Enzyme Inhibitors. Inhibition of Brain Choline Acetyltransferase by Derivatives of 4-Styrylpyrimidine†

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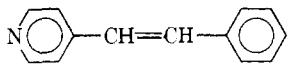
Twenty-two styrylpyrimidine derivatives were prepared and evaluated as inhibitors of choline acetyltransferase. The most effective inhibitors had two or four-amino substituents on the pyrimidine ring. Effectiveness of the inhibitors did not correlate well with the pK_a of the pyrimidine base. Kinetics of inhibition for the styrylpyrimidine and styrylpyridine inhibitors are similar.

In previous reports a large number of derivatives and analogs of 4-stilbazole (1) were evaluated as inhibitors of the enzyme choline acetyltransferase (acetyl-CoA: choline *O*-acetyltransferase, E.C. 2.3.1.6). In studies on quaternized derivatives of 1, Cavallito, *et al.*, presented extensive work on the structural and electronic requirements for potent and specific inhibition of choline acetyltransferase (ChAc).[1-3] Subsequently, we reported similar studies on nonquaternized derivatives of 1. The effects of substituents on the phenyl moiety were shown to differ markedly from those reported for the quaternary analogs.[4,5] Large increments in inhibition of ChAc were obtained by small, nonpolar meta substituents on the phenyl ring of 1, as large as 160-fold for *m*-iodo.[5] The contribution to binding by the meta substituents did not correlate well with the electronic nature or hydrophobic nature of the substituents.



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Studies on the contribution to binding by the vinyl bridge of 1 suggested that the double bond interacted directly with the enzyme.[4,5] This observation is supported by the presence of an irreversible component to ChAc inhibition by *N*-methyl-4-(1-naphthylvinyl)pyridinium iodide.[6] *In vivo* irreversible inhibition by 3′-chloro-4-stilbazole has also been reported.§

Although modification of the pyridyl moiety of 1, either by quaternization of the nitrogen or by the introduction of a 2-amino substituent, reportedly increased inhibition of ChAc,[1-3,7] no extensive study of the effects of changes in the heterocyclic system has been presented. We therefore undertook the synthesis of derivatives of 4-styrylpyrimidine and evaluation of these derivatives as inhibitors of ChAc. The results are the subject of this paper.

Results and Discussion

In the stilbazole series it has been shown that the introduction of small, nonpolar substituents in the 3′ position of the phenyl ring resulted in large increases in inhibition of ChAc. For this reason we chose the 3′-chloro substituent as the "constant" portion of the molecule and varied the structure of the heterocycle. The simplest pyrimidine derivative, 3′-chloro-4-styrylpyrimidine (3), had an I_{50} of 300 μM (Table I) which represents a loss of 38-fold in inhibition compared to 3′-chloro-4-stilbazole (2). This loss was recovered by the introduction of a 2-NH_2 (4) which exhibited an I_{50} of 15 μM. Similarly, the introduction of a 4-NH_2 (8) gave a 33-fold increase in inhibition compared to 3. The 4-amino-2-(3-chlorostyryl)pyrimidine (9) represents one of the best nonquaternized inhibitors of ChAc with I_{50} = 3 μM. The effects of addition of a second NH_2 substituent on the pyrimidine ring was dependent upon the position of the substitution. For example, the 2,4-diamino derivative 15 inhibited ChAc as effectively as 9, whereas the 4,5-diamino derivative 17 lost 300-fold in inhibition compared to 8.

The increase in inhibition observed with amino substitution may be attributed to the effects of the substituent on the pK_a of the heterocycle. Examination of the pK_a's in Table I indicates that there is no correlation between the basicity of the inhibitors and their I_{50}'s. Such comparisons may be misleading, however, since substitution on the ring may result in interactions with the enzyme other than those caused by redistribution of the electron density of the pyrimidine ring. An alternate explanation is that the amino substituents increase binding through point interactions with the enzyme.

