κ Opioid Receptor Selective Affinity Labels: Electrophilic Benzeneacetamides as κ-Selective Opioid Antagonists

An-Chih Chang,† Akira E. Takemori,‡ William H. Ojala,§ William B. Gleason,§ and Philip S. Portoghese*,†

Department of Medicinal Chemistry, College of Pharmacy, Department of Pharmacology, Medical School, Biomedical Engineering Center, Department of Laboratory Medicine and Pathology, Medical School, University of Minnesota, Minneapolis, Minnesota 55455

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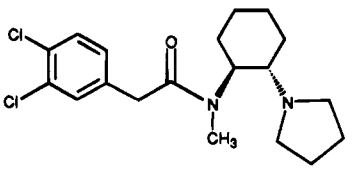
2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[1-(3- or 4-substituted phenyl)-2-(1-pyrrolidinyl)ethyl]-acetamides **3**–**6** were synthesized as κ-selective affinity labels and evaluated for opioid activity. In smooth muscle preparations, the non-electrophilic parent compound (+)-*S*-**2** and the affinity labels **3**–**6** behaved as κ agonists in that they were potently antagonized by norbinaltorphimine (norBNI). In addition to the high binding affinity and selectivity of the 3-isothiocyanate **3** (DIPPA) to κ opioid receptors, wash studies have suggested that this involves covalent binding. In the mouse tail-flick assay, the 3- and 4-substituted isomers (**3** and **5**, respectively) produced long-lasting antagonism of the antinociceptive effect of the κ opioid agonist, (±)-trans-2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]acetamide ((±)-U50,488). In contrast, the non-electrophilic parent compound (+)-*S*-**2** and the fumaramate derivative **4** were devoid of antagonist activity in the tail-flick assay. At substantially different doses, DIPPA (**3**) and the 4-isothiocyanate **5** also produced antinociception in the mouse abdominal stretch assay. In addition, DIPPA and the 3-fumaramate methyl ester **4** had improved in vivo κ-selectivities compared to the unsubstituted parent compound (+)-*S*-**2** and the para-substituted derivative **5**. The improved κ-selectivities of **3** and **4** and the different agonist and antagonist potencies of **3** and **5** may be explained respectively by the existence of multiple κ agonist binding sites and distinct agonist and antagonist binding sites. In view of the antagonist selectivity and the apparent irreversible binding of DIPPA to κ receptors, it may serve as a useful pharmacologic or biochemical tool to investigate κ opioid receptors.

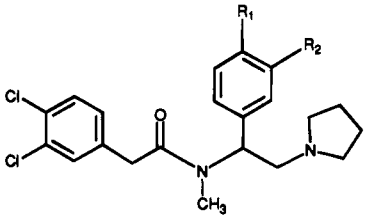
Introduction

Opioid ligands mediate their effects through at least three major types of opioid receptors: μ, κ, and δ.[1] Besides analgesia, opioid receptor activation produces many additional biological effects at both peripheral and central sites. In order to fully elucidate the functions of opioid receptors, agonists and antagonists selective for each type of opioid receptor are required as pharmacologic tools. κ opioid receptor-induced analgesia has been of special interest because it is associated with minimal physical dependence and respiratory depression.[2] Furthermore, the recent cloning and sequencing of the κ opioid receptor have heightened the need for additional κ ligands as pharmacologic and biochemical tools.[3–8]

Since the report of compound **1** (U50,488)[9,10] as a highly selective κ agonist, many structural analogues of improved affinity and selectivity for the κ opioid receptor have been reported.[11–14] A number of κ-selective affinity labels based on **1** have been reported to exhibit wash-resistant binding[15–17,19] as well as in vivo κ antagonism.[18] In particular, κ-selective antagonists are valuable pharmacologic tools to elucidate the functional role of the κ opioid receptor in vivo.[20] Moreover, affinity labels would be useful as probes to identify the binding locus on cloned κ receptors. There would be an advantage to having an array of structurally diverse in vivo-active antagonists as affinity labels because such agents may facilitate correlation of covalent receptor binding with loss of κ activity in vivo.



1, U50,488



2, (R) or (S), $R_1 = H, R_2 = H$

3 (DIPPA), (S), $R_1 = H, R_2 = -NCS$

4, (S), $R_1 = H, R_2 = (E)\text{-}NHCOCH{=}CHCO_2Me$

5, (RS), $R_1 = -NCS, R_2 = H$

6, (RS), $R_1 = (E)\text{-}NHCOCH{=}CHCO_2Me, R_2 = H$

We report here on compound **3** (DIPPA), an in vivo κ-selective antagonist belonging to the arylacetamide class of ligands,[21] and on the synthesis and biological evaluation of related potential affinity labels **4**–**6**.

* To whom correspondence should be addressed.
† Department of Medicinal Chemistry.
‡ Department of Pharmacology.
§ Biomedical Engineering Center, Dept. of Laboratory Medicine and Pathology.
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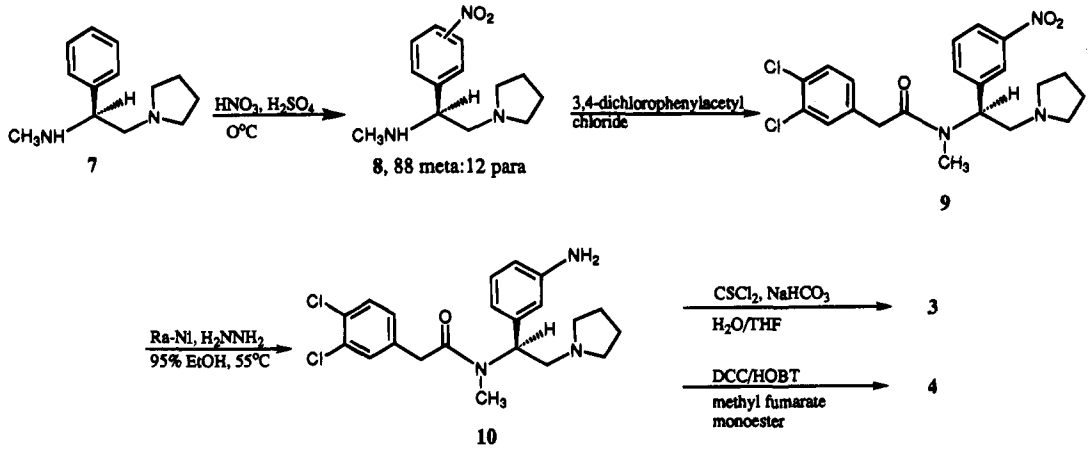
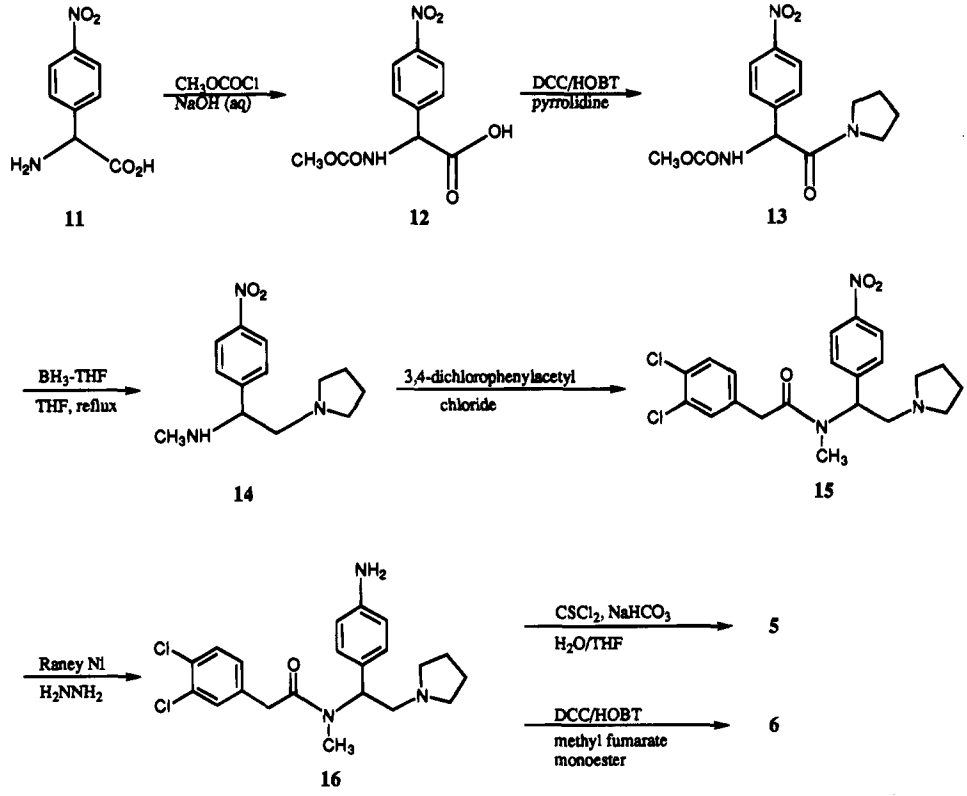
Scheme 1



