Acetazolamide-like Carbonic Anhydrase Inhibitors with Topical Ocular Hypotensive Activity

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New carbonic anhydrase (EC 4.2.1.1) inhibitors were synthesized as potential drugs for the topical treatment of glaucoma. They were obtained by substituting the acetyl group of acetazolamide and methazolamide with bicarboxylic acids of different chain length (C4–C6). The terminal carboxyl was either kept free or esterified with alcohols of different size (C1–C12). A γ-aminovaleric derivative was also prepared. All compounds proved active as carbonic anhydrase inhibitors in vitro, with an average IC_{50} of about 0.5 μM. Some proved also to be topically active in vivo in lowering the artificially elevated intraocular pressure in rabbits. The most active compound, carrying a succinic acid side chain, is the most soluble in aqueous buffers. Its duration of action is about 8 h and it is under evaluation as a topical antiglaucoma drug. It is hypothesized that the duration of action could be longer in compounds having both the same high water solubility and partition coefficient.

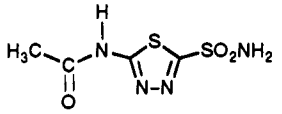
Introduction

High intraocular pressure (IOP) is most probably associated with the etiology of glaucoma, an optical disease evolving into the loss of peripheral vision, and eventually into blindness. Intraocular pressure is controlled primarily by the rate of aqueous humor formation and elimination, and any substance able to reduce the former or to increase the latter could be useful as an antiglaucoma drug.

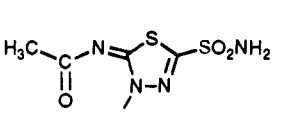
It is well-known that the systemic administration of inhibitors of carbonic anhydrase (CA),[1,2] an enzyme present in nonpigmented cells of ciliary structure, decreases the rate of aqueous humor formation and lowers the IOP. But all known carbonic anhydrase inhibitors (CAI), when orally administered at the large doses required to obtain a useful reduction of IOP, evoke side effects, which prevent or reduce their likelihood of use.[3-6] These side effects are probably caused by the inhibition of CA in extraocular tissues and could be reduced by topical administration of inhibitors.

When applied directly into the eye, the classical CAI, such as acetazolamide, methazolamide, and ethoxazolamide,[7] are poorly absorbed and ineffective in reducing the IOP.[8] Only recently have examples of topically effective inhibitors been reported.[9,10]

In this paper we describe a series of analogues of acetazolamide (1) and methazolamide (2) in which lipophilicity



1, acetazolamide



2, methazolamide

is varied systematically in order to change their water solubility and their partitioning coefficient and, possibly, to improve their ocular absorption. According to a rapid, primary screening test, some of them are topically effective in lowering the IOP.[11]

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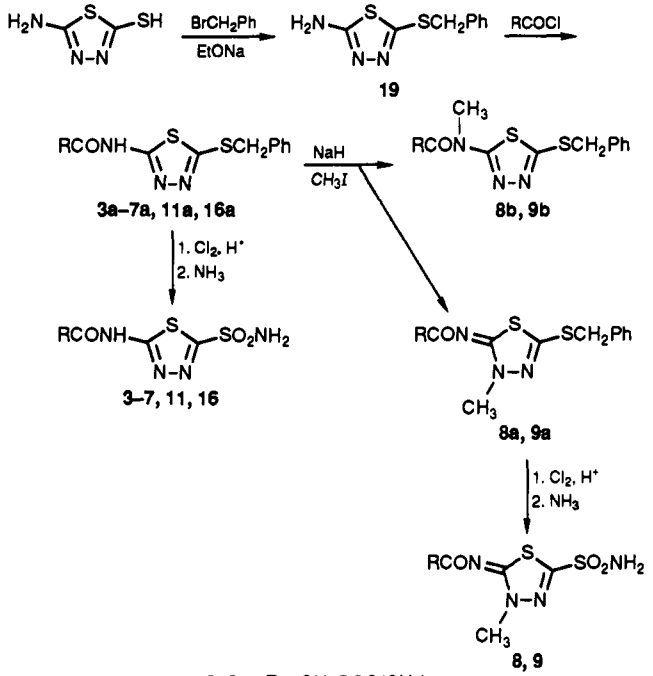
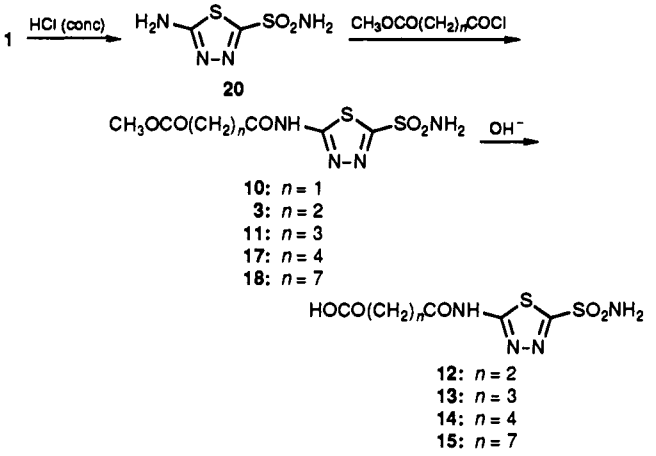
Scheme I



Scheme II



Strategy and Chemistry

In order to systematically vary the lipophilicity in a series of analogues of acetazolamide, without loss of the enzymatic inhibiting activity, the acetyl group of acetazolamide was substituted with other aliphatic acyl groups having a carbon chain of increasing length and carrying a second carboxylic group. These derivatives seemed particularly suitable for our study, because their synthesis was accomplished by acylating 5-amino-2-(benzylthio)-1,3,4-thiadiazole, **19**, with different monoester monochlorides of bicarboxylic acids and then applying a known procedure for conversion to the acetazolamide derivatives.[12-14] Only for a few derivatives was a different route necessary. Moreover, the physical–chemical properties of these new derivatives could be systematically varied by increasing the number of methylenes between the two carboxyls; the terminal carboxyl could be either kept free or esterified, and the nature and the size of the ester group could also be systematically varied. The presence of a free carboxyl group in some cases allowed for the preparation of suitable salts which increased water solubility.

Derivatives of succinic acid esterified with methyl (**3**), ethyl (**4**), *sec*-butyl (**5**), *n*-pentyl (**6**), and dodecyl (**7**) alcohols were prepared. Malonylamino (**10**), glutarylamino (**11**), adipoylamino (**17**), and azelaoylamino (**18**) methyl esters were synthesized as examples in a homologous series around **3**. As an example of a hydrophylic, basic terminal group, we synthesized a (4-aminovaleryl)amino derivative (**16**).

Derivatives with a free carboxylic group were prepared with succinic (**12**), glutaric (**13**), adipic (**14**), and azelaic (**15**) acids. The free malonic acid derivative was unstable and was prepared only as methyl ester **10**.

Methazolamide derivatives were prepared with succinic acid esterified with methyl (**8**) and pentyl (**9**) alcohols.

The new compounds were synthesized according either to Scheme I or II. Scheme I is similar to that used for the synthesis of acetazolamide and related compounds.[12-14] 5-Amino-2-(benzylthio)-1,3,4-thiadiazole, **19**, was acylated in Et_3N/4-DMAP with the desired monoester monochloride of a bicarboxylic acid prepared as described by Cason.[15,16] At this step methazolamide derivatives were prepared by methylation with CH_3I/NaH. Subsequently Cl_2 oxidation and treatment with liquid ammonia gave the final compound.

