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Inhibition of FAAH, TRPV1, and COX2 by NSAID-Serotonin Conjugates

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Abstract

Serotonin was linked by amidation to the carboxylic acid groups of a series of structurally diverse NSAIDs. The resulting NSAID-serotonin conjugates were tested in vitro for their ability to inhibit FAAH, TRPV1, and COX2. Ibuprofen-5-HT and Flurbiprofen-5-HT inhibited all three targets with approximately the same potency as *N*-arachidonoyl serotonin (AA-5-HT), while Fenoprofen-5-HT and Naproxen-5-HT showed activity as dual inhibitors of TRPV1 and COX2.

Keywords

NSAID; FAAH; fatty acid amide hydrolase; TRPV1; transient receptor potential vanilloid type 1; dual inhibition; multimodal inhibition; arachidonoyl serotonin

Fatty acid amide hydrolase (FAAH) is a membrane-associated, intracellular enzyme that degrades endocannabinoids, including anandamide (*N*-arachidonoyl ethanolamine), by amide hydrolysis.[1] Inhibition of FAAH induces cannabinoid (CB) receptor-dependent analgesia in rodents, often without causing the full tetrad of symptoms (anti-nociception, hypothermia, hypolocomotion, catalepsy) associated with direct CB receptor agonists.[2] This is thought to be due to the localized action of endocannabinoids, which are only synthesized as-needed in those regions of the body where they are required. As a result, inhibitors of FAAH have been aggressively pursued as a potentially new class of drugs for pain relief.[3]

Although many potent and selective FAAH inhibitors have been reported in the literature, the first phase II clinical trial with one such inhibitor, PF-04457845, was terminated early due to a lack of efficacy in treating osteoarthritis pain compared with naproxen.[4] This, in spite of a greater than 10-fold excess of anandamide in the blood of patients treated with the

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inhibitor[4] and pre-clinical data showing potent analgesic effects in rodent models.[5] A number of reasons for the lack of observed efficacy have been proposed, including the possibility that, in humans, CB-mediated anti-nociception by anandamide and other fatty acid amides may be negated by the concomitant initiation of pro-nociceptive pathways by the same molecules.[4,6] Such signaling may include activation of transient receptor potential vanilloid type 1 (TRPV1) receptors. Like capsaicin, the pain-evoking component of “hot” chili peppers, anandamide is also an agonist of TRPV1,[7] a ligand-gated calcium channel associated with thermal pain perception and inflammation-induced hyperalgesia.[8] Antagonists of TRPV1 have been shown to reduce pain in humans and other animals,[9] as well as in pain models that are refractory to NSAIDs (non-steroidal anti-inflammatory drugs).[10] Furthermore, anandamide may be converted to the pro-inflammatory prostamide $F_{2\alpha}$ by cyclooxygenase 2 (COX2).[6,11] Thus, in order to harness the therapeutic potential of the endocannabinoid system, a multi-modal approach may be required.

Combination inhibitors of FAAH, TRPV1, and/or COX2 may have the advantage of effective pain relief with a high therapeutic index. For example, arachidonoyl serotonin (AA-5-HT) inhibits both FAAH (IC50 = 1–12 μM)[12] and TRPV1 (IC50 = 37–270 nM against 100 nM capsaicin in HEK-293 cells).[12a,13] In mice, AA-5-HT had greater efficacy at relieving carrageenan-induced hyperalgesia than either a high-potency, FAAH-selective inhibitor or a TRPV1-selective inhibitor.[14] Similarly, AA-5-HT was more effective in an animal model of anxiety than selective FAAH or TRPV1 inhibitors.[15] Dual inhibitors of FAAH and TRPV1 that are more stable and drug-like than AA-5-HT have been pursued by others.[12a,16] Dual inhibition of COX2 and FAAH has also been explored, with early indications that greater analgesia can be achieved with fewer adverse effects than targeting each alone.[17]

NSAIDs treat pain by inhibiting COX, which catalyzes the first steps in the conversion of arachidonic acid (AA) into prostanoids associated with pain and inflammation. Most NSAIDs reversibly bind the COX active site, mimicking the unsaturated fatty chain and carboxylic acid head group of AA. Based on their ability to bind the AA site on COX enzymes, NSAIDs were hypothesized to be able to also effectively mimic the AA portion of AA-5-HT at its binding sites on FAAH and TRPV1. Evidence to support this hypothesis includes the ability of some NSAIDs to weakly inhibit FAAH,[18] as well as inhibition of FAAH by some analogues of ibuprofen.[17b,19] In this work, a series of NSAIDs were conjugated to serotonin by forming an amide bond between the serotonin amine and the carboxylic acid group of the NSAIDs. The resulting NSAID-5-HT analogues were tested for their ability to inhibit FAAH, TRPV1, and COX2.

