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Synthesis and Evaluation of *N*-(Phenylacetyl)trifluoromethanesulfonamides as Anticonvulsant Agents

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A series of *N*-(phenylacetyl)trifluoromethanesulfonamides (**3a**−**g**) was prepared according to the Topliss scheme in order to determine if aryl substitutents would influence anticonvulsant activity. In initial (phase I) screening and quantitative (phase II) evaluation, all seven compounds exhibited significant activity against MES- and scMet-induced seizures. *N*-(Phenylacetyl)trifluoromethanesulfonamide (**3a**) was then advanced through five additional testing phases (phases III−VII). Compound **3a** displayed good oral bioavailability, low toxicity, and a larger protective index in mice than the prototype drugs, phenytoin, phenobarbital, valproate, and ethosuximide. Additionally, **3a** exhibited a longer time to peak effect in all tests and a greater 24-h margin of safety (HD_{50}/ED_{50}) than the prototypes. Compound **3a** blocked picrotoxin-induced seizures but was ineffective against seizures induced by bicuculline or strychnine. *In vitro* receptor binding studies revealed that **3a** did not displace [^{3}H]-labeled γ-aminobutyric acid or [^{3}H]-labeled flunitrazepam, and tolerance did not develop during 5-day chronic administration.

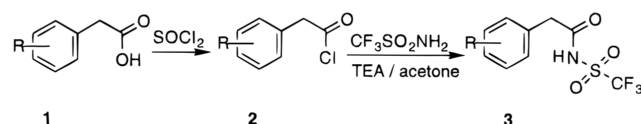
Introduction

Epilepsy has been found to have point prevalence rates in the range of 4−10/1000 in the general population.[1] The majority (60−70%) of these cases occur without clear etiology. Despite this, anticonvulsant drugs are estimated to be useful in treating 90% of all epileptic patients. However, all currently approved anticonvulsant agents have dose-related toxicity and idiosyncratic side effects.[2] Additionally, many antiepileptic drugs induce xenobiotic-metabolizing liver enzymes resulting in complex and undesired side effects. In response to the premise that major medical breakthroughs in nonpharmacologic therapies for the treatment of epilepsy in the near future seem remote, the search for new antiepileptic drugs with lower toxicities and fewer side effects continues.[3]

Sulfonamides currently represent a minor class of antiepileptic agents.[4] Early structure−activity studies of sulfonamides examined phenyl and heteroaromatic primary sulfonamides,[5] a number of which displayed anticonvulsant activity. Acetazolamide (2-(acetylamino)-1,3,4-thiadiazole-5-sulfonamide) has been used as an anticonvulsant for some time, and recently as an adjunct to carbamazepine in the treatment of partial seizures.[6] Studies[7] of 3-substituted 1,2-benzisoxazoles identified 1,2-benzisoxazole-3-methanesulfonamide, zonisamide, as a potent anticonvulsant which was able to suppress maximal electroshock (MES) induced seizures, but was ineffective against seizures induced by subcutaneous pentylenetetrazol (scMet). Zonisamide has recently been approved for use as an antiepileptic drug in Japan.[4]

Various secondary and tertiary alkyl and haloalkyl sulfonamides have been prepared and assayed for a broad spectrum of biological activity including anticonvulsant activity.[8−10] These studies identified trifluoromethanesulfonamides as the most active class of anticonvulsants in terms of their ability to inhibit MES- and scMet-induced seizures, although detailed pharmacological data for these compounds has not been published. *N*-(Phenylacetyl)trifluoromethanesulfonamide (**3a**) was cited as one of several compounds of this type which showed promising anticonvulsant potential.[10]

Scheme 1



In recent studies, we examined the effect of phenyl substitutents on the anticonvulsant activity of various 2-benzyl-substituted glutarimides[11] and succinimides.[12] In both series, the introduction of lipophilic ($+\pi$) electron withdrawing ($+\sigma$) substituents into the phenyl ring resulted in a significant increase in anticonvulsant potency compared to the unsubstituted benzyl compounds. These observations together with the reported[10] anticonvulsant activity of **3a** prompted us to prepare a series of substituted (*N*-(phenylacetyl)trifluoromethanesulfonamides using the Topliss[13] approach for the logical choice of phenyl substituents. In this paper we report details of the synthesis and evaluation of the anticonvulsant and toxicological profiles of these compounds.

