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ORIGINAL RESEARCH

Design, synthesis, and evaluation of anti-inflammatory and ulcerogenicity of novel pyridazinone derivatives

K. A. M. Abouzid · N. A. Khalil · E. M. Ahmed · A. Esmat · A. M. Al-Abd

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Abstract A series of pyridazinone-containing compounds were designed and synthesized as congeners for diclofenac, the most potent and widely used NSAID. The target compounds were evaluated for their anti-inflammatory activity on rat paw edema inflammation model against diclofenac as a reference compound. Seven of the tested compounds demonstrated more than 50% inhibition of carrageenan-induced rat paw edema at a dose 10 mg/kg. The compounds, 6-(2-bromophenylamino)pyridazin-3(2H)-one **2a** and 6-(2,6-dimethylphenylamino)pyridazin-3(2H)-one **2e**, displayed 74 and 73.5% inflammation-inhibitory activity, respectively, which is comparable to diclofenac (78.3%) at the same dose level after 4 h. The most active compounds as anti-inflammatory agents, **2a**, **2e**, and **6a**, displayed fewer number of ulcers and milder ulcer score than indomethacin in ulcerogenicity screening.

Keywords Pyridazinone · Anti-inflammatory · Ulcerogenicity · Diclofenac

K. A. M. Abouzid (✉)
Pharmaceutical Chemistry Department, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt
e-mail: abouzid@yahoo.com

N. A. Khalil · E. M. Ahmed
Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt

A. Esmat
Pharmacology Department, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

A. M. Al-Abd
Department of Pharmacology, Medical Division, National Research Center, Dokki, Cairo, Egypt

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medications for the treatment of pain, fever, and inflammation, however, their clinical usefulness is still restricted due to their gastrointestinal side effects as gastric irritation, ulceration, bleeding, and in some cases may lead to life-threatening conditions, (Lanas *et al.*, 2006) (Fig. 1a). Based on the side effects observed by NSAIDs, it has been suggested that selective COX-2 inhibitors (coxibs) may act as safer NSAIDs devoid of ulcerogenic side effects; however, long-term use of these drugs has shown kidney and hepatotoxicity as well as an increased risk of cardiovascular events (Dogne *et al.*, 2000). These observations raised serious concerns about safety of selective COX-2 inhibitors, since some of these agents have been withdrawn from the market (Graham *et al.*, 2005; Hsiao *et al.*, 2009). Therefore, development of novel classical anti-inflammatory agents with an improved safety profile is still a necessity. In this direction, the efforts of our research team were continued to develop safer and effective anti-inflammatory drug candidates that could be introduced as a substitute for coxibs. Our strategy was directed towards structural modification of the well-known anti-inflammatory/antipyretic/analgesic drug, diclofenac, which is the most potent and widely prescribed medication for the treatment of different inflammatory conditions such as osteoarthritis, rheumatoid arthritis, and ankylosing spondylitis (Allison *et al.*, 1992).

In previous research work in the anti-inflammatory area, pyridazinone-based compounds were discovered as promising candidates in this field, (Gleave *et al.*, 2010; Chintakunta *et al.*, 2002; Süküroglu *et al.*, 2005; Abouzid and Bekhit, 2008; Gökce *et al.*, 2009) (Fig. 1b).

In this study, and based on the above-mentioned findings, we attempt to design pyridazinone-containing

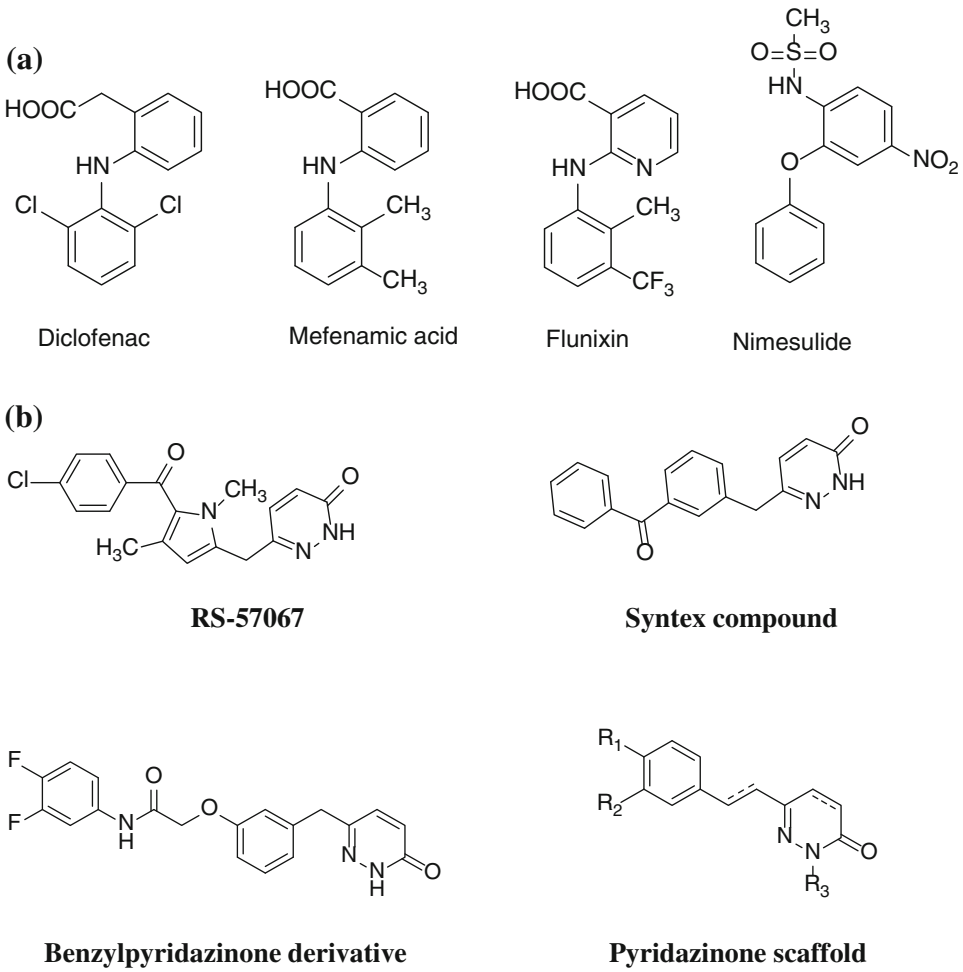


Fig. 1 **a** Chemical structures of representative NSAIDs. **b** Pyridazinone-containing compounds with anti-inflammatory activities

compounds as potential candidates for the treatment of inflammatory conditions (Fig. 2). To achieve this goal, we introduced certain modification to the skeleton of the lead compound, diclofenac, via the replacement of aryl acetic acid moiety with 3(2H)-pyridazinone or pyridazinone-3-acetic acid moieties. Another approach was to replace the NH between the two aryl groups of diclofenac with isosteric hetero atom like oxygen and addition of a carboxylic or carboxamide moieties on position-2 of the aryl group. Also, replacing the SP^3 carbon between pyridazinone and the aryl or heteroaryl ring in the pyridazinone anti-inflammatory drug candidate with oxygen and nitrogen hetroatoms.

