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Synthesis and Biological Activities of Some Indolyl Substituted Pyrazolines

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A series of 2-chloroacetyl methylene-3-substituted phenyl-6-halo/6,8-dihalo substituted quinazolin-4(3*H*)-ones, 2-hydrazinoacetylmethylene-3-substituted phenyl-6,8-dihalo substituted quinazolin-4(3*H*)-ones, 5-(2'-acetyl hydrazino-methyl-3'-substituted phenyl-6-halo/dihalo substituted) quinazolin-1'-yl-4' (3'*H*)-one-2-substituted phenyl-4-(2''-substituted)-indol-3''-yl-2-pyrazolines have been prepared. The structures of the compounds were established on the basis of elemental analysis and spectral studies. The newly synthesized compounds were evaluated for their antiinflammatory, analgesic, ulcerogenic liabilities and cyclooxygenase II-inhibitory activities. The structures of these compounds have been elucidated by IR, ^{1}H NMR, mass spectroscopy and elemental analysis.

Key Words: Synthesis, Indolyl substituted pyrazolines, Biological activities.

INTRODUCTION

Non-steroidal antiinflammatory drugs, usually abbreviated to NSAIDs, are drugs with analgesic, antipyretic and antiinflammatory effects. They reduce pain, fever and inflammation. The term non-steroidal is used to distinguish these drugs from steroids, which amongst a broad range of other effects have a similar eicosanoid-depressing, antiinflammatory action. NSAIDs are unusual in that they are non-narcotic. The most prominent members of this group of drugs are aspirin and ibuprofen. They exert their pharmacological action by inhibition both forms of cyclooxygenase isoenzyme. The GIT ulcerogenic effects are commonly associated with drugs which inhibit both types of isoenzyme. Classically, COX-II inhibitors are not acids, even then they possess equipotent analgesic, antipyretic and anti-inflammatory activities of the same degree as the classical NSAIDs. Another important feature of COX-II inhibitors is that they don't cause gastritis or gastric ulcers which is in fact an advantage over the drug which

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act non-selectively. Rofecoxib and celecoxib are frequently prescribed by the physicians for different type of antiinflammatory disorders all over the world. These two drugs produce hypertension, congestive heart failure in those patients who show hypersensitivity reactions similar to the aspirin.

Celecoxib is the derivative of pyrazole which in term is derivative of pyrazoline. Systematic variation around the pyrazoline nucleus by different aryl or heteroaryl derivatives at 1,3,5 position markedly modulate the anti-inflammatory activity.

Indolyl pyrazolines have a number of biological activities like anti-inflammatory[1-4], bactericidal[5], pesticidal[6], fungicidal[7], anticonvulsant[8], tuberculostatic[9], anti cancer[10,11], CNS depressant[12]. These findings prompted us to synthesize a new series of pyrazoline derivatives with a hope to get better biological activities.

EXPERIMENTAL

Melting points were determined in open capillaries and are uncorrected. The homogeneity of all compounds were checked by using silica gel-G plates. IR spectra were located in KBr on Beckman Acculab-10 spectrophotometer (ν_{max}, cm^{-1}) and ^1H NMR spectra in $CDCl_3$ on Bruker-400-FT and Bruker-300-FT instrument (Chemical shift in δ ppm). Elemental analysis (C, H, N) and spectral analysis and biological activities of the newly synthesized compounds were obtained from Central Drug Research Institute, Lucknow, India. The elemental analysis for all compounds was obtained within ± 0.4 % of the theoretical values.

