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Carbonic anhydrase inhibitors: aromatic and heterocyclic sulfonamides incorporating adamantyl moieties with strong anticonvulsant activity

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Abstract—A series of aromatic/heterocyclic sulfonamides incorporating adamantyl moieties were prepared by reaction of aromatic/heterocyclic aminosulfonamides with the acyl chlorides derived from adamantyl-1-carboxylic acid and 1-adamantyl-acetic acid. Related derivatives were obtained from the above-mentioned aminosulfonamides with adamantyl isocyanate and adamantyl isothiocyanate, respectively. Some of these derivatives showed good inhibitory potency against two human CA isozymes involved in important physiological processes, CA I, and CA II, of the same order of magnitude as the clinically used drugs acetazolamide and methazolamide. The lipophilicity of the best CA inhibitors was determined and expressed as their experimental $\log k'$ IAM and theoretical ClogP value. Their lipophilicity was propitious with the crossing of the blood-brain barrier ($\log k'$ IAM > 1.35). The anticonvulsant activity of some of the best CA inhibitors reported here has been evaluated in a MES test in mice. After intraperitoneal injection (30 mg kg^{-1}), compounds **A8** and **A9** exhibited a high protection against electrically induced convulsions (>90%). Their ED_{50} was 3.5 and 2.6 mg kg^{-1}, respectively.

1. Introduction

Epilepsy is a chronic neurological disorder characterized by seizures generated by the sudden, massive, synchronous excitation of neurons in the brain. In general, the amplitude of seizure can range from brief cessation of responsiveness (absence seizure) to severe tonic-clonic muscle spasms with a loss of consciousness (general seizure).[1–3] Several generations of anticonvulsants are available, such as phenytoin, phenobarbital, benzodiazepines, etosuximide, carbamazepine, and valproic acid (valproate) among others. Their use was also proved useful in the treatment of other diseases such as neuropathic pain and bipolar disorders, conditions associated with significant morbidity and mortality.[4–8] The newest generation of anticonvulsants, including vigabatrin, lamotrigine, gabapentin, tiagabine, topiramate, felbamate, zonisamide, or oxcarbazepine, represents a step forward toward better anticonvulsant drugs.[9–12] Nowadays the marketed anticonvulsants are estimated to be effective in approximatively 90% of the epileptic patients. However, their use is associated with dose-related toxicity and idiosyncratic side effects, and the presently available drugs should thus be optimized.[9–12]

Beside its ability to block the voltage Na^+-channel, to potentiate GABAergic transmission and to block the kainate/AMPA receptor, topiramate **TPM** occupies a

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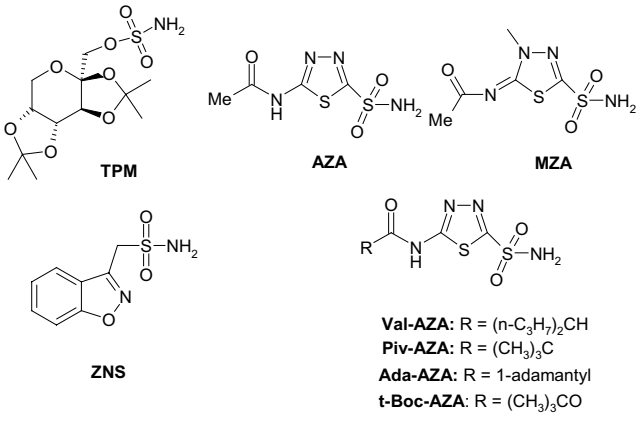
particular place among new anticonvulsants due to its ability to inhibit carbonic anhydrase.[13–20] The enzyme, catalyzing the interconversion of CO_2 and HCO_3^- is quite abundant in the brain, being present in the glia and neurons, mainly as the cytosolic isozymes CA II, CA VII and the membrane-bound isoform CA XIV.[16–18,21] Although their function in the brain was not well established, it is known that these proteins are involved in the secretion of cerebrospinal fluid (CSF), being present in the choroid plexus of vertebrates in high amount.[16–18,21,22] It also has been proven that inhibition of the brain CAs increases the cerebral blood flow with the concomitant raising of the CO_2 partial pressure.[16,22] Kaila and co-workers,[23–26] Rivera et al.,[27] and Grover et al.[28] revealed that CA inhibition might serve as an anticonvulsant mechanism (at least in some forms of epilepsy), taking into account that the contribution of HCO_3^- current to the ionic current through γ-aminobutirate A ($GABA_A$) receptors on dendrites is increased during periods of high-frequency receptor activation. The excitatory effect of HCO_3^- is blocked by membrane-permeant CA inhibitors.[14,25,28]



Classical CA inhibitors (CAIs) such as acetazolamide **AZA** and methazolamide **MZA** have been used for decades as anticonvulsants[18,29a] although they occupy a marginal role in therapy at present.[29b] Generally, the lipophilicity improve brain penetration of the drug, so that methazolamide **MZA** or the lipophilic *tert*-butoxycarbonyl derivative of acetazolamide **tBOC-AZA** are more effective as anticonvulsants than acetazolamide, which is very hydrophilic.[29] A similar effect is observed in the case of zonisamide **ZNS**, a derivative for which the CA inhibitory properties have not been investigate in detail.[18] Considering the interesting anticonvulsant activity of several CAIs, a series of aromatic/heterocyclic sulfonamides incorporating valproyl moieties were prepared recently.[30] The valproyl derivative of acetazolamide (5-valproylamido-1,3,4-thiadiazole-2-sulfonamide) **Val-AZA** was one of the best hCA I and hCA II inhibitors in this series and exhibited very strong anticonvulsant properties in a maximal electroshock seizure (MES) test in mice.[30] It was also observed that some structurally related derivatives such as 5-pivaloylamido-1,3,4-thiadiazole-2-sulfonamide **Piv-AZA**[31] and 5-adamantylcarboxamido-1,3,4-thiadiazole-2-sulfonamide **Ada-AZA**[32] also showed promising in vivo anticonvulsant properties.[30] Considering the last derivative as lead compound, we decided to further investigate this class of compounds. We report here a new series of aromatic/heterocyclic sulfonamides incorporating the adamantyl moiety in their molecule, as well as their CA inhibitory and anticonvulsant properties in the MES test in rats.

2. Results

2.1. Chemistry

The chemical structures of sulfonamides **1**–**9** on which the adamantyl-containing moieties **A**–**D** have been attached are shown below. These new sulfonamides obtained by attaching moieties **A**–**D** of to the amino/imino group of sulfonamides **1**–**9** will be designated as (**A**–**D**) **1**–**9** (Chart 1), as for other such derivatives synthesized by means of the tail approach.[18,33,34]

Two synthetic approaches have been used for the preparation of the new sulfonamides reported here: (i) reaction of 1-adamantyl carboxylic acid or 1-adamantylacetic acid chlorides with sulfonamides **1**–**9** in the presence of a base, as reported previously by this group,[33,34] leading to amides **A1**–**A9** and **B1**–**B9**, and (ii) reaction of adamantyl isocyanate or isothiocyanate with sulfonamides **1**–**9**, leading to derivatives **C1**–**C9** and **D6**, **D7**[35,36] (Scheme 1).

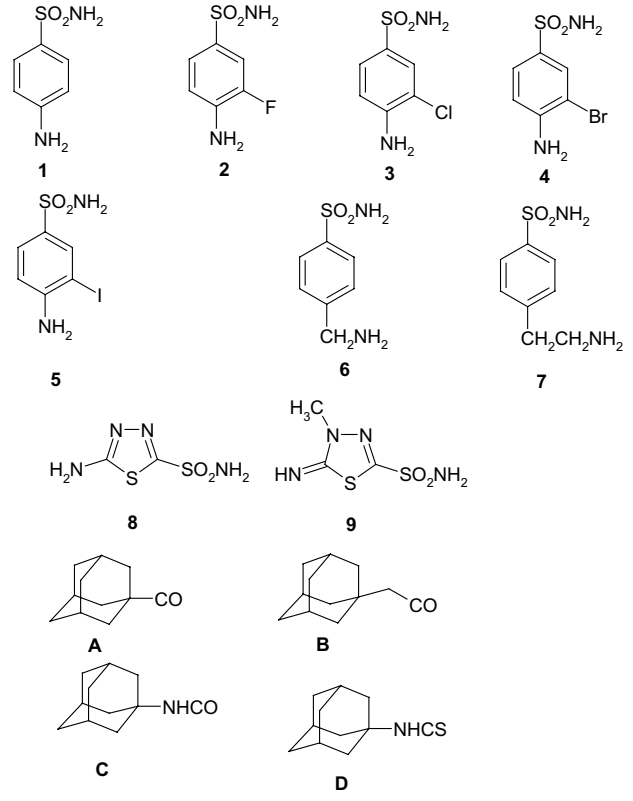
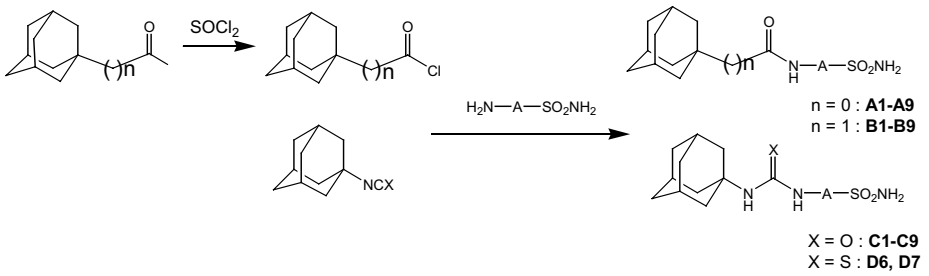


Chart 1.



