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Synthesis and SAR study of 4,5-diaryl-1*H*-imidazole-2(3*H*)-thione derivatives, as potent 15-lipoxygenase inhibitors

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ABSTRACT

A series of 4,5-diaryl-1*H*-imidazole-2(3*H*)-thione was synthesized and their inhibitory potency against soybean 15-lipoxygenase and free radical scavenging activities were determined. Compound **11** showed the best IC_{50} for 15-LOX inhibition (IC_{50} = 4.7 μM) and free radical scavenging activity (IC_{50} = 14 μM). Methylation of SH at C_2 position of imidazole has dramatically decreased the 15-LOX inhibition and radical scavenging activity as it can be observed in the inactive compound **14** (IC_{50} >250 μM). Structure activity similarity (SAS) showed that the most important chemical modification in this series was methylation of SH group and Docking studies revealed a proper orientation for SH group towards Fe core of the 15-LOX active site. Therefore it was concluded that iron chelating could be a possible mechanism for enzyme inhibition in this series of compounds.

1. Introduction

Eicosanoids are a family of lipid mediators derived from the metabolism of arachidonic acid (AA). Some types of eicosanoids such as prostanoids and leukotrienes showed to have a wide range of biological actions including potent effects on inflammation and immunity. AA is the substrate for various metabolic pathways that produces biological mediators with cyclooxygenase and lipoxygenase.[1,2] Lipoxygenases (LOX) constitute a family of dioxygenases, that catalyze dioxygenation of 1,4-*cis*,*cis*-pentadiene-containing polyunsaturated fatty acids to produce the corresponding hydroperoxy derivatives.[3] They are class of non-heme iron containing enzyme found in both plant and animals. Three main LOXs including 5, 12 and 15-lipoxygenases have been distinguished based on the peroxidation site of AA.[4] Comparing the two LOXs of the rabbit and soybean revealed similarities between the active site of animal LOX and that of plant in order to its sequence homology and structure.[5–10] The highest level of sequence identity between LOX from plants and mammals lies in the area of the catalytic domain containing the non-heme iron atom. Since the soybean enzyme is much more accessible than human, its use as a model for designing inhibitors could be considered as a tool in structural characterization, mechanism elucidation and discovery of new inhibitors.[11] 15-Lipoxygenase (15-LOX) has been implicated in several diseases such as cancer and chronic obstructive pulmonary diseases (COPD).[12,13] The role of 15-LOX in the progression of atherogenesis and vascular disease has been also verified.[14,15] It was also found that 15-LOX takes role in the oxidation of low-density protein (LDL) leading to the progression of atherosclerosis.[16] The immediate products of 15-LOX oxidation were shown to have pro-inflammatory and pro-thrombotic effects.[17,18] Furthermore, 15-LOX proposed to have a beneficial role in human airway carcinomas and promotes apoptotic pathway. Neoplastic tissues from human airway carcinomas demonstrated non-specific staining for human 15-LOX as compared with normal tissues.[19] In contrast, in human prostate tumors, 15-LOX_a was over expressed as compared with normal adjacent tissue, and 15-LOX_b was poorly expressed in prostate tumors.[12,20]

It was found that 15-LOX_a takes part in minimization of the brain damage after stroke. An important consequence of stroke is damage of the mitochondria, leading to the breakdown of the membrane potential, the cytochrome C release and production of ROS, which are the major features of neuronal cell death.[21]

The three different strategies to inhibit the LOX's pathway are as follows: (i) redox inhibitors or antioxidants, which interfere with the redox cycle of 15-LO, (ii) iron-chelator agents and (iii) non-redox competitive inhibitors, which compete with AA to bind the enzyme active site.[22]

It has been reported that the iron chelating property of sulfur atom could be essential for the activity of 15-LOX inhibitors. According to the later study, 4-Methyl-2-(4-methylpiperazinyl)

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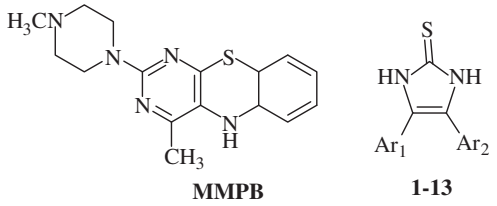
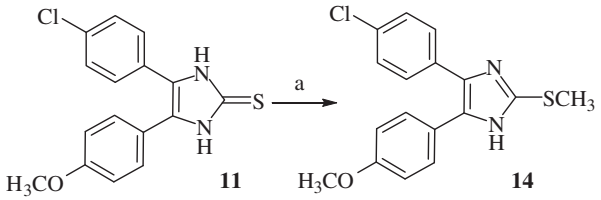


Figure 1. Chemical structure of 4-MMPB and designed compounds.



Scheme 2. Reagents and conditions: (a) CH_3I, CH_3OH, reflux.

pyrimido[4,5-*b*]benzothiazine (4-MMPB) was introduced as a potent 15-lipoxygenase[23] (Fig. 1).

