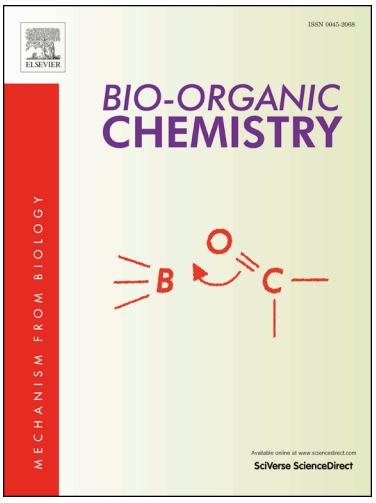

Synthesis, biological evaluation and docking study of 1,3,4-thiadiazole-thiazolidinone hybrids as anti-inflammatory agents with dual inhibition of COX-2 and 15-LOX

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Synthesis, biological evaluation and docking study of 1,3,4-thiadiazole-thiazolidinone hybrids as anti-inflammatory agents with dual inhibition of COX-2 and 15-LOX

Yasser M. Omar[a], Hajjaj H. M. Abdu-Allah[a*†,] Samia G. Abdel-Moty[a]

[a] Department Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Assiut University, Assiut 71526, Egypt. E-mail: hajjaj@aun.edu.eg

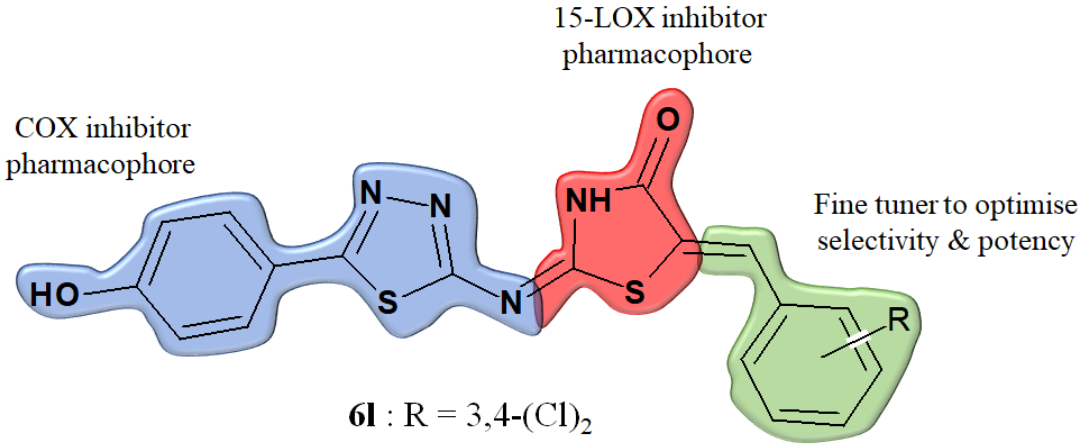
[†] Present address: Department of Applied Biochemistry, Tokai University, 4-1-1 Kitakaname, Hiratsuka, Kanagawa 259-1292, Japan

Abstract

Selective inhibition of both cyclooxygenase-2 (COX-2) and 15-lipooxygenase (15-LOX) may provide good strategy for alleviation of inflammatory disorders while minimizing side effects associated with current anti-inflammatory drugs. The present study describes the synthesis, full characterization and biological evaluation of a series of thiadiazole-thiazolidinone hybrids bearing 5-alk/arylidene as dual inhibitors of these enzymes. Our design was based on merging pharmacophores that exhibit portent anti-inflammatory activities in one molecular frame. 5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-amine (**3**) was efficiently synthesized, chloroacetylated and cyclized to give the key 4-thiazolidinone (**5**). Knovenagel condensation of **5** with different aldehydes afforded the final compounds **6a-m**, **7**, **8** and **9**. These compounds were subjected to *in vitro* COX-1/COX-2, 15-LOX inhibition assays. Compounds (**6a**, **6f**, **6i**, **6l**, **6m** and **9**) with promising potency (IC_{50} = 70-100 nM) and selectivity index (SI = 220-55) were further tested for *in vivo* anti-inflammatory activity and effect on gastric mucosa. The most promising compound (**6l**) inhibits COX-2 enzyme at a nanomolar concentration (IC_{50} = 70 nM, SI = 220) with simultaneous inhibition of 15-LOX (IC_{50} = 11 µM). These results are comparable to the potency and selectivity of the standard drugs of both enzymes; celecoxib (COX-2 IC_{50} = 49 nM, SI = 308) and zileuton (15-LOX IC_{50} = 15 µM) in one construct. Interestingly three compounds (**6a**, **6l** and **9**) exhibited equivalent to or even higher than that of celecoxib *in vivo* anti-inflammatory activity at 3 h interval with good GIT safety profile. Molecular docking study conferred binding sites of these compounds on COX-2 and 15-LOX. Such type of compounds would represent valuable leads for further investigation and derivatization.

Keywords: 1,3,4-thiadizole, 4-thiazolidinone, cyclooxygenase, lipoxygenase, anti-inflammatory.

Graphical abstract.



6l : R = 3,4-$(Cl)_2$

	COX-2 IC_{50}	Selectivity index	15-LOX IC_{50}
6l	**70 nM**	**220**	**11.8 µM**
Celecoxib	49 nM	308	-
Zileuton	-	-	15.6 µM

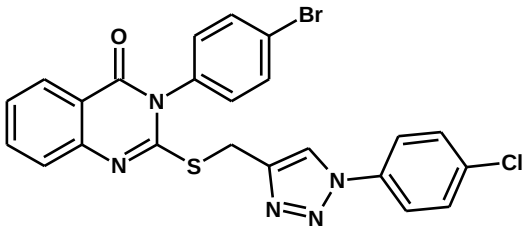
1. Introduction

Inflammation is involved in many diseases ranging from microbial infections, neural disorders to cancer metastasis [1]. A key step of inflammation is the activation of a cyclooxygenases (COX) and lipoxygenases (LOX) responsible for production of several inflammatory mediators from arachidonic acid [2]. There are two isoforms of cyclooxygenase enzyme: COX-1 and COX-2 [3]. While COX-1 is involved in the synthesis of prostaglandins responsible for maintaining normal body function in kidney, GIT and other organs, COX-2 is mainly induced during inflammation [4]. Classical non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, diclofenac and indomethacin inhibit both COX isoforms leading to effective anti-inflammatory response but with high risk of gastric ulceration and kidney damage upon prolonged use[5]. Efforts to design selective COX-2 inhibitors were crowned by approval of two products in the late nineties: Celebrex® (celecoxib)[6] and Vioxx® (rofecoxib)[7]. These selective COX-2 inhibitors demonstrate good anti-inflammatory activity with good gastric and kidney safety profile [5]. However, recent reports have been published correlating the new selective COX-2 inhibitors with cardiovascular complications leading to the withdrawal of the rofecoxib on 2004 [8]. On the other hand, overexpression of 15-LOX is associated with several inflammatory diseases such as osteoarthritis [9] and asthma [10-12]. For instance, 15-LOX is responsible for the production of 15(S)-hydroxy-eicosatetraenoic acid (15-HETE) and eoxins which contribute to airway inflammation and injury in asthmatic patient [13]. Prolonged use of COX inhibitors shunts the metabolism of arachidonic acid towards the LOX pathway and worsen the asthmatic patient condition as in aspirin-intolerant asthma [4, 14]. Zileuton is the only approved selective LOX inhibitor and was used in the treatment of chronic asthma [15]. Therefore, development of selective COX-2/COX-1 inhibitors with LOX inhibitory properties seems to deliver compounds with safe gastrointestinal and cardiovascular profiles.

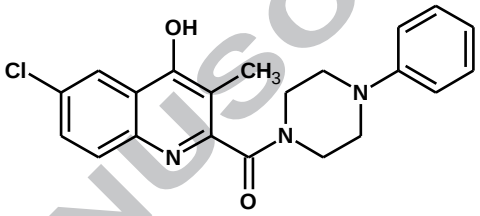
Accordingly, we envisage selective inhibition of both COX-2 and 15-LOX may provide a good strategy for alleviation of inflammatory disorders while minimizing side effects with potential application in asthmatic patients.

Recent trials to develop dual inhibitors of COX and LOX led to compounds **I-VI** (Fig. 1) which demonstrated moderate selectivity towards COX-2 at micromole level. for example, compound **I** incorporating quinazoline and triazole rings inhibits COX-2 and 15-LOX with IC_{50} = 0.16 and 5.21 µM, respectively [16] and selectivity index

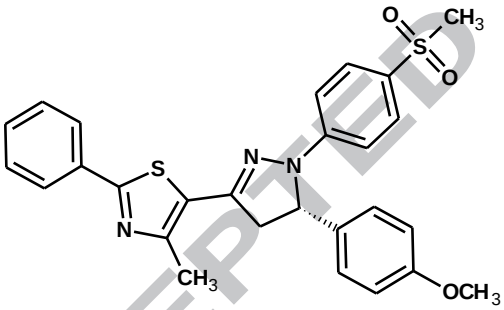
(SI) = 32. Similarly, quinoline derivative **II** [17] and celecoxib analogue **III** [18] were found to inhibit both COX-2 and 15-LOX with five to seven-fold selectivity towards COX-2 at low micromole potency. A hybrid molecule **IV** [19] containing 4-thiazolidinone and thiazole rings has been discovered using computer aided drug design as inhibitor of both COX-1 and 15-LOX without inhibitory activity on COX-2 till 200 µM. A very related compound **V** [20] was separately published containing 4-thiazolidinone with methoxyphenyl group showed better inhibitory activity against 15-LOX (IC_{50} = 17.11 µM) than quercetin.



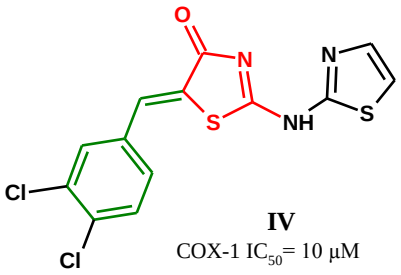
I
COX-2 IC_{50} = 0.16 µM
SI = 32.93
15-LOX IC_{50} = 5.21 µM



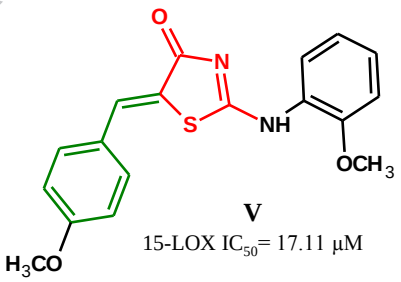
II
COX-2 IC_{50} = 1.13 µM
SI = 7.61
15-LOX IC_{50} = 5.91 µM



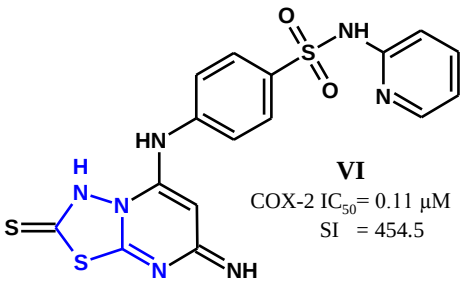
III
COX-2 IC_{50} = 1.14 µM
SI = 5.08
15-LOX IC_{50} = 4.71 µM



IV
COX-1 IC_{50}= 10 µM
15-LOX IC_{50} = 143 µM



V
15-LOX IC_{50}= 17.11 µM



VI
COX-2 IC_{50}= 0.11 µM
SI = 454.5

Fig. 1. Chemical structure of certain reported COX and/or 15-LOX inhibitors.

