Probes for Narcotic Receptor Mediated Phenomena. 17.[1] Synthesis and Evaluation of a Series of *trans*-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (U50,488) Related Isothiocyanate Derivatives as Opioid Receptor Affinity Ligands

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A series of U50,488 related isothiocyanates was synthesized from enantiomerically pure (*S,S*)-(+)-*trans*-2-pyrrolidinyl-*N*-methylcyclohexylamine [(+)-7] and (*R,R*)-(−)-*trans*-2-pyrrolidinyl-*N*-methylcyclohexylamine [(−)-7]. DCC coupling of (+)- and (−)-7 with nitrophenylacetic acids followed by catalytic hydrogenation and treatment with thiophosgene afforded a series of six isomeric aryl isothiocyanate analogues of U50,488. Similarly, DCC coupling of (+)- and (−)-7 with (+)- and (−)-*N*-*t*-Boc-protected phenylglycines afforded four isomeric alkyl isothiocyanates. Evaluation of the isothiocyanates for their capacity to produce wash-resistant inhibition of μ, δ, and κ sites in vitro was performed using rat and guinea pig brain membranes. None of the compounds was able to irreversibly inhibit binding of [^{3}H]bremazocine to guinea pig and rat brain membranes (depleted of functional μ and δ receptors by pretreatment with acylating agents BIT and FIT). However, (1*S*,2*S*)-*trans*-2-isothiocyanato-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(−)-1] was able to specifically and irreversibly inhibit κ receptors labeled by [^{3}H]-U69,593: Incubation of rat brain membranes for 60 min at 25 °C with 1 μM of (−)-1 resulted in a wash-resistant reduction of the binding to 11.2 ± 2.5% of the control. Binding analysis revealed the wash-resistant reduction in [^{3}H]-U69,593 binding by (−)-1 to be through an increase in the K_d without effect on the B_{max}. (−)-1 failed to effect μ or δ binding in rat or guinea pig brain under the same conditions. The enantiomer of (−)-1, (1*R*,2*R*)-*trans*-2-isothiocyanato-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(+)-1], failed to affect κ receptors labeled by [^{3}H]-U69,593 under the same conditions as for (−)-1. (1*S*,2*S*)-*trans*-3-Isothiocyanato-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(−)-2] inhibited to 49.6 ± 5.1% of the control, in a wash-resistant manner, κ receptors labeled by [^{3}H]-U69,593. However, (−)-2 was not as selective as (−)-1 since it also reduced [^{3}H]DADLE (δ) binding to 82.4 ± 8.0% of the control value. (1*S*,2*S*)-*trans*-4-Isothiocyanato-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide [(−)-3] exhibited selective wash-resistant inhibition of δ receptors labeled by [^{3}H]DADLE resulting in a reduction in binding to 42.9 ± 4.2% of control. In the alkyl isothiocyanate series, (1*S*,2*S*)-*trans*-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-(*S*)-2-phenyl-2-isothiocyanatoacetamide [(−)-11] also showed the capacity to selectively inhibit [^{3}H]-U69,593-sensitive κ sites, resulting in a reduction in binding to 72.2 ± 2.54% of control at 1 μM while (+)-11 was inactive. None of the amino precursors (−)-8, (+)-8, (−)-9, (+)-9, (−)-10, (+)-10, (−)-15, (+)-15, (−)-16, and (+)-16 of the isothiocyanates exhibited the capacity for wash-resistant inhibition of any of the receptor systems tested. Although intracerebroventricular (icv) injection of the most potent compound (−)-1 into guinea pig brain failed to produce any irreversible inhibition of κ receptors, icv injection of the less potent (−)-2 into guinea pig brain resulted in a significant reduction in the κ receptors that bind [^{3}H]-U69,593, but not those that bind [^{3}H]bremazocine. All of the compounds that showed the capacity to irreversibly inhibit κ receptors labeled by [^{3}H]-U69,593 in vitro possessed the 1*S*,2*S* absolute configuration.

Specific, site-directed irreversible agents for opioid receptors have found much use in the study of receptor subtypes.[2-5] In radiolabeled form, they facilitated the isolation and purification of opioid receptors.[6-9] Radiolabeled photoaffinity agents have been used to label and identify both opioid[10] and non-opioid receptors.[11,12] However, unlike electrophilic affinity ligands, they cannot be used to label receptors in vivo or in situations where the receptors are sensitive to UV light.

We have extensively employed the design, synthesis, and biological study of electrophilic affinity ligands as part of our strategy for gaining more insight into the structure and function of the opioid receptor–endorphin system. For example, using the etonitazene derived electrophilic ligand, BIT, we were able to specifically and irreversibly inhibit μ opioid receptor binding.[13] Similarly, FIT and FAO based on fentanyl and 3,14-dihydroxy-4,5-epoxymorphinan, respectively, have enabled us to selectively deplete δ opioid receptor subtypes.[13]

Labeling of crude receptor preparations with [^{3}H]SUPERFIT,[14] a more potent and selective analogue of FIT, allowed the purification to homogeneity of δ opiate receptors on NG108-15 neuroblastoma × glioma cells.[8] This

