Synthesis, Assignment of Absolute Configuration, and Receptor Binding Studies Relevant to the Neuroleptic Activities of a Series of Chiral 3-Substituted Cyproheptadine Atropisomers

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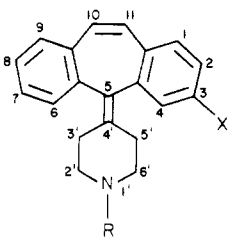
The syntheses and resolutions of chiral analogues of cyproheptadine bearing a cyano (1) and a trifluoromethylsulfonyl (8) substituent in the 3 position are described. The absolute configuration of (−)-3-bromocyproheptadine (5) was determined by X-ray crystal structure analysis to be pR_apS_b. The absolute configurations of 1, 8, and five other pairs of 3-substituted cyproheptadine analogues were related to the 3-bromo compound by circular dichroism spectrophotometry. The binding affinities of each member of the eight pairs of atropisomers as well as the parent unsubstituted cyproheptadine to membrane sites specifically binding tritiated ligands comprising the α-adrenergic agonist (−)-[^{3}H]norepinephrine, the α-adrenergic antagonist [[[2-(2′,6′-[^{3}H]dimethoxyphenoxy)ethyl]amino]-methyl]benzodioxane (WB-4101), the dopamine antagonist [^{3}H]spiroperidol, and the muscarinic cholinergic antagonist [^{3}H]quinuclidinyl benzilate (QNB) were measured by displacing these ligands from membrane preparations of mammalian brain. [^{3}H]QNB was uniformly more easily displaced by the pS_apR_b absolute configuration of each pair, while the remaining three ligands were more easily displaced by isomers of the pR_apS_b configuration. Quantitative structure–activity relationships demonstrate only modest correlations between the receptor binding data and physical parameters of the 3-substituents, indicating the complex nature of the drug–receptor interactions.

Chiral recognition of molecules has long been acknowledged as an important characteristic of many drug–receptor interactions.[1] Although the stereochemical dependence of numerous pharmacological responses in the central nervous system has been known for years, it is only recently that this has been established for the interaction of chiral molecules with membrane binding sites, as measured by the displacement of selected radioligands.[2-4] All previous data upon which the stereochemical dependence of receptor binding is based were derived from the study of molecules in which chirality arose from one or more specific asymmetric centers. Recently, the synthesis, resolution, and central nervous system pharmacology of the 3-substituted cyproheptadine-type compounds 1–4



1, R = CH_3; X = CN
2, R = CH_3; X = SCF_3
3, R = CH_2-c-C_3H_7; X = SCF_3
4, R = CH_3; X = OCH_3
5, R = CH_3; X = Br
6, R = CH_3; X = I
7, R = CH_2-c-C_3H_7; X = I
8, R = CH_3; X = SO_2CF_3
9, R = CH_3; X = H

have been reported.[5-7] These molecules are chiral by virtue of molecular asymmetry rather than by the presence of one or more asymmetric centers. We now wish to extend these previous results by demonstrating that pharmacologically relevant receptor sites recognize the molecular asymmetry in a series of these compounds. Based on the pharmacology of these compounds, we have selected the central α-adrenergic, dopaminergic, and cholinergic receptor binding sites to illustrate this point.

Since the pharmacological properties of chiral 3-substituted cyproheptadines are influenced markedly by the nature of the substituent, it was important to establish the relevance of these properties to their receptor binding affinities. The absolute configuration (−)-3-bromocyproheptadine [(−)-5] was determined by X-ray crystal structure analysis to be (pR_apS_b). The absolute configurations of the other 3-substituted cyproheptadine analogues reported in this paper, namely, 1–4 and 6–8, then were related to this 3-bromo compound using circular dichroism spectrophotometry. Therefore, in the current work, receptor binding studies were carried out with a series of compounds having known absolute stereochemistry and differing only in the nature of the 3-substituent.

Chemistry. Using procedures that were described previously for the resolutions of (±)-4,[7] (±)-6,[6] and (±)-7,[6] racemic 3-bromocyproheptadine [(±)-5][8] and 3-cyanocyproheptadine [(±)-1][9] were separated into their constituent enantiomers.

The levorotatory compound (−)-1 was also prepared from (−)-6. Reaction of this chiral iodo compound with cuprous cyanide in DMF on a steam bath for 10 h gave, after one recrystallization, 52% of (−)-1 that was 97% optically pure. The levorotatory compounds (−)-4 and (−)-2 have also been prepared from (−)-6.

Oxidation of the trifluoromethyl thioketone 10 with 30% H_2O_2 for 192 h in glacial acetic acid gave a mixture of the two trifluoromethylsulfonyl ketones 12 and 13 and the trifluoromethylsulfinyl ketone 11 (Scheme I). This mixture was separated by column chromatography on silica gel to afford each of the pure ketones. The relative proportions of these oxidation products is, of course, dependent on the time and temperature of the reaction. With respect to the oxidation state of the sulfur atom, the course of this and related oxidation reactions may be monitored by ^{19}F NMR spectroscopy directly on the reaction mixture.[10] Also, the oxidation state of the sulfur atom in compounds containing a trifluoromethyl group bound to sulfur can be determined by ^{19}F NMR.[10] Using fluorotrichloromethane as a standard, the fluorine resonance spectrum of the SCF_3 group in 10 occurs as a sharp

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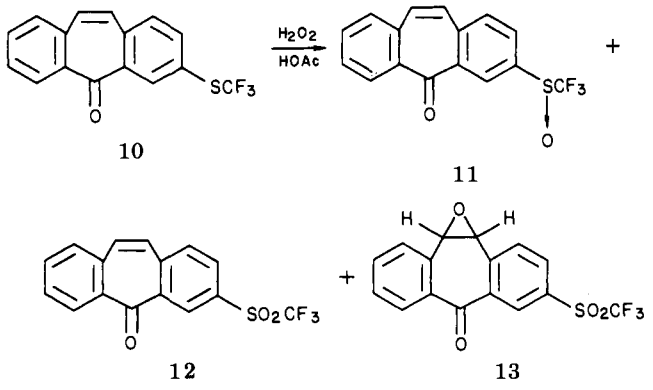
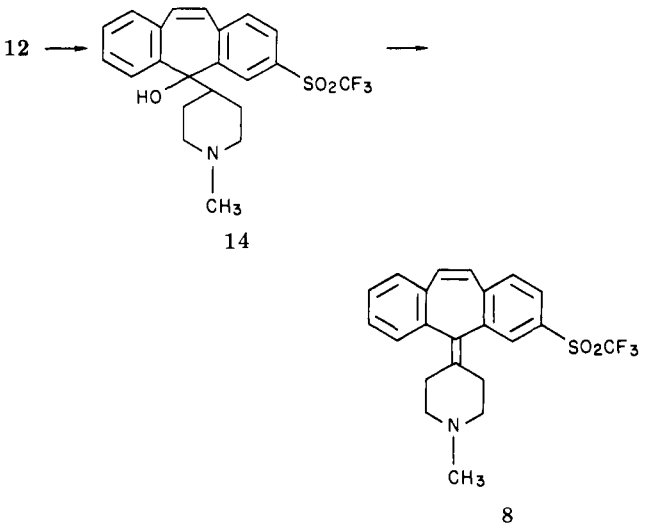
Scheme I



10 11 12 13

Scheme II



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singlet at δ 41.4, while that of the $SOCF_3$ group of **11** occurs at δ 73.4 and that of the SO_2CF_3 groups of **12** and **13** occurs at δ 77.5. Very similar chemical shifts are found for the fluorine resonances of the respective racemic and enantiomeric cyproheptadine analogues.

