mean blood pressure by 15 mmHg (peak effect).

Antiaggregation Activity in Vitro. Male Wistar rats (250–350 g) under ether anesthesia were used. Blood sample was taken from the abdominal aorta into a plastic syringe containing 0.1 volume of 3.13% sodium citrate solution. Platelet aggregation was measured by using an aggregometer (Chrono-Log Corp., Havertown, PA) at 37 °C under stirring. Aggregation was initiated by adding 5 μL of collagen (final concentration 5 μg/mL).

Antiaggregation Activity ex Vivo. Male Wistar rats (150–200 g) were orally given a test drug solubilized in 1.3% $NaHCO_3$ with 5% EtOH or the vehicle alone as a control. At the scheduled time after oral administration of the drug, the blood which was obtained by the same manner as in vitro was centrifuged at 250*g* for 7 min at room temperature. The supernatant fraction was used as platelet-rich plasma (PRP). Platelet-poor plasma (PPP) was obtained from the supernatant fraction of the residual blood by centrifugation at 1300*g* for 10 min. A 495-μL volume of PRP was placed in a cuvette and incubated at 37 °C for 3 min. After incubation, platelet aggregation was initiated by adding 5 μL of collagen (final concentration 5 μg/mL). Changes in the light transmission in the cuvette after addition of collagen were recorded.

Alterations in the Stereochemistry of the κ-Selective Opioid Agonist U50,488 Result in High-Affinity σ Ligands

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The synthesis and in vitro σ receptor activity of the two diastereomers of U50,488 [(±)-**2**], namely, (1*R*,2*S*)-(+)-*cis*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(+)-**1**] and (1*S*,2*R*)-(−)-*cis*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(−)-**1**], are described. (+)-**1** and (−)-**1** were synthesized from (±)-*trans*-*N*-methyl-2-aminocyclohexanol [(±)-**3**]. Pyridinium chlorochromate (PCC) oxidation of the *N*-*t*-Boc-protected derivative of (±)-**3** afforded (±)-2-[*N*-[(*tert*-butyloxy)carbonyl]-*N*-methylamino]cyclohexanone [(±)-**5**]. The sequence of enamine formation with pyrrolidine, catalytic reduction, N-deprotection, and optical resolution afforded (1*R*,2*S*)-(−)-*cis*-2-pyrrolidinyl-*N*-methylcyclohexylamine [(−)-**10**] and (1*S*,2*R*)-(+)-*cis*-2-pyrrolidinyl-*N*-methylcyclohexylamine [(+)-**10**]. The optical purity (>99.5%) of (−)-**10** and (+)-**10** was determined by HPLC analysis of the diastereomeric ureas formed by reaction with optically pure (*R*)-α-methylbenzyl isocyanate. The absolute configuration of (−)-**10** and (+)-**10** was determined by single-crystal X-ray diffractometry of the bis-(*R*)-mandelate salt. Condensation of optically pure (−)-**10** and (+)-**10** with 3,4-dichlorophenylacetic acid furnished (+)-**1** and (−)-**1**, respectively. Compounds (+)-**1**, (−)-**1**, (−)-**2**, and (+)-**2** were compared for their binding affinities at κ opioid, σ, D_2-dopamine, and phencyclidine (PCP) receptors in competitive binding assays using [^{3}H]bremazocine ([^{3}H]BREM) or [^{3}H]U69,593, [^{3}H]-(+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine [[^{3}H]-(+)-3-PPP], or [^{3}H]-1,3-di(*o*-tolyl)guanidine ([^{3}H]DTG), [^{3}H]-(−)-sulpiride [[^{3}H]-(−)-SULP], and [^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine ([^{3}H]TCP), respectively. In the systems examined, (−)-**2** exhibited the highest affinity for κ receptors, with a K_i of 44 ± 8 nM. However, (−)-**2** also showed moderate affinity for σ receptors, with a K_i of 594 ± 3 nM [[^{3}H]-(+)-3-PPP]. The (1*R*,2*R*)-(+)-enantiomer, (+)-**2**, had low affinity for both κ and σ receptors, exhibiting K_i values of 1298 ± 49 nM at κ ([^{3}H]BREM) and 1270 ± 168 nM at σ [[^{3}H]-(+)-3-PPP]. In contrast, the chiral cis compounds (+)-**1** and (−)-**1** showed high affinity for σ receptors and negligible affinity for κ opioid receptors in the [^{3}H]BREM assay. Compound (−)-**1** exhibited a K_i of 81 ± 13 nM at σ receptors [[^{3}H]-(+)-3-PPP] and 250 ± 8 nM ([^{3}H]DTG). The corresponding values for (+)-**1** were 221 ± 36 nM [[^{3}H]-(+)-3-PPP] and 118 ± 7 nM ([^{3}H]DTG). Compounds (−)-**2** and (+)-**2** lacked affinity for D_2-dopamine receptors. Compounds (+)-**1** and (−)-**1** bound only weakly to D_2-dopamine receptors, displaying K_i values of 14039 ± 1429 nM and 3762 ± 829 nM, respectively. All of the compounds lacked affinity for PCP receptors.

Psychotomimetic compounds have been shown to interact with σ and phencyclidine (PCP) receptors among others in the central nervous system. σ receptors are nonopioid, nondopaminergic receptors that bind antipsychotic drugs such as haloperidol and the (+)-enantiomer of opiate benzomorphans such as pentazocine and cyclazocine.[1-5] These receptors have been implicated in regulation of neurotransmitter release,[6] smooth muscle contraction,[6,7] control of motor behavior,[8,9] and modulation of phosphoinositide turnover.[10] However, many of the physiological functions of σ receptors remain to be elucidated. PCP receptors bind both PCP-related compounds and (+)-benzomorphans.[1] In addition to its psychotomimetic actions, the PCP receptor has recently been associated with anticonvulsant and neuroprotective activity.

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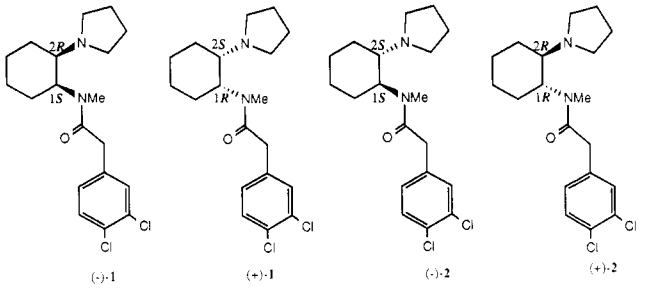
These effects result from antagonism of excitatory amino acid effects[1,11] via an apparent allosteric association of the PCP binding site with the binding site of the *N*-methyl-D-aspartic acid (NMDA) class of glutamate receptors.[12] Although (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine [(+)-3-PPP][3] and 1,3-di(*o*-tolyl)guanidine (DTG)[5] exhibit relative selectivity for σ receptors over PCP receptors, several ligands, including PCP, display significant cross-reactivity between σ and PCP receptors.[1] This suggests a close relationship between these two receptor macromolecules. Furthermore, several neuroleptic-related ligands that bind with high affinity to σ receptors also bind with high affinity to D_2-dopamine receptors. An example is the antipsychotic drug haloperidol. This cross-reactivity and the paucity of σ-selective agonists and antagonists have hampered the study of the physiological role of σ receptors.