Results and discussion

Chemistry

The synthetic pathways leading to the 6-substituted-pyridazine and 3(2H)-pyridazinone derivatives are outlined in Schemes 1 and 2.

In Scheme 1, the physical and spectral properties of 6-chloro-3-(4-chlorophenylamino)pyridazine **1b** and 6-(4-chlorophenylamino)pyridazine-3(2H)-one **2b** were in accordance with the literature (Boissier *et al*., 1963). Hydrolysis of **1a**–**h** was carried out upon heating in glacial acetic to afford **2a**–**h**. The formation of these compounds was confirmed by IR spectra which showed the appearance of C=O band in the range of 1,670–1,685/cm. ^{1}H-NMR spectra revealed two D_2O exchangeable signals corresponding to the two NH protons. Alkylation of pyridazinones **2a**–**h** was performed using ethyl bromoacetate in the presence of anhydrous K_2CO_3 in acetone at reflux temperature to provide the target acetic acid esters **3a**–**g**. The esters in their IR spectra displayed an additional C=O band of ester in the range of 1,728–1,743/cm. In ^{1}H-NMR spectra, additional signals derived from ester group were observed at 1.21 ppm (OCH_2CH_3) and 4.18 ppm (OCH_2CH_3) integrating for three protons and two protons, respectively. In Scheme 2, compounds **5a**, **b** were prepared via reaction of 3,6-dichloropyridazine with either salicylic acid or salicylamide in the presence of anhydrous

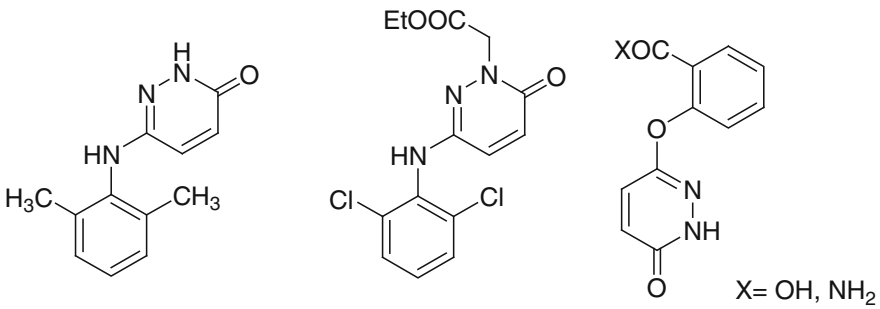
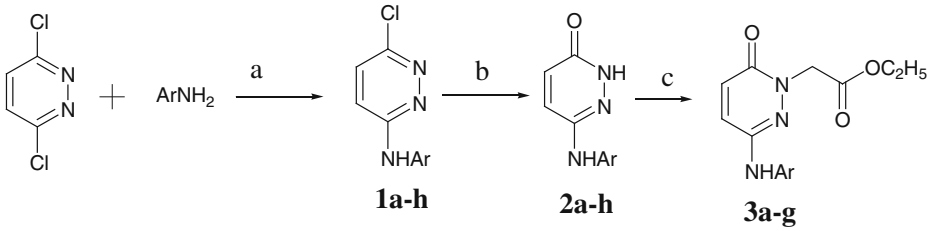
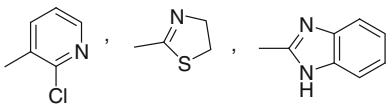


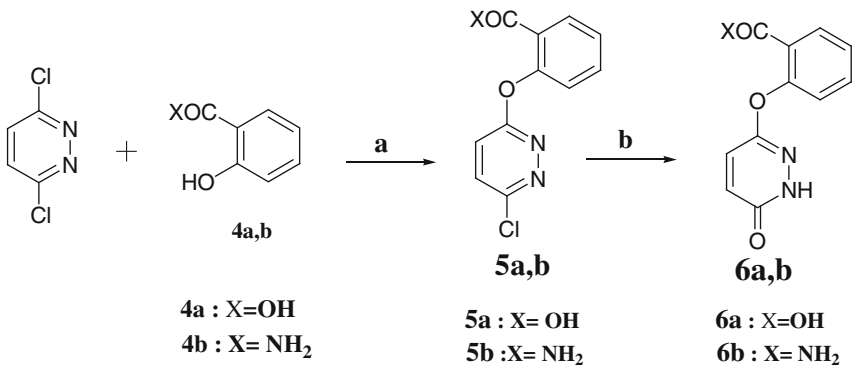
Fig. 2 Structures of representative designed target compounds

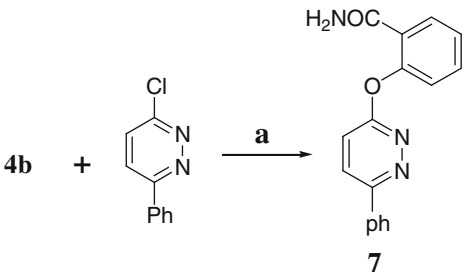


Scheme 1 Reagents and conditions: **a** isopropanol/K_2CO_3/reflux 4 h, **b** acetic acid/reflux 5 h, **c** $BrCH_2COOC_2H_5$/K_2CO_3, reflux 24 h

Ar = 2-BrC_6H_4, 4-ClC_6H_4 , 2,4-$Cl_2C_6H_3$, 2,6-$Cl_2C_6H_3$, 2,6-$(CH_3)_2C_6H_3$,







Scheme 2 Reagents and conditions: **a** isopropanol/K_2CO_3/reflux 4 h, **b** CH_3COOH/reflux 5 h

K_2CO_3. Hydrolysis of **5a**, **b** with acetic acid gave **6a**, **b**. Finally, compound **7** was synthesized by reacting 3-chloro-6-phenylpyridazine with salicylamide. The IR spectra of **5a**, **b**, **6a**, **b**, and **7** were very informative. The carboxylic acid derivatives, **5a** and **6a**, displayed the O–H stretching band of acid in the range of 2,515–3,200/cm. The amide derivatives **5b**, **6b**, and **7** showed the additional C=O of amide at 1,645–1,678/cm. Further spectroscopic details of these compounds are presented in "Experimental" section.

In vivo anti-inflammatory activity

Some of the synthesized compounds (**2a**, **2c–h**, **3a–g**, **6a**, **b**, and **7**) were screened for their anti-inflammatory activities against carrageenan-induced rat paw edema at a dose 10 mg/kg. The anti-inflammatory properties were compared to that of diclofenac (in a dose 10 mg/kg) as a reference standard. The test compounds **2a**, **2c–h** have elicited significant anti-inflammatory activity of variant degree (20.7–74%). Esterification of the latter compounds to their corresponding esters **3a–g** showed low to moderate anti-inflammatory activities (17.2–61.2%). In general, they exhibited lower degree of inhibition of edema compared to their parent compounds except for compound **3c** and **3d** (Table 1).

