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Design, synthesis and biological screening of new 4-thiazolidinone derivatives with promising COX-2 selectivity, anti-inflammatory activity and gastric safety profile

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ABSTRACT

Two series of new thiazolidin-4-one derivatives **4a–c** and **8a–e** were designed and prepared. All the synthesized compounds were evaluated for their *in vitro* COX-2 selectivity and anti-inflammatory activity *in vivo*. Compounds **8c** and **8d** showed the best overall *in vitro* COX-2 selectivity (selectivity indexes of 4.56 and 5.68 respectively) and *in vivo* activities (edema inhibition % = 61.8 and 67 after 3 h, respectively) in comparison with the reference drug celecoxib (S.I. = 7.29, edema inhibition % = 60 after 3 h). In addition, **8c** and **8d** were evaluated for their mean effective anti-inflammatory doses (ED_{50} = 27.7 and 18.1 μmol/kg respectively, celecoxib ED_{50} = 28.2 μmol/kg) and ulcerogenic liability (reduction in ulcerogenic potential versus celecoxib = 85%, 92% respectively. Molecular docking studies were performed and the results were in agreement with that obtained from the *in vitro* COX inhibition assays.

1. Introduction

Non steroidal anti-inflammatory drugs (NSAIDs) are considered one of the most widely used therapeutics to alleviate pain and inflammation, especially arthritis [1]. The anti-inflammatory activity of NSAIDs arises from their ability to inhibit cyclooxygenase (COX) enzyme which catalyzes the production of pro-inflammatory prostaglandins (PGs) and thromboxanes (TXs) [2,3]. Currently, It is well known that cyclooxygenase enzyme exists in at least two distinct isoforms, a constitutive form (COX-1) and an inducible form (COX-2) [4]. The constitutively expressed COX-1 isoform plays a critical role as a housekeeping enzyme which is responsible for the maintenance of physiological functions such as protection of gastric mucosa, vascular homeostasis and platelet aggregation. The inducible COX-2 isoform is significantly upregulated during acute and chronic inflammation, pain and oncogenesis [5,6]. Traditional nonselective NSAIDs such as aspirin, ibuprofen and indomethacin interact with both forms (COX-1 and COX-2); this broad inhibitory profile accounts for their anti-inflammatory activity in addition to their pronounced side effects resulting from the inhibition of gastro protective PGs synthesized through COX-1 pathway. Therefore, their long term administration even at low prophylactic doses resulting in gastro intestinal side effects ranging from ulcers to perforation and bleeding [7]. For these reasons, the synthesis of selective COX-2 inhibitory drugs (coxibs) takes much consideration in recent years that achieves the same anti-inflammatory efficacy as traditional NSAIDs, but with minimal risk of the concomitant gastric and renal toxicity mediated through the inhibition of COX-1 enzyme [4,6]. Hence, a number of selective COX-2 inhibitors such as celecoxib **I**, rofecoxib **II** and valdecoxib **III** (coxibs) has been developed and approved for marketing by virtue of their fewer gastrointestinal side effects compared to traditional NSAIDs (Fig. 1). However, when the coxibs were marketed, evidence for increased the risk of cardiovascular adverse effects appeared that led to rofecoxib ban in 2004 followed by the voluntary withdrawal of some other coxibs from the market [8]. In this respect, celecoxib has advantages of that it is not associated with an increased incidence of cardiovascular events compared with placebo and with nonselective NSAIDs [9]. However, there are some characteristics of celecoxib that need to be improved. For example, celecoxib has some gastrointestinal side effects and is not effective in all patients [10,11]. In earlier studies, we reported some derivatives of celecoxib **IV–VIII** [12–17] (Fig. 1) with comparable activities to celecoxib as COX-2 selective compounds.

In continuation with our work related to synthesis of safe anti-inflammatory agents, we now describe the synthesis, *in vitro* evaluation as COX-1/COX-2 inhibitors, *in vivo* anti-inflammatory (AI) activity, and ulcerogenic liability for two new groups of

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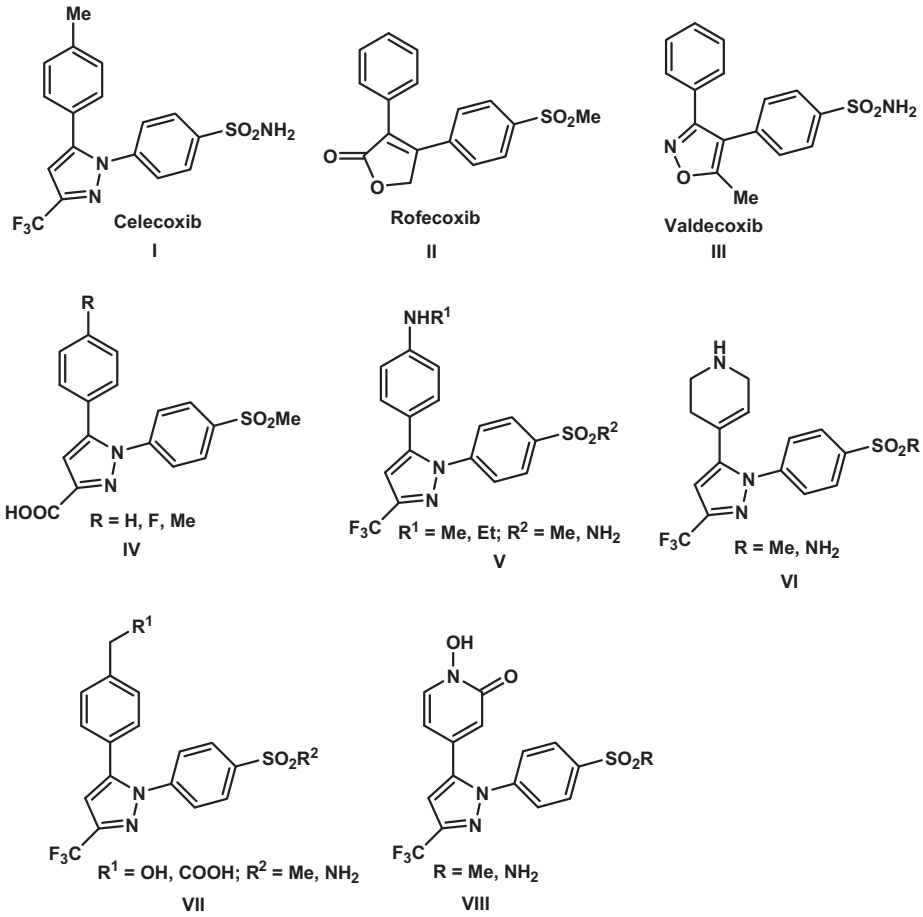


Fig. 1. Chemical structures of the selective cyclooxygenase-2 (COX-2) inhibitors celecoxib (**I**), rofecoxib (**II**), valdecoxib (**III**), and some reported celecoxib derivatives **IV**–**VIII**.

4-thiazolidinone derivatives **4a**–**c** and **8a**–**e** in which, (i) the pyrazole ring of celecoxib was replaced with thiazole moiety in compounds **4a**–**c** or 4-thiazolidinone nucleus in compounds **8a**–**e**, (ii) tolyl group was replaced with iminothiazolidin-4-one in compounds **4a**–**c** and maintained or replaced with different aryl moieties in compounds **8a**–**e**, (iii) the aminosulfonylphenyl group which is the major determinant for COX-2 selectivity and *in vivo* efficacy [18–20] was replaced with aryl moiety substituted at para position with bulky group in compounds **4a**–**c** or replaced with more bulky aminosulfonylphenylamino group in compounds **8a**–**e**. This bulky substitution could maximize the interaction with the hydrophobic residues within COX-2 active site and enhance COX-2 selectivity [21], and (iv) the trifluoromethyl group of celecoxib was replaced with methyl group (**8a**–**e**) since it was reported that the substituent at pyrazole C-3 has very few steric restrictions with respect to COX-2 suggesting that COX-2 inhibition should be retained [22] (Fig. 2).

2. Results and discussion

2.1. Chemistry

The target compounds 4-thiazolidinones **4a**–**c** were synthesized *via* a reaction sequence illustrated in Scheme 1 starting from the reaction of 4-substituted-acetophenone (**1a**–**c**) with iodine and thiourea at 60 °C in water bath to provide the corresponding 2-amino-4-(4-substituted-phenyl)thiazole (**2a**–**c**) in high yields (70–85%). Then, compounds **3a**–**c** were obtained in good yield (65–70%) *via* the reaction of **2a**–**c** with chloroacetyl chloride in dioxane under reflux conditions. Finally, cyclization of acetamide derivatives **3a**–**c** with ammonium thiocyanate in ethanol afforded thiazolidinone derivatives **4a**–**c** in moderate yield (40–50%).

All the newly synthesized compounds have been characterized by IR, ^{1}H NMR, ^{13}C NMR, mass spectra and elemental analyses. The suggested mechanism of heterocyclization of **3a**–**c** and the theoretically tautomeric forms of target compounds **4a**–**c** are shown in Scheme 2. The γ lactam structure of **4a**–**c** was confirmed based on the ^{1}H NMR spectra which exhibited an NH proton appeared at δ = 12.12–12.21 ppm which was in the same region as the previously reported NH appeared at $\delta \approx 12.35$ ppm. These findings proved that this proton accounts for a lactam proton but not for an imine proton which is expected to appear at a much higher field at δ = 9.0 ppm. [23–25].

