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Design, Synthesis and Analgesic/Anti-inflammatory Evaluation of Novel Diarylthiazole and Diarylimidazole Derivatives Towards Selective COX-1 Inhibitors with Better Gastric Profile

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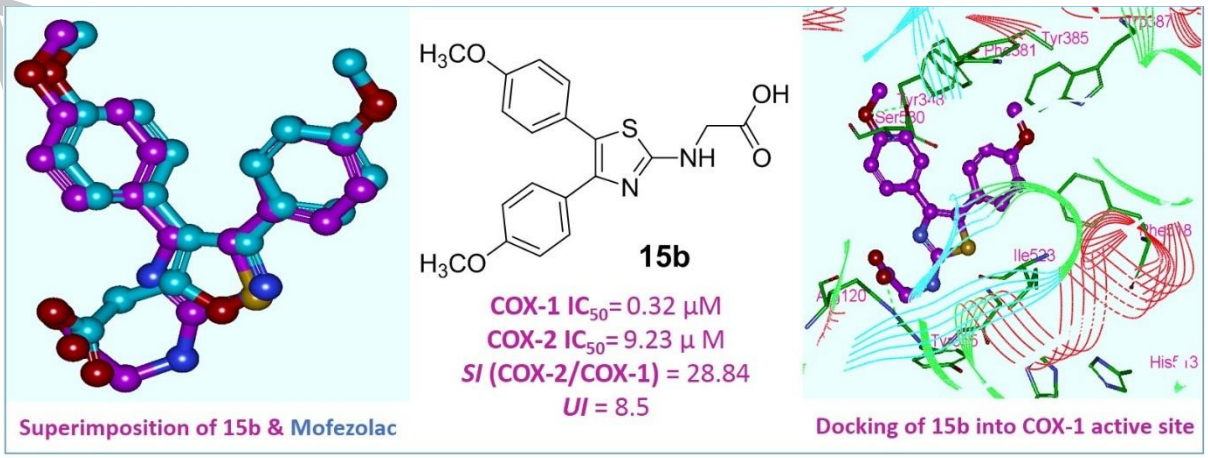
Abstract

The inhibition of gastric cyclooxygenase 1 (COX-1) enzyme was believed to be the major cause of non-steroidal anti-inflammatory drugs (NSAIDs)-induced gastric ulcer. Recent studies disproved this belief and showed that the gastric tissues vulnerability is not solely connected to COX-1 inhibition. This work aimed at exploring and rationalizing the differential analgesic and anti-inflammatory activities of novel selective COX-1 inhibitors with improved gastric profile. Two novel series of 4,5-diarylthiazole and diarylimidazole were designed, synthesized in analogy to selective COX-1 inhibitors (mofezolac and FR122047) which lack gastric damaging effects. The new compounds were evaluated *in vitro* for their COXs inhibitory activity and *in vivo* for their anti-inflammatory and analgesic potentials. Four compounds; diphenylthiazole glycine derivatives (**15a**, **15b)** and diphenylimidazolo acetic acid derivatives (**19a**, **19b)**, which possess carboxylic acid group exhibited significant activity and selectivity against COX-1 over COX-2. Of these compounds, (4,5-bis(4-methoxyphenyl)thiazol-2-yl)glycine **15b** was the most potent compound against COX-1 with an inhibitory half maximal concentration (IC_{50}) of 0.32 µM and a selectivity index (COX-2 IC_{50}/COX-1 IC_{50}) of 28.84. Furthermore, an ulcerogenicity study was performed where the tested compounds demonstrated a significant gastric tolerance. Interestingly, the most selective COX-1 inhibitor showed higher analgesic activity *in vivo* as expected compared to their moderate anti-inflammatory activity. This study underscores the need for further design and development of novel analgesic agents with low tendency to cause gastric damage based on improving their COX-1 affinity and selectivity profile.

Keywords: Selective COX-1; mofezolac, diarylthiazole; diarylimidazole; analgesic; anti-inflammatory; ulcerogenicity

Graphical abstract



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1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most prevalent drugs, either prescribed or non-prescribed, for alleviating pain, inflammation, fever and rheumatic disorders.[1] However, these drugs suffer from several associated drawbacks, including gastric ulceration, kidney injury and cardiotoxicity.[2,3] It's well-documented that the traditional NSAIDs exert their pharmacological effects through the inhibition of cyclooxygenases (COXs)-dependent prostaglandins biosynthesis. The COXs are a class of bifunctional enzymes which are responsible for bis-oxygenation followed by reduction of arachidonic acid to generate prostaglandin H_2 (PGH_2).[4] To date, three COX isoforms, COX-1, COX-2 and COX-3 have been identified. COX-1 enzyme is expressed constitutively generating PGE_2 and TXA_2 which are responsible for gastrointestinal protection and platelet aggregation, respectively.[5] On the other hand, COX-2 is an inducible isoform which is upregulated in response to inflammatory or any immunological stimuli.[6] Therefore, COX-2 attracted great attention to be a proper target for the development of powerful anti-inflammatory and analgesic agents with no tendency to cause gastric damage. Despite the widespread belief that the inhibition of COX-1 enzyme is the main cause of gastric ulcer, recent studies confirmed that the gastric tissues vulnerability is not connected solely to COX-1 inhibition.[7-9] Indeed, the pharmacological role of COX-1 in human was cloudy, except for platelet activation and gastrointestinal protection, until Langenbach and coworkers enlightened the horizons by confirmation that the inhibition of COX-1 alone is not sufficient to induce gastric ulceration.[7,8] Later, Wallace *et al.* disproved this accusation and ensured that COX-1 selective inhibition does not cause any gastric damage.[10] Moreover, studies by Tanaka *et al*. strengthened these findings by showing that the inhibition of COX-1 causes up-regulation of COX-2 expression which, in turn, increases the production of PGE2 to a level necessary for mucosal integrity.[11] Since then, COX-1 has represented a potential therapeutic target for the design of potent analgesic agents with improved gastric safety profile. However, only few selective COX-1 inhibitors have been discovered and introduced as potential analgesic and anti-platelets agents such as **1** (SC-560), **2** (Mofezolac), **3** (FR122047), **4** (P6), **5** (TFAP), **Fig. 1**.[9,12-15] Of these compounds, mofezolac, a selective COX-1 inhibitor, was developed and marketed in Japan as a powerful pain killer.[12,16]

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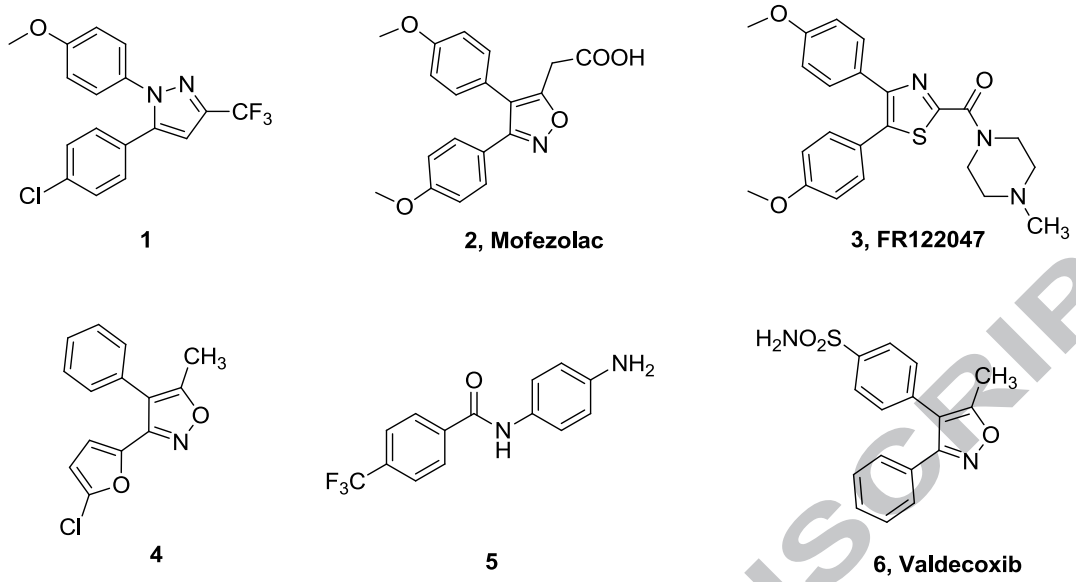

Fig. 1. Structures of some known COX-1 selective inhibitors (**1-5**) and valdecoxib **6**

It was conceptualized from comparing the structures of mofezolac **2** and its COX-2 selective analog valdecoxib **6** that there are some important keys for their converse COXs activities. Firstly, the replacement of the sulfonamide group in valdecoxib **6** with two methoxy groups in mofezolac **2** resulted in the reversal of COX-2 selectivity in favor of COX-1. Secondly, the presence of an acetic acid moiety attached to the five-membered ring in mofezolac **2** was found to be influential primarily on the COX-1 selectivity. However, the absence of acetic acid moiety in FR122047 **3** did not affect its COX-1 selectivity. Moreover, it seems that the 4-methylpiperazino moiety linked to the thiazolo heterocyclic nucleus in compound **3** with a carbonyl spacer has a great impact on COX-1 activity and selectivity as acetic acid moiety. Interestingly, the diaryl heterocyclic system as a known and frequently used scaffold in COX-2 selective inhibitors was likewise common among the majority of COX-1 selective compounds but with different substitutions.

In the light of these observations, two novel sets of compounds were designed and synthesized in analogy to mofezolac **2** and FR122047 **3** with preserving the structural features required for COX-1 selectivity that have been previously conceptualized including the two methoxy groups and acetic acid moiety or methylpiperazine one. Furthermore, an iterative structure activity relationship was conducted through varying the five-membered ring (thiazole and imidazole), using different linkers and replacement of methylpiprazine by various cyclic amines, **Fig. 2**. The novel compounds were evaluated *in vitro* against COX-1 and COX-2 and *in vivo* for their analgesic and anti-inflammatory potential. Finally, our main focus was to examine the effect of these new compounds on the gastric mucosa using acute ulcerogenicity studies.

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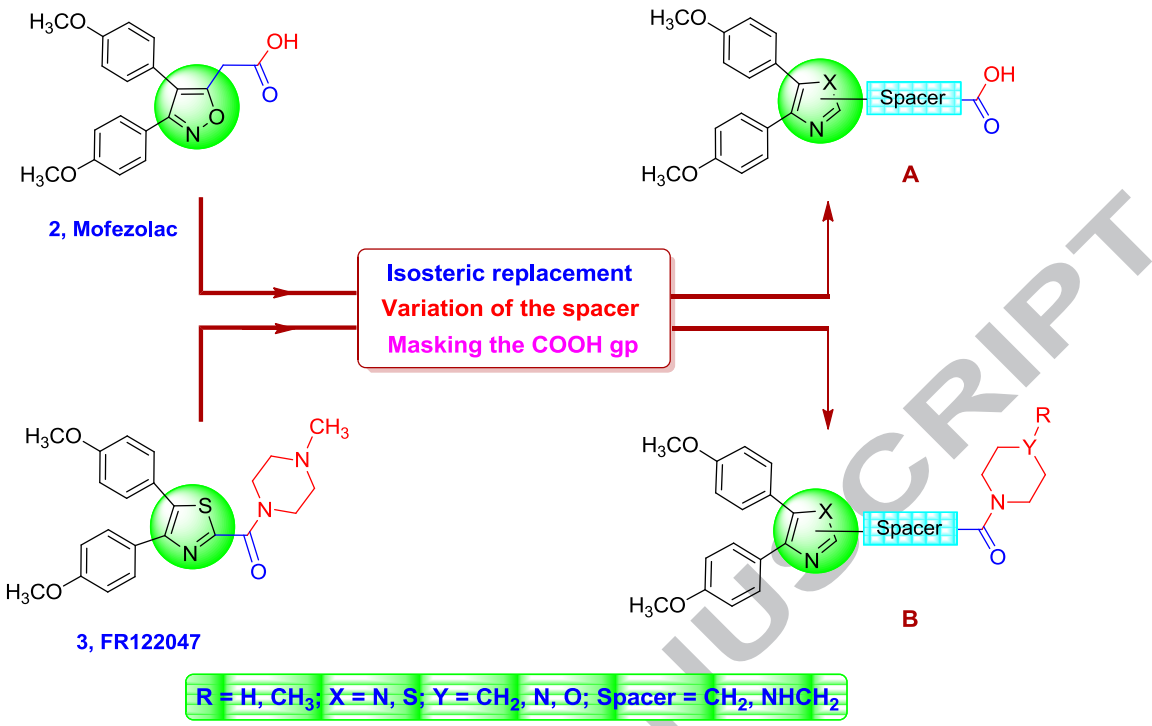

