Articles

Nonpeptide Analogues of Dynorphin A(1−8): Design, Synthesis, and Pharmacological Evaluation of κ-Selective Agonists

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Two novel series of κ opioid receptor agonist analogues of MPCB-GRRI and MPCB-RRI, hybrid ligands of MPCB ((−)-cis-*N*-(2-phenyl-2-carbomethoxy)cyclopropylmethyl-*N*-normetazocine) and of the C-terminal fragments of dynorphin A(1−8), have been synthesized. The critical functional groups of the peptide fragments of hybrid compounds were maintained, and the binding affinities and selectivities for compounds **1**−**40** to μ, δ, and κ opioid receptors were analyzed. Compounds **15** and **16**, MPCB-Gly-Leu-NH-$(CH_2)_n$-NH-C(=NH)-C_4H_9 ($n = 5, 6$), displayed high affinity and selectivity for κ opioid receptors ($K_i^{\kappa} = 6.7$ and 5.3 nM, $K_i^{\mu}/K_i^{\kappa} = 375$ and 408, and $K_i^{\delta}/K_i^{\kappa} = 408$ and 424, respectively). Since κ agonists may also cause psychotomimetic effects by interaction with σ sites, binding assays to σ_1 sites were performed where compounds **15** and **16** showed negligible affinity ($K_i > 10\,000$). Compounds **15** and **16** were further characterized in vivo and showed potent antinociceptive activity in mouse abdominal constriction tests ($ED_{50} = 0.88$ and 1.1 mg/kg, respectively), fully prevented by nor-BNI. Thus, these novel analogues open an exciting avenue for the design of peptidomimetics of dynorphin A(1−8).

Introduction

Opioid analgesics exert their actions through three types of opioid receptors: μ, κ, and δ. Research in the area of opioid receptors has focused on the development of selective ligands, aimed to clarify their anatomical distributions and functions.[1] Over the past two decades, a large number of novel κ-selective agonists have been developed. In fact, κ agonists are potentially safer as analgesic agents, without exhibiting many of the clinically limiting side effects that characterize morphine.[2] Recently, pharmacological studies which report activation of κ opioid followed by μ antagonist effects have provided new interest for the development of κ-selective ligands, opening a possible new therapeutic strategy in pain treatment.[3−5]

The κ opioid receptor was first cloned in 1993, and the analysis of its amino acid sequence identified this receptor to be a member of the G-protein-coupled receptor (GPCR) superfamily.[6−9] Dynorphin A (H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH) was then postulated to be the endogenous ligand for the κ opioid receptor.[10] Dynorphin A and its shorter homologues, dynorphin A(1−8) and dynorphin A(1−13), are reported to have high affinity for both natural and cloned κ opioid receptors.[11,12]

Studies on chimeric receptors composed of fragments of different opioid receptor types have suggested that the second extracellular loop region (EL2) plays an important role in the selective interaction of dynorphin A with the κ opioid receptor.[13,14] More specifically, the interaction between EL2 and the C-terminal region of dynorphin A may be relevant in receptor activation. In fact studies on the mechanism of activation of cloned κ opioid receptors have demonstrated the necessity of simultaneous interaction with the two binding domains of the κ opioid receptor.[15] This event implies that the peptidomimetic design of dynorphin A should require both the essential chemical fragments of the C-terminal region and the "message" sequence. Recently reported molecular simulations of dynorphin A(1−10) binding to EL2 does not exclude an interaction of dynorphin A with the κ opioid receptor in a conformation similar to that of an α-helical secondary structure as proposed by Tessmer and Kallick.[16,17]

Structure−activity relationship (SAR) studies on peptide and nonpeptide κ-selective ligands enabled us to design and synthesize MPCB and its *p*-chlorophenyl analogue (CCB), the first (−)-normetazocine agonist derivatives that bind to κ opioid receptors with high affinity and specificity (Figure 1).[18,19] The cyclopropylmethyl-normetazocine (CPM) nucleus represents a scaffold to support a phenolic ring, a basic nitrogen, and a phenyl ring in a fixed and an appropriate conformation, which then mimics the recognition process of the N-terminal fragment of dynorphin A (Figure 2A). To confirm this hypothesis, we later synthesized a series

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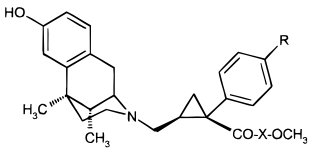


MPCB	X = nil	R = H
CCB	X = nil	R = Cl
MPCB-RRI	X = Arg-Arg-Ile	R = H
MPCB-GRRI	X = Gly-Arg-Arg-Ile	R = H

Figure 1. MPCB,[18] CCB,[19] and hybrid compounds.[20]

of hybrid ligands of MPCB and CCB, in which the heterocyclic nucleus was linked to various C-terminal fragments of dynorphin A(1−8) (Figure 1). In particular, MPCB-GRRI and MPCB-RRI exhibited high affinities to both cloned and native κ opioid receptors, further supporting the hypothesis that MPCB and CCB are appropriate mimetics of the N-terminal fragment of dynorphin A (Figure 2B).[20]

To further develop conformationally constrained peptidomimetics of dynorphin A(1−8), we herein report the design and synthesis of two novel series of analogues of MPCB-GRRI and MPCB-RRI with nonpeptide replacement at the C-terminus (Figure 3). Only highly flexible analogues were synthesized. The use of a flexible spacer chain might make impossible a rigorous interpretation of the results with respect to the stereochemical requirements of the pharmacophore; however, it will permit the alignment of critical functional groups with the receptor. A systematic evaluation of the distance among recognition features could in fact identify the functionalities capable of mimicking relevant groups in the C-terminal region of dynorphin A. In addition, a qualitative analysis of the observed activity trends can provide a basis to improve the design of the heterocyclic scaffold for the dynorphin A pharmacophores. We synthesized two series of compounds in which a spacer, constituted by Leu or Gly-Leu, was linked to the MPCB nucleus in order to mimic the amino acid fragments R and GR of the hybrid compounds MPCB-RRI and MPCB-GRRI. The amino acid residue Leu was chosen to replace the Arg^6 residue because it can be considered an analogue lacking the positive charge on the side chain. Previous structure−affinity studies of dynorphin A have determined that only the charged residue Arg^7 and the hydrophobic residue Ile^8 are necessary for κ affinity and selectivity.[21−23] The amino acid residue Arg^7 was replaced with a linear diamine, where the spacer chain length ranged from 3 to 6 methylene units to represent the distance between the pharmacophore and the charged nitrogen of Arg^7. Different basic groups were considered to explore the influence of basicity and optimal charge distribution. Thus we synthesized derivatives with a guanidine group and with a substituted amidine with lipophilic R groups of increasing hydrophobicity to mimic the side chain of the Ile^8 residue.

Chemistry

All of the compounds **1**−**40** listed in Figure 3 were synthesized according to Scheme 1. The following compounds were synthesized by published procedures: 2(*R*)-[2-(carbobenzoxy)aminoacetylamino]-4-methylpentanoic acid,[24] 2(*R*)-(carbobenzoxy)amino-4-methylpentanoic acid *p*-nitrophenyl ester,[25] *N*-(*tert*-butoxycarbonyl)-1,3-diaminopropane,[26] *N*-(*tert*-butoxycarbonyl)-1,4-diaminobutane, *N*-(*tert*-butoxycarbonyl)-1,5-diaminopentane, *N*-(*tert*-butoxycarbonyl)-1,6-diaminohexane,[27] ethyl propionimidate hydrochloride, ethyl butyrimidate hydrochloride, ethyl valerimidate hydrochloride,[28] butyramide, and valeramide.[29] *cis*-(−)-(2*R*,6*R*,11*R*)-*N*-Normetazocine was separated from a racemic mixture as reported by Brine et al.[30] Alkylation of *cis*-(−)-(2*R*,6*R*,-11*R*)-*N*-normetazocine with methyl (1*S*,2*R*)-2-(chloromethyl)-1-phenylcyclopropanecarboxylate[31] afforded methyl 1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxylate (MPCB) that was hydrolyzed to the corresponding 1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxylic acid.[18] 2(*R*)-[2-(Carbobenzoxy)aminoacetylamino]-4-methylpentanoic acid *p*-nitrophenyl ester was synthesized following the same procedure to synthesize 2(*R*)-(carbobenzoxy)amino-4-methylpentanoic acid *p*-nitrophenyl ester[25] (analytical data are available as Supporting Information).

The Cbz-Gly-Leu-NH-$(CH_2)_n$-NH-Boc compounds **41**−**44** and the Cbz-Leu-NH-$(CH_2)_n$-NH-Boc compounds **45**−**48** were prepared by coupling *mono*-Boc-diamines with *p*-nitrophenyl active esters Cbz-Gly-Leu-O-*p*NP and Cbz-Leu-O-*p*NP, respectively. The Cbz-α-amino protecting group was removed by hydrogenolysis to obtain the compounds H-Gly-Leu-NH-$(CH_2)_n$-NH-Boc **49**−**52** and the compounds H-Leu-NH-$(CH_2)_n$-NH-Boc **53**−**56**. The coupling reagents DCC and HOBt were employed to synthesize compounds **57**−**60** and **61**−**64** by reacting 1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]-cyclopropanecarboxylic acid with compounds **49**−**52** and **53**−**56**, respectively. Treatment of Boc-protected compounds with 90% TFA in the presence of anisole provided the final compounds **1**−**4** and **21**−**24**. Compounds **1**−**4** and **21**−**24,** with the terminal primary amino group, were transformed to N-terminal ethylamidino derivatives **5**−**8** and **25**−**28** by reaction with ethyl propionimidate hydrochloride in EtOH and DIEA at pH 10.[32−34] Similarly, N-terminal propylamidino derivatives **9**−**12** and **29**−**32** were obtained with ethyl butyrimidate hydrochloride and N-terminal butylamidino derivatives **13**−**16** and **33**−**36** with ethyl valerimidate hydrochloride. Finally, N-terminal guanidino derivatives **17**−**20** and **37**−**40** were synthesized by reacting compounds **1**−**4** and **21**−**24** with 3,5-dimethylpyrazole-1-carboxamidine nitrate in EtOH and DIEA at pH 10.[35]

Thin-layer chromatography (TLC) and analytical HPLC assessed the purity of the final compounds **1**−**40**. Molecular weights were confirmed by FAB-MS (Table 1).

Results

Binding affinities of the synthesized compounds in comparison with that of standard compounds (MPCB, U50,488, and dynorphin A(1−8)) are summarized in Tables 2 and 3.

