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Carbonic Anhydrase Inhibitors. Preparation of Potent Sulfonamides Inhibitors Incorporating Bile Acid Tails

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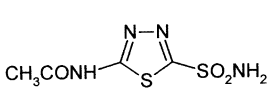
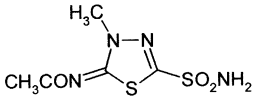
Abstract—Reaction of TBDMS-protected bile acids (cholic, chenodeoxycholic, deoxycholic, lithocholic, ursodeoxycholic acids) or dehydrocholic acid with aromatic/heterocyclic sulfonamides possessing free amino/hydroxy moieties, in the presence of carbodiimides, afforded after deprotection of the OTBDMS ethers, a series of sulfonamides incorporating bile acid moieties in their molecules. Many such derivatives showed strong inhibitory properties against three isozymes of carbonic anhydrase (CA, EC 4.2.1.1), that is CA I, II and IV, zinc enzymes playing critical roles in many pathologies, and which represent interesting targets for developing diverse pharmacological agents. Some of the most active derivatives, incorporating 1,3,4-thiadiazole-2-sulfonamide or benzothiazole-2-sulfonamide functionalities in their molecules, showed low nanomolar affinity for CA II and CAIV. Furthermore, the bioavailability of these derivatives in rabbits is comparable to that of acetazolamide, being in the range of 85–90%, showing them as promising candidates for systemically acting CA inhibitors.

Introduction

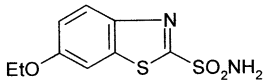
Sulfonamide inhibitors of carbonic anhydrase (CA, EC 4.2.1.1), such as acetazolamide **AAZ**, methazolamide **MZA**, ethoxzolamide **EZA**, dichlorophenamide **DCP**, dorzolamide **DZA** or brinzolamide **BRZ** have been clinically used for a long period in the treatment/prevention of a variety of diseases/pathological states among which open-angle glaucoma, epilepsy and other neuromuscular diseases, acid/base secretory disequilibria and osteoporosis are the most common ones.[1–3] The main drawback of systemically used agents (such as the first four sulfonamides mentioned above) is constituted by side effects such as augmented diuresis, fatigue, paresthesias, anorexia and so on due to CA inhibition in other tissues/organs than the target one



