trans-10,11-Dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine: A Highly Potent Selective Dopamine D_1 Full Agonist

William K. Brewster,[†] David E. Nichols,*[,†] Robert M. Riggs,[†] David M. Mottola,[‡] Timothy W. Lovenberg,[‡] Mark H. Lewis,[§] and Richard B. Mailman[‡,§]

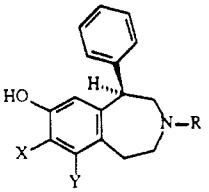
Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907, and Biological Sciences Research Center, Departments of Pharmacology and Psychiatry, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27599. *Received October 23, 1989*

trans-10,11-Dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine (**4a**, dihydrexidine) has been found to be a highly potent and selective agonist of the dopamine D_1 receptor in rat brain. Dihydrexidine had an EC_{50} of approximately 70 nM in activating dopamine-sensitive rat striatal adenylate cyclase and a maximal stimulation equal to or slightly greater than that produced by dopamine. Dihydrexidine had an IC_{50} of 12 nM in competing for [^{3}H]SCH23390 (**1a**) binding sites in rat striatal homogenate, and of 120 nM versus [^{3}H]spiperone. These data demonstrate that dihydrexidine has about ten-fold selectivity for D_1/D_2 receptors. More importantly, however, is the fact that dihydrexidine is a full agonist. Previously available agents, such as SKF38393 (**1b**), while being somewhat more selective for the D_1 receptor, are only partial agonists. The isomeric *cis*-dihydroxybenzo[*a*]-phenanthridine neither stimulated cAMP synthesis nor inhibited the cAMP synthesis induced by dopamine. The cis isomer also lacked appreciable affinity for [^{3}H]-**1a** binding sites. N-Methylation of the title compound decreased affinity for D_1 sites about 7-8-fold and markedly decreased ability to stimulate adenylate cyclase. Addition of an *N*-*n*-propyl group reduced affinity for D_1 sites by about 50-fold and essentially abolished the ability to stimulate adenylate cyclase. However, this latter derivative had twice the affinity of the D_2-selective agonist quinpirole for the D_2 receptor. The results are discussed in the context of a conceptual model for the agonist state of the D_1 receptor.

Dopamine neurotransmission is of physiological importance in both the central nervous system and the periphery. Not only do dopamine receptors mediate the actions of many psychotropic drugs, but dopamine has been hypothesized to play a role in several psychiatric and neurological illnesses, as well as in vascular regulation. Thus, ligands for dopamine receptors have use as basic research tools and potential therapeutic agents.

At present, the accepted classification of dopamine receptors is based on a hypothesis first proposed by Kebabian and Calne[1] in which these receptors are divided into two classes, D_1 and D_2. The D_1 class is linked biochemically to dopamine-mediated stimulation of cAMP synthesis, whereas the D_2 class may act via inhibition of cAMP synthesis or through less well-characterized mechanisms. It was the D_2 class that was originally believed to be the site of antipsychotic drug action, as well as most other important dopamine-mediated functional changes.[1,2] For this reason, until recently, most efforts in drug development were aimed at new ligands of the D_2 class.

While the role of D_1 receptors in the vasculature was clear, the availability of the selective D_1 antagonist **1a** led to an appreciation of new functional roles for central D_1 receptors, as well as the fact that D_1 and D_2 receptors could modulate each other.[3-10] Further investigation of such phenomena requires selective D_1 ligands. While various 1-phenyl-3-benzazepines (e.g., **1a**) are excellent antagonists,



1a R = CH_3, X = Cl, Y = H (SCH 23390)
1b R = Y = H, X = OH (SKF 38393)
1c R = $CH_2CH{=}CH_2$, X = OH, Y = Cl (SKF 82958)

the available agonist analogues are not entirely satisfactory. In general, these compounds are far less efficacious than dopamine in stimulating cAMP synthesis, and thus do not meet the biochemical criterion as full D_1 agonists. One member of this class, SKF 82958 (6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-3-benzazepine, **1c**), has been suggested to be a full agonist.[11] Unfortunately, there are two major confounds with SKF 82958. For one, the available data indicate that it causes 50% more stimulation of cAMP synthesis than does dopamine, suggesting that it may either act on several receptor systems or have other biochemical actions.[11] In addition, its potential utility is minimized by the fact that it is only slightly more potent than dopamine. It is clear, therefore, that a high-potency full D_1 agonist would be a powerful tool to study the many functional roles of D_1 receptors.

Our research has been directed toward the discovery and characterization of additional types of D_1 selective agents to study this important receptor subtype, and to use as tools to aid in describing its structure–activity relationships. Earlier, we reported on the selective dopamine D_1 agonist and antagonist properties of a series of substituted 4-phenyl-1,2,3,4-tetrahydroisoquinolines, e.g. **2**.[12-16] While

[†] Department of Medicinal Chemistry and Pharmacognosy, Purdue University.
[‡] Department of Pharmacology, University of North Carolina School of Medicine.
[§] Department of Psychiatry, University of North Carolina School of Medicine.

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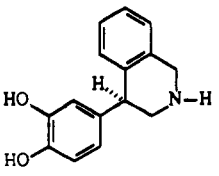

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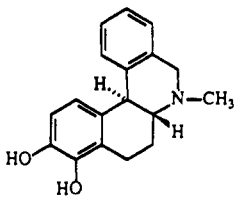
the resulting data were informative, these compounds are conformationally mobile. The search for rigid analogues, in which the conformation of the pharmacophore might be well-defined, led to a focus on the hexahydrobenzo[*a*]phenanthridines. A search of the literature revealed that the 9,10-dihydroxy compound **3** had been prepared



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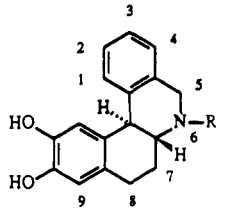
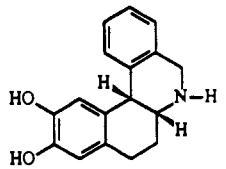


4a R = H
4b R = CH_3
4c R = n-C_3H_7



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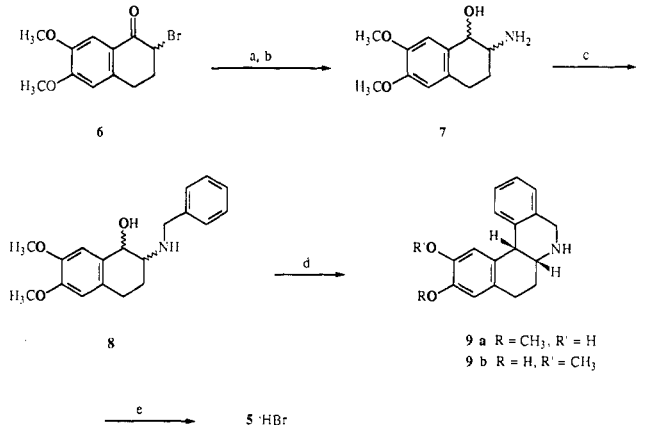
as an analogue of apomorphine but was reported to be inert at dopaminergic receptors.[17] This was not surprising, however, on the basis of the conceptual model of the dopamine D_2 receptor originally developed by McDermed[18] and later extended to include the D_1 receptor.[19]

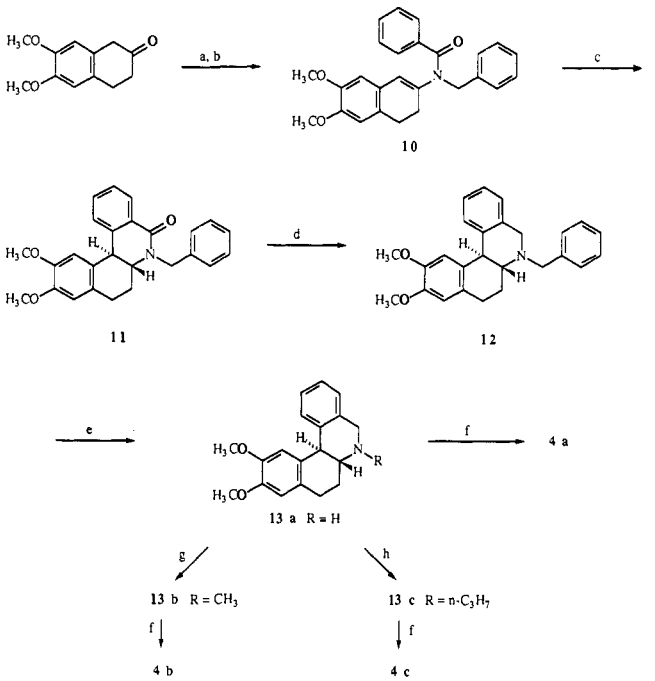
By contrast, using the McDermed-derived model as a basis, one could hypothesize that the 10,11-dihydroxy compound **4a** might be active. Compound **4a** is quite intriguing as a rigid analogue. Although we had designed it to be a congener of 4-phenyltetrahydroisoquinoline **2**, the molecular fragment used to do this, the C(7)–C(8) ethano bridge, is also a part of the potent dopamine agonist 6,7-dihydroxy-2-aminotetralin (ADTN).[20] Thus, this rigid analogue represents a sort of "hybrid" structure, which incorporates the elements of both the 4-phenyltetrahydroisoquinolines and the aminotetralins, with no apparent superfluous molecular bulk.

