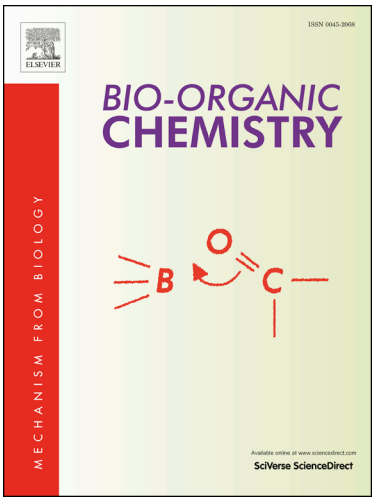


Design, synthesis, analgesic, anti-inflammatory activity of novel pyrazolones possessing aminosulfonyl pharmacophore as inhibitors of COX-2/5-LOX enzymes: Histopathological and docking studies

Mohamed A. Abdelgawad, Madlen B. Labib, Waleed A.M. Ali, Gehan Kamel, Amany A. Azouz, EL-Shaymaa EL-Nahass

PII: S0045-2068(17)30927-6
DOI: https://doi.org/10.1016/j.bioorg.2018.03.011
Reference: YBIOO 2301

To appear in: *Bioorganic Chemistry*

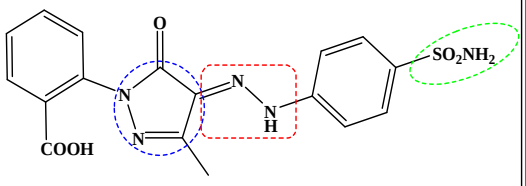
Received Date: 5 December 2017
Revised Date: 27 January 2018
Accepted Date: 7 March 2018

Please cite this article as: M.A. Abdelgawad, M.B. Labib, W.A.M. Ali, G. Kamel, A.A. Azouz, E-S. EL-Nahass, Design, synthesis, analgesic, anti-inflammatory activity of novel pyrazolones possessing aminosulfonyl pharmacophore as inhibitors of COX-2/5-LOX enzymes: Histopathological and docking studies, *Bioorganic Chemistry* (2018), doi: https://doi.org/10.1016/j.bioorg.2018.03.011

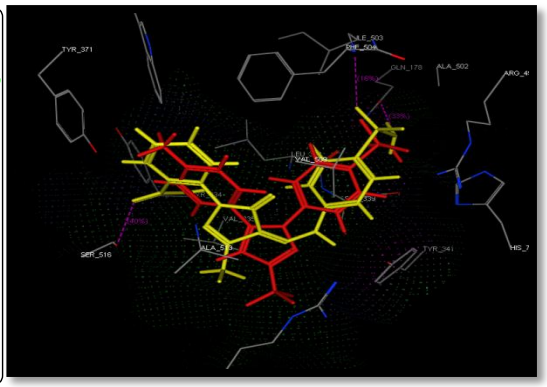
Graphical abstract

Design, synthesis, analgesic, anti-inflammatory activity of novel pyrazolones possessing aminosulfonyl pharmacophore as inhibitors of COX-2/5-LOX enzymes: Histopathological and docking studies

Mohamed A. Abdelgawad[1,2*], Madlen B. Labib[1], Waleed A.M. Ali[3], Gehan Kamel[4], Amany A. Azouz[5]and EL-Shaymaa EL-Nahass[6]



5f
COX-2 IC_{50} = 0.72 µM
5-LOX IC_{50} = 0.52 µM
COX-2 SI = 5.29
ED_{50} = 0.044 (2h), 0.064 (4h)mmol/Kg
Ulcer Index = 2.33



Design, synthesis, analgesic, anti-inflammatory activity of novel pyrazolones possessing aminosulfonyl pharmacophore as inhibitors of COX-2/5-LOX enzymes: Histopathological and docking studies

Mohamed A. Abdelgawad[1,2*], Madlen B. Labib[1], Waleed A.M. Ali[3], Gehan Kamel[4], Amany A. Azouz[5] and EL-Shaymaa EL-Nahass[6]

[1]*Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt;*

[2]*Department of Pharmaceutical Chemistry, College of Pharmacy, Aljouf University, Sakaka, Aljouf-2014, Saudi Arabia;*

[3] *Biochemistry Department, Cairo General Hospital, Egypt;*

[4] *Department of Pharmacology, Faculty of Veterinary, Cairo University, Cairo, Egypt;*

[5] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt;*

[6]*Department of Pathology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, 62511, Egypt.*

* Author to whom correspondence should be addressed;

Email mohamedabdelwahab976@yahoo.com;

Official email; mohamed.abdelgawad@pharm.bsu.edu.eg, mhmdgwd@ju.edu.sa

Tel.:0020238482600, mobile +966595435214

Address: Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Beni-Suef University, Beni-Suef 62514, Egypt. Pharmaceutical chemistry Department, College of Pharmacy, Aljouf University, Aljouf, KSA.

Abstract

A series of newly synthesized 4-aryl-hydrazonopyrazolones were designed and their structures were confirmed by spectral and elemental analyses. All synthesized compounds were evaluated for their *in vitro* COXs, 5-LOX inhibition, *in vivo* analgesic and anti-inflammatory activities. Compounds **5d**, **5f** and **5i** were found to be the most potent COX-2/5-LOX inhibitors with superior COX-2 selectivity index values (SI = 5.29 - 5.69) to reference standard celecoxib (SI = 3.52). Four compounds; **5b**, **5c**, **5d** and **5f** showed excellent anti-inflammatory activity (% edema inhibition = 72.72 - 54.54%) and perfect ED_{50} values (ED_{50} = 0.044 - 0.104 mmol/Kg) relative to celecoxib (ED_{50} = 0.032 mmol/Kg). To explore the most active compounds, ulcerogenic effect on stomach in comparison with indomethacin and celecoxib in addition to histopathological investigations were performed. Compound **5f** showed better gastric profile (UI = 2.33) than celecoxib (UI = 3.00). Also, **5f** caused 50% increase in thermal pain threshold close to reference drug indomethacin (53.13%). Docking study of all the target compounds into COX-2 and 5-LOX active sites was performed to rational their anti-inflammatory activities.

Key words: pyrazolone, analgesic, anti-inflammatory, COX-2, 5-LOX,

1. Introduction

The inflammatory mediators, prostaglandins (PGs), leukotrienes (LTs) and thromboxanes (TXs) are responsible for inflammation, other pathological and physiological processes. [1] They are generated from arachidonic acid (AA), a poly saturated fatty acid released from membrane phospholipids metabolism by the action of cyclooxygenase (COX-1, -2, -3) and lipoxygenase (5-LOX, 8-, 12-, 15-) enzymes. [2-4]

COX enzymes are responsible for the production of PGs and TXs. COX-1 is a constitutive enzyme found in the stomach, platelets and kidneys as a "house-keeper" enzyme and involved in gastric protection, platelet aggregation and normal kidney functions. COX-2 is an inducible enzyme found in macrophages, fibroblasts and leukocytes and stimulated in response to pro-inflammatory mediators. COX-3, the third cyclooxygenase is latterly discovered and present in central nervous system. [5-8]

Classical non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin exert their therapeutic action *via* suppressing PGs bio-synthesis through non-selective inhibition of COX-1 and COX-2 enzymes resulting in serious adverse effects like gastric pain, bleeding, ulcer and kidney complications. [9-10]

COX-2 selective NSAIDs (Coxibs) illustrated by celecoxib (Celebrex®), valdecoxib (Bextra®) and rofecoxib (Vioxx®) have no effect on gastric mucosal prostaglandin. But recent studies have shown the risk of some highly selective COX-2 inhibitors to increase the incidence of myocardial infarction leading to cardiac arrest due to alteration in the COX-1/COX-2 biochemical pathway. [3, 11-12]

5-LOX is a human non-heme enzyme responsible for the production of LTs involved in the inflammatory process. Zileuton, licofelone and meclofenamate sodium (Meclomen®) are examples of orally active 5-LOX inhibitors. [13-14]

The incident of NSAIDs side effects is thought to be due to inhibition of one enzyme pathway (COX) over the other (LOX) pathway leading to shift in AA metabolism [15], hence the development of new anti-inflammatory (AI) agents targeting both metabolic pathways of AA (COX-2 and 5-LOX) inhibition is a worthy rational approach to obtain effective and safe NSAIDs. [14, 16-17]

Pyrazolone ring system is a core structure in numerous drugs displaying analgesic and AI activities such as aminoantipytine, propyphenazone and famorofazone [18-20]

Several research studies reported the enhanced biological activities of heterocyclic compounds incorporating hydrazono pharmacophore as analgesic and AI agents with improved gastric profile due to its dual COX/5-LOX inhibitory activities. [18, 21-23]

Furthermore, structure activity relationship studies of selective COX-2 inhibitors demonstrated the importance of aminosulfonyl (SO_2NH_2) pharmacophore for COX-2 selectivity. [24-26]

On the basis of these findings and in continuation of our previous work [27-32] to develop effective AI agents devoid from adverse effects, we describe the design, synthesis, analgesic and AI activities of novel 4-aryl-hydrazonopyrazolone derivatives incorporating both sulfonamoyl and hydrazone pharmacophores as COX-2 and 5-LOX inhibitors. (Fig. 1) Ulcerogenic liability and histopathological screening were performed in order to identify the non-ulcerogenic AI active compounds. Docking studies were also performed to understand the possible binding modes of the synthesized compounds into both COX-2 and 5-LOX active sites in order to explain their AI activities.

2. Results and Discussion

2.1. Chemical Synthesis

The synthetic pathways adopted for starting materials **2a-e** and target compounds **3**, **4**, **5a-i** are illustrated in Scheme 1, 2.

Hydrazones **2a-d** were prepared *via* coupling the diazonium salt of different primary aromatic amines with the active methylene group of ethyl acetoacetate. [33]

3-Oxo-2-[(4-sulfamoylphenyl)-hydrazono]-butyric acid ethyl ester (**2e**) was prepared through diazotization of sulfanilamide **1e** and coupling the formed diazonium salt with ethyl acetoacetate in presence of sodium acetate. IR spectrum of compound **2e** demonstrated two absorption bands at 1690 and 1676 cm^{-1} corresponding to two C=O groups while ^1H NMR spectrum showed a D_2O exchangeable peak at δ 11.60 ppm indicating NH proton. Also, the presence of a singlet CH_3, a triplet CH_3 and a quartet CH_2 peaks at δ 2.47, 1.28 and 4.29 ppm corresponding to acetyl ($CO\mathit{CH_3}$) and ethoxy ($OC\mathit{H_2CH_3}$) protons respectively confirmed the structure**.** These spectral data demonstrated the existence of compounds **2a-f** in hydrazone form (*i*) rather than azo form (*ii*). (Scheme 1)

[Please insert Scheme 1 about here]

Cyclo-condensation ethanolic solution of **2e** with an equimolar amount of hydrazine hydrate afforded pyrazolone **3** in 74% yield. ^{1}H NMR spectrum of **3** revealed the disappearance of the signals due to ethoxy protons of the parent ester **2e**. The presence of additional D_2O exchangeable singlet signal at δ 13.22 ppm corresponding to pyrazolone NH proton, confirmed the reaction. In addition, the mass spectra of **3** displayed molecular ion peak at m/z 281(92.94%).

Heating pyrazolone **3** with acetyl chloride afforded the *N*-acetylpyrazolone **4** in a good yield of 63%. IR spectrum of **4** displayed an additional absorption band at 1749 cm^{-1} due to the carbonyl group of *N*-acetyl moiety. The absence of pyrazolone NH peak of the precursor **3** and presence of a peak of an additional signal of three protons integration due to acetyl moiety at δ 2.45 ppm in ^{1}H NMR spectrum of **4** confirmed the reaction. Also, ^{13}C NMR spectrum of compound **4** revealed the presence of two peaks at δ 24.20 and δ 171.61 ppm corresponding to $CO\underline{CH_3}$ and $\underline{CO}CH_3$, respectively.