7 8, 88 meta:12 para 9 10 3 4

Scheme 2



11 12 13 14 15 16 5 6

Chemistry

Since isothiocyanate substitution on the phenyl group of U50,488 has resulted in derivatives with relatively reduced potency,[15-17] we sought to introduce the electrophilic moiety in a different portion of the molecule in hope of producing κ-receptor affinity labels that are also more potent κ-selective antagonists. Because (+)-*S*-**2**[12] is a potent and selective κ agonist with a phenyl group in the middle portion of the molecule, it was chosen as the parent compound to be derivatized with electrophilic groups.

The preparation of the amino precursor **10** began with the nitration of optically pure **7**[12] to afford a regioisomeric mixture consisting of 88% *m*- and 12% *p*-nitro isomers (**8**) which could not be easily separated by a variety of separation techniques (Scheme **1**). The isomeric ratio was determined by ^{1}H NMR, and no peaks corresponding to the *o*-nitro isomer were observed. However, after coupling 3,4-dichlorophenylacetyl chloride to the isomeric mixture **8**, enantio- and regioisomerically pure **9** was obtained by chromatography and crystallization. Raney nickel/hydrazine reduction of the nitro group then gave the *m*-amino precursor **10**.

Because the corresponding para isomer **16** was not obtained in pure form using the above scheme, it was prepared by an alternate route (Scheme 2). Compounds in the para series were synthesized as racemates. (±)-2-(4-Nitrophenyl)glycine[22] (**11**) was protected by conversion to the carbamate ester **12**, which was converted to the pyrrolidine amide **13**, and then reduced to the diamine **14** with BH_3–THF. Coupling of **14** to 3,4-dichlorophenylacetyl chloride followed by reduction of the product **15** with Raney nickel/hydrazine then gave the desired aniline derivative **16**.

The target compounds in the meta series were obtained by treatment of **10** with either thiophosgene or methyl fumarate to afford **3** and **4**, respectively (Scheme

Table 1. Agonist Potencies in Smooth Muscle Preparations

compd	IC_{50} (nM) ± SEM[a] GPI	IC_{50} (nM) ± SEM[a] MVD	NorBNI K_e (nM)[b] GPI
(+)-*S*-**2**	0.27 ± 0.09	2.50 ± 1.7	0.05
(−)-*R*-**2**	198 ± 43	87.0 ± 10.7	
3	23.8 ± 4.2	14.9 ± 4.4	0.3
4	29.0 ± 14.4	5.4 ± 3.5	
5	38.6 ± 18	40.0 ± 14.0	
6	11.2 ± 4.9	22.7 ± 9.5	
10	0.17 ± 0.08	0.12 ± 0.01	
16	0.65 ± 0.20	4.5 ± 1.4	

[a] Values are arithmetic means of at least three experiments. [b] GPI was incubated with 5 and 20 nM norBNI for 15 min before **3** and **2** were tested, respectively.

Table 2. Wash-Resistant Activity of (+)-*S*-**2** and **3** in the GPI

treatment	% recovery[a] (+)-**2**	% recovery[a] **3**
wash[b]	20	0
naltrexone (500 nM)[c]	59	41
post-wash[b]	32	0
norBNI (20 nM)[c]	91	77
post-wash[b]	32	35

[a] Expressed as percent recovery of twitch height. [b] Forty washes with Kreb's buffer after incubation with agonist. [c] Incubation of treated (**2** or **3**) preparation for 15 min ($n = 3$ for **2**, $n = 1$ for **3**).

1). Target compounds **5** and **6** in the para series were obtained from **16** in a similar fashion (Scheme 2).

Biological Results

Smooth Muscle Preparations. Compounds **2-6**, **10**, and **16** were tested on the electrically stimulated guinea pig ileal longitudinal muscle[23] (GPI) and mouse vas deferens[24] (MVD) preparations as described previously.[25] The antagonist potencies of the κ antagonist, norbinaltorphimine[26] (norBNI), are expressed as K_e values which were calculated from the equation K_e = [antagonist]/(IC_{50} ratio − 1), where the IC_{50} ratio represents the IC_{50} of the agonist in the presence of the antagonist divided by the control IC_{50} of the agonist in the same preparation.

In both GPI and MVD, all ligands behaved as agonists, although the parent compound (+)-*S*-**2** and the *m*- and *p*-amino precursors (**10** and **16**) were substantially more potent than the target compounds (Table 1). Also the smooth muscle data confirmed that the opioid activity resides predominantly with the *S* isomer as reported previously.[27] In order to demonstrate that the agonist activities of (+)-*S*-**2** and **3** in the GPI were mediated through interaction with κ opioid receptors, the κ antagonist, norBNI, was employed. The low nanomolar K_e values strongly suggest that κ receptors are involved.

In attempts to detect the possible presence of an antagonist component of **3** in the GPI, the κ-selective agonists, ethylketazocine (EK) and **1**, were employed. It is noteworthy that a 30 min preincubation with **3** (0.001−1 nM) did not produce statistically significant antagonism of the inhibition of muscular twitch by morphine, EK, or **1**.

Both **2** and **3** also exhibited wash-resistant agonist activity (Table 2). While there was no recovery of twitch height after 40 washes with buffer, the electrically stimulated twitch was restored in the presence of naltrexone or norBNI. However, the agonist effect returned upon removal of the antagonists by washing with buffer.

Table 3. Opioid Receptor Binding Selectivity of DIPPA and Its Precursor **10**

compd	IC_{50} (nM)[a] κ	μ	δ
3 (DIPPA)	2.21	1799	>1000[b]
10	0.00032	30.7	88.5

[a] Values are geometric means of three replicate experiments. [b] Value is based on two experiments.

