ACS Medicinal Chemistry Letters

Letter

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ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.9b00193 • Publication Date (Web): 09 Sep 2019

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Chalcone-Thiazole Hybrids: Rational Design, Synthesis and Lead Identification against 5-Lipoxygenase

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KEYWORDS: Chalcone, thiazole, 5-LOX, pharmacophore, design, hybrid, synthesis, pseudoperoxidase

ABSTRACT: A hybrid pharmacophore approach is used to design and synthesize novel chalcone-thiazole hybrid molecules. Herein, thiazole has been hybridized with chalcone to obtain a new class of 5-LOX inhibitors. *In vitro* biological evaluation showed that most of the compounds were better 5-LOX inhibitor than the positive control, Zileuton (IC_{50} = 1.05 ± 0.03 μM). The best compounds in the series namely, **4k, 4n** and **4v** (**4k**: IC_{50} = 0.07 ± 0.02 μM, **4n**: IC_{50} = 0.08 ± 0.05 μM, **4v**: 0.12 ± 0.04 μM) are found to be 10 times more active than previously reported 2-amino thiazole (**2m**: IC_{50} = 0.9 ± 0.1 μM) by us. Further, **4k** has redox (non-competitive) while **4n** and **4v** act through competitive inhibition mechanism. SAR indicated that the presence of methoxy/methyl either in the vicinity of chalcone or both thiazole and chalcone contributed to the synergistic inhibitory effect.

5-Lipoxygenase (5-LOX) is an important enzyme which catalyzes arachidonic acid (AA) for the production of several leukotrienes (LTs) which are well-known mediators of inflammation related disorders such as rheumatoid arthritis, allergic rhinitis, atherosclerosis and cardiovascular diseases among which asthma is the major pathophysiological implication associated with it.[1,2] 5-LOX oxygenates essential polyunsaturated fatty acid (AA) to 5(S)-hydroperoxy-6-trans-8,11,14-cis-eicosatetraenoic acid (5-HpETE) and further dehydrates it to the unstable epoxide leukotriene A4 (LTA4). So far, Zileuton is the only marketed 5-LOX inhibitor against asthma.[2] But it has side-effects of hepatotoxicity and poor pharmacokinetics. Its hepatotoxicity is associated with its mechanism involving redox process in which N-hydroxyurea is involved in electron transfer through free radical-mediated lipid peroxidation of cell membranes and thiophene forms chemically reactive metabolites resulting in liver serum enzyme elevation and toxicity.[2] So, there is a need to develop inhibitors against 5-LOX.

Thiazoles are active constituents of various naturally occurring biologically significant compounds such as thiamine, mycothiazole as well as synthetic drugs which act as antimicrobial, anti-inflammatory, anticancer, antiviral, and antitubercular.[3,4]. Thiazol-4(5H)-one derivative, namely darbufelone and CI-987 are reported as dual COX/LOX inhibitors.[5] Recently, thiazole bearing scaffolds, namely 2-amino-4-aryl thiazole-5-phenylmethanones[6,7], N-aryl thiazole-2-amines[8] and 5-benzylidene-2-phenylthiazolinones[9] are identified as selective 5-LOX whereas aminothiazole pirinixic acids[10] as dual 5‑LOX/mPGES1 inhibitors.

Chalcone (α,β-unsaturated ketone, collectively defined as an aromatic ketone and enone) based analogues are found in many natural compounds including turmeric in the form of curcumin, liquorice as isoliquiritigenin and synthetic drugs such as homobutein.[11] Chalcones are known to exhibit biological properties, such as antibacterial, anti-inflammatory, antioxidant, anticancer, antimalarial and as carbonic anhydrase inhibitor.[11,12] Diarylsulfonylurea-chalcones[12], and di-*o*-prenylated chalcones[13] have been reported as 5-LOX whereas chalcone-triazole hybrids[14] as 15-LOX inhibitors. Phenylsulphonyl urenyl chalcones[15] and 3,4-dihydroxychalcones[16] act as dual 5-LOX/COX inhibitors.

Combining two bioactive pharmacophores to design a hybrid molecule has considerably attracted interest of medicinal chemists. This molecular hybridization is a useful tool to generate lead molecules with synergetic and superior biological activities.[17] Considering the anti-inflammatory potencies of thiazole and chalcone, it is envisaged that integrating both these moieties in one molecular platform could possibly produce compounds with synergistic properties. Therefore, in the present study, as an extension of our earlier work in the field of development of LT inhibitors, an attempt has been made to design and synthesize hitherto novel chalcone-thiazole structural hybrids along with elaboration of their mechanism to understand their redox and non-redox behavior to overcome pharmacokinetic problems as potential 5-LOX inhibitors.

Rational Design and Pharmacophore Elucidation. The molecular hybridization approach on the concept of combining two bioactive pharmacophores to achieve superior biological activities forms the basis of the designed prototype (**A**) in this study (Figure 1). Optimization for hybrid molecules (**A**) containing both chalcone and thiazole scaffolds have been pursued via pharmacophore elucidation towards 5-LOX inhibition. Pharmacophore is a group of essential features namely, H-bond acceptor, donor, hydrophobic and aromatic center for a particular biological activity required for enzyme-ligand interaction.[18] To elucidate structure activity relationship (SAR) of the designed structural hybrid (**A**), a pharmacophore model (Ph model) was generated from the set of known and reported inhibitors of 5-LOX (Figure 2). It included a known drug: Zileuton, clinical trials inhibitors: Atreleuton, Siteleuton[19] and reported inhibitors: di-o-prenylated and diarylsulfonylurea chalcones.[12,13]

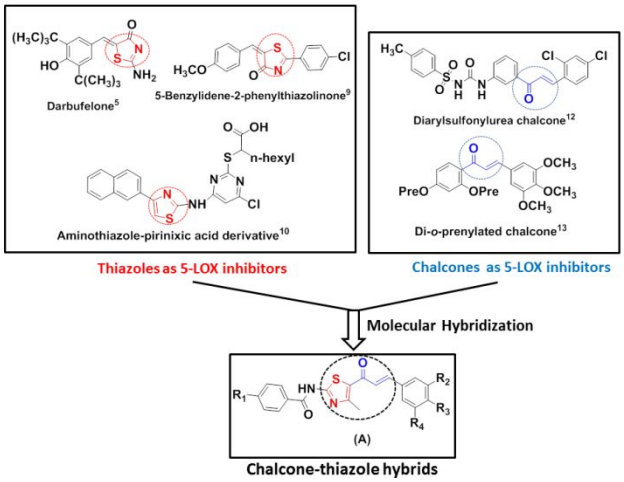


Figure 1: Design of chalcone-thiazole hybrids via molecular hybridization.

