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RESEARCH ARTICLE

Fluorinated pyrrolidines and piperidines incorporating tertiary benzenesulfonamide moieties are selective carbonic anhydrase II inhibitors

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Abstract

A series of substituted pyrrolidines and piperidines were synthesized using superacid HF/SbF_5 chemistry. Investigated as inhibitors of several human carbonic anhydrase (hCA, EC 4.2.1.1) isoforms, i.e. the cytosolic hCA I and II as well as the tumor-associated transmembrane isoforms hCA IX and XII, these compounds showed a never yet reported selectivity toward the human carbonic anhydrase hCA II. In the tertiary benzenesulfonamide family, this class of inhibitors points out a new mechanism of action for human carbonic anhydrase II inhibition.

Keywords

Carbonic anhydrase, fluorine, selectivity, superacid, tertiary benzenesulfonamides

History

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Introduction

Carbonic anhydrases (CAs, EC 4.2.1.1) are widely distributed metalloenzymes in the living world and are involved in many biochemical processes that depend on the hydration/dehydration of carbon dioxide/bicarbonate[1,2]. Human CAs (hCAs) exist in 15 isoforms, belonging to the α-class and differ in their cellular localization (cytosol, mitochondria and cell membrane) and catalytic activity[3]. They are involved in numerous cellular and physiological processes such as respiration, electrolyte secretion, pH homoeostasis and biosynthetic reactions which require bicarbonate as substrate including lipogenesis, glucogenesis and ureagenesis, as well as tumorigenicity among others[4]. As a consequence, CAs are well-established therapeutic targets to treat a wide range of disorders through the use of inhibitors or activators[5–7]. Classical CA inhibitors (CAIs) act by complexing the metal ion from the enzyme active site, as anions, and among the numerous families of CAIs, the primary sulfonamide is one of the most largely exploited, mainly considering the aromatic/heteroaromatic representatives of such compounds[8–11]. To prevent undesired side effects, designing selective CAIs is now largely accepted to be a privileged strategy[12,13]. In this context, the so-called family of ''non-zinc bonding inhibitors'' has deeply increased over the last few years[14–16], especially in the quest of potent and selective anti-cancer agents (targeting the cancer-associated isoforms hCA IX and XII)[17–21]. Our recent studies, focused on the synthesis of fluorinated compounds in superacid[22–26], allowed to identify a series of fluorinated tertiary benzenesulfonamides acting as selective hCA IX inhibitors[27–29]. The peculiar, never reported behavior of these tertiary benzenesulfonamides was attributed to a new mode of inhibition, comparable to that of non-zinc binding inhibitors (e.g. the coumarins)[30–32], and the evaluation of their ^{18}F-labeled analogue for imaging CA IX expression in tumors through PET imaging was recently reported[33]. As a consequence, this new class of hCA inhibitors might now be considered as an innovative tool in the design of selective CAIs for further applications in medicinal chemistry. A strong impact of the modification of the geometrical shape of the 4-aminobenzenesulfonamides on the selectivity between cytosolic (hCA I and II) and transmembrane isoforms (hCA IX and XII) was identified with compounds **A** and **B** (Table 1). The impact of the presence of fluorine atom(s) substituents and sterically crowded aromatic rings on hCA inhibition selectivity was also predominant (compounds **C** and **D**). Applied to *N*-substituted saccharin derivatives as selective hCA XII inhibitors, studies based on similar strategies were also recently conducted by one of our groups, showing a dramatic effect of the nitrogen atom substitution pattern on CA inhibition and selectivity towards various CA isoforms (compounds **E** and **F**)[34].

In this context, the X-ray crystallographic structure of the adduct of human isozyme II with the antipsychotic drug sulpiride

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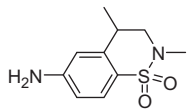
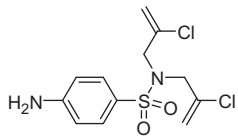
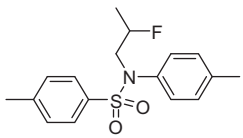
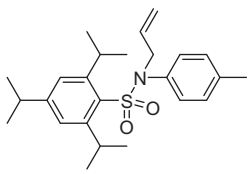
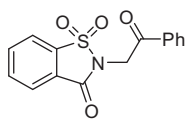
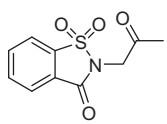
C. T. Supuran, Università degli Studi di Firenze, Laboratorio di Chimica Bioinorganica, Rm 188, Via della Lastruccia 3, I-50019 Sesto Fiorentino (Firenze), Italy. Tel: + 39-055-4573005. Fax: + 39-055-4573385. E-mail: claudiu.supuran@unifi.it

Table 1. Inhibition data of selected tertiary benzenesulfonamides and saccharins against isoforms hCA I, II, IX and XII.

Entry	Substrate		Ki (μM)*			
			hCA I†	hCA II†	hCA IX‡	hCA XII‡
1¶	**A**		0.637	0.431	0.136	0.298
2¶	**B**		6.796	4.995	0.073	0.080
3§	**C**		0.073	>50	0.009	0.033
4||	**D**		>100	>100	0.451	>100
5#	**E**		>50	>50	>50	1.780
6#	**F**		>50	0.042	0.150	0.440

*Errors in the range of ±5% of the reported data from three different assays.
†Recombinant isoforms[35–37].
‡Catalytic domain.
¶Values from Ref. [29].
§Values from Ref. [27].
||Values from Ref. [28].
#Values from Ref. [34].

strongly inspired this work[38]. Sulpiride has been shown to selectively inhibit hCA II versus hCA I and the membrane-bound hCA IV. Furthermore, the binding of sulpiride to hCA II revealed strong stacking interactions between the *N*-methyl-pyrrolidine ring and the Phe 131 residue of the carbonic anhydrase II, a moiety known to be critical for the binding of inhibitors possessing cyclic side chains (Figure 1)[39].

In addition, the role of fluorine in medicinal chemistry is well recognized[40,41], and the evaluation of its impact on modification of nitrogen-containing proximal functionalities behavior is considered as a common strategy in drug design studies[42–45]. Furthermore, recent studies revealed a strong effect of fluorine atom(s) on nitrogen containing heterocycles preferred conformations, with possible effect on bioactivities[46–48]. Thus, we report here the synthesis of new fluorinated *N*-pyrrolidino and piperidino tertiary benzenesulfonamides, and their screening for the inhibition of cytosolic CA isoforms hCA I and II and transmembrane isoforms IX and XII. For the first time, hCA II selective inhibitors with no measurable affinity for hCA I, IX and XII have been identified.

