An Efficient Preparation of the Pseudopeptide Endothelin-B Receptor Selective Antagonist BQ-788

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The endothelins are a family of bicyclic 21 amino acid peptides that are potent and prolonged vasoconstrictors. BQ-788 is a modified tripeptide that is the only known highly potent and selective antagonist for the endothelin-B (ET_B) receptor subtype discovered to date. Previous preparations of BQ-788 (*N*-(*cis*-2,6-dimethylpiperidinocarbonyl)-γ-methylleucyl-D-1-(methoxycarbonyl)tryptophanyl-D-norleucine sodium salt) have suffered from several synthetic difficulties, including formation of the sterically hindered *N*-(*cis*-2,6-dimethylpiperidinocarbonyl)-γ-methylleucine, racemization of tryptophan during carbamination, and the facile reduction of the indole ring of tryptophan during catalytic hydrogenation. In order to prepare sufficient quantities of BQ-788 for *in vitro* and *in vivo* evaluations of the physiological significance of the ET_B receptor, we have developed an efficient solution phase multiple-gram synthetic strategy.

Introduction

Endothelin-1 (ET-1, Figure 1) is a potent peptidic constrictor of vascular smooth muscle that was first isolated and characterized from the supernatant of porcine endothelial cells.[1] ET-1 is one member of a family of isopeptides that includes ET-2 and ET-3 along with the structurally and functionally related mouse vasoactive intestinal contractor (VIC),[2] the cardiotoxic sarafotoxins (SRTXs),[3] and bibrotoxin.[4] All members of this family possess disulfide bridges between positions 1–15 and 3–11, in addition to a highly conserved C-terminal hydrophobic hexapeptide, His^{16}-Xxx-Asp-Yyy-Ile-Trp^{21} (Xxx = Leu or Gln, Yyy = Ile or Val).[5] The recent identification of potent and specific endothelin receptor antagonists will undoubtedly assist in determining the physiological and/or pathophysiological roles of endothelin and its isopeptides.

Initially, two endothelin receptors were cloned, sequenced, and characterized from the bovine and rat lung, respectively.[6] The ET_A receptor is selective for ET-1 and ET-2 over ET-3, and the ET_B receptor possesses equal affinity for all of the ET isopeptides. Subsequently, the corresponding human receptors have been cloned.[7] The endothelin receptor subtype populations (ET_A/ET_B) are

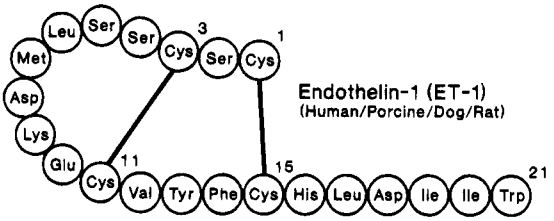

Figure 1.

widely distributed in several tissues and possess different functions dependent upon species and location. For example, utilizing a highly selective ET_B receptor ligand, sarafotoxin-6c (SRTX-6c),[8] the ET_B receptor has been linked to vasodilation in the rat aortic ring[9] while it is functionally linked to vasoconstriction in several other tissues.[10] Recently, the existence of additional ET_B receptor subtypes and/or species differences have been reported.[11]

The identification of these receptors has facilitated the development of peptidic and nonpeptidic endothelin antagonists for the ET_A receptor or combined antagonists for both the ET_A and ET_B receptors (for recent reviews, see ref 5). However, there is a paucity of ET_B receptor selective antagonists. For example, IRL 1038 (cyclo[Cys-Val-Tyr-Phe-Cys]-His^{16}-Leu-Asp-Ile-Ile-Trp^{21} (cyclic disulfide)) was initially reported to exhibit only micromolar affinity for the ET_A receptor in a variety of species but

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(1) (a) Hickey, K. A.; Rubanyi, G.; Paul, R. J.; Highsmith, R. F. *Am. J. Physiol.* **1985,** *248*, C550. (b) Yanagisawa, M.; Kurihara, H.; Kimura, S.; Tomobe, Y.; Kobayashi, M.; Mitsui, Y.; Goto, K.; Masaki, T. A. *Nature (London)* **1988,** *332*, 411. (c) Yanagisawa, M.; Inoue, A.; Ishikawa, T.; Kasuya, T.; Kimura, S.; Kumagaye, S.; Nakajima, K.; Watanabe, T. X.; Sakakibara, S.; Goto, K.; Masaki, T. *Proc. Natl. Acad. Sci. U.S.A.* **1989,** *86*, 6964. (d) Inoue, A.; Yanagisawa, M.; Kimura, S.; Kasuya, Y.; Miyauchi, T.; Goto, K.; Masaki, T. *Proc. Natl. Acad. Sci. U.S.A.* **1989,** *86*, 2863.

(2) Saida, K.; Mitsui, Y.; Ishida, N. *J. Biol. Chem.* **1989**, *264*, 14613.

(3) (a) Kloog, T.; Ambar, I.; Sokolovsky, M.; Kochva, E.; Wolberg, Z.; Bdolah, A. *Science* **1988**, *242*, 268. (b) Bdolah, A.; Wolberg, Z.; Fleminger, G.; Kochva, E. *FEBS Lett.* **1989**, *251*, 1.

(4) Becker, A.; Dowdle, E. B.; Hechler, U.; Kauser, K.; Donner, P.; Schleuning, W. D. *FEBS Lett.* **1993**, *315*, 100.

