Probes for Narcotic Receptor-Mediated Phenomena. 25.[1] Synthesis and Evaluation of *N*-Alkyl-Substituted (α-Piperazinylbenzyl)benzamides as Novel, Highly Selective δ Opioid Receptor Agonists

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A series of *N*-alkyl- and *N*,*N*-dialkyl-4-[α-{(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl}benzyl]-benzamides were synthesized and evaluated for binding affinities at μ, δ, and κ opioid receptor subtypes. Several compounds (**2e**,**f**,**h**,**i**,**m**) strongly bound to the δ receptor with IC_{50} values in the nanomolar range. On the other hand, the binding affinities of these compounds for the μ and κ receptors were in the micromolar or greater range indicating excellent δ opioid receptor subtype selectivities. In this series, two important structure−activity relationships were found for the δ receptor binding affinity. First, the spatial orientation of the α-benzylic position influenced the affinities with the α*R* derivatives **2a**−**n** generally showing more than 10-fold greater affinity than the α*S* derivatives **3a**−**n**. Second, the binding affinities were strongly influenced by the number of alkyl substituents on the amide nitrogen. *N*-Monoalkylbenzamide derivatives **2b**−**d** showed lower affinity than *N*,*N*-dialkylbenzamide derivatives **2e**−**n**, and the *N*-unsubstituted benzamide derivative **2a** had the lowest affinity for the δ receptor in the series. The dramatic effect of the amide group substitution pattern on the binding affinity for the δ receptor strongly suggests that the amide function is an important structural element in the interaction of this series of compounds at the δ receptor. Selective compounds in this series were examined for binding affinity in cloned human μ and δ receptors. The results obtained generally paralleled those from the rat brain binding assay. Compounds **2e**,**f** with potent δ binding affinities and high δ selectivities were shown to be δ agonists with high selectivity by studies in the guinea pig ileum (GPI) and mouse vas deferens (MVD) preparations. Compound **2f** was the most selective compound in the rat brain and GPI/MVD assays with 1755- and 958-fold δ *vs* μ selectivity, respectively.

Since the initial identification of opioid receptors[2−4] in the central nervous system and the subsequent discovery of mu, delta, and kappa (μ, δ, and κ) opioid receptor subtypes, extensive studies of each receptor subtype have revealed new physiological functions during the last 2 decades.[1,5−9] These studies have shown that δ receptor agonists produce analgesia in animal models and enhance the potency and efficacy of μ agonist analgesics, such as morphine.[10−13] Therefore, δ agonists may be useful as a novel class of analgesics. Recent studies on the role of the δ receptor have also revealed that δ agonists function as immunomodulators in the central nervous system and also through δ-like binding sites on the surface of immune cells.[14−20]

As in many other areas, advances in understanding the structure and function of the δ receptor have been largely dependent on the discovery of novel agents with the appropriate receptor subtype selectivity. A number of novel peptide and nonpeptide agonists and antagonists have been introduced in these studies including the racemic nonpeptide agonist BW373U86 (**1**; Chart 1) from the Burroughs Wellcome Laboratories.[21−25] Although these drugs have proven useful for the investigation of δ receptor-mediated pharmacological actions, optimum progress requires the development of agents which avoid the metabolic instability and low systemic availability associated with peptides and show exquisite receptor selectivity.

As a part of our continuing program aimed at obtaining novel probes for opioid receptors, we have recently found a highly selective and potent δ agonist, (+)-4-[(α*R*)-α-{(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl}-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (**2n**, SNC80),[26−28] related to **1**. In the preceding studies of the structure−activity relationships on **2n**, we have investigated the role of the piperazine nucleus[26] and the effect of several substituents on the aromatic site.[27] This paper describes our studies on the importance of the amide function group of **2n**. We initially examined the effect of placement of the *N*,*N*-diethylamide function at the meta and ortho positions verses the para position as in **2n**. Contrary to good δ receptor binding affinities of

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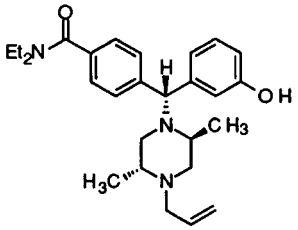
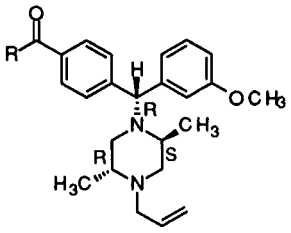
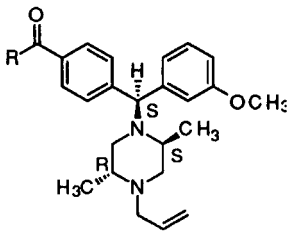
Chart 1

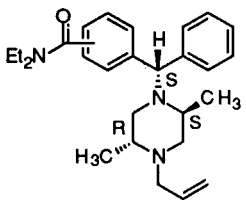


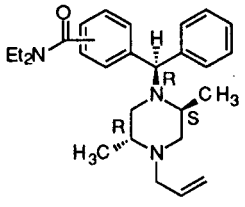
(±)-1 (BW373U86)





2 a.	R = H_2N	**3 a.**	R = H_2N
2 b.	R = EtNH	**3 b.**	R = EtNH
2 c.	R = Et_2CHNH	**3 c.**	R = Et_2CHNH
2 d.	R = t-BuNH	**3 d.**	R = t-BuNH
2 e.	R = Me_2N	**3 e.**	R = Me_2N
2 f.	R = Et(Me)N	**3 f.**	R = Et(Me)N
2 g.	R = n-Pr_2N	**3 g.**	R = n-Pr_2N
2 h.	R = i-Pr_2N	**3 h.**	R = i-Pr_2N
2 i.	R = Et(n-Bu)N	**3 i.**	R = Et(n-Bu)N
2 j.	R = n-Bu_2N	**3 j.**	R = n-Bu_2N
2 k.	R = 	**3 k.**	R = 
2 l.	R = 	**3 l.**	R = 
2 m.	R = 	**3 m.**	R = 
2 n.	R = Et_2N, (SNC80)	**3 n.**	R = Et_2N





4 a.	p-	**5 a.**	p-
4 b.	m-	**5 b.**	m-
4 c.	o-	**5 c.**	o-

p-(α-piperazinylbenzyl)benzamide derivatives, such as **2n** and its desmethoxy analog **4a**,[27] the meta and ortho position isomers **4b**,**c** did not show significant δ receptor binding. Therefore, in our search for potent and selective δ ligands, the position of the amide group was retained at the para position. The synthesis and the structure−activity relationships of a new series of (α-piperazinylbenzyl)benzamides are reported herein.

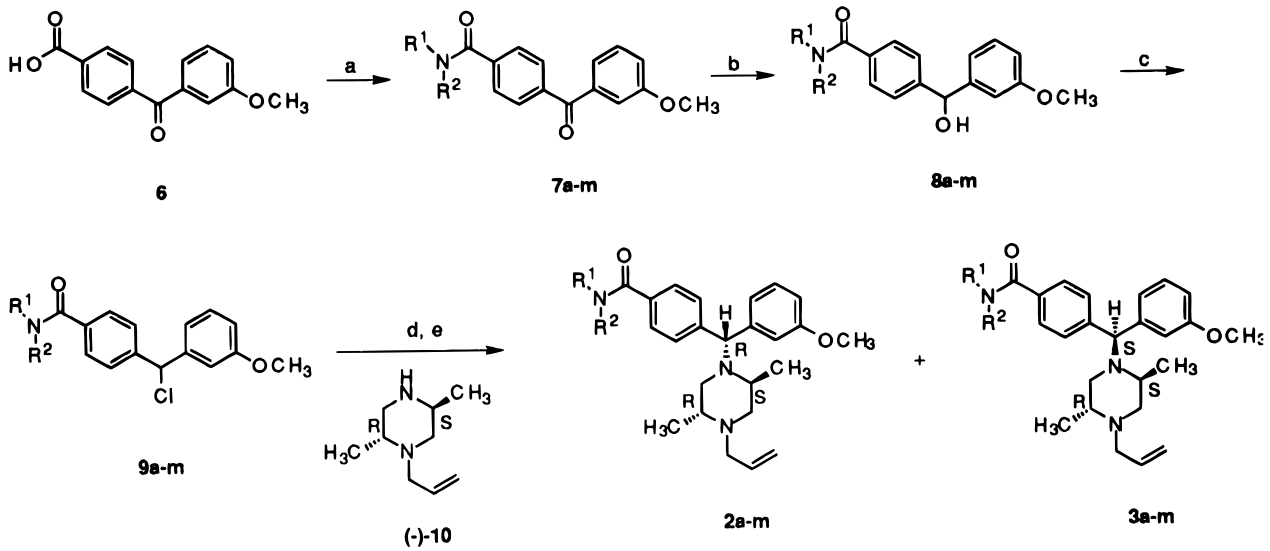
Chemistry

The synthetic pathways to the target compounds are outlined in Schemes 1−4 and paralleled our earlier route.[26,27] Condensation of 4-(3-methoxybenzoyl)benzoic acid (**6**)[27] with the appropriate amine in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBT) yielded the corresponding amido derivatives **7a**−**m**. After reduction with sodium borohydride, the solutions of the resulting alcohols **8a**−**m** in chloroform were treated with concentrated hydrochloric acid to generate the corresponding chlorides **9a**−**m**. The chlorides were reacted with optically pure (−)-*2R,5S*-**10**[27] in the presence of potassium carbonate and potassium iodide in acetonitrile to give a mixture of diastereomeric (diphenylmethyl)-piperazines, which were separated by flash column chromatography to afford the desired compounds **2** and **3** as shown in Scheme 1.

