in liquid nitrogen until required.

Test compounds were dissolved and diluted as necessary in dimethyl sulfoxide (DMSO). The drug solutions were then diluted in assay buffer (20 mM HEPES/NaOH, pH 7.1, containing 10 mM magnesium chloride, 100 mM sodium chloride and 0.2% (w/v) bovine serum albumin) such that the DMSO concentration was 3% (v/v). A 50 μL aliquot of each diluted drug solution was incubated for 90 min at room temperature (nominally 20 °C) with 50 μL of monoiodinated angiotensin II [(3-[^{125}I]iodotyrosyl)-angiotensin II, 74 TBq/mmol, (Amersham International plc)] and 50 μL of membrane suspension containing approximately 50 μg membrane protein. Following incubation, receptor-bound radioactivity was separated from free by rapid filtration over glass-fiber mats (Skatron Ltd, Newmarket, UK). Receptor-bound radioactivity trapped on the filter mats was quantified using a gamma counter (Pharmacia/Wallac 1277 Gammamaster).

Specific angiotensin binding was defined as the difference between total binding and the nonspecific binding which was determined in the presence of excess (0.3 μM) nonradioactive angiotensin II. Specific angiotensin binding in the presence of drug was compared to the control specific binding in the presence of vehicle. Results were calculated as percentage displacement relative to the control. IC_{50} values (concentration for 50% displacement of the specifically bound monoiodinated angiotensin II) were determined from the relationship between percentage inhibition and drug concentration. Test concentrations were chosen to bracket the expected IC_{50} and to cover at least 4 orders of magnitude.

Under the assay conditions used, the total binding and nonspecific binding were typically in the range 0.48–0.85 fmol and 0.02–0.04 fmol per 150 μL, respectively. The concentration of monoiodinated angiotensin used in the assay was 0.1 nM and the IC_{50} for its displacement by authentic angiotensin II was 0.77 ± 0.46 nM (mean ± standard deviation, n = 400). The presence of 1% DMSO in the incubation mixture had no significant effect on angiotensin binding to the adrenal receptor.

pA_2 Determination in the in Vitro Rabbit Aorta. New Zealand White rabbits (2–4 kg) were deeply anaesthetized with sodium pentobarbitone, the descending thoratic aorta was removed, cut into helical strips and mounted in 20-mL organ baths containing Krebs bicarbonate solution at 37 °C and bubbled with 5% CO_2. Resting tension was set at 1.0 g and the aortic strips were allowed to equilibrate for 120 min, after which a cumulative dose–response curve to angiotensin II was constructed. Following a 60-min washout period, an angiotensin II antagonist was added to the bath and 60 min later, a second angiotensin II dose–response curve was constructed. The pA_2 values of antagonists were determined according to the method of Schild.

Intravenous ED_{50} Determination in the Rat. Male Alderley Park Wistar rats (200–250 g) were prepared with indwelling carotid artery and jugular vein catheters under Saffan (alphaxolone/alphadolone) anaesthesia. The following day, arterial blood pressure was measured while angiotensin II was infused intravenously at a dose of 1 μg/kg^{-1} min^{-1}, which increased mean aterial pressure by approximately 50 mmHg. During the angiotensin II infusion, incremental doses of an angiotensin II antagonist were administered intravenously and the ED_{50} of the antagonist determined.

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Registry No. **2**, 133075-99-7; **3**, 137377-98-1; **4**, 138459-12-8; **5**, 138459-13-9; **6**, 133141-61-4; **7**, 133052-87-6; **8**, 133052-85-4; **9**, 133052-47-8; **10**, 133052-46-7; **11**, 133052-43-4; **12**, 133052-44-5; **13**, 133052-42-3; **14**, 138459-14-0; **15**, 133052-41-2; **16**, 133052-38-7; **17**, 138459-15-1; **18**, 133052-39-8; **19**, 133052-40-1; **20**, 133052-88-7; **21**, 138459-16-2; **22**, 138459-17-3; **23**, 138459-18-4; **24a**, 114772-38-2; **25a**, 133052-50-3; **25b**, 133052-52-5; **26a**, 591-78-6; **26b**, 26818-07-5; **27a**, 138459-19-5; **27b**, 138488-58-1; **28**, 138459-20-8; **29**, 18469-52-8; **30a**, 133052-61-6; **31a**, 133052-62-7; **31b**, 133052-63-8; **31c**, 138459-21-9; **31d**, 133052-69-4; **32**, 138459-22-0; **33**, 51436-99-8; **34a**, 138459-23-1; **35a**, 138459-24-2; **36a**, 138459-25-3; **36b**, 138459-26-4; 2-butylbenzimidazole, 5851-44-5; 2-aminopyridine, 504-29-0; 1,4-difluoro-2-nitrobenzene, 364-74-9; 2,4-difluoro-1-nitrobenzene, 446-35-5; 1-fluoro-2-nitrobenzene, 1493-27-2; valeric anhydride, 2082-59-9; tributyltin azide, 17846-68-3; methyl 2-iodobenzoate, 610-97-9; 2-butyl-4-chloro-5-(hydroxymethyl)-imidazole, 79047-41-9; angiotensin II, 11128-99-7.

