European Journal of Medicinal Chemistry 77 (2014) 155–165

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamides: Synthesis, molecular modeling, evaluation of their anti-inflammatory activity and ulcerogenicity

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ARTICLE INFO

Article history:
Received 17 September 2013
Received in revised form
26 February 2014
Accepted 2 March 2014
Available online 3 March 2014

Keywords:
1,2,4-Triazole
Anti-inflammatory
Ulcerogenicity
Histopathological investigation
Cyclooxygenase activity
Docking study

ABSTRACT

A series of novel 1-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamides were synthesized and confirmed with different spectroscopic techniques. The prepared compounds exhibited remarkable anti-inflammatory activity that represents 38%–100% of indomethacin activity and 44%–115% of celecoxib activity after 3 h. The anilides **5a**–**l** and hydrazide **6** exhibit low incidence of gastric ulceration compared to indomethacin which was confirmed with histopathological investigation. *In vitro* COX-1/COX-2 inhibition studies showed compounds **4b** (COX-1 IC_{50} = 45.9 μM; COX-2 IC_{50} = 68.2 μM) and **6** (COX-1 IC_{50} = 39.8 μM; COX-2 IC_{50} = 46.3 μM) are the most potent COX inhibitors in the tested compounds. The binding mode for some of the tested compounds to the enzymes was predicted using docking studies.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most useful clinical therapies for the treatment of pain, fever, and inflammation [1]. The major mechanism by which NSAIDs exert their anti-inflammatory activity is through inhibition of cyclooxygenase-derived prostaglandin synthesis, which is also responsible for the gastrointestinal [2–6], renal [7–9], and hepatic side effects [10] that are observed mainly in chronic use of NSAIDs. Diaryl-heterocycles have become the major class of selective COX-2 inhibitors, such as celecoxib, rofecoxib, parecoxib and valdecoxib (Fig. 1), which display improved gastrointestinal safety profile compared to the traditional NSAIDs [11–13]. However, the cardiovascular risk associated with "-coxibs" has become a concern since rofecoxib and valdecoxib were withdrawn from the market [14]. Therefore, the challenge still exists for the pharmaceutical industry to develop, effective anti-inflammatory agents with enhanced safety profile. The considerable biological importance of triazoles has stimulated a lot of interest, 1,2,4-triazoles and their heterocyclic derivatives have a wide range of therapeutic properties like antibacterial [15–17], antifungal [16–18] antitubercular [19–21], antiviral [22,23], analgesic [24,25], anti-inflammatory [25–27], anticonvulsant [28,29], antidepressant [30], anticancer [31,32], antihypertensive [33], hypoglycemic [34], insecticidal [35,36] and plant growth activities [37]. Besides, studies that described simple chemical derivatization (esterification or amidation) of the carboxylic function of representative NSAIDs showed that this approach resulted not only in the reduction of the ulcerogenic effect but also in an increased anti-inflammatory activity [38–40]. Various oxadiazole, triazole, thiadiazole and triazine derivatives of indomethacin have been synthesized and tested for anti-inflammatory activity. The test compounds inhibited the induction of gastric mucosal lesions which may be related to inhibition of lipid peroxidation in gastric mucosa [41]. Promoted with the above-mentioned studies and as a continuation of our research interest in the synthesis and biological activities of novel NSAIDs derivatives with lower ulcerogenic liability [42–44], the present study is concerned with the synthesis of novel 1-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamides with the objective of discovering novel and potent anti-inflammatory

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http://dx.doi.org/10.1016/j.ejmech.2014.03.001
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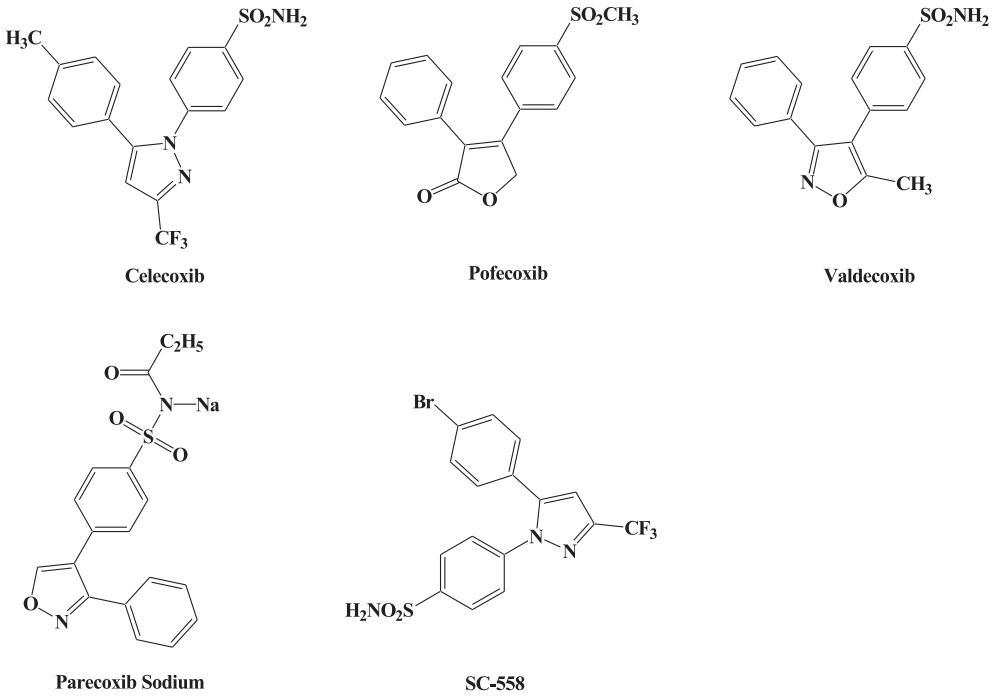


Fig. 1. Selective COX-2 inhibitors.

agents. The ability of the prepared compounds to induce gastric toxicity has been also evaluated. Among the synthesized series, some selected derivatives were tested for their inhibitory activity against COX-2 and COX-1 enzymes and their binding mode to the enzymes were predicted using docking studies.

2. Results and discussions

2.1. Chemistry

The synthetic route used to synthesized 1-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamides **4a**–**c**, **5a**–**l** and **6** is outlined in Scheme 1. Key starting compound, 2-(3,4,5-trimethoxybenzamido)acetic acid **1** was prepared in good yield (85%) by the reaction of glycine with 3,4,5-trimethoxybenzoyl chloride in 10% NaOH. Heating of compound **1** with acetic anhydride afforded the corresponding compound **2**. The synthesis of the key intermediate **3** was carried out using Kuskov like reaction through coupling of the diazonium salt of 4-methoxyaniline with the active methylene of **2** in presence of sodium acetate. According to Sawdey rearrangement [45], reaction of **3** with ammonia or appropriate amine in methanol gave the corresponding amides **4a**–**c** in 55–71% yield. Also, treatment of **3** with primary aromatic amines in acidic medium formed the target anilides **5a**–**l** in 35–62% yield. Moreover, reaction of **3** with hydrazine monohydrate in methanol afforded hydrazide **6** in 65% yield. The physical and spectral data are listed in the experimental part.

