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The Discovery of (2*S*,3*S*)-*cis*-2-(Diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]-octan-3-amine as a Novel, Nonpeptide Substance P Antagonist

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We describe the structure–activity relationship development of a series of quinuclidines which culminated in the first potent, selective, nonpeptide substance P (SP) antagonist, (2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine, **3** (CP-96,345). Compound **3** is a potent displacer of [^{3}H]SP binding in human IM-9 cells and blocks SP-induced and capsaicin-induced plasma extravasation, as well as SP-induced salivation in the rat in vivo. This compound may both help to further our understanding of the interactions of small molecules with peptide receptors and serve to evaluate the therapeutic potential of a SP antagonist.

Substance P (SP), a peptide neurotransmitter first discovered in 1931[1] and eventually characterized in 1970 as the undecapeptide Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2,[2] is a member of the tachykinin family of peptides, which includes neurokinins A and B (NKA and NKB). These peptides bind to a series of three neurokinin receptors, NK_1, NK_2, and NK_3, which have selective affinity for SP, NKA, and NKB, respectively.[3] SP has been shown to elicit a wide variety of physiological responses which suggest it plays a role in initiating the immune response and in transmitting pain and stress to the central nervous system (CNS). For example, SP has been reported to elicit IL-1 production in macrophages,[4] sensitize neutrophils,[5] and enhance dopamine release in the substantia nigra region in cat brain.[6] Its key position at the interface between the immune system and CNS suggests SP may be involved in a variety of important diseases. There is evidence, for example, for SP's role in rheumatoid arthritis,[7] ulcerative colitis,[8] and migraine.[9]

Recognizing that SP blockade represents a potentially important avenue for novel therapy, previous workers in the field have sought to discover NK_1 receptor antagonists by modifying SP's peptide structure. Two strategies were ultimately successful: the "linear" approach used by Folkers' group, which led to spantide II,[10] **1**, and the "conformational constraint" approach, which culminated in the recent announcement of GR71251,[11] **2**. Despite the impressive pA_2 value of 7.7 at the NK_1 receptor for both these compounds, their peptidic structure is expected to limit their oral bioavailability and hence their potential for investigating clinical applications of NK_1 antagonists.

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‡ Department of Medicinal Biology.

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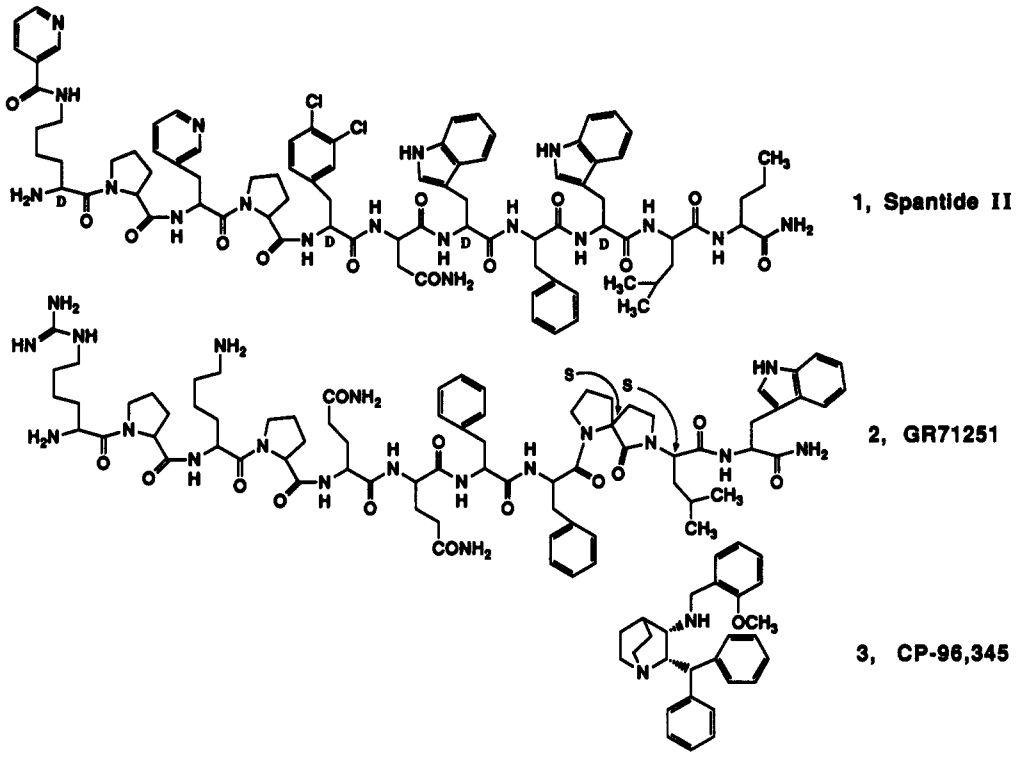
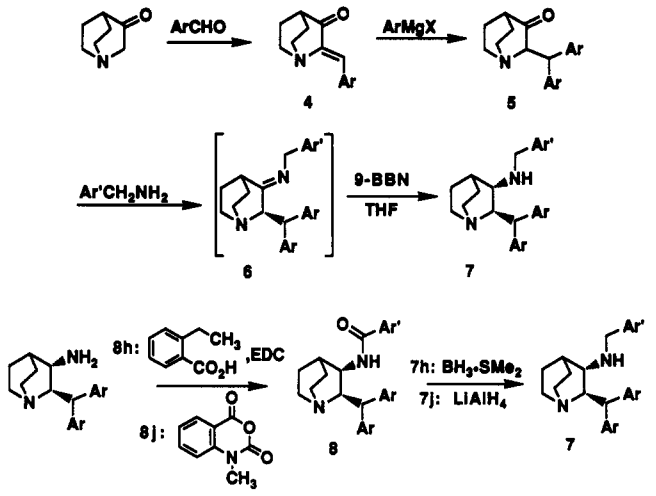


Figure 1. Substance P antagonists.

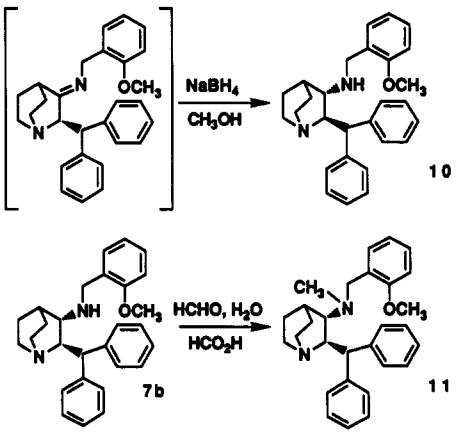
Scheme I. Preparation of Quinuclidine SP Antagonists



4-8	Ar	Ar'
a	Ph	Ph
b	Ph	(2-OMe)Ph
c	Ph	(3-OMe)Ph
d	Ph	(4-OMe)Ph
e	Ph	(2-Cl)Ph
f	Ph	(3-Cl)Ph
g	Ph	(4-Cl)Ph
h	Ph	(2-Et)Ph
i	Ph	(2-OEt)Ph
j	Ph	(2-NHMe)Ph
k	(4-Cl)Ph	(2-OMe)Ph

It was our intent to discover a nonpeptide SP antagonist which would surmount these difficulties, and to this end

Scheme II. Preparation of Compounds 10 and 11



we initiated a screening program using [^{3}H]SP binding in bovine caudate membranes. We describe herein the structure–activity relationship (SAR) development of **7a**, the parent compound from a lead series of quinuclidines from this effort, which led to the discovery of a potent NK_1 receptor antagonist (2*S*,3*S*)-*cis*-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (CP-96,345), **3** (Figure 1).

Chemistry

The quinuclidine compounds described herein were prepared by the method of Warawa et al.,[12] with the exception that 9-BBN was used for the final reduction of the intermediate imine in order to provide the desired cis stereochemistry, as shown in Scheme I.

Compound **7f** was prepared by acylation of the amino compound derived by debenzylation of **7a**, followed by borane/methyl sulfide reduction. The preparation of

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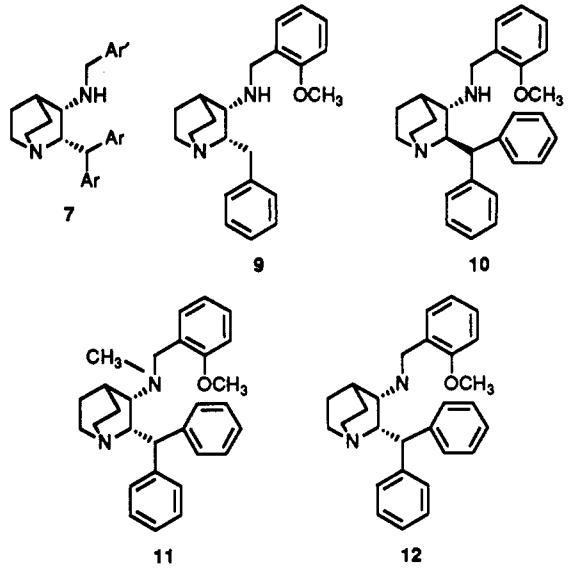
Table I. In Vitro SAR of Quinuclidine Substance P Antagonists



no.	Ar	Ar'	IC_{50}[a]
7a	Ph	Ph	16.0 ± 4.5
7b	Ph	2-OMePh	2.20 ± 0.50
7c	Ph	3-OMePh	20.0 ● 2.5
7d	Ph	4-OMePh	343 ± 82
7e	Ph	2-ClPh	33.3 ± 7.1
7f	Ph	3-ClPh	28000 ± 2310
7g	Ph	4-ClPh	1890 ± 440
7h	Ph	2-EtPh	16.7 ± 3.2
7i	Ph	2-OEtPh	16.0 ± 2.6
7j	Ph	2-(NHMe)Ph	67.7 ± 11
7k	4-ClPh	2-OMePh	67.7 ± 7.9
9			487 ± 150
10			41.0 ± 4.6
11			383 ● 146
12			>32000
CP-96,345			0.77 ± 0.24

[a] Binding affinity for the NK_1 receptor in human IM-9 cells using [^{3}H]substance P as ligand, given in nM units. IC_{50} values were determined from six-point concentration–response curves with each concentration in duplicate. Mean ± SEM values from three separate experiments are given for each compound. The value for CP-96,345 was determined from a four-point concentration–response curve.

analogue **9**, the trans compound **10**, and *N*-methyl analogue **11** is outlined in Scheme II. The N-methylation of compound **7b** to produce **11**, achieved by an Eschweiler–Clarke reaction,[13] is noteworthy for its success despite the steric congestion in compound **7b**. The desaza analogue, bicyclic compound **12**, was prepared in analogy with a literature route.[12]

The resolution of compound **7b** to provide **3** has already been described.[14] Full details of this procedure are provided in the Experimental Section.