The second series of the target 4-thiazolidinones **8a**–**e** were synthesized according to the method described by Neuenfeldt et al. [26]. This new method allows the synthesis of the 4-thiazolidinones derived from phanylhydrazines even if they have electron withdrawing groups on the phenyl ring. Accordingly, the hydrazones **7a**–**e** were prepared in high yields (80–90%) by heating 4-hydrazinobenzenesulfonamide hydrochloride (**5**) with 4-substituted-aldehydes (**6a**–**e**) in presence of sodium acetate in ethanol. Heating the formed hydrazones **7a**–**e** with excess thiolactic acid at 60 °C under solvent free conditions afforded the 4-thiazolidinone derivatives **8a**–**e** in moderate yields (35–60%) (Scheme 3). The ^{1}H NMR spectra of **8a**–**e** displayed a peak appeared as two doublets at δ 1.52–1.53 and 1.54–1.55 ppm corresponding to thiazolidinone CH_3. In addition, another peak appears as two singlets at δ 5.82–6.02 and 5.87–6.06 ppm corresponding to

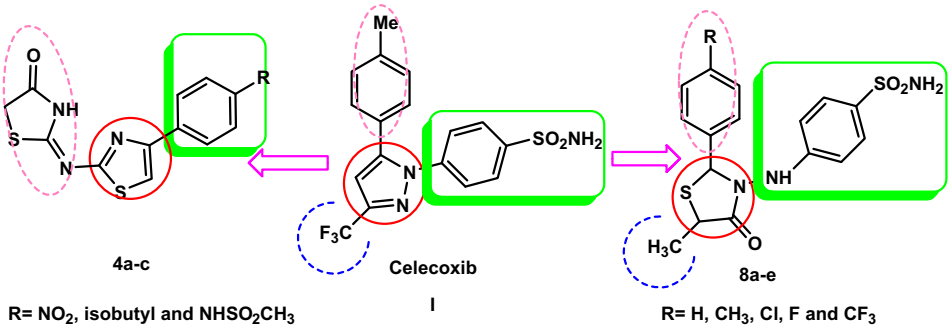
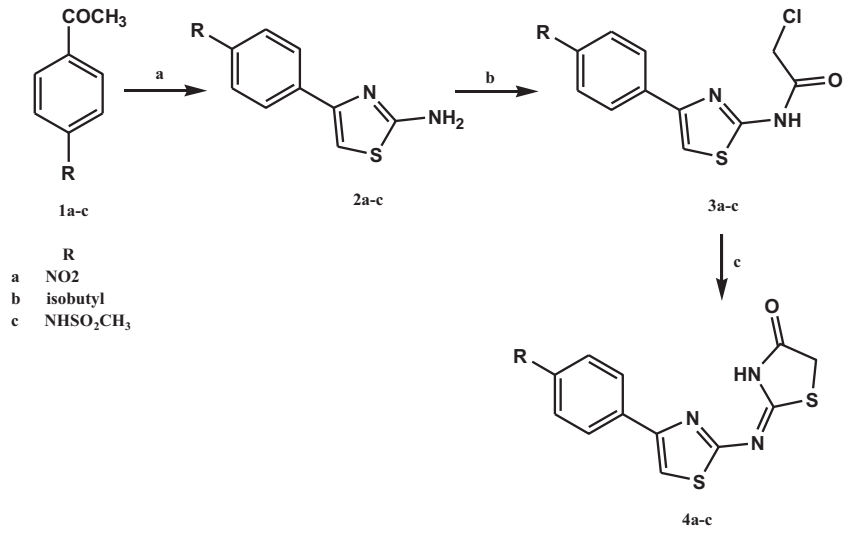


Fig. 2. Representative example of selective COX-2 inhibitor (celecoxib **I**) and the designed thiazolidinones **4a**–**c** and **8a**–**e**.



Scheme 1. General synthetic procedure for title compounds **4a**–**c**. Reagents and conditions: (a) I_2, thiourea, heat at 60 °C, 3–5 h; (b) chloroacetyl chloride, dioxan, reflux, 24 h; (c) ammonium thiocyanate, ethanol, reflux, 24 h.

thiazolidinone H-2. Another D_2O exchangeable peak appears as two singlets at δ 8.67–8.81 and 8.74–8.87 ppm corresponding to NH group and these findings are due to presence of two chiral centers at C_2 and C_5 as previously reported [27,28], so compounds **8a**–**e** are present as diastereoisomers. The separation of two isomers was not possible to be done by chromatography because of similar retention times between them in different solvents [29].

