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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of 2-substituted-4-aryl-6-phenylpyridazin-3(2*H*)-ones as potential anti-inflammatory and analgesic agents with cardioprotective and ulcerogenic sparing effects

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Abstract Several new 2-substituted-4-aryl-6-phenylpyridazin-3(2*H*)-ones were synthesized and evaluated for in vivo anti-inflammatory and analgesic activities and possible in vitro COX-2 selectivity. To critically evaluate the possibility of deleterious effects of newly synthesized agents on normal hemostasis, anti-platelet activity and whole blood clotting time were also assessed. The structures of the synthesized compounds were confirmed on the basis of spectral data and elemental analysis. The present study has led to the identification of 4-(4-methoxyphenyl)-2-(2-(4-phenylpiperazin-1-yl)-2-oxoethyl)-6-phenylpyridazin-3(2*H*)-one (**17**) as an ideal anti-inflammatory agent with good affinity and remarkable selectivity for COX-2 enzyme without any ulcerogenic and cardiovascular side effects. In addition, at lower doses all the pyridazinone derivatives do not seem to affect normal hemostatic balance.

Keywords Pyridazin-3(2*H*)-one · Anti-inflammatory · Analgesic · Hemostasis · Cyclooxygenase

Introduction

Inflammation can originate from both infectious and non-infectious processes of chronic injury or irritation (Gu *et al*., 2007). The majority of currently known nonsteroidal anti-inflammatory and analgesic drugs (NSAIDs) cause serious gastrointestinal side effects. The GIT damage from NSAIDs is because of non-selective inhibition of both COX-1 and COX-2. Thus, the selective inhibition of COX-2 over COX-1 is a useful strategy for development of new agents for treating inflammation. However, increasing selectivity for COX-2 also results in increased cardiovascular toxicity (Dannhardt and Kiefer, 2001). Therefore, search for more effective and less toxic drugs to treat inflammation has become mandatory.

Pyridazines represent an important class of biologically active compounds. Among pyridazine derivatives, 3(2*H*)-pyridazinones form an important class of compounds due to their diverse pharmacological properties. Easy functionalization of various ring positions of pyridazinone core structure makes it an attractive synthetic and therapeutic target for designing and synthesis of new drugs (Bansal and Thota, 2013). The phenomenal research efforts to develop pyridazinone-based anti-inflammatory agents have led to many interesting findings (Cesari *et al*., 2006). Emorfazone (Fig. 1), a 3(2*H*)-pyridazinone derivative, has been launched in Japan as an analgesic drug without any gastric complications (Takaya *et al*., 1979). This encouraged us to synthesize and assess new pyridazinone-based chemical entities for anti-inflammatory potential.

There has been much focus recently in respect to cardiovascular risks associated with long-term usage of both non-selective and selective NSAIDs (Chelucci *et al*., 2014). In 2007, the American Heart Association published a focused update discouraging the use of NSAIDs for

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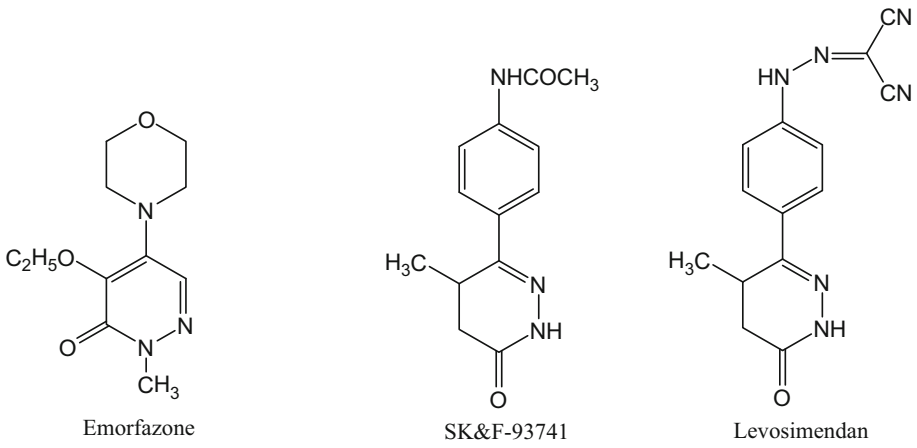


Fig. 1 Pyridazinone-based anti-inflammatory and vasodilatory agents

patients with established cardiovascular disease (Olsen *et al*., 2012). Keeping in mind the good properties of pyridazinone-based compounds (SK&F-93741 and levosimendan, Fig. 1) toward controlling cardiovascular diseases, particularly as vasodilatory and anti-platelet agents (Sur *et al*., 2003), we envisaged new structural modifications of the pyridazinone nucleus to produce derivatives with good anti-inflammatory potential without any cardiovascular toxicity.

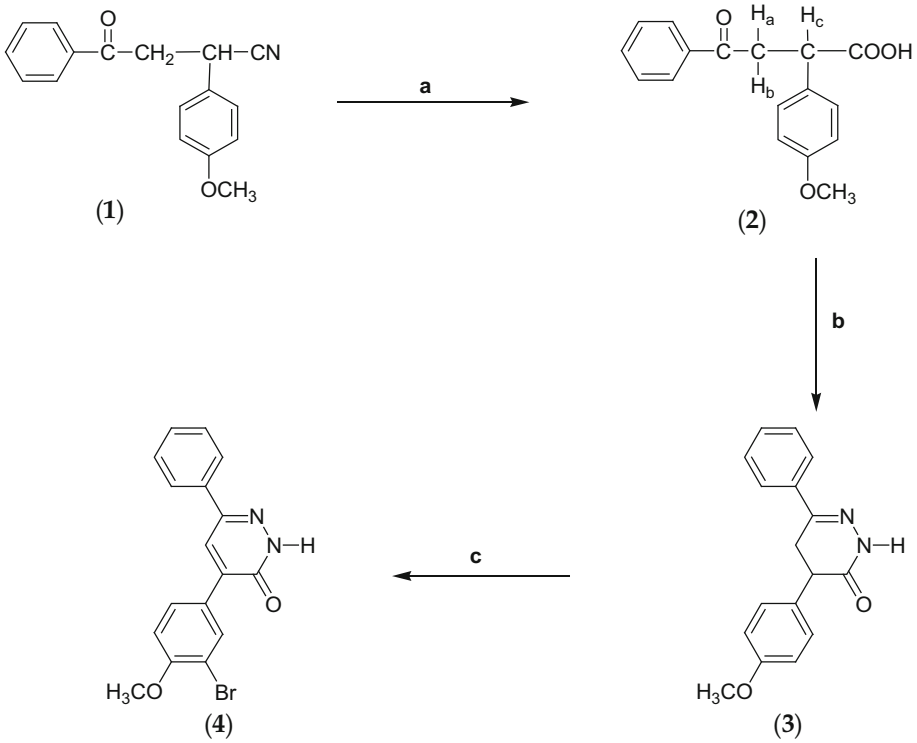
Results and discussion

Chemistry

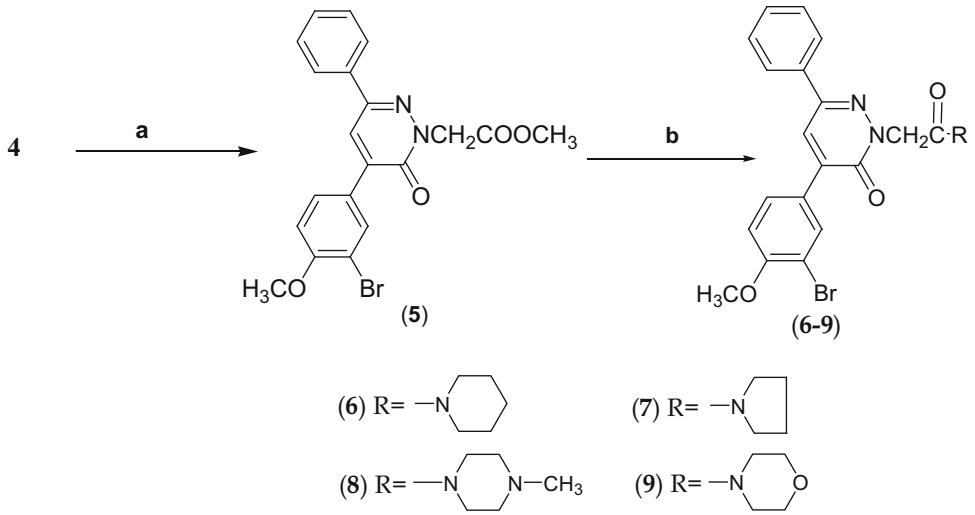
2-(4-Methoxyphenyl)-4-phenyl-4-oxobutyronitrile (**1**) was synthesized according to the reported procedure (Ellis *et al*., 1997). Hydrolysis of nitrile **1** in refluxing hydrochloric acid (10 N) yielded 2-(4-methoxyphenyl)-4-oxobutanoic acid (**2**). The formation of the acid was confirmed using ^{1}H NMR spectral analysis. For γ-keto acid **2**, NMR signals at δ 3.27 (dd, 1H, –COC(*H*)H–), 3.80 (s, 3H, –OCH_3), 3.87 (dd, 1H, –COC(H)*H*) and 4.25 ppm (dd, 1H, –COCH_2C*H*COOH–) were observed. Subsequent cyclization reaction of γ-keto acid **2** using hydrazine hydrate in 1-butanol afforded 4,5-dihydropyridazinone derivative **3**. Double doublets of *germinal* and *vicinal* protons of 4-C*H* and 5-CH_2 of pyridazinone ring appeared separately at δ 3.19 (dd, 1H, 5–C(*H*)H, pyridazinone, J_{gem} = 16.92 Hz, J_{vic} = 9.4 Hz), 3.29 (dd, 1H, 5–C(H)*H*, pyridazinone, J_{gem} = 16.9 Hz, J_{vic} = 7.18 Hz) and 3.79 ppm (m, 4H, –OCH_3 and 4–C*H*, pyridazinone). A signal for 2-unsubstituted –N*H* of dihydropyridazinone ring was observed at δ 8.89 ppm. The formation of pyridazinone nucleus was attempted by dehydrogenating the 4 and 5 positions of dihydropyridazinone **3** using bromine–acetic acid (Coudert *et al*., 1988). However, activation of the aryl ring due to the presence of *p*-methoxy electron-donating substituent led to bromination of aromatic ring by electrophilic substitution along with unsaturation at desired position to obtain compound 4-(3-bromo-4-methoxyphenyl)-6-phenylpyridazin-3(2*H*)-one (**4**) as shown in Scheme 1, the structure of which was confirmed by various spectral analyses. Unsaturated 5–C*H* proton appeared downfield at δ 7.78 in the nuclear magnetic resonance spectrum of compound **4** in comparison with its saturated counterpart **3**, where it appeared at 3.29 ppm. The presence of bromine *ortho* to –OCH_3 functionality was further confirmed by ^{1}H NMR analysis by appearance of doublet of *ortho* coupled aromatic protons at δ 7.85 with J_o = 7.38 Hz, while *meta* coupled aromatic proton *ortho* to bromo group resonated downfield at 8.13 ppm (J_m = 2.16 Hz).

