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Pentafluorosulfanyl-containing flufenamic acid analogs: Syntheses, properties and biological activities

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Abstract

Pentafluorosulfanyl-containing analogs of flufenamic acid have been synthesized in high yields. Computationally, pK_a , logP and logD values have been determined. Initial bioactivity studies reveal effects as ion channel modulators and inhibitory activities on aldo-keto reductase 1C3 (AKR1C3) as well as COX-1 and COX-2.

Graphical Abstract

Keywords

Flufenamic acid; Pentafluorosulfanyl group; Computational conformational analyses; Ion channel modulator; Bile acid-sensitive ion channel; AKR1C3 inhibitor

Compounds with fluorine-containing substituents such as trifluoromethyl (CF₃) and pentafluorosulfanyl (SF₅) groups have attractive chemical and biological properties.¹ As such groups affect the electronic and steric parameters of molecules, bioavailability and pharmacokinetics are often improved. Consequently, incorporating fluoro substituents into crop protection agents and pharmaceuticals^{2–3} is a rapidly expanding field of research.⁴

Flufenamic acid (**1a**, Figure 1), namely 2-[[3-(trifluoro-methyl)phenyl]amino]-benzoic acid, is a CF₃-containing anthranilic acid derivative with various applications in biology and medicine. It has been recognized as highly effective ion channel modulator, and a particularly useful tool in studying the mode of action of a variety of ion channels, including Cl⁻, Ca²⁺, Na⁺, K⁺ and GABA channels, and non-selective cation channels.⁵ In terms of pharmaceutical applications, an early discovery in 1963 revealed anti-inflammatory and analgesic properties of **1a**.⁶ Compound **1a** belongs to the fenamate class of non-steroidal anti-inflammatory drugs (NSAIDs) and inhibits cyclooxygenase.⁷ Recently, flufenamic acid (**1a**) has been successfully applied as lead compound in a study on new therapeutics for castration resistant prostate cancer (CRPC).⁸ Structural analogs of **1a**, such as 3-aminobenzoic acids **1b** and **1c** (Figure 1), have been prepared, showing potent inhibition of aldo-keto reductase 1C3 (AKR1C3),⁹ an enzyme over-expressed in CRPC and required for intratumoral androgen biosynthesis.

Due to its unique properties, the SF₅ group is of special interest among fluorine-containing substituents.¹⁰ Incorporation of this 'super-trifluoromethyl' group, as it is often called, into aromatic compounds involves high thermal stability and inertness to hydrolysis, superior to CF₃.¹¹ Particularly attractive is the high electronegativity of SF₅-containing compounds,¹² leading to an increased polarity in the respective molecules, in combination with superior lipophilicity.¹³ Consequently, the exchange of CF₃ to SF₅ in drugs and crop protection agents can be beneficial, resulting in improved biological profiles, as for example observed for SF₅-containing mefloquine,¹⁴ fenfluramine,¹⁵ trifluralin¹⁶ and fipronil.¹⁷

Surprisingly, to the best of our knowledge, SF₅-containing derivatives of flufenamic acid have not been reported up to now. Following our interest in SF₅-containing compounds¹⁸ and structurally modified NSAIDs,¹⁹ we herein present the synthesis and property assessment of SF₅-containing flufenamic acid analogs **2a–d** (Figure 1). The analysis includes computational investigations of conformations and ADME parameters (computed pK_a, LogP, and LogD values) and biological evaluations of ion channel modulation and inhibitory activity against AKR1C3.

Flufenamic acid SF₅-derivatives **2a–d** were prepared in high yields following two-step sequences (Scheme 1).⁸ First, palladium-catalyzed Buchwald-Hartwig-type coupling reactions of pentafluorosulfanyl anilines **3** and **4** with methyl bromobenzoates **5** and **6** were performed, leading to methyl esters **7a–d** in yields of 98–99%. Subsequent saponifications with KOH afforded products **2a–d** in yields ranging from 79% to 95%.²⁰

With the goal to reveal structural parameters possibly affecting reactivity patterns, the new compounds (**2a–d**) were investigated computationally, and the results were compared to the analogous data of the parent compound, flufenamic acid (**1a**).