Additionally, the coordination chemistry of heterocyclic thiones especially those possessing α-*N*-heteroatom is rich due to their versatility in chelating property.[24] Considering this fact that a thiourea moiety has constructed an electron rich site, evaluation of lipoxygenase properties of imidazole-2-thione scaffold has been of our interest. In continuation of our research work on design and synthesis of LOX inhibitors,[25–28] a new series of 4,5-diaryl-1*H*-imidazole-2(3*H*)-thione derivatives were synthesized and in vitro 15-LOX inhibitory and free radical scavenging activity of the synthesized compounds were studied. Structure activity similarity (SAS) and docking experiments were subsequently used to obtain a pattern of structure activity in this series of compounds. Furthermore, diarylheterocycle scaffold has been extensively studied as cyclooxygenase (COX) inhibitors.[29]

2. Results and discussion

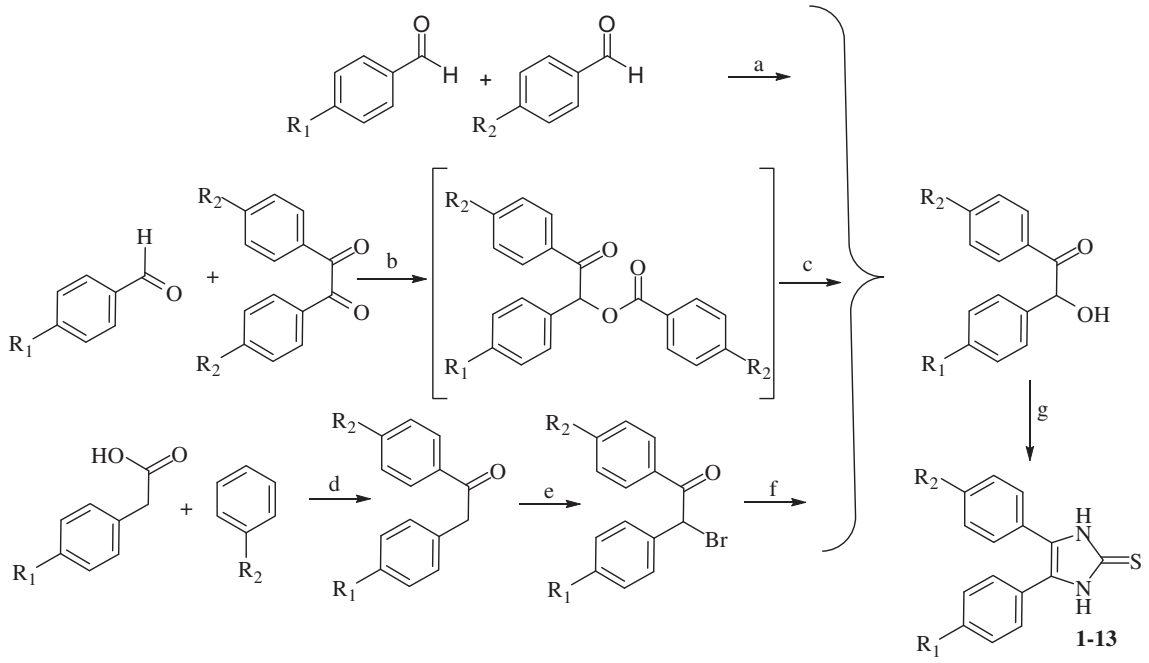
2.1. Chemistry

4,5-Diaryl-1*H*-imidazole-2(3*H*)-thiones were synthesized by the reaction of different benzoins and ammonium thiocyanate.[30] The cyanide ion-catalyzed condensation of aromatic aldehydes is a convenient synthetic method for the symmetrical benzoins. However, synthesis of unsymmetrical benzoins under traditional conditions imposes to the formation of four different benzoins.[31] In one approach for the preparation of unsymmetrical benzoins, cyanide ion-catalyzed cleavage of benzils derivatives was used to generate acyl intermediates. The reaction of these intermediates with various aldehydes furnishes the corresponding esters of unsymmetrical benzoins. Finally, the products were subjected to hydrolyze in a basic medium.[32] In another approach, various substituted aromatic hydrocarbons were reacted with appropriate phenyl acetic acid. The products were then subjected to bromination using bromine in glacial acetic acid. Subsequently, related α-bromodesoxybenzoin was refluxed with sodium methoxide in methanol and the reaction was finally quenched with cold 10% hydrochloric acid to obtain unsymmetrical benzoin.[33] Treatment of different benzoins with a ten-fold excess of ammonium thiocyanate in *n*-butanol afforded the desired 4,5-diaryl-1*H*-imidazole-2(3*H*)-thiones **1**–**13** in good yield (Scheme 1).

Finally, alkylation of **11** using methyl iodide in basic media afforded compounds **14** in good yield (Scheme 2).

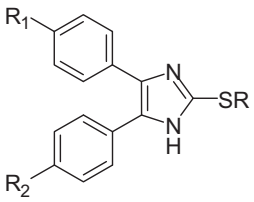
2.2. Inhibition of 15-lipoxygenase

The target compounds **1**–**14** were evaluated for their inhibitory activity against Soybean LOX using modified DMAB-MBTH method reported by Anthon et al.[34] In this method the basis of the lipoxygenase activity determination is peroxide measurement and the potent 15-LOX inhibitor 4-methyl-2-(4-methylpiperazinyl)pyrimido[4,5-*b*]benzothiazine (4-MMPB) was used as the reference compound. The results are summarized in Table 1.



Scheme 1. Reagents and conditions: (a) KCN, CH_3OH/H_2O, reflux; (b) KCN, DMF, rt; (c) NaOH, CH_3CN, rt; (d) H_3PO_4, $(CF_3CO)_2O$, 25 °C; (e) Br_2, glacial AcOH, rt; (f) CH_3ONa, CH_3OH, reflux, 10% HCl; (g) NH_4SCN, *n*-butanol, reflux.

