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Synthesis and Pharmacological Evaluation of Substituted Naphth[1,2,3-*de*]isoquinolines (Dinapsoline Analogues) as D_1 and D_2 Dopamine Receptor Ligands

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Abstract—Dinapsoline ((**2**); (±)-dihydroxy-2,3,7,11b-tetrahydro-1*H*-naphth[1,2,3-*de*]isoquinoline) is a full D_1 dopamine agonist that also has significant D_2 receptor affinity. Based on a similar pharmacophore, dinapsoline has pharmacological similarities to dihydrexidine ((**1**); (±)-*trans*-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo[*a*]phenanthridine), the first high affinity full D_1 agonist. Small alkyl substitutions on the dihydrexidine backbone are known to alter markedly the D_1:D_2 selectivity of dihydrexidine, and it was of interest to determine whether similar SAR exists within the dinapsoline series. This report describes the synthesis and pharmacological evaluation of six analogues of dinapsoline: *N*-allyl-(3);*N*-*n*-propyl- (**4**); 6-methyl- (**5**); 4-methyl- (**6**); 4-methyl-*N*-allyl- (**7**); and 4-methyl-*N*-*n*-propyl-dinapsoline (**8**). As expected from earlier studies with the dihydrexidine backbone, *N*-allyl (**3**) or *N*-*n*-propyl (**4**) analogues had markedly decreased D_1 affinity. Unexpectedly, and unlike the dihydrexidine series, these same substituents did not markedly increase D_2 affinity. The addition of a methyl group to position 6 (**5**) increased D_1:D_2 selectivity, but less markedly than did the analogous 2-methyl substituent added to 1. Unlike the analogous 4-methyl substituent of 1, the addition of a 4-methyl-group (**6**) actually decreased D_1 affinity without affecting D_2 affinity. These data demonstrate that the dinapsoline (**2**) backbone can be modified to produce dopamine agonists with novel properties. Moreover, as rigid ligands in which small substituents can cause significant changes in selectivity, they are important tools for deriving 'differential' SARs of the dopamine receptor isoforms.

Introduction

The receptors for dopamine are categorized on biochemical and pharmacological properties that divide them into two families.[1,2] The D_1-like receptor family in mammals includes D_{1A}[3] and D_{1B}[4] (alternatively named D_5[5]) while the D_2-like family includes the D_{2long}, D_{2short}, D_3, and D_4.[6–8] The D_1-like receptor subtypes have high sequence homology, very similar SAR profiles, and in many cell types lead to stimulation of adenylate cyclase.[9] The D_2-like receptor subtypes resemble each other in terms of SAR profiles, and in being coupled to inhibition of adenylate cyclase or mechanistically related second messenger systems.[9] All five receptor subtypes belong to the G protein-coupled receptor (GPCR) superfamily, also known as the seven-transmembrane (7TM) superfamily. At one time the D_2-receptor subtype was thought to mediate most of the behavioral effects of dopamine, and to be the subtype most implicated in the etiology or therapy of dopamine-related disease states.

Until recently, it had been a general consensus that the antiparkinsonian effects of levodopa and mixed dopamine agonists were due principally to stimulation of one or more of the D_2–like dopamine receptors.[10] After we had synthesized dihydrexidine (DHX 1), the first high affinity true full D_1 agonist,[11,12] we demonstrated that

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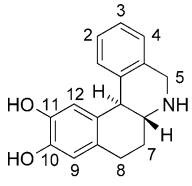
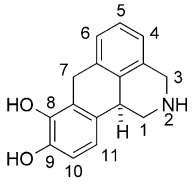
dihydrexidine could dramatically reduce MPTP-induced parkinsonism in monkeys (tremor, motor freezing, abnormal posture, rigidity and bradykinesia)[13] contrary to accepted dogma, but consistent with the notion that the location of D_1 receptors might make them of particular importance.[14] Because DHX has only 10-fold selectivity for D_1 versus D_2 receptors in brain, its D_2 activity might have been responsible for these profound antiparkinsonian effects. In acute studies, however, the antiparkinsonian actions of DHX were completely blocked by the D_1 antagonist SCH23390, but not significantly affected by the D_2 antagonist remoxepride.[2] Subsequent clinical trials demonstrated efficacy in Parkinson patients for both dihydrexidine and other D_1 full agonists.[15,16] Although these data demonstrated the importance of D_1 activation, the co-activation of both families of dopamine receptors may also be important.



Dihydrexidine (1)



Dinapsoline (2)

Since the development of DHX (**1**), we have continued to study the D_1-like receptors and to develop novel ligands with full D_1 agonist properties. One of the products of this research was the second-generation rigid dopamine D_1 agonist dinapsoline (**2**), first synthesized in 1996.[17] More recently, the in vivo pharmacology of **2** has been explored in greater detail, where it was found that **2** produced a robust response in the unilateral 6-OH-DA lesioned rat model of Parkinson's disease.[18] In addition, it now has been established that the predicted *R*-(+)-enantiomer (illustrated for **2**) possesses the dopaminergic effects of the racemate.[19]

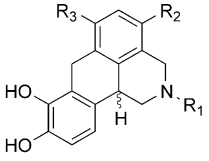
Dinapsoline is of similar affinity and potency to dihydrexidine, but is actually less selective for the D_1 versus D_2 receptors. With dihydrexidine, it was of particular interest that relatively subtle substitutions on its backbone caused marked, often unpredicted, changes in both the selectivity and affinity of these drugs for the different dopamine receptor isoforms.[20,21] For example, the addition of an *N*-*n*-propyl moiety markedly increased D_2-like affinity, with specific increases in the affinity for the D_3 receptor (Table 1). Further, the addition of a 2-methyl substituent led to a marked increase in D_1 selectivity. In view of the close structural similarity between **1** and **2**, as well as a high degree of complementarity of **2** to a D_1 dopamine full agonist model recently developed by Mottola et al.,[22] it was of great interest to examine structural modifications of **2** that were parallel to those reported earlier by Knoerzer et al.[20] In contrast to that work, however, there is no readily apparent divergent or combinatorial synthetic approach that allows the synthesis of a variety of dinapsoline (**2**) analogues. Rather, each ring-substituted compound requires the specific synthesis of the requisite

Table 1. Apparent affinity of dihydrexidine and analogues for dopamine receptors in rat striatal homogenates and cloned receptors[a]

Drug	Rat striatum (nM)		C-6 Glioma cells (nM)	
	D_1	D_2	D_{2L}	D_3
Dihydrexidine (DHX; 1)	6.2	58.1	1,490	170
N-propyl-DHX	180	25.7	434	8.70
2-Methyl-DHX	8.7	302	—	—
4-Methyl-DHX	6.6	18.0	674	85
4-Methyl-*N*-propyl-DHX	23.4	9.0	229	2.08

[a]From references.[20,21]

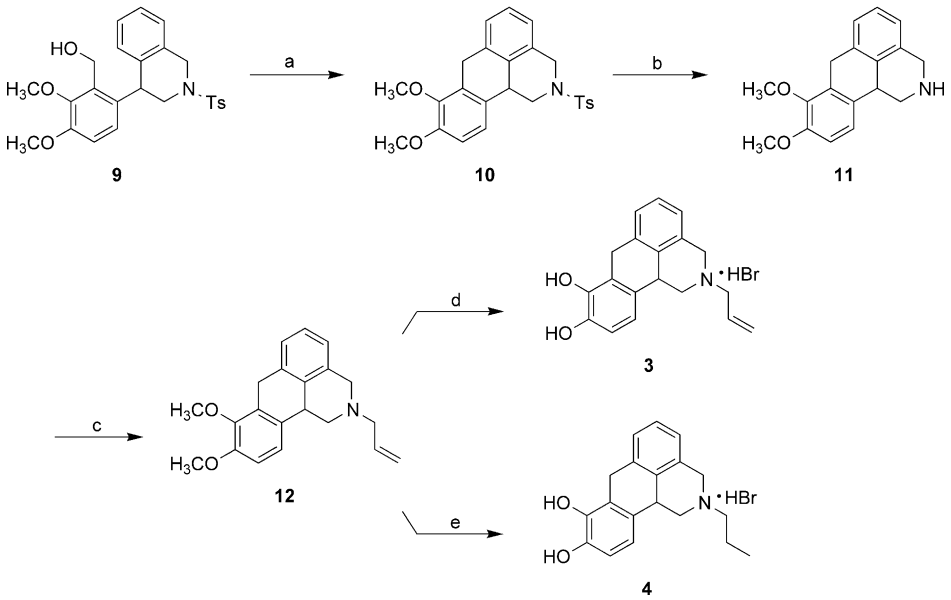
ring-substituted isoquinoline-derived synthon. Each substituted isoquinoline must then be carried through a parallel linear synthesis. Thus, we were forced to choose judiciously which ring substituents to examine. The data reported by Knoerzer et al.,[20] showed that a 2-methyl group in **1** (corresponding to a 6-methyl in **2**) gave enhanced D_1 activity, whereas a 4-methyl group in **1** (corresponding to a 4-methyl in **2**) led to enhanced D_3 potency (Table 1). Thus, we hypothesized that 6-methyl-**2** would have enhanced D_1 potency and that 4-methyl-**2** would possess enhanced D_3 activity. This report, therefore, describes the synthesis and dopaminergic properties of compounds **3**–**8**, and demonstrates that, surprisingly, analogous substitutions do not always cause changes in receptor affinities that are predictable based on the analogous backbone of **1**. Compounds were examined as the racemates because significant dopaminergic activity resides only in the illustrated (+)-11b*R* enantiomer of **2**.[19]



(±)-3: R_1 = allyl; R_2 = R_3 = H
(±)-4: R_1 = *n*-propyl; R_2 = R_3 = H
(±)-5: R_1 = R_2 = H; R_3 = CH_3
(±)-6: R_1 = H; R_2 = CH_3; R_3 = H
(±)-7: R_1 = allyl; R_2 = CH_3; R_3 = H
(±)-8: R_1 = *n*-propyl; R_2 = CH_3; R_3 = H

Chemistry and Pharmacology

We have recently reported an improved method for the synthesis of the key intermediate **9** in the preparation of dinapsoline.[23] Starting from **9** the syntheses of both *N*-*n*-allyl dinapsoline (**3**) and *N*-*n*-propyl dinapsoline (**4**) were accomplished (Scheme 1). After stirring **9** with sulfuric acid at −20 °C, the resulting intermediate **10** was treated with sodium amalgam to remove the *N*-(*p*-toluenesulfonyl) group. This afforded amine **11** that was immediately treated with allyl bromide to give

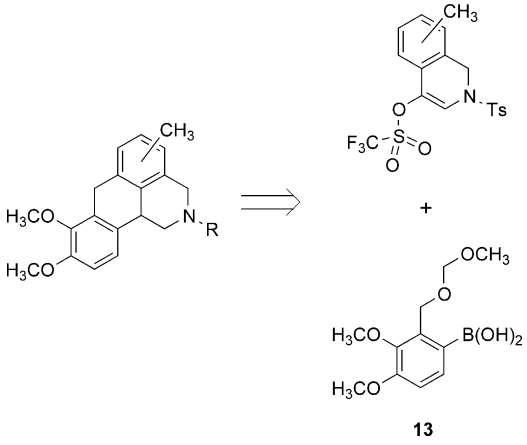


Scheme 1. Reagents and conditions: (a) concd H_2SO_4, −20 °C (31%); (b) Na(Hg), Na_2HPO_4, MeOH (74%); (c) allyl bromide, K_2CO_3, acetonitrile (94%); (d) BBr_3, CH_2Cl_2, −78 °C (89%); (e) (i) 10% Pd/C, ethanol; (ii) BBr_3, CH_2Cl_2, −78 °C (66%, two steps).

the *N*-allyl amine **12**. Cleavage of the *O*-methyl groups then was effected using boron tribromide to yield *N*-allyl dinapsoline hydrobromide (**3**). The same *N*-allyl amine **12** was catalytically reduced over hydrogen, and then treated with boron tribromide to give *N*-*n*-propyl dinapsoline hydrobromide (**4**).