Materials and methods

Chemistry

The authors draw the reader's attention to the dangerous features of superacidic chemistry. Handling of hydrogen fluoride and antimony pentafluoride must be done by experienced chemists with all the necessary safety arrangements in place. Reactions performed in superacid were carried out in a sealed Teflon® flask with a magnetic stirrer. No further precautions have to be taken to prevent mixture from moisture (test reaction worked out in anhydrous conditions leads to the same results as expected). Yields refer to isolated pure products. ^{1}H, ^{13}C and ^{19}F NMR were recorded on a 400-MHz Bruker Advance DPX spectrometer using $CDCl_3$ as solvent and C_6F_6 as external reference. COSY ^{1}H–^{1}H and ^{1}H–^{13}C experiments were used to confirm the NMR

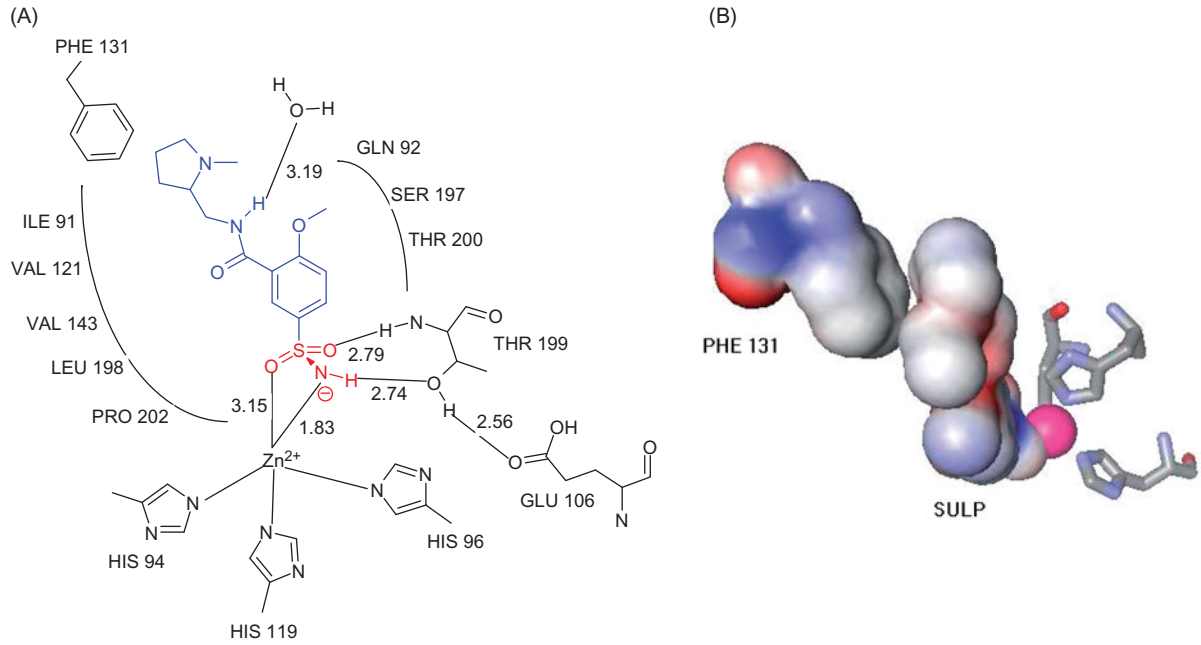


Figure 1. (A) Binding of sulpiride to hCA II active site. Figures represent distances in Å. (B) Representation of the π-stacking between the phenyl ring of Phe131 and the N-methylpyrrolidone fragment of the drug.

peaks assignments. Melting points were determined in a capillary tube with a device Büchi melting point B-545 and were uncorrected. All separations were done under flash-chromatography conditions on silica gel (15–40 µm). High-resolution mass spectrometry (HRMS) spectra were performed at the Institut Lavoisier de Versailles (ILV) of the University of Versailles St Quentin (LTQ-Orbitrap Velos Pro, THERMOFISHER SCIENTIFIC), at Centre Régional de Mesures Physiques de l'Ouest (CRMPO) of the University of Rennes (Q-TOF 2, Waters), at the Institut de Chimie Organique et Analytique (ICOA) of the University of Orléans (Q-Tof MaXis, Bruker), at the Institut de Chimie des Milieux et des Matériaux de Poitiers (IC2MP) of the University of Poitiers (LC-QTOF MaXis Impact, Bruker). Optical activities (α^D) were analyzed on a Schmidt+ Haensch polatronic HH8 polarimeter in MeOH at 20 °C.

Methyl-(2S)-1-(4-nosyl)pyrrolidine-2-carboxylate **(2)**

Into a round-bottom flask, methyl ester L-proline (165 mg, 1 mmol) was solubilized in 10 mL of dichloromethane then pyridine (1 mL, 12 eq.) was added followed by 4-nosyl chloride (665 mg, 3 eq.). The mixture was magnetically stirred at room temperature overnight, then water was added and the aqueous layer was extracted with dichloromethane (×3). The combined organic phases were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The reaction crude was purified over silica gel with the eluent ethyl acetate/petroleum ether: 30/70, thereby obtaining compound **2** (290 mg, 91%). Aspect: white solid. Mp: 111.5 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 8.35 (dm, 2H, $J = 9.0$ Hz), 8.06 (dm, 2H, $J = 9.0$ Hz), 4.44 (m, 1H), 3.69 (s, 3H), 3.44 (m, 2H), 2.15 (m, 1H), 2.08–1.84 (m, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 172.2, 150.2, 144.7, 128.7, 124.3, 60.7, 52.6, 48.4, 31.0, 24.8. $\alpha^D = -107.9$ °.