Scheme 1.

2.2. Carbonic anhydrase inhibitory activity

The new sulfonamides reported here were assayed for inhibition of two CA isozymes (CA I, and II) known to play a critical role in electrolyte secretion/CO_2 transport processes in a variety of tissues,[18] using the esterase activity of these enzymes (with 4-nitrophenylacetate as chromogenic substrate). The inhibition data are shown in Table 1.[37]

2.3. Lipophilicity

In each series of tail (**A**–**D**), the lipophilicity of the most active compounds **A6**–**A9**, **B6**–**B9**, **C6**–**C9**, and **D6**–**D7** was expressed as their capacity factor ($\log k'$) measured by liquid chromatography at pH 7.40 by using an immobilized artificial cellular membrane (IAM) stationary phase (Table 2).[37,38] Their ClogP has been also calculated with the software ChemDraw Ultra 6.0.1. (Table 2).[39]

Table 1. Inhibition data of CA isozymes I and II for derivatives reported in the present paper

Inhibitor	K_I (nM)	
	hCA I[a]	hCA II[a]
TPM Topiramate	250 ± 13	5 ± 0.2
AZA Acetazolamide	900 ± 40	12 ± 1
MZA Methazolamide	780 ± 33	14 ± 0.8
ZNS Zonisamide	Nt[b]	35[c] ± 2
A1	18,600 ± 235 (28,000)	265 ± 14 (300)
A2	3400 ± 123 (8300)	49 ± 3 (60)
A3	3500 ± 95 (9800)	62 ± 4 (110)
A4	1600 ± 42 (6500)	35 ± 3 (40)
A5	1550 ± 74 (6000)	41 ± 2 (70)
A6	5500 ± 240 (25,000)	87 ± 6 (170)
A7	4300 ± 200 (21,000)	69 ± 6 (160)
A8	850 ± 41 (8600)	10 ± 0.8 (60)
A9	770 ± 38 (9300)	12 ± 1 (19)
B1	15,100 ± 460 (28,000)	233 ± 21 (300)
B2	1800 ± 62 (8300)	36 ± 3 (60)
B3	2000 ± 95 (9800)	39 ± 2 (110)
B4	1300 ± 47 (6500)	32 ± 3 (40)
B5	1050 ± 58 (6000)	28 ± 3 (70)
B6	2300 ± 76 (25,000)	47 ± 1 (170)
B7	1000 ± 55 (21,000)	35 ± 3 (160)
B8	340 ± 13 (8600)	8 ± 1 (60)
B9	265 ± 14 (9300)	7 ± 0.6 (19)
C1	22,400 ± 755 (28,000)	276 ± 18 (300)
C2	3650 ± 120 (8300)	53 ± 4 (60)
C3	3700 ± 83 (9800)	68 ± 7 (110)
C4	1870 ± 94 (6500)	39 ± 2 (40)
C5	1700 ± 50 (6000)	48 ± 4 (70)
C6	5840 ± 230 (25,000)	93 ± 7 (170)
C7	4800 ± 200 (21,000)	76 ± 5 (160)
C8	810 ± 33 (8600)	13 ± 1 (60)
C9	800 ± 27 (9300)	14 ± 0.8 (19)
D6	7800 ± 400 (25,000)	134 ± 11 (170)
D7	6560 ± 330 (21,000)	125 ± 14 (160)

The data in parenthesis represent inhibition by the parent sulfonamide **1**–**9**. Mean ± standard deviation (from three different measurements).
[a] Human (cloned) isozymes.
[b] Not tested.
[c] Unpublished data from this laboratory.

Table 2. Extent of protected mice (in %) against convulsions in the MES test at 3 h following intraperitoneal injection of 30 mg/kg, phospholipophilicity (log k' IAM) and lipophilicity (ClogP) of selected CA inhibitors

Inhibitor	MES test % of protected mice	Lipophilicity	
		log k' IAM	ClogP
TPM Topiramate	83[a]		+0.04
AZA Acetazolamide	—		−1.13
MZA Methazolamide	100[a]		+0.09
A6	48	+1.35	+1.77
A7	50	+1.39	+1.84
A8	92	+1.55	+1.53
A9	96	+2.20	+2.99
B6	50	+1.75	+2.39
B7	67	+1.59	+2.71
B8	75	+2.22	+2.15
B9	42	+2.32	+3.37
C6	58	+1.66	+2.04
C7	75	+1.76	+2.37
C8	47	+2.16	+1.69
C9	73	+1.73	+2.01
D6	58	+2.21	+2.16
D7	44	+2.22	+2.65

[a] Values from Ref. 30.

2.4. Maximal electroshock seizures test

The anticonvulsant activity of the most active CA inhibitors reported here was examined at 30 mg/kg (ip) in mice by using the MES test, which detects drugs that prevent spread of tonic-clonic seizures.[40,41] Three hours following intraperitoneal administration of 30 mg/kg, the electrical stimulus was delivered and the extent of protected mice was noted (Table 2).

3. Discussion

3.1. Chemistry

In a previous work[30] it was showed that some sulfonamide CAIs incorporating lipophilic moieties, such as the valproyl, pivaloyl, or adamantyl residues connected to the structure of acetazolamide, exhibited good anticonvulsant activity in experimental animals. These data prompted us to extend our investigations in this field, reporting a new series of sulfonamides incorporating adamantyl moieties attached to the scaffolds of aromatic/heterocyclic sulfonamides of type **1**–**9**, known to act as good CAIs.[18,30]

The amides **A1**–**A9** and **B1**–**B9** incorporating the adamantyl/adamantylmethyl moieties were obtained from the corresponding acids, which were converted to the acyl chlorides (with $SOCl_2$), by reaction with aminosulfonamides **1**–**9** in the presence of base (triethylamine, pyridine, or sodium bicarbonate).[33,34] The amides were obtained in good yields by this simple method. It has been demonstrated that in such conditions only the acylation reaction of the amino moiety takes place, without the concomitant N-acylation of the sulfonamide group.[33,34]

Ureas **C1**–**C9** and thioureas **D6**, **D7** were analogously obtained by reaction of aminosulfonamides **1**–**9** with adamantyl isocyanate and adamantyl isothiocyanate, respectively, as reported for other CAIs incorporating these functionalities.[35,36]

3.2. Carbonic anhydrase inhibitory activity

The data of Table 1 show that the new inhibitors prepared by attaching diverse adamantyl-containing moieties to aromatic/heterocyclic sulfonamides **1**–**9**, are systematically more effective as compared to their parent sulfonamide from which they were prepared, toward the two investigated isozymes, hCA I and hCA II. The enhanced inhibitory power of these compounds is presumably due to the interaction of the relatively bulky adamantyl moiety with the enzyme active site. The following structure–activity relationships can be evidenced on hCA: (i) the adamantylacetamides **B1**–**B9** were more effective CAIs than the corresponding adamantylcarboxamides **A1**–**A9**, which were in turn more effective inhibitors than the corresponding ureas **C1**–**C9**. These were more effective CAIs as compared to the corresponding thioureas **D6**, **D7**. Thus, the best tail for inducing good CA inhibitory properties was the adamantylacetamide one of type **B**; (ii) heterocyclic sulfonamides incorporating heads **8** and **9** were more effective CAIs as compared to the aromatic derivatives incorporating heads **1**–**7**. Among these last derivatives, the scaffolds of halogenated sulfanilamides **2**–**5** and 4-aminoethyl-benzenesulfonamide **7** generally lead to better inhibitors than the simple sulfanilamide scaffold **1**; (iii) among the halogenated sulfonamides, the bromo **4** and the iodo **5** compounds are more active; (iv) CA II was much more sensitive to inhibition by these sulfonamides as compared to isozyme CA I, a fact well documented for many types of sulfonamide inhibitors.[18] It should also be mentioned that some of the new sulfonamides reported here, such as **A8**, **A9**, **B8**, **B9**, **C8**, and **C9** possess CA I and CA II inhibitory properties of similar or better potency than the clinically used

inhibitors acetazolamide **AZA** and methazolamide **MZA**, being slightly less effective than topiramate **TPM**. Due to the low expression of CA I and to the abundance of CA II in the brain, the selectivity toward CA II isozyme should be favorable to the anticonvulsant activity. The ratio of the K_I values (hCA I/hCA II) of **A8** (ratio=85) is higher than that of acetazolamide (ratio=75), the most specific hCA I inhibitor, whereas **A9** (ratio=64) and **C9** (ratio=57) were more selective for hCA II than topiramate (ratio=50).

