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Research paper

Exploring the role of chloro and methyl substitutions in 2-phenylthiomethyl-benzoindole derivatives for 5-LOX enzyme inhibition

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ABSTRACT

Following the results we previously reported on a series of ethyl 2-phenylthiomethyl 5-hydroxyindole-3-carboxylate derivatives as 5-lipoxygenase (5-LOX) inhibitors, in order to obtain a more selective compound with respect to the previous generation of derivatives, we decided to modify the structure of the core ligand.

The first level of structural modification involved the annelation of benzene to the indole, yielding corresponding benzo[*g*]indole derivatives, systematic optimization of methyl or chlorine groups in meta-, ortho- and ortho/para-position of 2-phenylthiomethyl moiety were applied. The reported results show that extension of the aromatic core led to a great enhancement of activity, especially in cell-free assay, and the accurate structure-based design provided compounds **6f**, **6g** and **6l** that block 5-LOX activity in cell-free assays with IC_{50} ranging from 0.17 to 0.22 μM, and suppress 5-LOX product synthesis in polymorphonuclear leukocytes with IC_{50} ranging from 0.19 to 0.37 μM. Moreover we have identified **6f** and **6l** as dual 5-lipoxygenase (5-LO) and microsomal prostaglandin E_2 synthase-1 (mPGES-1) inhibitors and compound **6l** significantly reduces inflammatory reactions in the carrageenan-induced mouse paw oedema. The reported *in vivo* analysis, together with the accessible synthetic procedure, stimulate for the generation of further potent antinflammatory benzoindoles-based agents.

1. Introduction

In the last decades, several studies have demonstrated the key role played by 5-lipoxygenase (5-LOX) in inflammation-related disorders. Leukotrienes (LT), formed from AA by catalysis of 5-LOX, are fast reacting pro-inflammatory mediators of the immune system [1,2]; besides their physiological roles, they primarily mediate inflammatory and allergic reactions [3] and are involved in the onset of inflammatory diseases such as asthma, allergic rhinitis, rheumatoid arthritis and also cardiovascular disorder [4]. However, numerous studies have demonstrated the overexpression of 5-LOX in tissue samples of primary tumor cells as well as in established cancer cell lines [5]. An increasing body of evidence suggests a crucial role for 5-LOX products in the early stage of pancreatic, prostate and colorectal carcinogenesis [6] and glioma cell lines [7]. Recent studies showed that 5-LOX expression appears to be upregulated in patients with neurodegenerative disease like AD, demonstrating the key role of leukotrienes promoting Aβ generation [8–10]. In other reports, the potent and selective 5-LOX inhibitor zileuton reduced the amyloid and tau pathology as well as memory impairments in different mouse models of AD [11,12].

Continuing our studies on small molecules able to block 5-LOX activity [13–18], we recently reported the design and synthesis of a small collection of differently decorated 5-hydroxyindole-3-carboxylates derivatives able to interact with 5-LOX at nanomolar

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concentration [14]. Our previous investigations have disclosed the influence of the substitution pattern on the phenylthiomethyl moiety, the simultaneous presence of chlorine in ortho-positions of the thiophenol ring as well as the introduction of three methyl groups (ortho, para) that led to compound with marked increase of 5-LOX inhibitory activity [14]. Among all tested compounds, ethyl 5-hydroxy-2-(mesitylthiomethyl)-1-methyl-1H-indole-3-carboxylate (**1**) showed good potency in inhibiting 5-LOX activity in cell-free assays with IC_{50} = 0.7 μM and suppressed 5-LOX product synthesis in polymorphonuclear leukocytes with IC_{50} = 0.23 μM, being equally potent to the well-recognized reference inhibitor zileuton, used as antiasthmatic drug in the clinics. With regard to other AA-metabolizing enzymes like COX-1, COX-2 and 12-LO, compound **1** was rather selective for 5-LOX with minor effects also on 15-LOX. Moreover, our previous works reported that the annelation of a [g]benzene ring to the indole led to more potent inhibitors, exemplified by compounds **2a** and **2b** with IC_{50} of 0.086 and 0.097 μM in cell-free and 0.23 and 1.2 μM in cell-based assays, respectively [19,20] (Fig. 1). We showed also that benzo[g]indol-3-carboxylates potently inhibit mPGES-1 and thus represent a novel class of dual 5-LO/mPGES-1 inhibitors.

With the goal of increasing potency and establishing a better understanding of the pharmacophore for 5-LO inhibition, we focused our investigation on the effects of introduction one or more chlorine and methyl substituents on the benzoindole ring. Moreover, introduction of a methoxy or ethoxy group at C5 position and N alkylation of the indole nitrogen atom (**7a-b**, **8a-b** and **11**) were also carried out in order to investigate the effect of OH and NH on the potency against 5-LOX.

Here we report the synthesis and the pharmacological profile of this second generation of benzoindole derivatives with anti-inflammatory effectiveness *in vivo*. While further studies are certainly needed to better describe the exact anti-inflammatory mode of action, this novel focused small molecules library exerts new interesting hits shedding further light on the structural requirements needed for an optimal ligand–enzyme interaction.