The retrosynthetic analysis for compounds **5**–**8** is shown in Scheme 2. This route represents a convergent synthesis that utilizes a Suzuki cross-coupling reaction[24,25] between the aryl boronic acid (**13**)[23] and a suitable vinyl triflate.[26] The triflates were synthesized as shown in Scheme 3. *N*-*p*-Toluenesulfonyl glycine methyl ester was alkylated with the appropriate benzyl chloride to afford the *N*-(methylbenzyl)-*N*-(*p*-toluenesulfonyl)-glycine methyl esters **14** and **15**.[27] The esters then were hydrolyzed with aqueous sodium hydroxide solution to give the acids **16** and **17** that were converted to acyl chlorides upon treatment with thionyl chloride. Following the precedent of Schlademan and Partch,[28] the acid chlorides were treated with three equivalents of aluminum chloride at −78–11 °C, to give the isoquinolones **18** and **19**. Treatment with lithium diisopropyl amide, followed by Comins reagent[29] [1-*N*,*N*-bis(trifluoromethylsulfonyl)amino-5-chloropyridine], furnished the triflates (**20** and **21**).

Initial attempts to effect a Suzuki cross-coupling between **20** or **21** and boronic acid **13** using classical conditions failed, but modified Suzuki conditions [exclusion of water, stronger base (KBr instead of LiCl), and toluene in place of 1,2-dimethoxyethane] were used to afford the coupling products **22** and **23** in excellent yield (Scheme 4). These protected alcohols were reduced catalytically with hydrogen over 50% (w/w) of 10%



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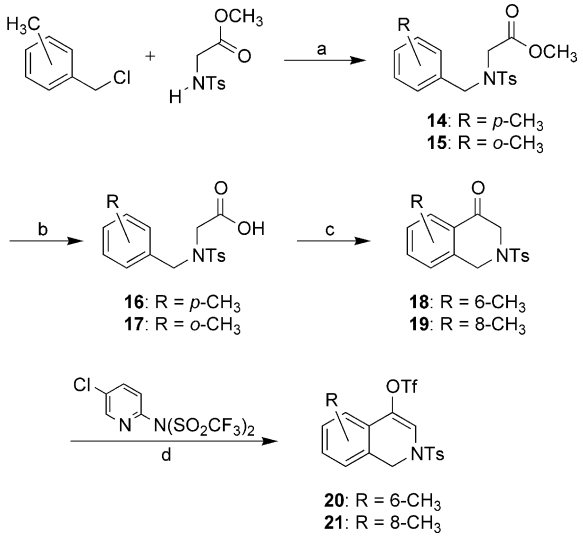
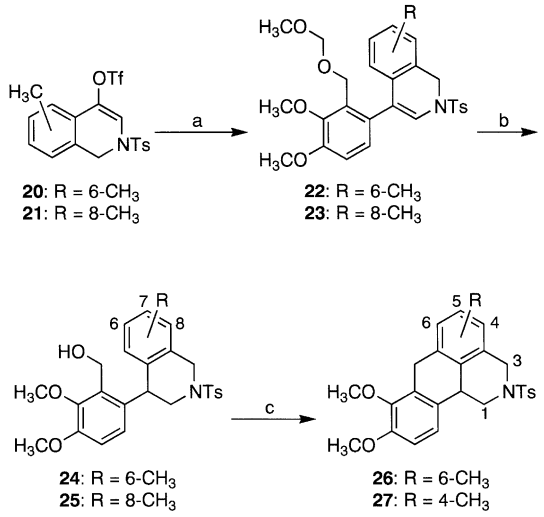
Scheme 2. Retrosynthetic analysis for methyl-substituted analogues of **2**.



20: R = 6-CH_3
21: R = 8-CH_3

Scheme 3. Reagents and conditions: (a) K_2CO_3, NaI, acetone, reflux (14: 76%, 15: 90%); (b) 10% NaOH (16: 85%, 17: 94%); (c) $SOCl_2$; $AlCl_3$, −78 →, −11 °C, CH_2Cl_2 (18: 70%, 19: 70%); (d) (*n*-BuLi, $HN(iPr)_2$, THF, −78–0 °C (20: 77%, 21: 77%).



Scheme 4. Reagents and conditions: (a) 13, $Pd(PPh_3)_4$, KBr, KOH, toluene, reflux (22: 91%, 23: 92%); (b) i. Pd/C (10%), H_2, ethanol, ii. 3N HCl–THF (1:1) (24: 75%, 25: 79%); (c) concd H_2SO_4, −20 °C (26: 42%, 27: 62%).

palladium on carbon, followed by heating to reflux in 3 N HCl/THF (1:1) to yield the deprotected benzyl alcohols **24** and **25** in good yields. Intermediates **24** and **25** then were treated with concentrated sulfuric acid at −20 °C to afford the cyclized products **26** and **27**.

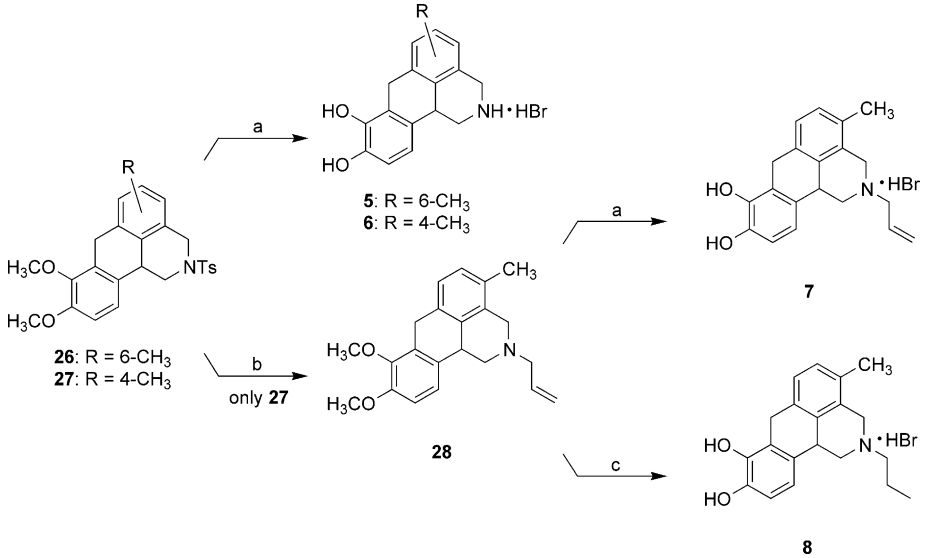
Treatment of the cyclized intermediate with sodium amalgam in buffered methanol effected the removal of the *N*-tosyl protecting group, then demethylation with boron tribromide gave good yields of **5** and **6** as the hydrobromide salts (Scheme 5). To prepare the *N*-alkyl compounds **7** and **8**, the amine intermediate resulting from the *N*-detosylation of **27** was treated with allyl bromide in acetone in the presence of potassium carbonate to yield **28** that then was demethylated using boron tribromide to give *N*-*n*-allyl-4-methyl dinapsoline hydrobromide (**7**). Catalytic reduction of the *N*-allyl amine **28** followed by treatment with boron tribromide afforded *N*-*n*-propyl-4-methyl dinapsoline hydrobromide (**8**).

Pharmacology

Each of the new compounds was evaluated for its affinity at D_1-like and D_2-like receptors in rat striatal homogenates, as described previously.[12] Competition assays were also carried out in cloned D_{1A}, D_{2L}, and D_3 receptors expressed in C-6 glioma cells.[21] In addition, functional assays were carried out both in striatal homogenate and in C-6 glioma cells to assess the ability of the new compounds to stimulate cAMP accumulation.

Results

The radioreceptor competition data for the dinapsoline analogues and reference compounds are presented in Table 2. As has been reported previously, dinapsoline has high affinity for either brain, or expressed primate D_1 receptors, with a similar pattern of activity seen in the rat brain and cloned primate D_1 receptors. With the brain receptors, dinapsoline (**2**) is relatively non-selective for D_1 versus D_2 receptors, having high affinity for both. The addition of either *N*-allyl (**3**) or *N*-*n*-propyl (**4**) substituents caused a significant decrease in D_1-like affinity, as well as a smaller decrease in D_2-like affinity. The addition of 4-methyl (**6**) or 6-methyl (**5**) substituents did not appreciably affect D_2-like affinity, but in the case of the 4-methyl (**6**) substituent, it actually decreased D_1-like affinity. The combination of the *N*-*n*-propyl and 4-methyl substitutions in **8** caused a dramatic reduction in D_1 affinity with no corresponding decrease in D_2 affinity. This result was predictable by the additive effects of each substituent alone. Studies in a cell line transiently transfected with the D_5 receptor



Scheme 5. Reagents and conditions: (a) (i) Na(Hg), Na_2HPO_4, methanol; (ii) BBr_3, CH_2Cl_2, −78 °C (5: 61%, 6:73%, 7:62%); (b) (i) Na(Hg), Na_2HPO_4, methanol; (ii) allyl bromide, K_2CO_3, acetonitrile; (c) (i) 10% Pd/C, ethanol; (ii) BBr_3, CH_2Cl_2, −78 °C, (59%, two steps).