3.3. Lipophilicity

Generally, the crossing of the blood-brain barrier is related to lipophilicity, this parameter has been determined for the best CA II inhibitors (**A6**–**A9**, **B6**–**B9**, **C6**–**C9**, and **D6**, **D7**), as well as for the reference CA inhibitors (**TPM**, **AZA**, **MZA**). On one hand, their lipophilicity was theoretically calculated and expressed as ClogP; on the other hand, their lipophilicity was experimentally determined by using IAM columns and expressed as their capacity factor ($\log k'$ IAM) (Table 2).[38] These IAM columns are coated with a monolayer of phosphatidylcholine immobilized on a silica support and mimic very closely the lipid environment of a fluid cell membrane. It has been demonstrated that the $\log k'$ IAM value represents the so called 'phospholipophilicity'. This value is better than the octanol–water partition coefficient ($\log P$) or than $\Delta \log P$ to predict the blood-brain barrier crossing. Indeed, IAM chromatography takes into account hydrophobic, ion pairing, and hydrogen bonding interactions. Considering the adamantyl side chain, the thioureas compounds (**D6**, **D7**) were more lipophilic than their ureas counterparts (**C6**, **C7**) whatever the method used. As expected, the $\log k'$ IAM and the ClogP of adamantylacetamides **B6**–**B9** were higher than that of adamantylcarboxamides **A6**–**A9** ($\Delta \log k'$ IAM $= +0.1$ to $+0.7$; ΔClogP $= +0.4$ to 0.9). By using the chromatographic method, the phospholipophilicity of both types of benzenesulfonamide moieties is quite similar (compare **A6**–**A7**, **B6**–**B7**, **C6**–**C7**, and **D6**–**D7**). On the contrary, the ClogP of aminoethyl-benzenesulfonamides **7** are higher than that of the aminomethylbenzenesulfonamides **6** (ΔClogP $= +0.1$ to $+0.5$). Similarly, the ClogP value of the 4-methylthiadiazoline derivatives (**A9**, **B9**, **C9**) is higher that that of their thiadiazole counterparts (**A8**, **B8**, **C8**; ΔClogP $= +0.1$ to $+0.5$). Taking all these results, a poor correlation ($p < 0.05$) is observed between $\log k'$ IAM and ClogP values. The ClogP value of all the adamantyl derivatives investigated are much higher (ClogP $> +1.53$) than that of methazolamide MZA (ClogP $= +0.09$) or topiramate (ClogP $= +0.04$) marketed as anticonvulsant. Thus both sets of values are features of compounds able to cross the blood-brain barrier well,[37] as was the case of the reference CA inhibitors TPM, AZA, or MZA. It should be mentioned that at the physiological pH, due to the acidity of the sulfonamide/sulfamate moieties, all these drugs are found as sulfonamidate/sulfamate anions. Still, as the anion is in equilibrium with the free acid (sulfonamide/sulfamate) that is able to cross membranes, a lipophilic enough such molecule would not have difficulties in crossing the blood-brain barrier and reach the brain CAs, which represent the target of these CAIs. Thus, the lipophilicity measurements/calculations are really critical for detecting good anticonvulsant candidates among the compounds synthesized in this work.

3.4. Maximal electroshock seizures test

Compounds characterized by a high CA II inhibitory potency and by a lipophilicity propitious to brain penetration were evaluated for their anticonvulsant properties in the MES-test. Intraperitoneally administered in mice at 30 mg kg^{-1}, their anticonvulsant activity was evaluated 3 h later and defined as a protective index against convulsions induced by an electrical stimulus. With a protective index ranging from 42% to 58%, compounds **A7**, **B9**, **C8**, and **D7** can be regarded as poor anticonvulsant drugs as well as the aminomethylbenzenesulfonamides (**A6**, **B6**, **C6**, **D6**). The molecules **B7**, **B8**, **C7**, and **C9** can be considered as moderately active (protective index: 67–75%). Methazolamide **MZA** and both adamantylcarboxamido derivatives **A8** and **A9** exhibited a high protection against induced convulsions (>90%). Dose–response curves (30, 20, 10, 5, 2.5 mg kg^{-1}) were performed with these two last compounds. The ED_{50} of 4-methylthiadiazolidine **A9** and thiadiazole **A8** were 2.6 and 3.5 mg kg^{-1}, respectively. Both molecules were the most selective inhibitors of the series for hCA II mainly located in the brain.

In conclusion this work led to the design, the synthesis and the discovery of two lipophilic anticonvulsant compounds characterized by a high inhibitory potency of hCA II and a selectivity for this isozyme when compared to their activity on hCA I. Further in vivo experiment (time-course activity, other epileptic models) are warranted to confirm their interest in the treatment of central nervous disease such as epilepsy, or depression among others.

4. Experimental

4.1. General

Melting points: heating plate microscope (not corrected); IR spectra: KBr pellets, 400–4000 cm^{-1} Karl Zeiss Jena UR-20 spectrometer; NMR spectra: Varian Gemini 300BB apparatus, operating at 300 MHz for ^{1}H NMR and at 75 MHz for ^{13}C NMR (chemical shifts are expressed as δ values relative to Me_4Si as internal standard for proton spectra and to the solvent resonance for carbon spectra). Assignments were made by means of chemical shifts, peak integrals, selective deuteration, model spectra, APT, COSY (^{1}H–^{1}H), and HETCOR (^{1}H–^{13}C) experiments. Elemental analysis ($\pm$0.4% of the theoretical values, calculated for the proposed structures): Carlo Erba Instruments CHNS Elemental Analyzer, Model 1106.

All reactions were monitored by thin-layer chromatography (TLC), using 0.25 mm-thick precoated silica gel plates (E. Merck) eluted with MeOH/$CHCl_3$ 1/4 v/v unless specified otherwise. 1-Adamantane carboxylic acid, 1-adamantyl-acetic acid, adamantyl isocyanate, adamantyl isothiocyanate, homosulfanilamide (hydrochloride), and 4-(2-aminoethyl)benzenesulfonamide were from Sigma-Aldrich Chemical Co. Thionyl chloride, triethylamine, pyridine, were from Aldrich, Fluka, or Merck. Acetonitrile, tetrahydrofurane, methanol, ethanol, chloroform, hexane (E. Merck, Darmstadt, Germany), or other solvents used in the synthesis were double distilled and kept on molecular sieves in order to maintain them in anhydrous conditions. 5-Amino-1,3,4-thiadiazole-2-sulfonamide was obtained from acetazolamide (Sigma) by deprotection with concentrated hydrochloric acid followed by neutralization, using a previously reported procedure.[42,43] 5-Imino-4-methyl-Δ^2-1,3,4-thiadiazoline-2-sulfonamide hydrochloride, was similarly prepared from methazolamide (Sigma). Halogenosulfonamides were prepared by literature procedures,[44–46] optimized in our laboratory. Preparative column chromatography was performed on silica gel 60 (0.063–0.200 mm)—from Merck—eluted with MeOH/$CHCl_3$ 2/8 v/v.

4.2. Chemistry

4.2.1. General procedure for the synthesis of adamantylcarboxamides A1–A9 and B1–B9. An amount of 5 mmol aminosulfonamide was suspended/dissolved in 20 mL anhydrous MeCN and 0.78 mL (0.56 g, 5.5 mmol) triethylamine was added under stirring. The mixture was cooled to 0–5 °C, then a solution of 5 mmol adamantanecarboxyl chloride/adamantaneacetyl chloride (generated from the corresponding acids and thionyl chloride), dissolved in 3 mL MeCN, was added dropwise during 15 min. The reaction was stirred at room temperature for 3 h or until a reasonable conversion was reached (TLC control). The solvent was evaporated in vacuum and the resulted product was treated with 20 mL water. The crude product was filtered off, washed with water, and dried. Yields were in the range of 50–90%, depending on the reactivity of the corresponding aminosulfonamide. The resulted compounds were further purified by recrystallization (from ethanol) or by column chromatography, when the fractions containing the desired compound were collected, evaporated to dryness, and the obtained product was recrystallized from the same solvent.

