Published in final edited form as:

ChemMedChem. 2016 June 20; 11(12): 1242–1251. doi:10.1002/cmdc.201500395.

A double whammy – targeting both fatty acid amide hydrolase (FAAH) and cyclooxygenase (COX) to treat pain and inflammation

Dr Rita Scarpelli^[a], Dr Oscar Sasso^[a], and Prof Daniele Piomelli^{[a],[b]}

Daniele Piomelli: daniele.piomelli@iit.it

^[a]Department of Drug Discovery and Development, Fondazione Istituto Italiano di Tecnologia, via Morego 30, 16163 Genoa, Italy

^[b]Departments of Anatomy and Neurobiology, Pharmacology and Biological Chemistry, University of California, Irvine, CA 92697-4625, USA

Abstract

Pain states that arise from non-resolving inflammation, such as inflammatory bowel disease or arthritis, pose an unusually difficult challenge for therapy because of the complexity and heterogeneity of their underlying mechanisms. It has been suggested that key nodes linking interactive pathogenic pathways of non-resolving inflammation might offer novel targets for the treatment of inflammatory pain. Non-steroidal anti-inflammatory drugs (NSAIDs), which inhibit the cyclooxygenase (COX)-mediated production of pain- and inflammation-inducing prostanoids, are a common first-line treatment for this condition, but their use is limited by mechanism-based side effects. The endogenous levels of anandamide, an endocannabinoid mediator with analgesic and tissue protective functions, are regulated by fatty acid amide hydrolase (FAAH). The present article outlines the pharmacological and chemical rationale for the simultaneous inhibition of COX and FAAH activities with designed multi-target agents. Preclinical studies indicate that such agents may combine superior anti-inflammatory efficacy with reduced toxicity.

Graphical Abstract

Accumulated evidence suggests that drugs that target both FAAH and COX should have substantial analgesic and anti-inflammatory efficacy combined with reduced gastrointestinal toxicity. Herein we review the rational basis of multitarget FAAH/COX inhibition and the state of the art of medicinal chemistry efforts.

Keywords

enzymes; drug design; multitarget inhibitors; pain; inflammation; structure-activity relationships

1. Introduction

Despite substantial advances made in the last decades, the identification of safe and effective therapeutics for the management of acute and chronic pain remains a formidable challenge in drug development. The current standards of care – opioids, non-steroidal anti-inflammatory drugs (NSAIDs) and gabapentinoids – help many patients, but it is estimated that only 25% of them receive adequate pain relief. Moreover, available analgesic drugs exert acute and long-term side effects and can have, as in the case of the opioids, a significant potential to induce dependence and addiction. Pain conditions that arise from non-resolving inflammatory pathologies – such as inflammatory bowel disease and arthritis – are particularly challenging owing to the complexity and heterogeneity of their underlying pathogenesis. In such conditions, key control nodes that link interactive molecular pathways involved in promoting and maintaining pain, inflammation and tissue damage might be exploited to achieve greater clinical benefits.^[1] In this context, attention has focused on two important families of lipid-derived mediators: prostanoids and endocannabinoids.

Accumulated evidence points to the existence of multiple biochemical and functional interactions between these signaling pathways, which might offer useful molecular targets for analgesic and anti-inflammatory therapy.^[2] In the present review, we outline the scientific rationale for simultaneously inhibiting two pivotal enzymes of endocannabinoid and prostanoid metabolism – fatty acid amide hydrolase (FAAH) and cyclooxygenase (COX) – and review medicinal chemistry approaches and structure-activity relationship (SAR) studies of the multi-target FAAH/COX inhibitors discovered to date.

2. Interacting lipid pathways

Fatty acid ethanolamides (FAEs) such as arachidonylethanolamide (anandamide), palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) (Figure 1), constitute a family of signaling lipids involved in the regulation of a wide range of physiological and pathological processes, including pain and inflammation.^[3]

The FAEs exert their biological actions by binding to selective protein receptors in cells. Anandamide is an endogenous agonist of G-protein-coupled CB₁ and CB₂ cannabinoid receptors, which mediates the majority of its biological effects.^[4] Other pharmacological targets for anandamide, such as transient receptor potential vanilloid type 1 (TRPV-1), have been identified, but their physiological significance remains unclear.^[5]

OEA and PEA do not productively interact with cannabinoid receptors, but act by engaging nuclear peroxisome proliferator-activated receptor- α (PPAR- α) and, to a lesser extent, GPR119.^[6]

The FAEs are produced from phospholipid precursors in membranes through either tonic or 'on demand' mechanisms. Therefore, the signaling activity of these substances is strictly dependent on the enzymes responsible for their formation and degradation.^[6d, 7] FAAH is the main enzyme involved in the degradative hydrolysis of anandamide into arachidonic acid (AA) and ethanolamine (Figure 2).^[8] It is a membrane-bound serine hydrolase localised to intracellular organelles such as microsomes and mitochondria, and is highly expressed in brain and liver, among other tissues.^[9]

Soon after its discovery,^[8a, 10] FAAH attracted attention as a potential target for indirect-acting cannabinoid agents, that is, for compounds that stimulate cannabinoid receptors by modulating endogenous anandamide levels.^[5b] There are multiple precedents that justify the adoption of this indirect strategy (e.g. neurotransmitter uptake inhibitors), which is further supported by the fact that direct cannabinoid receptor activation causes a variety of central and peripheral side effects.^[11] Conversely, protecting anandamide from FAAH-mediated degradation might result in a more physiological activation of cannabinoid receptors – similar to that caused by endogenously released anandamide – and might thus exert a restricted, and hopefully favorable, set of pharmacological effects compared to exogenous direct-acting agonists.^[12] One important piece of evidence in support of this theory comes from experiments with mice lacking the gene encoding for FAAH. These mutant mice display an analgesic and anti-inflammatory phenotype, yet do not show any of the typical signs of direct CB₁ receptor activation, such as decreased motor activity, lowered body temperature and catalepsy.^[13]

Another element in support of FAAH's value as a target for analgesic drugs is provided by the preclinical pharmacological profile of FAAH inhibitors. Several classes of such inhibitors, both reversible and irreversible, have been disclosed in the scientific and patent literature.^[14] A few of the most investigated classes are exemplified in Figure 3.