The presence of an acetamide side chain on lactam nitrogen of pyridazinone ring has been reported to positively modulate the anti-inflammatory activity of the compounds with less gastrointestinal complications (Sahina *et al*., 2004; Banoglu *et al*., 2004; Süküroglu *et al*., 2005); therefore, introduction of an acetamide linker was planned. Pyridazinone derivative **4** was treated with methyl chloroacetate in ethyl methyl ketone to afford 4-(3-bromo-4-methoxyphenyl)-2-(methoxycarbonylmethyl)-6-phenylpyridazin-3(2*H*)-one (**5**) as depicted in Scheme 2. Additional singlets for –NCH_2 at δ 5.04 and –COOCH_3 at 3.95 ppm appeared in the proton nuclear magnetic spectrum of compound **5**. The presence of a prominent $[MH+2]^+$ peak almost equal in intensity to that of the molecular ion peak $[MH]^+$ was also observed in the mass spectrum of product **5**, which is in agreement with the proposed structure.

Thermal fusion of 2-(methoxycarbonylmethyl)-substituted pyridazinone derivative **5** with heterocyclic amines such as morpholine, *N*-methylpiperazine, piperidine and pyrrolidine yielded desired 2-substituted-6-phenylpyri-



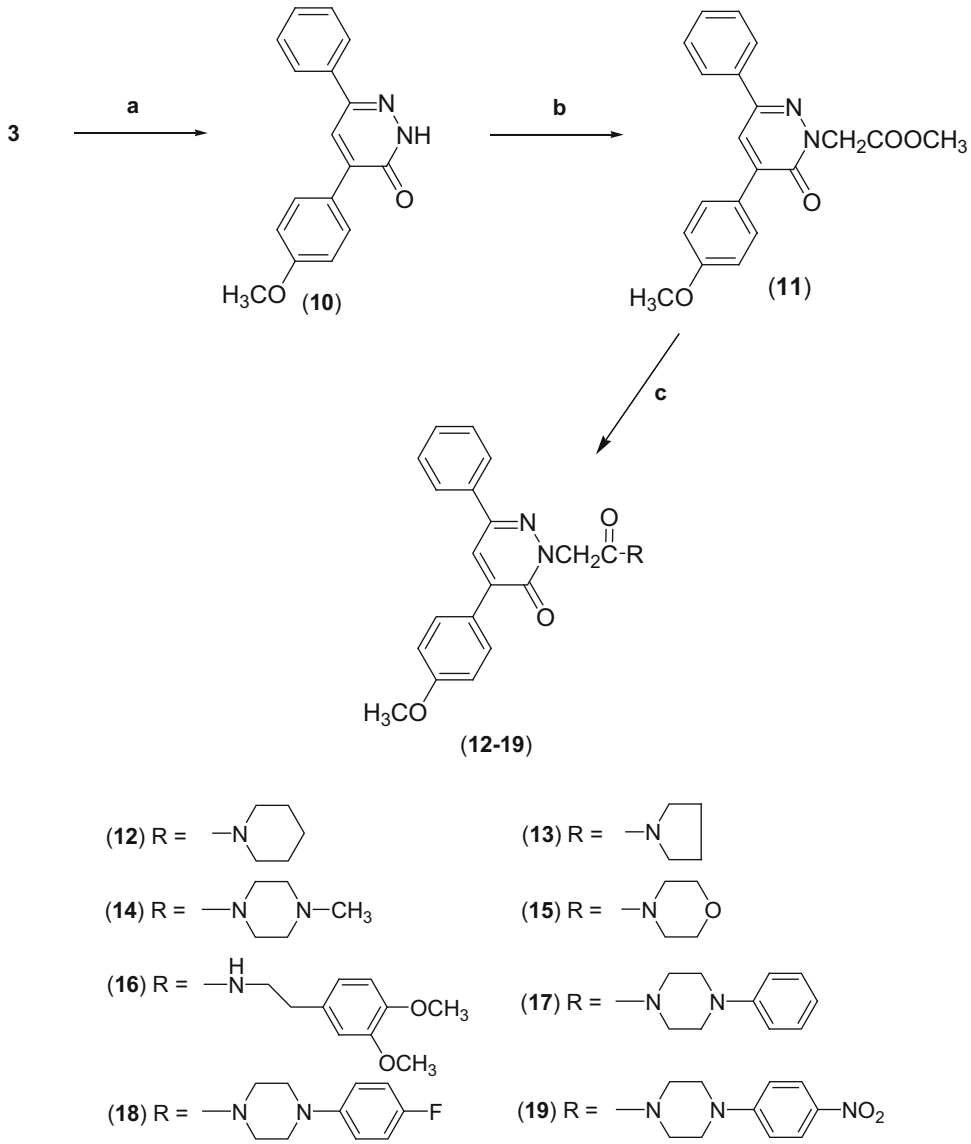
Scheme 1 Synthetic route to the formation of 4-(3-bromo-4-methoxyphenyl)-6-phenylpyridazin-3(2*H*)-one (**4**). Reagents and reaction conditions: **a** 10 N HCl, reflux; **b** *n*-butanol, hydrazine hydrate, reflux; **c** acetic acid, bromine, heat



Scheme 2 Synthetic route to the formation of pyridazinones **5**–**9**. Reagents and reaction conditions: **a** methyl chloroacetate, anhyd. K_2CO_3, ethyl methyl ketone; **b** requisite amine, heating

dazin-3(2*H*)-ones (**6**–**9**). A characteristic amidic shift to a lower wave number ~ 1649 was observed for carbonyl absorption bands in infrared spectra of compounds **6**–**9** in comparison with parent ester **5**, where it appeared at a higher frequency 1751 cm^{-1}. Almost equal intensity $[MH+2]^+$ and $[MH]^+$ peaks due to the presence of molecular ions containing Br^{81} and Br^{79} isotopes were prominently present in the mass spectra of all these derivatives **6**–**9**. This further confirmed the presence of bromine in these compounds.

The observation that bromination of phenyl ring occurs while introducing double bond at 4,5 positions of dihydropyridazinone nucleus by using bromine–acetic acid led us to adopt an alternative synthetic pathway (Sotelo *et al*., 2002) for aromatization to prepare targeted pyridazinones (Scheme 3). Copper (II) chloride was reported by Sotelo et al. as an extremely efficient and versatile reagent capable of carrying out successful aromatization of 4,5-dihydropyridazin-3(2*H*)-ones (Sotelo and Ravina, 2000). Consequently to serve the purpose, the intermediate **3** was



Scheme 3 Synthetic route to the formation of pyridazinones **11–19**. Reagents and reaction conditions: **a** acetonitrile, copper (II) chloride, reflux; **b** methyl chloroacetate, ethyl methyl ketone, anhyd. K_2CO_3; c requisite amine, fusion

reacted with copper (II) chloride in the presence of acetonitrile to afford compound **10**. A downfield singlet of 5-C*H* of pyridazinone (δ 7.81 ppm) was observed in the nuclear magnetic resonance spectrum of compound **10**. As mentioned earlier, substitution at N^2 position to afford pyridazinone methyl ester **11** and subsequent thermal fusion with requisite amines afforded aimed derivatives **12–19**.

A characteristic shift of vibrational frequency of carbonyl stretch of ester to lower wave number $\sim$ 1660 cm^{-1} due to carbonyl stretch of tertiary amides was observed. Two downfield signals at $\sim\delta$ 160 and 166 ppm representing carbonyl carbons were observed in ^{13}C NMR of all the final products.

Biological evaluation

Anti-inflammatory activity

The 2-substituted-4-aryl-6-phenylpyridazin-3(2*H*)-ones **5–9** and **11–19** were evaluated for anti-inflammatory activity using carrageenan-induced hind paw edema model (Abouzid and Bekhit, 2008) in male Wistar rats.

The compounds at 20 and 40 mg/kg were given orally as suspensions, and activity profile was compared with standard drugs indomethacin and celecoxib (20 mg/kg). Additionally, all compounds were also investigated for ulcerogenic side effects. The results of the studies are summarized in Table 1.

Table 1 Anti-inflammatory activity and gastric ulcer index (G.I.) of various newly synthesized pyridazinones and standard drugs

