1.5 mL/min over 35 min. Peak heights were used to determine the percent peptide remaining and were plotted as a function of incubation time.

Hypotensive Efficacy. Male cynomolgus monkeys weighing 4–6 kg were anesthetized, and polyvinyl catheters were implanted under sterile conditions in the abdominal aorta and the thoracic vena cava via an external iliac artery and vein respectively. At least 1 week was allowed for recovery from surgery before initiation of an experiment. The monkeys were fed a quantity of Standard SKF Monkey Diet deleting sodium (ICN Nutritional Biochemicals, Cleveland, OH) adequate to furnish potassium at 1 mequiv/kg per day. Intravenous 0.9% saline adequate to provide sodium at 1 mequiv/kg per day was administered to maintain the animals in a sodium-replete state. Sodium depletion was accomplished by omitting the daily intravenous administration of saline and substituting the intravenous administration of furosemide (1 mg/kg body weight per day) for 7 days prior to a study. Drinking water was allowed ad libitum. Experiments were carried out while the monkeys were seated in primate restraining chairs (Plas-Labs, Lansing, MI). Blood pressure was recorded continuously from the arterial catheter via a Statham pressure transducer and a Grass polygraph. Heart rate was continuously recorded from a Grass tachograph triggered by the arterial pressure pulse. Each animals received a bolus injection of saralasin (Peninsula Laboratories, Inc., Belmont, CA) at 1 mg/kg iv at least 5 h prior to the infusion of peptide XII to elucidate the magnitude of the renin-dependent blood pressure component. Blood samples were drawn at intervals, and plasma renin activity was determined via standard radioimmunoassay techniques at pH 7.4 using the Gamma Coat [^{125}I] Plasma Renin Activity Radioimmunoassay Kit (Travenol-Genentech Diagnostics, Cambridge, MA).

Acknowledgment. We are grateful to Ruth E. Ten-Brink for the preparation of peptide VI and to Dr. Jackson B. Hester, Jr., for the initial supply of Boc-Leuψ-[CHOTBSCH$_2$]Val-OH.

Neuroleptics from the 4a,9b-*cis*- and 4a,9b-*trans*-2,3,4,4a,5,9b-Hexahydro-1*H*-pyrido[4,3-*b*]indole Series. 2

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Received January 21, 1986

Compounds derived from 4a,9b-*trans*-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole are consistently efficacious in displacing [^{3}H]spiroperidol from striatal dopamine receptors in vitro. Derivatives bearing substituents at position 2, particularly those derived from butyrophenone moieties, are exceptionally potent in vivo. Compounds from the corresponding 4a,9b-cis series are substantially less potent in both in vivo and in vitro assays of neuroleptic activity. Although the cis and trans derivatives have, in some conformations, similar basic nitrogen atom to aromatic ring separations of about 5.1 Å, the distance at which the basic nitrogen atom lies above or below the plane of the aromatic ring differs substantially between the two series. Consideration of these results in terms of this and earlier work indicates that the out-of-plane distance for the basic nitrogen in neuroleptic molecules may range from about 0 to about 0.90 Å but may be optimized at about 0.55 Å.

A major objective of medicinal chemistry in recent years has been the elucidation of mechanisms by which naturally occurring substances interact with pharmacologically relevant sites in in vivo systems. Such an understanding would permit the design of synthetic compounds that could interact with the same sites to potentiate or antagonize the action of the naturally occurring substance in hopes of producing a therapeutic response. Since many naturally occurring substances are conformationally flexible and capable in theory of interacting in any one of multiple molecular conformations, chemists have sought compounds of defined geometry that are also potently active in specific biological assays as investigational tools.[2] Specificity of an optically active enantiomer for a receptor yields valuable information about the spatial and conformational requirements of the receptor, especially in those cases in which the disfavored enantiomer is substantially less potent.

Current theories maintain that schizophrenia derives from alterations in the impulse flow in dopaminergic neurons in the central nervous system[3] and that patients can be treated by the administration of dopamine (DA) receptor blocking agents. Several recently developed neuroleptic agents, including the optically active (+)-butaclamol (I)[4] and Ro-22-1319 (II)[5] are potent DA receptor blockers in in vivo and in vitro model systems, are capable of interacting in only one enantiomeric form, and are derived from relatively rigid frameworks. A third structure that possesses these properties of potent activity, optical specificity, and structural rigidity at the DA receptor is (−)-apomorphine (III)[6] which is, however, an agonist at the receptor. We became interested in two novel series of chiral hexahydro-γ-carbolines, the trans derivatives represented by IV and the cis derivatives represented by V, as a result of our earlier work in the achiral tetrahydro-γ-carboline series[7,8] that culminated in the discovery of flutroline (VI), a compound that has demonstrated clinical antipsychotic activity in man.[9,10]

The hexahydro series IV and V were of interest to us for several reasons: First, these compounds would serve

