Exploring the Structure−Activity Relationships of [1-(4-*tert*-Butyl-3′-hydroxy)benzhydryl-4-benzylpiperazine] (SL-3111), A High-Affinity and Selective *δ*-Opioid Receptor Nonpeptide Agonist Ligand†

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SL-3111 [1-(4-*tert*-butyl-3′-hydroxy)benzhydryl-4-benzylpiperazine] is a de novo designed, high-affinity and selective nonpeptide peptidomimetic agonist of the *δ*-opioid receptor. In a previous report we had described the unique biological characteristics of this ligand and also a need for further structural evaluation.[6] To pursue this, we have introduced a completely different heterocyclic template (**2** and **3**), which, based on molecular modeling studies, may present the required structural features to properly orient the pharmacophore groups. We also have made more subtle changes to the original piperazine scaffold (**5** and **11**). The biological activities of these compounds revealed an important participation of the scaffold in the ligand−receptor interaction. To further explore functional diversity on the scaffold, we have maintained the original piperazine ring and introduced four different functionalities at position 2 of the heterocyclic ring (**15a**−**d**; **a** = CH_2−O−CH_2−Ph; **b** = Me; **c** = CH_2Ph; **d** = CH_2OH). The biological activities observed for these compounds showed a very interesting trend in terms of the steric effects of the groups introduced at this position. A decrease of almost 2000-fold in affinity and potency at the *δ*-receptor was observed for **15c** compared with **15b**. This difference may be explained if we postulate that the bioactive conformation of these peptidomimetics is close to the minimal energy conformations calculated in our study. On the basis of these findings we have realized the importance of this position to further explore and simplify the structure of future generations of peptidomimetic ligands.

Introduction

Opiate drugs, which act primarily on the *μ*-opioid receptors, are used clinically to relieve severe pain. Countless morphine derivatives have been synthesized and examined with the aim of developing analgesics without the undesirable side effects of these drugs, such as euphoria, constipation, and respiratory depression. On the other hand, agonists acting at the *δ*-opioid receptor (DOR) have strong antinociceptive activity with relatively mild side effects, compared with agonists at the *μ*- or *κ*-receptors.[1] An important class of selective agonists for the *δ*-opioid receptors are the cyclic peptide ligands related to the enkephalins, such as the conformationally constrained peptide DPDPE and its halogenated analogues.[2] They are exceptionally selective for this opioid receptor and resistant to enzymatic degradation.[3] However, they are not very active when systemically administered because of their relative inability to cross the blood-brain barrier.

In the biological, chemical, and pharmacological areas peptidomimetics may offer advantages over native peptides.[4] These pharmacological properties include (1) increased effectiveness and selectivity that may decrease side effects and (2) metabolic stability and improved oral bioavailability. A systematic approach to peptide and peptidomimetic design has been presented by Hruby et al.[5] The major goal of this strategy is to define a specific pharmacophore of a particular receptor or acceptor molecule, and its validity, by specific design of a ligand with predictable agonist or antagonist activities. By applying this scheme to our ongoing research, which seeks to translate the information contained in an endogenous opioid peptide, such as enkephalin, into a small organic compound, we have recently reported a series of new peptidomimetic compounds.[6] As depicted in Figure 1 this design was based on the topographically constrained and highly selective peptide [(2*S*,3*R*)TMT[1]]DPDPE.[7] From the first generation of peptidomimetics based on this design criteria (Figure 1), 1-(4-*tert*-butyl-3′-hydroxy)benzhydryl-4-benzylpiperazine (SL-3111) emerged as a promising nonpeptidomimetic lead for further design.

In that first series, our intention was primarily to explore the functional roles of the two aromatic rings and the hydrophobicity of the R_1 group in the ligand−receptor interaction. SL-3111 showed 8 nM binding affinity and over 2000-fold selectivity for the *δ*- over the *μ*-opioid receptor. Binding studies of SL-3111 and [*p*-ClPhe[4]]DPDPE on the cloned wild type and mutated

† Abbreviations used for amino acids follow the rules of the IUPAC−IUB Joint Commission of Biochemical Nomenclature in *Biochem. J.* **1984**, *219*, 345−373. Other abbreviations are as follows: MEM, methoxyethoxymethyl ether; TFA, trifluoroacetic acid; TEA, triethylamine; MVD, mouse vas deferens; GPI, guinea pig illeum; OPLS, optimized potential for liquid simulations; HOBt, 1-hydroxybenzotriazole; DIC, diisopropylcarbodiimide; RP-HPLC, reverse-phase high-performance liquid chromatography; LAH, lithium aluminum hydride; RMS, root-mean-square; MeCN, acetonitrile; TLC, thin-layer chromatography; DPDPE, c[D-Pen[2],D-Pen[5]]enkephalin; Pen, *β*,*β*-dimethylcysteine; ESI, electrospray ionization.

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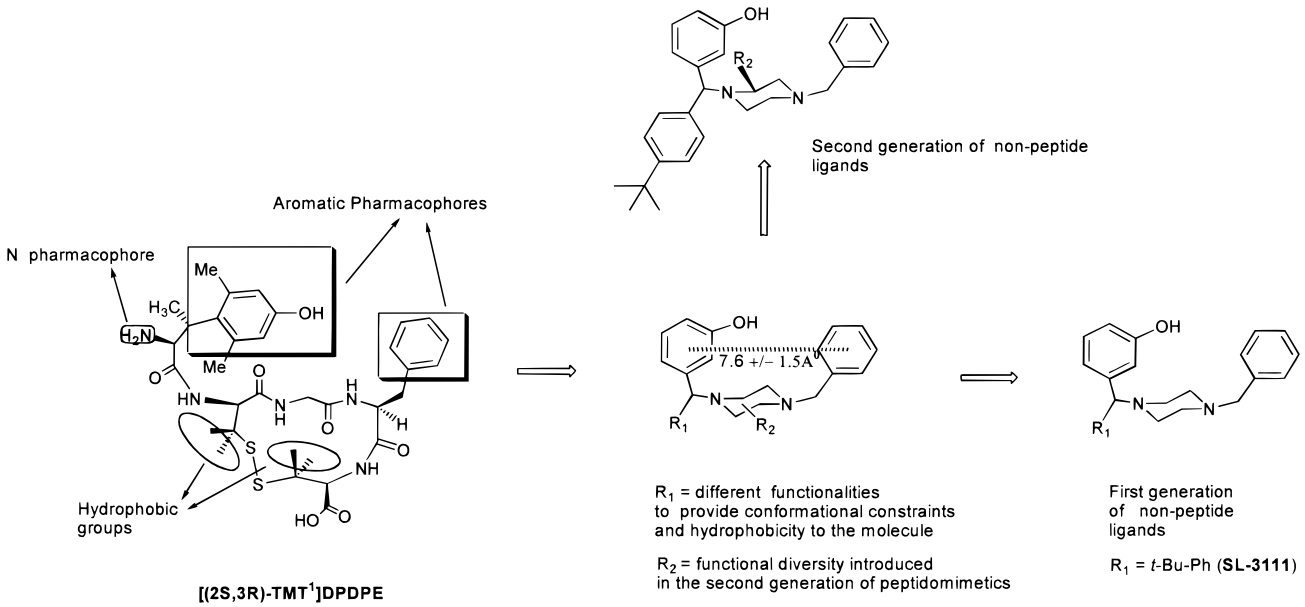

Figure 1. Rationale for the design of the first and second generation of peptidomimetic ligands based on [(2*S*,3*R*)TMT[1]]DPDPE.

human δ-opioid receptor suggested that SL-3111 has a binding profile similar to that of DPDPE, but different from that of SNC80, a potent δ-opioid selective nonpeptide agonist[6,8] that also contains a piperazine ring. However, when tested in MVD(δ) and GPI(μ) bioassays, SL-3111, despite having a moderate selectivity of 460-fold μ/δ, showed low potency (EC_{50} = 85 nM) compared to the 1.8 nM binding affinity observed for the peptide lead [(2*S*,3*R*)TMT[1]]DPDPE. Also, in contrast to the peptide lead, no in vivo antinociception was detected for SL-3111.

In an attempt to improve the biological profile of this peptidomimetic lead, we have pursued two different routes. First we looked for a modified scaffold. Thus, the piperazine ring in SL-3111 was substituted by two different heterocyclic systems: 1,3,5-thiadiazine-2-thione and piperidine. The biological activities of these compounds revealed the importance of a piperazine-like or specific-piperidine-like scaffold for the binding of this ligand toward the δ-opioid receptor. On the basis of those observations, we have directed our attention to the introduction of functional diversity on the R_2 group (Figure 1) as a possible way to improve the biological properties of SL-3111 while maintaining a piperazine ring as a template. In this paper we also report the design and synthesis of a second generation of peptidomimetics based on SL-3111, which display different functionalities at position 2 of the piperazine ring, as well as their pharmacological evaluation. Also, molecular modeling studies performed on these compounds have revealed interesting structural data, which suggested a possible bioactive conformation for these types of opioid ligands.

