2o, 124421-32-5; **2p**, 124421-33-6; **3a**, 124421-34-7; **3b**, 124421-35-8; **3c**, 124421-36-9; **3d**, 124421-37-0; **3e**, 124421-38-1; **4a**, 124421-44-9; **4b**, 124421-45-0; **4c**, 124421-46-1; **4d**, 124421-47-2; **5**, 117422-80-7; **8**, 33741-79-6; **9**, 117422-79-4; **10**, 117422-81-8; (*R*)-**10**, 124509-57-5; (*S*)-**10**, 124509-58-6; **12**, 124421-42-7; **13**, 124421-43-8; (*R*)-**13**, 124509-63-3; **14**, 124421-48-3; (*R*)-**14**, 124509-62-2; (±)-(BOC)-$NHCH(2-C_{10}H_7)CONHCH_2Ph$, 124421-39-2; (±)-$H_2NCH(2-C_{10}H_7)CONHCH_2Ph\cdot MeSO_3H$, 124421-41-6; 2-$FC_6H_4CH_2NH_2$, 89-99-6; 3-$FC_6H_4CH_2NH_2$, 100-82-3; 4-$FC_6H_4CH_2NH_2$, 140-75-0; 2,5-$F_2C_6H_3CH_2NH_2$, 85118-06-5; 2,6-$F_2C_6H_3CH_2NH_2$, 69385-30-4; (*R*)-$PhCHMeNH_2$, 3886-69-9; (*S*)-$PhCHMeNH_2$, 2627-86-3; 2-methylpyrrole, 636-41-9; (±)-α-amino-32-thiopheneacetic acid, 38150-49-1; furan, 110-00-9; (*R*)-(α-methylbenzyl)ammonium (*R*)-α-acetamido-2-furanacetate, 124509-59-7; (*S*)-(α-methylbenzyl)ammonium (*S*)-α-acetamido-2-furanacetate, 124509-60-0; (*R*)-(α-methylbenzyl)ammonium (*S*)-α-acetamido-2-furanacetate, 124509-61-1.

Supplementary Material Available: Tables listing data collection and processing parameters (Table IV), atomic coordinates and equivalent isotropic displacement parameters (Table V), bond lengths (Table VI), bond angles (Table VII), and hydrogen-bonding parameters (Table VIII) (4 pages); observed and calculated structure factors (Table IX) for compound (*R*)-**2g** (3 pages). Ordering information is given on any current masthead page.

Synthesis and Anticonvulsant Activity of 2-Benzylglutarimides

R. Richard Goehring, Thomas D. Greenwood, Godson C. Nwokogu, Jyothi S. Pisipati, Tommie G. Rogers, and James F. Wolfe*

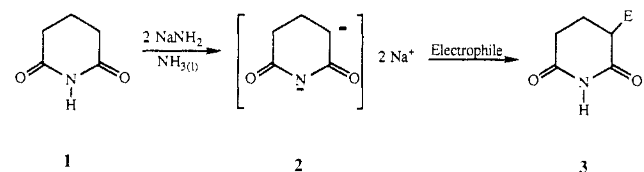
Department of Chemistry and The Harvey W. Peters Research Center for the Study of Parkinson's Disease and Disorders of the Central Nervous System, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.
Received August 21, 1989

A series of 2-benzylglutarimides (**4**) and their *N*-methyl analogues (**5**) were prepared according to the Topliss scheme for the selection of benzyl substituents to maximize anticonvulsant activity. A total of 22 such compounds were subjected to initial (phase I) screening in mice against seizures induced by maximal electroschock (MES) and pentylenetetrazol (scMet) and in the rotorod assay for neurotoxicity. From this series of test compounds, 10 were advanced to quantitative (phase II) testing. Of these, 2-(4-chlorobenzyl)glutarimide (**4b**) emerged as the most promising anticonvulsant drug candidate by demonstrating both good anti-scMet and anti-MES activity combined with low neurotoxicity after intraperitoneal administration in mice. In drug differentiation tests, **4b** was also effective in nontoxic doses against seizures induced by bicuculline, picrotoxin, and strychnine. When compared with the clinically useful drugs phenytoin, carbamazepine, phenobarbital, valproate, and ethosuximide, **4b** exhibited an overall pharmacological profile most closely resembling that of valproate.

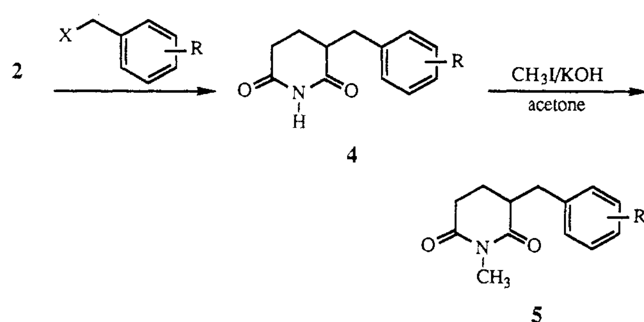
The past decade has witnessed a resurgence of interest in the development of new anticonvulsant drugs. Recent estimates indicate that 1% of the population is affected by some form of epilepsy and that 20–40% of epileptic patients fail to experience significant seizure control with the drugs currently available.[2] Furthermore, the anticonvulsant drugs presently used in clinical practice suffer from a broad range of adverse side effects.[2,3] No single drug is effective against the various forms and degrees of convulsive disorders and the necessity for repeat and combination therapy[3b] not only increases the danger of toxic effects but also contributes to the dissatisfaction of the patient. Consequently, there is a need for new antiepileptic substances having greater specificity and fewer toxic side effects.