Scheme I[a]



[a] (a) NaN_3, DMF; (b) $LiAlH_4$, THF; (c) (i) PhCHO, CH_3CH_2OH, reflux, (ii) $NaBH_4$; (d) $AlCl_3$, CH_2Cl_2, reflux; (e) 48% HBr, reflux.

Scheme II[a]



[a] (a) $PhCH_2NH_2$, toluene, reflux; (b) PhCOCl, $N(CH_2CH_3)_3$, CH_2Cl_2; (c) *hν*, THF; (d) BH_3, THF; (e) H_2, 10% Pd-C, 95% CH_3CH_2OH; (f) 48% HBr, reflux; (g) CH_2O, $NaCNBH_3$, CH_3OH; (h) CH_3CH_2CHO, $NaCNBH_3$, CH_3OH.

This report describes the synthesis of compounds **4a–c**, as well as the cis-fused isomer **5** and details pharmacological studies which demonstrate that compound **4a**, to which we have given the generic name "dihydrexidine", is an extremely potent, full agonist at the D_1 receptor. Although **4a** is selective and potent at the D_1 receptor, it also possesses modest dopamine D_2 agonist activity.

Chemistry

Our initial, but unsuccessful, approach to the synthesis of compound **4** is outlined in Scheme I. Reductive amination of amino alcohol **7** with benzaldehyde afforded the *N*-benzylamine **8**. Treatment of this with $AlCl_3$ in CH_2Cl_2 at reflux provided a mixture of the partially O-demethylated isomers **9a** and **9b**. Assignment of the correct structures was based on NOE enhancement of the H(9) aromatic proton in the ^1H NMR, following irradiation of the *O*-methyl absorption in **9a**. However, instead of producing the desired trans-fused product, which would have

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Table I. Comparison of **4a** (Dihydrexidine) and Its Analogues and Certain Other Dopamine Agonists at D_1 and D_2 Receptors in Rat Striatal Membranes[a]

drug	IC_{50}, nM: D_1 potency	IC_{50}, nM: D_2 potency	adenylate cyclase EC_{50}, nM	max stimulation of adenylate cyclase, % vs DA
dopamine	800 (7)	1500 (7)	5000	100
4a	12 (7)	120 (4)	70	120
4b	91 (2)	136 (2)	1000	63
4c	651 (2)	53 (3)	$>10^4$	32
5	$>5 \times 10^3$ (2)	$>5 \times 10^3$ (3)	$>10^4$	17
1b	35 (6)	3750 (1)	100[b]	30–50
14	120 (3)	285 (2)	700	120
16	$>5 \times 10^3$ (2)	68 (2)	$>10^3$	33
17	$>5 \times 10^3$ (2)	290 (2)	$>10^4$	25
quinpirole	$>3 \times 10^4$ (1)	100 (5)		
1a	1 (3)	$>10^4$ (1)	0	0

[a] All tests were performed as described in the methods on rat striatal membranes, using [^{3}H]-**1a** as the D_1 ligand and [^{3}H]-spiperone as the D_2 ligand. The adenylate cyclase assay is as described by Schulz and Mailman.[47] Hill coefficients for the agonist binding curves were significantly less than 1. Therefore K_i values cannot be determined until the number of binding sites is resolved. To minimize interassay variability, the values in the tables were from assays in which four or more compounds were run on the same day. The number of determinations on separate days is listed in parentheses. [b] Concentration at which stimulation was $^1/_2$ of the maximal value of 50%.

led ultimately to compound **4a**, the small coupling constant (4.7 Hz) between H(6a) and H(12b) indicated that the cis-fused product had been obtained. These isomeric ethers, therefore, when treated with 48% HBr at reflux, yielded catechol **5**.

Our successful efforts to produce **4**, outlined in Scheme II, were based on the earlier work of Wei and Teitel.[17] In this approach, the β-tetralone was first condensed with benzylamine, and the resulting enamine was acylated with benzoyl chloride. Enamide **10** was then subjected to photocyclization conditions to afford tetracyclic lactam **11**. Diborane reduction, followed by catalytic N-debenzylation, led to the O,O-diprotected compound **13a**, which was treated with 48% HBr at reflux to cleave the O-methyl ethers. The HBr salt could not be induced to crystallize, but was instead converted to the hydrochloride, which was crystallized to yield the desired *trans*-**4a**. The coupling constant between H(6a) and H(12b) was 11.1 Hz, confirming the expected trans B/C ring fusion. The N-alkylated derivatives **4b** and **4c** were prepared by treatment of the secondary amine **13a** with formaldehyde or propionaldehyde and $NaCNBH_3$ to afford the tertiary amines **13b** and **13c**, which were then O-demethylated with 48% HBr at reflux.

N-Benzylaminotetralin **17** was prepared by condensation of benzylamine with 6,7-dimethoxy-β-tetralone. The resulting enamine was reduced by low pressure hydrogenation over Pt–C in ethanol, followed by cleavage of the O-methyl ethers.

Pharmacology

All compounds were tested for the ability to stimulate adenylate cyclase from rat striatum. In addition, the ability to displace either [^{3}H]-**1a** or [^{3}H]-spiperone from binding sites in rat striatum was used to measure affinity for the D_1 or D_2 receptor subtype, respectively.

Results and Discussion

The data characterizing the pharmacology of compounds **4** and **5** are summarized in Table I and Figure 1. Dopamine and the partial dopamine D_1 agonist **1b**[21] are included for comparison. The racemic trans compound **4a** produced a full agonist response against dopamine-sensitive adenylate cyclase and was nearly 2 orders of magnitude more potent than dopamine. In our comparisons, **4a** is fully 10-fold more potent than the full agonists [4-

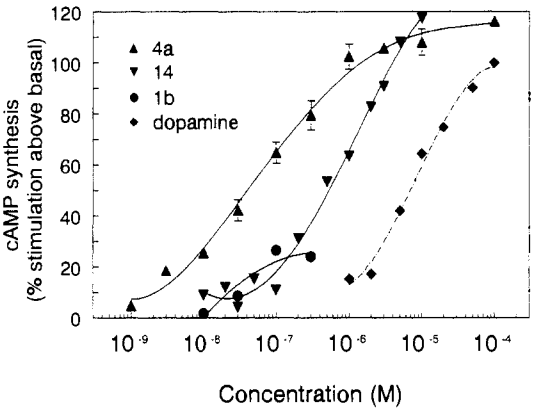
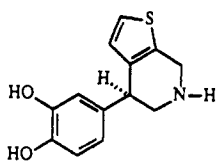


Figure 1. Dose–response curves for stimulation of rat striatal adenylate cyclase. Cyclase activation was measured in rat striatal membranes by the ability of the compounds to convert [α-^{32}P]ATP to [^{32}P]cAMP (see the Experimental Section). This graph shows that **4a** (▲) is a potent, full efficacy agonist, with comparisons to dopamine (◆), **1b** (●), and **14**[13,22] (▼).

(3,4-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline (**2**) and its thiophene bioisostere **14**,[13,22]] both in competing



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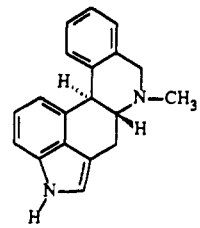

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for **1a** binding sites and in stimulating cAMP synthesis in striatal homogenates. In this regard, **4a** has the potential to be a powerful tool to explore D_1 receptor function. The prototypical D_1 agonists of the benzazepine series (e.g. **1b** and fenoldopam) are only partial agonists (see Figure 1) as is benzergoline CY 208-243 (**15**).[23-25] Thus, as can be seen in Figure 1, **1b** produces less than 50% stimulation

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of cyclase. The racemic cis isomer **5** was ineffective in stimulating cAMP synthesis.

