the product was hydrolyzed in 0.2 N KOH (25 μL) at 37 °C for 30 min. It was then subjected to electrophoresis as described above. Ac[^{14}C]PheOH and [^{14}C]PheOH were used as reference compounds. The radioactive peaks were located with a Packard radiochromatogram scanner Model 7201. The mobilities of **2i** and the corresponding hydrolysis product, Ac[^{14}C]Phe-L-phenylglycine were virtually identical with those of A-(AcPhePhe) and AcPheOH. The peak areas were cut into 1-cm wide strips, and the radioactivity was measured as described in section A. Further details are given in the legend of Figure 4. An aliquot of Ac[^{14}]Phe-L-phenylglycine, obtained by hydrolysis of 2′(3′)-*O*-peptidyl nucleoside **2i** and subsequent electrophoresis, as described above, was eluted from the paper with 0.1 M NH_4HCO_3 (0.1 mL), and the eluate was lyophilized. Carboxypeptidase A (Worthington Biochemical Corp., Freehold, NJ, 10 μg, 0.1 unit per 20 nmol of peptide) in 0.1 M NH_4HCO_3 (0.1 mL) was added, and the mixture was incubated at 37 °C for 6 h and then subjected to paper electrophoresis as specified above. Only a single peak corresponding to that of AcPheOH was observed.

Acknowledgment. The NMR spectra were measured by W. Brukwinski and S. Grunfeld. This investigation was supported in part by U.S. Public Health Service Research Grant GM-21093 from the National Institute of General Medical Sciences and in part by an institutional grant to the Michigan Cancer Foundation from the United Foundation of Greater Detroit. The measurements of NMR spectra were supported by Biomedical Research Support Grant SO-7-RR-05529 from the National Institutes of Health.

Registry No. **2a**, 83649-50-7; **2b**, 83649-51-8; **2c**, 83649-52-9; **2d**, 83649-53-0; **2e**, 83649-54-1; **2f**, 83649-55-2; **2g**, 83649-57-4; **2h**, 83649-56-3; **3a**, 83649-43-8; **3b**, 83649-44-9; *N*-benzylglycine hydrochloride, 7689-50-1; ethyl *N*-benzylglycinate, 6436-90-4; DL-3-amino-3-phenylpropionic acid, 3646-50-2; L-3-(*N*-formylamino)-3-phenylpropionic acid quinidine, 83649-45-0; L-3-(*N*-formylamino)-3-phenylpropionic acid, 3082-67-5; D-3-(*N*-formylamino)-3-phenylpropionic acid quinine, 83649-46-1; D-3-(*N*-formylamino)-3-phenylpropionic acid, 40856-45-9; D-3-amino-3-phenylpropionic acid, 83649-47-2; *N*-(benzyloxycarbonyl)DL-3-amino-3-phenylpropionic acid, 14440-98-3; benzyloxycarbonyl chloride, 501-53-1; *N*-(benzyloxycarbonyl)-L-3-amino-3-phenylpropionic acid, 14441-07-7; L-3-amino-3-phenylpropionic acid hydrochloride, 83649-48-3; *N*-(benzyloxycarbonyl)-D-3-amino-3-phenylpropionic acid, 14441-08-8; *N*-(benzyloxycarbonyl)-DL-phenylglycine, 5491-18-9; DL-phenylglycine, 2835-06-5; *N*-(benzyloxycarbonyl)-D-phenylglycine, 17609-52-8; D-phenylglycine, 875-74-1; *N*-benzyl-*N*-(*tert*-butoxycarbonyl)glycine, 76315-01-0; 2-[[(*tert*-butoxycarbonyl)oxy]imino]-2-phenylacetonitrile, 58632-95-4; *N*-benzylglycine hydrochloride, 7689-50-1; 9-(3,5-*O*-isopropylidene-β-D-xylofuranosyl)adenine, 7687-49-2; 9-β-D-xylofuranosyladenine, 524-69-6; 5′-*O*-(4-methoxytrityl)adenosine, 51600-11-4; *N*-(benzyloxycarbonyl)-L-phenylalanine, 1161-13-3; 9-[2-*O*-[[*N*-(benzyloxycarbonyl)-L-phenylalanyl]-3,5-*O*-isopropylidene-β-D-xylofuranosyl]adenine, 83649-49-4; peptidyltransferase, 9059-29-4.

1-(4-Aminobenzyl)- and 1-(4-Aminophenyl)isoquinoline Derivatives: Synthesis and Evaluation as Potential Irreversible Cyclic Nucleotide Phosphodiesterase Inhibitors

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In an effort to increase the specificity of the potent phosphodiesterase inhibitor papaverine, we synthesized two series of novel 1-(4-aminobenzyl)- and 1-(4-aminophenyl)isoquinoline derivatives, incorporating alkylating moieties on the amine substituents. These compounds were evaluated for their inhibitory action on phosphodiesterase preparations from bovine heart and rat cerebral cortex. Studies were also conducted to determine whether these compounds were reacting with the enzymes in an irreversible manner. The compounds were potent inhibitors of the phosphodiesterases; however, no evidence was found for an irreversible inhibition.

The role of cyclic nucleotides as intracellular mediators of the action of numerous physiological and pharmacological agents is well recognized. Certain defects in the metabolism of cyclic nucleotides may be involved in a wide variety of diseases, including cancer and cardiovascular disorders.[2] Intracellular concentrations of adenosine 3,5-monophosphate (cAMP) and guanosine 3,5-monophosphate (cGMP) are regulated, in part, by hydrolysis to their corresponding 5′-nucleotides by cyclic nucleotide phosphodiesterases. Multiple forms of phosphodiesterases differing in both structural and kinetic properties have been isolated from various tissues.[3] Enzymes with a relative substrate specificity for hydrolyzing either cAMP or cGMP have been described,[3,4] and previous work has also demonstrated the differential effects of various agents on the activity of these enzymes.[5] Thus, it should be possible to develop pharmacological agents that selectively alter intracellular levels of a specific cyclic nucleotide

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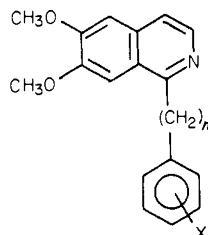
within discrete cell types or within the same tissue through inhibition of phosphodiesterase isozymes.

