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Study of the Structural Requirements for Dopa Potentiation and Oxotremorine Antagonism by L-Prolyl-L-leucylglycinamide

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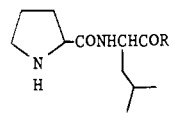
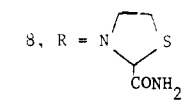
A number of analogues of the tripeptide L-prolyl-L-leucylglycinamide (1) were synthesized and evaluated in the Dopa potentiation and oxotremorine antagonism tests. The replacement of the glycinamide residue with either the glycine methylamide, glycine, aminoacetonitrile, amino-2-propanone, semicarbazide, or β-alaninamide residues resulted in a loss of activity in both tests. A 1:1 mixture of L-prolyl-L-leucyl-(−)-thiazolidine-2-carboxamide (8) and L-prolyl-L-leucyl-(+)-thiazolidine-2-carboxamide (9) showed marked activity in the Dopa potentiation test but was unable to antagonize the tremors induced by oxotremorine. L-Prolyl-L-leucyl-L-prolinamide (11), on the other hand, was active in the oxotremorine antagonism test but inactive in the Dopa potentiation test. The replacement of the pyrrolidine ring of 1 with either a thiazolidine or cyclopentane ring system caused a loss of activity. The cyclopentanecarboxylic acid analogue 13, however, was found to have moderate activity in the serotonin potentiation test.

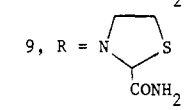
Several of the hypothalamic releasing and release-inhibiting hormones have recently been shown to have effects on the central nervous system which are independent of their endocrine effects.[2] Among these polypeptides is L-prolyl-L-leucylglycinamide (1). This tripeptide has been



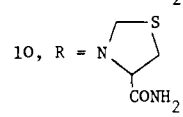
1, R = $NHCH_2CONH_2$
2, R = $NHCH_2CONHCH_3$
3, R = $NHCH_2COOH$
4, R = $NHCH_2CN$
5, R = $NHCH_2COCH_3$
6, R = $NHNHCONH_2$
7, R = $NHCH_2CH_2CONH_2$



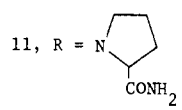
8, R = (−)-isomer



9, R = (+)-isomer



10, R =



11, R =

postulated to be the hypothalamic hormone that inhibits the release of melanocyte-stimulating hormone from the anterior pituitary gland.[3-6] The extra-endocrine effects of 1 were first demonstrated by Plotnikoff et al.[7] with Everett's Dopa potentiation test.[8] In this test 1 was found to potentiate the behavioral effects of L-3,4-dihydroxyphenylalanine (L-Dopa) in both normal and hypophysectomized mice. Subsequent studies with 1 have also shown that this tripeptide antagonizes the central and peripheral effects of oxotremorine,[9,10] reverses the sedative effects of deserpidine,[11] potentiates the behavioral effects of apomorphine,[12] attenuates puromycin-induced amnesia,[13] and facilitates the development of morphine dependence.[14] Moreover, a number of preliminary clinical studies have indicated that 1 may be of potential value in the treatment of Parkinson's disease.[15,16]

In an effort to determine the structural features of 1 that enable this tripeptide to potentiate the behavioral effects of L-Dopa and antagonize the tremors induced by oxotremorine, we have synthesized several analogues of 1 in which the prolyl and glycinamide residues have been modified. In order to determine the importance of the primary carboxamide moiety, the glycinamide residue has been replaced with the glycine methylamide, glycine, aminoacetonitrile, and amino-2-propanone residues, analogues 2–5, respectively. In the case of analogue 6 an imino group has been substituted for the methylene group while in compound 7 an additional methylene group has been inserted into the peptide chain. In addition to these modifications, several analogues (8–11) have been synthesized where either a thiazolidine or pyrrolidine ring system has been incorporated into the glycinamide portion of the molecule.

The prolyl residue has been modified in an attempt to determine the importance of the pyrrolidine ring system. Castensson et al.[10] have reported that the replacement of the prolyl residue with a pyroglutamyl residue provides a compound (14) which is an even better antagonist of oxotremorine-induced tremors than 1. We have thus synthesized compounds 12 and 13 whereby the pyrrolidine ring has been replaced by a thiazolidine and cyclopentane ring system, respectively.

Results and Discussion

Chemistry. The protected dipeptide Z-Pro-Leu-OH (15), which was prepared in a manner similar to that previously described by Cash,[17] served as the key intermediate in the synthesis of 2–11. Aminoacetonitrile, semicarbazide, β-alaninamide, L-prolinamide, and methyl

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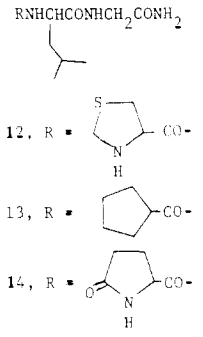

glycinate were coupled to **15** using the mixed anhydride method.[18,19] The benzyloxycarbonyl-protecting group was removed from Z-Pro-Leu-$NHCH_2CN$ (**19**), Z-Pro-Leu-$NHNHCONH_2$ (**22**), Z-Pro-Leu-β-Ala-NH_2 (**23**), and Z-Pro-Leu-Pro-NH_2 (**27**) by hydrogenolysis to give directly analogues **4**, **6**, **7**, and **11**. The protected ester Z-Pro-Leu-Gly-OCH_3 (**16**), on the other hand, was treated with either methylamine or aqueous sodium hydroxide to give Z-Pro-Leu-Gly-$NHCH_3$ (**17**) and Z-Pro-Leu-Gly-OH (**18**), respectively. Removal of the benzyloxycarbonyl group by hydrogenolysis provided **2** and **3**.