The series with the dipeptide spacer Gly-Leu exhibited the best values for κ binding affinity (Table 2). In this series, compounds **1**−**4**, without N-substitution on the diamine, displayed a positive trend from compound

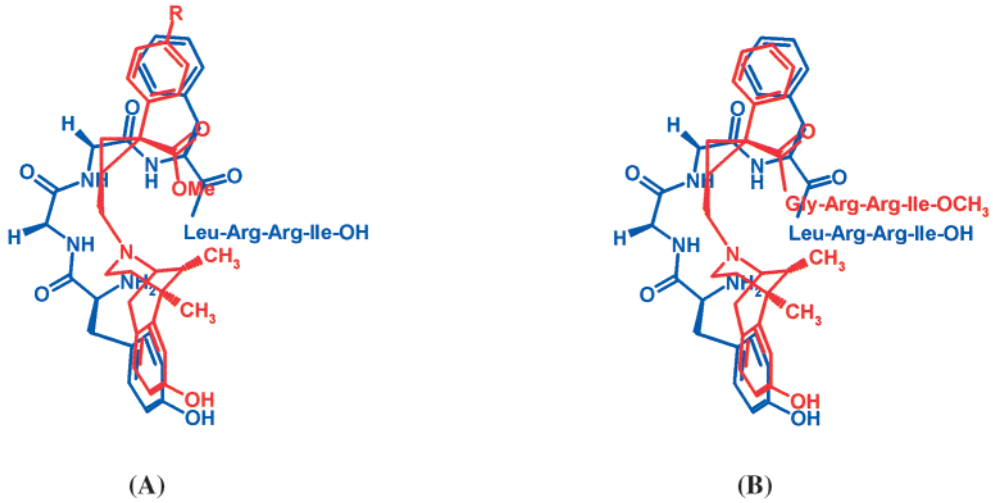
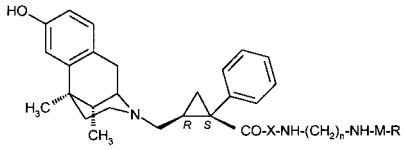


Figure 2. Superposition of (A) MPCB or CCB (red) and (B) hybrid compound MPCB-GRRI (red) with dynorphin A(1−8) (blue).



X = Gly-Leu	**M** = nil	**R** = H	**Compounds 1-4**
n = 3,4,5,6	**M** = C(=NH)	**R** = C_2H_5, C_3H_7, C_4H_9	**Compounds 5-16**
	M = C(=NH)	**R** = NH_2	**Compounds 17-20**
X = Leu	**M** = nil	**R** = H	**Compounds 21-24**
n = 3,4,5,6	**M** = C(=NH)	**R** = C_2H_5, C_3H_7, C_4H_9	**Compounds 25-36**
	M = C(=NH)	**R** = NH_2	**Compounds 37-40**

Figure 3. Structures of novel synthesized compounds **1**−**40**.

1 (trimethylene spacer chain) to compound **4** (hexamethylene spacer chain) for κ binding affinities. The K_i^{κ} values ranged from 282.4 to 79.6 nM. Similar analysis can be made for the other groups of compounds with N-terminal substitution. Particularly, in the compounds with alkylamidino substituents, lengthening of the alkyldiamine chain produced an increase in the κ opioid binding affinity. Compounds **5**−**8,** with the N-terminal ethylamidino substituent, have K_i^{κ} values ranging from 256.1 to 46.9 nM, compounds **9**−**12** (N-terminal propylamidino substituent) between 80.8 and 15.4 nM, and compounds **13**−**16** (N-terminal butylamidino substituent) between 18.0 and 5.3 nM. Compounds **17**−**20,** with the N-terminal guanidino substituent, have K_i^{κ} values ranging between 72.5 and 35.5 nM. N-Substitution of the diamine side chain with alkylamidino or guanidino substituents in the series of compounds also enhances κ binding affinities. Moreover, compounds with alkylamidino substituents exhibit parallel increases in binding affinities with increases in lipophilic character of the R group. Thus, the greatest κ opioid affinity occurred in compounds **13**−**16** with the N-terminal butylamidino substituent. In conclusion, among the synthesized compounds, the greatest κ opioid affinity is shown by compounds **15** and **16** (K_i^{κ} = 6.7 and 5.3 nM, respectively) that possess a pentamethylene and a hexamethylene spacer chain, respectively, and a butylamidino-modified N-terminus.

Similar observations can be made for compounds **21**−**40** which possess a Leu residue spacer, where their K_i^{κ} values were notably higher than the corresponding values of compounds **1**−**20** (Table 3).

The binding affinity data of compounds **1**−**20** and **21**−**40** are also shown in Figure 4. It is evident that there is a parallel trend of the κ opioid binding affinity values of the two series of compounds. Their binding affinity ratios for homologous compounds range from 1.3 to 15.

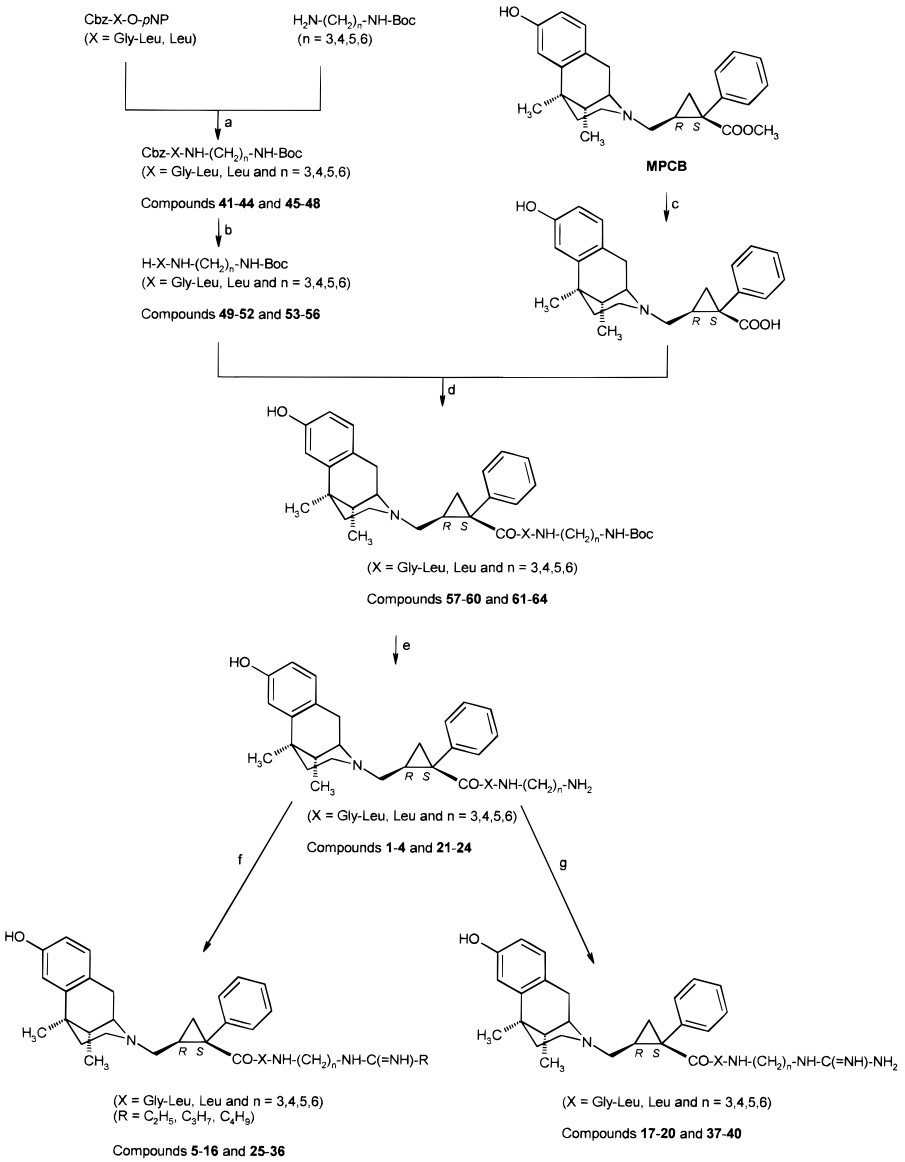
The series of compounds **1**−**20** shows higher values of μ/κ and δ/κ selectivity than the series **21**−**40**. In particular, for compounds **13**−**16**, with the butylamidine modified N-terminus, the K_i^{μ}/K_i^{κ} ratio ranges from 192 to 408 and the $K_i^{\delta}/K_i^{\kappa}$ ratio ranges from 275 to 424. Selectivity ratios of compounds **1**−**20** are represented in Figure 5. Compounds **13**−**16**, exhibiting the most potent values for κ opioid binding affinity, are also the compounds showing the greatest selectivity, mainly as a consequence of their higher κ affinity.

Since κ agonists may also interact with σ sites, binding assays for σ_1 sites were performed.[36] However, compounds **15** and **16** showed negligible affinity (K_i > 10 000).

Compounds **15**, **16**, and U50,488 (a selective κ opioid receptor agonist; obtained from RBI, Natick, MA) were effective in raising the nociceptive threshold in the mouse abdominal constriction test after subcutaneous administration. The nociceptive effect was fully antagonized by the selective κ opioid receptor antagonist nor-BNI (Table 4).

Compounds **15**, **16**, and U50,488 produced sedation (locomotor incapacitation), determined by visual observation and by the ability of mice to maintain their position at higher doses in the rotarod test than of those producing a full antinociceptive effect (Table 4). The ratio of the sedative dose (MPE_{50}) to the antinociceptive dose (MPE_{50}) was approximately 10. Moreover, compounds **15** and **16** did not cause any stereotyped behavior or ataxia in the mouse in doses up to 40 mg/kg, as evaluated adopting the scoring scale described by Tanaka et al. (data not shown).[37]

Scheme 1[a]



[a] Reagents: (a) DMF; (b) H_2, 10% Pd/C, CH_3OH/H_2O; (c) 1 N NaOH; (d) DCC, HOBt, DMF; (e) 90% TFA, anisole; (f) R-C(═NH)-O-C_2H_5·HCl, DIEA, EtOH (R = C_2H_5, C_3H_7, C_4H_9); (g) 1-amidino-3,5-dimethylpyrazole·HNO_3, DIEA, EtOH.

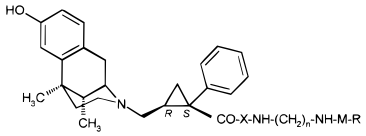
Discussion

The introduction of the Gly-Leu spacer in compounds **1**−**20** results in an increase in κ binding affinity and selectivity, as compared to compounds **21**−**40** which lack the Gly residue. These data present evidence that the Gly-Leu spacer allows for a better alignment of the cation-functionalized peptide fragments designed to mimic the "address" segment of the κ-selective dynorphins with the κ receptor.

