Synthesis of 6-(4-methanesulphonamidophenyl)-substituted dihydropyridazinone/ phthalazinone derivatives as potent anti-inflammatory and analgesic agents

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ABSTRACT

New 6-(4-methanesulphonamidophenyl)-substituted dihydropyridazinones and their phthalazinone analogues have been synthesized and evaluated for anti-inflammatory and analgesic activities using carrageenan induced paw edema and acetic acid induced writhing models, respectively. Most of the newly synthesized compounds displayed potent anti-inflammatory activity without ulcerogenic side effects. Both pyridazinone as well as phthalazinone analogues displayed moderate analgesic activity at 40 mg/kg. In general, all pyridazinone derivatives showed activity better than their phthalazinone counterparts.

Keywords: 3(2*H*)-pyridazinones, phthalazinones, analgesics, anti-inflammatory agents, ulcerogenic effects

1. INTRODUCTION

Inflammation is one of the most important natural defense mechanisms against internal and external threats. The first anti-inflammatory drug discovered with therapeutic benefits is aspirin, which is being used as a Non Steroidal Anti-Inflammatory Drug (NSAID) for almost a century. Since then NSAIDs have become the most widely used agents for treatment of pain and inflammation (Vane 2000, 573). In 1970s, Vane and coworkers further observed that these drugs exhibit their anti-inflammatory effects by inhibiting cyclooxygenase (COX) enzyme, which catalyses the bioconversion of arachidonic acid to prostaglandins (PGs) (Vane 1971, 232 and Whittle *et al*. 1978, 955). Majority of the NSAIDs so far developed to heal the inflammation inhibit both COX-1 and COX-2 with similar potency and hence result in adverse effects like gastric ulcer, kidney damage and sometimes hepatotoxicity. In view of this, selective cyclooxygenase-2 (COX-2) inhibitors were developed to minimize these side effects. However unfortunately, increasing selectivity for COX-2 also increased toxicity and recently their liberal use has come under scrutiny with alarming reports of cardiovascular side effects (Dannhardt *et al*. 2001, 109). Therefore, research aimed towards developing COX-2 selective NSAIDs devoid of unwanted cardiovascular effects has generated much interest in scientific community.

The pyridazinones are considered as privileged structures as substitutions at various positions of this wonder nucleus result in a variety of derivatives possessing diversified biological properties (Asif *et al*. 2010, 1112 and Bansal *et al*. 2013, 2539). Pyridazinone derivatives have been reported to exhibit pharmacological activities ranging from cardiovascular to anti-inflammatory effects (Khaled *et al*. 2010, 629 and Sircar *et al*. 1987, 1955). 3(2*H*)-pyridazinone derivative emorfazone (**1**) has been launched in Japan as an analgesic drug (Sato *et al*. 1981, 1738). Interestingly, the mechanism of action of emorfazone involves inhibitory effect

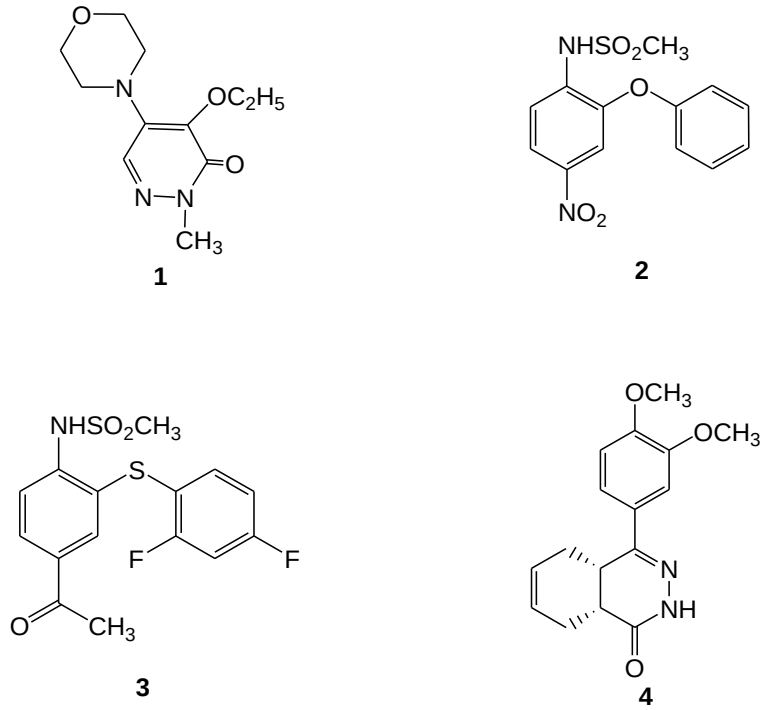
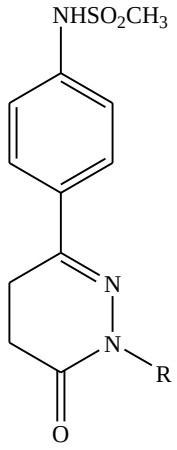


Figure 1: Important structural features of some known potent anti-inflammatory molecules

on the release of bradykinin-like substance into the extra vascular space, which is different from other currently used drugs (Sato *et al*. 1982, 379). Methanesulphonamide moiety has been found

to be an important pharmacophoric structural component present in many classical (nimesulide, **2**) (Garcia-Nieto *et al*., 1999, 14) and selective COX-2 inhibitors (FR115068, **3**) (figure 1) (Nakamura *et al*. 1993, 894). These observations *i.e*. presence of a methanesulphonamide moiety and pyridazinone skeleton as structural requirements for formation of therapeutically useful anti-inflammatory agents instigated us to further explore this area. Subsequently we screened some of our previously synthesized and reported cardiotonic pyridazinone derivatives **5a-c** (figure 2) (Kumar *et al*. 2008, 393) for anti-inflammatory and analgesic activities in order to find NSAIDs with better therapeutic profile.

Further extension of research work was carried out by introducing a nitro group *ortho* to methanesulphonamide moiety (**8**) and methyl group at C_5 of basic pyridazinone skeleton (**10**) to study structure activity relationship. It was also considered worthwhile to synthesize and study compounds **12** and **13** having an acetamide side chain on lactam nitrogen of pyridazinone ring as such molecules are reported to possess potent analgesic and anti-inflammatory activities (Dogruer *et al*. 2003, 727).



