Synthesis and Biological Evaluation of 2-substituted-6-(morpholinyl/piperidinyl)pyridazin-3(2*H*)-ones as Potent and Safer Anti-inflammatory and Analgesic Agents

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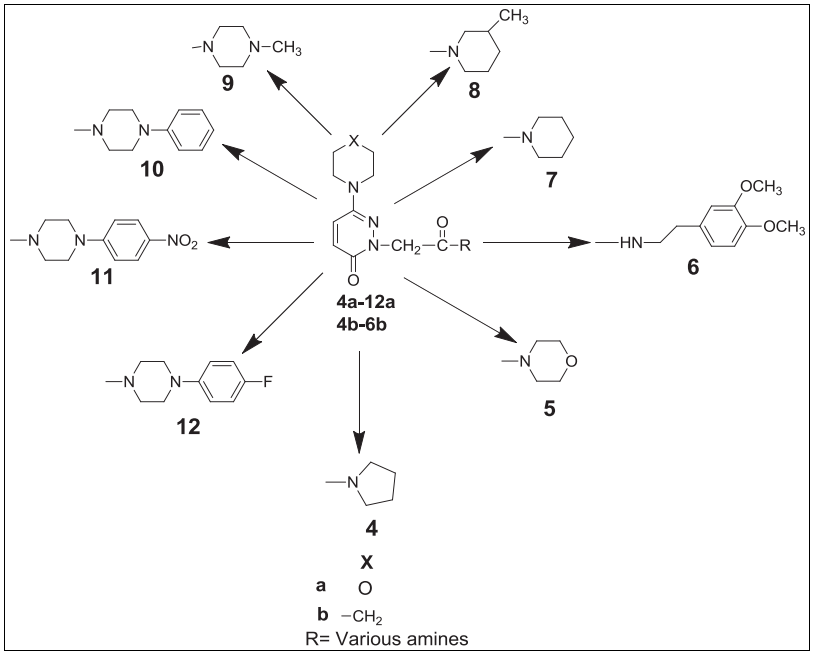
Received May 15, 2016
DOI 10.1002/jhet.2905
Published online 00 Month 2017 in Wiley Online Library (wileyonlinelibrary.com).



A series of 2-substituted-6-(morpholinyl/piperidinyl)pyridazin-3(2*H*)-ones was synthesized and the structures were established using various spectroscopic techniques. The target compounds were screened for anti-inflammatory and analgesic activities at 20 and 40 mg/kg. The safety of the synthesized derivatives was evaluated by assessing anti-platelet activity and ulcer index. The obtained pharmacological data revealed that 6-morpholinyl derivatives **4a–12a** were found to be somewhat more potent than 6-piperidinyl derivatives **4b–6b.** The 6-morpholinyl substituted pyridazinone **12a** exhibited maximum anti-inflammatory and analgesic activities. Homoveratrylamine substituted compounds **6a** and **6b** emerged as promising leads in both the series with good anti-inflammatory and analgesic activities without any ulcerogenicity. Anti-platelet activity results of the compounds of both the series showed significantly low bleeding time in comparison with standard drug aspirin indicating the cardiovascular safety of new pyridazinones.

INTRODUCTION

Inflammation is defined as a complex defensive process, in which body responds to different injuries. It is characterized by the accumulation of fluids and leukocytes with the objective of eliminating the noxious stimulus [1]. A large number of nonsteroidal anti-inflammatory drugs (NSAIDs) are available for the treatment of inflammation, pain, and fever [2,3]. These drugs act by inhibition of cyclooxygenase (COX), a key enzyme required for the prostaglandin biosynthesis [4]. COX enzyme exists in three isoforms: COX-1, COX-2, and COX-3. COX-1 is a constitutive enzyme that is known for its cytoprotection and maintains homeostasis while COX-2 is induced in response to inflammatory mediators. COX-3, produced by alternative splicing of COX-1, is considered as another target for anti-inflammatory drugs [5].

The long-term use of NSAIDs may produce gastrointestinal ulceration, bleeding, and nephrotoxicity because of the unwanted inhibition of COX-1 enzyme. Therefore, challenges are still there to develop potent and safer anti-inflammatory agents [6]. Preferential inhibition of COX-2 over COX-1 may prove to be an important

strategy for the development of safer NSAIDs. The identification of COX-2 as a prospective target has influenced the development of safer anti-inflammatory agents [7,8]. However, increasing selectivity for COX-2 may result in unwanted cardiovascular effects such as myocardial infarction, heart failure, and cardiac arrhythmia because of the altered balance between the prostacyclin and thromboxane that could promote the prothrombotic state.

Pyridazines and pyridazinones belong to an important category of heterocyclics, which have been reported to show wide range of biological activities, such as analgesic and anti-inflammatory [9–12], antihypertensive [13,14], anticancer [15], antiplatelet [16,17], anti-diabetic [18], anticonvulsant [19,20], and anti-microbial activities [21]. Literature has highlighted the importance of pyridazinone nucleus as an excellent template for the synthesis of anti-inflammatory agents [22] particularly in light of the platelet aggregation inhibitory and vasodilatory properties associated with this core structure. Emorfazone (Fig. 1), a 3(2*H*)-pyridazinone derivative, has been marketed in Japan as an analgesic and anti-inflammatory agent [9].

Structure activity relationship studies on pyridazinones have shown that *N*-substitution is an essential requirement for COX-2 selectivity [23]. It is also reported in literature that incorporation of morpholine, arylpiperidine and arylpiperazine moieties at the 6-position of the basic nucleus shows a positive influence on analgesic activity [9]. Keeping in mind the anti-platelet effects of pyridazinones, it was planned to introduce such moieties at the *N*-2 position of the 6-morpholinyl/piperidinyl substituted pyridazinones to obtain compounds with improved safety profile. The synthesized compounds were evaluated for their anti-inflammatory, analgesic, and anti-platelet potential.

RESULTS AND DISCUSSION

Chemistry. The title compounds were synthesized according to Scheme 1. 3-Chloro-6-substituted pyridazine derivatives **1a, 1b** were synthesized in accordance with the literature reports by nucleophilic displacement of 3,6-dichloropyridazine with morpholine and piperidine, respectively [24]. Synthesis of 6-substituted 3-oxo-pyridazinones derivatives **2a, 2b** is reported previously by simply refluxing 3-chloro-6-substituted pyridazines **1a, 1b** in glacial acetic acid for 6 h [25], but because of the noncompletion of the reaction even after 3 days, an alternative time saving synthetic route using microwave irradiation was adopted. In this method, pyridazines **1a** and **1b** were hydrolyzed at 140°C using glacial acetic acid in a microwave oven (Biotage, Sweden), which resulted in formation of 3-oxo products **2a**, **2b** in high yields in only 15 min. Esterification of **2a**, **2b** with methyl chloroacetate gave corresponding intermediate esters **3a** and **3b**, fusion of which with requisite cyclic amines afforded the target compounds **4a–12a** and **4b–6b**. The structure and purity of synthesized compounds were confirmed using various spectral and elemental analyses. Disappearance of ─NH peak and presence of characteristic singlets at ~ δ 3.7 ($COOCH_3$) and ~4.7 ppm ($─NCH_2$) confirmed the formation of 2-substituted products **3a** and **3b**. The methylenes attached to nitrogen of morpholine ring resonated at ~ δ 3.23 and those of piperidine ring at ~3.22 ppm in the ^{1}H-NMR spectra of all the target 6-morpholinyl (**4a–12a**) and 6-piperidinyl derivatives (**4b–6b**). Aromatic proton attached to C_4 of the pyridazinone ring was seen downfield (~ δ 7.1) as a doublet in comparison to the proton present at C5 position (~ δ 6.8). The carbonyl carbon of pyridazinone core structure was observed at ~ δ 160 in both the series of compounds in ^{13}C-NMR. The further detailed characterization of the synthesized compounds is given in the Experimental section.

