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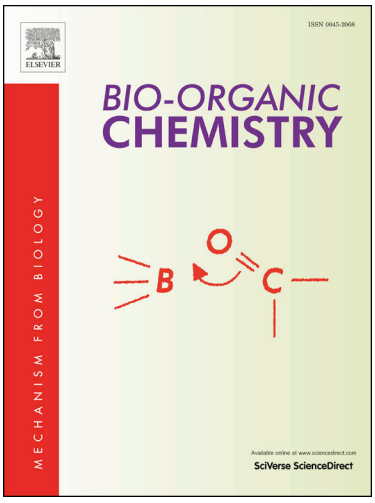

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Further insight into the dual COX-2 and 15-LOX anti-inflammatory activity of 1,3,4-thiadiazole-thiazolidinone hybrids: The contribution of the substituents at 5th positions is size dependent

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Abstract

The current study reports the design, synthesis, full characterization and biological investigation of new 15-LOX/COX dual inhibitors based on 1,3-thiazolidin-4-one (15-lipoxygenase pharmacophore) and 1,3,4-thiadiazole (COX pharmacophore) scaffolds. This series of molecular modifications is an extension of a previously reported series to further explore the structural activity relationship. Compounds **3a**, **4e**, **4n**, **4q, 7** and **8** capable of inhibiting 15-LOX at (2.74, 4.2, 3.41, 10.21, 3.71 and 3.36 μM, respectively) and COX-2 at (0.32, 0.28, 0.28, 0.1, 0.28 and 0.27 μM, respectively). The results revealed that binding to 15-LOX and COX is sensitive to the bulkiness of the substituents at the 5 positions. 15-LOX bind better with small substituents, while COXs bind better with bulky substituents. Compounds **3a**, **4r** and **4q** showed comparable *in vivo* anti-inflammatory activity to the reference drug (celecoxib). The ulcer liability test showed no sign of ulceration which ensures the safe gastric profile. Docking study was performed to explore the possible mode of interaction of the new compounds with the active site of human 15-LOX and COX-2. This study discloses some structural features for binding to 15-LOX and COX, thus pave the way to design anti-inflammatory agents with balanced dual inhibition of these enzymes.

Keywords: Anti-inflammatory; 15-LOX; COX; inhibitory potency; 1,3,4-thiadiazole; 1,3-thiazolidin-4-one; structure-activity; safety

1. **Introduction**

Lipoxygenases (LOX) belongs to the family of non-heme iron-containing enzymes which catalyze lipid peroxidation [1]. They classified according to the peroxidation site of arachidonic acid (AA) into 5-LOX, 12-LOX and 15-LOX [1]. They have been implicated in a number of physiological processes and in the pathogenesis of inflammatory, hyperproliferative and neurodegenerative diseases [1]. LOX enzymes which are well conserved among mammalian species [2], catalyze the production of pro-inflammatory mediators from arachidonic acid (AA) like leukotrienes, eoxins and lipoxins [3-5]. 15-Lipoxygenase (15-LOX) is one of the major metabolic pathways that converts arachidonic acid into 15-Hydroxyeicosatetraenoic acid (15-HETE) and other pro-inflammatory mediators [6, 7]. Evidence continues to reveal the role of 15-LOX and its metabolites in a plethora of diseases [6]. Recent literature showed the direct contribution of 15-HETE in airway inflammation [7], induced dysfunction of the retina in diabetic retinopathy [8], various types of cancer [9-11], osteoarthritis [12] and multiple sclerosis [13]. To date, zileuton which acts by chelating the iron metal located in the active site of the LOX enzyme, is the only approved LOX inhibitor [14]. However, it has unfavourable pharmacokinetic properties and has been related with liver toxicity [15, 16]. Moreover, the increased demand on anti-LOX therapies has enhanced the interest in developing new safe and effective LOX inhibitors. Although several lipoxygenase inhibitors have been reported [17, 18], it is proved to be challenging to generate potent small inhibitors with favourable physicochemical properties [19]. In addition to LOX enzymes, cyclooxygenases (COX-1 and COX-2) is the other metabolic pathway of arachidonic acid [20, 21]. They convert arachidonic acid into prostaglandin, thromboxane, and prostacyclin which play a major role in inflammation [21]. However, the use of cyclooxygenase inhibitors (NSAIDs) shunts the metabolism of arachidonic acid toward 15-LOX [22]. In fact, this can worsen the patient's condition if the 15-LOX enzyme has a significant role in the pathogenesis of the disease like in asthma [23-25]. Accordingly, the use of multitarget-directed ligands (MTDLs) to inhibit both enzymes (15-LOX and COX) is a promising strategy in drug therapy with maximum efficiency and minimal side effects [26-28]. On the other hand, 4-thiazolidinone and 1,3,4-thiadiazole are common scaffolds in anti-inflammatory agents. For example, a series of compounds containing 4-thiazolidinone moiety (**I** and **II** Fig.1) has been reported to inhibit 15-LOX enzyme with IC_{50} (17.7 and 52 μM, respectively) [29, 30]. However, 1,3,4-thiadiazole ring containing compounds (**III** and

IV Fig. 1) explored significant inhibition to COX-2 enzyme (IC_{50} = 0.42 and 0.09 µM, respectively) [31, 32]. Therefore, it seems interesting to investigate the anti-inflammatory activity of 1,3,4-thiadiazole-thiazolidinone hybrid and its structural modifications.

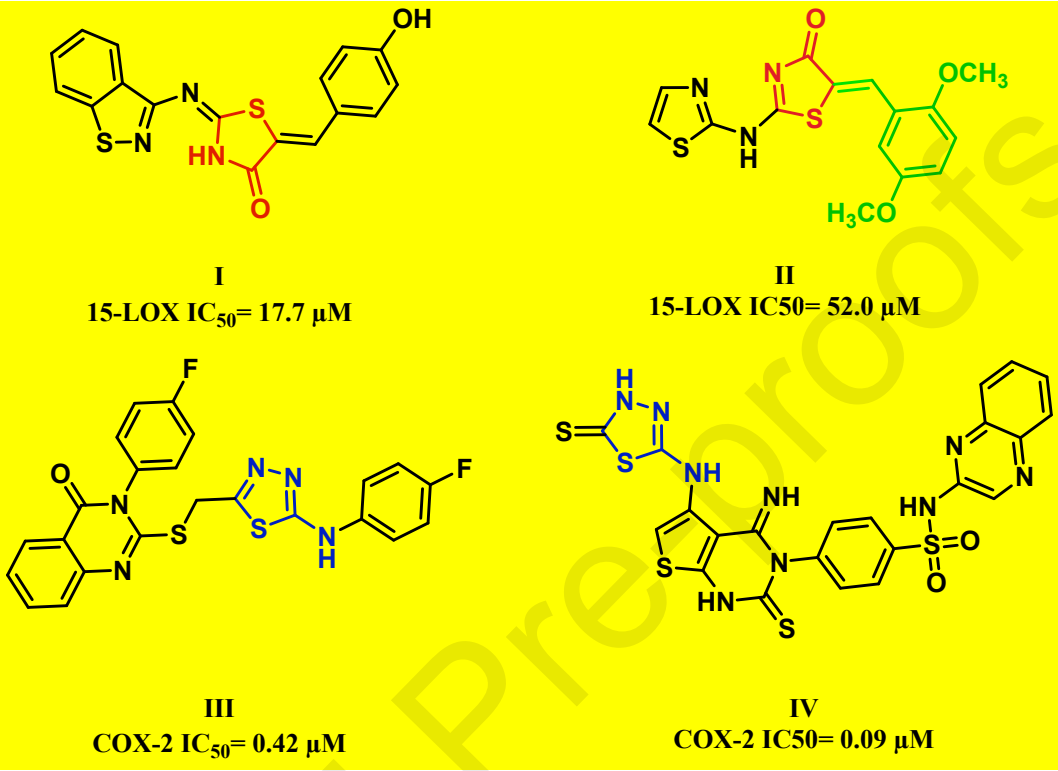


Fig. 1. Some reported compounds with 15-LOX and COX-2 inhibitory activities.

In a previous study we developed a series of 1,3,4-thiadiazole-thiazolidinone hybrids as potent dual COX-2 and 15-LOX inhibitors with good *in vivo* anti-inflammatory activity by structural modifications at the 5th position of thiazolidinone moiety [33]. In the current study, the contribution of the substituents at the 5th positions of thiadiazole and thiazolidinone moieties to the anti-inflammatory activity was investigated.

2. Result and discussion

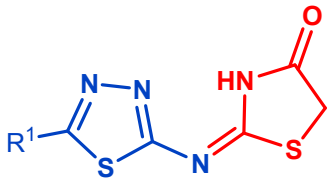
2.1. Design

As mentioned above, in a recent study from our group, hybrid compounds containing both 1,4-thiazoldin-4-one (15-LOX pharmacophore) and 1,3,4-thiadiazole (COX pharmacophore) were capable of inhibiting both 15-LOX and COX enzymes at low micromolar concentration [33]. Also, it was found that the 15-LOX inhibitory potency of the test compound was inversely proportional to the volume of the substituent at 5th

position of 1,4-thiazolidin-4-one ring, while the activity against COX-2 enzyme has a direct relation [33]. To get further insight into the SAR of this class of 15-LOX inhibitors, we developed compounds **3a-i** (**Scheme 1**). This series were synthesized to study the contribution of the substituent at the 5th position of 1,3,4-thiadiazole moiety to the anti-inflammatory activity. Accordingly, *p*-hydroxyphenyl moiety in the recently published series [33] was replaced with methyl **3a** or un/substituted phenyl **3b-i (**Fig. 2). The volume of the compounds was calculated using MOE software. Also, the new compounds were tested for their 15-LOX and COX inhibitory activity. From data shown in Table 1, it is clear that the inhibitory activities against 15-LOX are inversely proportional to the size of the substituents at the 5th position of the 1,3,4-thiadiazole ring. Whereas the unsubstituted phenyl and methyl group (**3a** and **3b**) showed the highest potencies, the 3,4,5-trimethoxyphenyl (**3i**) showed the lowest one. Despite the fact that the methyl (**3a**) and the unsubstituted phenyl group (**3b**) have almost similar potency against 15-LOX, the methyl group (**3a**) has better selectivity against COX-2 enzyme. For this reason, compound **3a** was chosen as lead compound for further optimization by using the substitutions on arylidene moiety (5th position of 1,4-thiazolidin-4-one) to modify the inhibitory potency against both 15-LOX and COX enzymes (Scheme 2). Additionally, ulcer liability test was performed to ensure the safety of gastric profile.

Table 1.

15-LOX, COX-1, and COX-2 IC50 of the synthesized compounds 3a-i and references drugs.



Compounds No.	R^1	15-LOX IC$_{50}$[a] (µM)	COX-1 IC$_{50}$ (µM)	COX-2 IC$_{50}$ (µM)	Selectivity index[b]	Volume[c]
3a	Methyl	**2.74**	3.98	0.32	**12.44**	**168.1**
3b	Phenyl	**2.54**	3.87	0.44	**8.80**	**231.3**
3c	2-hydroxyphenyl	4.13	5.21	0.45	11.58	236.3
3d	4-hydroxyphenyl	3.11	6.48	0.41	16.86	
3e	4-methylphenyl	4.86	6.78	0.42	16.14	247.1
3f	4-bromophenyl	7.64	8.74	0.34	25.71	256.4
3g	4-methoxyphenyl	4.33	5.41	0.36	15.03	257.3
3h	3,4- dimethoxyphenyl	5.22	6.33	0.29	21.83	282.1

3i	3,4,5- trimethoxyphenyl	**7.89**	9.11	0.19	**47.95**	**307.9**
Celecoxib	-	-	15.1	0.049	308.16	-
Diclofenac Sod.	-	-	5.37	0.32	16.78	-
Zileuton	-	15.6	-	-	-	-

[a] IC_{50} value represents the concentration of the compound that produce 50% inhibition of COX-1, COX-2, or 15-LOX 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}). [c]calculated by vol descriptor

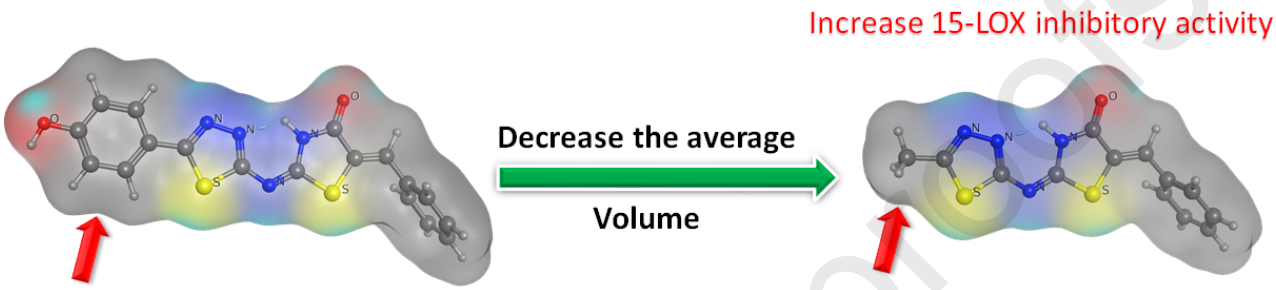
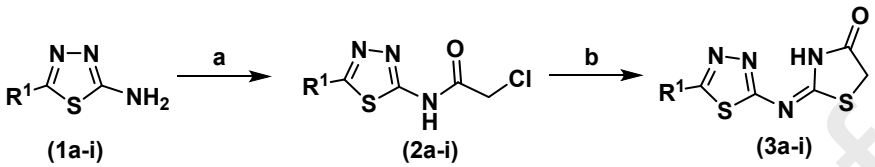


Fig. 2. The design of new compounds with higher 15-LOX activity.

