elemental analyses and M. Vartanian, P. Mickevicius, and B. Stieber (Warner-Lambert) for pharmacological test results. The help of the Anticonvulsant Drug Development Program, Epilepsy Branch, NINCDS (H. J. Kupferberg and G. Gladding) in the pharmacological evaluation of several of these compounds is gratefully acknowledged.

Registry No. 1, 108122-22-1; 1·HCl, 108122-23-2; 2, 108122-24-3; 3, 51047-54-2; 3·HCl, 38500-97-9; 4, 108122-25-4; 4·HBr, 108122-26-5; 5, 108122-27-6; 5·HCl, 108122-28-7; 6, 108122-29-8; 6·HCl, 108122-30-1; 7, 108148-27-2; 7·xHCl, 108122-31-2; 8, 108122-32-3; 9, 108122-33-4; 9·2HCl, 108122-34-5; 10, 108122-35-6; 10·HCl, 108122-36-7; 11, 108148-28-3; 12, 108122-37-8; 13, 108122-38-9; 14, 108122-39-0; 14·xHCl, 108122-40-3; $CH_3O^-Na^+$, 124-41-4; $CH_2CH_3O^-Na^+$, 141-52-6; $(CH_3)_2CHO^-Na^+$, 683-60-3; $(CH_3)_2CHCH_2O^-Na^+$, 13259-29-5; $(CH_3)_2N(CH_2)_2O^-Na^+$, 37616-36-7; $H_2N(CH_2)_2N(CH_3)_2$, 108-00-9; 2,6-dichloropyridine, 2402-78-0; cyclohexanol sodium salt, 22096-22-6; cyclopropanemethanol sodium salt, 34006-50-3; cyclohexanemethanol sodium salt, 108122-41-4; 2-cyclohexyloxy-6-chloropyridine, 108122-42-5; 2-methoxy-6-chloropyridine, 17228-64-7; 2-ethoxy-6-chloropyridine, 42144-78-5; 2-(1-methylethyloxy)-6-chloropyridine, 89481-98-1; 2-chloro-6-(2-methylpropoxy)pyridine, 108122-43-6; 2-cyclopropylmethoxy-6-chloropyridine, 108122-44-7; 2-cyclohexylmethoxy-6-chloropyridine, 108122-45-8; 2-[(2-dimethylamino)ethyl]-6-chloropyridine, 108122-46-9; piperazine, 110-85-0; *N*-methylpiperazine, 109-01-3; piperidine, 110-89-4; morpholine, 110-91-8; hexahydro-1*H*-1,4-diazepine, 505-66-8.

Synthesis and Anticonvulsant Activity of Analogues of 4-Amino-*N*-(1-phenylethyl)benzamide

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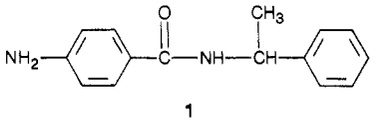
A group of amides and amines related to 4-amino-*N*-(1-phenylethyl)benzamide, 1, were prepared in a study on the relationship of structure to anticonvulsant activity in this compound. Acylation and alkylation of the amino group of 1 resulted in almost total loss of anticonvulsant activity. Insertion of a methylene between the 4-amino group and the aromatic ring of 1 produced a slight increase in anticonvulsant potency and a significant increase in toxicity. Hydride reduction of the amide carbonyl in 1 also yielded compounds having a slightly lower ED50 against convulsions induced by electroshock and a much lower TD50 in the rotorod assay. Modification of the 1-phenylethyl group of 1 also decreased anticonvulsant potency.

A series of prior reports have described the anticonvulsant effects of aminobenzamides of alkyl-, aryl- and arylalkylamines.[1-3] The initial studies on this series of amides have shown the 4-aminobenzamides to be more effective anticonvulsants than the 3-amino derivatives, with the 2-aminobenzamides essentially inactive in most anticonvulsant tests. Maximum anticonvulsant activity in the 4-aminobenzamides is observed in those amides derived from aryl- or arylalkylamines. Recent studies[4] on a series of 4-aminophenylacetamides have shown significant loss of anticonvulsant activity resulting from this insertion of a methylene between the aromatic ring and the amide carbonyl of the aminobenzamides.