Results and Discussion

Design. As illustrated in Figure 1, one of the most important pieces of pharmacophore information obtained from our efforts to close the gap between peptide and nonpeptide[9] is the distance vector of 7.6 ± 1.5 Å between the two aromatic side chains of Tyr[1] and Phe[4] on the peptide lead, an essential structural requirement for any potential opioid ligand. The tetrahydro-2*H*-1,3,5-thiadiazine-2-thione heterocyclic ring (Figure 2) is a well-known prodrug system.[10] An attractive feature of this scaffold is the fact that its relatively straightforward synthesis allows the introduction of at least two different R groups, using readily available starting materials (Figure 3). From previous research efforts we had obtained the X-ray structure of 3,5-diphenethyltetrahydro-2*H*-1,3,5-thiadiazine-2-thione (**1**) (Figure 2, Figure 4a). This structure was compared with the low-energy structure of 7-spiroindanyloxymorphone (SIOM), an agonist with modest δ-opioid receptor selectivity.[11] As shown in Figure 4b, an acceptable fit of the two aromatic moieties in both molecules was observed. Thus, following a similar criterion, we eliminated one methylene group in both side chains of compound **1** to obtain **2** and **3**. As depicted in Figure 4c, the superimposition of the corresponding minimal energy structures revealed again a good overlap between SIOM and compound **3** (RMS = 1.2 Å).

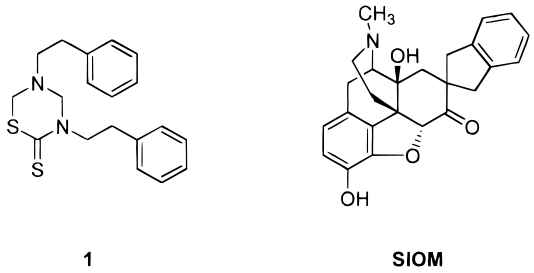

Figure 2. Structures of **1** and SIOM.

These observations prompted us to investigate the possibility of a novel, and simple to prepare, scaffold for a series of potential δ-opioid receptor ligands. We selected D-*p*-hydroxyphenylglycine as the amino acid whose phenolic side chain presented a good fit with the one displayed by SIOM (Figure 2, Figure 4c). Thus, compounds **2** and **3** were prepared as depicted in Figure 3. However, as shown in Table 1, both compounds, when assayed in radiolabeled competition binding experiments, showed very poor micromolar IC_{50} values at both μ- and δ-opioid receptors. On average, these two compounds were 3000- and 5000-fold less potent compared

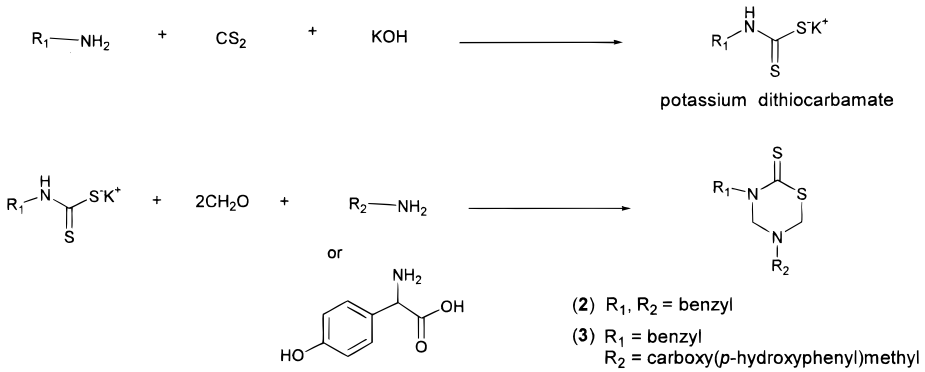

Figure 3. Synthesis of 3,5-disubstituted-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione derivatives.

Table 1. Binding Affinity of Synthesized SL-3111 Analogues

compound	binding affinity, IC_{50} (nM) ± SEM [^{3}H]DAMGO(μ)	[^{3}H]deltorphin II(δ)	selectivity μ/δ
[(2*S*,3*R*)TMT[1]]-DPDPE	4300 ± 820	5 ± 1.0[a]	860
2	27900 ± 7100	28600 ± 2900[a]	0.97
3	38300 ± 2700	44700 ± 8500[a]	0.86
5	22900	12600	1.8
11	4300	33	130
15d	7980 ± 1800	38 ± 3.7	210
15a	26000 ± 4100	38 ± 4.8	670
15b	3300 ± 300	11 ± 0.8	292
15c1	>80000	>10000	
15c2	46300	21300	2.2
SL-3111(*rac*)[6]	17000 ± 3000	8.4 ± 1.6[a]	2020

[a] Versus [^{3}H][p-ClPhe^4]DPDPE.

with the lead SL-3111. From these results an important question was raised: Does the receptor care about the nature of the scaffold? To further address this question we decided to introduce a more subtle modification on the original piperazine-like scaffold of SL-3111. As shown in Figure 5, compound **5** was synthesized from commercially available 4-benzylpiperidine and benzhydryl halide **4**, prepared by previously described methods.[6] In addition, compound **11** was synthesized as depicted in Figure 6. Table 1 shows the binding affinity data for these two analogues of SL-3111, which support the idea of the scaffold having importance for interaction with the δ-receptor. In compound **5**, the nitrogen atom bearing a benzyl group has been replaced with a CH, and it showed a dramatic loss of binding affinity (1500-fold) compared to SL-3111. However, when the nitrogen atom bearing the benzhydryl group was CH-substituted, compound **11** was obtained and found to be only 4-fold less potent. These results may indicate that the nitrogen atom linked to the benzyl group in SL-3111 is the one that mimics the basic amine group known to be essential in opioid-like drugs,[12] which is thought to imitate the amino terminus on our peptide lead [(2*S*,3*R*)TMT[1]]-DPDPE (Figure 1).

Therefore, based on the above results it was decided to maintain the original piperazine-based scaffold for the second generation of peptidomimetics and explore the functional diversity that can be introduced in such a template (R_2 in Figure 1). From the point of view of molecular diversity, diketopiperazines (DKPs) offer a highly attractive choice. The DKP skeleton has been one of the most investigated of the so-called "drug-like" types of scaffolds. Their synthesis goes back many years, and several recent reports have described their synthesis both in solution- and solid-phase formats.[13] To obtain the desired substituted piperazines, reduction of the corresponding DKP was performed. Figure 7 shows the synthetic scheme and the structure of the synthesized analogues of SL-3111 (**15a**−**d**), which display aromatic, hydrophobic, and potential donor/acceptor hydrogen bond moieties in the 2-position.

Synthesis. Compounds **2** and **3** were prepared as illustrated in Figure 3. Primary amines were reacted with carbon disulfide (CS_2) under basic conditions to afford the corresponding potassium dithiocarbamate salts. Reaction of the potassium salts with 2 equiv of formaldehyde and the corresponding primary amine (or amino acid for **3**) gave the desired compounds **2** and **3**. The synthetic route for compound **5** is shown in Figure 5. Commercially available 4-benzylpiperidine was reacted, under S_N2 conditions, with benzhydryl halide **4**[6] to yield **5** after the hydroxyl protecting group (MEM) was removed with 2 N HCl, in a 1:1 methanol/dioxane mixture by overnight reaction. As shown in Figure 6, compound **11** was prepared starting from commercially available isonipecotinic acid. Esterification using thionyl chloride and methanol and then N-alkylation with benzylbromide under basic conditions afforded compound **6**, which after LAH reduction produced primary alcohol **7**. Swern oxidation of alcohol **7** yielded aldehyde **8**, which after condensation with 3-methoxyphenylmagnesium bromide gave compound **9**.[14] Friedel−Crafts reaction of **9** in *tert*-butylbenzene yielded several products, from which we could isolate **10**, although in low yield. Compound **10** was then treated with BBr_3−DCM followed by acid treatment to remove *O*-methyl protection, to give the desired compound **11**.[15]

Figure 7 illustrates the new synthetic route for the preparation of the functionalized SL-3111 analogues **15a**−**d**. The synthesis starts with a rather simple peptide coupling between commercially available *N*-benzylglycine ethyl ester and a N^{α}-Boc amino acid, using HOBt/DIC as coupling agents in DCM from 0 °C to room temperature, in overnight stirring. Protected dipeptides **12a**−**c** were treated with 90% TFA/DCM for 1 h, monitoring the reaction by TLC using ninhydrin spray and heating to detect the primary amine[16] and by analytical RP-HPLC. After evaporation under high vacuum of solvent and TFA, the residue was redissolved in DCM and treated with 8 equiv of TEA, and the mixture was stirred at room temperature. In general, reactions were complete after a few hours (2−3). To reduce diketopiperazines **13a**−**c** to the corresponding

