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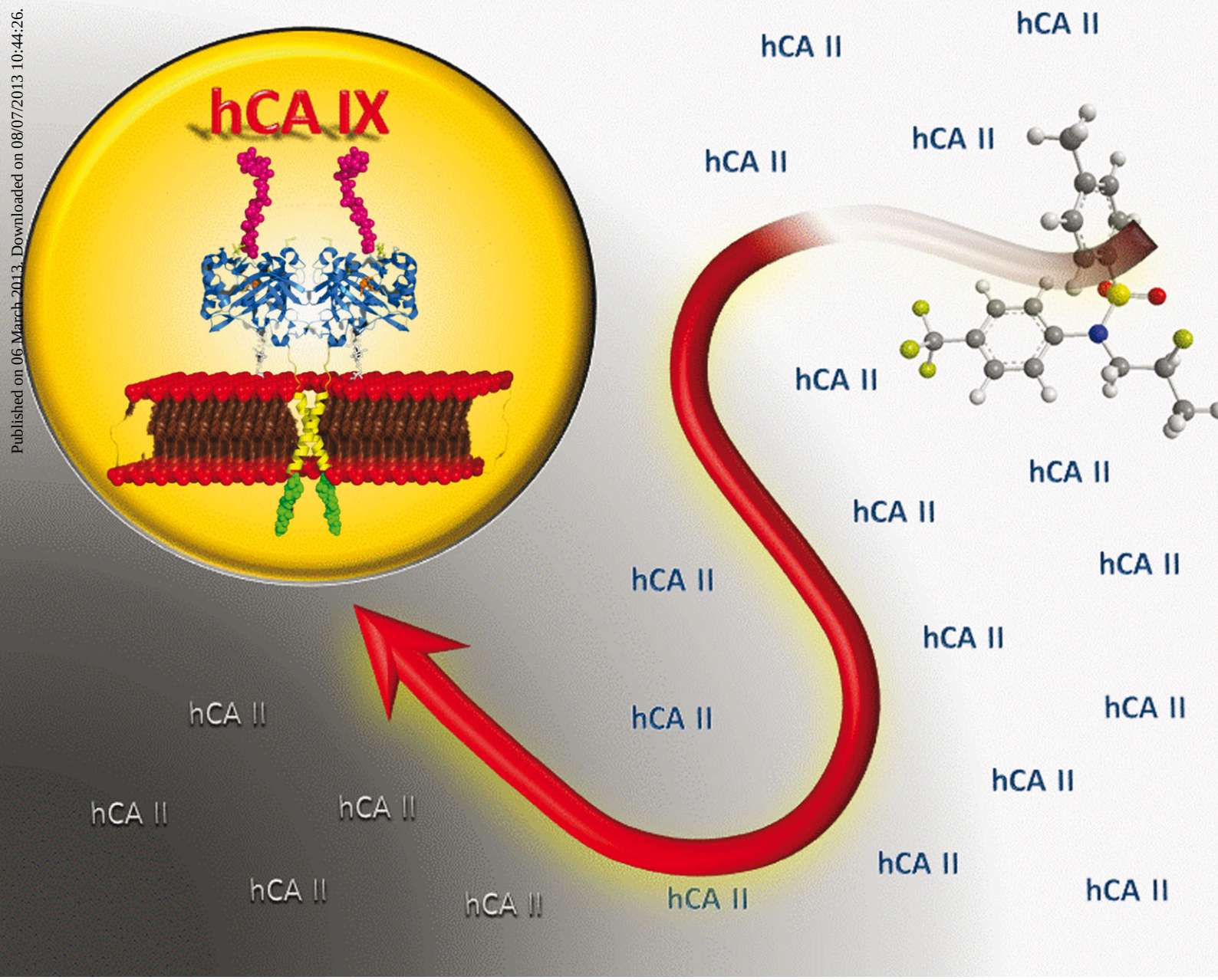

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Claudiu. T. Supuran, Sébastien Thibaudeau *et al.*

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New superacid synthesized (fluorinated) tertiary benzenesulfonamides acting as selective hCA IX inhibitors: toward a new mode of carbonic anhydrase inhibition by sulfonamides†

