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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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.

(3) Alexander, G. J.; Kopeloff, L. M. *Neuropharmacology* **1977**, *16*, 405–409. Crider, A. M.; Tita, T. T.; Tschappart, K. D.; Hinko, C. N.; Seibert, K. *J. Pharm. Sci.* **1984**, *73*, 1612–1616. Hinko, C. N.; Seibert, K.; Crider, A. M. *Neuropharmacology* **1984**, *23*, 1009–1014. Crider, A. M.; Tita, T. T.; Wood, J. D.; Hinko, C. N. *J. Pharm. Sci.* **1982**, *71*, 1214–1219. Yunger, L. M.; Fowler, P. J.; Zarevics, P.; Setler, P. E. *J. Pharmacol. Exp. Ther.* **1984**, *228*, 109–115. Beitz, A. J.; Larson, A. A. *Eur. J. Pharmacol.* **1985**, *114*, 181–187. Matsui, Y.; Deguchi, T. *Life Sci.* **1971**, *20*, 1291–1296. Erez, U.; Frenk, H.; Goldberg, O.; Cohn, A.; Teichberg, V. I. *Eur. J. Pharmacol.* **1985**, *110*, 31–39.

(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.

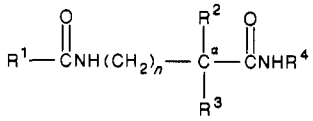
(5) Takahashi, T.; Ogui, K.; Fujimura, H.; Satoda, I.; Fukui, T.; Yamamato, Y. Swiss Patent 393 355, Oct 30, 1965. Thorne, D. E. U.S. Patent 3 657 341, April 18, 1972.

(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.

(7) Zetler, G. *Eur. J. Pharmacol.* **1980**, *65*, 297–300. Meldrum, B. S.; Menini, C.; Stutzmann, J. M.; Naquet, R. *Brain Res.* **1979**, *170*, 333–348. Tortella, F. C.; Cowan, A.; Adler, M. W. *Life Sci.* **1981**, *29*, 1039–1045. Tortella, F. C.; Cowan, A. *Life Sci.* **1982**, *31*, 2225–2228.

(8) Other models have been proposed. For a comprehensive discussion as well as review, see: Camerman, A.; Camerman, N. In *Antiepileptic Drugs: Mechanism of Action*; Glaser, G. H., Penry, J. K., Woodbury, D. M., Eds.; Raven: New York, 1980; pp 223–231. Wong, M. G.; Defina, J. A.; Andrews, P. A. *J. Med. Chem.* **1986**, *29*, 562–572.

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

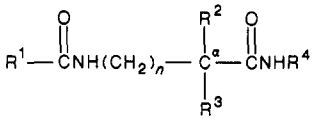


1

| 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.

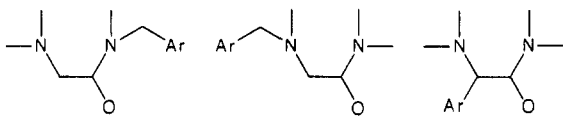
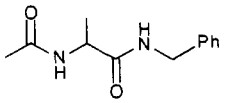


**Figure 1.** Structural unit present in many anticonvulsants.

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

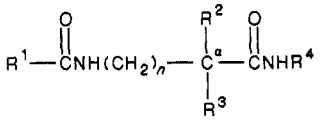
*N*-Acetyl-DL-alanine *N*-benzylamide (**1a**) served as the parent compound in this study (Tables I–III). Systematic structural variations have been conducted at three sites: the $\alpha$-carbon (Table I), the amide substituent (Table II), and the *N*-acyl group (Table III). In the selection of derivatives, 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 $\alpha$-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 $\alpha$-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 $\beta$-amino acid derivatives (**1h** and **1i**) in which the $\alpha$-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]

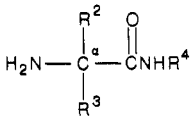


1

| 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

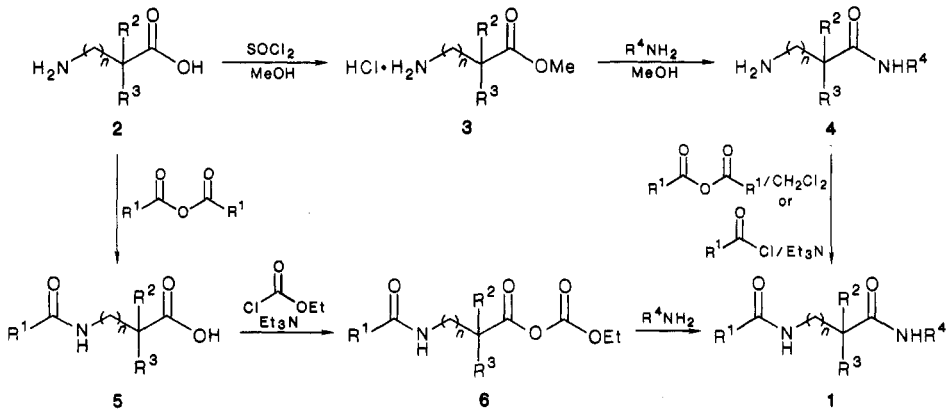


4

| no. | $R^2$ ($R^3$) | $R^3$ ($R^2$) | $R^4$ | yield[a] | mp[b] | IR[c] | $^1$H NMR,[d,e] $\alpha$-CH | $^{13}$C NMR[d] $\alpha$-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 $\delta$). [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.

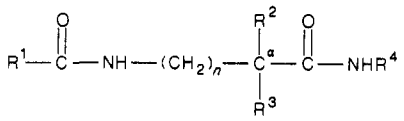
## Chemistry

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**



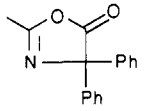
**1**

| 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

(11) Brenner, M.; Huber, W. *Helv. Chim. Acta* **1953**, *36*, 1109–1115. Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961.

(12) For *N*-acetyl-DL-α-amino acids, see: Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*, Wiley: New York, 1961. For *N*-acetyl-DL-β-amino acids, see: Fodor, P. J.; Price, V. E.; Greenstein, J. P. *J. Biol. Chem.* **1949**, *178*, 503–509.

(13) Hohenlohe-Oehringen, K. *Monatsch. Chem.* **1962**, *93*, 639–644.

(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 $\alpha$-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 $\alpha$-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 $\alpha$-carbon atom of the parent compound were replaced with phenyl groups (**1g**). Fifth, homologation of **1a** and **1b** giving the corresponding $\beta$-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

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