4.2.2. General procedure for the synthesis of adamantyl ureas C1–C9 and thioureas D6, D7. Aminosulfonamide (5 Mmol) dissolved in 15 mL anhydrous tetrahydrofurane, were treated under stirring with 5 mmol adamantyl isocyanate/isothiocyanate dissolved in 3 mL THF. The mixture was refluxed overnight or until a reasonable conversion was reached (TLC control). Then the solvent was evaporated in vacuum and the resulted crude product purified by recrystallization from ethanol or by column chromatography followed by recrystallization from the same solvent. Yields were in the range of 30–90%, depending on the reactivity of the corresponding aminosulfonamide. Moreover, due to the low reactivity of adamantyl isothiocyanate and its limited thermal stability, the corresponding thioureas could be obtained just in the case of very reactive aminosulfonamides.

4.2.2.1. 4-(Adamantan-1-yl-carboxamido)-benzenesulfonamide A1. White crystals, mp 281–284 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 9.43 (s, 1H, NH), 7.84 (d, $J = 8.9$ Hz, 2H, H3,5-Ph), 7.74 (d, $J = 8.9$ Hz, 2H, H2,6-Ph), 7.23 (s, 2H, SO_2NH_2), 2.01 (br s, 3H, 3CHβ-Ad), 1.91 (d, $J = 3.4$ Hz, 6H, $3CH_2$α-Ad), 1.69 (t, $J = 3.3$ Hz, 6H, $3CH_2$γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 176.4 (CO), 142.4 (C4-Ph), 138.2 (C1-Ph), 126.3 (2C, C3,C5-Ph), 119.6 (2C, C2,C6-Ph), 41.1 (Cq-Ad), 38.1 (3Cα-Ad), 36.0 (3Cγ-Ad), 27.6 (3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1325 (ν_{SO_2as}), 1530 (δ_{NH}), 1660 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{17}H_{22}N_2O_3S$) C, H, N.

4.2.2.2. 4-(Adamantan-1-yl-carboxamido)-3-fluorobenzenesulfonamide A2. White crystals, mp 163–166 °C (dec) (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 9.22 (s, 1H, NH), 7.73 (dd, 1H, $J = 8.5$, 7.4 Hz, H5-Ph), 7.66 (dd, 1H, $J = 8.4$, 2.2 Hz, H6-Ph), 7.61 (dd, 1H, $J = 9.2$, 2.2 Hz, H2-Ph), 7.43 (br s, 2H, SO_2NH_2), 2.02 (br s, 3H, 3CHβ-Ad), 1.92 (br s, 6H, $3CH_2$α-Ad), 1.70 (br s, 6H, $3CH_2$γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 176.06 (CO), 154.20 (d, $J_{Cipso\text{-}F} = 248.6$ Hz, C3-Ph), 140.9 (d, $J_{Cmeta\text{-}F} = 3.0$ Hz, C1-Ph), 129.45 (d, $J_{Cortho\text{-}F} = 11.8$ Hz, C4-Ph), 124.5 (C6-Ph), 121.63 (d, $J_{Cmeta\text{-}F} = 3.4$ Hz, C5-Ph), 113.15 (d, $J_{Cortho\text{-}F} = 23.02$ Hz, C2-Ph), 41.03 (d, $J = 6.6$ Hz, Cq-Ad), 38.52 (d, $J = 3.4$ Hz, 3Cα-Ad), 36.16 (3Cγ-Ad), 27.75 (3Cβ-Ad); IR (KBr), cm^{-1}: 1165 (ν_{SO_2sym}), 1330 (ν_{SO_2as}), 1540 (δ_{NH}), 1665 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{17}H_{21}FN_2O_3S$) C, H, N.

4.2.2.3. 4-(Adamantan-1-yl-carboxamido)-3-clorobenzenesulfonamide A3. White crystals, mp 222–224 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 9.06 (s, 1H, NH), 7.89 (d, $J = 1.8$ Hz, 1H, H2-Ph), 7.78 (d, $J = 8.6$ Hz, 1H, H5-Ph), 7.74 (dd, $J = 8.6$, 1.8 Hz, 1H, H6-Ph), 7.49 (s, 2H, SO_2NH_2), 2.03 (br s, 3H, 3CHβ-Ad), 1.94 (br s, 6H, $3CH_2$α-Ad), 1.71 (br s, 6H, $3CH_2$γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 176.01 (CO), 141.66 (C4-Ph), 138.32 (C1-Ph), 128.10 (C3-Ph), 127.16 (C2-Ph), 126.66 (C5-Ph), 124.82 (C6-Ph), 40.97 (Cq-Ad), 38.39 (3C, 3Cα-Ad), 36.00 (3C, 3Cγ-Ad), 27.63 (3C, 3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1335 (ν_{SO_2as}), 1540 (δ_{NH}), 1665 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{17}H_{21}ClN_2O_3S$) C, H, N.

4.2.2.4. 4-(Adamantan-1-yl-carboxamido)-3-bromobenzenesulfonamide A4. White crystals, mp 218–221 °C (dec) (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 9.01 (s, 1H, NH), 8.04 (d, $J = 1.8$ Hz, 1H, H2-Ph), 7.79 (dd, $J = 8.4$, 1.8 Hz, 1H, H6-Ph), 7.75 (d, $J = 8.4$ Hz, 1H, H5-Ph), 7.49 (s, 2H, SO_2NH_2), 2.03 (br s, 3H, 3CHβ-Ad), 1.94 (br s, 6H, $3CH_2$α-Ad), 1.71 (br s, 6H, $3CH_2$γ-

Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 175.79 (CO), 141.78 (C4-Ph), 139.55 (C1-Ph), 129.65 (C2-Ph), 126.77 (C5-Ph), 125.34 (C6-Ph), 118.35 (C3-Ph), 40.95 (Cq-Ad), 38.39 (3C, 3Cα-Ad), 35.93 (3C, 3Cγ-Ad), 27.56 (3C, 3Cβ-Ad); IR (KBr), cm^{-1}: 1165 (ν_{SO_2sym}), 1330 (ν_{SO_2as}), 1540 (δ_{NH}), 1665 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{17}H_{21}BrN_2O_3S$) C, H, N.

4.2.2.5. 4-(Adamantan-1-yl-carboxamido)-3-iodobenzenesulfonamide A5. White crystals, mp 250–253 °C (dec) (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 8.93 (s, 1H, NH), 8.24 (d, 1H, $J = 2.0$ Hz, H2-Ph), 7.78 (dd, 1H, $J = 8.4$, 2.0 Hz, H6-Ph), 7.63 (d, 1H, $J = 8.4$ Hz, H5-Ph), 7.44 (s, 2H, SO_2NH_2), 2.04 (br s, 3H, 3CHβ-Ad), 1.95 (br s, 6H, 3CH_2α-Ad), 1.71 (br s, 6H, 3CH_2γ-Ad); IR (KBr), cm^{-1}: 1165 (ν_{SO_2sym}), 1330 (ν_{SO_2as}), 1540 (δ_{NH}), 1665 ($\nu_{C=O}$), 2860 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{17}H_{21}IN_2O_3S$) C, H, N.

4.2.2.6. 4-(Adamantan-1-yl-carboxamido-methyl)-benzenesulfonamide A6. White crystals, mp 250–252 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 8.08 (t, 1H, $J = 5.8$ Hz, NH), 7.74 (d, $J = 8.3$ Hz, 2H, H3,5-Ph), 7.36 (d, $J = 8.3$ Hz, 2H, H2,6-Ph), 7.30 (s, 2H, SO_2NH_2), 4.29 (d, 2H, $J = 3.1$ Hz, CH_2-Ph), 1.97 (br s, 3H, 3CHβ-Ad), 1.81 (br s, 6H, 3CH_2α-Ad), 1.67 (br s, 6H, 3CH_2γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 177.0 (CO), 144.4 (C4-Ph), 142.3 (C1-Ph), 127.1 (2C, C2,C6-Ph), 125.6 (2C, C3,C5-Ph), 41.6 (Cq-Ad), 39.8 (CH_2-Ph), 38.7 (3Cα-Ad), 36.1 (3Cγ-Ad), 27.6 (3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1330 (ν_{SO_2as}), 1540 (δ_{NH}), 1615 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2920 (ν_{CH_2as}); Anal. ($C_{18}H_{24}N_2O_3S$) C, H, N.