Table 1
Enzyme inhibitory assessment and DPPH radical scavenging activity data of the synthetic compounds in comparison with 4-MMPB and NDGA respectively



Compounds	R	R_1	R_2	IC_{50} μM (DPPH radical scavenging activity)	IC_{50} μM (soybean 15-LOX)
1	H	H	H	49.2 ± 0.9	15.2 ± 0.8
2	H	CH_3O	CH_3O	15.5 ± 0.7	14.7 ± 0.5
3	H	F	F	143.1 ± 2.8	23.4 ± 1.5
4	H	Cl	Cl	107.4 ± 1.2	62.0 ± 2.5
5	H	CH_3O	H	87.4 ± 1.1	13.7 ± 0.8
6	H	Cl	H	30.3 ± 0.8	24.9 ± 1.3
7	H	CH_3S	H	90.0 ± 1.6	5.7 ± 0.4
8	H	$(CH_3)_2N$	Cl	15.9 ± 0.3	22.3 ± 0.6
9	H	$(CH_3)_2N$	CH_3O	15.2 ± 0.8	9.4 ± 0.4
10	H	CH_3O	F	57.2 ± 1.3	11.0 ± 0.9
11	H	CH_3O	Cl	14.0 ± 0.4	4.7 ± 0.3
12	H	CH_3S	F	49.0 ± 0.5	136 ± 4.3
13	H	CH_3S	Cl	21.2 ± 0.6	19.7 ± 0.7
14	CH_3	CH_3O	Cl	>250	>250
4-MMPB[a]				—	34.2 ± 1.2
NDGA[b]				6.2 ± 0.4	—

[a] 4-Methyl-2-(4-methylpiperazinyl)pyrimido[4,5-*b*]benzothiazine.
[b] Nordihydroguaiaretic acid.

In general, most of compounds demonstrated significant 15-LOX inhibitory potential. Two of the synthesized compounds, **7** (IC_{50} = 5.7 μM) and **11** (IC_{50} = 4.7 μM), had the best 15-LOX inhibition compared to reference compound 4-MMPB (IC_{50} = 34.2 μM). According to the substitution pattern on the phenyl rings, the synthesized compounds could be categorized in two series: 'Symmetrical derivatives' which contain the similar group on both phenyl rings and 'unsymmetrical derivatives' with different substituted moiety on the mentioned phenyl rings. Structure–activity relationship studies showed that the nature of substituted group on the phenyl ring (R_1 and R_2) had a profound influence on 15-LOX inhibitory activity of compounds in both series.

2.2.1. Symmetrical series

Substitution of electron withdrawing halogen groups into the *para* position of both phenyls resulted in drastic reduced 15-LOX-inhibitory potential of symmetrical derivatives; while introduction of *p*-methoxy group into the phenyl rings, eventuated in enhanced inhibitory activity. The symmetrical compound **2** containing *p*-dimethoxy phenyl substitutes on the central imidazole core, is the most potent agent of this series (IC_{50} = 14.7 μM). However; the halogen containing derivatives, demonstrated the lowest inhibitory potential against 15-LOX enzyme that is attributed to the size of halogen atom along with electron withdrawing property of substituted halogen. The difluorophenyl containing derivative **3** (IC_{50} = 23.4 μM) is more potent than the dichlorophenyl susbstituted compound **4** (IC_{50} = 62 μM) which could be due to smaller size of fluorine relation to chlorine group.

2.2.2. Unsymmetrical derivatives

Among monosubstituted derivatives of this category (compounds **5**–**7**), *p*-methylthio containing compound **7** showed significant LOX inhibitory activity (IC_{50} = 5.7 μM). Relying on the *para*-substituted moiety (R_1) of monosubstituted derivatives, the order of potency is $CH_3S > CH_3O >$ Cl.

As the employment of methoxy (OCH_3) and methylthio (SCH_3) substitutes into the *para* position of phenyl ring is favorable for potency, we substituted such moieties (R_1 = OCH_3, SCH_3 and $N(CH_3)_2$) into *p*-position of one of the phenyl rings and employed different substitutes (R_2 = Cl, F and OCH_3) on the other one. The IC_{50} value of resulted derivatives **8**–**13**, defined as disubstituted group, is varied in the range of 4.7–136 μM. Investigation of R_1 moiety in disubstituted derivatives revealed that the order of 15-LOX inhibitory potential is $OCH_3 > SCH_3 > N(CH_3)_2$. Comparison of compound **8** with **9** containing $N(CH3)_2$ group, compound **9** (R_2 = *p*-methoxy, IC_{50} = 9.4 μM) is more potent than **8** (R_2 = *p*-Cl, IC_{50} = 22.3 μM). Therefore, it seems that one methoxy group in *para* position was favorable for potency. Considering the R_2 in *p*-methoxy phenyl substituted compounds (**2**, **5**, **10** and **11**) demonstrated that the 15-LOX inhibitory potency is mainly dependent on the nature of R_2 moiety with the order of $Cl \gg F > H > CH_3O$. Thus, it can be deduced that the introduction of electron-donating OCH_3 in R_1 and electron-withdrawing Cl in R_2 were able to improve the activity of these series. It could be postulated that OCH_3 and Cl establish a balanced resonance in aryl rings in order to increase the iron chelating property of sulfur atom. Hofmann et al. reported similar substituents in 5-benzylidene-2-phenylthiazolinone scaffold as a potent 5-LOX inhibitor.[35] Furthermore, comparison of compounds **10**–**13** showed that replacement of the *p*-methoxy with a weak electron-releasing methylthio group (**12** vs **10**, **13** vs **11**) led to decrease of 15-LOX activity.