(**5a-c**)

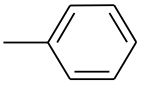
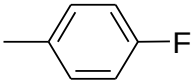
	R
a	H
b	
c	

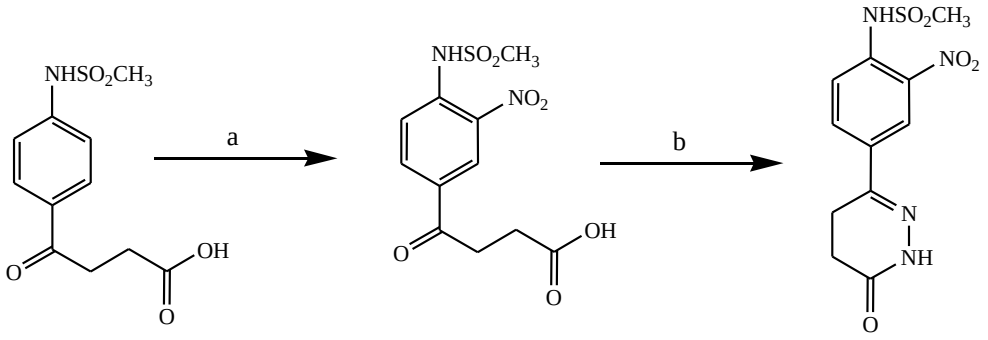
Figure 2: Structure of previously synthesized compounds **5a-c** reported as cardiotonic agents

Literature reports many phthalazinone derivatives, represented by structure **4** (figure 1), as potent phosphodiesterase (PDE) 4 inhibitors (Margaretha *et al*. 2001, 2511). PDE 4 isozymes have absolute specificity for cyclic adenosine-3′,5′-monophosphate and are considered potential therapeutic targets for the treatment of chronic inflammatory disorders such as chronic obstructive pulmonary disease (Kagayama *et al*. 2009, 6959). Thus in accordance with literature studies, another series of similar analogues **15a-c** with phthalazinone nucleus as basic heterocyclic skeleton has also been synthesized and studied.

2. RESULTS AND DISCUSSION

2.1 Chemistry

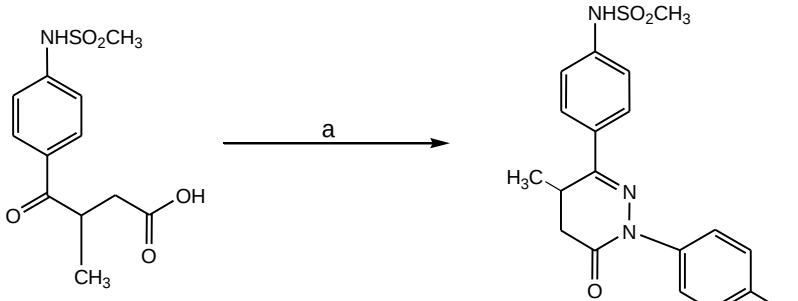
Synthesis of pyridazinones **5a-c** has been reported previously [Kumar *et al*., 2008] and their structures are presented in figure 2. General pathways followed for the synthesis of compounds **8**, **10-13** and **15a-c** are depicted in schemes 1-4. Key intermediates **6** and **9** were prepared by Friedel-Crafts acylation of the *N*-phenylmethanesulfonamide with methylsuccinic anhydride and succinic anhydride, respectively (Kumar *et al*. 2008, 393).



(**6**) (**7**) (**8**)

Scheme 1: Synthetic route to the formation of compound **8**. Reagents and reactions conditions: (a) ice cold mixture of conc. HNO_3-H_2SO_4 (3:1), 0 ^oC, 2 h; (b) Hydrazine hydrate, absolute alcohol, reflux.

To study the effect of introducing nitro group *ortho* to methanesulphonamide moeity, nitration of compound **6** in ice cold mixture of concentrated HNO_3-H_2SO_4 (3:1) was carried out to afford product **7**. Cyclization of γ-keto acids **7** and **9** with appropriate hydrazine derivative



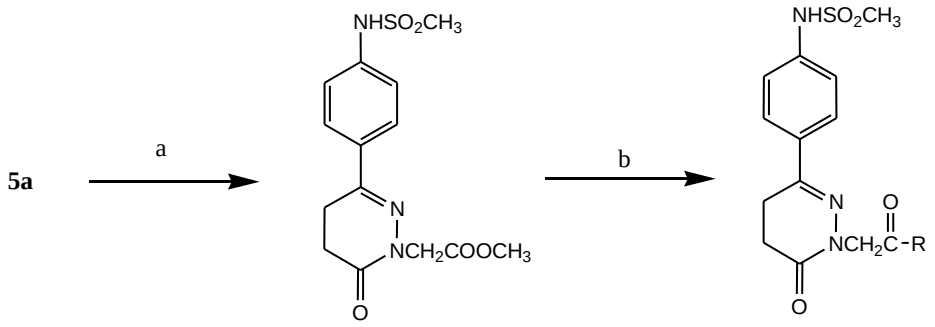
(**9**) (**10**)

Scheme 2: Synthetic route to the formation of compound **10**. Reagents and reactions conditions: (a) *p*-fluorophenylhydrazine hydrochloride, absolute alcohol, reflux.

resulted in the formation of aimed compounds **8** and **10**. The absence of acidic C=O stretching bands in the infrared spectra of **8** and **10** confirmed the cyclization. The characteristic ^{1}H NMR signals of 5-methyl substituted pyridazinone **10** appeared at δ 1.38 (d, 3H, 5-CH(C*H*$_3$)] and 3.15 ppm (m, 1H, 5-C*H*(CH$_3$)]. Distinguished exchangeable singlets for two -N*H* protons were observed at δ 9.88 (-N*H*SO$_2$CH$_3$) and 10.69 ppm (-N*H*, pyridazinone) in case of compound **8**.
Esterification of previously synthesized 6-(4-methanesulfonamidophenyl-4,5-dihydropyridazin-3(2*H*)-one (**5a)** with methyl chloroacetate afforded compound **11**, subsequent treatment of which with requisite amines in presence of dimethylformamide afforded products **12** and **13**. A prominent IR stretching vibrational band for C=O of ester functionality appeared at 1748 for derivative **11**, which shifted to lower wavenumber 1670 cm^{-1} in case of compounds **12** and **13** due to formation of tertiary amides.

For the preparation of phthalazinone derivatives, *N*-phenylmethanesulfonamide [Kumar *et al*. 2008, 393] was treated with phthalic anhydride in the presence of anhydrous aluminium chloride in purified carbon disulfide to afford 2-(4-methanesulfonamidobenzoyl)benzoic acid (**14**). The infrared bands appeared at 1709 due to C=O stretching vibrations of acid and at 1661 cm^{-1} due to highly conjugated ketonic C=O stretch. Aromatic protons of resulting γ-keto acid

resonated as multiplets at δ 7.30 and 8.03 ppm. Subsequent cyclocondensation of keto acid **14** with requisite hydrazine derivative in aldehyde free alcohol afforded final compounds **15a-c**. A very downfield -N*H* proton of phthalazinone residue was seen at δ 11.77 ppm for **15a** and this signal was missing in ^{1}H NMR spectra of 2-substituted phthalazinones **15b** and **15c**.

