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Short communication

Synthesis and biological evaluation of fluorinated 1,5-diarylpyrrole-3-alkoxyethyl ether derivatives as selective COX-2 inhibitors endowed with anti-inflammatory activity

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ABSTRACT

A series of substituted 1,5-diarylpyrrole-3-alkoxyethyl ethers were previously synthesized and the potential anti-inflammatory and antinociceptive activities of these compounds were evaluated *in vivo*. The compounds displayed a very good activity against both carrageenan-induced hyperalgesia and oedema in the rat paw test. Therefore, in a very preliminary test, compounds (**8a,b**) showed antiproliferative activity in the HaCaT (aneuploid immortal keratinocyte from adult human skin) cell models. On these basis, our research continued with the synthesis of fluorinated derivatives (**8c**,**d**, **9b-d**, and **10b-d**) with the aim of improving the pharmacokinetic profile of the previous active compounds. Substitution of a hydrogen atom by a fluorine atom may change the conformational preferences of the molecules due to stereoelectronic effects and also fluorine atom may be able to exert the metabolic obstruction reducing the "first-pass effect". Compound **10b** exhibited a prominent *in vivo* anti-inflammatory and antinociceptive activities, in addition its antiproliferative power in an *in vitro* model of human skin cancer is herein described.

1. Introduction

Prostaglandin G/H synthase enzymes (PGHS), commonly known as cyclooxygenases (COX), are responsible for the formation of important biological mediators called prostanoids, which play a critical role in various biological processes [1]. Arachidonic acid (AA) is a 20 carbon unsaturated fatty acid distributed throughout the lipid bilayer of the cell membranes. Phospholipase A2 (PLA_2) enzymes cleave membrane bound arachidonate for the conversion into bioactive precursors. AA can be metabolized through COX pathway via a two-step process: the first step involves conversion of AA to prostaglandin G2 (PGG_2), a 9,11-endoperoxide-15-hydroperoxide, and in the second step peroxidase reduces PGG_2 to prostaglandin H_2 (PGH_2). Specific PG synthases further metabolize PGH_2 to give structurally related bioactive lipids, including PGs PGE_2, PGD_2, $PGF_{2\alpha}$, prostacycline PGI_2 and thromboxane A_2 (TxA2) [2].

Non-Steroidal Anti-Inflammatory Drugs (NSAID), which include both traditional NSAIDs (*t*NSAIDs) and selective inhibitors of COX-2 (COXib) (Fig. 1), relieve pain and inflammation suppressing the COX function of PGHS and the consequent formation of PGE_2 and

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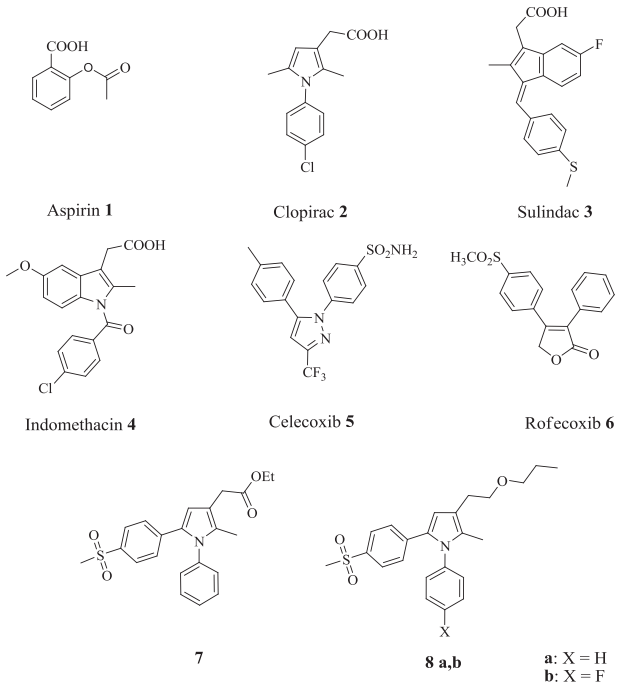

Fig. 1. Structures of reference compounds.

prostacyclin (PGI_2), along with other prostanoids [1].

Long-term therapy with *t*NSAIDs generates adverse gastrointestinal complications ranging from stomach erosions and silent intestinal ulcerations to life-threatening complications [3]. Selective inhibitors of COX-2 depress prostacyclin (PGI_2), an atheroprotective agent, but not COX-1-derived thromboxane A_2 (TXA_2), a pro-aggregatory and vasoconstrictor mediator, which might predispose patients to heart attack and stroke [4]. In 2004, Rofecoxib, (4-[4-(methylsulfonyl)phenyl]-3-phenyl-2(5*H*)-furanone), marketed as Vioxx was withdrawn from the market due to increased risk for cardiovascular thrombotic events in a long term-therapy [5].