Chemistry

The *N*-(phenylacetyl)trifluoromethanesulfonamides (**3a**−**g**) were synthesized in two steps from the corresponding carboxylic acids **1a**−**g** *via* the acid chlorides **2** followed by treatment with trifluoromethanesulfonamide in the presence of triethylamine (Scheme 1). Physical property data for compounds **3a**−**g** are summarized in Table 1.

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Table 1. Physical Properties of *N*-(Phenylacetyl)trifluoromethanesulfonamides (**3**)[a]

compd	R	% yield[b]	mp, °C	recryst solvent	formula[c]
3a	H	50	126−127	$CHCl_3$	$C_9H_8F_3NO_3S$
3b	*p*-Cl	52	170−171	acetone−hexanes	$C_9H_7ClF_3NO_3S$
3c	*p*-Br	61	181−182	acetone−hexanes	$C_9H_7BrF_3NO_3S$
3d	*p*-OMe	39	124	$CHCl_3$	$C_{10}H_{10}F_3NO_4S$
3e	*p*-NO_2	35	184−185	$CHCl_3$−acetone	$C_9H_7F_3N_2O_5S$
3f	*m*-Cl	30	114	CH_2Cl_2	$C_9H_7ClF_3NO_3S$
3g	*m*-OMe	46	109	$CHCl_3$	$C_{10}H_{10}F_3NO_4S$

[a] ^{1}H NMR spectra were consistent with assigned structures. [b] Reported yields are for analytically pure compounds. [c] All compounds gave satisfactory C, H, N analyses (±0.4%).

Table 2. Phase I Anticonvulsant Testing Data[a]

		MES[b]		sc Met[c]		toxicity[d]	
compd	R	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
3a	H	++++	++++	+	++++	+	−
3b	4-Cl	++	++++	+++	++++	+	++
3c	4-Br	+++	++++	+	+++	+	+
3d	4-OMe	++	++++	−	++	++	+
3e	4-NO_2	+++	++++	++	++++	++	+
3f	3-Cl	+++	++++	+++	++	++	+
3g	3-OMe	+++	+++	++	++	+	+

[a] ++++, +++, ++, and + denote antiseizure activity or toxicity at 30, 100, 300, and 600 mg/kg ip, respectively; − denotes no activity or no toxicity. [b] Maximal electroshock seizure test. [c] Subcutaneous pentylenetetrazol seizure test. [d] Neurologic toxicity (rotorod test).

Results and Discussion

Pharmacological testing of the *N*-(phenylacetyl)trifluoromethanesulfonamides **3a**−**g** was obtained through the Epilepsy Branch of the National Institute of Neurological Disorders and Stroke (NINDS) following the protocol adopted by the Antiepileptic Drug Development (ADD) program.[14] The results of preliminary (Phase I)[14c] screening of **3a-g** are summarized in Table 2. All seven compounds were effective in blocking scMet-induced seizures and especially MES-induced seizures.[15] With the exception of **3g**, all compounds exhibited anticonvulsant activity at dosages of 30 mg/kg in the MES assay 4 h after drug administration, whereas a minimum dose of 300 mg/kg was required to elicit motor impairment.

On the basis of the considerable anticonvulsant promise suggested in phase I testing, compounds **3a**−**g** were subjected to phase II[14c] evaluation to determine their median effective dose (ED_{50}) values against MES- and scMet-induced seizures and their median toxic dose (TD_{50}) values at the time of peak effect (TPE). These pharmacological parameters are presented in Table 3 for compounds **3a**−**g** and the prototype anticonvulsant drugs phenytoin, phenobarbital, valproate, and ethosuximide.[14c] The ED_{50} values confirmed phase I findings that the test compounds were more effective in controlling MES-induced seizures than those induced chemically. For example, sulfonamides **3a**, **3b**, and **3f** all possessed ED_{50} values against MES-induced seizures of less than 8 mg/kg. Of these three compounds, **3a** was the most potent against both MES-induced seizures (ED_{50} = 6.2 mg/kg) and scMet-induced seizures (ED_{50} = 10 mg/kg). Thus, the phenyl substituents of **3b**−**g** did not enhance the anticonvulsant activity of these Topliss analogs. Relatively large doses of **3a** were required to produce motor impairment (TD_{50} = 329 mg/kg) resulting in extremely attractive protective index (PI) values of 53 and 34 against MES- and scMet-induced seizures, respectively. The TPEs of **3a** for anticonvulsant activity and motor impairment were much longer than those of the prototype anticonvulsant drugs. As a result of its impressive phase II anticonvulsant profile, **3a** was chosen for advanced testing in phases III−VII.[14c]