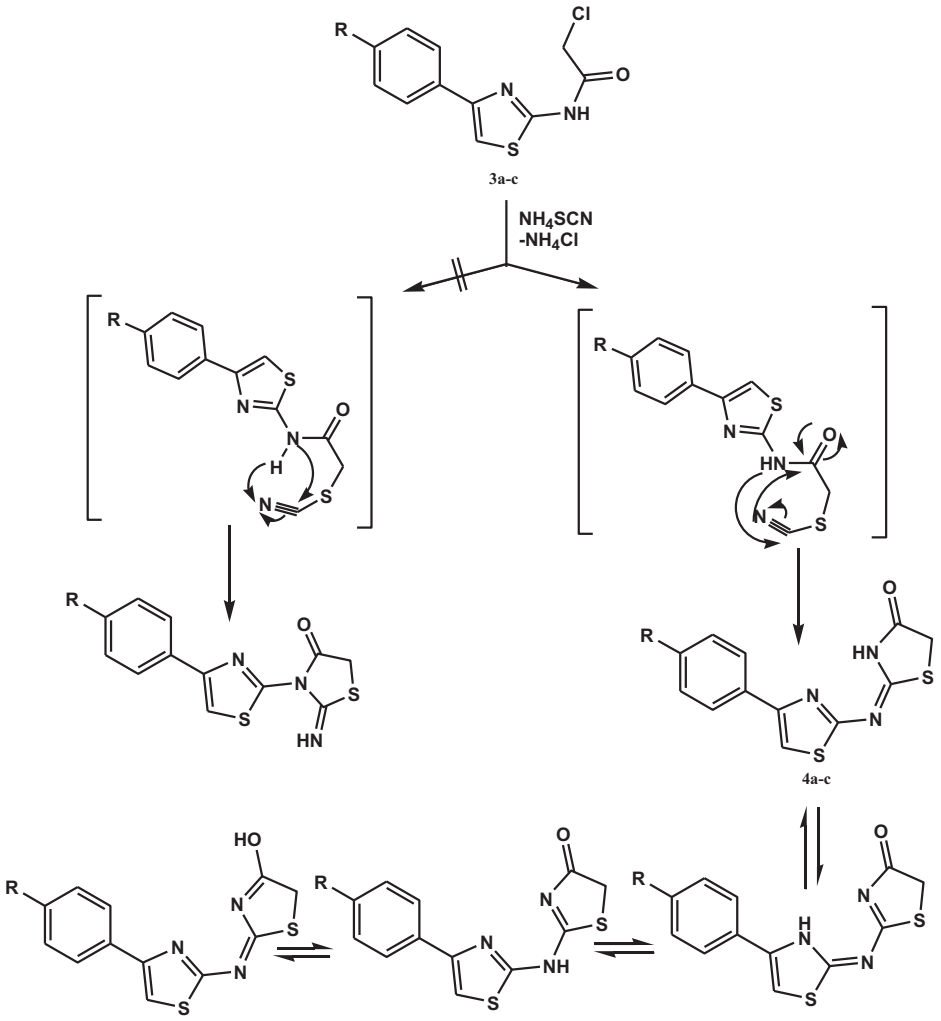
2.2. Biological evaluation

2.2.1. In vitro cyclooxygenase (COX) inhibition assay

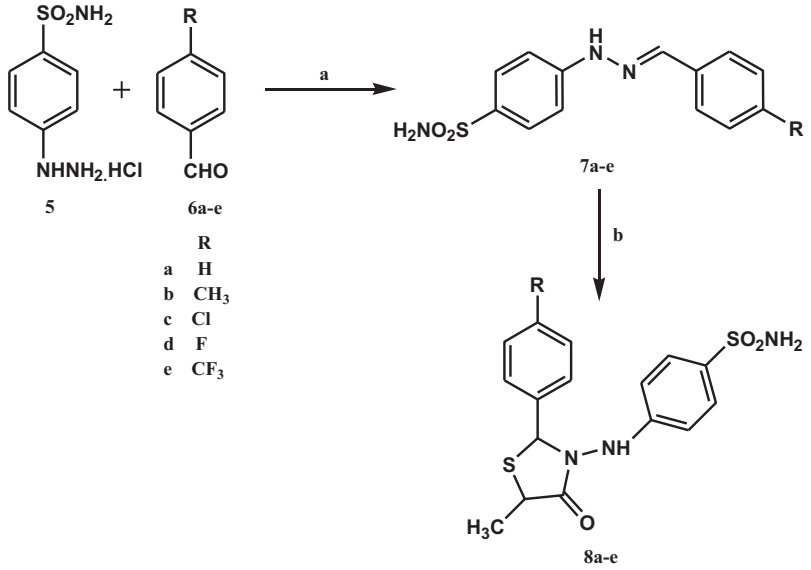
The target of the *in vitro* biological activity tests was to study the ability of tested compounds to inhibit ovine COX-1 and human recombinant COX-2 using an enzyme immunoassay (EIA) kit. The obtained data (Table 1) showed that all the tested compounds (**4a**–**c** and **8a**–**e**) were weak inhibitors of COX-1 isozyme (IC_{50} = 10.5–29.1 μM range) and exhibited moderate COX-2 isozyme inhibitory activities (IC_{50} = 1.9–8.7 μM). Also, the results showed COX-2 selectivity indexes in the 2.84–5.68 range (celecoxib COX-2 S.I. = 7.29). The structure activity data acquired showed that when the two bulky moieties attached to the central heterocyclic thiazole ring are not vicinal (**4a**–**c**), lower inhibitory activity against both COX-1 (IC_{50} = 13.4–17.6 μM) and COX-2 (IC_{50} = 4.6–6.1 μM) than celecoxib (IC_{50} = 9.7 and 1.33 μM for COX-1 and COX-2, respectively) were obtained. While, when the two bulky moieties attached to the central heterocyclic 4-thiazolidinone moiety are vicinal (**8a**–**e**) comparable inhibitory activity against COX-1 (IC_{50} = 10.5–29.1 μM) and against COX-2 (IC_{50} = 1.9–8.7 μM) with celecoxib (IC_{50} = 9.7 and 1.33 μM for COX-1 and COX-2, respectively). Within the biologically active series (**8a**–**e**), the chloro (**8c**) and fluoro (**8d**) analogs were more selective COX-2 inhibitors (selectivity indexes of 4.56 and 5.68 respectively) than the unsubstituted (**8a**), methyl (**8b**) and trifluoromethyl (**8e**) analogs (selectivity indexes of 3.34, 3.50 and 3.47 respectively) in comparison with celecoxib (S.I. = 7.29).

2.2.2. In vivo anti-inflammatory activity

The anti-inflammatory (AI) activities exhibited by compounds **4a**–**c** and **8a**–**e** using dose 10 mg/kg according to a previously reported procedures [30] are listed in Table 2. Compounds **4a** and **4b** showed moderate COX-2 inhibitory activity (IC_{50} = 4.6 and 6.1 μM, respectively) with moderate selectivity index (S.I. = 2.91 and 2.88, respectively) were found to possess anti-inflammatory activity (52.7% and 56.7% reduction in inflammation after 3 h, respectively) close to celecoxib (60% reduction in inflammation after 3 h). While, 4-thiazolidinone derivatives **8c** and **8d** which showed COX-2 inhibitory activity *in vitro* comparable to celecoxib, showed *in vivo* anti-inflammatory activity (edema inhibition % = 61.8 and 67 after 3 h, respectively) higher than the reference drug celecoxib (edema inhibition % = 60 after 3 h). Moreover, compounds **8b** and **8e** with hydrophobic methyl or trifluoromethyl groups that had good COX-2 inhibitory activity exhibited moderate anti-inflammatory effect *in vivo* (edema inhibition % = 22.3 and 23.7 after 3 h, respectively).



Scheme 2. Mechanistic pathway for compounds **4a–c** and their tautomers.



Scheme 3. General synthetic procedure for compounds **8a–e**. Reagents and conditions: (a) sodium acetate, ethanol, reflux, 12–18 h; (b) thiolactic acid, heat at 60 °C, 3 h.

Table 1
In vitro COX-1 and COX-2 inhibitory activity and COX-2 selectivity index of thiazoldinones **4a–c**, **8a–e** as well as celecoxib.

Compounds	IC_{50} (μM)[a]		S.I.[b]
	COX-1	COX-2	
4a	13.4	4.6	2.91
4b	17.6	6.1	2.88
4c	15.1	5.3	2.84
8a	29.1	8.7	3.34
8b	16.8	4.8	3.5
8c	10.5	2.3	4.56
8d	10.8	1.9	5.68
8e	11.8	3.4	3.47
Celecoxib	9.7	1.33	7.29

[a] IC_{50} value represents the concentration of the compound required to produce 50% inhibition of COX-1 or COX-2 which is the mean value of two determinations where the deviation from the mean is <10% of the mean value.
[b] Selectivity index (COX-1 IC_{50}/COX-2 IC_{50}).

Table 2
Anti-inflammatory activity at different time intervals of the tested compounds **4a–c**, **8a–e** and celecoxib using 10 mg/kg dose employing carrageenan-induced paw edema method in mice.

Compounds	Edema inhibition %		
	1 h	3 h	5 h
4a	42.2	52.7	57
4b	43.1	56.7	60
4c	2.9	22.36	29.1
8a	8.7	17	22.8
8b	11.6	22.3	30.4
8c	48.3	61.8	64.5
8d	50.7	67	69.6
8e	4.3	23.7	30.4
Celecoxib	50.1	60	62.5

On the basis of the aforementioned *in vitro* and *in vivo* results, compounds **8c** and **8d** which showed COX-2 inhibitory potency and selectivity indexes very close to celecoxib and *in vivo* anti-inflammatory activity higher than celecoxib, their corresponding mean effective anti-inflammatory doses (ED_{50}) were calculated according to the method of Litchfield and Wilcoxon [31], Table 3. The oral anti-inflammatory activity (ED_{50} value) exhibited by the chloro derivative **8c** (ED_{50} = 27.7 mg/kg) was found to be nearly equipotent to celecoxib (ED_{50} = 28.2 μmol/kg) and < the fluoro derivative **8d** (ED_{50} = 18.1 μmol/kg). The ED_{50} data obtained confirmed the high *in vitro* and *in vivo* results of **8c** and **8d** suggesting the promising activity of those two derivatives as selective COX-2 inhibitors.

2.2.3. Ulcerogenic liability

Compounds **8c** and **8d** which showed the most potent COX-2 inhibitory activity and showed higher anti-inflammatory activity than celecoxib were evaluated for their ulcerogenic potential according to a previously reported method [32]. The ulcerogenic effect was compared to both celecoxib (50 mg/kg) and the classical NSAID ibuprofen (50 mg/kg). From the obtained data in Table 3, it had been observed that compounds **8c** and **8d** caused much less gastric ulceration effect with nearly negligible ulcerogenic liability (ulcer index of compound **8c**: 1.2 and **8d**: 0.6) in the experimental animals, compared to that of the standards, ibuprofen (ulcer index: 14.6) and celecoxib (ulcer index: 7.8). Therefore, the potential value of these compounds as anti-inflammatory agents is that they have highly better safety margin on gastric mucosa than celecoxib and ibuprofen. These promising ulcer protective properties of the designed 4-thiazolidinone derivatives **8c** and **8d** greatly supported our main objective to avoid gastric injuries caused by COX-1 inhibition. Moreover, compound **8c** showed about 85% reduction in ulcerogenic potential versus celecoxib. While, compound **8d** with best overall profile in animal efficacy model showed about 92% reduction in ulcerogenic potential versus celecoxib. Hence, this successful result supported the aim of the present work to develop novel series of celecoxib analogs bearing thiazolidinone moiety as COX-2 inhibitors with diminished gastrointestinal side effects.

Table 3
Mean effective anti-inflammatory doses (ED_{50}) and ulcerogenic liability **8c**, **8d**, celecoxib and ibuprofen.

Compounds	ED_{50} (mg/kg)[a]	ED_{50} (μmol/kg)[a]	Ulcer index[b]
8c	11.0	27.7	1.2
8d	6.9	18.1	0.6
Celecoxib	10.8	28.2	7.8
Ibuprofen	ND[c]	ND[c]	14.6

[a] Anti-inflammatory activity represented by ED_{50} which was the effective dose calculated after 3 h.
[b] Ulcerogenic liability using 50 mg/kg dose.
[c] Not determined.