Bulk tolerance studies on the 4-stilbazole molecule indicated that the inhibitor fits into a pocket on the enzyme which allows only small substituents in the 2′, 3′, and 4′ positions of the phenyl ring.[2-5] Investigation of the bulk tolerance adjacent to the pyrimidine ring also showed severe restrictions on the size of the substituent. Substituents in the 2, 5, and 6 positions (10-14, 18-23) other than the simple amino substituents led to large losses in inhibition. Although bulk tolerance has been demonstrated in the 1 position by a large number of quaternized derivatives,[2,3] compound 20 was not an inhibitor at test concentrations.

The lack of bulk tolerance adjacent to the pyrimidine ring is somewhat surprising in view of the reported inhibition of ChAc by 4-styrylquinolinium methiodide[2] and 2,4-distyrylpyridinium methiodide.[2] 2-Amino-4,6-di(3-methylstyryl)pyrimidine (24) was prepared and was shown to be ineffective as an inhibitor. To further examine this reported bulk tolerance, 2,6-di(3-chlorostyryl)pyridine (25) was evaluated as an inhibitor of ChAc. There was no detectable inhibition at 1 mM concentration of 25 when choline bromide concentration was 2.5 mM (see Experimental Section).

The introduction of 3′-Cl on the 4-stilbazole inhibitor resulted in a 20-fold increase in inhibition of ChAc. Similarly, substitution of 3′-Cl on 2-amino-4-styrylpyrimidine (5) resulted in a 50-fold increase in inhibition compared to 5. That this increase was not peculiar to the Cl group was demonstrated by compound 6 in which the 3′-CH_3 increased inhibition 21-fold compared to 5. However, the introduction of a 3′-NO_2 (7) led to a loss of inhibition. The substituent effects parallel those of 4-stilbazole indicating that the styrylpyrimidine and styrylpyridine inhibitors bind similarly to the enzyme.

The increases in inhibition by the 3′ substituents are far too large to be explained by arguments involving electronic or hydrophobic interactions. Two alternate explanations may be (1) a small increase in actual binding may result

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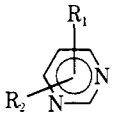
§R. D. Krell and A. M. Goldberg, personal communication.

Table I. Inhibition of Choline Acetyltransferase and Acetylcholinesterase by 

No.	R_1	R_2	Choline acetyltransferase: Inhibn, μM	Choline acetyltransferase: % inhibn	Choline acetyltransferase: I_{50},[a] μM	pK_a	Acetylcholinesterase: Inhibn, μM	Acetylcholinesterase: % inhibn	Acetylcholinesterase: I_{50},[a] μM
1					150		125	0	
2					8		500	33	
3	4-(3-ClC_6H_4CH=CH)	H			300	2.1	590[b]	37	1000[c]
4	4-(3-ClC_6H_4CH=CH)	2-NH_2			15	4.0	200[b]	20	875[c]
5	4-C_6H_4CH=CH	2-NH_2			750		500[b]	0	800[c]
6	4-(3-$CH_3C_6H_4CH$=CH)	2-NH_2			35		25[b]	0	
7	4-(3-$NO_2C_6H_4CH$=CH)	2-NH_2	500	0					
8	6-(3-ClC_6H_4CH=CH)	4-NH_2			9	4.8	50[b]	0	
9	2-(3-ClC_6H_4CH=CH)	4-NH_2			3	5.5	25[b]	0	
12	4-(3-CNC_6H_4CH=CH)	6-Me_2N							40
13	4-(3-ClC_6H_4CH=CH)	2-MeNH	50[b]	40	120[c]	4.3	19[b]	0	
14	4-(3-ClC_6H_4CH=CH)	2-CH_3S	60[b]	0			10[b]	0	
15	4-(3-ClC_6H_4CH=CH)	2,6-$(NH_2)_2$			2.5	6.7			
16	5-(3-ClC_6H_4CH=CH)	2,4-$(NH_2)_2$	1000	0					635
17	2-(3-ClC_6H_4CH=CH)	4,5-$(NH_2)_2$			900	5.9			500
18	2-(3-ClC_6H_4CH=CH)	4-NH_2-5-NO_2			10	(2.4)[d]	5[b]	0	
19	4-(3-ClC_6H_4CH=CH)	2-NH_2-6-Ph	60[b]	0			5[b]	0	
20	2-(3-ClC_6H_4CH=CH)	4-NH_2-5-Ph	50[b]	0			25[b]	0	
21	4-(3-ClC_6H_4CH=CH)	2-NH_2-6-CH_3O			260		50	0	
22	4-(3-ClC_6H_4CH=CH)	2-NH_2-6-$C_6H_5CH_2O$	50[b]	0			5[b]	0	
23	4-(3-ClC_6H_4CH=CH)	2-NH_2-5-Bu	25[b]	0					
24	4-(3-$CH_3C_6H_4CH$=CH)	2-NH_2-6-(3-$CH_3C_6H_4CH$=CH)		0					