† Department of Medicinal Chemistry.
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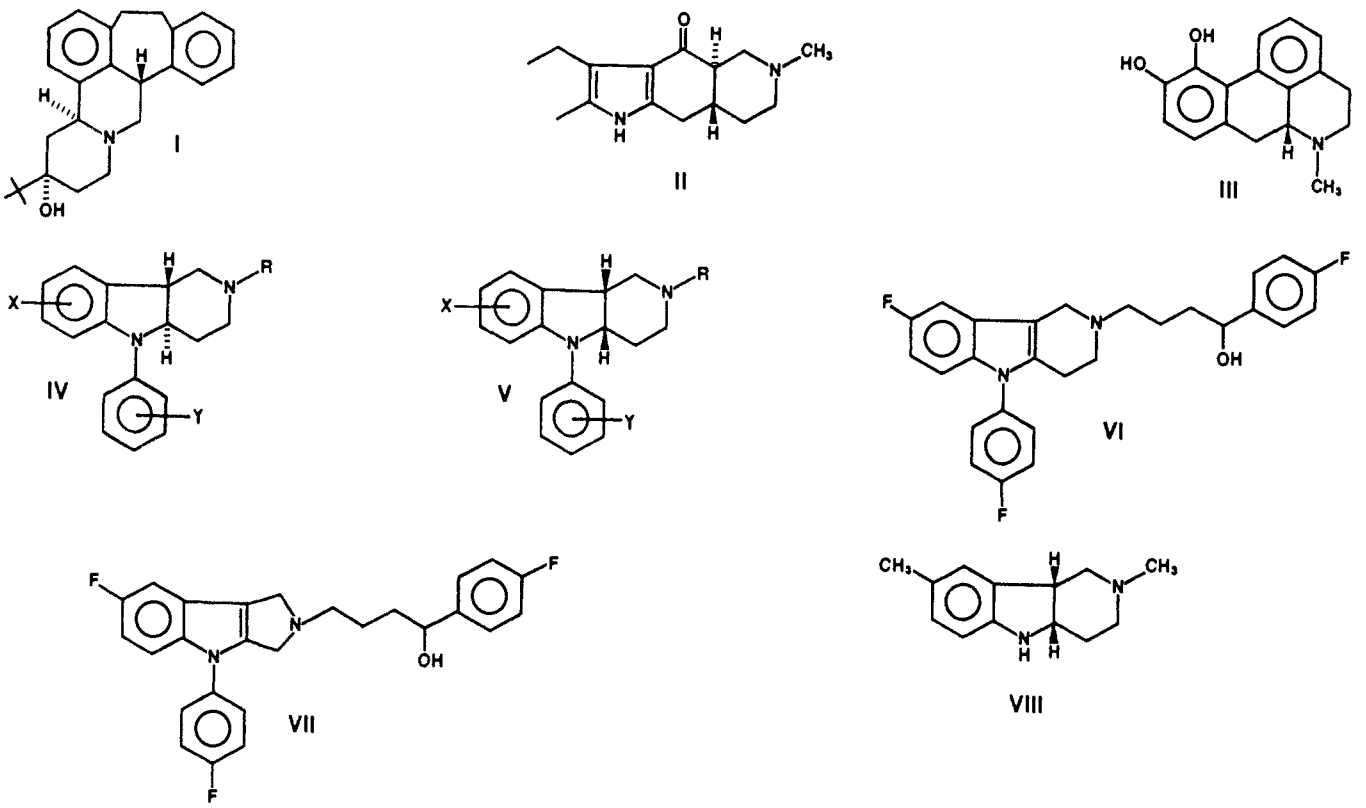
0022-2623/86/1829-2093$01.50/0

Chart I



as conformationally novel probes of the DA receptor, yielding information leading to a more thorough understanding of the requirements of the receptor and potentially useful in the design of new agents. Second, these structurally novel compounds might yield derivatives from which clinically improved neuroleptics might be derived. The increasing understanding of the regiospecific and stereospecific requirements of the DA receptor engendered by the discovery of (+)-butaclamol and other agents[2] and the similar requirements for the DA agonist (-)-apomorphine[7] suggested that one conformer of the achiral tetrahydro-γ-carboline nucleus might preferentially interact with this receptor. Hexahydro compounds from the 4a,9b-cis and the 4a,9b-trans series are chiral and, if prepared optically pure, should show the same stereospecific dependence of other enantiomeric DA receptor agonists and antagonists. Such compounds might have enhanced lipophilicity and thus penetrate the blood–brain barrier better than the tetrahydro species. In addition, reduction of the carboline double bond removes one potential site for metabolic deactivation of the compounds and might thus extend the already quite long duration of action displayed by the tetrahydro analogues.[7]

When this work was initiated, we had little information as to which (cis or trans) series would be expected to be more active. Comparisons of X-ray and/or Dreiding model structures show that (+)-butaclamol, (-)-apomorphine, tetrahydro-γ-carbolines such as VI, *trans*-hexahydro-γ-carbolines represented by IV, and *cis*-hexahydro-γ-carbolines represented by V share aromatic ring to basic nitrogen distances in the plane of these molecules of approximately 5.1A.[6,11] Such model structures also show that the spatial volume that the basic nitrogen atom occupies lies above (or below) the plane of the molecule and that substantial differences in this out-of-plane distance are seen among the different structures. One possibility for an optimum out-of-plane distance appeared to be on the order of 0.5–0.9 Å, since the out-of-plane distance for one of the two conformers of both (+)-butaclamol (0.19, 0.90 Å) and (-)-apomorphine (0.9, 1.23 Å) is 0.9 Å and the tetrahydro-γ-carboline VI has an out-of-plane distance of about 0.55 Å.[7] On the other hand we had earlier reported on a somewhat less active series of pyrrolo[4,3-*b*]indoles VII,[12] which are essentially planar but bind potently to the dopamine receptor in vitro and demonstrate neuroleptic properties in animals. Further, the potent pyrrolo[2,3-*g*]isoquinoline II has an out-of-plane distance of only 0.1 Å.[5] Dreiding models suggest that *trans*-hexahydro-γ-carboline derivatives IV should have out-of-plane distances very close to the 0.55 Å of tetrahydro-γ-carboline VI and the energy barrier for ring conformational change should be considerably higher. The *cis*-γ-carboline series V is less conformationally rigid and should show out-of-plane distances in the range of about 1.4–2.4 Å for various chair and boat conformations (Figure 1). While these observations combined to suggest that the trans series would yield the more active derivatives, we were aware of a report that a *cis*-hexahydro-γ-carboline, carbidine (VIII), had been shown to possess neuroleptic activity.[13] Thus, we decided to undertake an investigation of both series.

