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Synthesis and Activity of a New Methoxytetrahydropyran Derivative as Dual Cyclooxygenase-2/5-Lipoxygenase Inhibitor

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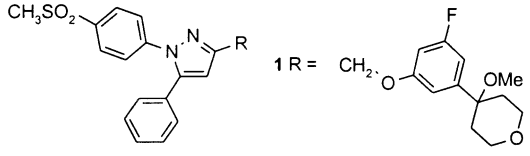
Abstract—Dual COX-2/5-LO inhibitors are described as potential new therapeutic agents for inflammatory diseases. A surprisingly potent effect of a 5-LO pharmacophoric group on the COX-2 inhibition is presented as well as pharmacological in vitro and in vivo results.

Introduction

Non-steroidal anti-inflammatory drugs are widely used in the treatment of pain and inflammation.[1] These compounds non selectively inhibit the two isoforms of the cyclooxygenase (COX-1 and COX-2) and thus prevent the upregulation of prostaglandin formation, which otherwise lead to an increase of vascular permeability, edema, hyperalgesia, pyrexia and inflammation.[2–5] Recently, it was demonstrated that COX-2 selective inhibitors may relieve the symptoms in these pathologies while exhibiting a safer toxicity profile.[6–9] The other major route of arachidonic acid (AA) metabolism is the lipoxygenase pathway which generates leukotrienes (LTs). LTB_4 and the cysteinyl-leukotrienes have powerful pro-inflammatory properties and the inhibition of this metabolic pathway led to the development of new therapeutic treatments for pathologies such as asthma, allergies and other inflammatory disorders.[10–12] It has been pointed out that inhibiting only one of these biosynthetic ways could shunt the metabolism of AA towards the other pathway, thus leading to potential side effects.[13] Pharmacologically active compounds that inhibit both enzymes at similar concentrations would have the potential to provide more complete relief for patients suffering from arthritis and inflammatory, hypersensitivity, dermatological or cardiovascular disorders.[14] Only a few compounds were designed to inhibit both COX-2 and 5-LO enzymes and this should have a synergistic effect on the reduction of the inflammatory process.[15,16] Our effort to find potent in vitro but also orally active inhibitors has led us to explore the new series of compounds **1**–**5**.



2 R = CH_3
3 R = C_4H_9
4 R = COOEt
5 R = CH_2OH

In compound **1**, the pyrazole of the COX-2 inhibitor Celecoxib is substituted at position 3 by the pharmacophoric group 4-(3-fluoro-5-oxy)phenyl-4-methoxytetrahydropyran of ZD-2138, a 5-LO inhibitor from Zeneca. This compound presents an excellent overall biological profile.

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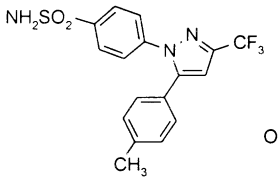
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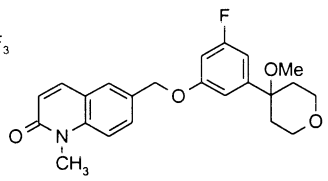
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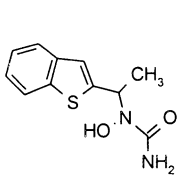
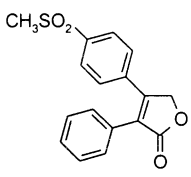
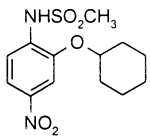

Zileuton


Rofecoxib


NS-398

Structure–Activity Relationships and Drug Design

The original idea was to verify that the combination of the pharmacophores of the COX-2 and 5-LO inhibitors on a molecule would give a dual inhibitor. The chosen groups were the classic tricyclic sulfonamide (Celecoxib-like) as the COX-2 part[17,18] and the 4-methoxytetrahydropyran substituent of ZD-2138. This compound was selected because it was devoid of redox and iron-chelating properties. It should be less prone to cause undesirable side effects. Comparison with the reference compounds, Zileuton (a 5-LO inhibitor) and Rofecoxib or NS-398 (COX-2 selective agents) showed a potent COX/5-LO inhibition and a high COX-2 selectivity of compound **1** (Table 1).

The idea of pharmacophoric combination was already developed by Searle[26] for pyrazoles (Searle 1, Table 2) and oxazoles (Searle 2, Table 2), possessing a selectivity COX-2/COX-1 but a weak 5-LO inhibition.

Nevertheless, the in vitro results were not confirmed in vivo. Indeed, the most active compound (Searle 2) is weakly active on the rat paw edema model (15% inhibition at 10 mg/kg per os).[28] To rationalise the importance of the various substituents, compounds **2**–**5** were prepared (Table 3). They do not inhibit the cyclooxygenases and have no or low potency as 5-LO inhibitors. Incorporation of the methoxytetrahydropyran moiety increases 5-LO potency which might be expected but surprisingly also provide potent and selective COX-2 inhibition.

