European Journal of Medicinal Chemistry 45 (2010) 1225–1229

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis and investigation of inhibition effect of fluorinated sulfonamide derivatives on carbonic anhydrase

Zohra Benfodda [a], Franck Guillen [b], Bernard Romestand [c], Abdelkader Dahmani [a], Hubert Blancou [a,*]

[a] Institut des Biomolécules Max Mousseron (IBMM) UMR CNRS 5247 Université de Montpellier I et de Montpellier II, CC1706, Place Eugène Bataillon 34095 Montpellier cedex 05, France
[b] S3F Chimie Université de Montpellier II, CC1706, Place Eugène Bataillon 34095 Montpellier cedex 05, France
[c] Laboratoire Ecosystèmes lagunaires, UMR 5554 Université de Montpellier II, CC, Place Eugène Bataillon 34095 Montpellier cedex 05, France

ARTICLE INFO

Article history:
Received 27 August 2009
Received in revised form
20 October 2009
Accepted 27 November 2009
Available online 5 December 2009

Keywords:
Perfluoroalkanesulfonamides
Sodium salt of perfluoroalkanesulfonamides
Carbonic anhydrase
Cellular toxicity

ABSTRACT

Series of perfluoroalkanesulfonamides **1**, sodium salt of perfluoroalkanesulfonamides **2** and polyfluoroalkanesulfonamides **3** derivatives were synthesized and characterized by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, IR and HRMS. Inhibition effects of these compounds on bovine carbonic anhydrase (bCA) and human carbonic anhydrase isoenzyme II (hCA) have been investigated. Comparing IC_{50} values of the synthesized molecules **1**, **2** and **3**, it has been found that compound **2b** is a more potent inhibitor than acetazolamide on hCA. Moreover **2b** does not present cellular toxicity on sheep red globules.

© 2009 Elsevier Masson SAS. All rights reserved.

1. Introduction

Sulfonamides of the type RSO_2NH_2 occur in numerous biologically active compounds which include antimicrobial drugs, saluretics, insulin releasing sulfonamides, anthyroid agents, antitumour drugs, carbonic anhydrase inhibitors and number of other biological activities [1–9]. However investigations on polyfluoroalkanesulfonamides or perfluoroalkanesulfonamides with a long chain have not been carried out in the biological area.

In our laboratory we studied perfluoroalkanesulfonamides with a long chain and their derivatives in order to inhibit carbonic anhydrase. Indeed, our working idea is that on one hand the perfluoroalkyl chain is very hydrophobic and we thought that perfluoroalkanesulfonamides could push back the water which is formed at the time of the enzymatic catalyse. On the other hand we showed that perfluoroalkanesulfonamides could bind different metal ions in particular divalents cations and the hypothesis is that perfluoroalkanesulfonamides could complex the Zn metal of the metalloenzyme [10].

Moreover, It was shown previously that $CF_3SO_2NH_2$ could inhibit carbonic anhydrase, these findings comfort us to prepare and to evaluate the different perfluoroalkanesulfonamides on carbonic anhydrase [11,12].

We herein report the synthesis of different perfluoroalkanesulfonamides, sodium salts of perfluoroalkanesulfonamides and polyfluoroalkanesulfonamides in order to study their inhibition on bovine carbonic anhydrase (bCa) and human carbonic anhydrase isoenzyme II (hCa). Finally, the cellular toxicity of these compounds was evaluated on sheep red globules.

2. Results and discussion

2.1. Chemistry

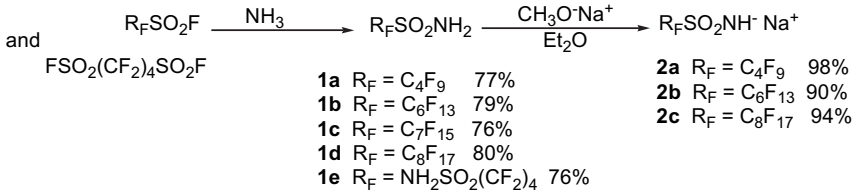
In this study, the perfluoroalkanesulfonamides **1** were prepared in good yields by reaction of perfluoroalkanesulfonyl fluorides with an excess of ammonia without any solvent at room temperature (Scheme 1).

The perfluoroalkanesulfonamides were easily purified by crystallisation in ethanol/water media. They were characterized by ^{1}H, ^{13}C, ^{19}F NMR spectroscopy, HRMS and IR.