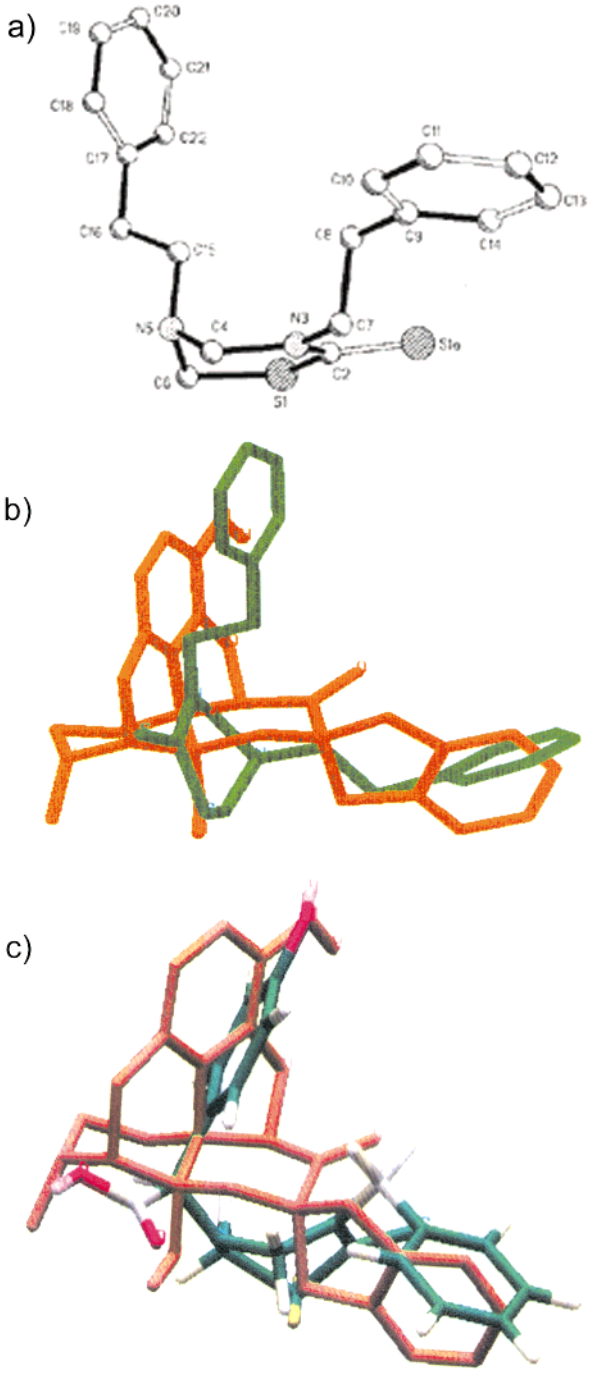

Figure 4. (a) X-ray structure of compound **1**. (b) Overlap of the X-ray structure of **1** and SIOM (orange). (c) Overlap of SIOM (orange) and the low-energy conformation of compound **3**.

1-benzyl-3-substituted piperazines **14a**−**c**, we chose LAH using slightly modified reaction conditions to those reported by Pohlmann.[17] Compounds **15a**−**d** were obtained by subsequent S_N2 reaction between the corresponding functionalized piperazine **14a**−**c** with benzhydryl halide **4**, followed by treatment with 2 N HCl to remove the MEM group. Compound **15d** was obtained from **15a** by hydrogenation using Pd/C catalyst after 16 h at room temperature and 1 atm, followed by in situ deprotection of the MEM group.

Structure−Activity Relationship Studies. Radiolabeled ligand in vitro binding assays were made using rat brain membranes,[6] and, in addition, the classical

Table 2. Biological Potencies of the Synthesized Peptidomimetics

	bioassay data, IC_{50} (nM) ± SEM		selectivity
compound	GPI(μ)	MVD(δ)	μ/δ
[(2*S*,3*R*)TMT[1]]-DPDPE	0% at 60 μM (antagonist, IC_{50} = 5 μM)	1.8 ± 3	>33000
11	15600 ± 2100	390 ± 140	40
15d[a]	36% at 10 μM	2263 ± 150	NA
15a[a]	0% at 10 μM	44% at 30 μM	NA
15b[a]	0% at 30 μM	290 ± 50	>104
15c1[a]	0% at 30 μM	10% at 1 μM	NA
15c2			
SL-3111(*rac*)[6]	39000 ± 2600	85 ± 10	460

[a] CTAP (μ-opioid antagonist) insensitive, ICI-174864 (δ-selective antagonist) sensitive.

MVD(δ) and GPI(μ) bioassays[18] were run. This provided information about the functional group requirements in our peptidomimetics that may be important for binding and recognition toward the δ-receptor. Table 1 shows the binding affinity data for these compounds. As discussed before, although a favorable three-dimensional fit was observed when compared with SIOM (Figure 4), compounds **2** and **3** were 3000- and 5000-fold less potent at the δ-opioid receptor compared with SL-3111. These results stress the importance of a piperazine-like scaffold, which may present a key interaction with the receptor. Furthermore, as shown in Table 1, compounds **5** and **11**, bearing a more subtle change on the scaffold with both having piperidine rather than piperazine rings but with different nitrogens being replaced by CH groups, revealed that the nitrogen atom on SL-3111 that may mimic the amino terminus on the peptide lead is the one bearing the benzyl group. This also was reflected in the bioassay experiments shown in Table 2; compound **11** was shown to be an agonist in the MVD(δ), with very low activity at the GPI(μ). In addition, compounds **15a**−**d** showed a very interesting trend in terms of both selectivity and recognition for the δ-opioid receptor, depending on the nature of the functionality attached at position 2 of the piperazine scaffold. Thus, as shown in Table 1, compound **15d** was shown to be only 4.5-fold less potent than SL-3111, and 12-fold less selective. Also, **15a**, like **15d**, shows only 4.5-fold less affinity, and only 3-fold less selectivity. On the other hand, compound **15b** possesses almost the same binding affinity as SL-3111, with 7-fold less selectivity. Such good binding may be consistent with the presence of methyl groups in several nonpeptide ligands reported for this receptor,[19] including the BW-series of piperazine derivatives,[19a] but as we reported in detail previously for SL-3111 and its precursor analogues,[6] SL-3111 has a different structure−activity relationship with the δ-opioid receptor than the BW-series of piperazines. The results reported here are consistent with these earlier results.

The most interesting data is the dramatic decrease observed in both affinity and selectivity when the substituent is a benzyl group (**15c2**, Table 1). The two diastereoisomers of compound **15c** were separated by RP-HPLC, and no difference in binding affinity is observed as shown in Table 1. Both analogues presented a nearly complete loss of activity at both μ- and δ-receptors. Furthermore, as shown in Table 2, in vitro

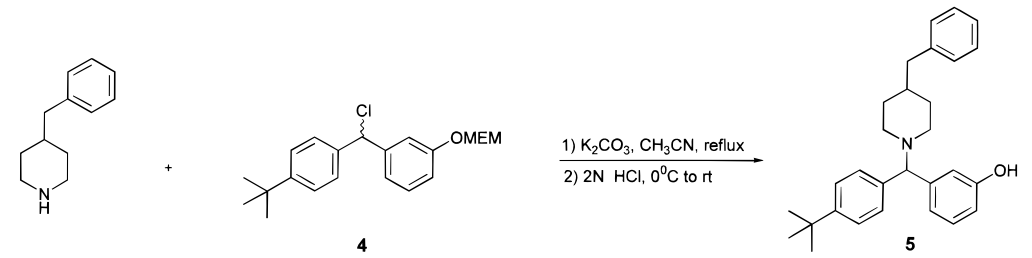

Figure 5. Synthesis of compound **5**.

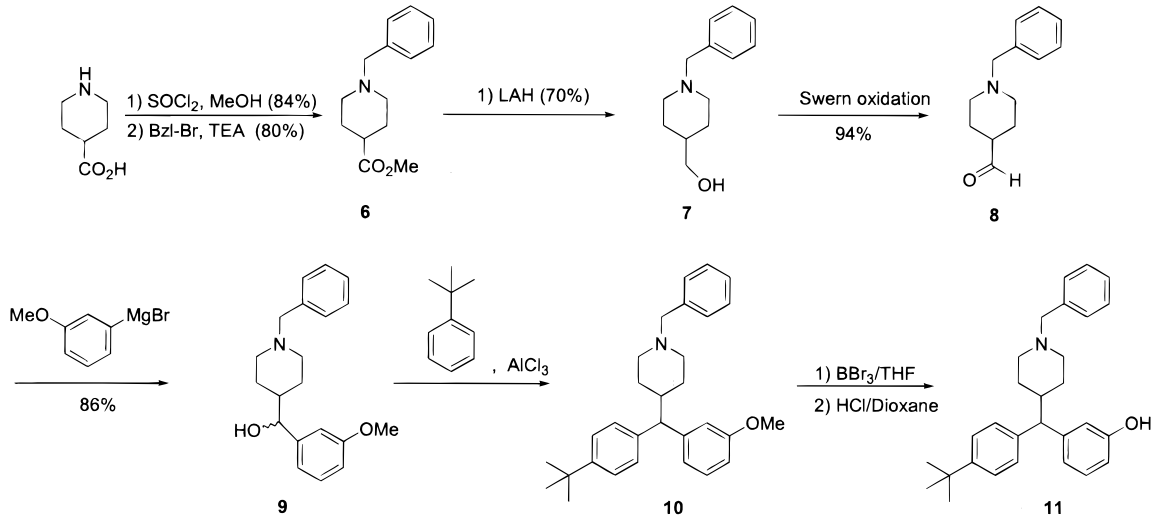

Figure 6. Synthetic route for the preparation of **11**.