Amino-2-propanone semicarbazone was obtained from glycine by way of the Dakin–West reaction[20] and coupled to **15** via the mixed anhydride method to give Z-Pro-Leu-$NHCH_2C(CH_3)$=$NNHCONH_2$ (**20**). Treatment of **20** with aqueous hydrochloric acid afforded Z-Pro-Leu-$NHCH_2COCH_3$ (**21**) which was subsequently converted to **5** with hydrobromic acid in acetic acid.

The synthesis of diastereoisomers **8** and **9** required the prior synthesis of the thiazolidine amino acid residue. Thus, 2-aminoethanethiol was condensed with glyoxylic acid to give racemic thiazolidine-2-carboxylic acid (**24**). Treatment of **24** with thionyl chloride and ethanol yielded racemic ethyl thiazolidine-2-carboxylate (**25**) which was easily resolved using the *d*- and *l*-tartaric acids. Previous attempts by us to resolve the primary amide of **24** with the tartaric and di-*p*-toluoyltartaric acids proved unsuccessful. Both (−)- and (+)-**25** were coupled to **15** using *N*,*N*-dicyclohexylcarbodiimide. The resulting tripeptide esters were converted to primary amides and then decarbobenzoxylated with hydrobromic acid to give **8** and **9**, respectively. The isomeric thiazolidine analogue **10** was prepared from L-thiazolidine-4-carboxamide (**26**) using similar coupling and deprotection procedures employed for the synthesis of **8** and **9**.

In the synthesis of **12**, methyl L-leucylglycinate was coupled to *N*-*tert*-butyloxycarbonyl-L-thiazolidine-4-carboxylic acid by the mixed anhydride method. After the resulting ester was converted to a primary amide with ammonia, the *tert*-butyloxycarbonyl protecting group was removed with hydrobromic acid in acetic acid to give **12**. Compound **13**, on the other hand, was prepared by reacting the *N*-hydroxysuccinimide ester of cyclopentanecarboxylic acid with L-leucylglycinamide.

Pharmacology. Compounds **2**–**13** were evaluated in the Dopa potentiation[8] and oxotremorine antagonism tests.[9,21] The results of these tests are depicted in Tables I and II, respectively. They indicate that there are strict structural requirements for the ability of **1** to potentiate the behavioral effects of L-Dopa and antagonize the central and peripheral effects of oxotremorine. In particular, the primary carboxamide moiety appears to be necessary for the activity of **1** since the replacement of this group with the $-CONHCH_3$, $-COOH$, $-CN$, and $-COCH_3$ moieties, analogues **2**–**5**, respectively, results in a loss of activity in both the Dopa potentiation test and the oxotremorine antagonism test. These results suggest that the carboxamide group is important for the interaction of **1** with its receptor. The results also show that the substitution of a semicarbazide or β-alaninamide residue for the glycinamide residue, as was done in compounds **6** and **7**, also results in a loss of pharmacological activity.

Table I. Activity of L-Prolyl-L-leucylglycinamide (**1**) and Its Analogues in the Dopa Potentiation Test[a]

Compd	Dose, mg/kg ip	Rating	Compd	Dose, mg/kg ip	Rating
1	0.1	3	**8**	2.5	1
	1	3		10	2
	10	3	**9**	10	1
2	10	1	**8** + **9**[b]	0.625	1
3	10	1		2.5	2
4	2.5	1		10	3
	10	2	**10**	10	1
5	2.5	2	**11**	10	1
	10	2	**12**	10	1
6	10	1	**13**	2.5	1
7	10	1		10	2

[a] See Experimental Section for a description of the test and ratings. [b] Ratio of **8** and **9** is 1:1.

Table II. Activity of L-Prolyl-L-leucylglycinamide (**1**) and Its Analogues in the Oxotremorine Antagonism Test[a]

Compd	Dose, mg/kg ip	Rating	Compd	Dose, mg/kg ip	Rating
1	1	22	**8**	20	19
	4	12	**9**	20	22
	20	7	**8** + **9**[b]	20	22
2	20	22	**10**	20	22
3	20	20	**11**	10	22
4	20	22		20	14
5	20	22	**12**	10	22
6	20	22		20	17
7	20	19	**13**	20	22

[a] See Experimental Section for a description of the test and ratings. [b] Ratio of **8** and **9** is 1:1.