Table 4. Wash-Resistant Binding Studies of DIPPA (**3**) and Its Precursor **10**

compd	treatment	K_d[a] (nM) [^{3}H]U69593	B_{max}[a] (fmol/mg) [^{3}H]U69593
none	no wash	2.46 ± 0.18	27.0 ± 3.8
none	three washes[b]	2.79 ($n = 1$)	31.5 ($n = 1$)
3	three washes[b]	2.43 ± 1.1	3.55 ± 1.2
10	three washes[b]	3.36 ± 0.93	23.4 ± 5.8

[a] Values are the means of at least three replicate experiments ± SEM. [b] The membrane preparation was incubated with or without drug for 1 h at 37 °C, and incubation was terminated by centrifugation at 27000*g* for 15 min at 4 °C. The suspended pellet was then washed three times by centrifugation at 27000*g* for 15 min at 4 °C and resuspended in ice-cold buffer to 2% w/v for eceptor binding assay using Werling's procedure.[28]

Binding. The opioid receptor affinities of **3** and its precursor **10** were determined by competition with radioligands in guinea pig brain membranes employing a modification of the method of Werling et al.[28] Binding to κ receptors was evaluated with 1 nM [^{3}H]-(5α,7α,8β)-(−)-*N*-methyl-*N*-1-pyrrolidinyl-1-oxaspiro[4.5]dec-8-yl-benzeneacetamide[29] (U69,593), to μ receptors with 2 nM [^{3}H][D-Ala^2,MePhe^4,Gly-ol^6]enkephalin[30] (DAMGO), and to δ receptors with 5 nM [^{3}H][D-Pen^2,D-Pen^5]enkephalin[31] (DPDPE). Both **3** and **10** exhibited high affinity and selectivity for the κ opioid receptor (Table 3).

Wash-resistant binding studies were performed to test for covalent binding of **3** to κ opioid receptors (Table 4). In control studies, the wash treatment was found to produce no significant changes in the guinea pig brain membrane binding characteristics of [^{3}H]U69,593. Preincubation with the amino precursor **10** (0.1 nM) followed by washing resulted in only minor changes in the binding parameters. However, preincubation with **3** (10 nM) at 37 °C for 1 h decreased the number of [^{3}H]-U69,593 binding sites (B_{max}) by 87% while having no significant effect on its binding affinity.

Surprisingly, while (+)-*S*-**2** afforded reasonable IC_{50} values from competition studies with μ- and δ-selective radioligands ([^{3}H]DAMGO and [^{3}H]DPDPE), with IC_{50} values of 0.2 μM and > 1μM, respectively, no concentration-dependent competition was observed with [^{3}H]-U69,593.

In Vivo Studies. The target compounds also were evaluated for in vivo activity using either the mouse tail-flick[32] or the mouse abdominal stretch[33] assays via the sc route. Compound **6** was not evaluated in vivo because it was not expected to produce antagonism in view of the results obtained with **4**. In evaluating agonist selectivity, norBNI, naltrindole[34] (NTI), and β-funaltrexamine[35] (β-FNA) were employed as κ-, δ-, and μ-selective antagonists, respectively. The determination of antagonist selectivity was carried out using U50,488, DPDPE, and morphine as κ-, δ-, and μ-selective agonists, respectively.

Table 5. Antinociceptive Potency and Selectivity in the Mouse Abdominal Stretch Assay

compd	ED_{50}, μmol/kg	ED_{50} ratio[a] norBNI (κ)	NTI (δ)	β-FNA (μ)
(+)-*S*-**2**	0.017 (0.013–0.022)	*2.31 (1.6–3.3)[b]	1.19 (0.55–2.77)[c]	1.72 (1.00–2.86)[d]
3	1.53 (1.11–2.06)	*16.7 (10.0–25.0)[e]	1.25 (0.76–2.04)[c]	*3.03 (1.89–5.26)[f]
4	1.41 (1.24–1.57)	*9.09 (7.69–10.0)[e]	1.02 (0.86–1.19)[c]	*2.08 (1.79–2.44)[f]
5	0.71 (0.55–0.88)	*2.78 (1.96–4.00)[b]	0.48 (0.34–0.68)[c]	0.78 (0.56–1.15)[f]

[a] The ED_{50} of the agonist (sc) in the antagonist-treated mice divided by the control ED_{50}; numbers in parentheses are 95% confidence levels. [b] NorBNI (12.25 μmol/kg sc) was administered 1.5 h prior to agonist. [c] NTI (44.44 μmol/kg sc) was administered simultaneously with agonist. [d] β-FNA (20.36 μmol/kg sc) was administered 24 h prior to agonist. [e] NorBNI (12.25 μmol/kg sc) was administered 3.5 h prior to agonist. [f] β-FNA (10.18 μmol/kg sc) was administered 24 h prior to agonist.

Table 6. Antagonist Potency and Selectivity of **3** and **5** in the Mouse Tail-Flick Assay

compd	dose (μmol/kg sc)	ED_{50} ratio[a] U50,488 (κ)[b]	morphine (μ)[c]	DPDPE (δ)[d]
3 (DIPPA)	0.53	9.1 (6.7–14.3)	1.8 (0.8–2.9)	1.3 (0.8–2.1)
5	16	4.8 (3.4–6.2)	3.1 (2.6–3.8)	1.2 (0.74–2.0)

[a] ED_{50} of agonist in the antagonist-treated mice divided by the control ED_{50}; numbers in parentheses are 95% confidence levels. [b] U50,488 was administered sc using a 20 min peak time. [c] Morphine was administered sc using a 30 min peak time. [d] DPDPE was administered icv using a 20 min peak time.

Table 7. Equiactive Potencies[a] and Duration of Action of DIPPA (**3**) and **5**

compd	antagonism (tail-flick assay) dose (nmol/kg)	peak time (h)	duration (h)	agonism (abdominal-stretch assay) ED_{50} (nmol/kg)	peak time (min)	duration (h)
3 (DIPPA)	65.4	4	48–72	1,530	30	<4
5	4,260	18	72–96	710	30	<4

[a] Dose required to produce equivalent antagonism of U50,488 antinociception.

In the mouse abdominal stretch assay, compounds **2-5** produced κ-selective antinociception which peaked at 30 min and disappeared within 4 h of administration (Table 5). The parent compound (+)-*S*-**2** was 40–90-fold more potent than its substituted derivatives. However, **2** appeared to have a low in vivo κ-selectivity, as indicated by the ED_{50} ratio of 2.3 in the presence of norBNI. The highest κ-selectivity ratios were associated with compounds **3** and **4**.

In the mouse tail-flick assay, isothiocyanates **3** and **5** behaved as κ-selective antagonists (Table 6). In addition, **5** exhibited significant μ-antagonism. When tested at doses ranging from 16.5 nmol/kg to 2.11 μmol/kg and at times from 15 min to 6 h after injection, **3** did not exhibit antinociceptive activity. Similarly, **5** did not produce antinociception in the mouse tail-flick assay when tested at 4 μmol/kg and 16 μmol/kg at 15 min to 4 h after administration.

A comparison of the antagonist (tail-flick) and agonist (abdominal stretch) selectivities of **3** and **5** is presented in Table 7. While the ED_{50} of **3** was higher than its antagonist dose (0.53 μmol/kg sc, 90% antagonism), the ED_{50} of **5** was lower than its antagonist dose. Both **3** and **5** produced long-lasting antagonism of U50,488 antinociception in the mouse tail-flick assay (Figure 1). The antagonist activity of **3** peaked at 4 h and persisted at 48 h after administration (Table 7). On the other hand, the antagonist activity of **5** peaked at 18 h and persisted 96 h after administration.

Because of the antinociceptive activity of DIPPA in the mouse abdominal stretch assay, it failed to produce detectable antagonism in the same assay at doses ranging from 0.02 to 33.7 μmol/kg at times from 20 min to 24 h.