We were aided in our efforts by novel carboline chemistry that had been recently reported in the literature. The first of these involved reduction of a tetrahydro-γ-carboline species with borane and acid to give *trans*-hexahydro-γ-carbolines IV[14] and the second involving reduction of similar tetrahydro species with hydrogen and a catalyst or dissolving metal to give the cis series represented by V.[15]

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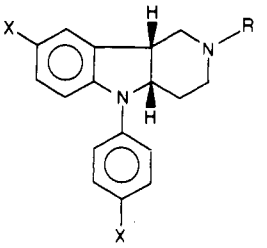
Table I. In Vivo and in Vitro Neuroleptic Activities of Cis Derivatives



compd	R	X	mp, °C	formula[a]	antag of amphetamine (rat): ED_{50},[b] mg/kg ip 1 h	5 h	24 h	inhibn of [^{3}H]-spiroperidol binding: IC_{50},[c] nM
1	H	H	180–182	$C_{17}H_{18}N_2 \cdot C_4H_4O_4$	>32	>32	NT	>1000
2	CH_3	H	136–139	$C_{18}H_{20}N_2 \cdot C_4H_4O_4$	>32	>32	NT	>1000
3	$CH_2C_6H_5$	H	248–250	$C_{24}H_{24}N_2 \cdot HCl$	>32	>32	NT	>1000
4	$(CH_2)_3CHOH(4\text{-}FC_6H_4)$	H	124–125	$C_{27}H_{29}N_2OF \cdot 0.5H_2O$	>32	>32	NT	140
5	$(CH_2)_3CHOH(4\text{-}FC_6H_4)$	F	215–220	$C_{27}H_{27}N_2OF_3 \cdot HCl$	22	1.1	9.1	52
carbidine					>32	>32	NT	NT
chlorpromazine					5.3	8.5	>32	51
VI					0.1–0.32	0.1–0.32	0.32–1.0	14

[a] All compounds were analyzed for C, H, and N; values were within ±0.4% of calculated values. [b] The effect of each compound on prominent amphetamine-elicited symptoms were studied in rats by using the rating scale and method reported by Weissman et al.[24] Groups of five rats were treated with compounds at doses separated by 0.5 log unit (i.e., ..., 0.32, 1.0, 3.2, 10, ... mg/kg) and were then treated with *d*-amphetamine sulfate, 5 mg/kg ip, 1, 5, and 24 h later. ED_{50} values were determined by using the tables of Weil.[25] [c] [^{3}H]Spiroperidol binding to rat striatal membranes using 0.5 nM ligand was performed by the method of Burt et al.[18] IC_{50} values were determined graphically from four drug concentrations separated by 0.5 log unit. Entries are means of two to three determinations.

Chemistry

The reported reduction[15] of tetrahydro-γ-carbolines to give *cis*-hexahydro-γ-carbolines utilizing lithium and liquid ammonia was successfully applied to *N*-benzyl-5-phenyltetrahydro-γ-carboline to give compound **3**, from which compounds **1**, **2**, and **4**, unsubstituted in the aromatic rings (Table I), were prepared by previously reported methods.[7] However, reduction of the difluorinated species using these conditions resulted in hydrogenolysis of the fluorine substituents concurrent with reduction of the double bond. The trifluorinated compound **5**, which was of particular interest, was successfully prepared in two ways. Reduction of flutroline VI using the bis(trifluoroacetoxy)borane complex of Maryanoff[16] was neither complete nor stereospecific, but the desired cis product **5** could be separated from starting material by careful column chromatography. Alternatively, reduction of 5-(4-fluorophenyl)-8-fluorotetrahydro-γ-carboline with excess bis(trifluoroacetoxy)borane gave the cis hexahydro nucleus (**1**; X = F) in low but adequate yield. This nucleus could be then acylated with 3-(4-fluorobenzoyl)propionic acid and the resulting amide reduced with LAH to give the desired **5**, in all respects identical with that prepared from flutroline.

Trans compounds were prepared by reduction of the 5-phenyl- or 8-fluoro-5-(4-fluorophenyl)-2-benzyl derivatives using modifications of the previously reported procedure utilizing borane and acid.[1] Both the nonfluorinated and the 4′,8-difluoro-*N*-benzylhexahydro derivatives could be debenzylated catalytically under mild conditions, with no detectable loss of fluorine in the latter case. This step provided racemic trans hexahydro starting materials for both the substituted and unsubstituted series.

Compounds that have chiral centers in the side chain such as **4**, **5**, **13**, and **26** were initially isolated as 1:1 mixtures of racemic diastereoisomers and were tested as such without separation. We have previously reported that,

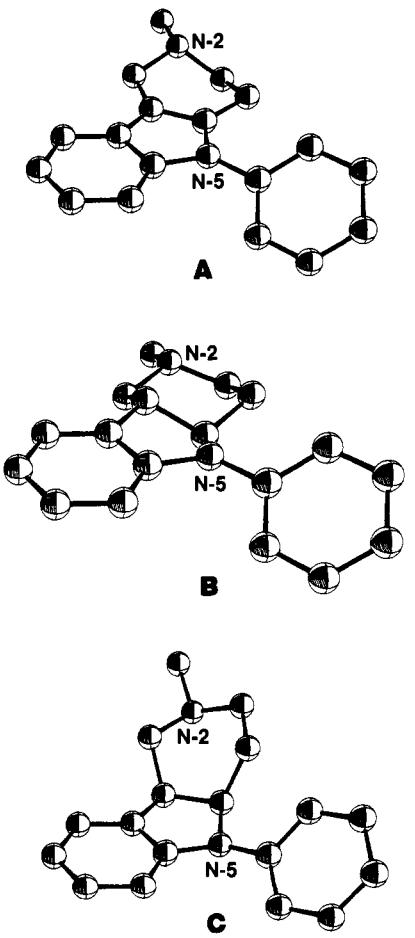


Figure 1. One lowest energy conformer of N^2-methyl-substituted (A) tetrahydro-γ-carboline, (B) *trans*-hexahydro-γ-carboline, and (C) *cis*-hexahydro-γ-carboline. Hydrogen atoms are absent to facilitate depiction of three-dimensional features.