(5) Doherty, A. M. *J. Med. Chem.* **1992**, *35*, 1493. (b) Huggins, J. P.; Pelton, J. T.; Miller, R. C. *Pharmacol. Ther.* **1993**, *59*, 55. (c) Cheng, X. M.; Nikam, S. S.; Doherty, A. M. *Curr. Med. Chem.* **1995**, *1*, 271.

(6) (a) Arai, H.; Hori, T.; Aramori, I.; Ohkubo, H.; Nakanishi, S. *Nature (London)* **1990**, *348*, 730. (b) Sakurai, T.; Yanagisawa, M.; Takuwa, Y.; Miyazaki, H.; Kimura, S.; Goto, K.; Masaki, T. *Nature (London)* **1990**, *348*, 732.

(7) (a) Sakamoto, A.; Yanagisawa, M.; Sakurai, T.; Takuwa, Y.; Yanagisawa, H.; Masaki, T. *Biochem. Biophys. Res. Commun.* **1991**, *178*, 656. (b) Hosoda, K.; Nakao, K.; Arai, H.; Suga, S.; Ogawa, Y.; Mukoyama, M.; Shirakami, G.; Saito, T.; Nakanishi, S.; Imura, H. *FEBS Lett.* **1991**, *287*, 23.

(8) Williams, D. L.; Jones, K. L.; Pettibone, D. L.; Lis, E. V.; Clineschmidt, B. V. *Biochem. Biophys. Res. Commun.* **1991**, *175*, 556.

(9) Takayanagi, R.; Kitazumi, K.; Takasaki, C.; Ohnaka, K.; Aimoto, S.; Tasaka, K.; Ohashi, M.; Nawata, H. *FEBS. Lett.* **1991**, *282*, 103.

(10) (a) Saeki, T.; Ihara, M.; Fukuroda, T.; Yamagiwa, M.; Yano, M. *Biochem. Biophys. Res. Commun.* **1991,** *179*, 286. (b) Panek, R. L.; Major, T. C.; Hingorani, G. P.; Doherty, A. M.; Taylor, D. G.; Rapundalo, S. T. *Biochem. Biophys. Res. Commun.* **1992**, *183*, 566.

(11) (a) Sudjarwo, S. A.; Hori M.; Takai, M.; Urade, Y.; Okada, T.; Karaki, H. *Life Sci.* **1993**, *53*, 431. (b) Warner, T. D.; Allcock, G. H.; Corder, R.; Vane, J. R. *Br. J. Pharmacol.* **1993**, *110*, 777. (c) Reynolds, E. E.; Hwang, O.; Flynn, M. A.; Welch, K. M.; Cody, W. L.; Steinbaugh, B.; He, J. X.; Chung, F. Z.; Doherty, A. M. *Biochem. Biophys. Res. Commun.* **1995**, *209*, 506.

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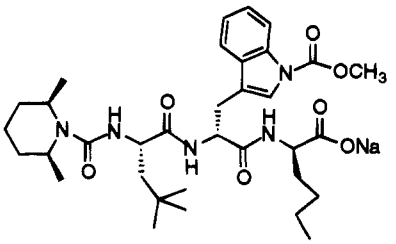


BQ-788, 1

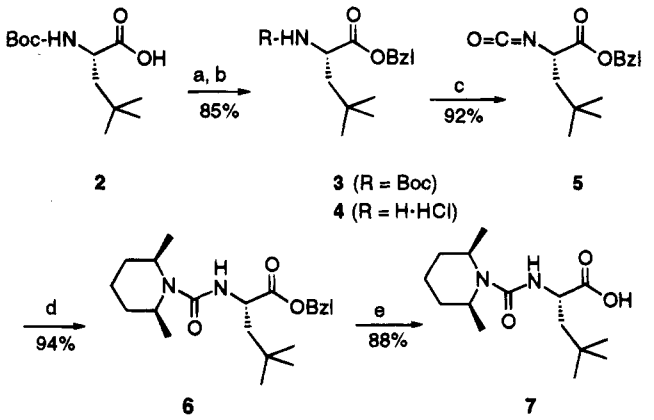
Figure 2.

low nanomolar affinity for the ET_B receptor.[12] However, current studies have shown that IRL 1038 only possesses low micromolar affinity for either the ET_A or ET_B receptor subtypes, in similar systems/tissues.[13]

Recently, RES-701-1 (cyclo[Gly-Asn-Trp-His-Gly-Thr-Ala-Pro-Asp]-Trp-Phe-Phe-Asn-Tyr-Tyr-Trp (cyclized via a lactam bridge between the α-amine of Gly and the β-carboxylate of Asp)) was isolated from *Streptomyces* sp. This compound was reported to exhibit 10 nM affinity for the human ET_B receptor but greater than 5 μM affinity for the human ET_A receptor, both stably expressed in Chinese hamster ovary (CHO) cells.[14] However, we have shown that synthetic preparations of RES-701-1 only possess low micromolar affinity for each of the human endothelin receptor subtypes.[15]