Table 2. Affinities of dinapsoline analogues for dopamine receptors

Drug	Rat striatum (nM)		Clonal lines (nM)				
	D_1-like	D_2-like	D_{1A} C-6	D_{2L} C-6	D_3 C-6	D_4 CHO	D_5 HEK
SCH23390	0.69	—	0.32	—		—	1.0
Chlorpromazine	—	1.2	—	0.74	0.9	20	—
Dihydrexidine	5.5	24	2.2[b]	180	18	13	16
SKF38393	20[a]	—	8.6[b]	—	—	—	80
2 *(DNS)*	12.1	25	6.1	53	10	60	5.0
(±)-3 > (*N*-allyl-DNS)	210	110	23	81	14	48	290
(±)-4 (*N*-propyl-DNS)	660	60	170	39	2.8	17	1500
(±)-5 (*6*-Me-DNS)	11	57	9.8	190	28	220	8.1
(±)-6 (*4*-Me-DNS)	90	19	32	36	18	83	20
(±)-7 (*4*-Me-*N*-allyl-DNS)	140	49	56	36	4.3	63	690
(±)-8 (*4*-Me-*N*-propyl-DNS)	1400	21	200	23	2.5	28	2400

All receptor data are apparent affinities (K0.5) in nM units. All standard errors were within 15% of the values reported.
[a]From[21]
[b]From[31]

indicated that none of these compounds had selectivity for D_1 vs. D_5 receptors.

Neither the parent compound, nor the *N*-substituted compounds **3**, **4**, and **7** had significant selectivity for D_{2L} versus D_3 receptors. Rather, most of the new compounds possessed selectivity for D_3 over D_{2L} receptors, with compounds **4**, **7**, and **8** all having about 10-fold selectivity for D_3 over D_{2L}. It is also noteworthy that at the D_4 receptor, there was relative insensitivity of the dinapsoline structural motif to the types of substituents that were examined.

The addition of substituents affected the functional interaction of these drugs with D_1 receptors (Figs. 1 and 2). The 6-methyl substitution **5** actually increased potency relative to the parent, while the 4-methyl substitution somewhat decreased potency, and surprisingly rendered the compound a partial agonist. The *N*-substituted compounds **3**, **4** and **7** had reduced potency and efficacy, with the 4-methyl-*N*-allyl DNS **7** compound being inactive.

Discussion

The study of these dinapsoline (**2**) analogues represents an important continuation of our efforts to develop a complete and unambiguous picture of the D_1 agonist pharmacophore. As noted earlier, these substitutions were selected to parallel previously synthesized analogues of dihydrexidine (**1**) in which the selectivity of the parent drug for different dopamine receptor isoforms was altered in unexpected ways.[20,21] In the present study, the parent molecule **2** was, as previously reported, of affinity similar to **1**. The addition of either an *N*-allyl (**3**) or an *N*-*n*-propyl (**4**) substituent caused a significant decrease in D_1–like affinity, consistent with the effects seen with the dihydrexidine series and as predicted by the D_1 pharmacophore we have developed. The pattern with the cloned D_{1A} receptor paralleled that of the rat brain D_1 receptor, not surprisingly, because the rodent striatum contains no detectable D_{1B}/D_5 binding sites.[30] We further tested the affinity at cloned D_5 receptors and found the pattern of affinities to be similar to that observed at D_1 receptors. As noted, these

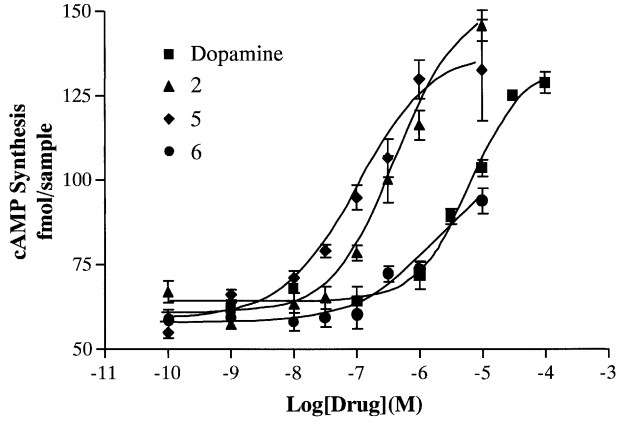


Figure 1. Functional effects of DNS and analogues at rat striatal D_1 receptors. Some agonists are not depicted because they showed minimal stimulation (see Table 3). Dopamine is included for reference. Note the significantly greater potency and intrinsic activity of 5 versus 6. Stimulation of D_1 receptors by agonists was blocked by the D_1 antagonist SCH23390 (data not shown).

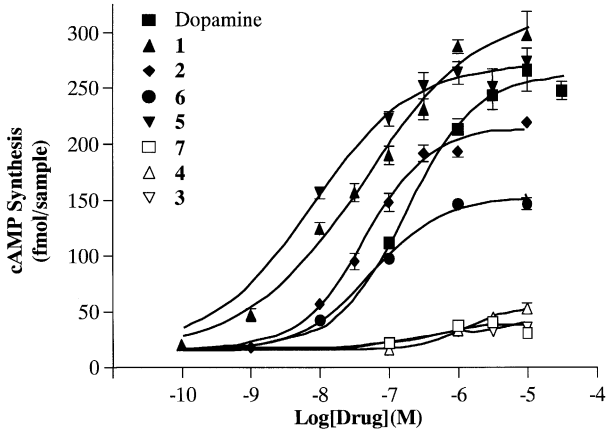


Figure 2. Functional effects of DNS and analogues at C-6-mD1 receptors. Dopamine and dihydrexidine are included for reference. Consistent with data from striatal membranes, notice the unexpected and significantly greater potency and intrinsic activity of 5 versus 6. Stimulation of D_1 receptors by agonists was blocked by the D_1 antagonist SCH23390 (data not shown).

Table 3. Functional effects (EC_{50}s) of dinapsoline analogues at D_1 and D_2 dopamine receptors

Drug	Rat striatum D_1-like (nM)	D_{1A} C-6 glioma cells (nM)
Dopamine	1000	220
Dihydrexidine	100	34
SKF38393	100[a]	520[b]
2 (DNS)	350	55
(±)-3 (*N*-allyl-DNS)	~500	500
(±)-4 (*N*-propyl-DNS)	NR	1200
(±)-5 (*6*-Me-DNS)	170	10
(±)-6 (*4*-Me-DNS)	900	48
(±)-7 (*4*-Me-*N*-allyl-DNS)	NR	NR
(±)-8 (*4*-Me-*N*-propyl-DNS)	ND	ND

All receptor data are EC_{50}s in nM units. All standard errors were within 15% of the values reported.
NR = Non-responsive.
ND = Not determined.
[a]From .[12]
[b]From .[31]

results with the D_1 receptors provide additional validation for the well-defined D_1 agonist pharmacophore that has been developed in our laboratories.[12,20,22]

Conversely, although we had expected that substituent effects on D_2-like affinity would be very similar to those seen with the dihydrexidine backbone, there were many unexpected findings. Knoerzer et al.[20] demonstrated that addition of a 2-methyl to **1** caused a marked decrease in D_2 affinity, whereas a methyl substituent at the 4-position in **2** increased D_2 affinity (Table 1). The compound analogous to 2-Me-DHX, **5**, thus was predicted to have lower D_2 affinity and higher D_1 selectivity than the parent drug **2**. In fact, whereas there was some increase in D_1:D_2 selectivity, it was of smaller magnitude than seen with the dihydrexidine series. Similarly, **6**, analogous to 4-methyl-DHX, was predicted to have higher D_2 affinity and thus lower D_1:D_2 selectivity than the parent. While lower D_1:D_2 selectivity was found, this was a result solely of an unexpected loss of D_1 affinity, a change that was paralleled by a loss of full agonist character.

Differences in how these two series of rigid dopamine agonists interact with D_2-like receptors also were seen when comparing their interactions with the cloned D_{2L}, D_3, and D_4 receptors. Dinapsoline (**2**) had much higher D_{2L} affinity than dihydrexidine (**1**), and slightly higher D_3 affinity (compare Table 2 with Table 1). The addition of an *N*-*n*-propyl substituent increased affinity with both series, but somewhat more with the dihydrexidine than dinapsoline backbones (Tables 1 and 4 vs. 2). Similarly, compounds with the highest D_3 affinity in both series were the 4-methyl-*N*-*n*-propyl derivatives, yet both the absolute D_3 affinity, and the D_3 versus D_2 selectivity were higher within the dihydrexidine series. The pattern of affinities of the dinapsoline series at cloned D_4 receptors was similar to that observed at D_{2L} receptors, as seen in Table 2. These results suggest that these rigid analogues may be useful for developing agonist pharmacophores for these two receptor subtypes.

Functional characterization of these compounds provided other unexpected results. Previous studies had determined dinapsoline (**2**) to be a full agonist at rat striatal[23] and cloned[31] D_1 receptors. Characterization of the analogues of **2** in both these preparations revealed that a 6-methyl addition to dinapsoline (**5**) increased the potency of the drug 5-fold, while a 4-methyl addition (**6**) decreased the potency as expected from the affinity, but changed the compound to a partial agonist (Figs. 1 and 2). Interestingly, while the addition of the *N*-*n*-allyl **3** decreased the potency of the molecule, the combination of the 4-methyl and the *N*-*n*-allyl-**7** rendered the molecule inactive. As expected, the inclusion of the *N*-*n*-propyl-**4** greatly diminished the activity at D_1 receptors. The functional activity of **8** was not determined due to its low affinity for these receptors.

Together, these data indicate that a valid pharmacophore is available to describe adequately the recognition characteristics of both the rat and human D_1 receptor. Yet while this pharmacophore has been highly predictive, it is clear that it has certain aspects that can be probed more accurately with rigid analogues of these types. By contrast, the differences in substituent effects for these two rigid backbones clearly indicate that a different pharmacophore will need to be developed to accommodate the interaction of these ligand families with the D_2-like receptors, including the D_2 and D_3 receptors. Thus, dinapsoline and dihydrexidine analogues should be extremely useful for testing hypotheses about the docking of ligands to these D_2-like receptors for several reasons: (1) they are both based on a rigid backbone; (2) one or more members of each family have high affinity for the receptors in question; and (3) small substituents can cause substantial changes in affinity.

It also has been reported that dihydrexidine and several of its D_2-selective analogues were atypical agonists at D_2-like receptors, having a property we have termed 'functional selectivity' (e.g., they could bind the D_{2L} receptor in an expression system and cause agonist effects at one function, and antagonist effects at another).

In preliminary studies, dinapsoline has been shown to differ from dihydrexidine in notable ways, but like dihydrexidine, appears to have this unique property of functional selectivity. The preliminary data suggest that detailed and careful functional characterization is necessary for the D_2-like properties of these drugs. It is clear, however, that whereas useful predictions can be made using our D_1 pharmacophore, at the D_2-like receptors analogous substituents clearly lead to unexpected effects.

Conclusion

In summary, although dihydrexidine and dinapsoline arose from the same D_1 agonist pharmacophore parentage, they have significant differences in both their pharmacology, and in the pharmacology of their substituted analogues. Such differences provide avenues for

extremely interesting uses as research tools and in clinical applications. Not only can one design drugs with different degrees of relative receptor activation, but simple substituents may also affect pharmacokinetic properties if they can intrinsically inhibit specific oxidative or conjugative enzymes.