(2S)-1-(4-nosyl)pyrrolidine-2-carbaldehyde **(3)**

In an argon-flushed round-bottom flask, compound **2** (5.66 g, 18 mmol) was solubilized in 45 mL of dichloromethane, cooled to −78 °C then DIBALH (1 M in CH_2Cl_2, 25.2 mL, 1.4 eq.) was slowly added and magnetically stirred for 1 h 40 min at the same temperature. MeOH was then added and stirred overnight at room temperature after which the mixture was concentrated *in vacuo*. At the crude residue, an aqueous solution of HCl (1 M) and ice were added, the mixture was stirred 15 min then ethyl acetate was added, followed by Na_2CO_3 up to pH 9–10 and extracted with ethyl acetate (×3). The combined organic phases were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography over silica gel with the eluent ethyl acetate/petroleum ether: 30/70 thereby obtaining compound **3** (4.67 g, 91%). Aspect: pale yellow solid. Mp: degradation. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 9.66 (d, 1H, $J = 2.0$ Hz), 8.41 (d, 2H, $J = 9.0$ Hz), 8.05 (d, 2H, $J = 9.0$ Hz), 4.01 (ddd, $J = 8.1$ Hz, $J = 4.9$ Hz, $J = 2.0$ Hz, 1H), 3.60 (m, 1H), 3.28 (m, 1H), 2.12 (m, 1H), 2.00–1.85 (m, 2H), 1.78 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 198.8, 150.6, 143.1, 129.0, 124.7, 66.9, 49.3, 27.7, 24.8.

(2S)-1-(4-nosyl)-2-vinylpyrrolidine **(4)**

In an argon-flushed double-necked round-bottom flask, methyltriphenylphosphine bromide (11.29 g, 31.6 mmol) was dissolved in THF (100 mL), cooled to −78 °C, then n-BuLi (1.6 M in hexane, 20 mL, 32.0 mmol) was slowly added. The reaction mixture was stirred 1 h at the same temperature, then a solution of compound **3** (4.49 g, 15.8 mmol) in THF (100 mL) was slowly added via cannula. The mixture was allowed to warm to room temperature and stirred for 1 h, after which pentane (110 mL) was added, followed by MeOH (220 mL). Solvents were removed *in vacuo*, then water was added and the aqueous layer was extracted with ethyl acetate (×3). The combined organic phases were washed with brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography over silica gel with the eluent ethyl acetate/petroleum ether 20/80 thereby obtaining compound **4** (2.43 g, 54%). Aspect: pale yellow solid. Mp: 82 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 8.35 (d, 2H, $J = 9.0$ Hz), 8.01 (d, 2H, $J = 9.0$ Hz), 5.71 (ddd, 1H, $J = 16.8$ Hz, $J = 10.2$ Hz, $J = 6.4$ Hz), 5.25 (dt, 1H, $J = 16.9$ Hz, $J = 1.2$ Hz), 5.13 (dt, 1H, $J = 10.2$ Hz, $J = 1.2$ Hz), 4.24 (m, 1H), 3.47 (m, 1H), 3.33 (m, 1H), 1.81 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.1, 144.7, 137.8,

128.7, 124.4 116.4, 62.5, 48.9, 32.6, 24.0. $\alpha^D = -75.8°$. HRMS (Q-TOF 2, ES$^+$, MeOH): *m/z* calc. for [M+H]$^+$: $C_{12}H_{15}N_2O_4S$ 283.0747 found 283.0745.

Procedure A: general procedure for selective reduction of aromatic nitro compound to aromatic amine

Into a round-bottom flask, the aromatic nitro containing compound was solubilized in absolute ethanol. $SnCl_2 \cdot 2H_2O$ (5 eq.) was added. The mixture was magnetically stirred at 70 °C for 30 min, then poured into ice. A solution of Na_2CO_3 (5% aq.) was slowly added up to pH 7 then extracted with ethyl acetate (×3). The combined organic phases were treated with activated charcoal, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The reaction crude was purified over silica gel.

(2S)-1-(4-aminobenzenesulfonyl)-2-vinylpyrrolidine **(5)**

This compound was obtained from compound **4** (34 mg, 0.12 mmol) following the general procedure A. The reaction crude was purified over silica gel with the eluent ethyl acetate/petroleum ether 40/60 thereby obtaining compound **5** (28 mg, 93%). Aspect: pale yellow solid. MP: 109.5 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 7.56 (d, 2H, $J = 8.7$ Hz), 6.66 (d, 2H, $J = 8.7$ Hz), 5.80 (ddd, 1H, $J = 16.9$ Hz, $J = 10.2$ Hz, $J = 5.9$ Hz), 5.24 (dt, 1H, $J = 17.0$ Hz, $J = 1.4$ Hz), 5.08 (dt, 1H, $J = 10.2$ Hz, $J = 1.3$ Hz), 4.21 (s_{broad}, 2H), 4.05 (m, 1H), 3.39 (ddd, 1H, $J = 10.0$ Hz, $J = 7.1$ Hz, $J = 4.4$ Hz), 3.20 (dt, 1H, $J = 10.0$ Hz, $J = 7.4$ Hz), 1.81–1.54 (m, 4H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.8, 139.1, 129.6, 125.8, 115.1, 114.1, 61.9, 48.9, 32.3, 23.8. $\alpha^D = -128.4°$. HRMS (Q-TOF 2, ES$^+$, MeOH): *m/z* calc. for [M+H]$^+$: $C_{12}H_{17}N_2O_2S$ 253.1005 found 253.1005.

(2S)-1-(4-aminobenzenesulfonyl)-2-ethylpyrrolidine **(6)**

In a round-bottom flask, compound **4** (100 mg, 0.35 mmol) was solubilized in dichloromethane (10 mL), the mixture was placed under vacuum before being placed under inert atmosphere, then palladium on carbon 5% (catalytic amount) was added and stirred under H_2 atmosphere (1 atm.) for 5 h. The reaction mixture was filtered on celite powder and concentrated *in vacuo*, thereby obtaining compound **6** without further purification (87 mg, 96%). Aspect: brown solid. Mp: 145.3 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 7.54 (d, $J = 8.6$ Hz, 2H), 6.65 (d, $J = 8.6$ Hz, 2H), 4.27 (s_{broad}, 2H), 3.46 (m, 1H), 3.30 (ddd, 1H, $J = 10.5$ Hz, $J = 7.0$ Hz, $J = 5.4$ Hz), 3.13 (dt, 1H, $J = 10.4$ Hz, $J = 7.3$ Hz), 1.80–1.69 (m, 2H), 1.57–1.35 (m, 4H), 0.86 (t, 3H, $J = 7.5$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.7, 129.5, 126.1, 114.1, 61.8, 49.1, 30.2, 29.3, 24.2, 10.5. $\alpha^D = -91.5°$.