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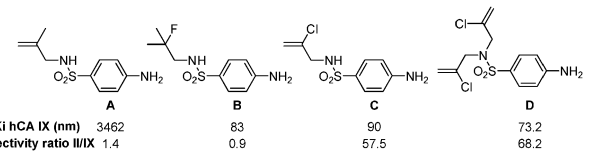
Benoît Métayer,[a] Agnès Mingot,[a] Daniella Vullo,[b] Claudiu. T. Supuran*[b] and Sébastien Thibaudeau*[a]

Tertiary substituted (fluorinated) benzenesulfonamides were synthesized in superacid HF/SbF_5 and tested as inhibitors of human carbonic anhydrases (hCAs, EC 4.2.1.1). Strong selectivity toward tumor-associated hCA IX, without inhibiting the offtarget hCA II, was observed, pointing out to a new mechanism of action compared to classical sulfonamides.



Scheme 1 4-Aminobenzenesulfonamide inhibition data.

Carbonic anhydrases (CAs, EC 4.2.1.1) are widespread metalloenzymes that catalyse the reversible hydratation of carbon dioxide to bicarbonate and protons.[1] Human CAs (hCAs) exist in sixteen isoforms, belonging to the so-called α-class and differ widely in their cellular localization (cytosol, mitochondria, cell membrane).[2] Sulfonamides and their bioisosteres (sulfamates, sulfamides, *etc.*) constitute the most investigated inhibitors of these enzymes,[3] with useful therapeutic applications.[4] Crystallographic studies of many sulfonamide adducts with several CA isozymes largely show the anchoring properties of these types of inhibitors.[3,5] In their deprotonated forms, sulfonamides bind the Zn^{2+} ion of the active site, disrupting the catalytic process.[6] Most of the side-effects of the sulfonamide-based CA inhibitors[7] are due to the inhibition of the cytosolic catalytically highly active isoform CA II, which is abundant in many tissues/organs and involved in numerous physiological functions (pH homeostasis, secretion of electrolytes, transport of anions, *etc.*).[4,8] Among CAs, being hypoxia overexpressed, the plasma membrane associated isoforms hCA IX and XII are now well-established anticancer drug targets.[9]As a consequence, in the quest for new anti-cancer agents, the selective inhibition of hCA IX and XII over the offtarget hCA II is essential. Therefore, much effort was dedicated in the last few years to the development of new chemotypes, with lower affinity for the CA zinc binding site, in order to be more selective toward the different isoforms.[4] Such a strategy led to the discovery of new chemotypes that selectively inhibit some targeted hCA isoforms. Recently, several classes of such agents have been detected. For example, coumarins, thiocoumarins[10] and more recently sulfocoumarins,[11] located at the entrance of the enzyme active site, were found to act as selective inhibitors of the hCA IX isozyme. In the sulfonamide series, despite the recently discovered zinc-binding ureido-substituted benzenesulfonamides,[12] few other benzenesulfonamides were reported to be hCA IX selective inhibitors. In due course of our work on the discovery of new sulfonamide type inhibitors,[13] a series of 4-aminobenzenesulfonamides were recently reported to be selective nanomolar inhibitors of tumor-associated isoforms IX and XII (Scheme 1).[14]

Whereas the allylic substituted benzenesulfonamide **A** showed only a micromolar level of inhibition toward hCA IX, its fluorinated analogue **B** inhibited hCA IX at the nanomolar level, but also the offtarget hCA II. Interestingly, a chlorinated analogue showed a similar level of inhibition for hCA IX with selectivity toward hCA II. Even more surprisingly, the secondary sulfonamide **C** and its tertiary substituted analogue **D** showed similar inhibition properties.[14] This similarity between secondary sulfonamides and tertiary ones (which cannot act on a deprotonated form) and the high level of inhibition and selectivity for hCA IX could not come from the inhibition of the enzyme through the "classical" zinc binding mode.

