the residue was crystallized from EtOAc/petroleum ether (bp 60–80 °C): yield 0.3 g (12%); mp 90–91 °C.

2-[*N*-(2-Aminopropyl)-*N*-benzylamino]-1-phenylethanol Hydrochloride. A mixture of 2-(benzylamino)-1-phenylethanol (11.35 g, 0.05 mol), MeCN (100 mL), KI (0.1 g), and chloroacetone (2.32 g, 0.025 mol) was heated under reflux for 1.5 h, then cooled, and filtered, and the filtrate was evaporated to dryness.

A mixture of the residue, hydroxylamine hydrochloride (3.0 g, 0.05 mol), K_2CO_3 (7.9 g, 0.05 mol), H_2O (10 mL), and EtOH (50 mL) was refluxed for 2 h and then evaporated to near dryness. Water (100 mL) was added, the mixture was extracted with EtOAc (3 × 50 mL), and the combined EtOAc extracts were dried (Na_2SO_4) and evaporated to dryness. A 70%, w/v, solution of sodium bis(2-methoxyethoxy)aluminum hydride ("Red-Al"; 29.8 mL, 0.1 mol) in benzene was added over 0.5 h to a stirred solution of the residue in toluene (100 mL), and the solution was stirred at room temperature for 18 h.

The stirred mixture was acidified with 2 N HCl, the aqueous phase was separated, and the toluene phase was extracted again with 2 N HCl (100 mL). The combined aqueous acid extracts were basified with 10 N NaOH and extracted with $CHCl_3$ (3 × 100 mL), and the combined chloroform extracts were dried ($MgSO_4$) and evaporated to dryness. The residue was dissolved in Et_2O, ethereal HCl was added, the Et_2O was decanted, and the residual gum was crystallized from 5% MeOH/acetonitrile: yield 0.44 g (5%); mp 197–198 °C. Anal. ($C_{18}H_{24}ON_2$) C, H, N.

The mother liquors were evaporated to dryness, and the residue was stirred with water (50 mL) and filtered. The filtrate was basified with 10 N NaOH and extracted with Et_2O, and the extracts were dried (Na_2SO_4) and evaporated to give crude base (1.7 g, 24%), which was used without further purification to prepare compound **44**.

2-[[2-(Phenylacetamido)propyl]amino]-1-phenylethanol (44). Phenylacetyl chloride (0.98 g, 0.0063 mol) was added to a stirred solution of 2-[*N*-(2-aminopropyl)-*N*-benzylamino]-1-phenylethanol (1.7 g, 0.006 mol) and triethylamine (1 mL) in toluene (50 mL), and the mixture was stirred for 0.5 h and then washed successively with 1 N NaOH (20 mL) and water (20 mL). The toluene phase was dried (Na_2SO_4) and evaporated to dryness.

A solution of the residue in EtOH was hydrogenated over 30% Pd/C at room temperature and atmospheric pressure and then filtered, and the filtrate was evaporated to dryness. The residue was crystallized from EtOAc: yield 0.4 g (21%); mp 136–137 °C.

***N*-(2-Aminoethyl)cyclopentanecarboxamide (58).** A mixture of methyl cyclopentanecarboxylate and (12.8 g, 0.1 mol) ethylenediamine (24 g, 0.4 mol) was heated at 100 °C for 18 h, cooled, and then poured into water (100 mL). The resulting suspension was filtered, and the filtrate was evaporated to dryness. A solution of the residue in EtOAc was added to a solution of oxalic acid in EtOAc, and the precipitated oxalate was collected and crystallized from EtOH: yield 12 g (49%); mp 164–165 °C.