[a] Concentration for 50% inhibition. [b] Maximum solubility. [c] Estimated from inhibition at maximum solubility. [d] pK_a for 4-amino-5-nitro-2-styrylpyrimidine; see M. E. Biffin, D. J. Brown, and T. C. Lee, *Aust. J. Chem.*, **20**, 1041 (1967).

in a large increase in observed inhibition through allosteric effects, and (2) the unsubstituted 4-stilbazole and 4-styrylpyrimdine bind at a site different from that of the 3′-substituted derivatives. If either were the case, structure-activity relationships of the inhibitors would be meaningless.

To test the first possibility, the inhibition patterns and apparent K_i's for **1**, **2**, **4**, and **5** were determined. All the inhibitors gave double reciprocal plots characteristic of noncompetitive inhibition when choline was the varying substrate. The K_i's for **1**, **2**, **4**, and **5** were determined from the Dixon plots[8] and found to be 175, 6, 23, and 675 μM, respectively. Analysis of the inhibition of ChAc by **2** using slope and intercept replots[9] showed that the inhibition was linearly noncompetitive with K_i = 3 μM. The inhibition pattern of **2** was determined with acetyl-CoA as the varying substrate and was also shown to be noncompetitive. These results are in agreement with the kinetics of inhibition reported by White and Cavallito for *N*-methyl-4-(1-naphthylvinyl)pyridinium iodide (NVP^+).[6]

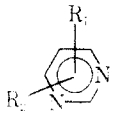
The kinetic data above suggest that the increments in observed inhibition are attributable to large increments in binding. It is interesting that the introduction of the 3′-Cl increased the apparent K_i's of both 4-stilbazole (**1**) and 2-amino-4-styrylpyrimidine (**5**) by 29-fold; the increments in I_{50} were 20- and 50-fold, respectively. Although the structure-activity relationships and the kinetic data suggest that the styrylpyrimidine and styrylpyridine inhibitors are binding to the same site, a definite conclusion is not warranted.

All the styrylpyrimidine derivatives described above were also tested as inhibitors of acetylcholinesterase (AChE, acetylcholine acetyl hydrolase, E.C. 3.1.1.7). Specificity between enzymes has been maintained where solubility of the inhibitors in the AChE assay made comparisons possible. Of the compounds which showed good inhibition of AChE, the dimethylamino derivative **11** was the most effective, showing an I_{50} of 40 μM. In the stilbazole series, 3′-cyano-4-stilbazole inhibited the esterase tenfold more effectively than **2**.[4] The 3′-cyano derivative **12** did not show a similar increase in inhibition for the styrylpyrimidine inhibitor. This suggests that the phenyl moieties of **2** and **11** bind differently to AChE.

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each sample has an ir spectrum compatible with its structure and moved as one spot on tlc on Brinkman silica gel GF with EtOAc or $CHCl_3$. The uv spectrum of each compound was compatible with the trans isomer and was stable to photoisomerization at the assay pH of 7.4.[10] The reported yields are for analytically pure material and are minimum. All analytical samples gave combustion values for C, H, and N within 0.4% of theoretical values (Galbraith Laboratories, Knoxville, Tenn.). The pK_a's were determined from uv spectra by the method of Albert and Sergeant.[11]

4-(3-Chlorostyryl)pyrimidine (3). Method A. A mixture of 1.0 g (10.6 mmol) of 4-methylpyrimidine and 1.5 g (10.6 mmol) of 3-chlorobenzaldehyde was condensed in acetic anhiydride as described by Loader and Timmons.[12] After 18 hr of reflux under N_2, the cooled mixture was poured into water, basified, and extracted into 3 × 50 ml of benzene. The solvent was washed free of base