The first selective and systemically active FAAH inhibitor to be disclosed, the *O*-arylcarbamate derivative URB597,^[12c, 15] exerts mild anti-hyperalgesic and anti-allodynic

effects in animal models of acute and chronic pain.^[12c, 16] Interestingly, its peripherally restricted analogue, URB937, is markedly more effective in the same models.^[17] This is consistent with the presence of a pro-algesic anandamide-dependent circuit in the dorsal spinal cord, which is potentiated by global FAAH blockade.^[18]

The compound PF-3845 provides an example of a structurally different class of FAAH inhibitors, based on a urea scaffold, which effectively reduces nociceptive responses in a rat model of chronic inflammatory joint pain (Complete Freund's Adjuvant, CFA).^[19] Improvements on PF-3845 yielded PF-04457845, which shows excellent pharmacokinetics and is also active in the rat CFA model.^[20] PF-04457845 progressed into Phase II clinical trials for the treatment of osteoarthritic pain, but was found to be ineffective.^[21] While disappointing, this negative result is consistent with animal studies suggesting that FAAH inhibitors are only weakly active in pain conditions – such as osteoarthritic and neuropathic pain – that are characterized by profound neuroplastic changes in the central nervous system.^[22] Acute pain states, such as those occurring after surgery or during treatment with antineoplastic drugs, might provide better indications for these agents.^[23]

In addition to anandamide, FAAH catalyzes the hydrolysis of PEA, OEA (Figure 1) and other bioactive FAEs.^[24] Thus, FAAH inhibition might produce beneficial effects in models of pain and inflammation through the contribution of PEA- and OEA-mediated activation of PPAR- α .^[13b, 25] Moreover, other endogenous signaling molecules, such as *N*-acyl glycines and *N*-acyl taurines, may also contribute.^[26]

Anandamide does not only modulate nociception, but can also attenuate neutrophil migration and immune-cell recruitment during inflammation, through activation of CB₁ and CB₂ receptors.^[27] This may be a factor in the ability of FAAH inhibitors to alleviate inflammatory symptoms in animal models of intestinal inflammation.^[13c, 28]

Innate immune and other host-defense cells produce a variety of pro-algesic and inflammatory eicosanoids through the action of the bifunctional membrane-bound enzyme, cyclooxygenase (COX) (Figure 2).^[29] There are two COX isoforms, COX-1 and COX-2, which share ~60% sequence homology and are located on the luminal surfaces of the endoplasmatic reticulum and the inner and outer membranes of the nuclear envelope. COX-1 is considered to be a constitutive enzyme and is expressed in most cells of the body. COX-2 is inducible in many tissues, but is also normally expressed in the brain, spinal cord and kidney.^[30] COX-2 induction occurs during tissue damage or inflammation in response to cytokines, mitogens and growth factors.^[31]

Both COX isoforms catalyze the first committed step in the pathway that converts membrane-derived AA into a large family of prostanoids that includes prostaglandin E₂ (PGE₂), prostacyclin (PGI₂) and thromboxane A₂ (TXA₂) (Figure 4).^[29] These signaling molecules play important roles in biology through the activation of selective G-protein-coupled receptors. Cardiovascular homeostasis, mucosal protection, inflammation, and cancer, are among the diverse physiological and pathological processes modulated by these agents.^[32]

Conventional NSAIDs – such as ibuprofen, flurbiprofen, diclofenac and indomethacin (Figure 5) – are non-selective inhibitors of both COX isoforms.^[33] Although they are widely prescribed to treat pain, inflammation and fever, their chronic use can produce serious gastrointestinal (GI) and renal damage.^[34] Inhibition of constitutive COX-1 activity in the gut mucosa is thought to underpin, at least in part, the toxic effects of NSAIDs on the GI tract.^[34a] This is due to the fact that the tonic production of prostanoids (e.g., PGE₂) by epithelial cells in the GI mucosa protects these cells from injury, a property that is also shared by anandamide (Figure 6).^[35]

In order to avoid COX-1-dependent GI toxicity, various COX-2-preferring inhibitors ('coxibs') (Figure 5) have been developed.^[29] These compounds are highly effective at reducing pain and inflammation, but exert their own set of potentially severe adverse events, which are mostly localized to the renal and cardiovascular systems.^[36]

COX-2, but not COX-1, converts anandamide into a family of biologically active prostaglandin ethanolamides ('prostamides').^[37] These agents are pro-algesic and do not interact with cannabinoid or prostanoid receptors, but their mechanism of action is still under investigation.^[38] Nevertheless, they represent an interesting point of intersection between endocannabinoid and prostanoid systems. The COX-2-mediated transformation of anandamide into pro-algesic prostamides has led to hypothesize the existence of deeper functional connections between the endocannabinoid and prostanoid systems. Confirming this idea, it was shown that genetic deletion or pharmacological blockade of CB₁ receptors prevents the antinociceptive effects produced by spinal administration of indomethacin in the mouse formalin test.^[39] Comparable results were obtained with another NSAID, flurbiprofen, given by intrathecal injection.^[40]

Further supporting the existence of a tight link between endocannabinoid and prostanoid signaling, local co-administration of ibuprofen and anandamide in the rat hind paw produced synergistic antinociceptive effects in the formalin test.^[41] The CB₁ receptor antagonist AM251 prevented this synergy.^[41] Another study, in which anandamide was used along with the selective COX-2 inhibitor rofecoxib or the non-selective COX inhibitor ibuprofen showed that these combinations reduce mechanical and thermal hyperalgesia in the rat sciatic nerve ligation model.^[42] In addition, co-administration of the NSAID ketorolac and the non-selective cannabinoid agonist WIN-55,212-2 exerted additive analgesic effects in the mouse acetic acid test of visceral pain.^[43] Collectively, these findings strongly imply that the endocannabinoid and prostanoid systems interact at multiple levels to regulate nociception.

Additional evidence in support of this hypothesis comes from experiments in which various combinations of FAAH inhibitors and NSAIDs were tested in animal models. These experiments have consistently shown that simultaneous FAAH and COX blockade produces synergistic analgesic responses, along with a marked reduction of NSAID-induced GI damage. In one study, the FAAH inhibitor URB597 synergistically enhanced the effect of diclofenac in the acetic acid test.^[44] In the same report, URB597 was also able to reduce gastric hemorrhages caused by the NSAID.^[44] This protective effect was likely due to FAAH blockade – because it was reproduced by genetic FAAH deletion – and required CB₁

receptor activation – because it was absent in mice lacking CB₁, but not CB₂, receptors.^[44] Like URB597, its peripherally restricted derivative URB937 reduced the number and severity of gastric lesions produced by indomethacin.^[17b] In addition to this gastroprotective effect, URB937 synergistically enhanced the antinociceptive effects of indomethacin in mouse models of inflammatory (carrageenan) and neurogenic (sciatic nerve ligation) pain. Notably, the antinociceptive and gastroprotective effects of URB937 were superior to those of URB597.^[44] Finally, co-administration of diclofenac and PF-3845 attenuated mechanical allodynia in models of inflammatory (carrageenan) and neurogenic pain (sciatic nerve ligation).^[45] Blockade of either CB₁ or CB₂ receptors attenuated the effects produced by the combined administration of FAAH and COX inhibitors.

These findings have provided a rationale for the design of molecules that carry multi-target FAAH and COX inhibitory activities. Single molecules with such a profile are expected to have several advantages over combination therapies, including more predictable pharmacokinetic profile and a reduced potential for drug-drug interactions.

3. Approaches to the design of multi-target FAAH/COX inhibitors

3.1. From single to multiple target inhibitors

FAAH is a homodimer of 64 kDa subunits and belongs to the amidase signature family, which is characterized by an unusual Ser241-Ser217-Lys142 catalytic triad. The residues of the catalytic triad interact through a network of H-bonds that facilitates proton exchange, activating the nucleophilic residue Ser241 (via deprotonation) and the leaving group of the substrate (via protonation). Adjacent to the nucleophilic serine side chain is a circle of four amide N-H bonds that form the oxyanion hole of FAAH. In addition to the catalytic core, FAAH is characterized by a series of channels and cavities, which include the membrane access channel (MAC) connecting the active site to an opening located at the membrane anchoring face of the enzyme, the cytosolic port (CP) that allows exit of hydrophilic products from the active site, and the acyl chain-binding pocket (ABP) – a narrower, longer and more hydrophobic channel that interacts with the acyl chain of the substrate during the catalytic reaction.^[14a, 46]

COX-1 and COX-2 are homodimers of 70 and 72 kDa subunits, respectively. Dimerization is required for structural integrity and catalytic activity. Each subunit contains one cyclooxygenase and one peroxidase active sites, which are located on opposite sides of the catalytic domain, with the heme prosthetic group positioned at the base of the peroxidase site.