Although the opium alkaloid papaverine (**1b**) is a potent

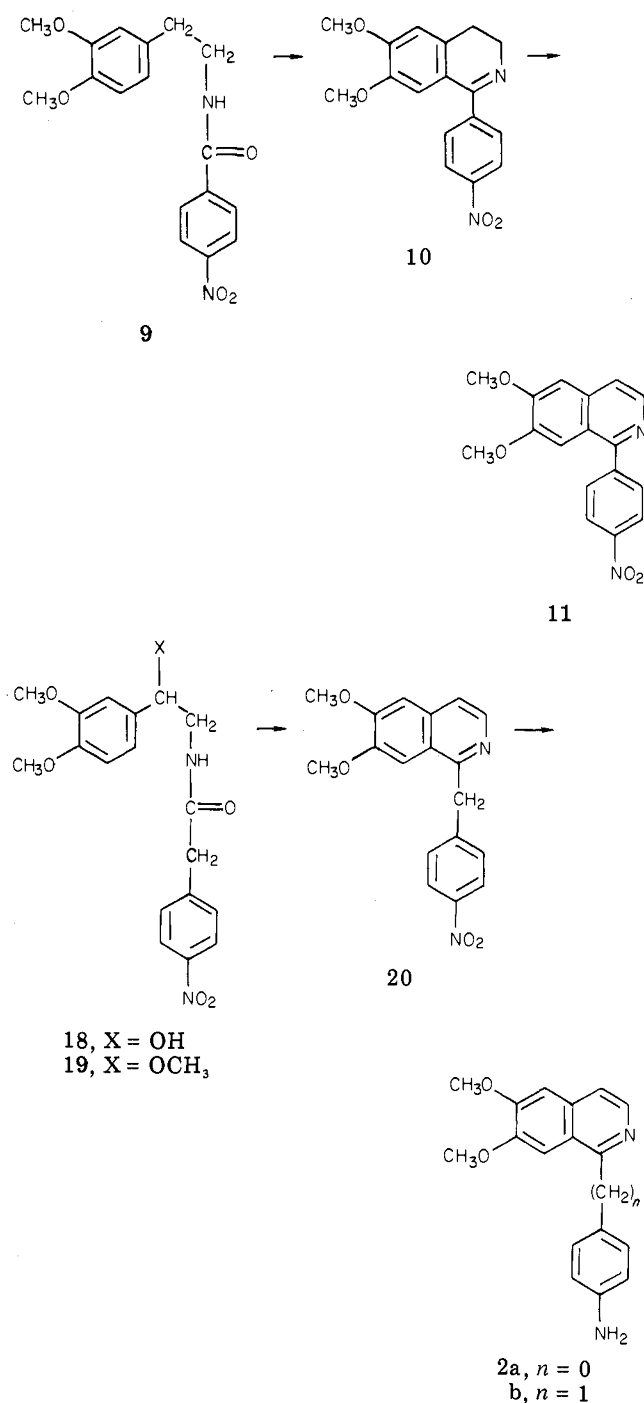


a series, $n = 0$; b series, $n = 1$
1, X = 3,4-$(OCH_3)_2$
2, X = 4-NH_2
3, X = 4-$NHCOCH{=}CH_2$
4, X = 4-$NHCOCH_2Cl$
5, X = 4-$NHCH_2CH_2Cl$
6, X = 4-$NHCOCH{=}CHCOOCH_3$ (cis)
7, X = 4-$NHCOCH{=}CHCOOCH_3$ (trans)
8, X = 4-$N(CH_2CH_2Cl)_2$

inhibitor of phosphodiesterases, it rarely shows appreciable tissue selectivity in its actions.[5,6] Baker has suggested that a potent inhibitor of an enzyme might be made more specific by addition of a moiety that could form a covalent bond with a nucleophilic group on the enzyme adjacent to the site where the inhibitor is reversibly complexed.[7] This phenomenon of active-site-directed irreversible inhibition might make it possible to inhibit selectively any group of enzymes that are closely related by the nature of their substrate. Since papaverine is a potent inhibitor of phosphodiesterases, incorporation of an alkylating group on the molecule could potentiate its enzyme specificity. In the present report the synthesis of papaverine analogues **2–8** containing different types of alkylating moieties and their inhibitory activity on phosphodiesterase preparations isolated from bovine heart and rat cerebral cortex are described.

Since SAR studies have shown that the 6,7-dimethoxy groups of papaverine are necessary for effective phosphodiesterase inhibition,[8] these substituents were retained in the target molecules. It has also been demonstrated that although a 1-phenyl or 1-benzyl substituent is essential for good activity,[8a,9] the methoxy groups on this ring do not significantly contribute to activity.[8a,b] However, various substitutions on the 1-phenyl and 1-benzyl ring have greatly increased enzymatic inhibition.[9b,10a,c,11] Therefore, introduction of alkylating moieties on this portion of the molecule might be efficacious for developing irreversible phosphodiesterase inhibitors. Although no clear trend has been demonstrated relating potency with partial saturation of the nitrogen-containing ring,[8a,9a,c] in this study only the

Scheme I



fully aromatic isoquinolines were examined.

In order to probe for a possible nucleophilic site on the phosphodiesterase enzyme, we synthesized two series of papaverine analogues with functional groups in the para position of the 1-phenyl or 1-benzyl substituent. The substituent groups were chosen so as to allow possible covalent bond formation by nucleophilic groups at varying distances from the papaverine-binding site and with differing electronic and steric requirements. Differences in any of these factors among isoenzymes could be the basis for the desired selectivity by one or more of the compounds. The chloroacetamido, **4a,b**, and chloroethylamino, **5a,b**, alkylating derivatives were prepared, and based on Tisdale's observation that the two-armed nitrogen mustard chlorambucil inhibits the high-affinity form of phosphodiesterase in Walker carcinoma cells,[11] the *N,N*-bis(chloroethyl) derivatives, **8a,b**, were also prepared. Since the

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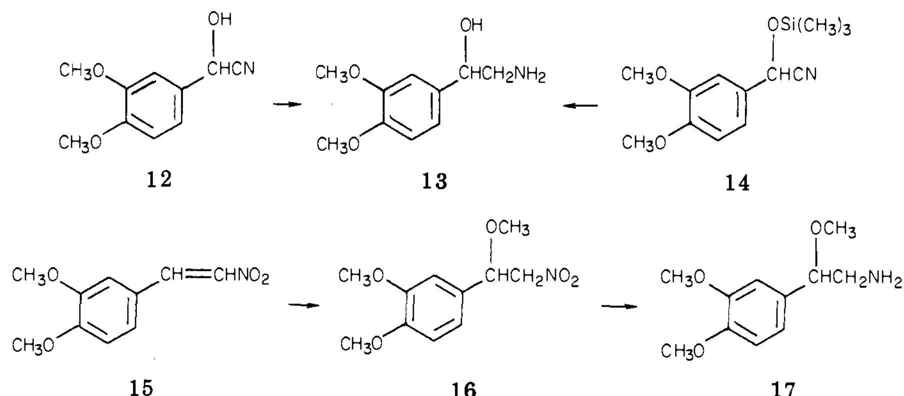
Scheme II



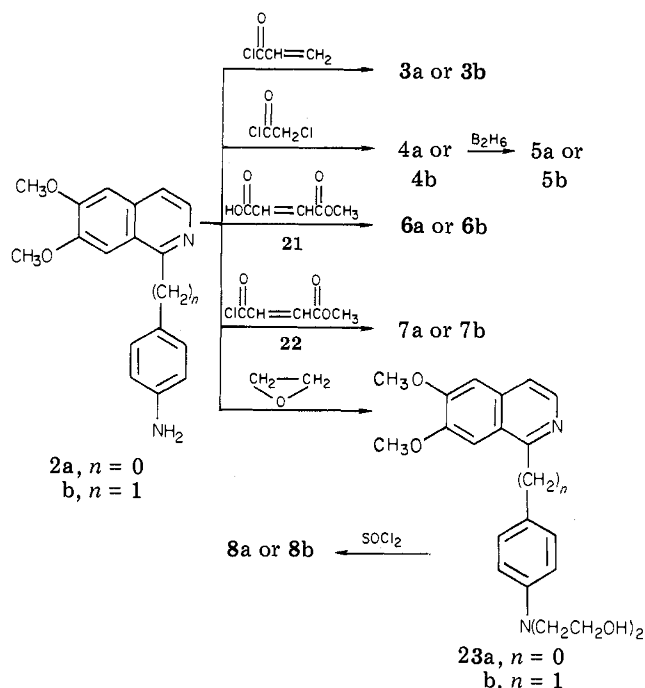
aziridinium ions formed from the nitrogen mustards, **5a,b**, and **8a,b** are so highly reactive, Michael acceptors might confer greater selectivity in covalent bond formation due to their lower reactivity; therefore, the α,β-unsaturated carbonyl compounds, **3a,b**,**6a,b**, and **7a,b** were synthesized. This approach has previously been successful in the development of selective irreversible narcotic antagonists by Portoghese and co-workers.[12]

Chemistry. The synthesis of the 1-(4-aminophenyl)isoquinolines was carried out via the classical Bischler–Napieralski cyclization reaction[13] as shown in Scheme I. It was not possible to obtain the 1-(4-aminobenzyl)isoquinolines by the Bischler–Napieralski reaction. Attempts to dehydrogenate the 6,7-dimethoxy-1-(4-nitrobenzyl)-3,4-dihydroisoquinoline to the isoquinoline compound with palladium on charcoal were unsuccessful. Other workers have also reported difficulties in the dehydrogenation of certain dihydroisoquinolines to their isoquinoline analogues with palladium on charcoal, as well as with other catalysts.[14] Therefore, the 1-benzylisoquinoline derivatives were obtained via the Pictet–Gams modification[15] of the Bischler–Napieralski reaction shown in Scheme I, thus circumventing the dehydrogenation step.

Several synthetic routes were employed in the preparation of the appropriate 1-benzylisoquinoline precursors. The amino alcohol **13** was prepared by the two methods shown in Scheme II. The first method involved the facile synthesis of the cyanohydrin **12** by the reaction of 3,4-dimethoxybenzaldehyde with potassium cyanide and hydrochloric acid.[16] Subsequent reduction of the cyanohydrin with $LiAlH_4$ yielded **13**. Although this method was satisfactory for the synthesis of the amino alcohol **13**, the isolation and purification of the cyanohydrin were tedious. Alternatively, 3,4-dimethoxybenzaldehyde was reacted with trimethylsilyl cyanide.[17] The resulting trimethylsilyl cyanohydrin ether **14** did not have to be isolated and was added directly to a suspension of $LiAlH_4$/THF, affording **13** in good yields.