Biology

The human IM-9 cell NK_1 receptor binding assay was carried out in analogy with a previously described method.[15] The hamster urinary bladder NK_2 receptor binding assay[16] and guinea pig cortex NK_3 binding assay[17] were based on previous methods. The SP- and capsaicin-induced plasma leakage assays used to assess the in vivo efficacy of the test compounds were carried out by measuring the extent of Evans Blue dye leakage induced by SP and capsaicin, respectively.[18] SP-induced salivation in the rat has previously been shown to involve the NK_1 receptor,[19] and was measured following SP challenge 30 min after administration of the test compounds.

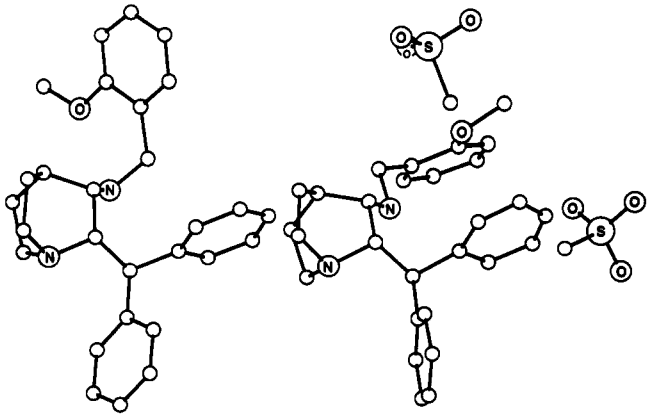


Figure 2. ORTEP rendering of single crystal X-ray data for **7b** and its dimesylate salt. Hydrogens are omitted for clarity.

Discussion

Our SAR investigation of compound **7a** began with a study of substituent effects in the benzylamino side chain at the C-3 position of the quinuclidine. As shown in Table I, potent activity was found with substituents in the ortho position, such as in compound **7b**, while both electron-donating (**7c** and **7d**) and electron-withdrawing (**7f** and **7g**) groups at the meta and para positions showed substantially reduced potency. In the ortho position, the electron-donating methoxy group was significantly more potent than a number of other groups which resemble it in size (**7h**), electron-donating capability (**7i**), or hydrogen-bonding ability (**7j**). These results led to the hypothesis that the 2-methoxy group induces a conformation of the benzylamino side chain which allows it to bind to a site in the receptor where groups larger than methoxy are not tolerated (vide infra). This hypothesis is supported by the reduced potency of *N*-methyl analogue **10**, which cannot assume the same conformation as the parent compound due to steric hindrance, and the loss of potency in the trans isomer, **9**, which does not place the benzylamino side chain in the same spatial orientation relative to the benzhydryl group and bridgehead nitrogen. The exact orientation

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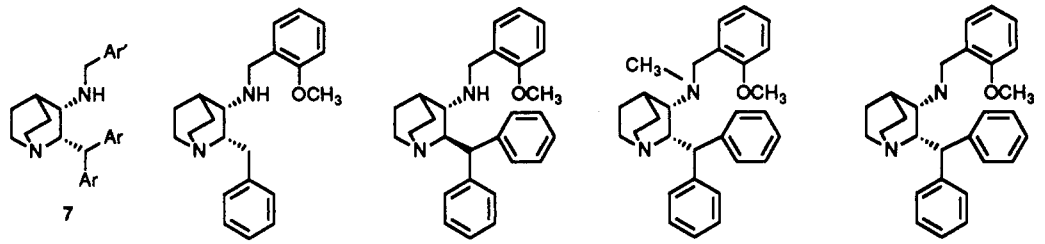
Table II. In Vivo SAR of Quinuclidine Substance P Antagonists



			% inhibition [ED_{50}, mg/kg]		
no.	Ar	Ar'	SP-induced plasma extravasation[a]	capsaicin-induced plasma extravasation[b]	SP-induced salivation[c]
7a	Ph	Ph	-9	-21	0 (3)
7b	Ph	2-OMePh	39	[6 (0-19)]	66.9 ± 4.4 (4)**
7c	Ph	3-OMePh	16	-8	51.5 ± 4.4 (4)*
7d	Ph	4-OMePh	31	-14	24.7 ± 14 (3)
7e	Ph	2-ClPh	38	[7 (1-13)]	43.9 ± 4.9 (5)*
7f	Ph	3-ClPh	-6	23	52.8 ± 4.3 (3)*
7g	Ph	4-ClPh	8	34	27.0 ± 6.5 (3)*
7h	Ph	2-EtPh	79	[4 (0-12)]	16.7 ± 5.2 (3)
7i	Ph	2-OEtPh	41	[3 (0-7)]	42.3 ± 8.5 (3)*
7k	4-ClPh	2-OMePh	44	21	0 (5)
9			35	-30	0 (2)
10			28	18	11.3 ± 6.1 (5)
11			13	-13	ND[d]
12			-9	-67	ND
CP-96,345			[4.4 ± 2.3 (3)]	[2.3 ± 0.4 (3)]	76.2 ± 2.8 (11)**

[a] Plasma extravasation, measured as spreading of Evans Blue dye, induced by injection of 1 pmol/site of substance P into the dorsal skin of guinea pigs. The test compound was administered at 15 mg/kg po, and the percent inhibition or ED_{50} value in mg/kg determined. [b] Plasma extravasation into guinea pig ureter, measured as spreading of Evans Blue dye, induced by 10 mL injection of 30 μM capsaicin into the peritoneal cavity. The test compound was administered at 10 mg/kg po, and the % inhibition determined. For compounds with >50% inhibition, an ED_{50} value (95% confidence limits) in mg/kg was determined and is in brackets. [c] Salivation in rats induced by injection of 4.7 nmol/kg substance P into the tail vein. The test compound was administered ip at 50 mg/kg 30 min prior to substance P challenge, and the percent inhibition determined. Compounds designated are considered significantly active at the $p < 0.01$* or $p < 0.001$** level. [d] ND, not determined.

assumed by the 2-methoxybenzyl side chain in **7b** cannot yet be determined, since X-ray structures (Figure 2) on the free base and mesylate salt forms of **7b** show different side-chain conformations, and the degree of protonation of **7b** at the receptor active site has not yet been ascertained.

The remaining determinants of binding potency are, first, an unsubstituted benzhydryl group, as shown by **7g** and **9**, and, second, the bridgehead nitrogen, as shown by **12**. These two structural features, which are important for potent in vitro activity, together with the benzylamino side chain make up the three points of a binding model describing the interactions of these compounds with the NK_1 receptor which is outlined in the conclusion.

The in vivo activity of the quinuclidine analogues, shown in Table II, also emphasizes the importance of an ortho substituent on the benzylamino side chain. Thus compounds **7b**, **7e**, **7h**, and **7i** showed greater than 50% inhibition of the capsaicin-induced plasma leakage model in the guinea pig, which translated to ED_{50} values of 3–7 mg/kg. Since the potency of these four compounds was considered equivalent, the SP-induced salivation model in the rat was used to differentiate them further. As shown in Table II, **7b** proved to be the most potent, consistent with its superiority in vitro. Finally, a time-course study of the inhibition of SP-induced salivation in the rat was carried out as shown in Figure 3, demonstrating the duration of action of **7b** and **3** administered orally.

With the demonstration that 2-methoxy analogue **7b** possesses the optimal activity in this series, resolution was carried out to afford the 2*S*,3*S* enantiomer, compound **3**, as previously described.[14] The pharmacological characterization of this compound, demonstrating its credentials as an NK_1 receptor antagonist, has been described in detail elsewhere.[20,21] Briefly, **3** was shown to be a competitive,

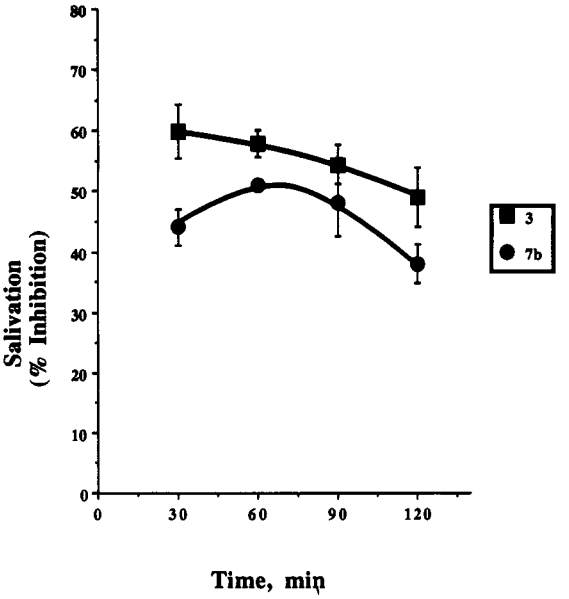


Figure 3. Time course for inhibition of SP-induced salivation in the rat by compounds **3** and **7b**. Data points represent the mean percent inhibition of SP-induced salivation +/- SEM of three to seven animals, where compounds were administered po at a dose of 50 mg/kg following the protocol given in the Experimental Section. The data point without an error bar is the mean of two animals.