2.2. Chemistry

The synthesis of thiazolidinones **3a-i** and 5-arylidenes of **3a** (**4a-r** and **5-8)** is illustrated in Schemes 1 and 2, respectively. The synthesis of compound **3d** was recently reported by our group [33]. Firstly, 1,3,4-thiadiazole-2-amines (**1a-i**) were synthesized from the appropriate carboxylic acid by reaction with thiosemicarbazide and phosphorus oxychloride as reported [33-35]. These were chloroacetylated by refluxing with chloroacetyl chloride in dry benzene to give **2a-i** [36, 37]. Heterocyclization of **2a-i** by refluxing with ammonium thiocyanate in ethanol afforded the key thiazolidinones **3a-i**. Cyclization was confirmed by the slight shifting of the CH_2 signal to lower δ 4.05-4.10 in ^{1}H-NMR spectra. Their IF-IR spectra showed increase of the C=O frequency to 1712-1737 cm^{-1} due to formation of five-membered ring. These compounds are fully characterized, and their spectral data and the elemental analysis correlate very well with the proposed structures. As shown in Scheme 2, Knoevenagel condensation of the 1,3-thiazolidin-4-one active methylene of **3a** with different aldehydes using acetic acid/ sodium acetate buffer afforded compounds (**4a-r** and **5-8**). The structures of these compounds were confirmed by shifting of the amidic carbonyl peaks to lower

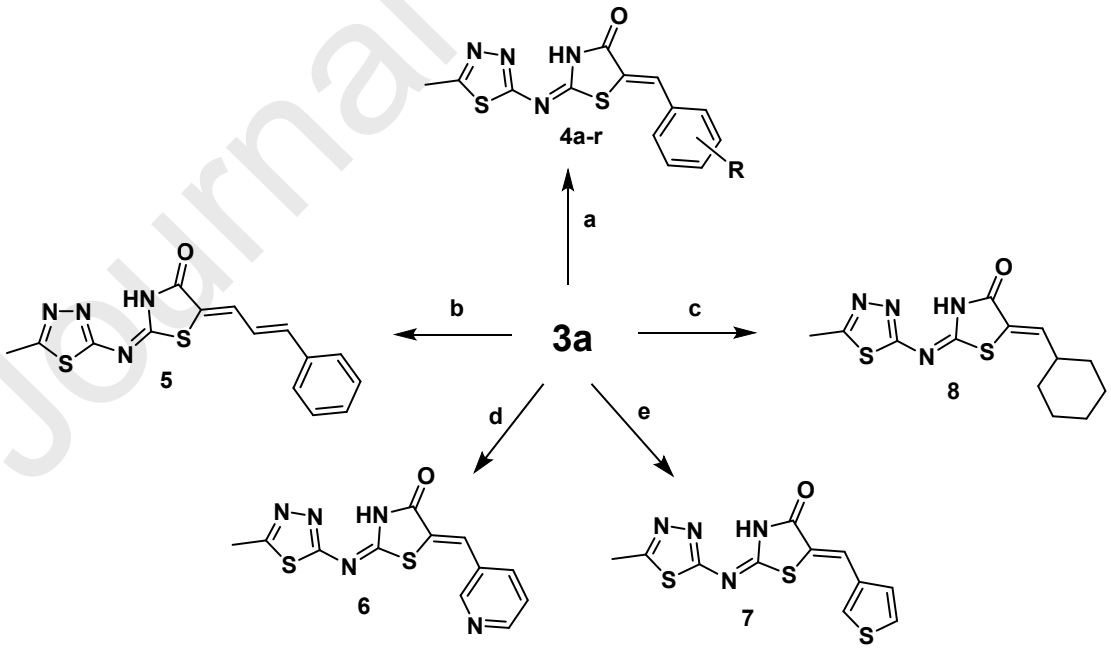
wavenumber due to conjugation (range from 1724 to 1683 to cm^{-1}) in the IR spectrum and the disappearance of the active methylene signal at 4.08 ppm in the ^{1}H-NMR. Moreover, the disappearance of the active methylene carbon signal (at 35.90 ppm) in ^{13}C-NMR spectrum confirms cyclization. Verification of all synthesized compounds purity was performed using elemental analysis and their chemical structures were fully



R^1	2-amino	2-Chloroacetamido	Thiazolidinone
CH_3	1a	2a	3a
Ph	1b	2b	3b
2-OH-Ph	1c	2c	3c
4-OH-Ph	1d	2d	3d
4-CH_3-Ph	1e	2e	3e
4-Br-Ph	1f	2f	3f
4-CH_3O-Ph	1g	2g	3g
3,4-$(CH_3O)_2$-Ph	1h	2h	3h
3,4,5-$(CH_3O)_3$Ph	1i	2i	3i

characterized by IR, NMR and mass spectrometry.

Scheme 1. Synthesis of 1,3,4-thiadiazole-thiazolidinone hybrids. Reagents and conditions: (a) $ClCH_2COCl$, benzene, reflux, 3-5 h, 76-96% yield; (b) NH_4SCN, EtOH, reflux, 3-5 h, 83-93% yield.



Scheme 2. Synthesis of target compounds **4a-r** and **5–8**. Reagents and conditions: Appropriate aldehyde (a: substituted benzaldehyde, b: cinnamaldehyde, c:

cyclohexanecarboxaldehyde, d: pyridine-3-carboxaldehyde, e: thiophene-3-carboxaldehyde), sodium acetate/ acetic acid buffer, reflux, 24 h, 55–78% yield.

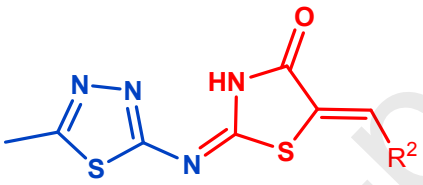
2.3. Biology

2.3.1. In vitro Lipoxygenase inhibitory assay

The synthesized compounds were tested in vitro for their inhibitory potencies against 15-LOX using 15-LOX enzyme of soybean. The IC50s are shown in Table 2.

Table 2

15-LOX, COX-1, and COX-2 IC_{50} of the synthesized compounds **4a-r**, **5-8** and references drugs



Compounds No.	R^2	15-LOX IC_{50}[a] (µM)	COX-1 IC_{50} (µM)	COX-2 IC_{50} (µM)	Selectivity index[b]
4a	4-methylphenyl	**3.11**	4.98	0.33	15.09
4b	4-propylphenyl	5.24	6.33	0.26	24.35
4c	4-isopropylphenyl	8.33	9.24	0.17	54.35
4d	4-hydroxyphenyl	5.47	6.25	0.37	16.89
4e	2- hydroxyphenyl	4.21	5.42	0.28	19.36
4f	3,4-dihydroxyphenyl	10.24	11.42	0.11	103.82
4g	4-methoxyphenyl	5.23	6.41	0.34	18.85
4h	3,4-dimethoxyphenyl	5.87	7.24	0.41	17.66
4i	3,4,5-trimethoxyphenyl	10.94	12.34	**0.1**	**123.40**
4j	4-hydroxy-3-methoxyphenyl	4.62	5.63	0.38	14.82
4k	4-dimethylaminophenyl	8.42	9.71	0.13	74.69
4l	4-chlorophenyl	6.17	7.42	0.32	23.19
4m	3-chlorophenyl	4.65	7.42	0.31	23.94
4n	2-chlorophenyl	3.41	5.87	0.28	20.96
4o	2,4-dichlorophenyl	7.36	8.42	0.42	20.05
4p	3,4-dichlorophenyl	6.98	8.41	0.2	42.05
4q	4-bromophenyl	10.21	11.42	0.1	114.20
4r	pentafluorophenyl	13.11	15.11	**0.1**	**151.10**
5	2-phenylethenyl	6.55	8.71	0.36	24.19
6	3-pyridyl	4.62	5.74	0.25	22.96
7	3-thienyl	3.71	4.98	0.28	17.79
8	Cyclohexyl	3.36	4.52	0.27	16.74
Celecoxib	-	-	15.1	0.049	308.16

Diclofenac sod.	-	-	5.37	0.32	16.78
Zileuton	-	15.6	-	-	-

[a] IC_{50} value represents the concentration of the compound that produce 50% inhibition of COX-1, COX-2, or 15-LOX 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}). The most promising results are highlighted in bold.

All the synthesized compounds revealed higher potency than zileuton. Compound **3a** devoted from arylidene group showed the highest activity with IC_{50} = 2.74 μM. Within the arylidene series, compound **4a** with 4-methylphenyl exhibited the highest potency (IC_{50} = 3.11 μM) which is slightly lower than **3a**. That means 4-methylphenyl is tolerated. Increasing the length of the aliphatic substituent on the phenyl ring decreased the LOX inhibitory activity (**4b** and **4c**). Compound **4e** with 2-hydroxyphenyl showed higher potency than **4d** with 4-hydroxyphenyl group. Increasing the number of hydroxyl groups led to decrease in the inhibitory activity (**4f**). Similar observation obtained by increasing the number of methoxy groups (**4h**, **4i,** and **4j**). These results may indicate that the interaction at this position is mainly lipophilic in nature. It is worth mentioning that compound **4j** containing 3-methoxy-4-hydroxyphenyl group showed higher potency then both **4d** (with 4-hydroxyphenyl group) and **4h** (with mono methoxyphenyl group). Moreover, Compound **4n** containing 2-chlorophenyl group explored the highest potency among the halogenated phenyl series. Any attempt to change the position (**4l** and **4m**), increase the number of chlorine atom (**4o** and **4p**) or replace chlorine by bromine atom (**4q**) led to decrease in the 15-LOX inhibitory potency. Additionally, Compounds (**7** and **8**) containing (3-thienyl and cyclohexyl group, respectively) exhibited similar activity to **4a**. Finally, the insertion of an ethenyl spacer (**5**) or the replacement of phenyl group with 3-pyridyl group (**6**) decreased the inhibitory activity. The results clearly show that small hydrophobic group, such as 4-methylphenyl, 3-thienyl or cyclohexyl, at the 5th position of thiazolidinone is required for good activity.

2.3.2. In vitro cyclooxygenase inhibitory assay

All the tested compounds showed inhibitory potencies against COX-2 ranging from 0.1 to 0.42 μM and selectivity index ranging from 12.44 to 151.10 (Table 2). The most selective compounds were **4r**, **4i**, **4q,** and **4f** with selectivity index of 151.10, 123.40, 114.20 and 103.82, respectively. Also, these compounds showed higher potency than

Diclofenac sodium. Moreover, compound **3a** that lack of arylidene moiety showed the lowest potency and selectivity among all synthesized compounds. The structure-activity relationship of compound **4i** showed that, decreasing the number of methoxy group resulted in marked decrease in the potency and selectivity (**4g** and **4h**). Similar observation obtained when decreasing the number of hydroxy groups as for compound **4f**. Additionally, replacement of the bromine atom in compound **4q** by chlorine atom results in marked decrease in potency and selectivity (**4l**). Therefore, bulky substituent (4-bromo or trimethoxyphenyl) is required at the 5th position of thiazolidinone moiety for good activity and selectivity. Compounds with phenyl (**4a**), 3-thienyl (**7**) or cyclohexyl (**8**) are potent dual inhibitors of 15-LOX and COX-2 due to presence of moderately bulky substituents. These compounds were also less selective than celecoxib. This property, dual balanced inhibition of COX-2 and 15-LOX, is the basis for developing safe anti-inflammatory agents [27, 28].