Condensation of 3,4-dimethoxybenzaldehyde with nitromethane yielded the β-nitrostyrene **15** as shown in

Scheme III



Scheme II. The nitrostyrene was converted to the nitroethane compound **16** by the Michael-type addition of the methoxide group. Catalytic reduction of **16** over Raney nickel afforded the β-methoxyphenethylamine **17**.

The N-(β-hydroxy- and β-methoxyphenethyl) amides **18** and **19**, were prepared by the reaction of the appropriate amine **13** or **17** with 4-nitrobenzeneacetyl chloride.[15] The 1-benzylisoquinoline derivative **20** was prepared by reaction of either **18** or **19** with phosphorus oxychloride.[15] Catalytic reduction of the 1-(4-nitrobenzyl)isoquinoline over Raney nickel afforded the amine derivative **2b**.

As illustrated in Scheme III, the amines **2a** and **2b** were reacted with acryloyl chloride to give **3a** and **3b**.[18] Treatment of the amines with chloracetyl chloride yielded the chloracetamido derivatives **4a** and **4b**.[19] Reduction of these chloracetamido compounds with diborane afforded the one-armed nitrogen mustards **5a** and **5b**.[18] The maleamate (**6a** and **6b**) and fumaramate (**7a** and **7b**) analogues were prepared by reaction of the appropriate amines with methyl hydrogen maleate (**21**) or ethyl 3-(chloro-

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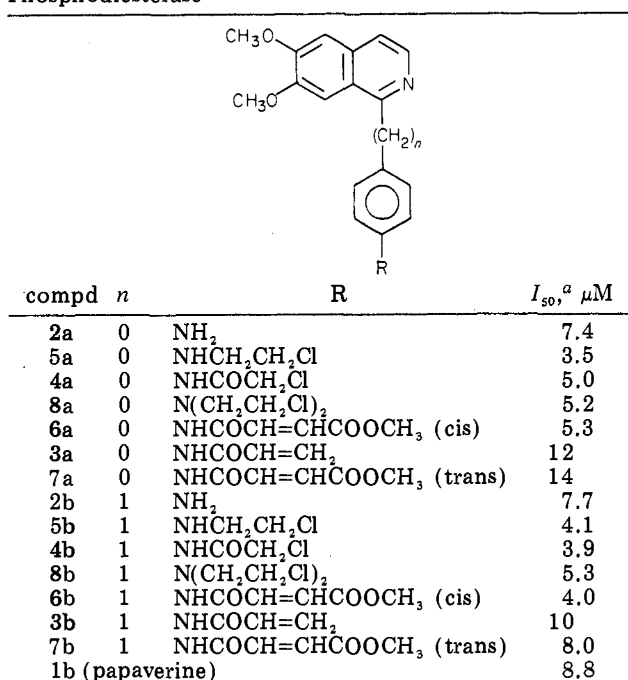
Table I. I_{50} Values for 1-Phenyl- and 1-Benzylisoquinoline Derivatives against Bovine Heart Phosphodiesterase



compd	n	R	I_{50},[a] μM
2a	0	NH_2	7.4
5a	0	$NHCH_2CH_2Cl$	3.5
4a	0	$NHCOCH_2Cl$	5.0
8a	0	$N(CH_2CH_2Cl)_2$	5.2
6a	0	$NHCOCH{=}CHCOOCH_3$ (cis)	5.3
3a	0	$NHCOCH{=}CH_2$	12
7a	0	$NHCOCH{=}CHCOOCH_3$ (trans)	14
2b	1	NH_2	7.7
5b	1	$NHCH_2CH_2Cl$	4.1
4b	1	$NHCOCH_2Cl$	3.9
8b	1	$N(CH_2CH_2Cl)_2$	5.3
6b	1	$NHCOCH{=}CHCOOCH_3$ (cis)	4.0
3b	1	$NHCOCH{=}CH_2$	10
7b	1	$NHCOCH{=}CHCOOCH_3$ (trans)	8.0
1b (papaverine)			8.8

[a] The reactions were carried out at 37 °C with cAMP at 1 M as substrate. The concentrations of the inhibitors used ranged from 0.5 to 25 μM. All assays were performed in duplicate and the average of the two separate determinations are presented, which were within ±10%.

formyl)-2-propenoate (**22**), respectively.[20] The two-armed nitrogen mustard derivatives **8a** and **8b** were prepared from their respective bisalcohol precursors, **23a** and **23b**, as shown in Scheme III.[19]

Results and Discussion

Table I shows the effect of the 1-(4-aminobenzyl)- and 1-(4-aminophenyl)isoquinoline derivatives on the cAMP-hydrolyzing enzymes present in Sigma bovine heart phosphodiesterase preparations. In both series, all the compounds were effective inhibitors of the enzyme. Furthermore, the inhibition profiles of the derivatives in both series are almost identical, indicating that enzyme inhibition can be effected with either the 1-(4-aminobenzyl) or 1-(4-aminophenyl) derivatives. The unsubstituted amines **2a** and **2b** had I_{50} values of 7.4 and 7.7, respectively, compared with papaverine, which had an I_{50} value of 8.8. The substituted amine derivatives exhibited only about a 4-fold difference in I_{50} values throughout the series. This is a surprisingly small difference in activity considering the variations in structure that are present. In the phenyl series, the fumaramate analogue **7a** was the least potent inhibitor, with an I_{50} of 14 μM. In the phenyl series, the least potent inhibitor was the propenamido derivative **3b**, with an I_{50} of 10 μM. The most potent inhibitors were the chloroethylamine derivative **5a** in the phenyl series with an I_{50} of 3.5 and the chloroacetamido derivative **4b** in the benzyl series with an I_{50} of 3.9. In both series the maleamate analogues **6a** and **6b** were more effective inhibitors than their corresponding trans isomers **7a** and **7b**.

Table II shows the effect of the 1-(4-aminophenyl)isoquinoline derivatives on the cAMP- and cGMP-hydrolyzing enzymes present in crude extracts from rat

(20) P. S. Portoghese, V. G. Telang, A. E. Takemori, and G. Hayashi, *J. Med. Chem.*, 14, 144 (1971).

Table II. Effect of Derivatives of 1-Phenylisoquinoline on Hydrolysis of Cyclic AMP and Cyclic GMP by Phosphodiesterases Present in Crude Extracts of Rat Cerebral Cortex[a]

	I_{50}, μM	
derivative	cAMP hydrolysis	cGMP hydrolysis
2a	3.4 ± 0.2	2.1 ± 0.1
4a	2.3 ± 0.5	6.8 ± 0.8
7a	35 ± 2.6	>>100[b]
6a	1.7 ± 0.4	1.1 ± 0.3
3a	10 ± 1.3	30 ± 4.5
5a	2.9 ± 0.2	12 ± 0.7
8a	7.3 ± 0.8	15 ± 1.5
1[c]	12 ± 1.2	21 ± 3.6

[a] The assay for phosphodiesterase activity was according to the method of Davis and Daly[26] with 1 μM cyclic AMP or 1 μM cyclic GMP as substrate. I_{50} is defined as that concentration of agent that inhibited the hydrolysis of the corresponding cyclic nucleotide by 50% and was calculated by interpolating from at least five values of inhibition, ranging from to to 100%, against the logarithm of inhibitor concentrations (10^{-7} to 10^{-4} M). Assays were performed in triplicate, and the values are the means plus or minus standard error from three to five separate experiments. [b] Less than 15% inhibition at 100 μM. [c] Papaverine.

cerebral cortex. In general, all the compounds tested were more potent than papaverine and demonstrated greater specificity for cAMP hydrolysis than for cGMP hydrolysis. The fumaramate analogue **7a** was ineffective in inhibiting the hydrolysis of cGMP, although it demonstrated moderate inhibitory action against cAMP hydrolysis. This is in sharp contrast to the potent inhibition of both cAMP and cGMP hydrolysis by the cis isomer **6a**. In a separate experiment, further confirmation of the marked differences in potencies of the cis and trans isomers was obtained. As shown in Table III, the cis isomer at a concentration of 2 μM inhibited cGMP hydrolysis by the purified calcium-dependent enzyme about 80%, while the trans isomer had no effect at a concentration of 25 μM. All the 1-(4-aminophenyl)isoquinoline compounds were more active against the cAMP phosphodiesterase activity present in rat cerebral cortex than the bovine heart, except for the fumaramate derivative, which was more active against the bovine heart cAMP-hydrolyzing enzyme. Furthermore, the rank order of potency of these compounds was nearly identical with either the heart or cerebral cortical enzyme preparations.

