Identification of an Opioid κ Receptor Subtype-Selective N-Substituent for (+)-(3*R*,4*R*)-Dimethyl-4-(3-hydroxyphenyl)piperidine

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A three-component library of compounds was prepared in parallel using multiple simultaneous solution-phase synthetic methodology. The compounds were biased toward opioid receptor antagonist activity by incorporating (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (a potent, nonselective opioid pure antagonist) as one of the monomers. The other two monomers, which included N-substituted or unsubstituted Boc-protected amino acids and a range of substituted aryl carboxylic acids, were selected to add chemical diversity. Screening of these compounds in competitive binding experiments with the κ opioid receptor selective ligand [^{3}H]-U69,593 led to the discovery of a novel κ opioid receptor selective ligand, *N*-{(2′*S*)-[3-(4-hydroxyphenyl)propanamido]-3′-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**8**, RTI-5989-29). Additional structure−activity relationship studies suggested that **8** possesses lipophilic and hydrogen-bonding sites that are important to its opioid receptor potency and selectivity. These sites appear to exist predominantly within the κ receptor since the selectivity arises from a 530-fold loss of affinity of **8** for the μ receptor and an 18-fold increase in affinity for the κ receptor relative to the μ-selective ligand, (+)-*N*-[*trans*-4-phenyl-2-butenyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**5a**). The degree of selectivity observed in the radioligand binding experiments was not observed in the functional assay. According to its ability to inhibit agonist stimulated binding of [^{35}S]GTPγS at all three opioid receptors, compound **8** behaves as a μ/κ opioid receptor pure antagonist with negligible affinity for the δ receptor.

Introduction

The discovery of potent, highly receptor-selective opioid pure antagonists has been a goal of medicinal chemists for many years.[1,2] As molecular probes, antagonists have served as useful tools in the study of both the structure as well as the physiological functions of the highly complex opioid receptor system. Much has been accomplished as evidenced by the elegant work of Portoghese and co-workers over the past decade which ultimately has led to the discovery of the naltrexone-based κ and δ receptor subtype-selective antagonists norbinaltorphimine[3] (**1**, nor-BNI) and naltrindole[4] (**2**, NTI), respectively. Following Portoghese's lead, workers at SmithKline Beecham recently reported that the octahydroisoquinoline (**3**, SB 205588) was a second-generation, highly potent and selective δ antagonist formally derived from naltrindole fragmentation.[5] One of our specific research aims has been the discovery of opioid receptor selective reversibly binding ligands from the N-substituted (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**4a**) class of compounds that display pure antagonist activity.[6] These compounds will be useful as molecular probes for the opioid receptor as well as potential drug candidates for the treatment of substance abuse.[7] While μ antagonists have for years been used in drug abuse therapy, recent findings suggest that κ antagonists could provide a more effective, long-lasting treatment strategy.[8] A great variety of N-substituted derivatives of **4a** has been prepared, but until the recent demonstration of μ selectivity for **5a**,[9] none had shown selectivity between the opioid receptor subtypes. Since the pure antagonist activity of these compounds is not dependent on the N-substituent, multiple changes to this part of the molecule would be expected to affect binding affinity and possibly receptor selectivity but not alter its fundamental antagonist character. This feature distinguishes this class of antagonist from the morphone-based compounds, which display pure antagonist behavior only with N-substituents such as allyl or cyclopropylmethyl but not methyl, ethyl, or phenethyl.[10] It is currently believed that the N-substituent in **4a** interacts with a lipophilic binding domain which has been described as either very large or quite malleable since a multitude of different types of N-substituent changes provided ligands displaying high binding affinity.[11] It has also been determined that maximum potency and selectivity for the μ opioid receptor is achieved when the N-substituent incorporates a lipophilic entity (phenyl or cyclohexyl ring) separated from the piperidine nitrogen by three atoms as illustrated by compounds **5a**−**d**.[9,11]

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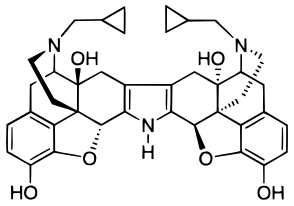

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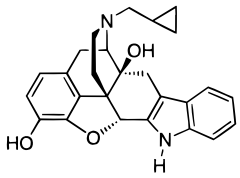

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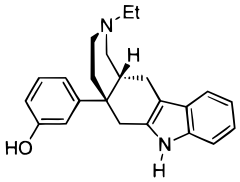

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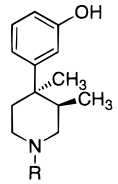
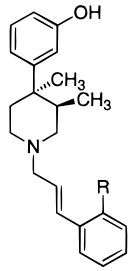
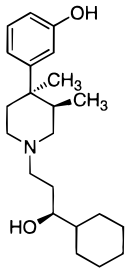


4a, R = substituent
4b, R = H (LY272922)



5a, R = H
5b, R = CH_3
5c, R = Cl



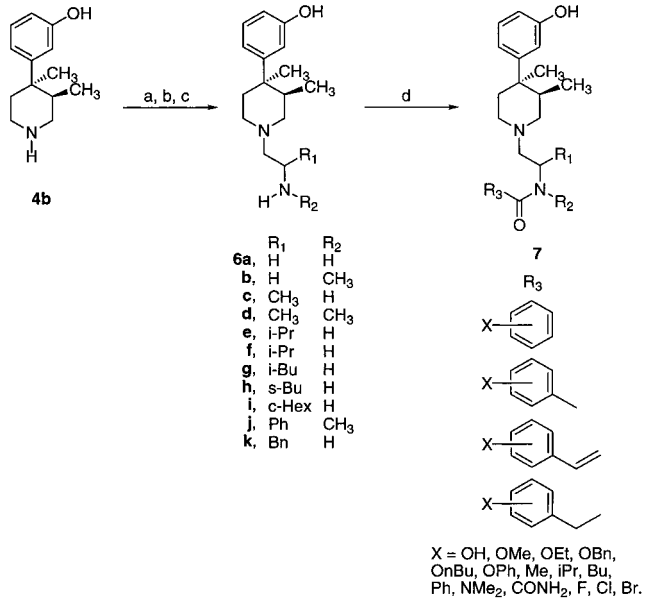
5d

During the past decade, combinatorial chemistry has become widely practiced by medicinal chemists for the optimization of drug leads.[12−14] This library-based approach to drug discovery has spurred the development of methodologies for both solid- and solution-phase chemistry with a central theme of multiple, simultaneous compound synthesis. In our search for κ-selective opioid antagonists, a library biased for opioid receptor antagonist activity was designed by utilizing an N-substituent structure for **4b** (LY272922) which allowed incorporation of diversity elements while simultaneously avoiding features resembling the μ-favoring N-substituent structure mentioned previously.[9,11] Thus, the basic structural unit expressed in the library is illustrated in the general structure **7** where the groups R_1, R_2, and R_3 are varied to obtain a highly diverse set of compounds (see Scheme 1). This strategy used commercially available protected amino acids for the R_1 and R_2 diversity elements which avoided the undesired μ-favoring substitution pattern in all amino acids except phenylalanine, which was prepared for comparative purposes. Using this strategy, 288 compounds were prepared for screening in a relatively short period of time from commercially available carboxylic acids for the R_3 diversity element. In this study, we report the application of this strategy to discover the opioid κ receptor selective compound, N-{(2′*S*)-[3-(4-hydroxyphenyl)propanamido]-3′-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**8**).

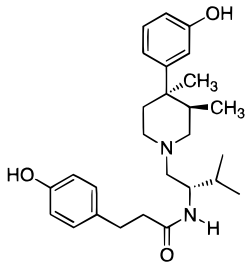
Chemistry

Coupling of (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (**4b**) with an appropriate *tert*-butoxycarbonyl-protected amino acid (Boc-protected) followed by

Scheme 1[a]



[a] Reagents: (a) Boc-amino acid, BOP, TEA, THF; (b) TFA, CH_2Cl_2; (c) borane/dimethyl sulfide; (d) R_3CO_2H, BOP, TEA, THF.



8

removal of the Boc-protecting group with trifluoroacetic acid (TFA) in methylene chloride followed by reduction using a tetrahydrofuran (THF) solution of borane−dimethyl sulfide complex gave the intermediate amines (**6a**−**k**) in 15−78% yields (Scheme 1). These intermediates **6** were subjected to column chromatography or crystallization as necessary to obtain pure compounds. The final products (**7**) were prepared in scintillation vials via amide bond formation by coupling with a wide variety of commercially available carboxylic acids. Benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) in THF was employed as the coupling reagent which provided very clean products after aqueous workup. These compounds were used directly in screening without additional purification. Pure compounds for further structure−activity relationship (SAR) analysis were obtained by purification of library samples or by single-compound synthesis by conventional synthetic methodology and characterized by MS, ^{1}H NMR, and elemental analyses.

