RESEARCH ARTICLE

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2019, 10, 1775Discovery of new non-acidic lonazoloc analogues
with COX-2 selectivity as potent anti-
inflammatory agentsMarwa F. Harras,^a Rehab Sabour^a and Omkulthom Mohamed Alkamali^b

Herein, the design and synthesis of some novel 1,3,4-trisubstituted pyrazole derivatives was carried out through the structural modification of lonazoloc. All the synthesized compounds were investigated for *in vitro* COX-1 & COX-2 inhibition and *in vivo* anti-inflammatory activity by a carrageenan rat paw edema model. Among them, the chalcones **2a** and **2b** were the most COX-2 selective derivatives (S.I. = 8.22 and 9.31, respectively) and revealed very good *in vivo* anti-inflammatory potency. Similarly, the compounds **4a**, **6b**, **7a** and **8a** exhibited good COX-2 selectivity and *in vivo* anti-inflammatory activity. The active compounds were selected to further investigate their ulcerogenic activity, and they were found to be less ulcerogenic (ulcer indices = 2.4–8.4) as compared to indomethacin (ulcer index = 17.6) and nearly as ulcerogenic as celecoxib (ulcer index = 8.1). Moreover, histological studies were performed to evaluate the safety of these compounds on the stomach, liver and kidney. Furthermore, a docking study was performed to determine possible binding of the most active compounds **2a** and **2b**, which showed high docking scores (−9.461 and −7.962 kcal mol^{−1}, respectively) that were comparable to that of celecoxib (−8.692 kcal mol^{−1}).

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1. Introduction

Inflammation is considered a physiological response generated by the body as a result of injury, microbial infection, or irritation. Conventional pharmacological management of inflammation involves treatment with non-steroidal anti-inflammatory drugs (NSAIDs).^{1,2} To date, NSAIDs have been speculated to be the most extensively used therapeutics to relieve pain and inflammation, especially in arthritis.^{3–5} The anti-inflammatory activity of NSAIDs is attributed to the inhibition of cyclooxygenase (COX)-mediated production of pro-inflammatory prostaglandins (PGs) and thromboxanes (TXs) by them.⁶ There are two different isoforms of the cyclooxygenase enzyme: a constitutive form (COX-1) and an inducible form (COX-2).⁷

The COX-1 isoform plays a significant role as the organizing enzyme, which aids in the maintenance of physiological functions such as the protection of gastric mucosa. On the other hand, the COX-2 isoform is expressed in both acute and chronic inflammation.^{8,9} Currently, most of the used NSAIDs exert their anti-inflammatory effect *via* the inhibition of both isoforms of COX. However, the absence of selectivity together with long-term clinical use leads to serious adverse

effects such as gastrointestinal bleeding, gastric ulceration and nephrotoxicity, which are mostly associated with the reported non-selective NSAIDs.^{10,11}

Consequently, the selective inhibitory action of the inducible COX-2 form over the constitutive COX-1 form is considered an effective treatment of inflammatory disorders associated with low gastrointestinal toxicities when compared with the case of non-selective NSAIDs.^{12,13} The synthesis of the selective inhibitors of COX-2 has attracted significant interest recently since these inhibitors can achieve the same anti-inflammatory effect as NSAIDs but with minimal threat of gastric toxicity mediated through the inhibition of COX-1.¹⁴

The tri-substituted pyrazole prototype is one of the preferred scaffolds due to its broad biological spectrum and affinity to various biological targets;¹⁵ lonazoloc¹⁶ is a 1,3,4-trisubstituted-pyrazole anti-inflammatory drug but is non-selective; therefore, its use is accompanied by gastrointestinal disorders resembling those caused by traditional NSAIDs.

Taking into consideration the previous findings about the significance of COX-2-selective inhibitors for reducing the gastric side effects associated with non-selective COX inhibitors, we were encouraged to design and synthesize a new series of 1,3,4-trisubstituted-pyrazoles (**2–8a,b**) *via* some modifications in the structure of lonazoloc by retaining the phenyl group at the N1 of the pyrazole ring and the aryl group at the position 3 of the pyrazole ring, which was substituted with the benzyloxy group at the 4-position instead of chlorine. This bulky hydrophobic group was inserted to increase the

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COX-2 affinity and selectivity since the proposed hits were too bulky to fit into the smaller COX-1 active site and they had an additional COX-2 pharmacophoric moiety.¹⁷

The significant modification was actually carried out at the 4-position where the $-\text{CH}_2\text{COOH}$ moiety was replaced with a more bulky moiety (differently substituted five or six-membered heterocyclic rings) (Fig. 1). This replacement was predicted to reduce the acidity of our compounds, which would consequently reduce the local effect exerted by their direct contact with gastric mucosa. The new compounds **2–8a,b** were synthesized as outlined in Schemes 1 and 2 and evaluated for their *in vitro* COX-1/COX-2 inhibitory activities, *in vivo* anti-inflammatory activities and ulcerogenic liabilities.

2. Results and discussion

2.1. Chemistry

The synthetic route of our target compounds is described in Schemes 1 and 2. The starting material 3-(4-(benzyloxy)-phenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde (**1**) was prepared by the Vilsmeier–Haack reaction, as reported previously.¹⁸ The Claisen–Schmidt condensation of **1** with acetophenone or 4-Cl acetophenone in the presence of 10% NaOH afforded

the chalcones **2a,b**.¹⁹ The treatment of **2a,b** with hydroxylamine HCl in ethanolic NaOH (ref. 20 and 21) yielded the isoxazole derivatives **3a,b**. Furthermore, the cyclocondensation of the unsaturated ketones **2a,b** with hydrazine hydrate in glacial acetic acid²² produced the *N*-acetyl pyrazoline derivatives **4a,b**. On the other hand, when **2a,b** were heated with thiosemicarbazide in ethanolic NaOH,²³ the 1-thiocarbamoyl pyrazole derivatives **5a,b** were obtained (Scheme 1).

The presence of a pyrazoline ring system in **4a,b** and **5a,b** was confirmed by the appearance of a signal due to the methane proton (H_X) in addition to two signals corresponding to the non-equivalent geminal pair²⁴ of the methylene protons (H_A , H_B) in their ¹HNMR spectra. For example, the ¹HNMR spectrum of **4b** revealed two pairs of doublets at δ 3.16 ppm (dd, 1H, H_A , $J_{\text{A,B}} = 13.5$ and $J_{\text{A,X}} = 3.9$) and 3.81 ppm (dd, 1H, H_B , $J_{\text{B,A}} = 13.5$ and $J_{\text{B,X}} = 8.7$) for the methylene protons of the pyrazoline ring. The methane proton on the same ring (H_X) appeared as a doublet of doublet at 5.62 ppm with $J_{\text{X,A}} = 3.9$ and $J_{\text{X,B}} = 8.7$.