Fig. 2. The design strategy of our novel compounds based on Mofezolac and FR122047 structures

2. Result and discussion

2.1. Chemistry

The synthetic pathways used for preparing the novel compounds were outlined in schemes **1** and **2**. The substituted benzoin **8a** or **8b** were synthesized as starting materials from substituted benzaldehyde in presence of NaCN as a catalyst using the reported method.[17] The intermediates **8a** and **8b** were subjected to a chlorination process using thionyl chloride in pyridine forming desyl chloride derivatives **9a** and **9b**, respectively which in turn were cyclized upon condensation reaction with thiourea in ethanol to afford 4,5-diphenylthiazol-2-amine derivatives **10a** and **10b**, respectively in a good yield according to Hantez-thiazole synthesis protocol.[18-20] In order to prepare the intermediates **13a** and **13b**, we firstly tried the direct *N*-alkylation reaction with ethyl chloroacetate using NaH as a base and it surprisingly resulted in the cyclized product **11a** or **11b** due to the incessant attack of the thiazole's nitrogen on the acetate group. Thus, efficient *N*-formylation of the amino group followed by reaction with ethyl chloroacetate, as reported,[21] was used to prevent the second attack and it successfully afforded the targeted intermediates **13a** and **13b** as shown in **Scheme 1**. ^{1}H NMR spectrum of the cyclized derivatives **11a** and **11b** revealed a singlet signal at 4.42 and 4.40 ppm, respectively attributed to the methylene group. Also, the mass spectrum of **11a** endorsed that interpretation via the appearance of its molecular ion peak at *m/z* 294. On the other hand, ^{1}H NMR spectra of compounds **13a** and **13b** revealed the presence of the characteristic triplet and quartet patterns of the ethyl moiety at

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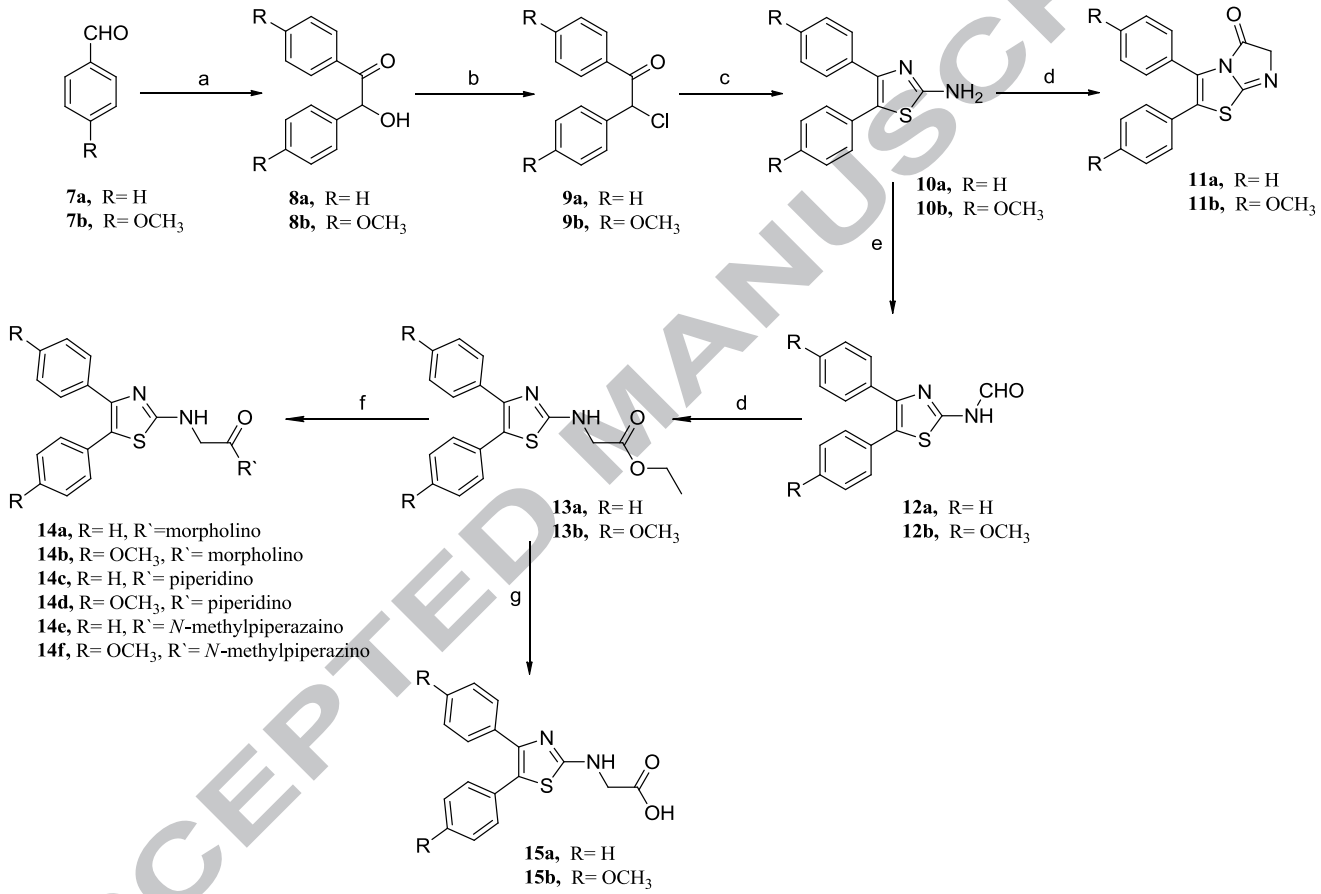
1.32 and 4.25 ppm, respectively. The final targeted compounds **14a-h** were obtained in excellent yield from the condensation reaction of **13a** and **13b** with various secondary amines. Finally, the alkaline hydrolysis of **13a** and **13b** afforded the acetic acid containing compounds **15a** and **15b**, respectively. The IR spectrum showed a broad absorption band at 3300 cm^{-1} assigned to the carboxylic OH group. Moreover, ^{1}H NMR spectrum displayed a singlet peak at 12.89 ppm that disappeared upon deuteration attributed to carboxylic acid proton.^{13}C NMR revealed a peak at 171 ppm assigned to the carbonyl group of the carboxylic acid.



Scheme 1. Reagents and reaction conditions: (a) NaCN, ethanol, reflux, 1 h; (b) $SOCl_2$, pyridine, rt, 1 h; (c) thiourea, ethanol, reflux, 3 h; (d) ethyl chloroacetate, NaH, DMF, rt, overnight; (e) formic acid, acetic anhydride, diethyl ether, rt, overnight; (f) appropriate secondary amine, reflux, 4 h; (g) KOH, MeOH, reflux, 12 h.

A similar approach was used for the synthesis of 4,5-diarylimidazole derivatives **18a-h** as shown in **Scheme 2**.[22,23] First, the substituted benzoins **8a** and **8b** were cyclized using formamide to form the imidazole intermediates **16a** and **16b** which were subsequently reacted with ethyl chloroacetate followed by condensation with various amines to afford the final targeted compounds **18a-h**. On the other hand, the final acetic acid containing compounds **19a** and **19b**

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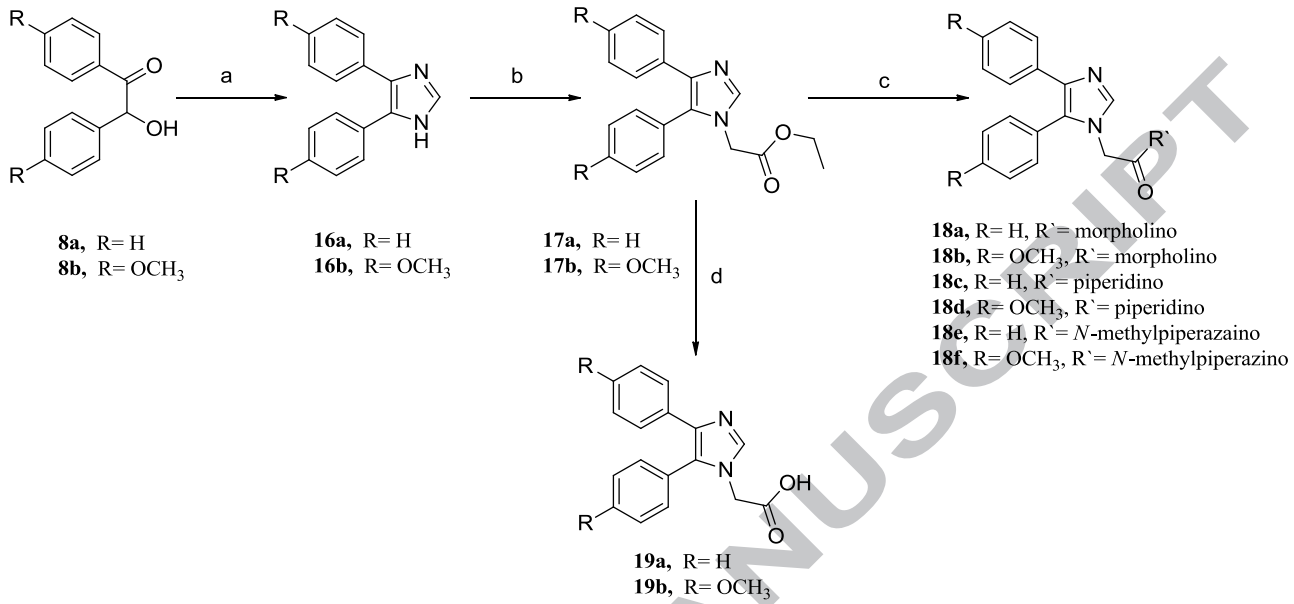
were obtained upon alkaline hydrolysis of the acetate intermediates **17a** and **17b** using potassium hydroxide in methanol.



Scheme 2. Reagents and reaction conditions: (a) Formamide, reflux, 3 h; (b) ethyl chloroacetate, NaH, DMF, rt, overnight; (c) appropriate secondary amine, reflux, 4 h; (d) KOH, MeOH, reflux, 12 h.

2.2. Biological activity

Considering the previous reports indicating COX-1 inhibition as a molecular mechanism of the analgesic activity of mofezolac **2**,[16] our novel diarylthiazole and diarylimidazole derivatives as mofezolac analogs were assessed *in vitro* for their COXs inhibitory activity and *in vivo* for their analgesic and anti-inflammatory potential in addition to an acute ulcerogenicity study. The results of COXs inhibitory assay were expressed in terms of IC_{50} values, **Table 1**. Two animal models were utilized in the *in vivo* studies; the acetic acid-induced writhing test was employed to assess the anti-nociception activity and the carrageenan-induced paw edema assay was used for evaluating the anti-inflammatory potential.

