Eur J Med Chem (1996) 31, 597–606

Carbonic anhydrase activators. Part 14*. Syntheses of mono and bis pyridinium salt derivatives of 2-amino-5-(2-aminoethyl)- and 2-amino-5-(3-aminopropyl)-1,3,4-thiadiazole and their interaction with isozyme II

CT Supuran[1], M Barboiu[2], C Luca[2], E Pop[3], ME Brewster[3], A Dinculescu[3]

[1]*Università degli Studi, Dipartimento di Chimica, Laboratorio di Chimica Inorganica e Bioinorganica, Via Gino Capponi 7, 50121 Florence, Italy;*
[2]*Polytechnic University, Department of Analytical Chemistry, Polizu 1 78126 Bucharest, Romania;*
[3]*Pharmos Corporation, Two Innovation Drive, Alachua, FL 32615, USA*

(Received 31 October 1995; accepted 14 February 1996)

Summary — Reaction of 2-amino-5-(2-aminoethyl)- and 2-amino-5-(3-aminopropyl)-1,3,4-thiadiazole with 2,4,6-trisubstituted pyrylium salts in molar ratios of 1:1 and 1:2 afforded three series of derivatives which were investigated for their abilities to activate the enzyme carbonic anhydrase (CA). Only compounds possessing free aminoalkyl moieties behaved as strong CA II activators, presumably via a mechanism involving the shuttling of protons between the enzyme active site and the environment.

carbonic anhydrase / activator / isozyme II / 1,3,4-thiadiazole / pyrylium salt

Introduction

Carbonic anhydrase (CA, EC 4.2.1.1.) catalyzes a very simple physiological reaction, the interconversion between carbon dioxide and the hydrogen carbonate anion [2].

$$CO_2 + 2H_2O \Leftrightarrow H_3O^+ + HCO_3^- \quad (1)$$

CA inhibitors of the sulfonamide type [3] are widely used pharmaceuticals in the treatment of a variety of disorders such as glaucoma [4], epilepsy [5], and gastro-doudenal ulcers [6]. Activators of this enzyme, on the other hand, have only recently been reported but may have important medical uses such as in the treatment of genetic CA deficiencies [7, 8]. The mechanism of action for enzyme activation is thought to involve formation of a complex between the enzyme and activator facilitating an 'intramolecular' proton transfer (which is the rate-determining process in the catalytic turnover) [9, 10]. Under normal circumstances:

$$EZn^{2+}\text{-}OH\text{-} + CO_2 \Leftrightarrow EZn^{2+}\text{-}HCO_3\text{-} \overset{H_2O}{\Leftrightarrow} EZn^{2+}\text{-}OH_2 + HCO_3^- \quad (2)$$

$$EZn^{2+}\text{-}OH_2 + B \Leftrightarrow EZn^{2+}\text{-}OH\text{-} + BH^+ \quad (3)$$

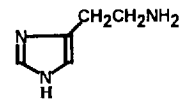
where B represents a proton acceptor which can be either an amino acid residue within the enzyme active site (such as His-64) [9] or a general base in the surrounding medium. In the presence of activators (A), eq (3) becomes [9]:

$$EZn^{2+}\text{-}OH_2 + A \Leftrightarrow [EZn^{2+}\text{-}OH_2\text{---}A] \Leftrightarrow [EZn^{2+}\text{-}OH^-\text{---}AH^+] \Leftrightarrow EZn^{2+}\text{-}OH^- + AH^+ \quad (4)$$

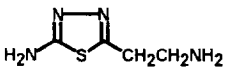
where $[EZn^{2+}\text{-}OH_2\text{---}A]$ represents the enzyme–activator complex prior to the proton transfer and $[EZn^{2+}\text{-}OH\text{---}AH^+]$ represents the enzyme–activator complex subsequent to the proton transfer.

As shown in previous papers [1, 7, 9] activators of isozymes I and II of CA must possess groups capable of protonation (generally primary, secondary or tertiary amino groups) attached to a bulky aromatic or heterocyclic moiety. Activators include, for example, histamine **1** [7, 9], 2-amino-5-(2-aminoethyl)-1,3,4-thiadiazole **2** [7], as well as ω-aminoethyl substituted pyridinium cations **3** [11].

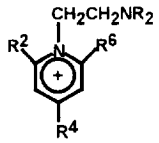
*For part 13, see [1].



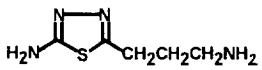
1



2



3



4

Recently it was shown that amines **1–3** act via non-competitive (with the substrate CO_2, as well as 4-nitrophenyl acetate) mechanisms of action, consistent with the proposed reactions [1]. The particularly potent activities observed for the 1,3,4-thiadiazole derivative **2** prompted an extended investigation of this class of compounds. The synthesis, characterization and biological activity of a series of positively-charged derivatives of 2-amino-5-(2-aminoethyl)- and 2-amino-5-(3-aminopropyl)-1,3,4-thiadiazole are described herein.

Results and discussion

Chemistry

2-Amino-5-(2-aminoethyl)-1,3,4-thiadiazole **2** was reported by Ohta [12] who prepared several histamine agonists of this structural type. It was shown that this compound stimulates gastric acid secretion in man [13], but more detailed experiments regarding its mechanism of action were not reported. Only later was it recognized that **2** is a powerful CA activator, and this might also account for its gastric acid-stimulating properties [14].

The preparation of **2**, as well as its homologue, 2-amino-5-(3-aminopropyl)-1,3,4-thiadiazole **4**, was accomplished by a slightly modified procedure of the original synthesis [12]. Unfortunately attempts to prepare 2-amino-5-(2-aminomethyl)-1,3,4-thiadiazole (starting from glycine) were unsuccessful, with the reaction giving rise to resins from which the desired compound could not be isolated.