On the other hand, a fused system containing 1,3,4-thiadiazole ring **VI** [21] exhibited good inhibitory activity against COX-2 (IC_{50} = 0.11 μM) with high selectivity (SI = 454.5). These data encouraged us to design a new series of compounds combining both 4-thiazolidinone and 1,3,4-thiadiazole in order to achieve selectivity and dual targeting of both COX-2 and 15-LOX.

2. Result and discussion

2.1. Design

In continue of our interest in developing thiazolidinone hybrids [22], anti-inflammatories with dual inhibition of COX-2 and LOX [23] and based on the above-mentioned examples (Fig. 1), we surmised that the hybridization of the pharmacophores 1,3,4-Thiadiazole and 4-thiazolidinone in one molecular frame could show highly effective anti-inflammatory with broad spectrum and minimum side effects. Combining both scaffolds was expected to inhibit both COX-2 (1,3,4-thiadiazole), LOX (4-thiazolidinone) and provide better selectivity towards COX-2 over COX-1 enzyme due to their large volume which will not fit in the smaller COX-1 binding pocket [24]. To test these hypotheses, we have synthesized compounds **1, 3** and **6a** (Fig. 2). The new hybrid compound incorporating both 4-thiazolidinone and 1,3,4-thiadiazole **6a** was compared with its building blocks; 4-thiazolidinone (**1**) and 1,3,4-thiadiazole (**3**) in terms of molecular volume, potency and selectivity against both COX-2 and 15-LOX enzymes (Fig. 2). Molecular volume was calculated using MOE program vsurf_D1 descriptor. The hybrid **6a** was about two-folds larger, three-folds more potent and two-folds more selective than **3** against COX-2. The hybrid **6a** also demonstrates better inhibitory activity against 15-LOX (about 1.5 folds more potent than **1)**. Based on these results, we designed a library of new compounds **6-9** to elaborate other structural features of the hybrid molecules. The arylidene moiety attached to the 4-thiazolidinone ring is varied as a fine tuner to optimise both selectivity and potency against COX-2 and 15-LOX enzymes.

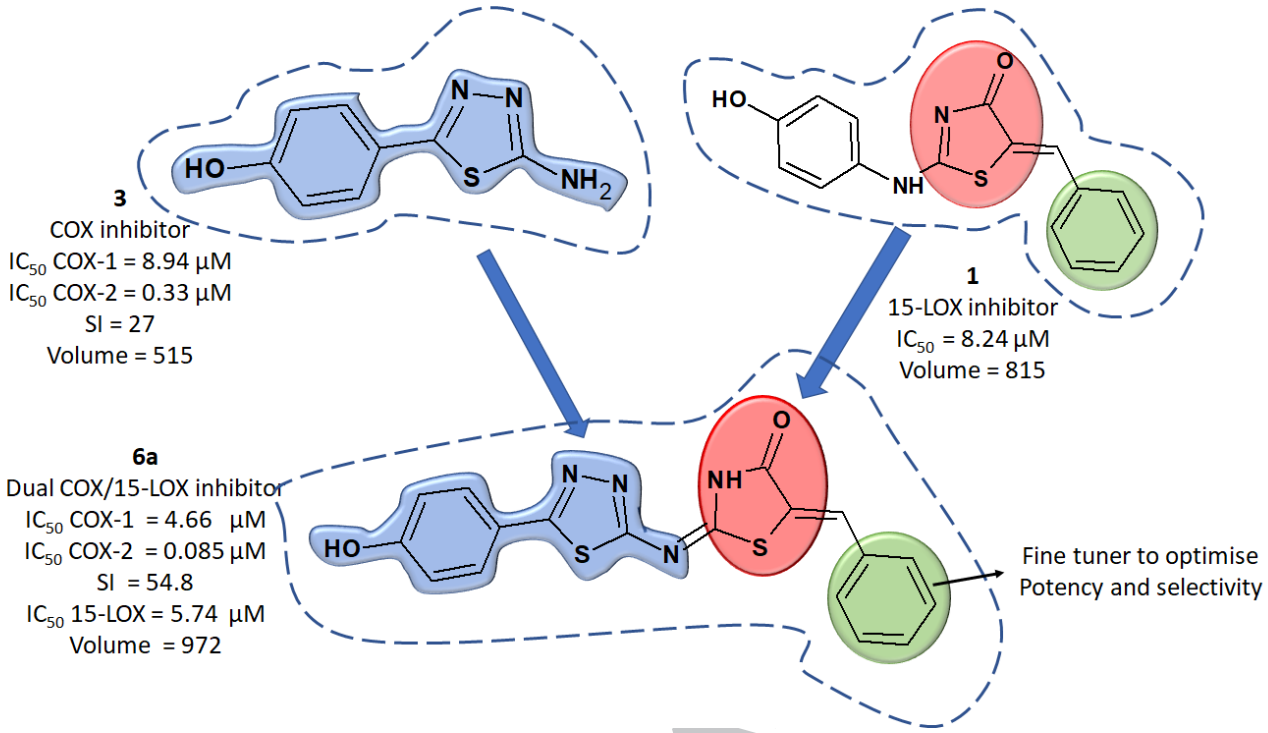
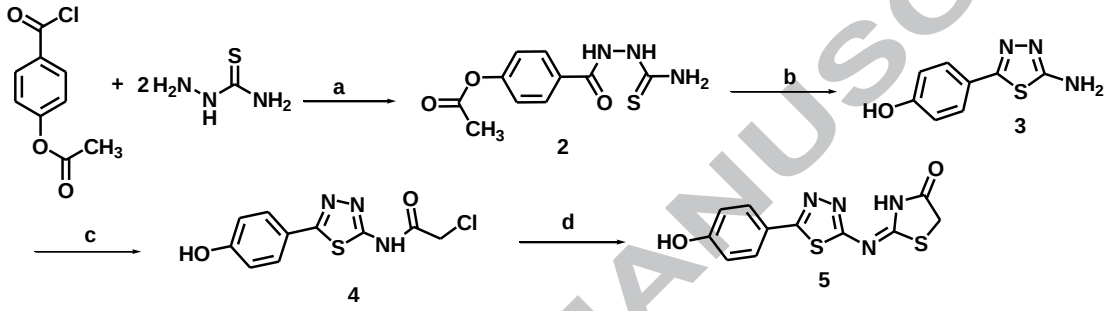


Fig. 2. Diagram represents the new design of synthetic dual COX-2/15-LOX inhibitors.

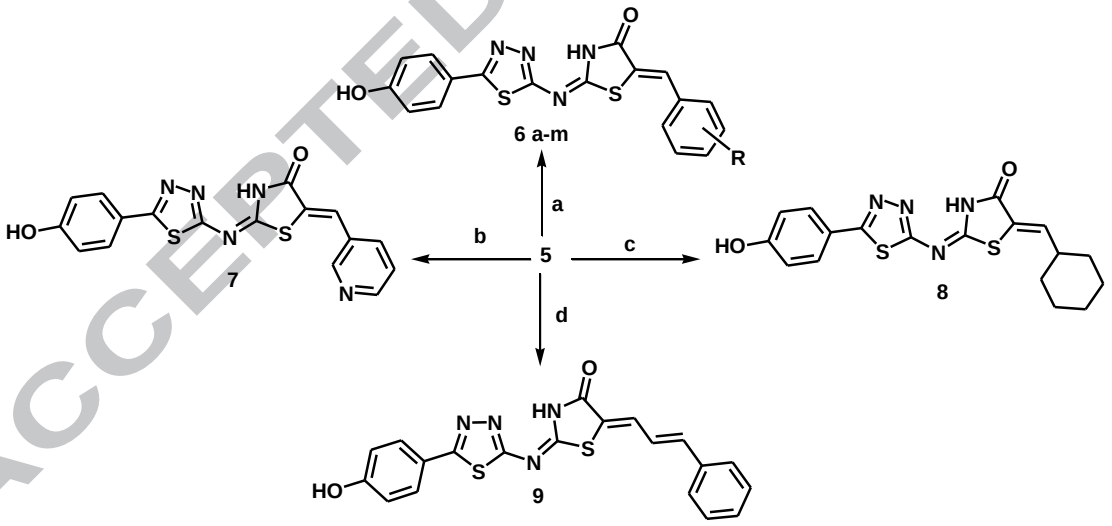
2.2. Chemistry

Compound **1** (Fig. 2) which is (5Z)-5-benzylidene-2-(4-hydroxyanilino)-1,3-thiazol-4(5H)-one was prepared using a previously reported procedure [25]. The new intermediate **5** was prepared via 4 steps with overall yield 50%, (Scheme 1). Firstly, 4-acetoxybenzoyl chloride was condensed with thiosemicarbazide in dry THF to produce **2**, which was simultaneously deacetylated and cyclized to give **3** upon reflux with phosphorus oxychloride. This was confirmed by disappearance of the ester and amidic carbonyl groups of **2** (1749 and 1623 cm^{-1}) in the IR spectrum after cyclization to give **3.** Additionally, the methyl protons (δ 2.3 ppm) and the two NH protons of the acylated thiosemicarbazide **2** at δ 9.3 and 10.3 ppm disappear in the ^{1}H-NMR spectrum of **3**. Chloroacetylation of **3** was achieved by controlled addition of chloroacetyl chloride over 5 h to yield **4**. The chloroacetamido group of **4** was proved by the appearance of amidic carbonyl band at 1693 cm^{-1} and the appearance of $C\underline{H}_2$ protons singlet signal at δ 4.35 in the IR and ^{1}H-NMR spectrum of **4,** respectively. Uncontrolled addition of chloroacetyl chloride in this reaction resulted in partial esterification of the phenolic hydroxyl group and difficulty in purification. Heterocyclization to yield **5** was performed by reflux of **4** with ammonium thiocyanate in ethanol. Formation of 4-thiazolidinone ring was confirmed by the shift of amidic carbonyl band to a higher frequency (1720 cm^{-1}) in the IR spectrum and the

$C\underline{H}_2$ upfield shift to δ 4.11 in the ^{1}H-NMR spectrum. The purity of all synthesized compounds was checked by elemental analyses. Efficient synthesis of intermediate **5** enabled us to explore the Knoevenagel condensation reaction of various aldehydes with the active methylene of the 4-thiazolidinone ring, (Scheme 2). This reaction was catalysed by the addition of piperidine to the ethanolic solution of **5** and the appropriate aldehyde to give the ylidene derivatives **6-9** in fairly good yields 50-83%. All products were characterized by IR, NMR, mass spectrometry and elemental analysis.



Scheme 1. Synthesis of 1,3,4-thiadiazole-thiazolidinone hybrids. Reagents and conditions: (a) dry THF, rt, 24 h, 89%yield; (b) (i) $POCl_3$, reflux, 2 h, (ii) H_2O, reflux, 4 h, (ii) NH_4OH, 73%yield in 3 steps; (c) $ClCH_2COCl$, benzene, reflux, 3-5 h, 91.8% yield; (d) NH_4SCN, EtOH, reflux, 3-5 h, 83% yield.



Scheme 2. Synthesis of target compounds **6a-m** and **7-9**. Reagents and conditions: Appropriate aldehyde **(a**: substituted benzaldehyde, **b**: pyridine-3-carboxaldehyde, **c**: cyclohexanecarboxaldehyde, **d**: cinnamaldehyde**)**, Piperidine, EtOH, reflux, 24 h, 50-83% yield.