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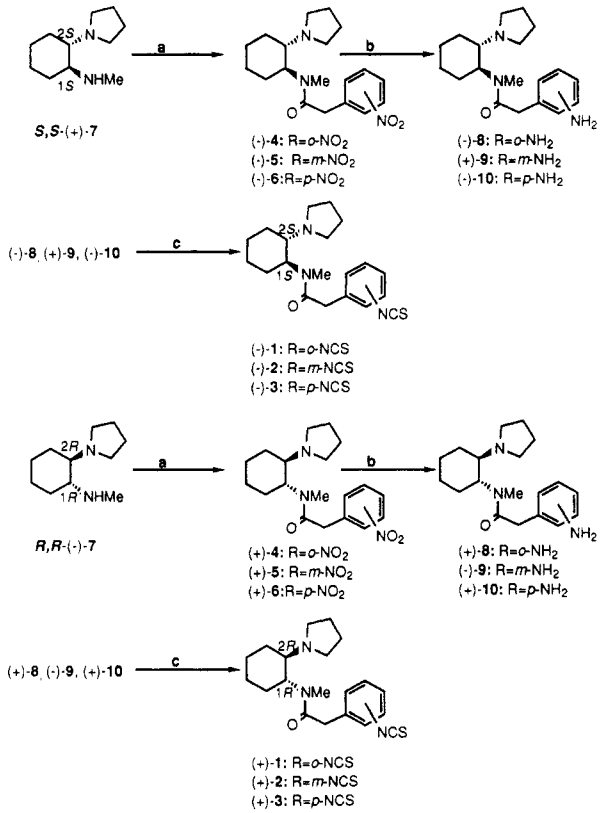
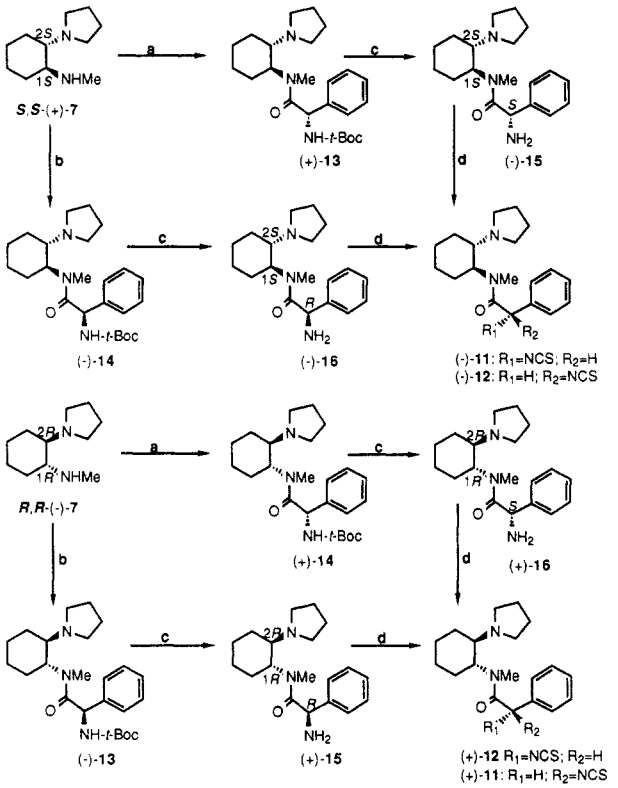
Scheme I. Synthesis of Aryl Isothiocyanate Derivatives of (1*S*,2*S*)- and (1*R*,2*R*)-U50,488[a]



[a] Reagents and conditions: (a) *o*-, *m*-, or *p*-nitrophenylacetic acid, pyridine, DCC, CH_2Cl_2, room temperature. (b) 10% Pd–C, MeOH, H_2, room temperature. (c) $CSCl_2$, $CHCl_3$, aqueous $NaHCO_3$, room temperature, 10 min.

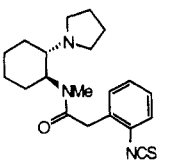
Scheme II. Synthesis of Alkyl Isothiocyanate Derivatives of (1*S*,2*S*)-U50,488 and (1*R*,2*R*)-U50,488[a]



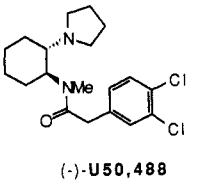
[a] Reagents and conditions: (a) *N*-*t*-Boc-(*S*)-phenylglycine, pyridine, DCC, CH_2Cl_2, room temperature; (b) *N*-*t*-Boc-(*R*)-phenylglycine, pyridine, DCC, CH_2Cl_2, room temperature; (c) 3 M HCl, 60 °C, 10 min; (d) $CSCl_2$, $CHCl_3$, aqueous $NaHCO_3$, room temperature, 10 min.

tool has also proven useful in identification of δ like opioid receptors on cells of the immune system.[9] Another important application of specific site-directed affinity ligands is illustrated by an autoradiographic study in which a combination of affinity ligands was employed to increase the apparent binding selectivity of a nonselective ligand:[2,15] treatment of brain slices with a combination of BIT and FIT depleted μ and δ receptors, respectively, and thus made possible direct autoradiographic analysis of the κ receptor distribution in guinea pig brain with [^{3}H]bremazocine, even though bremazocine binds with high affinity to μ, δ, and κ receptor subtypes.

We recently reported the synthesis of (1*S*,2*S*)-*trans*-2-isothiocyanato-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(-)-1], an enantioselective and specific acylating agent for the κ opioid receptor type,[16] structurally related to the κ selective agent (-)-U50,488.[24] In that study (see also Table II), (-)-1 irreversibly inhibited the binding of the κ selective ligand [^{3}H]-U69,593 but not that of [^{3}H]bremazocine to guinea pig brain κ receptors,[16] thus providing independent evidence for heterogeneity of κ opioid receptors.[17-21]



(-)-1



(-)-U50,488

We now report on attempts to improve the selectivity and potency of (-)-1 by synthesis of a series of isomeric analogues of (-)-1 and analysis of their capacity to acylate μ, δ, and κ ([^{3}H]bremazocine and [^{3}H]-U69,593) opioid receptor subtypes in vitro and then studying in vitro acylators for in vivo activity by intracerebroventricular (icv) injection into guinea pig brain.

