

## Design, Synthesis, and Opioid Receptor Binding of Some Novel Benzazepine Constrained Leucine Enkephalin Mimetics

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An *N*-substituted 2-benzazepine, previously reported to possess morphine-like analgesic activity *in vivo*, was adapted for use as a constrained mimic of the *N*-terminal residues of leucine enkephalin. Molecular modeling was used to evaluate the suitability of the 2-benzazepine nucleus for this purpose. The principle peptide constraint structure, 2-(7-methoxy-2,3,4,5-tetrahydro-1H-2-benzazepin-2-yl)-ethanoic acid (**8**) and some structurally related benzazepine analogues were synthesized and incorporated into peptides using solid-phase protocols. Radioligand receptor binding studies were used to evaluate the general opioid receptor affinity of the target compounds. The target constrained peptide (**21**) demonstrated an affinity for [<sup>3</sup>H]naloxone-labeled sites (IC<sub>50</sub> 16 μM) comparable to the corresponding leucine enkephaline analogue. © 1994 Academic Press, Inc.

### INTRODUCTION

Constrained peptides and peptide analogues have been utilized to great effect in the elucidation of peptide biologically active conformations (1–4). Furthermore, they provide clinically interesting alternatives to the use of peptides as pharmaceuticals due to their decreased susceptibility to degradation *in vivo*, better absorption, slower excretion, and lower incidence of undesirable cross-reactivities at alternate receptors due to the conformational flexibility inherent to peptides (5).

The endogenous neuropeptides, the enkephalins (6), have been the subject of many studies (7) using peptide mimetics and constraints in attempts to provide a common structural basis for the analgesic activity they share with the alkaloid morphine (**1**). Many endeavours to exploit the apparent structural similarities which exist between the two have focused upon the highly mobile *N*-terminal domain (8) of the endogenous peptides which have been constrained in several analogues in attempts to deduce the active conformation of the tyramine moiety and correlate this to the corresponding region present in **1** (9–15).

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The 2-benzazepine system **2** has been reported (16) to affect an *in vivo* analgesic activity in rats 8.4 times that of morphine (**1**). Scheme 2 possesses the fundamental requirements for central nervous system (CNS) activity, namely, an aromatic ring and a basic nitrogen substituent (17), in conjunction with a phenolic oxygen and a second hydrophobic domain, both considered necessary for conferring analgesic activity (18). The benzazepines have, as a class, been demonstrated to possess a diverse range of CNS activities (1, 16, 19–23). As part of a general program aimed at producing novel CNS-active agents (24–29) and investigating peptidomimetic structures (25, 30), the structure of **1** was considered as a constrained mimic of the *N*-terminal domain of leucine enkephalin (**3**).

In this paper we describe a molecular modeling evaluation of the 2-benzazepine nucleus as a mimic of the tyramine moiety of leucine enkephalin (**3**) and the design and synthesis of several peptides containing benzazepine-based structures as constrained mimics of the two *N*-terminal residues of **3**. Radioligand receptor binding studies were used to provide an evaluation of opioid receptor affinities for the target peptides.

## MATERIALS AND METHODS

### *Molecular Modeling*

Molecular Modeling was carried out using the Macromodel package (version 1.5) (31). Structures not deposited in the Cambridge Crystallographic data base were constructed from standard bond lengths and angles from measurements made on Drieding models. Dihedral angles were varied systematically using increments of 20°; bond angle variation was permitted over the range  $\pm 10^\circ$  and a ring closure tolerance of  $1.5 \pm 0.5 \text{ \AA}$  employed. A  $84 \text{ kJ mol}^{-1}$  energy filter was used to reject nonfeasible conformations.

### *Synthesis*

Melting points were determined on a Reichert hot-stage microscope and are uncorrected. Microanalyses were carried out by AMDEL (Australian Microanalytical Service, Melbourne). Amino acid analyses were performed by Auspep (Melbourne, Australia). Infrared spectra were recorded as potassium bromide discs or as films on sodium chloride plates using an Hitachi 270-30 grating spectrophotometer.  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were recorded at 300 and 75 MHz, respectively using a Bruker AM-300 MHz instrument; tetramethylsilane was used as internal standard. Mass spectra were recorded at 70 eV on a Jeol JMS-DX300 mass spectrometer. Flash-column and thin-layer chromatography were performed using Merck silica No. 9385 and 1-mm layers of Kieselgel (GF254) on glass, respectively. Radial chromatography was performed on a Harrison Research (U.S.A.) "Chromatotron" system using either 1-, 2-, or 4-mm-thick silica-coated plates, prepared as per the manufacturers specifications. High-performance liquid chromatography (HPLC) was performed on an ICI system which was composed of two model LC 1500 pumps, an ICI AS 2000 automatic injector, an ICI Kortec K45 automatic

gradient controller, and a Knauer variable wavelength spectrophotometer. Detection was at 254 nm. The column used was a  $\mu$ -Bondapak RP-C18, 10  $\mu$ m, 7.8 mm  $\times$  30 cm with a Brownlee  $\mu$ -Bondapak C18-RP guard column. A flow rate of 5.0 ml min<sup>-1</sup> was used with a gradient-controlled mobile phase of the following composition: [Time (min), % buffer A (H<sub>2</sub>O/0.1% TFA), % buffer B (60% CH<sub>3</sub>CN (aq.)/0.1% TFA)] [0, 100, 0]; [5, 100, 0]; [35, 0, 100]; [45, 0, 100]; [50, 100, 0]. Reagents were purchased from Aldrich, Sigma, or Fluka and were of HPLC or analytical grade. Peptide synthesis grade *N,N'*-dimethylformamide (DMF) and fluorenylmethoxycarbonyl (Fmoc) amino acids were purchased from Novabiochem (Switzerland) and Auspep (Melbourne, Australia).