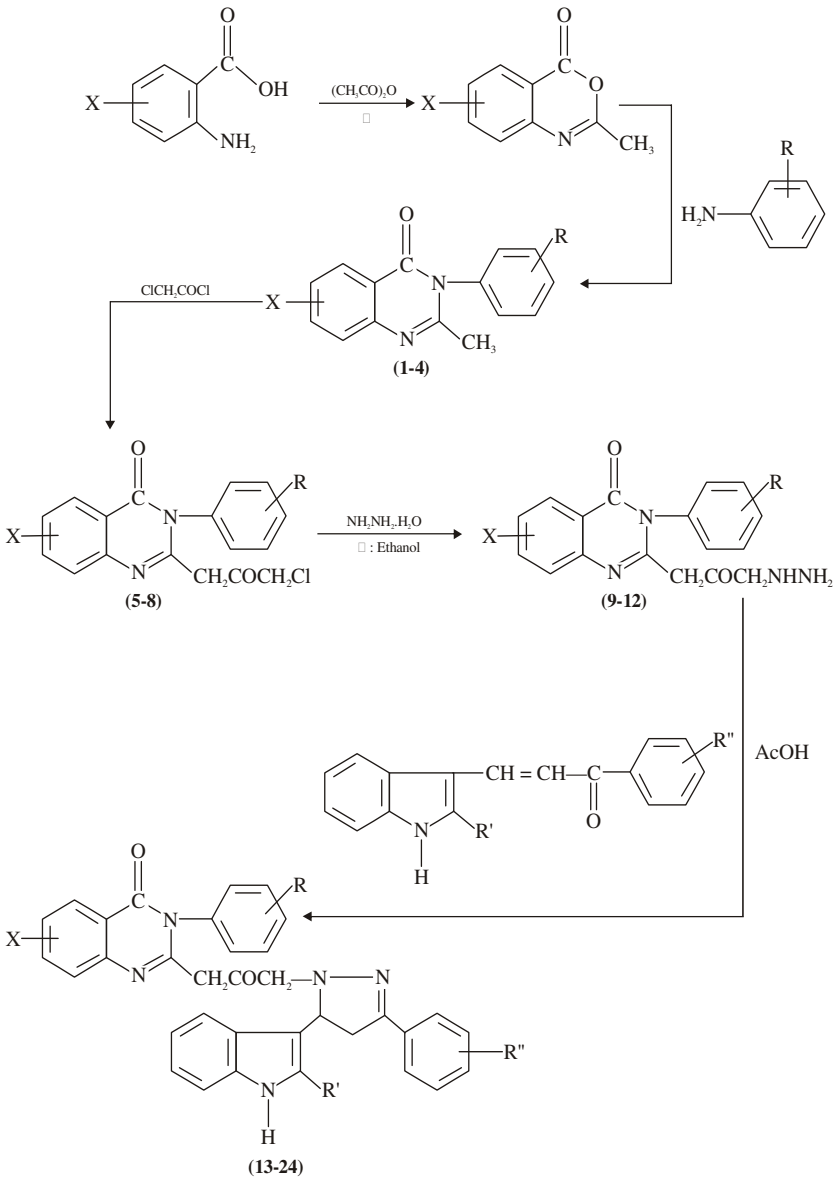
Synthesis of compounds

5-Bromoanthranilic acid and 3,5-dibromoanthranilic acids: These compounds were prepared according to the method of Wheeler and Oats[13].

Acetanthranils: These were prepared according to the method of Bogert and Seil[14]. A mixture of appropriate anthranilic acid (0.01 mol) and acetic anhydride (0.02 mol) were refluxed for 2-3 h with constant stirring. The excess of acetic anhydride was distilled off. On cooling, a solid separated out which was filtered, washed with petroleum ether (60-80 ºC) and dried *in vacuo*.