4.2.2.7. 4-[(Adamantan-1-yl-carboxamido)-2-ethyl]-benzenesulfonamide A7. White crystals, mp 268–271 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.73 (d, $J = 8.3$ Hz, 2H, H3,5-Ph), 7.47 (t, 1H, $J = 5.6$ Hz, NH), 7.35 (d, $J = 8.3$ Hz, 2H, H2,6-Ph), 7.30 (s, 2H, SO_2NH_2), 3.27 (q, 2H, $J = 5.6$ Hz, 2-Et), 2.77 (t, 2H, $J = 7.1$ Hz, 1-Et), 1.94 (br s, 3H, 3CHβ-Ad), 1.72 (d, 6H, $J = 2.5$ Hz, 3CH_2α-Ad), 1.64 (br s, 6H, 3CH_2γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 176.9 (CO), 143.9 (C4-Ph), 141.9 (C1-Ph), 129.1 (2C, C2,C6-Ph), 125.6 (2C, C3,C5-Ph), 39.9 (2-Et), 39.8 (1-Et), 38.7 (3Cα-Ad), 36.1 (3Cγ-Ad), 34.8 (Cq-Ad), 27.6 (3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1335 (ν_{SO_2as}), 1540 (δ_{NH}), 1635 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2900 (ν_{CH_2as}); Anal. ($C_{19}H_{26}N_2O_3S$) C, H, N.

4.2.2.8. 5-(Adamantan-1-yl-carboxamido)-1,3,4-thidiazole-2-sulfonamide A8. White crystals, mp 246–248 °C (dec) (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 12.66 (s, 1H, NH), 8.29 (s, 2H, SO_2NH_2), 2.00 (br s, 3H, 3CHβ-Ad), 1.94 (br s, 6H, 3CH_2α-Ad), 1.68 (br s, 6H, 3CH_2γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 176.7 (CO), 164.5 (C–NH), 161.9 (C–SO_2NH_2), 40.8 (Cq-Ad), 37.4 (3Cα-Ad), 35.7 (3Cγ-Ad), 27.4 (3Cβ-Ad); IR (KBr), cm^{-1}: 1170 (ν_{SO_2sym}), 1360 (ν_{SO_2as}), 1515 (δ_{NH}), 1670 ($\nu_{C=O}$), 2865 (ν_{CH_2sym}), 2915 (ν_{CH_2as}); Anal. ($C_{13}H_{18}N_4O_3S_2$) C, H, N.

4.2.2.9. 5-(Adamantan-1-yl-carboximido)-4-methyl-Δ^2-1,3,4-thidiazoline-2-sulfonamide A9. White crystals, mp 230–231 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 8.43 (s, 2H, SO_2NH_2), 3.94 (s, 3H, N–CH_3), 2.00 (br s, 3H, 3CHβ-Ad), 1.88 (br s, 6H, 3CH_2α-Ad), 1.69 (br s, 6H, 3CH_2γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 186.6 (CO), 164.8 (C=N), 157.5 (C–SO_2NH_2), 42.0 (Cq-Ad), 38.9 (3Cα-Ad), 38.0 (N–CH_3), 36.2 (3Cγ-Ad), 27.6 (3Cβ-Ad); IR (KBr), cm^{-1}: 1175 (ν_{SO_2sym}), 1360 (ν_{SO_2as}), 1600 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2900 (ν_{CH_2as}); Anal. ($C_{14}H_{20}N_4O_3S_2$) C, H, N.

4.2.2.10. 4-(Adamantan-1-yl-methylcarboxamido)-benzenesulfonamide B1. White crystals, mp 260–262 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 10.10 (s, 1H, NH), 7.74 (s, 4H, AA′BB′, Ph), 7.23 (s, 2H, SO_2NH_2), 2.08 (s, 2H, CH_2-Ad), 1.93 (br s, 3H, 3CHβ-Ad), 1.63 (m, 6H, 3CH_2α-Ad), 1.61 (m, 6H, 3CH_2γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 169.73 (CO), 142.05 (C4-Ph), 138.07 (C1-Ph), 126.60 (2C, C3,C5-Ph), 118.55 (2C, C2,C6-Ph), 50.80 (CH_2-Ad), 42.01 (3Cα-Ad), 36.37 (3Cγ-Ad), 32.79 (Cq-Ad), 28.01 (3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1335 (ν_{SO_2as}), 1530 (δ_{NH}), 1640 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2900 (ν_{CH_2as}); Anal. ($C_{18}H_{24}N_2O_3S$) C, H, N.

4.2.2.11. 4-(Adamantan-1-yl-methylcarboxamido)-3-clorobenzenesulfonamide B3. White crystals, mp 196–199 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 9.61 (s, 1H, NH), 7.95 (d, $J = 8.5$ Hz, 1H, H5-Ph), 7.88 (d, $J = 2.1$ Hz, 1H, H2-Ph), 7.74 (dd, $J = 8.5$, 2.1 Hz, 1H, H6-Ph), 7.47 (s, 2H, SO_2NH_2), 2.20 (s, 2H, CH_2-Ad), 1.93 (br s, 3H, 3CHβ-Ad), 1.64 (m, 12H, 3CH_2α-Ad-+3CH_2γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 169.75 (CO), 140.87 (C4-Ph), 138.07 (C1-Ph), 126.84 (C2-Ph), 125.74 (C3-Ph), 125.67 (C5-Ph), 124.82 (C6-Ph), 50.00 (CH_2-Ad), 41.84 (3C, 3Cα-Ad), 36.43 (3C, 3Cγ-Ad), 32.80 (Cq-Ad), 28.10 (3C, 3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1330 (ν_{SO_2as}), 1540 (δ_{NH}), 1665 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{18}H_{23}ClN_2O_3S$) C, H, N.

4.2.2.12. 4-(Adamantan-1-yl-methylcarboxamido)-3-iodobenzenesulfonamide B5. White crystals, mp 222–225 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 9.56 (s, 1H, NH), 8.24 (br s, 1H, H6-Ph), 7.67 (m, 1H, H5-Ph), 7.42 (s, 2H, SO_2NH_2), 2.15 (s, 2H, CH_2-Ad), 1.95 (br s, 3H, 3CHβ-Ad), 1.64 (m, 12H, 3CH_2 α-Ad+3CH_2γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 169.89 (CO), 143.29 (C4-Ph), 142.16 (C1-Ph), 136.68 (C2-Ph), 126.85 (C5-Ph), 126.31 (C6-Ph), 50.53 (CH_2-Ad), 42.70 (3Cα-Ad), 36.90 (3Cγ-Ad), 33.32 (Cq-Ad), 28.58 (3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1330 (ν_{SO_2as}), 1535 (δ_{NH}), 1660 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{18}H_{23}IN_2O_3S$) C, H, N.

4.2.2.13. 4-(Adamantan-1-yl-methylcarboxamido-methyl)-benzenesulfonamide B6. White crystals, mp 204–208 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 8.31 (t, 1H, $J = 5.9$ Hz, NH), 7.76 (d, $J = 8.2$ Hz, 2H, H3,5-Ph), 7.42 (d, $J = 8.2$ Hz, 2H, H2,6-Ph), 7.31 (s, 2H, SO_2NH_2), 4.30 (d, 2H, $J = 5.9$ Hz, NH–CH_2), 1.91 (br s,

6H, 3CH$_2$α-Ad), 1.68 (br s, 1H, 1CHβ-Ad), 1.64 (br s, 2H, 2CHβ-Ad), 1.57 (br s, 6H, 3CH$_2$γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 170.14 (CO), 143.95 (C4-Ph), 142.55 (C1-Ph), 127.53 (2C, C3,C5-Ph), 125.61 (2C, C2,C6-Ph), 49.90 (CH$_2$-Ad), 42.16 (3Cα-Ad), 41.67 (Cq-Ad), 38.96 (2Cγ-Ad), 32.33 (Cγ-Ad), 28.05 (3Cβ-Ad); IR (KBr), cm^{-1}: 1175 (ν_{SO_2sym}), 1365 (ν_{SO_2as}), 1530 (δ_{NH}), 1665 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2900 (ν_{CH_2as}); Anal. ($C_{19}H_{26}N_2O_3S$) C, H, N.