Different substituted phenylhydrazine hydrochlorides were heated with hydrazones **2a-e** under reflux in absolute ethanol to give the target compounds **5a-i** in excellent yield (68-85%). The reaction proceeds *via* addition of the more nucleophilic NH_2 hydrazine group to the reactive acetyl carbonyl group ($COCH_3$) followed by intra-molecular cyclization through nucleophilic substitution of the good leaving ethoxy group and loss of an ethanol molecule. (Scheme 2)

The structure of diarylpyrazolones **5a**–**i** was investigated by elemental and spectral analyses. The IR spectra of compounds **5a-i** indicated the presence of two absorption bands at 3460-3313 cm^{-1} and 3279-3247 cm^{-1} corresponding to NH_2 and NH groups in addition to an absorption band at 1672-1650 cm^{-1} indicating C=O group at C-5 of pyrazolone ring. Carboxy compounds, **5b, 5c, 5d, 5f, 5g, 5h** displayed two additional absorption bands at 3440-3416 cm^{-1} and 1701-1680 cm^{-1} due to carboxylic OH and C=O groups, respectively. ^{1}H NMR spectra of **5a-i** showed the presence of a singlet signal at δ 2.29-2.34 ppm corresponding to methyl protons at C-3 of pyrazolone ring, in addition to two exchangeable singlet signals at δ 7.22-7.42 corresponding to NH_2 protons of aminosulfonyl moiety, and at δ 13.03-13.66 for NH protons. Carboxy compounds **5b, 5c, 5d, 5f, 5g** and **5h** displayed carboxyl protons at δ 13.03-15.05 ppm. Also, ^{13}C NMR

spectra of **5a-i** exhibited the methyl carbon at δ 12.14-12.30 ppm and carbonyl carbons at δ 154.71-156.99 ppm. In Addition, ^{13}C NMR spectra of carboxy compounds **5b, 5c, 5d, 5g, 5h** displayed carboxy carbons at δ 167.17-168.67 ppm which confirmed the structure.

[Please insert Scheme 2 about here]

2.2. Biological activity

2.2.1. Analgesic activity (Hot plate latency test)

Analgesic activity of compounds **3**, **4** and diarylpyrazolones **5a-i** was evaluated applying hot plate latency test [34]. Oral administration of tested compounds produced a significant delay in the latency time relative to basal values except for compounds **3**, **4**, **5e** and **5i** which displayed weak delay in latency time and didn't reach statistical significance (Table 1). Compound **5f** caused the highest percentage increase in pain threshold (50%) compared to reference drug indomethacin (PIP = 53.13%). Mice treated with compounds **5a**, **5b** and **5c** showed moderate analgesic activity on thermal pain (PIP = 28.13, 31.25, 21.88 % sequentially). Three compounds **5d**, **5g** and **5h** were less potent in order of **5h** (PIP = 18.75%) > **5d** = **5g** (PIP = 15.63%)

[Please insert Table 1 about here]

2.2.2. *In vitro* cyclooxygenase (COX-1/2) and lipoxygenase (5-LOX) inhibition assays

(IC_{50} values, μM) were measured to evaluate the potency of prepared compounds **3**, **4**, **5a-i**, indomethacin and celecoxib to inhibit COX-1/2 enzymes as the concentration causing 50% enzyme inhibition using a colorimetric EIA kit [35-36]. Also, COX-2 SI (COX-2 selectivity index) values were calculated as [IC_{50} (COX-1) / IC_{50} (COX-2)] and compared to standard drugs: a non-selective COX inhibitor; indomethacin and a selective COX-2 inhibitor; celecoxib. (Table 2)

All the tested compounds inhibited COX-1 enzyme at higher concentrations (IC_{50} = 1.32 - 4.66 μM) than indomethacin (IC_{50} = 1.14 μM). Compounds **3**, **5b**, **5d**, **5f** and **5i** showed COX-1 inhibitory activity at a higher dose range (IC_{50} = 3.76 - 4.66 μM) than celecoxib (IC_{50} = 3.14 μM), while compounds **4**, **5a**, **5c**, **5e**, **5g** and **5h** were close in potency to celecoxib with IC_{50} range of (1.32 - 2.68 μM). Accordingly, all target compounds are considered as weak COX-1 enzyme inhibitors and expected to have a safe gastric profile.

Alternatively, all tested compounds inhibited COX-2 enzyme at lower dose range of (IC_{50} = 0.66 - 2.04 μM) than indomethacin (IC_{50} = 7.24 μM). Three compounds; **5d** (IC_{50} = 0.77 μM), **5f** (IC_{50} = 0.72 μM) and **5i** (IC_{50} = 0.66 μM) were more potent than celecoxib (IC_{50} = 0.89 μM) while compounds **3**, **4**, **5b**, **5c**, **5h** showed moderate COX-2 inhibitory activity (IC_{50} = 1.00 – 1.42 μM). Three compounds (**5a**, **5e**, **5g**) were weak COX-2 inhibitors (IC_{50} = 1.59 - 2.04 μM).

Regarding COX-2 selectivity index values, all target compounds were more selective to COX-2 enzyme than COX-1 (SI = 1.32 - 5.69) except **5g** (SI = 0.82). Compounds **5d**, **5f** and **5i** (SI = 5.29- 5.69) exhibited superior SI values than celecoxib (SI = 3.52) while compounds **3**, **5b** showed selectivity indices of (3.41 and 3.47) respectively close to that of celecoxib. The rest of compounds displayed SI values (SI = 1.32 - 2.17) in order of **5c**>**5e**>**5a**>**5h**>**4**. Presence of two aminosulfonyl (SO_2NH_2) moieties; the most important pharmacophore for COX-2 selectivity in pyrazolone **5i** may explain its maximum COX-2 selectivity (SI = 5.96).

Concerning data acquired from *in vitro* lipoxygenase (5-LOX) inhibition assay demonstrated that three compounds (**5d**, **5f** and **5i**) showed higher 5-LOX inhibitory activity (IC_{50} = 0.53, 0.52 and 0.57 μM in sequent) than standard drug, Zileuton (IC_{50} = 0.77 μM). Six compounds (**3**, **4**, **5b**, **5c**, **5e**, and **5h**) displayed moderate activities (IC_{50} = 0.84 – 1.17 μM) close to that of Zileuton. Two compounds; **5a** (IC_{50} = 1.42 μM) and **5g** (IC_{50} = 1.59 μM) exhibited the least 5-LOX inhibitory activity. (Table 2)

Collectively, three derivatives (**5d**, **5f**, and **5i**) of the designed target compounds were found to have superior inhibitory activity against both COX-2 and 5-LOX with excellent COX-2 SI values and can be considered as promising safe AI agents *via* dual COX-2/5-LOX inhibition.

[Please insert Table 2 about here]

2.2.3. *In vivo* anti-inflammatory activity

2.2.3.1. Carrageenan-induced rat paw edema assay)

AI activities of all synthesized compounds were evaluated employing carrageen-induced rat foot paw edema model using celecoxib as a reference drug [37-38]. Increase in paw thickness, edema inhibition percentage (EIP) and ED_{50} values displayed by the

prepared pyrazolones and celecoxib at 2h and 4h after carrageenan injection are listed in Table 3.

Tested compounds **3**, **4**, **5a-i** showed a wide range of EIP in the range of 18.18 to 72.72% after both time intervals used. The benzoic acid derivatives **5b** and **5f** that possess carboxyl moiety at position 2 displayed the highest AI activity (EIP = 72.72%; 2h and 67.67%; 4h). This could be due to the possibility of intra-molecular hydrogen bond formation which enhances membrane permeability and absorption. (Fig. 2) Compounds **5a**, **5c**, **5d** and **5h** showed good AI activity (EIP = 58.18 - 49.09%) in order of **5c** = **5h** >**5a** = **5d** after 2h and **5d** >**5c** >**5h** >**5a** after 4h. Five derivatives **3**, **4**, **5e**, **5g**, **5i** displayed lower AI activity in sequence of **3** = **5i** >**4** = **5e** >**5g** at two different time intervals.

[Please insert Fig. 2 about here]

Regarding ED_{50} values, compound **5f** was the most potent AI agent (ED_{50} = 0.044 (2h) and 0.064 (4h) mmol/Kg) relative to celecoxib (ED_{50} = 0.032 mmol/Kg). Compounds **5b**, **5c** and **5d** exhibited promising degree of AI activity (ED_{50} = 0.079 – 0.084 mmol/Kg; 2h and 0.072 - 0.089 mmol/Kg; 4h). Compounds **3**, **4**, **5a**, **5e**, **5g**, **5h** and **5i** showed moderate AI activity with ED_{50} values in the range of (0.104 – 0.588 mmol/Kg) and compounds' potency were in order of **5h** >**5a**>**5i**>**3**>**5g**>**4**>**5e**.

[Please insert Table 3 about here]

Ulcerogenic liability

Test compounds **5b**, **5c**, **5d** and **5f** that showed potent *in vivo* AI activity (ED_{50} = 0.044 – 0.104 mmol/kg) were further evaluated for their ulcerogenic liability and compared to indomethacin; an ulcerogenic drug and celecoxib; a safe drug [39]. Ulcer index values were calculated and tabulated (Table 4). The data obtained showed that celecoxib and tested compounds caused a much smaller number of ulcers with ulcer index (UI = 2.33 – 9.67) when compared to indomethacin (UI = 21.33). Compound **5f** was less ulcerogenic (UI = 2.33) than celecoxib (UI = 3.00) while the other analog, **5b** (UI = 4.67) showed a close ulcerogenic effect to celecoxib. Compounds **5c** and **5d** caused higher gastric ulceration effect (UI = 9.67, 6.67 in sequent).

[Please insert Table 4 about here]

Although **5b**, **5c**, **5d** and **5f** hold an acidic center; carboxyl group and was expected to have a local ulcerogenic effect, they were much safer than indomethacin.

Moreover, the most preferable pyrazolone **5f** with the carboxyl group at position 2 of phenyl ring was safer than celecoxib. This may be due to the formation of intra-molecular hydrogen bond between the carboxyl group and pyrazolone N-2 forming a pseudo-ring that withdraws topical irritant effect of the carboxyl group on the epithelium (Fig. 2).

[Please insert Fig. 2 about here]

2.2.3.3. Histopathological study

Examining histopathological lesions produced after drug intake was performed to explore the ulcerogenic effect of compounds **5b**, **5c**, **5d** and **5f** on stomach in comparison to celecoxib or indomethacin (Table 5). In control negative group, more or less normal histological structure of glandular gastric mucosa, and submucosa. The non-glandular portion showed normal histological structure (Fig. 3 (i), A-I, A-II). Administrations of indomethacin led to severe degenerative changes and necrosis in both glandular and non-glandular portions of the stomach. The glandular stomach showed variable degrees of erosive and ulcerative changes accompanied by submucosal leukocytic infiltration, edema, and congestion. The muscular layer suffered from severe degenerative changes, hyalinosis, and massive leukocytic infiltration. The non-glandular stomach showed focal erosive gastritis and hyperkeratosis (Fig. 3 (i), B-I— B-IV). The celecoxib treated rats showed moderate pathological lesions in the glandular stomach in form of degenerative changes of mucosal lining, submucosal congestion and mild leukocytic infiltration. Additionally, the mucosal lining of the non-glandular stomach portion was more or less normal and mild congestion could be detected (Fig. 3 (i), C-I, C-II).

[Please insert Table 5 about here]

Treatment with compound **5b** caused mild to moderate degeneration of mucosal lining of the glandular portion of the stomach. The degenerative changes were mainly in form of hydropic degeneration which consequently led to microvesicle formation in certain areas and presence of mild congestion and edema in the submucosal blood vessels. In addition, the muscular layer showed mild hyalinosis. More or less normal histological structure of the non-glandular stomach with moderate hyperkeratosis in small focal areas was also detected (Fig. 3 (ii), 5b-I, 5b-II).

Compound **5c** intake caused severe degenerative changes and sloughing of glandular portion of stomach in certain areas accompanied with massive leukocytic

infiltration while the muscular layer showed moderate hyalinosis. Also, moderate degenerative changes of mucosa and submucosa were detected in other areas. Besides, the presence of focal areas of hyperkeratosis in the non-glandular stomach was also observed (Fig. 3 (ii), 5c-I, 5c-II).

Scanning stomach of rats treated with compound **5d**, showed presence of multifocal areas of mucous degeneration in the lining epithelium associated with some necrotic changes in another area. The submucosa was suffering from moderate congestion and leukocytic infiltration while the muscular layer suffered from mild to moderate degenerative changes and hyalinosis. Also, mild hyperkeratosisof the non-glandular stomach was detected (Fig. 3 (ii), 5d-I, 5d-II).