Results and Discussion

The results from the screening of the 288-compound library in competitive binding against the κ opioid receptor selective ligand [^{3}H]U69,593 are illustrated graphically in Figure 1. It is evident from this representation that few compounds showed significant inhibition of radioligand binding at 100 nM with **8** (plate 4,

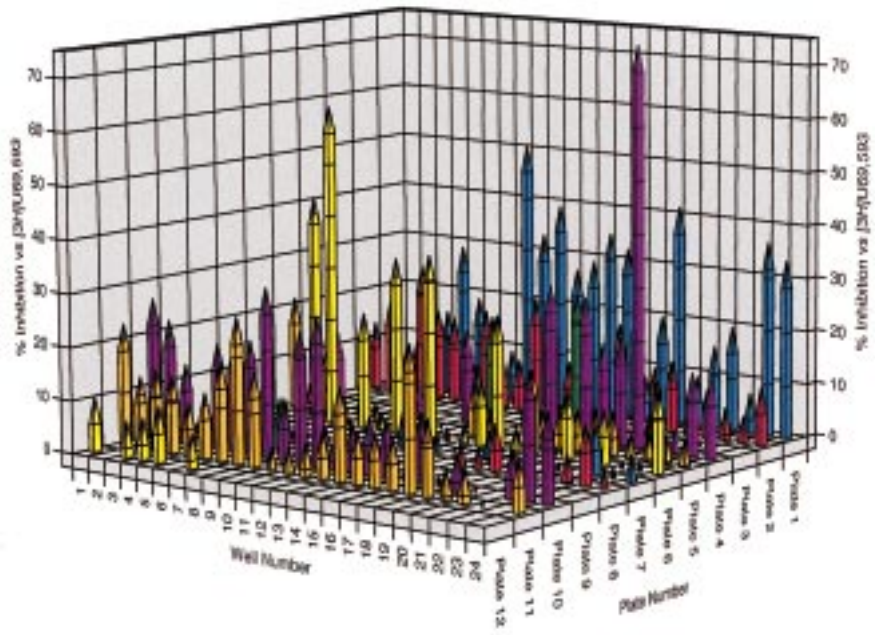
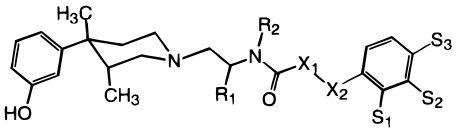


Figure 1. Data from screening of library at 100 nM against the κ-selective ligand [^{3}H]U69,593 (percent inhibition).

Table 1. Results of Inhibition Screening of Selected Structural Isomers of Compound **8** Taken from the Library versus κ Opioid Selective Ligand [^{3}H]U69,593



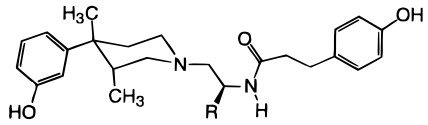
compd	R_1	R_2	X_1	X_2	S_1	S_2	S_3	% inhibition at 100 nM
8	*i*-Pr	H	CH_2	CH_2	H	H	OH	71
9	*i*-Pr[a]	H	CH_2	CH_2	H	H	OH	11
10	*i*-Pr	H	CH_2	CH_2	H	H	H	28
11	*i*-Pr	H	CH_2	CH_2	H	OH	H	20
12	*i*-Pr	H	CH_2	CH_2	OH	H	H	25
13	*i*-Pr	H	CH_2		H	H	OH	6
14	*i*-Pr	H	CH[b]	CH[b]	H	H	OH	15
15	*i*-Pr	H	CH_2	CH_2	H	H	F	26
16	*i*-Pr	H	CH_2	CH_2	H	OH	OH	31
17	*i*-Pr	H	CH_2	CH_2	H	OCH_3	OH	42
18	*i*-Pr	H	CH_2	CH_2	H	H	OCH_3	16
19	H	H	CH_2	CH_2	H	H	OH	11
20	CH_3	H	CH_2	CH_2	H	H	OH	20
21	H	CH_3	CH_2	CH_2	H	H	OH	0
22	CH_3	CH_3	CH_2	CH_2	H	H	OH	1
23	C_6H_5	CH_3	CH_2	CH_2	H	H	OH	7
DMSO								4

[a] The carbon to which the *i*-Pr group is attached has the opposite stereochemistry from that in **8**. [b] Trans double bond.

well 20, 71%) being the best (Figure 1). The data for percent inhibition of [^{3}H]U69,593 binding by selected library compounds **8**−**23** at 100 nM are listed in Table 1.

A comparative analysis of the structures related to compounds **9**−**23**, which lack significant binding affinity relative to **8**, readily illustrates the importance for κ receptor binding of each structural subunit of group R_3 (Table 1). Compound **9**, a diastereomer of **8**, where the carbon to which the R_1 isopropyl group is connected has the opposite stereochemistry, shows no appreciable binding affinity (11%) for the opioid κ receptor. The sensitivity to orientation (*R* or *S*) at this stereogenic center suggests that the isopropyl group may be working in tandem with another structural feature of the R_3 unit to both increase binding in **8** and decrease binding in **9**. The difference in affinity of compounds **8** (71%) and **10** (28%) indicates that the 4-hydroxyl substituent in **8** is essential for high κ binding affinity. Furthermore, the weak inhibition displayed by compounds **11** (20%) and **12** (25%) possessing *m*- and *o*-hydroxyl substituents, respectively, pinpoints the para placement of the *p*-hydroxyl group as the optimum position. The fact that compound **19**, which lacks the isopropyl group but has the *p*-hydroxyphenylpropionic substituent, shows little affinity (11% vs 71%) relative to **8** adds additional support to the importance of the R_1 isopropyl and 4-hydroxyphenyl groups to the κ-selective binding. The low affinity of compound **20** (20%) which has a methyl substituent in position (R_1) shows that a methyl group may not substitute for the isopropyl group. This strengthens the notion that both the isopropyl group (R_1) and the 4-hydroxyphenyl group for R_3 are working together to elicit high affinity binding at the κ opioid receptor in compound **8**. The results for compound **13** (6%) illustrate that two methylene groups are necessary between the phenyl ring and the amide carbonyl in diversity element R_3, presumably because the *p*-hydroxyl group cannot reach its site of interaction in the truncated derivative. Furthermore, the poor inhibition of binding for compound **14** (15%) which incorporates a trans double bond in the connecting chain shows that the length of the chain is not sufficient to impart high binding affinity, implying that flexibility is also required in this carbon chain to provide proper ligand and receptor alignment. The low affinity of the 4-fluoro derivative **15** (26%) and the 4-methoxy derivative **18** (16%) supports the suggestion that a hydrogen bond exists between ligand **8** and the receptor with compound **8** donating the hydrogen. This is further supported by the low affinity of the 3,4-dihydroxyl derivative **16** (31%) which can hydrogen bond internally and the 3-methoxy-4-hydroxy derivative **17** (42%) whose hydrogen bond could be sterically encumbered by interference from an adjacent methoxy

Table 2. Radioligand Binding Data for **8** and Related Compounds at μ, δ, and κ Opioid Receptor Assays



		K_i (nM ± SD) ($-n_H$)				
compd	R	[^{3}H]DAMGO	[^{3}H]DADLE	[^{3}H]U69, 593	μ/κ	δ/κ
8	*i*-Pr	393 ± 13.3 (0.89 ± 0.02)	>5700	6.91 ± 0.55 (0.81 ± 0.05)	57	>824
24	*i*-Bu	398 ± 72.3 (0.91 ± 0.16)	NA	89.3 ± 7.03 (0.78 ± 0.05)	4.5	
25	*sec*-Bu	421 ± 30.5 (0.91 ± 0.06)	NA	8.84 ± 0.30 (0.87 ± 0.02)	47	
26	c-Hex	234 ± 25.2 (0.84 ± 0.08)	NA	83.1 ± 5.7 (0.79 ± 0.04)	2.8	
27	benzyl	9.6 ± 1.18 (0.89 ± 0.09)	NA	54.6 ± 3.5 (0.86 ± 0.04)	0.17	
5a[a]		0.74 ± 0.05 (0.89 ± 0.09)	322 ± 38.1 (0.75 ± 0.09)	122 ± 11.9 (0.52 ± 0.07)	0.006	2.6
1 (nor-BNI)[b,c]		47.2 ± 3.3	42.9 ± 11	0.28 ± 0.07	181	150
naltrexone[b]		1.39 ±0.40 (0.94 ± 0.08)	94.9 ± 6.6 (1.01 ± 0.09)	4.71 ± 0.12 (1.05 ± 0.08)	0.30	20.1

[a] Data taken from ref 9. [b] Data provided for reference; compound is not a derivative of **8**. [c] Data taken from ref 15. [^{3}H]DAMGO, [^{3}H]DPDPE, and [^{3}H]U69,593 were used as the radioligands for the μ, δ, and κ assays, respectively. Guinea pig brain membranes were used.

group. Interestingly, all compounds having methyl and not hydrogen as the second diversity element R_2 (**21** (0%), **22** (1%), and **23** (7%)) displayed very low binding affinity usually at baseline (DMSO blank) levels. Apparently, position R_2 should remain unsubstituted. These results suggest that the amide group may be part of a separate hydrogen-bonding interaction to place the key R_1 isopropyl and R_3 *p*-hydroxyphenyl rings in their correct positions for strong interaction with the receptor. Alternatively, the *N*-methyl substituent may be decreasing ligand affinity through repulsive steric interactions.

Taken together, the data suggests that the high binding affinity displayed by **8** results from a combination of several structural features present in its N-substituent. These include a 4-hydroxyl group in the pendant phenyl ring of group R_3, the length and flexibility of the carbon chain connecting this ring to the amide carbonyl, and the presence of a β (position R_1) isopropyl group with an *S* configuration at the adjacent stereogenic center. The data analysis suggests that the principal stabilizing interactions could be related to binding of the hydroxyl and isopropyl substituents with the other atoms of the N-substituent substructure acting to hold these two binding elements in optimum overlapping positions within the receptor site. Alternatively, the isopropyl group could be acting to bias the conformation of molecule to provide the best alignment of the 4-hydroxyphenylpropionic acid side chain with its binding site.