A similar pattern was seen when competition for D_1 and D_2 antagonist binding sites was examined. As shown in Table I, **4a** has about 3 times higher affinity for [^{3}H]-**1a**-labeled sites than does **1b**. The IC_{50} for **4a** of 120 nM at D_2 sites is comparable to that observed with the selective and potent D_2 agonist quinpirole. Thus, **4a** demonstrates about a 10-fold selectivity for D_1 versus D_2 sites. In preliminary behavioral studies, subcutaneous injection of 1 mg/kg of **4a** into either control or 6-hydroxydopamine lesioned rats led to extensive bouts of grooming, as well as vacuous chewing. Although these behaviors are characteristic of D_1 receptor activation, some involvement of a D_2 component cannot be ruled out.

In a preliminary communication of this work,[26] we reported that **4a** had more than 60-fold selectivity for D_1 vs D_2 receptors. As can be seen in Table I, the true selectivity is only 10-fold. In that preliminary communication we inadvertently used HEPES buffer with 4 mM $MgCl_2$ for the D_1 studies, and HEPES buffer with 0.1 mM $MgCl_2$ for the D_2 studies.[26] The IC_{50} of agonists in competing for D_1 or D_2 antagonist binding sites is markedly decreased in buffer containing 4 mM $MgCl_2$. Thus, an incorrect estimate of selectivity was obtained in our earlier work. The present data, obtained with 4 mM $MgCl_2$ in both assays, accurately reflect the relative potencies of these drugs.

We have also recently reported[27] that competition binding assays of **4a** at [^{3}H]-**1a** labeled sites gave indirect Hill plots with pseudo Hill coefficients of about 0.7, and shallow competition curves suggestive of binding to multiple D_1 sites. Nonlinear regression analysis of competition curves using LIGAND gave a significant fit for a two-site model, with a K_H of 0.5–3 nM and a K_L of about 50–100 nM. While addition of guanine nucleotides caused a decrease in potency, they did not appreciably change the Hill coefficients. These data are consistent with the hypothesis we have proffered that there are multiple D_1 dopamine receptors,[28] although they do not provide direct evidence for it.

To examine the probable conformations of **4a** and **5**, computer modeling was done and energy-minimized structures were generated with the CHARMm software,[29] implemented on an IRIS 3030 workstation. Stereopair representations of **4a** and **5** are shown in Figure 2, parts A and B, respectively. Although we have so far only assayed racemic **4a**, the figure illustrates what is anticipated to be the more active enantiomer of **4a** (6a*R*,12b*S*), on the basis of the known stereochemistry of *R*-(+)-6,7-ADTN.[18]

If binding occurs to the α (underside) face of the molecule, the unsubstituted phenyl in *trans*-**4a** lies slightly above the plane of the catechol ring. There are several low-energy conformations for **4a**, all within about 2 kcal/mol of each other. The lowest energy of these is illustrated in Figure 2A, with the B ring in a pseudochair conformation, the C ring in a pseudoboat conformation, and defined by the torsional angles $\tau_{12,12a,12b,12c} = 42.8°$ and $\tau_{12a,12b,12c,1} = 0°$. The ethylamine fragment in this conformer approaches an antiperiplanar relationship to the

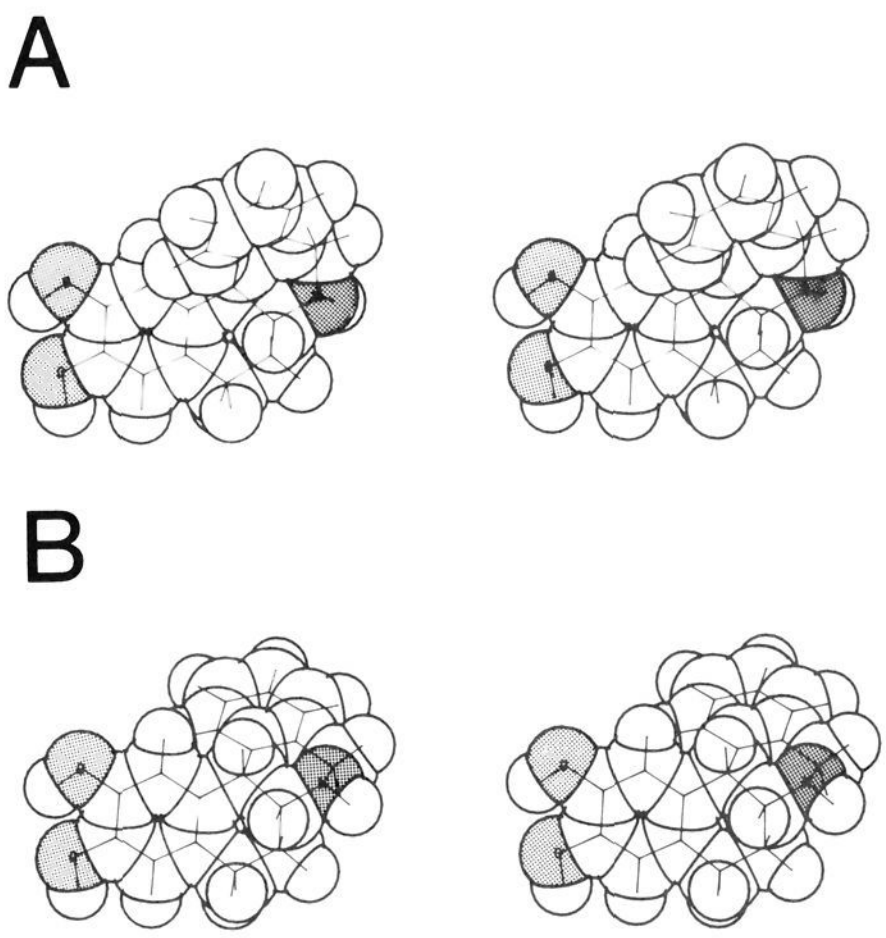

Figure 2. Stereopair representations of the low-energy conformations of (A) **4a** and (B) **5**. The aromatic D ring is slightly above the catechol ring in **4a**, but is projected below, in a plane nearly orthogonal to the catechol ring plane in **5**. The nitrogen and oxygen atoms have been shaded.

catechol ring, with $\tau_{12a,12b,6a,6} = 170.2°$. This conformation allows the N(6) electron pair to adopt either a pseudoequatorial or an α-pseudoaxial orientation. A second low-energy conformation exists for **4a** within about 0.5 kcal/mol, with the C ring in a pseudochair conformation, where the unsubstituted phenyl ring projects slightly below the plane of the catechol ring, with $\tau_{12,12a,12b,12c} = 6°$, and $\tau_{12a,12b,12c,1} = -59°$.

By contrast, in *cis*-**5**, when C(6a) has the *R* absolute configuration (homochiral with *R*-(+)-6,7-ADTN), the unsubstituted phenyl ring projects downward, toward the α-face of the molecule, with the two aromatic ring planes at nearly right angles. The torsion angles defining this conformation are $\tau_{12,12a,12b,12c} = -80.5°$ and $\tau_{12a,12b,12c,1} = 33.5°$. The ethylamine fragment of the embedded dopamine moiety is again nearly antiperiplanar ($\tau_{12a,12b,6a,6} = 177°$), and the nitrogen lone pair can adopt a pseudoequatorial or a β-pseudoaxial orientation. The dramatically different shape for **5**, however, suggests that steric effects may account for its lack of activity.

A number of workers have described the probable importance of the amine lone pair directionality for optimal dopamine receptor binding. Nichols[19] first proposed that the lone pair or the N–H vector in D_1 agonists might preferentially reside in a pseudoequatorial orientation, while the lone pair in D_2 agonists might prefer to orient pseudoaxially. While evidence seems to be accumulating to support this notion, at least for D_2 agonists (e.g., Tonani et al.[30] and Froimowitz and Baldessarini[31]), there is as yet no clear evidence for a specific electron-pair orientation for D_1 agonists. Tonani et al.[30] do suggest that it may differ for D_1 and D_2 agonists. The strongest previous evidence was the progressive loss of D_1 activity upon N-alkylation

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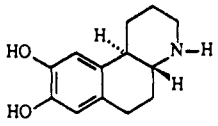
of **2**, where an *n*-propyl substitution nearly abolished activity.[12] It would be expected that the larger *N*-alkyl group would adopt the pseudoequatorial orientation, while the electron pair would be forced pseudoaxial. In the secondary amine or the *N*-methyl tertiary amine, the barrier to nitrogen inversion would be relatively small.