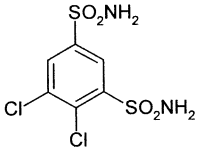
AAZ



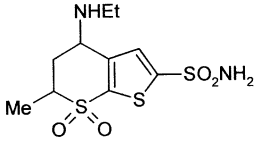
MZA



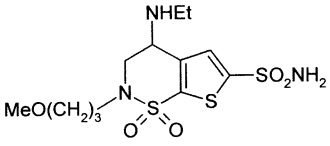
EZA



DCP



DZA



BRZ

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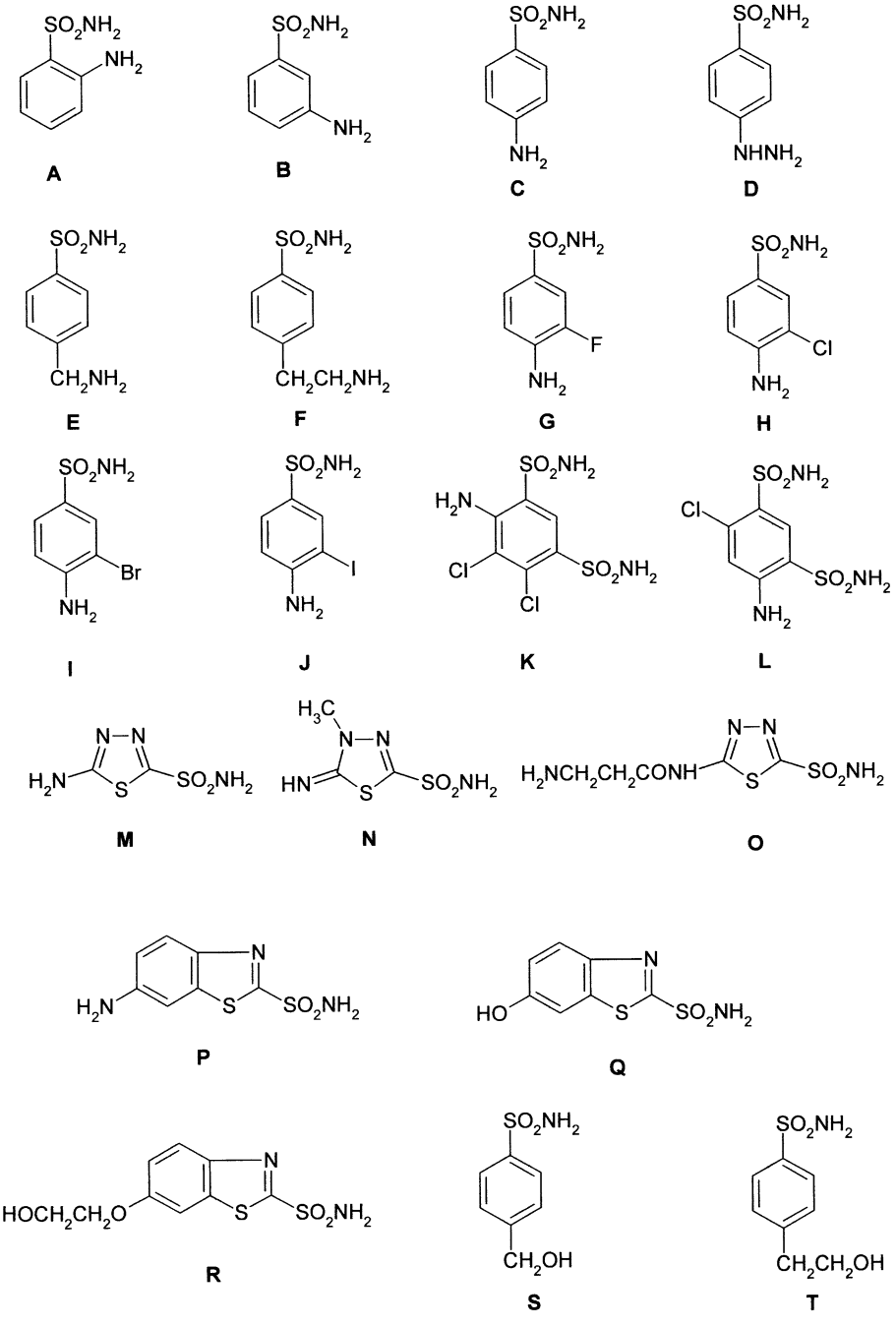
(CA, in the form of 14 isozymes, is ubiquitous in vertebrates).[4] The recently developed topically acting agents **DZA** and **BRZ**, on the other hand, show better selectivity, generally inhibiting only eye tissues CAs responsible for the enhanced aqueous humor secretion and increased intraocular pressure characteristic of glaucoma, which represents the main risk factor of blindness in the western countries.[1–5] The ubiquity of these enzymes in many tissues of higher vertebrates constantly prompts much research for discovering novel biomedical applications which may exploit their differential inhibition.[6–16]

It has been reported that bile acids/salts are inhibitors of several CA isozymes, with speculation that this interaction may contribute to the regulation of the enzymatic activity along the enterohepatic circulation as well as to some metabolic derangements in patients with cholestatic liver disease.[17] Since several CA isozymes (such as CA I, II, IV and V) are highly abundant along the gastro-intestinal tract (where they play important roles in the control of ion and fluid transport processes as well as acid-base homeostasy),[3,18,19] it appeared of interest to investigate in more detail whether the presence of bile acid moieties in the molecules of sulfonamide CA inhibitors may lead to novel biomedical applications of these agents. It should be mentioned that bile acids per se (such as derivatives **1**–**6**) play important physiological functions such as induction of bile flow, feedback inhibition of bile acid synthesis,