Table II. Physical Properties of 

No.	R_1	R_2	HX	Method	% yield	Mp, °C	Formula
3	4-(3-ClC_6H_4CH=CH)	H	HCl	A	44[a]	252–254	$C_{12}H_{10}Cl_2N_2$
4	4-(3-ClC_6H_4CH=CH)	2-NH_2	Base	B	50[b]	156–158	$C_{12}H_{10}ClN_3$
5	4-C_6H_5CH=CH	2-NH_2	Base	B	63[c]	147–149	$C_{12}H_{11}N_3$
6	4-(3-$CH_3C_6H_4CH$=CH)	2-NH_2	Picrate	B	43[b]	229–231	$C_{19}H_{16}N_6O_7$
7	4-(3-$NO_2C_6H_4CH$=CH)	2-NH_2	Base	B	73[d]	160–164	$C_{12}H_{10}N_4O_2$
8	6-(3-ClC_6H_4CH=CH)	4-NH_2	Base	C[e]	20[f]	237–238	$C_{12}H_{10}ClN_3$
9	2-(3-ClC_6H_4CH=CH)	4-NH_2	Picrate	A	17[b]	252–254	$C_{18}H_{13}ClN_6O_7$
10	6-(3-ClC_6H_4CH=CH)	4-MeNH	Base	A[g]	20[h]	115–116	$C_{13}H_{12}ClN_3$
11	6-(3-ClC_6H_4CH=CH)	4-Me_2N	Base	A[i]	30[h]	130–131	$C_{14}H_{14}ClN_3$
12	6-(3-CNC_6H_4CH=CH)	4-Me_2N	Base	A[i]	60[h]	156–157	$C_{15}H_{14}N_4$
13	4-(3-ClC_6H_4CH=CH)	2-MeNH	Picrate	B[j]	14[b]	227–229	$C_{19}H_{15}ClN_6O_7$
14	4-(3-ClC_6H_4CH=CH)	2-CH_3S	Base	C[e]	86[a]	105–107	$C_{13}H_{11}ClN_2S$
15	6-(3-ClC_6H_4CH=CH)	2,4-$(NH_2)_2$	Picrate	A[l]	23[m]	317–320 dec	$C_{18}H_{14}ClN_7O_7$
16	5-(3-ClC_6H_4CH=CH)	2,4-$(NH_2)_2$	HCl	Exptl	47[c]	295–298	$C_{12}H_{12}Cl_2N_4$
17	2-(3-ClC_6H_4CH=CH)	4,5-$(NH_2)_2$	HCl		52[d,n]	290–292	$C_{12}H_{12}Cl_2N_4$
18	2-(3-ClC_6H_4CH=CH)	4-NH_2-5-NO_2	Base	Exptl	60[d]	181–182	$C_{12}H_9ClN_4O_2$
19	4-(3-ClC_6H_4CH=CH)	2-NH_2-6-C_6H_5	Base	A[o]	33[a]	140–142	$C_{18}H_{14}ClN_3$
20	2-(3-ClC_6H_4CH=CH)	4-NH_2-5-C_6H_5	Base	A[p]	34[d]	204–205	$C_{18}H_{14}ClN_3$
21	4-(3-ClC_6H_4CH=CH)	2-NH_2-6-CH_3O	Base	Exptl	53[b]	115–116	$C_{13}H_{12}ClN_3O$
22	4-(3-ClC_6H_4CH=CH)	2-NH_2-6-$C_6H_5CH_2O$	Picrate	Exptl	4[b]	200–201	$C_{25}H_{19}ClN_6O_8$
23	4-(3-ClC_6H_4CH=CH)	2-NH_2-5-*n*-Bu	Picrate	A[q]	58[b]	210–211	$C_{22}H_{20}ClN_6O_7$
24	4-(3-$CH_3C_6H_4CH$=CH)	2-NH_2-6-(3-CH_3-C_6H_4CH=CH)	Base	A	41[c]	141–143	$C_{22}H_{21}N_3$