2.3.3. In vivo anti-inflammatory activity

Carrageenan-induced paw edema in rats was chosen as an animal model to study the *in vivo* anti-inflammatory activity of the selected compounds (**3a, 4f, 4i, 4q,** and **4r**) against celecoxib as a reference drug (Table 3). The synthesized compounds and celecoxib were administered i.p at a dose of 28 µg/Kg. All the tested compounds reached its maximal activity after 3 h and showed comparable activity (non-significant statistical difference) to the reference drug (Table 3). Compounds (**3a, 4q** and **4r**) exhibited the highest percent of inhibition among the tested compounds.

Table 3

In vivo anti-inflammatory in rats using celecoxib as a reference drug.

Compounds	**% Inhibition of Edema ± SE**			
No.	**1h**	**2 h**	**3 h**	**4 h**
Celecoxib	24.1 ± 2.5 **c**	44.0 ± 3.4**d**	56.1 ± 7.1 **d**	40.0 ± 3.1 **d**
3a	17.2 ± 4.2 **a**	36.0 ± 2.5 **d**	49.5 ± 4.8 **d**	44.8 ± 7.0 **d**
4f	21.8 ± 5.6	52.0 ± 5.8 **d**	45.8 ± 3.5 **d**	27.6 ± 2.3 **d**
4i	8.0 ± 3.6	38.0 ± 3.7 **d**	42.1 ± 1.9 **d**	27.6 ± 2.3 **d**
4q	17.2 ± 5.6	42.0 ± 6.6 **d**	60.7 ± 3.5 **d**	27.6 ± 2.3 **d**
4r	21.4 ± 8.4 **a**	48.0 ± 7.3 **d**	57.9 ± 4.8 **d**	25.7 ± 1.9 **d**

a $P < 0.05$, **b** $P < 0.01$, **c** $P < 0.001$, **d** $P < 0.0001$.

The calculated LogP (Table 4) reflects the high lipophilicity of compounds **4q** and **4r** which may contribute to their high *in vivo* anti-inflammatory activity.

2.3.4. Ulcerogenic liability

Ulcerogenic liability test was performed to ensure the safety of gastric profile for the most active compounds (**3a**, **4i** and **4r**) in the *in vivo* anti-inflammatory test. Double the dose (20 mg/kg) of the *in vivo* test was chosen to measure the tolerability of the stomach at a higher dose. Also, indomethacin at 10 mg/kg was selected as a positive control. After oral administration of the tested compounds and indomethacin, gastric mucosa was examined using light microscope. In compare to the negative control, the gastric mucosa following the administration of the selected compounds showed no sign of ulceration edema or desquamation of the epithelial cells in contrast to indomethacin which showed edema, dilatation of the blood vessel and detached of the epithelial cells (Fig. 3 and 4).

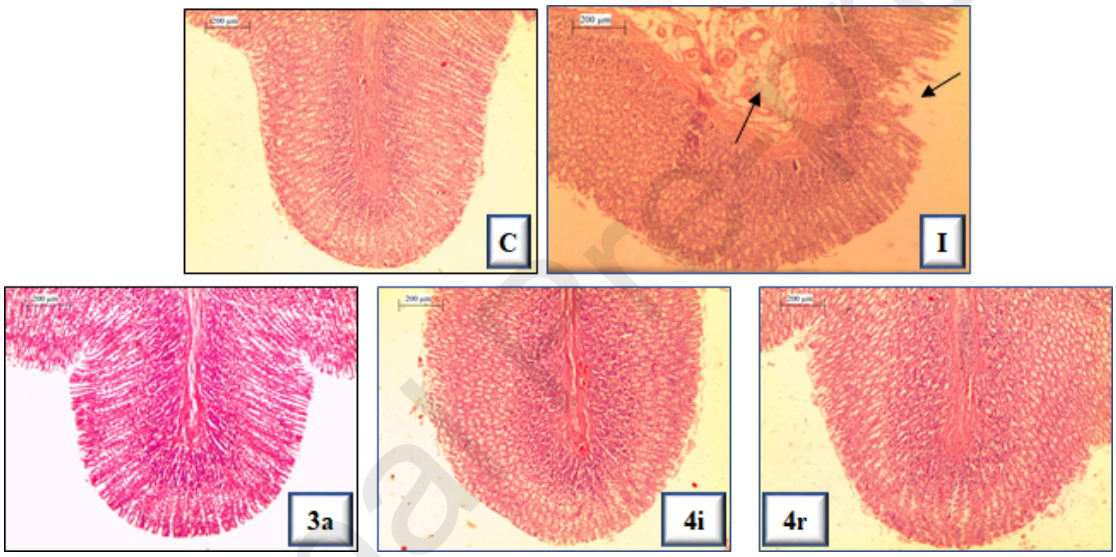


Fig. 3. Gastric mucosa at 4x magnification and 200µm scale bare of the negative control (**C**), tested compounds (**3a**, **4i** and **4r**) and indomethacin (**I**). The arrows in the indomethacin Fig clarify the edema and detached epithelial cells.

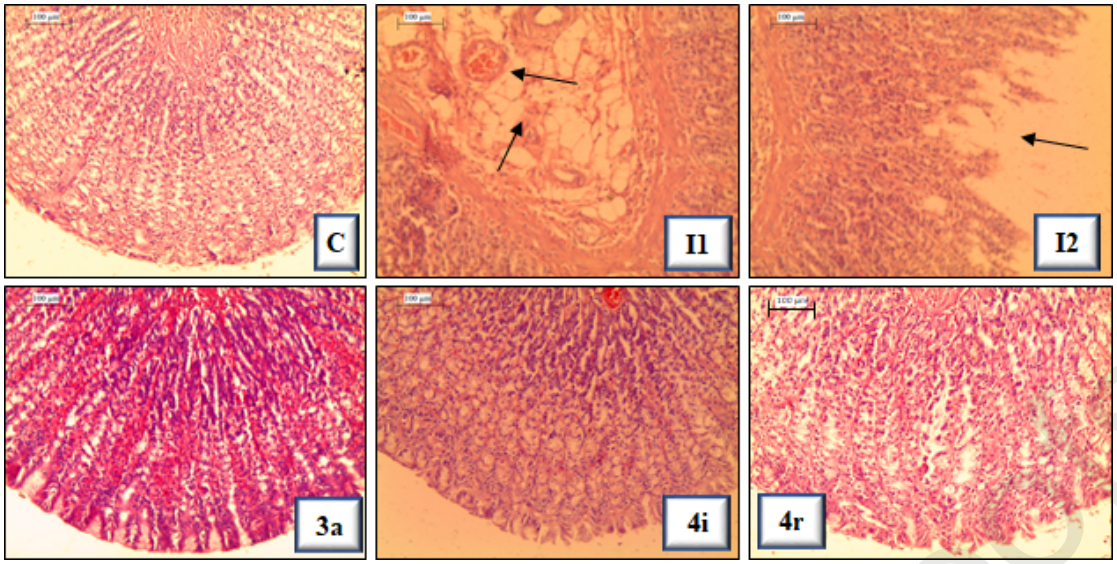


Fig. 4. Gastric mucosa at 10x magnification and 100 µm scale bare of the negative control (**C**), tested compounds (**3a**, **4i** and **4r**) and indomethacin (**I1** and **I2**). The arrows in the indomethacin figures clarify the edema and detached epithelial cells.

2.4. Docking and molecular modelling studies

Descriptors and docking scores were calculated using MOE 2019 software to explain the activity of the synthesized compounds (Table 3). Also, Human 15-LOX enzyme with PDB code (4NRE) and human COX-2 enzyme with PDB code (5KIR) were chosen to perform the docking study.

Table 4. Descriptors and docking scores of the synthesized compounds.

Compound No.	Volume [a]	LogP [b]	M.Wt	ΔG COX-2 [c]	ΔG 15-LOX [c]
3a	168.3	1.08	214.27	-5.87	-5.89
4a	278.4	3.93	316.40	-7.37	-7.58
4b	313.0	4.84	344.45	-6.72	-8.46
4c	312.4	4.77	344.45	-7.52	-8.4
4d	267.4	3.32	318.37	-7.11	-7.32
4e	265.0	3.32	318.37	-7.15	-7.46
4f	272.8	3.05	334.37	-7.23	-7.58
4g	284.5	3.58	332.40	-7.63	-7.81
4h	311.5	3.33	362.43	-6.18	-8.38

4i	340.4	3.07	392.45	-7.46	-8.78
4j	292.8	3.31	348.40	-7.87	-7.52
4k	310.4	3.54	345.44	-7.67	-8.28
4l	275.4	4.22	336.82	-6.98	-7.56
4m	276.9	4.26	336.82	-7.28	-7.06
4n	276.1	4.22	336.82	-7.25	-7.05
4o	289.6	4.85	371.26	-7.14	-7.37
4p	291.1	4.85	371.26	-7.54	-7.78
4q	285.4	4.43	381.27	-7.67	-7.78
4r	275.4	4.38	392.33	-7.15	-7.32
5	290.4	4.27	328.41	-7.50	-7.6
6	253.9	2.39	303.36	-6.98	-7.23
7	249.0	3.28	308.40	-6.48	-7.1
8	278.1	3.64	308.42	-7.19	-7.49
Rofecoxib	-	-	-	-8-49	-

a: calculated by vol descriptor **b:** calculated by LogP (o/w) descriptor **c:** ΔG (Kcal/mol)

2.4.1. Docking study on human 15-LOX

The calculated volume and molecular weight of the synthesized compounds showed a rough inverse correlation with their $IC_{50.}$ This may explain the high potency of compound **3a** among the synthesized compound and match with the proposed design. Docking study was performed to explore the possible interaction between the synthesized compounds and the active site of the 15-LOX enzyme which consists of iron metal complexed with four amino acids (His 373, His 378, His 553 and Ile 676) (Fig. 5).

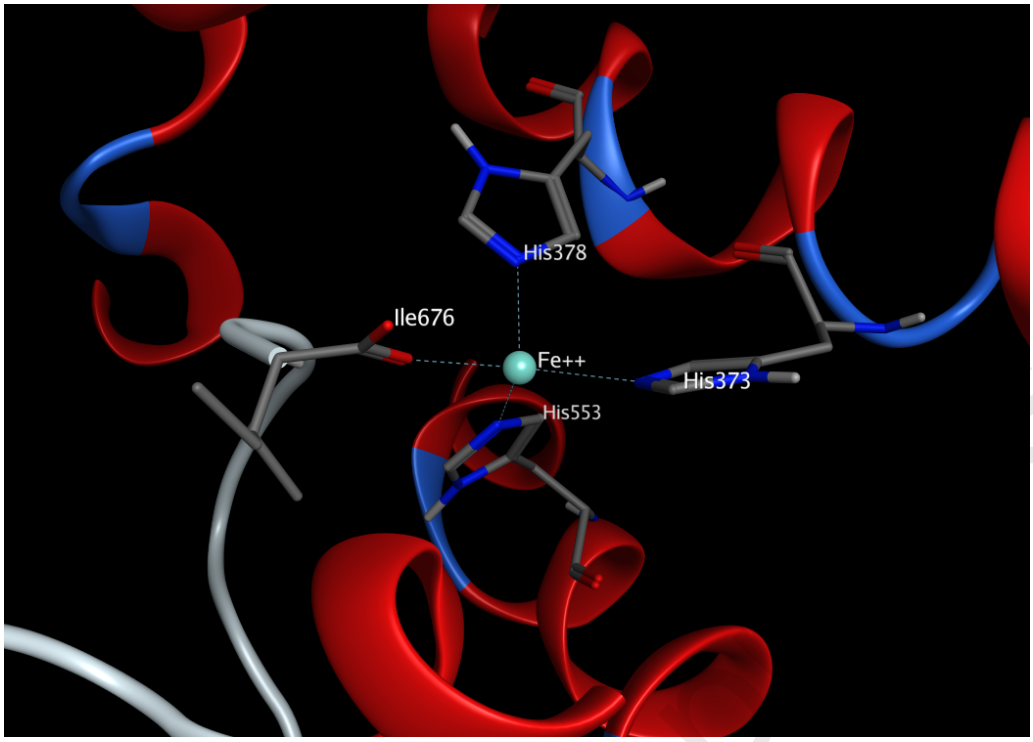


Fig. 5. The active site of 15-LOX active site.