(±)-3-[(Trifluoromethyl)sulfonyl]cyproheptadine [(±)-**8**] was prepared by addition of 1-methyl-4-piperidylmagnesium chloride to ketone **12**, followed by subsequent dehydration using $(CF_3CO)_2O$ and CF_3COOH (Scheme II). Resolution of (±)-**8** was effected using the procedure described previously for the resolution of (±)-**3**.[6]

Results and Discussion

X-Ray Crystal Structure and Absolute Configuration of (−)-5. An X-ray diffraction experiment was performed on a single crystal of (−)-**5** to establish its absolute configuration. Figure 1 is a perspective drawing with the correct conformation and configuration of the molecule,[11] while bond lengths and angles for (−)-**5** are given under the Supplementary Material (see paragraph at the end of this paper concerning Supplementary Material). The X-ray diffraction analysis of (−)-**5** has shown that the absolute configuration is pR_apS_b using the Cahn–Ingold–Prelog conventions for planar chirality.[12] Using these conventions, plane "a" is defined as that plane containing the bromine-substituted aromatic ring and its attachments, namely, carbon atoms C_5 and C_{11}; plane "b" is defined as that plane containing the unsubstituted aromatic ring and its attachments, namely, carbon atoms C_5 and C_{10}. Carbon atom $C_{4'}$ is off of both planes and is the pilot atom to both planes, while C_5 is the common first atom of the two in-plane sequences. Because (−)-**5** has

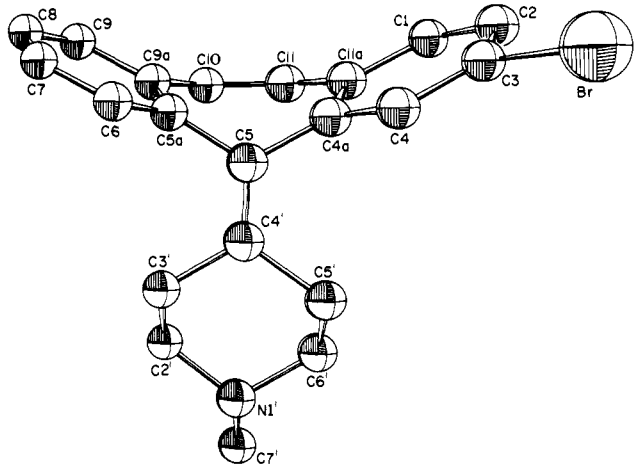


Figure 1. (pR_apS_b)-(−)-1′-Methyl-4′-(3-bromo-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine [(−)-**5**] (structure according to X-ray analysis).

nonequivalent chiral planes, the Cahn–Ingold–Prelog conventions recommend not only that the planar chirality be specified by the italic letter *p* (for planar) before the corresponding *R* or *S* but that the chirality about each plane be specified separately and, further, that plane-identifying characters be associated with each chiral specification. Thus, (−)-**5** is (pR_apS_b)-(−)-1′-methyl-4′-(3-bromo-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidine)piperidine.

Except for the bromine atom, the molecule is quite symmetrical with respect to a mirror plane bisecting the central cycloheptene ring; the distortions from absolute mirror symmetry appear to involve both experimental error as well as lattice energy effects. The molecule could in principle be converted to its mirror image by rotations around the C_{4a}–C_5 and C_5–C_{5a} single bonds. However, this ring flipping requires a substantial amount of energy, as can be seen from models, because of the effort required to both enlarge the C_{4a}–C_5–C_{5a} angle, as well as to bring hydrogen on C_4 and C_6 past those on $C_{5'}$ and $C_{3'}$. The two benzene rings "a" and "b" are both planar to within 0.015 Å, while the exocyclic double-bond system is slightly distorted from planarity as is evidenced by the torsion angle of 4.7° for C_{5a}–C_5–$C_{4'}$–$C_{3'}$. In keeping with the requirement for neuroleptic activity of a nonplanar, tricyclic nucleus,[13,14] the benzene rings "a" and "b" have an interplanar angle of 125°. The interplanar angles between the best plane defined by C_{4a}, C_5, C_{5a}, $C_{3'}$, $C_{4'}$, and $C_{5'}$ and the benzene rings a and b are 112° and 111°, respectively. The piperidine ring is in a chair conformation with the methyl group on the lone nitrogen in an equatorial position. The nitrogen atom is 6.1 Å from each of the centers of rings a and b and 3.6 and 3.8 Å, respectively, below the best planes formed by these rings.

Chemical and Spectroscopic Correlations of Configurations. Inasmuch as the question of bond breaking at an asymmetric carbon atom does not arise with the nuclear substitution reaction described for the conversion of the chiral iodo derivative (−)-**6** to the chiral cyano-cyproheptadine (−)-**1**, the product of the reaction must have the same relative configuration as the starting material, since only racemization or retention of configuration is possible.[15] The chiral derivatives (−)-**2** and (−)-**4** must also have the same relative configuration as (−)-**1**, since they too were prepared from (−)-**6** by analogous nuclear substitution reactions.[6,7] A similar situation obtains for the relative configurational correlation of (−)-**7** and (−)-**3**.[6]

Circular dichroism (CD) spectra of the (+) isomers of compounds **1** to **8** were recorded in order to link the

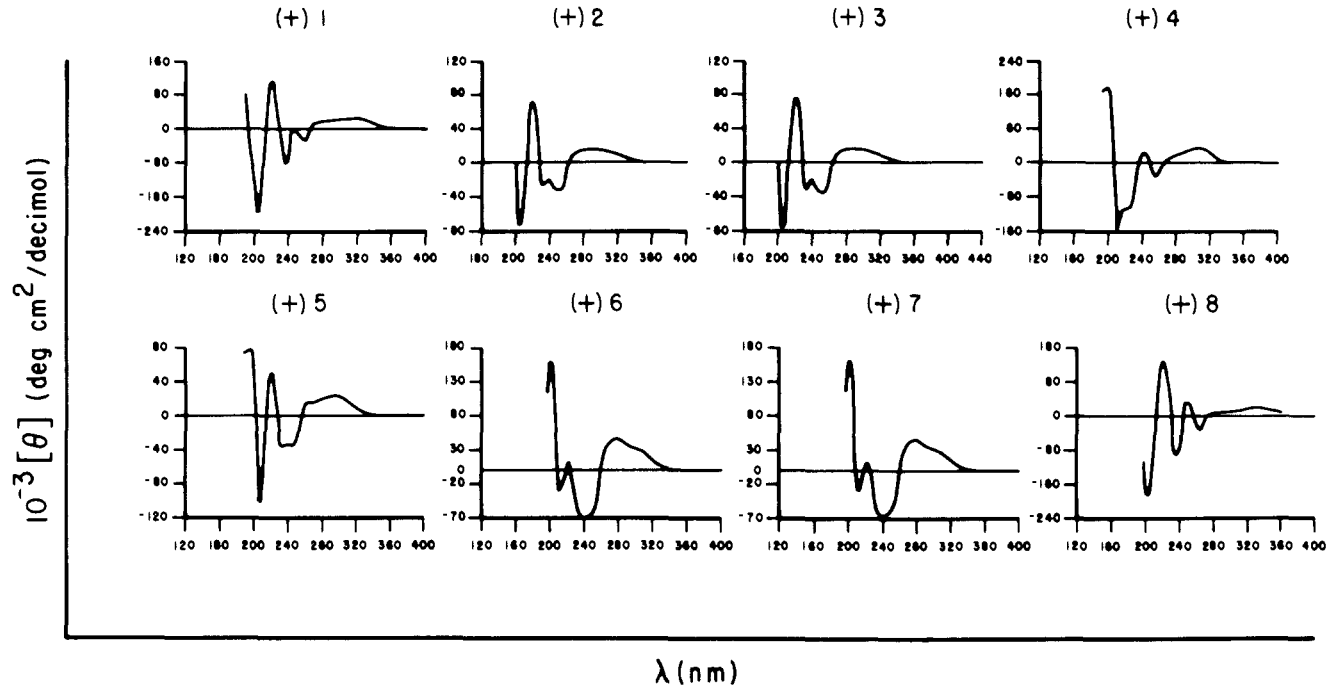


Figure 2. Circular Dichroism Spectra.