2-Methyl-3-(aryl substituted)-6-mono halo/6,8-dihalo substituted quinazolin-4(3*H*)-ones (1-4): These compounds were synthesized according to the method of Bogert and Seil[14]. The physical, analytical and spectral data of compounds **1-4** are given below:

2-Methyl-3-(3'-chlorophenyl)-4(3*H*)-one (1): m.p. 152 ºC; yield 85 %, recrystallization solvent ethanol. m.f. $C_{15}H_{11}ON_2Cl$; Calcd. (%) C, 66.54; H, 4.07; N, 10.35, Found (%): C, 66.32; H, 4.16; N, 10.46. The compound



Scheme

1 showed characteristic bands at 1720 ν(C=O), 1590 ν(C=N), 1480 ν(C-N), 3040 aromatic ν(C=H), 2840, 2960 ν(aliphatic C-H), 760 ν(C-Cl) cm^{-1} in its IR (KBr) spectrum. ^1H NMR ($CDCl_3$): δ 7.60-6.70 (m, 8H, ArH); 2.45 (s, 3H, CH_3) (ppm). The presence of molecular ion peak at m/z 270 confirmed its structure.

2-Methyl-3-(2', 4'-di-chlorophenyl) quinazolin-4(3*H*)-one (2): m.p. 156 ºC; yield 80 % recrystallization solvent ethanol. m.f. $C_{15}H_{10}ON_2Cl_2$ Calcd. (%): C, 59.02; H, 3.28, N, 9.18, Found (%): C, 59.19; H, 3.26; N, 9.25. The compound **2** showed characteristic bands at 1710 ν(C=O), 1580 ν(C=N), 1490 ν(C-N), 780 ν(C-Cl) 2850, 2950 ν(aliphatic C-H) in its IR (KBr) spectrum. ^{1}H NMR ($CDCl_3$) δ: 7.65-6.75 (m, 8H, Ar-H), 2.50 (s, 3H, CH_3) (ppm). Molecular ion peak at m/z 305 further confirmed the formation of compound **2**.

2-Methyl-3-(3'-methoxyphenyl)-6-bromoquinazolin-4(3*H*)-one (3): m.p. 176 ºC; yield 90 %; recrystallization solvent methanol. m.f. $C_{16}H_{13}O_2N_2Br$ Calcd. (%) C, 55.65; H, 3.76; N, 8.11; Found (%) C, 55.73; H, 3.67; N, 8.22. The compound **3** exhibited characteristic bands at 1680 ν(C=O), 1560 ν(C=N), 1480 ν(C-N), 2840, 2990 ν(aliphatic C-H) in its IR KBr spectrum. ^{1}H NMR ($CDCl_3$) δ: 8.86 (dd, 2H, Ha & Hb, 2H, Ar-H), 8.70 (dd, Hc, Ar-H); 7.80-7.40 (m, 4H, Ar-H), 2.25 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3) (ppm). Molecular ion peak at m/z 345 confirmed its structure.

2-Methyl-3-(3'-methoxyphenyl)-6,8-dibromoquinazolin-4(3*H*)-one (4): m.p. 172 ºC, recrystallization solvent methanol. The compound **4** m.f. $C_{16}H_{12}O_2N_2Br_2$; Calcd. (%): C, 45.28; H, 2.83; N, 6.60; Found (%): C, 45.37, H, 2.71; N, 6.67. IR (KBr) spectrum of this compound showed characteristic bands at 1690 ν(C=O), 1570 ν(C=N), 1500 ν(C-N), 2850, 2960 ν(aliphatic C-H). ^{1}H NMR ($CDCl_3$) δ: 8.90 (dd, 2H, Ha & Hb, 2H, Ar-H), 7.90-7.50 (m, 4H, Ar-H), 3.45 (s, 3H, OCH_3), 2.30 (s, 3H, CH_3) (ppm). The presence of molecular ion at m/z 424 confirmed its structure.