2.3. DPPH radical scavenging activity

The free radical scavenging activity of the compounds **1**–**14** was evaluated by DPPH colorimetric method. Several dilutions of the compounds were prepared for calculation of the IC_{50} through regression analysis of the mean values. Among the tested compounds, compound **11** (IC_{50} = 14.0 μM) showed the best DPPH radical scavenging activity in comparison with nordihydroguaiaretic acid (NDGA) as a reference compound. This observation suggests that the synthetic compounds could possibly inhibit the lipoxygenase activity by their radical scavenging properties and chelating the iron atom. Finally, *S*-methylation of compound **11** led to inactive compound **14** (15-LOX inhibition and radical scavenging potency >250 μM).

2.4. Structure activity similarity

In order to obtain a correlation between the activity and structure of the compounds, structure activity similarity map (SAS) was generated for this series of compounds.[36]

SAS map is a comprehensive tool in developing structure–activity relationship (SAR) of the synthetic structures. By the aforementioned method, it is possible to obtain an overview of the chemical activity space prior to visual inspection of the modifications in each pair of ligands. In addition, by using SAS maps, it is possible to cluster the data in to four regions of different SAR, which makes it easier to design more potent compounds based on the similarities of the active structures.

The plot of the activity and similarity coefficients for the synthesized structures is depicted in Figure 2. According to the pattern shown in this Figure, most of the points are present in the upper right part of the plot, which is anticipated for the analogue series with similarities in the structure and activity. The highest similarity in terms of both activity and structural features was in compounds **5** and **2**, with one and two OCH_3 substitutions at para position of the phenyl rings, respectively (Fig. 2a. Tanimoto similarity = 1, Activity similarity = 0.99). On the other hand, the lowest similarity in the structural features was in compounds **12** and **9**

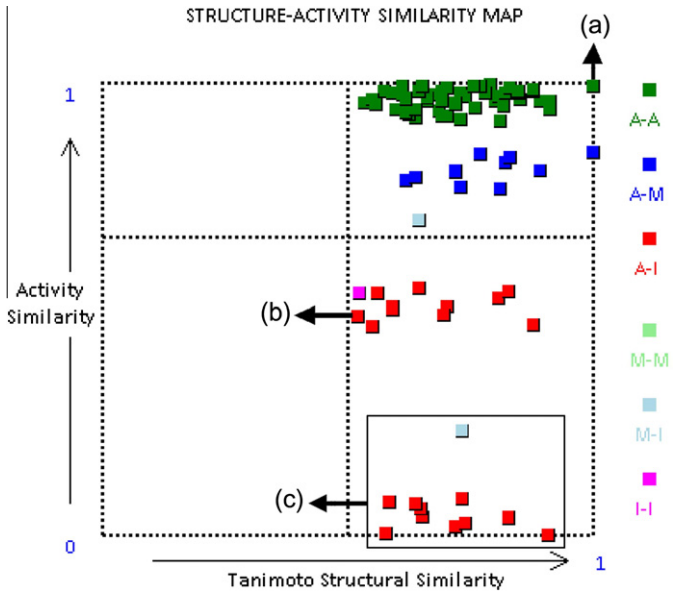


Figure 2. SAS map of the synthesized structures, the color coding represent activity alterations for each pair of ligands. 'A' denounce for Active, 'M' for moderate and 'I' for the Inactive compounds. (a) Pair comparison between activity similarity and structural similarity of the compounds **5** and **2**. (b) Pair comparison between compound **9** and **12**. (c) Pair comparisons between compound **14** and other synthesized structures.

with SCH_3 replaced by OCH_3 and F replaced by $N(CH_3)_2$ (Fig. 2b. Tanimoto similarity = 0.52)

As it can be observed in Figure 2, most of the points are colored green and are located in the upper right area of the SAS plot. This means that most compounds are structurally relevant and are more potent than the control drug. On the other hand, the points in the lower right side of the plot accounts for the activity cliffs, compounds with high structural similarity but low similarity in the activity. Most of the data in this area are related to pair comparisons between compounds **14** with other compounds (Fig. 2c). The inactive compound **14** is showing the lowest similarity in the activity (<0.1) compared to other structurally similar compounds of high potency in this series. Since SH group is present in all other structures of this series this dramatic decrease in the activity can be related to methylation of SH in compound **14**. Based on the SAS map, reasonable range of structural–activity diversity (tanimoto similarity 0.5–1, activity similarity 0–1) suggested that the number of synthesized compounds was suitable for studies of structure–activity relationship.

2.5. Molecular modeling study

Based on the results, the most important chemical modification in this series of compounds was methylation of SH at the C_2 of imidazol ring, which led to the inactive compound, **14**. It was postulated that a possible mechanism for the activity of the SH containing compounds might be through chelating the iron atom in the binding site of the lipooxygenase enzyme. In order to investigate the orientation of the synthesized structures towards the iron atom in the active site pocket, some docking studies using autodock vina were performed. After running vina in case of two active structures (**1** and **5**), the best docking poses of the ligands were visualized using VMD. According to Figure 3 (left), a 4.84 Å distance between the sulfur atoms of the ligands and the iron atom in the active site was observed. This verifies that the possible mechanism for the activity of the synthesized compounds could be through iron chelating activity. Therefore, it looks reasonable that a decrease in the activity of compound **14** might be caused by sulfur methylation and reduction of its chelating potency.

