was 52 ± 2 nmol of O_2 min^{-1} mg^{-1}.

2. Enzyme Assays. Enzyme activity was determined by measuring oxygen consumption in solution with a Yellow Springs polarographic electrode (Clark oxygen electrode, YSI-5331) in conjunction with a Gilson oxygraph (Model K-1C) as previously described.[37] The YSI reaction chamber was modified by uniformly rounding the bottom to permit smaller sample volumes. The temperature of the reaction chamber was maintained at 37 ± 1 °C with a Haake FG water circulator. The enzyme reaction was initiated by the addition of sodium arachidonate solution (5 mg/mL; Nu-Check Preps, Elysian, MN) to provide a 100 μM final concentration (K_m = 5.9 μM)[30] in the 2-mL reaction chamber. All inhibitors were added as 10 or 100 mM solutions in Me_2SO for enzymes assays. The levels of Me_2SO used in the enzyme-inhibition experiments had no effect on enzyme activity. Initial enzyme velocities (dO_2/dt) were obtained by measuring the slopes of the resulting oxygen concentration vs. time curves and are reported as a percent of uninhibited control.

(37) Rome, L. H.; Lands, W. E. M. *Prostaglandins* **1975**, *10*, 813.

Acknowledgment. We gratefully acknowledge the partial support of this work through a National Institute of Health Pre-doctoral Training Grant Award to J.L.V. for the period 1984-1985.

Functionalized DL-Amino Acid Derivatives. Potent New Agents for the Treatment of Epilepsy

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Department of Chemistry, University of Houston—University Park, Houston, Texas 77004. *Received September 17, 1986*

Structural analogues of the potent known anticonvulsant agent *N*-acetyl-DL-alanine *N*-benzylamide (**1a**) have been prepared (16 examples). The pharmacological activities of these products were evaluated in the maximal electroshock seizure (MES), the subcutaneous pentylenetetrazole seizure threshold (sc Met), and the rotorod (Tox) tests. The median effective doses (ED50) and the median toxic doses (TD50) for the most active compounds by both intraperitoneal and oral administration are reported. The most active compounds were *N*-acetyl-DL-phenylglycine *N*-benzylamide (**1d**) and *N*-acetyl-DL-alanine *N*-*m*-fluorobenzylamide (**1m**) along with the parent compound **1a**. The ED50 values in the MES test for these three compounds compared well with phenobarbital, while their high TD50 values contributed to their large protective indexes, which approached that of phenytoin. When tested against four convulsant agents, compounds **1a** and **1d** displayed activity profiles significantly different from those reported for conventionally used antiepileptic drugs.

Amino acids and their derivatives have not had a significant impact in the development of new agents for the treatment of epilepsy. The lack of interest in amino acid type compounds stems from the inability of many of these polar compounds to readily penetrate the blood-brain barrier.[2] Despite this concept, several types of amino acids and their derivatives have demonstrated the ability to prevent chemically, audiogenically, and photically induced seizures. These include derivatives of alicyclic and aromatic amino acids,[3] phosphono derivatives of aliphatic amino acids,[4] *N*-benzoyl- and *N*-phenylacetylglycine amides,[5] and structural analogues of the inhibitory neurotransmitter γ-aminobutyric acid (GABA).[6] The endogenous neuropeptides Met- and Leu-enkephalin have also exhibited anticonvulsant activity in a variety of test animals and may play an important role in the prevention of a static convulsive state or in the maintenance of normal brain function.[7]

Inspection of chemotherapeutic agents possessing central nervous system (CNS) depressant and anticonvulsant activity reveals a common structural pattern (Figure 1). Three functionalities are prevalent in many of these compounds: (1) a vicinal diamine linkage, (2) an oxygen atom on the ethylene chain bridging the two amino groups, and (3) an aromatic ring one carbon removed from an amino residue.[8] Representatives of this structural design are

(1) Abstracted from the Ph.D. dissertation of this author. Additional structure proof, discussion, and experimental and spectral data may be found in this reference.

(2) Callery, P. S.; Geelhaar, L. A.; Nayar, M. S. B.; Stogniew, M.; Rao, K. G. *J. Neurochem.* **1982**, *38*, 1063-1067.

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(4) Croucher, M. J.; Collins, J. E.; Meldrum, B. S. *Science (Washington, D.C.)* **1982**, *216*, 899-901. Cates, L. A.; Li, V-S.; Hu, Z-S.; Lehmann, J.; Coyle, J. T.; Ferkany, J. W. *J. Pharm. Sci.* **1984**, *73*, 1550-1553.

(5) Takahashi, T.; Ogui, K.; Fujimura, H.; Satoda, I.; Fukui, T.; Yamamato, Y. Swiss Patent 393 355, Oct 30, 1965. Thorne, D. E. U.S. Patent 3 657 341, April 18, 1972.

(6) Krogsgaard-Larsen, P.; Scheel-Krüger, J.; Kofod, H. *GABA-Neurotransmitters, Pharmacochemical, Biochemical, and Pharmacological Aspects*; Academic: New York, 1979. Enna, S. J. *Biochem. Pharmacol.* **1981**, *30*, 907-913. Scheechter, P. J.; Tranier, Y.; Jung, M. J.; Sjoerdsma, A. *J. Pharmacol. Exp. Ther.* **1977**, *201*, 606-612. Scheechter, P. J.; Tranier, Y.; Jung, M. J.; Böhlen, P. *Eur. J. Pharmacol.* **1977**, *45*, 319-328. Galzigna, L.; Garbin, L.; Bianchi, M.; Marzotto, A. *Arch. Int. Pharmacodyn.* **1978**, *235*, 73-85. Chambon, J. P.; Molimard, J. C.; Calassi, R.; Maruani, J.; Rodier, D.; Sigault, G.; Legris, R.; Roncucci, R.; Bizière, K. *Arzneim.-Forsch.* **1984**, *34*, 1017-1021.

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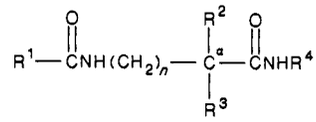
Table I. Phase I Pharmacological Evaluation of Functionalized DL-Amino Acid Derivatives 1[a]

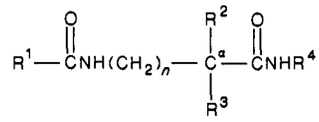


1

no.	n	R^1	R^2	R^3	R^4	ASP[b]	MES,[c] 0.5 h	sc Met,[d] 0.5 h	Tox,[e] 0.5 h
1a	0	CH_3	CH_3	H	Bn[f]	I	3	0	1
1b	0	CH_3	H	H	Bn	II	2	0	2
1c	0	CH_3	$CH(CH_3)_2$	H	Bn	II	2	0	0
1d	0	CH_3	C_6H_5	H	Bn	I	4	0	2
1e	0	CH_3	$CH_2CH_2SCH_3$	H	Bn	II	2	0	0
1f	0	CH_3	Bn	H	Bn	III	0	0	0
1g	0	CH_3	C_6H_5	C_6H_5	Bn	III	0	0	0
1h	1	CH_3	H	H	Bn	III	0	0	0
1i	1	CH_3	CH_3	H	Bn	II	2	0	0

[a] The following code has been adopted: 0 = no activity at dose levels of 600 mg/kg; 1 = noticeable activity at dose levels of 600 mg/kg; 2 = noticeable activity at dose levels of 300 mg/kg; 3 = noticeable activity at dose levels of 100 mg/kg; 4 = noticeable activity at dose levels of 30 mg/kg. [b] ASP Results Classification. [c] MES = maximal electroshock seizure test. [d] sc Met = subcutaneous pentylenetetrazole (Metrazol) seizure test. [e] Tox = neurologic toxicity (the rotorod test). [f] Bn = benzyl.