In contrast, it has been reported by Banyu Pharmaceutical Co. Ltd. that *N*-(*cis*-2,6-dimethylpiperidinocarbonyl)-γ-methylleucyl-D-1-(methoxycarbonyl)tryptophanyl-D-norleucine sodium salt (BQ-788, compound **1**, Figure 2) is highly selective for the human ET_B receptor [IC_{50} = 1300 nM for ET_A receptors in a human neuroblastoma cell line (SKN-N-MC) and IC_{50} = 1.2 nM for ET_B receptors in human Girardi heart cells].[16] We have subsequently confirmed the potency and selectivity [ET_A = 370 nM (Ltk cells stably transfected with the human receptor); ET_B = 1.8 nM (CHO cells stably transfected with the human receptor)];[17] thus, BQ-788 represents the first compound known that is truly a highly ET_B receptor selective antagonist. Therefore, we have developed a highly efficient synthesis of BQ-788 that is capable of producing multiple-gram quantities to assist in further *in vitro* and *in vivo* evaluations of the physiological/pathophysiological role of the ET_B receptor. All manipulations are high yielding and have overcome several synthetic problems that have been encountered in previously reported preparations of BQ-788, including the formation of the sterically hindered *N*-(*cis*-2,6-dimethylpiperidinocarbonyl)-γ-methylleucine,[16a] the racemization of tryptophan during carbamination,[16a] and the facile reduction of the indole ring of tryptophan during catalytic hydrogenation.

Scheme 1[a]



[a] Conditions: (a) $PhCH_2Br$, $CsCO_3$, DMF (91%); (b) HCl/*p*-dioxane (94%); (c) diphosgene, charcoal, toluene, relux; (d) *cis*-dimethyldipiperidine, EtOAc; (e) H_2, 20%Pd/C, MeOH, 50 psi.

Results and Discussion

Preparation of *N*-(*cis*-2,6-Dimethylpiperidinocarbonyl)-γ-methylleucine (7, Scheme 1). Starting with N^{α}-(*tert*-butyloxycarbonyl)-γ-methylleucine[18] (N^{α}-tBoc-γ-MeLeu, **2**) the title compound was prepared in 64% overall yield. Initially, N^{α}-tBoc-γ-MeLeu was converted to its benzyl ester (**3**) via the cesium salt and the N^{α}-tBoc protecting group was removed with saturated HCl in *p*-dioxane (**4**). The corresponding isocyanate (**5**) prepared from reaction with diphosgene and activated charcoal in refluxing toluene could be readily condensed with *cis*-2,6-dimethylpiperidine to yield the benzyl ester of the title compound (**6**). *N*-(*cis*-2,6-dimethylpiperidinocarbonyl)-γ-methylleucine (**7**) was isolated after catalytic hydrogenation with 20% palladium on carbon (Pd/C) in methanol. An alternative strategy for the preparation of **7** involved the reaction of *cis*-2,6-dimethylpiperidine with the phenyl carbamate of γ-methylleucine benzyl ester, however no reaction was observed.[16a]

Preparation of D-1-(Methoxycarbonyl)tryptophan-benzyl Ester Hydrochloride (11, Scheme 2). Starting with N^{α}-(*tert*-butyloxycarbonyl)-D-tryptophan (N^{α}-tBoc-D-Trp, **8**) the title compound was prepared in 68% overall yield. Compound **8** was converted to its benzyl ester (**9**) by condensation of benzyl alcohol with the activated ester of N^{α}-tBoc-D-Trp, formed by the reaction of the water soluble carbodiimide ethyl(3-(3-dimethylamino)propyl)carbodiimide hydrochloride (EDC·HCl), *N*-methylmorpholine (NMM), and *N*-hydroxybenzotriazole (HOBt) in DMF. Carbamination of the indole nitrogen of compound **9** was achieved using dimethyl dicarbonate in the presence of 4-(dimethylamino)pyridine (DMAP) in acetonitrile (90%). The N^{α}-tBoc group was removed with saturated HCl in dioxane to yield D-1-(methoxycarbonyl)-tryptophan benzyl ester hydrochloride (**11**).

(12) Urade, Y.; Fujitani, Y.; Oda, K.; Watakabe, T.; Umemura, I.; Takai, M.; Okada, T.; Sakata, K.; Karaki, H. *FEBS Lett.* **1993**, *311*, 12.

(13) (a) Cody, W. L.; He, J. X.; DePue, P. L.; Rapundalo, S. T.; Hingorani, G. P.; Dudley, D. T.; Hill, K. E.; Reynolds, E. E.; Doherty, A. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 567. (b) Urade, Y.; Fujitani, T.; Oda, K.; Watakabe, T.; Umemura, I.; Takai, M.; Okada, T.; Sakata, K.; Karaki, H. *FEBS Lett.* **1994**, *342*, 103.

(14) (a) Morishita, Y.; Chiba, S.; Tsukuda, E.; Tanaka, T.; Ogawa, T.; Yamasaki, M.; Yoshida, M.; Kawamoto, I.; Matsuda, Y. *J. Antibiot.* **1994**, *47*, 269. (b) Yamasaki, M.; Yano, K.; Yoshida, M.; Matsuda, Y.; Yamaguchi, K. *J. Antibiot.* **1994**, *47*, 276.

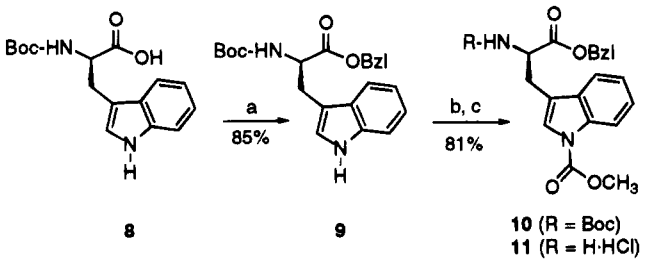
(15) He, J. X.; Cody, W. L.; Flynn, M. A.; Welch, K. M.; Reynolds, E. E.; Doherty, A. M. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 621.