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Cerebrovasodilatation through Selective Inhibition of the Enzyme Carbonic Anhydrase. 2. Imidazo[2,1-*b*]thiadiazole and Imidazo[2,1-*b*]thiazolesulfonamides

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A series of imidazo[2,1-*b*]thiadiazole and imidazo[2,1-*b*]thiazolesulfonamide carbonic anhydrase inhibitors is described and their anticonvulsant activities are listed. Many of the compounds have the same degree of ionization as acetazolamide and methazolamide, but their higher lipophilicity means that they are more able to penetrate into the central nervous system. One compound, 6-*tert*-butyl-2-sulfamoylimidazo[2,1-*b*]-1,3,4-thiadiazole (**8**, UK-15 454) had an anticonvulsant ED_{50} of 2.6 mg/kg when administered orally to mice. **8** selectively increased cerebral blood flow in animals without producing a high level of metabolic acidosis.

Because of the increase in the number of elderly people, cerebrovascular disease is now becoming a major medical and social problem; there is, therefore, an urgent need to discover an effective therapy that will improve the function of the aging brain. It is known that the degree and extent of the neurological deficit correlate with decreases in both cerebral blood flow and oxygen utilization,[1-3] but it is not known whether either of these constitutes the primary defect. Current therapy is mainly confined to vasodilator drugs,[4] even though these are usually unselective in their vasodilator action and frequently lower blood pressure.

In the previous publication,[5] we described how 4-[(4-methoxypiperidino)sulfonyl]-2-chlorobenzenesulfonamide (UK-12 130) caused a selective increase in cerebral blood flow in man and animals by raising CO_2 levels locally through inhibition of brain and/or erythrocyte carbonic anhydrase. By this means, autoregulation[6,7] was surmounted and, since blood pressure was not decreased,[8] CO_2 achieved a dilation limited only by the responsiveness of the cerebral vasculature.

The carbonic anhydrase inhibitor acetazolamide is also known to increase cerebral blood flow in patients with cerebrovascular disease.[8-10] However, acetazolamide is a potent inhibitor of carbonic anhydrase in the kidney, and the increase in cerebral blood flow is always accompanied by a marked diuresis[11] which eventually leads to a metabolic acidosis.

The related carbonic anhydrase inhibitor methazolamide is able to penetrate erythrocytes very readily and to enter

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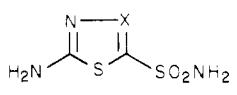
the central nervous system much more easily than acetazolamide.[12] Two factors contributing to this superior penetration into the central nervous system by methazolamide are (i) higher lipophilicity and (ii) lower plasma protein binding.

The aim of our synthetic program was to obtain carbonic anhydrase inhibitors which possessed a selectivity in vivo for the enzyme in brain (and erythrocytes). It seemed worthwhile, therefore, to investigate lipophilic sulfonamides which shared some common structural features with methazolamide. Accordingly, we now describe a series of imidazo[2,1-*b*]-1,3,4-thiadiazole and related imidazo-[2,1-*b*]thiazolesulfonamides.

Chemistry. 2-Amino-5-sulfamoyl-1,3,4-thiadiazole (**1**, X = N) was prepared by acid hydrolysis of acetazolamide



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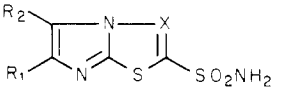

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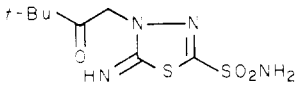
according to the method of Petrow.[13] The sulfonamides **2** (X = N) were obtained in yields ranging from 14 to 75% by heating **1** for 24 h in EtOH at reflux temperature with the appropriate α-bromo ketone. 2-Amino-5-sulfamoylthiazole (**1**, X = CH) was prepared from 2-acetamidothiazole according to the method of Backer and Keverling,[14] and the sulfonamides **2** (X = CH) were prepared as described above. Condensation of a bromo ketone, e.g., α-bromopinacolone, with an amino azole such as **1** is normally reckoned to involve alkylation on the ring nitrogen to give an intermediate such as **3**, which usually is



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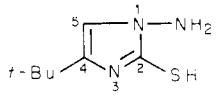

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not isolated and which readily cyclizes to the bicyclic structure **2** (R_1 = *t*-Bu; R_2 = H; X = N). X-ray crystallography[15] on this *tert*-butyl compound (listed as **8**, Table I) confirmed the structural assignment to be correct. Heating **8** in 5 N NaOH caused cleavage of the thiadiazole ring system and gave 1-amino-2-mercapto-4-*tert*-butylimidazole (**4**) in 91% yield.