2.2. Biological investigations

2.2.1. Screening of anti-inflammatory activity

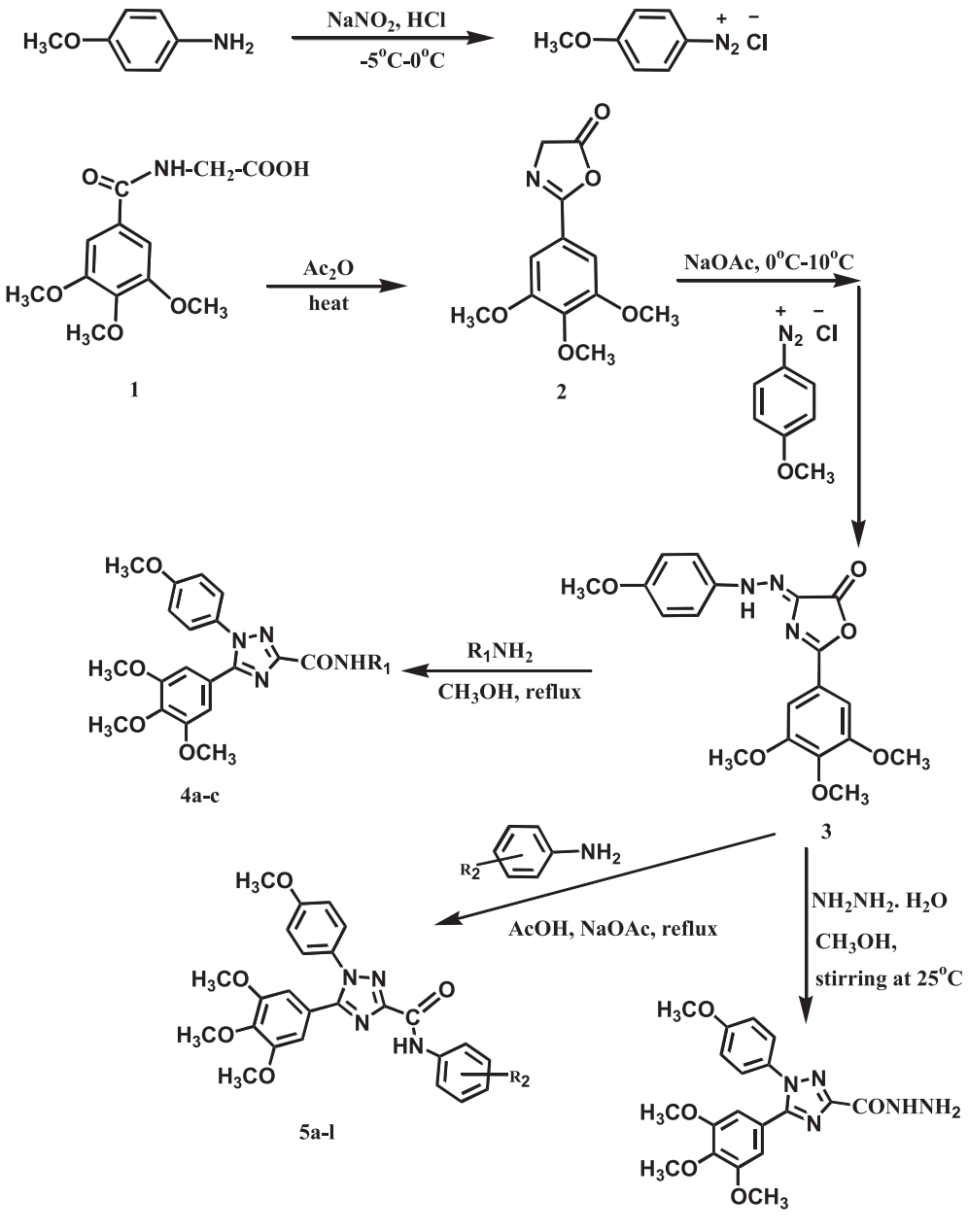
The synthesized compounds **4a**–**c**, **5a**–**l** and **6** were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema method in rats described by Winter *et* al [46]. The test compounds, celecoxib and indomethacin (reference drugs) were administered orally at a dose level of (0.28 mmol/kg) 30 min before carrageenan injection at the right hind paw of Albino male rats. The thickness of both paws was measured at different time intervals of 1, 2, 3, 4 and 5 h after carrageenan injection. The anti-inflammatory activity of the tested compounds, celecoxib and indomethacin was calculated as the percentage decrease in edema thickness induced by carrageenan and was determined using the following formula:

$$\%\ \text{of edema inhibition} = \frac{(V_R - V_L)_{control} - (V_R - V_L)_{treated}}{(V_R - V_L)_{control}} \times 100$$

where V_R represents the mean right paw thickness and V_L represents the mean left paw thickness.

$(V_R–V_L)_{control}$ represents the mean increase in paw thickness in the control group of rats.
$(V_R–V_L)_{treated}$ represents the mean increase in paw thickness in rats treated with the test compounds.

The results listed in Table 1 show the percentage of edema inhibition induced by carrageenan for all of the test compounds and indomethacin versus time in h. The obtained results listed in Table 1 indicated that most of the test compounds showed significant ($p < 0.01$) inhibition against carrageenan-induced paw edema in rats and the maximum anti-inflammatory activity was obtained after 3 h which is the time required for reaching the maximum activity and then the activity decreased gradually for the next 2 h. Indomethacin showed an inhibitory activity of 78% against carrageenan-induced paw edema after 3 h while celecoxib exhibited an inhibitory activity of 68% against carrageenan-induced paw edema after 3 h. Compounds **4a**–**c** exhibited good anti-inflammatory activity of 72%, 74% and 69%, respectively, which represents 92%, 95% and 88% of indomethacin activity and 106%, 109% and 101% of celecoxib activity, respectively, after 3 h (Table 2).



4a: R_1 = H, **4b:** R_1 = CH_3, **4c:** R_1 = CH_2CH_3.
5a: R_2 = 4-OCH_3, **5b:** R_2 = 2-OCH_3, **5c:** R_2 = 4-CH_3, **5d:** R_2 = 2-CH_3, **5e:** R_2 = 4-Br, **5f:** R_2 = 4-Cl, **5g:** R_2 = 3-F, **5h:** R_2 = 4-CF_3, **5i:** R_2 = 3,4-Di-OCH_3, **5j:** R_2 = 4-COOH, **5k:** R_2 = 4-OH, **5l:** R_2 = H.

Scheme 1. Synthesis of the target compounds **4a–c, 5a–l** and **6**.

The anti-inflammatory activity of amide derivatives **4a–c** revealed that the unsubstituted amide **4a** was found to possess good anti-inflammatory activity (72%). The substitution on the amide nitrogen by methyl group **4b** lead to a slight increase of the anti-inflammatory activity (74%), while substitution on the amide nitrogen by ethyl group **4c** lead to a slight decrease of the anti-inflammatory activity (69%). The anilides **5a–l** showed a variable anti-inflammatory activity ranging from 30% to 78% and these results represent 38%–100% of indomethacin activity and 44%–115% of celecoxib activity, respectively (Table 2). The results indicated that substitution on the amide nitrogen by phenyl **5l**, 4-methoxyphenyl **5a**, 2-methoxyphenyl **5b**, 4-methylphenyl **5c**, 2-methylphenyl **5d**, 4-bromophenyl **5e**, 3-trifluoromethylphenyl **5h** and 4-hydroxyphenyl **5k** moiety resulted in a dramatic decrease of the anti-inflammatory activity (30%–67%), while substitution on the amide nitrogen by 4-fluorophenyl moiety **5g** and 4-carboxyphenyl **5j** moieties increases the anti-inflammatory activity (72%–74%). Moreover, substitution on the amide nitrogen by 4-chlorophenyl **5f**, 3,4-dimethoxyphenyl **5i** moieties resulted in an increase of the anti-inflammatory activity of the test compounds and resulted in equipotent activity compared to indomethacin (78%). The hydrazide derivative **6** showed an anti-inflammatory activity of 57% which represents 73% and 84% of indomethacin and celecoxib activity, respectively.

Table 1
The anti-inflammatory activity at different time intervals and ulcer indices of compounds **4a**–**c**, **5a**–**l** and **6** compared to **celecoxib** and **indomethacin**.