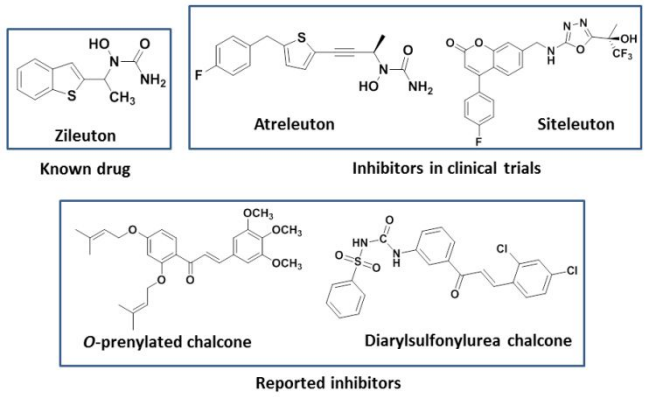


Figure 2: Structures of reported 5-LOX inhibitors.

A Ph model obtained gave a four-point pharmacophoric feature consisting of three hydrophobic centroids and one H-bond acceptors (Figure 3a, b). All the in-house designed compounds are used as query molecules and subjected to conformational search to create a library of database. A pharmacophore search is performed for the in-house database against Ph model to examine mapping of the designed molecules to get the hit molecules. Surprisingly, all the 23 compounds designed in the series hit the query and found to map exactly with the four-point common features of Ph model such as **4k** and **4n** (Figure 3c, d). It can be noticed that the double bond of chalcone is one of the important reasons for the generation of hydrophobic centroids, contributing synergistic anti-inflammatory effect. Thus, pharmacophore elucidation gave a preliminary indication that this designed hybrid (**A**) could be a good lead against 5-LOX and hence these were synthesized and biologically tested.

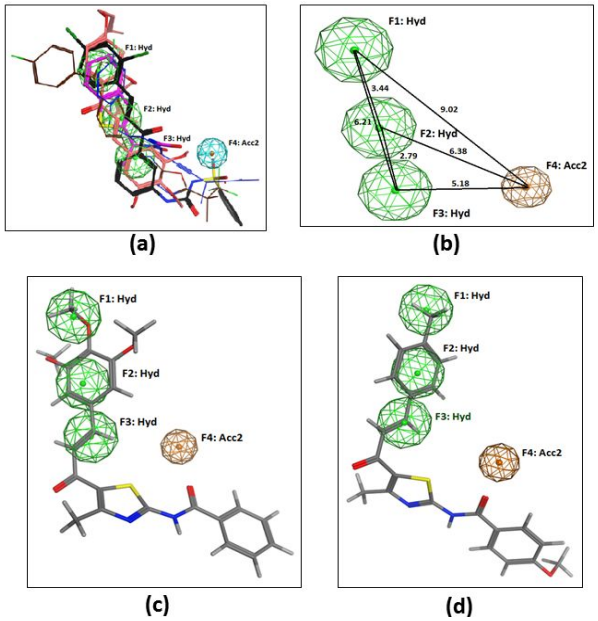


Figure 3: (a) Pharmacophoric model (Ph model) generated from known 5-LOX inhibitors in software MOE 2016.0801; (b) Ph model with geometric distances (A^0) in 3D spatial relationship; Pharmacophore mapping of (c) **4k** and (d) **4n** with Ph model. Pharmacophoric features: Hydrophobic (Hyd - green), H-bond acceptor (Acc2 - orange) and (blue in (Figure 3a)). Inhibitors superimposed in (Figure 3a) are: Zileuton- magenta, Atreleuton- navy blue, Siteleuton- brown, di-o-prenylated- pink and diarylsulfonylurea chalcone- black.

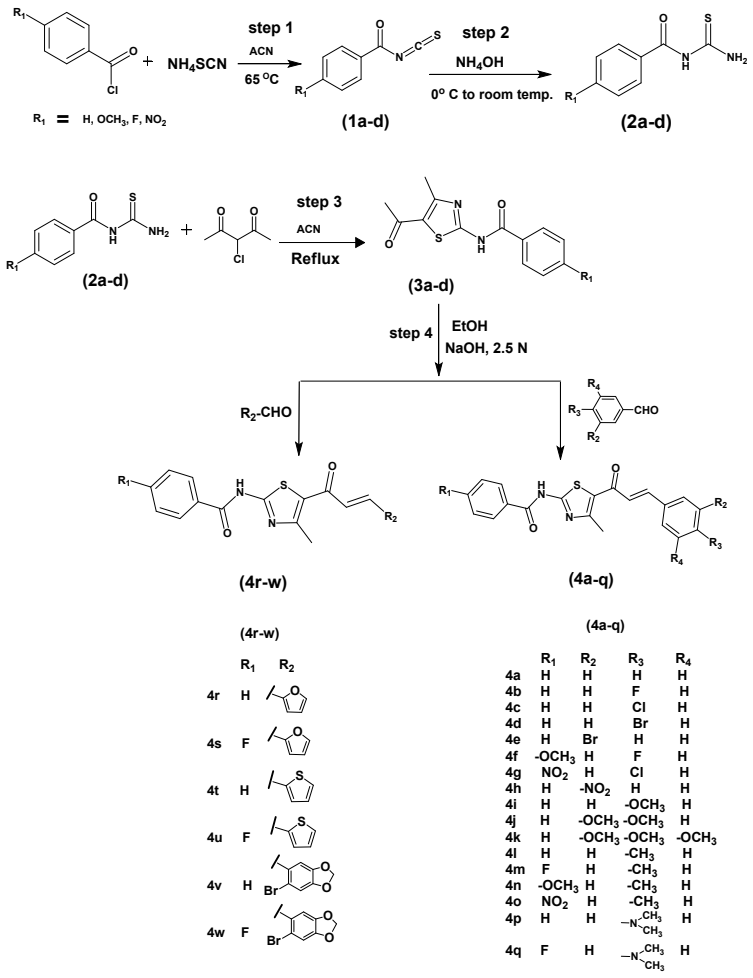
The synthetic pathways for the novel chalcone-thiazoles are illustrated in Scheme 1. The key intermediates (**2a-d**) and (**3a-d**) are prepared as per previously reported procedures with some modifications.[20,21] Briefly, benzoyl isothiocyanates (**1a-d**) in the 1st step are synthesized by treating substituted benzoyl chloride with ammonium thiocyanate-acetonitrile. The product (**1**) in acetonitrile (ACN) layer in the 2nd step is treated with required amount of ammonium hydroxide at 0 °C to yield various N-carbamothioyl substituted benzamides (**2a-d**). In the 3rd step, 3-chloropentane-2,4-dione is added to the 2nd step intermediate, N-carbamothioyl substituted benzamide (**2**), followed by reflux to afford the desired intermediates, N-(5-acetyl-4-methylthiazol-2-yl) substituted benzamides (**3a-d**) via Hantzsch thiazole synthesis. The final step for the synthesis of the target chalcone-thiazole hybrids (**4a-w**), is achieved using base catalyzed Claisen–Schmidt condensation reaction between benzamide thiazoyl ketones (**3a-d**) and various substituted aromatic/heteroaromatic aldehydes in ethanol, and 2.5 N NaOH.[14] All the structures were confirmed using ^{1}H NMR, ^{13}C NMR and HRMS (Supporting Information).