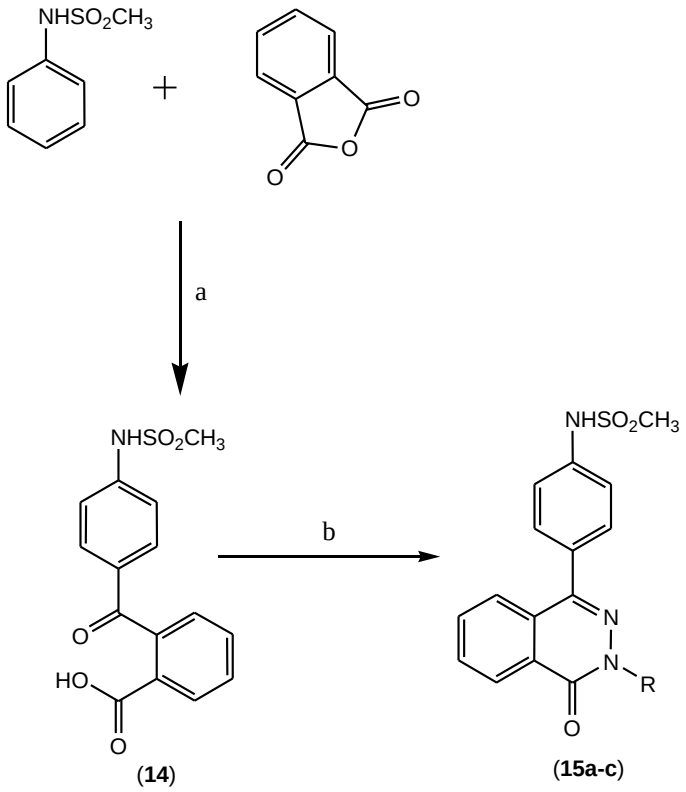


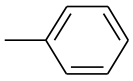
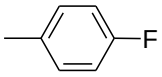
Comp. No.	R
12	—N
13	—N O

Scheme 3: Synthetic route to the formation of 2-substituted-6-(4-methanesulphonamidophenyl)-4,5-dihydropyridazin-3(2*H*)-ones **12** and **13**. Reagents and reactions conditions: a) methyl chloroacetate, ethyl methyl ketone, anhyd. K_2CO_3, reflux; b) requisite amine, dimethylformamide, heating.

Biological evaluation

Anti-inflammatory and analgesic activities of the newly synthesized pyridazinone (**8, 10-13**) and phthalazinone derivatives (**15a-c**) at 20 and 40 mg/kg were assessed by carrageenan-induced hind paw edema model and acetic acid induced writhing test, respectively. Additionally, all compounds were also investigated for ulcerogenic side effects. In general, newly synthesized compounds displayed dose dependent inhibition of rat paw edema, being more effective at 40 mg/kg than 20 mg/kg as shown in table 1. The anti-inflammatory activity of methanesulfonamide substituted derivatives at 40 mg/kg was found comparable to that of indomethacin (80.8%) as well as celecoxib (82.61%) at 20 mg/kg. It seems that N^2-aryl substitution of pyridazinone ring as in compounds **5b** and **5c** results in marginal reduction of anti-inflammatory potency as the 2-unsubstituted derivative **5a** produced more inhibition of edema (84.78%) in comparison to the substituted ones **5b** and **5c**. Introduction of a nitro group in 6-phenyl ring as in compound **8** maintains the activity, however presence of methyl group at C_5 of pyridazinone ring in compound **10** resulted in reduced activity. Presence of an acetamidic linkage at *N*-2 of pyridazinone scaffold resulted in derivatives **12** and **13** with a longer duration of action but with substantially reduced anti-inflammatory potential. A moderate activity was displayed by the phthalazinone analogs **15a-c**. Compounds substituted with phenyl (**15b**) and *p*-fluorophenyl (**15c**)



Comp. No.	R
a	H
b	
c	

Scheme 4: Synthetic route to the formation of N-{4-[3-substituted-4-oxo-1-phthalazinyl]phenyl}methanesulfonamides **15a-c**. Reagents and reactions conditions: (a) aluminium chloride, carbon disulphide, 48 h; (b) Hydrazine hydrate/phenylhydrazine hydrochloride/*p*-fluorophenylhydrazine hydrochloride, absolute alcohol, reflux.

groups at 3-position of phthalazinone skeleton showed slightly improved inhibition (~50%) of edema compared to unsubstituted phthalazinone derivative **15a** (40.2%) at 40 mg/kg after 3 h of study. Overall pyridazinone core structure seems to be better suited for anti-inflammatory activity in comparison to phthalazinone nucleus (table 1).

Table 1. Anti-inflammatory activity and Gastric Ulcer Index (G.I.) of various newly synthesized pyridazinones and standard drugs

	Dose	Edema volume (ml±SEM) (inhibition %)						Gastric Ulcer
	(mg/kg)	30min	60min	90min	120min	180min	240min	index
Control		0.21±0.01	0.39±0.04	0.43±0.04	0.44±0.03	0.46±0.05	0.46±0.06	0/6
5a	20	0.22±0.06 (-4.76)	0.34±0.05 (12.82)	0.27±0.07 (37.21)	0.22±0.04 (50.00)**	0.23±0.06 (50.00)*	0.25±0.06 (45.65)	0/6
	40	0.18±0.06 (14.3)	0.30±0.05 (23.1)	0.18±0.04 (58.1)**	0.1±0.03 (77.27)**	0.07±0.06 (84.78)**	0.07±0.01 (84.78)***	0/6
5b	20	0.18±0.03 (14.29)	0.32±0.05 (17.95)	0.36±0.04 (16.28)	0.29±0.05 (34.09)	0.29±0.05 (36.96)*	0.34±0.02 (26.09)*	0/6
	40	0.15±0.03 (28.6)*	0.26±0.04 (33.3)*	0.25±0.03 (41.9)**	0.18±0.06 (59.09)**	0.13±0.05 (71.74)**	0.16±0.03 (65.22)***	0/6
5c	20	0.22±0.05 (-4.76)	0.25±0.06 (35.90)	0.24±0.04 (44.19)**	0.23±0.08 (47.73)	0.19±0.04 (58.70)**	0.23±0.03 (50)**	0/6
	40	0.17±0.05 (19.0)	0.16±0.04 (59.0)**	0.14±0.04 (67.4)**	0.13±0.08 (70.45)**	0.09±0.04 (80.43)**	0.14±0.02 (69.57)***	0/6
8	20	0.22±0.04 (-4.76)	0.25±0.04 (35.90)*	0.22±0.06 (48.84)*	0.21±0.05 (52.27)**	0.22±0.04 (52.17)**	0.25±0.02 (45.65)**	0/6
	40	0.2±0.04 (4.8)	0.21±0.06 (46.2)*	0.21±0.06 (51.2)**	0.16±0.06 (63.64)**	0.13±0.04 (71.74) ***	0.17±0.04 (63.04)***	0/6
10	20	0.21±0.04 (0.00)	0.33±0.04 (15.38)	0.3±0.03 (30.23) *	0.36±0.05 (18.18)	0.38±0.05 (17.49)	0.42±0.05 (8.70)	0/6
	40	0.17±0.04 (19.0)	0.26±0.04 (33.3)*	0.22±0.03 (48.8)**	0.21±0.05 (52.27) **	0.15±0.03 (67.39)***	0.22±0.04 (42.17)*	0/6
11	20	0.21±0.03 (0.00)	0.32±0.05 (17.95)	0.34±0.03 (20.93)	0.33±0.06 (25.00)	0.34±0.04 (26.09)	0.33±0.05 (28.26)	0/6
	40	0.19±0.02 (9.52)	0.31±0.05 (20.51)	0.31±0.03 (27.91)*	0.3±0.05 (31.82)*	0.29±0.03 (36.96)*	0.28±0.02 (39.13)*	0/6
12	20	0.2±0.04 (4.76)	0.3±0.04 (23.08)	0.36±0.04 (16.28)	0.35±0.05 (20.45)	0.32±0.06 (30.43)	0.28±0.03 (39.13)*	0/6
	40	0.18±0.03 (14.29)	0.29±0.05 (25.64)	0.3±0.02 (30.23) *	0.28±0.04 (36.36) *	0.27±0.03 (41.30)*	0.26±0.04 (43.48)*	0/6
13	20	0.19±0.03 (9.52)	0.29±0.06 (25.64)	0.35±0.05 (18.60)	0.36±0.05 (18.18)	0.34±0.05 (26.09)	0.34±0.04 (26.09)	0/6
	40	0.2±0.01 (4.76)	0.26±0.02 (33.33)*	0.31±0.02 (27.91)*	0.26±0.03 (40.91)*	0.26±0.05 (43.48)*	0.25±0.03 (45.65)**	0/6
15a	20	0.2±0.05 (4.76)	0.34±0.06 (12.82)	0.33±0.04 (23.26)	0.32±0.08 (27.27)	0.29±0.04 (36.96)*	0.32±0.05 (30.43)	0/6
	40	0.2±0.05 (4.8)	0.33±0.06 (15.4)	0.31±0.04 (27.9)	0.30±0.08 (31.82)	0.27±0.03 (40.22)*	0.28±0.05 (39.13)	0/6
15b	20	0.18±0.04 (14.29)	0.26±0.06 (33.33)	0.27±0.04 (37.21)*	0.26±0.03 (40.91)*	0.31±0.05 (32.61)	0.32±0.03 (30.43)*	0/6
	40	0.16±0.04 (23.8)	0.24±0.05 (38.5)*	0.2±0.04 (53.5)**	0.21±0.05 (52.27)**	0.21± 0.04 (54.35)**	0.24±0.01 (47.83)**	0/6
15c	20	0.21±0.04 (0.00)	0.32±0.03 (17.95)	0.26±0.05 (39.53)*	0.28±0.04 (36.36)*	0.28±0.02 (39.13)**	0.32±0.05 (30.43)	0/6
	40	0.16±0.04	0.27±0.03	0.22±0.02	0.23±0.05	0.22±0.02	0.23±0.03	0/6