modulation of cholesterol synthesis, elimination of cholesterol (by solubilizing it in the bile and promoting its intestinal excretion) and facilitation of adsorption of lipids and fat-soluble vitamins, and are also used therapeutically for gallstone dissolution, the treatment of primary biliary cyrrhosis and hepatobiliary complications of cystic fibrosis.[20,21] Here, we report the synthesis and CA inhibitory properties of a large series of sulfonamides incorporating cholic, chenodeoxycholic, deoxycholic, lithocholic, ursodeoxycholic and dehydrocholic acid (**1**–**6**) moieties in their molecules, as well as preliminary in vivo bioavailability data of several such compounds.

Chemistry

The chemical structure of the carboxylic acids **1**–**6** as well as those of sulfonamides **A**–**T** used in the synthesis of the new compounds reported here are shown.

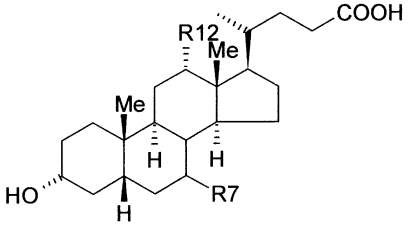
As a large number of such derivatives (amides/esters) have been prepared, in the following they will be abbreviated by using both a figure designating the carboxylic acid from which they were derived, as well as a letter designating the sulfonamides to which the bile acid moiety has been attached. For example: **1A** is the amide of cholic acid **1** with orthanilamide **A**; **6M** is the amide of dehydrocholic acid **6** with 5-amino-1,3,4-thiadiazole-2-sulfonamide **M**; **4S** is the ester of lithocholic acid **4** with 4-(2-hydroxymethyl)-benzenesulfonamide **S** and so on. The whole series of sulfonamides containing the six carboxylic acids **1**–**6** and the 20 sulfonamides **A**–**T** have been prepared (Table 1).

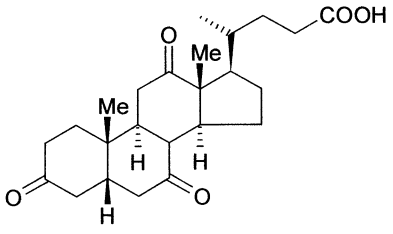
The synthesis has been done by applying previously reported procedures,[2,7,10–12] involving the reaction of appropriately protected carboxylic acids with amino/hydroxy-sulfonamides **A**–**T**, in the presence of carbodiimides (Scheme 1). The *tert*-butyl-dimethylsilyl ethers were chosen as protection of the reactive hydroxyl moieties present in bile acids **1**–**5**,[22] whereas no protecting groups were necessary for the reaction of **6**, which was simply coupled with **A**–**T** in the presence of carbodiimides. The deprotection step has thereafter been done by the standard procedure, that is in the presence of tetraalkylammonium fluoride,[23] leading to the desired products (**1**–**6**)**A**–**T** with excellent yields.[24]

Carbonic Anhydrase Inhibitory Activity

The data of Table 1 show that the new inhibitors prepared by attaching bile acid moieties to aromatic/heterocyclic sulfonamides **A**–**T**, are more effective as compared to the parent sulfonamides from which they were prepared, towards the three investigated isozymes, hCA I, hCA II and bCA IV. The enhanced inhibitory power of these compounds is presumably due to the interaction of the (long) bile acid moiety incorporated in their molecules with hydrophilic/hydrophobic patches at the entrance of the enzyme active site as observed for inhibitors previously reported by Whitesides'[25] and our groups,[1,2,7–16] and explained by detailed QSAR models.[26]