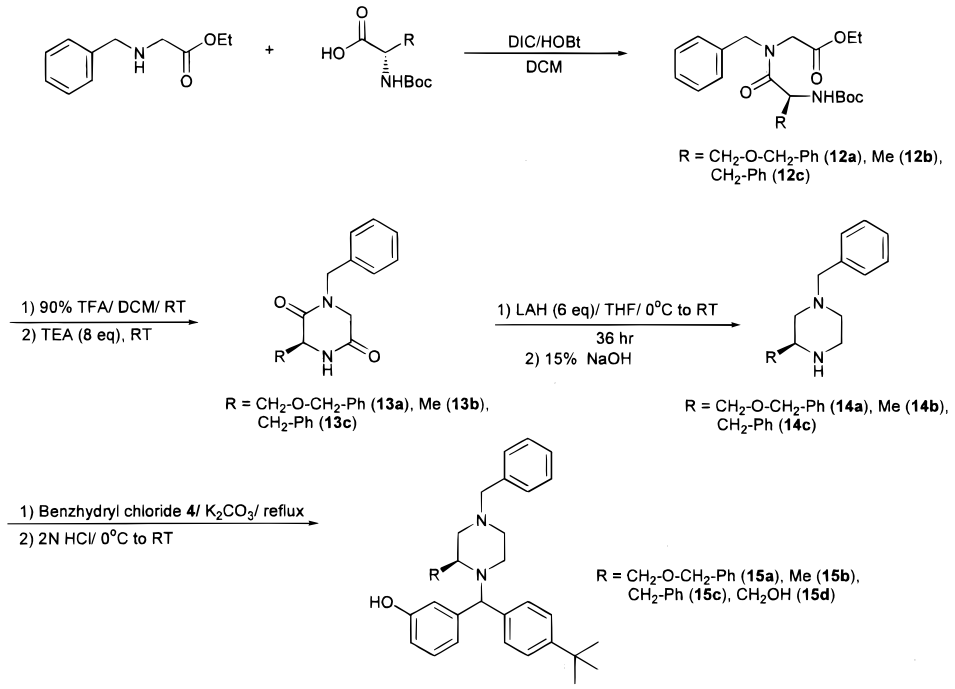

Figure 7. Synthetic route for the preparation of compounds **15a**−**d**.

bioassay experiments also revealed a similar trend to the binding affinity data. Compound **15d** showed poor biological activity in both tissues. A similar profile was presented by **15a**, showing only minor agonist activity at the δ-receptor (MVD) and also higher binding potency and selectivity for the δ-receptor. Compound **15b** showed no biological activity (neither agonist or antagonist) in the GPI(μ) bioassay. However, it was shown to be a δ-agonist only 3-fold less potent than SL-3111 and possibly more selective. It is difficult to compare these results with those for BW- and SNC80-related compounds because similar changes in the piperazine ring have not been made, though a recent report on patent applications[20] not yet granted indicates that another class of compounds, the diarylalkenylamines, may be related. Unfortunately nothing has appeared in the peer-reviewed literature, and further discussion is thus not possible at this time.

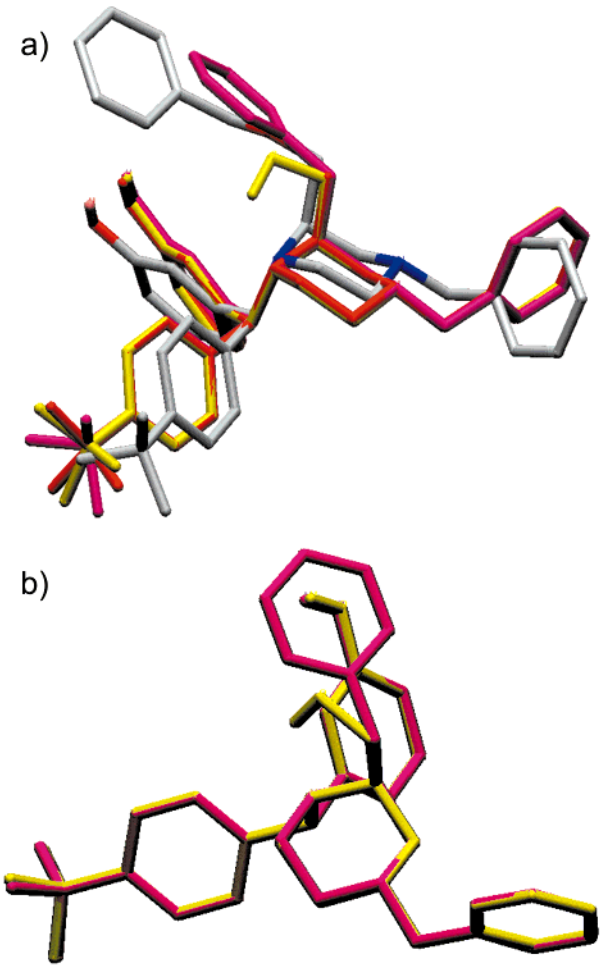

Figure 8. (a) Overlap of the calculated minimal energy conformations for compounds **15d** (yellow), **15a** (gray), **15b** (red), and **15c** (pink). (b) "Top view" of the superimposition of **15d** and **15c**.

Finally, similarly to that observed in the rat brain binding experiments, compound **15c1** was shown to completely lack biological activity at both μ- and δ-receptors (GPI and MVD).

Molecular Modeling. In an attempt to explain the dramatic differences (up to 2000-fold) in binding affinity and potency between compounds **15a,b,d** and **15c**, we have performed a molecular modeling study on this series of peptidomimetics. Each ring system was subjected to a conformational search (20000 iterations) using the OPLS force field[21] and the Monte Carlo[22] minimization gradient. Figure 8 illustrates the overlap of the calculated minimal energy conformations for compounds **15a**−**d**, using an atom-by-atom correspondence. As shown in Figure 8a, an almost perfect fit of the three main pharmacophores in the minimized structures is observed. However, the most interesting observation, which also agrees with the lack of bioactivity of compound **15c1**, is shown in Figure 8b. In this "top view" it is evident how the phenyl ring at position 2 of the piperazine scaffold resides in a region of the molecule that "blocks" the phenol group, which is known to mimic the Tyr[1] residue in our peptide lead known as the "message" moiety in opioid peptides.[23]

Furthermore, with the aid of Drieding models, we also have observed that with an *S* configuration at position 2, interconversion of the chair (found as a minimal energy conformation depicted in Figure 8a) toward the other possible one results in highly sterically crowded structures, specially in the case of **15c**. Thus, we postulate that the chair conformation alternate to the 3D structure calculated for compounds **15a**−**d** (Figure 8a) may be close to their actual bioactive conformation. Such observations suggest that functionalization of the piperazine scaffold at position 2, as depicted in Figure 1, with a proper functionality may produce a new generation of peptidomimetic ligands for this receptor. Thus, the use of a suitable amino acid such as β-isopropyltyrosine (a chimera of Tyr and Leu)[24] may provide two of the functionalities known to be essential pharmacophores of any opioid mimetic ligand. These and other studies on solid supports are being pursued in our laboratory.

Conclusions

The biological profile observed for compounds **2** and **3** suggest that the original piperazine-based scaffold is important in this type of peptide mimetic ligand for interaction with the δ-receptor. By preparation of compounds **5** and **11** (Table 1), we have found which of the two nitrogen atoms present in the piperazine template of SL-3111 is the one that may engage a noncovalent interaction with a counterpart on the δ-receptor. These observations have revealed information about the three-dimensional features of these ligands, compared with our peptide lead.

In addition, a new series of analogues of SL-3111, with different side chains incorporated at position 2 of the piperazine ring, has been synthesized. Unlike the other analogues prepared, incorporation of a benzyl group at that position produced compound **15c** which has more than 2000-fold less affinity for the δ-opioid receptor compared with the lead SL-3111. On the basis of such a dramatic difference in biological activity and on molecular modeling studies performed on these compounds, we have postulated a possible bioactive conformation for these ligands.