All of the compounds tested demonstrated a relatively high degree of affinity for the different phosphodiesterases present in either a bovine heart or rat cerebral cortex—the one important exception being **7a**, which was inactive against the cGMP enzymes from the rat cerebral cortex. However, the small differences in the I_{50} values exhibited by these analogues suggest that none of the alkylating groups are actually interacting with the enzymes by covalent-bond formation. The large differences in reactivity, stereochemistry, and bulk of these alkylating moieties makes it unlikely that all of these compounds are nearly equipotent irreversible inhibitors. Support for this comes from an experiment in which the 1-(4-aminophenyl)isoquinolines were incubated prior to the assay with a calcium-dependent phosphodiesterase purified from rat cerebral cortex (Table III). After the preincubation, the enzyme preparation was diluted to concentrations at which the inhibitors should have no reversible effect on phosphodiesterase activity, and then the enzyme was assayed with 1 μM cGMP as substrate. Under these conditions, the activity of the enzyme preincubated with concentra-

Table III. Alterations in the Activity of the Calcium-Dependent Phosphodiesterases Purified from Rat Cerebral Cortex Preincubated with 1-(4-Aminophenyl)isoquinoline Derivatives[a]

compd	concn, μM	% phosphodiesterase act.	
		drug absent	drug present
control		100	
2a	2	103	16
4a	2	105	19
7a	25	100	104
6a	2	99	14
3a	25	103	50
5a	25	102	21
8a	25	100	45

[a] Phosphodiesterase (80 ng) was preincubated with either 2 or 25 μM concentrations of the indicated derivative for 24 h at 4 °C and 20 min at 37 °C. The enzyme was then diluted 1000-fold and assayed for phosphodiesterase activity with 1 μM cGMP, either in the absence of additional drug or with the addition of each drug in the indicated preincubation concentrations.

tions of inhibitors that should have inhibited enzyme activity 50–90% was not found to be significantly different from the control.

These data indicated that although these inhibitors are acting as potent reversible inhibitors of the enzyme, they are not interacting with the enzymes in an irreversible fashion. However, the addition of the alkylating groups in the 4-position of the benzyl or phenyl ring does not seem to interfere with the binding of the 3,4-dimethoxy-1-phenyl- or 1-benzylisoquinoline portion of the molecule to the receptor site. The most significant exception to this is when the substituent on the phenyl ring is the fumaramate moiety; all activity against the calcium-dependent, cGMP-hydrolyzing enzyme from the rat was lost. The corresponding cis isomer, the maleamate moiety, showed no such loss of activity. Thus, the trans structure must be interfering with the binding of the isoquinoline to the active site of the enzyme. The alkylating groups in the 4-position of the substituted rings may be able to bind irreversibly to other isozymes of phosphodiesterases, owing to structural variations in these enzymes. The effects of these inhibitors on different forms of phosphodiesterases need to be examined further in order to elucidate possible differences in the structural requirements of different isozymes.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. The infrared spectra were recorded on a Beckman AccuLab 8 grating infrared spectrophotometer. The NMR spectra were determined on a Hitachi Perkin-Elmer R-24 spectrophotometer with Me_4Si as an internal standard. Elemental analyses were performed by the A. H. Robins Co., Richmond, VA. All analyses (C, H, and N) are within ±0.4% of the calculated values.

***N*-[2-(3,4-Dimethoxyphenyl)ethyl]-4-nitrobenzamide (9).** A solution of 3,4-dimethoxyphenethylamine (100 g, 0.55 mol) in 400 mL of chloroform and 400 mL of 20% K_2CO_3 was cooled to 0 °C, and a solution of 4-nitrobenzoyl chloride (137 g, 0.83 mol), dissolved in chloroform, was added dropwise with rapid stirring. The reaction mixture was stirred at room temperature for 3 h. The organic layer was separated and washed sequentially with 200 mL of H_2O, 200 mL of saturated $NaHCO_3$, and 200 mL of H_2O. After the solution was dried over $MgSO_4$, the solvent was removed under reduced pressure to give a viscous yellow oil, which solidified on standing. Recrystallization from methanol afforded 155 g (83%) of pale yellow crystals: mp 144–145 °C; IR ($CHCl_3$) 3420 (NH), 1670 (C=O), 1530 (NO_2) cm^{-1}; NMR ($CDCl_3$) δ 8.15 (d, 2, Ar*H*, *J* = 8 Hz), 6.9 (s, 3, Ar*H*), 7.15 (s, 1, NH), 3.7–3.9 (m, 8, OCH_3, ArCH_2), 2.85 (t, 2, CH_2NH, *J* = 6 Hz). Anal. ($C_{17}H_{18}N_2O_5$) C, H, N.

6,7-Dimethoxy-1-(4-nitrophenyl)-3,4-dihydroisoquinoline (10). A mixture of **9** (41.5 g, 0.120 mol) and phosphorous oxychloride (27.5 g, 0.180 mol) in 500 mL of acetonitrile was heated at reflux for 2 h. The solvent was evaporated under reduced pressure, and the resultant orange residue was dissolved in 400 mL of 4 N HCl. The aqueous solution was washed twice with 200-mL portions of chloroform. The aqueous layer was made alkaline with 50% NaOH and extracted twice with 100-mL portions of chloroform. The combined chloroform extracts were dried over $MgSO_4$, and the solvent was removed under reduced pressure, yielding 31 g (82%) of a yellow solid. Recrystallization from chloroform afforded bright yellow crystals: mp 154–155 °C; IR ($CHCl_3$) 1530 (NO_2) cm^{-1}; NMR ($CDCl_3$) δ 8.3 (d, 2, Ar*H*, *J* = 9 Hz), 7.7 (d, 2, Ar*H*, *J* = 9 Hz), 6.8 (s, 1, Ar*H*), 6.6 (s, 1, Ar*H*), 3.9 (s, 3, OCH_3), 3.7 (s, 5, OCH_3, ArCH_2), 2.65 (t, 2, CH_2NH, *J* = 6 Hz). Anal. ($C_{17}H_{16}N_2O_4$) C, H, N.

6,7-Dimethoxy-1-(4-nitrophenyl)isoquinoline (11). A mixture of **10** (20 g, 0.06 mol) and 2.0 g of 10% Pd/C in 500 mL of decalin was heated at reflux under nitrogen for 15 h. The catalyst was removed from the hot solution by filtration. The filtrate was allowed to cool to room temperature, and the resultant orange precipitate was removed by filtration and dissolved in 400 mL of 4 N HCl. The aqueous solution was washed with two 100-mL portions of chloroform. The aqueous layer was made alkaline with 50% NaOH and extracted twice with 100-mL portions of chloroform. The combined chloroform extracts were dried over $MgSO_4$, and the solvent was evaporated under reduced pressure, yielding 17 g (85%) of a yellow solid. Recrystallization from chloroform afforded bright yellow needles: mp 214–215 °C; IR ($CHCl_3$) 1530 cm^{-1}; NMR ($CDCl_3$) δ 8.5 (d, 2, Ar*H*, *J* = 9 Hz), 8.1 (d, 2, Ar*H*, *J* = 9 Hz), 7.3–7.7 (m, 4, Ar*H*), 4.1 (s, 3, OCH_3). Anal. ($C_{17}H_{14}N_2O_4$) C, H, N.

4-(6,7-Dimethoxy-1-isoquinolinyl)benzenamine (2a). Catalytic hydrogenation at 55 psi of **11** (3.1 g, 0.01 mol) over Raney nickel in absolute ethanol afforded 1.5 g (53%) of **2a** as a white solid. Recrystallization from acetone yielded fine white needles: mp 200–201 °C; IR (Nujol) 3200–3400 (NH) cm^{-1}; NMR ($CDCl_3$) δ 8.5 (d, 1, Ar*H*), 6.8–7.65 (m, 7, Ar*H*), 4.05 (s, 3, OCH_3), 3.9 (s, 3, OCH_3), 1.8 (s, 2, NH_2). Anal. ($C_{17}H_{16}N_2O_2$) C, H, N.