Compound	% Of edema inhibition (% mean ± S.E.M)					UI mean ± S.E.M
	1 h	2 h	3 h	4 h	5 h	
Control	0	0	0	0	0	0.5 ± 0.06
4a	40.00 ± 1.45***	59.00 ± 2.07***	72.00 ± 1.08***	71.00 ± 2.52***	64.00 ± 2.21***	9.0 ± 1.03**
4b	56.00 ± 1.18***	74.00 ± 2.62***	74.00 ± 2.31***	65.00 ± 1.16***	50.00 ± 1.13***	7.5 ± 0.18**
4c	60.00 ± 2.07***	68.00 ± 1.22***	69.00 ± 1.24***	58.00 ± 1.24***	52.00 ± 1.20***	9.0 ± 0.79***
5a	39.00 ± 1.72***	45.00 ± 1.47***	67.00 ± 2.10***	63.00 ± 2.75***	51.00 ± 2.51***	4.5 ± 0.92***
5b	52.00 ± 1.07***	57.00 ± 1.13***	30.00 ± 2.44***	24.00 ± 1.94***	10.00 ± 2.32***	5.0 ± 1.31**
5c	38.00 ± 1.16***	60.00 ± 1.63***	60.00 ± 3.41***	58.00 ± 2.31***	42.00 ± 1.73***	5.5 ± 1.63**
5d	19.00 ± 1.12***	30.00 ± 2.16***	50.00 ± 2.60***	46.00 ± 1.20***	47.00 ± 1.16***	1.5 ± 0.86**
5e	40.00 ± 2.38***	50.00 ± 2.11***	66.00 ± 1.71***	59.00 ± 1.08***	57.00 ± 3.69***	1.8 ± 0.18***
5f	55.00 ± 1.26***	70.00 ± 2.02***	78.00 ± 2.05***	75.00 ± 1.57***	70.00 ± 1.80***	5.0 ± 0.47***
5g	48.00 ± 2.16***	67.00 ± 1.47***	72.00 ± 1.89***	70.00 ± 1.47***	53.00 ± 2.11***	13 ± 0.25***
5h	48.00 ± 1.41***	55.00 ± 1.46***	65.00 ± 1.42***	56.00 ± 2.03***	50.00 ± 2.16***	5.0 ± 0.50***
5i	57.00 ± 1.41***	68.00 ± 2.25***	78.00 ± 3.34***	70.00 ± 2.23***	61.00 ± 3.09***	7.0 ± 0.70***
5j	50.00 ± 1.38***	57.00 ± 3.08***	74.00 ± 1.65***	69.00 ± 1.86***	60.00 ± 1.18***	12 ± 1.46**
5k	36.00 ± 1.69***	35.00 ± 1.49***	50.00 ± 2.71***	37.00 ± 4.47***	27.00 ± 1.64***	1.0 ± 1.0***
5l	45.00 ± 1.60**	51.00 ± 1.79***	54.00 ± 2.39***	50.00 ± 2.44***	46.00 ± 2.06***	4.0 ± 2.0**
6	36.00 ± 1.80***	47.00 ± 1.07***	57.00 ± 1.98***	54.00 ± 2.53***	56.00 ± 2.24***	2.0 ± 2.67**
Celecoxib	42.00 ± 1.60***	48.00 ± 0.60***	68.00 ± 1.20***	57.00 ± 0.80***	51.00 ± 1.40***	0.50 ± 0.60***
Indomethacin	55.00 ± 0.78***	68.00 ± 2.23***	78.00 ± 1.14***	81.00 ± 2.36***	86.00 ± 1.47***	41 ± 0.38***

**Significantly different from control group at $P < 0.01$.
***Significantly different from control group at $P < 0.001$.
Note. One way ANOVA test was applied to determine the significance of the difference between the control group and rats treated with the tested compounds. ($n = 4$), $^{**}p < 0.01$,$^{***}p < 0.001$, significant difference from control group.

2.2.2. Screening of ulcerogenicity

The *in vivo* ulcerogenic liability was evaluated for the synthesized compounds **4a**–**c**, **5a**–**l** and **6** relative to both celecoxib and indomethacin according to a reported procedure [47]. Ulcers were classified into levels, level I, ulcer area less than 1 mm^2, level II, ulcer area from 1 to 3 mm^2 and level III, ulcer area more than 3 mm^2, and the ulcer index (UI) was calculated as follows:

$$\begin{aligned} UI = {} & 1 \times (\text{number of ulcers level I}) + 2 \\ & \times (\text{number of ulcers level II}) + 3 \\ & \times (\text{number of ulcers level III}), \text{ etc…} \end{aligned}$$

The UI of compounds **4a**–**c**, **5a**–**l** and **6** were calculated and listed in Table 1 as (mean ± S.E.M). The results of ulcerogenic liability revealed that indomethacin caused significant ulcerogenic toxicity with UI of **41**, while celecoxib exhibited very low UI of 0.5. The majority of the synthesized compounds exhibited much lower UIs compared to indomethacin. Compounds **4a**–**c** exhibited lower ulcerogenic liability relative to indomethacin with UIs of 9, 7.5 and 9, respectively, (Fig. 2A). The anilides **5a**–**l** also exhibited lower gastric toxicity relative to indomethacin with UI ranging from 1 to 13 (Fig. 2B–D). The hydrazide derivative **6** showed lower gastric toxicity relative to indomethacin with UI of 2 (Fig. 2A). The obtained results revealed that all of the tested compounds exhibit safer ulcerogenic liability relative to indomethacin.

Table 2
% Activity of the tested compounds **4a**–**c**, **5a**–**l** and **6** relative to **celecoxib** and **indomethacin** after 3 h.

Compound	% Activity relative to indomethacin after 3 h	% Activity relative to celecoxib after 3 h
Control	0	0
4a	92.00	106.00
4b	95.00	109.00
4c	88.00	101.00
5a	86.00	99.00
5b	38.00	44.00
5c	77.00	88.00
5d	64.00	74.00
5e	85.00	97.00
5f	100.00	115.00
5g	92.00	106.00
5h	83.00	96.00
5i	100.00	115.00
5j	95.00	109.00
5k	64.00	74.00
5l	69.00	79.00
6	73.00	84.00
Celecoxib		100.00
Indomethacin	100.00	

2.2.3. Histopathological investigation

Different stomach sections of the ulcers including the control and the treated groups were stained by standard hematoxylin and eosin stains. The produced slides were subjected to microscopical examination and pictures were picked for these slides.

The control (Fig. 3A) showed no lesions and characterized by the presence of continuous mucosal layer. Indomethacin (Fig. 3B) exhibited marked loss of mucosal membrane at the areas of ulceration where certain areas of fundic glands were totally degenerated and cellular details were lost. Capillary inflammatory cells were also found and apoptotic glandular epithelial cells could be detected. The stomach sections of the ulcers treated with anilide **5d** showed normal morphology for the fundic glands and the results are in consistence with the previous results [42–44]. The edema was very minimal and also the vasodilatation of blood capillaries was not marked and this was confirmed by the low UI of 1.5 (Fig. 3C). A very low incidence of gastric ulceration was induced by the anilide **5k** (Fig. 3D) where a continuous mucosal layer was observed with the absence of capillary inflammatory cells and the ulcerative damage of the gastric mucosa was markedly decreased which was proved by the very low UI of 1.

2.3. Cyclooxygenase inhibitory activity

Compounds **4b**, **5e**, **5i**, **5f** and **6** were evaluated for their ability to inhibit COX-1 and COX-2 enzymatic activity using COX Inhibitor Screening Assay Kit. The potency (IC_{50} values) of test compounds was determined and compared to that of reference molecules NS-398 and indomethacin; their IC_{50} values and selectivity indices (SI) are given in Table 3.