Compd. no.	Dose (mg/kg)	Edema volume (ml) ± SEM (% inhibition)						G.I.
		30 min	60 min	90 min	120 min	180 min	240 min	
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	0/5
5	20	0.21 ± 0.04	0.38 ± 0.04	0.37 ± 0.02	0.38 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0/5
		(0)	(2.56)	(13.9)	(13.64)	(19.57)	(19.57)	
	40	0.22 ± 0.04	0.36 ± 0.05	038 ± 0.05	0.35 ± 0.03	0.32 ± 0.03	0.30 ± 0.04	0/5
		(−4.7)	(7.69)	(11.63)	(20.45)	(30.43)*	(34.78)*	
6	20	0.20 ± 0.04	0.30 ± 0.05	0.32 ± 0.06	0.34 ± 0.05	0.32 ± 0.03	0.31 ± 0.04	0/5
		(4.76)	(23.08)	(25.58)	(22.73)	(30.43)*	(32.61)	
	40	0.24 ± 0.02	0.32 ± 0.04	0.31 ± 0.03	0.27 ± 0.06	0.25 ± 0.04	0.23 ± 0.04	0/5
		(−14.29)	(17.95)	(27.91)	(38.64)	(45.65)**	(50.00)**	
7	20	0.21 ± 0.02	0.35 ± 0.04	0.34 ± 0.02	0.37 ± 0.06	0.33 ± 0.04	0.30 ± 0.02	0/5
		(0)	(10.26)	(20.93)	(15.91)	(28.26)	(34.78)	
	40	0.18 ± 0.01	0.27 ± 0.05	0.25 ± 0.04 (41.86)**	0.23 ± 0.03	0.23 ± 0.04	0.22 ± 0.05	0/5
		(14.29)	(30.77)		(47.73)**	(50.0)**	(52.17)**	
8	20	0.22 ± 0.05	0.36 ± 0.04	0.38 ± 0.07	0.38 ± 0.04	0.38 ± 0.02	0.36 ± 0.06	0/5
		(−4.76)	(7.69)	(11.63)	(13.64)	(17.36)	(21.74)	
	40	0.23 ± 0.05	0.28 ± 0.05	0.26 ± 0.04	0.25 ± 0.06	0.25 ± 0.04	0.23 ± 0.03	0/5
		(−9.52)	(28.21)	(39.53)	(43.18)*	(45.65)**	(50.00)*	
9	20	0.19 ± 0.0	0.32 ± 0.05	0.34 ± 0.04	0.33 ± 0.06	0.32 ± 0.03	0.30 ± 0.04	0/5
		(9.52)	(17.95)	(20.93)	(25.00)	(30.43)	(34.78)*	
	40	0.17 ± 0.04 (19.05)	0.29 ± 0.03	0.28 ± 0.05	0.26 ± 0.05	0.26 ± 0.04	0.24 ± 0.03	0/5
			(25.64)	(34.88)	(40.91)*	(43.48)	(47.83)**	
11	20	0.21 ± 0.05	0.34 ± 0.04	0.37 ± 0.07	0.35 ± 0.04	0.32 ± 0.05	0.31 ± 0.06	0/5
		(0)	(12.8)	(13.9)	(20.45)	(30.43)	(32.61)	
	40	0.22 ± 0.04	0.35 ± 0.06	0.36 ± 0.04	0.32 ± 0.06	0.27 ± 0.04	0.27 ± 0.04	0/5
		(−4.7)	(10.2)	(16.2)	(27.27)	(41.3)*	(41.30)*	
12	20	0.21 ± 0.04	0.35 ± 0.0	0.32 ± 0.06	0.29 ± 0.05	0.28 ± 0.04	0.26 ± 0.04	0/5
		(0)	(10.26)	(25.58)	(34.09)	(39.13)*	(43.48)*	
	40	0.21 ± 0.03	0.3 ± 0.04	0.27 ± 0.04	0.21 ± 0.05	0.16 ± 0.03	0.15 ± 0.05	0/5
		(0)	(23.08)	(37.21)	(52.27)**	(65.22)***	(67.39)**	
13	20	0.19 ± 0.0	0.33 ± 0.05	0.30 ± 0.07	0.28 ± 0.04	0.27 ± 0.04	0.27 ± 0.06	0/5
		(9.52)	(15.38)	(30.23)	(36.36)**	(41.3)	(41.30)	
	40	0.2 ± 0.04	0.30 ± 0.04	0.27 ± 0.05	0.23 ± 0.02	0.2 ± 0.03	0.18 ± 0.04	0/5
		(4.76)	(23.08)	(37.21)	(47.73)***	(56.52)**	(60.87)**	

Table 1 continued

Compd. no.	Dose (mg/kg)	Edema volume (ml) ± SEM (% inhibition)						G.I.
		30 min	60 min	90 min	120 min	180 min	240 min	
14	20	0.2 ± 0.05 (4.76)	0.34 ± 0.06 (12.82)	0.37 ± 0.04 (13.95)	0.33 ± 0.08 (25.00)	0.31 ± 0.04 (32.6)*	0.29 ± 0.04 (36.96)*	0/5
	40	0.23 ± 0.03 (−9.52)	0.31 ± 0.06 (20.51)	0.31 ± 0.04 (27.91)	0.26 ± 0.06 (40.91)*	0.19 ± 0.03 (58.70)**	0.17 ± 0.05 (63.04)**	0/5
15	20	0.20 ± 0.0 (4.76)	0.33 ± 0.04 (15.38)	0.31 ± 0.03 (27.91)	0.27 ± 0.05 (38.64)*	0.22 ± 0.03 (52.17)**	0.21 ± 0.05 (54.35)**	0/5
	40	0.19 ± 0.0 (9.52)	0.26 ± 0.04 (33.33)	0.23 ± 0.04 (46.51)**	0.21 ± 0.06 (52.27)*	0.17 ± 0.03 (63.04)***	0.14 ± 0.02 (69.57)***	0/5
16	20	0.23 ± 0.05 (−9.52)	0.35 ± 0.01 (10.26)	0.33 ± 0.02 (23.26)	0.32 ± 0.05 (27.27)	0.30 ± 0.02 (34.7)**	0.31 ± 0.02 (32.61)*	0/5
	40	0.20 ± 0.05 (4.76)	0.28 ± 0.05 (28.21)	0.21 ± 0.04 (51.16)**	0.17 ± 0.06 (61.36)**	0.11 ± 0.02 (76.09)***	0.09 ± 0.02 (80.43)***	0/5
17	20	0.21 ± 0.0 (0.00)	0.33 ± 0.05 (15.38)	0.31 ± 0.04 (27.91)	0.29 ± 0.05 (34.09)	0.28 ± 0.04 (39.13)*	0.27 ± 0.05 (41.30)*	0/5
	40	0.22 ± 0.04 (−4.76)	0.28 ± 0.05 (28.21)	0.24 ± 0.06 (44.19)*	0.18 ± 0.05 (59.09)**	0.16 ± 0.03 (65.22)***	0.16 ± 0.04 (65.22)**	0/5
18	20	0.18 ± 0.04 (14.29)	0.31 ± 0.06 (20.51)	0.30 ± 0.04 (30.23)	0.28 ± 0.03 (36.36)*	0.27 ± 0.04 (41.3)*	0.26 ± 0.03 (43.48)*	0/5
	40	0.18 ± 0.03 (14.29)	0.25 ± 0.02 (35.90)	0.21 ± 0.03 (51.16)**	0.16 ± 0.04 (63.64)***	0.14 ± 0.03 (69.57)***	0.11 ± 0.03 (76.09)***	0/5
19	20	0.21 ± 0.05 (0)	0.37 ± 0.06 (5.13)	0.36 ± 0.04 (16.28)	0.33 ± 0.08 (25.00)	0.30 ± 0.04 (34.7)*	0.29 ± 0.04 (36.96)*	0/5
	40	0.22 ± 0.02 (−4.76)	0.25 ± 0.04 (35.90)	0.20 ± 0.05 (53.49)**	0.16 ± 0.06 (63.64)*	0.13 ± 0.02 (71.74)***	0.12 ± 0.04 (73.91)***	0/5
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 ± 0.00 (80.8)*	1/5
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)***	0/5

Data are represented as mean ± SEM ($n = 5$). 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 at respective time point

Table 2 Analgesic activity of various pyridazinones and standard drugs

Compound no.	20 mg/kg		40 mg/kg	
	No. of writhes	% protection	No. of writhes	% protection
Control/VEHICLE	35.4 ± 1.2			
Indomethacin	13.3 ± 1.3***	62.3		
Celecoxib	9.83 ± 1.07***	72.16		
5	31.67 ± 2.23	10.37	30.8 ± 2.21	12.73
6	28.83 ± 1.30**	18.4	22.67 ± 1.50***	35.8
7	31.83 ± 2.15	9.9	28.67 ± 1.89*	18.9
8	30.33 ± 1.31*	10.8	30.33 ± 1.20*	14.1
9	32.50 ± 1.54	8.0	28.83 ± 2.15*	18.4
11	33.17 ± 1.96	6.12	30.5 ± 3.06	13.67
12	26.83 ± 2.01**	24.04	24.17 ± 2.17**	31.6
13	28.50 ± 1.38**	19.3	25.33 ± 1.56***	28.3
14	32.33 ± 1.02	8.5	29.50 ± 1.23**	16.5
15	25.50 ± 1.38**	27.82	23.83 ± 2.23**	32.5
16	27.67 ± 1.43**	21.7	17.67 ± 1.80***	50.0
17	29.17 ± 2.86	17.4	24.00 ± 2.08**	32.1
18	30.83 ± 3.05	12.7	20.00 ± 1.73***	43.4
19	30.33 ± 1.61	14.1	22.50 ± 2.51***	36.3

Data are represented as mean ± SEM ($n = 5$). 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 general, most of the tested compounds (40 mg/kg) displayed anti-inflammatory activity comparable to the reference compound indomethacin (20 mg/kg). The compounds produced dose-dependent inhibition of rat paw edema, being more effective at 40 than 20 mg/kg. The intermediate esters **5** and **11** were found to be less active than their corresponding acetamide derivatives. All the pyridazinone derivatives except compounds **5**, **9** and **11** showed more than 50 % inhibition of edema at 40 mg/kg. Homoveratrylamine-substituted derivative **16** exhibited 80.43 % inhibition of rat paw edema (40 mg/kg), which is comparable to standard drugs indomethacin and celecoxib (20 mg/kg). Also *p*-fluorophenylpiperazine (**18**) and *p*-nitrophenylpiperazine (**19**) analogs showed remarkably potent anti-inflammatory activity. These results are in accordance with the literature reports where the presence of an arylpiperazine moiety at 2 position of pyridazinone ring has been shown to increase anti-inflammatory activity of pyridazinone derivatives (Dogruer *et al*., 2004). It was interesting to note that bromo-unsubstituted derivatives **11**–**19** displayed better potency than bromo-substituted counterparts **5**–**9**.

Gastric ulcerogenic activity

Rats were killed under deep ether anesthesia 24 h after the anti-inflammatory activity experiment, and their stomachs were removed (Chan *et al*., 1995). At the maximum tested dose (40 mg/kg), none of the synthesized derivative showed any sign of gastric complications.

Analgesic activity

All the newly synthesized compounds were also evaluated for analgesic activity at 20 and 40 mg/kg by acetic acid-induced writhing test as reported (Ozkay *et al*., 2011). Mouse abdominal constrictions were measured as writhing response, and % protection was calculated. The obtained results are summarized in Table 2.

Analgesic activity of the compounds was in good correlation with their anti-inflammatory activity profile. In accordance with literature reports, introduction of arylpiperazine moieties on the amide side chain of pyridazinones **17**–**19** seems to be contributing positively toward anti-nociceptive activity, with *p*-fluorophenyl-substituted derivative **18** displaying maximum protection of 43.4 % at 40 mg/kg in 2-substituted-4-aryl-6-phenylpyridazin-3(2*H*)-one series. Furthermore, it is also noticed that the presence of bromine on the phenyl ring (except compound **6**) resulted in a remarkable decrease in analgesic activity. The presence of *N*-methylpiperazine group as a substituent in compounds **8** and **14** led to drastic decrease in % protection against acetic acid-induced writhing in mice at both the tested doses.