Experimental

Chemistry

General procedures. Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian VXR-5000s (500 MHz) or a Bruker-AXR (300 MHz) NMR instrument in $CDCl_3$, DMSO-d_6 or CD_3OD and chemical shifts are reported in δ values (ppm) relative to an internal reference of $CHCl_3$ (δ 7.24), DMSO-d_5 (δ 2.49) or CD_3OH (δ 3.3) respectively. Chemical ionization (CI) and electron ionization (EI) mass spectra were obtained with a Finnegan 4000 quadrupole mass spectrometer. High resolution CI and EI mass spectra were obtained on a Kratos MS 50 spectrometer and were within 0.0015 *m/z*, unless otherwise noted. Ionization gas for CIMS and high resolution CIMS was isobutane, unless otherwise noted. Elemental analyses were performed by the microanalysis laboratory in the Chemistry Department at Purdue University.

8,9-Dimethoxy-(*N*-*p*-toluenesulfonyl)-2,3,7,11b-tetrahydro-1*H*-naphth[1,2,3-*de*]isoquinoline (10). To sulfuric acid (50 mL) cooled to −20 °C and stirred with a mechanical stirrer under argon was added the alcohol **9**[23] (500 mg, 1.1 mmol) as a solid in portions. After 30 min at −20 °C the reaction mixture was poured into 150 g of ice and the aqueous solution was extracted with dichloromethane (3×150 mL). The combined extracts were washed with aqueous 1 N NaOH solution (2×50 mL), dried ($MgSO_4$), filtered and the solvent was evaporated. The residue was subjected to column chromatography (silica gel, 30% ethylacetate–hexane) to afford 150 mg (31%) of a white solid. The reaction was repeated several times to accumulate more of the product: mp 210–212 °C (lit.[17] 204–206 °C); ^{1}H NMR and chemical ionization MS were identical to material prepared by the method of Ghosh et al.[17]

8,9-Dimethoxy-2,3,7,11b-tetrahydro-1*H*-naphth[1,2,3-*de*]isoquinoline (11). The cyclized compound **10** (1 g, 2.29 mmol) was dissolved in 100 mL of anhydrous methanol and 11.2 g of 6% sodium-amalgam and 1.3 g (6.87 mmol) of Na_2HPO_4 was added. The reaction mixture was maintained at reflux overnight, then it was decanted into an Erlenmeyer flask and diluted with 200 mL of water. The mixture was extracted with dichloromethane (3×200 mL) and the combined organic layers were dried (Na_2SO_4). The solvent was filtered and evaporated, and the residue was purified by rotary chromatography (50% ethylacetate–hexane, under ammonia) to yield 480 mg (75%) of a yellow oil that was immediately carried into the next reaction. The ^{1}H NMR of the product was identical to material prepared by the method of Ghosh et al.[17]

***N*-Allyl-8,9-dimethoxy-2,3,7,11b-tetrahydro-1*H*-naphth[1,2,3-*de*]isoquinoline (12).** To the amine **11** (480 mg, 1.71 mmol) in 50 mL of acetone, 236 mg (1.71 mmol) of potassium carbonate and 0.15 mL (210 mg, 1.73 mmol) of allyl bromide were added. The mixture was allowed to stir under argon for 5 h and then was diluted with 100 mL of diethyl ether, filtered and concentrated. The residue was purified with rotary chromatography (silica gel, 10% ethylacetate–hexane under ammonia) to afford 516 mg (94%) of a clear oil that was used immediately in the next reaction: ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.56 (t, 1H, Ar_2–CH–*CH2*–N, *J 32;* = *32;* 11.5 Hz); 3.31 (dt, 1H, N–CH_2–CH=CH_2, *J 32;* = 6.5 Hz, 1 Hz); 3.41 (d, 1H, Ar–CH_2–N, *J* = 15 Hz); 3.49 (dd, 1H, Ar–CH_2–Ar, *J 32;* = 17.5 Hz, 3 Hz); 3.80 (m, 1H, Ar–CH–*CH*$_2$–Ar); 3.83 (s, 6H, OCH_3); 3.86 (m, 1H, Ar–*CH*–CH_2–Ar); 3.98 (d, 1H, Ar–CH_2–N, *J* = 15 Hz); 4.32 (d, 1H, Ar–CH_2–Ar, *J* = 17.5 Hz); 5.24 (dd, 1H, , N–CH_2–CH=*CH*$_2$, *J* = 10.0 Hz, 1.5 Hz); 5.32 (dd, 1H, , N–CH2–CH=*CH*$_2$, *J* = 17.0 Hz, 1.5 Hz); 6.01 (m, 1H, , N-CH_2–*CH*=CH_2); 6.76 (d, 1H, ArH, *J* = 8.0 Hz); 6.90 (d, 1H, ArH, *J* = 8.5 Hz); 6.92 (d, 1H, ArH, *J* = 8.5 Hz); 7.09 (t, 1H, ArH, *J* = 7.5 Hz); 7.17 (d, 1H, ArH, *J* = 8 Hz). CIMS *m/z*: 322 ($M+H^+$, 100%). HRMS: calcd: 321.1729. Found: 321.1741.

***N*-Allyl-8,9-dihydroxy-2,3,7,11b-tetrahydro-1*H*-naphth[1,2,3-*de*]isoquinoline hydrobromide (3).** The *N*-allyl amine 12 (280 mg, 0.87 mmol) was dissolved in 100 mL of dichloromethane and stirred at −78 °C under argon. To this solution 4.5 mL (4.5 mmol) of boron tribromide (1 M solution in dichloromethane) was added and the mixture was allowed to stir at −78 °C for 2 h, then the cooling bath was removed and the reaction mixture was stirred at room temperature overnight. The reaction mixture was re-cooled to −78 °C, and 15 mL of anhydrous methanol were added. The mixture was stirred for 15 min at room temperature and then evaporated to dryness. This was repeated three times to quench all the boron species in the reaction mixture. The residue was crystallized from ethanol–ethyl acetate to afford 290 mg (89%) of a tan solid: mp 228 °C dec; ^{1}H NMR: δ 3.48 (dd, 1H, Ar–CH_2–Ar, *J* = 17.5 Hz, 3 Hz); 3.546 (t, 1H, Ar_2–CH–*CH*$_2$–N, *J* = 11.5 Hz); 4.08 (m, 3H, Ar_2–CH–*CH*$_2$–N and N–*CH*$_2$–CH=CH_2); 4.38–4.59 (m, Ar–CH_2–Ar and Ar–CH_2–N); 4.46 (m, 1H, Ar_2–CH–N); 4.59 (d, 1H, Ar–CH_2–N, *J* = 15.5); 5.72 (d, 1H, N–CH_2–CH=*CH*$_2$, *J* = 10.5 Hz); 5.75 (d, 1H, , N–CH_2–CH=*CH*$_2$, *J* = 16.5 Hz); 6.15 (m, 1H, N–CH_2–*CH*=CH_2); 6.59 (d, 1H, ArH, *J* = 8.0 Hz); 6.71 (d, 1H, ArH, *J* = 8.0 Hz);7.11 (d, 1H, ArH, *J* = 8.0 Hz);7.27 (t, 1H, ArH, *J* = 7.5 Hz);7.35 (d, 1H, ArH, *J* = 7.5 Hz). PDMS *m/z*: 294. Anal. ($C_{19}H_{20}BrNO_5$) C, H, N.

***N*-*n*-Propyl-8,9-dihydroxy-2,3,7,11b-tetrahydro-1*H*-naphth[1,2,3-*de*]iso-quinoline hydrobromide (4).** The *N*-allyl amine 12 (180 mg, 0.56 mmol) was dissolved in 100 mL of ethanol and then shaken with 18 mg of Pd/C (10%) in a Parr hydrogenator at 50 psi for 3 h. The catalyst was filtered and the solvent was evaporated. The residue was placed under high vacuum for 8 h, then it was dissolved in 100 mL CH_2Cl_2 and stirred under argon at −78 °C. To the solution, 2.8 mL (2.8 mmol) of

boron tribromide (1 M solution in dichloromethane) was added and the mixture was stirred for 2 h, then the cooling bath was removed and the reaction was stirred at room temperature overnight. The reaction was re-cooled to −78 °C, 15 mL of anhydrous methanol was added, and the mixture was stirred for 15 min and then evaporated to dryness. This was repeated three times to quench the boron species in the reaction. The residue was crystallized from ethanol–ethyl acetate to afford 150 mg (66%) of a tan solid: mp 228 °C dec; ^{1}H NMR (300 MHz, CD_3OD): δ1.11 (t, 1H, N-CH_2–CH_2–CH_3, J = 8 Hz); δ1.97 (m, 1H, N–CH_2–CH_2–CH_3, J = 8.0 Hz); 3.3–3.39 (m, 4H, Ar–CH_2–Ar, Ar_2–CH–CH_2–N and N–CH_2–CH_2–CH_2); 4.08 (m, 1H, Ar_2–CH–CH_2–N); 4.36–4.56 (m, 3H, Ar_2–CH–CH_2–N, Ar–CH_2–Ar and Ar–CH_2–N); 4.65 (d, 1H, Ar–CH_2–N, J = 17.5 Hz); 6.61 (d, 1H, ArH, J = 8.5 Hz); 6.72 (d, 1H, ArH, J = 8.5 Hz); 7.12 (d, 1H, ArH, J = 8.0 Hz); 7.27 (t, 1H, A–H, J = 8.0 Hz); 7.35 (d, 1H, ArH, J = 8.0 Hz). CIMS m/z: 296 (M + H$^+$, 100%).

***N*-(*p*-Methylbenzyl)-*N*-(*p*-toluenesulfonyl) glycine methyl ester (14).** *N*-(*p*-Toluene-sulfonyl) glycine methyl ester (80 g; 0.329 mol) was dissolved in 1000 mL of acetone. The solution was placed under argon and 2.46 g (5 mol%) of sodium iodide, and 45.47 g (0.329 mol) of potassium carbonate were added. After 30 min at reflux, 52.3 mL (55.49 g, 0.395 mol) of 4-methylbenzyl chloride was added. Reflux was maintained for 6 h, and then the mixture was cooled, filtered, and concentrated. The residue was dissolved in 300 mL of dichloromethane and washed extensively with water. The organic layer was then dried ($MgSO_4$), filtered and evaporated. The residue was crystallized from ethyl acetate–hexane to yield 87.3 g (76%): mp 62–64 °C; ^{1}H NMR (500, $CDCl_3$): δ 2.30 (s, 3H, Ar–CH_3); δ 2.42 (s, 3H, Ar–CH_3); δ 3.52 (s, 3H, O–CH_3); δ 3.88 (s, 2H, CO–CH_2–N); δ 4.42 (s, 2H, Ar–CH_2–N); δ 7.09 (s, 4H, ArH); δ 7.31 (d, 2H, ArH, J = 8 Hz); δ7.75 (d, 2H, ArH, J = 8 Hz). CIMS m/z: 348, (M + H, 50%); 192 (M + H–$SO_2C_7H_7$, 22%), 105, (C8H9, 100%). Anal. ($C_{18}H_{21}NO_4S$) C, H, N.