2.3. Molecular modeling

With the aim to understand the protein-inhibitor interaction and the structure features of COX-2 active site taking into account that COX-2 is the most relevant enzymatic system for inflammation and the target of our compounds, a molecular modeling study was performed using the crystal structure of COX-2 (3LN1) [33]. Docking of compounds **4a**, **4b**, **8c**, **8d** and celecoxib as the reference ligand into the crystal structure of COX-2 enzyme was performed using the MOE 2008.10 modeling software (Molecular Operating Environment) [34]. The docking results of compounds **4a**, **4b**, **8c**, **8d** and celecoxib are presented in Table 4. The docking score of celecoxib = −26.44 kcal/mol, while the tolyl moiety is bound in the primary hydrophobic pocket, the trifluoromethyl group is bound in an adjacent pocket formed by Val335, Tyr341, Leu345 and Leu517. The phenyl sulfonamide moiety is inserted in the secondary pocket where the sulfonamide group exhibited three hydrogen bonding interaction with Gln178, Leu338 and Ser339 (distance = 3.11, 2.76 and 2.97 Å, respectively), such interactions are almost essential for COX-2 inhibitory activity [33,35]. Also, the selectivity of celecoxib seems to result from the binding of the sulfonamide group to this secondary pocket which is more restricted in COX-1 [35]. The docking models of 4-thiazolidione compounds **4a** and **4b** that exhibited the highest anti-inflammatory activities *in vivo* in Scheme 1 showed that they are oriented in COX-2 active site in a close manner to celecoxib (Figs. 3 and 4). These compounds had moderate binding affinities (docking scores: −13.30 and − 6.05 kcal/mol, respectively), Table 4. The iminothiazolidin-4-one is positioned in the primary hydrophobic pocket occupied by the tolyl moiety of celecoxib. Also, the 4-nitro/isobutylphenyl moiety is oriented toward the secondary pocket, in which it doesn't exhibit any hydrogen bond interactions with the amino acids present in COX-2 secondary pocket. Accordingly, the lack of the H-bond interactions with COX-2 side pocket for the analogs **4a** and **4b** might be responsible for their reduced aforementioned *in vitro* COX-2 inhibitory activities.

The geometries of the top ranked poses of 4-thiazolidinone compounds with the best overall *in vivo* and *in vitro* profiles **8c** and **8d** indicate that celecoxib and those two compounds adopt nearly superimposed orientations in the binding site and interact with many of the same amino acid residues in the crystal structure 3LN1 (Figs. 5 and 6). It is interesting to note that 4-sulfamoylphenylamino moiety of **8c** and **8d** is positioned in the COX-2 secondary pocket, surrounded by His75, Ser339, Arg499 and Gln178. As aforementioned, this secondary pocket is considered to be responsible for the selectivity and occupied by the *P*-SO_2NH_2 pharmacophore of selective COX-2 inhibitors [35].

Table 4
Docking score and bond interactions of celecoxib and synthesized compounds **4a**, **4b**, **8c** and **8d** with amino acids of COX-2.

Compounds	Docking score (kcal/mol)	No. of H-bonds	Distance	Amino acids involved	Molecular structure	*c* log *P*[a]
4a	−13.30	–	–	–	–	2.76
4b	−6.05	–	–	–	–	4.05
8c	−16.40	3	3.16	His75	H of SO_2NH_2	3.07
			2.02	Ser339	H of SO_2NH_2	
			2.69	Arg499	O of SO_2NH_2	
8d	−17.15	2	2.14	Ser339	H of SO_2NH_2	2.55
			2.78	Arg499	O of SO_2NH_2	
Celecoxib	−26.44	3	2.97	Ser339	N of SO_2NH_2	3.82
			2.76	Leu338	N of SO_2NH_2	
			3.11	Gln78	N of SO_2NH_2	

[a] Calculated using Ligand Properties tool in MOE (The Molecular Operating Environment, Version 2008.10).

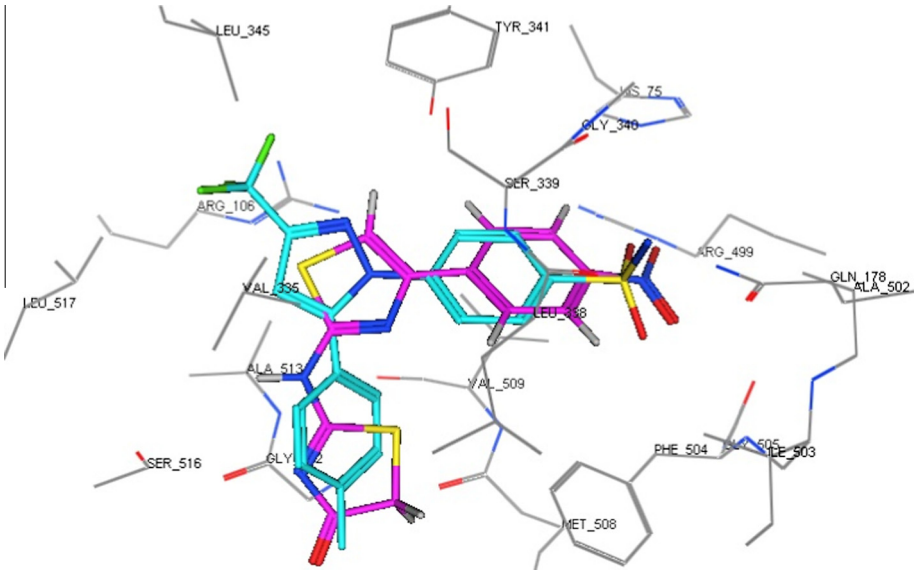


Fig. 3. The orientation of **4a** (pink) in COX-2 active site and celecoxib (turquoise) top ranked docking pose in the COX-2 active site. (For interpretation of the references to color in this figure legend, the reader is to the web version of this article).

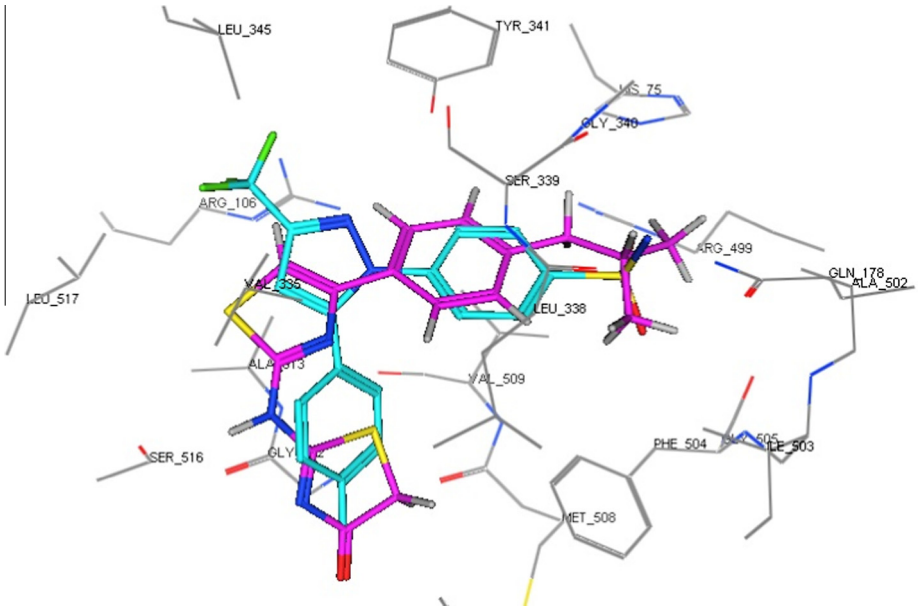


Fig. 4. The orientation of **4b** (pink) in COX-2 active site and celecoxib (turquoise) top ranked docking pose in the COX-2 active site. (For interpretation of the references to color in this figure legend, the reader is to the web version of this article).

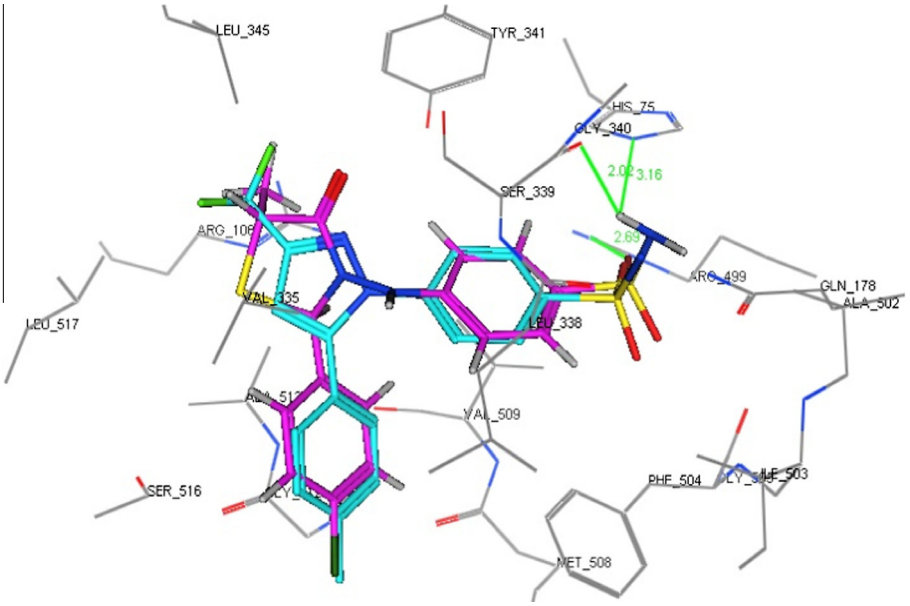


Fig. 5. Overlay of **8c** (pink) and celecoxib (turquoise) top ranked docking pose in the COX-2 active site and hydrogen bonds are shown in green. (For interpretation of the references to color in this figure legend, the reader is to the web version of this article).