Abbreviations: bCA, Bovine carbonic anhydrase; hCA, Human carbonic anhydrase isoenzyme II.

* Corresponding author. Institut des Biomolécules Max Mousseron (IBMM) UMR CNRS 5247 Université de Montpellier I et de Montpellier II, CC1706, Place Eugène Bataillon 34095 Montpellier cedex 05, France. Tel.: +33 4 67 14 39 19; fax: + 33 4 67 63 10 46.
E-mail address: hubert.blancou@univ-montp2.fr (H. Blancou).

0223-5234/$ – see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2009.11.052



Scheme 1. Synthesis of perfluoroalkanesulfonamides and sodium salts derivatives.

Then, the perfluoroalkanesulfonamides underwent a reaction with sodium methylate in a Et_2O/CH_3OH media to give in excellent yields with the corresponding sodium sulfonamides (**2** as described in Scheme 1). These compounds were characterized by ^{19}F NMR spectroscopy and HRMS.

The polyfluoroalkanesulfonamides **3** were prepared, in good yields by reaction of polyfluoroalkanesulfonyl chlorides with gaseous NH_3 in AcOEt at 0 °C (Scheme 2).

The polyfluoroalkanesulfonamides were easily purified by crystallisation in ethanol/water media or by sublimation. These compounds were characterized by ^1H, ^{13}C, ^{19}F NMR spectroscopy, HRMS and IR.

Indeed, we have synthesized two alkanesulfonamides **4** from corresponding alkanesulfonyl chorides using the same method (Scheme 3) in order to compare them with the perfluoroalkanesulfonamides conterparts in their biological activities.

2.2. Carbonic anhydrase inhibition

Among the zinc enzymes extensively studied recently, the carbonic anhydrases (CAs) have a special place by the reasons that these enzymes are ubiquitous in all kingdoms. They catalyse the reversible hydration of carbon dioxide ($H_2O + CO_2 = H^+ + HCO_3^-$) and as such are vital to many biological and physical functions. The inhibition of these enzymes may be clinically exploited in the treatment or prevention of a variety of disorders [13,14].

2.2.1. Bovine carbonic anhydrase (bCA) inhibition

As can be seen from data in Table 1, there is a very sharp difference in inhibition activity on bCA, between the perfluoroalkanesulfonamides **1a**–**e**, the sodium salts of perfluoroalkanesulfonamides **2a**–**c**, the polyfluoroalkanesulfonamides **3a**–**c** on the one hand and the carbohydrogenated compounds **4a**–**b** on the other hand. We can deduce that the substitution of hydrogen by fluorine increases the inhibitory activity of these compounds. Thus, perfluoroalkanesulfonamides $CF_3SO_2NH_2$, **1a**,**b**,**d** and the sodium salt of perfluorohexanesulfonamide **2b** showed that inhibition constants (IC_{50}) are comparable to that of acetazolamide (a clinically used sulfonamide) e.g in the range of 1.380–1.585 μM and 1.351 μM.

In our experiment, the sodium salt of perfluoroalkanesulfonamides (**2a**–**c**) were solubilized in water; they did not need the addition of any cosolvents (DMSO in particular) for the determination of the inhibition activity on bCA comparatively to the others synthesized compounds. The perfluoroalkanesulfonamides **1c**,**e** and the polyfluoroalkanesulfonamide **3a**,**b** have shown rather weak inhibitory properties against this enzyme with inhibition constants in the range of 1.901–2.089 μM which is less than that of acetazolamide (1.351 μM).

For the polyfluoroalkanesulfonamides (**3**) bCA inhibition varied with the perfluorinated alkyl chain length. The polyfluoroalkanesulfonamides (**3**) with the C_8F_{17} chain (**3c**) were more inhibitor than those with the C_6F_{13} chain (**3b**), which were also more inhibitor than those with C_4F_9 chain (**3a**).

2.2.2. Human carbonic anhydrase isoenzyme II (hCA)

hCA II is generally considered as the main therapeutic target of sulfonamides carbonic anhydrase inhibitors.

To confirm the study model realised on bCA, we have evaluated the inhibition of hCA II by the compound which present the best inhibition properties (IC_{50}) on bCA in particular the sodium salt of perfluorohexanesulfonamides (**2b**, IC_{50}:1.380 μM). We found that compound **2b** (IC_{50}: 0.122 μM) was a more potent inhibitor than acetazolamide (IC_{50}: 0.152 μM) on hCa. (Table 1).

