NEW INDOLINE-BASED 5-LOX/sHE DUAL INHIBITORS: *IN SILICO* ASSISTED STUDY, *IN VITRO* AND *IN VIVO* PHARMACOLOGICAL CHARACTERIZATION

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The role of inflammation as a driver of pathology is linked to the involvement of several converging signaling pathways that coordinate the action of different mediators and the recruitment of inflammatory cells. The arachidonic acid (AA) cascade represents a key biochemical route for targeting inflammatory pathological conditions. AA is synthetized from membrane phospholipids by cytosolic phospholipase A2 (cPLA2) and transformed via three separate enzymatic systems; a) COXs lead the conversion into prostaglandins (PG) and thromboxane, b) cytochrome P450 (CYP450) transforms AA to anti-inflammatory epoxyeicosatrienoic acids (EETs), that are converted to the pro-inflammatory dihydroxyeicosatrienoic acids (DiHETrEs) by epoxide hydrolase (sEH) c) lipoxygenases (LOs) drive AA conversion to leukotriens. Therefore, the simultaneous inhibition of 5-LOX and sEH represents a valid approach for the development of a new class of anti-inflammatory drugs. The present work describes a medicinal chemistry workflow leading to the development of a new series of indoline-based 5-LOX/sEH double inhibitors.[1] The design of these molecules was carried out starting from a virtual screening protocol performed using an in-house molecular library. Since the synthesized compounds nine different zileuton-inspired[2] molecules were initially selected. The *in vitro* analysis of these molecules revealed compound **43** as a suitable, indoline-based hit-compound with low micromolar inhibitory potencies against 5-LOX. Therefore, a structure-based design of compound **43** analogues was carried out, leading to the synthesis of 19 new molecules. The second developed series of compounds underwent to extensive in vitro testing revealing a remarkable dual inhibitory profile over 5-LOX and sEH, rationalized by molecular modelling studies. Compound **73** emerged as the most potent compound of the library (IC_{50s}= 0.41±0.01 □M and 0.43±0.10 □M against isolated 5-LOX and sEH, respectively). This is the reason why derivative **73** was challenged for its anti-inflammatory activity in two in vivo murine models (zymosan-induced peritonitis and ovoalbumin-induced asthma). Compound **73** displayed remarkable *in vivo* anti-inflammatory properties, suggesting that 5-LOX/sEH dual inhibitors could be considered an important starting point for the development of therapeutic tools in inflammatory diseases.

References

[1] Cerqua, I.; Musella, S.; *et* al. J. Med. Chem. **2022**; 65(21):14456-14480.
[2] Rossi, A.; Pergola, C.; Koeberle, A. *et* al. Br J Pharmacol. **2010**; 161(3):555-570.