To gain additional SAR information, a pure sample of **8** along with compounds **24**−**27** which vary at the R_1 position alone was prepared for study. Table 2 lists the K_i values for these derivatives at the μ and κ opioid receptors along with the K_i values for the μ-selective reference compound **5a**, naltrexone, and the κ-selective antagonist nor-BNI.[15] The δ receptor assay was not performed for compounds **24**−**27** as all previous derivatives of **8** had shown no affinity for this receptor subtype. This study revealed that **8** not only actively binds the κ receptor (K_i = 6.9 nM) but also possesses a 57-fold selectivity for the κ vs the μ receptor (K_i = 393 nM) and >870-fold selectivity for the κ vs the δ receptor (K_i > 5700 nM). Compound **8** is thus the only derivative of **4b** to display this magnitude of opioid κ receptor subtype selectivity.[9,11] Nor-BNI (**1**) has a higher affinity for the κ receptor than **8** and has a greater κ selectivity relative to the μ receptor. However, **8** is more selective for the κ receptor relative to the δ receptor. A part of these differences could be due to the use of different tissues and radioligands.

The data for the β isobutyl substituent compound **24**, which results formally from insertion of a methylene between the isopropyl group and its adjacent stereogenic center of compound **8**, displays a significant loss of affinity for the κ receptor while maintaining the same affinity for the μ receptor as compound **8**. The net effect is a loss of selectivity between the μ and κ receptor subtypes. Compound **26** (R_1 = cyclohexyl) shows a similar loss of affinity for the κ receptor with a gain in affinity for the μ receptor, resulting in a similar loss of selectivity. Compound **25** with an R_1 *sec*-butyl group shows a slight decrease in both κ and μ potency but retains selectivity, though its magnitude is lower relative to **8**. Compound **27** (R_1 = benzyl) displayed a binding profile completely different from that seen in **8** with a tremendous increase in μ potency and concomitant loss of κ potency. This was not unexpected since compound **27**, prepared from the amino acid phenylalanine, possesses an N-substituent with a phenyl ring separated from the piperidine ring by three methylene groups which are known to favor μ binding.[9,11] It was for this reason that phenylalanine was excluded from use in the library synthesis. Overall, the behaviors of the various R_1 derivatives of **8** indicate that the size of the lipophilic group in position R_1 is critical to both the potency and receptor subtype selectivity of the ligand. Furthermore, the data supports the hypothesis that the isopropyl group in **8** is not simply biasing the conformation of side chain but is instead interacting with the receptor directly in a ligand-stabilizing interaction.

Table 3. Inhibition by Antagonists of [^{35}S]GTPγS Binding in Guinea Pig Caudate Stimulated by DAMGO (μ), SNC80 (δ), and U69,593 (κ) Selective Opioid Agonists

	K_i (nM ± SD) ($-n_H$)[a]		
compd	DAMGO[b]	SNC80[c]	U69,593[d]
8	7.25 ± 0.52 (1.11 ± 0.08)	450 ± 74.1 (1.05 ± 0.17)	4.70 ± 0.56 (1.38 ± 0.19)
5a[e]	0.039 ± 0.003 (1.06 ± 0.07)	1.48 ± 0.094 (1.19 ± 0.08)	1.04 ± 0.061 (1.07 ± 0.06)
1, nor-BNI	16.75 ± 1.47 (1.02 ± 0.09)	10.14 ± 0.96 (1.18 ± 0.12)	0.038 ± 0.005 (0.97 ± 0.12)
naltrexone	0.93 ± 0.21 (1.03 ± 0.22)	19.3 ± 2.25 (1.05 ± 0.17)	2.05 ± 0.21 (1.38 ± 0.19)

[a] See footnote *a* from Table 2. [b] DAMGO [(D-Ala^2,MePhe^4,Gly-ol^5)enkephalin]. Agonist selective for μ opioid receptor. [c] SNC-80 ([(+)-4-[(αR)-α-(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl]-*N*,*N*-diethylbenzamide). Agonist selective for δ opioid receptor. [d] U69,593 [(5α,7α,8β)-(−)-*N*-methyl-*N*-[7-(1-pyrrolidinyl)-1-oxaspiro[4,5]dec-8-yl]benzeneacetamide]. Agonist selective for κ opioid receptor. [e] Data taken from ref 9.

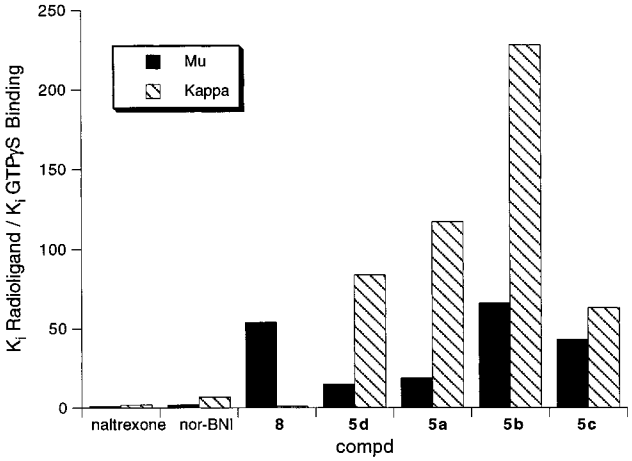


Figure 2. Comparison of ratios of radioligand binding and GTPγS assays for compound **8**, naltrexone, nor-BNI, **5d**, and **5a**−**c**, the *N*-*trans*-cinnamyl derivatives of **4b**. The radioligand and GTPγS binding data for **5a**−**d** were taken from ref 9.

The agonist/antagonist activity of compound **8** was measured by determining its ability to either stimulate or reverse opioid agonist stimulated binding of the nonhydrolyzable GTP analogue, [^{35}S]GTPγS, in all three opioid receptor assays (Table 3).[16] Table 3 includes data obtained for naltrexone, the standard nonselective opioid pure antagonist, nor-BNI, the prototypical κ-selective antagonist, and the potent, μ-favoring opioid antagonist (**5a**). The κ selectivity displayed by compound **8** in the inhibition of radioligand binding assay was not observed in the [^{35}S]GTPγS functional assay. This is not an atypical situation; radioligand binding results often differ substantially from those seen in functional assays, but this typically involves agonists. The antagonists, naltrexone, normally display K_i (radioligand)/K_i (GTPγS) binding ratios near unity whereas ratios greater than unity have been observed for antagonists of the N-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)-piperidine series.[9] This phenomenon is illustrated graphically in Figure 2. The *trans*-cinnamoyl derivatives **5a**−**c** and compound **5d** display K_i (radioligand)/K_i (GTPγS) binding ratios greater than unity in the μ and κ receptor assays which is distinctly different from the response demonstrated by naltrexone. In the present case, compound **8** is found to behave like naltrexone in the κ receptor assays with a ratio near unity which is far different from the behavior seen for **5a**−**c** and **5d**, which show ratios of 118, 228, 63, and 85, respectively. In the μ receptor assay on the other hand, compound **8** with a ratio of 54 behaves like **5a**−**c** and **5d** which give ratios of 19, 66, 43, and 15. This differential response of **8** in the [^{35}S]GTPγS assay is sufficiently large so as to erode the κ receptor selectivity observed for **8** in the radioligand binding assays. Note that the K_i (radioligand)/K_i (GTP) binding ratios for nor-BNI at the μ and κ receptor are 2.8 and 7.36, respectively.

Conclusions

The discovery of compound **8**, which displays a highly selective κ vs μ receptor inhibition of radioligand binding profile and a potent μ/κ opioid antagonist profile, demonstrates the effectiveness of the biased library approach to lead compound generation. Though **8** does not retain the same degree of selectivity in the [^{35}S]-GTPγS assay as compared with its inhibition radioligand binding at opioid receptor assays, its atypical behavior sets it apart from all previously reported derivatives of **4b**.[9,11] Moreover, since recent reports[8] show that both the μ and κ receptors may be important in heroin abuse, compound **8** will be a useful ligand to further these studies and could lead to a better treatment medication for heroin abuse.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary tube apparatus and are not corrected. Elemental analyses were obtained by Atlantic Microlabs, Inc., and are within ±0.4% of the calculated values. All optical rotations were determined at the sodium D line using a Rudolph Research Autopol III polarimeter (1 dm cell). ^{1}H NMR spectra were determined on a Bruker WM-250 spectrometer using tetramethylsilane as an internal standard. Silica gel 60 (230−400 mesh) was used for all column chromatography. Mass spectral data was obtained using a Finnegan LCQ electrospray mass spectrometer in positive ion mode at atmospheric pressure. All reactions were followed by thin-layer chromatography using Whatman silica gel 60 TLC plates and were visualized by UV, charring using 5% phosphomolybdic acid in ethanol and/or exposure to iodine vapor. All solvents were reagent grade. Tetrahydrofuran and diethyl ether were dried over sodium benzophenone ketyl and distilled prior to use. Methylene chloride was distilled from calcium hydride prior to use.

General Method for the Introduction of Diversity Elements R_1 and R_2 into Structure 6. (+)-(3*R*,4*R*)-Dimethyl-4-(3-hydroxyphenyl)piperidine (**4b**) (11.5 mmol), the appropriate Boc-protected amino acid (11.5 mmol), and BOP reagent (11.5 mmol) were combined in THF (150 mL) at room temperature, and to this was immediately added triethylamine (TEA) or diisopropylethylamine (25.3 mmol). After being stirred for 1 h, the reaction mixture was poured into ethyl ether (500 mL) and water (150 mL) in a separatory funnel. The mixture was shaken and the aqueous layer removed. This procedure was repeated using 150 mL of saturated $NaHCO_3$ and 150 mL of brine. The organic layer was diluted with hexane until cloudy and dried (Na_2SO_4), concentrated under reduced pressure, then dissolved in 100 mL of chloroform (stored over K_2CO_3), and concentrated again. This was placed on a high-vacuum system to remove residual solvent, yielding a foamy yellow/white solid.