Comparison of the binding profiles of compounds **1**−**20** with that of the hybrid compounds MPCB-GRRI and MPCB-RRI suggests that the presence of only one mimetic structure of the two arginine residues is sufficient to maintain binding affinity and selectivity for the κ receptor. This result may be well-correlated to previous SAR studies that have identified Arg[7] as the critical basic residue in dynorphin A(1−8).[21,22]

For Arg-mimicking structures, the length of the linear diamine is important for the binding properties of compounds **1**−**20** of which the highest affinity values occur in compounds with the pentamethylene and hexamethylene spacer chains. Nevertheless, the perception of optimal cation placement is difficult to infer because it may be overshadowed by the effect of increased hydrophobicity. It should also be noted that the lipophilicity of these linear chains might favor the interaction with the receptor. N-Substitution of the diamine side chain with alkylamidino or guanidino substituents is optimal with regard to κ binding affinity, reflecting the possibility for a high basic fragment to efficiently dock to κ opioid receptors. In fact, compounds **3** and **4** and the relative alkylamidino or guanidino derivatives display binding affinity ratios ranging from 3.6 to 36.7 (pentamethylene spanner chain) and from 1.7 to 15.0 (hexamethylene spanner chain). It is evident that the lipophilic character of the R group of alkylamidino substituents increases both κ binding affinity and selectivity. In fact, butylamidino compounds **15** and **16** display 7.1 and 6.7 times higher κ binding affinity than the guanidino compounds **19** and **20**, respectively.

Table 1. Analytical Properties of Compounds **1**−**40**



					TLC R_f values[a]		HPLC[b] (t_R)		
no.	X	*n*	M	R	I	II	1 (min)	2 (min)	FAB-MS
1	Gly-Leu	3	nil	H	0.35	0.60	2.30	2.94	618 $[M + H]^+$
2	Gly-Leu	4	nil	H	0.36	0.59	2.40	2.97	632 $[M + H]^+$
3	Gly-Leu	5	nil	H	0.37	0.58	2.45	3.00	646 $[M + H]^+$
4	Gly-Leu	6	nil	H	0.38	0.57	2.50	3.12	660 $[M + H]^+$
5	Gly-Leu	3	C(=NH)	C_2H_5	0.40	0.56	2.40	3.15	673 $[M + H]^+$
6	Gly-Leu	4	C(=NH)	C_2H_5	0.41	0.55	2.50	3.35	687 $[M + H]^+$
7	Gly-Leu	5	C(=NH)	C_2H_5	0.42	0.54	2.70	3.48	701 $[M + H]^+$
8	Gly-Leu	6	C(=NH)	C_2H_5	0.43	0.53	2.85	3.59	715 $[M + H]^+$
9	Gly-Leu	3	C(=NH)	C_3H_7	0.43	0.54	2.60	3.38	687 $[M + H]^+$
10	Gly-Leu	4	C(=NH)	C_3H_7	0.44	0.53	2.70	3.47	701 $[M + H]^+$
11	Gly-Leu	5	C(=NH)	C_3H_7	0.45	0.52	2.80	3.57	715 $[M + H]^+$
12	Gly-Leu	6	C(=NH)	C_3H_7	0.46	0.51	2.95	3.80	729 $[M + H]^+$
13	Gly-Leu	3	C(=NH)	C_4H_9	0.46	0.51	2.85	3.49	701 $[M + H]^+$
14	Gly-Leu	4	C(=NH)	C_4H_9	0.47	0.50	3.00	3.67	715 $[M + H]^+$
15	Gly-Leu	5	C(=NH)	C_4H_9	0.48	0.49	3.10	3.88	729 $[M + H]^+$
16	Gly-Leu	6	C(=NH)	C_4H_9	0.49	0.48	3.20	3.93	743 $[M + H]^+$
17	Gly-Leu	3	C(=NH)	NH_2	0.38	0.58	2.45	3.04	660 $[M + H]^+$
18	Gly-Leu	4	C(=NH)	NH_2	0.39	0.57	2.50	3.18	674 $[M + H]^+$
19	Gly-Leu	5	C(=NH)	NH_2	0.40	0.56	2.55	3.30	688 $[M + H]^+$
20	Gly-Leu	6	C(=NH)	NH_2	0.41	0.55	2.70	3.47	702 $[M + H]^+$
21	Leu	3	nil	H	0.43	0.57	2.55	3.53	561 $[M + H]^+$
22	Leu	4	nil	H	0.44	0.56	2.60	3.67	575 $[M + H]^+$
23	Leu	5	nil	H	0.45	0.55	2.70	3.88	589 $[M + H]^+$
24	Leu	6	nil	H	0.46	0.54	2.90	3.96	603 $[M + H]^+$
25	Leu	3	C(=NH)	C_2H_5	0.46	0.54	2.85	3.62	616 $[M + H]^+$
26	Leu	4	C(=NH)	C_2H_5	0.47	0.53	2.90	3.80	630 $[M + H]^+$
27	Leu	5	C(=NH)	C_2H_5	0.48	0.52	3.00	4.00	644 $[M + H]^+$
28	Leu	6	C(=NH)	C_2H_5	0.49	0.51	3.20	4.20	658 $[M + H]^+$
29	Leu	3	C(=NH)	C_3H_7	0.47	0.51	2.90	3.98	630 $[M + H]^+$
30	Leu	4	C(=NH)	C_3H_7	0.48	0.50	3.00	4.07	644 $[M + H]^+$
31	Leu	5	C(=NH)	C_3H_7	0.50	0.49	3.30	4.20	658 $[M + H]^+$
32	Leu	6	C(=NH)	C_3H_7	0.52	0.48	3.70	4.43	672 $[M + H]^+$
33	Leu	3	C(=NH)	C_4H_9	0.50	0.49	3.30	4.21	644 $[M + H]^+$
34	Leu	4	C(=NH)	C_4H_9	0.51	0.48	3.60	4.30	658 $[M + H]^+$
35	Leu	5	C(=NH)	C_4H_9	0.52	0.47	3.70	4.50	672 $[M + H]^+$
36	Leu	6	C(=NH)	C_4H_9	0.53	0.46	3.90	4.64	686 $[M + H]^+$
37	Leu	3	C(=NH)	NH_2	0.46	0.55	2.80	3.62	603 $[M + H]^+$
38	Leu	4	C(=NH)	NH_2	0.47	0.54	2.85	3.75	617 $[M + H]^+$
39	Leu	5	C(=NH)	NH_2	0.48	0.52	2.90	3.88	631 $[M + H]^+$
40	Leu	6	C(=NH)	NH_2	0.50	0.50	3.15	4.15	645 $[M + H]^+$

[a] Solvent systems: I, 1-butanol/water/acetic acid (45:45:10); II, acetonitrile/water/TFA (50:50:2). [b] 1 and 2 refer to the HPLC systems as described in the Experimental Section.

Moreover, compound **16** also show μ/κ and δ/κ selectivity ratios 4.3 and 5.0 times higher than the corresponding guanidino compound **20**.

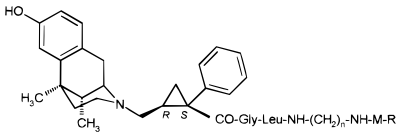
Compounds **15** and **16** show higher affinity and similar selectivity with respect to the hybrid compounds MPCB-RRI and MPCB-GRRI and also to dynorphin A(1−8). Compounds **15** and **16** also have affinity and selectivity comparable to that of the standard compound U50,488. A representative superposition of compound **16** and the previously reported peptidic analogue MPCB-GRRI is presented in Figure 6.

Compounds **15** and **16** also show very low affinity for σ_1 binding sites. Therefore, contrary to other benzomorphan derivatives,[36,38] compounds **15** and **16** are capable of recognizing κ opioid receptors from σ_1 binding sites. Compounds **15** and **16** display typical in vivo profiles as that of the κ opioid receptor agonist U50,488. In fact, both compounds effectively increase the nociceptive threshold and exhibit locomotor impairment at higher doses. These effects are fully inhibited by the selective κ opioid receptor antagonist nor-BNI.[39]

Contreras et al.[40] have observed that several benzomorphan derivatives cause stereotyped behavior and ataxia in rodents by interacting with binding sites other than opioid receptors. Compounds **15** and **16** do not cause these effects in the mouse; therefore, it is unlikely that they may bind to σ or phencyclidine sites to induce psychotomimetic effects.

In conclusion, compounds **15** and **16** have κ binding affinities higher than dynorphin A(1−8) and κ selectivity higher than dynorphin A(1−17). Moreover, all the compounds derived from nonpeptide ligands MPCB and CCB,[18,19] i.e., hybrid compounds MPCB-GRRI and MPCB-RRI[20] and compounds **15** and **16**, show μ/κ and δ/κ selectivity ratios higher than those shown by κ endogenous ligands (μ/κ and δ/κ values higher than 42.4 and higher than 407.8 with respect to 10.9 and 1.9 for dynorphin A(1−8) and 3.38 and 6.8 for dynorphin A(1−

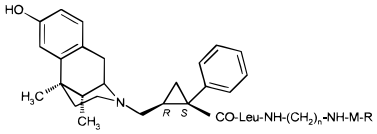
Table 2. Binding Affinities and Selectivities of Compounds **1**−**20** toward κ, μ, and δ Opioid Receptors



				$K_i \pm$ SEM (nM)[a,b]			selectivity ratio	
no.	*n*	M	R	κ	μ	δ	μ/κ	δ/κ
MPCB[c]				240 ± 39	>25000	>25000	nc[d]	nc[d]
MPCB-GRRI[e]				54.3 ± 8.5	2300 ± 356	>25000	42.36	nc[d]
MPCB-RRI[e]				78.4 ± 6.4	4600 ± 817	>25000	58.67	nc[d]
1	3	nil	H	282.4 ± 19.7	16100 ± 805.0	10610 ± 604.7	57.0	37.6
2	4	nil	H	269.6 ± 13.5	13230 ± 661.5	10450 ± 543.4	49.1	38.7
3	5	nil	H	246.0 ± 12.3	10920 ± 567.8	9580 ± 507.7	44.4	38.9
4	6	nil	H	79.6 ± 5.6	8070 ± 468.1	6050 ± 326.7	101.4	76.0
5	3	C(=NH)	C_2H_5	256.1 ± 20.5	10890 ± 620.7	10370 ± 591.1	42.5	40.5
6	4	C(=NH)	C_2H_5	175.6 ± 8.8	7480 ± 433.8	9700 ± 514.1	42.6	55.2
7	5	C(=NH)	C_2H_5	68.4 ± 4.1	4820 ± 250.6	5580 ± 284.6	70.5	81.6
8	6	C(=NH)	C_2H_5	46.9 ± 3.3	4210 ± 214.7	5060 ± 268.2	89.8	107.9
9	3	C(=NH)	C_3H_7	80.8 ± 4.8	10730 ± 579.4	9540 ± 534.2	132.8	118.1
10	4	C(=NH)	C_3H_7	67.8 ± 4.7	6780 ± 372.9	7280 ± 393.1	100.1	107.4
11	5	C(=NH)	C_3H_7	65.3 ± 3.3	7190 ± 359.5	5210 ± 270.9	110.1	79.8
12	6	C(=NH)	C_3H_7	15.4 ± 0.9	2270 ± 120.3	2430 ± 136.0	124.9	157.9
13	3	C(=NH)	C_4H_9	18.0 ± 1.1	3460 ± 197.2	4950 ± 267.3	192.1	275.0
14	4	C(=NH)	C_4H_9	10.2 ± 0.6	3310 ± 172.1	3660 ± 204.9	325.3	359.2
15	5	C(=NH)	C_4H_9	6.7 ± 0.4	2530 ± 136.6	2750 ± 145.7	375.0	407.8
16	6	C(=NH)	C_4H_9	5.3 ± 0.3	2150 ± 111.8	2230 ± 113.7	408.0	423.8
17	3	C(=NH)	NH_2	72.5 ± 5.1	9240 ± 517.4	3720 ± 200.8	127.5	51.4
18	4	C(=NH)	NH_2	67.2 ± 4.0	6160 ± 334.9	3500 ± 185.5	91.7	52.1
19	5	C(=NH)	NH_2	47.5 ± 2.4	3850 ± 204.0	3480 ± 117.5	81.1	73.1
20	6	C(=NH)	NH_2	35.5 ± 2.1	3350 ± 170.8	2990 ± 155.5	94.3	84.2
U50,488				5.01 ± 0.3	716 ± 37.9	8100 ± 162.0	142.9	1616
Dyn A(1−8)				123.8 ± 6.2	1350 ± 71.5	240.7 ± 12.5	10.9	1.9