#### Peptide Constraint (Con) Syntheses

**7-Methoxy-2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepine (15) and 7-methoxy-1-oxo-2,3,4,5-tetrahydro-1*H*-2-benzazepine (14).** The title compounds were prepared simultaneously using an optimized procedure which will be described elsewhere (32). A 60°C solution of 6-methoxy- $\alpha$ -tetralone (**13**) (29.2 g, 166 mmol) in trichloroacetic acid (200 g) was carefully treated with sodium azide (16.2 g, 249 mmol) over 30 min, then maintained at this temperature with stirring to afford a mixture of the regioisomeric lactams **14** and **15**. The products were separated by radial chromatography using chloroform/methanol (49:1) as eluent. The chromatographically more mobile component, *R<sub>f</sub>* 0.25, yielded **15** as pale orange plates after recrystallization from chloroform/petrol (15.2 g, 35%) mp 128–129°C (Lit. (33) 128–129°C).  $\nu$  3200 br, 2944, 1676, 1626, 1586 cm<sup>-1</sup>. <sup>1</sup>H-NMR (CHCl<sub>3</sub>-d<sub>1</sub>)  $\delta$  2.17–2.26 (m, 2, 2  $\times$  H4); 2.33 (t, *J* = 7.1 Hz, 2, 2  $\times$  H3); 2.77 (t, *J* = 6.9 Hz, 2, 2  $\times$  H5); 3.80 (s, 3, CH<sub>3</sub>O); 6.74–6.77 (m, 2, H6 and H9); 6.93 (dd, *J* = 7.7, 1.4 Hz, 1, H8); 7.92 (br s, 1, NH). <sup>13</sup>C-NMR (CHCl<sub>3</sub>-d<sub>1</sub>)  $\delta$  28.3, 32.6, 55.6, 112.3, 115.3, 123.2, 130.9, 136.0, 157.6, 175.2. *m/z* 191 (M<sup>+</sup>, 53%), 162 (23), 136 (100). The regioisomeric lactam (**14**), *R<sub>f</sub>* 0.23, crystallized from chloroform (11.1 g, 48%) mp 159.0–160.5°C (Lit. (33) 159–160°C).  $\nu$  3204, 2952, 1662, 1608 cm<sup>-1</sup>. <sup>1</sup>H-NMR (CHCl<sub>3</sub>-d<sub>1</sub>) [300 K]  $\delta$  2.02 (p, *J* = 6.7 Hz, 2, 2  $\times$  H4); 2.85 (t, *J* = 6.7 Hz, 2, 2  $\times$  H3); 3.14 (q, *J* = 6.7 Hz, 2, 2  $\times$  H4); 3.85 (s, 3, CH<sub>3</sub>O); 6.72 (d, *J* = 2.3 Hz, 1, H6); 6.73 (br s, 1, NH); 6.86 (dd, *J* = 8.5, 2.3 Hz, 1, H8); 7.69 (d, *J* = 8.5 Hz, 1, H9). <sup>13</sup>C-NMR (CHCl<sub>3</sub>-d<sub>1</sub>)  $\delta$  30.4, 30.8, 55.3, 111.8, 127.5, 130.9, 140.6, 161.9, 174.1. *m/z* 191 (M<sup>+</sup>, 74%), 163 (22), 162 (57), 161 (100), 148 (29), 134 (33), 91 (33), 77 (28), 63 (27), 51 (22).

**Ethyl 2-(7-methoxy-2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl)ethanoate (20).** A solution of **15** (109 mg, 0.57 mmol) in dry tetrahydrofuran (THF) (5 ml) was treated with sodium hydride (20.5 mg, 0.86 mmol) and the suspension was stirred at room temperature for 1 h. Ethyl-2-bromoacetate (63 ml, 0.57 mmol) was added and stirring continued for 1 h. The reaction mixture was concentrated, the residue was treated with water (30 ml) and was extracted with chloroform (3  $\times$  30 ml). The combined organic extracts were washed sequentially with saturated brine (50 ml) and water (2  $\times$  50 ml), and then dried and concentrated to yield an orange oil. This was subjected to radial chromatography using chloroform/methanol (49:1) as eluent. The component of *R<sub>f</sub>* 0.76 afforded **20** (155 mg, 98%) as a

clear viscous oil which decomposed on attempted vacuum distillation (195°C/0.1 Torr). Found:  $M^{+}$  277.1339;  $C_{15}H_{19}NO_4$  requires  $M^{+}$  277.1315. IR  $\nu$  3400 br, 2964, 1750, 1668, 1582, 1502  $cm^{-1}$ .  $^1H$ -NMR ( $CHCl_3$ - $d_1$ ) [330K]  $\delta$  1.24 (t,  $J$  = 7.2 Hz, 3,  $CH_3CH_2$ ); 2.16 (p,  $J$  = 6.5 Hz, 2,  $2 \times H_4$ ); 2.32 (t,  $J$  = 6.5 Hz, 2,  $2 \times H_3$ ); 2.89 (t,  $J$  = 6.5 Hz, 2,  $2 \times H_5$ ); 3.80 (s, 3,  $CH_3O$ ); 4.17 (q,  $J$  = 7.2 Hz, 2,  $CH_3CH_2$ ); 4.45 (s, 2,  $\alpha CH_2$ ); 6.74 (d,  $J$  = 3.0 Hz, 1,  $H_6$ ); 6.77 (dd,  $J$  = 8.5, 3.0 Hz, 1,  $H_8$ ); 7.05 (d,  $J$  = 8.5 Hz, 1,  $H_9$ ).  $^{13}C$ -NMR ( $d_1$ - $CHCl_3$ ) [295K]  $\delta$  14.1, 28.5, 30.0, 32.7, 50.0, 55.4, 61.2, 112.4, 114.8, 123.2, 135.3, 137.4, 157.8, 169.2, 173.4. MS  $m/z$  277 ( $M^{+}$ , 34%), 204 (100), 176 (24), 57 (24), 43 (23).