After remaining under vacuum on the pump overnight, this unpurified material was dissolved in methylene chloride (45 mL) and cooled to −20 °C (methanol/ice). To this was added neat trifluoroacetic acid in 10 mL portions over 2 min to give a total addition of 30 mL. The entire mixture was stirred for exactly 30 min, and then the cooling bath was removed for exactly 30 min. At this point, the reaction mixture was poured

into a 1 L beaker containing a large stir bar and a rapidly agitated mixture of saturated bicarbonate solution (400 mL) and chloroform (150 mL). After completed addition, the pH of the mixture was verified to be 10 and adjusted with solid sodium bicarbonate if necessary. This mixture was poured into a separatory funnel. Any precipitated organic compounds were rinsed into the separatory funnel using a small amount of methanol. The beaker was then rinsed with a small amount of water which was added to the separatory funnel. The layers were agitated and separated, and the aqueous layer was extracted five additional times using 3:1 methylene chloride: THF. It was observed that compounds with small groups R_1 required additional extractions and/or sodium chloride saturation of the aqueous layer. The combined organic layers were dried over sodium sulfate, and the solvent was removed at reduced pressure. The material was then placed on a high vacuum pump to yield a yellow foamy solid.

Unpurified material from the deprotection step was dissolved in THF (150 mL) and cooled to −20 °C (methanol/ice). To this stirred mixture was added a solution of borane dimethyl sulfide complex (2 M) in THF (110 mmol) dropwise. The solution was then heated to reflux and held for 3 h after which time the solution was cooled to −20 °C, and to this was carefully added methanol (72 mL) dropwise. This mixture was stirred for 1 h at room temperature, 16.4 mL of 1 M HCl in ethyl ether was added, the solution was allowed to stir for 30 min, and the solvents were removed on a rotary evaporator. The resulting residue was partitioned between 3:1 methylene chloride:tetrahydrofuran and water, the pH was adjusted to 10 with saturated sodium bicarbonate, and the aqueous layer was saturated with sodium chloride and extracted several times with 3:1 methylene chloride:tetrahydofuran. The combined organic layers were dried over sodium sulfate, and the solvent was removed. This material was purified by flash chromatography on a silica gel column which was prepared by slurry packing with chloroform. The impure compounds were loaded on the column as a chloroform solution. Elution proceeded with neat chloroform followed by 3% methanol up to 10% methanol in chloroform as needed to elute the desired compounds. Product fractions were combined, and the solvent was removed on a rotary evaporator. This material was dissolved in a minimum of hot ethyl acetate and allowed to crystallize. Crystalline material was isolated by filtration followed by washing with a small amount of ice-cold ethyl acetate and used directly in the next step after drying overnight in a vacuum oven.

Introduction of Diversity Element R_3 into Structure 7. The appropriate pure diamine **6**, produced in the previous step (0.05 mmol × the number of derivatives being prepared), was dissolved in THF (2 mL × the number of derivatives being prepared) and to this was added TEA (0.1 mmol × the number of derivatives being prepared). Then, into prelabeled, 20 mL scintillation vials containing a stir bar was added one of the chosen carboxylic acids (0.05 mmol). To this was added the appropriate fraction of the diamine/TEA/THF mixture followed by 50 μL of a 1 M solution of BOP reagent in dimethylformamide (DMF). The vial was then capped with a Teflon-lined lid and stirred for 1 h at room temperature. After this time, 4 mL of ethyl ether and 2 mL of water were added to the vial. After the vial was shaken and the layers were allowed to settle, the aqueous layer was withdrawn with a pipet. Next, 2 mL of saturated sodium bicarbonate solution was added and the procedure repeated. This was followed by a similar wash with saturated sodium chloride solution. Sodium sulfate was added to the vial, and after drying, the mixture was pipetted into a preweighed, prelabeled 20 mL scintillation vial via a 6 in. Pasteur pipet containing a small cotton plug. Following this, 2 mL of chloroform was added to the drying agent and the vial shaken, after which the chloroform rinse was filtered as above. The collecting vials were placed under a nitrogen outlet and allowed to evaporate. Once the solvent was removed, the vials were placed in a high-vacuum desiccator and allowed to remain overnight. The vials were reweighed, and the crude yield determined by difference. Since pilot studies showed that the BOP-coupling reaction produced very clean samples, the products were used without further purification, and the purity was taken to be 100%.

Prior to screening, all compounds were diluted to a concentration of 10 mM in dimethyl sulfoxide (DMSO). Dilution was accomplished by determining the mean mmol/vial for each batch of 20 reactions using an Excel 3.0 spreadsheet. Weights deviating from the mean by >±10% were grouped into a second and third set above and below the mean. These were also averaged within the same parameters. Any compounds not falling within the above sets were diluted individually according to their weight. This procedure permitted sample dilution to be accomplished using a minimum number of different volume deliveries of DMSO. Once diluted to 10 mM, 1 mL samples from each vial were withdrawn and placed in rows A and E (1 compound/well) of a 1 mL × 96-well polypropylene microtiter plate. Serial dilution was then performed using Matrix multichannel pipettors which provided a 1 mM solution in rows B and F and a 0.1 mM solution in rows C and G. Once all of the compounds were transferred to plates and diluted to the proper concentration, the plates were placed in the refrigerator prior to assay.

***N*-(2′-Aminoethyl)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (6a).** Compound **6a** was prepared from *N*-(*tert*-butoxy)-glycine and (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine according to the general procedure in 15% yield: ^{1}H NMR (MeOH-d_4) δ 7.13−7.062 (t, 1H, J = 8.1 Hz), 6.77−6.74 (m, 2H), 6.59−6.55 (m, 1H), 3.31−3.29 (m, 1H), 2.83−2.70 (m, 3H), 2.5 (d, 2H, J = 3.1 Hz), 2.46−2.27 (m, 3H), 2.00 (s, 1H), 1.6 (d, 2H, J = 3.1 Hz), 1.68 (d, 1H, J = 13.7 Hz), 1.29 (s, 3H), 0.89 (d, 3H, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 158.5, 152.9, 130.0, 117.9, 113.9, 113.3, 61.6, 57.1, 51.5, 40.2, 39.5, 39.1, 32.0, 28.2, 16.7. MS (electrospray) M + 1 = 249. Calculated = 249.

***N*-(2′-Methylaminoethyl)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (6b).** Compound **6b** was prepared from *N*-(*tert*-butoxy)-sarcosine and (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine according to the general procedure in 32% yield: ^{1}H NMR (MeOH-d_4) δ 7.9 (t, 1H, J = 7.7 Hz), 6.77 (d, 1H), 6.74 (s, 1H), 6.58 (d, 1H), 2.95−2.90 (m, 1H), 2.87−2.82 (m, 2H), 2.66 (dd, 1H), 2.61−2.55 (m, 2H), 2.54 (s, 3H), 2.52 (td, 1H), 2.37 (td, 1H), 2.03−2.00 (m, 1H), 1.69 (brd, 1H), 1.30 (s, 3H), 0.89 (d, 3H, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 130.0, 118.0, 113.8, 113.3, 57.4, 56.7, 51.1, 48.2, 40.2, 39.4, 35.0, 31.9, 28.1, 16.6. MS (electrospray) M + 1 = 263. Calculated = 263.

***N*-[(2′*S*)-Aminopropyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (6c).** Compound **6d** was prepared from *N*-(*tert*-butoxy)-L-alanine and (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine according to the general procedure in 56% yield: ^{1}H NMR (MeOH-d_4) δ 7.11−7.08 (t, 1H, J = 7.7), 6.78−6.76 (d, 1H), 6.74 (s, 1H), 6.59−6.57 (d, 1H), 2.953−2.902 (m, 1H), 2.874−2.826 (m, 2H), 2.676−2.647 (dd, 1H), 2.618−2.559 (m, 2H), 2.548 (s, 3H), 2.541−2.400 (td, 1H), 2.342−2.284 (td, 1H), 2.030−2.002 (m, 1H), 1.613−1.587 (brd, 1H), 1.303 (s, 3H), 0.800−0.786 (d, 3H, J = 7.0); ^{13}C NMR (MeOH-d_4) δ 130.0, 118.0, 113.8, 113.3, 57.4, 56.7, 51.1, 48.2, 40.2, 39.4, 35.0, 31.9, 28.1, 16.6. MS (electrospray) M + 1 = 263. Calculated = 263.

***N*-[(2′*S*)-(Methylamino)propyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (6d).** Compound **6d** was prepared from *N*-(*tert*-butoxy)-*N*-methyl-L-alanine[17] and (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine according to the general procedure in 33% yield: ^{1}H NMR (MeOH-d_4) δ 7.18 (t, 1H, J = 7.9 Hz), 6.76 (d, 1H), 6.73 (s, 1H), 6.57 (d, 1H), 2.72−2.64 (m, 2H), 2.61−2.47 (m, 3H), 2.36 (s, 3H), 2.34−2.20 (m, 3H), 2.00−1.99 (m, 1H), 1.56 (dd, 1H), 1.29 (s, 3H), 1.03 (d, 3H, J = 6.2 Hz), 0.65 (d, 3H, J = 6.9 Hz); ^{13}C NMR (MeOH-d_4) δ 158.4, 153.3, 130.1, 117.9, 113.7, 113.3, 65.1, 56.0, 52.9, 52.9, 40.0, 39.5, 33.7, 31.9, 28.0, 17.3, 16.7. MS (electrospray) M + 1 = 277. Calculated = 277.