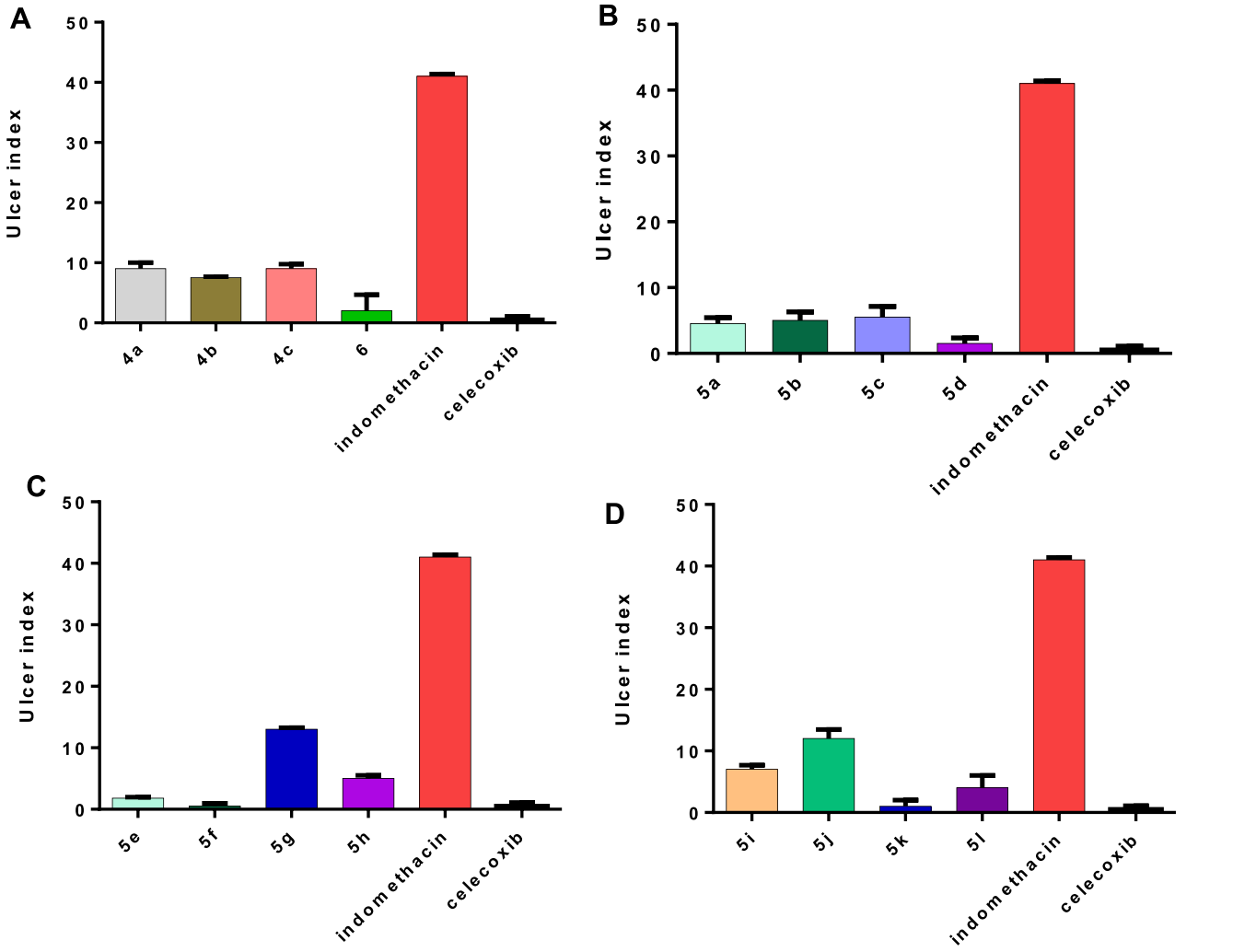


Fig. 2. A. UI of compounds **4a**–**c** and **6** compared to indomethacin and celecoxib expressed as mean ± S.E.M. B. UI of compounds **5a**–**d** compared to indomethacin and celecoxib expressed as mean ± S.E.M. C. UI of compounds **5e**–**h** compared to indomethacin and celecoxib expressed as mean ± S.E.M. D. UI of compounds **5i**–**l** compared to indomethacin and celecoxib expressed as mean ± S.E.M.

In vitro COX-1/COX-2 inhibition studies showed that the most potent COX inhibitors in this series were **4b** (COX-1 $IC_{50} = 45.9$ μM; COX-2 $IC_{50} = 68.2$ μM) and **6** (COX-1 $IC_{50} = 39.8$ μM; COX-2 $IC_{50} = 46.3$ μM) whereas they did not have selectivity (SI ~ 1). Compounds **5e**, **5f** and **5i** did not inhibit cyclooxygenase enzymes at the highest test compound concentration (100 μM).

2.4. Docking studies

With the aim of getting insights into the structural basis for their activities, compound **4b, 5e, 5i**, **5f** and **6** were docked into the active site of COX-1 and COX-2 enzyme by using MOE software program. Docking studies in COX-2 showed that the trimethoxyphenyl ring of all the compounds fitted into the hydrophobic cavity formed by Phe381, Leu384, Tyr385, Trp387, Phe518 and Ser530. In the crystal structure of COX-2, this hydrophobic cavity was occupied by bromophenyl ring of SC-558. **4b** and **6** were oriented so that their methoxyphenyl group fitted into the adjunct pocket which is responsible for COX-2 inhibitory activity (Fig. 4A). The oxygen atom of methoxy group of **4b** and **6** formed weak bonds with Arg513 and His90 approximately at distances of 3.8 and 3.1 Å respectively. Furthermore, hydrogen bonds were observed between Arg120 and C═O group of **4a** and **6** at distances of 2.24 and 2.12 Å respectively. On the other hand, it was seen that **5e**, **5i** and **5f** oriented in a different way compared to **4a** and **6**. These compounds were unable to fill the adjunct pocket because of their bulky groups (Fig. 4B). This may explain why **4b** and **6** are the active derivatives whereas **5e**, **5i** and **5f** are not.

In COX-1 enzyme, it was observed that the methoxy group, at meta position of trimethoxyphenyl ring of **4a** and **6**, formed strong hydrogen bonds with hydroxyl group of Ser530. It can be assumed that the increase in COX-1 potency of **4a** and **6** (Fig. 4C) is due to the presence of this interaction.

3. Conclusions

A group of novel 1-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamide derivatives was prepared and characterized by different spectroscopic and elemental analysis techniques. Most of the synthesized compounds showed significant anti-inflammatory activity using carrageenan-induced rat paw edema method and the tested compounds exhibit safer UI relative to indomethacin. Histopathological examination indicated that the novel anilides **5a**–**l** have very low incidence of gastric ulceration. Considering the COX inhibitory activity and docking study together, it is observed that substitution of less

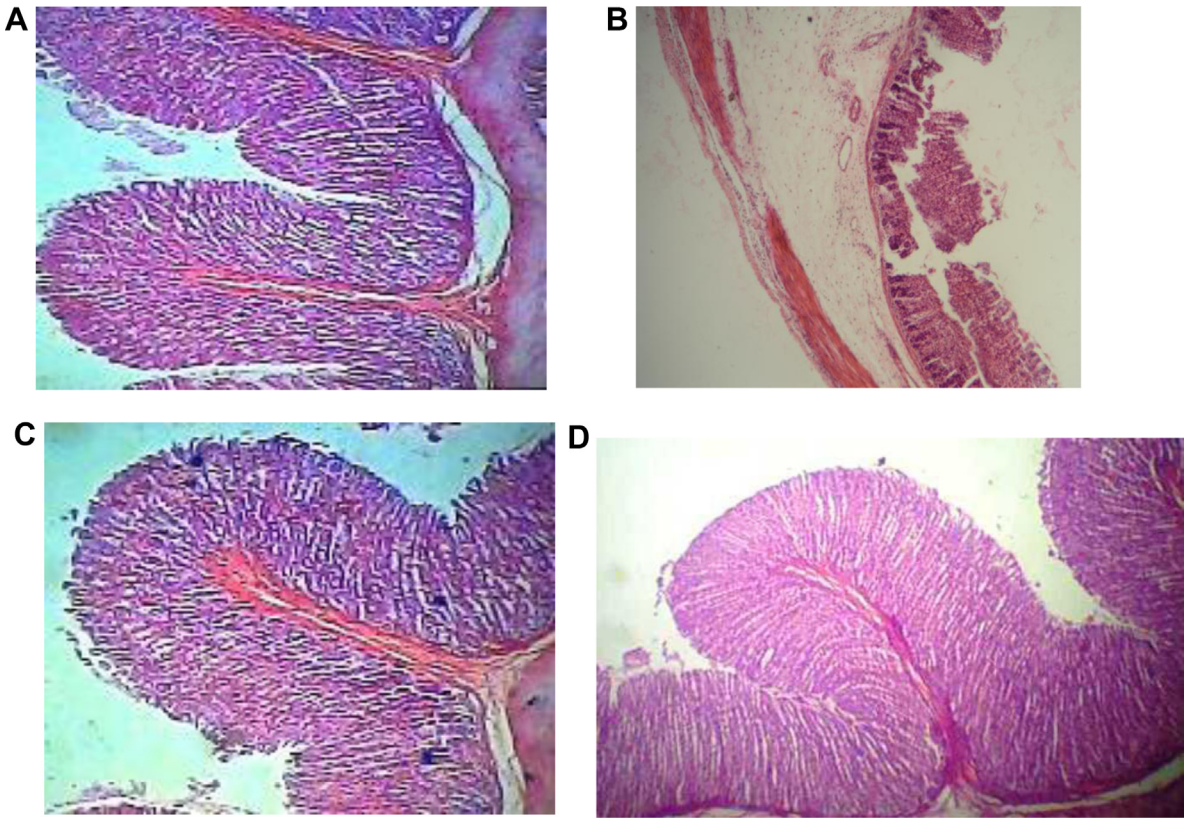


Fig. 3. A. Photomicrograph of the mucosa of fundic stomach of control. B. Photomicrograph of the mucosa of fundic stomach of indomethacin. C. Photomicrograph of the mucosa of fundic stomach of compound **5d**. D. Photomicrograph of the mucosa of fundic stomach of compound **5k**.

bulky groups such as CH_3 and NH_2 on the amide nitrogen of 1-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamide increased the COX activity. In conclusion the prepared 1-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1*H*-1,2,4-triazole-3-carboxamide derivatives looks as a promising approach to improve the safety of NSAIDs without altering their effectiveness.