**2-(7-Methoxy-2-oxo-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl)ethanoic acid (12).** A suspension of **20** (102 mg, 0.38 mmol) in aqueous sodium hydroxide (2 M, 10 ml) and THF (10 ml) was heated at reflux for 2.5 h. The reaction mixture was cooled to room temperature and acidified to pH 1 with aqueous hydrochloric acid (2 M), and the solution was extracted with chloroform ( $3 \times 30$  ml). The combined organic extracts were dried and concentrated and the residue was recrystallized from aqueous methanol to yield the title compound (65 mg, 69%) as colorless microneedles mp 186–188°C. Found:  $M^{+}$  249.1009;  $C_{13}H_{15}NO_4$  requires  $M^{+}$  249.1009. IR  $\nu$  2950 br, 2964, 1749, 1664, 1582, 1502  $cm^{-1}$ .  $^1H$ -NMR ( $CHCl_3$ - $d_1$ ) [300K]  $\delta$  2.22–2.26 (m, 2,  $2 \times H_4$ ); 2.31 (t,  $J$  = 6.5 Hz, 2,  $2 \times H_3$ ); 2.80–2.84 (m, 2,  $2 \times H_5$ ); 3.77 (s, 3,  $CH_3O$ ); 4.45 (s, 2,  $\alpha CH_2$ ); 6.71 (d,  $J$  = 2.0 Hz, 1,  $H_6$ ); 6.76 (dd,  $J$  = 9.0, 2.0 Hz, 1,  $H_8$ ); 7.06 (d,  $J$  = 9.0 Hz, 1,  $H_9$ ).  $^{13}C$ -NMR ( $CHCl_3$ - $d_1$ ) [295K]  $\delta$  28.5, 30.0, 32.6, 50.1, 55.4, 61.2, 112.4, 114.9, 123.3, 135.3, 137.4, 158.1, 171.9, 174.2.

**Ethyl 2-(7-methoxy-1-oxo-2,3,4,5-tetrahydro-1H-2-benzazepin-2-yl)ethanoate (19).** A solution of **14** (109 mg, 0.57 mmol) in dry THF (5 ml) was treated with sodium hydride (20.5 mg, 0.86 mmol) and ethyl 2-bromoacetate (63 ml, 0.57 mmol) as described for (**20**) above. After workup, the component of  $R_f$  0.76 afforded the *N*-alkylated lactam (155 mg, 98%) as a clear viscous oil. Found:  $M^{+}$  277.1337;  $C_{15}H_{19}NO_4$  requires  $M^{+}$  277.1315. IR  $\nu$  (film) 3400 br, 2964, 1750, 1668, 1582  $cm^{-1}$ .  $^1H$ -NMR ( $CHCl_3$ - $d_1$ ) [330K]  $\delta$  1.31 (t,  $J$  = 7.2 Hz, 3,  $CH_3CH_2$ ); 2.08 (p,  $J$  = 6.8 Hz, 2,  $2 \times H_4$ ); 2.89 (t,  $J$  = 6.8 Hz, 2,  $2 \times H_3$ ); 3.31 (t,  $J$  = 6.8 Hz, 2,  $2 \times H_5$ ); 3.85 (s, 3H),  $CH_3O$ ; 4.23 (q,  $J$  = 7.2 Hz, 2,  $CH_3CH_2$ ); 4.35 (s, 2,  $2 \times H\alpha$ ); 6.70 (d,  $J$  = 2.5 Hz, 1,  $H_6$ ); 6.64 (dd,  $J$  = 8.5, 2.5 Hz, 1,  $H_8$ ); 7.66 (d,  $J$  = 8.5 Hz, 1,  $H_9$ ).  $^{13}C$ -NMR ( $CHCl_3$ - $d_1$ ) [295K]  $\delta$  14.2, 29.5, 30.6, 48.1, 49.6, 55.3, 61.3, 111.7, 114.1, 127.9, 130.9, 140.2, 161.7, 169.8, 171.7. MS  $m/z$  277 ( $M^{+}$ , 31%), 204 (100), 177 (24).

**2-(7-Methoxy-1-oxo-2,3,4,5-tetrahydro-1H-2-benzazepin-2-yl)ethanoic acid (II).** A suspension of (**19**) (150 mg, 1.26 mmol) in aqueous sodium hydroxide (2 M, 10 ml) and THF (10 ml) was heated at reflux for 5 h. The reaction mixture was treated as for (**12**) to yield the title compound (65 mg, 69%) as colorless microneedles after recrystallization from methanol, mp 183–185°C. Found: C, 62.3; H, 6.6; N, 5.8 ( $M^{+}$  249.1024);  $C_{13}H_{15}NO_4$  requires  $M^{+}$  C, 62.6; H, 6.1; N, 5.6 ( $M^{+}$  249.1001). IR  $\nu$  3600–2500, 2872, 1746, 1641, 1608, 1575  $cm^{-1}$ .  $^1H$ -NMR ( $MeOH$ - $d_4$ ) [293K]  $\delta$  2.09 (p,  $J$  = 6.8 Hz, 2,  $2 \times H_4$ ); 2.87 (t,  $J$  = 6.8 Hz, 2,  $2 \times H_3$ ); 3.30 (t,  $J$  = 6.8 Hz, 2,  $2 \times H_5$ ); 3.83 (s, 3,  $CH_3O$ ); 4.31 (s, 2,  $2 \times H\alpha$ ); 6.79

(d,  $J = 2.5$  Hz, 1, H6); 6.88 (dd,  $J = 8.5, 2.5$  Hz, 1, H8); 7.53 (d,  $J = 8.5$  Hz, 1, H9).

