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

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i.

 $k R^2 = NHC(S)NHPh$

 $I = R^2 = NHC(O)Ph(2'CO_2H)$

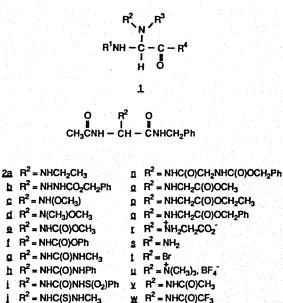
Abstract
Recent studies have demonstrated that functionalized α, α -diamino acids (1) display excellent activity when evaluated in the maximal electroshock seizure (MES) test in mice. The synthesis and pharmacological evaluation of 14 select analogues within this series of compounds are detailed. Included in this survey were 10 N-acyl derivatives in which the basic $C(\alpha)$ 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 $C(\alpha)$ 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 -N²-(benzyloxycarbonyl)hydrazino (2b) (55.6 mg/kg) derivatives approached the ED_{50} of phenobarbital⁶ (21.8 mg/kg), whereas the ED_{50} s of the α -methoxyamino (2c) (6.2 mg/kg) and the α -[(methoxymethyl)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-acvl derivatives (2e-n) were evaluated to determine the effect of conversion of the basic $C(\alpha)$ -amino group in 2a-d to a neutral $C(\alpha)$ -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 20-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 (8) 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



Physical Chemistry Department of the Lilly Research Laboratories. All compounds gave satisfactory elemental analyses (C, H, N) that were within ±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 × 20 cm; Analtech 11187).

Chemical Synthesis-General Procedure for the Synthesis of Functionalized Amino Acid Derivatives 2e-k-A tetrahydrofuran (THF) solution containing 2s⁵ 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, 22 h; (2j) 65 °C, 4 h, EtOH; (2k) 65 °C, 3 h, EtOH.

Synthesis of N-[Acetamido(benzylcarbamoyl)methyl]phthalamic Acid (21). 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 (2×20 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.

Synthsis of 2-Acetamido-N-benzyl-2-(N-succinimidyl)acetamide (2m). A cooled (-78 °C) THF solution (150 mL) of 2t⁵ [prepared from 2-acetamido-N-benzyl-2-ethoxyacetamide^{7,8} (2.00 g, 8.0 mmol) and BBrz (951 o 1005 mmal)] was added slowly into a cooled (-78 °C) THE

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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 NaHCO₃ solution, and the reaction mixture extracted with EtOAc (3 × 100 mL). The combined extracts were dried (Na₂SO₄), and the volatile materials were removed by distillation *in vacuo*. The residue was purified by flash column chromatography on SiO₂ gel (6% MeOH/CHCl₃) to give 1.10 g (45%) of **2m**; mp 181–183 °C (recrystallized from EtOH).

Synthesis of N-(Benzyloxy)-N-[acetamido(benzylcarbamoy])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 H₂O, and dried to give 2n. The initial insoluble material on trituration with H₂O gave an additional 0.40 g of 2n to give a combined yield of 1.00 g (68%); mp 177-179 °C (recrystallized from EtOH).

Synthesis of Methyl N-[Acetamido(benzylcarbamoyl)methyl]glycinate (20). A methanolic solution (50 mL) containing $2u^5$ (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 SiO₂ gel (3% MeOH/CHCl₃) to give 0.60 g (72%) of 20; mp 144-146 °C (recrystallized from EtOAc).

Synthesis of Ethyl N-[Acetamido(benzylcarbamoyl)methyl]glycinate (2p). A methanolic solution (70 mL) containing $2u^5$ (1.50 g, 4.28 mmol) and ethyl glycinate [prepared from ethyl glycinate hydrochloride (3.10g, 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 SiO₂ gel (5% MeOH/CHCl₃) to give 0.60 g (46%) of **2p**; mp 125–127 °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 Et₃N (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 2t⁵ [prepared from 2-acetamido-N-benzyl-2-ethoxyacetamide^{7.8} (4.00 g, 16.0 mmol) and BBr₃ (1 M in CH₂Cl₂, 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 chromatrgraphy on SiO₂ gel (3% MeOH/CHCl₃) to give 1.56 g (26%) of 2q as a white solid; mp 133-135 °C (recrystallized from EtOH).