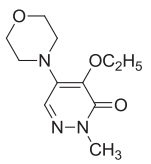
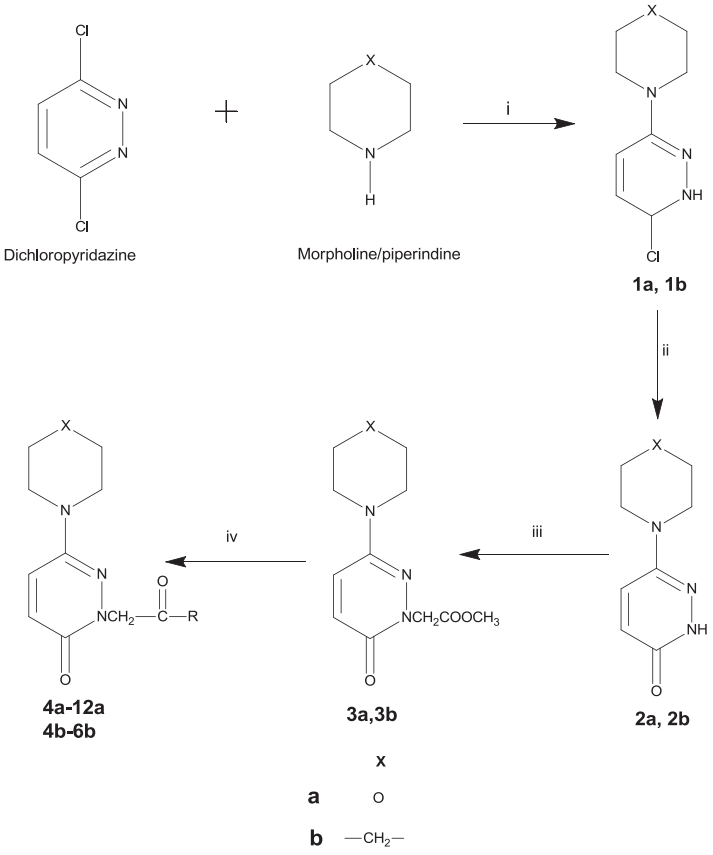


Figure 1. Chemical structure of emorfazone.

Anti-inflammatory activity. The pyridazinones **4a–12a** and **4b–6b** were evaluated for *in vivo* anti-inflammatory activity using carrageenan-induced rat paw edema model at 20 and 40 mg/kg. The results obtained are summarized in Table 1. In general, all the tested compounds produced dose dependent inhibition of edema, being more effective at 40 mg/kg than 20 mg/kg. The intermediate esters **3a** and **3b** were found to be less active than their acetamide derivatives. All compounds showed significant anti-inflammatory effects showing 30–70.96% inhibition of edema at 40 mg/kg after 240 min. In general, 6-morpholinyl derivatives **4a–12a** were found to possess marginally better anti-inflammatory activity than 6-piperidinyl derivatives **4b–6b**. Therefore, only three compounds of piperidinyl series were prepared. In morpholinyl series replacement of methyl group of piperazine nucleus (**9a**) by bulkier groups like phenyl (**10a**), p-nitrophenyl (**11a**) and fluorophenyl (**12a**) have shown positive influence on anti-inflammatory activity of these compounds. Substitution of esters **3a** and **3b** with homoveratrylamine group resulted in synthesis of two promising leads **6a** and **6b** with good anti-inflammatory activity in both the series. A moderate activity was

Scheme 1. Synthetic scheme for the formation of 2-substituted-6-(morpholinyl/piperidinyl)-pyridazin-3(2*H*)-ones. Reaction conditions and reagents: (i) thermal fusion, 120°C; (ii) glacial acetic acid, microwave, 140°C; (iii) ethyl methyl ketone, methyl chloroacetate, anhyd. K_2CO_3, 90–100°C; (iv) thermal fusion, 100–110°C.



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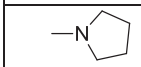
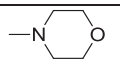
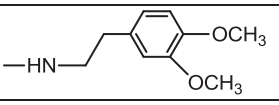
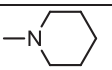
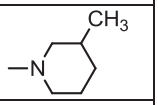
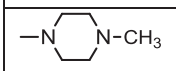
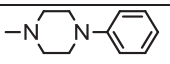
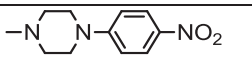
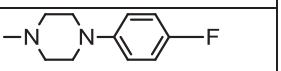
					
	9	**10**	**11**	**12**	
					

generally shown by all remaining compounds at a twofold dose when compared with indomethacin.

Gastric ulcerogenic activity. All the compounds were evaluated for ulcerogenic potential as the long-term administration of NSAIDs may produce gastrointestinal erosions and ulcers as common side effects. The results listed in Table 2 revealed that all the synthesized compounds have shown superior gastrointestinal safety at both tested doses in comparison with standard drug indomethacin.

Analgesic activity. The analgesic activity of the synthesized pyridazinone derivatives was evaluated by acetic acid-induced writhing model (Table 3), which is an established procedure to evaluate the peripherally acting analgesics. Analgesic activity of the compounds was in good correlation with their anti-inflammatory activity profile. Intermediate esters of both morpholinyl and piperidinyl substituted pyridazinones **3a** and **3b** displayed marginally lower percentage protection against acetic acid-induced writhes in comparison with their final carboxylate amide derivatives at both tested doses. In accordance with literature reports, introduction of arylpiperazine moieties on the side chain at 2- position of pyridazinones seems to be contributing positively toward anti-nociceptive activity, with *p*-fluorophenyl substituted derivative **12a** displaying maximum protection (44.06%) followed by *p*-nitrophenyl substituted derivative **11a** with 41.80% protection at

Table 1

Anti-inflammatory activity of various newly synthesized pyridazinones and standard drugs.