2.2.1. In vitro COXs Inhibitory Assay

The COXs inhibitory activity of the newly synthesized compounds was examined as reported before.[24,25] The activity in terms of IC_{50} values in addition to the selectivity index were summarized in **table 1**. The results revealed that compounds possessing acetic acid moiety, either thiazole or imidiazole derivatives, were the most potent and selective COX-1 ligands as presented in compounds **15a,b** and **19a,b** with IC_{50} values between 0.32-0.67 µM. However, the presence of the two methoxy groups improved the COX-1 activity to some extent where compounds **15b** and **19b** were more potent than **15a** and **19a**, respectively. In general, the

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diarylthiazole derivatives showed more activity and selectivity than for COX-1 over COX-2 subtype in both scaffolds **A** and **B**. On the other hand, compounds **14a-f** and **18a-f** having scaffold **B** as FR122047 analogs exerted lower activity than compounds bearing free carboxylic groups (**15a,b** and **19a,b**) in scaffold **A**. It was worth noting that compounds possessing methylpiperazine moiety **14e,f** and **18e,f** were the most active derivatives with IC_{50} values between 2.72 and 4.17 μM. Upon replacement of methylpiperazine with morpholine or piperidine moieties, the COX-1 activities were remarkably decreased. Comparing the activity of these compounds with that of the parent compound, FR122047, it was suggested that the change of the length of the spacer between the heterocyclic nucleus and the secondary amine has a great effect on decreasing COX-1 activity and selectivity. However, the piperidine containing derivatives **14a,b** and **18a,b** showed the least activity between these FR122047 analogs with scaffold **B**. On the contrary, almost all the tested compounds showed weak activity with no selectivity against COX-2 subtype owing to the absence of sulfonamide or methylsulfone groups as essential structural basis for COX-2 selectivity where they form H-bonding with Arg513 residue in the side pocket of COX-2 active site as reported.[19,26] Taken together, it could be conceptualized from these findings that there are some structural requirements that are optimal for COX-1 activity and selectivity. First, the free carboxylic acid attached to the five-membered heterocyclic ring is pivotal for COX-1 inhibitory activity and selectivity as represented in compounds **15a**,**b** and **19a,b**. Secondly, the isosteric replacement of isoxazole ring in mofezolac **2** with thiazole ring preserved the COX-1 activity and selectively while the replacement with imidazole resulted in a slight decrease in activity as demonstrated in compounds **19a** and **19b**. Furthermore, the removal of the two methoxy groups from both scaffolds **A** and **B** did not abolish the COX-1 activity however, their presence enhanced the potency. Finally, the spacer length in the second series with scaffold **B** was crucial for COX-1 activity and selectivity where the carbonyl group as a linker in FR122047 was optimal and largely contributed to their significant COX-1 selectivity. Interestingly, compound **15b** containing all these structural features showed the most COX-1 affinity and selectivity with IC_{50} of 0.32 μM and the selectivity index (COX-2 IC_{50}/COX-1 IC_{50}) of 28.84.

Table 1. The results of *in vitro* COXs inhibition assay in terms of IC_{50} values and selectivity index (*SI*)

Compound	COXs inhibition (IC_{50} μM)[a]		Selectivity Index (*SI*)[b]
	COX-1	COX-2	
14a	5.76 ± 1.34	8.28 ± 2.06	1.44
14b	4.01± 1.56	6.89 ± 1.42	1.72

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14c	7.99 ± 2.31	15.12 ± 1.74	1.89
14d	6.01 ± 1.70	16.12 ± 2.21	2.68
14e	3.04 ± 1.98	11.02 ± 2.04	3.63
14f	2.72 ± 1.02	7.95 ± 2.10	2.92
15a	0.42 ± 1.03	10.71 ± 1.08	25.50
15b	0.32 ± 1.12	9.23 ± 1.12	28.84
18a	6.32 ± 1.76	11.12 ± 1.75	1.76
18b	5.09 ± 1.04	9.12 ± 1.03	1.79
18c	8.51 ± 1.04	13.12 ± 1.15	1.54
18d	7.34 ± 1.04	14.12 ± 2.23	1.92
18e	4.17 ± 1.74	10.23 ± 1.94	2.45
18f	3.06 ± 1.51	8.32 ± 1.43	2.72
19a	0.67 ± 1.56	13.45 ± 1.97	20.07
19b	0.54 ± 1.77	12.57 ± 1.43	23.28
Celecoxib	16.20 ± 1.21	0.34 ± 0.02	0.02
Indomethacin	0.71 ± 0.03	11.07 ± 2.0	15.59

[a]*Values are expressed as mean ± SEM (n = 3)*

[b]*Selectivity index (SI) = COX-2 IC_{50}/COX-1 IC_{50}*

2.2.2. Acetic acid-induced writhing test

In order to measure the analgesic activity of the newly synthesized compounds, the acetic acid-induced writhing test was employed using diclofenac as a positive control according to the reported method.[27] The activity of each compound was determined based on the reduction in the number of acetic acid-induced writhing. At a glance, the results were in concordance with that of the *in vitro* COXs inhibitory assay where the most active and COX-1 selective compounds **15a,b** and **19a,b** bearing the free carboxylic group showed the *in vivo* highest potency with number of writhes between 10.67-14.40 compared to the nonselective COXs diclofenac (reference drug) with number of writhes 24. Moreover, almost all compounds possessing thiazole ring were more active than that derivatives having an imidazole ring. However, the compounds (FR122047 analogs) with scaffold **B**, either thiazole or imidazole derivatives, exhibited low to moderate analgesic activity. Compounds **14a,b** and **18a,b** bearing morpholine revealed lower activity compared to *N*-methylpiperazine containing compounds **14e,f** and **18e,f**, **Fig. 3**. These results affirmed the important role of the free carboxylic group, the two methoxy groups and the spacer length in the *in vitro* and *in vivo* activities of our compounds as well.

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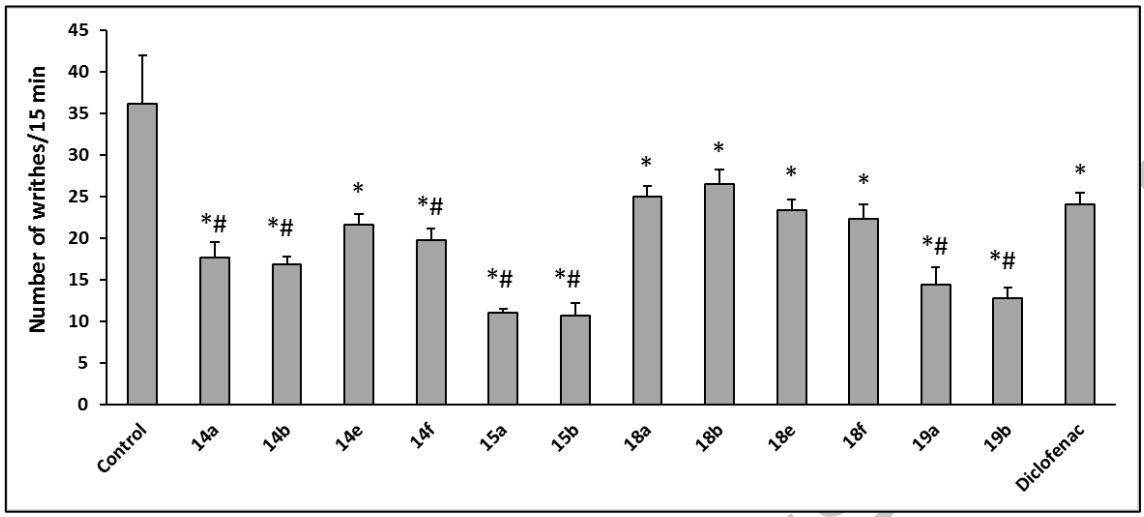

Fig. 3. The results of acetic acid-induced writhing assay. Statistical analysis was performed using one-way ANOVA followed by Dunnett Multiple Comparisons Test, Data are mean ± S.D., *Significant different from control at $p<0.05$, #Significant different from diclofenac at $p<0.05$, N=5.

2.2.3. Carrageenan-induced rat paw edema assay

The anti-inflammatory activity of these new compounds was evaluated using the reported carrageenan-induced rat paw edema assay where diclofenac sodium was a positive reference drug.[28,29] The tested compounds were suspended in DMSO and administered orally to the experimental animals. The mean sizes of the resulted edema thickness of rats pretreated with the tested compounds were observed and measured at 0, 1, 3 and 7 h from the induction of inflammation. The percentage of inhibition in thickness of edema was calculated in comparison to diclofenac sodium. Conversely to the analgesic assay results, all tested compounds exhibited modest anti-inflammatory activity with average edema inhibition percentages between 50.30-61.91% compared to the reference drug with average edema inhibition percentage of 84.52% as shown in **Table 2**. It was clear from the results that compounds **14a**, **14b**, **14f**, **18e** and **18f** showed slightly higher activity than the rest of other derivatives. Compound **14f** bearing the methylpiperazine moiety was the most potent compounds with average edema inhibition percentage of 61.91%. Interestingly enough, the most potent and COX-1 selective compound **15b** possessing an acetic acid moiety exerted the least *in vivo* anti-inflammatory activity with average edema inhibition percentage of 50.30%. These results reflected the great influence of the significant COX-1 affinity and selectivity on improving the analgesic activity at expense of anti-inflammatory activity.

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Table 2. The results of carrageenan-induced rat paw edema assay of the tested compounds compared to diclofenac as a reference drug.

Compound	Mean edema thickness (mm) ± SEM				Average edema inhibition%
	0 h	1 h	3 h	7 h	
Control	0.171 ± 0.017	0.394 ± 0.220	0.401 ± 0.019	0.464 ± 0.023	---
14a	0.148 ± 0.007	0.148 ± 0.067	0.167 ± 0.008	0.192 ± 0.012	59.80
14b	0.170 ± 0.016	0.170 ± 0.164	0.190 ± 0.017	0.213 ± 0.018	54.52
14e	0.173 ± 0.016	0.173 ± 0.160	0.197 ± 0.017	0.205 ± 0.010	54.26
14f	0.144 ± 0.007	0.144 ± 0.066	0.163 ± 0.004	0.172 ± 0.009	61.91
15a	0.147 ± 0.006	0.166 ± 0.008	0.185 ± 0.008	0.231 ± 0.009	53.99
15b	0.155 ± 0.007	0.165 ± 0.008	0.213 ± 0.009	0.251 ± 0.006	50.30
18a	0.155 ± 0.008	0.166 ± 0.009	0.186 ± 0.010	0.218 ± 0.016	54.55
18b	0.142 ± 0.008	0.146 ± 0.010	0.159 ± 0.008	0.190 ± 0.012	60.78
18e	0.147 ± 0.006	0.147 ± 0.006	0.168 ± 0.007	0.182 ± 0.006	60.52
18f	0.147 ± 0.006	0.170 ± 0.012	0.191 ± 0.010	0.152 ± 0.008	58.82
19a	0.148 ± 0.007	0.161 ± 0.005	0.189 ± 0.008	0.235 ± 0.009	53.79
19b	0.149 ± 0.008	0.149 ± 0.008	0.180 ± 0.011	0.215 ± 0.009	56.98
Diclofenac	0.162 ± 0.008	0.090 ± 0.008	0.060 ± 0.004	0.040 ± 0.003	84.52

All test compounds were given orally in a dose of 100 mg/kg while diclofenac sodium in a dose of 60 mg/kg. Treatments began 1 h before induction of inflammation by the injection of 1% carrageenan-sodium gel into the sub-planter region of the right hind paw. The mean size of the induced paw edema thickness of rats pretreated with the tested compounds were observed and measured at 0, 1, 3 and 7 h from the induction of inflammation. The percentage of inhibition in thickness of edema was calculated in comparison to diclofenac sodium. (N=5).

2.2.4. Acute ulcerogenicity study

Based on the *in vitro* COXs assay results, the most active and COX-1 selective compounds **15a,b** and **19a,b** were selected to be examined for their gastric-ulcerogenic potential using indomethacin as a reference drug.[30] Observation of the gastric mucosa for the presence of lesions following oral administration of 20 mmol/kg of the tested compounds or the reference drug was used as an indication for the ulcerogenic effects. The results revealed that the four compounds have superior safety profile compared to indomethacin as shown in **Table 3** and **Fig. 4**. Obviously, compound **15b** demonstrated a remarkable improvement in ulcer index (UI = 8.5) in comparing with indomethacin (UI =19.5).