The presence of bromine atom at position 2 (**2a**) or 2,6-dimethyl group (**2e**) in the aromatic ring gave rise to an increased anti-inflammatory activity (74 and 73.5%), respectively. It is also obvious that adopting a 2-chloro-pyridyl function at the 6-aminopyridazinone **2f** seems preferable for obtaining an effective anti-inflammatory agent.

On the other hand, the pyridazinone derivative **6a** carrying acid function in the 2-position of the aromatic ring exhibited higher anti-inflammatory activity (65.6%) compared to that of the corresponding amide, **6b** (29.3%). The low anti-inflammatory activity observed by the amide **6b** was substantiated by the comparable anti-inflammatory effect of the amide derivative **7** (31.3%).

Ulcerogenicity studies

Three of the test compounds that exhibited the most potent anti-inflammatory activity were tested for gastric ulcerative effect on rat stomach.

The ulcerative effect of test compounds (**2a**, **2e**, and **6a**) has been inspected visually and histopathologically relative to the known ulcerogenic drug, indomethacin. After gross visual inspection, all test compounds showed fewer number of ulcers than indomethacin and milder ulcer score as well. Compound **2a** showed the strongest gross visual ulcerogenic score (4.2 ± 1.34); yet, milder than indomethacin (7.2 ± 0.65). All test compounds showed less than two gross ulcers per stomach, while indomethacin showed average of 2.6 ± 0.27 ulcers per stomach (Table 2).

However, microscopically examined sections of all test compounds and indomethacin showed considerable signs of gastric ulcerogenic activity such as thinning of gastric mucosa layer and microscopic cone shape mucosal ulcers. Thinning of the gastric mucosa is considered early gastric

Table 1 Anti-inflammatory activity of the test compounds assessed in comparison to diclofenac as reference

Group	1 h		2 h		4 h		4 h
	Paw vol. (mL)	% Edema inhibition	Paw vol. (mL)	% Edema inhibition	Paw vol. (mL)	% Edema inhibition	Potency
Control	$1.530^{a} \pm 0.032$	0.00	$1.605^{a} \pm 0.015$	0.00	$1.817^{a} \pm 0.088$	0.00	–
Diclofenac	$1.340^{a,b} \pm 0.034$	65.3	$1.302^{a,b} \pm 0.013$	73.5	$1.279^{a,b} \pm 0.019$	78.3	100
2a	$1.473^{a,b} \pm 0.005$	19.6	$1.461^{a,b} \pm 0.024$	34.9	$1.308^{a,b} \pm 0.02$	74	94.4
2c	$1.484^{a,b} \pm \pm 0.024$	15.8	$1.558^{a} \pm 0.018$	11.4	$1.6^{a,b} \pm 0.021$	31.6	40.35
2d	$1.504^{a} \pm 0.023$	8.9	$1.584^{a,b} \pm 0.009$	5	$1.675^{a,b} \pm 0.065$	20.7	25.54
2e	$1.405^{a,b} \pm 0.009$	42.9	$1.372^{a,b} \pm 0.006$	56.6	$1.312^{a,b} \pm 0.038$	73.5	93.86
2f	$1.473^{a,b} \pm 0.005$	19.6	$1.480^{a,b} \pm 0.007$	30.3	$1.388^{a,b} \pm 0.022$	62.4	79.69
2g	$1.488^{a} \pm 0.012$	14.4	$1.561^{a} \pm 0.017$	10.7	$1.603^{a,b} \pm 0.026$	31.1	39.71
2h	$1.465^{a,b} \pm 0.021$	22.3	$1.523^{a,b} \pm 0.022$	19.9	$1.479^{a,b} \pm 0.021$	49.1	62.7
3a	$1.476^{a,b} \pm 0.013$	18.6	$1.495^{a,b} \pm 0.004$	26.9	$1.454^{a,b} \pm 0.065$	52.8	67.43
3b	$1.483^{a,b} \pm 0.018$	16.2	$1.514^{a,b} \pm 0.013$	22.1	$1.460^{a,b} \pm 0.025$	51.9	66.16
3c	$1.474^{a,b} \pm 0.007$	19.2	$1.481^{a,b} \pm 0.007$	30.1	$1.396^{a,b} \pm 0.027$	61.2	78.16
3d	$1.457^{a,b} \pm 0.024$	25.1	$1.533^{a,b} \pm 0.02$	17.5	$1.476^{a,b} \pm 0.073$	49.6	63.34
3e	$1.502^{a} \pm 0.018$	9.6	$1.552^{a,b} \pm 0.019$	12.8	$1.596^{a,b} \pm 0.025$	32.1	40.99
3f	$1.504^{a} \pm 0.015$	8.9	$1.591^{a} \pm 0.01$	3.4	$1.699^{a,b} \pm 0.016$	17.2	21.96
3g	$1.498^{a} \pm 0.018$	11	$1.561^{a} \pm 0.026$	10.7	$1.617^{a,b} \pm 0.027$	29.1	37.16
6a	$1.471^{a,b} \pm 0.005$	20.3	$1.483^{a,b} \pm 0.006$	29.6	$1.387^{a,b} \pm 0.019$	65.6	83.78
6b	$1.495^{a} \pm 0.02$	12	$1.564^{a} \pm 0.019$	9.9	$1.616^{a,b} \pm 0.029$	29.3	37.42
7	$1.5^{a} \pm 0.016$	10.3	$1.561^{a} \pm 0.028$	10.8	$1.602^{a,b} \pm 0.027$	31.3	39.97

[a] Statistically significant difference from the control group at $p < 0.05$

[b] Statistically significant difference from the carrageenan-induced group at $p < 0.05$

Table 2 Gross visual gastric ulcerative assessment

Compound number	Ulcer number	Ulcer score
Control	0.0±0.0	0.0±0.0
Indomethacin	2.6±0.27	7.2±0.65
2a	1.8±0.55	4.2±1.34
2e	1.4±0.89	1.8±0.42
6a	1.6±1.04	2.0±1.27

ulceration sign; herein, gastric mucosal thickness has been calculated microscopically after treatment with test compounds and compared to normal gastric mucosal tissues. All test compounds showed significant gastric mucosal thinning down to 20% of the original mucosal thickness. Again, compound **2a** showed the most profound gastric mucosal thinning among all test compounds. Besides mucosal thickness, the microscopic depth of ulcer has been evaluated after treatment with test compounds and compared to control untreated group. All test compounds showed narrower and shallower gastric ulcers than indomethacin. Surprisingly, indomethacin-induced gastric mucosal thinning was less than all test compounds. However, indomethacin-induced ulcers were much deeper than all other test compounds (Fig. 3).

Histopathological assessment for gastric ulceration was carried out in fundus glandular region of gastric tissues by routine H&E staining for paraffin embedded sections. Normal group (A) showed normal gastric mucosal appearance. Group treated with the test compounds **2a** (B), **2e** (C), **6a** (D), and indomethacin (E) showed thinning in the mucosal layer, cone-shape ulcers (star), and mucosal dislodgments (arrows). Gastric mucosal thickness and depth of ulcers were calculated using image-J™ software and compared to control untreated group (F). Scale bar equals 100 μm. Data are presented as mean ± SEM.