[a] Values are the mean of three separate experiments each carried out in duplicate. [b] K_i values were obtained as [^{3}H]U69,593 displacement for κ receptor and [^{3}H]diprenorphine displacement for μ and δ receptors. [c] Ronsisvalle et al.[18] [d] nc = not calculated. [e] Ronsisvalle et al.[20]

Table 3. Binding Affinities and Selectivities of Compounds **21**−**40** toward κ, μ, and δ Opioid Receptors



				$K_i \pm$ SEM (nM)[a,b]			selectivity ratio	
no.	*n*	M	R	κ	μ	δ	μ/κ	δ/κ
21	3	nil	H	367.3 ± 22.0	4220 ± 223.6	nd[c]	11.5	nd[c]
22	4	nil	H	382.1 ± 19.7	3400 ± 193.8	nd[c]	8.9	nd[c]
23	5	nil	H	276.5 ± 14.6	3250 ± 169.0	nd[c]	11.8	nd[c]
24	6	nil	H	242.1 ± 14.7	3080 ± 308.0	3420 ± 181.2	12.7	14.1
25	3	C(=NH)	C_2H_5	254.1 ± 15.2	4980 ± 278.8	nd[c]	19.6	nd[c]
26	4	C(=NH)	C_2H_5	191.5 ± 10.9	2200 ± 118.8	nd[c]	11.5	nd[c]
27	5	C(=NH)	C_2H_5	149.0 ± 10.4	2210 ± 112.7	nd[c]	14.8	nd[c]
28	6	C(=NH)	C_2H_5	116.5 ± 6.5	2940 ± 149.9	3550 ± 191.7	25.3	30.5
29	3	C(=NH)	C_3H_7	237.6 ± 16.1	3570 ± 192.7	nd[c]	15.0	nd[c]
30	4	C(=NH)	C_3H_7	203.5 ± 10.3	2280 ± 120.8	nd[c]	11.2	nd[c]
31	5	C(=NH)	C_3H_7	155.8 ± 8.72	2000 ± 108.0	nd[c]	12.8	nd[c]
32	6	C(=NH)	C_3H_7	121.9 ± 6.94	1900 ± 108.3	3460 ± 179.9	15.6	28.4
33	3	C(=NH)	C_4H_9	138.9 ± 8.6	2230 ± 124.8	nd[c]	16.0	nd[c]
34	4	C(=NH)	C_4H_9	116.1 ± 6.6	2130 ± 112.8	nd[c]	18.3	nd[c]
35	5	C(=NH)	C_4H_9	98.1 ± 5.5	2380 ± 128.5	3590 ± 186.6	24.3	36.6
36	6	C(=NH)	C_4H_9	81.0 ± 4.7	2260 ± 117.5	3260 ± 177.3	27.9	40.2
37	3	C(=NH)	NH_2	279.7 ± 16.5	2430 ± 131.2	nd[c]	8.7	nd[c]
38	4	C(=NH)	NH_2	249.5 ± 13.2	2950 ± 156.3	nd[c]	11.8	nd[c]
39	5	C(=NH)	NH_2	216.1 ± 12.3	2790 ± 159.0	nd[c]	12.9	nd[c]
40	6	C(=NH)	NH_2	138.9 ± 7.3	2630 ± 136.7	2830 ± 155.6	18.9	20.3

[a] Values are the mean of three separate experiments each carried out in duplicate. [b] K_i values were obtained as [^{3}H]U69,593 displacement for κ receptor and [^{3}H]diprenorphine displacement for μ and δ receptors. [c] nd = not determined.

17),[20] respectively). These results further support the hypothesis that the κ-selective nonpeptide ligands MPCB and CCB already have all the structural and conformational requirements permitting a κ-selective interaction. The attachment of a moiety mimicking the C-terminal fragment of dynorphin A(1−8) does not reduce κ binding affinity and maintains high κ preference, thus supporting the idea that these molecules might interact with κ opioid receptors possibly occupying binding sites very close to that of endogenous ligands. Compounds **15** and

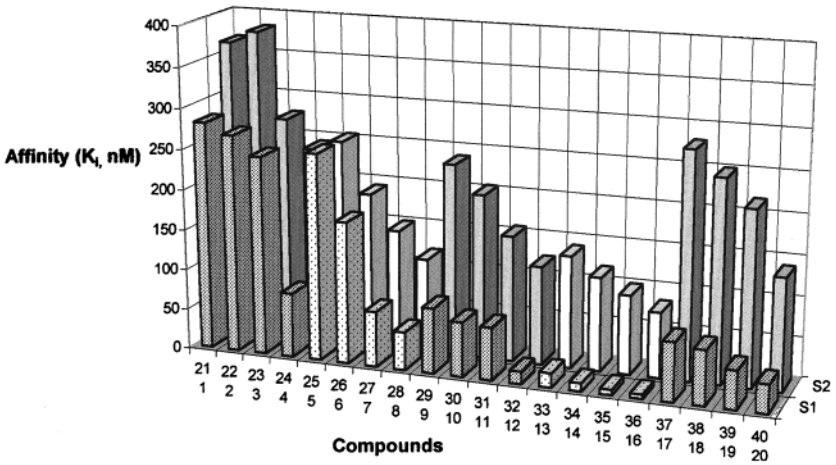


Figure 4. κ opioid binding affinities of compounds **1**−**20** (dotted tonality) and compounds **21**−**40** (gray tonality).

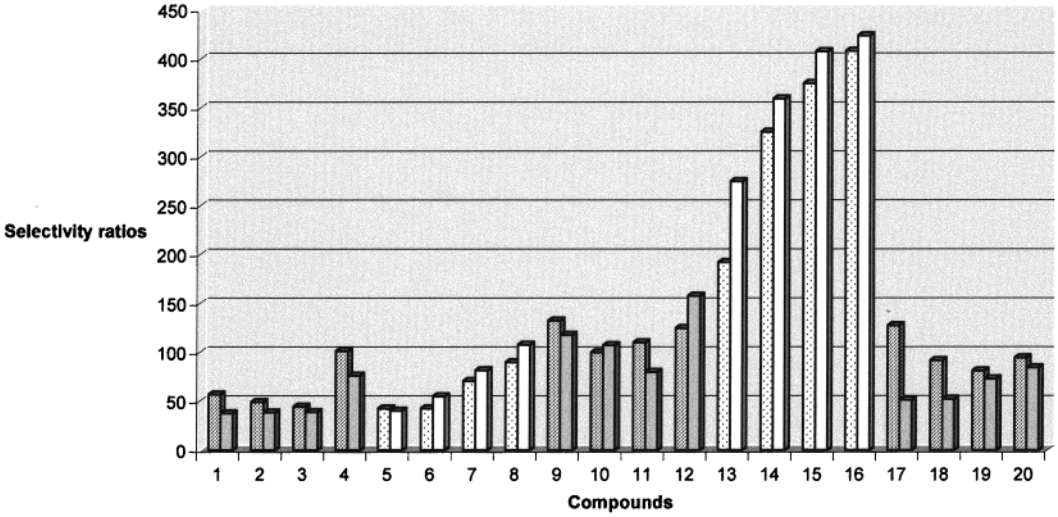


Figure 5. Selectivity ratios K_i^{μ}/K_i^{κ} (dotted tonality) and $K_i^{\delta}/K_i^{\kappa}$ (gray tonality) of compounds **1**−**20**.

Table 4. Antinociceptive and Sedative Activity of Compounds **15**, **16**, and U50,488 in the Mouse

compound	ED_{50} (95% confidence limits), mg/kg sc		rotarod value MPE_{50}/ antinocicpetive activity MPE_{50}
	antinociceptive activity	rotarod value	
15	0.88 (0.5−1.3)	9.2 (4.5−13.8)	10.45
15 + nor-BNI (10 mg/kg; administered 10 min before agonist)	*a*	*a*	
16	1.1 (0.6−1.5)	11.9 (7.0−18.7)	10.81
16 + nor-BNI (10 mg/kg; administered 10 min before agonist)	*a*	*a*	
U50,488	0.76 (0.4−1.1)	8.2 (3.7−11.4)	10.78
U50,488 + nor-BNI (10 mg/kg; administered 10 min before agonist)	*a*	*a*	

[a] Full antagonism of the pharmacological effect produced by the ED_{50} dose of the agonist.

16, therefore, allowing to identify the functionalities relevant in the C-terminal region, provide insights for future design of peptidomimetics of dynorphin A.