The unsubstituted parent compound (+)-*S*-**2** and the fumaramate **4** failed to antagonize U50,488 in the mouse tail-flick assay. At 0.6 μmol/kg, (+)-*S*-**2** (ED_{50} = 0.24 μmol/kg, sc) did not exhibit antagonist activity at 4, 18, 24, and 48 h after administration. In a dose range of 0.46–1.85 μmol/kg, **4** did not antagonize the actions of U50,488 from 15 min to 4 h after administration. Antinociceptive activity (ED_{50} = 6.1 μmol/kg) was observed for **4** in the mouse tail-flick assay.

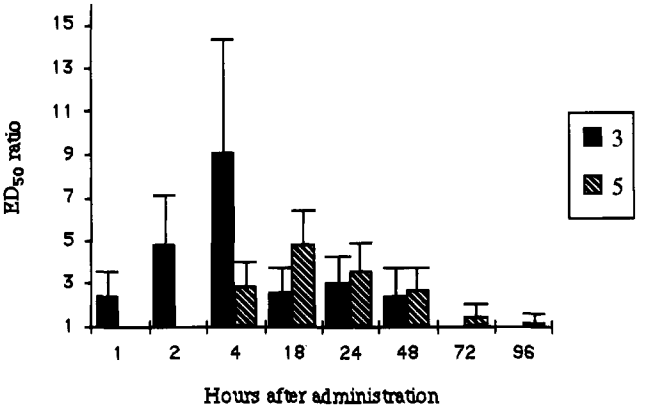


Figure 1. Time course of antagonism of U50,488 antinociception by DIPPA (0.53 μmol/kg sc) and its para isomer (16 μmol/kg sc). Antinociception was measured by the tail-flick assay 20 min after sc administration of U50, 488 in mice. The ED_{50} ratio is the ED_{50} of U50,488 in the presence of antagonist divided by the control ED_{50}. Error bar indicates the upper limit of the 95% confidence intervals.

Crystal Structure and Modeling Studies

X-ray crystal analysis (see the Experimental Section) of two different samples revealed two different conformations (polymorphs I and II) of (+)-*S*-**2**·HCl that are different in the rotation of the phenyl group and the positioning of the dichlorophenyl moiety (Figure 2). Crystal structures have also been reported for four other arylacetamide κ agonists: **17**,[11] **18**,[36] **19**,[14] **20**.[13] It is noteworthy that the non-cyclohexyl-containing ligands (+)-*S*-**2**, **19**, and **20** have improved κ-affinities and

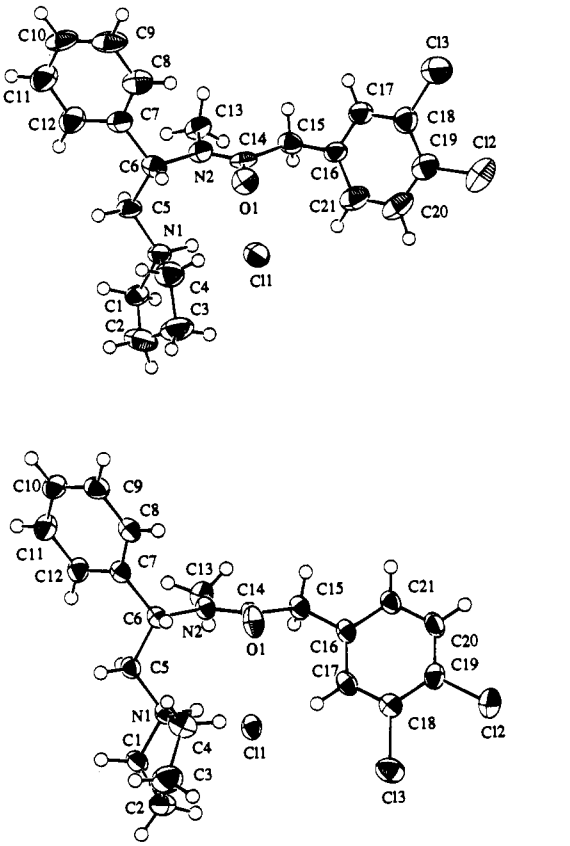
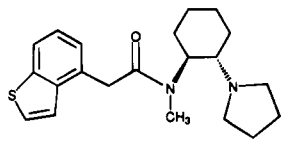
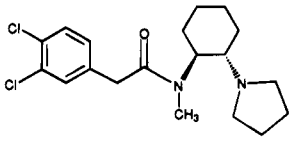


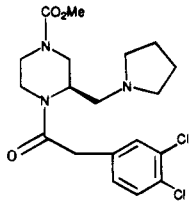
Figure 2. ORTEP drawings of the two X-ray crystal conformations of (+)-*S*-**2**·HCl with crystallographic numbering system. The non-hydrogen atoms are shown at the 50% probability level. The upper conformer has been designated polymorph I, while the lower conformer is polymorph II.



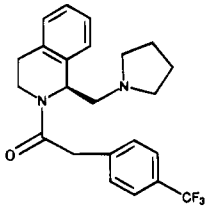
17, PD117302



18, (-) U50,488



19, GR89696



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κ-selectivities compared to the cyclohexyl-containing ligands **17** and **18**. Since the isothiocyanate derivatives (**3**, **5**) have potent and selective κ-antagonist activity which is absent in the parent compound (+)-*S*-**2**, it was of interest to compare crystal structures of (+)-*S*-**2** with **17**–**20** using Biosym software (InsightII v.2.3.0) in an effort to determine whether or not conformational differences play a role in the covalent binding of **3** and **5** to produce antagonism of U50,488.

The RMS deviations of the superimposition of the pyrrolidine N and the amide (NC=O) atoms of (+)-*S*-**2**, **17**-**20** ranged from 0.0169 to 0.1450 Å. The conformations of the inactive (−)-*R*-**2** were overlapped by superimposing the pyrrolidine N, the chiral carbon, and the amide N.

Superposition of the crystal structures of (+)-*S*-**2**, (−)-*R*-**2**, **17**, and **18** revealed that the backbone conformations and the pyrrolidine rings of the biologically active molecules overlap closely while the less potent (−)-*R*-**2** adopts different conformations (Figure 3). The backbone and the pyrrolidine ring of (+)-*S*-**2** also overlap with the corresponding groups in **19** and **20** while the conformers corresponding to the (−)-*R*-**2** isomer fail to achieve overlap (Figure 4).

Discussion

The observation that sc administration of DIPPA or **5**, but not (+)-*S*-**2**, produced a transient κ agonist effect followed by long-lasting κ antagonism in the mouse using the tail-flick assay is reminiscent of the action of the μ opioid antagonist, β-FNA, and the δ opioid antagonist, [D-Ala^2, Leu^5, Cys^6]enkephalin[37] (DALCE). Both of these affinity labels also produce transient agonism that precedes persistent antagonism that presumably is due to covalent modification of their respective opioid receptors.[38,39]

In the present case, the agonist and antagonist effects of DIPPA and **5** might be mediated through agonist and antagonist sites on the same κ receptor,[40] or, alternately, multiple κ opioid receptor subtypes might explain the biphasic effects.[41] In regard to this latter possibility, there is evidence that arylacetamide κ agonists may interact with different κ receptor subtypes in spite of their structural similarities.[42] One of the subtypes would interact noncovalently with DIPPA or **5** to afford agonism of short duration, while the second subtype would be inactivated through covalent binding to produce long-lasting antagonism. This would be similar to the pharmacology of β-FNA[43] and DALCE[37] in that a second receptor system is involved in the transient agonism. The inability of **4** to produce antagonism may signify that its Michael acceptor fumaramate moiety is not sufficiently reactive to form a covalent linkage with the receptor-based nucleophile.

