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Research paper

Potent multitarget FAAH-COX inhibitors: Design and structure-activity relationship studies

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) exert their pharmacological effects by inhibiting cyclooxygenase (COX)-1 and COX-2. Though widely prescribed for pain and inflammation, these agents have limited utility in chronic diseases due to serious mechanism-based adverse events such as gastrointestinal damage. Concomitant blockade of fatty acid amide hydrolase (FAAH) enhances the therapeutic effects of the NSAIDs while attenuating their propensity to cause gastrointestinal injury. This favorable interaction is attributed to the accumulation of protective FAAH substrates, such as the endocannabinoid anandamide, and suggests that agents simultaneously targeting COX and FAAH might provide an innovative strategy to combat pain and inflammation with reduced side effects. Here, we describe the rational design and structure-active relationship (SAR) properties of the first class of potent multitarget FAAH-COX inhibitors. A focused SAR exploration around the prototype **10r** (ARN2508) led to the identification of achiral (**18b**) as well as racemic (**29a-c** and **29e**) analogs. Absolute configurational assignment and pharmacological evaluation of single enantiomers of **10r** are also presented. (*S*)-(+)-**10r** is the first highly potent and selective chiral inhibitor of FAAH-COX with marked *in vivo* activity, and represents a promising lead to discover novel analgesics and anti-inflammatory drugs.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely utilized to treat pain and inflammation [1], but their chronic use is hindered by a variety of potentially serious adverse events that include gastrointestinal (GI) mucosal lesions, bleeding and perforations [2–5]. Conventional NSAIDs inhibit the two isoforms of cyclooxygenase (COX), COX-1 and COX-2, which catalyze the first committed steps in the biosynthetic pathway that converts arachidonic acid (AA) into inflammatory prostanoids such as prostaglandin E_2 (PGE_2) and thromboxane A_2 (TXA_2) [6]. The dual role of COX-1-derived PGE_2 as inflammation promoter and mucosal tissue protectant explains, at least in part, why NSAIDs cause damage to the GI tract [7–10]. Efforts to overcome this problem have led to the development of selective COX-2 inhibitors, which combine a high level of anti-inflammatory efficacy with a reduced propensity to cause injury to the GI mucosa [6]. Nevertheless, the use of COX-2 inhibitors has been linked to a distinctive set of adverse cardiovascular effects [11,12]. Thus, the need for safe and effective drugs that can be used in the treatment of chronic inflammatory disorders remains urgent.

A promising approach to meet this need is offered by targeting with a single agent more than one component of the inflammatory cascade [13–15]. Agents designed to achieve this objective include nitric oxide (NO) donors-NSAIDs [16,17], COX-2 inhibitors–NO–donors [18,19], hydrogen sulfide (H_2S) donors-NSAIDs [20–22], as well as compounds that block distinct enzymes of the AA pathway, such as COX/lipoxygenase [23,24] and COX-2/soluble epoxy hydrolase (sEH) [25]. Another potential multitarget strategy to treat inflammation is the concomitant inhibition of COX and fatty acid amide hydrolase (FAAH) [26] [27–33], a serine hydrolase

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that deactivates a family of analgesic and anti-inflammatory lipid amides that are produced by host-defense cells and other cells in the body [34,35]. These lipid mediators include the endocannabinoid anandamide (arachidonoylethanolamide) – which engages cannabinoid-1 (CB_1) and CB_2 receptors to suppress neutrophil migration [36] and prevent immune-cell recruitment [37,38] – as well as the endogenous peroxisome proliferator-activate receptor-α (PPAR-α) agonists, palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) [39–41]. In addition to opposing pain and inflammation, these FAAH substrates are also protective of the GI mucosa [42,43]. Indeed, studies in animal pain models have shown that co-administration of FAAH and COX inhibitors results in a synergistic potentiation of analgesia along with reduced gastric damage [44–46].

In several chronic inflammatory conditions, including inflammatory bowel disease (IBD), FAAH [47–49] and COX-2 [50] are expressed at abnormally high levels. This simultaneous up-regulation may help establish a pathological state that exacerbates inflammation by amplifying inflammatory COX-dependent signals at the expense of defensive FAAH-regulated mediators. This hypothesis predicts that drugs targeting both COX and FAAH should have substantial anti-inflammatory efficacy combined with reduced GI toxicity. In a recent study, we provided support to this hypothesis using a multitarget modulator based on the hybrid scaffold **1** (Fig. 1) [51]. This scaffold merges key pharmacophores of two known classes of FAAH and COX inhibitors – *O*-aryl carbamates [52–58] such as [3-(3-carbamoylphenyl)phenyl] *N*-cyclohexylcarbamate (URB597, **2**) [54,57], and 2-aryl propionic acids [6] such as flurbiprofen, **3a** [59–61] – which share a biphenyl core as a common structural motif (*A* and *B* rings, Fig. 1). Moreover, structure-activity relationship (SAR) studies of these scaffolds supported the hypothesis of additional elements of structural overlapping, such as the oxygenated substituents at the 3′-position of the *A* phenyl ring, corresponding to the carbamate functionality of **2** [53,54,56] and the ether moieties of **3b** or **3c** [61], respectively (Fig. 1).