The cyclocondensation of the chalcones **2a,b** with thiourea in catalytic amount of NaOH (ref. 25) yielded the pyrimidine-2-thione derivatives **6a,b**. The results of the elemental and spectral analyses of these compounds were in agreement with

Fig. 1 Lonazolac and structure-based design of the 1,3,4-trisubstituted pyrazole derivatives **2–8a,b**.

Scheme 1

Scheme 2

their structures. Their ^1H NMR spectra revealed a singlet at δ 4.51–4.52 ppm, corresponding to the H-5 of the pyrimidine ring. On the other hand, the reaction of **2a,b** with guanidine sulfate in ethanolic sodium hydroxide provided the 2-aminopyrimidine derivatives **7a,b**. Moreover, the cyclocondensation of the chalcones **2a,b** with malononitrile in the presence of piperidine²⁶ produced the corresponding 2-amino pyran-3-carbonitrile derivatives **8a,b** (Scheme 2). The IR spectra of these compounds revealed the presence of a $\text{C}\equiv\text{N}$ band at 2188–2204 cm^{-1} and an NH_2 band at 3361–3199 cm^{-1} , and the $\text{C}=\text{O}$ band corresponding to the parent compound was absent.

2.2. Biological evaluation

2.2.1. *In vitro* cyclooxygenase inhibition assay. The main conventional mechanism of action of anti-inflammatory drugs is mediated through the inhibition of the cyclooxygenase enzyme and thus the inhibition of prostaglandin synthesis.²⁷

To study the *in vitro* COX-1/COX-2 isozyme inhibition activities of the target compounds **2–8a,b**, we examined their ability to inhibit the human recombinant COX-1 and COX-2 forms using an enzyme immunoassay (EIA). Celecoxib was chosen as a reference drug with IC_{50} = 8.75 and 1.07 μM against COX-1 and COX-2, respectively. The results (Table 1) revealed that the pyrazole derivatives **2a,b**, **4a**, **6b**, **7a** and **8a** possessed weak COX-1 isozyme inhibitory activities (IC_{50} = 4.02–16.5 μM), whereas they had high inhibitory activities against the COX-2 isozyme (IC_{50} = 0.6–2.8 μM). Therefore, it was obviously concluded that these compounds exhibited more potent inhibitory activity against COX-2 than against COX-1. Furthermore, the selectivity indices (S.I.) of all the compounds were calculated and compared to those of the reference drug celecoxib (S.I. = 8.17). Among all the derivatives, the chalcones **2a** and **2b** displayed highest COX-2 selectivity indices (S.I. = 8.22 and 9.31, respectively).

2.2.2. *In vivo* anti-inflammatory activity. The *in vivo* anti-inflammatory effect (AI) of **2–8a,b** and celecoxib was evalu-

ated using the carrageenan-induced rat paw edema method by following the reported procedure²⁸ using a dose of 100 mg kg^{-1} body weight. The anti-inflammatory activity was calculated at 1, 3, 4, 8 and 12 h after injection using carrageenan, as displayed in Table 2 and Fig. 2A and B. It was observed that most compounds significantly decreased the inflammation when compared with carrageenan.

A comparative study of the anti-inflammatory activity of the target compounds relative to that of celecoxib at different time intervals showed that after 1 h, 3 h, 4 h, 8 h and 12 h, the compounds **2a,b**, **3b**, **4a**, **6b**, **7a** and **8a** showed high *in vivo* anti-inflammatory activities at all the time intervals (AI = 42.2–89.7%, 54.4–77.1%, 41.8–69.9%, 24.7–55.2% and 15.5–57.7%, respectively) as compared to celecoxib (AI = 56.1%, 58.5%, 48.1%, 44.9% and 34.6%, respectively). Moreover, the compounds **3a**, **4b**, **5a,b**, **6a**, **7b** and **8b** displayed low to moderate anti-inflammatory activities.

2.2.3. Ulcerogenic liability. The compounds **2a,b**, **3b**, **4a**, **6b**, **7a** and **8a** were chosen on the basis of the *in vivo* anti-inflammatory results for the ulcerogenic studies²⁹ (Table 3). Celecoxib and indomethacin were used as the reference drugs for this study. The results showed that all the tested compounds were markedly less ulcerogenic (ulcer indices = 2.4–8.4) than indomethacin (ulcer index = 17.6). Comparatively, the chalcones **2a** and **2b** exhibited lesser ulcerogenic activity (ulcer index = 2.8 and 2.4, respectively) than celecoxib (ulcer index = 3.2). Moreover, the compound **7a** displayed the same ulcer index as celecoxib. On the other hand, the compounds **3b**, **4a**, **6b** and **8a** demonstrated higher ulcerogenic potential as compared to celecoxib.

Interestingly, the chalcones **2a** and **2b** exhibited significant *in vitro* COX-2 inhibitory activity with high selectivity index together with potent *in vivo* anti-inflammatory activities and low ulcerogenic liabilities.

2.2.4. Histopathological study. Histopathological study was performed to further examine the ulcerogenic effect³⁰ of the compounds **2a,b**, **3b**, **4a**, **6b**, **7a** and **8a** on the stomach of rats as compared to that of the controls indomethacin or celecoxib.

In the control group, the gastric wall showed average mucosa with a preserved mucous layer, average parietal and chief cells, average submucosa, and average musculosa (Fig. 3A). After indomethacin intake, the gastric wall showed complete mucosal ulceration, intra-mucosal congested blood vessels, relative increase in the parietal cells, and marked submucosal edema with significantly dilated congested blood vessels (Fig. 3B). In the case of the celecoxib group, the gastric wall revealed superficial mucosal ulceration, some intra-mucosal dilated congested blood vessels, intra-mucosal inflammatory infiltrate, average parietal cells, and mild submucosal edema (Fig. 3C). In the case of the chalcone **2a**, the gastric wall showed intact mucosa, relative increase in the parietal cells, and mild submucosal edema (Fig. 3D). Moreover, in the case of the chalcone **2b**, the gastric wall showed average mucosa, average parietal cells, average submucosa, and musculosa (Fig. 3E); on the other hand, in the case of

Table 1 *In vitro* COX-1 and COX-2 inhibition activity of pyrazole derivatives **2–8a,b** and the reference drug celecoxib

Compound	COX-1	COX-2	SI (COX-1/COX-2)
2a	15.46	1.88	8.223
2b	5.59	0.6	9.317
3a	9.57	2.11	4.535
3b	2.89	1.25	2.312
4a	13.01	1.93	6.741
4b	2.72	2.79	0.975
5a	5.2	5.2	1
5b	6.07	4.69	1.315
6a	0.31	1	0.31
6b	7.2	1.1	6.545
7a	16.5	2.8	5.892
7b	0.6	3.99	0.15
8a	4.02	0.72	5.583
8b	9.68	11.64	0.831
Celecoxib	8.75	1.07	8.177

Table 2 *In vivo* anti-inflammatory activity of the pyrazole derivatives **2–8a,b** and the reference drugs celecoxib and indomethacin

