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Research paper

4,5-Diarylisoxazol-3-carboxylic acids: A new class of leukotriene biosynthesis inhibitors potentially targeting 5-lipoxygenase-activating protein (FLAP)

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ABSTRACT

In this article, we report novel leukotriene (LT) biosynthesis inhibitors that may target 5-lipoxygenase-activating protein (FLAP) based on the previously identified isoxazole derivative (**8**). The design and synthesis was directed towards a subset of 4,5-diaryl-isoxazole-3-carboxylic acid derivatives as LT biosynthesis inhibitors. Biological evaluation disclosed a new skeleton of potential anti-inflammatory agents, exemplified by **39** and **40**, which potently inhibit cellular 5-LO product synthesis ($IC_{50} = 0.24$ μM, each) seemingly by targeting FLAP with weak inhibition on 5-LO ($IC_{50} \geq 8$ μM). Docking studies and molecular dynamic simulations with 5-LO and FLAP provide valuable insights into potential binding modes of the inhibitors. Together, these diaryl-isoxazol-3-carboxylic acids may possess potential as leads for development of effective anti-inflammatory drugs through inhibition of LT biosynthesis.

1. Introduction

Leukotrienes (LTs) are potent lipid mediators that play important roles in the pathophysiology of inflammatory, fibrotic and hyperproliferative diseases such as asthma, chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), arthritis, allergic diseases, autoimmune diseases, cardiovascular disease (CVD) and certain cancer types [1,2]. In the first step of LT biosynthesis, 5-lipoxygenase (5-LO) catalyzes the production of the unstable epoxide LTA_4 from arachidonic acid (AA), which is further metabolized to LTB_4 or cysteinyl LTs (cys-LTs) such as LTC_4, D_4 and E_4 [3,4]. This first step also requires the involvement of 5-LO-activating protein (FLAP), which acts as a regulatory protein by interaction with 5-LO for the transfer of AA to 5-LO for efficient metabolism [5].

Despite intensive efforts to develop 5-LO/FLAP inhibitors as anti-LTs, zileuton (**1**), the only 5-LO inhibitor, reached the market for the treatment of asthma and/or allergic rhinitis, but has experienced limited use due to its poor pharmacokinetics and observed idiosyncratic hepatotoxicity [6]. Additionally, the 5-LO inhibitor setileuton (**2**, MK-0633) [7–9] and several FLAP inhibitors such as AM803 (**3**, GSK2190915), AZD6642 (**4**) and BI665915 (**5**) were reported to be in various stages of preclinical and clinical studies for treatment of asthma and COPD [10–13].

Recent studies have also implicated LTs in CVD such as atherosclerosis, myocardial infarction (MI), and stroke [14,15]. 5-LO and FLAP are considered to be important components of the LT cascade found in atherosclerotic lesions, thus implicating their involvement in atherogenesis [16–18]. The 5-LO inhibitor **2** recently completed phase II clinical trials for atherosclerosis (Clinical trials ID: NCT00421278) as well as for asthma and COPD [7,19], implying the value of LT inhibition for CVD besides respiratory disorders.

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Pharmacological intervention with FLAP has also been reported to decrease atherosclerotic lesion size in animal models [20–23] and the FLAP inhibitor veliflapon (**6**, DG-031/BAY-X1005) has successfully passed a phase II human clinical trial for MI, although participant recruitment in phase III was suspended due to unexpected formulation issues. In addition, there is a growing interest in 5-LO or FLAP inhibitors as broad-spectrum LT biosynthesis inhibitors in cancer therapy, especially in prostate cancer [24,25] and certain types of leukemia [26–28].

Inspired by the therapeutic potential of LT biosynthesis inhibitors, we have recently introduced two promising chemotypes, a benzimidazole derivative 1-(2-chlorobenzyl)-2-(1-(4-isobutylphenyl)ethyl)-1*H*-benzimidazole (**7,** BRP-7) and an isoxazole derivative 2-[4-(4-chlorophenyl)-3-methyl-1,2-oxazol-5-yl]-5-[(2-methyl phenyl)methoxy]phenol (**8**) as anti-LT agents [29,30] (see Fig. 1). From the SARs [29,31] and detailed pharmacological studies [32], **7** was revealed as potent inhibitor of LT biosynthesis targeting FLAP in a cell-based assay (IC_{50} = 0.15–0.31 μM) without affecting isolated 5-LO (IC_{50} > 10 μM), whereas **8** was moderately effective in a cell-based assay (IC_{50} = 4.4 μM) and directly inhibited isolated 5-LO (IC_{50} = 1.9 μM), which might be the primary cause for the decrease of cellular LT formation [29]. However, whether **8** also interferes with FLAP was not explored in those studies. Therefore, the present work describes the structure-guided design, synthesis, structure–activity relationship (SAR), and biological evaluation of novel 4,5-diarylisoxazol-3-carboxylic acids as potent inhibitors of LT biosynthesis, potentially targeting FLAP.