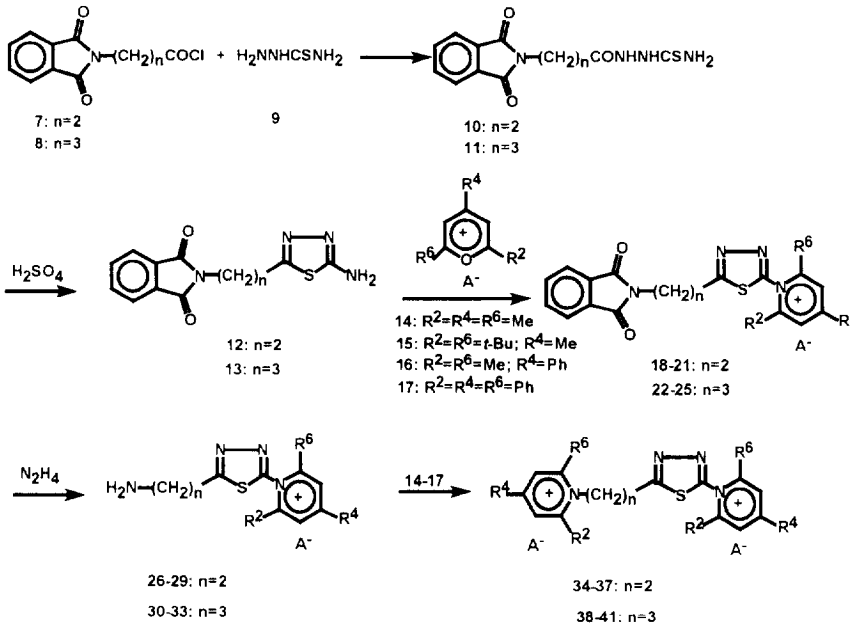
The starting materials in the preparation of the thiadiazole pyridinium derivatives included β-alanine and γ-aminobutyric acid (GABA), which were converted to the corresponding phthalimide derivatives **5** and **6** respectively with phthalic anhydride in refluxing toluene [15]. The corresponding acyl chlorides **7** and **8** were subsequently obtained by reaction of the above-mentioned amino acid derivatives with $SOCl_2$ in benzene. Acylation of thiosemicarbazide **9** with **7** and **8** in the presence of pyridine afforded compounds **10** and **11** [16] which were cyclized in concentrated sulfuric acid and gave the thiadiazoles **12** and **13** respectively [12, 13]. These were the key intermediates in the preparation of other derivatives. Treatment of the two thiadiazoles with various 2,4,6-trisubstituted pyrylium salts **14–17**, which were prepared according to literature procedures [17], gave rise to a group of intermediates **18–25**. In the presence of hydrazinium hydroxide these were deprotected at the phthalimido moiety, affording the amines **26–33**. Reaction of the generated amines (**26–33**) with another equivalent of pyrylium salt afforded the bis-pyridinium salts **34–41**.

Conversely, deprotection of **12** or **13** with hydrazinium hydroxide afforded the free amines **2** and **4** (the latter has not been described previously), which, by reaction with one equivalent of pyrylium salt, led to derivatives **42–49**. Reaction of two equivalents of pyrylium salt with **2** or **4** afforded the already prepared bispyridinium derivatives **34–41**, by an alternative route.

Compounds **4** and **12–49** were characterized by elemental analysis (C, H, N, within ±0.4% of the theoretical values, see the *Experimental protocols*), IR spectrophotometry and ^{1}H-NMR spectroscopy.

In the IR spectra of the prepared derivatives the main features were the following: (i) the strong CO vibrations (at 1700 and 1610 cm^{-1}) in the case of compounds possessing phthalimido moieties (**18–25**) and the lack of such bands in the other derivatives; (ii) the presence of anion bands (ClO_4^- at 625 and 1100 cm^{-1}; BF_4^- at 610 and 1075 cm^{-1}) for the salt-type compounds (**18–45**), together with bands characteristic of the substituted pyridinium moiety (C=C, C=N in the range of 1480, 1535 and 1600 cm^{-1}); (iii) the presence of the free amino bands in derivatives **4**, **26–33**, and **42–49** which absorbed at 3300 cm^{-1} (see the *Experimental protocols* for details).

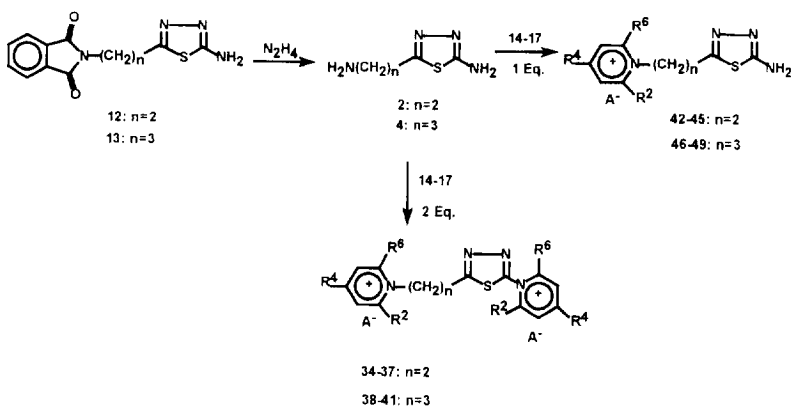
The ^{1}H-NMR spectra are presented in tables I–III. For compounds **18–33**, where the substituted pyridinium group is attached directly to an aromatic ring, the ring current of the thiadiazolic moiety is readily evidenced, due to the shielding of the signals of the 2,6-dimethyl groups, as described earlier for related systems by Dinculescu and Balaban [17, 18]. In such cases, the signals of these groups appear at 2.42–2.43 ppm, whereas those of the 4-methyl groups appear at 2.69–2.70 ppm. For compounds **18–45**, the signals of the polymethylene moiety appear as broad multiplets in the region 3.05–3.30 ppm. The same is not true when the phthalimido group is removed, meaning that for compounds **26–33** the CH_2 group adjacent to the $-NH_3^+$ moiety (the protonations achieved due to the presence of trifluoracetic acid as NMR solvent) appears at a resonance of 3.80–3.90 ppm. The remaining $(CH_2)_{n-1}$ protons appear as triplets for derivatives **30–33** (see table I).



Scheme 1.

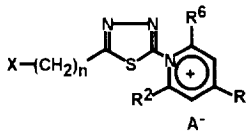
The ^{1}H-NMR spectra of compounds **34–41** are more complicated (table II) due to the fact that the two substituted pyridinium moieties are not equivalent. Thus, the pyridinium moiety associated with the 2-amino group shows signals similar to those of derivatives **18–33**, due to the fact that this pyridinium moiety is orthogonal to the thiadiazole ring. On the other hand, the pyridinium moiety related with the 5-position presents signals typical of *N*-alkyl pyridinium salts [17, 18]. Thus, the 4-methyl groups appear as singlets at 2.56–2.65 ppm, and the 2,6-dimethyl groups as singlets at 2.71–2.72 ppm.

In the derivatives **34–37**, additional effects are seen, such as the shielding of the CH_2 protons adjacent to the heterocyclic ring in the 2,4,6-triphenyl derivative **37**, as well as the shielding of the 2,6-dimethyl groups. The effects are probably due to through-space charge transfer between the (α-methylene group and



Scheme 2.

Table I. ^{1}H-NMR spectra of compounds **18–33**, at 100 MHz in trifluoracetic acid (TFA) as solvent.