Table II. Phase I Pharmacological Evaluation of Functionalized DL-Amino Acid Derivatives 1[a]



1

no.	n	R^1	R^2	R^3	R^4	ASP[b]	MES,[c] 0.5 h	sc Met,[d] 0.5 h	Tox,[e] 0.5 h
1a	0	CH_3	CH_3	H	Bn[f]	I	3	0	1
1j	0	CH_3	CH_3	H	CH_3	III	0	0	0
1k	0	CH_3	CH_3	H	$CH(C_6H_5)_2$	III	0	0	0
1l	0	CH_3	CH_3	H	$CH_2C_6H_4$-*m*-OCH_3	II	2	0	0
1m	0	CH_3	CH_3	H	$CH_2C_6H_4$-*m*-F	I	3	0	0
1n	0	CH_3	CH_3	H	$CH_2CONHBn$	III	0	0	0

[a] The following code has been adopted: 0 = no activity at dose levels of 600 mg/kg; 1 = noticeable activity at dose levels of 600 mg/kg; 2 = noticeable activity at dose levels of 300 mg/kg; 3 = noticeable activity at dose levels of 100 mg/kg; 4 = noticeable activity at dose levels of 30 mg/kg. [b] ASP Results Classification. [c] MES = maximal electroshock seizure test. [d] sc Met = subcutaneous pentylenetetrazole (Metrazol) seizure test. [e] Tox = neurologic toxicity (the rotorod test). [f] Bn = benzyl.

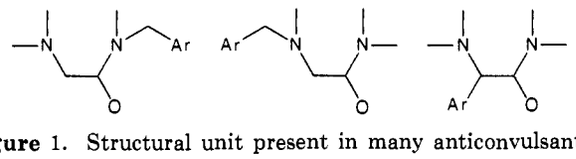
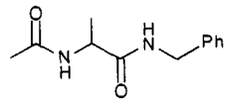


Figure 1. Structural unit present in many anticonvulsants.

substituted hydantoins, piperazines, and benzodiazepines.

Recognition of this empirical blueprint in anticonvulsant drugs led to the hypothesis that functionalized amino acids should provide a rich source for future antiepileptic agents. This rationale was supported by a recent report from this laboratory that *N*-acetyl-DL-alanine *N*-benzylamide (**1a**) displayed potent anticonvulsant activity.[9]



1a

In this paper, the syntheses, physical properties, and anticonvulsant activities of a select series of functionalized amino acid derivatives are described. Evidence is presented that these simple compounds comprise a new and novel class of antiepileptic agents.

Selection of Compounds

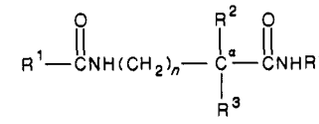
N-Acetyl-DL-alanine *N*-benzylamide (**1a**) served as the parent compound in this study (Tables I–III). Systematic structural variations have been conducted at three sites: the α-carbon (Table I), the amide substituent (Table II), and the *N*-acyl group (Table III). In the selection of derivatives, we have attempted to adhere to the molecular blueprint cited previously and to test the validity of the empirical relationship between this molecular pattern and anticonvulsant activity. In all cases, where appropriate, the DL racemates were synthesized.

In compounds **1b–f**, the α-carbon substituent has been systematically changed from methyl to hydrogen to isopropyl to phenyl to a thio alkyl group to benzyl, while in compound **1g** both α-carbon sites have been substituted with phenyl groups. Interestingly, **1g** represents an open-chained analogue of the potent antiepileptic phenytoin. Remaining compounds in this first category included the β-amino acid derivatives (**1h** and **1i**) in which the α-carbon moiety has been homologated by one carbon atom.

The second category of substituents were structural variants of **1a** in which the amide group has been altered. Included in this list were the *N*-methylamide (**1j**), the *N*-benzhydrylamide (**1k**), and the two derivatives of **1a** in

(9) Cortes, S.; Liao, Z-K.; Watson, D.; Kohn, H. *J. Med. Chem.* 1985, *28*, 601–606.

Table III. Phase I Pharmacological Evaluation of Functionalized DL-Amino Acid Derivatives 1[a]

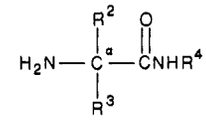


1

no.	n	R^1	R^2	R^3	R^4	ASP[b]	MES,[c] 0.5 h	sc Met,[d] 0.5 h	Tox,[e] 0.5 h
1a	0	CH_3	CH_3	H	Bn[f]	I	3	0	1
1o	0	$(CH_3)_2CH$	CH_3	H	Bn	III	0	0	0
1p	0	$(CH_3)_3C$	CH_3	H	Bn	II	2	2	0
1q	0	$CH_3CONHCH_2$	CH_3	H	Bn	III	0	0	0

[a] The following code has been adopted: 0 = no activity at dose levels of 600 mg/kg; 1 = noticeable activity at dose levels of 600 mg/kg; 2 = noticeable activity at dose levels of 300 mg/kg; 3 = noticeable activity at dose levels of 100 mg/kg; 4 = noticeable activity at dose levels of 30 mg/kg. [b] ASP Results Classification. [c] MES = maximal electroshock seizure test. [d] sc Met = subcutaneous pentylenetetrazole (Metrazol) seizure test. [e] Tox = neurologic toxicity (the rotorod test). [f] Bn = benzyl.

Table IV. Selected Physical and Spectral Data of DL-Amino Acid N-Substituted Amides 4