Experimental Section

Materials and Methods. All reagents, unless otherwise noted, were purchased from Aldrich Chemical Co. and were used without further purification. All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded with a Varian Gemini-200 spectrometer, using tetramethylsilane (TMS) or D_2O (4.66 ppm downfield from TMS) as an internal standard. Flash column chromatography was performed using E. Merck silica gel (230 mesh). Analytical RP-HPLC was performed in a Hewlett-Packard instrument model 1090 using a C_{18}-bonded silica column (VYDAC 218 TBP-16, 4.6 × 250 mm) with a linear gradient elution of 10−90% MeCN in 0.1% TFA aqueous solution over 40 min at a flow rate of 1 mL/min. Retention times (t_R) are reported in minutes. Thin-layer chromatography was performed on silica plates (0.25 mm; Analtech, Newark, DE). Preparative reverse-phase high-performance liquid chromatography (RP-HPLC) was performed on a Perkin-Elmer instrument model 410-Bio with a C_{18}-bonded silica column (VYDAC 218TP1010, 12 × 275 mm), eluting with a linear gradient of 10−90% MeCN in 0.1% aqueous TFA over 40 min at a flow rate of 5 mL/min. High-resolution mass spectra (HRMS) were obtained at the University of Arizona Chemistry Department Mass Spectroscopy Facility, with a JEOL mass spectrometer model HX-110. All analytical (C, H, N) and spectroscopic (^{1}H NMR, ^{13}C NMR, MS) data are in agreement with the proposed structures.

Preparation of 3,5-Dibenzyl-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione (2). A solution of benzylamine (2.14 g, 20 mmol) and KOH (1 equiv) in 30 mL of ethanol was cooled to 15 °C, and then carbon disulfide (0.76 g, 10 mmol) was added dropwise. The yellow mixture was stirred at 30−35 °C (gentle reflux); after a few minutes a heavy precipitate was formed and the mixture stirred for 3 h. The mixture was cooled to ∼10 °C, and formaldehyde (1.72 mL, 20 mmol) was added. The mixture was refluxed for three more hours. After 1 h the solution became clear, and after 3 h a white precipitate formed again. The mixture was filtered, and 4.85 g of a white solid (77.2% yield) was collected. TLC (hexanes/AcOEt, 8:2) showed only one product, $R_f = 0.283$, mp = 83−85 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.41−7.09 (m, 10H), 5.32 (s, 2H), 4.36 (s, 2H), 4.31 (s, 2H), 3.75 (s, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 135.86, 135.23, 128.95, 128.86, 128.58, 128.17, 127.94, 68.17, 57.53, 54.56, 53.83. FAB/HRMS calcd for $C_{17}H_{19}N_2S_2$ $[M + 1]^+$ = 315.4814; found 315.0990. Anal. Calcd for $C_{17}H_{18}N_2S_2$: C, 64.93; H, 5.77; N, 8.91. Found: C, 65.02; H, 5.64; N, 8.96.

Preparation of 3-Benzyl-5-carboxy(*p*-hydroxyphenyl)-methyl-tetrahydro-2*H*-1,3,5-thiadiazine-2-thione (3). Into a three-necked round-bottom flask containing a solution of benzylamine (1.07 g, 10 mmol) dissolved in H_2O (20 mL) was added, simultaneously and dropwise from two addition funnels, 2.8 mL of a 20% solution of KOH and carbon disulfide (0.76 g, 10 mmol). After complete addition, the white suspension was stirred at room temperature. After 3 h formaldehyde (0.64 g, 20 mmol) was added, and the pink solution turned into a yellow suspension. The reaction was stirred for one more hour. Then, the slurry was filtered, and the obtained yellow solution was immediately added dropwise to a solution of D-*p*-hydroxyphenylglycine (1.67 g, 20 mmol) in 20 mL of a phosphate buffer of pH 7.8 (prepared from Na_2HPO_4 and KH_2PO_4). The mixture was stirred for two more hours and then filtered to obtain 2.9 g of a white solid (79% yield); mp = 151−152 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 7.29−7.26 (m, 5H), 7.07 (d, 2H, J = 8.46 Hz), 6.68 (d, 2H, J = 8.42 Hz), 5.29 (d, 1H, J = 16.03 Hz), 4.85 (d, 1H, 16.0 Hz), 4.60−4.41 (m, 3H), 4.33 (s, 2H). ^{13}C NMR (DMSO, 50 MHz): δ 193.21, 171.91, 157.88, 135.75, 130.22, 130.03, 129.19, 128.28, 125.92, 115.97, 67.19, 65.27, 54.12. FAB/HRMS calcd for $C_{18}H_{19}N_2S_2O_3$ $[M + 1]^+$ = 375.0837; found 375.0842. Anal. Calcd for $C_{18}H_{18}N_2O_3S_2$: C, 57.73; H, 4.84; N, 7.48. Found: C, 57.86; H, 4.77; N, 7.53.

Preparation of 1-(4-*tert*-Butyl-3′-hydroxy)-4-benzylpiperidine (5). A round-bottom flask equipped with a reflux condenser was loaded with 4-benzylpiperazine (0.2 g, 1.14 mmol), chloride **4** (0.41 g, 1.14 mmol), and K_2CO_3 (3.5 equiv), dissolved in MeCN (10 mL). The reaction mixture was refluxed under N_2 atmosphere and monitored by TLC. Filtration of the crude and evaporation of the solvent yielded a yellow oil. The residue was redissolved in a 1:1 solution of MeOH/dioxane. The solution was cooled to 0 °C and then 2 mL of 4 N HCl was added and stirred overnight at room temperature. The residue was triturated in cold ether, washed with ether (3 × 10 mL), and then purified by RP-HPLC. t_R = 26.12 min. ^{1}H NMR (CD_3OD, 200 MHz): δ 7.48 (s, 4H), 7.32−6.09 (m, 8H), 5.15 (s, 1H), 6.78 (d, 1H), 3.35 (t, 2H, J = 12.1 Hz), 2.90 (t, 2H, J = 12.3 Hz), 2.59 (d, 2H, J = 13.7 Hz), 1.84 (m, 3H), 1.55 (t, 2H, J = 13.7 Hz), 1.29 (s, 9H). ^{13}C NMR (CD_3OD, 50 MHz): δ 137.63, 131.87, 130.17, 124.46, 128.83, 127.70, 127.37, 117.33, 60.09, 35.54, 33.74, 31.51, 30.18, 30.12. FAB/HRMS calcd for $C_{29}H_{36}NO$ $[M + 1]^+$ = 414.2797; found 414.2807. Anal. Calcd for $C_{29}H_{35}NO\text{-}2CF_3CO_2H-H_2O$: C, 63.8; H, 6.5; N, 2.3. Found: C, 64.4; H, 6.3; N, 2.3

Preparation of *N*-Benzyl-4-carbomethoxypiperidine (6). Thionyl chloride (119 g, 1.00 mol) was added dropwise over 10 min to 300 mL of anhydrous MeOH at −10 °C. To this solution was added isonipecotinic acid (38.7 g, 300 mmol) at the same temperature. The mixture was allowed to stir at room temperature for 20 h. The obtained clear solution was concentrated in vacuo, and the remaining white solid was suspended in ether, washed, and filtered off. The residue was dried in vacuo to afford 45.3 g (84.0%) of methyl isonipecotate hydrochloride. To a suspension of methyl isonipecotate hydrochloride (35.9 g, 200 mmol) in 200 mL of DCM was added 34.2 g (200 mmol) of benzylbromide. To this mixture was added dropwise 56.0 mL (400 mmol) of triethylamine over 13 min to maintain the temperature below 20 °C in an ice−water bath. The mixture was allowed to stir at room temperature overnight. The solution was washed with water and saturated $NaHCO_3$ solution and dried over $MgSO_4$. Concentration of the solution gave 49.2 g of crude product as a yellow oil. Distillation under reduced pressure gave 37.2 g (79.7%) of **6** as a pale yellow oil: bp = 137−140 °C (2 mmHg) [lit.[25] bp = 115 °C (0.3 mmHg)].

Preparation of *N*-Benzyl-4-hydroxymethylpiperidine (7). To a suspension of $LiAlH_4$ (17.1 g, 450 mmol) in 300 mL of anhydrous THF was added, dropwise over 20 min, a solution of methyl ester (**6**) in 300 mL of anhydrous THF while maintaining the temperature below 10 °C in an ice−water bath. After the addition was completed, the reaction mixture was allowed to stir at room temperature for 30 min and then refluxed at 50 °C for 30 min. The reaction mixture was cooled to 0 °C, and 500 mL of ice−water was added with vigorous stirring. Insoluble material was filtered off, and the filtrate was concentrated in vacuo to remove THF. The resulting aqueous suspension was extracted with EtOAc and dried over $MgSO_4$. Concentration of the solution followed by distillation under reduced pressure gave 10.7 g (69.5%) of **7** as a colorless oil: bp = 158−159 °C (2 mmHg) [lit.[25] bp = 140 °C (0.4 mmHg)]. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.36−7.22 (m, 5H), 3.49 (s, 2H), 3.43 (dd, 2H, J = 6.2 Hz), 2.95−2.85 (m, 2H), 2.00−1.15 (m, 8H).