If there are multiple κ receptor subtypes in the GPI preparation, the latter mechanism would explain why DIPPA did not act as a κ opioid antagonist in this preparation. We are unable to distinguish between the above possibilities because washing experiments failed to remove the agonist effect of DIPPA or its parent compound (+)-*S*-**2**.

Support for covalent binding of κ receptors by DIPPA was obtained from binding studies, in that DIPPA displayed wash resistant binding with a substantial decrease in B_{max} while its amine precursor **10** bound reversibly. In light of the known chemical reactivity of aryl isothiocyanates for amine and thiol groups, lysine and cysteine residues are likely candidates for covalent modification.

In contrast to DIPPA and its precursor **10**, the parent compound (+)-*S*-**2** exhibited no concentration dependence in the inhibition of [^{3}H]U69,593 binding. Thus (+)-*S*-**2** inhibited binding over a wide concentration range. These results are puzzling and suggest that perhaps [^{3}H]U69,593 and the meta-substituted derivatives, DIPPA and **10**, bind to a domain of the κ receptor that is different from that which binds (+)-*S*-**2**. The

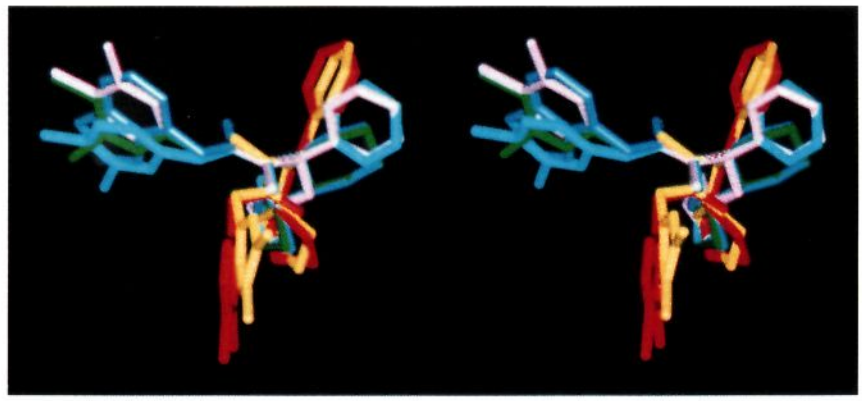

Figure 3. Stereoview of the superimposition of the crystal structures of (+)-*S*-**2**·HCl, (−)-*R*-**2**·HCl, **17**, and **18**. Note that the dichlorophenyl ring of (−)-*R*-**2**·HCl does not overlay with the corresponding group in (+)-*S*-**2**·HCl, **17**, and **18**. (**17** and **18** are blue and green, respectively. Polymorphs of (+)-*S*-**2** are cyan and magenta, and polymorphs of (−)-*R*-**2** are yellow and red. The structures of (−)-*R*-**2** were obtained by reversing the signs of the *z*-axis coordinates of the reported/less potent structures.)

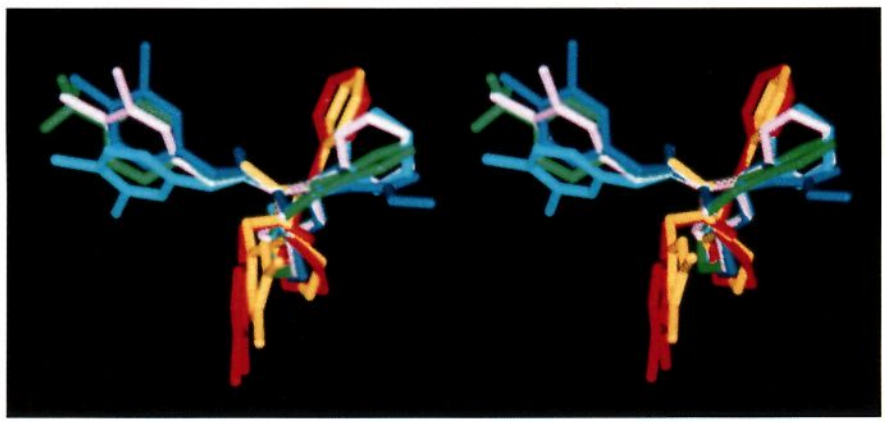

Figure 4. Stereoview of the superimposition of the crystal structures of (+)-*S*-**2**·HCl, (−)-*R*-**2**·HCl, **19**, and **20**. Note that the dichlorophenyl ring of (−)-*R*-**2**·HCl does not overlay with the corresponding group in (+)-*S*-**2**·HCl, **19**, and **20**. (**19** and **20** are blue and green, respectively. Polymorphs of (+)-*S*-**2** are cyan and magenta, and polymorphs of (−)-*R*-**2** are yellow and red. The structures of (−)-*R*-**2** and **20** were obtained by reversing the signs of the *z*-axis coordinates of the reported/less potent structures.)

existence of multiple agonist binding domains also was suggested by studies of (1*S*,2*S*)-*trans*-2-isothiocyanato-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide, which was reported[15] to irreversibly inhibit [^{3}H]-U69,593 binding without inhibiting [^{3}H]bremazocine binding in guinea pig membranes devoid of functional μ and δ receptors.

The possible change in agonist binding domain upon meta substitution of (+)-*S*-**2** also is indicated by the antinociception studies. The high potency and relatively low κ-selectivity of (+)-*S*-**2** in mice may be explained by high affinity binding to a κ receptor site that is not effectively antagonized by norBNI. Substitution at the meta position of (+)-*S*-**2** with an isothiocyanate or fumaramate group (**3** and **4**, respectively) may shift the binding to a different site on the same receptor that is more readily antagonized by norBNI. Evidence for the involvement of different recognition sites was also observed in the GPI preparation, where (+)-*S*-**2** was 90-fold more potent than DIPPA and was antagonized to a different extent by norBNI. However, (+)-*S*-**2** was antagonized more potently by norBNI in the GPI preparation while DIPPA was antagonized more potently by norBNI in mice, suggesting species- or tissue-related differences in the κ opioid receptor. Furthermore, the similar κ-selectivities of (+)-*S*-**2** and the para substituted isothiocyanate **5** suggest that para-substitution does not cause this possible change in binding site.

The fact the DIPPA-induced antagonism of U50,488 was not detected in the mouse abdominal stretch assay reflects the greater sensitivity of this assay over the tail-flick assay for κ agonists.[44] If the tail-flick and abdominal stretch assays involve different neuronal pathways, it is possible that different κ opioid receptor subtypes may mediate the antinociceptive effect. Also, if a greater number of spare κ receptors are involved in the abdominal stretch assay, the threshold dose of agonist for antinociceptive effect might be more detectable.

In view of the possible involvement of multiple sites in the antinociception and antagonism of DIPPA, the X-ray crystal structure of its parent compound (+)-*S*-**2** was determined in order to evaluate whether there is any difference in conformation between (+)-*S*-**2** and other κ agonists that might contribute to our inability to obtain binding data for this compound. Superimposition of four benzeneacetamide agonists of known crystal structure upon (+)-**2** revealed no clear conformational differences that would account for the data. If it is assumed that the *m*-amino (**10**) and *m*-isothiocyanate (**3**) substitutions cause no changes in the conformational preference of (+)-*S*-**2**, then the direct effect of substitution on the phenyl group of (+)-*S*-**2** may be responsible for the observed changes in binding behavior rather than conformational effects.

Conclusions

In conclusion, the affinity label, DIPPA (**3**), has been documented to possess selective κ opioid receptor antagonist activity in vivo. Its long duration of action and receptor binding characteristics suggest that the κ antagonist effect is due to covalent binding to the κ receptor. Because DIPPA produced no observable toxicity at a dose that produced significant antagonism, it may serve as a useful pharmacologic or biochemical tool to inactivate κ opioid receptors.