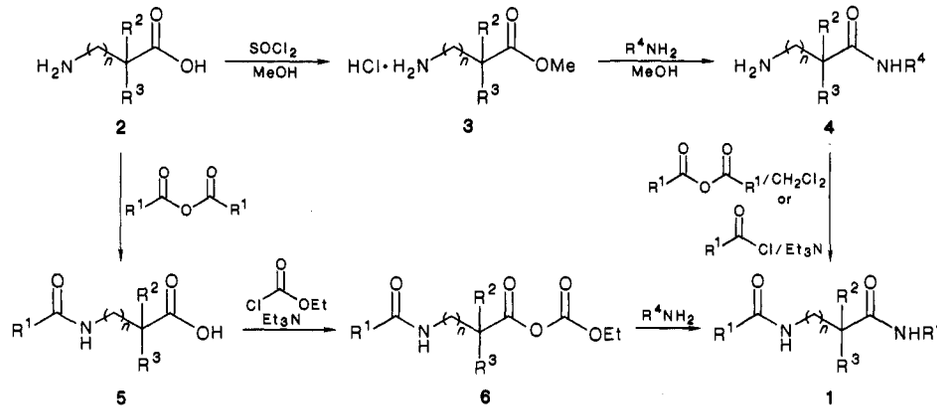


4

no.	R^2 (R^3)	R^3 (R^2)	R^4	yield[a]	mp[b]	IR[c]	^{1}H NMR,[d,e] α-CH	^{13}C NMR[d] α-C	^{13}C NMR[d] C=O
4a	CH_3	H	Bn[f]	40	oil	1655, 1525[g]	3.50 (q, 6.0, 1 H)	50.4	174.3
4b	H	H	Bn	36	44–48[h]	1630, 1545 (br)	3.15 (s, 2 H)	44.7	173.1
4c	$(CH_3)_2CH$	H	Bn	55	oil[h]	1650 (br), 1515	3.17 (d, 4.0, 1 H)	60.2	174.6
4d	C_6H_5	H	Bn	82	oil	1670 (br), 1520 (br)	4.13 (s, 1 H)	59.3	173.3
4e	Bn	H	Bn	60	64–65	1655, 1535	3.49 (dd, 8.5, 4.4, 1 H)	56.4	174.1
4f	CH_3	H	CH_3	33	oil[h]	1650 (br), 1550 (br)	3.47 (q, 6.9, 1 H)	50.7	176.9

[a] Purified yields (%) from the methyl ester hydrochloride **3**. All compounds gave satisfactory analyses for C, H, N (±0.4%) unless otherwise indicated. [b] Melting points (°C) are uncorrected. [c] Infrared peak positions are recorded in centimeters (cm^{-1}) vs. the 1601-cm^{-1} band in polystyrene. Solids were taken in KBr disks and oils were taken neat (NaCl). [d] NMR spectra were taken in $CDCl_3$ (in δ). [e] The information in parentheses is the multiplicity of the signal, followed by the coupling constant in hertz (Hz), followed by the number of protons attributed to the signal. [f] Bn = benzyl. [g] Reference 9. [h] Elemental composition was verified by high-resolution mass spectroscopy.

Scheme I



which an electron-donating (1l) or an electron-withdrawing (**1m**) group has been placed in the meta position of the aromatic ring. The remaining member of this class of compounds was the dipeptide **1n** in which the amide substituent has been extended by a glycyl moiety.

In the next group of drug candidates, the *N*-acyl substituent in **1a** has been modified. Compounds selected for synthesis included the dimethyl- and the trimethylacetyl derivatives (**1o** and **1p**) of **1a**. As the amide substituent of **1a** was extended with a glycyl moiety in the dipeptide **1n**, the *N*-acyl group was lengthened with a glycyl group in the dipeptide **1q**. Of note, dipeptides **1n** and **1q** are isomeric.

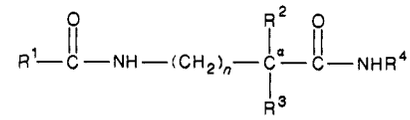
Chemistry

The strategies employed in the synthesis of the racemic functionalized amino acid derivatives were patterned after procedures common to peptide synthesis.[10] Two general methods were utilized for the preparation of these compounds as depicted in Scheme I. No major effort was made to optimize the yields.

In the first procedure (method A), the starting DL-amino acid **2** was initially converted to the corresponding methyl

(10) Bodanszky, M.; Klausner, Y. S.; Ondetti, M. A. *Peptide Synthesis*, 2nd ed.; Wiley: New York, 1976.

Table V. Selected Physical and Spectral Data of *N*-Acyl-DL-amino Acid N-Substituted Amides and Their Analogues 1



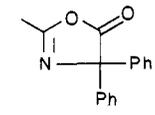
1

no.	*n*	R^1	R^2 (R^3)	R^3 (R^2)	R^4	method[a]	yield[b]	mp[c]	IR[d]	^{1}H NMR,[e,f] α-CH	^{13}C NMR[e] R^1-CO	α-C	C$_\alpha$-CO
1a	0	CH_3	CH_3	H	Bn[g]	A	60	138–139	1660, 1515[h]	4.26–4.34 (m, 1 H)	168.9	48.2	172.3
1b	0	CH_3	H	H	Bn	A	81	140–142	1640, 1545	3.74 (d, 5.3, 2 H)	168.9	42.4	172.3
1c	0	CH_3	$CH(CH_3)_2$	H	Bn	A	86	192–193	1625, 1535	4.11 (d, 8.8, 1 H)	169.2	57.8	171.1
1d	0	CH_3	C_6H_5	H	Bn	A	66	202–203	1635, 1540	5.50 (d, 7.9, 1 H)	168.9	56.3	169.9
1e	0	CH_3	CH_2CH_2-SCH_3	H	Bn	B	43	134–135	1630, 1545	4.10–4.53 (m, 1 H)	169.5	52.0	171.4
1f	0	CH_3	Bn	H	Bn	A	84	161–162	1630, 1545	4.36–4.72 (m, 1 H)	169.0	54.1	171.2
1g	0	CH_3	C_6H_5	C_6H_5	Bn	B	72[i]	189–190	1645, 1530		169.2	68.8	170.6
1h	1	CH_3	H	H	Bn	B	27	166–167	1640, 1545	2.40 (t, 6.5, 2 H)	169.3	35.4	170.4
1i	1	CH_3	CH_3	H	Bn	B	79	130–131	1640, 1560	2.58 (q, 6.9, 1 H)	169.5	42.0	174.3
1j	0	CH_3	CH_3	H	CH_3	A	90	158–159	1635, 1565	3.95–4.48 (m, 1 H)	169.1	48.2	172.8
1k	0	CH_3	CH_3	H	$CH(C_6H_5)_2$	B	67	193–194	1635, 1540	4.30–4.60 (m, 1 H)	169.0	48.1	171.8
1l	0	CH_3	CH_3	H	$CH_2C_6H_4$-*m*-OCH_3	B	69	111–113	1630, 1540	4.24-4.37 (m, 1 H)	169.1	48.3	172.5
1m	0	CH_3	CH_3	H	$CH_2C_6H_4$-*m*-F	B	54	120–121	1645, 1545	4.23–4.41 (m, 1 H)	169.6	48.5	172.8
1n	0	CH_3	CH_3	H	CH_2CON-HBn	B	47	185–186	1685, 1640, 1545	3.93–4.38 (m, 1 H)	168.7	48.7	172.8
1o	0	$(CH_3)_2CH$	CH_3	H	Bn	A	40	164–165	1635, 1545	4.03–4.48 (m, 1 H)	175.9	48.0	172.4
1p	0	$(CH_3)_3C$	CH_3	H	Bn	A	40	123–124	1630, 1535	4.23–4.42 (m, 1 H)	177.1	48.4	172.5
1q	0	$CH_3CONHCH_2$	CH_3	H	Bn	B	69[j]	184–186	1685, 1640, 1545	4.00–4.18 (m, 1 H)	169.5	48.2	172.1

