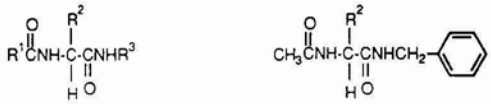
Preparation and Anticonvulsant Activity of a Series of Functionalized α-Aromatic and α-Heteroaromatic Amino Acids

Harold Kohn,*,† Kailash N. Sawhney,† Philippe LeGall,† Judith D. Conley,† David W. Robertson,‡ and J. David Leander‡

Department of Chemistry, University of Houston, Houston, Texas 77204-5641, and Lilly Research Laboratories, Eli Lilly Company, Indianapolis, Indiana 46285. *Received July 28, 1989*

We recently reported the potent anticonvulsant activity of (*R*,*S*)-α-acetamido-*N*-benzyl-α-phenylacetamide (**2b**). Selectively substituted derivatives of this compound have now been prepared (23 examples) and evaluated in the maximal electroshock seizure (MES) and horizontal screen (tox) tests in mice. In several key cases, replacement of the α-phenyl substituent in **2b** by a relatively small, electron-rich, heteroaromatic moiety led to a substantial improvement in the anticonvulsant potency of the drug candidate. The most active compounds were (*R*,*S*)-α-acetamido-*N*-benzyl-2-furanacetamide (**2g**) and (*R*,*S*)-α-acetamido-*N*-benzyl-2-pyrroleacetamide (**2i**). After ip administration, the MES ED_{50} values for **2g** (10.3 mg/kg) and **2i** (16.1 mg/kg) compared well with phenytoin (9.50 mg/kg). Evaluation of the two individual enantiomers of **2g** demonstrated that the anticonvulsant activity resided in the *R* stereoisomer. The low ED_{50} value (3.3 mg/kg) for (*R*)-**2g** contributed to the large protective index (TD_{50}/ED_{50}) observed for this drug candidate, which approached that of phenytoin.

Recently we have reported the excellent anticonvulsant activities of functionalized amino acid derivatives **1**.[1–5]



1 **2** a $R^2 = CH_3$, b $R^2 = Ph$

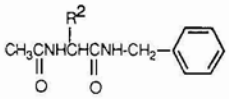
The pharmacological data suggested that these compounds comprised a new and important class of anticonvulsant agents.[6] The structure–activity profile for **1** indicated that stringent steric and electronic requirements existed for optimal anticonvulsant activity. Excellent protection against maximal electroshock seizures (MES) in mice was observed for functionalized amino acid racemates containing an *N*-benzylamide moiety, an acetylated amino group, and either a methyl (**2a**) or a phenyl (**2b**) substituent on the α-carbon. The median effective doses (ED_{50}) for **2a** and **2b** in mice (ip) were 76.5 and 20.3 mg/kg, respectively.[3] These values compared favorably with the corresponding ED_{50} value obtained for the proven antiepileptic phenobarbital (21.8 mg/kg).[7] Significantly, evaluation of the individual enantiomers of **2a** and **2b** showed that the anticonvulsant activity resided primarily in the *R* stereoisomers.[1,4,5] In both cases, the *R* isomer was over 10 times more effective in the MES test than the corresponding *S* enantiomer. This difference in activity represented the greatest eudismic ratio[8] reported to date for MES-selective anticonvulsants.

The pronounced activity observed for **2b** prompted our investigation of the anticonvulsant properties of a select series of functionalized amino acids in which the α-substituent is either an aromatic or a heteroaromatic group. In this paper, the synthesis, physical properties, and anticonvulsant activities of these compounds are described. Evidence is presented that placement of a relatively small, electron-rich, heteroaromatic moiety at the α-site leads to a substantial enhancement in the anticonvulsant activity of the drug candidate and that the high eudismic ratio observed for **2a** and **2b** is preserved for the most active member of this series of compounds.

Selection of Compounds

(*R*,*S*)-α-Acetamido-*N*-benzyl-α-phenylacetamide[3] (**2b**) served as the parent compound in this study (Table I). In the first series of functionalized amino acid derivatives

Table I. Selected Physical and Pharmacological Data in Mice for *R*,*S*-α-Aromatic and α-Heteroaromatic Substituted Functionalized Amino Acid Derivatives **2**[a]



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no.	R^2	mp[b]	MES[c] ED_{50}	tox[d] TD_{50}
2b[e]	C_6H_5	202–203	32.1 (27.5–40.2)	>40
2c	4-C_6H_4OH	232–235	>300	*f*
2d	4-$C_6H_4OCH_3$	196–198	>300	*f*
2e	2-OH-5-$CH_3C_6H_3$	183–185	>300	*f*
2f	2-$C_{10}H_7$	210–211	>300	>300
2g	2-furanyl	178–179	10.3 (9.1–11.6)	~40
2h	5-CH_3-2-furanyl	148–150	19.2 (16.4–23.8)	75.4
2i	2-pyrrolyl	174–175	16.1 (13.2–19.9)	>30, <100
2j	5-CH_3-2-pyrrolyl	167–168	36.5 (30.6–57.1)	*f*
2k	1-CH_3-2-pyrrolyl	179–181	~300	*f*
2l	2-thienyl	167–169	44.8 (38.9–51.4)	>30, <100
2m	3-thienyl	198–199	87.8 (69.9–150)	>100
2n	benzo[*b*]furan-2-yl	195–196	>100, <300	>100, <300
2o	indol-3-yl	213–214	>300	<300
2p	benzo[*b*]thien-2-yl	226–227	>100, <300	>100, <300
phenytoin[g]			9.5 (8.1–10.4)	65.5 (52.5–72.1)
phenobarbital[g]			21.8 (15.0–22.5)	69.0 (62.8–72.9)
valproate[g]			272 (247–338)	426 (369–450)