Even though the search for antiepileptic drugs has recently been extended to novel structural types,[4] the classical group of cyclic imides, because of their demonstrated anticonvulsant potency, continue to receive considerable attention.[5] Within the last 20 years, however, there are surprisingly few reports concerning the observation and evaluation of anticonvulsant properties for substituted glutarimides.[5e,6–8]

Scheme I



Scheme II



Our earlier discovery that the dianion (**2**) prepared from glutarimide (**1**) and sodium amide in liquid ammonia reacted with electrophiles such as alkyl halides, aldehydes, ketones, and esters exclusively at the carbanion site provided a convenient, one-step route to 2-substituted glu-

(1) Shorvon, S. D.; Reynolds, E. H. *Br. Med. J.* **1982**, *285*, 1699.
(2) Liebman, J. M.; Schneider, J. A. *Annu. Rep. Med. Chem.* **1985**, *20*, 11.
(3) (a) Schmidt, D. *Epilepsia* **1984**, *25*, 544. (b) Lindhout, D.; Höppener, R. J. E. A.; Meinardi, H. *Ibid.* **1984**, *25*, 77.
(4) Meldrum, B. S.; Porter, R. J. *New Anticonvulsant Drugs. Current Problems in Epilepsy 4*; John Libbey: London, 1986; Chapter 3.
(5) For more recent examples, see: (a) Kornet, M. J. *J. Pharm. Sci.* **1984**, *73*, 405. (b) Poupaert, J. H.; Vandervorst, D.; Guiot, P.; Moustafa, M. M. M.; Dumont, P. *J. Med. Chem.* **1984**, *27*, 76. (c) Cortes, S.; Liao, Z.-K.; Watson, D.; Kohn, H. *J. Med. Chem.* **1985**, *28*, 601. (d) Tarver, M. L.; Nicholson, J. M.; Scott, K. R. *J. Pharm. Sci.* **1985**, *74*, 785. (e) Piatak, D. M.; Tang, P. L.; Yen, C.-C. *J. Med. Chem.* **1986**, *29*, 50. (f) Borenstein, M. R.; Doukas, P. H. *J. Pharm. Sci.* **1987**, *76*, 300.
(6) Witiak, D. T.; Seth, S. K.; Baizman, E. R.; Weibel, S. L.; Wolf, H. H. *J. Med. Chem.* **1972**, *15*, 1117.
(7) Aboul-Enein, H. Y.; Schauberger, C. W.; Hansen, A. R.; Fischer, L. J. *J. Med. Chem.* **1975**, *18*, 736.
(8) Witiak, D. T.; Cook, W. L.; Gupta, T. K.; Gerald, M. C. *J. Med. Chem.* **1976**, *19*, 1419.

0022-2623/90/1833-0926$02.50/0 © 1990 American Chemical Society

Table I. Physical Properties of 2-Benzylglutarimides 4 and N-Methyl-2-benzylglutarimides 5[a]

compd	R	% yield	mp, °C	formula[b]	recryst solvent
4a	H	80	142–144[c]	$C_{12}H_{13}NO_2$	EtOH
4b	4-Cl	56	158–159[d]	$C_{12}H_{12}ClNO_2$	EtOH
4c	4-OMe	83	152–153[d]	$C_{13}H_{14}NO_3$	EtOH
4d	2-Br	43	184–185	$C_{12}H_{12}BrNO_2$	toluene
4e	4-Br	41	162–164	$C_{12}H_{12}BrNO_2$	$CHCl_3$–hexane
4f	4-I	61	158–160	$C_{12}H_{12}INO_2$	$CHCl_3$–hexane
4g	4-CF_3	70	149–151	$C_{13}H_{12}F_3NO_2$	toluene–hexane
4h	2,4-Cl_2	58	152–155	$C_{12}H_{11}Cl_2NO_2$	toluene–hexane
4i	3,4-Cl_2	38	131–133	$C_{12}H_{11}Cl_2NO_2$	MeOH
4j	4-F	20	166–168	$C_{12}H_{12}FNO_2$	$CHCl_3$–hexane
4k	4-Ph	56	181–182	$C_{18}H_{17}NO_2$	ether–EtOAc
4l	4-$SiMe_3$	54	150–152	$C_{15}H_{21}NO_2Si$	toluene–hexane
4m	4-OPr	54	125–126	$C_{15}H_{19}NO_3$	EtOH–hexane
4n	4-OiPr	59	132–133	$C_{15}H_{19}NO_3$	EtOH–hexane
5a	H	66	61–63	$C_{13}H_{15}NO_2$	ether–hexane
5b	4-Cl	67	61–64	$C_{13}H_{14}ClNO_2$	ether–hexane
5c	2-Br	75	68–72	$C_{13}H_{14}BrNO_2$	EtOH–H_2O
5d	4-Br	87	67–69	$C_{13}H_{14}BrNO_2$	ether–hexane
5e	4-I	84	106–107	$C_{13}H_{14}INO_2$	THF–hexane
5f	4-CF_3	95	99–101	$C_{14}H_{14}F_3NO_2$	ether–hexane
5g	2,4-Cl_2	63	89–91	$C_{13}H_{13}Cl_2NO_2$	hexane
5h	4-$SiMe_3$	91	74–75	$C_{16}H_{23}NO_2Si$	$CHCl_3$–hexane

[a] The infrared and ^1H NMR spectra were consistent with assigned structures. [b] All compounds gave satisfactory C, H, N analyses (±0.4%). [c] Lit.[18] mp 143–144 °C. [d] Reference 9.

tarimides (3)[9] (Scheme I). Primary (Phase I) anticonvulsant screening of 16 2-substituted glutarimides prepared in this way showed that those glutarimides with a 2-benzyl substituent possessed some efficacy against maximal electroshock (MES)- and/or Metrazol (scMet)-induced seizures. This prompted us to prepare a series of 2-benzylglutarimides with the goal of maximizing anticonvulsant efficacy. The substitution pattern suggested by Topliss[10] was followed for rational choice of phenyl substituents. In this paper, we wish to report details of the synthesis and evaluation of anticonvulsant properties of these compounds.

Results and Discussion

Chemistry. The 2-benzylglutarimides (**4a–n**) were synthesized by treatment of disodioglutarimide (**2**) in liquid ammonia with the appropriately substituted benzyl halide. *N*-methyl analogues (**5a–h**) of certain 2-benzylglutarimides were prepared by the method of Pachter and Kloetzel[11] (Scheme II). Physical property data for these compounds are summarized in Table I.