Our approach here was to synthesize all the possible isothiocyanate positional isomers in the benzeneacetamide moiety of (+)-1 and its enantiomer (-)-1 and then to de-

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Table I. Synthetic Data on U50,488-Related Isothiocyanate Derivatives and Synthetic Intermediates

compound	synthetic procedure	mp, °C	recrystn solvent	yield, %	$[\alpha]^{25}_D$, deg (c, g/100 mL of MeOH)	formula[a]
(−)-1·HCl	C	179–181	*i*PrOH–Et_2O	54	−63.1 (0.52)	$C_{20}H_{28}ClN_3OS·0.5H_2O$
(−)-2·HBr	C	100–105	*i*PrOH–Et_2O	63	−34.2 (0.61)	$C_{20}H_{28}BrN_3OS$
(−)-3·HBr	C	205–206	*i*PrOH–Et_2O	48	−41.2 (0.89)	$C_{20}H_{28}BrN_3OS$
(+)-1·HCl	C	179–181	*i*PrOH–Et_2O	58	+64.4 (0.89)	$C_{20}H_{28}ClN_3OS·0.5H_2O$
(+)-2·HBr	C	100–105	*i*PrOH–Et_2O	58	+32.3 (1.29)	$C_{20}H_{28}BrN_3OS$
(+)-3·HBr	C	205–206	*i*PrOH–Et_2O	33	+40.8 (0.71)	$C_{20}H_{28}BrN_3OS$
(−)-4·HBr	A	236–237	*i*PrOH–Et_2O	89	−14.6 (0.82)	$C_{19}H_{28}BrN_3O_3S$
(−)-5·HI	A	206.5–207.5	EtOH	86	−23.8 (0.42)	$C_{19}H_{28}IN_3O_3$
(−)-6·HCl	A	218–220	*i*PrOH–Et_2O	81	−38.3 (0.33)	$C_{19}H_{28}ClN_3O_3$
(−)-8·HBr	B	127–129	*i*PrOH	97	−19.9 (0.83)	$C_{19}H_{30}BrN_3O·C_3H_8O$
(+)-9 (base)	B	79–80	EtOAc–hexane	75	+11.7 (1.32)	$C_{19}H_{29}N_3O$
(−)-10·HCl	B	225–226	*i*PrOH–Et_2O	77	−29.7 (0.99)	$C_{19}H_{30}ClN_3O$
(+)-4·HBr	A	236–237	*i*PrOH–Et_2O	88	+15.1 (0.62)	$C_{19}H_{28}BrN_3O_3S$
(+)-5·HI	A	206.5–207.5	EtOH	80	+22.6 (0.66)	$C_{19}H_{28}IN_3O_3$
(+)-6·HCl	A	218–220	*i*PrOH–Et_2O	78	+40.0 (0.58)	$C_{19}H_{28}ClN_3O_3$
(+)-8·HBr	B	127–129	*i*PrOH	100	+22.5 (0.78)	$C_{19}H_{30}BrN_3O·C_3H_8O$
(−)-9 (base)	B	80–81	EtOAc–hexane	75	−13.5 (0.76)	$C_{19}H_{29}N_3O$
(+)-10·HCl	B	225–226	*i*PrOH–Et_2O	86	+32.0 (0.83)	$C_{19}H_{30}ClN_3O$
(−)-11·HCl	C	195–196	EtOAc	80	−79.6 (0.56)	$C_{20}H_{28}ClN_3OS$
(−)-12 (base)	F	114–115	isooctane	82	−63.0 (0.45)	$C_{20}H_{27}N_3OS·0.25H_2O$
(+)-12 (base)	F	195–196	isooctane	82	+61.1 (0.48)	$C_{20}H_{27}N_3OS·0.25H_2O$
(+)-11·HCl	C	195–196	EtOAc	72	+80.4 (0.39)	$C_{20}H_{28}ClN_3OS$
(+)-13 (base)	D	87–88	hexane	100	+81.9 (0.57)	$C_{24}H_{37}N_3O_3$
(−)-14 (base)	D	oil		97	−74.7 (0.38)[b]	$C_{24}H_{37}N_3O_3$[d]
(−)-15 (base)	E	92–93	isooctane	87	−73.6 (0.64)	$C_{19}H_{29}N_3O$
(−)-16·2HCl	E	193–195	MeOH/EtOAc	84	−44.2 (0.56)	$C_{19}H_{31}Cl_2N_3O$
(+)-14 (base)	D	oil		93	+69.2 (0.75)[c]	$C_{24}H_{37}N_3O_3$[e]
(−)-13 (base)	D	87–88	hexane	97	−81.0 (0.48)	$C_{24}H_{37}N_3O_3$
(+)-16·2HCl	E	193–195	MeOH/EtOAc	83	+45.0 (0.48)	$C_{19}H_{31}Cl_2N_3O$
(+)-15 (base)	E	90–91	isooctane	86	+73.0 (1.11)	$C_{19}H_{29}N_3O$

[a] All compounds (with the exception of oily (+)-14 and (−)-14) were determined to have elemental analyses within ±0.4% of the theoretical value for C, H, and N. [b] Due to solvent retention in oil, this is an approximate value. [c] Due to the possibility of solvent retention in oil, this is an approximate value. [d] M^+ (found) 415.2810, calculated 415.2834. [e] M^+ (found) 415.2829, calculated 415.2835.

termine their ability to produce wash-resistant inhibition at μ, δ, and κ receptors in vitro. We considered this the optimum approach since structurally related benzeneacetamides devoid of the aromatic chlorine atoms are more easily prepared and are known to be active as selective κ agonists.[22] Because enantiomeric drugs can produce different and in some cases opposite effects,[23] it was imperative to synthesize all of the isothiocyanate derivatives in enantiomeric form in order to obtain the most meaningful results in this study.