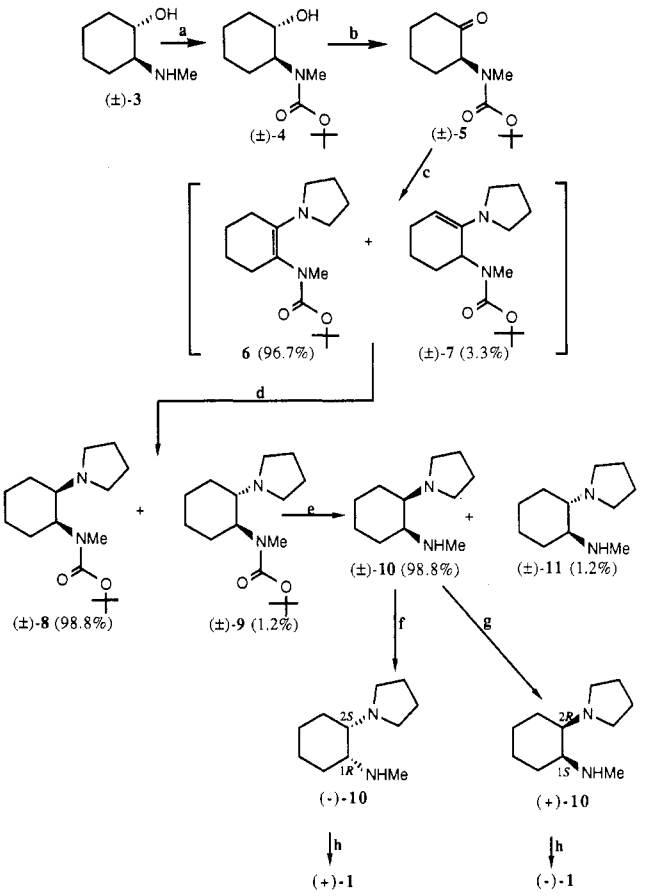
The benzeneacetamide derivative (±)-*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide (U50,488) [(±)-**2**] has been shown to be a highly selective ligand for κ opioid receptors.[13] We and others have demonstrated that (1*S*,2*S*)-(−)-**2** is the more κ-active component of (±)-**2**.[14,15] Among the effects displayed by in vivo administration of (±)-**2** are blockade of convulsions induced by electroshock,[16] blockade of convulsions induced by intracerebroventricular injection of excitatory amino acids (kainic, *N*-methylaspartic, and quisqualic acids) or the calcium channel activator Bay K 8644,[17,18] protection from cerebral ischemia,[19] and protection from hippocampal cell degeneration due to systematically injected kainic acid.[20] In vitro, (±)-**2** has been shown to block depolarization-induced calcium uptake in synaptosomes and to block enhancement of kainic acid binding induced by calcium.[17,18]



Due to the κ selectivity of (±)-**2**, these effects have been attributed to interaction with κ receptors. However, while most evidence points to the opioid nature of these effects, some anomalies point to the possible involvement of other mechanisms. For example, some of the effects of (±)-**2** are only partially antagonized by opioid antagonists,[16] require high concentrations,[16] or are otherwise insensitive to naloxone.[17] Furthermore, Von Voigtlander et al.[18] have described anticonvulsant and biochemical effects of U54,494A, a structural analogue of (±)-**2**. This compound exhibits in vivo anticonvulsant and calcium-related in vitro biochemical activities similar to those of (±)-**2** and yet lacks the κ receptor mediated analgesic and sedative effects of (±)-**2**.

In light of the neuroprotective and anticonvulsant properties of both (±)-**2** and PCP-related compounds, we initially set out to determine whether the enantiomers (−)-**2** and (+)-**2** and their cis diastereomers (+)-**1** and (−)-**1** interact with PCP receptors. Because of the possibility of PCP, σ, and dopamine receptor cross-reactivity, binding of these compounds to σ and D_2-dopamine receptors was also investigated. σ, PCP, and dopamine receptor activity was then compared to κ activity in the population of κ binding sites labeled by [^{3}H]bremazocine ([^{3}H]BREM) in guinea pig brain membranes.

Scheme I[a]



[a] (a) *t*-Boc dicarbonate, aqueous $NaHCO_3$; (b) PCC, CH_2Cl_2; (c) pyrrolidine, *p*-toluenesulfonic acid, benzene; (d) H_2/10% Pd–C, EtOAc; (e) 6 M HCl; (f) 2 equiv of (*R*)-(−)-mandelic acid; (g) 2 equiv of (*S*)-(+)-mandelic acid; (h) 1.5 equiv of 3,4-dichlorophenylacetic acid, DCC, pyridine, CH_2Cl_2.

Chemistry

The starting material (Scheme I), (±)-*trans*-2-(*N*-methylamino)cyclohexanol [(±)-**3**], was readily synthesized in quantitative yield by methylaminolysis of cyclohexene oxide.[21] Protection of (±)-**3** by treatment with di-*tert*-butyl dicarbonate (1.2 equiv)[22] in excess aqueous $NaHCO_3$