[a] Compounds by method A were prepared from the DL-amino acid N-substituted amides, while those by method B from the *N*-acyl-DL-amino acids. All compounds gave satisfactory analyses for C, H, N (±0.4%) unless otherwise indicated. [b] The purified yields (%) are from the DL-amino acid N-substituted amides **4** for compounds synthesized by method A and from the *N*-acetyl-DL-amino acids **5** for compounds prepared by method B unless otherwise indicated. [c] Melting points (°C) are uncorrected. [d] Infrared peak positions are recorded in reciprocal centimeters (cm^{-1}) vs. the 1601-cm^{-1} band in polystyrene and were taken in KBr disks. [e] All NMR spectra were taken in Me_2SO-d_6 (in δ). [f] The information in parentheses is the multiplicity of the signal, followed by the coupling constant in hertz (Hz), followed by the number of protons attributed to the signal. [g] Bn = benzyl. [h] Reference 9. [i] The yield is from *N*-acetyldiphenylglycine. [j] Elemental composition was verified by high-resolution mass spectroscopy.

ester hydrochloride **3** by the addition of $SOCl_2$ to a suspension of the DL-amino acid in cold methanol.[11] Yields were quantitative, and no further purification was required. Conversion of the DL-amino acid methyl ester hydrochloride **3** to the corresponding N-substituted amide **4** was accomplished with an excess of the appropriate amine. At least 2 equiv of the amine was used in order to permit the isolation of **4** as the free base. Yields ranged from 33% to 82% (Table IV). Reaction of the DL-amino acid N-substituted amide **4** with an acid anhydride or an acid halide produced the final product **1** in yields from 28% to 90% (Table V). This procedure was employed in the synthesis of **1a-d**, **1f**, **1j**, **1o**, **1p**, **1r**, and **1s**.

Low yields were encountered in the conversion of several DL-amino acid methyl ester hydrochlorides **3** to the corresponding DL-amino acid N-substituted amides **4**, necessitating the use of method B. In this route, the DL-amino acid or dipeptide **2** was initially acylated with the acid anhydride (1.1–3.0 equiv in refluxing acetic acid, dichloromethane, or water) to give the N-protected DL-amino acid **5** in moderate to high yields (56–99%).[12] Protection of the amino terminus permitted the subsequent reaction with triethylamine and ethyl chloroformate to proceed at the terminal carboxyl group to generate the mixed *N*-acyl-DL-amino acid–carbonic ester anhydride intermediate **6**. The activated mixed anhydride was not isolated but directly treated in situ with 1.1 equiv of the appropriate amine at −5 °C to produce the *N*-acyl-DL-amino acid N-substituted amides **1** in 43–79% yields. Compounds **1e**, **1h**, **1i**, **1k–n**, and **1q** were prepared by this route. *N*-Acetyldiphenylglycine *N*-benzylamide (**1g**) was synthesized by a slightly different method. Diphenylglycine or *N*-acetyldiphenylglycine was heated with acetic anhydride at reflux (5–30 min) to give the oxazolone intermediate **7** (80%).[13] Treatment of the oxazolone with benzylamine yielded product **1g** in 90% yield.



7

Pharmacological Evaluation

All *N*-acyl-DL-amino acid N-substituted amides **1** prepared in this study were submitted to the National Institutes of Health Antiepileptic Drug Development Program for pharmacological evaluation. Each compound was tested for anticonvulsant activity by using the procedures described by Krall et al.[14]

The phase I test results are summarized in Tables I–III. All compounds were administered intraperitoneally at three doses (30, 100, and 300 mg/kg). The only exception was the parent compound **1a**, which was evaluated at 600 mg/kg as well. The smallest dose that produced activity was noted for separate tests involving maximal-induced

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(14) Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. *Epilepsia* **1978**, *19*, 409–428.

Table VI. Phase II Pharmacological Evaluation of Functionalized DL-Amino Acid Derivatives 1[a]

compound	MES,[b] ED50	sc Met,[c] ED50	Tox,[d] TD50	PI[e]
N-acetyl-DL-alanine *N*-benzylamide (**1a**)	76.54 (66.58–89.04)	*f*	453.86 (416.56–501.01)	5.93
N-acetyl-DL-phenylglycine *N*-benzylamide (**1d**)	20.31 (16.85–24.45)	*f*	96.92 (79.80–118.39)	4.77
N-acetyl-DL-alanine *N*-*m*-fluorobenzylamide (**1m**)	77.38 (62.55–91.01)	142.73 (61.53–237.97)	*g*	>6.46
phenytoin[h]	9.50	*i*	65.46	6.89
phenobarbital[h]	21.78	13.17	69.01	3.17
ethosuximide[h]	*j*	130.35	440.83	<0.44
valproate[h]	271.66	148.59	425.84	1.57

[a] ED50 and TD50 are in mg/kg. Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] sc Met = subcutaneous pentylenetetrazole (Metrazol) seizure test. [d] Tox = neurologic toxicity (the rotorod test). [e] PI = protective index (TD50/MES ED50). [f] The ED50 value was not computed for this substrate. [g] No toxicity observed up to 500 mg/kg. [h] Reference 15. [i] Not effective. [j] No activity observed up to 1000 mg/kg.

Table VII. Phase IV Pharmacological Evaluation of Functionalized DL-Amino Acid Derivatives 1[a]

compound	MES,[b] ED50	sc Met,[c] ED50	Tox,[d] TD50	PI[e]
N-acetyl-DL-alanine *N*-benzylamide (**1a**)	122.68 (106.82–138.40)	266.29 (242.19–289.75)	*f*	>8.15
N-acetyl-DL-phenylglycine *N*-benzylamide (**1d**)	46.71 (30.76–76.40)	*g*	241.38 (194.39–284.08)	5.17
phenytoin[h]	9.04 (7.39–10.62)	*i*	86.71 (80.39–96.09)	9.59
phenobarbital[h]	20.09 (14.78–31.58)	12.59 (7.99–19.07)	96.78 (79.88–115.00)	4.82
ethosuximide[h]	*j*	192.21 (158.59–218.44)	879.21 (839.89–933.51)	<0.44
valproate[h]	664.80 (605.33–718.00)	388.31 (348.87–438.61)	1264.39 (800–2250)	1.90

[a] ED50 and TD50 are in mg/kg. Numbers in parentheses are 95% confidence intervals. [b] MES = maximal electroshock seizure test. [c] sc Met = subcutaneous pentylenetetrazole (Metrazol) seizure test. [d] Tox = neurologic toxicity (the rotorod test). [e] PI = protective index (TD50/MES ED50). [f] No toxicity was observed for doses up to 1000 mg/kg. [g] The ED50 value was not computed for this substrate. [h] Reference 15. [i] No protection up to 300 mg/kg. [j] No protection up to 2000 mg/kg.