Conclusion

The anti-inflammatory activity evaluation was carried out using carrageenan-induced paw edema assay. The screening data revealed that all investigated compounds exhibit considerable anti-inflammatory properties with the percentage inhibition of edema ranging from 17.2 to 74, while the reference diclofenac showed 78.3 inhibition. The test compounds, 6-(substitutedamino)pyridazin-3(2H)-ones, except **2c** and **2d** were found to be more potent than their corresponding esters **3a**–**g**. Compounds **2a** and **2e** showed remarkable activities with potency of 94.5 and 93.86%, respectively. The esters **3a**–**g** exhibited anti-inflammatory activity of the potency range from 21.96 to 78.16%. The carboxylic acid derivative **6a** displayed higher potency than the corresponding amide **6b**.

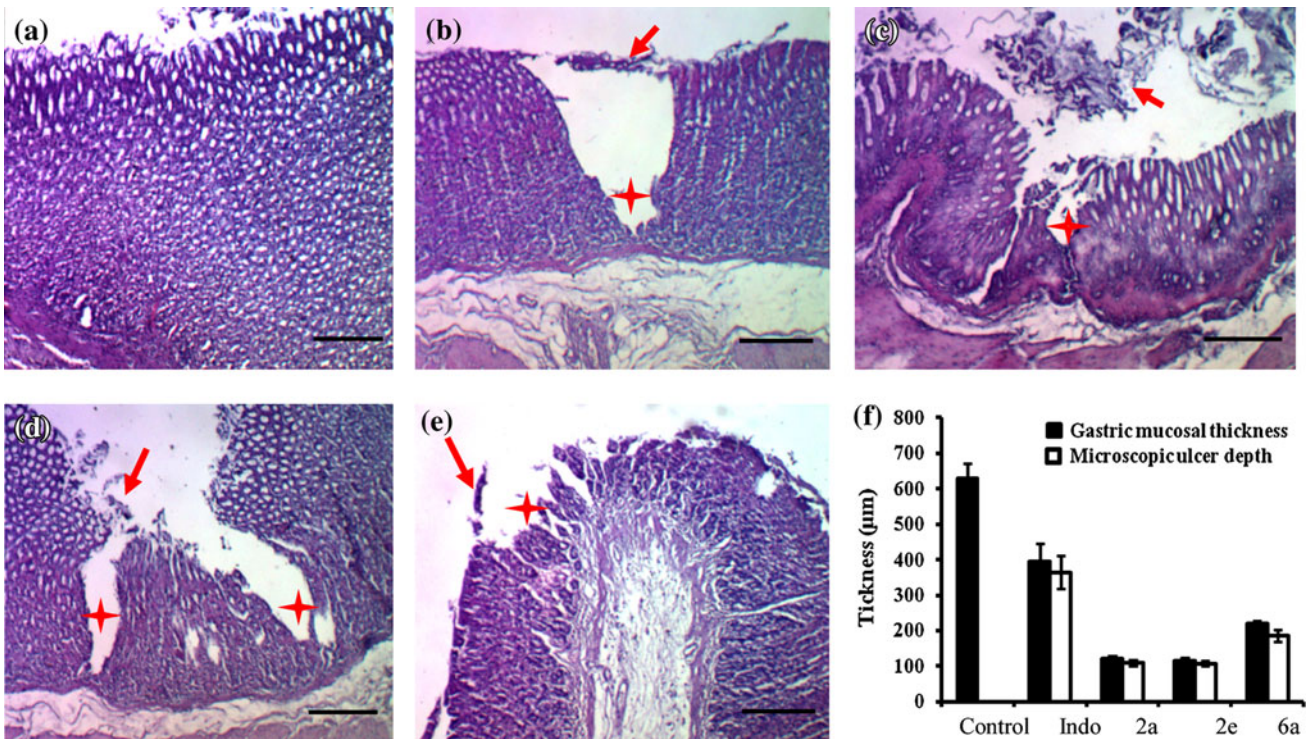


Fig. 3 Histological assessment of gastric ulceration

The ulcerogenicity studies revealed that compounds **2a**, **2e**, and **6a** showed fewer number of ulcers and milder ulcer score than indomethacin.

Since our findings are preliminary results; further studies need to be carried out to investigate the other specifications such as in vitro assays, toxicological studies, or side effect-activity profiles of these compounds.

Experimental

Melting points were determined on Griffin apparatus and the values given are uncorrected. IR spectra were determined on Shimadzu IR 435 spectrophotometer (KBr, /cm). ^{1}H-NMR spectra were carried out using a Varian Gemini 200 MHz Spectrophotometer and Varian Mercury-300 (300 MHz) Spectrophotometer using TMS as internal standard. Chemical shift values are recorded in ppm on δ scale, Microanalytical Center, Cairo University, Egypt. Mass spectra were recorded on a GCMP-QP1000 EX Mass spectrometer, Microanalytical Center, Cairo University, Egypt. Elemental analyses were carried out at the Microanalytical Center, Cairo University, Egypt. Progress of the reactions was monitored using TLC sheets precoated with UV fluorescent silica gel Merck 60F 254 using acetone/benzene (1:9) and were visualized using UV lamp.

All chemicals were obtained from Aldrich, Fluka, or Merck chemicals.

6-Chloro-3-(4-chlorophenylamino)pyridazine **1b** and 6-(4-chlorophenylamino)pyridazine-3(2H)-one **2b** were prepared according to reported procedure (Boissier *et al*., 1963).

6-Chloro-3-substituted-aminopyridazines (**1a–h**)

General procedure

A mixture of 3,6-dichloropyridazine (1.48 g, 0.01 mol), anhydrous K_2CO_3 (0.02 mol) and the appropriate substituted amine (0.01 mol) in isopropanol (30 mL) was heated under reflux for 4 h. The reaction mixture was concentrated under reduced pressure to half its volume, poured onto water (50 mL) and extracted by methylene chloride (3 × 10 mL). The combined extract was dried (Na_2SO_4) and distilled off under diminished pressure to give **1a–h**.

3-(2-Bromophenylamino)-6-chloropyridazine ***1a*** yield 60%; oil; IR (KBr)/cm: 3315 (N–H), 3135, 3085 (C–H aromatic), 1615 (C=N); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 5.10 (br s, 1H, NH, D_2O exchangeable); 6.43 (d, 1H, Ar–H); 6.77- 6.81 (m, 2H, Ar–H); 7.06 (d, 1H, Ar–H); 7.31 (d, 1H, pyridazine H-4); 7.99 (d, 1H, pyridazine H-5). Anal calcd for $C_{10}H_7BrClN_3$ (284.54): C, 42.21; H, 2.48; N, 14.77; Found: C, 42.51; H, 2.60; N, 14.85.

6-Chloro-3-(2,4-dichlorophenylamino)pyridazine ***1c*** yield 75%; oil; IR(KBr)/cm: 3310 (N–H), 3155, 3033 (C–H aromatic), 1617 (C=N);^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 5.44 (br s, 1H, NH, D_2O exchangeable); 6.76 (d, 1H, pyridazine H-4); 7.01 (d, 1H, Ar–H); 7.05 (d, 1H, Ar–H); 7.23 (d, 1H, pyridazine H-5); 8.00 (s, 1H, Ar–H). Anal calcd for $C_{10}H_6Cl_3N_3$ (274.53): C, 43.75; H, 2.20; N, 15.31; Found: C, 43.90; H, 2.45; N, 15.50.