Selection of Compounds. As mentioned previously, Phase I screening of the various 2-substituted glutarimides initially prepared in our laboratory revealed that 2-benzylglutarimides **4a–c** appeared to be most promising. Of these compounds, 2-(4-chlorobenzyl)glutarimide (**4b**) was chosen for phase II screening where it showed good ED_{50}'s in both electrically and chemically induced seizures, being most potent in the scMet test. On the basis of these test results and considering the approach developed by Topliss[10] for assessing the influence of aromatic substitution on biological activity, it appeared that other electron withdrawing ($+\sigma$) substituents appropriately placed on the benzyl moiety might lead to further enhancement of anticonvulsant activity for other members of this congeneric series. Thus, compounds **4d–k** were prepared by using the Topliss scheme as a guide for choice of substituents. Compound **4l** (R = 4-$Si(Me)_3$) was added to the pool of drug candidates as a result of the discovery by Belsky et al.[12] that [4-(trimethylsilyl)phenyl]acetyl]urea was found

(9) Wolfe, J. F.; Rogers, T. G. *J. Org. Chem.* **1970**, *35*, 3600.
(10) Topliss, J. G. *J. Med. Chem.* 1972, *15*, 1006.
(11) Pachter, I. J.; Kloetzel, M. C. *J. Am. Chem. Soc.* **1952**, *74*, 1321.

Table II. Phase I Anticonvulsant Testing Data[a]

compd	R	MES[b] 0.5 h	MES[b] 4 h	scMet[c] 0.5 h	scMet[c] 4 h	tox[d] 0.5 h	tox[d] 4 h
4a	H	+	–	–	–	–	–
4b	4-Cl	+	+	+++	+	–	–
4c	4-OMe	–	–	–	+	–	–
4e	4-Br	+	+	+++	+	+	–
4f	4-I	–	–	+	+	–	–
4g	4-CF_3	+	+	++	+	+	+
4h	2,4-Cl_2	–	–	+	++	+	+
4j	4-F	+	–	+	–	+	–
5a	4-H	–	–	+	–	–	–
5b	4-Cl	+	–	++	–	+	–
5c	2-Br	++	–	+	++	–	–
5d	4-Br	+	–	++	–	–	–
5e	4-I	–	–	+++	+	–	–
5f	4-CF_3	–	+	+	+	–	–
5g	2,4-Cl_2	–	–	++	++	–	–

[a] +++, ++, and + denote activity or toxicity at 30, 100, and 300 mg/kg, respectively, – denotes no activity or toxicity up to 300 mg/kg. [b] Maximal electroshock seizure test. [c] Subcutaneous Metrazol seizure test. [d] Neurologic toxicity (rotorod test). Compounds **4d**, 4i, **4k–4n**, and **5h** were inactive in the MES and scMet tests at 300 mg/kg. Compounds **4d**, 4m, and **4n** exhibited neurotoxicity at 300 mg/kg.

to be both more effective and longer acting than (phenylacetyl)urea (phenacemide) in the MES model. 2-Benzylglutarimides **4m** (R = 4-OPr) and **4n** (R = 4-OiPr) were synthesized after a preliminary quantitative structure–activity relationship (QSAR) study[13] limited to compounds **4b** and **4e–h** and as a result of a report[14] that certain analogous 4-alkoxy-2-benzylsuccinimides exhibited anticonvulsant activity similar to that of methsuximide and phensuximide. Finally, *N*-methyl derivatives **5a–h** of the more promising 2-benzylglutarimides were prepared in order to assess the effect of increasing lipophilicity and loss of hydrogen-bonding capability on anticonvulsant activity.

Pharmacology. All 2-benzylglutarimides prepared during the course of this study were submitted to the Epilepsy Branch of the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) for pharmacological testing. Each compound was evaluated for anticonvulsant activity and toxicity by using the testing protocol adopted by the Antiepileptic Drug Development (ADD) Program.[15] Primary anticonvulsant screening data (phase I), which are summarized in Table II, indicate that the glutarimides in general show greater efficacy against scMet-induced seizures than those induced by MES. Only in the case of **5c** was protection against MES-induced seizures achieved at dosages <300 mg/kg. In contrast, compounds **4b**, **4e**, and **5e** were all effective in the scMet test at 30 mg/kg. Furthermore, as predicted earlier, the data show that the most impressive activity resides in those

(12) Belsky, I.; Gertner, D.; Zilkha, A. *J. Med. Chem.* **1968**, *11*, 451.
(13) Pisipati, J. S. Ph.D. dissertation, Virginia Polytechnic Institute and State University, June 1984.
(14) Babiyan, N. A.; Mnatsakanyan, V. A.; Arutyunyan, R. G.; Akopyan, N. E.; Mndzhoyan, O. L. *Izv. Akad. Nauk Arm. SSR, Khim. Nauki* 1964, *17* (5), 549.
(15) (a) "*Anticonvulsant Screening Project, Antiepileptic Drug Development Program*"; National Institutes of Health, DHEW Publ (NIH) (U.S.) 1978, NIH 78-1093. (b) Porter, R. J.; Cereghino, J. J.; Gladding, G. D.; Hessie, B. J.; Kupferberg, H. J.; Scoville, B.; White, B. G. *Cleveland Clin. Q* 1984, *51*, 293. (c) Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. *Epilepsia* 1978, *19*, 409. (d) Swinyard, E. A.; Wolf, H. H.; Shenoy, A. K.; Kupferberg, H. J.; Gladding, G. D. "The Early Evaluation of Anticonvulsant Drugs"; Technical Report No. 21 on Contract No. N01-NS-1-2347; Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke: Bethesda, MD, 1981.

