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Ergoline Congeners as Potential Inhibitors of Prolactin Release. 3. Derivatives of 3-Phenylpiperidine

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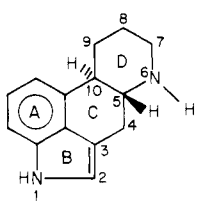
In a continuation of our attempts to elucidate the prolactin release inhibiting pharmacophore within the ergoline structure, we have prepared several derivatives of 3-phenylpiperidine. These congeners have been evaluated for inhibition of prolactin release in vivo and are for the most part inactive.

The ability of certain ergoline (1) derived compounds to inhibit the release of prolactin has been known and studied for some time.[1] The nidation-blocking action[2,3] as well as the lactation-inhibiting[4] and tumor-regression effects[5,6] of various ergoline derivatives are direct consequences of their prolactin release inhibiting activity. More recent findings have indicated that prolactin release is under the influence of a dopaminergic system in the brain.[7-9] Since potent dopaminergic activity has been demonstrated for several ergoline derivatives,[10,11] it is likely that these compounds inhibit prolactin release via an action on a dopaminergic system.[12]



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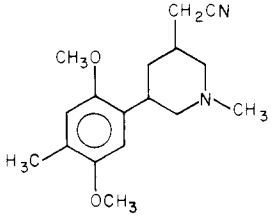


2

Examination of the ergoline structure reveals that the A and D rings comprise a 3-phenylpiperidine moiety. Derivatives of 3-phenylpiperidine have previously been prepared as analogues of ergoline derived compounds;[13-15] however, none of these have been evaluated for prolactin release inhibiting or dopaminergic activity. In a continuation of our efforts[16,17] to elucidate the smallest fragment within the ergoline structure that still retains prolactin release inhibiting activity, we have prepared several derivatives of 3-phenylpiperidine. In the design of potential prolactin release inhibitors several modifications of the basic 3-phenylpiperidine structure were desired: (1) a tertiary amine correlating with N-6 of ergoline, (2) an activated aromatic nucleus, and (3) a functional group that mimics the C-8 substituent of an ergoline derivative known to have prolactin release inhibiting activity. Congener **2** fulfills these criteria and now we report the synthesis and evaluation of this target compound and several intermediates for inhibition of prolactin release in vivo.

Chemistry. The synthetic sequence that was chosen for the preparation of **2** is shown in Scheme I. The 3-phenylpiperidine ring skeleton is formed by a Dieckmann cyclization of **9**, followed by appropriate alteration of the functional groups attached to the ring. This approach is somewhat similar to that used by Hohenlohe-Ochringen et al.[14] for their preparation of 3-phenylpiperidine derivatives. Although it is somewhat lengthy, this sequence was used since it permits the possibility of preparing congeners which have unsaturation in a position analogous to the unsaturation found in the D ring of some ergoline derivatives.

The unsaturated ester **8** was prepared from **3** in about 40% overall yield. Cyclization of **9** was accomplished with sodium hydride in benzene and initially gave **10** as a viscous oil. Interestingly, this oil showed four absorptions in the carbonyl region of the infrared spectrum (1620, 1660, 1720, and 1740 cm^{-1}) indicating the presence of both β-keto ester and hydrogen-bonded enol ester tautomers. After **10** was crystallized or converted to its crystalline hydrochloride salt, only the absorptions at 1620 and 1660 cm^{-1} remained. Since the hydrogen-bonded enolized tautomer of **10** has only one asymmetric center, the tendency for **10** to exist predominantly in this form eliminated the need for a separation of diastereomers which might have been encountered at this stage.