6-Chloro-3-(2,6-dichlorophenylamino)pyridazine ***1d*** yield 70%; oil; IR (KBr)/cm: 3325 (N–H), 3145, 3068 (C–H aromatic), 1616 (C=N); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 5.34 (s, 1H, NH, D_2O exchangeable); 6.53 (d, 1H, pyridazine H-4); 6.56 (d, 1H, Ar–H); 7.16 (d, 2H, Ar–H); 7.92 (d, 1H, pyridazine H-5). Anal calcd for $C_{10}H_6Cl_3N_3$ (274.53): C, 43.75; H, 2.20; N, 15.31; Found: C, 43.80; H, 2.05; N, 15.65.

6-Chloro-3-(2,6-dimethylphenylamino)pyridazine ***1e*** yield 50%; oil; IR (KBr)/cm: 3312 (N–H), 3150, 3050 (C–H aromatic), 2950, 2844 (C–H aliphatic), 1618 (C=N); (DMSO-d_6, 300 MHz) δ (ppm): 2.04 (s, 6H, $2CH_3$); 4.70 (br s, 1H, NH, D_2O exchangeable); 6.42 (d, 1H, pyridazine H-4); 6.58 (d, 1H, Ar–H); 6.89 (d, 2H, Ar–H); 7.94 (d, 1H, pyridazine H-5). Anal Calcd for $C_{12}H_{12}ClN_3$ (233.70): C, 61.67; H, 5.18; N, 17.98; Found: C, 61.95; H, 5.43; N, 18.25.

6-Chloro-3-(2-chloropyridin-3-ylamino)pyridazine ***1f*** yield 50%; mp 45–46 °C; IR (KBr)/cm: 3309 (N–H), 3178, 3030 (C–H aromatic), 1616 (C=N); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 4.11 (br s, 1H, NH, D_2O exchangeable); 7.00 (d, 1H, pyridazine H-4); 7.03–7.75 (m, 4H, 3H pyridine and 1H pyridazine H-5). Anal Calcd for $C_9H_6Cl_2N_4$, (241.08): C, 44.84; H, 2.51; N, 23.24; Found: C, 44.93; H, 2.65; N, 23.50.

N-(6-Chloropyridazin-3-yl)-4,5-dihydrothiazol-2-amine ***1g*** yield 60%; mp 43–44 °C; IR (KBr)/cm: 3305 (N–H), 3066, 3039 (C–H aromatic), 2943, 2854 (C–H aliphatic), 1623 (C=N); (DMSO-d_6, 300 MHz) δ (ppm): 3.43 (t, 2H, CH_2–thiazoline); 3.84 (t, 2H, CH_2–thiazoline); 3.96 (br s, 1H, NH, D_2O exchangeable); 7.72 (d, 1H, pyridazine H-4); 8.03 (d, 1H, pyridazine H-5). MS (EI) *m/z* (% rel. Int.): 214 ($M\cdot^+$, 0.44); 216 ($M+2^+$, 0.97). Anal Calcd for $C_7H_7ClN_4S$ (214.68): C, 39.16; H, 3.29; N, 26.10; Found: C, 39.40; H, 3.45; N, 26.35.

N-(6-Chloropyridazin-3-yl)-1H-benzo[d]imidazol-2-amine 1h yield 50%; mp 183–184 °C; IR (KBr)/cm: 3317 (N–H), 3143, 3066 (C–H aromatic), 1620 (C=N); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 6.05 (s, 1H, NH, D_2O exchangeable); 6.80–7.09 (m, 5H, 4 Ar–H, and 1H pyridazine H-4); 8.01 (d, 1H, pyridazine H-5); 10.62 (br s, 1H, NH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 245 (M^+, 0.15). Anal Calcd for $C_{11}H_8ClN_5$ (245.67): C, 53.78; H, 3.28; N, 28.51; Found: C, 53.80; H, 3.40; N, 28.73.

6-(Substituted-amino)pyridazin-3(2H)-ones (**2a–h**)

General procedure

Compounds **1a–h** (0.01 mol) were heated under reflux in glacial acetic acid (25 mL) for 5 h. The reaction mixture was concentrated to half its volume then cooled. The precipitated crystalline solid was filtered, washed with water, and recrystallized from ethanol to give **2a–h**.

6-(2-Bromophenylamino)pyridazin-3(2H)-one ***2a*** yield 60%; mp 175–176 °C; IR (KBr)/cm: 3398, 3221(N–H), 3035, 3005 (C–H aromatic), 1685 (C=O), 1589 (C=N); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 7.15 (d, 1H, $J = 9.6$ Hz, pyridazine H-4); 7.29–7.61 (m, 4H, Ar–H); 7.65 (d, 1H, $J = 9.6$ Hz, pyridazine H-5); 8.00 (s, 1H, NH, D_2O exchangeable); 8.91 (s, 1H, NH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 265 ($M^{\cdot+}$, 1.22), 267(M+2, 1.36); Anal Calcd for $C_{10}H_8BrN_3O$ (266.09): C, 45.14; H, 3.03; N, 15.79; Found: C, 45.30; H, 3.33; N, 15.90.

6-(2,4-Dichlorophenylamino)pyridazin-3(2H)-one ***2c*** yield 65%; mp 159–160 °C; IR (KBr)/cm: 3398, 3282 (N–H), 3078, 3032 (C–H aromatic), 1670 (C=O), 1585 (C=N); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.84 (d, 1H, $J = 9.9$ Hz, pyridazine H-4); 7.36–7.65 (m, 3H, Ar–H); 7.96 (d, 1H, $J = 9.9$ Hz, pyridazine H-5); 8.26 (s, 1H, NH, D_2O exchangeable); 9.05 (s, 1H, NH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 256 ($M^{\cdot+}$, 8.81), 258 (M+2, 4.30), 260 (M+4, 1.33). Anal Calcd for $C_{10}H_7Cl_2N_3O$ (256.09): C, 46.90; H, 2.76; N, 16.41; Found: C, 47.10; H, 2.82; N, 16.51.

6-(2,6-Dichlorophenylamino)pyridazin-3(2H)-one ***2d*** yield 62%; mp 64–65 °C; IR (KBr)/cm: 3444, 3352 (N–H), 3059, 3032 (C–H aromatic), 1681 (C=O), 1585 (C=N); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 5.43 (s, 1H, NH, D_2O exchangeable); 6.55–6.60 (m, 1H, Ar–H); 6.98 (d, 1H, $J = 10.5$ Hz, pyridazine H-4); 7.20 (d, 2H, Ar–H); 7.51 (d, 1H, $J = 10.5$ Hz, pyridazine H-5); 13.15 (s, 1H, NH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 256 (M^+, 0.35), 258 (M+2, 0.08). Anal Calcd for $C_{10}H_7Cl_2N_3O$ (256.09): C, 46.90; H, 2.67; N, 16.41; Found: C, 47.23; H, 2.73; N, 16.55.