The nature of the sulfonamide attached to the bile acid moiety in the new derivatives reported here greatly influenced the CA inhibitory power of these compounds. Among the synthesized compounds, the heterocyclic sulfonamide derivatives were the most active, followed by the aromatic sulfonamide derivatives. The efficiency of the obtained inhibitor generally varied in the following way, based on the parent sulfonamide from which it was prepared: the derivatives of *p*-hydrazino-benzenesulfonamide < the orthanilamides < the metanilamides < the sulfanilamides < the homosulfanilamides < the *p*-aminoethyl-benzenesulfonamides ≅ the halogeno-substituted sulfanilamides ≅ the 1,3-benzene-disulfonamides < the 1,3,4-thiadiazole-2-sulfonamides ≅ 4-methyl-δ^2-1,3,4-thiadiazoline-2-sulfonamide ≅ the benzothiazole-2-sulfonamides. The nature of the acylating moiety also influenced the inhibitory properties of these new sulfonamides, with the following range of inhibitory effects for the corresponding sulfonamides incorporating polyamino-polycarboxylic acid moieties: cholic acid derivatives < chenodeoxycholic acid derivatives < deoxycholic acid derivatives < lithocholic acid derivatives < ursodeoxycholic acid derivatives < dehydrocholic acid derivatives. All three CA isozymes investigated here were susceptible to





1: R7 = R12 = α-OH (cholic acid)

2: R7 = α-OH; R12 = H (chenodeoxycholic acid)

3: R7 = H; R12 = α-OH (deoxycholic acid)

4: R7 = R12 = H (lithocholic acid)

5: R7 = β-OH; R12 = H (ursodeoxycholic acid)

6: dehydrocholic acid

Table 1. Inhibition data for derivatives (**1**–**6**)**A**–**T** reported in the present paper (data in parentheses represent inhibition by the parent sulfonamide **A**–**T**) and standard sulfonamide CA inhibitors

Inhibitor	K_I (nM)		
	hCA I[a]	hCA II[a]	bCA IV[b]
Acetazolamide	900	12	220
Methazolamide	780	14	240
Ethoxzolamide	25	8	13
Dichlorophenamide	1200	38	380
Dorzolamide	>50,000	9	43
Brinzolamide	—	3	45
1A	25,500 (45,400)	275 (295)	1200 (1310)
1B	23,000 (25,000)	218 (240)	1350 (2200)
1C	21,000 (28,000)	210 (300)	430 (3000)
1D	32,100 (78,500)	315 (320)	1050 (3200)
1E	3780 (25,000)	120 (170)	254 (2800)
1F	3400 (21,000)	86 (160)	220 (2500)
1G	1280 (8300)	50 (60)	133 (180)
1H	970 (9800)	82 (110)	168 (320)
1I	600 (6500)	35 (40)	60 (66)
1J	510 (6000)	53 (70)	104 (125)
1K	400 (6100)	21 (28)	97 (175)
1L	400 (8400)	56 (75)	130 (160)
1M	420 (8600)	50 (60)	205 (540)
1N	700 (9300)	12 (19)	115 (355)
1O	250 (455)	1.2 (3)	31 (125)
1P	35 (70)	5 (9)	10 (19)
1Q	32 (55)	4 (8)	9 (17)
1R	39 (50)	3 (7)	10 (15)
1S	1200 (24,000)	97 (125)	280 (560)
1T	1150 (18,000)	80 (110)	300 (450)
2A	22,100 (45,400)	245 (295)	900 (1310)
2B	21,400 (25,000)	210 (240)	1250 (2200)
2C	20,000 (28,000)	186 (300)	420 (3000)
2D	27,500 (78,500)	295 (320)	850 (3200)
2E	3450 (25,000)	96 (170)	244 (2800)
2F	3300 (21,000)	86 (160)	235 (2500)
2G	1050 (8300)	50 (60)	128 (180)
2H	950 (9800)	80 (110)	150 (320)
2I	617 (6500)	34 (40)	53 (66)
2J	521 (6000)	55 (70)	110 (125)
2K	343 (6100)	21 (28)	95 (175)
2L	300 (8400)	62 (75)	143 (160)
2M	410 (8600)	52 (60)	240 (540)
2N	514 (9300)	13 (19)	100 (355)
2O	268 (455)	2 (3)	45 (125)
2P	62 (70)	5.6 (9)	13 (19)
2Q	47 (55)	6.1 (8)	12 (17)
2R	44 (50)	5.2 (7)	9 (15)
2S	1230 (24,000)	98 (125)	340 (560)
2T	1100 (18,000)	90 (110)	320 (450)
3A	20,100 (45,400)	280 (295)	975 (1310)
3B	12,700 (25,000)	230 (240)	980 (2200)
3C	12,000 (28,000)	210 (300)	355 (3000)
3D	21,300 (78,500)	310 (320)	920 (3200)
3E	1710 (25,000)	80 (170)	233 (2800)
3F	1500 (21,000)	77 (160)	245 (2500)
3G	780 (8300)	42 (60)	96 (180)
3H	890 (9800)	77 (110)	151 (320)
3I	365 (6500)	31 (40)	50 (66)
3J	415 (6000)	47 (70)	110 (125)
3K	285 (6100)	20 (28)	100 (175)
3L	225 (8400)	55 (75)	121 (160)
3M	200 (8600)	50 (60)	130 (540)
3N	240 (9300)	11 (19)	63 (355)
3O	200 (455)	2.1 (3)	30 (125)
3P	56 (70)	6.5 (9)	12 (19)
3Q	39 (55)	4.8 (8)	9 (17)
3R	37 (50)	4.1 (7)	8 (15)
3S	1000 (24,000)	106 (125)	350 (560)
3T	885 (18,000)	78 (110)	300 (450)
4A	9200 (45,400)	270 (295)	750 (1310)
4B	9500 (25,000)	210 (240)	740 (2200)
4C	6300 (28,000)	106 (300)	280 (3000)
4D	15,000 (78,500)	280 (320)	650 (3200)