Conformational analyses of **1a** and the flufenamic acid analogs **2a–2d** were performed in water, applying the M06-2X level of theory.²¹ These studies indicated that regardless of the substitution pattern, all compounds favor very analogous minimum energy conformations (see Figure 2), featuring a slight twist between the aryl moieties and, where possible, a stabilizing intramolecular O...H-N interaction (*i.e.* in **2a** and **2d**). Similarly, our calculations

of the ADME parameters (pK_a , LogP and LogD values) using *Cosmotherm*²² predicted similar properties for flufenamic acid and its analogues.²³ See Figure S2 in the SI for pK_a ,

LogP, LogD of **2a–d** relative to **1a**. These data therefore suggest that the origin of activity differences of the compounds is not likely due to their conformational or physical properties.

The bile acid-sensitive ion channel (BASIC) is a cation channel sensitive to alterations of its membrane environment.^{24,25} Like naturally occurring bile acids, flufenamic acid activates rat BASIC,²⁶ presumably by interacting with the cell membrane.²⁵ Therefore, rBASIC was used as a model to investigate ion channel modulation by the SF₅-containing flufenamic acid analogs (compounds **2a–d**).

Similar to flufenamic acid (**1a**), all four analogs **2a–d** induced rapid and reversible increases in current amplitude when applied at 1 mM to *Xenopus* oocytes heterologously expressing rBASIC (Figure 3a). Interestingly, all compounds activated rBASIC more strongly than flufenamic acid. While the amplitude of rBASIC currents induced by compounds **2b–2d** was 1.5- to 3-fold larger than the amplitude induced by flufenamic acid, it was 8-fold larger for compound **2a** (Figure 3b). Due to the limited solubility of flufenamic acid and its analogs in the aqueous bath solution, apparent affinities could not be determined precisely. With this reservation, the EC₅₀ of flufenamic acid for rBASIC was 2.6 ± 0.3 mM, similar to previous reports.²⁶ While apparent affinities of compounds **2b**, **2c** and **2d** were not significantly different from that of flufenamic acid (EC₅₀: 2.9 ± 0.1 mM, 2.8 ± 0.4 mM and 2.6 ± 0.2 mM, respectively; $n = 9$), apparent affinity of compound **2a** was significantly higher (EC₅₀: 1.4 ± 0.1 mM; $p < 0.005$, $n = 9$) (Figure 3c). These results suggest that the SF₅-substitution of the CF₃-group of flufenamic acid increases efficacy of rBASIC activation. Compound **2a** may additionally have a higher potency (increased affinity) at rBASIC. Considering the similar chemical properties of compounds **2a–d** and flufenamic acid (**1a**), this suggests that these new compounds activate rBASIC not solely via a membrane-based mechanism but via a specific interaction with the ion channel.

AKR1C3 is a potential therapeutic target for the treatment of CRPC because of its pivotal role in converting 4-androstene-3,17-dione and 5 α -androstane-3, 17-dione to testosterone and dihydrotestosterone which are potent ligands for the androgen receptor in the prostate.²⁷ An important consideration in the development of AKR1C3 inhibitors is selectivity. AKR1C1 and AKR1C2 share > 86% sequence identity with AKR1C3, and are also involved in dihydrotestosterone inactivation, so their inhibition would be undesirable. The inhibitory potencies of SF₅-analogs for both AKR1C2 and AKR1C3 were determined, and their selectivities were compared by using the ratio of IC₅₀ values observed for AKR1C2 and AKR1C3, where a high ratio shows high selectivity for AKR1C3. Compound **2a** displayed 4.7-fold selectivity for AKR1C3 (IC₅₀: 57 nM) over AKR1C2 (IC₅₀: 270 nM), which is comparable to that seen with flufenamic acid (**1a**).⁸ When the carboxyl group was moved from the *ortho* position in **2a** to the *meta* position in **2b**, a 1.5-fold decrease of inhibitory potency for AKR1C3 (IC₅₀: 86 nM) was observed accompanied by a loss of inhibitory activity for AKR1C2 (IC₅₀: 14 μ M), which resulted in 35-fold increase in inhibitor selectivity (IC₅₀ ratio: 163). This shows that the movement of carboxyl group did not have a remarkable effect on the inhibitory potency for AKR1C3 but decreased the inhibitory