As depicted in Figure 3 (right), in the binding pocket formed by the residues Ile 770, Gln 514, Val 566, Leu 773 and Trp 519, a π–π interaction is seen between Trp 519 and one of the phenyl rings of compound **1**. The other phenyl ring is oriented towards the hydrophobic pocket shaped by Ile 770 and Leu 773. This conformation stabilizes the molecule so that the more hydrophilic mercapto imidazole moiety orients towards hydrophilic area around the iron core.

3. Conclusions

A series of 4,5-diaryl-1*H*-imidazole-2(3*H*)-thione was synthesized and their inhibitory potency against soybean 15-lipoxygenase and free radical scavenging activities were determined. In general, most of compounds demonstrated significant 15-LOX inhibitory potential. Structure–activity relationship studies showed that the nature of substituent of two arly (R_1 and R_2) on the imidazole ring had a profound influence on LOX activity. A possible mechanism for the activity of the SH containing compounds

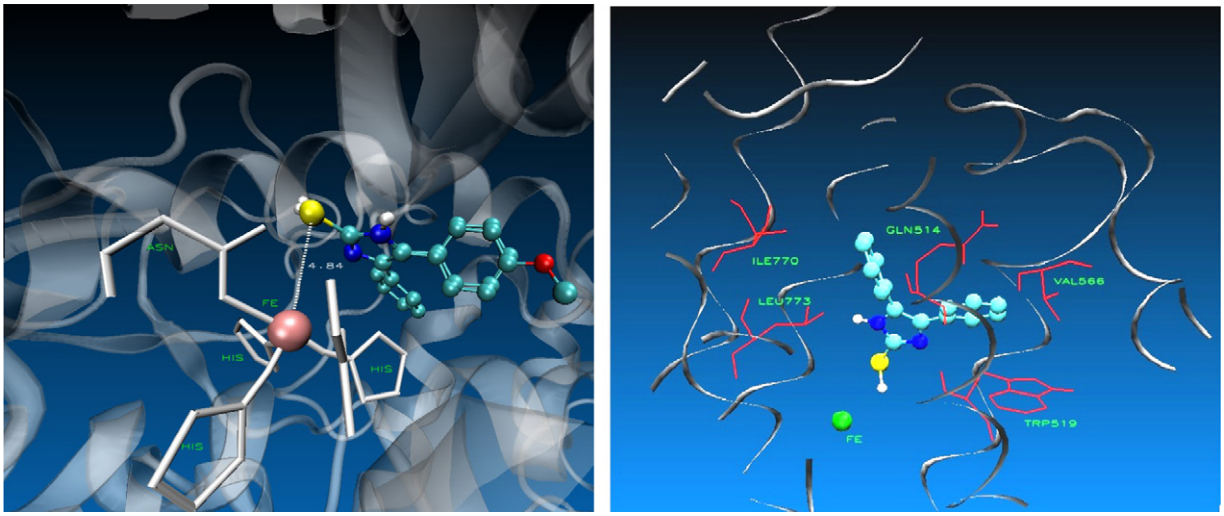


Figure 3. (Left) Superposition of the best docked poses for the two active structures **1** and **5** in the active site. (Right) Ball and stick representation of the best docked pose for compound **1** in the active site of 15-LOX. All visualizations were done using VMD.

might be through radical scavenging properties and chelating the iron atom in the binding site of the lipooxygenase enzyme.

4. Experimental

4.1. Chemistry

^{1}H NMR spectra were recorded on a 500 MHz Bruker spectrometer using $CDCl_3$ or DMSO-d_6 as solvent. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane (TMS) as internal standard. Infrared spectra were acquired on a Nicolet Magna 550-FT spectrometer. IR spectra of solids were recorded in KBr and the absorption band was given in wave numbers v in cm^{-1}. Mass spectra were obtained with a Finnigan Mat TSQ-70 spectrometer. Elemental microanalyses were within ±0.4 of the theoretical values for C, H and N. All measurements of lipoxygenase activities were carried out using an Spekol 1500 spectrophotometer. The soybean 15-lipoxygenase and other chemicals were purchased from Sigma, Aldrich and Merck Co. respectively.

4.1.1. 4,5-Diphenyl-1*H*-imidazole-2(3*H*)-thione (1)

Yield, 78%; mp 226–229 °C; IR (KBr, cm^{-1}): v 3441, 1598, 1495, 1214, 758, 691, 558; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.29–737 (m, 10H, phenyl), 12.50 (br s, 2H, NH); MS, m/z (%) 252 (M^+, 100), 218 (14), 193 (20), 165 (21), 104 (10), 77 (8). Anal. Calcd. for $C_{15}H_{12}N_2S$: C, 71.40; H, 4.79; N, 11.10. Found: C, 71.28; H, 4.61; N, 11.23.

4.1.2. 4,5-bis(4-Methoxyphenyl)-1*H*-imidazole-2(3*H*)-thione (2)

Yield, 71%; mp 261–264 °C; IR (KBr, cm^{-1}): v 3426, 1613, 1506, 1250, 1168, 1029, 840; ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.75 (s, 6H, OCH_3), 6.91 (d, J = 8.5, 4H, $H_{3,5}$-methoxyphenyl), 7.27 (d, J = 8.5, 4H, $H_{2,6}$-methoxyphenyl), 12.34 (br s, 2H, NH); MS, m/z (%) 312 (M^+, 100), 297 (30), 238 (11), 156 (5), 134 (4). Anal. Calcd. for $C_{17}H_{16}N_2O_2S$: C, 65.36; H, 5.16; N, 8.97. Found: C, 65.52; H, 5.27; N, 8.81.