The compounds reported in this study were prepared in an investigation of the structure–activity relationships for the anticonvulsant 4-aminobenzamide 1. Racemic 1



1

has been identified in a previous report[1] as an effective anticonvulsant in seizures induced by electroshock and pentylenetetrazole in mice and rats. The preliminary anticonvulsant activity profile for 1 is similar to that for phenobarbital and phenytoin in the same assays. Compound 1 is more effective against electroshock-induced convulsions, ED50 = 18.02 mg/kg, than against pentylenetetrazole-induced seizures, ED50 = 41.72 mg/kg. Rats pretreated orally twice daily for 7 days with 28 mg/kg of 1 showed no significant increase in hexobarbital sleep time. The rotorod toxicity for 1 is TD50 = 170.78 mg/kg while the hypnotic dose (HD50) and lethal dose (LD50) are 461.76 and 718.18 mg/kg, respectively. The molecular modifications of 1 reported in this study include alkylation and acylation of the aromatic amino group, methylene insertion between the amino and aromatic groups to produce compounds of enhanced basicity and conformational degrees of freedom, reduction of the amide carbonyl to yield the corresponding amines, and modifications of the 1-phenylethyl group.

Chemistry

The 4-(alkylamino)- and 4-(acylamino)benzamides prepared in this study are listed in Table I. The monomethyl analogues were prepared by condensation of methyl 4-(*N*-methylamino)benzoate and 1-phenylethylamine in a sealed tube at 180 °C. The methyl 4-(*N*-methylamino)benzoate was prepared by treating the tosylate of 4-aminobenzoic acid with dimethyl sulfate followed by hydrolysis according to the methods of Iwanami et al.[5] Dimethylation of the aromatic amino group was accomplished by treating the primary amine 1 with formaldehyde and sodium cyanoborohydride under acidic conditions via the procedure of Borch et al.[6] Acylation of 1 by reaction with the appropriate acyl chloride under nonaqueous conditions gave compounds 8–10.

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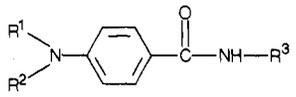
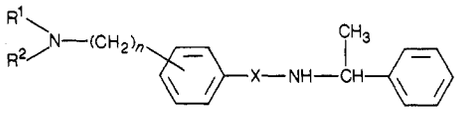
Table I. (Alkylamino)- and (Acylamino)benzamides



no.	R^1	R^2	R^3	mp, °C	recrystn solvent	% yield	formula	anal.
2	CH_3	H	$CH(CH_3)C_6H_5$	132–133	C_6H_6–pet. ether	69	$C_{16}H_{18}N_2O$	C, H, N
3	CH_3	H	$CH_2C_6H_5$	123–124	C_6H_6–pet. ether	69	$C_{15}H_{16}N_2O$	C, H, N
4	CH_3	CH_3	$CH(CH_3)C_6H_5$	156–157	C_6H_6	82	$C_{17}H_{20}N_2O$	C, H, N
5	CH_3	CH_3	$CH_2C_6H_5$	164–166	C_6H_6	76	$C_{16}H_{18}N_2O$	C, H, N
6	CH_3	CH_3	C_6H_5	166–168	C_6H_6	80	$C_{15}H_{16}N_2O$	C, H, N
7	CH_3	CH_3	$CH_2CH_2C_6H_5$	109–112	C_6H_6	79	$C_{17}H_{20}N_2O$	C, H, N
8	CH_3CO	H	$CH(CH_3)C_6H_5$	227–229	acetone	55	$C_{17}H_{18}N_2O_2$	C, H, N
9	C_2H_5OCO	H	$CH(CH_3)C_6H_5$	184–186	CH_2Cl_2	64	$C_{18}H_{20}N_2O_3$	C, H, N
10	$C_2H_5O_2CCO$	H	$CH(CH_3)C_6H_5$	208–211	THF	41	$C_{19}H_{20}N_2O_4$	C, H, N