In vitro COX inhibitory activity

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). In the present study, the involvement of COX enzyme toward anti-inflammatory activity is expected as all the tested compounds were found to be effective even after 240 min of the oral administration in rat paw edema assay. Moreover, structure activity relationship (SAR) studies employing pyridazinones have shown *N*-substitution as a structural requirement for COX-2 selectivity (Saeed *et al*., 2012).These observations motivated us to study in vitro COX enzyme inhibitory screening of the newly synthesized compounds. The literature suggests the possibility of a weak in vitro–in vivo correlation between biological activity and enzyme selectivity (Chintakunta *et al*., 2002; Unsal-Tan *et al*., 2010). Thus, compounds with maximum structural diversity were selected for in vitro COX activity irrespective of their in vivo analgesic and anti-inflammatory profile. This would further help in understanding the mechanism of action of pyridazinones as anti-inflammatory agents. The inhibitory activity of selected compounds on both isoforms of cyclooxygenase enzymes, COX-1 and COX-2, was assayed using the COX Inhibitor Screening Assay Kit (Cayman No: 560101, Table 3) according to the protocol recommended by the supplier.

Screening of the selected compounds and reference drugs (indomethacin and celecoxib) was performed at 40 μM to determine the percent inhibition of the COX-1 and COX-2 isoforms. Results of COX inhibitor screening assay are given in Table 3. It is evident from the in vitro assay that many of the synthesized derivatives demonstrated COX inhibitory potency and reasonable selectivity for COX-2 isoform, but none showed activity superior to that of standard drug celecoxib. Intermediate esters **5** and **11** displayed negligible affinity for either form of cyclooxygenase enzyme, which is in strong agreement with their poor in vivo anti-inflammatory activity. The compound **17** carrying a phenylpiperazine substituent at 2-position of the pyridazinone ring showed good affinity and remarkable selectivity for COX-2 enzyme being 6.37 times more selective for COX-2 in comparison with COX-1 isoform. However, addition of a nitro group at *para* position of phenylpiperazine as in compound **19** leads to marginal reduction in the affinity as well as selectivity toward COX-2 enzyme. COX-2 specificity was also observed to a good extent in case of homoveratrylamine-substituted (**16**) and pyrrolidine-derived (**13**) diarylpyridazinones as they displayed preferential inhibition of COX-2 enzyme with selectivity of the order of 3.89 and 3.63, respectively, versus COX-1 enzyme. In addition to this, we noticed that compounds **9** and **15** having morpholine ring in side chain inhibited both the enzymes with similar potency. Only compound **7** showed more potency and selectivity toward COX-1 enzyme.

Table 3 In vitro COX-1 and COX-2 enzyme inhibition data

Compound no.	% Inhibition (40 μM)[a]	
	COX-1	COX-2
5	1.88	8.21
7	66.84	39.60
9	17.33	26.25
11	16.54	9.07
13	26.25	95.45
15	76.24	64.89
16	23.38	91.02
17	12.44	79.83
19	21.91	59.50
Indomethacin	79.99	97.40
Celecoxib	6.44	98.18

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

Anti-platelet activity

All the synthesized compounds were also examined for cardiovascular safety profile. A diversity of effects was observed in tail transaction bleeding test (Table 4). In general, treatment with test compounds at 20 mg/kg did not affect the platelet function in comparison to control except compounds **15**, **17** and **19**, which produced dose-dependent reduction in the bleeding time. However, at 40 mg/kg, out of 2-substituted-4-aryl-6-phenylpyridazin-3(2*H*)-ones **5**–**9** and **11**–**19**, piperidinyl-substituted compound **12** significantly increased the bleeding time. On the contrary, aryl piperazine-substituted compounds **17** and **19** and homoveratrylamine-based derivative **16**, which emerged as selective COX-2 inhibitors with potent analgesic and anti-inflammatory activity, tend to shorten the bleeding time at both the tested doses in comparison with control. Interestingly, treatment with *p*-fluorophenylpiperazine derivative **18** brought no change in bleeding time at both the tested doses. Marginal shifts in bleeding time were observed after oral administration of rest of the compounds of the series at 40 mg/kg. 4-(4-Methoxyphenyl)-2-(2-pyrrolidin-1-yl-2-oxoethyl)-6-phenylpyridazin-3(2*H*)-one (**13**) although depicted selective inhibition of COX-2 (3.89 times) versus COX-1, but displayed no change in bleeding time with respect to control. Therefore,

Table 4 Effect on bleeding time (sec) after oral administration of test compounds

Compound no.	Bleeding time (s)	
	20 mg/kg	40 mg/kg
Control	220.0 ± 13.0	
Aspirin (20 mg/kg)	290.0 ± 11.8**	
5	216.0 ± 11.7	244.0 ± 13.3
6	216.0 ± 17.2	240.0 ± 14.1
7	228.0 ± 16.2	252.0 ± 10.2
8	212.0 ± 21.5	196.0 ± 16.0
9	216.0 ± 11.7	228.0 ± 8.0
11	216.0 ± 11.7	208.0 ± 12.0
12	252.0 ± 10.2	260.0 ± 8.9*
13	220.0 ± 16.7	208.0 ± 16.2
14	236.0 ± 11.7	248.0 ± 10.2
15	184.0 ± 11.7	176.0 ± 7.5*
16	208.0 ± 10.2	154.0 ± 10.8**
17	196.0 ± 7.5	180.0 ± 14.1
18	204.0 ± 14.1	204.0 ± 11.7
19	192.0 ± 10.2	172.0 ± 10.2*

Data are represented as mean ± SEM ($n = 5$). Results were analyzed using one-way ANOVA followed by post hoc Dunnett's test

* $p < 0.05$ and ** $p < 0.01$ as compared to control value

Table 5 Effect on clotting time (sec) after oral administration of test compounds

Compound no.	Clotting time (s)	
	20 mg/kg	40 mg/kg
Control	158 ± 7.8	
Aspirin (20 mg/kg)	192 ± 9.6*	
5	154 ± 8.4	156 ± 9.7
6	158 ± 4.6	150 ± 10.1
7	152 ± 3.4	182 ± 10.4
8	160 ± 4.0	154 ± 11.8
9	170 ± 5.4	160 ± 10.4
11	162 ± 4.4	160.0 ± 6.3
12	152 ± 6.3	152 ± 11.1
13	172 ± 9.6	150 ± 6.6
14	166 ± 3.7	158 ± 6.1
15	146 ± 10.7	142 ± 8.7
16	154 ± 6.2	124 ± 4.6**
17	154 ± 7.2	162 ± 4.7
18	154 ± 4.6	150 ± 5.6
19	156 ± 2.3	180.0 ± 11.5

Data are represented as mean ± SEM ($n = 5$). Results were analyzed using one-way ANOVA followed by post hoc Dunnett's test

* $p < 0.01$ and ** $p < 0.01$ as compared to control value

compound **13** could be considered as a lead compound of the series for further structural exploration for development of therapeutically useful anti-inflammatory agents.

Whole blood clotting time assay

The discovery of cardioprotective NSAIDs remains a challenge in the field of medicinal chemistry and may prove beneficial in the treatment of chronic inflammatory diseases. Thus, in addition to bleeding time studies, effect on whole blood clotting time was also checked for all the synthesized pyridazinone-based anti-inflammatory agents to further evaluate their safety . The clotting time observed in the mice, treated with various compounds at different doses, was compared with aspirin and vehicle control. The results obtained are summarized in Table 5.

Most of the tested pyridazinone derivatives did not affect clotting time significantly except compound 2-(2-(3,4-dimethoxyphenethylamine)-2-oxoethyl)-4-(4-methoxyphenyl)-6-phenylpyridazin-3(2*H*)-one (**16**), which showed reduction in clotting time in contrast to compounds **7**, **17** and **19** which displayed increase in clotting time as compared to control at 40 mg/kg. Most of the synthesized compounds showed cardiovascular safety profile in both anti-platelet and whole blood clotting time assays.

Conclusions

The preliminary structure activity relationship (SAR) data designate pyridazinone ring as a suitable template to design a new class of analgesic and anti-inflammatory compounds. In addition, these compounds displayed excellent gastrointestinal safety profile. Further, most of the pyridazinone derivatives did not affect normal hemostatic balance. However, these results were obtained following short-term exposure with the drugs, other sub-chronic and chronic level studies are required to confirm the results. It is further interesting to mention that unlike anti-inflammatory and analgesic activity, bleeding time and clotting time assays did not show dose-dependent effects in majority of cases.

Materials and methods

Experimental

Chemistry

Melting points were determined on a Veego melting point apparatus and are uncorrected. Infrared (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}). Proton (^1H) and

carbon (^{13}C) nuclear magnetic resonance spectra were recorded on a Bruker Avance II 400 MHz spectrometer using deuterated chloroform ($CDCl_3$) or deuterated dimethylsulfoxide (DMSO-d_6) as solvents containing tetramethylsilane as internal standard (chemical shifts in δ, ppm). The spin multiplicities are indicated by symbols, s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), p (pentet), m (multiplet) and br (broad). Mass spectra were obtained on an Applied Biosystems API 2000^{TM} mass spectrophotometer. The purity of compounds was established by thin-layer chromatography (TLC) and elemental analyses. Plates for TLC were prepared with silica gel G according to Stahl's method (E. Merck) using ethyl acetate as solvent and activated at 110 °C for 30 min. Iodine was used to develop the TLC plates. Elemental analyses were carried out on a Perkin-Elmer-2400 model CHN analyzer. All solvents were distilled prior to use according to standard procedures. Anhydrous sodium sulfate was used as drying agent.

2-(4-Methoxyphenyl)-4-phenyl-4-oxobutanoic acid ***(2)*** A solution of 2-(4-methoxyphenyl)-4-phenyl-4-oxobutyronitrile (**1**, 1 g, 3.77 mmol) in hydrochloric acid (10 N, 23 ml) was stirred at room temperature for 2 h and then heated on a steam bath for 3 h. The reaction mixture was cooled, and the product obtained was filtered, washed with water and dried.

Yield = 37.38 %; mp 135–137 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.56$ (d, 2H, $J_o = 8.56$ Hz, Ar*H*), 7.56 (t, 1H, $J_o = 6.8$ Hz, Ar*H*), 7.45 (t, 2H, $J_o = 7.64$ Hz, Ar*H*), 7.29 (d, 2H, $J_o = 8.68$ Hz, Ar*H*), 6.88 (d, 2H, $J_o = 8.72$ Hz, Ar*H*), 4.25 (dd, 1H, $J_{ac} = 4.28$ Hz, $J_{bc} = 10.00$ Hz, –$COCH_2CHCOOH$), 3.87 (dd, 1H, $J_{bc} = 9.98$ Hz, $J_{ab} = 18.06$ Hz, –COC(H)*H*–), 3.80 (s, 3H,–OCH_3), 3.27 (dd, 1H, $J_{ac} = 4.32$ Hz, $J_{ab} = 18.04$ Hz, –COC(*H*)*H*–) ppm; Anal. Calcd. For $C_{17}H_{16}O_4$: C, 71.82; H, 5.67. Found: C, 71.51; H, 5.79.