In previous studies, the absolute configurations of **2n**, its benzylic epimer **3n**, and a number of closely related pairs epimeric at the α-benzylhydryl position differing only in replacement of the methoxy group with other substituents were assigned based on X-ray crystallographic analysis.[26,27] Invariably, the α*R* epimer showed a substantially larger R_f value than the α*S* epimer on silica gel thin layer chromatography (TLC). In the present study, we applied an empirical correlation between the diastereomeric structures (**2a**−**n** and **3a**−**n**) and the aromatic NMR pattern in methanol-d_4 for the assignment of the benzylic configuration. Similar method was applied by Boswell *et al.*[29] to determine the epimeric structures of the heterocyclic analogs of **1**. Resonances for the protons of the methoxy-substituted aromatic ring were observed to have different characteristic patterns for the pairs of studied epimers. For **2n** with the α*R* configuration determined by X-ray crystallographic analysis,[26] resonances of three of the methoxy aromatic protons were overlapping in the range of 6.7−6.9 ppm, and the resonance of the fourth proton appeared downfield and was partially overlapped with those on the *N*,*N*-diethylamide-substituted aromatic ring. For the benzylic epimer of **2n**, analog **3n** with the α*S* configuration, resonances of the four methoxy aromatic protons were distinct and evenly spread from 6.7 to 7.2 ppm, appearing consecutively as doublet of doublet, doublet, singlet, and triplet peaks. On the other hand, the four protons on the *N*,*N*-disubstituted aromatic ring appeared as two sets of doublets as expected. Spectra for the epimeric pairs of the *p*-(α-piperazinylbenzyl)benzamide derivatives **2a**−**m** and **3a**−**m** conformed nicely to the foregoing pattern, permitting assignment of the relative stereochemistry. The absolute configuration of **2a**−**m** and **3a**−**m** then followed from the assignment of the benzylic configuration and the known absolute stereochemistry of (−)-(2*R*,5*S*)-**10**.[26]

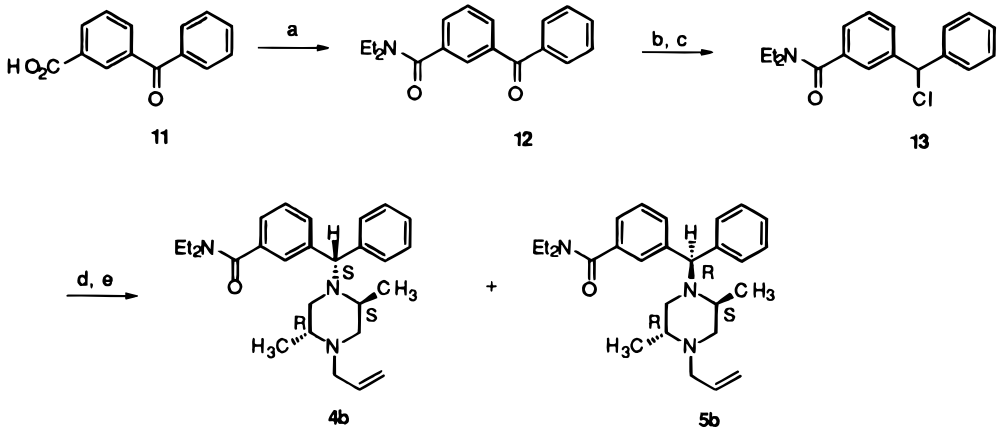
The 3-benzylbenzamide analogs **4b** and **5b** were synthesized in a straightforward manner similar to that of 4-benzylbenzamide derivatives **2a**−**m** and **3a**−**m** (Scheme 2). However, preparation of the 2-benzylbenzamide derivatives **4c** and **5c** proved problematic (Scheme 3). Treatment of **16** with HCl produced 3-phe-

Scheme 1[a]



[a] Reagents: (a) R^1R^2NH/EDCl−HOBT/DMF; (b) $NaBH_4$/MeOH; (c) concd HCl/$CHCl_3$; (d) K_2CO_3−KI/MeCN; (e) flash chromatography.

Scheme 2[a]



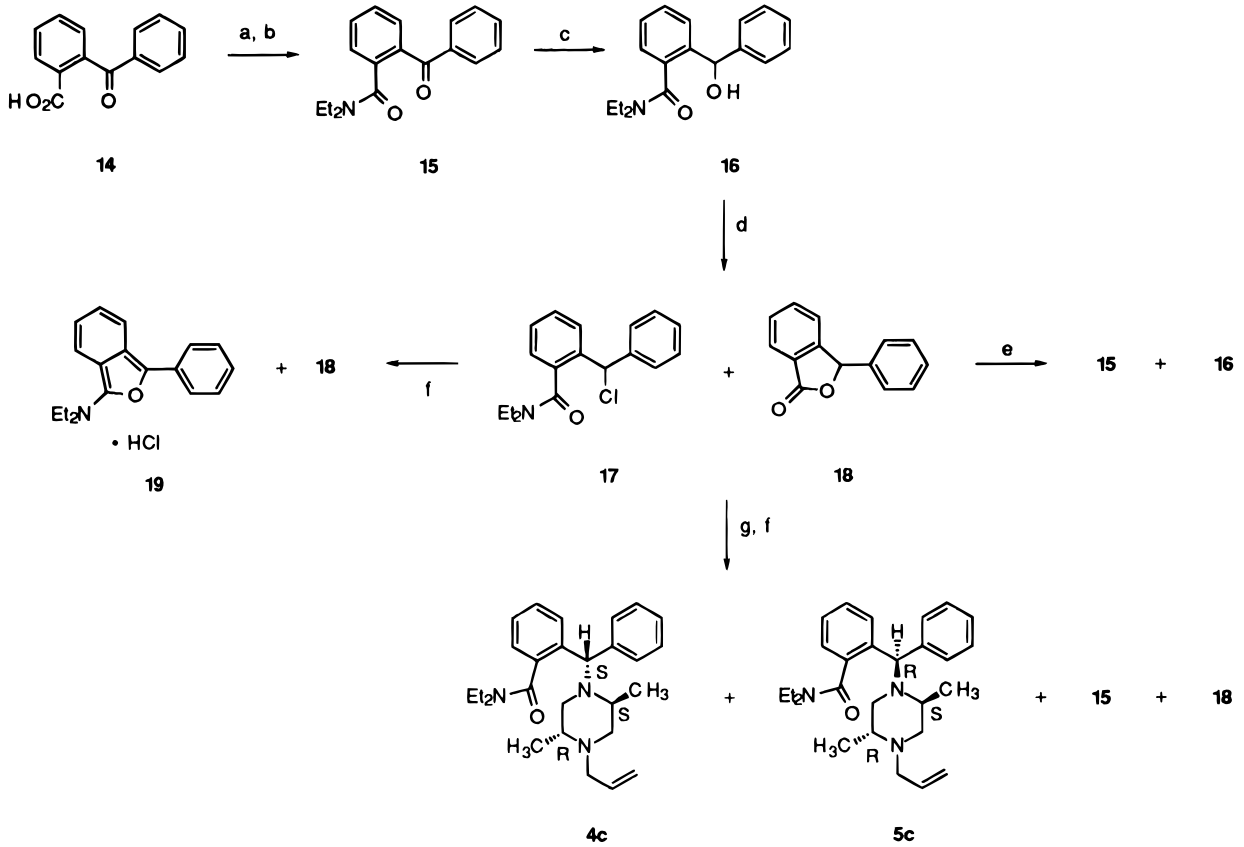
[a] Reagents: (a) Et_2NH/EDCl−HOBT/DMF; (b) $NaBH_4$/MeOH; (c) concd HCl/$CHCl_3$; (d) (−)-**10**/K_2CO_3−KI/MeCN; (e) flash chromatography.

nylphthalide (**18**) along with the desired product 2-(α-chlorobenzyl)-*N*,*N*-diethylbenzamide (**17**) in a ratio of 4:6 by NMR. Purification of **17** by recrystallization was not successful due to its instability. Attempts to purify **17** by flash chromatography led to the formation of 1-(diethylamino)-3-phenylisobenzofuran hydrochloride (**19**) in a yield of 81% from **17**. Reaction of the crude mixture of **17** and **18** with **10** according to the protocol described above generated only degradation compounds **15** (in a yield of 35% from **17**) and **16** (in a yield of 53% from **17**). Alternatively, neat reaction of the mixture of **17** and **18** with **10** at 40 °C for 3 days provided the desired compounds **4c** and **5c** in a low yield (12%) from **16**.