Compd. no.	Dose (mg/kg)	Edema volume (mL) ± SEM (% inhibition)					
		30 min	60 min	90 min	120 min	180 min	240 min
3a	20	0.22 ± 0.04 (0)	0.39 ± 0.04 (2.53)	0.37 ± 0.02 (12.80)	0.38 ± 0.03 (13.85)	0.36 ± 0.02 (18.00)	0.36 ± 0.03 (18.00)
	40	0.28 ± 0.03 (2.00)	0.45 ± 0.03 (8.70)	0.48 ± 0.02 (13.80)	0.46 ± 0.2 (17.85)	0.44 ± 0.03 (30.00)	0.44 ± 0.03 (30.00)
4a	20	0.23 ± 0.01 (3.95)	0.32 ± 0.02 (4.34)	0.34 ± 0.03 (7.14)	0.32 ± 0.02 (14.28)*	0.31 ± 0.02 (25.80)**	0.29 ± 0.03 (32.25)***
	40	0.26 ± 0.02 (6.70)	0.35 ± 0.01 (8.69)	0.31 ± 0.02 (25.36)	0.29 ± 0.01 (32.21)*	0.28 ± 0.01 (41.93)**	0.26 ± 0.01 (48.38)**
5a	20	0.30 ± 0.02 (0)	0.45 ± 0.03 (8.69)	0.48 ± 0.02 (14.28)	0.48 ± 0.01 (17.85)	0.47 ± 0.02 (29.03)	0.44 ± 0.02 (38.56)
	40	0.26 ± 0.03 (0)	0.31 ± 0.02 (13.04)	0.38 ± 0.00 (23.91)	0.32 ± 0.02 (25.0)	0.28 ± 0.02 (45.16)**	0.25 ± 0.02 (54.83)***
6a	20	0.16 ± 0.01 (0)	0.35 ± 0.03 (8.69)	0.34 ± 0.03 (13.04)	0.34 ± 0.02 (14.28)	0.31 ± 0.02 (32.25)*	0.29 ± 0.03 (38.70)**
	40	0.24 ± 0.01 (6.66)	0.29 ± 0.02 (17.39)	0.31 ± 0.02 (25)	0.29 ± 0.01 (32.14)*	0.27 ± 0.02 (45.16)**	0.22 ± 0.03 (61.29)***
7a	20	0.21 ± 0.05 (0)	0.34 ± 0.05 (12.14)	0.36 ± 0.03 (13.28)	0.33 ± 0.02 (20.43)	0.32 ± 0.04 (30.43)**	0.31 ± 0.02 (32.00)**
	40	0.21 ± 0.04 (0)	0.35 ± 0.05 (10.2)	0.36 ± 0.02 (16.00)	0.33 ± 0.05 (27.22)	0.27 ± 0.06 (41.30)**	0.27 ± 0.05 (41.30)**
8a	20	0.28 ± 0.02 (−5.00)	0.33 ± 0.02 (9.48)	0.36 ± 0.01 (13.04)	0.36 ± 0.04 (16.1)	0.34 ± 0.03 (28.1)*	0.32 ± 0.02 (34.64)**
	40	0.26 ± 0.03 (4.60)	0.32 ± 0.01 (13.79)	0.34 ± 0.02 (20.3)	0.33 ± 0.02 (26.57)	0.31 ± 0.01 (37.9)*	0.28 ± 0.04 (47.7)**
9a	20	0.26 ± 0.03 (−5.30)	0.32 ± 0.02 (5.17)	0.35 ± 0.02 (9.04)	0.33 ± 0.01 (19.5)	0.33 ± 0.04 (24.8)*	0.31 ± 0.02 (31.37)**
	40	0.24 ± 0.01 (7.89)	0.30 ± 0.03 (13.8)	0.32 ± 0.02 (20.3)	0.31 ± 0.02 (26.57)	0.30 ± 0.03 (34.6)*	0.28 ± 0.02 (41.2)**
10a	20	0.19 ± 0.02 (9.52)	0.32 ± 0.05 (17.96)	0.35 ± 0.06 (21.03)	0.33 ± 0.05 (25.00)	0.32 ± 0.05 (30.43)*	0.32 ± 0.05 (30.43)*
	40	0.17 ± 0.05 (19.42)	0.28 ± 0.03 (25.55)	0.27 ± 0.05 (34.88)*	0.27 ± 0.05 (41.36)**	0.26 ± 0.04 (43.37)**	0.24 ± 0.05 (47.84)***
11a	20	0.24 ± 0.02 (4)	0.39 ± 0.03 (12.06)	0.40 ± 0.02 (23.94)	0.40 ± 0.01 (23.94)	0.40 ± 0.02 (31.37)	0.39 ± 0.02 (34.64)
	40	0.26 ± 0.02 (6.57)	0.32 ± 0.02 (8.89)	0.32 ± 0.03 (21.40)	0.29 ± 0.01 (32.14)*	0.26 ± 0.02 (48.38)**	0.23 ± 0.02 (58.06)***
12a	20	0.21 ± 0.02 (0)	0.38 ± 0.04 (4.34)	0.37 ± 0.04 (23.80)	0.37 ± 0.02 (25.35)	0.34 ± 0.03 (38.56)*	0.32 ± 0.02 (47.05)**
	40	0.27 ± 0.03 (0)	0.27 ± 0.03 (34.48)	0.28 ± 0.02 (42.85)*	0.24 ± 0.03 (57.14)**	0.22 ± 0.03 (67.74)***	0.21 ± 0.02 (70.96)***
3b	20	0.21 ± 0.02 (0)	0.37 ± 0.03 (5.30)	0.36 ± 0.04 (16.28)	0.37 ± 0.04 (15.91)	0.38 ± 0.04 (17.39)	0.35 ± 0.03 (20.91)
	40	0.20 ± 0.02 (4.76)	0.33 ± 0.04 (15.38)	0.35 ± 0.02 (18.60)	0.35 ± 0.03 (18.45)	0.33 ± 0.0 4 (28.26)	0.29 ± 0.03 (36.96)*
4b	20	0.23 ± 0.03 (−9.52)	0.38 ± 0.04 (2.56)	0.36 ± 0.03 (16.28)	0.35 ± 0.03 (20.45)	0.35 ± 0.05 (23.91)	0.29 ± 0.03 (36.96)*
	40	0.2 ± 0.03 (4.76%)	0.34 ± 0.04 (12.82)	0.32 ± 0.02 (25.58)*	0.3 ± 0.04 (31.82)*	0.27 ± 0.06 (41.30)*	0.25 ± 0.03 (45.65)*
5b	20	0.21 ± 0.04 (0)	0.36 ± 0.03(7.69)	0.37 ± 0.06(13.95)	0.34 ± 0.04 (22.73)	0.34 ± 0.03 (26.09)	0.30 ± 0.02 (34.78)*
	40	0.21 ± 0.05 (0	0.37 ± 0.06 (5.13)	0.34 ± 0.04 (20.93)	0.33 ± 0.05 (25.00)	0.28 ± 0.03 (39.13)	0.23 ± 0.04 (50.00)**
6b	20	0.22 ± 0.02 (−4.76%)	0.35 ± 0.05 (10.26)	0.36 ± 0.06 (16.28)	0.35 ± 0.04 (20.45)	0.34 ± 0.03 (26.09)	0.32 ± 0.04 (30.43)
	40	0.2 ± 0.02 (4.76)	0.34 ± 0.04 (12.82)	0.34 ± 0.03 (20.93)	0.32 ± 0.05 (27.27)	0.26 ± 0.04 (43.48)***	0.22 ± 0.03 (52.17)**
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
Indomethacin	20	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 ± 0.05 (82.6)***	0.05 ± 0.00 (80.8)**
Celecoxib	20	0.20 ± 0.03 (4.76)	0.20 ± 0.05 (48.72)**	0.21 ± 0.01 (60.47)***	0.15 ± 0.001 (70.45)***	0.08 ± 0.05 (82.61)***	0.05 ± 0.00 (82.61)***

Data is 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 with control value at respective time point.

Table 2

The ulcerogenic activity of the pyridazinones and standard drug.

Compd. no.	Ulcerogenic activity SI ± SEM	
	20 mg/kg	40 mg/kg
3a	0.20 ± 0.12**	0.50 ± 0.22
4a	0.30 ± 0.12*	0.40 ± 0.20
5a	0.40 ± 0.10	0.60 ± 0.20
6a	0.0	0.0
7a	0.30 ± 0.12*	0.50 ± 0.16
8a	0.20 ± 0.12**	0.40 ± 0.20
9a	0.10 ± 0.20***	0.20 ± 0.20**
10a	0.20 ± 0.12	0.30 ± 0.13*
11a	0.10 ± 0.12***	0.20 ± 0.16**
12a	0.30 ± 0.11*	0.40 ± 0.10
3b	0.20 ± 0.12**	0.40 ± 0.11
4b	0.30 ± 0.20*	0.40 ± 0.10
5b	0.20 ± 0.13**	0.50 ± 0.0
6b	0.0	0.0
Control	0.0	
Indomethacin	1.6 ± 0.20	

Severity index (SI): mean score of each treated group minus the mean score of the control group. Relative to standard and data were analyzed by ANOVA followed by Dunnett's multiple comparison test.
*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

Table 3

Analgesic activity of various pyridazinones and standard drugs.

Compd. no.	Dose (mg/kg)	No. of writhes ± SEM	% Protection
3a	20	29.0 ± 1.22*	18.08
	40	25.8 ± 1.70***	27.12
4a	20	28.80 ± 1.28*	19.00
	40	22.20 ± 1.30***	37.28
5a	20	26.20 ± 1.39**	26.00
	40	21.60 ± 1.00***	39.00
6a	20	27.60 ± 1.63**	22.03
	40	20.40 ± 1.33***	42.37
7a	20	28.6 ± 2.71**	19.20
	40	24.8 ± 1.39***	29.94
8a	20	26.83 ± 1.36**	24.04
	40	24.18 ± 1.30***	31.60
9a	20	30.40 ± 1.20	14.12
	40	23.40 ± 1.25***	34.00
10a	20	27.60 ± 1.72**	22.03
	40	21.60 ± 1.07***	38.98
11a	20	26.80 ± 2.03**	24.29
	40	20.60 ± 2.60***	41.80
12a	20	26.20 ± 1.39**	26.0**
	40	19.80 ± 1.60***	44.06
3b	20	30.5 ± 1.90	13.84
	40	28.16 ± 1.42	20.45
4b	20	29.0 ± 2.29	18.0
	40	25.61 ± 2.31***	28.9
5b	20	29.16 ± 2.47	17.6
	40	23.83 ± 2.43***	32.0
6b	20	29.16 ± 2.1	17.6
	40	23.5 ± 2.14***	33.61
Control	20	35.4 ± 1.2	
Celecoxib	20	9.83 ± 1.07***	72.16
Aspirin	20	13.4 ± 1.14***	62.3

Data is 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 with control value.