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Table 3. Ulcerogenic effects of synthesized compounds and indomethacin as a reference drug

Compound	Average number of ulcers	Ulcer index[a]
15a	2.25	9.39
15b	1.5	8.5
19a	4	13.3
19b	2.5	9.75
Control	--	Nil
Indomethacin	11	19.5

[a]*The ulcer index is the sum of % incidence, average severity and average number of ulcers following oral administration of 20 mmol/kg of the tested compounds as well as the reference drug. (N=5).*

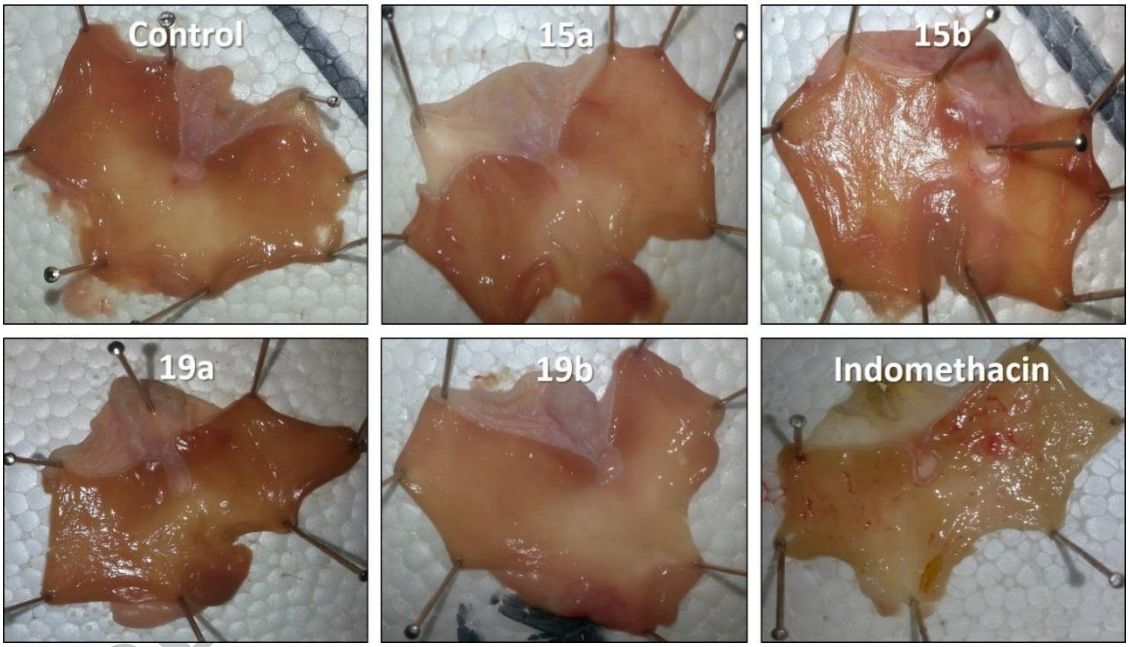

Fig. 4. Representative pictures of the stomachs of rats treated with tested compounds **15a**, **15b**, **19a** and **19b** compared to indomethacin indicating the ulcer lesions. All test compounds and indomethacin were used orally (20 mmol/kg).

2.3. Molecular Docking studies

In order to explore some clues on the most significant structural features governing COX-1 affinity and selectivity, a docking simulation was conducted using LIGANDFIT embedded in Discovery Studio software.[31] The results of this *in silico* study would correlate the *in vitro* COXs inhibitory activity and the difference in selectivity profiles of the newly synthesized with their chemical structures based on their orientation and binding patterns inside the COXs active sites. In doing so, two representative compounds **15b** and **14f** were docked into the active site of COX-1 where the 3D crystal structure complex (PDB codes: 1PGF) was employed for this study. In addition, mofezolac **2** and FR122047 **3** were used for comparison as two well-known

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COX-1 selective agents. It was found that compound **15b** with the highest COX-1 activity and selectivity exhibited a binding pattern and interactions similar to that of mofezolac **2**, **Fig. 5**. Clearly from **Fig. 5(A)**, the diphenyl rings bearing the two methoxy groups were oriented towards the hydrophobic region lined by Tyr348, Phe381, Tyr385 and Trp387 while the acetic acid moiety was leaned towards Arg120 with establishing an important H-bond interaction likewise the free carboxylic group of mofezolac, **Fig. 5(B)**. In addition, one of the two methoxy groups formed H-bond with the backbone of Ser530 residue. The superimposition of the docked poses **15b** and Mofezolac **2** within COX-1 active site as shown in **Fig. 5(C,D)** pointed out the high similarity between both compounds and explained the significant activity and selectivity of **15b** against COX-1.

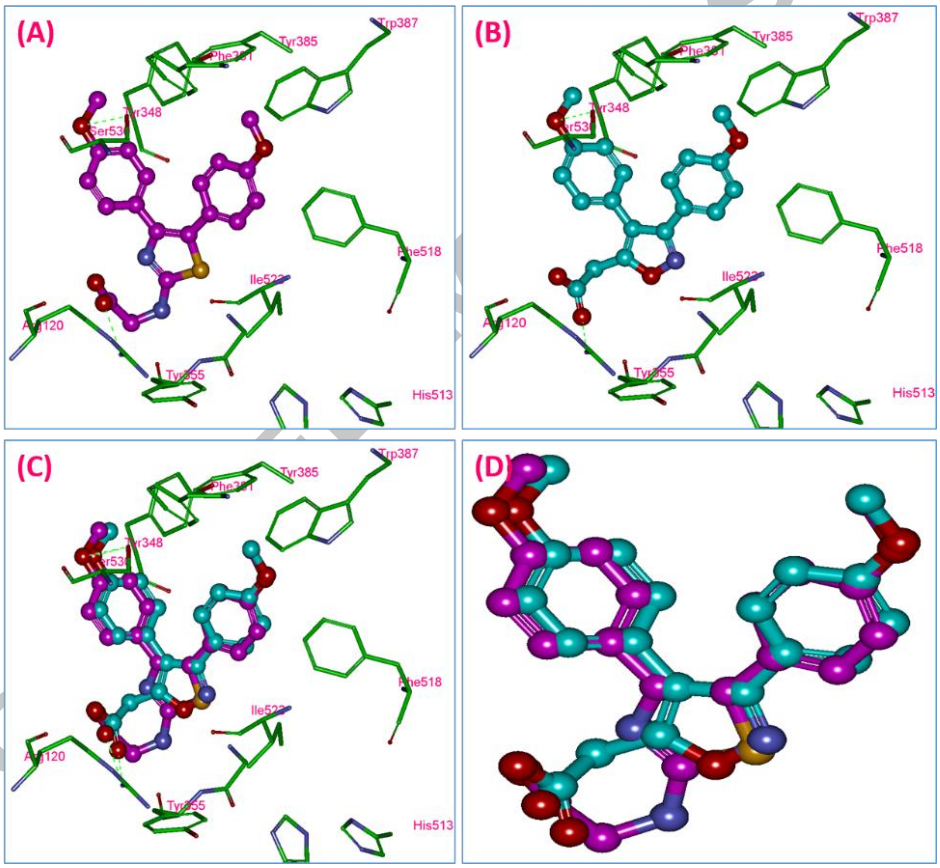

Fig. 5. (A) Docking and binding pattern of compound **15b** into COX-1 active site (PDB code: 1PGF); (B) Docking and binding pattern of Mofezolac **2** into the same COX-1 binding pocket; (C) The superimposition of the docked pose **15b** (violet) and Mofezolac **2** (cyan) within active site of COX-1; (D) The superimposition of the docked pose **15b** (violet) and Mofezolac **2** (cyan) without active site residues. The poses were rendered as ball and stick models. Hydrogen bonds were represented as dashed green lines. All hydrogens were removed for the purposes of clarity.

On the other hand, the docking results of compound **14f** possessing methylpiperazine moiety into COX-1 active site indicated that this compound was forced to adopt a longitudinal binding pattern and it was pushed to the bottom of the active site, **Fig. 6**. However, the disposition of the diphenyl bearing the two methoxy groups was in the same hydrophobic room formed of Tyr348,

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Phe381, Tyr385 and Trp387 residues, the methylpiperazine moiety seems to extremely protrude outside the COX-1 active site, compared to FR122047 due to the variation in the spacer length between methylpiperazine and the thiazole heterocycle, **Fig. 6(A-C)**. This observation could be the reason behind the inferior affinity of **14f** against COX-1 subtype, which reflected its low *in vivo* analgesic potential.

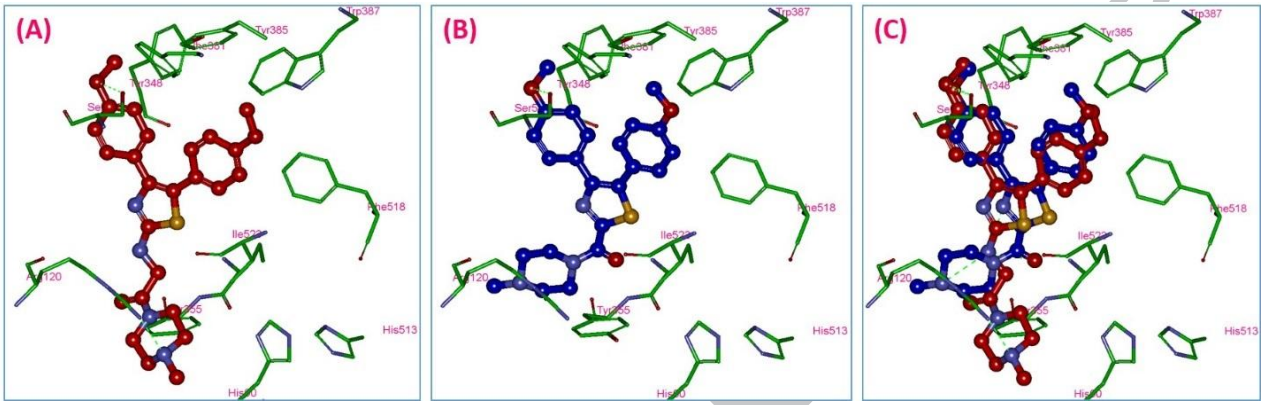

Fig. 6. (A) Docking and binding pattern of compound **14f** into COX-1 active site (PDB code: 1PGF); (B) Docking and binding pattern of FR122047 **3** into the same COX-1 binding pocket; (C) The superimposition of the docked pose **14f** (red) and Mofezolac **2** (blue) within active site of COX-1. The poses were rendered as ball and stick models. Hydrogen bonds were represented as dashed green lines. All hydrogens were removed for the purposes of clarity.

3. Conclusion

In the present investigation, a series of diarythiazole and diarylimidazole have been designed and synthesized as mofezolac and FR122047 analogs. The newly synthesized compounds were evaluated *in vitro* against COXs subtypes and *in vivo* for their analgesic, anti-inflammatory and ulcerogenicity potential. The *in vitro* assays results revealed that compounds **15a**,**b** and **19a**,**b** possessing acetic acid moiety were the most potent and COX-1 selective inhibitors with IC_{50} values of 0.42, 0.32, 0.67 and 0.54 µM, respectively. From the structure activity relationship study, it could be concluded that the presence of free carboxylic or methylpiperazine moiety in addition to the two methoxy groups are optimal for COX-1 affinity and selectivity. However, the spacer length was crucial for COX-1 activity and selectivity especially in the FR122047 analogs. Conversely, the tested compounds showed weak activity with no selectivity against COX-2 subtype due to the absence of sulfonamide or methylsulfone groups which highly contribute to COX-2 activity and selectivity. On the other hand, the *in vivo* study results were consistent with that of the *in vitro* COXs inhibitory assay where the most potent and COX-1 selective compounds, especially **15b**, exhibited the highest analgesic activity and least anti-inflammatory potential. Meanwhile, compound **15b** revealed a much better gastric tolerance profile (UI = 8.5) than indomethacin (UI = 19.5). In the docking simulation, compound **15b** adopted binding pattern and H-bonding interactions inside COX-1 active site similar to that of mofezolac

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explaining its remarkable activity and selectivity. This study indicates the potential medicinal value of these compounds as analgesic agents with better safety margin less gastric damage.