In phase III testing the toxicity profile was determined by administering TD_{50}, $2 \times TD_{50}$, and $4 \times TD_{50}$ doses of **3a** to mice, intraperitoneally (ip) (Table 4). The TD_{50} dose of **3a** produced decreased motor activity, ataxia, rotorod toxicity, sedation, ptosis, decreased respiration, and dyspnea. At doses of $2 \times TD_{50}$, one of two mice suffered a loss of righting reflex after 8 h and subsequently died within 24 h. Both animals given $4 \times TD_{50}$ doses experienced hypnosis and death within 20 min. Toxicity studies utilizing groups of eight mice established the 24 h median lethal dose (LD_{50}) as 522 mg/kg. The median hypnotic dose (HD_{50}) was indistinguishable from the LD_{50}, since hypnosis and death occurred within the same dosing range. Compound **3a** exhibited a greater margin of safety (HD_{50}/ED_{50}) against either MES- or scMet-induced seizures than any of the prototype drugs.

Phase IV and VI testing evaluated the ED_{50} and the TD_{50} values for compound **3a** following oral (po) administration in mice and rats, respectively (Table 5). These data indicate that **3a** is nearly as effective in blocking MES-induced seizures whether administered po or ip. However, a dramatic decrease in anticonvulsant potency in the scMet test and a similar decrease in rotorod toxicity resulted when **3a** was administered po vs ip in mice. In mice, the calculated $poED_{50}/ipED_{50}$ ratio of 6.1 for the scMet test and an $poTD_{50}/ipTD_{50}$ ratio > 3.0 indicate that **3a** is adequately absorbed when administered po. In oral tests in rats, **3a** again showed good anti-MES activity but was ineffective in nontoxic doses against scMET-induced seizures.[16] Comparison of the po TD_{50}'s for mice and rats identifies **3a** as 25 times more neurotoxic po in rats than in mice resulting in much smaller MES and scMet PI values in rats. The MES safety ratios (TD_3/ED_{97}) of 33 and 2.2 calculated from the oral test data for mice and rats, respectively, also reflect the greater degree of motor impairment of **3a** in rats compared to mice.[14d] Regardless, the PI and safety ratios based on the MES assay following po administration of **3a** compare favorably with those of the prototype anticonvulsant drugs. However, the longer TPE of **3a** compared with the prototypes could be an important factor in evaluating its overall anticonvulsant potential.

In drug differentiation tests (phase V) **3a** inhibited seizures induced in mice by a convulsant dose (CD_{97}) of picrotoxin (ED_{50} = 56 mg/kg) yet afforded no protection against seizures induced by bicuculline or strychnine in doses up to 300 mg/kg (Table 6). This is in contrast to the prototypes where those drugs which were active against picrotoxin also blocked bicuculline and styrchnine induced convulsions.

In vitro receptor binding studies revealed that a 40 μM concentration of **3a** had no significant inhibitory effect on [^{3}H]flunitrazepam binding, and at 100 μM **3a** did not alter [^{3}H]GABA receptor binding. These assays

Table 3. Phase II Quantitative Anticonvulsant Testing Data

compd	MES ED_{50}[a–c]		scMet ED_{50}[a–c]		TD_{50}[a,b,d]		PI[e]		TPE[f]	
							MES	scMet	activity	toxicity
3a	6.2	(5.7–7.0)	10	(3.3–19)	329	(275–370)	53	34	6.0	24.0
3b	7.6	(6.9–9.1)	49	(34–66)	206	(150–289)	27	4.2	4.0	4.0
3c	23	(18–28)	55	(32–73)	291	(249–359)	13	5.3	4.0	2.0
3d	33	(32–34)	53	(29–87)	557	(522–599)	17	10	2.0	0.5
3e	12	(11–15)	20	(11–33)	482	(424–514)	39	24	4.0	6.0
3f	6.8	(5.4–8.1)	35	(22–68)	249	(186–307)	37	7.2	2.0	0.5
3g	19	(16–21)	77	(44–129)	325	(255–437)	17	4.2	2.0	2.0
phenytoin	9.5	(8–10)	*g*		65	(52–72)	6.9	<0.22	2.0	2.0
phenobarbital	22	(15–26)	13	(5.9–16)	69	(63–73)	3.2	5.2	1.0	0.5
valproate	272	(247–338)	149	(123–177)	426	(369–450)	1.6	2.9	0.25	0.25
ethosuximide	>1000		130	(111–150)	441	(383–485)	<0.44	3.4	0.5	0.5