It was known that F-alkylated chain has two main properties, they are very hydrophobic (from C_4F_9 chain) and they have an electron-attracting effect (–I) very important and relatively constant with the length of the F-alkylated chain studied. These properties can play two roles on one hand the electron-acceptor effect (–I) stabilize the sodium salt of perfluoroalkanesulfonamide (**2**) and this ion seems to be very good complexing metal ion by its delocalization of charge. On the other hand the hydrophobic F-alkylated chain could have a favorable positioning in the enzymatic site (interaction between the hydrophobic F-alkylated chain with the hydrophobic pocket of the enzyme) with the stabilization possibility by hydrogen bond.

2.2.3. Cellular toxicity on sheep red globules

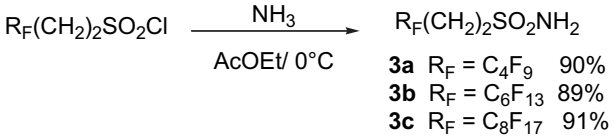
We knew that some perfluoroalkanesulfonamides in particular **2d** present toxicity [10,12]. We decided to evaluate the cellular toxicity on sheep red globules of some sulfonamides. The results were summarized in the Graph 1.

The sodium salt of perfluorohexanesulfonamide **2b** did not present a cellular cytotoxicity whatever the concentration studied.

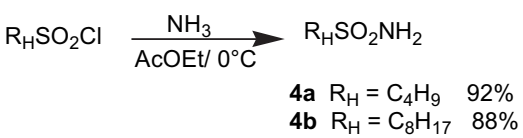
Trifluoromethanesulfonamide and perfluorohexanesulfonamide **1b** present a cellular cytotoxicity at concentration of 1000 μM. The sodium salt of perfluorobutanesulfonamide (2a) and the perfluorobutanesulfonamide (1b) (data not shown) did not present a cellular cytototoxicity whatever the concentration studied.

The sodium salt of perfluorooctanesulfonamide **2c** afford to the hemolysis at concentration of 31.25 μM. The perfluorooctanesulfonamide **1d** present a cellular cytotoxicity at concentration of 62.5 μM.

These results seems to be promising to continue to explore the sodium salt of perfluoroalkanesulfonamide and their derivatives in



Scheme 2. Synthesis of polyfluoroalkanesulfonamides.



Scheme 3. Synthesis of alkanesulfonamides.

Table 1

Inhibitor	IC_{50} (μM)[a]	
	bCA[b]	*hCA II*[c]
Acétazolamide	1.351[a]	0.152[a]
$CF_3SO_2NH_2$	1.495[a]	ND[e]
1a	1.486[a]	ND[e]
1b	1.473[a]	ND[e]
1c	1.722[a]	ND[e]
1d	1.585[a]	ND[e]
1e	1.735[a]	ND[e]
2a	1.670[a]	ND[e]
2b	1.380[a]	0.122[a]
2c	1.789[a]	ND[e]
3a	2.089[a]	ND[e]
3b	1.901[a]	ND[e]
3c	1.658[a]	ND[e]
4a	NI[d]	ND[e]
4b	NI[d]	ND[e]

[a] Mean ± Errors in the range 5–10% of the reported value (from 3 different assays).
[b] Bovine carbonic anhydrase.
[c] Human carbonic anhydrase isoenzyme II.
[d] NI: non inhibitor at these inhibition concentrations.
[e] ND: not determined.

particular **2b** because of their inhibition properties on carbonic anhydrase and the absence of cellular toxicity.

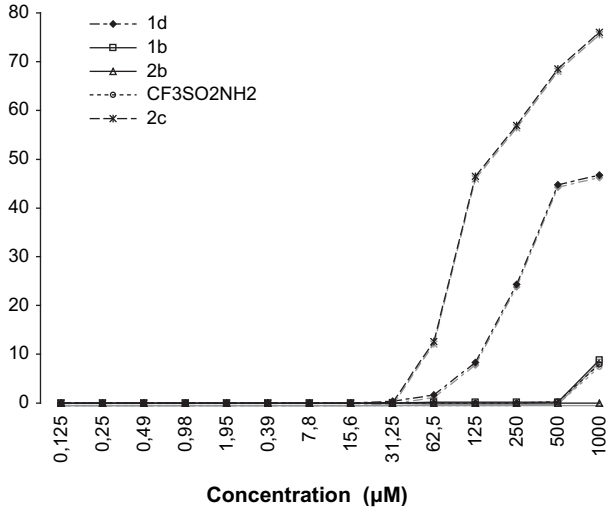
3. Conclusion

In summary, series of perfluoroalkanesulfonamides **1**, sodium salt of perfluoroalkanesulfonamide **2**, polyfluoroalkanesulfonamides **3** were synthesized and their inhibitory activities on carbonic anhydrase have been evaluated. All compounds demonstrated potent inhibition against bovine carbonic anhydrase. Moreover, compound **2b** was also evaluated on human carbonic anhydrase isoenzyme II and it presented better inhibitory properties than the commercial drug acetazolamide. Finally the cellular toxicity of these compounds was evaluated on sheep red globules. We found that **2b** did not present cellular cytotoxicity.

