH), 2.05(d, 1H, ,N–NH), 8.12(s, 1H, –N–NH), 3.08(d, 1Ha), 3.36(d, 1Hb), 6.41(t, 1Hx), 6.45–7.94(m, 6H, Ar–H), **MS (m/z):** 409 (M^++2), 408(M^+).

Synthesis of 6',8'-Dibromo-3'-(4-chlorophenyl)-5-methyl-2,4-dihydro-1'H-spiro[pyrazole-3,2'-quinazolin]-4'(3'H)-one (4d).

IR (KBr, cm^{-1}): 3481 (NH), 3069 (CH-aromatic), 2890 (CH-aliphatic), 1671 (CO,quinazolinone), 1341(CN str),549 (Br), **^{1}HNMR (CDCl3) δppm;** 9.72(s, 1H, –NH), 2.29(d, 1H, ,N–NH), 8.19(s, 1H, –N–NH), 3.12(d, 1Ha), 3.36(d, 1Hb), 6.59(t, 1Hx), 6.35–7.91(m, 6H, Ar–H), **MS (m/z):** 489(M^++2), 488 (M^+).

Synthesis of 6'-Bromo-5-methyl-3'-(4-nitrophenyl)-2,4-dihydro-1'H-spiro[pyrazole-3,2'-quinazolin]-4'(3'H)-one (4e).

IR (KBr, cm^{-1}): 3469 (NH), 3054 (CH-aromatic), 2891 (CH-aliphatic), 1689 (CO,quinazolinone), 1328(CN str),556 (Br), **^{1}HNMR (CDCl3) δppm;** 9.72(s, 1H, –NH), 2.25(d, 1H, ,N–NH), 8.36(s, 1H, –N–NH), 3.14(d, 1Ha), 3.49(d, 1Hb), 6.42(t, 1Hx), 6.04–7.14(m, 6H, Ar–H), **MS (m/z):** 419 (M+), 420 (M+2).

Synthesis of 6',8'-Dibromo-5-methyl-3'-(4-nitrophenyl)-2,4-dihydro-1'H-spiro[pyrazole-3,2'-quinazolin]-4'(3'H)-one (4f).

IR (KBr, cm^{-1}): 3486 (NH), 3071 (CH-aromatic), 2892 (CH-aliphatic), 1676 (CO,quinazolinone), 1343(CN str),552 (Br), **^{1}HNMR (CDCl3) δppm;** 9.02(s, 1H, –NH), 2.25(d, 1H, ,N–NH), 8.29(s, 1H, –N–NH), 3.11(d, 1Ha), 3.08(d, 1Hb), 6.42(t, 1Hx), 6.05–7.83(m, 6H, Ar–H), **MS (m/z):** 500 (M^++2), 499 (M^+).

Synthesis of 6'-bromo-3'-(4-bromophenyl)-5-methyl-2,4-dihydro-1'H-spiro[pyrazole-3,2'-quinazolin]-4'(3'H)-one (4g).

IR (KBr, cm^{-1}): 3472 (NH), 3059 (CH-aromatic), 2891 (CH-aliphatic), 1661 (CO,quinazolinone), 1335(CN str), 589 (Br), **^{1}HNMR (CDCl3) δppm;** 9.56(s, 1H, –NH), 2.08(d, 1H, ,N–NH), 8.01(s, 1H, –N–NH), 2.78(d, 1Ha), 3.24(d, 1Hb), 6.56(t, 1Hx), 6.31–7.94(m, 6H, Ar–H), **MS (m/z):** 453 (M+).

Synthesis of 6',8'-Dibromo-3'-(4-bromophenyl)-5-methyl-2,4-dihydro-1'H-spiro[pyrazole-3,2'-quinazolin]-4'(3'H)-one (4h).

IR (KBr, cm^{-1}): 3477 (NH), 3061 (CH-aromatic), 2886 (CH-aliphatic), 1669 (CO,quinazolinone), 1325(CN str),559 (Br), **^{1}HNMR (CDCl3) δppm;** 9.70(s, 1H, –NH), 2.25(d, 1H, ,N–NH), 7.98(s, 1H, –N–NH), 2.89(d, 1Ha), 3.06(d, 1Hb), 6.48(t, 1Hx), 6.48–7.87(m, 6H, Ar–H), **MS (m/z):** 534 (M^++2), 533 (M+).

Synthesis of 6'-Bromo-5-methyl-3'-(4-methylphenyl)-2,4-dihydro-1'*H*-spiro[pyrazole-3,2'-quinazolin]-4'(3'*H*)-one (4i).