***N*-(*o*-Methylbenzyl)-*N*-(*p*-toluenesulfonyl) glycine methyl ester (15).** *N*-(*p*-Toluenesulfonyl) glycine methyl ester (70 g; 0.288 mol) was dissolved in 1000 mL of acetone. The solution was placed under argon and then 2.15 g (5 mol%) of sodium iodide and 39.70 g (0.288 mol) potassium carbonate were added. After 30 min at reflux, 45.6 mL (48.47 g, 0.344 mol) of 2-methylbenzyl chloride was added. Reflux was maintained for 6 h, and then the mixture was cooled, filtered, and concentrated. The residue was dissolved in 300 mL of dichloromethane and washed extensively with water. The organic layer was then dried ($MgSO_4$), filtered and concentrated. The residue was crystallized from ethyl acetate–hexane to yield 90.2 g (90%): mp 66–68 °C; ^{1}H NMR (500, $CDCl_3$): δ 2.30 (s, 3H, Ar–CH_3); 2.42 (s, 3H, Ar–CH_3); 3.52 (s, 3H, O–CH_3); 3.88 (s, 2H, CO–CH_2–N); 4.42 (s, 2H, Ar–CH_2–N); 7.09 (s, 4H, ArH); 7.31 (d, 2H, ArH, J = 8 Hz); 7.75 (d, 2H, ArH, J = 8.0 Hz). CIMS m/z: 348, (M + H, 100%); 192 (M + H–$SO_2C_7H_7$, 22%), 105, (C8H9, 62%). Anal. ($C_{18}H_{21}NO_4S$) C, H, N.

***N*-(*p*-Methylbenzyl)-*N*-(*p*-toluenesulfonyl) glycine (16).** To the ester **14** (70 g; 0.2 mol), 400 mL of 10% sodium hydroxide solution was added and the mixture was stirred vigorously at room temperature overnight with a mechanical stirrer. The mixture was then acidified and extracted with dichloromethane (3×150 mL). The organic layers were dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was crystallized from ethyl acetate–hexane to yield 57.2 g (85%): mp 142–144 °C; ^{1}H NMR (500, $CDCl_3$): δ 2.26 (s, 3H, Ar–CH_3); 2.439 (s, 3H, Ar–CH_3); 3.76 (s, 2H, CO–CH_2–N); 4.33 (s, 2H, Ar–CH_2–N); 7.07 (d, 2H, ArH, J = 8 Hz); 7.12 (d, 2H, ArH, J = 8.0 Hz); 7.39 (d, 2H, ArH, J = 8.0 Hz); 7.73 (d, 2H, ArH, J = 8.0 Hz). CIMS m/z: 334 (M + H, 40%); 178 (M + H-$SO_2C_7H_7$, 19%); 157 ($SO_2C_7H_7$, 18%); (105 (C_8H_9, 100%). Anal. ($C_{17}H_{19}NO_4S$) C, H, N.

***N*-(*o*-Methylbenzyl)-*N*-(*p*-toluenesulfonyl) glycine (17).** To the ester **15** (70 g; 0.2 mol), 400 mL of 10% sodium hydroxide solution was added and the mixture was stirred vigorously overnight at room temperature with a mechanical stirrer. The mixture was then acidified and extracted with dichloromethane (3×150 mL). The organic layers were dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was crystallized from ethyl acetate–hexane to yield 59.9 g (94%): mp 116–118 °C; ^{1}H NMR (500, $CDCl_3$): δ 2.28 (s, 3H, Ar–CH3); δ 2.41 (s, 3H, Ar–CH_3); δ 3.82 (s, 2H, CO–CH_2–N); 4.46 (s, 2H, Ar–CH_2–N); 7.06 (d, 1H, ArH, J = 8 Hz); 7.12 (t, 1H, ArH, J = 7.5 Hz); 7.136 (d, 2H, ArH, J = 7.5 Hz); 7.19 (d, 2H, Ar-H, J = 8 Hz); 7.296 (d, 2H, ArH, J = 8.5 Hz); 7.73(d, 2H, ArH, J = 8.5 Hz); 9.1 (bs, 1H, COOH). CIMS m/z: 334 (M + H, 63%); 178 (M + H–$SO_2C_7H_7$, 19%); 157 ($SO_2C_7H_7$, 100%); (105 (C_8H_9, 87%). Anal. ($C_{17}H_{19}NO_4S$) C, H, N.

6-Methyl-*N*-(*p*-toluenesulfonyl)-1,2,3,4-tetrahydroisoquinol-4-one (18). The acid **16** (62 g; 0.186 mol) was dissolved in 35 mL of thionyl chloride under an atmosphere of argon. After dissolution was complete the excess thionyl chloride was distilled off under vacuum. The residue was triturated with 500 mL of hexane. The solid residue was then dissolved in 200 mL of dry dichloromethane and the solution was added slowly to a stirred suspension of 74.4 g (0.558 mol) of aluminum chloride in 200 mL of dry dichloromethane cooled to −78 °C under argon. The mixture was allowed to warm to −11 °C over 4 h and it was then carefully added to 2.0 L of an HCl–ice mixture and stirred for 30 min. The layers was separated, and the aqueous layer was extracted with dichloromethane (3×300 mL). The organic layers then were washed with water, and dried ($MgSO_4$). The solvent was filtered, evaporated, and the residue was crystallized from dichloromethane–diethyl ether to yield 41 g (70%): mp 150–152 °C; ^{1}H NMR (500, CDCl3): δ 2.32 (s, 3H, Ar–CH_3); 2.37 (s, 3H, Ar–CH_3); 3.96 (s, 2H, CO–CH_2–N); 4.44 (s, 2H, Ar–CH_2–N); 7.11 (d, 1H, ArH, J = 8 Hz); 7.23 (d, 1H, ArH, J = 8 Hz); 7.23 (d, 1H, ArH, J = 8 Hz); 7.61 (d, 2H, ArH, J = 8 Hz); 7.66 (d, 2H, ArH, J = 8 Hz). Anal. ($C_{17}H_{17}NO_3S$) C, H, N.

8-Methyl-*N*-(p-toluenesulfonyl)-1,2,3,4-tetrahydroisoquinol-4-one (19). The acid **17** (58 g; 0.174 mol) was dissolved in 35 mL of thionyl chloride under an atmosphere of argon. After dissolution was complete the excess thionyl chloride was distilled off under vacuum. The residue was triturated with 500 mL of hexane. The solid residue then was dissolved in 200 mL of dry dichloromethane and the solution was added slowly to a stirred suspension of 69.6 g (0.522 mol) of aluminum chloride in 200 mL of dry dichloromethane cooled to −78 °C under argon. The mixture was allowed to warm to −11 °C over 4 h and was then carefully added to 2.0 L of an HCl–ice mixture and stirred for 30 min. The layers was separated and the aqueous layer was extracted with dichloromethane (3×300 mL). The organic layers then were washed with water and dried ($MgSO_4$). The solvent was filtered, evaporated, and the residue was crystallized from dichloromethane–diethyl ether to yield 38.3 g (70%): mp 148–150 °C; ^{1}H NMR (500, $CDCl_3$): δ 2.31 (s, 3H, Ar–CH_3); 2.35 (s, 3H, Ar–CH_3); 3.97 (s, 2H, CO–CH_2–N); 4.43 (s, 2H, Ar–CH_2–N); 7.21 (m, 4H, ArH,); 7.34 (d, 1H, ArH, J = 7.5 Hz); 7.605 (d, 2H, ArH, J = 8 Hz); 7.72 (d, 1H, ArH, J = 8 Hz). CIMS m/z: 316 (M + H, 100%); 160 (M + H– $SO_2C_7H_7$, 36%). Anal. ($C_{17}H_{17}NO_3S$) C, H, N.

***N*-(*p*-Toluenesulfonyl)-6-methyl-4-(trifluoromethylsulfonyloxy)-1,2-dihydro-isoquinoline (20).** A round bottomed flask was charged with 200 mL of freshly distilled THF under argon. Next, 4 mL (2.89 g, 28.56 mmol) of diisopropylamine was added, followed by 17.8 mL of *n*-BuLi (1.6 M solution in hexane). After 5 min, the mixture was cooled to −78 °C, and a solution of isoquinolone **18** (7.5 g; 23.8 mmol) in 100 mL of dry THF was added dropwise. The mixture was stirred for 1 h and then a solution of 1-[*N,N*-bis(trifluoromethylsulfonyl)]amino-5-chloropyridine (10 g, 25.47 mmol) in 100 mL of dry THF was slowly added. The mixture was stirred, and left to warm to room temperature overnight. The reaction was quenched with 15 mL of water and concentrated, and then diluted with 500 mL of diethyl ether. The organic layers were washed with 2 N sodium hydroxide solution, dried ($MgSO_4$), then filtered. The residue after solvent evaporation was subjected to column chromatography (neutral alumina, 5% ethyl acetate–hexane) to afford 8.2 g (77%) of the triflate: mp 70–72 °C; ^{1}H NMR (500, DMSO–d_6): δ2.32 (s, 3H, Ar–CH_3); 2.37 (s, 3H, Ar–CH_3); 4.59 (s, 2H, Ar–CH_2–N); 6.91 (s, 1H, CO(Tf)=CH-N); 7.14 (d, 1H, ArH, J = 8 Hz); 7.21 (d, 1H, ArH, J = 8 Hz); 7.33 (d, 1H, ArH, J = 8 Hz); 7.43 (d, 2H, ArH, J = 8 Hz); 7.78 (d, 2H, Ar–H, J = 8 Hz). CIMS m/z: 448 (M + 1); 292 (M–$SO_2C_7H_7$); 276 (M–OSO_2CF_3). Anal. ($C_{16}H_{16}F_3NO_5S_3$) C, H, N.