4. Experimental

4.1. Chemistry

Moisture sensitive reactions were carried out under dry nitrogen. Polyfluoroalkanesulfonyl chlorides and alkanesulfonyl chlorides were synthesized according to a new method developed in our laboratory (publication under review). Perfluoroalkanesulfonyl fluorides were a gift from Elf Atochem. Solvents were distilled from the appropriate drying agents immediately prior to use.



Graph 1. Determination of cellular toxicity of sulfonamides ($CF_3SO_2NH_2$, **1b**, **1d**, **2b**, **2c**) on sheep red globules.

^1H, ^{19}F, and ^{13}C NMR spectra were recorded at 300.13 MHz, 282.37 MHz and 75.46 MHz respectively with a Brucker Avance 300 spectrometer; the chemical shifts are given in ppm relative to Me_4Si for the ^1H and ^{13}C, and CCl_3F for ^{19}F, as internal standards. Coupling constants are given in Hz. Mass spectra and HRMS were recorded on a Jeol SX 102 spectrometer. IR Spectra were obtained using a Nicolet 205 FT-IR instrument (ν cm^{-1}). Compounds **4a** and **4b** were subjected to a quantitative elemental (C, H, N) analysis by a Flash EA 1112 (Thermo Finnigan) instrument. Melting points were recorded at atmospheric pressure unless otherwise stated on a Stuart scientific SMP3 apparatus and were uncorrected.

4.1.1. General procedure for the synthesis of perfluoroalkanesulfonamides ***1a–e***

A typical procedure for perfluoroalkanesulfonamides is described. In a 100 mL dried stainless steel bomb, perfluoroalkanesulfonyl fluoride (1 mol) was placed with magnetic stirrer, at 25 °C. Then ammonia (3 mol) was transferred into the bomb through the vacuum line within 1 h. The excess of ammonia was then removed with a flow of dry N_2. The solid product ($R_FSO_2NH^-NH_4^+$ and NH_4F) was acidified with an aqueous HCl followed by the addition of Et_2O. The organic layer was extracted and dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by recrystallization from EtOH/H_2O (90/10, V/V) to give white crystals of the corresponding sulfonamides.

The synthesis of perfluorobutanesulfonamide **1a** and perfluorooctanesulfonamide **1c** were described previously [15].

*4.1.1.1. Synthesis of 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluoro-n-hexane-1-sulfonamide **1b**.* Yield: 79%; IR (KBr): 3458 (ν_{NH2}), 1375 (ν_{SO2}), 1147 (ν_{CF}); Mp: 119–121 °C; ^1H NMR (300.13 MHz, d_6-acetone): δ 4.90 (m, 2H, $SO_2N\mathbf{H_2}$); ^{19}F NMR (282.37 MHz, d_6-acetone): δ −125.85 (m, 2F, ($CF_3C\mathbf{F_2}(CF_2)_4$)), −122.37 (m, 2F, ($CF_3CF_2C\mathbf{F_2}(CF_2)_3$)), −121.45 (m, 2F, ($CF_3(CF_2)_2C\mathbf{F_2}(CF_2)_2$)), −119.79 (m, 2F, ($CF_3(CF_2)_3C\mathbf{F_2}CF_2$)), −113.79 (m, 2F, ($CF_3(CF_2)_4C\mathbf{F_2}$)), −80.77 (m, 3F, ($C\mathbf{F_3}(CF_2)_5$)); MS (FAB$^-$, NBA): $[M-H^+]=398$; HRMS calcd for $C_6F_{13}HNO_2S$: 397.9520, found: 397.9521.