Docking of **1** in the active site of human COX-2, modelled from the corresponding crystallised one of mouse, was performed with DISCOVER module (CVFF forcefield) of Biosym software.[29] In one of the most stable (−52.87 kcal/mol) binding mode (Fig. 1), the sulfone group fills the specific hydrophilic pocket (with Val523) and forms H bond with Arg513. These molecular modelling studies correlate well with established results[30] and more recent data.[31] In addition, the 5-LO pharmacophoric group fits well into the N/E region of the active site. This small lipophilic cavity, where are situated Phe209, Val228, Ile377, Phe381, Phe529, Leu534, is identical in COX-1 and 2. H bond is observed between the methoxy group and Ser530.

Chemistry

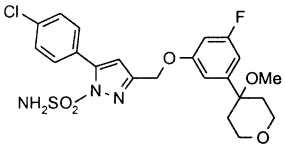
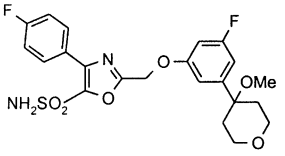
The diarylpyrazole backbone was prepared from an phenylalkynone for compounds **2** and **3** and the corresponding 4-methylsulfonylphenylhydrazine and acetic acid in refluxing EtOH (Scheme 1). The second method

Table 1. Comparison of COX-1, COX-2 and 5-LO inhibitory activities

Compd	COX-1 IC_{50} (μM)[a]	COX-2 IC_{50} (μM)[b]	COX-1/COX-2 selectivity	5-LO IC_{50} (μM)[c]
Rofecoxib	> 10	0.001	> 1000	> 10
NS-398	3.0	0.001	> 1000	> 10
Zileuton	> 10	> 10	ns[d]	2.9
1	> 10	0.05	> 200	0.003

[a]Values determined using monocytes-like cells.
[b]Values determined using osteosarcomes.
[c]Values determined using granulocytes-like cells. Each IC_{50} value corresponds to an average of at least two independent experiments performed in duplicate. Experiments were performed using the classical procedures described in the literature.[19–25]
[d]Non selective.

Table 2. In vitro potency

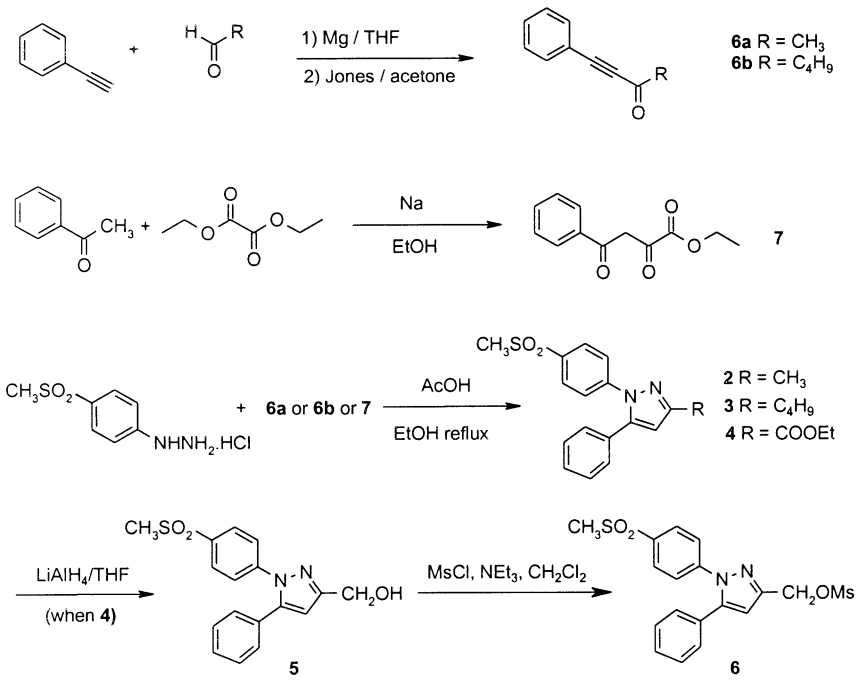
Compd	COX-1 IC_{50} (μM)[a]	COX-2 IC_{50} (μM)[a]	5-LO IC_{50} (μM)[b]
 **Searle 1**	38	< 0.1	0.15
 **Searle 2**	1.3	< 0.1	0.30

[a]Prostaglandin E_2 inhibition was measured using recombinant COX-1 and COX-2 prepared as described by Gierse.[27]
[b]The 5-LO activity of the compounds was determined by the inhibition of calcium ionophore-induced leukotriene B_4 produced in human blood.

Table 3. Comparison of COX-1, COX-2 and 5-LO inhibitory activities[a] of compounds **1**–**5** using monocytes-like, osteosarcomes and granulocytes-type cells, respectively

Compd	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)	COX-1/COX-2 selectivity	5-LO IC_{50} (μM)
1	> 10	0.05	> 200	0.003
2	> 10	> 10	ns[b]	40.0
3	> 10	> 10	ns[b]	6.0
4	> 10	> 10	ns[b]	> 10
5	> 10	10.0	ns[b]	> 10

[a]Each IC_{50} value corresponds to an average of at least two independent experiments performed in duplicate.
[b]Non selective.