In the case of the thiazolidine-2-carboxamide analogues, diastereoisomer **8** was found to be moderately active in the Dopa potentiation test at a dose of 10 mg/kg while **9** was completely inactive. Interestingly, a 1:1 mixture of **8** and **9** markedly potentiated the behavioral effects of L-Dopa. The reason for this surprising result is not known although a possible explanation may be that the inactive diastereoisomer inhibits the metabolism of the active one. Neither of the diastereoisomers nor the mixture antagonized the effects of oxotremorine, however. In contrast, the isomeric thiazolidine analogue **10** was inactive in both tests while the prolinamide analogue, compound **11**, was found to be capable of antagonizing the effects of oxotremorine but incapable of potentiating the behavioral effects of L-Dopa. The observed differences in activity between the thiazolidine-2-carboxamide and prolinamide analogues suggest that the mechanism of action of these analogues is different.

Although Castensson et al.[10,22] have shown that the replacement of the prolyl residue with the pyroglutamyl residue yields a compound with enhanced activity in the oxotremorine antagonism test, our results indicate that the pyrrolidine ring cannot be replaced by the larger thiazolidine ring system or by the cyclopentane ring system without losing biological activity. Surprisingly, the cyclopentanecarboxylic acid analogue **13** at a dose of 10 mg/kg was found by us to have moderate activity in the serotonin potentiation test.[23] Although thyrotropin releasing hormone (<Glu-His-Pro-NH_2) has previously been shown to be active in the serotonin potentiation test, **1** has

been found to be inactive.[24] None of the other compounds prepared in this study were found to have serotonin potentiating activity.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt apparatus and on a Mettler FP2-FP51 system. Specific rotations were measured with a Perkin-Elmer 141 polarimeter. Microanalyses were performed either on a Hewlett-Packard 185B C, H, N analyzer, University of Kansas, by Midwest Microlab Inc., Indianapolis, Ind., or by the Australian Microanalytical Service, University of Melbourne, Melbourne, Australia. Where analyses are indicated by symbols of the elements, analytical results obtained were within ±0.4% of the theoretical values. IR spectra were recorded on either a Beckman IR-33 or a Perkin-Elmer 257 spectrophotometer. NMR spectra were recorded on either a Perkin-Elmer R-12 spectrophotometer or Varian Associates T-60 and EM-360 spectrophotometers with either Me_4Si or sodium trimethylsilylpropanesulfonate as the internal standards. TLC was carried out on Merck 0.25-mm silica gel 60 F-254 plates.

***N*-Benzyloxycarbonyl-L-prolyl-L-leucine (15).** The method of Cash[17] was modified in the following manner. After the THF was removed in vacuo from the acidified reaction mixture, the aqueous mixture remaining was extracted with chloroform. The chloroform extract was washed with water and dried over Na_2SO_4. Removal of the chloroform in vacuo gave a viscous oil which when dissolved in ether and allowed to stand at room temperature afforded crystalline product: mp 136.5–137 °C; $[\alpha]^{24}_D$ –65.2° (*c* 0.8, MeOH) [lit.[17] mp 138.5–139.5 °C; $[\alpha]^{22}_D$ –63.0° (*c* 5, MeOH)].

***N*-Benzyloxycarbonyl-L-prolyl-L-leucylglycine Methylamide (17).** Methyl *N*-benzyloxycarbonyl-L-prolyl-L-leucylglycinate[25] (**16**, 3.5 g, 8 mmol) was dissolved in 30 mL of ethanol saturated with CH_3NH_2. The solution was stirred at room temperature for 24 h and then stripped of solvent and excess CH_3NH_2 under reduced pressure to give a colorless oil. Trituration in Et_2O afforded 3.4 g (98%) of the methylamide: mp 148–150 °C. An analytical sample was obtained by recrystallizing a portion of this material from EtOAc–acetone: mp 149–151 °C; $[\alpha]^{22}_D$ –60.5° (*c* 0.5, MeOH). Anal. ($C_{22}H_{32}N_4O_5$) C, H, N.

L-Prolyl-L-leucylglycine Methylamide (2). A solution of **17** (2.0 g, 4.6 mmol) in 65 mL of MeOH was added to a flask containing 400 mg of 5% Pd/C and hydrogen was bubbled into the reaction mixture for 30 min. The mixture was filtered and the filtrate stripped of solvent in vacuo to yield 1.3 g of crude product as a colorless oil. This material was chromatographed on a silica gel dry column (Woelm activity III, 75 × 2.5 cm) with MeOH as the developing solvent. The portion of the column between the R_f values of 0.4 and 0.2 was isolated and extracted with $CHCl_3$. Evaporation of the $CHCl_3$ yielded 700 mg (51%) of **2** as a hygroscopic solid: mp 63–66 °C; $[\alpha]^{27}_D$ –37.6° (*c* 0.9, MeOH). Anal. ($C_{14}H_{26}N_4O_3 \cdot H_2O$) C, H, N.