2.3. Biology

2.3.1 In vitro cyclooxygenase lipoxygenase inhibitory activity.

All compounds were tested for inhibitory activity of ovine COX-1 and human recombinant COX-2. The concentration of the tested compounds causing 50% inhibition (IC_{50}, µM) and selectivity index (COX-1 IC_{50}/COX-2 IC_{50}) were calculated (Table 1).

Table 1.
In vitro COX-1, COX-2 and 15-LOX inhibitory results

Compounds No.	R	COX-1 IC_{50} (µM)	COX-2 IC_{50} (µM)	Selectivity index	15-LOX IC_{50} (µM)
1	-	-	-	-	8.24
3	-	8.94	0.33	27.09	-
5	-	6.48	0.41	16.86	**3.11**
6a	H	4.66	0.085	54.82	5.74
6b	4-Methyl	10.42	0.11	94.73	8.96
6c	4-Propyl	9.81	0.18	54.50	7.63
6d	4-Isopropyl	10.21	0.12	85.08	8.78
6e	4-Hydroxy	10.74	0.14	76.71	9.74
6f	2-hydroxy	12.31	0.11	111.91	9.87
6g	4-Methoxy	11.42	0.11	103.82	9.74
6h	3,4-Dimethoxy	14.62	0.10	146.20	12.67
6i	3,4,5-Trimethoxy	16.11	0.09	179.00	11.74
6j	4-Dimethylamino	13.52	0.10	135.20	12.87
6k	4-Chloro	11.34	0.18	63.00	10.52
6l	3,4-Dichloro	15.42	0.07	**220.29**	11.87
6m	4-Bromo	14.11	0.10	141.10	10.84
7	-	8.47	0.41	20.66	7.22
8	-	7.89	0.22	35.86	5.97
9	-	15.23	0.08	190.38	13.2
Celecoxib	-	15.1	0.049	**308.16**	-
Diclofenac Sod.	-	5.29	0.3	17.63	-
Zileuton	-	-	-	-	**15.6**

The hybrid **6a** combining scaffolds targeting both COX-2 and 15-LOX exhibited better selectivity and potency in comparison with analogues containing each scaffold alone (compounds **1** and **3**). COX-2 IC_{50} of **6a** is 0.085 μM (IC_{50} of **3** = 0.33 μM) with selectivity index of 54 for COX-2 enzyme (SI of **3** = 27). 15-LOX IC_{50} of **6a** is 5.74 μM (IC_{50} of **1** = 8.24 μM). Encouraged by these subtle differences, we envisaged further modification of the arylidene moiety of **6a** may be a fine tuner of the activity of hybrids towards COX-2 and 15-LOX enzyme. We have introduced different modifications including ring substitutions on the arylidene moiety or its replacement with pyridene (**7**) or cyclohexyl (**8**). This resulted in significant changes in the potency and selectivity for COX-2 enzyme and the potency towards 15-LOX enzyme. For instance, arylidene ring substitution with 3,4-dichloro atoms in **6l** led to a little increase in enzyme inhibition (IC_{50} = 0.070 μM) with a marked effect on selectivity (COX-2 SI = 220). Compound **6l** is four folds more selective than the parent hybrid **6a**. In contrast, compound **6k** with only 4-chloro substitution on the arylidene moiety showed marked decrease in potency and selectivity. On the other hand, among the arylidene ring substitution with methoxy groups (**6g-i**) was tolerated. Therefore, **6i** which has three methoxy groups is 3 folds more selective than the parent hybrid **6a**. In general, introducing aliphatic substituent on the phenyl ring of the arylidene moiety (**6b-d**) led to decrease in potency while increasing selectivity in comparison with the parent hybrid **6a**. Methyl derivative **6b** showed higher potency and selectivity than propyl or isopropyl. Furthermore, hydroxyl substitution (**6e** and **6f**) enhanced the selectivity of the hybrid molecule. Compound **6f** with 2-hydroxy group was more potent and selective than **6e** with 4-hydroxy one. The absence of the arylidene moiety (**5)** or its replacement with pyridyl **7** or cyclohexyl moiety **(8)** decreased both potency and selectivity. The lower activity of compounds **6f** with 2-hydroxyphenyl, **6e** with 4-hydroxyphenyl and **7** with pyridyl than **6a** with phenyl suggests that the interaction at phenyl binding site is hydrophobic in nature. An interesting observation was that insertion of ethylene spacer in compound **9** had a slight effect on enzyme inhibitory activity (IC_{50} = 0.080 μM) and 3.5 folds increase in selectivity than the parent hybrid **6a**. Most compounds have similar potency but significantly different selectivity. Therefore, the preliminary structure activity relationship could be concluded: the arylidene moiety is required for high potency and selectivity, substitution on arylidene ring affect the selectivity more than potency through hydrophobic and electronic interactions.

2.3.2. In vitro lipoxygenase inhibitory activity.

All compounds were tested for 15-LOX enzyme of soybean. The new synthesized compounds are more potent than the reference drug zileuton against 15-LOX enzyme. The most active compound was **5** which lacks arylidene moiety. Compound **5** has an IC_{50} of 3.11 μM. Among arylidene containing compounds, **6a** with unsubstituted phenyl ring was the most potent derivative (IC_{50} of 5.74 μM). All trialled substitutions on the phenyl ring led to a decrease in potency. Replacement of the phenyl group **6a** with a cyclohexyl moiety **8** retain activity. Compound **9** with ethylene spacer showed marked decrease in potency.

2.3.3. In vivo anti-inflammatory activity.

Compounds (**6a**, **6f**, **6i**, **6l**, **6m** and **9)** were selected for *in vivo* study by carrageenan induced paw edema method in rats at 28 μmol/kg. Celecoxib and diclofenac sodium were used as reference drugs. The results are presented as a percentage of edema inhibition at time interval of 1, 2, 3, 4 h (Table 2). All tested compounds showed a gradual increase of the anti-inflammatory activity up to its maximum after 3 h. Compounds (**6a**, **6l** and **9**) were the most active derivatives and exhibited comparable inhibitory activity to the two reference drugs (Fig. 3).

Table 2.
% of edema inhibition of the synthesized compounds.

Compounds	**% Inhibition of Edema**			
No.	**1h**	**2 h**	**3 h**	**4 h**
Celecoxib	18.18 ± 6.63	41.51 ± 3.53^{d}	42.11 ± 7.13^{d}	40.35 ± 4.29^{d}
Diclofenac Sod.	22.73 ± 6.63^{a}	41.51 ± 4.62^{d}	40.35 ± 6.45^{d}	33.33 ± 5.95^{d}
6a	40.91 ± 8.35^{d}	47.17 ± 6.40^{d}	52.63 ± 5.95^{d}	36.84 ± 4.30^{d}
6f	25.00 ± 2.78^{a}	37.74 ± 3.77^{d}	38.60 ± 3.92^{d}	17.54 ± 3.51^{a}
6i	13.64 ± 5.79	33.96 ± 5.97^{d}	47.37 ± 6.20^{d}	29.83 ± 4.80^{d}
6l	29.55 ± 6.63^{b}	52.83 ± 6.67^{d}	54.39 ± 1.75^{d}	35.09 ± 2.15^{d}
6m	31.82 ± 5.08^{c}	45.28 ± 6.26^{d}	45.61 ± 5.12^{d}	35.09 ± 6.56^{d}
9	38.63 ± 2.78^{d}	64.15 ± 1.89^{d}	50.88 ± 5.95^{d}	40.35 ± 4.30^{d}

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

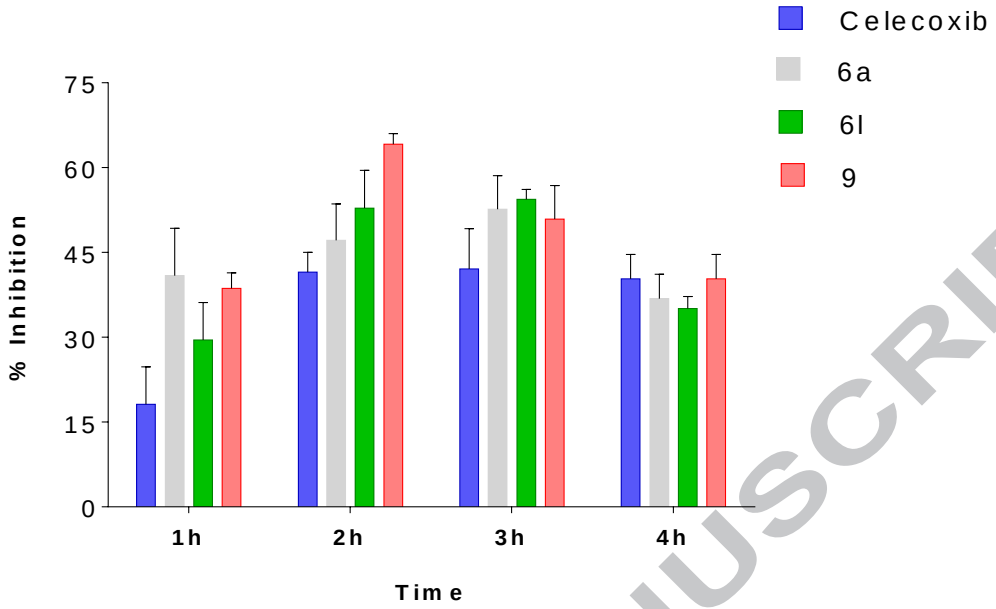


Fig. 3. % Edema inhibition of tested compounds (**6a, 6l** and **9)** in comparison with celecoxib.

2.3.4. Ulcerogenic liability.

Gastrointestinal (GI) complications are the most serious side effects of NSAIDs. Microscopic examination of gastrointestinal mucosa for the presence of lesions following oral administration of a single dose of tested compound as well as the reference drug has been taken as an indication for the ulcerogenic effect. Compounds (**6f, 6l, 6m** and **9**) were selected for testing of their ulcerogenic effect at a dose of 20 mg/kg which is double the dose of *in vivo* anti-inflammatory test. Increasing the amount of tested compounds was made to ensure the safety at a higher dose. Indomethacin was the reference drug at a dose of 10 mg/kg (as a positive control). Microscopic examination of gastric mucosa following administration of tested compounds and control group showed normal glandular architecture and gastric pits (Fig. 4 and 5) in comparison with indomethacin which induced desquamation of surface epithelial cells, marked loss of normal glandular architecture, edema and dilation of blood vessel (Fig. 4 and 5).