α-Hydroxy-3,4-dimethoxybenzeneacetonitrile (12). Potassium cyanide (11.2 g, 0.17 mol) dissolved in 25 mL of H_2O was added to 3,4-dimethoxybenzaldehyde (16.6 g, 0.1 mol). The reaction mixture was cooled in an ice bath, and 17 mL of concentrated HCl was added with rapid stirring at a rate such that the temperature did not exceed 45 °C. The reaction mixture was allowed to come to room temperature, and the resultant yellow solid was separated by filtration. Recrystallization from benzene yielded 17 g (87%) of **12** as a white solid: mp 104–105 °C (lit.[16] mp 87–90 °C); IR ($CHCl_3$) 3520 (OH) cm^{-1}; NMR (acetone-d_6) δ 6.7–6.9 (m, 3, Ar*H*), 5.4 (s, 1, CH), 3.65 (s, 6, OCH_3), 3.1 (s, 1, OH). Anal. ($C_{10}H_{11}NO_3$) C, H, N.

α-(Aminomethyl)-3,4-dimethoxybenzenemethanol (13). Method A. All glassware was flame dried. $LiAlH_4$ (7.6 g, 0.20 mol) was suspended in dry THF and cooled under an atmosphere of nitrogen to 0–5 °C with a methanol/ice bath. The cyanohydrin **12** dissolved in 200 mL of dry THF was added dropwise to the reaction mixture over a 1-h period. The reaction mixture was heated at reflux for 36 h. Excess hydride was destroyed by the slow successive addition of 7.6 mL of H_2O, 7.6 mL of 15% NaOH, and 32 mL of H_2O. The inorganic salts were removed by filtration, and the organic filtrate was dried over $MgSO_4$. Excess solvent was removed under reduced pressure, leaving a yellow oil that solidified on standing. Distillation under reduced pressure afforded a clear colorless oil that solidified on standing at room temperature to a white solid: yield 7.1 g (35%); mp 70–74 °C (lit.[21] mp 72–76 °C).

Method B. Trimethylsilyl cyanide (33 g, 0.33 mol) was added via syringe in 1-mL aliquots over a 1-h period to 3,4-dimethoxybenzaldehyde (50 g, 0.3 mol) and a catalytic amount of ZnI_2

(21) G. I. Poos, J. R. Carson, J. D. Rosenau, A. P. Rozekowski, N. M. Kelley, and J. McGowin, *J. Med. Chem.*, 6, 266 (1963).

(1.0 mg). The resultant orange-colored solution was warmed gently (30 °C) until the IR spectrum of sample aliquots showed no absorption in the carbonyl region of the spectrum (approximately 2 h). The trimethylsilyl cyanohydrin ether 14 was not isolated but was diluted with 200 mL of dry THF and added to a suspension of $LiAlH_4$ (23 g, 0.16 mol) in dry THF under nitrogen. After the addition was complete, the reaction mixture was heated at reflux for 15 h. After the mixture was cooled, the reaction was hydrolyzed and isolated as previously described in method A. Distillation under reduced pressure afforded a clear colorless oil that solidified on standing to a white solid: yield 21.5 g (36%); mp 70–74 °C.

1,2-Dimethoxy-4-(2-nitroethenyl)benzene (15). A stirred mixture of 3,4-dimethoxybenzaldehyde (100 g, 0.60 mol), nitromethane (160 mL, 3.0 mol), ammonium acetate (54 g, 0.60 mol), and 150 mL of benzene was heated at reflux for 6 h. Heating was discontinued, and the reaction mixture was stirred at room temperature for 12 h. The resultant yellow precipitate was separated. The filtrate was washed twice with 200 mL of water and twice with 200 mL of saturated NaCl and dried over $MgSO_4$. Additional product was recovered by evaporation of the solvent. Recrystallization of the combined solids from methanol afforded 112 g (89%) of 15 as yellow needles, mp 140–141 °C (lit.[22] mp 141–142 °C).

1,2-Dimethoxy-4-(1-methoxy-2-nitroethyl)benzene (16). A commercially prepared (Aldrich Chemical Co.) 25% $NaOCH_3$/MeOH solution (110 mL) was diluted to 250 mL with MeOH and added in one portion to 15 (50 g, 0.24 mol) suspended in 250 mL of MeOH. The reaction mixture was stirred for 5 min, 80 mL of glacial acetic acid was added, and stirring was continued for an additional 3 min. The resultant precipitate was separated and recrystallized from MeOH, affording 25.3 g (44%) of 16, mp 104–106 °C (lit.[15] mp 104–105 °C).

3,4-Dimethoxy-β-methoxybenzeneethanamine (17). Catalytic hydrogenation at 55 psi of 16 (2.41 g, 0.01 mol) over Raney nickel in absolute ethanol afforded 1.87 (78%) of 17 as a clear colorless oil: IR (neat) 3380 (NH) cm^{-1}; NMR ($CDCl_3$) δ 6.9–7.1 (m, 3, Ar*H*), 4.15 (t, 1, Ar*H*, J = 6 Hz), 3.85 and 3.9 (2 s, 6, $ArOCH_3$), 3.3 (s, 3, OCH_3), 2.9 (d, 2, CH_2, J = 6 Hz), 2.6 (s, 2, NH_2). Anal. ($C_{11}H_{17}NO_3$) C, H, N.

***N*-[2-Hydroxy-2-(3,4-dimethoxyphenyl)ethyl]-4-nitrobenzeneacetamide (18).** Compound 18 was prepared from the reaction of 13 with 4-nitrobenzeneacetyl chloride in the same manner employed for the preparation of 9. Recrystallization from methanol afforded 18 in 65% yield: mp 97–98 °C; IR ($CHCl_3$) 3100–3500 (OH, NH), 1685–1690 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 7.9 (d, 2, Ar*H*, J = 4 Hz), 7.6 (d, 2, Ar*H*, J = 4 Hz), 6.5 (s, 3, Ar*H*) 4.8 (s, 1, NH), 4.6 (s, 2, CH_2), 3.4 (s, 6, OCH_3), 3.3 (s, 1, OH), 3.1 (d, CH_2NH). Anal. ($C_{18}H_{20}N_2O_6$) C, H, N.

***N*-[2-Methoxy-2-(3,4-dimethoxyphenyl)ethyl]-4-nitrobenzeneacetamide (19).** Compound 19 was prepared from the reaction of 17 with 4-nitrobenzeneacetyl chloride in the same manner employed for the preparation of 9. Recrystallization from methanol/diethyl ether afforded 19 in 53% yield: mp 104–106 °C; IR ($CHCl_3$) 3420 (NH), 1670 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 8.1 (d, 2, Ar*H*, J = 4 Hz), 7.9 (d, 2, Ar*H*, J = 4 Hz), 6.8 (s, 3, Ar*H*), 4.7 (s, 1, NH), 4.5 (s, 2, CH_2), 3.7–3.9 (m, 7, 2 OCH_3), ArCH_3), 3.5 (s, 3, OCH_3), 3.0–3.3 (d, 2 , CH_2NH).

6,7-Dimethoxy-1-(4-nitrobenzyl)isoquinoline (20). Compound 20 was prepared from 18 or 19 in the same manner employed for the preparation of 10. The reaction mixture was heated for 30 min. After the mixture was cooled, the solvent was removed under reduced pressure. The isoquinoline was purified by column chromatography (silica; chloroform/methanol/ammonium hydroxide, 94:5:1). The material was recrystallized from chloroform/diethyl ether, affording 20 in 63% yield: mp 195–196 °C; IR ($CHCl_3$) 1530 (NO_2) cm^{-1}; NMR ($CDCl_3$) δ 8.6 (d, 2, Ar*H*, J = 9 Hz), 8.0 (d, 2, Ar*H*, J = 9 Hz), 7.2–7.5 (m, 4, Ar*H*), 4.4 (s, 2, CH_2), 4.1 (s, 3, OCH_3), 3.8 (s, 3, OCH_3). Anal. ($C_{18}H_{16}N_2O_4$) C, H, N.