Similar ring cleavage of fused thiadiazoles has been previously reported.[16,17] The unambiguous structural identification of **8**, by means of X-ray crystallography, now enables the ring carbon atoms of **4** to be correctly identified in the ^{13}C NMR spectrum. Thus, in Me_2SO-d_6, C(2) appears as a singlet at 158.17 ppm, C(4) as a singlet at 135.11 ppm, and C(5) as a doublet centered on 112.32 ppm (J_{CH} = 197.3 Hz).

Biology. The anticonvulsant effect of the primary sulfonamides is mediated through carbonic anhydrase inhibition.[12] Direct correlation between in vitro carbonic anhydrase activity and a specific in vivo effect is unfortunately not possible, since the latter depends on an adequate concentration of drug reaching the desired site of action. In rats, carbonic anhydrase inhibitors as a class do not readily pass the blood–brain barrier, and in this species anticonvulsant activity depends almost entirely on inhibition of the enzyme in erythrocytes.[18] In mice, the enzyme in brain, in erythrocytes, or at both sites is involved, the determining factors being the potency of the drug and its comparative ease of entry into both brain and erythrocytes.[18]

Harper[19] has suggested that control of cerebral blood flow is mediated by a predominantly metabolic mechanism via changes in intraparenchymal resistance (precapillary arterioles and small pial vessels). Alterations in pH, with consequent changes in intraparenchymal resistance, occur either generally as a consequence of changes in blood gas composition or locally from CO_2 and, to a small extent, from lactate generated by cerebral glucose metabolism. In theory, inhibition of carbonic anhydrase in brain and/or erythrocytes should induce local increases in CO_2 in the vicinity of the intraparenchymal vessels. In order to identify compounds that might increase cerebral blood flow by raising CO_2 levels via carbonic anhydrase inhibition, the mouse electroshock test was used as the primary screen. The results are presented in Table I.

The in vitro inhibition of carbonic anhydrase was determined using enzymes prepared from mouse brain, kidney, and erythrocytes. The assay was based on the method of Philpot and Philpot.[20] None of the compounds showed any selectivity for enzyme from a particular tissue, the differences in ID_{50} values for each compound being within the limits of variation. Table II shows the results using enzyme from erythrocytes.

Results and Discussion

It has been established that the ionized form of a primary sulfonamide is the active species in the inhibition of the carbonic anhydrase enzyme.[21] However, since compounds pass through lipid barriers mostly in their un-ionized form, high ionization, coupled with low lipid solubility, prevents penetration of drug through the blood–brain barrier. For example, benzolamide (pK_a = 3.2) does not enter the brain of mice[22] and acetazolamide (pK_a = 7.4) does so only poorly. By contrast, methazolamide (pK_a = 7.2) enters the brain very readily, and the superior anticonvulsant activity of this drug, compared to that of acetazolamide, is directly attributable to this greater ability to cross the blood–brain barrier.[18] Lipid solubility is of great significance in determining membrane penetration rates, and the higher lipophilicity of methazolamide (log P = 0.2) compared to that of acetazolamide (log P = −0.25) is one important factor influencing the difference in activity between the two compounds. In seeking carbonic anhydrase inhibitors that would readily cross the blood–brain barrier, we decided to employ 2-sulfonamido-5-amino-1,3,4-thiadiazole (**1**, X = N) as a standard unit upon which to base compound design. Because of the structural relationship with methazolamide, the sulfonamide group on the thiadiazole ring could be expected to be at least 50% ionized at physiological pH. Addition of suitable substituents to the other parts of the ring system would