Experimental Section

Reagents were purchased from Aldrich Chemical Co. unless otherwise indicated. NMR spectra were recorded on a Varian Inova-200 spectrometer, using tetramethylsilane (TMS) as an internal standard. Infrared spectra were recorded on 1600 FTIR Perkin-Elmer instruments and are consistent with the assigned structures. Elemental analyses were measured on an elemental analyzer (model 1106, Carlo Erba) and were within 0.4% of the theoretical values. Mass spectra (EI) were recorded on a Kratos 2S RFA spectrometer using a Tektroniks 4205 computer. Molecular weights of the final products were determined by FAB-MS on a Finnigan MHT 90 mass spectrometer. Analytical RP-HPLC was performed on a Waters model 600E, operating at a flow rate of 1.5 mL/min using a Lichrosorb RP-18 10 μm column, and monitoring at 280 and 220 nm with a Knauer UV/VIS filter photomer. The HPLC solvent systems were (1) an isocratic system of 80% A and 20% B, where solvent A is 10% water in acetonitrile with 0.1% TFA and solvent B is 10% acetonitrile in water with 0.1% TFA, and (2) a linear gradient system of 30% A and 70% B to 0% A and 100% B over 10 min, where solvent A is water with 0.1% TFA and solvent B is acetonitrile with 0.1% TFA. Melting points were determined on a Büchi 530 capillary apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on precoated silica gel 60 RP-18 F_{254} plates (Merck) and precoated silica gel F_{254} plates (Merck). Merck silica gel

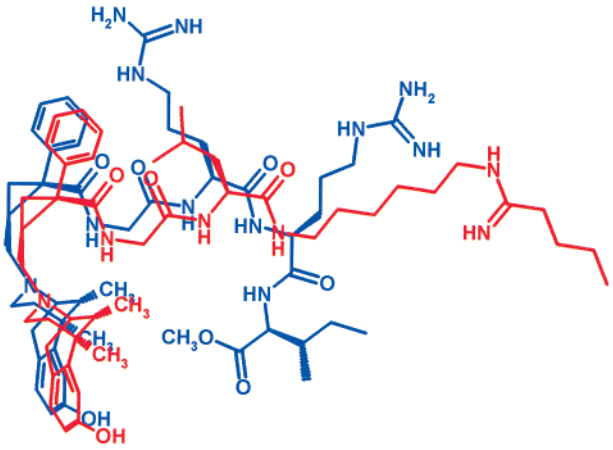


Figure 6. Superposition of novel synthesized compound **16** (red) with MPCB-GRRI (blue).

60 (mesh 230−400) was used for flash column chromatography. All compounds exhibited NMR, IR, and MS spectral data consistent with those of the assigned structures.

***N*-[3-(*tert*-Butoxycarbonyl)aminopropyl]-2(*R*)-[2-(carbobenzoxy)aminoacetylamino]-4-methylpentanamide (41).** To a solution of *N*-(*tert*-butoxycarbonyl)-1,3-diaminopropane (575 mg, 3.3 mmol) in DMF (3 mL), cooled at 0 °C, was added 2(*R*)-[2-(carbobenzoxy)aminoacetylamino]-4-methylpentanoic acid *p*-nitrophenyl ester (1.33 g, 3 mmol) portionwise over 1 h. The reaction mixture was then stirred for 5 h at room temperature and the residue was dissolved in EtOAc and washed consecutively with 5% citric acid, 5% $NaHCO_3$ and brine. The organic layer was separated and dried over anhydrous Na_2SO_4 and evaporated to dryness to give 1.19 g of the pure desired compound **41** as a white solid: 83% yield; mp 113−115 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, d, Leu-CH_3, J = 5.8 Hz), 0.91 (3H, d, Leu-CH_3, J = 5.8 Hz), 1.40 (9H, s, Boc-CH_3), 1.75−1.49 (5H, m, CH_2 + Leu-$C_\beta H_2$ + Leu-C_γH), 3.16−3.06 (2H, m, Leu-NH-*CH_2*), 3.29−3.20 (2H, m, *CH_2*-NH-Boc), 3.82 (1H, dd, Gly-CH_a, J = 5.0, 17.0 Hz), 4.03 (1H, dd, Gly-CH_b, J = 6.2, 17.0 Hz), 4.53−4.41 (1H, m, Leu-C_αH), 4.96 (1H, br s, Leu-*NH*), 5.12 (2H, s, Cbz-CH_2), 5.99 (1H, br s, Gly-N_αH), 6.69 (1H, d, Leu-N_αH, J = 8.0 Hz), 7.11 (1H, br s, *NH*-Boc), 7.37−7.30 (m, 5H, Cbz-aromatic-H); ^{13}C NMR ($CDCl_3$) δ 21.8, 22.9, 24.8, 28.4, 29.8, 35.5, 36.9, 40.8, 44.6, 51.8, 67.2, 79.5, 128.0, 128.2, 128.5, 136.1, 156.2, 156.7, 169.4, 171.9; MS *m*/*z* (EI) 478 $[M]^+$. Anal. ($C_{24}H_{38}N_4O_6$) C, H, N.

The following compounds **42**−**44** were prepared from 2(*R*)-[2-(carbobenzoxy)aminoacetylamino]-4-methylpentanoic acid *p*-nitrophenyl ester and *N*-(*tert*-butoxycarbonyl)-1,4-diaminobutane, *N*-(*tert*-butoxycarbonyl)-1,5-diaminopentane, and *N*-(*tert*-butoxycarbonyl)-1,6-diaminohexane, respectively, following the same methodology as that described for compound **41**.

***N*-[4-(*tert*-Butoxycarbonyl)aminobutyl]-2(*R*)-[2-(carbobenzoxy)aminoacetylamino]-4-methylpentanamide (42):** 80% yield; mp 112−114 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, d, Leu-CH_3, J = 5.8 Hz), 0.90 (3H, d, Leu-CH_3, J = 5.8 Hz), 1.42 (9H, s, Boc-CH_3), 1.74−1.40 (7H, m, -$(CH_2)_2$- + Leu-$C_\beta H_2$ + Leu-C_γH), 3.13−3.06 (2H, m, Leu-NH-*CH_2*), 3.29−3.21 (2H, m, *CH_2*-NH-Boc), 3.81 (1H, dd, Gly-CH_a, J = 5.4, 17.0 Hz), 3.92 (1H, dd, Gly-CH_b, J = 6.0, 17.0 Hz), 4.53−4.40 (1H, m, Leu-C_αH), 4.83 (1H, br s, Leu-*NH*), 5.11 (2H, s, Cbz-CH_2), 5.90 (1H, br s, Gly-N_αH), 6.82 (1H, br s, *NH*-Boc), 6.92 (1H, d, Leu-N_αH, J = 8.0 Hz), 7.34−7.29 (m, 5H, Cbz-aromatic-H); ^{13}C NMR ($CDCl_3$) δ 22.0, 22.8, 24.7, 26.3, 27.4, 28.4, 39.1, 40.1, 40.9, 44.5, 51.8, 67.2, 79.2, 128.0, 128.2, 128.5, 136.1, 156.2, 156.8, 169.3, 171.8; MS *m*/*z* (EI) 492 $[M]^+$. Anal. ($C_{25}H_{40}N_4O_6$) C, H, N.

***N*-[5-(*tert*-Butoxycarbonyl)aminopentyl]-2(*R*)-[2-(carbobenzoxy)aminoacetylamino]-4-methylpentanamide (43):** 85% yield; mp 112−113 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, d, Leu-CH_3, J = 5.8 Hz), 0.91 (3H, d, Leu-CH_3, J = 5.8 Hz), 1.42 (9H, s, Boc-CH_3), 1.76−1.21 (9H, m, -$(CH_2)_3$- + Leu-$C_\beta H_2$ + Leu-C_γH), 3.14−3.06 (2H, m, Leu-NH-*CH_2*), 3.26−3.19 (2H, m, *CH_2*-NH-Boc), 3.81 (1H, dd, Gly-CH_a, J = 5.4, 17.0 Hz), 3.93 (1H, dd, Gly-CH_b, J = 6.0, 17.0 Hz), 4.50−4.39 (1H, m, Leu-C_αH), 4.75 (1H, br s, Leu-*NH*), 5.11 (2H, s, Cbz-CH_2), 5.85 (1H, br s, Gly-N_αH), 6.59 (1H, br s, *NH*-Boc), 6.83 (1H, d, Leu-N_αH, J = 8.0 Hz), 7.34−7.27 (m, 5H, Cbz-aromatic-H); ^{13}C NMR ($CDCl_3$) δ 22.0, 22.8, 23.8, 24.7, 28.4, 28.8, 29.5, 39.2, 40.3, 40.8, 44.5, 51.8, 67.2, 79.1, 128.0, 128.2, 128.5, 136.1, 156.1, 156.8, 169.2, 171.7; MS *m*/*z* (EI) 506 $[M]^+$. Anal. ($C_{26}H_{42}N_4O_6$) C, H, N.

***N*-[6-(*tert*-Butoxycarbonyl)aminohexyl]-2(*R*)-[2-(carbobenzoxy)aminoacetylamino]-4-methylpentanamide (44):** 89% yield; mp 99−101 °C; ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, d, Leu-CH_3, J = 5.8 Hz), 0.91 (3H, d, Leu-CH_3, J = 5.8 Hz), 1.33−1.21 (8H, m, -$(CH_2)_4$-), 1.43 (9H, s, Boc-CH_3), 1.77−1.40 (3H, m, Leu-$C_\beta H_2$ + Leu-C_γH), 3.13−3.03 (2H, m, Leu-NH-*CH_2*), 3.24−3.15 (2H, m, *CH_2*-NH-Boc), 3.81 (1H, dd, Gly-CH_a, J = 5.4, 17.0 Hz), 3.93 (1H, dd, Gly-CH_b, J = 6.0, 17.0 Hz), 4.48−4.37 (1H, m, Leu-C_αH), 4.65 (1H, br s, Leu-*NH*), 5.12 (2H, s, Cbz-CH_2), 5.76 (1H, br s, Gly-N_αH), 6.49 (1H, br s, *NH*-Boc), 6.70 (1H, d, Leu-N_αH, J = 8.0 Hz), 7.34−7.28 (m, 5H, Cbz-aromatic-H); ^{13}C NMR ($CDCl_3$) δ 22.0, 22.8, 24.7, 25.8, 28.4, 29.0, 29.7, 39.1, 40.0, 40.9, 44.6, 51.7, 67.2, 79.1, 128.0, 128.2, 128.5, 136.1, 156.1, 156.7, 169.1, 171.6; MS *m*/*z* (EI) 520 $[M]^+$. Anal. ($C_{27}H_{44}N_4O_6$) C, H, N.

The following compounds **45**−**48** were prepared from 2(*R*)-(carbobenzoxy)amino-4-methylpentanoic acid *p*-nitrophenyl ester and *N*-(*tert*-butoxycarbonyl)-1,3-diaminopropane, *N*-(*tert*-butoxycarbonyl)-1,4-diaminobutane, *N*-(*tert*-butoxycarbonyl)-1,5-diaminopentane, and *N*-(*tert*-butoxycarbonyl)-1,6-diaminohexane, respectively, following the same methodology as that described for compound **41**.