4. Experimental

4.1. Chemistry

Melting points were determined on Stuart electrothermal melting point apparatus and were uncorrected. IR spectra were recorded on Nicolet iS5 FT-IR spectrometer at Minia University. NMR spectra were carried out using a Bruker Advance 300 MHz NMR spectrometer and a Bruker Advance 400 MHz NMR spectrometer using TMS as internal reference. Chemical shifts (δ) values are given in parts per million (ppm) relative to $CDCl_3$ (7.29 for proton and 76.9 for carbon) or DMSO-d_6 (2.50 for proton and 39.50 for carbon) and coupling constants (J) in Hertz. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; m, multiplet. Accurate masses were obtained on a Bruker Daltonics BioTOF Mass Spectrometer, in high resolution mode using cesium acetate solution (1 mM) in methanol as internal standard in a positive ion mode with flow injection electron spray ionization (ESI) with a flow rate of 120 μL/h and the EI-MS was carried out using Jeol JMS 600 spectrometers. High resolution mass spectra (HRMS) were obtained on a JEOL JMS D-300 mass spectrometer. The progress of reactions and the purity of the prepared compounds were monitored by thin-layer chromatography (TLC) using Merck 9385 pre-coated aluminum plate silica gel (Kieselgel 60) 5 × 20 cm plates with a layer thickness of 0.2 mm. The spots were detected by exposure to UV-lamp at $\lambda = 254$ nm. Elemental analysis was performed on Vario El Elementar CHN Elemental analyzer, organic microanalysis section, national research center, Giza, Egypt and the results were within ±0.4% of the theoretical values.

Table 3
In vitro COX-1 and COX-2 enzyme inhibition data for compounds **4b**, **5e**, **5f**, **5i** and **6** compared to **NS-398** and **indomethacin**.

Compound	R	COX-1 IC_{50} (μM)[a]	COX-2 IC_{50} (μM)[a]	SI[b]
4b	CH_3	45.9	68.2	0.67
5e	4-Br-C_6H_4	>100	>100	nd[c]
5f	4-Cl-C_6H_4	>100	>100	nd[c]
5i	3,4-diOCH_3-C_6H_4	>100	>100	nd[c]
6	NH_2	39.8	46.3	0.86
NS-398		213.2	2.1	101.52
Indomethacin		0.6	18.5	0.03

The result (IC_{50}, μM) is the mean of two determinations acquired using the COX Inhibitor Screening Assay Kit (Catalog No. 560131, Cayman Chemicals Inc., Ann Arbor, MI, USA).

[a] The in vitro test compound concentration required to produce 50% inhibition of enzymatic activity.

[b] In vitro COX-2 selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

[c] Not determined.

4.1.1. Synthesis of 4-[(4-methoxyphenyl)hydrazono]-2-(3,4,5-trimethoxyphenyl)-4H-oxazol-5-one 3

Trimethoxyhippuric acid **1** (0.13 mol, 35.00 g) in acetic anhydride (75 mL) was heated until a clear solution of **2** was obtained; this solution was cooled to room temperature (solution A). To a cold solution of p-methoxyaniline (0.1 mol, 12.31 g) in 5N HCl (35 mL) in

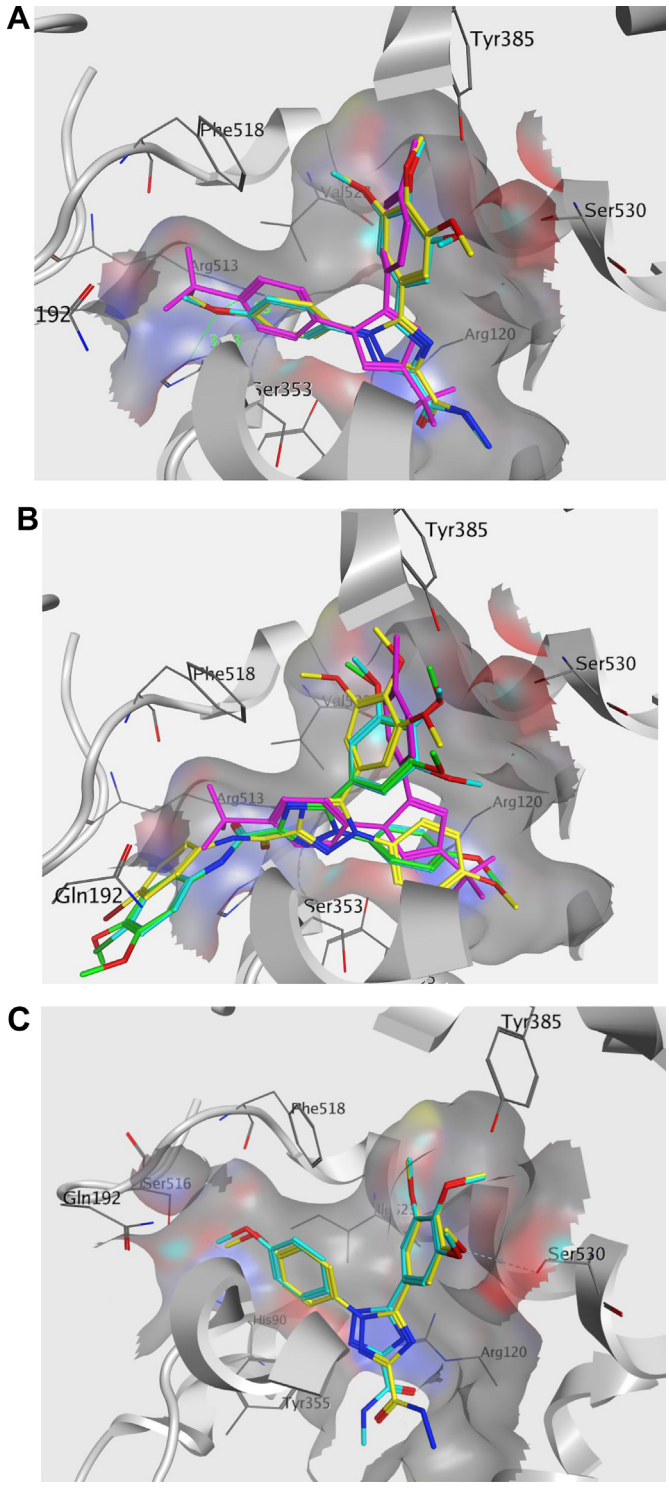


Fig. 4. A. The orientation of compounds **4b**, **6** and **SC-558** (shown as blue, yellow and purple respectively) in COX-2 active site. B. The orientation of **5e**, **5f**, **5i** and **SC-558** (shown as yellow, blue, green and purple respectively) in COX-2 active site. C. The orientation of **4b** and **6** (shown as blue and yellow respectively) in COX-1 active site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

an ice-salt bath −5 to 0 °C, a solution of sodium nitrite (0.13 mol, 8.97 g) in water (15 mL) was added in a dropwise manner. The reaction mixture was left for 10 min (solution B). Solution A was added to solution B in presence of anhydrous sodium acetate (0.18 mol, 15 g). The reaction mixture was stirred at 0–10 °C for 2 h, the formed precipitate was filtered off and dried (yield 70%).

*4.1.2. General procedure for the synthesis of 1-(4-methoxyphenyl)-5-(3,4,5-trimethoxy-phenyl)-1H-1,2,4-triazole-3-carboxamides (**4a**–**c**)*

A suspension of compound **3** (0.01 mol, 3.85 g) in methanol (30 mL) was treated with aqueous solution of ammonium hydroxide (25%, 50 mL) or methyl amine solution 41% (0.02 mol, 0.62 g) or ethyl amine (0.02 mol, 0.90 g) was refluxed for 30 min. The solvent was evaporated in vacuo. The formed precipitate solid was crystallized from ethanol.

*4.1.2.1. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid amide **4a**.* Pale yellow crystals (2.53 g; 65.90% yield); mp 180 °C; IR (KBr, cm^{-1}): 3329 (NH_2), 1669 (C=O) 1588 (C=N); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.69 (s, 6H, $2OCH_3$), 3.86 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.65 (br.s, 2H, $CONH_2$), 6.78 (s, 2H, Ar–H), 6.98 (d, 2H, J = 9.00 Hz, Ar–H), 7.35 (d, 2H, J = 9.00 Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 57.68 (CH_3), 58.04 ($2CH_3$), 62.85 (CH_3), 107.74 (CH), 115.88 (CH), 122.81 (C), 128.36 (CH), 131.79 (C), 140.85 (C), 153.91 (C), 155.67 (C), 156.18 (C), 161.08 (C), 162.00 (CO); EI-MS (70 eV) m/z (%): 384 (100) (M^+), 372 (13), 371 (31), 340 (54), 339 (30), 324 (30); Anal. Calcd for $C_{19}H_{20}N_4O_5$ (384.14): C, 59.37; H, 5.24; N, 14.58. Found: C, 59.40; H, 5.22; N, 14.33.