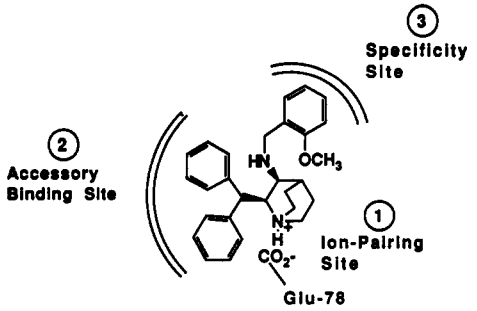


Figure 4. Three-point binding model for interaction of CP-96,345 with the NK_1 receptor.

reversible NK_1 antagonist by studies in the dog isolated carotid artery with a pA_2 value of 8.7 and to block SP-induced excitation of neurons in the locus coeruleus in guinea pig brain (IC_{50} value of 90 nM); in vivo, **3** blocks SP-induced salivation in the rat[21]; finally, [$^3H_2$]-**3** displays an autoradiographic binding pattern identical to that for [^3H]-SP in guinea pig brain.[21] Finally, we report here that compound **3** has negligible affinity (IC_{50} values > 10 μM) for the NK_2 receptor in hamster urinary bladder and the NK_3 receptor in guinea pig cortex, demonstrating its selectivity for the NK_1 receptor. Thus **3** not only provides characterization of many aspects of SP pharmacology but also establishes possible therapeutic utilities for a SP antagonist.

Conclusion

Based on the preceding SAR analysis, the essential elements involved in recognition of **3** by the NK_1 receptor can be assembled into a three-point binding model. As depicted in Figure 4, these three points are (1) the ion-pair site interaction with the bridgehead nitrogen, (2) the accessory binding site interaction with the benzhydryl group, and (3) the specificity site interaction with the (2-methoxybenzyl)amino side chain. There is precedent not only for a three-point binding model for small molecule interactions with a peptide receptor[22] but also for the first two of the proposed binding sites.

The ion-pair site is proposed to be an acidic residue within the transmembrane domain of the NK_1 receptor. The glutamic acid residue at position 78[23] is a likely candidate for this site, since the homologous residue within the transmembrane domain of the β-adrenergic receptor has been shown to be important for ligand binding.[24] The accessory binding site has been proposed by Ariens to recognize the "double-ring" motif, exemplified by the benzhydryl group, which is a common structural feature of G protein-coupled receptor antagonists.[25] An example of a binding model based on these two points was recently described for compounds binding to the dopamine D_2 receptor.[26] The specificity site, however, seems to be unique to the NK_1 receptor and is proposed to be an important aspect of antagonist binding to G protein-coupled receptors for peptides.[27]

The three points of the binding model in Figure 4 provide a paradigm for the design of antagonists at other peptide receptors belonging to the G protein-coupled receptor family. While the first two points are familiar from previous work on receptor antagonists, the specificity site interactions may well be unique for each peptide receptor, and thus require the most SAR investigation. Compound **3** will be useful for constructing photoaffinity reagents and other tools to help delineate the portions of the NK_1 receptor responsible for these interactions. Until such studies reveal the molecular basis for these interactions, however, simple models such as that shown in Figure 4 are the most reliable for the design of new peptide receptor antagonists.

CP-96,345, **3**, should thus be useful in understanding the interactions of antagonists at peptide receptors, and may well help to unravel details of the structure and function of the NK_1 receptor. In the meantime, it is already proving valuable as a tool for characterization of SP pharmacology, and at the same time suggests considerable potential for the therapeutic utility of a SP antagonist.

Table III. Physical Properties of Compounds **7**, **8h**, **8j**, **9**, **10**, **11**, and **12**

no.	formula	mp, °C	% yield	anal.
7b	$C_{28}H_{32}N_2O$	132–135	71	C,H,N
7c	$C_{28}H_{32}N_2O$	156–158	58	C,H,N
7d	$C_{28}H_{32}N_2O \cdot {}^1/_4H_2O$	154–157	28	C,H,N
7e	$C_{27}H_{29}N_2Cl \cdot {}^1/_4H_2O$	172–174	58	C,H,N
7f	$C_{27}H_{29}N_2Cl \cdot {}^1/_4H_2O$	186–188	64	C,H,N
7g	$C_{27}H_{29}N_2Cl$	157–160	37	C,H,N
7h	$C_{29}H_{34}N_2 \cdot {}^1/_4H_2O$	145–150	71	C,H,N
7i	$C_{29}H_{34}N_2O \cdot H_2O$	159–166	79	C,H,N
7j	$C_{28}H_{32}N_3 \cdot {}^3/_4H_2O$	173–176	71	C,H,N
7k	$C_{28}H_{30}N_2OCl_2$	172–174	14	C,H,N
8h	$C_{29}H_{32}N_2O \cdot {}^1/_2H_2O$	180–186	39	C,H,N
8j	$C_{28}H_{31}N_3O \cdot {}^1/_4H_2O$	242–246	66	C,H,N
9	$C_{22}H_{28}N_2O \cdot 2HCl \cdot {}^1/_2H_2O$	140–160	29	C,H,N
10	$C_{28}H_{32}N_2O \cdot H_2O$	140–155	22	C,H,N
11	$C_{29}H_{34}N_2O$	125–132	80	C,H,N
12	$C_{29}H_{33}NO$	153–157	55	C,H,N

Experimental Section

Melting points were obtained on a Hoover Melting Point Apparatus and are uncorrected. NMR spectra were obtained on a

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Varian XL-300 or a Bruker AM-300, with trimethylsilane as internal standard; all J values are in hertz. IR spectra were obtained on Perkin-Elmer 283B and 1420 spectrometers. Mass spectra were obtained on a Finnegan 4510 mass spectrometer, and high-resolution mass spectra were obtained on an AE-9 instrument. TLC analysis was carried out on EM Kieselgel 60 F_{254} 5 × 20 cm plates. Elemental analyses were carried out by the Analytical Laboratory of Pfizer Central Research, and are within ±0.4% of theory unless otherwise noted.

Syntheses. The examples presented below illustrate the methods for preparation of the compounds which are listed in Table III along with their physical data. The following compounds were prepared by literature methods: **7a**,[12] *cis*-2-(diphenylmethyl)-1-azabicyclo[2.2.2]octan-3-amine,[12] *cis*-2-(diphenylmethyl)bicyclo[2.2.2]octan-3-amine,[12] *cis*-2-[bis(4-chlorophenyl)methyl]-1-azabicyclo[2.2.2]octan-3-one,[28] *cis*-2-[bis(4-bromophenyl)methyl]-1-azabicyclo[2.2.2]octan-3-one,[29] and 2-(phenylmethyl)-1-azabicyclo[2.2.2]octan-3-one.[30]

Method A. *cis*-2-(Diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (7b). To a 50-mL round-bottomed flask equipped with a Dean–Stark trap, condenser, and N_2 inlet were added 1.33 g (4.56 mmol) of 2-(diphenylmethyl)-1-azabicyclo[2.2.2]octan-3-one, 938 mg (6.84 mmol) of 2-methoxybenzylamine, 2 mg of camphorsulfonic acid, and 23 mL of toluene. The solution was refluxed 18 h and cooled, and the solvent evaporated. The residue was taken up in 3 mL of dry tetrahydrofuran and treated with 18 mL (9.2 mmol) of a 0.5 M solution of 9-BBN in tetrahydrofuran. The solution was stirred at room temperature 3 days, poured into 1 N hydrochloric acid, and extracted with methylene chloride. The aqueous layer was adjusted to pH 14 with solid sodium hydroxide, then extracted with methylene chloride. The organic layer was dried over sodium sulfate and evaporated. The residue was crystallized from 2-propanol to afford 1.33 g (71%) of a white solid: mp 132–135 °C; ^{1}H NMR (δ, $CDCl_3$) 1.2–2.1 (series of 5 multiplets, 5 H), 2.7–2.9 (m, 4 H), 3.2–3.3 (m, 3 H), 3.67 (dd, 1 H), 3.78 (s, 3 H), 4.45 (d, J = 12, 1 H), 6.6–6.9 and 7.1–7.4 (m, 13 H); ^{13}C NMR (δ, $CDCl_3$) 20.0, 24.6, 25.1, 42.2, 46.3, 49.4, 49.6, 54.4, 55.2, 61.8, 111.0, 120.0, 126.3, 126.7, 127.6, 127.7, 127.9, 128.3, 128.6, 129.4, 129.6, 143.0, 146.4, 159.6; MS m/e 413 (parent + 1, <1), 216 (34), 215 (100), 96 (13), 92 (15), 91 (91), 65 (15). Anal. ($C_{28}H_{32}N_2O$) C, H, N.

The dimesylate salt was prepared using excess methanesulfonic acid in acetone, mp 248–251 °C.