*4.1.1.2. Synthesis of 1,1,2,2,3,3,4,4,5,5,6,6,7,7,7-pentadecafluoro-n-heptane-1-sulfonamide **1c**.* Yield: 76%; IR (KBr): 3460 (ν_{NH2}), 1380 (ν_{SO2}), 1147 (ν_{CF}); Mp: 128–130 °C; ^1H NMR (300.13 MHz, d_6-acetone): δ 8.2 (m, 2H, $SO_2N\mathbf{H_2}$); ^{19}F NMR (282.37 MHz, d_6-acetone): δ −126.73 (m, 2F, $CF_3C\mathbf{F_2}(CF_2)_5$), −123.31 (m, 2F, $CF_3CF_2C\mathbf{F_2}(CF_2)_4$), −122.29 (m, 4F, $CF_3(CF_2)_2(C\mathbf{F_2})_2(CF_2)_2$), −114.34 (m, 2F, $CF_3(CF_2)_5C\mathbf{F_2}$), −81.72 (m, 3F, $C\mathbf{F_3}(CF_2)_6$); MS (FAB$^-$, NBA): $[M-H^+]=448$; HRMS calcd for $C_7F_{15}HNO_2S$: 447.9488, found: 447.9500.

*4.1.1.3. Synthesis of 1,1,2,2,3,3,4,4 octafluoro-n-butane-1-4-bis-sulfonamide **1e**.* Yield: 76%; IR (KBr): 3462 (ν_{NH2}), 1375 (ν_{SO2}), 1145 (ν_{CF}); Mp: 114–116 °C; ^1H NMR (300.13 MHz, d_6-acetone): δ 8.10 (m, 4H, $(SO_2N\mathbf{H_2})_2$); ^{19}F NMR (282.37 MHz, d_6-acetone): δ −114.24 (m, 4F, $H_2NSO_2C\mathbf{F_2}(CF_2)_2C\mathbf{F_2}SO_2NH_2$); −120.76 (m, 4F, $H_2NSO_2CF_2(C\mathbf{F_2})_2CF_2SO_2NH_2$); MS (FAB$^-$, NBA): $[M-H^+]=359$; HRMS calcd for $C_4F_8H_3N_2O_4S_2$: 358.9407, found: 358.9390.

4.1.2. General procedure for the synthesis of sodium perfluoroalkanesulfonamides (***2a–c***)

A solution of perfluoroalkanesulfonamides (1 mol), dissolved in anhydrous diethyl ether was added dropwise to a suspension of

sodium methylate (95%) in anhydrous methanol (0.9 mol). The mixture was refluxed for 3–5 h and then filtered. The filtrate was concentrated in vacuo and the residue was washed three times with diethyl ether to remove the excess of perfluoroalkanesulfonamide and then dried under vacuum.

The synthesis of sodium perfluorobutanesulfonamide **2a**, sodium perfluorohexanesulfonamide **2b**, and perfluorooctanesulfonamide **2c** were described previously [15,16].

*4.1.3. General procedure for the synthesis of polyfluoroalkanesulfonamides and alkanesulfonamides (**3** and **4**)*

Polyfluoroalkanesulfonyl chlorides or alkanesulfonyl chlorides (1 mol) was dissolved in AcOEt and gaseous NH_3 was bubbled through the solution for 30 min at 0 °C. The mixture was stirred at room temperature for 15 min, then filtered and evaporated. The residue was dissolved with AcOEt and washed with water and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by recrystallization from EtOH/H_2O (90/10) to give white crystals of the corresponding sulfonamides.

*4.1.3.1. Synthesis of 3,3,4,4,5,5,6,6,6-nonafluoro-n-hexanesulfonamide **3a**.* Yield: 90%; IR (KBr): 3478 (ν_{NH2}), 1380 (ν_{SO2}), 1147 (ν_{CF}); Mp: 96–98 °C; ^{1}H NMR (300.13 MHz, d_6-acetone): δ 2.72 (m, 2H, $C_4F_9C\mathbf{H_2}$), 3.45 (m, 2H, $C_4F_9CH_2C\mathbf{H_2}$), 8.1 (m, 2H, $SO_2N\mathbf{H_2}$); ^{19}F NMR (282.37 MHz, d_6-acetone): δ −126.72 (m, 2F, $CF_3C\mathbf{F_2}(CF_2)_2$), −124.88 (m, 2F, $CF_3CF_2C\mathbf{F_2}CF_2$), −114.44 (m, 2F, $CF_3(CF_2)_2C\mathbf{F_2}$), −82.01 (m, 3F, $\mathbf{CF_3}(CF_2)_3$); ^{13}C NMR (75.46 MHz, d_6-acetone): δ 22.28 (m, 2H, $C_4F_9\mathbf{C}H_2$), 46.81 (m, 2H, $\mathbf{C}H_2$), 110–120 (C_4F_9); MS (FAB$^-$, NBA): $[M-H^+]=326$; HRMS calcd for $C_6F_9H_5NO_2S$: 325.9897, found: 325.9888.