absolute configuration of compound (−)-**5**, determined by X-ray crystallography, to the absolute configurations of the other compounds. These spectra, shown in Figure 2, while obviously consisting of complex patterns of overlapping transitions, reveal several common features for all the (+) isomers: two broad positive extrema at long wavelengths (330–260 nm), a strong overlapping region consisting of approximately three negative and one positive extrema at shorter wavelength (260–210 nm), and an intense conservative pair of extrema at short wavelength (220–190 nm) whose longest wavelength peak is positive. The shorter wavelength member of this pair is not always detectable, but its presence can be inferred from the rotatory strength, R, calculated from the observed spectrum. Since this integral must be zero over all wavelengths, a positive value of R calculated by integration of the spectrum down to the shortest wavelengths examined implies the existence of a negative extremum at still shorter wavelengths.

Wavelengths and molecular ellipticities of the extrema of the cyproheptadine derivatives 1 through 8 having a positive (+) sign of rotation at the sodium D line (589 nm) are listed in the Supplementary Material.

On the basis of these data, we concluded that the cyproheptadine derivatives (+)-1 through (+)-8 exhibiting dextrorotatory behavior at 589 nm have the same absolute configurations. It therefore follows that the enantiomeric derivatives (−)-1 through (−)-8, exhibiting levorotatory behavior at 589 nm, share a common absolute configuration. Since the absolute configuration of (−)-**5** has been shown to be pR_apS_b by X-ray crystallographic studies, all of the levorotatory enantiomers, therefore, have this absolute configuration.

Receptor Binding Studies. Determinations of the in vitro affinities of each member of this series of atropisomers for four relevant central pharmacological receptors were based on the displacement of low concentrations of high specific activity radioligands from binding sites on membrane preparations obtained from various regions of mammalian brain. The results are shown in Table I.

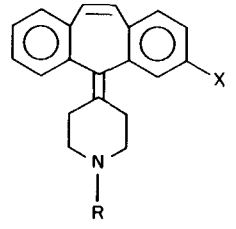
Binding to the central α-adrenergic receptor was determined using (−)-[^{3}H]norepinephrine[16] and [[[2-(2′,6′-[^{3}H]dimethoxyphenoxy)ethyl]amino]methyl]benzodioxane[17] (WB-4101) as radioligands. The apparent K_i values obtained in this manner are shown in columns A and B of Table I. Agonists are known to displace ^{3}H agonists more readily than antagonists and vice versa, a fact attributed to the existence of noninterconverting "agonist" and "antagonist" receptor subclasses.[18]

The ratio of apparent dissociation constants, K_i (WB-4101)/K_i (clonidine), has been correlated with the relative agonist/antagonist ratios of compounds which interact with α-adrenergic binding sites.[18] Thus "pure" agonists were shown to have ratios greater than 10, while "pure" antagonists had ratios smaller than 1, and mixed agonist/antagonists showed intermediate ratios. Since the apparent dissociation constants for displacement of (−)-[^{3}H]norepinephrine have been shown to agree reasonably well with those for clonidine,[2] the ratio K_i (WB-4101)/K_i (norepinephrine), which is shown in column E of Table I, should have the same significance as the ratio K_i (WB-4101)/K_i (clonidine). It can be seen that, while the (−) atropisomers of the cyproheptadine analogues are less potent than relatively "pure" α-antagonists, such as WB-4101, they display little agonist activity. The (+) isomers are considerably less active in displacing α-adrenergic ligands than their optical antipodes.

With the exception of the butyrophenones, compounds with neuroleptic activity have been reported to demonstrate positive cooperativity when displacing ^{3}H-labeled WB-4101 from rat brain membranes as evidenced by Hill coefficients substantially greater than 1.0.[19] Only two of the (−) atropisomers in the present study show positive cooperativity, namely, (−)-**6** with an n_H of 1.5 and (−)-**2** with an n_H of 1.7.

Antidopaminergic activity of the atropisomers of cyproheptadine was measured using the dopamine antagonist [^{3}H]spiroperidol,[20] the displacement of which by a number of compounds with neuroleptic activity has been correlated with their clinical efficacies.[21] As can be seen from column C of Table I, the (−) atropisomers are more potent in displacing [^{3}H]spiroperidol than their (+) antipodes, with K_i values ranging between 4 and 24 nM. The potent neuroleptic (+)-butaclamol has a K_i value of 2.0 nM.

Table I. Displacement of Radioligand Binding by 3-Substituted Cyproheptadine



				K_i, nM,[a] of displaced radioligand					
compd	R	X	stereochem[c]	(−)-[^{3}H]-norepinephrine A	[^{3}H]WB-4101[b] B	[^{3}H]spiroperidol C	[^{3}H]QNB[d] D	K_i (WB)[b]/ K_i (norep) E	K_i (WB)[b]/ K_i (spiro) F
1	$-CH_3$	-CN	(−)	4 200 ± 800	100 ± 30	5 ± 1	570 ± 140	0.024	20.0
			(+)	7 000 ± 800	1 870 ± 65	700 ± 140	1.0 ± 0.4	0.267	
2	$-CH_3$	$-SCF_3$	(−)	1 100 ± 360	120 ± 20	11 ± 1	1 220 ± 110	0.109	10.9
			(+)	>20 000[e]	>10 000[e]	1 182 ± 11	8 ± 5		
3	$-CH_2$-c-C_3H_5	$-SCF_3$	(−)	2 200 ± 740	38 ± 2	24 ± 2	5 200 ± 1300	0.017	1.6
			(+)	>15 000[e]	>10 000[e]	2 080 ± 550	16 ± 3		
4	$-CH_3$	$-OCH_3$	(−)	2 200 ± 200	39 ± 5	16.0 ± 0.2	153 ± 25	0.018	2.4
			(+)	2 600 ± 100	220 ± 30	310 ± 150	4 ± 1	0.085	
5	$-CH_3$	-Br	(−)	1 000 ± 100	58 ± 2	4.0 ± 0.2	96 ± 9	0.058	14.5
			(+)	1 500 ± 300	190 ± 20	75 ± 4	2.0 ± 0.7	0.127	
6	$-CH_3$	-I	(−)	640 ± 60	52 ± 4	5.0 ± 0.6	350 ± 25	0.081	10.4
			(+)	1 100 ± 110	142 ± 6	90 ± 2	7 ± 1.6	0.129	
7	$-CH_2$-c-C_3H_5	-I	(−)	1 300 ± 200	52 ± 5	10 ± 3	265 ± 35	0.040	5.2
			(+)	7 400 ± 400	116 ± 6	89 ± 3	20 ± 4	0.016	
8	$-CH_3$	$-SO_2CF_3$	(−)	750 ± 30	28 ± 12	8 ± 1	1 550 ± 350	0.037	3.5
			(+)	16 000 ± 1000	3 940 ± 450	2 550 ± 730	13 ± 1.0	0.246	
9	$-CH_3$	-H	NA	1 500 ± 250	123 ± 3	63 ± 11	4.0 ± 0.1	0.082	1.95
(+)-butaclamol				41 ± 1	37 ± 9	2 ± 0.6	49 600 ± 660		
spiroperidol				750 ± 110	10 ± 2	0.2 ± 0.02	5 200 ± 990	0.013	
dopamine				69 ± 1		1 019 ± 108			
WB-4101[b]				32 ± 12	0.52 ± 0.13			0.016	
oxymethazoline				4.8 ± 1.5	37 ± 2			7.7	

[a] The apparent inhibition constant found from displacement of the indicated radioligand, K_i (WB), for instance refers to the apparent inhibition constant found from the displacement of ^{3}H-labeled WB-4101. Values reported are the averages of two runs plus or minus the range. [b] [[[2-(2′,6′-Dimethoxyphenoxy)ethyl]amino]methyl]benzodioxane. [c] As indicated in the text, all (−) atropisomers are of pR_apS_b absolute configuration and all (+) atropisomers are of pR_bpS_a absolute configuration. [d] Quinuclidinyl benzilate. [e] The solubility of the compound was insufficient to determine an I_{50} value.