Comp.	Mean value of paw edema thickness (mm) $\pm$ SEM ^a (% of inhibition)				
	1 h	3 h	4 h	8 h	12 h
2a	0.57 $\pm$ 0.15 (81.7)	1.38 $\pm$ 0.29 (58.2)	1.51 $\pm$ 0.26 (51.2)	1.65 $\pm$ 0.13 (23.8)	1.23 $\pm$ 0.20 (32.5)
2b	0.32 $\pm$ 0.13 (89.7)	0.75 $\pm$ 0.12 (77.1)	0.93 $\pm$ 0.08 (69.9)	1.11 $\pm$ 0.07 (48.6)	1.07 $\pm$ 0.06 (41.4)
3a	0.82 $\pm$ 0.01 (73.8)	1.90 $\pm$ 0.24 (42.5)	2.22 $\pm$ 0.32 (28.4)	2.02 $\pm$ 0.34 (6.9)	1.83 $\pm$ 0.34 (—)
3b	0.95 $\pm$ 0.12 (69.8)	0.96 $\pm$ 0.29 (70.9)	1.77 $\pm$ 0.27 (42.9)	1.11 $\pm$ 0.26 (48.7)	1.00 $\pm$ 0.23 (45.0)
4a	0.68 $\pm$ 0.13 (78.3)	1.17 $\pm$ 0.10 (64.6)	1.25 $\pm$ 0.07 (59.8)	0.97 $\pm$ 0.06 (55.2)	0.77 $\pm$ 0.06 (57.7)
4b	0.95 $\pm$ 0.14 (69.8)	1.66 $\pm$ 0.17 (49.7)	2.30 $\pm$ 0.19 (25.8)	2.17 $\pm$ 0.20 (—)	1.82 $\pm$ 0.33 (—)
5a	3.02 $\pm$ 0.29 (3.97)	3.07 $\pm$ 0.17 (7.29)	3.04 $\pm$ 0.25 (2.15)	2.20 $\pm$ 0.34 (—)	1.80 $\pm$ 0.37 (—)
5b	1.65 $\pm$ 0.29 (47.5)	2.50 $\pm$ 0.33 (24.6)	2.34 $\pm$ 0.32 (24.5)	2.04 $\pm$ 0.33 (5.9)	1.89 $\pm$ 0.42 (—)
6a	1.62 $\pm$ 0.28 (48.5)	2.19 $\pm$ 0.08 (33.8)	2.65 $\pm$ 0.13 (14.6)	2.19 $\pm$ 0.09 (—)	1.88 $\pm$ 0.11 (—)
6b	1.82 $\pm$ 0.25 (42.2)	1.51 $\pm$ 0.15 (54.4)	1.80 $\pm$ 0.14 (41.8)	1.63 $\pm$ 0.11 (24.7)	1.55 $\pm$ 0.09 (15.5)
7a	0.56 $\pm$ 0.20 (82)	0.85 $\pm$ 0.37 (74.4)	0.50 $\pm$ 0.31 (83.9)	1.30 $\pm$ 0.36 (40.2)	1.21 $\pm$ 0.31 (33.6)
7b	1.55 $\pm$ 0.05 (50.8)	2.30 $\pm$ 0.29 (30.4)	2.47 $\pm$ 0.18 (20.4)	2.17 $\pm$ 0.45 (—)	1.80 $\pm$ 0.39 (—)
8a	0.97 $\pm$ 0.09 (69)	1.34 $\pm$ 0.20 (59.5)	1.40 $\pm$ 0.15 (54.5)	1.24 $\pm$ 0.23 (42.9)	1.31 $\pm$ 0.32 (28.2)
8b	2.24 $\pm$ 0.37 (28.8)	2.05 $\pm$ 0.51 (38.2)	2.54 $\pm$ 0.35 (18.2)	2.31 $\pm$ 0.11 (—)	1.85 $\pm$ 0.16 (—)
Celecoxib	1.38 $\pm$ 0.06 (56.1)	1.37 $\pm$ 0.01 (58.5)	1.61 $\pm$ 0.04 (48.1)	1.19 $\pm$ 0.04 (44.9)	1.19 $\pm$ 0.02 (34.6)
Indomethacin	0.62 $\pm$ 0.25 (80.2)	0.64 $\pm$ 0.09 (79.8)	1.09 $\pm$ 0.15 (65.2)	1.18 $\pm$ 0.17 (62.5)	1.39 $\pm$ 0.08 (55.6)

Values represent mean $\pm$ SEM of six animals for each group. ^a The anti-inflammatory activity (inhibition %) was calculated according to the equation: % inhibition = $(W_c - W_t / W_c) \times 100$, where W_t is the main increase in paw thickness in the rats treated with the tested compounds and W_c is the mean increase in paw thickness in the control group.

the compounds **3b**, **4a**, **6b**, **7a**, and **8a**, superficial mucosal ulceration with an increase in the number of parietal cells and mild submucosal oedema were observed.

Many NSAIDs were withdrawn from the market due to their hepatic adverse reactions. Infrequent acute hepatitis was reported in the case of almost all NSAIDs. The mechanism of NSAID hepatotoxicity has not yet been explained and may comprise different factors (genetic factors, mitochondrial injury, inhibition of COX, *etc.*).³¹ Thus, it was important to study the effect of our new compounds on the liver of rats as compared to that of the controls indomethacin and celecoxib.

The liver of the control group exhibited average central veins with average hepatocytes arranged in single cell cords and average intervening blood sinusoids as well as average portal tracts with average portal veins, average bile ducts and average hepatocytes in the periportal area (Fig. 4A). The hepatic alterations in the indomethacin group were manifested by markedly dilated congested central veins, congested portal veins, and moderate apoptosis of hepatocytes in the peri-venular area, in addition to marked areas of hemorrhage (Fig. 4B). In the case of celecoxib, the liver showed average central veins, scattered apoptotic hepatocytes in the peri-venular and peri-portal areas, mild bile stasis, and average portal tracts (Fig. 4C). After the intake of **2a**, the liver presented average central veins, few scattered apoptotic hepatocytes in the peri-venular area, and average portal tracts (Fig. 4D). After the intake of **2b**, mildly dilated central veins were observed with average portal tracts and average hepatocytes (Fig. 4E). The rest of the tested compounds showed mild to moderate dilated central veins and few scattered apoptotic hepatocytes.

The COX-1 isozyme is expressed constitutively in the kidney. Moreover, COX-2 exists at detectable levels in normal

adult kidneys.³² Nephrotoxicity has been reported as one of the serious side effects of NSAIDs.¹⁰ Therefore, the effects of indomethacin, celecoxib and the compounds **2a,b**, **3b**, **4a**, **6b**, **7a** and **8a** on the kidney of rats were examined through the histopathological study.