This SAR work led to the identification of compound **10r** ((±)-2-[3-fluoro-4-[3-(hexylcarbamoyloxy)phenyl]phenyl]propanoic acid, ARN2508) [51] as a potent *in vivo* active inhibitor of intracellular FAAH and COX activities, which exerts profound anti-inflammatory effects in mouse models of IBD without causing COX-dependent gastric toxicity [51]. In the present study, (a) we outline the in-depth SAR investigations that led to the discovery of compound **10r** [51]; (b) we report an expansion of this SAR work, which culminated in the identification of several new and potent multitarget inhibitors (**18b**, **29a-c** and **29e**); and, finally (c) we describe the absolute configurational assignment and pharmacological properties of single enantiomers of **10r**, identifying (*S*)-(+)-**10r** as the first chiral inhibitor of FAAH-COX with marked *in vivo* activity.

2. Results and discussion

2.1. Chemistry

Compounds **10a-t** were synthesized from the corresponding phenol **8** through a carbamoylation reaction, using commercially available isocyanates, followed by the hydrolysis of the methyl esters **9a-t**, under acidic conditions (Scheme 1).

The intermediate **8** was prepared in four steps, starting from the acid **4**, obtained as previously described [62]. Compound **4** was converted to the corresponding methyl ester **5**, under standard acidic conditions, to afford, after catalytic hydrogenation with ammonium formate in the presence of Pd/C, the resulting aniline **6**. Compound **6** was then transformed into the corresponding diazonium salt, that was reacted *in situ* with NaI to obtain the phenyl iodide **7** in good yield, which was converted, under ligand less Suzuki cross coupling conditions [63], to the biphenyl derivatives **8** and **13a-c** in excellent yield (Schemes 1–3).

3-Hydroxypropyl derivative **12** was synthesized by reduction of the methyl ester **8** to the alcohol **11** (Scheme 1). Although lithium aluminum hydride succeeded in reducing the ester **8**, a significant *des*-fluorinated side product was observed and separation of the two compounds was troublesome. Therefore, a milder reducing agent, such as zirconium borohydride generated *in situ*, was used to afford a clean conversion of **8** to **11** [64], which was then converted to **12** under standard carbamoylation reaction conditions (Scheme 1).

Carbamates **15a-b** and urea **15c** were prepared from the corresponding phenols **13a-b** and aniline **13c**, respectively, through a carbamoylation reaction using *n*-hexyl-isocyanate, followed by acidic hydrolysis of the methyl esters **14a-c** (Schemes 2 and 3). The reverse carbamate **15d** was prepared upon activation of the aniline **13c** with triphosgene, and, then, reaction with *n*-hexanol, followed by acidic hydrolysis of the methyl ester **14d** (Scheme 3).

Compounds **18a** and **21a-b** were synthesized by reacting the phenyl iodides **16b** and **19a-b**, with (3-hydroxyphenyl)boronic acid under Suzuki cross coupling conditions, followed by carbamoylation reaction of phenols **17** and **20a-b** under standard conditions (Schemes 4 and 5).

Compounds **18a** and **21b** were then transformed into the corresponding acids **18b** and **21c** under standard acidic hydrolysis (Schemes 4 and 5).

Compounds **29a-g** were synthesized following the synthetic sequence described in Scheme 6 *p*-Nitrofluorobenzenes **22a-d** were reacted with diethyl methylmalonate followed by decarboxylation to the corresponding acids **23a-d**. **23a-d** and the commercially available **23e** were converted into methyl esters **24a-e** in acidic MeOH. In addition, the phenolic intermediate **24d** was directly converted into the corresponding *O*-Bn protected **24f**,

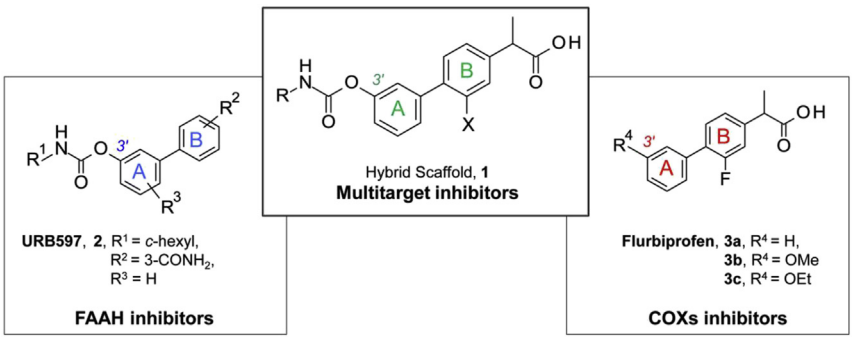

Fig. 1. Rational design of a 'hybrid scaffold' for FAAH and COX inhibition.