[a] The compounds were administered intraperitoneally. ED_{50} and TD_{50} values are in mg/kg. Numbers in parentheses are 95% confidence intervals. [b] Melting points (°C) are uncorrected. [c] MES = maximal electroshock seizure test. [d] Tox = neurologic toxicity determined from horizontal screen. [e] Reference 3. [f] Not determined. [g] Reference 7.

* Author to whom correspondence should be addressed to at the University of Houston.

(1) Kohn, H.; Conley, J. D. *Chem. Br.* **1988**, *24*, 231.
(2) Cortes, S.; Liao, Z.-K.; Watson, D.; Kohn, H. *J. Med. Chem.* **1985**, *28*, 601.
(3) Conley, J. D.; Kohn, H. *J. Med. Chem.* **1987**, *30*, 567.
(4) Kohn, H.; Conley, J. D.; Leander, J. D. *Brain Res.* **1988**, *457*,

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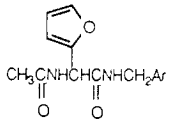
Table II. Selected Physical and Pharmacological Data in Mice for Fluoro-Substituted (*R,S*)-α-Acetamido-*N*-substituted-benzyl-2-furanacetamides 3



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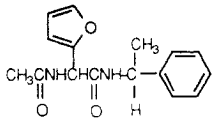
no.	Ar	mp[b]	MES[c] ED_{50}	tox[d] TD_{50}
2g	C_6H_5	178–179	10.3 (9.1–11.6)	~40
3a	2-FC_6H_4	193–195	40.0	*e*
3b	3-FC_6H_4	163–165	13.3 (11.5–15.3)	136 (115–162)
3c	4-FC_6H_4	188–190	12.7 (10.4–15.1)	144 (123–171)
3d	2,5-$F_2C_6H_3$	177–178	23.8 (20.2–28.4)	*e*
3e	2,6-$F_2C_6H_3$	237–239	>25, <100	*e*

[a] The compounds were administered intraperitoneally. ED_{50} and TD_{50} values are in mg/kg. Numbers in parentheses are 95% confidence intervals. [b] Melting points (°C) and uncorrected. [c] MES = maximal electroshock seizure test. [d] Tox = neurologic toxicity determined from horizontal screen. [e] Not determined.

selected for synthesis, the α-substituent was systematically varied. Both aromatic (**2c–f**) and heteroaromatic (**2g–p**) moieties were incorporated into the amino acid backbone. In all cases, the functionalized amino acid racemates were prepared and tested.

The pharmacological properties observed for **2g** warranted further investigation of this compound. Accordingly, two different types of structural modifications of the N-terminal benzyl moiety were made. First, a series of racemic fluorine-substituted benzylamides **3a–e** were synthesized (Table II). Impetus for this study was provided by an earlier observation that a modest improvement of the overall activity of **2a** in mice (ip) was obtained upon incorporation of a fluorine atom at the meta position of the aromatic ring.[3] The second structural modification examined for **2g** involved the replacement of the *N*-benzylamide group by the corresponding *N*-α-methylbenzylamides. Use of (*R*)-α-methylbenzylamine and (*S*)-α-methylbenzylamine in the synthesis permitted the preparation and pharmacological evaluation of each of the four individual diastereomers of 4.



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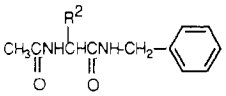
The final group of drug candidates synthesized were the individual *R*-(−) and *S*-(+) stereoisomers of **2g** (Table III). The marked selectivity previously noted[1,4,5] for the individual enantiomers of **2a** and **2b** prompted this investigation.

(6) For the pharmacological properties of the related α,α-dialkyl-α-phthalimidoacetamides and α,α-dialkyl-α-benzamidoacetamides, see: Upham, S. D.; Dermer, O. C. *J. Org. Chem.* **1957**, *22*, 799.
(7) 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.
(8) Eudismic ratio = ratio of activities of the two enantiomers. See: Lehmann, P. A. *Trends Pharmacol. Sci.* **1982**, *3*, 103.