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afforded 94% yield of (±)-*trans*-2-(1-pyrrolidinyl)-*N*-[(*tert*-butyloxy)carbonyl]-*N*-methylcyclohexylamine [(±)-4]. Oxidation[23] of (±)-4 in CH_2Cl_2 with 1.55 equiv of pyridinium chlorochromate (PCC) followed by distillation of the product under reduced pressure gave pure ketone **5** in 95% yield. Condensation of this ketone with excess pyrrolidine in the presence of a catalytic quantity of *p*-toluenesulfonic acid gave a quantitative crude yield of enamine mixture **6** and (±)-**7**.[24] This was achieved by refluxing in benzene under a Dean–Stark trap until the theoretical amount of water had been collected and following the reaction progress by capillary GC (decomposition of the enamine occurred during examination by TLC). Integration of the olefinic proton of enamine (±)-**7** and comparison with the integration of the *tert*-butyl signal for the mixture indicated the presence of 3.3% of (±)-**7** in the enamine mixture [**6** and (±)-**7**]. Because of its instability, the mixture of enamines was hydrogenated immediately. Reduction at 50 psi H_2 over PtO_2 (1:20 weight of catalyst to substrate) afforded a 12.3:87.7 mixture of unwanted (±)-*trans*-*N*-[(*tert*-butyloxy)carbonyl]-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine [(±)-**9**] to the desired cis isomer (±)-**8**. Reduction under identical conditions over 10% Pd/C (1:10 w/w catalyst:substrate) gave a much more favorable 98.8:1.2 ratio of (±)-**8** to (±)-**9** in quantitative overall yield from (±)-**5**. This reduction was scaled up to 200 g without any loss in the high (98.8%) ratio of cis product formed. For chemical comparison, (±)-**9** was synthesized by treatment of an authentic sample of (±)-*trans*-*N*-methyl-2-(1-pyrrolidinyl)cyclohexylamine [(±)-**11**][14] with 1.2 equiv of di-*tert*-butyl dicarbonate in aqueous $NaHCO_3$[22] and was found to be identical by GC, TLC, and ^1H NMR to the side product formed during the above hydrogenations. Deprotection of (±)-**8** by brief treatment at 60 °C with 6 M aqueous HCl[25] afforded 91% redistilled yield of free amine (±)-**10**. Analysis of redistilled (±)-**10** by capillary GC (oven temp 100 °C) indicated the presence of 1.2% impurity of the corresponding *trans*-diamine (±)-**11**, which was identical (GC, TLC, and spectroscopically) with authentic (±)-**11**. This impurity was readily separated by one crystallization of the bis-HCl salt of (±)-**10**. Optical resolution of (±)-**10** (Scheme I) was achieved via the mandelate salt [which also removed the 1.2% (±)-**11** impurity]. Initial treatment of (±)-**10** with 1 mol equiv of (*R*)-(−)-mandelic acid formed (−)-**10**·bis-(*R*)-(−)-mandelate as needles from 2-propanol. Two recrystallizations of this salt from 2-propanol afforded diastereomerically pure crystals in 58% of theoretical yield. This low yield and the observation that the salt gave a combustion analysis corresponding to the bis-(*R*)-mandelate salt suggested that 2 equiv of mandelic acid were necessary for a better yield of resolved compound. Thus, formation of the salt of (±)-**10** with 2 mol equiv of (*S*)-(+)-mandelic acid followed by two recrystallizations from 2-propanol afforded a better (91.5%) yield of diastereomerically pure (+)-**10**·bis-(*S*)-(+)-mandelate, which on treatment with aqueous NH_3 afforded (+)-**10** in greater than 99.5% enantiomeric purity.

Identical treatment of the mixed bases recovered from the above resolution with 2 mol equiv of (*R*)-(−)-mandelic acid gave 91.6% yield of (1*R*,2*S*)-(−)-*cis*-2-pyrrolidinyl-*N*-methylcyclohexylamine·bis-(*R*)-(−)-mandelate, from which (−)-**10** was obtained, also in 99.5+% optical purity. The optical purity of (+)-**10** and (−)-**10** was established by normal-phase HPLC analysis of the corresponding diastereomeric ureas formed by treatment of each enantiomer with optically pure (*R*)-α-methylbenzyl isocyanate in dry $CHCl_3$.[14]

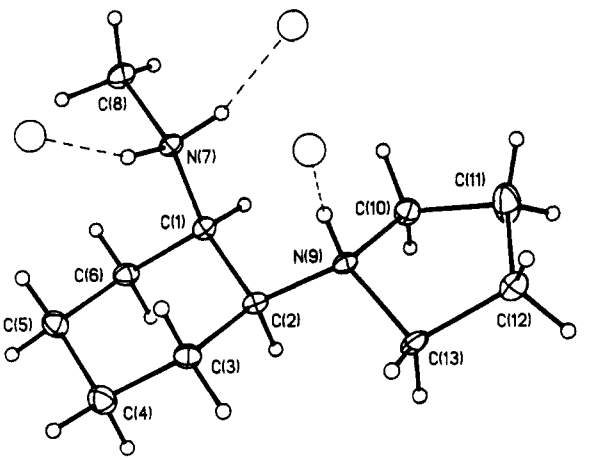

Figure 1. Thermal ellipsoid plot drawn from experimental coordinates of (1*R*,2*S*)-(−)-*cis*-2-pyrrolidinyl-*N*-methylcyclohexylamine dication. Thermal ellipsoids are drawn at the 20% probability level. The dashed lines represent hydrogen bonds to the mandelate anions, which were omitted except for the oxygens involved in the hydrogen bonds.

The target 3,4-dichlorophenylacetamides (±)-**1**, (+)-**1**, and (−)-**1** were formed by *N*,*N*′-dicyclohexylcarbodiimide (DCC) mediated coupling of (±)-**10**, (−)-**10**, and (+)-**10** with 3,4-dichlorophenylacetic acid.

X-ray Analysis of (−)-10. An X-ray crystallographic analysis of the bis-(*R*)-(−)-mandelate salt of (−)-**10**, which established the absolute configuration as 1*R*,2*S*, was based on the chirality of (*R*)-(−)-mandelate as referenced to the chiral centers C(1) and C(2). Figure 1 shows the configuration of the (−)-**10** dication. Bond distances and angles are normal. The pyrrolidinium ring is in a half-chair conformation, and the cyclohexane ring is in a chair conformation (absolute values for ring torsion angles range from 53.9° to 57.1°). The two cyclohexane substituents are cis, with the C–N bonds from the ring being axial for the methylammonium and equatorial for the pyrrolidinyl substituents. In the diastereomer (1*S*,2*S*)-(+)-*trans*-2-pyrrolidinyl-*N*-methylcyclohexylammonium[14] of (−)-**10**, the C–N bonds are both equatorial. The relative orientation of the two rings in (−)-**10** can be described by the torsion angle C(1)–C(2)–N(9)–C(10) = −52.1°. The values for the equivalent torsion angles in the previously cited diastereomer are 77.0° and 74.4° for the two crystallographically independent molecules present. The relative orientation of the two rings is affected by the strong hydrogen bonding present in each. In (−)-**10**, the quadrivalent nitrogens are involved in three intermolecular hydrogen bonds to the mandelate oxygens, and N–O distances range from 2.66 to 2.69 Å. Intermolecular and intramolecular hydrogen bonding also occurs between the mandelate anions.