4. Experimental protocols

4.1. Chemistry

Generally, reagents and solvents were of commercial quality and were used without further purification. Melting points were uncorrected and were carried out by open capillary tube method using IA 9100MK-Digital melting point apparatus. Microanalyses were carried out at the micro-analytical center, Faculty of Science, Cairo University. Infrared spectra were done on Bruker FT-IR spectrophotometer Vector 22 and expressed in wave number (cm^{-1}) using KBr discs at the micro-analytical center, Faculty of Science, Cairo University. The proton magnetic resonance ^{1}H NMR and ^{13}C NMR spectra were recorded on BRUKER APX400 spectrometer at 400 MHz and 100 MHz, respectively in the specified solvent at the Faculty of Pharmacy, Beni-Suef University. The chemical shifts were reported on the *d* scale and were related to that of the solvent and *J* values were given in Hz. Mass spectra were recorded on Fennigan MAT, SSQ 7000, Mass spectrometer, at 70 eV (EI) at the micro-analytical center, Faculty of Science, Cairo University. Thin layer chromatography (TLC) was done using Macherey–Nagel Alugram Sil G/UV254 silica gel plates and Hexane-Ethyl acetate (8:2) as the eluting system.

2-Hydroxy-1,2-diphenylethanone ***(8a)***.[18] A mixture of benzaldehyde (50 g, 4.7 mol) and sodium cyanide (5 g) were heated for one-half hour in a mixture of absolute ethanol (62.5 mL) and water (50 mL). The solution was cooled and the precipitate was filtered and washed with water. The crude solid was recrystallized from ethanol to afford **9a** as a white solid (80%) m.p.129 ^{o}C.

2-Hydroxy-1,2-bis(4-methoxyphenyl)ethanone **(8b)**.[17] Yield 35%, bright yellow solid, m.p. 113-114 ^{o}C.

2-Chloro-1,2-diphenylethanone ***(9a)***.[19] Compound **2a** (10 g, 0.047 mol) was heated in pyridine (5.7 mL) until a solution was obtained, then cooled till solidification has been occurred. The mass was coarsely ground and thionyl chloride (7.5 g, 0.063 mol) was added slowly with vigorous stirring in cold water bath for 1 h and at room temperature for another 1 h. Water was added and the solid was filtered and recrystallized from ethanol to obtain compound **3a** as colorless crystals (74%), m.p. 66-67 ^{o}C.

2-Chloro-1, 2-bis(4-methoxyphenyl)ethanone ***(9b)***.[32] Yield 36% as yellow solid, m.p. 80-81 ^{o}C.

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4,5-Diphenylthiazol-2-amine ***(10a)***.[19] A mixture of compound **3a** (2 g, 8.3 mmol) and thiourea (0.7 g, 9 mmol) in ethanol was refluxed for 2 h. After cooling, the resulting precipitate was collected by filtration. The solid was filtered, washed with water and then recrystallized from ethanol to furnish compound **4a** as a grey, needle-shaped solid (72%). m.p. 188-189 ^{o}C.

4,5-Bis(4-methoxyphenyl)thiazol-2-amine ***(10b)***.[20] Yield 89% as yellow solid, m.p. 178-180 ^{o}C.

2,3-Diphenylimidazo[2,1-b]thiazol-5(6H)-one ***(11a)***. To a stirred suspension of sodium hydride 60% (0.2 g, 5 mmol) in DMF (5 mL), compound **10a** (1.4 g, 5 mmol) was added portionwise. The stirring was continued for 30 minutes, and then a solution of ethyl chloroacetate (0.92 g, 5.5 mmol) was added. The reaction mixture was stirred at rt for 4 h and subsequently was poured onto ice-water mixture. The precipitate was filtered off and recrystallized from ethanol to afford compound **11a** as brown solid (65%), m.p. 125-126 ^{o}C. IR (cm^{-1}) 3050 (CH aromatic), 2945 (CH aliphatic), 1688 (amide C=O), 1607, 1511 (C=C, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.42 (s, 2H, C$\underline{H}_2$), 7.16-7.49 (m, 10H, aromatic). ^{13}C NMR (101 MHz, DMSO-d_6) δ 22.9, 125.6, 128.1, 128.3, 128.7, 128.8, 129.4, 129.7, 132.3, 135.1, 144.1, 156.3, 169.1. MS (EI) *m/z* 294 (M^{+}+1). Anal. Calcd. For $C_{17}H_{12}N_2OS$: C, 69.84; H, 4.14; N, 9.58. Found: C, 69.47; H, 4.84; N, 9.68.

2,3-Bis(4-methoxyphenyl)imidazo[2,1-b]thiazol-5(6H)-one ***(11b)***. Yield 76% as brown solid, m.p. 177-180 ^{o}C. IR (cm^{-1}): 3040 (CH aromatic), 2956 (CH aliphatic), 1662 (amide C=O), 1607, 1511 (C=C, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.7 (s, 3H, OC$\underline{H}_3$), 3.8 (s, 3H, OC$\underline{H}_3$), 4.40 (s, 2H, C$\underline{H}_2$), 6.85-7.38 (m, 8H, aromatic). ^{13}C NMR (101 MHz, DMSO-d_6) δ 22.8, 55.4, 55.5, 114.13, 114.8, 124.1, 124.5, 127.7, 130.0, 131.0, 143.2, 155.6, 159.0, 159.3, 169.0. MS (EI) *m/z* 354 (M^{+}+1). Anal. Calcd. For $C_{19}H_{16}N_2O_3S$: C, 64.76; H, 4.58; N, 7.95. Found: C, 64.53; H, 5.19; N, 8.11.

N-(4,5-diphenylthiazol-2-yl)formamide ***(12a)***. To a mixture of the formic acetic anhydride (4.4 g, 0.05 mol) prepared by heating a mixture of acetic anhydride and 98 % formic acid at 60 ^{o}C for 2 h, **10a** (6.3 g, 0.025 mol) in ether (12 mL) was added. The mixture was stirred at rt for 12 h where the formed precipitate was filtered off, washed with ether and recrystallized from ethanol to obtain compound **12a** as white solid (67%), m.p. 110 ^{o}C. IR (cm^{-1}): 3400 (NH), 3100 (CH aromatic), 2964 (CH aliphatic), 1674 (amide C=O), 1572, 1512 (C=C, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.15-8.16 (m, 10H, aromatic), 8.56 (s, 1H, C$\underline{H}$), 12.49 (S, 1H, N$\underline{H}$ exchangeable with D_2O). ^{13}C NMR (100 MHz, DMSO) δ 126.3, 128.3, 128.5, 128.7, 128.94,

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129.4, 129.7, 132.0, 134.9, 144.4, 154.5, 160.3. MS (EI) *m/z* 280 (M^++1). Anal. Calcd. For $C_{16}H_{12}N_2OS$: C, 68.55; H, 4.31; N, 9.99. Found: C, 68.79; H, 4.37; N, 10.12.

N-(4,5-bis(4-methoxyphenyl)thiazol-2-yl)formamide ***(12b)***. Yield 69% as light yellow solid, m.p. 125-126 ^oC. IR (cm^{-1}): 3392 (NH), 3100 (CH aromatic), 2993 (CH aliphatic), 1692 (amide C=O), 1609, 1511 (C=C, C=N). ^1H NMR (400 MHz, $CDCl_3$) δ 3.83 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 6.85-7.42 (m, 8H, aromatic), 7.58 (s, 1H, CH), 12.84 (s, 1H, NH, exchangeable with D_2O). ^{13}C NMR (100 MHz, DMSO-d_6) δ 55.2, 55.3, 114.1, 114.2, 123.7, 126.4, 126.9, 130.3, 130.5, 143.0, 155.4, 158.9, 159.3, 159.6. MS (EI) *m/z* 340 (M^++1). Anal. Calcd. For $C_{18}H_{16}N_2O_3S$: C, 63.51; H, 4.74; N, 8.23. Found: C, 63.64; H, 4.81; N, 8.41.

Ethyl 2-((4,5-diphenylthiazol-2-yl)amino)acetate ***(13a)***. To a stirred suspension of 60% sodium hydride (0.2 g, 5 mmol) in DMF (5 mL), *N*-(4,5-diphenylthiazol-2-yl)formamide **12a** (1.4 g, 5 mmol) was added. The stirring was continued for 1 h and then a solution of ethyl chloroacetate (0.92 g, 5.5 mmol) in DMF (2 mL) was added dropwise. The reaction mixture was left to stirred overnight at rt. The mixture was poured onto ice-water and the precipitate was filtered off and recrystallized from ethanol to give compound **13a** as brown solid (66%), m.p. 98-99 ^oC. IR (cm^{-1}): 3372 (NH), 3056 (CH aromatic), 2980 (CH aliphatic), 1744 (amide C=O), 1597, 1495 (C=C, C=N). ^1H NMR (400 MHz, $CDCl_3$) δ 1.31 (t, *J* = 7.1 Hz, 3H, CH_3), 4.11 (s, 1H, NH exchangeable with D_2O), 4.26 (q, *J* = 7.1 Hz, 2H, CH_2), 4.40 (s, 2H, CH_2), 7.57-7.18 (m, 10H, aromatic). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3, 46.5, 61.6, 126.3, 126.7, 128.6, 128.8, 129.4, 129.5, 130.2, 131.0, 134.9, 137.1, 138.9, 168.6. MS (EI) *m/z* 338 (M^++1). Anal. Calcd. For $C_{19}H_{18}N_2O_2S$: C, 67.43; H, 5.36; N, 8.28. Found: C, 67.52; H, 5.41; N, 8.37.

Ethyl-2-((4,5-bis(4-methoxyphenyl)thiazol-2-yl)amino)acetate ***(13b)***. Yield 65% as yellow solid, m.p. 102-103 ^oC. IR (cm^{-1}): 3432 (NH), 3100 (CH aromatic), 2959 (CH aliphatic), 1745 (ester C=O), 1608, 1511 (C=C, C=N). ^1H NMR (400 MHz, $CDCl_3$) δ 1.31 (t, J = 6.9 Hz, 3H, CH_3), 3.79 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.25 (q, *J* = 6.8 Hz, 2H, CH_2), 4.37 (s, 2H, CH_2), 6.80-7.44 (m, 8H, aromatic), 9.1 (1H, NH, exchangeable with D_2O). ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.0, 46.2, 55.1, 55.3, 61.9, 113.5, 114.5, 121.9, 127.1, 127.4, 127.6, 132.2, 137.1, 137.7, 158.2, 160.0, 167.8. MS (EI) *m/z* 398 (M^++1). Anal. Calcd. For $C_{21}H_{22}N_2O_4S$: C, 63.30; H, 5.56; N, 7.03. Found: C, 63.45; H, 5.64; N, 7.11.

2-((4,5-diphenylthiazol-2-yl)amino)-1-morpholinoethanone ***(14a)***. A mixture of ethyl-2-((4,5-diphenylthiazol-2-yl)amino)acetate **13a** (1.6 g, 0.005 mol) and morpholine (0.86 g, 0.01 mol) was refluxed for 4 h. The mixture was cooled to rt and then poured onto ice cold water. The formed precipitate was filtered and recrystallized from aqueous ethanol to give compound

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14a as off-white solid (70%), m.p. 165-166 ^{o}C. IR (cm^{-1}): 3431 NH), 3056 (CH aromatic), 2968 (CH aliphatic), 1648 (amide C=O), 1558, 1492 (C=C, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.44 (s, 1H, NH exchangeable with D_2O), 3.56 (t, *J* = 4, 2 H, CH_2), 3.65 (t, J=4; 2 H, CH_2), 3.71-3.80 (m, 4H, 2*CH_2), 4.42 (s, 2H, CH_2), 7.18-7.47 (m, 10H, aromatic). ^{13}C NMR (100 MHz, DMSO-d_6) δ 42.2, 45.5, 51.5, 66.8, 121.5, 127.1, 127.5, 128.0, 128.5, 129.0, 129.4, 132.7, 135.4, 146.2, 167.0, 167.3. MS (EI) *m/z* 379 (M^+-1). Anal. Calcd. for $C_{21}H_{21}N_3O_2S$: C, 66.47; H, 5.58; N, 11.07. Found: C, 66.59; H, 5.64; N, 11.25.