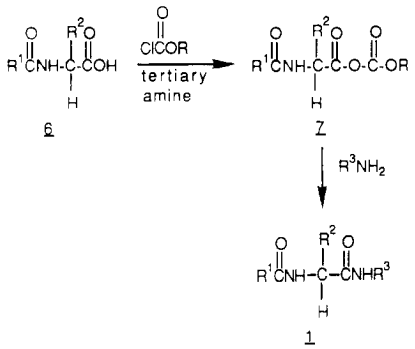
Table III. Selected Physical and Pharmacological Data in Mice for Functionalized Amino Acid Stereoisomers



no.	R^2	mp[b]	MES[c] ED_{50}	tox[d] TD_{50}
(*R,S*)-**2g**	2-furanyl	178–179	10.3 (9.1–11.6)	~40
(*R*)-**2g**	2-furanyl	196–197	3.3 (2.8–3.9)	23.8
(*S*)-**2g**	2-furanyl	196–197	>25	>200
(*R,S*)-**2a**[e]	CH_3	139–141	76.5 (66.6–89.0)	454 (417–501)
(*R*)-**2a**[e]	CH_3	139–141	54.8 (50.3–59.7)	214 (148–262)
(*S*)-**2a**[e]	CH_3	139–142	548 (463–741)	841 (691–954)
(*R,S*)-**2b**[f]	C_6H_5	202–203	32.1 (27.5–40.2)	>40
(*R*)-**2b**[f]	C_6H_5	219–221	26.4 (21.1–32.0)	>80
(*S*)-**2b**[f]	C_6H_5	221–222	>300	>100, <300

[a] The compounds were administered intraperitoneally. ED_{50} and TD_{50} values are in mg/kg. Numbers in parentheses are 95% confidence intervals. [b] Melting points (°C) are uncorrected. [c] MES = maximal electroshock seizure test. [d] Tox = neurologic toxicity determined from horizontal screen. [e] Values determined at the Epilepsy Branch, NINCD, NIH; see ref 4. The median toxic dose (TD_{50}) was determined by using the rotorod test (see: Durham, N. W.; Miya, T. S. *J. Am. Pharm. Assoc.* **1957**, *46*, 208). [f] Reference 4.

Scheme I. Preparation of Compound 1 via the Mixed Carbonic Anhydride Method



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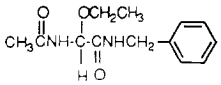
Chemistry

The strategies employed in the synthesis of the functionalized amino acid derivatives were patterned after procedures previously employed. The preparation of compounds **2c–e,g–i,k,l,n–p** have been reported.[9] Introduction of the aromatic or heteroaromatic group at carbon 2 in compounds **2c–e,g–l,n–p** was accomplished by an amidoalkylation reaction using 2-acetamido-*N*-benzyl-2-ethoxyacetamide (**5**), BF_3, and the appropriate aromatic substrate. The reactions proceeded in moderate yields (28–67%) with excellent regioselectivity.



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The remaining compounds in this study were prepared by the mixed carbonic anhydride method (Scheme I).[10] In

(9) LeGall, P.; Sawhney, K. N.; Conley, J. D.; Kohn, H. *Int. J. Pept. Protein Res.* **1988**, *32*, 279.

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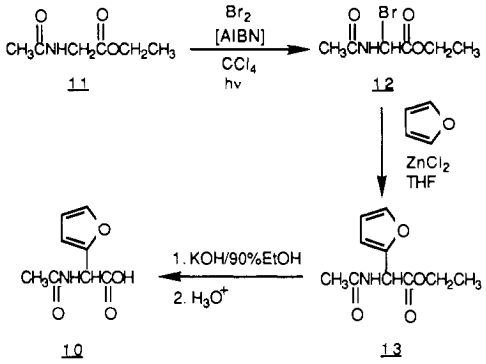
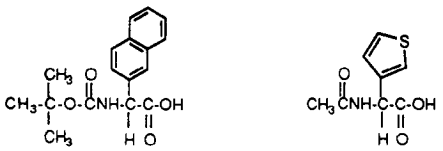
Scheme II. Improved Procedure for the Preparation of (*R*,*S*)-α-Acetamido-2-furanacetic Acid (**10**)



this procedure, the N-acylated amino acid **6** was treated with an alkyl chloroformate in the presence of a tertiary amine to generate the mixed N-acyl amino acid carbonic ester anhydride **7**. This intermediate was not isolated but reacted in situ with the appropriate amine (R^3NH_2) to produce the N-acyl amino acid N-substituted amide **1**. The starting N-acylated amino acid selected for the synthesis of **2f** was *N*-*t*-Boc-(*R*,*S*)-2-naphthylglycine[11] (**8**).



8 **9**

Subsequent removal (CF_3CO_2H) of the N-protecting group after the mixed carbonic anhydride coupling step, followed by acetylation with acetyl chloride and triethylamine, yielded **2f**. In the synthesis of (*R*,*S*)-**2g**, (*R*)-**2g**, (*S*)-**2g**, **2m**, **3**, and **4** the appropriate N-acetyl amino acid was directly employed, thereby simplifying the experimental procedure. Synthesis of (*R*,*S*)-α-acetamido-3-thiopheneacetic acid (**9**) was readily accomplished beginning with (*R*,*S*)-α-amino-3-thiopheneacetic acid and acetic anhydride. An improved procedure (Scheme II) was developed for the synthesis of (*R*,*S*)-α-acetamido-2-furanacetic acid (**10**). This functionalized amino acid served as the starting material for compounds **2g**, **3**, and **4**. The method adopted took advantage of the recent report on the employment of protected α-bromo amino acid derivatives as electrophilic glycine templates in amino acid synthesis.[12] Accordingly, ethyl acetamido-2-bromoacetate[13] (**12**) was prepared from ethyl acetamidoacetate (**11**) and then treated with furan and $ZnCl_2$ to give **13**. Hydrolysis of the ethyl ester yielded **10**, the necessary precursor for the mixed carbonic anhydride coupling procedure (Scheme I). The overall yield for **10** in this three-step sequence was 65%.