***cis*-2-(Diphenylmethyl)-*N*-[(3-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (7c)** was prepared according to method A in 58% yield: mp 156–158 °C; ^{1}H NMR (δ, $CDCl_3$) 1.3–2.1 (m, 5 H), 2.70 (m, 1 H), 2.84 (m, 2 H), 2.94 (m, 1 H), 3.25 (m, 1 H), 3.45 (AB q, J_{AB} = 13, $\Delta\nu$ = 114, 2 H), 3.76 (m, 1 H), 3.79 (s, 3 H), 4.50 (d, J = 12, 1 H), 6.2–7.4 (m, 14 H); ^{13}C NMR (δ, $CDCl_3$) 20.2, 24.8, 25.5, 42.1, 49.4, 49.6, 52.2, 54.7, 55.2, 61.8, 112.0, 113.8, 120.1, 126.0, 126.6, 127.5, 127.6, 128.4, 129.3, 141.6, 143.3, 145.2, 159.5; IR (cm^{-1}, KBr): 1607, 1582 (C=C); MS m/e 413 (1, parent + 1), 245 (100), 121 (89), 96 (51), 91 (95), 77 (72). Anal. ($C_{28}H_{32}N_2O$) C, H, N.

***cis*-2-(Diphenylmethyl)-*N*-[(4-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (7d)** was prepared according to method A in 28% yield: mp 154–157 °C; ^{1}H NMR (δ, $CDCl_3$) 1.2–2.0 (m, 5 H), 2.6–2.9 (m, 4 H), 3.1–3.3 and 3.55 (m, 3 H), 3.69 (dd, 1 H), 3.77 (s, 3 H), 4.42 (d, 1 H), 6.57 (m, 4 H), 7.0–7.4 (m, 10 H); ^{13}C NMR (δ, $CDCl_3$) 20.1, 24.6, 25.4, 42.1, 49.4, 49.6, 51.4, 54.4, 55.3, 61.8, 113.6, 126.0, 126.5, 127.5, 127.6, 128.5, 128.9, 129.3, 132.0, 143.3, 145.2, 158.4; MS m/e 413 (<1, parent + 1), 245 (58), 121 (100), 91 (33). Anal. ($C_{28}H_{32}N_2O \cdot {}^1/_4H_2O$) C, H, N.

***cis*-2-(Diphenylmethyl)-*N*-[(2-chlorophenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (7e)** was prepared according to method A in 58% yield: mp 172–174 °C; ^{1}H NMR (δ, $CDCl_3$) 1.2–2.1 (m, 5 H), 2.68 (m, 1 H), 2.86 (m, 2 H), 2.97 (m, 1 H), 3.31 (m, 1 H), 3.43 and 3.72 (m, 2 H), 3.78 (dd, 1 H), 4.55 (d, 1 H), 6.65 (m, 1 H), 7.1–7.5 (m, 13 H); ^{13}C NMR (δ, $CDCl_3$) 20.2, 25.1, 25.6, 42.1, 49.1, 49.4, 49.6, 54.8, 61.9, 126.0, 126.6, 127.6, 127.7, 128.0, 128.4, 129.3, 129.8, 133.9, 137.5, 143.3, 145.4; MS m/e 417 (<1, parent + 1), 251 (42), 249 (100), 127 (26), 125 (86), 96 (40), 91 (29). Anal. ($C_{27}H_{29}N_2Cl \cdot {}^1/_4H_2O$) C, H, N.

***cis*-2-(Diphenylmethyl)-*N*-[(3-chlorophenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (7f)** was prepared according to method A in 64% yield: mp 186–188 °C; ^{1}H NMR (δ, $CDCl_3/CD_3OD$) 1.2–2.0 (m, 5 H), 2.6–2.9 (m, 4 H), 3.2–3.4 and 3.62 (m, 3 H), 3.81 (dd, 1 H), 4.41 (d, 1 H), 6.6 (m, 2 H), 7.1–754 (m, 12 H); ^{13}C NMR (δ, $CDCl_3/CD_3OD$) 23.8, 28.3, 29.0, 45.8, 53.3, 53.4, 55.4, 58.3, 65.6, 131.0, 131.1, 131.2, 131.4, 131.5, 131.6, 131.7, 131.9, 132.5, 138.0, 145.5, 147.0, 148.6; MS m/e 417/419 (2.1/<1, parent + 1), 291 (34), 251 (42), 249 (100), 127 (31), 125 (64). Anal. ($C_{27}H_{29}N_2Cl \cdot {}^1/_4H_2O$) C, H, N.

***cis*-2-(Diphenylmethyl)-*N*-[(4-chlorophenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (7g)** was prepared according to method A in 37% yield: mp 157–160 °C; ^{1}H NMR (δ, $CDCl_3$) 1.2–2.0 (m, 5 H), 2.6–2.9 (m, 4 H), 3.16 and 3.54 (m, 3 H), 3.67 (dd, 1 H), 4.37 (d, 1 H), 6.45 (m, 2 H), 7.0–7.4 (m, 12 H); ^{13}C NMR (δ, $CDCl_3$) 20.1, 24.6, 25.3, 42.1, 49.4, 49.5, 51.2, 54.3, 61.7, 126.1, 126.6, 127.5, 127.6, 128.3, 128.5, 129.2, 129.4, 132.3, 138.3, 143.3, 145.0; MS m/e 417 (<1, parent + 1), 251 (62), 249 (100), 127 (57), 125 (94), 96 (60), 91 (60). Anal. ($C_{27}H_{29}N_2Cl$) C, H, N.

***cis*-2-(Diphenylmethyl)-*N*-[(2-ethoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (7i)** was prepared according to method A in 79% yield; mp 159–166 °C; ^{1}H NMR (δ, $CDCl_3$) 1.33 (t, 3 H), 1.3–2.2 (m, 5 H), 2.6–3.9 (8 H), 4.54 (d, 1 H), 6.6–7.4 (m, 14 H), ^{13}C NMR (δ, $CDCl_3$) 15.0, 19.9, 24.8, 25.3, 42.0, 45.9, 49.2, 49.5, 54.2, 62.2, 63.3, 111.1, 120.0, 126.0, 126.5, 127.6, 127.8, 128.0, 128.1, 128.6, 128.7, 129.1, 129.2, 129.4, 143.0, 145.4, 156.7; MS m/e 427 (parent + 1, 3), 292 (11), 291 (41), 274 (26), 260 (48), 259 (100), 165 (11), 136 (11), 135 (70), 107 (36), 96 (17), 91 (54), 79 (15), 77 (20). Anal. ($C_{29}H_{34}N_2O \cdot H_2O$), C, H, N.

***cis*-2-[Bis(4-chlorophenyl)methyl]-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (7k)** was prepared according to method A in 14% yield; mp 172–174 °C; ^{1}H NMR (δ, $CDCl_3$) 1.25 (m, 1 H), 1.45 (m, 1 H), 1.62 (m, 1 H), 1.89 (m, 1 H), 2.08 (m, 1 H), 2.59 (m, 1 H), 2.71 (m, 3 H), 2.82 (m, 1 H), 3.12 (m, 1 H), 3.44 (AB q, J_{AB} = 13, $\Delta\nu$ = 99, 2 H), 3.55 (s, 3 H), 3.6 (m, 1 H), 4.44 (d, J = 12, 1 H), 6.7–7.4 (m, 12 H), ^{13}C NMR (δ, $CDCl_3$) 19.9, 24.7, 25.5, 42.0, 46.7, 49.5, 49.6, 53.9, 55.2, 61.8, 110.0, 120.1, 127.7, 128.2, 128.4, 128.5, 128.6, 128.8, 128.9, 129.1, 129.7, 131.6, 132.2, 141.3, 143.7, 157.5; IR (cm^{-1}, KBr) 1601, 1590 (C=C); MS m/e 245 (100), 121 (72), 91 (43). Anal. ($C_{28}H_{30}N_2OCl_2$) C, H, N.

***cis*-2-(Diphenylmethyl)-*N*-(2-ethylbenzoyl)-1-azabicyclo[2.2.2]octan-3-amine (8h).** To a 50-mL round-bottomed flask equipped with condenser and N_2 inlet were added 0.78 g (5.188 mmol) of 2-ethylbenzoic acid, 0.84 g (5.188 mmol) of carbonyldiimidazole, and 17 mL of dry tetrahydrofuran. The reaction was stirred at room temperature for 1 h, then 1.01 g (3.459 mmol) of *cis*-2-(diphenylmethyl)-1-azabicyclo[2.2.2]octan-3-amine was added, and the reaction was refluxed for 18 h. It was then poured into water and extracted into methylene chloride. The organic extracts were dried over sodium sulfate and evaporated, and the residue was chromatographed on silica gel using methanol/methylene chloride as eluent to afford 576 mg (39%) of a white solid: mp 180–186 °C; ^{1}H NMR (δ, $CDCl_3$) 1.27 (t, J = 7, 3 H), 1.4–1.5 (m, 1 H), 1.7–1.9 (m, 3 H), 2.24 (m, 1 H), 2.4–2.6 (m, 2 H), 2.73 (m, 1 H), 2.84 (m, 2 H), 3.03 (m, 1 H), 4.04 (dd, J = 2, 12, 1 H), 4.21 (d, J = 12, 1 H), 4.62 (m, 1 H), 6.17, 6.45, and 7.0–7.4 (m, 14 H); ^{13}C NMR (δ, $CDCl_3$) 5.7, 20.2, 25.4, 26.0, 27.4, 41.3, 48.4, 49.6, 50.2, 60.5, 125.4, 126.3, 126.5, 127.4, 127.5, 128.5, 128.9, 129.1, 129.6, 136.5, 141.8, 142.3, 144.8, 169.6; IR (cm^{-1}, KBr) 1633 (C=O); MS m/e 424 (39, parent), 257 (86), 180 (83), 133 (100). Anal. ($C_{29}H_{32}N_2O \cdot {}^1/_2H_2O$) C, H, N.