Here we investigate in detail this hypothesis, and report the evaluation of superacid synthesized (fluorinated) tertiary substituted benzenesulfonamide inhibitors. For the first time in the benzenesulfonamide family, hCA IX nanomolar selective

[a] *Superacid Group in "Organic Synthesis" Team – Université de Poitiers, CNRS UMR 7285 IC2MP, 4, avenue Michel Brunet, F-86022 Poitiers Cedex, France. E-mail: sebastien.thibaudeau@univ-poitiers.fr; Fax: +33-549453501; Tel: +33-549454588*

[b] *Università degli Studi di Firenze, Laboratorio di Chimica Bioinorganica, Rm 188, Via della Lastruccia 3, I-50019 Sesto Fiorentino (Firenze), Italy*

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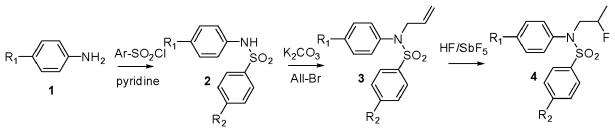

Scheme 2 Synthesis of tertiary substituted benzenesulfonamides.

CA inhibitors with no measurable affinity for the off target hCA II have been identified.

Starting from commercially available aniline derivatives **1**, sulfonylation with sulfonylchloride derivatives allowed the synthesis of benzenesulfonamide derivatives **2** (Scheme 2).

Then, allylic substitution allowed the synthesis of a large variety of tertiary substituted benzenesulfonamides **3**. Using a superacid catalyzed[15] hydrofluorination process,[16] a series of fluorinated analogues **4** could be obtained. The *N*-allylic derivatives **3a–i** and the fluorinated analogues **4b–i** were then tested as hCA inhibitors against hCA I, hCA II, hCA IX and hCA XII and compared to the previously reported compounds **A–D** (Table 1). All tertiary benzenesulfonamides were found to be ineffective as hCA II inhibitors (or a very weak inhibitor in the case of compounds **3b** and **3g**). Exceptionally, whereas they do not inhibit the highly catalytically active hCA II, all the tested compounds inhibited the tumor associated isoform IX at the nanomolar level (K_Is of 8.5–95.3 nM). To the best of our knowledge, these new tertiary benzenesulfonamide inhibitors are the first sulfonamide type inhibitors showing such a selectivity between the widespread offtarget isoform II and the targeted cancer-related isoform IX, making them new lead structures in the design of potent new anticancer agents. What also has to be noticed is the impact of the substitution pattern of the aromatic ring with halogen atoms on the efficiency of these molecules as hCA IX and XII inhibitors. For example, for the *N*-allylic derivatives, whereas the chlorinated analogue **3d** inhibits almost equally hCA I, IX and XII, its brominated analogue **3c** is less effective as an inhibitor of hCA XII (Table 1, entries 7 and 8). Interestingly the fluorinated analogue **3e** possesses a higher affinity for the tumor associated isozymes over the cytosolic isozyme CA I (Table 1, entry 9). Analogously, for the β-fluorinated derivatives, the aromatic substitution with a fluorine atom strongly alters hCA XII inhibition (Table 1, entry 17). This effect of the halogen on the inhibitor activity against hCAs is difficult to be explained at this stage, but can be compared to similar reported effects of halogen on sulfanilamide derivatives.[17] Fluorination of the allylic side chain also strongly influences the inhibitory profile of the tested inhibitors. Fluorination of the aliphatic chain increases hCA IX inhibition selectivity for derivatives **4d**, **4f** and **4h–i**, whereas a similar modification of the brominated analogue **3c** improved hCA XII inhibition (Table 1, entry 15). The strongest effect of side chain fluorination can be noticed for substrates **3e** and **4e**. Derivative **3e** can be considered as an excellent selective hCA IX inhibitor, whereas the inhibitory profile of **4e** places it in the not yet reported class of selective benzenesulfonamide hCA I inhibitors. The role of fluorine in medicinal chemistry is well recognized.[18] For the nitrogen containing compounds, the modification of the basicity of the nitrogen containing proximal functions, by using fluorine's strong electron withdrawing effect,[19] has been largely used in medicinal chemistry SAR studies.[20] It is also accepted that through a fluorine *gauche* effect and through electrostatic interactions, the fluorine atoms can strongly modify the preferred conformation of nitrogen containing biomolecules.[21] However, the reported fluorine effect could not be attributed to a modification of the interaction of the molecule with the Zn^{2+} ion, as tertiary benzenesulfonamides cannot interact with the metal ion due to steric impairment. In addition, a strong influence of the modifications of the aromatic ring on the inhibition properties has been noticed. This strong effect of aromatic substitution is comparable to what was recently reported on non-zinc-binding inhibitors such as (thio)coumarin derivatives.[10,22] As a consequence, the reported behavior of tertiary substituted benzenesulfonamides reported here probably correlates with a new mode of inhibition for these "classical" inhibitors.