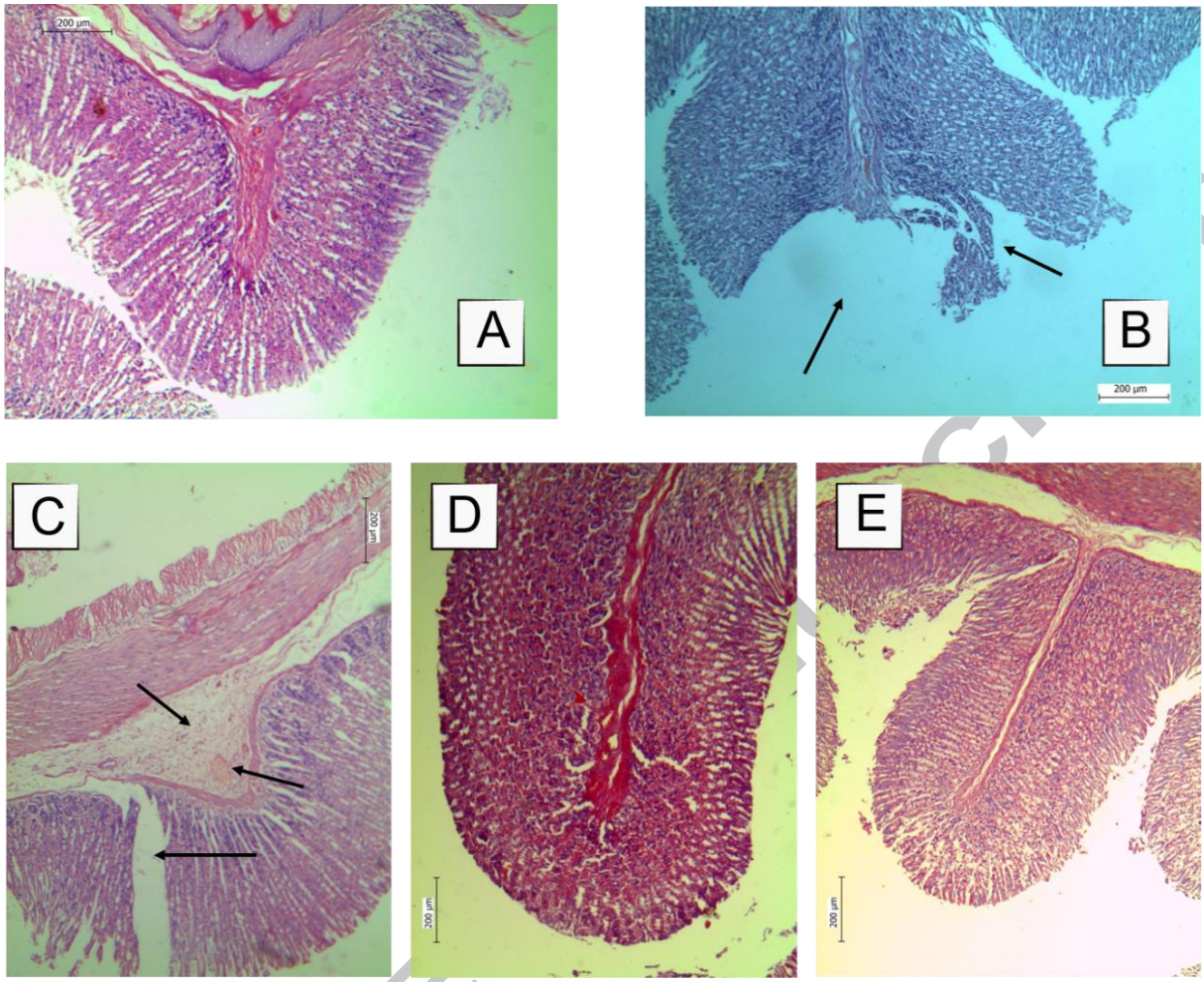


Fig. 4. Light microscopic pictures of gastric mucosa of the control group (Fig. **A**), tested compounds (**6l** (Fig. **D**) and **6f** (Fig. **E**)**)** showed normal glandular architecture and gastric pits. The indomethacin group (Fig. **B** and **C**) showed induced desquamation of surface epithelial cells edema and dilation of blood vessel. Magnification 4x; scale bar 200 µm.

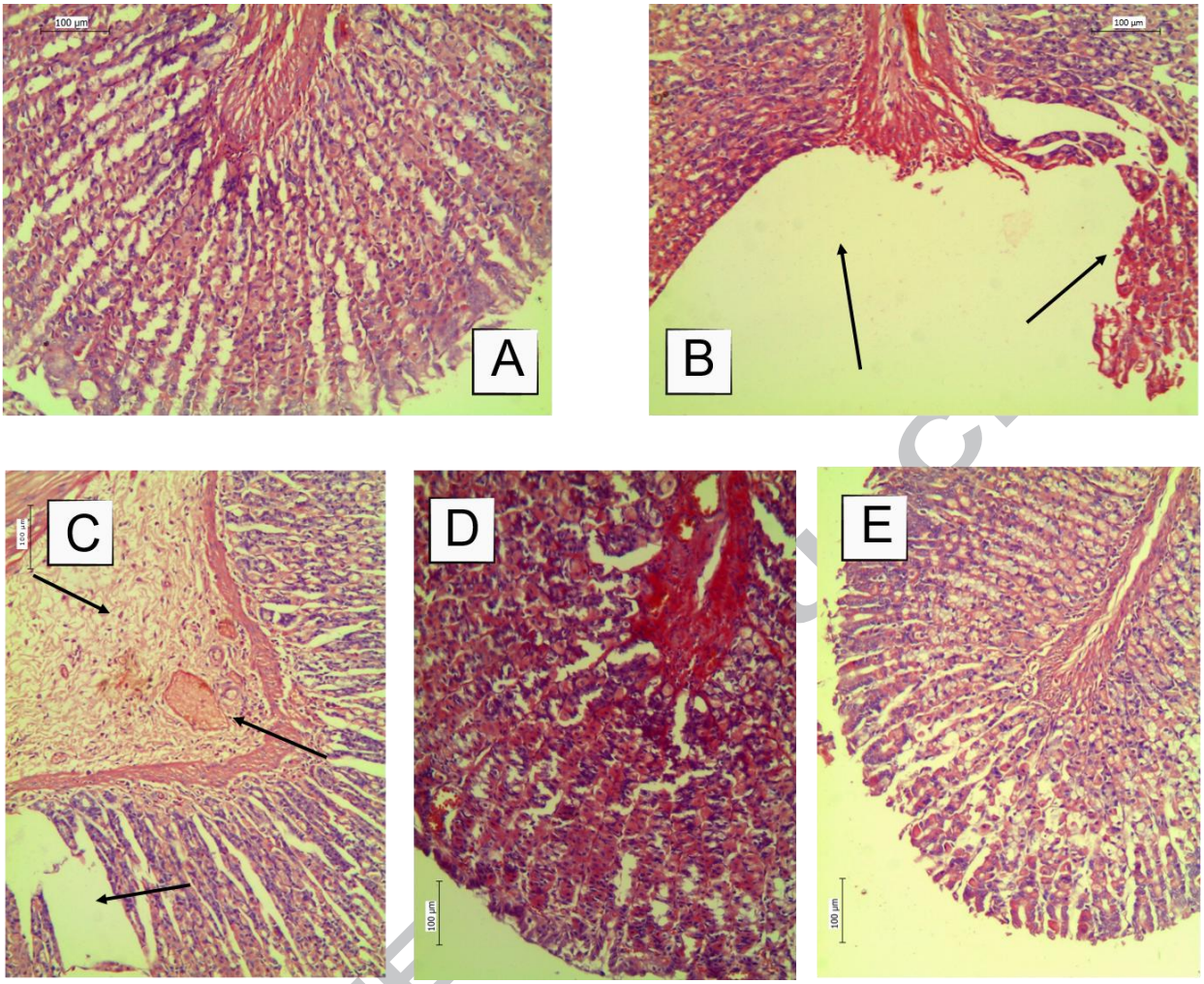


Fig. 4. Light microscopic pictures of gastric mucosa of the control group (Fig. **A**), tested compounds (**6l** (Fig. **D**) and **6f** (Fig. **E**)**)** showed normal glandular architecture and gastric pits. The indomethacin group (Fig. **B** and **C**) showed induced desquamation of surface epithelial cells edema and dilation of blood vessel. Magnification 10x; scale bar 100 µm.

2.3.5. Molecular modelling.

2.3.5.1. Docking study on COX-2.

Volume, LogP and docking scores (Table 3) were calculated using MOE 2016.09 software and the human COX-2 enzyme (5KIR) was used for the docking study. The docking scores ranged from -7.018 to -7.948 showing rough correlation with the IC_{50} of the synthesized compounds. As mentioned earlier, the size of COX-2 pocket is bigger than COX-1 which allows selective binding of larger molecules. The most selective compound **6l** showed the largest calculated volume among the series (Fig. 6) and the least selective compound **5** showed the least calculated volume. Additionally, **6l** possesses the highest LogP which may also have contributed to its high *in vivo* anti-inflammatory activity.

Table 3.
Volume, LogP and docking score of the new synthesis compounds.

Compound No.	Volume [a]	LogP [b]	ΔG COX-2 [c]	ΔG 15-LOX [c]
5	672.4	2.433	-7.232	-5.269
6a	972.0	4.981	-7.018	-5.038
6b	1005.1	5.280	-7.221	-5.083
6c	1090.4	6.197	-7.948	-5.016
6d	1077.1	6.125	-7.416	-5.334
6e	950.8	4.674	-7.162	-5.206
6f	939.6	4.672	-7.239	-4.794
6g	1007.0	4.938	-7.359	-4.481
6h	1058.9	4.680	-7.886	-5.257
6i	1026.4	4.423	-7.911	-5.542
6j	1090.9	4.897	-7.404	-4.695
6k	1058.4	5.574	-7.762	-4.747
6l	1118.6	6.201	7.612	-4.478
6m	1098.9	5.780	-7.134	-4.698
7	919.3	3.749	-7.112	-5.052
8	957.6	4.997	-7.371	-4.898
9	1087.5	5.626	-7.815	-3.672
Rofecoxib	-	-	-8.375	-

a. Volume calculated using vsurf_D1. **b.** LogP (o/w). **c.** ΔG (Kcal/mol)

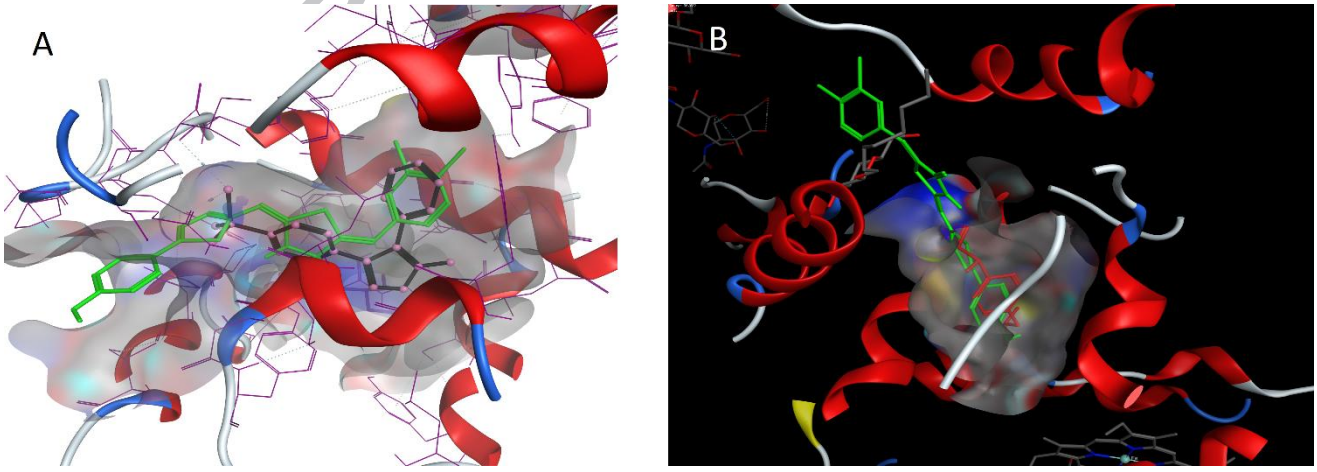


Fig. 5. Compound **6l** (showed in green) binds effectively to the pocket of COX-2 enzyme (Fig. **A**) in compare with COX-1 (Fig. **B**). The co-crystalized ligand rofecoxib (showed in black Fig. **A**) and Ibuprofen® (showed in red Fig. **B**) are also explored.