The oxalate salt of **2** provided a suitable nonhygroscopic derivative and was prepared by treating a solution of oxalic acid (40 mg, 0.44 mmol) in THF with a solution of **2** (100 mg, 0.34 mmol) in THF. Recrystallization of the resulting precipitate from isopropyl alcohol yielded 100 mg (76%) of the oxalate salt: mp 168–169.5 °C; $[\alpha]^{22}_D$ –40.2° (*c* 0.54, H_2O). Anal. ($C_{16}H_{28}N_4O_7$) C, H, N.

L-Prolyl-L-leucylglycine (3). *N*-Benzyloxycarbonyl-L-prolyl-L-leucylglycine[26] (**18**, 700 mg, 1.67 mmol) was dissolved in MeOH (40 mL). This solution was added to a suspension of 500 mg of 5% Pd/C in 5% AcOH (10 mL). Hydrogen was bubbled into the reaction mixture until TLC analysis revealed no starting material remaining (5 h). The reaction mixture was filtered and the filtrate evaporated to dryness under reduced pressure to give a white solid. Recrystallization of this material from a mixture of isopropyl alcohol and H_2O afforded 360 mg (76%) of **3**: mp 222–222.5 °C; $[\alpha]^{20}_D$ –73° (*c* 0.2, H_2O). Anal. ($C_{13}H_{23}N_3O_4$) C, H, N.

***N*-Benzyloxycarbonyl-L-prolyl-L-leucylaminoacetonitrile (19).** The mixed anhydride prepared from **15** (15 g, 41 mmol), NEt_3 (4.15 g, 41 mmol), and isobutyl chloroformate (5.65 g, 41 mmol) was treated with a solution of aminoacetonitrile hydrochloride (3.8 g, 41 mmol) and NEt_3 (4.15 g, 41 mmol) in H_2O (30 mL). The reaction mixture was stirred at room temperature for 2 h, acidified with concentrated HCl, stripped of THF in vacuo, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with 5% $NaHCO_3$ and dried ($MgSO_4$). The $CHCl_3$ was evaporated to give a colorless oil. An ethereal solution of this material on standing at room temperature provided 12.5 g (76%) of crystalline product: mp 120–122 °C. An analytical sample was obtained by recrystallizing a portion of this material from $CHCl_3$–Et_2O: mp 121–122 °C; $[\alpha]^{24}_D$ –86.0° (*c* 0.5, $CHCl_3$). Anal. ($C_{21}H_{28}N_4O_4$) C, H, N.

L-Prolyl-L-leucylaminoacetonitrile (4). A solution of **19** (8 g, 20 mmol) and AcOH (1.2 g, 20 mmol) in MeOH (75 mL) was hydrogenated for 2 h over 1.5 g of 5% Pd/C. The mixture was filtered and the filtrate evaporated to dryness. The residue was partitioned between $CHCl_3$ and 10% HCl. The aqueous layer was made basic with 2 N NaOH and extracted with $CHCl_3$ (4 × 20 mL). The combined $CHCl_3$ extracts were dried (Na_2SO_4) and the solvent was evaporated to give the desired product as a yellow oil. Trituration in Et_2O yielded 3.2 g (60%) of crystalline nitrile: mp 95–100 °C. Recrystallization of this material from $CHCl_3$–Et_2O provided 2.4 g of pure product: mp 101–103 °C; $[\alpha]^{20}_D$ –125.8° (*c* 1.24, $CHCl_3$). Anal. ($C_{13}H_{22}N_4O_2$) C, H, N.

***N*-Benzyloxycarbonyl-L-prolyl-L-leucylamino-2-propanone Semicarbazone (20).** A solution of amino-2-propanone semicarbazone hydrochloride[20] (1.7 g, 11 mmol) and NEt_3 (1.1 g, 11 mmol) in H_2O (25 mL) was added to the mixed anhydride prepared in THF from **15** (3.6 g, 10 mmol), *N*-methylmorpholine (1.0 g, 10 mmol), and isobutyl chloroformate (1.4 g, 10 mmol). After the mixture was stirred at room temperature for 2 h, it was stripped of THF in vacuo and the residue partitioned between $CHCl_3$ and 10% Na_2CO_3. The organic phase was washed with 10% HCl and H_2O and dried (Na_2SO_4). Removal of the $CHCl_3$ in vacuo yielded 4.43 g (93%) of **20**: mp 102–104 °C; $[\alpha]^{21}_D$ –54.4° (*c* 0.3, MeOH). Anal. ($C_{23}H_{34}N_6O_5$) C, H, N.

***N*-Benzyloxycarbonyl-L-prolyl-L-leucylamino-2-propanone (21).** A solution of **20** (1 g, 2 mmol) in MeOH (10 mL) was treated with 10% HCl (15 mL). The mixture was stirred at room temperature for several hours and then extracted with $CHCl_3$ (4 × 20 mL). The combined $CHCl_3$ extracts were dried ($MgSO_4$) and the $CHCl_3$ was evaporated to give 790 mg (90%) of the ketone: mp 142.5–143 °C; $[\alpha]^{20}_D$ –89.3° (c 1.0, MeOH). Anal. ($C_{22}H_{31}N_3O_5$) C, H, N.