2-Chloroacetyl methylene-3-(2',4'-dichlorophenyl)-quinazolin-4(3*H*)-one (6): To the solutions of 2-methyl-3-(2',4'-dichlorophenyl)-quinazolin-4(3*H*)-one (0.32 mol) in dry THF (100 mL) was added a solution of chloroacetylchloride (0.64 mol) in dry THF (200 mL) at 0 ºC drop by drop along with manual stirring for 2 h. The reaction mixture was further stirred for 2-4 h on the mechanical stirrer and excess of solvent was distilled off, cooled and poured onto ice. The solid thus obtained was filtered and recrystallized from methanol. Compound **6**, m.p. 160 ºC, yield 75 %. m.f. $C_{17}H_{11}O_2N_2Cl_3$. Calcd. (%): C, 53.47; H, 2.88; N, 7.34. Found (%): C, 53.64; H, 2.90; N, 7.31. IR (KBr, ν_{max}, cm^{-1}): 1730 (C=O), 2580 (CH_2), 3055 (C-H aromatic), 1590 (C=N), 740 (C-Cl). ^{1}H NMR ($CDCl_3$) δ : 8.10-8.70 (m, 7H, Ar-H), 3.40 (s, 2H, CH_2CO, 2.52 (s, 2H, $COCH_2Cl$). MS: [M^+] m/z 381. Compounds (**5-8**) were prepared similarly and their characterization data are given in Table-1.

2-Hydrazinoacetylmethylene-3-(2',4'-dichlorophenyl)quinazolin-4(3*H*)-ones (10): The mixtures of 2-chloroacetyl hydrazino methylene-3-(2',4'-dichlorophenyl)quinazolin-4(3*H*)-one (0.01 mol) and hydrazine hydrate (99 %) (0.01 mol) in absolute ethanol (50 mL) was refluxed for

10-12 h and the completion of reaction was monitered by TLC. The excess of solvent was distilled off and the reaction mixture was poured onto ice, the products thus obtained were recrystallized from ethanol compound (**10**). m.p. 222 ºC, yield 68 %. m.f. $C_{17}H_{14}O_2N_2Cl_2$. Elemental analysis: Calcd. (%): C, 54.11; H, 3.71; N, 14.85; Found (%): C, 54.23; H, 3.69; N, 14.88. IR (KBr, ν_{max},cm^{-1}): 1715 (C=O), 3440 (NH_2), 3380 (NH), 3065 (C-H aromatic), 1580 (C=N), 760 (C-Cl). ^{1}H NMR ($CDCl_3$) δ ppm : 8.15-8.62 (m, 7H, Ar-H), 5.55 (br, 1H, NH exchangeable), 4.55 (hump, 2H, NH_2 exchangeable), 2.35 (s, 2H, CH_2CO), 2.47 (d, 2H, CH_2NH). MS: [M^+] m/z 377. The compounds (**9-12**) were prepared similarly and their characterization data are given in Table-1.

5-(2′-Acetylmethylene)-3′-(2″,4″-dichlorphenyl)-quinazolin-4′-(3′*H*)-one-2-(*o*-methoxyphenyl)-4-(2‴-methyl indol-3‴-yl)-2-pyrazoline (14): The mixture of compounds **10** (0.02 mol) in glacial acetic acid (100 mL) and 2-substituted indol-3-yl-substituted chalcones was refluxed for 16 h, distilled in vacuum and cooled. The separated solids were washed with ether and recrystallized from ethanol compound **14**, m.p. 237ºC, yield 48 %. m.f. $C_{36}H_{29}O_3N_5Cl_2$. Elemental analysis: Calcd. (%): C, 66.46; H, 4.46; N, 10.77. Found (%): C, 66.59; H, 4.44; N, 10.82. IR (KBr, ν_{max}, cm^{-1}): 3065 (C-H aromatic), 1720 (C=O), 2485 (CH_2), 1590 (C=N), 2995, 2960 (C-H, aliphatic), 760 (C-Cl). ^{1}H NMR ($CDCl_3$) δ ppm: 7.25-8.67 (m, 15H, Ar-H), 2.40 (s, 3H, CH_3 attached to indole nucleus), 8.75 (br, 1H, NH, indole, exchangeable), 5.82 (d, 2H, CH_2 of pyrazoline ring), 3.95 (t, 1H, CH_2 of pyrazoline ring), 2.91 (s, 2H, CH_2 CO), 3.82 (s, 2H, $COCH_2$), 3.39 (s, 3H, OCH_3). MS: [M^+] m/z 650. The compounds (**13-24**) were prepared similarly and their characterization data are given in Table-1.