In terms of binding and interaction, the three most potent compounds (**6l**, **9** and **6i**) explore good binding with COX-2 enzyme. For instance, compound **6l** (Fig.7) form

three hydrogen bonds with Arg513 (2.62 Å), Phe518 (2.05 Å) and Ile517 (2.42 Å) and one hydrophobic interaction with Ala527. Also, **9** (Fig. 8) forms two hydrogen bonds with Arg513 (3.52Å) and Phe518 (2.51 Å). Lastly, **6i** (Fig. 9) forms two hydrogen bonds with Arg513 (2.79Å) and Leu352 (1.92 Å) and one hydrophobic interaction with Ser353.

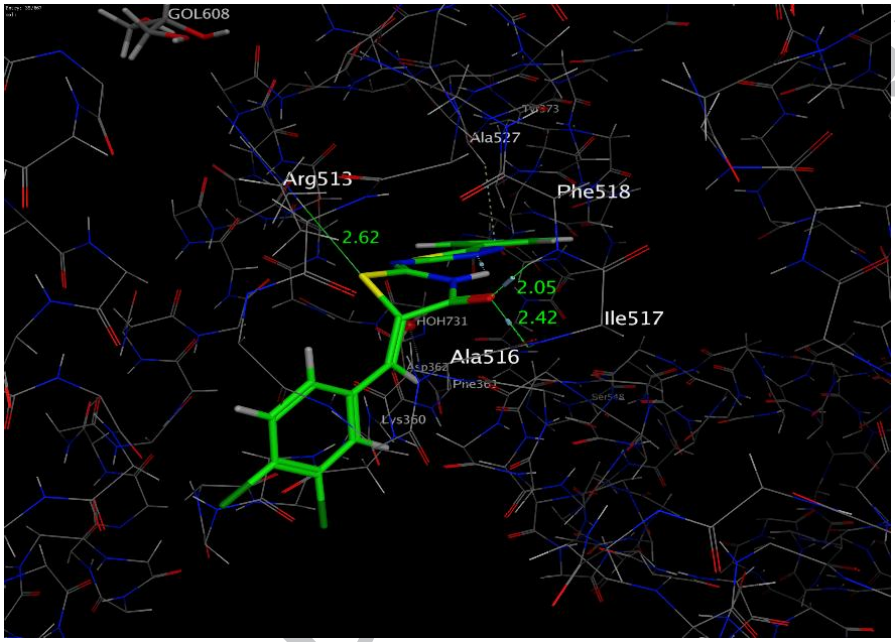


Fig. 6. Interaction of **6l** with COX-2 enzyme.

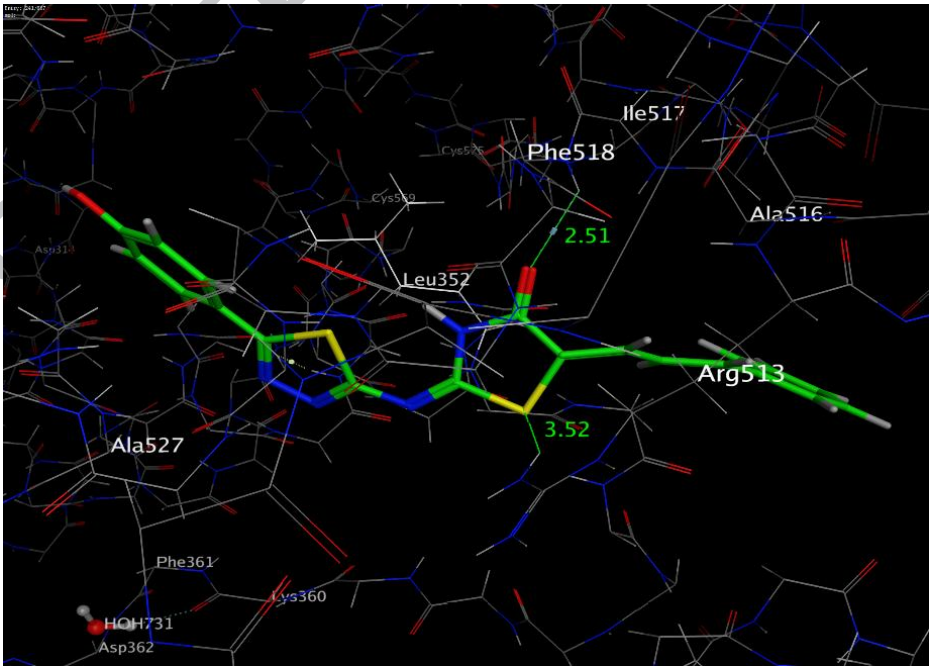


Fig. 7. Interaction of **9** with COX-2 enzyme.

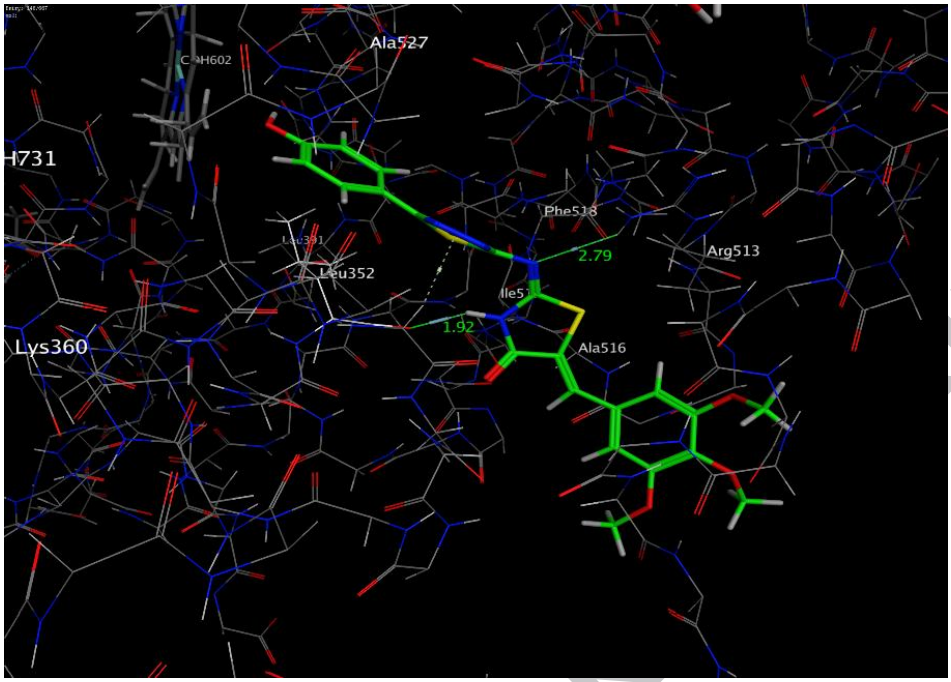


Fig. 8. Interaction of **9** with COX-2 enzyme.

2.3.5.2. Docking study on 15-LOX.

Human 15-LOX enzyme (4NRE) was used for the docking study. The docking scores ranged from -3.672 to -5.542 showing rough correlation with the IC_{50} of the synthesized compounds. There is a good correlation between the molecular volume and IC_{50} of the synthesized compounds where smaller molecules have higher potency than larger one (Table 3).

In terms of binding and interaction, the three most potent compounds (**5**, **6a** and **8**) form a hydrogen bond with Glu 613 (Fig. 10 to 12) with a distance of (2.26 Å, 2.25 Å and 2.28 Å, respectively). Compound **8** form additional hydrogen bond with Lys 612 (2.36 Å)

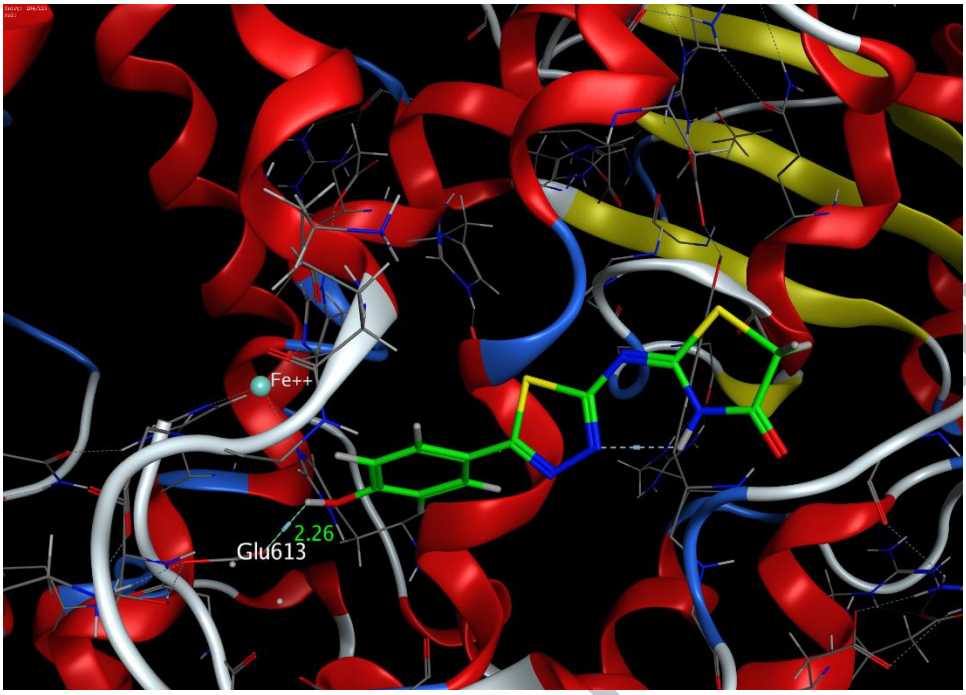


Fig. 9. Interaction of **5** with 15-LOX enzyme.

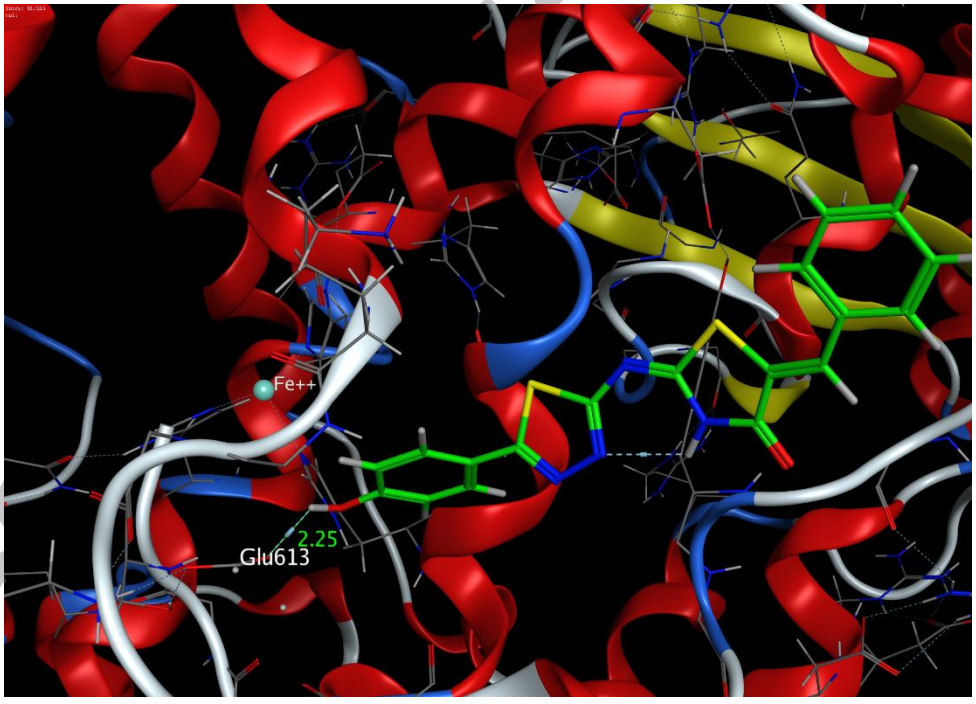


Fig. 10. Interaction of **6a** with 15-LOX enzyme.