The ratio K_i (WB-4101)/K_i (haloperidol) has been correlated with the relative propensity of a neuroleptic to elicit orthostatic hypotension and sedation,[22] with a ratio value greater than 10 indicating a low propensity and a value less than 2 indicating a high propensity. The ligand used in the present study, spiroperidol, has binding properties similar to haloperidol and, therefore, the ratio K_i (WB-4101)/K_i (spiroperidol) should also possess the same predictive measure. This latter ratio is shown in column F of Table I. Especially interesting in this regard are compounds (−)-**1**, (−)-**2**, (−)-**5**, and (−)-**6** which have ratios of 10 or greater.

The measurement of antimuscarinic cholinergic activity using [^{3}H]quinuclidinyl benzilate (QNB) as ligand,[23] column D of Table I, showed that the anticholinergic activity of the cyproheptadine derivatives and its analogues resides almost exclusively in the (+) enantiomers, the (−) enantiomers being essentially devoid of such activity. The ratio of anticholinergic to antidopaminergic activities for a potential neuroleptic has been used as a predictive measure regarding the incidence of extrapyramidal side effects (EPS), such as tardive dyskinesia.[24] Thus, a neuroleptic with intrinsic anticholinergic activity, such as clozapine or thioridazine, has a low incidence of EPS, while chlorpromazine, which has little intrinsic anticholinergic activity, has a high incidence of EPS. In this respect, therefore, (−)-**4** and (−)-**5** may be considered to be of particular interest, since both the antidopaminergic and anticholinergic activities of these compounds are fairly high.

The in vitro receptor binding data presented in this study are consistent with those reported earlier for (+)- and (−)-**3**[25] and with the in vivo pharmacological data previously obtained for the optical antipodes of **1**,[5] **2**,[6] and **3**[6] and **4**.[7] Thus, antidopaminergic activities as observed by blockade of avoidance responses, striatal homovanillic acid elevation, and blockade of apomorphine-induced stereotypies were observed only for the (−) isomers of **1**–**3**, while the central anticholinergic activity assessed by antagonism to physostigmine-induced lethality in mice was observed predominately with (+)-**3** but with only little such activity in (−)-**3**. The (+) enantiomers of **2** and **4**, but not the corresponding (−) isomers, were observed to be peripheral anticholinergics as measured by mydriatic potency in mice.

Structure–Activity Considerations. The data in Table I clearly show that the receptor binding of chiral cyproheptadine analogues is stereospecific. Since the 3-substituent gives rise to this type of molecular chirality, it must play a role in the receptor–substrate recognition phenomenon. For this reason, it is of interest to see if any conclusions can be obtained as to the nature of the substituent contributions to the binding processes. The following discussion involves comparison of the apparent strength of binding of **9** with the six pairs of 3-substituted, *N*-methyl atropisomers **1**, **2**, **4**–**6**, and **8**. The relative strength of binding to a given receptor for any compound can be represented in a quantitative fashion by −ln [K_i/K_i (**9**)], where K_i and K_i (**9**) represent the apparent receptor–compound dissociation constants for the compound of interest and **9**, respectively. These values are collected in Table II, along with physical parameters which could be of importance in the contributions of the substituents to the binding of these compounds to the various receptors. The parameters selected were π, representing the hydrophobic character of the substituents,[26] the field, $\mathcal{F}$, and resonance, $\mathcal{R}$, terms representing electronic aspects of the substituent,[26,27] and a molecular volume term, V, calculated

Table II. Substituent Constants and Relative Strengths of Binding

		substituent constants				−ln [K_i/K_i (9)] determined for displacement of the indicated radioligand													
						(−)-[^{3}H]norepinephrine				[^{3}H]WB-4101[a]				[^{3}H]spiroperidol				[^{3}H]QNB[b]			
						pR_aS_b		pS_aR_b		pR_aS_b		pS_aR_b		pR_aS_b		pS_aR_b		pR_aS_b		pS_aR_b	
no.	substit	π	$\mathcal{F}$	$\mathcal{R}$	V[c]	obsd[d]	pred,[e] eq 1	obsd[d]	pred,[e] eq 5	obsd[d]	pred,[e] eq 2	obsd[d]	pred,[e] eq 6	obsd[d]	pred,[e] eq 3	obsd[d]	pred,[e] eq 7	obsd[d]	pred,[e] eq 8	obsd[d]	pred,[e] eq 4
9	H	0.0	0.0	0.0	2.06	0.0	−0.24	0.0	0.49	0.0	−0.27	0.0	0.54	0.0	0.60	0.0	−0.23	0.0	1.09	0.0	0.76
1	CN	−0.57	0.51	0.19	10.83	−1.03	−0.67	−1.54	−1.06	0.17	0.60	2.73	−3.00	2.53	2.27	2.42	−2.21	−4.96	4.94	1.39	0.39
2	SCF_3	1.44	0.35	0.18	44.03	0.31	0.84		0.57	0.05	0.26		0.38	1.75	1.74	2.93	2.91	−5.72	−3.73	−0.69	−1.03
4	OCH_3	−0.02	0.26	−0.51	21.70	−0.38	−0.26	−0.55	−0.30	1.15	1.04	−0.58	0.93	1.37	1.45	1.60	1.63	−3.64	−3.05	0.00	0.07
5	Br	0.86	0.44	−0.17	14.56	0.41	0.41	0.00	−0.84	0.75	0.96	−0.42	0.85	2.76	2.04	−0.17	0.12	−3.18	−4.41	0.69	0.23
6	I	1.12	0.40	−0.19	19.55	0.85	0.60	0.31	0.72	0.86	0.90	−0.15	0.29	2.53	1.91	0.36	−0.26	−4.47	−4.41	−0.56	0.02
8	SO_2CF_3	0.55	0.73	0.26	39.94	0.69	0.17	−2.37	−1.72	1.49	0.99	−3.47	−2.83	2.06	2.99	3.70	−3.82	−5.96	−6.59	−1.39	−0.86

[a] [[[2-(2′,6′-Dimethoxyphenoxy)ethyl]amino]methyl]benzodioxane. [b] Quinuclidinyl benzilate. [c] The volume was calculated as the product $L{\cdot}B_3{\cdot}B_4$ using values listed in ref 28. [d] Observed values. [e] The predicted values were calculated from the equations listed in Table III.