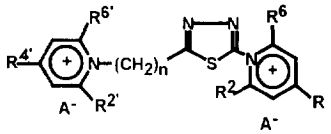


18–21: n = 2; X = phthalimido
22–25: n = 3; X = phthalimido
26–29: n = 2; X = NH_2
30–33: n = 3; X = NH_2

Compound	*n*	R^2	R^4	R^6	R^2	R^4	R^6	*3,5-H*	$(CH_2)_n$	*X*
18	2	Me	Me	Me	2.43 (s, 3H)	2.69 (s, 3H)	2.43 (s, 3H)	7.60 (s, 2H)	3.28 (m, 4H)	7.20 (m, 4H)
19	2	*t*Bu	Me	*t*Bu	1.58 (s, 9H)	2.70 (s, 3H)	1.58 (s, 9H)	7.60 (s, 2H)	3.27 (m, 4H)	7.20 (m, 4H)
20	2	Me	Ph	Me	2.42 (s, 3H)	7.68 (m, 5H)	2.42 (s, 3H)	7.60 (s, 2H)	3.30 (m, 4H)	7.20 (m, 4H)
21	2	Ph	Ph	Ph	7.00–8.30 (m, 15H, ArH)			7.70 (s, 2H)	3.15 (m, 4H)	7.20 (m, 4H)
22	3	Me	Me	Me	2.42 (s, 3H)	2.69 (s, 3H)	2.42 (s, 3H)	7.60 (s, 2H)	3.08 (m, 6H)	7.20 (m, 4H)
23	3	*t*Bu	Me	*t*Bu	1.59 (s, 9H)	2.70 (s, 3H)	1.59 (s, 9H)	7.60 (s, 2H)	3.10 (m, 6H)	7.20 (m, 4H)
24	3	Me	Ph	Me	2.42 (s, 3H)	7.68 (m, 5H)	2.42 (s, 3H)	7.60 (s, 2H)	3.10 (m, 6H)	7.20 (m, 4H)
25	3	Ph	Ph	Ph	7.00–8.35 (m, 15H, ArH)			7.70 (s, 2H)	3.05 (m, 6H)	7.20 (m, 4H)
26	2	Me	Me	Me	2.42 (s, 3H)	2.68 (s, 3H)	2.42 (s, 3H)	7.60 (s, 2H)	3.90 (m, 2H), 3.20 (t, 2H)	[a]
27	2	*t*Bu	Me	*t*Bu	1.59 (s, 9H)	2.70 (s, 3H)	1.59 (s, 9H)	7.60 (s, 2H)	3.90 (m, 2H), 3.21 (t, 2H)	[a]
28	2	Me	Ph	Me	2.42 (s, 3H)	7.70 (m, 5H)	2.42 (s, 3H)	7.60 (s, 2H)	3.90 (m, 2H), 3.18 (t, 2H)	[a]
29	2	Ph	Ph	Ph	7.00–8.30 (m, 15H, ArH)			7.70 (s, 2H)	3.90 (m, 2H), 3.10 (t, 2H)	[a]
30	3	Me	Me	Me	2.43 (s, 3H)	2.68 (s, 3H)	2.43 (s, 3H)	7.60 (s, 2H)	3.90 (m, 2H), 3.20 (t, 2H)	[a]
31	3	*t*Bu	Me	*t*Bu	1.58 (s, 9H)	2.70 (s, 3H)	1.58 (s, 9H)	7.60 (s, 2H)	3.84 (m, 2H), 3.21 (t, 2H)	[a]
32	3	Me	Ph	Me	2.42 (s, 3H)	7.70 (m, 5H)	2.42 (s, 3H)	7.60 (s, 2H)	3.85 (m, 2H), 3.17 (t, 2H)	[a]
33	3	Ph	Ph	Ph	7.00–8.35 (m, 15H, ArH)			7.70 (s, 2H)	3.80 (m, 2H), 3.16 (t, 2H)	[a]

[a] In TFA this group is NH_3^+ and these protons, being in fast exchange with the solvent, are not seen in the ^{1}H-NMR spectra.

Table II. ^{1}H-NMR spectra of compounds **34–41**, at 100 MHz in trifluoroacetic acid (TFA).



34–37: $n = 2$
38–41: $n = 3$

*Compound**	*n*	R^2=$R^{2'}$	R^4=$R^{4'}$	R^6=$R^{6'}$	R^2	R^4	R^6	$R^{2'}$	$R^{4'}$	$R^{6'}$	N^+-CH_2	$(CH_2)_{n-1}$
34	2	Me	Me	Me	2.43 (s, 3H)	2.69 (s, 3H)	2.43 (s, 3H)	2.71 (s, 3H)	2.57 (s, 3H)	2.71 (s, 3H)	4.78 (t, 2H)	3.25 (t, 2H)
35	2	*t*Bu	Me	*t*Bu	1.58 (s, 9H)	2.68 (s, 3H)	1.58 (s, 9H)	1.05 (s, 9H)	2.65 (s, 3H)	1.05 (s, 9H)	4.80 (t, 2H)	3.20 (t, 2H)
36	2	Me	Ph	Me	2.42 (s, 3H)	7.70 (m, 5H)	2.42 (s, 3H)	2.72 (s, 3H)	7.70 (m, 5H)	2.72 (s, 3H)	4.65 (t, 2H)	3.18 (t, 2H)
37	2	Ph	Ph	Ph	7.00–8.30 (m, 30H, ArH)			7.00–8.30 (m, 30H, ArH)			4.95 (t, 2H)	3.00 (t, 2H)
38	3	Me	Me	Me	2.43 (s, 3H)	2.68 (s, 3H)	2.43 (s, 3H)	2.66 (s, 3H)	2.56 (s, 3H)	2.66 (s, 3H)	4.75 (t, 2H)	3.17 (m, 4H)
39	3	*t*Bu	Me	*t*Bu	1.58 (s, 9H)	2.69 (s, 3H)	1.58 (s, 9H)	1.03 (s, 9H)	2.65 (s, 3H)	1.03 (s, 9H)	4.77 (t, 2H)	3.15 (m, 4H)
40	3	Me	Ph	Me	2.43 (s, 3H)	7.70 (m, 5H)	2.43 (s, 3H)	2.64 (s, 3H)	7.70 (m, 5H)	2.64 (s, 3H)	4.75 (t, 2H)	3.16 (m, 4H)
41	3	Ph	Ph	Ph	7.00–8.20 (m, 30H, ArH)			7.00–8.20 (m, 30H, ArH)			4.77 (t, 2H)	3.16 (m, 4H)

*In all compounds **34–41**, the 3,5-protons of the pyridinium moieties appear as sharp singlets at 7.60–7.70 ppm, except for the triphenyl-substituted derivative (**37** and **41**) where they appeared at 8.10 ppm.

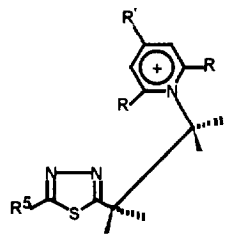
pyridinium ring, which favors the conformation **50** illustrated below (also evidenced previously for the related histamine-pyridium derivatives) [19].

These effects are not seen for the pyridinium propyl derivatives **38–41**. In **34–41** the $^+NCH_2$ signals appear as triplets at 4.65–4.95 ppm, whereas the $(CH_2)_{n-1}$ signals occur as triplets for **34–37** and multiplets for **38–41**, at 3.00–3.25 ppm.

Biochemistry and bioassays

All new compounds prepared in this study, **2**, **4**, and **26–33** were assayed as CA activators, using purified bovine CA isozyme II in the concentration range 10^{-9}–10^{-4} M as described by Maren [20].