Compounds **3–9**, **11**, and **16** were synthesized according to Scheme I. Compound **10**, which is unstable under chlorine action, and compounds **12–15** and **17–18** were prepared by following Scheme II. The same procedure was applied also for the synthesis of compounds **3** and **11**. According to Scheme II, 5-amino-2-sulfamyl-1,3,4-thiadiazole (**20**), obtained by hydrolysis of **1**,[17] was directly acylated with the acid monochlorides of the methyl hemiesters. The free acids were subsequently obtained by mild alkaline hydrolysis of the corresponding esters.

Product **12** was also directly synthesized by reacting **20** with succinic anhydride that had been already used, bound to dextran, as a macromolecular CAI.[18]

Biological and Pharmacological Activity

All synthesized compounds were tested in vitro as CA (Boehringer) inhibitors. Eleven of them, the most representative, were tested in vivo as topical IOP lowering agents; they were dissolved (or suspended with methyl cellulose (200–400 cps)) in 66 mM phosphate buffer pH

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Table I. Comparison of Partitioning Ability, Solubility in Phosphate Buffer (66 mM), pH 7.6, and IC_{50} on CA for 1–18

compd	R	R_m	sol, mM	IC_{50}, μM
1	CH_3	−0.18	9.9	0.44
3	$CH_2CH_2COOCH_3$	−0.49	3.2	0.55
4	$CH_2CH_2COOCH_2CH_3$	−0.16	8.9	0.73
5	CH_2CH_2COO-*sec*-Bu	−0.05	3.7	0.37
6	$CH_2CH_2COO(CH_2)_4CH_3$	0.18	1.3	0.39
7	$CH_2CH_2COO(CH_2)_{11}CH_3$	0.90		0.52
8	methazol-$CH_2CH_2COOCH_3$	0.25	7.3	0.45
9	methazol-$CH_2CH_2COO(CH_2)_4CH_3$	0.37	1.4	0.31
10	CH_2COOCH_3	−0.63	16	0.94
11	$(CH_2)_3COOCH_3$	−0.39	11.5	0.91
12	CH_2CH_2COOH	−0.60	60.7[a]	0.55
13	$(CH_2)_3COOH$	−0.45	31.0[b]	0.52
14	$(CH_2)_4COOH$	−0.41	25.0[c]	0.52
15	$(CH_2)_7COOH$	−0.33	21.6	0.48
16	$(CH_2)_4NH_2$	−0.14	8.8	0.44
17	$(CH_2)_4COOCH_3$	0.00	<1	0.23
18	$(CH_2)_7COOCH_3$	0.18	<1	0.91

[a] pH of solution 5.8. [b] pH of solution 6.6. [c] pH of solution 6.8.

Table II. Effects of the Compounds on Intracellular Pressure (IOP)[a]

	IOP: base			IOP: t = 0 min			IOP: t = 10 min			IOP: t = 20 min			IOP: t = 40 min		
compd	RE	Δ_{IOP}	LE	RE	Δ_{IOP}	LE	RE	Δ_{IOP}	LE	RE	Δ_{IOP}	LE	RE	Δ_{IOP}	LE
3	15.67	(0.16)	15.83	15.17	(1.00)	16.17	25.33	(0.17)	25.50	19.50	(0.67)	20.17	16.50	(0.17)	16.67
4	15.33	(0.00)	15.33	13.33	(2.17)	15.50	20.33	(5.34)	25.67	17.33	(3.34)	20.67	15.67	(2.00)	17.67
5	15.67	(−0.34)	15.33	14.67	(0.83)	15.50	21.50	(4.17)	25.67	19.00	(1.17)	21.17	16.67	(0.50)	17.17
6	15.83	(−0.16)	15.67	15.00	(0.67)	15.67	22.67	(1.83)	24.50	20.00	(1.33)	21.33	17.83	(1.00)	18.83
8	15.50	(−0.17)	15.33	14.83	(1.17)	16.00	24.67	(1.16)	25.83	20.83	(0.67)	21.50	17.33	(0.50)	17.83
9	15.67	(0.16)	15.83	15.50	(0.17)	15.33	24.00	(1.17)	25.17	20.67	(1.33)	22.00	17.83	(0.17	18.00
10	16.17	(0.16)	16.33	15.87	(0.30)	16.17	23.17	(4.00)	27.17	19.67	(2.83)	22.50	17.00	(2.17)	19.17
12	15.67	(−0.17)	15.50	14.33	(1.17)	15.50	19.33	(6.64)	26.00	17.17	(4.50)	21.67	15.67	(3.33)	19.00
13	15.50	(0.00)	15.50	14.67	(0.66)	15.33	21.17	(4.33)	25.50	19.00	(3.00)	22.00	16.50	(2.33)	17.83
14	16.83	(−0.50)	16.33	16.33	(−0.16)	16.17	22.00	(3.17)	25.17	20.00	(1.67)	21.67	17.17	(0.83)	18.00
15	15.83	(0.17)	16.00	16.17	(−0.17)	16.00	25.17	(1.33)	26.50	19.50	(1.17)	20.67	17.00	(0.50)	17.50
16	15.83	(−0.33)	15.50	15.00	(0.83)	15.83	24.00	(2.17)	26.17	20.83	(0.67)	21.50	16.83	(0.50)	17.33
timolol[b]	18.53	(0.00)	18.53	15.77	(1.07)	16.84	18.57	(7.27)	25.84	19.38	(2.59)	21.97	18.77	(0.00)	18.77

[a] IOP, mmHg, lowering was determined by a transition ocular hypertension model. Each sample (0.1 mL of a 2% solution) was unilaterally instilled into the right eye of male New Zealand rabbits 2 h before intravenous injection of 20 mL/kg of a 5% glucose solution in water. Six animals were treated with each compound. Tonometryes were measured at 0, 10, 20, and 40 min from infusion. RE, right eye; LE, left eye. [b] Data from ref 22.

Table III. Topical Activity of Compound 12 as a Function of Time from Administration[a]

	IOP: base			IOP: t = 0 min			IOP: t = 10 min			IOP: t = 20 min			IOP: t = 40 min		
time (h)	RE	Δ_{IOP}	LE	RE	Δ_{IOP}	LE	RE	Δ_{IOP}	LE	RE	Δ_{IOP}	LE	RE	Δ_{IOP}	LE
4	15.50	(0.00)	15.50	13.33	(2.00)	15.33	18.50	(7.33)	25.83	18.33	(3.67)	22.00	16.33	(2.50)	18.83
8	15.67	(0.00)	15.67	14.83	(0.84)	15.67	22.23	(3.84)	26.17	18.67	(2.83)	21.50	16.50	(2.17)	18.67

[a] IOP, mmHg, measured as in Table II. RE, right eye; LE, left eye.

7.6; the resulting solution was made isotonic with NaCl and aseptic with benzalkonium chloride.

The results are summarized in Tables I–III together with the R_m values and solubility of the derivatives measured according to Biagi.[19]

Discussion

In Table I are reported IC_{50} values, R_m values as a measure of the partition coefficient, and solubility in a water buffers at pH 7.6 of the new compounds.

All tested compounds exhibited a good intrinsic inhibitory activity against CA in the range of potency of acetazolamide (1), in agreement with previously reported data.[1,2] This is shown also by those derivatives with a long carbon chain in the ester moiety. The variation of lipophilicity in these acetazolamide analogues has thus little influence on their CA inhibitory power.

On the contrary, as clear from Table II, the in vivo activity, after topical administration, is strongly dependent on the physical-chemical characteristics of the tested compounds.