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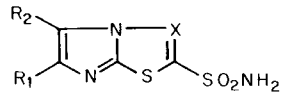
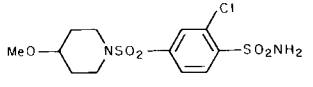
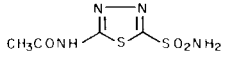
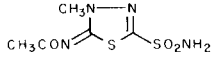
Table I. Chemical Data and Anticonvulsant Activity



no.	X	R_1	R_2	mp, °C	recrystn solvent[a]	mol formula[b]	% yield	ED_{50}, mg/kg po, protection against max electroshock in mice[c]	ED_{50} of 24/ED_{50} of compd anticonvulsant potency ratio rel to standard compd[d]
5	N	H	H	207-210[f]	EtOH	$C_4H_4N_4O_2S_2 \cdot HBr$	26	8	1.4
6	N	CH_3	H	237-239[f]	MeOH-EtOH	$C_5H_6N_4O_2S_2 \cdot HBr$	38	5	2.2
7	N	C_2H_5	H	214[f]	EtOH	$C_6H_8N_4O_2S_2 \cdot HBr$	27	5	1.6
8	N	$(CH_3)_3C$	H	254-255	EtOH	$C_8H_{12}N_4O_2S_2$	75	2.6 ± 0.15[g]	3.2
9	N	$C_{10}H_{15}$	H	278	EtOH	$C_{14}H_{18}N_4O_2S_2 \cdot HBr$	65	6	1.0
10	N	CH_3	CH_3	235-237[f]	MeOH-Et_2O	$C_6H_8N_4O_2S_2 \cdot HBr$	37	12	0.6
11	N	C_2H_5	CH_3	238-240[f]	EtOH	$C_7H_{10}N_4O_2S_2 \cdot HBr$	17	8	1.0
12	N	$-CH_2CH_2CH_2CH_2-$		241-242[f]	EtOH	$C_8H_{16}N_4O_2S_2 \cdot HBr$	42	>16	<0.5
13	N	C_6H_5	H	267-269[f]	acetone	$C_{10}H_8N_4O_2S_2$	48	16	0.6
14	N	4-OHC_6H_4	H	283-284[f]	MeOH-H_2O	$C_{10}H_8N_4O_3S_2$	54	10	0.9
15	N	4-$CH_3C_6H_4$	H	266-268[f]	acetone	$C_{11}H_{10}N_4O_2S_2$	44	>16	<0.6
16	N	2-OHC_6H_4	H	254-255[f]	MeOH	$C_{10}H_8N_4O_3S_2$	39	>16	<0.6
17	N	4-$AcNHC_6H_4$	H	317-319[f]	DMF-H_2O	$C_{12}H_{11}N_5O_3S_2$	14	>16	<0.4
18	N	4-$CH_3OC_6H_4$	H	269-271[f]	MeOH	$C_{11}H_{10}N_4O_3S_2$	26	>16	<0.6
19	N	2-C_4H_3S	H	284-286	DMF-H_2O	$C_8H_6N_4O_2S_3$	35	>16	<0.4
20	N	H	C_6H_5	230-232	EtOH-H_2O	$C_{10}H_8N_4O_2S_2$	28	>16	0.4
21	CH	H	H	>305[f]	H_2O	$C_5H_5N_3O_2S_2 \cdot HBr$	36	16	0.7
22	CH	CH_3	H	>255[f]	H_2O	$C_6H_7N_3O_2S_2 \cdot HBr$	20	6	1.3
23	CH	$(CH_3)_3C$	H	197-198	EtOH	$C_9H_{13}N_3O_2S_2$[e]	10	7	1.7
24	 (UK-12 130)							8.40 ± 0.19[h]	1.0
25	 (acetazolamide)							36	0.2
26	 (methazolamide)							12	0.75

[a] DMF, dimethylformamide. [b] All compounds were analyzed for C, H and N. [c] Compound 24 used as the standard in each determination: standard deviation ± 1.68. [d] The ratio ED_{50} of 24/ED_{50} of compound is based upon the ED_{50} value obtained for 24 during the separate electroshock experiments on each compound. [e] N: calcd, 16.2; found, 15.7. [f] Decomposition. [g] $n = 7$. [h] $n = 70$.