[a] ED_{50} and TD_{50} values are in units of mg/kg of test drug delivered intraperitoneally (ip). [b] 95% confidence intervals are presented in parentheses. [c] Measured at the time of peak effect. [d] Measured at the time of peak neurologic deficit. [e] Protective index: PI = (TD_{50}/ED_{50}). [f] Time to peak effect (TPE) in hours; TPE for activity based on the MES test. [g] No protection up to 300 mg/kg.

Table 4. Phase III Quantitative Toxicity Profile of **3a** and Prototype Anticonvulsant Drugs

compd	HD_{50}[a,b]		LD_{50}[b,c]		HD_{50}/ED_{50}	
					MES	scMet
3a	522	(477–581)	*d*		84.0	53.5
phenytoin	178	(153–195)	230	(216–259)	18.8	
phenobarbital	135	(115–147)	265	(242–286)	6.22	10.3
valproate	885	(821–947)	1100	(1020–1250)	3.26	5.96
ethosuximide	850	(751–918)	1810	(614–1920)		6.53

[a] Median hypnotic dose (HD_{50}) in mg/kg; determined by loss of righting reflex. [b] 95% confidence intervals in parentheses. [c] Median lethal dose (LD_{50}) in mg/kg; mortality was determined 24 h after ip injection. [d] Hypnosis and death occurred over the same dosing range.

Table 5. Phase IV and VI Pharmacological Data for **3a** and Prototype Compounds (po) in Mice and Rats

compd	test animal	TPE (h)			MES ED_{50}[a]		sc Met ED_{50}[a]		toxicity	TD_{50}[a]	PI	
		MES	scMET	Tox							MES	scMet
3a	mice	8	8	24	8.4	(7.6–9.0)	60	(17–129)		>1000	>119	>17
3a	rats	8	8	24	6.1	(5.2–7.3)	*b*		40	(30–49)	6.6	<1.0
phenytoin	mice	2	2	2	9.0	(7.4–11)	*c*		87	(80–96)	9.6	<0.29
phenytoin	rats	4	4	0.5	30	(22–39)	*d*		*e*		>100	
phenobarbital	mice	2	2	2	20	(15–32)	13	(8.0–19)	97	(80–115)	4.8	7.7
phenobarbital	rats	5	5	0.5	9.1	(7.6–12)	12	(7.7–15)	61	(44–96)	6.7	5.3
ethosuximide	mice	0.5	0.5	1	*f*		193	(159–218)	879	(840–933)	<0.44	4.6
ethosuximide	rats	2	2	2	*g*		54	(46–61)	1010	(902–1110)	<0.84	19
valproate	mice	1	1	2	665	(605–718)	388	(349–439)	1260	(800–2250)	1.9	3.3
valproate	rats	0.5	0.5	1	490	(351–728)	180	(147–210)	280	(191–353)	0.57	1.6

[a] ED_{50} and TD_{50} values are in units of mg/kg and determined at the indicated time. [b] No protection up to 40 mg/kg. [c] No protection up to 300 mg/kg. [d] No protection up to 800 mg/kg. [e] No ataxia up to 3000 mg/kg. [f] No protection up to 2000 mg/kg. [g] No protection up to 1200 mg/kg.