Only compounds **2**, **4**, and **26–33**, which possess a free ω-amino alkyl side chain, were highly effective CA activators (table IV). Other derivatives demonstrated no increase in CA activity. As seen from the above data, amines **2** and **4** are strong activators of isozyme II, following the general formula of other known activators previously described [7]. It seems that the polymethylene bridge between the NH_2 group and the heterocyclic moiety may contain either two or three



50

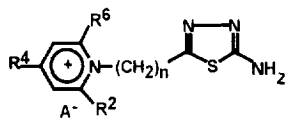
carbon atoms, in order to generate powerful activators. This is also true for the positively charged derivatives **26–33**.

As for CA inhibitors which possess substituted pyridinium moieties in their structure [21], biological activity is highly influenced by the nature of the substituents at the pyridinium ring. Thus, the presence of methyl or *t*-butyl groups in the 2- and 6-positions of the positively-charged activators, generally leads to strong activities, whereas 2,6-diphenylation leads to compounds with poor activity. This may be due to the fact that polyphenyl derivatives are too bulky and cannot easily be accommodated within the enzyme active site. On the other hand, those containing 2,6-dimethyl-4-phenylpyridinium moieties, ie, **28** and **32**, are highly active. The same situation was previously reported for CA inhibitors derivatives of 5-amino-1,3,4-thiadiazolesulfonamide possessing the same substituted pyridinium moieties [21]. In the more recent case, the 5-(2,6-dimethyl-4-phenyl)pyridinium derivative was one of the most powerful inhibitors of this class [22]. This observation has also been rationalized theoretically, in several QSAR studies on both CA inhibitors [22] and activators [23].

It therefore appears likely that these compounds are able to form enzyme–activator complexes, and, by means of their amino groups, to shuttle protons between the active site and the environment.

The presence of positive charges within these activators might favor the formation of such complexes by means of interactions with negatively-charged amino acid side chains within the active site cavity. On the other hand, this salt-like character suggests that these derivatives will poorly penetrate the plasma membranes (data not shown: CT Supuran and TH Maren, unpublished results) and may therefore provide some selectivity for the membrane-bound CA isozymes CA IV or the mitochondrial one CA V, without affecting the cytosolic isozymes. A few compounds have been reported to possess selectivity for certain CA isozymes, both among inhibitors as well as activators of these enzymes [24].

Table III. ^{1}H-NMR spectra of compounds **42–49**, at 100 MHz in trifluoroacetic acid (TFA).



42–45: $n = 2$
46–49: $n = 3$

Compound	*n*	R^2	R^4	R^6	R^2	R^4	R^6	*3,5-H*	N^+-CH_2	$(CH_2)_{n-1}$
42	2	Me	Me	Me	2.73 (s, 3H)	2.57 (s, 3H)	2.73 (s, 3H)	7.60 (s,2H)	4.77 (t, 2H)	3.20 (t, 2H)
43	2	*t*Bu	Me	*t*Bu	1.02 (s, 9H)	2.65 (s, 3H)	1.02 (s, 9H)	7.60 (s, 2H)	4.78 (t, 2H)	3.18 (t, 2H)
44	2	Me	Ph	Me	2.72 (s, 3H)	7.70 (m, 5H)	2.72 (s, 3H)	7.60 (s, 2H)	4.66 (t,2H)	3.15 (t,2H)
45	2	Ph	Ph	Ph	7.00–8.20 (m, 15H, ArH)			8.15 (s, 2H)	4.94 (t, 2H)	2.95 (t, 2H)
46	3	Me	Me	Me	2.72 (s, 3H)	2.57 (s, 3H)	2.72 (s, 3H)	7.60 (s, 2H)	4.75 (t, 2H)	3.16 (m, 4H)
47	3	Bu	Me	Bu	1.05 (s, 9H)	2.66 (s, 3H)	1.05 (s, 9H)	7.60 (s, 2H)	4.76 (t,2H)	3.15 (m,4H)
48	3	Me	Ph	Me	2.72 (s, 3H)	7.70 (m, 5H)	2.72 (s, 3H)	7.60 (s, 2H)	4.75 (t, 2H)	3.15 (m, 4H)
49	3	Ph	Ph	Ph	7.00–8.20 (m, 15H, ArH)			7.70 (s, 2H)	4.77 (t, 2H)	3.15 (m, 4H)

Conclusion

The synthesis and characterization of novel heterocyclic derivatives of 2-amino-5-(2-aminoethyl)-1,3,4-thiadiazole and 2-amino-5-(3-aminopropyl)-1,3,4-thiadiazole, as well as their reactions with 2,4,6-trisubstituted pyrylium salts, have been reported. The different reactivities of the two amino groups within the title compounds allowed us to obtain several series of derivatives, which proved to possess divergent biological activities. Derivatives possessing free ω-aminoalkyl side-chains were effective activators of isozyme CA II. Due to their membrane-impermeant profile, they may show selectivity towards membrane-bound CA isozymes.

Experimental protocols

Chemistry

Melting points were determined on a heating stage microscope-type apparatus and were not corrected. IR spectra were recorded in KBr pellets, with a Beckman 4260 instrument; ^{1}H-NMR spectra were recorded with a Varian EM 360 L spectrometer or a Bruker C&P 100 spectrometer, using trifluoracetic acid (TFA) as solvent. Chemical shifts are reported as δ values, relative to Me_4Si which served as an internal standard. Elemental analyses (C, H, N) were performed by microcombustion, with an automatic Carlo Erba analyzer. β-Alanine, γ-aminobutyric acid, thiosemicarbazide, phthalic anhydride, $SOCl_2$ and hydrazinium hydroxide (80%) were obtained from Merck Chemical Co and were used without further purification. Pyrylium salts (**14–17**) were prepared by literature procedures, generally by bisacylation of alkenes or their precursors, as originally described by Balaban and Nenitzescu [25]. Bovine red blood cell carbonic anhydrase (isozyme II) was obtained from Sigma Chemical Co (St Louis, MO, USA).

*Preparation of 1-(2-phthalimidopropionyl)- and 1-(3-phthalimidobutiryl)thiosemicarbazide **10** and **11***

β-Alanine or GABA (20 mmol) and phthalic anhydride (20 mmol) were suspended in 150 mL dry toluene and refluxed under Dean–Stark conditions until water was separated (generally 5–6 h). The solvent was evaporated in vacuo, the residue dissolved in 50 mL anhydrous benzene and treated with a two- or threefold excess of $SOCl_2$. The solution was refluxed until no more SO_2 and HCl evolved. The solvent and excess $SOCl_2$ were distilled in vacuo and the acid chlorides **7** and **8** were treated with 21 mmol thiosemicarbazide **9** in pyridine (100 mL) as a solvent, at 0 °C for 3 h. After standing overnight, the mixture was poured into 800 mL water and ice, and the white precipitate thus obtained was filtered, washed with 500 mL aqueous acetic acid solution (50%, v/v), and then with water. The products were recrystallized from acetic acid, generating white needles with yields of 80–85%. Compound **7** melted at 240 °C (lit [12] mp 238–239 °C), and **8** melts at 253 °C (dec).

