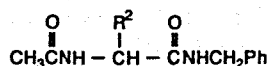
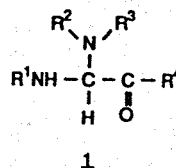


Anticonvulsant Properties of *N*-Substituted α,α -Diamino Acid Derivatives

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Abstract □ Recent studies have demonstrated that functionalized α,α -diamino acids (**1**) display excellent activity when evaluated in the maximal electroshock seizure (MES) test in mice. The synthesis and pharmacological evaluation of 14 select analogues within this series of compounds are detailed. Included in this survey were 10 *N*-acyl derivatives in which the basic C(α) *N*-group in **1** was replaced by a neutral *N*-substituent and four dipeptides where the amino acid fusion point was the α -carbon site. *N*-Acylation of **1** led to decreased anticonvulsant activity. The importance of these findings in relation to the requirements of the C(α) substituent for anticonvulsant activity in **1** are briefly discussed.



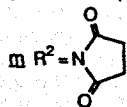
Introduction

Recent studies have shown that α,α -diamino acid derivatives (**1**) and related compounds are surprisingly stable and readily accessible materials.¹⁻⁵ Moreover, we have demonstrated that α -amino, α -hydrazino, and α -*N*-hydroxylamino adducts display excellent anticonvulsant activity when evaluated in the maximal electroshock seizure (MES) test in mice.⁵ For example, the median effective dose (ED₅₀) values after intraperitoneal injection for the α -*N*-ethylamino (**2a**) (42.4 mg/kg) and α -*N*-(benzyloxy-carbonyl)hydrazino (**2b**) (55.6 mg/kg) derivatives approached the ED₅₀ of phenobarbital⁶ (21.8 mg/kg), whereas the ED₅₀s of the α -methoxyamino (**2c**) (6.2 mg/kg) and the α -[(methoxy-methyl)amino] (**2d**) (6.7 mg/kg) adducts exceeded the ED₅₀ of phenytoin⁶ (9.5 mg/kg). Both **2c** and **2d** exhibited these potent anticonvulsant effects at doses much lower than those which produced neuromotor impairment on the horizontal screen (HS) test (46.0 and 50.5 mg/kg were the ED₅₀ doses for **2c** and **2d** on the HS test).⁵ These findings prompted our investigation of the pharmacological activity of the racemic *N*-substituted α,α -diamino acid derivatives (**2e-r**) (Table 1). The *N*-acyl derivatives (**2e-n**) were evaluated to determine the effect of conversion of the basic C(α)-amino group in **2a-d** to a neutral C(α)-carbamate (**2e, 2f**), urea (**2g-2i**), thiourea (**2j, 2k**), amide (**2l, 2n**), or succinimide (**2m**) substituent on anticonvulsant activity. Also included in our study were the unique dipeptides **2o-r**, where the amino acid fusion point was the α -carbon site.

Experimental Section

Chemical Methods—Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra (IR) were run on a 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 (¹³C 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 Me₄Si and coupling constants (*J* values) are in hertz. Low-resolution mass spectra (MS) were recorded at an ionizing voltage of 70 eV from a Varian MAT CH-5 spectrometer at the Lilly Research Laboratories. Microanalyses were provided by the

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|--|---|
| 2a R ² = NHCH ₂ CH ₃ | n R ² = NHC(O)CH ₂ NHC(O)OCH ₂ Ph |
| b R ² = NHNHCO ₂ CH ₂ Ph | o R ² = NHCH ₂ C(O)OCH ₃ |
| c R ² = NH(OCH ₃) | p R ² = NHCH ₂ C(O)OCH ₂ CH ₃ |
| d R ² = N(CH ₃)OCH ₃ | q R ² = NHCH ₂ C(O)OCH ₂ Ph |
| e R ² = NHC(O)OCH ₃ | r R ² = NH ₂ CH ₂ CO ₂ |
| f R ² = NHC(O)OPh | s R ² = NH ₂ |
| g R ² = NHC(O)NHCH ₃ | t R ² = Br |
| h R ² = NHC(O)NHPH | u R ² = N(CH ₃) ₃ , BF ₄ ⁻ |
| i R ² = NHC(O)NHS(O) ₂ Ph | v R ² = NHC(O)CH ₃ |
| j R ² = NHC(S)NHCH ₃ | w R ² = NHC(O)CF ₃ |
| k R ² = NHC(S)NHPH | |
| l R ² = NHC(O)Ph(2'CO ₂ H) | |



Physical Chemistry Department of the Lilly Research Laboratories. All compounds gave satisfactory elemental analyses (C, H, N) that were within $\pm 0.4\%$ of theoretical values. Thin- and thick-layer chromatography were run on precoated silica gel GHLF microscope slides (2.5 \times 10 cm; Analtech No. 21521) or silica gel GHLF (20 \times 20 cm; Analtech 11187).