Table 6. Phase V Testing of **3a** and Prototype Drugs; Anticonvulsant Drug Differentiation Testing

compd	ED_{50} scBic[a,b]		ED_{50} scPic[a,b]		ED_{50} scStrych[a,b]		PI[c]		
							scBic	scPic	scStrych
3a	*d*		56	(30–127)	*d*		*e*	5.9	*e*
phenytoin	*f*		*f*		***g***		*e*	*e*	*e*
phenobarbital	38	(26–47)	28	(21–35)	95	(91–100)	1.9	2.5	0.72
valproate	360	(294–439)	387	(341–444)	293	(261–323)	1.8	1.1	1.5
ethosuximide	459	(350–633)	243	(228–255)	*h*		0.96	1.8	<0.44

[a] ED_{50} values are in units of mg/kg of compound administered ip at the time of peak therapeutic effect (TPE). [b] 95% confidence intervals are presented in parentheses. [c] Calculated from TD_{50} (ip mice) values in Table 3. [d] No protection up to 300 mg/kg. [e] Cannot be determined accurately with the data. [f] No protection up to 100 mg/kg. [g] Maximum 50% protection at 55–100 mg/kg. [h] Maximum 62.5% protection at 250–1000 mg/kg.

suggest that the anticonvulsant action of **3a** does not directly involve benzodiazepine or GABA receptors.

Phase VII testing examined the effect of prolonged oral administration on the anticonvulsant activity of **3a**. Tolerance was assessed *via* the MES test following 5-day chronic administration of **3a** and subsequently by the hexobarbital sleep time test and by assaying for increases in liver microsomal enzymes (Table 7). Five-day chronic studies in rats demonstrate that no tolerance to **3a** was induced by five daily doses of 6 mg/kg (MES ED_{50}).[17] Instead, an increase in the anticonvulsant activity of **3a** was observed for chronically treated rats compared to the control (acute) group which suggests accumulation of **3a** over time (Table 7). Following 5-day chronic administration of **3a**, test animals then treated with hexobarbital (ip 100 mg/kg) on day 6 experienced an increase in hexobarbital-induced sleep time relative to the control group. These results are consistent with the absence of tolerance as indicated by the MES assay.[18] Further evidence for the lack of

Table 7. Phase VII testing of **3a**: 5-Day Tolerance and 7-Day Enzyme Induction Studies in Rats

test groups[a-c]	animals protected	hexobarbital sleep time[d]	body weight[e]	liver weight[e]	total liver protecin[f]	cytochrome P-450[g]	*p*-nitroanisole *O*-demethylase[h]	NADPH cytochrome C reductase[i]
treated (chronic)	8/8	51 ± 3	128.8 ± 3.1	6.35 ± 0.34	40.4 ± 3 .8	0.41 ± 0.02	0.36 ± 0.05	109.9 ± 3.6
control (acute)	6/8	33 ± 2						
control	0/8	32 ± 2	138.8 ± 1.3	7.38 ± 0.3	37.3 ± 1.1	0.40 ± 0.03	0.32 ± 0.05	96.9 ± 4.0

[a] Treated (chronic) rats were administered 6 mg/kg po of **3a** (MES ED_{50}) once a day for 5 consecutive days. [b] Control (acute) rats were given the requisite volume of vehicle po for 4 days and a single 6 mg/kg po dose of **3a** on day 5. [c] Control rats were administered vehicle po for 5 days. [d] The length of time in minutes that the loss of righting reflex persisted following an injection of a hypnotic dose (100 mg/kg) of hexobarbital. Test conducted on day six. [e] Mass in grams on day 7: average and deviation. [f] In units of mg/liver. [g] In units of nmol of enzyme/mg liver. [h] In units of nmol/min per mg liver. [i] In units of nmol/min per mg liver.

metabolic or physiological tolerance to **3a** was demonstrated by liver microsomal assays that detected no significant differences in cytochrome P-450 levels or liver microsomal enzyme activity in the livers of chronically treated vs control animals.

Conclusions

Of the seven *N*-(phenylacetyl)trifluoromethanesulfonamides **3a**−**g** examined for anticonvulsant activity, aryl substituents did not enhance efficacy, and the unsubstituted phenyl compound **3a** emerged as the most attractive drug candidate on the basis of its favorable ED_{50} values in the MES and scMet assays combined with favorable PI values. The principal merits of **3a** compared to the prototype anticonvulsant drugs include large PI values (using the MES assay), specificity in blocking scPic-induced seizures, a strong correlation of the dose−response regression line against MES-induced seizures, and a large MES safety ratio. The disadvantages of **3a** include flat regression lines[19] (scMet and scPic), a low safety ratio as determined by the scMet test and long TPE's in all tests. Overall, the pharmacological profile of **3a** is unique and compares favorably with the prototype drugs.