***N*-[3-(*tert*-Butoxycarbonyl)aminopropyl]-2(*R*)-(carbobenzoxy)amino-4-methylpentanamide (45):** 84% yield; mp 118−119 °C; ^{1}H NMR ($CDCl_3$) δ 0.94 (6H, d, Leu-CH_3, J = 6.0 Hz), 1.43 (9H, s, Boc-CH_3), 1.75−1.49 (5H, m, CH_2 + Leu-$C_\beta H_2$ + Leu-C_γH), 3.16−3.04 (2H, m, Leu-NH-*CH_2*), 3.33−3.24 (2H, m, *CH_2*-NH-Boc), 4.22−4.11 (1H, m, Leu-C_αH), 4.95 (1H, br s, Leu-*NH*), 5.10 (2H, s, Cbz-CH_2), 5.30 (1H, d, Leu-N_αH, J = 7.8 Hz), 6.79 (1H, br s, *NH*-Boc), 7.37−7.29 (m, 5H, Cbz-aromatic-H); ^{13}C NMR ($CDCl_3$) δ 21.9, 22.9, 24.7, 28.4, 30.0, 35.8, 36.8, 41.7, 53.7, 67.0, 79.3, 128.0, 128.1, 128.5, 136.2, 156.1, 156.5, 172.5; MS *m*/*z* (EI) 421 $[M]^+$. Anal. ($C_{22}H_{35}N_3O_5$) C, H, N.

***N*-[4-(*tert*-Butoxycarbonyl)aminobutyl]-2(*R*)-(carbobenzoxy)amino-4-methylpentanamide (46):** 88% yield; mp 117−119 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (6H, d, Leu-CH_3, J = 6.0 Hz), 1.43 (9H, s, Boc-CH_3), 1.70−1.40 (7H, m, -$(CH_2)_2$- + Leu-$C_\beta H_2$ + Leu-C_γH), 3.16−3.06 (2H, m, Leu-NH-*CH_2*), 3.29−3.20 (2H, m, *CH_2*-NH-Boc), 4.21−4.10 (1H, m, Leu-C_αH), 4.66 (1H, br s, Leu-*NH*), 5.10 (2H, s, Cbz-CH_2), 5.34 (1H, d, Leu-N_αH, J = 7.8 Hz), 6.40 (1H, br s, *NH*-Boc), 7.37−7.29 (m, 5H, Cbz-aromatic-H); ^{13}C NMR ($CDCl_3$) δ 21.9, 22.9, 24.7, 26.3, 27.5, 28.4, 39.1, 39.9, 41.4, 53.6, 67.0, 79.2, 128.0, 128.2, 128.5, 136.1, 156.1, 156.2, 172.2; MS *m*/*z* (EI) 435 $[M]^+$. Anal. ($C_{23}H_{37}N_3O_5$) C, H, N.

***N*-[5-(*tert*-Butoxycarbonyl)aminopentyl]-2(*R*)-(carbobenzoxy)amino-4-methylpentanamide (47):** 89% yield; mp 109−110 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (6H, d, Leu-CH_3, J = 6.0 Hz), 1.43 (9H, s, Boc-CH_3), 1.70−1.25 (9H, m, -$(CH_2)_3$- + Leu-$C_\beta H_2$ + Leu-C_γH), 3.14−3.05 (2H, m, Leu-NH-*CH_2*), 3.25−3.16 (2H, m, *CH_2*-NH-Boc), 4.20−4.09 (1H, m, Leu-C_αH), 4.70 (1H, br s, Leu-*NH*), 5.10 (2H, s, Cbz-CH_2), 5.30 (1H, d, Leu-N_αH, J = 7.8 Hz), 6.24 (1H, br s, *NH*-Boc), 7.37−7.29 (m, 5H, Cbz-aromatic-H); ^{13}C NMR ($CDCl_3$) δ 21.9, 22.9, 23.8, 24.7, 28.4, 29.0, 29.4, 39.1, 40.3, 41.4, 53.6, 67.0, 79.1, 128.0, 128.2, 128.5, 136.1, 156.0, 156.3, 172.1; MS *m*/*z* (EI) 449 $[M]^+$. Anal. ($C_{24}H_{39}N_3O_5$) C, H, N.

***N*-[6-(*tert*-Butoxycarbonyl)aminohexyl]-2(*R*)-(carbobenzoxy)amino-4-methylpentanamide (48):** 85% yield; mp 108−111 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (6H, d, Leu-CH_3, J = 6.0 Hz), 1.33−1.25 (8H, m, -$(CH_2)_4$-), 1.43 (9H, s, Boc-CH_3), 1.70−1.25 (3H, m, Leu-$C_\beta H_2$ + Leu-C_γH), 3.14−3.04 (2H, m, Leu-NH-*CH_2*), 3.26−3.17 (2H, m, *CH_2*-NH-Boc), 4.19−4.09 (1H, m,

Leu-$C_\alpha H$), 4.60 (1H, br s, Leu-*NH*), 5.10 (2H, s, Cbz-CH_2), 5.27 (1H, d, Leu-$N_\alpha H$, $J = 7.8$ Hz), 6.24 (1H, br s, *NH*-Boc), 7.37−7.29 (m, 5H, Cbz-aromatic-H); ^{13}C NMR ($CDCl_3$) δ 21.9, 22.9, 24.7, 25.9, 28.4, 29.2, 29.8, 39.1, 40.1, 41.4, 53.6, 67.0, 79.1, 128.0, 128.2, 128.5, 136.1, 156.0, 156.2, 172.0; MS *m/z* (EI) 463 $[M]^+$. Anal. ($C_{25}H_{41}N_3O_5$) C, H, N.

***N*-[3-(*tert*-Butoxycarbonyl)aminopropyl]-2(*R*)-(2-aminoacetylamino)-4-methylpentanamide (49).** A mixture of **41** (850 mg, 1.77 mmol) and 10% Pd−C (212.5 mg) in MeOH (15 mL) and water (0.75 mL) was allowed to stir under hydrogen (1 atm) at room temperature for 45 min. The catalyst was filtered and the filtrate was evaporated and dried to give 573.1 mg of the desired product **49** as a pale yellow oil: 94% yield; ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, d, Leu-CH_3, $J = 5.6$ Hz), 0.94 (3H, d, Leu-CH_3, $J = 5.6$ Hz), 1.43 (9H, s, Boc-CH_3), 1.75−1.41 (5H, m, CH_2 + Leu-$C_\beta H_2$ + Leu-$C_\gamma H$), 2.18 (2H, br s, NH_2), 3.16−3.05 (2H, m, Leu-NH-CH_2), 3.27−3.20 (2H, m, CH_2-NH-Boc), 3.44 (2H, s, Gly-CH_2), 4.48−4.40 (1H, m, Leu-$C_\alpha H$), 4.80 (1H, br s, Leu-*NH*), 6.83 (1H, br s, *NH*-Boc), 7.71 (1H, d, Leu-$N_\alpha H$, $J = 8.0$ Hz); ^{13}C NMR ($CDCl_3$) δ 22.1, 22.8, 24.7, 28.4, 29.7, 35.5, 36.8, 40.7, 44.5, 51.4, 79.2, 156.1, 172.1, 173.0; retention time (t_R) (HPLC system 1) 1.85 min.

Compounds **50**−**52** were prepared from compounds **42**−**44**, respectively, following the same methodology as that described for compound **49**.

***N*-[4-(*tert*-Butoxycarbonyl)aminobutyl]-2(*R*)-(2-aminoacetylamino)-4-methylpentanamide (50):** 92% yield; ^{1}H NMR ($CDCl_3$) δ 0.92 (3H, d, Leu-CH_3, $J = 5.6$ Hz), 0.94 (3H, d, Leu-CH_3, $J = 5.6$ Hz), 1.44 (9H, s, Boc-CH_3), 1.75−1.38 (7H, m, -$(CH_2)_2$- + Leu-$C_\beta H_2$ + Leu-$C_\gamma H$), 2.16 (2H, br s, NH_2), 3.16−3.09 (2H, m, Leu-NH-CH_2), 3.26−3.22 (2H, m, CH_2-NH-Boc), 3.39 (2H, s, Gly-CH_2), 4.43−4.40 (1H, m, Leu-$C_\alpha H$), 4.74 (1H, br s, Leu-*NH*), 6.79 (1H, br s, *NH*-Boc), 7.69 (1H, d, Leu-$N_\alpha H$, $J = 8.0$ Hz); ^{13}C NMR ($CDCl_3$) δ 22.1, 22.8, 24.7, 26.3, 27.3, 28.4, 38.9, 40.0, 40.6, 44.4, 51.4, 79.1, 156.1, 172.0, 172.9; retention time (t_R) (HPLC system 1) 1.90 min.

***N*-[5-(*tert*-Butoxycarbonyl)aminopentyl]-2(*R*)-(2-aminoacetylamino)-4-methylpentanamide (51):** 98% yield; ^{1}H NMR ($CDCl_3$) δ 0.91 (3H, d, Leu-CH_3, $J = 5.6$ Hz), 0.94 (3H, d, Leu-CH_3, $J = 5.6$ Hz), 1.44 (9H, s, Boc-CH_3), 1.75−1.26 (9H, m, -$(CH_2)_3$- + Leu-$C_\beta H_2$ + Leu-$C_\gamma H$), 2.13 (2H, br s, NH_2), 3.14−3.04 (2H, m, Leu-NH-CH_2), 3.27−3.17 (2H, m, CH_2-NH-Boc), 3.45 (2H, s, Gly-CH_2), 4.47−4.36 (1H, m, Leu-$C_\alpha H$), 4.74 (1H, br s, Leu-*NH*), 6.71 (1H, br s, *NH*-Boc), 7.73 (1H, d, Leu-$N_\alpha H$, $J = 8.0$ Hz); ^{13}C NMR ($CDCl_3$) δ 22.1, 22.8, 23.8, 24.7, 28.4, 28.9, 29.5, 39.1, 40.3, 40.6, 44.4, 51.4, 79.0, 156.0, 171.9, 172.8; retention time (t_R) (HPLC system 1) 1.95 min.

***N*-[6-(*tert*-Butoxycarbonyl)aminohexyl]-2(*R*)-(2-aminoacetylamino)-4-methylpentanamide (52):** 95% yield; ^{1}H NMR ($CDCl_3$) δ 0.91 (3H, d, Leu-CH_3, $J = 5.6$ Hz), 0.94 (3H, d, Leu-CH_3, $J = 5.6$ Hz), 1.32−1.20 (8H, m, -$(CH_2)_4$-), 1.44 (9H, s, Boc-CH_3), 1.75−1.40 (3H, m, Leu-$C_\beta H_2$ + Leu-$C_\gamma H$), 2.08 (2H, br s, NH_2), 3.13−3.04 (2H, m, Leu-NH-CH_2), 3.26−3.16 (2H, m, CH_2-NH-Boc), 3.40 (2H, s, Gly-CH_2), 4.45−4.36 (1H, m, Leu-$C_\alpha H$), 4.71 (1H, br s, Leu-*NH*), 6.65 (1H, br s, *NH*-Boc), 7.70 (1H, d, Leu-$N_\alpha H$, $J = 8.0$ Hz); ^{13}C NMR ($CDCl_3$) δ 22.1, 22.8, 24.7, 25.8, 28.4, 29.0, 29.6, 39.1, 40.2, 40.6, 44.3, 51.3, 79.0, 156.0, 171.8, 172.7; retention time (t_R) (HPLC system 1) 2.00 min.