All the compounds (**4a-w**) at a concentration of 10 μM are screened for 5-LOX inhibition activity *in vitro* with Zileuton as a reference drug in cell free system using human recombinant 5-LOX enzyme (Table 1,2). The inhibition is measured by determining the conversion of substrate AA to product, 5-HPETE at λ_{236}.[22] 2% DMSO was taken as negative control which did not show any inhibition while standard drug, Zileuton showed an IC_{50} of 1.05 ± 0.03 μM as expected according to the literature[37] (Table 3). Most of the compounds exhibited good inhibition. The best active compounds were tested further at different concentrations (below 10 μM) against 5-LOX keeping substrate concentration constant to find out their IC_{50} (using GraphPad Prism, 5.01). It is heartening to note that many compounds exhibited better potency than the reference drug, with IC_{50}s ranging from 0.05 to 0.5 μM (**4k, 4n** and **4v,** being the most potent in the series) (Table 3).

In Vitro 5-LOX Inhibition and SAR. To explore the influence of hydrophobic centroids of chalcones with H-bond acceptor property of the substituted thiazoles on 5-LOX inhibition, a series of hybrids (**A**), (**4a-w**) have been synthesized. Substitutions on both thiazole (at R_1) and chalcone (at R_2, R_3, R_4) pharmacophores of prototype (**A**) are done to analyse their effect on the inhibition and develop a tentative SAR profile.

The basic chalcone-thiazole core, **4a** having no substitutions exhibited moderate inhibition, 73.5 ± 2.8 % at 10 μM. It is interesting to note that the introduction of highly electronegative electron withdrawing halogen, fluoro at *para* position (R_3) in phenyl ring attached to chalcone, **4b** enhanced the inhibition to 98.0 ± .7 % (Table 1) with IC_{50} of 0.14 ± 0.06 μM (Table 3). The activities for other *para* halogen substituted compounds, **4c** (63.7 ± 2.8 %) and **4d** (52.3 ± 2.1 %) are found in the decreasing order of electronegativity of halogens indicative **4b** > **4c** > **4d** (*p*-F > *p*-Cl > *p*-Br). Again a slight decrease in the potency is observed when *p*-Br is replaced with *m*-Br, **4e** (42.5 ± 3.6 %) as compared to **4d**. Insertion of electron donating substituent, methoxy at R_1 in the phenyl ring of benzamido group attached to thiazole, **4f** (IC_{50} = 0.56 ± 0.15 μM, 88.2 ± 7.8 %) resulted in a fold decrease in the efficiency with respect to **4b** (IC_{50} = 0.14 ± 0.06 μM). However, even addition of electron withdrawing group, $-NO_2$ at R_1, **4g** (48.8 ± 0.7 %) could not improve the potency, instead reduced it by 1.3 fold, as compared to **4c**. Surprisingly, replacing bromo by another electron withdrawing group, $-NO_2$, at the *meta* position, **4h** (IC_{50} = 0.24 ± 0.07 μM, 98.0 ± 2.1 %) increased the inhibitory activity to more than double when compared to **4e** (42.5 ± 3.6 %). The inhibition activity seems to increase with increasing number of electron donating methoxy substituent (R_2, R_3, R_4) in the phenyl ring adjacent to the vicinity of chalcone, **4i** < **4j** < **4k** which might be due to increase in electron density on the substituted phenyl**.** Trimethoxy substituted compound, **4k**, is a highly active inhibitor (IC_{50} of 0.07 ± 0.02 μM). Existence of electron donating substituents, $-OCH_3$ (R_1) and $-CH_3$ (R_3) in the vicinity of both thiazole and chalcone phenyl rings respectively is well tolerated in **4n** which contributed to a synergistic effect exhibiting an IC_{50} of 0.08 ± 0.05 μM. A marginal decrease in potency is observed with the replacement of $-OCH_3$ by an electron withdrawing fluoro (R_1), **4m** (0.54 ± 0.10 μM).

It was encouraging to substitute targeted hybrid (**A**) with hetero-aromatic groups in the vicinity of chalcone. Methylenedioxy group is known to be widely found in natural products and drugs. Addition of methylenedioxy bromophenyl group at R_2 yielded **4v** (Table 2) which resulted in significantly promising inhibitory activity (IC_{50} of 0.12 ± 0.04 μM). But replacement of -H at R_1 with fluoro, **4w** (50.6 ± 1.4 %) is not tolerated and potency reduced to half. Other heteroaromatic groups such as furan, thiophene substitutions at R_2 resulted in moderate to good inhibition (60 to 80 %).