In the control group, the kidneys revealed average glomeruli surrounded by average Bowman's spaces, average proximal tubules with preserved brush borders and average distal tubules (Fig. 5A). After the intake of indomethacin, the kidneys showed an average renal capsule, markedly congested glomeruli with average Bowman's spaces, scattered proximal tubules with the loss of brush borders, as well as congested blood vessels in the cortex and medulla with areas of hemorrhage (Fig. 5B). In the case of celecoxib, the kidneys exhibited an average renal capsule, scattered small-sized glomeruli with widened Bowman's spaces, scattered proximal tubules with the loss of brush borders, and areas of hemorrhage in the cortex and medulla (Fig. 5C). After the intake of **2a** and **2b**, normal kidneys with no notable changes were found (Fig. 5D and E, respectively). On the other hand, the rest of the compounds presented mild to moderately congested glomeruli and dilated congested blood vessels with areas of hemorrhage.

2.3. Molecular docking study

The molecular docking study was applied to determine the best binding pose of the compounds **2a** and **2b** using the molecular operating environment (MOE) software.³³ To examine the selectivity of **2a** and **2b** based on their docking with COX-1 and COX-2, both compounds together with standards drugs, *i.e.* celecoxib as the selective COX-2 drug and lonazolac as the non-selective drug,³⁴ were prepared for docking. The crystal structures of COX-1 (PDB code: 5WBE)

Fig. 2 *In vivo* anti-inflammatory activity of the pyrazole derivatives **2a–8a,b** and the reference drugs celecoxib and indomethacin. A: Represents the paw edema thickness. B: Represents the % inhibition of edema.

Table 3 Ulcerogenic liability for the pyrazole derivatives **2a,b**, **3b**, **4a**, **6b**, **7a** and **8a** and the reference drugs celecoxib and indomethacin

Compound	% incidence/10	Average no. of ulcers ^a	Average severity ^a	Ulcer index
2a	2	0.4 ± 0.017	0.4 ± 0.015	2.8
2b	2	0.2 ± 0.013	0.2 ± 0.09	2.4
3b	6	1.2 ± 0.09	1.2 ± 0.12	8.4
4a	4	0.8 ± 0.10	1.2 ± 0.015	6
6b	4	1.2 ± 0.04	1.2 ± 0.08	6.4
7a	2	0.6 ± 0.12	0.6 ± 0.02	3.2
8a	4	0.8 ± 0.02	0.4 ± 0.13	5.2
Celecoxib	2	0.6 ± 0.017	0.6 ± 0.05	3.2
Indomethacin	10	5 ± 0.015	2.6 ± 0.02	17.6

^a Values represent mean ± SEM of ten animals for each group.

and COX-2 (PDB code: 3LN1) were downloaded from the Protein Data Bank.

2.3.1. Docking against COX-2. The promising results of the compounds **2a** and **2b** with respect to their *in vitro* COX2-

inhibition directed us to perform molecular docking studies to understand their ligand–protein binding modes.

The results of the docking study showed that the compounds **2a** and **2b** were able to fit in the binding pocket of

Fig. 3 A: **Control**: The gastric wall showing the average mucosal thickness with the preservation of the mucous layer (black arrow), average submucosa (red arrow), and average muscosa (blue arrow); B: **indomethacin**: the gastric wall showing complete mucosal ulceration (black arrows), with average submucosa (red arrow); C: **celecoxib**: gastric wall showing superficial mucosal ulceration (black arrows), intra-mucosal dilated congested blood vessels (red arrows), and mild submucosal edema (blue arrow); D: **2a**: gastric wall showing intact mucosa (black arrow), mild submucosal edema (red arrow), and average muscosa (blue arrow); and E: **2b**: gastric wall showing intact mucosa (black arrow), and average submucosa and muscosa (red arrow) (H&E X 200).

Fig. 4 A: **Control**: The liver showing the average central veins (black arrows), average portal tracts (blue arrows), and average hepatocytes (red arrow); B: **indomethacin**: liver showing markedly dilated congested central vein (CV) and marked areas of hemorrhage (black arrows); C: **celecoxib**: liver showing average central veins (CV) and average hepatocytes (black arrow); D: **2a**: liver showing average central veins (black arrows), average portal tracts (blue arrow), and average hepatocytes (red arrow); E: **2b**: liver showing mildly dilated central veins (CV), average portal tracts (black arrow), and average hepatocytes (yellow arrow) (H&E X 200).

COX-2 with the high binding energies of -9.461 and -7.962 kcal mol $^{-1}$, respectively. Interestingly, both showed similar binding modes; they formed two hydrogen bonds between the oxygen atom of the chalcone moiety and the side chains of Arg106 and Tyr341. In addition, they formed the arene-H interaction between Ser339 and the pyrazole ring.

It was found that celecoxib was oriented inside the binding pocket of COX-2 by establishing three important H-bond interactions between the sulfonamide functionality and Gln178, Arg499 and Phe504. In addition, the arene-H interaction was observed with Ser 339.

The docking of lonazolac revealed the same hydrophobic arene-H interaction with Ser339 (Fig. 6 and 7).

2.3.2. Docking against COX-1. Next, we focused on examining the selectivity of our designed compounds **2a** and **2b** by understanding their docking with COX-1. Interestingly, both compounds were docked on the enzyme with the weak scores of -1.938 and -2.193 kcal mol $^{-1}$.

The bulky benzyloxyphenyl group attached to the C3 position of the pyrazole ring was directed outside the receptor and could not interact with the active site. This was due to the fact that the active site of COX-2 was nearly 25% larger than that of COX-1; therefore, the active site of COX-2 was liable to accommodate a bigger structure; for the same reason, the arylsulfonamide group in celecoxib was also incapable of being docked on COX-1 because it couldn't reach the

Fig. 5 A: **Control**: Kidney showing an average renal capsule (black arrow), average glomeruli (red arrows), and average tubules (blue arrow); B: **indomethacin**: kidney showing an average renal capsule (black arrow), average glomeruli (yellow arrows), mildly dilated tubules (blue arrow), and areas of hemorrhage (green arrow); C: **celecoxib**: kidney showing an average renal capsule (black arrow), scattered small-sized glomeruli (red arrow), mildly dilated tubules (blue arrow), and areas of hemorrhage (yellow arrows); D: **2a**: kidney showing an average renal capsule (black arrow), average glomeruli (yellow arrows), and average tubules (blue arrow); E: **2b**: kidney showing an average renal capsule (black arrow), average glomeruli (blue arrows), average tubules (red arrow), and average blood vessels (green arrow) (H&E X 200).

Fig. 6 Docking of celecoxib and lonazolac at the binding pocket of COX-2.

Fig. 7 Docking of 2a and 2b at the binding pocket of COX-2.

active site of COX-1 or fit in the smaller space of the COX-1 site.³⁵

Moreover, lonazolac was docked inside the active site, forming 2 hydrogen bonds with Tyr355 and Phe356 with the

binding energy of about $-4.676 \text{ kcal mol}^{-1}$. These findings could explain the selectivity of our compounds as they showed good binding affinity to COX-2, whereas they were not docked completely on the COX-1 active site (Fig. 8 and 9).