	(23.8)*	(30.8)	(48.8)**	(47.73)**	(52.17) **	(50.0)*	
Indomethacin 20 mg/kg	0.13±0.03 (38.1)*	0.22±0.02 (43.6)**	0.21±0.01 (51.2)***	0.15±0.001 (65.1)***	0.08±0.005 (82.6)***	0.05±0.01 (80.8)*	1/6
Celecoxib 20 mg/kg	0.20±0.03 (4.76)	0.20±0.05 (48.72)**	0.17±0.04 (60.47)***	0.13±0.02 (70.45)***	0.08±0.03 (82.61)***	0.08±0.02 (82.61)***	0/6

Data presented as mean± S.E.M. (% of inhibition). n=5, *p<0.05;**p<0.01; ***p<0.001 as compared to control at respective time point.

It is interesting to note that compound **15a** with an unsubstituted nitrogen is found to be less active in phthalazinone series, which is in sharp contrast to the activity pattern observed in case of pyridazinone counterpart **5a**. In addition, none of the synthesized derivatives showed any symptoms of gastric lesions in rats and thus are free from ulcerogenic effects.

A good correlation was observed between anti-inflammatory and analgesic potential of the newly synthesized compounds. Both pyridazinone as well as phthalazinone analogues displayed moderate analgesic activity at 40 mg/kg. As given in table 2, the highest analgesic effect was observed for compound **5a** with 47.7% protection.

Table 2. Analgesic activity of various pyridazinones and standard drugs

	20 mg/kg		40 mg/kg	
	No. of writhes	% protection	No. of writhes	% protection
Control	35.4±1.2			
Indomethacin	13.3±1.3***	62.3		
Celecoxib	9.83±1.07***	72.16		
5a	26.8±1.4***	24.2	18.5±1.02***	47.7
5b	31.0±1.6	12.4	22.2±1.2**	37.3
5c	26.1±1.02***	26.0	21.5±1.5***	39.2
8	30.8±2.1	12.9	26.3±1.08**	25.6
10	29.66±1.7*	16.2	24.7±1.7***	30.3
11	32.5±1.2	8.2	26.8±2.14**	24.2
12	26.8±1.3***	18.5	23.0±1.1***	35.0
13	25.2±1.1***	28.9	20.2±1.5***	43.0
15a	31.5±2.5	11.0	27.8±1.4*	21.8
15b	32±1.0	9.6	22.5±2.05***	36.4
15c	32.2±1.6	8.6	19.7±1.9***	44.4

Significance against control group *p< 0.05, **p < 0.01, ***p <0.001. Values are given as mean ± SEM. n = 5.

A drop in percentage protection was observed for both series of compounds at 20 mg/kg in comparison to their effects at 40 mg/kg. Figures 3 and 4 show the comparison between inflammatory and writhing responses of various compounds at 20 and 40 mg/kg, respectively.

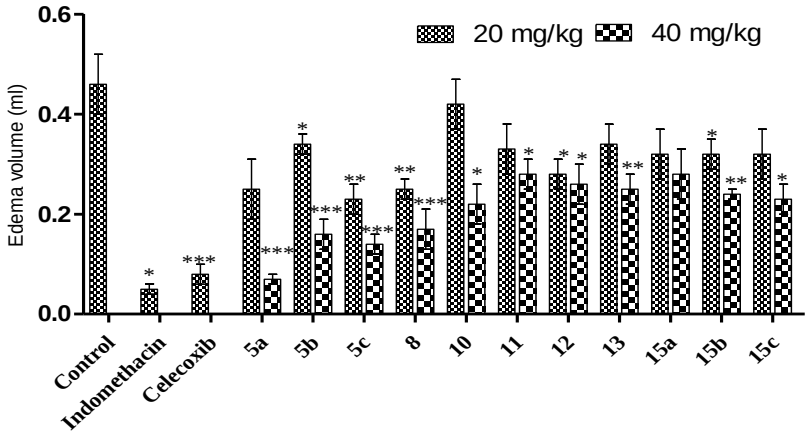


Figure 3: In paw edema test after oral ad s represented as mean±S.E.M Dunnett's test. *p<0.05, **p

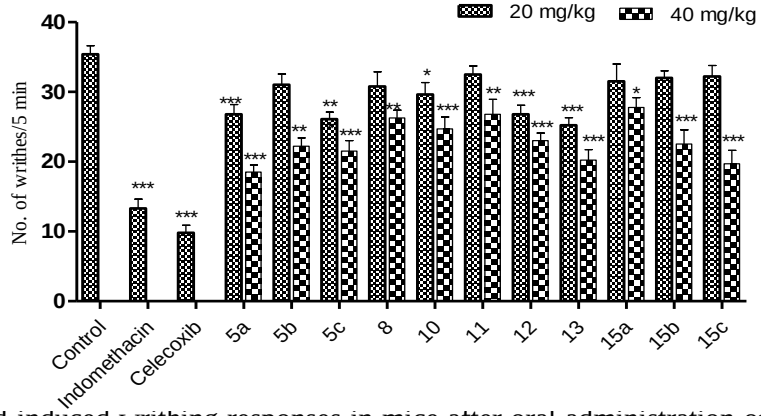


Figure 4: Acetic acid-induced writhing responses in mice after oral administration of test compounds at 20 and 40 mg/kg. Data is represented as mean±S.E.M. (n=5). Results were analyzed using one way ANOVA followed by post hoc Dunnett's test. *p<0.05, **p<0.01 and ***p<0.001 as compared to control value.