(16) (a) Ishikawa, K.; Fukami, T.; Nagase, T.; Mase, T.; Ihara, M.; Yano, M.; Nishikibe, M. Eur. Patent Appl. 555 537 A2, 1993. (b) Karaki, H.; Sudjarwo, S. R.; Hori, M. *Biochem. Biophys. Res. Commun.* **1994**, *205*, 168. (c) Ishikawa, K.; Ihara, M.; Noguchi, K.; Mase, T.; Mino, N.; Saeki, T.; Fukuroda, T.; Fukami, T.; Ozaki, S.; Nagase, T.; Nishikibe, M.; Yano, M. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 4892. (d) D'Orleans-Juste, P.; Claing, A.; Telemaque, S.; Maurice, M. C.; Yano, M.; Gratton, J. P. *J. Pharmacol.* **1994**, *113*, 1257.

(17) Reynolds, E. E.; Hwang, O.; Flynn, M. A.; Welch, K. M.; Cody, W. L.; Steinbaugh, B.; He, J. X.; Chung, F. Z.; Doherty, A. M. *Biochem. Biophys. Res. Commun.* **1995**, *209*, 506.

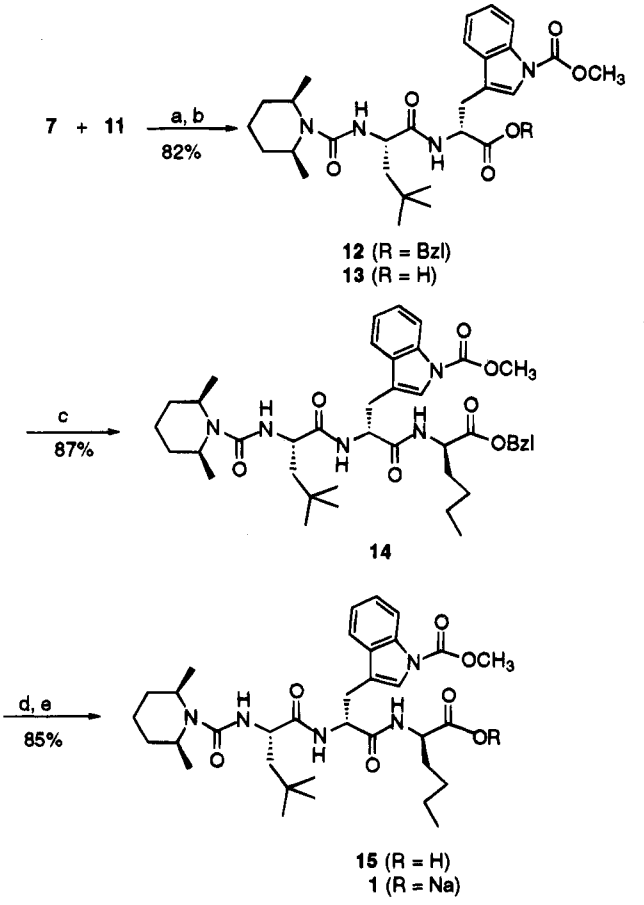
(18) N^{α}-(*tert*-Butyloxycarbonyl)-γ-methylleucine (**2**) was obtained from Bachem BioScience Inc., King of Prussia, PA.

Scheme 2[a]



[a] Conditions: (a) $PhCH_2OH$, EDC·HCl, HOBt, NMP; (b) dimethyl dicarbonate, DMAP,CH_3CN (90%); (c) HCl/*p*-dioxane (90%).

Scheme 3[a]



[a] Conditions: (a) EDC·HCl, HOBt, NMM, DMF (92%); (b) H_2, 5% Pd/C, Et_2O (89%); (c) HCl·D-Nle-OBzl, HBTU, DIEA, DMF; (d) H_2, 5%Pd/C, Et_2O (85%); (e) 5% $NaHCO_3/H_2O$ (>99%).

Several additional conditions were evaluated for the carbamination of N^{α}-tBoc-D-Trp benzyl ester (**9**), including the literature procedure.[16a] Utilizing methyl chloroformate under phase-transfer conditions (45% aqueous sodium hydroxide and tetrabutylammonium hydrogen sulfate in dichloromethane) resulted in complete racemization at the α carbon of tryptophan.[16a] Unfortunately, this side reaction could not be detected until **11** was coupled to **7** to form the diastereomeric dipeptides **12** (Scheme 3). The diastereomeric dipeptides were readily resolved by analytical reversed-phase (RP) HPLC, but these hydrophobic dipeptides could not be purified in reasonable quantities by preparative RP-HPLC without significant losses of the desired compound. Other attempts at carbamination with methyl chloroformate with sodium hydride (NaH) in THF or DMAP in DMF resulted in low yields of the desired product (25% and 0% yield, respectively). In addition, other conditions utilizing dimethyl dicarbonate were examined such as NaH/THF (25% yield), *n*-butyllithium (nBuLi)/THF (20% yield), and DMAP/DMF (25% yield), but all resulted in relatively low yields and difficult-to-purify complex mixtures. Clearly, the use of dimethyl dicarbonate with DMAP in acetonitrile is the condition of choice for preparation of **11**.