5-(Aminomethyl)-3-aryldihydrofuran-2(3*H*)-ones, a New Class of Monoamine Oxidase-B Inactivators

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Both *cis*- and *trans*-5-(aminomethyl)-3-aryldihydrofuran-2(3*H*)-one hydrochloride salts (**9** and **10**) were synthesized efficiently in a 5-step sequence from arylacetic acids. Both compounds were found to be irreversible inactivators of monoamine oxidase B. These compounds constitute the first members of a new class of monoamine oxidase inactivators.

5-(Aminomethyl)-3-aryl-2-oxazolidinones (**1**) were reported to be selective inactivators of monoamine oxidase B (EC 1.4.3.4)[1] and therefore to have the potential to be useful adjuncts to L-dopa treatment for Parkinson's disease.[2] Recently, we investigated the mechanism of inactivation of monoamine oxidase by **1**[3] and concluded that inactivation arises from attachment of the enzyme to the α-position of the aminomethyl side chain (**2**, X = amino acid residue). The stability of this seemingly unstable adduct **2** was proposed to be the result of the electron-

Table I. Kinetic Constants for the Inactivation of Monoamine Oxidase-B by 5-(Aminomethyl)-3-aryldihydrofuran-2(3*H*)-ones

compd	k_{inact}, min^{-1}	K_I, mM	k_{inact}/K_I min^{-1} mM^{-1}
9a	2.57×10^{-3}	4.2	6.1×10^{-4}
10a	2.14×10^{-3}	5.1	4.2×10^{-4}
9b	4.44×10^{-3}	6.67	6.7×10^{-4}
10b	23×10^{-3}	18.2	12.6×10^{-4}
1 (R = Me, R′ = H)[11]	0.8×10^{-3}	12.5	0.71×10^{-4}

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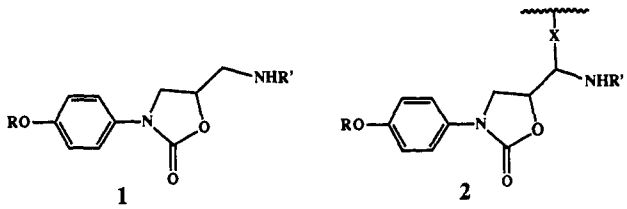
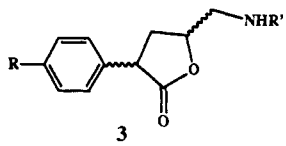


withdrawing effect of the oxazolidinone heteroatoms. Just as the electron-withdrawing effect of halogens α to an aldehyde functionality changes the equilibrium of the keto form in favor of the hydrate,[4] it was thought that the sp^3 hybridization of the enzyme adduct also would be stabilized by the oxygen of the oxazolidinone heterocycle. If that is the case, then the corresponding lactones (3) should