L-Prolyl-L-leucylamino-2-propanone Oxalate (5). A 30% solution of HBr in AcOH (3 mL) was added to **21** (700 mg, 1.7 mmol) and the mixture stirred for 30 min. The addition of Et_2O gave a hygroscopic precipitate which was collected, washed with Et_2O, and dissolved in 10 mL of H_2O. The aqueous solution was washed with EtOAc (10 mL), made alkaline with 2 N NaOH, saturated with NaCl, and extracted with $CHCl_3$ (3 × 8 mL). The combined $CHCl_3$ extracts were dried (Na_2SO_4) and the $CHCl_3$ was removed in vacuo to give 450 mg (95%) of **5** as a hygroscopic glassy solid. This material was converted to the oxalate salt by treating a solution of the free base with a solution of oxalic acid (200 mg) in THF. The precipitate was washed with Et_2O and recrystallized from isopropyl alcohol to give 450 mg of the oxalate salt: mp 166–168 °C; $[\alpha]^{23}_D$ –58.2° (*c* 1.0, H_2O). Anal. ($C_{16}H_{27}N_3O_7$) C, H, N.

***N*-Benzyloxycarbonyl-L-prolyl-L-leucylsemicarbazide (22).** The mixed anhydride prepared from **15** (3.6 g, 10 mmol), NEt_3 (1.0 g, 10 mmol), and isobutyl chloroformate (1.4 g, 10 mmol) was treated with a solution of semicarbazide hydrochloride (1.5 g, 13.5 mmol) and NEt_3 (1.4 g, 13.5 mmol) in H_2O (20 mL). The product was isolated in the same manner as described for **19**. Recrystallization from MeOH–Et_2O afforded 4.15 g (99%) of semicarbazide: mp 193–195 °C; $[\alpha]^{25}_D$ –80.0° (*c* 0.36, MeOH). Anal. ($C_{20}H_{29}N_5O_5$) C, H, N.

L-Prolyl-L-leucylsemicarbazide Acetate (6). The benzyloxycarbonyl-protecting group was removed from **22** (2.4 g, 8 mmol) in a manner analogous to that used for **3** to yield 2.6 g of crude material. Recrystallization from MeOH–Et_2O gave 1.8 g (64%) of the acetate salt: mp 160.5–161.5 °C; $[\alpha]^{23}_D$ –54.3° (*c* 0.76, MeOH). Anal. ($C_{14}N_{27}N_5O_5$) C, H, N.

***N*-Benzyloxycarbonyl-L-prolyl-L-leucyl-β-alaninamide (23).** The mixed anhydride prepared in the THF from **15** (3.6 g, 10 mmol), NEt_3 (1.0 g, 10 mmol), and isobutyl chloroformate (1.4 g, 10 mmol) was mixed with an aqueous solution of β-alaninamide hydrochloride (1.3 g, 10 mmol) and NEt_3 (1.0 g, 10 mmol). The product was isolated in the same manner as described

for **19**. After evaporation of the solvent, the residue was recrystallized from isopropyl alcohol to give 2.3 g (53%) of product: mp 177–179 °C; $[\alpha]^{24}_D$ –79.1° (*c* 1.0, EtOH). Anal. ($C_{22}H_{32}N_4O_5$) C, H, N.

L-Prolyl-L-leucyl-β-alaninamide Hydrochloride (7). A solution of the protected amide **23** (1.3 g, 3 mmol) in 30 mL of MeOH containing concentrated HCl (0.5 mL) was hydrogenated for 1 h over 70 mg of 5% Pd/C. After the mixture was filtered, the filtrate was stripped of solvent in vacuo and the residue recrystallized from isopropyl alcohol to afford 830 mg (83%) of the hydrochloride salt: mp 198–200 °C; $[\alpha]^{26}_D$ –62.0° (*c* 1.0, MeOH). Anal. ($C_{14}H_{27}N_3O_6Cl$) C, H, N.

(±)-Thiazolidine-2-carboxylic Acid (24). The method used was analogous to that reported by Yurev et al.[27] for the synthesis of substituted thiazolidine-2-carboxylic acids. A solution of 2-aminoethanethiol hydrochloride (19.2 g, 0.17 mmol) in MeOH was treated with $NaOCH_3$ (9.1 g, 0.17 mmol). After the solvent was removed in vacuo, the free base was treated with a solution of glyoxylic acid (12.5 g, 0.17 mmol) in absolute EtOH (100 mL). This mixture was shaken vigorously for several minutes and then cooled in an ice bath. The yellow precipitate was collected and recrystallized from 70% EtOH to afford 11.2 g (50%) of the amino acid: mp 190–191 °C dec (lit.[28] 181 °C); NMR ($CDCl_3$) δ 5.22 (s, 1 H, NCHS), 3.58–3.92 (m, 2 H, SCH_2), 3.15–3.40 (m, 2 H, NCH_2). Anal. ($C_4H_7NO_2S$) C, H, N.