(*continued on next page*)

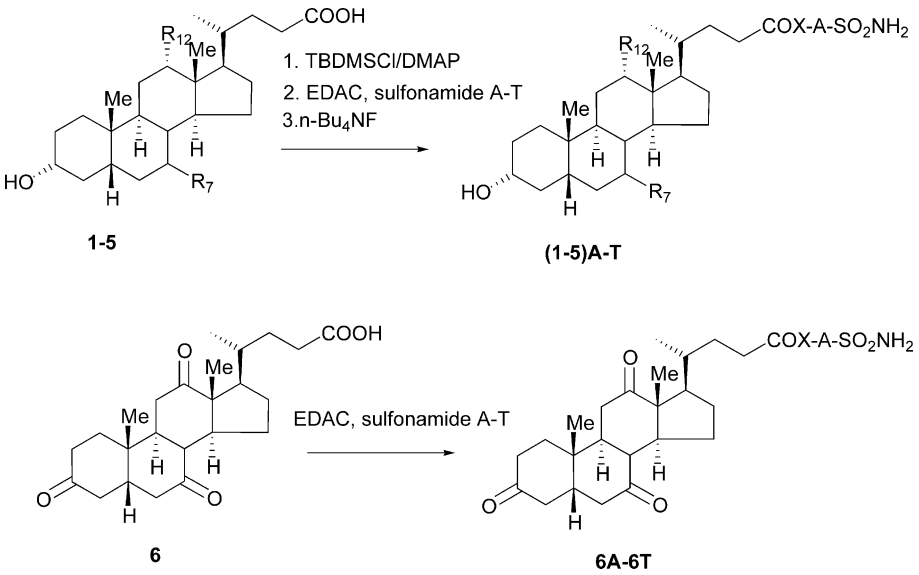
Table 1 (*continued*)