Preparation of *N*-(*cis*-2,6-Dimethylpiperidinocarbonyl)-γ-methylleucyl-D-1-(methoxycarbonyl)tryptophanyl-D-norleucine Sodium Salt [1 (BQ-788), Scheme 3]. The target compound was assembled using solution phase peptide synthetic strategies in 60% overall yield from compound **7**. The dipeptide **12** was formed by coupling the protected amino acids **7** and **11** with a water soluble carbodiimide (EDC·HCl) in the presence of HOBt and NMM. The dipeptide free acid **13** was obtained by catalytic hydrogenation of **12**. Care must be exercized in that the C2–C3 bond of the indole ring of tryptophan was also partially reduced (~30%) using 20% Pd/C in methanol at 50 psi. However, 5% Pd/C in diethyl ether proved to be effective for this transformation. The protected tripeptide **14** was prepared by coupling HCl·D-norleucine benzyl ester (HCl·D-Nle-OBzl)[19] with 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) in the presence of diisopropylethylamine (DIEA). *N*-(*cis*-2,6-dimethylpiperidinocarbonyl-γ-methylleucyl-D-1-methoxy-carbonyl)tryptophanyl-D-norleucine (**15**) was obtained following catalytic hydrogenation of the C-terminal benzyl ester. In this case, the use of 20% Pd/C in methanol at 50 psi resulted in complete reduction of the C2–C3 bond of the indole ring of tryptophan. Once again, 5% Pd/C in diethyl ether proved to be effective for this transformation. In its fully protonated form, compound **15** is very insoluble in aqueous solvents and not suitable for *in vitro* and *in vivo* evaluations; therefore, **15** was quantitatively converted to its sodium salt (**1, BQ-788**).[20]

Conclusions

Herein, we have reported an efficient synthesis of the ET_B receptor selective antagonist BQ-788 (**1**), and to our knowledge this is the first report of a high-yielding racemization-free preparation. This preparation of BQ-788 is analytically (HPLC, MS, and NMR) and pharmacologically [ET_A = 370 nM (Ltk cells stably transfected with the human receptor); ET_B = 1.8 nM (CHO cells stably transfected with the human receptor)][17] indistinguishable from the originally described compound.[16] The synthetic strategy described has alleviated several problems that have been encountered in the preparation of this compound, including formation of the sterically hindered *N*-(*cis*-2,6-dimethylpiperidinocarbonyl)-γ-methylleucine,[16a] racemization of tryptophan during carbamination,[16a] and the facile reduction of the indole ring of tryptophan during catalytic hydrogenation. The availability of multiple-gram quantities of BQ-788 will assist

(19) HCl·D-Nle-OBzl was prepared from N^{α}-tBoc-D-Nle using the same procedure as for the preparation of compound **4** in 86% overall yield: ^1H NMR (400 MHz, DMSO-d_6) δ 0.90 (t, 3H), 1.12–1.40 (m, 4H), 1.80 (m, 2H), 4.02 (m, 1H), 5.02 (dd, 2H), 7.35–7.45 (m, 5H), 8.62 (br s, 3H); CIMS *m/z* 222 (M + H).

(20) He, J. X.; Cody, W. L.; Doherty, A. M. In *Innovation and Perspectives in Solid Phase Synthesis; Peptides, Proteins and Nucleic Acids; Biological and Biomedical Applications*; Epton, R., Ed.; Mayflower Worldwide Ltd., Birmingham, England, 1994; pp 365–70.

in the full characterization of the physiological/pathophysiological function of the ET_B receptor subtype.

Experimental Section

General Methods. ^1H NMR spectra were recorded on a 400 MHz NMR spectrometer with DMSO-d_6 or $CDCl_3$ as the solvent. Chemical ionization mass spectral (CIMS) data were obtained in acetonitrile:water (1:1) with 0.1% ammonium hydroxide, and electrospray mass spectral (ESMS) data were obtained using 1% ammonia in methane as the reagent gas. For analytical RP-HPLC two gradients were used; gradient A, 90:10 to 24:76, 0.1% aqueous TFA:0.1% TFA in acetonitrile, linear gradient over 22 min at 1.5 mL/min (λ = 214 and 280 nm); and gradient B, 80:20 to 14:86, 0.1% aqueous TFA:0.1% TFA in acetonitrile, linear gradient over 22 min at 1.5 mL/min (λ = 214 and 280 nm) on a Vydac 218TP54 (0.46 × 25.0 cm) C18 column. For preparative RP-HPLC, compounds were eluted utilizing a linear gradient of 0.1% trifluoroacetic acid (TFA)/water and increasing concentrations of 0.1% TFA/acetonitrile on a Vydac 218TP1022 (2.2 × 25.0 cm) C18 column. Synthetic reagents were obtained from commercial sources and used without further purification unless otherwise noted. Solvents for chemical synthesis were of reagent grade and solvents for RP-HPLC were of HPLC grade. All amino acids were of the L configuration unless otherwise indicated.