Stomach specimens isolated from rats treated with **5f** showed more or less normal mucosal lining of glandular stomach accompanied with minimal congestion, edema and leukocytic infiltration of the submucosa and very mild degenerative changes of the musculosa. The normal histological structure of the non-glandular stomach was also observed (Fig. 3 (ii), 5f-I, 5f-II).

[Please insert Fig. 3 about here]

In summary, the preferable pyrazolone **5f** induced minimal pathological lesions of both glandular and non-glandular stomach and can be considered as a safe AI agent while the use of **5b** caused mild lesions. Alternatively, compound **5c** caused severe degenerative and necrotic changes in the glandular stomach associated with degenerative changes and hyperkeratosis in the non-glandular stomach relative to the less severe lesions detected in glandular and non-glandular stomach by the use of **5d**. On the other hand, severe ulcerative changes, congestion, and leukocytic infiltration could be detected with indomethacin treatment.

2.3. Molecular docking study

In order to investigate the possible binding conformations of all synthesized compounds with either COX-2 (PDB code 3LN1) or 5-LOX (PDB code 3V99) receptors, molecular docking study was carried out using *MOE* (version 2008.10) modeling software [41-42]. Docking scores, amino acid residues forming Hydrogen bonding (H-bonds) interactions and their lengths were summarized in Table 6.

[Please insert Table 6 about here]

Regarding COX-2 enzyme, Celecoxib aminosulfonyl moiety (NH_2 and SO_2 groups) made two H-bonding interactions with Gln178 and Arg499 amino acids in a distance of 3.20 and 2.95 A^o in sequent, with an energy score equals to -12.96 Kcal/Mol.

All the compounds subjected to molecular docking fitted well to COX-2 active site inside the pocket and showed good binding energy scores ranged from -11.22 to -16.35 Kcal/Mol. Also, aminosulfonyl moiety of all compounds showed H-bond interactions with Gln178, Arg499, Ser516, Tyr341, Tyr371, Arg106 and Phe504 amino acids of the active site which proved the importance of SO_2NH_2 as a pharmacophoric moiety in COX-2 selectivity. The most active AI compounds **5b**, **5c**, **5d** and **5f** made one to four H-bonds with distance of (2.32 – 3.17 A^o) showing excellent docking scores (-14.18 to -16.35 Kcal/Mol).

For 5-LOX enzyme, AA made one H-bond with His367 amino acid with distance of 2.77 A^o and energy score of -9.00 Kcal/Mol. The synthesized compounds made one or two H-bond interactions with distance in range of 1.53 to 3.03 A^o exhibiting energy scores (-6.46 — -8.29 Kcal/Mol). The proposed binding modes of compound **5f** inside COX-2 and 5-LOX receptor active sites are displayed in Fig. 4 (I and II).

[Please insert Fig. 4 about here]

3. Conclusion

In this work, we represent the design and synthesis of novel 4-aryl-hydrazonopyrazolones **3**, **4**, **5a-i** as analgesic and anti-inflammatory agents. All target compounds were screened for their *in vitro* COX-1, COX-2 and 5-LOX inhibitory activity in addition to their *in vivo* analgesic and anti-inflammatory activities. Most s compounds were found to be good inhibitors of both COX-2 (IC_{50} = 0.66 -2.04 μM, reference celecoxib IC_{50} = 0.89 μM) and 5-LOX (IC_{50} = 0.52 - 1.59 μM, reference zileuton IC_{50} = 0.77 μM). *In vivo* biological testing showed that compounds **5b**, **5c**, **5d** and **5f** [ED_{50} = 0.044 – 0.084 mmol/Kg (2h) and 0.064 – 1.104 mmol/Kg (4h)] were the most potent AI agents and were further screened for their ulcerogenic liability and histopathological studies to give an evidence for the most preferable AI agent. pyrazolone **5f** (UI = 2.33, references: celecoxib UI = 3.00 and indomethacin UI = 21.33) caused minimal pathological lesions on both glandular and non-glandular stomach. Also, **5f** showed 50% delay in latency time to thermal pain induced by hot plate close to reference

drug indomethacin (53.13%). Docking studies showed the importance of aminosulfonyl moiety in celecoxib and prepared compounds in hydrogen bond formation with the COX-2 active site.

4. Experimental

4.1. Chemistry

General Information: Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded as films on KBr desks using a Nicolet 550 Series II Magna FT-IR spectrometer (Middleton, WI). ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker Avance III 400 MHz (Bruker BioSpin AG, Fa¨llanden, Switzerland) for ^{1}H and 100 MHz for ^{13}C with BBFO Smart Probe and Bruker 400 AEON Nitrogen-Free Magnet, Faculty of Pharmacy, Beni-Suef University, Egypt, in DMSO-d_6 with TMS as the internal standard, where *J* (coupling constant) values are estimated in Hertz (Hz) and chemical shifts were recorded in ppm on δ scale. Mass spectra (MS) were recorded on a Hewlett Packard 5988 spectrometer (Palo Alto, CA). Microanalyses for C, H and N were carried out on Perkin-Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT) at the Micro analytical unit of Cairo University, Egypt, and all compounds were within ± 0.4% of the theoretical values.

General Procedure for synthesis of *3-oxo-2-aryl-hydrazonobutyric acid ethyl ester (2a-e)*: To an ice cooled solution of various substituted aromatic amines **1a-e** (0.01 mol) in hydrochloric acid (2.5 mL) and distilled water (5 mL), a solution of sodium nitrite (0.9 g, 0.013 mol) in distilled water (5 mL) was added portion-wise. The resulting diazonium salt solution was then added to a cold solution of ethyl acetoacetate (1.3 g, 0.01 mol) in aqueous ethanol (50%, 10 mL) containing sodium acetate (0.9 g, 0.011 mol). The reaction mixture was stirred in ice bath for 2 h and then filtered, dried and crystallized from ethanol.

3-Oxo-2-[(4-sulfamoylphenyl)-hydrazono]-butyric acid ethyl ester (**2e**). Yellow solid; 94% yield; mp 239-241 ^{o}C; IR (KBr, υ cm^{-1}): 3344, 3253 (NH_2), 3232(NH), 3080 (C-H aromatic), 2964 (C-H aliphatic), 1690 (<u>*CO*</u>OCH_2CH_3), 1676 (<u>*CO*</u>CH_3), 1551 (C=N), 1343, 1155 (SO_2); ^{1}H NMR (DMSO-d_6, δ ppm): 1.28 (t, *J* = 7.2 Hz, 3H, CH_2<u>*CH_3*</u>), 2.47 (s, 3H, CO<u>*CH_3*</u>), 4.29 (q, *J* = 7.2 Hz, 2H, <u>*CH_2*</u>CH_3), 7.30 (s, 2H, NH_2, exchangeable with D_2O), 7.55 (d, *J* = 8.4 Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.81 (d, *J* = 8.4 Hz, 2H,

aminosulfonylphenyl H-2, H-6), 11.61 (s, 1H, NH, exchangeable with D_2O); MS (m/z, %): 313 ($M^{+\cdot}$, 46.26). Anal. Calcd. for $C_{12}H_{15}N_3O_5S$: C, 46.00; H, 4.83; N, 13.41;. Found: C, 46.35; H, 4.92; N, 13.54.

Procedure for synthesis of *4-[(3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-ylidene)-hydrazin-1-yl]-benzenesulfonamide* (3): A mixture of 3-oxo-2-[(4-sulfamoylphenyl)-hydrazono]-butyric acid ethyl ester (**2e**, 7.8 g, 0.025 mol) and hydrazine hydrate 99% (0.8 g, 0.025 mol) in absolute ethanol (30 mL) was heated under reflux for 4 h. After cooling to room temperature, the separated solid was filtered off. The obtained crude product was crystallized from aqueous ethanol to afford **3** as orange solid; 74% yield; mp 135-137 ^oC; IR (KBr, υ cm^{-1}): 3460, 3340 (NH_2), 3311, 3214(2NH), 3090 (C-H aromatic), 2927 (C-H aliphatic), 1671 (C=O), 1551 (C=N), 1337, 1150 (SO_2); ^1H NMR (DMSO-d_6, δ ppm): 2.16 (s, 3H, CH_3), 7.35 (s, 2H, NH_2, exchangeable with D_2O), 7.67 (d, *J* = 8.4 Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.84 (d, *J* = 8.4 Hz, 2H, aminosulfonylphenyl H-2, H-6), 11.65 (s, 1H, NH, exchangeable with D_2O), 13.22 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 12.07 (CH_3), 115.99 (CH, aminosulfonylphenyl C-3, C-5), 127.83 (CH, aminosulfonylphenyl C-2, C-6), 130.23 (C, aminosulfonylphenyl C-1), 140.10 (C, aminosulfonylphenyl C-4), 144.54 (C, pyrazole C-4), 147.59 (C, pyrazole C-3), 160.35 (C, C=O); MS (m/z, %): 281 ($M^{+\cdot}$, 92.94). Anal. Calcd. for $C_{10}H_{11}N_5O_3S$: C, 42.70; H, 3.94; N, 24.90. Found: C, 42.45; H, 4.12; N, 24.81.

Procedure for synthesis of *4-[(1-Acetyl-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-4-ylidene)-hydrazin-1-yl]-benzenesulfonamide* (4): A well-stirred mixture of 4-[(3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-4-ylidene)-hydrazin-1-yl]-benzenesulfonamide (**3**, 0.807 g, 0.0025 mol) and glacial acetic acid (15 mL) was heated under reflux for 6 h. After cooling, the formed solid was filtered off, dried and crystallized from n-butanol to afford **4** as yellow solid; 63% yield; mp 245-247 ^oC; IR (KBr, υ cm^{-1}): 3422, 3329 (NH_2), 3235(NH), 3118 (C-H aromatic), 2927 (C-H aliphatic), 1749, 1672 (2C=O), 1549 (C=N), 1346, 1157 (SO_2); ^1H NMR (DMSO-d_6, δ ppm): 2.25 (s, 3H, CH_3), 2.45 (s, 3H, COCH_3), 7.39 (s, 2H, NH_2, exchangeable with D_2O), 7.78 (d, *J* = 7.6 Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.87 (d, *J* = 7.6 Hz, 2H, aminosulfonylphenyl H-2, H-6), 13.03 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 12.81 (CH_3), 24.20 (COCH_3), 116.88 (CH, aminosulfonylphenyl C-3, C-5), 127.84 (CH, aminosulfonylphenyl C-2, C-

6), 128.31(C, aminosulfonylphenyl C-1), 141.06 (C, aminosulfonylphenyl C-4), 151.28 (C, pyrazole C-4), 157.71 (C, pyrazole C-3), 166.59 (C, C=O), 171.61 (C, *CO*CH_3); Anal. Calcd. for $C_{12}H_{13}N_5O_4S$: C, 44.58; H, 4.05; N, 21.66. Found: C, 44.49; H, 4.02; N, 21.62.

General procedure for preparation of 1,4-diarylpyrazolones (5a-i): A mixture of the appropriate hydrazone **2a-e** (0.035 mol), anhydrous sodium acetate (0.5 g) and the proper phenyl hydrazine hydrochloride (0.035 mol) in absolute ethanol (25 mL) was heated under reflux for 10 h. The separated solid was filtered off, dried and crystallized from aqueous ethanol to afford **5a-i** in excellent yield (68 – 85%).