Compounds 7, 17, and 18 were not tested because of their very low solubility. Only a few compounds, among those with a water solubility higher than 3.5 mM (4, 5, 10, 12, 13), elicited a significant IOP reduction.

Therefore, water solubility seems to be a necessary, but obviously not sufficient, condition for in vivo topical activity.

This conclusion was already reached by the researchers of the Merck group, which selected and developed compounds MK-927 and MK-417,[10] new potent, topically active CAI, with a thienothiopynen-2-sulfonamide structure.

Of the five compounds with a significant topical activity, three (4, 5, 10) are esters and two (12, 13) have a free carboxylic group. These latter ones are more effective than the former.

SAR[20] on compounds carrying a free carboxylic end

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group and tested in vivo indicate that only those derivatives with a short methylenic side chain have significant topical activity. By increasing of the length of the side chain, the partition coefficient increases, while both water solubility and topical activity decrease in parallel.

The available data are too few and neither a generalization nor a quantitative evaluation are possible; however, a direct dependence of activity on water solubility is clear.

By comparing the compounds carrying an esterified side chain and tested in vivo (**3**, **4**, **5**, **6**, **10**), those that have shown a significant level of activity (**4**, **5**, **10**) are more water soluble than the less active ones (**3**, **6**), but there is no apparent correlation between water solubility, partition coefficient, and activity. For example compound **10** has the highest solubility in water and the lowest partition coefficient, but it is less active in vivo than **4** and **5**, which have lower solubility and higher R_m.

Furthermore the comparison of the water solubility and in vivo activity of **4** and **12**, the most active compounds of the two series, shows that **12**, carrying a free carboxylic group, is much more water soluble than its ethyl ester **4** (60.7 vs 8.9 mM), while the in vivo activity of the former is only slightly higher than that of the latter. Therefore the solubility in water of these compounds is an essential requisite for their activity, but a certain degree of lipophilicity is a concomitant requisite as well.

In general the IOP lowering action of the active compounds is local because unilateral instillation of the examined compounds into the right eye had no effect on the artificially elevated IOP of the left eye.

On the basis of these results compound **12** was chosen for the study of its topical activity as a function of time. IOP was measured at the 4th and 8th hour after administering **12** to groups of rabbits (Table III). After the 4th hour the lowering of the IOP is coincident with that reported in Table II within the limits of experimental errors. After the 8th hour the lowering of the IOP is still measurable but of little intensity. It can be then concluded that the drug is effective for a time shorter than 8 h.

Compound **12** has been submitted to further pharmacological and toxicological evaluation. Its duration of action is similar to that of the recent Merck CAI mentioned above,[10] and its possible use as topical antiglaucoma agent requires multiple daily administrations.

This unfavorable feature of the pharmacological behavior of **12** can be explained by considering that it is very soluble in the aqueous humor, as it is in water buffers. Since the aqueous humor has a quick turnover, it is highly probable that **12** be rapidly cleared up, while substances that could bind to the internal ocular structures would be retained for a longer time and exert a depot effect.

The search for analogues of **12** having a higher affinity for the ocular tissues can be a probable development of this research for the discovery of longer lasting, topically active CAI.

Experimental Section

Melting points were determined with a Reichert-Jung Thermovar melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained with a Bruker VP 80 SY spectrometer for the benzyl compounds and with a Varian XL 300 spectrometer for sulfonamide compounds. ^{13}C-NMR spectra were obtained on a Varian XL 300 instrument operating at 75.4 MHz. Chemical shifts are given in ppm from TMS and were referenced against solvent residual signals. IR spectra were obtained with a Varian EM-360A IR spectrophotometer. A UV–vis DU Beckman 65 spectrophotometer was used for the determination of the solubility of compounds. Mass spectra were obtained with a Kratos MS 80 instrument, using the FAB MS technique (Gly + TDGE matrix). For analyzed compounds (C, H, S, N), values obtained were within 0.4% of theoretical values. Silica gel 60 (Merck, 70–230 mesh) was used for column chromatography and silica gel 60 F_{254} TLC sheets (Merck) were used to examine the purity of compounds and obtain R_f and R_m values.

All commercial acid chloride compounds were obtained from Fluka and Aldrich and used without any further purification. Triethylamine was dried on KOH before use. Methylene chloride was dried by distilling on P_2O_5. THF was dried by refluxing on sodium. Organic solutions were dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness on a Buchi rotary evaporator under water-aspirator pressure.

Acid–base treatment consists of solubilization of a product in NH_4OH, 30%, filtration of insoluble residues, and acidification with HCl to pH 4.

Preparation of Monoester Monochlorides. All monoester monochlorides have been obtained by following the Cason[15] method, opening the succinic anhydride with alcohols, and reacting them with $SOCl_2$ in order to obtain the respective monoester monochlorides. In the case of ethyl, *sec*-butyl, *n*-pentyl, and *n*-dodecyl alcohols, the reaction needs acid catalysis.

5-((Methoxysuccinyl)amino)-2-(benzylthio)-1,3,4-thiadiazole (3a). To a suspension of 5-amino-2-(benzylthio)-1,3,4-thiadiazole[12] (**19**) (4 g, 18 mmol) in 20 mL of dry CH_2Cl_2 were added Et_3N (3.7 mL, 27 mmol), succinyl chloride monomethyl ester (2.7 g, 18 mmol), and 4-DMAP (0.1 g, 0.8 mmol). The solution was stirred at room temperature under N_2 for 4 h. The reaction was monitored by TLC (10% CH_3OH–$CHCl_3$). To the mixture was added 2 N HCl (10 mL), and after extraction with CH_2Cl_2, the organic layers were washed with water, dried on Na_2SO_4, and concentrated to dryness. The solid product, crystallized from EtOH, gave 4.2 g of **3a** (70%): mp 156–161 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.8 (dt, 4 H, CH_2CH_2), 3.6 (s, 3 H, OCH_3), 4.4 (s, 2 H, CH_2 benzyl), 7.6 (s, 5 H, aryl); IR ($CHCl_3$) 3170, 1740, 1700, 1550, 1300, 1160 cm^{-1}.

5-((Ethoxysuccinyl)amino)-2-(benzylthio)-1,3,4-thiadiazole (4a). To a suspension of **19** (4.5 g, 20 mmol) in 40 mL of CH_2Cl_2 ware added Et_3N (4.4 mL, 30 mmol), succinyl chloride monoethyl ester (3.3 g, 20 mmol), and 4-DMAP (0.1 g, 0.8 mmol). The preparation is similar to that of compound **3a**, and the reaction was completed in 3 h (TLC: 5% CH_3OH–$CHCl_3$). Crystallization from EtOH gave 4.9 g of **4a** (75%): mp 140–143 °C; ^{1}H NMR ($CDCl_3$) δ 1.2 (t, 3 H, CH_3), 2.9 (m, 4 H, CH_2CH_2), 4.1 (q, 2 H, OCH_2), 4.5 (s, 2 H, CH_2 benzyl), 7.3 (s, 5 H, aryl); IR ($CHCl_3$) 3165, 1735, 1700, 1555, 1310, 1160 cm^{-1}.