4.2.2.14. 4-[(Adamantan-1-yl-methylcarboxamido)-2-ethyl]-benzenesulfonamide B7. White crystals, mp 219–222 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.78 (t, 1H, J = 5.8 Hz, NH), 7.73 (d, J = 8.2 Hz, 2H, H3,5-Ph), 7.39 (d, J = 8.2 Hz, 2H, H2,6-Ph), 7.79 (s, 2H, SO_2NH_2), 3.24 (dd, 2H, J = 7.1, 5.8 Hz, NH–CH_2), 2.78 (t, 2H, J = 7.1 Hz, CH$_2$-Ph), 1.88 (br s, 3H, 3CHβ-Ad), 1.79 (s, 2H, CH$_2$-Ad), 1.65 (br s, 2H, CH$_2$α-Ad), 1.61 (br s, 2H, CH$_2$α-Ad), 1.56 (br s, 2H, CH$_2$α-Ad), 1.49 (m, 6H, 3CH$_2$γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 169.92 (CO), 143.76 (C4-Ph), 142.01 (C1-Ph), 129.08 (2C, C3,C5-Ph), 125.59 (2C, C2,C6-Ph), 50.04 (CH$_2$-Ad), 42.09 (3Cα-Ad), 39.50 (NH-CH$_2$), 36.45 (3Cγ-Ad), 34.92 (CH$_2$-Ph), 32.11 (Cq-Ad), 28.01 (3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1320 (ν_{SO_2as}), 1535 (δ_{NH}), 1625 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2900 (ν_{CH_2as}); Anal. ($C_{20}H_{28}N_2O_3S$) C, H, N.

4.2.2.15. 5-(Adamantan-1-yl-methylcarboxamido)-1,3,4-thidiazole-2-sulfonamide B8. White crystals, mp 232–234 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 12.94 (s, 1H, NH), 8.30 (s, 2H, SO_2NH_2), 2.28 (s, 2H, CH$_2$-Ad), 1.92 (br s, 3H, 3CHβ-Ad), 1.67 (s, 2H, CH$_2$α-Ad), 1.62 (br s, 2H, CH$_2$α-Ad), 1.58 (br s, 6H, 3CH$_2$γ-Ad), 1.55 (br s, 2H, CH$_2$α-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 170.00 (CO), 164.25 (C–NH), 160.78 (C–SO_2NH_2), 48.83 (CH$_2$-Ad), 41.82 (3Cα-Ad), 36.21 (3Cγ-Ad), 33.04 (Cq-Ad), 27.95 (3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1320 (ν_{SO_2as}), 1530 (δ_{NH}), 1640 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{14}H_{20}N_4O_3S_2$) C, H, N.

4.2.2.16. 5-(Adamantan-1-yl-methylcarboximido)-4-methyl-Δ^2-1,3,4-thidiazoline-2-sulfonamide B9. White crystals, mp 216–217 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 8.43 (s, 2H, SO_2NH_2), 3.92 (s, 3H, N–CH$_3$), 2.31 (s, 2H, CH$_2$-Ad), 1.91 (br s, 3H, 3CHβ-Ad), 1.61 (br s, 12H, 3CH$_2$α-Ad+3CH$_2$γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 180.5 (CO), 163.5 (C=N), 157.5 (C–SO_2NH_2), 53.5 (*C*H$_2$–CO), 42.1 (3Cα-Ad), 38.2 (N–CH$_3$), 36.3 (3Cγ-Ad), 33.0 (Cq-Ad), 28.1 (3Cβ-Ad); IR (KBr), cm^{-1}: 1180 (ν_{SO2sym}), 1325 (ν_{SO_2as}), 1590 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2900 (ν_{CH_2as}); Anal. ($C_{15}H_{22}N_4O_3S_2$) C, H, N.

4.2.2.17. 4-(3-Adamantan-1-yl-ureido)-benzenesulfonamide C1. White crystals, mp 277–279 °C (dec) (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 8.64 (s, 1H, NH-Ph), 7.64 (d, J = 8.8 Hz, 2H, H3,5-Ph), 7.47 (d, J = 8.8 Hz, 2H, H2,6-Ph), 7.14 (s, 2H, SO_2NH_2), 6.02 (s, 1H, NH-Ad), 2.03 (br s, 3H, 3CHβ-Ad), 1.93 (br s, 6H, 3CH$_2$α-Ad), 1.63 (br s, 6H, 3CH$_2$γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 153.5 (NHCO), 143.7 (C4-Ph), 135.8 (C1-Ph), 126.8 (2C, C2,C6-Ph), 116.5 (2C, C3,C5-Ph), 50.1 (Cq-Ad), 41.5 (3Cα-Ad), 36.0 (3Cγ-Ad), 28.9 (3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1325 (ν_{SO_2as}), 1540 (δ_{NH}), 1660 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2915 (ν_{CH_2as}); Anal. ($C_{17}H_{23}N_3O_3S$) C, H, N.

4.2.2.18. 4-(3-Adamantan-1-yl-ureido)-3-clorobenzenesulfonamide C3. White crystals, mp 156–159 °C (dec) (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 8.37 (d, J = 8.8 Hz, 1H, H5-Ph), 8.20 (s, 1H, NH-Ph), 7.78 (d, J = 2.0 Hz, 1H, H2-Ph), 7.64 (dd, J = 8.8, 2.0 Hz, 1H, H6-Ph), 7.30 (s, 2H, SO_2NH_2), 7.04 (s, 1H, Ad-NH), 2.03 (br s, 3H, 3CHβ-Ad), 1.95 (br s, 6H, 3CH$_2$α-Ad), 1.63 (br s, 6H, 3CH$_2$γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 152.88 (CO), 140.01 (C4-Ph), 136.71 (C1-Ph), 126.62 (C2-Ph), 125.14 (C6-Ph), 119.93 (C5-Ph), 119.18 (C3-Ph), 50.31 (Cq-Ad), 41.39 (3C, 3Cγ-Ad), 36.02 (3C, 3Cα-Ad), 28.91 (3C, 3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1320 (ν_{SO_2as}), 1535 (δ_{NH}), 1660 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{17}H_{22}ClN_3O_3S$) C, H, N.

4.2.2.19. 4-[(3-Adamantan-1-yl-ureido)-methyl]-benzenesulfonamide C6. White crystals, mp 226–228 °C (dec) (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.75 (d, J = 8.3 Hz, 2H, H3,5-Ph), 7.38 (d, J = 8.3 Hz, 2H, H2,6-Ph), 7.29 (s, 2H, SO_2NH_2), 6.20 (t, 1H, J = 6 Hz, N*H*–CH$_2$), 5.68 (s, 1H, NH-Ad), 4.21 (d, 2H, J = 6 Hz, NH–CH_2), 1.99 (br s, 3H, 3CHβ-Ad), 1.87 (br s, 6H, 3CH$_2$α-Ad), 1.60 (br s, 6H, 3CH$_2$γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 156.9 (NHCO), 145.4 (C4-Ph), 142.3 (C1-Ph), 127.2 (2C, C2,C6-Ph), 125.6 (2C, C3,C5-Ph), 49.6 (CH$_2$-Ph), 42.2 (Cq-Ad), 42.0 (3Cα-Ad), 36.1 (3Cγ-Ad), 28.9 (3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1335 (ν_{SO_2as}), 1550 (δ_{NH}), 1640 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{18}H_{25}N_3O_3S$) C, H, N.

4.2.2.20. 4-[2-(3-Adamantan-1-yl-ureido)-ethyl]-benzenesulfonamide C7. White crystals, mp 218–220 °C (dec) (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.74 (d, J = 8.3 Hz, 2H, H3,5-Ph), 7.37 (d, J = 8.3 Hz, 2H, H2,6-Ph), 7.29 (s, 2H, SO_2NH_2), 5.65 (t, 1H, J = 5.8 Hz, N*H*–CH$_2$), 5.55 (s, 1H, NH-Ad), 3.19 (q, 2H, J = 6.6 Hz, NH–CH_2), 2.72 (t, 2H, J = 7 Hz, CH_2-Ph), 1.98 (br s, 3H, 3CHβ-Ad), 1.84 (br s, 6H, 3CH$_2$α-Ad), 1.59 (br s, 6H, 3CH$_2$γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 157.0 (NHCO), 144.1 (C4-Ph), 141.9 (C1-Ph), 129.1 (2C, C2,C6-Ph), 125.7 (2C, C3,C5-Ph), 49.4 (CH$_2$-NH), 42.0 (3Cα-Ad), 40.1 (Cq-Ad), 36.1 (3Cγ-Ad), 35.9 (CH$_2$-Ph), 28.9 (3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1340 (ν_{SO_2as}), 1530 (δ_{NH}), 1640 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{19}H_{27}N_3O_3S$) C, H, N.