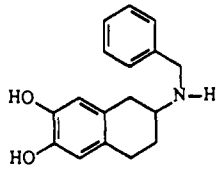
Analogous reasoning can be applied to the activity of **4a** and its N-alkylated derivatives **4b** and **4c**. Thus, the high D_1 activity of compound **4a**, which is also a secondary amine, the attenuated potency of its N-methyl derivative **4b**, and the dramatic loss of activity in its N-*n*-propyl derivative **4c** (Table I) all provide additional support for this idea.

The necessity for a relatively planar structure for D_2 receptor activation has already been demonstrated by Cannon et al.[32] for the structurally related benzo[*f*]-quinolines, where the trans, but not the cis, compounds were active. It may be that the D_1 receptor has a similar requirement.

The selectivity for the D_1 receptor appears to be largely attributable to the addition of the unsubstituted aromatic ring. We confirmed this by resynthesis and biological evaluation of compound **16**.[32] Radioligand binding assays



16



17

against [^{3}H]-**1a** and [^{3}H]spiperone (Table I) clearly show that the additional phenyl ring in **4a** not only imparts selectivity for the D_1 receptor but also *increases* affinity for this site. Indeed, the "fusion" of the aromatic D ring to **16** to afford **4a** results in a more than 400-fold increase in affinity for the D_1 site.

It is clear that this phenyl ring must interact with some important accessory binding site on the D_1 receptor. The influence of this feature is also evident in the novel D_1-selective benzergoline partial agonist (**15**).[23-25] This structure is an ergoline which has a phenyl ring "fused" onto the ergoline 8,9-positions. The molecule lacks a catechol function, yet still demonstrates significant D_1 agonist activity, although it is only a partial agonist. It also possesses significant in vivo D_1 agonist activity, although metabolism of the indole ring would lead to a hydroxylated species that might be expected to have higher dopaminergic activity than the parent molecule.

However, simply appending an *N*-benzyl to 6,7-ADTN to give **17** did not result in a compound with significant affinity for the D_1 receptor (Table I). Our earlier work did show that β-phenyl dopamine was a more potent agonist than was β-methyldopamine,[33] probably indicating that the β-phenyl moiety must be tethered in closer proximity to the catechol ring.

Although the aromatic D ring increases affinity for the D_1 receptor, it may also be acting as a spacer, or "wedge", that can be accommodated by the D_1, but not the D_2 receptor. Furthermore, the lack of activity for **17** and low potency of β-phenyldopamine[33] show that for optimal activity the phenyl ring must be held in a particular orientation by incorporation into a relatively rigid framework. Since the D_1 receptor can be presumed to be conformationally mobile, the insertion of the β-phenyldopamine moiety into the receptor may also facilitate the orientation of the amine binding site of the receptor; this relates to the postulated requirement for a pseudoequatorial electron pair on the basic nitrogen.

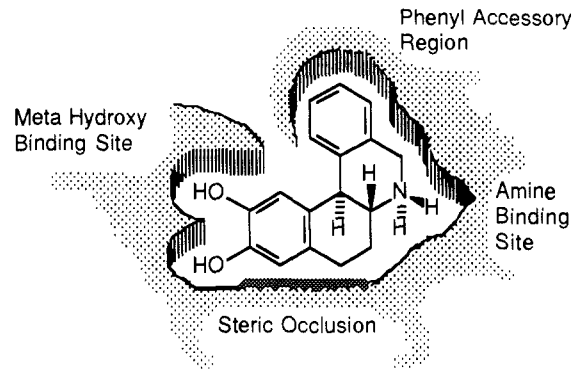


Figure 3. Schematic representation of the agonist-complementary D_1 receptor. The phenyl accessory binding site is envisioned as approaching coplanarity with the catechol ring binding site. A binding site for the more critical "meta" hydroxy is illustrated, but for D_1 agonists a requirement for both catechol hydroxys seems apparent. The steric boundary originally incorporated in the McDermed model[18] is included. It is proposed that the electron pair on the nitrogen, or the N–H vector in the protonated form, must occupy a pseudoequatorial orientation for agonist binding. Although the absolute configuration for the more active enantiomer of 4a has not been determined, the illustrated stereochemistry is based on analogies to known enantiomerically pure agonists.

Of particular significance is the fact that **1b** is only a partial agonist whereas **4a** is a full agonist. This suggests either that the ethylamine moiety within the structure should be in a trans extended conformation or that the two aromatic rings should be able to more nearly approach coplanarity than is possible in **1b**. It is clear that the two phenyl rings, in terms of the centroid distances and the relative angle of the ring planes, should have a specific orientation that may be critical both for D_1 affinity and for full agonist efficacy. Specifically, changes in the orientation of the phenyl rings may be sufficient to cause loss of agonist properties. Thus, our tentative conceptual working model for the agonist state of the D_1 receptor, complementary to the new agonist **4a**, is shown in Figure 3.

Although this model generally incorporates features of earlier models, a major finding of significance in the present work is the near coplanarity of the "β-phenyl" moiety. We had earlier proposed that a hydrophobic or accessory region existed on the receptor to accommodate this phenyl ring, and approximately in the plane of the catechol ring.[19] On the other hand, Kaiser and co-workers[34-36] have speculated, on the basis of the low-energy conformations of **1b** and of the 8-amino derivative of **2**, that this accessory site may be orthogonal to the catechol ring. However, because **1b** is only a partial agonist and because **4a** (a full agonist) has a relatively rigid geometry, it now seems reasonable to conclude that this accessory site prefers the rings to be closer to coplanarity than in

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1b. If the complex pharmacology of **1c**[11] is ultimately shown to be due solely to agonist effects on D_1 receptors (vide supra), this compound may provide further insight into this model.

By contrast, for dopamine D_1 antagonists, it may well be that the appended phenyl ring should preferentially be twisted out of the plane of the substituted aryl moiety. Further, the conformation of **1a**, or the recently reported more conformationally rigid SCH 39166,[37,38] may indicate that a nonplanar, more cisoid ethylamine fragment is optimal for antagonist activity.

On the basis of current models of the dopamine D_2 receptor, it is possible to identify certain similarities and differences between the D_1 and D_2 receptors. With respect to the catechol ring, the D_1 receptor requires both catechol hydroxy groups for maximal activation, while the D_2 receptor is much less demanding, being fully activated by a single hydroxy or even by hydroxy isosteres such as an indole N(1)H, a sulfonamido, or an acetamido group. In addition, it appears that the D_1 receptor may prefer the dopamine moiety to be in the so-called "β-rotameric" form for optimal activity, while the more potent D_2 receptor agonists generally incorporate an "α-rotameric" dopamine element.

The dopamine D_2 receptor also can accommodate large *N*-alkyl groups, including a β-phenethyl moiety. Indeed, addition of the *N*-*n*-propyl group to give **4c** increases D_2 affinity by a factor of 2–3 (Table I). The receptor models described by Liljefors and Wikstrom[39] and extended by Van de Waterbeemd et al.[40] describe a postulated receptor geometry that can accommodate these substituents. It seems possible that the "cleft" described by these workers to accommodate large *N*-alkyl groups on the D_2 receptor may in fact be structurally similar to the hydrophobic accessory site on the D_1 receptor, but which has become topographically displaced through evolution.

Because of the differences that exist between any of the proposed pharmacophoric maps of D_1 and D_2 receptors, it might have been predicted that a potent and full D_1 agonist might also be very selective. Therefore, it is noteworthy that the selectivity of dihydrexidine for D_1 versus D_2 binding sites is only 1 order of magnitude. While it is true that this is greater selectivity than is seen for the other full agonist **14** (Table I), this will require that D_2 activity be accounted for in pharmacological studies with dihydrexidine.

The possibility does exist that resolution of the enantiomers of dihydrexidine (work now underway) may reveal that the D_1 and D_2 activities reside in opposite antipodes. Should this prove to be the case, it would provide not only a selective high-potency full agonist, but also a new class of D_2 agonist with potency at least comparable to the present prototype, quinpirole.

However, if both the D_1 and D_2 activities are possessed by the same enantiomer, dihydrexidine becomes of particular interest as a possible antiparkinsonian drug. In lesion models of brain dopamine systems, it is generally accepted that the normal functional interaction of D_1 and D_2 receptors[10] is disrupted.[4,6,41-43] It may be hypothesized that an agent with simultaneous high affinity for both classes of dopamine receptors would be more efficacious pharmacotherapy than would an agent of similar potency toward only one class of dopamine receptor. Although dihydrexidine is modestly selective toward the D_1 receptor, it is still nearly as potent as quinpirole at D_2 receptors.