Serotonin conjugates were prepared as shown in Scheme 1 by treating a stirred solution of the NSAID in DMF with hydroxybenzotriazole (HOBt) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC) at 0°C. The solution was then brought to room temperature, followed by addition of serotonin-HCl and triethylamine. After stirring overnight, products were extracted into ethyl acetate, subjected to aqueous workup, and purified by flash chromatography.

The serotonin conjugates shown in Figure 1 were synthesized and tested as inhibitors against FAAH, TRPV1, and COX2. To assist in identifying structure-related trends in activity, the NSAID components were selected from each of the major structural classes of carboxylic acid containing NSAIDs: salicylates (salicylate-5-HT and ASA-5-HT), arylacetic acids (Diclofenac-5-HT), heteroarylacetic acids (Indomethacin-5-HT), *N*-arylanthranilic acids (Flufenamate-5-HT), 2-arylpropionic acids (Flurbiprofen-5-HT, Ibuprofen-5-HT, Naproxen-5-HT, Fenoprofen-5-HT, and Ketoprofen-5-HT), and a cyclized heteroarylpropionic acid (Ketrolac-5-HT). The results from inhibition assays of these NSAIDs conjugated with serotonin are shown in Table 1.

AA-5-HT was purchased and used as a positive control. AA-5-HT is a synthetic compound originally identified in a screen for novel FAAH inhibitors.[12b] Subsequent work showed AA-5-HT is also an antagonist of TRPV-1 and it was reported to be the first dual inhibitor of FAAH and TRPV1 to appear in the literature.[13] As the prototype for dual FAAH/TRPV1 inhibition, AA-5-HT has been the reference compound of choice for drug discovery efforts in this area.[12a,16b]

All the NSAID-5-HT analogues and AA-5-HT significantly inhibited COX2 using an inhibitor concentration of 10 μM in activity screens. At 10 μM, AA-5-HT was among the least potent inhibitors of COX2 while, consistent with observations made by other investigators who have studied COX inhibition by NSAID amides, indomethacin-5-HT was most potent.[20]

Fenoprofen-5-HT and Naproxen-5-HT appear to be able to inhibit both TRPV1 and COX2 with approximately the same potency as AA-5-HT, but do not inhibit FAAH, even at concentrations of 50 μM. Only Ibuprofen-5-HT and Flurbiprofen-5-HT seem to inhibit all three targets with potencies similar to AA-5-HT. None of the parent NSAIDs, which are known COX inhibitors, showed significant inhibition of FAAH in DMSO at 10 μM (data not shown). The parent NSAIDs were also less active than their serotonin counterparts at reducing TRPV1 activity. Flufenamic acid and diclofenac sodium at 50 μM reduced residual TRPV1 activity to 66% and 61%, respectively, and indomethacin at 250 μM reduced TRPV1 activity to 82% (Supplementary Information). None of the other NSAIDs showed activity at these concentrations.

Several observations about structure-activity relationships can be made from this series, including that: 1) all lead compounds are NSAIDs of the 2-arylpropionic acid class, 2) analogues with *para*-oriented aryl substituents showed the best inhibition of FAAH, and 3) replacing the ether linkage of fenoprofen with a ketone (in ketoprofen) significantly reduced TRPV1 inhibition. Furthermore, an amide linker, rather than a carbamate or urea, was present in all analogues, consistent with the observation that amide linkers are better suited to provide dual inhibition of FAAH and TRPV1.[12a]

There was greater inhibition of FAAH by the NSAID analogues and AA-5-HT in the IC50 assays using ethanol as a stock solvent than there was in initial screens where the analogues were delivered in DMSO (Table 1). The use of ethanol allowed AA-5-HT to be aliquoted on ice without freezing the solvent and the effect on inhibition may be due to improved

solubility or dispersal of the compounds in buffer when delivered in ethanol. In the IC50 assays, AA-5-HT was aliquoted on ice and flushed with argon to minimize loss of inhibitory activity, presumably due to oxidation of its unsaturated fatty acid chain. The NSAID-5-HT analogues, which did not require aliquoting on ice or the use of an inert gas during handling, are likely to have better drug properties than AA-5-HT in regard to stability and oral bioavailability.