[a] Recrystallized from EtOH-hexane. [b] Recrystallized from EtOH-H_2O. [c] Recrystallized from EtOH. [d] Recrystallized from EtOH-EtOAc. [e] For starting 4-amino-6-methylpyrimidine, see H. C. van der Plas, *Recl. Trav. Chim. Pays-Bas*, 84, 1101 (1965). [f] Recrystallized from EtOAc. [g] The 4-methyl-2-methylaminopyrimidine was prepared by the method of van der Plas (see ref in footnote *e*) and used without further purification. [h] Recrystallized from EtOAc-hexane. [i] For 4-dimethylamino-6-methylpyrimidine (**26**) see the Experimental Section. [j] For 4-methyl-2-methylaminopyrimidine see Johnson and MacKenzie, *Amer. Chem. J.*, 42, 353 (1909); D. J. Brown "The Pyrimidines," Wiley, New York, N. Y., 1962. [k] For 4-methyl-2-methylthiopyrimidine see D. J. Brown and R. V. Foster, *Aust. J. Chem.*, 19, 2321 (1966). [l] For 2,4-diamino-6-methylpyrimidine see the Experimental Section. [m] Recrystallized from EtOH-acetone. [n] Obtained by the reduction of 18 with $SnCl_2$ and HCl by the method of Biffin, *et al.* (see ref in footnote *d*, Table I). [o] For 2-amino-4-methyl-6-phenylpyrimidine see K. D. Kulkani, S. S. Sabris, and B. S. Kulkani, *J. Sci. Ind. Res., Sect. C*, **19**, 6 (1960); *Chem. Abstr.*, 54, 22576 (1960). [p] For 4-amino-2-methyl-5-phenylpyrimidine see the Experimental Section. [q] For 2-amino-5-*n*-butyl-4-methylpyrimidine see D. J. Brown, B. T. England, and J. M. Lyoll, *J. Chem. Soc. C*, 226 (1966).

and dried over Na_2SO_4 and a stream of dry HCl was passed through the solution. The tan precipitate was collected and recrystallized twice from EtOH-petroleum ether (bp 60–90°) to give the analytical sample. See Table II for additional data.

2-Amino-4-(3-chlorostyryl)pyrimidine (4). Method B. A mixture of 1.5 g (14 mmol) of 2-amino-4-methylpyrimidine and 2.0 g (14 mmol) of 3-chlorobenzaldehyde was condensed in refluxing formic acid as described by Matsukow and Suikawa.[13] After 18 hr, the cooled reaction mixture was basified with 1 *N* NaOH and the product collected by filtration. Two recrystallizations from EtOH-H_2O gave the analytical sample. See Table II for additional data.

4-Amino-6-(3-chlorostyryl)pyrimidine (8). Method C. A mixture of 0.3 g (2.8 mmol) of 4-amino-6-methylpyrimidine and 1.26 g (9 mmol) of 3-chlorobenzaldehyde was condensed in 5 ml of acetic acid with 2 g of H_2SO_4 as described by Saikawa and Wada.[14] After 18 hr of reflux, the cooled reaction mixture was poured into 50 ml of cold H_2O and extracted with 3 × 50 ml of benzene. The aqueous layer was basified with 1 *N* NaOH and extracted with 3 × 50 ml of EtOAc. The solvent was washed free of base and dried over Na_2SO_4 and the solvent was removed *in vacuo*. The residue was recrystallized twice from EtOH-hexane to give the analytical sample. See Table II for additional data.

2,4-Diamino-5-(3-chlorostyryl)pyrimidine (16). To a mixture of 3.4 g (8 mmol) of 3-chlorotriphenylphosphonium chloride[4] and 1.1 g (8 mmol) of 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) in 50 ml of dimethylformamide was added 1.0 g (4.5 mmol) of 2,4-diacetamido-5-pyrimidinecarboxaldehyde.[15] The mixture was stirred overnight at ambient temperature and then poured into 50 ml of cold 2 *N* HCl. After extraction with 3 × 50 ml of EtOAc, the aqueous phase was basified with 50% NaOH with cooling. The product was extracted into 3 × 50 ml of EtOAc and a stream of dry HCl passed through the solution. The precipitate was collected and recrystallized twice from EtOH to give the analytical sample. For additional data see Table II.