***N*-[(2′*S*)-Amino-3′-methylbutyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (6e).** Compound **6e** was prepared from *N*-(*tert*-butoxy)-L-valine and (+)-(3*R*,4*R*)-dimethyl-

4-(3-hydroxyphenyl)piperidine according to the general procedure in 78% yield: ^{1}H NMR (MeOH-d_4) δ 7.126−7.062 (t, 1H), 6.769−6.735 (m, 2H), 6.603−6.558 (m, 1H), 2.657−2.179 (m, 8H), 2.000 (brs, 1H), 1.583−1.502 (m, 2H), 1.294 (s, 3H), 0.978−0.912 (q, 6H), 0.789−0.761 (d, 3H); ^{13}C NMR (MeOH-d_4) δ 158.5, 153.3, 130.1, 117.8, 113.8, 113.3, 63.4, 55.8, 54.1, 53.3, 40.0, 39.5, 33.1, 31.9, 28.1, 19.6, 19.2, 16.8. MS (electrospray) M + 1 = 291. Calculated = 291.

***N*-[(2′*R*)-Amino-3′-methylbutyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (6f).** Compound **6f** was prepared from *N*-(*tert*-butoxy)-D-valine and (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine according to the general procedure in 62% yield: ^{1}H NMR (MeOH-d_4) δ 7.11−7.08 (t, 1H), 6.78−6.76 (d, 1H), 6.74 (s, 1H), 6.59−6.57 (dd, 1H), 3.139−3.097 (m, 1H), 2.953 (brs, 1H), 2.894−2.865 (dd, 1H), 2.546−2.500 (m, 2H), 2.401−2.292 (m, 3H), 2.046−2.034 (brm, 1H), 1.894−1.827 (sext, 1H), 1.62−1.30 (m, 1H), 1.311 (s, 3H), 1.042−1.006 (dd, 6H), 0.834−0.820 (d, 3H); ^{13}C NMR (MeOH-d_4) δ 152.9, 130.1, 118.0, 113.8, 113.3, 59.8, 58.8, 55.2, 50.0, 40.4, 39.4, 31.6, 31.1, 28.0, 18.8, 18.5, 16.5. MS (electrospray) M + 1 = 291. Calculated = 291.

***N*-[(2′*S*)-Amino-4′-methylpentyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (6g).** Compound **6g** was prepared from *N*-(*tert*-butoxy)-L-leucine and (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine according to the general procedure in 56% yield: ^{1}H NMR (MeOH-d_4) δ 7.09 (t, 1H, J = 7.9 Hz), 6.76 (d, 1H, J = 7.9 Hz), 6.73 (s, 1H), 6.57 (dd, 1H, J = 2.2, 7.9 Hz), 3.03−2.97 (m, 1H), 2.73 (d, 1H, J = 11.2 Hz), 2.64 (d, 1H, J = 11.1 Hz), 2.56 (td, 1H, J = 2.5, 12.0 Hz), 2.48 (dd, 1H, J = 2.7, 11.4 Hz), 2.33 (td, 1H, J = 4.5, 12.7 Hz), 2.25 (dd, 1H, J = 3.6, 12.4 Hz), 2.19−2.15 (m, 1H), 2.01−2.00 (m, 1H), 1.75 (sept, 1H, J = 6.6 Hz), 1.56 (d, 1H, J = 13.0 Hz), 1.29 (s, 3H), 1.27−1.15 (m, 2H), 0.94−0.91 (m, 6H), 0.07 (d, 3H, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 158.3, 153.3, 130.1, 117.9, 113.7, 113.2, 65.7, 56.0, 53.1, 46.5, 45.2, 40.0, 39.5, 31.9, 28.0, 25.8, 23.7, 22.6, 16.7. MS (electrospray) M + 1 = 305. Calculated = 305.

***N*-[(2′*S*)-Amino-3′-methylpentyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (6h).** Compound **6h** was prepared from *N*-(*tert*-butoxy)-L-isoleucine and (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine according to the general procedure in 47% yield: ^{1}H NMR (MeOH-d_4) δ 7.19 (t, 1H, J = 7.9 Hz), 6.76 (d, 1H, J = 8.1 Hz), 6.73−6.73 (m, 1H), 6.58−6.56 (dd, 1H, J = 2.1, 7.9 Hz), 2.86−2.82 (m, 1H), 2.75−2.73 (m, 1H), 2.65−2.57 (m, 2H), 2.502−2.474 (dd, 1H, J = 2.8, 11.4 Hz), 2.40−2.23 (m, 3H), 2.02−2.00 (m, 1H), 1.59−1.50 (m, 2H), 1.46−1.41 (m, 1H), 1.30 (s, 3H), 1.24−1.17 (m, 1H), 0.98−0.87 (m, 6H), 0.78 (d, 3H, J = 7.0 Hz); ^{13}C NMR (MeOH-d_4) δ 158.3, 153.2, 130.1, 117.9, 113.7, 113.3, 61.9, 55.9, 53.1, 52.9, 49.0, 40.0, 39.5, 39.3, 31.9, 28.0, 26.6, 16.7, 15.1, 11.8. MS (electrospray) M + 1 = 305. Calculated = 305.

***N*-[(2′*S*)-Amino-2′-cyclohexylethyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (6i).** Compound **6i** was prepared from *N*-(*tert*-butoxy)-L-cyclohexylglycine and (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine according to the general procedure in 63% yield: ^{1}H NMR (MeOH-d_4) δ 7.18 (t, 1H, J = 7.9), 6.76 (d, 1H, J = 7.8 Hz), 6.75 (s, 1H), 6.57 (d, 1H, J = 7.8 Hz), 2.74−2.70 (m, 2H), 2.63−2.55 (m, 2H), 2.47−2.45 (d, 1H, J = 10.0 Hz), 2.48 (dd, 1H, J = 2.9, 12.4 Hz), 2.36 (td, 1H, J = 4.3, 12.6 Hz), 2.23 (t, 1H, J = 11.6 Hz), 2.00 (m, 1H), 1.76−1.74 (m, 3H), 1.67 (d, 2H, J = 11.9 Hz), 1.57 (d, 1H, J = 13.0 Hz), 1.39−1.16 (m, 7H), 1.09 (quint, 2H, J =12.4 Hz), 0.77 (d, 3H, J = 6.8 Hz); ^{13}C NMR (MeOH-d_4) δ 158.3, 153.3, 130.1, 117.9, 113.7, 113.3, 162.6, 55.8, 53.4, 53.1, 42.9, 40.0, 39.5, 31.9, 30.9, 30.5, 30.2, 28.0, 27.6, 27.4, 16.7. MS (electrospray) M + 1 = 331. Calculated = 331.

***N*-[(2′*S*)-Methylamino-2′-phenylethyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (6j).** Compound **6j** was prepared from *N*-(*tert*-butoxy)-*N*-methyl-phenylglycine[17] and (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine according to the general procedure in 44% yield: ^{1}H NMR (MeOH-d_4) δ 7.34−7.22 (m, 5H), 7.13 (t, 1H, J = 8.2 Hz), 6.80−6.77 (m, 2H), 6.61−6.69 (m, 1H), 3.63 (dd, 1H, J = 3.7, 12.6 Hz), 2.73 (brd, 2H, J = 7.6 Hz), 2.64−2.52 (m, 3H), 2.38 (dd, 2H, J = 3.6, 12.6 Hz), 2.25 (s, 3H), 2.04 (brd, 1H, J = 6.3 Hz), 1.59 (d, 1H, J = 12.9), 1.312 (s, 3H), 0.818−0.790 (d, 3H, J = 6.9); ^{13}C NMR (MeOH-d_4) δ 147.3, 142.5, 131.5, 119.5, 119.0, 118.0, 107.4, 103.2, 102.7, 68.7, 68.233, 67.7, 55.2, 52.9, 45.1, 42.5, 42.5, 29.2, 28.9, 24.2, 21.3, 17.7. MS (electrospray) M + 1 = 339. Calculated = 339.

***N*-[(2′*S*)-Amino-3′-phenylpropyl]-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (6k).** Compound **6k** was prepared from *N*-(*tert*-butoxy)-L-phenylalanine and (+)-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine according to the general procedure in 44% yield: ^{1}H NMR (MeOH-d_4) δ 7.29 (t, 1H, J = 7.4 Hz), 7.24−7.06 (m, 5H), 6.75−6.71 (m, 2H), 6.57−6.55 (m, 1H), 3.86−3.84 (m, 5H), 3.22−3.94 (m, 1H), 2.83−2.69 (m, 2H), 2.63−2.39 (m, 5H), 2.35−2.24 (m, 2H), 1.97 (t, 1H, J = 6.4 Hz), 1.54 (t, 1H, J = 12.7 Hz), 1.27 (s, 3H), 0.74 (dd, 3H, J = 6.95, 21.04 Hz); ^{13}C NMR (MeOH-d_4) δ 158.3, 153.3, 139.9, 130.6, 130.3, 130.0, 129.6, 129.2, 127.5, 127.1, 118.0, 117.9, 113.8, 113.7, 113.2, 65.0, 64.7, 61.0, 57.3, 56.1, 52.9, 52.1, 50.5, 49.5, 49.3, 49.2, 49.0, 48.8, 48.7, 48.5, 41.9, 41.5, 40.3, 40.0, 39.4, 31.9, 28.0, 16.7. MS (electrospray) M + 1 = 339. Calculated = 339.