4.1.3. 4,5-bis(4-Fluorophenyl)-1*H*-imidazole-2(3*H*)-thione (3)

Yield, 64%; mp 244–247 °C; IR (KBr, cm^{-1}): v 3436, 1603, 1511, 1229, 1157, 840; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.20 (t, J = 8.5, 4H, $H_{3,5}$-fluorophenyl), 7.37 (dd, J = 8.5, J = 5.5, 4H, $H_{2,6}$-fluorophenyl), 12.56 (br s, 2H, NH); MS, m/z (%) 288 (M^+, 100), 254 (10), 229 (21), 201 (20), 122 (15), 95 (9). Anal. Calcd. for $C_{15}H_{10}F_2N_2S$: C, 62.49; H, 3.50; N, 9.72. Found: C, 62.24; H, 3.36; N, 9.58.

4.1.4. 4,5-bis(4-Chlorophenyl)-1*H*-imidazole-2(3*H*)-thione (4)

Yield, 68%; mp 287–290 °C; IR (KBr, cm^{-1}): v 3395, 1501, 1091, 830; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.35 (d, J = 8.5, 4H, chlorophenyl), 7.44 (d, J = 8.5, 4H, chlorophenyl), 12.64 (br s, 2H, NH); MS, m/z (%) 322 (M^++2, 67), 420 (M^+, 100), 300 (15), 227 (14), 138 (16), 111 (15), 69 (25). Anal. Calcd. for $C_{15}H_{10}Cl_2N_2S$: C, 56.09; H, 3.14; N, 8.72. Found: C, 56.27; H, 3.32; N, 8.63.

4.1.5. 4-(4-Methoxyphenyl)-5-phenyl-1*H*-imidazole-2(3*H*)-thione (5)

Yield, 74%; mp 255–257 °C; IR (KBr, cm^{-1}): v 3400, 1608, 1506, 1255, 1024, 830, 763, 691; ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.76 (s, 3H, OCH_3), 6.93 (d, J = 8.5, 2H, $H_{3,5}$-methoxyphenyl), 7.28 (d, J = 8.5, 2H, $H_{2,6}$-methoxyphenyl), 7.25–7.38 (m, 5H, phenyl), 12.43 (br s, 1H, NH), 12.44 (br s, 1H, NH). MS, m/z (%) 282 (M^+, 100), 267 (23), 223 (10), 180 (11), 152 (6), 77 (5). Anal. Calcd. for $C_{16}H_{14}N_2OS$: C, 68.06; H, 5.00; N, 9.92. Found: C, 68.22; H, 5.17; N, 9.84.

4.1.6. 4-(4-Chlorophenyl)-5-phenyl-1*H*-imidazole-2(3*H*)-thione (6)

Yield, 76%; mp 264–267 °C; IR (KBr, cm^{-1}): v 3452, 1603, 1506, 1219, 1091, 830, 763, 697; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.31–7.39 (m, 7H, aromatic), 7.43 (d, J = 8.5, 2H, chlorophenyl), 12.59 (br s, 2H, NH); MS, m/z (%) 288 (M^++2, 33), 286 (M^+, 100), 252 (20), 227 (15), 193 (15), 168 (18), 104 (10). Anal. Calcd. for $C_{15}H_{11}ClN_2S$: C, 62.82; H, 3.87; N, 9.77. Found: C, 62.94; H, 3.68; N, 9.58.

4.1.7. 4-(4-(Methylthio)phenyl)-5-phenyl-1*H*-imidazole-2(3*H*)-thione (7)

Yield, 72%; mp 241–244 °C; IR (KBr, cm^{-1}): v 3416, 1506, 1209, 1101, 825, 768, 691; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.47 (s, 3H, SCH_3), 7.22 (d, J = 8.5, 2H, $H_{2,6}$-methylthiophenyl), 7.27 (d, J = 8.5, 2H, $H_{3,5}$-methylthiophenyl), 7.29–7.39 (m, 5H, phenyl), 12.50 (br s, 2H, NH); MS, m/z (%) 298 (M^+, 100), 283 (25), 250 (9), 193 (7), 165 (6), 77 (4). Anal. Calcd. for $C_{16}H_{14}N_2S_2$: C, 64.39; H, 4.73; N, 9.39. Found: C, 64.56; H, 4.85; N, 9.53.

4.1.8. 4-(4-Chlorophenyl)-5-(4-(dimethylamino)phenyl)-1*H*-imidazole-2(3*H*)-thione (8)

Yield, 62%; mp 258–261 °C; IR (KBr, cm^{-1}): v 3441, 1608, 1531, 1367, 1193, 825; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.92 (s, 6H, NCH_3), 6.68 (d, J = 8.5, 2H, $H_{3,5}$-dimethylaminophenyl), 7.15 (d, J = 8.5, 2H, $H_{2,6}$-dimethylaminophenyl), 7.35 (d, J = 8.5, 2H, chlorophenyl), 7.40 (d, J = 8.5, 2H, chlorophenyl), 12.36 (br s, 1H, NH), 12.42 (br s, 1H, NH); MS, m/z (%) 331 (M^++2, 33), 329 (M^+, 100), 270 (7), 164 (8). Anal. Calcd. for $C_{17}H_{16}ClN_3S$: C, 61.90; H, 4.89; N, 12.74. Found: C, 61.78; H, 4.98; N, 12.86.