2-((4,5-bis(4-methoxyphenyl)thiazol-2-yl)amino)-1-morpholinoethanone ***(14b)***. Yield 71% brown solid, m.p. 140-142 ^{o}C. IR (cm^{-1}): 3435 (NH), 3071 (CH aromatic), 2959 (CH aliphatic), 1650 (amide C=O), 1606, 1511 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.49 (s, 1H, NH exchangeable with D_2O), 3.58 (t, *J* = 4 Hz, 2H, CH_2), 3.65 (t, *J* = 4 Hz, 2H, CH_2), 3.70-3.75 (m, 4H, 2* CH_2), 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3) 4.51 (s, 2H, CH_2), 6.77-7.41 (m, 8H, aromatic). ^{13}C NMR (100 MHz, $CDCl_3$) δ 42.25, 45.52, 51.45, 55.20, 55.25, 66.85, 113.39, 113.97, 120.14, 125.17, 128.21, 130.10, 130.76, 145.39, 158.74, 158.82, 166.76, 167.13. MS (EI) *m/z* 439 (M^++1). Anal. Calcd. for $C_{23}H_{25}N_3O_4S$: C, 62.85; H, 5.73; N, 9.56. Found: C, 63.01; H, 5.791; N, 9.67.

2-((4,5-diphenylthiazol-2-yl)amino)-1-(piperidin-1-yl)ethanone ***(14c)***. Yield 73% brown solid, m.p. 133-135 ^{o}C. IR (cm^{-1}): 3423 (NH), 3053 (CH aromatic), 2930 (CH aliphatic), 1645 (amide C=O), 1551, 1532 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59-1.63 (m, 6H, 3*CH_2), 3.38 (s, 1H, NH exchangeable with D_2O), 3.45 (t, *J* = 4 Hz, 2H, CH_2), 3.58 (t, *J* = 4 Hz, 2H, CH_2), 4.54 (s, 2H, CH_2), 7.18-7.50 (m, 10H, aromatic). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.45, 25.59, 26.38, 43.17, 46.04, 51.81, 121.14, 126.99, 127.30, 127.92, 128.45, 129.10, 129.48, 133.02, 135.70, 146.26, 166.57, 167.72. MS (EI) *m/z* 377 (M^++1). Anal. Calcd. for $C_{22}H_{23}N_3OS$: C, 70.00; H, 6.14; N, 11.13. Found: C, 70.17; H, 6.22; N, 11.29.

2-((4,5-bis(4-methoxyphenyl)thiazol-2-yl)amino)-1-(piperidin-1-yl)ethanone ***(14d)***. Yield 72%, brown solid, m.p. 144-145 ^{o}C. IR (cm^{-1}): 3432 (NH), 3052 (CH aromatic), 2994 (CH aliphatic), 1646 (amide C=O), 1623, 1511 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59-1.68 (m, 6H, 3*CH_2), 1.68 (s, 1H, NH exchangeable with D_2O), 3.47 (t, *J* = 2 Hz, 2H, CH_2), 3.59 (t, *J* = 4 Hz, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.81(s, 3H, OCH_3), 4.53 (s, 2H, CH_2), 6.64-7.46 (m, 8H, aromatic). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.48, 25.59, 26.40, 43.15, 46.06, 51.68, 55.19, 55.24, 113.28, 113.89, 119.64, 125.55, 128.52, 130.18, 130.82, 145.38, 158.59, 158.67, 166.70, 167.15. MS (EI) *m/z* 437 (M^++1). Anal. Calcd. for $C_{24}H_{27}N_3O_3S$: C, 65.88; H, 6.22; N, 9.60. Found: C, 66.03; H, 6.31; N, 9.78.

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2-((4,5-diphenylthiazol-2-yl)amino)-1-(4-methylpiperazin-1-yl)ethanone ***(14e)***. Yield 82%, off-white solid, m.p. 143-145 ^{o}C. IR (cm^{-1}): 3383 (NH), 3053 (CH aromatic), 2934 (CH aliphatic), 1645 (amide C=O), 1533, 1440 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$) 2.27 (s, 3H, C$\underline{H}_3$), 2.32 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 3.21 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$) 3.60 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 4.53 (s, 2H, C$\underline{H}_2$), 7.14-7.47 (m, 10H, aromatic), 7.69 (s, 1H, N$\underline{H}$ exchangeable with D_2O). ^{13}C NMR (100 MHz, $CDCl_3$): δ 42.3, 44.8, 45.6, 45.9, 54.4, 54.6, 126.3, 126.6, 128.0, 128.3, 128.9, 129.1, 130.4, 131.0, 134.5, 137.9, 138.0, 164.8. MS (EI) *m/z* 392 (M^++1). Anal. Calcd. for $C_{22}H_{24}N_4OS$: C, 67.32; H, 6.16; N, 14.27. Found: C, 67.59; H, 6.28; N, 14.49.

2-((4,5-bis(4-methoxyphenyl)thiazol-2-yl)amino)-1-(4-methylpiperazin-1-yl)ethanone ***(14f)***. Yield 70%, brown solid, m.p. 170-171 ^{o}C. IR (cm^{-1}): 3384 (NH), 3000 (CH aromatic), 2934 (CH aliphatic), 1647 (amide C=O), 1608, 1510 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21-2.23 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 2.27 (s, 3H, C$\underline{H}_3$), 2.33-2.35 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 3.24-3.26 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 3.59-3.61 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 3.76 (s, 3H, OC$\underline{H}_3$), 3.86 (s, 3H, OC$\underline{H}_3$), 4.51 (s, 2H, C$\underline{H}_2$), 6.76-7.43 (m, 8H, aromatic), 7.68 (s, 1H, N$\underline{H}$ exchangeable with D_2O). ^{13}C NMR (100 MHz, $CDCl_3$) δ 42.2, 44.8, 45.6, 45.9, 54.4, 54.6, 55.1, 55.3, 113.5, 114.5, 122.3, 127.2, 127.7, 130.9, 132.3, 137.5, 137.6, 158.1, 159.9, 165.0. MS (EI) *m/z* 452 (M^++1). Anal. Calcd. for $C_{24}H_{28}N_4O_3S$: C, 63.69; H, 6.24; N, 12.38. Found: C, 63.84; H, 6.31; N, 12.53.

(4,5-diphenylthiazol-2-yl)glycine ***(15a)***. A solution of potassium hydroxide (0.112 g, 2 mmol) in methanol (18 mL) was added to a mixture of ethyl2-((4,5-diphenylthiazol-2-yl)amino)acetate derivatives **13a** (0.33 g, 1 mmol) in methanol (25 mL). The resulting mixture was heated under reflux overnight. The mixture was allowed to cool to room temperature and then poured onto water and acidified with 1N HCl. The precipitate was filtered, washed with water and recrystallization occurred from aqueous ethanol to afford compound **15a** as yellow crystals (77%), m.p. 98-99 ^{o}C. IR (cm^{-1}): 3406 (NH), 3243 (acidic OH), 3054 (CH aromatic), 2924 (CH aliphatic), 1727 (acidic C=O), 1624, 1500 (C=C, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.05 (s, 1H, N$\underline{H}$ exchangeable with D_2O), 4.27 (s, 2H, C$\underline{H}_2$), 7.13-7.52 (m, 10H, aromatic), 12.89 (s, 1H, COO$\underline{H}$ exchangeable with D_2O). ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.43, 126.38, 126.67, 128.59, 128.90, 129.39, 129.57, 130.40, 131.03, 134.95, 136.88, 138.92, 170.10. MS (EI) *m/z* 311 (M^++1). Anal. Calcd. For $C_{17}H_{14}N_2O_2S$: C, 65.79; H, 4.55; N, 9.03. Found: C, 65.94; H, 4.52; N, 9.17.

(4,5-bis(4-methoxyphenyl)thiazol-2-yl)glycine ***(15b)***. Yield 70% yellow solid, m.p. 100-101 ^{o}C. IR (cm^{-1}): 3404 (NH), 3200 (acidic OH), 3100 (CH aromatic), 2934 (CH aliphatic), 1685

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(amide C=O), 1608, 1509 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73 (s, 3H, OC<u>H</u>$_3$) 3.83 (s, 3H, OC<u>H</u>$_3$), 3.87 (s, 1H, N<u>H</u> exchangeable with D_2O), 4.2 (s, 2H, C<u>H</u>$_2$), 6.91-7.30 (m, 8H, aromatic), 9.30 (s, 1H, COO<u>H</u> exchangeable with D_2O). ^{13}C NMR (100 MHz, $CDCl_3$) δ 47.3, 55.6, 55.7, 113.5, 114.5, 122.3, 127.1, 127.3, 127.7, 132.2, 137.5, 137.6, 158.1, 159.9, 165.2. MS (EI) *m/z* 370 (M^+). Anal. Calcd. For $C_{19}H_{18}N_2O_4S$: C, 61.61; H, 4.90; N, 7.56. Found: C, 61.75; H, 4.98; N, 7.69.

4,5-diphenyl-1H-imidazole ***(16a)***.[22] A mixture of compound **8a** (4.8 g, 0.023 mol) and formamide (30 mL) was heated to reflux for 3 h. The reaction mixture was poured onto water and stirred vigorously to dissolve the gummy product. The resulting solid was filtered, washed with water and suspended in 5% HCl (200 mL). The solution was heated to 80-90 ^{o}C and filtered while hot. The filtrate was treated with excess NH_4OH where a white precipitate which was formed, filtered and washed with water to afford compound **16a** as white solid (80%), m.p. 230-234 ^{o}C.

4,5-bis(4-methoxyphenyl)-1H-imidazole ***(16b)***.[23] Yield 67% a white solid, m.p. 178-180 ^{o}C.

Ethyl-2-(4,5-diphenyl-1H-imidazol-1-yl)acetate ***(17a)***. Yield 71% as yellowish green solid. m.p. 163-164 ^{o}C. IR (cm^{-1}): 3100 (CH aromatic), 2970 (CH aliphatic), 1747 (C=O), 1667, 1600 (C=C, C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.06 (t, *J* = 7.0 Hz, 3H, C<u>H</u>$_3$), 4.02 (q, *J* = 7.1 Hz, 2H, C<u>H</u>$_2$), 4.75 (s, 2H, C<u>H</u>$_2$), 7.13-7.49 (m, 10H, aromatic), 7.85 (s, 1H, C<u>H</u>). ^{13}C NMR (100 MHz, DMSO-d_6) δ 14.3, 46.5, 61.6, 126.3, 126.7, 128.6, 128.8, 129.4, 129.5, 130.2, 131.0, 134.9, 137.1, 138.9, 168.6. MS (EI) *m/z* 306 (M^++1). Anal. Calcd. for $C_{22}H_{24}N_4O$: C, 73.31; H, 6.71; N, 15.54. Found: C, 73.54; H, 6.76; N, 15.72.

Ethyl-2-(4,5-bis(4-methoxyphenyl)-1H-imidazol-1-yl)acetate ***(17b)***. Yield 67% as off-white solid, m.p. 169-170 ^{o}C. IR (cm^{-1}): 3100 (CH aromatic), 2900 (CH aliphatic), 1755 (C=O), 1666, 1611 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21 (t, *J* = 6.7 Hz, 3H, C<u>H</u>$_3$), 3.80 (s, 3H, OC<u>H</u>$_3$), 3.81(s, 3H, OC<u>H</u>$_3$), 4.16 (q, *J* = 8 Hz, 2H, C<u>H</u>$_2$), 4.48 (s, 2H, C<u>H</u>$_2$), 6.76-7.43 (m, 8H, aromatic), 8.00 (s, 1H, C<u>H</u>). ^{13}C NMR (101 MHz, $CDCl_3$) δ 14.0, 46.2, 55.1, 55.3, 61.9, 113.5, 114.5, 121.9, 127.1, 127.4, 127.6, 132.2, 137.1, 137.7, 158.2, 160.0, 167.8. MS (EI) *m/z* 366 (M^++1). Anal. Calcd. for $C_{21}H_{22}N_2O_4$: C, 68.84; H, 6.05; N, 7.65. Found: C, 68.67; H, 6.80; N, 13.45.