5-((*sec*-Butoxysuccinyl)amino)-2-(benzylthio)-1,3,4-thiadiazole (5a). To a suspension of **19** (3.2 g, 14 mmol) in 20 mL of CH_2Cl_2 were added Et_3N (2.9 mL, 22 mmol), succinyl chloride *sec*-butyl ester (2.8 g, 14.5 mmol), and 4-DMAP (0.09 g, 0.7 mmol). The reaction was completed in 5 h (TLC: 6% CH_3OH–$CHCl_3$), and crystallization from EtOH gave 2.7 g of **5a** (50%): mp 133–135 °C; ^{1}H NMR ($CDCl_3$) δ 0.8 (d, 6 H, 2 CH_3), 1.9 (m, 1 H, CH), 2.9 (m, 4 H, CH_2CH_2), 3.8 (d, 2 H, OCH_2), 4.3 (s, 2 H, CH_2 benzyl), 7.3 (s, 5 H, aryl); IR ($CHCl_3$) 3160, 1730, 1700, 1555, 1305, 1160 cm^{-1}.

5-((Pentoxysuccinyl)amino)-2-(benzylthio)-1,3,4-thiadiazole (6a). To a suspension of **19** (5 g, 22 mmol) in 30 mL of CH_2Cl_2 were added Et_3N (4.6 mL, 34 mmol), succinyl chloride monopentyl ester (4.6 g, 22 mmol), and 4-DMAP (0.15 g, 1.2 mmol). After the mixture was stirred for 5 h (TLC: 6% CH_3OH–$CHCl_3$), the reaction was worked up as described for **3a**. Crystallization from EtOH gave 5.3 g of **6a** (60%): mp 128–130 °C; ^{1}H NMR ($CDCl_3$) δ 0.8 (t, 3 H, CH_3), 1.32 [m, 6 H, $(CH_2)_3$], 2.8 (m, 4 H, $-CH_2CH_2-$), 4.0 (t, 2 H, OCH_2), 4.35 (s, 2 H, CH_2 benzyl), 7.3 (s, 5 H, aryl); IR ($CHCl_3$) 3160, 1730, 1700, 1550, 1300, 1160 cm^{-1}.

5-((Dodecoxysuccinyl)amino)-2-(benzylthio)-1,3,4-thiadiazole (7a). To a suspension of **19** (3.7 g, 16 mmol) in 25 mL of CH_2Cl_2 were added Et_3N (3.4 mL, 25 mmol), succinyl chloride monododecyl ester (5 g, 16 mmol), and 4-DMAP (0.09 g, 0.7 mmol). The reaction was completed in 6 h. Crystallization from EtOH gave 4.4 g of **7a** (55%): mp 130–133 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H, CH_3), 1.3 [s, 20 H, $(CH_2)_{10}$], 2.9 (m, 4 H, CH_2CH_2), 4.1 (t, 2 H, OCH_2), 4.35 (s, 2 H, CH_2 benzyl), 7.25 (s, 5 H, aryl); IR ($CHCl_3$) 3165, 2920, 1732, 1700, 1300, 1160 cm^{-1}.

5-((Methoxyglutaryl)amino)-2-(benzylthio)-1,3,4-thiadiazole (11a). To a suspension of **19** (2 g, 9 mmol) in 15 mL of

CH_2Cl_2 were added Et_3N (0.9 mL, 13 mmol), glutaryl chloride monomethyl ester (1.4 g, 8.3 mmol), and 4-DMAP (0.09 g, 0.7 mmol). The reaction was completed in 4 h (TLC: 6% CH_3OH–$CHCl_3$). Crystallization from EtOH gave 3 g of **11a** (96%): mp 121–123 °C; ^1H NMR ($CDCl_3$) δ 1.9 (m, 2 H, CH_2), 2.5 [m, 4 H, $(CH_2)_2$], 3.5 (s, 3 H OCH_3), 4.3 (s, 2 H, CH_2 benzyl), 7.3 (s, 5 H, aryl); IR ($CHCl_3$) 3160, 1735, 1700, 1555, 1300 cm^{-1}.

5-((Methoxysuccinyl)amino)-2-mercapto-1,3,4-thiadiazole (3b). To a stirred suspension of 5-amino-3-mercapto-1,3,4-thiadiazole (5 g, 37 mmol) in 30 mL of CH_2Cl_2 at room temperature under a nitrogen atmosphere were added Et_3N (6 mL, 43 mmol), succinyl chloride monomethyl ester (5.6 mL, 37 mmol), and 4-DMAP (0.1 g, 0.8 mmol). The reaction was completed in 3 h (TLC: 10% CH_3OH–$CHCl_3$). To the mixture were added 100 mL of NH_4OH (30%) and 20 mL of CH_2Cl_2, and after extraction the aqueous layer was acidified with HCl to pH 4. The obtained product was filtered, and crystallization from EtOH gave 7 g of **3b** (76%): mp 183–186 °C; ^1H NMR (Py) δ 2.8 (m, 4 H, CH_2CH_2), 3.62 (s, 3 H, OCH_3); IR (KBr) 3225, 1695, 1580, 1310, 1160 cm^{-1}.

Methylation of 5-((Methoxysuccinyl)amino)-2-(benzylthio)-1,3,4-thiadiazole (8a,b). An oil suspension of NaH (50%) (0.6 g, 13 mmol) was added to **3a** (3 g, 9 mmol) dissolved in 90 mL of dried THF. Under N_2, CH_3I (8 mL, 0.09 mmol) was slowly added to the stirring mixture, at room temperature, and after 3 h (TLC: 5% CH_3OH–$CHCl_3$) the reaction was neutralized with 2 N HCl and extracted with diethyl ether; the organic extracts were washed with water, dried, filtered, and concentrated to dryness. The residue, chromatographed on silica gel (40% EtOAc–petroleum ether), gave 1.86 g (60%) of 5-((methoxysuccinyl)amino)-4-methyl-2-(benzylthio)-1,3,4-thiadiazole (**8a**), mp 76–78 °C, and 0.8 g (26%) of 5-((methoxysuccinyl)-*N*-methylamino)-2-(benzylthio)-1,3,4-thiadiazole (**8b**). **8a**: ^1H NMR ($CDCl_3$) δ 2.8 (dt, 4 H, CH_2CH_2), 3.6 (s, 3 H, OCH_3), 3.8 (s, 3 H, CH_3), 4.3 (s, 2 H, CH_2 benzyl), 7.2 (s, 5 H, aryl); IR ($CHCl_3$) 3090, 1740, 1620, 1500, 1300, 1160 cm^{-1}. **8b**: ^1H NMR ($CDCl_3$) δ 2.8 (dt, 4 H, $-CH_2CH_2-$), 3.6 (s, 3 H, OCH_3), 3.7 (s, 3 H, CH_3), 4.4 (s, 2 H, CH_2 benzyl), 7.3 (s, 5 H, aryl); IR ($CHCl_3$) 2970, 1735, 1675, 1410 cm^{-1}.

5-((Methoxysuccinyl)amino)-4-methyl-2-sulfamyl-1,3,4-thiadiazole (8). A suspension of **8a** (1 g, 2.8 mmol) in 15 mL of CH_3COOH (33%) was cooled at 0 °C, and chlorine gas was introduced in a fine stream for 2 h. The sulfonyl chloride was extracted with 50 mL of CH_2Cl_2, and the organic extracts were washed with saturated $NaHCO_3$ and water, dried, and evaporated. Since this product is very unstable it is better to maintain the temperature below 10 °C during the workup. The sulfonyl chloride obtained in this way (0.9 g, 2.7 mmol), dissolved in dried THF, was slowly added under N_2 atmosphere to 25 mL of freshly condensed liquid NH_3 at −78 °C. After remotion of NH_3 the solid residue purified by acid–base treatment gave 0.32 g of **8** (38%): mp 135–137 °C; λ_{max} 289; ^1H NMR (DMSO-d_6) δ 2.62 (t, 2 H, CH_2), 2.81 (t, 2 H, CH_2), 3.6 (s, 3 H, OCH_3), 3.97 (s, 3 H, CH_3), 8.23 (s′, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 28.7 (C-9), 33.7 (C-8), 38.1 (NCH_3), 51.2 (C-11), 157.6 (C-5), 164.2 (CSO_2NH_2), 172.7 (CO), 180.9 ppm (CONH); IR (KBr) 3320, 3220, 3120, 1715, 1642, 1500, 1330, 1180 cm^{-1}. Anal. ($C_8H_{12}N_4O_5S_2$) H, S, N; C: calcd 31.16; found 31.70.