Table II. In Vitro Carbonic Anhydrase Activity (Erythrocytes) and pK_a Values

compd	concn, M, producing 50% inhibn	pK_a
8	$6.5 \times 10^{-10} \pm 0.12$[a]	6.94
13	$9.0 \times 10^{-10} \pm 0.13$	7.12
14	$6.0 \times 10^{-10} \pm 0.13$	8.16
23	$4.6 \times 10^{-8} \pm 0.12$	8.37
24	$1.9 \times 10^{-7} \pm 0.12$	8.76
acetazolamide	$2.0 \times 10^{-8} \pm 0.10$	7.4
methazolamide	$4.0 \times 10^{-8} \pm 0.10$	7.2

[a] Standard deviation.

then enable lipophilicity to be varied as required. The imidazo[2,1-*b*]thiadiazole and the related imidazo[2,1-*b*]-thiazolesulfonamides that were accordingly prepared are listed in Table I. The oral anticonvulsant ED_{50} values of these compounds in mice are given, together with the ED_{50} values obtained for the standard compound, 4-[(4-methoxypiperidino)sulfonyl]-2-chlorobenzenesulfonamide.[5]

As may be seen from Table I, compounds **5–7** all have anticonvulsant activity greater than that of the standard compound. This superior activity is most marked in the case of the *tert*-butyl analogue **8** (log P = 2.15). Increasing the lipophilicity of the system still further by use of an adamantyl moiety (**9**) lowered activity. 5,6-Disubstitution (**10** and **11**) was not especially favorable, nor was cyclization to the tricyclic sulfonamide **12**. The 6-phenyl compound **13** was some six times less potent than the 6-*tert*-butyl analogue **8**, but activity was slightly improved by incorporation of a *p*-hydroxyl group into the phenyl ring (**14**). Other substituents on this phenyl ring led to substantial loss of activity (**15–18**). Both replacement of phenyl by thienyl **19** and transferance of the phenyl group to the 5-position (**20**) of the imidazo[2,1-*b*]thiadiazole system were unfavorable. Evaluation of the related imidazo[2,1-*b*]thiazolesulfonamides **21–23** showed that good activity was also achieved with these compounds, the *tert*-butyl analogue **23** being the most noteworthy.

Experiments in rats were carried out on selected compounds, measuring urine volume and electrolyte excretion. ED_{50} values for urine output after oral administration were 5 (**6** and **8**) and 6 mg/kg (**9** and **23**). Values for the standard compound **24** and acetazolamide were 13 and 5 mg/kg, respectively. The imidazo[2,1-*b*]thiadiazole-sulfonamides, e.g., **8**, **13**, and **14**, are potent in vitro inhibitors of the carbonic anhydrase enzyme and are superior to both acetazolamide and methazolamide (Table II). Compound **23**, an imidazo[2,1-*b*]thiazolesulfonamide, is less potent in this respect and, in addition, is not as strongly ionized (pK_a = 8.37).

In four conscious beagle dogs, a 2.5 mg/kg intravenous dose of **8** (UK-15 454) increased vertebral blood flow by a maximum of 39 ± 7% (mean ± SEM), the effect lasting 2.5 h. Based on the area under the flow curves, **8** was some five times more effective than an equipotent (8.0 mg/kg iv) dose of 4-[(4-methoxypiperidino)sulfonyl]-2-chlorobenzenesulfonamide (**24**).[5] Oral administration of **8** to four conscious dogs produced maximum increases in vertebral blood flow of 31 ± 9% (2.5 mg/kg) and 49 ± 12% (5 mg/kg) after 2 h, the effects lasting for 4 and 4.5 h, respectively. Oral diuretic activity of **8** in dogs at 2.5 mg/kg was similar to that produced by 10 mg/kg of **24**.[5] Thus, while the selectivity (blood flow vs. diuresis) was comparable to that of the standard **24**, compound **8** was some five times more effective (based on the area under the flow curve) in increasing vertebral blood flow.