Chemical Synthesis—General Procedure for the Synthesis of Functionalized Amino Acid Derivatives 2e-k—A tetrahydrofuran (THF) solution containing **2s** and either the acylating agent (1.06–1.10 equiv) and triethylamine (1.20 equiv) or the isocyanate (isothiocyanate) (1.0–1.1 equiv) was heated. The reaction was then filtered to remove any salts formed and purified, and the product was recrystallized if necessary. The reaction temperatures, times, and recrystallization solvents (if appropriate) were as follows: (**2e**) 55–60 °C, 2 h, EtOH; (**2f**) 45–50 °C, 2 h, MeOH; (**2g**) 45–50 °C, 2 h, MeOH; (**2h**) 45–50 °C, 2 h; (**2i**) 50–55 °C, 2 h; (**2j**) 65 °C, 4 h, EtOH; (**2k**) 65 °C, 3 h, EtOH.

Synthesis of *N*-(Acetamido(benzylcarbamoyl)methyl)phtalamic Acid (2l**)**. To a warm pyridine solution (7.0 mL) containing **2s** (0.63 g, 2.83 mmol) was added phthalic anhydride (0.43 g, 2.87 mmol), and the reaction was stirred at 50–55 °C (5 h). Pyridine was removed by distillation *in vacuo* and the residue was treated with H₂O (20 mL). The aqueous mixture was extracted with EtOAc (2 \times 20 mL) and then acidified with aqueous 1 N HCl solution. The white solid (0.70 g, 70%) that precipitated was filtered, washed with H₂O (10 mL), and dried; mp 186–188 °C.

Synthesis of 2-Acetamido-*N*-benzyl-2-(*N*-succinimidyl)acetamide (2m**)**. A cooled (–78 °C) THF solution (150 mL) of **2t**⁷ [prepared from 2-acetamido-*N*-benzyl-2-ethoxyacetamide^{7,8} (2.00 g, 8.0 mmol) and BB₃ (2.51 g, 10.05 mmol)] was added slowly into a cooled (–78 °C) THF

reaction mixture was stirred at -78°C (30 min) and at room temperature (90 min), and then treated with a 10% aqueous citric acid solution (50 mL). The resulting solution was neutralized with a saturated aqueous NaHCO_3 solution, and the reaction mixture extracted with EtOAc (3×100 mL). The combined extracts were dried (Na_2SO_4), and the volatile materials were removed by distillation *in vacuo*. The residue was purified by flash column chromatography on SiO_2 gel (6% $\text{MeOH}/\text{CHCl}_3$) to give 1.10 g (45%) of **2m**; mp $181\text{--}183^{\circ}\text{C}$ (recrystallized from EtOH).

Synthesis of *N*-(Benzyloxy)-*N*-[acetamido(benzylcarbamoyl)methyl]malondiamide (2n). 4-Methylmorpholine (0.35 g, 3.56 mmol) was added to a solution of *N*-CBZ-glycine (0.74 g, 3.55 mmol) in THF (75 mL) at -10 to -15°C . The solution was stirred (5 min), and then isobutyl chloroformate (0.49 g, 3.55 mmol) was added and the mixture was stirred for an additional 20 min. A cooled (-10°C) solution of **2s** (0.79 g, 3.55 mmol) in THF (125 mL) was then added slowly (30 min). The reaction mixture was stirred at this temperature (2 h) and then at room temperature (2 h). The insoluble materials were filtered, and the filtrate was concentrated *in vacuo*. The residue was triturated with EtOAc (20 mL) and the white solid (0.60 g) that remained was filtered, washed with H_2O , and dried to give **2n**. The initial insoluble material on trituration with H_2O gave an additional 0.40 g of **2n** to give a combined yield of 1.00 g (63%); mp $177\text{--}179^{\circ}\text{C}$ (recrystallized from EtOH).