Inhibitor	K_I (nM)		
	hCA I[a]	hCA II[a]	bCA IV[b]
4E	910 (25,000)	48 (170)	83 (2800)
4F	650 (21,000)	41 (160)	70 (2500)
4G	360 (8300)	29 (60)	36 (180)
4H	720 (9800)	47 (110)	54 (320)
4I	120 (6500)	21 (40)	53 (66)
4J	120 (6000)	32 (70)	69 (125)
4K	130 (6100)	17 (28)	87 (175)
4L	200 (8400)	54 (75)	82 (160)
4M	210 (8600)	10 (60)	28 (540)
4N	205 (9300)	8 (19)	21 (355)
4O	200 (455)	2.1 (3)	19 (125)
4P	48 (70)	2.8 (9)	9 (19)
4Q	40 (55)	2.0 (8)	9 (17)
4R	36 (50)	1.4 (7)	8 (15)
4S	800 (24,000)	92 (125)	313 (560)
4T	625 (18,000)	81 (110)	310 (450)
5A	8400 (45,400)	180 (295)	500 (1310)
5B	7050 (25,000)	157 (240)	480 (2200)
5C	4000 (28,000)	71 (300)	164 (3000)
5D	12,000 (78,500)	240 (320)	615 (3200)
5E	550 (25,000)	21 (170)	40 (2800)
5F	295 (21,000)	14 (160)	34 (2500)
5G	180 (8300)	11 (60)	24 (180)
5H	190 (9800)	17 (110)	30 (320)
5I	91 (6500)	10 (40)	23 (66)
5J	67 (6000)	13 (70)	20 (125)
5K	59 (6100)	10 (28)	29 (175)
5L	71 (8400)	9 (75)	46 (160)
5M	48 (8600)	0.9 (60)	16 (540)
5N	54 (9300)	2.0 (19)	17 (355)
5O	98 (455)	1.3 (3)	11 (125)
5P	30 (70)	0.6 (9)	9 (19)
5Q	31 (55)	0.7 (8)	6 (17)
5R	30 (50)	0.6 (7)	5 (15)
5S	500 (24,000)	75 (125)	245 (560)
5T	350 (18,000)	58 (110)	160 (450)
6A	8100 (45,400)	170 (295)	505 (1310)
6B	7440 (25,000)	156 (240)	440 (2200)
6C	3700 (28,000)	70 (300)	147 (3000)
6D	12,750 (78,500)	260 (320)	550 (3200)
6E	600 (25,000)	26 (170)	40 (2800)
6F	285 (21,000)	18 (160)	29 (2500)
6G	210 (8300)	15 (60)	28 (180)
6H	200 (9800)	13 (110)	30 (320)
6I	66 (6500)	9 (40)	28 (66)
6J	61 (6000)	10 (70)	19 (125)
6K	45 (6100)	9 (28)	24 (175)
6L	77 (8400)	11 (75)	50 (160)
6M	41 (8600)	0.5 (60)	9 (540)
6N	29 (9300)	1.0 (19)	8 (355)
6O	22 (455)	1.1 (3)	7 (125)
6P	39 (70)	0.8 (9)	7 (19)
6Q	33 (55)	0.6 (8)	5 (17)
6R	30 (50)	0.8 (7)	6 (15)
6S	450 (24,000)	71 (125)	200 (560)
6T	415 (18,000)	50 (110)	123 (450)

[a]Human (cloned) isozymes.
[b]From bovine lung microsomes, by the esterase method.[27]

inhibition with this type of sulfonamides, with hCA II and bCA IV the most sensitive, whereas hCA I was generally less susceptible to inhibition as compared to the first two isozymes.

Some of the most active inhibitors reported here, such as **6M** and **6Q** were administered (via gavage) to albino rabbits in order to investigate their systemic bioavailability. Acetazolamide, a widely clinically used CA inhibitor has been used as standard in these experiments (Table 2). Both at 1 h, as well as 2 h post administration, red blood cells (containing 150 μM CA I and 10 μM CA II)[29] were saturated with sulfonamide, as determined both by an HPLC as well as an electronic spectroscopic method.[28] Due to the increased liposolubility of the new inhibitors as compared to acetazolamide, already at 1 h



