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Design, synthesis, and biological evaluation of some novel indolizine derivatives as dual cyclooxygenase and lipoxygenase inhibitor for anti-inflammatory activity

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ABSTRACT

Some novel indolizine derivatives were synthesized by bioisosteric modification of imidazo[1,2-*a*]pyridine for anti-inflammatory activity. The physicochemical characterization and structure of compounds were elucidated by state of the art spectroscopic technique. Induced fit docking was performed for initial screening to elucidate the interactions with corresponding amino acids of cyclooxygenase (COX-1, COX-2) and lipoxygenase (LOX) enzymes. The target compounds **53**–**60** were then evaluated against *in vivo* carrageenan and arachidonic acid induced rat paw edema models for anti-inflammatory activity. Amongst all the synthesized derivatives, compound **56** showed the significant anti-inflammatory activity in both rat paw edema models with very less ulcerogenic liability in comparison to standard diclofenac, celecoxib, and zileuton. The compounds **56** was further assessed to observe *in vitro* enzyme inhibition assay on both cyclooxygenase and lipoxygenase enzyme where it showed a preferential and selective non-competitive enzyme inhibition towards the COX-2 (IC_{50} = 14.91 μM, Ki = 0.72 μM) over COX-1 (IC_{50} > 50 μM) and a significant non-competitive inhibition of soybean lipoxygenase enzyme (IC_{50} = 13.09 μM, Ki = 0.92 μM). Thus, *in silico, in vivo*, and *in vitro* findings suggested that the synthesized indolizine compound **56** has a dual COX-2 and LOX inhibition characteristic and parallel *in vivo* anti-inflammatory activity in comparison to the standard drugs.

1. Introduction

The prostaglandins and leukotrienes are naturally occurring twenty carbon fatty acid derivatives produced through biochemical oxidation of arachidonic acid (AA), which play an essential modulatory role in many normal and disease-related cellular processes.[1] In fact, much of the inflammation, pain, fever, nausea, asthmatic and allergic reaction occurs due to excessive production of prostaglandins and leukotrienes.[2]

The primary enzyme involved in the first step of the AA cascade is cyclooxygenase (COX), which exists in three isoforms as COX-1, COX-2, and COX-3. COX-1 and COX-2 are structurally 63% identical and 77% similar at the amino acid level.[3] The COX-1 is ubiquitous form typically produced in normal, quiescent condition and remains as a constitutive protein of normal cell. It is also important in the production of prostaglandins that regulates cellular homeostasis, such as renal blood flow, and in circumstances where prostaglandins have a protective function such as gastric mucous production.[4] COX-2 is the inducible form of the enzyme, expressed in the endothelial cell, chondrocytes, and osteoblast of traumatic tissue after tissue trauma and therefore plays a major role in inflammation. COX-3 is an enzyme mostly present in the brain, expressed under the influence of COX-1 gene, but not functional in the human being.[5]

There are some other important AA metabolites like leukotriene produced by Lipoxygenase (LOX) enzyme activity. LOXs are the member of non-iron containing dioxygenases family and be available for animals, plants, and fungi. In humans, three functional isoforms of LOX exist as 5-, 12-, and 15-LOXs, whereas two isoforms 9- and 13-LOX exist in plants.[6]

The crystal structures of two COXs suggested that the active site has a narrow hydrophobic channel extending from the membrane-binding region to the protein core. Initially, the binding of the substrate at COX enzyme occurs at the channel opening pocket lined with Arg120 and Ile345 in both the enzymes.[7] The key difference between the COX-1 and COX-2 isozyme active site is the exchange of isoleucine in COX-1 for valine in COX-2 at positions 434 and 523.[8] The differences in the amino acid sequence make the COX-2 substrate-binding site more flexible and somewhat larger by

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creating a secondary pocket. The COX-2 selective inhibitors explicitly bind to this secondary binding pocket (lined by His90, Arg513, and Val523) resulting in specific inhibition of COX-2 activity. Apart from this secondary pocket, another critical region in the COX-2 active site lined by Trp387, Tyr385, Phe518, Phe381, Met522, and Leu352 is known as the hydrophobic pocket. The selective COX-2 inhibitors acquire a pharmacophore which can selectively bind to the secondary pocket and bring enough steric bulk to block the hydrophobic channel of COX-2.[8] The active site of 5-LOX is an elongated cavity, with no clear access to bulk solvent, lined with both invariant (Leucines 368, 373, 414, and 607 and Ile406) and 5-LOX-specific amino acids (Tyr181, Ala603, Ala606, His600, and Thr364).[9] Further, alignment studies of five isoforms of LOX and two isoforms of COX suggested that pharmacophoric interaction with amino acid Tyr181, Phe359, Phe421, and Trp599 at 5-LOX binding site may increase specificity towards COX-2 and 5-LOX.[10]

Non-steroidal anti-inflammatory drugs (NSAIDs) as COX inhibitors are the leading prescription medicine worldwide for the cure of inflammation, but their long-term use is restricted due to gastrointestinal, bronchoconstriction and hepatotoxic side effects.

Indeed significant NSAIDs are fluxed in the world market, but the blockade of arachidonate cascade at the COX level diverts the substrate towards increased production of LOX-derived eicosanoids such as leukotrienes (LTs) that cause bronchoconstriction, ulceration, and inflammation, which exists as a big challenge for medicinal chemists.