***cis*-2-(Diphenylmethyl)-*N*-[(2-ethylphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (7h).** To a 50-mL round-bottomed flask equipped with condenser and N_2 inlet were added 432 mg (1.02 mmol) of *cis*-2-(diphenylmethyl)-*N*-[(2-ethylphenyl)carbonyl]-1-azabicyclo[2.2.2]octan-3-amine, 5 mL of dry

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tetrahydrofuran, and 2.5 mL (5.1 mmol) of a 2 M solution of borane–methyl sulfide in tetrahydrofuran. The reaction was refluxed for 3 days, cooled, and evaporated. The residue was taken up in 20 mL of ethanol, treated with 1 g of sodium carbonate and 1 g of cesium fluoride, and then refluxed for 4 days. The reaction was cooled, concentrated, taken up in water, and extracted into methylene chloride. The organic layer was dried over sodium sulfate and evaporated, and the residue crystallized from 2-propanol to afford a white solid: 418 mg (71%); mp 145–150 °C; ^{1}H NMR (δ, $CDCl_3$) 1.27 (t, 3 H), 1.3–2.2 (m, 5 H), 2.49 (q, 2 H), 2.6–3.9 (8 H), 4.55 (d, 1 H), 6.73 (d, 1 H), 7.1–7.6 (m, 13 H); ^{13}C NMR (δ $CDCl_3$) 15.6, 20.4, 25.2, 2.4, 25.6, 42.3, 49.4, 49.7, 49.9, 56.5, 61.9, 125.9, 126.1, 126.6, 127.1, 127.7, 128.3, 128.5, 129.2, 129.3, 137.7, 142.2, 143.3, 145.4; MS *m/e*, 411 (parent + 1, 3.5), 291 (53), 244 (48), 243 (100), 165 (23), 119 (69), 117 (27), 103 (21), 96 (31), 91 (53). Anal. ($C_{29}H_{34}N_2 \cdot {}^1/_4H_2O$) C, H, N.

***cis*-2-(Diphenylmethyl)-*N*-[(2-methylamino)benzoyl]-1-azabicyclo[2.2.2]octan-3-amine (8j).** To a 50-mL round-bottomed flask equipped with condenser and N_2 inlet were added 1.00 g (3.42 mmol) of *cis*-2-(diphenylmethyl)-1-azabicyclo-[2.2.2]octan-3-amine, 0.91 g (5.14 mmol) of *N*-methylisatoic anhydride, and 17 mL of dry tetrahydrofuran. The reaction was refluxed for 18 h, cooled, and partitioned between water and methylene chloride. The organic layer was washed with brine, dried over sodium sulfate, and evaporated. The residue was crystallized from 2-propanol to give a white solid: 966 mg (66%); mp 242–246 °C; ^{1}H NMR (δ, $CDCl_3$) 1.3–1.5 (m, 1 H), 1.5–1.8 (m, 3 H), 2.01 (m, 1 H), 2.69 (d, J = 6, 3 H), 2.6–2.9 (m, 3 H), 2.9–3.0 (m, 1 H), 3.93 (dd, J = 2, 12, 1 H), 4.09 (d, J = 12, 1 H), 4.43 (m, 1 H), 6.11, 6.4–6.6, and 6.9–7.5 (m, 14 H); IR (cm^{-1}, KBr) 1638 (C=O); MS *m/e* 425 (30, parent), 258 (80), 134 (100). Anal. ($C_{28}H_{31}N_3O \cdot {}^1/_4H_2O$) C, H, N.

***cis*-2-(Diphenylmethyl)-*N*-[[2-(methylamino)phenyl]-methyl]-1-azabicyclo[2.2.2]octan-3-amine (7j).** To a 50-mL round-bottomed flask equipped with condenser and N_2 inlet were added 929 mg (2.19 mmol) of *cis*-2-(diphenylmethyl)-*N*-[2-(methylamino)benzoyl]-1-azabicyclo[2.2.2]octan-3-amine, 11 mL of tetrahydrofuran, and 250 mg (6.59 mmol) of lithium aluminum hydride. The reaction was refluxed for 72 h, an additional 300 mg (7.90 mmol) of lithium aluminum hydride added, and the reaction again refluxed for 72 h. The reaction was cooled and poured into water, and the mixture extracted with methylene chloride. The organic layer was dried over sodium sulfate and evaporated, and the residue chromatographed on silica gel using methanol/methylene chloride as eluent. The product fractions were collected and crystallized from 2-propanol to afford a white solid: 296 mg (71%); mp 173–176 °C; ^{1}H NMR (δ, $CDCl_3$) 1.4–2.2 (m, 5 H), 2.8–3.2 and 3.6 (m, 7 H), 2.82 (s, 3 H), 3.91 (dd, J = 8, 12, 1 H), 4.37 (d, J = 12, 1 H), 6.6–7.6 (m, 14 H); ^{13}C NMR (δ, $CDCl_3$) 20.5, 25.4, 25.6, 30.6, 42.2, 49.6, 49.7, 52.1, 57.1, 61.9, 109.7, 116.4, 124.4, 126.2, 126.6, 127.5, 127.7, 128.6, 129.2, 129.5, 143.2, 145.2, 148.9; MS *m/e* 411 (5.6, parent) 291 (57), 274 (27), 245 (34), 244 (100), 120 (83), 91 (23). Anal. ($C_{28}H_{33}N_3/\cdot {}^3/_4H_2O$) C, H, N.

***cis*-2-(Phenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine (9)** was prepared from 2-(phenylmethyl)-1-azabicyclo[2.2.2]octan-3-one[30] according to method A in 27% yield: mp 140–160 °C; ^{1}H NMR (δ, $CDCl_3$) 1.42 (m, 1 H), 1.61 (m, 1 H), 1.78 (m, 1 H), 2.04 (m, 1 H), 2.16 (m, 1 H), 2.83 (m, 1 H), 2.99 (m, 4 H), 3.3–3.4 (m, 3 H), 3.78 (AB q, J_{AB} = 13, $\Delta\nu$ = 82, 2 H), 3.79 (s, 3 H), 6.9–7.4 (m, 5 H); ^{13}C NMR (δ, $CDCl_3$) 19.8, 24.8, 25.6, 34.0, 42.0, 47.4, 49.3, 55.1, 55.3, 60.3, 110.1, 120.3, 125.9, 128.1, 128.37, 128.45, 128.6, 129.6, 140.5, 157.6; IR (cm^{-1}, KBr) 1600 (C=C); MS *m/e* 337 (56, parent + 1), 336 (19, parent), 245 (100), 215 (91), 198 (62), 176 (78), 160 (53), 121 (93), 91 (87). Anal. ($C_{22}H_{28}N_2O \cdot 2HCl \cdot {}^1/_2H_2O$) C, H, N.

***trans*-2-(Diphenylmethyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine (10).** To a 50-mL round-bottomed flask equipped with N_2 inlet were added 517 mg (1.26 mmol) of 2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-imine, prepared as described in method A, and 6 mL methanol. The solution was cooled to 0 °C, and 191 mg (5.04 mmol) of sodium borohydride added. The reaction was allowed to warm to room temperature and stirred for 24 h. It was then poured into water and the mixture extracted with methylene chloride. The organic layer was dried over sodium sulfate and evaporated, and the residue chromatographed on silica gel using methylene chloride/methanol as eluent to afford a white solid after crystallization from 2-propanol: 113 mg (22%); mp 140–155 °C; ^{1}H NMR (δ, $CDCl_3$) 1.3–2.1 (m, 5 H), 2.6, (m, 2 H), 2.9–3.2 (m, 2 H), 3.36 (AB q, J_{AB} = 13, $\Delta\nu$ = 130, 2 H), 3.31 (dd, J = 5, 12, 1 H), 3.73 (s, 3 H), 4.01 (d, J = 12, 1 H), 6.7–7.4 (m, 14 H); ^{13}C NMR (δ, $CDCl_3$) 19.4, 24.7, 26.0, 40.9, 45.5, 55.3, 60.0, 67.1, 110.3, 120.3, 126.4, 126.8, 128.0, 128.6, 128.8, 129.5, 142.4, 143.2, 157.2; MS *m/e* 413 (<1, parent), 291 (21), 246 (38), 237 (100), 121 (74), 96 (26), 91 (72). Anal. ($C_{28}H_{30}N_2O \cdot H_2O$) C, H, N.

***cis*-2-(Diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-*N*-methyl-1-azabicyclo[2.2.2]octan-3-amine (11).** To a 25-mL round-bottomed flask equipped with condenser and N_2 inlet were added 1000 mg (2.43 mmol) of *cis*-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine, 6 mL of formic acid, and 6 mL of 37% aqueous formaldehyde solution. The reaction was heated at 80 °C for 1 h, cooled, and poured into aqueous sodium hydroxide solution. The aqueous layer was extracted twice with methylene chloride, and the organic layer dried over sodium sulfate and evaporated. The residue was crystallized from 2-propanol to afford a white solid: 820 mg (80%); mp 125–132 °C; ^{1}H NMR (δ, $CDCl_3$) 1.3–2.1 (m, 5 H), 2.29 (s, 3 H), 2.60 (m, 1 H), 2.82 (m, 2 H), 3.0–3.2 (m, 2 H), 3.39 (AB q, J_{AB} = 13, $\Delta\nu$ = 117, 2 H), 3.77 (s, 3 H), 4.04 (dd, J = 1, 12, 1 H), 4.46 (d, J = 12, 1 H), 6.3, 6.6–6.7, and 7.0–7.3 (m, 14 H); ^{13}C NMR (δ, $CDCl_3$) 22.9, 24.4, 30.9, 40.2, 41.8, 49.8, 51.6, 55.1, 55.7, 63.2, 64.6, 109.8, 120.2, 125.1, 125.7, 127.3, 127.9, 128.0, 128.2, 128.4, 130.1, 146.0, 146.4, 157.4; MS *m/e* 426 (4, parent), 305 (59), 274 (32), 260 (39), 259 (100), 121 (85), 110 (41), 91 (89). Anal. ($C_{29}H_{34}N_2O$) C, H, N.