because the neuroleptic activity of compounds of this type derives almost wholly from the carboline portion of the molecule, the activities of these racemic diastereoisomers

(15) Heath-Brown, B. *Chem. Ind.* **1969**, 1595.
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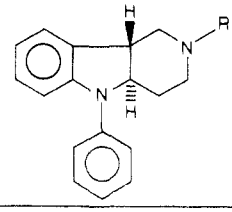
Table II. In Vivo and in Vitro Neuroleptic Activities of Trans Derivatives



compd	R	mp, °C	formula[a]	antag of amphetamine (rat): ED_{50},[b] mg/kg ip 1 h	5 h	24 h	inhibn of [^{3}H]-spiroperidol binding: IC_{50},[c] nM
6	H	218–222	$C_{17}H_{18}N_2$·HCl	>3.2	>3.2	NT	100
7	CH_3	253–255	$C_{18}H_{20}N_2$·HCl·H_2O	0.56	>1	NT	4.9
8	C_2H_5	239–241	$C_{19}H_{22}N_2$·HCl	>1	>1	NT	23
9	$CH_2C_6H_5$	248–250	$C_{24}H_{24}N_2$·HCl	1.0–3.2	1.0–3.2	NT	20
10	$(CH_2)_3CO(4\text{-}FC_6H_4)$	244–246	$C_{27}H_{27}N_2OF$·HCl	0.56	0.18	~1.0	8.0
11	$(CH_2)_3CHOHC_6H_5$	224–225	$C_{27}H_{30}N_2O$·HCl·0.5H_2O	0.07	0.056	>1.0	7.8
12	$(CH_2)_3CHOH(4\text{-}OCH_3C_6H_4)$	amorph	$C_{28}H_{32}N_2O_2$·HCl·0.5H_2O	0.22	0.14	>1.0	21
13	$(CH_2)_3CHOH(4\text{-}FC_6H_4)$	239–245	$C_{27}H_{29}N_2OF$·HCl	0.10	0.03–0.10	>0.10	11
(±)-butaclamol				0.38	0.37	>3.2	12
haloperidol				0.1–0.32	0.1–0.32	>3.2	9
chlorpromazine				5.3	8.5	>32	51
VI				0.1–0.32	0.1–0.32	0.32–1.0	14

[a] All compounds were analyzed for C, H, and N; values were within ±0.4% of calculated values. [b] The effects of each compound on prominent amphetamine-elicited symptoms were studied in rats by using the rating scale and method reported by Weissman et al.[24] Groups of five rats were treated with compounds at doses separated by 0.5 log unit (i.e., ..., 0.32, 1.0, 3.2, 10, ... mg/kg) and were then treated with *d*-amphetamine sulfate, 5 mg/kg ip, 1, 5, and 24 h later. ED_{50} values were determined from the tables of Weil.[25] [c] [^{3}H]Spiroperidol binding to rat striatal membranes using 0.5 nM ligand was performed by the method of Burt et al.[18] IC_{50} values were determined graphically from four drug concentrations separated by 0.5 log unit. Entries are means of two to three determinations.

are equivalent to each other (or to an equivalent amount of the 1:1 mixture).[1] For this reason, we felt that for initial SAR studies the occasionally tedious separation of racemic diastereoisomers in each present case was unnecessary. Part 1[1] of this series discloses the methodology by which racemic diastereoisomers could be separated chromatographically. We have more recently reported the resolution of the tricyclic nucleus and the preparation of derivatives thereof, demonstrating that neuroleptic activity in these molecules derives from the nucleus with the 4a*S*,9b*S* absolute configuration.[17]

Results and Discussion

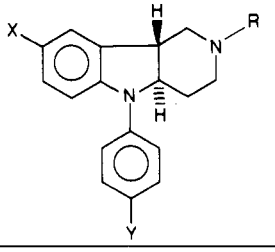
Neuroleptic activity of the compounds listed in Tables I–III was assessed in vitro by their ability to displace [^{3}H]spiroperidol from labeled receptors of corpus striatum by a method adapted from that of Burt, Creese, and Snyder[18] and in vivo by their ability to antagonize the stereotypy induced in rats by *d*-amphetamine sulfate (5 mg/kg, ip). Both methods have been described fully in an earlier paper from these laboratories.[7]

Cis Series. *cis*-Hexahydro-γ-carboline testing data are presented in Table I and suggest that the *cis*-hexahydro-γ-carboline moiety has low affinity for the DA receptor. Compounds **1–3** bearing simple alkyl substituents at position 2 of the indole nucleus were essentially inactive in the in vitro [^{3}H]spiroperidol displacement study. Previous studies in the earlier tetrahydro-γ-carbolines series had shown that simple alkyl substitution at position 2 of the tetrahydro-γ-carboline nucleus did not lead to in vivo neuroleptic activity in animal models, and the lack of in vivo activity (Table I) among compounds **1–3** is consistent with this previous observation. On the other hand, potent in vitro and most importantly in vivo activity had been observed in tetrahydro-γ-carboline derivatives substituted with a 4-hydroxy-4-(substituted aryl)butyl moiety in this position, particularly the 4-(4-fluorophenyl) derivatives. By way of contrast, in the cis-hexahydro-unsubstituted series bearing this same 2-substituent, compound **4** was found to be much less active in vivo than might have been expected on the basis of the tetrahydro results although weak affinity for the [^{3}H]spiroperidol site was seen for this compound. Since, in the tetrahydro series, activity in both the in vitro and in vivo screens had peaked in compounds having the 4′,8-difluoro substitution pattern, it was of obvious interest to determine how the same substitution pattern would affect the present cis series. Therefore, compound **5** was synthesized and, as shown in Table I, was found to interact with the DA receptor in vitro at a level equal to that of chlorpromazine as shown by the equivalent IC_{50} values in the [^{3}H]-spiroperidol binding assay. Compound **5** is also superior to chlorpromazine in the amphetamine model at 1-, 5-, and 24-h end points. Thus, the enhancement of activity upon addition of fluorine substituents in the 4′- and 8-position as had previously been observed in the tetrahydro-γ-carboline series clearly plays a role in the binding of the cis species to the DA receptor. The limited in vivo and in vitro potency observed in the cis-hexahydro series, however, clearly demonstrates that this molecule does not bind to the receptor in an optimal manner and that other conformational factors play a much larger role in binding ability and in vivo activity than does substitution.