2. Results and discussion

2.1. Chemistry

The synthesis of desired compounds **6a-l** (Scheme 1) has been accomplished in three steps of reaction. The final Nenitzescu reaction has proven to be the simplest entry into 5-hydroxy-1*H*-benzo[*g*]indoles. Essentially, stirring of the enaminoesters **5a-l** with 1.0 equiv of 1,4-naphthoquinone in presence of catalytic amount of ZnI_2 at room temperature led to the expected 5-hydroxy-1*H*-benzo[*g*]indoles. Nenitzescu reactants **5a-l** were prepared starting from thiophenols **3a-l**, which were first reacted with ethyl 4-chloroacetoacetate to give the *β*-ketoesters **4a-l**. *β*-Ketoesters **4a-l** were converted into their appropriate enaminoesters **5a-l** by refluxing them in toluene with an excess of ammonium acetate. **7a-b** and **8a-b** were prepared by alkylation of **6b** with iodoalcanes

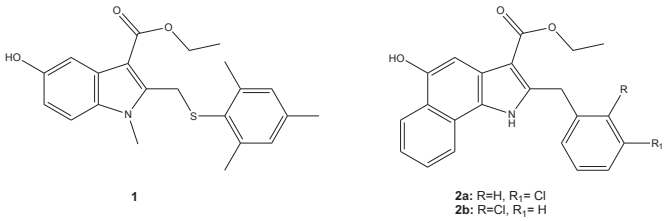
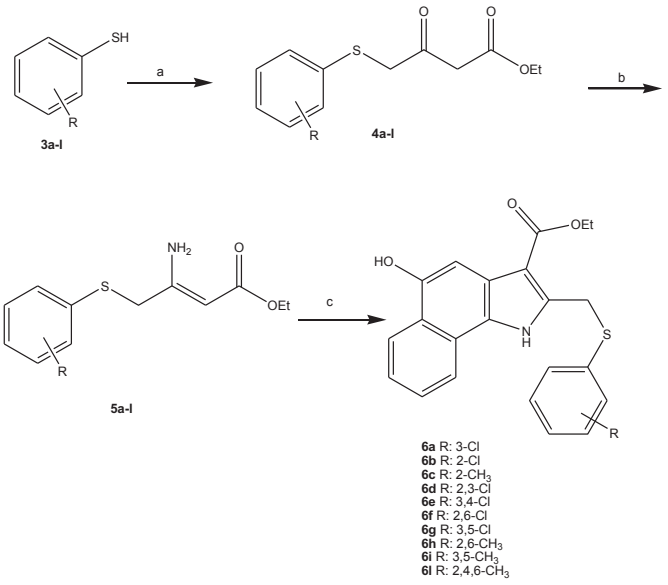


Fig. 1. Chemical structure of compounds **1, 2a** and **2b.**

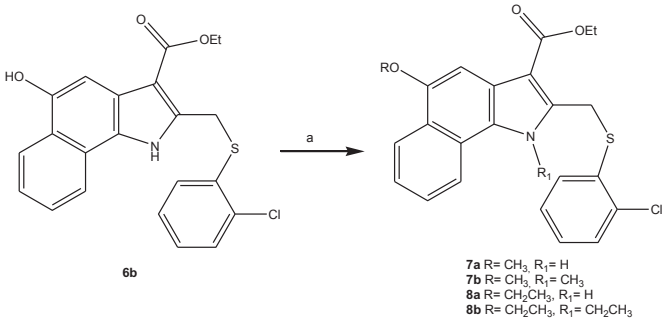


Scheme 1. Synthesis of compounds **6a-l**. Reagents and conditions: a) 4-chloroacetoacetate, NEt_3, CH_2Cl_2, 0 °C, 30 min; b) NH_4OAc, HOAc toluene, reflux, 6h; c) 1,4-naphtoquinone, ZnI_2, CH_2Cl_2, reflux, 40 min.

under basic conditions using microwave irradiation. Protection of the hydroxy group of **6b** with tertbutyl dimethyl silyl chloride (TBDSCl), alkylation with iodomethane and final deprotection with TBAF in THF allowed to obtain desired compound **11** in good yield (Scheme 2) (see Scheme 3)

2.2. Evaluation of 5-LOX activity and structure-activity Relationships

In order to assess the effects of the synthesized compounds on 5-LOX product synthesis, a cell-free assay using isolated human recombinant 5-LOX and a cell-based assay using human neutrophils was applied. The cell-free assay allows identifying compounds that directly interfere with 5-LOX catalytic activity, whereas the cell-based test system considers cellular regulatory aspects of 5-LOX product synthesis, and as such offers several points of attack of a given compound (e.g., inhibition of FLAP or coactosine-like protein (CLP), interference with 5-LOX-activating lipid hydroperoxides, protein kinases or Ca^{2+} mobilization, and 5-LOX translocation/membrane association) [21]. The reference 5-LOX inhibitor N-[1-(1-benzothien-2-yl)ethyl]-N-hydroxyurea (zileuton) was used to control the 5-LOX activity assays. As shown in Table 1, all tested compounds (**6a-l**) which maintain OH and NH groups in



Scheme 2. Synthesis of compounds **7a-b** and **8a-b**. Reagents and conditions: a) Iodomethane or iodoethane K_2CO_3, Acetone, MW, 30 min, 130 °C.