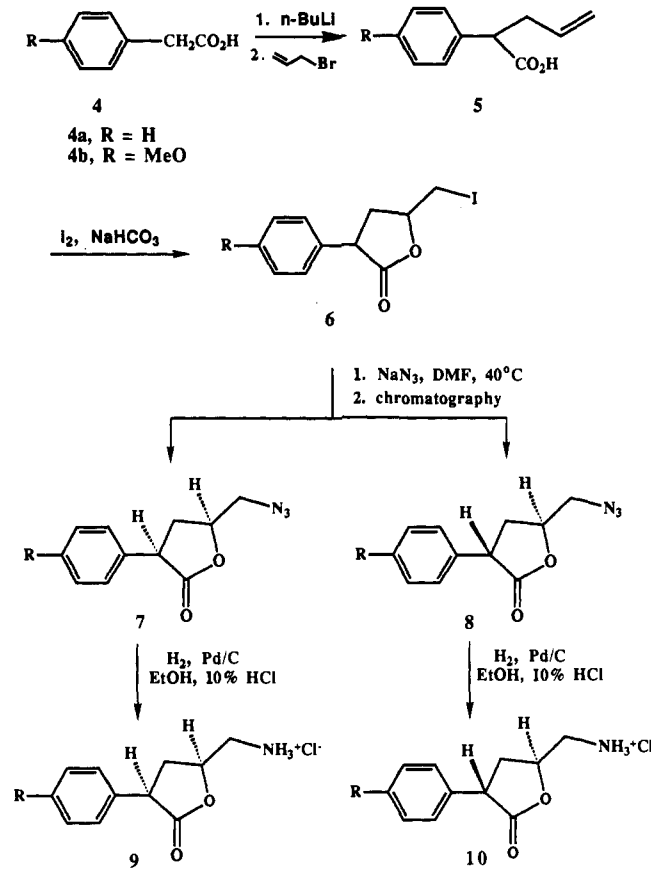


act as inactivators of monoamine oxidase as well as the oxazolidinones. As a test of this hypothesis we report an efficient synthesis of *cis*- and *trans*-3 (R = R′ = H; R = MeO, R′ = H) and their evaluation as inactivators of monoamine oxidase B. A series of 5-(aminomethyl)-3-phenyldihydrofuran-2(3*H*)-ones was synthesized previously as potential GABA antagonists; however, the synthesis proceeded with low yields and the diastereoisomers were not separated.[5]

Results and Discussion

Chemistry. The syntheses of *cis*- and *trans*-3 (R = R′ = H; R = MeO, R′ = H) are summarized in Scheme I. Dilithiation of arylacetic acids (4) with *n*-butyllithium at 0 °C in THF under argon gave the dianion, which was alkylated with allyl bromide to give 2-aryl-5-pentenoic

Scheme I. Synthetic Route to the 5-(Aminomethyl)-3-aryldihydrofuran-2(3*H*)-ones



acids (5).[6] Iodolactonization of 5 was effected by treatment with iodine in saturated sodium bicarbonate.[7] This results in a mixture of cis and trans diastereoisomers of iodolactones 6 in a ratio of 2:1, respectively.[8] The two diastereoisomers can be separated by careful silica gel column chromatography, but it was observed that when a single diastereoisomer of the iodolactone (either cis or trans), was treated with sodium azide in DMF at 40 °C, it was transformed into a mixture of cis and trans dia-

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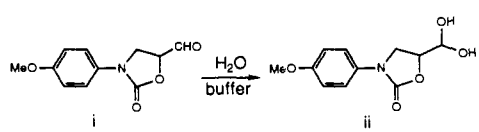

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stereoisomers of azidolactones 7 and 8.[9] Therefore, the mixture of iodolactone diastereoisomers was directly converted into the diastereomeric mixture of azidolactones 7 and 8 in a ratio of 3:1 in favor of 7, and these were separated by silica gel chromatography. The *cis*- and *trans*-azidolactones were hydrogenated separately in ethanol in the presence of 10% HCl to give diastereomerically pure *cis*- and *trans*- aminolactone hydrochlorides **9** and **10**, respectively.[10] It was found that hydrogenation of diastereomerically pure azidolactones in ethanol in the absence of aqueous HCl resulted in a diastereoisomeric mixture of aminolactones, which again indicates the strong acidity of the C-3 protons.

Enzymology. All four of the lactones that were synthesized were time-dependent inactivators of beef liver mitochondrial monoamine oxidase (Table I). The kinetic constants were determined by the method of Kitz and Wilson.[12] The inactivation of MAO-B by the four lactones was not reversed by short-term dialysis (2–3 h) against sodium phosphate buffer (50 mM, pH 7.40) at 0 °C; however, over an extended period of time it was found that the enzyme activity slowly returned. The rate of inactivation of MAO-B by the four compounds was not altered by the addition of β-mercaptoethanol. This suggests that the inactivation is not the result of attachment of an electrophile that had escaped the active site prior to its interaction with MAO. However, the inactivation was protected by a 10-fold excess of benzylamine, indicating that the inactivation is an active site-directed process. All four lactones are more potent inactivators of MAO-B than is the corresponding oxazolidinone (see Table I). These results support the hypothesis that the key feature contributing to the inactivation properties of the oxazolidinone inactivators is the electron-withdrawing ability of the heteroatoms in the ring.