In-vitro COX inhibitory assay

It is known that carrageenan induced edema is a biphasic event where histamine, bradykinin and serotonin like mediators are involved in the first stage of inflammation. On the contrary, second stage of inflammation is inhibited by NSAIDs indicating contribution of COX enzyme (Dogruer *et al*. 2004, 303). In the present study, involvement of COX enzyme towards anti-inflammatory activity is expected as compounds **11**-**13** were found to be active even after 240 min of the oral administration in rat paw edema assay (table 1). This observation motivated us to study *in-vitro* COX enzyme inhibitory action of some selected compounds. Results of COX inhibitory screening assay are shown in table 3.

Table 3. *In vitro* COX-1 and COX-2 enzyme inhibition data of some selected compounds

Compd. No	%Inhibition (40 µM)[a]	
	COX-1	COX-2
11	19.65	21.28
13	93.93	91.44
Indomethacin	79.99	97.40
Celecoxib	6.44	98.18

[a]The determination was performed in duplicate for two independent experiments.

It is evident from *in vitro* assay results that intermediate ester **11** displayed little affinity for either form of cyclooxygenase enzyme, which is in strong agreement with its poor *in vivo* anti-inflammatory activity profile. In addition to this, we noticed that compound **13** having morpholine ring in side chain attached at N^2 of pyridazinone nucleus inhibited both the enzymes with similar potency.

3. EXPERIMENTAL

The m.p. reported are uncorrected, ^{1}H NMR spectra were recorded on Brucker AC-300F, 300 MHz instrument using Me_4Si (TMS) as the internal standard (chemical shifts in δ, ppm). The IR spectra were recorded on a Perkin–Elmer 882 and Perkin–Elmer spectrum RX 1, FT-IR spectrophotometer models using potassium bromide pellets (υ_{max} in cm^{-1}). Elemental analyses were carried out on a Perkin–Elmer-2400 model CHN analyzer. Plates for TLC were prepared with silica gel G according to Stahl's method (E. Merck) using EtOAc as solvent (activated at 110°C for 30 min) and were visualized by exposure to iodine vapours. Anhydrous sodium sulfate was used as drying agent. All solvents were dried and freshly distilled prior to use according to standard procedures. 4-(4-(Methanesulfonamido)phenyl)-4-oxobutyric acid (**6**), 3-methyl-4-(4-(methanesulfonamido)phenyl)-4-oxobutyric acid (**9**) and pyridazinones **5a-c** were synthesized according to previously reported procedure from our laboratory (Kumar *et al*. 2008, 393).

3.1. 4-(4-(Methanesulfonamido)-3-nitrophenyl)-4-oxobutyric acid (7)

The compound **6** (0.6 g, 2.21 mmol) was added in small portions to the ice cold solution of nitrating mixture of conc. HNO_3-H_2SO_4 (3:1, 6 ml) with constant stirring while maintaining the temperature at 0 ^oC. The stirring was further continued for 2 h. The reaction mixture was poured onto crushed ice. The precipitate obtained was filtered, washed thoroughly with cold water and dried.

Yield: 45.97%; mp 185-188 ºC; ir (KBr): NH 3281, 3089, 2934, CO 1743, 1697, 1533, 1349 cm^{-1}; ^{1}H nmr: δ 2.67 (t, 2H, -$COCH_2CH_2$COOH), 3.27 (s, 3H, -$NHSO_2CH_3$), 3.31 (t, 2H, -$COCH_2CH_2$COOH), 8.24 (d, 1H, Ar*H*, J_o = 8.72 Hz), 8.52 (dd, 1H, Ar*H*, J_o = 9.12Hz, J_m = 2.25 Hz), 8.63 (s, 1H, -N*H*SO_2CH_3), 8.87 (d, 1H, Ar*H*, J_m = 2.39 Hz), 10.08 (s, 1H, -COO*H*).

3.2. General procedure for synthesis of 6-(4-methanesulfonamidophenyl)-substituted-4,5-dihydropyridazin-3(2*H*)-ones (**8** and **10**)

To a stirred and refluxing solution of γ-keto acids **7** and **9** (3.8 mmol) in absolute alcohol, requisite hydrazine derivative was added. The reaction mixture was further refluxed with stirring for 7 h. The completion of the reaction was monitored by TLC. The resulting solution was concentrated to half of the volume and left overnight in refrigerator to afford crystals of corresponding dihydropyridazinone derivatives **8** and **10**. The crystals obtained were washed with cold ethanol, dried and recrystallized.

3.2.1. 6-(4-Methanesulfonamido-3-nitrophenyl)-4,5-dihydropyridazin-3(2*H*)-one (**8**)

Recrystallization: ethanol; yield: 38.23%; mp 230-232 ºC; ir (KBr): NH 3304, C-H, aromatic 3096, 2943, CO 1672, 1526, 1384, SO_2 1341, 1277, 863 cm^{-1}; ^1H nmr: δ 2.58 (t, 2H, 5-C*H*$_2$), 2.99 (t, 2H, 4-C*H*$_2$), 3.27 (s, 3H, -NHSO$_2$C*H*$_3$), 7.83 (d, 1H, Ar*H ortho* to methanesulfonamide, J_o = 8.82 Hz), 8.05 (dd, 1H, Ar*H meta* to methanesulfonamide, J_o = 8.82 Hz, J_m = 2.16 Hz), 8.48 (d, 1H, Ar*H ortho* to nitro, J_m = 2.01 Hz), 9.88 (s, 1H, -N*H*SO$_2$CH$_3$, disappeared on deuterium exchange), 10.69 (s, 1H, -N*H*, pyridazinone, disappeared on deuterium exchange); Anal. calcd. for $C_{11}H_{12}N_4O_5S$: C, 42.30; H, 3.87; N, 17.94. Found C, 41.99; H, 3.84; N, 17.93.

3.2.2. 2-(4-Fluorophenyl)-5-methyl-6-(4-methanesulfonamidophenyl)-4,5-dihydro-pyridazin-3(2*H*)-one (**10**)

Recrystallization: ethanol; yield: 48.23%; mp 215-217 ºC; ir (KBr): NH 3211, 2933, CO 1658, 1510, 1398, 1337 (SO_2), 1147, 969, 836 cm^{-1}; ^1H nmr: δ 1.38 (d, 3H, 5-CH(C*H*$_3$), J = 6.52 Hz), δ 2.78-2.81 (m, 2H, 4-C*H*$_2$), 3.05 (s, 3H, -NHSO$_2$C*H*$_3$), 3.13-3.19 (m, 1H, 5-C*H*(CH$_3$)), 6.67 (br(s), 1H, N*H*), 7.09 (t, 2H, Ar*H*, *ortho* to fluoro, J_o = 8.66 Hz), 7.25 (dd, 2H, Ar*H*, *ortho* to methanesulfonamide, J_o = 6.64 Hz, J_m = 1.88 Hz), 7.51-7.54 (m, 2H, Ar*H*, *meta* to fluoro) and 7.78 ppm (dd, 2H, Ar*H*, *meta* to methanesulfonamide, J_o = 6.84 Hz, J_m = 2.00 Hz); Anal. calcd. for $C_{18}H_{18}FN_3O_3S$: C, 57.59; H, 4.83; N, 11.19. Found C, 56.25; H, 4.49; N, 11.03.

