Quinazolines as Inhibitors of Dihydrofolate Reductase. 3. Analogs of Pteroic and Isopteroic Acids[1,†]

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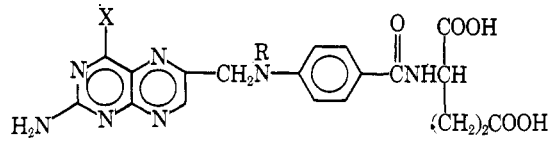
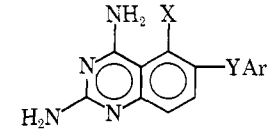
A series of 19 quinazoline analogs of pteroic and isopteroic acid was prepared with particular emphasis being placed upon carboxylic acid esters. Each compound was evaluated as an inhibitor of the dihydrofolate reductases from rat liver as well as from *Streptococcus faecium*. Several of the more potent inhibitors were found to be inactive against L1210 leukemia in mice at low dose levels and were lethal to mice at 100 mg/kg. Six compounds were also evaluated for antimalarial activity against *Plasmodium berghei* in mice. Three of these were found to be curative at higher levels, while the remaining compounds were found to be toxic.

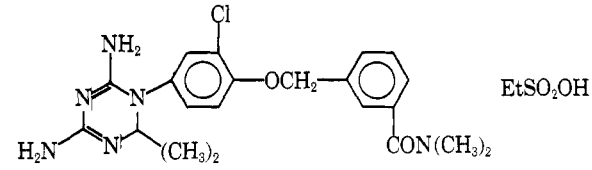
Antagonists of folic acid metabolism can be divided into two distinct classes which are generally referred to as classical and nonclassical. The former type closely resembles folic acid (**1a**) in structure, cross biological membranes by active transport, and is generally targeted for cancer chemotherapy. From a clinical standpoint the most important drug of this class is methotrexate (**1b**) which has been used with varying degrees of success in the treatment of choriocarcinoma, acute lymphoblastic leukemia, and Burkitt's lymphoma.[2] The cytotoxicity of **1b** has been shown by numerous investigations to be due primarily to potent inhibition of the enzyme dihydrofolate reductase. A number of tumors, however, are naturally resistant to this agent, while others become refractory despite initial sensitivity. Murine leukemia cells resistant to **1b** have been shown to have substantially elevated levels of dihydrofolate reductase.[3] Nevertheless, other factors, in particular alterations in the transport mechanism normally utilized by this drug, are no doubt involved with certain unresponsive cell lines. A second limitation with **1b** is that only low levels of drug appear in the central nervous system following systemic administration.[4] Therefore, it must be given intrathecally in the treatment of meningeal leukemias or neuroblastomas. Nonclassical antagonists of folic acid are devoid of an amino acid residue, enter cells by passive diffusion, and are often effective against pathogenic microorganisms such as plasmodia and certain folate synthesizing bacteria. Some important examples of this type are pyrimethamine and trimethoprim, as well as a variety of 2,4-diaminoquinazolines as exemplified in structure **2**.[5] Other nonclassical inhibitors of dihydrofolate reductase have been synthesized in an effort to develop agents which are effective against tumors resistant to **1b**. For example, the 4,6-diamino-1,2-dihydro-*s*-triazine derivative, **3**, as its ethanesulfonate salt, was highly effective in inhibiting Walker 256 carcinosarcoma and Dunning leukemia and yet was inactive against L1210 leukemia in mice.[6] Recent studies have demonstrated that the uptake of **3** by tumor cells is not affected by the presence of a large excess of **1b**, implying that these compounds enter cells via different pathways.[7]