We next investigated an alternative pathway to obtain **4c** and **5c** as shown in Scheme 4. According to the method described in Scheme 1, 2-bromobenzophenone (**20**) was converted to 2-(α-piperazinylbenzyl)-bromobenzene (**22**) which was obtained as a mixture of benzylic epimers. Treatment of the mixture with *n*-butyllithium followed by carbon dioxide yielded the corresponding appropriate benzoic acid derivatives which were separated by flash chromatography to give optically pure **23** and **24**. The relative configuration of **24** was determined by X-ray crystallographic analysis. Amidation of **23** and **24**, respectively, with diethylamine afforded the desired compounds **4c** and **5c**.

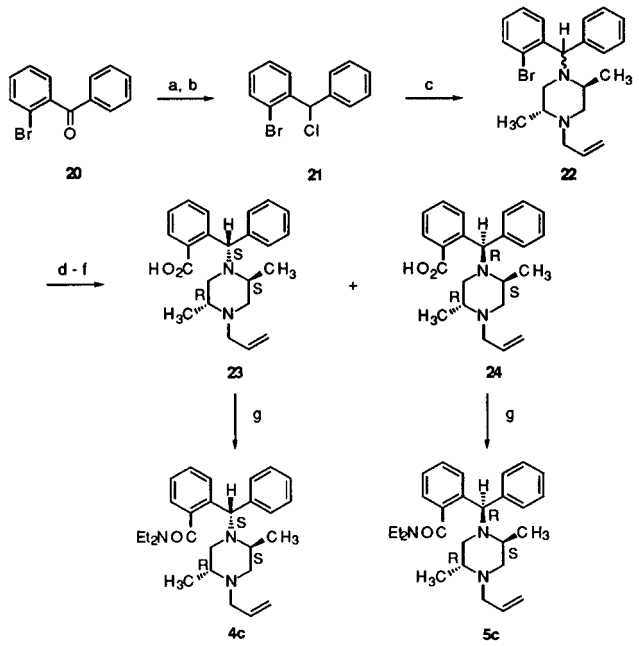
The relative stereochemistries of *o*-(α-piperazinylbenzyl)benzamide derivatives **4c** and **5c** were assigned based on the X-ray crystallographic analysis of **24** (Figure 1) which allowed the assignment of the stereochemistry of **23**. The absolute stereochemistry of **4c**, **5c**, **23**, and **24** then followed from the previously determined absolute stereochemistry of (−)-2*R*,5*S*-**10**. The configuration of the para diastereomeric pair, **4a** and **5a**, was unequivocally assigned in the early studies.[26] However, comparison of the NMR spectra of meta epimers **4b** and **5b** with those of the para and ortho epimers (**4a** and **5a**, **4c** and **5c**) gave ambiguous results. The benzylic configuration of **4b** and **5b** was assigned based on the relative R_f values on TLC. In this and the previous study,[27] the epimer with the benzylic spatial arrangement as in **2** and **4** invariably showed the higher R_f values on TLC. Thus, the higher running meta epimer was assigned as (α*S*)-**4b** and the lower running epimer as (α*R*)-**5c**. It should be noted that the spatial orientation at the α position of **2a**−**n** is the same as in **4a**−**c** and that of **3a**−**n** is the same as in **5a**−**c**. However, due to the priority of the groups defined by Cahn, Ingold, and Prelog nomenclature,[30] the α position

Scheme 3[a]



[a] Reagents: (a) $SOCl_2$; (b) Et_2NH/THF; (c) $NaBH_4$/MeOH; (d) concd HCl/$CHCl_3$; (e) (−)-**10**/K_2CO_3−KI/MeCN; (f) flash chromatography; (g) (−)-**10** (neat).

Scheme 4[a]



[a] Reagents: (a) $NaBH_4$; (b) concd HCl/$CHCl_3$; (c) (−)-**10**/K_2CO_3−KI/MeCN; (d) *n*-BuLi; (e) CO_2; (f) flash chromatography; (g) Et_2NH/EDCl−HOBT.

of **2a**−**n** is arbitrarily defined as α*R* and the α position of **4a**−**c** as α*S*. Similarly, **3a**−**n** are designated as α*S* and **5a**−**c** as α*R*.

The physical data of the newly synthesized (diphenylmethyl)piperazines are summarized in Table 1.

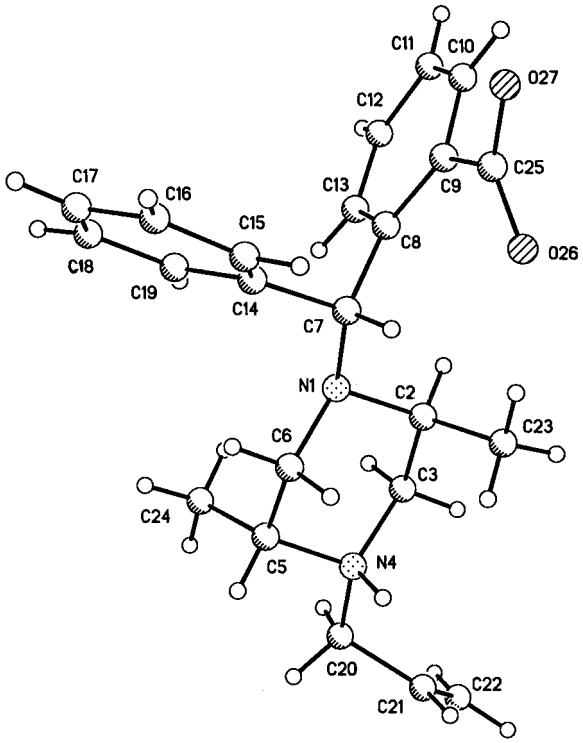
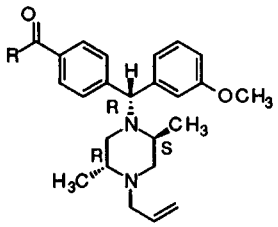


Figure 1. X-ray crystallographic structure of compound **24**. The figure is drawn using the experimentally determined coordinates with arbitrary thermal parameters.

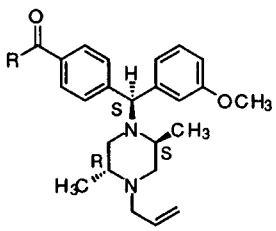
Results and Discussion

The binding affinities of the new analogs of **2n** for μ and δ receptors were determined by inhibition of binding

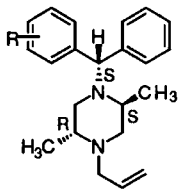
Table 1. Physical Data of the Newly Synthesized (α-Piperazinylbenzyl)benzamide Derivatives



2a-m



3a-m



4b-c

5b-c

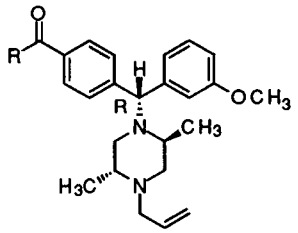
compd.	R	benzylic config.	yield (%)	mp (°C)	recrystn solvent	$[\alpha]^{20}_{D}$ [c] (deg)	c	salt formula[e]
2a	H_2N	R	26	171-172	EtOH	+19.0[d]	(1.0)	$C_{24}H_{31}N_3O_2$ ·2HCl·0.75H_2O
3a	H_2N	S	26	160-162	Me_2CO	+20.6[d]	(1.0)	$C_{24}H_{31}N_3O_2$·2HCl
2b	EtNH	R	26	175-176	Et_2O-Me_2CO	+23.7	(1.0)	$C_{26}H_{35}N_3O_2$ ·2HCl·0.5H_2O
3b	EtNH	S	27	153-155	Et_2O-Me_2CO	+27.4	(1.0)	$C_{26}H_{35}N_3O_2$·2HCl
2c	Et_2CHNH	R	17	142-144	Et_2O-Me_2CO	+22.5	(1.0)	$C_{29}H_{41}N_3O_2$ ·2HCl·0.5H_2O
3c	Et_2CHNH	S	26	148-150	Et_2O-Me_2CO	+24.1	(1.0)	$C_{29}H_{41}N_3O_2$·2HCl
2d	t-BuNH	R	22	145-147	Et_2O-Me_2CO	+18.7	(1.1)	$C_{28}H_{39}N_3O_2$·2HCl
3d	t-BuNH	S	25	148-150	Et_2O-Me_2CO	+24.3	(1.0)	$C_{28}H_{39}N_3O_2$·2HCl
2e	Me_2N	R	28	166-168	Me_2CO	+25.3	(1.1)	$C_{26}H_{35}N_3O_2$ ·2HCl·1.5H_2O
3e	Me_2N	S	23	147-149	Et_2O-Me_2CO	+25.1	(1.3)	$C_{26}H_{35}N_3O_2$·2HCl
2f	Et(Me)N	R	26	161-162	Me_2CO	+21.9	(1.0)	$C_{27}H_{37}N_3O_2$ ·2HCl·1.5H_2O
3f	Et(Me)N	S	32	144-146	Et_2O-Me_2CO	+27.1	(1.0)	$C_{27}H_{37}N_3O_2$·2HCl
2g	n-Pr_2N	R	35	softens 79[a]	b	+20.9	(1.0)	$C_{30}H_{43}N_3O_2$ ·HCl·H_2O
3g	n-Pr_2N	S	29	softens 79[a]	b	+25.0	(0.7)	$C_{30}H_{43}N_3O_2$ ·HCl·1.2H_2O
2h	i-Pr_2N	R	27	175-176	Me_2CO	+20.6	(1.1)	$C_{30}H_{43}N_3O_2$ ·2HCl·0.5H_2O
3h	i-Pr_2N	S	22	169-170	Me_2CO	+24.0	(0.5)	$C_{30}H_{43}N_3O_2$·2HCl
2i	Et(n-Bu)N	R	33	softens 79[a]	b	+17.9	(0.4)	$C_{30}H_{43}N_3O_2$ ·HCl·1.2H_2O
3i	Et(n-Bu)N	S	29	softens 79[a]	b	+23.9	(0.6)	$C_{30}H_{43}N_3O_2$ ·HCl·H_2O
2j	n-Bu_2N	R	33	softens 71[a]	b	+17.2	(1.0)	$C_{32}H_{47}N_3O_2$ ·$C_2H_2O_4$·0.5H_2O
3j	n-Bu_2N	S	28	softens 71[a]	b	+27.4	(1.0)	$C_{32}H_{47}N_3O_2$ ·$C_2H_2O_4$