We have previously argued that the DA receptor site consists of an aromatic ring binding site and a hydrogen-bonding site to which basic nitrogen binds, these two primary sites being separated by about 5.1 Å.[6,7] We have also suggested that the basic nitrogen binding site can be anywhere from coplanar with the aromatic ring to about 0.9 Å above such a plane but should preferably be about 0.5–0.6 Å out of the plane of the indole aromatic ring. In the *cis*-hexahydro-γ-carboline series, the tricyclic portion

(17) Sarges, R.; Howard, H. R.; Donahue, K. M.; Welch, W. M.; Dominy, B. W.; Weissman, A.; Koe, B. K.; Bordner, J. *J. Med. Chem.* **1986**, *29*, 8.
(18) Burt, D. R.; Creese, I.; Snyder, S. H. *Mol. Pharmacol.* **1976**, *12*, 800.

Table III. In Vivo and in Vitro Neuroleptic Activities of Fluorinated Trans Derivatives



compd	R	X	Y	mp, °C	formula[a]	antag of amphetamine (rat): ED_{50},[b] mg/kg ip 1 h	5 h	24 h	inhibn of [^{3}H]-spiroperidol binding: IC_{50},[c] nM
14	H	F	F	115–117	$C_{17}H_{16}N_2F_2$	>3.2	>3.2	NT	14
15	CH_3	F	F	amorph	$C_{18}H_{18}N_2F_2$·HCl·0.75H_2O	0.14	0.23	>3.2	8
16	C_2H_5	F	F	267–269	$C_{19}H_{20}N_2F_2$·HI	0.14	0.18	>10	2.3
17	$(CH_2)_2CH{=}CH(4\text{-}OCH_3C_6H_4)$	F	F	224–229	$C_{28}H_{28}N_2OF_2$·HCl·0.5H_2O	>3.2	~0.32	~0.32	110
18	$(CH_2)_2CH{=}CH(4\text{-}FC_6H_4)$	F	F	278–280	$C_{27}H_{25}N_2F_3$·HCl	>1.0	0.57	0.57	70
19	$(CH_2)_3CO(4\text{-}FC_6H_4)$	F	F	265–267	$C_{27}H_{25}N_2OF_3$·HCl	>1.0	0.10	0.03–0.10	15
20	$(CH_2)_3CH_2OH$	F	F	220–223	$C_{21}H_{24}N_2OF_2$·HCl	0.03	0.009	~1.0	7.4
21	$(CH_2)_3CHOHC_6H_5$	F	F	220–223	$C_{27}H_{28}N_2OF_2$·HCl	0.18	0.18	0.18	20.5
22	$(CH_2)_3CHOH(4\text{-}OCH_3C_6H_4)$	F	F	45–48	$C_{28}H_{30}N_2O_2F_2$·0.5H_2O	0.36	0.01–0.03	0.10	18
23	$(CH_2)_3CHOH(4\text{-}FC_6H_4)$	F	F	241–245	$C_{27}H_{27}N_2OF_3$·HCl	0.41	0.03	0.03	21
24	$(CH_2)_3CHOH(4\text{-}FC_6H_4)$	F	H	193–195	$C_{27}H_{28}N_2OF_2$·HCl	0.23	0.08	0.11	22
25	$(CH_2)_3CHOH(4\text{-}FC_6H_4)$	F	H	199–201	$C_{27}H_{28}N_2OF_2$·HCl	0.1–0.32	~0.032	0.032–0.10	15
26	$(CH_2)_3CHOH(4\text{-}FC_6H_4)$	H	F	219–220	$C_{27}H_{28}N_2OF_2$·HCl·1.5H_2O	~0.32	0.032–0.10	0.032–0.10	26
(±)-butaclamol						0.38	0.37	>3.2	12
haloperidol						0.1–0.32	0.1–0.32	>3.2	9
chlorpromazine						5.3	8.5	>32	51
VI						0.1–0.32	0.1–0.32	0.32–1.0	14

[a] All compounds were analyzed for C, H, and N; values were within ±0.4% of calculated values. [b] The effects of each compound on prominent amphetamine-elicited symptoms were studied in rats by using the rating scale and method reported by Weissman et al.[24] Groups of five rats were treated with compounds at doses separated by 0.5 log unit (i.e., ..., 0.32, 1.0, 3.2, 10, ... mg/kg) and were then treated with *d*-amphetamine sulfate, 5 mg/kg ip, 1, 5, and 24 h later. ED_{50} values were determined from the tables of Weil.[25] [c] [^{3}H]Spiroperidol binding to rat striatal membranes using 0.5 nM ligand was performed by the method of Burt et al.[18] IC_{50} values were determined graphically from four drug concentrations separated by 0.5 log unit. Entries are means of two to three determinations.

of the molecule can exist in several chair and boat conformers, none of which closely resemble the tetrahydro-γ-carboline series in both out-of-plane distance and the distance between the aromatic ring center and the basic nitrogen atom.[19] Although two low-energy boat conformers of the cis series show nearly the same aromatic ring to basic nitrogen distances, the differences in N out-of-plane distance are clearly detrimental to potency and hence to the binding ability of these molecules.