Preparation of 1-Benzyl-4-formylpiperidine (8).[26] To a solution of oxalyl chloride (6.98 mL, 80.0 mmol) in 200 mL of anhydrous DCM was added dropwise dimethyl sulfoxide (6.82 mL, 96.0 mmol) over 7 min at −48 °C. The reaction mixture was stirred at the same temperature for 10 min. A solution of alcohol (**7**) (8.21 g, 40.0 mmol) in 20 mL of anhydrous DCM was added dropwise over 15 min, and then the reaction mixture was stirred at −55 °C for 15 min. To the mixture was added dropwise 24.0 mL (170 mmol) of triethylamine at −55 °C, over 3 min. The reaction mixture was warmed to ambient temperature over 1 h and then poured into water. The organic phase was separated, and the aqueous phase was extracted two times with DCM. The combined organic phase was dried over $MgSO_4$ and concentrated in vacuo. The residual oil was purified by flash chromatography (EtOAc/hexane, 3:7 to 1:1), and 7.67 g (94.3%) of aldehyde (**8**) was obtained as a yellow oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 9.65 (s, 1H), 7.40−7.20 (m, 5H), 3.50 (s, 2H), 2.90−2.70 (m, 2H), 2.35−1.55 (m, 7H).

Preparation of *N*-Benzyl-4-[hydroxy(3-methoxyphenyl)methyl]piperidine (9). Magnesium mesh (2.80 g, 115 mmol) was suspended in 100 mL of anhydrous THF with stirring under nitrogen atmosphere. To this solution was added 3-bromoanisole (19.6 g, 105 mmol) in one portion, and then the solution was refluxed for 30 min. The solution was cooled to 0 °C, and then aldehyde (**8**) (7.12 g, 35.0 mmol) dissolved in 25 mL of anhydrous THF was added dropwise over 8 min at the same temperature. The resulting mixture was stirred at room temperature for 2 h and poured into 400 mL of ice−water, followed by exhaustive extraction with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual oil was purified by flash chromatography (EtOAc/hexane, 2:1 to EtOAc), and 7.67 g (94.3%) of compound **9** was yielded as a white solid: mp = 99−100 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.35−7.18 (m, 6H), 6.90−6.75 (m, 3H), 4.32 (d, J = 8.9 Hz, 1H), 3.79 (s, 3H), 3.45 (s, 2H), 3.02−2.75 (m, 2H), 2.20−1.75 (m, 5H), 1.70−1.18 (m, 3H). FAB/MS calcd for $C_{20}H_{26}NO_2$ $[M+H]^+$ = 312.20; found 312.13.

Preparation of *N*-Benzyl-4-(4-*tert*-butyl-3′-methoxybenzhydryl)piperidine (10). To a solution of carbinol (**9**) (934 mg, 3.00 mmol) in 12 mL of *tert*-butylbenzene was added 800 mg (6.00 mmol) of $AlCl_3$ in one portion. The mixture was heated to reflux for 3 min. The resulting dark brown solution

was cooled to 0 °C, and then ice−water was added to form an insoluble material. To the mixture were added 30 mL of 2 N NaOH and 50 mL of EtOAc. The organic phase was separated and the aqueous phase extracted two times with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residual oil was purified by flash chromatography (EtOAc/hexane, 4:1 to 3:1) to yield 7.67 g (94.3%) of compound **10** as a colorless oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.40−7.10 (m, 10H), 6.92−6.80 (m, 2H), 6.67 (dd, J = 1.7, 7 Hz, 1H), 3.77 (s, 3H), 3.49 (s, 2H), 3.44 (d, J = 11 Hz, 1H), 2.84 (br d, J = 12 Hz, 2H), 2.20−1.83 (m, 3H), 1.65−1.20 (m, 4H), 1.27 (s, 9H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 160.07, 149.22, 146.38, 141.01, 138.87, 129.77, 129.67, 128.54, 128.06, 127.31, 125.77, 121.03, 114.88, 111.14, 78.12, 63.82, 58.96, 55.56, 54.28, 40.16, 34.76, 31.84. FAB/MS calcd for $C_{30}H_{38}NO$ $[M+H]^+$ = 428.63; found 428.29.

Preparation of *N*-Benzyl-4-(4-*tert*-butyl-3′-hydroxybenzhydryl)piperidine (11). Compound **10** (0.24 g, 0.56 mmol) was dissolved in 1.15 mL of 0.1 M BBr_3/DCM solution at −10 °C, and the mixture was allowed to stir at room temperature for 1 h. To the solution was added an excess amount of ice-cooled $NaHCO_3$ solution, and then the mixture was extracted two times with DCM. The combined organic phases were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography ($CHCl_3$/MeOH, 50:1). The purified free amine was dissolved in 0.20 mL of 4 N HCl/dioxane, and the hydrochloride salt was precipitated by adding ether to this solution. The resulting solid was filtered off and dried in vacuo to yield 78 mg (31.0%) of compound **11** as an off-white solid: mp = 186−190 °C (decomp). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.70−7.30 (m, 4H), 7.20−6.90 (m, 7H), 6.80−6.65 (m, 2H), 4.20−3.85 (m, 2H), 3.45−3.00 (m, 3H), 2.70−1.40 (m, 8H), 1.18 (s, 9H). FAB/HRMS calcd for $C_{29}H_{36}NO$ $[M + H]^+$ = 414.2797; found 414.2796. Anal. Calcd for $C_{29}H_{35}NO$−HCl: C, 77.39; H, 8.06; N, 3.11. Found: C, 77.15; H, 7.66; N, 3.11.

Preparation of N^{α}-Boc-Ser(OBzl)-N^{α}-benzylglycine Ethyl Ester (12a). Into a solution of *N*-benzylglycine ethyl ester (1 g, 5.17 mmol), *N*-Boc-Ser(OBzl)-OH (1.52 g, 5.17 mmol), and HOBT (1.46 g, 10.8 mmol) dissolved in 20 mL of DCM and at 0 °C was added dropwise dicyclohexylcarbodiimide (DCC) (1.12 g, 5.40 mmol) dissolved in 5 mL of DCM. The reaction was stirred and allowed to warm to room temperature overnight. TLC (hexanes/AcOEt, 4:6) showed total consumption of the benzylglycine ester. The crude mixture was diluted with 100 mL of DCM, filtered twice to eliminate most of the urea byproduct to yield, after evaporation of the solvent, a slightly green oil. Flash chromatography purification gave 1.96 g (80.6% yield) of a colorless oil. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.3−7.22 (m, 10H), 5.43 (d, 1H, J = 7.9 Hz), 4.98 (d, 1H, J = 14.5 Hz), 4.73 (d, 1H, J = 13.1 Hz), 4.51 (s, 2H), 4.42 (d, 1H, J = 14.7 Hz), 4.17−4.07 (m, 3H), 3.74−3.63 (m, 2H), 1.43 (s, 9H), 1.22 (t, 3H, J = 6.9 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 171.65, 168.90, 168.65, 155.02, 135.98, 128.79, 128.30, 128.01, 127.89, 127.82, 127.62, 127.39, 79.78, 73.38, 71.31, 61.55, 51.96, 50.14, 46.93, 28.23, 14.04. ESI/MS calcd for $C_{26}H_{34}N_2O_6$ $[M + Na]^+$ = 493.28; found 493.30.

Preparation of N^{α}-Boc-Ala-N^{α}-benzylglycine Ethyl Ester (12b). Following the same procedure outlined for **12a**, but using Boc-Ala-OH, led to **12b,** after flash chromatography purification (AcOEt/hexanes, 1:1), as a colorless oil (93% yield). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.37−7.20 (m, 5H), 5.48 (bs, 1H), 4.80−4.50 (m, 3H), 4.18−4.11 (dd, 3H), 1.44−1.42 (bs, 9H), 1.39−1.34 (dd, 3H), 1.28−1.20 (td, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 173.80, 168.89, 168.72, 155.11, 154.86, 136.06, 135.25, 128.87, 128.55, 128.05, 127.97, 127.54, 127.17, 79.56, 51.53, 61.07, 60.24, 51.70, 49.86, 48.23, 46.70, 46.06, 28.21, 19.21, 13.98. FAB/MS calcd for $C_{19}H_{29}N_2O_5$ $[M + 1]^+$ = 365.20; found 365.10.

Preparation of N^{α}-Boc-Phe-N^{α}-benzylglycine Ethyl Ester (12c). Following the same procedure outlined for **12a**, but using Boc-Phe-OH, led to **12c** (2.116 g), after flash chromatography purification (AcOEt/hexanes, 1:1), as a white solid (93% yield). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.28−7.21 (m, 8H), 7.11−7.01 (m, 2H), 5.27 (dd, 1H, J = 4.38 Hz), 4.75−4.61 (m, 1H), 4.56−4.48 (m, 1H), 4.22−4.06 (m, 2H), 3.14−2.91 (m, 2H), 1.38 (s, 9H), 1.28−1.18 (m, 5H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 172.71, 168.74, 135.95, 129.63, 128.68, 128.57, 128.43, 127.94, 127.65, 127.27, 126.76, 79.72, 61.63, 61.12, 51.88, 51.67, 48.21, 47.02, 39.62, 28.24, 14.01. ESI/MS calcd for $C_{25}H_{33}N_2O_5$ $[M + 1]^+$ = 441.23; found 441.26.