(±)-Ethyl Thiazolidine-2-carboxylate Hydrochloride (25). To an ice-cold suspension of **24** (15.8 g, 0.12 mmol) in 50 mL of absolute EtOH was added in a dropwise manner $SOCl_2$ (14.5 g, 0.12 mmol). The reaction mixture was heated with constant stirring at 60 °C for 14 h after which time the solvent was removed under reduced pressure to yield an oil which solidified on standing. Recrystallization of this solid from a mixture of EtOH and Et_2O yielded 18.95 g (81%) of **25**: mp 103–104.5 °C; NMR ($CDCl_3$) δ 5.56 (s, 1 H, NCHS). Anal. ($C_6H_{12}NO_2SCl$) C, H, N.

Resolution of (±)-Ethyl Thiazolidine-2-carboxylate. An aqueous solution of **25** (7.5 g, 38 mmol) was made basic with 10% Na_2CO_3 and extracted with Et_2O (4 × 15 mL). The combined ethereal extracts were dried (Na_2SO_4) and stripped of solvent in vacuo to give 5.91 g (36.7 mmol) of the free base. A solution of this material in anhydrous Et_2O (10 mL) was added to a hot solution of (+)-tartaric acid (5.5 g, 36.7 mmol) in absolute EtOH (30 mL). On cooling a white crystalline product was obtained. Recrystallization of this material to optical purity from EtOH–Et_2O (3:1 v/v) gave 5.39 g (94% of theory) of the (+)-tartrate salt of (–)-ethyl thiazolidine-2-carboxylate: mp 120–122 °C; $[\alpha]^{18}_D$ –65.5° (*c* 0.2, MeOH). Anal. ($C_{10}H_{17}NO_8S$) C, H, N, S.

An aqueous solution of the (+)-tartrate salt of (–)-ethyl thiazolidine-2-carboxylate (1.75 g, 5.6 mmol) was made alkaline with 10% Na_2CO_3 and extracted with Et_2O (4 × 15 mL). After the combined ethereal extracts were dried (Na_2SO_4), the Et_2O was removed in vacuo to afford 0.87 g (97%) of (–)-ethyl thiazolidine-2-carboxylate: mp 47–49 °C; $[\alpha]^{25}_D$ –107.0° (*c* 1.7, EtOAc). Anal. ($C_6H_{11}NO_2S$) C, H, N.

The mother liquors from the above recrystallization were combined and stripped of solvent in vacuo. The residue was dissolved in 10% Na_2CO_3 and extracted with Et_2O (2 × 20 mL). The combined ethereal extracts were dried ($MgSO_4$) and the Et_2O was removed in vacuo to give 1.85 g (11.5 mmol) of crude (+)-ethyl thiazolidine-2-carboxylate. An ethereal solution of this material was added to a hot solution of (–)-tartaric acid (1.73 g, 11.5 mmol) in absolute EtOH. The crude (–)-tartrate salt which precipitated on cooling was treated in the same manner as the (+)-tartrate salt to give 2.07 g (36% of theory) of the (–)-tartrate salt of (+)-ethyl thiazolidine-2-carboxylate: mp 121–123 °C; $[\alpha]^{18}_D$ +65.0° (*c* 0.2, MeOH). Anal. ($C_{10}H_{17}NO_8S$) C, H, N, S.

(+)-Ethyl thiazolidine-2-carboxylate was isolated from its (–)-tartrate salt (0.75 g, 2.4 mmol) in the same manner as was the (–) isomer to yield 330 mg (85%) of (+) ester: mp 51–53 °C; $[\alpha]^{25}_D$ +109.7° (*c* 2.0, EtOAc).

L-Prolyl-L-leucyl-(–)-thiazolidine-2-carboxamide (8). To an ice-cold solution of **15** (1.82 g, 5 mmol) and (–)-ethyl thiazolidine-2-carboxylate (0.81 g, 5 mmol) in EtOAc (50 mL) was added *N,N*-dicyclohexylcarbodiimide (1.03 g, 5 mmol). The mixture was stirred at 0 °C for 6 h and then overnight at room temperature. The mixture was filtered and the filtrate washed with 10% Na_2CO_3, 10% HCl, and saturated NaCl solution. After the organic layer was dried (Na_2SO_4), the solvent was evaporated to give 2.16 g (85%) of ester as an oil. This material was dissolved in MeOH (50 mL) saturated with NH_3. When TLC [$CHCl_3$–CH_3OH (9:1)] showed no starting material to be present, the solvent was removed in vacuo to give 2.0 g of amide which was used without further purification.

The amide (1.1 g, 2.3 mmol) was treated with 4 mL of a 30% solution of HBr in acetic acid. After 30 min at room temperature Et_2O was added. The salt was collected by filtration, washed with Et_2O, and dissolved in H_2O (10 mL). The aqueous solution was washed with EtOAc (10 mL), made basic with 2 N NaOH, saturated with NaCl, and extracted with $CHCl_3$ (4 × 10 mL). The combined $CHCl_3$ extracts were dried (Na_2SO_4) and the solvent was removed in vacuo to give 610 mg (77%) of **8**: mp 75–78 °C; $[\alpha]^{25}_D$ –90.9° (*c* 0.55, $CHCl_3$); NMR ($CDCl_3$) δ 5.57 (s, 1 H, NCHS). Anal. ($C_{15}H_{26}N_4O_3S$) C, H; N: calcd, 16.36; found, 15.74.