Table 1 (Continued)

compd.	R	benzylic config.	yield (%)	mp (°C)	recrystn solvent	$[\alpha]^{20}_D$ [c] (deg)	c	salt formula[e]
2k		R	26	softens 69[a]	b	+24.0	(1.0)	$C_{28}H_{37}N_3O_2$ ·1.75$C_2H_2O_4$
3k		S	21	softens 70[a]	b	+26.3	(1.0)	$C_{28}H_{37}N_3O_2$ ·1.75$C_2H_2O_4$
2l		R	41	softens 70[a]	b	+22.1	(1.0)	$C_{29}H_{39}N_3O_2$ ·1.5$C_2H_2O_4$
3l		S	38	softens 70[a]	b	+25.4	(1.0)	$C_{29}H_{39}N_3O_2$ ·1.5$C_2H_2O_4$
2m		R	35	147-150	Et_2O-Me_2CO	+13.2[d]	(0.8)	$C_{30}H_{41}N_3O_2$ ·2HCl
3m		S	30	190-192	Me_2CO	+18.0[d]	(1.7)	$C_{30}H_{41}N_3O_2$ ·2HCl
4b	m-Et_2NCO	S	31	142-145	Et_2O-Me_2CO	+19.3	(1.0)	$C_{27}H_{37}N_3O$·2HCl
5b	m-Et_2NCO	R	29	166-168	Et_2O-Me_2CO	+19.0	(1.0)	$C_{27}H_{37}N_3O$·2HCl
4c	o-Et_2NCO	S	65	176-177	Me_2CO	+19.5	(0.8)	$C_{27}H_{37}N_3O$ ·2HCl·1/3H_2O
5c	o-Et_2NCO	R	59	177-178	Me_2CO	-38.3	(0.7)	$C_{27}H_{37}N_3O$·2HCl

[a] Amorphous powder. [b] Purified by precipitation with Et_2O from a solution of Me_2CO. [c] $[\alpha]^{20}_D$ values were determined for the free base in MeOH. [d] $[\alpha]^{20}_D$ values were determined for the free base in MeOH/$CHCl_3$. [e] Analyses for C, H, and N were within ±0.4% of the theoretical values.

Table 2. Binding Affinities of *p*-(α-Piperazinylbenzyl)benzamide Derivatives **2a**−**n** for *μ*, *δ*, and *κ* Receptors

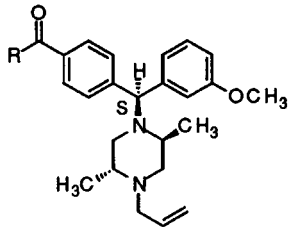


2a-n

compd.	R	IC_{50}(nM±SEM) μ binding[b]	δ binding[c]	κ binding[d]	μ/δ ratio	κ/δ ratio
2a	H_2N	>2500	451±44	>2500	>5.5	>5.5
2b	EtNH	242±38	150±23	345±52	1.6	2.3
2c	Et_2CHNH	>2500	176±23	>2500	>14	>14
2d	t-BuNH	>2500	281±30	>2500	>8.9	>8.9
2e	Me_2N	3261±719	4.8±0.69	>10000	679	>2083
2f	Et(Me)N	7373±558	4.2±0.47	>10000	1755	>2380
2g	n-Pr_2N	3992±313	13.1±2.1	>10000	305	>763
2h	i-Pr_2N	>2500	7.1±0.70	>2500	>352	>352
2i	Et(n-Bu)N	1889±141	6.6±0.73	>10000	286	>1515
2j	n-Bu_2N	>2500	34.5±3.6	>2500	>71	>71
2k		>2500	18.1±2.5	>10000	>138	>552
2l		>2500	14.6±2.1	>10000	>171	>684
2m		>2500	8.2±0.91	>10000	>305	>1219
2n[a]	Et_2N	2467±200	2.9±0.35	7070±3682	857	2438

[a] Reference 25. [b] Inhibitory effect to [^{3}H]DAMGO in rat brain membranes. [c] Inhibitory effect to [^{3}H]DADAL in rat brain membranes. [d] Inhibitory effect to [^{3}H]U69,593 in guinea pig brain membranes.

Table 3. Binding Affinities of *p*-(α-Piperazinylbenzyl)benzamide Derivatives **3a**−**n** for μ, δ, and κ Receptors



3a-n

compd.	R	IC_{50}(nM±SEM) μ binding[b]	δ binding[c]	κ binding[d]	μ/δ ratio	κ/δ ratio
3a	H_2N	>2500	389±140	>2500	>6.4	>6.4
3b	EtNH	169±34	34.4±5.0	1634±129	4.9	48
3c	Et_2CHNH	NT[e]	NT	NT	-	-
3d	t-BuNH	NT	NT	NT	-	-
3e	Me_2N	>2500	234±24	>2500	>11	>11
3f	Et(Me)N	>2500	60.6±9.7	>2500	>41	>41
3g	n-Pr_2N	4342±949	153±18.5	>10000	28	>65
3h	i-Pr_2N	>2500	344±70	>2500	>7.3	>7.3
3i	Et(n-Bu)N	5879±518	151±28.9	>10000	39	>66
3j	n-Bu_2N	>2500	129±10	>2500	>5.5	>5.5
3k		>2500	265±58	>10000	>9.4	>38
3l		>2500	450±117	>10000	>5.5	>22
3m		>2500	124±25	>2500	>20	>81
3n[a]	Et_2N	5712±457	63.3±13.2	NT	90	-

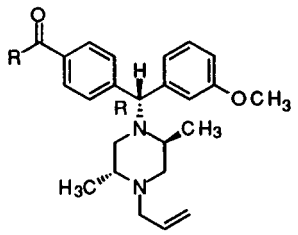
[a] Reference 25. [b] Inhibitory effect to [^{3}H]DAMGO in rat brain membranes. [c] Inhibitory effect to [^{3}H]DADAL in rat brain membranes. [d] Inhibitory effect to [^{3}H]U69,593 in guinea pig brain membranes. [e] Not tested.

of [^{3}H]DAMGO (Tyr-D-Ala-Gly-(Me)-Phe-Gly-ol)[31] and [^{3}H]DADLE (Tyr-D-Ala-Gly-Phe-D-Leu)[32] at rat brain membranes. The affinity of these derivatives for κ receptors was determined by inhibition of binding of [^{3}H]-U69,593[33] at guinea pig brain membranes. The IC_{50} values and the selectivities for the δ receptor are listed in Tables 2 and 3. Compounds with an α*R* configuration at the benzylic positions (Table 2) showed higher affinity for the δ receptor than their epimers (Table 3). The δ binding affinities of the new derivatives were dramatically influenced by the type of substitution of the amide group. Compound **2b**, the monoethyl analog of **2n**, showed a remarkable loss of affinity for the δ receptor. The unsubstituted amide **2a** exhibited even lower affinity than **2b**. The relative affinities of **2a**,**b** were approximately 1/150 and 1/50 that of **2n**, respectively. Replacement of ethyl group in **2b** with a bulky monoalkyl group (**2c**,**d**) did not change the affinity for the δ receptor suggesting that the loss of activity in **2b** was not simply due to the lack of lipophilicity or steric hindrance on the amide function. The observed structure−activity relationships indicate that the two-alkyl substituent on the amide group was critical for the high δ binding activity. Among the series of *N*,*N*-disubstituted benzamide derivatives, compounds with small alkyl groups (**2e**,**f**) showed higher affinities in the δ binding assays. Increasing the steric bulkiness of the alkyl substituent tended to reduce the affinities at the δ receptor. Cyclization of the alkyl substituent also resulted in reduced affinity at the δ receptor as in **2k** *vs* **2n**. The opioid receptor subtype selectivity in this series was also dependent on the amide substitution as shown in Table 2. The *N*-monoalkyl derivatives **2b**−**d** showed poor subtype selectivities, while the *N*,*N*-dialkyl derivatives showed good to excellent μ/δ and κ/δ ratios. Compound **2f** was the most δ selective binding ligand in this series with a ratio of 1755 for μ/δ receptors and >2380 for κ/δ receptors.

