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Research paper

SAR studies on truxillic acid mono esters as a new class of antinociceptive agents targeting fatty acid binding proteins

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ABSTRACT

Fatty acid binding proteins (FABPs) serve as critical modulators of endocannabinoid signaling by facilitating the intracellular transport of anandamide and whose inhibition potentiates anandamide signaling. Our previous work has identified a novel small-molecule FABP inhibitor, α-truxillic acid 1-naphthyl monoester (SB-FI-26, **3**) that has shown efficacy as an antinociceptive and anti-inflammatory agent in rodent models. In the present work, we have performed an extensive SAR study on a series of **3**-analogs as novel FABP inhibitors based on computer-aided inhibitor drug design and docking analysis, chemical synthesis and biological evaluations. The prediction of binding affinity of these analogs to target FABP3, 5 and 7 isoforms was performed using the AutoDock 4.2 program, using the recently determined co-crystal structures of **3** with FABP5 and FABP7. The compounds with high docking scores were synthesized and evaluated for their activities using a fluorescence displacement assay against FABP3, 5 and 7. During lead optimization, compound **3l** emerged as a promising compound with the Ki value of 0.21 μM for FABP 5, 4-fold more potent than 3 (Ki, 0.81 μM). Nine compounds exhibit similar or better binding affinity than **3**, including compounds **4b** (Ki, 0.55 μM) and **4e** (Ki, 0.68 μM). Twelve compounds are selective for FABP5 and 7 with >10 μM Ki values for FABP3, indicating a safe profile to avoid potential cardiotoxicity concerns. Compounds **4f**, **4j** and **4k** showed excellent selectivity for FABP5 and would serve as other new lead compounds. Compound **3a** possessed high affinity and high selectivity for FABP7. Compounds with moderate to high affinity for FABP5 displayed antinociceptive effects in mice while compounds with low FABP5 affinity lacked *in vivo* efficacy. *In vivo* pain model studies in mice revealed that exceeding hydrophobicity significantly affects the efficacy. Thus, among the compounds with high affinity to FABP5 *in vitro*, the compounds with moderate hydrophobicity were identified as promising new lead compounds for the next round of optimization, including compounds **4b** and **4j**. For select cases, computational analysis of the observed SAR, especially the selectivity of new inhibitors to particular FABP isoforms, by comparing docking poses, interaction map, and docking energy scores has provided useful insights.

1. Introduction

The fatty acid binding proteins (FABPs) are a family of small

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chaperone proteins that act as cytosolic transporters for a wide variety of lipophilic substances including fatty acids, *N*-acylethanolamines (NAE), eicosanoids, and cannabinoids [1,2]. FABPs are widely expressed throughout the body and play an integral role in a multitude of physiological processes such as lipid metabolism, inflammation and neuronal signaling [3]. FABPs of the mammalian central and peripheral nervous systems have been shown to facilitate the intracellular transport of NAEs, particularly the endocannabinoid arachidonoyl ethanolamide (anandamide, AEA), as well as catabolism by the endoplasmic reticulum-localized enzyme fatty acid amide hydrolase (FAAH) [4]. Genetic or pharmacological inhibition of the FAAH enzyme or the FABPs results in a marked elevation of brain AEA levels, which acts upon type-1 cannabinoid receptors (CB_1R) and thereby cause a suppression of pain transmission and other therapeutically beneficial effects [5–7]. As such, designing inhibitors of AEA inactivation is desirable. Over the years numerous FAAH inhibitors have been developed and have generally been well tolerated in the clinical setting [8,9]. However, focused medicinal chemistry efforts on targeting FABPs may hold advantages over direct FAAH inhibition because unlike FAAH, which is distributed throughout the body, humans express multiple FABP isoforms that exhibit tissue-specific expression patterns. Designing small molecule inhibitors that selectively bind target FABP isoforms will allow for drugs to preferentially act upon target tissues of interest rather than systemic NAE upregulation, which may increase the likelihood of off-target adverse events. This has led to the pursuit of identifying novel compounds that are capable of selectively inhibiting the FABP isoforms that are expressed in the mammalian central and peripheral nervous systems. Three FABP isoforms have been identified in these tissues, i.e., FABP3 (heart FABP, H-FABP), FABP5 (epidermal FABP, keratinocyte FABP, E-FABP), and FABP7 (brain FABP, B-FABP) [10].

Previous work from our group has led to the identification of a novel competitive FABP inhibitor, α-truxillic acid 1-naphthyl monoester (SB-FI-26, **3**) [11]. It has been shown that **3** is a potent inhibitor of FABP5 and FABP7, with sub-micromolar affinities reported *in vitro* ($K_i = 0.9 \pm 0.1$ μM and 0.4 ± 0.0 μM, respectively), with weaker binding to FABP3 ($K_i = 3.9 \pm 0.7$ μM) [6]. Selective inhibition for FABP5 over FABP3 is deemed desirable, as mice bearing a knockout for FABP3 exhibited age-related cardiac hypertrophy, and thus pharmacological inhibition of this protein may have a potential to cause undesirable side effects [12].