Docking score ranging from -5.89 to -8.78. All the compounds bearing arylidene moiety (**4-8**) showed a pi-pi interaction between the 1,3thiazolidin-4-one ring (the 15-LOX pharmacophore) and His 378. Moreover, the sulfur atom of the 1,3-thiazolidin-4-one ring forms a hydrogen bond with the C-terminal amino acid Ile 676. For example, compounds (**4a** and **8**) explore a pi-pi interaction with His 373 at a distance of (3.80, 3.79 Å) and formation of a hydrogen bond with Ile 676 at a distance of (3.17, 3.39 Å), respectively (Fig. 6 and 7). In addition to the previous interaction, compound **4a** showed a pi- hydrogen interaction with Glu 369 (at 4.60 Å) which may explain its high potency (IC_{50} = 3.11 µM) (Fig. 6).

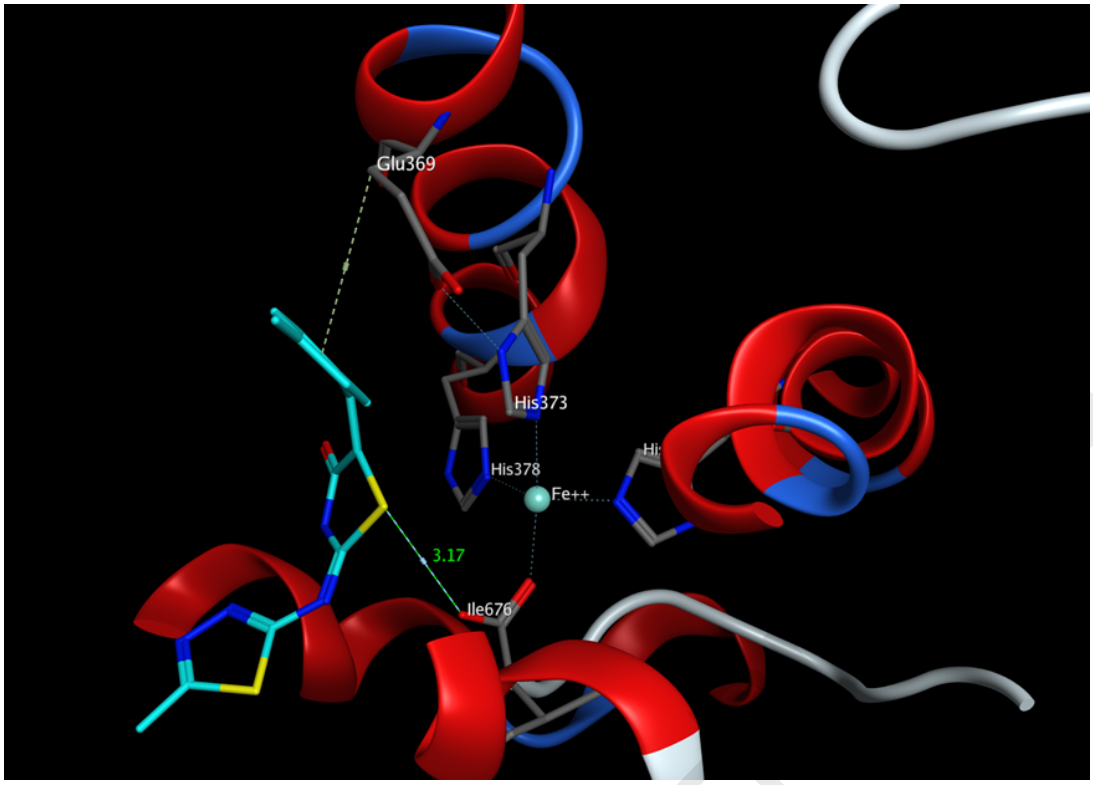


Fig. 6. Interaction between **4a** and 15-LOX active site.

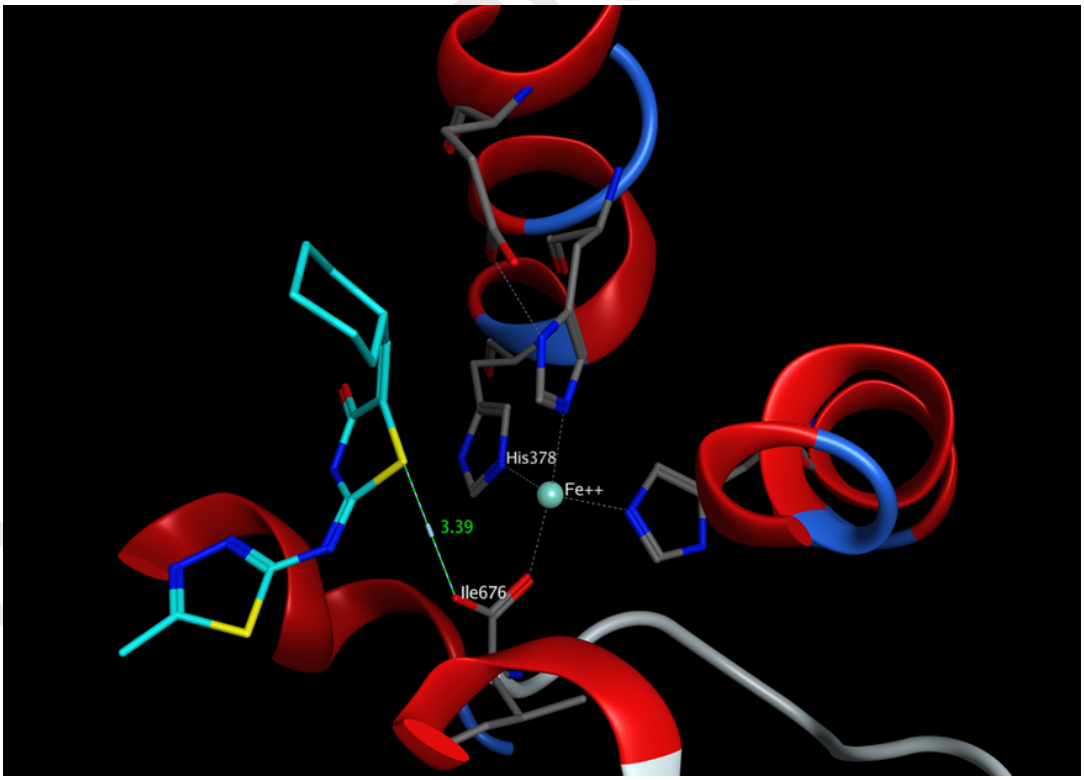


Fig. 7. Interaction between **8** and 15-LOX active site.

Compound **3a** which lacks arylidene moiety showed different types of interaction with the 15-LOX active site (Fig. 8). Besides the formation of hydrogen bonding between the amidic NH of the 1,3-thiazolidin-4-one ring (at 2.93 Å), The carbonyl group interacts with the iron metal to form a metal complex (at 2.48 Å). In fact, these types of interactions give a reasonable explanation to the high activity of compound **3a** (IC_{50} = 2.74 µM) which consider the highest among the newly synthesized compounds.

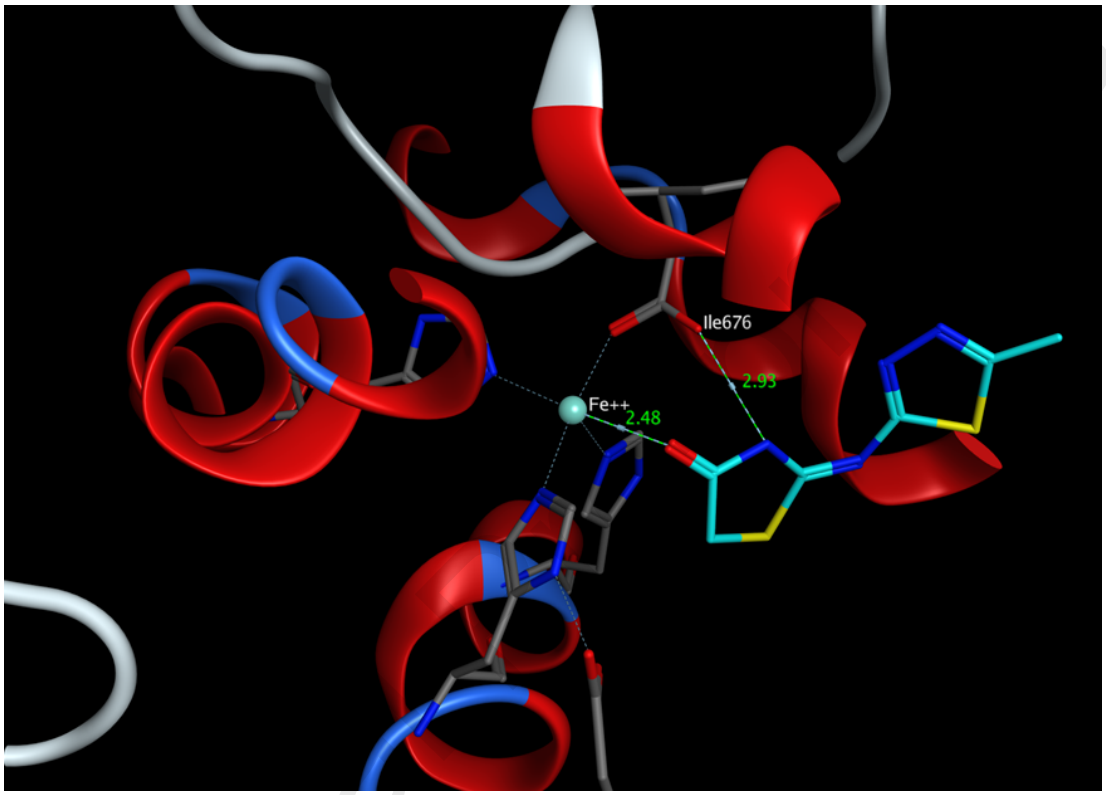


Fig. 8. Interaction between **3a** and 15-LOX active site.

2.4.2. Docking study on human COX-2

Despite the reduction of the new synthesized compounds volume and molecular weight, they still maintain relative selectivity towards COX-2 enzyme as predicted from the design. The docking scores (ranging from -5.87 to -7.87) reflect the good binding of the newly synthesized compounds with the COX-2 enzyme. All the new compounds explore a hydrogen bonding between the 1,3,4-thiadiazole ring (COX-2 pharmacophore) and the amino acid Arg 513 (except compounds **4c**, **4l,** and **4q** which bind with Arg 120). For example, compounds **4i** and **4r** bind with Arg 513 at a distance of (2.88 and 3.14 Å) respectively (Fig. 9 and 10). Also, compound **4i** shows additional binding with Gln192.

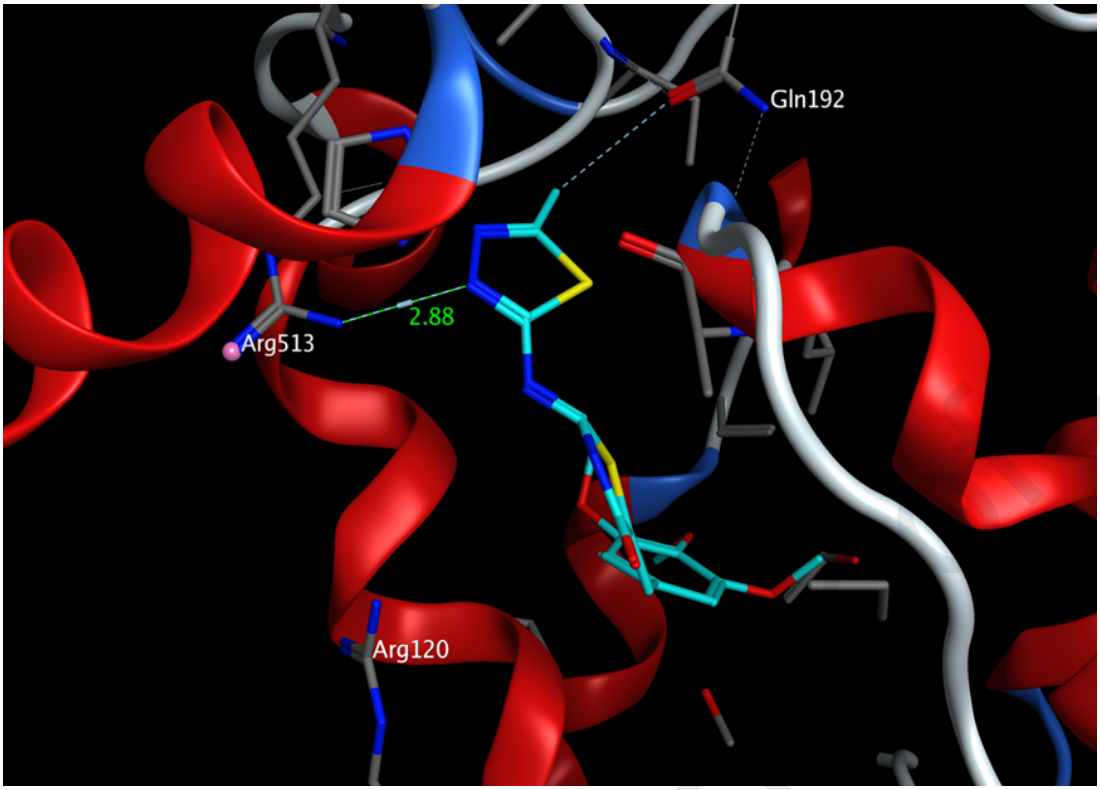


Fig. 9. Interaction between **4i** and COX-2 enzyme.