3. Conclusion

Herein, a novel series of 1,3,4-trisubstituted-pyrazoles as lonazolac analogues were synthesized and evaluated for their COX-1 and COX-2 inhibitory activity, *in vivo* anti-inflammatory activity and ulcerogenic effect. The results revealed that the compounds **2a,b**, **4a**, **6b**, **7a** and **8a** possessed a higher COX-2 selectivity (S.I. = 5.58–9.31) than the reference drug celecoxib (S.I. = 3.41). Moreover, the compounds **2a,b**, **3b**, **4a**, **6b**, **7a** and **8a** exhibited good *in vivo* anti-inflammatory activities at all the time intervals. The ulcerogenic liability screening of the most active compounds showed that they were markedly less ulcerogenic (ulcer indexes = 2.4–8.4) than indomethacin (ulcer index = 17.6). Among all the compounds, the chalcones **2a** and **2b** displayed best *in vivo* and *in vitro* results. Moreover, the histopathological examination of the rats' stomach, liver and kidney revealed that these two chalcones caused minimal degenerative changes, indicating the safety of these derivatives. Finally, a docking study was performed to confirm the previous results.

4. Experimental

All chemicals were purchased from VWR International Merck, Germany or Sigma-Aldrich and used without further purification. The melting points were determined by the open capillary tube method using the Stuart SMP3 Melting Point apparatus, and they were uncorrected. Elemental microanalysis was carried out at the Regional Center for Mycology and Biotechnology, Al-Azhar University. The infrared spectra were obtained using potassium bromide discs *via* the Bruker ATR/FTIR Spectrophotometer at the Armed Forces Laboratories. The ^1H NMR and ^{13}C NMR spectra were obtained using the Varian Gemini 300 MHz Spectrophotometer; the spectra were

obtained at 300 MHz in deuterated dimethyl sulfoxide (DMSO- d_6) at the Armed Forces Laboratories. Chemical shifts were expressed in δ units and related to those of the solvents. For the proton magnetic resonance, D_2O was used for NH and OH exchangeable protons. The mass spectra were obtained using the Shimadzu Gas Chromatograph Mass spectrometer-Qp 2010 plus (Japan) at the Regional Center for Mycology and Biotechnology, Al-Azhar University. All the reactions were followed by thin layer chromatography using silica gel F254 plates (Merck) and visualized by a UV-lamp. The compounds **1** and **2a,b** were prepared according to the reported procedures.^{19,36}

4.1. Synthesis

4.1.1. General procedure for the synthesis of the compounds 3a,b. A mixture of the chalcones **2a** or **2b** (0.01 mol) and hydroxylamine hydrochloride (0.69 g, 0.01 mol) in ethanol (30 mL) containing sodium hydroxide (1 g, 0.025 mol) was refluxed for 4 h. The solid product formed upon pouring the abovementioned mixture onto ice/water was obtained by filtration, washed with water, and then crystallized from ethanol.

5-(3-(4-(Benzyloxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-3-phenylisoxazole (3a). Yield 52%; m.p. > 300 °C; IR (KBr, cm^{-1}): 3047 (CH aromatic), 2921 (CH aliphatic), 1590 ($\text{C}=\text{C}$ & $\text{C}=\text{N}$); ^1H NMR (DMSO- d_6) δ (ppm): 5.14 (s, 2H, OCH_2), 7.00–8.46 (m, 20H, 19 aromatic H+ CH of isoxazole), 8.46 (s, 1H, CH of pyrazole); ^{13}C NMR (DMSO- d_6) δ (ppm): 68.76, 107.62, 109.52, 112.80 (2C), 121.16 (2C), 125.95, 126.04, 127.15 (2C), 127.50 (2C), 128.20, 128.38 (2C), 128.71 (2C), 128.92, 129.12 (2C), 129.43 (2C), 133.51, 134.64, 135.13, 141.51, 143.53, 156.56, 157.76, 166.98; MS, m/z : 469 (M^+ , 10.87%); anal. calcd. for $\text{C}_{31}\text{H}_{23}\text{N}_3\text{O}_2$ (469.18): C, 79.30; H, 4.94; N, 8.95, found: C, 78.92; H, 5.12; N, 9.18.

5-(3-(4-(Benzyloxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-3-(4-chlorophenyl)isoxazole (3b). Yield 65%; m.p. > 300 °C; IR (KBr, cm^{-1}): 3064 (CH aromatic), 2922 (CH aliphatic), 1598 ($\text{C}=\text{C}$ & $\text{C}=\text{N}$); ^1H NMR (DMSO- d_6) δ (ppm): 5.14 (s, 2H, OCH_2), 7.01–8.10 (m, 19H, 18 aromatic H+ CH of isoxazole),

Fig. 8 Docking of celecoxib and lonazolac at the binding pocket of COX-1.

Fig. 9 Docking of **2a** and **2b** at the binding pocket of COX-1.

8.88 (s, 1H, CH of pyrazole); ^{13}C NMR (DMSO- d_6) δ (ppm): 69.77, 107.01, 110.48, 113.11 (2C), 115.32 (2C), 123.71, 124.85, 125.38, 125.56 (2C), 127.39, 128.21 (2C), 128.50 (2C), 128.79 (2C), 129.33 (2C), 129.38 (2C), 131.06, 132.40, 136.60, 146.05, 150.61, 155.24, 156.97, 167.33; MS, m/z : 503 (M^+ , 3.18%), 505 $[(\text{M} + 2)^+]$, 0.76%]; anal. calcd. for $\text{C}_{31}\text{H}_{22}\text{ClN}_3\text{O}_2$ (503.14): C, 73.88; H, 4.40; N, 8.34, found: C, 73.89; H, 4.64; N, 8.49.

4.1.2. General procedure for the synthesis of the compounds 4a,b. A mixture of the chalcones **2a** or **2b** (0.01 mol) and hydrazine hydrate (0.5 g, 0.01 mol) in glacial acetic acid (20 mL) was refluxed for 6–8 h. After cooling, the resulting solid formed was obtained by filtration, washed with water, and crystallized from ethanol.

1-(3'-(4-(Benzyloxy)phenyl)-1',5-diphenyl-3,4-dihydro-1'H,2H-[3,4'-bipyrazol]-2-yl)ethanone (4a). Yield 70%; m.p. 200–202 °C; IR (KBr, cm^{-1}): 3059 (CH aromatic), 2919 (CH aliphatic), 1655 (C=O), 1589 (C=C & C=N); ^1H NMR (DMSO- d_6) δ (ppm): 2.29 (s, 3H, COCH_3), 3.15 (dd, 1H, H_A , $J_{A,B} = 13.4$, $J_{A,X} = 3.9$), 3.30 (dd, 1H, H_B , $J_{B,A} = 13.4$, $J_{B,X} = 9$), 5.14 (s, 2H, OCH_2), 5.62 (dd, 1H, H_X , $J_{X,A} = 3.9$, $J_{X,B} = 9$), 7.08–7.85 (m, 19H, aromatic H), 8.30 (s, 1H, CH of pyrazole); ^{13}C NMR (DMSO- d_6) δ (ppm): 22.38, 43.38, 52.81, 69.73, 115.33 (2C), 118.52, 121.01 (2C), 123.63, 126.76, 126.89, 127.04 (2C), 128.15, 128.32 (2C), 128.92 (2C), 129.14 (2C), 129.75 (2C), 129.85 (2C), 130.62, 131.74, 137.47, 139.81, 149.63, 154.36, 158.69, 168.06; MS, m/z : 512 (M^+ , 3.71%); anal. calcd. for $\text{C}_{33}\text{H}_{28}\text{N}_4\text{O}_2$ (512.22): C, 77.32; H, 5.51; N, 10.93, found: C, 77.69; H, 5.68; N, 11.21.