2. Results and discussion

2.1. Docking studies and molecular dynamic (MD) simulations with 5-LO

Based on the favorable profile of **8**, we focused on the preparation of further derivatives in order to understand SARs for interference with 5-LO product synthesis. First, we addressed the necessity of the *o*-hydroxyl group on C5-phenyl for the bioactivity of **8**. As we previously showed the importance of hydrophobic contacts over hydrophilic interactions as a general determinant for many known 5-LO inhibitors [33], our initial hypothesis was the removal of the *o*-hydroxyl group to improve the potency. Therefore, we synthesized two derivatives of **8** by either completely removing the polar *o*-hydroxyl moiety (**15**) or by replacing it by a less polar methoxy group (**16**) (Scheme 1). Interestingly, removal of the *o*-hydroxyl group from **8** or *O*-methylation abolished the inhibition of cellular LT biosynthesis (**15**–**16**, Table 1).

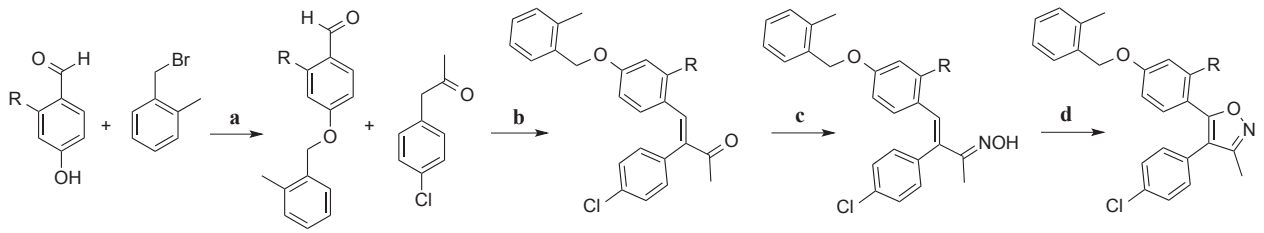
For rationalizing the inhibitory activity of **8** on 5-LO by means of molecular docking, we used the recently reported apo form of the 5-LO crystal structure (PDB code 3O8Y). The unavailability of a holo 5-LO conformation represented the first major difficulty to elucidate the ligand-active site interactions. Therefore, we referred to the conserved or unconserved active site interaction models [33,34]. Grounding on those results, we combined docking studies with MD simulations (see Supporting information for detail) to investigate the binding mode of inhibitors taking into account ligand-induced conformational changes of 5-LO. As a result of the docking studies, **8** interacts with the binding site engaging residues Tyr181, Trp599, Phe177, His372, Phe421, Asn425 and His600 (Fig. S1). The 5-LO/**8** complex obtained by docking was submitted to a 10 ns MD study (Fig. 2A). As a result of MD simulations, the *o*-hydroxyl group is engaged in a stable hydrogen bonding interaction with the side-chain of Asn425 (occupancy, 94%, Fig. 2A) orienting the phenyl moiety to engage π-π contacts with Tyr181 (occupancy, 38%), His372 (occupancy, 39%) and Phe421 (occupancy, <30%), which is in agreement with previously reported data on the catalytic site of 5-LO [35].

These findings led us to turn our attention into a structure-based design of new derivatives of **8** involving the isoxazole-3-carboxylic acid core (**32**, Fig. 2B), where the carboxyl arm on isoxazole might influence the pattern of more strong interactions with neighboring polar amino acids or with amino acid backbones. Indeed, from the analysis of molecular docking and MD results, both the isoxazol-3-carboxylic acid analog **32** and **8** disclosed a similar binding mode at the 5-LO active site (Fig. 2A–B). Compound **32**, as already seen for the compound **8**, engaged a strong hydrogen bond reinforced by charge assistance with Asn425 (occupancy, 99%), as well as maintaining the key hydrophobic contacts with Tyr181 (occupancy, <30%), His372 (occupancy, 34%) and Phe421 (occupancy, 32%). These results suggested the synthesis of a focused library of compounds with decorations on an isoxazole-3-carboxylic acid core that was designed taking into account both synthetic accessibility, and the compatibility of new skeleton with the binding requirements of the active pocket to obtain preliminary SAR (Scheme 2). The biological evaluation of this small set of compounds might be helpful for the comprehension of the key features of new isoxazole-3-carboxylic acids as LT biosynthesis inhibitors.

2.2. Chemistry

Compounds **15**–**16** were prepared following the reaction sequence shown in Scheme 1 using general methods previously reported [36]. Hence, the α,β-unsaturated ketone intermediates (**11**–**12**), which were prepared by condensation of 4-chlorophenylacetone with benzaldehydes (**9**–**10**) in the presence of piperidine, were converted to α,β-unsaturated oximes (**13**–**14**). Subsequent cyclization of the oximes (**13**–**14**) generated the 3-methylisoxazole derivatives (**15**–**16,** Scheme 1).

For the synthesis of compounds **32**–**40**, we utilized the



9 R = H
10 R = OCH_3
11 R = H
12 R = OCH_3
13 R = H
14 R = OCH_3
15 R = H
16 R = OCH_3

Scheme 1. Synthesis of 4,5-diaryl-3-methylisoxazoles. Reagents and conditions: (a) K_2CO_3, MeCN, reflux; (b) piperidine, 90 °C; (c) $NH_2OH.HCl$, NaOAc, EtOH, reflux; (d) KI, I_2, $NaHCO_3$, THF, H_2O, reflux.