Trans Series. *trans*-Hexahydro-γ-carbolines without substituents in the aromatic rings were prepared for direct comparison with cis compounds **1–4** in Table I. The results of in vitro and in vivo testing of the these and other related analogues are presented in Table II. Compounds **6**, **7**, **9**, and **13** are capable of potent interaction with the DA receptor as measured by their ability to displace [^{3}H]-spiroperidol from striatal homogenates, contrasting with the lack of activity of compounds **1–3** and the limited activity of compound **4** in this test. Again it will be noted that secondary amines (compound **6**) or simple alkyl-substituted compounds (compounds **7–9**) have limited activity in the in vivo screen, but the intrinsic activity of the trans tricyclic nucleus can be deduced from their relatively potent binding activities in the in vitro assay. When trans compounds **7** and **9** are compared directly with cis compounds **2** and **3**, it is obvious that the trans derivatives are capable of much better interaction with the receptor. Further elaboration of this nucleus by incorporation of butyrophenone-derived side chains at position 2 led to compounds **10–13** that were potently active in vivo as well as in vitro. Among the limited series examined, the butyrophenone **10** is about as active as (±)-butaclamol in vivo and appears to be of similar duration of action. As seen previously in the tetrahydro-γ-carboline series, reduction of the carbonyl moiety (**10** → **13**) enhances potency without having much effect in duration of action. Thus, the presence of auxillary binding site(s) in the side chain contributes little to in vitro binding but elicits potent in vivo activity from the nucleus, probably assisting penetration of these more lipophilic compounds into the central nervous system or slowing metabolic degradation.

The in vivo activity of compounds **10–13** in the anti-amphetamine test appears to peak at around 5 h and to decrease by the 24-h test. We had observed this result in tetrahydro-γ-carboline derivatives that were unsubstituted in the 4′- and 8-positions and found that substitution of fluorine atoms in these two positions optimized both potency and duration of action in that series.[7] In Table III, it will be noted that substitution of fluorine in the 4′- and 8-positions in the hexahydro series again enhances both potency and duration and that compounds derived from this nucleus display exceedingly potent in vitro and in vivo activity. The simple alkyl-substituted derivatives **15** and **16** are somewhat more potent than the secondary amine **14** in the in vitro [^{3}H]spiroperidol binding assay, and the potent activity of **14** in vitro illustrates again the intrinsic ability of this nucleus to interact with the DA receptor. The decrease in potency seen between 1 and 5 h for compounds **7**, **14**, and **15** suggests that these compounds are subject to more rapid metabolism than are compounds with more elaborate side chains, e.g. **10–13** and **17–26**. The in vivo activity of compounds **15** and **16** at the 5-h end point not seen in compounds **7** or **8** probably reflects the

(19) Dominy, B. W., unpublished results.

blockade of two important metabolic sites para to the indole nitrogen atom. The alkane side chains of compounds **17** and **18** decrease activity in vitro, suggesting a possible decrease of binding due to increased chain length; these compounds show good activity in vivo, comparable to the methyl and ethyl derivatives **15** and **16**, although they are not active at the 1-h end point.

All hexahydro-γ-carboline derivatives bearing a hydroxyl group in the side chain four carbon atoms removed from the tricyclic nucleus displayed potent in vitro and in vivo neuroleptic activity. Compound **20**, bearing the simple 4-hydroxybutyl substituent, was found to exceed in potency even the 4-hydroxy-4-(4-fluorophenyl)butyl derivative **23** in both of these tests and was the most potent derivative found in the present series. We interpret this result to indicate that binding to the DA receptor and related auxilliary sites is more related to the presence of nonbasic unshared electron pairs of oxygen or nitrogen substituents than it is to aromatic rings or lipophilic hydrocarbon residues. The observation that the half-life of compound **20** is considerably shorter in vivo than that of the corresponding 4-fluorophenyl derivative **23** suggests that the role of the 4-fluorophenyl substituent is that of an inhibitor of metabolism rather than being of functional significance with regard to binding. Other investigators using (+)-butaclamol as a ligand for investigating the three-dimensional characteristics of the DA receptor site have suggested that an auxiliary lipophilic binding site exists about 4.5 Å from the basic nitrogen hydrogen-bonding site.[20] We note that hexahydro-γ-carboline derivatives lacking a hydroxyl group in the side chain (e.g., **17** and **18**) but possessing the lipophilic 4-fluorophenyl group are much less active than are compounds **20–26**. In addition, compound **20** is substantially more active at 1- and 5-h end points than compounds bearing both the aromatic ring and the hydroxyl group (e.g., **21** and **23**). This evidence supports the existence of an auxiliary site in this region but argues strongly that its nature is more likely that of a hydrogen-bonding site rather than a lipophilic group acceptor. Such a hydrogen-bonding functionality can be found in every class of neuroleptic with in vivo activity and may be an oxygen substituent as in butaclamol, haloperidol, or compounds from the present series, but may also be a nitrogen substituent as illustrated by the neuroleptic activity of thiothixene, octoclothepine, and fluspirilene as well as by the benzamides and clozapine. Tertiary amine substituents found in these latter compounds furthermore, suggest strongly that the receptor auxilliary binding site is hydrogen-bond accepting rather than donating. This result has been translated into a second series of highly active hexahydro-γ-carbolines.[21]

In summary, the results of the present studies add the following points to the definition of the DA receptor site: (1) The pharmacophore binding to the receptor auxilliary binding site must contain a hydrogen-bond donating group, but an aromatic ring is not critical. (2) The DA receptor is relatively flexible in accepting molecules with a variety of out-of-plane distances as long as the aromatic ring–basic nitrogen distance and the stereospecificity parameters are met. (3) Out-of-plane distances of 1.4 Å, as seen in one possible conformer of cis compounds **5** above, appear to be just marginally capable of binding to the DA receptor site, but compounds with out-of-plane distances of around 0.5 Å (±0.5 Å) are required for potent activity. The results derived from the cis and trans series discussed here, from the tetrahydro-γ-carboline and the pyrrolo[4,3-*b*]indole series, and from the recent report of neuroleptic activity in compounds from the isobutaclamol series, which have an aromatic ring–basic nitrogen separation of 6.4 Å,[22] provide additional support for the concept of a rather flexible, stereoselective DA receptor.

