In mammals, lipoxygenases (LOXs) are enzymes containing iron that are biological targets for essential fatty acids. These fatty acids are eicosanoids, derived from arachidonic acid, play important roles in implication and development of multifactorial inflammatory diseases. The 5-Lipoxynase (5-LO) catalysates two-step reaction that is from arachidonic acid to leukotriene A4 (LTA4). In the first step, oxidation of arachidonic acid to an unstable reactive intermediate 5-hydroperoxyeicosatetraenoic acid (5-HPETE), followed by in the second step, dehydration of 5-HPETE to form LTA4. These reactions are initially leading to the biosynthesis of various classes of leukotrienes and plays a significant role in regulating leukotriene production. 5-LO has progressed its role through multiple mechanisms by its function, structure, cofactors, and the other regulatory mechanisms controlling its expression. Based on these functions, lipoxygenases were identified as potential drug-target associated with pathogenesis of inflammatory diseases.

Zileuton is the only reversible direct inhibitor of 5-lipoxynase that was marketed, due to its hepatotoxic effects the 300 mg immediate release tablet was withdrawn from the U.S. market on February 12, 2008. Since there is an enormous demand for new 5-LO inhibitors, a series of 5-benzylidene-1,3-thiazolidine-2,4-dione-chalcone hybrids (1-24) which earlier synthesized and characterized in our study were subjected to High-throughput Screening (HTS) by 5-LO inhibition assay (UV-Kinetic method) as described by: The exploration of in vitro 5-LO inhibitory activity screening data (Figure 1) revealed that the compounds 20 and 18 were appeared to be demonstrated the most potent inhibitory activity, with IC\textsubscript{50} values of 7.88 ± 0.14 and 11.77 ± 0.21 µg/mL. It is remarkable to note that the compounds 16, 17 and 12 also showed considerable inhibitory activity with IC\textsubscript{50} values of 15.32 ± 0.16, 18.12 ± 0.42 and 18.12 ± 0.32 µg/mL, respectively. The other compounds such as 2-4, 9-11, 13-15, 21 and 24 showed modest level of activity at concentrations (IC\textsubscript{50}) ranging from 22.18 ± 0.11 to 33.31 ± 0.22 µg/mL. The compounds 1, 5-8, 19, 22 and 23 showed relatively less activity with IC\textsubscript{50} values ranging from 35.11 ± 0.23 to 46.22 ± 0.12 µg/mL in comparison with the standard (abietic acid (L101020), IC\textsubscript{50}: 4.34 ± 0.37 µg/mL).