4-Amino-5-nitro-2-(3-chlorostyryl)pyrimidine (18). A mixture of 1 g (3.3 mmol) of 4-amino-2-(3-chlorostyryl)-6-hydrazino-5-nitropyrimidine (**26**) and 8.4 g of Ag_2O was heated to reflux in 100 ml of ethylene glycol monomethyl ether for 1 hr. The mixture was filtered hot and the solvent removed *in vacuo*. The residue was recrystallized twice from EtOH-EtOAc. See Table II for additional data.

4-Amino-2-(3-chlorostyryl)-6-hydrazino-5-nitropyrimidine (26). A mixture of 1 g (3.2 mmol) of **27** and 1 g of hydrazine hydrate in 100 ml of EtOH was refluxed for 1 hr and cooled and the precipitate was collected. The product was recrystallized once from EtOH-acetone and used without further purification.

4-Amino-6-chloro-2-(3-chlorostyryl)-5-nitropyrimidine (27). To a suspension of 3.4 g (10.3 mmol) of **28** in 50 ml of Et_2O was added dropwise a solution of ammonia in MeOH prepared by saturating 4 ml of MeOH at 4° with NH_3 followed by dilution to 10 ml with cold MeOH. The mixture was stirred for 2 hr at room temperature, filtered, and washed with Et_2O. The product was recrystallized twice from EtOAc-hexane: yield, 2.3 g (72%); mp 188–188.5°. *Anal.* ($C_{12}H_8Cl_2N_4O_2$) C, H, N.

2-(3-Chlorostyryl)-4,6-dichloro-5-nitropyrimidine (28). A mixture of 1 g (3.4 mmol) of **29**, 20 ml of phosphorus oxychloride, and 5 ml of diethylaniline was refluxed under N_2 for 1 hr, cooled, and poured over ice. The solid was collected, washed with water, and air-dried. The brick red product was dissolved in benzene

and filtered through silica gel. Evaporation of the solvent gave a light tan residue which was recrystallized twice from EtOAc-hexane to give the analytical product: yield, 0.51 g (45%); mp 167-168°. *Anal.* ($C_{12}H_6Cl_3N_3O_2$) C, H, N.

2-(3-Chlorostyryl)-5-nitro-6-hydroxy-4-pyrimidone (29) Piperidine Salt. A mixture of 1 g (5.9 mmol) of 2-methyl-5-nitro-6-hydroxy-4-pyrimidone, 8 g of 3-chlorobenzaldehyde (53 mmol), and 4 ml of piperidine was heated to 90° for 90 min and then to 150-160° for 20 min. The mixture was cooled and diluted with 20 ml of MeOH-Et_2O (1:1) and the precipitate collected by filtration. Two recrystallizations from MeOH gave the analytical product: yield, 1.87 g (84%); mp 230-234° dec. *Anal.* ($C_{17}H_{19}ClN_4O_4$) C, H, N.

2-Amino-4-(3-chlorostyryl)-6-methoxypyrimidine (21). A mixture of 0.5 g (1.9 mmol) of 2-amino-6-(3-chlorostyryl)-4-chloropyrimidine (prepared by the method of Henze, *et al.*;[16] the product was a dark red amorphous powder used without further purification) and 1 g of NaOMe in 20 ml of MeOH was refluxed for 1 hr. The mixture was poured into cold water and extracted into 3 × 50 ml of EtOAc. The solvent was removed *in vacuo* and a saturated EtOH-picric acid solution added to the residue. The precipitate was collected and recrystallized three times from EtOH-H_2O. The free base was recovered by treating with 50 ml of 2 *N* NaOH and extracted into $CHCl_3$. A saturated HCl-EtOH solution was added to the residue and the solvent removed *in vacuo*. The residue was recrystallized twice from EtOH-hexane. The product was then taken up in a minimum of EtOH, placed on a 10-cm silica gel column, and eluted with $CHCl_3$. The product isolated from the column was recrystallized once from EtOH-H_2O to give the analytical sample. See Table II for additional data.