L-Prolyl-L-leucyl-(+)-thiazolidine-2-carboxamide (9). Following the same procedures that were used for **8**, (+)-ethyl thiazolidine-2-carboxylate (330 mg, 2 mmol) was coupled to **15** (725 mg, 2 mmol) with *N,N*-dicyclohexylcarbodiimide. The same reaction conditions and work-up as above gave 300 mg (44%) of **9** after recrystallization from EtOAc: mp 158–158.5 °C; $[\alpha]^{25}_D$ –23.9° (*c* 1.5, $CHCl_3$); NMR ($CDCl_3$) δ 5.50 (s, 1 H, NCHS). Anal. ($C_{15}H_{26}N_4O_3S$) C, H, N, S.

L-Thiazolidine-4-carboxamide (26). Methyl L-thiazolidine-4-carboxylate (12.5 g, 85 mmol) was treated with a methanolic solution of NH_3 for 1 day. The solvent was removed in vacuo and the residue recrystallized from EtOH to give 8 g (71%) of amide: mp 97–98.5 °C; $[\alpha]^{21}_D$ –125.4° (*c* 1.0, MeOH). Anal. ($C_4H_8N_2OS$) C, H, N, S.

L-Prolyl-L-leucyl-L-thiazolidine-4-carboxamide (10). Compound **26** (390 mg, 3 mmol) in EtOAc (40 mL) was coupled to **15** (1.09 g, 3 mmol) with *N,N*-diclohexylcarbodiimide (620 mg, 3 mmol). After the reaction was stirred for 5 h at 0° C, it was filtered. The filtrate was washed with 10% HCl, 5% $NaHCO_3$, and saturated NaCl solution. The organic layer was dried (Na_2SO_4) and the solvent removed in vacuo to yield 940 mg (66%) of protected tripeptide: mp 77–80 °C. Without further purification, the benzyloxycarbonyl-protecting group was removed with a 30% solution of HBr in acetic acid. The reaction conditions and work-up were the same as those described for **8**. Recrystallization of the crude product from EtOAc gave 348 mg (50%) of **10**: mp 168–170 °C; $[\alpha]^{29}_D$ –181.2° (*c* 0.5, MeOH). Anal. ($C_{15}H_{26}N_4O_3S$) C, H, N, S.

L-Prolyl-L-leucyl-L-prolinamide (11). The mixed anhydride prepared in THF from **15** (1.9 g, 5.3 mmol), NEt_3 (0.53 g, 5.3 mmol), and isobutyl chloroformate (0.72 g, 5.3 mmol) was treated with a solution of L-prolinamide (0.6 g, 5.3 mmol) in THF (10 mL). The mixture was stirred at room temperature for 2 h, filtered, and stripped of THF under reduced pressure. The residue was dissolved in EtOAc (50 mL) and washed successively with 10% Na_2CO_3, 10% HCl, and H_2O. After the organic layer was dried (Na_2SO_4), the solvent was removed in vacuo to give 2.0 g (87%) of Z-Pro-Leu-Pro-NH_2 (**27**) as a clear colorless oil. This material was hydrogenated for 1.5 h over 750 mg of 5% Pd/C in MeOH (75 mL) containing concentrated HCl (0.8 mL). The mixture was filtered and the salt which was obtained after evaporation of the solvent was dissolved in $CHCl_3$. Ammonia was bubbled into the solution and the precipitate of NH_4Cl was removed by filtration. The residue which remained after the $CHCl_3$ was evaporated was recrystallized from EtOAc to give 530 mg (38%) of pure **11**: mp 192–193.5 °C; $[\alpha]^{21}_D$ –120° (*c* 0.1, MeOH). Anal. ($C_{16}H_{28}N_4O_3$) C, H, N.

Methyl *N*-*tert*-Butyloxycarbonyl-L-thiazolidine-4-carbonyl-L-leucylglycinate (28). The mixed anhydride prepared in THF from 1.17 g (5 mmol) of *tert*-butyloxycarbonyl-L-thiazolidine-4-carboxylic acid [prepared by the general procedure of Hofmann et al.:[29] mp 130–130.5 °C; $[\alpha]^{19}_D$ –114.1° (*c* 1.0, MeOH)], *N*-methylmorphine (0.5 g, 5 mmol), and isobutyl chloroformate (0.7 g, 5 mmol) was treated with a solution of Leu-Gly-OCH_3·HBr (1.7 g, 6 mmol) and NEt_3 (0.6 g, 6 mmol) in DMF (7 mL). The mixture was stirred at 25 °C for 30 min and then evaporated to dryness. A solution of the residue in $CHCl_3$ (30 mL) was washed with 5% $NaHCO_3$, 10% HCl, and saturated NaCl solution. The organic layer was dried (Na_2SO_4) and stripped

of solvent in vacuo to give 1.0 g (50%) of product: mp 133–135 °C. Recrystallization from MeOH–hexane provided an analytically pure sample: mp 135–137 °C; $[\alpha]^{27}_D$ −131.8° (c 1.2, MeOH). Anal. ($C_{18}N_{31}N_3O_6S$) C, H, N.