4.1.9. 4-(4-(Dimethylamino)phenyl)-5-(4-methoxyphenyl)-1*H*-imidazole-2(3*H*)-thione (9)

Yield, 51%; mp 231–233 °C; IR (KBr, cm^{-1}): v 3416, 1618, 1516, 1362, 1244, 1173, 819; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.89 (s, 6H, NCH_3), 3.75 (s, 3H, OCH_3), 6.68 (d, J = 8.5 Hz, 2H, $H_{3,5}$-dimethylaminophenyl), 6.91 (d, J = 8.5 Hz, 2H, $H_{3,5}$-methoxyphenyl), 7.16 (d, J = 8.5, 2H, $H_{2,6}$-dimethylaminophenyl), 7.28 (d, J = 8.5, 2H, $H_{2,6}$-methoxyphenyl), 12.24 (br s, 2H, NH); MS, m/z (%) 325 (M^+, 100), 315 (21), 266 (10), 251 (8), 162 (8). Anal. Calcd. for $C_{18}H_{19}N_3OS$: C, 66.43; H, 5.88; N, 12.91. Found: C, 66.56; H, 5.67; N, 12.75.

4.1.10. 4-(4-Fluorophenyl)-5-(4-methoxyphenyl)-1*H*-imidazole-2(3*H*)-thione (10)

Yield, 72%; mp 253–256 °C; IR (KBr, cm^{-1}): v 3426, 1608, 1511, 1250, 1029, 835; ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.76 (s, 3H, OCH_3), 6.93 (d, J = 8.5 Hz, 2H, $H_{3,5}$-methoxyphenyl), 7.20 (t, J = 8.5, 2H, $H_{3,5}$-fluorophenyl), 7.26 (d, J = 8.5, 2H, $H_{2,6}$-methoxyphenyl), 7.36 (dd, J = 8.5, J = 5.5, 2H, $H_{2,6}$-fluorophenyl), 12.44 (br s, 1H, NH), 12.46 (br s, 1H, NH); MS, m/z (%) 300 (M^+, 100), 286 (70), 241 (25), 226 (22), 198 (20), 171 (10), 150 (11), 122(8), 95(6). Anal. Calcd. for $C_{16}H_{13}FN_2OS$: C, 63.98; H, 4.36; N, 9.33. Found: C, 63.64; H, 4.54; N, 9.56.

4.1.11. 4-(4-Chlorophenyl)-5-(4-methoxyphenyl)-1*H*-imidazole-2(3*H*)-thione (11)

Yield, 74%; mp 247–250 °C; IR (KBr, cm^{-1}): v 3426, 1598, 1516, 1250, 758, 830; ^{1}H NMR (500 MHz, DMSO-d_6): δ 3.77 (s, 3H, OCH_3), 6.95 (d, J = 8.5, 2H, $H_{3,5}$-methoxyphenyl), 7.27 (d, J = 8.5, 2H, $H_{2,6}$-methoxyphenyl), 7.35 (d, J = 8.5, 2H, chlorophenyl), 7.41 (d, J = 8.5, 2H, chlorophenyl), 12.47 (br s, 1H, NH), 12.50 (br s, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 55.65, 114.74, 121.07, 123.05, 123.75, 128.12, 129.19, 129.41, 129.73, 132.75, 159.71, 161.50; MS, m/z (%) 318 (M^++2, 33), 316 (M^+, 100), 302 (22), 252 (15), 180 (10), 152 (8), 69 (7). Anal. Calcd. for $C_{16}H_{13}ClN_2OS$: C, 60.66; H, 4.14; N, 8.84. Found: C, 60.86; H, 4.25; N, 8.67.

4.1.12. 4-(4-Fluorophenyl)-5-(4-(methylthio)phenyl)-1*H*-imidazole-2(3*H*)-thione (12)

Yield, 74%; mp 242–245 °C; IR (KBr, cm^{-1}): v 3436, 1629, 1506, 1209, 1157, 845; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.48 (s, 3H,

SCH_3), 7.20–7.24 (m, 4H, aromatic), 7.26 (d, J = 8.5, 2H, $H_{3,5}$-methylthiophenyl), 7.39 (dd, J = 8.5, J = 5.5, 2H, $H_{2,6}$-fluorophenyl), 12.52 (br s, 2H, NH); MS, m/z (%) 316 (M^+, 100), 301 (23), 268 (6), 242 (5), 211 (5), 183 (4), 158 (4). Anal. Calcd. for $C_{16}H_{13}FN_2S_2$: C, 60.73; H, 4.14; F, N, 8.85. Found: C, 60.89; H, 4.26; N, 8.63.