*4.1.2.2. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid methylamide **4b**.* Yellowish brown powder (2.82 g; 70.85% yield); mp 175 °C; IR (KBr, cm^{-1}): 3359 (NH), 1676 (C=O) 1584 (C=N); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.04 (d, 3H, J = 5.1 Hz, NH–$\underline{CH_3}$), 3.66 (s, 6H, $2OCH_3$), 3.83 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.73 (s, 2H, Ar–H), 6.95 (d, 2H, J = 9.00 Hz, Ar–H), 7.31 (d, 2H, J = 9.00 Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 28.46 (CH_3), 57.65 (CH_3), 57.66 (CH_3), 57.98 (CH_3), 62.82 (CH_3), 107.63 (CH), 115.81 (CH), 122.99 (C), 128.37 (CH), 131.85 (C), 140.74 (C), 153.86 (C), 155.34 (C), 156.75 (C), 160.50 (C), 161.00 (C=O); EI-MS (70 eV) m/z (%): 398 (17) (M^+), 388 (40), 386 (100), 370 (15), 367 (14), 340 (33); Anal. Calcd for $C_{20}H_{22}N_4O_5$ (398.16): C, 60.29; H, 5.57; N, 14.06. Found: C, 59.90; H, 5.60; N, 13.80.

*4.1.2.3. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid ethylamide **4c**.* Brown powder (2.27 g; 55.10% yield); mp 155 °C; IR (KBr, cm^{-1}): 3350 (NH), 1675 (CO), 1586 (C=N); ^1H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.29 (t, 3H, J = 7.50 Hz, CH_3), 3.56 (*p*, 2H, CH_2), 3.69 (s, 6H, $2OCH_3$), 3.85 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.75 (s, 2H, Ar–H), 6.97 (d, 2H, J = 8.50 Hz, A-H), 7.20 (t, 1H, J = 8.00 Hz, NH), 7.33 (d, 2H, J = 9.00 Hz, ArH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 15.25 (CH_3), 34.04 (CH_2), 56.09 (CH_3), 56.16 (CH_3), 60.59 (CH_3), 106.70 (CH), 115.08 (CH), 122.50 (C), 128.32 (CH), 131.00 (C), 139.36 (C), 153.07 (C), 154.68 (C), 156.53 (C), 158.98 (C), 160.42 (C=O); HRMS (FAB) m/z Calcd for $C_{21}H_{25}N_4O_5$ (M^+ + 1): 413.1825; found: 413.1846; Anal. Calcd for $C_{21}H_{24}N_4O_5$ (412.17): C, 61.15; H, 5.87; N, 13.58. Found: C, 61.00; H, 5.70; N, 13.40.

*4.1.3. General procedure for the synthesis of 1-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-1,2,4-triazole-3-carboxyanilides (**5a**–**l**)*

A mixture of compound **3** (3.85 g, 0.01 mol) and appropriate primary aromatic amine (0.01 mol) was refluxed in acetic acid (50 mL) in the presence of anhydrous sodium acetate (1.5 g, 0.018 mol) for 2 h. The reaction mixture was cooled and poured into ice water (50 mL). The formed precipitate was filtered off, dried and recrystallized from aqueous methanol.

4.1.3.1. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid (4-methoxyphenyl)amide ***5a***. Dark brown crystals (2.40 g, 48.98% yield); mp 190 °C; IR (KBr, cm^{-1}): 3380 (NH), 1686 (CO), 1588 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.75 (s, 6H, $2OCH_3$), 3.86 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.83 (s, 2H, Ar–H), 6.94–7.04 (m, 4H, Ar–H), 7.41 (d, 2H, J = 9.00 Hz, Ar–H), 7.71 (d, 2H, J = 9.00 Hz, Ar–H), 9.03 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 57.40 (CH_3), 58.10 (CH_3), 62.90 (CH_3), 107.78 (CH), 115.56 (CH), 115.91 (CH), 122.80 (CH), 128.41 (CH), 131.72 (C), 131.81 (C), 141.50 (C), 154.01 (C), 156.50 (C), 157.10 (C), 157.41 (C), 161.13 (C=O); EI-MS (70 eV) m/z (%): 490 (15) (M^+), 451 (14), 444 (20), 422 (15), 421 (29), 403 (31), 389 (100), 374 (16); Anal. Calcd for $C_{26}H_{26}N_4O_6$ (490.19): C, 63.66; H, 5.34; N, 11.42. Found: C, 63.54; H, 5.60; N, 11.20.

4.1.3.2. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid (2-methoxyphenyl)amide ***5b***. Golden yellow crystals (2.69 g, 54.90% yield); mp 131–132 °C; IR (KBr, cm^{-1}): 3381 (NH), 1696 (CO), 1588 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.75 (s, 6H, $2OCH_3$), 3.91 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 6.84 (s, 2H, Ar–H), 6.97–7.17 (m, 5H, Ar–H), 7.41 (d, 2H, J = 9.00 Hz, Ar–H), 8.63 (dd, 1H, J = 7.80 and 1.80 Hz, Ar–H), 9.62 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 57.71 (CH_3), 57.83 (CH_3), 58.08 (CH_3), 62.89 (CH_3), 107.87 (CH), 111.38 (CH), 115.86 (CH), 121.51 (CH), 122.37 (CH), 123.14 (C), 125.40 (C), 128.39 (CH), 128.48 (CH), 131.97 (C), 140.85 (C), 149.23 (C), 153.94 (C), 155.69 (C), 157.23 (C), 157.50 (C), 161.07 (C=O); EI-MS (70 eV) m/z (%): 490 (12) (M^+), 403 (67), 388 (22), 387 (100), 371 (13), 339 (12); Anal. Calcd for $C_{26}H_{26}N_4O_6$ (490.19): C, 63.66; H, 5.34; N, 11.42. Found: C, 63.50; H, 5.20; N, 11.10.

4.1.3.3. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid p-tolylamide ***5c***. Pale brown powder (2.60 g, 54.85% yield); mp 167 °C; IR (KBr, cm^{-1}): 3351 (NH), 1691 (CO), 18589 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.39 (s, 3H, CH_3), 3.74 (s, 6H, $2OCH_3$), 3.90 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 6.82 (s, 2H, Ar–H), 7.01 (d, 2H, J = 8.70 Hz, Ar–H), 7.21 (d, 2H, J = 8.10 Hz, Ar–H), 7.40 (d, 2H, J = 8.70 Hz, Ar–H), 7.68 (d, 2H, J = 8.10 Hz, Ar–H), 9.01 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 23.37 (CH_3), 57.71 (CH_3), 58.09 (CH_3), 62.89 (CH_3), 107.77 (CH), 115.89 (CH), 121.15 (CH), 122.95 (C), 128.40 (CH), 130.69 (CH), 131.86 (C), 135.31 (C), 135.96 (C), 140.91 (C), 153.99 (C), 155.62 (C), 156.92 (C), 157.39 (C), 161.09 (C=O); EI-MS (70 eV) m/z (%): 474 (16) (M^+), 469 (16), 450 (10), 424 (21), 421 (49), 390 (35), 389 (74), 375 (34), 324 (65); Anal. Calcd for $C_{26}H_{26}N_4O_5$ (474.19): C, 65.81; H, 5.52; N, 11.81. Found: C, 65.50; H, 5.20; N, 11.60.

4.1.3.4. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid o-tolylamide ***5d***. Yellowish brown powder (2.80 g, 59.10% yield); mp 173 °C; IR (KBr, cm^{-1}): 3386 (NH), 1700 (CO), 1588 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.40 (s, 3H, CH_3), 3.70 (s, 6H, $2OCH_3$), 3.86 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 6.79 (s, 2H, Ar–H), 6.99 (d, 2H, J = 8.50 Hz, Ar–H), 7.12 (t, 1H, J = 7.50 Hz, Ar–H), 7.23–7.30 (m, 2H, Ar–H), 7.37 (d, 2H, J = 9.00 Hz, Ar–H), 8.13 (d, 1H, J = 8.00 Hz, Ar–H), 8.95 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 20.98 (CH_3), 56.13 (CH_3), 56.21 (CH_3), 60.62 (CH_3), 106.83 (CH), 115.12 (CH), 121.09 (CH), 122.39 (C), 128.41 (CH), 129.52 (CH), 130.91 (C), 133.63 (C), 136.20 (C), 139.45 (C), 153.10 (C), 154.99 (C), 156.47 (C), 157.66 (C), 160.51 (C=O); EI-MS (70 eV) m/z (%): 474 (12) (M^+), 469 (18), 450 (20), 424 (18), 421 (49), 390 (40), 389 (80), 375 (34), 324 (55); HRMS (FAB) m/z Calcd for $C_{26}H_{27}N_4O_5$ (M^+ + 1): 475.1981; found: 475.1972; Anal. Calcd for $C_{26}H_{26}N_4O_5$ (474.19): C, 65.81; H, 5.52; N, 11.81. Found: C, 65.70; H, 5.20; N, 11.80.