In summary, we have developed a new selective dopamine D_1 agonist that has high potency and full agonist activity. The relatively rigid nature of this compound has allowed a further refinement of the structure–activity requirements for D_1 receptor activation. We anticipate that further studies of dihydrexidine (**4a**) and its congeners will lead to significant advances in our knowledge of the functional importance of the dopamine D_1 receptor(s).

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian FT-80, Chemagnetics A200, or Varian VXR-500S NMR instrument in $CDCl_3$ with TMS as an internal standard, except as noted. High-resolution mass spectra were obtained on a Kratos MS50 and chemical ionization mass spectra were obtained on a Finnigan 4000. Elemental analyses were performed by the Purdue University microanalysis laboratory and are within ±0.4% of the calculated values.

***cis*- and *trans*-2-Amino-6,7-dimethoxy-1,2,3,4-tetrahydronaphthalen-1-ol (7).** A solution of bromo ketone **6**[44] (3.6 g, 12.6 mmol) in 50 mL of DMF plus 1 mL of water was cooled to 0 °C and a solution of 2.10 g (37.8 mmol) of NaN_3 in 10 mL of water was added dropwise over 2 min. The reaction was stirred under N_2 for 0.5 h, then poured into ice water. The product was extracted into CH_2Cl_2, the organic extract was washed with water and dried ($MgSO_4$). After filtration and solvent removal, the residue was dissolved in 40 mL of THF and added to a suspension of 1.2 g (31.5 mmol) of $LiAlH_4$ in THF. The reaction was stirred at reflux for 1 h and was then decomposed by dropwise addition of 3.6 mL of water. The alumina salts were removed by filtration. The solvent was removed by rotary evaporation. The basic residue was dissolved in ether and the ether was extracted with 3 × 30 mL of 1% acetic acid. This aqueous extract was basified with concentrated NH_4OH and extracted with 3 × 30 mL of CH_2Cl_2. After drying ($MgSO_4$) and filtration, the solvent was removed by rotary evaporation. The crude free base was crystallized from THF–hexane to yield 1.03 g (37% from bromo ketone): mp 118–122 °C (lit.[45] mp 135 °C, cis; mp 101 °C, trans); ^{1}H NMR ($CDCl_3$) δ 6.93 (s, 1, ArH), 6.58 (s, 2, ArH), 4.47 (d, 1, cis-CH, J = 3.9 Hz), 4.40 (d, 1, trans-CH, J = 6.7 Hz; integration gave 82:18 cis/trans), 3.88, 3.84 (2 s, 6, OCH_3), 3.18 (m, 1, CH), 2.79 (m, 2, CH_2), 1.91 (s, 2, NH, OH), 1.80 (m, 2, CH_2).

***cis*- and *trans*-*N*-Benzyl-2-amino-6,7-dimethoxy-1,2,3,4-tetrahydronaphthalen-1-ol (8).** Amino alcohol **7** (0.57 g, 2.55 mmol) and 0.3 g (2.81 mmol) of benzaldehyde in 15 mL of EtOH were stirred at reflux under N_2 for 4 h. Sodium borohydride (0.2 g, 5.2 mmol) was added and the mixture was stirred overnight at room temperature. The reaction was concentrated to dryness and the residue was stirred with 25 mL of ether. The ether was extracted several times with 1% acetic acid. Following basification of the aqueous acidic extract with concentrated NH_4OH, the base was extracted into CH_2Cl_2. The organic extract was dried ($MgSO_4$), filtered, and concentrated under vacuum to afford 0.78 g (95%) of a viscous oil: ^{1}H NMR ($CDCl_3$, free base) δ 7.33 (m, 5, ArH), 6.95 (s, 1, ArH), 6.57 (s, 1, ArH), 4.62 (d, 1, cis-CH, J = 3.8 Hz), 4.40 (d, 1, trans-CH, J = 7.9 Hz, ca. 18%), 3.90 (s, 2, CH_2), 3.87, 3.83 (2 s, 6, OCH_3), 3.05 (m, 1, CH), 2.92–2.69 (m, 2, CH_2), 2.31–1.46 (m, 4, CH_2, NH, OH). Conversion to the HCl salt and

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crystallization gave the following: mp 193–195 °C; MS calcd for $C_{19}H_{23}NO_3$ 313.1678, found 313.1682.

***cis*-10(11)-Hydroxy-11(10)-methoxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrochlorides (9a and 9b).** A solution of 0.78 g (2.5 mmol) of *N*-benzylamines **8** in 20 mL of CH_2Cl_2 was added dropwise over 10 min to a stirring suspension of 1.0 g (7.5 mmol) of $AlCl_3$ in 60 mL of CH_2Cl_2 under N_2. The reaction was heated at reflux for 2.5 h and then poured onto 100 g of crushed ice. The aqueous mixture was made basic with 5 N NaOH, the two phases were carefully separated, and the aqueous phase was extracted with 2 × 50 mL of ethyl acetate. The combined organic phases were washed with saturated NaCl and dried ($MgSO_4$). TLC analysis indicated two major products, with R_f = 0.3 (**9a**) and R_f = 0.2 (**9b**) (silica gel, CH_2Cl_2–NH_3 atmosphere). The two compounds were separated by centrifugal thin-layer chromatography on a silica rotor using the same developing solvent. The purified bases were converted to their hydrochloride salts and recrystallized from EtOH–ether to afford 174 mg (22%) of **9a**, mp 217–219 °C, and 155 mg (19%) of **9b**, mp 280–283 °C. Both compounds had (M + 1) = 282 in the CIMS.

9a: ^{1}H NMR (DMSO-d_6, HCl salt) δ 8.90 (s, 1, ArOH), 7.33 (m, 3, ArH), 7.19 (m, 1, ArH), 6.74 (s, 1, ArH(12)), 6.59 (s, 1, ArH(9)), 4.32 (d, 1, C_5Ha), 4.20 (d, 1, C_5Hb), 4.18 (d, 1, CH), 3.98 (m, 1, CH), 3.74 (s, 3, OCH_3), 2.76 (m, 2, CH_2), 2.10 (m, 1, C_7Ha), 1.73 (m, 1, C_7Hb). NOE experiments were carried out on the free base, dried under high vacuum for 24 h. A $CDCl_3$ solution was degassed with N_2 for 10 min. Irradiation of the absorption at δ 3.74 gave a 2.7% enhancement of ArH(12) and 14.9% enhancement of ArH(9); MS calcd for $C_{18}H_{19}NO_2$ 281.1416, found 281.1415.

9b: ^{1}H NMR (DMSO-d_6, HCl salt) δ 9.02 (s, 1, ArOH), 7.29 (m, 3, ArH), 7.20 (m, 1, ArH), 6.76 (s, 1, ArH(12)), 6.59 (s, 1, ArH(9)), 4.31 (d, 1, C_5Ha), 4.23 (dd, 2, C_5Hb, CH), 3.94 (m, 1, CH), 3.71 (s, 3, OCH_3), 2.70 (m, 2, CH_2), 2.04 (m, 1, C_7Ha), 1.68 (m, 1, C_7Hb). Anal. ($C_{18}H_{20}ClNO_2$) C, H, N.

***cis*-10,11-Dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrobromide (5).** Amine salts **9a** or **9b** (0.14 g, 0.44 mmol) in 1.5 mL of 48% HBr were heated at reflux under N_2 for 2 h. After removal of the excess HBr by rotary evaporation, the residue was recrystallized from EtOH to yield 106 mg (69%): mp 277–279 °C; CIMS (M + 1) 268; ^{1}H NMR (DMSO-d_6) δ 8.89, 8.80 (2 s, 2, ArOH), 7.36–7.30 (m, 3, ArH), 7.26–7.10 (m, 1, ArH), 6.56 (s, 1, ArH), 6.53 (s, 1, ArH), 4.27 (dd, 2, CH_2), 4.14 (d, 1, CH, J = 4.7 Hz), 3.95 (m, 1, CH), 2.68 (m, 2, CH_2), 2.07 (m, 1, C_7Ha), 1.70 (m, 1, C_7Hb). Anal. ($C_{17}H_{18}BrNO_2$) C, H, N.