Each monomer of COX consists of three structural domains: a short N-terminal epidermal growth factor domain, a membrane-binding domain (MBD), and a large, globular C-terminal catalytic domain. The catalytic domain constitutes the majority of the COX monomer and is the site of substrate binding and NSAID action. The entrance to the COX active site occurs at the base of the MBD and leads to a long hydrophobic channel that extends deep into the interior of the catalytic domain to form a constriction composed of three residues (Arg-120, Tyr-355, and Glu-524) that separate the entrance from the active site. Tyr385 is in the COX active site for both isoforms and is positioned at the end of the channel on the opposite side

of Arg-120. The peroxidase reaction converts Tyr385 to a tyrosyl radical species, which is necessary for activation of the COX reaction cycle. COX-1 and COX-2 active sites are very similar, but differ in the presence of a side pocket in COX-2 located above the Arg-120/Tyr-355/Glu-524 region. This COX-2 side pocket contains Val-523 (Ile-523 in COX-1) and a conserved Arg-513 (His-513 in most COX-1) at the base of the pocket and it has been proposed to be essential for selective COX-2 binding recognition.^[47]

Despite being evolutionary unrelated, FAAH and COX have some commonalities, possibly linked to the structural closeness of their substrates, which might be exploited in the design of multitarget inhibitors. In addition, knowledge of the chemical structures of the many FAAH and COX inhibitors now available may be helpful in the rational design of potential binders with desired multitarget pharmacology and drug-like properties.^[14a, 29]

Different drug design approaches have been developed for the identification of multi-target inhibitors leading to hybrid, fused or chimeric compounds.^[48] The following section illustrates the most relevant strategies and representative examples reported in the scientific and patent literature for the identification of FAAH and COX inhibitors.

One strategy for the design of multi-target FAAH/COX inhibitors has been the identification of known single target inhibitors (e.g., COX) that also show weak activity at the other target (e.g. FAAH), and are amenable to structural modifications aimed at enhancing activities on that target. Some NSAIDs directly bind to FAAH and inhibit its catalytic activity. In 1996, Paria et al. reported that indomethacin reduced anandamide hydrolysis in mouse uterus.^[49] Subsequent *in vitro* studies showed that suprofen, ibuprofen, fenoprofen, naproxen, ketoprofen, diclofenac,^[50] ketorolac, flurbiprofen^[51] and indomethacin^[52] inhibit FAAH activity, albeit with weak potencies (median inhibitory concentrations, IC₅₀, in the low to high μ M range).^[50a, 53]

These observations stimulated interest in identifying NSAID- based compounds that also express FAAH-inhibitory activity.^[50a, 51] In 2003, Cocco et al. described a series of heteroaromatic ibuprofen anilides, bearing substituted pyridine or pyrimidine groups, which showed improved analgesic activity and reduced GI side effects relative to the parent acid.^[54] This exploration identified compound **1** (ibu-am5) as the best analog. This compound produced a complete inhibition of acetic acid-induced writhing after oral administration in rats, which was associated with very low ulcerogenic effects compared to its parent molecule, ibuprofen (Figure 7).

The reduced GI toxicity of **1** was initially attributed to the amidation of the free carboxylic acid group present in ibuprofen, and the consequent reduction of its local irritating action^[55].

Alternatively, weaker GI effects were ascribed to a potential inhibitory action of this set of amide derivatives on COX-2, as previously reported for ester and amide analogs of aryl acetic and fenamic acids.^[56] Nevertheless, no additional data were reported in this study to support either of these hypotheses.

A more complete biochemical evaluation of compound **1**, together with 13 additional heteroaromatic amides of ibuprofen and indomethacin, was reported^[57] and later integrated with additional comparative data with novel analogs (Figure 7).^[58] In the former report,^[57] none of the six indomethacin amide analogs caused a complete inhibition of FAAH activity at the concentration tested and, for this reason, were not further profiled in COX assays. Within the ibuprofen amide series, compound **1** was confirmed to be the most promising analog, showing a more potent inhibitory activity against rat FAAH, but with a similar inhibitory profile against ovine COX-1 and COX-2 compared to ibuprofen. It was found that **1** inhibits FAAH activity in a non-competitive manner with IC₅₀ values of 4.7 μ M and 2.5 μ M at pH 6 and 8, respectively (for the FAAH assay conditions used, see^[59]). In comparison, the IC₅₀ values of ibuprofen for FAAH were 130 μ M and 750 μ M at pH 6 and 8, respectively. In intact C6 glioma cells, **1** inhibited FAAH with an IC₅₀ of 1.2 μ M at pH 6 and 8 (for the FAAH assay conditions used, see^[60]). In addition, **1** showed a good selectivity profile against other hydrolases, such as *N*-acylethanolamine acid amidase and monoacylglycerol lipase. The effects of **1**, ibuprofen and indomethacin on the specific binding of [³H]-CP55,940 to rat CB₁ and human CB₂ receptors were also investigated.^[57] The compound displayed little or no ability to interact with cannabinoid receptors (IC₅₀ = 41 μ M and 24 μ M for **1** towards CB₁ and CB₂, respectively).

In light of the overall *in vitro* pharmacological profile of **1**, a retrospective interpretation of its *in vivo* efficacy in the acetic acid model and its reduced ulcerogenic properties^[54] was proposed. The lower GI toxicity of **1** compared to ibuprofen was ascribed more to differences in the physicochemical properties of the two compounds, rather than to their inhibitory potencies toward COX-1. On the other hand, the different analgesic effect between the two molecules was linked to the ability of **1** to inhibit both COX and FAAH.

In a subsequent study,^[58] **1** was compared to ibuprofen and eight additional amide analogs of **1**. Compounds **1** and **2** were the most potent inhibitors of rat FAAH compared to ibuprofen (IC₅₀ = 134 μ M), showing IC₅₀ = 0.65 μ M and 3.6 μ M, respectively (Figure 7). Differently from previous reports from the same group,^[57] COX activity was measured using an oxygen electrode assay with commercially available ovine COX-1 and human recombinant COX-2 as enzyme sources (for the COX assay conditions used, see^[61]). Under those assay conditions, ibuprofen inhibited the activity of ovine COX-1 towards AA with an IC₅₀ of ~29 μ M (using ethanol as a vehicle) and ~77 μ M (using DMSO as a vehicle). Compound **1** was less potent than ibuprofen at inhibiting COX-1, with an IC₅₀ of ~60 μ M (ethanol) and ~240 μ M (DMSO). Compound **2** inhibited COX-1 with an IC₅₀ of ~50 μ M (ethanol). Ibuprofen, **1** and **2** also showed substrate-selective inhibition of COX-2, being poor inhibitors of the cyclooxygenation of AA by COX-2 and producing 36, 41 and 18% inhibition at the highest concentrations tested (300, 300 and 100 μ M, respectively). However, when anandamide was used as substrate, these compounds were relatively potent inhibitors of COX-2, with IC₅₀ of ~6 μ M (ibuprofen), ~19 (**1**) and ~10 μ M (**2**).