(2S)-1-(4-nosyl)-2-((1S)-fluoroethyl)pyrrolidine/(2R)-1-(4-nosyl)-2-((1R)-fluoroethyl)pyrrolidine **(7)**

To a HF/SbF_5 mixture (8 mL, 3.8 mol% SbF_5) cooled at −20 °C, the substrate **4** (100 mg, 0.35 mmol) was slowly added. The mixture was magnetically stirred at the same temperature for 30 min, after which the reaction mixture was neutralized with water/ice/Na_2CO_3 up to pH 9–10 and extracted with ethyl acetate (×3). The combined organic phases were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The crude residue was purified by column chromatography over silica gel with the eluent diethyl ether/petroleum ether/triethylamine: 29/70/1 thereby obtaining compound **7** as a racemic mixture of enantiomers mixed with compound **8** 1/1, also obtained as a racemic mixture of enantiomers (473 mg, 78%). Aspect: white solid. Mp: 95.7 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 8.38 (m, 2H), 8.03 (m, 2H), 5.00 (dqd, 1H, $J = 49.1$ Hz, $J = 6.5$ Hz, $J = 2.6$ Hz), 3.73 (dm, 1H, $J = 26.1$ Hz), 3.41 (m, 1H), 3.31 (m, 1H), 2.05–1.85 (m, 2H), 1.72–1.55 (m, 2H), 1.34 (dd, 3H, $J = 23.6$ Hz, $J = 6.5$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.3, 144.4, 128.6, 124.6, 91.5 (d, $J = 174.1$ Hz), 64.0 (d, $J = 21.2$ Hz), 49.4, 25.4 (d, $J = 4.3$ Hz), 25.0 (d, $J = 1.4$ Hz), 18.07 (d, $J = 22.0$ Hz). ^{19}F {1H} NMR ($CDCl_3$, 376 MHz, ppm) δ: −190.7. HRMS (Q-TOF 2, ES$^+$, MeOH): *m/z* calc for [M+Na]$^+$: $C_{12}H_{15}FN_2NaO_4S$ 325.0629 found 325.0631.

(2R)-1-(4-nosyl)-2-((1S)-fluoroethyl)pyrrolidine/(2S)-1-(4-nosyl)-2-((1R)-fluoroethyl)pyrrolidine **(8)**

Aspect: white solid. Mp: 87.7 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 8.38 (m, 2H), 8.04 (m, 2H), 4.82 (dqd, 1H, $J = 46.5$ Hz, $J = 6.3$ Hz, $J = 4.9$ Hz), 3.92 (m, 1H), 3.39 (m, 2H), 1.87 (m, 2H), 1.65 (m, 2H), 1.38 (dd, 3H, $J = 24.4$ Hz, $J = 6.4$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.3, 143.9, 128.9, 124.5, 91.6 (d, $J = 171.1$ Hz), 62.5 (d, $J = 25.0$ Hz), 49.7, 27.0 (d, $J = 2.3$ Hz), 24.8, 16.5 (d, $J = 22.3$ Hz). ^{19}F {1H} NMR ($CDCl_3$, 376 MHz, ppm) δ: −180.0. HRMS (Q-TOF 2, ES$^+$, MeOH): *m/z* calc. for [M+Na]$^+$: $C_{12}H_{15}FN_2NaO_4S$ 325.0629 found 325.0631.

(2S)-1-(4-aminobenzenesulfonyl)-2-(1S-fluoroethyl)pyrrolidine and (2R)-1-(4-aminobenzenesulfonyl)-2-(1R-fluoroethyl) pyrrolidine **(9)**

This compound was obtained from compound **7** (13 mg, 43 µmol) following the general procedure A. The reaction crude was purified over silica gel with the eluent ethyl acetate/petroleum ether: 30/70 thereby obtaining compound **9** (7 mg, 60%). Aspect: orange oil. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 7.61 (d, 2H, $J = 6.1$ Hz), 6.71 (d, 2H, $J = 6.1$ Hz), 4.94 (dqd, 1H, $J = 49.0$ Hz, $J = 6.5$ Hz, $J = 3.2$ Hz), 3.60 (dm, 1H, $J = 24.7$ Hz), 3.34 (ddd, 1H, $J = 10.5$ Hz, $J = 7.3$ Hz, $J = 5.2$ Hz), 3.20 (dt, 1H, $J = 10.4$ Hz, $J = 7.1$ Hz), 1.93 (m, 1H), 1.83 (m, 1H), 1.53 (m, 2H), 1.34 (dd, 3H, $J = 23.8$ Hz, $J = 6.5$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.5, 129.7, 126.4, 114.4, 92.1 (d, $J = 172.9$ Hz), 63.6 (d, $J = 21.9$ Hz), 49.3, 25.4 (d, $J = 4.3$ Hz), 24.8 (d, $J = 1.2$ Hz), 18.3 (d, $J = 22.1$ Hz). ^{19}F {1H} NMR ($CDCl_3$, 376 MHz, ppm) δ: −189.7. HRMS (Q-TOF 2, ES$^+$, MeOH): *m/z* calc. for [M+H]$^+$: $C_{12}H_{18}FN_2O_2S$ 273.1068 found 273.1076.

(2R)-1-(4-aminobenzenesulfonyl)-2-(1S-fluoroethyl)pyrrolidine and (2S)-1-(4-aminobenzenesulfonyl)-2-(1R-fluoroethyl) pyrrolidine **(10)**

This compound was obtained from compound **8** (21 mg, 69 µmol) following the general procedure A. The reaction crude was purified over silica gel with the eluent ethyl acetate/petroleum ether: 30/70 thereby obtaining compound **10** (17 mg, 90%). Aspect: orange solid. Mp: 116.2 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 7.61 (m, 2H), 6.70 (m, 2H), 4.93 (dqd, 1H, $J = 46.3$ Hz, $J = 6.4$ Hz, $J = 4.2$ Hz), 3.80 (ddt, 1H, $J = 12.7$ Hz, $J = 7.8$ Hz, $J = 3.7$ Hz), 3.35 (m, 1H), 3.22 (m, 1H), 1.86 (m, 1H), 1.76 (m, 1H), 1.60 (m, 1H), 1.49 (m, 1H), 1.37 (dd, 3H, $J = 24.5$ Hz, $J = 6.4$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.7, 129.8, 125.6, 114.4, 91.9 (d, $J = 169.0$ Hz), 61.5 (d, $J = 26.6$ Hz), 49.7, 26.5 (d, $J = 1.2$ Hz), 24.7, 15.9 (d, $J = 22.1$ Hz). ^{19}F {1H} NMR ($CDCl_3$, 376 MHz, ppm) δ: −181.1. HRMS (Q-TOF 2, ES$^+$, MeOH): *m/z* calc. for [M+H]$^+$: $C_{12}H_{18}FN_2O_2S$ 273.1068 found 273.1076.