Methylation of 5-((Pentoxysuccinyl)amino)-2-(benzylthio)-1,3,4-thiadiazole (9a,b). An oil suspension of NaH (50%) (0.5 g, 10.5 mmol) was added to **6a** (3 g, 7.8 mmol) in 100 mL of dried THF. Under N_2, 10 mL of CH_3I (10:1 m/m) were added to the stirred mixture. After 3 h (TLC: 2% CH_3COOH–$CHCl_3$) the reaction was neutralized with 2 N HCl and extracted with diethyl ether; the organic extracts were washed with water, dried, and evaporated. The residue purified by silica gel column gave 1.94 g (62%) of 5-((pentoxysuccinyl)amino)-4-methyl-2-(benzylthio)-1,3,4-thiadiazole (**9a**), mp 95–97 °C, and 0.87 g (27%) of 5-((pentoxysuccinyl)-*N*-methylamino)-2-(benzylthio)-1,3,4-thiadiazole (**9b**). **9a**: ^1H NMR ($CDCl_3$) δ 0.85 (t, 3 H, CH_3), 1.4 [m, 6 H, $(CH_2)_3$], 2.8 (m, 4 H, CH_2CH_2), 3.9 (s, 3 H, CH_3), 4.1 (t, 2 H, OCH_2), 4.3 (s, 2 H, CH_2 benzyl), 7.3 (s, 5 H, aryl); IR ($CHCl_3$) 1730, 1620, 1380, 1160, 975 cm^{-1}. **9b**: ^1H NMR ($CDCl_3$) δ 0.9 (t, 3 H, CH_3), 1.4 [m, 6 H, $(CH_2)_3$], 2.8 (m, 4 H, $-CH_2CH_2-$), 3.8 (s, 3 H, CH_3), 4.2 (t, 2 H, OCH_2), 4.5 (s, 2 H, CH_2 benzyl), 7.4 (s, 5 H, aryl); IR ($CHCl_3$) 1730, 1670, 1310, 1115 cm^{-1}.

5-((Pentoxysuccinyl)amino)-4-methyl-2-sulfamyl-1,3,4-thiadiazole (9). A suspension of **9a** (1.94 g, 4.7 mmol) in 30 mL of CH_3COOH (33%) was cooled at 0 °C, and chlorine gas was introduced in a fine stream for 2 h. The sulfonyl chloride was extracted with 100 mL of CH_2Cl_2, and the organic layer was washed with saturated $NaHCO_3$ and water to neutral pH, dried, and evaporated at low temperature. The crude sulfonyl chloride (1.7 g), dissolved in 6 mL of dry THF, was added in small portions, under N_2, to 40 mL of freshly condensed NH_3. The residue obtained after removing of excess NH_3, chromatographed on silica gel column (5% CH_3OH–$CHCl_3$), gave 1.12 g of **9** (64%): mp 95–97 °C; λ_{max} 290; ^1H NMR (DMSO-d_6) δ 0.85 (t, 3 H, CH_3), 1.27 (m, 4 H, $-CH_2CH_2-$), 1.55 (m, 2 H, CH_2), 2.65 (t, 2 H, CH_2CO), 2.82 (t, 2 H, $COCH_2$), 3.92 (s, 3 H, CH_3), 4.0 (t, 2 H, OCH_2), 8.26 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 13.7 (CH_3), 21.6 (C-14), 27.4 (C-12), 27.7 (C-13), 29.1 (C-9), 33.7 (C-8), 38.1 (C-4), 63.7 (C-11), 157.6 (C-5), 164.2 (CSO_2NH_2), 172.2 (CO), 181 ppm (CON); IR ($CHCl_3$) 3425, 3345, 1730, 1625, 1490, 1375, 1160 cm^{-1}. Anal. ($C_{12}H_{20}N_4O_5S_2$) C, H, S, N.

5-((Methoxysuccinyl)amino)-2-sulfamyl-1,3,4-thiadiazole (3). A suspension of **3a** (4 g, 11.7 mmol) in 50 mL of CH_3COOH (33%) was cooled at 0 °C, with stirring, and chlorine gas was introduced in a fine stream for 2 h. During this time the character of precipitate changed. The solid was filtered off, washed with cold water, and dried in vacuo. Under an atmosphere of dry N_2, sulfonyl chloride was slowly added to 60 mL of freshly condensed liquid NH_3 at −78 °C. The excess of NH_3 was removed by putting the flask in a water bath, and the colorless solid residue was redissolved in 2 N NH_4OH (5 mL) and precipitated by HCl at pH 4. The solid was filtered, washed with water, and dried and gave 1.6 g of **3** (50%): mp 173–175 °C; λ_{max} 298; ^1H NMR (DMSO-d_6) δ 2.76 (m, 4 H, CH_2CH_2), 3.59 (s, 3 H, OCH_3), 8.29 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 27.8 (C-9), 29.7 (C-8), 51.5 (CH_3), 161 (C-5), 164.2 (CSO_2NH_2), 171.7 (CONH), 172.8 ppm (CO); IR (KBr) 3320, 3220, 1718, 1700, 1360, 1335, 1160, 925 cm^{-1}. Anal. ($C_7H_{10}N_4O_5S_2$) C, H, S, N.

5-((Ethoxysuccinyl)amino)-2-sulfamyl-1,3,4-thiadiazole (4). A suspension of **4a** (4 g, 10 mmol) in 70 mL of CH_3COOH (33%) was cooled at 0 °C and stirred under chlorine gas for 3 h. The sulfonyl chloride was treated with liquid NH_3, and after evaporation of NH_3 the product obtained directly by crystallization from EtOH 95% gave 1.5 g of **4** (50%): mp 197–199 °C; λ_{max} 297; ^1H NMR (DMSO-d_6) δ 1.15 (t, 3 H, CH_3), 2.68 (t, 2 H, CH_2), 2.79 (t, 2 H, CH_2), 4.05 (q, 2 H, OCH_2), 8.35 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 14 (CH_3), 28.1 (C-9), 29.7 (C-8), 60 (C-11), 161 (C-5), 164.2 (CSO_2NH_2), 171.2 (CONH), 171.8 ppm (CO); IR (KBr) 3330, 1720, 1700, 1555, 1340, 1170 cm^{-1}. Anal. ($C_8H_{12}N_4O_5S_2$) C, H, S, N.