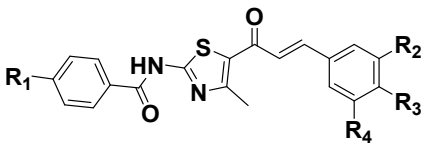


Scheme 1: Synthesis of chalcone-thiazole hybrid (**4a-w**) derivatives.

5-LOX Kinetic studies. The mode of action of the best active compounds (**4k**, **4n** and **4v**) is estimated by measuring the initial rate of formation of product for different substrate (AA) concentrations with three concentrations of these inhibitors (0, 2 and 5 μM).[23] Lineweaver-Burke (L-B) plot indicates that the most active analogue, **4k**, acts as a non-competitive inhibitor against the enzyme (Figure 4a) with constant K_m (1.9 ± 0.4, 1.8 ± 0.4, 2.1 ± 0.4 μM) and decreasing V_{max} (0.38 ± 0.02, 0.29 ± 0.02, 0.25 ± 0.01 nmol/min) with increase in inhibitor concentration (0, 2 and 5 μM respectively). So, it can be concluded that, **4k** can bind either enzyme or enzyme-substrate complex with equal affinity. L-B plots for the other two compounds, **4n** and **4v,** showed competitive inhibition (Figure 4b, c) namely at these three different concentration, the lines intersect the y-axis indicating constant V_{max} (**4n**: 0.33 ± 0.01, 0.32 ± 0.01, 0.31 ± 0.01; **4v**: 0.45 ± 0.01, 0.43 ± 0.02,

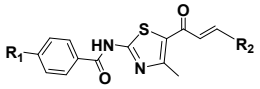
0.42 ± 0.02 nmol/min) while K_m increases for both compounds (**4n**: 0.35 ± 0.10, 0.43 ± 0.10, 0.53 ± 0.13; **4v**: 0.31 ± 0.08, 1.41 ± 0.33, 2.91 ± 0.55 μM with increasing concentrations 0, 2 and 5 μM respectively). It suggested that both these inhibitors probably bind to the enzyme's active site and thus hinder the substrate binding site.

Table 1. 5-LOX inhibition and DPPH radical scavenging activities of N-(5-(3-(substituted phenyl)acryloyl)-4-methylthiazol-2-yl) benzamide derivatives (**4a-q**) at 10 μM. Data is expressed as means ± SD from three independent experiments.



Compd code	R_1	R_2	R_3	R_4	% 5-LOX inhibition ± SD	% DPPH radical scavenging ± SD
4a	H	H	H	H	73.5 ± 2.8	2.5 ± 1.5
4b	H	H	F	H	98.0 ± .7	3.0 ± 1.7
4c	H	H	Cl	H	63.7 ± 2.8	8.8 ± 1.2
4d	H	H	Br	H	52.3 ± 2.1	2.4 ± 1.2
4e	H	Br	H	H	42.5 ± 3.6	5.7 ± 1.1
4f	$-OCH_3$	H	F	H	88.2 ± 7.8	2.1 ± 1.6
4g	NO_2	H	Cl	H	48.8 ± 0.7	2.4 ± 1.9
4h	H	$-NO_2$	H	H	98.0 ± 2.1	8.7 ± 3.5
4i	H	H	$-OCH_3$	H	58.8 ± 2.1	7.9 ± 1.0
4j	H	$-OCH_3$	$-OCH_3$	H	70.2 ± 8.5	6.4 ± 1.8
4k	H	$-OCH_3$	$-OCH_3$	$-OCH_3$	~ 100 ± 1.4	5.7 ± 2.9
4l	H	H	$-CH_3$	H	75.1 ± 2.1	3.0 ± 0.2
4m	F	H	$-CH_3$	H	91.5 ± .7	15.0 ± 2.6
4n	$-OCH_3$	H	$-CH_3$	H	~ 100 ± 2.8	5.5 ± 0.2
4p	H	H	$-N(CH_3)_2$	H	88.2 ± 3.6	~ 0 ± 0.9
4q	F	H	$-N(CH_3)_2$	H	70.2 ± 2.8	3.9 ± 0.8
Zileuton	-	-	-	-	86.6 ± 2.8	10.3 ± 0.5
Ascorbic acid	-	-	-	-	-	60.5 ± 2.3

Table 2. 5-LOX inhibition and DPPH radical scavenging activities of N-(5-(3-substituted acryloyl)-4-methylthiazol-2-yl) benzamide derivatives (**4r-w**) at 10 µM. Data is expressed as means ± SD from three independent experiments.



Compd code	R_1	R_2	% 5-LOX Inhibition ± SD	% DPPH radical scavenging ± SD
4r	H		57.2 ± 5.7	3.4 ± 1.6
4s	F		65.3 ± 3.6	8.2 ± 0.8
4t	H		71.9 ± 6.4	2.2 ± 0.7
4u	F		81.7 ± 0.7	~ 0 ± 1.8
4v	H		~ 100 ± 2.8	5.4 ± 2.3
4w	F		50.6 ± 1.4	5.1 ± 0.8
Zileuton	-	-	86.6 ± 2.8	10.3 ± 0.5
Ascorbic acid	-	-	-	60.5 ± 2.3

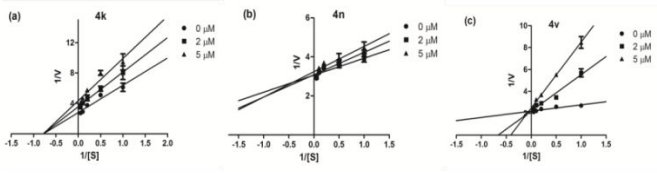


Figure 4: Lineweaver-Burk plots for inhibitors at 0, 2 and 5 µM concentrations (a) **4k** (b) **4n** (c) **4v**. [S] = concentration of the substrate (AA, in µM), V = reaction rate.