2-Amino-4-benzyloxy-6-(3-chlorostyryl)pyrimidine (22). To 0.38 g (8 mmol) of NaH in 10 ml of DMF was added 1 g (9.3 mmol) of benzyl alcohol over 1 hr. When evolution of H_2 ceased, 0.5 g (2.0 mmol) of 2-amino-4-chloro-6-(3-chlorostyryl)pyrimidine was added at once and the mixture heated to 60° for 1 hr. The cooled mixture was poured into 100 ml of cold water and the product extracted into 3 × 50 ml of EtOAc. The solvent was washed free of base, dried over Na_2SO_4, and removed *in vacuo*. A saturated EtOH-picric acid solution was added to the residue and the crystalline product recrystallized twice from EtOH-H_2O. See Table II for additional data.

2,6-Di(3-chlorostyryl)pyridine (25) Hydrochloride. This compound was prepared from 3-chlorobenzaldehyde and 2,6-lutidine by method A. The product was isolated as the HCl salt and recrystallized twice from EtOH to give the analytical sample: yield, 5.3 g (43%); mp 157-159°. *Anal.* ($C_{21}H_{16}Cl_3N$) C, H, N.

2,4-Diamino-6-methylpyrimidine (26). A mixture of 2 g (14 mmol) of 2-amino-4-chloro-6-methylpyrimidine and 50 ml of saturated NH_3-EtOH was heated to 180° for 6 hr in a stainless steel bomb. The cooled reaction mixture was evaporated *in vacuo* and 10 ml of 10 *N* NaOH was added to the residue. The mixture was heated to 60° for 1 hr, cooled, and filtered, and the filtrate was allowed to stand at room temperature overnight. The crystals were collected and recrystallized from acetone-hexane: yield, 0.53 g (31%); mp 184-186°.

2-Acetamido-6-(3-chlorostyryl)-4-pyrimidol (31). To a mixture of 2.3 g (5.4 mmol) of 3-chlorobenzyltriphenylphosphonium chloride and 1 g of DBN in 50 ml of DMF was added 1 g (5.5 mmol) of 2-acetamido-6-hydroxy-4-pyrimidinecarboxaldehyde.[&] After 2 hr the solution was poured into 50 ml of H_2O and the precipitate collected. The sample was recrystallized twice from EtOH to give the analytical sample: yield, 9.8 g (62%); mp 270-271°. *Anal.* ($C_{14}H_{12}ClN_3O_2$) C, H, N.

[&] J. Joordan and D. V. Santi, unpublished results.

4-Amino-2-methyl-5-phenylpyrimidine (32). A mixture of 11 g (67 mmol) of α-cyano-β-ethoxystyrene and 5.0 g (53 mmol) of acetamidine hydrochloride previously treated with 3.5 g (62 mmol) of NaOMe was refluxed in 75 ml of EtOH overnight. The solvent was removed *in vacuo*. The solid was recrystallized twice from EtOH-EtOAc-hexane to give the analytical sample: yield, 2.6 g (29%); mp 193-194°. *Anal.* ($C_{11}H_{11}N_3$) C, H, N.

Enzyme Assays. Choline acetyltransferase (ChAc) and acetylcholinesterase (AChE) were isolated from rabbit brain acetone powder by the method of Potter, *et al.*[17] ChAc was assayed with 1 m*M* choline bromide (ChBr) and 0.05 m*M* [^{14}C]acetyl-CoA by a modification of the method of McCaman and Hunt[18] described earlier.[4] AChE was assayed by a modification of the method of Potter[19] previously reported.[4] Substrate concentrations used for kinetic studies were ChBr, 0.28-2.5 m*M*, and acetyl-CoA, 0.08-0.75 m*M*. Inhibitors were added to the ChAc assay in dimethyl sulfoxide (DMSO) and to the AChE assay in methoxyethanol (MeOEtOH) to final concentrations of 10% DMSO and 25% MeOEtOH. Enzyme activity at these solvent concentrations remained linear over the times used for these studies. All I_{50}'s were performed in duplicate and kinetic data are the average of four runs.

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