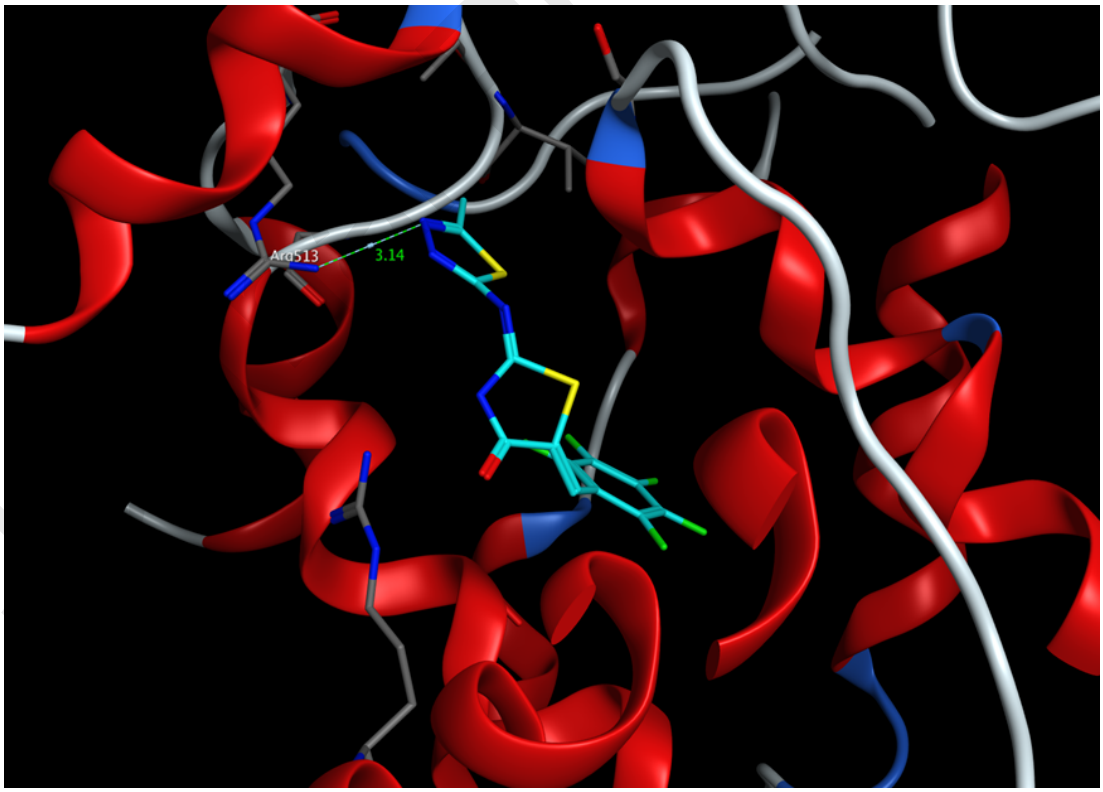


Fig. 10. Interaction between **4r** and COX-2 enzyme.

Compound **4q** showed a hydrogen bonding with Arg 120 instead of Arg 513 and a pi hydrogen interaction with Tyr 355 (Fig. 11).

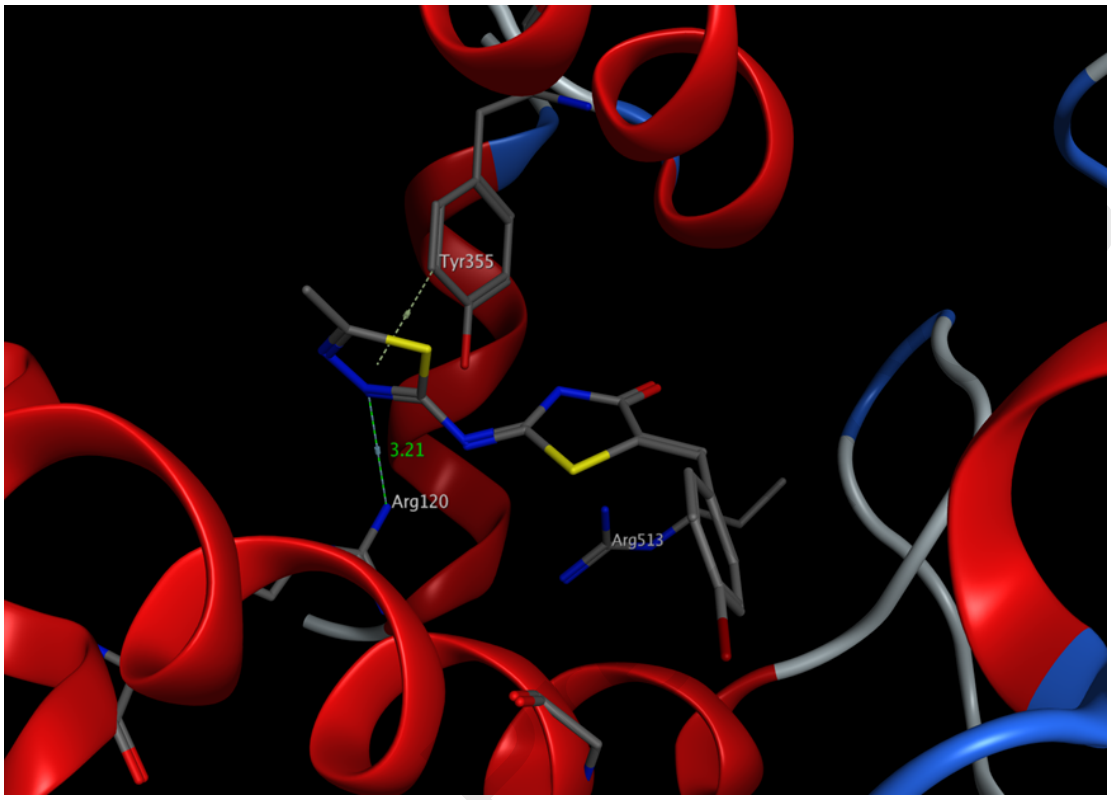


Fig. 11. Interaction between **4q** and COX-2 enzyme.

3. Conclusion

In this study, we gained further insight into the SAR of thiadiazole-thiazolidinone hybrids as dual inhibitors of 15-LOX and COX-2. For good activity against 15-LOX, small hydrophobic group (methyl) should be introduced at the 5th positions of thiadiazole moiety, while the 5th position of thiazolidinone moiety should be unsubstituted (**3a**) or substituted by small hydrophobic groups, such as 4-methylphenyl (**4a**), 3-thienyl (**7**) or cyclohexyl (**8**). In contrary introducing a bulky hydrophobic substituent at that position improved the binding for COX-2 as shown by 3,4,5-trimethoxyphenyl (**4i**), 4-bromophenyl (**4q**) and pentafluorophenyl (**4r**). The balance between 15-LOX binding and COX-2 binding was achieved by introducing methyl group at the 5th position of thiadizole moiety and phenyl, 3-thienyl or cyclohexyl at the 5th position of thiazolidinone moiety. Therefore, compounds **4a**, **7** and **8** are balanced and potent dual inhibitors of 15-LOX and COX-2. The *in vivo* anti-inflammatory evaluation revealed that compounds **3a**, **4q** and **4r** are equipotent to the reference drug

celecoxib and exhibited good gastric safety profile. Molecular docking studies disclosed important binding modes of these compounds with COX-2 and 15-LOX.

4. Experimental

4.1. Chemistry

Commercial grade solvents and reagents were used to synthesize the specified compounds without further treatment except for benzene which dried using sodium metal. Cole-Parmer -Electrothermal [model IA9100, UK] melting point apparatus was used to determined melting points and they were uncorrected. Pre-coated TLC sheets (kieselgel 0.25 mm, 60G F254, Merck, Germany) were used to monitor the progress of reactions. estimate the reaction time. Spots were visualized by an ultraviolet lamp at 254 nm wavelength (Spectroline, model CM-10, USA). Thermo Scientific Nicolet IS10 FT IR spectrometer was used to generate IR spectrum (Thermo Fischer Scientific, USA) at Faculty of Science, Assiut University, Assiut, Egypt. ^{1}H-NMR and ^{13}C-NMR spectra were scanned on AVANCE-III High-Performance FT-NMR spectrometer (400 MHz), (Brucker) at Faculty of Science, Sohag University, Sohag, Egypt. While that of compounds (**1a**, **3b**, **3e**, **3f** and **3g**) were performed on a Varian EM-390 NMR spectrometer (60 MHz, Varian, CA, USA) at Faculty of Pharmacy, Assiut University, Assiut, Egypt and compounds (**2a**, **3c**, **3h** and **3i**) were performed on a Varian EM-390 NMR spectrometer (90 MHz, Varian, CA, USA) at Faculty of Science, Assiut University, Assiut, Egypt. Mass spectra were performed on Direct Probe Controller Inlet Part TO Single Quadrupole mass analyzer in Thermo Scientific GCMS model ISQ LT using Thermo X-Calibur software at the Regional Center for Mycology and Biotechnology (RCMB), Faculty of Science, Al-Azhar University, Cairo, Egypt. Elemental microanalyses were carried out on elemental analyzer model flash 2000 Thermo-fisher at the Regional Center for Mycology and Biotechnology (RCMB), Faculty of Science, Al-Azhar University, Cairo, Egypt.

*4.1.1. Synthesis of 2-amino-5-methyl-1,3,4-thiadiazole (**1a**)*

A mixture of acetic acid (1 mL, 16.6 mmol,), thiosemicarbazide (1.5 g, 16.6 mmol, 1 equv.) and phosphorus oxychloride (7.5 mL, 80.2 mmol, 8.2 equv.) were refluxed for 2 h. The reaction mixture was cooled to room temperature, ice water (25 mL) was added, and the reaction was refluxed for further 4 h. After cooling the reaction mixture was alkalinized by conc ammonium hydroxide solution (20%), the formed precipitate

was filtered off and washed with cold water. The product used for the next step without further purification. Brownish solid; Yield (52%); mp 224-225 ^{o}C; IR (cm^{-1}, KBr): 3259, 3069 (NH_2), 1640 (C=N); ^{1}H-NMR (60 MHz, δ ppm DMSO-d_6): 2.6 (s, 3H, C<u>H</u>$_3$), 7.1 (br. s, 2H, N<u>H</u>$_2$), EI-MS [m/z (%)]: 115.29 (M+, 48.11%), 45.98 (100% base peak); Elemental analysis for $C_3H_5N_3S$ (115.16): Calculated/Found: 31.29/31.53 (%C), 4.38/4.61(%H), 36.49/36.70 (%N) and 27.84/27.69 (%S).

*4.1.2. Synthesis of 2-chloro-N-(5-methyl-1,3,4-thiadiazol-2-yl)acetamide (**2a**)*

Compound **1a** (1.15 g, 10 mmol) was stirred in dry benzene (15 mL). Chloroacetyl chloride (1.3 mL, 16 mmol, 1.6 equv.) in dry benzene (10 mL) was added in a dropwise manner. The reaction mixture was refluxed for 3-5 h. The reaction was monitored by TLC using ethyl acetate: hexane (1:1 v/v) system. The products were filtered while hot and washed with a small amount of cold methanol. The The reaction mixture was filtered while hot, washed with a small amount of cold methanol and recrystallized from dioxane-water to give white crystalline solid. Yield (89.5%); mp decompose at 236 ^{o}C; IR (cm^{-1}, KBr): 3434(NH), 1698 (C=O), 1580 (C=N); ^{1}H-NMR (90 MHz, δ ppm DMSO-d_6): 2.6 (s, 3H, C<u>H</u>$_3$), 4.4 (s, 2H, C<u>H</u>$_2$), 12.9 (br. s, 1H, N<u>H</u>), Elemental analysis for $C_5H_6ClN_3OS$ (191.64): Calculated/Found: 31.34/31.49 (%C), 3.16/3.28 (%H), 21.93/21.87 (%N) and 16.73/16.94 (%S).