3.3. 2-(Methoxycarbonylmethyl)-6-(4-methanesulphonamidophenyl)-4,5-dihydro-pyridazin-3(2*H*)-one (**11**)

Methyl chloroacetate (1 ml, 9.26 mmol) was added to a stirred and refluxing suspension of 6-(4-methanesulfonamidophenyl-4,5-dihydropyridazin-3(2*H*)-one (**5a**, 1g, 3.69 mmol) and anhydrous potassium carbonate (1.0 g) in ethyl methyl ketone (50 ml). The reaction mixture was further refluxed for 6 h with continuous stirring. The completion of the reaction was monitored by TLC. On completion, the reaction mixture was cooled, filtered and the excess solvent was removed under reduced pressure. Ice cold water was added to reaction mixture. The solid thus separated was filtered off, washed with water and dried.

Recrystallization: ethanol+hexane; yield: 48.33%; mp 188-190 ºC; ir (KBr): NH 3329, 3058, 2959, CO 1748, 1695, 1510, SO_2 1327, COC 1224, CN 1150, 967, 885 cm^{-1}; ^1H nmr: δ 2.48 (t, 2H, 4-C*H*$_2$, J = 8.08 Hz), 2.96 (t, 2H, 5-C*H*$_2$, J = 8.08 Hz), 3.09 (s, 3H, -NHSO$_2$C*H*$_3$), 3.71 (s, 3H, -COOC*H*$_3$), 4.50 (s, 2H, -NC*H*$_2$CO), 7.48 (d, 2H, Ar*H*, *ortho* to methanesulfonamide, J_o = 8.60 Hz), 7.76 (d, 2H, Ar*H*, *meta* to methanesulfonamide, J_o = 8.64 Hz) and 10.93 ppm (s, 1H, -N*H*); ms: m/z 340.1 $[M+1]^+$

3.4. General Procedure for synthesis of 6-(4-methanesulfonamidophenyl)-2-substituted-4,5-dihydropyridazin-3(2*H*)-ones (**12** and **13)**

A mixture of 2-(methoxycarbonylmethyl)-6-(4-methanesulphonamidophenyl)-4,5-dihydropyridazin-3(2*H*)-one (**11**, 0.5 g, 1.55 mmol) and piperidine (0.5ml, 5.87 mmol) or morpholine (0.5ml, 5.74 mmol) was heated at 100°C in DMF (*N*,*N*-dimethylformamide) (5 ml). The reaction mixture was further stirred with heating till completion of reaction as monitored by TLC. On completion, the reaction mixture was cooled and the excess of DMF was removed

under reduced pressure. The obtained residue was then recrystallized to obtain corresponding products **12** and **13**.

3.4.1. 6-(4-Methanesulfonamidophenyl)-2-(2-piperidin-1-yl-2-oxoethyl)-4,5-dihydro-pyridazin-3(2*H*)-one(12)

Recrystalization: ether; Yield: 41.19%; mp 212-214 ºC; ir (KBr): NH 3267, 3058, 2926, 1681, CO 1636, SO_2 1333, CN 1145, 959, 769 cm^{-1}; ^1H nmr: δ 1.65 (s(br), 6H, -(CH_2)$_3$-, piperidine), 2.61 (t, 2H, 4-CH_2, J = 8.48 Hz), 2.98 (t, 2H, 5-CH_2, J = 8.48 Hz), 3.19 (s, 3H, -$NHSO_2CH_3$), 3.34 (t, 2H, -NCH_2-, piperidine, J = 5.80 Hz), 3.55 (t, 2H, -NCH_2-, piperidine, J = 5.24 Hz), 4.57 (s, 2H, -NCH_2), 7.60 (d, 2H, Ar*H*, *ortho* to methanesulfonamide, J_o = 8.36 Hz), 7.71 (d, 2H, C*H*, Ar*H*, *meta* to methanesulfonamide, J_o = 8.36 Hz) and 8.49 ppm (s, 1H, -N*H*); Anal. calcd. for $C_{18}H_{24}N_4O_4S$: C, 55.08; H, 6.16; N, 14.28. Found C, 54.81; H, 6.99; N, 14.07.

3.4.2. 6-(4-Methanesulfonamidophenyl)-2-(2-morpholin-4-yl-2-oxoethyl)-4,5-dihydro-pyridazin-3(2*H*)-one (**13**)

Recrystalization: ether; yield: 41.15%; mp 218-220 ºC; ir (KBr): NH 3329, 3058, 2979, 1694, CO 1649, 1398, SO_2 1324, CN 1151, COC 1105, 963, 886 cm^{-1}; ^1H nmr : δ 2.52 (t, 2H, 4-CH_2, J = 8.52 Hz), 2.96 (t, 2H, 5-CH_2, J = 8.00 Hz), 3.14 (s, 3H, -$NHSO_2CH_3$), 3.47 (s(br), 2H, -NCH_2-, morpholine), 3.54 (s(br), 2H, -NCH_2-, morpholine), 3.63 (s(br), 4H, -(OCH_2)$_2$-, morpholine), 4.64 (s, 2H, -NCH_2), 7.51 (d, 2H, Ar*H*, *ortho* to methanesulfonamide, J_o = 8.68 Hz), 7.74 (d, 2H, Ar*H*, *meta* to methanesulfonamide, J_o = 8.68 Hz) and 10.84 ppm (s, 1H, -N*H*); Anal. calcd. for $C_{17}H_{22}N_4O_5S$: C, 51.76; H, 5.62; N, 14.20. Found C, 51.61; H, 5.45; N, 14.09.

3.5. 2-(4-Methanesulfonamidobenzoyl)benzoic acid (**14**)

A mixture of *N*-phenylmethanesulfonamide (3.0 g, 17.50 mmol) and phthalic anhydride (2.6 g, 17.50 mmol) was added to a stirred solution of aluminium chloride (10 g) in purified carbon disulphide (20 ml). The reaction mixture was stirred manually under the anhydrous conditions for 20 min and was allowed to stand at room temperature for 48 h. The mixture was decomposed with crushed ice. The solid product obtained was filtered and washed thoroughly with distilled water. The residual solid was dissolved in 5% aqueous sodium bicarbonate solution and filtered off insoluble part. Acidification with concentrated hydrochloric acid afforded precipitate, which was filtered, washed with ice cold water and dried. The solid obtained was sufficiently pure for further reaction.

Recrystallization: methanol; yield: 36.30%; m.p.: 180-182 °C; ir (KBr): NH 3234, 3016, 2928, CO 1709, 1661, 1284 cm^{-1}; ^1H nmr: δ 3.03 (s, 3H, -$NHSO_2CH_3$), 7.30 (m, 3H, Ar*H*), 7.56 (dd, 2H, Ar*H*, *ortho* to methanesulfonamide, J_o = 7.33 Hz, J_m = 1.14 Hz), 7.63 (m, 2H, Ar*H*, *meta* to methanesulfonamide), 8.03 (d, 1H, Ar*H*, J_o = 7.35 Hz) and 10.11 ppm (s, 1H, -N*H*SO_2CH_3, disappeared on deuterium exchange).