***cis*-2-(Diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-bicyclo[2.2.2]octan-3-amine (12)** was prepared according to method A in 55% yield: mp 153–157 °C; ^{1}H NMR (δ, $CDCl_3$) 1.1–2.0 (series of m, 10 H), 2.61 (m, 1 H), 2.95 (m, 1 H), 3.23 (m, 1 H), 3.57 (s, 3 H), 3.58 (m, 1 H), 4.36 (d, J = 12.5, 1 H), 6.5–7.4 (m, 14 H); ^{13}C NMR (δ, $CDCl_3$) 19.6, 20.9, 24.2, 26.4, 27.0, 27.1, 43.1, 46.0, 49.4, 55.2, 109.9, 120.1, 125.8, 126.1, 127.6, 127.8, 128.4, 128.5, 128.9, 129.4, 144.6, 146.1, 157.4; IR (cm^{-1}, KBr) 1599 (C=C); MS *m/e* 411 (13, parent), 167 (33), 136 (33), 121 (100), 91 (73). Anal. ($C_{29}H_{33}NO$) C, H, N.

Preparation of CP-96,345, 3. *cis*-2-[Bis(4-bromophenyl)-methyl]-*N*-[3-(4-methoxyphenyl)methyl]-1-azabicyclo-[2.2.2]octan-3-amine was prepared according to method A in 59% yield: mp 166–169 °C; ^{1}H NMR (δ, $CDCl_3$) 1.2–2.0 (multiplets, 5 H), 2.59 and 2.73 (2 m, 4 H), 3.1 (m, 1 H), 3.36 (AB q, J_{AB} = 12, $\Delta\nu$ = 120, 2 H), 3.5–3.6 (m, 1 H), 3.77 (s, 3 H), 4.31 (d, 1 H), 6.5–7.4 (series of d with fine splitting, 12 H); IR (cm^{-1}, KBr) 1610 (aromatic C=C); MS *m/e* 449 (1, parent – (4-methoxybenzyl)), 246 (21), 245 (100), 121 (83). Anal. Calcd for $C_{28}H_{30}N_2OBr_2$: C, 58.96; H, 5.30; N, 4.91. Found: C, 59.27; H, 5.20; N, 4.89.

***cis*-2-[Bis(4-bromophenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine.** To a 250-mL round-bottomed flask equipped with condenser and N_2 inlet were added 4.91 g (8.61 mmol) of *cis*-2-[bis(4-bromophenyl)methyl]-*N*-[3-(4-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine and 100 mL of concentrated (48%) hydrobromic acid. The reaction was stirred at a bath temperature of 130–140 °C for 2 h, cooled very briefly to prevent crystallization of the hydrobromide salt, and poured onto ice. The mixture was adjusted to pH 13 with solid sodium hydroxide under cooling and extracted twice with methylene chloride. The organic layer was dried over sodium sulfate and evaporated to a white solid, 3.20 g (82.6%), which could be used directly in the following reaction, or chromatographed on silica gel using 2/1 methylene chloride/methanol as eluent to afford a white solid after trituration with hexane: mp 172–174 °C; ^{1}H NMR (δ, $CDCl_3$) 0.80 (br s, 2 H, NH_2), 1.1–1.8 (multiplets, 5 H), 0.54, 2.73, and 3.06 (multiplets, 4 H), 3.16 (dd, J = 7, 5, 1 H), 3.43 (dd, J = 12, 7, 1 H), 4.44 (d, J = 12, 1 H), 7.0–7.5 (series of d with fine splitting, 8 H); IR (cm^{-1}, KBr) 1600 (aromatic C=C); MS *m/e* 450 (<1, parent), 165 (10), 126 (13), 125 (100), 56 (9). Anal. Calcd for $C_{20}H_{22}N_2Br_2 \cdot H_2O$: C, 51.30; H, 5.17; N, 5.98. Found: C, 51.51; H, 4.78; N, 5.79.

(+)-*cis*-2-[Bis(4-bromophenyl)methyl]-*N*-[[1-(1-naphthyl)ethyl]carbamoyl]-1-azabicyclo[2.2.2]octan-3-amine. To a 125-mL round-bottomed flask equipped with condenser and N_2 inlet were added 2.28 g (5.07 mmol) of *cis*-2-[bis(4-bromophenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine, 1.00 g (5.07

mmol) of (*S*)-(+)-1-(1-naphthyl)ethyl isocyanate, and 40 mL of toluene. The solution was refluxed 4 h and then filtered while hot. The solid was washed with toluene and dried to afford 1.17 g (35.7% of total theoretical) of a white solid, mp 284–285 °C, shown by ^{1}H NMR to be >95% one diastereomer. This material could be recrystallized by dissolving it in a mixture of 50 mL of methylene chloride and enough methanol to afford solution at reflux, followed by addition of methanol at reflux until most of the methylene chloride had been evaporated and a white solid just started to appear. The solid that crystallized on standing was collected with methanol to give 0.78 g, mp 285–286 °C, $[\alpha]_D$ +67.4° (c = 1, DMSO). Further crystallization did not increase the optical purity as indicated by the rotation value: ^{1}H NMR (δ, $CDCl_3$) 0.87 (m, 1 H), 1.3–1.8 (multiplets, 5 H), 1.45 (d, J = 7, 3 H, the methyl group from the chiral auxiliary, which is used to judge the diastereomeric purity; the other methyl doublet is at 1.53), 2.11 (m, 1 H), 2.50 (m, 2 H), 3.5 (m, 2 H), 3.87 (br s, 1 H, NH), 4.24 (d, J = 12, 1 H), 4.44 (br s, 1 H, NH), 5.05 (m, 1 H), 6.7–8.2 (m, 15 H); IR (cm^{-1}, KBr) 1635 (C=O); MS 647 (8, parent), 322 (100), 155 (55), 125 (37). Anal. Calcd for $C_{33}H_{33}N_3OBr_2 \cdot {}^3/_2H_2O$: C, 58.77; H, 5.38; N, 6.23. Found: C, 58.91; H, 5.20; N, 6.23.

(−)-*cis*-2-[Bis(4-bromophenyl)methyl]-1-azabicyclo-[2.2.2]octan-3-amine. To a 125-mL round-bottomed flask equipped with condenser and N_2 inlet were added 1.10 g (1.70 mmol) of (+)-*cis*-2-[bis(4-bromophenyl)methyl]-*N*-[[1-(1-naphthyl)ethyl]carbamoyl]-1-azabicyclo[2.2.2]octan-3-amine and 4 mL of water. To the stirring mixture was added carefully 8 mL of concentrated sulfuric acid, and the mixture refluxed (bath about 160 °C) for 22 h. The dark reaction was then cooled, poured into ice, and the pH adjusted to 12 with 6 N aqueous sodium hydroxide solution. The mixture was extracted twice with methylene chloride, and the organic layer dried over sodium sulfate and evaporated. The residue was chromatographed on silica gel using 2/1 methylene chloride/methanol as eluent to afford an oil, which was decolorized with charcoal in hot ethyl acetate to afford a white solid, 643 mg (84.0%): mp 185–187 °C: spectral data identical to those of the racemate; $[\alpha]_D$ −38.8° (c = 1.0, CH_2Cl_2). Anal. Calcd for $C_{20}H_{22}N_2Br_2$: C 53.36, H 4.93, N 6.22. Found: C 53.16, H 4.99, N 6.16.

(−)-*cis*-2-(Diphenylmethyl)-1-azabicyclo[2.2.2]octan-3-amine. To a solution of 545 mg (1.21 mmol) of (−)-*cis*-2-[bis(4-bromophenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine and 2 mL of 6 N hydrochloric acid in 40 mL of ethanol was added under nitrogen 100 mg of 10% palladium on carbon, and reaction shaken under 40 psi of hydrogen for 7 h. The reaction was filtered through Celite with ethanol and evaporated. The residue was treated with 1 N sodium hydroxide and extracted into methylene chloride. The organic layer was dried and evaporated to afford a white solid, which was triturated with hexane to give 175 mg (49.5%): mp 153–160 °C; the ^{1}H NMR spectrum, mass spectrum, and IR spectrum all matched those of an authentic sample of the racemate; $[\alpha]_D$ −57.3° (c = 1.0, CH_2Cl_2). Anal. Calcd for $C_{20}H_{24}N_2 \cdot {}^1/_2H_2O$: C, 79.69; H, 8.36, N, 9.29. Found: C, 79.95; H, 8.06; N, 9.14.