2-(4,5-diphenyl-1H-imidazol-1-yl)-1-morpholinoethanone ***(18a)***. Yield 71% brown solid, m.p. 109-110 ^{o}C. IR (cm^{-1}): 3116 (CH aromatic), 2970, 2917 (CH aliphatic), 1643 (amide C=O), 1607, 1507 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.19 (t, *J* = 4 Hz, 2H, C<u>H</u>$_2$), 3.48 (t, *J* =

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4 Hz, 2H, C$\underline{H}_2$), 3.57 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 3.61 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 4.53 (s, 2H, C$\underline{H}_2$), 7.14-7.51 (m, 10H, aromatic), 7.66 (s, 1H, C$\underline{H}$).^{13}C NMR (100 MHz, $CDCl_3$) δ 42.5, 45.3, 45.5, 66.1, 66.7, 126.4, 126.6, 128.1, 128.3, 128.9, 129.1, 129.3, 130.3, 130.9, 134.4, 138.04, 165.1. MS (EI) *m/z* 347 (M^++1). Anal. Calcd. for $C_{21}H_{21}N_3O_2$: C, 72.60; H, 6.09; N, 12.10. Found: C, 72.69; H, 6.17; N, 12.34.

2-(4,5-bis(4-methoxyphenyl)-1H-imidazol-1-yl)-1morpholinoethanone ***(18b)***. Yield 80% off-white solid, m.p. 97-98 ^{o}C. IR (cm^{-1}): 3020 (CH aromatic), 2930, 2835 (CH aliphatic), 1643 (amide C=O), 1614, 1577 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.21 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 3.52 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 3.59 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 3.64 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 3.76 (s, 3H, OC$\underline{H}_3$), 3.86 (s, 3H, OC$\underline{H}_3$), 4.51 (s, 2H, C$\underline{H}_2$), 6.76-7.42 (m, 8H, aromatic), 7.62 (s, 1H, C$\underline{H}$).^{13}C NMR (100 MHz, $CDCl_3$) 42.5, 45.3, 45.5, 55.1, 55.3, 66.2, 66.7, 113.5, 114.5, 122.3, 127.1, 127.3, 127.7, 132.2, 137.5, 137.6, 158.1, 159.9, 165.2. MS (EI) *m/z* 407 (M^++1). Anal. Calcd. For $C_{23}H_{25}N_3O_4$: C, 67.80; H, 6.18; N, 10.31. Found: C, 67.96; H, 6.24; N, 10.44.

2-(4,5-diphenyl-1H-imidazol-1-yl)-1-(piperidin-1-yl)ethanone ***(18c)***. Yield 84% off-white solid, m.p. 120-122 ^{o}C. IR (cm^{-1}): 3116 (CH aromatic), 2937, 2865 (CH aliphatic), 1646 (amide C=O), 1604, 1506 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34-1.37 (m, 2H, C$\underline{H}_2$), 1.50-1.53 (m, 2H, C$\underline{H}_2$), 1.59-1.62 (m, 2H, C$\underline{H}_2$), 3.15 – 3.17 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 3.51-3.53 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 4.53 (s, 2H, C$\underline{H}_2$), 7.16-7.49 (m, 10H, aromatic), 7.66 (s, 1H, C$\underline{H}$).^{13}C NMR (100 MHz, $CDCl_3$) δ 24.2, 25.4, 26.1, 43.5, 45.9, 46.0, 126.2, 126.6, 128.0, 128.5, 128.8, 129.0, 130.4, 131.0, 134.5, 137.8, 138.0, 164.5. MS (EI) m/z 345 (M^++1). Anal. Calcd. for $C_{22}H_{23}N_3O$: C, 76.49; H, 6.71; N, 12.16. Found: C, 76.56; H, 6.78; N, 12.31.

2-(4,5-bis(4-methoxyphenyl)-1H-imidazol-1-yl)-1-(piperidin-1-yl)ethanone ***(18d)***. Yield 86% off-white solid, m.p. 123-125 ^{o}C. IR (cm-1): 3129 (CH aromatic), 2935, 2858 (CH aliphatic), 1650 (amide C=O), 1612, 1577 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 2H, C$\underline{H}_2$), 1.52 (s, 2H, C$\underline{H}_2$), 1.60 (s, 2H, C$\underline{H}_2$), 3.18 (s, 2H, C$\underline{H}_2$), 3.53 (s, 2H, C$\underline{H}_2$), 3.76 (s, 3H, OC$\underline{H}_3$), 3.86 (s, 3H, OC$\underline{H}_3$), 4.50 (s, 2H, C$\underline{H}_2$), 6.75-7.43 (m, 8H, aromatic), 7.61 (s, 1H, C$\underline{H}$).^{13}C NMR (100 MHz, $CDCl_3$): 24.2, 25.4, 26.1, 43.5, 45.8, 46.0, 55.1, 55.3, 113.5, 114.5, 122.3, 127.1, 127.3, 127.7, 132.2, 137.5, 137.6, 158.1, 159.9, 165.2. MS (EI) *m/z* 405 (M^++1). Anal. Calcd. for $C_{24}H_{27}N_3O_3$: C, 71.09; H, 6.71; N, 10.36. Found: C, 71.31; H, 6.80; N, 10.51.

2-(4,5-diphenyl-1H-imidazol-1-yl)-1-(4-methylpiperazin-1-yl)ethanone ***(18e)***. Yield 71% brown solid, m.p. 145-146 ^{o}C. IR (cm^{-1}): 3115 (CH aromatic), 2973-2942 (CH aliphatic), 1643 (amide C=O), 1600, 1506 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.19-2.21 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 2.27 (s, 3H, C$\underline{H}_3$), 2.32-2.35 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$), 3.21-3.23 (t, *J* = 4 Hz, 2H, C$\underline{H}_2$)

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3.59-3.61 (t, J = 4 Hz, 2H, C<u>H</u>$_2$), 4.53 (s, 2H, C<u>H</u>$_2$), 7.14-7.47 (m, 10H, aromatic), 7.66 (s, 1H, C<u>H</u>).^{13}C NMR (100 MHz, $CDCl_3$): δ 42.3, 44.8, 45.6, 45.9, 54.4, 54.6, 126.3, 126.6, 128.0, 128.3, 128.9, 129.1, 130.4, 131.0, 134.5, 137.9, 138.0, 164.8. MS (EI) *m/z* 360 (M^++1). Anal. Calcd. for $C_{22}H_{24}N_4O$: C, 73.31; H, 6.71; N, 15.54. Found: C, 73.54; H, 6.76; N, 15.72.

2-(4,5-bis(4-methoxyphenyl)-1H-imidazol-1-yl)-1-(4-methylpiperazin-1-yl)ethanone ***(18f)***. Yield 79% brown solid, m.p. 163-165 ^{o}C. IR (cm-1): 3100 (CH aromatic), 2930, 2835 (CH aliphatic), 1657 (amide C=O), 1666, 1611 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.21-2.23 (t, *J* = 4 Hz, 2H, C<u>H</u>$_2$), 2.27 (s, 3H, C<u>H</u>$_3$), 2.33-2.35 (t, *J* = 4 Hz, 2H, C<u>H</u>$_2$), 3.24-3.26 (t, *J* = 4 Hz, 2H, C<u>H</u>$_2$), 3.59-3.61 (t, *J* = 4 Hz, 2H, C<u>H</u>$_2$), 3.76 (s, 3H, OC<u>H</u>$_3$), 3.86 (s, 3H, OC<u>H</u>$_3$), 4.51 (s, 2H, C<u>H</u>$_2$), 6.76-7.43 (m, 8H, aromatic), 7.62 (s, 1H, C<u>H</u>).^{13}C NMR (100 MHz, $CDCl_3$) δ 42.2, 44.8, 45.6, 45.9, 54.4, 54.6, 55.1, 55.3, 113.5, 114.5, 122.3, 127.2, 127.7, 130.9, 132.3, 137.5, 137.6, 158.1, 159.9, 165.0. MS (EI) *m/z* 420 (M^++1). Anal. Calcd. for $C_{24}H_{28}N_4O_3$: C, 68.55; H, 6.71; N, 13.32. Found: C, 68.67; H, 6.80; N, 13.45.

2-(4,5-diphenyl-1H-imidazol-1-yl)acetic acid ***(19a)***. Yield 77% yellow solid, m.p. 125-128 ^{o}C. IR (cm^{-1}): 3112 (CH aromatic), 2972 (CH aliphatic), 1753 (acidic C=O), 1625, 1546 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.65 (s, 2H, C<u>H</u>$_2$), 6.75-7.30 (m, 8H, aromatic), 8.68 (s, 1H, C<u>H</u>), 10.40 (s, 1H, CO<u>OH</u> exchangeable with D_2O). ^{13}C NMR (100 MHz, DMSO-d_6) δ 46.4, 126.3, 126.6, 128.5, 128.9, 129.3, 129.5, 130.4, 131.0, 134.9, 136.8, 138.9, 170.1. MS (EI) *m/z* 278 (M^+). Anal. Calcd. for $C_{17}H_{14}N_2O_2$: C, 73.37; H, 5.07; N, 10.07. Found: C, 73.52; H, 5.14; N, 10.31.

2-(4,5-bis(4-methoxyphenyl)-1H-imidazol-1-yl)acetic acid ***(19b)***. Yield 70% yellow solid, m.p. 154-155 ^{o}C. IR (cm^{-1}): 3300 (OH acidic), 3100 (CH aromatic), 2900 (CH aliphatic), 1724 (C=O), 1629, 1575 (C=C, C=N). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.73 (s, 3H, OC<u>H</u>$_3$) 3.83 (s, 3H, OC<u>H</u>$_3$), 4.76 (s, 2H, C<u>H</u>$_2$), 6.75-7.30 (m, 8H, aromatic), 8.68 (s, 1H, C<u>H</u>), 9.40 (s, 1H, CO<u>OH</u> exchangeable with D_2O). ^{13}C NMR (100 MHz, $CDCl_3$) δ 47.3, 55.6, 55.7, 114.5, 115.21, 119.63, 123.08, 128.42, 128.47, 132.68, 137.29, 159.36, 160.55, 169.18. MS (EI) *m/z* 338 (M^+). Anal. Calcd. For $C_{19}H_{18}N_2O_4$: C, 67.44; H, 5.36; N, 8.28. Found: C, 67.59; H, 5.41; N, 8.37.

4.2. Biological screening

4.2.1. *In vitro* COXs inhibitory assay

The ability of the newly synthesized compounds to inhibit COX subtypes activity was evaluated using COXs Colorimetric Inhibitor Screening Kit from Cayman Chemical Company (Ann Arbor, MI) according manufacturer's directions and as mentioned before.[24,25] This assay is based on measuring the peroxidase component of COXs colorimetrically by monitoring the production

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of oxidized *N,N,N',N'*-tetramethyl-p-phenylenediamine (TMPD) at 590 nm. The assay includes both ovine COX-1 and human recombinant COX-2. The enzymes were pre-incubated for 5 min at 25 ^{o}C with the test compounds prior to addition of arachidonic acid (final concentration 1.1 mM) and TMPD and incubation for 5 min at 25 ^{o}C. Sixteen compounds were screened for their COX-1/COX-2 affinity and selectivity. All assays were performed in triplicates and IC_{50} values are the average of three determinations for each compound.

4.2.2. Acetic acid-induced writhing test

The analgesic activity of the tested compounds was estimated using acetic acid-induced writhing method as described by Koster *et al.*.[27] The writhing test was performed in groups of five mice each. One hour after the administration of the test compound, 0.01 ml/g of 0.6% acetic acid solution was injected intra-peritoneal in each mouse. The writhing movements of each animal were counted for 15 min (between the fifth and 20th min after the injection of the irritant). Diclofenac (6 mg/kg) was used as reference drug. The number of abdominal constrictions was cumulatively counted over a period of 20 min.