Table III. Phase II Pharmacological Evaluation Data

compd	ED_{50}[a,b] MES	scMet	tox, TD_{50}[a,c]	PI[d] MES	scMet	TPE[e] activity	toxicity
4b	136.8 (113.9–158.4)[f]	72.76 (52.15–110.52)	1223.9 (954.6–1479.9)	8.95	16.82	0.5	1.0
4e	278.8 (103.4–421.7)	56.33 (18.56–92.88)	513.1 (470.5–560.3)	1.84	9.11	1.0	1.0
4f	*g*	87.19 (22.28–294.8)	>1050		>12.04	1.0	
4g	126.3 (113.2–139.5)	119.3 (85.02–158.4)	348.6 (319.2–382.3)	2.76	2.92	2.0	1.0
4h	*h*	160.1 (123.7–188.4)	687.3 (619.8–780.0)		4.29	1.0	0.5
5b	247.9 (219.2–269.0)	123.7 (78.66–178.7)	348.1 (315.2–380.1)	1.40	2.81	0.5	1.0
5c	474.1 (400.0–700.0)	255.9 (206.8–342.1)	617.4 (531.0–694.1)	1.30	2.41	1.0	0.5
5d	162.0 (149.1–187.4)	143.3 (119.6–167.1)	533.8 (490.5–588.2)	3.80	3.72	1.0	1.5
5c	*i*	174.9 (114.1–262.9)	>900		>5.15	1.0	
5g	*h*	153.4 (90.35–259.1)	650.4 (444.6–768.2)		4.24	0.5	2.0
phenytoin	9.50 (8.13–10.44)	*j*	65.46 (52.49–72.11)	6.89		2.0	2.0
carbamazepine	8.81 (5.45–14.09)	*j*	71.56 (45.91–134.7)	8.12		0.25	0.25
phenobarbital	21.78 (14.99–25.52)	13.17 (5.87–15.93)	69.01 (62.84–72.89)	3.17	5.24	1.0	0.5
valproate	271.7 (247.0–337.9)	148.6 (122.6–177.0)	425.8 (368.9–450.4)	1.57	2.87	0.25	0.25
ethosuximide	>1000	130.4 (111.0–150.5)	440.8 (383.1–485.3)		3.38	0.5	0.5

[a] ED_{50} and TD_{50} values are in mg/kg of test drug delivered intraperitoneally (ip). [b] Measured at time of peak effect. [c] Measured at time of peak neurologic deficit. [d] PI = protective index (TD_{50}/ED_{50}). [e] Time of peak effect. TPE for activity determined in the MES test for compounds **4b**, **4e**, **4g**, and **5b–d** and in the scMet test for MES-inactive compounds **4f**, **4h**, **5e**, and **5g**. [f] Numbers in parentheses are 95% confidence intervals. [g] No protection up to 750 mg/kg. [h] No protection up to 1050 mg/kg. [i] No protection up to 800 mg/kg. [j] Not effective.

glutarimides bearing an electron withdrawing (+σ) substituent in the 4-position. The inactivity of compound **4i** (R = 3,4-Cl_2) might be due to some unfavorable steric interaction with a receptor site[10] or lack of suitable lipophilicity.[16]

On the basis of the results of phase I screening, compounds **4b**, **4e–h**, **5b–e**, and **5g** were selected for phase II quantification of their pharmacological parameters. Median effective doses (ED_{50}'s) were evaluated against MES- and scMet-induced convulsions and median toxic doses (TD_{50}'s) were determined by using the rotorod procedure for assessing neurologic deficit. These results are summarized in Table III along with similar information for the clinically useful antiepileptic drugs phenytoin, carbamazepine, phenobarbital, valproate, and ethosuximide.[15] Comparison of the activities of the 2-benzylglutarimides (**4**) with those of their corresponding *N*-methyl analogues (**5**) reveals that methylation tends to decrease anticonvulsant potency except for the MES activity of **4e** vs **5d** (R = 4-Br) and the scMet activity of **4h** vs **5g** (R = 2,4-Cl_2). The data in Table III also confirm the phase I findings that all test compounds are more effective at controlling scMet-type seizures than those induced by MES, with compounds **4b**, **4e**, and **4f** all having ED_{50}'s < 100 mg/kg. Of these three compounds, only **4b** showed reasonably good protection against MES seizures. Moreover, **4b** was the least neurotoxic of all compounds tested (TD_{50} = 1224 mg/kg), giving it very attractive protective index (PI) values of 8.95 and 16.82 in the MES and scMet tests, respectively. Compared to the prototype drugs, **4b** proved to be less effective than phenytoin, carbamazepine, and phenobarbital in the MES test. However, **4b** was superior to all but phenobarbital in the scMet assay and exhibited much lower neurotoxicity than any of the prototypes. The MES and scMet safety ratios (TD_3/ED_{97}) for **4b** compared favorably with those of the prototype agents, indicating that after intraperitoneal (ip) administration, nontoxic doses of **4b** protected 97% of mice subjected to these tests.[15d] In light of its considerable anticonvulsant potential, compound **4b** was progressed through advanced stages of testing (phases III–VI).

The toxicity profile of **4b** (Table IV) was determined in phase III testing by administering the TD_{50}, 2 × TD_{50}, and 4 × TD_{50} doses ip to mice. The toxicity induced by **4b** was characterized by decreased motor activity, ataxia, sedation,

Table IV. Phase III Toxicity Profile of **4b** and Prototype Drugs

compound	HD_{50}[a]	LD_{50}[b]	LD_{50}/HD_{50}
4b	1282.47 (1153.99–1379.18)[c]	>2400[d]	>1.87
phenytoin	178.34 (152.93–195.45)	229.61 (216.44–259.10)	1.29
carbamazepine	172.24 (134.12–197.79)	628.70 (555.77–707.67)	3.65
phenobarbitol	135.45 (114.90–117.42)	264.70 (241.55–285.52)	1.95
valproate	885.53 (820.86–947.04)	1104.62 (1021.54–1253.66)	1.25
ethosuximide	850.61 (751.19–917.93)	1752.2	2.06

[a] Median hypnotic dose in mg/kg as determined by loss of righting reflex. [b] Median lethal dose in mg/kg measured after 24 h. [c] See footnote *f*, Table III. [d] At 2400 mg/kg, no mice were dead after 24 h; 2/8 mice were deat at 36 h.