Chemistry

The structures and synthesis of the compounds in Table I are shown in Schemes I and II. Compounds (−)-1, (−)-2, and (−)-3 (Scheme I) based on (*S,S*)-(−)-U50,488 were prepared via the DCC coupling of (*S,S*)-(+)-7[16,24] with, respectively, 2-, 3-, and 4-nitrophenylacetic acids. The resulting 2-, 3-, and 4-nitro-substituted benzeneacetamides (−)-4, (−)-5, and (−)-6 were catalytically reduced (10% Pd/C) to the corresponding anilines (−)-8, (+)-9, and (−)-10. Treatment of (−)-8, (+)-9, and (−)-10 with thiophosgene[25] afforded the corresponding isothiocyanates (−)-1, (−)-2, and (−)-3. Compounds (+)-1, (+)-2, and (+)-3 (Scheme I), based on (*R,R*)-(+)-U50,488 were prepared similarly, with (*R,R*)-(−)-7 as the starting material.[16,24] Compounds (−)-11 and (−)-12 were prepared (Scheme II) by DCC coupling of *t*-Boc-protected (*S*)-(+)-2-phenylglycine and *t*-Boc-protected (*R*)-(−)-2-phenylglycine, respectively, with (*S,S*)-(+)-7 to give the corresponding amides (+)-13 and (−)-14. Conversion of (+)-13 and (−)-14 to their primary amines (−)-15 and (−)-16 with aqueous HCl[26] followed by treatment with thiophosgene afforded (−)-11 and (−)-12.

Similarly, (+)-11 and (+)-12 were synthesized (Scheme II) by DCC coupling of *t*-Boc-protected (*S*)-(+)-2-phenylglycine and *t*-Boc-protected (*R*)-(−)-2-phenylglycine with (*R,R*)-(−)-7. No evidence for racemization of the N-protected 2-phenylglycines was observed during the above DCC coupling reactions or in subsequent steps. Since the absolute configurations of the enantiomers of (±)-7 have been previously established and their optical purity demonstrated,[24] the use of (*S,S*)-(+)-7 and (*R,R*)-(−)-7 defined the absolute stereochemistry of all the intermediates and final products tested in this series of compounds.

Results and Conclusion

We previously showed (−)-1 was a specific and relatively potent irreversible inhibitor of guinea pig brain κ ([^{3}H]-U69,593) receptors resulting in irreversible inhibition of [^{3}H]-U69,593 binding with an IC50 of 100 nM.[16] The present study revealed that (−)-1 was the most potent member of the series at irreversibly inhibiting the binding of [^{3}H]-U69,593 to the guinea pig brain κ receptor, resulting in a significant increase (doubling) of the K_d value after incubation of the membranes at 25 °C with 100 nM of (−)-1. Interestingly, (−)-1 failed to significantly reduce the B_{max},[16] a change not necessarily produced by irreversible inhibitors of drug receptors. The effect of (−)-1 on increasing the K_d without any effect on the B_{max} suggests that (−)-1 may acylate a site which allosterically decreases

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Table II. Summary of Effects of Amine and Isothiocyanate Derivatives on Wash-Resistant Binding of Radioligands to μ, δ, and κ Receptors in Rat and Guinea Pig (gp) Brain[a]

	in vitro % (±SD) of control binding				
		κ		δ:	μ:
compound	[^{3}H]-U69,593 (gp)	[^{3}H]BREM (gp)	[^{3}H]BREM (rat)	[^{3}H]DADLE (rat)	[^{3}H]FOXY (rat)
		Effect of Amino Precursors (to Isothiocyanates)			
control	100 ± 18				
(−)-8	96.6 ± 4.7				
(+)-8	95.0 ± 2.4				
(−)-9	109.7 ± 3.6				
(+)-9	86.8 ± 7.2				
(−)-10	88.0 ± 8.8				
(+)-10	94.6 ± 7.0				
(−)-15	85.6 ± 4.6				
(+)-15	86.5 ± 7.8				
(−)-16	114 ± 9.6				
(+)-16	120 ± 1.9[b]				
		Effect of Isothiocyanate Derivatives			
control	100 ± 9.3	100 ± 4.0	100 ± 8.3	100 ± 8.7	100 ± 15.9
(−)-1	11.2 ± 2.5[b]	97.6 ± 5.6	98.1 ± 14.6	100.3 ± 2.9	118.7 ± 2.5
(+)-1	95.9 ± 6.5	108.0 ± 1.8	91.9 ± 13.1	89.0 ± 3.0[f]	69.0 ± 17.0[e]
(−)-2	49.6 ± 5.1[b]	101.8 ± 3.2	93.0 ± 4.4	82.4 ± 8.0[e]	107.0 ± 5.0
(+)-2	93.6 ± 2.1	110.0 ± 5.7	97.1 ± 4.0	92.3 ± 11.6	103.6 ± 14.9
(−)-3	70.4 ± 3.6[c]	92.1 ± 7.6	79.7 ± 9.7[e]	42.9 ± 4.2[b]	109.0 ± 5.5
(+)-3	94.7 ± 8.5	108.0 ± 3.9	87.6 ± 1.9	113.4 ± 19.3	123.9 ± 22.3
(−)-11	72.2 ± 2.5[c]	96.0 ± 2.3	91.2 ± 2.8	91.6 ± 6.5	108.2 ± 8.5
(+)-11	108.5 ± 9.6	101.2 ± 19.9	92.7 ± 8.2	117.3 ± 2.1	131.7 ● 7.6[d]
(−)-12	106 ± 2.1	99.7 ± 5.2	95.4 ± 2.1	94.6 ± 6.6	112 ± 8.2
(+)-12	119 ± 1.9[b]	94.2 ± 5.4	95.5 ± 1.8	102.0 ± 9.3	86.0 ± 26.0