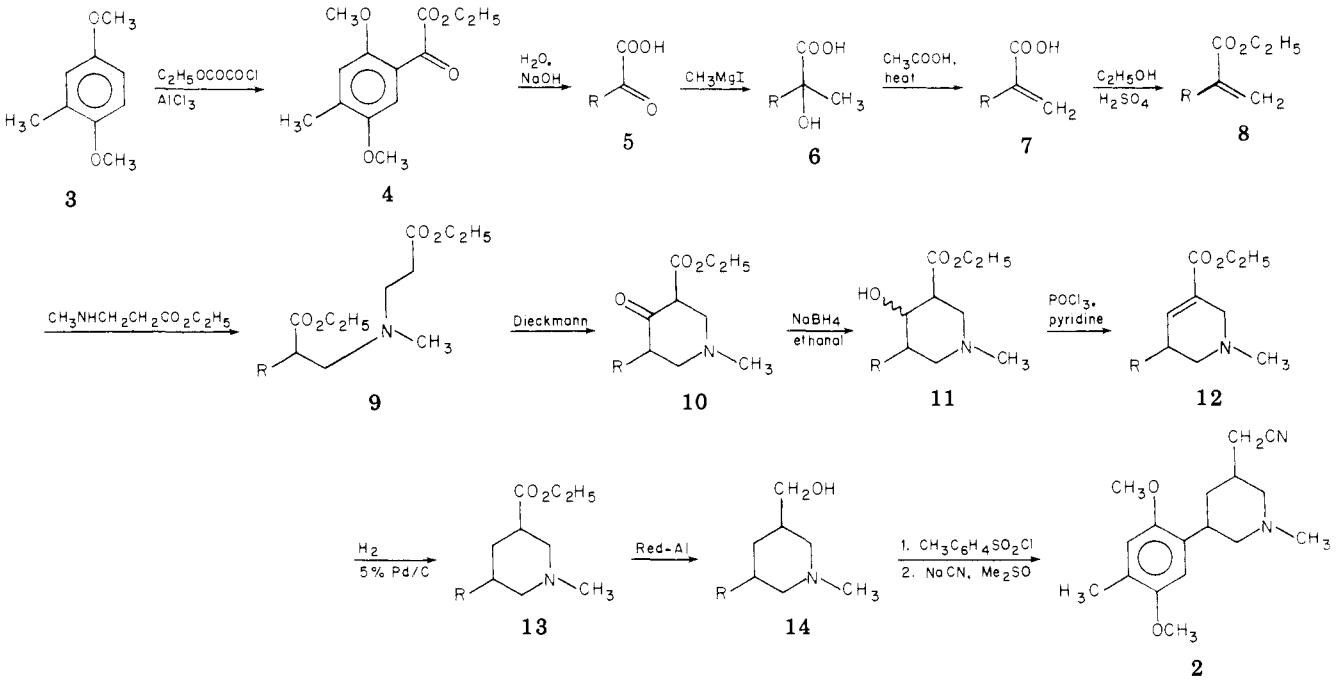
Extraction of **10** from aqueous base into an organic solvent gave only a 75–83% recovery of this material. Improved yields were obtained from the Dieckmann cyclization when the hydrochloride salt of **10** was extracted from the work-up mixture into chloroform.

Treatment of **10** with sodium borohydride in ethanol gave **11**, which was presumed to be a mixture of isomers. Initial attempts to dehydrate **11** with $POCl_3$ in pyridine at steam bath temperature resulted in nearly complete loss of basic material; however, when the reaction was carried out at 5–10 °C a good yield of **12** was obtained.

Catalytic hydrogenation of **12** over 5% Pd/C proceeded to completion in several hours. Chromatographic analysis revealed that the product was a single diastereomer and a 220-MHz NMR spectrum (Morgan Schaffer Corp., Montreal, Canada) was in accord with the assignment of a cis-diequatorial arrangement of the substituents in **13**. This interpretation was confirmed by a computer-generated NMR spectrum. Entering chemical shift and coupling constant values from the 220-MHz NMR spectrum into a LAOCOON III program[18] in the format appropriate for *cis*-**13**, a splitting pattern for the piperidine protons was generated which was superimposable on the original spectrum.

Modification of the ester functional group proceeded without difficulty. It is assumed that the target compound **2** has the same cis-diequatorial arrangement of the C-3 and

Scheme I



C-5 substituents that was established for **13**.

Experimental Section

All boiling points are uncorrected. Melting points were determined in open glass capillaries using a Thomas-Hoover Uni-Melt apparatus and are uncorrected. Elemental analysis were performed by Midwest Microlab, Ltd., Indianapolis, Ind., and by the Division of Medicinal Chemistry and Natural Products, University of Iowa. Infrared spectra were recorded on a Beckman IR-10 spectrophotometer or a Perkin-Elmer 267 grating infrared spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian Associates T-60 spectrometer using tetramethylsilane as an internal standard. Mass spectra were obtained on a Finnigan Model 1015 mass spectrometer. Where analyses are indicated by symbols of the elements, the analytical results were with ±0.4% of the theoretical values.

Ethyl 2,5-Dimethoxy-4-methylphenylglyoxylate (4). Following the procedure of Stiller et al.,[19] a solution of 24 g (0.16 mol) of 2,5-dimethoxytoluene (**3**) and 25 g (0.18 mol) of ethyloxalyl chloride (Aldrich) in 150 ml of CH_2Cl_2 was added dropwise to a stirred suspension of 37.3 g (0.28 mol) of $AlCl_3$ in 100 ml of CH_2Cl_2 with the temperature maintained below 5 °C throughout. When addition was complete, the mixture was stirred at 25 °C for 2.5 h and then poured onto 600 g of ice–H_2O. The aqueous layer was washed once with CH_2Cl_2. The combined organic extracts were washed with 3 M HCl, 1 M HCl, H_2O, and saturated NaCl, dried ($MgSO_4$), and evaporated. The product was crystallized from MeOH: yield 31.6 g (78%); mp 92–94 °C. Anal. ($C_{13}H_{16}O_5$) C, H.