Moreover, the involvement of COX-2 in cancer development was previously evidenced by pharmacological analysis of PGs in different human cancers. PGE_2 specifically exerts carcinogenic effects in the human body. It was found that premalignant lesions and established cancers produce excessive quantities of PGE_2 and this enhances tumor cell growth and increases tumor invasiveness [1]. COX-2 is up-regulated in a number of epithelial cancers, including those originating in the colon and rectum, stomach, breast, prostate, and lung [6]. In the context of cutaneous tissues, increased expression of COX-2 and production of its primary product PGE_2 have been reported to increase cell growth and decrease apoptosis. PGE_2 is also thought to be responsible for different skin cancers, such as squamous cell and basal cell carcinoma [7].

During the last decade a large number of COX inhibitors have been described. In particular, very recently novel chemical entities have been identified by different approaches such as: i) rational design providing novel scaffolds based on 4-phenylpyrimidine-2(1*H*)-thiones [8] and, indole based peptidomimetics [9], or ii) computational procedures providing novel and structurally unrelated COX-2 inhibitors [10].

Within a large programme devoted to the design and synthesis of new anti-inflammatory agents based on a diaryl heterocyclic scaffold, we previously synthesized different series of 1,5-diarylpyrrole-3-acetic esters **7** [11] and 3-alkoxyethyl ethers **8a,b** [12] as new selective COX-2 inhibitors, in which the pyrroloacetic subunit, reminiscent of indomethacine and clopirac was conjugated with vicinal diaryl moiety mimicking the main feature of COXib (Fig. 1).

These compounds were shown to be potent and highly selective COX-2 inhibitors in *in vitro* cell culture assays [12–14]. The potential anti-inflammatory and antinociceptive activities of these compounds were also evaluated *in vivo*, demonstrating a very good activity against both carrageenan induced hyperalgesia and oedema in rat paw test. Furthermore, compounds **8a,b** showed, in a very preliminary screening, a clear inhibition of cell proliferation on HaCaT cells with non-toxic effects in the range between 10 and 100 μM [7].

Encouraged by these data, we focused our attention on the preparation of two homologous series of differently fluorinated 3-substituted-1,5-diarylpyrrole alkoxyethyl ethers (**8c,d**; **9b-d** and **10b-d**) (Fig. 2) with the aim of investigating the biological effects that can be elicited by the insertion of a fluorine atom on different portion of the previously synthesized COX-2 inhibitors. Compound **10b**, which displays the best biological profile in terms of affinity and selectivity toward COX-2, as well as percent inhibition of the enzyme (cell culture assay), was also investigated for its *in vivo* anti-inflammatory, analgesic and antiproliferative activity, displaying interesting results.

2. Results and discussion

2.1. Chemistry

The synthetic procedure used to obtain final compounds **8c,d**, **9b-d** and **10b-d** is depicted in Scheme 1. Briefly, the suitable alcohols **11c-d** were treated with propyl iodide in NaOH 50% (w/w) solution, following the previously reported procedure [12] to obtain final compounds **8c,d**. Using the same procedure, derivatives **11b-d** were treated with the proper hydroxyalkyl bromide in its tetrahydropyranyl protected form [15] (see Supplementary Material) to yield compounds **12b-d** and **13b-d** that were successively deprotected to give hydroxyalkyl ethers **14b-d** and **15b-d**. These derivatives were then activated by tosylation and treated with $Bu_4N^+F^-$ to afford fluorinated compounds **9b-d** and **10b-d** in satisfactory yield. Purity of compounds **8c,d**, **9b-d, 10b-d** was assessed by RP-HPLC and was found to be higher than 95% (see Supplementary Material for further details).

2.2. Structure-activity relationship, molecular modeling studies and biological evaluation

In this limited series of compounds the presence of an additional fluorine atom in the 3-alkoxyalkyl side-chain with respect to compounds **8a,b** would allow us to assess if the contemporary presence of fluorine atoms in two different portions of the molecule could influence the interaction of these new inhibitors with the COX-2 active site. In particular, compounds **8c,d** were synthesized

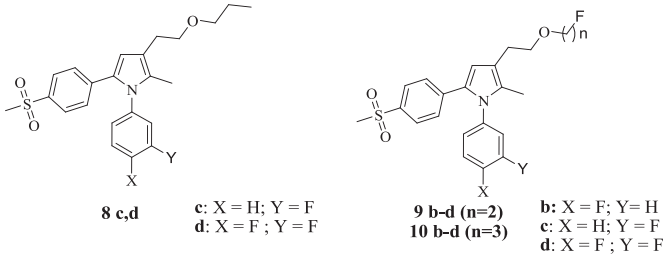

Fig. 2. Structure of fluorinated compounds **8c,d**, **9b-d** and **10b-d**.