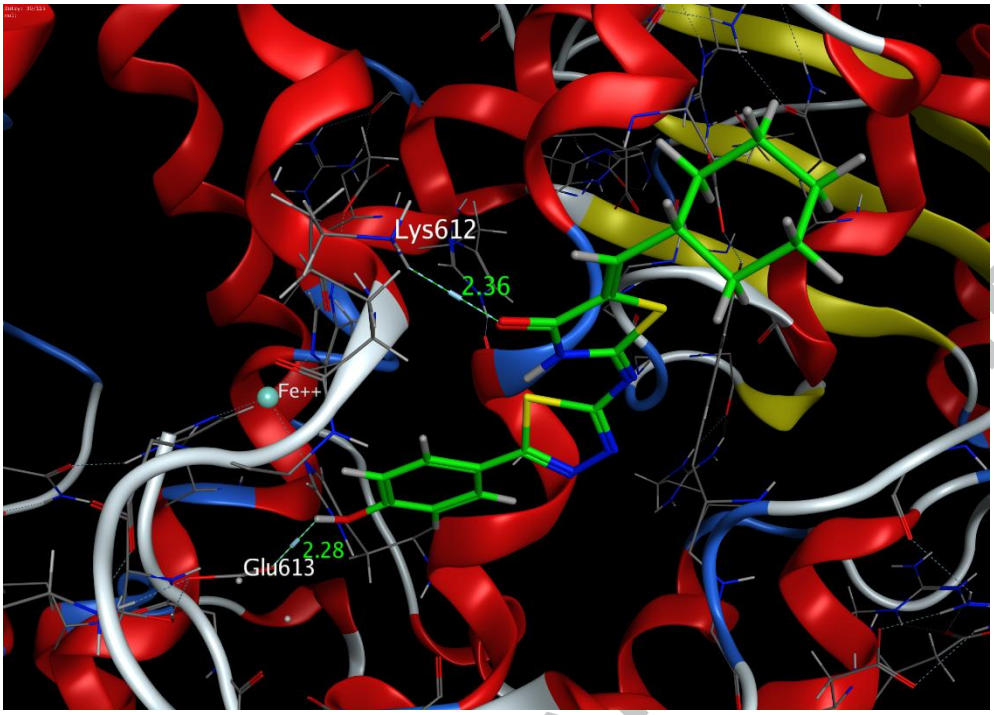


Fig. 11. Interaction of 6a with 15-LOX enzyme.

2.3. Conclusion

The current study proposed a new rational design of dual inhibitors of COX-2 and 15-LOX based on combining 2-amino-1,3,4-thiadiazole as a COX inhibitor pharmacophore and 4-thiazolodine as a 15-LOX inhibitor pharmacophore into one construct. The obtained results clearly revealed that the hybrids are much more potent and selective COX-2 and 15-LOX than their individual components. Moreover, compounds **6a**, **6f**, **6i**, **6l**, **6m** and **9** showed anti-inflammatory activity equivalent to or even higher than that of celecoxib. In vitro COX-1/COX-2 inhibition study revealed that among the synthesized compounds, compound 6l showed the highest inhibitory activity against COX-2 with an IC50 values of 70 nM and selectivity index 220. Additionally, the active compounds showed significant *in vitro* LOX inhibitory activity higher than that of zileuton and good GIT safety profile and are well tolerated by experimental animals. The docking experiments attempted to investigate the binding mode of the most active compounds in the binding site of COX-2 and 15-LOX and confirmed the high selectivity binding towards COX-2 enzyme over COX-1. Consequently, The in *vitro* and *in vivo* anti-inflammatory profiles of the synthesized compounds can be a base for developing new anti-inflammatory drugs.

3. Experimental.

3.1. Chemistry

All solvents and reagents used for the synthesis of target compounds were of commercial grade without further purification before use except THF dried by benzophenone/sodium metal method and benzene dried over sodium metal. Melting points were determined on an electrothermal melting point apparatus [Cole-Parmer - Electrothermal IA9100, UK], and were uncorrected. Pre-coated silica gel plates (TLC) (kieselgel 0.25 mm, 60G F254, Merck, Germany) were used for monitoring of the chemical reactions. Spots were detected by using ultraviolet lamp at 254 nm wavelength (Spectroline, model CM-10, USA). (IR) spectra (KBr discs) were recorded on thermo scientific nicolet IS10 FT IR spectrometer (thermo Fischer scientific, USA) at Faculty of science, Assiut University, Assiut, Egypt. Most of ^{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 (**2, 3** and **4**) 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 carried out on Direct Probe Controller Inlet Part TO Single Quadropole 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, Nasr city, Cairo, Egypt. Elemental microanalyses were performed on elemental analyzer model flash 2000 thermo fisher at the Regional Center for Mycology and Biotechnology (RCMB), Faculty of Science, Al-Azhar University, Nasr city, Cairo, Egypt.

*3.1.1. Synthesis of 4-(2-thiocarbamoylhydrazinocarbonyl) phenyl acetate (**2**).*

4-Acetoxy benzoyl chloride (11 g, 55 mmol) was dissolved in dry THF (70 mL) and the obtained solution was poured to a stirred suspension of thiosemicarbazide (10 g, 109.8 mmol, 2 equv) in dry THF (240 mL). The reaction mixture was stirred at room temperature for 24 h. The THF was evaporated, distilled water (50 mL) was added to the dry solid and vigorously stirred for 5 min. The product was filtered to give white solid. The product was used in the next step without further purification as white solid; Yield 89%; mp 205-207 °C; IR (cm^{-1}, KBr): 3353, 3296 (NH_2, NH), 1749 (C=O ester) 1623 (C=O amidic); ^{1}H-NMR (90 MHz, δ ppm DMSO- d_6): 2.3 (s, 3H, C$\underline{H}_3$), 7.15,7.9 (dd, *J*= 9 Hz, 4H, Ar-H), 7.65 (br. s, 2H, N$\underline{H}_2$), 9.3 (br. s, 1H, N$\underline{H}$),

10.3 (br. s, 1H, NH); Elemental analysis for $C_{10}H_{11}N_3O_3S$ (253.28)**:** Calculated/Found: 47.42/47.69 (%C), 4.38/4.61 (%H), 16.59/16.75 (%N) and 12.66/12.49 (%S).

*3.1.2. Synthesis of 2-amino-5-(4-hydroxyphenyl)-1,3,4-thiadiazole (**3**).*

Compound **2** (10 g, 39.5 mmol) and phosphorus oxychloride (39.5 mL, 157.1 mmol, 4 equv.) were refluxed for 1 h. The excess of phosphorus oxychloride was evaporated under vacuum. cold water (50 mL) was added and the reaction mixture was stirred under reflux overnight. After cooling to room temperature, the solution was basified by conc. ammonium hydroxide solution (20%) and the separated solid was filtered and washed with cooled water to obtain a yellow solid; Yield 73%; mp 209-211 °C; IR (cm^{-1}, KBr): 3396, 3242 (OH, NH_2), 1568 (C=N); ^{1}H-NMR (90 MHz, δ ppm DMSO-d_6): 6.85,7.55 (dd, *J*= 9 Hz, 4H, Ar-H), 7.2 (br. s, 2H, NH_2), 9.9 (br. s, 1H, OH); EI-MS [*m/z* (%)]: 192.89 (M^+, 75.3%), 119.96 (100% base peak); Elemental analysis for $C_8H_7N_3OS$ (193.23)**:** Calculated/Found: 49.73/49.58 (%C), 3.65/3.82 %H), 21.75/21.93 (%N) and 16.59/16.72 (%S).

*3.1.3. Synthesis of 2-chloro-N-[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]acetamide (**4**).*

Compound **3** (2.69 g, 10 mmol) was stirred in dry benzene (15 mL). Chloroacetyl chloride (0.88 mL, 11 mmol, 1.1 equv.) in dry benzene (10 mL) was divided into three equal parts and added within 30 min time interval. After 1 h from the addition of the third part, additional amounts of chloroacetyl chloride (0.08 mL, 1 mmol, 0.1 equv.) were added every 1 h until the start consume. The reaction mixture was filtered while hot and washed with cold methanol. The product recrystalized from dioxane/ water as white solid; Yield 91.8%; mp 256-258 °C; IR (cm^{-1}, KBr): 3396 (NH), 1693 (C=O), 1595,1574 (C=N); ^{1}H-NMR (90 MHz, δ ppm DMSO- d_6): 4.35 (s, 2H, CH_2), 6.79,7.09 (dd, *J*= 9 Hz, 4H, Ar-H), 9.8 (br. s, 1H, OH). EI-MS [*m/z* (%)]:270.92 (M^++2, 36.93%), 268.93 (M^+, base beak); Elemental analysis for $C_{10}H_8ClN_3O_2S$ (269.71)**:** Calculated/Found: 44.53/44.81 (%C), 2.99/3.08 (%H), 15.58/15.7 (%N) and 11.89/11.97 (%S).

*3.1.4. 2-[(5-(4-Hydroxyphenyl)-1,3,4-thiadiazol-2-yl)imino]-1,3-thiazolidin-4-one (**5**)*

Compound **4** (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 product was filtered while hot, washed with cooled water and recrystallize from from DMSO/water. The product

was brownish solid; Yield 83%; mp >300 °C; IR (cm^{-1}, KBr): broad band at 3409 (NH, OH), 1720 (C=O), 1574 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO- d_6)**:** 4.11 (s, 2H, C$\underline{H}_2$), 6.9 ,7.75 (dd, *J*= 8.56 Hz, 4H, Ar-H), 10.15 (s, 1H, O$\underline{H}$), 12.31 (br. s, 1H, N$\underline{H}$); ^{13}C-NMR (100 MHz, δ ppm DMSO- d_6)**:** 36.06 ($\underline{C}H_2$),116.59, 121.68, 129.27,160.54 (Ar-C), 174.34($\underline{C}$=O); EI-MS [*m/z* (%)]:294.82 (M^++2, 9.70%), 292.07 (M^+, 70.84%), 274.06 (96.58%), 271.28 (77.25%), 58.98 (100% base beak); Elemental analysis for $C_{11}H_8N_4O_2S_2$ (292.34)**:** Calculated/Found: 45.19/45.42 (%C), 2.76/2.79 (%H), 19.17/19.35 (%N) and 21.94/22.18 (%S).

*3.1.5. General procedure for Knoevenagel condensation of compound (**5**) with aldehydes to give compounds (**6a-m** and **7-9**).*

Compound **5** (0.29 g, 1 mmol) and the appropriate aldehyde (1.5 mmol, 1.5 equv.) were stirred in absolute ethanol (10 mL). Piperidine (8 drops) was added and the reaction mixture was refluxed for 16-18 h, cooled and acidified with glacial acetic acid. The solid product was collected by filtration and washed with cold water.