***N*-{(2′*S*)-[3-(4-Hydroxyphenyl)propanamido]-3′-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (8).** Compound **8** was prepared from compound **6e** and 3-(4-hydroxyphenyl)propionic acid according to the general procedure above in 74% yield and purified by silica gel chromatography. The hydrochloride salt was prepared using 1 M HCl in ethyl ether/methanol and precipitated from ethyl acetate: mp 136−140 °C; ^{1}H NMR (free base, CD_3OD) δ 7.16 (t, J = 7.94, Hz, 1H), 7.04 (d, J = 8.45 Hz, 2H), 6.76 (d, J = 7.78 Hz, 1H), 6.72−6.69 (m, 2H), 6.65 (dd, J = 8.04, 1.76 Hz, 1H), 4.02−3.98 (m, 1 H), 3.57 (d, J = 12.5 Hz, 1H), 3.40 (ddd, J = 2.90, 11.6, 13.4 Hz, 2 H), 3.03 (dd, J = 10.5, 13.4 Hz, 1 Hz), 2.84 (t, 7.07 Hz, 2H), 2.60 (t, 7.58 Hz, 2H), 2.43 (dt, J = 13.21, 4.9 Hz, 1H), 2.36−2.35 (m, 1H), 1.85 (d, J = 14.5 Hz, 1H), 1.87−1.76 (m, 1H), 1.42 (s, 3H), 0.92 (t, J = 6.98 Hz, 6H), 0.815 (d, J = 7.53, 3H); ^{13}C NMR (CD_3OD) δ 176.3, 159., 157.7, 153.8, 133.8, 131.3, 131.0, 118.9, 117.1, 114.6, 114.2, 62.0, 57.2, 53.2, 52.8, 40.9, 40.3, 33.1, 33.1, 32.5, 31.7, 28.8, 20.6, 18.9, 17.3. MS (electrospray) M + 1 = 439. Anal. ($C_{27}H_{39}ClN_2O_3$·1.5H_2O): C, H, N.

Compounds cited in Table 1 were removed from the library and purified by silica gel chromatography. The purity of the library sample was determined according to the following formula: (mg isolated sample/mg crude mass sample) × 100.

***N*-{(2′*R*)-[3-(4-Hydroxyphenyl)propanamido]-3′-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (9).** Compound **9** was prepared from compound **6f** and 3-(4-hydroxyphenyl)propionic acid according to the general procedure: purity 85%; ^{1}H NMR (MeOH-d_4) δ 7.83 (s, 3H), 7.13−7.00 (m, 3H), 6.77−6.67 (m, 4H), 6.61−6.57 (m, 1H), 3.96−3.89 (m, 1H), 2.86−2.78 (m, 3H), 2.62−2.58 (m, 1H), 2.48 (d, 3H, J = 8.0 Hz), 2.36−2.14 (m, 4H), 1.94 (brd, 1H, J = 6.3 Hz), 1.76 (sept, 1H, J = 5.5 Hz), 1.51 (brd, 1H, J = 11.0 Hz), 1.26 (s, 3H), 0.84−0.74 (m, 9H). MS (electrospray) M + 1 = 439. Calculated = 439.

***N*-{(2′*S*)-[(3-Phenylpropanamido)-3′-methyl]butyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (10).** Compound **10** was prepared from compound **6e** and 3-phenylpropionic acid according to the general procedure: purity 87%; ^{1}H NMR (MeOH-d_4) δ 7.25−7.22 (m, 2H), 7.17−7.13 (m, 4H), 6.82 (s, 1H), 6.76 (d, 1H, J = 7.8 Hz), 6.70−6.68 (m, 1H), 5.74 (s, 1H), 4.02−3.97 (m, 1H), 2.99−2.87 (m, 2H), 2.74−2.69 (m, 1H), 2.64 (brd, 1H, J = 1.3 Hz), 2.57−2.40 (m, 6H), 2.27−2.21 (m, 2H), 2.17 (s, 3H), 1.92−1.87 (m, 2H), 1.56 (d, 1H, J = 13.0 Hz), 1.28 (s, 3H), 0.81 (t, 6H, J = 6.8 Hz), 0.69 (d, 3H, J = 6.8 Hz). MS (electrospray) M + 1 = 423. Calculated = 423.

***N*-{(2′*S*)-[3-(3-Hydroxyphenyl)propanamido]-3′-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (11).** Compound **11** was prepared from compound **6e** and 3-(3-hydroxyphenyl)propionic acid according to the general procedure: purity 84%; ^{1}H NMR (MeOH-d_4) δ 7.24−7.23 (m, 1H), 7.13−7.03 (m, 3H), 6.76−6.57 (m, 5H), 3.32−3.29 (m, 4H), 2.85−2.17 (m, 8H), 1.97 (brs, 1H), 1.75−1.73 (m, 1H), 1.57 (brd,

1H, J = 12.3 Hz), 1.28 (s, 3H), 0.863 (t, 6H, J = 6.5 Hz), 0.72 (d, 3H, J = 7.0). MS (electrospray) M + 1 = 439. Calculated = 439.

***N*-{(2′*S*)-[3-(2-Hydroxyphenyl)propanamido]-3′-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (12).** Compound **12** was prepared from compound **6e** and 3-(2-hydroxyphenyl)propionic acid according to the general procedure: purity 85%; ^{1}H NMR ($CDCl_3$-d) δ 7.04−6.82 (m, 3H), 6.66−6.65 (m, 2H), 6.48−6.39 (m, 3H), 3.97−3.94 (m, 1H), 2.87−2.84 (m, 2H), 2.76 (d, 1H, J = 11 Hz), 2.56−2.22 (m, 8H), 1.94−1.93 (brm, 1H), 1.80 (sextet, 1H, J = 6.9 Hz), 1.52 (d, 1H, J = 13.3 Hz), 1.26 (s, 3H), 0.84 (dd, 6H, J = 13.1 Hz), 0.75 (d, 3H, J = 6.9 Hz). MS (electrospray) M + 1 = 439. Calculated = 439.

***N*-{(2′*S*)-[(4-Hydroxyphenyl)acetamido]-3′-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (13).** Compound **13** was prepared from compound **6e** and 4-hydroxyphenylacetic acid according to the general procedure: purity 88%; ^{1}H NMR (MeOH-d_4) δ 7.14−7.06 (m, 3H), 6.67−6.69 (m, 4H), 6.58 (d, 1H, J = 8.1 Hz), 3.95−3.92 (m, 1H), 3.32−3.30 (m, 2H), 2.70−2.60 (m, 1H), 2.56−2.47 (m, 1H), 2.41−2.15 (m, 6H), 1.90 (brs, 1H), 1.81−1.74 (m, 1H), 1.51 (d, 2H, J = 12.5 Hz), 1.25 (s, 3H), 0.86 (t, 6H, J = 6.7 Hz), 0.67 (d, 3H, J = 6.9 Hz). MS (electrospray) M + 1 = 425. Calculated = 425.

***N*-{(2′*S*)-[*trans*-3-(4-Hydroxyphenyl)acrylamido]-3′-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (14).** Compound **14** was prepared from compound **6e** and *trans*-3-(4-hydroxyphenyl)cinnamic acid according to the general procedure: purity 85%; ^{1}H NMR (MeOH-d_4) δ 7.25−7.37 (m, 3H), 7.11−7.04 (m, 1H), 6.79−6.72 (m, 4H), 6.56 (d, 1H, J = 9.5 Hz), 6.47 (d, 1H, J = 12.7 Hz), 4.10 (m, 1H), 2.80 (m, 1H), 2.64 (m, 1H), 2.54−2.26 (m, 5H), 1.95 (m, 2H), 1.56 (d, 1H, J = 13.1), 1.28 (s, 3H), 0.94 (t, 6H, J = 6.8 Hz), 0.70 (d, 3H, J = 6.9). MS (electrospray) M + 1 = 437. Calculated = 437.

***N*-{(2′*S*)-[3-(4-Fluorophenyl)propanamido]-3′-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (15).** Compound **15** was prepared from compound **6e** and 3-(4-fluorophenyl)propionic acid according to the general procedure: purity 89%; ^{1}H NMR (MeOH-d_4) δ 7.23−7.17 (m, 2H), 7.69 (t, 1H, J = 8.0 Hz), 6.99−6.92 (m, 2H), 6.76−6.73 (m, 2H), 6.60−6.54 (m, 1H), 3.96−3.90 (m, 1H), 2.88 (t, 2H, J = 7.7), 2.76 (d, 1H, J = 10.3 Hz), 2.65−2.32 (m, 8H), 1.97 (brs, 1H), 1.73−1.69 (m, 1H), 1.54 (d, 1H, J = 12.1 Hz), 1.27 (s, 3H), 0.80 (t, 6H, J = 5.8 Hz), 0.71 (d, 3H, J = 6.9 Hz). MS (electrospray) M + 1 = 441. Calculated = 441.

***N*-{(2′*S*)-[3-(3,4-Dihydroxyphenyl)propanamido]-3′-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (16).** Compound **16** was prepared from compound **6e** and 3-(3,4-dihydroxyphenyl)propionic acid according to the general procedure: purity 78%; ^{1}H NMR (MeOH-d_4) δ 7.09 (t, 1H, J = 7.9 Hz), 6.76−6.73 (m, 2H), 6.67−6.49 (m, 4H), 3.92 (brs, 1H), 2.74 (t, 3H, J = 7.6 Hz), 2.63−2.59 (m, 1H), 2.51−2.15 (m, 7H), 1.94 (brs, 1H), 1.75−1.70 (m, 1H), 1.55 (d, 1H, J = 12.1 Hz), 1.27 (s, 3H), 0.82 (t, 6H, J = 6.4 Hz), 0.71 (d, 3H, J = 6.9 Hz). MS (electrospray) M + 1 = 455. Calculated = 455.