(2S)-1-(4-nosyl)-2-(2-chloro-(1R)-fluoroethyl)pyrrolidine and (2R)-1-(4-nosyl)-2-(2-chloro-(1S)-fluoroethyl)pyrrolidine **(11)**

To a HF/SbF_5 mixture (1.3 mL, 21.6 mol% SbF_5) was added NCS (140 mg, 1.05 mmol). After 1 min of stirring at −10 °C, the substrate **4** (100 mg, 0.35 mmol) was slowly added. The mixture

was magnetically stirred at the same temperature for 1 h, then neutralized with water/ice/Na_2CO_3 up to pH 10 and extracted with ethyl acetate (×3). The combined organic phases were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The reaction crude was purified over silica gel with the eluent diethyl ether/pentane/diethylamine: 20/79/1, thereby obtaining compounds **11** and **12** (3/2) as a racemic mixture of their enantiomers (84 mg, 71%). Aspect: brown oil. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 8.39 (d, 2H, $J = 8.8$ Hz), 8.04 (d, 2H, $J = 8.8$ Hz), 4.67 (ddt, 1H, $J = 46.4$ Hz, $J = 7.6$ Hz, $J = 3.7$ Hz), 4.10 (ddt, 1H, $J = 21.7$ Hz, $J = 9.0$ Hz, $J = 3.1$ Hz), 3.89 (ddd, 1H, $J = 26.7$ Hz, $J = 12.3$ Hz, $J = 3.9$ Hz), 3.76 (ddd, 1H, $J = 14.9$ Hz, $J = 12.3$ Hz, $J = 7.7$ Hz), 3.41 (m, 2H), 1.93 (m, 1H), 1.93 (m, 1H), 1.70 (m, 1H), 1.56 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.3, 143.4, 128.8, 124.5, 94.6 (d, $J = 182.7$ Hz), 60.3 (d, $J = 21.2$ Hz), 49.8, 43.1 (d, $J = 25.2$ Hz), 28.2, 24.5. ^{19}F {1H} NMR ($CDCl_3$, 376 MHz, ppm) δ: −192.0. HRMS (Q-TOF 2, ES$^+$, MeOH): *m/z* calc. for $[M+Na]^+$: $C_{12}H_{14}ClFN_2NaO_4S$ 359.0239 found: 359.0239.

(2S)-1-(4-nosyl)-2-(2-chloro-(1S)-fluoroethyl)pyrrolidine and (2R)-1-(4-nosyl)-2-(2-chloro-(1R)-fluoroethyl)pyrrolidine **(12)**

Aspect: brown oil. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 8.41 (d, 2H, $J = 9.0$ Hz), 8.04 (d, 2H, $J = 9.0$ Hz), 4.92 (dm, 1H, $J = 47.8$ Hz), 3.95 (dm, 1H, $J = 20.6$ Hz), 3.77 (ddd, 1H, $J =$ 16.3 Hz, $J = 12.1$ Hz, $J = 6.3$ Hz), 3.71 (ddd, 1H, $J = 16.1$ Hz, $J = 12.1$ Hz, $J = 5.5$ Hz), 3.48 (ddd, 1H, $J = 10.3$ Hz, $J = 7.2$ Hz, $J = 4.5$ Hz), 3.26 (dt, 1H, $J = 10.2$ Hz, $J = 7.2$ Hz), 2.02 (m, 1H), 1.93 (m, 1H), 1.62 (m, 1H), 1.59 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.3, 143.1, 128.8, 124.5, 92.7 (d, $J = 182.8$ Hz), 60.4 (d, $J = 22.9$ Hz), 49.4, 42.8 (d, $J = 25.6$ Hz), 26.0 (d, $J = 4.7$ Hz), 24.5. ^{19}F {1H} NMR ($CDCl_3$, 376 MHz, ppm) δ: −193.8.

(2S)-1-(4-nosyl)-2-((1R)-chloro-2-fluoroethyl)pyrrolidine and (2R)-1-(4-nosyl)-2-((1S)-chloro-2-fluoroethyl)pyrrolidine **(13)**

To a HF/SbF_5 mixture (2 mL, 12.2 mol% SbF_5) was added NCS (208 mg, 1.56 mmol). After 1 min of stirring at −20 °C, the substrate **4** (122 mg, 0.52 mmol) was slowly added. The mixture was magnetically stirred at the same temperature for 10 min, then neutralized with water/ice/Na_2CO_3 up to pH 10 and extracted with ethyl acetate (×3). The combined organic phases were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The reaction crude was purified over silica gel with the eluent diethyl ether/pentane/diethylamine: 20/79/1, thereby obtaining compounds **13** and **13′** (7/3) as a racemic mixture of their enantiomers (154 mg, 88%), **13′** could not be isolated. Aspect: yellow solid. Mp: 125.4 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 8.40 (d, 2H, $J = 9.0$ Hz), 8.04 (d, 2H, $J = 9.0$ Hz), 4.64 (ddd, 1H, $J = 47.5$ Hz, $J = 5.2$ Hz, $J = 8.9$ Hz), 4.64 (m, 1H), 4.51 (ddd, 1H, $J = 47.5$ Hz, $J = 9.6$ Hz, $J = 6.6$ Hz), 4.06 (ddd, 1H, $J = 4.0$ Hz, $J = 5.0$ Hz, $J = 8.5$ Hz), 3.42 (dt, 1H, $J = 10.6$ Hz, $J = 6.9$ Hz), 3.36 (ddd, 1H, $J = 10.6$ Hz, $J = 7.3$ Hz, $J = 5.7$ Hz), 2.08 (m, 1H), 1.96 (m, 1H), 1.76 (m, 1H), 1.52 (dtt, 1H, $J = 12.5$ Hz, $J = 7.3$ Hz, $J = 7.2$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.3, 143.3, 128.7, 124.5, 83.1 (d, $J = 176.6$ Hz), 60.9 (d, $J = 19.8$ Hz), 60.8 (d, $J = 3.1$ Hz), 49.6, 26.7, 24.7. ^{19}F {1H} NMR ($CDCl_3$, 376 MHz, ppm) δ: −219.2. HRMS (Q-TOF 2, ES$^+$, MeOH): *m/z* calc. for $[M+Na]^+$: $C_{12}H_{14}ClFN_2NaO_4S$ 359.0239 found: 359.0239.