Preparation of 1-Benzyl-3-benzyloxymethyl-2,5-diketopiperazine (13a). Compound **12a** (1.15 g, 2.61 mmol) was treated with 14 mL of 95% TFA−DCM at room temperature and was monitored by TLC (MeOH/AcOEt, 4:6). After 1 h TLC indicated total consumption of starting material. The reaction was evaporated off, and the residue (a yellow oil) was dried under high vacuum for 2 h to remove most of the TFA. The residue was redissolved in 10 mL of DCM, and 2 mL of TEA was added at room temperature; upon addition of TEA the clear solution became cloudy. The reaction was stirred at room temperature for 2 h. The reaction was monitored by TLC by spraying the TLC with an ethanolic solution of ninhydrin and heating; the product was ninhydrin inactive. The mixture was evaporated off and the residue purified with two solvent systems of increasing polarity (hexanes/AcOEt, 4:6) until all the hydrophobic contaminant was eliminated and then (MeOH/AcOEt, 4:6) to elute the desired compound. Concentration of the collected pure fractions gave 0.853 g of compound **13a** as a white solid (96% yield): mp = 149−150 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.32−7.20 (m, 10H), 4.75 (d, 1H, J = 14.6 Hz), 4.52 (s, 2H), 4.43 (d, 1H, J = 14.6 Hz), 3.97−3.88 (m, 2H), 3.79−3.68 (m, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 166.34, 164.43, 137.20, 134.90, 128.83, 128.49, 128.17, 127.97, 127.58, 73.59, 71.98, 55.93, 49.67, 49.13. ESI/MS calcd for $C_{13}H_{21}N_2O_3$ $[M + 1]^+$ = 325.08; found 325.00.

Preparation of 1-Benzyl-3-methyl-2,5-diketopiperazine (13b). Following the same procedure outlined above for **13a**, but using **12b**, led to **13b**, after flash chromatography purification (MeOH/AcOEt, 4:6), as a white solid (75% yield): mp = 137−138 °C. ^{1}H NMR (CD_3OD, 200 MHz): δ 7.36−7.26 (m, 5H), 4.60 (d, 2H, J = 3.1 Hz), 4.11 (q, 1H, J = 7 Hz), 3.88 (s, 2H), 1.45 (d, 3H, J = 6.96 Hz). ^{13}C NMR (CD_3OD, 50 MHz): δ 169.46, 167.95, 136.96, 129.92, 129.11, 128.99, 128.91, 52.02, 19.84. ESI/MS calcd for $C_{12}H_{15}N_2O_2$ $[M + 1]^+$ = 219.10; found 219.01.

Preparation of 1,3-Dibenzyl-2,5-diketopiperazine (13c). Following the same procedure outlined above for **13a**, but using **12c**, led to **13c,** after flash chromatography purification, as a white solid (96% yield): mp = 172−173 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.32−7.15 (m, 10H), 4.46 (s, 2H), 4.3 (bs, 1H), 3.49 (d, 1H, J = 17.6 Hz), 3.17 (m, 2H), 2.92 (d, 1H, J = 17.6 Hz). ^{13}C NMR (DMSO, 50 MHz): δ 165.49, 165.00, 135.70, 130.14, 128.54, 128.35, 128.12, 127.58, 126.83, 55.60, 48.49, 48.25, 39.24. ESI/MS calcd for $C_{18}H_{19}N_2O_2$ $[M + 1]^+$ = 295.13; found 295.10.

Preparation of 1-Benzyl-3-benzyloxymethylpiperazine (14a). A solution of compound **13a** (0.60 g, 1.85 mmol) dissolved in 15 mL of dry THF was flushed with nitrogen for 20 min. The solution was cooled to 0 °C in an ice bath, and LAH (0.35 g, 9.25 mmol) was added in portions (slowly at the beginning). The reaction was stirred at room temperature for 1 h, then LAH (0.210 g, 5.5 mmol) was added, and the reaction was stirred at room temperature for 36 h. The reaction was cooled to −10 °C, and 0.15 mL of a 15% aqueous NaOH solution was added. After 1 h, 2 mL of H_2O was added and the reaction stirred overnight. The white precipitate was filtered out and washed with DCM (3 × 20 mL). The solution was dried over $MgSO_4$, filtered, and concentrated to give 0.46 g of a slightly green oil (84% yield). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.30−7.25 (m, 10H), 4.47 (s, 2H), 3.48 (s, 2H), 3.39−3.33 (m, 2H), 3.1−2.9 (m, 1H), 2.9−2.85 (m, 2H), 2.08 (td, 1H, J = 4.04 Hz), 1.82 (t, 1H, J = 10.2 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 137.99, 137.81, 129.08, 128.29, 128.09, 127.62, 127.58, 126.94, 73.27, 72.52, 63.30, 55.74, 54.47, 53.61, 45.03. FAB/MS calcd for $C_{19}H_{25}N_2O$ $[M + 1]^+$ = 297.4219; found 297.1967.

Preparation of 1-Benzyl-3-methylpiperazine (14b). Following the same procedure outlined above for **14a**, but

using **13b**, led to **14b** as a yellow oil (98% yield). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.33−7.24 (m, 5H), 3.49 (s, 2H), 2.92 (dd, 2H, J = 2.5 Hz), 2.90−2.82 (m, 1H), 2.76 (dd, 2H, J = 2.2 Hz), 2.03−1.97 (m, 2H), 1.01 (d, 3H, J = 6.3 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 137.95, 129.04, 128.03, 126.84, 63.25, 61.14, 53.47, 50.36, 45.73, 19.82. ESI/MS calcd for $C_{12}H_{19}N_2$ $[M + 1]^+$ = 191.105; found 191.20.

Preparation of 1,3-Dibenzylpiperazine (14c). Following the same procedure outlined above for **14a**, but using **13c**, led to **14c** as a brownish oil (97% yield). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.33−7.17 (m, 10H), 3.50 (d, 2H, J = 5.8 Hz), 3.09−2.82 (m, 1H), 2.88−2.70 (m, 4H), 2.67−2.50 (m, 1H), 2.09 (td, 2H, J = 3.9 Hz, 10.8 Hz), 1.91 (t, 1H, J = 10.3 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 138.45, 137.98, 129.14, 128.45, 128.13, 128.04, 126.96, 126.31, 63.26, 59.54, 56.23, 53.25, 45.62, 40.71. ESI/MS calcd for $C_{18}H_{23}N_2$ $[M + 1]^+$ = 267.14; found 267.20.

Preparation of 1-(4-*tert*-Butyl-3′-hydroxybenzhydryl)-2-benzyloxymethyl-4-benzylpiperazine (15a). Compound **14a** (0.430 g, 1.45 mmol) and K_2CO_3 (0.60 g, 4.3 mmol) were dissolved in 10 mL of MeCN; complete dissolution was obtained after a few minutes of heating. Then, chloride **4** (0.526 g, 1.45 mmol) was added, dissolved in 1.5 mL of MeCN. After 2 h of reflux under nitrogen atmosphere, TLC (hexanes/AcOEt, 4:6) indicated no presence of **4**. The reaction was filtered and the precipitate washed several times with DCM. After concentration of the solution, 1.028 g of a brown oil was obtained. Flash chromatography purification of the crude gave 0.67 g of a yellow oily residue. The residue was dissolved in 0.5 mL of a 1:1 solution of MeOH/dioxane. The solution was cooled to 0 °C, then 1.5 mL of 4 N HCl was added and stirred overnight, allowing to warm to room temperature. The mixture was evaporated off to dryness, and the residue was treated with cold ether. The white−yellow precipitate was triturated with ether several times and then dried under high vacuum to give **15a** as a slightly red solid (40% yield): mp = 85−86 °C. ^{1}H NMR (CD_3OD, 200 MHz): δ 7.28−7.15 (m, 15H), 6.90−6.83 (m, 2H), 6.7 (m, 1H), 4.87 (s, 2H), 4.37−4.30 (m, 2H), 3.88 (td, 2H, J = 3.08 Hz), 3.75 (td, 2H, J = 2.92 Hz), 3.58−3.96 (m, 2H), 3.04−2.87 (m, 1H), 2.79−2.74 (m, 1H), 2.54 (t, 2H, J = 8.88 Hz), 2.38 (d, 2H), 1.25 (s, 9H). ^{13}C NMR (CD_3OD, 50 MHz): δ 157.02, 147.34, 141.79, 139.62, 130.33, 129.43, 129.27, 128.83, 128.76, 128.57, 128.41, 128.22, 126.16, 110.43, 74.01, 63.93, 55.33, 54.02, 38.99, 31.78. FAB/HRMS calcd for $C_{36}H_{43}N_2O_2$ $[M + 1]^+$ = 535.7524; found 535.3325. Anal. Calcd for $C_{36}H_{42}N_2O_2$−1.5HCl: C, 73.2; H, 7.42; N, 4.74. Found: C, 73.0; H, 7.35; N, 4.64. t_R = 29.66 and 30.19 min (both diastereoisomers, 1:1 ratio).