***N*-Benzoyl-*N*-benzyl-6,7-dimethoxy-3,4-dihydro-2-naphthylamine (10).** To a solution of 4.50 g (21.8 mmol) of 6,7-dimethoxy-β-tetralone in 100 mL of toluene was added 2.46 g (23 mmol) of benzylamine. The reaction was heated at reflux overnight under N_2 with continuous water removal. The reaction was cooled, and the solvent was removed by rotary evaporation to yield the crude *N*-benzyl enamine as a brown oil.

This residue was dissolved in 80 mL of CH_2Cl_2. To this was added 2.43 g (24 mmol) of triethylamine, and the solution was cooled in an ice bath. Benzoyl chloride (3.37 g, 24 mmol) was then dissolved in 15 mL of CH_2Cl_2 and this solution was added dropwise to the cold stirring *N*-benzyl enamine solution. After complete addition the reaction was allowed to warm to room temperature and was left to stir overnight. The mixture was then washed successively with 2 × 50 mL of 5% aqueous HCl, 2 × 50 mL of 1 N NaOH, saturated NaCl solution, and was then dried over $MgSO_4$. After filtration through silica, the filtrate was concentrated under vacuum. Crystallization from diethyl ether gave 5.6 g (64%) of the enamide: mp 119–120 °C; IR (KBr) 1620 cm^{-1}; CIMS (isobutane): M + 1 400; ^{1}H NMR ($CDCl_3$) δ 7.64 (m, 2, ArH), 7.33 (m, 8, ArH), 6.52 (s, 1, ArH), 6.38 (s, 1, ArH), 6.05 (s, 1, ArCH), 4.98 (s, 2, $ArCH_2N$), 3.80 (s, 3, OCH_3), 3.78 (s, 3, OCH_3), 2.47 (t, 2, CH_2, J = 8.1 Hz), 2.11 (t, 2, CH_2, J = 8.1 Hz). Anal. ($C_{26}H_{25}NO_3$) C, H, N.

***trans*-6-Benzyl-10,11-dimethoxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridin-5-one (11).** A solution of 3.14 g (7.85 mmol) of the 6,7-dimethoxy enamide prepared above, in 300 mL of THF, was introduced into an Ace Glass 250-mL photochemical reactor. This solution was stirred and irradiated for 5 h with a 450-W Hanovia medium-pressure, quartz, mercury-vapor lamp seated in a water-cooled, quartz immersion well. The solution was concentrated in vacuo and crystallized from ether to provide 1.35 g (42.9%) of the 10,11-dimethoxylactam: mp 183–186°; IR (KBr) 1655, 1640 cm^{-1}; CIMS (isobutane) M + 1 400; ^{1}H NMR ($CDCl_3$) δ 8.19 (m, 1, ArH), 7.52 (m, 1, ArH), 7.46 (m, 2, ArH), 7.26 (m, 5, ArH), 6.92 (s, 1, ArH), 6.63 (s, 1, ArH), 5.35 (d, 1, $ArCH_2N$, J = 16.0 Hz), 4.78 (d, 1, ArCHN, J = 16.0 Hz), 4.37 (d, 1, Ar_2CH, J = 11.3 Hz), 3.89, 3.88 (2 s, 6, OCH_3), 3.80 (m, 1, CHN), 2.67 (m, 2, $ArCH_2$), 2.25 (m, 1, CHCN), 1.75 (m, 1, CHCN). Anal. ($C_{26}H_{25}NO_3$) C, H, N.

***trans*-6-Benzyl-10,11-dimethoxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrochloride (12).** A solution of 1.20 g (3 mmol) of the lactam prepared above, in 100 mL of dry THF, was cooled in an ice–salt bath and 6.0 mL of a 1 M solution of BH_3 in THF was added via a syringe. The reaction was heated at reflux overnight. Water (10 mL) was added dropwise, and the reaction mixture was concentrated by distillation at atmospheric pressure. The residue was stirred with 50 mL of toluene, 1.0 mL of methane sulfonic acid was added, and the mixture was heated with stirring on the steam bath for 1 h. The mixture was diluted with 40 mL of water and the aqueous layer was separated. The toluene was extracted several times with 6 N HCl, and the aqueous layers were combined. After basification of the aqueous phase with concentrated ammonium hydroxide, the free base was extracted into 5 × 25 mL of CH_2Cl_2. This organic extract was washed with saturated NaCl solution, and dried over $MgSO_4$. After filtration, the organic solution was concentrated under vacuum; the residue was taken up into ethanol and carefully acidified with concentrated HCl. After drying several times by azeotropic distillation of ethanol, the product was crystallized from ethanol to afford 0.97 g (76.5%) of the salt: mp 235–237 °C; CIMS (NH_3) M + 1 386; ^{1}H NMR ($CDCl_3$, free base) δ 7.37 (m, 9, ArH), 6.89 (s, 1, ArH), 6.74 (s, 1, ArH), 4.07 (d, 1, Ar_2CH, J = 10.7 Hz), 3.90 (s, 3, OCH_3), 3.82 (m, 2, $ArCH_2N$), 3.79 (s, 3, OCH_3), 3.52 (d, 1, ArCHN, J = 15.3 Hz), 3.30 (d, 1, ArCHN, J = 13.1 Hz), 2.86 (m, 2, CHN, ArCH), 2.30 (m, 2, ArCH, CHCN), 1.95 (m, 1, CHCN). Anal. ($C_{26}H_{28}ClNO_2$) C, H, N.

***trans*-10,11-Dimethoxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrochloride (13a).** A solution of 201 mg (0.48 mmol) of the 6-benzyl hydrochloride salt prepared above in 50 mL of 95% ethanol containing 50 mg of 10% Pd–C catalyst was shaken at room temperature under 50 psig of H_2 for 8 h. After removal of the catalyst by filtration, the solution was concentrated to dryness under vacuum and the residue was recrystallized from acetonitrile to afford 119 mg (75%) of crystalline salt: mp 243–244 °C; CIMS (NH_3) M + 1 296; ^{1}H NMR ($CDCl_3$, free base) δ 7.46 (d, 1, ArH, J = 6.1 Hz), 7.24 (m, 3, ArH), 6.91 (s, 1, ArH), 6.74 (s, 1, ArH), 4.09 (s, 2, $ArCH_2N$), 3.88 (s, 3, OCH_3), 3.78 (m, 4, OCH_3, Ar_2CH), 2.87 (m, 3, CHN, $ArCH_2$), 2.17 (m, 1, CHCN), 1.61 (m, 2, NH, CHCN). Anal. ($C_{19}H_{22}ClNO_2$) C, H, N.

***trans*-10,11-Dimethoxy-6-methyl-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrochloride (13b).** A solution of 144 mg (0.434 mmol) of amine hydrochloride **13a**, 0.22 mL (2.71 mmol) of 37% formalin solution, and 112 mg (1.69 mmol) of 95% sodium cyanoborohydride in 4 mL of methanol was stirred overnight at room temperature. After removal of the volatiles in vacuo, the residue was partitioned between 5% HCl and ether. The layers were separated, the aqueous layer was washed once more with ether, and the organic fractions were discarded. The aqueous layer was made basic with concentrated NH_4OH, and the alkylated amine was then extracted into 3 × 30 mL of dichloromethane which was dried ($MgSO_4$) and then filtered. The solvent was removed in vacuo to give the free base as a colorless oil. This was dissolved in absolute ethanol, acidified with concentrated HCl, and dried by azeotropic distillation (ethanol) of residual water. The salt was crystallized from acetonitrile–ether to provide 95 mg (63.3% yield) of pale yellow crystals: mp 209–210 °C, CIMS (NH_3) M + 1 310; ^{1}H NMR ($CDCl_3$, free base) δ 7.39 (m, 1, ArH), 7.18 (m, 3, ArH), 6.83 (s, 1, ArH), 6.74 (s, 1, ArH), 3.97 (m, 2, ArCH), 3.88, 3.75 (2 s, 6, OCH_3), 3.54 (d, 1, ArCH, J = 14.7 Hz), 2.92 (m, 2, $ArCH_2$), 2.39 (s, 3, NCH_3), 2.08 (m, 2, CHN, CHCN), 1.89 (m, 1, CHCN). Anal. ($C_{20}H_{24}ClNO_2$) C, H, N.

***trans*-10,11-Dimethoxy-6-*n*-propyl-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrochloride (13c).** A solution of 133 mg (0.40 mmol) of amine hydrochloride **13a**, 150 mg (2.56 mmol) of propionaldehyde, and 104 mg (1.57 mmol) of sodium cyanoborohydride in 3.5 mL of methanol was stirred overnight and the product was isolated as described for **13b**.