Although the K_I values for the lactone analogues are relatively high (in the mM range), it should be kept in mind that the optimal 3-aryl substituent in the oxazolidinone series is a (*m*-chlorobenzyl)oxyl group not a methoxyl group.[1] Presumably, when that substituent is appended to the 3-phenyl substituent of the lactone analogue, the K_I value also will be lowered below that for the corresponding oxazolidinone.

Experimental Section

Chemistry. NMR spectra were recorded either on a Varian EM-390 90-MHz or on a Varian XL-400 400-MHz spectrometer. Chemical shifts are reported as δ values in parts per million downfield from Me_4Si as the internal standard in $CDCl_3$. Thin-layer chromatography was performed on EM/UV silica gel plates with a UV indicator. Melting points were obtained with a Fisher-Johns melting point apparatus and are uncorrected. Elemental combustion analyses were performed by Searle Laboratory, Skokie, IL. Mass spectra were recorded on a VG Instruments VG70-250SE high-resolution spectrometer. Column chromatography was performed with Merck silica gel (230–400 mesh). All chemicals were purchased from Aldrich Chemical Co. and were used without further purification. *n*-Butyllithium solution was titrated by the method of Ronald.[13] THF was freshly distilled from sodium. DMF was distilled from BaO before use. DMSO was distilled from CaH. Glassware was dried in the oven overnight when dry conditions were required. All reactions were carried out in an atmosphere of inert gas (nitrogen or argon) except hydrogenations.

2-(4-Methoxyphenyl)-4-pentenoic Acid (5b). To a solution of (*p*-methoxyphenyl)acetic acid (15 g, 90 mmol) in 300 mL of anhydrous THF in an ice bath, under argon, a solution of *n*-butyllithium (2.5 M, 72 mL, 180 mmol) was added dropwise over 45 min. After the addition was completed, the mixture was stirred at 0 °C for 1 h, then at room temperature for 2 h. Upon addition of hexamethylphosphoramide (15 mL) a yellowish homogeneous solution resulted, which was stirred for 30 min, then cooled to 0 °C. Allyl bromide (8 mL, 92.4 mmol) was added via syringe, and then the resulting mixture was stirred at 0 °C for 1 h and at room temperature for 4 h. The reaction was quenched by addition of an aqueous 10% HCl solution (200 mL) at 0 °C. The resulting two layer mixture was concentrated in vacuo, then was extracted with ethyl acetate (2 × 200 mL). The combined organic extracts were washed with brine and dried over sodium sulfate. The solvent was removed in vacuo, and the residue was distilled at 175–180 °C (5 mmHg). The product was obtained as a colorless oil, which solidified to a semisolid upon standing (17.5 g, 95%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.50 (broad, 1 H), 7.35 (d, 2 H), 6.90 (d, 2 H), 5.80 (m, 1 H), 5.10 (dd, 2 H), 3.80 (s, 3 H), 3.62 (t, 1 H), 2.85 (m, 1 H), 2.55 (m, 1 H); IR (neat) 3100 (broad, s), 1714 (s), 1614 (s).

2-Phenyl-4-pentenoic Acid (5a). The same procedure for the preparation of 2-(4-methoxyphenyl)-4-pentenoic acid was employed, and the title compound was obtained as a liquid: bp 105 °C (0.5 mmHg); ^{1}H NMR (90 MHz, $CDCl_3$) δ 10.70 (broad, 1 H), 7.25 (s, 5 H), 5.65 (m, 1 H), 5.05 (m, 2 H), 3.60 (t, 1 H), and 2.60 ppm (m, 2 H); FTIR (neat) ν_{max} 3150 (broad, s), 1713 (s), and 1615 cm^{-1} (s).

