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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.

Compounds with fluorine-containing substituents such as trifluoromethyl (CF_3) and pentafluorosulfanyl (SF_5) 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**, Fig. 1), namely 2-{[3-(trifluoromethyl)phenyl]amino}-benzoic acid, is a CF_3-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^-, Ca^{2+}, 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** (Fig. 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 SF_5 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 CF_3.[11] Particularly attractive is the high electronegativity of SF_5-containing compounds,[12] leading to an increased polarity in the respective molecules, in combination with superior lipophilicity.[13] Consequently, the exchange of CF_3 to SF_5 in drugs and crop protection agents can be beneficial, resulting in improved biological profiles, as for example observed for SF_5-containing mefloquine,[14] fenfluramine,[15] trifluralin[16] and fipronil.[17]

Surprisingly, to the best of our knowledge, SF_5-containing derivatives of flufenamic acid have not been reported up to now. Following our interest in SF_5-containing compounds[18] and structurally modified NSAIDs,[19] we herein present the synthesis and property assessment of SF_5-containing flufenamic acid analogs **2a–d** (Fig. 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.

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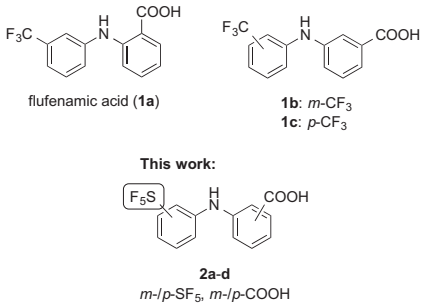


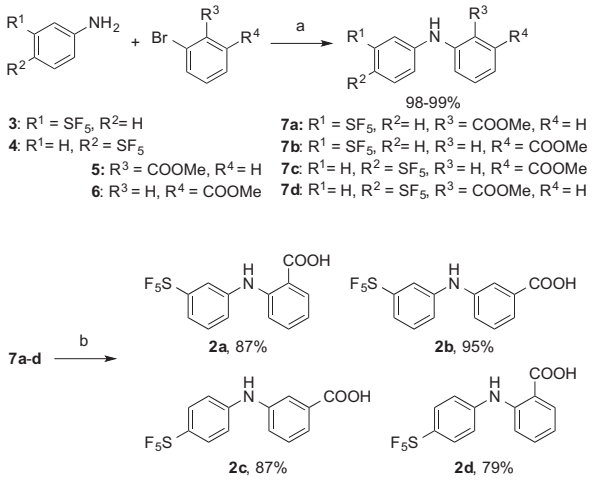
Figure 1. Flufenamic acid (**1a**), structural CF_3-analogs **1b** and **1c** and new SF_5-analogs **2a–d**, presented in this work.

Flufenamic acid SF_5-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–d** 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 Fig. 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 Table S2 in the SI for ΔpK_a, ΔLogP, ΔLogD 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,



Scheme 1. Syntheses of SF_5-containing flufenamic acid analogs **2a–d**. Reagents and conditions: (a) $Pd(OAc)_2$ (5 mol %), BINAP (8 mol %), Cs_2CO_3, toluene, 110 °C, 18 h; (b) KOH, EtOH, H_2O, 100 °C, 2 h.

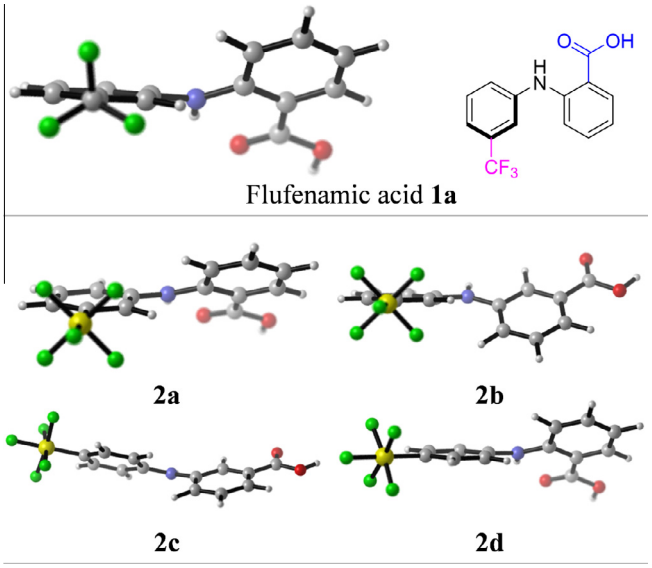


Figure 2. Favored minimum energy conformations of **1a** (top) and synthetic analogues **2a–d** (bottom), calculated at SMD (H_2O) M062X/6-311++G(d, p)//SMD (H_2O) B3LYP/6-31G(d) level of theory.[21]

rBASIC was used as a model to investigate ion channel modulation by the SF_5-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 (Fig. 3a). Interestingly, all compounds activated rBASIC more strongly than flufenamic acid. While the amplitude of rBASIC currents induced by compounds **2b–d** was 1.5- to 3-fold larger than the amplitude induced by flufenamic acid, it was 8-fold larger for compound **2a** (Fig. 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 EC_{50} 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 (EC_{50}: 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 (EC_{50}: 1.4 ± 0.1 mM; $p < 0.005$, $n = 9$) (Fig. 3c). These results suggest that the SF_5-substitution of the CF_3-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 5α-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 SF_5-analogs for both AKR1C2 and AKR1C3 were determined, and their selectivities were compared by using the ratio of IC_{50} values observed for AKR1C2 and AKR1C3, where a high ratio shows high selectivity for AKR1C3. Compound **2a** displayed 4.7-fold selectivity for AKR1C3 (IC_{50}: 57 nM) over AKR1C2 (IC_{50}: 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