***N*-{(2′*S*)-[3-(3-Methoxy-4-hydroxyphenyl)propanamido]-3′-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)-piperidine (17).** Compound **17** was prepared from compound **6e** and 3-(3-methoxy-4-hydroxyphenyl)propionic acid according to the general procedure: purity 87%; ^{1}H NMR (MeOH-d_4) δ 7.15 (t, 1H, J = 7.7 Hz), 6.81−6.76 (m, 3H), 6.67 (d, 3H, J = 3.3 Hz), 3.98 (brm, 1H), 3.80 (s, 3H), 2.86−2.69 (m, 3H), 2.53−2.22 (m, 8H), 1.89 (brs, 2H), 1.55 (d, 1H, J = 12.0 Hz), 1.27 (s, 3H), 0.82 (dd, 6H, J = 6.6, 3.2 Hz), 0.67 (d, 3H, J = 6.9 Hz). MS (electrospray) M + 1 = 469. Calculated = 469.

***N*-{(2′*S*)-[3-(3-Methoxyphenyl)propanamido]-3′-methylbutyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (18).** Compound **18** was prepared from compound **6e** and 3-(3-methoxyphenyl)propionic acid according to the general procedure: purity 88%; ^{1}H NMR (MeOH-d_4) δ 7.30−7.12 (m, 4H), 6.9−6.8 (m, 4H), 3.95 (brs, 1H), 3.76 (s, 3H), 2.96 (d, 2H, J = 6.8 Hz), 2.86−2.72 (m, 5H), 2.65−2.61 (m, 1H), 2.56−2.14 (m, 7H), 1.91 (brs, 1H), 1.73−1.71 (m, 1H), 1.52 (d, 1H, J = 13.0 Hz), 1.26 (s, 3H), 0.81 (t, 6H, J = 6.7 Hz), 0.67 (d, 3H, J = 6.9 Hz). MS (electrospray) M + 1 = 453. Calculated = 453.

***N*-{2′-[3-(4-Hydroxyphenyl)propanamido]ethyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (19).** Compound **19** was prepared from compound **6a** and 3-(4-hydroxyphenyl)-propionic acid according to the general procedure: purity 82%; ^{1}H NMR (MeOH-d_4) δ 7.13−6.99 (m, 3H), 6.79−6.67 (m, 4H), 6.59 (dd, 1H, J = 7.3, 1.8 Hz), 3.32−3.25 (m, 3H), 2.83−2.77 (m, 3H), 2.58 (s, 2H), 2.46−2.15 (m, 6H), 1.98 (brs, 1H), 1.58 (brd, 1H, J = 12.8 Hz), 1.29 (s, 3H), 0.76 (d, 3H, J = 7.0 Hz). MS (electrospray) M + 1 = 397. Calculated = 397.

***N*-{(2′*S*)-[3-(4-Hydroxyphenyl)propanamido]propyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (20).** Compound **20** was prepared from compound **6c** and 3-(4-hydroxyphenyl)propionic acid according to the general procedure: purity 88%; ^{1}H NMR (MeOH-d_4) δ 7.77 (s, 1H), 7.08 (t, 1H, J = 8.1 Hz), 6.98 (d, 2H, J = 8.4 Hz), 6.74−6.67 (m, 4H), 6.7 (d, 1H, J = 7.5 Hz), 4.03 (dd, 1H, J = 6.4 Hz), 2.81−2.70 (m, 3H), 2.49 (s, 2H), 2.44−2.26 (m, 4H), 2.16 (td, 2H, J = 3.7, 10.9 Hz), 1.92−1.89 (m, 1H), 1.50 (d, 1H, J = 12.3 Hz), 1.23 (s, 3H), 1.04 (d, 3H, J = 6.4 Hz), 0.71 (d, 3H, J = 6.9 Hz). MS (electrospray) M + 1 = 411. Calculated = 411.

***N*-{2′-[3-(4-Hydroxyphenyl)-*N*-methylpropanamido]-ethyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (21).** Compound **21** was prepared from compound **6b** and 3-(4-hydroxyphenyl)propionic acid according to the general procedure: purity 78%; ^{1}H NMR (MeOH-d_4) δ 7.84 (s, 1H), 7.18−7.00 (m, 3H), 6.77−6.69 (m, 4H), 6.60 (d, 1H, J = 8.1 Hz), 3.47−3.27 (m, 2H), 2.92−2.90 (m, 3H), 2.82−2.77 (m, 3H), 2.67−2.54 (m, 3H), 2.47−2.18 (m, 3H), 1.96 (brs, 1H), 1.58−1.49 (m, 3H), 1.27 (d, 3H, J = 2.91 Hz), 0.73 (t, 3H, J = 6.5 Hz). MS (electrospray) M + 1 = 411. Calculated = 411.

***N*-{(2′*S*)-[3-(4-Hydroxyphenyl)-*N*-methylpropanamido]-propyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (22).** Compound **22** was prepared from compound **6d** and 3-(4-hydroxyphenyl)propionic acid according to the general procedure: purity 89%; ^{1}H NMR (MeOH-d_4) δ 7.09 (t, 1H, J = 7.9 Hz), 6.99 (d, 2H, J = 8.2 Hz), 6.78−6.66 (m, 4H), 6.58−6.56 (m, 1H), 4.92−4.86 (m, 1H), 2.74 (s, 3H), 2.27−2.17 (m, 2H), 1.96−1.95 (brm, 1H), 1.55 (brd, 1H, J = 14.3 Hz), 1.27 (s, 3H), 1.02 (d, 3H, J = 6.7 Hz), 0.66 (d, 3H, J = 6.9 Hz). MS (electrospray) M + 1 = 425. Calculated = 425.

***N*-{(2′*S*)-[3-(4-Hydroxyphenyl)-*N*-methylpropanamido]-2′-phenylethyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)-piperidine (23).** Compound **23** was prepared according to the general procedure using compound **6j** and 3-(4-hydroxyphenyl)propionic acid according to the general procedure: purity 86%; ^{1}H NMR (MeOH-d_4) δ 7.69−7.66 (m, 1H), 7.45−7.42 (m, 1H), 7.32−6.97 (m, 7H), 6.76 (d, 1H, J = 9.4 Hz), 6.73 (s, 1H), 6.66−6.64 (m, 1H), 6.59−6.57 (m, 1H), 6.05 (q, 1H, J = 5.5 Hz), 3.00−2.71 (m, 9H), 2.65−2.63 (m, 2H), 2.29 (td, 1H, J = 4.3, 8.4 Hz), 2.01−2.00 (brm, 1H), 1.59 (brd, 1H, J = 12.0 Hz), 1.32−1.28 (m, 6H), 0.71 (d, 3H, J = 6.9 Hz). MS (electrospray) M + 1 = 487. Calculated = 487.

***N*-{(2′*S*)-[3-(4-Hydroxyphenyl)propanamido]-4′-methylpentyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (24).** Compound **24** was prepared according to the general coupling procedure (though on a 3 mmol scale) using compound **6g** and 3-(4-hydroxyphenyl)propionic acid in 85% yield. Crude products were then purified by silica gel chromatography using 10−25% methanol in chloroform: ^{1}H NMR (MeOH-d_4) δ 7.85 (s, 1H), 7.26−7.06 (m, 6H), 6.97 (d, 2H, J = 8.5 Hz), 6.76−6.66 (m, 3H), 6.58 (d, 1H, J = 7.2 Hz), 4.27 (t, 1H, J = 7.3 Hz), 2.84−2.23 (m, 10H), 1.93 (brd, 1H, J = 7.2 Hz), 1.52 (d, 1H, J = 12.0 Hz), 1.25 (s, 3H), 1.05 (t, 1H, J = 7.2 Hz), 0.74 (d, 3H, J = 6.9 Hz); ^{13}C NMR (MeOH-d_4) δ 164.0, 147.5, 143.0, 142.6, 129.0, 122.3, 119.9, 119.6, 119.3, 118.5, 116.5, 107.3,105.5, 103.0, 102.2, 51.6, 46.1, 40.8, 29.4, 29.3, 29.3, 28.7, 21.4, 21.0, 17.3. Anal. ($C_{28}H_{40}N_2O_3$): C, H, N.

***N*-{(2′*S*)-[3-(4-Hydroxyphenyl)propanamido]-3′-methylpentyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (25).** Compound **25** was prepared according to the general procedure (though on a 3 mmol scale) using compound **6h** and 3-(4-hydroxyphenyl)propionic acid in 81% yield. Crude

products were then purified by silica gel chromatography using 10−25% methanol in chloroform: ^{1}H NMR (MeOH-d_4) δ 7.59 (s, 1H), 6.90−6.76 (m, 3H), 6.52−6.45 (m, 3H), 6.36 (d, 1H, J = 7.6 Hz), 3.89 (brs, 1H), 2.56−2.54 (m, 3H), 2.39−1.95 (m, 9H), 1.70 (brs, 1H), 1.32−1.10 (m, 3H), 1.03 (s, 5H), 0.65−0.61 (m, 8H), 0.52−0.42 (m, 3H); ^{13}C NMR (MeOH-d_4) δ 163.8, 147.5, 146.0, 142.6, 122.2, 119.7, 119.4, 107.4, 105.5, 103.1, 102.6, 68.7, 53.7, 46.2, 41.0, 39.4, 39.1, 35.4, 33.4, 29.5, 28.9, 28.7, 21.5, 21.2, 17.5, 15.1, 13.3, 11.9. Anal. ($C_{28}H_{40}N_2O_3$): C, H, N.