4-[3-methyl-5-oxo-4-(2-phenylhydrazin-1-ylidene)-4,5-dihydro-1H-pyrazol-1-yl]benzene-1-sulfonamide **(5a)**. Orange solid; 81% yield; mp 163-165 ^oC; IR (KBr, υ cm^{-1}): 3426, 3348 (NH_2), 3258 (NH), 3070 (C-H aromatic), 2924 (C-H aliphatic), 1665 (C=O), 1592 (C=N), 1339, 1164 (SO_2); ^1H NMR (DMSO-d_6, δ ppm): 2.34 (s, 3H, CH_3), 7.27-7.29 (m, 1H, phenyl H-4), 7.36 (s, 2H, NH_2, exchangeable with D_2O), 7.46-7.48 (m, 2H, phenyl H-2, H-6), 7.65 (d, *J* = 7.2 Hz, 2H, phenyl H-3, H-5), 7.91 (d, *J* = 8.4 Hz, 2H, aminosulfonylphenyl H-3, H-5), 8.13 (d, *J* = 8.4 Hz, 2H, aminosulfonylphenyl H-2, H-6), 13.23 (s, 1H, NH, exchangeable with D_2O); MS (m/z, %): 357 ($M^{+\cdot}$, 1.87). Anal. Calcd. for $C_{16}H_{15}N_5O_3S$: C, 53.77; H, 4.23; N, 19.60. Found: C, 53.63; H, 4.19; N, 19.73

2-{2-[3-methyl-5-oxo-1-(4-sulfamoylphenyl)-4,5-dihydro-1H-pyrazol-4-ylidene]hydrazin-1-yl}benzoic acid **(5b)**. Orange red solid; 88% yield; mp 183-185 ^oC; IR (KBr, υ cm^{-1}): 3422-3313 (OH and NH_2), 3247(NH), 3066 (C-H aromatic), 2925 (C-H aliphatic), 1701, 1664 (2C=O), 1588 (C=N), 1329, 1156 (SO_2); ^1H NMR (DMSO-d_6, δ ppm): 2.33 (s, 3H, CH_3), 7.26-7.28 (m, 1H, benzoic acid H-3), 7.39 (s, 2H, NH_2, exchangeable with D_2O), 7.66-7.68 (m, 1H, benzoic acid H-5), 7.90 (d, *J* = 8.8 Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.99-8.02 (m, 2H, benzoic acid H-4, H-6), 8.13 (d, *J* = 8.8 Hz, 2H, aminosulfonylphenyl H-2, H-6), 13.66 (s, 1H, NH, exchangeable with D_2O), 15.08 (s, 1H, COOH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 12.30 (CH_3), 115.22 (CH, benzoic acid C-3), 117.38 (CH, benzoic acid C-5), 119.12 (C, benzoic acid C-1), 125.03 (CH, aminosulfonylphenyl C-3, C-5), 127.37 (CH, aminosulfonylphenyl C-2, C-6), 129.27 (C, aminosulfonylphenyl C-1), 131.91 (CH, benzoic acid C-6), 134.28 (CH, benzoic acid C-4), 139.87 (C, aminosulfonylphenyl C-4), 140.98 (C, benzoic acid C-2),

144.21 (C, pyrazole C-4), 150.19 (C, pyrazole C-3), 156.54 (C, C=O), 168.67 (C, COOH); MS (m/z, %): 401 ($M^{+\cdot}$, 10.52). Anal. Calcd. for $C_{17}H_{15}N_5O_5S$: C, 50.87; H, 3.77; N, 17.45. Found: C, 50.63; H, 3.69; N, 17.43

3-{2-[3-methyl-5-oxo-1-(4-sulfamoylphenyl)-4,5-dihydro-1H-pyrazol-4-ylidene]hydrazin-1-yl}benzoic acid **(5c)**. Orange solid; 68% yield; mp 190-192 ^{o}C; IR (KBr, υ cm^{-1}): 3416-3353 (OH and NH_2), 3263(NH), 3097 (C-H aromatic), 2981 (C-H aliphatic), 1693, 1672 (2C=O), 1554 (C=N), 1337, 1159 (SO_2); ^{1}H NMR (DMSO-d_6, δ ppm): 2.29 (s, 3H, CH_3), 7.37 (s, 2H, NH_2, exchangeable with D_2O), 7.67-7.69 (m, 3H, benzoic acid H-4, H-5, H-6), 7.92-7.97 (m, 4H, aminosulfonylphenyl H-2, H-3, H-5, H-6), 8.08 (s, 1H, benzoic acid H-2), 13.05 (s, 2H, NH and COOH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 12.14 (CH_3), 115.82 (CH, benzoic acid C-2), 116.41 (CH, benzoic acid C-6), 117.56 (CH, benzoic acid C-4), 121.46 (CH, aminosulfonylphenyl C-3, C-5), 127.41 (CH, aminosulfonylphenyl C-2, C-6), 127.78 (C, benzoic acid C-1), 129.31 (C, aminosulfonylphenyl C-1), 131.40 (CH, benzoic acid C-5), 140.21 (C, aminosulfonylphenyl C-4), 140.63 (C, benzoic acid C-4), 145.29 (C, pyrazole C-4), 150.10 (C, pyrazole C-3), 156.99 (C, C=O), 167.17 (C, COOH); MS (m/z, %): 401 ($M^{+\cdot}$, 20.84). Anal. Calcd. for $C_{17}H_{15}N_5O_5S$: C, 50.87; H, 3.77; N, 17.45. Found: C, 51.03; H, 3.79; N, 17.48

4-{2-[3-methyl-5-oxo-1-(4-sulfamoylphenyl)-4,5-dihydro-1H-pyrazol-4-ylidene]hydrazin-1-yl}benzoic acid **(5d)**. Orange red solid; 77% yield; mp 198-200 ^{o}C; IR (KBr, υ cm^{-1}): 3425-3354 (OH and NH_2), 3263(NH), 3100 (C-H aromatic), 2925 (C-H aliphatic), 1693, 1672 (2C=O), 1604 (C=N), 1337, 1160 (SO_2); ^{1}H NMR (DMSO-d_6, δ ppm): 2.33 (s, 3H, CH_3), 7.37 (s, 2H, NH_2, exchangeable with D_2O), 7.72 (d, J = 8.4 Hz, 2H, benzoic acid H-3, H-5), 7.92 (d, J = 8.4 Hz, 2H, benzoic acid H-2, H-6), 8.00 (d, J = 8 Hz, 2H, aminosulfonylphenyl H-3, H-5), 8.11 (d, J = 8 Hz, 2H, aminosulfonylphenyl H-2, H-6), 13.09 (s, 2H, NH and COOH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 12.18 (CH_3), 116.53 (CH, benzoic acid C-3, C-5), 117.59 (CH, aminosulfonylphenyl C-3, C-5), 127.42 (CH, aminosulfonylphenyl C-2, C-6), 127.80 (C, benzoic acid C-1), 129.28 (C, aminosulfonylphenyl C-1), 131.42 (CH, benzoic acid C-2, C-6), 140.13 (C, aminosulfonylphenyl C-4), 140.71 (C, benzoic acid C-4), 145.93 (C, pyrazole C-4), 150.23 (C, pyrazole C-3), 156.97 (C, C=O), 167.23 (C, COOH); MS (m/z, %): 401 ($M^{+\cdot}$,

5.16). Anal. Calcd. for $C_{17}H_{15}N_5O_5S$: C, 50.87; H, 3.77; N, 17.45. Found: C, 50.78; H, 3.84; N, 17.57

4-{2-[3-methyl-5-oxo-1-phenyl-4,5-dihydro-1H-pyrazol-4-ylidene]hydrazin-1-yl}benzene-1-sulfonamide **(5e)**. Dark yellow solid; 68% yield; mp 184-186 ^oC; IR (KBr, υ cm^{-1}): 3440, 3320 (NH_2), 3247(NH), 3070 (C-H aromatic), 2922 (C-H aliphatic), 1655 (C=O), 1601 (C=N), 1329, 1155 (SO_2); ^1H NMR (DMSO-d_6, δ ppm): 2.32 (s, 3H, CH_3), 7.24 (t, *J* = 7.6 Hz, 1H, phenyl H-4), 7.39 (s, 2H, NH_2, exchangeable with D_2O), 7.47 (t, *J* = 7.6 Hz, 2H, phenyl H-3, H-5), 7.78 (d, *J* = 8.4 Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.87 (d, *J* = 8.4 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.92 (d, *J* = 8 Hz, 2H, phenyl H-2, H-6), 13.24 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 12.14 (CH_3), 116.62 (CH, aminosulfonylphenyl C-3, C-5), 118.21 (CH, phenyl C-2, C-6), 125.47 (CH, phenyl C-4), 127.81 (CH, aminosulfonylphenyl C-2, C-6), 129.87 (CH, phenyl C-3, C-5), 129.87 (C, aminosulfonylphenyl C-1), 138.23 (C, phenyl C-1), 140.72 (C, aminosulfonylphenyl C-4), 144.48 (C, pyrazole C-4), 149.26 (C, pyrazole C-3), 154.71 (C, C=O); MS (m/z, %): 357 ($M^{+\cdot}$, 9.34). Anal. Calcd. for $C_{16}H_{15}N_5O_3S$: C, 53.77; H, 4.23; N, 19.60. Found: C, 53.64; H, 4.19; N, 19.63.

2-[3-methyl-5-oxo-4-[2-(4-sulfamoylphenyl)hydrazin-1-ylidene]-4,5-dihydro-1H-pyrazol-1-yl]benzoic acid **(5f)**. Orange solid, 80% yield; mp 185-187 ^oC; IR (KBr, υ cm^{-1}): 3460-3330 (OH and NH_2), 3279(NH), 3081 (C-H aromatic), 2923 (C-H aliphatic), 1692, 1650 (2C=O), 1601 (C=N), 1338, 1153 (SO_2); ^1H NMR (DMSO-d_6, δ ppm): 2.30 (s, 3H, CH_3), 7.32 (s, 2H, NH_2, exchangeable with D_2O), 7.49 (d, *J* = 8 Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.56 (t, *J* = 7.6 Hz, 1H, benzoic acid H-5), 7.68-7.71 (m, 3H, benzoic acid H-3, H-4, H-6), 7.81 (d, *J* = 8 Hz, 2H, aminosulfonylphenyl H-2, H-6), 13.22 (s, 2H, NH and COOH, exchangeable with D_2O); MS (m/z, %): 401 ($M^{+\cdot}$, 1.48). Anal. Calcd. for $C_{17}H_{15}N_5O_5S$: C, 50.87; H, 3.77; N, 17.45. Found: C, 50.80; H, 3.56; N, 17.19

3-[3-methyl-5-oxo-4-[2-(4-sulfamoylphenyl)hydrazin-1-ylidene]-4,5-dihydro-1H-pyrazol-1-yl]benzoic acid **(5g)**. Orange red solid; 74% yield; mp 180-182 ^oC; IR (KBr, υ cm^{-1}): 3435-3350 (OH and NH_2), 3253(NH), 3090 (C-H aromatic), 2925 (C-H aliphatic), 1680, 1671 (2C=O), 1594 (C=N), 1337, 1159 (SO_2); ^1H NMR (DMSO-d_6, δ ppm): 2.29 (s, 3H, CH_3), 6.86 (d, *J* = 8.4 Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.22 (s, 2H, NH_2, exchangeable with D_2O), 7.51-7.57 (m, 2H, benzoic acid H-3, H-4), 7.76 (d, *J* = 7.2 Hz,

1H, benzoic acid H-6), 7.90 (d, *J* = 8.4 Hz, 2H, aminosulfonylphenyl H-2, H-6), 8.21 (s, 1H, benzoic acid H-2), 13.06 (s, 2H, NH and COOH exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 12.29 (CH_3), 120.04 (CH, aminosulfonylphenyl C-3, C-5), 121.32 (CH, benzoic acid C-2), 123.93 (CH, benzoic acid C-4), 124.46 (CH, benzoic acid C-6), 126.95 (CH, aminosulfonylphenyl C-2, C-6), 129.87 (C, aminosulfonylphenyl C-1), 130.55 (CH, benzoic acid C-5), 131.91 (CH, benzoic acid C-1), 136.18 (C, benzoic acid C-3), 144.43 (C, pyrazole C-4), 150.82 (C, aminosulfonylphenyl C-4), 151.21 (C, pyrazole C-3), 156.34 (C, C=O), 168.05 (C, COOH); MS (m/z, %): 401 ($M^{+\cdot}$, 1.34). Anal. Calcd. for $C_{17}H_{15}N_5O_5S$: C, 50.87; H, 3.77; N, 17.45. Found: C, 50.92; H, 3.62; N, 17.23