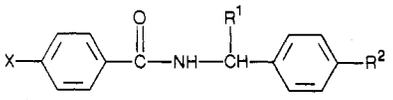
Table II. (Aminomethyl)benzamides and Aminobenzylamines



no.	position	n	R^1	R^2	X	mp or bp (mm), °C	recrystn solvent	% yield	formula	anal.
11	4	1	CH_3	CH_3	CO	223–225	2-propanol	66	$C_{18}H_{22}N_2O$·HCl	C, H, N
12	4	1	H	CH_3	CO	233–235	2-propanol	58	$C_{17}H_{20}N_2O$·HCl	C, H, N
13	2	0	H	H	CH_2	133–134 (0.8)		94	$C_{15}H_{18}N_2$	C, H, N
14	3	0	H	H	CH_2	175–176 (0.8)		86	$C_{15}H_{18}N_2$	C, H, N
15	4	0	H	H	CH_2	*a*		75	$C_{15}H_{18}N_2$	C, H, N

[a] Purified by column liquid chromatography on silica gel with stepwise gradient elution using petroleum ether (30–60 °C) and ether.

Table III. Benzamides of Substituted Benzylamines



no.	X	R^1	R^2	mp, °C	recrystn solvent	% yield	formula	anal.
16	H	CH_3	H	115–119	toluene	84	$C_{15}H_{15}NO$	C, H, N
17	H	CH_3	NH_2	94–96	2-propanol	59	$C_{15}H_{16}N_2O$	C, H, N
18	NH_2	C_2H_5	H	132–135	toluene	64	$C_{16}H_{18}N_2O$	C, H, N
19	NH_2	C_3H_7	H	150–151	C_6H_6	78	$C_{17}H_{20}N_2O$	C, H, N
20	NH_2	C_4H_9	H	115–117	C_6H_6	75	$C_{18}H_{22}N_2O$	C, H, N
21	NH_2	CH_3	CH_3	141–143	C_6H_6	81	$C_{16}H_{18}N_2O$	C, H, N
22	NH_2	CH_3	OCH_3	138–140	C_6H_6–THF	68	$C_{16}H_{18}N_2O_2$	C, H, N
23	NH_2	CH_3	Cl	153–156	C_6H_6	55	$C_{15}H_{15}N_2OCl$	C, H, N
24	NH_2	CH_3	F	143–145	C_6H_6–THF	47	$C_{15}H_{15}N_2OF$	C, H, N

The acid chloride of 4-carboxybenzaldehyde was prepared with thionyl chloride and the amide was prepared in the usual manner.[1] Reductive amination of the 4-aldehyde group with methyl- or dimethylamine and sodium borohydride produced the desired (aminomethyl)-benzamides **11** and **12** (Table II). The aminobenzylamines **13–15** were prepared by hydride reduction of the amide carbonyl of the 2-, 3-, and 4-aminobenzamides, respectively, with sodium bis(2-methoxyethoxy)aluminum hydride (Red Al).

The 1-phenylalkylamines and most of the 4-substituted 1-phenylethylamines required for the preparation of amides **16–24** were obtained by the Leukhart reductive amination reaction.[7] The synthesis of 4-nitro-1-phenylethylamine was accomplished by reduction of the corresponding oxime acetate with diborane–THF.[8] The oxime of 4-nitroacetophenone was prepared from hydroxylamine hydrochloride and pyridine. Direct reduction (Red Al) of the oximes of the 4-substituted acetophenones gave the *N*-ethylanilines as the major product, perhaps through a Beckman-type[9] rearrangement during the reduction. Conversion of the oxime to the acetate ester by refluxing in acetic anhydride followed by diborane reduction gave the amines which could not be obtained via the Leukhart method. The required amides (Table III) of these amines were prepared from 4-nitrobenzoyl chloride by Schotten–Baumann[10] acylation of the amines and subsequent catalytic reduction of the aromatic nitro group.[1]

Results and Discussion

The initial anticonvulsant and toxicity screening results for compounds **2–24** are presented in Table IV. The first series of compounds prepared in this study were amides **2–10**, which have alkyl and acyl substitutions on the aromatic amino group. Compound **2**, the *N*-methyl analogue of **1**, prevented MES-induced convulsions in one of three mice 30 min after dosing at 100 mg/kg, and anti-scMet