*Preparation of 2-amino-5-(2-phthalimidoethyl)- and 2-amino-5-(3-phthalimidopropyl)-1,3,4-thiadiazole **12** and **13***

Compound **10** or **11** (15 mmol) was dissolved (while stirring) in 50 mL of 98% H_2SO_4 then the solution was warmed to 100 °C for 15 min. After cooling, the solution was poured over ice and neutralized with 30% NaOH solution to pH = 7.5. The precipitate obtained was filtered and washed with 800 mL water (in order to remove the coprecipitated Na_2SO_4), and recrystallized from dioxane/water (50%, v/v). Mp 231 °C (dec) (lit [12] mp 226–227 °C) for **12**, and 238 °C (dec) for **13**.

Table IV. CA II activation data for compounds **2**, **4**, **26–33**, at a concentration of activator of 10^{-5} M ($[E_0] = 5 \times 10^{-10}$ M).

Compound	*% CA activity[a] (mean ± SE)*
2	158 ± 3
4	157 ± 4
26	164 ± 5
27	172 ± 3
28	184 ± 5
29	108 ± 4
30	161 ± 3
31	169 ± 6
32	178 ± 5
33	105 ± 3

[a]Control CA activity in the absence of activator was taken as 100%. The data represent the mean ± standard error, determined from five different assays.

*General procedure for the preparation of compounds **18–25***

Compounds **12** or **13** (3 mmol) and 3.1 mmol of the appropriate pyridinium salt **14–18** were suspended in 50 mL absolute ethanol and refluxed for 3 h. After cooling, 1 g active charcoal was added and refluxing continued for another 15 min. The filtered solution was treated with 1 mL conc ammonia, extracted with 2 × 30 mL diethylether, evaporated to dryness, and the crude products recrystallized from acetic acid or ethanol. Yields were 60–85%.

*1-[5-(2-Phthalimidoethyl)-1,3,4-thiadiazol-2-yl]-2,4,6-trimethylpyridinium tetrafluoroborate **18***. Mp 282–284 °C (dec), from AcOH, IR (KBr), cm^{-1}: 609, 770, 1090, 1310, 1480, 1610, 1700, 2980 (underlined bands are due to the anion). ^{1}H-NMR (TFA), ppm: 2.43 (s, 3H), 2.69 (s, 3H), 3.28 (m, 4H), 7.20 (m, 4H), 7.60 (s, 2H). Anal $C_{20}H_{19}N_4O_2S^+ \cdot BF_4^-$ (C, H, N).

*1-[5-(2-Phthalimidoethyl)-1,3,4-thiadiazol-2-yl]-2,6-di-tert-butyl-4-methylpyridinium perchlorate **19***. Mp 252–254 °C (dec), from AcOH. IR (KBr), cm^{-1}: 625, 660, 760, 1100, 1320, 1485, 1610, 1700, 2980. 1H-NMR (TFA), ppm: 1.58 (s, 9H), 2.70 (s, 3H), 3.27 (m, 4H), 7.20 (m, 4H), 7.60 (s, 2H). Anal $C_{26}H_{31}N_4O_2S^+ \cdot ClO_4^-$ (C, H, N).

*1-[5-(2-Phthalimidoethyl)-1,3,4-thiadiazol-2-yl]-2,6-dimethyl-4-phenylpyridinium perchlorate **20***. Mp 293–295 °C (dec), from AcOH. IR (KBr), cm^{-1}: 625, 690, 795, 1100, 1340, 1485, 1605, 1710, 2900. ^{1}H-NMR (TFA), ppm: 2.42 (s, 3H), 3.30 (m, 4H), 7.10 (m, 4H), 7.60 (s, 2H), 7.68 (m, 5H). Anal $C_{25}H_{35}N_4O_2S^+ \cdot ClO_4^-$ (C, H, N).

*1-[5-(2-Phthalimidoethyl)-1,3,4-thiadiazol-2-yl]-2,4,6-triphenylpyridinium perchlorate **21***. Mp 311–313 °C (dec), from

AcOH. IR (KBr), cm^{-1}: 600, 625, 680, 770, 995, 1100, 1450, 1480, 1610, 1700, 2970. ^{1}H-NMR (TFA), ppm: 3.15 (m, 4H), 7.20 (m, 4H), 7.70 (s, 2H), 7.00–8.30 (m, 15H, ArH). Anal $C_{35}H_{25}N_4O_2S^+ \cdot ClO_4^-$ (C, H, N).

1-[5-(3-Phthalimidopropyl)-1,3,4-thiadiazol-2-yl]-2,4,6-trimethylpyridinium tetrafluoroborate ***22***. Mp 276–279 °C (dec), from AcOH. IR (KBr), cm^{-1}: 610, 760, 1090, 1315, 1480, 1600, 1615, 1700, 2985. ^{1}H-NMR (TFA), ppm: 2.42 (s, 3H), 2.69 (s, 3H), 3.08 (m, 6H), 7.20 (m, 4H), 7.60 (s, 2H). Anal $C_{21}H_{21}N_4O_2S^+ \cdot BF_4^-$ (C, H, N).

1-[5-(3-Phthalimidopropyl)-1,3,4-thiadiazol-2-yl]-2,6-di-tert-butyl-4-methylpyridinium perchlorate ***23***. Mp 246–248 °C (dec), from AcOH. IR (KBr), cm^{-1}: 625, 650, 810, 1100, 1315, 1485, 1610, 1705, 2980. ^{1}H-NMR (TFA), ppm: 1.59 (s, 9H), 2.70 (s, 3H), 3.10 (m, 6H), 7.20 (m, 4H), 7.60 (s, 2H). Anal $C_{27}H_{33}N_4O_2S^+ \cdot ClO_4^-$ (C, H, N).

1-[5-(3-Phthalimidopropyl)-1,3,4-thiadiazol-2-yl]-2,6-dimethyl-4-phenylpyridinium perchlorate ***24***. Mp 264–269 °C (dec), from AcOH. IR (KBr), cm^{-1}: 625, 685, 890, 950, 1100, 1345, 1470, 1600, 1710, 2890. ^{1}H-NMR (TFA), ppm: 2.42 (s, 3H), 3.10 (m, 6H), 7.20 (m, 4H), 7.60 (s, 2H), 7.68 (m, 5H). Anal $C_{26}H_{23}N_4O_2S^+ \cdot ClO_4^-$ (C, H, N).

1-[5-(3-Phthalimidopropyl)-1,3,4-thiadiazol-2-yl]-2,4,6-triphenylpyridinium perchlorate ***25***. Mp 295–296 °C (dec), from EtOH. IR (KBr), cm^{-1}: 625, 675, 770, 990, 1100, 1440, 1485, 1590, 1610, 1700, 2975. ^{1}H-NMR (TFA), ppm: 3.05 (m, 6H), 7.20 (m, 4H), 7.70 (s, 2H), 7.00–8.35 (m, 15H, ArH). Anal $C_{36}H_{27}N_4O_2S^+ \cdot ClO_4^-$ (C, H, N).

Hydrazinolysis of compounds ***18–25***.
Compound **18–25** (3 mmol) was dissolved in 80 mL ethanol and 3.5 mL hydrazinium hydroxide was added. The mixture was refluxed for 3 h, and the solvent was evaporated in vacuo. A 2 N solution of HCl was added in a slight excess, the phthalhydrazide which precipitated was filtered and the free amine was obtained after evaporation (in vacuo) of the solvent. Pure derivatives **26–33** were obtained after recrystallization as described for individual compounds below.