Compounds **53**−**56** were prepared from compounds **45**−**48**, respectively, following the same methodology as that described for compound **49**.

***N*-[3-(*tert*-Butoxycarbonyl)aminopropyl]-2(*R*)-amino-4-methylpentanamide (53):** 91% yield; ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, d, Leu-CH_3, $J = 6.0$ Hz), 0.96 (3H, d, Leu-CH_3, $J = 6.0$ Hz), 1.44 (9H, s, Boc-CH_3), 1.75−1.44 (5H, m, CH_2 + Leu-$C_\beta H_2$ + Leu-$C_\gamma H$), 1.94 (2H, br s, NH_2), 3.18−3.09 (2H, m, Leu-NH-CH_2), 3.32−3.25 (2H, m, CH_2-NH-Boc), 3.44−3.35 (1H, m, Leu-$C_\alpha H$), 4.66 (1H, br s, Leu-*NH*), 7.53 (1H, br s, *NH*-Boc); ^{13}C NMR ($CDCl_3$) δ 22.1, 23.2, 24.5, 28.4, 29.9, 35.6, 36.4, 44.6, 53.0, 77.5, 155.9, 175.8; retention time (t_R) (HPLC system 1) 1.95 min.

***N*-[4-(*tert*-Butoxycarbonyl)aminobutyl]-2(*R*)-amino-4-methylpentanamide (54):** 93% yield; ^{1}H NMR ($CDCl_3$) δ 0.93 (3H, d, Leu-CH_3, $J = 6.0$ Hz), 0.96 (3H, d, Leu-CH_3, $J = 6.0$ Hz), 1.44 (9H, s, Boc-CH_3), 1.75−1.35 (7H, m, -$(CH_2)_2$- + Leu-$C_\beta H_2$ + Leu-$C_\gamma H$), 1.90 (2H, br s, NH_2), 3.17−3.10 (2H, m, Leu-NH-CH_2), 3.30−3.24 (2H, m, CH_2-NH-Boc), 3.43−3.38 (1H, m, Leu-$C_\alpha H$), 4.62 (1H, br s, Leu-*NH*), 7.41 (1H, br s, *NH*-Boc); ^{13}C NMR ($CDCl_3$) δ 22.1, 23.2, 24.5, 26.2, 27.3, 28.4, 38.3, 39.5, 44.5, 53.0, 77.5, 155.9, 175.6; retention time (t_R) (HPLC system 1) 2.10 min.

***N*-[5-(*tert*-Butoxycarbonyl)aminopentyl]-2(*R*)-amino-4-methylpentanamide (55):** 95% yield; ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, d, Leu-CH_3, $J = 6.0$ Hz), 0.97 (3H, d, Leu-CH_3, $J = 6.0$ Hz), 1.45 (9H, s, Boc-CH_3), 1.75−1.25 (9H, m, -$(CH_2)_3$- + Leu-$C_\beta H_2$ + Leu-$C_\gamma H$), 1.83 (2H, br s, NH_2), 3.16−3.07 (2H, m, Leu-NH-CH_2), 3.30−3.20 (2H, m, CH_2-NH-Boc), 3.43−3.38 (1H, m, Leu-$C_\alpha H$), 4.60 (1H, br s, Leu-*NH*), 7.34 (1H, br s, *NH*-Boc); ^{13}C NMR ($CDCl_3$) δ 22.1, 23.3, 23.8, 24.3, 28.4, 28.9, 29.3, 38.3, 39.9, 44.4, 53.0, 77.6, 155.8, 175.5; retention time (t_R) (HPLC system 1) 2.20 min.

***N*-[6-(*tert*-Butoxycarbonyl)aminohexyl]-2(*R*)-amino-4-methylpentanamide (56):** 95% yield; ^{1}H NMR ($CDCl_3$) δ 0.94 (3H, d, Leu-CH_3, $J = 6.0$ Hz), 0.97 (3H, d, Leu-CH_3, $J = 6.0$ Hz), 1.35−1.20 (8H, m, -$(CH_2)_4$-), 1.44 (9H, s, Boc-CH_3), 1.75−1.40 (3H, m, Leu-$C_\beta H_2$ + Leu-$C_\gamma H$), 1.80 (2H, br s, NH_2), 3.14−3.05 (2H, m, Leu-NH-CH_2), 3.29−3.18 (2H, m, CH_2-NH-Boc), 3.41−3.36 (1H, m, Leu-$C_\alpha H$), 4.58 (1H, br s, Leu-*NH*), 7.30 (1H, br s, *NH*-Boc); ^{13}C NMR ($CDCl_3$) δ 22.1, 23.3, 24.3, 25.8, 28.4, 29.2, 29.7, 38.0, 39.8, 44.4, 53.0, 77.6, 155.8, 175.4; retention time (t_R) (HPLC system 1) 2.30 min.

***N*-{{1-[3-(*tert*-Butoxycarbonyl)aminopropylcarbamoyl]-3-methyl-1(*R*)-butylcarbamoyl}methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (57) and *N*-{[1-(3-Aminopropylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide·2TFA (1).** To a solution of 1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxylic acid (563.8 mg, 1.44 mmol) and compound **49** (413.3 mg, 1.2 mmol) in DMF (5 mL) was added HOBt (201.6 mg, 1.44 mmol). The reaction mixture was cooled to 0 °C and DCC (297.1 mg, 1.44 mmol) was added. The reaction mixture was kept at 0−5 °C for 48 h, then the DCU precipitated was filtered and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc and washed with H_2O, 5% $NaHCO_3$ and brine. The separated organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness to give compound **57** as a yellow pale solid, which was used without further purification. The *tert*-butoxycarbonyl protecting group was removed by treating crude compound **57** with aqueous 90% TFA (1:10 mL, w/v) and anisole (1:100 mL, w/v). The mixture was stirred at room temperature for 30−40 min, then the solvent was evaporated in vacuo at 0 °C, and the residue was triturated with *n*-hexane/Et_2O (2:1). The resulting solid was purified by flash column chromatography (LiChroprep RP-18, 40−63 μm, eluted with acetonitrile/water/TFA, 30:70:0.25). The fractions containing the desired product **1** were collected and lyophilized to constant weight: 42% yield. Anal. ($C_{36}H_{51}N_5O_4 \cdot 2CF_3COOH \cdot 3H_2O$) C, H, N.

Compounds **2**−**4** were prepared from 1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxylic acid and compounds **50**−**52**, respectively, following the same methodology as that described for compound **1**.

***N*-{{1-[4-(*tert*-Butoxycarbonyl)aminobutylcarbamoyl]-3-methyl-1(*R*)-butylcarbamoyl}methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (58) and *N*-{[1-(4-Aminobutylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]-**

cyclopropanecarboxamide·2TFA (2): 45% yield. Anal. ($C_{37}H_{53}N_5O_4$·$2CF_3COOH$·$3H_2O$) C, H, N.

***N*-{{1-[5-(*tert*-Butoxycarbonyl)aminopentylcarbamoyl]-3-methyl-1(*R*)-butylcarbamoyl}methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (59) and *N*-{[1-(5-Aminopentylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide·2TFA (3):** 40% yield. Anal. ($C_{38}H_{55}N_5O_4$·$2CF_3$-$COOH$·$3H_2O$) C, H, N.

***N*-{{1-[6-(*tert*-Butoxycarbonyl)aminohexylcarbamoyl]-3-methyl-1(*R*)-butylcarbamoyl}methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (60) and *N*-{[1-(6-Aminohexylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide·2TFA (4):** 50% yield. Anal. ($C_{39}H_{57}N_5O_4$·$2CF_3COOH$·$3H_2O$) C, H, N.

Compounds **21**−**24** were prepared from 1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxylic acid and compounds **53**−**56**, respectively, following the same methodology as that described for compound **1**.

***N*-{1-[3-(*tert*-Butoxycarbonyl)aminopropylcarbamoyl]-3-methyl-1(*R*)-butyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),-11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (61) and *N*-[1-(3-Aminopropylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]-cyclopropanecarboxamide·2TFA (21):** 43% yield. Anal. ($C_{34}H_{48}N_4O_3$·$2CF_3COOH$·$3H_2O$) C, H, N.

***N*-{1-[4-(*tert*-Butoxycarbonyl)aminobutylcarbamoyl]-3-methyl-1(*R*)-butyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),-11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (62) and *N*-[1-(4-Aminobutylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]-cyclopropanecarboxamide·2TFA (22):** 47% yield. Anal. ($C_{35}H_{50}N_4O_3$·$2CF_3COOH$·$3H_2O$) C, H, N.

***N*-{1-[5-(*tert*-Butoxycarbonyl)aminopentylcarbamoyl]-3-methyl-1(*R*)-butyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),-11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (63) and *N*-[1-(5-Aminopentylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]-cyclopropanecarboxamide·2TFA (23):** 44% yield. Anal. ($C_{36}H_{52}N_4O_3$·$2CF_3COOH$·$3H_2O$) C, H, N.

***N*-{1-[6-(*tert*-Butoxycarbonyl)aminohexylcarbamoyl]-3-methyl-1(*R*)-butyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),-11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (64) and *N*-[1-(6-Aminohexylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]-cyclopropanecarboxamide·2TFA (24):** 49% yield. Anal. ($C_{37}H_{54}N_4O_3$·$2CF_3COOH$·$3H_2O$) C, H, N.

***N*-{[1-(3-Amidinoethylpropylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (5).** To a solution of compound **1** (30 mg, 0.0354 mmol) in EtOH (0.5 mL) was added ethyl propionimidate hydrochloride (5.85 mg, 0.0425 mmol) and the pH was adjusted to 10 by adding DIEA. The reaction mixture was stirred for 24 h at room temperature and then evaporated to dryness. The crude compound was purified by flash column chromatography (silica gel, eluted with $CHCl_3$/MeOH/NH_4OH, 9:1:0.1), followed by trituration with diethyl ether to give 18.65 mg of the pure desired compound **5** as a white solid with a 64% yield.

Compounds **6**−**8** were prepared from compounds **2**−**4**, respectively, following the same methodology as that described for compound **5**.

***N*-{[1-(4-Amidinoethylbutylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (6):** 62% yield.

***N*-{[1-(5-Amidinoethylpentylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (7):** 61% yield.

***N*-{[1-(6-Amidinoethylhexylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (8):** 64% yield.

Compounds **25**−**28** were prepared from compounds **21**−**24**, respectively, following the same methodology as that described for compound **5**.