Table VIII. Phase V Pharmacological Evaluation of Functionalized DL-Amino Acid Dervatives 1[a]

compound	sc Met,[b] ED50	sc Bic,[c] ED50	sc Pic,[d] ED50	sc Strych,[e] ED50
N-acetyl-DL-alanine *N*-benzylamide (**1a**)	*f*	204.66 (157.50–286.49)	133.61 (115.92–153.22)	*g*
N-acetyl-DL-phenylglycine *N*-benzylamide (**1d**)	*g*	*g*	*g*	*g*
phenytoin[h]	*g*	*g*	*g*	*i*
phenobarbital[h]	13.17 (5.87–15.93)	37.72 (26.49–47.39)	27.51 (20.88–34.82)	95.30 (91.31–99.52)
ethosuximide[h]	130.35 (110.99–150.45)	459.01 (349.92–633.13)	242.69 (227.84–255.22)	*j*
valproate[h]	148.59 (122.64–177.02)	359.95 (294.07–438.54)	387.21 (341.37–444.38)	262.96 (261.12–323.43)

[a] ED50 and TD50 are in mg/kg. Numbers in parentheses are 95% confidence intervals. [b] sc Met = subcutaneous Metrazol test (CD97 = 85 mg/kg). [c] sc Bic = subcutaneous bicuculline test (CD97 = 2.70 mg/kg). [d] sc Pic = subcutaneous picrotoxin test (CD97 = 3.15 mg/kg). [e] sc Strych = subcutaneous strychnine test (CD97 = 1.20 mg/kg). [f] Maximum protection: 50% at 800 mg/kg. [g] The ED50 value was not computed for this substrate. [h] Reference 15. [i] Maximum protection: 50% at 55–100 mg/kg. [j] Maximum protection: 62.5% at 250–1000 mg/kg.

convulsions (MES), subcutaneous Metrazol-induced convulsions (sc Met), and a rotorod toxicity test (Tox). The overall effect of the three tests were then given by one of four different ratings (ASP Results Classification I–IV). Compounds with a rating of I were designated as promising and were considered for phase II (quantification) testing (Table VI). This stage involved the same tests previously described, except under stricter monitoring of dosages and activity time spans and included an evaluation of the median effective dose (ED50) and the median toxic dose (TD50). If the anticonvulsant activity of the test compound was satisfactory, the amino acid derivative was then subjected to phase IV and V trials. Phase IV entailed the same tests described for phase I and II, except the test compound was administered to mice orally (Table VII). The in vivo antiepileptic potential was further delineated in phase V (antiepileptic drug differentiation in mice), and the results are summarized in Table VIII. Phase V examined the ability of the drug candidate to protect mice against seizures induced by a CD97 subcutaneous injection of Metrazol, bicuculline, picrotoxin, and strychnine. These convulsants have CD97 values of 85, 2.70, 3.15, and 1.20 mg/kg, respectively.[15]

(15) 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*, 293–305.

Table I lists the pharmacological phase I results of the parent compound **1a** and those analogues where only the α-carbon moiety has been modified. Evaluation of this set of results revealed several significant observations. First, the principal biological activity of these compounds resided in their ability to prevent seizures in the MES test. Second, reduced CNS activity was noted as the size of the substituent on the α-carbon atom in **1a** was decreased from a methyl group to a hydrogen (**1b**) or increased to either an isopropyl group (**1c**) or a thio alkyl group (**1e**). Each of these analogues possessed anticonvulsant activity but were not as effective as the parent compound. Third, pronounced activity was observed for *N*-acetyl-DL-phenylglycine *N*-benzylamide (**1d**). Of note, *N*-acetyl-DL-phenylglycine *N*-benzylamide (**1d**) contains two aromatic rings both of which are one carbon atom removed from an amino residue. Fourth, loss of activity was observed when the phenyl group of **1d** was extended by a CH_2 to a benzyl moiety (**1f**) and when both substituents on the α-carbon atom of the parent compound were replaced with phenyl groups (**1g**). Fifth, homologation of **1a** and **1b** giving the corresponding β-alanine derivatives **1i** and **1h**, respectively, led to reduced CNS activity. Both compounds possess a 1,3-diamine linkage.

The second category of compounds tested for anticonvulsant activity involved analogues of **1a** where the benzyl moiety of the amide group was altered. The phase I results

for these compounds are shown in Table II. Assessment of these results revealed a significant pattern in the ability of these amino acid derivatives to prevent seizures. The anticonvulsant activity in the MES test was abolished when the size of the benzyl substituent in **1a** was decreased to a methyl (**1j**) or increased to a benzhydryl (**1k**) or a glycine *N*-benzylamide (**1n**) group. A similar trend can be ascertained by comparing the MES activities of *N*-acetyl-DL-phenylglycine *N*-benzylamide (**1d**) with *N*-acetyl-DL-phenylglycine *N*-methylamide.[9] Substitution on the benzyl group of the amide in **1a** with either an electron-donating (i.e., *m*-methoxy) (**1l**) or an electron-withdrawing (i.e., *m*-fluoro) (**1m**) moiety led to compounds yielding different activities. Of the two, the latter analogue (**1m**) displayed higher activity in the MES test. Moreover, the toxicity of *N*-acetyl-DL-alanine *N*-*m*-fluorobenzylamide (**1m**) was lower than the parent compound, **1a**. Finally, all drug candidates listed in Table II were devoid of activity in the sc Met test at the administered doses.

Table III lists the phase I results for **1a** and its analogues where the *N*-acetyl moiety has been changed. Evaluation of this set of results revealed that any increase in the size of the methyl group of the *N*-acetyl moiety in **1a** led to compounds that possessed decreased activity in the MES test. Furthermore, we note that compound **1p**, unlike the other derivatives examined, displayed significant activity in the sc Met test.

To further probe the structural parameters needed for anticonvulsant activity, two of our most active compounds, **1a** and **1d**, were compared to the known isomeric compounds, *N*-phenylacetyl-DL-alanine *N*-methylamide (**1r**) and *N*-phenylacetyl-DL-phenylglycine *N*-methylamide (**1s**).[5] In this series, the methyl group of the *N*-acetyl moiety and the benzyl group of the *N*-benzylamide moiety have been interchanged. Transposition of these two terminal groups resulted in compounds that were devoid of anticonvulsant activity under the ADD screening protocol. The activity profiles exhibited by **1a** and **1d** vs. **1r** and **1s** are in agreement with our empirical molecular blueprint.

The pharmacological activities for compounds **1d** and **1m** warranted their further evaluation in phase II trials. The data obtained from these studies are summarized in Table VI along with similar information for **1a** and several proven antiepileptic drugs.[15] The ED50 values in the MES test for *N*-acetyl-DL-phenylglycine *N*-benzylamide (**1d**) and *N*-acetyl-DL-alanine *N*-*m*-fluorobenzylamide (**1m**) compared well with phenobarbital. The high TD50 values of these amino acid analogues contributed significantly to their large protective indexes, which approach that of phenytoin.

Phase IV tests provided information concerning the test candidate when administered orally (po). The results observed for compounds **1a** and **1d** are summarized in Table VII with similar data for currently used antiepileptic agents.[15] The ED50 and the TD50 values disclosed both *N*-acetyl-DL-alanine *N*-benzylamide (**1a**) and *N*-acetyl-DL-phenylglycine *N*-benzylamide (**1d**) were less potent in the MES test and less toxic after oral administration than after intraperitoneal injection (Table VI). Significantly, compound **1a** was effective in the sc Met test after oral administration but not after intraperitoneal injection (Table VI). A variety of phenomena (i.e., adsorption, metabolism) may be responsible for the differences observed in activity depending upon the mode of administration.