Table III. Regression Equations Relating Apparent Strengths of Binding to Substituent Constants

eq. no.	abs config	radioligand displaced	regression eq:[a] $-\ln [K_i/K_i(9)] =$	N	S	R^2
1	pR_apS_b	(−)-norepinephrine	$0.24 + (0.76 \pm 0.23)\pi$	7	0.191	0.682
2	pR_apS_b	WB-4101[b]	$0.265 + (2.2 \pm 0.8)\mathcal{F} - (1.4 \pm 0.6)R$	7	0.194	0.701
3	pR_apS_b	spiroperidol	$0.597 + (3.3 \pm 1.2)\mathcal{F}$	7	0.297	0.597
4	pS_apR_b	QNB[c]	$0.854 - (0.043 \pm 0.019)V$	7	0.319	0.501
5	pS_apR_b	(−)-norepinephrine	$0.49 - (3.0 \pm 1.5)\mathcal{F}$	6	0.413	0.504
6	pS_apR_b	WB-4101[b]	$0.536 + (1.2 \pm 0.4)\pi - (5.5 \pm 1.1)\mathcal{F}$	6	0.348	0.901
7	pS_apR_b	spiroperidol	$-0.14 + (1.4 + 0.2)\pi - (1.4 \pm 0.3)R - (0.105 \pm 0.007)V$	7	0.118	0.990
8	pR_apS_b	QNB[c]	$-1.09 - (7.5 \pm 2.2)\mathcal{F}$	7	0.549	0.695

[a] Coefficients are listed plus or minus the standard error. [b] [[[2-(2′,6′-Dimethoxyphenoxy)ethyl]amino]methyl]benzodioxane. [c] Quinuclidinyl benzilate.

from the parameters of Verloop et al.,[28] which represents possible steric contributions of the substituents to the interaction with the receptors.

In order to investigate possible relationships between the affinities of these compounds for their receptors and the physical parameters of Table II, the technique of stepwise regression analysis was employed.[29] This technique allows one to investigate the contribution of each variable to the regression equation, selecting only those terms which make statistically significant contributions. The results of these analyses are shown in eq 1–8, which are collected in Table III.

Since for a given absolute configuration and receptor six to seven observations are being fitted by up to four variables, a condition which can lead to spurious correlations,[30] any conclusions based on these regression analyses must be rather tentative. It is of interest, nevertheless, to note some common features indicated by these equations. In those cases where the π term is significant, its sign is positive, indicating that hydrophobic substituents promote binding to the receptor. This, in turn, would suggest that the regions of the receptors in contact with the substituents in these cases would likewise be hydrophobic. For the more active series of isomers (pR_apS_b when displacing (−)-norepinephrine, WB-4101, and spiroperidol and pS_apR_b when displacing QNB, eq 1–4 on Table III), if $\mathcal{F}$ enters the regression equation it has a positive sign, indicating that an electron-withdrawing ability of the substituents promotes binding to the receptor, while the negative sign of $\mathcal{F}$ for the less active series of isomers (pS_apR_b when displacing (−)-norepinephrine, WB-4101, and spiroperidol and pR_apS_b when displacing QNB, eq 5, 6, and 8) indicates that electron-donating groups are favored here. These effects could, of course, result either from a direct reaction between the substituent and the receptor or they could represent a more indirect result of perturbation of the entire cyproheptadine structure.

The small values of the multiple correlation coefficients are indicative of poor correlations in most of these equations. Since the interaction process for these compounds with the various receptors undoubtedly is complex, it is not surprising that it cannot be fully explained by a single linear correlation with these physical parameters. Even when a good fit is obtained, as in eq 7 and 8, the small number of data points employed dictate a cautious view of these correlations.[30] Nevertheless, these results suggest that quantitative data on receptor binding may be used to identify certain of the principal contributions of the substituents to the strength of receptor binding.

Conclusions. Using chiral 3-substituted cyproheptadine analogues as models, it has been shown that these atropisomers are stereospecifically recognized by four central nervous system receptors believed to be pharmacologically significant. The receptor sites binding the α-adrenergic agonist (−)-[^{3}H]norepinephrine, the α-adrenergic antagonist ^{3}H-labeled WB-4101, and the dopamine antagonist [^{3}H]spiroperidol were stereoselective for the pR_apS_b absolute configuration.

The receptor site binding the muscarinic cholinergic antagonist [^{3}H]quinuclidinyl benzilate displayed a preference for the opposite (pS_apR_b) absolute configuration. An attempt to explain the relative binding affinities using quantitative structure–activity relationships has met with limited success, indicating the complex nature of these receptor–drug interactions.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt capillary melting point apparatus and are uncorrected. Optical rotation measurements were determined with a Perkin-Elmer 141 automatic polarimeter. At least two readings were recorded at each wavelength and showed a deviation of ±0.005°. ^{1}H and ^{19}F NMR spectra were determined on a Varian T-60 spectrometer in $CDCl_3$, and all shifts are relative to tetramethylsilane and $CFCl_3$, respectively, as internal standards. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. X-Ray measurements were performed on a Syntex P_{2_1} diffractometer with Cu radiation.

All CD spectra were recorded over a wavelength range of 360–195 nm on a Jasco J-41A circular dichroism spectropolarimeter in 0.10-cm path-length cells containing 3×10^{-4} M ethanolic solutions of the cyproheptadine atropisomers. The observed extrema were subsequently slowly scanned manually to determine more precisely their positions and intensities. The ethanol base line was subtracted from each of the spectra for a pair of isomers, and the observed ellipticities at selected wavelengths were then converted to the appropriate sign of the (+) isomer, averaged, converted to molar ellipticities and plotted. The spectropolarimeter was calibrated with a reference aqueous solution of 0.600 mg/mL *d*-10-camphorsulfonic acid, using published values for the molar ellipticities at 290.5 and 192.5 nm.[31]

Resolution of (±)-1′-Methyl-4′-(3-bromo-5*H*-dibenzo[*a*,*d*]cyclohepten-5-ylidene)piperidine. A. Levorotatory Isomer (−)-5. To a solution of 12.42 g (0.0339 mol) of (±)-5 in 250 mL of hot EtOH was added 13.11 g (0.0339 mol) of di-*p*-toluoyl-*d*-tartaric acid dissolved in 50 mL of warm EtOH. The solution was stirred and allowed to cool to room temperature. The salt that crystallized was removed by filtration and was recrystallized from EtOH six times to afford 2.62 g of material having a constant rotation: $[\alpha]^{25}_{589}$ −111°, $[\alpha]^{25}_{578}$ −116°, $[\alpha]^{25}_{546}$ −137°, $[\alpha]^{25}_{436}$ −306° (*c* 0.531, pyridine). This salt was converted to the free base with saturated sodium bicarbonate solution and extracted into ether. The ether phase was washed with water, dried over $MgSO_4$, and filtered, and the ether was removed. Recrystallization from CH_3CN gave (−)-5 as TLC homogeneous (fl. alumina/$CHCl_3$), sparkling white prisms: mp 189–190 °C; $[\alpha]^{25}_{589}$ −100°, $[\alpha]^{25}_{578}$ −106°, $[\alpha]^{25}_{546}$ −127°, $[\alpha]^{25}_{436}$ −304° (*c* 0.731, $CHCl_3$). Anal. ($C_{21}H_{20}BrN$) C, H, Br, N.

B. Dextrorotatory Isomer (+)-5. Starting with 5.76 g (0.0157 mol) of (±)-5 in 110 mL of hot EtOH and 6.36 g (0.0157 mol) of di-*p*-toluoyl-*l*-tartaric acid monohydrate in 25 mL of EtOH and using the procedure as described above, 1.60 g of crystalline salt was obtained: $[\alpha]^{25}_{589}$ +110°, $[\alpha]^{25}_{578}$ +116°, $[\alpha]^{25}_{546}$ +137°; $[\alpha]^{25}_{436}$ +302 (*c* 0.403, pyridine). Conversion to the free base and

crystallization from CH_3CN gave (+)-**5**: mp 189–191 °C; $[\alpha]^{25}_{589}$ +100°, $[\alpha]^{25}_{578}$ +107°, $[\alpha]^{25}_{546}$ +127°, $[\alpha]^{25}_{436}$ +307° (*c* 0.651, $CHCl_3$). Anal. ($C_{21}H_{20}BrN$) C, H, Br, N.