In addition, **1** was confirmed to be active at the dose of 30 mg kg⁻¹ (subcutaneous) in a murine model of visceral nociception, but this effect was not blocked by either CB₁ or CB₂ receptor antagonists. Two possible explanations were proposed: (a) the contribution of analgesia due to the COX inhibitory properties of **1** dominates at the dose and in the model

used, or that (b) **1** is metabolized *in vivo* to a compound that retains (or even improves) its COX-inhibitory properties, but loses its FAAH inhibitory activity. The authors excluded ibuprofen being the metabolite, considering that in rats at the lower dose of 20 mg kg⁻¹ (*i.p.*) ibuprofen is significantly less effective than **1** in the acetic acid test.^[54]

A small series of racemic flurbiprofen and naproxen amide derivatives were also investigated.^[62] Within this limited set, **3** (flu-am1) and **4** (nap-am1) demonstrated increased inhibitory potency for FAAH, relative to the parent NSAIDs (flurbiprofen and **3**, FAAH IC₅₀ values of 29 μM and 0.44 μM, respectively; naproxen and **4**, FAAH IC₅₀ values of >100 μM and 0.74 μM, respectively) (for FAAH assay conditions, see.^[59])

Compounds **3** and **4** were also investigated for their ability to inhibit COX-1 and COX-2, using the oxygen electrode assay and two different substrates, AA (for both isoforms) and 2-AG (for COX-2) (for COX assay conditions, see^[61]). The study confirmed that flurbiprofen inhibited the cyclooxygenation of AA catalyzed by ovine COX-1 (IC₅₀ = 3.6 μM) and had little effect on the cyclooxygenation of AA catalyzed by human recombinant COX-2 (IC₅₀ = 103 μM). Flurbiprofen also inhibited the cyclooxygenation of 2-AG catalyzed by human recombinant COX-2 (IC₅₀ = 1.3 μM). A similar pattern of COX-1 and COX-2 inhibition was observed with **3**, although in general 2–3 fold higher concentrations were required to produce the same effect as flurbiprofen [COX-1 (AA) IC₅₀ = 6.6 μM; COX-2 (AA) IC₅₀ = 42 μM and COX-2 (2-AG) IC₅₀ = 1.7 μM, respectively]. By contrast, **4** was a rather poor inhibitor of both COX isoforms [COX-1 (AA) IC₅₀ = 56 μM; COX-2 (AA) IC₅₀ > 100 μM and COX-2 (2-AG) IC₅₀ = 6.3 μM, respectively].

Compound **3**, which reversibly inhibits FAAH, was further investigated *in vivo* for its ability to inhibit the enzyme in the brain. The compound was administered by intraperitoneal injection in rats 30 min prior to the administration of [¹⁸F]-3-(4,5-dihydrooxazol-2-yl)phenyl (5-fluoropentyl)carbamate (¹⁸F-DOPP) an irreversible and brain penetrant ¹⁸F-labelled FAAH inhibitor.^[63] It was postulated that pretreatment with **3** would prevent the interaction of ¹⁸F-DOPP with FAAH.^[64] However, administration of **3** at two different dosages had no effect on brain labeling with ¹⁸F-DOPP, which is suggestive of limited penetration.

Using a computational strategy,^[65] Favia et al. identified the 2-arylpropionic acid, carprofen, as a multitarget FAAH, COX-1 and COX-2 inhibitor^[66] (Figure 9). The compound was shown to inhibit rat FAAH with IC₅₀ = 79 μM, ovine COX-1 with an IC₅₀ = 22 μM, and human COX-2 with an IC₅₀ = 4 μM (for assay conditions, see^[12c, 66]). Medicinal chemistry efforts focused on a diversified SAR exploration around different regions of the carprofen scaffold were also reported in that study. In particular, the functionalization at the nitrogen atom of the carbazole ring of carprofen led to the identification of novel analogs, compounds **5** and **6** (Figure 9), which showed an improved potency profile for both FAAH and COX (IC₅₀, μM: compound **5**: FAAH = 22; COX-1 = 74; COX-2 = 72; compound **6**: FAAH = 85; ovine COX-1 = 30; human COX-2 = 28). *In vitro* profile studies showed that only the (*S*)-enantiomer of carprofen retained multitarget FAAH and COX inhibition, whereas the enantiomers of **5** and **6** lost activity for one of the two targets.

Docking analysis of the putative binding modes of carprofen on FAAH and COX-2 were also reported.^[66] In COX-2, it was proposed that carprofen may preferentially interact, *via* H-bonds formed by its carboxylic group with Arg120 and Tyr238, while the carbazole scaffold may lie deep in the pocket, facing Tyr385. This putative binding mode is consistent with the experimentally observed modes of interaction of several NSAIDs in complex with either COX-1 or COX-2. In FAAH, carprofen was shown to fit the acyl chain binding channel and establish H-bond interactions with the oxyanion hole through its carboxylic group, although other binding modes could not be excluded. In addition, accurate computational analysis of the structural similarities between FAAH and COX-2 were also reported, mapping hydrophobic (*i.e.*, acyl chain binding channel in FAAH; catalytic binding site in COX-2) and hydrophilic (*i.e.*, oxyanion hole and cytosolic channel in FAAH; exit of catalytic binding site in COX-2) portions of the two enzymes, by the use of two known probes in complex with FAAH (methyl arachidonyl fluorophosphonate) and COX-2 (AA).

In 2013, Bertolacci et al. solved the X ray crystal structure of rat FAAH in complex with carprofen at 2.25Å resolution.^[67] This was the first structural determination of the molecular interactions of an NSAID in complex with FAAH. Diffraction-quality crystals of the FAAH-carprofen complex were obtained by preincubating the enzyme with the *O*-arylcarbamate inhibitor, URB597.^[15] The molecular details of the interactions were investigated using a combination of site-directed mutagenesis, WaterLOGSY and FABS NMR spectroscopy, with the goal of providing structural insights that might be useful to design novel FAAH/COX inhibitors.

A number of dual FAAH and COX inhibitors (mainly COX-2 inhibitors) with indomethacin-like structures are described in patent applications from Microbia Inc. (changed to Ironwood Pharmaceuticals on April 7, 2008). These compounds are exemplified by **7** and **8** (Figure 10).^[68] However, a systematic SAR analysis for this class of molecules has not been reported.