IR (KBr, cm^{-1}): 3495 (NH), 3082 (CH-aromatic), 2879 (CH-aliphatic), 1678 (CO,quinazolinone), 1341(CN str), 592 (Br), **^{1}HNMR (CDCl3) δppm;** 9.12(s, 1H, –NH), 2.05(d, 1H, ,N–NH), 8.71(s, 1H, –N–NH), 3.51(d, 1Ha), 3.08(d, 1Hb), 6.25(t, 1Hx), 6.21–7.87(m, 6H, Ar–H), **MS (m/z):** 388 (M^++2), 387 (M^+).

Synthesis of 6',8'-Dibromo-5-methyl-3'-(4-methylphenyl)-2,4-dihydro-1'*H*-spiro[pyrazole-3,2'-quinazolin]-4'(3'*H*)-one (4j).

IR (KBr, cm^{-1}): 3489 (NH), 3076 (CH-aromatic), 2872 (CH-aliphatic), 1651 (CO,quinazolinone), 1336(CN str),558 (Br), **^{1}HNMR (CDCl3) δppm:** 9.03(s, 1H, –NH), 2.21(d, 1H, ,N–NH), 8.69(s, 1H, –N–NH), 3.31(d, 1Ha), 3.19(d, 1Hb), 6.19(t, 1Hx), 6.31–7.97(m, 6H, Ar–H). **MS (m/z):** 468 (M^++2), 467 (M^+).

Table 1: physicochemical properties of synthesized compounds

Sr No	R	R_1	Molecular formula	M.P (^{0}C)	% yield	Percentage of CHN Calcd			Percentage of CHN Found		
						C	H	N	C	H	N
1	6-Br	H	$C_{17}H_{15}N_4OBr$	119-121	68	52.87	3.37	12.33	52.84	3.32	12.35
2	6,8-Br	H	$C_{17}H_{14}N_4OBr_2$	145-147	71	51.65	3.29	12.05	51.61	3.23	12.01
3	6-Br	Cl	$C_{17}H_{14}N_4OBrCl$	159-161	63	51.65	3.29	12.05	51.60	3.30	12.04
4	6,8-Br	Cl	$C_{17}H_{13}N_4OBr_2Cl$	173-175	65	51.65	3.29	12.05	51.59	3.30	12.05
5	6-Br	NO_2	$C_{17}H_{14}N_5O_3Br$	128-130	67	50.32	3.07	11.74	50.31	3.05	11.69
6	6,8-Br	NO_2	$C_{17}H_{13}N_5O_3Br_2$	136-138	61	50.32	3.07	11.74	50.29	3.10	11.71
7	6-Br	Br	$C_{17}H_{14}N_4OBr_2$	151-153	71	50.32	3.07	11.74	50.30	3.07	11.73
8	6,8-Br	Br	$C_{16}H_{13}N_4OBr_3$	178-182	69	49.59	3.03	13.49	49.52	3.00	13.44
9	6-Br	CH_3	$C_{17}H_{17}N_4OBr$	191-194	73	49.59	3.03	13.49	49.56	3.01	13.46
10	6,8-Br	CH_3	$C_{17}H_{16}N_4OBr_2$	202-204	70	49.59	3.03	13.49	49.54	3.04	13.48

PHARMACOLOGICAL EVALUATION

All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of College, constituted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Government of India.

Registration number and date of registration
648/02/c/CPCSEA/08 date: 30/10/2012

ANTI-INFLAMMATORY ACTIVITY[14]

The anti-inflammatory activity of ten newly synthesized compounds was evaluated by applying carrageenan-induced paw oedema bioassay in rats using indomethacin as a reference standard. Results were expressed as mean ± S.E. Difference between vehicle control and treatment groups were tested using one way ANOVA followed by the least significant difference (L.S.D.). Methods of statistical analysis were done according to Armitage et al.

Antiinflammatory testing

The carrageenan rat paw oedema model of inflammation was used to evaluate the anti-inflammatory properties of the tested compounds. Rats were randomly assigned to treatment groups and sterile carrageenan (100 ml of a 1% solution in saline) was injected sub-planter into right hind paw of the rat. Carrageenan caused visible redness and pronounced swelling that was well developed by 4 h and persisted formore than 48 h. Right hind paws measured with a planimeter before, and at 1, 2, 3 and 4 h after carrageenan injection. Due to water insolubility of the tested compounds, they were dissolved in tween 80(2%) then given orally (10 mg/kg body wt). The control animals were given tween 80 with appropriate volume. The standard drug was Indomethacin (10 mg/kg body wt). Different compounds or Indomethacin were given 1h before carrageenan injection.