1a, X = OH; R = H
b, X = NH_2; R = CH_3



2, X = H, Cl, CH_3; Y = S, SO, SO_2, CH_2NH, O



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In view of these considerations, a series of quinazoline analogs of pteroic and isopteroic acids was synthesized. Each of these was evaluated as an inhibitor of dihydrofolate reductase from rat liver as well as from *Streptococcus faecium*. Particular emphasis was placed upon ethyl and *n*-butyl esters since it was anticipated that these more lipophilic derivatives could readily gain entry into the central nervous system. In addition, nonclassical antagonists of this type might be expected to be efficacious against tumor lines resistant to **1b** because of impaired transport of that drug. Finally, a potent inhibitor of the mammalian dihydrofolate reductase which is absorbed percutaneously could prove to be of value as a topical antipsoriatic agent. It should be noted that **1b** can produce dramatic remissions in severe cases of psoriasis but that this drug must be administered systemically, thereby introducing the possibility of severe side effects.[8]

Chemistry. The physical properties and chemical structures of the compounds prepared for this study are summarized in Table I. The ethyl esters of the quinazolines having the isopteroic acid configuration (**4–6** and **16–18**) were prepared by condensing the appropriately substituted 6-NH_2 quinazoline[9] with ethyl 4-formylbenzoate.[10] The resulting anils were subsequently reduced with dimethylamine–borane in acetic acid.[11] The condensation reaction with 5-chloro-2,4,6-triaminoquinazoline proved to be the most difficult of these transformations. Even after heating for 26 hr at 110° in the presence of excess aldehyde together with 5A molecular sieves to remove water formed during the reaction, TLC analysis showed that unreacted amine was still present. A similar phenomenon was encountered with reac-

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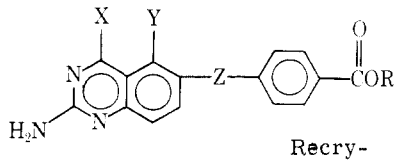
Table I. Properties of Quinazoline Analogs of Pteroic and Isopteroic Acids



No.	X	Y	Z	R	Mp, °C	Method	Yield, %	Recry-stn[a] medium	Formula[b]	I_{50}, μM[c] Rat liver[d]	I_{50}, μM[c] *S. faecium*[e]
4	NH_2	H	$NHCH_2$	C_2H_5	222–224[f]	A	58	I	$C_{18}H_{19}N_5O_2 \cdot H_2O$	0.073	0.046
5	NH_2	CH_3	$NHCH_2$	C_2H_5	204–205	A	26	I	$C_{19}H_{21}N_5O_2 \cdot H_2O$	0.0045	0.0014
6	NH_2	Cl	$NHCH_2$	C_2H_5	172–175	B	11	II	$C_{18}H_{18}ClN_5O_2$	0.013	0.0063
7	NH_2	H	CH_2NH	C_2H_5	216–218[g]	C	67	II	$C_{18}H_{19}N_5O_2 \cdot H_2O$	0.0063	0.021
8	NH_2	CH_3	CH_2NH	C_2H_5	256–258	C	28	III	$C_{19}H_{21}N_5O_2 \cdot 2H_2O$	0.0057	0.0051
9	NH_2	Cl	CH_2NH	C_2H_5	209–211	C	35	IV	$C_{18}H_{18}ClN_5O_2$	0.014	0.0023
10	NH_2	H	CH_2NH	n-C_4H_9	187–190	C	33	III	$C_{20}H_{23}N_5O_2$	0.026	0.030
11	NH_2	CH_3	CH_2NH	n-C_4H_9	213–215	C	15	III	$C_{21}H_{25}N_5O_2$	0.017	0.0071
12	NH_2	Cl	CH_2NH	n-C_4H_9	193–195	C	22	V	$C_{20}H_{20}ClN_5O_2$	0.014	0.021
13	NH_2	H	CH_2NH	H	235–240 dec	D	64	III	$C_{16}H_{15}N_5O_2 \cdot 2H_2O$[h]	0.019	0.0016
14	NH_2	CH_3	CH_2NH	H	285–290 dec	D	54		$C_{17}H_{17}N_5O_2 \cdot 2H_2O$	0.0083	0.011
15	NH_2	Cl	CH_2NH	H	232–235 dec	D	71		$C_{16}H_{14}ClN_5O_2 \cdot 2H_2O$	0.0067	0.0054
16	OH	H	$NHCH_2$	C_2H_5	258–260	B	53	I	$C_{18}H_{18}N_4O_3$	1.7	2.7
17	OH	CH_3	$NHCH_2$	C_2H_5	268–271 dec	B	36		$C_{19}H_{20}N_4O_3 \cdot 0.5H_2O$	3.1	30
18	OH	Cl	$NHCH_2$	C_2H_5	252–254	B	17	I	$C_{18}H_{17}ClN_4O_3$[i]	0.73	0.81
19	OH	H	$NHCH_2$	H	>235 dec	E	34	III	$C_{16}H_{14}N_4O_3 \cdot 0.75H_2O$	1.1	28
20	OH	CH_3	$NHCH_2$	H	335–337 dec	F	55		$C_{17}H_{16}N_4O_3 \cdot 1.5H_2O$	2.6	85
21	OH	H	CH_2NH	C_2H_5	297–299 dec	C	39	III	$C_{18}H_{18}N_4O_3$	0.30	0.085
22	OH	H	CH_2NH	H	>200 dec	F	22	III	$C_{16}H_{14}N_4O_3 \cdot 1.5H_2O$	0.17	1.82