4.1.13. 4-(4-Chlorophenyl)-5-(4-(methylthio)phenyl)-1*H*-imidazole-2(3*H*)-thione (13)

Yield, 71%; mp 259–262 °C; IR (KBr, cm^{-1}): ν 3390, 1634, 1501, 1219, 1086, 819; ^1H NMR (500 MHz, DMSO-d_6): δ 2.48 (s, 3H, SCH_3), 7.24 (d, J = 8.5, 2H, $H_{2,6}$-methylthiophenyl), 7.28 (d, J = 8.5, 2H, $H_{3,5}$-methylthiophenyl), 7.35 (d, J = 8.5, 2H, chlorophenyl), 7.43 (d, J = 8.5, 2H, chlorophenyl), 12.56 (br s, 2H, NH); ^{13}C NMR (DMSO-d_6): δ 14.56, 122.12, 126.24, 127.55, 128.50, 129.05, 129.27, 129.66, 133.86, 141.02, 141.40, 177.40; MS, m/z (%) 334 (M^++2, 33), 332 (M^+, 100), 317 (19), 284 (5), 227 (4), 166 (4). Anal. Calcd. for $C_{16}H_{13}ClN_2S_2$: C, 57.73; H, 3.94; N, 8.42. Found: C, 57.92; H, 3.75; N, 8.53.

4.1.14. 5-(4-Chlorophenyl)-4-(4-methoxyphenyl)-2-(methylthio)-1*H*-imidazole (14)

Yield, 85%; mp 179–181 °C; IR (KBr, cm^{-1}): ν 3446, 1613, 1506, 1250, 1168, 1091, 830; ^1H NMR (500 MHz, $CDCl_3$): δ 2.67 (s, 3H, SCH_3), 3.83 (s, 3H, OCH_3), 6.89 (d, J = 8.5, 2H, $H_{3,5}$-methoxyphenyl), 7.26 (d, J = 8.5, 2H, $H_{2,6}$-methoxyphenyl), 7.37 (d, J = 8.5, 2H, chlorophenyl), 7.47 (d, J = 8.5, 2H, chlorophenyl); MS, m/z (%) 332 (M^++2, 33), 330 (M^+, 100), 297 (61), 257 (38), 243 (19), 133 (7). Anal. Calcd. for $C_{17}H_{15}ClN_2OS$: C, 61.72; H, 4.57; N, 8.47. Found: C, 61.58; H, 4.36; N, 8.71.

4.2. Biological assays

4.2.1. 15-Lipoxygenase inhibition assay

For 15-LO Inhibitory assessment, Linoleic acid and two assay solutions (A and B) were prepared in advance. Solution A was 50 mM DMAB (3-dimethylaminobenzoic acid) in a l00 mM phosphate buffer (pH 7.0) while solution B was a mixture of l0 mM MBTH (3-methyl-2-benzothiazolonhydrazone) (3 mL), hemoglobin (5 mg/mL, 3 mL) in 50 mM phosphate buffer at pH 5.0 (25 ml). A linoleic acid solution was prepared by mixing 5 mg of linoleic acid with 0.5 mL ethanol and then diluting with KOH (100 mM) to a final volume of 5 mL. In the standard assay, the sample in ethanol (25 μL), SLO (40,000 units/mL in 50 mM phosphate buffer pH 7.0; 25 μL) and phosphate buffer pH 7.0 (50 mM; 900 μL) were mixed in a test tube and preincubation was carried out for 5 min at room temperature. A control test was done with the same volume of ethanol. After preincubation, linoleic acid solution (50 μL) was added to initialize the peroxidation reaction, and, 7 min later, solution A (270 μL) and then solution B (130 μL) was added. Five minutes later, 200 μL of a 2% SDS solution was added to terminate the reaction. The absorbance at 598 nm was compared with the control test.

4.2.2. DPPH radical scavenging activity

For Determination of DPPH radical scavenging activity, 25 μM solution of DPPH in absolute ethanol was prepared. This solution was added to an equal volume of the solution of the test compounds (dissolved in ethanol) to obtain a desired concentration. Ethanol was used as control solution in the experiments. Being 30 min at room temperature, the absorbance was recorded at 517 nm and compared to NDGA (nordihydroguaiaretic acid).

4.3. Structure activity similarity (SAS) study

In order to obtain an SAS pattern for this series of compounds, the structures of the ligands were saved as SMILES and entered into our application implemented in .NET together with their corresponding activity vector. Tanimoto similarity index for each pair of compounds was calculated based on FP_2 fingerprint of the two compounds using Open Babel.[37] In the SAS maps, pair wise Tanimoto similarity coefficients on horizontal axis represent the structural similarities between the two ligands, meanwhile experimental activity similarity (vertical axis) for each ligand pair (i,j) is calculated as follows:

$$\mathrm{Sim}_{\mathrm{Activity}}(i,j) = 1 - [(|\mathrm{Activity}_i - \mathrm{Activity}_j|)/(\mathrm{Activity}_{\max} - \mathrm{Activity}_{\min})]$$

The two metrics $\mathrm{Activity}_{\max}$ and $\mathrm{Activity}_{\min}$ denounce the IC_{50}s of the most and the least potent compounds, respectively. A new color coding system was implemented in our application to show all possible activity alterations in each pair of ligands. In this study for color coding system, the two cutoff values 34.2 and 64 were used to show the range of activity for moderate structures. The value 34.2 was selected based on the activity of the positive control compound (IC_{50} = 34.2).

4.4. Molecular modeling studies

Docking simulation studies were performed using Autodock Vina.[38] For this purpose, two active structures (**1** and **5**) were converted to pdbqt using open babel 2.3.1. The PDB of the soybean lipoxygenase was also retrieved from protein data bank (1IK3) and converted to into pdbqt via autodock tools. The exhaustiveness parameter was set to 100 and the box center was set to the dimensions of iron atom in the protein file (x = 23.944, y = 2.975, z = 15.444). The box size in all directions was set to 50 Å, num of modes was set to 9 and energy range was set to 3 in autodock vina conf.txt.

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