***N*-*tert*-Butyloxycarbonyl-L-thiazolidine-4-carbonyl-L-leucylglycinamide (29).** Compound **28** (596 mg, 1.4 mmol) was dissolved in MeOH (30 mL) saturated with NH_3 and placed in a refrigerator for 1 week. The solvent was removed in vacuo and the residue recrystallized from EtOAc–Et_2O to give 445 mg (77%) of **29**: mp 149–151 °C; $[\alpha]^{25}_D$ −122.6° (c 0.73, MeOH). Anal. ($C_{17}H_{20}N_4O_5S$) C, H, N.

L-Thiazolidine-4-carbonyl-L-leucylglycinamide (12). Compound **29** (550 mg, 1.4 mmol) was deprotected with 2 mL of 30% HBr in acetic acid. The reaction conditions and work-up were similar to those described for **8**. Recrystallization of the crude material from MeOH–EtOAc gave 140 mg (33%) of pure **12**: mp 154–155 °C; $[\alpha]^{23}_D$ −51.7° (c 0.6, MeOH). Anal. ($C_{12}H_{27}N_4O_3S$) C, H, N.

***N*-Cyclopentanecarbonyl-L-leucylglycinamide (13).** *N*,*N*-Dicyclohexylcarbodiimide (4.1 g, 20 mmol) was added to a solution of cyclopentanecarboxylic acid (2.3 g, 20 mmol) and *N*-hydroxysuccinimide (2.3 g, 20 mmol) in dioxane (50 mL). The mixture was stirred at 25 °C for 6 h. The solid was removed by filtration and the filtrate evaporated to dryness under reduced pressure. A solution of the residue in EtOAc was washed with 5% $NaHCO_3$ and dried (Na_2SO_4). Evaporation of the solvent gave a semisolid which was recrystallized from isopropyl alcohol to give 2.25 g (53%) of the activated ester: mp 68–70 °C.

A solution of 640 mg (3 mmol) of the *N*-hydroxysuccinimide ester of cyclopentanecarboxylic acid, NEt_3 (300 mg, 3 mmol), and L-leucylglycinamide acetate (750 mg, 3 mmol) in DMF (10 mL) was stirred overnight at 25 °C. The DMF was removed in vacuo and the residue partitioned between $CHCl_3$ and 10% HCl. The $CHCl_3$ layer was washed with 10% Na_2CO_3 and saturated NaCl solution. The organic layer was dried (Na_2SO_4) and stripped of solvent in vacuo to give after recrystallization from MeOH–EtOAc 370 mg (44%) of **13**: mp 170–172 °C; $[\alpha]^{24}_D$ −20.6° (c 1.0, MeOH). Anal. ($C_{14}H_{25}N_3O_3$) C, H, N.

Dopa Potentiation Test. Male ICR mice (A. R. Schmidt, Sprague–Dawley), weighing 16–20 g, were pretreated orally 4 h before the test with a low dose of the monoamine oxidase inhibitor, pargyline hydrochloride (40 mg/kg). The test compound was then administered intraperitoneally. Four mice were tested at each dose. After 1 h a challenge dose of *dl*-Dopa (200 mg/kg) was given intraperitoneally and the mice were observed for 1 h. The changes in activity and aggressive behavior were recorded and rated according to the following system: (1) slight—piloerection, slight salivation, slight increased motor activity; (2) moderate—piloerection, salivation, marked increased activity, and irritability; and (3) marked—piloerection, profuse salivation, marked increased irritability and reactivity, jumping, squeaking, and aggressive fighting. Mice which were given only pargyline hydrochloride (40 mg/kg) and *dl*-Dopa (200 mg/kg) served as the controls. These mice had a behavioral rating of 1.

Oxotremorine Antagonism Test. Normal (ICR) male mice (16–20 g) were pretreated intraperitoneally with varying doses of the test drug 1 h before the administration of oxotremorine (0.5 mg/kg ip). Four mice were tested at each dose of the test drug. The responses of the mice were recorded by observation techniques and were compared with those observed in mice which received only oxotremorine as a control. The responses were graded as 0 (no effect), 1 (slight), 2 (moderate), and 3 (marked) for the effect of oxotremorine on each group of mice. The graded responses for the central effects of oxotremorine (including decreased motor activity, tremors, head twitch, limb abduction, and ataxia) were added together with the graded responses of the peripheral effects (lachrymation, salivation, and diarrhea) to produce a total score. Oxotremorine itself results in an average total score of 22 out of a possible maximum total score of 24. A compound is considered active if the total score is 16/22 or less.

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