4-[3-methyl-5-oxo-4-[2-(4-sulfamoylphenyl)hydrazin-1-ylidene]-4,5-dihydro-1H-pyrazol-1-yl]benzoic acid **(5h)**. Orange red solid; 71% yield; mp 190-192 ^oC; IR (KBr, υ cm^{-1}): 3430-3340 (OH and NH_2), 3253(NH), 3060 (C-H aromatic), 2923 (C-H aliphatic), 1680, 1666 (2C=O), 1592 (C=N), 1332, 1154 (SO_2); ^1H NMR (DMSO-d_6, δ ppm): 2.31 (s, 3H, CH_3), 7.42 (s, 2H, NH_2, exchangeable with D_2O), 7.77 (d, *J* = 8.4 Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.88 (d, *J* = 8.4 Hz, 2H, aminosulfonylphenyl H-2, H-6), 8.02 (d, *J* = 8.8 Hz, 2H, benzoic acid H-3, H-5), 8.06 (d, *J* = 8.8 Hz, 2H, benzoic acid H-2, H-6), 13.03 (s, 2H, NH and COOH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 12.24 (CH_3), 116.96 (CH, aminosulfonylphenyl C-3, C-5), 117.19 (CH, benzoic acid C-3, C-5), 126.92 (C, benzoic acid C-1), 127.75 (CH, aminosulfonylphenyl C-2, C-6), 129.20 (C, aminosulfonylphenyl C-1), 131.11 (CH, benzoic acid C-2, C-6), 140.88 (CH, benzoic acid C-4), 141.81 (C, aminosulfonylphenyl C-4), 144.82 (C, pyrazole C-4), 150.18 (C, pyrazole C-3), 156.54 (C, C=O), 167.24 (C, COOH); MS (m/z, %): 401 ($M^{+\cdot}$, 17.01). Anal. Calcd. for $C_{17}H_{15}N_5O_5S$: C, 50.87; H, 3.77; N, 17.45. Found: C, 50.79; H, 3.71; N, 17.52

4-[3-methyl-5-oxo-4-[2-(4-sulfamoylphenyl)hydrazin-1-ylidene]-4,5-dihydro-1H-pyrazol-1-yl]benzene-1-sulfonamide **(5i)**. Dark orange solid; 83% yield; mp 215-217 ^oC; IR (KBr, υ cm^{-1}): 3425-3340 ($2NH_2$), 3260(NH), 3098 (C-H aromatic), 2925 (C-H aliphatic), 1670 (C=O), 1590 (C=N), 1331, 1157 ($2SO_2$); ^1H NMR (DMSO-d_6, δ ppm): 2.34 (s, 3H, CH_3), 7.40 (s, 2H, NH_2, exchangeable with D_2O), 7.43 (s, 2H, NH_2, exchangeable with D_2O), 7.79 (d, *J* = 8.4 Hz, 2H, aminosulfonylphenyl H-3, H-5), 7.88 (d, *J* = 8.4 Hz, 2H,

aminosulfonylphenyl H-2, H-6), 7.93 (d, *J* = 8.8 Hz, 2H, aminosulfonylphenyl H-2', H-6'), 8.11 (d, *J* = 8.8 Hz, 2H, aminosulfonylphenyl H-3', H-5'), 13.21 (s, 1H, NH, exchangeable with D_2O); ^{13}C NMR (DMSO-d_6, δ ppm): 12.21 (CH_3), 116.79 (CH, aminosulfonylphenyl C-3', C-5'), 117.56 (CH, aminosulfonylphenyl C-3, C-5), 127.47 (CH, aminosulfonylphenyl C-2, C-6), 127.81 (CH, aminosulfonylphenyl, C-2', C-6'), 129.47 (C, aminosulfonylphenyl C-1'), 140.28 (C, aminosulfonylphenyl C-1), 140.64 (C, aminosulfonylphenyl C-4), 140.97 (C, aminosulfonylphenyl C-4'), 144.43 (C, pyrazole C-4), 150.22 (C, pyrazole C-3), 156.99 (C, C=O); MS (m/z, %): 436 ($M^{+\cdot}$, 10.67). Anal. Calcd. for $C_{16}H_{16}N_6O_5S_2$: C, 44.03; H, 3.69; N, 19.25. Found: C, 44.14; H, 3.62; N, 19.40

4.2. Biological activity

Animals: Adult male Swiss albino mice (20–25 g) and Wistar albino rats (100 - 150 g) were used all through the study to examine analgesic activity, AI activity and ulcerogenic liability. The animals (five per cage) were housed under controlled laboratory conditions (12/12 h light/dark cycle, humidity 60 ± 10 % and temperature 23 ± 2 ^oC), and were allowed standard chow and water *ad libitum*. All experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations of Faculty of Pharmacy, Beni-Suef University, Beni-Suef, Egypt.

4.2.1. Analgesic activity (hot plate latency test)

Hot plate latency (seconds) was evaluated in animals receiving normal saline or test agents (50 mg/kg) at 2 h after administration [34]. Celecoxib and indomethacin (10 mg/kg) were used as reference standards. Temperature was adjusted at 50 ± 1^oC and test compounds were administered using an oral gavage 1 h before starting the experimental protocol. A cut-off latency of 30 seconds was used to prevent heat-induced tissue damage. Time taken for each mouse to jump out or lick the paw was recorded as latency time and pain inhibition percentage (PIP) was calculated (Table 1).

4.2.2. *In vitro* cyclooxygenase (COX-1/2) and lipoxygenase (5-LOX) inhibition assays

Enzyme immune assay (EIA) kits; *i)* catalog no. 560131, Cayman Chemical, Ann Arbor, MI, USA and *ii)* catalogue no 760709, Cayman Chemical, Ann Arbor, MI, USA, were used to evaluate the ability of test compounds to inhibit ovine COX-1/2 and 5-LOX enzymes, respectively according to the manufacturer's procedure [35-36]. For COX-1/2 assay, incubation of the enzymes with different concentrations of test compounds for a

period of 5 min at 25 ^{o}C was carried out before addition of arachidonic acid and the colorimetric substrate; absorbance was measured at 590 nm using plate reader (Table 2).

Considering 5-LOX assay, stock solutions were freshly prepared before use and a buffer solution (0.1 M Tris HCl, PH, 7.4) was used. 10 µL of different compounds were dissolved in the least amount of DMSO and diluted with the stock solution to prepare (0.001, 0.1, 1, 5, 10 µM) concentrations in a final volume of 210 µL, Table 2.

4.2.3. *In vivo* anti-inflammatory activity

4.2.3.1. Carrageenan-induced rat paw edema assay: The AI activity of test compounds was determined *in vivo* applying carrageenan-induced rat paw edema model [37-38]. After oral administration of test compounds and celecoxib, induction of paw edema was performed by subcutaneous injection of a 1% saline solution of carrageenan into the right hind paw of each rat. Paw thickness of each rat was measured using plethysmometer and Percentage Inhibition in edema thickness (EIP, %) was determined after 2 h and 4 h of carrageenan injection. ED_{50} values for each tested compound were also calculated using three different doses (Table 3).

4.2.3.2. Ulcerogenic liability

The ulcerogenic effect of the most active compounds **5b**, **5c**, **5d**, **5f** and celecoxib was evaluated and compared to that of indomethacin. 21 rats were used in this study, divided into 7 groups and fasted for 18 h before drug administration. The control group received the vehicle (2.5 % Tween 80). Other groups received test compounds, celecoxib, or indomethacin at a dose of 50 mg/kg, then the animals were fed after 2 h. Rats were given the required dose orally for three successive days. After 2 h of the last dose, rats were sacrificed; the stomach of each rat was removed then opened along the greater curvature and rinsed with saline. In order to examine the stomach, it was stretched by pins on a corkboard. The gastric mucosa was carefully inspected for the occurrence of ulcers with the aid of an illuminated magnifying lens (l0x), the ulcer index was then calculated according to the previously described method [39]. Lesions were counted and measured along the greater diameter using the transparent ruler. Every five hemorrhagic spots were considered equivalent to 1 mm of an ulcer. The ulcer index (mm) was calculated from the sum of the total length of ulcers and hemorrhagic spots in each stomach (Table 4).

4.2.3.3. Histopathological study

Tissue specimens were collected from rat stomach (glandular and non-glandular portions). They were fixed in 10% buffered formalin for 72 h followed by routine histological processing and paraffin embedding according to Bancroft and Gamble (2008). Five-micron tissue sections were stained with routine Hematoxylin and Eosin stain [40]. (Table 5)

4.3. Molecular docking study

Molecular Operating Environment, Montreal, QC, Canada (MOE version 2008.10) was chosen as the computer software in this docking study. Crystal structure of celecoxib in complex with COX-2 active side (PDB code 3LN1) and AA bound to 5-LOX (PDB code 3V99) were downloaded from protein data bank at Research Collaboration for Structural Bioinformatics (RCSB) [41-42].

Docking of the co-crystallized ligands was performed to determine the binding energy score, amino acid interactions and relative mean square deviation (rmsd) in order to confirm the validity of the docking steps. 3D structures of synthesized compounds to be docked were built by *MOE* and docking steps were accomplished using London DG force, structures were protonated using the 3D protonation application and energy was minimized using the MMFF94x force field energy to produce the lowest energy conformer in order to obtain refined results. For each docked compound, one pose was selected based on superposition with the original ligand, docking scores and the number of binding interactions with amino acid residues forming H-bonds (Table 6).

Acknowledgments

The authors would like to thank all members of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Beni-Suef University for their great support and encouragement during the whole work.

Conflicts of Interest

The authors have no conflict of interest to declare.

References

1- G.F. Meresman, C.N. Olivares. Involvement of prostaglandins in the pathophysiology of endometriosis, in: K. Chaudhury, B. Chakravarty (Eds.), Endometriosis - Basic concepts and current research trends, ISBN: 978-953-51-0524-4, E-Publishing Inc.,

InTech, 2012, pp. 115-132, Available from: http://www.intechopen.com/books/endometriosis-basicconcepts-and-current-research-trends/involvement-of-prostaglandins-in-the-pathophysiology-of-endometriosis

2- S. Koontongkaew and K. Leelahavanichkul. Arachidonic acid metabolism and its implication on head and neck cancer, in: M. Agulnik (Ed.), Head and neck cancer, ISBN: 978-953-51-0236-6, E-Publishing Inc., InTech, 2012, pp. 167-184. Available from: http://www.intechopen.com/books/head-and-neck-cancer/arachidonic-acid-metabolism-and-its-implication-on-head-and-neck-cancer

3- M.A. Chowdhury, K.R.A. Abdellatif, Y. Dong, D. Das, M.R. Suresh, E.E. Knaus, Synthesis of celecoxib analogs that possess a N-hydroxypyrid-2(1H)one 5-lipoxygenase pharmacophore: Biological evaluation as dual inhibitors of cyclooxygenases and 5-lipoxygenase with anti-inflammatory activity. Bioorg. Med. Chem. Lett. 18 (2008) 6138.

4- G. Szabó, J. Fischer, Á. Kis-Varga, K. Gyires. New celecoxib derivatives as anti-inflammatory agents J. Med. Chem., 51 (2008), 142.

5- T. Zebardast, A. Zarghi, B. Daraie, M. Hedayati, O.G. Dadrass. Design and synthesis of 3-alkyl-2-aryl-1,3-thiazinan-4-one derivatives as selective cyclooxygenase (COX-2) inhibitors. Bioorg. Med. Chem. Lett. 19 (2009) 3162.

6- M. Gao, M. Wang, K. D. Miller, Q. Zheng, Synthesis and preliminary in vitro biological evaluation of new carbon-11-labeled celecoxib derivatives as candidate PET tracers for imaging of COX-2 expression in cancer. Eur. J. Med. Chem. 46 (2011) 4760.

7- M. Blankenbuehler, S. Parkin. Synthesis of 4-(1,4-diaryl-2*H*-cyclopent[*d*]pyridazin-2-yl)-benzenesulfonamides. Heterocycles 57 (2002) 2383.

8- G.S. Hassan, S.M. Abou-Seri, G. Kamel, M.M. Ali. Celecoxib analogs bearing benzofuran moiety as cyclooxygenase-2 inhibitors: Design, synthesis and evaluation as potential anti-inflammatory agents. Eur. J. Med. Chem. 76 (2014) 482.

9- U. Salgın-Goksen,N. Gokhan-Kelekci,O. Goktas,Y. Koysal,E. Klc¸S. Isk,G. Aktay, M. Ozalp. 1-Acylthiosemicarbazides, 1,2,4-triazole-5(4H)-thiones, 1,3,4-thiadiazoles and hydrazones containing 5-methyl-2-benzoxazolinones: Synthesis, analgesic-anti-inflammatory and antimicrobial activities. Bioorg. Med. Chem. 15 (2007) 5738.

10- S.M. El-Moghazy, F.F. Barsoum, H.M. Abdel-Rahman, A.A. Marzouk. Synthesis and anti-inflammatory activity of some pyrazole derivatives. Med. Chem. Res. 21 (2012) 1722.