With regard to the δ binding of the amide positional isomers, both 2-benzylbenzamide (**4c**, $IC_{50} = 2348 \pm 771$ nM) and 3-benzylbenzamide **4b**, $IC_{50} = 83.4 \pm 11.9$ nM) derivatives showed 2500- and 90-fold lower affinity than the corresponding 4-benzylbenzamide derivative **4a** ($IC_{50} = 0.94 \pm 0.09$ nM).[27]

N,*N*-Disubstituted benzamide derivatives with an α*R* configuration at the benzylic positions (**2e**−**i**,**k**−**n**) were further studied at cloned human μ and δ opioid receptors. Results from the radioligand binding inhibition studies for the human μ and δ opioid receptor preparations are presented in Table 4. All of the compounds examined showed poor binding affinities at the human μ receptor and good to excellent binding affinities at the

Table 4. Binding Affinities of Selected Compounds for Cloned Human μ and δ Receptors



2

compd.	R	IC_{50}(nM±SEM) μ binding[a]	IC_{50}(nM±SEM) δ binding[b]	μ/δ ratio
2e	Me_2N	9,724 ± 826	18 ± 1	540
2f	Et(Me)N	12,110 ± 1,470	8.0 ± 1	1,514
2g	n-Pr_2N	6,083 ± 1400	7.5 ± 0.2	811
2h	i-Pr_2N	6,837 ± 160	5.0 ± 1	1,367
2i	Et(n-Bu)N	3,340 ± 533	10.2 ± 2.2	327
2k		6,186 ± 1,669	26.6 ± 1.69	233
2l		5,999 ± 951	38 ± 8.4	158
2m		4,470 ± 306	13 ± 4	344
2n	Et_2N	4,785 ± 1,854	1.3 ± 0.5	3,681

[a] Inhibitory effect to [^{3}H]DAMGO in cloned human μ receptors. [b] Inhibitory effect to [^{3}H]-*p*-Cl-DPDPE in cloned human δ receptors.

human δ receptor, which was in agreement with the results seen in the rat brain membrane assay. The structure−activity relationships associated with the binding affinities of these compounds at the human δ receptor were also consistent with the studies in rat brain membranes. Compound **2n** showed the highest affinity and selectivity at the human δ receptor in this series. These observations further support the conclusion drawn by Knapp *et al.*[34] that existing opioid ligands do not distinguish human opioid receptors from those of other species.

The compounds **2e**,**f** which showed high affinity and selectivity at the δ receptor were evaluated for opioid activities in the mouse vas deferens (MVD) and guinea pig ileum (GPI) preparations.[35] Both compounds showed potent δ agonist activity in MVD assay with IC_{50} values of 8.92 nM (**2e**) and 4.45 nM (**2f**) and weak μ agonist activities in GPI assay with IC_{50} values of 7468 nM (**2e**) and 4267 nM (**2f**). The activity ratios of GPI/MVD for **2e**,**f** were 837 and 958, respectively. The agonistic activities of these compounds in the MVD assay were inhibited by 1 μM ICI 174,864, the δ antagonist.[36]

Conclusions

We have investigated a series of *N*-alkyl- and *N*,*N*-dialkyl-4-[α-{(2*S*,5*R*)-4-allyl-2,5-dimethyl-1-piperazinyl}-benzyl]benzamides as novel δ opioid receptor ligands. Although maximal δ binding affinity was observed for the lead compound of this series (**2n**), a more selective compound (**2f**) with nearly the same δ binding affinity was identified by studies in rat brain tissue. Compound **2f** also exhibited potent δ agonist activity with approximately 1000-fold δ/μ selectivity in the isolated tissue preparations.

In a preceding paper,[27] we reported that δ binding activities showed little change when the methoxy group in **2n** was removed or replaced by the other substituents. In contrast to this result, the alkyl substituent pattern on the amide group dramatically affected the δ binding affinity strongly suggesting that the amide function is an important structural element in the binding of the (diphenylmethyl)piperazine series of ligands to the δ receptor.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded in $CDCl_3$ (unless otherwise noted) with tetramethylsilane (TMS) as the internal standard on a Varian Gemini-300 spectrometer. Chemical ionization mass spectra (MS, CI-NH_3) were recorded on a Finnigan 4600 spectrometer. Polarimetric measurements were taken using a Perkin-Elmer 241 MC polarimeter. Thin layer chromatography (TLC) was performed on Analtech silica gel GHLF 0.25 mm plates. Flash column chromatography was performed with Fluka silica gel 60 (mesh 220−240). Elemental analyses were performed by Atlantic Microlabs, Inc., Norcross, GA, and the results were within ±0.4% of the theoretical values. All extracted solutions were dried over magnesium sulfate and concentrated to dryness on a rotary evaporator under reduced pressure.

General Procedures for Preparation of Benzoylbenzamides. 4-(3-Methoxybenzoyl)-*N*-ethyl-*N*-methylbenzamide (7f). Method A. 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.74 g, 3.9 mmol) was added to a solution of 4-(3-methoxybenzoyl)benzoic acid (**6**)[27] (0.90 g, 3.5 mmol), ethylmethylamine (0.21 g, 3.5 mmol), and 1-hydroxybenzotriazole (0.52 g, 3.9 mmol) in *N*,*N*-dimethylformamide (DMF) (9 mL) at 0 °C with stirring. After being stirred for 2 h, the mixture was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed

three times with water, dried, and concentrated to give **7f** (1.0 g, 96%). This compound was used for the next reaction without further purification. A sample was obtained by recrystallization from Et_2O: mp 59−60 °C; ^{1}H NMR δ 1.15, 1.26 (two t, J = 7 Hz, 3H), 2.95, 3.11 (two s, 3H), 3.28, 3.62 (two q, J = 7 Hz, 2H), 3.87 (s, 3H), 7.15 (dd, J = 2, 8 Hz, 1H,), 7.34 (d, J = 8 Hz, 1H), 7.36 (d, J = 2 Hz, 1H), 7.40 (t, J = 8 Hz, 1H), 7.49 (d, J = 8 Hz, 2H), 7.84 (d, J = 8 Hz, 2H); MS (CI-NH_3) m/z 298 (MH^+).

Compounds **7c**,**d**,**g**,**i**−**m** and **12** were prepared according to method A and characterized by ^{1}H NMR.

4-(3-Methoxybenzoyl)-*N*,*N*-dimethylbenzamide (7e). Method B. Triethylamine (0.61 g, 6 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (0.63 g, 3.3 mmol) were successively added to a solution of **6** (0.77 g, 3 mmol), dimethylamine hydrochloride (0.24 g, 3 mmol), and 1-hydroxybenzotriazole (0.45 g, 3.3 mmol) in DMF (5 mL) at 0 °C with stirring. After being stirred for 2 h, the mixture was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed three times with water, dried, and concentrated to give **7e** (0.76 g, 89%). This compound was used for the next reaction without purification. A sample was obtained by recrystallization from EtOAc/hexane: mp 65−66 °C; ^{1}H NMR δ 3.00 (s, 3H), 3.15 (s, 3H), 3.87 (s, 3H), 7.15 (dd, J = 2.5, 8 Hz, 1H), 7.33 (d, J = 8 Hz, 1H), 7.34 (d, J = 2.5 Hz, 1H), 7.40 (t, J = 8 Hz, 3H), 7.53 (d, J = 8 Hz, 2H), 7.84 (d, J = 8 Hz, 2H); MS (CI-NH_3) m/z 284 (MH^+).

Compounds **7a**,**b** were prepared according to method B and characterized by ^{1}H NMR.

2-Benzoyl-*N*,*N*-diethylbenzamide (15). Method C. A solution of 2-benzoylbenzoic acid (**14**) (4.0 g, 18 mmol) in thionyl chloride (40 mL) was refluxed for 1 h. After concentration, the residue was dissolved in tetrahydrofuran (THF) (10 mL). The solution was added dropwise to a solution of diethylamine (5.5 mL, 57 mmol) in THF (20 mL) at 0 °C, and the mixture was stirred for a further 1 h. The resulting precipitate was removed by filtration, and the filtrate was concentrated. The residue was added to water and extracted with EtOAc. The extract was washed with water, dried, and concentrated to give **15** (4.5 g, 90%). This compound was used for the next reaction without purification. A sample was obtained by recrystallization from EtOAc/hexane: mp 49−50 °C; ^{1}H NMR δ 1.06 (t, J = 7 Hz, 3H), 1.11 (t, J = 7 Hz, 3H), 3.26 (q, J = 7 Hz, 2H), 3.42 (q, J = 7 Hz, 2H), 7.39−7.57 (m, 7H), 7.79 (d, J = 8 Hz, 2H); MS (CI-NH_3) m/z 282 (MH^+).

Compound **7h** was prepared according to method C and characterized by ^{1}H NMR.