**7-Methoxy-2,3,4,5-tetrahydro-1H-1-benzazepine (16).** A solution of the lactam (**15**) (497 mg, 2.60 mmol) in dry THF (30 ml) was carefully added to a room-temperature suspension of lithium aluminium hydride (200 mg, 5.20 mmol) in THF (30 ml). After being heated at reflux for 12 h, the reaction mixture was cooled to room temperature and cautiously treated with a saturated solution of ammonium chloride. The mixture was filtered and extracted with diethyl ether ( $3 \times 100$  ml) and the combined extracts were washed with saturated brine (100 ml) and water (100 ml) and then dried and concentrated. The residue was subjected to radial chromatography using chloroform/methanol/ammonia (aq) (94:5:1) as eluent. Isolation of the major component (TLC  $R_f$  0.61) afforded a clear oil (428 mg, 93%). The amine was used directly in subsequent steps. A small quantity of **16** was treated with ethereal hydrogen chloride to yield the hydrochloride salt, mp 201.5–203°C (Lit. (**16**) 203–204°C). Found:  $M^{+}$  177.1131,  $C_{11}H_{15}NO$  requires  $M^{+}$  177.1153. IR  $\nu$  3382, 2932, 1611  $cm^{-1}$ .  $^1H$ -NMR ( $CHCl_3$ - $d_1$ )  $\delta$  1.62–1.68 (m, 2,  $2 \times H4$ ); 1.77–1.84 (m, 2,  $2 \times H3$ ); 2.74–2.77 (m, 2,  $2 \times H2$ ); 2.99 (t,  $J = 5.3$  Hz, 2,  $2 \times H5$ ); 3.50 (br s, 1, NH); 3.76 (s, 3,  $OCH_3$ ); 6.60 (dd,  $J = 8.4, 2.7$  Hz, 1, H8); 6.68 (d,  $J = 8.4$  Hz, 1, H9); 6.72 (d,  $J = 2.7$  Hz, 1, H6).  $m/z$  177 ( $M^{+}$ , 26%), 149 (26), 148 (29), 147 (31), 117 (27), 97 (31), 95 (32), 81 (31), 71 (46), 69 (59), 57 (94), 55 (79), 43 (100).

**Benzyl 2-(7-methoxy-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl)ethanoate (18).** The amine (**16**) (159 mg, 0.90 mmol) in dry THF (5 ml) was treated with sodium hydride (158 mg, 1.26 mmol) and benzyl-2-bromoacetate (0.90 mmol). The reaction mixture was stirred at room temperature under nitrogen for 1 h. After removal of solvent *in vacuo*, the residue was partitioned between chloroform ( $3 \times 100$  ml) and water (50 ml). The combined organic extracts were dried and concentrated and the residue was purified by column chromatography using chloroform/methanol (95:5) as eluent. Isolation of the major component (TLC  $R_f$  0.73) afforded **18** as a viscous colorless oil (265 mg, 91%). Found  $M^{+}$  325.1670,  $C_{20}H_{23}NO_3$  requires  $M^{+}$  325.1676. IR  $\nu$  2936, 1742, 1610  $cm^{-1}$ .  $^1H$ -NMR ( $CHCl_3$ - $d_1$ ) [300K]  $\delta$  1.47–1.55 (m, 2,  $2 \times H4$ ); 1.82–1.90 (m, 2,  $2 \times H3$ ); 2.69–2.71 (m, 2,  $2 \times H2$ ); 2.91–2.95 (m, 2,  $2 \times H5$ ); 3.65 (s, 3,  $OCH_3$ ); 3.77 (s, 2,  $\alpha CH_2$ ); 5.10 (s, 2,  $BzI-CH_2$ ); 6.67 (dd,  $J = 8.6, 3.0$  Hz, 1, H8); 6.74 (d,  $J = 3.0$  Hz, 1, H6); 6.93 (d,  $J = 8.6$  Hz, 1, H9); 7.25–7.39 (m, 5,  $BzI-Ph$ ).  $^{13}C$ -NMR ( $CHCl_3$ - $d_1$ ) [300K]  $\delta$  25.6, 29.7, 35.0, 55.0, 55.4, 56.9, 66.3, 111.1, 116.1, 118.9, 128.2, 128.5, 128.7, 137.8, 144.6, 154.7, 171.5.  $m/z$  325 ( $M + 1^{+}$ , 7%), 325 ( $M^{+}$ , 32), 190 (100), 91 (34).