[a] I, EtOH–H_2O; II, MeCN; III, DMF–H_2O; IV, 2-methoxyethanol–H_2O; V, THF–H_2O. [b] Analyzed for C, H, and N. Several of these compounds were analyzed correctly as hydrates or fractional hydrates even after drying in vacuo at 100°. Similar findings have been reported with related 2,4-disubstituted quinazolines.[9,12] [c] Assayed spectrophotometrically at 340 nm. [d] Conditions: dihydrofolate, 9 μM; NADPH, 30 μM; KCl, 0.15 M; in 0.05 M Tris buffer (pH 7.4) I_{50} for pyrimethamine, 0.07 μM.[1] [e] Conditions: dihydrofolate, 30 μM; NADPH, 50 μM; KPO_4, 0.05 M (pH 5.6); I_{50} for pyrimethamine, 3.0 μM.[13] [f] J. Davoll and A. M. Johnson [U.S. Patent 3505330 (1970)] reported mp 229–230°. [g] J. Davoll and A. M. Johnson[9] reported mp 224–225° for the hemihydrate. [h] H: calcd, 5.54; found, 5.06. [i] Cl: calcd, 9.51; found, 9.46.

tions of this triaminoquinazoline with acyl chlorides.[12] Apparently the presence of the electron-withdrawing chlorine atom in the 5 position reduces the nucleophilic character of the amino group located at position 6.

The pteroic acid analogs were synthesized by the reductive condensation of ethyl or n-butyl 4-aminobenzoate with the requisite 6-CN (**8–12** and **21**) or 6-CHO (**7**) quinazoline according to the method of Davoll and Johnson.[9] Saponification of the ethyl esters (**7–9**) with dilute sodium hydroxide and dimethyl sulfoxide as the cosolvent afforded the free acids **13–15.** However, when compound **4** was treated with base under reflux, deamination and deesterification occurred concurrently yielding 5,8-deazaisopteroic acid (**19**). As expected, the esters of the 4-OH quinazolines **17** and **21** were rapidly converted into the free carboxylic acids **20** and **22,** respectively, by heating in the presence of dilute hydrochloric acid. This procedure was not employed in the case of 4-NH_2 compounds since hydrolysis of the 4-NH_2 group occurs readily under these conditions.