Scheme 1. Synthesis of the diarylpyrazoles.

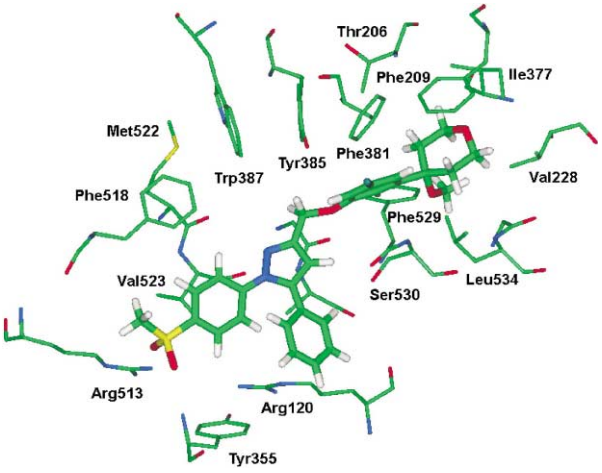


Figure 1. Molecule **1** in the COX-2 active site.

involved the same hydrazine and ethyl (2,4-dioxo-4-phenyl)butanoate **7**.

Compound **4** was reduced in the presence of $LiAlH_4$ in anhydrous THF yielding the 1-(4-methylsulfonylphenyl)-5-phenyl-1*H*-pyrazole-3-methanol **5**. The alcohol was then mesylated in the presence of NEt_3 in CH_2Cl_2 and reacted with 3-fluoro-5-[4-(4-methoxytetrahydropyranyl)]phenol[26] and Cs_2CO_3 in DMF at 80 °C (Scheme 2) to yield 3-(3-fluoro-5-[4-(4-methoxytetrahydropyranyl)]phenoxymethyl)-1-(4-methylsulfonylphenyl)-5-phenyl-1*H*-pyrazole **1**.

Pharmacology

In vivo efficacy was evaluated in the model of arachidonic acid-induced ear edema in rat.

Animals

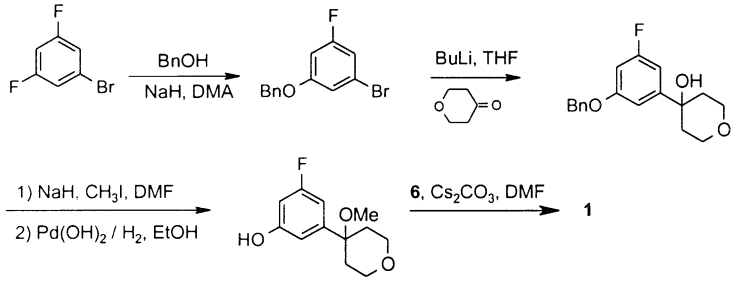
Male Wistar rats 150±20 g body weight were used. The rats were divided in three groups of nine. Group 1: control; group 2: reference compound (NS-398); and group 3 received compound **1**. Inflammation was induced by topical application of 25 μL of 25% AA in acetone to the internal face of the left ear. The right ear (control) received acetone (25 μL). Either AA solution or acetone were applied with an automatic pipette.

Treatment

For oral administration, the drugs were dissolved in carboxymethylcellulose 1%. The treatment was administered 2 h before AA application. For intravenous administration, the drugs were dissolved in dimethyl sulfoxide and injected (via penile vein) 15 min before AA application. In that case, intravenous administration was carried out under isoflurane anaesthesia.

Experiments and results

One hour after AA application, the animals were anaesthetised. A 5-mm punch biopsy was performed on each ear. Edema induced by AA was assessed as the difference on weight between left and right ears. Intravenous administration of compound **1** led to a strong reduction of AA-induced ear edema. Indeed, compound **1** (0.01 or 0.1 μg/kg) decreased edema by 41 and 44%, respectively. Oral administration of **1** (0.1 and 5 mg/kg) showed a similar effect because the edema was reduced by 54 and 41%, respectively. The implication of both 5-LO and COX-2 pathways were checked in animals pretreated with Zileuton and Rofecoxib. In such cases, the maximal inhibitory effect reached about 40%.



Scheme 2. Synthesis of the 3-(3-fluoro-5-[4-(4-methoxytetrahydropyranyl)]phenoxymethyl)-1-(4-methylsulfonylphenyl)-5-phenyl-1*H*-pyrazole **1**.

Conclusion

A new dual inhibitor of COX-2 and 5-LO has been prepared. A powerful pharmacophoric groupement that can improve the inhibitory activity of a molecule on both COX-2 and 5-LO in vitro and in vivo has been demonstrated. This finding is in accordance with the previous observations made on Searle sulfonamide-substituted pyrazoles. We have also pointed out that 3-alkyl -1-(4-methylsulfonylphenyl)-5-phenyl-1*H*-pyrazoles do not show any activity against COX-2 in our tests.

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