Biological Results

The ability of the stereoisomers of (±)-**2** to compete for guinea pig brain κ ([^{3}H]BREM), σ ([^{3}H]-(+)-3-PPP and [^{3}H]DTG), PCP ([^{3}H]TCP) and guinea pig brain κ ([^{3}H]-(−)-U69,593) and rat brain D_2-dopamine receptors ([^{3}H]-(−)-sulpiride, [^{3}H]-(−)-SULP) was determined by using competition binding assays (Table I). The racemic

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Table I. Differential Affinities (K_i) of U50,488 Stereoisomers at κ, σ, D_2-Dopamine, and Phencyclidine (PCP) Receptors[a]

	compound [configuration]				
	U50, 488 [(±)-2] [(±)-trans]	(+)-1 [(1*R*,2*S*)-(+)-cis]	(−)-1 [(1*S*,2*R*)-(−)-cis]	(−)-2 [(1*S*,2*S*)-(−)-trans]	(+)-2 [(1*R*,2*R*)-(+)-trans]
κ (g.p.) ([^{3}H]BREM]	109 ± 40	no inhibn[b]	no inhibn[b]	44 ± 8	1298 ± 49
κ (g.p.) [[^{3}H]-(−)U69,593]	ND	2715 ± 130	167 ± 4	0.89 ± 0.05	299 ± 16
σ (g.p.) [[^{3}H]-(+)-3-PPP]	874 ± 283	221 ± 36	81 ± 13	594 ± 3	1270 ± 168
σ (g.p.) ([^{3}H]DTG)	ND	118 ± 7	250 ± 8	ND	ND
D_2-dopamine (rat) ([^{3}H]SULP)	ND	14039 ±1429	3726 ± 829	no inhibn[b]	no inhibn[b]
PCP (g.p.) ([^{3}H]TCP)	no inhibn[b]	no inhibn[b]	no inhibn[b]	no inhibn[b]	no inhibn[b]
ratio of κ ([^{3}H]BREM) to σ[[^{3}H]-(+)-3-PPP]	0.12	c	c	0.07	1.02

[a] Competition binding assays were carried out under the conditions described under Experimental Section, using various concentrations of unlabeled test ligand, ranging from 0.05 to 10000 nM (0.05–500000 nM for dopamine receptor assay). Data were analyzed by using the iterative curve fitting program CDATA (EMF Software, Inc., Baltimore, MD). K_i values were then calculated from the IC_{50} values by using the Cheng–Prusoff[32] equation. Values are the averaged results of two or three determinations ± SEM. Each experiment was carried out in duplicate. ND indicates not determined. g.p. refers to guinea pig brain. The κ/σ ratio refers to the K_i for κ binding against [^{3}H]bremazocine divided by the K_i for σ binding against [^{3}H]-(+)-3-PPP. The K_d values of the radioligands used to calculate K_i were either determined in independent experiments or obtained from the literature:[1] [^{3}H]BREM (κ), 0.64 nM; [^{3}H]-(+)-3-PPP (σ), 22 nM; [^{3}H]TCP (PCP), 25 nM; (D_2-dopamine), 10.3 nM. [b] No K_i could be determined in these cases since the compounds produced no inhibition of radioligand binding at a concentration of 10000 nM. [c] The κ/σ ratio could not be determined in these cases because it was not possible to calculate the K_i for κ ([^{3}H]BREM) binding for these compounds.

trans compound [(±)-2] (U50,488) is the compound widely used as a highly selective κ ligand. However, (±)-2 was found to exhibit weak affinity for σ receptors and as a result is only 8-fold selective for κ receptors ([^{3}H]BREM) over σ receptors (Table I). Of the U50,488 enantiomers, (−)-2 was the most potent at κ receptors and was 14-fold selective for κ receptors ([^{3}H]BREM) over σ receptors (Table I). (+)-2 was found to bind with low affinity to κ receptors, confirming earlier findings.[14,15] Interestingly, (+)-2 bound almost equipotently to both κ and σ receptors.

Neither of the optically pure cis diastereomers showed affinity at κ receptors labeled by [^{3}H]BREM. However, in an independent study of κ receptors labeled by [^{3}H]-U69,593, (+)-1 was found to bind with a K_i of 2715 ± 130 nM and (−)-1 with a K_i of 167 ± 4 nM (see Table I).[26] In the same study[26] (−)-2 bound with a K_i of 0.89 ± 0.05 nM and (+)-2 bound with a K_i of 299 ± 16 nM.

For σ receptors labeled by [^{3}H]-(+)-3-PPP (Table I), (−)-1 was the most potent, with a K_i of 81 ± 13 nM, while the value for (+)-1 was 221 ± 36 nM. Similar results were obtained when [^{3}H]-1,3-di(*o*-tolyl)guanidine ([^{3}H]DTG) was used to label σ receptors: (+)-1, 118 ± 7 nM, and (−)-1, 250 ± 8 nM. The significance of the reverse order of potency of the cis diastereomers when [^{3}H]DTG is used to label σ receptors is not known.

The trans diastereomers (−)-2 and (+)-2 displayed no affinity for D_2-dopamine receptors. The cis diastereomers (+)-1 and (−)-1 displayed only low affinity. (+)-1 bound to dopamine receptors with a K_i of 14039 ± 1429 nM, and (−)-1 bound with a somewhat higher affinity of 3726 ± 829 nM. None of the enantiomeric stereoisomers of (±)-2 showed any affinity for PCP receptors (Table I).

Discussion

Racemic **2**, a mixture of trans enantiomers and in wide use as a selective κ opiate receptor agonist, exhibited weak affinity for σ receptors (Table I). The κ receptor active enantiomer (−)-2 possessed significant σ activity. However, the cis enantiomers of this compound exhibited the highest affinity for σ receptors. By changing the orientation of the pyrrolidine ring from the *S* configuration in (−)-2 to the *R* configuration in (−)-1, the result was a total loss of affinity for κ receptors labeled by [^{3}H]BREM in the presence of μ and δ blockers, and an increase in affinity for σ receptors labeled by both [^{3}H]-(+)-3-PPP and [^{3}H]DTG. Similar κ and σ receptor affinity changes were obtained when the cis enantiomer was generated by changing the orientation of the benzeneacetamide group from *S* in (−)-2 to *R* in (+)-1.

It should be noted that an independent study[26] revealed that all of these compounds consistently showed substantially higher affinity for κ receptors labeled by [^{3}H]-U69,593 [(−)-enantiomer] in the presence of μ and δ blockers than κ receptors labeled in this study by [^{3}H]-BREM in the presence of μ and δ blockers. This suggests the existence of different populations of κ receptors. Evidence for κ receptor heterogeneity has also been described previously[27,28] and will be further discussed.[26] However, as with κ receptors labeled by [^{3}H]BREM, changing the orientation from trans to cis resulted in marked decreases in affinity for κ receptors labeled by [^{3}H]U69,593. Importantly, while (−)-1 was relatively potent at the [^{3}H]-U69,593-labeled κ site (K_i = 167 ± 4 nM), the affinity of (+)-1 at this site was weak (K_i = 2715 ± 30 nM). Taking this into account, (+)-1 is 12 times more potent at σ sites labeled by [^{3}H]-(+)-3-PPP and 23 times more potent at σ sites labeled by [^{3}H]DTG compared with κ receptors labeled by [^{3}H]U69,593.