*3.1.5.1. (5Z)-5-benzylidene-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**6a**).*

Orange solid; Yield 58.4%; mp >300 °C; IR (cm^{-1}, KBr): broad band at 3409 (NH, OH), 3021 (sp^2C-H), 1721 (C=O), 1606 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 6.90 (d, *J* = 8.44 Hz, 2H, Ar-$\underline{H}$), 7.50 (d, *J* = 6.72 Hz, 1H, Ar-$\underline{H}$), 7.57 (t, *J* = 6.97 Hz, 2H, Ar-$\underline{H}$), 7.61-7.80 (m, 5H, Ar-$\underline{H}$), 10.13 (br. s, 1H, O$\underline{H}$), 12.89 (br. s, 1H, N$\underline{H}$); ^{13}C-NMR (100 MHz, δ ppm DMSO- d_6)**:** 116.61, 121.49, 124.58, 129.35, 129.81, 130.63, 130.89, 133.15, 133.66, 158.50, 160.68, 165.69, 167.39, 168.95 ($\underline{C}$=O); Elemental analysis for $C_{18}H_{12}N_4O_2S_2$ (380.44): Calculated/Found: 56.83/57.07 (%C), 3.18/3.31 (%H), 14.73/15.02 (%N) and 16.86/16.79 (%S).

*3.1.5.2.(5Z)-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-5-[(4-methylphenyl)methylidene]-1,3-thiazolidin-4-one(**6b**).*

Yellow solid; Yield 83.7%; mp >300 °C; IR (cm^{-1}, KBr): broad band at 3115 (NH, OH), 3021 (sp^2C-H), 2909 (sp^3C-H), 1708 (C=O), 1594 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO- d_6)**:** 2.38 (s, 3H, C$\underline{H}_3$), 6.91,7.77 (dd, *J* =8.31-8.68, 4H, Ar-$\underline{H}$), 7.40, 7.57 (dd, *J* =7.46-7.58, 4H, Ar-$\underline{H}$), 7.75 (s, 1H, =C-$\underline{H}$), 10.13 (br. s, 1H, O$\underline{H}$), 12.85 (br. s, 1H, N$\underline{H}$); Elemental analysis for $C_{19}H_{14}N_4O_2S_2$ (394.47): Calculated/Found: 57.85/58.02 (%C), 3.58/3.80 (%H), 14.20/14.37 (%N) and 16.26/16.39 (%S).

*3.1.5.3.(5Z)-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-5-[(4-propylphenyl)methylidene]-1,3-thiazolidin-4-one (**6c**).*

Yellow solid; Yield 71%; mp 263-265 °C; IR (cm^{-1}, KBr): broad band at 3421 (NH, OH), 3022 (sp^2C-H), 2927 (sp^3C-H), 1719 (C=O), 1597 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO- d_6)**:** 0.90 (t, *J* =7.34, 3H, C<u>H</u>$_3$), 1.61 (sxt, *J* =7.41, 2H, C<u>H</u>$_2$), 2.61 (t, *J* =7.52, 2H, C<u>H</u>$_2$), 6.91, 7.77 (dd, *J* =8.56-8.68, 4H, Ar-<u>H</u>), 7.40, 7.58 (dd, *J* = 7.95, 4H, Ar-<u>H</u>), 7.75 (s, 1H, =C-<u>H</u>) 10.19 (br. s, 1H, O<u>H</u>), 12.89 (br. s, 1H, N<u>H</u>); Elemental analysis for $C_{21}H_{18}N_4O_2S_2$ (422.52): Calculated/Found: 59.69/60.02 (%C), 4.29/4.37 (%H), 13.26/13.43 (%N) and 15.18/15.29 (%S).

*3.1.5.4.(5Z)-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-5-{[4-(propan-2-yl)phenyl]methylidene}-1,3-thiazolidin-4-one (**6d**).*

Yellow solid; Yield 73.4%; mp 182-184 °C; IR (cm^{-1}, KBr): broad band at 3372 (NH, OH), 3022 (sp^2C-H), 2958 (sp^3C-H), 1696 (C=O), 1595 (C=N). ^{1}H-NMR (400 MHz, δ ppm DMSO- d_6)**:** 1.23 (d, *J* =6.85, 6H, (C<u>H</u>$_3$)$_2$), 2.69 (m, 1H, C<u>H</u>), 6.92 (d, *J* =8.56, 2H, Ar-<u>H</u>), 7.47, 7.61 (dd, *J* = 7.70, 4H, Ar-<u>H</u>), 7.74-7.80 (m, 3H, Ar-<u>H</u>, 1H, =C-<u>H</u>) 10.19 (br. s, 1H, O<u>H</u>), 12.90 (br. s, 1H, N<u>H</u>); Elemental analysis for $C_{21}H_{18}N_4O_2S_2$ (422.52): Calculated/Found: 59.69/59.98 (%C), 4.29/4.41 (%H), 13.26/13.19 (%N) and 15.18/15.31 (%S).

*3.1.5.5.(5Z)-5-[(4-hydroxyphenyl)methylidene]-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**6e**).*

Yellow solid; Yield 75.7%; mp >300 °C; IR (cm^{-1}, KBr): broad band at 3404 (NH, OH), 3012 (sp^2C-H), 1694 (C=O), 1580 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 6.92 (br. s, 2H, Ar-<u>H</u>), 6.96 (br. s, 2H, Ar-<u>H</u>), 7.53, 7.69, 7.75 (m, 5H, Ar-<u>H</u>, =C-<u>H</u>), 10.04 (br. s, 1H, O<u>H</u>), 10.22 (br. s, 1H, O<u>H</u>), 12.66 (br. s, 1H, N<u>H</u>); Elemental analysis for $C_{18}H_{12}N_4O_3S_2$ (396.44): Calculated/Found: 54.53/54.81 (%C), 3.05/3.22 (%H), 14.13/14.5 (%N) and 16.18/16.35 (%S).

*3.1.5.6.(5Z)-5-[(2-hydroxyphenyl)methylidene]-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**6f**).*

Yellow solid; Yield 65.6%; mp 264-266 °C; IR (cm^{-1}, KBr): broad band at 3377 (NH, OH), 3032 (sp^2C-H), 1690 (C=O), 1592 (C=N).^{1}H-NMR (400 MHz, δ ppm DMSO-d_6) 6.92,7.76 (dd, *J* = 6.97-7.21, 4H, Ar-<u>H</u>), 7.00 (br. s, 2H, Ar-<u>H</u>), 7.33 (br. s, 1H, Ar-<u>H</u>), 7.47 (d, 2H, *J* = 5.99, Ar-<u>H</u>), 8.03 (s, 1H, =C-<u>H</u>), 10.04 (br. s, 1H, O<u>H</u>), 10.44

(br. s, 1H, OH), 12.71 (br. s, 1H, NH); ^{13}C-NMR (100 MHz, δ ppm DMSO- d_6)**:** 116.36, 121.62, 129.32,160.55,116.76, 120.24, 120.73, 122.91, 128.79,129.32 (br. signal), 132.75, 157.75, 158.99, 165.55, 167.58, 169.05(C=O); Elemental analysis for $C_{18}H_{12}N_4O_3S_2$ (396.44): Calculated/Found: 54.53/54.79 (%C), 3.05/3.11 (%H), 14.13/14.37 (%N) and 16.18/16.32 (%S).

*3.1.5.7. (5Z)-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-5-[(4-methoxyphenyl)methylidene]-1,3-thiazolidin-4-one (**6g**).*

Yellow solid; Yield 70.7%; mp > 300 °C; IR (cm^{-1}, KBr): 3348 (NH, OH), 3013 (sp^{2}C-H), 2814 (sp^{3}C-H), 1712 (C=O), 1588 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO- d_6)**:** 3.83 (s, 3H, OCH$_3$), 6.91, 7.75 (dd, *J* =8.44-8.56, 4H, Ar-H), 7.15, 7.63 (dd, *J* = 8.19, 4H, Ar-H), 7.73 (s, 1H, =C-H), 10.12 (br. s, 1H, OH), 12.78 (br. s, 1H, NH); Elemental analysis for $C_{19}H_{14}N_4O_3S_2$ (410.47): Calculated/Found: 55.60/55.94 (%C), 3.44/3.70 (%H), 13.65/13.87 (%N) and 15.62/15.89 (%S).

*3.1.5.8. (5Z)-5-[(3,4-dimethoxyphenyl)methylidene]-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**6h**).*

Yield 61.3%; mp 286-288 °C; IR (cm^{-1}, KBr): 3423 (NH, OH), 3010 (sp^{2}C-H), 2939 (sp^{3}C-H), 1692 (C=O), 1588 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO- d_6)**:** 3.825 (s, 3H, OCH$_3$), 3.834 (s, 3H, OCH$_3$), 6.91, 7.75 (dd, *J* = 8.56, 4H, Ar-H), 7.19 (d, *J* = 8.22, 1H, Ar-H), 7.22-7.32 (m, 3H, Ar-H), 7.73 (s, 1H, =C-H), 10.12 (br. s, 1H, OH), 12.77 (br. s, 1H, NH); Elemental analysis for $C_{20}H_{16}N_4O_4S_2$ (440.50): Calculated/Found: 54.53/54.8 (%C), 3.66/3.67 (%H), 12.72/12.59 (%N) and 14.56/14.32 (%S).

*3.1.5.9.(5Z)-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-5-[(3,4,5-trimethoxyphenyl)methylidene]-1,3-thiazolidin-4-one (**6i**).*

Yellow solid; Yield 72%; mp decompose at 280 °C; IR (cm^{-1}, KBr): 3435 (NH, OH), 3010 (sp^{2}C-H), 2944 (sp^{3}C-H), 1699 (C=O), 1600 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO- d_6)**:** 3.75 (s, 3H, OCH$_3$), 3.85 (s, 6H, O(CH$_3$)$_2$), 6.89, 7.74 (dd, *J* = 8.56, 4H, Ar-H), 6.96 (s, 2H, Ar-H), 7.70 (s, 1H, =C-H), 10.11 (br. s, 1H, OH), 12.82 (br. s, 1H, NH); Elemental analysis for $C_{21}H_{18}N_4O_5S_2$ (470.52): Calculated/Found: 53.61/53.89 (%C), 3.86/3.94 (%H), 11.91/12.23 (%N) and 13.63/13.78 (%S).

*3.1.5.10.(5Z)-5-{[4-(dimethylamino)phenyl]methylidene}-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**6j**).*

Yellow solid; Yield 79.2%; mp 294-297 °C; IR (cm^{-1}, KBr): broad bande at 3323 (NH, OH), 3000 (sp^2C-H), 2902 (sp^3C-H), 1659 (C=O), 1566 (C=N).^{1}H-NMR (400 MHz, δ ppm DMSO- d_6)**:** 3.00 (s, 6H, N($C\underline{H}_3$)$_2$), 6.83,7.47 (dd, *J* =8.56-8.44, 4H, Ar-H), 6.90, 7.74 (dd, *J* =8.44, 4H, Ar-H), 7.61 (s, 1H, =C-H), 10.15 (br. s, 1H, OH), 12.31 (br. s, 1H, NH); ^{13}C-NMR (100 MHz, δ ppm DMSO- d_6)**:** (2 $\underline{C}H_3$ signals overlapped with DMSO signal), 116.56, 121.62, 129.29,160.55, 112.53, 132.89, 116.61, 120.41,134.43, 151.94, 159.04, 165.06, 167.65, 169.16 (C=O); Elemental analysis for $C_{20}H_{17}N_5O_2S_2$ (423.51): Calculated/Found: 56.72/56.89 (%C), 4.05/4.12 (%H), 16.54/16.80 (%N) and 15.14/15.23 (%S).