5-((*sec*-Butoxysuccinyl)amino)-2-sulfamyl-1,3,4-thiadiazole (5). A suspension of **5a** (2 g, 5.2 mmol) in 20 mL of CH_3COOH (33%) was cooled at 0 °C and stirred under chlorine gas for 3 h. The sulfonyl chloride was added to 35 mL of liquid NH_3, and after evaporation of NH_3 the product submitted to an acid–base treatment gave 1.5 g of **5** (85%): mp 201–203 °C; λ_{max} 275; ^1H NMR (DMSO-d_6) δ 0.85 (d, 6 H, 2 CH_3), 1.85 (m, 1 H, CH), 2.7 (t, 2 H, CH_2), 2.82 (t, 2 H, CH_2), 3.81 (d, 2 H, OCH_2); ^{13}C (DMSO-d_6) 18.7 (2 CH_3), 27.1 (C-12), 28.1 (C-9), 29.8 (C-8), 69.8 (C-11), 161 (C-5), 164.2 (CSO_2NH_2), 171.2 (CONH), 171.7 ppm (CO); IR (KBr) 3315, 3225, 1720, 1700, 1555, 1370, 1175 cm^{-1}. Anal. ($C_{10}H_{18}N_4O_5S_2$) C, H, S, N.

5-((Pentoxysuccinyl)amino)-2-sulfamyl-1,3,4-thiadiazole (6). A suspension of **6a** (2 g, 5 mmol) in 20 mL of CH_3COOH (33%) was cooled at 0 °C and stirred under chlorine gas for 4 h. The sulfonyl chloride was treated with liquid NH_3 and, after evaporation, the product, submitted to an acid–base treatment, gave 0.8 g of **6** (48%): mp 189–191 °C; λ_{max} 292; ^1H NMR (DMSO-d_6) δ 0.80 (t, 3 H, CH_3), 1.2 [s, 4 H, $(CH_2)_2$ pent], 1.55 (t, 2 H, OCH_2CH_2), 2.75 (dt, 4 H, $-CH_2CH_2-$), 4.0 (t, 2 H, OCH_2), 8.4 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 13.7 (CH_3), 21.7 (C-14), 27.5 (C-12), 28 (C-9), 28.1 (C-13), 29.8 (C-8), 64 (C-11), 161 (C-5), 164.2 (CSO_2NH_2), 171.2 (CONH), 171.8 ppm (CO); IR (KBr) 3340, 3240, 1720, 1710, 1550, 1365, 1165 cm^{-1}. Anal. ($C_{11}H_{18}N_4O_5S_2$) C, H, S, N.

5-((Dodecoxysuccinyl)amino)-2-sulfamyl-1,3,4-thiadiazole (7). A suspension of **7a** (4 g, 8 mmol) in 40 mL of CH_3COOH (33%) was cooled at 5 °C and stirred under chlorine gas for 2 h.

The sulfonyl chloride was added to 60 mL of liquid NH_3, and after evaporation of NH_3 the residue crystallized from 95% EtOH gave 2.5 g of 7 (70%): mp 184–186 °C; λ_{max} 290; ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3 H, CH_3), 1.25 [m, 20 H, $(CH_2)_{10}$], 2.65 (t, 2 H, CH_2CO), 2.8 (t, 2 H, $COCH_2$), 4.0 (t, 2 H, OCH_2), 8.32 (s', 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 13.3 (CH_3), 28.1 (C-9), 29.8 (C-8), 64 (C-11), 161 (C-5), 164.2 (CSO_2NH_2), 171.2 (CONH), 171.8 ppm (CO); IR (KBr) 3340, 3240, 1740, 1720, 1550, 1340, 1175 cm^{-1}. Anal. ($C_{16}H_{32}N_4O_5S_2$) C, S; H: calcd 7.19; found 7.60; N: calcd 12.49; found 12.02.

5-((Methoxyglutaryl)amino)-2-sulfamyl-1,3,4-thiadiazole (11). A suspension of 11a (3 g, 8.4 mmol) in 50 mL of CH_3COOH (33%) was cooled at 0 °C and stirred under chlorine gas for 2 h. The sulfonyl chloride was added to 50 mL of liquid NH_3 and after evaporation of NH_3 the product submitted to an acid–base treatment gave 1.85 g of 11 (70%): mp 181–184 °C; λ_{max} 272; ^{1}H NMR (DMSO-d_6) δ 1.85 (m, 2 H, CH_2), 2.4 (t, 2 H, CH_2), 2.55 (t, 2 H, CH_2), 3.6 (s, 3 H, OCH_3), 8.3 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 19.6 (C-9), 32.3 (C-10), 33.7 (C-8), 51.2 (C-12), 161 (C-5), 164.2 (CSO_2NH_2), 171.6 (CONH), 172.8 ppm (CO); IR (KBr) 3400, 3145, 1735, 1680, 1560, 1380, 1300, 1170 cm^{-1}. Anal. ($C_8H_{12}N_4O_5S_2$) C, H, S, N.

5-((5-Chlorovaleryl)amino)-2-(benzylthio)-1,3,4-thiadiazole (16a). To a suspension of 19 (2 g, 8.9 mmol) in 5 mL of dry CH_2Cl_2 at room temperature were added Et_3N (1.5 mL, 13 mmol), 5-chlorovaleryl chloride (1.4 g, 9 mmol), and 4-DMAP (0.09 g, 0.7 mmol). The reaction was stirred under N_2 for 3 h (TLC: 10% CH_3OH–$CHCl_3$). The solid obtained was filtered off, washed with water, and crystallized from EtOH and gave 2.7 g of 16a (90%): mp 146–148 °C. ^{1}H NMR ($CDCl_3$) δ 1.9 (m, 4 H, $-CH_2CH_2-$), 2.8 (t, 2 H, CH_2CO), 3.7 (t, 2 H, CH_2Cl), 4.5 (s, 2 H, CH_2 benzyl), 7.35 (s, 5 H, aryl); IR ($CDCl_3$) 3160, 1700, 1555, 1305 cm^{-1}.

5-((5-Aminovaleryl)amino)-2-sulfamyl-1,3,4-thiadiazole (16). A suspension of 16a (2 g, 6.1 mmol) in 40 mL of CH_3COOH (33%) was cooled at 0 °C and stirred under chlorine gas for 3 h. The sulfonyl chloride was added to 40 mL of liquid NH_3 and after evaporation of NH_3 crystallization from EtOH (95%) gave 0.75 g of 16 (45%): mp 209–212 °C; λ_{max} 269; ^{1}H NMR (DMSO-d_6) δ 1.7 (m, 4 H, CH_2CH_2), 2.5 (t, 2 H, CH_2CO), 3.6 (t, 2 H, CH_2NH_2), 8.3 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 22.4 (3 CH_2), 27.9 (2 CH_2CH_2CON), 33.9 (CH_2CON), 34.6 (CH_2NH_2), 161 (C-5), 164.3 (CSO_2NH_2), 172 ppm (CONH); IR (KBr) 3360, 3200, 1690, 1565, 1380, 1175 cm^{-1}. Anal. ($C_7H_{13}N_5O_3S_2$) C, H, N; S: calcd 22.96; found 21.90.

5-((Methoxymalonyl)amino)-2-sulfamyl-1,3,4-thiadiazole (10). To a suspension of 5-amino-2-sulfamyl-1,3,4-thiadiazole (20) (0.68 g, 3.8 mmol) in 2.5 mL of CH_2Cl_2 were added Et_3N (0.44 g, 4.3 mmol) malonyl chloride monomethyl ester (0.64 g, 4.6 mmol), and 4-DMAP (0.04 g, 0.3 mmol). The reaction was complete in 3 h (TLC: 10% CH_3OH–$CHCl_3$). To the mixture was added 3 mL of NH_4OH (30%), and after extraction with CH_2Cl_2, the aqueous layer was acidified with HCl to pH 4. The obtained solid filtered and dried gave 0.39 g of 10 (37%): mp 189–191 °C; λ_{max} 296; ^{1}H NMR (DMSO-d_6) δ 3.6 (s, 3 H, CH_3), 3.7 (s, 2 H, CH_2), 8.3 (s', 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 42 (CH_2), 52.3 (Ch_3), 161 (C-5), 164.7 (C-SO_2NH_2) 165.4 (CONH), 167 ppm (CO); IR (Nujol) 3260, 1740, 1680, 1560, 1260, 1175; FAB-MS m/z 281 (100) [M + H]$^+$, 202 (27), 108 (53). Anal. ($C_5H_8N_4O_5S_2$) C, H, S, N.