Several different alkyl chloroformates and tertiary amines were examined for the mixed carbonic anhydride reaction beginning with **10**. Higher conversion rates were generally obtained with isobutyl chloroformate and 4-methylmorpholine.[10] The yields for the final coupling step

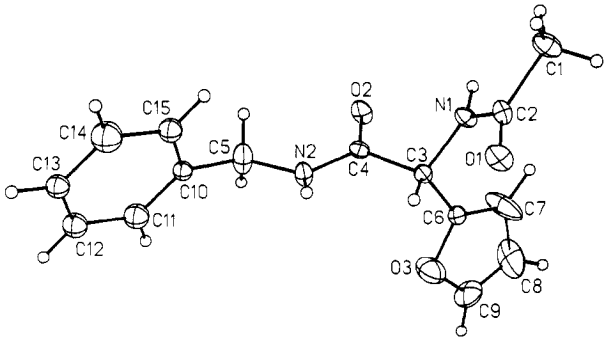
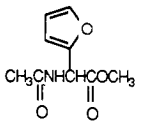


Figure 1. ORTEP view of compound (*R*)-**2g** with atom labeling scheme. The thermal ellipsoids are 20% equiprobability envelopes, with hydrogens as spheres of arbitrary diameter. Only one orientation of the disordered phenyl ring is shown.

for the preparation of the fluorine-substituted aryl amides **3** (Table II) ranged from 50 to 88%. A comparable synthetic protocol was adopted for methylbenzyl amides **4**. The configuration at C-2 in each of the four individual diastereomers of **4** was not determined.

Synthesis of the two enantiomeric forms of **2g**, (*R*)-**2g**, and (*S*)-**2g** (Table III) was achieved by resolution of racemic **10** via fractional recrystallization of the diastereomeric salts formed with (*R*)- and (*S*)-α-methylbenzylamine, respectively, and then coupling the individual stereoisomers with benzylamine. Use of isobutyl chloroformate and 4-methylmorpholine in the mixed carbonic anhydride coupling procedure did not lead to significant amounts of racemization of **2g**. An X-ray crystallographic structural determination of (*R*)-**2g** was conducted to provide basic information concerning the solid-state structure of this compound, and the ORTEP view is presented in Figure 1. Thermal disorder limited the amount of data obtainable in this determination, but some useful observations concerning the structure of the molecular backbone of (*R*)-**2g** in the solid state can still be made. Significant double bond character in the C–N peptide linkages were indicated by unusually short N1–C2 (1.338 (9) Å) and N2–C4 (1.323 (8) Å) bond lengths and unusually long C1–C2 (1.509 (9) Å) and C3–C4 (1.534 (8) Å) bond lengths [for C(sp^2)–C(sp^3)]. Comparable observations have been previously noted in related compounds.[14] The torsion angles about N1–C2 and N2–C4 are essentially 0°, as would be expected in this highly conjugated system, and the sum of the angles about both nitrogens is 359°, indicating virtual planarity and substantial delocalization of the lone electron pairs.

The absolute configurations of the enantiomers of **10** were determined by converting an enriched sample of (*R*)-**10** to the corresponding methyl ester (*R*)-**14** with diazomethane. The optical rotation observed for this adduct [$[\alpha]^{26}_D = -95°$ (c = 1, MeOH)] was comparable to a sample obtained after treatment of racemic **14**[9] with papain



14

in aqueous DMF. This enzymatic system has been re-

(10) For an excellent discussion and review of this method, see: Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012.
(11) Kukolja, S.; Draheim, S. E.; Pfeil, J. L.; Cooper, R. D. G.; Graves, B. J.; Holmes, R. E.; Neel, D. A.; Huffman, G. W.; Webber, J. A.; Kinneck, M. D.; Vasileff, R. T.; Foster, B. J. *J. Med. Chem.* **1985**, *28*, 1886.
(12) Williams, R. M.; Sinclair, P. J.; Zhai, D.; Chen, D. *J. Am. Chem. Soc.* **1988**, *110*, 1547.
(13) Kuber, R.; Steglich, W. *Liebigs Ann. Chem.* **1983**, 599.
(14) (a) Ishida, T.; Tanabe, N.; Inoue, M. *Acta Crystallogr.* **1983**, *C39*, 110. (b) Kojima, T.; Tanaka, I.; Ashida, T. *Ibid.* **1982**, *B38*, 221. (c) Hansen, L. K.; Hagen, E. A.; Loennechen, T.; Aasen, A. J. *Acta Chem. Scand.* **1982**, *B36*, 327. (d) Aubry, A.; Vitoux, B.; Boussard, G.; Marraud, M. *Int. J. Pept. Protein Res.* **1981**, *18*, 195 and references therein.