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Table IV. Anticonvulsant and Toxicity Screening Data[a]

	sc-Met[b]		MES[b]		rotorod toxicity[b]	
no.	30 min	4 h	30 min	4 h	30 min	4 h
2	2	2	3	2	*c*	*c*
5	*c*	*c*	*c*	2	*c*	*c*
10	*c*	*c*	*c*	*c*	2	*c*
11	*e*	*e*	4	*e*	3	*e*
12	*d*	*d*	4	3	3	3
13	*e*	*e*	2	*e*	1	*e*
14	*c*	*c*	3	*c*	2	*c*
15	*c*	*c*	4	*c*	3	*c*
17	2	*c*	3	2	2	*c*
18	2	2	3	3	3	2
19	*c*	*c*	3	2	*c*	*c*
20	2	*c*	3	2	2	*c*
22	*c*	3	*c*	*c*	*c*	*c*
23	2	2	2	*c*	*c*	*c*
24	*c*	*c*	2	3	*c*	2

[a] Compounds **3**, **4**, **6**, **7**, **8**, **9**, **16**, and **21** were inactive in all tests at a dose of 300 mg/kg. [b] 4 = activity at 30 mg/kg, 3 = activity at 100 mg/kg, 2 = activity at 300 mg/kg, 1 = activity at 600 mg/kg. [c] No activity at 300 mg/kg. [d] No activity at 100 mg/kg. [e] No activity at 30 mg/kg.

Table V. Quantitative Anticonvulsant Data

		MES		sc-Met	
no.	TD50[a,b]	ED50[b]	PI[c]	ED50[b]	PI[c]
1	170.78 (153.02–189.96)[d]	18.02 (13.41–21.43)[d]	9.5	41.72 (38.83–46.00)[d]	4.1
5		233.45 (197.12–262.41)			
11	34.28 (28.66–38.69)	13.04 (10.54–16.39)	2.63		
12	22.40 (15.92–32.39)	13.50 (8.92–17.17)	1.66		
14	49.70 (42.6–57.6)	32.39 (29.2–37.0)	1.53		
15	15.53 (12.2–18.5)	11.07 (9.1–13.7)	1.40		
17	190.02 (163.9–208.8)	68.18 (57.4–81.3)	2.79	125.98 (112.2–142.3)	1.51
18	95.38 (78.4–110.3)	54.28 (47.7–58.8)	1.76	54.06 (47.6–60.2)	1.76
19	377.85 (295.6–488.0)	93.86 (89.8–102.1)	4.03	78.79 (44.4–119.3)	4.80
20	331.88 (273.9–377.8)	73.69 (58.9–87.0)	4.50	161.42 (122.3–200.1)	2.06

[a] Rotorod procedure. [b] Doses are in milligrams/kilogram. [c] PI = TD50/ED50. [d] Ninety-five percent confidence interval.

activity was observed at 300 mg/kg in four of five animals after 30 min. The limited activity of **2** and the lack of significant anticonvulsant effects for most of the other alkyl and acyl derivatives of **1** indicates the significance of the primary aromatic amino group to the activity of **1**. Compound **8**, the *N*-acetyl derivative of **1**, showed no activity in any of the testing procedures even at doses of 600 mg/kg. Compound **8** was prepared as a potential metabolite of **1**, and its lack of activity shows that metabolic acetylation of **1** would result in loss of anticonvulsant activity. Acetylation of primary aromatic amines is a common metabolic pathway in mammalian systems and, in the case of procainamide, does not totally eliminate the biological activity observed in the parent molecule. Compound **8** has been identified as a major metabolite of **1** in preliminary studies conducted in rats in this laboratory.