5-LOX Pseudoperoxidase Activity and Mechanism. Known mechanisms for 5-LOX inhibition are redox, non-redox and iron chelating.[10] The inhibitors acting via redox mechanism disrupt the redox cycle by affecting the iron's ionic state from Fe^{3+} to Fe^{2+}. Deactivated ferrous ion has a tendency to consume lipid peroxide for activating the redox cycle of 5-LOX. Here, the substrate, 13(S)-hydroperoxyoctadecadienoic acid (13(S) HpODE) in the pseudoperoxidase assay is consumed in the presence of a redox inhibitor. The decrease in absorbance (at 234 nm) with respect to time is the measure of consumption of the substrate.[7] Pseudoperoxidase activities of **4k, 4n, 4v, 4b, 4h, 4m, 4f** and **4p** are performed and reported here (Figure 5). The most active inhibitor, **4k**, decreases 56.6 % of absorbance in 300 s (Table 3, Figure 5b) depicting significant consumption of the hydroperoxide, exhibiting redox nature. Zileuton is a well known reported redox inhibitor. When analysed experimentally it showed 63.4 % hydroperoxide consumption (Figure 5a). **4h** and **4m** also show significant decrease in the peroxide by 40.2 and 56.3 % respectively suggesting they also interrupt the redox cycle of the enzyme. While compounds **4n, 4v, 4b, 4f** and **4p** do not show significant fall (Table 3) in the absorbance and the lines are almost paralell to the x-axis suggesting that they act by non-redox process (Figure 5c, d, e, h, i). Therefore, these compounds probably interact in the active site. These pseudoperoxidase activity results are supported by L-B plots also.

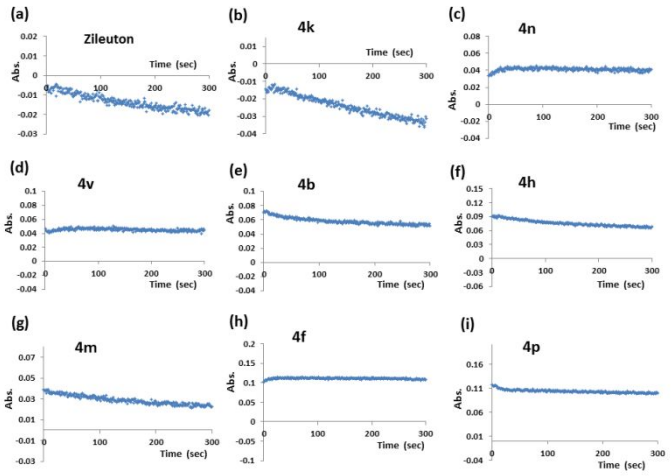
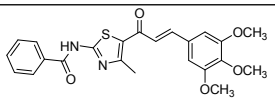
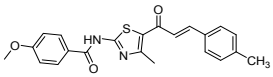
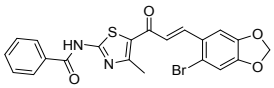
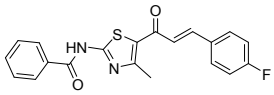
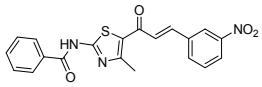
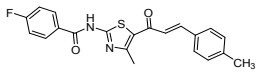
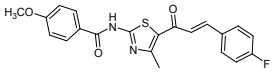
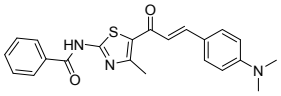
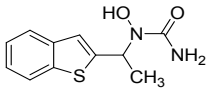


Figure 5: Pseudoperoxidase activity plots for the consumption of 13(S)-HpODE (at 234 nm; control substracted) by (a) Zileuton, (b) **4k** (c) **4n**, (d) **4v**, (e) **4b**, (f) **4h,** (g) **4m,** (h) **4f**, (i) **4p** as a function of time (in sec).

DPPH Radical Scavenging Activity. 5-LOX contains Fe^{3+} in the activated state and most of the 5-LOX inhibitors are found to be good scavenging radicals. Therefore, scavenging property of all the derivatives is determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) methodology as per previously reported procedure.[24] Positive control (ascorbic acid) showed 60.5 ± 2.3 % antioxidant activity (Table 1). Results showed that almost all compounds are poor radical scavengers, showing below ~10 % activity and Zileuton exhibited 10.3 ± 0.5 % at 10 µM. Whereas **4m** posseses about 15 % of antioxidant activity for which pseudoperoxidase assay also suggested that it acts by disrupting the redox cycle of iron. The other best inhibitors namely **4n** and **4v,** showed antioxidant activities in the range of ~5 %. In support, pseudoperoxidase assay also indicated that both compounds act through non-redox mechanism.

Docking Studies. The binding interactions of competitive inhibitors, **4n** and **4b**, were evaluated by docking them into the active site of 5-LOX. The Molecular Operating Environment (MOE 2017.0801) software was used and 5-LOX protein (pdb ID 3O8Y) was obtained from Protein Data Bank (PDB). **4n** showed a binding score of -9.09 kcal/mol along with H-bond and *pi*-H interactions (Figure 6a,b). The carbon in the double bond of chalcone interacted with the polar amino acids, Gln363, forming a H-bond donor with a distance of 2.62 Å. Thiazole ring forms a *pi*-H interaction with the Leu414 (4.7 Å) in the hydrophobic pocket of the enzyme. **4v** with a binding score of -8.03 kcal/mol showed side chain H-bond interaction with the polar Gln557 (3.25 Å) and bromo group of benzo ethylenedioxide substituent. Other two *pi*-H interactions are seen with the polar residues of thiazole (Leu414) and benzene rings of the benzoamide group attached to thiazole (Tyr181) (Figure 6c,d).

Table 3. 5-LOX inhibition IC_{50}, Lipinski parameters, pseudoperoxidase activity and type of mechanism for the best compounds of the series.