3.6. General procedure for the synthesis of *N*-[4-(3-substituted-4-oxo-1-phthalazinyl)phenyl]methanesulfonamides (15a-c)

Requisite hydrazine derivative was added to a stirred and refluxing solution of 2-(4-methanesulfonamidobenzoyl)benzoic acid (**14**, 0.5 g, 1.56 mmol) in aldehyde free ethanol. The reaction mixture was further refluxed with stirring for 7 h. The completion of the reaction was monitored by TLC. On completion, the resulting solution was concentrated and left overnight in refrigerator to afford crystals of corresponding phthalazinone derivative **15a-c**. The crystals obtained were washed with cold ethanol, dried and recrystallized from suitable solvent.

3.6.1. *N*-[4-(4-oxo-1-phthalazinyl)phenyl]methanesulfonamide (15a)

Recrystallization: methanol; yield: 61.22%; mp 290-292 ºC; ir (KBr): NH 3248, 3017, 2896, CO 1681, 1609, 1516, 1419, SO_2 1336 cm^{-1}; ^1H nmr: δ 3.02 (s, 3H, -$NHSO_2CH_3$), 7.42 (dd, 2H, Ar*H*, *ortho* to methanesulfonamide, J_o = 6.62 Hz, J_m = 1.76 Hz), 7.53 (dd, 2H, Ar*H*, *meta* to methanesulfonamide J_o = 8.65 Hz, J_m = 2.28 Hz), 7.77 (m, 3H, Ar*H*, phthalazinone), 8.48 (m, 1H, Ar*H*, phthalazinone), 9.62 (s, 1H, -N*H*SO_2CH_3) and 11.77 ppm (s, 1H, -N*H*, phthalazinone, disappeared on deuterium exchange); Anal. calcd. for $C_{15}H_{13}N_2SO_3$: C, 57.13; H, 4.15; N, 13.33. Found C, 57.11; H, 4.13; N, 13.30.

3.6.2. *N*-[4-(3-phenyl-4-oxo-1-phthalazinyl)phenyl]methanesulphonamide (15b)

Recrystallization: ethanol; yield: 30.55%; mp 235-238 ºC; ir (KBr): 3157, CO 1643, 1607, 1513, 1492, SO_2 1338, CN 1153, 788 cm^{-1}; ^1H nmr: δ 3.03 (s, 3H, -$NHSO_2CH_3$), 7.39 (d, 1H, Ar*H*), 7.47 (m, 4H, Ar*H*), 7.60 (d, 2H, Ar*H*, *ortho* to methanesulfonamide, J_o = 8.39 Hz), 7.69 (d, 2H, Ar*H*, *meta* to methanesulfonamide J_o = 6.66 Hz), 7.84 (m, 3H, Ar*H*, phthalazinone), 8.54 (m, 1H, Ar*H*, phthalazinone) and 9.58 ppm (s, 1H, -N*H*SO_2CH_3); Anal. calcd. for $C_{21}H_{17}N_3SO_3$: C, 64.43; H, 4.38; N, 10.73. Found C, 64.40; H, 4.34; N, 10.69.

3.6.3. *N*-[4-{3-(4-fluorophenyl)-4-oxo-1-phthalazinyl}phenyl]methanesulphonamide (15c)

Recrystallization: ethanol; yield: 33.70%; mp 239-241 ºC; ir (KBr): NH 3238, CO 1656, 1509, SO_2 1332, 1147, 839 cm^{-1}; ^1H nmr: δ 3.02 (s, 3H, -$NHSO_2CH_3$), 7.16 (m, 2H, Ar*H*, *N*-*p*-fluorophenyl), 7.45 (dd, 2H, Ar*H*, *ortho* to methanesulfonamide, J_o = 6.69 Hz, J_m = 1.94 Hz), 7.58 (dd, 2H, Ar*H*, *meta* to methanesulfonamide J_o = 6.66 Hz, J_m = 1.96 Hz), 7.71 (m, 2H, Ar*H*, *N*-*p*-fluorophenyl), 7.82 (m, 3H, Ar*H*, phthalazinone), 8.55 (m, 1H, Ar*H*, phthalazinone) and 9.75 ppm (s, 1H, -N*H*SO_2CH_3); Anal. calcd. for $C_{21}H_{16}N_3SO_3F$: C, 61.60; H, 3.93; N, 10.26. found C, 61.58; H, 3.92; N, 10.28.

3.7. Biological evaluation

3.7.1. Anti-inflammatory activity

All the synthesized derivatives were evaluated for anti-inflammatory activity using carrageenan induced hind paw edema model (Abouzid *et al*. 2008, 5547) in male wistar rats (120-130 g). Animals were provided with regular rodent pellet diet (Ashirwad Industries, Chandigarh) and purified water ad libitum. The food was withdrawn one day before the experiment, but allowed free access to water. The experimental study protocol was duly approved by institutional animal ethics committee (IAEC), Panjab University and strictly carried out in accordance with the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Government of India. Acute edema in the hind paws of the rats was induced by injecting 0.1 ml of freshly prepared 1% solution of carrageenan in distilled water under the plantar aponeurosis of right hind paw. The suspensions of 20 and 40 mg/kg of the respective compounds, uniformly dispersed in distilled water by adding 0.1 ml of Tween 80, were given to test animals orally an hour prior to the administration of carrageenan. The control group received the same experimental handling as test group except that equivalent doses of vehicle alone were administered by the same route in place of test compounds. The paw volumes were measured using plethysmometer (UGO BASILE) before and after 30, 60, 90, 120, 180 and 240 min of injecting carrageenan. Indomethacin was used as the standard anti-inflammatory drug. The percent inhibition of inflammation was calculated using following formula:

$$\%\ inhibition\ of\ inflammation = 100[1 - \frac{a - x}{b - y}]$$

where x and a are the mean foot volumes of the rats before and after the administration of carrageenan injection respectively, treated with test compounds or standard drug, whereas y and b are the mean foot volumes of the rats before and after the administration of carrageenan respectively, in the control group. Animals were also observed for 24 h and the mortality rate was recorded for each group at the end of the observation period.

3.7.2. Analgesic activity

Anti-nociceptive activity of compounds against noxious chemical stimuli was evaluated by acetic acid-induced writhing test in mice (20-25 g) as described previously (Umide *et al*. 2011, 152). Sixty min after the p.o. administration of saline or compounds, mice were treated with an aqueous solution of acetic acid (0.6% v/v, i.p.) at 10 ml/kg to induce contractions. After 5 min, the number of abdominal constrictions and stretches during the following 10 min were recorded. After treatments, a significant reduction in the number of writhes was considered as a positive anti-nociceptive response. The percentage protection against writhing was calculated according to following equation:

$$Protection\ \% = \frac{(control\ mean - treated\ mean)}{Control\ mean} \times 100$$

3.7.3. Gastric ulcerogenic effect

Rats were killed under deep ether anesthesia 24 h after the anti-inflammatory experiment, and their stomachs were removed. The abdomen of each rat was opened through great curvature and examined for lesions or bleedings using a hand lens. For each stomach the mucosal damage was assessed according to the following scoring system: 0.5: redness; 1.0: spot ulcers; 1.5: hemorrhagic streaks; 2.0: ulcers >3 but ≤5; 3.0: ulcers >5. The mean score of each treated group minus the mean score of the control group was regarded as severity index of the gastric mucosal damage (Chan *et al*. 1995, 1531).