Experimental Section

Melting points (uncorrected) were taken with a Thomas-Hoover capillary apparatus. NMR spectra were recorded on Varian T-60 and XL-100 spectrometers with Me_4Si as an internal standard. IR spectra were determined with a Perkin-Elmer Model 21 spectrometer. Mass spectra were obtained with a Perkin-Elmer RMU-6E mass spectrometer. Microanalysis were performed by the Pfizer Analytical Department.

***cis*-(±)-2-Benzyl-5-phenyl-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole Hydrochloride (3).** Sodium metal was added in small portions to a solution of 5.45 g (16.1 mmol) of 2-benzyl-5-phenyl-1,2,3,4-tetrahydropyrido[4,3-*b*]indole[23] in 60 mL of THF and about 80 mL of liquid NH_3 until a blue color was sustained for 15 min. The reaction mixture was stirred 30 min and then quenched with excess NH_4Cl. The excess ammonia was allowed to evaporate overnight; the residues were partitioned between water and $CHCl_3$. The $CHCl_3$ layer was combined with one additional wash of the aqueous, dried over $MgSO_4$, and evaporated to give 5.40 g (98%) of a pale yellow oil that was used for the next step without purification. For testing and analysis, 1 g of this oil was dissolved in 5 mL of acetone, and then a slight excess of ethereal HCl was added. The product that crystallized was isolated and recrystallized from CH_3CN/CH_3OH to give 410 mg of product hydrochloride, mp 248–250 °C.

***cis*-(±)-5-Phenyl-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole Maleate (1).** A solution of 4.5 g (13.2 mmol) of compound **3** in 200 mL of absolute EtOH was made slightly acidic with HCl(g) and then reduced at 55 °C over 5 g of 5% Pd/C at an initial pressure of 55 psi. After about 6 h, the reaction was complete. The reaction mixture was filtered and evaporated to a foam. This was chromatographed on silica gel, eluting with 5% $HOAc/CH_3OH$. The fractions containing product were combined, the solvent was evaporated, and the residue was dissolved in ether. This solution was washed with 10% NaOH to remove residual HOAc and then dried and evaporated to give 3.56 g (71%) of an oil that was used for further reactions. A 950-mg sample of this oil in ethyl acetate was treated with a slight excess of maleic acid to give a crystalline product that was recrystallized from hot acetonitrile; mp 180–182 °C.

***cis*-(±)-2-Methyl-5-phenyl-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole Maleate (2).** A 0.455-g (4.2-mmol) portion of ethyl chloroformate was added to a stirred solution of 1.0 g (4.0 mmol) of compound **1** and 440 mg (4.4 mmol) of triethylamine in 20 mL of CH_2Cl_2. This solution was stirred overnight and was then washed with dilute HCl and dilute $NaHCO_3$. The solvent was evaporated, and the residue was dissolved in 50 mL of THF. This solution was heated to reflux, and an excess of solid LAH was added portionwise. The reaction mixture was refluxed 5 min, and then excess LAH was decomposed with Glauber's salt and filtered. The filtrate was evaporated, and the residue was dissolved in EtOAc. A solution of 4 mmol of maleic acid in EtOAc was added, and the product was crystallized by addition of ether to give 493 mg (32%) of the desired product, mp 136–139 °C.

***cis*-(±)-5-(4-Fluorophenyl)-2-[4-hydroxy-4-(4-fluorophenyl)butyl]-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole (4).** A 1.14-g (5.54-mmol) portion of dicyclohexylcarbodiimide was added to a solution of 1.08 g (5.54 mmol) of (4-fluorobenzoyl)propionic acid in 20 mL of CH_2Cl_2 stirred in an ice bath. After 25 min, 1.26 g (5.04 mmol) of compound **1** in 5 mL of CH_2Cl_2 was added, and the reaction mixture was allowed to warm to room

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temperature. After 2 h, the reaction mixture was again cooled to 5 °C and filtered. The filtrate was evaporated to a foam that was chromatographed on silica gel with 1:1 hexane/EtOAc. Product-containing fractions were combined, the solvent was evaporated, and the residue was dissolved in THF. This solution was heated to reflux, and the keto amide was reduced by portionwise addition of a slight excess of solid LAH. After a further 5-min reflux, the reaction mixture was cooled, decomposed with Glauber's salt, and filtered. After evaporation of the solvent, the residue crystallized from ether to give 570 mg (27%) of colorless crystals, mp 124–125 °C.

***trans*-(±)-2-Benzyl-5-phenyl-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole Hydrochloride (9).** To 150 mL of a stirred solution of 0.93 M borane in THF (140 mmol) at 0–5 °C was added 23.9 g (71 mmol) of 2-benzyl-5-phenyl-1,2,3,4-tetrahydropyrido-[4,3-*b*]indole[23] in 460 mL of THF. The reaction mixture was then refluxed 1 h. Most of the solvent and excess borane were removed on a rotary evaporator, leaving a white precipitate. This was mixed with 40 mL of dry THF, and then 180 mL of 1:1 5 N HCl/HOAc was run in rapidly. This suspension was heated at reflux 1 h. The cooled reaction mixture was partially evaporated, and the resulting white solid was filtered off and washed with water. This material was suspended in THF and stirred 30 min and then filtered, washed with THF and ether, and air-dried to give 16.7 g (63%) of a white, crystalline product, mp 256–260 °C.

