CHAPTER V

SCREENING OF SYNTHESIZED DIARYLSULFONYLUREA-CHALCONE HYBRIDS FOR THEIR *IN VITRO* 5-LIPOXYGENASE ACTIVITY

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5.1 INTRODUCTION

Lipoxygenases are a set of non-heme, iron-containing enzymes that catalyze the merging of molecular oxygen into 1,4,-*cis*,*cis*-pentadiene-containing fatty acids (e.g. linoleic and arachidonic acids) to appear hydroperoxide products (Kenyon et al., 2006). The human isozymes, 5-, 12- and 15-Lipoxygenases are associated with different disease states, which suggest that selective inhibition may be important in targeting them for therapeutic purposes. 5-Lipoxygenase (5-LO), which was first discovered in 1976, plays an essential role in the biosynthesis of leukotrienes (LTs) that exert a large number of different biological activities mediated by specific G-protein coupled receptors. LTB_4 is a typical proinflammatory mediator that recruits and activates leukocytes, whereas cysteinyl-leukotrienes C_4, D_4 and E_4 effect vascular permeability and smooth muscle contraction. In view of these properties, development of drugs with 5-LO inhibitory activity has been hypothesized to possess therapeutic potential for cure of asthma, allergic disorders and supplementary inflammatory diseases (Werz, 2002). Based on the mechanism of action, the lipoxygenase inhibitors have been classified into four distinct classes:

(i) Iron chelating inhibitors,

(ii) Competitive reversible inhibitors,

(iii) Inhibitors of the 5-LO activating protein (FLAP) and

(iv) Anti-oxidative [3].

Intensive discovery efforts in the development of clinically useful drugs from the inhibitors of 5 LO enzyme have led to one marketed drug; Zileuton (A-64066) and others, namely MK-3000, MK-886, MK-0591, ZM 211965, AKBA, BW A4C, LDP-

977, Bay-X-1005, and Abt-761, which are evaluated at different stages of drug development (Werz , 2002 , Babu et al., 2002).

5.2 MATERIALS AND METHODS

The 5-LO inhibitory potential of the synthesized compounds (**4a**-**4y**) was determined by 5-LO inhibition assay (UV-Kinetic method) as described by Sircar et al. (Sircar et al., 1983). For the evaluation of 5-LO inhibitory activity, the enzymatic activity of 5-LO was calculated spectrophotometrically using potato 5-LO (Reddenna et al., 1990) and an incubation mixture containing 80 mM linoleic acid and 50 mM sodium phosphate buffer (pH = 6.3).

The reaction was initiated by the addition of an enzyme buffer mix to substrate (Linoleic acid) and the enzyme activity was monitored as an increase in rate of absorbance at 234 nm on a UV/visible spectrophotometer (Varion Cary-50 UV-Visible spectrophotometer) for 120 sec. Each experiment was conducted by incubating along with control at various concentrations of the test substances with enzyme buffer mix for 2 min before addition of the substrate. The percentage inhibition was measured by evaluating the slope or identification of increase in absorbance of test substance with that of control enzyme activity. The assay was performed in triplicate and the mean±SEM values were used for the calculation. The IC_{50} values were measured using the fenny probed analysis software. The obtained result to the tested compounds were compared with positive control like abietic acid (LI01020) (Ulusu et al., 2002).

5.3 RESULTS AND DISCUSSION

The results of 5-LO inhibitory activity are given in Table 1. The investigation of *in vitro* 5-LO inhibitory activity screening data (Table 1) revealed that the compounds **4r** and **4o** demonstrated comparatively the most potent inhibitory activity, with IC_{50} values of 7.88±0.14 µg/mL and 11.77±0.21 µg/mL, respectively. It is interesting to note that the compounds **4y**, **4q**, **4t** and **4n** also showed appreciable inhibitory activity with IC_{50} values of 14.91±0.77, 15.32±0.16, 18.12±0.32 and 18.12±0.42 µg/mL, respectively. The other compounds such as **4b**, **4d**, **4i**-**4l**, **4p**, **4s**, **4u** and **4x** showed moderate level of activity at concentrations (IC_{50}) ranging from 22.18±0.11 to

33.31±0.22 µg/mL. The compounds **4a**, **4c**, **4f-h**, **4m**, **4v** and **4w** exhibited comparatively less activity with IC_{50} values ranging from 35.11±0.23 to 46.22±0.12 µg/mL in comparison with the standard drug (Abietic acid (LI01020), IC_{50}: 4.34±0.37 µg/mL).

Table 5.1: 5-LO inhibitory activity data of diarylsulfonylurea-chalcone hybrids 4a-4y produced via Scheme 1

Compound	R	IC_{50} (µg/mL) (mean±SEM) [a]
4a	C_6H_5	38.66±0.25
4b	4-MeC_6H_4	25.24±0.45
4c	4-$NMe_2C_6H_4$	35.11±0.23
4d	2,4-di$OMeC_6H_3$	23.11±0.32
4e	3,4, 5-tri$OMeC_6H_2$	22.18±0.11
4f	2-OHC_6H_4	35.13±0.45
4g	3-OHC_6H_4	46.22±0.12
4h	4-OHC_6H_4	39.24±0.34
4i	3-OEt,4-OHC_6H_3	26.31±0.52
4j	3-OMe,4-OHC_6H_3	22.18±0.17
4k	2-$NO_2C_6H_4$	24.28±0.13
4l	3-$NO_2C_6H_4$	33.66±0.61
4m	5-OH,2-$NO_2C_6H_3$	44.18±0.53
4n	3-FC_6H_4	18.12±0.42
4o	4-FC_6H_4	11.77±0.21
4p	2-ClC_6H_4	24.81±0.51
4q	4-ClC_6H_4	15.32±0.16
4r	2,4-diClC_6H_3	7.88±0.14
4s	3-BrC_6H_4	29.41±0.27
4t	4-BrC_6H_4	18.12±0.32
4u	4-Allyl-OC_6H_4	29.13±0.23
4v	Phenylethene-yl	44.38±0.13
4w	Pyridin-3-yl	41.22±0.49
4x	Pyridin-4-yl	33.31±0.22
4y	Anthracen-9-yl	14.91±0.77
Abietic acid (LI01020)		4.34±0.37

[a] SEM = Standard error of the mean.