Biological Results. Each of the target compounds was evaluated for inhibition of the dihydrofolate reductases from rat liver as well as from *S. faecium.* The results expressed as concentrations required to produce 50% inhibition of the enzymatic reaction are presented in Table I. Of all the 2,4-diaminoquinazolines studied, compound **4** is the least effective inhibitor. The introduction of a chloro or methyl group at position 5 causes a significant elevation in potency against either enzyme, with the 5-CH_3 compound (**5**) being the best inhibitor of those compounds having the isopteroic acid configuration. An analogous enhancement of potency resulting from nonpolar substituents in position 5 has been observed with quinazolines bearing a variety of other substituents at the 6 position.[14,15] A similar pattern exists with regard to the isomeric ethyl esters **7–9** for the bacterial enzyme only. In this case, however, the 5-Cl derivative **9** is the most effective inhibitor. Compound **7** is an 11-fold better inhibitor of the mammalian enzyme than its reverse-bridged counterpart **4,** and the presence of 5-CH_3 (**8**) or 5-Cl (**9**) causes only modest alterations in activity. It appears likely, therefore, that with respect to the mammalian enzyme, there is a limiting level of inhibitory potency in the neighborhood of 5 nM for compounds of this type. The n-butyl esters (**10–12**), in general, are moderately less effective than the corresponding ethyl esters with the exception of the 5-Cl derivative **12,** which is equally effective against the rat liver enzyme but is tenfold less potent against the reductase from *S. faecium.*

Among the 4-NH_2 compounds which contain a free carboxyl group (**13–15**), the inhibitory potencies are similar to those of the corresponding ethyl esters with one exception. Thus, although **13** is, when compared to **7,** a threefold poorer inhibitor of the rat liver enzyme, it is 13 times more potent against the *S. faecium* enzyme. This suggests that the partially ionized carboxyl group of bound **13** extends into a polar region of the bacterial enzyme. This contention is supported by the fact that the n-butyl esters **10** and **12** are less inhibitory toward the bacterial enzymes than are the corresponding free acids **13** and **15.** As has been shown with a related series of quinazolines, the 4-OH modifications are much poorer inhibitors of the dihydrofolate reductases from either source.[1] With the exception of the ester derivatives **18** and **21,** these compounds are substantially less ef-

Table II. Compounds Tested against *Plasmodium berghei* in Mice

Compd	Δ MST (days) after single sc dose (mg/kg)[a,b]					
	20	40	80	160	320	640
4		0.3		0.3		0.9 (2T)
5	0.3	1.5	6.5	8.7	10.9 (3C)	5C
6	1.1	2.9	3.9	13.3	16.9 (4C)	5C
7		8.1		15.7 (1C)		3C (2T)
9[c]	6.1	7.9	4C (1T)	5T	5T	
12	3.3	8.3	10.7	11.4 (3T)	5T	5T

[a] Δ MST represents the increase in mean survival time vs. controls. Mean survival time for controls, 6.1 days. [b] Mice surviving for 60 days are considered cured and are designated C. Deaths occurring prior to controls are presumably due to drug toxicity and are designated T. [c] 10 mg/kg. 4.9 days.

fective against the bacterial enzyme. However, it is surprising that against this isozyme **21** is only fourfold less inhibitory that the 4-NH_2 counterpart **7**.

In general, the compounds studied do not display the high degree of isozyme specificity which has been reported for drugs such as trimethoprim.[16] This implies that compounds which closely resemble folic acid even though devoid of an amino acid residue bind to dihydrofolate reductase in a similar configuration to that of classical inhibitors such as **1b**.

Based upon their potent inhibition of the rat liver enzyme coupled with partial to complete solubility in a variety of organic solvents, compounds **5–8** were selected for testing against L1210 leukemia in mice at 10 and 100 mg/kg.[17,§] At the lower dose level none of these afforded a statistically significant increase in survival time (>25%), while at the higher level, each was lethal to all animals within 12 hr. Since death due to **1b** did not occur until 4 to 5 days following single injection in this test system, it appears that toxicity of these esters is not due to the depletion of tetrahydrofolate coenzymes.