Following acidification of the free base, the amine hydrochloride was recrystallized from THF–ether to provide 91 mg (60.7% yield) of pale yellow crystals: mp 142–143 °C; CIMS (NH_3) M + 1 338; ^{1}H NMR ($CDCl_3$, free base) δ 7.37 (m, 1, ArH), 7.24 (m, 3, ArH), 6.86 (s, 1, ArH), 6.73 (s, 1, ArH), 4.08 (d, 1, ArCHN, J = 15.3 Hz), 3.88 (m, 4, ArCH, OCH_3), 3.78 (s, 3, OCH_3), 3.66 (d, 1, ArCHN, J = 15.3 Hz), 2.83 (m, 2, $ArCH_2$), 2.59 (m, 1, CHN), 2.26 (m, 3, CH_2N, CHCN), 1.87 (m, 1, CHCN), 1.60 (m, 2, 2 x CH), 1.00 (m, 3, CH_3). Anal. ($C_{22}H_{28}ClNO_2$) C, H, N.

***trans*-10,11-Dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrochloride (4a).** A suspension of 580 mg (1.75 mmol) of *O,O*-dimethyl salt **13a**, in 10 mL of 48% HBr, was heated at reflux under N_2, for 3 h. The reaction mixture was concentrated to dryness under high vacuum. This material was dissolved in 50 mL of water and was neutralized to the free base with $NaHCO_3$, under N_2, while the solution was cooled in an ice bath. The free base, a white solid, was removed by suction filtration and washed with cold water. The filtrate was extracted with dichloromethane and ether; the organic extracts were combined, dried, filtered, and concentrated in vacuo. The residue was combined with the filter cake, dissolved in ethanol, and carefully acidified with concentrated HCl. After removal of the volatiles, the salt was recrystallized from methanol (20 mL). The colorless crystals were collected by suction filtration and washed successively with cold 2-propanol, ethyl acetate and ether to afford 340 mg (57.9%) of the desired salt, solvated with a 1:1 stoichiometry of amine salt and CH_3OH, mp 205 °C (desolvation caused softening up to 195 °C). A second crop of 92 mg was obtained following concentration of the filtrate and recrystallization, for a total yield of 73.6%: CIMS (isobutane) M + 1 268; ^{1}H NMR (DMSO-d_6, HCl salt) δ 9.46 (b s, 2, NH_2), 8.74 (b s, 2, OH), 7.42 (m, 4, ArH), 6.74 (s, 1, ArH), 6.62 (s, 1, ArH), 4.39 (s, 2, $ArCH_2N$), 4.16 (d, 1, Ar_2CH, J = 11.1 Hz), 2.95 (m, 1, CHN), 2.74 (m, 2, $ArCH_2$), 2.17 (m, 1, CHCN), 1.93 (m, 1, CHCN). Anal. ($C_{17}H_{18}ClNO_2$) C, H, N.

***trans*-10,11-Dihydroxy-6-methyl-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrobromide (4b).** A mixture of 82 mg (0.24 mmol) of amine hydrochloride **13b** and 3 mL of 48% HBr was heated at reflux for 3 h, during which time a solid material precipitated. The reaction mixture was concentrated and the residue was dissolved in hot water, filtered, and cooled. After crystallization, the supernatant was decanted and the solid was then washed with cold 2-propanol and ether, to afford 47 mg (54.7% yield) of **4b**: mp 289–290 °C; CIMS (isobutane) M + 1 282; ^{1}H NMR (DMSO-d_6, HBr salt, 500 MHz) δ 10.58 (b s, 1, NH), 8.62 (s, 1, OH), 8.53 (s, 1, OH), 7.48 (m, 2, ArH), 7.37 (m, 2, ArH), 6.72 (s, 1, ArH), 6.68 (s, 1, ArH), 4.68 (d, 1, ArCH, J = 15.0 Hz), 4.38 (d, 1, ArCH, J = 15.0 Hz), 4.30 (d, 1, ArCH, J = 11.1 Hz), 2.98 (m, 1, ArCH), 2.93 (s, 3, NCH_3), 2.80 (m, 2, ArCH), 2.34 (m, 1, CHCN), 2.13 (m, 1, CHCN). Anal. ($C_{18}H_{20}BrNO_2$) C, H, N.

***trans*-10,11-Dihydroxy-6-*n*-propyl-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine Hydrobromide (4c).** A mixture of 78 mg (0.21 mmol) of amine hydrochloride **13c** and 3 mL of 48% HBr was heated at reflux for 2.5 h. The product crystallized from the reaction mixture upon cooling. After refrigeration, the crystals were collected and washed with cold 2-propanol and ether, affording 15 mg (18.5% yield) of **4c**, mp 259–260°. The filtrate and washes were combined, concentrated, and recrystallized from 2-propanol–ethyl acetate to afford an additional 32 mg of product, for a total yield of 58%: CIMS (isobutane) M + 1 310; ^{1}H NMR (DMSO-d_6, HBr salt) δ 10.14 (s, 1, NH), 8.68 (b s, 2, OH), 7.41 (m, 2, ArH), 7.38 (m, 1, ArH), 7.32 (m, 1, ArH), 6.67 (s, 1, ArH), 6.58 (s, 1, ArH), 4.59 (d, 1, ArCHN, J = 14.6 Hz), 4.40 (d, 1, ArCHN, J = 14.6 Hz), 4.23 (d, 1, ArCH, J = 11.0 Hz), 3.15 (m, 1, CHN), 2.87 (m, 2, $ArCH_2$), 2.74 (m, 2, CH_2N), 2.29 (m, 1, CHCN), 2.04 (m, 1, CHCN), 1.74 (m, 2, CH_2), 0.93 (t, 3, CH_3, J = 7.32 Hz). Anal. ($C_{20}H_{24}BrNO_2$) C, H, N.

***N*-Benzyl-6,7-dihydroxy-1,2,3,4-tetrahydro-2-naphthylamine Hydrobromide (17).** A mixture of 127 mg (0.38 mmol) of *N*-benzyl-6,7-dimethoxy-1,2,3,4-tetrahydro-2-naphthylamine hydrochloride[46] and 2 mL of 48% HBr was heated at reflux overnight. The solvent was removed in vacuo and the product was recrystallized from absolute ethanol–ether to afford 70 mg (53%) of a pale gray solid: mp 239–240 °C; CIMS (isobutane) M + 1 270; ^{1}H NMR (500 MHz; DMSO-d_6, HBr salt) δ 8.25 (b s, 2, NH_2), 8.59, 8.56 (2 s, 2, OH), 7.51 (m, 2, ArH), 7.39 (m, 3, ArH), 6.45, 6.43 (2 s, 2, ArH), 4.22 (d, 1, ArCH, J = 13 Hz), 4.19 (d, 1, ArCH, J = 13 Hz), 3.32 (m, 1, CHN), 3.01 (dd, 1, ArCH, J = 4.85 Hz, 15.5 Hz), 2.72 (dd, 1, ArCH, J = 10.6 Hz, 15.5 Hz), 2.66 (m, 2, ArCH), 2.22 (m, 1, CHCN), 1.71 (m, 1, CHCN). Anal. ($C_{17}H_{20}BrNO_2$) C, H, N.

Pharmacological Methods. A. Dopamine-Sensitive Adenylate Cyclase. The method used separates cAMP from other labeled nucleotides by automated HPLC.[47] Rat striatal tissue was homogenized at 50 mL/g of tissue in 5 mM HEPES buffer (pH 7.5) containing 2 mM EGTA. After eight manual strokes with a Wheaton Teflon–glass homogenizer, an additional 50 mL/g of 100 mM HEPES–2 mM EGTA was added and mixed with one additional stroke. A 20-μL aliquot of this tissue homogenate was added to a prepared reaction mixture, yielding a final volume of 100 μL, containing 0.5 mM ATP, [α-^{32}P]ATP (0.5 μCi), 1 mM cAMP, 2 mM $MgCl_2$, 0.7 mM HEPES, 2 μM GTP, 0-100 μM dopamine and/or drug, 10 mM phosphocreatine, and 5 units of creatine phosphokinase. The reaction is initiated by placing the samples in a water bath at 30 °C and terminated 15 min later by addition of 100 μL of 3% sodium dodecyl sulfate (SDS). Proteins and much of the noncyclic nucleotides are precipitated by addition of 300 μL each of 4.5% $ZnSO_4$ and 10% $Ba(OH)_2$ (pH 2.0) to each incubation tube. The samples were centrifuged at 10000*g* for 6 min, and the supernatants were immediately removed and loaded into an ISIS (Isco Inc., Lincoln, NB) autoinjector.