Biological study: The experiments were performed with albino rats of Charles Foster species of either sex, excluding pregnant females, of 60 to 90 d weighing 120-160 g. Food (chaw pallet) and water was given to the animals *ad libitum*. The test compounds were dissolved in propylene glycol. Phenyl butanone and aspirin were used as reference drugs for the comparison of antiinflammatory property. Phenylbutazone was also used for comparison of analgesic activity.

Antiinflammatory activity: This study was done by following the procedure of Winter *et al.*[15]. The rats were divided into three groups (control, drug treated and standard drug) of six rats each. A freshly prepared suspension of carrageenin (1 % in 0.9 % saline). 0.2 mL was injected under the planter aponeurosis of the right hind paw of each rat. Test compounds and standard drug were administered orally to the animals of drug treated groups and the standard drug group, respectively, one hour before the carrageenin injection. The paw volume of each rat was measured before 1 h and after 3 h of carrageenin treatment with the help of plethysmometer.

TABLE-1
PHYSICAL AND ANALYTICAL DATA OF COMPOUNDS **5-24**

Compd. no.	X	R	R′	R″	m.p. (ºC)	Yield (%)	Recrystallization solvent	m.f.	Elemental analysis (%): Found (Calcd.)		
									C	H	N
5	H	3-Cl	–	–	156	82	Benzene	$C_{17}H_{12}O_2N_2Cl_2$	58.78 (58.92)	3.45 (3.46)	8.07 (8.10)
6	H	2,4-Cl_2	–	–	160	75	Methanol	$C_{17}H_{11}O_2N_2Cl_3$	53.47 (53.64)	2.88 (2.90)	7.34 (7.31)
7	6-Br	3-OCH_3	–	–	185	60	Ethanol	$C_{18}H_{14}O_3N_2ClBr$	51.24 (51.43)	3.32 (3.30)	6.64 (6.62)
8	6,8-Br_2	3-OCH_3	–	–	196	72	DMF	$C_{18}H_{13}O_3N_2ClBr_2$	43.16 (43.18)	2.59 (2.57)	5.59 (5.62)
9	H	3-Cl	–	–	205	71	Ethanol	$C_{17}H_{15}O_2N_4Cl$	59.56 (59.72)	4.38 (4.42)	16.35 (16.29)
10	H	2,4-Cl_2	–	–	222	68	Ethanol	$C_{17}H_{14}O_2N_4Cl_2$	54.11 (54.23)	3.71 (3.69)	14.85 (14.88)
11	6-Br	3-OCH_3	–	–	275	52	Methanol	$C_{18}H_{17}O_3N_4Br$	51.79 (51.46)	4.07 (4.08)	13.42 (13.49)
12	6,8-Br_2	3-OCH_3	–	–	260	53	Benzene	$C_{18}H_{16}O_3N_4Br_2$	43.54 (43.67)	3.22 (3.25)	11.29 (11.32)
13	H	3-Cl	CH_3	2-OCH_3	224	52	Methanol	$C_{36}H_{30}O_3N_5Cl$	70.18 (70.29)	4.87 (4.92)	11.37 (11.31)
14	H	2,4-Cl_2	CH_3	2-OCH_3	237	48	Ethanol	$C_{36}H_{29}O_3N_5Cl_2$	66.46 (66.59)	4.46 (4.44)	10.77 (10.82)
15	6-Br	3-OCH_3	CH_3	H	212	51	Acetic acid	$C_{36}H_{30}O_3N_5Br$	65.45 (65.31)	4.54 (4.51)	10.60 (10.54)
16	6,8-Br_2	3-OCH_3	CH_3	H	244	60	Benzene	$C_{36}H_{29}O_3N_5Br_2$	58.45 (58.57)	3.92 (3.88)	9.47 (9.52)
17	H	3-Cl	C_6H_5	2-OCH_3	210	39	Methanol	$C_{41}H_{32}O_3N_5Cl$	72.61 (72.85)	4.72 (4.73)	10.33 (10.29)
18	H	2,4-Cl_2	C_6H_5	2-OCH_3	255	50	Methanol	$C_{41}H_{31}O_3N_5Cl_2$	69.10 (69.23)	4.35 (4.33)	9.83 (9.80)
19	6-Br	3-OCH_3	C_6H_5	H	241	42	Ethanol	$C_{41}H_{32}O_3N_5Br$	68.14 (68.19)	4.43 (4.41)	9.69 (9.72)
20	6,8-Br_2	3-OCH_3	C_6H_5	H	252	44	DMF	$C_{41}H_{31}O_3N_5Br_2$	61.42 (60.30)	3.87 (3.88)	8.74 (8.71)
21	H	3-Cl	H	2-OCH_3	256	47	Benzene	$C_{35}H_{28}O_3N_5Cl$	69.82 (69.99)	4.65 (4.67)	11.63 (11.59)
22	H	2,4-Cl_2	H	2-OCH_3	260	51	Methanol	$C_{35}H_{27}O_3N_5Cl_2$	66.04 (66.18)	4.24 (4.26)	11.01 (11.09)
23	6-Br	3-OCH_3	H	H	198	42	Ethanol	$C_{35}H_{28}O_3N_5Br$	65.01 (65.18)	4.33 (4.31)	10.83 (10.88)
24	6,8-Br_2	3-OCH_3	H	H	214	60	Benzene	$C_{35}H_{27}O_3N_5Br_2$	57.93 (58.12)	3.72 (3.70)	9.65 (9.69)