*4.1.3.2. Synthesis of 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluoro-n-octanesulfonamide **3b**.* Yield: 89%; IR (KBr): 3478 (ν_{NH2}), 1377 (ν_{SO2}), 1147 (ν_{CF}); Mp: 131–133 °C; ^{1}H NMR (300.13 MHz, d_6-acetone): δ 2.72 (m, 2H, $C_6F_{13}C\mathbf{H_2}$), 3.42 (m, 2H, $C_6F_{13}CH_2C\mathbf{H_2}$), 8.2 (m, 2H, $SO_2N\mathbf{H_2}$); ^{19}F NMR (282.37 MHz, d_6-acetone): δ −126.81 (m, 2F, $CF_3C\mathbf{F_2}(CF_2)_4$), −123.93 (m, 2F, $CF_3CF_2C\mathbf{F_2}(CF_2)_3$), −123.50 ($CF_3(CF_2)_2C\mathbf{F_2}(CF_2)_2$), −122.49 (m, 2F, $CF_3(CF_2)_3C\mathbf{F_2}CF_2$), −114.19 (m, 2F, $CF_3(CF_2)_4C\mathbf{F_2}$), −81.79 (m, 3F, $C\mathbf{F_3}(CF_2)_5$); ^{13}C NMR (75.46 MHz, d_6-acetone): δ 26.65 (m, 2H, $C_6F_{13}\mathbf{C}H_2$), 46.39 (m, 2H, $\mathbf{C}H_2$), 110–120 (C_6F_{13}); MS (FAB$^-$, NBA): $[M-H^+]=426$; HRMS calcd for $C_8F_{13}H_5NO_2S$: 425.9912, found: 425.9905.

*4.1.3.3. Synthesis of 3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-heptadecafluoro-n-decanesulfonamide **3c**.* Yield: 91%; IR (KBr): 3475 (ν_{NH2}), 1377 (ν_{SO2}), 1150 (ν_{CF}); Mp: 152–154 °C; ^{1}H NMR (300.13 MHz, d_6-acetone): δ 2.73 (m, 2H, $C_8F_{17}C\mathbf{H_2}$); 3.41 (m, 2H, $C_8F_{17}CH_2C\mathbf{H_2}$), 8.2 (m, 2H, $SO_2N\mathbf{H_2}$); ^{19}F NMR (282.37 MHz, d_6-acetone): δ −126.80 (m, 2F, $CF_3C\mathbf{F_2}(CF_2)_6$); −123.84 (m, 2F, $CF_3CF_2C\mathbf{F_2}(CF_2)_5$); −123.33 (m, 2F, $CF_3(CF_2)_2C\mathbf{F_2}(CF_2)_4$); −122.32 (m, 6F, $CF_3(CF_2)_3(C\mathbf{F_2})_3CF_2$); −114.16 (m, 2F, $CF_3(CF_2)_6C\mathbf{F_2}$); −81.70 (m, 3F, $\mathbf{CF_3}(CF_2)_7$); ^{13}C NMR (75.46 MHz, d_6-acetone): δ 26.68 (m, 2H, $C_8F_{17}\mathbf{C}H_2$); 46.10 (m, 2H, $\mathbf{C}H_2$); 110–120 (C_8F_{17}); MS (FAB$^-$, NBA): $[M-H^+]=526$; HRMS calcd for $C_{10}F_{17}H_5NO_2S$: 525.9848, found: 525.9860.