***N*-(*p*-Toluenesulfonyl)-8-methyl-4-(trifluoromethylsulfonyloxy)-1,2-dihydro-isoquinoline (21).** A round bottomed flask was charged with 300 mL of freshly distilled THF and was flushed with argon. Next, 4 mL (5.78 g, 57.12 mmol) of diisopropylamine was added, followed by 35.6 mL of *n*-BuLi (1.6 M solution in hexane). After 5 min, the mixture was cooled to −78 °C and a solution of isoquinolone **19** (15 g; 47.6 mmol) in 200 mL of dry THF was added dropwise. The mixture was stirred for 1 h and then a solution of 1-[*N,N*-bis(trifluoro-methylsulfonyl)]amino-5-chloropyridine (20 g, 50.94 mmol) in 150 mL of dry THF was added slowly. The mixture was stirred and left to warm to room temperature overnight. The reaction was quenched with 50 mL of water and concentrated, and then diluted with 500 mL of diethyl ether. The organic layers were washed with cold 2 N sodium hydroxide solution and then dried ($MgSO_4$). The residue after filtration and solvent evaporation was subjected to column chromatography over neutral alumina (5% ethyl acetate–hexane) to afford 16.35 g (77%) of the triflate: mp 76–78 °C; ^{1}H NMR (500, DMSO–$d6$): δ 2.32 (s, 3H, Ar–CH_3); 2.37 (s, 3H, Ar–CH_3); 4.59 (s, 2H, Ar–CH_2–N); 6.91 (s, 1H, CO(Tf) = CH–N); 7.14 (d, 1H, ArH, J = 8 Hz); 7.21 (d, 1H, ArH, J = 8 Hz); 7.33 (d, 1H, ArH, J = 8 Hz); 7.43 (d, 2H, ArH, J = 8 Hz); 7.78 (d, 2H, ArH, J = 8 Hz). CIMS m/z: 448 (M + 1); 292 (M–$SO_2C_7H_7$); 276 (M–OSO_2CF_3). Anal. ($C_{16}H_{16}F_3NO_5S_2$) C, H, N.

4-(3,4-Dimethoxy-2-methoxymethoxymethylphenyl)-6-methyl-(*N*-*p*-toluene-sulfonyl)-1,2-dihydroisoquinoline (22). To a solution of triflate 20 (4 g, 8.94 mmol) in 50 mL of degassed toluene, tetrakis(triphenylphosphine)palladium(0) (0.5 g, 5 mol%) was added. After 5 min, the boronic acid 13 (2.98 g, 11.62 mmol), potassium bromide (3.19 g, 26.8 mmol), and potassium hydroxide (1.5 g, 26.8 mmol) were added and the mixture was heated to 90 °C for 1 h. The mixture was then allowed to cool to room temperature, it was diluted with 500 mL of toluene, and the resulting suspension was washed with 2 N sodium hydroxide solution (3×70 mL). The organic layer was dried ($MgSO_4$) and then filtered. The residue after evaporation of the solvent was subjected to column chromatography (neutral alumina, 30% ethyl acetate-hexane) to afford 4.1 g (90%) as a white solid. An analytical sample was crystallized from ethyl acetate–hexane: mp 116–118 °C; ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.08 (s, 3H, Ar–CH_3); 2.36 (s, 3H, Ar–CH_3); 2.95 (s, 3H, OCH_3); 3.77 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 3.97 (d, 1H, Ar–CH_2–O, J = 9.5 Hz); 4.3 (d, 1H, O–CH_2–O, J = 4.0 Hz); 4.6 (m, 2H, Ar–CH_2–N and O–CH_2–O); 4.64 (d, 1H, Ar–CH_2–N, J = 14.0 Hz); 6.26 (s, 1H, Ar–C=CH–N); 6.65 (s, 1H, ArH); 6.91 (d, 1H, ArH, J = 8.5 Hz); 6.98 (d, 1H, ArH, J = 7.0 Hz); 7.08 (d, 1H, ArH, J = 8.5 Hz); 7.155 (2, 1H, ArH, J = 7.5 Hz); 7.41 (d, 2H, Ar–H, J = 7.5 Hz); 7.75 (d, 2H, Ar–H, J = 7.5 Hz). CIMS m/z: 509 (M^+, 32%); 478 (M^+-OCH_3, 32%); 448 (M^+–OCH_2OCH_3, 77%); 292, (M^+–OCH_2OCH_3–$SO_2C_7H_7$, 100%); 276. Anal. ($C_{28}H_{31}NO_6S$) C, H, N.

4-(3,4-Dimethoxy-2-methoxymethoxymethylphenyl)-8-methyl-(*N*-*p*-toluenesulfonyl)-1,2-dihydroisoquinoline (23). To a solution of triflate 21 (14.6 g, 32.63 mmol) in 150 mL of degassed toluene, tetrakis (triphenylphosphine) palladium(0) (1.82 g, 5 mol%) was added. After 5 min, the boronic acid 13 (10.86 g, 42.42 mmol), potassium bromide (11.5 g, 96.6 mmol), and potassium hydroxide (4.5 g, 80.2 mmol) were added and the mixture was heated to 90 °C for 1 h. The reaction mixture was then allowed to cool to room temperature, it was diluted with 1000 mL of toluene, and the resulting suspension was

washed with 2 N sodium hydroxide solution (3×100 mL). The organic layer was dried ($MgSO_4$) and then filtered. The residue after evaporation of the solvent was subjected to column chromatography (neutral alumina, 30% ethyl acetate–hexane) to afford 15.35 g (92%) of a white solid. An analytical sample was crystallized from ethyl acetate–hexane: mp 112–114 °C; ^{1}H NMR (500 MHz, DMSO–d_6): δ 2.26 (s, 3H, Ar–CH_3); 2.35 (s, 3H, Ar–CH_3); 2.97 (s, 3H, OCH_3); 3.76 (s, 3H, OCH_3); 3.84 (s, 3H, OCH_3); 3.97 (d, 1H, Ar–CH_2–O, J = 10.5 Hz); 4.32 (s, 1H, O–CH_2–O); 4.36 (d, 1H, O–CH_2–O, J = 10 Hz); δ4.41 (d, 1H, Ar–CH_2–N, J = 14.0 Hz); 4.71 (d, 1H, Ar–CH_2–N, J = 14.0 Hz); 6.24 (d, 1H, ArH, J = 7.5 Hz); 6.63 (s, 1H, Ar–C=CH–N); 6.89 (dd, 1H, ArH, J = 8.5 Hz, 1 Hz); 6.96 (t, 1H, ArH, J = 7.5 Hz); 7.02 (d, 1H, ArH, J = 7.5 Hz); 7.09 (dd, 1H, ArH, J = 8.5 Hz, 2 Hz); 7.4 (2, 1H, ArH, J = 7.5 Hz); 7.41 (d, 2H, ArH, J = 7.5 Hz); 7.75 (d, 2H, ArH, J = 7.5 Hz). CIMS m/z: 509 (M^+, 18%); 478 (M^+–OCH_3, 32%); 448 (M^+–OCH_2OCH_3, 95%); 292, (M^+–OCH_2OCH_3–$SO_2C_7H_7$, 100%); 276. Anal. ($C_{28}H_{31}NO_6S$) C, H, N.

4-(3,4-Dimethoxy-2-hydroxymethylphenyl)-6-methyl-(*N*-*p*-toluenesulfonyl)-1,2,3,4-tetrahydroisoquinoline (24). The enamide **22** (4 g, 7.85 mmol) was dissolved in 200 mL of ethanol containing 2 g of Pd/C (10%). The mixture was shaken at 50 psi in a Parr hydrogenator for 72 h. The catalyst was removed by filtration and the solvent was evaporated. The residue was taken up into 30 mL of THF, 30 mL of 3 N HCl was added, and the reaction was maintained at 50 °C for 7 h. The reaction was then allowed to cool and was diluted with 50 mL of water and extracted with dichloromethane (3×100 mL). The combined organic extracts were then dried ($MgSO_4$), filtered and the solvent was evaporated. The residue was crystallized from ethyl acetate–hexane to afford 2.75 g (75%) as a white solid: mp 138–149 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.15 (s, 3H, Ar-CH_3); 2.38 (s, 3H, Ar-CH_3); 3.19 (dd, 1H, Ar_2CH-CH_2-N, J = 12.0 Hz, 7.0 Hz); 3.59 (dd, 1H, Ar_2CH-CH_2-N, J = 12.0 Hz, 5.0 Hz); 3.82 (s, 3H, OCH_3); 3.89 (s, 3H, OCH_3); 4.26 (d, 1H, Ar-CH_2-N, J = 15.0 Hz); 4.32 (d, 1H, Ar-CH_2-N, J = 15 Hz); 4.53 (t, 1H, Ar-CH-Ar, J = 6.5 Hz); 4.72 (bs, 2H, Ar-CH_2-OH); 6.57 (d, 1H, ArH, J = 8.5 Hz); 6.64 (s, 1H, ArH); 6.73 (d, 1H, ArH, J = 8.5 Hz); 6.96 (s, 2H, ArH); 7.26 (d, 2H, ArH, J = 8.0 Hz); 7.63 (d, 2H, ArH, J = 8.0 Hz). CIMS m/z: 450 ($M+H^+-H_2O$, 100%). Anal. ($C_{26}H_{29}NO_5S$) C, H, N.

4-(3,4-Dimethoxy-2-hydroxymethylphenyl)-8-methyl-(*N*-*p*-toluenesulfonyl)-1,2,3,4-tetrahydroisoquinoline (25). The enamide **23** (4 g, 7.85 mmol) was dissolved in 200 mL of ethanol and to the solution was added 2 g Pd/C (10%). The mixture was shaken at 50 psi in a Parr hydrogenator for 72 h. The catalyst was removed by filtration and the solvent was then evaporated. The residue was taken up into 30 mL of THF, 30 mL of 3 N HCl was added, and the reaction was maintained at 50 °C for 7 h. The reaction was then allowed to cool and was diluted with 50 mL of water and extracted with dichloromethane (3×100 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was crystallized from ethyl acetate–hexane to afford 2.89 g (79%) as a white solid: mp 162–164 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.23 (s, 3H, Ar–CH_3); 2.39 (s, 3H, Ar–CH_3); δ 3.23 (dd, 1H, Ar_2CH–CH_2–N, J = 12.0 Hz, 7.0 Hz); 3.6 (dd, 1H, Ar_2CH–CH_2–N, J = 12.0, 5.0 Hz); 3.81 (s, 3H, OCH_3); 3.89 (s, 3H, OCH_3); 4.18 (d, 1H, Ar–CH_2–N, J = 15.0 Hz); 4.28 (d, 1H, Ar–CH_2–N, J = 15.0 Hz); 4.56 (t, 1H, Ar–CH–Ar, J = 6.5 Hz); 4.71 (bs, 2H, Ar–CH_2–OH); 6.56 (d, 1H, ArH, J = 8.5 Hz); 6.69 (t, 1H, ArH, J = 8.0 Hz); 6.72 (d, 1H, ArH, J = 8.5 Hz); 6.99 (m, 2H, ArH); 7.27 (d, 2H, ArH, J = 8.0 Hz); 7.65 (d, 2H, ArH, J = 8.0 Hz). CIMS m/z: 458 ($M+H^+$, 35%); 450 ($M+H^+$–H_2O, 100%). Anal. ($C_{26}H_{29}NO_5S$). C, H, N.