Synthesis of N-[Acetamido(benzylcarbamoyl)methyl]glycine (2r). A solution of methyl N-[acetamido(benzylcarbamoyl)methyl]glycinate (20) (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 H₂O (10 mL). The aqueous solution was extracted with EtOAc ($2 \times$ 20 mL), and the aqueous layer was acidified to pH \sim 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 H₂O. The acidic aqueous reaction solution was added to the top of the column, and the column was eluted with H₂O (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 124-126 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. Initia 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 Derivatives⁴

О R² О II I II CH₃CNH — CH — CNHCH₂Ph

No.	as a the R² and a second	mp ^b	MES ^c ED ₅₀	
2e	NHC(O)OCH ₃	202-204	48.0	
			(37.7-56.7)	
21	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(O2)Ph	188191	>100	
2]	NHC(S)NHCH ₃	162-163	>100d	
2k	NHC(S)NHPh	196-197	>100 ^d	
21	NHC(O)Ph(2'-CO ₂ H)	186-188	>100	
2m	NC(0)CH ₂ CH ₂ C(0)	181–183	>100	
2n	NHC(O)CH2NHC(O)OCH2Ph	177-179	~30°	
20	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	
phenytoin ^r			9.5	
			(8.1–10.4)	
phenobarbital		n ta sata di General	21.8	
valproate ^r			(15.0–22.5) 272	
and a second			(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 (°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 Antiepilepitc Drug Development Program of the Epilepsy Branch of NINCDS, NIH.⁶⁹

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 × 13 cm) wire screen which was mounted on a vertical rod. The rod was rotated 180°, 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 Table 2—Key Spectral Properties of N-Substituted α, α -Diamino Acid Derivatives

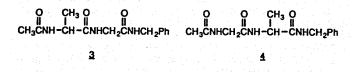
	IRª	¹ H NMR ^b		¹³ C NMR ^c		
No.		Сан	CH₂Ph	C _α H		MS₫
2e	1650	5.56 (t, 7.8)	4.27 (d, 5.6)	58.57	@	279
2f	1630, 1700	5.66 (t, 7.6)	4.29-4.35 (m)	58.69	° <u> </u>	341
2g	1630	5.59 (t, 7.8)	4.26 (d, 5.8)	57.92	157.30	279
2h	1600 (br)	5.67 (t, 7.6)	4.30 (d, 5.9)	57.59	153.98	340
21	1630 (br)	5.47 (t, 7.7)	4.24 (d, 5.7)	57.14	150.36	405
2]	1620	6.10 (br, s)	4.27 (d, 5.8)	61.33	 g i	294
2k	1620	5.24 (t, 6.9)	4.32 (d, 5.8)	61.18	180.02	356
21	1620 (br)	5.92 (t, 7.2)	4.36 (d, 6.0)	57.44	. <u></u>	370
2m	1620 (br)	6.31 (d, 9.0)	4.23-4.36 (m)	55.19	176.33	304
2n	1640 (br)	5.79 (t, 7.7)	4.28 (d, 5.8)	56.77	167.86	413
20	1610, 1710	5.00 (t, 7.8)	4.28 (d, 6.0)	63.98	46.09	294
2p	1600, 1710	5.01 (t. 8.2)	4.28 (d, 5.8)	63.96	46.22	342
2q	1620, 1710	5.02 (t, 8.2)	4.27 (d. 6.1)	63.94	46.22	370
2r	1630	4.98 (d, 8.2)	• • •	64.08	47.48	