40 mg/kg. In strong agreement with anti-inflammatory activity, compounds **6a** and **6b** having homoveratrylamino group as the side chain emerged as potent compounds with percentage protection of 42.37 and 33.61 at 40 mg/kg in the respective series. 6-(Piperidin-1-yl)pyridazin-3(2*H*)-ones **3b**–**6b** displayed comparatively lower analgesic activity profile than their corresponding morpholinyl substituted structural analogues.

Antiplatelet activity. There has been much focus recently with respect to cardiovascular risk associated with long-term usage of both COX-2 and non-selective NSAIDs [26]. The American Heart Association in 2007 issued a new guidance discouraging the use selective COX-2 inhibitors and NSAIDs in patients with heart diseases and those with high risk of heart diseases. Hence, we evaluated the cardiovascular safety profile of the synthesized compounds by tail transaction bleeding test in mice. The data for the tail transaction bleeding are given in Table 4. Both the series have shown different effects in tail transaction bleeding test. The intermediate ester of 6-morpholinyl substituted derivative **3a** has shown marginal increase in bleeding time at 40 mg/kg in comparison with 6-piperidinyl substituted ester **3b**. 6-Morpholinyl substituted pyridazinone derivatives showed dose dependent increase in bleeding time except compounds **4a**, **6a, 8a,** and **11a** whereas the 6-piperdinyl substituted analogues did not produce significant changes in bleeding time in respect to control. Among the 6-morpholinyl substituted compounds, pyrrolidinyl (**4a**) and 3-methylpiperidinyl (**8a**) pyridazinones displayed negligible change in bleeding time with respect to control and emerged as safest derivatives of the series at both tested doses in terms of cardiovascular risk. Although 6-morpholinylpyridazinones showed good cardiovascular safety, substitution of pyridazinones with piperidine ring at 6- position is even better in this regard. The platelet aggregation effects reported with selective COX-2 inhibitors are not observed in the current series as depicted by significantly low bleeding time in comparison with standard drug aspirin and marginally high bleeding time in comparison with control.

Looking at the chemical structure of the synthesized compounds, 2-substitution with flourophenylpiperazine moiety resulted in increased anti-inflammatory and analgesic activities. Compound 2-(2-(4-(4-fluorophenyl) piperazin-1-yl)-2-oxoethyl)-6-morpholino-pyridazin-3(2*H*)-one (**12a)** exhibited potent anti-inflammatory (70.96% inhibition of edema) and analgesic activities (44.06% protection) with lower ulcerogenicity.

Table 4

Anti-platelet activity of the pyridazinones and standard drug.

Compd. no.	Bleeding time (s)	
	20 mg/kg	40 mg/kg
3a	230.0 ± 12.24	239.0 ± 8.80
4a	217.0 ± 9.69	212 ± 14.57
5a	218.0 ± 3.74	246.0 ± 13.64
6a	282.0 ± 10.67*	244.0 ± 12.83
7a	234.0 ± 12.77	250.0 ± 12.20
8a	220.0 ± 15.7	198.0 ± 18.4
9a	237.0 ± 13.11	250.0 ± 8.92
10a	238.0 ± 10.67	248.0 ± 15.50
11a	248.0 ± 11.13	218.0 ± 28.70
12a	236.0 ± 9.27	254.0 ± 10.93
3b	228.0 ± 13.6	224.0 ± 11.7
4b	204.0 ± 11.7	192.0 ± 10.2
5b	220.0 ± 14.1	200.0 ± 12.6
6b	216.0 ± 9.8*	212.0 ± 18.5
Control	220.0 ± 13.0	
Aspirin	290.0 ± 11.8**	

Data is 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 with control value.

CONCLUSION

In the present study, we report the synthesis, spectral characterization, and pharmacological evaluation of a novel series of 2-substituted-6-(morpholinyl/piperidinyl) pyridazin-3(2*H*)-one derivatives. The compounds have been designed to develop safer anti-inflammatory drugs. The compounds showed potent anti-inflammatory and analgesic activities with cardioprotective and ulcerogenic sparing effects.

EXPERIMENTAL

Instrumentation and chemicals. All the chemicals and reagents used in the synthesis were purchased from commercial supplier (Sigma Aldrich). Melting points of synthesized compounds were determined on a Veego melting point apparatus and are uncorrected. IR spectra were recorded on Perkin-Elmer RX1 FTIR spectrophotometer model as potassium bromide pellets (υ_{max} in cm^{-1}). The ^{1}H-NMR and ^{13}C-NMR were recorded on Bruker AC-400F, 400 MHz spectrophotometer using either DMSO-d_6 or $CDCl_3$ as solvent. Chemical shifts are reported in δ ppm units with respect to TMS as internal standard. Mass spectra were determined on an Applied Biosystems API 2000 mass spectrometer. The purity of the compounds was established by thin layer chromatography (TLC) and elemental analysis. The monitoring of the rate of reaction was carried out on TLC plates prepared according to Stahl's method using ethyl acetate as solvent, and plates were activated at temperature of 110°C for 30 min. The elemental analysis (C, H, N) of the compounds was performed on Perkin-Elmer-2400 CHN elemental analyzer. Results of elemental analysis were within ±0.4% of the theoretical values. The spin multiplicities are denoted as singlet (s), broad singlet (br s), doublet (d), double doublet (dd), triplet (t), doublet of triplets (dt), and so on. The *in vivo* acute anti-inflammatory activity was carried out using digital plethysmometer (Ugo-Basile, Italy).

Synthesis. ***General procedure for synthesis of 6-substitutedpyridazin-3(2H)-ones (2a, 2b).*** A solution of 3-chloro-6-substitutedpyridazine (1.2 g, 6.09 mmol) in glacial acetic acid (15 mL) was prestirred for 20 s and then irradiated in a microwave oven at 140°C for 15 min. The completion of reaction was monitored by TLC. On completion of the reaction, acetic acid was removed under reduced pressure. Remaining liquid residue was dissolved in water and extracted with chloroform (3 × 25 mL). The collected organic layer was finally washed with water, dried over sodium sulfate, and evaporated under reduced pressure. The residue so obtained was purified by recrystallization from ethyl acetate to afford 6-substitutedpyridazin-3(2*H*)-ones **2a** and **2b**.

6-Morpholinopyridazin-3(2H)-one (2a). Yield: 0.5 g, (72.92%); mp. 181–182°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.24 (t, 4H, ─N(C*H*$_2$)$_2$, morpholine, J = 4.86 Hz), 3.80 (t, 4H, ─O(C*H*$_2$)$_2$, morpholine, J = 4.84 Hz), 6.91 (d, 1H, 5-C*H*, pyridazinone, J = 10.04 Hz), 7.17 (d, 1H, 4-C*H*, pyridazinone, J = 10.08 Hz) and 11.17 ppm (s(br), 1H, ─N*H* pyridazinone); FTIR (KBr, cm^{-1}) υ_{max}: 3324 (N─H), 3059 (C─H, aromatic), 2922 (C─H, aliphatic), 2862 (C─H, aliphatic), 1663 (C═O, amide), 1584 (C═C, aromatic), 1440 (CH_2, bending), and 1112 (C─O─C); *Anal*. calcd. for $C_8H_{11}N_3O_2$: C, 52.44; H, 7.15; N, 22.94. Found: C, 52.78; H, 6.92; N, 23.15.