The percent antiinflammatory activity was calculated according to the formula given below:

$$\% \text{ Antiinflammatory activity} = 1 - \frac{D_t}{D_c} \times 100$$

where D_t and D_c are paw volumes of oedema in tested and control group, respectively.

Analgesic activity: Acetic acid writhing test was performed on mice following the method of Davis *et al.*[16]. Groups of five mice, body weight (200-300 g) of both sexes, pregnant females excluded, were given a dose of test compound. 0.5 h later the animals were injected intraperitoneally with 0.25 mL/mouse of 0.5 % acetic acid during the following 25 min. The mean number of writhes for each experimental group and percentage decrease compared with the control group (five mice not treated with test compounds) were calculated after 1 h.

$$\% \text{ Protection} = 1 - \frac{\text{Mean no. of writhes in mice of test group}}{\text{Mean no. of writhes in mice of control group}} \times 100$$

Ulcerogenic activity: Ulcerogenic activity of newly synthesized compounds was checked by the method of Verma *et al.*[17]. Albino rats were fasted 24 h prior to drug administration. All animals were sacrificed 8 h after drug treatment and their stomachs and small intestines were microscopically examined to assess the incidence of hyperemia, shedding of epithelium, petechial and frank hemorrhages and erosion or discrete ulceration with or without perforation. The presence of any one of these criteria was considered to be an evidence of ulcerogenic activity.

Acute toxicity study: The test compounds were investigated for their acute toxicity in albino mice according to the method of Smith[18]. The test compounds were given orally at different dose levels in separate groups of animals. After 24 h of drug administration, per cent mortality in each group was observed. ALD_{50} (approximate lethal dose) was calculated from the data obtained.

RESULTS AND DISCUSSION

Antiinflammatory, analgesic, ulcerogenic, cyclooxygenase and toxicity studies of compounds (5-24): Percentage of oedema inhibition relative to control and % decrease of writhes in 1 h after treatment relative to control of compounds (**5-24**) have been mentioned in Table-2. All the compounds of the series have shown varying degree of statistically significant anti-inflammatory (6.3 to 63.8 %) and analgesic activities (5-50 %). The Table-2 shows the activities of three types of quinazolinones. The compounds for first step are characterized by the presence of chloroacetyl-ethylene moiety at 2 position of quinazolinone nucleus besides other substitutents at position 6 and 3 which were varied also.