1-(3'-(4-(Benzyloxy)phenyl)-5-(4-chlorophenyl)-1'-phenyl-3,4-dihydro-1'H,2H-[3,4'-bipyrazol]-2-yl)ethanone (4b). Yield 67%; m.p. 145–146 °C; IR (KBr, cm^{-1}): 3060 (CH aromatic), 2927 (CH aliphatic), 1664 (C=O), 1598 (C=C & C=N); ^1H NMR (DMSO- d_6) δ (ppm): 2.30 (s, 3H, COCH_3), 3.16 (dd, 1H, H_A , $J_{A,B} = 13.5$, $J_{A,X} = 3.9$), 3.81 (dd, 1H, H_B , $J_{B,A} = 13.5$, $J_{B,X} = 8.7$), 5.13 (s, 2H, OCH_2), 5.62 (dd, 1H, H_X , $J_{X,A} = 3.9$, $J_{X,B} = 8.7$), 7.07–7.85 (m, 18H, aromatic H), 8.32 (s, 1H, CH of pyrazole); ^{13}C NMR (DMSO- d_6) δ (ppm): 22.37, 42.53, 54.01, 69.73, 115.31 (2C), 118.51, 120.34 (2C), 123.50, 126.53, 126.86, 127.29 (2C), 127.92, 128.16 (2C), 128.50 (2C), 128.92 (2C), 129.23 (2C), 129.77 (2C), 130.68, 135.15, 137.64, 139.80,

149.65, 153.39, 158.69, 167.56; MS, m/z : 546 (M^+ , 3.54%), 548 $[(\text{M} + 2)^+]$, 1.66%]; anal. calcd. for $\text{C}_{33}\text{H}_{27}\text{ClN}_4\text{O}_2$ (546.18): C, 72.45; H, 4.97; N, 10.24, found: C, 72.61; H, 4.89; N, 10.47.

4.1.3. General procedure for the synthesis of the compounds 5a,b. A mixture of the chalcones **2a** or **2b** (0.01 mol) and thiosemicarbazide (0.91 g, 0.01 mol) in absolute ethanol (30 mL) containing sodium hydroxide (1 g, 0.025 mol) was refluxed for 2 h. The solid product formed upon pouring the abovementioned mixture onto ice/water containing a few drops of hydrochloric acid was obtained by filtration, washed with water and recrystallized from ethanol.

3'-(4-(Benzyloxy)phenyl)-1',5-diphenyl-3,4-dihydro-1'H,2H-[3,4'-bipyrazol]-2-carbothioamide (5a). Yield 72%; m.p. 254–255 °C; IR (KBr, cm^{-1}): 3273 (NH_2), 3063 (CH aromatic), 2927 (CH aliphatic), 1593 (C=C & C=N); ^1H NMR (DMSO- d_6) δ (ppm): 3.19 (dd, 1H, H_A , $J_{A,B} = 13.4$, $J_{A,X} = 3$), 3.88 (dd, 1H, H_B , $J_{B,A} = 13.4$, $J_{B,X} = 8.4$), 5.14 (s, 2H, OCH_2), 6.01 (dd, 1H, H_X , $J_{X,A} = 3$, $J_{X,B} = 8.4$), 7.11–7.86 (m, 19H, aromatic H), 8.53 (s, 1H, CH of pyrazole), 9.85 (2H, NH_2 ; exchangeable with D_2O); ^{13}C NMR (DMSO- d_6) δ (ppm): 42.67, 56.14, 69.74, 115.31 (2C), 118.41, 120.32 (2C), 124.37, 126.13, 126.44, 127.62 (2C), 128.18, 128.33 (2C), 128.50 (2C), 128.92 (2C), 129.61 (2C), 129.89 (2C), 130.88, 131.54, 137.48, 139.78, 149.43, 155.23, 158.66, 176.41; MS, m/z : 529 (M^+ , 11.12%); anal. calcd. for $\text{C}_{32}\text{H}_{27}\text{N}_5\text{OS}$ (529.19): C, 72.56; H, 5.14; N, 13.22, found: C, 72.34; H, 5.03; N, 13.48.

3'-(4-(Benzyloxy)phenyl)-5-(4-chlorophenyl)-1'-phenyl-3,4-dihydro-1'H,2H-[3,4'-bipyrazol]-2-carbothioamide (5b). Yield 68%; m.p. > 300 °C; IR (KBr, cm^{-1}): 3285 (NH_2), 3060 (CH aromatic), 2923 (CH aliphatic), 1601 (C=C & C=N); ^1H NMR (DMSO- d_6) δ (ppm): 3.19 (dd, 1H, H_A , $J_{A,B} = 13.5$, $J_{A,X} = 3$), 3.88 (dd, 1H, H_B , $J_{B,A} = 13.5$, $J_{B,X} = 8.7$), 5.13 (s, 2H, OCH_2), 6.01 (dd, 1H, H_X , $J_{X,A} = 3$, $J_{X,B} = 8.7$), 7.07–8.10 (m, 18H, aromatic H), 8.51 (s, 1H, CH of pyrazole); ^{13}C NMR (DMSO- d_6) δ (ppm): 42.52, 64.90, 69.74, 115.29 (2C), 118.43, 120.52 (2C), 124.26, 126.11, 126.47, 127.92 (2C), 128.19, 128.41 (2C), 128.53 (2C), 128.93 (2C), 129.64 (2C), 129.90 (2C), 131.55, 137.47, 139.79, 143.84, 149.47, 154.16, 158.67, 176.51; MS, m/z : 563 (M^+ , 9.34%), 565 $[(\text{M} + 2)^+]$, 3.76%]; anal. calcd. for $\text{C}_{32}\text{H}_{26}\text{ClN}_5\text{OS}$ (563.15): C, 68.13; H, 4.65; N, 12.42, found: C, 68.41; H, 4.51; N, 12.67.

4.1.4. General procedure for the synthesis of the compounds 6a,b. A mixture of the chalcones 2a or 2b (0.01 mol) and thiourea (0.76 g, 0.01 mol) in ethanol (30 mL) containing (1 g, 0.025 mol) NaOH was refluxed for 6–8 h. The solid product formed upon pouring the abovementioned mixture onto ice/water containing few drops of HCl was filtered off, washed with water and crystallized from ethanol.