6-(Piperidin-1-yl)pyridazin-3(2H)-one (2b). Yield: 0.6 g, (75.55%); mp. 183–185°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.62–1.65 (m, 6H, ─(C*H*$_2$)$_3$─, piperidine), 3.22–3.24 (m, 4H, ─N(C*H*$_2$)$_2$, piperidine), 6.86 (d, 1H, 5-C*H*, pyridazinone, J = 10.04 Hz), 7.19 (d, 1H, 4-C*H*, pyridazinone, J = 10.20 Hz), and 10.88 ppm (s, 1H, ─N*H*, pyridazinone); FTIR (KBr, cm^{-1}) υ_{max}: 3246 (N─H), 3057 (C─H, aromatic), 2932 (C─H, aliphatic), 1667 (C═O, amide), 1585 (C═C, aromatic), 1445 (CH_2, bending), and 1236 (C─O─C); *Anal*. calcd. for $C_9H_{13}N_3O$: C, 60.32; H, 7.31; N, 23.45. Found: 60.41; H, 7.45; N, 23.29.

General procedure for synthesis of 2-(methoxycarbonylmethyl)-6-(substituted)pyridazin-3(2H)-ones (3a, 3b). Methyl chloroacetate (1 mL, 9.21 mmol) was added to the stirred and refluxing suspension of desired 6-substituted pyridazin-3(2*H*)-one (**2a, 2b**) (0.60 g, 3.34 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 at a

temperature of 90–100°C. 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. Thus, obtained oily residue was purified by recrystallization in diethyl ether to afford corresponding 2-substituted pyridazinones **3a** and **3b**.

2-(Methoxycarbonylmethyl)-6-(morpholin-4-yl)pyridazin-3(2H)-one (3a). Yield: 0.50 g (60.54%); mp. 92–94°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.23 (t, 4H, ─N(C*H*$_2$)$_2$, morpholine, *J* = 4.88 Hz), 3.77 (s, 3H, COOC*H*$_3$), 3.79 (t, 4H, ─O(C*H*$_2$)$_2$, morpholine, *J* = 4.86), 4.76 (s, 2H, ─NC*H*$_2$), 6.90 (d, IH, 5-C*H*, pyridazinone, *J* = 10.00 Hz), and 7.13 ppm (d, 1H, 4-C*H*, pyridazinone, *J* = 10.04 Hz); FTIR (KBr, cm^{-1}) υ_{max}: 3057 (C─H, aromatic), 2962 (C─H, aliphatic), 2854 (C─H, aliphatic), 1753 (C═O, ester), 1663 (C═O, amide), 1587 (C═C, aromatic), 1455 (CH_2, bending), 1358, and 1114 (C─O─C). *Anal*. calcd. for $C_{11}H_{15}N_3O_4$: C, 52.16; H, 5.97; N, 16.95. Found: C, 51.98; H, 6.12; N, 16.73.

2-(Methoxycarbonylmethyl)-6-(piperidin-1-yl)pyridazin-3(2H)-one (3b). ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.46 (s(br), 6H, ─(C*H*$_2$)$_3$─, piperidine), 3.15 (s(br), 4H, ─N(C*H*$_2$)$_2$, piperidine), 3.69 (s, 3H, ─COOC*H*$_3$), 4.67 (s, 2H, ─NC*H*$_2$), 6.77 (d, 1H, 5-C*H*, pyridazinone, *J* = 10 Hz), and 7.11 ppm (d, 1H, 4-C*H*, pyridazinone, *J* = 10 Hz,).

General procedure for synthesis of 2-substituted-6-(morpholin-4-yl/piperidin-1-yl)pyridazin-3(2H)-ones (4a-12a and 4b-6b). A mixture of desired 2-(methoxycarbonylmethyl)-6-(substituted)pyridazin-3*(2H)*-one (**3a**, **3b**) (0.7 g, 2.27 mmol) and requisite amine (1 mL, in excess) was heated at 100–110°C with continuous stirring. The mixture was further stirred with heating for 5–7 h for all, except for compound **10a** (heating time 10 h), and for **6a** and **6b** (heating time 13 h), the reaction being monitored by TLC. On completion, ice cold water was added to the reaction mixture. The clear aqueous solution thus obtained was evaporated under reduced pressure. The residual liquid was crystallized by refluxing in ethyl acetate and hexane to afford corresponding target compounds **4a**–**12a** and **4b**–**6b**.

2-(2-(Pyrrolidin-1-yl)-2-oxoethyl)-6-(morpholin-4-yl)-pyridazin-3(2H)-one (4a). Yield: 0.43 g (53.75%); mp. 182–184°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.87 (p, 2H, ─C*H*$_2$─, pyrrolidine, *J* = 6.90 Hz), 2.01 (p, 2H, ─C*H*$_2$─, pyrrollidine, *J* = 6.56 Hz,), 3.23 (t, 4H, ─N(C*H*$_2$)$_2$, morpholine, *J* = 4.84 Hz), 3.48–3.53 (m, 4H, ─N(C*H*$_2$)$_2$, pyrrolidine), 3.78 (t, 4H, ─O(C*H*$_2$)$_2$, morpholine, *J* = 4.84 Hz), 4.75 (s, 2H, ─NC*H*$_2$), 6.90 (d, 1H, 5-C*H*, pyridazinone, *J* = 9.92 Hz), and 7.12 ppm (d, 1H, 4-C*H*, pyridazinone, *J* = 9.96 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 24.09 (**C**H$_2$), 26.18 (**C**H$_2$), 45.71 (**C**H$_2$), 46.05 (**C**H$_2$), 46.95 (2× **C**H$_2$), 53.70 (**C**H$_2$CO), 66.40 (2× **C**H$_2$), 125.37 (Ar**C**H), 131.12 (Ar**C**H), 148.96 (Ar**C**), 158.65 (**C**═O), and 164.68 ppm (**C**═O); FTIR (KBr, cm^{-1}) υ_{max}: 3058 (C─H, aromatic), 2964 (C─H, aliphatic), 1656 (C═O, amide), 1587 (C═C, aromatic), 1445 (CH_2, bending), 1338, 1258 (C─N), and 1116 (C─O─C); MS (ESI) m/z: 293.2 [M + H]$^+$; *Anal*. calcd. for $C_{14}H_{20}N_4O_3$: C, 57.52; H, 6.90; N, 19.17. Found: C, 57.46; H, 6.99; N, 18.94.

2-(2-(Morpholin-4-yl)-2-oxoethyl)-6-(morpholin-4-yl)pyridazin-3(2H)-one (5a). Yield: 0.50 g (58.28%); mp. 200–202°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.24 (t, 4H, ─N(C*H*$_2$)$_2$, morpholine, *J* = 4.82 Hz), 3.50 (t, 2H, ─NC*H*$_2$, morpholine, *J* = 4.78 Hz), 3.63 (t, 2H, ─NC*H*$_2$, morpholine, *J* = 4.64 Hz), 3.72 (m, 4H, ─O(C*H*$_2$)$_2$, morpholine), 3.78 (t, 4H, ─O(C*H*$_2$)$_2$, morpholine, *J* = 4.82 Hz), 4.83 (s, 2H, ─NC*H*$_2$), 6.90 (d, 1H, 5-C*H,* pyridazinone, *J* = 9.96 Hz), and 7.13 ppm (d, 1H, 4-C*H,* pyridazinone, *J* = 9.96 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 42.30 (**C**H$_2$), 45.31 (**C**H$_2$), 46.87 (2× **C**H$_2$), 52.64 (**C**H$_2$CO), 66.38 (2× **C**H$_2$), 66.77 (2× **C**H$_2$), 125.49 (Ar**C**H), 131.09 (Ar**C**H), 149.12 (Ar**C**), 158.61 (**C**═O), and 165.05 ppm (**C**═O); FTIR (KBr, cm^{-1}) υ_{max}: 2961 (C─H, aromatic), 2924 (C─H, aliphatic), 2864 (C─H, aliphatic), 1657 (C═O, amide), 1588 (C═C, aromatic), 1453 (CH_2, bending), 1251 (C─N), and 1117 (C─O─C); MS (ESI) m/z: 309.3 [M + H]$^+$; *Anal*. calcd. for $C_{14}H_{20}N_4O_4$: C, 54.53; H, 6.54; N, 18.17. Found C, 54.86; H, 6.35; N, 18.32.