ptosis, muscular relaxation, loss of righting reflex, decreased respiration, and cyanosis. Animals given doses of 2 × TD_{50} and 4 × TD_{50} also experienced hypnosis, analgesia, and anesthesia. Two of eight mice died after 36 h at doses of 2 × TD_{50}. All surviving animals were free of overt toxic effects at the end of 24 h. The median hypnotic dose (HD_{50}) for **4b** was established as 1282.5 mg/kg, which is very close to the TD_{50} (1223.9 mg/kg). The 24-h median lethal dose (LD_{50}) could not be accurately determined, but it was in excess of 2400 mg/kg. Although the HD_{50} and LD_{50} of **4b** are significantly greater than those of the prototype drugs mentioned above, the margin between the hypnotic and lethal doses of **4b** are comparable to those of the prototypes.

As in phase II, phase IV involved the evaluation of ED_{50} and TD_{50} for **4b**, except that the candidate drug was administered orally (po) rather than ip in mice. Phase VI testing measured these same pharmacological parameters after po administration in rats. For comparison purposes, the results of these two phases of testing (Table V) will be discussed together. The data in Tables III and V clearly reflect a decrease in anticonvulsant potency and neurotoxicity when **4b** was administered po as opposed to ip in mice. Nevertheless, the PI values in these studies with mice are comparable, regardless of the mode of drug delivery. Calculation of the po ED_{50}/ip ED_{50} ratios for **4b** are > 2.45, 4.23, and 3.77 by the rotorod, MES, and scMet tests, respectively. These ratios suggest that **4b** is adequately absorbed in mice after oral administration. Some

(16) Krogsgaard-Larsen, P. *J. Med. Chem.* 1981, *24*, 1377.

Table V. Phase IV and Phase VI Pharmacological Evaluation of **4b** and Prototype Drugs

compound	rodent type	ED_{50}[a] MES	ED_{50}[a] scMet	toxicity, TD_{50}[a]	PI MES	PI scMet
4b	mice	578.99 (412.67–748.73)	274.00 (183.20–360.60)	>3000	>5.18	>10.95
4b	rats	58.26 (46.57–70.83)	36.42 (28.79–43.33)	68.24 (51.87–83.83)	1.17	1.87
phenytoin	mice	9.04 (7.39–10.62)	*g*	86.71 (80.39–96.09)	9.59	
phenytoin	rats	29.82 (21.92–38.91)	*b*	>3000	>100	
carbamazepine	mice	15.44 (12.44–17.31)	48.07 (40.75–57.35)	217.21 (131.49–270.11)	14.06	4.52
carbamazepine	rats	8.50 (3.39–10.53)	*b*	813.06 (488.76–1233.87)	95.65	
phenobarbitol	mice	20.09 (14.78–31.58)	12.59 (7.99–19.07)	96.78 (79.88–115.00)	4.82	7.69
phenobarbitol	rats	9.14 (7.58–11.86)	11.55 (7.74–15.00)	61.09 (43.72–95.85)	6.68	5.29
valproate	mice	664.80 (605.33–718.00)	388.31 (348.87–438.61)	1264.39 (800–2250)	1.90	3.26
valproate	rats	489.54 (351.14–728.37)	179.62 (146.75–210.35)	280.26 (191.32–352.76)	0.57	1.56
ethosuximide	mice	>2000	192.21 (158.59–218.44)	879.21 (839.89–933.51)		4.57
ethosuximide	rats	>1200	53.97 (45.57–60.85)	1012.31 (901.66–1109.31)		18.75

[a] ED_{50} and TD_{50} values are in mg/kg of drugs delivered orally. [b] Not effective.

Table VI. Anticonvulsant Drug Differentiation Tests for **4b** and Prototype Drugs

compound	ED_{50}[a] scMet[b]	ED_{50}[a] scBic[c]	ED_{50}[a] scPic[d]	ED_{50}[a] scStrych[e]
4b	72.76 (52.15–110.52) [16.82][f]	248.92 (172.13–359.59) [4.92]	158.94 (74.67–326.19) [7.70]	231.88 (116.02–369.17) [5.28]
phenytoin	*g* [<0.22]	*h* [<0.65]	*h* [<0.65]	*i* [<0.65]
carbamazepine	*h* [<0.72]	*j* [<0.72]	37.20 (25.32–59.69) [1.92]	78.83 (39.39–132.03) [0.91]
phenobarbitol	13.17 (5.87–15.93) [5.24]	37.72 (26.49–47.39) [1.83]	27.51 (20.88–34.82) [2.51]	95.30 (91.31–99.52) [0.72]
valproate	148.59 (122.64–177.02) [2.87]	359.95 (294.07–438.54) [1.18]	387.21 (341.37–444.38) [1.10]	292.96 (261.12–323.43) [1.45]
ethosuximide	130.4 (111.0–150.5) [3.39]	459.0 (349.9–633.1) [0.96]	242.7 (227.8–255.2) [1.82]	*k*

[a] ED_{50} values are in mg/kg of drug delivered intraperitoneally (ip). [b] Subcutaneous Metrazol test (CD_{97} = 85 mg/kg). [c] Subcutaneous bicuculline test (CD_{97} = 2.70 mg/kg). [d] Subcutaneous picrotoxin test (CD_{97} = 3.15 mg/kg). [e] Subcutaneous strychnine test (CD_{97} = 1.20 mg/kg). [f] Numbers in brackets are protective indices, PI = TD_{50}/ED_{50}. [g] No protection up to 300 mg/kg. [h] No protection up to 100 mg/kg. [i] Maximum 50% protection at 55–100 mg/kg. [j] One of two protected at 100 mg/kg. [k] Maximum 62% protection at 250–1000 mg/kg.

striking differences in drug response between mice and rats are apparent following po administration of **4b**. The candidate drug is almost 10 times more potent in rats than mice in the MES and scMet tests and nearly 50 times more neurotoxic, resulting in a much narrower margin of safety (PI < 2). A variety of phenomena (e.g. absorption, metabolism) may account for the observed differences in response of mice and rats to **4b** or for changes in potency depending on the mode of administration of **4b** to the same animal species.