5-(Methoxyadipoyl)amino)-2-sulfamyl-1,3,4-thiadiazole (17). To a suspension of 20 (0.5 g, 2.8 mmol) in 3 mL of CH_2Cl_2 were added Et_3N (0.29 g, 2.9 mmol), adipoyl chloride monomethyl ester (0.6 g, 3.3 mmol), and 4-DMAP (26 mg, 0.2 mmol). The reaction was stirred for 3 h (TLC: 10% CH_3OH–$CHCl_3$). To the mixture was added 4 mL of NH_4OH (25%), and after extraction with CH_2Cl_2, the aqueous layer was acidified with HCl to pH 4. The obtained solid was filtered and dried and gave 0.32 g of 17 (35%): mp 172–174 °C; λ_{max} 267; ^{1}H NMR (DMSO-d_6) δ 1.55 (m, 4 H, 2 CH_2), 2.28 (t, 2 H, CH_2CO), 2.48 (t, 2 H, $OCOCH_2$), 3.57 (s, 3 H, CH_3O), 8.27 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 23.8 (2 CH_2), 32.9 (C-11), 34.5 (C-8), 51.3 (OCH_3), 161.1 (C-5), 164.3 (CSO_2NH_2), 172 (CONH), 173.2 ppm (CO); IR (Nujol) 3200, 1710, 1680, 1525, 1300, 1170 cm^{-1}; FAB-MS m/z 323 (100) [M + H]$^+$, 244 (23), 181 (23), 160 (76). Anal. ($C_9H_{14}N_4O_5S_2$) C, H, S, N.

5-((Methoxyazelaoyl)amino)-2-sulfamyl-1,3,4-thiadiazole (18). To a suspension of 20 (1 g, 5.5 mmol) in 3 mL of CH_2Cl_2 were added Et_3N (0.8 g, 7.9 mmol), azelaoyl chloride monomethyl ester (2.1 g, 9.5 mmol), and 4-DMAP (0.05 g, 0.4 mmol). The reaction was stirred for 3 h (TLC: 20% CH_3OH–$CHCl_3$). To the mixture was added 4 mL of NH_4OH (30%), and after extraction with CH_2Cl_2, the aqueous layer was acidified with HCl to pH 4. The product obtained, filtered, and crystallized from EtOH–H_2O (1:1) gave 0.95 g of 18 (47%): mp 176–178 °C; λ_{max} 267; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 6 H, 3 CH_2), 1.6 (m, 4 H, 2 CH_2CH_2CO), 2.3 (t, 2 H, CH_2CONH), 2.5 (t, 2 H, CH_2COO), 3.6 (s, 3 H, OCH_3), 8.3 (s, 2 H, SO_2NH_2); ^{13}C NMR (DMSO-d_6) 34.7 (CH_2CO), 33.2 (CH_2CON), 51 (OCH_3), 28 (2 CH_2CH_2CO), 24 (3 CH_2), 161 (C_5), 164 (CSO_2NH_2), 172 (CONH), 173 ppm (CO); IR (Nujol) 3280, 1740, 1680, 1540, 1320, 1170 cm^{-1}; FAB-MS m/z 365 (100) [M + H]$^+$, 286 (30), 181 (51). Anal. ($C_{12}H_{20}H_4O_5S_2$) C, H, S, N.

5-((Hydroxysuccinyl)amino)-2-sulfamyl-1,3,4-thiadiazole (12). Compound 3 (5 g, 17 mmol) was dissolved in 20 mL of NaOH (5%), and the reaction was heated with stirring at 60 °C for 30 min. After cooling, the solution was acidified with HCl to pH 4. The solid obtained, filtered, and washed with water gave 3.3 g of 12 (70%): mp 196–198 °C; λ_{max} 269; ^{1}H NMR (DMSO-d_6) δ 2.68 (dt, 4 H, CH_2CH_2), 8.3 (s, 2 H, SO_2NH_2), 12.7 (s', 1 H, OH); ^{13}C NMR (DMSO-d_6) 27.8 (C-9), 29.5 (C-8), 160.6 (C-5), 163.7 (CSO_2NH_2), 171 (CONH), 172.9 ppm (CO); IR (KBr) 3520, 3220, 1732, 1723, 1370, 1170 cm^{-1}. Anal. ($C_6H_8N_4O_5S_2$) C, H, S, N.

5-((Hydroxyglutaryl)amino)-2-sulfamyl-1,3,4-thiadiazole (13). Compound 11 (0.95 g, 3 mmol) was dissolved in 20 mL of NaOH (5%), and the reaction was heated at 60 °C under stirring for 30 min. After cooling the solution was acidified with HCl to pH 4. The solid obtained, filtered, and washed with water gave 0.5 g of 13 (55%): mp 242–244 °C; λ_{max} 268; ^{1}H NMR (DMSO-d_6) δ 1.9 (q, 2 H, CH_2), 2.3 (t, 2 H, CH_2), 2.6 (t, 2 H, CH_2), 8.4 (s, 2 H, SO_2NH_2), 12.6 (s', 1 H, COOH); ^{13}C NMR (DMSO-d_6) 19.7 (C-9), 32.7 (C-10), 33.9 (C-8), 161 (C-5), 164.3 (CSO_2NH_2), 171.8 (CONH), 173.9 ppm (CO); IR (Nujol) 3250, 3170, 1710, 1670, 1510, 1335, 1220, 1180 cm^{-1}; FAB-MS m/z 295 (48), 181 (100) [M + H]$^+$, 114 (81), 99 (88). Anal. ($C_7H_{10}N_4O_5S_2$) C, H, S, N.

5-((Hydroxyadipoyl)amino)-2-sulfamyl-1,3,4-thiadiazole (14). Compound 17 (0.4 g, 1.2 mmol) was dissolved in 10 mL of NaOH (5%), and the reaction was warmed at 60 °C under stirring for 30 min. After cooling the solution was acidified with HCl to pH 4. The solid obtained, filtered, and washed with water gave 0.26 g of 14 (68%): mp 215–217 °C. λ_{max} 267; ^{1}H NMR (DMSO-d_6) δ 1.54 (m, 4 H, 2 CH_2), 2.2 (t, 2 H, CH_2CONH), 2.5 (t, 2 H, CH_2COO), 8.4 (1, 2 H, SO_2NH_2), 12 (s', 1 H, COOH); ^{13}C NMR (DMSO-d_6) 24 (2 CH_2), 33 (C-11), 34.5 (C-8), 161.1 (C-5), 164.3 (CSO_2NH_2), 172 (CONH), 174.3 ppm (CO); IR (Nujol) 3200, 1740, 1680, 1540, 1310, 1170 cm^{-1}; FAB-MS m/z 309 (57), 207 924), 145 (31), 114 (100) [M + H]$^+$. Anal. ($C_8H_{12}N_4O_5S_2$) C, H, S, N.