1-(4-nosyl)piperidine-4-ol **(15)**

In a round-bottom flask, 4-nosyl chloride (512 mg, 2.3 mmol) was solubilized in dichloromethane (8 mL), 4-hydroxypiperidine (404 mg, 4.0 mmol) and triethylamine (0.4 mL, 3.0 mmol) were added. The mixture was magnetically stirred at room temperature overnight, then water was added and extracted with ethyl acetate (×3). The combined organic phases were dried over magnesium sulfate, filtered and concentrated *in vacuo*, thereby obtaining compound **15** without further purification (575 mg, 87%). Aspect: yellow solid. Mp: 177.2 °C. ^{1}H NMR (CD_3OD, 400 MHz, ppm) δ: 8.44 (d, 2H, $J = 9.0$ Hz), 8.03 (d, 2H, $J = 9.0$ Hz), 3.66 (m, 1H), 3.40 (m, 2H), 2.88 (ddd, 2H, $J = 12.0$ Hz, $J = 8.8$ Hz, $J = 3.4$ Hz), 1.88 (m, 2H), 1.57 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.3, 142.7, 128.9, 124.5, 65.3, 43.0, 33.2.

1-(4-nosyl)-4-methanesulfonatepiperidine **(16)**

Into a round-bottom flask, compound **15** (400 mg, 1.4 mmol) was solubilized in acetonitrile (5 mL), triethylamine (0.38 mL, 2.8 mmol) and mesyl chloride (0.16 mL, 2.1 mmol) were added. The mixture was magnetically stirred at 80 °C overnight, then water and an aqueous solution of NaOH 2M were added. The mixture is stirred 5 min and extracted with ethyl acetate (×3). The combined organic phases were dried over magnesium sulfate, filtered and concentrated *in vacuo*, thereby obtaining compound **15** without further purification (504 mg, 99%). Aspect: yellow solid. ^{1}H NMR (CD_3OD, 400 MHz, ppm) δ: 8.38 (d, 2H, $J = 9.0$ Hz), 7.98 (d, 2H, $J = 9.0$ Hz), 4.70 (m, 1H), 3.24–3.05 (m, 4H), 2.97 (s, 3H), 2.03 (m, 2H), 1.88 (m, 2H). ^{13}C NMR (CD_3OD, 100 MHz, ppm) δ: 151.5, 143.1, 129.9, 125.5, 77.0, 43.6, 38.7, 31.7. HRMS (Q-TOF 2, ES$^+$, MeOH): *m/z* calc. for $[M+H]^+$: $C_{12}H_{17}N_2O_7S_2$ 365.0471 found 365.0473.

1-(4-nosyl)-1,2,5,6-tetrahydropyridine **(17)**

Into a round-bottom flask, compound **16** (300 mg, 0.82 mmol) was solubilized in DBU (5 mL). The mixture was magnetically stirred at 80 °C for 2 h, then water was added and extracted with ethyl acetate (×3). The combined organic phases were washed with an aqueous solution of HCl 0.5 M and brine, dried over magnesium sulfate, filtered and concentrated *in vacuo*. The reaction crude was purified over silica gel with the eluent ethyl acetate/petroleum ether: 30/70 thereby obtaining compound **17** (86 mg, 39%). Aspect: yellow solid. Mp: 134.3 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 8.37 (d, 2H, $J = 9.0$ Hz), 7.98 (d, 2H, $J = 9.0$ Hz), 5.78 (dm, 1H, $J = 10.2$ Hz), 5.63 (dm, 1H, $J = 10.2$ Hz), 3.67 (m, 2H), 3.28 (t, 2H, $J = 5.7$ Hz), 2.21 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.3, 143.2, 128.8, 125.4, 124.4, 122.5, 44.8, 42.7, 25.2. HRMS (Q-TOF 2, ES$^+$, MeOH): *m/z* calc. for $[M+H]^+$: $C_{11}H_{13}N_2O_4S$ 269.0590 found 269.0593.

(3S,4S)-1-(4-nosyl)-4-chloro-3-fluoropiperidine and (3R,4R)-1-(4-nosyl)-4-chloro-3-fluoropiperidine **(18)**

To a HF/SbF_5 mixture (1 mL, 12.2 mol% SbF_5) was added NCS (72 mg, 0.54 mmol). After 1 min of stirring at −20 °C, compound **17** (50 mg, 0.18 mmol) was slowly added. The mixture was magnetically stirred at the same temperature for 10 min, then neutralized with water/ice/Na_2CO_3 up to pH 10 and extracted with ethyl acetate (×3). The combined organic phases were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The reaction crude was purified over silica gel with the eluent petroleum ether/ethyl acetate: 90/10, thereby obtaining compound **18** (30 mg, 50%) and **19** (4 mg, 6%) as a racemic mixture of enantiomers. Aspect: white solid. Mp: 157.1 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 8.39 (d, 2H, $J = 8.9$ Hz), 7.98 (d, 2H, $J = 8.9$ Hz), 4.63 (dm, 1H, $J = 45.9$ Hz), 4.09 (m, 1H), 3.58 ($_{broad}$dd, 1H, $J = 13.7$Hz, $J = 4.7$ Hz), 3.50 (ddd, 1H, $J = 27.3$ Hz, $J = 13.5$ Hz, $J = 2.5$ Hz), 3.38 (m, 1H), 3.26 (m, 1H), 2.30 (m,

1H), 1.92 (dtd, 1H, $J = 14.2$ Hz, $J = 5.3$ Hz, $J = 3.3$ Hz). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.4, 143.4, 128.7, 124.6, 87.1 (d, $J = 184.4$ Hz), 53.7 (d, $J = 26.7$ Hz), 45.5 (d, $J = 23.6$ Hz), 41.3, 29.3. ^{19}F {1H} NMR ($CDCl_3$, 376 MHz, ppm) δ: −178.8. HRMS (Q-TOF 2, ES^+, CH_3CN): *m/z* calc. for $[M+H]^+$: $C_{11}H_{13}ClFN_2O_4S$ 323.0263 found: 323.0264.