HPLC separations were carried out with a Waters Z-module or RCM 8 × 10 module equipped with a C18, 10-μm cartridge, using a mobile phase of 150 mM sodium acetate–20% MeOH, adjusted to pH 5.0 with concentrated HCl prior to filtration (0.2 μm) and degassing under vacuum. A flow rate of about 4 mL/min was used for separation. The autoinjector was programmed for a 2-min injection interval, with a rinse between samples. A UV detector equipped for 254-nm detection triggered collection of the cAMP fractions via a FOXY fraction collector (ISCO, Inc., Omaha, NB) with a three-way diversion valve. Unlabeled cAMP added to the samples provided the source of UV absorbance and served as an internal standard. Peak areas were quantitated with a Nelson Analytical chromatography data system, and each fraction's radioactivity was determined with an LKB liquid scintillation counter. Percent stimulation was calculated from peak areas and radioactivity. This separation procedure is a major improvement over previously used techniques and was particularly useful when dealing with very small amounts of tissue.

B. Competition Assays for D_1 Receptors. After dissection, individual rat striata were homogenized by seven manual strokes in a Wheaton Teflon–glass homogenizer with ice-cold 50 mM HEPES buffer with 4.0 mM $MgCl_2$ at pH 7.4. Tissue was centrifuged at 27000*g* for 10 min, the supernatant was discarded, and the pellet was homogenized (five strokes) and resuspended in ice-cold buffer and centrifuged again. The final pellet was suspended at a concentration of approximately 2 mg wet weight/mL.

The assay buffer was 50 mM HEPES with 4 mM $MgCl_2$ (pH 7.4). Assay tubes containing a final volume of 1.0 mL were incubated at 37 °C for 15 min. Nonspecific binding of [^{3}H]-**1a** was defined by adding unlabeled 1 μM **1a**. Incubations were filtered through glass-fiber filter mats with a 15-mL ice-cold buffer wash using a Skatron or Brandel cell harvester. Filters were allowed to dry and 3.0 mL of Scintiverse E (Fischer Scientific) was added. After shaking for 30 min, radioactivity was counted on an LKB Betarack liquid-scintillation counter. Tissue-protein levels were estimated by using the Folin reagent method of Lowry et al.[48] adapted to a Technicon Autoanalyzer I (Terrytown, NY).

C. Competition Assays for D_2 Receptors. The binding procedure and protein analysis were identical with that described above except that [^{3}H]spiperone was used as the radioligand. Nonspecific binding of [^{3}H]spiperone was defined by adding unlabeled 1 μM chlorpromazine. Ketanserin tartrate (50 nM) was added to mask binding of [^{3}H]spiperone to serotonin receptors.

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Sources of Chemicals. [^{3}H]SCH 23390 was synthesized as described previously.[49] [^{3}H]spiperone was purchased from New England Nuclear (Boston, MA). The following drugs were obtained as gifts: SCH 23390 (Schering Inc., Bloomfield, NJ), quinpirole (Eli Lilly, Indianapolis, IN), ketanserin tartrate (Janssen Pharmaceutica, New Brunswick, NJ), and SKF 38393 and chlorpromazine (Smith, Kline and French, Philadelphia, PA). All other compounds were purchased from commercial sources.

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Registry No. **4a**, 126295-91-8; **4a**·HCl, 126327-49-9; **4b**, 126295-92-9; **4b**·HBr, 126295-93-0; **4c**, 126295-94-1; **4c**·HBr, 126295-95-2; **5**, 126295-96-3; **5**·HBr, 126295-97-4; **6**, 126295-98-5; *cis*-**7**, 126295-99-6; *trans*-**7**, 126296-00-2; *cis*-**8**, 126296-01-3; *trans*-**8**, 126296-02-4; **9a**, 126296-03-5; **9a**·HCl, 126296-04-6; **6b**, 126296-05-7; **9b**·HCl, 126296-06-8; **10**, 126296-07-9; **11**, 126296-08-0; **12**·HCl, 126296-09-1; **13a**·HCl, 126296-10-4; **13b**, 126296-11-5; **13b**·HCl, 126296-12-6; **13c**, 126296-13-7; **13c**·HCl, 126296-14-8; **17**, 126296-15-9; **17**·HBr, 126296-16-0; $PhCH_2NH_2$, 100-46-9; CH_3CH_2CHO, 123-38-6; benzaldehyde, 100-52-7; 6,7-dimethoxy-β-tetralone, 2472-13-1; 6,7-dimethoxy-β-tetralone *N*-benzyl enamine derivative, 126296-17-1; *N*-benzyl-6,7-dimethoxy-1,2,3,4-tetrahydro-2-naphthylamine hydrochloride, 126296-18-2.

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Structure–Activity Relationships for Metal-Labeled Blood Flow Tracers: Comparison of Keto Aldehyde Bis(thiosemicarbazonato)copper(II) Derivatives

Elizabeth K. John and Mark A. Green*

Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy, Purdue University, West Lafayette, Indiana 47907. *Received September 26, 1989*

Radiocopper-labeled pyruvaldehyde bis(N^4-methylthiosemicarbazonato)copper(II), Cu[PTSM], is under investigation as a radiopharmaceutical for evaluation of regional blood flow in the brain, heart, and kidneys because it affords relatively high levels of radioactivity in these organs upon intravenous injection, followed by prolonged tissue retention of the radiolabel. To probe and differentiate the physicochemical properties that are critical for blood–brain barrier (BBB) penetration and tissue retention in complexes of this type, 17 ^{67}Cu-labeled copper(II) bis(thiosemicarbazone) derivatives of Cu[PTSM] have been prepared and characterized, focusing on the bis(thiosemicarbazone), bis(N^4-methylthiosemicarbazone), bis(N^4-dimethylthiosemicarbazone), and bis(N^4-ethylthiosemicarbazone) derivatives of several alkylglyoxals ($R_{(1)}$ = Me, Et, *n*-Pr, *i*-Pr, *n*-Bu, or Me(EtO)CH) and phenylglyoxal. The compounds studied varied in lipophilicity from log P = 0.75 to log P = 3.5 (where P is the octanol/water partition coefficient). In rat biodistribution studies the N^4-methylthiosemicarbazone ($R_{(1)}$TSM) and N^4-dimethylthiosemicarbazone ($R_{(1)}TSM_2$) complexes always show comparable cerebral uptake at 1 min postinjection (iv) for any given $R_{(1)}$ group, while the thiosemicarbazone ($R_{(1)}$TS) complex always penetrates the BBB less efficiently. Comparison of the various Cu[$R_{(1)}$TS] derivatives shows that their brain uptake does tend to increase with increasing lipophilicity over the range 0.75 < log P < 2.4, although it never reaches that of the N^4-alkylated derivatives. The Cu[$R_{(1)}$TS] and Cu[$R_{(1)}$TSM] complexes are found to exhibit prolonged cerebral retention of activity, consistent with their known susceptibility to reductive decomposition by intracellular sulfhydryl groups, while the more inert Cu[$R_{(1)}TSM_2$] complexes clear from the brain relatively rapidly. Tracer clearance kinetics in the heart and kidney are similar to those observed for the brain with each of the tracers examined.

Introduction

Radionuclide imaging remains a technique that uniquely addresses the frequent need of the medical community for measurements of tissue perfusion at the capillary level. However, despite the importance of perfusion imaging, especially in the diagnosis of cardiovascular[1-3] and cerebrovascular[4,5] disease, the ability of nuclear medicine to provide this information often remains limited by the radiopharmaceuticals that are now available. A radiotracer that is completely extracted from blood into tissue in its first pass through the capillary system will map regional perfusion and will also allow calculation of absolute regional blood flow (mL min^{-1} g^{-1}), if both the tissue concentration of tracer and the concentration of tracer in the arterial blood supplied to the organ are known.[6]

Radiolabeled microspheres, sized such that they will be mechanically trapped in the capillary bed of an organ, serve as one standard to which possible new blood flow agents can be compared.[7] Biodegradable denatured albumin particles (macroaggregated albumin or MAA) that can be labeled[8-10] with ^{99m}Tc, ^{68}Ga, or ^{11}C are routinely used in the clinical assessment of pulmonary blood flow following intravenous injection. Unfortunately, the use of particulate tracers to evaluate perfusion in other organs

* Author to whom correspondence should be addressed.

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