2-(2-(3,4-Dimethoxyphenethylamino)-2-oxoethyl)-6-(morpholin-4-yl)pyridazin-3(2H)-one (6a). Yield: 0.35 g (44.30%); mp. 142–145°C; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 2.68 (t, 2H, ─NHCH$_2$C*H*$_2$─, *J* = 7.06 Hz), 3.15 (t, 4H, ─N(C*H*$_2$)$_2$, morpholine, *J* = 4.86 Hz), 3.42 (q, 2H, ─NHC*H*$_2$CH$_2$─, *J* = 6.64 Hz), 3.71 (t, 4H, ─O(C*H*$_2$)$_2$, morpholine, *J* = 4.82 Hz), 3.78 (s, 3H, ─OC*H*$_3$), 3.79 (s, 3H, ─OC*H*$_3$), 4.57 (s, 2H,─NC*H*$_2$), 6.43 (s(br), 1H, ─N*H*), 6.62 (m, 2H, Ar*H*), 6.69 (d, 1H, Ar*H, J*$_o$ = 8.68 Hz), 6.79 (d, 1H, 5-C*H,* pyridazinone, *J* = 9.96 Hz), and 7.04 ppm (d, 1H, 4-C*H,* pyidazinone, *J* = 9.96 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 35.00 (**C**H$_2$), 40.69 (**C**H$_2$), 46.70 (2× **C**H$_2$), 55.89 (**C**H$_2$CO), 55.92 (O**C**H$_3$), 56.19 (O**C**H$_3$) 66.28 (2× **C**H$_2$), 111.33 (Ar**C**H), 111.95 (Ar**C**H), 120.60 (Ar**C**H), 125.29 (Ar**C**H), 131.12 (Ar**C**H), 131.24 (Ar**C**), 147.57 (Ar**C**), 148.93 (Ar**C**), 149.36 (Ar**C**), 158.60 (**C**═O), and 167.39 ppm (**C**═O); FTIR (KBr, cm^{-1}) υ_{max}: 3307 (N─H), 3097 (C─H, aromatic), 2925 (C─H, aliphatic), 1661 (C═O, amide), 1585 (C═C, aromatic), 1460 (CH_2, bending), 1265, and 1160 (C─O─C); MS (ESI) m/z: 403.3 [M + H]$^+$; *Anal*. calcd. for $C_{20}H_{26}N_4O_5$: C, 59.68; H, 6.51; N, 13.92. Found: C, 59.86; H, 6.40; N, 13.98.

2-(2-(Piperidin-1-yl)-2-oxoethy)-6-(morpholin-4-yl)pyridazin-3(2H)-one (7a). Yield: 0.18 g (43.90%); mp. 164–166°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.57–1.68 (m(br), 6H, ─(C*H*$_2$)$_3$─, piperidine), 3.23 (t, 4H, −N(C*H*$_2$)$_2$, morpholine, *J* = 4.82 Hz), 3.41 (t, 2H, ─NC*H*$_2$, piperidine, *J* = 5.16 Hz), 3.56 (t, 2H, ─NC*H*$_2$, piperidine, *J* = 5.50 Hz,), 3.78 (t, 4H, ─O(C*H*$_2$)$_2$, morpholine, *J* = 4.82 Hz) 4.83 (s, 2H, ─NC*H*$_2$), 6.90 (d, 1H, 5-C*H,* pyridazinone, *J* = 10.00 Hz,), and 7.13 ppm (d, 1H, 4-C*H,* pyridazinone, *J* = 10.00 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 24.41 (**C**H$_2$), 25.35 (**C**H$_2$), 26.19 (**C**H$_2$), 43.19 (**C**H$_2$), 45.91 (**C**H$_2$), 46.89 (2× **C**H$_2$), 52.89 (**C**H$_2$CO), 66.40 (2× **C**H$_2$), 125.32 (Ar**C**H), 131.11 (Ar**C**H), 148.98 (Ar**C**),

158.65 (**C**═O), and 164.44 ppm (**C**═O); FTIR (KBr, cm^{-1}) υ_{max}: 3053 (C─H, aromatic), 2947 (C─H, aliphatic), 2863 (C─H, aliphatic), 1654 (C═O, amide), 1585 (C═C, aromatic), 1448 (CH_2 bending), 1256 (C─N), and 1117 (C─O─C); MS (ESI) m/z: 307.2 $[M + H]^+$; *Anal*. calcd. for $C_{15}H_{22}N_4O_3$: C, 58.81; H, 7.24; N, 18.29. Found: C, 59.06; H, 6.99; N, 18.29.

2-(2-(3-Methylpiperidin-1-yl)-2-oxoethyl)-6-(morpholin-4-yl) pyridazin-3(2H)-one (8a). Yield: 0.42 g (50.00%); mp. 138–140°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.92 (m, 3H, ─C(C*H*$_3$)H-, piperidine), 1.15–1.19 (m, 1H, ─C*H(*H)─, piperidine), 1.46–1.76 (m, 3H, ─C*H(*H)─ and ─C*H*$_2$─, piperidine), 1.83 (d, 1H, ─C(CH$_3$)*H,* piperidine J = 13.12 Hz), 2.29 (t) and 3.02 (t) (1:1, 1H, ─NCH(*H),* piperidine J = 12.44 Hz), 2.69 (p, 1H, ─NCH(*H*), J = 13.12 Hz), 3.23 (t, 4H, ─N(C*H*$_2$)$_2$, morpholine, J = 4.64 Hz), 3.67 (dd, 1H, ─NCH(*H*), J = 13.28 Hz), 3.77 (t, 4H, ─O(C*H*$_2$)$_2$, morpholine, J = 4.64 Hz), 4.38 (d, 1H, ─NCH(*H*), J = 13.0 Hz), 4.83 (m, 2H, ─NC*H*$_2$), 6.90 (d, 1H, 5-C*H*, pyridazinone, J = 9.92 Hz), and 7.12 ppm (d, 1H, 4-C*H*, pyridazinone, J = 9.96 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 19.00 (**C**H$_3$), 25.69 (**C**H$_2$), 30.85 (**C**H$_2$), 31.61 (**C**H$_2$), 45.46 (**C**H$_2$), 46.91 (2× **C**H$_2$), 49.60 (**C**H$_2$), 52.40 (**C**H$_2$CO), 66.40 (2× ─**C**H$_2$), 125.33 (Ar**C**H), 131.11 (Ar**C**H), 148.98 (Ar**C**), 158.64 (**C**═O), and 164.44 ppm (**C**═O); FTIR (KBr, cm^{-1}) υ_{max}: 3066 (C─H, aromatic), 2933 (C─H, aliphatic), 2854 (C─H, aliphatic), 1653 (C═O, amide), 1594 (C═C, aromatic), 1455 (CH_2, bending), 1258 (C─N), and 1116 (C─O─C); MS (ESI) m/z: 321.3 $[M + H]^+$; *Anal*. calcd. for $C_{16}H_{24}N_4O_3$: C, 59.98; H, 7.54; N, 17.48. Found: C, 60.23; H, 7.25; N, 17.67.