4-[(6,7-Dimethoxy-1-isoquinolinyl)methyl]benzenamine (2b). Catalytic hydrogenation of 20 (3.2 g, 0.01 mol) over Raney nickel in absolute ethanol afforded the amine 2b. Recrystallization from acetone yielded 1.5 g (54%) of a white powder: mp 200–202 °C; IR (Nujol) 3200–3400 (NH) cm^{-1}; NMR ($CDCl_3$) δ 8.4 (d, 1, Ar*H*, J = 6 Hz), 6.8–7.6 (m, 7, Ar*H*), 4.3 (s, 2, CH_2), 4.05 (s, 3, OCH_3) 3.9 (s, 3, OCH_3), 1.8 (s, 2, NH_2). Anal. ($C_{18}H_{18}N_2O_2$) C, H, N.

***N*-[4-(6,7-Dimethoxy-1-isoquinolinyl)phenyl]propenamide (3a).** Acryloyl chloride (0.34 g, 0.38 mol), dissolved in 8 mL of chloroform, was added dropwise to a cooled stirring suspension of 2a (1.0 g, 3.6 mmol), triethylamine (0.75 g, 75 mmol), and 30 mL of chloroform. Following the addition, the solution was stirred at room temperature for 15 min, washed thrice with 100-mL portions of H_2O, and dried over $MgSO_4$. The solvent was removed under reduced pressure, leaving 0.7 g (58%) of crude product. Recrystallization from ethanol/diethyl ether afforded the product as yellow needles: mp (HCl salt) 230–233 °C; IR ($CHCl_3$) 3300 (NH), 1680 (C=O), 1620 (CH=CH) cm^{-1}; NMR ($CDCl_3$) δ 9.7 (br s, 1, NH), 7.5–8.6 (m, 8, Ar*H*), 6.65 (t, 1, CH, J = 8 Hz), 5.9 (d, 2, CH_2, J = 8 Hz), 3.9 (s, 3, OCH_3), 3.7 (s, 3, OCH_3). Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

***N*-[4-[(6,7-Dimethoxy-1-isoquinolinyl)methyl]phenyl]propenamide (3b).** Compound 3b was prepared from 2b in the same manner employed for the preparation of 3a to yield 0.8 g (59%) of 3b as crude product. The material was recrystallized from absolute ethanol/diethyl ether, affording pale yellow crystals: mp (HCl salt) 210–213 °C; IR ($CHCl_3$) 3360 (NH), 1680 (C=O), 1620 (CH=CH) cm^{-1}; NMR ($CDCl_3$) δ 9.8 (br s, 1, NH), 7.5–8.0 (m, 8, Ar*H*), 6.65 (t, 1, C*H*, J = 6 Hz), 5.95 (d, 2, CH_2, J = 6 Hz), 5.3 (s, 2, CH_2), 3.9 (s, 3, OCH_3), 3.7 (s, 3, OCH_3). Anal. ($C_{21}H_{20}N_2O_3$) C, H, N.

2-Chloro-*N*-[4-(6,7-dimethoxy-1-isoquinolinyl)phenyl]acetamide (4a). Chloroacetyl chloride (0.45 g, 4.0 mmol) was added dropwise to 2a (1.0 g, 3.6 mmol) suspended in 150 mL of dry acetonitrile. The reaction mixture was stirred at room temperature for 2 h. The resultant yellow precipitate was separated and stirred in 20 mL of a 10% $NaHCO_3$ solution for 10 min. The free base was extracted into chloroform. The organic layer was washed and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield 1.1 g (85%) of 4a as yellow crystals. Recrystallization from 2-propanol afforded fine white needles: mp (HCl salt) 211–213 °C; IR (Nujol) 3320 (NH), 1690 (C=O) cm^{-1}; NMR ($CDCl_3$) δ 8.85 (br s, 1, NH), 8.6 (d, 1, Ar*H*, J = 6 Hz), 7.2–7.85 (m, 7, Ar*H*), 4.1 (s, 2, CH_2Cl), 3.9 (s, 3, OCH_3), 3.7 (s, 3, OCH_3). Anal. ($C_{19}H_{17}ClN_2O_3$) C, H, N.

2-Chloro-*N*-[4-[(6,7-dimethoxy-1-isoquinolinyl)methyl]phenyl]acetamide (4b). Compound 4b was prepared from 2b in the same manner employed for the preparation of 4a to yield 0.9 g (85%) of the crude product. The material was recrystallized from 2-propanol, affording pale yellow crystals: mp (HCl salt) 201–203 °C; IR (Nujol) 3750 (NH), 1695 (C=O) cm^{-1}; NMR ($CDCl_3$) 8.85 (br s, 1, NH), 8.6 (d, 1, Ar*H*, J = 6 Hz), 7.0–7.5 (m, 7, Ar*H*), 4.45 (s, 2, CH_2), 4.1 (s, 2, CH_2Cl), 3.9 (s, 3, OCH_3), 3.7 (s, 3, OCH_3). Anal. ($C_{20}H_{19}ClN_2O_3$) C, H, N.

***N*-(2-Chloroethyl)-4-(6,7-dimethoxyl-1-isoquinolinyl)benzenamine (5a).** Following a procedure reported by Filer et al.,[19] 0.94 M diborane/THF (12.5 mL, 11.8 mmol) was added via syringe to 4a (700 mg, 1.96 mmol) suspended in 25 mL of dry THF under nitrogen at −78 °C. Stirring was continued for 2 h at −78 °C, and the reaction mixture was then stirred for 24 h at 4–10 °C. The reaction was cautiously quenched by addition of 45 mL of a 2% HCl solution. The THF was evaporated under reduced pressure and 50 mL of chloroform was added to the aqueous solution. The rapidly stirring mixture was neutralized to pH 7.1 with a saturated $NaHCO_3$ solution. The layers were separated, and the aqueous layer was extracted with two additional portions of chloroform (20 mL each). The chloroform extracts were combined, and the solvent was evaporated under reduced pressure to afford a viscous orange oil. The oil was heated at 50 °C for 20 min with 0.5 mL of pyridine in 20 mL of THF. The THF was evaporated, and the residue dissolved in chloroform. The chloroform was washed with water and dried over $MgSO_4$. Evaporation of the chloroform yielded 5a as a yellow solid. The material was purified by column chromatography (silica; chloroform/methanol/diethyl ether, 94:5:1). Recrystallization from 2-propanol/diethyl ether afforded 120 mg (17%) of 5a as bright yellow needles: mp (HCl salt) 201–202 °C; IR ($CHCl_3$) 3650 (NH) cm^{-1}; NMR ($CDCl_3$) δ 8.5 (d, 1, Ar*H*, J = 6 Hz), 6.5–7.7 (m, 7,

(22) C. B. Gairaud and G. R. Lappin, *J. Org. Chem.*, 18, 1 (1953).

Ar*H*), 3.8 (s, 3, OCH_3), 3.7 (s, 3, OCH_3), 3.1–3.7 (m, 4, CH_2). Anal. ($C_{19}H_{19}ClN_2O_2$) C, H, N.

***N*-(2-Chloroethyl)-4-[(6,7-dimethoxy-1-isoquinolinyl)methyl]benzenamine (5b).** Compound **5b** was prepared from **4b** in the same manner employed for the preparation of **5a** to yield 132 mg (18%) of **5b** as crude product. The material was purified by column chromatography with the same solvent system described for **5a**. Recrystallization from 2-propanol/diethyl ether afforded the product as yellow crystals: mp (HCl salt) 180–182 °C; IR ($CHCl_3$) 3660 (NH) cm^{-1}; NMR ($CDCl_3$) δ 8.5 (d, 1, Ar*H*, *J* = 6 Hz), 6.1–7.5 (m, 7, Ar*H*), 4.1 (s, 3, OCH_3), 3.9 (s, 3, OCH_3), 3.1–3.7 (m, 6, CH_2). Anal. ($C_{20}H_{21}ClN_2O_2$) C, H, N.

Methyl Hydrogen (*Z*)-Butenedioate (21). Maleic anhydride (25 g, 0.25 mol) and 10 mL of absolute methanol (0.31 mol) were stirred and heated at 50 °C for 2 h. The excess methanol was removed under reduced pressure, yielding a viscous, colorless oil, which was used without further purification: IR (neat) 3000–3600 (OH), 1780 (acid C=O), 1730 (ester C=O) cm^{-1}; NMR ($CDCl_3$) δ 7.2 (br s, 1, OH), 6.4 (s, 2, CH=CH), 3.8 (s, 3, OCH_3).