TABLE-2
ANTIINFLAMMATORY, ANALGESIC, ULCEROGENIC, CYCLOXYGENASE AND TOXICITY DATA OF COMPOUNDS (**5-24**)

Compd. no.	AA	BB	AA	Analgesic activity		Ulcerogenic activity		FF	ED_{50} mg/kg p.o.	ALD_{50} mg/kg p.o.
				CC	AA	DD	EE			
5	50	18.5	50	5	200	70	20	Nil	–	>1000
6	50	16.3	50	10	200	60	30	Nil	–	>1000
7	50	17.2	50	10	200	40	20	Nil	–	>1000
8	50	19.2	50	15	200	60	30	Nil	–	>1000
9	50	9.2	50	10	200	30	10	Nil	–	>1000
10	50	6.3	50	5	200	20	30	Nil	–	>1000
11	50	13.2	50	5	200	10	10	Nil	–	>1000
12	50	10.2	50	5	200	10	10	Nil	–	>1000
13	50	21.7	50	30	200	100	20	Nil	–	>1000
	25	46.4	25	10	100	60	10			
14	50	63.8	50	20	200	90	20	90	26.3	>1500
	100	84.7	100	50	300	100	30			
15	50	30.2	50	50	200	80	30	Nil	–	>1000
16	50	21.7	50	50	200	70	20	Nil	–	>1000
17	50	33.0	50	30	200	50	30	Nil	–	>1000
18	50	35.0	50	40	200	60	70	Nil	–	>1000
19	50	20.0	50	21	200	70	10	Nil	–	>1000
20	50	21.3	50	10	200	90	20	Nil	–	>1000
	25	28.2	25	10	100	30	20			
21	50	50.0	50	20	200	60	30	Nil	50.0	>1000
	100	70.8	100	40	300	80	40			
	25	24.0	25	20	100	50	10			
22	50	37.0	50	30	200	60	20	Nil	63.1	>1000
	100	74.4	100	50	300	90	40			
	25	26.6	25	20	100	40	5			
23	50	36.6	50	30	200	50	10	Nil	63.1	>1000
	100	86.6	100	50	300	60	30			
24	50	32.8	50	30	200	30	20	Nil		
Phenyl buta-none	25	29.3								
	50	38.2								
	100	64.4								

AA = Dose mg/kg p.o.; BB = Antiinflammatory activity-% Oedema inhibition relative to control; CC = %Decrease of writhes in 1 h after treatment relative to control; DD = % of animal with hyperemia; EE = % of animal with ulcer; FF = Cyclooxygeanse activity assay inhibitory action of some selected compound % inhibition 10 μM.

All the four compounds (**5-8**) of step 1 exhibited only mild to moderate antiinflammatory as well as analgesic activities at a dose of 50 mg/kg p.o. In the corresponding hydrazino products (step 2), all the four (9-12) exhibited either less or complete loss of both the activities.

Furthermore, when hydrazinoacetylmethylene moiety is converted into 2,4,5 trisubstitutedpyrazolines (stage 3, Table-2), all the compounds (**13-24**) showed potent activities. The compound **14** has shown the most potent antiinflammatory (63.8 %) activity at a dose of 50 mg/kg. Interestingly this compound was also associated with promising analgesic activity (50 %). Considering it potentiality it was further studied at three dose level 25, 50 and 100 mg/kg and was found to possess more activity than phenyl butanone 25, 50 and 100 mg/kg and was found to possess more activity than phenyl butanone (25, 50, 100 mg/kg p.o.) and aspirin. In other words, it can be concluded that when the compound (quinazolino-2-pyrazoline; **14**) was substituted with 2-methoxyphenyl group and 2-methylindole moeity at 2 and 4 position of pyrazoline hetero system, then compound is most active. Fig. 1 shows the bar diagram results of this compound.