*4.1.3.4. Synthesis of n-hexanesulfonamide **4a**.* Yield: 90%; IR (KBr): 3460 (ν_{NH2}), 1360 (ν_{SO2}), 2970 (ν_{CH}); Mp: 46–48 °C; ^{1}H NMR (300.13 MHz, d_6-acetone): δ 0.85 (t, 3H, $C\mathbf{H_3}(CH_2)_5$), 1.32 (m, 4H, $CH_3(C\mathbf{H_2})_2(CH_2)_3$), 1.45 (m, 2H, $CH_3(CH_2)_2C\mathbf{H_2}(CH_2)_2$), 1.85 (m, 2H, $CH_3(CH_2)_3C\mathbf{H_2}CH_2$), 2.1 (m, 2H, SO_2NH_2), 3.05 (t, 2H, $CH_3(CH_2)_4C\mathbf{H_2}$); ^{13}C NMR (75.46 MHz, d_6-acetone): δ 14.30 ($\mathbf{C}H_3$), 23.07 et 32.10 ($\mathbf{CH_2}$)$_2$, 24.77 ($\mathbf{C}H_2CH_2S$), 28.64 $(CH_2)_2\mathbf{C}H_2(CH_2)_2$, 55.67 ($\mathbf{C}H_2S$); MS (FAB$^-$, NBA): $[M-H^+]=164$; HRMS calcd for $C_6H_{14}NO_2S$: 164.0745, found: 164.0745. Anal. Calcd. for $C_6H_{15}NO_2S$: C, 43.61; H, 9.15; N, 8.48. Found: C, 43.50; H, 9.25; N, 8.30

*4.1.3.5. Synthesis of n-octanesulfonamide **4b**.* Yield: 82%; IR (KBr): 3465 (ν_{NH2}), 1365 (ν_{SO2}), 2975 (ν_{CH}); Mp: 76–78 °C; ^{1}H NMR (300.13 MHz, d_6-acetone): δ 0.9 (t, 3H, $C\mathbf{H_3}(CH_2)_5$), 1.30 (m, 8H, $CH_3(C\mathbf{H_2})_4(CH_2)_3$), 1.42 (m, 2H, $CH_3(CH_2)_4C\mathbf{H_2}(CH_2)_2$), 1.91 (m, 2H, $CH_3(CH_2)_5C\mathbf{H_2}CH_2$), 2.0 (m, 2H, SO_2NH_2), 3.05 (m, 2H, $CH_3(CH_2)_6C\mathbf{H_2}$); ^{13}C NMR (75.46 MHz, d_6-acetone): δ 14.34 ($\mathbf{C}H_3$), 23.29, 29.78, 29.89 et 32.47 ($\mathbf{CH_2}$)$_4$, 24.84 ($\mathbf{C}H_2CH_2S$), 28.60 $(CH_2)_2\mathbf{C}H_2(CH_2)_2$, 55.69 ($\mathbf{C}H_2S$); MS (FAB$^-$, NBA): $[M-H^+]=192$; HRMS calcd for $C_8H_{18}NO_2S$: 192.1058; found: 192.1054. Anal. Calcd. for $C_8H_{19}NO_2S$: C, 49.71; H, 9.91; N, 7.25. Found: C, 49.60; H, 9.95; N, 7.26

4.2. Biological

Bovine carbonic anhydrase (bCA) and human carbonic isoenzyme 2 (hCA) were purchased as a lyophilised powder from Sigma Chemical Co. All reagents used were of analytical grade. $CF_3SO_2NH_2$ and acetazolamide were purchased from aldrich.

4.2.1. Evaluation of inhibition activity on bCA and hCA

Native enzyme concentrations were determined from the absorbance at 280 nm, using a molar absorbance of 5.7×10^4 M^{-1} cm^{-1} for bCA and 5.5×10^4 M^{-1} cm^{-1} for the hCA. All enzyme preparations were stored in a mixture of 0.05 M TrisSO_4^{2-}/1 mM mercaptoethanol pH 8.7 at 4 °C. Enzymes concentrations were 2.9×10^{-6} M for the bCA and 0.82×10^{-6} M for the hCA.

Initial rate of 4-nitrophenyl acetate (4-NPA) hydrolysis was estimated by a modification of the method of Verpoorte et al. [17,18]. The increase in absorbance was followed at 348 nm for approximatively 20 min. Steady state measurements were made at 25 °C in a Kontron Uvikon 860 spectrophotometer. Solutions of substrate were prepared in acetone/H_20 media; the substrate concentrations varied from 1.04×10^{-3} M to 1.875×10^{-4} M for the bCA and 9.4×10^{-4} M for hCA. A molar absorption coefficient ε of 16.3×10^3 M^{-1} cm^{-1} was used for the 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 8). Non enzymatic hydrolysis rates were always subtracted from the observed rates. Triplicate experiments were done for each substrate concentration and for each inhibitor concentration. Solutions of inhibitor were prepared in distilled–deionized water for $R_FSO_2NH^-Na^+$ (**2**) and in distilled–deionized water with 10% (v/v) of DMSO (which is not inhibitory at these concentrations) for the others inhibitors (**1**,**3**,**4**). Dilutions were done thereafter with distilled–deionized water; the inhibitor concentrations varied between 2.07×10^{-6} M to 0.27×10^{-6} M for the bCA and 6.75×10^{-7} M to 0.675×10^{-7} M for the hCA. Inhibitor and enzyme solutions were preincubated together for 5 min at room temperature prior to assay. Acetazolamide was used as a specific inhibitor in our studies, its inhibitory effectiveness was determined using 2.9×10^{-6} M of bCA, 0.82×10^{-6} M of hCA and varying acetazolamide concentration from 64×10^{-6} M to 0.27×10^{-6} M for the bCA and 6.75×10^{-7} to 0.675×10^{-7} M for the hCA.