When compared with the clinically useful drugs listed in Table V, in oral tests with mice, **4b** exhibited an anticonvulsant profile similar to that of valproate. The low neurotoxicity (TD_{50} > 3000 mg/kg) of **4b** translated to PI values that compared very favorably with those of the prototypes. The oral efficacy of **4b** in rats by the MES test was ca. 8 and 20 times that of the prototype drugs, valproate and ethosuximide, respectively. With regard to anti-scMet activity, **4b** ranked second only to phenobarbital. Unfortunately, its relatively high neurotoxicity (TD_{50} = 68.24 mg/kg) dictated narrow safety margins similar to those of valproate.

In phase V, **4b** were tested for its ability to protect mice aganst seizures induced by convulsant doses (CD_{97}) of pentylenetetrazol (Metrazol), bicuculline (Bic), picrotoxin (Pic), and strychnine (Strych) (Table VI).[15d,16] The profile of anticonvulsant activity of **4b** resembled that of valproate in that both drugs protect, in nontoxic doses, against all four chemically induced seizures. It is also worthy of note that the PI values for **4b** in the four tests are at least 3 times higher than those of all five prototype drugs.

Conclusions. The most promising drug candidate, 2-(4-chlorobenzyl)glutarimide (**4b**), to emerge from pharmacological screening of the 2-benzylglutarimides prepared in this study exhibited an anticonvulsant profile resembling that of valproate. The principle advantages of **4b** are related to its considerable anticonvulsant potency and low toxicity in tests with mice, which translate into fa-

vorable 24-h margins of safety (LD_{50}/ED_{50}) by the MES and scMet tests, and high PI values by the MES, scMet, scBic, scPic, and scStrych tests. The disadvantages include a narrow margin between TD_{50} and HD_{50}, low PI values in oral tests with rats, and relatively flat regression lines by the MES and scMet tests. Overall, however, the pharmacological data suggest that **4b** has significant antiepileptic potential.

Experimental Section

General Methods. 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 or by Galbraith Laboratories, Knoxville, TN. IR spectra were obtained on samples as 5% chloroform solutions using a Perkin-Elmer 710 B spectrophotometer. ^1H NMR spectra were recorded on either a Varian EM-390 spectrometer or a Bruker WP 270 SY spectrometer. Thin-layer chromatography was performed with Eastman chromatogram sheets, type 13181 (silica gel) with fluorescent indicator. Preparative column chromatography was run with 60–200 mesh Davidson silica gel. Experimental data for all new compounds are provided in Table I. Typical experiments illustrating the general procedures for the preparation of the 2-benzylglutarimides (4) and *N*-methyl-2-benzylglutarimides (5) are described below.

2-(2,4-Dichlorobenzyl)glutarimide (4h). The disodium salt of glutarimide (1) was prepared according to the procedure of Wolfe and Rogers.[9] Thus, a 1-L three-necked flask, purged with dry nitrogen and equipped with a dry-ice/acetone condenser, a bare-metal magnetic stirring bar, and a pressure-equalizing addition funnel, was charged with 350 mL of anhydrous liquid ammonia. To this was added 1.50 g (65 mmol) of sodium metal and a catalytic amount of ferric nitrate nonahydrate. After the blue color was discharged, 3.40 g (30 mmol) of solid, powdered glutarimide was added and the white suspension was stirred for 30 min. To the resulting thick white suspension of the dianion (2) was added rapidly a solution of 6.45 g (33 mmol) of 2,4-dichlorobenzyl chloride in 25 mL of anhydrous ether. The addition funnel was rinsed with 10 mL more of ether and the reaction mixture was stirred for 1 h and then quenched by pouring onto 5.35 g (100 mmol) of solid ammonium chloride contained in a 1-L beaker. The ammonia was allowed to evaporate while being replaced with 200 mL of ether (CAUTION: too rapid addition of the ether may cause the ammonia to overheat and erupt). A mixture of 25 mL of concentrated HCl and 250 mL of ice was added, and the resulting mixture was stirred until the ice melted. The ethereal layer was set aside, and the remaining solid and aqueous layer were extracted with methylene chloride (3 × 150 mL). These extracts, combined with the ethereal layer, were washed with an equal volume of water, dried ($MgSO_4$), filtered, and concentrated on a rotary evaporator to give 7.69 g of a cream-colored solid. Chromatography of this solid on 100 g of silica gel using methylene chloride gave 2.12 g of 2,2′,4,4′-tetrachlorostilbene, mp 158–159.5 °C (lit.[17] mp 161–161.5 °C), after recrystallization from THF–hexane. The column was then washed with methanol to give 5.55 g of a white solid. Recrystallization from toluene–hexane afforded 4.77 g (58%) of **4h** as a white powder: mp 152–155 °C, ^1H NMR ($CDCl_3$) δ 1.57–1.97 (m, 2 H, CH_2), 2.33–3.00 (m, 4 H, $COCH_2$ + $ArCH_2$), 3.13–3.57 (m, 1 H, methine), 7.10–7.50 (m, 3 H, aromatic), 10.65 (br s, 1 H, NH); IR (5% solution in $CHCl_3$) 3360 (NH), 1730 (C=O), 1710 (C=O) cm^{-1}.