***N*-{(2′*S*)-[3-(4-Hydroxyphenyl)propanamido]-2′-cyclohexylethyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (26).** Compound **26** was prepared according to the general procedure (though on a 3 mmol scale) using compound **6i** and 3-(4-hydroxyphenyl)propionic acid in 87% yield. Crude products were then purified by silica gel chromatography using 10−25% methanol in chloroform: ^{1}H NMR (MeOH-d_4) δ 7.85−7.82 (m, 2H), 7.11−6.97 (m, 3H), 6.74−6.56 (m, 5H), 3.99−3.97 (m, 1H), 2.81−2.75 (m, 3H), 2.54 (m, 1H), 2.44−2.12 (m, 7H), 1.94 (brs, 1H), 1.54−1.26 (m, 3H), 1.25 (s, 3H), 1.02−0.68 (m, 10H); ^{13}C NMR (MeOH-d_4) δ 164.1, 147.5, 146.0, 142.6, 122.2, 119.7, 119.4, 107.3, 105.5, 103.1, 102.5, 68.7, 49.4, 45.5, 41.3, 40.9, 29.4, 28.8, 28.4, 0.21.5, 21.1, 17.4, 15.4. Anal. ($C_{30}H_{24}N_2O_3$): C, H, N.

***N*-{(2′*S*)-[3-(4-Hydroxyphenyl)propanamido]-3′-phenylpropyl}-(3*R*,4*R*)-dimethyl-4-(3-hydroxyphenyl)piperidine (27).** Compound **27** was prepared according to the general procedure (though on a 3 mmol scale) using compound **6k** and 3-(4-hydroxyphenyl)propionic acid in 82% yield. Crude products were then purified by silica gel chromatography using 10−25% methanol in chloroform: ^{1}H NMR (MeOH-d_4) δ 7.88 (s, 1H), 7.12−7.00 (m, 3H), 6.76−6.66 (m, 4H), 6.59−6.55 (m, 1H), 3.90 (m, 1H), 2.78 (q, 3H, J = 7.0 Hz), 2.62−2.56 (m, 1H), 2.47−2.24 (m, 6H), 1.66−1.50 (m, 6H), 1.26 (s, 3H), 1.16−1.03 (m, 3H), 0.88−0.84 (m, 2H), 0.71 (d, 3H, J = 6.9 Hz); ^{13}C NMR (MeOH-d_4) δ 164.1, 147.5, 146.0, 142.6, 122.1, 119.8, 119.4, 107.3, 105.5, 103.1, 102.6, 68.7, 50.1, 45.6, 41.2, 41.1, 31.7, 29.4, 28.8, 21.5, 21.1, 20.3, 18.4, 17.4, 16.8. Anal. ($C_{31}H_{28}N_2O_3$): C, H, N.

Opioid Binding Assays. μ binding sites were labeled using [^{3}H][D-Ala^2-MePhe^4,Gly-ol^5]enkephalin ([^{3}H]DAMGO) (2.0 nM, SA = 45.5 Ci/mmol), and δ binding sites were labeled using [^{3}H][D-Ala^2,D-Leu^5]enkephalin (2.0 nM, SA = 47.5 Ci/mmol) using rat brain membranes prepared as described.[18] κ-1 binding sites were labeled using [^{3}H]U69,593 (2.0 nM, SA = 45.5 Ci/mmol) and guinea pig membranes pretreated with BIT and FIT to deplete the μ and δ binding sites.[19]

[^{3}H]DAMGO binding proceeded as follows: 12 × 75 mm polystyrene test tubes were prefilled with 100 μL of the test drug which was diluted in binding buffer (BB = 10 mM Tris-HCl, pH 7.4, containing 1 mg/mL BSA), followed by 50 μL of BB, and 100 μL of [^{3}H]DAMGO in a protease inhibitor cocktail (10 mM Tris-HCl, pH 7.4, which contained bacitracin (1 mg/mL), bestatin (100 μg/mL), leupeptin (40 μg/mL), and chymostatin (20 μg/mL)). Incubations were initiated by the addition of 750 μL of the prepared membrane preparation containing 0.2 mg/mL of protein and proceeded for 4 to 6 h at 25 °C. The ligand was displaced by 10 concentrations of test drug, in triplicate, 2×. Nonspecific binding was determined using 20 μM levallorphan. Under these conditions, the K_d of [^{3}H]-DAMGO binding was 4.35 nM. Brandel cell harvesters were used to filter the samples over Whatman GF/B filters, which were presoaked in wash buffer (ice-cold 10 mM Tris-HCl, pH 7.4).

[^{3}H][D-Ala^2,D-Leu^5]enkephalin binding proceeded as follows: 12 × 75 mm polystyrene test tubes were prefilled with 100 μL of the test drug which was diluted in BB, followed by 100 μL of a salt solution containing choline chloride (1 M, final concentration of 100 mM), $MnCl_2$ (30 mM, final concentration of 3.0 mM), and to block μ sites, DAMGO (1000 nM, final concentration of 100 nM), followed by 50 μL of [^{3}H][D-Ala^2,D-Leu^5]enkephalin in the protease inhibitor cocktail. Incubations were initiated by the addition of 750 μL of the prepared membrane preparation containing 0.41 mg/mL of protein and proceeded for 4 to 6 h at 25 °C. The ligand was displaced by 10 concentrations of test drug, in triplicate, 2×. Nonspecific binding was determined using 20 μM levallorphan. Under these conditions the K_d of [^{3}H][D-Ala^2,D-Leu^5]enkephalin binding was 2.95 nM. Brandel cell harvesters were used to filter the samples over Whatman GF/B filters, which were presoaked in wash buffer (ice-cold 10 mM Tris-HCl, pH 7.4).

[^{3}H]U69,593 binding proceeded as follows: 12 × 75 mm polystyrene test tubes were prefilled with 100 μL of the test drug which was diluted in BB, followed by 50 μL of BB, followed by 100 μL of [^{3}H]U69,593 in the standard protease inhibitor cocktail with the addition of captopril (1 mg/mL in 0.1 N acetic acid containing 10 mM 2-mercapto-ethanol to give a final concentration of 1 μg/mL). Incubations were initiated by the addition of 750 μL of the prepared membrane preparation containing 0.4 mg/mL of protein and proceeded for 4 to 6 h at 25 °C. The ligand was displaced by 10 concentrations of test drug, in triplicate, 2×. Nonspecific binding was determined using 1 μM U69,593. Under these conditions the K_d of [^{3}H]U69,593 binding was 3.75 nM. Brandel cell harvesters were used to filter the samples over Whatman GF/B filters, which were presoaked in wash buffer (ice-cold 10 mM Tris-HCl, pH 7.4) containing 1% PEI.

For all three assays, the filtration step proceeded as follows: 4 mL of the wash buffer was added to the tubes, was rapidly filtered, and was followed by two additional wash cycles. The tritium retained on the filters was counted, after an overnight extraction into ICN Cytoscint cocktail, in a Taurus β counter at 44% efficiency.

[^{35}S]-GTP-γ-S Binding Assay. Ten frozen guinea pig brains (Harlan Bioproducts for Science, Inc., Indianapolis, IN) were thawed, and the caudate putamen were dissected and homogenized in buffer A (3 mL/caudate) (buffer A = 10 mM Tris-HCl, pH 7.4, at 4 °C containing 4 μg/mL leupeptin, 2 μg/mL chymostatin, 10 μg/mL bestatin, and 100 μg/mL bacitracin) using a polytron (Brinkman) at setting 6 until a uniform suspension was achieved. The homogenate was centrifuged at 30000*g* for 10 min at 4 °C and the supernatant discarded. The membrane pellets were washed by resuspension and centrifugation twice more with fresh buffer A, aliquotted into microfuge tubes, and centrifuged in a Tomy refrigerated microfuge (model MTX 150) at maximum speed for 10 min. The supernatants were discarded, and the pellets were stored at −80 °C until assayed.

For the [^{35}S]GTP-γ-S binding assay, all drug dilutions were made up in buffer B [50 mM Tris-HCl, pH 7.7/0.1% BSA]. Briefly, 12 × 75 mm polystyrene test tubes received the following additions: (a) 50 μL of buffer B with or without an agonist, (b) 50 μL of buffer B with or without 60 μM GTP-γ-S for nonspecific binding, (c) 50 μL of buffer B with or without an antagonist, (d) 50 μL of a salt solution which contained in buffer B 0.3 nM [^{35}S]GTP-γ-S, 600 mM NaCl, 600 μM GDP, 6 mM dithiothreitol, 30 mM $MgCl_2$, and 6 mM EDTA, and (e) 100 μL of membranes in buffer B to give a final concentration of 10 μg per tube. The final concentration of the reagents were 100 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 100 μM GDP, 0.1% BSA, 0.05−0.1 nM [^{35}S]GTP-γ-S, 500 nM or 10 μM agonists, and varying concentrations (at least 10 different concentrations) of antagonists. The reaction was initiated by the addition of membranes and terminated after 4 h by addition of 3 mL of ice-cold (4 °C) purified water (Milli-Q UV-Plus, Millipore) followed by rapid vacuum filtration through Whatman GF/B filters presoaked in purified water. The filters were then washed once with 5 mL of ice-cold water. Bound radioactivity was counted by liquid scintillation spectroscopy using a Taurus (Micromedic) liquid scintillation counter at 98% efficiency after an overnight extraction in 5 mL of Cytoscint scintillation fluid. Nonspecific binding was determined in the presence of 10 μM GTP-γ-S. Assays were performed in triplicate, and each experiment was performed at least 3 times.

Data Analysis. The data of the two separate experiments (opioid binding assays) or three experiments ([^{35}S]-GTP-γ-S assay) were pooled and fit, using the nonlinear least-squares curve-fitting language MLAB-PC (Civilized Software, Be-

thesda, MD), to the two-parameter logistic equation[20] for the best-fit estimates of the IC_{50} and slope factor. The K_i values were then determined using the equation: $IC_{50}/1 + ([L]/K_d)$.

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Supporting Information Available: The percent inhibition of [^{3}H]U69,593 binding for the compounds at 100 nM in Figure 1 and the acids used in the library synthesis (13 pages). Ordering information is given on any current masthead page.

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