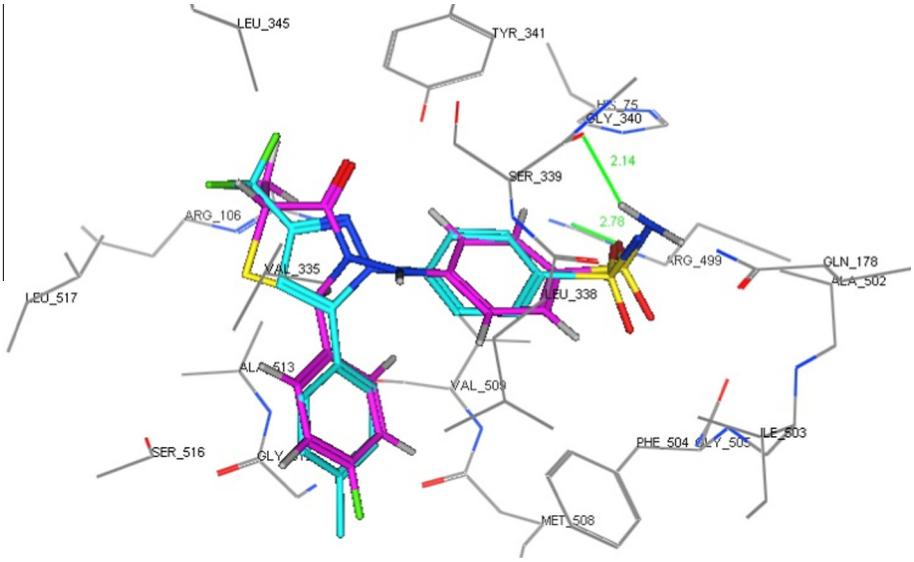


Fig. 6. Overlay of **8d** (pink) and celecoxib (turquoise) top ranked docking pose in the COX-2 active site and hydrogen bonds are shown in green. (For interpretation of the references to color in this figure legend, the reader is to the web version of this article).

Furthermore, the CH_3 group of compounds **8c** and **8d** is placed within the same region of the CF_3 moiety of celecoxib. Compound **8c** forms three hydrogen bonds with the amino acids present in the COX-2 secondary pocket in which one of the sulfonamide hydrogen atoms forms two hydrogen bonds with the nitrogen atom of His75 (distance = 3.16 Å) and the oxygen atom of Ser339 (distance = 2.02 Å) and one of the sulfonamide oxygen atoms forms a hydrogen bond with the NH of Arg499 (distance = 2.69 Å) (Fig. 5). While, compound **8d** forms two hydrogen bonds with the amino acids present in the COX-2 secondary pocket in which one of the sulfonamide hydrogen atoms forms a hydrogen bond with the oxygen atom of Ser339 (distance = 2.14 Å) and one of the sulfonamide oxygen atoms forms a hydrogen bond with the NH of Arg499 (distance = 2.78 Å) (Fig. 6).

The profound enhancement in COX-2 inhibitory effect of the chlorophenyl and fluorophenyl derivatives **8c** and **8d** suggested that they bound to the enzyme in such way to maximize their interaction with the binding site. As seen in Table 4, these compounds exhibited high binding affinities (docking scores: −16.40 and −17.15 kcal/mol, respectively) with docking scores close to that of celecoxib, (docking score: −26.44 kcal/mol). This may be attributed to the highly electronegative nature of chlorine or fluorine atom which led to a more efficient π–π stack interaction of the phenyl ring with Tyr371 and Trp373 [36,37]. Moreover, the difference in electronegativity between chlorine or fluorine and carbon creates a large dipole moment in this bond which may contribute to the molecule's ability to be engaged in intermolecular interactions with the COX-2 active site [38,39].

Table 5
ADME of celecoxib and synthesized compounds **4a**, **4b**, **8c** and **8d** using mipc-Molinspiration property calculator.

Compounds	MW	No. of H-bond donors	No. of H-bond acceptors	milogP	No. of rotatable bonds	TPSA	No. of violations
4a	320.355	1	7	2.1	4	100.178	0
4b	331.466	1	4	3.442	5	54.354	0
8c	397.909	3	6	2.811	4	92.501	0
8d	381.454	3	6	2.297	4	92.501	0
Celecoxib	381.379	2	5	3.611	4	77.991	0

MW: molecular weight.
milogP: octanol–water partition coefficient (log*P* predicted at Molinspiration).
TPSA: topological polar surface area.

2.4. ADME profiling

The bioavailability of the reference drug celecoxib and the most biologically active compounds **4a**, **4b**, **8c** and **8d** was assessed using mipc-Molinspiration Property Calculator [40]. In particular, we calculated the compliance of compounds to Lipinski's "rule of five" to evaluate the drug-likeness [41]. The rule describes molecular properties which are important for drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and elimination (ADME) and is used to make sure that drug-like physicochemical properties are maintained during drug design. This simple rule states that orally active drug has no more than one violation of the following criteria: molecular weight less than 500 Da; no more than five hydrogen bond donors; no more than 10 hydrogen bond acceptors; and calculated octanol–water partition coefficient (*c* log*P*) not greater than 5 [42]. Moreover, topological polar surface area (TPSA) together with the number of rotatable bonds have been considered to be very good descriptors of oral bioavailability of drugs. Compounds which meet the following two criteria: ten or fewer rotatable bonds and polar surface area equal to or less than 140 Å^2 are predicted to exhibit good oral bioavailability [43]. The calculated parameters presented in Table 5 showed good bioavailability of studied compounds. The most active compounds **4a**, **4b**, **8c** and **8d** fulfilled all rules, similar to the clinically used drug celecoxib. Theoretically, these four compounds should exhibit good passive oral absorption and differences in their bioactivity cannot be attributed to these properties. Compounds **4a** and **4b** exhibited anti-inflammatory activity with edema inhibition % (42.2 and 43.1, respectively) after 1 h indicating their high anti-inflammatory activity and rapid onset of action which might be attributed to their enhanced aqueous solubility (miLogP: 2.1 and 3.442, respectively). Also, the determined log*P* values were 2.76 and 4.05, respectively, which affirmed the hydrophilic characteristics of these compounds. In the same context, compounds **8c** and **8d** which showed the highest inhibitory activity and selectivity for COX-2 enzyme in the *in vitro* assay (IC_{50} = 1.9 and 2.3 μM, respectively), exhibited anti-inflammatory activity with edema inhibition % (48.3 and 50.7, respectively) after 1 h. The high potency and the rapid onset of action of 3-(4-aminosulfonylphenylamino)-2-(4-chloro/fluorophenyl)-5-methyl-4-thiazolidinone derivatives (**8c** and **8d**) might be attributed to their enhanced aqueous solubility (miLogP: 2.81 and 2.29, respectively). In this context, aqueous solubility controls the rate of dissolution of the compound together with the maximum concentration reached in the gastrointestinal fluid and may be the main factor ensuring a good level of distribution of these compounds *in vivo* [44]. The determined log*P* values were 3.07 and 2.55, respectively, which affirmed the hydrophilic characteristics of compounds.

3. Conclusion

The present study reported the design and synthesis of novel thiazoldinone derivatives as selective COX-2 inhibitors. The synthesized compounds were evaluated for their COX-1/COX-2 inhibitory activity *in vitro* and anti-inflammatory activity *in vivo*. Compounds **8c** and **8d** showed COX-2 inhibitory potency (IC_{50} = 1.9 and 2.3 μM, respectively) and selectivity indexes (S.I. = 4.56 and 5.68, respectively) very close to celecoxib (IC_{50} = 1.33 μM and S.I. = 7.29). Moreover, compounds **8c** and **8d** were found to be potent and much more selective toward COX-2 isozyme (IC_{50} = 1.9 and 2.3 μM, respectively) than COX-1 isozyme (IC_{50} = 10.5 and 10.8 μM, respectively). In addition, the synthesized compounds were evaluated for their *in vivo* anti-inflammatory activity. Compounds **4a**, **4b**, **8c** and **8d** were found to have close to higher anti-inflammatory activity (edema inhibition % = 52.7, 56.7, 61.8 and 67 after 3 h, respectively) than celecoxib (edema inhibition % = 60 after 3 h). ED_{50} was calculated for the most potent compounds **8c** and **8d** that showed the highest *in vitro* and *in vivo* results. It is further interestingly confirmed their promising anti-inflammatory activities with ED_{50} values indicating that compound **8c** is equipotent to celecoxib and compound **8d** is much more potent than celecoxib (ED_{50} = 27.7, 18.1 and 28.2 μmol/kg for **8c**, **8d** and celecoxib, respectively). The ulcerogenic liability of compounds **8c** and **8d** was also determined which was another proof of their promising activity as safe anti-inflammatory compounds with nearly negligible ulcerogenic liabilities compared to celecoxib and ibuprofen (Ulcer index = 1.2, 0.6, 7.8 and 14.6 for **8c**, **8d**, celecoxib and ibuprofen, respectively).

Molecular docking simulations and analysis of the binding modes of the new inhibitors within COX-2 active site were also performed to rationalize the highly obtained anti-inflammatory activity results. The H-bonding capability of sulfonamide group in the COX-2 active site and the electronic effect (electronegativity) of halogen atom on the phenyl ring seems to be the most crucial factors affecting the anti-inflammatory activity and exerted the major influence on the COX-2 inhibitory potency of 3-(4-aminosulfonylphenylamino)-2-(4-chloro/fluorophenyl)-5-methyl-4-thiazolidinone (**8c** and **8d**). The promising anti-inflammatory activity of **8c** and **8d** which was higher than celecoxib as well as their reduced ulcerogenic potential by 85% and 92%, respectively compared to celecoxib, make them good lead candidates for further optimization and development of potent and safe anti-inflammatory agents.