Intriguingly, compound **3** shares the same α-truxillic acid skeleton with that of (−)-incarvillateine (Fig. 1), a natural monoterpene alkaloid isolated from the Chinese herbal *Incarvillei sinensis,* that has been used as a pain-reliever in traditional Eastern medicine (as the dried plant matter 'Jiaohao'), and more recently has been shown to produce potent analgesic and anti-inflammatory effects in formalin-induced mouse models [13,14]. Structure-antinociceptive activity studies on (−)-incarvillateine have found that the cyclobutane moiety is required for its analgesic properties [15]. To our knowledge, the mechanism of action for (−)-incarvillateine-induced analgesia has not been formally elucidated in full, though adenosine receptors likely play a role and there is some contention in the literature pertaining to the involvement of the opioid system [16,17]. Considering the structural similarity and essentially overlapping reported pharmacological profiles of (−)-incarvillateine and **3**, it is tempting to speculate a possibility that its effects are mediated, at least in part, by FABP inhibition and subsequent NAE/endocannabinoid potentiation.

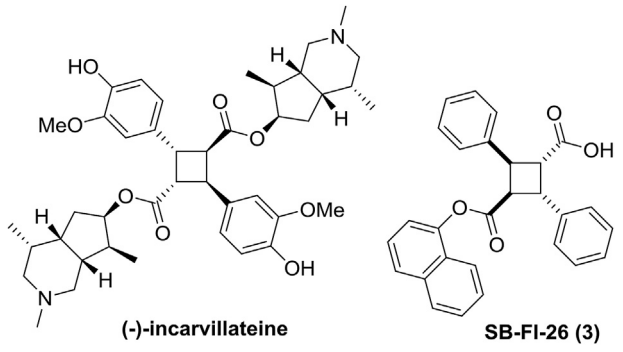

Fig. 1. Structures of (−)-incarvillateine and **3**.

Compound **3** was further shown to be biologically active in a FABP and CB_1R-dependent manner. The compound has a half-life of ~3 h *in vivo* and is efficacious in producing anti-inflammatory and anti-nociceptive effects in rodent models of visceral, thermal, neuropathic, and inflammatory pain [6]. Furthermore, intraperitoneal (i.p.) administration of **3** up to 40 mg/kg in mice showed no conditioned place preference nor conditioned place aversion, indicating a relatively low potential for addiction [18].

Despite promising efficacy in pain models, **3** requires further preclinical optimization to improve potency, solubility, selectivity and *in vivo* stability. To this end, the α-truxillic acid monoester core structure was used as the scaffold for optimization in the present study. Based on the prediction using the Autodock 4.2 program [19], a series of novel **3**-analogs have been designed, synthesized, and their potencies evaluated to investigate SAR for enhanced potency and selectivity.

2. Results and discussion

2.1. Optical resolution of 3

In the previous study, we examined the potency of racemic **3**. However, our recent protein X-ray structure determination of FABP5/**3** as well as FABP7/**3** cocrystals revealed that (*S,S,S,S*)-**3** was incorporated into the canonical binding site. Accordingly, there is a good possibility that the (*S,S,S,S*)-enantiomer may be substantially more potent than the (*R,R,R,R*)-enantiomer. Thus, we set out to optically resolve the two enantiomers of **3**.

(*1R,2S*)-2-amino-1,2-diphenylethanol, (*S*)-1-(1-naphthyl)ethylamine, and (*S*)-phenylalaninol (Fig. 2), were examined as resolving agents. A mixture of **3** and a resolving agent was dissolved in common lab solvents (i.e., ethanol, isopropanol, and acetonitrile) and allowed to recrystallize as diastereomeric salts. Samples showing crystal formation were acidified with 4 M HCl, extracted with ethyl acetate, and analyzed by chiral HPLC using a Chiralcel ODH column (iPrOH/hexane). The attempts using (*1R,2S*)-2-amino-1,2-diphenylethanol, and (*S*)-1-(1-naphthyl)ethylamine resulted in recovering only racemic **3**. Fortunately, (*S*)-phenylalaninol was found to be a suitable resolving agent in combination with methanol. Thus, we were able to isolate two enantiomers, **3-A** and **3-B**. (See Experimental for details.)

Since **3-A** gave better crystals, it was subjected to an X-ray crystallographic analysis and unambiguously determined to be the (1*R*,2*R*,3*R*,4*R*)-enantiomer (Fig. 3). (See Supporting Information for crystallographic data.) Thus, **3-B** was assigned to the (1*S*,2*S*,3*S*,4*S*)-enantiomer.

It should be noted, however, the fluorescence displacement assay of the two enantiomers showed little difference in activity. Ki values of (*R,R,R,R*)-**3** were 0.71 ± 0.08 μM and 0.92 ± 0.22 μM for FABP5 and FABP7, respectively, while Ki values of (*S,S,S,S*)-**3** were 0.79 ± 0.15 μM and 0.45 ± 0.01 μM for FABP5 and FABP7, respectively. The Ki values of two enantiomers for FABP5 are within an error, although there is a recognizable difference in their values for FABP7. Based on these data, we concluded that synthesis of