3.7.4. *In-vitro* COX inhibitory activity

The inhibitory activity of selected compounds **11** and **13** on COX-1 and COX-2 was assayed using the COX Inhibitor Screening Assay Kit (Cayman No: 560101) according to procedure mentioned by supplier. Screening of the selected compounds and references (indomethacin and celecoxib) was performed at 40 μM to determine the percent inhibition of the COX-1 and COX-2 isoforms.

3.7.5. Statistical analysis

Data obtained from the animal experiments were expressed as the mean standard error (±SEM). Statistical differences between the treatments and the control were tested by ANOVA. Data with $p < 0.05$ value was considered to be significant.

4. CONCLUSION

Development of a new series of 6-(4-methanesulphonamidophenyl)-substituted dihydro-pyridazinone and phthalazinone derivatives as potential anti-inflammatory and analgesic agents is presented in this work. 6-(4-Methanesulfonamidophenyl)-4,5-dihydropyridazin-3(2*H*)-one (**5a**), previously reported as a cardiotonic agent, showed good analgesic and anti-inflammatory profile at 40 mg/kg. The compound was found almost equipotent to standard drug indomethacin. In general, all pyridazinone derivatives showed better anti-inflammatory and analgesic potential than their phthalazinone counterparts. The study indicates the possibility of developing molecules with multiple beneficial effects such as anti-inflammatory, analgesic and desired cardiotonic activities within the same molecule using pyridazinone core structure.

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6. CONFLICT OF INTEREST

The authors have declared no conflict of interest.

7. REFERENCES

Abouzid, K.; Bekhit, S. "Novel anti-inflammatory agents based on pyridazinone scaffold; design, synthesis and in vivo activity." *Bioorg Med Chem* 16: (2008) 5547-56.

Asif, M.; Singh, A. "Exploring potential, synthetic methods and general chemistry of pyridazine and pyridazinone: a brief introduction." *Int J Chem Tech Res* 2(2) (2010): 1112-28.

Bansal, R.; Thota, S. "Pyridazin-3(2*H*)-ones: the versatile pharmacophore of medicinal significance." *Med Chem Res* 22 (2013): 2539-52.

Chan, C. C.; Boyce, S.; Brideau, C.; Ford-Hutchinson, A. W.; Gordon, R.; Guay, D.; Hill, R. G.; Li, C. S.; Mancini, J.; Penneton, M. "Pharmacology of a selective cyclooxygenase-2 inhibitor, L-745,337: a novel nonsteroidal anti-inflammatory agent with an ulcerogenic sparing effect in rat and nonhuman primate stomach." *J Pharmacol Exp Therap* 274 (1995): 1531-37.

Dannhardt, G.; Kiefer, W. "Cyclooxygenase inhibitors--current status and future prospects." *Eur J Med Chem* 36 (2001): 109-26.

Dogruer, D. S.; Kupeli, E.; Yeşilada, E.; Sahin, M. F. "Synthesis of new 2-[1(2*H*)-phthalazinon-2-yl]acetamide and 3-[1(2*H*)-phthalazinon-2-yl]propanamide derivatives as antinociceptive and anti-inflammatory agents." *Arch Pharm Pharm Med Chem* 337 (2004): 303-10.

Dogruer, D. S.; Sahin, M. F.; Küpeli, E.; Yeşilada, E. "Synthesis and analgesic and anti-inflammatory activity of new pyridazinones." *Turk J Chem* 27 (2003): 727-38.

Garcia-Nieto, R.; Perez, C.; Checa, A.; Gago, F. "Molecular model of the interaction between nimesulide and human cyclooxygenase-2." *Rheumatology* 38 (1999): 14-18.

Kagayama, K.; Morimoto, T.; Nagata, S.; Katoh, F.; Zhang, X.; Inoue, N.; Hashino, A.; Kageyama, K.; Shikaura, J.; Niwa, T. **"**Synthesis and biological evaluation of novel phthalazinone derivatives as topically active phosphodiesterase 4 inhibitors." *Bioorg Med Chem* 17 (2009): 6959-70.

Khaled, A. M. A.; Nadia, A. K.; Eman, M. A.; Hekmat, A. A.; Moustafa, E. E. "Structure-based molecular design, synthesis, and in vivo anti-inflammatory activity of pyridazinone derivatives as nonclassic COX-2 inhibitors." *Med Chem R*es 19 (2010): 629-42.

Kumar, D.; Carron, R.; Calle, C. D. L.; Jindal, D. P.; Bansal, R. "Synthesis and evaluation of 2-substituted-6-phenyl-4,5-dihydropyridazin-3(2*H*)-ones as potent inodilators." *Acta Pharm* 58 (2008): 393-405.

Margaretha, V. M.; Armin, H.; Ivonne, J. V. L.; Geert, J. S.; Ulric, T.; Hendrik, T. Novel selective PDE4 inhibitors. "1. Synthesis, structure-activity relationships, and molecular modeling of 4-(3,4-dimethoxyphenyl)-2*H*-phthalazin-1-ones and analogues." *J Med Chem* 44 (2001): 2511-22.

Nakamura, K.; Tsuji, K.; Konishi, N.; Okumura, H.; Matsuo, M. "Studies on antiinflammatory agents. II. Synthesis and pharmacological properties of 2'-(phenylthio)methanesulfonanilides and related derivatives." *Chem Pharm Bull* 41 (1993): 894-906.

Sato, M.; Ishizuka, Y.; Yamaguchi, A. "Pharmacological investigations of 4-ethoxy-2-methyl-5-morpholino-3(2*H*)-pyridazinone (M 73101), a new analgesic and anti-inflammatory drug." *Arzneim.-Forsch Drug Res* 31 (1981): 1738-45.

Sato, M.; Yamaguchi, A. **"**Pharmacological investigations of 4-ethoxy-2-methyl-5-morpholino-3(2*H*)-pyridazinone (M 73101), a new analgesic and anti-inflammatory drug." *Arzneim Forsch* 32 (1982): 379-82.

Sircar, I.; Weishaar, R. E.; Kobylarz, D.; Moos, W. H.; Bristol, J. A. "Cardiotonic agents. 7. Inhibition of separated forms of cyclic nucleotide phosphodiesterase from guinea pig cardiac muscle by 4,5-dihydro-6-[4-(1*H*-imidazol-1-yl)phenyl]-3(2*H*)-pyridazinones and related compounds. Structure-activity relationships and correlation with in vivo positive inotropic activity." *J Med Chem* 30 (1987): 1955-62.

Umide, D. O.; Yusuf, O.; Ozgur, D. C. "Synthesis and analgesic effects of 2-(2-carboxyphenylsulfanyl)-*N*-(4-substitutedphenyl) acetamide derivatives." *Med Chem Res* 20 (2011): 152-57.

Vane, J. R. "The fight against rheumatism: from willow bark to COX-1 sparing drugs." *J Physiol Pharmacol* 51 (2000): 573-86.

Vane, J. R. "Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs." *Nature (New Biology)* 231 (1971): 232-35.

Whittle, B. J. R.; Boughton-Smith, N. K.; Moncada, S.; Vane, J. R. "Actions of prostacyclin (PGI_2) and its product, 6-oxo-$PGF_{1\alpha}$ on the rat gastric mucosa *in vivo* and *in vitro.*" *Prostaglandins* 15 (1978): 955-68.