Compounds **11** and **12** represent methylene insertion between the aromatic ring and the amino group in **1**. Both compounds showed activity against MES induced convulsions 30 min after ip administration of 30 mg/kg in mice. However, both compounds displayed strong rotorod toxicity at 30 min in eight of eight animals given a dose of 100 mg/kg. Quantitation (Table V) of the rotorod toxicity gave TD50 values of 34.28 and 22.40 mg/kg for **11** and **12**, respectively. The anti-MES activity of the two compounds is quite similar at about 13 mg/kg, producing a slightly higher protective index (PI = TD50/ED50) for **11**. The increase in basicity in **11** and **12** produces a slight increase in anti-MES activity over **1**; however, the toxic effects are significantly enhanced and result in a low PI valve.

The amines (**13–15**) produced by hydride reduction of the 2-, 3- and 4-aminobenzamides showed activity in the anti-MES screen with a profile similar to that of **11** and **12**. Compound **13** showed anti-MES activity at 300 mg/kg whereas **14** and **15** protected at doses of 100 and 30 mg/kg, respectively. Quantitation (Table V) of the anti-MES and neurologic deficit for **14** and **15** yielded very little separation between activity and toxicity. Compound **15** was the more potent with an ED50 of 11.07 mg/kg and TD50 = 15.53 mg/kg. Thus, the relationship between anti-MES activity and the relative position of the aromatic amino group for these benzylamines is 4-amino > 3-amino > 2-amino. This same order of potency was observed for the aminobenzamides; i.e., compound **1** is more potent than its 3-amino isomer, etc. Both sets of aliphatic amines **11** and **12** and **13–15** displayed greatly increased toxicity relative to the aminobenzamides.

The substituted benzamides described in Table III were moderately active in the initial anticonvulsant screening procedures. Compound **16** was inactive at 300 mg/kg in both the anti-MES test and the rotorod assay. This can be compared to the activity of **1** and demonstrates the significance of the aromatic amino group in the anticonvulsant activity of **1**. Compound **17**, the benzamide of 4-aminobenzylamine, was active in both the MES and scMet test 30 min after dosing. Compound **17** represents a shifting of the amino group to the other aromatic ring when compared to **1**. Quantitation of the activity for **17** confirmed both anti-MES and scMet activity, however, yielding much higher ED50 values when compared to **1**.

The extension of the methyl group in **1** to ethyl, propyl, and butyl, compounds **18–20**, revealed activity against both MES- and sc-Met-induced convulsions. Quantitation of the activity showed **18** to be essentially equipotent in both the MES and sc-Met test. The ED50 values for **19** and **20** were higher than those for **18**; however, the low toxicity observed in **19** and **20** yielded higher PI values. Compound

19 showed good separation between ED50 and TD50 for both anti-MES and sc-Met activity. These data describe the activity for the racemic mixture of each of these compounds. The data in Table V describing the activity for 1 is also for the racemic mixture.

The 4-aminobenzamides of 4-substituted 1-phenylethylamines showed generally weak activity compared to 1. The 4-fluoro derivative 24 displayed an interesting anti-MES profile upon initial evaluation. The compound was more active at 4 h after administration than at 30 min. This compound is undergoing additional anticonvulsant and toxicity testing.

In summary, the structural modifications described in this study failed to produce a compound superior to 1. Although several compounds of enhanced basicity displayed lower ED50 values against MES-induced convulsions, this potency was offset by significantly lower TD50 values yielding low PI values. Thus, the anticonvulsant activity profile of 1 remains superior to the compounds explored in this study.

Experimental Section

General Procedures. All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were recorded on a Beckman 4230 or AccuLab 6 spectrophotometer. Proton NMR spectra were recorded on a Varian T-60A spectrometer, using Me_4Si as the internal standard. Samples were dissolved in deuteriated chloroform, and when necessary, deuteriated Me_2SO was added to aid dissolution. NMR and IR spectral data were consistent with assigned structures. Elemental analyses (C, H, N) were performed by Atlantic Microlabs, Inc., Atlanta, GA, and the results obtained were within ±0.4 of the calculated percentage. Compound 1 and related amides were prepared according to previously reported methods.[1]