(−)-*cis*-2-(Diphenylmethyl)-*N*-[(2-methoxyphenyl)-methyl]-1-azabicyclo[2.2.2]octan-3-amine, CP-96,345. To a 65-mL round-bottomed flask equipped with condenser and N_2 inlet were added 320 mg (1.09 mmol) of (−)-*cis*-2-(diphenylmethyl)-1-azabicyclo[2.2.2]octan-3-amine, 6 mL of methanol, 0.55 mL (1.10 mmol) of 2 N HCl in methanol, 300 mg (2.18 mmol) of 2-methoxybenzaldehyde, and 137 mg (2.18 mmol) of sodium cyanoborohydride. The reaction was stirred at room temperature for 24 h and quenched with 6 N HCl to maintain a pH of 1, and the methanol removed by evaporation. The residue was taken up in 20 mL of 1 N HCl and extracted with ethyl acetate, and the pH adjusted to 14 with 1 N sodium hydroxide. The aqueous layer was extracted with ethyl acetate, and the organic layer washed with brine, dried over sodium sulfate, and evaporated. The residue was crystallized by dissolving in a little methylene chloride, adding 2-propanol, and evaporating until crystals appeared, giving 326 mg (72.6%): mp 153–155 °C; ^{1}H NMR (δ, $CDCl_3$) 1.2–2.1 (series of 5 m 5 H), 2.73 (m, 1 H), 2.87 (m, 2 H), 3.01 (m, 1 H), 3.35 (m, 1 H), 3.43 and 3.71 (m, 2 H), 3.62 (s, 3 H), 3.77 (dd, 1 H), 4.61 (m, 1 H), 6.7–6.9 and 7.1–7.5 (m, 14 H); ^{13}C NMR (δ, $CDCl_3$) 20.2, 25.0, 25.7, 42.2, 46.3, 49.4, 49.7, 54.5, 55.3, 61.9, 110.1, 120.2, 125.9, 126.4, 127.67, 127.73, 127.9, 128.2, 128.4, 129.1, 129.4, 143.4, 145.7, 157.5; MS m/e 413 (<1, parent + 1), 291 (30), 274 (21), 246 (37), 245 (100), 165 (11), 121 (55), 96 (12), 91 (58); $[\alpha]_D$ −23.8° (c = 1.0, CH_2Cl_2). Anal. Calcd for $C_{28}H_{32}N_2O$: C, 81.51; H, 7.82; N, 6.79. Found: C, 81.56; H, 7.86; N, 6.68.

The mesylate salt was prepared by dissolving the free base in acetone, adding 2 equiv of methanesulfonic acid, evaporating, and dissolving the residue in 2-propanol. The resulting crystalline precipitate (342 mg) had mp 243–245 °C, $[\alpha]_D$ −25.8° (c = 1, MeOH).

Alternative Preparation of CP-96,345. **(−)-*cis*-2-(Diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine.** To a 65-mL round-bottomed flask equipped with condenser and N_2 inlet were added 175 mg (0.60 mmol) of (−)-*cis*-2-(diphenylmethyl)-1-azabicyclo[2.2.2]octan-3-amine, 122 mg (0.90 mmol) of 2-methoxybenzaldehyde, 2 mg of camphorsulfonic acid, and 10 mL of toluene. The reaction was refluxed 24 h, cooled, and evaporated. The residue was taken up in 3 mL of dry tetrahydrofuran and treated with 1.50 mL (3.00 mmol) of a 2.0 M solution of borane–methyl sulfide in tetrahydrofuran. The reaction was refluxed for 24 h, cooled, and evaporated. The residue was taken up in 20 mL of ethanol, treated with 0.5 g of sodium carbonate and 0.5 g cesium fluoride, and refluxed for 3.5 days. The reaction was then cooled, evaporated, and taken up in ethyl acetate/water, and the layers were separated. The organic layer was washed with saturated aqueous sodium bicarbonate and brine, dried over sodium sulfate, and evaporated. The residue was chromatographed on silica gel using methylene chloride/methanol as eluent to afford 130 mg (52.5%) of a white solid, which was triturated with 2-propanol to give a crystalline solid: mp 154–155 °C; The ^{1}H NMR spectrum, mass spectrum, and IR spectrum matched those above; $[\alpha]_D$ −23.4° (c = 0.9, CH_2Cl_2).

Table IV. Single Crystal X-ray Crystallographic Analysis of 7b

A. Crystal Parameters	
formula	$C_{28}H_{32}N_2O$ (412.6)
crystallization medium	2-propanol and dichloromethane
crystal size, mm	0.24 × 0.40 × 0.44
cell dimensions	a = 9.355 (4) Å
	b = 11.013 (4) Å
	c = 11.769 (4) Å
	α = 73.64 (3)°
	β = 76.61 (3)°
	τ = 88.28 (3)°
	v = 1131.0 (8) Å^3
space group	$P\bar{1}$
molecules/unit cell	2
density calcd, g/cm^3	1.21
linear absorption factor, cm^{-1}	5.31
B. Refinement Parameters	
number of reflections	2316
nonzero reflections ($I > 3.0\sigma$)	2088
R index[a]	0.040
GOF[b]	1.07
scale factor	1.605 (1)
secondary extinction factor	none

[a] R index = $\sum||F_o| - |F_c||/\sum|F_o|$. [b] GOF = $[\sum w(F_o^2 - F_c^2)^2/(m - s)]^{1/2}$ where $w = [\sigma^2(F) + |g|F^2|]^{-1}g$ = 0.00000.

Single Crystal X-ray Analysis of 7b. A representative crystal was surveyed and a 1-Å data set (maximum sin θ/λ = 0.5) was collected on a Nicolet R3m/μ diffractometer. Atomic scattering factors were taken from *International Tables for X-ray Crystallography*.[31] All crystallographic calculations were facilitated by the SHELXTL[32] system. All diffractometer data were collected at room temperature. Pertinent crystal, data collection, and refinement parameters are summarized in Table IV.

A trial structure was obtained by direct methods. This trial structure refined routinely. Hydrogen positions were calculated wherever possible. The methyl hydrogens and the hydrogen on

(31) *International Tables for X-ray Crystallography*; Birmingham: Kynoch Press, 1974; Vol. IV, pp 55, 99, 149.
(32) Sheldrick, G. M. SHELXTL. User Manual, Nicolet Instrument Co., 1981.

Table V. Single Crystal X-ray Crystallographic Analysis of 7b Dimesylate

A. Crystal Parameters	
formula	$C_{28}H_{32}N_2O \cdot 2CH_3SO_3H$ (604.8)
crystallization medium	2-propanol
crystal size, mm	0.12 × 0.29 × 0.36
cell dimensions	a = 10.527 (5) Å
	b = 11.331 (7) Å
	c = 13.820 (9) Å
	α = 77.53 (5)°
	β = 71.53 (2)°
	τ = 85.33 (2)°
	v = 1527 (1) Å^3
space group	$P\bar{1}$
molecules/unit cell	2
density calcd, g/cm^3	1.32
linear absorption factor, cm^{-1}	11.65
B. Refinement Parameters	
number of reflections	3089
nonzero reflections ($I > 3.0\sigma$)	1379
R index[a]	0.123
GOF[b]	1.34
scale factor	1.587 (1)
secondary extinction factor	3 (1) × 10^{-3}

[a] R-index = $\sum||F_o| - |F_c||/\sum|F_o|$. [b] GOF = $\sum w(F_o^2 - F_c^2)^2/(m - s)]^{1/2}$ where $w = [\sigma^2(F) + |g|F^2|]^{-1}g = 0.00000$.

nitrogen were located by difference Fourier techniques. The hydrogen parameters were added to the structure factor calculations but were not refined. The shifts calculated in the final cycle of least squares refinement were all less than 0.1 of their corresponding standard deviations. The final R index was 0.040. A final difference Fourier revealed no missing or misplaced electron density.

The refined structure was plotted using the SHELXTL plotting package and redrawn for comparison with the dimesylate salt as shown in Figure 2 by the ORTEP routine. Coordinates, anisotropic temperature factors, distances and angles are available as supplementary material (Tables S1–S5).

Single Crystal X-ray Analysis of 7b as Its Dimesylate Salt. A representative crystal was surveyed and a 1-Å data set (maximum sin θ/λ = 0.5) was collected on a Nicolet R3m/μ diffractometer. *These crystals were very sensitive to the atmosphere (probably water vapor) and had to be sealed quickly.* Unfortunately, most sealing agents also destroyed the crystal. Sealing the crystals in glass capillaries also proved unsuccessful. Finally, a marginal crystal was obtained for data collection using Duro Super Glue, which is a cyano acrylate ester based glue. Atomic scattering factors were taken from *International Tables for X-ray Crystallography*.[31] All crystallographic calculations were facilitated by the SHELXTL[30] system. All diffractometer data were collected at room temperature. Pertinent crystal, data collection, and refinement parameters are summarized in Table V.

A trial structure was obtained by direct methods. This trial structure refined routinely. Hydrogen positions were calculated wherever possible. The methyl hydrogens were located by difference Fourier techniques. The acidic hydrogens could not be located either on oxygen or nitrogen. The hydrogen parameters were added to the structure factor calculations but were not refined. The shifts calculated in the final cycle of least squares refinement were all less than 0.1 of their corresponding standard deviations. The final R index was 0.123. *This larger than normal R index was not surprising when the quality of the crystal is taken into consideration.* A final difference Fourier revealed no missing or misplaced electron density.

The refined structure was plotted using the SHELXTL plotting package and redrawn for comparison with the free base as shown in Figure 2 using ORTEP. Coordinates, anisotropic temperature factors, distances, and angles are available as supplementary material (Tables S6–S9). *It should be emphasized that the accuracy of the bond lengths and angles are not up to the usual standards of a routine X-ray analysis because of the marginal nature of the diffracting crystal. However, the stereochemistry shown in the drawings is not in doubt.*

Biological Methods. [^{3}H]Substance P Binding in Human IM-9 Cells. The procedure was based on a literature protocol.[15] Tissue was thawed, weighed, and homogenized in 50 volumes (w/v) of ice-cold 50 mM TRIS buffer (pH 7.7), then centrifuged twice at 30000g for 20 min at 2–4 °C. The pellet was suspended in assay buffer (50 mM TRIS-HCl (pH 7.7), 1 mM $MnCl_2$, 0.02% BSA, 40 mg/mL bacitracin, 4 mg/mL leupeptin, 2 mg/mL chymotrypsin, 30 mg/mL phosphoramidon), and the assay conducted in 5-mL polystyrene tubes with 100 μL of test compound, 100 μL of ligand (0.5 nM final concentration, 36–55 Ci/mmol), and 800 μL of tissue preparation (20 mg original wet weight/tube). After incubation in the dark at room temperature for 20 min, the assay was terminated by filtration onto GF/B filters which had been presoaked in 0.2% polyethylenimine for 1–2 h. The filters were washed (5 × 1 s) with ice-cold mM TRIS-HCl buffer (pH 7.7) using a Brandell harvesting system, and the filters quantified for radioactivity by liquid scintillation counting. Standard errors are indicated following the IC_{50} values.