(3S,4S)-N-(4-nosyl)-3-chloro-4-fluoropiperidine and (3R,4R)-N-(4-nosyl)-3-chloro-4-fluoropiperidine **(19)**

Aspect: white solid. Mp: 126.6 °C. ^{1}H NMR ($CDCl_3$, 400 MHz, ppm) δ: 8.40 (d, 2H, $J = 9.0$ Hz), 7.98 (d, 2H, $J = 9.0$ Hz), 4.58 (dtd, 1H, $J = 47.0$ Hz, $J = 6.4$ Hz, $J = 3.4$ Hz), 4.07 (m, 1H), 3.62 (dt, 1H, $J = 13.2$ Hz, $J = 3.1$ Hz), 3.35 (dd, 1H, $J = 13.1$ Hz, $J = 6.2$ Hz), 3.25 (m, 2H), 2.31 (m, 1H), 1.93 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz, ppm) δ: 150.5, 143.2, 128.8, 124.7, 89.2 (d, $J = 180.6$ Hz), 53.9 (d, $J = 24.5$ Hz), 48.7 (d, $J = 2.8$ Hz), 42.0 (d, $J = 6.5$ Hz), 27.76 (d, $J = 20.0$ Hz). ^{19}F {1H} NMR ($CDCl_3$, 376 MHz, ppm) δ: −179.0.

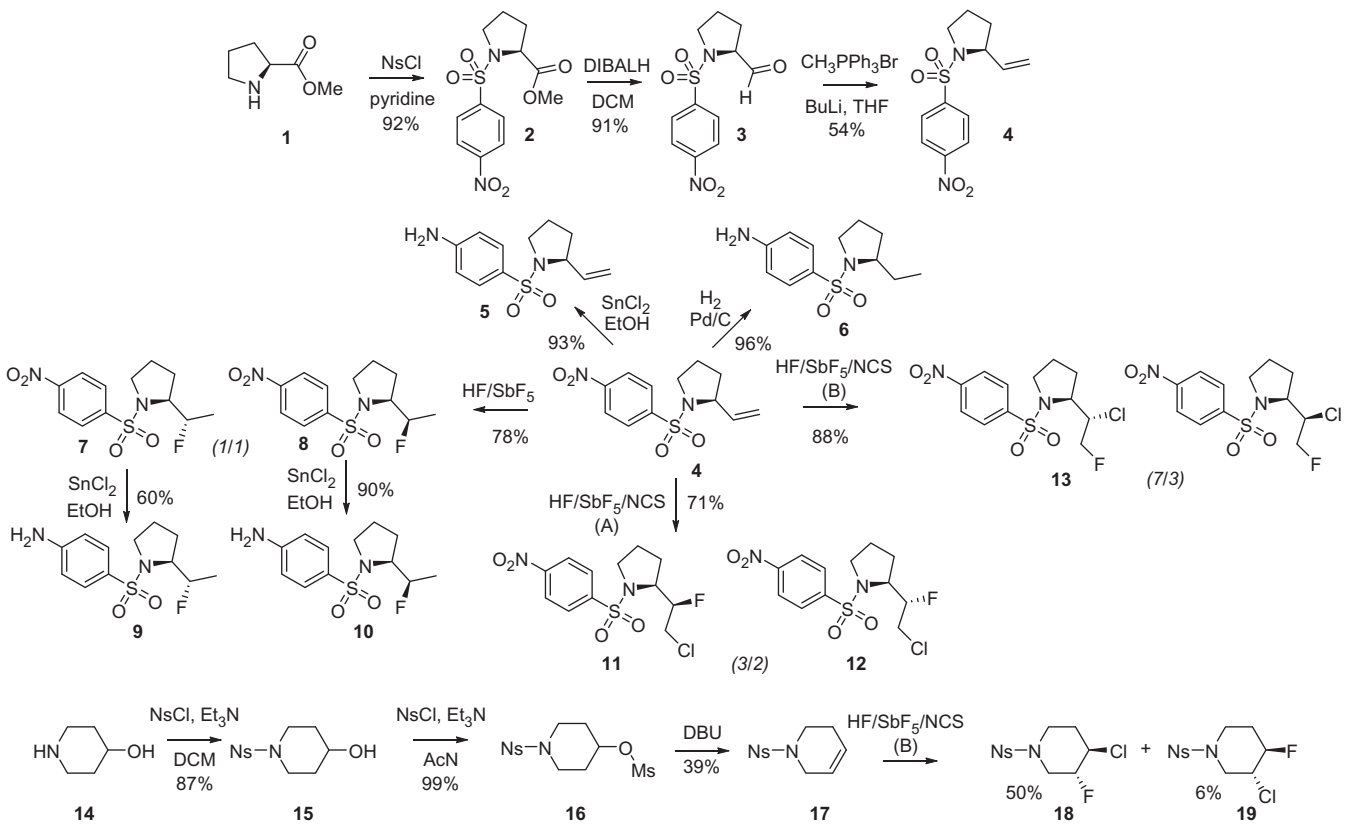
CA inhibition assay

An applied photophysics stopped-flow instrument has been used for assaying the CA catalysed CO_2 hydration activity[49]. Phenol red (at a concentration of 0.2 mM) has been used as indicator, working at the absorbance maximum of 557 nm, with 20 mM Hepes (pH 7.5) as buffer, and 20 mM Na_2SO_4 (for maintaining constant the ionic strength), following the initial rates of the CA-catalyzed CO_2 hydration reaction for a period of 10–100 s. The CO_2 concentrations ranged from 1.7 to 17 mM for the determination of the kinetic parameters and inhibition constants. For each inhibitor, at least six traces of the initial 5–10% of the reaction have been used for determining the initial velocity. The uncatalyzed rates were determined in the same manner and subtracted from the total observed rates. Stock solutions of inhibitor (0.1 mM) were prepared in distilled–deionized water and dilutions up to 0.01 nM were done thereafter with distilled–deionized water. Inhibitor and enzyme solutions were preincubated together for 15 min at room temperature prior to assay, in order to allow for the formation of the E-I complex. The inhibition constants were obtained by non-linear least-squares methods using the Cheng–Prusogg equation, as reported earlier, and represent the mean from at least three different determinations[50–55]. CA isoforms were recombinant ones obtained in house as reported earlier[50–55].