3.2. Rational drug design: merging strategy

An effective approach to the design of multi-target inhibitors is to exploit the presence of structural similarities present in selective binders.^[48] Compounds with overlapping or integrated pharmacophores are likely to have lower molecular weights and potentially more drug-like physicochemical properties. The rational design of compound **9**, designed using this strategy, was recently reported.^[69] This agent combines structural commonalities and pharmacophore groups of two classes of known FAAH and COX inhibitors – *O*-aryl carbamate FAAH inhibitors such as URB597,^[15] and 2-aryl propionic acid NSAIDs such as flurbiprofen^[47c, 70] – which share a biphenyl core as common structural motif (Figure 11). Moreover, the previously reported SAR surrounding these scaffolds support 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 URB597^[15, 71] and the ether moieties of flurbiprofen analogs **10a** or **10b**, respectively.^[70b]

An initial SAR exploration of the hybrid scaffold **9** around the alkyl substituent of the carbamate group, starting from the hit **11** (IC₅₀, μM; FAAH = 8.2; COX-1 = 7.9; COX-2 > 100) led to the identification of compound **12** (ARN2508) as the first highly potent and

selective multi-target FAAH/COX inhibitor (IC_{50} ; FAAH = 31 nM; COX-1 = 12 nM; COX-2 = 430 nM). The pharmacological activity of this compound was evaluated in mouse models of inflammatory bowel disease, in which it is known that both FAAH and COX-2 are expressed at abnormally high levels.^[72] After oral administration, **12** fully engaged its intended targets and elicited a profound anti-inflammatory response, without any overt signs of gastric toxicity. Pharmacological and genetic experiments provided evidence that such response was caused by dual FAAH/COX inhibition. The results suggest that multitarget FAAH/COX inhibitors such as **12** might achieve marked anti-inflammatory efficacy while protecting the GI tract from NSAID-induced damage.^[69] The ‘double whammy’ these compounds produce by blocking both FAAH and COX activities might interrupt the pathological loop established in inflamed tissues by the concomitant up-regulation of these enzymes (Figure 12).

4. Future perspectives

Where do we go from here? It is reasonable to expect that multi-target ligands such as ARN2508 (**12**) will continue to be useful in probing the functional interactions between FAAH and COX, and as a means to explore the potential therapeutic value of FAAH/COX blockade. In addition to inflammatory bowel disease, where ARN2508 appears to be more effective than standards of therapy and single-target FAAH inhibitors,^[69] multi-target FAAH/COX agents might prove valuable in other chronic inflammatory conditions in which FAAH and COX-2 (but also COX-1) are expressed at pathologically high levels. These include a variety of peripheral and central inflammatory states as well as certain forms of cancer.^[23, 73] The wide potential applicability of these agents provides a great therapeutic opportunity, but also poses interesting challenges to medicinal chemistry. In addition to balanced potency for FAAH and COX, future molecules should be able to engage these proteins in all compartments of the body, including the central nervous system, and to selectively target FAAH/COX-1 versus FAAH/COX-2.

Acknowledgments

This work was supported in part by grants from the National Institutes of Health.

References

1. a) Hopkins AL. *Nature chemical biology*. 2008; 4(11):682–690. [PubMed: 18936753] b) Peters JU. *J Med Chem*. 2013; 56(22):8955–8971. [PubMed: 23919353]
2. a) Fowler CJ. *Br J Pharmacol*. 2007; 152(5):594–601. [PubMed: 17618306] b) Rouzer CA, Marnett LJ. *Chem Rev*. 2011; 111(10):5899–5921. [PubMed: 21923193] c) Piscitelli F, Di Marzo V. *ACS Chem Neurosci*. 2012; 3(5):356–363. [PubMed: 22860203] d) Maione S, Costa B, Di Marzo V. *Pain*. 2013; 154(Suppl 1):S87–93. [PubMed: 23623250]
3. a) Piomelli D. *Trends Endocrinol Metab*. 2013; 24(7):332–341. [PubMed: 23567058] b) Piomelli D, Sasso O. *Nat Neurosci*. 2014; 17(2):164–174. [PubMed: 24473264]
4. a) Axelrod J, Felder CC. *Neurochem Res*. 1998; 23(5):575–581. [PubMed: 9566594] b) Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA. *Pharmacol Rev*. 2010; 62(4):588–631. [PubMed: 21079038]
5. a) Di Marzo V, De Petrocellis L. *Curr Med Chem*. 2010; 17(14):1430–1449. [PubMed: 20166923] b) Piomelli D. *Neuropharmacol*. 2014; 76(Pt B):228–234.

6. a) Fu J, Gaetani S, Oveisi F, Lo Verme J, Serrano A, Rodriguez De Fonseca F, Rosengarth A, Luecke H, Di Giacomo B, Tarzia G, Piomelli D. *Nature*. 2003; 425(6953):90–93. [PubMed: 12955147] b) LoVerme J, Russo R, La Rana G, Fu J, Farthing J, Mattace-Raso G, Meli R, Hohmann A, Calignano A, Piomelli D. *J Pharmacol Exp Ther*. 2006; 319(3):1051–1061. [PubMed: 16997973] c) Schwartz GJ, Fu J, Astarita G, Li X, Gaetani S, Campolongo P, Cuomo V, Piomelli D. *Cell Metab*. 2008; 8(4):281–288. [PubMed: 18840358] d) Ueda N, Tsuboi K, Uyama T. *Prog Lipid Res*. 2010; 49(4):299–315. [PubMed: 20152858]
7. a) Ahn K, McKinney MK, Cravatt BF. *Chemical reviews*. 2008; 108(5):1687–1707. [PubMed: 18429637] b) Magotti P, Bauer I, Igarashi M, Babagoli M, Marotta R, Piomelli D, Garau G. *Structure*. 2015; 23(3):598–604. [PubMed: 25684574]
8. a) Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB. *Nature*. 1996; 384(6604):83–87. [PubMed: 8900284] b) Giang DK, Cravatt BF. *Proc Natl Acad Sci U S A*. 1997; 94(6):2238–2242. [PubMed: 9122178]
9. Alexander SP, Kendall DA. *Br J Pharmacol*. 2007; 152(5):602–623. [PubMed: 17876303]
10. Desarnaud F, Cadas H, Piomelli D. *J Biol Chem*. 1995; 270(11):6030–6035. [PubMed: 7890734]
11. Chaperon F, Thiebot MH. *Crit Rev Neurobiol*. 1999; 13(3):243–281. [PubMed: 10803637]
12. a) Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D. *Nat Med*. 2003; 9(1):76–81. [PubMed: 12461523] b) Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, Cassano T, Morgese MG, Debonnel G, Duranti A, Tontini A, Tarzia G, Mor M, Trezza V, Goldberg SR, Cuomo V, Piomelli D. *Proc Natl Acad Sci U S A*. 2005; 102(51):18620–18625. [PubMed: 16352709] c) Justinova Z, Mangieri RA, Bortolato M, Chefer SI, Mukhin AG, Clapper JR, King AR, Redhi GH, Yasar S, Piomelli D, Goldberg SR. *Biol Psychiatry*. 2008; 64(11):930–937. [PubMed: 18814866]
13. a) Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH. *Proc Natl Acad Sci U S A*. 2001; 98(16):9371–9376. [PubMed: 11470906] b) Lichtman AH, Shelton CC, Advani T, Cravatt BF. *Pain*. 2004; 109(3):319–327. [PubMed: 15157693] c) Massa F, Marsicano G, Hermann H, Cannich A, Monory K, Cravatt BF, Ferri GL, Sibaev A, Storr M, Lutz B. *J Clin Invest*. 2004; 113(8):1202–1209. [PubMed: 15085199] d) Cravatt BF, Saghatelian A, Hawkins EG, Clement AB, Bracey MH, Lichtman AH. *Proc Natl Acad Sci U S A*. 2004; 101(29):10821–10826. [PubMed: 15247426] e) Karsak M, Gaffal E, Date R, Wang-Eckhardt L, Rehne J, Petrosino S, Starowicz K, Steuder R, Schlicker E, Cravatt B, Mechoulam R, Buettner R, Werner S, Di Marzo V, Tuting T, Zimmer A. *Science*. 2007; 316(5830):1494–1497. [PubMed: 17556587]
14. a) Seierstad M, Breitenbucher JG. *J Med Chem*. 2008; 51(23):7327–7343. [PubMed: 18983142] b) Otrubova K, Ezzili C, Boger DL. *Bioorg Med Chem Lett*. 2011; 21(16):4674–4685. [PubMed: 21764305] c) Feledziak M, Lambert DM, Marchand-Brynaert J, Muccioli GG. *Recent Pat CNS Drug Discov*. 2012; 7(1):49–70. [PubMed: 22280341]
15. Mor M, Rivara S, Lodola A, Plazzi PV, Tarzia G, Duranti A, Tontini A, Piersanti G, Kathuria S, Piomelli D. *J Med Chem*. 2004; 47(21):4998–5008. [PubMed: 15456244]
16. Russo R, Loverme J, La Rana G, Compton TR, Parrott J, Duranti A, Tontini A, Mor M, Tarzia G, Calignano A, Piomelli D. *J Pharmacol Exp Ther*. 2007; 322(1):236–242. [PubMed: 17412883]
17. a) Clapper JR, Moreno-Sanz G, Russo R, Guijarro A, Vacondio F, Duranti A, Tontini A, Sanchini S, Sciolino NR, Spradley JM, Hohmann AG, Calignano A, Mor M, Tarzia G, Piomelli D. *Nat Neurosci*. 2010; 13(10):1265–1270. [PubMed: 20852626] b) Sasso O, Bertorelli R, Bandiera T, Scarpelli R, Colombano G, Armirotti A, Moreno-Sanz G, Reggiani A, Piomelli D. *Pharmacol Res*. 2012; 65(5):553–563. [PubMed: 22420940]
18. Zeilhofer HU, Benke D, Yevenes GE. *Annu Rev Pharmacol Toxicol*. 2012; 52:111–133. [PubMed: 21854227]
19. Ahn K, Johnson DS, Mileni M, Beidler D, Long JZ, McKinney MK, Weerapana E, Sadagopan N, Liimatta M, Smith SE, Lazerwith S, Stiff C, Kamtekar S, Bhattacharya K, Zhang Y, Swaney S, Van Becelaere K, Stevens RC, Cravatt BF. *Chem Biol*. 2009; 16(4):411–420. [PubMed: 19389627]
20. Johnson DS, Stiff C, Lazerwith SE, Kesten SR, Fay LK, Morris M, Beidler D, Liimatta MB, Smith SE, Dudley DT, Sadagopan N, Bhattachar SN, Kesten SJ, Nomanbhoy TK, Cravatt BF, Ahn K. *ACS Med Chem Lett*. 2011; 2(2):91–96. [PubMed: 21666860]