***cis*- and *trans*-5-(Iodomethyl)-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-ones (6b)**. A solution of 2-(4-methoxyphenyl)-4-pentenoic acid (**5b**, 7.5 g, 36.4 mmol) in ether (45 mL) was added to saturated sodium bicarbonate (180 mL) at 0 °C under nitrogen. Iodine (27.7 g, 109 mmol) in 75 mL of THF was added at 0 °C over 45 min, and then the mixture was stirred at 0 °C for 4 h. The reaction was quenched by addition of saturated sodium thiosulfate at 0 °C, and the resulting clear solution was concentrated in vacuo. The aqueous layer was extracted with ethyl acetate (3 × 150 mL), and the combined extracts were washed with brine and dried over magnesium sulfate. Removal of the solvent resulted in a yellowish oil (10 g, 83%), which was identified by NMR spectral analysis as a mixture of the *cis*- and *trans*-iodolactones in a ratio of 2:1. The diastereomeric iodolactones could be separated by careful silica gel column chromatography eluting with a mixture of hexane and ethyl acetate (2:1) to give two products.

***cis*-6b**: A colorless oil; R_f = 0.33; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, 2 H), 6.90 (d, 2 H), 4.48 (m, 1 H), 3.90 (dd, 1 H), 3.80 (s, 3 H), 3.50 (dd, 1 H), 3.37 (dd, 1 H), 2.90 (m, 1 H), and 2.10 (q, 1 H); IR ν_{max} 1771 cm^{-1}.

***trans*-6b**: A colorless oil; R_f = 0.44; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, 2 H), 6.90 (d, 2 h), 4.70 (m, 1 H), 3.97 (t, 1 H), 3.80 (s, 3 H), 3.43 (dd, 1 H), 3.35 (dd, 1 H), and 2.55 (m, 1 H); IR ν_{max} 1772 cm^{-1}.

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(11) These kinetic values are different from those reported in ref 3 for the same compound (compound 17 (R = H) in ref 3). The reason for this disparity is that in ref 3 general conditions for the inactivation of monoamine oxidase by the inactivators was given, but these conditions were not used for the inactivation of the enzyme by compound 17 (R = H). Actually, the values reported in ref 3 for the inactivation of MAO by 17 (R = H) were obtained in 100 mM Tris-HCl buffer, pH 9.0 in the presence of 15% DMSO. The difference in conditions for the two inactivation experiments accounts for the large difference in kinetic constant values for the same compound reported in ref 3 (17, R = H) and here (1, R = Me, R′ = H). We thank the referee who pointed out this disparity.

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***cis*- and *trans*-5-(Iodomethyl)-3-phenyldihydrofuran-2(3*H*)-ones (6a).** The same procedure for the preparation of *cis*- and *trans*-5-(iodomethyl)-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-ones (**6b**) was employed, and the title compounds were obtained in a 91% yield as a mixture of diastereomers, which could be separated by column chromatography eluting with a mixture of hexane and ethyl acetate (2:1) to give the following.

***cis*-6a**: A colorless oil; R_f = 0.35; ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.35 (s, 5 H), 4.50 (m, 1 H), 4.00 (m, 1 H), 3.40 (m, 2 H), 2.90 (m, 1 H), and 2.10 ppm (q, 1 H); FTIR (neat) 1775 cm^{-1}; HRMS (EI) calcd for $C_{11}H_{11}O_2I$ 301.9804, found 301.9790; MS(EI) *m/z* 302 (M^+, 16.9), 131 (100.0), 129 (11.8), 116 (12.5), 115 (10.2), 104 (10.6), 103 (13.2), 91 (39.3), 77 (12.0).

***trans*-6a**: A colorless oil; R_f = 0.50; ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.35 (s, 5 H), 4.60 (m, 1 H), 4.05 (m, 1 H), 3.35 (dd, 2 H), and 2.60 (t of dd, 2 H); FTIR (neat) 1772 cm^{-1}; HRMS (EI) calcd for $C_{11}H_{11}O_2I$ 301.9804, found 301.9784.

***cis*- and *trans*-5-(Azidomethyl)-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one (7b and 8b).** To a solution of the diastereomeric iodolactones (**6b**, 0.65 g, 1.95 mmol) in 10 mL of anhydrous DMF at room temperature under nitrogen was added sodium azide (0.51 g, 7.8 mmol). The mixture was heated in an oil bath at 40 °C overnight then the resulting solid was removed by filtration, and the filtrate was poured into a mixture of *n*-hexane and ethyl acetate (100 mL, 3:1). The solid that formed was filtered through a pad of magnesium sulfate, and the filtrate was concentrated in vacuo, giving a mixture of the diastereoisomeric azidolactones (**7b** and **8b**) (0.45 g, 94%) in a ratio of 3:1. Separation of the diastereoisomers was accomplished by silica gel column chromatography eluting with a mixture of hexane and ethyl acetate (2:1) to afford two products.