X-Ray Structure Determination of (−)-5. Crystals of (−)-3-bromocyproheptadine ($C_{21}H_{20}NBr$) formed as large parallelepipeds from acetonitrile. Preliminary diffraction experiments indicated the space group of the crystals was $P_{2_12_12_1}$ with a = 9.533(1), b = 11.297(1), c = 16.389(2) Å and Z = 4. X-Ray diffraction data were collected with graphite monochromated Cu K_α radiation (λ 1.5418 Å) and $2\theta/\theta$ scans for all unique reflections with $2\theta \leq 115°$. Of the 1402 measured reflections, 1327 (95%) were considered observed ($I \geq 3\sigma_I$) and corrected for Lorentz and polarization effects. Structure solution was undertaken with standard Patterson techniques which provided coordinates for the single Br atom. Least-squares refinements minimizing $\Sigma\omega(|F_o| - |F_c|)^2$ with $\omega = 1/(\sigma F_o)^2$ and difference Fourier analyses generated positions for the remaining non-hydrogen atoms.[32] Full-matrix least-squares refinments incorporating anomalous dispersion contributions from the Br atom and using anisotropic temperature parameters lowered the conventional residual index to 0.076 for one enantiomer and 0.084 for the other—a statistically significant difference.[33] Careful remeasurement of 15 enantiomorph-sensitive reflections also confirmed the above result. Hydrogen atoms with fixed isotropic temperature parameters were added and six reflections judged to be suffering from secondary extinction were removed.[34] The final unweighted residual index is 0.060. Final fractional coordinates and thermal parameters may be obtained from the Supplementary Material.

Resolution of (±)-1′-Methyl-4′-(3-cyano-5*H*-dibenzo[*a*,*d*]cycloheptan-5-ylidene)piperidine [(±)-1]. Using the same procedure as described for the resolution of the 3-bromo analogue (±)-**5**, 24.10 g of (±)-3-cyanocyproheptadine [(±)-1][9] was resolved. Each pure enantiomer was recrystallized from CH_3CN.

The levorotatory isomer (−)-1, 2.75 g, had mp 178–179 °C; $[\alpha]^{25}_{589}$ −195°, $[\alpha]^{25}_{578}$ −208°, $[\alpha]^{25}_{546}$ −252°, $[\alpha]^{25}_{436}$ −676° (*c* 0.927, $CHCl_3$); IR 2225 cm^{-1} (CN); NMR ($CDCl_3$) δ 1.9–2.8 [11 H, m, with a peak at 2.25 (N-CH_3), aliphatic CH)], 7.0 (d, J = 3 Hz, 2 H, vinyl CH), 7.3–7.6 (m, 7 H, Ar H). Anal. ($C_{22}H_{20}N_2$) C, H, N.

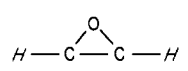
The dextrorotatory isomer (+)-1, 3.14 g, had mp 178–179 °C; $[\alpha]^{25}_{589}$ +193°, $[\alpha]^{25}_{578}$ +206°, $[\alpha]^{25}_{546}$ +251°, $[\alpha]^{25}_{436}$ +670° (*c* 0.839, $CHCl_3$); IR 2225 cm^{-1} (CN); NMR spectrum was identical with the NMR spectrum of (−)-1. Anal. ($C_{22}H_{20}N_2$) C, H, N.

(−)-1′-Methyl-4′-(3-cyano-5*H*-dibenzo[*a*,*d*]cycloheptan-5-ylidene)piperidine [(−)-1] from Reaction of (−)-1-Methyl-4-(3-iodo-5*H*-dibenzo[*a*,*d*]cycloheptan-5-ylidene)-piperidine [(−)-6] with Cuprous Cyanide. A mixture of 1.00 g (0.0024 mol) of (−)-**6**, $[\alpha]^{25}_{589}$ −142°, 0.433 g (0.0048 mol) of cuprous cyanide, and 8 mL of DMF was stirred and heated on a steam bath for 7.5 h. An additional 0.15 g of cuprous cyanide and 2 mL of DMF was added, and the mixture was stirred and heated on the steam bath for an additional 2.5 h. The mixture was cooled, 25 mL of a saturated solution of NaCN and 25 mL of benzene were added, and the mixture was stirred vigorously. The benzene phase was removed and the aqueous phase was extracted with an additional 25 mL of benzene. The combined benzene extracts were washed with a dilute solution of NaCN, H_2O, dried ($MgSO_4$), and filtered, and the benzene was evaporated. The residue was triturated with CH_3CN and collected to afford 0.50 g (66%) of (−)-1, $[\alpha]^{25}_{589}$ −177°. Recrystallization of this material from CH_3CN gave 0.39 g (52%) of (−)-1, mp 178–179 °C, that had $[\alpha]^{25}_{589}$ −187° (*c* 0.528, $CHCl_3$) and was, therefore, 97% optically pure.

Oxidation of 3-[(Trifluoromethyl)thio]-5*H*-dibenzo[*a*,*d*]cycloheptan-5-one (10). A solution of 26.90 g (0.0877 mol) of **10** in 1 L of HOAc was cooled in an ice bath and, while stirring, 240 mL of 30% H_2O_2 was added dropwise. After stirring at room temperature for 192 h, the solution was diluted with 6 L of H_2O and then was extracted with five 750-mL portions of $CHCl_3$. The combined $CHCl_3$ extracts were washed with H_2O, saturated Na_2CO_3 solution, and H_2O. After drying over $MgSO_4$, the $CHCl_3$ was removed on a rotatory evaporator to give 29.34 g of an oil that consisted of three components as determined by TLC (fl. silica gel/benzene). These three components were separated by column chromatography on silica gel using benzene as an eluting solvent.

Product 1. 3-[(Trifluoromethyl)sulfonyl]-5*H*-dibenzo[*a*,*d*]cycloheptan-5-one (12) was the first product eluted from the column. Evaporation of the benzene gave 15.25 g (51%) of TLC-homogeneous material. An analytical sample was prepared by recrystallization from CH_3OH: mp 145–149 °C; NMR ($CDCl_3$) δ 7.0–9.0 (m, vinyl and Ar H), 77.5 (s, SO_2CF_3). Anal. ($C_{16}H_9F_3O_3S$) C, H, F.

Product 2. 3-[(Trifluoromethyl)sulfonyl]-10,11-epoxy-5*H*-dibenzo[*a*,*d*]cycloheptan-5-one (13) was the second product eluted from the column. Evaporation of the benzene from the pooled fractions that were TLC homogeneous in this component gave 4.00 g (13%) of crystalline material. An analytical sample of this epoxide was prepared by recrystallization from CH_3OH: mp 142–145 °C; NMR ($CDCl_3$) δ 4.55 (s, 2 H, i), 7.5–8.3 (m, 7 H,



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Ar H), 77.5 (s, SO_2CF_3). Anal. ($C_{16}H_9F_3O_4S$) C, H, F.

Product 3. 3-[(Trifluoromethyl)sulfinyl]-5*H*-dibenzo[*a*,*d*]cycloheptan-5-one (11) was the third product eluted from the column with benzene. Concentration of the pooled fractions containing this component gave 2.50 g (9%) of a light yellow solid. Recrystallization from EtOH gave pure sulfoxide **11**: mp 101–105 °C; NMR ($CDCl_3$) δ 7.2–8.2 (m, vinyl and Ar H), 73.4 (s, $SOCF_3$). Anal. ($C_{16}H_9F_3O_2S$) C, H, F.