11- M.A. Chowdhury, K.R.A. Abdellatif, Y. Dong, G. Yu, Z. Huang, M. Rahman, D. Das, C.A. Velzquez, M.R. Suresh, E.E. Knaus. Celecoxib analogs possessing a N-(4-nitrooxybutyl)piperidin-4-yl or N-(4-nitrooxybutyl)-1,2,3,6-tetrahydropyridin-4-yl nitric oxide donor moiety: Synthesis, biological evaluation and nitric oxide release studies. Bioorg. Med. Chem. Lett. 20 (2010) 1324.

12- R.J. Bing, M. Lomnicka. Why do cyclo-oxygenase-2 inhibitors cause cardiovascular events? JACC 39 (2002) 521.

13- H. A. Abd El Razik, M. H. Badr, A. H. Atta, S. M. Mouneir, and M. M. Abu-Serie; Benzodioxole–Pyrazole hybrids as anti-inflammatory and analgesic agents with COX-1,2/5-LOX inhibition and antioxidant potential, Archiv der Pharmzie 350 (2017), doi: 10.1002/ardp.201700026

14- J. Martel-Pelletier, D. Lajeunesse, P. Reboul, J. Pelletier. Therapeutic role of dual inhibitors of 5-LOX and COX, selective and non-selective non-steroidal anti-inflammatory drugs. Ann. Rheum. Dis. 62 (2003) 501.

15- K.K. Reddy, V.K.V. Rajan, A. Gupta, P. Aparoy, P. Reddanna. Exploration of binding site pattern in arachidonic acid metabolizing enzymes, Cyclooxygenases and Lipoxygenases. BMC Research Notes 8 (2015) 152. doi: 10.1186/s13104-015-1101-4

16- C. Charlier, C. Michaux. Dual inhibition of cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) as a new strategy to provide safer non-steroidal anti-inflammatory drugs. Eur. J. Med. Chem. 38 (2003) 645.

17- M.A. Chowdhury, H. Chen, K.R.A. Abdellatif, Y. Dong, K.C. Petruk, E.E. Knaus. Synthesis of new 1-(2-, 3-, or 4-methanesulfonylphenyl)-2-[5-(N-hydroxypyridin-2(1H)-one)]acetylene regioisomers: A Search for novel cyclooxygenase and lipoxygenase inhibitors. J. Het. Chem. 46 (2009) 58.

18- K.K. Sivakumar, A. Rajasekaran. Synthesis, *in-vitro* antimicrobial and antitubercular screening of some schiff base of 5-amino-4-[2-(4-phenyl-1,3-thiazol-2-yl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one. IJDDD 4 (2013) 1065.

19- F.A. Ragab, N. M. Abdel-Gawad, H. H. Georgey, M. F. Said. Pyrazolone derivatives: synthesis, anti-inflammatory, analgesic, quantitative structure–activity relationship and in vitro studies. Chem. Pharm. Bull. 61 (2013) 834.

20- P. Gupta, J. K. Gupta, A. K. Halve. Synthesis and biological significance of pyrazolones: a review. IJPSR 6 (2015) 2291.

21- P. Manojkumar, T.K. Ravi, S.Gopalakrishnan. Antioxidant and antibacterial studies of arylazopyrazoles and arylhydrazonopyrazolones containing coumarin moiety. Eur. J. Med. Chem. 44 (2009) 4690.

22- A. Almasirad, M. Tajik, D. Bakhtiari, A. Shafiee, M. Abdollahi, M. J. zamani, R. khorasani, H. Esmaily, Synthesis and analgesic activity of N-arylhydrazone derivatives of mefenamic acid. J. Pharm. Pharmaceut. Sci. 8 (2005) 419.

23- M. Asif, A. Husain. Analgesic, anti-inflammatory, and antiplatelet profile of hydrazones containing synthetic molecules. J. Appl. Chem. 2013 (2013) 1. http://dx.doi.org/10.1155/2013/247203

24- A.G. Habeeb, P.N.P. Rao, E.E. Knaus. design and synthesis of celecoxib and rofecoxib analogues as selective cyclooxygenase-2 (cox-2) inhibitors: replacement of sulfonamide and methylsulfonyl pharmacophores by an azido bioisostere. J. Med. Chem. 44 (2001) 3039.

25- K.O. Mohammed, Y. M. Nissan. Synthesis, molecular docking, and biological evaluation of some novel hydrazones and pyrazole derivatives as anti-inflammatory agents. Chem. Biol. Drug Des. 84 (2014) 473.

26- G. Mustafa, I.U. Khan, M. Ashraf, I. Afzal, S.A. Shahzad, M. Shafiq. Synthesis of new sulfonamides as lipoxygenase inhibitors. Bioorg. Med. Chem. 20 (2012) 2535.

27- K.R.A. Abdellatif, M.A. Abdelgawad, M.B. Labib, T.H. Zidan; Synthesis, cyclooxygenase inhibition, anti-inflammatory evaluation and ulcerogenic liability of novel triarylpyrazoline derivatives as selective COX-2 inhibitors; Bioorg. Med. Chem. Lett., 25 (2015) 5787.

28- K.R.A. Abdellatif, M.A. Abdelgawad, M.B. Labib, T.H. Zidan, Synthesis and biological evaluation of new diarylpyrazole and triarylimidazoline derivatives as selective COX-2 Inhibitors. Archiv der Pharmzie 350 (2017), doi: 10.1002/ardp.201600386

29- M.A. Abdelgawad, M.B. Labib, M. Abdel-Latif. Pyrazole-hydrazone derivatives as anti-inflammatory agents: Design, synthesis, biological evaluation, COX-1,2/5-LOX inhibition and docking study. Bioorg. Chem.74 (2017) 212.

30- A. Belal, M.A. Abdelgawad. New benzothiazole/benzoxazole-pyrazole hybrids with potential as COX inhibitors: design, synthesis and anticancer activity evaluation. Res. Chem. Intermediat. (2017) doi:10.1007/s11164-016-2851-x

31- K.R.A. Abdellatif, M.A. Abdelgawad, H.A.H. Elshemy, S.S.R. Alsayed, G. Kamel. Synthesis and anti-inflammatory evaluation of new 1,3,5-triaryl-4,5-dihydro-1H-pyrazole derivatives possessing an aminosulphonyl pharmacophore. Arch. Pharm. Res. 38 (2015) Doi: 10.1007/s12272-015-0606-7

32- M.H. Abdelrahman, B.G.M. Youssif,M. A. Abdelgawad,A.H. Abdelazeem,H.M. Ibrahim, A.A. Moustafa, L.T.Syed, N.A. Bukhari. Synthesis, biological evaluation, docking study and ulcerogenicity profiling of some novel quinoline-2-carboxamides as dual COXs/LOX inhibitors endowed with anti-inflammatory activity. Eur. J. Med. Chem. 127 (2017) 972.

33- H.C. Yao, P. Resnick. Azo-hydrazone conversion. I. The Japp-Klingemann reaction. J. Am. Chem. Soc. 84 (1962) 3514.

34- I.M. Fakhr, M.A. Radwan, S. el-Batran, O.M. Abd el-Salam, S.M.el-Shenawy. Synthesis and pharmacological evaluation of 2-substituted benzo[b]thiophenes as anti-inflammatory and analgesic agents. *Eur. J. Med. Chem*. 44 (2009) 1718.

35- P.N.P Rao, M. Amini, H. Li, A. Habeeb, E.E. Knaus. J. Med. Chem. 46 (2003) 4872.

36- S. Yamamoto, Mammalian lipoxygenases: Molecular structures and functions. Biochim. Biophys. acta 1128 (1992) 117

37- C.A. Winter, E.A. Risley, G.W. Nuss. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. 111 (1962) 544.

38- R.A. Turner, P. Hebban, Analgesic Screening Methods in Pharmacology, vol. 100, Academic Press, New York, 1965.

39- C.H. Cho, C.W. Ogle. Cholinergic-mediated gastric mast cell degranulation with subsequent histamine H1-and H2-receptor activation in stress ulceration in rats. Eur. j. pharmacol. 55 (1979) 23.

40- J. Bancroft, M. Gamble, M. 2008. Theory and Practice of Histological Techniques. 6th. Edition, Churchill Livingstone Elsevier, Philadelphia.

41- RCSB Protein Data Bank. Available online: http://www.rcsb.org/pdb (accessed on 1Dec 2017).

42- J.L. Wang, D. Limburg, M.J. Graneto, J. Springer, J.R. Hamper, S. Liao, J.L. Pawlitz, R.G. Kurumbail, T. Maziasz, , J.J. Talley, J.R. Kiefer, J. Carter. Thenovel benzopyran class of selective cyclooxygenase-2 inhibitors. Part 2: the second clinical candidate having a shorter and favorable human half-life. Bioorg. Med. Chem. Lett. 20 (2010) 7159.

Tables, Figures and Schemes captions

Table 1: Analgesic activity of test compounds using a hotplate latency test

Table 2: *In vitro* COX-1, COX-2 and 5- LOX enzyme inhibition, and COX-2 selectivity index (SI) data for **3**, **4**, **5a-i,** reference drugs indomethacin, celecoxib and zileuton.

Table 3: *In vivo* anti-inflammatory activities for pyrazolones **3**, **4**, **5a-i** and celecoxib against Carrageenan-induced rat paw edema.

Table 4: Ulcerogenic liability of compounds **5b**, **5c**, **5d**, **5f,** indomethacin, and celecoxib.

Table 5: Scoring of different pathological lesions for groups treated with compounds **5b**, **5c**, **5d**, **5f,** indomethacin, and celecoxib.

Table 6: The docking scores and binding interactions of **3, 4, 5a-i** inside COX-2 and 5-LOX active sites.

Fig. 1: Structure of aminoantipyrine, CBS 1108, celecoxib, and general structure of targeted pyrazolones (A) and (B).

Fig. 2: Intra-molecular hydrogen bonds in **5b**and **5f**

Fig. 3: Histological structure of glandular stomach (first row) and non-glandular stomach (second row) in: (i) negative control (A-I, A-II), indomethacin (B-I—B-IV), celecoxib (C-I, C-II) and (ii) compounds **5b** (I, II), **5c** (I, II), **5d** (I, II), **5f** (I, II). L: leukocytic infiltration; H: hyalinosis; Ed: edema; C: congestion; K: hyperkeratosis

Fig. 4: I) The proposed binding modes of the most active pyrazolone **5f** inside COX-2 receptor active site. IA: 2D interaction of **5f** with Ser516, Gln178 and Phe504 amino acids residues forming H-bonds, IB: 3D interaction of **5f** (yellow) with ligand (red). II) The proposed binding modes of the most active pyrazolone **5f** inside 5-LOX receptor active site (PDB code 3V99). IIA: 2D interaction of **5f** with His367 and Ser171 amino acids residues forming H-bonds, IIB: 3D interaction of **5f** (red) with ligand (yellow).

Scheme 1: Synthesis of starting materials **2a-e**

Scheme 2: Synthesis of target pyrazolones **3, 4, 5a-i**.

Table 1: Analgesic activity of test compounds using the hotplate latency test

Compound no	Latency time (sec) [a]	PIP	Compound no	Latency time (sec) [a]	PIP
3	3.5 ± 0.16	9.38	**5f**	4.8 ± 0.26***	50
4	3.4 ± 0.19	6.25	**5g**	3.6 ± 0.20*	15.63
5a	4.1 ± 0.22***	28.13	**5h**	3.8 ± 0.23*	18.75
5b	4.2 ± 0.29***	31.25	**5i**	3.5 ± 0.16	9.38
5c	3.9 ± 0.23***	21.88	**Celecoxib[b]**	6.2 ± 0.27***	93.75
5d	3.7 ± 0.21*	15.63	**Indomethacin[b]**	4.9 ± 0.25***	53.13
5e	3.4 ± 0.19	6.25	**control**	3.2 ± 0.16	----

[a] The results are expressed as means ± SD (n = 5)

*Significantly different from normal control group at $p < 0.05$

*** Significantly different from normal control group at $p < 0.001$

[b] values are determined using10mg/Kg dose

Dose = 50mg/Kg

Table 2: *In vitro* COX-1, COX-2, 5- LOX enzyme inhibition, and COX-2 selectivity index (SI) data for **3**, **4**, **5a-i** and reference drugs indomethacin, celecoxib and zileuton.