1-[5-(2-Aminoethyl)-1,3,4-thiadiazol-2-yl]-2,4,6-trimethylpyridinium tetrafluoroborate ***26***. Mp 162–163 °C (dec), from AcOH. IR (KBr), cm^{-1}: 610, 770, 1090, 1310, 1485, 1535, 2985, 3390. ^{1}H-NMR (TFA), ppm: 2.43 (s, 3H), 2.68 (s, 3H), 3.20 (t, 2H), 3.90 (m, 2H), 7.60 (s, 2H). Anal $C_{12}H_{17}N_4O_2S^+ \cdot BF_4^-$ (C, H, N).

1-[5-(2-Aminoethyl)-1,3,4-thiadiazol-2-yl]-2,6-di-tert-butyl-4-methylpyridinium perchlorate ***27***. Mp 175–177 °C (dec), from EtOH. IR (KBr) cm^{-1}: 625, 660, 820, 1100, 1335, 1485, 2980, 3380. ^{1}H-NMR (TFA), ppm: 1.58 (s, 9H), 2.70 (s, 3H), 3.21 (t, 2H), 3.84 (m, 2H), 7.60 (s, 2H). Anal $C_{18}H_{29}N_4O_2S^+ \cdot ClO_4^-$ (C, H, N).

1-[5-(2-Aminoethyl)-1,3,4-thiadiazol-2-yl]-2,6-dimethyl-4-phenylpyridinium perchlorate ***28***. Mp 193–195 °C (dec), from EtOH. IR (KBr), cm^{-1}: 625, 680, 790,890, 945, 1100, 1340, 1480, 2900, 3385. ^{1}H-NMR (TFA), ppm: 2.42 (s, 3H), 3.18 (t, 2H), 3.90 (m, 2H), 7.60 (s, 2H), 7.70 (m, 5H). Anal $C_{17}H_{19}N_4O_2S^+ \cdot ClO_4^-$ (C, H, N).

1-[5-(2-Aminoethyl)-1,3,4-thiadiazol-2-yl]-2,4,6-triphenylpyridinium perchlorate ***29***. Mp 211–213 °C (dec), from EtOH. IR (KBr), cm^{-1}: 625, 680, 775, 990, 1100, 1450, 1485, 1590, 2970, 3390. ^{1}H-NMR (TFA), ppm: 3.05 (t, 2H), 3.90 (m, 2H), 7.76 (s, 2H), 7.00–8.30 (m, 15H, ArH). Anal $C_{27}H_{23}N_4O_2S^+ \cdot ClO_4^-$ (C, H, N).

1-[5-(3-Aminopropyl)-1,3,4-thiadiazol-2-yl]-2,4,6-trimethylpyridinium tetrafluoroborate ***30***. Mp. 177–179 °C (dec), from EtOH. IR (KBr), cm^{-1}: 609, 770, 1090, 1310, 1480, 2980, 3395. ^{1}H-NMR (TFA), ppm: 2.43 (s, 3H), 2.68 (s, 3H), 3.18 (m, 4H), 3.85 (m, 2H), 7.60 (s, 2H). Anal $C_{13}H_{19}N_4O_2S^+ \cdot BF_4^-$ (C, H, N).

1-[5-(3-Aminopropyl)-1,3,4-thiadiazol-2-yl]-2,6-di-tert-butyl-4-methylpyridinium perchlorate ***31***. Mp 164–166 °C (dec), from EtOH. IR (KBr), cm^{-1}: 625, 660, 790, 1100, 1320, 1485, 2980, 3390. ^{1}H-NMR (TFA), ppm: 1.58 (s, 9H), 2.70 (s, 3H), 3.17 (m, 4H), 3.84 (m, 2H), 7.60 (s, 2H). Anal $C_{19}H_{31}N_4O_2S^+ \cdot ClO_4^-$ (C, H, N).

1-[5-(3-Aminopropyl)-1,3,4-thiadiazol-2-yl]-2,6-dimethyl-4-phenylpyridinium perchlorate ***32***. Mp 188–190 °C (dec), from EtOH. IR (KBr), cm^{-1}: 625, 680, 880, 950, 1100, 1340, 1480, 2890, 3395. ^{1}H-NMR (TFA), ppm: 2.42 (s, 3H), 3.17 (m, 4H), 3.85 (m, 2H), 7.60 (s, 2H), 7.70 (m, 5H). Anal $C_{18}H_{21}N_4O_2S^+ \cdot ClO_4^-$ (C, H, N).

1-(5-(3-Aminopropyl)-1,3,4-thiadiazol-2-yl]-2,4,6-triphenylpyridinium perchlorate ***33***. Mp 191–193 °C (dec), from EtOH. IR (KBr), cm^{-1}: 625, 670, 775, 980, 1100, 1390, 1445, 1485, 1590, 2975, 3400. ^{1}H-NMR (TFA), ppm: 3.16 (m, 4H), 3.80 (m, 2H), 7.70 (s, 2H), 7.00–8.35 (m, 15H, ArH). Anal $C_{28}H_{25}N_4O_2S^+ \cdot ClO_4^-$ (C, H, N).

General procedure for the preparation of compounds ***34–41***
Method A. The compound **26–33** (2 mmol) and 2 mmol of the appropriate pyrylium salt **14–17** (as the perchlorate or tetrafluoroborate salt) were suspended in 50 mL ethanol and refluxed for 2 h. Upon cooling, compound **34–41** precipitated, was filtered and recrystallized from acetic acid or aqueous perchloric acid. Yields were over 80%.

Method B. The diamine **2** or **4** (2 mmol) was dissolved in 50 mL methanol and 4 mmol of pyrylium salt **14–17** was added. The mixture was refluxed for 2 h and thereafter was worked up as described above.

2-(2,4,6-Trimethylpyridinium-1-yl)-5-[2-(2,4,6-trimethylpyridinium-1-yl)ethyl]-1,3,4-thiadiazole bis(tetrafluoroborate) ***34***. Mp 307–309 °C (dec), from AcOH. IR (KBr), cm^{-1}: 609, 770, 1015, 1090, 1480, 1535, 2985. ^{1}H-NMR (TFA), ppm: 2.43 (s, 3H), 2.57 (s, 3H), 2.69 (s, 3H), 2.71 (s, 3H), 3.25 (t, 2H), 4.78 (t, 2H). Anal $C_{20}H_{26}N_4O_2S^{2+} \cdot 2BF_4^-$ (C, H, N).

2-(2,6-Ditertbutyl-4-methylpyridinium-1-yl)-5-[(2,6-ditertbutyl-4-methylpyridinium-1-yl)ethyl]-1,3,4-thiadiazolbis(perchlorate) ***35***. Mp 299–300 °C (dec), from AcOH. IR (KBr), cm^{-1}: 625, 665, 760, 1100, 1375, 1400, 1475, 2980. ^{1}H-NMR (TFA), ppm: 1.05 (s, 9H), 1.58 (s, 9H), 2.65 (s, 3H), 2.68 (s, 3H), 3.20 (t, 2H), 4.80 (t, 2H). Anal $C_{23}H_{29}N_4O_2S^{2+} \cdot 2ClO_4^-$ (C, H, N).