(±)-1′-Methyl-4′-[3-[(trifluoromethyl)sulfonyl]-5*H*-dibenzo[*a*,*d*]cycloheptan-5-ylidene]piperidine [(±)-8]. To an ice-cooled solution of 2.50 g (0.0074 mol) of **12** in 35 mL of THF was added dropwise 36 mL of 0.41 M 1-methyl-4-piperidylmagnesium chloride. The solution was stirred for 1 h and then the THF was removed on a rotary evaporator. The red oily residue was dissolved in benzene and H_2O was added dropwise until a clear benzene supernatant and a gelatinous aqueous phase were obtained. The benzene phase was decanted and the gelatinous aqueous phase was extracted with four 50-mL portions of hot benzene. The combined benzene phases were washed with water, dried ($MgSO_4$), and filtered, and the benzene was removed. The residue was triturated with CH_3CN and the product was collected by filtration to afford 1.30 g (40%) of 1′-methyl-4′-[3-[(trifluoromethyl)sulfonyl]-5-hydroxy-5*H*-dibenzo[*a*,*d*]cycloheptan-5-yl]piperidine (**14**), mp 213–216 °C.

A solution of 1.05 g of this alcohol in 38 mL of trifluoroacetic acid and 19 mL of trifluoroacetic anhydride was stirred and refluxed for 149 h. The solution was concentrated on a rotary evaporator and the residue was made basic with 20% NaOH solution. The oil that precipitated was extracted into $CHCl_3$, washed with H_2O, dried ($MgSO_4$), and filtered. After removing the $CHCl_3$, the residue was triturated with CH_3CN and the product was collected by filtration. An analytical sample of (±)-**8** was prepared by recrystallization from acetonitrile: mp 108–110 °C; NMR δ ($CDCl_3$) 2.0–2.6 [11 H, m, with a peak at 2.26 (NCH_3), aliphatic CH], 6.9–7.9 (9 H, m, vinyl CH and Ar H), 77.7 (s, SO_2CF_3). Anal. ($C_{22}H_{20}F_3NO_2S$) C, H, F, N.

Resolution of (±)-1′-Methyl-4′-[3-[(trifluoromethyl)-sulfonyl]-5*H*-dibenzo[*a*,*d*]cycloheptan-5-ylidene]piperidine [(±)-8]. To a solution of 4.62 g (0.0110 mol) of (+)-**8** in 40 mL of benzene was added 2.13 g (0.0055 mol) of di-*p*-toluoyl-*d*-tartaric acid. The mixture was stirred and warmed until solution was achieved, and, on cooling, crystallization occurred. The solid was collected by filtration, washed with benzene, and dried. The clear filtrate was allowed to evaporate slowly at room temperature to about one-half its original volume, whereupon additional crystallization occurred. This solid was collected, washed with benzene, and dried. The solid materials were combined to give 3.54 g of a pale-yellow solid, designated A. The benzene filtrates and washings were combined and the benzene was removed on a rotary evaporator to give an oily residue, designated B.

A. Levorotatory Isomer (−)-8. The solid A was suspended in a small amount of water and was treated with a saturated solution of Na_2CO_3. The oil that precipitated was extracted into Et_2O. The Et_2O phase was washed with water, dried ($MgSO_4$), and filtered, and the Et_2O was evaporated. The residue was recrystallized from CH_3CN five times to afford 0.69 g of (−)-**8** having a constant rotation: $[\alpha]^{25}_{589}$ −132°, $[\alpha]^{25}_{578}$ −138°, $[\alpha]^{25}_{546}$

−170°, $[\alpha]^{25}_{436}$ −475° (c 0.331, $CHCl_3$); mp 139–141 °C; NMR ($CDCl_3$) δ 2.0–2.6 [11 H, m, with a peak at 2.23 (NCH_3), aliphatic CH], 7.0 (d, J = 2 Hz, 2 H, vinyl CH), 7.2–7.9 (m, 7 H, Ar H), 78.1 (s, SO_2CF_3). Anal. ($C_{22}H_{20}F_3NO_2S$) C, H, N, F.

B. Dextrorotatory Isomer (+)-8. The oily residue B was stirred and partitioned between a saturated solution of sodium carbonate and benzene. The benzene layer was separated, washed with water, dried ($MgSO_4$), and filtered, and the benzene was removed on a rotary evaporator. The residue was recrystallized four times from CH_3CN to afford 0.50 g of (+)-8: mp 139–141 °C; $[\alpha]^{25}_{589}$ +132°, $[\alpha]^{25}_{578}$ +140°, $[\alpha]^{25}_{546}$ +170°, $[\alpha]^{25}_{436}$ +467° (c 0.36, $CHCl_3$); NMR spectrum was identical with the NMR spectrum of (−)-8. Anal. ($C_{22}H_{20}F_3NO_2S$) C, H, N, F.

Receptor Binding Assays. α-Adrenergic Receptor Binding. Assays for the competitive binding of test compounds to central α-adrenergic receptors employed (−)-[^{3}H]norepinephrine[16] and ^{3}H-labeled WB-4101.[17] ^{3}H-labeled WB-4101 (specific activity 2.9 Ci/mmol) was custom synthesized by New England Nuclear, Boston, Mass., and stored as an ethanol solution at 4 °C. (−)-[^{3}H]norepinephrine (specific activity 28 Ci/mmol) was also obtained from New England Nuclear and stored in 0.2 N acetic acid–ethanol (9:1) at 4 °C. Glass and polyethylene laboratory ware were used, respectively, for the ^{3}H-labeled WB-4101 and (−)-[^{3}H]norepinephrine studies, since it was found that the radioligands bound least to these surfaces.

Both α-adrenergic binding assays were run using calf neocortical membranes. These were prepared by homogenizing (Brinkmann Polytron PT 10/35; setting 6; 10 s) calf neocortex in 20 volumes (w/v) of ice-cold 50 mM, pH 7.7, Tris-HCl buffer. The resultant homogenate was centrifuged twice at 49000g (Sorvall SS-34 rotor) for 10 min at 4 °C, with rehomogenization of the intermediate pellet in 20 volumes of fresh buffer. The final pellet was resuspended in 50 volumes of ice-cold, pH 7.7, Tris-HCl buffer for the WB-4101 assay and in 49 volumes of an ice-cold, pH 7.7, Tris-HCl buffer containing 1 μM pargyline for the norepinephrine assay. The displacement of ^{3}H-labeled WB-4101 was measured as follows. Triplicate assay tubes contained ^{3}H-labeled WB-4101 to a final concentration of 0.22 nM, 100 μL of various concentrations of the compound being investigated, 1 mL of calf neocortical homogenate, and 50 mM, pH 7.7, Tris-HCl buffer to a final volume of 2 mL. The reaction was initiated by the addition of tissue and incubation continued for 15 min at 25 °C, at which time it was terminated by rapid filtration through Whatman GF/B glass-fiber filters under vacuum. Each filter was then rapidly washed with 2 × 5 mL aliquots of ice-cold buffer. Samples were counted and results calculated as described below.