2-(2-(4-Methylpiperazin-1-yl)-2-oxoethyl)-6-(morpholin-4-yl) pyridazin-3(2H)-one (9a). Yield: 0.42 g (61.76%); mp. 192–194°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.25 (s, 3H, ─NC*H*$_3$), 2.35 (t, 2H, ─NC*H*$_2$, piperazine, J = 4.80 Hz), 2.40 (t, 2H, ─NC*H*$_2$, piperazine, J = 4.62 Hz), 3.16 (t, 4H, ─N(C*H*$_2$)$_2$, morpholine, J = 4.70 Hz), 3.44 (t, 2H, ─NC*H*$_2$, piperazine, J = 4.70 Hz), 3.59 (s(br), 2H, ─NC*H*$_2$, piperazine), 3.71 (t, 4H, ─O(C*H*$_2$)$_2$, morpholine, J = 4.66 Hz), 4.77 (s, 2H, ─NC*H*$_2$─), 6.83 (d, 1H, 5-C*H,* pyridazinone, J = 9.96 Hz), and 7.06 ppm (d, 1H, 4-C*H,* pyridazinone, J = 9.96 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 41.91 (**C**H$_3$), 44.68 (**C**H$_2$), 45.97 (**C**H$_2$), 46.87 (2× **C**H$_2$), 52.72 (**C**H$_2$), 54.42 (**C**H$_2$), 54.70 (**C**H$_2$CO), 66.38 (2× **C**H$_2$), 125.43 (Ar**C**H), 131.09 (Ar**C**H), 149.05 (Ar**C**), 158.61 (**C**═O), and 164.75 ppm (**C**═O); FTIR (KBr, cm^{-1}) υ_{max}: 2851 (C─H, aliphatic), 1659 (C═O, amide), 1583 (C═C, aromatic), 1450 (CH_2, bending), 1254 (C─N), and 1113 (C─O─C); MS (ESI) m/z: 322.2 $[M + H]^+$; *Anal*. calcd. for $C_{15}H_{23}N_5O_3$: C, 56.06; H, 7.21; N, 21.79. Found: C, 56.32; H, 6.97; N, 21.66.

2-(2-(4-Phenylpiperazin-1-yl)-2-oxoethyl)-6-(morpholin-4-yl) pyridazin-3(2H)-one (10a). Yield: 0.31 g (41.33%); mp. 204–206°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.21 (s, 2H, ─NC*H*$_2$, piperazine), 3.24 (t, 6H, ─N(C*H*$_2$)$_2$, morpholine and N─C*H*$_2$, piperazine, J = 4.84 Hz), 3.68 (s, 2H, ─NC*H*$_2$, piperazine), 3.78 (t, 4H, ─O(C*H*$_2$)$_2$, morpholine, J = 5.60 Hz), 3.81 (s(br), 2H, ─NC*H*$_2$, piperazine) 4.89 (s, 2H, ─NC*H*$_2$), 6.94 (m, 4H, Ar*H*, phenylpiperazine and 5-C*H*, pyridazinone), 7.14 (d, 1H, 4-C*H,* J = 9.96 Hz, pyridazinone), and 7.30 ppm (t, 2H, Ar*H,* phenylpiperazine, J_o = 7.92 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 42.03 (**C**H$_2$), 44.89 (**C**H$_2$), 46.89 (2× **C**H$_2$), 49.32 (**C**H$_2$), 49.56 (**C**H$_2$), 52.71 (**C**H$_2$CO), 66.38 (2× **C**H$_2$), 116.75 (Ar**C**H), 116.86 (Ar**C**H), 120.68 (Ar**C**H), 125.43 (Ar**C**H), 129.29 (2× Ar**C**H), 131.13 (Ar**C**H), 149.06 (Ar**C**), 150.87 (Ar**C**), 158.57 (**C**═O), and 164.89 ppm (**C**═O); FTIR (KBr, cm^{-1}) υ_{max}: 3050 (C─H, aromatic), 2970 (C─H, aliphatic), 2849 (C─H, aliphatic), 1660 (C═O, amide), 1584 (C═C, aromatic), 1456 (CH_2 bending), 1237 (C─N), and 1113 (C─O─C); MS (ESI) m/z: 384.3 $[M + H]^+$; *Anal*. calcd. for $C_{20}H_{25}N_5O_3$: C, 62.67; H, 6.54; N, 18.27. Found: C, 62.90; H, 6.72; N, 18.01.

2-(2-(4-(4-Nitrophenyl)piperazin-1-yl)-2-oxoethyl)-6-(morpholin-4-yl)pyridazin-3(2H)-one (11a). Yield: 0.42 g (50.00%); mp. 206–208°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.23 (t, 4H, ─N(C*H*$_2$)$_2$, morpholine, J = 4.82 Hz), 3.48 (t(br), 2H, ─N(C*H*$_2$), piperazine, J = 4.72 Hz), 3.53 (s (br), 2H, ─NC*H*$_2$, piperazine), 3.72 (t(br), 2H, ─NC*H*$_2$, piperazine, J = 4.68 Hz), 3.77 (t, 4H, ─O(C*H*$_2$)$_2$, morpholine, J = 4.80 Hz), 3.80 (t(br), 2H, ─NC*H*$_2$, piperazine J = 4.68 Hz), 4.87 (s, 2H, ─NC*H*$_2$), 6.82 (d, 2H, Ar*H*, phenylpiperazine, J_o = 9.36 Hz), 6.89 (d, 1H, 5-C*H*, pyridazinone, J = 9.96 Hz), 7.14 (d, 1H, 4-C*H*, pyridazinone, J = 9.96 Hz), and 8.13 ppm (d, 2H, Ar*H*, phenylpiperazine, J_o = 9.32 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 41.35 (**C**H$_2$), 44.22 (**C**H$_2$), 46.79 (2× **C**H$_2$), 46.87 (2× **C**H$_2$), 52.71 (**C**H$_2$CO), 66.36 (2× **C**H$_2$), 113.02 (2× Ar**C**H), 125.58 (Ar**C**H), 125.99 (2× Ar**C**H), 131.07 (Ar**C**H), 139.17 (Ar**C**), 149.17 (Ar**C**), 154.31 (Ar**C**), 158.56 (**C**═O), and 165.23 ppm (**C**═O); FTIR (KBr, cm^{-1}) υ_{max}: 3059 (C─H, aromatic), 2943 (C─H, aliphatic), 1666 (C═O, amide), 1588 (C═C, aromatic), 1448, 1325 (NO_2 stretch), 1234 (C─N), and 1110 (C─O─C); MS (ESI) m/z: 429.3 $[M + H]^+$; *Anal*. calcd. for $C_{20}H_{24}N_6O_5$: C, 56.06; H, 5.64; N, 19.61. Found: C, 55.84; H, 5.35; N, 19.42.

2-(2-(4-(4-Fluorophenyl)piperazin-1-yl)-2-oxoethyl)-6-(morpholin-4-yl)pyridazin-3(2H)-one (12a). Yield: 0.12 g (48.00%); mp. 172–174°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.18 (t, 4H, ─N(C*H*$_2$)$_2$, morpholine, J = 4.78 Hz), 3.25 (s(br), 8H, 2× ─N(C*H*$_2$)$_2$, piperazine), 3.74 (t, 4H, ─O(C*H*$_2$)$_2$, morpholine, J = 4.76 Hz), 4.59 (s, 2H, ─NC*H*$_2$─), 6.81 (d, 1H, 5-C*H,* pyridazinone, J = 9.88 Hz), 6.85–6.89 (m, 2H, Ar*H*), 6.98 (t, 2H, Ar*H,* J_o = 8.70 Hz,), and 7.04 ppm (d, 1H, 4-C*H,* pyridazinone, J = 9.88 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 43.37 (2× **C**H$_2$), 46.89 (2× **C**H$_2$), 47.94 (2× **C**H$_2$), 56.50 (**C**H$_2$CO), 66.37 (2× **C**H$_2$), 115.64 (Ar**C**H), 115.86 (Ar**C**H), 118.81 (Ar**C**H), 118.89 (Ar**C**H), 124.99 (Ar**C**H), 131.04 (Ar**C**H), 147.17 (Ar**C**), 147.19 (Ar**C**), 148.83 (Ar**C**), 156.59

(Ar**C**), 158.55 (Ar**C**), 158.98 (**C**═O), and 173.44 ppm (**C**═O); FTIR (KBr, cm^{-1}) υ_{max}: 3067 (C─H, aromatic), 2958 (C─H, aliphatic), 2849 (C─H, aliphatic), 1658 (C═O, amide), 1586 (C═C, aromatic), 1515, 1381 (C─F), 1259 (C─N), and 1116 (C─O─C); MS (ESI) m/z: 402.2 $[M + H]^+$; *Anal*. calcd. for $C_{20}H_{24}FN_5O_3$: C, 59.83; H, 6.02; N, 17.44. Found: C, 59.61; H, 6.25; N, 17.62.