6-(3-(4-(Benzyloxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-4-phenylpyrimidine-2(1H)-thione (6a). Yield 56%; m.p. 130–132 °C; IR (KBr, cm^{-1}): 3285 (NH), 3036 (CH aromatic), 2922 (CH aliphatic), 1600 (C=N), 1580 (C=C); ^1H NMR (DMSO- d_6) δ (ppm): 4.51 (s, 1H, CH of pyrimidine), 5.17 (s, 2H, OCH_2), 7.04–8.07 (m, 19H, aromatic H), 8.88 (s, 1H, CH of pyrazole), 9.32 (1H, NH; exchangeable with D_2O); ^{13}C NMR (DMSO- d_6) δ (ppm): 69.90, 115.29, 118.91 (2C), 119.20, 121.25 (2C), 124.27, 126.79, 127.15 (2C), 127.83, 128.30 (2C), 128.53 (2C), 128.91 (2C), 129.32 (2C), 130.01 (2C), 131.57, 135.26, 137.83, 138.20, 139.75, 149.62, 152.76, 156.08, 169.67, 181.63; MS, m/z : 512 (M^+ , 2.75%); anal. calcd. for $\text{C}_{32}\text{H}_{24}\text{N}_4\text{OS}$ (512.17): C, 74.98; H, 4.72; N, 10.93, found: C, 75.22; H, 4.89; N, 11.24.

6-(3-(4-(Benzyloxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-4-(4-chlorophenyl)pyrimidine-2(1H)-thione (6b). Yield 65%; m.p. 112–114 °C; IR (KBr, cm^{-1}): 3293 (NH), 3063 (CH aromatic), 2923 (CH aliphatic), 1597 (C=C & C=N); ^1H NMR (DMSO- d_6) δ (ppm): 4.52 (s, 1H, CH of pyrimidine), 5.16 (s, 2H, OCH_2), 7.06–8.46 (m, 18H, aromatic H), 8.46 (s, 1H, CH of pyrazole), 9.34 (1H, NH; exchangeable with D_2O); ^{13}C NMR (DMSO- d_6) δ (ppm): 69.95, 115.32, 118.39 (2C), 119.57, 124.36 (2C), 125.97, 127.91, 127.55 (2C), 127.93, 128.06 (2C), 128.56 (2C), 128.92 (2C), 129.19 (2C), 129.99 (2C), 131.67, 134.64, 136.01, 138.60, 140.86, 149.43, 152.07, 155.90, 174.05, 181.37; MS, m/z : 546 (M^+ , 6.10%), 548 [$(\text{M} + 2)^+$, 2.74%]; anal. calcd. for $\text{C}_{32}\text{H}_{23}\text{ClN}_4\text{OS}$ (546.13): C, 70.25; H, 4.24; N, 10.24, found: C, 70.41; H, 4.30; N, 10.43.

4.1.5. General procedure for the synthesis of the compounds 7a,b. An aqueous solution of 5 mL of 40% sodium hydroxide was added gradually during a period of 3 h to a mixture of chalcone 2a or 2b (0.01 mol) and guanidine sulfate (1.57 g, 0.01 mol) in ethanol (25 mL). The reaction mixture was refluxed for 7 h, and the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was poured onto ice-cold water, and the solid product formed was obtained by filtration, washed with water and then recrystallized from ethanol.

4-(3-(4-(Benzyloxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-6-phenylpyrimidin-2-amine (7a). Yield 66%; m.p. > 300 °C; IR (KBr, cm^{-1}): 3340, 3120 (NH_2), 3061 (CH aromatic), 2918 (CH aliphatic), 1597 (C=C & C=N); ^1H NMR (DMSO- d_6) δ (ppm): 4.96 (2H, NH_2 ; exchangeable with D_2O), 5.14 (s, 2H, OCH_2), 7.08–7.86 (m, 20H, aromatic H), 8.50 (s, 1H, CH of pyrazole); ^{13}C NMR (DMSO- d_6) δ (ppm): 69.70, 101.23, 114.15, 115.32 (2C), 118.80 (2C), 121.96, 126.72, 127.12 (2C), 127.53 (2C), 128.20, 128.34 (2C), 128.70, 128.91 (2C), 129.88 (2C), 129.99 (2C), 130.74, 134.12, 137.49, 148.98, 151.86, 159.20, 161.96, 163.32, 165.41; MS, m/z : 495 (M^+ , 7.86%); anal. calcd. for

$\text{C}_{32}\text{H}_{25}\text{N}_5\text{O}$ (495.21): C, 77.56; H, 5.08; N, 14.13, found: C, 77.48; H, 5.31; N, 14.29.

4-(3-(4-(Benzyloxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-6-(4-chlorophenyl)pyrimidin-2-amine (7b). Yield 60%; m.p. 165–167 °C; IR (KBr, cm^{-1}): 3306, 3193 (NH_2), 3064 (CH aromatic), 2921 (CH aliphatic), 1582 (C=C & C=N); ^1H NMR (DMSO- d_6) δ (ppm): 5.15 (s, 2H, OCH_2), 5.41 (2H, NH_2 ; exchangeable with D_2O), 7.06–7.99 (m, 19H, aromatic H), 8.45 (s, 1H, CH of pyrazole); ^{13}C NMR (DMSO- d_6) δ (ppm): 69.69, 104.57, 114.93, 115.31 (2C), 118.16 (2C), 118.94, 125.87, 127.18 (2C), 127.63 (2C), 128.03, 128.14 (2C), 128.31, 128.89 (2C), 129.08 (2C), 129.24 (2C), 129.98, 130.59, 135.60, 136.55, 137.49, 158.92, 161.62, 163.06, 164.24; MS, m/z : 529 (M^+ , 1.86%), 531 [$(\text{M} + 2)^+$, 1.02%]; anal. calcd. for $\text{C}_{32}\text{H}_{24}\text{ClN}_5\text{O}$ (529.17): C, 72.51; H, 4.56; N, 13.21, found: C, 72.84; H, 4.69; N, 13.48.

4.1.6. General procedure for the synthesis of the compounds 8a,b. A mixture of chalcones 2a or 2b (0.01 mol) and malononitrile (0.66 g, 0.01 mol) in ethanol containing few drops of piperidine was refluxed for 4 h. The reaction mixture was reduced to half of its volume and cooled. The precipitated crude product was filtered, washed with cold water, and crystallized from ethanol.