4.1.3.5. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid (4-bromophenyl)amide ***5e***. Brown powder (3.18 g, 59.10% yield); mp 135 °C; IR (KBr, cm^{-1}): 3310 (NH), 1692 (CO), 1589 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.74 (s, 6H, $2OCH_3$), 3.91 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.82 (s, 2H, Ar–H), 7.02 (d, 2H, J = 7.20 Hz, Ar–H), 7.39 (d, 2H, J = 7.20 Hz, Ar–H), 7.51 (d, 2H, J = 8.10 Hz, Ar–H), 7.72 (d, 2H, J = 8.40 Hz, Ar–H), 9.17 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 57.75 (CH_3), 58.14 (CH_3), 62.92 (CH_3), 107.83 (CH), 115.62 (C), 115.97 (CH), 118.55 (C), 122.70 (CH), 128.41 (CH), 131.65 (C), 133.15 (CH), 137.55 (C), 141.11 (C), 154.03 (C), 155.64 (C), 157.31 (C), 157.42 (C), 161.22 (C=O); EI-MS (70 eV) m/z (%): 540 (10) (M^+ + 2), 538 (9) (M^+), 403 (96), 387 (100); Anal. Calcd for $C_{25}H_{23}BrN_4O_5$ (538.09): C, 55.67; H, 4.30; N, 10.39. Found: C, 55.40; H, 4.20; N, 10.00.

4.1.3.6. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid (4-chlorophenyl)amide ***5f***. Dark brown powder (3.06 g, 61.94% yield); mp 120–121 °C; IR (KBr, cm^{-1}): 3360 (NH), 1691 (CO), 1588 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 3.75 (s, 6H, $2OCH_3$), 3.91 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.84 (s, 2H, Ar–H), 7.02 (d, 2H, J = 8.70 Hz, Ar–H), 7.39–7.45 (m, 4H, Ar–H), 7.81 (d, 2H, J = 7.80 Hz, Ar–H), 9.20 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 57.73 (CH_3), 58.12 (CH_3), 62.91 (CH_3), 107.84 (CH), 115.95 (CH), 121.20 (CH), 122.47 (C), 125.85 (C), 128.42 (C), 130.21 (CH), 131.68 (CH), 138.45 (C), 141.11 (C), 154.03 (C), 155.50 (C), 156.44 (C), 157.19 (C), 161.20 (C=O); ESI-MS: m/z (%) 495 (100) (M^+ + 1). Anal. Calcd for $C_{25}H_{23}ClN_4O_5$ (494.14): C, 60.67; H, 4.68; N, 11.32. Found: C, 60.40; H, 4.40; N, 11.10.

4.1.3.7. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid (3-fluorophenyl)amide ***5g***. Pale brown powder (2.96 g, 61.92% yield); mp 126 °C; IR (KBr, cm^{-1}): 3310 (NH), 1693 (CO), 1588 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.74 (s, 6H, $2OCH_3$), 3.90 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.81 (s, 2H, Ar–H), 7.01 (d, 2H, J = 8.70 Hz, Ar–H), 7.10 (t, 2H, J = 8.70 Hz, Ar–H), 7.39 (d, 2H, J = 9.00 Hz, Ar–H), 7.74–7.79 (m, 2H, Ar–H), 9.14 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 57.71 (CH_3), 58.08 (CH_3), 62.89 (CH_3), 107.76 (CH), 115.92 (CH), 116.90 (C), 117.19 (C), 122.82 (CH), 122.92 (CH), 128.37 (CH), 131.77 (C), 134.53 (C), 140.98 (C), 154.01 (C), 155.71 (C), 156.68 (C), 157.50 (C), 161.15 (CO), 161.88 (C); EI-MS (70 eV) m/z (%): 480 (5) (M^+ + 2), 478 (10) (M^+), 405 (10), 386 (87), 371 (100), 352 (24), 332 (22), 325 (41), 275 (18); Anal. Calcd for $C_{25}H_{23}FN_4O_5$ (478.17): C, 62.76; H, 4.85; N, 11.71. Found: C, 62.50; H, 5.20; N, 11.30.

4.1.3.8. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid (4-trifluoromethylphenyl)amide ***5h***. Yellowish brown crystals (2.42 g, 45.83% yield); mp 151 °C; IR (KBr, cm^{-1}): 3350 (NH), 1696 (CO), 1588 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.75 (s, 6H, $2OCH_3$), 3.91 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 6.82 (s, 2H, Ar–H), 7.03 (d, 2H, J = 8.70 Hz, Ar–H), 7.39–7.58 (m, 4H, Ar–H), 8.00 (s,1H, Ar–H), 8.10 (d, 1H, J = 8.40 Hz, Ar–H), 9.19 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 57.72 (CH_3), 58.09 (CH_3), 62.90 (CH_3), 107.77 (CH), 115.94 (CH), 117.78 (C), 122.79 (CH), 124.20 (CH), 128.37 (CH), 130.86 (C), 131.75 (C), 138.97 (C), 141.01 (C), 154.02 (C), 155.84 (C), 156.46 (C), 157.74 (C),161.19 (C=O); EI-MS (70 eV) m/z (%): 528 (14) (M^+), 403 (42), 387 (40), 370 (12), 300 (16); Anal. Calcd for $C_{26}H_{23}F_3N_4O_5$ (528.16): C, 59.09; H, 4.39; N, 10.60. Found: C, 58.80; H, 4.10; N, 10.30.

4.1.3.9. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid (3,4-dimethoxyphenyl)amide ***5i***. Pale brown powder (2.91 g, 55.96% yield); mp 175 °C; IR (KBr, cm^{-1}): 3320 (NH), 1686 (CO), 1588 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.75 (s, 6H, $2OCH_3$), 3.90 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3),

3.92 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.83 (s, 2H, Ar–H), 6.89 (d, 1H, J = 8.70 Hz, Ar–H), 7.02 (d, 2 H, J = 8.70 Hz, Ar–H), 7.22 (dd, 1H, J = 6.90 and 1.80 Hz, Ar–H), 7.40 (d, 2H, J = 9.00 Hz, Ar–H), 7.60 (d, 1H, J = 2.40 Hz, Ar–H), 9.08 (s, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 57.71 (CH_3), 58.04 (CH_3), 58.10 (CH_3), 61.90 (CH_3), 62.89 (CH_3), 106.21 (CH), 107.80 (CH), 112.73 (CH), 113.25 (CH), 115.93 (CH), 122.62 (C), 128.37 (CH), 131.74 (C), 132.17 (C), 141.05 (C), 147.03 (C), 149.98 (C), 154.02 (C), 155.50 (C), 156.61 (C), 157.14 (C), 161.16 (C=O); EI-MS (70 eV) m/z (%): 520 (14) (M^+), 403 (100), 387 (91), 371 (14); Anal. Calcd for $C_{27}H_{28}N_4O_7$ (520.20): C, 62.30; H, 5.42; N, 10.76. Found: C, 62.10; H, 5.40; N, 10.60.

4.1.3.10. 4-{[1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4]triazole-3-carbonyl]amino}benzoic acid ***5j***. Brown powder (2.72 g, 53.97% yield); mp 259 °C; IR (KBr, cm^{-1}): 3301 (OH), 1696 (CO), 1593 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.70 (s, 6H, $2OCH_3$), 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.78 (s, 2H, Ar–H), 6.86 (d, 2H, J = 9.00 Hz, Ar–H), 6.99 (d, 2H, J = 9.00 Hz, Ar–H), 7.37 (d, 2H, J = 9.50 Hz, Ar–H), 7.62 (d, 2H, J = 9.00 Hz, Ar–H), 8.90 (s, 1H, NH), 10.22 (s, 1H, COOH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 56.13 (CH_3), 56.22 (CH_3), 60.63 (CH_3), 105.76 (CH), 106.86 (CH), 115.13 (CH), 120.36 (CH), 122.29 (C), 126.50 (C), 128.43 (C), 130.67 (CH), 130.84 (CH), 139.50 (C), 142.81 (C), 153.11 (C), 153.93 (C), 155.19 (C), 156.12 (C), 158.12 (C), 160.56 (C=O), 167.42 (C=O); HRMS (FAB) m/z Calcd for $C_{26}H_{25}N_4O_7$ (M^+ + 1): 505.1723; found: 505.1751; Anal. Calcd for $C_{26}H_{24}N_4O_7$ (504.16): C, 61.90; H, 4.80; N, 11.11. Found: C, 61.70; H, 5.20; N, 11.00.