The lack of affinity of (+)-1 for the [^{3}H]BREM-labeled κ site, the low affinity for the [^{3}H]U69,593-labeled site, and the increased affinity for σ receptors demonstrates that the trans to cis orientation change in U50,488-related compounds produces relatively selective σ ligands with respect to κ receptors.

The two cis enantiomers (+)-1 and (−)-1 had weak affinity for D_2-dopamine receptors (Table I). However, compared to σ receptors, the binding affinity at D_2-dopamine receptors was 60-fold lower for (+)-1 and 50-fold lower for (−)-1. Because (+)-1 and (−)-1 lack affinity for PCP receptors, they represent the first example of an enantiomeric pair of ligands structurally related to (±)-2 that exhibit very high selectivity for σ receptors over D_2-dopamine and PCP receptors. Since (+)-1 exhibits low affinity for both putative κ receptor subpopulations, it represents the more σ-selective compound in the enantiomeric pair.

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The observation that compounds related to (±)-2 bind to σ receptors may have important pharmacological implications. Although some biological effects of σ receptor ligands have been demonstrated,[6-10] the physiological roles of this receptor are still largely unknown. It is possible, therefore, that some of the effects of (±)-2, especially those not readily attributed to κ receptors,[18] are mediated by σ receptors. Furthermore, preliminary studies show that, in addition to other σ receptor ligands such as (+)-pentazocine, haloperidol, and DTG,[10] (−)-1 exhibits the ability to block carbachol-stimulated phosphoinositide metabolism (data not shown). This suggests that arylacetamides related to (±)-2 can exert biological effects through σ receptor interaction.

In conclusion, studies of σ receptor pharmacology have been greatly hampered by the limited number of ligands that do not cross-react with PCP or dopamine receptors. The identification of arylacetamides as high-affinity σ ligands that lack affinity for PCP receptors and have low affinity for dopamine receptors provides a novel class of compounds in which to develop tools for the study of σ receptors.

Experimental Section

Biological Materials and Methods. κ, σ, and PCP receptor binding assays were performed by using the crude synaptosomal (P_2) membrane fraction of frozen guinea pig brain (minus cerebellum) (Pel-Freeze, Rogers, AR), prepared as described previously for rat brain.[29] Assays for D_2-dopamine receptor binding utilized rat brain P_2 fraction (minus cerebellum) prepared as previously described.[29]

σ Receptors. σ receptors were labeled with [^{3}H]-(+)-3-PPP (110.0 Ci/mmol). Incubations were carried out in 50 mM Tris-HCl, pH 8.0, for 120 min at 25 °C in a volume of 0.5 mL with 500 μg of membrane protein and 3 nM [^{3}H]-(+)-3-PPP. All assays contained 1 μM dopamine to preclude any labeling of dopamine receptors. Nonspecific binding was determined in the presence of 1 μM haloperidol. Assays were terminated by addition of 5 mL of ice-cold 10 mM Tris-HCl, pH 8.0, and filtration through glass fiber filters (Schleicher & Schuell), which were soaked in 0.5% poly(ethylenimine) for at least 30 min at 25 °C prior to use. Filters were then washed twice with 5 mL of ice-cold Tris-HCl buffer.

σ receptors were also labeled with [^{3}H]DTG (SA = 52 Ci/mmol, New England Nuclear). Guinea pig membranes were prepared essentially as described for rat brains.[30] Twenty frozen guinea pig brains, including the cerebellum, were homogenized with a Brinkman Polytron (setting 6) in 5 mL of ice-cold 5 mM Tris-HCl, pH 8.2 (buffer), 10 mL per brain. The homogenates were pooled and centrifuged at 4 °C for 10 min at 39000*g*. The supernatants were decanted, and the pellets were resuspended by vigorous vortexing, using 10 mL of buffer per brain. The homogenate was recentrifuged, and the pellets were resuspended in 0.8 mL of buffer per brain. One-milliliter aliquots of the pooled homogenates were distributed to 1.5-mL microfuge tubes, which were centrifuged for 5 min. The supernatants were aspirated and the pellets stored at −70 °C until assayed.

Membranes were incubated in a final volume of 1 mL (approximately 1 mg of protein) for 3 h at 25 °C (steady state) in 5 mM *N*-(2-hydroxyethyl)piperazine-*N*′-hydroxypropanesulfonic acid (HEPPSO), pH 8.1 (buffer), containing EDTA (10 μM), ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) (10 μM), chymostatin (2.5 μg/mL), and leupeptin (2.5 μg/mL). Nonspecific binding was determined by incubations in the presence of 50 μM haloperidol. Incubations were terminated by rapid filtration over glass fiber filters presoaked in 2% poly(ethylenimine), which were washed twice with ice-cold buffer. Each point was assayed in quadruplicate (with less than 5% variation). Typical total and nonspecific cpm's (±SD) using 1 nM and 5 nM [^{3}H]DTG were 3245 ± 119 and 512 ± 34, and 16234 ± 410 and 2310 ± 143, respectively.

Phencyclidine (PCP) Receptors. PCP receptors were labeled by using [^{3}H]TCP (42.2 Ci/mmol). Incubations were carried out in 5 mM Tris-HCl, pH 7.4, for 60 min at 4 °C in a volume of 0.5 mL with 500 μg of membrane protein and 5 nM [^{3}H]TCP. Assays were terminated by addition of 5 mL of ice-cold buffer and filtration through glass fiber filters under reduced pressure. Filters were then washed twice with 5 mL of ice-cold buffer. Filters were soaked in 0.3% poly(ethylenimine) for at least 30 min at 25 °C prior to use. Nonspecific binding was determined in the presence of 10 μM cyclazocine.

κ Receptors. κ receptors were labeled with [^{3}H]BREM (21.3 Ci/mmol) in the presence of DAGO and DSTLE as μ and δ blockers, respectively. Incubations were carried out in 0.5 mL of 10 mM Tris-HCl, pH 7.4, for 90 min at 25 °C with 500 μg of membrane protein, 100 nM DSTLE, 100 nM DAGO, and 2 nM [^{3}H]BREM. Assays were terminated by addition of 5 mL of ice-cold buffer and filtration through glass fiber filters under reduced pressure. Filters were then washed twice with 5 mL of ice-cold buffer. Nonspecific binding was determined in the presence of 10 μM levallorphan.