[a] Frozen brain membranes were thawed, resuspended in 10 mM MOPS (3-morpholinopropanesulfonic acid) buffer, pH 7.4, containing 3 mM $MnCl_2$, and then incubated for 60 min at 25 °C in the presence or absence of 1 μM of various drugs. The incubation was terminated by centrifugation (11000g for 10 min), and membranes were washed three additional times by resuspension and centrifugation. The pellets were then resuspended in 50 mM Tris-HCl, pH 7.0, incubated for 60 min at 25 °C, and washed twice by centrifugation. The final pellets were resuspended in the appropriate buffer for assay. This washing protocol was sufficient to prevent any inhibition of [^{3}H]-U69,593 binding when the membranes were incubated with 1 μM of the amine precursors of isothiocyanates (−)-1, (−)-2, (−)-3, (+)-1, (+)-2, (+)-3 and (+)-11, (+)-12, (−)-11, (−)-12. (1) κ binding sites were measured with use of [^{3}H]-U69,593 (1.18 nM, sp act. = 40 Ci/mmol).[27] Incubations took place for 60 min at 37 °C (equilibrium) in 50 mM Tris-HCl, pH 7.4, containing 3 mM $MnCl_2$, 0.1 mg/mL bovine serum albumin, and several protease inhibitors: bacitracin (0.1 mg/mL), bestatin (0.01 mg/mL), leupeptin (0.004 mg/mL), chymostatin (0.002 mg/mL), and captopril (0.001 mg/mL). Incubations were terminated by rapid filtration over glass fiber filters presoaked in 1% polyethylenimine. The nonspecific binding was determined by incubations in the presence of 1 μM U69,593. (2) μ binding sites were measured with [^{3}H]FOXY (1.16 nM, sp act. = 53 Ci/mmol) as previously described.[28] (3) [^{3}H]DADLE (1.34 nM, sp act. = 46.9 Ci/mmol) binding sites (higher and lower affinity binding sites) were measured by using previously described methods.[29] With this ligand, 76% of the cpm were to the higher affinity (δ) site while 24% of the cpm were due to the μ binding site. (4) κ binding sites were also measured with use of [^{3}H]bremazocine (1.31 nM, sp act. = 21.3 Ci/mmol) with the following modifications of published procedure:[2] incubations were in the absence of NaCl, and the same protease inhibitors used in the [^{3}H]-U69,593 binding assay were included in the assay; nonspecific binding was determined in the presence of 1 μM bremazocine. Guinea pig membranes used for the [^{3}H]-U69,593 and [^{3}H]bremazocine binding assays were pretreated with the site-directed acylating agents 2-(*p*-ethoxybenzyl)-1-[(diethylamino)ethyl]-5-isothiocyanatobenzimidazole hydrochloride (BIT) and *N*-phenyl-*N*-[1-[2-(*p*-isothiocyanatophenyl)ethyl]-4-piperidinyl]propanamide hydrochloride (FIT), with minor modifications of published methods.[30] BIT and FIT completely eliminate μ and δ binding sites, respectively.[2] [^{3}H]DADLE and [^{3}H]FOXY binding assays used frozen, lysed P2 membranes prepared from rat brain as described elsewhere.[27,30] Each value is the mean ± SD of three independent experiments. [b] $p < 0.001$ when compared to the control. [c] $p < 0.01$ when compared to the control. [d] $p < 0.02$ when compared to the control. [e] $p < 0.05$ when compared to the control. [f] $p < 0.1$ when compared to the control.

[^{3}H]-U69,593 binding. Alternatively, (−)-1 may acylate a site just outside of the κ receptor in such a way that it sterically impedes binding to the κ receptor. The selectivity of (−)-1 for κ receptors was apparent from the failure to observe inhibition of δ ([^{3}H]DADLE) or μ ([^{3}H]FOXY) (Table II) binding. The observation that (−)-1 failed to irreversibly inhibit [^{3}H]bremazocine binding in guinea pig brain membranes pretreated with BIT and FIT (to deplete μ and δ receptors[2]) (Table II)[16] offers an independent line of evidence for heterogeneity of κ receptors.[17-21] Compounds (−)-1, (−)-2, and (−)-3 (Scheme I) and (−)-11 (Scheme II) derived from the diamine precursor (*S,S*)-(+)-7 were found to acylate the κ receptor binding site labeled by [^{3}H]-U69,593 (Table II) in guinea pig brain membranes after incubation of the membranes at 25 °C with 1 μM of the drugs followed by a wash protocol sufficient to remove the amino precursors of the isothiocyanate and analysis of residual binding with [^{3}H]-U69,593. Compound (−)-12 (the epimer of (−)-11 and also derived from (*S,S*)-(+)-7), had no effect on μ, δ, or κ receptor binding (Table II). Although compounds (−)-2, (−)-3, and (−)-11 also irreversibly inhibited [^{3}H]-U69,593 binding (Table II) and failed to have any effect on [^{3}H]bremazocine binding (Table II), the extent of this inhibition was less than that observed for (−)-1. In the case of (−)-2 and (−)-3, the inhibition was not as selective as for (−)-1 (Table II). For example, (−)-2 at 1 μM resulted in an irreversible reduction of guinea pig brain [^{3}H]-U69,593 binding to 49.6% of the control value, and also caused a wash-resistant inhibition of [^{3}H]DADLE binding (Table II). Compound (−)-3 was more selective as an irreversible δ receptor inhibitor, reducing [^{3}H]DADLE binding to 42.9 ± 4.2% of the control at 1 μM. Compound (−)-11 was the