[^{125}I]Neurokinin A Binding in Hamster Urinary Bladder. According to the method of Burcher and Buck,[16] bladder tissue was homogenized in 20 vol of 50 mM Tris-HCl (pH 7.4), 120 mM NaCl, and 5 mM KCl for 1 min using a Brinkman Polytron (setting 10). The homogenate was centrifuged at 17 000 rpm for 30 min and the pellet resuspended in 10 mM Tris-HCl, 300 mM KCl, and 10 mM EDTA at 4 °C for 1 h. The tissue was then centrifuged, washed with 50 mM Tris-HCl, centrifuged again, and washed twice. The final pellet was resuspended in 50 mM Tris-HCl, and incubated (200 μg/mL) with 50 pmol [^{125}I]neurokinin A (NKA, giving a final ligand concentration of 0.05 nM) in the presence of the test compound in 50 mM Tris-HCl (pH 7.4), 3 mM $MgCl_2$, 0.02% BSA, 40 μg/mL bacitracin, 4 μg/mL chymotrypsin, and 4 μg/mL leupeptin for 90 min at 22 °C. Nonsaturable binding was determined using 100 nM NKA. The assay was terminated by filtration onto Skatron filters. After washing, the filters were quantified for radioactivity on an LKB counter. Data were analyzed using Lundon2 (Lundon Software, Cleveland, OH).

[^{125}I]BH-eledoisin Binding in Guinea Pig Cortex. According to the procedure of Foster and Tridgett,[17] guinea pig cortical tissue was homogenized in 0.32 M sucrose and then centrifuged at 17 000 rpm for 25 min. The resulting pellet was resuspended and lysed for 10 min in ice-cold 5 mM Tris acetate (pH 7.4). The tissue was then washed twice, and the final pellet resuspended in 50 mM Tris-HCl and incubated (6 mg/mL) with 1 mM $MnCl_2$, 50 μg/mL chymostatin, and 0.02% BSA and a final concentration of 0.1 nM [^{125}I]BH-eledoisin in the presence of the test compounds for 60 min at 22 °C. Nonsaturable binding was defined as the radioactivity remaining in the presence of 1 μM eledoisin. The assay was terminated by filtration onto Skatron filters. After washing, the filters were quantified for radioactivity on an LKB counter. Data were analyzed using Lundon2 (Lundon Software, Cleveland, OH).

Substance P-Induced Plasma Extravasation. Male Hartley guinea pigs, 350–400 g, shaved 2 days prior to testing, were anesthetized with sodium pentobarbitol, 25 mg/kg. Evans Blue dye, 30 mg/kg in 0.02% BSA saline, was injected iv, then after 5 min, SP (20 nM solution in 0.1% BSA saline) was injected at 50 μL per site intradermally. After 10 min, the animals were sacrificed by dislocation, and the dorsal skin removed. Samples were punched out (12 mm), extracted with formamide for 24 h at 60 °C with shaking, and OD at 600 nm determined. Test compounds were administered 1 h before SP challenge at 15 mg/kg. Results are expressed as percent inhibition of the SP effect or ED_{50} (n) with 95% confidence limits.

Capsaicin-Induced Plasma Extravasation. Male Hartley guinea pigs, 300–400 g, fasted overnight, were anesthetized with sodium pentobarbitol, 25 mg/kg. Evans Blue dye was injected iv at 30 mg/kg, followed after 5 min by capsaicin [a 30 mM solution of capsaicin in 70% ethanol was diluted in 0.1% BSA buffer (Krebs' bicarbonate solution: 118 mM NaCl, 4.6 mM KCl, 1.17 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.17 mM NaH_2PO_4, 25 mM $NaHCO_3$, 10 mM glucose), giving a 30 μM solution of capsaicin which was administered at 10 mL per animal]. The animals were killed 10 min later by exsanguination, and the ureter removed and extracted with 1 mL formamide at 60 °C for 24 h. OD at 600 nm was then determined. Results are expressed as a percent inhibition of the capsaicin effect or ED_{50} (n) with 95% confidence limits following dose–response determination. The compounds

for test were administered at 10 mg/kg po 1 h before capsaicin challenge.

Substance P-Induced Salivation in Rats. Fasted male Sprague-Dawley rats were administered test compound (50 mg/kg ip or po dissolved in 0.05 N hydrochloric acid) or saline 30 min prior to SP challenge. Sodium pentobarbitol (V-pento 65 mg/mL at 1 μL/g ip, 10 min prior to SP challenge) anesthetized animals were challenged with 4.7 nmol/kg (2.0 μL/g) SP by tail vein injection. Saliva was then absorbed into preweighed Q-tips and the saliva weight was calculated by subtraction and recorded until the salivation response subsided (about 4 min). Results are expressed as percent inhibition of the SP response.

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Supplementary Material Available: Parameters, atomic coordinates, bond lengths, bond angles, anisotropic thermal parameters, and H-atom coordinates from single crystal X-ray data for **7b** as its free base and mesylate salt (11 pages). Ordering information is given on any current masthead page.

Methoxytetrahydropyrans. A New Series of Selective and Orally Potent 5-Lipoxygenase Inhibitors

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Investigation of the SAR of the lead (methoxyalkyl)thiazole 1-[3-(naphth-2-ylmethoxy)phenyl]-1-thiazol-2-ylpropyl methyl ether (1, ICI 211965) led to the methoxytetrahydropyrans, a new series of 5-lipoxygenase (5-LPO) inhibitors exemplified by the parent compound 4-[3-(naphth-2-ylmethoxy)phenyl]-4-methoxy-3,4,5,6-tetrahydro-2*H*-pyran (**4f**). In vitro **4f** inhibited leukotriene C_4 (LTC_4) synthesis in zymosan-stimulated plasma-free mouse macrophages and LTB_4 synthesis in A-23187-stimulated human whole blood (IC_{50}s 0.5 nM and 0.07 μM, respectively). In the rat **4f** inhibited LTB_4 synthesis in blood ex vivo and in zymosan-inflamed air pouch exudate with an ED_{50} 3 h after oral dosing of 10 mg/kg in each system. In seeking more potent orally active compounds, strategies were explored in congeners of **4f** for reducing lipophilicity without sacrificing potency. For example, replacement of 2-naphthyl of **4f** by various aza- and oxoheterocycles afforded compounds in which log *P* is reduced by 1.7-2.3 units while potency in human whole blood in vitro was maintained or enhanced relative to **4f**. In addition, the oxoheterocyclic replacements provided compounds with improved oral potency and the preferred compound from this group is 6-[[3-fluoro-5-(4-methoxy-3,4,5,6-tetrahydro-2*H*-pyran-4-yl)phenoxy]methyl]-1-methylquinol-2-one (**4y**). In the in vitro systems, **4y** inhibited LT formation with IC_{50}s in mouse macrophages and human whole blood of 3 nM and 0.02 μM, respectively. **4y** did not inhibit the synthesis of cyclooxygenase (CO) products at concentrations up to 500 μM in human blood, a selectivity for 5-LPO over CO of >20 000-fold. In the rat **4y** inhibited the formation of LTB_4 in blood ex vivo and in inflammatory exudate with ED_{50}s 3 h after oral dosing of 0.9 and 0.3 mg/kg, respectively. **4y** was more potent in vitro in human whole blood and in rat blood ex vivo at 3 h than either the 5-LPO inhibitor A-64077 or the FLAP antagonist MK-886. Based on these data **4y** (ICI D2138) has been entered into development as an orally active, selective 5-LPO inhibitor for clinical evaluation in inflammatory conditions in which LTs are believed to play a role.

Introduction

Arachidonic acid is metabolized to inflammatory mediators by two major oxidative pathways. 5-Lipoxygenase (5-LPO) is the first enzyme in a cascade which produces the leukotrienes (LTs) while cyclooxygenase (CO) initiates the cyclic pathway leading to prostaglandins and thromboxanes. Inhibition of CO is a well-established clinical treatment for inflammation although this mechanism of action is associated, inter alia, with ulceration of the gastrointestinal tract.

The LTs are a family of important biologically active molecules. LTB_4 is a potent chemotactic agent and inflammatory mediator[1] and the peptidoleukotrienes LTC_4 and LTD_4 are powerful spasmogens in vascular and bronchial tissues.[2] Elevated levels of LTs are associated with a number of inflammatory conditions and indeed LTs have been recovered from various pathological tissues. For these reasons it is believed that restricting LT synthesis by inhibition of 5-LPO will have therapeutic utility for the treatment of a variety of inflammatory conditions including asthma, rheumatoid arthritis, inflammatory bowel disease, and psoriasis. Encouraging preliminary clinical results in some of these indications have been reported for one such compound, A-64077.[3-5] However, only when selective, orally active inhibitors of 5-LPO capable of sustained suppression of LT synthesis are evaluated clinically will the value of 5-LPO inhibition in the treatment of inflammatory conditions become clear.

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