D_2-Dopamine Receptors. D_2-dopamine receptors were labeled with 5 nM [^{3}H]-(−)-sulpiride (60 Ci/mmol). Incubations were carried out for 1 h at 25 °C in 0.5 mL of 50 mM Tris-HCl, pH 7.7, containing 120 mM NaCl and 500 μg of membrane protein. Nonspecific binding was determined in the presence of 1 μM haloperidol. Assays were terminated by addition of ice-cold incubation buffer and vacuum filtration through glass fiber filters. Filters were then washed twice with ice-cold incubation buffer.

All scintillation counting was performed with a Packard Model 4450 scintillation spectrometer using Ecoscint cocktail (National Diagnostics, Manville, NJ) after an overnight extraction of counts from the filters. Radioligands were obtained from Du Pont/New England Nuclear (Boston, MA). Haloperidol, poly(ethylenimine), and Tris were obtained from Sigma Chemicals (St. Louis, MO). Cyclazocine and levallorphan were obtained from the National Institute on Drug Abuse (Rockville, MD).

Synthesis. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. High-performance liquid chromatography (HPLC) was performed with a Gilson Model 303 with a solvent mixer (Model 811) and a Data Master (Model 620). Specific rotation measurements at the sodium D line ($[\alpha]^{25}_D$) were obtained in a 1 dM cell using a Perkin-Elmer 241-MC polarimeter. Gas chromatography (GC) was determined on a Hewlett-Packard 5880A instrument with a 30-m SE-30 capillary column using flame ionization detection. A Nicolet Model l.c.m automatic X-ray diffractometer in $\theta/2\theta$ collection mode was used for X-ray crystallography. Elemental analyses were performed at Atlanta Microlabs, Atlanta, GA. Chemical ionization mass spectra (CIMS) were obtained on a Finnigan 1015 mass spectrometer. Electron ionization mass spectra (EIMS) and high-resolution mass measurements (HRMS) were obtained by using a V.G. Micro Mass 7070F mass spectrometer. ^{1}H NMR spectra were obtained from $CDCl_3$ solutions using a Varian XL-300 spectrometer. Infrared (IR) spectra were determined on a Beckman 4230 IR spectrophotometer using KBr pellets for crystalline compounds and liquid films for compounds that were liquids or oils. Thin-layer chromatography (TLC) was performed on 250-μm Analtech GHLF silica gel plates. TLC systems used were EtOAc–hexane, 1:4 (system 1), and $CHCl_3$–MeOH–concentrated aqueous NH_3, 90:10:1 (system 2).

(±)-*trans*-2-[*N*-[(*tert*-Butyloxy)carbonyl]-*N*-methylamino]cyclohexanol [(±)-4]. A mixture of (±)-*trans*-*N*-methyl-2-aminocyclohexanol (141.6 g, 1.1 mol), *tert*-butyl dicarbonate (239.5 g, 1.1 mol), $KHCO_3$ (458 g), and water (1000 mL) was stirred overnight at room temperature. Extraction (3 × 500 mL) with CH_2Cl_2, drying of the organic extract (Na_2SO_4), and evaporation of the solvent under reduced pressure gave a crystalline residue, which on recrystallization from hot isooctane afforded 236.8 g (94%) of (±)-4: mp 82–83 °C. Anal. ($C_{12}H_{23}NO_3$) C, H, N.

(±)-2-[*N*-[(*tert*-Butyloxy)carbonyl]-*N*-methylamino]cyclohexanone [(±)-5]. A solution of (±)-4 (236.8 g, 1.03 mol) in CH_2Cl_2 (500 mL) was added dropwise over 15 min to a me-

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chanically stirred solution of PCC (334.3 g, 1.55 mol) in CH_2Cl_2 (1000 mL). The mixture began to reflux gently. After 3 h at room temperature, the reaction mixture was diluted with 1500 mL of ether and filtered through a 4-in. pad of florisil. Evaporation of solvent in vacuo afforded crude (±)-**5** in quantitative yield as a pale green viscous oil. Distillation (115 °C at 0.9 mmHg) afforded 222.7 g (95%) of pure (GC at 150 °C and TLC in system 1) (±)-**5** as a viscous oil. Anal. ($C_{12}H_{21}NO_3$) C, H, N.

1-[*N*-[(*tert*-Butyloxy)carbonyl]-*N*-methylamino]-2-(*N*-pyrrolidinyl)cyclohexene (6) and 3-[*N*-[(*tert*-Butyloxy)-carbonyl]-*N*-methylamino]-2-(*N*-pyrrolidinyl)cyclohexene [(±)-7]. To a solution of **5** (202.7 g, 0.89 mol) and pyrrolidine (95 mL, 1.27 equiv) in 3000 mL of dry benzene was added *p*-toluenesulfonic acid (9.8 g, 0.06 equiv), and the mixture was stirred at room temperature until all of the acid had dissolved. The reaction was refluxed under a nitrogen atmosphere for 24 h when capillary GC (150 °C) indicated 23% of (±)-**5** remaining together with 77% of enamine product. A further 95 mL of pyrrolidine was added and refluxing continued for a total of 36 h. GC (150 °C) of the mixture indicated 0.7% of (±)-**5** remaining and 99.3% of desired product. The solvent and excess pyrrolidine were distilled at atmospheric pressure and final traces of solvent removed in vacuo to give the desired mixture [**6** and (±)-**7**] in quantitative yield. ^1H NMR analysis indicated a 96.7:3.3 mixture of **6** and (±)-**7** by direct comparison of the integration values for the olefinic signal for (±)-**7** at 4.74 ppm and the *tert*-butyl signal for the mixture at 1.40 ppm. No attempt was made to further purify this mixture because of its instability to air and water vapor. It was therefore used immediately for the next reaction step. HRMS (EI mode), *m/z* 280.2157 (280.2151 calcd for $C_{16}H_{28}N_2O_2$).

(±)-*cis*-2-Pyrrolidinyl-*N*-[(*tert*-butyloxy)carbonyl]-*N*-methylcyclohexylamine [(±)-8]. Enamine mixture **6** and (±)-**7** (100 g, 0.36 mol) in dry ethyl acetate (300 mL) together with 10 g of 10% Pd/C was placed in a 500-mL Parr bottle. After purging with nitrogen, the reaction mixture was shaken in the presence of hydrogen at 50 psi for 4 h at room temperature. GC (150 °C) indicated that the reaction was complete. Filtration through Celite followed by washing with a further 300 mL of ethyl acetate and evaporation of the solvent in vacuo afforded 100 g of (±)-**8** as a colorless oil. GC (150 °C) of this product indicated it to be greater than 95% pure. Coinjection with authentic (±)-**9** [see synthesis of (±)-**9** below] indicated that it contained 1.2% of (±)-**9**. Authentic (±)-**9** also comigrated with the 1.2% impurity on TLC (system 2) and was identical by ^1H NMR comparison and CIMS. The fumarate salt of (±)-**8** (ethyl acetate) afforded an analytically pure sample [which was free of traces of (±)-**9**]: mp 127–128 °C. Anal. ($C_{20}H_{34}N_2O_6$) C, H, N.