***N*-Methyl-2-[4-(trifluoromethyl)benzyl]glutarimide (5f).** To a 100-mL flask, equipped with a magnetic stirring bar and condenser, was added 1.42 g (5 mmol) of 2-[4-(trifluoromethyl)benzyl]glutarimide (**4g**), 2.30 g (16 mmol) of iodomethane, and 30 mL of spectrograde acetone. The mixture was warmed slightly to achieve solution and 0.84 g (15 mmol) of powdered potassium hydroxide was added. The reaction mixture was refluxed for 30 min and filtered while hot, and the solvent was removed on a rotary evaporator. The resulting solid residue was partitioned between water and ether, and the aqueous layer was extracted with ether (3 × 50 mL). The combined ethereal layers were washed once with water, dried ($MgSO_4$), and filtered. Removal of the ether gave 1.49 g of crude product as a white, crystalline solid. Recrystallization from ether–hexane gave 1.42 g (95%) of **5f** as white crystals: mp 99–101 °C; ^1H NMR ($CDCl_3$) δ 1.40–2.07 (m, 2 H, CH_2), 2.27–3.00 (m, 4 H, $COCH_2$ + $ArCH_2$), 3.17 (s, 3 H, NCH_3), 3.30–3.70 (m, 1 H, methine), 7.45 (q, 4 H, aromatic); IR (5% solution in $CHCl_3$) 1725 (C=O), 1670 (C=O) cm^{-1}.

Pharmacology. Pharmacological evaluation of the candidate drugs was performed by the Epilepsy Branch of NINCDS using established protocols.[15] Tests were conducted with either Carworth Farms No. 1 mice of Sprague–Dawley rats. Solutions of the test compounds were prepared in 30% polyethylene glycol 400 and were administered intraperitoneally (ip) or orally (po) in a volume of 0.01 mL/g of body weight in mice and 0.004 mL/g in rats. In phase I screening (Table II), each compound was given in three dose levels (30, 100, and 300 mg/kg) with anticonvulsant activity and neurotoxicity being assessed 30 min and 4 h after administration.

Anticonvulsant efficacy was measured by the maximal electroshock (MES) test and the subcutaneous Metrazol (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 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 the 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 of at least 5-s duration during 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 and gait and stance tests.

The pharmacological parameters estimated in phase I were quantified for the most promising compounds (**4b**, **4e**–h, **5b**–**e**, and **5g**) in phase II (Table III). Anticonvulsant activity was expressed in terms of the median effective dose (ED_{50}) and neurotoxicity as the median toxic dose (TD_{50}). For the determination of the ED_{50} and TD_{50}, groups of 6–12 mice were given a range of doses of the test drug ip until at least three points were established in the range of 10–90% seizure protection or minimal neurotoxicity. From the plot of this data, the respective ED_{50} and TD_{50} values, the 95% confidence intervals, the slope of the regression line, and the SE of the slope were calculated by means of a computer program written by NINCDS.

In phase III testing, the general behavior of mice was assessed at regular time intervals up to 24 h following ip administration of the TD_{50}, 2 × TD_{50}, and 4 × TD_{50} 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 IV) by using the procedure described previously for the evaluation of ED_{50} and TD_{50}.

Phases IV and VI (Table V) involved the same procedures for determining ED_{50} and TD_{50} as used in phase II, except the test drug was administered po to mice (phase IV) and po to rats (phase VI).

In the phase V drug differentiation tests (Table VI), 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 Metrazol, bicuculline, and strychnine tests and for 45 min in the picrotoxin test. Protection was defined as the absence of clonic spasms persisting for at least 5 s in the Metrazol test, complete absence of seizures in the bicuculline and picrotoxin tests, and abolition of the hind-leg tonic-extensor component of a seizure in the strychnine test.

Acknowledgment. We are pleased to acknowledge the generous financial support of this work by the Harvey W. Peters Research Center for Parkinson's Disease and Disorders of the Central Nervous System Foundation and by the National Institute of Neurological and Communicative Disorders and Stroke, Grant Number NS10197. The authors also wish to thank Gill Gladding and James Stables

(17) Hoeg, D. F.; Lusk, D. I. *J. Organomet. Chem.* **1966**, *5*, 1.
(18) Yu, T. Y.; Huanq, M. Y. *Hua Hsueh Hsueh Pao* **1959**, *25*, 146.

for providing pharmacological data through the Antiepileptic Drug Development Program, National Institute of Health.

Supplementary Material Available: Complete anticonvulsant and toxicity screening data for all compounds submitted to the National Institute of Health's Antiepileptic Drug Development (ADD) Program protocol is available from the authors.

Registry No. 1, 1121-89-7; **4a**, 97938-45-9; **4b**, 24866-79-3; **4c**, 24866-80-6; **4d**, 124482-59-3; **4e**, 124482-60-6; **4f**, 124482-61-7; **4g**, 124482-62-8; **4h**, 124482-63-9; **4i**, 124482-64-0; **4j**, 124482-65-1; **4k**, 124482-66-2; **4l**, 124482-67-3; **4m**, 51180-38-2; **4n**, 51180-39-3; **5a**, 124482-68-4; **5b**, 124482-69-5; **5c**, 124482-70-8; **5d**, 124482-71-9; **5e**, 124482-72-0; **5f**, 124482-73-1; **5g**, 124482-74-2; **5h**, 124482-75-3; $PhCH_2Br$, 100-39-0; p-$ClC_6H_4CH_2Br$, 622-95-7; p-$MeOC_6H_4CH_2Br$, 2746-25-0; o-$BrC_6H_4CH_2Br$, 3433-80-5; p-$BrC_6H_4CH_2Br$, 589-15-1; p-$IC_6H_4CH_2Br$, 16004-15-2; p-$CF_3C_6H_4CH_2Br$, 402-49-3; 2,4-$Cl_2C_6H_3CH_2Br$, 94-99-5; 3,4-$Cl_2C_6H_3CH_2Br$, 18880-04-1; p-$FC_6H_4CH_2Br$, 459-46-1; p-$PhC_6H_4CH_2Br$, 2567-29-5; p-$TMSC_6H_4CH_2Br$, 17903-42-3; p-$PrOC_6H_4CH_2Br$, 2606-58-8; p-i-$PrOC_6H_4CH_2Br$, 72729-52-3; 2,2′,4,4′-tetrachlorostilbene, 6271-50-7.