***trans*-(±)-8-Fluoro-5-(4-fluorphenyl)-2-[4-(4-fluorophenyl)-4-oxobutyl]-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole Hydrochloride (19).** A solution of 383 mg (4.91 mmol) of Me_2SO in 15 mL of CH_2C_2 in a flame-dried flask was cooled under dry N_2 to −75 °C. A solution of 286 mg (2.25 mmol) of oxalyl chloride in 5 mL of CH_2Cl_2 was added to this such that the internal temperature remained below −70 °C. The resulting reaction mixture was then stirred 10 min at −75 °C, and then 0.814 g (1.80 mmol) of compound **23**[1] in 10 mL of CH_2Cl_2 was added at such a rate as to maintain the temperature below −70 °C. After 20 min at −75 °C, 1.42 ml (10.2 mmol) of triethylamine was added, and the reaction mixture was warmed to room temperature. After 45 min, the reaction mixture was washed with dilute HCl, with water, and then with dilute $NaHCO_3$ and was then dried and evaporated. The residue was taken up in ether, filtered, and treated with a solution of HCl(g) in ether to give a solid. The ether was evaporated, and the residue was suspended in methanol and filtered to give **19**: 460 mg (52%); mp 265–267 °C.

***trans*-(±)-8-Fluoro-5-(4-fluorophenyl)-2-(4-hydroxybutyl)-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole Hydrochloride (20).** A suspension of the free base of compound **14** (500 mg, 1.7 mmol), ethyl 4-bromobutyrate (340 mg, 1.7 mmol), 1.1 g (10.5 mmol) of anhydrous Na_2CO_3, and a catalytic amount of KI in 50 mL of methyl isobutyl ketone was heated at reflux overnight. Solvent was removed from the reaction mixture, and the residues were partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2 solution was dried ($MgSO_4$) and evaporated to a yellow gum. This material was chromatographed on silica gel, eluting with ethyl acetate, giving the desired intermediate ester as a pale yellow gum in 72% yield.

A solution of 208 mg (0.48 mmol) of this product in dry ether (5 mL) was added dropwise to a suspension of 36 mg (0.96 mmol) of LAH in 5 mL of dry ether under N_2, and the resulting reaction mixture was stirred at room temperature for 2 h. Then, an excess of Glauber's salt was added, and the mixture was stirred until decomposition of excess LAH was complete. The solids were filtered and washed with dry ether, and then a solution of dry HCl(g) in ether was added to the filtrate to give compound **20**: 157 mg (82%); mp 220–223 °C.

***trans*-(±)-8-Fluoro-5-(4-fluorophenyl)-2-[4-(4-fluorophenyl)but-3-enyl]-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido[4,3-*b*]indole Hydrochloride (18).** A solution of 1.00 g (2.05 mmol) of compound **23** in 30 mL of 1-propanol and 10 mL of concentrated HCl was heated at reflux for 20 h at which time no starting material remained. The alcohol was evaporated from the reaction mixture, and the residual crystalline solid was suspended in EtOAc, filtered off, and washed with EtOAc. After air-drying, the product (**18**) weighed 1.475 g (76%), mp 270 °C dec.

Registry No. (±)-**1**, 103383-85-3; (±)-**1**·$C_4H_4O_4$, 103383-86-4; (±)-**2**, 103383-87-5; (±)-**2**·$C_4H_4O_4$, 103383-88-6; (±)-**3**, 103384-02-7; **3**(tetrahydro deriv.), 6208-47-5; (±)-**3**·HCl, 103383-89-7; (±)-**4** (isomer 1), 103475-87-2; (±)-**4** (isomer 2), 103475-91-8; (±)-**5** (isomer 1), 103529-66-4; (±)-**5** (isomer 2), 103475-92-9; (±)-**5**·HCl (isomer 1), 103475-88-3; (±)-**5**·HCl (isomer 2), 103529-93-7; (±)-**6**, 56757-37-0; (±)-**6**·HCl, 103383-90-0; (±)-**7**, 72650-63-6; (±)-**7**·HCl, 72650-62-5; (±)-**8**, 72650-88-5; (±)-**8**·HCl, 72650-64-7; (±)-**9**, 75738-62-4; (±)-**9**·HCl, 75738-61-3; (±)-**10**, 75738-69-1; (±)-**10**·HCl, 69623-14-9; **11**, 75738-65-7; (±)-**11**·HCl, 103383-91-1; **12**, 69623-12-7; (±)-**12**·HCl, 103383-92-2; (±)-**13**·HCl (isomer 1), 103475-89-4; (±)-**13**·HCl (isomer 2), 103476-87-5; (±)-**14**, 69623-07-0; (±)-**15**, 103384-03-8; (±)-**15**·HCl, 98634-82-3; (±)-**16**, 103384-09-4; (±)-**16**·HI, 103383-93-3; (±)-**17**, 103384-04-9; (±)-**17**·HCl, 103383-94-4; (±)-**18**, 103384-05-0; (±)-**18**·HCl, 103383-95-5; (±)-**19**, 103384-06-1; (±)-**19**·HCl, 103383-96-6; (±)-**20**, 103384-07-2; (±)-**20** (ethyl ester), 98634-64-1; (±)-**20**·HCl, 103383-97-7; **21**, 69645-82-5; (±)-**21**·HCl, 103383-98-8; **22**, 69645-81-4; **23**, 69623-05-8; (±)-**23**·HCl, 103383-99-9; **24**, 103384-08-3; (±)-**24**·HCl, 103384-00-5; (±)-**26** (isomer 1), 103475-90-7; (±)-**26** (isomer 2), 103475-93-0; (±)-**26**·HCl (isomer 1), 103384-01-6; (±)-**26**·HCl (isomer 2), 103529-67-5; 4-$FC_6H_4CO(CH_2)_2CO_2H$, 366-77-8; $Br(CH_2)_3CO_2CH_2CH_3$, 2969-81-5.