(±)-*trans*-2-Pyrrolidinyl-*N*-[(*tert*-butyloxy)carbonyl]-*N*-methylcyclohexylamine [(±)-9]. To an aqueous solution of (±)-**11**[14] (0.85 g, 4.66 mmol) and $NaHCO_3$ (1.17 g, 3 equiv) was added di-*tert*-butyl dicarbonate (1.12 g, 1.2 equiv), and the solution was stirred for 24 h at room temperature. Extraction with ether (3 × 30 mL), drying over Na_2SO_4, and evaporation of the solvent under reduced pressure afforded a quantitative yield of (±)-**9** as a colorless oil. This product was identical chromatographically and spectroscopically to the 1.2% side product formed during the hydrogenation of enamine mixture (**6**) and (±)-**7** above. An analytically pure sample was obtained by crystallization of the oxalate salt of (±)-**9** from ethyl acetate: mp 123–124 °C. Anal. ($C_{18}H_{32}N_2O_6$) C, H, N.

(±)-*cis*-2-Pyrrolidinyl-*N*-methylcyclohexylamine [(±)-10]. 247 g (0.87 mol) of (±)-**8** was added slowly (10 min) to a rapidly stirred aqueous solution of HCl (6 M) at 60 °C. During the addition, vigorous evolution of gas occurred. After the addition was complete, the solution was left to stir for 5 min more at 60 °C and then monitored by TLC (concentrated aqueous NH_3–MeOH–$CHCl_3$, 1:9:90). Crushed ice (200 g) was added and the solution basified by addition of excess concentrated aqueous NH_3. After extraction with 5 × 200 mL of CH_2Cl_2, the combined extracts were dried with anhydrous Na_2SO_4 and the solvent was evaporated in vacuo. Distillation under reduced pressure (78 °C at 0.3 mmHg) afforded 145.8 g (91%) of (±)-**10**, which was 98.8% pure by GC (100 °C). The 1.2% impurity was identical with authentic (±)-**11** by GC (100 °C) and TLC (system 2). Recrystallization of (±)-**10**·2HCl from 2-propanol afforded a pure sample: mp 240–242 °C. Anal. ($C_{11}H_{24}N_2Cl_2$) C, H, N.

(1*S*,2*R*)-(+)-*cis*-2-Pyrrolidinyl-*N*-methylcyclohexylamine [(+)-10]. A mixture of (±)-**10** (45.63 g, 250.3 mmol) and (*S*)-(+)-mandelic acid (76.16 g, 500.6 mmol) in 200 mL of MeOH was distilled, keeping the volume constant at 350 mL by addition of 2-propanol. Crystallization occurred spontaneously after most of the MeOH component had been distilled. On standing at room temperature for 1 h, the crystals were filtered and washed twice with cold (0 °C) 2-propanol followed by ether and recrystallized two further times from 350 mL of 2-propanol, affording 55.7 g (91.5%) of (+)-**10**·bis-(*S*)-(+)-mandelate: mp 143–144 °C, $[\alpha]^{25}_D$ +90.2° (*c* 0.89, MeOH). Anal. ($C_{27}H_{38}N_2O_6$) C, H, N.

Conversion of 78.4 g of (+)-**10**·bis-(*S*)-(+)-mandelate to (+)-**10** free base (10% NaOH, ether extraction with 3 × 200 mL) followed by distillation under reduced pressure afforded 29.1 g of optically pure[14] (+)-**10**: bp 77 °C at 1.2 mmHg, $[\alpha]^{25}_D$ +33° (*c* 1.34, MeOH).

(1*R*,2*S*)-(−)-*cis*-2-Pyrrolidinyl-*N*-methylcyclohexylamine [(−)-10]. Identical treatment of the recovered redistilled mixed bases (24.45 g, 134.1 mmol) from the above resolution with 40.81 g (268.2 mmol) of (*R*)-(−)-mandelic acid followed by two recrystallizations from 350 mL of 2-propanol as described above afforded 55.8 g (91.6% of theoretical yield) of (−)-**10**·bis-(*R*)-(−)-mandelate: mp 143–144 °C, $[\alpha]^{25}_D$ −91.2° (*c* 0.86, MeOH). Anal. ($C_{27}H_{38}N_2O_6$) C, H, N.

Conversion of 77.8 g of (−)-**10**·bis-(*R*)-(−)-mandelate to free (−)-**10** base as described above afforded 28.8 g of optically pure[14] (−)-**10**: bp 77 °C at 1.2 mmHg, $[\alpha]^{25}_D$ −31° (*c* 1.49, MeOH).

(1*R*,2*S*)-(+)-*cis*-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(+)-1]. To a stirred solution of (−)-**10** (1.00 g, 5.48 mmol), 3,4-dichlorophenylacetic acid (1.69 g, 1.5 equiv), and dry pyridine (0.22 g, 0.5 equiv) in alcohol-free CH_2Cl_2 (20 mL) was added DCC (2.26 g, 2.0 equiv), and the reaction mixture was stirred for 10 min at room temperature. TLC (system 2) of the reaction mixture after this time indicated that reaction was complete. The *N*,*N*′-dicyclohexylurea (DCU) was filtered off and washed with ether (50 mL). The filtrate was diluted to 100 mL with ether and then shaken well with 50 mL of 10% aqueous citric acid solution. The organic layer was discarded, and the aqueous layer was washed with a further 2 × 50 mL of ether. Addition of excess aqueous NH_3 liberated (+)-**1** (base). Extraction with 2 × 50 mL of CH_2Cl_2 followed by drying of the organic extract through Na_2SO_4 and evaporation of the solvent afforded 2.12 g (100%) of (+)-**1** as a dextrorotatory (MeOH D line) oil. Treatment of this oily base (1.37 g, 3.71 mmol) with (+)-tartaric acid (0.56 g, 3.71 mmol) furnished 1.82 g (91%) of (+)-**1**·(+)-tartrate·H_2O after crystallization from 10 mL of 5% aqueous 2-propanol: mp 115–117 °C, $[\alpha]^{25}_D$ +56.7° (*c* 0.71, MeOH). Anal. ($C_{23}H_{34}Cl_2N_2O_8$) C, H, N.