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Table III. Effects of Isothiocyanates (-)-1 and (-)-2 on [^{3}H]-U69,593 and [^{3}H]Bremazocine Binding after Intracerebroventricular Injection into Guinea Pigs[a]

	[^{3}H]bremazocine		[^{3}H]-U69,593	
compd	specific binding, fmol/mg of protein	% control	specific binding, fmol/mg of protein	% control
control	145.2 ± 8.94	100	13.16 ● 0.71	100
(-)-1	167.4 ± 16.67	115.2	12.31 ± 1.78	93.5
(-)-2	128.2 ± 0.065	88.2	10.1 ± 0.785	76.7[b]

[a] Intracerebroventricular injections: male Hartley guinea pigs weighing 300 g were anesthetized with halothane. Following placement in a stereotactic apparatus, an incision was made that exposed the cisterna magnum. An injection cannula consisting of 30-gauge thin wall stainless steel tubing extending 2 mm beyond a 24-gauge outer shell was inserted into this structure. A Gilmont microinjector was used to deliver agents through polyethylene tubing attached to the injection cannula. Twenty-four hours after injection, animals were decapitated, and brain tissue was immediately frozen in -80 °C isopentane. The frozen tissue was later prepared for binding assay with [^{3}H]-U69,593 and [^{3}H]bremazocine as described in the footnote to Table I.[27] Injections consisted of 100 μg each of (-)-1 and (-)-2, each dissolved in 10 μL of dimethyl sulfoxide. [b] $p < 0.05$ when compared with the control.

next most selective compound to (-)-1 as an irreversible inhibitor of [^{3}H]-U69,593 binding (Table II), but was less potent since it reduced [^{3}H]-U69,593 binding to only 72.2 ± 2.5% of the control at 1 μM (Table II). The precursors of (-)-1, (-)-2, (-)-3, (-)-11, and (-)-12, compounds (-)-8, (+)-9, (-)-10, (-)-15, and (-)-16, respectively, all failed to produce a significant irreversible effect on guinea pig brain [^{3}H]-U69,593 binding (Table II).

When membranes were pretreated with the corresponding enantiomers of the above isothiocyanates, compounds (+)-1, (+)-2, (+)-3, and (+)-11, respectively (Schemes I and II), a significant decrease in [^{3}H]-U69,593 binding was not produced, indicating that the irreversible inhibition of [^{3}H]-U69,593 by (-)-1, (-)-2, (-)-3, and (-)-11 is enantiospecific under the conditions employed. Pretreatment of guinea pig brain membranes with 1 μM of either compound (+)-12 or its amine precursor (+)-16 resulted in an apparent irreversible increase in [^{3}H]-U69,593 binding. The reason for this effect is unclear at the present time. The amino precursors of (+)-1, (+)-2, (+)-3, and (+)-11, compounds (+)-8, (-)-9, (+)-10, and (+)-15, had no significant irreversible effect on [^{3}H]-U69,593 binding (Table II). Compounds (+)-1, (+)-2, (+)-3, (+)-11, and (+)-12 failed to have any significant effect on the κ binding site labeled by [^{3}H]bremazocine in either the rat or the guinea pig (Table II). However, (+)-1 exhibited irreversible inhibition of μ ([^{3}H]FOXY) binding in rat brain (Table II), reducing the binding to 69.0 ± 17% of the control value at 1 μM. Also of interest is the apparent increase in μ binding resulting from treatment of rat brain membranes with (+)-11 (Table II).

Compounds (-)-1 and (-)-2 and their aniline precursors (-)-8 and (+)-9 were tested for their capacity to cause κ ([^{3}H]BREM and [^{3}H]-U69,593) inhibition in vivo after icv injection into guinea pig brain (Table III). Thus, icv injection of 100 μg each of (-)-1, (-)-2, (-)-8, and (+)-9 into guinea pig brain followed by decapitation after 24 h and analysis of the brain tissue for κ receptor binding revealed the extent of in vivo acylation. The isothiocyanate anilino precursors (-)-8 and (+)-9 failed to affect κ binding. Of the isothiocyanates, the more potent (-)-1 failed to show a significant reduction in [^{3}H]BREM or [^{3}H]-U69,593 binding. However, surprisingly, the less potent (-)-2, although having no effect on [^{3}H]BREM binding, resulted in a small but significant wash-resistant inhibition of [^{3}H]-U69,593 binding (Table III) to 76.7 ± 6.0% of control. This result can be rationalized by the possibility of different κ receptor conformations between in vivo and in vitro situations which may result in enhanced acylation by the less potent (in vitro) (-)-2 compared with (-)-1. Furthermore, differences in the metabolism and biodistribution of (-)-1 and (-)-2 in vivo may significantly alter the relative concentrations of these drugs reaching the κ receptors. The results of this study indicate that compounds derived only from (*S,S*)-(+)-7 exhibit the capacity to acylate κ receptors labeled by [^{3}H]-U69,593. Furthermore, the results show that of the compounds derived from (*S,S*)-(+)-7, only (-)-1 and (-)-11 are selective in this effect. This study also concludes that (-)-1[16] is the most potent member of the series in its ability to selectively acylate [^{3}H]-U69,593 sensitive κ receptors in vitro in guinea pig brain, while (-)-2 shows some wash-resistant inhibition of [^{3}H]-U69,593 binding in vivo (Table III). Since none of the compounds that irreversibly depleted [^{3}H]-U69,593 binding was able to affect the binding of [^{3}H]bremazocine in rat or guinea pig brain, the results confirm earlier observations of heterogeneity of κ receptors.[16-21]