2-(2,6-Dimethyl-4-phenylpyridinium-1-yl)-5-[(2,6-dimethyl-4-phenylpyridinium-1-yl)ethyl]-1,3,4-thiadiazolebis(perchlorate) ***36***. Mp > 350 °C, from AcOH. IR (KBr), cm^{-1}: 580, 625, 695, 790, 880, 950, 1100, 1380, 1480, 1590, 2900. ^{1}H-NMR (TFA), ppm: 2.42 (s, 3H), 2.72 (s, 3H), 3.18 (t, 2H), 4.65 (t, 2H), 7.70 (m, 5H). Anal $C_{22}H_{19}N_4O_2S^{2+} \cdot 2ClO_4^-$ (C, H, N).

2-(2,4,6-Triphenylpyridinium-1-yl)-5-[(2,4,6-triphenylpyridinium-1-yl)ethyl]-1,3,4-thiadiazole bis(perchlorate) ***37***. Mp > 350 °C (dec), from AcOH. IR (KBr), cm^{-1}: 480, 625, 680, 675, 995, 1100, 1450, 1480, 1595, 2880. ^{1}H-NMR (TFA), ppm: 3.00 (t, 2H), 4.95 (t, 2H), 7.00–8.30 (m, 30H, ArH). Anal $C_{32}H_{23}N_4O_2S^{2+}\cdot 2ClO_4^-$ (C, H, N).

2-(2,4,6-Trimethylpyridinium-1-yl)-5-[(2,4,6-trimethylpyridinium-1-yl)propyl]-1,3,4-thiadiazole bis(tetrafluoroborate) ***38***. Mp 303–305 °C (dec), from AcOH. IR (KBr), cm^{-1}: 610, 750, 780, 1010, 1090, 1475, 1530, 2985. ^{1}H-NMR (TFA), ppm: 2.43 (s, 3H), 2.56 (s, 3H), 2.66 (s, 3H), 2.68 (s, 3H), 3.17 (m, 4H), 4.75 (t, 2H). Anal $C_{21}H_{28}N_4O_2S^{2+}\cdot 2BF_4^-$ (C, H, N).

2-(2,6-Di-tert-butyl-4-methylpyridinium-1-yl)-5-[(2,6-di-tert-butyl-4-methylpyridinium-1-yl)propyl]-1,3,4-thiadiazole bis(perchlorate) ***39***. Mp 308–309 °C (dec), from AcOH. IR (KBr), cm^{-1}: 600, 625, 665, 770, 1100, 1380, 1475, 1540, 2985. ^{1}H-NMR (TFA), ppm: 1.03 (s, 9H), 1.58 (s, 9H), 2.65 (s, 3H), 2.69 (s, 3H), 3.15 (m, 4H), 4.77 (t, 2H). Anal $C_{24}H_{31}N_4O_2S^{2+}\cdot 2ClO_4^-$ (C, H, N).

2-(2,6-Dimethyl-4-phenylpyridinium-1-yl)-5-[3-(2,6-dimethyl-4-phenylpyridinium-1-yl)propyl]-1,3,4-thiadiazol bis(perchlorate) ***40***. Mp 346–348 °C (dec), from AcOH. IR (KBr), cm^{-1}: 625, 690, 790, 875, 940, 1100, 1390, 1475, 2900. ^{1}H-NMR (TFA), ppm: 2.43 (s, 3H), 2.64 (s, 3H), 3.16 (m, 4H), 4.75 (t, 2H), 7.70 (m, 5H). Anal $C_{23}H_{21}N_4O_2S^{2+}\cdot 2ClO_4^-$ (C, H, N).

2-(2,4,6-Triphenylpyridinium-1-yl)-5-[3-(2,4,6-triphenylpyridinium-1-yl)propyl]-1,3,4-thiadiazol bis(perchlorate) ***41***. Mp > 350 °C (dec), from AcOH. IR (KBr), cm^{-1}: 480, 625, 680, 770, 985, 1100, 1450, 1475, 1590, 2890. ^{1}H-NMR (TFA), ppm: 3.16 (m, 4H), 4.77 (t, 2H), 7.00–8.20 (m, 30H, ArH). Anal $C_{33}H_{25}N_4O_2S^{2+}\cdot 2ClO_4^-$ (C, H, N).

General procedure for the preparation of compounds ***42–49***
The amine **2** or **4** (2 mmol) was suspended in 30 mL freshly distilled CH_2Cl_2, and 2 mmol pyrylium salt **14–17** was added while maintaining the temperature at **10–15** °C (cooling with an ice-water bath if necessary). The mixture was evaporated and the crude product was recrystallized from acetic acid or ethanol. Yields were 70–85%.

2-Amino-5-(3-aminopropyl)-1,3,4-thiadiazole ***4***. Mp 230–233 °C (dec). IR (KBr), cm^{-1}: 680, 770, 985, 1100, 1450, 1475, 1590, 2890. ^{1}H-NMR (TFA), ppm : 3.06 (m, 4H), 4.87 (t, 2H). Anal $C_5H_{10}N_4O_2\cdot 2HCl$ (C, H, N).

1-[(5-Amino-1,3,4-thiadiazol-2-yl)ethyl]-2,4,6-trimethylpyridinium tetrafluoroborate ***42***. Mp 234–236 °C (dec), from EtOH. IR (KBr), cm^{-1}: 609, 670, 820, 1090, 1320, 1480, 1535, 2985, 3395. 1H-NMR (TFA), ppm: 2.57 (s, 3H), 2.73 (s, 3H), 3.20 (t, 2H), 4.77 (t, 2H), 7.60 (s, 2H). Anal $C_{12}H_{17}N_4O_2S^+\cdot BF_4^-$ (C, H, N).

1-[(5-Amino-1,3,4-thiadiazol-2-yl)ethyl]-2,6-di-tert-butyl-4-methylpyridinium perchlorate ***43***. Mp 212–215 °C (dec), from EtOH. IR (KBr), cm^{-1}: 625, 640, 810, 885, 1100, 1340, 1480, 2985, 3395. ^{1}H-NMR (TFA), ppm: 1.02 (s, 9H), 2.65 (s, 3H), 3.18 (t, 2H), 4.78 (m, 2H), 7.60 (s, 2H). Anal $C_{18}H_{29}N_4O_2S^+\cdot ClO_4^-$ (C, H, N.

1-[(5-Amino-1,3,4-thiadiazol-2-yl)ethyl]-2,6-dimethyl-4-phenylpyridinium perchlorate ***44***. Mp 239–241 °C (dec), from EtOH. IR (KBr), cm^{-1}: 625, 675, 770, 830, 880, 950, 1100, 1335, 1475, 2895, 3390. 1H-NMR (TFA), ppm: 2.72 (s, 3H), 3.15 (t, 2H), 4.66 (t, 2H), 7.60 (s, 2H), 7.70 (m, 5H). Anal $C_{19}H_{19}NO_2S^+\cdot ClO_4^-$ (C, H, N).