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ported to selectively hydrolyze racemic N-protected furylglycine methyl esters to the free (*S*)-acids and unreacted (*R*)-esters in high enantiomeric excess.[15]

Several attempts were conducted to directly employ chiral (*R*)-**13** or (*R*)-**14** (obtained by papain-mediated hydrolysis of the corresponding racemic esters) for the preparation of (*R*)-**2g**. Unfortunately, treatment of either ester with benzylamine in the absence or presence of NaCN gave racemic **2g**.[16]

Pharmacological Evaluation

The *N*-acetyl amino acid N-substituted amides **2–4** were tested for anticonvulsant activity by using the procedures described by Krall et al.[17] All compounds were administered intraperitoneally (ip) in mice. Tables I–III list the median effective dose (ED_{50}) values required to prevent seizures in the MES test by racemic **2**, racemic **3**, and the individual enantiomers of **2g**, respectively. Included in these tables are the median toxic dose (TD_{50}) values determined for select compounds by using the horizontal screen test.[18]

Table I lists the pharmacological data for those compounds in which only the α-carbon moiety has been modified. Evaluation of this subset of results revealed several important observations. First, addition of electron-releasing hydroxy (i.e., **2c** and **2e**) or methoxy (i.e., **2d**) groups to the α-substituted phenyl group in **2b** or expansion of the aromatic ring from the phenyl group in **2b** to the naphthyl residue in **2f** led to a precipitous drop in anticonvulsant potency. Second, replacement of the α-phenyl ring in **2b** with an electron-rich, five-membered heteroaromatic ring resulted in a substantial improvement in the potency of the compound in the MES test. Notable protection against seizures were observed for the racemates of **2g–j** and **2l**. The ED_{50} values for these compounds compared favorably with the reported data for phenytoin.[7] Third, placement of a methyl substituent on the five-membered heteroaromatic ring was accompanied by a decrease in the potency of the drug candidate versus the unsubstituted compounds (i.e., **2h** versus **2g**; **2j**, **2k** versus **2i**). Fourth, replacement of the α-heteroaromatic substituent by the corresponding benzoheteroaromatic group led to a reduction in biological activity (i.e., **2n–p**). This observation paralleled the results obtained for **2b** versus **2f**.

The second and third series of compounds tested for anticonvulsant activity were adducts in which the terminal *N*-benzylamide moiety in **2g** was altered. Table II lists the comparative data obtained for five fluorine-substituted derivatives. All the compounds exhibited pronounced activity in the MES test. The meta (**3b**) and para (**3c**) fluoro adducts displayed activities comparable to that of **2g**, while a small reduction in activity versus **2g** was noted for ortho derivative **3a**, and the two difluoro analogues **3d** and **3e**. These data contrasted with the pharmacological results secured from the α-methylbenzylamides **4a–d**. Increasing the size of the benzylamide moiety by incorporation of an α-methyl group resulted in a significant decrease in the anticonvulsant potency of the drug candidate regardless of the stereochemical relationship between the two asymmetric centers. The MES ED_{50} values for these compounds were all greater than 100 mg/kg. A similar trend was previously noted in the 2-acetamido-*N*-benzylpropionamide (**2a**) series.[3]

Table III lists the pharmacological data for the two individual isomers of **2g** along with the racemic mixture, as well as the corresponding data for **2a** and **2b**.[4] In all three series of compounds a pronounced improvement in anticonvulsant potency was noted for the *R* enantiomer versus either the *S* isomer or the racemate. Moreover, in each case little activity in the MES test was observed for the *S* enantiomer. The ED_{50} value of (*R*)-**2g** was 3.3 mg/kg, which was considerably lower than that reported for phenytoin[7] (ED_{50} = 9.5 mg/kg). The enhanced potency of (*R*)-**2g** contributed to the observed high protective index (TD_{50}/ED_{50} = 7.2) for this compound, which compared favorably with the value observed for phenytoin (TD_{50}/ED_{50} = 6.9).

Conclusions

The pharmacological data obtained in this investigation significantly extended the structure–activity profile previously reported for functionalized amino acid derivatives.[2,3] The observed data supported our hypothesis that stringent steric and electronic requirements exist for maximal anticonvulsant activity in this novel class of compounds.[3] The outstanding potencies noted for **2g** and **2i** in the MES test suggested that the placement of relatively small, electron-rich groups at the α-position in **1** was beneficial for anticonvulsant activity. Furthermore, our finding that the primary activity of **2g** resided in the *R* enantiomer provided additional evidence for the marked stereospecificity exhibited in this new class of anticonvulsants. Additional studies are in progress investigating the generality of this class of compounds as well as their mode of action.