A series of new tertiary (fluorinated) benzenesulfonamide inhibitors were synthesized and explored as inhibitors of several physiologically/pathologically relevant CA isozymes with the aim of discovering inhibitors that could selectively target the tumor associated isozymes CA IX and XII. All the reported compounds showed good nanomolar inhibition of hCA IX and XII. In addition, all these compounds are ineffective inhibitors of the widespread hCA II isoform (offtarget), making them excellent candidates in the quest for hCA IX inhibitors with no side-effects. A strong influence of both aliphatic and aromatic modifications on CA inhibition selectivity has also been shown, presumably due to a new mode of action of tertiary benzenesulfonamide inhibitors. This study led to the discovery of a new hCA IX highly selective fluorinated inhibitor which could be considered as a lead compound in the discovery of benzenesulfonamide non-zinc-binding inhibitors, with an anticancer pharmacological profile.

Table 1 hCA inhibition data of benzenesulfonamides **A–D**, **3** and **4**

		Substrate		K_I[a] (nM)				Selectivity[b,c]			
Entry		R_1	R_2	hCA I	hCA II	hCA IX	hCA XII	I/ IX	I/ XII	II/ IX	II/ XII
1	**A**			7136	4708	3462	1277	2.1	5.6	1.4	3.7
2	**B**			174	75	83	39.7	2.1	4.4	0.9	1.9
3	**C**			5626	5176	90	96	62	58.6	57.5	54
4	**D**			6796	4995	73.2	79.8	92.8	85.2	68.2	62.6
5	**3a**	NO_2	Me	9.20	/[d]	95.3	52.0	0.1	0.18	/[e]	/[e]
6	**3b**	Me	NO_2	83.9	975	73.3	1327	1.1	0.06	13.3	0.7
7	**3c**	Br	Me	33.2	/[d]	34.4	837	0.9	0.04	/[e]	/[e]
8	**3d**	Cl	Me	81.7	/[d]	71.9	195.6	1.1	0.42	/[e]	/[e]
9	**3e**	F	Me	238	/[d]	8.31	82.5	28	2.8	/[e]	/[e]
10	**3f**	Me	Me	75.1	/[d]	70.1	17.7	1.1	4.2	/[e]	/[e]
11	**3g**	CF_3	Me	58.7	366	86.9	83.6	0.7	0.7	4.2	4.4
12	**3h**	Ome	Me	72.5	/[d]	49.6	96.4	1.5	0.7	/[e]	/[e]
13	**3i**	COMe	Me	81.7	/[d]	33.3	60.7	2.4	1.3	/[e]	/[e]
14	**4b**	Me	NO_2	3.8	/[d]	4.2	75.9	0.9	0.05	/[e]	/[e]
15	**4c**	Br	Me	7.5	/[d]	8.5	71.6	0.9	0.1	/[e]	/[e]
16	**4d**	Cl	Me	77.3	/[d]	9.1	100.0	8.5	0.8	/[e]	/[e]
17	**4e**	F	Me	9.8	/[d]	86.1	3511	0.1	0.003	/[e]	/[e]
18	**4f**	Me	Me	73.1	/[d]	9.3	33.6	7.8	2.2	/[e]	/[e]
19	**4g**	CF_3	Me	9.3	/[d]	81.2	80.4	0.1	0.1	/[e]	/[e]
20	**4h**	Ome	Me	78.6	/[d]	33.3	60.7	2.4	1.3	/[e]	/[e]
21	**4i**	COMe	Me	89.1	/[d]	9.6	83.8	9.3	1.1	/[e]	/[e]

[a] Errors in the range of ±5% of the reported data from 3 different assays. [b] Selectivity ratio between hCA I (hCA II) and IX. [c] Selectivity ratio between hCA I (hCA II) and XII. [d] /: Not active. [e] /: Not determined >1000.

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