Compounds **1a** and **1d** were tested for the ability to protect mice against a variety of chemically induced seizures. The findings from these tests (phase V evaluations) are summarized in Table VIII along with similar results for phenytoin, phenobarbital, ethosuximide, and valproate. The mode of action of these convulsants (Metrazol, bicuculline, picrotoxin, and strychnine) is different.[15] *N*-Acetyl-DL-alanine *N*-benzylamide (**1a**) was moderately effective in preventing seizures induced in mice by bicuculline and picrotoxin, was partially effective (maximum protection, 50% at 800 mg/kg) in the sc Met test, and was ineffective against strychnine-induced convulsions. On the other hand, *N*-acetyl-DL-phenylglycine *N*-benzylamide (**1d**) did not provide mice with any protection against seizures induced by the four convulsants. The anticonvulsant activity profiles of both **1a** and **1d** differ significantly from those of phenytoin, phenobarbital, ethosuximide, and valproate.

Conclusions

The pharmacological profiles exhibited by the functionalized DL-amino acid derivatives establishes a new class of anticonvulsant agents. The specific activities of these compounds in the MES, sc Met, and toxicity tests can be independently modulated by alteration of the substitution pattern at the α-carbon atom, the *N*-acyl, and the *N*-amido moieties. The observed structure–activity profile suggests that stringent steric and electronic requirements exist for maximal anticonvulsant activity. Additional studies in progress are aimed at investigating the generality of this class of compounds as well as their mode of action.

Experimental Section

General Methods. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Satisfactory IR (Perkin-Elmer 1330 spectrophotometer, solids in KBr and oils neat on NaCl plates) and NMR (Varian Associates Model FT-80A or Nicolet NT-300, Me_4Si as internal standard, $CDCl_3$ or Me_2SO-d_6 as noted) spectral data were obtained for all new compounds and are listed in Tables IV and V. Mass spectral data [Bell-Howell 21-491 mass spectrometer (low resolution) and a CEC21-110B double-focusing magnetic-sector spectrometer (high resolution)] were performed by Dr. John Chinn at the University of Texas—Austin. Elemental analyses were obtained at Spang Microanalytical Laboratories, Eagle Arbor, MI. Preparative column chromatography was run with Merck silica gel, grade 60, 230–400 mesh, 60 Å, from Aldrich Chemical Co., Milwaukee, WI. General procedures followed by results of typical experiments are described below.

Method A. Preparation of DL-Amino Acid *N*-Benzylamides (4). General Procedure. The DL-amino acid methyl ester hydrochloride[11] **3** (25 mmol) was dissolved in a minimal amount of methanol (15–25 mL) and then benzylamine (10.72 g, 10.93 mL, 100 mmol) was added dropwise. The solution was heated to reflux (24 h) and then cooled to room temperature. The resulting mixture was concentrated in vacuo, leaving a mixture of an oil and a precipitate. The residual mixture was washed with chloroform (3 × 25 mL). The white precipitate was collected, dried in vacuo, and identified as benzylamine hydrochloride. The chloroform washings were combined and concentrated in vacuo. The oily residue was distilled under high vacuum (40 °C, 10 μm) to produce a clear oil identified as benzylamine (^{1}H NMR). Vacuum distillation (100 °C, 10 μm) and then further purification by flash column chromatography (90:10 chloroform/methanol) of the remaining residue gave the desired product. For **4d**: yield 4.93 g (82%); ^{1}H NMR (80 MHz, $CDCl_3$) δ 2.13 (s, 2 H), 4.13 (s, 1 H), 4.28 (d, J = 5.4 Hz, 2 H), 7.15 (s, 5 H), 7.23 (s, 5 H), 7.95 (t, J = 5.4 Hz, 1 H); ^{13}C NMR (80 MHz, $CDCl_3$) 42.8, 59.3, 126.7 (2 C), 127.0, 127.2 (2 C), 127.5 (2 C), 128.3, 128.4 (2 C), 138.5, 141.3, 173.3 ppm; IR (neat, NaCl) 3300, 1670 (br), 1520 (br), 1450, 730, 700 cm^{-1}; mass spectrum, m/e (relative intensity) 241 (16), 134 (8), 106 (100), 91 (62), 77 (56), 65 (43). Anal. ($C_{15}H_{16}N_2O$) C, H, N. Table IV lists other new compounds.

Preparation of DL-Alanine *N*-Methylamide (4f). DL-Alanine methyl ester hydrochloride (4.46 g, 25 mmol) was placed into a 50-mL round-bottomed flask and a 40% aqueous solution of

methylamine (31.1 mL, 400 mmol) was added dropwise. The solution was heated to reflux (50 °C, 6 h) and then cooled to room temperature. The aqueous solution was extracted with chloroform (3 × 25 mL). The chloroform washings were combined, dried (Na_2SO_4), and concentrated to dryness. The oily residue was purified by flash column chromatography (90:10 chloroform/methanol) (TLC R_f 0.80, 90:10 chloroform/methanol): yield 0.84 g (33%); ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.30 (d, J = 6.9 Hz, 3 H), 2.07 (s, 2 H), 2.79 (d, J = 4.9 Hz, 3 H), 3.47 (q, J = 6.9 Hz, 1 H), 7.61 (br s, 1 H); ^{13}C NMR (80 MHz, $CDCl_3$) 21.7, 25.8, 50.7, 176.9 ppm; IR (neat, NaCl) 3320, 1650 (br), 1550 (br), 1450 cm^{-1}; mass spectrum, m/e (relative intensity) 103 (12), 102 (12), 87 (9), 69 (18), 58 (100); M_r 102.079 09 (calcd for $C_4H_{10}N_2O$ 102.07931).

Preparation of *N*-Acyl-DL-amino Acid *N*-Benzyl- and *N*-Methylamides (1). General Procedure. The DL-amino acid N-substituted amide **4** (11 mmol) was dissolved in dichloromethane (15 mL) and then the appropriate acid anhydride (12 mmol) was added dropwise. The solution was stirred at room temperature (4–6 h) and then concentrated to dryness. The residue was recrystallized from chloroform/hexane. For **1d**: yield 2.05 g (66%); mp 202–203 °C; ^{1}H NMR (80 MHz, Me_2SO-d_6) δ 1.91 (s, 3 H), 4.27 (d, J = 5.6 Hz, 2 H), 5.50 (d, J = 7.9 Hz, 1 H), 7.36 (s, 10 H), 8.38–8.86 (m, 2 H); ^{13}C NMR (80 MHz, Me_2SO-d_6) 22.3, 42.0, 56.3, 126.6 (2 C), 127.0, 127.1 (2 C), 127.4 (2 C), 128.1 (3 C), 138.9, 139.0, 168.9, 169.9 ppm; IR (KBr) 3290, 1635, 1540, 1450, 745, 690 cm^{-1}; mass spectrum, m/e (relative intensity) 283 (20), 264 (21), 149 (100), 131 (20), 118 (34), 106 (92), 91 (70), 77 (54), 65 (45). Anal. ($C_{17}H_{18}N_2O_2$) C, H, N. Table V lists other new compounds.