Compd code	Structure	5-LOX inhibition, IC_{50} (μM)[a]	Lipophilicity *C* log *P*[b]	Total polar surface area (TPSA)[c] Å^2	Pseudoperoxidase[d] activity in terms of % change in absorbance (at 10 μM of 13(S)-HpODE and inhibitor)	Type of mechanism
4k		0.07 ± 0.02	3.56	114.99	- 56.6	Redox (Non competitive)
4n		0.08 ± 0.05	4.84	96.53	+ 5.6	Non-redox (Competitive)
4v		0.12 ± 0.04	5.16	105.76	+ 1.2	Non-redox (Competitive)
4b		0.14 ± 0.06	4.40	87.30	- 12.5	Non-redox
4h		0.24 ± 0.07	4.00	133.12	- 40.2	Redox
4m		0.54 ± 0.10	4.91	87.30	- 56.3	Redox
4f		0.56 ± 0.15	4.49	96.53	+ 0.3	Non-redox
4p		0.64 ± 0.08	4.42	90.54	- 12.2	Non-redox
Zileuton		1.05 ± 0.03	2.48	94.80	- 63.4	Redox

[a]determined experimentally
[d]determined experimentally. Negative values indicate % decrease in absorbance due to consumption of 13(S)-HpODE and positive values indicate % increase in absorbance.
[b]calculated using ChemBioDraw Ultra 11.0
[c]calculated using Swiss ADME software

The active inhibitors **4k, 4n** and **4v** have higher total polar surface area (TPSA) (Table 3) than Zileuton indicating chances of lesser adverse effects (www.asteris-app.com/technical-info/core-properties/logp.htm). The Swiss ADME prediction indicated that these inhibitors satisfy Lipinski rule of five as well as Ghose, Veber, Egan, Muegge rules signifying that they have good druglikeness properties along with *C* log *P* value more than that of Zileuton indicating their higher lipophilic nature.[25]

In conclusion, a series of novel chalcone-thiazole molecules were rationally designed and synthesized using pharmacophore hybridization approach and evaluated against enzyme 5-LOX. All the 23 compounds synthesized are completely new molecules, verified by Scifinder. In analogy to our previous study[7], it was possible to incorporate chalcone group along with thiazole for 5-LOX inhibition with a synergistic effect. As a result, molecules (**4k**: IC_{50} = 0.07 ± 0.02 μM, **4n**: IC_{50} = 0.08 ± 0.05 μM) synthesized here are found to be 10-100 times better potent than previously reported thiazole molecules (**2m**: IC_{50} = 0.9 ± 0.1 μM, **1d**: IC_{50} = 10 μM) by us and also better than Zileuton (IC_{50} = 1.05 ± 0.03 μM). [6,7] SAR indicated that the presence of more number of electron donating groups namely, methoxy and methyl either in the vicinity of chalcone or both thiazole and chalcone contributed synergistic effect and yielded highly active molecules. Pharmacophore elucidation revealed that the double bond of chalcone is one of the important reasons for hydrophobic centroid generation for inhibition. Further, pseudoperoxidase assay and kinetic studies revealed the mechanism of **4k** has a redox type (non-competitive) while **4n** and **4v** act through competitive inhibition. Docking studies of **4n** and **4v** gave molecular binding interactions with the active site amino acids. Therefore, the results has laid a foundation

for the discovery of novel hybrids which will help further to develop a new class of next generation 5-LOX inhibitors targeting inflammation.

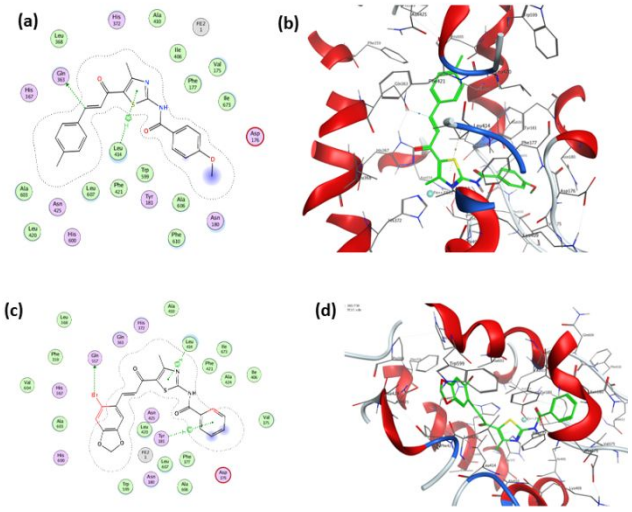


Figure 6: Binding modes of compounds with amino acid residues of 5-LOX (pdb ID 3O8Y). (a) Ligplot interaction of **4n** (b) Docked pose of **4n** (c) Ligplot interaction of **4v** (d) Docked pose of **4v**. Dotted lines indicate interactions. Colors depicted are: hydrogen: cyan, carbon: grey, nitrogen: blue, oxygen: red, sulphur: yellow.

ASSOCIATED CONTENT

Supporting Information

Synthetic procedure, characterization, experimental procedures for biological evaluation (*in vitro* assays), docking methods; spectral details including ^{1}H, ^{13}C NMR, HRMS spectra, pharmacokinetic parameters from Swiss ADME (*in silico* studies).

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Author Contributions

SS performed the experiments and written the manuscript. MD and SLM designed the experiments and corrected the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

Shweta Sinha acknowledges DST, New Delhi, WOS-A (SR/WOS-A/CS-126/2013), GoI for funding and fellowship for the research. The authors thank SAIF, IIT-Madras for NMR and IR spectral analysis and Department of Biotechnology, IIT-Madras for HRMS analysis. The authors thank VIT, Vellore for providing 'VIT Seed Grant' for the research.