Several 4-NH_2 derivatives (**4–7, 9,** and **12**) were also submitted for evaluation as potential antimalarial agents. The testing results against *Plasmodium berghei* in mice are summarized in Table II.[18,&] Of those compounds having the isopteroic acid configuration, the presence of a 5-CH_3 or 5-Cl (**5** and **6**) was essential for activity since **4** was inactive and displayed some toxicity at the highest level. Compound **7**, which is isomeric with **4**, is more active at lower doses but also displayed toxicity at 640 mg/kg. The 5-Cl modifications (**9** and **12**) displayed good activity at lower dose levels but were significantly more toxic than **7**.

Experimental Section

All analytical samples were dried at 100° (P_2O_5) and gave combustion values for C, H, and N within ±0.4% of the theoretical values. Melting points were determined with a Fisher-Johns or a Mel-Temp apparatus and are uncorrected. All target compounds had ir spectra (Beckman IR 8) in agreement with their assigned structures and were free of significant impurities on TLC (Gelman SAF). Ethyl and *n*-butyl 4-aminobenzoates were obtained from Sigma Chemical Co. Ethyl 4-formylbenzoate was prepared from ethyl 4-cyanobenzoate (Aldrich Chemical Co.) by a modification of the method of Slotta and Kethur.[10] As has been observed with other aromatic nitriles, better results were obtained using anhydrous $SnCl_2$ (dried in vacuo over H_2SO_4) in the absence of moisture.

Method A. 6-(4-Carbethoxybenzylamino)-2,4-diamino-5-methylquinazoline (5). A mixture of 4.53 g (0.024 mol) of 2,4,6-triaminoquinazoline[9] and 4.49 g (0.0252 mol) of ethyl 4-formylbenzoate in 40 ml of EtOH was heated at reflux for 18 hr. The resulting solid was separated by filtration, washed with EtOH and acetone, and then dried in air. This was then dissolved in 150 ml of glacial HOAc and a solution of 1.54 g (0.026 mol) of $(CH_3)_2NH{\cdot}BH_3$ in 15 ml of glacial HOAc was added dropwise with stirring below 20°. After stirring for 0.5 hr, the solution was neutralized with concentrated NH_4OH in the cold to pH 9. The resulting solid was separated by filtration, washed with H_2O, dried in vacuo, and then recrystallized from EtOH. There was obtained 2.17 g (26%) of yellow crystals, mp 204–205° (TLC in DMF).

Method B. 6-(4-Carbethoxybenzylamino)-5-chloro-2,4-diaminoquinazoline (6). A mixture of 2.1 g (10 mmol) of 5-chloro-2,4,6-triaminoquinazoline[9] and 1.96 g (11 mmol) of ethyl 4-formylbenzoate in 20 ml of DMF was heated at ca. 100° for 20 hr. TLC (1:3 DMF–MeCN) indicated the presence of a substantial amount of triaminoquinazoline so 0.32 g (2.0 mmol) of aldehyde and 1 g of 5A molecular sieves were added and the heating was continued for 6 hr. The solution was filtered and then cooled to produce a yellow solid. This was isolated on a filter, washed with MeOH, and dried to yield 0.95 g (25%) of anil which showed no significant impurities on TLC. The reduction was carried out with $(CH_3)_2NH{\cdot}BH_3$ as in method A and after recrystallization from MeCN there was obtained 0.42 g (11% overall) of yellow crystals, mp 172–175°.