Method B. Preparation of *N*-Acetyldipeptides (5). General Procedure. The dipeptide **2** (1.46 g, 10 mmol) was combined with dichloromethane (25 mL) and acetic anhydride (1.12 g, 1.04 mL, 11 mmol) was added dropwise. The mixture was heated to reflux (18 h), cooled to room temperature, concentrated to dryness, and then recrystallized from absolute ethanol.

***N*-Acetyl-DL-alanylglycine (5a)**: yield 1.50 g (80%); mp 184–185 °C; ^{1}H NMR (80 MHz, Me_2SO-d_6) δ 1.21 (d, J = 7.5 Hz, 3 H), 1.85 (s, 3 H), 3.75 (d, J = 5.7 Hz, 2 H), 4.06–4.53 (m, 1 H), 7.88–8.25 (m, 2 H); ^{13}C NMR (80 MHz, Me_2SO-d_6) 18.3, 22.4, 40.6, 47.9, 169.1, 171.1, 172.8 ppm; IR (KBr) 3250, 1670, 1635, 1590, 1550, 1445 cm^{-1}; mass spectrum, m/e (relative intensity) 189 (7), 170 (2), 126 (2), 114 (11), 100 (11), 86 (100), 72 (16). Anal. ($C_7H_{12}N_2O_4$) C, H, N.

***N*-Acetylglycyl-DL-alanine (5b)**: yield 1.18 g (63%); mp 178–180 °C; ^{1}H NMR (80 MHz, Me_2SO-d_6) δ 1.27 (d, J = 7.3 Hz, 3 H), 1.85 (s, 3 H), 3.71 (d, J = 5.7 Hz, 2 H), 3.91–4.38 (m, 1 H), 7.64–8.28 (m, 2 H); ^{13}C NMR (80 MHz, Me_2SO-d_6) 17.2, 22.4, 41.6, 47.4, 168.6, 169.5, 173.9 ppm; IR (KBr) 3260, 1660, 1635, 1615, 1560, 1450 cm^{-1}; mass spectrum, m/e (relative intensity) 189 (11), 143 (38), 128 (61), 100 (62), 90 (27), 88 (51), 85 (84), 73 (100), 70 (48), 56 (72); M_r 188.08034 (calcd for $C_7H_{12}N_2O_4$ 188.079 70).

Preparation of *N*-Acetyl-DL-amino Acid N-Substituted Amides and *N*-Acetyl-DL-dipeptide N-Substituted Amides (1). The starting *N*-acetyl-DL-amino acid[12] or dipeptide **5** (1 equiv) was combined with acetonitrile (0.25 M) and the mixture was placed into an ice/salt water bath (−5 °C). Triethylamine (1 equiv) was added dropwise, followed by 1 equiv of ethyl chloroformate. All additions were done slowly so that the temperature of the mixture did not rise above 0 °C. The mixture was then stirred at −5 °C (20 min). The appropriate amine (1.1 equiv) in acetonitrile (5 mL) was added dropwise and the mixture was stirred at −5 °C (1 h) and then at room temperature (18 h). The mixture was filtered and a white precipitate was collected and dried in vacuo and identified as the desired product (^{1}H and ^{13}C NMR analyses). The filtrate was concentrated in vacuo and the residue was combined with hot tetrahydrofuran (50 mL) and cooled in the freezer (3 h), resulting in the formation of a white precipitate. The mixture was filtered and the precipitate was collected, dried in vacuo, and identified as triethylammonium hydrochloride. The filtrate containing tetrahydrofuran was concentrated in vacuo and the resulting residue was purified by flash column chromatography. A white solid was isolated and identified as the desired product (^{1}H and ^{13}C NMR analyses). The two solids identified as the product were combined and recrystallized. This workup was performed on **1e**, **1k**, **1n**, and **1q**. A second workup procedure for **1h**, **1i**, **1l**, and **1m** was as follows. The reaction mixture was concentrated in vacuo and the residue was combined with hot tetrahydrofuran (50 mL) and was cooled in the freezer (3 h), resulting in the formation of a white precipitate. The mixture was filtered and the precipitate was collected, dried in vacuo, and identified as triethylammonium hydrochloride. The filtrate was concentrated in vacuo and the resulting solid was recrystallized. For **1i**: yield 79%; mp 130–131 °C; ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 1.02 (d, J = 6.9 Hz, 3 H), 1.80 (s, 3 H), 2.58 (q, J = 6.9 Hz, 1 H), 3.12–3.16 (m, 2 H), 4.25 (dd, J = 15.0 Hz, 5.7 Hz, 1 H), 4.35 (dd, J = 15.0 Hz, 6.1 Hz, 1 H), 7.20–7.30 (m, 5 H), 7.97 (t, J = 5.6 Hz, 1 H), 8.44 (t, J = 5.8 Hz, 1 H); ^{13}C NMR (300 MHz, Me_2SO-d_6) 15.8, 22.5, 39.8, 42.0, 42.1, 126.7, 127.1 (2 C), 128.3 (2 C), 139.7, 169.5, 174.3 ppm; IR (KBr) 3265, 1640, 1560, 1450, 740, 700 cm^{-1}; mass spectrum, m/e (relative intensity) 234 (2), 206 (5), 128 (25), 106 (100), 91 (70), 77 (8), 65 (9). Anal. ($C_{13}H_{18}N_2O_2$) C, H, N. Table V lists other new compounds.

Preparation of *N*-Acetyldiphenylglycine *N*-Benzylamide (1g). 4,4-Diphenyl-2-methyl-2-oxazolin-5-one[13] (1.95 g, 7.76 mmol) was dissolved in acetonitrile (25 mL). Benzylamine (0.83 g, 0.85 mL, 7.76 mmol) was added dropwise and the solution was stirred at 50 °C (3 h), during which time a precipitate formed. The mixture was concentrated in vacuo and the residue was recrystallized from acetonitrile to give **1g**: yield 2.50 g (90%); mp 189–190 °C; ^{1}H NMR (80 MHz, Me_2SO-d_6) δ 1.95 (s, 3 H), 4.28 (d, J = 5.9 Hz, 2 H), 7.03–7.48 (m, 15 H), 8.31 (t, J = 5.9 Hz, 1 H), 8.51 (s, 1 H); ^{13}C NMR (80 MHz, Me_2SO-d_6) 23.4, 42.7, 68.8, 126.3, 126.8 (2 C), 127.3 (2 C), 127.8 (2 C), 128.3 (2 C), 139.4, 141.8, 169.2, 170.6 ppm; the signal for the remaining aromatic carbon atom was not detected; IR (KBr) 3430, 1645, 1530, 1445, 740, 695 cm^{-1}; mass spectrum, m/e (relative intensity) 340 (34), 225 (15), 207 (35), 182 (45), 165 (73), 131 (19), 106 (7), 91 (100), 77 (22), 65 (10); mass spectrum (CI mode, CH_4), m/e 359 (P + 1). Anal. ($C_{23}H_{22}N_2O_2$) C, H, N.