4.1.3. General procedure for heterocyclization

Compound (**2a-i**) (10 mmol) and ammonium thiocyanate (1.5 g, 20 mmol, 2 equv.) were stirred in absolute ethanol (20 mL) under reflux for 3 h. The reaction mixture was filtered while hot, the collected product was washed with cooled water and recrystallized from dioxane-water.

*4.1.3.1. Synthesis of 2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**3a**)*

Brown crystals. Yield (91%); mp 269-271 ^{o}C; IR (cm^{-1}, KBr): 3429 (NH), 1734 (C=O), 1556 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6): 2.64 (s, 3H, C<u>H</u>$_3$), 4.08 (s, 2H, C<u>H</u>$_2$), 12.24 (br. s, 1H, N<u>H</u>); ^{13}C-NMR (100 MHz, δ ppm DMSO-d_6): 16.14 (<u>C</u>H$_3$), 35.90 (<u>C</u>H2), 162.49, 165.41, 170.55, 174.33(<u>C</u>=O); EI-MS [m/z (%)] 215.96 (M^{+}+2, 2.42%), 213.99 (M^{+},21.93%), 140.01 (80.25%), 141.04 (93.40%), 58.95 (100.00); Elemental analysis for $C_6H_6N_4OS_2$ (214.27)**:** Calculated/Found: 33.63/33.39 (%C), 2.82/2.48 (%H), 26.15/26.12 (%N) and 29.93/29.79 (%S).

*4.1.3.2. 2-[(5-Phenyl -1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**3b**)*

White crystalline solid; Yield 92%; mp 280-282 °C (reported mp 128 – 130 °C [32]). IR (cm^{-1}, KBr): 3100 (NH), 1726 (C=O), 1566 (C=N); ^{1}H-NMR (60 MHz, δ ppm DMSO-d_6): 4.1 (s, 2H, C$\underline{H}_2$), 7.3-8.2 (m, 5H, Ar-H); Elemental analysis for $C_{11}H_8N_4OS_2$ (276.34)**:** Calculated/Found: 47.81/48.12 (%C), 2.92/2.96 (%H), 20.27/20.54 (%N) and 23.21/23.37 (%S).

*4.1.3.3. 2-[(5-(2-Hydroxyphenyl) -1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**3c**)*

White crystalline solid; Yield 85%; mp decompose at 263 °C (reported mp 160 – 161 °C [32]); IR (cm^{-1}, KBr): 3438 (NH, OH), 1729 (C=O), 1564 (C=N); ^{1}H-NMR (90 MHz, δ ppm DMSO-d_6): 4.1 (s, 2H, C$\underline{H}_2$), 6.8-7.15 (m, 2H, Ar-H),7.2-7.5 (m, 1H, Ar-H), 8.1 (d, J = 9, 1H, Ar-H) off set (br. s, 1H, O$\underline{H}$), off set (br. s, 1H, N$\underline{H}$); Elemental analysis for $C_{11}H_8N_4O_2S_2$ (292.34)**:** Calculated/Found: 45.19/45.00 (%C), 2.76/2.73 (%H), 19.17/19.35 (%N) and 21.94/21.85 (%S).

*4.1.3.4 2-[(5-(4-Methylphenyl) -1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**3e**)*

White crystalline solid; Yield 92%; mp decompose at 260 °C (reported mp 122 – 123 °C [32]); IR (cm^{-1}, KBr): 3100 (NH), 1740 (C=O), 1589 (C=N); ^{1}H-NMR (60 MHz, δ ppm DMSO-d_6): 2.3 (s, 3H, C$\underline{H}_3$), 4.05 (s, 2H, C$\underline{H}_2$), 7.33 (d, J = 8.4, 2H, Ar-H); ,7.80 (d, J = 8.4, 2H, Ar-H); elemental analysis for $C_{12}H_{10}N_4OS_2$ (290.36)**:** Calculated/Found: 49.64/49.51 (%C), 3.47/3.75 (%H), 19.30/19.53 (%N) and 22.09/21.78 (%S).

*4.1.3.5. 2-[(5-(4-Bromophenyl) -1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**3f**)*

White crystalline solid; Yield 93%; mp 295-296 °C (reported mp 135 – 136 °C [32]); IR (cm^{-1}, KBr): 3437 (NH), 1722 (C=O), 1548 (C=N); ^{1}H-NMR (60 MHz, δ ppm DMSO-d_6): 4.4 (s, 2H, C$\underline{H}_2$), 7.55-8.1 (m, 4H, Ar-H); Elemental analysis for $C_{11}H_7BrN_4OS_2$ (355.23)**:** Calculated/Found: 37.19/37.54 (%C), 1.99/1.82 (%H), 15.77/15.73 (%N) and 18.05/17.84 (%S).

*4.1.3.6 2-[(5-(4-Methoxyphenyl) -1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**3g**)*

White crystalline solid; Yield 92.5%; mp decompose at 268 °C (reported mp 108 – 110 °C [32]); IR (cm^{-1}, KBr): 3100 (NH), 1719 (C=O), 1589 (C=N); ^{1}H-NMR (60 MHz, δ

ppm DMSO-d_6): 3.9 (s, 3H, O-C$\underline{H}_3$), 4.1 (s, 2H, C$\underline{H}_2$), 7.10 (d, J = 9, 2H, Ar-H) ,7.88 (d, J = 9, 2H, Ar-H);; Elemental analysis for $C_{12}H_{10}N_4O_2S_2$ (306.36)**:** Calculated/Found: 47.04/47.19 (%C), 3.29/3.35 (%H), 18.29/18.48 (%N) and 20.93/21.04 (%S).

*4.1.3.7. 2-[(5-(3,4-Dimethoxyphenyl) -1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**3h**)*

White crystalline solid; Yield 91%; mp decompose at 258 °C; IR (cm^{-1}, KBr): 3435 (NH), 1712 (C=O), 1571 (C=N); ^{1}H-NMR (90 MHz, δ ppm DMSO-d_6): 3.8 (s, 6H, 2 O-C$\underline{H}_3$), 4.05 (s, 2H, C$\underline{H}_2$), 6.9-7.5 (m, 3H, Ar-H), 12.4 (br. s, 1H, N$\underline{H}$); Elemental analysis for $C_{13}H_{12}N4O_3S_2$ (336.39)**:** Calculated/Found: 46.42/46.54 (%C), 3.60/3.78 (%H), 16.66/16.86 (%N) and 19.06/19.11 (%S).

*4.1.3.8. 2-[(5-(3,4,5-Trimethoxyphenyl)-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**3i**)*

White crystalline solid; Yield 92.6%; mp decompose at 280 °C; IR (cm^{-1}, KBr): 3446 (NH), 1737 (C=O), 1583 (C=N); ^{1}H-NMR (90 MHz, δ ppm DMSO-d_6): 3.7 (s, 3H, O-C$\underline{H}_3$), 3.8 (s, 6H, 2 O-C$\underline{H}_3$), 4.05 (s, 2H, C$\underline{H}_2$), 7.05 (s, 2H, Ar-H), 12.5 (br. s, 1H, N$\underline{H}$); Elemental analysis for $C_{14}H_{14}N_4O_4S_2$ (366.42)**:** Calculated/Found: 45.89/45.61 (%C), 3.85/4.04 (%H), 15.29/14.99 (%N) and 17.5/17.46 (%S).

*4.1.4. General procedure for Knoevenagel condensation of an appropriate aldehyde with compound (**3a**) to give compounds (**4a-r** and **6-8**)*

Compound (**3a**) (0.21 g, 1 mmol) and an aldehyde derivative (1.5 mmol, 1.5 equv.) were stirred in glacial acetic acid (8 mL). Sodium acetate (0.16 g, 2 mmol, 2 equv.) was added and the reaction mixture was heated under reflux for 24 h. The precipitate was filtered off while hot from the reaction mixture (except compounds **4r**, **6** and **7** were collected after adding few drops of distilled water and cooling).

*4.1.4.1. (5Z)-5-[(4-methylphenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4a**)*

Yellow solid; Yield (69.5%); mp > 300 ^{o}C; IR (cm^{-1}, KBr): 3412 (NH), 2821 (sp^{3}C-H), 1712 (C=O), 1568 (C=N). ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 2.37 (s, 3H, C$\underline{H}_3$), 2.67 (s, 3H, C$\underline{H}_3$),), 7.38 (d, J = 7.7 Hz, 2H, Ar-$\underline{H}$), 7.54 (d, J = 7.7 Hz, 2H, Ar-$\underline{H}$), 7.72 (s, 1H, C=C$\underline{H}$), 12.78 (br. s, 1H, N$\underline{H}$); ^{13}C-NMR (100 MHz, δ ppm DMSO-d_6): 16.30 (C$\underline{H}_3$), 21.61, 123.23, 130.5, 130.73, 130.88, 133.18, 141.27,

158.37, 163.28, 167.53, 170.38 (C=O); Elemental analysis for $C_{14}H_{12}N_4OS_2$ (316.40): Calculated/Found: 53.14/53.45 (%C), 3.82/3.97 (%H), 17.71/17.82 (%N) and 20.27/20.35 (%S).

*4.1.4.2. (5Z)-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-5-[(4-propylphenyl)-methylidene]-1,3-thiazolidin-4-one (**4b**)*

Yellow solid; Yield (77%); mp decompose at 292 °C; IR (cm^{-1}, KBr): 3415 (NH), 2951 (sp^3C-H), 1716 (C=O), 1565 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 0.92 (t, *J* = 7.09 Hz, 3H, CH$_3$), 1.63 (m, 2H, CH$_2$), 2.63 (t, *J* = 7.46 Hz, 2H, CH$_2$), 2.67 (s, 3H, CH$_3$), 7.40 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.57 (d, *J* = 7.2 Hz, 2H, Ar-H), , 75 (br. s, 1H, NH); Elemental analysis for $C_{16}H_{16}N_4OS_2$ (344.45): Calculated/ Found: 55.79/55.96 (%C), 4.68/4.80 (%H), 16.27/16.34 (%N) and 18.62/18.84 (%S).

*4.1.4.3. (5Z)-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-5-{[4-(propan-2-yl)phenyl]-methylidene}-1,3-thiazolidin-4-one (**4c**)*

Yellow solid; Yield (78%); mp 286-288 °C; IR (cm^{-1}, KBr): 3435 (NH), 2958 (sp^3C-H), 1715 (C=O), 1573 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 1.24 (d, *J* = 6.72 Hz, 6H, 2CH$_3$), 2.68 (s, 3H, CH$_3$), 2.92-2.99 (m, 1H, CH), 7.46 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.59 (d, *J* = 7.2 Hz, 2H, Ar-H), 7.7 (s, 1H, C=CH), 12.75 (br. s, 1H, NH); Elemental analysis for $C_{16}H_{16}N_4OS_2$ (344.45): Calculated/Found: 55.79/56.04 (%C), 4.68/4.89 (%H), 16.27/16.38 (%N) and 18.62/18.91 (%S).

*4.1.4.4. (5Z)-5-[(4-hydroxyphenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4d**)*

Yellow solid; Yield 67%; mp > 300 °C; IR (cm^{-1}, KBr): broad band at 3385 (NH, OH), 2825 (sp^3C-H), 1702 (C=O), 1583 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 2.67 (s, 3H, CH$_3$), 6.97 (d, *J* = 8.4 Hz, 2H, Ar-H), 7.53 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.69 (s, 1H, C=CH), 10.36 (s, 1H, OH), 12.74 (br. s, 1H, NH); EI-MS [m/z (%)]: 317.91 (M+, 57.85%), 149.92 (100% base peak); Elemental analysis for $C_{13}H_{10}N_4O_2S_2$ (318.37): Calculated/Found: 49.04/49.38 (%C), 3.17/3.19 (%H), 17.60/17.68 (%N) and 20.14/20.41 (%S).

*4.1.4.5. (5Z)-5-[(2-hydroxyphenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4e**)*

Yellow solid; Yield 56.5%; mp Decompose at 258 °C; IR (cm^{-1}, KBr): broad band at 3418 (NH, OH), 2825 (sp^3C-H), 1707 (C=O), 1588 (C=N); ^{1}H-NMR (400 MHz, δ ppm

DMSO-d_6)**:** 2.66 (s, 3H, C<u>H</u>$_3$), 6.92-7.07 (m, 2H, Ar-<u>H</u>), 7.27-7.38 (m, 1H, Ar-<u>H</u>), 7.45 (d, *J* = 7.4 Hz, 1H, Ar-<u>H</u>), 8.01 (s, 1H, C=C<u>H</u>), 10.51 (s, 1H, O<u>H</u>), 12.72 (br. s, 1H, N<u>H</u>); Elemental analysis for $C_{13}H_{10}N_4O_2S_2$ (318.37): Calculated/Found: 49.04/49.31 (%C), 3.17/3.44 (%H), 17.60/17.89 (%N) and 20.14/20.33 (%S).