Selective Inhibition of γ-Aminobutyric Acid Aminotransferase by (3*R*,4*R*),(3*S*,4*S*)- and (3*R*,4*S*),(3*S*,4*R*)-4-Amino-5-fluoro-3-phenylpentanoic Acids

Richard B. Silverman* and Shrenik M. Nanavati

Department of Chemistry and Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois 60208-3113. *Received July 31, 1989*

(3*R*,4*R*),(3*S*,4*S*)- and (3*R*,4*S*),(3*S*,4*R*)-4-amino-5-fluoro-3-phenylpentanoic acid (**1a** and **1b**) were synthesized and studied as selective inactivators of γ-aminobutyric acid (GABA) aminotransferase. Neither compound caused time-dependent inactivation of the enzyme. Neither compound underwent enzyme-catalyzed transamination nor was fluoride ion eliminated from either compound by the enzyme. No 3-phenyllevulinic acid, the product of elimination of HF followed by enamine hydrolysis, was detected. However, both **1a** and **1b** were competitive reversible inhibitors of GABA aminotransferase; the K_i for **1a** was smaller than the K_m for GABA. These results suggest that **1a** and **1b** bind to the active site of GABA aminotransferase, but γ-proton removal does not occur. Whereas (*S*)-4-amino-5-fluoropentanoic acid (AFPA) is a potent inhibitor of L-glutamic acid decarboxylase (GAD), neither **1a** nor **1b** at concentrations 40 times the K_i of AFPA caused any detectable competitive inhibition of GAD. Therefore, the incorporation of a phenyl substituent at the 3-position of AFPA confirms selective inhibition of GABA aminotransferase over GAD.

The concentrations of the excitatory and inhibitory neurotransmitters L-glutamate and γ-aminobutyric acid (GABA),[1] respectively, are regulated in the central nervous system (CNS) principally by two PLP-dependent enzymes, L-glutamic acid decarboxylase, the enzyme that catalyzes the conversion of L-glutamate to GABA and GABA aminotransferase, the enzyme that degrades GABA to succinic semialdehyde.[2] Convulsive states have been observed in systems where GABA is prevented from functioning normally[3,4] either by the lowering of its concentration in the CNS below a certain level[5] or by blocking its effect.[6] An increase in GABA levels above the threshold limit usually results in protection against these seizures.[3,4,7] The simplicity of administering GABA directly as an anticonvulsant agent is complicated by the fact that it does not permeate through the blood–brain barrier, i.e., a membrane that surrounds the capillaries of the circulatory system in the brain and protects it from passive diffusion of undesirable compounds from the circulating blood. Although GABA aminotransferase is present in cerebral blood vessel endothelial cells,[8,9] inhibition of its activity does not lead to permeability of GABA into the brain.[10] Therefore, GABA degradation at the blood–brain barrier is not the mechanism for the inability of GABA to cross it; presumably, the lipophobicity is the primary mechanism.

Since GABA administration is ineffective for increasing the GABA concentration in the brain, an alternative approach would be administration of a more lipophilic compound that crosses the blood–brain barrier and then once inside the brain selectively inhibits GABA aminotransferase. This would block the degradation of GABA, and provided that inhibition of L-glutamate decarboxylase, the enzyme that catalyzes the biosynthesis of GABA, does not occur, GABA levels should rise, leading to an anticonvulsant effect. This approach has been shown to be effective; various in vitro inhibitors of GABA aminotransferase increase whole-brain GABA levels in vivo and possess anticonvulsant properties.[7,11-14]

γ-Vinyl-GABA (vigabatrin) is a GABA aminotransferase inactivator that is used clinically for the treatment of ep-

* Author to whom correspondence should be sent at the Department of Chemistry.

(1) Abbreviations GABA, γ-aminobutyric acid; PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; (*S*)-AFPA, (*S*)-4-amino-5-fluoropentanoic acid.

(2) Baxter, C. F.; Roberts, E. *J. Biol. Chem.* **1958**, *233*, 1135.
(3) Tower, D. B. In GABA *in Nervous System Function*; Roberts, E., Chase, T. N., Tower, D. B.; Eds.; Raven Press: New York, 1976; pp 461–478.
(4) Roberts, E., Ed. *Inhibition in the Nerous System and γ-Aminobutyric Acid*; Pergamon Press: Oxford, 1960.
(5) Karlson, A.; Fonnum, F.; Malthe-Sorrensen, D.; Storm-Mathisen, J. *Biochem. Pharmacol.* **1974**, *23*, 3053.
(6) Cutris, D. R.; Duggan, A. W.; Felix, D.; Johnston, G. A. R. *Brain Res.* **1971**, *32*, 69.
(7) Anlezark, G.; Horton, R. W.; Meldrum, B. S.; Sawaya, M. C. *Biochem. Pharmacol.* **1976**, *25*, 413.
(8) Frey, H. H.; Loscher, W. *Neuropharmacology* **1980**, *19*, 217.
(9) Krause, D. N.; Roberts, E.; Wong, E.; Degener, P.; Rogers, K. *Brain Res. Bull.* **1980**, *5*, 173.
(10) Krantis, A. *Neurosci. Lett.* **1986**, *67*, 48.
(11) Metcalf, B. W.; Lippert, B.; Casara, P. In *Enzyme-Activated Irreversible Inhibitors*; Seiler, N., Jung, M. J., Koch-Weser, J., Eds.; Elsevier/North-Holland Press: Amsterdam, 1978; pp 123–133.
(12) Jung, M. J.; In *Enzyme-Activated Irreversible Inhibitors*; Seiler, N., Jung, M. J., Koch-Weser, J., Eds.; Elsevier/North-Holland Press: Amsterdam, 1978; pp 135–148.
(13) Schechter, P. J.; And Tranier, Y. In *Enzyme-Activated Irreversible Inhibitors*, Seiler, N.; Jung, M. J.; Koch-Weser, J., Eds.; Elsevier/North-Holland Press: Amsterdam, 1978; pp 149–162.
(14) Loscher, W. *Biochem. Pharmacol.* **1982**, *31*, 837.

0022-2623/90/1833-0931$02.50/0 © 1990 American Chemical Society