Experimental Section

General Methods. Unless otherwise specified all chemicals were commercial reagent grade and were used without further purification. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by Analytical Services of Virginia Polytechnic Institute and State University using a Perkin-Elmer 240 C, H, and N analyzer, Galbraith Laboratories, Knoxville, TN, or Atlantic Microlabs, Norcross, GA. ^{1}H NMR spectra were obtained on either a Varian EM-390 or a Bruker WP 270 spectrophotometer. Experimental data for all new compounds are provided in Table 1. Typical experiments illustrating the preparation of the target compounds **3a**−**g** are described below.

3-Chlorophenylacetyl chloride (2f). Thionyl chloride (86.5 g, 0.727 mol), previously distilled from linseed oil, was added to 10.0 g (0.0586 mol) of 3-chlorophenylacetic acid (**1f**) under N_2 atmosphere. The reaction mixture was gently warmed to reflux temperature, and the progress of the reaction was monitored by the evolution of gas. After ca. 15 min gas evolution ceased and the thionyl chloride was distilled off at ambient pressure leaving 13.6 g of a red oil. The residual oil was vacuum distilled (bp 79−82 °C, 0.5 mmHg) to yield 10.26 g (93%) of a purple-red oil: bp 150 °C (ambient pressure); ^{1}H NMR ($CDCl_3$) δ 4.12 (s, 2H, benzyl CH_2), 7.17 (t, J = 2 Hz, 1H, C-5 arom H), 7.26 (s, 1H, C-2 arom H), 7.30−7.35 (m, 2H, C-4 and C-6 arom H).

***N*-[(3-Chlorophenyl)acetyl]trifluoromethanesulfonamide (3f).** Trifluoromethanesulfonamide (3.73 g, 0.025 mol) and 9.5 mL of triethylamine (TEA) (0.05 mol) were dissolved in 150 mL of acetone in a dry 500 mL round-bottom flask under N_2 atmosphere. Compound **2f** (4.73 g, 0.025 mol) was added dropwise to the reaction mixture. When the addition was complete, the reaction mixture was maintained at reflux temperature for 1 h. The solvent was removed under reduced pressure and the residue was dissolved in 200 mL of EtOAc. The organic phase was washed twice with 100 mL portions of 1 N HCl and twice with 50 mL portions of brine and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the resulting yellow solid was recrystallized twice from CH_2Cl_2 to yield 1.49 g (30%) of **3f** as fine white needles: mp 114 °C; ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 2H, benzyl CH_2), 7.14 (t, J = 3 Hz, 1 H, C-5 arom CH), 7.26 (s, 1H, C-2 arom CH), 7.35 (m, 2H, C-4 and C-6 arom CH), 7.87 (bs, 1H, NH). Anal. ($C_9H_7NO_3SClF_3$) C, H, N.

Pharmacology. Pharmacological evaluation of the candidate compounds, **3a**−**g**, was performed by the Epilepsy Branch of NINDS using established protocols.[14c] Tests were conducted with either Carworth Farms No. 1 mice or Sprague−Dawley rats. Solutions of the test compounds were prepared in 30% polyethylene glycol 400 and were administered ip or po in a volume of 0.01 mL/g body weight for mice and 0.004 mL/g body weight in rats.

In phase I screening (Table 2) each compound was administered as an ip injection at four dose levels (30, 100, 300, and 600 mg/kg) with anticonvulsant activity and neurotoxicity assessed at 30 min and 4 h intervals after administration. Anticonvulsant efficacy was measured by the maximal electroshock (MES) test and the subcutaneous pentylenetetrazol (scMET) test. In the MES test, seizures were elicited with a 60-Hz alternating current of 50 mA intensity in mice and 150 mA intensity in rats. The current was applied *via* corneal electrodes for 0.2 s. Abolition of the hind-leg tonic-extensor component of the seizure indicated protection against the spread of MES-induced seizures. The scMet test involved subcutaneous injection of a convulsant dose (CD_{97}) of pentylenetetrazol (85 mg/kg in mice, 70 mg/kg in rats). Elevation of the pentylenetetrazol-induced seizure threshold was indicated by the absence of clonic spasms for at least a 5-s duration over a 30-min period following administration of the test compound. Anticonvulsant drug-induced neurologic deficit was detected in mice by the rotorod ataxia test and in rats by the positional sense, gait, and stance tests.