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR spectra were recorded in $CDCl_3$ solution with a Varian XL-300 MHz spectrometer. Infrared (IR) spectra were recorded with a Beckman IR 4230 spectrophotometer from KBr pellets. Chemical-ionization mass spectra (CIMS) were determined with a Finnegan 1015D mass spectrometer with a Model 6000 data collection system. Electron-ionization mass spectra (EIMS) and accurate molecular ion determinations were made with a VG-Analytical Micromass 7070F mass spectrometer. All compounds had IR, NMR, and mass spectral data in full accordance with their structure. Physical and chemical data are reported in Table I. Specific rotation measurements were determined with a Perkin-Elmer 241 MC polarimeter. All determinations were made in MeOH solution in a 1-dM cell at the indicated concentration (g/100 mL). Thin-layer chromatography (TLC) was performed on Analtech GHLF 250 μM plates in a solvent system consisting of concentrated aqueous NH_3–MeOH–$CHCl_3$ (1:9:90). Amine salts were converted to their free bases by partitioning between 10% aqueous NaOH and ether. Elemental compositions for all crystalline compounds reported in Table I were determined at Atlantic Microlabs, Atlanta, GA, and are within 0.4% of the theoretical values calculated for C, H, and N.

(1*S*,2*S*)-*trans*-2-Nitro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide [(-)-4]. Procedure A. To a solution of (+)-7 (0.51 g, 2.79 mmol), 2-nitrophenylacetic acid (0.76 g, 4.19 mmol), and dry pyridine (110 μL, 1.40 mmol) in alcohol-free CH_2Cl_2 (20 mL) was added DCC (1.15 g, 5.57 mmol), and the reaction mixture was stirred overnight at room temperature. Filtration of the precipitated *N,N'*-dicyclohexylurea and evaporation of the solvent afforded an oily residue. Addition of 50 mL of 10% aqueous citric acid followed by extraction with ether (3 × 50 mL) removed any neutral products from the aqueous layer. The ether extract was discarded, and the aqueous layer was brought to pH 9 by addition of excess concentrated aqueous NH_3. Extraction with CH_2Cl_2 (3 × 50 mL), drying of the organic extract through a plug of Na_2SO_4, and evaporation of the solvent afforded (-)-4 as a colorless oil. Crystallization of the HBr salt from 2-propanol–ether afforded an analytically pure sample of (-)-4·HBr (Table I). Compounds (-)-5, (-)-6 and (+)-4, (+)-5, (+)-6 (Scheme I) were similarly prepared (Table I).

(1*S*,2*S*)-*trans*-2-Amino-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide [(-)-8]. Procedure B. A solution of (-)-4·HBr (0.5 g, 1.17 mmol) in MeOH (25 mL) was hydrogenated in a Parr bottle under 50 psi H_2 over 10% Pd/C (100 mg). TLC of the reaction mixture indicated that the reaction was complete after 1 h. Filtration of the catalyst through a pad of Celite and evaporation of the solvent afforded crystalline (-)-8·HBr in quantitative yield. Purification from 2-propanol afforded an analytically pure sample (Table I). Compounds (+)-9,

(−)-**10**, (+)-**8**, (−)-**9**, and (+)-**10** (Scheme I) were similarly prepared (Table I).

(1*S*,2*S*)-*trans*-2-Isothiocyanato-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(−)-1]. Procedure C. To a rapidly stirred solution of (−)-**8**·HBr (100 mg, 0.23 mmol) in a mixture of $CHCl_3$ (10 mL) and aqueous $NaHCO_3$ (191 mg, 10 equiv in 10 mL of water) was added a solution of freshly redistilled thiophosgene (19.1 μL, 1.1 equiv) in $CHCl_3$ (1 mL). After 20 min at room temperature, reaction was complete (TLC). The organic layer was separated, diluted to 50 mL with $CHCl_3$, and washed once with water. Drying by filtration through a short column of Na_2SO_4 followed by evaporation of the solvent afforded (−)-**1** as a viscous oil. Recrystallization of (−)-**1**·HBr from 2-propanol–ether afforded an analytically pure sample (Table I). Compounds (−)-**2**, (−)-**3** and (+)-**1**, (+)-**2**, (+)-**3** (Scheme I), and (+)-**11** and (−)-**11** (Scheme II) were similarly prepared (Table I).