Compound no	COX-1 IC_{50} (μM)[a]	COX-2 IC_{50} (μM)[a]	SI[b]	5-LOX IC_{50} (μM)[a]
3	4.48	1.31	3.41	1.03
4	1.32	1.00	1.32	1.06
5a	2.63	1.93	1.36	1.42
5b	4.66	1.34	3.47	1.17
5c	2.54	1.17	2.17	0.89
5d	4.08	0.77	5.29	0.53
5e	2.68	1.59	1.68	1.09
5f	3.81	0.72	5.29	0.52
5g	1.69	2.04	0.82	1.59
5h	1.89	1.42	1.33	0.84
5i	3.76	0.66	5.69	0.57
Celecoxib	3.14	0.89	3.52	ND[c]
Indomethacin	1.14	7.24	0.15	ND[c]
Zileuton	ND[c]	ND[c]	ND[c]	0.77

[a] *In vitro* test compound concentration that produce 50% inhibition of COX-1, COX-2 and 5-LOX enzyme, the result (IC_{50}, μM) is the mean of two values obtained using an ovine COX-1/COX-2 assay Kits (Cayman Chemicals Inc., Ann Arbor, MI, USA). the deviation from the mean is <10% of the mean value.

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

[c] ND: Not determined.

Table 3. *In vivo* anti-inflammatory activities for pyrazolones **3**, **4**, **5a-i** and celecoxib against Carrageenan-induced rat paw edema.

	Increase in paw thickness (mm) [a]		EIP %		ED_{50} (mmol/Kg) [c]	
Compound no	**2h**	**4h**	**2h**	**4h**	**2h**	**4h**
3	3.5±0.18*	3.6±0.15*	36.36	34.54	0.232	0.217
4	4.1±0.19*	3.8±0.18*	25.45	30.9	0.518	0.546
5a	2.5±0.11*	2.8±0.13*	54.54	49.09	0.126	0.145
5b	1.5±0.07*	1.8±0.10*	72.72	67.27	0.079	0.072
5c	2.3±0.14*	2.5±0.13*	58.18	54.54	0.082	0.104
5d	2.5±0.13*	2.3±0.10*	54.54	58.18	0.084	0.089
5e	4.1±0.19*	3.8±0.18*	25.45	30.9	0.588	0.546
5f	1.5±0.09*	1.8±0.08*	72.72	67.27	0.044	0.064
5g	4.5±0.19*	4.5±0.19*	18.18	18.18	0.239	0.229
5h	2.3±0.12*	2.6±0.13*	58.18	52.73	0.104	0.119
5i	3.5±0.18*	3.6±0.15*	36.36	34.54	0.220	0.217
control	5.5±0.29	5.5±0.29	0	0	ND[d]	ND[d]
celecoxib [b]	1.8±0.08*	2.1±0.10*	67.27	61.81	0.032	0.032

[a] The results are expressed as means ± SEM (n = 5) Significance levels *p< 0.05 as compared with the respective celecoxib.
[b] Values are determined at 15 mg/ Kg dose
[c] Effective dose calculated at 2h and 4h
[d] Not determined

Table 4: Ulcerogenic liability of compounds **5b**, **5c**, **5d** and **5f,** indomethacin, and celecoxib.

Compound no	Ulcer no.	Ulcer index	Compound no	Ulcer no.	Ulcer index
5b	2.67[b]	4.67[b]	**Celecoxib**	3.00[b]	3.00 [b]
5c	7.33[a, b]	9.67 [a, b]	**Indomethacin**	14.00[a]	21.33 [a]
5d	5.00[a, b]	6.67 [b]	**Control**	0.67	0.67
5f	2.33[b]	2.33 [b]			

[a] Significantly different from control at $p<0.05$.

[b] Significantly different from indomethacin at $p<0.05$.

Table 5: Scoring of different pathological lesions in different groups treated with compounds **5b**, **5c**, **5d** and **5f,** indomethacin, and celecoxib.

Lesion	5b	5c	5d	5f	Celecoxib	Indomethacin	negative
1- Glandular stomach							
a) Mucosa							
Degenerative changes	++	++	++	+	++	+++	-/+
Nuclear pyknosis	-/+	++	+	-	+	+++	-
Erosion	+	++	++	-	+	+++	-
Ulcer	-	+++	+	-	-	+++	-
b) Submucosa							
Congestion	+	++	++	-/+	++	+++	-
Leuckocytic infiltration	+	++	++	-/+	+	+++	-
Edema	-/+	++	+	-/+	+	+++	-
c) Muscolosa							
Degenerative changes	+	++	+	-/+	+	+++	-
Hyalinosis	+	+	+	-	+	+++	-
Leuckocytic infiltration	-	-/+	-	-	+	+++	-
2- Non-glandular stomach							
Erosion	-	+	-	-	-	++	-
Ulcer	-	-	-	-	-	-/+	-
Hyperkeratosis	-	++	+	-	+	+++	-

-/+ minimal, +/mild, ++/moderate, +++/severe

Table 6: The docking scores and binding interactions of **3, 4, 5a-i** inside COX-2 and 5-LOX active site.

	COX-2			5-LOX		
Comp. no.	**Docking Score (Kcal/mol)**	**Main residues (Distance A°)**	**Functional group**	**Docking Score (Kcal/mol)**	**Main residues (Distance A°)**	**Functional group**
3	-11.22	Ser516(2.84) Arg499(2.92)	PyrazolN-2 SO_2	-7.08	Gln557(2.51)	Pyrazol NH
4	-11.39	Arg499(2.34) Tyr371 (2.84) Tyr341(2.75)	$COCH_3$ NH_2 Pyrazol N-2	-7.88	Gln363(2.55) His367(2.80)	NH_2 Pyrazol CO
5a	-12.94	Ser516(2.52) Tyr371(2.76) Tyr341(2.59)	SO_2 NH_2 NH=N	-6.46	Ala672(2.85)	NH_2
5b	-14.40	Ser516(2.35) Tyr371(2.65) Gln178(3.17) Arg499(3.01)	COO- COO^- NH_2 SO_2	-7.74	Asn554(3.03)	NH_2
5c	-14.04	Phe504(3.02) Gln178 (2.98) Tyr371(3.01) Tyr341(2.54)	SO_2 NH_2 COO^- Pyrazol N-2	-7.25	Gln363(2.40)	NH_2
5d	-14.18	Arg499(2.73)	SO_2	-8.22	Ala672(2.45)	NH_2
5e	-12.98	Phe504(2.89)	SO_2	-7.52	Ala672(2.57)	NH_2
5f	-15.06	Ser516(2.54) Gln178 (2.92) Phe504(2.93)	COOH NH_2 SO_2	-8.29	His367(3.13) Gln363(1.53)	NH=N NH_2
5g	-13.94	Phe504(3.01) Tyr341 (2.83)	SO_2 NH=N	-6.57	His367(2.62) Ser171(2.62)	COOH NH_2
5h	-14.77	Phe504(2.93) Tyr341(2.72)	SO_2 NH=N	-7.78	Lys409(2.77) Ser171(2.92)	SO_2 NH_2
5i	-14.67	Arg499(2.76)	SO_2	-8.08	Arg596(2.77)	SO_2

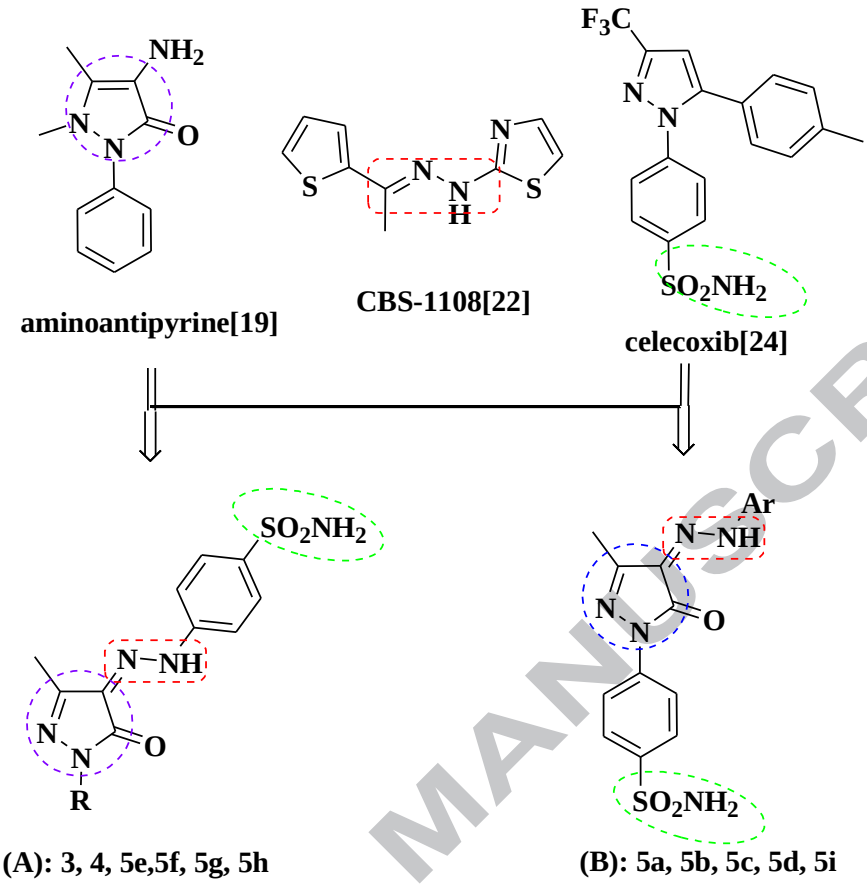
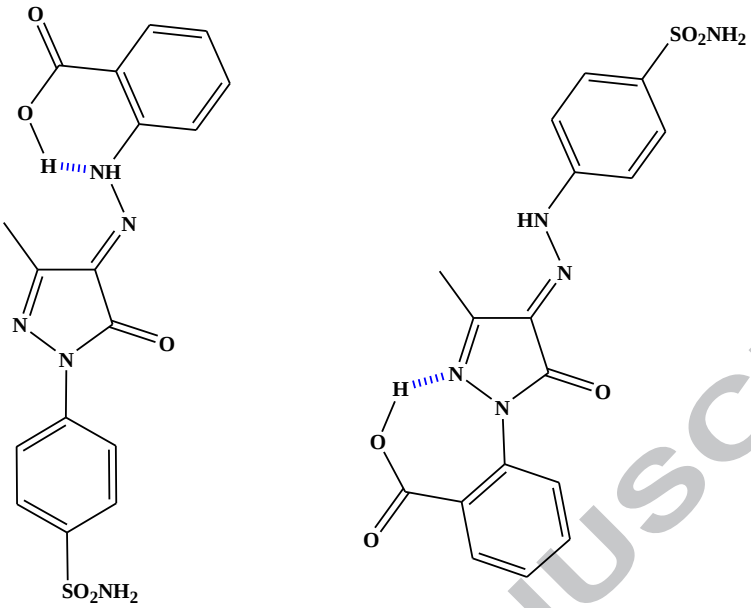


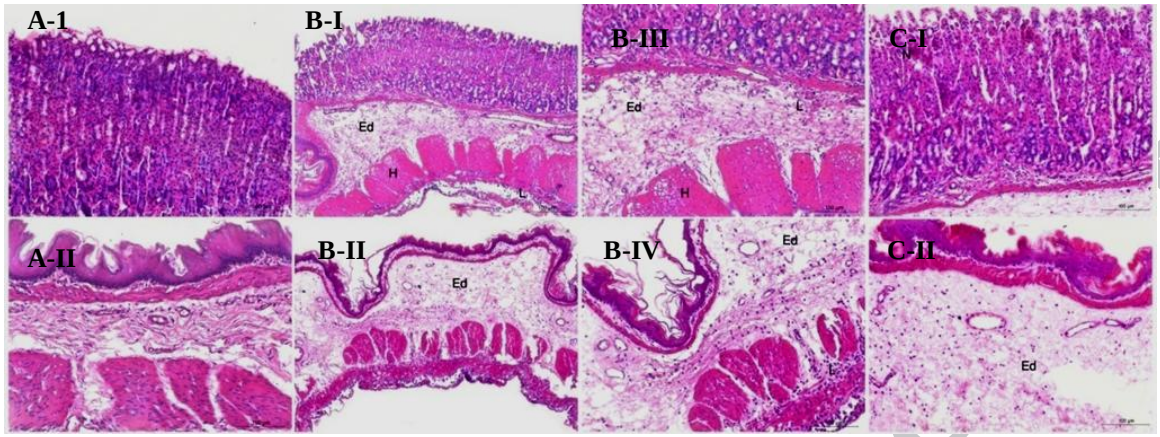
Fig. 1: Structures of aminoantipyrine, CBS 1108, celecoxib, and general structure of the targeted pyrazolones (A) and (B).