Results and discussion

Chemistry

The synthesis of the unsaturated product **4** was first achieved (Scheme 1). Starting from L-proline methyl ester derivative **1**, a three steps procedure, including a nosyl protection of the nitrogen atom, a selective reduction of the methyl ester to the corresponding aldehyde, and a Wittig reaction led to the synthesis of the tertiary benzenesulfonamide **4** in 46% overall yield. This procedure could be conducted on a gram scale. This product served as the key intermediate for the divergent synthesis of a wide range of (fluorinated) pyrrolidines containing tertiary benzenesulfonamides. The tin (II) chloride selective reduction of the nitro group of substrate **4** to the corresponding aminobenzenesulfonamide **5** was performed in 93% yield. A complete reduction of the same substrate was also achieved in 96% yield after hydrogenation over Pd/C catalyst (formation of product **6**). The hydrofluorination of substrate **4** in superacid HF/SbF_5 (% mol $SbF_5 = 3.8$, −20 °C, 10 min) led to the formation of a mixture of *like* and *unlike* diastereoisomers **7** and **8**. By adopting the same reduction procedure than the previously described, the corresponding 4-aminobenzenesulfonamides **9** and **10** have been synthesized in good yields. Regioselective superacid catalyzed



Scheme 1. Synthesis of carbonic anhydrase inhibitors.

Table 2. Inhibition data of tertiary (fluorinated) benzenesulfonamides.

		Ki (μM)*			
Entry	Compound	hCA I†	hCA II†	hCA IX‡	hCA XII‡
1	**2**	>100	>100	>100	>100
2	**4**	>100	0.5	>100	>100
3	**5**	>100	6.3	>100	>100
4	**6**	>100	7.3	>100	>100
5	**7**	>100	4.7	>100	>100
6	**8**	>100	3.9	>100	>100
7	**9**	>100	4.8	>100	>100
8	**10**	>100	3.4	>100	>100
9	**11**	>100	7.6	>100	>100
10	**12**	>100	6.3	>100	>100
11	**13**	>100	6.6	>100	>100
12	**18**	>100	11.0	>100	>100
13	**19**	>100	10.0	>100	>100

*Errors in the range of ±5% of the reported data from three different assays.
†Recombinant isoforms.
‡Catalytic domain.

chlorofluorination of substrate **4** in conditions A (% mol $SbF_5 = 21.7$, NCS (3eq.), −10 °C, 60 min) allowed the synthesis of the β-fluoro-γ-chlorinated diastereoisomers **11** and **12** in overall 71% yield. An adapted procedure (conditions B: % mol $SbF_5 = 12.1$, NCS (3eq.), −20 °C, 10 min) led to the synthesis of the corresponding regioisomers in 86% yield, the isomer **13** being cleanly separated from the mixture. It has to be noted that unfortunately these superacid catalyzed reactions suffer from epimerization of the pyrollidines chiral carbon center, thus delivering in all cases only racemic mixture of *like* and *unlike* isomers. The relative configuration of the products was determined by careful analysis of the ^1H and ^{19}F NMR spectrum. To evaluate the impact of the ring shape on carbonic anhydrases inhibition selectivity, analogous piperidines were synthesized. Starting from piperidin-4-ol **14**, after successive protection of the amino group and activation of the alcohol through mesylation, elimination with DBU led to the synthesis of the unsaturated substrate **17**. The chlorofluorination of this compound led to the formation of products **18** and **19** showing a relative anticonfiguration.

Carbonic anhydrase inhibition

All the tested tertiary benzenesulfonamides were found to be ineffective as hCA I, hCA IX and hCA XII inhibitors (Table 2). Exceptionally, whereas they do not inhibit these isoforms, all the tested (despite compound **2**) substrates inhibited the highly catalytically active hCA II at the micromolar level. When these tertiary benzenesulfonamides act as selective hCA II inhibitor, the compound **2** was found to be inactive toward all the tested isoforms, revealing a strong impact of the pyrrolidine substituents on the inhibition profile. The amino group located in the position 4 of the aromatic benzenesulfonamides inhibitors was previously shown to be essential for the inhibition selectivity of primary benzenesulfonamides[55]. In the case of these tertiary benzenesulfonamides, the impact of the presence of the amino group on the inhibition potential was difficult to predict. Indeed when compound **4** was around 10 times more efficient than its amino analogue **5**, nitroderivative **7** (or **8**) and its amino analogue **9** (or **10**) showed similar activities. In addition, the unsaturation was found not to be primordial for the selectivity of the inhibition, as compounds **5** and **6** showed a similar inhibition profile. The introduction of the fluorine atom in the β position of the nitrogen atom was found to increase the inhibition potency (Table 2, entries 4, 7 and 8), but this effect was very small, or absent for the chlorofluorinated analogues. The disparity of this effect confirms the impact of the geometrical shape of the molecule on the hCA II selective inhibition and reinforced the hypothesis of a non-zinc binding mode of action of these inhibitors and a strong influence of the structural shape on the inhibition efficiency. In addition, no significant effect of the inversion of the relative configuration of the two stereogenic centers on the inhibition potency appeared. Interestingly, the piperidine analogues **18** and **19** also act as hCA II selective inhibitors, confirming the importance of a cyclic core for the inhibition selectivity.

Conclusions

In conclusion, through the use of superacid chemistry, a set of variously substituted pyrrolidine and piperidine were synthesized and evaluated as specific carbonic anhydrase inhibitors. This work allowed to identify a new family of hCA II inhibitors in the tertiary benzenesulfonamides family, a class of inhibitors which is expanding rapidly. This class of CAIs points out a new mechanism of action for inhibition of hCA II, the physiologically most dominant isoform, being present in virtually all human cells. Indeed, CA II is a cytoplasmic enzyme, which is the most widespread of all CA isozymes and the catalytically fastest member of the α-class family (together with CA IX) and thus implicated in numerous physiological functions. To get further insights into various pathologies implicating CA II in autoimmune reactions[56] or deficiency[57], it makes no doubt that specific hCA II inhibitors are needed and should be welcome for further advances in these fields.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article. We are indebted to the Université de Poitiers, the CNRS, @rtMolecule and ANRT (CIFRE scholarship for A.LD) and an EU FP7 grant (Dynano to CTS) for financial support.

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