Methyl (*E*)-4-Chloro-4-oxo-2-butenoate (22). Thionyl chloride (33 g, 0.28 mol) dissolved in 30 mL of benzene was added dropwise to **21** (33 g, 0.25 mol). The mixture was heated to reflux for 18 h. The solvent and excess thionyl chloride were removed under reduced pressure, leaving a faintly yellow oil, which was used without further purification: IR (neat) 1760 (acid C=O), 1730 (ester C=O) cm^{-1}; NMR ($CDCl_3$) δ 7.2 (d, 2, CH, *J* = 20 Hz), 3.8 (s, 3, OCH_3).

Methyl (*Z*)-4-[[4-(6,7-Dimethoxy-1-isoquinolinyl)phenyl]amino]-4-oxo-2-butenoate (6a). To a stirring mixture of **2a** (1.0 g, 3.6 mmol), DCC (0.74 g, 3.6 mmol), and 100 mL of dry acetonitrile, methyl hydrogen maleate (**21**; 0.50 g, 3.6 mmol) dissolved in 30 mL of methylene chloride was added dropwise. The solution was stirred at room temperature for 2 h and filtered. The solvent was removed under reduced pressure, leaving 1.4 g (100%) of yellow crystals. Recrystallization from absolute ethanol/hexane afforded the product **6a** as pale yellow crystals: mp (HCl salt) 219–220 °C; IR ($CHCl_3$) 3420 (NH), 1720 (ester C=O), 1680 (amide C=O) cm^{-1}; NMR ($CDCl_3$) δ 7.0–8.3 (m, 11, Ar*H*, NH, CH=CH), 4.0 (s, 3, OCH_3), 3.9 (s, 3, $ArOCH_3$), 3.7 (s, 3, $ArOCH_3$). Anal. ($C_{22}H_{20}N_2O_5$) C, H, N.

Methyl (*Z*)-4-[[4-[(6,7-Dimethoxy-1-isoquinolinyl)methyl]phenyl]amino]-4-oxo-2-butenoate (6b). Compound **6b** was prepared from **2b** in the same manner employed for the preparation of **6a** to yield 1.1 g (100%) of **6b** as crude product. The material was recrystallized from absolute ethanol/hexane: mp (HCl salt) 196–199 °C; IR ($CHCl_3$) 3430 (NH), 1720 (ester C=O), 1675 (amide C=O) cm^{-1}; NMR ($CDCl_3$) δ 6.9–8.3 (m, 10, Ar*H*, NH, CH=CH), 4.5 (s, 2, CH_2), 4.0 (s, 3, OCH_3), 3.9 (s, 3, $ArOCH_3$), 3.17 (s, 3, $ArOCH_3$). Anal. ($C_{23}H_{22}N_2O_5$) C, H, N.

Methyl (*E*)-4-[[4-(6,7-Dimethoxy-1-isoquinolinyl)phenyl]amino]-4-oxo-2-butenoate (7a). Methyl 3-(chloroformyl)-2-propenoate (**22**; 0.5 g, 3.6 mmol), dissolved in 4 mL of chloroform, was added dropwise to a stirring suspension of **2a** (1.0 g, 3.6 mmol) and 20 mL of chloroform. The reaction mixture was stirred at reflux for 1 h. The solvent was removed under reduced pressure, yielding 1 g (71%) of a fine yellow powder. The solid was stirred in 20 mL of a 10% $NaHCO_3$ solution. The free base was extracted into chloroform, and the solvent was evaporated under reduced pressure. Recrystallization from methanol/diethyl ether afforded pale yellow crystals: mp 209 °C; IR (Nujol) 3400 (NH), 1720 (ester C=O), 1690 (amide C=O), 965–970 (CH=CH, trans) cm^{-1}; NMR ($CDCl_3$) δ 7.1–8.5 (m, 10, Ar*H*, CH=CH), 4.3 (s, 3, OCH_3), 4.1 (s, 6, $ArOCH_3$). Anal. ($C_{22}H_{20}N_2O_5$) C, H, N.

Methyl (*E*)-4-[[4-[(6,7-Dimethoxy-1-isoquinolinyl)methyl]phenyl]amino]-4-oxo-2-butenoate (7b). Compound **7b** was prepared from **2b** in the same manner employed for the preparation of **7a** to yield 0.95 g (70%) of **7b** as the crude product. The material was recrystallized from methanol/diethyl ether: mp (HCl salt) 187–189 °C; IR (Nujol) 3400 (NH), 1730 (ester C=O), 1690 (amide C=O), 956–970 (CH=CH, trans) cm^{-1}; NMR ($CDCl_3$) δ 7.1–8.5 (m, 10, Ar*H*, CH=CH), 4.3 (s, 2, CH_2), 4.1 (s, 3, OCH_3), 3.9 (s, 6, $ArOCH_3$). Anal. ($C_{23}H_{22}N_2O_5$) C, H, N.

***N*-[4-(6,7-Dimethoxy-1-isoquinolinyl)phenyl]-2,2′-aminobis[ethanol] (23a).** Modifying a procedure reported by Filer et al.,[19] we cooled a solution of **2a** (2.0 g, 7.1 mmol) in 40 mL of glacial acetic acid to −4 °C and added ethylene oxide (8.9 g, 0.20 mol) in one portion to the reaction mixture. The reaction flask was stoppered, and the mixture was allowed to stand at room temperature for 20 h. The solution was poured into 100 g of ice and made alkaline by the dropwise addition of a 20% NaOH solution. The free base was extracted with chloroform, the extract was dried over $MgSO_4$, and the solvent was removed under reduced pressure to yield 2.0 g (77%) of the crude product as a yellow solid. The material was purified by column chromatography (silica; chloroform/methanol/ammonium hydroxide, 95:4:1): mp (HCl salt) 211–213 °C; IR ($CHCl_3$) 3725 (OH) cm^{-1}; NMR ($CDCl_3$) δ 6.5–8.2 (m, 9, Ar*H*, NH), 4.5 (s, 2, 2 OH), 3.8 (s, 3, OCH_3), 3.65 (s, 3, OCH_3), 3.5 (t, 8, 4 CH_2). Anal. ($C_{21}H_{24}N_2O_4$) C, H, N.

***N*-[4-[(6,7-Dimethoxy-1-isoquinolinyl)methyl]phenyl]-2,2′-aminobis[ethanol] (23b).** Compound **23b** was prepared from **2b** in the same manner employed for the preparation of **23a** to yield 1.8 g (72%) of **27b** as crude product. The material was purified by column chromatography with the same solvent system described for **23a**: mp (HCl salt) 193–195 °C; IR ($CHCl_3$) 3690–3025 (OH) cm^{-1}; NMR ($CDCl_3$) δ 6.8–8.3 (m, 9, Ar*H*, NH), 4.65 (s, 2, CH_2), 4.5 (s, 2, 2 OH), 3.9 (s, 3, OCH_3), 3.8 (s, 3, OCH_3), 3.5 (t, 8, 4 CH_2). Anal. ($C_{22}H_{26}N_2O_4$) C, H, N.

***N*,*N*-Bis(2-chloroethyl)-4-(6,7-dimethoxy-1-isoquinolinyl)benzenamine (8a).** A mixture of **23a** (1.0 g, 2.7 mmol) dissolved in 50 mL of dry acetonitrile was cooled to 4 °C, and freshly distilled thionyl chloride (1.5 mL, 21.6 mmol) was added in one portion to the rapidly stirring solution. The reaction mixture was stirred at 4 °C for 1 h, and then the solvent was evaporated under reduced pressure. The residue was dissolved in 15 mL of chloroform and stirred with 20 mL of a saturated $NaHCO_3$ solution for 5 min. The chloroform layer was separated, and the aqueous layer was extracted with two 10-mL portions of chloroform. The combined extracts were dried over $MgSO_4$. The solvent was evaporated under reduced pressure to give 0.79 g (82%) of crude product as a yellow solid. Column chromatography (silica; chloroform/methanol/ammonium hydroxide, 94:5:1) afforded **8a** as fine yellow needles: mp (HCl salt) 206–208 °C; NMR ($CDCl_3$) δ 6.7–8.2 (m, 8, Ar*H*), 4.1 (s, 3, OCH_3), 3.9 (s, 3, OCH_3), 3.4–3.7 (m, 8, 4 CH_2). Anal. ($C_{21}H_{22}Cl_2N_2O_2$) C, H, N.