Hyperthermia has been a hindrance to the development of TRPV1 antagonists. This side-effect has been hypothesized to be due to inhibition of TRPV1 by molecules that bind the proton binding site, as opposed to the capsaicin binding site, since hyperthermia seems to be associated only with antagonists that inhibit activation of TRPV1 by acidic pH.[21] The effect of NSAID-5-HT analogues on body temperature has not been determined. However, systemic administration of AA-5-HT did not cause hyperthermia in mice.[14]

Because previous work has indicated that multi-target inhibitors with modest activity may provide equivalent therapeutic outcomes with fewer adverse effects compared to highly potent, single-target inhibitors, the NSAID-5-HT analogues reported here may be not only useful tools for studying the effects of multi-target inhibition in animals, but also therapeutic leads themselves.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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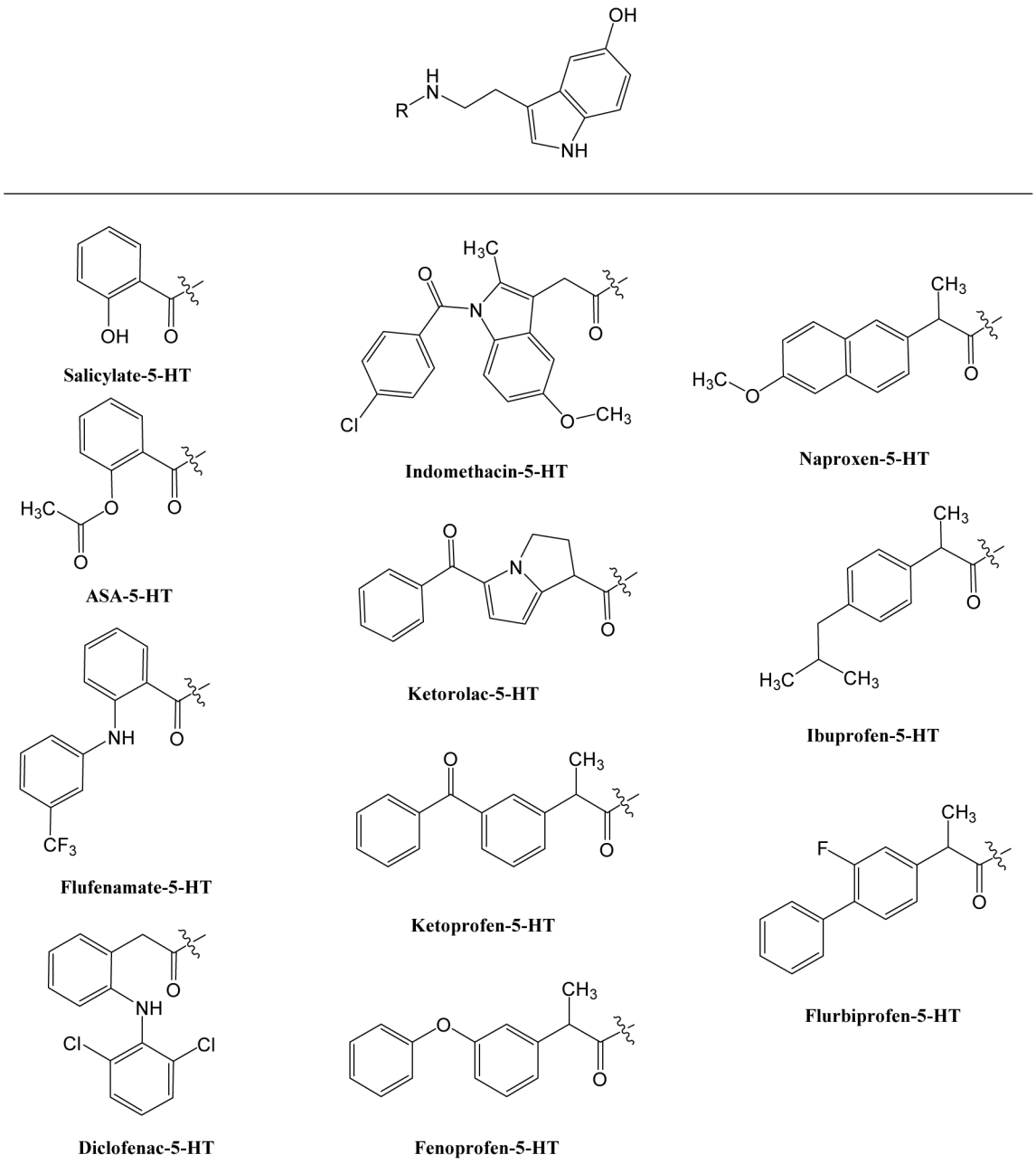
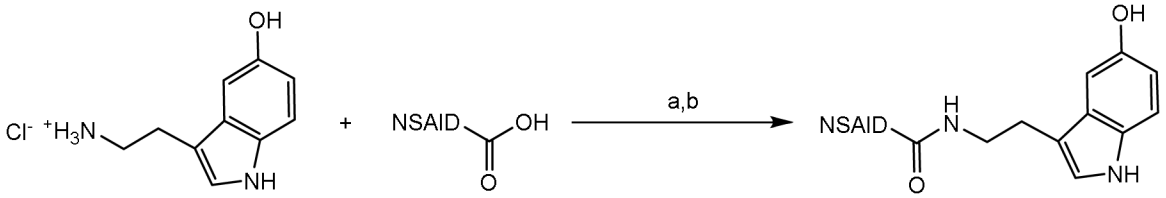