4. Experimental

4.1. Chemistry

Melting points were determined on a Griffin apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Shimadzu 435 spectrometer using KBr discs. ^{1}H NMR was measured on a Bruker 400 MHz spectrometer and ^{13}C NMR spectra were measured on a Bruker 100 MHz spectrometer (Faculty of Pharmacy, Beni Suef University, Beni Suef, Egypt) in D_2O or DMSO-d_6 with TMS as the internal standard, where *J* (coupling constant) values were estimated in hertz (Hz). Mass spectra were run on Hewlett Packard 5988 spectrometer. Microanalyses were performed for C, H, N at the (Micro Analytical Center, Cairo University, Egypt) and were

within ±0.4% of theoretical values. All other reagents, purchased from the Acros Chemical Company, were used without further purification; celecoxib was prepared while ibuprofen was purchased from the Aldrich Chemical Company (Milwaukee, WI), Compounds **2a** [45], **2b** [46], **2c** [47], **3a** [48], **5** [49] and **7a–e** [50] were prepared according to reported procedures.

*4.1.1. General method for preparation of 2-chloroacetamido-4-(4-substituted-phenyl)thiazoles (**3b–c**)*

To a solution of 2-amino-4-(4-substituted-phenyl)thiazole (**2b** or **2c**) (2 mmol) in dioxane (20 mL), chloroacetyl chloride (4 mmol, 0.44 gm) was added dropwise then the reaction mixture was heated under reflux for 24 h. The solid precipitated on hot was filtered, washed with aqueous sodium carbonate followed by cold water, dried and crystallized from ethanol to give the respective 2-chloroacetamido-4-(4-substituted-phenyl)thiazoles (**3b–c**). Physical and spectral data for **3b–c** are listed below.

*4.1.1.1. 2-Chloroacetamido-4-(4-isobutylphenyl)thiazole (**3b**).* Yield: 65%; buff solid; mp 143–145 °C; IR (KBr): 3425 (NH), 3047 (CH aromatic), 2923, 2867 (CH aliphatic), 1698 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.87 (d, J = 6.8 Hz, 6H, $CH_2CH(CH_3)_2$), 1.83–1.89 (m, 1H, $CH_2CH(CH_3)_2$), 2.47 (d, J = 7.2 Hz, 2H, $CH_2CH(CH_3)_2$), 4.41 (s, 2H, CH_2Cl), 7.22 (d, J = 8 Hz, 2H, isobutylphenyl H-3, H-5), 7.62 (s, 1H, thiazole H-5), 7.80 (d, J = 8 Hz, 2H, isobutylphenyl H-2, H-6), 12.63 (s, 1H, N*H*, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 22.6 ($CH_2CH(CH_3)_2$), 30.0 ($CH_2CH(CH_3)_2$), 42.7 (CH_2Cl), 44.7 ($CH_2CH(CH_3)_2$), 108.2 (thiazole C-5), 125.9 (isobutylphenyl C-2, C-6), 129.7 (isobutylphenyl C-3, C-5), 132.2 (isobutylphenyl C-1), 141.4 (isobutylphenyl C-4), 149.6 (thiazole C-4), 157.7 (thiazole C-2), 165.5 (C=O); MS (m/z): 308 ($M^{+\cdot}$, 47%), 309 (M^{+1}, 9%), 310 (M^{+2}, 18%), 265 (100%); Anal. Calcd for $C_{15}H_{17}ClN_2OS$: C, 58.34; H, 5.55; N, 9.07. Found: C, 58.57; H, 5.62; N, 9.31.

*4.1.1.2. 2-Chloroacetamido-4-(4-methanesulfonamidophenyl)thiazole (**3c**).* Yield: 70%; buff solid; mp 230–232 °C; IR (KBr): 3441, 3394 (2 NH), 3041 (CH aromatic), 2937 (CH aliphatic), 1690 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.33 (s, 3H, CH_3), 4.10 (s, 1H, methanesulfonamido N*H*, D_2O exchangeable), 4.43 (s, 2H, CH_2Cl), 7.58 (d, J = 8.4 Hz, 2H, 4-methanesulfonamidophenyl H-3, H-5), 7.86 (s, 1H, thiazole H-5), 8.00 (d, J = 8.4 Hz, 2H, 4-methanesulfonamidophenyl H-2, H-6), 12.68 (s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 42.7 (CH_3), 43.5 (CH_2Cl), 110.8 (thiazole C-5), 127.0 (4-methanesulfonamidophenyl C-3, C-5), 131.8 (4-methanesulfonamidophenyl C-2, C-6), 133.6 (4-methanesulfonamidophenyl C-1), 136.0 (4-methanesulfonamidophenyl C-4), 148.2 (thiazole C-4), 158.1 (thiazole C-2), 165.7 (C=O); MS (m/z): 344 (M^{-1}, 100%), 345 ($M^{+\cdot}$, 20%), 346 (M^{+1}, 44%), 347 (M^{+2}, 10%); Anal. Calcd for $C_{12}H_{12}ClN_3O_3S_2$: C, 41.68; H, 3.50; N, 12.15. Found: C, 41.85; H, 3.55; N, 12.52.

4.1.2. General method for preparation of 2-(4-(4-substituted-phenyl) thiazol-2-ylimino)thiazolidin-4-ones (4a–c)

To a solution of 2-chloroacetamido-4-(4-substituted-phenyl)th iazole (**3a–c**) (2 mmol) in ethanol (20 mL), ammonium thiocyanate (4 mmol, 0.3 gm) is added then the reaction mixture was heated under reflux for 24 h. The separated solid was filtered, washed with water, dried then crystallized from ethanol to afford corresponding compounds **4a–c**. Physical and spectral data for **4a–c** are listed below.

*4.1.2.1. 2-(4-(4-Nitrophenyl)thiazol-2-ylimino)thiazolidin-4-one (**4a**).* Yield: 50%; yellow solid; mp 300–302 °C; IR (KBr): 3428 (NH), 3100, 3052 (CH aromatic), 2920 (CH aliphatic), 1716 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.07 (s, 2H, CH_2), 8.18–8.19 (m, 3H, nitrophenyl H-2, H-6, thiazole H-5), 8.31 (d, J = 8.4 Hz, 2H, nitrophenyl H-3, H-5), 12.21(s, 1H, NH, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 35.5 (thiazolidinone C-5), 115.5 (thiazole C-5), 124.7 (nitrophenyl C-3, C-5), 127.0 (nitrophenyl C-2, C-6), 140.3 (nitrophenyl C-1), 147.0 (nitrophenyl C-4), 149.1 (thiazole C-4), 164.7 (thiazolidinone C-2), 170.1 (thiazole C-2), 174.6 (C=O); MS (m/z): 320 ($M^{+\cdot}$, 100%); Anal. Calcd for $C_{12}H_8N_4O_3S_2$: C, 44.99; H, 2.52; N, 17.49. Found: C, 44.96; H, 2.33; N, 17.20.

*4.1.2.2. 2-(4-(4-Isobutylphenyl)thiazol-2-ylimino)thiazolidin-4-one (**4b**).* Yield: 40%; brown solid; mp 250–252 °C; IR (KBr): 3136 (NH), 3030 (CH aromatic), 2935, 2821 (CH aliphatic), 1725 (C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.87 (d, J = 6 Hz, 6H, $CH_2CH(CH_3)_2$), 1.83–1.86 (m, 1H, $CH_2CH(CH_3)_2$), 2.47 (d, J = 6.4 Hz, 2H, $CH_2CH(CH_3)_2$), 4.03 (s, 2H, CH_2), 7.22 (d, J = 8 Hz, 2H, isobutylphenyl H-3, H-5), 7.74 (s, 1H, thiazole H-5), 7.85 (d, J = 8 Hz, 2H, isobutylphenyl H-2, H-6), 12.12 (s, 1H, N*H*, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 22.5 ($CH_2CH(CH_3)_2$), 30.0 ($CH_2CH(CH_3)_2$), 35.4 (thiazolidinone C-5), 44.7 ($CH_2CH(CH_3)_2$), 110.1 (thiazole C-5), 125.9 (isobutylphenyl C-2, C-6), 129.8 (isobutylphenyl C-3, C-5), 132.0 (isobutylphenyl C-1), 141.7 (isobutylphenyl C-4), 151.5 (thiazole C-4), 164.0 (thiazolidinone C-2), 169.3 (thiazole C-2), 174.9 (C=O); MS (m/z): 331 ($M^{+\cdot}$, 100%); Anal. Calcd for $C_{16}H_{17}N_3OS_2$: C, 57.98; H, 5.17; N, 12.68. Found: C, 57.66; H, 5.36; N, 12.58.