ANALGESIC ACTIVITY[14]

The analgesic activity of the above mentioned ten derivatives was also evaluated by applying Hot plate test using tramadol as a standard reference. Results were expressed as mean±S.E. Difference between vehicle control and treatment groups were tested using one way ANOVA followed by the least significant difference (L.S.D.). Methods of statistical analysis were done according to Armitage et al.

Analgesia testing

The hot-plate test was performed on mice (25–30 mg) by using an electronically controlled hot-plate heated to 52°C (±0.1°C), for possible centrally mediated analgesic effect of the drugs. Twelve groups of mice each were given vehicle and/or the different compounds and the last group received tramadol (20 mg/Kg body wt) 60 min prior to testing. Latency to lick a hind paw or jumping was recorded sequentially before and at 1, 2 h post treatment.

The results of anti-inflammatory activity and analgesic activities are given in diagram I and II.

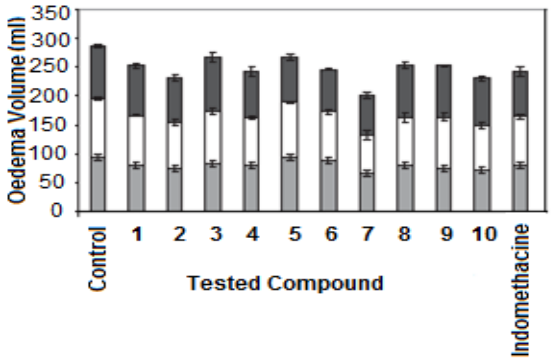


Diagram I: Anti-inflammatory Activity

Note:

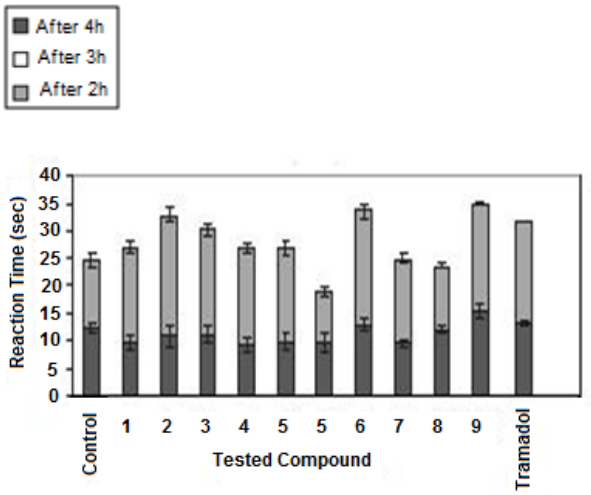
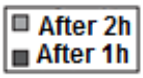


Diagram II: Analgesic Activity

Note:



Result and Discussion

All compounds synthesized are obtained in crystalline form and with good practical yield. The purity and homogeneity of compounds synthesized were determined by sharp melting points and TLC method. All the synthesized compounds were analyzed by TLC, MP, FTIR, 1HNMR, MASS and elemental analysis. All derivatives showed a broad absorbance band at about 1320 cm^{-1},1620 $cm^{-1}$and 3477 cm^{-1}associated with stretching vibrations of bonded aromatic C-N ,C=N and N-H indicating presence of nitrogen containing group in the structure. The compound also explain a strong absorbance band at 1420 $cm^{-1}$of –OH stretching vibration, absorbance at 800 cm^{-1}stretching vibration indicating present of Cl group, 530 cm^{-1}stretching vibration indicating present of Br group and 1361 cm^{-1}stretching vibration indicating present of NO_2 group. Preliminary pharmacological screening includes approximate toxicity testing (LD_{50}) on both rats as per the OECD guidelines for selecting the dose. The LD_{50} of all the derivatives was found >200mg/kg. The anti-inflammatory activity of test compounds was performed on the Albino rats of SD and Wister strain. The anti-inflammatory activity of compounds was done by using of Carageenan Induced Rat Paw Method.

Conclusion

All derivatives were synthesized by convenient route and prepared in good yield. The pharmacological screening showed that the test compounds 1, 3, 5, 6, 8 and 9 possess good anti-inflammatory activity whereas, compound 2, 3, 7, and 10 possess good analgesic activity.

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