Experimental Section

All NMR spectra were recorded on a GE 300 MHz or a Varian 500 MHz spectrometer at room temperature. IR spectra were recorded with a Nicolet 5DXC FT-IR spectrometer. Optical rotations were measured using a Rudolph Research Autopol III automatic polarimeter. Melting points were uncorrected and were determined using a Thomas-Hoover capillary melting point apparatus. Elemental analyses were conducted by M-H-W Laboratories in Phoenix, AZ. Mass spectra were recorded by the Chemistry Mass Spec Labs at the University of Minnesota's Chemistry Department.

(*S*)-1-[2-(Methylamino)-2-(3/4-nitrophenyl)ethyl]pyrrolidine (8). To an ice-cold solution of **7**[12] (21.3 g, 104 mmol) in concentrated sulfuric acid (150 mL) was added concentrated nitric acid (10 mL, 157 mmol) with vigorous stirring. The stirring with ice-cooling continued for 45 min, and the reaction mixture was made basic by careful addition of 5 N NaOH and NaOH(s) pellets and water with stirring and ice-cooling. The precipitate was extracted into EtOAc (3 L), which was dried ($MgSO_4$), filtered, evaporated under reduced pressure, and dried in vacuo to yield 22.06 g (85%) of an oil, which was used without further purification. On the basis of ^{1}H NMR integration ratios, the product was determined to be a mixture of 88% *m*- and 12% *p*-nitro regioisomers, and no peaks corresponding to the o-nitro isomer were observed: ^{1}H NMR (free base, $CDCl_3$) δ 1.75 (br s, 4H, CH_2CH_2), 2.26 (s, 3H, NCH_3), 2.23–2.79 (complex, 7H, 3 CH_2N and 1 NH), 3.64–3.69 (dd, J = 11, 3.6 Hz, 1H, CH), δ 7.47 (m, 1H, *m*-$NO_2C_6H_4$), δ 7.52 (d, J = 8.4 Hz, *p*-$NO_2C_6H_4$, 12%), δ 7.71 (d, J = 8.7 Hz, 1H, *m*-$NO_2C_6H_4$), δ 8.08 (d, J = 8.4 Hz, 1H, *m*-$NO_2C_6H_4$), δ 8.15 (d, J = 8.7 Hz, *p*-$NO_2C_6H_4$, 12%), δ 8.21 (br s, 1H, *m*-$NO_2C_6H_4$); MS (EI) *m/z* 250.2.

2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-(3-nitrophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide (9). A solution of 3,4-dichlorophenylacetic acid (23.59 g, 115 mmol) in thionyl chloride (66 mL, 905 mmol) was heated at 76 °C for 2.5 h and then evaporated under reduced pressure to yield 3,4-dichlorophenylacetyl chloride. A solution of 3,4-dichlorophenylacetyl chloride in cold, dry CH_2Cl_2 (90 mL) was added dropwise to an ice-cold solution of **8** (26.025 g, 104 mmol) and Et_3N (16 mL, 115 mmol) in dry CH_2Cl_2 (150 mL) with stirring under argon and ice-cooling over 30 min. The mixture was stirred at 25 °C for 21 h before the solvent was removed under reduced pressure. The residue was then dissolved in EtOAc and washed with 3 N NaOH. The organic fraction was dried ($MgSO_4$), filtered, and evaporated to yield 43.5 g (95%) of crude product. Two crystallizations from boiling MeOH yielded 10.24 g (21%) of pure **9**·HCl: mp (**9**·HCl) >260 °C; $[\alpha]^{25}_D$ +137° (c = 0.2, **9**·HCl, MeOH); ^{1}H NMR (**9**·HCl, DMSO-d_6) δ 1.89–2.01 (m, 4H, CH_2CH_2), 2.83 (s, 3H, NCH_3), 3.15–4.19 (complex, 6H, 3 CH_2N), 3.85 (d, J = 17 Hz, 1H, $ArCH_2CO$), 3.99 (d, J = 16 Hz, 1H, $ArCH_2CO$), 6.21 (m, 1H, CH), 7.28–7.30 (m, 1H, $Cl_2C_6H_3$), 7.56–7.57 (m, 2H, $Cl_2C_6H_3$), 7.71–7.72 (m, 2H, *m*-$NO_2C_6H_4$), δ 8.08 (s, 1H, *m*-$NO_2C_6H_4$), δ 8.21–8.22 (m, 1H, *m*-$NO_2C_6H_4$); MS (FAB) *m/z* 436.1. Anal. ($C_{21}H_{23}N_3O_3Cl_2$·HCl·0.5 H_2O) C, H, N, Cl.

2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-(3-aminophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide (10). A mixture of **9** (1.17 g, 2.68 mmol), hydrazine (1 mL, 32 mmol), and Raney Ni in 95% EtOH (75 mL) was heated at 55 °C. Small portions of Raney Ni and hydrazine were added until TLC indicated completion of reaction (2.5 h). The reaction mixture was filtered through Celite, and the Raney Ni was washed with hot MeOH. The combined filtrates were evaporated under reduced pressure to yield **10** in quantitative yield. Further purification was achieved by gravity column chromatography with $CHCl_3$:1% MeOH:2% NH_3: mp (**10**·2HCl) softens at 149 °C and melts at 223–224 °C; $[\alpha]^{25}_D$ +143.7° (c = 0.45, **10**·2HCl, MeOH); ^{1}H NMR (**10**·2HCl, DMSO-d_6) δ 1.9–2.0 (m, 4H, CH_2CH_2), 2.82 (s, 3H, NCH_3), 3.07–4.07 (complex, 6H, 3 CH_2N), 3.77 (d, J = 16 Hz, 1H, $ArCH_2CO$), 4.10 (d, J = 16 Hz, 1H, $ArCH_2CO$), 6.05 (dd, J = 12.5 and 2.5 Hz, 1H, CH), 6.87 (br s, 2H, *m*-$NH_2C_6H_4$), 6.92 (d, J = 8 Hz, 1H, *m*-$NH_2C_6H_4$), 7.26 (t, J = 8 Hz, 1H, *m*-$NH_2C_6H_4$), 7.29–7.31 (m, 1H, $Cl_2C_6H_3$), 7.55–7.59 (m, 2H, $Cl_2C_6H_3$); MS (FAB) *m/z* 406.2. Anal. ($C_{21}H_{25}N_3OCl_2$·2HCl) C, H, N, Cl.

(±)-*N*-(Methoxycarbonyl)-2-(4-nitrophenyl)glycine (12). To a stirred mixture of **11**[22] (3.2400 g, 16.5 mmol) and NaOH (0.6611 g, 16.5 mmol) in water (150 mL) were added methyl chloroformate (1.404 mL, 18.2 mmol), 2 N NaOH (9.1 mL), and water (6 mL) at 0 °C. After adjusting the pH to 9 with 2 N NaOH (1 mL) and stirring at 25 °C for 17 h, the mixture was basified to pH 10 with 2 N NaOH and washed with diethyl ether before it was acidified with 5 N HCl in the presence of EtOAc. The organic fractions (1 L) were dried ($MgSO_4$) and evaporated to yield 3.62 g (86%) of **12**: mp 152 °C; ^{1}H NMR (DMSO-d_6) δ 3.53 (s, 3H, OCH_3), 5.35 (d, J = 8.7 Hz, 1H, CH), 7.66 (d, J = 8.4 Hz, 2H, aromatic), 8.20 (d, J = 8.4 Hz, 3H, aromatic and NH); MS (FAB) *m/z* 255.0.