***N*$^\alpha$-(*tert*-Butyloxycarbonyl)-γ-methylleucine Benzyl Ester (*N*$^\alpha$-tBoc-γ-MeLeu-OBzl, 3).** To a solution of *N*$^\alpha$-tBoc-γ-MeLeu[18] (**2**) (4.0 g, 16.3 mmol) in DMF (10 mL) were added cesium carbonate (10.0 g, 31.6 mmol) and benzyl bromide (2.0 mL, 16.5 mmol). The reaction was stirred for 2 h at 23 °C, and 100 mL of ethyl acetate:water was added (1:1). The aqueous layer was separated and reextracted with ethyl acetate (2 × 50 mL). The organic layers were combined, washed with 10% $NaHCO_3$, brine, and water, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was triturated with hexane to yield **3** (5.10 g, 91%) as a white solid: HPLC t_R = 17.2 min (gradient B, >99%); ^1H NMR (400 MHz, DMSO-d_6) δ 0.90 (s, 9H), 1.48 (s, 9H), 1.55 (m, 2H), 3.95 (m, 1H), 5.15–5.25 (dd, 2H), 7.08 (d, 1H), 7.35–7.50 (m, 5H); CIMS *m/z* 236 (M − Boc). Anal. Calcd for $C_{19}H_{29}NO_4$: C, 68.02; H, 8.73; N, 4.17. Found: C, 68.25; H, 8.80; N, 4.20.

γ-Methylleucine Benzyl Ester Hydrochloride (HCl·γ-MeLeu-OBzl, 4). To a solution of *N*$^\alpha$-tBoc-γ-MeLeu-OBzl (**3**) (5.0 g, 14.9 mmol) in *p*-dioxane (100 mL) was bubbled anhydrous HCl gas for 30 min. The solution was then refluxed for 1 h. After cooling to 23 °C, the solution was evaporated to dryness under reduced pressure. Additional *p*-dioxane (3 × 50 mL) was added and evaporated under reduced pressure repeatedly to remove the excess HCl. The resulting solid was recrystallized from methanol–diethyl ether to yield **4** (3.30 g, 94%): HPLC t_R = 13.7 min (gradient B, >99%); ^1H NMR (400 MHz, DMSO-d_6) δ 0.90 (s, 9H), 1.68 (dd, 1H), 1.80 (dd, 1H), 3.94 (m, 1H), 5.20–5.30 (dd, 2H), 7.35–7.50 (m, 5H), 8.65 (br s, 3H); CIMS *m/z* 236 (M + H). Anal. Calcd for $C_{14}H_{22}NO_2Cl\cdot{}^1/_2H_2O$: C, 59.99; H, 8.02; N, 5.02. Found: C, 60.35; H, 7.76; N, 5.38.

Isocyanato-γ-methylleucine Benzyl Ester (5). To a suspension of HCl·γMeLeu-OBzl (**4**) (3.0 g, 11.0 mmol) in toluene (10 mL) was added activated charcoal (0.30 g), followed by diphosgene (1.6 mL, 12.1 mmol). After stirring for 1 h at 23 °C, the solution was brought to reflux for an additional 4 h. The reaction mixture was allowed to cool to 23 °C and filtered through Celite, and the filtrate was evaporated to dryness under reduced pressure to yield **5** (2.65 g, 92%) as an oil. This oil was used without further purification.

***N*-(*cis*-2,6-Dimethylpiperidinocarbonyl)-γ-methylleucine Benzyl Ester (6).** Isocyanato-γ-methylleucine benzyl ester (**5**) (2.65 g, 10.1 mmol) was dissolved in ethyl acetate (20 mL) and allowed to stir under a nitrogen atmosphere at 0 °C. *cis*-2,6-Dimethylpiperidine (1.5 mL, 11.2 mmol) was added, and resulting solution was stirred for 1 h at 0 °C and an additional 1 h at 23 °C. The solution was diluted with ethyl acetate (30 mL), washed consecutively with 1 N HCl, water, and brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to an oil. The oil was recrystallized from ethyl acetate–hexane to yield **6** (3.55, 94%): HPLC t_R = 20.5 min (gradient B, >99%); ^1H NMR (400 MHz, $CDCl_3$) δ 0.95 (s, 9H), 1.18 (d, 3H), 1.22 (d, 3H), 1.45–1.80 (m, 8H), 4.18 (m, 2H), 4.60–4.72 (m, 2H), 5.08–5.22 (dd, 2H), 7.35 (m, 5H); CIMS *m/z* 375 (M + H). Anal. Calcd for $C_{22}H_{34}N_2O_3$: C, 70.54; H, 9.08; N, 7.48. Found: C, 70.84; H, 9.12; N, 7.53.

***N*-(*cis*-2,6-Dimethylpiperidinocarbonyl)-γ-methylleucine (7).** *N*-(*cis*-2,6-Dimethylpiperidinocarbonyl)-γ-methylleucine benzyl ester (**6**) (3.0 g, 8.3 mmol) was dissolved in methanol (75 mL), treated with 20% Pd/C (0.20 g), and placed under a hydrogen atmosphere at 50 psi (2 h, 23 °C). The reaction mixture was filtered through Celite and the solvent was evaporated under reduced pressure to yield **7** (2.05 g, 88%) as a white powder: HPLC t_R = 14.2 min (gradient A, >99%); ^1H NMR (400 MHz, $CDCl_3$) δ 0.95 (s, 9H), 1.15 (dd, 6H), 1.45–1.55 (m, 2H), 1.58–1.82 (m, 5H), 2.05–2.15 (dd, 1H), 4.10–4.20 (br, 2H), 4.25 (m, 1H), 4.70 (d, 1H); CIMS *m/z* 285 (M + H). Anal. Calcd for $C_{15}H_{28}N_2O_3$: C, 63.35; H, 9.92; N, 9.85. Found: C, 63.39, H, 10.02, N, 9.93.