***N*-[1-(3-Amidinoethylpropylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (25):** 47% yield.

***N*-[1-(4-Amidinoethylbutylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (26):** 53% yield.

***N*-[1-(5-Amidinoethylpentylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (27):** 56% yield.

***N*-[1-(6-Amidinoethylhexylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (28):** 74% yield.

***N*-{[1-(3-Amidinopropylpropylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (9).** To a solution of compound **1** (30 mg, 0.0354 mmol) in EtOH (0.5 mL) was added ethyl butyrimidate hydrochloride (6.44 mg, 0.0425 mmol) and the pH was adjusted to 10 by adding DIEA. The reaction mixture was stirred for 24 h at room temperature and then evaporated to dryness. Purification by flash column chromatography (silica gel, eluted with $CHCl_3$/MeOH/NH_4OH, 9:1:0.1), followed by trituration with diethyl ether, gave 17.8 mg of the pure desired compound **9** as a white solid with a 60% yield.

Compounds **10**−**12** were prepared from compounds **2**−**4**, respectively, following the same methodology as that described for compound **9**.

***N*-{[1-(4-Amidinopropylbutylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (10):** 63% yield.

***N*-{[1-(5-Amidinopropylpentylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (11):** 70% yield.

***N*-{[1-(6-Amidinopropylhexylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (12):** 70% yield.

Compounds **29**−**32** were prepared from compounds **21**−**24,** respectively, following the same methodology as that described for compound **9**.

***N*-[1-(3-Amidinopropylpropylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (29):** 60% yield.

***N*-[1-(4-Amidinopropylbutylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (30):** 72% yield.

***N*-[1-(5-Amidinopropylpentylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (31):** 53% yield.

***N*-[1-(6-Amidinopropylhexylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (32):** 65% yield.

***N*-{[1-(3-Amidinobutylpropylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (13).** To a solution of compound **1** (30 mg, 0.0354 mmol) in EtOH (0.5 mL) was added ethyl valerimidate hydrochloride (7.04 mg, 0.0425 mmol) and the pH was adjusted to 10 by adding DIEA. The reaction mixture was stirred for 24 h at room temperature and then evaporated to dryness. Purification by flash column chromatography (silica gel, eluted with $CHCl_3$/MeOH/NH_4OH, 9:1:0.1), followed by trituration with diethyl ether, gave 20.2 mg of the pure desired compound **13** as a white solid with a 67% yield.

Compounds **14**−**16** were prepared from compounds **2**−**4**, respectively, following the same methodology as that described for compound **13**.

***N*-{[1-(4-Amidinobutylbutylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (14):** 50% yield.

***N*-{[1-(5-Amidinobutylpentylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (15):** 59% yield. Anal. ($C_{43}H_{64}N_6O_4 \cdot 7H_2O$) C, H, N.

***N*-{[1-(6-Amidinobutylhexylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (16):** 61% yield. Anal. ($C_{44}H_{66}N_6O_4 \cdot 5.5H_2O$) C, H, N.

Compounds **33**−**36** were prepared from compounds **21**−**24**, respectively, following the same methodology as that described for compound **13**.

***N*-[1-(3-Amidinobutylpropylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (33):** 57% yield.

***N*-[1-(4-Amidinobutylbutylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (34):** 63% yield.

***N*-[1-(5-Amidinobutylpentylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (35):** 69% yield.

***N*-[1-(6-Amidinobutylhexylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (36):** 72% yield.

***N*-{[1-(3-Guanidinopropylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (17).** To a solution of compound **1** (25 mg, 0.0295 mmol) in EtOH (1 mL) was added 3,5-dimethylpyrazole-1-carboxamidine nitrate (7.17 mg, 0.0354 mmol) and the pH was adjusted to 10 by adding DIEA. The reaction mixture was stirred for 48 h at 60 °C, under N_2 atmosphere, and then evaporated to dryness. Purification by flash column chromatography (silica gel, eluted with $CHCl_3$/MeOH/NH_4OH, 9:1:0.1), followed by trituration with diethyl ether, gave 15.8 mg of the pure desired compound **17** as a white solid with a 64% yield.

Compounds **18**−**20** were prepared from compounds **2**−**4**, respectively, following the same methodology as that described for compound **17**.

***N*-{[1-(4-Guanidinobutylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),-11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (18):** 67% yield.

***N*-{[1-(5-Guanidinopentylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (19):** 58% yield.

***N*-{[1-(6-Guanidinohexylcarbamoyl)-3-methyl-1(*R*)-butylcarbamoyl]methyl}-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),-11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)methyl]cyclopropanecarboxamide (20):** 60% yield.

Compounds **37**−**40** were prepared from compounds **21**−**24**, respectively, following the same methodology as that described for compound **17**.

***N*-[1-(3-Guanidinopropylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (37):** 60% yield.

***N*-[1-(4-Guanidinobutylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (38):** 50% yield.

***N*-[1-(5-Guanidinopentylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (39):** 52% yield.

***N*-[1-(6-Guanidinohexylcarbamoyl)-3-methyl-1(*R*)-butyl]-1(*S*)-phenyl-2(*R*)-[(8-hydroxy-6(*R*),11(*R*)-dimethyl-1,2,5,6-tetrahydro-4*H*-2(*R*),6-methanobenzazocin-3-yl)-methyl]cyclopropanecarboxamide (40):** 51% yield.

Radioligand Binding Assays. Binding to κ, μ, and δ Opioid Receptors. Male Hartley guinea pigs and Sprague−Dawley rats were purchased from Charles River (Como, Italy). Guinea pig cerebellum or rat brain membrane fractions were prepared following a procedure reported by Bowen[41] and the protein content was evaluated.[42] For radioligand binding assays on κ receptors, aliquots of homogenate obtained from guinea pig cerebella (1 mg protein/tube) were incubated in 50 mM Tris·HCl (pH 7.4) containing 0.5 μg/mL aprotinin, 10 μg/mL leupeptin, 200 μg/mL bacitracin at 25 °C for 60 min with 2 nM [^{3}H]U69,593 (Amersham; 60 Ci/mmol, $K_d = 1.98 \pm 0.04$ nM, $n = 3$). The concentration of the tested compounds ranged from 10^{-11} to 10^{-5} M. Binding studies were performed in the presence of [D-Ala^2,N-Me-Phe^4,Gly^5-ol]-enkephalin (DAMGO; 100 nM) and [D-Ala^2,D-Ala^5]-enkephalin (DADLE; 100 nM) to eliminate the interaction with μ and δ receptors, respectively. Nonspecific binding was determined by addition of U50,488 (Upjohn; 10 μM).

Binding to μ and δ sites was carried out on crude membrane fractions obtained from the whole rat brain minus the cerebellum as previously reported.[18] For radioligand binding assays to μ receptors, aliquots of homogenate (1 mg protein/tube) were incubated in 50 mM Tris·HCl (pH 7.4) containing 0.5 μg/mL aprotinin, 10 μg/mL leupeptin, 200 μg/mL bacitracin at 25 °C for 60 min with 1 nM [^{3}H]diprenorphine (Amersham; 30 Ci/mmol, $K_d = 0.22 \pm 0.03$ nM, $n = 5$). The concentration of the tested compounds ranged from 10^{-11} to 10^{-5} M. Binding studies were performed in the presence of U50,488 (300 nM) and DADLE (300 nM) which were added to saturate the κ and δ receptors, respectively. Nonspecific binding was determined by the addition of DAMGO (1 μM). For radioligand binding assays to δ receptors, aliquots of homogenate (1 mg protein/

tube) were incubated in the same conditions with 1 nM [^{3}H]-diprenorphine ($K_d = 0.44 \pm 0.03$ nM, $n = 5$). The concentration of the tested compounds ranged from 10^{-11} to 10^{-5} M. Binding studies were performed in the presence of U50,488 (300 nM) and DAMGO (300 nM) which were added to saturate the κ and μ receptors, respectively. Nonspecific binding was determined by the addition of DPDPE (1 μM).

The incubation was interrupted by rapid filtration through Whatman GF/B glass filters which were presoaked in a 0.1% poly(ethylenimine) solution for 1 h. Filters were washed twice with 4 mL of ice-cold 50 mM Tris·HCl solution.

Binding to σ_1 Sites. σ_1 binding assays were carried out on guinea pig brain membranes prepared according to a method reported by Matsumoto et al.[43] Binding assays were performed as described by DeHaven et al.[44] Briefly, each tube contained 500 μg of membrane protein, 3 nM [^{3}H]pentazocine (NEN Life Science; 31.6 Ci/mmol, $K_d = 4.3 \pm 0.8$ nM, $n = 3$). Nonspecific binding was determined by the addition of haloperidol (10 μM). The reaction was performed for 150 min at 37 °C and terminated by filtration through Whatman GF/B glass filters which were presoaked in a 0.5% poly(ethylenimine) solution.

Radioactivity on the filters was measured by a liquid scintillation cocktail. Scatchard parameters and inhibition constants were calculated using the EBDA-LIGAND program,[45] purchased from Elsevier/Biosoft.

Antinociception. Male albino Swiss mice (20−25 g; Charles River, Calco, Italy) were used. Test compounds were dissolved in the vehicle (saline containing 0.5% carboxymethylcellulose, w/v) and administered subcutaneously (sc) in a dose-volume of 10 mL/kg (0.1−10 mg/kg) 30 min before testing. The opioid antagonist nor-binaltorphimine (nor-BNI; RBI, Natik, MA) was administered sc 10 min before the test compound at a 10 mg/kg dosage. Antinociception was measured by the acetylcholine-induced abdominal constriction test.[19,46] Mice were injected intraperitoneally with 0.25 mL of a solution of acetylcholine iodine (0.75 mg/mL) and were then observed for 5 min during which the number of abdominal constrictions was counted. The ED_{50} value was defined as the dosage that reduced the mean number of constrictions to 50% of the vehicle-treated mice.

Behavioral Studies. Male albino Swiss mice (20−25 g) were placed individually into clear acrylic cages and left for 1 h to acclimatize to the new environment. Test compounds were dissolved in the vehicle and administered sc 30 min later. After 10 min, behavioral scores were recorded in 5-min intervals for 40 min. The scoring scale is modeled after by Tanaka et al;[37] 10 mice/group were used. The total score of the vehicle-treated group was defined as 100% and ED_{50} values for treated mice were determined using log-probit conversion of data.

Rotarod Test. The test compounds were administered (as previously described), and after 30 min, male albino Swiss mice were placed individually on an accelerated rotarod (Ugo Basile, Milan, Italy) with three opportunities to maintain balance on the bar. Mice that failed to maintain balance in at least one of three separate trials were considered impaired. The ED_{50} value was defined as the dosage that halved the latency of the vehicle-treated mice for falling off the rotarod.

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Supporting Information Available: Elemental analyses of compounds **1**−**4**, **15**, **16**, **21**−**24**, and **41**−**48**. This material is available free of charge via the Internet at http://pubs.acs.org.

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