(1*S*,2*S*)-*trans*-*N*-Methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-*N*-*t*-Boc-(*S*)-2-amino-2-phenylacetamide [(+)-13]. Procedure D. To a stirred solution of *N*-*t*-Boc-(*S*)-phenylglycine (4.13 g, 3.0 equiv), (*S*,*S*)-(+)-**7** (1.00 g, 5.48 mmol), and pyridine (0.43 g, 0.5 equiv) in alcohol-free CH_2Cl_2 (20 mL) was added DCC (4.52 g, 4.0 equiv) and the solution stirred for 10 min at room temperature. Precipitated DCU was filtered and washed with ether 50 mL. The organic layer and ether washings were diluted to 200 mL with ether and washed with 100 mL of 10% aqueous citric acid. The citric acid layer was back-washed with 3 × 50 mL of ether, and the ether washings were discarded. Addition of excess aqueous NH_3 to the citric acid extract precipitated free (+)-**13**. Extraction with CH_2Cl_2 (2 × 100 mL) afforded pure (TLC) (+)-**13** as a colorless foam. An analytically pure sample of (+)-**13** was obtained by crystallization of the foam from cold (0 °C) *n*-hexane (Table I). Compounds (−)-**13**, (+)-**14**, and (−)-**14** (Scheme II) were similarly prepared (Table I).

(1*S*,2*S*)-*trans*-*N*-Methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-(*S*)-2-amino-2-phenylacetamide [(−)-15]. Procedure E. (+)-**13** (2.00 g, 4.82 mmol) was dissolved in 3 M HCl (50 mL) and the solution stirred for 10 min at 60 °C. Addition of ice (50 g), followed by enough excess NH_3 to make the solution basic, liberated free (−)-**15**. Extraction with 3 × 30 mL of CH_2Cl_2 followed by drying of the organic extract through a short column of Na_2SO_4 afforded (−)-**15** as a crystalline solid. Recrystallization from isooctane yielded an analytically pure sample (Table I). Compounds (+)-**15**, (−)-**16**, and (+)-**16** (Scheme II) were similarly prepared (Table I).

(1*S*,2*S*)-*trans*-*N*-Methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-(*R*)-2-phenyl-2-isothiocyanatoacetamide [(−)-12]. Procedure F. To a rapidly stirred solution of (−)-**16**·2HCl (0.40 g, 1.03 mmol) in a mixture of saturated $NaHCO_3$ (20 mL) and $CHCl_3$ (20 mL) was added freshly redistilled thiophosgene (86.5 μL, 1.1 equiv). After 10 min at room temperature, reaction was complete by TLC. The organic layer was separated, diluted to 50 mL with $CHCl_3$, and washed with water (50 mL). Filtration through a column of Na_2SO_4, followed by evaporation of the solvent, afforded the crude product as an impure dark red oil. Addition of ether (100 mL) followed by trituration gave a dark red insoluble precipitate. The ether triturate was filtered through a pad of Celite and evaporated to give purified (−)-**12** as a crystalline solid. Recrystallization from isooctane afforded an analytically pure sample (Table I). Compound (+)-**12** (Scheme II) was similarly prepared (Table I).

Registry No. (−)-**1**, 118243-28-0; (+)-**1**, 118243-30-4; (−)-**1**·HCl, 118170-30-2; (−)-**1**·HBr, 125074-70-6; (+)-**1**·HCl, 118331-98-9; (−)-**2**, 124942-76-3; (+)-**2**, 125132-66-3; (−)-**2**·HBr, 125072-94-8; (+)-**2**·HBr, 125072-96-0; (−)-**3**, 124942-77-4; (+)-**3**, 125072-85-7; (−)-**3**·HBr, 125072-95-9; (+)-**3**·HBr, 125132-67-4; (−)-**4**, 125073-02-1; (+)-**4**, 125073-04-3; (−)-**4**·HBr, 118245-43-5; (+)-**4**·HBr, 118170-32-4; (−)-**5**, 125073-03-2; (+)-**5**, 125132-73-2; (−)-**5**·HI, 124942-82-1; (+)-**5**·HI, 125072-98-2; (−)-**6**, 125132-72-1; (+)-**6**, 125073-05-4; (−)-**6**·HCl, 125074-69-3; (+)-**6**·HCl, 124942-83-2; (−)-**7**, 67347-43-7; (+)-**7**, 67198-53-2; (−)-**8**, 118243-27-9; (+)-**8**, 118243-29-1; (−)-**8**·HBr, 118170-31-3; (+)-**8**·HBr, 118331-97-8; (−)-**9**, 124942-79-6; (+)-**9**, 125072-89-1; (−)-**10**, 124942-80-9; (+)-**10**, 125072-90-4; (−)-**10**·HCl, 125072-97-1; (+)-**10**·HCl, 125132-68-5; (−)-**11**, 124942-78-5; (+)-**11**, 125072-86-8; (−)-**11**·HCl, 124942-84-3; (+)-**11**·HCl, 125132-69-6; (−)-**12**, 125072-87-9; (+)-**12**, 125072-88-0; (−)-**13**, 125073-01-0; (+)-**13**, 124942-85-4; (−)-**14**, 125072-99-3; (+)-**14**, 125073-00-9; (−)-**15**, 124942-81-0; (+)-**15**, 125072-91-5; (−)-**16**, 125072-92-6; (+)-**16**, 125072-93-7; (−)-**16**·2HCl, 125132-70-9; (+)-**16**·2HCl, 125132-71-0; *o*-nitrophenylacetic acid, 3740-52-1; *m*-nitrophenylacetic acid, 1877-73-2; *p*-nitrophenylacetic acid, 104-03-0; *N*-*t*-Boc-(*S*)-phenylglycine, 2900-27-8; *N*-*t*-Boc-(*R*)-phenylglycine, 33125-05-2.