In three anesthetized cats, intravenous administration of 1.9 mg/kg of **8** increased vertebral blood flow by a maximum of 54 ± 9%, the effect lasting for 2 h. At this dose there were no appreciable changes in femoral blood flow, blood pressure, or heart rate.

Experimental Section

Pharmacology. a. Electroshock Test.[20] Male mice weighing between 18 and 28 g were used, ensuring that in any one assay the weight range about the mean was within ±2 g. The electroshock stimulus was applied via electrodes placed on the corneal surface of the eyes for a duration of 200 ms. The current necessary to ensure the production of a maximal (tonic/tonic extensor) convulsion in all untreated mice was 20 (where the mean weight was 20 g or less) or 25 mA (where the mean weight exceeded 20 g).

The compounds to be tested were ball-milled with glass beads for up to 24 h in 0.1% (v/v) Tween-80 in saline, and the suspension was administered by gavage. Three dose levels (2.5, 6.25, and 16 mg/kg) were administered to groups of ten mice 2 h prior to electroshock. A control group was similarly given 0.1% (v/v) Tween-80 in saline (0.1 mL/10 g of body weight). The convulsions were graded as no effect, clonic/clonic, clonic/tonic, or tonic/tonic (maximal). Anticonvulsant activity was expressed as the percentage protection from the maximal seizure effect at each dose level. Table I shows the protective ED_{50} values (in mg/kg) for each compound.

b. Diuretic Activity. The diuretic effect of selected compounds in rats was investigated. Male rats, 150–200 g, were deprived of food overnight. A fluid load of isotonic saline (2.5 mL/100 g of body weight po) was given immediately before administration of drug. The animals were divided into groups of eight, and the compounds to be tested were administered at three dose levels of 5, 12.5, and 25 mg/kg in separate experiments (acetazolamide was also tested at 5 mg/kg). Vehicle was administered to control groups (0.5 mL/100 g of body weight of 0.1% Tween-80 in saline). The animals were placed individually in metabolism cages, and 4 h later urine volume and electrolyte outputs were determined. The diuretic effects of **8** and 4-[(4-methoxypiperidino)sulfonyl]-2-chlorobenzenesulfonamide (**24**) were also assessed in dogs during the vertebral flow measurements. Urine was collected via a urethral catheter at 30-min intervals for 2 h prior to and 3 h after the administration of test compound or vehicle, and volume and electrolyte outputs were determined.

c. Blood Flow. Right vertebral artery blood flow was monitored in conscious dogs after the chronic implantation of a Doppler ultrasonic flow probe around the artery. The artery was approached via a central midline incision in the neck and the probe placed on the artery distal to its exit point from the thoracic cavity and proximal to its entry into the cervical vertebrae. The probe was exteriorized via a path under the skin to the dorsal surface of the neck. Flow and heart rate measurements were made for 2 h prior to and 3 h after administration of the test compound or vehicle.

Vertebral and femoral artery blood flows were monitored using electromagnetic flow probes in anesthetized cats [induction, halothane, nitrous oxide/oxygen (4:1 v/v); maintenance, chloralose (70 mg/kg iv)] and positive pressure ventilation. Blood flow, blood pressure, and heart rate were monitored continuously.

d. Carbonic Anhydrase Inhibition Methodology. In vitro carbonic anhydrase activity was determined by a modification of the colorimetric method of Philpot and Philpot.[20] The activity was expressed in terms of enzyme units and calculated from the expression

$$EU = (T_0 - T)/T$$

where EU represents enzyme units, T_0 the time of the uncatalyzed reaction in seconds, and T the time of the catalyzed reaction in seconds. All reactions were carried out in an ice bath. The inhibitors were preincubated with the enzyme for 5 min prior to the addition of substrate. This procedure allowed for enzyme–inhibitor equilibrium to take place. The concentration of compound required to inhibit 50% of the enzyme activity was determined graphically. Approximately 2 units of enzyme activity was utilized in each experiment.