(±)-Methyl *N*-[(4-Nitrophenyl)(1-pyrrolidinylcarbonyl)methyl]carbamate (13). To a suspension of **12** (1.0049 g, 3.95 mmol) in dry CH_2Cl_2 was added 1-hydroxybenzotriazole (HOBT) (0.5394 g, 3.99 mmol) with ice-cooling and stirring under a N_2 atmosphere. A CH_2Cl_2 solution of dicyclohexylcarbodiimide (DCC) (0.8314 g, 4.03 mmol) was added dropwise, and the reaction mixture was stirred in ice bath for 1 h before pyrrolidine (0.330 mL, 3.95 mmol) was added. After stirring at 25 °C for 5 days, the reaction mixture was filtered through Celite, and the residue remaining after evaporation of the filtrate was dissolved in EtOAc and washed sequentially with saturated $NaHCO_3$, brine, 2 N HCl, and brine. The organic fraction was dried ($MgSO_4$) and evaporated to yield 1.193 g (98%) of **13**: mp 143–145 °C; ^{1}H NMR ($CDCl_3$) δ 1.69–1.94 (m, 4H, CH_2CH_2), 3.00–3.60 (complex, 4H, CH_2NCH_2), 3.63 (s, 3H, OCH_3), 5.45 (d, J = 6 Hz, 1H, CH), 6.42 (d, J = 6 Hz, 1H, NH), 7.61 (d, J = 8.4 Hz, 2H, aromatic), 8.20 (d, J = 8.4 Hz, 2H, aromatic); MS (FAB) *m/z* 307.1.

(±)-1-[2-(Methylamino)-2-(4-nitrophenyl)ethyl]pyrrolidine (14). A solution of **13** (3.0354 g, 9.88 mmol) in dry THF (66 mL) was added dropwise to 1 M BH_3–THF in THF (49.5 mL, 49.5 mmol) with stirring under N_2 and cooling in ice bath. After refluxing for 96 h, excess BH_3–THF was hydrolyzed with concentrated HCl, and the reaction mixture was stirred at 40 °C for 2.5 h before it was evaporated. The residue was then partitioned between 2 N HCl and $CHCl_3$, followed by basification of the aqueous fraction with NaOH(s) and extraction with diethyl ether. After drying ($MgSO_4$) and evaporating the ether fractions, the product was purified by flash column chromatography ($CHCl_3$:1% MeOH:2% NH_3, R_f = 0.19). The yield after purification was 1.22 g (49.7%): ^{1}H NMR ($CDCl_3$) δ 1.75 (br s, 4H, CH_2CH_2), 2.22–2.76 (complex, 6H, 3 CH_2N), 2.26 (s, 3H, NCH_3), 3.66 (dd, J = 9.9 Hz and 3.6 Hz, 1H, CH), 7.52 (d, J = 8.4 Hz, 2H, aromatic), 8.15 (d, J = 8.7 Hz, 2H, aromatic); MS (FAB) *m/z* 249.2.

2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*R*,*S*)-1-(4-nitrophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide (15). Compound **15** was prepared from **14** by a method analogous to the preparation of **9** using 3,4-dichlorophenylacetyl chloride and Et_3N in dry CH_2Cl_2. The crude product was purified by flash column chromatography using $CHCl_3$:2% NH_3:0.9% MeOH as solvent (R_f = 0.2). Crude products from two experiments were combined for the purification, which resulted in recrystallization of 0.90 g (30%) of **15**·HCl (based on a combined theoretical yield of both experiments): mp 254 °C (**15**·HCl); ^{1}H NMR (free base, $CDCl_3$) δ 1.75 (br s, 4H, CH_2CH_2), 2.42–3.14 (complex, 6H, 3 CH_2N), 2.74 (s, 3H, NCH_3), 3.69 (d, J = 15.6 Hz, 1H, $ArCH_2CO$), 3.78 (d, J = 15.9 Hz, 1H, $ArCH_2CO$), 6.10 (dd, J = 9.8 and 6 Hz, 1H, CH), 7.12–7.35 (complex, 3H, $C_6H_3Cl_2$), 7.46

(d, J = 8.4 Hz, 2H, $C_6H_4NO_2$), 8.16 (d, J = 8.7 Hz, 2H, $C_6H_4NO_2$); ^{13}C NMR (**15**·HCl, DMSO-d_6) δ 23.8, 24.2, 31.4, 52.3, 53.6, 56.3, 124.7, 129.8, 130.0, 131.0, 131.4, 131.8, 133.2, 138.4, 145.3, 148.2, 173.3; MS (FAB) m/z 436.2. Anal. ($C_{21}H_{23}N_3O_3Cl_2$·HCl) C, H, N, Cl.

2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*R*,*S*)-1-(4-aminophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide (16). Compound **16** was prepared from **15** by a method analogous to the preparation of **10** using Raney nickel and hydrazine to yield 0.11 g (59%) of **16**: mp (**16**·2HCl) 159–161 °C; ^1H NMR (free base, $CDCl_3$) δ 1.77 (br s, 4H, CH_2CH_2), 2.46–3.22 (complex, 6H, 3 CH_2N), 2.69 (s, 3H, NCH_3), 3.67 (d, J = 15.9, 1H, $ArCH_2CO$), 3.78 (d, J = 14.4 Hz, 1H, $ArCH_2CO$), 6.03 (dd, J = 11.7 and 6 Hz, 1H, CH), 6.64 (d, J = 8.4 Hz, 2H, $C_6H_4NH_2$), 7.09 (d, J = 8.4 Hz, 2H, $C_6H_4NH_2$), 7.15–7.39 (complex, 3H, $C_6H_3Cl_2$); ^{13}C NMR (**16**·2HCl, DMSO-d_6) δ 23.75, 24.15, 51.86, 53.54, 53.60, 56.24, 122.28, 122.36, 129.55, 129.92, 131.00, 131.34, 131.78, 133.18, 133.23, 138.61, 173.04; MS (FAB) m/z 406.2. Anal. ($C_{21}H_{25}N_3OCl_2$·2HCl) C, H, N, Cl.

2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide (3). A solution of freshly distilled thiophosgene (10 drops) in dry THF (16 mL) was added dropwise to an ice-cold solution of **10** (0.47 g, 1.16 mmol) and $NaHCO_3$ (1.02 g, 12 mmol) in H_2O (13 mL) and THF (6.6 mL). The reaction mixture was allowed to slowly warm up to 25 °C as the ice bath melted and stirred at 25 °C overnight. The reaction mixture was extracted with ether and $CHCl_3$, which was dried (Na_2SO_4), filtered, and evaporated. The residue was flash-column chromatographed with $CHCl_3$: 10% acetone:1% Et_3N, and treated with Et_2O·HCl to yield the HCl salt, which was crystallized from CH_3CN–Et_2O to yield 140.7 mg (25%) of **3**·HCl in the first crop: mp (**3**·HCl) 209–210 °C; $[\alpha]^{25}_D$ +149° (c = 0.48, **3**·HCl, CH_3CN); IR (**3**·HCl, KBr) 2126 cm^{-1} (N=C=S); ^1H NMR (**3**·HCl, CD_3CN) δ 2.04 (br s, 4H, CH_2CH_2), 2.83 (s, 3H, NCH_3), 3.0–3.9 (complex, 6H, 3 CH_2N), 3.68 (d, J = 16.5 Hz, 1H, $ArCH_2CO$), 4.23 (d, J = 16.8 Hz, 1H, $ArCH_2CO$), 6.24 (dd, J = 12 and 3 Hz, 1H, CH), 7.18–7.20 (m, 2H, m-$NCSC_6H_4$), 7.25–7.27 (m, 1H, m-$NCSC_6H_4$), 7.28–7.32 (m, 1H, $Cl_2C_6H_3$), δ 7.38–7.45 (m, 2H, $Cl_2C_6H_3$), 7.57 (br s, 1H, m-$NCSC_6H_4$); MS (FAB) m/z 448.1. Anal. ($C_{22}H_{23}N_3OCl_2S$·HCl) C, H, N, Cl.