ABBREVIATIONS

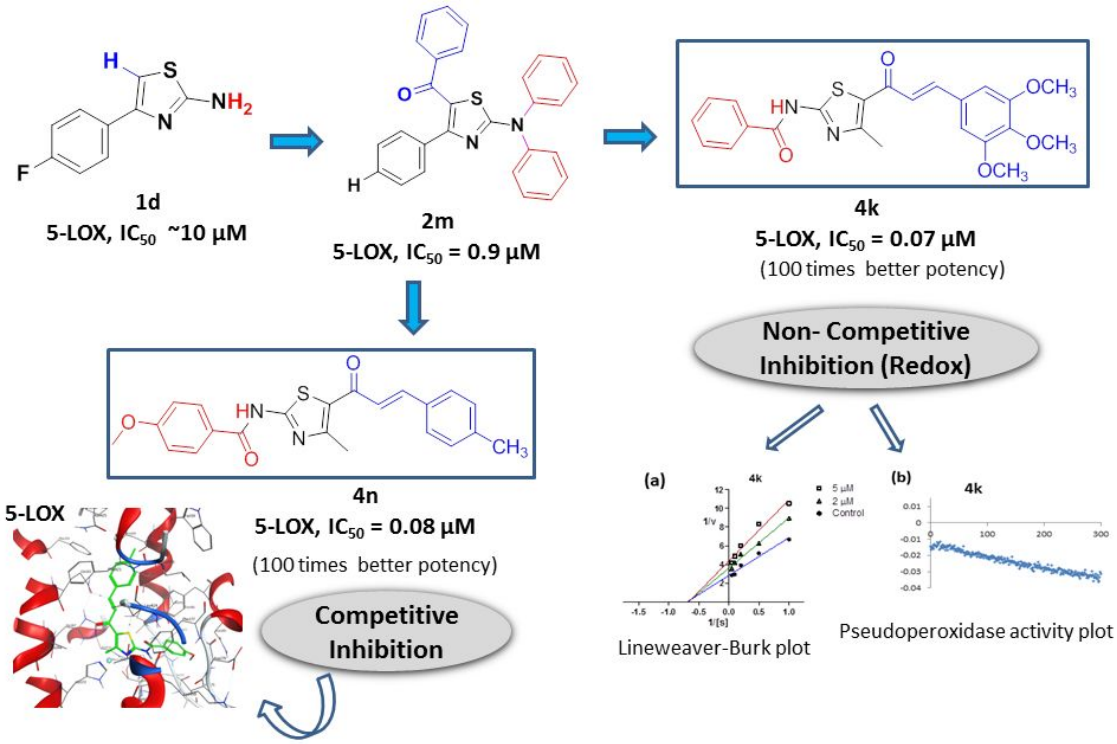
AA, Arachidonic acid; LT, Leukotriene; 5-LOX, 5-Lipoxygenase; LTA4, Leukotriene A4; DPPH, 2,2 -diphenyl-1-picrylhydrazyl; 5-HPETE, 5(S) hydroperoxyeicosatetraenoic acid; 13(S) HpODE, 13(S)-hydroperoxyoctadecadienoic acid; LB, Lineweaver–Burk; PMSF, Phenylmethyl sulphonyl fluoride; Ph, Pharmacophore; TPSA, Total polar surface area.