21. Huggins JP, Smart TS, Langman S, Taylor L, Young T. Pain. 2012; 153(9):1837–1846. [PubMed: 22727500]
22. a) Jaggi AS, Singh N. Brain Res. 2011; 1381:187–201. [PubMed: 21238432] b) Pelletier R, Higgins J, Bourbonnais D. Phys Ther. 2015
23. Guindon J, Hohmann AG. Br J Pharmacol. 2011; 163(7):1447–1463. [PubMed: 21410463]
24. a) Boger DL, Henriksen SJ, Cravatt BF. Curr Pharma Des. 1998; 4(4):303–314. b) Muccioli GG. Drug Discov Today. 2010; 15(11–12):474–483. [PubMed: 20304091]
25. a) Fegley D, Gaetani S, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D. J Pharmacol Exp Ther. 2005; 313(1):352–358. [PubMed: 15579492] b) Jayamanne A, Greenwood R, Mitchell VA, Aslan S, Piomelli D, Vaughan CW. Br J Pharmacol. 2006; 147(3):281–288. [PubMed: 16331291]
26. a) Saghatelian A, McKinney MK, Bandell M, Patapoutian A, Cravatt BF. Biochem. 2006; 45(30):9007–9015. [PubMed: 16866345] b) Desroches J, Guindon J, Lambert C, Beaulieu P. Br J Pharmacol. 2008; 155(6):913–924. [PubMed: 18695638] c) Bradshaw HB, Rimmerman N, Hu SS, Burstein S, Walker JM. Vitam Horm. 2009; 81:191–205. [PubMed: 19647113] d) Alhouayek M, Masquelier J, Muccioli GG. Drug Discov Today. 2014; 19(3):295–304. [PubMed: 23891880]
27. a) Cencioni MT, Chiurciu V, Catanzaro G, Borsellino G, Bernardi G, Battistini L, Maccarrone M. PLoS one. 2010; 5(1):e8688. [PubMed: 20098669] b) Chiurciu V, Cencioni MT, Bisicchia E, De Bardi M, Gasperini C, Borsellino G, Centonze D, Battistini L, Maccarrone M. Ann Neurol. 2013; 73(5):626–636. [PubMed: 23447381]
28. Storr MA, Keenan CM, Emmerdinger D, Zhang H, Yuce B, Sibaev A, Massa F, Buckley NE, Lutz B, Goke B, Brand S, Patel KD, Sharkey KA. J Med Chem. 2008; 86(8):925–936.
29. Blobaum AL, Marnett LJ. J Med Chem. 2007; 50(7):1425–1441. [PubMed: 17341061]
30. Patrignani P, Tacconelli S, Sciulli MG, Capone ML. Brain Res Rev. 2005; 48(2):352–359. [PubMed: 15850674]
31. Ghilardi JR, Svensson CI, Rogers SD, Yaksh TL, Mantyh PW. J Neurosci. 2004; 24(11):2727–2732. [PubMed: 15028765]
32. a) Iniguez MA, Cacheiro-Llaguno C, Cuesta N, Diaz-Munoz MD, Fresno M. Arch Physiol Biochem. 2008; 114(3):201–209. [PubMed: 18629685] b) Wallace JL. Physiol Rev. 2008; 88(4):1547–1565. [PubMed: 18923189] c) Yang Y, Tang LQ, Wei W. J Recept Signal Transduct Res. 2013; 33(1):14–27. [PubMed: 23327583]
33. Steinmeyer J. Arthritis Res. 2000; 2(5):379–385. [PubMed: 11094452]
34. a) Davies NM, Wallace JL. J Gastroenterol. 1997; 32(1):127–133. [PubMed: 9058309] b) Cheng HF, Harris RC. Hypertension. 2004; 43(3):525–530. [PubMed: 14732722]
35. a) Wilson DE. J Clin Gastroenterol. 1991; 13(Suppl 1):S65–71. [PubMed: 1940199] b) Wright K, Rooney N, Feeney M, Tate J, Robertson D, Welham M, Ward S. Gastroenterol. 2005; 129(2):437–453.
36. Zhang J, Ding EL, Song Y. Jama. 2006; 296(13):1619–1632. [PubMed: 16968832]
37. a) Yu M, Ives D, Ramesha CS. J Biol Chem. 1997; 272(34):21181–21186. [PubMed: 9261124] b) Kozak KR, Prusakiewicz JJ, Rowlinson SW, Prudhomme DR, Marnett LJ. Biochem. 2003; 42(30):9041–9049. [PubMed: 12885237]
38. a) Ross RA, Craib SJ, Stevenson LA, Pertwee RG, Henderson A, Toole J, Ellington HC. J Pharmacol Exp Ther. 2002; 301(3):900–907. [PubMed: 12023517] b) Matias I, Chen J, De Petrocellis L, Bisogno T, Ligresti A, Fezza F, Krauss AH, Shi L, Protzman CE, Li C, Liang Y, Nieves AL, Kedzie KM, Burk RM, Di Marzo V, Woodward DF. J Pharmacol Exp Ther. 2004; 309(2):745–757. [PubMed: 14757851] c) Fowler CJ, Tiger G. Biochem Pharmacol. 2005; 69(8):1241–1245. [PubMed: 15794945]
39. Guhring H, Hamza M, Sergejeva M, Ates M, Kotalla CE, Ledent C, Brune K. Eur J Pharmacol. 2002; 454(2–3):153–163. [PubMed: 12421642]
40. Ates M, Hamza M, Seidel K, Kotalla CE, Ledent C, Guhring H. Eur J Neurosci. 2003; 17(3):597–604. [PubMed: 12581177]
41. Guindon J, De Lean A, Beaulieu P. Pain. 2006; 121(1–2):85–93. [PubMed: 16480822]
42. Guindon J, Beaulieu P. Neuropharmacol. 2006; 50(7):814–823.