4-(4-Methoxyphenyl)-6-phenyl-4,5-dihydropyridazin-3(2H)-one ***(3)*** Hydrazine hydrate (2 ml) was added to a solution of 2-(4-methoxyphenyl)-4-phenyl-4-oxobutanoic acid (**2**, 0.5 g, 1.76 mmol) in 1-butanol (9.5 ml). The reaction mixture was refluxed for 6 h, and the reaction was monitored by TLC for completion. Then, the mixture was concentrated and cooled. The solid product separated was filtered off, washed with water, dried and recrystallized from ethanol (95 %).

Yield 70 %; mp 152–155 °C; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.89$ (s, 1H, –N*H,* pyridazinone), 7.69–7.74 (m, 2H, Ar*H*), 7.42 (t, 3H, $J_o = 7.16$ Hz, Ar*H*), 7.21 (d, 2H, $J_o = 8.68$ Hz, Ar*H*), 6.88 (d, 2H, $J_o = 8.76$ Hz, Ar*H*), 3.81–3.77 (m, 4H, –OCH_3 and 4-C*H*, pyridazinone), 3.29 (dd, 1H, $J_{gem} = 16.92$ Hz, $J_{vic} = 7.18$ Hz, 5-C(H)*H*, pyridazinone), 3.19 (dd, 1H, $J_{gem} = 16.92$ Hz, $J_{vic} = 9.4$ Hz, 5-C(*H*)H, pyridazinone) ppm; Anal. Calcd. for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.51; H, 5.59; N, 9.88.

4-(3-Bromo-4-methoxyphenyl)-6-phenylpyridazin-3(2H)-one ***(4)*** A vigorously stirred solution of 4-(4-methoxyphenyl)-6-phenyl-4,5-dihydro-3(2*H*)-pyridazinone (**3**, 0.5 g, 1.78 mmol) in acetic acid (9 ml) was heated to about 60–70 °C. Bromine (0.5 ml) was added drop wise for 15 min. The mixture was stirred for 3 h and then poured into ice water. The solid that separated was filtered off, washed with water, dried and recrystallized from ethanol (95 %).

Yield 96.8 %; mp 220–224 °C; IR (KBr)υ_{max} 3286 (N–H), 3009 (C–H, aromatic), 2845 (C–H, aliphatic), 1645 (C=O), 1590 (C=C, aromatic), 1497 (C=N), 1018 (C–Br), 700 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 12.4$ (s, 1H, –N*H*, pyridazinone), 8.13 (d, 1H, $J_m = 2.16$ Hz, Ar*H*, *ortho* to bromine), 7.94 (dd, 1H, Ar*H*, $J_o = 8.6$ Hz, $J_m = 2.10$ Hz, *meta* to methoxy), 7.85 (d, 2H, $J_o = 7.38$ Hz, Ar*H*), 7.78 (s, 1H, 5-C*H*, pyridazinone), 7.49–7.43 (m, 3H, Ar*H*), 7.00 (d, 1H, $J_o = 8.40$ Hz, Ar*H*, *ortho* to methoxy), 3.96 (s, 3H, –OCH_3) ppm; Anal. Calcd. for $C_{17}H_{13}N_2O_2Br$: C, 57.16; H, 3.67; N, 7.84. Found: C, 57.41; H, 3.59; N, 7.58.

4-(3-Bromo-4-methoxyphenyl)-2-(methoxycarbonylmethyl)-6-phenylpyridazin-3(2H)-one ***(5)*** Methyl chloroacetate (1 ml, in excess) was added to a stirred and refluxing suspension of 4-(3-bromo-4-methoxyphenyl)-6-phenylpyridazin-3(2*H*)-one (**4**, 0.57 g, 1.38 mmol) and anhydrous potassium carbonate (1.0 g) in ethyl methyl ketone (40 ml). The reaction mixture was further refluxed for 2 h with continuous stirring. The completion of the reaction was monitored by TLC. On completion, the reaction mixture was cooled and filtered and the solvent was removed under reduced pressure. Ice cold water was added to the reacting mixture. The solid thus separated was filtered off, washed with water, dried and recrystallized from methanol.

Yield 44.11 %; mp 120–122 °C; IR (KBr)υ_{max} 3049 (C–H, aromatic), 2954 (C–H, aliphatic), 1751 (C=O, ester), 1647 (C=O), 1599 (C=C, aromatic), 1439 (CH_2 bending), 1232 (C–O–C), 1178 (C–N, amide), 1025 (C–Br, aryl bromide), 1098 (C–O–C), 833 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.10$ (d, 1H, $J_m = 2.20$ Hz, Ar*H*, *ortho* to bromine), 7.93 (dd, 1H, $J_o = 8.68$ Hz, $J_m = 2.24$ Hz, Ar*H*, *meta* to methoxy), 7.81 (d, 2H, $J_o = 6.52$ Hz, Ar*H*), 7.78 (s, 1H, 5-C*H*, pyridazinone), 7.48–7.46 (m, 3H, Ar*H*), 6.97 (d, 1H, Ar*H*, *ortho* to methoxy, $J_o = 8.72$ Hz), 5.04 (s, 2H, –NCH_2), 3.95 (s, 3H, –$COOCH_3$), 3.81 (s, 3H, –OCH_3) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 168.1$

(**C**=O), 160.9 (**C**=O), 159.5 (Ar**C**), 145.4 (Ar**C**), 139.3 (Ar**C**), 135.0 (Ar**C**), 130.3 (Ar**C**), 129.4 (Ar**C**), 128.9 (Ar**C**), 128.8 (Ar**C**), 128.7 (Ar**C**), 126.1 (2xAr**C**), 126.0 (Ar**C**), 125.8 (Ar**C**), 114.4 (Ar**C**), 113.9 (Ar**C**), 55.4 (O**C**H_3), 54.4 (O**C**H_3), 52.5 (**C**H_2CO) ppm; Anal. Calcd. for $C_{20}H_{17}BrN_2O_4$: C, 55.96; H, 3.99; N, 6.53. Found: C, 55.51; H, 3.89; N, 6.97. ESIMS *m/z* 429 $[MH]^+$, 431 $[MH+2]^+$.

*General procedure for the synthesis of 2-(2-amino-2-oxoethyl)-4-(3-bromo-4-methoxyphenyl)-6-phenylpyridazin-3(2H)-one derivatives (**6–9**)* A mixture of 4-(3-bromo-4-methoxyphenyl)-2-(methoxycarbonylmethyl)-6-phenylpyridazin-3(2*H*)-one (**5**, 0.2 g, 0.47 mmol) and requisite amine (1 ml, in excess) was heated at 100 °C with continuous stirring. The mixture was further stirred with heating for 6 h, reaction being monitored by TLC. On completion, ice cold water was added to the reaction mixture. The precipitated compound was filtered, washed several times with distilled water to remove any unreacted amine and recrystallized using methanol to provide desired compounds **6–9**.

*4-(3-Bromo-4-methoxyphenyl)-2-(2-piperidin-1-yl-2-oxoethyl)-6-phenylpyridazin-3(2H)-one **(6)*** Yield 44.49 %; mp 190–192 °C; IR (KBr)υ_{max} 3014 (C–H, aromatic), 2937 (C–H, aliphatic), 1649 (C=O, amide), 1594 (C=C, aromatic), 1441 (CH_2 bending), 1256 (C–O–C), 1174 (C–N, amide), 1049 (C–Br, aryl bromide), 1012 (C–O–C), 889, 694 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11 (d, 1H, J_m = 2.20 Hz, Ar*H*, *ortho* to bromine), 7.92 (dd, 1H, J_o = 8.66 Hz, J_m = 2.20 Hz, Ar*H*, *meta* to methoxy), 7.81 (dd, 2H, J_o = 6.48 Hz, J_m = 1.72 Hz, Ar*H*), 7.77 (s, 1H, 5-C*H*, pyridazinone), 7.47–7.42 (m, 3H, C*H*, aromatic), 6.95 (d, 1H, J_o = 8.72 Hz, Ar*H*, *ortho* to methoxy), 5.13 (s, 2H, –NCH_2), 3.95 (s, 3H, –OCH_3), 3.60 (t, 2H, J = 5.42 Hz, –NCH_2–, piperidine), 3.48 (s(br), 2H, –NCH_2–, piperidine), 1.68 (s(br), 4H, –CH_2–, piperidine), 1.59 (s(br), 2H, –CH_2–, piperidine) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 164.15 (**C**=O), 159.45 (**C**=O), 156.88 (Ar**C**), 145.11 (Ar**C**), 137.68 (Ar**C**), 135.16 (Ar**C**), 133.56 (Ar**C**), 129.52 (Ar**C**), 129.31 (2xAr**C**), 128.84 (Ar**C**), 127.72 (Ar**C**), 126.36 (Ar**C**), 126.22 (2xAr**C**), 111.56 (Ar**C**), 111.36 (Ar**C**), 56.36 (O**C**H_3), 54.28 (**C**H_2CO), 45.94 (**C**H_2), 43.29 (**C**H_2), 26.25 (**C**H_2), 25.33 (**C**H_2), 24.43 (**C**H_2) ppm; Anal. Calcd. for $C_{24}H_{24}BrN_3O_3$: C, 59.76; H, 5.01; N, 8.71 %. Found: C, 59.51; H, 5.39; N, 8.97. ESIMS *m/z* 482.2 $[MH]^+$, 484.2 $[MH+2]^+$.