potency for AKR1C2 significantly, a similar trend was observed for compounds **2c** and **2d**. The pronounced decrease of inhibitory activity for AKR1C2 by the movement of carboxyl group has been reported when **1a** and **1b** were compared. It is reasoned that the *meta*-carboxyl compounds alter the orientation of the phenylamino- group and prevent its binding to the smaller SP1 pocket in AKR1C2.⁸ When the SF₅ group was moved from the *meta* in **2b** to the *para* position in **2c**, the inhibitory potency for AKR1C3 was increased by 2.5-fold (IC₅₀: 35 nM). In addition, compound **2c** retained its inhibitory activity for AKR1C2 (IC₅₀: 20 μM), which led to further 3.5-fold increase in selectivity for AKR1C3 (IC₅₀ ratio: 571). The positional effect of moving the SF₅ group around the phenyl ring was also observed when the CF₃ group is moved in compound **1b** (IC₅₀: 320 nM) to give compound **1c** (IC₅₀: 60 nM).⁸ This effect was related to the electron withdrawing properties of CF₃. In comparison to **2a**, compound **2d** displayed a similar increase in inhibitory potency for AKR1C3 (IC₅₀: 36 nM) when the position of SF₅ group was changed. However, in contrast to **2c**, the inhibitory potency of **2d** for AKR1C2 was increased (IC₅₀: 150 nM), resulting in little change in the inhibitor selectivity for AKR1C3 (IC₅₀ ratio: 4.2). Based on these data, both **2b** and **2c** displayed high inhibitory potency (IC₅₀ < 100 nM) and high inhibitory selectivity (IC₅₀ ratio > 150) for AKR1C3, and could be considered as new drug candidates for CRPC related to AKR1C3.

Flufenamic acid analogs have been used as the inhibitors of cyclooxygenase (COX-1 and COX-2) as NSAIDs. The inhibitory potencies of compounds **2a–2d** to COX enzymes were also analyzed. As shown in Table S4, **2a** displayed the inhibitory activities towards both COX-1 (IC₅₀: 1.8 μM) and COX-2 (IC₅₀: 12 μM) for the catalysis of the conversion of arachidonic acid to prostaglandin H₂ (PGH₂). Compound **2d** showed the inhibitory effect on COX-1 (22 μM) and no inhibitory effect on COX-2 at 100 μM, and **2b** and **2c** displayed little or no inhibitory potencies to COX-1 and COX-2 at 100 μM. The data shows again the high inhibitory selectivity of **2b** and **2c** to AKR1C3.

In conclusion, we prepared SF₅-containing analogs of flufenamic acid and analyzed their structural parameters and ADME properties computationally. Biological activities were investigated with respect to ion channel modulation and AKR1C3 inhibition. While all flufenamic acid analogs activated the ion channel rBASIC more strongly than flufenamic acid itself, compound **2a** had highest potency, inducing 8-fold larger currents at 1 mM. SF₅-containing analogs of flufenamic acid should therefore be further explored as possible modulators of ion channels. In another context AKR1C3 inhibition, compounds **2b** and **2c** are of interest, as they show both high inhibitory potency and high inhibitory selectivity identifying them as potential new drug candidates for CRPC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Flufenamic acid (**1a**), structural CF₃-analogs **1b** and **1c** and new SF₅-analogs **2a-d**, presented in this work.

Figure 2. Favored minimum energy conformations of **1a** (top) and synthetic analogues **2a–d** (bottom), calculated at SMD (H₂O) M062X/6-311++G(d,p)//SMD (H₂O) B3LYP/6-31G(d) level of theory.²¹

Figure 3.

rBASIC is potently activated by SF₅-containing flufenamic acid analogs. A) Representative current trace from a *Xenopus* oocyte expressing rBASIC. Successive application of 1 mM flufenamic acid (FFA) and 1 mM of the flufenamic acid analogs **2a**, **2d**, **2b** and **2c** rapidly and reversibly induced inward currents. B) Quantitative comparison of peak current amplitudes induced by the application of 1 mM flufenamic acid or compounds **2a**, **2d**, **2b** and **2c** as shown in (A). Currents were normalized to the peak current induced by flufenamic acid. Error bars represent S.E.M. (n = 6; **, p < 0.005; ***, p < 0.001). The order of compound application was varied in different experiments. C) Concentration-response curves for flufenamic acid and its analogs. Error bars represent S.E.M. and lines fits to the Hill equation (n = 9)

Scheme 1.

Syntheses of SF_5 -containing flufenamic acid analogs **2a–d**.

Reagents and conditions: (a) $\text{Pd}(\text{OAc})_2$ (5 mol%), BINAP (8 mol%), Cs_2CO_3 , toluene, 110 °C, 18 h; (b) KOH, EtOH, H_2O , 100 °C, 2 h.