6-(2,6-Dimethylphenylamino)pyridazin-3(2H)-one ***2e*** yield 55%; mp 159–160 °C; IR (KBr)/cm: 3452, 3390 (N–H), 3062, 3039 (C–H aromatic), 2924, 2800 (C–H aliphatic), 1680 (C=O), 1585 (C=N); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 2.21 (s, 6H, $2CH_3$); 6.77 (d, 1H, $J = 9$ Hz, pyridazine H-4); 7.00–7.12 (m, 3H, Ar–H); 7.45 (d, 1H, $J = 9$ Hz, pyridazine H-5); 8.09 (s, 1H, NH, D_2O exchangeable); 8.68 (s, 1H, NH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 215 ($M^{\cdot+}$, 0.37). Anal Calcd for $C_{12}H_{13}N_3O$ (215.25): C, 66.96; H, 6.09; N, 19.52; Found: C, 66.75; H. 5.98; N, 19.65.

6-(2-Chloropyridin-3-ylamino)pyridazin-3(2H)-one ***2f*** yield 50%; mp 84–85 °C; IR (KBr)/cm: 3421, 3300 (N–H), 3120, 3055 (C–H aromatic), 1674 (C=O), 1581 (C=N); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 5.50 (s, 1H, NH, D_2O exchangeable); 6.95 (d, 1H, $J = 9.9$ Hz, pyridazine H-4); 7.07–7.11 (m, 2H, Ar–H); 7.40 (d, 1H, $J = 9.9$ Hz, pyridazine H-5); 7.56 (d, 1H, Ar–H); 13.14 (s, 1H, NH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 222 ($M^{\cdot+}$, 33.85), 224 (M+2, 23.05). Anal Calcd for $C_9H_7ClN_4O$ (222.63): C, 48.55; H, 3.17; N, 25.17; Found: C, 48.77; H, 3.50; N, 25.45.

6-(4,5-Dihydrothiazol-2-ylamino)pyridazin-3(2H)-one ***2g*** yield 55%; mp 117–118 °C; IR (KBr)/cm: 3432, 3350 (N–H), 3120, 3055 (C–H aromatic), 2985, 2850 (C–H aliphatic), 1670 (C=O), 1581 (C=N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 3.42 (t, 2H, CH_2 thiazoline), 4.05 (t, 2H, CH_2 thiazoline), 6.92 (d, 1H, $J = 9.9$ Hz, pyridazine H-4); 7.48 (d, 1H, $J = 9.9$ Hz, pyridazine H-5); 13.09 (br s, 2H, 2NH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 197 (M+H, 2.76). Anal Calcd for $C_7H_8N_4OS$ (196.23): C, 42.85; H, 4.11; N, 28.55; Found: C, 42.75; H, 4.45; N, 28.65.

6-(1H-Benzo[d]imidazol-2-ylamino)pyridazin-3(2H)-one ***2h*** yield 58%; mp 119–120 °C; IR (KBr)/cm: 3433, 3350 (N–H), 3140, 3055 (C–H aromatic), 1674 (C=O), 1581 (C=N); ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.92 (d, 1H, $J = 9.6$ Hz, pyridazine H-4); 7.16–7.37 (m, 4H, Ar–H); 7.48 (d, 1H, $J = 9.6$ Hz, pyridazine H-5); 13.13 (br s, 3H, 3NH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 228 (M+H, 4.83). Anal Calcd for $C_{11}H_9N_5O$ (227.22): C, 58.14; H, 3.99; N, 30.82; Found: C, 58.45; H, 4.25; N, 30.65.

Ethyl 2-[3-(substitutedamino)-6-oxo-1,6-dihydropyridazin-1-yl] acetates (**3a–g**)

General procedure

A mixture of the appropriate **2a–g** (0.01 mol), anhydrous K_2CO_3 (0.04 mol), and ethyl bromoacetate (0.02 mol) and in dry acetone (40 mL) was heated under reflux for 24 h. Water (10 mL) was added and the mixture was extracted with methylene chloride (3 × 5 mL). The combined organic layers were washed with water (2 × 5 mL), dried (Na_2SO_4), filtered, and concentrated in vacuo to give **3a–g**.

Ethyl 2-[3-(2-bromophenylamino)-6-oxo-1,6-dihydropyridazin-1-yl] acetate ***3a*** yield 60%; mp 109–110 °C; IR (KBr)/cm: 3221 (N–H); 3120, 3035 (C–H aromatic); 2954, 2854 (C–H aliphatic); 1743, 1670 (C=O); 1589 (C=N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.18 (t, 3H, CH_3), 4.13 (q, 2H, CH_2); 4.33 (s, 2H, N–CH_2), 7.07–7.76 (m, 6H, 4H, 3 Ar–H, and 2H pyridazine); 8.90 (s, 1H, NH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 351 ($M^{\cdot+}$, 0.11), 353 (M+2, 0.06). Anal Calcd for $C_{14}H_{14}BrN_3O_3$ (352.18):

C, 47.74; H, 4.01; N, 11.93; Found: C, 47.89; H, 4.30; N, 11.85.

Ethyl 2-[3-(4-chlorophenylamino)-6-oxo-1,6-dihydropyridazin-1-yl] acetate ***3b*** yield 62%; mp 129–130 °C; IR (KBr)/cm: 3278 (N–H), 3089, 3051 (C–H aromatic); 2980, 2835 (C–H aliphatic); 1728, 1666 (C=O), 1581 (C=N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.20 (t, 3H, CH_3); 4.14 (q, 2H, CH_2); 4.73 (s, 2H, N–CH_2); 7.18–7.76 (m, 6H, 4H Ar–H, and 2H pyridazine); 9.59 (s, 1H, NH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 307 ($M^{\cdot+}$, 34.94), 309 (M+2, 16.36). Anal Calcd for $C_{14}H_{14}ClN_3O_3$ (307.73): C, 54.64; H, 4.59; N, 13.65; Found: C, 54.75; H, 4.80; N, 13.85.

Ethyl 2-[3-(2,4-dichlorophenylamino)-6-oxo-1,6-dihydropyridazin-1-yl] acetate ***3c*** yield 65%; mp 105–106 °C; IR (KBr)/cm: 3390 (N–H), 3109, 3062 (C–H aromatic), 2927, 2862 (C–H aliphatic); 1739, 1670 (C=O); 1589 (C=N). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.28 (t, 3H, CH_3); 4.24 (q, 2H, CH_2); 4.80 (s, 2H, N–CH_2); 6.94 (d, 1H, $J = 9.6$ Hz, pyridazine-H4); 7.04–7.45 (m, 2H, Ar–H); 7.53 (d, 1H, $J = 9.6$ Hz, pyridazine-H5); 8.10 (s, 1H, Ar–H); 9.30 (s, 1H, NH, D_2O exchangeable). Anal Calcd for $C_{14}H_{13}Cl_2N_3O_3$ (342.18): C, 49.14; H, 3.83; N, 12.28; Found: C, 49.30; H, 3.75; N, 12.55.