43. Ulugol A, Ozyigit F, Yesilyurt O, Dogrul A. *Anesth Analg*. 2006; 102(2):443–447. [PubMed: 16428540]
44. Naidu PS, Booker L, Cravatt BF, Lichtman AH. *J Pharmacol Exp Ther*. 2009; 329(1):48–56. [PubMed: 19118134]
45. Grim TW, Ghosh S, Hsu KL, Cravatt BF, Kinsey SG, Lichtman AH. *Pharmacol Biochem Beh*. 2014; 124:405–411.
46. Bracey MH, Hanson MA, Masuda KR, Stevens RC, Cravatt BF. *Science*. 2002; 298(5599):1793–1796. [PubMed: 12459591]
47. a) Picot D, Loll PJ, Garavito RM. *Nature*. 1994; 367(6460):243–249. [PubMed: 8121489] b) Luong C, Miller A, Barnett J, Chow J, Ramesha C, Browner MF. *Nat Struct Biol*. 1996; 3(11): 927–933. [PubMed: 8901870] c) Kurumbail RG, Stevens AM, Gierse JK, McDonald JJ, Stegeman RA, Pak JY, Gildehaus D, Miyashiro JM, Penning TD, Seibert K, Isakson PC, Stallings WC. *Nature*. 1996; 384(6610):644–648. [PubMed: 8967954]
48. Morphy R, Kay C, Rankovic Z. *Drug Discov Today*. 2004; 9(15):641–651. [PubMed: 15279847]
49. Paria BC, Deutsch DD, Dey SK. *Mol Reprod Dev*. 1996; 45(2):183–192. [PubMed: 8914076]
50. a) Fowler CJ, Tiger G, Stenstrom A. *J Pharmacol Exp Ther*. 1997; 283(2):729–734. [PubMed: 9353392] b) Fowler CJ, Stenstrom A, Tiger G. *Pharmacol Toxicol*. 1997; 80(2):103–107. [PubMed: 9060042]
51. Fowler CJ, Janson U, Johnson RM, Wahlstrom G, Stenstrom A, Norstrom K, Tiger G. *Arch Biochem Biophys*. 1999; 362(2):191–196. [PubMed: 9989926]
52. Fowler CJ, Borjesson M, Tiger G. *Br J Pharmacol*. 2000; 131(3):498–504. [PubMed: 11015300]
53. a) Omeir RL, Chin S, Hong Y, Ahern DG, Deutsch DG. *Life Sci*. 1995; 56(23–24):1999–2005. [PubMed: 7776824] b) Tiger G, Stenstrom A, Fowler CJ. *Biochem Pharmacol*. 2000; 59(6):647–653. [PubMed: 10677581]
54. Cocco MT, Congiu C, Onnis V, Morelli M, Cauli O. *Eur J Med Chem*. 2003; 38(5):513–518. [PubMed: 12767601]
55. a) Kavvada KM, Murray JG, Moore VA, Coombes AG, Hanson PJ. *Eur J Pharmacol*. 2006; 549(1–3):41–49. [PubMed: 16996496] b) Wallace JL. *Gastroenterol*. 1997; 112(3):1000–1016. c) Hawkey CJ. *Gastroenterol*. 2000; 119(2):521–535.
56. a) Kalgutkar AS, Marnett AB, Crews BC, Rimmel RP, Marnett LJ. *J Med Chem*. 2000; 43(15): 2860–2870. [PubMed: 10956194] b) Kalgutkar AS, Crews BC, Rowlinson SW, Marnett AB, Kozak KR, Rimmel RP, Marnett LJ. *Proc Natl Acad Sci U S A*. 2000; 97(2):925–930. [PubMed: 10639181]
57. Holt S, Paylor B, Boldrup L, Alajakku K, Vandevoorde S, Sundstrom A, Cocco MT, Onnis V, Fowler CJ. *Eur J Pharmacol*. 2007; 565(1–3):26–36. [PubMed: 17397826]
58. Fowler CJ, Bjorklund E, Lichtman AH, Naidu PS, Congiu C, Onnis V. *J Enzyme Inhib Med Chem*. 2013; 28(1):172–182. [PubMed: 22225576]
59. Boldrup L, Wilson SJ, Barbier AJ, Fowler CJ. *J Biochem Biophys Met*. 2004; 60(2):171–177.
60. Paylor B, Holt S, Fowler CJ. *Pharmacol Res*. 2006; 54(6):481–485. [PubMed: 16997568]
61. a) Meade EA, Smith WL, DeWitt DL. *J Biol Chem*. 1993; 268(9):6610–6614. [PubMed: 8454631] b) Onnis V, Congiu C, Bjorklund E, Hempel F, Soderstrom E, Fowler CJ. *J Med Chem*. 2010; 53(5):2286–2298. [PubMed: 20143779]
62. Cipriano M, Bjorklund E, Wilson AA, Congiu C, Onnis V, Fowler CJ. *Eur J Pharmacol*. 2013; 720(1–3):383–390. [PubMed: 24120370]
63. Sadoski O, Hicks JW, Parkes J, Raymond R, Nobrega J, Houle S, Cipriano M, Fowler CJ, Vasdev N, Wilson AA. *Bioorg Med Chem*. 2013; 21(14):4351–4357. [PubMed: 23712084]
64. Horita A, Mc GW. *Biochem Pharmacol*. 1960; 3:206–211. [PubMed: 14403109]
65. a) Huang N, Shoichet BK, Irwin JJ. *J Med Chem*. 2006; 49(23):6789–6801. [PubMed: 17154509] b) Trott O, Olson AJ. *J Comput Chem*. 2010; 31:455–461. [PubMed: 19499576] c) Knox C, Law V, Jewison T, Liu P, Ly S, Frolkis A, Pon A, Banco K, Mak C, Neveu V, Djoumbou Y, Eisner R, Guo AC, Wishart DS. *Nucleic Acids Res*. 2011; 39(Database issue):D1035–1041. [PubMed: 21059682]