***N*$^\alpha$-(*tert*-Butyloxycarbonyl)-D-tryptophan Benzyl Ester (*N*$^\alpha$-tBoc-D-Trp-OBzl, 9).** To a solution of *N*$^\alpha$-D-tBoc-Trp (5.0 g, 16.4 mmol) in DMF (15 mL) were added EDC·HCl (3.2 g, 16.7 mmol), HOBt (2.3 g, 17.0 mmol), NMM (1.9 mL, 17.3 mmol), and benzyl alcohol (2.0 mL, 19.3 mmol). The reaction mixture was allowed to stir overnight at 23 °C. The reaction mixture was diluted with ethyl acetate (100 mL), washed in turn with 10% $NaHCO_3$, brine, and water, dried with $MgSO_4$, filtered, and evaporated. Crystallization from ethyl acetate-hexane yielded **9** (5.50 g, 85%) as a white powder: HPLC t_R = 18.6 min (gradient B, >99%); ^1H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, 9H), 3.30 (m, 2H), 4.73 (m, 1H), 5.15 (dd, 2H), 6.80 (br s, 1H), 7.08 (t, 1H), 7.20 (t, 1H), 7.22–7.38 (m, 6H), 7.65, (d, 1H), 8.05 (br s, 1H); CIMS *m/z* 394 (M + H). Anal. Calcd for $C_{23}H_{26}N_2O_4$: C, 70.02; H, 6.66; N, 7.10. Found: 70.15; H, 6.68; N, 7.15.

***N*$^\alpha$-(*tert*-Butyloxylcarbonyl)-D-1-(methoxycarbonyl)-tryptophan Benzyl Ester (10).** To a solution of *N*$^\alpha$-tBoc-D-Trp-OBzl (**9**) (5.0 g, 12.7 mmol) in acetonitrile (20 mL) was added dimethyl dicarbonate (1.9 mL, 18.0 mmol), followed by DMAP (0.20 g, 1.62 mmol), and the solution was allowed to stir (3 h, 23 °C). The reaction mixture was diluted with ethyl acetate (100 mL), and the organic phase was washed with cold 1 N HCl, brine, and water, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. Trituration with hexane yielded **10** (5.15 g, 90%) as a white powder: HPLC t_R = 18.8 min (gradient B, >99%); ^1H NMR (400 MHz, $CDCl_3$) δ 1.40 (s, 9H), 3.15–3.30 (m, 2H), 4.00 (s, 3H), 4.73 (m, 1H), 5.05–5.15 (m, 3H), 7.18–7.38 (m, 8H), 7.55 (d, 1H), 8.15 (br, 1H); CIMS *m/z* 452 (M). Anal. Calcd for $C_{25}H_{28}N_2O_6$: C, 66.36; H,6.24; N, 6.19. Found: C, 66.19; H, 6.24; N, 6.20.

D-1-(Methoxycarbonyl)tryptophan Benzyl Ester Hydrochloride (11). To a solution of *N*$^\alpha$-tBoc-D-1-(methoxycarbonyl)tryptophan benzyl ester (**11**) (4.0 g, 8.8 mmol) in *p*-dioxane (50 mL) was bubbled anhydrous HCl gas for 30 min, and the solution was allowed to stir (1 h, 23 °C). The reaction mixture was evaporated to dryness under reduced pressure. The residue was taken up in *p*-dioxane (50 mL) and evaporated again to remove excess HCl. Crystallization from methanol–diethyl ether yielded **11** (3.19 g, 90%) as white powder: HPLC t_R = 12.8 min (gradient B, 96%); ^1H NMR (400 MHz, DMSO-d_6) δ 3.25–3.35 (m, 2H), 3.95 (s, 3H), 4.20 (t, 1H), 5.10 (s, 2H), 7.18–7.40 (m, 7H), 7.58 (s, 1H), 7.60 (d, 1H), 8.12 (d, 1H), 8.68 (br s, 3H); CIMS *m/z* 353 (M). Anal. Calcd for $C_{20}H_{20}N_2O_4\cdot$HCl: C, 61.78; H, 5.44; N, 7.20. Found: C, 61.40; H, 5.42; N, 7.20.

***N*-(*cis*-2,6-Dimethylpiperidinocarbonyl)-γ-methylleucyl-D-1-(methoxycarbonyl)tryptophan Benzyl Ester (12).** The protected amino acids **7** (3.0 g, 10.6 mmol) and **11** (4.5 g, 11.6 mmol) were dissolved in DMF (20 mL). To this solution were added EDC·HCl (2.05 g, 10.7 mmol), HOBt (1.44 g, 10.7 mmol), and NMM (1.9 mL, 20.1 mmol). The mixture was allowed to stir (12 h, 23 °C) and poured into 100 mL of ethyl acetate: water (1:1). The aqueous phase was separated and reextracted with ethyl acetate (2 × 50 mL). The organic layers were

combined, washed with 1N HCl, brine, and water, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. Crystallization from ethyl acetate–hexane yielded **12** (6.06 g, 92%) as a white powder: HPLC t_R = 19.4 min (gradient B, 98%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (s, 9H), 1.10 (br d, 3H), 1.22 (br d, 3H), 1.45–1.85 (m, 10H), 3.18 (dd,1H), 3.30 (dd, 1H), 4.30 (br, 1H), 4.90 (q, 1H), 5.15 (s, 2H), 7.22–7.38 (m, 10H), 7.50 (d, 1H), 8.18 (br d, 1H); ESMS *m/z* 619.6 (M).