*4.1.4.6. (5Z)-5-[(3,4-dihydroxyphenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4f**)*

Brown solid; Yield 63%; mp >300 ^oC; IR (cm^{-1}, KBr): broad band at 3492 (NH, OH), 2964 (sp^3C-H), 1702 (C=O), 1586 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 2.67 (s, 3H, C<u>H</u>$_3$), 6.91(d, *J* = 8.07 Hz, 1H, Ar-<u>H</u>), 7.03 (d, *J* = 7.82 Hz, 1H, Ar-<u>H</u>), 7.09 (s, 1H, Ar-<u>H</u>) 7.60 (s, 1H, C=C<u>H</u>), 9.57 (s, 1H, O<u>H</u>), 9.78 (s, 1H, O<u>H</u>), 12.66 (br. s, 1H, N<u>H</u>); Elemental analysis for $C_{13}H_{10}N_4O_3S_2$ (334.37): Calculated/Found: 46.70/46.52 (%C), 3.01/3.07 (%H), 16.76/16.54 (%N) and 19.18/19.42 (%S).

*4.1.4.7. (5Z)-5-[(4-methoxyphenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4g**)*

Yellow solid; Yield 60%.; mp 290-292 ^oC; IR (cm^{-1}, KBr): 3400 (NH), 2817 (sp^3C-H), 1710 (C=O), 1564, 1592 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 2.71 (s, 3H, C<u>H</u>$_3$), 3.89 (s, 3H, OC<u>H</u>$_3$), 7.18 (d, *J* = 8.4 Hz, 2H, Ar-<u>H</u>), 7.66 (d, *J* = 8.5 Hz, 2H, Ar-<u>H</u>), 7.76 (s, 1H, C=C<u>H</u>), 12.70 (br. s, 1H, N<u>H</u>); ^{13}C-NMR (100 MHz, δ ppm DMSO-d_6): 16.29 (<u>C</u>H$_3$), 55.97 (O<u>C</u>H$_3$), 115.49, 121.24, 126.11, 132.75, 133.17, 158.46, 161.53, 163.14, 167.59, 170.41(<u>C</u>=O); EI-MS [m/z (%)]: 332.48 (M+, 18.91%), 123.89 (100% base peak); Elemental analysis for $C_{14}H_{12}N_4O_2S_2$ (332.40): Calculated/Found: 50.59/50.82 (%C), 3.64/3.78 (%H), 16.86/17.01 (%N) and 19.29/19.38 (%S).

*4.1.4.8. (5Z)-5-[(3,4-dimethoxyphenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4h**)*

Yellow solid; Yield 55%; mp 274-276 ^oC; IR (cm^{-1}, KBr): 3432 (NH), 2813 (sp^3C-H), 1697 (C=O), 1568, 1580,1593 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 2.71 (s, 3H, C<u>H</u>$_3$), 3.88 (s, 3H, OC<u>H</u>$_3$), 3.90 (s, 3H, OC<u>H</u>$_3$), 7.24 (d, *J* = 8 Hz, 1H, Ar-<u>H</u>),7.29, 7.32 (br. s, 2H, Ar-<u>H</u>), 7.78 (s, 1H, C=C<u>H</u>), 12.71 (br. s, 1H, N<u>H</u>); Elemental analysis for $C_{15}H_{14}N_4O_3S_2$ (362.43): Calculated/Found: 49.71/49.97 (%C), 3.89/3.94 (%H), 15.46/15.75 (%N) and 17.69/17.52 (%S).

*4.1.4.9. (5Z)-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-5-[(3,4,5-trimethoxyphenyl) methylidene]-1,3-thiazolidin-4-one (**4i**)*

Yellow solid; Yield 68.8%; mp 233-236 ^{o}C; IR (cm^{-1}, KBr): 3435 (NH), 2836 (sp^{3}C-H), 1694 (C=O), 1578 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 2.70 (s, 3H, C$\underline{H}_3$), 3.81 (s, 3H, OC$\underline{H}_3$), 3.90 (s, 6H, 2 (OC$\underline{H}_3$)), 7.01 (s, 2H, Ar-$\underline{H}$), 7.76 (s, 1H, C=C$\underline{H}$), 12.73 (br. s, 1H, N$\underline{H}$); ^{13}C-NMR (100 MHz, δ ppm DMSO-d_6): 16.23 ($\underline{C}H_3$), 56.57, 60.71, 108.43, 123.54, 129.18, 133.4, 140.09, 153.66, 158.27, 163.21, 167.35, 170.31 ($\underline{C}$=O); Elemental analysis for $C_{16}H_{16}N_4O_4S_2$ (392.45): Calculated/Found: 48.97/49.13 (%C), 4.11/4.15 (%H), 14.28/14.54 (%N) and 16.34/16.49 (%S).

*4.1.4.10. (5Z)-5-[(4-hydroxy-3-methoxyphenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4j**)*

Yellow solid; Yield 73%; mp Decompose at 281 ^{o}C; IR (cm^{-1}, KBr): 3512, 3400 (NH, OH), 2825 (sp^{3}C-H), 1706 (C=O), 1575 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 2.67 (s, 3H, C$\underline{H}_3$), 3.84 (s, 3H, OC$\underline{H}_3$), 7.00 (d, J = 8.31, 1H, Ar-$\underline{H}$), 7.14 (d, J = 8.68, 1H, Ar-$\underline{H}$), 7.28 (br. s, 1H, Ar-$\underline{H}$), 7.71 (s, 1H, C=C$\underline{H}$), 10.02 (s, 1H, O$\underline{H}$), 12.75 (br. s, 1H, N$\underline{H}$); Elemental analysis for $C_{14}H_{12}N_4O_3S_2$ (348.40): Calculated/Found: 48.26/48.42 (%C), 3.47/3.43 (%H), 16.08/16.28 (%N) and 18.41/18.63 (%S).

*4.1.4.11. (5Z)-5-{[4-(dimethylamino)phenyl]methylidene}-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4k**)*

Reddish solid; Yield 70%; mp > 300 ^{o}C; IR (cm^{-1}, KBr): 3100 (NH), 2822 (sp^{3}C-H), 1683 (C=O), 1577 (C=N);^{1}H-NMR (400 MHz, δ ppm DMSO-d_6): 2.67 (s, 3H, C$\underline{H}_3$), 3.03 (s, 6H, N(C$\underline{H}_3$)$_2$), 6.88 (d, J = 8.7 Hz, 2H, Ar-$\underline{H}$), 7.51 (d, J = 8.7 Hz, 2H, Ar-$\underline{H}$), 7.66 (s, 1H, C=C$\underline{H}$), 12.63 (br. s, 1H, N$\underline{H}$); EI-MS [m/z (%)]: 344.89 (M+, 17.79%), 177.19 (100% base peak); Elemental analysis for $C_{15}H_{15}N_5OS_2$ (345.44): Calculated/Found: 52.15/52.38 (%C), 4.38/4.44 (%H), 20.27/20.48 (%N) and 18.56/18.70 (%S).

*4.1.4.12. (5Z)-5-[(4-chlorophenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4l**)*

Yellow solid; Yield 72.5%; mp > 300 ^{o}C; IR (cm^{-1}, KBr): 3417 (NH), 2901 (sp^{3}C-H), 1711 (C=O), 1568 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6): 2.67 (s, 3H, C$\underline{H}_3$), 7.60 – 7.75 (m, , 4H, Ar-$\underline{H}$), 7.78 (s, 1H, C=C$\underline{H}$), 12.93 (br. s, 1H, N$\underline{H}$); Elemental

analysis for $C_{13}H_9ClN_4OS_2$ (336.82): Calculated/Found: 46.36/46.09 (%C), 2.69/2.88 (%H), 16.63/16.97 (%N) and 19.04/19.42 (%S).

*4.1.4.13. (5Z)-5-[(3-chlorophenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4m**)*

Yellow solid. Yield 75%. mp 263-265 °C; IR (cm^{-1}, KBr): 3435 (NH), 2899 (sp^3C-H), 1711 (C=O), 1587 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6): 2.67 (s, 3H, C<u>H</u>$_3$), 7.60 – 7.75 (m, 5H, C=C<u>H</u>, Ar-<u>H</u>), 12.87 (br. s, 1H, N<u>H</u>); ^{13}C-NMR (100 MHz, δ ppm DMSO-d_6) 16.24 (<u>C</u>H$_3$), 126.37, 128.42, 130.37 (br. peak), 131.35, 131.61, 134.44, 135.88, 157.82, 163.43, 167.18, 170.24(<u>C</u>=O). Elemental analysis for $C_{13}H_9ClN_4OS_2$ (336.82): Calculated/Found: 46.36/46.50 (%C), 2.69/2.77 (%H), 16.63/16.89 (%N) and 19.04/19.27 (%S).

*4.1.4.14. (5Z)-5-[(2-(chlorophenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4n**)*

Yellow solid; Yield 74%; mp > 300 °C; IR (cm^{-1}, KBr): 3435 (NH), 2989 (sp^3C-H), 1710 (C=O), 1574 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6): 2.67 (s, 3H, C<u>H</u>$_3$), 7.45 – 7.75 (m, 4H, Ar-<u>H</u>), 7.92 (s, 1H, C=C<u>H</u>) 12.96 (br. s, 1H, N<u>H</u>); Elemental analysis for $C_{13}H_9ClN_4OS_2$ (336.82): Calculated/Found: 46.36/46.54 (%C), 2.69/2.62 (%H), 16.63/16.75 (%N) and 19.04/19.38 (%S).

*4.1.4.15. (5Z)-5-[(2,4-(dichlorophenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4o**)*

Yellow solid; Yield 68%; mp 262-264 °C; IR (cm^{-1}, KBr): 3412 (NH, OH), 2918 (sp^3C-H), 1717 (C=O), 1584 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6): 2.66 (s, 3H, C<u>H</u>$_3$), 7.62-7.93 (M, 4H, Ar-<u>H</u>, C=C<u>H</u>), 13.03 (br. s, 1H, N<u>H</u>); Elemental analysis for $C_{13}H_8Cl_2N_4OS_2$ (371.26): Calculated/Found: 42.06/42.19 (%C), 2.17/2.2 (%H), 15.09/15.34 (%N) and 17.27/17.38 (%S).

*4.1.4.16. (5Z)-5-[(3,4-dichlorophenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4p**)*

Yellow solid. Yield 77.5%; mp > 300 °C; IR (cm^{-1}, KBr): 3412 (NH), 2895 (sp^3C-H), 1713 (C=O), 1586 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6): 2.67 (s, 3H, C<u>H</u>$_3$), 7.61 (d, J = 8.3 Hz, 1H, Ar-<u>H</u>), 7.75 (s, 1H, C=C<u>H</u>), 7.84 (d, J = 8.07 Hz, 1H, Ar-<u>H</u>), 7.92 (s, 1H, Ar-<u>H</u>), 12.88 (br. s, 1H, N<u>H</u>); Elemental analysis for $C_{13}H_8Cl_2N_4OS_2$

(371.26): Calculated/Found: 42.06/42.19 (%C), 2.17/2.22 (%H), 15.09/15.34 (%N) and 17.27/17.38 (%S).

*4.1.4.17. (5Z)-5-[(4-bromophenyl)methylidene]-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**4q**)*

Yellow solid. Yield 70%; mp >300 ^{o}C; IR (cm^{-1}, KBr): 3413 (NH), 2899 (sp^{3}C-H), 1712 (C=O), 1569 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6): 2.68 (s, 3H, C$\underline{H}_3$), 7.61 (d, J = 8.2 Hz, 2H, Ar-$\underline{H}$), 7.80 (d, J = 8.2 Hz, 2H, Ar-$\underline{H}$), 7.76 (s, 1H, C=C$\underline{H}$), 12.92 (br. s, 1H, N$\underline{H}$; Elemental analysis for $C_{13}H_9BrN_4OS_2$ (381.27): Calculated/Found: 40.95/41.31 (%C), 2.38/2.52 (%H), 14.69/14.87 (%N) and 16.82/17.01 (%S).

*4.1.4.18. (5Z)-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-5-[(pentafluorophenyl)-methylidene]-1,3-thiazolidin-4-one (**4r**)*

White solid. Yield 76.5%; mp decompose at 230 ^{o}C; IR (cm^{-1}, KBr): 3434 (NH), 2932 (sp^{3}C-H), 1724 (C=O), 1594 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6): 2.65 (s, 3H, C$\underline{H}_3$), 7.48 (s, 1H, C=C$\underline{H}$), 12.92 (br. s, 1H, N$\underline{H}$); Elemental analysis for $C_{13}H_5F_5N_4OS_2$ (392.33): Calculated/Found: 39.80/40.03 (%C), 1.28/1.26 (%H), 14.28/14.45 (%N) and 16.35/16.44 (%S).