2-Amino-4-(3-(4-(benzyloxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-6-phenyl-4H-pyran-3-carbonitrile (8a). Yield 53%; m.p. 170–172 °C; IR (KBr, cm^{-1}): 3336, 3199 (NH_2), 3058 (CH aromatic), 2934 (CH aliphatic), 2188 (C≡N), 1584 (C=C & C=N); ^1H NMR (DMSO- d_6) δ (ppm): 5.07 (d, 1H, CH of pyran), 5.14 (s, 2H, OCH_2), 5.82 (1H, NH; exchangeable with D_2O), 7.01–7.95 (m, 20H, 19 aromatic H+ CH of pyran), 8.83 (s, 1H, CH of pyrazole); ^{13}C NMR (DMSO- d_6) δ (ppm): 22.66, 45.41, 69.72, 79.63, 115.27 (2C), 118.47, 118.67, 119.00 (2C), 124.01, 125.54 (2C), 126.44, 127.31, 128.30 (2C), 128.40, 128.85, 128.88 (2C), 129.01 (2C), 129.43 (2C), 129.56 (2C), 129.75, 130.09, 130.13, 137.35, 139.65, 150.21, 158.56; MS, m/z : 522 (M^+ , 5.92%); anal. calcd. for $\text{C}_{34}\text{H}_{26}\text{N}_4\text{O}_2$ (522.21): C, 78.14; H, 5.01; N, 10.72, found: C, 78.01; H, 5.23; N, 10.89.

2-Amino-4-(3-(4-(benzyloxy)phenyl)-1-phenyl-1H-pyrazol-4-yl)-6-(4-chlorophenyl)-4H-pyran-3-carbonitrile (8b). Yield 60%; m.p. 129–131 °C; IR (KBr, cm^{-1}): 3361, 3226 (NH_2), 3067 (CH aromatic), 2937 (CH aliphatic), 2204 (C≡N), 1612 (C=N), 1591 (C=C); ^1H NMR (DMSO- d_6) δ (ppm): 5.09 (d, 1H, CH of pyran), 5.15 (s, 2H, OCH_2), 6.34 (1H, NH; exchangeable with D_2O), 7.05–8.03 (m, 19H, 18 aromatic H+ CH of pyran), 8.83 (s, 1H, CH of pyrazole); ^{13}C NMR (DMSO- d_6) δ (ppm): 23.51, 45.81, 69.34, 80.10, 115.36 (2C), 118.68, 118.56, 119.57 (2C), 124.16, 125.52 (2C), 126.56, 127.72, 128.41 (2C), 128.64, 128.71, 128.98 (2C), 129.32 (2C), 129.62 (2C), 129.92 (2C), 130.50, 131.77, 132.54, 137.89, 142.61, 154.67, 159.32; MS, m/z : 556 (M^+ , 4.87%); 558 [$(\text{M} + 2)^+$, 1.44%]; anal. calcd. for $\text{C}_{34}\text{H}_{25}\text{ClN}_4\text{O}_2$ (556.17): C, 73.31; H, 4.52; N, 10.06, found: C, 73.57; H, 4.68; N, 10.41.

4.2. Biological evaluation

4.2.1. In vitro cyclooxygenase inhibition assay. The ability of the synthesized compounds 2–8a,b, indomethacin and

celecoxib to inhibit human recombinant COX-1 and human recombinant COX-2 (IC_{50} value, μM) was estimated using enzyme immunoassay (ELISA) kits (catalog no. 701070 and 701018, respectively, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions as previously reported.³⁷

4.2.2. *In vivo* anti-inflammatory activity. Adult male albino rats (100–150 g) were used in this study. The animals (six per group) were maintained under standard laboratory conditions with access to water and food. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations. The new compounds **2–8a,b** and the reference drugs indomethacin and celecoxib were evaluated for their *in vivo* anti-inflammatory activity using the carrageenan-induced rat paw edema method. In this method, the edema is induced by injection of 0.05 mL of 1% solution of carrageenan into the plantar tissues of the hind paw of the rat. The developed edema is inhibited by pretreatment of the animals by single oral doses of the tested compounds and the reference drugs (100 mg kg⁻¹). The paw thickness was measured after 1 h, 3 h, 4 h, 8 h and 12 h of the carrageenan injection as reported before.²⁸

4.2.3. Ulcerogenic liability. The ulcerogenic effect of the compounds **2a,b**, **3b**, **4a**, **6b**, **7a** and **8a** as well as indomethacin and celecoxib was evaluated following the Meshali's method.³⁸ Herein, fifty adult albino rats were used and divided into 10 groups. After fasting for 18 h, the animals were administered with drugs. The first control group received a 10% DMSO aqueous solution (v/v), the second group received indomethacin in a dose of 50 mg kg⁻¹, and the third group received celecoxib in a dose of 50 mg kg⁻¹. The other groups received **2a,b**, **3b**, **4a**, **6b**, **7a** and **8a** in a dose of 50 mg kg⁻¹. The ulcer index was determined according to the Robert's method.³⁹ The degree of the ulcerogenic effect was expressed in terms of the percentage incidence of ulcers in each group of animals divided by 10, the average number of ulcers per stomach and the average severity of ulcers (ulcer score) by visual observation. The ulcer scores were as follows: 0 means no ulcer, 1 means mucosal erythema only, 2 means mild mucosal edema, slight bleeding, or slight erosion, 3 means moderate edema, bleeding ulcers, or erosions, and 4 means severe ulceration, erosions, edema and tissue necrosis. The ulcer index is the sum value of the abovementioned three values.

4.2.4. Histopathological study. Whole rat stomachs, livers, and kidneys were obtained and examined completely for monitoring any pathological changes; the selected samples from each rat were fixed in 10% neutral buffered formalin for 48 h, washed with running tap water, dehydrated in ascending grades of alcohol, cleaned in xylene, embedded in paraffin wax, and then blocked. The blocks were cut at 5 μm thickness, and the sections were stained with hematoxylin and eosin for histopathological examination.⁴⁰

4.3. Molecular docking study

The molecular docking study was conducted using the molecular operating environment (MOE-Dock 2014.09) software.³³

The structures of the compounds celecoxib, lonazolac, **2a** and **2b** were drawn using the builder button. Then, these compounds were exposed to energy minimization by utilizing the default MMFF94x force field in the MOE program. Herein, the low energy 3D conformers of the tested compounds were docked into the active site of the COX-1 and COX-2 proteins (PDB codes: 5WBE⁴¹ and 3LN1 (ref. 42), respectively). During the docking process, the water molecules were removed. The missing hydrogen atoms were added in the order of the correct ionization states to be assigned to the protein structure. The “docking” module in MOE was run to perform the molecular docking. The docking procedure was applied with default settings. The top 30 poses as ranked by London dG were kept and minimized using MMFF94x within a rigid receptor. The GBVI/WSA dG (Generalized-Born Volume Integral/Weighted Surface area) scoring function was then applied to score the resulting poses. The “Ligand Interactions” MOE tool was then used to analyze the molecular docking results by visualization of the protein–ligand interactions in the active site of the complex. The tool presents in the diagram form an identification and visualization of the interactions between the ligand and the receptor interacting entities, solvent molecules, and ions in the active site of the protein. The molecular docking protocol was validated by docking mofezolac in the active site of 5WBE and celecoxib in the active site of 3LN1, where they showed 0.8 and 0.7 Å root mean square deviation (RMSD), respectively.

5. Animal rights

All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Al-Azhar University and approved by the Scientific Research Ethics Committee of Faculty of Pharmacy (Girls), Al-Azhar University.

Conflicts of interest

The authors confirm that this article content has no conflicts of interest.

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