Ethyl 2-[3-(2,6-dichlorophenylamino)-6-oxo-1,6-dihydropyridazin-1-yl] acetate ***3d*** yield 52%; oil; IR (KBr)/cm: 3340 (N–H); 3110, 3054 (C–H aromatic); 2980, 2820 (C–H aliphatic); 1740, 1675 (C=O), 1585 (C=N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.20 (t, 3H, CH_3); 4.16 (q, 2H, CH_2); 4.95 (s, 2H, N–CH_2); 6.58 (d, 1H, Ar–H); 7.10 (d, 1H, $J = 9.6$ Hz, pyridazine-H4); 7.23 (d, 2H, Ar–H); 7.64 (d, 1H, $J = 9.6$ Hz, pyridazine-H5); 9.80 (s, 1H, NH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 342 ($M^{\cdot+}$, 1.95), 344 (M+2, 1.04), 346 (M+4, 1.09); Anal Calcd for $C_{14}H_{13}Cl_2N_3O_3$ (342.18): C, 49.14; H, 3.83; N, 12.28; Found: C, 49.45; H, 3.95; N, 12.45.

Ethyl 2-[3-(2,6-dimethylphenylamino)-6-oxo-1,6-dihydropyridazin-1-yl] acetate ***3e*** yield 50%; mp 115–116 °C; IR (KBr)/cm: 3468 (N–H); 3093, 3055 (C–H aromatic); 2962, 2800 (C–H aliphatic); 1743, 1678 (C=O); 1585 (C=N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 1.20 (t, 3H, CH_3); 2.02 and 2.21 (2s, 6H, $2CH_3$); 4.14 (q, 2H, CH_2); 4.82 (s, 2H, N–CH_2); 7.10–7.64 (m, 5H, 3Ar–H, and 2H pyridazine); 9.20 (s, 1H, NH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 301 ($M^{\cdot+}$, 0.37). Anal Calcd for $C_{16}H_{19}N_3O_3$ (301.34): C, 63.77; H, 6.36; N, 13.94; Found: C, 63.85; H, 6.60; N, 13.82.

Ethyl 2-[3-(2-chloropyridin-3-yl)amino-6-oxo-1,6-dihydropyridazin-1-yl] acetate ***3f*** yield 54%; oil; IR (KBr)/cm: 3442 (N–H); 3098, 3052 (C–H aromatic); 2980, 2844 (C–H aliphatic); 1738, 1670 (C=O), 1584 (C=N). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.24 (t, 3H, CH_3); 4.02 (s, 1H, NH, D_2O exchangeable); 4.21 (q, 2H, CH_2); 4.90 (s, 2H, N–CH_2); 6.89 (d, 1H, $J = 9.6$ Hz, pyridazine-H4); 6.93–7.27 (m, 3H, Ar–H); 7.73 (d, 1H, $J = 9.6$ Hz, pyridazine-H5). Anal Calcd for $C_{13}H_{13}ClN_4O_3$ (308.72): C, 50.58; H, 4.24; N, 18.15; Found: C, 50.80; H,4.62; N, 18.33.

Ethyl 2-[3-(4,5-dihydrothiazol-2-yl)amino-6-oxo-1,6-dihydropyridazin-1-yl] acetate ***3g*** yield 60%; oil; IR (KBr)/cm: 3432 (N–H); 3120, 3055 (C–H aromatic); 2985, 2854 (C–H aliphatic); 1735, 1670 (C=O); 1581 (C=N). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ (ppm): 1.27 (t, 3H, CH_3); 3.81 (t, 2H, CH_2–thiazoline); 3.90 (s, 1H, NH, D_2O exchangeable); 3.93 (t, 2H, CH_2–thiazoline); 4.23 (q, 2H, CH_2); 4.80 (s, 2H, N–CH_2); 6.91 (d, 1H, $J = 9.6$ Hz, pyridazine-H4); 7.23 (d, 1H, $J = 9.6$ Hz, pyridazine-H5). Anal Calcd for $C_{11}H_{14}N_4O_3S$ (282.32): C, 46.80; H, 5.00; N, 19.85; Found: C, 46.95; H, 5.22; N, 19.98.

2-(6-Chloropyridazine-3-yloxy)benzoic acid and benzamide (**5a**, **b**)

A mixture of 3,6-dichloropyridazine (1.48 g, 0.01 mol), anhydrous K_2CO_3 (0.02 mol) and the desired acid or amide (0.01 mol) in isopropanol (30 mL) was heated under reflux for 4 h. The reaction mixture was concentrated under reduced pressure to half its volume, cooled, and poured into ice-cold water. The separated solid was filtered, dried, and crystallized from isopropanol.

2-(6-Chloropyridazine-3-yloxy)benzoic acid ***5a***

Yield 80%; mp 94–95 °C; IR (KBr)/cm: 3200-2534(O–H); 3047 (C–H aromatic); 1681 (C=O); 1581 (C=N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.89–6.90 (m, 2H, 1H aromatic, and 1H pyridazine); 7.44–7.49 (m, 2H, 1H aromatic, and 1H pyridazine); 7.75–7.77 (m, 1H, Ar–H); 7.97-8.00 (m, 1H, Ar–H); 11.26 (br. s, 1H, OH, D_2O exchangeable). Anal Calcd for $C_{11}H_7ClN_2O_3$ (250.64): C, 52.71; H, 2.82; N, 11.18; Found: C, 52.80; H, 2.53; N, 11.30.

2-(6-Chloropyridazine-3-yloxy)benzamide ***5b***

Yield 70%; mp 199–200 °C; IR (KBr)/cm: 3487, 3336 (NH_2); 3066 (C–H aromatic); 1670 (C=O); 1582 (C=N). ^{1}H-NMR (DMSO-d_6, 200 MHz) δ (ppm): 6.40 (d, 1H, $J = 9.6$ Hz, pyridazine-H4); 6.18–6.22 (m, 1H, Ar–H); 6.96–7.01 (m, 1H, Ar–H); 7.65–7.72 (m, 2H, Ar–H); 8.04 (s, 2H, NH_2, D_2O exchangeable); 8.63 (d, 1H, $J = 9.4$ Hz, pyridazine-H5). Anal Calcd for $C_{11}H_8ClN_3O_2$ (249.65): C, 52.92; H, 3.23; N, 16.83; Found: C, 53.10; H, 3.45; N, 16.94.

2-(6-Oxo-1,6-dihydropyridazin-3-yloxy)benzoic acids and benzamides (**6a**, **b**)

Either of **5a** or **5b** (0.01 mol) in glacial acetic acid (25 mL) was heated under reflux for 5 h. The reaction mixture was concentrated to half its volume under reduced pressure then cooled. The precipitated crystalline solid was filtered, washed with water, and recrystallized from ethanol to give **6a**, **b**.