The pharmacological parameters estimated in phase I screening were quantified for compounds **3a**−**g** in phase II screening (Table 3). Anticonvulsant activity was expressed in terms of the median effective dose (ED_{50}), and neurotoxicity was expressed as the median toxic dose (TD_{50}). For determination of the ED_{50} and TD_{50}, groups of 6−12 mice were given a range of ip doses of the test drug until at least three points were established in the range of 10−90% seizure protection or minimal observed neurotoxicity. From the plot of this data, the respective ED_{50}, TD_{50} values, 95% confidence intervals, slope of the regression line, and the standard error of the slope were calculated by means of a computer program written at NINDS.

In phase III testing, the general behavior of mice was assessed at regular time intervals up to 24 h following ip administration of TD_{50}, 2 × TD_{50}, and 4 × TD_{50} doses of the test compound. The median hypnotic dose (HD_{50}), assessed by loss of righting reflex, and the 24-h median lethal dose (LD_{50}) were determined (Table 4) using the procedure described previously for evaluation of the ED_{50} and TD_{50} values.

Phase IV and phase VI testing (Table 5) involved the same procedures for determining ED_{50} and TD_{50} as used in phase

II screening, except that the test drug was administered po to mice (phase IV) and po to rats (phase VI).

In the phase V drug differentiation tests (Table 6), the CD_{97} of each of four convulsants were administered ip as a 0.5% solution to mice. The animal was then observed for 30 min in the scMet, bicuculline, and strychnine tests and for 45 min in the picrotoxin test. Protection was defined as the absence of clonic spasms of seizures in the bicuculline and picrotoxin tests, and abolition of the hind-leg tonic-extensor component of a seizure in the strychnine test. *In vitro* receptor binding studies were performed as follows. The crude synaptic membranes were prepared according to the method of Enna and Snyder[20] for use in the benzodiazepine receptor binding studies and by the method of Ticku and Burch[21] in the GABA receptor binding studies. [^{3}H]Flunitrazepam binding studies were performed by a modified method of Braestrup and Squires,[22] and GABA binding studies were done using the method of Zukin *et al.*[23] and by a centrifugation assay according to Enna and Snyder.[24]

Phase VII chronic studies measured the development of tolerance to the candidate drug following po administration to rats. To determine the effect of 5-day chronic treatment on anticonvulsant activity against MES-induced seizures, three groups of eight animals each were subjected to the following dosing regimens. Group one, control, was furnished the vehicle po daily for 5 days. Group two, control (acute), was given the vehicle for 4 days, and a single dose (MES ED_{50}) of the test drug was administered on the fifth day. Group three, treated (chronic), was administered the MES ED_{50} dose of the test drug each day for 5 days. All groups were then subjected to the MES test at the time of peak drug effect on day 5, and the number of animals protected was determined. On day 6 all rats were administered hypnotic doses of hexobarbital (100 mg/kg) ip. The sleep time of each was determined to the nearest minute, and the mean sleep time for each group was calculated. After remaining on their original treatment regimen for an additional 2 days, the rats were decapitated on day 8 and the livers were perfused with 0.9% sodium chloride solution, removed, weighed, and homogenized in 0.25 M sucrose. Preparations of the microsomes and subsequent tests were performed according to the procedure described by Franklin and Estabrook.[25]

Note: Complete anticonvulsant and toxicity screening data for all compounds submitted to the Antiepileptic Drug Development (ADD) program is available from the authors.

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(15) Greater efficacy against MES-induced seizures than against scMet-induced convulsions appears to be a phenomenon common to many anticonvulsant primary sulfonamides, see refs 5−7.
(16) This lack of activity against scMet seizures was verified by ip administration of doses of 25, 50, and 100 mg/kg of **3a** to three groups of two rats each. This resulted in neurotoxicity in all animals and no protection by the scMet test.
(17) These results are similar to those reported for zonisamide after 7-day tolerance studies (see ref 7b) but contrast with the rapid development of tolerance observed with acetazolamide, see ref 6.
(18) Development of tolerance is indicated by a shorter sleep time in chronically treated animals, see ref 14c.
(19) Flat regression lines are indicative of a wide dosage range between zero and 100% anticonvulsant effect of the test drug.
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