*4.1.4.19. (5Z)-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-5-[(2E)-3-phenylprop-2-en-1-ylidene]-1,3-thiazolidin-4-one (**5**)*

Yellow solid. Yield 66%; mp 258-260 ^{o}C; IR (cm^{-1}, KBr): 3428 (NH), 3026 (sp^{2}C-H), 2890 (sp^{3}C-H), 1708 (C=O), 1610 (C=C), 1557 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6): 2.67 (s, 3H, C$\underline{H}_3$), 7.12 (t, J = 13.0 Hz, 1H, =CH-C$\underline{H}$=CH-), 7.30 (d, J = 15.2 Hz, 1H, =CH-CH=C$\underline{H}$-), 7.35-7.45 (m, 3H, Ar-$\underline{H}$), 7.48 (d, J = 11.1 Hz, 1H, =C$\underline{H}$-CH=CH-), 7.71 (br. s, 2H, Ar-$\underline{H}$), 12.60 (br. s, 1H, N$\underline{H}$); ^{13}C-NMR (100 MHz, δ ppm DMSO-d_6): 16.28 ($\underline{C}H_3$), 123.82, 125.63, 128.44, 129.37, 130.19, 133.43, 136.17, 144.31, 158.27, 162.98, 167.03, 170.42 ($\underline{C}$=O); Elemental analysis for $C_{15}H_{12}N_4OS_2$ (328.41): Calculated/Found: 54.86/55.13 (%C), 3.68/3.79 (%H), 17.06/17.28 (%N) and 19.53/19.67 (%S).

*4.1.4.20. (5Z)-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-5-[(pyrid-3-yl)methyli-dene]-1,3-thiazolidin-4-one (**6**)*

Greenish solid. Yield 70.5%; mp 282-285 ^{o}C; IR (cm^{-1}, KBr): 3416 (NH), 2900 (sp^{3}C-H), 1715 (C=O), 1587 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6):2.67 (s, 3H,

C<u>H</u>$_3$), 7.62 (dd, *J* = 7.78- 4.72 Hz, 1H, Ar-<u>H</u>), 7.81 (s, 1H, C=C<u>H</u>), 8.03 (d, *J* = 8.15 Hz, 1H, Ar-<u>H</u>), 8.65 (d, *J* = 4.08 Hz, 1H, Ar-<u>H</u>), 8.88 (d, *J* = 1.59 Hz, 1H, Ar-<u>H</u>), 13.01 (br. s, 1H, N<u>H</u>); Elemental analysis for $C_{12}H_9N_5OS_2$ (303.36): Calculated/Found: 47.51/47.69 (%C), 2.99/3.02 (%H), 23.09/23.40 (%N) and 21.14/21.29 (%S).

4.1.4.5.21. (5Z)-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]-5-[(thien-3-yl)methylidene] -1,3-thiazolidin-4-one (7)

White solid. Yield 71%; mp 282-284 ^{o}C; IR (cm^{-1}, KBr): 3413 (NH), 2825 (sp^{3}C-H), 1712 (C=O), 1596 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO- d_6): 2.68 (s, 3H, C<u>H</u>$_3$), 7.31 (t, J = 4.22 Hz, 1H, Ar-<u>H</u>), 7.71 (br. s, 1H, Ar-<u>H</u>), 8.04 (s, 2H, Ar-<u>H</u>, C=C<u>H</u>) 12.83 (br. s, 1H, N<u>H</u>); ^{13}C NMR (100 MHz δ ppm DMSO-*d_6*): 16.27 (<u>C</u>H$_3$), 137.73, 157.65, 126.19, 121.84, 167.19, 135.27, 133.76, 129.43, 170.30, 163.21 (<u>C</u>=O): Elemental analysis for $C_{11}H_8N_4OS_3$ (308.40): Calculated/Found: 42.84/43.08 (%C), 2.61/2.67 (%H), 18.17/18.43 (%N) and 31.19/31.28 (%S).

*4.1.4.22. (5Z)-5-(cyclohexylmethylidene)-2-[(5-methyl-1,3,4-thiadiazol-2-yl)imino]- 1,3-thiazolidin-4-one (**8**)*

White solid. Yield 75%; mp 219-222 ^{o}C; IR (cm^{-1}, KBr): 3425 (NH), 2935 (sp^{3}C-H), 1717 (C=O), 1594 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-*d_6*): 1.15-1.42 (m, 5H, cyclohexyl), 1.57-182 (m, 5H, cyclohexyl), 2.24 (m, 1H, C<u>H</u>), 2.67 (s, 3H, C<u>H</u>$_3$), 6.77 (d, J = 8.68 Hz, 1H, C=C<u>H</u>), 12.58 (br. s, 1H, N<u>H</u>); ^{13}C-NMR (100 MHz, δ ppm DMSO-*d_6*): 16.25 (<u>C</u>H$_3$), 25.2, 25.63, 31.11, 40.88, 125.72,142.54, 158.46, 163.05, 166.46, 170.45 (<u>C</u>=O); Elemental analysis for $C_{13}H_{16}N_4OS_2$ (308.42): Calculated/Found: 50.63/50.71 (%C), 5.23/5.4 (%H), 18.17/18.53 (%N) and 20.79/20.82 (%S).

5. Biological screening

5.1. In vitro Lipoxygenase inhibition assay

Lipoxygenase inhibitory activity was performed at the Department of Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt. The prepared compounds screened against LOX enzyme using LOX inhibitor screening assay kit (Catalog No. 760700, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions and the deviation from the mean is < 10% of the mean value.

5.2. In vitro cyclooxygenase inhibition assay

Cyclooxygenase inhibitory activity was performed at the Department of Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt. The prepared compounds were

screened against ovine COX-1 and human recombinant COX-2 using a COX inhibitor screening assay kit (Catalog No. 560131, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions and the deviation from the mean is < 10% of the mean value.

5.3. In vivo anti-inflammatory activity

Compounds (**3a, 4f, 4i, 4q,** and **4r**) were chosen to examine their *in vivo* anti-inflammatory activity using the Carrageenan-induced paw edema model in rats. The study was performed according to the reported procedures [38]. Briefly, seven groups each of five male albino rats (120-150 g) were injected subplantar at right hind of the paw by carrageenan suspension (0.2 mL of 1% solution in normal saline). The paw thickness was measured by a Vernier caliper (SMIEC, China) before and 30 min after the carrageenan injection. The selected compounds were injected i.p at a dose of 28 μM/Kg (dissolved in 1 % sodium carboxymethyl cellulose solution in normal saline). The reference drugs (Celecoxib) were injected at the same dose while the negative control group injected by the vehicle (1 % sodium carboxymethyl cellulose solution in normal saline). The thickness of the right paw was measured at 1, 2, 3 and 4 h and the percentage inhibition of edema was calculated by the following equation. Statistical analysis was made using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA.

$$\%\ Edema\ inhibition = \frac{(VR - Vl)control - (VR - Vl)\ treated}{(VR - Vl)control}$$

Where, VR: Average right paw thickness, VL: Average left paw thickness.

5.4. Ulcerogenic liability

Compounds (**3a**, **4i** and **4r**) were examined for their ulcerogenic liability according to the reported method on adult male albino rats [39]. Briefly, five groups each of two rats fasted for 12 h with free access to water. The selected compounds were administered orally at doses of 20 mg/Kg (dissolved in 1 % sodium carboxymethyl cellulose solution in normal saline). The positive control group received indomethacin orally at 10 mg/kg while the negative control group received the vehicle only (dissolved in 1 % sodium carboxymethyl cellulose solution in normal saline). The animals were sacrificed after 6 h, the stomachs were isolated and washed with saline. Stomachs were preserved in a

10 % w/v formalin solution. Afterward, they were divided into sections and stained by hematoxylin and eosin dyes and were examined using a light microscope.

5.5. Molecular modelling

Calculation of descriptors and docking study was performed using MOE 2019 software at the Medicinal Chemistry Department, Faculty of Pharmacy, Assiut University. For human 15-LOX (PDB code 4NRE), the enzyme was prepared by the removal of water. The docking study was performed using the default options of the MOE 2019 software except changing the placement from triangle matcher into alpha triangle. For human COX-2 (PDB Code 5KIR, the docking study was performed using the default software options. Rofecoxib (the co-crystallized ligand) docked into the COX-2 enzyme to validate the docking parameters and the root mean square deviation (RMSD) was 0.843. All the compounds were subjected to energy minimization at root mean square cut off equal to 0.005 using MMFF94x force field. Lipophilicity of the compounds and volumes were calculated using logP(o/w) and vol descriptors, respectively.

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Declaration of Competing Interest

The authors have declared no conflict of interest

Supplementary material

Supplementary data to this article can be found online at

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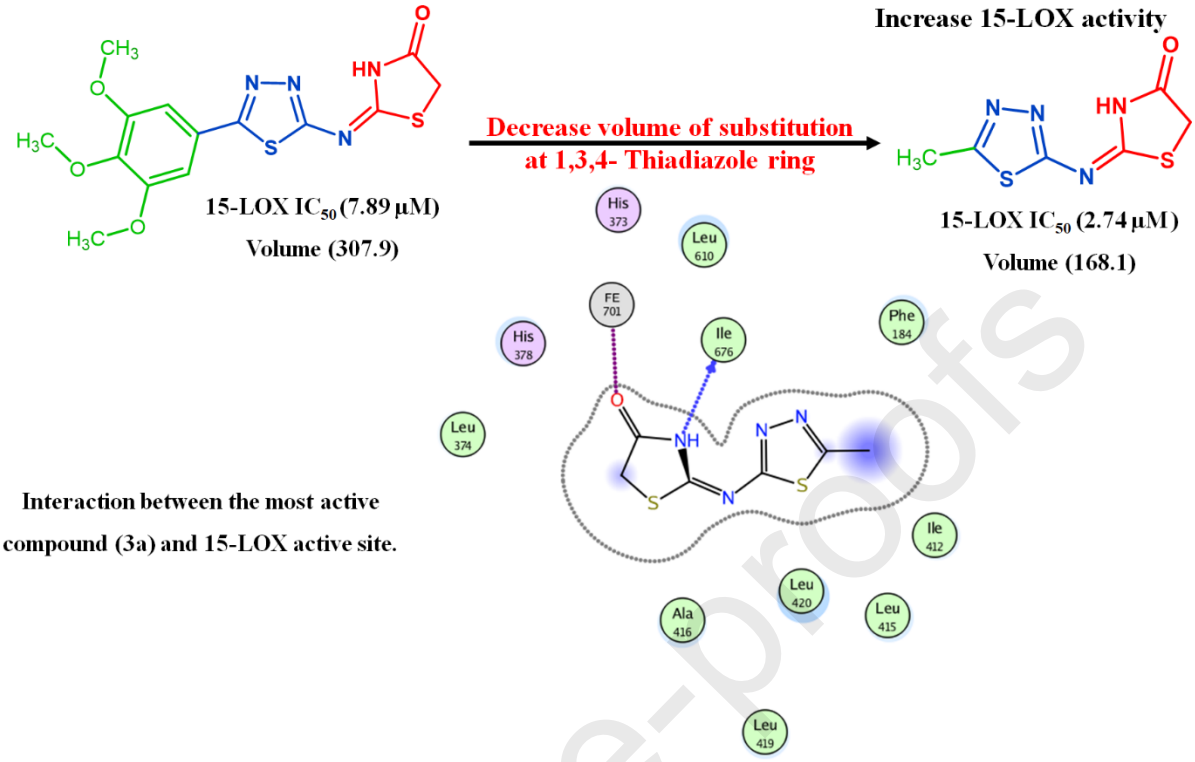
Graphical abstract



Interaction between the most active compound (3a) and 15-LOX active site.

Highlights

- Synthesis of 1,3,4-thiadiazole-4-thiazolidinone hybrid modified at the 5th position of thiadiazole and/or thiazolidinone.
- The inhibitory potency and selectivity of these hybrids against COX and 15-LOX were determined, in addition to their *in vivo* anti-inflammatory activity and gastric safety profile.
- The results revealed that the activity depends on the size of the substituent at the 5th position.
- The study provided new compounds that are potent dual COX-2/15-LOX inhibitors with good *in vivo* anti-inflammatory activity and good gastric safety profile.

Declaration of Competing Interest

The authors have declared no conflict of interest