2-(6-Oxo-1,6-dihydropyridazin-3-yloxy)benzoic acid ***6a***

Yield 85%; mp 148–149 °C; IR (KBr)/cm: 3228-2515 (O–H); 1678, 1639 (C=O); 1581 (C=N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.89–6.95 (m, 3H, 2H aromatic, and 1H pyridazine); 7.20 (s, 1H, NH, D_2O exchangeable); 7.47–7.53 (m, 2H, Ar–H); 7.80 (d, 1H, pyridazine); 10.58 (br s, 1H, OH, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 232 ($M^{\cdot+}$, 41.07). Anal Calcd for $C_{11}H_8N_2O_4$ (232.19): C, 56.90; H, 3.47; N, 12.06; Found: C, 56.98; H, 3.55; N, 12.40.

2-(6-Oxo-1,6-dihydropyridazin-3-yloxy)benzamide ***6b***

Yield 80%; mp 229–230 °C; IR (KBr)/cm: 3421, 3271 (NH, NH_2); 3082 (C–H aromatic); 1678, 1645 (C=O); 1593 (C=N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.94 (d, 1H, $J = 9.3$ Hz, pyridazine-H4); 6.99–7.08 (m, 2H, Ar–H); 7.92-8.05 (m, 2H, Ar–H); 8.54 (d, 1H, $J = 9.3$ Hz, pyridazine-H5); 10.73 (s, 1H, NH, D_2O exchangeable); 11.69 (s, 2H, NH_2, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 231 ($M^{\cdot+}$, 11.28), 232 (M+H, 2.45). Anal Calcd for $C_{11}H_9N_3O_3$ (231.21): C, 57.14; H, 3.92; N, 18.17; Found: C, 57.25; H, 4.10; N,18.35.

2-(6-Phenylpyridazin-3-yloxy)benzamide **7**

An equimolar mixture of 3-chloro-6-phenylpyridazine and salicylamide (0.01 mol each) and anhydrous K_2CO_3 (0.02 mol) in isopropanol (30 mL) was heated under reflux for 4 h. The reaction mixture was concentrated under reduced pressure to half its volume, cooled, and poured into ice-cold water (20 mL). The solid separated was filtered, dried, and crystallized from isopropanol.

Yield 78%; mp 139–140 °C; IR (KBr)/cm: 3398, 3363 (NH, NH_2); 3049 (C–H aromatic); 1674, 1650 (C=O); 1593 (C=N). ^{1}H-NMR (DMSO-d_6, 300 MHz) δ (ppm): 6.85 (d, 1H, $J = 9$ Hz, pyridazine-H4); 7.37-8.01 (m, 9H, Ar–H); 8.30 (d, 1H, $J = 9$ Hz, pyridazine-H5); 13.00 (s, 2H, NH_2, D_2O exchangeable). MS (EI) *m/z* (% rel. Int.): 291 ($M^{\cdot+}$, 68), 292(M+H, 20.62). Anal Calcd for $C_{17}H_{13}N_3O_2$ (291.30): C, 70.09; H, 4.50; N, 14.42; Found: C, 70.44; H, 4.65; N, 14.60.

Pharmacology

Anti-inflammatory activity

Materials and methods

Inflammatory-grade carrageenan was purchased from FMC (Rockland, ME, USA). All other chemicals were of the highest available commercial grade. All test compounds were dissolved in carboxymethylcellulose (CMC) before being injected into the animals. Throughout the experiments, adult male Sprague–Dawley rats weighing 180–200 g were used (Pharmacological Department, Faculty of Pharmacy, Ain Shams University). They were housed at a temperature of 23 ± 2 °C with free access to water and standard food pellets. Rats were acclimatized in our animal facility for at least 1 week prior to the experiment.

Measurement of paw volume in carrageenan-induced rat edema model

The experimental tests on animals have been performed in accordance with the Institutional Ethical Committee approval. The anti-inflammatory effect of the test compounds was evaluated in correspondence to the carrageenan-induced paw edema method (Winter *et al*., 1962). The rats were equally divided into 14 groups (six animals/group). The first group was injected with 0.05 mL of 1% carrageenan in the subplantar tissue of the right-hind paw and served as untreated control. The positive control group was given 10 mg/kg diclofenac 1 h before carrageenan injection.

The test compounds were suspended in 0.5% carboxymethylcellulose (CMC) and given to the rats at a dose of 10 mg/kg 1 h prior to carrageenan injection. The paw volume of each rat was measured before 1 h and after 1, 2, and 4 h of carrageenan treatment using UGO-BASILE 7140 plethysmometer (Comerio, Italy).

Statistical analysis of data

Quantitative variables from normal distribution were expressed as means ± SE (standard error). The significant difference between groups was tested using one-way ANOVA and the chosen level of significance was $p < 0.05$. Further comparisons among groups were made according to post hoc Tukey's test. All statistical analyses were performed using Graph Pad Instat software version 3 (ISI® software, CA, USA).

The anti-inflammatory activity was expressed as percentage inhibition of edema volume in treated animals in comparison with the control group (Table 1).

% Inhibition of edema $= V_c - V_t/V_c \times 100$ where V_c and V_t are the volumes of edema for the control and drug-treated animal groups, respectively.

Potency of the tested compounds was calculated 4 h after treatment with a single dose (10 mg/kg) of the tested compounds relative to diclofenac-treated group.

According to the following equation:

$$\text{Potency} = \%\ \text{edema inhibition of test compounds-treated group/ \%edema inhibition of diclofenac-treated group.} \quad (1)$$

Gastric ulcerative effect

Materials and methods

Male Sprague–Dawley rats (120–130 g wt) were purchased from the animal house facility the National Research Center (Dokki, Giza, Egypt). Animals were acclimatized in the animal house unite facility, Faculty of pharmacy, Ain Shams University, Cairo, Egypt for at least 1 week prior to experimentation. Animals were kept at 22 ± 3 °C and 55 ± 5% relative humidity during the whole experiment. Standard food pellets and water were supplied ad labium. All test compounds were dispensed in 0.5% CMC solution in distilled water. Animals' treatment protocol was approved by Ain Shams University Animal Rights committee.

Ulcerative effect (Gastric hemorrhagic gross lesions) of test compounds was assessed after intragastric administration of 50 mg/kg in 0.5% CMC solution as previously described; briefly, 6 h after drug administration, rats were killed by cervical dislocation, and the stomachs were removed, inflated, and opened along the greater curvature. The hemorrhagic lesions were stretched out, and scored from 0 to 5 according to the method of Clementi *et al.* (1998). Gastric tissue samples were fixed in neutral buffered formalin for 24 h. Stomach sections were dehydrated with graded ethanol, passed through xylene, and embedded in paraffin. The paraffin sections (5-μm thick) were stained with hematoxylin and eosin (H&E) (Oyagi *et al.*, 2010). Mucosal membrane thinning and mucosal microscopic ulcer depth were further quantified using Image-J™ software indomethacin was used as positive control drug.

Statistical analysis

Data are presented as mean ± SEM. Analysis of variance (ANOVA) with Dunnet's post hoc test was used for testing the significance of data using SPSS® for windows, version 17.0. $p < 0.05$ was taken as a cut off value for significance.

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