66. Favia AD, Habrant D, Scarpelli R, Migliore M, Albani C, Bertozzi SM, Dionisi M, Tarozzo G, Piomelli D, Cavalli A, De Vivo M. *J Med Chem.* 2012; 55(20):8807–8826. [PubMed: 23043222]
67. Bertolacci L, Romeo E, Veronesi M, Magotti P, Albani C, Dionisi M, Lambruschini C, Scarpelli R, Cavalli A, De Vivo M, Piomelli D, Garau G. *J Am Chem Soc.* 2013; 135(1):22–25. [PubMed: 23240907]
68. a) Bartolini, W.; Cali, BM.; Chen, B.; Chien, Y-T.; Currie, MG.; Milne, TG.; Pearson, JP.; Talley, JJ.; Zimmerman, C. *PCT Int Appl.* WO002525 (A2). 2005. b) Bartolini, W.; Cali, BM.; Chen; Chen, B.; Chen, Y-T.; Currie, MG.; Milne, GT.; Pearson, JP.; Talley, JJ.; Yang, JJ.; Zimmerman, C.; Monreal, A. *PCT Int Appl.* WO022501 (A2). 2007. c) Bartolini, W.; Cali, BM.; Chen; Chen, B.; Chen, Y-T.; Currie, MG.; Milne, GT.; Pearson, JP.; Talley, JJ.; Yang, JJ.; Zimmerman, C.; Kim, C.; Sprott, K.; Barden, T.; Lundigran, R.; Mermerian, A. *PCT Int Appl.* WO070892 (A2). 2007. d) Talley, JJ.; Sprott, K.; Pearson, JP.; Milne, GT.; Schairer, W.; Yang, JJ.; Kim, C.; Barden, T.; Lundigran, R.; Mermerian, A.; Currie, MG. *PCT Int Appl.* WO019357 (A2). 2008.
69. Sasso O, Migliore M, Habrant D, Armirotti A, Albani C, Summa M, Moreno-Sanz G, Scarpelli R, Piomelli D. *FASEB J.* 2015; 29(6):2616–2627. [PubMed: 25757568]
70. a) Bayly CI, Black WC, Leger S, Ouimet N, Ouellet M, Percival MD. *Bioorg Med chem Lett.* 1999; 9(3):307–312. [PubMed: 10091674] b) Selinsky BS, Gupta K, Sharkey CT, Loll PJ. *Biochem.* 2001; 40(17):5172–5180. [PubMed: 11318639]
71. a) Tarzia G, Duranti A, Tontini A, Piersanti G, Mor M, Rivara S, Plazzi PV, Park C, Kathuria S, Piomelli D. *J Med Chem.* 2003; 46(12):2352–2360. [PubMed: 12773040] b) Mor M, Lodola A, Rivara S, Vacondio F, Duranti A, Tontini A, Sanchini S, Piersanti G, Clapper JR, King AR, Tarzia G, Piomelli D. *J Med Chem.* 2008; 51(12):3487–3498. [PubMed: 18507372]
72. a) Singer D II, Kawka W, Schloemann S, Tessner T, Riehl T, Stenson WF. *Gastroenterol.* 1998; 115(2):297–306. b) Richardson D, Pearson RG, Kurian N, Latif ML, Garle MJ, Barrett DA, Kendall DA, Scammell BE, Reeve AJ, Chapman V. *Arthritis Res Ther.* 2008; 10(2):R43. [PubMed: 18416822] c) Di Sabatino A, Battista N, Biancheri P, Rapino C, Rovedatti L, Astarita G, Vanoli A, Dainese E, Guerci M, Piomelli D, Pender SL, MacDonald TT, Maccarrone M, Corazza GR. *Mucosal immunol.* 2011; 4(5):574–583. [PubMed: 21471961] d) Suarez J, Romero-Zerbo Y, Marquez L, Rivera P, Iglesias M, Bermudez-Silva FJ, Andreu M, Rodriguez de Fonseca F. *PLoS one.* 2012; 7(5):e37729. [PubMed: 22662201]
73. a) Collaco-Moraes Y, Aspey B, Harrison M, de Belleruche J. *J Cereb Blood Flow Metab.* 1996; 16(6):1366–1372. [PubMed: 8898713] b) Berger C, Schmid PC, Schabitz WR, Wolf M, Schwab S, Schmid HH. *J Neurochem.* 2004; 88(5):1159–1167. [PubMed: 15009671] c) Izzo AA, Camilleri M. *Pharmacol Res.* 2009; 60(2):117–125. [PubMed: 19442536] d) Pisanti S, Bifulco M. *Pharmacol Res.* 2009; 60(2):107–116. [PubMed: 19559362] e) Fowler CJ, Gustafsson SB, Chung SC, Persson E, Jacobsson SO, Bergh A. *Curr Top Med Chem.* 2010; 10(8):814–827. [PubMed: 20370711] f) Freimuth N, Ramer R, Hinz B. *J Pharmacol Exp Ther.* 2010; 332(2):336–344. [PubMed: 19889794] g) Malfitano AM, Ciaglia E, Gangemi G, Gazzero P, Laezza C, Bifulco M. *Expert Opin Ther Targets.* 2011; 15(3):297–308. [PubMed: 21244344] h) Khan MN, Lee YS. *Med Res Rev.* 2011; 31(2):161–201. [PubMed: 19967720] i) Hermanson DJ, Gamble-George JC, Marnett LJ, Patel S. *Trends Pharmacol Sci.* 2014; 35(7):358–367. [PubMed: 24845457] j) Adinolfi B, Romanini A, Vanni A, Martinotti E, Chicca A, Fogli S, Nieri P. *Eur J Pharmacol.* 2013; 718(1–3):154–159. [PubMed: 24041928]

anandamide, **AEA**

palmitoylethanolamide, **PEA**

oleoylethanolamide, **OEA**

Figure 1.
Chemical structures of representative FAEs.

Figure 2.
Simplified scheme of the endocannabinoid and prostanoid signaling pathways with some nodes of biological interactions.

URB597, R = H

URB937, R = OH

PF-3845

PF-04457845

Figure 3.
Chemical structures of representative FAAH inhibitors.

Figure 4. Chemical structures of representative prostanoids: prostaglandin E₂ (PGE₂), prostacyclin (PGI₂) and thromboxane (TXA₂).

Figure 5.
Chemical structures of representative COX inhibitors.

Figure 6.

Prostanoids and FAEs, such as anandamide, exert antagonistic effect on pain and inflammation, but act synergistically to promote cytoprotection.

Figure 7.
Ibuprofen and representative amide analogs, **1** and **2**.

flurbiprofen **3, flu-am1** **naproxen** **4, nap-am1**

Figure 8.
Flurbiprofen, naproxen and their amide analogs, compounds **3** and **4**.

Figure 9.
Carprofen and some representative *N*-functionalized analogs, **5** and **6**.

indomethacin

Figure 10.
Representative dual FAAH/COX inhibitors, **7** and **8** from Microbia Inc.

Figure 11.
Rational design of the 'hybrid scaffold' **9** and initial SAR results.

Figure 12.

Concomitant up-regulation of FAAH and COX may establish a pathological loop that exacerbates or prolongs inflammation by amplifying inflammatory COX-dependent signals at the expense of defensive anandamide (AEA)-dependent responses. This hypothesis predicts that drugs targeting both FAAH and COX should have substantial anti-inflammatory efficacy combined with reduced GI toxicity.