4.2.2.21. 5-(Adamantan-1-yl-ureido)-1,3,4-thidiazole-2-sulfonamide C8. White crystals, mp 278–280 °C (dec) (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 10.86 (s, 1H, NH-thiadiazole), 8.23 (s, 2H, SO_2NH_2), 6.50 (s, 1H, NH-Ad), 2.04 (br s, 3H, 3CHβ-Ad), 1.93 (br s, 6H, 3CH$_2$α-Ad), 1.63 (br s, 6H, 3CH$_2$γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 163.3 (C–NH), 162.7 (C–SO_2NH_2), 151.5 (NHCO), 50.9 (Cq-Ad), 41.2 (3Cα-Ad), 35.8 (3Cγ-Ad), 28.9 (3Cβ-Ad); IR (KBr), cm^{-1}: 1180 (ν_{SO_2sym}),

1340 (ν_{SO_2as}), 1540 (δ_{NH}), 1680 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{13}H_{19}N_5O_3S_2$) C, H, N.

4.2.2.22. 5-(Adamantan-1-yl-ureido)-4-methyl-Δ^2-1,3,4-thidiazoline-2-sulfonamide C9. White crystals, mp 209–211 °C (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 8.29 (s, 2H, SO_2NH_2), 7.18 (s, 1H, NH-Ad), 3.77 (s, 3H, N–CH_3), 2.01 (br s, 3H, 3CHβ-Ad), 1.94 (br s, 6H, 3CH_2α-Ad), 1.62 (br s, 6H, 3CH_2γ- Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 161.8 (C=N), 159.9 (C–SO_2NH_2), 156.0 (NHCO), 50.3 (Cq-Ad), 41.1 (3Cα-Ad), 36.1 (3Cγ-Ad), 28.9 (3Cβ-Ad); IR (KBr), cm^{-1}: 1175 (ν_{SO_2sym}), 1300 (ν_{SO_2as}), 1595 ($\nu_{C=O}$), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{14}H_{21}N_5O_3S_2$) C, H, N.

4.2.2.23. 4-[(3-Adamantan-1-yl-thioureido)-methyl]-benzenesulfonamide D6. White crystals, mp 203–205 °C (dec) (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.76 (d, $J = 8.3$ Hz, 2H, H3,5-Ph), 7.69 (t, $J = 5.6$ Hz, 1H, NH–CH_2), 7.41 (d, $J = 8.3$ Hz, 2H, H2,6-Ph), 7.31 (s, 2H, SO_2NH_2), 7.11 (s, 1H, NH-Ad), 4.70 (d, 2H, $J = 5.6$ Hz, CH_2-Ph), 2.14 (br s, 6H, 3CH_2α-Ad), 2.03 (br s, 3H, 3CHβ-Ad), 1.62 (br s, 6H, 3CH_2γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 181.01 (C=S), 143.82 (C4Ph), 142.53 (C1-Ph), 127.51 (2C, C3,C5-Ph), 125.67 (2C, C2,C6-Ph), 52.90 (CH_2-Ph), 45.88 (Cq-Ad), 43.01 (Cα-Ad), 41.15 (2C, 2Cα-Ad), 35.95 (2C, 2Cγ-Ad), 34.91 (Cγ-Ad), 29.04 (3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1300 ($\nu_{C=S}$), 1325 (ν_{SO_2as}), 1540 (δ_{NH}), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{18}H_{25}N_3O_2S_2$) C, H, N.

4.2.2.24. 4-[2-(3-Adamantan-1-yl-thioureido)-ethyl]-benzenesulfonamide D7. White crystals, mp 186–188 °C (dec) (EtOH); ^{1}H NMR (DMSO-d_6), δ, ppm: 7.75 (d, $J = 8.2$ Hz, 2H, H3,5-Ph), 7.40 (d, $J = 8.2$ Hz, 2H, H2,6-Ph), 7.30 (s, 2H, SO_2NH_2), 7.20 (t, $J = 5.0$ Hz, 1H, N*H*–CH_2), 6.96 (br s, 1H, NH-Ad), 3.59 (dt, $J = 7.0$, 5.0 Hz, 2H, NH–CH_2), 2.85 (t, $J = 7.1$ Hz, 2H, CH_2-Ph), 2.11 (br s, 6H, 3CH_2α-Ad), 2.01 (br s, 3H, 3CHβ-Ad), 1.60 (br s, 6H, 3CH_2γ-Ad); ^{13}C NMR (DMSO-d_6), δ, ppm: 180.66 (C=S), 143.77 (C4-Ph), 142.07 (C1-Ph), 129.14 (2C, C3,C5-Ph), 125.77 (2C, C2,C6-Ph), 52.72 (CH_2-NH), 43.96 (CH_2-Ph), 41.19 (3C, 3Cα-Ad), 35.98 (3C, 3Cγ-Ad), 35.44 (Cq-Ad), 29.04 (3C, 3Cβ-Ad); IR (KBr), cm^{-1}: 1160 (ν_{SO_2sym}), 1310 ($\nu_{C=S}$), 1320 (ν_{SO_2as}), 1550 (δ_{NH}), 2850 (ν_{CH_2sym}), 2910 (ν_{CH_2as}); Anal. ($C_{19}H_{27}N_3O_2S_2$) C, H, N.

4.3. Enzyme preparations

Human CA I and CA II cDNAs were expressed in *Escherichia coli* strain BL21 (DE3) from the plasmids pACA/hCA I and pACA/hCA II described by Lindskog et al.[47] Cell growth conditions were those described by this group,[48] and enzymes were purified by affinity chromatography according to the method of Khalifah et al.[49] Enzyme concentrations were determined spectrophotometrically at 280 nm, utilizing a molar absorptivity of 49 mM^{-1} cm^{-1} for CA I and 54 mM^{-1} cm^{-1} for CA II, respectively, based on $M_r = 28.85$ kDa for CA I, and 29.30 kDa for CA II, respectively.[50,51]

Initial rates of 4-nitrophenyl acetate hydrolysis catalyzed by different CA isozymes were monitored spectrophotometrically, at 400 nm, with a Cary 3 instrument interfaced with an IBM compatible PC.[52] Solutions of substrate were prepared in anhydrous acetonitrile; the substrate concentrations varied between 2×10^{-2} and 1×10^{-6} M, working at 25 °C. A molar absorption coefficient ε of 18,400 M^{-1} cm^{-1} was used for the 4-nitrophenolate formed by hydrolysis, in the conditions of the experiments (pH 7.40), as reported in the literature.[52] Nonenzymatic hydrolysis rates were always subtracted from the observed rates. Triplicate experiments were done for each inhibitor concentration, and the values reported throughout the paper are the mean of such results. Stock solutions of inhibitor (1 mM) were prepared in distilled–deionized water with 10–20% (v/v) DMSO (which is not inhibitory at these concentrations) 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 constant K_I was determined as described by Pocker and Stone.[52] Enzyme concentrations were 3.3 nM for hCA II, and 9 nM for hCA I.

4.4. Lipophilicity

The retention factor was determined with a immobilized artificial membrane (IAM) column (1 cm, $\varnothing = 3$ mm, Regis Technologies, Morton Grove, IL, USA) connected to a HPLC system (HP 1100 series, Agilent Technologies) equipped with a diode-array detector. The mobile phase was 0.01 M PBS (pH 7.4) at a flow of 0.7 mL/min. The column was maintained at 37 °C and the drugs detected at 254 nm. The retention factor ($\log k'$ IAM) is expressed as $(t_R - t_0)/t_0$ were t_R is the retention time of the drug and t_0 the void time.[37,38] Drugs (10^{-4} M) were dissolved in the mobile phase containing 1% DMSO as void marker. Values are means of three injections, the standard deviation is less than 1%. For each drug, the ClogP value was calculated by using the program ChemDraw Ultra 6.0.1.[39]

4.5. Maximal electroshock seizures test

Each compound was intraperitoneally injected to OF1 male mice (26–29 g; Iffa-Credo, Brusseles, Belgium) at a dose volume of 3 mL/kg. Three hours later, an electrical stimulus (50 mA; 60 Hz) was delivered for 0.2 s through corneal electrodes. Protection against seizures was defined as the abolition of the hind limb tonic extension and results expressed as the percentage of animals who did not convulse.[40,41] The preliminary screening was conducted with groups of 8–12 mice at an intraperitoneal dose of 30 mg/kg. For compounds **A8** and **A9**, dose–response were performed at a dosage of: 20, 10, 5 and 2.5 mg/kg. Nonlinear regression (GraphPad prism software 3.0) was used to determine the intraperitoneal dose (ED50) protecting 50% of mice against electrically induced convulsions.