Scheme 1. Preparation of sulfonamides (**1**–**6**)**A**–**T**. Reagents and conditions are: TBDMSCl, *tert*-butyl-dimethylsilylchloride; DMAP, 4-dimethylaminopyridine; DMF, room temperature, 4 h; EDAC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide, sulfonamide **A**–**T**, in DMF, room temperature, 12–15 h; *n*-Bu_4NF, tetra-*n*-butylammonium fluoride, 2 equivalents, in THF, room temperature, 2 h (in the above formulas X = O or NH; A as in sulfonamides **A**–**T**).

post administration the maximal sulfonamide levels were attained in the blood of the experimental animals in the case of the new inhibitors **6M** and **6Q**, whereas for acetazolamide, the same levels are attained only after 2 h. Since the bioavailability of acetazolamide is estimated to be of 85–90%,[29] we speculate the same order of magnitude for this parameter in the case of the new sulfonamides reported here, which makes them interesting leads for the investigation of different types of biomedical applications employing systemic CA inhibitors.

Conclusions

We report here a novel class of sulfonamides obtained by the 'tail' approach,[1] which incorporate bile acid moieties. They were obtained by coupling TBDMS-protected bile acids with amino/hydroxysulfonamides in the presence of carbodiimides, followed by deprotection in the presence of tetraalkylammonium fluoride. Some of these new CA inhibitors possessed affinities in the low nanomolar range for isozymes hCA I, hCA II and bCA IV, acting as effective enzyme inhibitors in vitro. In vivo, in rabbits, the bioavailability of some of these derivatives was in the range of 85–90%, proving that such new compounds reported here might lead to the development of other type of drugs from the class of the sulfonamide CA inhibitors.

Table 2. Levels of sulfonamides (μM) in red blood cells of albino rabbits, at 60 and 120 min post-administration of 50 mg/kg sulfonamides **AAZ**, **6M** and **6Q**

Inhibitor	[sulfonamide], μM*			
	$t = 60$ min		$t = 120$ min	
	HPLC[a]	ES[b]	HPLC[a]	ES[b]
Acetazolamide	136 ± 7	140 ± 4	160 ± 8	167 ± 5
6M	155 ± 4	151 ± 5	162 ± 4	159 ± 3
6Q	156 ± 6	158 ± 9	159 ± 5	163 ± 5

[a]Mean ± standard deviation (from three determinations) by the HPLC method.
[b]Mean ± standard deviation (from three determinations) by the electronic spectroscopic method.

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24. An example of synthesis is illustrated below: an amount of 180 mg (1 mmol) 5-amino-1,3,4-thiadiazole-2-sulfonamide M, 402 mg (1 mmol) carboxylic acid **6** and 192 mg (1 mmol) EDAC·HCL were dissolved in 50 mL DMF. The stoichiometric amount of Et_3N (79 μL) was then added and the reaction mixture stirred at room temperature for 12 h (TLC control). The solvent was evaporated in vacuo, the residue taken up with 25 mL of water, filtered, dried and crystallized from EtOH (502 mg, yield of 89%). Colorless crystals, mp 191–193 °C, IR(KBr), cm^{-1}: 1164 (SO_2^{sym}), 1347 (SO_2^{as}), 1561 (amide II), 1607 (amide I), 1723 (C=O); 3330 (NH, NH_2); ^1H NMR ($CDCl_3$), 300 MHz, δ, ppm: 0.98 (s, 3H); 1.32 (s, 3H); 0.74–2.71 (m, 27H); 7.35 (2H, br s, SO_2NH_2); 8.10 (br s, CONH); ^{13}C NMR (DMSO-d_6), δ, ppm: 13.24; 20.31; 22.89; 26.34; 28.97; 32.13; 32.82; 36.30; 36.75; 37.45; 37.90; 40.21; 44.25; 45.83; 46.30; 47.22; 47.78; 49.80; 53.12; 57.89; 173.49; 175.11; 176.50; 211.20; 211.32; 213.55. Anal. found C, 55.61; H, 6.24; N, 9.89%; $C_{26}H_{36}N_4O_6S_2$ requires: C, 55.30; H, 6.43; N, 9.92%.
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