*4-(3-Bromo-4-methoxyphenyl)-2-(2-pyrrolidin-1-yl-2-oxoethyl)-6-phenylpyridazin-3(2H)-one **(7)*** Yield 45.83 %; mp 150–151 °C; IR (KBr)υ_{max} 3051 (C–H, aromatic), 2948 (C–H, aliphatic), 1643 (C=O, amide), 1593 (C=C, aromatic), 1444 (CH_2 bending), 1255 (C–O–C), 1182 (C–N), 1054 (C–Br, aryl bromide), 1020 (C–O–C), 822, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11(d, 1H, J_m = 2.20 Hz, Ar*H*, *ortho* to bromine), 7.89 (dd, 1H, J_o = 8.66 Hz, J_m = 2.20 Hz, Ar*H*, *meta* to methoxy), 7.83 (dd, 2H, J_o = 8.32 Hz, J_m = 1.74 Hz, Ar*H*), 7.77 (s, 1H, 5-C*H*, pyridazinone), 7.46–7.43 (m, 3H, Ar*H*), 6.96 (d, 2H, J_o = 8.72 Hz, Ar*H*, *ortho* to methoxy), 5.04 (s, 2H, –NCH_2), 3.95 (s, 3H, –OCH_3), 3.58–3.56 (m, 4H, –N(CH_2)$_2$–, pyrrolidine), 2.04 (p, 2H, –CH_2–, pyrrolidine), 1.89 (p, 2H, –CH_2–, pyrrolidine) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 164.40 (**C**=O), 159.39 (**C**=O), 156.87 (Ar**C**), 145.09 (Ar**C**), 137.67 (Ar**C**), 135.12 (Ar**C**), 133.55 (Ar**C**), 129.49 (Ar**C**), 129.31 (2xAr**C**), 128.83 (Ar**C**), 127.68 (Ar**C**), 126.37 (2xAr**C**), 126.21 (Ar**C**), 111.56 (Ar**C**), 111.37 (Ar**C**), 56.36 (O**C**H_3), 55.15 (**C**H_2CO), 46.14(**C**H_2), 45.80 (**C**H_2), 26.19 (**C**H_2), 24.12 (**C**H_2) ppm; Anal. Calcd. for $C_{23}H_{22}BrN_3O_3$: C, 58.98; H, 4.73; N, 8.97. Found: C, 58.51; H, 4.89; N, 8.47. ESIMS *m/z* 468.2 $[MH]^+$, 470.2 $[MH+2]^+$.

*4-(3-Bromo-4-methoxyphenyl)-2-(2-(4-methylpiperazin-1-yl)-2-oxoethyl)-6-phenylpyridazin-3(2H)-one **(8)*** Yield 43.29 %;mp 140–141 °C; IR (KBr)υ_{max} 2953 (C–H, aliphatic), 1640 (C=O, amide), 1597 (C=C, aromatic), 1445 (CH_2 bending), 1256 (C–O–C), 1165 (C–N), 1052 (C–Br, aryl bromide), 1017 (C–O–C), 768, 691 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.21 (m, 2H, Ar*H*, *ortho* to bromine), 7.92–7.85 (m, 3H, Ar*H*), 7.71 (s, 1H, 5-C*H*, pyridazinone), 7.48–7.43 (m, 3H, Ar*H*), 7.02 (d, 2H, J_o = 8.60 Hz, Ar*H*, *ortho* to methoxy), 4.93 (s, 2H, –NCH_2), 3.94 (s, 3H, –OCH_3), 3.60 (s(br), 2H, –NCH_2–, piperazine), 3.43 (s(br), 2H, –NCH_2–, piperazine), 2.74 (t, 2H, J = 4.00 Hz,–NCH_2–, piperazine), 2.52 (t, 2H, J = 4.52 Hz, –NCH_2–, piperazine), 2.36 (s, 3H, N–CH_3) ppm; Anal. Calcd. for $C_{24}H_{25}BrN_4O_3$: C, 57.95; H, 5.07; N, 11.26. Found: C, 57.58; H, 5.19; N, 11.57.

*4-(3-Bromo-4-methoxyphenyl)-2-(2-morpholin-4-yl-2-oxoethyl)-6-phenylpyridazin-3(2H)-one (**9**)* Yield 22.22 %; mp 180–186 °C; IR (KBr)υ_{max} 3065 (C–H, aromatic), 2925 (C–H, aliphatic), 1646 (C=O, amide), 1591 (C=C, aromatic), 1446 (CH_2 bending), 1239 (C–O–C), 1178 (C–N), 1053 (C–Br, aryl bromide), 1023 (C–O–C), 891, 692 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11 (d, 1H, J_m = 2.20 Hz, Ar*H*, *ortho* to bromine), 7.92 (dd, 1H, J_o = 8.68 Hz, J_m = 2.20 Hz, Ar*H*, *meta* to methoxy), 7.81 (dd, 2H, J_o = 8.16 Hz, J_m = 1.72 Hz, Ar*H*), 7.78 (s, 1H, 5-C*H*, pyridazinone), 7.42–7.49 (m, 3H, Ar*H*), 6.96 (d, 1H, J_o = 8.72 Hz, Ar*H*, *ortho* to methoxy), 5.12 (s, 2H, –NCH_2), 3.95 (s, 3H, –OCH_3), 3.78–3.72 (m, 4H, –O(CH_2)$_2$–, morpholine), 3.67 (t, 2H, J = 4.92 Hz, –NCH_2–, morpholine), 3.57 (t, 2H, J = 4.54 Hz, –NCH_2–, morpholine) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 164.75 (**C**=O), 159.41 (**C**=

O), 156.96 (Ar**C**), 145.35 (Ar**C**), 137.73 (Ar**C**), 134.99 (Ar**C**), 133.53 (Ar**C**), 129.49 (Ar**C**), 129.43 (2xAr**C**), 128.88 (Ar**C**), 127.51 (Ar**C**), 126.45 (Ar**C**), 126.20 (2xAr**C**), 111.61 (Ar**C**), 111.38 (Ar**C**), 66.75 (**C**H_2), 66.35 (**C**H_2), 56.36 (O**C**H_3), 54.03 (**C**H_2CO), 45.32 (**C**H_2), 42.39 (**C**H_2) ppm; Anal. Calcd. For $C_{23}H_{22}BrN_3O_4$: C, 57.04; H, 4.58; N, 8.68. Found: C, 57.41; H, 4.19; N, 8.59. ESIMS *m/z* 484.2 $[MH]^+$, 486.2 $[MH+2]^+$.

4-(4-Methoxyphenyl)-6-phenylpyridazin-3(2H)-one ***(10)*** Copper (II) chloride (0.30 g, 2.25 mmol) was added to a solution of 4-(4-methoxyphenyl)-6-phenyl-4,5-dihydro-3(2*H*)-pyridazinone (**3**, 0.25 g, 0.89 mmol) in acetonitrile (10 ml). The reaction mixture was refluxed for 3 h, and the reaction was monitored by TLC. On completion of the reaction, the mixture was added to crushed ice. The resulting solid was filtered off, washed with water and recrystallized from ethanol.

Yield 60.43 %; mp 212–214 °C; IR (KBr)υ_{max} 3290 (N–H), 3025 (C–H, aromatic), 2911 (C–H, aliphatic), 1650 (C=O), 1593, 1511, 1262 (C–O–C), 1178 (C–N), 1018 (C–O–C), 841, 603 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.92$ (d, 2H, $J_o = 6.88$ Hz, Ar*H*, *meta* to methoxy), 7.84–7.82 (m, 2H, Ar*H*), 7.81 (s, 1H, 5-C*H*, pyridazinone), 7.48–7.46 (m, 3H, Ar*H*), 7.01 (d, 2H, $J_o = 6.84$ Hz, Ar*H*, *ortho* to methoxy), 3.87 (s, 3H, –OCH_3), ppm; Anal. Calcd. for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.61; H, 4.89; N, 10.39.

2-(Methoxycarbonylmethyl)-4-(4-methoxyphenyl)-6-phenylpyridazin-3(2H)-one ***(11)*** Methyl chloroacetate (1 ml, in excess) was added to a stirred and refluxing suspension of 4-(4-methoxyphenyl)-6-phenylpyridazin-3(2*H*)-one (**10**, 1.0 g, 3.59 mmol) and anhydrous potassium carbonate (1.0 g) in ethyl methyl ketone (40 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 and filtered and the excess solvent was removed under reduced pressure. Ice cold water was added to the reaction mixture. The solid thus separated was filtered off, washed with water, dried and recrystallized using methanol.

Yield 62.50 %; mp 108–110 °C; IR (KBr)υ_{max} 3049 (C–H, aromatic), 2951 (C–H, aliphatic), 1754 (C=O, ester), 1640 (C=O), 1594 (C=C, aromatic), 1441 (CH_2 bending), 1224 (C–O–C), 1180 (C–N), 1032 (C–O–C), 829 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.88$ (d, 2H, $J_o = 8.88$ Hz, Ar*H*, *meta* to methoxy), 7.83–7.80 (m, 2H, Ar*H*), 7.78 (s, 1H, 5-C*H*, pyridazinone), 7.47–7.45 (m, 3H, Ar*H*), 6.98 (d, 2H, $J_o = 9.00$ Hz, Ar*H*, *ortho* to methoxy), 5.04 (s, 2H, –NCH_2), 3.86 (s, 3H, –COOCH_3), 3.80 (s, 3H, –OCH_3) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 167.9$ (**C**=O), 159.2 (**C**=O), 157.1 (Ar**C**), 145.3 (Ar**C**), 137.9 (Ar**C**), 134.8 (Ar**C**), 133.4 (Ar**C**), 129.5 (Ar**C**), 129.4 (Ar**C**), 128.9 (Ar**C**), 127.3 (2xAr**C**), 126.4 (Ar**C**), 126.1 (Ar**C**), 111.7 (Ar**C**), 111.4 (Ar**C**), 56.4(O**C**H_3), 54.5 (O**C**H_3), 52.6 (**C**H_2CO) ppm; Anal. Calcd. for $C_{20}H_{18}N_2O_3$: C, 71.84; H, 5.43; N, 8.38. Found: C, 71.51; H, 5.59; N, 8.57.

General procedure for the synthesis of 2-(2-amino-2-oxoethyl)-4-(4-methoxyphenyl)-6-phenylpyridazin-3(2H)-one derivatives ***(12–19)*** A mixture of 2-(methoxycarbonylmethyl)-4-(4-methoxyphenyl)-6-phenylpyridazin-3(2*H*)-one (**11**, 0.2 g, 0.51 mmol) and requisite amine (1 ml, in excess) was heated at 100 °C with continuous stirring. The mixture was further stirred with heating for 6 h, reaction being monitored by TLC. On completion, ice cold water was added to the reaction mixture. The precipitated compound was filtered, washed several times with distilled water to remove any unreacted amine and recrystallized using ethyl acetate to provide desired compounds **12**–**19**.