**2-(7-Methoxy-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl)ethanoic acid (10).** A solution of **18** (127 mg, 0.39 mmol) in dry ethanol (20 ml) was stirred at room temperature under an atmosphere of hydrogen in the presence of palladium on activated charcoal (10%, 100 mg) for 12 h. The reaction mixture was then filtered through a short column of celite and rinsed with portions of ethanol ( $3 \times 10$  ml). Concentration of the filtrate afforded a white solid which was recrystallized from methanol/diethyl ether to furnish **10** (100 mg, 89%) mp 161–165°C (dec). Found:  $M^{+}$  235.1180,  $C_{13}H_{17}NO_3$  requires  $M^{+}$  235.1208. IR  $\nu$  3500–2500 br, 2944, 1743, 1610  $cm^{-1}$ .  $^1H$ -NMR ( $MeOH$ - $d_4$ ) [300K]  $\delta$  1.75–1.85 (m, 2,  $2 \times H4$ ); 1.94–2.11

(m, 2, 2  $\times$  H<sub>3</sub>); 2.97–3.01 (m, 2, 2  $\times$  H<sub>2</sub>); 3.78–3.82 (m, 2, 2  $\times$  H<sub>5</sub>); 3.78 (s, 2,  $\alpha$ CH<sub>2</sub>); 3.80 (s, 3, OCH<sub>3</sub>); 6.89–6.97 (m, 3, H<sub>6</sub>, H<sub>8</sub>, and H<sub>9</sub>). <sup>13</sup>C-NMR (MeOH-d<sub>4</sub>) [300K]  $\delta$  26.1, 29.1, 30.0, 34.8, 55.2, 56.1, 111.6, 116.5, 120.2, 125.2, 138.2, 157.0, 172.7. *m/z* 235 (M<sup>+</sup>, 34%), 191 (44), 190 (100), 177 (22), 176 (27), 162 (32), 148 (26), 135 (38), 117 (37), 91 (68).

**7-Methoxy-2,3,4,5-tetrahydro-1H-2-benzazepine (7).** A solution of the 2-aza lactam (**14**) (503 mg, 2.65 mmol) in dry THF (30 ml) was carefully added to a room-temperature suspension of lithium aluminium hydride (254 mg, 6.68 mmol) in THF (30 ml). After being heated at reflux for 16 h, the reaction mixture was cooled to room temperature and cautiously treated with a saturated solution of ammonium chloride. The mixture was filtered and extracted with diethyl ether (3  $\times$  100 ml) and the combined extracts were washed with saturated brine (100 ml) and water (100 ml) and then dried and concentrated. The residue was subjected to radial chromatography using chloroform/methanol/ammonia (aq) (94:5:1) as eluent. Isolation of the major component (TLC R<sub>f</sub> 0.25) afforded **7** as a clear oil (377 mg, 81%), which was used without further purification in subsequent steps. A small quantity of **7** was treated with ethereal hydrogen chloride to yield the hydrochloride salt, mp 208–209°C (Lit. (16) 208–210°C). Found: M<sup>+</sup> 177.1165, C<sub>11</sub>H<sub>15</sub>NO requires M<sup>+</sup> 177.1154. IR  $\nu$  3450, 2938, 2848, 1611 cm<sup>-1</sup>. <sup>1</sup>H-NMR (CHCl<sub>3</sub>-d<sub>1</sub>)  $\delta$  1.71 (p, *J* = 5.2 Hz, 2, 2  $\times$  H<sub>4</sub>); 2.89 (t, *J* = 5.2 Hz, 2, 2  $\times$  H<sub>3</sub>); 3.18 (t, *J* = 5.2 Hz, 2, 2  $\times$  H<sub>5</sub>); 3.78 (s, 3, OCH<sub>3</sub>); 3.87 (s, 2, 2  $\times$  H<sub>1</sub>); 6.61 (dd, *J* = 8.1, 2.6 Hz, 1, H<sub>8</sub>); 6.71 (d, *J* = 2.6 Hz, 1, H<sub>6</sub>); 7.02 (d, *J* = 8.1 Hz, 1, H<sub>9</sub>). *m/z* 178 (M + 1<sup>+</sup>, 14%), 177 (M<sup>+</sup>, 96), 176 (70), 148 (100), 147 (60), 117 (27), 91 (29).

**Benzyl 2-(7-methoxy-2,3,4,5-tetrahydro-1H-2-benzazepin-2-yl)ethanoate (17).** The amine (315 mg, 1.78 mmol) in dry THF (10 ml) was treated with sodium hydride (128 mg, 1.98 mmol) and benzyl 2-bromoacetate (1.98 mmol). The reaction mixture was stirred at room temperature under nitrogen for 12 h. After removal of solvent *in vacuo*, the residue was partitioned between chloroform (3  $\times$  100 ml) and water (50 ml). The combined organic extracts were dried and concentrated and the residue was purified by radial chromatography using chloroform/methanol (95:5) as eluent. Isolation of the major component (TLC R<sub>f</sub> 0.60) afforded **17** as a viscous oil (376 mg, 65%). Found: M<sup>+</sup> 325.1669, C<sub>11</sub>H<sub>15</sub>NO requires M<sup>+</sup> 325.1667. IR  $\nu$  3500–3300 br, 2942, 1746, 1611 cm<sup>-1</sup>. <sup>1</sup>H-NMR (CHCl<sub>3</sub>-d<sub>1</sub>) [300K]  $\delta$  1.68–1.72 (m, 2, 2  $\times$  H<sub>4</sub>); 2.82–2.87 (m, 2, 2  $\times$  H<sub>3</sub>); 3.18–3.22 (m, 2, 2  $\times$  H<sub>5</sub>); 3.24 (s, 2,  $\alpha$ CH<sub>2</sub>); 3.77 (s, 3, OCH<sub>3</sub>); 3.97 (s, 2, 2  $\times$  H<sub>1</sub>); 5.16 (s, 2, Bzl-CH<sub>2</sub>); 6.57 (dd, *J* = 8.1, 2.6 Hz, 1, H<sub>8</sub>); 6.68 (d, *J* = 2.6 Hz, 1, H<sub>6</sub>); 6.88 (d, *J* = 8.1 Hz, 1, H<sub>9</sub>); 7.31–7.35 (m, 5, Bzl-Ph). <sup>13</sup>C-NMR (CHCl<sub>3</sub>-d<sub>1</sub>) [300K]  $\delta$  25.4, 29.7, 36.1, 54.7, 55.2, 58.6, 59.1, 66.2, 110.2, 115.4, 128.2, 128.4, 128.5, 130.6, 135.9, 144.2, 158.9, 171.0, *m/z* 325 (M<sup>+</sup>, 6%), 190 (100), 147 (46).