Synthesis of Methyl *N*-[Acetamido(benzylcarbamoyl)methyl]glycinate (2o). A methanolic solution (50 mL) containing **2n**⁵ (1.00 g, 2.85 mmol) and methyl glycinate (prepared from methyl glycinate hydrochloride (1.01 g, 8.55 mmol), and NaOMe (0.38 g, 7.10 mmol)) was heated to reflux (2 h). The reaction was concentrated *in vacuo* to give an oily residue that was purified by flash column chromatography on SiO_2 gel (3% $\text{MeOH}/\text{CHCl}_3$) to give 0.60 g (72%) of **2o**; mp $144\text{--}146^{\circ}\text{C}$ (recrystallized from EtOAc).

Synthesis of Ethyl *N*-[Acetamido(benzylcarbamoyl)methyl]glycinate (2p). A methanolic solution (70 mL) containing **2n**⁵ (1.50 g, 4.28 mmol) and ethyl glycinate [prepared from ethyl glycinate hydrochloride (3.10 g, 22.2 mmol) and NaOMe (1.17 g, 21.74 mmol)] was heated to reflux (2 h). The reaction was concentrated *in vacuo* to give an oily residue that was purified by flash column chromatography on SiO_2 gel (5% $\text{MeOH}/\text{CHCl}_3$) to give 0.60 g (46%) of **2p**; mp $125\text{--}127^{\circ}\text{C}$ (recrystallized from EtOAc).

Synthesis of Benzyl *N*-[Acetamido(benzylcarbamoyl)methyl]glycinate (2q). A suspension of benzyl glycinate hydrochloride (5.00 g, 24.8 mmol) in THF (400 mL) containing Et_3N (4.90 g, 48.5 mmol) was stirred (4 h) at room temperature. The reaction mixture was cooled (-78°C) and then a cooled (-78°C) THF solution (150 mL) of **2t**⁵ [prepared from 2-acetamido-*N*-benzyl-2-ethoxyacetamide^{7,8} (4.00 g, 16.0 mmol) and BBr_3 (1 M in CH_2Cl_2 , 20.0 mL, 20.0 mmol)] was added (30 min). The reaction mixture was stirred at -78°C (30 min) and then at room temperature (16 h). The insoluble materials were filtered, the filtrate was concentrated *in vacuo*, and the residue was purified by flash column chromatography on SiO_2 gel (3% $\text{MeOH}/\text{CHCl}_3$) to give 1.56 g (26%) of **2q** as a white solid; mp $133\text{--}135^{\circ}\text{C}$ (recrystallized from EtOH).

Synthesis of *N*-[Acetamido(benzylcarbamoyl)methyl]glycine (2r). A solution of methyl *N*-[acetamido(benzylcarbamoyl)methyl]glycinate (**2o**) (0.60 g, 2.05 mmol) and KOH (0.30 g, 5.36 mmol) in 90% aqueous EtOH (50 mL) was stirred at room temperature (48 h). The volatile materials were then removed *in vacuo*, and the residue dissolved in H_2O (10 mL). The aqueous solution was extracted with EtOAc (2×20 mL), and the aqueous layer was acidified to pH ~ 2.0 with aqueous 1 N HCl . A column containing ion-exchange resin Dowex 50X W4 was prepared using 10% aqueous pyridine. The column was thoroughly washed with H_2O . The acidic aqueous reaction solution was added to the top of the column, and the column was eluted with H_2O (300 mL or until the eluate was neutral). The column was then eluted with 10% aqueous pyridine (400 mL). The aqueous pyridine fraction was concentrated *in vacuo* to give a white solid, dried *in vacuo* and then triturated with absolute EtOH (7 mL). The insoluble materials that remained were filtered and dried to give 0.29 g (50%) of **2r**; mp $124\text{--}126^{\circ}\text{C}$ dec.