Experimental Section

Chemistry. General Methods. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (IR) were run on Perkin-Elmer 1330 and 283 spectrometers and calibrated against the 1601 cm^{-1} band of polystyrene. Absorption values are expressed in wavenumbers (cm^{-1}). Proton (^1H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were taken on Nicolet NT-300 and General Electric QE-300 NMR instruments. Chemical shifts (δ) are in parts per million (ppm) relative to Me_4Si and coupling constants (*J* values) are in hertz. Low-resolution mass spectra (MS) were recorded at an ionizing voltage of 70 eV with a Varian MAT CH-5 spectrometer at the Lilly Research Laboratories. Microanalyses were provided by the Physical Chemistry Department of the Lilly Research Laboratories. Thin- and thick-layer chromatography were run on precoated silica gel GHLF microscope slides (2.5 × 10 cm; Analtech No. 21521) or silica gel GHLF (20 × 20 cm; Analtech 11187).

Preparation of (*R*,*S*)-2-Acetamido-*N*-benzyl-2-(5-methylpyrrolyl)acetamide (2j). 2-Acetamido-*N*-benzyl-2-ethoxyacetamide[9] (5, 2.00 g, 8 mmol) was suspended in anhydrous Et_2O (175 mL), and then $BF_3 \cdot Et_2O$ (1.38 g, 9.7 mmol) was added and the resulting solution was stirred (15 min). 2-Methylpyrrole[19] (0.85 g, 10 mmol) was then added and the reaction mixture was stirred under N_2 (6 days), during which time the color of the reaction mixture turned reddish brown and a dark-brown deposit formed at the bottom of the flask. The clear solution was decanted and treated with an aqueous saturated $NaHCO_3$ solution containing ice (100 mL) for 30 min. The aqueous reaction mixture was extracted with EtOAc (3 × 30 mL). The combined extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The brown oily residue was purified by flash column chromatography using 2% $MeOH/CHCl_3$ as the eluent to yield 0.20 g (9%) of the

(15) Drueckhanmer, D. G.; Barbas, C. F.; Nozaki, K.; Wong, C.-H.; Wood, C. Y.; Ciufolini, M. A. *J. Org. Chem.* **1988**, *53*, 1607.
(16) Hogberg, T.; Strom, P.; Ebner, M.; Ramsby, S. *J. Org. Chem.* **1987**, *52*, 2033.
(17) Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. *Epilepsia* **1978**, *19*, 409.
(18) Coughenour, L. L.; McLean, R. R.; Parker, R. B. *Pharmacol. Biochem. Behav.* **1977**, *6*, 351.
(19) Castro, A. J.; Deck, J. F.; Hugo, M. T.; Marsh, J. P.; Pfiffer, R. J. *J. Org. Chem.* **1963**, *28*, 857.

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desired product. Compound **2j** was recrystallized from ethyl acetate/hexane to give a light yellow amorphous solid: R_f 0.44 (95:5, $CHCl_3$/MeOH); mp 167–168 °C; IR (KBr) 3250, 1630, 1520, 1420, 1360, 1300, 1260, 1230, 1160, 1110, 1020 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.87 (s, 3 H), 2.13 (s, 3 H), 4.27 (br s, 2 H), 5.33 (d, J = 7.4 Hz, 1 H), 5.60 (s, 1 H), 5.77 (s, 1 H), 7.19–7.30 (m, 5 H), 8.22 (d, J = 7.4 Hz, 1 H), 8.45 (t, J = 5.5 Hz, 1 H), 10.38 (s, 1 H); ^{13}C NMR (DMSO-d_6) 12.74, 22.49, 42.11, 51.21, 105.09, 106.07, 126.16, 126.64, 126.85, 127.09 (2 C), 128.17 (2 C), 139.33, 168.88, 169.79 ppm. Anal. ($C_{16}H_{19}N_3O_2$) C, H, N.

Preparation of (*R*,*S*)-2-Acetamido-*N*-benzyl-2-(2-naphthyl)acetamide (2f). *N*-*t*-Boc-(*R*,*S*)-2-naphthylglycine *N*-Benzylamide. *N*-*t*-Boc-(*R*,*S*)-2-naphthylglycine[11] (8, 7.53 g, 25 mmol) was combined with CH_3CN (100 mL) and the mixture was placed into an ice/salt water bath (−5 °C). Et_3N (2.53 g, 3.50 mL, 25 mmol) was added dropwise, followed by ethyl chloroformate (2.71 g, 2.40 mL, 25 mmol). 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). Benzylamine (2.95 g, 3.0 mL, 27.5 mmol) in CH_3CN (10 mL) was added dropwise and the mixture was stirred at −5 °C (1 h) and then room temperature (18 h). The brown mixture was concentrated in vacuo and the residue was combined with hot THF 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 Et_3NHCl. The filtrate was concentrated in vacuo and the resulting solid was recrystallized from chloroform/hexane: yield 4.18 g (43%); mp 127–129 °C; IR (KBr) 3240, 1635, 1520, 1505, 1460, 1370, 720, 705 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.31 (s, 9 H), 4.32 (s, 2 H), 5.42 (s, 1 H), 7.14–7.79 (m, 12 H), the N-H protons were not detected; ^{13}C NMR (DMSO-d_6) 28.2 (3C), 43.3, 58.3, 80.0, 124.6, 126.1, 126.2, 126.3, 127.1, 127.2 (2 C), 127.5, 127.9, 128.3 (2 C), 128.6, 133.0, 133.2, 135.9, 137.7, 155.3, 170.3 ppm. Anal. ($C_{24}H_{26}N_2O_3$) C, H, N.