Chemistry. All melting points are uncorrected and were obtained using an Electrothermal capillary melting point apparatus.

The structures of all compounds were confirmed by their IR, UV, and NMR spectra, the latter being determined as a solution in Me_2SO-d_6. The IR spectra were obtained with a Perkin-Elmer 237 spectrophotometer and the UV spectra with a Perkin-Elmer 137 spectrophotometer. The ^1H NMR spectra were obtained with a Varian Associates spectrometer Model A-60A and the ^{13}C NMR spectra were recorded on a JEOL PS100 spectrometer.

6-*tert*-Butylimidazo[2,1-*b*]-1,3,4-thiadiazole-2-sulfonamide (8). 5-Amino-1,3,4-thiadiazole-2-sulfonamide (195.7 g, 1.087 mol) and 1-bromo-3,3-dimethyl-2-butanone (207.3 g, 1.087 mol) were heated under reflux in EtOH (1.5 L) for 60 h. After the mixture was cooled to 10 °C, **8** crystallized out from the reaction mixture as a hydrobromide salt and was collected by filtration and dried in vacuo at 50 °C: yield 290 g (78%); mp 278–280 °C.

The HBr salt was slurried in water (2.0 L) at room temperature and NH_4OH was added until solution was obtained. HCl (5 N) was added until the pH was 7.5, whereupon the precipitated solid was collected, recrystallized from EtOH, and dried in vacuo at 50 °C: yield 211.9 g (75%); mp 254–256 °C. Anal. ($C_8H_{12}N_4O_2S_2$) C, H, N. Pertinent spectral data for **8** are as follows: UV (MeOH) λ_{max} 262 nm; IR (Nujol) 3335, 3158, 1175, 1030, 920, 756, 709, 635 cm^{-1}; ^1H NMR 1.2 (9 H, s), 7.9 (1 H, s), 8.5 (2 H, m) ppm. With the exceptions of **5**, **21**, and **20**, all the compounds listed in Table I were made by this route using either of the aminoazoles **1** and an appropriate bromo ketone.

Imidazo[2,1-*b*]thiazole-6-sulfonamide (21). 2-Amino-4-sulfamoylthiazole hydrobromide (2.6 g, 0.01 mol) and diethyl bromoacetal (2.0 g, 0.01 mol) were heated under reflux for 12 h in EtOH (40 mL). Upon cooling, the crystalline solid was filtered, washed, and recrystallized from H_2O to give **21** as the HBr salt: yield 1.0 g (36%); mp >305 °C dec. Anal. ($C_5H_5N_3O_2S_2$·HBr) C, H, N.

Compound **5** was prepared in a similar manner using the appropriate thiadiazole and diethyl bromoacetal.

5-Phenylimidazo[2,1-*b*]-1,3,4-thiadiazole-2-sulfonamide (20). 5-Amino-1,3,4-thiadiazole-2-sulfonamide (5.4 g, 0.03 mol) and redistilled α-bromophenylacetaldehyde (6.2 g, 0.03 mol) were heated under reflux for 12 h in EtOH (180 mL). Upon cooling, the crystalline solid was filtered, washed, and recrystallized from EtOH–H_2O to give **20**: yield 2.5 g (28%); mp 230–232 °C. Anal. ($C_{10}H_8N_4O_2S_2$) C, H, N.

1-Amino-2-mercapto-4-*tert*-butylimidazole (4). 6-*tert*-Butylimidazo[2,1-*b*]-1,3,4-thiadiazole-2-sulfonamide (**8**; 13.4 g, 0.052 mol) was slurried in 5 N NaOH (100 mL), and the mixture was heated to 95 °C for 6 h. The solution was cooled and 5 N HCl was added until pH 7.0 was achieved. The resultant precipitate was collected, washed, dried, and recrystallized from EtOAc to give **4** as the HCl salt: yield 8.0 g (91%); mp 196 °C.