A close look at the SAR (Structure-Activity Relationship) of these compounds clearly exhibited the inherent phenomenon of 5-LO inhibitory activity associated with the basic skeleton consisting of diarylsulfonylurea and α,β-unsaturated ketone moieties as seen in case of the unsubstituted compound **4a** with IC_{50} value of 38.66±0.25 µg/mL, which in some cases was enhanced by the influence of some substituents and decreased by some other substituents. For example, the compounds **4r** (2,4-diCl, IC_{50}: 7.88±0.14 µg/mL) > **4o** (4-F, IC_{50}: 11.77±0.21 µg/mL) > **4q** (4-Cl, IC_{50}: 15.32±0.16 µg/mL) > **4n** (3-F, IC_{50}: 18.12±0.42 µg/mL) > **4t** (4-Br, IC_{50}: 18.12±0.32 µg/mL) > **4p** (2-Cl, IC_{50}: 24.81±0.51 µg/mL) > **4s** (3-NH_2, IC_{50}: 29.41±0.27 µg/mL) having halogen substituents either at ortho or meta or para positions significantly enhanced the activity. A reduction in the activity was observed when the substituted phenyl ring B was replaced by a cinnamyl moiety, as seen in the case of compound **4v** (IC_{50} value 44.38±0.13 µg/mL). The presence of a 3-pyridyl ring in compound **4w** in the place of substituted phenyl ring B of α,β- unsaturated carbonyl system enhanced the activity compared to the one possessing cinnamyl moiety, but less than that of the one having substituted phenyl ring. It is also interesting to see the presence of 4-pyridyl ring in the place of substituted phenyl ring B contributed to an increase in activity compared to the one possessing 3-pyridyl ring, respectively as seen in the case of compounds **4x** and **4w** with IC_{50} values 33.31±0.22 and 41.22±0.49 µg/mL, respectively. It was identified that the replacement of substituted phenyl ring B with allyloxy group at para position enhanced 5-LO inhibitory activity (**4u**, IC_{50}: 29.13±0.23 µg/mL). The presence of a 9-anthracenyl ring in compound **4y** (IC_{50}: 14.91±0.77 µg/mL) in the place of substituted phenyl ring B of α,β- unsaturated carbonyl system significantly increased the activity compared to the one possessing 3-pyridyl and 4-pyridyl ring systems.

However, it was noticed that various aromatic/heteroaromatic rings substituted at position 3 of α,β-unsaturated carbonyl system followed its activity order as anthreacen-9-yl > pyridin-4-yl > phenyl > pyridin-3-yl moieties, respectively. It was also noted that the compounds substituted with electron releasing groups was found to be biologically relevant and the activity order was (**4e** (3,4,5-triOCH_3, IC_{50}: 22.18±0.11 µg/mL) > **4d** (2,4-diOCH_3, IC_{50}: 23.11±0.32±0.23 µg/mL) > **4b** (4-CH_3, IC_{50}: 25.24±0.45 µg/mL) > **4c** (4-$N(CH_3)_2$, IC_{50}: 35.11±0.23 µg/mL)), respectively. It is important that less activity was observed when the hydroxyl groups are substituted

at different positions on the phenyl ring as seen in the case of compounds **4f**-**4h** and the order of activity was **4f** (2-OH, IC_{50}: 35.13±0.45 µg/mL) > **4h** (4-OH, IC_{50}: 39.24±0.34 µg/mL) > **4g** (3-OH, IC_{50}: 44.18±0.53 µg/mL) respectively. The compounds **4j** (IC_{50}: 22.18±0.17 µg/mL) having methoxyl group at substitution on the phenyl ring B at position 3, **4i** (IC_{50}: 26.31±0.52 µg/mL) having ethoxy group at substitution on the phenyl ring B at position 3 and **4m** (IC_{50}: 44.18±0.53 µg/mL) having nitro group at substitution on the phenyl ring B at position 2 along with the hydroxyl group substitution at positions 4 (in case of **4j** and **4i**) and 5 (in case of **4m**), respectively showed enhanced level of 5-LO inhibitory activity when compared with that of the compounds (**4f**-**4h**) possessing only hydroxyl group substitution. It is notable that enhanced level of activity was observed when the nitro group introduced on the phenyl ring B of α,β-unsaturated carbonyl system at 2 and 3 positions as seen in the case of compounds **4k** and **4l** with IC_{50} values 24.28±0.13 and 33.66±0.6 µg/mL, respectively.

5.4 CONCLUSION

In summary, we synthesized and characterized a series of diarylsulfonylurea-chalcone hybrids (**4a**-**4y**). For the first time, this class of compounds were screened for 5-LO inhibitory activity and the results revealed the positive contribution of halogen substituents on the phenyl ring B of α,β-unsaturated ketone towards the observed 5-LO inhibitory activity. The observed activity may also be due to diarylsulfonylurea and α,β-unsaturated ketone moieties forming part of the basic structure of these molecules. The results indicated that further scope of compounds might show biological interest like control of ageing diseases.