1-[(5-Amino-1,3,4-thiadiazol-2-yl)ethyl]-2,4,6-triphenylpyridinium perchlorate ***45***. Mp. 254–257 °C (dec), from EtOH. IR (KBr), cm^{-1}: 600, 625, 680, 770, 980, 1100, 1445, 1475, 1580, 3395. ^{1}H-NMR (TFA), ppm: 2.95 (t, 2H), 4.94 (t, 2H), 8.15 (s, 2H), 7.00–8.20 (m, 15H, ArH). Anal $C_{27}H_{23}N_4O_2S^+\cdot ClO_4^-$ (C, H, N).

1-[(5-Amino-1,3,4-thiadiazol-2-yl)propyl]-2,4,6-trimethylpyridinium tetrafluoroborate ***46***. Mp. 241–243 °C (dec), from EtOH. IR (KBr), cm^{-1}: 610, 750,810, 930, 1100, 1320, 1480, 2985, 3400. ^{1}H-NMR (TFA), ppm: 2.57 (s, 3H), 2.72 (s, 3H), 3.16 (m, 4H), 4.75 (t, 2H), 7.60 (s, 2H). Anal $C_{13}H_{19}N_4O_2S^+\cdot BF_4^-$ (C, H, N).

1-[(5-Amino-1,3,4-thiadiazol-2-yl)propyl]-2,6-di-tert-butyl-4-methylpyridinium perchlorate ***47***. Mp 197–198 °C (dec), from EtOH. IR (KBr), cm^{-1}: 625, 650, 760, 930, 1100, 1315, 1480, 2985, 3400. ^{1}H-NMR (TFA), ppm: 1.05 (s, 9H), 2.66 (s, 3H), 3.15 (m, 4H), 4.76 (t, 2H), 7.60 (s, 2H). Anal $C_{19}H_{31}O_2S^+\cdot ClO_4^-$ (C, H, N).

1-[(5-Amino-1,3,4-thiadiazol-2-yl-propyl]-2,6-dimethyl-4-phenylpyridinium perchlorate ***48***. Mp 212–215 °C (dec), from EtOH. IR (KBr), cm^{-1}: 625, 650, 760, 930, 1100, 1315, 1480, 2895, 3400. ^{1}H-NMR (TFA), ppm: 2.72 (s, 3H), 3.15 (m, 4H), 4.75 (t, 2H), 7.60 (s, 2H), 7.70 (m, 5H). Anal $(C_{18}H_{21}N_4O_2S^+\cdot ClO_4^-$ (C, H, N).

1-[(5-Amino-1,3,4-thiadiazol-2-yl)propyl]-2,4,6-triphenylpyridinium perchlorate ***49***. Mp 251–254 °C (dec), from EtOH. IR (KBr), cm^{-1}: 625, 675, 750, 990, 1100, 1385, 1450, 1475, 1585, 3400. ^{1}H-NMR (TFA), ppm: 3.15 (m, 4H), 4.77 (t, 2H), 7.70 (s, 2H), 7.00–8.20 (m, 15H, ArH). Anal $C_{28}H_{25}N_4O_2S^+\cdot ClO_4^-$ (C, H, N).

Assay of biological activity of the new compounds

The activity of all derivatives reported here against bovine CA II was studied by the micromethod of Maren [20], working at 0 °C. Stock solutions of activator (10^{-3} M) were prepared in distilled deionized water (with the addition of 10–20% (v/v) DMSO for poorly soluble derivatives). Dilutions were obtained thereafter (with distilled deionized water) until 10^{-9} M was reached. In a special CO_2 bubbler cell, 0.3 mL distilled water was added, followed by 0.4 mL phenol red indicator solution (1%), 0.1 mL activator solution and 0.1 mL of the enzyme solution (2 enzyme units). The hydration reaction was initiated by addition of 0.1 mL barbital buffer. The time to obtain a color change was recorded with a stopwatch. Enzyme-specific activity was then calculated as described by Maren [20].

References

1 Part 13 of this series: Coltau M, Puscas I, Supuran CT (1994) *Rev Roum Chim* 34, 457–462
2 Maren TH (1967) *Physiol Rev* 47, 595–781
3 Supuran CT (1993) *Roum Chem Quart Rev* 1, 77–116
4 Sugrue MF (1989) *Pharm Ther* 43, 91–133

5 Woodbury DM (1980) Inhibitors of carbonic anhydrase. In: *Antiepileptic Drugs. Mechanisms of Action* (Glaser GH, Perry JK, Woodbury DM, eds) Raven, New York, 617–633
6 Puscas I (1984) *Ann NY Acad Sci* 429, 587–591
7 Puscas I, Supuran CT, Manole G (1990) *Rev Roum Chim* 35, 683–688
8 Rowlett RS, Gargiulo NJ, III, Santoli FA, Jackson M, Corbett H (1991) *J Biol Chem* 266, 933–945
9 Supuran CT (1992) *Rev Roum Chim* 37, 411–421
10 Silverman DN, Lindskog S (1988) *Acc Chem Res* 21, 30–36
11 Supuran CT, Baciu I, Balaban AT (1993) *Rev Roum Chim* 38, 725–731
12 Otha M (1952) *J Pharm Soc Jpn* 72, 1636–1639
13 Grechishkin LL, Gavrovskaya LK, Goldfarb VL (1978) US Patent 885,367
14 Supuran CT, Puscas I (1994) Carbonic anhydrase activators. In: *Carbonic Anhydrase and Modulation of Physiologic and Pathologic Processes in the Organism*, Helicon Publishing House, Timisoara, Roumania, 107–139
15 Gabriel S (1908) *Ber Dtsch Chem Ges* 41, 242–248
16 Ainsworth C, Jones RG (1953) *J Am Chem Soc*75, 4915–4918
17 Balaban AT, Dinculescu A, Dorofeenko GN et al (1982) Pyrylium salts; syntheses, reactions and physical properties. In: *Adv Heterocycl Chem* (Katritzky AR, ed) Academic Press, New York, 14–280
18 Balaban AT, Dinculescu A, Kontrakis HN, Chiraleu F (1979) *Tetrahedron Lett* 437–440
19 Dinculescu A, Balaban AT (1980) *Rev Roum Chim* 25, 1505–1528
20 Maren TH (1960) *J Pharmacol Exp Ther* 130, 26–30
21 Supuran CT, Dinculescu A, Manole G et al (1992) *J Pharm Sci* 81, 716–719
22 Supuran CT, Clare BW (1995) *Eur J Med Chem* 30, 687–696
23 Supuran CT, Clare BW (1994) *J Pharm Sci* 83, 768–773
24 Supuran CT, Puscas I (1994) *Roum Chem Quart Rev* 2, 313–341
25 Balaban AT, Nenitzescu CD (1973) *Org Synth Coll*, Vol 5, 1106–1108