**2-(7-Methoxy-2,3,4,5-tetrahydro-1H-2-benzazepin-2-yl)ethanoic acid (8).** A solution of **17** (155 mg, 0.48 mmol) in dry ethanol (20 ml) was stirred at room temperature under an atmosphere of hydrogen in the presence of palladium on activated charcoal (10%, 100 mg) for 12 h. The reaction mixture was then filtered through a short column of celite and rinsed with portions of ethanol (3  $\times$  10 ml). Concentration of the filtrate afforded a white solid which was recrystallized from methanol/diethyl ether to furnish **8** (100 mg, 89%) mp 150–158° (dec). Found: M<sup>+</sup>

235.1202,  $C_{13}H_{17}NO_3$  requires  $M^{+}$  235.1208. IR  $\nu$  3500–2500 br, 1744, 1611  $cm^{-1}$ .  $^1H$ -NMR ( $CHCl_3$ - $d_1$ ) [300K]  $\delta$  1.99–2.05 (m, 2,  $2 \times H_4$ ); 2.56 (t,  $J = 7.5$  Hz, 2,  $2 \times H_3$ ); 2.97 (t,  $J = 8.0$  Hz, 2,  $2 \times H_5$ ); 3.54 (s, 2,  $\alpha CH_2$ ); 3.70 (s, 3,  $OCH_3$ ); 6.60 (dd,  $J = 8.3, 2.6$  Hz, 1, H8); 6.67 (d,  $J = 2.6$  Hz, 1, H6); 6.98 (d,  $J = 8.3$  Hz, 1, H9).  $^{13}C$ -NMR ( $D_2O$ ) [300K]  $\delta$  25.9, 35.7, 36.1, 56.1, 58.8, 61.1, 61.7, 115.0, 118.6, 136.2, 148.0, 163.3, 170.7.  $m/z$  235 ( $M^{+}$ , 31%), 190 (91), 148 (41), 147 (100), 135 (38), 117 (37), 91 (68).

### Peptide Synthesis

Solid-phase synthesis (34) was carried out using Pepsin K (Milligen) resin derivatized with Fmoc leucine. Construction of the peptide chain was carried out by the successive coupling of the appropriate amino acid symmetrical anhydride under standard conditions. Completeness of coupling was determined (35) before commencement of the subsequent cycles. Constraint structures were incorporated onto the resin-bound peptide chain as their corresponding 1-hydroxybenzotriazole (HOBT) esters. The general procedure for the preparation and coupling of the active esters is described below. The appropriate constraint acid in dichloromethane (0.01 M) at 0°C was treated with a solution of 1-hydroxybenzotriazole in dichloromethane (0.5 M, 1 eq) and a solution of *N,N'*-dicyclohexylcarbodiimide in dichloromethane (0.5 M, 1 eq) and then stirred for 1 h. The HOBT esters were used in subsequent coupling reactions without further purification. The resin-bound peptide [previously deprotected by treatment with piperazine (20% in DMF) and washed exhaustively with DMF] was treated with the HOBT ester (2 eq) and the reaction mixture agitated for 2.5–3 h. After filtration, the resin was washed with DMF ( $3 \times 10$  ml), dichloromethane ( $3 \times 10$  ml), methanol ( $3 \times 10$  ml), and diethyl ether ( $3 \times 10$  ml). Cleavage of the peptide from the resin was achieved by treatment with trifluoroacetic acid (TFA). A suspension of the resin in TFA at 0°C was stirred for 1.5 h; the reaction mixture was then filtered and the filtrate concentrated to yield the crude peptide. Purification of the crude peptides was achieved by RP-HPLC, in each instance the major fraction collected contained the desired peptide.

2-(7-Methoxy-2-oxo-2,3,4,5-tetrahydro-1*H*-1-benzazepin-1-yl)ethanyl-1-*L*-glycyl-*L*-phenylalanyl-*L*-leucine (**24**). The HOBT ester of **12** was prepared and coupled to the resin-bound glycyl-*L*-phenylalaninyl-*L*-leucine as described above. Isolation by RP-HPLC of the major component (RT 25 min 25 s) afforded the title compound (183 mg, 78%). HR-FABMS—Found:  $M^{+}$  567.2835;  $C_{30}H_{39}N_4O_7$  requires  $M^{+}$  567.2819.  $^1H$ -NMR ( $MeOH$ - $d_4$ ) [330K]  $\delta$  0.88 (d,  $J = 6.4$  Hz, 3, Leu- $\delta CH_3$ ); 0.91 (d,  $J = 6.9$  Hz, 3, Leu- $\delta CH_3$ ); 1.60–1.80 (m, 2, Leu- $\beta CH_2$ ); 1.62–1.78 (m, 1, Leu- $\gamma CH$ ); 2.10–2.19 (m, 2,  $2 \times$  Con- $H_4$ ); 2.29 (t,  $J = 7.0$  Hz, 2,  $2 \times$  Con- $H_3$ ); 2.80–2.84 (m, 2,  $2 \times$  Con- $H_5$ ); 2.95 (dd,  $J = 14.1, 9.1$  Hz, 1, Phe- $\beta CH_2$ ); 3.18 (dd,  $J = 14.1, 5.1$  Hz, 1, Phe- $\beta CH_2$ ); 3.76 (d,  $J = 16.8$  Hz, 1, Gly- $\alpha CH$ ); 3.78 (s, 3,  $CH_3O$ ); 3.86 (d,  $J = 16.8$  Hz, 1, Gly- $\alpha CH$ ); 4.35 (br s, 2,  $2 \times$  Con- $\alpha CH_2$ ); 4.40–4.45 (m, 1, Leu- $\alpha CH$ ); 4.65 (dd,  $J = 9.1, 5.1$  Hz, 1, Phe- $\alpha CH$ ); 6.79 (d,  $J = 28.6$  Hz, 1, Con- $H_6$ ); 6.84 (dd,  $J = 8.6, 2.8$  Hz, 1, Con  $H_8$ ); 7.16 (d,  $J = 8.7$  Hz, 1, Con  $H_9$ ); 7.15–7.23 (m, 5,  $5 \times$  Phe ArH). Amino acid analy-