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References and notes

1. Levy, R. H.; Mattson, R. H.; Meldrum, B. S. *Antiepileptic Drugs*; 4th ed.; Raven: New York, 1995.
2. Unverferth, K.; Rundfeldt, C. *Pharmaceuticals* **2000**, *2*, 469–488.
3. Barrios, I. A.; Bruno-Blanch, L. E.; Estiu, G. L. *J. Mol. Struct. (Theochem)* **2002**, *580*, 243–250.
4. Soderpalm, B. *Eur. J. Pain* **2002**, *6*, 3–9.
5. Berk, M.; Segal, J.; Janet, L.; Vorster, M. *Drugs* **2001**, *61*, 1407–1414.
6. Nicholson, B. *Int. Congress Symp. Ser.—Royal Soc. Med.* **2001**, *248*, 33–41.
7. MacDonald, K. J.; Young, L. T. *CNS Drugs* **2002**, *16*, 549–562.
8. Taylor, C. P. *Mech. Med. Neuropathic Pain* **2002**, 211–232.
9. Bialer, M.; Johannessen, S. I.; Kupferberg, H. J.; Levy, R. H.; Loiseau, P., et al. *Epilepsy Res.* **1999**, *34*, 1–41.
10. Duncan, J. S. *Br. J. Clin. Pharmacol.* **2002**, *53*, 123–131.
11. Perucca, E. *Fund. Clin. Pharmacol.* **2001**, *15*, 405–417.
12. Tomson, T. *Curr. Sci.* **2002**, *82*, 698–706.
13. Perucca, E. *Pharmacol. Res.* **1997**, *35*, 241–256.
14. Dodgson, S. J.; Shank, R. P.; Maryanoff, B. E. *Epilepsia* **2000**, *41*, S35–S39.
15. Shank, R. P.; Gardocki, J. F.; Streeter, A. J.; Maryanoff, B. E. *Epilepsia* **2000**, *41*, S3–S9.
16. Supuran, C. T.; Scozzafava, A. *Exp. Opin. Ther. Pat.* **2000**, *10*, 575–600.
17. Supuran, C. T.; Scozzafava, A. *Exp. Opin. Ther. Pat.* **2002**, *12*, 217–242.
18. Supuran, C. T.; Scozzafava, A.; Casini, A. *Med. Res. Rev.* **2003**, *23*, 146–189.
19. Casini, A.; Antel, J.; Abbate, F.; Scozzafava, A.; David, S.; Waldeck, H.; Schäfer, S.; Supuran, C. T. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 841–845.
20. Herrero, A. I.; Del Olmo, N.; Gonzalez-Escalada, J. R.; Solis, J. M. *Neuropharmacology* **2002**, *42*, 210–220.
21. *Carbonic Anhydrase—Its Inhibitors and Activators*; Supuran, C. T., Scozzafava, A., Conway, J., Eds.; CRC (Taylor & Francis Group): Boca Raton, FL, 2004; pp 1–368.
22. Maren, T. H. *Physiol. Rev.* **1967**, *47*, 595–781.
23. Pasternack, M.; Voipio, J.; Kaila, K. *Acta Physiol. Scand.* **1993**, *148*, 229–231.
24. Lee, J.; Taira, T.; Pihlaja, P.; Ransom, B. R.; Kaila, K. *Brain Res.* **1996**, *706*, 210–216.
25. Voipio, J.; Kaila, K. *Prog. Brain Res.* **2000**, *125*, 329–338.
26. Uusisaari, M.; Smirnov, S.; Voipio, J.; Kaila, K. *Neuropharmacology* **2002**, *43*, 563–572.
27. Rivera, C.; Voipio, J.; Payne, J. A.; Ruusuvuori, E.; Lahtinen, H.; Lamsa, K.; Pirvola, U.; Saarma, M.; Kaila, K. *Nature* **1999**, *397*, 251–255.
28. Grover, L. M.; Lambert, N. A.; Schwartzkroin, P. A.; Teyler, T. J. *J. Neurophysiol.* **1993**, *69*, 1541–1555.
29. (a) Tanimukai, H.; Inui, M.; Hariguchi, S.; Kaneko, Z. *Biochem. Pharmacol.* **1965**, *14*, 961–970; (b) Chufan, E. E.; Pedregosa, J. C.; Baldini, O. N.; Bruno-Blanch, L. *Farmaco* **1999**, *54*, 838–841.
30. Masereel, B.; Rolin, S.; Abbate, F.; Scozzafava, A.; Supuran, C. T. *J. Med. Chem.* **2002**, *45*, 312–320.
31. Almajan, L. G.; Supuran, C. T. *Rev. Roumaine Chim.* **1997**, *42*, 593–597.
32. Supuran, C. T.; Mincione, F.; Scozzafava, A.; Briganti, F.; Mincione, G.; Ilies, M. A. *Eur. J. Med. Chem.* **1998**, *33*, 247–254.
33. Scozzafava, A.; Menabuoni, L.; Mincione, F.; Briganti, F.; Mincione, G.; Supuran, C. T. *J. Med. Chem.* **1999**, *42*, 2641–2650.
34. Scozzafava, A.; Briganti, F.; Mincione, G.; Menabuoni, L.; Mincione, F.; Supuran, C. T. *J. Med. Chem.* **1999**, *42*, 3690–3700.
35. Casini, A.; Scozzafava, A.; Mincione, F.; Menabuoni, L.; Ilies, M. A.; Supuran, C. T. *J. Med. Chem.* **2000**, *43*, 4884–4892.
36. Scozzafava, A.; Supuran, C. T. *J. Enzyme Inhib.* **1999**, *14*, 343–363.
37. Ducarme, A.; Neuwels, M.; Goldstein, S.; Massingham, R. *Eur. J. Med. Chem.* **1998**, *33*, 215–223.
38. Taillardat-Bertschinger, A.; Galland, A.; Carrupt, P. A.; Testa, B. *J. Chromatogr. A* **2002**, *953*, 39–53.
39. ChemDraw Ultra 6.0.1, available from CambridgeSoft Com, Cambridge, MA.
40. Masereel, B.; Lambert, D.; Dogné, J. M.; Poupart, J. H.; Delarge, J. *Epilepsia* **1997**, *38*, 334–337.
41. Porter, R. J.; Cereghino, J. J.; Gladding, G. D.; Hesie, B. J.; Kupferberg, H. J.; Scoville, B.; White, B. G. *Cleve. Clin. Q* **1984**, *51*, 293–305.
42. Jitianu, A.; Ilies, M. A.; Briganti, F.; Scozzafava, A.; Supuran, C. T. *Metal-Based Drugs* **1997**, *4*, 1–7.
43. Jitianu, A.; Ilies, M. A.; Scozzafava, A.; Supuran, C. T. *Main Group Metal Chem.* **1997**, *20*, 151–156.
44. Cingolani, E. *Gazz. Chim. Ital.* **1948**, *78*, 275–282.
45. Scudi, J. V. *J. Am. Chem. Soc.* **1937**, *59*, 1480–1483.
46. Ilies, M. A.; Vullo, D.; Pastorek, J.; Scozzafava, A.; Ilies, M.; Caproiu, M. T.; Pastorekova, S.; Supuran, C. T. *J. Med. Chem.* **2003**, *46*, 2187–2196.
47. Lindskog, S.; Behravan, G.; Engstrand, C.; Forsman, C.; Jonsson, B. H.; Liang, Z.; Ren, X.; Xue, Y. Structure–Function Relations in Human Carbonic Anhydrase II as Studied by Site-Directed Mutagenesis. In *Carbonic Anhydrase—from Biochemistry and Genetics to Physiology and Clinical Medicine*; Botrè, F., Gros, G., Storey, B. T., Eds.; VCH: Weinheim, 1991; pp 1–13.
48. Behravan, G.; Jonsson, B. H.; Lindskog, S. *Eur. J. Biochem.* **1990**, *190*, 351–357.
49. Khalifah, R. G.; Strader, D. J.; Bryant, S. H.; Gibson, S. M. *Biochemistry* **1977**, *16*, 2241–2247.
50. Lindskog, S.; Coleman, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1964**, *70*, 2505–2508.
51. Steiner, H.; Jonsson, B. H.; Lindskog, S. *Eur. J. Biochem.* **1975**, *59*, 253–259.
52. Pocker, Y.; Stone, J. T. *Biochemistry* **1967**, *6*, 668–678.