Method C. 6-(4-Carbethoxyanilinomethyl)-5-chloro-2,4-diaminoquinazoline (9). A solution containing 2 g (9.1 mmol) of 5-chloro-6-cyano-2,4-diaminoquinazoline,[9] 1.8 g (11 mmol) of ethyl 4-aminobenzoate, and 0.8 g of moist Raney nickel in glacial HOAc was hydrogenated at low pressure until H_2 uptake had ceased (90% of theory). After treatment with charcoal, the reaction mixture was filtered and then neutralized with concentrated NH_4OH. The precipitate was isolated by filtration, washed with H_2O, and dried in air. Recrystallization from 2-methoxyethanol–H_2O (charcoal) and then drying in vacuo over P_2O_5 yielded 1.2 g (35%), mp 209–211° (TLC in DMF–MeCN, 1:3).

Method D. 5-Chloro-6-(4-carboxyanilinomethyl)-2,4-diaminoquinazoline (15). A 0.1-g (0.27 mmol) sample of **9** was dissolved in 2 ml of Me_2SO and then treated with 1 ml of 0.5 *N* NaOH. After heating at reflux for 10 min, the solution was treated with charcoal, filtered, and then adjusted to pH 6 with HCl. The solid was isolated by filtration, washed with H_2O and then $(Me)_2CO$, and finally dried at 100° in vacuo (P_2O_5) to yield 66 mg (71%), mp 232–235° dec (TLC in DMF–MeCN, 1:3).

Method E. 6-(4-Carboxybenzylamino)-2-amino-4-hydroxyquinazoline (5,8-Deazaisopteroic Acid) (19). A 1.0-g (2.97 mmol) sample of **4** was heated at reflux in 25 ml of 2 *N* NaOH for 8 hr. Neutralization with 2 *N* HCl to pH 6 yielded a light tan solid which was recrystallized from DMF–H_2O (charcoal) yielding 0.35 g (34%), mp >235° dec.

Method F. 6-(4-Carboxybenzylamino)-2-amino-4-hydroxy-5-methylquinazoline (20). A 0.45-g (1.3 mmol) sample of **17** in 12 ml of 2 *N* HCl was heated at reflux for 2.25 hr. After cooling, the reaction mixture was adjusted to pH 6 with 2 *N* NaOH. The resulting solid was separated by filtration, washed with H_2O, and then dried in vacuo (P_2O_5), mp 335–337° dec (TLC in DMF).

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§ Testing was conducted under the direction of Dr. Glen R. Gale, Veteran's Administration Hospital, Charleston, S.C., using 10^6 rather than 10^5 cells for inoculum.[17] Compounds were administered in single doses on day 1 and were dissolved in Me_2SO.

& Antimalarial testing was carried out by the Rane Laboratory, University of Miami. Compounds were tested through the courtesy of Walter Reed Army Institute of Research, to which the samples were submitted.

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Cholinergic Effects of Molecular Segments of Apomorphine and Dopaminergic Effects of N,N-Dialkylated Dopamines†

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The hydrochlorides of molecular segments of apomorphine [2-(3′,4′-dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline, 2-(3′,4′-dihydroxybenzyl)piperidine, and 1,2,3,4-tetrahydroisoquinoline with their respective *N*-methyl and *N*-*n*-propyl homologs] and N,N-dialkylated dopamine compounds were synthesized and studied for (1) LD_{50} in intact mice; (2) stereotypy in intact mice; (3) curving of the body in unilaterally caudectomized mice; (4) rotation in 6-hydroxydopamine-lesioned rats, and (5) activation of adenylate cyclase in homogenates of mouse caudate nuclei. Instead of dopaminergic effects 1-(3′,4′-dihydroxybenzyl)-2-methyl-1,2,3,4-tetrahydroisoquinoline and 2-methyl-1,2,3,4-tetrahydroisoquinoline showed cholinergic ones. These effects were blocked in atropine-pretreated animals. Of the N,N-dialkylated dopamine compounds synthesized, the *N*-*n*-propyl-*N*-*n*-butyldopamine ranked in all tests as the strongest dopamine-receptor agonist and *N*-methyl-*N*-*n*-propyldopamine as the weakest. In contrast, *N,N*-dimethyldopamine and 1-(3,4-dihydroxyphenylethyl)piperidine showed no dopaminergic effects. The effectiveness of the dopaminergic agonists depended on the length of the *N*-alkyl substituents suggesting interactions with hydrophobic regions of the receptor site.