***N*-(*cis*-2,6-Dimethylpiperidinocarbonyl)-γ-methylleucyl-D-1-(methoxycarbonyl)tryptophan (13).** Pd/C (5%, 0.40 g) was added to a solution of **12** (5.5 g, 8.9 mmol) in diethyl ether (100 mL). The mixture was stirred under an atmosphere of hydrogen (2 h, 23 °C). The reaction mixture was filtered through Celite, and the filtrate was evaporated under reduced pressure to yield a white solid. Recrystallization from ethyl acetate–hexane yielded **13** (4.17 g, 89%): HPLC t_R = 17.5 min (gradient A, 98%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.79 (s, 9H), 1.05 (d, 6H), 1.18–1.30 (m, 2H), 1.35–1.50 (m, 6H), 1.60–1.72 (m, 2H), 3.02 (dd, 1H), 3.15 (dd, 1H), 3.95 (s, 3H), 4.10 (br, 2H), 4.30 (m, 1H), 4.52 (m, 1H), 5.95 (d, 1H), 7.25 (t, 1H), 7.35 (t, 1H), 7.50 (s, 1H), 7.62 (d, 1H), 8.07 (d, 1H), 8.12 (d, 1H); ESMS *m/z* 527.4 (M).

***N*-*cis*-2,6-Dimethylpiperidinocarbonyl-γ-methylleucyl-D-1-(methoxycarbonyl)tryptophanyl-D-norleucine Benzyl Ester (14).** To a solution of the dipeptide **13** (4.0 g, 7.6 mmol), HCl·D-Nle-OBzl[19] (2.9 g, 8.0 mmol), and DIEA (2.8 mL, 16.1 mmol) in DMF (15 mL) was added dropwise HBTU (3.0 g, 8.0 mmol) in DMF (10 mL) over 20 min. The solution was stirred (3 h, 23 °C) and poured into 200 mL of ethyl acetate: water (1:1). The aqueous phase was separated and reextracted with ethyl acetate (2 × 50 mL). The organic layers were combined, washed with cold 1 N HCl, brine, and water, dried with $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting oil **14** (4.80 g, 87%) was used without further purification.

***N*-(*cis*-2,6-Dimethylpiperidinocarbonyl)-γ-methylleucyl-D-1-(methoxycarbonyl)tryptophanyl-D-norleucine (15).** To a solution of **14** (4.8 g, 6.6 mmol) in diethyl ether (100 mL) was added 5% Pd/C (0.50 g), and the solution was stirred under an atmosphere of hydrogen gas (2 h, 23 °C). The reaction mixture was filtered through Celite and the filtrate evaporated to dryness to yield crude **15** as a white powder. The crude peptide was then purified by preparative RP-HPLC on a Vydac C18 column (218TP1015, 2.2 × 25.0 cm, 15 mL/min) with a mobile phase of 0.1% TFA in water with increasing concentrations of 0.1% TFA in acetonitrile. Individual fractions were collected and analyzed by analytical RP-HPLC. The appropriate fractions were combined, concentrated under reduced pressure, and lyophilized to yield **15** (3.57 g, 85%) as a white powder: HPLC t_R = 16.4 (gradient A, >99%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.75 (s, 9H), 0.85 (t, 3H), 1.05 (dd, 3H), 1.22–1.50 (m, 11H) 1.65–1.78 (m, 3H), 2.88 (dd, 1H), 3.25 (dd, 1H), 3.95 (s, 3H), 4.04 (m, 1H), 4.08–4.18 (m, 3H), 4.65 (m, 1H), 6.08 (d, 1H), 7.26 (t, 1H), 7.34 (t, 1H), 7.53 (s, 1H), 7.68 (d, 1H), 8.08 (d, 1H), 8.23 (d, 2H); ESMS *m/z* 640.4 (M).

***N*-(*cis*-2,6-Dimethylpiperidinocarbonyl)-γ-methylleucyl-D-1-(methoxycarbonyl)tryptophanyl-D-norleucine Sodium Salt (1, BQ-788).** To a solution of **15** (3.5 g, 5.5 mmol) in methanol (50 mL) was slowly added 5% aqueous $NaHCO_3$ (300 mL) over a period of 30 min. The solution was stirred until clarity was achieved (30 min, 23 °C). The solution was diluted with water (200 mL), and the resulting solution was passed through a C18 (60 mL) cartridge preequilbrated in water. BQ-788 (**1**) was eluted with methanol (2 × 50 mL), concentrated under reduced pressure, resuspended in water (50 mL), and lyophilized to quantitatively yield compound **1** as a white powder: HPLC t_R = 16.4 (gradient A, >99%); ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.80 (s, 9H), 0.74–0.85 (m, 3H), 1.00 (d, 3H), 1.02 (d, 3H), 1.10–1.25 (m, 6H), 1.30–1.55 (m, 6H), 1.60–1.75 (m, 2H), 2.92 (dd, 1H), 3.12 (dd, 1H), 3.78 (m, 1H), 3.95 (s, 3H), 4.08 (m, 1H), 4.13 (m, 1H), 4.29 (m, 1H), 4.50 (m, 1H), 5.98 (d, 1H), 7.22 (t, 1H), 7.32 (t, 1H), 7.50 (s, 1H), 7.58 (br d, 1H), 7.65 (d, 1H), 8.05 (d, 1H), 8.15 (br d, 1H) ESMS *m/z* 640.6 (M).

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