Considering the pro-inflammatory properties of LTs and prostanoids, the drugs able to block the synthesis of both eicosanoids, should prove itself as a better anti-inflammatory drug molecule with fewer side effects in comparison to established classical NSAIDs and selective COX-2 inhibitors.[11] The COX and LOX drug inhibitors are expected to enhance anti-inflammatory potency without risks of serious side effects. Hence the discovery of dual COX and LOX enzymes inhibitors with reduced toxicity and side effects is the need of pharmacotherapeutics in the modern age.[12]

1.1. Designing considerations

In the field of drug development and therapeutics, bioisosterism is a successful analog designing strategy over the years and translated into the development of drugs like Alloxanthine and Procainamide, etc.[13] Taking a cue from this, we have studied some Imidazo[1,2-*a*]pyridine derivatives having an aromatic ring at 4th position, and cyclohexanamine or cyclopentanamine at 5th position reported for LOX inhibition (IC_{50} = 0.21 μM) at micromolar concentration.[14] In the designing, the Imidazo[1,2-*a*]pyridine nucleus was chosen, where bioisosteric replacement of = N- with = CH- (ring equivalent) leads to flanged bicyclic nucleus 'indolizine' that could be considered as a novel class under non-classical bioisostere design.

Compounds with indolizine ring have received attention in recent years like Curindolizine, a chemical generated from Curvularia (species: IFB-Z10) reported for anti-inflammatory activity in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages (IC_{50} = 5.31 μM).[15] Licofelone is another molecule having 3H-pyrrolizine fragment like Curindolizine, reported for 5-LOX (IC_{50} = 0.21 μM), COX-1 (IC_{50} = 0.16 μM) and COX-2 (IC_{50} = 0.37 - μM) inhibitory activity and had been passed the safety level in the clinical trial, but the challenge of the ulceration persists in licofelone at doses of 30 and 100 mg/kg.[16] It may be due to the blocking of COX-1 enzyme and the strategy to block COX-2, and LOX enzyme selectively may produce a new dual COX-2 and LOX inhibitor to treat inflammatory disorders.

The primary objective of this study was to design and synthesize some novel 3-(aminomethyl)indolizine-1-carboxylic acid derivatives using the bioisosteric modification of imidazo[1,2-*a*] pyridine to indolizine (Fig. 1) and further evaluated for their particular COX and LOX enzyme inhibition, anti-inflammatory activity, and ulcerogenic liability.

2. Results and discussion

2.1. Chemistry

The targeted compounds were synthesized as per Scheme 1. The known malonate derivatives (**10**–**17**) were synthesized through the Knoevenagel condensation by reacting substituted aldehydes (**1**–**8**) with diethyl malonate (**9**) in the presence of catalytic amount of piperidine.[17] The reported pyridinium bromide salt (**20**) was then prepared by the addition of Bromo acetonitrile (**19**) in pyridine (**18**). Pyridinium bromide salt (**20**) then conjugated with malonate derivatives (**10**–**17**) through Huisgen [3+2]-cycloaddition reaction in dichloromethane to give unstable intermediate compounds (**21**–**28**) which than cyclized to five-membered heterocyclic indolizine compounds (**29**–**36**). Intermediate compounds (**21**–**28**) are unstable, so we made no attempts to isolate them. Also, indolizine compounds (**29**–**36**) were carried forward in next step without further purification and reacted with chloranil or chromium trioxide in the presence of the strong base and resulted in the corresponding aromatized indolizine derivatives (**37**–**44**),[18,19] which was initially confirmed by positive Dragendorff test on TLC.[20] Further, the nitrile was reduced by nickel boride, generated from in-situ sodium borohydride and nickel (II) chloride reaction in dry ethanol, resulted in the corresponding aminomethyl carboxylate (45–52),[21] which was confirmed by positive Ninhydrin Test on TLC.[22] The novel target 3-(aminomethyl)indolizine-1-carboxylic acid derivatives (53–60) were generated by base-catalysed hydrolysis of the ester. Preliminary identification of acid was confirmed by positive Bromo Cresol Green test on TLC.[23]

The FT-IR spectra of nitrile compounds (**37**–**44**) showed the characteristic medium C-N stretching of nitrile peak in the range of 2300–2350 cm^{-1} and C-O stretching of ester peak in the range of 1735–1755 cm^{-1}. Structures of compounds (**37**–**60**) were also confirmed by NMR spectroscopy. In all compounds, ^1H NMR spectra showed a peak of acid at down field (at 11 δ value). The characteristic amine peak was observed at 3–5 δ value with integration value of 2. Another peak of the CH_2 proton with integration value of 2 as triplet was observed at near to 3 δ value. Further, ^{13}C NMR spectra also confirmed the presence of carboxylate group by showing a peak near to 200 δ at down field.

2.2. Log P determination and prediction of drug-like properties

The lipophilicity (log P) of a molecule is considered as a characteristic to establish a relationship between the pharmacodynamic and pharmacokinetic property of drugs.[24] We have determined the partition coefficient of the synthesized compounds and standards by octanol/water system (Supplementary Table 1). Almost, the log P values of compounds were observed under suitable lipophilic range (2–4). The drug-likeness of all the compounds and standard were also calculated through QikProp software tool (Maestro 10.5.014, Schrödinger, LLC, and New York-1), *in silico* ADME/Tox Predictions ensured that all compounds follows Lipinski's rule of five and could be considered as a drug-like molecule (Table 1).

2.3. Docking study

The synthesized compounds (**37**–**60**) were subject for Rigid Docking on COX-1, COX-2, and LOX enzymes (PDB Code: 1CX2,[25] 3N8Y,[26] and 3V99[27] respectively) using Glide-XP protocol in the