***N*,*N*-Bis(2-chloroethyl)-4-[(6,7-dimethoxy-1-isoquinolinyl)methyl]benzenamine (8b).** Compound **8b** was prepared from **23b** in the same manner employed for the preparation of **8a** to yield 0.75 g (79%) of **8b** as crude product. The material was purified by column chromatography employing the same solvent system used for **8a**: mp (HCl salt) 194–196 °C; NMR ($CDCl_3$) δ 6.8–8.4 (m, 8, Ar*H*), 4.2 (s, 2, CH_2), 3.9 (s, 3, OCH_3), 3.8 (s, 3, OCH_3), 3.3–3.7 (m, 8, 4 CH_2). Anal. ($C_{22}H_{24}Cl_2N_2O_2$) C, H, N.

Enzyme Assays. Assay of Phosphodiesterase Activity in Bovine Heart. Crude bovine heart phosphodesterase containing protein activator and calcium was obtained from the Sigma Chemical Co., St. Louis, MO. Assay of bovine heart phosphodiesterase activity was by the method of Glennon et al.[23] Linear reaction kinetics were obtained by appropriately adjusting the incubation time and protein concentrations such that the hydrolysis of cyclic AMP was limited to less than 25% under the assay conditions. None of the agents were found to alter the elution profile of cyclic AMP on 5′-AMP on the Dowex resin.

Preparation of Enzymes and Assay of Phosphodiesterase Activity in Rat Cerebral Cortex. Female Sprague–Dawley rats (Harland Sprague Dawley, Madison, WI), 200–250 g, were used in these experiments. Crude extracts of rat cerebral cortex were prepared as described previously.[24] The calcium-dependent phosphodiesterase was obtained following Sephadex G-200 gel filtration as described by Kakiuchi et al.[25] Assay of rat cerebral cortical phosphodiesterase activity was by the method of Davis and Daly using polyacrylamide–boronate affinity gel chromatography.[26] Linear reaction kinetics were obtained by appro-

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priately adjusting the incubation time and protein concentrations such that the hydrolysis of either cyclic nucleotide was limited to less than 25% under the assay conditions. None of the agents were found to alter the elution profile of the nucleotides and nucleosides during the polyacrylamide–boronate gel chromatography.

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Registry No. **1b**, 58-74-2; **2a**, 83633-12-9; **2b**, 83633-13-0; **3a**, 83633-14-1; **3a**·HCl, 83649-36-9; **3b**, 83633-15-2; **3b**·HCl, 83633-16-3; **4a**, 83633-17-4; **4a**·HCl, 83633-18-5; **4b**, 83633-19-6; **4b**·HCl, 83633-20-9; **5a**, 83633-21-0; **5a**·HCl, 83633-22-1; **5b**, 83633-23-2; **5b**·HCl, 83633-24-3; **6a**, 83633-25-4; **6a**·HCl, 83633-26-5; **6b**, 83633-27-6; **6b**·HCl, 83633-28-7; **7a**, 83633-29-8; **7b**, 83633-30-1; **7b**·HCl, 83633-31-2; **8a**, 83633-32-3; **8a**·HCl, 83633-33-4; **8b**, 83633-34-5; **8b**·HCl, 83633-35-6; **9**, 10268-50-5; **10**, 10268-35-6; **11**, 83633-36-7; **12**, 6309-18-8; **13**, 6924-15-8; **15**, 4230-93-7; **16**, 57542-90-2; **17**, 4722-08-1; **18**, 83633-37-8; **19**, 26193-61-3; **20**, 25932-34-7; **21**, 3052-50-4; **22**, 17081-97-9; **23a**, 83633-38-9; **23a**·HCl, 53633-39-0; **23b**, 83633-40-3; **23b**·HCl, 83633-41-4; 3,4-dimethoxyphenethylamine, 120-20-7; 4-nitrobenzoyl chloride, 122-04-3; 3,4-dimethoxybenzaldehyde, 120-14-9; nitromethane, 75-52-5; 4-nitrobenzeneacetyl chloride, 50434-36-1; acryloyl chloride, 814-68-6; chloroacetyl chloride, 79-04-9; ethylene oxide, 75-21-8; maleic anhydride, 108-31-6; phosphodiesterase, 9025-82-5.

Antitumor Amino-Substituted Pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinolines and Pyrido[4,3-*b*]carbazole Derivatives: Synthesis and Evaluation of Compounds Resulting from New Side Chain and Heterocycle Modifications

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New modifications of 10-[[3-(diethylamino)propyl]amino]-6-methyl-5*H*-pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinoline (**1b**) and 1-[[3-(diethylamino)propyl]amino]-9-methoxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole (**4b**), which display important antitumor properties, were performed either on the side chain or on the intercalating heterocycle. Side chains were introduced by direct substitution of the corresponding chloro derivatives and 6-*N*-methyl-9-hydroxypyrido[4,3-*b*]carbazoles analogues were prepared via 9-*O*-benzoyl-1-chloroellipticines. Evaluation of all new compounds shows no significant increase of in vitro cytotoxicity and percent ILS on the L1210 leukemia system by comparison with the model compounds **1b** and **4b**.

Recently, we reported the synthesis of various [[(dialkylamino)alkyl]amino]pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinolines (9-azaellipticines) (**1** and **2**) and 1-amino-substituted ellipticines (**3** and **4**) [Chart I; R = $NH(CH_2)_nN(R_2)R_3$].[1-5] Among these compounds, **1b** and **4b** exhibit high antitumor activities.[3,6]

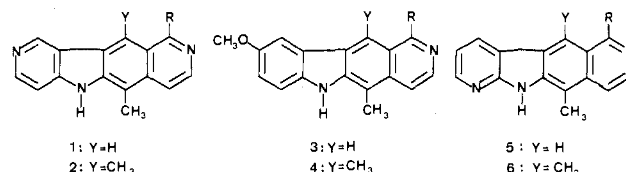
However, the biological results appeared closely dependent upon the nature of the side chain.[5] Furthermore, the biological properties could depend upon modifications of the heterocyclic intercalating ring, as suggested by the lack of antitumor activity of 7-azaellipticine derivatives (**5** and **6**).[7] Therefore, we decided to carry out further modifications of compounds **1–4** by introducing either new hydrophilic and lipophilic side chains or a 6-methyl group on the pyrido[4,3-*b*]carbazole heterocycle.

The synthesis and biological properties of these new compounds are reported in this paper.

Chemistry. Amino-substituted compounds **1–10** were obtained by substitution of the corresponding chloro derivatives, **1a–10a**, with appropriate amines. Bis(chloroethyl)amino derivative **3l** was obtained from [bis(hydroxyethyl)amino]propylamino compound **3b** by a standard method.[8]

The key intermediates **1a**, **3a**, and **4a** were already described,[1,4] and compounds **7a** and **8a** were prepared by methylation of the corresponding **3a** and **4a** (Scheme I). However, preparation of 6-methyl-9-hydroxypyrido[4,3-*b*]carbazoles **9b** and **10b** required an appropriate route to 9-*O*-benzoylpyrido[4,3-*b*]carbazoles **19a** and **20a**. This route involves demethylation of **11** and **12** to **13** and **14**,

Chart I



Substituent key:

a) R = Cl
b) R = $NH-(CH_2)_3-N(CH_2CH_3)_2$
c) R = $NH-(CH_2)_3-NH-CH_2CH_3$
d) R = $NH-CH_2-CH(OH)-CH_2-N(CH_2CH_3)_2$
e) R = $NH-CH_2-CH(OH)-CH_2OH$
f) R = $NH-(CH_2)_3-N$ (pyrrolidinone ring, C=O)
g) R = $NH-(CH_2)_3-N(CH_2CH_3)-(CH_2)_3-NH_2$
h) R = $NH-(CH_2)_3-N(CH_2CH_2OH)_2$
i) R = $NH-(CH_2)_3-N(CH_3)_2$
j) R = $NH-(CH_2)_3-N(CH_2CH_2CH_2CH_3)_2$
k) R = $NH-(CH_2)_2-N(CH_2CH_3)_2$
l) R = $NH-(CH_2)_3-N(CH_2CH_2Cl)_2$
m) R = $NH-C_6H_4-NH-SO_2CH_3$

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