7b: A colorless oil; R_f = 0.26; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (d, 2 H), 6.96 (d, 2 H), 4.69 (m, 1 H), 3.93 (dd, 1 H), 3.74 (dd, 1 H), 3.58 (dd, 1 H), 2.75 (m, 1 H), and 2.30 (q, 1 H); IR 2105 and 1770 cm^{-1}; HRMS (EI) calcd for $C_{12}H_{13}N_3O_3$ 247.0956, found 247.0957.

8b: A colorless oil; R_f = 0.39; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, 2 H), 6.92 (d, 2 H), 4.80 (m, 1 H), 4.03 (t, 1 H), 3.73 (dd, 1 H), 3.60 (dd, 1 H), 2.60 (m, 1 H), and 2.52 (m, 1 H); IR 2107 and 1770 cm^{-1}; HRMS (EI) calcd for $C_{12}H_{13}N_3O_3$ 247.0956, found 247.0955.

***cis*- and *trans*-5-(Azidomethyl)-3-phenyldihydrofuran-2(3*H*)-one (7a and 8a).** The same procedure for the preparation of *cis*- and *trans*-5-(azidomethyl)-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one (**7b** and **8b**) was employed, and the title compounds were obtained in an 89% yield as a mixture of diastereomers, which were separated by column chromatography eluting with a mixture of hexane and ethyl acetate (2:1) to afford two products.

7a: A colorless oil; R_f = 0.16; ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.35 (s, 5 H), 4.65 (m, 1 H), 4.00 (dd, 1 H), 3.60 (t, 2 H), 2.75 (m, 1 H), and 2.33 ppm (dd, 1 H); FTIR (neat), 2105 (s), and 1775 cm^{-1} (s); HRMS (EI) calcd for $C_{11}H_{11}O_2N_3$ ($-N_2$) 189.0790, found 189.0785; MS (EI) *m/z* 218 (M^+ + 1, 1.0), 189 (16.9), 161 (21.7), 145 (41), 133 (52), 117 (57.5), 105 (100), 91 (24), 77 (34), and 51 (18.3).

8a: A colorless oil; R_f = 0.29; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (m, 5 H), 4.82 (m, 1 H), 4.08 (t of dd, 1 H), 3.74 (dd, 1 H), 3.60 (dd, 1 H), 2.64 (m, 1 H), and 2.57 ppm (m, 1 H); FTIR (neat) 2098 (s), and 1770 cm^{-1} (s); HRMS (EI) calcd for $C_{11}H_{11}O_2N_3$ ($-N_2$) 189.0790, found 189.0788.

***cis*- and *trans*-5-(Aminomethyl)-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one Hydrochloride (9b and 10b).** Hydrogenation of **7b** and **8b** was carried out separately. Both isomers were hydrogenated to give crystalline solids in excellent yields. A representative example is given below.

The *cis*-azidolactone **7b** (180 mg, 0.73 mmol) was dissolved in 10 mL of a mixture of ethanol and 10% HCl (9:1). After addition of 10% palladium on carbon (50 mg) the reaction mixture was hydrogenated at room temperature and atmospheric pressure. After 4 h, the catalyst was removed by filtration through a pad of Celite and the filtrate was concentrated in vacuo, giving the product as a white solid, which was recrystallized from ethanol/ether (170 mg, 91%).

9b: mp 210–212 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.35 (d, 2 H), 7.08 (d, 2 H), 5.00 (m, 1 H), 4.28 (dd, 1 H), 3.55 (d, 1 H), 3.40 (m, 1 H), 3.88 (s, 3 H), 2.96 (m, 1 H), and 2.25 (q, 1 H); IR 1775 cm^{-1}. MS (EI) *m/z* 221 (M^+, free amine, 100), 178 (73), 163 (32), 149 (49), 135 (61), 119 (22), 105 (18), 91 (43), 77 (24), 65 (19), and 59 (32); HRMS (EI) calcd for $C_{12}H_{15}NO_3$ 221.1052, found 221.1048. Anal. for $C_{12}H_{16}O_3NCl$; C: Calcd, 55.93; found, 56.01; H: calcd, 6.27; found, 6.25; N: calcd, 5.45, found, 5.35.