*4.1.2.3. 2-(4-(4-Methanesulfonamidophenyl)thiazol-2-ylimino)thiazolidin-4-one (**4c**).* Yield: 45%; buff solid; mp 260–262 °C; IR (KBr): 3155, 3113 (2 NH), 3063 (CH aromatic), 2923, 2867 (CH aliphatic), 1730(C=O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.35 (s, 3H, CH_3), 4.05 (s, 2H, CH_2), 7.15 (s, 1H, methanesulfonamido NH, D_2O exchangeable), 7.59 (d, J = 8.4 Hz, 2H, 4-methanesulfonamidophenyl H-3, H-5), 7.98 (s, 1H, thiazole H-5), 8.04 (d, J = 8.4 Hz, 2H, 4-methanesulfonamidophenyl H-2, H-6), 12.12 (s, 1H, N*H*, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 35.4 (thiazolidinone C-5), 43.5 (CH_3), 112.8 (thiazole C-5), 127.1 (4-methanesulfonamidophenyl C-3, C-5), 131.9 (4-methanesulfonamidophenyl C-2, C-6), 133.7 (4-methanesulfonamidophenyl C-1), 135.9 (4-methanesulfonamidophenyl C-4), 150.1 (thiazole C-4), 164.3 (thiazolidinone C-2), 169.7 (thiazole C-2), 174.7 (C=O); MS (m/z): 367 (M^{-1}, 100%), 368 ($M^{+\cdot}$, 21%); Anal. Calcd for $C_{13}H_{12}N_4O_3S_3$: C, 42.38; H, 3.28; N, 15.21. Found: C, 42.49; H, 3.25; N, 15.45.

*4.1.3. General method for preparation of 3-(4-aminosulfonyl-phenylamino)-2-aryl-5-methyl-4-thiazolidinones (**8a–e**)*

A mixture of the appropriate phenyl hydrazones (**7a–e**, 2 mmol) and excess of thiolactic acid (2 mL) was heated at 60 °C for 3 h. The reaction was cooled then saturated solution of sodium carbonate (3 × 20 mL) was added and the precipitate was filtered. The residue was washed with water (1 × 10 mL), dried and crystallized from aqueous ethanol to afford 4-thiazolidinone derivatives **8a–e**. Physical and spectral data for **8a–e** are listed below.

*4.1.3.1. 3-(4-Aminosulfonylphenylamino)-5-methyl-2-phenyl-4-thiazolidinone (**8a**).* Yield: 50%; white solid; mp 138–140 °C; IR (KBr): 3267 (NH), 3091 (CH aromatic), 2928 (CH aliphatic), 1689 (C=O), 1328, 1154 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.52, 1.54 (2d, J = 7.2 Hz, 3H, thiazolidinone CH_3), 4.14–4.28 (m, 1H, thiazolidinone H-5), 5.87, 5.92 (2s, 1H, thiazolidinone H-2), 6.70–6.74 (m, 2H, aminosulfonylphenyl H-2, H-6), 7.07 (s, 2H, NH_2, D_2O exchangeable), 7.33–7.43 (m, 5H, phenyl H-2, H-3, H-4, H-5, H-6), 7.56–7.61 (m, 2H, aminosulfonylphenyl H-3, H-5), 8.73, 8.80 (2s, 1H, N*H*, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 14.6, 19.6 (thiazolidinone CH_3), 17.7, 20.3 (thiazolidinone C-5), 37.7, 38.6 (thiazolidinone C-2), 111.6, 111.8 (aminosulfonylphenyl C-2, C-6), 126.5 (phenyl C-4), 127.6, 127.7 (phenyl C-2, C-6), 127.9 (phenyl C-3, C-5), 129.1, 129.3 (aminosulfonylphenyl C-3, C-5),

133.9, 134.6 (aminosulfonylphenyl C-4), 134.7, 135.7 (phenyl C-1), 148.2, 149.9 (aminosulfonylphenyl C-1), 172.1 (C=O); MS (*m*/*z*): 363 ($M^{+\cdot}$, 39%), 77 (100%); Anal. Calcd for $C_{16}H_{17}N_3O_3S_2$: C, 52.87; H, 4.71; N, 11.56. Found: C, 52.99; H, 4.79; N, 11.71.

*4.1.3.2. 3-(4-Aminosulfonylphenylamino)-5-methyl-2-(4-methylphenyl)-4-thiazolidinone (**8b**).* Yield: 40%; white solid; mp 145–147 °C; IR (KBr): 3285 (NH), 3096 (CH aromatic), 2925 (CH aliphatic), 1690 (C=O), 1330, 1154 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.52, 1.54 (2d, J = 7.2 Hz, 3H, thiazolidinone CH_3), 2.29 (s, 3H, methylphenyl CH_3), 4.12–4.26 (m, 1H, thiazolidinone H-5), 5.82, 5.87 (2s, 1H, thiazolidinone H-2), 6.69–6.73 (m, 2H, aminosulfonylphenyl H-2, H-6), 7.06 (s, 2H, NH_2, D_2O exchangeable), 7.17–7.30 (m, 4H, methylphenyl H-2, H-3, H-5, H-6), 7.56–7.60 (m, 2H, aminosulfonylphenyl H-3, H-5), 8.67, 8.74 (2s, 1H, N*H*, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 19.7, 20.2 (thiazolidinone CH_3), 21.23 (methylphenyl CH_3), 21.21, 21.4 (thiazolidinone C-5), 37.7, 38.6 (thiazolidinone C-2), 111.6, 111.7 (aminosulfonylphenyl C-2, C-6), 126.5 (methylphenyl C-3, C-5), 127.6, 127.9 (methylphenyl C-2, C-6), 129.6, 129.7 (aminosulfonylphenyl C-3, C-5), 134.5, 134.6 (aminosulfonylphenyl C-4), 138.5, 138.6 (methylphenyl C-1), 138.8 (methylphenyl C-4), 149.8, 149.9 (aminosulfonylphenyl C-1), 171.7 (C=O); MS (*m*/*z*): 377 ($M^{+\cdot}$, 62%), 137 (100%); Anal. Calcd for $C_{17}H_{19}N_3O_3S_2$: C, 54.09; H, 5.07; N, 11.13. Found: C, 54.23; H, 5.18; N, 11.30.

*4.1.3.3. 3-(4-Aminosulfonylphenylamino)-2-(4-chlorophenyl)-5-methyl-4-thiazolidinone (**8c**).* Yield: 60%; white solid; mp 150–152 °C; IR (KBr): 3276 (NH), 3095 (CH aromatic), 2929 (CH aliphatic), 1692 (C=O), 1328, 1154 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.52, 1.54 (2d, J = 7.2 Hz, 3H, thiazolidinone CH_3), 4.15–4.28 (m, 1H, thiazolidinone H-5), 5.89, 5.93 (2s, 1H, thiazolidinone H-2), 6.69–6.73 (m, 2H, aminosulfonylphenyl H-2, H-6), 7.08 (s, 2H, NH_2, D_2O exchangeable), 7.42–7.47 (m, 4H, 4-chlorophenyl H-2, H-3, H-5, H-6), 7.56–7.61 (m, 2H, aminosulfonylphenyl H-3, H-5), 8.72, 8.79 (2s, 1H, N*H*, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 17.7, 19.6 (thiazolidinone CH_3), 17.8, 20.3 (thiazolidinone C-5), 37.6, 38.6 (thiazolidinone C-2), 111.6, 111.8 (aminosulfonylphenyl C-2, C-6), 127.6 (chlorophenyl C-3, C-5), 127.73, 127.75 (chlorophenyl C-2, C-6), 129.1, 129.2 (aminosulfonylphenyl C-3, C-5), 133.6 (aminosulfonylphenyl C-4), 133.8 (chlorophenyl C-4), 134.7, 134.8 (chlorophenyl C-1), 149.6, 149.7 (aminosulfonylphenyl C-1), 171.7 (C=O); MS (*m*/*z*): 397 ($M^{+\cdot}$, 48%), 398 (M^{+1}, 10%), 399 (M^{+2}, 21%) 213 (100%); Anal. Calcd for $C_{16}H_{16}ClN_3O_3S_2$: C, 48.30; H, 4.05; N, 10.56. Found: C, 48.47; H, 4.13; N, 10.67.

*4.1.3.4. 3-(4-Aminosulfonylphenylamino)-2-(4-fluorophenyl)-5-methyl-4-thiazolidinone (**8d**).* Yield: 35%; white solid; mp 140–142 °C; IR (KBr): 3281 (NH), 3089 (CH aromatic), 2929 (CH aliphatic), 1693 (C=O), 1329, 1154 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.53, 1.55 (2d, J = 7.2 Hz, 3H, thiazolidinone CH_3), 4.15–4.30 (m, 1H, thiazolidinone H-5), 5.89, 5.93 (2s, 1H, thiazolidinone H-2), 6.68–6.72 (m, 2H, aminosulfonylphenyl H-2, H-6), 7.07 (s, 2H, NH_2, D_2O exchangeable), 7.19–7.23 (m, 2H, fluorophenyl H-3, H-5), 7.49–7.61 (m, 4H, flourophenyl H-2, H-6, aminosulfonylphenyl H-3, H-5), 8.70, 8.78 (2s, 1H, N*H*, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 17.5, 19.6 (thiazolidinone CH_3), 17.7, 20.2 (thiazolidinone C-5), 37.7, 38.6 (thiazolidinone C-2), 111.6, 111.8 (aminosulfonylphenyl C-2, C-6), 115.8, 116.0 (flourophenyl C-3, C-5), 127.6, 127.7 (flourophenyl C-2, C-6), 130.0, 130.1 (aminosulfonylphenyl C-3, C-5), 134.6, 134.7 (aminosulfonylphenyl C-4), 149.6, 149.8 (flourophenyl C-1), 161.3, 161.4 (aminosulfonylphenyl C-1), 163.8, 163.9 (flourophenyl C-4), 171.9 (C=O); MS (*m*/*z*): 381 ($M^{+\cdot}$, 33%), 213 (100%); Anal. Calcd for $C_{16}H_{16}FN_3O_3S_2$: C, 50.38; H, 4.23; N, 11.02. Found: C, 50.49; H, 4.31; N, 11.17.