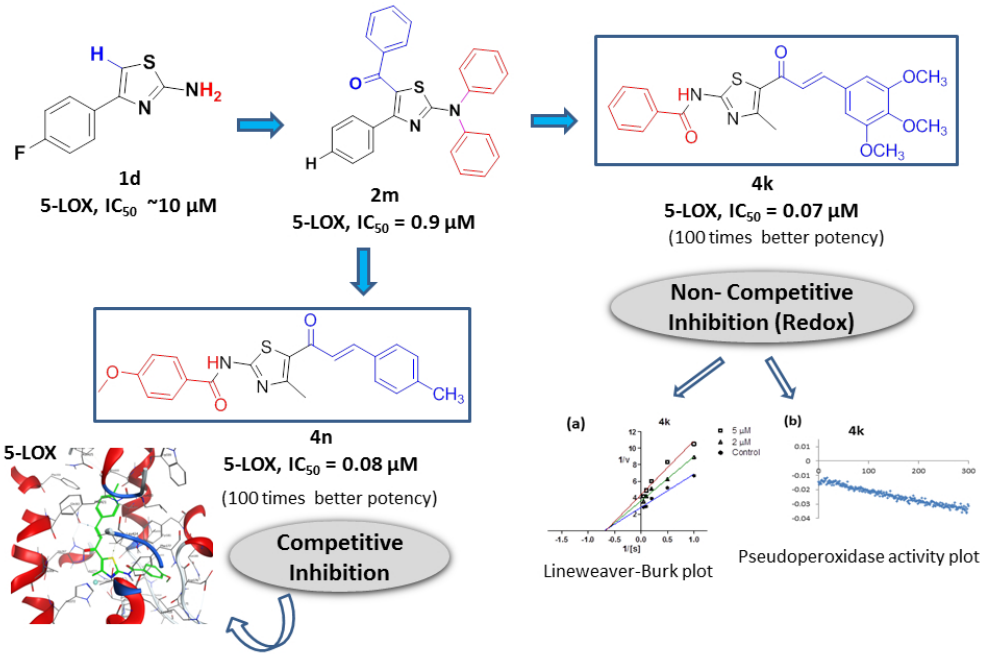
REFERENCES

1. Haeggström, J. Z.; Rinaldo-Matthis, A.; Wheelock, C. E.; Wetterholm, A. Advances in eicosanoid research, novel therapeutic implications. *Biochemical and biophysical research communications* **2010,** 396, 135-139.
2. Werz, O.; Steinhilber, D. Therapeutic options for 5-lipoxygenase inhibitors. *Pharmacology & therapeutics* **2006**, 112,701-718.
3. De Souza, M. V. N. Synthesis and biological activity of natural thiazoles: An important class of heterocyclic compounds. *Journal of Sulfur Chemistry* **2005,** 26, 429-449.
4. Chhabria, M.T.; Patel, S.; Modi, P.; Brahmkshatriya P.S. Thiazole: A Review on Chemistry, Synthesis and Therapeutic Importance of its Derivatives. *Current Topics in Medicinal Chem*istry **2016,** 16 (26), 2841-2862.
5. Liaras, K.; Fesatidou, M.; Geronikaki, A. Thiazoles and Thiazolidinones as COX/LOX Inhibitors. *Molecules* **2018**, 23, 685-706.
6. Sinha, S.; Sravanthi, T.; Yuvaraj, S.; Manju, S.; Doble, M. 2-Amino-4-aryl thiazole: a promising scaffold identified as a potent 5-LOX inhibitor. *RSC Advances* **2016,** 6, 19271-19279.
7. Sinha, S.; Doble, M.; Manju, S. Design, synthesis and identification of novel substituted 2-amino thiazole analogues as potential anti-inflammatory agents targeting 5-lipoxygenase. *European journal of medicinal chemistry* **2018,** 158, 34-50.
8. Suh, J.; Yum, E. K.; Cheon, H. G.; Cho, Y. S. Synthesis and Biological Evaluation of N-aryl-4-aryl-1, 3-Thiazole-2-Amine Derivatives as Direct 5-Lipoxygenase Inhibitors. *Chemical biology & drug design* **2012,** 80, 89-98.
9. Hofmann, B.; Barzen, S.; Rödl, C. B.; Kiehl, A.; Borig, J.; Zivkovic, A.; Stark, H.; Schneider, G.; Steinhilber, D. A class of 5-benzylidene-2-phenylthiazolinones with high potency as direct 5-lipoxygenase inhibitors. *Journal of medicinal chemistry* **2011,** 54, 1943-1947.
10. Hanke, T.; Dehm, F.; Liening, S.; Popella, S.-D.; Maczewsky, J.; Pillong, M.; Kunze, J.; Weinigel, C.; Barz, D.; Kaiser, A. Aminothiazole-featured pirinixic acid derivatives as dual 5-lipoxygenase and microsomal prostaglandin E2 synthase-1 inhibitors with improved potency and efficiency in vivo. *Journal of medicinal chemistry* **2013,** 56, 9031-9044.
11. Nowakowska, Z. A review of anti-infective and anti-inflammatory chalcones. *European journal of medicinal chemistry* **2007,** 42, 125-137.
12. Bugata, B. K.; Dowluru, S. V. G. K. K.; Avupati, V. R.; Gavalapu, V. R.; Nori, D. L. S.; Barla, S. Synthesis, characterization and in vitro biological evaluation of some new diarylsulfonylurea-chalcone hybrids as potential 5-lipoxygenase inhibitors. *European Journal of Chemistry* **2013,** 4, 396-401.
13. Reddy, N. P.; Aparoy, P.; Reddy, T. C. M.; Achari, C.; Sridhar, P. R.; Reddanna, P. Design, synthesis, and biological evaluation of prenylated chalcones as 5-LOX inhibitors. *Bioorganic & medicinal chemistry* **2010,** 18, 5807-5815.

14. Asadipour, A.; Noushini, S.; Moghimi, S.; Mahdavi, M.; Nadri, H.; Moradi, A.; Shabani, S.; Firoozpour, L.; Foroumadi, A. Synthesis and biological evaluation of chalcone-triazole hybrid derivatives as 15-LOX inhibitors. *Zeitschrift für Naturforschung B* **2018,** 73, 77-83.
15. Araico, A.; Terencio, M.; Alcaraz, M.; Dominguez, J.; Leon, C.; Ferrandiz, M. Phenylsulphonyl urenyl chalcone derivatives as dual inhibitors of cyclo-oxygenase-2 and 5-lipoxygenase. *Life sciences* **2006,** 78, 2911-2918.
16. Sharma, M. C. Molecular modeling studies of substituted 3, 4-dihydroxychalcone derivatives as 5-lipoxygenase and cyclooxygenase inhibitors. *Medicinal Chemistry Research* **2014,** 23, 1797-1818.
17. Viegas-Junior, C.; Danuello, A.; da Silva Bolzani, V.; Barreiro, E. J.; Fraga, C. A. M. Molecular hybridization: a useful tool in the design of new drug prototypes. *Current medicinal chemistry* **2007,** 14, 1829-1852.
18. Wu, Y.; He, C.; Gao, Y.; He, S.; Liu, Y.; Lai, L. Dynamic modeling of human 5-lipoxygenase–inhibitor interactions helps to discover novel inhibitors. *Journal of medicinal chemistry* **2012,** 55, 2597-2605.
19. Sinha, S.; Doble, M.; Manju, S .L. 5-Lipoxygenase as a drug target: A review on trends in inhibitors structural design, SAR and mechanism based approach. *Bioorganic & Medicinal Chemistry* **2019,** 27, 3745–3759.
20. Liaras, K.; Geronikaki, A.; Glamočlija, J.; Ćirić, A.; Soković, M. Thiazole-based chalcones as potent antimicrobial agents. Synthesis and biological evaluation. *Bioorganic & medicinal chemistry* **2011,** 19, 3135-3140.
21. Darji, D. N.; Pasha, T.; Bhandari, A.; Molvi, K.; Desai, S. A.; Makwana, M. V. Synthesis of some novel 2, 4, 5–trisubstituted thiazoles as possible antibacterial agents. *J. Chem. Pharm. Res* **2012,** 4, 2148-2152.
22. Werz, O.; Steinhilber, D. Development of 5-lipoxygenase inhibitors—lessons from cellular enzyme regulation. *Biochemical pharmacology* **2005,** 70, 327-333.
23. Wisastra, R.; Kok, P. A. M.; Eleftheriadis, N.; Baumgartner, M. P.; Camacho C. J.; Haisma, H. J.; Dekker, F. J. Discovery of a novel activator of 5-lipoxygenase from an anacardic acid derived compound collection. *Bioorganic & Medicinal Chemistry* **2013**, 21, 7763–7778.
24. Koeberle, A.; Muñoz, E.; Appendino, G. B.; Minassi, A.; Pace, S.; Rossi, A.; Weinigel, C.; Barz, D.; Sautebin, L.; Caprioglio D.; Collado J. A.; Werz, O. SAR Studies on Curcumin's Pro-inflammatory Targets: Discovery o Prenylated Pyrazolocurcuminoids as Potent and Selective Novel Inhibitors of 5‑Lipoxygenase. *Journal of Medicinal Chem*istry **2014**, 57, 5638−5648.
25. Singh, P.; Kaur, J.; Singh, G.; Bhatti, R. Anti-inflammatory conjugates: Identification of a highly potent anti-inflammatory agent. *Journal of Medicinal Chemistry* **2015**, 58, 5989−6001.

Table of Contents Graphic





Graphical abstract

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