5b **5f**

Fig. 2: Intra-molecular hydrogen bond in **5b** and **5f**

(i)



(ii)

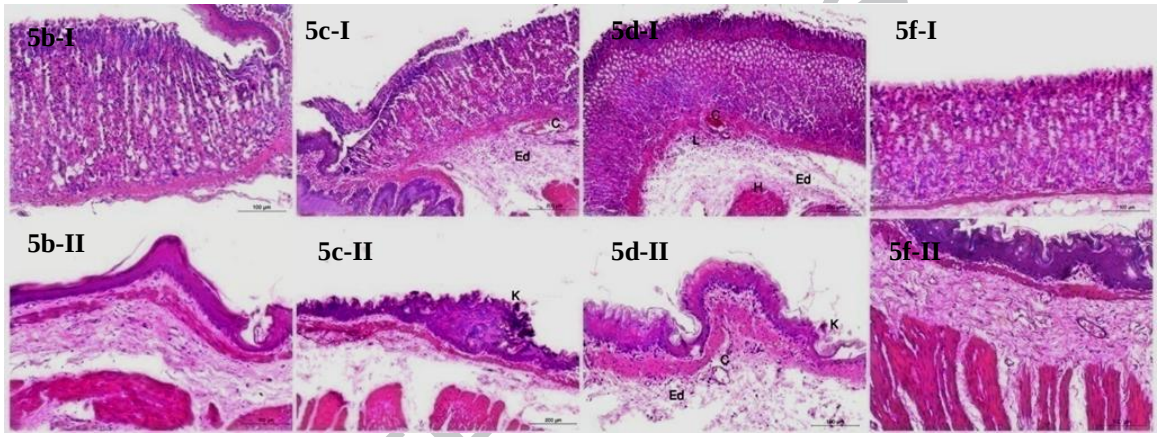


Fig. 3: Histological structure of glandular stomach (first row) and non-glandular stomach (second row) in: (i) negative control (A-I, A-II), indomethacin (B-I—B-IV), celecoxib (C-I, C-II) and (ii) compounds **5b** (I, II), **5c** (I, II), **5d** (I, II), **5f** (I, II). L: leukocytic infiltration; H: hyalinosis; Ed: edema; C: congestion; K: hyperkeratosis

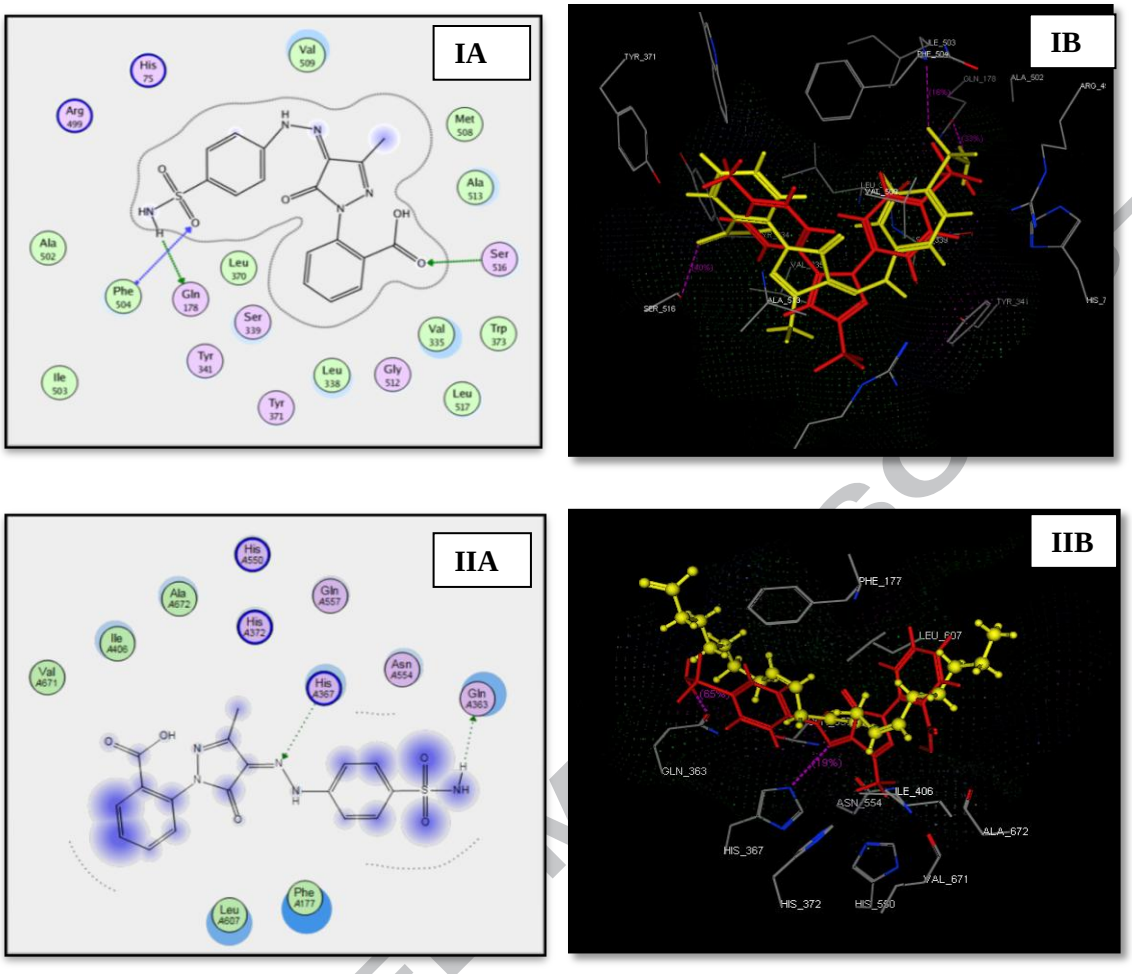
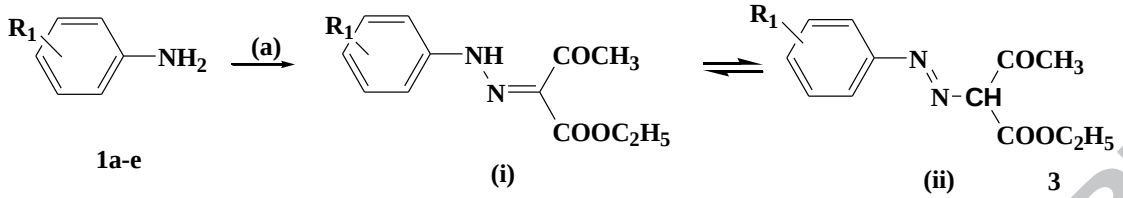


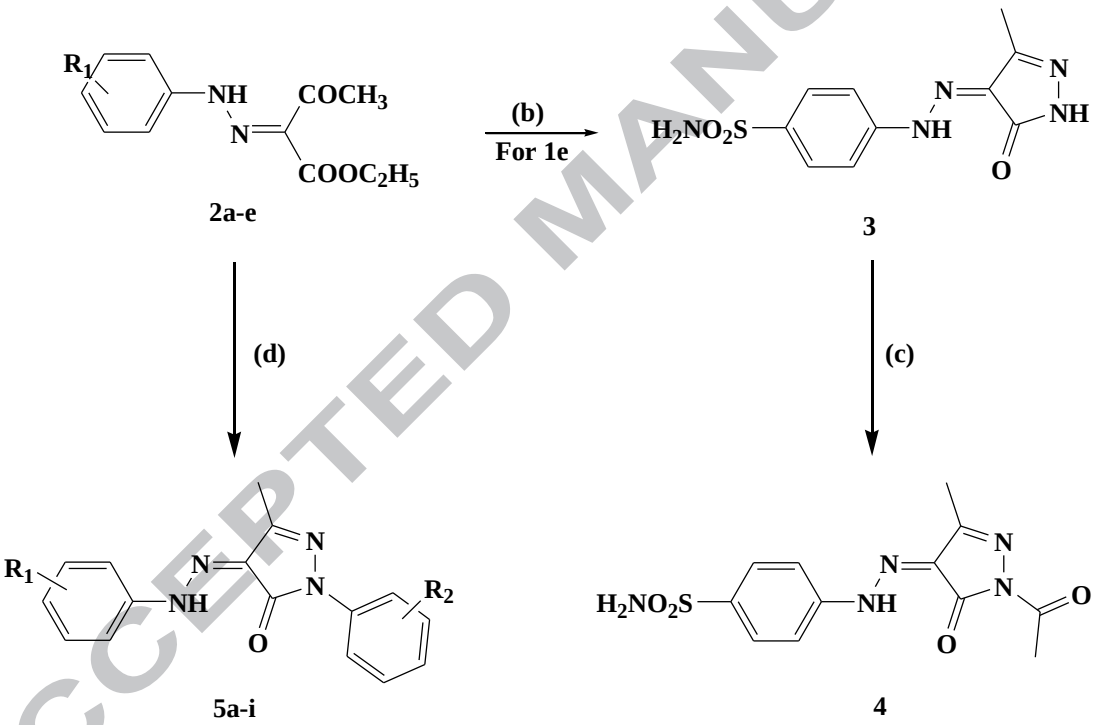
Fig. 4: I) The proposed binding modes of the most active pyrazolone **5f** inside COX-2 receptor active site (PDB code 3LN1). IA: 2D interaction of **5f** with Ser516, Gln178 and Phe504 amino acids residues forming H-bonds, IB: 3D interaction of **5f** (yellow) with ligand (red) inside COX-2, the dotted lines represent H-bonds.

II) The proposed binding modes of the most active pyrazolone **5f** inside 5-LOX receptor active site (PDB code 3V99). IIA: 2D interaction of **5f** with His367 and Ser171 amino acids residues forming H-bonds, IIB: 3D interaction of **5f** (red) with ligand (yellow) inside 5-LOX, the dotted lines represent H-bonds.



1a, 2a: R_1 = H,
1b, 2b: R_1 = 2-COOH,
1c, 2c: R_1 = 3-COOH,
1d, 2d: R_1 = 4-COOH,
1e, 2e: R_1 = 4-SO_2NH_2

Scheme 1: Reagents and conditions: a)i- $NaNO_2$, conc. HCl, 0^0C; ii- CH_3COONa, $CH_3COCH_2COOC_2H_5$



5a: R_1 = H, R_2 = 4-SO_2NH_2,
5b: R_1 = 2-COOH, R_2 = 4-SO_2NH_2,
5c: R_1 = 3-COOH, R_2 = 4-SO_2NH_2,
5d: R_1 = 4-COOH, R_2 = 4-SO_2NH_2,
5e: R_1 = 4-SO_2NH_2, R_2 = H,
5f: R_1 = 4-SO_2NH_2, R_2 = 2-COOH,
5g: R_1 = 4-SO_2NH_2, R_2 = 3-COOH,
5h: R_1 = 4-SO_2NH_2, R_2 = 4-COOH,
5i: R_1 = 4-SO_2NH_2, R_2 = 4-SO_2NH_2

Scheme 2: Reagents and conditions: b) NH_2NH_2, C_2H_5OH, reflux, 4h; c) CH_3COCl, $(Et)_3N$, reflux, 6h; d) $ArNHNH_2HCl$, CH_3COONa, ethanol, reflux, 10h.

Highlights

- Compounds **5d**, **5f** and **5i** were found to be the most potent COX-2/5-LOX inhibitors with superior COX-2 selectivity index values (SI = 5.29 - 5.69) to reference standard celecoxib (SI = 3.52).
- Four compounds; **5b**, **5d**, **5c** and **5f** showed excellent anti-inflammatory activity (% edema inhibition = 72.72 - 54.54%) and perfect ED_{50} values (ED_{50} = 0.044 - 0.104 mmol/Kg) relative to celecoxib (ED_{50} = 0.032 mmol/Kg).
- To explore, the most active compounds ulcerogenic effect on stomach in comparison with indomethacin and celecoxib, ulcerogenic liability and histopathological investigation were performed.
- Compound **5f** showed better gastric profile (UI = 2.33) than celecoxib (UI = 3.00).
- In addition, **5f** caused 50% increase in thermal pain threshold close to reference drug indomethacin (53.13%).
- Docking study of all the target compounds ducking study into COX-2 active site was also performed to rational their anti-inflammatory activities.