General Procedure for Preparation of (α-Hydroxybenzyl)benzamides. 4-(3-Methoxy-α-hydroxybenzyl)-*N*-ethyl-*N*-methylbenzamide (8f). Method D. Sodium borohydride (110 mg, 2.9 mmol) was added portionwise to a solution of **7f** (860 mg, 2.9 mmol) in MeOH (10 mL) at room temperature with stirring. After being stirred for 1 h, the mixture was concentrated. The residue was added to water and extracted with EtOAc. The extract was washed with water, dried, and concentrated to give **8f** (850 mg, 98%) as an oil: ^{1}H NMR δ 1.17−1.23 (m, 3H), 2.38 (d, J = 3.5 Hz, 1H), 2.92, 3.05 (two s, 3H), 3.26−3.58 (m, 2H), 3.79 (s, 3H), 5.82 (d, J = 3.5 Hz, 1H), 6.82 (dd, J = 2.5, 8 Hz, 1H), 6.94 (d, J = 8 Hz, 1H), 6.95 (d, J = 2.5 Hz, 1H), 7.26 (t, J = 8 Hz, 1H), 7.35 (d, J = 8 Hz, 2H), 7.42 (d, J = 8 Hz, 2H); MS (CI-NH_3) m/z 300 (MH^+).

Compounds **8a**−**e**,**g**−**m** were prepared according to method D and characterized by ^{1}H NMR.

General Procedure for Preparation of (α-Chlorobenzyl)benzamides. 4-(3-Methoxy-α-chlorobenzyl)-*N*-ethyl-*N*-methylbenzamide (9f). Method E. Concentrated hydrochloric acid (5 mL) was added to a solution of **8f** (810 mg, 2.7 mmol) in $CHCl_3$ (10 mL) at room temperature with stirring. After being stirred for 4 h, the organic layer was separated, washed with water, dried, and concentrated to give **9f** (840 mg, 98%) as an oil: ^{1}H NMR δ 1.13−1.23 (m, 3H), 2.94, 3.06 (two s, 3H), 3.27−3.59 (m, 2H), 3.80 (s, 3H), 6.09 (s, 1H), 6.84 (dd, J = 2.5, 8 Hz, 1H), 6.96 (d, J = 8 Hz, 1H), 6.97 (d, J = 2.5 Hz, 1H), 7.26 (t, J = 8 Hz, 1H), 7.37 (d, J = 8 Hz, 2H), 7.45 (d, J = 8 Hz, 2H); MS (CI-NH_3) m/z 320 (MH^+).

Compounds **9a**−**e**,**g**−**m** were prepared according to method E and characterized by ^{1}H NMR.

General Procedure for Preparation of (Diphenylmethyl)piperazines. (+)-4-[(α*R*)-α-{(2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl}-3-methoxybenzyl]-*N*-ethyl-*N*-methylbenzamide Dihydrochloride (2f·2HCl) and (+)-4-[(α*S*)-α-{(2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl}-3-methoxybenzyl]-*N*-ethyl-*N*-methylbenzamide Dihydrochloride (3f·2HCl). Method F. A mixture of **9f** (710 mg, 2.2 mmol), (−)-(2*R*,5*S*)-1-allyl-2,5-dimethylpiperazine (**10**)[27] (690 mg, 4.4 mmol), K_2CO_3 (300 mg, 2.2 mmol), and KI (370 mg, 2.2 mmol) in MeCN (10 mL) was refluxed for 15 h with stirring. After removal of the solvent, the residue was added to water and extracted with EtOAc. The extract was washed with water, dried, and concentrated to give 750 mg of residue, which was subjected to flash column chromatography on silica gel (50 g) eluting with EtOAc/hexane (1:1) to yield **2f** as the relatively less polar product (430 mg, 45%) and **3f** as the more polar product (410 mg, 43%). These free bases were converted to the hydrochloride salt and the salts were recrystallized from acetone (**2f**·2HCl) or acetone/Et_2O (**3f**·2HCl) to afford 290 mg of **2f**·2HCl (26%) and 360 mg of **3f**·2HCl (32%) as dihydrochlorides.

(+)-4-[(α*R*)-α-{(2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl}-3-methoxybenzyl]-*N*-ethyl-*N*-methylbenzamide dihydrochloride (2f·2HCl): ^{1}H NMR δ 1.15 (bs, 3H), 1.40 (d, J = 6 Hz, 3H), 1.49 (d, J = 6 Hz, 3H), 2.88−3.05 (m, 4H), 3.19−3.31 (m, 3H), 3.45−3.52 (m, 2H), 3.89 (s, 3H), 3.96−4.16 (m, 2H), 4.42−4.53 (m, 2H), 5.57 (d, J = 17 Hz, 1H), 5.64 (d, J = 10.5 Hz, 1H), 5.68 (s, 1H), 6.03−6.09 (m, 1H), 7.00 (dd, J = 2, 8 Hz, 1H), 7.12 (d, J = 2 Hz, 1H), 7.27 (d, J = 8 Hz, 1H), 7.45 (d, J = 8 Hz, 2H), 7.47 (t, J = 8 Hz, 1H), 7.90 (d, J = 8 Hz, 2H); MS (CI-NH_3) m/z 436 (MH^+).

(+)-4-[(α*S*)-α-{(2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl}-3-methoxybenzyl]-*N*-ethyl-*N*-methylbenzamide dihydrochloride (3f·2HCl): ^{1}H NMR δ 1.19 (bs, 3H), 1.42 (s, 3H), 1.48 (s, 3H), 2.90−3.39 (m, 7H), 3.55 (bs, 2H), 3.88 (s, 3H), 3.93−4.18 (m, 2H), 4.32−4.40 (m, 2H), 5.57 (d, J = 17 Hz, 1H), 5.68 (d, J = 12 Hz, 1H), 5.72 (s, 1H), 6.02−6.07 (m, 1H), 6.91 (d, J = 8 Hz, 1H), 7.11 (s, 1H), 7.27 (d, J = 8 Hz, 1H), 7.57 (bs, 3H), 7.72 (bs, 2H); MS (CI-NH_3) m/z 436 (MH^+).

Compounds **2a**−**e**,**g**−**m** and **3a**−**e**,**g**−**m** were prepared according to method F and fully characterized. The physical data of these compounds are summarized in Table 1.

3-(α-Chlorobenzyl)-*N*,*N*-diethylbenzamide (13). This compound was synthesized from compound **12** in two steps according to methods D and E. The overall yield from **12** to **13** was 87%: ^{1}H NMR δ 1.07 (bs, 3H), 1.23 (bs, 3H), 3.21 (bs, 2H), 3.52 (bs, 2H), 6.13 (s, 1H), 7.30−7.45 (m, 9H).

(+)-3-[(α*S*)-α-{(2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl}benzyl]-*N*,*N*-diethylbenzamide Dihydrochloride (4b·2HCl) and (+)-3-[(α*R*)-α-{(2*S*,5*R*)-3-Allyl-2,5-dimethyl-1-piperazinyl}benzyl]-*N*,*N*-diethylbenzamide Dihydrochloride (5b·2HCl). These were prepared from **13** according to method F. The salt **4b**·2HCl was recrystallized from acetone/Et_2O: yield 31%; mp 142−145 °C; $[\alpha]^{20}_D$ (free base in MeOH, c 1.0) = +19.3°; ^{1}H NMR δ 0.94 (bs, 3H), 1.21 (t, J = 6.5 Hz, 3H), 1.41 (d, J = 6 Hz, 3H), 1.47 (d, J = 6 Hz, 3H), 3.12−3.17 (m, 3H), 3.29 (d, J = 13 Hz, 1H), 3.44−3.54 (m, 3H), 3.97 (dd, J = 4.5, 13.5 Hz, 1H), 4.06 (bs, 2H), 4.38 (bs, 2H), 5.57 (d, J = 17 Hz, 1H), 5.64 (d, J = 10.5 Hz, 1H), 5.77 (s, 1H), 6.02−6.08 (m, 1H), 7.40−7.56 (m, 6H), 7.64 (d, J = 7.5 Hz, 2H), 8.14 (bs, 1H). Anal. ($C_{27}H_{37}N_3O_2$·2HCl) C, H, N.

The salt **5b**·2HCl was recrystallized from acetone/Et_2O: yield 29%; mp 166−168 °C; $[\alpha]^{20}_D$ (free base in MeOH, c 1.0) = +19.0°; ^{1}H NMR δ 1.09 (bs, 3H), 1.25 (bs, 3H), 1.40 (d, J = 6.5 Hz, 3H), 1.46 (d, J = 6.5 Hz, 3H), 3.15−3.20 (m, 3H), 3.28 (d, J = 13.5 Hz, 1H), 3.44−3.54 (m, 3H), 3.96 (dd, J = 4, 13.5 Hz, 1H), 4.05−4.17 (m, 2H), 4.38−4.50 (m, 2H), 5.57 (d, J = 17 Hz, 1H), 5.64 (d, J = 10 Hz, 1H), 5.77 (s, 1H), 6.01−6.10 (m, 1H), 7.38−7.48 (m, 4H), 7.52 (s, 1H), 7.62 (t, J = 8 Hz, 1H), 7.78 (d, J = 6.5 Hz, 2H), 7.93 (d, J = 8 Hz, 1H). Anal. ($C_{27}H_{37}N_3O_2$·2HCl) C, H, N.