4.1.8.1.4-(4-Methoxyphenyl)-2-(2-piperidin-1-yl-2-oxoethyl)-6-phenylpyridazin-3(2H)-one ***(12)*** Yield 34.73 %, mp 160–161 °C; IR (KBr)υ_{max} 3037 (C–H, aromatic), 2940 (C–H, aliphatic), 1643 (C=O, amide), 1604 (C=C, aromatic), 1449 (CH_2 bending), 1252 (C–O–C), 1188 (C–N), 1020 (C–O–C), 835, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.87$ (d, 2H, $J_o = 8.80$ Hz, Ar*H*, *meta* to methoxy), 7.82–7.80 (m, 2H, Ar*H*), 7.76 (s, 1H, 5-C*H*, pyridazinone), 7.47–7.38 (m, 3H, Ar*H*), 6.95 (d, 2H, $J_o = 8.80$ Hz, Ar*H*, *ortho* to methoxy), 5.12 (s, 2H, –NCH_2), 3.86 (s, 3H, –OCH_3), 3.60 (t, 2H, $J = 4.01$ Hz, –NCH_2–, piperidine), 3.48 (s(br), 2H, –NCH_2–, piperidine), 1.67 (s(br), 4H, –(CH_2)$_2$–, piperidine), 1.58 (s(br), 2H, –CH_2–, piperidine) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 164.3$ (**C**=O), 160.7 (**C**=O), 159.7 (Ar**C**), 145.1 (Ar**C**), 139.1 (Ar**C**), 135.4 (Ar**C**), 130.3 (2xAr**C**), 129.1 (Ar**C**), 128.8 (2xAr**C**), 126.5 (Ar**C**), 126.2 (2xAr**C**), 125.9 (Ar**C**), 113.7 (2xAr**C**), 55.3 (O**C**H_3), 54.1 (**C**H_2CO), 45.9 (**C**H_2), 43.2 (**C**H_2), 26.2 (**C**H_2), 25.3 (**C**H_2), 24.4 (**C**H_2) ppm; Anal. Calcd. for $C_{24}H_{25}N_3O_3$: C, 71.44; H, 6.25; N, 10.41. Found: C, 71.51; H, 6.59; N, 10.67. ESIMS *m/z* 404.3 $[MH]^+$.

4.1.8.2.4-(4-Methoxyphenyl)-2-(2-pyrrolidin-1-yl-2-oxoethyl)-6-phenylpyridazin-3(2H)-one ***(13)*** Yield 54.05 %; mp 128–132 °C; IR (KBr)υ_{max} 3051 (C–H, aromatic), 2945 (C–H, aliphatic), 1651 (C=O, amide), 1600 (C=C, aromatic), 1439 (CH_2 bending), 1254 (C–O–C), 1182 (C–N, amide), 1024 (C–O–C), 830, 694 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.89$ (d, 2H, $J_o = 8.72$ Hz, Ar*H*, *meta* to methoxy), 7.83 (dd, 2H, $J_o = 7.80$ Hz, $J_m = 1.26$ Hz, Ar*H*), 7.79 (s, 1H, 5-C*H*, pyridazinone), 7.49–7.43 (m, 3H, Ar*H*), 6.98 (d, 2H, $J_o = 8.84$ Hz, Ar*H*, *ortho* to methoxy), 5.06 (s, 2H, –NCH_2), 3.87 (s, 3H, –OCH_3), 3.59 (p, 4H, $J = 6.84$ Hz, –N(CH_2)$_2$–, pyrrolidine), 2.04 (p, 2H,

$J = 6.76$ Hz, –CH_2–, pyrrolidine), 1.91 (p, 2H, $J = 6.80$ Hz, –CH_2–, pyrrolidine) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 164.5$ (**C**=O), 160.7 (**C**=O), 159.7 (Ar**C**), 145.1 (Ar**C**), 139.1 (Ar**C**), 135.4 (Ar**C**), 130.3 (2xAr**C**), 129.2 (Ar**C**), 128.8 (2xAr**C**), 126.4 (Ar**C**), 126.2 (2xAr**C**), 126.0 (Ar**C**), 113.8 (2xAr**C**), 55.3 (O**C**H_3), 55.1 (**C**H_2CO), 46.1 (**C**H_2), 45.8 (**C**H_2), 26.2 (**C**H_2), 24.1 (**C**H_2) ppm; Anal. Calcd. for $C_{23}H_{23}N_3O_3$: C, 70.93; H, 5.95; N, 10.79. Found: C, 70.51; H, 5.89; N, 10.97. ESIMS *m/z* 390.4 $[MH]^+$.

4-(4-Methoxyphenyl)-2-(2-(4-methylpiperazin-1-yl)-2-oxoethyl)-6-phenylpyridazin-3(2H)-one ***(14)*** Yield 60.69 %; mp 166–168 °C; IR (KBr)υ_{max} 2937 (C–H, aliphatic), 1649 (C=O, amide), 1598 (C=C, aromatic), 1458 (CH_2 bending), 1244 (C–O–C), 1175 (C–N), 1028 (C–O–C), 833, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.79$ (d, 2H, $J_o = 8.27$ Hz, Ar*H*, *meta* to methoxy), 7.73 (d, 2H, $J_o = 7.36$ Hz, Ar*H*), 7.69 (s, 1H, 5-C*H*, pyridazinone), 7.39–7.33 (m, 3H, Ar*H*), 6.88 (d, 2H, $J_o = 8.76$ Hz, Ar*H*, *ortho* to methoxy), 5.05 (s, 2H, –NCH_2), 3.77 (s, 3H, –OCH_3), 3.61 (t, 2H, $J = 4.60$ Hz, –NCH_2–, piperazine), 3.50 (t, 2H, $J = 4.28$ Hz, –NCH_2–, piperazine), 2.42 (t, 2H, $J = 4.36$ Hz, –NCH_2–, piperazine), 2.36 (t, 2H, $J = 4.84$ Hz, –NCH_2–, piperazine), 2.35 (s, 3H, N–CH_3) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 164.5$ (**C**=O), 160.8 (**C**=O), 159.7 (Ar**C**), 145.3 (Ar**C**), 139.1 (Ar**C**), 135.3 (Ar**C**), 130.3 (Ar**C**), 130.1 (Ar**C**), 129.2 (Ar**C**), 128.9 (Ar**C**), 128.8 (Ar**C**), 126.3 (Ar**C**), 126.2 (Ar**C**), 126.0 (Ar**C**), 126.0 (Ar**C**), 114.0 (Ar**C**), 113.8 (Ar**C**), 55.3 (O**C**H_3), 54.7 (**C**H_2CO), 54.4 (**C**H_2), 54.0 (**C**H_2), 46.0 (**C**H_2), 44.7 (**C**H_2), 42.0 (**C**H_3) ppm; Anal. Calcd. for $C_{24}H_{26}N_4O_3$: C, 68.88; H, 6.26; N, 13.39. Found: C, 68.59; H, 5.89; N, 12.97. ESIMS *m/z* 419.4 $[MH]^+$.

4-(4-Methoxyphenyl)-2-(2-morpholin-4-yl-2-oxoethyl)-6-phenylpyridazin-3(2H)-one ***(15)*** Yield 43.30 %; mp 152–156 °C; IR (KBr)υ_{max} 3040 (C–H, aromatic), 2957 (C–H, aliphatic), 1648 (C=O, amide), 1600 (C=C, aromatic), 1450 (CH_2 bending), 1240 (C–O–C), 1176 (C–N), 1028 (C–O–C), 835, 698 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.79$ (d, 2H, $J_o = 6.80$ Hz, Ar*H*), 7.75–7.72 (m, 2H, Ar*H*), 7.70 (s, 1H, 5-C*H*, pyridazinone), 7.40–7.34 (m, 3H, Ar*H*), 6.90 (d, 2H, $J_o = 6.84$ Hz, Ar*H*), 5.05 (s, 2H, –NCH_2), 3.78 (s, 3H, –OCH_3), 3.69 (t(br), 2H, –OCH_2–, morpholine), 3.64 (t(br), 2H, –OCH_2–, morpholine), 3.60 (t(br), 2H, –NCH_2–, morpholine), 3.50 (t(br), 2H, –NCH_2–, morpholine) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 164.9$ (**C**=O), 160.8 (**C**=O), 159.7 (Ar**C**), 145.4 (Ar**C**), 139.2 (Ar**C**), 135.2 (Ar**C**), 130.3 (2xAr**C**), 129.3 (Ar**C**), 128.8 (2xAr**C**), 126.3 (Ar**C**), 126.2 (2xAr**C**), 126.0 (Ar**C**), 113.8 (2xAr**C**), 66.7 (**C**H_2), 66.4 (**C**H_2), 55.3 (O**C**H_3), 53.9 (**C**H_2CO), 45.3 (**C**H_2), 42.4 (**C**H_2) ppm; Anal. Calcd. for $C_{23}H_{23}N_3O_4$: C, 68.13; H, 5.72; N, 10.36. Found: C, 68.51; H, 5.89; N, 9.97. ESIMS *m/z* 406.3 $[MH]^+$.

2-(2-(3,4-Dimethoxyphenethylamine)-2-oxoethyl)-4-(4-methoxyphenyl)-6-phenylpyridazin-3(2H)-one ***(16)*** Yield 35.06 %; mp 162–164 °C; IR (KBr)υ_{max} 3287 (N–H), 3095 (C–H, aromatic), 2935 (C–H, aliphatic), 1654 (C=O, amide), 1599 (C=C, aromatic), 1513, 1454 (CH_2 bending), 1266 (C–O–C), 1178 (C–N), 1023 (C–O–C), 841, 690 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.75$ (d, 4H, $J_o = 8.60$ Hz, Ar*H*), 7.69 (s, 1H, 5-C*H*, pyridazinone), 7.42–7.37 (m, 3H, Ar*H*), 6.92 (d, 2H, $J_o = 8.68$ Hz, Ar*H*, *ortho* to methoxy), 6.57–6.51 (m, 3H, Ar*H*, homoveratrylamine), 6.41 (s, 1H, –N*H*), 4.85 (s, 2H, –NCH_2), 3.79 (s, 3H, –OCH_3), 3.68 (s, 3H, –OCH_3), 3.63 (s, 3H, –OCH_3), 3.43 (q, 2H, $J = 6.76$ Hz, –NHCH_2CH$_2$), 2.67 (t, 2H, $J = 6.90$ Hz, –NHCH$_2$CH_2,) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 167.1$ (**C**=O), 161.0 (**C**=O), 159.9 (Ar**C**), 148.9 (Ar**C**), 147.5 (Ar**C**), 145.6 (Ar**C**), 139.3 (Ar**C**), 134.7 (Ar**C**), 131.1 (Ar**C**), 130.2 (2xAr**C**), 129.6 (Ar**C**), 128.9 (2xAr**C**), 126.1 (2xAr**C**), 125.9 (Ar**C**), 125.8 (Ar**C**), 120.5 (Ar**C**), 113.9 (2xAr**C**), 111.8 (Ar**C**), 111.1 (Ar**C**), 57.3 (O**C**H_3), 55.7 (O**C**H_3), 55.7 (O**C**H_3), 55.4 (**C**H_2CO), 40.7 (**C**H_2), 34.9 (**C**H_2) ppm; Anal. Calcd. for $C_{29}H_{29}N_3O_5$: C, 69.72; H, 5.85; N, 8.41. Found: C, 69.59; H, 5.59; N, 8.80. ESIMS *m/z* 500.3 $[MH]^+$.