Pharmacology. Each compound listed in Tables I–III was tested for anticonvulsant activity (phase I evaluation) with male Carworth Farms No. 1 mice. All compounds were given in three dose levels (30, 100, and 300 mg/kg). The parent compound **1a** was also tested at 600 mg/kg. Seizures were then artificially induced by either electroshock or pentylenetetrazole. Maximal electroshock seizures (MES) were elicited with a 60-cycle alternating current of 50-mA intensity (5–7 times that was necessary to elicit minimal electroshock seizures) delivered for 0.2 s via corneal electrodes. A drop of 0.9% saline was instilled in the eye prior to application of the electrodes so as to prevent the death of the animal. Protection in this test was defined as the abolition of the hind-limb tonic extension component of the seizure. The subcutaneous pentylenetetrazole (Metrazol) seizure threshold test (sc Met) entailed the administration of 85 mg/kg of pentylenetetrazole as a 0.5% solution subcutaneously in the posterior midline of mice. This amount of pentylenetetrazole was expected to produce seizures in greater than 97% of mice. The animal was observed for 30 min. Protection was defined as the failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5-s duration). The effects of the compounds on forced and spontaneous motor activity were evaluated in mice by the rotorod test (Tox). The animal was placed on an 1-in.-diameter knurled plastic rod rotating at 6 rpm after the administration of the drug. Normal mice can remain on a rod rotating at this speed indefinitely. Neurologic toxicity was defined as the failure of the animal to remain on the rod for 1 min. The MES and the sc Met tests were conducted on single animals while four mice were utilized for the Tox test at each dose.

The overall effect of the drug in these three tests was then given by one of four different ratings (ASP Results Classification I–IV). The number I indicated anticonvulsant activity at 100 mg/kg or less, II designated activity at doses greater than 100 mg/kg, III denoted no anticonvulsant activity at doses up to and including 300 mg/kg, and IV indicated that anticonvulsant activity and toxicity or toxicity alone was demonstrated at 30 mg/kg or that anticonvulsant activity was displayed at 100 mg/kg or less, but that the test results were not consistent.

Compounds with a rating of I were considered promising and were submitted for phase II (quantification) testing. The dose-effect behavior of the three compounds listed in Table VI was evaluated by using the previously described procedures by the administration of varying dose levels of each compound, treating

normally eight mice at each dose.

Phase IV tests (Table VII) involved the same procedures described previously for phase II, except the test drug was administered to mice orally. Phase V entailed the administration of 85 mg/kg of Metrazol, 2.70 mg/kg of bicuculline, 3.15 mg/kg of picrotoxin, or 1.20 mg/kg of strychnine as a 0.5% solution subcutaneously in the posterior midline of mice. This amount of each convulsant was expected to produce seizures in 97% of the mice tested. The animal was isolated and observed for 30 min in the Metrazol, bicuculline, and strychnine tests and for 45 min in the case of the picrotoxin test. Protection in each of these tests was defined as the failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5-s duration) in the Metrazol test, complete absence of a seizure in the bicuculline and picrotoxin tests, and abolition of the hind-leg tonic extensor component of the seizure in the strychnine test. Eight mice were normally treated at each dose for both phase IV and V testing. The results obtained in this test are listed in Table VIII.

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Supplementary Material Available: Detailed experimental procedures and physical data for all new compounds (12 pages). Ordering information is given on any current masthead page.

Hydroxamic Acid Inhibitors of 5-Lipoxygenase

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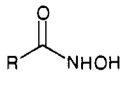
The hydroxamic acid functionality can be incorporated in a variety of simple molecules to produce potent inhibitors of 5-lipoxygenase. As an example of this, the structure–activity relationships in a series of ω-phenylalkyl and ω-naphthylalkyl hydroxamic acids are presented. Among the features described are the influence of hydrophobicity, aryl substitution, and modifications of the hydroxamate group on enzyme inhibitory potency. To assist in the selection of more potent hydroxamic acid inhibitors, a simple hypothesis about the nature of enzyme–inhibitor binding was devised. In this hypothesis, the structures of compounds were matched to a proposed geometry of arachidonic acid when bound to the enzyme. Compounds that match best without extending into disfavored regions were predicted to be the best inhibitors. Three series of hydroxamates selected according to this approach are described. Within these series are some of the most potent inhibitors of 5-lipoxygenase reported to date.

The enzyme 5-lipoxygenase catalyzes the first step of a biochemical pathway in which arachidonic acid is converted into the leukotrienes. Numerous biochemical effects have been associated with the leukotrienes and they have been implicated as important mediators in a variety of disease states including asthma, arthritis, psoriasis, and allergy.[1] As the first dedicated enzyme in the biosynthetic cascade leading to these important mediators, 5-lipoxygenase clearly represents an exciting target for therapeutic intervention.[2]

Hydroxamic acids are well-known to form strong complexes with a variety of transition metals. This property has been exploited in the use of hydroxamates as inhibitors of several metalloenzymes.[3] Since it is generally believed that 5-lipoxygenase contains a catalytically important iron atom,[4] this enzyme is a logical candidate for inhibition by hydroxamic acid containing molecules. In fact, Corey and co-workers[5] have reported that the hydroxamic acid of arachidonic acid is an inhibitor of 5-lipoxygenase. Kerdesky et al.[6] showed that when a hydroxamic acid group is positioned at C_5 of arachidonic acid, an inhibitor with a 10-fold enhancement in potency is obtained. This demonstrated that not only the presence but also the position of the hydroxamic acid moiety is important for 5-lipoxygenase inhibition. Corey et al.[5] has also shown that the full eicosenoid chain is not required for inhibition. A hydroxamate bearing the first 10 carbons of the arachidonate chain and capped by a phenyl ring inhibits 5-lipoxygenase.

While the compounds reported by Corey and Kerdesky are extremely potent inhibitors, they are unlikely to be of therapeutic value. As analogues of arachidonic acid they are prone to chemical oxidation and would almost certainly be degraded rapidly in vivo. It was our objective to identify simple, stable molecules containing the hydroxamic acid functionality that maintained the potent inhibition demonstrated by the arachidonic acid analogues referred to above. We report simple hydroxamates that not only inhibit 5-lipoxygenase, but surpass the potency of many of the commonly cited reference inhibitors of this enzyme.

Table I. ω-Arylalkyl Hydroxamic Acids: In Vitro RBL-1 5-Lipoxygenase Inhibitory Activities



no.	R	IC_{50},[a] μM
1	C_6H_5	110 (94–120)
2	$C_6H_5CH_2$	300 (250–400)
3	$C_6H_5CH_2CH_2$	87 (81–93)
4	$C_6H_5CH_2CH_2CH_2$	27 (25–29)
5	2-naphthyl	14 (12–16)
6	(2-naphthyl)CH_2	19 (16–21)
7	(2-naphthyl)CH_2CH_2	9.7 (8.5–11)
8	1-naphthyl	43 (37–53)
9	(1-naphthyl)CH_2	27 (26–29)
10	*trans*-C_6H_5CH=CH-	12 (8–14)
11	*cis*-C_6H_5CH=CH-	31 (27–36)
12	C_6H_5C≡C-	46 (42–49)

[a] IC_{50} with 95% confidence limits calculated by the method of Finney[16] in parentheses.

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