Pharmacological Evaluation—All tests were performed with male CF-1 mice from Charles River Breeding Laboratories (Portage, MI). All compounds were dissolved in 30% poly(ethylene glycol) 400 and administered in an injection volume of 1 mL/100 g of body weight. Initial anticonvulsant evaluation of **2e**–**r** was conducted with two dose levels (30, 100 mg/kg) administered intraperitoneally. Four mice at each dose level were tested at 0.5, 1, and 4 h after administration, unless otherwise

Table 1—Physical and Pharmacological Data in Mice for *N*-Substituted α,α -Diamino Acid Derivatives^a

$$\text{CH}_3\text{CNH} - \overset{\text{O}}{\parallel} \text{C} - \overset{\text{R}^2}{\text{CH}} - \overset{\text{O}}{\parallel} \text{C} - \text{NHCH}_2\text{Ph}$$

| No. | R ² | mp ^b | MES ^c ED ₅₀ |
|----------------------------|---|-----------------|-----------------------------------|
| 2e | NHC(O)OCH ₃ | 202–204 | 48.0 (37.7–56.7) |
| 2f | NHC(O)OPh | 201–203 | > 100 |
| 2g | NHC(O)NHCH ₃ | 229–230 | > 100 |
| 2h | NHC(O)NHPh | 242–244 | > 100 |
| 2i | NHC(O)NHS(O) ₂ Ph | 188–191 | > 100 |
| 2j | NHC(S)NHCH ₃ | 162–163 | > 100 ^d |
| 2k | NHC(S)NHPh | 196–197 | > 100 ^d |
| 2l | NHC(O)Ph(2'-CO ₂ H) | 186–188 | > 100 |
| 2m | NC(O)CH ₂ CH ₂ C(O) | 181–183 | > 100 |
| 2n | NHC(O)CH ₂ NHC(O)OCH ₂ Ph | 177–179 | $\sim 30^e$ |
| 2o | NHCH ₂ C(O)OCH ₃ | 144–146 | > 100 |
| 2p | NHCH ₂ C(O)OCH ₂ CH ₃ | 125–127 | > 100 |
| 2q | NHCH ₂ C(O)OCH ₂ Ph | 133–135 | > 100 |
| 2r | ⁺ NH ₂ CH ₂ CO ₂ ⁻ | 124–126 | > 100 ^d |
| phenytoin ^f | | | 9.5 (8.1–10.4) |
| phenobarbital ^f | | | 21.8 (15.0–22.5) |
| valproate ^f | | | 272 (247–338) |

^a The compounds were administered intraperitoneally. ED₅₀ values are in milligrams per kilogram. Numbers in parentheses are 95% confidence intervals. A dose–response curve was generated for all compounds that displayed sufficient activity. The dose–effect data for these compounds were obtained at 0.5 h (“time of peak effect”).

^b Melting points ($^{\circ}\text{C}$) are uncorrected. ^c MES = maximal electroshock seizure test. All compounds were suspended in 30% PEG. ^d The compound was tested only at 0.5 and 1 h due to insufficient supply of sample. ^e Unable to definitively define an ED₅₀ value and confidence limits because of insufficient supply of sample. ^f Reference 6.

MES seizures were elicited by electrical current (ac, 60 cps, 50 mA, 0.2 s) applied via corneal electrodes. A drop of 0.9% saline was instilled on each eye prior to application of the electrodes to ensure electrical contact. Abolition of the hind limb tonic extension component of the seizure was defined as protection in the MES test. This is the identical protocol used by the Antiepileptic Drug Development Program of the Epilepsy Branch of NINCDS, NIH.^{6,9}

After the time of peak anticonvulsant activity and the approximate dose range were determined, a dose–response curve was generated at the time of peak activity with at least three or four doses and 10–12 mice per dose. The MES ED₅₀ is the calculated dose required to protect 50% of the mice in the MES test. For those compounds with significant anticonvulsant activity, the doses that caused neuromotor impairment on the horizontal screen (HS) test also were determined.¹⁰ Previously trained mice were dosed with the compound and then placed individually on top of a square (13 \times 13 cm) wire screen which was mounted on a vertical rod. The rod was rotated 180 $^{\circ}$, and the number of mice that returned to the top of the screen in one minute were counted.

Results and Discussion

Chemistry—Several preparative routes were utilized for the construction of the targeted compounds. In most cases, 2-acetamido-*N*-benzyl-2-aminoacetamide⁵ (**2s**) served as the starting material. Treatment of **2s** with the appropriate chloroformate, isocyanate, isothiocyanate, or anhydride, or use of the mixed anhydride protocol advanced for peptide synthesis,¹¹ led to the