Figure 1.
Structures of the NSAID-5-HT conjugates tested against FAAH, TRPV1, and COX2.



Scheme 1.

General synthesis of NSAID-5-HT analogues. Reagents and conditions: (a) DMF, HOBT, EDC, 0°C, then 1 h at rt; (b) serotonin hydrochloride, NEt_3, overnight at rt.

Table 1

Results from FAAH, TRPV1, and COX2 inhibition assays.[a]

Compound	FAAH	COX2	TRPV1
	% activity (SD), DMSO	% activity (SD), EtOH	--
Name, MW	IC50 (95% CI), EtOH	IC50 (95% CI), EtOH	IC50 (95% CI), DMSO
AA-5-HT, 462.67	78% (6)	66% (2)	--
	16 μM (8–31 μM)	5 μM (2–11 μM)	10 μM (7.3–14.4)
Salicylate-5-HT, 296.32	101% (10)	51% (8)	--
	--	--	70 μM (33.8–144.2)
ASA-5-HT, 338.36	99% (5)	60% (9)	--
	--	--	203 μM (128.9–317.9)
Flufenamate-5-HT, 439.43	107% (18)	38% (4)	--
	--	--	10 μM[b]
Diclofenac-5-HT, 454.35	98% (5)	43% (7)	--
	--	--	19 μM (14.6–25.7)
Indomethacin-5-HT, 515.99	94% (7)	7% (1)	--
	--	--	48 μM (37.5–60.9)
Ketorolac-5-HT, 413.47	98% (6)	43% (3)	--
	--	--	70 μM (60.8–80.1)
Ketoprofen-5-HT, 412.48	88% (15)	51% (3)	--
	--	--	45 μM (31.6–64.6)
Fenoprofen-5-HT, 400.47	105% (9) [50 μM]	49% (2)	--
	--	7 μM (2–25 μM)	8 μM (6.5–9.6)
Naproxen-5-HT, 388.46	73% (6) [50 μM]	48% (3)	--
	--	18 μM (12–25 μM)	13 μM (10.4–16.7)
Ibuprofen-5-HT, 364.48	75% (9)	42% (2)	--
	5 μM (3–8 μM)	10 μM (8–13 μM)	6 μM (5.2–7.6)
Flurbiprofen-5-HT, 402.46	85% (15)	38% (1)	--
	15 μM (11–20 μM)	8 μM (6–9 μM)	9 μM (7.8–10.5)

[a] Values from positive controls containing no inhibitor were used as the denominator in % activity calculations. All % activity measurements were taken at 10 μM inhibitor, unless noted otherwise in brackets.

[b] This concentration only provided approximately 40% inhibition. Flufenamate-5-HT appears to stimulate TRPV1 activity at concentrations higher than 10 μM.