(*N*,*N*-Dimethylamino)benzamides 4–7. A solution of 13.6 mL of 37% formaldehyde (0.17 mol) and 0.017 mol of primary amine in 70 mL of acetonitrile was added to a 250-mL three-necked flask. To this stirred solution was added 2.0 g (0.03 mol) of $NaBH_3CN$, followed by 2.0 mL of acetic acid. The mixture was allowed to stir for 30 min, after which an additional 1.2 g of $NaBH_3CN$ (0.05 mol total) was added, followed by the slow dropwise addition of an additional 2.0 mL of acetic acid. The mixture was stirred overnight at ambient temperature, the solvent removed at reduced pressure, and the resulting residue suspended in 50 mL of water. This mixture was made basic (pH >10) with saturated NaOH and extracted with $CHCl_3$ (3 × 50 mL). The extracts were combined, dried, and evaporated to give a solid residue, which was recrystallized from benzene.

4-(Acylamino)benzamides 8–10. A solution of 0.05 mol of acyl chloride in 25 mL of THF was added to a flask and cooled to −15 °C in an acetone–ice bath. To this was added dropwise a solution of 0.02 mol of 1 and 2.0 mL (0.05 mol) of triethylamine in 25 mL of THF. The mixture was allowed to stir overnight and warm to ambient temperature. The mixture was transferred to a separatory funnel diluted with 200 mL of $CHCl_3$ and washed with water (2 × 100 mL). The organic layer was dried with $MgSO_4$ and evaporated and the resulting residue purified by recrystallization.

4-(Aminomethyl)benzamides 11 and 12. A 2.0-g (0.008 mol) sample of 4-formyl-*N*-(1-phenylethyl)benzamide was suspended in 50 mL of 40% aqueous methyl- or dimethylamine and the mixture added to a 125-mL flask. This mixture was brought to reflux, and 1 g of $NaBH_4$ was added portionwise followed 20 min later by an additional 1 g of $NaBH_4$ (0.05 mol, total). The resulting mixture was stirred at gentle reflux for 3 h. The mixture was cooled and 50 mL of 1 N NaOH was added, and then the mixture was stirred overnight and extracted with $CHCl_3$ (3 × 50 mL). The combined extracts were dried with $MgSO_4$ and evaporated, leaving a gummy residue. This was taken up in ether (40 mL) and mixed with 5 mL of HCl–ether to precipitate a solid, which was collected by filtration and recrystallized from 2-propanol.

Aminobenzylamines 13–15. A solution of 25 mL of Red Al (0.18 mol of hydride) in 100 mL of toluene was added to a 500-mL, three-necked flask equipped with a magnetic stirrer, a heating mantle, and a reflux condenser fitted with a drying tube. A 6.4-g (0.026 mol) sample of the aminobenzamide was added portionwise with a spatula. The resulting mixture was refluxed overnight and then cooled to 0 °C in an ice bath and excess hydride destroyed with ethanol and water. The organic layer was decanted and stirred for 20 min with 125 mL of 1 N HCl. The layers were separated, and the organic fraction was extracted twice with 50-mL portions of 1 N HCl. The combined aqueous portions were made basic (pH 10) with KOH pellets and extracted with $CHCl_3$ (3 × 50 mL). The combined extracts were dried with $MgSO_4$ and evaporated to yield a yellow oil.

1-Phenylalkylamines (Leukhart Method[7]). A 0.10-mol sample of alkylphenone and 50 g of ammonium formate were added to a flask fitted with a distillation head and water-cooled condenser. The mixture was warmed slowly over a period of 90 min to 220 °C. Water and phenone distilled into a collecting flask as the reaction proceeded, and the phenone was separated and returned to the reaction vessel. The mixture was stirred overnight at 200 °C, then cooled, and washed with water (2 × 50 mL). The combined water extracts were washed with benzene (2 × 20 mL), the benzene extracts were combined with the original reaction mixture and returned to the flask along with 40 mL of concentrated HCl, and the resulting mixture was refluxed for 2 h. The acid mixture was then washed with 20 mL of benzene, made strongly basic with saturated NaOH, and steam distilled. About 250 mL of distillate was collected and then extracted with $CHCl_3$ (5 × 35 mL). The combined extracts were dried with $MgSO_4$ and evaporated under vacuum to yield a clear, viscous liquid.