10b: mp 217–220 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.37 (d, 2 H), 7.10 (d, 2 H), 5.16 (m, 1 H), 4.24 (t, 1 H), 3.90 (s, 3 H), 3.46 (m, 2 H), 2.75 (m, 1 H), and 2.67 (m, 1 H); IR 1775 cm^{-1}. MS (EI) *m/z* 221 (M^+, free amine, 100), 178 (23), 163 (26), 148 (16), 135 (43), 121 (19), 105 (13), 91 (24), and 77 (12); HRMS (EI) calcd for $C_{12}H_{15}NO_3$ 221.1052, found 221.1044. Anal. for $C_{12}H_{16}O_3NCl$; C: calcd, 55.93; found, 55.80; H: calcd, 6.27; found, 6.24; N: calcd, 5.45; found, 5.43.

***cis*- and *trans*-8-(Aminomethyl)-3-phenyldihydrofuran-2(3*H*)-one (9a and 10a).** The same procedure for the preparation of *cis*- and *trans*-5-(aminomethyl)-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one hydrochloride (**9b** and **10b**) was employed, and the title compounds were obtained after recrystallization as white solids.

9a: mp 215–216 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.45 (m, 5 H), 5.02 (m, 1 H), 4.32 (dd, 1 H), 3.52 (dd, 1 H), 3.40 (dd, 1 H), 2.97 (m, 1 H), 2.30 (dd, 1 H); FTIR (KBr pellet) 3000 (broad, s), 1774 cm^{-1} (s); HRMS (EI) calcd for $C_{11}H_{13}NO_2$ 191.0946, found 191.0942. Anal. for $C_{11}H_{14}O_2NCl$; C: calcd, 58.02; found, 58.20; H: calcd, 6.20; found, 6.07; N: calcd, 6.15; found 6.13.

10a: mp 216–218 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.43 (m, 5 H), 5.10 (m, 1 H), 4.20 (t, 1 H), 3.37 (m, 2 H), 2.66 (m, 1 H), 2.57 (m, 1 H); FTIR (KBr pellet) 2900 (broad, s), 1775 cm^{-1} (s); HRMS (EI) calcd for $C_{11}H_{13}NO_2$ 191.0946, found 191.0936. Anal. for $C_{11}H_{14}O_2NCl$; C: calcd, 58.02; found 58.11; H: calcd, 6.20; found 6.10; N: calcd, 6.15; found 6.20.

Enzyme and Assays. Bovine liver MAO-B was isolated according to the method of Salach.[14] MAO activity was assayed by a modified published procedure[15] in Tris buffer (100 mM, pH 9.0) at 25 °C with benzylamine or cinnamylamine as substrates.

Time-Dependent Inactivation Experiments (General Methods). Solutions (180 μL each) of 5-(aminomethyl)-3-aryldihydrofuran-2(3*H*)-one hydrochlorides (11.11, 5.55, 3.70, and 2.78 mM) in sodium phosphate buffer (100 mM, pH 7.40) containing 10% DMSO were preincubated at 25 °C. To these solutions, was added MAO-B (20 μL of 3 mg/mL). After being mixed, the samples were incubated at 25 °C, periodically agitated, and assayed for MAO activity by removing 10 μL of the mixture and adding to 490 μL of a benzylamine or cinnamylamine solution in Tris buffer (1.02 mM, pH 9.0) as described above. The enzyme activity thus determined was corrected against a control containing no inactivator. Kinetic constants (K_I and k_{inact}) were determined as described by Kitz and Wilson.[11] It was shown that 10% DMSO had no adverse effect on the activity of MAO-B; the control enzyme also contained 10% DMSO.

Effects of β-Mercaptoethanol on the Rate of Inactivation of MAO-B by 5-(Aminomethyl)-3-aryldihydrofuran-2(3*H*)-ones Hydrochloride. The following solutions (180 μL each) were prepared in sodium phosphate buffer (100 mM, pH 7.40) containing 10% DMSO and preincubated at 25 °C: 5-(aminomethyl)-3-aryldihydrofuran-2(3*H*)-one hydrochloride (5.55 mM), β-mercaptoethanol (0.55 mM), 5-(aminomethyl)-3-aryldihydrofuran-2(3*H*)-one hydrochloride with β-mercaptoethanol (0.55 mM), and a control with only the buffer. To these solutions was added MAO-B (20 μL, 3 mg/mL). The mixtures were incubated at 25 °C, and the MAO activity was assayed as described above.