2-(α-Hydroxybenzyl)-*N,N*-diethylbenzamide (16). This compound was prepared from **15** according to method D in a yield of 96%: ^{1}H NMR δ 0.86 (bs, 3H), 0.94 (bs, 3H), 2.92 (bs, 2H), 3.19 (bs, 2H), 5.44 (bs, 1H), 5.74 (bs, 1H), 7.19−7.54 (m, 9H).

3-Phenylphthalide (18) and 1-(Diethylamino)-3-phenylisobenzofuran Hydrochloride (19). These two compounds were the undesired products in the preparation of **17** from **16** as discussed above, and the structures were confirmed by the following experiment. Concentrated hydrochloric acid (8 mL) was added to a solution of **16** (800 mg, 2.82 mmol) in $CHCl_3$ (16 mL) at room temperature with stirring. After being stirred for 1 h, the organic layer was separated, washed with water, dried, and concentrated to give a mixture (750 mg) of **17** and **18** as an oil. The mixture was then dissolved in 15 mL of $CHCl_3$ and stirred for 3 h. After filtration, the filter cake was washed with $CHCl_3$. The combined filtrate was concentrated to yield 200 mg (34%) of **18** as a solid: mp 109−110 °C (lit.[37] mp 112−113 °C); ^{1}H NMR δ 6.41 (s, 1H), 7.29−7.40 (m, 6H), 7.56 (t, J = 7.38 Hz, 1H), 7.65 (t, J = 7.92 Hz, 1H), 7.97 (d, J = 7.60 Hz, 1H); MS (CI-NH_3) m/z 211 (MH^+). Anal. ($C_{14}H_{10}O_2$) C, H.

The filter cake was further washed with $CHCl_3$/MeOH (1:1, 30 mL × 2), and the filtrate was concentrated to give 530 mg of **19** (62%) as a solid: mp 100−102 °C; ^{1}H NMR δ 1.04−1.26 (m, 6H), 3.26 (q, J = 7.05 Hz, 2H), 3.43 (q, J = 7.16 Hz, 2H), 7.39−7.46 (m, 4H), 7.50−7.57 (m, 3H), 7.80 (d, J = 8.25 Hz, 2H); MS (free base, CI-NH_3) m/z 266 (MH^+). Anal. ($C_{18}H_{19}NO\cdot HCl\cdot H_2O$) C, H, N.

2-Bromobenzyhydryl Chloride (21). Compound **21** was prepared from 2-bromobenzophenone (**20**) in two steps according to methods D and E. The intermediate, 2-bromobenzyhydrol, was characterized by ^{1}H NMR. The overall yield from **20** to **21** was 95%: ^{1}H NMR δ 6.58 (s, 1H), 7.17 (dt, J = 2, 8 Hz, 1H), 7.30−7.45 (m, 6H), 7.57 (dd, J = 2, 8 Hz, 1H), 7.65 (dd, J = 2, 8 Hz, 1H).

2-[(α*R* and α*S*)-α-{(2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl}benzyl]bromobenzene (22). A mixture of **21** (2.0 g, 7 mmol), **10** (2.2 g, 14 mmol), K_2CO_3 (0.98 g, 7 mmol), and KI (1.2 g, 7 mmol) in MeCN (20 mL) was refluxed for 48 h with stirring. After removal of the solvent, the residue was added to water and extracted with EtOAc. The extract was washed with water, dried, and concentrated to give 4.0 g of residue, which was subjected to flash column chromatography on silica gel (80 g) eluting with $CHCl_3$/MeOH (20:1) to afford the epimeric mixture **22** (2.5 g, 89%) as an oil: ^{1}H NMR δ 1.04 (d, J = 7 Hz, 3H), 1.08 (d, J = 7 Hz, 3H), 2.13−2.76 (m, 2H), 2.58−2.70 (m, 2H), 2.79 (d, J = 11.5 Hz, 1H), 2.87−3.01 (m, 2H), 3.21 (dt, J = 7, 15 Hz, 1H), 5.07 (d, J = 12.5 Hz, 1H), 5.33 (d, J = 11 Hz, 1H), 5.41 (s, 1H), 5.81−5.87 (m, 1H), 7.05 (t, J = 8 Hz, 1H), 7.17−7.57 (m, 7H), 7.84 (d, J = 8 Hz, 1H); MS (CI-NH_3) m/z 399 (MH^+).

(−)-2-[(α*S*)-α-{(2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl}benzyl]benzoic Acid (23) and (−)-2-[(α*R*)-α-{(2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl}benzyl]-benzoic Acid (24). A 1.6 M solution of *n*-butyllithium in hexane (16 mL, 25 mmol) was added dropwise to a solution of **22** (1.0 g, 2.5 mmol) in THF (20 mL) under an N_2 atmosphere at −78 °C. After being stirred for 2 h at −78 °C, the reaction solution was poured onto crushed dry ice. The mixture was warmed to room temperature, poured into water, and extracted three times with EtOAc. The extracts were combined, dried, and concentrated to give 1.3 g of oil, which was subjected to flash column chromatography on silica gel (50 g) eluting with $CHCl_3$/MeOH (20:1) to yield **23** as the less polar product (270 mg, 30%) and **24** as the more polar product (220 mg, 24%). The assignment of configuration of **23** and **24** was made by their relative chromatographic mobility and verified by single-crystal X-ray analysis of **24** as described below. Compound **23** was recrystallized from Et_2O/hexane after chromatography: mp 115−117 °C; $[\alpha]^{20}_D$ (MeOH, c 1.0) = −69.4°; ^{1}H NMR δ 1.00 (d, J = 5.5 Hz, 3H), 1.26 (d, J = 7 Hz, 3H), 2.31−2.47 (m, 3H), 2.75 (d, J = 12 Hz, 1H), 2.82−2.92 (m, 3H), 3.43 (dd, J = 4, 14.5 Hz, 1H), 5.19 (d, J = 9.5 Hz, 1H), 5.21 (d, J = 17.5 Hz, 1H), 5.75−5.80 (m, 1H), 5.78 (s, 1H), 6.90 (d, J = 8 Hz, 1H), 7.29−7.46 (m, 7H), 8.21 (d, J = 8.5 Hz, 1H); MS (CI-NH_3) m/z 365 (MH^+). Anal. ($C_{23}H_{28}N_2O_2\cdot{}^1/_5H_2O$) C, H, N.

Compound **24** was recrystallized from Et_2O/hexane after chromatography: mp 128−130 °C; $[\alpha]^{20}_D$ ($CHCl_3$/MeOH, 1:1, c 0.3) = −109.2°; ^{1}H NMR δ 1.18 (bs, 3H), 1.26 (bs, 3H), 2.34−2.41 (m, 2H), 2.65 (bs, 2H), 2.90 (bs, 1H), 3.07 (bs, 2H), 3.29 (bs, 1H), 4.84−5.31 (m, 3H), 5.78 (bs, 1H), 7.20−7.35 (m, 7H), 7.56 (bs, 2H); MS (CI-NH_3) m/z 365 (MH^+). Anal. ($C_{23}H_{28}N_2O_2\cdot{}^1/_2(C_2H_5)_2O$) C, H, N.

(+)-2-[(α*S*)-α-{(2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl}benzyl]-*N,N*-diethylbenzamide Dihydrochloride (4c·2HCl). 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (130 mg, 0.70 mmol) was added to a solution of **23** (230 mg, 0.63 mmol), diethylamine (50 mg, 0.63 mmol), and 1-hydroxybenzotriazole (95 mg, 0.70 mmol) in DMF (3 mL) at 0 °C with stirring. After being stirred for 7 h at room temperature, the mixture was poured into saturated aqueous $NaHCO_3$ and extracted with EtOAc. The extract was washed with water, dried, and concentrated to give a residue, which was converted to the dihydrochloride in the usual manner, and the salt was recrystallized from acetone to afford **4c**·2HCl (200 mg, 65%): mp 176−177 °C; $[\alpha]^{20}_D$ (free base in MeOH, c 0.8) = +19.5°; ^{1}H NMR δ 0.85 (bs, 3H), 1.16 (d, J = 5 Hz, 3H), 1.32 (t, J = 7 Hz, 3H), 1.40 (d, J = 5 Hz, 3H), 3.07 (d, J = 12.5 Hz, 1H), 3.27 (d, J = 12 Hz, 1H), 3.47−3.64 (m, 5H), 3.95 (d, J = 9 Hz, 1H), 4.10 (bs, 1H), 4.53 (bs, 2H), 4.80 (bs, 1H), 5.57 (d, J = 16.5 Hz, 1H), 5.63 (d, J = 11 Hz, 1H), 5.74 (s, 1H), 6.02−6.19 (m, 1H), 7.16 (d, J = 8 Hz, 1H), 7.33−7.42 (m, 5H), 7.56−7.61 (m, 3H). Anal. ($C_{27}H_{37}N_3O_2\cdot 2HCl\cdot{}^1/_3H_2O$) C, H, N.