8,9-Dimethoxy-6-methyl-(*N*-*p*-toluenesulfonyl)-2,3,7,11b-tetrahydro-1*H*-naphth-[1,2,3-*de*]isoquinoline (26). To 50 mL of sulfuric acid at −20 °C stirred with a mechanical stirrer was added the solid alcohol **24** (500 mg, 1.07 mmol) in portions. After 30 min at −20 °C the mixture was poured into 150 mL of ice, extracted with dichloromethane (3×100 mL), and the combined extracts were washed with aqueous 1 N NaOH solution (2×50 mL). The organic layers were dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was subjected to column chromatography (silica gel, 30% ethyl acetate–hexane) to afford 202 mg (42%) as a white solid. This reaction was repeated two times to accumulate more of the product: mp 192–194 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ2.39 (s, 3H, Ar–CH_3); 2.41 (s, 3H, Ar–CH_3); 2.8 (t, 1H, Ar_2–CH–CH_2–N, J = 11.0 Hz); 3.23 (dd, 1H, Ar–CH_2–Ar, J = 18.0, 3.5 Hz); 3.834 (s, 3H, OCH_3); 3.837 (s, 3H, OCH_3); 3.88 (m, 2H, Ar_2CH–CH_2–N and Ar–CH_2–N); 4.48 (d, 1H, Ar–CH_2–Ar, J = 18.0 Hz); 4.70 (m, 2H, Ar–CH_2–N and Ar–CH–Ar); 6.78 (d, 1H, ArH, J = 9.0 Hz); 6.84 (d, 1H, ArH, J = 8.0 Hz); 6.88 (d, 1H, ArH, J = 8.5 Hz); 7.00 (d, 1H, ArH, J = 8.0 Hz); 7.33 (d, 2H, ArH, J = 8.0 Hz); 7.8 (d, 2H, ArH, J = 8.0 Hz). CIMS m/z: 450 ($M+H^+$, 29%); 198 ($C_9H_{12}O_2S$, 100%). Anal. ($C_{26}H_{29}NO_5S$) C, H, N.

8,9-Dimethoxy-4-methyl-(*N*-*p*-toluenesulfonyl)-2,3,7,11b-tetrahydro-1*H*-naphth[1,2,3-*de*]isoquinoline (27). To 50 mL of sulfuric acid at −20 °C stirred with a mechanical stirrer was added the alcohol **25** (500 mg, 1.07 mmol) as a solid in portions. After 30 min at −20 °C the mixture was poured into 150 mL of ice, extracted with dichloromethane (3×100 mL) and the combined extracts were washed with aqueous 1 N NaOH solution (2×50 mL). The organic layers were dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was subjected to column chromatography (silica gel, 30% ethyl acetate-hexane) to afford 300 mg (62%) as a white solid. This reaction was repeated several times to accumulate more of the product: mp 160–162 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.19 (s, 3H, Ar–CH_3); 2.44 (s, 3H, Ar–CH_3); 2.79 (t, 1H, Ar_2–CH–CH_2–N, J = 11.5 Hz); 3.496 (dd, 1H, Ar–CH_2–Ar, J = 18.0 Hz, 3.5 Hz); 3.76 (d, 1H, Ar–CH_2–Ar, J = 16.0 Hz); 3.84 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 3.93 (m, 1H, Ar_2CH–CH_2–N); 4.29 (d, 1H, Ar–CH_2–Ar, J = 18.0 Hz); 4.73 (m, 2H, Ar_2CH–CH_2–N and Ar–CH–Ar); 6.78 (d, 1H, ArH, J = 8.5 Hz); 6.89 (d, 1H, ArH, J = 8.0 Hz); 6.99 (d, 1H, ArH, J = 7.0 Hz); 7.13 (d, 1H, ArH, J = 7.5 Hz); 7.36 (d, 2H, ArH,

$J=8.0$ Hz); 7.82 (d, 2H, ArH, $J=8.0$ Hz). CIMS m/z: 450 ($M+H^+$, 35%); 198 ($C_9H_{12}O_2S$, 100%). Anal. ($C_{26}H_{29}NO_5S$) C, H, N.

8,9-Dihydroxy-6-methyl-2,3,7,11b-tetrahydro-1*H*-naphth[1,2,3-*de*]isoquinoline Hydrobromide (5). The cyclized compound **26** (400 mg, 0.89 mmol) was dissolved in 100 mL of anhydrous methanol and 5.5 g of 6% sodium-amalgam and 505 mg (3.56 mmol) of Na_2HPO_4 were added. The reaction mixture was maintained at reflux overnight and the mixture was then decanted into an Erlenmeyer flask, diluted with 200 mL of water, and extracted with dichloromethane (3×200). The combined extracts were dried (Na_2SO_4), and the solvent was filtered and evaporated. The residue was purified with radial chromatography (Chromatotron; silica gel, 50% ethyl acetate–hexane, under ammonia) to yield 211 mg (73%) of a yellow oil that was immediately used for the next reaction. The amine (150 mg, 0.51 mmol) was dissolved in 50 mL of dichloromethane under argon and the solution was cooled to −78 °C. To this was added 2.6 mL (2.6 mmol) of boron tribromide (1 M solution in dichloromethane) and the mixture was stirred for 2 h. The cooling bath was removed and stirring was continued overnight at room temperature. The suspension was re-cooled to −78 °C, 20 mL of anhydrous methanol was added, the solution was stirred for 30 min, then the solvents were evaporated. Three more portions of anhydrous methanol were added, and evaporated to destroy all the remaining BBr_3. The residue then was crystallized from ethanol:ethyl acetate to afford 109 mg (61%): mp ^{1}H NMR (500, CD_3OD): δ 2.42 (s, 3H, Ar–CH_3); 3.27 (dd, 1H, Ar–CH_2–Ar, $J=18.0$ Hz, 3.0 Hz); 3.39 (t, 1H, Ar_2–CH–CH_2–N, $J=13.0$ Hz); 4.04 (m, 1H, Ar–CH–Ar); 4.33 (dd, 1H, Ar_2–CH–CH_2–N, $J=13.0$ Hz, 12.0 Hz); 4.38 (s, 2H, Ar–CH_2–N); 4.53 (d, 1H, Ar–CH_2–Ar, $J=18.0$ Hz); 4.81 (d, 1H, Ar–CH_2–Ar, $J=17.5$ Hz; 1.5 Hz); 6.6 (d, 1H, ArH, $J=8.5$ Hz); 6.73 (d, 1H, ArH, $J=8.5$ Hz); 7.03 (d, 1H, Ar-H, $J=8.0$ Hz); 7.16 (d, 1H, ArH, $J=8.0$ Hz); 7.17 (d, 1H, ArH, $J=8$ Hz). CIMS m/z: 268 ($M+H^+$, 100%). Anal. ($C_{17}H_{18}BrNO_2$) C, H, N.

8,9-Dihydroxy-4-methyl-2,3,7,11b-tetrahydro-1*H*-naphth[1,2,3-*de*]isoquinoline Hydrobromide (6). The cyclized compound **27** (600 mg, 1.33 mmol) was dissolved in 100 mL of anhydrous methanol and to the solution was added 8.2 g of 6% sodium-amalgam and 755 mg (5.32 mmol) of Na_2HPO_4 was added. The reaction was maintained at reflux overnight and the mixture was then decanted into an Erlenmeyer flask, diluted with 200 mL of water, and extracted with dichloromethane (3×200). The combined extracts were dried (Na_2SO_4), the solvent was filtered, and evaporated. The residue was purified with radial chromatography (silica gel, 50% ethyl acetate–hexane, over ammonia) to yield 298 mg (76%) of a yellow oil that was immediately used for the next reaction. This reaction was repeated two times to accumulate more of the amine. The amine (300 mg, 1.02 mmol) was dissolved in 100 mL of dichloromethane, and cooled to −78 °C under argon. To this was added 5.1 mL (5.1 mmol) of boron tribromide (1 M solution in dichloromethane). After stirring for 2 h at −78 °C the cooling bath was removed, and the reaction mixture was allowed to stir at room temperature overnight. The suspension was then re-cooled to −78 °C, 20 mL of anhydrous methanol was added, and the solution was stirred for 15 min at room temperature. The solvents were evaporated, and three more portions of anhydrous methanol were added and evaporated to destroy all the remaining BBr_3. The residue was then crystallized from ethanol–ethyl acetate to afford 260 mg (73%): mp 266 °C dec; ^{1}H NMR (500, CD_3OD): δ 2.26 (s, 3H, Ar–CH_3); 3.4 (t, 1H, Ar_2–CH–CH_2–N, $J=11.5$ Hz); 4.34 (d, 1H, Ar–CH_2–Ar, $J=19.0$ Hz); 4.03 (m, 1H, Ar–CH–Ar); 4.35 (m, 3H, Ar_2–CH–CH_2–N, Ar–CH_2–Ar and Ar–CH_2–Ar); 4.43 (d, 1H, Ar–CH_2–N, $J=15.5$ Hz); 6.58 (d, 1H, ArH, $J=8.5$ Hz); 6.7 (d, 1H, ArH, $J=8.0$ Hz); 7.1 (d, 1H, ArH, $J=8.0$ Hz); 7.22 (d, 1H, ArH, $J=8.0$ Hz); 7.17 (d, 1H, ArH, $J=8.0$ Hz). PDMS m/z: 268. Anal. ($C_{17}H_{18}BrNO_2$) C, H, N.

***N*-Allyl-8,9-dimethoxy-4-methyl-2,3,7,11b-tetrahydro-1*H*-naphth[1,2,3-*de*]-isoquinoline (28).** To the amine intermediate resulting from the detosylation of 27 (300 mg, 1.02 mmol) in 50 mL of acetone, 141 mg (1.02 mmol) of potassium carbonate and 0.1 mL (139 mg, 1.15 mmol) of allyl bromide were added. The reaction mixture was allowed to stir under argon for 5 h, then it was diluted with 100 mL of diethyl ether, filtered, and concentrated. The residue was purified with radial chromatography (silica gel, 10% ethyl acetate–hexane under ammonia) to afford 320 mg (94%) of a clear oil that was used immediately in the next reaction: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.19 (s, 3H, CH_3); 2.56 (t, 1H, Ar_2–CH–CH_2–N, $J=11.5$ Hz); 3.39 (dt, 1H, N–CH_2–CH=CH_2, $J=7.0$, 1.0 Hz); 3.26 (d, 1H, Ar–CH_2–N, $J=17.0$ Hz); 3.49 (dd, 1H, Ar–CH_2–Ar, $J=18.0$ Hz, 3.0 Hz); 3.82 (m, 1H, Ar_2–CH–CH_2–N); 3.86 (s, 6H, OCH_3); 3.91 (dd, 1H, Ar_2CH–CH_2–N, $J=11.5$ Hz, 6.0 Hz); 4.01 (d, 1H, Ar–CH_2–N, $J=17.0$ Hz); 4.32 (d, 1H, Ar–CH_2–Ar, $J=18.0$ Hz); 5.28 (dd, 1H, N–CH_2–CH=CH_2, $J=11$ Hz, 1.5 Hz); 5.38 (dd, 1H, N–CH_2–CH=CH_2, $J=17.5$ Hz, 1.5 Hz); 6.06 (m, 1H, N–CH_2–CH=CH_2); 6.77 (d, 1H, ArH, $J=8.0$ Hz); 6.94 (d, 1H, ArH, $J=8.0$ Hz); 6.98 (d, 1H, ArH, $J=8.0$ Hz); 7.12 (d, 1H, ArH, $J=8.0$ Hz). CIMS m/z: 336, ($M+H^+$, 100%), HRMS: calcd: 335.1885. Found: 335.1876.