4.2.2. Evaluation of cellular toxicity on sheep red globules

To determine cellular toxicity we used an hemolytic assay described [19]. The hemolytic activity of the compounds was determined using sheep blood cells. The cells were washed three times in buffered saline (PBS solution pH 7.4) just prior to the assay. The final cell concentration used was 5% in the same buffer. The cell suspension 10 μL and 100 μl of varying amounts of inhibitors solution (concentration 750 μM–0.125 μM in PBS) were added in each tube. The resulting suspension was incubated for 20 min at 37 °C, then centrifugated (5 min at 1000 rpm at room temperature).

After centrifugation 50 μl of each supernatant was introduced in the plates Nunc Elisa and the absorbance monitored at 405 nm.

Two controls 0% and 100% of hemolysis were determined using the supernatant after centrifugation of 10 μL of the erythrocyte stock suspension added respectively with PBS or distillate water and analyzed after the same conditions.

Acknowledgements

We gratefully acknowledge the « Ministère de l'Education Nationale et de la Recherche » for its financial support (Ph.D. grant) and the CNRS.

References

[1] N. Anand, In Burger's medicinal chemistry and drug discovery. in: M.E. Wolff (Ed.), fifth ed., Therapeutic agents, vol. 2 J. Wiley & Sons, New York, 1996 pp. 527–544.
[2] L. Bouissane, S. El Kazzouli, S. Leonce, B. Pfeiffer, E.M. Rakib, M. Khouili, Bioorg. Med. Chem. 14 (2006) 1078–1088.
[3] G. Melagraki, A. Afantitis, H. Sarimveis, O. Igglessi-Markopoulou, C.T. Supuran, Bioorg. Med. Chem. 14 (2006) 1108–1114.
[4] D. Mandloi, S. Joshi, P.V. Khadikar, N. Khosla, Bioorg. Med. Chem. Lett. 17 (2005) 15–40.
[5] D. Vullo, B. Steffansen, B. Brodin, C.T. Supuran, A. Scozzafava, C.U. Nielsen, Bioorg. Med. Chem. 14 (2006) 2418–2428.
[6] A. Sherif, F. Rostom, Bioorg. Med. Chem. 14 (2006) 6475–6485.
[7] M. A Santos, S.M. Marques, T. Tuccinardi, P. Carelli, L. Panelli, A. Rossello, Bioorg. Med. Chem. 14 (2006) 7539–7550.
[8] S. Joshi, N. Khosla, P. Tiwari, Bioorg. Med. Chem. 12 (2004) 571–576.
[9] A. Kalir, H.H. Kalir, Chem. Sulphonic Acids Esters Their Deriv. (1991) 767–787.
[10] H. Blancou, F. Guillen, WO2004046093.
[11] T. Maren, C. Conroy, J. Biol. Chem. 268 (1993) 26233–26239.
[12] D. Kvam, EP0277814.
[13] C.T. Supuran, A. Scozzafava, A. Casini, Med. Res. Rev. 23 (2003) 146–189.
[14] C.T. Supuran, A. Scozzafava, Expert Opin. Ther. Patents 10 (2000) 575–600.
[15] Z. Benfodda, L. Delon, F. Guillen, H. Blancou, J. Fluor Chem. 128 (2007) 1353–1358.
[16] Z. Benfodda, F. Guillen, H. Blancou, Heteroat. Chem. 19 (2008) 542–548.
[17] J.A. Verpoorte, S. Mehta, J.T. Edsall, J. Biol. Chem. 242 (1967) 4221–4229.
[18] J.B. Shelton, W.R. Chegwidden, Comp. Biochem. Physiol., A 114A (1996) 283–289.
[19] T. Niidome, M. Tsuiki, Y. Tokunaga, T. Hatakeyama, H. Aoyagi, Bull. Chem. Soc. Jpn. 73 (2000) 1397–1402.