Displacement of (−)-[^{3}H]norepinephrine utilized triplicate assay tubes which contained (−)-[^{3}H]norepinephrine at a final concentration of 2.0 nM; 10 μL of various concentrations of the compound being investigated; 0.95 mL of the calf neocortical homogenate; 20 μL of a solution containing 0.05% ascorbic acid, 5 mM Na_2EDTA, 50 mM pyrocatechol, and 0.5 mM dithiothreitol; and 0.1% ascorbic acid solution to a final volume of 1 mL. The reaction was initiated by addition of the tissue and continued for 40 min at 25 °C, at which time it was terminated by rapid filtration under vacuum onto Whatman GF/B glass-fiber filters. The filters were rapidly washed with a 5-mL and then with a 10-mL aliquot of ice-cold 50 mM, pH 7.7, Tris-HCl buffer containing 1 mM pyrocatechol and 0.1% ascorbic acid. Samples were counted and results calculated as described below.

Dopamine Receptor Binding. Dopamine receptors in rat caudate were characterized by the binding of [^{3}H]spiroperidol using a previously described methodology.[35] Male Sprague–Dawley rats (120–150 g; Charles River) were decapitated and their brains removed. The striata were dissected out and homogenized in 100 volumes of ice-cold 50 mM Tris-HCl buffer, pH 7.7, at 25 °C, using a Polytron PCU-2-110 with a PT-20-ST generator (Brinkmann Instruments, Westbury, N.Y.) at a setting of 5.3 for 10 s. The resultant homogenate was centrifuged twice at 48000g (21 000 rpm) in the SS-34 rotor of the Sorvall RC 5 centrifuge with rehomogenization of the intermediate pellet in 100 volumes of fresh buffer. The final pellet was resuspended in 150 volumes of ice-cold Tris–cation buffer (pH 7.6 at 37 °C) of the following composition: 50 mM Tris-HCl, 0.01 mM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 0.2% mM $MgCl_2$, 0.1% ascorbate. Following incubation at 37 °C for 5 min, the tissue suspension was placed on ice prior to use in the binding assay.

Incubation tubes were run in triplicate and contained [^{3}H]-spiroperidol (specific activity 23.6 Ci/mmol) to a final concentration of 0.1 nM, 100 μL of various concentrations of the compounds under study dissolved in 50 mM Tris-HCl, 0.01 mM pargyline, 0.1% ascorbate (pH 7.6 at 25 °C), and tissue (approximately 5 mg of original wet weight of striata) to a final volume of 1 mL. The binding reaction was initiated by the addition of the tissue, and, after a 10-min incubation at 37 °C, the contents of the tube were rapidly filtered under vacuum through Whatman GF/B glass-fiber filters held in a Millipore Filtration Manifold (Model 1225), followed by two 5-mL washes with ice-cold 50 mM Tris-HCl buffer (pH 7.7 at 25 °C). Filtration and washing were completed within 10 s. Samples were counted and results calculated as described below.

Muscarinic Cholinergic Receptor Binding. The muscarinic cholinergic receptor of rat brain was characterized by binding of [^{3}H]quinuclidinyl benzilate (QNB) as described previously.[23] Male Sprague–Dawley rats (120–150 g) were decapitated, and the brain (minus the cerebellum) was removed and homogenized in 10 volumes of ice-cold 0.32 M sucrose in a Teflon–glass homogenizer (A. H. Thomas, Philadelphia, Pa., Type B) with eight up and down strokes using a motor-driven pestle rotating at 500 rpm. The resultant homogenate was centrifuged at 1000g (3000 rpm) in the Beckman J21-C to give a nuclear pellet and the S_1 supernatant. The supernatant was decanted and immediately before use in the binding assay was homogenized with the Polytron (setting 5.0; 60 s) and then diluted 1:20 with 50 mM $NaKH_2PO_4$ buffer (pH 7.4).

Triplicate assay tubes contained [^{3}H]QNB (specific activity 16 Ci/mmol) to a final concentration of 0.06 nM, 100 μL of various concentrations of the compounds under study dissolved in phosphate buffer, 1 mL of tissue homogenate, and phosphate buffer to a final volume of 2 mL. The binding reaction was initiated by addition of the diluted tissue homogenate, and, after standing at 22 °C for 60 min, was terminated by the addition of 3 mL of ice-cold phosphate buffer. The contents of the tubes were filtered under vacuum, as described above, followed immediately with three 3-mL aliquots of phosphate buffer. Samples were counted and results calculated as described below.

Counting of Samples and Calculation of Results. Glass-fiber filters with the membrane-bound ligand complexes were placed in polyethylene scintillation vials. The procedural details then varied slightly for the various binding assays. For [^{3}H]spiroperidol and [^{3}H]QNB, a 10-mL aliquot of Aquasol 2 (NEN) scintillation cocktail was added, and the vials were capped, mechanically shaken for 30 min, equilibrated in the dark at 4 °C for at least 2 h, and counted in a Packard Model 3255 scintillation spectrometer at an efficiency of approximately 38%. For ^{3}H-labeled WB-4101 and (−)-[^{3}H]norepineprine, a 10 mL aliquot of PCS (Amersham) scintillation cocktail was added, and the vials were capped, mechanically shaken for 30 min, equilibrated in the dark at room temperature for at least 2 h, and counted in a Packard Model 2425 scintillation spectrometer at an efficiency of approximately 35%.

Specific binding for each of the tritiated ligands was determined as follows: for ^{3}H-labeled WB-4101, difference in binding in the absence and presence of 100 nM unlabeled WB-4101; for (−)-[^{3}H]norepinephrine, difference in the binding in the absence and presence of 100 nM unlabeled oxymetazoline; for [^{3}H]spiroperidol, difference in binding in the absence and presence of 100 nM unlabeled (+)-butaclamol; for [^{3}H]QNB, difference in binding in the absence and presence of 100 μM unlabeled oxotremorine.

Regression analysis was used on the resulting sets of specific radioligand binding vs. the concentration of test compounds to obtain the IC_{50} concentrations. Apparent inhibition constants are then calculated using the equation:

$$K_i = \frac{IC_{50}}{1 + [c]/K_d}$$

where [c] is the concentration of radioligand employed in the binding assay and K_d is its receptor dissociation constant.

The stepwise regression analyses were conducted using the STEPWISE procedure of the SAS.76 system.[36] The 0.50 significance level was used for entry of variables into the regression equations.

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Supplementary Material Available: Bond distances, bond angles, fractional coordinates, and temperature factors of (−)-3-bromocyproheptadine (5) and circular dichroism spectral features of the (+)-cyproheptadine atropisomers (6 pages). Ordering information is given on any current masthead page.

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Synthesis and Antitumor Activity of 5-Azacytosine Arabinoside[1]

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5-Azacytosine arabinoside (*ara*-AC) can be considered a combination of structural elements derived from the antitumor nucleosides cytosine arabinoside (*ara*-C) and 5-azacytidine (5-AC). The synthesis of *ara*-AC, for which standard methods were inadequate, was accomplished using the stable dihydro derivative as a synthetic intermediate. A novel dehydrogenation of the latter through the application of a trimethylsilylation–oxidation procedure gave *ara*-AC in good yield. Using murine L1210 leukemia as a test system, *ara*-AC was evaluated for antitumor properties in parallel determinations with 5-AC and *ara*-C. Although higher dose levels were necessary, *ara*-AC demonstrated a reproducibly greater efficacy in the L1210 system (% ILS = 144–148) than that shown by 5-AC (% ILS = 126–124) or *ara*-C (% ILS = 127–121). Moreover, initial data suggest that *ara*-AC exhibits less host toxicity than either 5-AC or *ara*-C. Although *ara*-AC can equally be considered an analogue of either 5-AC or *ara*-C, preliminary results indicate that *ara*-AC is chemically similar to 5-AC but biologically more closely related to *ara*-C.

The subtlety of a stereochemical inversion at the 2′ position of cytidine results in cytosine arabinoside (*ara*-C), which is an important agent in the treatment of leukemia.[3]

Bioisosteric replacement of the carbon-5 in cytidine with