4-(4-Methoxyphenyl)-2-(2-(4-phenylpiperazin-1-yl)-2-oxoethyl)-6-phenylpyridazin-3(2H)-one ***(17)*** Yield 36.45 %; mp 142–144 °C; IR (KBr)υ_{max} 3049 (C–H, aromatic), 2955 (C–H, aliphatic), 1643 (C=O, amide), 1595 (C=C, aromatic), 1449 (CH_2 bending), 1234 (C–O–C), 1184 (C–N, amide), 1025 (C–O–C), 845, 690 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.89$ (d, 2H, $J_o = 8.92$ Hz, Ar*H*, *meta* to methoxy), 7.83 (dd, 2H, $J_o = 8.24$ Hz, $J_m = 1.72$ Hz, Ar*H*), 7.80 (s, 1H, 5-C*H*, pyridazinone), 7.49–7.43 (m, 3H, Ar*H*), 7.31 (t, 2H, Ar*H*, phenylpiperazine), 7.00–6.92 (m, 5H, Ar*H*, phenylpiperazine and aromatic *ortho* to methoxy), 5.20 (s, 2H, –NCH_2), 3.87 (s, 3H, –OCH_3), 3.85 (t, 2H, $J = 4.84$ Hz, –NCH_2–, piperazine), 3.75 (t, 2H, $J = 5.04$ Hz, –NCH_2–, piperazine), 3.30 (t, 2H, $J = 4.76$ Hz, –NCH_2–, piperazine), 3.23 (t, 2H, $J = 4.87$ Hz, –NCH_2–, piperazine) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): $\delta = 164.7$ (**C**=O), 160.8 (**C**=O), 159.7 (Ar**C**), 145.4 (Ar**C**), 139.1 (Ar**C**), 135.2 (Ar**C**), 130.3 (2xAr**C**), 129.3 (2xAr**C**), 128.8 (2xAr**C**), 126.3 (Ar**C**), 126.2 (2xAr**C**), 126.0 (Ar**C**),

116.8 (Ar**C**), 113.8 (2xAr**C**), 55.4 (O**C**H_3), 54.0 (**C**H_2CO), 49.6 (**C**H_2), 49.3 (**C**H_2), 44.9 (**C**H_2), 42.0 (**C**H_2) ppm; Anal. Calcd. for $C_{29}H_{28}N_4O_3$: C, 72.48; H, 5.87; N, 11.66. Found: C, 72.59; H, 5.59; N, 11.97. ESIMS *m/z* 481.4 $[MH]^+$.

2-(2-(4-(4-Fluorophenyl)piperazin-1-yl)-2-oxoethyl)-4-(4-methoxyphenyl)-6-phenylpyridazin-3(2H)-one ***(18)*** Yield 50.30 %; mp 158–160 °C; IR (KBr)υ_{max} 2955 (C–H, aliphatic), 1642 (C=O, amide), 1591 (C=C, aromatic), 1509, 1457 (CH_2 bending), 1386 (C–F), 1247 (C–O–C), 1176 (C–N, amide), 1035 (C–O–C), 870, 697 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.80–7.78 (m, 4H, Ar*H* and Ar*H*, *meta* to methoxy), 7.69 (s, 1H, 5-C*H*, pyridazinone), 7. 45–7.41 (m, 3H, Ar*H*), 6.94–6.87 (m, 4H, Ar*H*, phenylpiperazine and Ar*H*, *ortho* to methoxy), 6.73–6.70 (m, 2H, Ar*H*, phenylpiperazine), 4.90 (s, 2H, –NCH_2), 3.80 (s, 3H, –OCH_3), 3.16 (t, 4H, J = 3.30 Hz, –N(CH_2)$_2$–, piperazine), 3.09 (t, 4H, J = 3.32 Hz, –N(CH_2)$_2$–, piperazine) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 185.6 (**C**=O), 160.6 (**C**=O), 147.2 (Ar**C**), 144.5 (Ar**C**), 138.8 (Ar**C**), 135.2 (Ar**C**), 130.2 (2xAr**C**), 129.2 (2xAr**C**), 128.8 (2xAr**C**), 126.4 (Ar**C**), 126.0 (2xAr**C**), 125.8 (Ar**C**), 118.7 (ArC), 118.7 (ArC), 115.7 (ArC), 115.5 (Ar**C**), 113.7 (2xAr**C**), 55.3 (**C**H_2), 47.7 (**C**H_2), 43.3 (**C**H_2) ppm; Anal. Calcd. for $C_{29}H_{27}N_4O_3F$: C, 69.86; H, 5.46; N, 11.24. Found: C, 69.99; H, 5.59; N, 10.97. ESIMS *m/z* 499.3 $[MH]^+$.

2-(2-(4-(4-Nitrophenyl)piperazin-1-yl)-2-oxoethyl)-4-(4-methoxyphenyl)-6-phenylpyridazin-3(2H)-one ***(19)*** Yield 38.17 %; mp 176–178 °C; IR (KBr)υ_{max} 3049 (C–H, aromatic), 2841 (C–H, aliphatic), 1636 (C=O, amide), 1592 (C=C, aromatic), 1457 (CH_2 bending), 1383, 1332 (NO_2), 1256 (C–O–C), 1171 (C–N, amide), 1033 (C–O–C), 834, 695 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06 (d, 2H, J_o = 9.40 Hz, Ar*H*, *meta* to methoxy), 7.91–7.88 (m, 4H, Ar*H*), 7.86 (s, 1H, 5-C*H*, pyridazinone), 7.47–7.41 (m, 3H, Ar*H*), 6.95 (d, 2H, J_o = 8.84 Hz, Ar*H*, phenylpiperazine, *meta* to nitro), 6.88 (d, 2H, J_o = 9.44 Hz, Ar*H*, *ortho* to methoxy), 4.85 (s, 2H, –NCH_2), 3.84 (s, 3H, –OCH_3), 3.50 (t, 4H, J = 4.84 Hz, –N(CH_2)$_2$–, piperazine), 3.07 (t, 4H, J = 5.04 Hz, –N(CH_2)$_2$–, piperazine) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.5 (**C**=O), 164.1 (**C**=O), 160.1 (Ar**C**), 158.7 (Ar**C**), 154.3 (Ar**C**), 143.5 (Ar**C**), 138.0 (Ar**C**), 134.8 (Ar**C**), 129.9 (2xAr**C**), 128.6 (Ar**C**), 128.3 (2xAr**C**), 126.1 (Ar**C**), 125.6 (2xAr**C**), 125.3 (2xAr**C**), 125.1 (Ar**C**), 113.2 (2xAr**C**), 112.4 (2xAr**C**), 55.5 (O**C**H_3), 54.8 (**C**H_2CO), 45.8 (**C**H_2), 44.8 (**C**H_2), 43.6 (2x**C**H_2) ppm; Anal. Calcd. for $C_{29}H_{27}N_5O_5$: C, 66.27; H, 5.18; N, 13.32. Found: C, 66.59; H, 5.29; N, 12.97. ESIMS *m/z* 526. 2 $[MH]^+$.

Biological activity

Anti-inflammatory activity

All the synthesized derivatives were evaluated for anti-inflammatory activity using carrageenan-induced hind paw edema model (Abouzid and Bekhit, 2008) 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 1 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 dose 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 and celecoxib were used as the standard anti-inflammatory drugs.

The percent inhibition of inflammation was calculated using following formula:

$$\% \text{ Inhibition of Inflammation} = 100\left[1 - \frac{a-x}{b-y}\right]$$

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.

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).

Analgesic activity

Anti-nociceptive activity of compounds against noxious chemical stimuli was evaluated by acetic acid-induced writhing test in Laca mice (20–25 g) as described previously (Ozkay *et al*., 2011). 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 a dose of 10 ml/kg to induce contractions. After 5 min, the number of abdominal constrictions and stretches during the following 10 min was 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 the following equation:

$$\text{Protection } \% = \frac{(\text{Control mean} - \text{Treated mean})}{\text{Control mean}} \times 100$$

In vitro COX Inhibitory activity

The inhibitory activities of compounds for COX-1 and COX-2 enzymes were assayed using the COX Inhibitor Screening Assay Kit (Cayman No: 560101) according to the protocol recommended by the supplier. This assay is based on the competition between PGs and a PG-acetylcholinesterase (AChE) conjugate (PG tracer) for a limited amount of PG antiserum. Because the concentration of the PG tracer is held constant while the concentration of PG varies, the amount of PG tracer that is able to bind to the PG antiserum will be inversely proportional to the concentration of PG in the well. This rabbit antiserum-PG (either free or tracer) complex binds to a mouse monoclonal anti-rabbit antibody that has been previously attached to the well. The plate is washed to remove any unbound reagents, and then Ellman's reagent (which contains the substrate to AChE) is added to the well. The product of this enzymatic reaction has a distinct yellow color and absorbs strongly at 412 nm. The intensity of this color, determined spectrophotometrically, is proportional to the amount of PG tracer bound to the well, which is inversely proportional to the amount of free PG present in the well during the incubation.

$$\text{Absorbance} \propto [\text{Bound PG Tracer}] \propto 1/[\text{PG}]$$

Anti-platelet activity

Anti-platelet activity was determined by using the tail transaction bleeding test as reported (Wang *et al*., 2004; Tanaka *et al*., 1998). Bleeding time of male mice (Laca strain) weighing 20–24 g was determined by administering compounds and aspirin (standard drug) suspended in carboxy methyl cellulose orally at 20 and 40 mg/kg or vehicle control. After 1 h, 3 mm of the tails of the mice under light diethyl ether anesthesia was transected and blood was dripped on filter paper. The duration of bleeding was recorded.

Whole blood clotting time assay

The whole blood clotting time test was essentially performed as described by Mackenzie et al. (Mackenzie *et al*., 1971). After the tail bleeding test was terminated, the tail was wiped dry. One of the tail veins was sliced with a razor blade, and the first drop of blood was allowed to fill a non-siliconized glass capillary tube. The tube was regularly tilted and checked for fluidity of the blood. When the blood ceased to flow by tilting or when fragmented blood coagula were observed to adhere to the inner walls, the tube was broken at 10-s intervals until a fibrin strand was seen between the broken ends. The whole blood clotting time was measured from the moment of filling the tube to the appearance of the first fibrin strands.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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