2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*S*)-1-[3-[(methoxyfumaroyl)amino]phenyl]-2-(1-pyrrolidinyl)ethyl]acetamide (4). To an ice-cold mixture of methyl fumarate monoester (0.1468 g, 1.13 mmol) and HOBT (0.1543 g, 1.14 mmol) in dry CH_2Cl_2 (10 mL) was added a solution of DCC (0.2358 g, 1.14 mmol) in dry CH_2Cl_2 (6 mL). After the mixture was stirred in ice bath for 1 h, a solution of **10** (0.3046g , 0.75 mmol) in CH_2Cl_2 (8 mL) was added, and the reaction mixture was stirred at 25 °C for 3 days before it was filtered. After evaporation of the filtrate, the residue was dissolved in EtOAc and washed with saturated $NaHCO_3$ and brine. The dried organic fraction (Na_2SO_4) was filtered, evaporated, and subjected to flash-column chromatography ($CHCl_3$:2% NH_3:2.25% MeOH, silica gel) to yield 0.3033 g (78%) of **4**, which was converted to the HCl salt with Et_2O·HCl. The twice crystallized (MeOH–Et_2O) salt (**4**·HCl) melted at 240 °C; $[\alpha]^{25}_D$ +135.6° (c = 0.28, **4**·HCl, MeOH); ^1H NMR (**4**·HCl, DMSO-d_6) δ 1.95 (br s, 4H, CH_2CH_2), 2.78 (s, 3H, NCH_3), 3.42 (complex, 6H, 3 CH_2N), 3.72 (s, 3H, OCH_3), 3.76 (d, J = 18.6 Hz, 1H, $ArCH_2CO$), 4.0 (d, J = 16.8 Hz, 1H, $ArCH_2CO$), 6.1 (m, 1H, CH), 6.70 (d, J = 15.6 Hz, 1H, trans HC=CH), 7.01 (d, J = 7.8 Hz, 1H, m-$NHRC_6H_4$), 7.21 (d, J = 15.3 Hz, 1H, trans HC=CH), 7.28 (dd, J = 8 and 2 Hz, 1H, $Cl_2C_6H_3$), 7.35 (t, J = 7.8 Hz, 1H, m-$NHRC_6H_4$), 7.54–7.55 (m, 2H, $Cl_2C_6H_3$), 7.56 (br s, 1H, m-$NHRC_6H_4$), 7.64 (d, J = 7.8 Hz, 1H, m-$NHRC_6H_4$); MS (FAB) m/z 518.1. Anal. ($C_{26}H_{29}N_3O_4Cl_2$·HCl) C, H, N, Cl.

2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*R*,*S*)-1-(4-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide (5). To a mixture of **16** (0.4779 g, 1.176 mmol) and $NaHCO_3$(s) (1.00 g, 11.9 mmol) in $CHCl_3$ (50 mL) and H_2O (50 mL) was added freshly distilled $CSCl_2$ (0.1 mL, 1.3 mmol) in $CHCl_3$ (1 mL) with vigorous stirring at 25 °C. After 2.5 h, the organic fraction was separated, and the aqueous fraction was washed with $CHCl_3$. The combined organic fractions were washed with H_2O, dried (Na_2SO_4), and acidified with Et_2O·HCl. The residue obtained by evaporation of the above was suspended in iPrOH, and an insoluble solid was removed by filtration. The HCl salt was then converted back to the free base and purified on 2 mm chromatotron plates in two equal portions ($CHCl_3$:10% acetone, silica gel). The pure product was converted to the HCl salt with Et_2O·HCl and dried to yield 0.159 g (28%) of **5**·HCl: mp >206–208 °C; IR (KBr) ν NCS 2101 cm^{-1}; ^1H NMR ($CDCl_3$–CD_3CN) δ 2.0–2.1 (m, 4H, CH_2CH_2), 2.83 (s, 3H, NCH_3), 3.00–3.06 (m, 2H, NCH_2 of pyrrolidine), 3.40 (m, 1H, NCH_2 next to stereocenter), 3.65 (d, J = 16.5 Hz, 1H, $PhCH_2CO$), 3.7–3.8 (m, 2H, NCH_2 of pyrrolidine), 3.91 (m, 1H, NCH_2 next to stereocenter), 4.23 (d, J = 16.5 Hz, 1H, $PhCH_2CO$), 6.26 (dd, J = 13 and 3 Hz, 1H, CH), 7.23–7.54 (complex, 7H, aromatic); MS (FAB) m/z 448.1. Anal. ($C_{22}H_{23}N_3OSCl_2$·HCl) C, H, N, Cl.

2-(3,4-Dichlorophenyl)-*N*-methyl-*N*-[(1*R*,*S*)-1-[4-[(methoxyfumaroyl)amino]phenyl]-2-(1-pyrrolidinyl)ethyl]acetamide (6). Target compound **6** was prepared in a manner analogous to that of **4** by treatment of **16** with 2.4 equiv of methyl fumarate monoester, DCC, and HOBT for 7 days at 25 °C. The crude product was flash-column chromatographed ($CHCl_3$:1% Et_3N:2% MeOH, silica gel) and then purified on 2 mm chromatotron plates ($CHCl_3$:4% MeOH, silica gel) followed by conversion to the HCl salt with Et_2O·HCl and dried to yield 37.8 mg (13%) of **6**·HCl: mp >250 °C; ^1H NMR (DMSO-d_6) δ 1.95 (br s, 4H, CH_2CH_2), 2.75 (s, 3H, NCH_3), 3.1–4.0 (complex, 8H, 4 CH_2), 3.72 (s, 3H, OCH_3), 6.06 (m, 1H, CH), 6.69 (d, J = 15.3 Hz, 1H, trans alkene), 7.20–7.68 (complex, 8H, 7 aromatic, 1 trans alkene); MS (FAB) m/z 518.2. Anal. ($C_{26}H_{29}N_3O_4Cl_2$·HCl) C, H, N.

X-ray Crystallography. Colorless crystals of (+)-*S*-**2**·HCl were grown from methanol/anhydrous ethyl ether by vapor diffusion. Polymorphs I and II were obtained from analyses of two different samples. All measurements were made on an Enraf-Nonius CAD-4 diffractometer with graphite monochromated Cu Kα radiation (λ = 1.541 78 Å) using the $\omega - 2\theta$ scan mode. The crystal temperature was maintained at 173(1) K by using a Molecular Structure Corporation low-temperature device. Intensities were corrected for Lorentz and polarization effects. Equivalent reflections were merged; absorption effects were corrected using the ψ-scan method.[45] The structures were solved by direct methods using SHELXS86[46] and refined using the TEXSAN[47] structure analysis package. All nonhydrogen atoms were refined anisotropically. Hydrogens bonded to carbon atoms were placed in calculated positions (0.95 Å) and were not refined. Hydrogens bonded to nitrogen were located in difference Fourier maps and their positional parameters were refined. Crystal data are given in the supplementary material.

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Supplementary Material Available: Tables of positional parameters, bond distances, and bond angles for polymorphs I and II (13 pages). Ordering information is given on any current masthead page.

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