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Inhibitors of Polyamine Biosynthesis. 8. Irreversible Inhibition of Mammalian *S*-Adenosyl-L-methionine Decarboxylase by Substrate Analogues

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Analogues of *S*-adenosyl-L-methionine (**1**) were synthesized and evaluated as inhibitors of the enzyme *S*-adenosyl-L-methionine decarboxylase from rat liver. The compounds synthesized were *S*-(5′-deoxy-5′-adenosyl)-(±)-2-methylhomocysteine (**10**), *S*-(5′-deoxy-5′-adenosyl)-(±)-2-methylmethionine dihydrogen sulfate (**11**), *S*-(5′-deoxy-5′-adenosyl)-(±)-2-methylhomocysteine sulfoxide (**12**), *S*-(5′-deoxy-5′-adenosyl)-(±)-1-methyl-3-thiopropylamine hydrogen sulfate (**16**), *S*-(5′-deoxy-5′-adenosyl)-(±)-1-methyl-3-(methylthio)propylamine dihydrogen sulfate (**17**), *S*-(5′-deoxy-5′-adenosyl)-(±)-1-methyl-3-thiopropylamine sulfoxide hydrogen sulfate (**18**), *N*-(cyanoethyl)-*N*-methyl-5′-amino-5′-deoxyadenosine (**20**), and *N*-(aminopropyl)-*N*-methyl-5′-amino-5′-deoxyadenosine dihydrochloride (**21**). *S*-Adenosyl-L-methionine decarboxylase was partially purified from rat liver homogenate using a methylglyoxal bis(guanylhydrazone) linked Sepharose column in the final purification step. At the highest concentration used (2.5×10^{-4} M), compounds **10**, **12**, **16**, **18**, and **20** did not produce inhibition of the enzymatic decarboxylation of [^{14}C]carboxyl-labeled *S*-adenosyl-L-methionine. Compounds **11**, **17**, and **21** were competitive inhibitors of *S*-adenosyl-L-methionine decarboxylase, and the apparent K_i values for these compounds were calculated to be 1.8×10^{-4}, 1.2×10^{-4}, and 1.1×10^{-4} M, respectively. Compounds **11** and **17** formed azomethine bonds with an essential carbonyl group in the enzyme active site which was reducible with sodium cyanoborohydride. These two inhibitors also caused a time-dependent inactivation of the enzyme, which was also dependent on the concentration of the inhibitor in the incubation media. Compound **21** did not form an azomethine bond with the enzyme and did not cause inactivation of the enzyme. These results suggest that the sulfonium analogues **11** and **17** have a binding mode to the enzyme active site which is different than that for the nitrogen analogue **21**.

The enzyme *S*-adenosyl-L-methionine decarboxylase (EC 4.1.1.50) is an essential enzyme in the biosynthesis of the polyamines spermidine and spermine. It catalyzes the decarboxylation of *S*-adenosyl-L-methionine (**1**) to give *S*-(5′-deoxy-5′-adenosyl)-1-(methylthio)propylamine (**2**). The enzyme spermidine synthase catalyzes the transfer of the aminopropyl moiety from **2** to putrescine to give spermidine. Similarly, the enzyme spermine synthase catalyzes the formation of spermine from **2** and spermidine. Recently, the enzyme *S*-adenosyl-L-methionine decarboxylase was purified to homogeneity from mammalian tissues,[1,2] yeast,[3] and bacteria.[4] A pyruvyl residue was identified as the prosthetic group essential for the catalytic activity of the enzymes from these three sources.[1-4] Only two other amino acid decarboxylases are known to contain covalently bound pyruvate as the catalytically essential carbonyl group in place of the commonly present pyridoxal 5′-phosphate.[5]

Methylglyoxal bis(guanylhydrazone) (**3**) and methylglyoxal bis(aminoguanylhydrazone) (**4**) are powerful inhibitors of the mammalian and yeast *S*-adenosyl-L-methionine decarboxylases. Compound **3** produced readily

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