Preparation of 1-(4-*tert*-Butyl-3′-hydroxybenzhydryl)-2-methyl-4-benzylpiperazine (15b). Following the same procedure outlined above for **15a**, but using **14b**, led to **15b** as a slightly yellow solid (43% overall yield in two steps): mp = 173−175 °C. After several experiments it was found that, in order to achieve better yields from the S_N2 reaction, the reflux temperature had to be around 100 °C and longer reaction times (9 h) must be used, compared with compound **15a**. ^{1}H NMR (CD_3OD, 200 MHz): δ 7.48−7.12 (m, 13H), 6.65 (bs, 1H), 4.41−4.23 (m, 2H), 3.7−3.1 (m, 7H), 1.17 (d, 12H). ^{13}C NMR (CD_3OD, 50 MHz): δ 159.44, 132.73, 131.73, 131.65, 131.44, 130.38, 129.73, 129.54, 129.34, 129.28, 127.46, 127.32, 78.26, 61.93, 61.84, 35.45, 31.73, 31.55. FAB/HRMS calcd for $C_{29}H_{37}N_2O$ $[M + 1]^+$ = 429.6256; found, 429.2906. Anal. Calcd for $C_{29}H_{36}N_2O$−2CF_3CO_2H: C, 60.41; H, 5.85; N, 4.27. Found: C, 60.2; H, 5.89; N, 4.10. t_R = 25.21 min (only one diastereoisomer).

Preparation of 1-(4-*tert*-Butyl-3′-hydroxybenzhydryl)-2,4-dibenzylpiperazine (15c). Following the same procedure outlined above for **15a**, but using **14c**, led to **15c** as a slightly yellow solid (41.6% overall yield in two steps): mp = 170−172 °C. Also, it was also found after several experiments that, in order to get better yields from the S_N2 reaction, the reflux temperature had to be around 100 °C and longer reaction times (9 h) must be used, compared with compound **15a**. ^{1}H NMR (CD_3OD, 200 MHz): δ 7.42−7.47 (m, 10H), 7.14−7.03 (m, 3H), 6.90−6.82 (m, 3H), 6.72−6.78 (m, 1H), 6.35 (d, 1H), 6.72 (d, 1H, J = 12.8 Hz), 4.02 (d, 1H, J = 12.5 Hz), 3.59−3.50 (m, 2H), 3.37−3.24 (m, 3H), 3.03−2.91 (m, 4H), 2.77−2.66 (m, 1H). ^{13}C NMR (CD_3OD, 50 MHz): δ 171.49, 138.57, 138.35, 138.04, 137.17, 132.24, 131.56, 131.32, 130.32, 129.49, 129.83, 129.60, 129.23, 128.47, 126.97, 116.06, 115.90, 78.77, 61.84, 57.35, 53.57, 50.95, 43.32, 35.21, 31.99, 31.63. FAB/HRMS calcd for $C_{35}H_{41}N_2O$ $[M + 1]^+$ = 505.3219; found 505.3230. Anal. Calcd for $C_{35}H_{40}N_2O$−2HCl: C, 72.97; H, 7.36; N, 4.86. Found: C, 72.90; H, 7.52; N, 4.54. t_R = 28.31 and 29.85 min (both diastereoisomers).

Preparation of 1-(4-*tert*-Butyl-3′-hydroxybenzhydryl)-2-hydroxymethyl-4-benzylpiperazine (15d). Compound **13a** (0.10 g, 0.16 mmol) was dissolved in 3 mL of dry THF. The mixture was flushed with nitrogen for 25 min, then 0.05 g of Pd−C was added, and the reaction was capped with a balloon filled with H_2. The reaction was monitored by TLC (hexanes/AcOEt, 1:1). After 7 h, total consumption of starting material was observed. The mixture was filtered out through celite, and the catalyst was washed with EtOH several times. After concentration of the solution, 0.082 g of a yellow oil was obtained (96% yield). The above residue was dissolved in 0.5 mL of a 1:1 solution of MeOH/dioxane. The solution was cooled to 0 °C, then 1.5 mL of 4 N HCl was added, and the mixture was stirred overnight, allowing to warm to room temperature. The mixture was evaporated off to dryness, and the residue was treated with cold ether. The white−yellow precipitate was triturated with ether several times and then dried under high vacuum. Analytical HPLC showed two peaks in a 1:1 ratio which, after HPLC separation, were shown to be the two corresponding diastereoisomers. Flash chromatography purification of the residue using two solvent systems of increasing polarity, hexanes/AcOEt, 8:2, then MeOH/AcOEt, 1:1, gave 0.042 g of compound **15d** as a slightly yellow solid (65% yield): mp = 139−140 °C. ^{1}H NMR (CD_3OD, 200 MHz): δ 7.43−6.69 (m, 13H), 4.85 (s, 2H), 4.66 (d, 1H, J = 12.1 Hz), 4.44 (d, 1H, J = 12.1 Hz), 3.97 (m, 1H), 3.65−3.50 (m 3H), 3.37−3.30 (m,3H), 2.99 (m, 1H), 1.26 (s, 9H). ^{13}C NMR (CD_3OD, 50 MHz): δ 130.26, 130.29, 130.06, 129.92, 129.12, 129.08, 127.28, 121.23, 74.38, 35.40, 31.54. FAB/HRMS calcd for $C_{29}H_{37}N_2O_2$ $[M + 1]^+$ = 445.6272; found 445.2855. Anal. Calcd for $C_{29}H_{36}N_2O_2$−HCl: C, 67.49; H, 7.43; N, 5.43. Found: C, 67.16; H, 7.45; N, 5.30. t_R = 25.49 min.

Radiolabeled Ligand Binding Assays. Rat brain membranes were used to study the synthesized compounds and were prepared by the literature method.[6] Binding affinities of the compounds were measured against [^{3}H][*p*-Cl-Phe^4]DPDPE ([^{3}H]Tyr-c[D-Pen-Gly-(*p*-Cl)Phe-D-Pen-OH]) (41.0 Ci/mmol) (δ-ligand) and [^{3}H]DAMGO ([^{3}H]Tyr-D-Ala-Gly-*N*-methyl-Phe-Glyol) (48.9 Ci/mmol) (New England Nuclear) (μ-ligand) by a rapid filtration technique. A solution of 0.75 nM [^{3}H][p-Cl-Phe^4]-DPDPE or 1.0 nM [^{3}H]DAMGO in a total volume of 1 mL of 50 mM Tris-HCl buffer (pH 7.4) containing bovine serum albumin (1.0 mg/mL), bacitracin (50 μg/mL), bestatin (30 μg/mL), captopril (10 μM), and phenylmethylsulfonyl fluoride (0.1 mM) was used. Assays were done in duplicate with 10 μM naltrexone hydrochloride to define nonspecific tissue binding. The binding reaction was terminated by rapid filtration through presoaked (0.5% polyethylenimine solution) GF/B Whatman glass fiber strips with a Brandel cell harvester followed immediately by three rapid washes with 4 mL aliquots of ice-cold saline solution. The filters were removed and soaked in 10 mL scintillation fluid at 4 °C for at least 6 h before bound radioactivity was measured. The data were analyzed by nonlinear least squares regression analysis.

In Vitro Bioassays. Electrically induced smooth muscle contractions from mouse vas deferens (MVD) and guinea pig ileum (GPI) longitudinal muscle-myenteric plexus were used for bioassays. Tissues came from male ICR mice weighing 25−30 g and from male Hartley guinea pigs weighing 150−400 g. The tissues were tied to gold chains with suture silk, suspended in 20 mL baths containing 37 °C oxygenated (95% O_2, 5% CO_2) Krebs bicarbonate solution (magnesium-free for the MVD), and allowed to equilibrate for 15 min. The tissues were then stretched to the optimal length previously determined

to be 1 g tension (0.5 g for MVD) and allowed to equilibrate for 15 min. The tissues were stimulated transmurally between platinum plate electrodes at 0.1 Hz for 0.4 ms pulses (2.0 ms pulses for MVD) and supramaximal voltage. Drugs were added to the baths in 20−60 μL volumes. The agonists remained in tissue baths for 3 min and were removed by rinsing several times with fresh Krebs solution. Tissues were reequilibrated to regain predrug contraction height. Antagonists were added to the bath 2 min prior to the addition of the agonists. Percent inhibition was calculated by dividing height for 1 min preceding the addition of the agonist by the contraction height 3 min after exposure to the agonist. The IC_{50} values represent the mean of not less than four tissues. Relative potency estimates were determined by fitting the mean data to the Hill equation by using a computerized nonlinear least-squares method. In some cases, the weak μ-agonist action of these analogues did not permit completion of full dose−response curves in the GPI.

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