4.2.2. Carrageenan-induced rat paw edema assay

The tested compounds were evaluated for their ant-inflammatory activity using carrageenan-induced rat paw edema model described by Winter *et al.*.[28,29] Albino rats of either sex weighing 120-150 g were divided into 15 groups of five animals each. Rats were uniformly hydrated by giving 3 mL water/rat through gastric inoculation to reduce variability to edema response. The control group was given 10% DMSO aqueous solution (v/v). Diclofenac sodium was taken as a reference standard (60 mg/kg) while the tested compounds in the form of 10% DMSO aqueous solutions were administered orally to the rest groups at a dose of 100 mg/kg body weight, treatments began 1 h before induction of inflammation. Paw edema was induced by subcutaneous injection of 50 µl of 1% carrageenan-sodium gel (Sigma-Aldrich, USA), into the subplantar region of the right hind paw. The thickness of the right and left hind paw of each rat was measured using a pair of dial thickness gauge calipers accurate to 0.001 cm 1, 3 and 7 hours after induction of inflammation. The left hind paw diameter served as a control for the degree of inflammation in the right hind paw.

4.2.3. Acute ulcerogenicity study

The ulcerogenic potential of the most active compounds **15a**, **15b**, **19a** and **19b** and indomethacin as a reference drug was examined in rats as reported by Cioli *et al.*.[30] Adult albino rats of both sexes weighing 120-150 g were used. Rats randomly divided into 6 groups each of five animals. Fasted animals administered the tested compounds and indomethacin orally in dose

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of 20 mmol/Kg suspended in 1% tween while one group received vehicle. Fasting rats for 2 hours, feeding for another 2 hours followed by fasting for another 20 hours. Two doses were given to rats in second and third days. Finally, rats were scarified to remove stomach and rinsing it in 0.9% saline. The mucosal damage was determined according to the following scores:

- **Zero** for normal (no injury).
- **1** latent small red spot.
- **2** wide red spots.
- **3** slight injuries.
- **4** severe injuries.

The average of number of ulcers was determined by dividing the number of ulcers in the group to the total number of rats in the group. The ulcer index is the sum of % incidence, average severity and average of number of ulcer. To determine the ulcer index, we calculated the % incidence/10 and average severity as shown in the following equations:

$$\%\frac{incidence}{10} = \left[\frac{number of\ rats\ showing\ ulcers}{total\ number\ of\ rats\ in\ the\ group} \times 100\right] \Big/ 10$$

$$Average\ severity = \frac{sum(each\ ulcer\ \times score\ of\ severity)}{number\ of\ ulcers}$$

4.2.4. Statistical analysis

All data are presented as means ± SEM (standard error of the mean). Statistical analysis was done using Statistical Package for Social Science (SPSS) software (version 22). One-way analysis of variance (ANOVA) test was used to detect significance among group means, followed by Tukey's post-hoc test for pair-wise comparison between means of groups. Differences were considered significant at $p < 0.05$ (*) or $p < 0.001$(**).

4.3. Molecular docking study

This study was performed using LIGANDFIT imbedded into Discovery Studio Software where the binding site was generated from the co-crystallized ligands IMM within COX-1 protein structure (PDB codes: 1PGF).[31] Two selected compounds, **14f** and **15b** were sketched and energy minimized using CHARMm Force Field and then docked into the aforementioned prepared protein active site. The number of Monte Carlo search trials in the utilized docking protocol was set to 15000 and torsional step size for polar hydrogens was equal to 30^{o}. Moreover, the Root Mean Square Difference (RMS) threshold for ligand-to-binding site shape

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match was set to 2.0 employing a maximum of 1.0 binding site partitions and 1.0 site partition seed. In addition, the Force Field used for evaluating and calculating ligand-receptor interaction energies was set to DREIDING force field with a dielectric constant of 1.0 and a non-bonded cutoff distance of 10.0 Å. An energy grid extending 3.0 Å from the binding site was implemented. Furthermore, a maximum of 10 diverse docked conformations/poses of optimal interaction energies were saved. The saved conformers/ poses were further energy-minimized within the binding site for a maximum of 1000 rigid-body iterations. Finally, docking results were analyzed and the pictures were generated using Discover Studio Visualizer software.

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References

1. Marnett, L. J. *Annu. Rev. Pharmacol. Toxicol.* **2009**, *49*, 265.

2. Wolfe, M. M.; Lichtenstein, D. R.; Singh, G. *N. Engl. J. Med*. **1999**, *340*, 1888.

3. Abdelazeem, A. H.; Khan, S. I.; White, S. W.; Sufka, K. J.; McCurdy, C. R. *Bioorg. Med. Chem*. **2015**, *23*, 3248.

4. Perrone, M. G.; Scilimati, A.; Simone, L.; Vitale, P. *Curr. Med. Chem*. **2010**, *17*, 3769.

5. Fu, J. Y.; Masferrer, J. L.; Seibert, K.; Raz, A.; Needleman, P. *J. Biol. Chem*. **1990**, *265*, 16737.

6. Laneuville, O.; Breuer, D. K.; Dewitt, D. L.; Hla, T.; Funk, C. D.; Smith, W. L. *J. Pharmacol. Exp. Ther*. **1994**, *271*, 927.

7. Kakuta, H.; Zheng, X.; Oda, H.; Harada, S.; Sugimoto, Y.; Sasaki, K.; Tai, A. *J. Med. Chem*. **2008**, *51*, 2400.

8. Langenbach, R.; Morham, S. G.; Tiano, H. F.; Loftin, C. D.; Ghanayem, B. I.; Chulada, P. C.; Mahler, J. F.; Lee, C. A.; Goulding, E. H.; Kluckman, K. D.; Kim, H. S.; Smithies, O. *Cell*. **1995**, *83*, 483.

9. Zheng, X.; Oda, H.; Takamatsu, K.; Sugimoto, Y.; Tai, A.; Akaho, E.; Ali, H. I.; Oshiki, T.; Kakuta, H.; Sasaki, K. *Bioorg. Med. Chem*. **2007**, *15*, 1014.

10. Wallace, J. L.; McKnight, W.; Reuter, B. K.; Vergnolle, N. *Gastroenterology*. **2000**, *119*, 706.

11. Tanaka, A.; Hase, S.; Miyazawa, T.; Takeuchi, K. *J. Pharmacol. Exp. Ther*. **2002**, *300*, 754.

12. Kitamura, T.; Kawamori, T.; Uchiya, N.; Itoh, M.; Noda, T.; Matsuura, M.; Sugimura, T.; Wakabayashi, K. *Carcinogenesis*. **2002**, *23*, 1463.

13. Ochi, T.; Motoyama, Y.; Goto, T. *Eur. J. Pharmacol*. **2000**, *391*, 49.

ACCEPTED MANUSCRIPT

14. von der Weid, P. Y.; Hollenberg, M. D.; Fiorucci, S.; Wallace, J. L. *Circulation*. **2004**, *110*, 1320.

15. Di Nunno, L.; Vitale, P.; Scilimati, A.; Tacconelli, S.; Patrignani, P. *J. Med. Chem*. **2004**, *47*, 4881.

16. Goto, K.; Ochi, H.; Yasunaga, Y.; Matsuyuki, H.; Imayoshi, T.; Kusuhara, H.; Okumoto, T. *Prostaglandins Other Lipid Mediat*. **1998**, *56*, 245.

17. Sumrell, G.; Stevens, J. I.; Goheen, G. E. *J. Org. Chem*. **1957**, *22*, 39.

18. Abdelazeem, A. H.; Habash, M.; Maghrabi, I. A.; Taha, M. O. *Med. Chem. Res*. **2015**, *24*, 3681.

19. Abdelazeem, A. H.; Gouda, A. M.; Omar, H. A.; Tolba, M. F. *Bioorg. Chem*. **2014**, *57*, 132.

20. Tanaka, A.; Sakai, H.; Motoyama, Y.; Ishikawa, T.; Takasugi, H. *J. Med. Chem*. **1994**, *37*, 1189.

21. Behr, A. P.; Kapui, Z.; Aranyi, P.; Batori, S.; Bodor, V. B.; Nagy, L. T.; Santa, M.; Varga, M.; Mikus, E.; Urban-Szabo, K.; Szeredi, J. V.; Szabo, T.; Susan, E.; Kovacs, M. *US 20080280963 A1*. **2008**.

22. Liu, W.; Bensdorf, K.; Proetto, M.; Abram, U.; Hagenbach, A.; Gust, R. *J. Med. Chem*. **2011**, *54*, 8605.

23. Liu, W.; Bensdorf, K.; Hagenbach, A.; Abram, U.; Niu, B.; Mariappan, A.; Gust, R. *Eur. J. Med. Chem*. **2011**, *46*, 5927.

24. Kulmacz, R. J.; Lands, W. E. *Prostaglandins*. **1983**, *25*, 531.

25. Abdelazeem, A. H.; Abdelatef, S. A.; El-Saadi, M. T.; Omar, H. A.; Khan, S. I.; McCurdy, C. R.; El-Moghazy, S. M. *Eur. J. Pharm. Sci*. **2014**, *62*, 197.

26. Kurumbail, R. G.; Stevens, A. M.; Gierse, J. K.; McDonald, J. J.; Stegeman, R. A.; Pak, J. Y.; Gildehaus, D.; Miyashiro, J. M.; Penning, T. D.; Seibert, K.; Isakson, P. C.; Stallings, W. C. *Nature*. **1996**, *384*, 644.

27. Koster, R.; Anderson, M.; Ej, D. B., Acetic acid for analgesic screening, Fed. Proc. **1959**, *18*, 412.

28. Winter, C. A.; Risley, E. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med*. **1962**, *111*, 544.

29. Abdelazeem, A. H.; Salama, S. A.; Maghrabi, I. A. *Arch. Pharm. (Weinheim)*. **2015**, *348*, 518.

30. Cioli, V.; Putzolu, S.; Rossi, V.; Scorza Barcellona, P.; Corradino, C. *Toxicol. Appl. Pharmacol*. **1979**, *50*, 283.

31. Accelrys Software Inc., **2007**. Discovery Studio Modeling Environment, Release 2.5. Accelrys Software Inc., San Diego.

32. Abdelazeem, A. H.; El-Saadi, M. T.; Safi El-Din, A. G.; El-Moghazy, S. M. *J. Chem. Pharm. Res*. **2015**, *7*, 1073.

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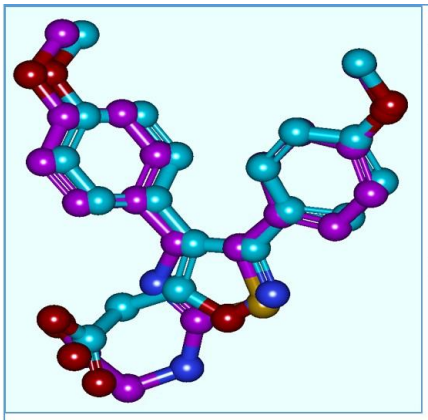
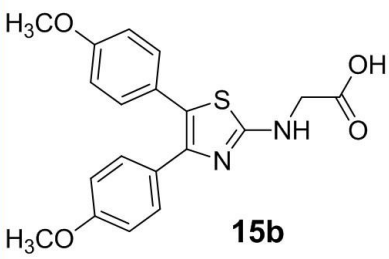
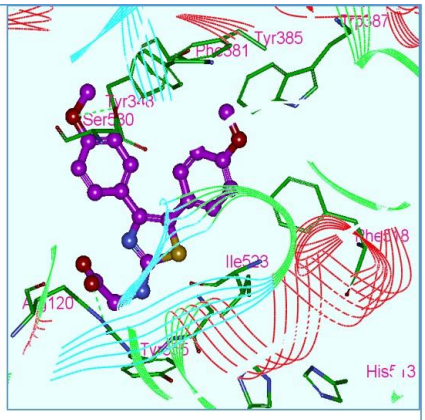


Superimposition of 15b & Mofezolac



15b

COX-1 IC_{50}= 0.32 µM
COX-2 IC_{50}= 9.23 µ M
***SI* (COX-2/COX-1) = 28.84**
***UI* = 8.5**



Docking of 15b into COX-1 active site

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