(*R*,*S*)-2-Naphthylglycine *N*-Benzylamide Methanesulfonate. The Boc-protected amino acid *N*-benzylamide (3.91 g, 10 mmol) was dissolved in trifluoroacetic acid (25 mL) and was stirred at room temperature (30 min), during which time gas evolved. The solution was concentrated in vacuo and the residue was redissolved in MeOH (50 mL). Methanesulfonic acid (0.96 g, 0.65 mL, 10 mmol) was added dropwise and stirred (5 min). After concentrating the solution in vacuo, the residue was repeatedly dissolved in MeOH and the solvent was removed (3 × 50 mL). The residue was then dried under vacuum (18 h), leaving a yellow oil. Trituration with CH_2Cl_2 gave a white solid: yield 2.48 g (83%); mp 180–182 °C; IR (KBr) 3245, 1655, 1460, 1385, 730, 700 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.35 (s, 3 H), 4.33 (d, J = 5.5 Hz, 2 H), 5.18 (s, 1 H), 7.15–8.09 (m, 12 H), 8.78 (s, 1 H), 9.06 (t, J = 5.5 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 39.5, 42.3, 55.7, 124.8, 126.6, 126.7, 127.0, 127.5, 127.8, 128.0 (2 C), 128.3, 131.4, 132.4, 132.8, 138.3, 167.1 ppm. The resonances for the remaining aromatic carbons were not detected. Anal. ($C_{20}H_{22}N_2O_4S$) C, H, N.

(*R*,*S*)-2-Acetamido-*N*-benzyl-2-(2-naphthyl)acetamide (2f). (*R*,*S*)-2-Naphthylglycine *N*-benzylamide methanesulfonate (1.59 g, 4.1 mmol) was suspended in CH_3CN (25 mL) and was then cooled in an ice bath. Et_3N (0.83 g, 1.20 mL, 8.2 mmol) was added dropwise, followed by acetyl chloride (0.32 g, 0.30 mL, 4.1 mmol). The ice bath was removed and stirring was continued at room temperature (18 h). The solution was concentrated in vacuo and the residue was recrystallized from 1:1 95% EtOH/H_2O: yield 1.31 g (95%); mp 210–211 °C; IR (KBr) 3230, 1710, 1625, 1535, 1465, 760, 710 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.94 (s, 3 H), 4.30 (d, J = 5.2 Hz, 2 H), 5.86 (d, J = 7.9 Hz, 1 H), 7.15–7.91 (m, 12 H), 8.63 (d, J = 7.9 Hz, 1 H), 8.33 (t, J = 5.2 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 22.5, 42.2, 56.6, 125.5, 126.0, 126.1, 126.3, 126.8, 127.1 (2 C), 127.5, 127.7, 127.9, 128.2 (2 C), 132.4, 132.8, 136.5, 139.1, 169.2, 170.0 ppm. Anal. ($C_{21}H_{20}N_2O_2$) C, H, N.

Preparation of (*R*,*S*)-α-Acetamido-*N*-benzyl-3-thiopheneacetamide (2m). (*R*,*S*)-α-Acetamido-3-thiopheneacetic Acid (9). (*R*,*S*)-α-Amino-3-thiopheneacetic acid (3.92 g, 25 mmol) was combined with H_2O (55 mL) and was cooled in an ice water bath. Solid NaOH (1.00 g, 25 mmol) was added in one portion and the reaction mixture was stirred until homogeneous. Ac_2O (5.10 g, 4.70 mL, 50 mmol) was added dropwise, followed by aqueous NaOH (5 M, 15 mL). The solution was stirred at a temperature below 25 °C (15 min). Acidification of the reaction solution with concentrated HCl (pH 1, 15 mL) led to the formation of a precipitate. The mixture was filtered and the collected white solid was recrystallized from 1:1 95% EtOH/H_2O, producing light yellow crystals: yield 3.55 g (75%); mp 190–192 °C; ^1H NMR (DMSO-d_6) δ 1.90 (s, 3 H), 5.42 (d, J = 7.6 Hz, 1 H), 7.13 (d, J = 5.0 Hz, 1 H), 7.50–7.55 (m, 2 H), 8.69 (d, J = 7.6 Hz, 1 H), 12.89 (s, 1 H); ^{13}C NMR (DMSO-d_6) 22.3, 52.2, 123.3, 126.5, 127.2, 137.3, 169.3, 171.8 ppm. Anal. ($C_8H_9NO_3S$) C, H, N.