*4.1.3.5. 3-(4-Aminosulfonylphenylamino)-5-methyl-2-(4-trifluoromethylphenyl)-4-thiazol-idinone (**8e**).* Yield: 45%; white solid; mp 143–145 °C; IR (KBr): 3269 (NH), 3095 (CH aromatic), 2931 (CH aliphatic), 1696 (C=O), 1326, 1157 (SO_2) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.53, 1.55 (2d, J = 7.6 Hz, 3H, thiazolidinone CH_3), 4.19–4.32 (m, 1H, thiazolidinone H-5), 6.02, 6.06 (2s, 1H, thiazolidinone H-2), 6.72–6.76 (m, J = 8.8 Hz, 2H, aminosulfonylphenyl H-2, H-6), 7.09 (s, 2H, NH_2, D_2O exchangeable), 7.58–7.71 (m, 4H, trifluoromethylphenyl H-2, H-3, H-5, H-6), 7.73–7.78 (m, 2H, aminosulfonylphenyl H-3, H-5), 8.81, 8.87 (2s, 1H, N*H*, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) δ 15.5, 19.4 (thiazolidinone CH_3), 18.0, 20.4 (thiazolidinone C-5), 37.6, 38.7 (thiazolidinone C-2), 111.7, 111.9 (aminosulfonylphenyl C-2, C-6), 123.1, 125.8 (trifluoromethyl), 126.1, 126.9 (trifluoromethylphenyl C-3, C-5), 127.7, 127.8 (trifluoromethylphenyl C-2, C-6), 127.9, 128.9 (aminosulfonylphenyl C-3, C-5), 129.4, 129.7 (trifluoromethylphenyl C-4), 134.6, 134.9 (aminosulfonylphenyl C-4), 145.2, 147.8 (trifluoromethylphenyl C-1), 149.5, 149.7 (aminosulfonylphenyl C-1), 172.0 (C=O); MS (*m*/*z*): 431 ($M^{+\cdot}$, 1%), 64 (100%); Anal. Calcd for $C_{17}H_{16}F_3N_3O_3S_2$: C, 47.32; H, 3.74; N, 9.74. Found: C, 47.45; H, 3.78; N, 9.88.

4.2. Biological evaluation

4.2.1. In vitro cyclooxygenase inhibition assay

The ability of the tested compounds to inhibit both COX-1 and COX-2 isozymes was measured using colorimetric COX (ovine) Inhibitor Screening Assay Kit (Kit catalog number 760111, Cayman Chemical, Ann Arbor, MI, USA) following the manufacturer's instructions and as mentioned before [51]. Different concentrations of celecoxib or tested compounds were incubated with the enzymes for a period of 5 min at 25 °C. After the incubation period an addition of the colorimetric substrate and arachidonic acid was done then the absorbance was measured at 590 nm using plate reader.

4.2.2. In vivo anti-inflammatory activity

White albino adult female mice, weighing approximately 18–22 g were used throughout the study of anti-inflammatory activity. While, white adult male albino rats weighing approximately 100–150 g were used throughout the study of ulcerogenic liability. The animals were housed in micro isolator cages (five per cage) at laboratory temperature of 24 ± 1 °C with 40–80% relative humidity with access to food and water. The animals were allowed to adapt to the experimental environment for 7 days before experimentation. All procedures relating to animal care and treatments were performed following the protocols approved by the Research Ethical Committee, Beni-Suef University (2014-Beni-Suef, Egypt). The white albino mice were divided into ten groups of ten animals each. The first group was acting as a negative control given 5% aqueous solution of DMSO (v/v). The second groups were orally administered celecoxib as a reference standard (10 mg/kg). The eight tested compounds **4a**–**c** and **8a**–**e** in the form of 5% aqueous solution of DMSO were given orally to the rest groups, one group for each compound, in which each compound was administered in a dose 100 mg/kg body weight and treatment began 1 h before induction of inflammation. Induction of Paw edema was performed by single sub-plantar injection of 0.02 ml of freshly prepared 1% carrageenan (Sigma) in normal saline [30]. The paw thickness was measured using vernier calipers and the difference between paw volumes was calculated after 1, 3 and 5 h of carrageenan injection. The anti-inflammatory activity was calculated as percentage inhibition of edema thickness in treated animals in comparison to the control group, Table 2 according to the following formula

$$\text{Edema inhibition } (\%) = ((N_a - N_b)/N_a) \times 100$$

In which, N_a was the average difference in thickness between the left and right hind paw of control group and N_b was that of drug-treated group.

Also, the mean effective anti-inflammatory doses (ED_{50}) for the prepared compounds showed the highest *in vitro* COX-2 inhibitory potency and *in vivo* anti-inflammatory activity **8c** and **8d** were determined. White albino mice were divided into ten groups of ten animals each. In all groups, induction of inflammation was performed by single sub-plantar injection of 0.02 mL of freshly prepared 1% carrageenan (Sigma) in normal saline [30]. The first group was acting as a negative control given 5% aqueous solution of DMSO (v/v). The second, third and fourth groups were orally administered celecoxib as a reference standard in three different doses 5, 10 and 15 mg/kg, one dose for each group, to calculate ED_{50}. The two tested compounds **8c** and **8d** in the form of 5% aqueous solution of DMSO were given orally to the rest groups, three groups for each compound, in which each compound was administered in three different doses 5, 10 and 15 mg/kg body weight to calculate ED_{50} for each compound and treatment began 1 h before induction of inflammation. The paw thickness was measured using vernier calipers and the difference between paw volumes was calculated after 3 h of carrageenan injection. On the basis of the obtained results, the corresponding mean effective anti-inflammatory doses (ED_{50}) were calculated according to the method of Litchfield and Wilcoxon [31].

4.2.3. Ulcerogenic liability

The ulcerogenic effect of **8c**, **8d**, celecoxib and ibuprofen was evaluated according to a previously reported method [32]. Twenty-five adult male albino rats were used in this study and divided into five groups. All animals were allowed to fast for 18 h before the administration of the drugs. The first group received 10% aqueous solution of DMSO (v/v) and kept as a control group. The second group received celecoxib as the first reference drug in a dose 50 mg/kg. The third group received Ibuprofen as the second reference drug in a dose 50 mg/kg. The other groups received the prepared compounds **8c** and **8d** in a dose 50 mg/kg. After 2 h of administration of drugs, Animals were fed. Rats were given orally the required dose for three successive days. After 2 h of the last given dose, rats were sacrificed; the stomach of each rat was removed and opened along the greater curvature then rinsed with sodium chloride 0.9%. In order to examine the stomach, stretching it by pins on a corkboard was done. With the aid of a magnifying lens (10 xs) ulcers were searched in the stretched stomach. Calculation of the ulcer index was done as previously reported [32] in which, ulcers were classified into levels, level I, ulcer area less than 1 mm^2, level II, ulcer area is 1–3 mm^2 and level III, ulcer area more than 3 mm^2, and the ulcer index was calculated according to the following formula:

$$\text{Ulcer index (UI)} = 1 \times (\text{number of ulcers level I}) + 2 \times (\text{number of ulcers level II}) + 3 \times (\text{number of ulcers level III}), \text{etc.}$$

4.3. Molecular modeling studies

All molecular modeling and docking studies were performed by Molecular Operating Environment MOE version 2008.10 [34]. The Structures of **4a**, **4b**, **8c** and **8d** were built in MOE. They were 3D protonated and subjected to energy minimization using MMFF94 × forcefield with 0.05 gradient. The X-ray crystal structure of celecoxib bound to the *musmusculus* COX-2 active site (PDB: 3LN1) was obtained from the protein data bank [33]. Preparation of the enzyme for docking was achieved as follows: (1) The Co-crystallized ligand and water molecules were removed. (2) The enzyme was 3D protonated, in which hydrogen atoms were added to their standard geometry, the partial charges were computed and the system was optimized. The conformers generated were docked into the COX-2 receptor with MOE-DOCK using the triangle matcher placement method and the London dG scoring function. A molecular mechanics force field refinement was carried out on the top 100 poses generated. In order to validate the docking procedure, celecoxib was docked into the active site of 3LN1. The highest docking score for each ligand–enzyme complex was selected.

5. Conflict of Interest

The authors have declared no conflict of interest.

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