A closer investigation at the Structure-Activity Relationship (SAR) of these compounds openly showed the inherent mechanism of 5-LO inhibitory potential related with the basic scaffold comprising of 1,3-thiazolidine-2,4-dione and α,β-unsaturated ketone moieties as observed in case of the compound 1 which is an unsubstituted compound, with IC\textsubscript{50} value of 38.66 ± 0.25 µg/mL, which in some compounds, improved by the influence of some functional group substituents and decreased by some other functional group substituents. For instance, the compounds 18 (p-F, IC\textsubscript{50}: 11.77 ± 0.21 µg/mL) > 16 (p-Cl, IC\textsubscript{50}: 15.32 ± 0.16 µg/mL) > 17 (m-F, IC\textsubscript{50}: 18.12 ± 0.42 µg/mL) > 15 (m-Cl, IC\textsubscript{50}: 24.81 ± 0.51 µg/mL) having halogen substituents either at para or meta positions relatively enhanced the activity. A decrease in the activity was seen when the substituted phenyl ring was changed by a naphthalene ring, as observed in the case of compound 23 with IC\textsubscript{50} value 44.38 ± 0.13 µg/mL. The existence of a 3-pyridyl ring in compound 22 in the place of substituted phenyl ring of α,β-unsaturated carbonyl moiety improved the activity compared to the compound that possessing naphthalene ring as substituent, but less than that of the compound having phenyl ring substituted with functional groups. Likewise, it is also remarkable to understand that the presence of 2-pyridyl ring in the place of substituted phenyl ring contributed to an increase in activity compared to the one possessing pyridin-3-yl ring, respectively as observed in the case of compounds 21 and 22 with IC\textsubscript{50} values 33.31 ± 0.22 and 41.22 ± 0.49 µg/mL, respectively. It is also remarkable to observe that the replacement of substituted phenyl ring with thiophene (20, IC\textsubscript{50}: 7.88 ± 0.14 µg/mL) and fluorene (24, IC\textsubscript{50}: 29.13 ± 0.23 µg/mL) rings improved 5-LO inhibitory activity. Conversely, it was revealed that various aromatic/heteroaromatic rings substituted at position 3 of α,β-unsaturated carbonyl system followed its activity order as thiophen-2-yl > fluorene-2-yl > pyridin-2-yl > phenyl > pyridin-3-yl > naphthalen-2-yl moieties, respectively. It is also reported that the compounds substituted with electron releasing groups were found to be biologically more significant and the order of inhibitory potential was (12 (4-NH\textsubscript{2}, IC\textsubscript{50}: 18.12 ± 0.32 µg/mL) > 4 (4-OCH\textsubscript{3}, IC\textsubscript{50}: 22.18 ± 0.11 µg/mL) > 3 (3-OCH\textsubscript{3}, IC\textsubscript{50}: 23.11 ± 0.32 ± 0.23 µg/mL) > 2 (4-OH, IC\textsubscript{50}: 25.24 ± 0.45 µg/mL) > (3-NH\textsubscript{2}, IC\textsubscript{50}: 29.41 ± 0.27 µg/mL)), respectively. It is essential that relatively less activity was seen when the hydroxyl groups are substituted at different positions on the phenyl ring as observed in the case of compounds 5-8 and the order of activity was (5 (2-OH, IC\textsubscript{50}: 35.13 ± 0.45 µg/mL) > 6 (4-OH, IC\textsubscript{50}: 39.24 ± 0.34 µg/mL) > (2,4-diOH, IC\textsubscript{50}: 44.18 ± 0.53 µg/mL) > 7 (2,5-diOH, IC\textsubscript{50}: 46.22 ± 0.12 µg/mL), respectively. The compounds 10 (IC\textsubscript{50}: 22.18 ± 0.17 µg/mL) and 9 (IC\textsubscript{50}: 26.31 ± 0.52 µg/mL) having substituted with the methyl group on the phenyl ring at position 5 along with the hydroxyl group substitution at 6 (10) and 2 (9) positions, respectively showed enhanced level of 5-LO inhibitory potential when compared with that of the compounds (5-8) possessing only hydroxyl group substitution. It is unexpected that improved level of activity was observed when the nitro group hosted on to the phenyl ring of α,β-unsaturated ketone system at 3 and 4 positions as observed in case of compounds 14 and 13 with IC\textsubscript{50} values 24.28 ± 0.13 and 33.66 ± 0.6 µg/mL, respectively. The compound 19 (IC\textsubscript{50}: 35.11 ± 0.23 µg/mL) having substituted with dibenzylxy group on the phenyl ring at...
positions 3 and 5 displayed lower level of 5-LO inhibitory activity. In summary, the SAR revealed the positive contribution of α,β-unsaturated ketone and 5-benzylidene-1,3-thiazolidine-2,4-dione moieties towards the observed activity. The results indicated that further development of thiophene ring substitution could be of biological interest.

ACKNOWLEDGEMENT

One of the authors Vasudeva Rao Avupati is thankful to the Dean, Faculty of Pharmacy and to the Vice-chancellor, Asia Metropolitan University, Malaysia for providing research lab space and instrumentation facilities to carry out the research work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


Article History: Submission Date : 19-10-2017 ; Revised Date : 05-12-2017; Acceptance Date : 09-12-2017. 
Cite this article: Avupati VR, Rani Y, Singh A. Discovery of a Series of 5-benzylidene-1,3-thiazolidine-2,4-dione-linked Chalcone Hybrids as a Novel Class of 5-Lipoxygenase (5-LO) Inhibitors. J Young Pharm. 2018;10(2):241-2.