(−)-2-[(α*R*)-α-{(2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl}benzyl]-*N,N*-diethylbenzamide Dihydrochloride (5c·2HCl). This compound was prepared from **24** in a yield of 59% using a similar procedure to that of **4c**·2HCl. The salt **5c**·2HCl was recrystallized from acetone: mp 177−178 °C; $[\alpha]^{20}_D$ (free base in MeOH, c 0.7) = −38.3°; ^{1}H NMR δ 0.67 (bs, 3H), 1.11 (bs, 3H), 1.47 (d, J = 6 Hz, 3H), 1.51 (bs, 3H), 3.05 (d, J = 11 Hz, 1H), 3.18 (bs, 2H), 3.33 (d, J = 14 Hz, 1H), 3.45−3.63 (m, 3H), 3.98 (dd, J = 4.5, 14 Hz, 2 H), 4.38 (bs, 2H), 4.63 (bs, 1H), 5.57 (d, J = 17 Hz, 1H), 5.64 (d, J = 11 Hz, 1H), 6.00−6.12 (m, 1H), 6.29 (s, 1H), 7.26 (t, J = 8 Hz, 2H), 7.39−7.49 (m, 4H), 7.49 (bs, 2H), 7.72 (t, J = 8 Hz, 1H). Anal. ($C_{27}H_{37}N_3O_2\cdot 2HCl$) C, H, N.

Assignment of the Relative Stereochemistry of the Diastereomeric Pairs of the *p*-(α-Piperazinylbenzyl)-benzamide Derivatives 2 and 3 by ^{1}H NMR. (+)-4-[(α*R*)-α-{(2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl}-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (**2n**): ^{1}H NMR (CD_3OD) δ 0.99 (d, J = 6.6 Hz, 3H), 1.12−1.23 (m, 9H), 1.86−1.92 (m, 1H), 2.16 (t, J = 11 Hz, 1H), 2.50−2.68 (m, 3H), 2.79−2.92 (m, 2H), 3.33−3.40 (m, 5H), 3.76 (s, 3H, OCH_3), 5.17−5.25 (m, 2H, C=CH_2), 5.31 (s, 1H, α-CH), 5.80−5.93 (m, 1H, C=CH), 6.75 (bs, 1H), 6.80 (d, J = 7.7 Hz, 1H), 6.87 (dd, J = 2.2, 8.8 Hz, 1H), 7.25−7.32 (m, 3H), 7.50 (d, J = 7.7 Hz, 2H). (+)-4-[(α*S*)-α-{(2*S*,5*R*)-4-Allyl-2,5-dimethyl-1-piperazinyl}-3-methoxybenzyl]-*N*,*N*-diethylbenzamide (**3n**): ^{1}H NMR (CD_3OD) δ 1.01 (d, J = 6 Hz, 3H), 1.14−1.24 (m, 9H), 1.87−1.94 (m, 1H), 2.19 (t, J = 8.8 Hz, 1H), 2.45−2.62 (m, 3H), 2.73 (dd, J = 2.7, 11.5 Hz, 1H), 2.81−2.94 (m, 1H), 3.31−3.56 (m, 5H), 3.74 (s, 3H, OCH_3), 5.18−5.28 (m, 3H, C=CH_2, α-CH), 5.81−5.87 (m, 1H, C=CH), 6.77 (dd, J = 2.7, 8.3 Hz, 1H), 6.91 (d, J = 7.7 Hz, 1H), 7.01 (bs, 1H), 7.18 (t, J = 7.6 Hz, 1H), 7.35 (d, J = 8.8 Hz, 2H), 7.39 (d, J = 8.8 Hz, 2H).

Resonances for the protons on the methoxy-substituted aromatic ring of **2n** and **3n** were observed to have different characteristic patterns as described above. All of the spectra for the epimeric pairs of the N,N-disubstituted *p*-(α-piperazinylbenzyl)benzamide derivatives **2a**−**m** and **3a**−**m** followed this pattern, thus enabling the assignment of the relative stereochemistry of these epimers.

Single-Crystal X-ray Analysis of Compound 24. Data were collected on a computer-controlled automatic Siemens P4 diffractometer and corrected for Lorentz and polarization effects. The structure was solved by direct methods with the aid of the program SHELXTL[38] and refined by full-matrix least-squares on F^2 values using the program SHELXLS.[38] The parameters refined included the coordinates and anisotropic

thermal parameters for all non-hydrogen atoms. Crystals were orthorhombic (space group $Pna2_1$) with $a = 14.667(1)$ Å, $b = 9.122(2)$ Å, and $c = 15.232(1)$ Å. Hydrogen atoms were included using a riding model in which the coordinate shifts of their covalently bonded atoms were applied to the attached hydrogens with C−H = 0.96 Å. H angles were idealized and $U_{iso}(H)$ set at fixed ratios of U_{iso} values of bonded atoms. Tables of crystal coordinates, bond distances, bond angles, and hydrogen bonds are available as Supporting Information as well as from the Cambridge Crystallographic Database.[39]

Biological Assays. 1. Radioligand Binding Assays for μ, δ, and κ Receptors. μ Binding sites were labeled using [^{3}H]DAMGO (1−3 nM) and rat brain membranes as previously described.[31] Briefly, incubations proceeded for 4 h at 25 °C in 50 mM Tris-HCl, pH 7.4, along with a protease inhibitor cocktail (PIC). The nonspecific binding was determined using 20 μM levallorphan. δ Binding sites were labeled using [^{3}H]-DADLE (1.7−2.5 nM) and rat brain membranes as previously described.[32] Incubations proceeded for 3−4 h at 25 °C in 10 nM Tris-HCl, pH 7.4, containing 100 mM choline chloride, 3 mM $MnCl_2$, and 100 nM DAMGO to block binding to μ sites and PIC. Nonspecific binding was determined using 20 μM levallorphan. κ_1 Binding sites were labeled using [^{3}H]U69,-593 (3.9 nM) and guinea pig brain membranes depleted of μ and δ binding sites by pretreatment with irreversible ligand BIT and FIT as previously described,[33] except that the incubation temperature was 25 °C. Briefly, incubations proceeded for 4−6 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing PIC and 1 mg/mL captopril. Nonspecific binding was determined using 1 μM U69,593. Each [^{3}H]ligand was displaced by nine concentrations of test drug, two times. All drug dilutions were done in 10 mM Tris-HCl, pH 7.4, containing 1 mg/mL bovine serum albumin. The IC_{50} and slope factor (N) were obtained by using the program MLAB.

2. GPI and MVD Bioassays.[35] Electrically induced smooth muscle contractions of mouse vas deferens and strips of guinea pig ileum longitudinal muscle myenteric plexus were used. Tissues came from male ICR mice weighing 25−40 g and male Hartley guinea pig weighing 250−500 g. The tissues were tied to gold chain with suture silk, suspended in 20 mL baths containing 37 °C oxygenated (95% O_2, 5% CO_2) Krebs bicarbonate solution (magnesium free for the MVD), and allowed to equilibrate for 15 min. The tissues were then stretched to optimal length previously determined to be 1 g tension (0.5 g for MVD) and allowed to equilibrate for 15 min. The tissues were stimulated transmurally between platinum wire electrodes at 0.1 Hz, 0.4-ms pulses (2-ms pulses for MVD), and supramaximal voltage. Drugs were added to the baths in 14−60 mM volumes. The agonists remained in contact with the tissue until maximum inhibition was reached before the addition of the next cumulative dose. Percent inhibition was calculated by using the average contraction height for 1 min preceding the addition of the agonist divided by the contraction height at maximal inhibition after exposure to the dose of agonist. IC_{50} estimates and their associated standard errors were determined by using a computerized nonlinear least-squares method.[40] Sensitivity to the antagonists ICI-174,864 were tested by adding a 1 μM concentration of the antagonist to the tissue bath at the completion of the agonist dose−response curve. Partial or complete restoration of contraction height on addition of the antagonist was used as an indicator of the agonistic action at the δ receptor in the case of ICI-174,864.

3. Radioligand Binding Studies for Human μ and δ Receptors. Crude membranes were prepared from B82 mouse fibroblast cells giving stable expression of the human μ opioid receptor or from CHO cells giving stable expression of the human δ opioid receptor. Radioligand binding inhibition studies were performed using 1.0 nM [^{3}H]DAMGO (New England Nuclear, Boston, MA; specific activity: 48.9 Ci/mmol) for binding the B82 μ receptor or 0.75 nM [^{3}H]-*p*-Cl-DPDPE (New England Nuclear, Boston, MA; specific activity: 47 Ci/mmol) for binding the CHO δ receptor.[34] Assay samples included duplicate total binding (no inhibitor), nonspecific binding (10 μM naltrexone), and 10 concentrations of inhibitor. All samples were prepared in a final volume of 1.0 mL and incubated for 3 h at 25 °C before separation of bound radioligand by filtration through GF/B glass-fiber filter material pretreated with 0.05% poly(ethylenimine) (Sigma Chemical Co., St. Louis, MO). The filter-trapped tissue was washed three times with normal saline chilled to 4 °C. Bound radioactivity was measured in EcoLite liquid scintillation cocktail (ICN Pharmaceuticals, Inc., Costa Mesa, CA) using a Beckman model LS6000SC liquid scintillation counter (Fullerton, CA) at about 65% efficiency. The IC_{50} values were estimated by nonlinear regression analysis using the curve-fitting program Prism (GraphPad Software) as previously described.[41]

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Supporting Information Available: Crystallographic data including bond lengths, bond angles, and atomic coordinates for compound **24** (6 pages). Ordering information can be found on any current masthead page.

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