***N*-Allyl-8,9-dihydroxy-4-methyl-2,3,7,11b-tetrahydro-1H-naphth[1,2,3-*de*]-isoquinoline Hydrobromide (7).** The *N*-allyl amine 30 (162 mg, 0.48 mmol) was dissolved in 40 mL of dichloromethane under argon. The solution was cooled to −78 °C, and 2.5 mL (2.5 mmol) of boron tribromide (1 M solution in CH_2Cl_2) was added. The mixture was allowed to stir for 2 h, then the cooling bath was removed and the reaction was stirred at room temperature overnight. The reaction mixture was then re-cooled to −78 °C, 10 mL of anhydrous methanol was added, the mixture was stirred for 15 min, then evaporated to dryness. Methanol was added and evaporated three times to quench the boron species. The residue was crystallized from ethanol–ethyl acetate to afford 115 mg (62%) of a tan solid: mp 258 °C dec; ^{1}H NMR (300 MHz, CD_3OD): δ 1.97 (s, 3H, CH_3); 3.12 (m, 2H, Ar_2–CH–CH_2–N and Ar–CH_2–Ar); 3.84 (m, 2H, N–

CH_2–CH=CH_2 and Ar_2–CH–CH_2–N); 3.97 (d, 1H, Ar–CH_2–N, J = 17.0 Hz); 4.06 (d, 1H, Ar–CH_2–Ar, J = 18.0 Hz); 4.19 (dd, 1H, Ar_2–CH–CH_2–N, J = 12.5 Hz, 6.0 Hz); 4.32 (d, 1H, Ar–CH_2–N, J = 17.0 Hz); 5.45 (dd, 1H, N–CH_2–CH=CH_2, J = 11 Hz); 5.51 (d, 1H, , N–CH_2–CH=CH_2, J = 19.0 Hz); 5.9 (m, 1H, N–CH_2–CH=CH_2); 6.29 (d, 1H, ArH, J = 9.0 Hz); 6.41 (d, 1H, ArH, J = 9.0 Hz); 6.82 (d, 1H, ArH, J = 7.5 Hz); 6.92 (d, 1H, ArH, J = 7.5 Hz). CIMS m/z: 308, ($M+H^+$, 100%). Anal. ($C_{20}H_{22}BrNO_2$) C, H, N.

_N_-_n_-Propyl-8,9-dihydroxy-4-methyl-2,3,7,11b-tetrahydro-1H-naphth[1,2,3-_de_]isoquinoline Hydrobromide (8). The _N_-allyl amine 30 (148 mg, 0.44 mmol) was dissolved in 100 mL of ethanol, then shaken in a Parr hydrogenator at 50 psi for 3 h over 15 mg of Pd/C (10%). The catalyst was then filtered and the solvent was evaporated. After placing the residue under high vacuum for 8 h, it was dissolved in 100 mL of dichloromethane, placed under argon, then cooled to −78 °C. To the solution 2.2 mL (2.2 mmol) of boron tribromide (1M solution in dichloromethane) was added, and the mixture was stirred for 2 h. Then the cooling bath was removed, and the reaction was stirred at room temperature overnight. The reaction mixture was re-cooled to −78 °C, 15 mL of anhydrous methanol was added, the mixture was stirred for 15 min and then evaporated to dryness. Methanol was added, and evaporated three times to quench all the boron species in the reaction. The residue was crystallized from ethanol–ethyl acetate to afford 101 mg (59%) of a tan solid: mp 240 °C dec; ^{1}H NMR (300 MHz, CD_3OD): δ 1.10 (t, 1H, N–CH_2–CH_2–CH_3, J = 8.0 Hz); 1.97 (m, 1H, N–CH_2–CH_2–CH_3, J = 8.0 Hz); 2.23 (s, 3H, Ar–CH_3); 3.38–3.52 (m, 4H, Ar–CH_2–Ar, Ar_2–CH–CH_2–N and N–CH_2–CH_2–CH_2); 4.06 (m, 1H, Ar_2–CH–CH_2–N); δ4.25 (d, 1H, Ar–CH_2–N, J = 17.5 Hz); 4.32 (d, 1H, Ar–CH_2–Ar, J = 19.0 Hz); 4.47 (dd, 1H, Ar_2–CH–CH_2–N, J = 13.0 Hz, 6.0 Hz); 4.59 (d, 1H, Ar–CH_2–N, J = 17.5 Hz); 6.57 (d, 1H, ArH, J = 9.0 Hz); 6.65 (d, 1H, ArH, J = 9.0 Hz); 7.07 (d, 1H, ArH, J = 8.0 Hz); 7.2 (d, 1H, ArH, J = 8.0 Hz). CIMS m/z: 310 ($M+H^+$, 100%), HRMS: calcd: 309.1729. Found: 309.1721.

Pharmacology Methods

Materials

[^{3}H]-SCH23390 was synthesized according to the method of Wyrick et al.[32] Dihydrexidine, dinapsoline and their analogues were synthesized according to published methods.[17,20] R(+)-SCH23390 was purchased from Research Biochemicals, Inc. (Natick, MA), whereas chlorpromazine HCl was a gift of SmithKline Beecham. [^{3}H]-Spiperone was purchased from Amersham (Piscataway, NJ).

Cell cultures

D_{1A},[33] D_{2L},[34] or D_3[35] receptors expressed in C-6 glioma cells (courtesy of Dr. Kim Neve, Portland VA Medical Center) were used. The C-6 cells were grown in DMEM-H media containing 4500 mg/L glucose, L-glutamine, 5% fetal bovine serum supplemented with 1.9 μg/mL puromycin (D_{1A}) or 600 ng/mL G418 (D_{2L} and D_3). CHO-D_4 cells were a gift from NIH and grown in Ham's/F12 media containing 10% bovine serum, 1% MEM and supplemented with 50,000 U penicillin, 50 mg/L streptomycin, 10 mg/L gentamycin and 0.5 mg/mL G418. HEK cells were grown in DMEM containing 10% bovine serum. Cells were transiently transfected using Lipofectamine according to the manufacturer's protocol. All cells were maintained in a humidified incubator at 37 °C with 95% O_2 and 5% CO_2.

Membrane preparation for receptor binding studies

Cells were rinsed with hypoosmotic buffer (HOB; 1 mM Hepes, and 2 mM EDTA, pH 7.4), and then incubated with 7 mL HOB for 5–10 min at 4 °C. Cells were then scraped off the bottom of the flask using a rubber policeman, collected into 50 mL tubes, and centrifuged at 28,000 g at 4 °C for 20 min. The resulting pellet was resuspended in binding buffer (50 mM Hepes, pH 7.4), homogenized with a Brinkman Polytron on a setting of 5, and either used immediately or stored in 1 mL aliquots at −80 °C until use. Protein content was assessed using the BCA protein assay reagent (Pierce, Rockford, IL).

Radioreceptor binding studies

Competition binding studies were done to evaluate the affinity of the different agonists cell lines. Membranes were incubated with 0.3 nM [^{3}H]-SCH23390 or 0.07 nM [^{3}H]spiperone in 50 mM Hepes, 4 mM $MgCl_2$, 0.01% ascorbic acid, pH 7.4 and increasing concentrations of competing drug. Tubes were run in triplicate in a final volume of 500 μL. After incubation at 37 °C for 15 min, 96-tube plates were filtered rapidly through Packard 96 GF/C filters, and rinsed with 5 mL of ice-cold wash buffer using a Packard Micro Cell Harvester (Packard Instruments, Downers Grove, IL). Filters were allowed to dry, and then 30 μL of Microscint-20 scintillation cocktail was added to each filter well. Each plate was sealed using a microplate heat sealing film on a microplate sealer and then counted on a Packard Topcount Microplate scintillation counter (Packard, Downers Grove, IL).

Functional assays; measurement of cAMP production

Adenylate cyclase assays in rat striatal or cell homogenates were performed as described by Watts et al.[21] A 20 μL aliquot of a 2.5 mg/mL membrane suspension was added to each reaction tube. Baseline values of cAMP were subtracted from the total amount of cAMP produced for each drug condition. Data for each drug were expressed as fmol cAMP per sample.

Data analysis

Dose–response curves were analyzed by nonlinear regression using an algorithm for sigmoid curves in the curve-fitting program Prism (Graphpad, Inc; San

Diego, CA). For all of the assays incorporated into these studies, analysis of the residuals indicated an excellent fit with r values being greater than 0.90. Data are expressed as apparent inhibition constants ($K_{0.5}$), and where the Hill slopes were significantly less than 1, this was so noted.

Elemental analysis data

Compd	C (%)		H (%)		N (%)	
	Calcd	Found	Calcd	Found	Calcd	Found
(±)-**3**	60.97	60.82	5.39	5.01	3.74	3.73
(±)-**4**	60.65	60.28	5.89	6.06	3.72	3.59
(±)-**5**	58.63	58.33	5.21	5.17	4.02	3.84
(±)-**6**	58.63	58.28	5.21	5.04	4.02	3.99
(±)-**7**	61.86	61.50	5.71	5.68	3.61	3.51
(±)-**8**	61.54	59.11	6.20	5.87	3.59	3.39
14	62.23	62.22	6.09	6.05	4.03	4.08
15	62.23	62.31	6.09	6.05	4.03	4.07
16	61.24	61.00	5.47	5.66	4.20	4.25
17	61.24	61.38	5.74	5.76	4.20	4.12
18	64.74	64.74	5.43	5.33	4.44	4.44
19	64.74	64.48	5.43	5.29	4.44	4.57
20	48.32	48.41	3.60	3.25	3.13	3.47
21	48.32	48.48	3.60	3.55	3.13	3.21
22	65.99	65.84	6.13	6.03	2.75	2.60
23	65.99	65.60	6.13	6.09	2.75	2.69
24	66.79	66.60	6.25	6.45	3.00	2.97
25	66.79	66.67	6.25	6.27	3.00	2.93
26	69.46	69.49	6.05	5.91	3.12	3.06
27	69.46	69.48	6.05	6.01	3.12	3.08

Disclosure: Drs. Mailman and Nichols have a significant financial interest in DarPharma Inc, Chapel Hill, NC, the company that currently holds license rights to dinapsoline and its analogues. The interpretation and discussion in this article are those of the authors alone and do not reflect the views of DarPharma Inc, the University of North Carolina at Chapel HIll, or Purdue University.

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