*3.1.5.11. (5Z)-5-[(4-chlorophenyl)methylidene]-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**6k**).*

Yellow solid; Yield 74.7%; mp >300 °C; IR (cm^{-1}, KBr): broad band at 3376 (NH, OH), 3019 (sp^2C-H), 1715 (C=O), 1606 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6): 6.91 (d, *J* = 6.36, 2H, Ar-H), 7.55-7.85 (m, 7H, Ar-H, =C-H), 10.13 (br. s, 1H, OH), 12.92 (br. s, 1H, NH); Elemental analysis for $C_{18}H_{11}ClN_4O_2S_2$ (414.89): Calculated/Found: 52.11/52.40 (%C), 2.67/2.75 (%H), 13.50/13.78 (%N) and 15.46/15.57 (%S).

*3.1.5.12. (5Z)-5-[(3,4-dichlorophenyl)methylidene]-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**6l**).*

Orange solid; Yield 64.3%; mp >300 °C; IR (cm^{-1}, KBr): broad band at 3371 (NH, OH), 3011 (sp^2C-H), 1729 (C=O), 1604 (C=N). ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 6.91 (br. s, 2H, Ar-H), 7.45-8.05 (m, 6H, Ar-H, =C-H), 10.19 (br. s, 1H, OH), 12.00 (br. s, 1H, NH); ^{13}C-NMR (100 MHz, δ ppm DMSO- d_6): 116.60, 121.44, 126.88, 129.35, 129.50, 130.38, 131.94, 132.53, 132.69, 133.2, 134.37, 157.73, 160.74, 165.85, 167.09, 168.79 (C=O); Elemental analysis for $C_{18}H_{10}Cl_2N_4O_2S_2$ (449.33): Calculated/Found: 48.11/48.39 (%C), 2.24/2.31 (%H), 12.47/12.8 (%N) and 14.27/14.56 (%S).

*3.1.5.13. (5Z)-5-[(4-bromophenyl)methylidene]-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**6m**).*

Yield 50.1%; sublimate at 290; IR (cm^{-1}, KBr): 3392 (NH, OH), 3109 (sp^2C-H), 1705 (C=O), 1603 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO- d_6)**:** 6.90 (d, *J* =8.19, 2H, Ar-H), 7.58 (d, *J* =7.46, 2H, Ar-H), 7.65-7.87 (m, 5H, Ar-H, =C-H), 10.06 (br. s, 1H, OH), 12.88 (br. s, 1H, NH); Elemental analysis for $C_{18}H_{11}BrN_4O_2S_2$ (459.34): Calculated/Found: 47.07/47.26 (%C), 2.41/ 2.44 (%H), 12.2/12.46 (%N) and 13.96/14.05 (%S).

*3.1.5.14. (5Z)-2-{[5-(4-Hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-5-[(pyridin-3-yl)-methylidene]-1,3-thiazolidin-4-one (**7**).*

Orange solid; Yield 78.4%; mp >300 °C; IR (cm^{-1}, KBr): broad band at 3409 (NH, OH), 3012 (sp^2C-H), 1721 (C=O), 1605 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO-d_6)**:** 6.91,7.77 (dd, *J* = 8.31-8.19 Hz, 4H, Ar-H), 7.36 (br. s, 1H, Ar-H), 7.82 (s, 1H, =C-H), 8.04 (d, *J* = 6.97 Hz, 1H, Ar-H), 8.65 (br. s, 1H, Ar-H), 8.89 (br. s, 1H, Ar-H), 10.21 (br. s, 1H, OH), 13.05 (br. s, 1H, NH); Elemental analysis for $C_{17}H_{11}N_5O_2S_2$ (381.43): Calculated/Found: 53.53/53.71 (%C), 2.91/2.98 (%H), 18.36/18.52 (%N) and 16.81/16.93 (%S).

*3.1.5.15.(5Z)-5-(cyclohexylmethylidene)-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-1,3-thiazolidin-4-one (**8**).*

White solid; Yield 77.6%; mp Decompose at 281 °C; IR (cm^{-1}, KBr): 3114 (NH, OH), 3025 (sp^2C-H), 2926 (sp^3C-H), 1703 (C=O), 1580 (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), 2.25 (m, 1H, CH), 6.78 (d, *J* = 9.41 Hz, 1H, C=CH), 6.91, 7.75 (dd, *J* =8.68, 4H, Ar-H), 10.18 (br. s, 1H, OH), 12.58 (br. s, 1H, NH); Elemental analysis for $C_{18}H_{18}N_4O_2S_2$ (386.49): Calculated/Found: 55.94/56.21 (%C), 4.69/4.78 (%H), 14.50/14.63 (%N) and 16.59/16.71 (%S).

*3.1.5.16.(5Z)-2-{[5-(4-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]imino}-5-[(2E)-3-phenylprop-2-en-1-ylidene]-1,3-thiazolidin-4-one (**9**).*

Orange solid; Yield 65%; mp 277-280 °C; IR (cm^{-1}, KBr): broad band at 3393 (NH, OH), 3023 (sp^2C-H), 2958 (sp^3C-H), 1712 (C=O), 1582 (C=N); ^{1}H-NMR (400 MHz, δ ppm DMSO- d_6)**:** 6.92 (d, *J* =5.62, 2H, Ar-H), 7.12- 8.00 (m, 10H, Ar-H, CH=CH, =C-H), 10.06 (br. s, 1H, OH), 12.63 (br. s, 1H, NH); Elemental analysis for

$C_{20}H_{14}N_4O_2S_2$ (406.48): Calculated/Found: 59.10/59.42 (%C), 3.47/3.60 (%H), 13.78/13.96 (%N) and 15.78/16.01 (%S).

3.2. Biological screening.

3.2.1 In vitro cyclooxygenase inhibition assay.

Cyclooxygenase inhibition studies were carried out at the department of Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt. Synthesized compounds were tested for their ability to inhibit 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.

3.2.2. In vitro Lipoxygenase inhibition assay.

Lipoxygenase inhibition studies were carried out at the department of Biochemistry, Faculty of Medicine, Cairo University, Cairo, Egypt. Synthesized compounds tested for their ability to inhibit 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.

3.2.3. In vivo Anti-inflammatory activity by carrageenan induced paw edema method.

The *in vivo* anti-inflammatory activity of the selected compounds (**6a**, **6f**, **6i**, **6l**, **6m** and **9**) were tested by carrageenan induced paw edema [26] method in rats in comparison to Celecoxib® and Diclofenac sodium® as a reference drugs. The test is based on the pedal inflammation in rat paws induced by subplantar injection of carrageenan suspension (0.2 mL of 1% solution in normal saline) into the right hind paw of the rats. Male adult albino rats (120-150 g) were divided into groups, each of five animals. The thickness of rat paw was measured by a Vernier calliper (SMIEC, China) before and 30 min after carrageenan injection to detect the carrageenan induced inflammation. Each test compound at a dose of 28 µmol/Kg (dissolved in 1 % sodium carboxymethyl cellulose solution in normal saline) was injected i.p. to different groups of rats. Control group received a vehicle (1 % sodium carboxymethyl cellulose solution in normal saline), while the two reference groups received Celecoxib and Diclofenac sodium i.p. at the same dose of the tested compounds. The difference between the thicknesses of the two paws was taken as a measure of edema.

The measurement was carried out at 1, 2, 3 and 4 h after injection of the test compounds, reference drugs. The percentages of edema inhibition were calculated according to the following equation. Two-way ANOVA followed by Dunnett's multiple comparisons test was performed 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.

3.2.4. Ulcerogenic liability.

Compounds (**6f, 6l, 6m** and **9**) were selected and tested for their ulcerogenic activity according to a reported method on adult male albino rats [27]. The rats were fasted for 12 h. The test compounds were administered orally at doses of 20 mg/Kg and indomethacin at 10 mg/kg to groups of rats each of 2 animals. After 6 h, the animals were sacrificed, the stomachs were removed and washed with saline. Stomachs were kept in 10 % w/v formalin solution. The surface of stomachs was examined using light microscope.

3.2.5. Molecular modelling.

Molecular modelling study carried out using MOE 2016.09 (medicinal chemistry department, faculty of pharmacy, Assiut university, Egypt) using human COX-2 enzyme (PDB Code 5KIR) and human 15-LOX-2 (PDB code 4NRE). The Rofecoxib® was redocked on human COX-2 enzyme and gave root mean square deviation (RMSD) equal to 0.843. A data base of 17 new compounds was minimized at root mean square of 0.005 using MMFF94x force field. Compounds volume and Log P was calculated using vsurf_D1 and Log P (o/w) descriptors, respectively.

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Conflict of interest

The authors declare no conflict of interest.

Ethical conduct of research

The protocols used in the present study followed the guidelines set in "The Guide for the Care and Use of Laboratory Animals" as found in the European Community Guidelines (Tan 2004) and Institutional Ethical Committee Approval was obtained.

Appendix A. Supplementary data

Supplementary data related to this article can be found online at ……

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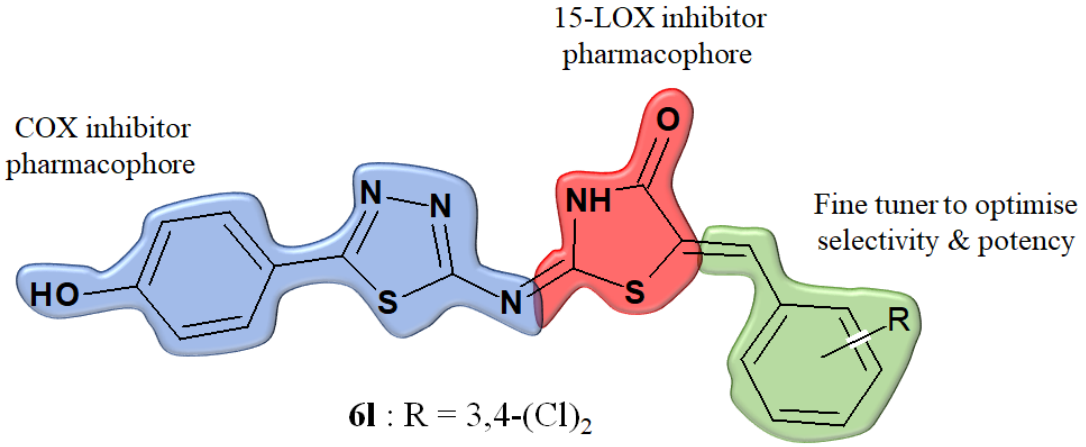
Highlights

- Synthesis of 1,3,4-thiadiazole-thiazolidinone hybrids and their alk/arylidene derivatives
- The potency and selectivity of these hybrids are higher than their individual components and comparable to standard drugs toward COX-2 and 15-LOX enzymes.
- Development of Dual COX-2/15-LOX inhibitors that have potent *in vivo* anti-inflammatory activity equivalent to clinically used drugs with good gastric safety profile.

Graphical abstract.



6l : R = 3,4-$(Cl)_2$

	COX-2 IC_{50}	Selectivity index	15-LOX IC_{50}
6l	**70 nM**	**220**	**11.8 µM**
Celecoxib	49 nM	308	-
Zileuton	-	-	15.6 µM