2-(2-(Pyrrolidin-1-yl)-2-oxoethyl)-6-(piperidin-1-yl)-pyridazin-3(2H)-one (4b). Yield: 0.25 g (25.72%); mp. 162–164°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.60–1.67 (m, 6H, ─(CH_2)$_3$─, piperidine), 1.88 (p, 2H, ─CH_2─, pyrrolidine, J = 6.56 Hz), 2.00 (p, 2H, ─CH_2─, pyrrolidine, J = 6.72 Hz), 3.23 (t, 4H, ─N(CH_2)$_2$, piperidine, J = 6.98 Hz), 3.49–3.55 (m, 4H, ─N(CH_2)$_2$─, pyrrolidine), 4.76 (s, 2H, ─NCH_2), 6.86 (d, 1H, 5-C*H*, pyridazinone, J = 9.96 Hz), and 7.16 ppm (d, 1H, 4-C*H*, pyridazinone, J = 10.00 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 24.11 (**C**H$_2$), 24.20 (**C**H$_2$), 25.32 (2× **C**H$_2$), 26.20 (**C**H$_2$), 45.70 (**C**H$_2$), 46.03 (**C**H$_2$), 47.81 (2× **C**H$_2$), 53.72 (**C**H$_2$CO), 126.28 (Ar**C**H), 130.64 (Ar**C**H), 149.63 (Ar**C**H), 158.02 (**C**═O), and 164.89 ppm (**C**═O); FTIR (KBr, cm^{-1}) υ_{max}: 3058 (C─H, aromatic), 2938 (C─H, aliphatic), 1658 (C═O, amide), 1587 (C═C, aromatic), 1443 (CH_2, bending), 1337, 1257 (C─N), 1178, and 1117 (C─O─C); MS (ESI) m/z: 291.3 $[M + H]^+$; *Anal*. calcd. for $C_{15}H_{22}N_4O_2$: C, 62.05; H, 7.64; N, 19.30. Found: C, 62.41; H, 7.45; N, 19.09.

2-(2-(Morpholin-4-yl)-2-oxoethyl)-6-(piperidin-1-yl)pyridazin-3(2H)-one (5b). Yield: 0.26 g (25.49%); mp. 166–168°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.58–1.65 (m, 6H, ─(CH_2)$_3$─, piperidine), 3.22 (t, 4H, ─N(CH_2)$_2$, piperidine, J = 4.74 Hz), 3.48 (t, 2H, ─NCH_2, morpholine, J = 4.76 Hz), 3.64 (t, 2H, ─NCH_2, morpholine, J = 5.16 Hz), 3.70 (s(br), 4H, ─(OCH_2)$_2$, morpholine), 4.82 (s, 2H, ─NCH_2), 6.85 (d, 1H, 5-C*H*, pyridazinone, J = 9.96 Hz), and 7.16 ppm (d, 1H, 4-C*H*, pyridazinone, J = 9.96 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 24.17 (**C**H$_2$), 25.31 (**C**H$_2$), 42.30 (**C**H$_2$), 45.35 (**C**H$_2$), 47.72 (2× **C**H$_2$), 52.58 (**C**H$_2$CO), 66.39 (─O**C**H$_2$), 66.80 (─O**C**H$_2$), 126.80 (Ar**C**H), 130.59 (Ar**C**H), 149.73 (Ar**C**), 158.42 (**C**═O), and 165.27 ppm (**C**═O); FTIR (KBr, cm^{-1}) υ_{max}: 2991 (C─H, aromatic), 2939 (C─H, aliphatic), 1658 (C═O, amide), 1586 (C═C, aromatic), 1437 (CH_2 bending), 1238 (C─N, amide), and 1115 (C─O─C); MS (ESI) m/z: 307.3 $[M + H]^+$; *Anal.* calcd. for $C_{15}H_{22}N_4O_3$: C, 58.81; H, 7.24; N, 18.29. Found: C, 58.51; H, 7.45; N, 18.09.

2-(2-(3,4-Dimethoxyphenethylamino)-2-oxoethyl)-6-(piperidin-1-yl) pyridazin-3(2H)-one (6b). Yield: 0.24 g (17.90%); mp. 118–120°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.60 (s(br), 6H, ─(CH_2)$_3$─, piperidine), 2.74 (t, 2H, ─NHCH_2CH_2, J = 7.06 Hz), 3.22 (s(br), 4H, ─N(CH_2)$_2$─, piperidine, J = 6.98 Hz), 3.47 (q, 2H, ─NHCH_2CH_2, J = 6.99 Hz), 3.48 (t, 4H, ─N(CH_2)$_2$, J = 4.72 Hz), 3.84 (s, 3H, ─OCH_3), 3.85 (s, 3H, ─OCH_3), 4.64 (s, 2H, ─NCH_2), 6.54 (t, 1H, ─N*H*), 6.65–6.68 (m, 2H, Ar-H), 6.75 (d, 1H, Ar-H, J_o = 8.64 Hz), 6.80 (d, 1H, 5-C*H*, pyridazinone, J = 9.96 Hz), and 7.14 ppm (d, 1H, 4-C*H*, pyridazinone, J = 9.96 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 24.11 (**C**H$_2$), 25.22 (2× **C**H$_2$), 35.06 (**C**H$_2$), 40.68 (**C**H$_2$), 47.56 (**C**H$_2$), 55.86 (**C**H$_2$CO), 55.89 (2× O**C**H$_3$), 56.15 (O**C**H$_3$), 111.25 (Ar**C**H), 111.90 (Ar**C**H), 120.58 (Ar**C**H), 126.13 (Ar**C**), 130.66 (Ar**C**) 131.30 (Ar**C**), 147.54 (Ar**C**), 148.92 (Ar**C**), 149.88 (Ar**C**), 158.46 (**C**═O), and 167.67 ppm (**C**═O); FTIR (KBr, cm^{-1}) υ_{max}: 3321 (N─H), 3070 (C─H, aromatic), 2927 (C─H, aliphatic), 1666 (C═O, amide), 1593 (C═C, aromatic), 1516, 1455 (CH_2 bending), 1265, and 1155 (C─O─C); MS (ESI) m/z: 401.1 $[M + H]^+$; *Anal.* calcd. for $C_{21}H_{28}N_4O_4$: C, 62.98; H, 7.05; N, 13.99. Found: C, 62.51; H, 7.45; N, 13.69.

Pharmacological evaluation. ***Animals.*** The experiments were carried out on male albino mice (20–25 gm) and male Wistar rats (180–200 gm). 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. Animal were housed in polypropylene cages and provided a constant temperature 25 ± 2°C and a relative humidity of 35–60%. All the animals were used only once, and they were killed after the experiment by cervical dislocation.

Anti-inflammatory activity. All the synthesized derivatives were evaluated for anti-inflammatory activity using carrageenan-induced hind paw edema model [27] in male Wistar rats (120–130 g). 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:

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

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 was recorded for each group at the end of observation period.

Gastric ulcerogenic activity. 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 [28].

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). Sixty minutes 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 [29].

The percentage protection against writhing was calculated according to following equation:

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

Antiplatelet activity. Antiplatelet activity was determined by using the tail transaction bleeding test as reported [30]. Bleeding time of male mice (Laca strain) weighing 20–24 g was determined by administering compounds and aspirin (standard drug) suspended in carboxymethyl 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.

Acknowledgment. The authors are thankful to the University Grants Commission, India, for the financial support [F.5-94/2007(BSR)].

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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