4.1.3.11. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4] triazole-3-carboxylic acid (4-hydroxyphenyl)amide ***5k***. Black powder (2.62 g, 55.04% yield); mp 205 °C; IR (KBr, cm^{-1}): 3316 (OH), 1683 (CO), 1588 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.69 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.76 (s, 2H, Ar–H), 6.98 (d, 2H, J = 9.00 Hz, Ar–H), 7.36 (d, 2H, J = 8.70 Hz, Ar–H), 7.88 (d, 2H, J = 8.40 Hz, Ar–H), 8.13 (d, 2H, J = 8.70 Hz, Ar–H), 9.19 (s, 1H, NH), 10.33 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 56.12 (CH_3), 56.21 (CH_3), 60.62 (CH_3), 106.83 (CH), 115.12 (CH), 121.13 (CH), 122.37 (C), 124.61 (C), 126.09 (C), 128.42 (CH), 129.13 (CH), 130.89 (C), 138.71 (C), 139.47 (C), 153.10 (C), 155.05 (C), 156.39 (C), 157.83 (C), 160.52 (C=O); HRMS (FAB) m/z Calcd for $C_{25}H_{25}N_4O_6$ (M^+ + 1): 477.1774; found: 477.1760. Anal. Calcd for $C_{25}H_{24}N_4O_6$ (476.17): C, 63.02; H, 5.08; N, 11.76. Found: C, 62.90; H, 5.10; N, 11.70.

4.1.3.12. 1-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-1H-[1,2,4]triazole-3-carboxylic acid phenylamide ***5l***. Brown powder (1.61 g, 35.00% yield); mp 135 °C; IR (KBr, cm^{-1}): 3312 (NH), 1691 (CO), 1593 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 3.65 (s, 6H, $2OCH_3$), 3.72 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 6.84 (s, 2H, Ar–H), 7.12–7.17 (m, 3H, Ar–H), 7.37–7.56 (m, 4H, Ar–H), 7.86 (d, 2H, J = 8.10 Hz, Ar–H), 10.50 (s, 1H, NH); ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 57.73 ($2CH_3$), 62.07 ($2CH_3$), 107.76 (CH), 115.91 (CH), 121.83 (CH), 123.32 (C), 125.29 (C), 129.01 (CH), 129.73 (CH), 131.47 (CH), 139.16 (C), 140.01 (C),153.42 (C), 155.33 (C), 156.65 (C), 158.08 (C), 160.75 (C=O); ESI-MS: m/z (%) 461 (100) (M^+ + 1); Anal. Calcd for $C_{25}H_{24}N_4O_5$ (460.17): C, 65.21; H, 5.25; N, 12.17. Found: C, 65.00; H, 5.20; N, 12.10.

4.1.4. General procedure for the synthesis of 1-(4-methoxyphenyl)-5-(3,4,5-trimethoxy-phenyl)-1H-[1,2,4]triazole-3-carboxylic acid hydrazide ***6***

To a stirred suspension of compound **3** (11.55 g, 0.03 mol) in methanol (100 mL), hydrazine hydrate 95% (4.5 g, 0.09 mol) was added gradually, stirring was continued overnight at 25 °C, solvent was evaporated and the obtained oily product was then purified using silica gel column eluted with chloroform/methanol (9.5:0.5), solvent was evaporated and the obtained product was recrystallized from ethanol give compound **6** as pale yellow powder (7.78 g, 65.00% yield); mp 210 °C; IR (KBr, cm^{-1}): 3380 and 3150 (NH and NH_2), 1661 (CO), 1590 (C=N); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 4.01 (s, 6H, $2OCH_3$), 4.02 (s, 6H, $2OCH_3$), 7.09 (d, 2H, J = 9.00 Hz, Ar–H), 7.54 (s, 2H, Ar–H), 8.18 (d, 2H, J = 9.00 Hz, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 58.31 (CH_3), 58.49 (CH_3), 63.01 (CH_3), 107.10 (CH), 115.89 (CH), 119.86 (C), 127.94 (CH), 143.52 (C), 148.14 (C), 154.44 (C), 165.28 (C), 176.07 (C), 177.28 (C); ESI-MS: m/z (%) 400 (100) [M^+ + 1]; Anal. Calcd for $C_{19}H_{21}N_5O_5$ (399.15): C, 57.14; H, 5.30; N, 17.53. Found: C, 56.80; H, 5.21; N, 17.38.

4.2. Biology

4.2.1. Screening of the anti-inflammatory activity

The experiments were performed on adult male albino rats, weighing (120–140 g), obtained from the animal house, Minia University. The animals were housed in stainless steel cages, divided into groups of four animals each and deprived of food but not water 24 h before the experiment. The anti-inflammatory activity of the compounds under investigation was studied using carrageenan. A suspension of the tested compounds **4a**–**c**, **5a**–**l** and **6**, celecoxib and indomethacin in carboxy methyl cellulose (CMC) solution (0.5% w/v in water) was administered orally in a dose level of (0.28 mmol/kg). Control animals were similarly treated with CMC solution (0.5% w/v in water). After 30 min, 0.1 mL of freshly prepared 1% carrageenan solution in normal saline was injected into the subplantar region of the right hind paw of rats according to the method of Winter et al. An equal volume of saline was injected into the left hind paw of each rat. The right paw thickness was measured by a Vernier caliper (SMIEC) directly before and after 1, 2, 3, 4 and 5 h intervals after carrageenan injection. The anti-inflammatory activity of the tested compounds, celecoxib and indomethacin was calculated as the percentage decrease in edema thickness induced by carrageenan.

4.2.2. Screening of ulcerogenicity

After measuring the anti-inflammatory activity the rats were sacrificed by decapitation. The stomachs were removed, collected, opened along the greater curvature, washed with distilled water and cleaned gently by dipping in saline. The mucosal damage for each stomach was examined with a magnifying lens for the presence of macroscopically visible lesions. The number of lesions in each stomach, if any, was counted and recorded. Ulcers were classified into levels, level I, in which the ulcer area is less than 1 mm^2, level II, in which ulcer area is in the range from 1 to 3 mm^2 and level III, in which the ulcer area more than 3 mm^2 and this rated according to their areas in mm^2.

The data are expressed as mean ± S.E.M, *one way* ANOVA test was applied to determine the significance of the difference between the control group and rats treated with the tested compounds.

4.2.3. Histopathological investigation

The histological slides were prepared according to the reported procedures for examination of ulcers under light microscope [48]. Identify site of the slide on which the section was applied by scratching wax around section with a needle. Dewax hydrated sections by using graded alcohols to water. Slides were stained with haematoxylin for 5–7 min, washed with tap water until sectioning for 5 min and immersed for 5–10 s in solution of (1% HCl in 70% alcohol), then washed well with tap water for 10–15 min followed by staining with 1% Eosin for 10 min, and washed with running tap water for 1–5 min. The slide was then dehydrated using alcohols,

cleaned by using xylene, covered by glass cover using Canda balsam then examined under microscope.

4.3. Cyclooxygenase inhibitory assay [49]

The effect of the compounds on ovine COX-1 and human recombinant COX-2 (IC_{50} value, μM) enzymes were determined by measuring prostaglandin F2α (PGF2α) using a COX Inhibitor Screening Kit (Catalog No 560131) from Cayman Chemical, Ann Arbor Michigan USA following the procedure suggested by manufacturer. The IC_{50} values (the concentration of the test compound causing 50% inhibition) were calculated from the concentration-inhibition response curves (duplicate determinations).

4.4. Docking studies

Molecular modeling studies were performed with MOE (The Molecular Operating Environment) Version 2011.10, software available from Chemical Computing Group Inc., 1010 Sherbrooke Street West, Suite 910, Montreal, Canada H3A2R7, http://www.chemcomp.com. The ligands were built using the builder tool of the MOE program and subjected to energy minimization (MMFF94x, gradient: 0.05). The X-ray crystallographic structures of COX-1 (PDB: 3KK6) [50] and COX-2 (PDB: 1CX2) [51] were obtained from the Protein Data Bank. The errors of the protein were corrected by the *Structure Preparation* process in MOE. After correction, hydrogens were added and partial charges (Gasteiger methodology) were calculated. The default Triangle Matcher placement method was used for docking. GBVI/WSA dG scoring function which estimates the free energy of binding of the ligand–enzyme complex with lowest *S* score was selected.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.03.001.

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