Pharmacology.[11] Initial anticonvulsant evaluation of these compounds was conducted by using at least three dose levels (30, 100, 300 mg/kg) and in some cases a fourth dose of 600 mg/kg. All tests were performed on male Carworth Farms number-one mice. Test solutions of all compounds were prepared in 30% polyethylene glycol 400, and animals were dosed intraperitoneally 30 min prior to testing.

Maximal electroshock seizures (MES) were elicited with a 60-cycle ac of 50-mA intensity delivered for 0.2 s via corneal electrodes. A drop of 0.9% saline was instilled in the eye prior to application of electrodes. Abolition of the hind limb tonic extension component of the seizure was defined as protection in the MES test.

The subcutaneous pentylenetetrazole (Metrazol) seizure threshold test (scMet) was conducted by administering 85 mg/kg of pentylenetetrazole as a 0.5% solution in the posterior midline. Protection in this test was defined as a failure to observe a single episode of clonic spasms of at least 5-s duration during a 30-min period following administration of the test compound.

Neurological deficit was measured in mice by the rotorod test. The dosed animal was placed on a 1-in.-diameter knurled plastic rod rotating at 6 rpm. Neurologic toxicity was defined as the failure of the animal to remain on the rod for 1 min. The median anticonvulsant (ED50) and toxicity (TD50) were determined by the graphical method.

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Registry No. 1, 85592-75-2; 2, 108191-10-2; 3, 108191-11-3; 4, 108191-12-4; 5, 108191-13-5; 5 (R^1, R^2 = H), 54977-92-3; 6, 3765-62-6; 6 (R^1, R^2 = H), 782-45-6; 7, 108191-14-6; 7 (R^1, R^2 = H), 61251-99-8; 8, 108267-41-0; 9, 108191-15-7; 10, 108191-16-8; 11, 108191-17-9; 12, 108191-18-0; 13, 108191-19-1; 13 (X = CO), 85592-80-9; 14, 108191-20-4; 14 (X = CO), 85592-79-6; 15, 108191-21-5; 15 (X = CO), 85592-75-2; 16, 28623-68-9; 17, 108191-22-6; 18, 108191-23-7; 19, 108191-24-8; 20, 108191-25-9; 21, 108191-26-0; 22, 108191-27-1; 23, 108191-28-2; 24, 108191-29-3; CH_3COCl, 75-36-5; C_2H_5OCOCl, 79-03-8; $C_2H_5O_2CCOCl$, 4755-77-5; 4-nitroacetophenone (oxime), 10342-64-0; 4-nitroaceto-

(11) The pharmacological evaluation of these compounds was conducted in the laboratories of the Anticonvulsant Drug Development Program, Epilepsy Branch, NINCDS, Bethesda, MD.

phenone, 100-19-6; hydroxylamine amine hydrochloride, 5470-11-1; formaldehyde, 50-00-0; 4-formyl-*N*-(1-phenylethyl)benzamine, 108191-30-6; methylamine, 74-89-5; dimethylamine, 124-40-3; *N*-benzyl-α-methylbenzenemethanamine, 19302-20-6; *N*-(4-aminobenzyl)-α-ethylenzenemethanamine, 108191-31-7; *N*-(4-aminobenzyl)-α-propylbenzenemethanamine, 108191-32-8; *N*-(4-aminobenzyl)-α-butylbenzenemethanamine, 108191-33-9; acetophenone, 98-86-2; ethyl phenyl ketone, 93-55-0; propyl phenyl ketone, 495-40-9; butyl phenyl ketone, 1009-14-9; benzylamine, 100-46-9; *p*-aminobenzenemethanamine, 4403-71-8.