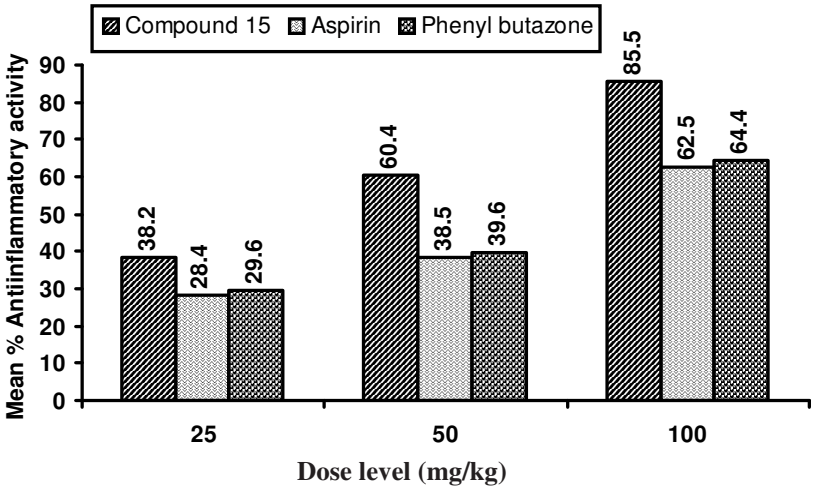


Fig. 1. Bar diagram showing antiinflammatory activity of compound **15**, aspirin and phenyl butazone

Ulcerogenicpotential activity: As these compounds have shown promising activities, they were also tested for ulcerogenic activity. Although these compounds (**13-24**) have shown varying degree of hyperemia (30 to 100 % of animals) but were associated with low degree of ulcer production (10-30 % of animals). Out of the 12 compounds some compounds (**14** and **23**) have

shown very low degree of ulcer production. Compound **14** being most potent at three dose levels was tested and compared with acetyl salicylic acid standard drug for ulcerogenic activity and was found to be less ulcerogenic.

Biochemical study: Arachidonic acid is converted by cyclooxygenase and lipoxygenase enzymes to biologically active metabolite which play a major role in pathophysiological responses such as inflammation[19]. The prostaglandins, which are derived from arachidonic acid *via* the cyclooxygenase pathway, have been shown to be responsible for many of the early signs of inflammation such as peralgesia, increase in vascular permeability leading to oedema and pyrexia. One group of drugs, the NSAIDs, are capable of alleviating on reducing these signs and symptoms are really thought to do so their inhibition of cyclooxygenase enzymes resulting in decrease levels of prostaglandins[20]. Blockage or inhibition of cyclooxygenase enzymes appears to be particularly attractive in terms of developing agents with therapeutic potential in inflammatory states[21]. In the light of these observations, it was considered worth while to test these compounds for *in vitro* cyclooxygenase activity so that the possible mechanism of action of these compounds as inflammation inhibitor could be established. The cyclooxygenase assay was carried out by measuring the rate of conversion of [1-^{14}C] arachidonic acid to PGE in the microsomal fraction of mucosa-preparation of rabbit distal colon, after incubation with test compounds. In this test compound **14**, displayed percentage inhibitory activity of 90 at (10 mM) (Table-2). It is interesting to note that compound **14** showed equipotent % inhibitory cycloxygenase activity as that of standard drug at the same concentration, while rest of the quniazolinopyrazolines are completely devoid of activity, despite their antiinflammatory action.

On the basis of these results it seems logical to conclude that the *in vivo* activity of compound **14** is supported by multiple mechanism of action, among which only the inhbition of prostaglandin biosynthesis is of prime importance. ALD_{50} value of compound **14** is quite high (> 1500 mg/kg p.o.) suggesting good safety margin, while rest of the compounds are also safe (ALD_{50} > 1000).

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