sis—Found: Con, 1.0; Gly, 1.0; Leu, 1.0; Phe, 0.9. Structure **24** requires Con, 1.0; Gly, 1.0; Leu, 1.0; Phe, 1.0.

*2-(7-Methoxy-1-oxo-2,3,4,5-tetrahydro-1H-2-benzazepin-2-yl)ethanyl-1-L-phenylalanyl-L-leucine (23)*. The HOBt ester of **11** was prepared and reacted with the resin-bound glycyl-L-phenylalaninyl-L-leucine as described above. Isolation by RP-HPLC of the component of RT 27 min 17 s afforded the title compound (51.0 mg, 72%). HR-FABMS—Found:  $M + 1^{+}$  567.2814;  $C_{30}H_{39}N_4O_7$  requires  $M + 1^{+}$  567.2815.  $^1H$ -NMR (DMSO- $d_6$ ) [300K]  $\delta$  0.82 (d,  $J = 6.2$  Hz, 3, Leu- $\delta CH_3$ ); 0.87 (d,  $J = 6.2$  Hz, 3, Leu- $\delta CH_3$ ); 1.50–1.56 (m, 2, Leu- $\beta CH_2$ ); 1.59–1.65 (m, 1, Leu- $\gamma CH$ ); 1.89–1.95 (m, 2, 2  $\times$  Con H4); 2.73–2.81 (m, 3, 2  $\times$  Con-H3 and Phe- $\beta CH$ ); 3.05 (dd,  $J = 13.9, 3.5$  Hz, 1, Phe- $\beta CH$ ); 3.15 (t,  $J = 6.0$  Hz, 2, 2  $\times$  Con-H5); 3.58–3.73 (m, 2H, Gly- $\alpha CH_2$ ); 3.78 (s, 3,  $CH_3O$ ); 4.12 (s, 2, Con- $\alpha CH_2$ ); 4.18–4.22 (m, 1, Leu- $\alpha CH$ ); 4.50–4.55 (m, 1H, Phe- $\alpha CH$ ); 6.79 (d,  $J = 2.5$  Hz, 1, Con-H6); 6.86 (dd,  $J = 8.4, 2.5$  Hz, 1H, Con-H8); 7.16–7.27 (m, 5, 5  $\times$  Phe-ArH); 7.44 (d,  $J = 8.5$  Hz, 1, Con-H9); 7.80–8.00 (m, 1, Gly-NH); 8.16–8.21 (m, 2H, Leu-NH and Phe-NH). Amino acid analysis—Found: Con, 1.0; Gly, 1.1; Leu, 1.1; Phe, 1.0. Structure **23** requires Con, 1.0; Gly, 1.0; Leu, 1.0; Phe, 1.0.

*2-(7-Methoxy-2,3,4,5-tetrahydro-1H-1-benzazepin-1-yl)ethanyl-1-L-glycyl-L-phenylalanyl-L-leucine (22)*. The HOBt ester of **10** was prepared and reacted with the resin-bound glycyl-L-phenylalaninyl-L-leucine as described above. Isolation by RP-HPLC of the major component (RT 22 min 35 s) afforded the title compound (17 mg, 73%). HR-FABMS Found  $M + 1^{+}$  553.3029;  $C_{30}H_{40}N_4O_6$  requires  $M + 1^{+}$  553.3025.  $^1H$ -NMR (MeOH- $d_4$ ) [300K]  $\delta$  0.89 (d,  $J = 6.3$  Hz, 3, Leu- $\delta CH_3$ ); 0.92 (d,  $J = 6.3$  Hz, 3, Leu- $\delta CH_3$ ); 1.57–1.75 (m, 5, 2  $\times$  Con-H4, Leu- $\beta CH_2$ , Leu- $\gamma CH$ ); 1.78–1.86 (m, 2, 2  $\times$  Con-H3); 2.72–2.84 (m, 2H, 2  $\times$  Con-H2); 2.90 (dd,  $J = 12.5, 4.5$  Hz, 1, Phe- $\beta CH$ ); 3.15 (dd,  $J = 12.5, 3.2$  Hz, 1, Phe- $\beta CH$ ); 3.29–3.35 (m, 2H, 2  $\times$  Con-H5); 3.73 (s, 3,  $CH_3O$ ); 3.79 (s, 2, Con- $\alpha CH_2$ ); 4.06 (s, 2, Gly- $\alpha CH_2$ ); 4.55–4.60 (m, 1, Leu- $\alpha CH$ ); 4.71–4.78 (m, 1, Phe- $\alpha CH$ ); 6.64–6.72 (m, 2, Con-H8 and Con-H9); 6.93 (br s, 1, Con-H6); 7.16 (d,  $J = 8.7$  Hz, 1, Con-H9); 7.15–7.23 (m, 5, Phe-ArH). Amino acid analysis. Found Con, 1.0; Gly, 1.0; Leu, 1.1; Phe, 0.9. Structure **22** requires Con, 1.0; Gly, 1.0; Leu, 1.0; Phe, 1.0.