Infrared spectra were taken with KBr discs and values are reported. in cm⁻¹. ^b All spectra were recorded with DMSO-d₆. The number in each entry is the chemical shift value (δ) observed in ppm relative to DMSO-d₆, followed by the multiplicity of the signal and the coupling constant in hertz. ¹H NMR spectra were recorded at 300 MHz. ^{c 13}C NMR spectra were obtained at 75 MHz and the values referenced to DMSO-d₈. ^d All spectra were recorded using FD-MS unless otherwise indicated. Molecular ions reported refer to either M⁺ or M⁺ + 1 ions. * The carbamate carbonyl carbon resonance was not detected. The attached NHC(0)OCH₃ signal was observed at δ 51.46. ¹ The carbamate carbonyl carbon resonance was not detected. The attached NHC(0)C₆H₅ signals were observed at δ 121.70, 125.18, 129.30, and 150.91. 9 The thiocarbonyl carbon resonance was not detected. The attached NHC(S)NHCH₃ signal was observed at δ 30.92. ^ The amide carbonyl resonance could not be readily assigned. Signals corresponding to four carbonyl peaks were observed at δ 167.85, 167.93, 168.48, and 169.47. Value refers to M⁺ + 1 ion observed by FAB-MS. / Spectrum was not taken.

employed as the immediate precursor for 2m and 2q, while 2-acetamido-N-benzyl-2-(trimethylammonio)acetamide tetrafluoroborate⁵ (2u) was utilized for the synthesis of 2o and 2p. Finally, alkaline hydrolysis of 2o, followed by neutralization of the dipeptide by passage through an ion-exchange resin yielded 2r. Key spectral data (i.e., IR, ¹H NMR, ¹³C NMR, MS) observed for 2e-r are recorded in Table 2 and were consistent with both the proposed structures and previously reported trends.⁵

Pharmacological Evaluation—The N-substituted α, α -diamino acid derivatives 2e-r were tested for anticonvulsant activity by using the procedures described by Krall et al.,9 which have become standard testing procedures for the Antiepileptic Drug Development Program of the Epilepsy Branch of the National Institutes of Health. All compounds were administered intraperitoneally (ip) to mice. Table 1 lists the median effective dose (ED₅₀) values required to prevent seizures in the MES test by racemic 2. Inspection of the results revealed several important observations. First, most of the N-acyl substituted derivatives (2e-n) evaluated were inactive in the MES test at doses of 100 mg/kg or less, and all were significantly less potent than 2c and 2d. Only 2e and 2n displayed noticeable activity. One noteworthy difference about the anticonvulsant effect of 2n is that it also produced neuromotor impairment on the HS test at the same doses that blocked MES seizures. Thus, this anticonvulsant effect is analitatively different than that previously seen with

derivatives 2e-n, unlike 2a-d, are not protonated at physiological pH values, thereby demonstrating that replacement of the basic $C(\alpha)$ -amino group in functionalized amino acids 2a-d by the neutral $C(\alpha)$ N-substituents in 2e-n did not lead to improved anticonvulsant activity. This observation was consistent with results previously obtained for two other N-acyl derivatives, 2,2-diacetamido-N-benzylacetamide (2w) and 2-acetamido-N-benzyl-2-(trifluoroacetamido)acetamide (2w).⁵ Both 2v and 2w did not prevent maximal electroshock seizures at doses of 100 mg or less. Of the two active N-acyl derivatives, 2n can also be viewed as a functionalized dipeptide as well as an N-acyl α, α -diamino acid adduct. Previously, we prepared and evaluated functionalized dipeptides 3 and 4.¹¹ These compounds differed



from 20-r in that the two amino acid units were joined through an amide bond. Both 3 and 4 were inactive in both the maximal electroshock and subcutaneous pentylenetetrazole seizure tests^{6,9} when administered to mice at dose levels up to and including 600 mg/kg.^{12}

Conclusions

The composite data indicated that most structural modifications at the α -amino site in functionalized α, α -diamino acids led to a decrease in the anticonvulsant activity after intraperitoneal administration to mice when compared to the simple *N*-ethylamino adduct 2a,⁵ while none of the compounds approached the superior activity observed for the *N*-hydroxylamino derivatives 2c and 2d.⁵ These results documented that excellent protection against MES-induced seizures by 1 can be achieved by incorporation of a basic C(α)-amino substituent.

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