Effects of Benzylamine on the Rate of Inactivation of MAO-B by 5-(Aminomethyl)-3-aryldihydrofuran-2(3*H*)-ones Hydrochloride. The following solutions (180 μL each) were prepared in sodium phosphate buffer (100 mM, pH 7.40) containing 10% DMSO and preincubated at 25 °C: 5-(aminomethyl)-3-aryldihydrofuran-2(3*H*)-one hydrochloride (5.55 mM),

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benzylamine hydrochloride (55.55 mM), 5-(aminomethyl)-3-aryldihydrofuran-2(3H)-one hydrochloride (5.55 mM) with benzylamine hydrochloride (55.55 mM), and a control containing only the buffer. To these solutions, was added MAO-B (20 μL, 3 mg/mL). The mixtures were incubated at 25 °C, and the MAO activity was assayed as described above.

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Analgesic Dipeptide Derivatives. 7. 3,7-Diamino-2-hydroxyheptanoic Acid (DAHHA) Containing Dipeptide Analogues of the Analgesic Compound H-Lys-Trp(Nps)-OMe

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A series of diastereomeric dipeptides, analogues of the analgesic compound H-Lys-Trp(Nps)-OMe (**2**), containing 3,7-diamino-2-hydroxyheptanoic acid (DAHHA) and 2-[(*o*-nitrophenyl)sulfenyl]tryptophan [Trp(Nps)] has been synthesized. These compounds were tested as enkephalin-degrading aminopeptidases (APs), AP-M and AP-B inhibitors, and analgesics. The inhibitory potencies and the antinociceptive effects depended on the stereochemistry of the compounds. (2S,3R)-DAHHA-L-Trp(Nps)-OMe (**26d**) was a highly potent and selective enkephalin-degrading APs inhibitor, with an IC_{50} value in the 10^{-8} M range. Although this derivative was about 10^3-fold more potent than **2** against these enzymes, their antinociceptive effects were completely similar. These results indicate that the inhibitory capacity of this series of Trp(Nps)-containing dipeptides against enkephalin-degrading enzymes is not an important factor for their antinociceptive effects.

In previous papers,[1,2] it was reported that the synthetic dipeptide H-Lys-Trp(Nps)-OH [Nps = (*o*-nitrophenyl)-sulfenyl] (**1**) and its methyl ester **2** exhibited a naloxone-reversible analgesia in mice, comparable with that of the enkephalin analogue D-Ala^2-Met-enkephalinamide (DAME), regarding both maximum effect and the time-course of analgesia. Studies to establish the structural requirements for the antinociceptive effect of **1** and **2** showed the need for a basic amino acid,[1,3] the importance of the Nps moiety,[1,2,4] since no analgesia was found with the unsubstituted dipeptide H-Lys-Trp-OH (**3**), and the dependence on the absolute configuration of each amino acid of the activity.[5] Studies on the mechanism of action of **1** appear to indicate that these Trp(Nps)-containing dipeptides do not act directly on opioid receptors, but their antinociceptive effects could be possible explained by a mixture of a moderate enkephalin-degrading aminopeptidase inhibition and Met-enkephalin-releasing properties.[1] We considered that a structural modification able to increase the aminopeptidase (AP) inhibitory potency of these dipeptide derivatives could help to clarify the participation of this inhibition in the observed analgesic effect and, therefore, in the mode of action. With this aim, the lysine residue of **2** has been replaced by the hydroxy-substituted homologue, 3,7-diamino-2-hydroxyheptanoic acid (DAHHA) whose α-hydroxy group could mimic the tetrahedral intermediate formed during the substrate hydrolysis by APs. This concept has been applied to explain the potent inhibition of APs by the natural compound [(2S,3R)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine [(2S,3R)-AHPBA-Leu, bestatin (**4**)],[6-9] which protects the endogenous enkephalins, released from K^+ depolarized brain slices, from degradation by this type of enzymes.[10,11] This paper deals with the synthesis, inhibitory properties against enkephalin-degrading APs, and the antinociceptive activity of a series of stereoisomeric dipeptide derivatives **5**, in which the absolute configuration of each asymmetric

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