(1*S*,2*R*)-(−)-*cis*-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide [(−)-1]. (+)-**10** (1.00 g, 5.48 mmol) was coupled with 3,4-dichlorophenylacetic acid as described for the synthesis of (+)-**1** above to give 2.13 g (100%) of (−)-**1** as a colorless oil which was levorotatory (D line) in MeOH solution. Treatment of (−)-**1** (1.95 g, 5.28 mmol) with (−)-tartaric acid (0.79 g, 5.28 mmol) afforded 2.62 g (92%) of (−)-**1**·(−)-tartrate·H_2O from 10 mL of 5% aqueous 2-propanol: mp 115–117 °C, $[\alpha]^{25}_D$ −55.8° (*c* 0.61, MeOH). Anal. ($C_{23}H_{34}Cl_2N_2O_8$) C, H, N.

(±)-*cis*-3,4-Dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]benzeneacetamide [(±)-1]. (±)-**10** (5.00 g, 27.4 mmol), pyridine (1.08 g, 0.5 equiv), 3,4-dichlorophenylacetic acid (8.43 g, 1.5 equiv), and DCC (11.32 g, 2 equiv) in CH_2Cl_2 (50 mL) were reacted as described for (+)-**1** above to give 10.6 g (100%) of (±)-**1** as a colorless oil. Treatment of an ethyl acetate solution (50 mL) of (±)-**1** with a solution of HCl gas in ethyl acetate afforded 10.40 g (91%) of (±)-**1**·HCl: mp 165–170 °C. Anal. ($C_{19}H_{29}Cl_3N_2O$) C, H, N.

Determination of Absolute Configuration by Single-Crystal X-ray Analysis of (−)-10·Bis-(*R*)-(−)-mandelate. Crystals of the (−)-**10**·bis-(*R*)-(−)-mandelate salt, $C_{11}H_{24}N_2^{2+}\cdot(C_8H_7O_3)_2^-$, fw = 486.61, were grown from 2-propanol by slow cooling of a saturated solution. A clear, colorless 0.05 × 0.23 × 0.56 mm^3 crystal was selected for data collection. Data were collected on a computer-controlled diffractometer with an incident beam monochromator (Nicolet R3m/V with Mo Kα radiation, λ = 0.71073 Å, *T* = 200 K). A least-squares refinement using 25 centered reflections within 20° < 2θ < 28° gave the triclinic cell

a = 6.130 (6) Å, b = 8.224 (6) Å, c = 13.108 (11) Å, α = 95.71 (6), β = 102.16 (7), and γ = 99.74 (7)°, with V = 630.4 (9) Å^3, Z = 1, and d_{calcd} = 1.282 g/cm^3. A total of 1743 independent reflections were measured in the $\theta/2\theta$ mode to $2\theta_{max}$ = 45°. Corrections were applied for Lorentz and polarization effects, but not for absorption. The structure was solved by direct methods with the aid of the program SHELXTL[31] and refined with a full matrix least squares.[31] The 336 parameters refined include the coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Carbon hydrogens used a riding model in which the coordinate shifts of the carbons were applied to the attached hydrogens, and C-H = 0.96 Å, angle H-C-H = 109.5°, and U(H) = 1.1U_{eq}(C). The remaining hydrogens were refined isotropically. The final R values for the 1652 observed reflections with $F_o > 3\sigma(|F_o|)$ were R = 0.036 and wR = 0.041, where $s = 1/[\sigma^2(|F_o| + g(F_o)^2]$ and g = 0.00023. Tables of coordinates, bond distances, and bond angles, and anisotropic thermal parameters, have been deposited with the Crystallographic Data Center, Cambridge CB2 1EW, England.

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Lipophilic Analogues of Sparsomycin as Strong Inhibitors of Protein Synthesis and Tumor Growth: A Structure-Activity Relationship Study

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Fourteen derivatives of sparsomycin (1) were synthesized. Six of them were prepared following a novel synthetic route starting from the L-amino acid alanine. Some physicochemical properties, viz. lipophilicity and water solubility, of selected derivatives were measured. The biological activity was tested in vitro in cell-free protein synthesis inhibition assays, in bacterial and tumor cell growth inhibition assays, and in the L1210 leukemia in vivo model in mice. Also for selected drugs the acute toxicity in mice was determined. Ribosomes from both an eukaryotic and a prokaryotic organism were used in the protein synthesis inhibition systems. A linear correlation between the lipophilicity parameters measured was observed. Water solubility and drug toxicity in mice were found to be linearly correlated with lipophilicity. All the derivatives studied are more lipophilic than 1. The deshydroxysparsomycin analogues (**30–33**) showed an interesting phenomenon: increase in hydrophobicity was accompanied by a considerable increase in water solubility. We found that an increase in hydrophobicity of the drug as a result of replacing the SMe group of 1 with larger alkylthio groups causes an increase in the biological activity of the drug. However, not only the hydrophobicity but also shape and size of the substituent are important; in the homologous series **1–9–10–11–12**, **21–22–23–24**, and **30–31–32–33**, highest protein synthesis inhibitory and in vitro cytostatic activity is found with compounds **11**, **23**, and **32**, respectively, and in comparison with the highly active *n*-butyl compound **10**, the isomeric *tert*-butyl compound **13** is rather inactive. Polar substituents replacing the SMe group, i.e. Cl in **17** and **35**, also render the molecule inactive. Substituting the bivalent sulfur atom for a methylene group decreases the drug's activity. This effect can be compensated for by increasing the length of the alkylsulfinyl side chain. The agreement between the resuts derived from cell-free and "in vivo" tests is good. The assays using ribosomes of bacterial and eukaryotic organisms give similar results although the latter seem to be more sensitive to changes in hydrophobicity of the drug. Our results confirm the presence of a hydrophobic region at the peptidyl transferase center of the ribosome; the interaction of sparsomycin with this region is more pronounced in the eukaryotic particles. The sparsomycin analogues **11**, **23**, and **30** show the highest antitumor activity against L1210 leukemia in mice, their median T/C values are 386, 330, and 216%, respectively. Sparsomycin (1), showing a T/C value of 117%, is only marginally active against this tumor. The analogues tested are 5–100 times less toxic than 1.

Sparsomycin (1)[1] is a broad-spectrum antibiotic active against parasites,[2] viruses,[3,4] fungi,[5] and bacteria.[6,7] The antibiotic has achieved prominence in the past 25 years as a tool to study the protein biosynthesis machinery. Since the isolation of sparsomycin from *Streptomyces sparsogenes* in 1962, much attention was focused on its early observed antitumor activity.[5]

Recently, both we[8,9] and Helquist[10] succeeded in developing total syntheses of sparsomycin. In 1984, our synthetic route to sparsomycin allowed the preparation of the first analogue, i.e octylsparsomycin (**12**), which proved to be 3 times more active against L1210 leukemia in vitro

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