5-((Hydroxyazelaoyl)amino)-2-sulfamyl-1,3,4-thiadiazole (15). Compound 18 (0.95 g, 2.6 mmol) was dissolved in 20 mL of NaOH (5%), and the reaction was heated at 60 °C with stirring for 45 min. After cooling the solution was acidified with HCl to pH 4. The solid obtained, filtered, and washed with water gave 0.7 g of 15 (77%): mp 199–200 °C; λ_{max} 269; ^{1}H NMR (DMSO-d_6) δ 1.25 (s, 6 H, 3 CH_2), 1.54 (m, 4 H, 2 CH_2CH_2CO), 2.2 (t, 2 H, CH_2CONH), 2.5 (t, 2 H, CH_2COO), 8.4 (s, 2 H, SO_2NH_2), 12 (s', 1 H, COOH); ^{13}C NMR (DMSO-d_6) 24 (3 CH_2), 28 (2 CH_2CH_2CO), 33.6 (CH_2CON), 34.8 (CH_2CO), 161 (C-5), 164 (CSO_2NH_2), 172 (CONH), 174 ppm (CO); IR (Nujol) 3250, 1725, 1680, 1545, 1170 cm^{-1}; FAB-MS m/z 351 (100) [M + H]$^+$, 272 (40), 181 (72). Anal. ($C_{11}H_{18}N_4O_5S_2$) C, H, S, N.

Solubility. The solubility of each compound was determined by measuring the UV absorbance, at each λ_{max} (see the Experimental Section), of saturated solutions prepared by stirring for 3 h at 25 °C an excess of the compound in 66 mM phosphate buffer (pH 7.6). The insoluble matter was filtered off and the solution diluted to give measurable absorbance values. The solubility was determined by linear extrapolation.

Activity Measurements. The ability of compounds 3–18 to inhibit CA (CAII, 2000 units/mg; Boehringer) in vitro was measured in comparison to acetazolamide by potentiometric analysis using a CO_2 electrode, according to the method of Maren.[21]

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The samples were dissolved in phosphate buffer 20 mM (pH 7.0) at concentrations 0.1, 0.3, 0.6, and 1.0 μM.

The inhibition effect was monitored for 60 min, and the IC_{50} was derived from the inhibition curves.

The ability of the selected compounds to lower the IOP in vivo was measured in New Zealand rabbits by the method of Bonomi[22] after artificial elevation of the IOP by intravenous injection of a 5% glucose solution in water.

Registry No. 1, 59-66-5; 3, 129504-06-9; 3a, 129504-20-7; 3b, 59133-61-8; 4, 129504-07-0; 4a, 129504-21-8; 5, 140696-97-5; 5a, 140696-98-6; 6, 129504-08-1; 6a, 129504-23-0; 7, 129504-10-5; 7a, 129504-28-5; 8, 129504-15-0; 8a, 129504-38-7; 8b, 129504-39-8; 9, 129504-17-2; 9a, 129524-72-7; 9b, 129504-42-3; 10, 140696-99-7; 11, 129504-11-6; 11a, 129504-30-9; 12, 78851-85-1; 13, 140697-00-3; 14, 138080-12-3; 15, 140697-01-4; 16, 129504-13-8; 16a, 140833-97-2; 17, 140697-02-5; 18, 140697-03-6; 19, 25660-71-3; 20, 14949-00-9; $CH_3OCO(CH_2)_3COCl$, 1490-25-1; $C_2H_5OCO(CH_2)_2COCl$, 14794-31-1; sec-$C_4H_9OCO(CH_2)_2COCl$, 140697-04-7; n-$C_5H_{11}OCO(CH_2)_2COCl$, 35444-35-0; $C_{12}H_{25}OCO(CH_2)_2COCl$, 41086-58-2; $CH_3OCO(CH_2)_3COCl$, 1501-26-4; $Cl(CH_2)_4COCl$, 1575-61-7; CH_3OCOCH_2COCl, 37517-81-0; $CH_3OCO(CH_2)_4COCl$, 35444-44-1; $CH_3OCO(CH_2)_7COCl$, 56555-02-3; 5-amino-2-mercapto-1,3,4-thiadiazole, 2349-67-9; 5-[(methoxysuccinyl)amino]-4-methyl-1,3,4-thiadiazole-2-sulfonyl chloride, 129504-40-1; 5-[(pentoxysuccinyl)amino]-4-methyl-1,3,4-thiadiazole-2-sulfonyl chloride, 129520-47-4; 5-[(methoxysuccinyl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 129504-19-4; 5-[(ethoxysuccinyl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 129504-22-9; 5-[(sec-butoxysuccinyl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 140697-05-8; 5-[(pentoxysuccinyl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 129504-24-1; 5-[(dodecoxysuccinyl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 129504-29-6; 5-[(methoxyglutaryl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 129504-31-0; 5-[(5-aminovaleryl)amino]-1,3,4-thiadiazole-2-sulfonyl chloride, 140697-06-9; carbonic anhydrase, 9001-03-0.

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Synthesis of the Acridone Alkaloids Glyfoline and Congeners. Structure–Activity Relationship Studies of Cytotoxic Acridones

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Glyfoline (4, 1,6-dihydroxy-10-methyl-2,3,4,5-tetramethoxyacridin-9-one) and its congeners were synthesized for evaluation of their cytotoxicity. A detailed structure–activity relationships (SAR) of these acridone derivatives were also studied. To study the SAR of glyfoline analogues, substituent(s) at C-1 and C-6 and at the heterocyclic nitrogen of glyfoline nucleus were modified. Nitro- and amino-substituted glyfoline analogues were also synthesized to study the effects of substituent(s) (electron-withdrawing vs electron-donating) on their cytotoxicity. These compounds were synthesized via the Ullmann condensation of anthranilic acids with iodobenzenes or 2-chlorobenzoic acids with aniline derivatives. The SAR studies showed that 1-hydroxy-9-acridones were more active than their 1-OMe derivatives against cell growth of human leukemic HL-60 cells in culture. Replacement of NMe of glyfoline with NH or $N(CH_2)_2NEt_2$ resulted in either total loss or dramatic reduction of cytotoxicity. Glyfoline congeners with nitro function at the A-ring were inactive, while compounds with amino substituent were shown to be cytotoxic in vitro.

Several 9-acridones have been found to exhibit anticancer activity. 9-Acridone derivatives with or without an alkyl side chain attached to the N-position were synthesized and studied for their antitumor activity.[1] Among these, *N*-[2-(dialkylamino)ethyl]-1-nitro-9-acridones (1, Figure 1) were shown to have antitumor activity in the S-180 system in vivo, and these have undergone extensive preclinical testing.[2] It was also reported that the biscationic side-chain-substituted 9-acridone (2, Figure 1) can act as an acceptable chromophore for DNA intercalation.[3]

Recently, the structure–activity relationships of 50 natural acridone alkaloids have been studied for their effects on the inhibition of cell growth and macromolecule biosynthesis of human promyelocytic leukemic HL-60 cells.[4] It was found that 23 out of the 50 alkaloids were more active than acronycine (3, Figure 1), an antineoplastic alkaloid used in clinical trials.[5] For inhibition of cell growth of human leukemic HL-60 cells in vitro, the most potent compound in this series was found to be glyfoline (4, 1,6-dihydroxy-10-methyl-2,3,4,5-tetramethoxyacridin-9-one), with an IC_{50} of 1.1 μM, while acronycine had an IC_{50} of 26.2 μM.

Glyfoline was originally isolated from *Glycosmis citrifolia* (Willd.) (Rutaceae).[6] The structure of this alkaloid,

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