An attempt to develop drugs effective against Parkinson's disease but having side effects different from those of the dopamine (DA) precursor *l*-Dopa[2a] led to studies of apomorphine **1b** because of some structural similarities between **1b** and DA.[2b] The dopaminergic effects of **1b** seem to be explained by these similarities, but some opposing effects encountered during these studies have been only tentatively explored.[3] The potentiation of the therapeutic effects of oral *l*-Dopa by injected **1b** is compatible with a dopaminergic function, but the diminution of some *l*-Dopa side effects suggests antidopaminergic or even cholinergic properties in **1b**. To clarify these effects we have synthesized molecular segments of **1b** and determined their LD_{50} in mice, and we have studied their effects on unilaterally caudectomized mice,[4] on nigral-lesioned rats,[5] and, when indicated, on the dopamine-activated adenylate cyclase activity of homogenized mouse caudate nuclei.[6,7]

In the present study, elimination of rings A and C from **1b** with retention of the catechol (ring D) and the piperidine (ring B) resulted in loss of recognizable dopaminergic or cholinergic effects (**17a–c**). When the bond joining ring D to ring A was eliminated (**21a–c**), dopaminergic effects disappeared and cholinergic ones appeared so that it was necessary to dissociate the tetrahydroisoquinoline from the piperidine moieties. After synthesizing a series of 1,2,3,4-tetrahydroisoquinolines (**19a–c**) and piperidines we found that some of the former had central cholinergic activities. To preserve dopaminergic while eliminating cholinergic activity we synthesized and investigated *N,N*-dialkyl-substituted dopamines (DA) (**9, 22–25**). When we found that three relatively nontoxic alkyl-substituted DA had dopaminergic activity in lesioned mice and rats, we deemed them worthy of testing on DA-activated adenylate cyclase in homogenized mouse caudate nuclei[6,7] to confirm this activity.

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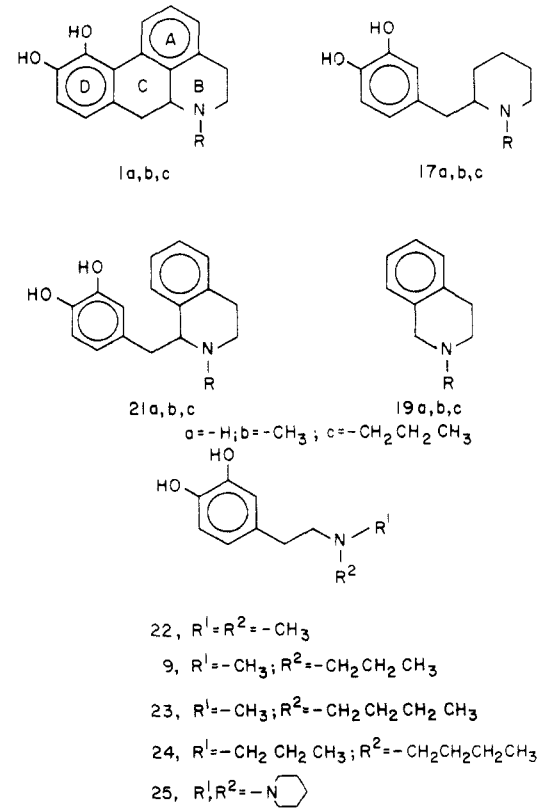

Results and Discussion

Chemistry. 1-Methyl-2-(3′,4′-dihydroxybenzyl)piperidine hydrochloride (**17b**) was synthesized by two different routes as illustrated by Schemes I and II. In Scheme I, hydrogenolysis in step B removed both the *O*-benzyl protective groups and the benzylic hydroxyl,[8] while in Scheme II