*2-(7-Methoxy-2,3,4,5-tetrahydro-1H-2-benzazepin-2-yl)ethanyl-1-L-glycyl-L-phenylalanyl-L-leucine (21)*. The HOBt ester of **8** was prepared and coupled to the resin-bound glycyl-L-phenylalaninyl-L-leucine as described above. Isolation by RP-HPLC of the major component (RT 23 min 30 s) afforded the title compound (9.5 mg, 69%). HR-FABMS—Found:  $M + 1^{+}$  553.2994;  $C_{30}H_{40}N_4O_6$  requires  $M + 1^{+}$  553.3025.  $^1H$ -NMR (MeOH- $d_4$ ) [305K]  $\delta$  0.91 (d,  $J = 6.1$  Hz, 3H, Leu- $\delta CH_3$ ); 0.95 (d,  $J = 6.1$  Hz, 3, Leu- $\delta CH_3$ ); 1.63 (t,  $J = 6.9$  Hz, 2, Leu- $\beta CH_2$ ); 1.62–1.67 (m, 1, Leu- $\gamma CH$ ); 1.95–2.05 (m, 2, 2  $\times$  Con-H4); 2.89 (dd,  $J = 9.2, 4.9$  Hz, 1, Phe- $\beta CH$ ); 3.21 (dd,  $J = 9.2, 4.8$  Hz, 1, Phe- $\beta CH$ ); 3.24–3.25 (m, 2, 2  $\times$  Con-H3); 3.53–3.65 (m, 2, 2  $\times$  Con H5); 3.71 (s, 2, Gly- $\alpha CH_2$ ); 3.79 (s, 3,  $CH_3O$ ); 4.42 (s, 2, 2  $\times$  Con-H1); 4.68–4.72 (m, 1, Leu- $\alpha CH$ ); 4.89–4.95 (m, 1, Phe- $\alpha CH$ ); 6.77 (dd,  $J = 8.2, 2.4$  Hz, 1, Con-H8); 6.84 (d,  $J = 2.4$  Hz, 1, Con-H6); 7.19 (d,  $J = 8.2$  Hz, 1, Con-H9); 7.23–7.27 (m, 5, 5  $\times$  Phe-ArH); 8.2 (br m, 1, Phe-NH); 8.3 (br m, 1, Leu-NH); 8.6 (br m, 1, Gly-NH); 10.0 (br s, 1, COOH). Amino acid

analysis—Found: Con, 1.0; Gly, 1.0; Leu, 1.0; Phe, 1.0. Structure **21** requires Con, 1.0; Gly, 1.0; Leu, 1.0; Phe, 1.0.

### *Receptor Binding Studies*

Radioligand-receptor binding experiments were conducted in triplicate and the results shown are derived from a minimum of two experiments. Radioligand, [<sup>3</sup>H]naloxone (42.7 Ci mmol<sup>-1</sup>), was purchased from New England Nuclear.

*Membrane preparation.* Male Sprague–Dawley rats (150–200 g) were killed by a swift blow to the head and the cerebral cortices isolated. The tissues were homogenized in 20 vol (w/v) of ice-cold 5 mM Tris/EDTA buffer (pH 7.2). Homogenates were centrifuged three times, each for 15 min at 1800 rpm using a Sorvall RC/5B centrifuge (SS34 head) at 4°C. The pellet was resuspended in fresh buffer between centrifugations and the final pellet of cerebral cortex resuspended in 30 vol (w/v) of assay buffer.

*Assay conditions and procedures.* Each incubation tube contained [<sup>3</sup>H]naloxone (cold ligand IC<sub>50</sub> 0.0045 ± 0.0002 μM), freshly resuspended membrane preparation (125 μl), and the test compound at a concentration in the range 3 × 10<sup>-3</sup> to 1 × 10<sup>-7</sup> M (five concentrations per experiment). The assay mixtures were filtered through Whatman GF/B glass fiber filters under vacuum after incubation (30 min). Filters were washed three times with aliquotes of ice-cold Tris buffer (2 ml, 50 mM; pH 7.5 at 25°C), dried, and placed in scintillation vials together with scintillation cocktail [10 ml, 5-diphenyloxazole (0.4%) and 1,4-bis(5-phenyloxazol-2-yl)benzene (0.1%) in toluene]. The vials were allowed to stand overnight before being counted in a Packard Tri-carb 460C scintillation counter with automatic quench correction at 55–58% efficiency. The specific binding of a test compound was calculated as the deficit over the blanks containing the appropriate concentration (36) of the defining ligand (laevorphanol). The specific binding was found to be within 50–65% of the total binding of the ligands. The computer program EBDA (37) was employed for the generation of Scatchard and Hill plots of receptor binding data and for the analysis of saturation experiments. Standard deviations of data cited all fell within ±0.4.

## RESULTS AND DISCUSSION

### *Design and Molecular Modeling*

The opioid analgesic-like activity of **2** suggested its use as a mimic of the tyrosine residue of leucine enkephalin (**3**). It was proposed that the *N*-alkyl side chain of **2** could be elaborated with a suitable functional handle, e.g., a carboxyl group, for its ready incorporation into a peptide chain. As the primary objective was to ascertain whether the 2-benzazepine moiety per se functioned in a peptidic context, the synthetically more accessible *O*-methyl derivatives of **2** were selected as the target structures. A correlation between the opioid receptor affinities of these and their phenolic equivalents was deemed possible by comparison to those of leucine