(*R*,*S*)-α-Acetamido-*N*-benzyl-3-thiopheneacetamide (2m). With the procedure previously described for the preparation of *N*-*t*-Boc-(*R*,*S*)-2-naphthylglycine *N*-benzylamide, compound **9** (2.99 g, 15 mmol) was treated with Et_3N (1.51 g, 2.10 mL, 15 mmol) and ethyl chloroformate (1.63 g, 1.43 mL, 15 mmol) and benzylamine (1.77 g, 16.5 mmol). The filtrate upon workup was concentrated in vacuo and the resulting yellow solid was recrystallized from 1:1 95% EtOH/H_2O: yield 1.91 g (44%); mp 198–199 °C; IR (KBr) 3460, 1675, 1570, 1400, 720, 695 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.91 (s, 3 H), 4.29 (d, J = 5.2 Hz, 2 H), 5.61 (d, J = 7.9 Hz, 1 H), 7.14–7.50 (m, 8 H), 8.55 (d, J = 7.9 Hz, 1 H), 8.74 (t, J = 5.2 Hz, 1 H); ^{13}C NMR (DMSO-d_6) 22.3, 42.0, 52.5, 122.4, 126.1, 126.7, 127.0 (3 C), 128.2 (2 C), 139.0, 139.2, 169.0, 169.8 ppm. Anal. ($C_{15}H_{16}N_2O_2S$) C, H, N.

Synthesis of (*R*,*S*)-Ethyl α-Acetamido-2-furanacetate (13). An ethereal solution of $ZnCl_2$ (1 N, 28.00 mL, 0.028 mol) was added to a stirred solution of **12**[13] (4.40 g, 0.019 mol) and furan (11.23 g, 0.165 mol) in dry THF (100 mL), and allowed to stir at room temperature (5 h). The mixture was then treated with H_2O (50 mL), the organic phase was separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 100 mL). The organic layers were combined and dried (Na_2SO_4), and the volatile materials were removed by distillation in vacuo to give approximately 4.00 g (97%) of light-brown semisolid material. TLC analysis showed a major spot at R_f 0.30 (1% MeOH/$CHCl_3$). The desired compound was purified by flash column chromatography on silica gel using 1% MeOH/$CHCl_3$ as the eluent to give 3.60 g (87%) of a beige solid: mp 68–70 °C (lit.[9] mp 69–70 °C).

Preparation of (*R*,*S*)-α-Acetamido-2-furanacetic Acid (10). Compound **13** (4.00 g, 19 mmol) was dissolved in 90:10 EtOH/H_2O (150 mL) and then KOH (2.00 g, 35 mmol) was added and the resulting solution was stirred at room temperature (48 h). The reaction was concentrated in vacuo and the residue was diluted with H_2O and then washed with Et_2O (3 × 50 mL). The aqueous layer was then made acidic with 8.5% H_3PO_4 and extracted with EtOAc (3 × 150 mL). The organic layers were combined, dried (Na_2SO_4), and evaporated to dryness in vacuo to give **10**: yield 2.65 g (76%), mp 172–174 °C (lit.[9] mp 171–172 °C); R_f 0.37 (8:1:1 2-propanol/NH_4OH/H_2O).

Synthesis of (*R*,*S*)-α-Acetamido-*N*-benzyl-substituted-2-furanacetamides (2–4). General Procedure. 4-Methylmorpholine (1 equiv) was added to a solution of **10** (1 equiv) in dry THF (75 mL/10 mmol) at −10 to −15 °C under N_2. After stirring (2 min), isobutyl chloroformate (1 equiv) was added, leading to the precipitation of a white solid. The reaction was allowed to proceed for two additional minutes and then a solution of the substituted benzylamine (1 equiv) in THF (10 mL/10 mmol) was added over 5 min at −10 to −15 °C. The reaction mixture was allowed to stir at room temperature for 5 min and then the 4-methylmorpholine hydrochloride salt was filtered. The organic layer was concentrated in vacuo, the residue was triturated with EtOAc, and the remaining white solid was filtered. Concentration of the EtOAc layer led to additional amounts of the white solid. The desired product was purified by recrystallization or flash chromatography of the combined solid material.

Using this procedure the following compounds were prepared.

(*R*,*S*)-α-Acetamido-*N*-benzyl-2-furanacetamide (2g). Using benzylamine (0.27 g, 2.56 mmol) and racemic **10** (0.47 g, 2.56 mmol) gave 0.46 g (65%) of **2g**. The product was recrystallized from EtOAc to give a white solid: mp 177–178 °C (lit.[9] mp 178–179 °C); R_f 0.30 (2% MeOH/$CHCl_3$); ^1H NMR (DMSO-d_6) δ 1.90 (s, 3 H), 4.31 (d, J = 6.0 Hz, 2 H), 5.58 (d, J = 8.1 Hz, 1 H), 6.27–6.33 (m, 1 H), 6.40–6.44 (m, 1 H), 7.20–7.36 (m, 5 H), 7.60–7.64 (m, 1 H), 8.57 (d, J = 8.1 Hz, 1 H), 8.73 (t, J = 6.0 Hz, 1 H).

(*R*,*S*)-α-Acetamido-*N*-(2-fluorobenzyl)-2-furanacetamide (3a). Using 2-fluorobenzylamine (1.13 g, 9.0 mmol) and racemic

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