Quantitative Structure–Activity Relationship of Triazine–Antifolate Inhibition of *Leishmania* Dihydrofolate Reductase and Cell Growth

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Quantitative structure–activity relationships have been formulated for the inhibition of *Leishmania major* dihydrofolate reductase (DHFR) and for inhibition of promastigote cell growth by a series of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(3-substituted-phenyl)-*s*-triazines. The inhibition of DHFR is best correlated by a modified variable for hydrophobicity of the 3-X substituent (π'_3), an alkoxy group indicator variable (I_{OR}), a disposable parameter (β) obtained by iteration, and a variable that parameterizes steric effects (MR) in the equation, $\log 1/K_i = 0.65\pi'_3 - 1.22 \log (\beta \cdot 10^{\pi'_3} + 1) - 1.12I_{OR} + 0.58MR_Y + 5.05$ ($r = 0.965$). The EC_{50} values for triazine inhibition of *L. major* cell growth in culture are correlated by the equation $\log 1/EC_{50} = 0.21\pi_3 + 0.44 \log 1/K_i + 0.53$ ($r = 0.960$). When compared to DHFR from human, other vertebrates, and *E. coli*, *L. major* DHFR differs in that it optimally binds triazine congeners that are much more hydrophobic. Furthermore, in contrast to other DHFR's studied, triazine binding to *L. major* DHFR does not seem to be influenced by the electronic characteristics of the 3-X substituent of the parent triazine molecule. However, *L. major* DHFR is more sensitive to the steric effects and polarizability of the 3-X substituent. Our results indicate that triazines inhibit *L. major* promastigote growth via direct inhibition of DHFR as is shown by the good correlation between $\log 1/K_i$ values for inhibition of the purified enzyme and $\log 1/EC_{50}$ values for inhibition of cell culture growth. Two lipophilic, sterically large analogues of this triazine series showed selectivity for *L. major* DHFR over human DHFR. Further optimization of the MR and I_{OR} terms in the above QSAR equations may provide even more selective inhibitors.

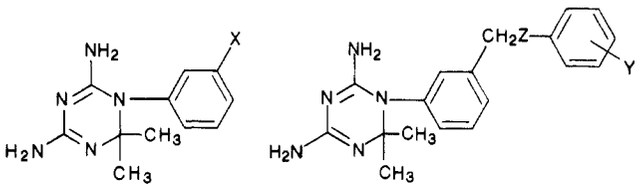
Dihydrofolate reductase (DHFR, EC 1.5.1.3) catalyzes the reduction of 7,8-dihydrofolate (H_2folate) to 5,6,7,8-tetrahydrofolate (H_4folate) by NADPH as follows:

$$H_2\text{folate} + \text{NADPH} + H^+ \rightleftarrows H_4\text{folate} + \text{NAD}^+$$

Structural studies show that there are large differences in the primary sequences of DHFR from protozoan,[1a,b] bacteriophage,[2] bacterial,[3] and mammalian[3] sources, and indeed, the enzyme from different sources shows wide variation in its sensitivity to inhibitors. These differences, coupled with its biochemical importance in folate metabolism, make DHFR an attractive target for design of selective inhibitors in pathogens with respect to their hosts.[4] The clinical effectiveness of the antibacterial drug trimethoprim and the antimalarial drug pyrimethamine attest to the utility of selective DHFR inhibitors.

The DHFR's in *Leishmania major* and other parasitic protozoan are unique from mammalian and other sources in that the protozoan DHFR's exist coupled to thymidylate synthetase (TS, EC 2.1.1.45) as a bifunctional protein.[5a–c] This makes the enzyme a promising target for design of selective inhibitors. The parasite causes cutaneous leishmaniases, a disfiguring disease endemic to Latin and South American, Mediterranean, and Middle Eastern countries. Currently, treatment of the infection employs the use of antimonial compounds that have demonstrated cardiovascular and other toxicity as well as emerging resistance.[6]

Correlation analysis[7] has been used to develop quantitative structure–activity relationships (QSAR) for 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-(3-substituted-phenyl)-*s*-triazine (triazine, **1a** and **1b**) inhibition of DHFR from human and other species.[8,9] A set of congeners is



1a **1b**

used to probe the enzyme to obtain information about the active site in terms of its hydrophobic, steric, and electronic requirements for ligand interaction. Using the QSAR approach, one can quantify differences in the free energy of binding of less than 0.5 kcal/mol. Furthermore, the method allows a comparison of the enzyme–ligand effect

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