2,5-Dimethoxy-4-methylphenylglyoxylic Acid (5). A solution of 70 g (0.28 mol) of **4** and 11.5 g (0.29 mol) of NaOH in 200 ml of 95% EtOH and 300 ml of H_2O was heated to reflux for 1 h. The clear solution was cooled in an ice bath and 100 ml of 2 M HCl was added followed by 300 ml of H_2O all at once. The product was removed by filtration and dried in vacuo at 100 °C. Crystallization from MeOH–H_2O gave 59.1 g (95%): mp 174–176 °C (lit.[20] mp 170 °C).

2-(2′,5′-Dimethoxy-4′-methylphenyl)-2-hydroxypropanoic Acid (6). A solution of methylmagnesium iodide in 150 ml of Et_2O made from 90 g (0.63 mol) of methyl iodide and 15 g (0.6 mol) of Mg was added dropwise with stirring to a suspension of 40 g (0.18 mol) of **5** in 300 ml of Et_2O at ice bath temperature. When addition was complete, the mixture was allowed to warm to room temperature while stirring for 2 h. The Et_2O layer was decanted and 200 ml of 3 M H_2SO_4 in 100 g of ice was added and the slurry was stirred vigorously. The solid precipitate was extracted into $CHCl_3$ and the aqueous solution was washed. The combined organic phases were washed with H_2O, dried ($MgSO_4$), and evaporated. The product was crystallized from MeOH: yield 35.4 g (82%); mp 151–153 °C. Anal. ($C_{12}H_{16}O_5$) C, H.

2-(2′,5′-Dimethoxy-4′-methylphenyl)-2-propenoic Acid (7). A solution of 10 g (0.042 mol) of **6** in 300 ml of glacial HOAc was heated to reflux for 4 h. The solvent was removed by evaporation and the residue was crystallized from $CHCl_3$: yield 7.8 g (84%); mp 167–168.5 °C (lit.[20] mp 164 °C).

Ethyl 2-(2′,5′-Dimethoxy-4′-methylphenyl)-2-propenoate (8). A solution of 44 g (0.198 mol) of **7** in 1 l. of EtOH with 2 ml of concentrated H_2SO_4 was heated to reflux for 60 h. The EtOH was removed in vacuo and the residue was taken up in Et_2O, washed with 1 M Na_2CO_3 and H_2O, dried ($MgSO_4$), and evaporated. The product crystallized on standing: yield 41.7 g (84%); mp 44.5–46 °C; bp 138 °C (0.2 mm). Anal. ($C_{14}H_{18}O_4$) C, H.

Ethyl 3-(Methylamino)propanoate. To a chilled solution of 45 g (1.45 mol) of methylamine in 200 ml of EtOH was added slowly with cooling and stirring 121 g (1.21 mol) of ethyl acrylate. The mixture was placed in a 500-ml pressure bomb, sealed, and allowed to stand at room temperature for 5 days. The bomb was opened and the EtOH was removed by roto-evaporation. The residue was fractionally distilled using a 3-in. Vigreux distillation head: yield 41 g (26%); bp 89–92 °C (44 mm) [lit.[21] bp 68–68.5 °C (18 mm)].

Ethyl 2-(2′,5′-Dimethoxy-4′-methylphenyl)-3-[*N*-methyl-*N*-(2-carboethoxyethyl)amino]propanoate (9). A mixture of 4 g (0.016 mol) of **8**, 2 g (0.016 mol) of ethyl 3-(methylamino)-propanoate, and 0.3 g of glacial HOAc was heated on a steam bath for 16 h. The mixture was taken up in Et_2O and extracted with 0.5 M HCl. The acid solution was washed with Et_2O, made basic with 3 M NaOH, and extracted with Et_2O. The Et_2O extracts were dried ($MgSO_4$) and evaporated to give a yellow oil: yield 3.6 g (59%). Anal. ($C_{20}H_{31}NO_6$) C, H, N.

***N*-Methyl-3-(2′,5′-dimethoxy-4′-methylphenyl)-5-carboethoxy-4-piperidone (10).** In a 1-l. three-necked flask fitted with an overhead stirrer, N_2 inlet, and condenser, 11.0 g (0.26 mol) of an NaH 57% oil dispersion was washed three times with 15 ml of dry hexane. The NaH was submerged in 250 ml of dry benzene and 15.0 g (0.038 mol) of **9** in 80 ml of benzene was added dropwise. The mixture was then heated to reflux for 3 h, cooled, and pipetted dropwise into 180 ml of ice-cold 3 M HCl with vigorous stirring. The benzene layer was separated and washed with 50 ml of 3 M HCl. The aqueous layers were combined and extracted with 3 × 150 ml of $CHCl_3$. Any remaining solid was dissolved in the $CHCl_3$. The $CHCl_3$ extracts were dried ($MgSO_4$) and evaporated. The residue was dissolved in a minimal volume of $CHCl_3$ and diluted carefully with Et_2O. Scratching induced crystallization:

Table I. Effect of Ergoline Congeners on Prolactin Release in Vivo

Test compd	Dose, mg	Serum prolactin levels (ng/ml ± SE)		% inhibn rel to control	Significance
		Control	After treatment		
Apomorphine	1	25.67 ± 3.06	18.3 ± 3.06	29	$p < 0.05$
2	2	43.7 ± 4.6	49.3 ± 13.0	−13	N.S.
10	1	34.1 ± 2.3	26.7 ± 2.4	22	$p < 0.05$
12	2	43.7 ± 4.6	46.1 ± 5.6	−5	N.S.
13	2	32.1 ± 2.3	30.6 ± 3.4	5	N.S.
14	2	43.7 ± 4.6	44.5 ± 5.1	−2	N.S.

yield 9.5 g (65%); mp 158–160 °C dec; ir (KBr) 1620 (C=C) and 1660 cm^{-1} (C=O). To 50 ml of 1 M Na_2CO_3 was added 2 g (0.0054 mol) of **10**·HCl. The suspension was vigorously extracted with 3 × 50 ml of Et_2O whereupon all solid dissolved. The extracts were dried ($MgSO_4$), filtered, and evaporated to give an oil: 1.5 g (83%); ir (neat) 1620 (C=C), 1660 (conj C=O), 1720 (ketone C=O), and 1740 cm^{-1} (ester C=O). The oil began to crystallize on standing and the process was hastened by the addition of hexane with a few drops of Et_2O: mp 95–99 °C; ir (KBr) 1620 (C=C) and 1660 cm^{-1} (C=O). A solution of the product in $CHCl_3$ gave a red coloration when a few drops of dilute ferric chloride in $CHCl_3$ were added. Anal. ($C_{18}H_{26}NO_4Cl$) C, H, N.

***N*-Methyl-3-(2′,5′-dimethoxy-4′-methylphenyl)-4-hydroxy-5-carboethoxypiperidine (11).** To a solution of 4.0 g (0.011 mol) of **10** in 50 ml of EtOH was added 0.2 g (0.0053 mol) of $NaBH_4$. The mixture was stirred for 4 h at room temperature and then the solvent was removed in vacuo. The residue was taken up in H_2O, made basic (NaOH), and extracted with Et_2O. The Et_2O was dried ($MgSO_4$), filtered, and evaporated to give a sticky gum: yield 3.4 g (84.5%). Anal. ($C_{18}H_{27}NO_5$) C, H, N.

***N*-Methyl-3-(2′,5′-dimethoxy-4′-methylphenyl)-5-carboethoxy-1,2,3,6-tetrahydropyridine (12) Hydrochloride.** A solution of 0.8 g (0.0024 mol) of **11** in 20 ml of KOH-dried pyridine with 0.2 ml of 85% H_3PO_4 was stirred magnetically and chilled to 5 °C in an ice bath. Then 3 ml (0.033 mol) of $POCl_3$ was added dropwise. The mixture was stirred overnight at 9 °C. The suspension was pipetted into 250 ml of ice–H_2O, made basic with 6 M NaOH, and extracted with Et_2O. The extracts were washed repeatedly with H_2O and twice with saturated NaCl. The Et_2O was dried ($MgSO_4$), filtered, and roto-evaporated. The residue was dissolved in toluene and roto-evaporated several times to remove traces of pyridine. The product was obtained as a yellow oil: yield 0.5 g (65%). The hydrochloride salt was precipitated with Et_2O–HCl and crystallized from 2-propanol–Et_2O: mp 183–185 °C; ir (KBr) 1710 cm^{-1} (conj ester C=O).

***N*-Methyl-3-(2′,5′-dimethoxy-4′-methylphenyl)-5-carboethoxypiperidine (13) Hydrochloride.** A slurry of 2.0 g (0.0063 mol) of **12** in 50 ml of EtOH with 0.25 g of 5% Pd/C was hydrogenated at 25 °C and 45 psig. After 9.5 h the reaction was stopped, the catalyst removed by filtration, and the solvent roto-evaporated to leave a yellow oil: yield 1.4 g (66%); ir (neat) 1725 cm^{-1} (ester C=O). The HCl salt was precipitated from Et_2O and crystallized from 2-propanol–Et_2O: mp 164–166.5 °C; mass spectrum (70 eV) m/e 321 (M^+). Anal. ($C_{18}H_{28}NO_4Cl$) H, N; C: calcd, 60.41; found, 59.78.

***N*-Methyl-3-(2′,5′-dimethoxy-4′-methylphenyl)-5-hydroxymethylpiperidine (14) Hydrochloride.** A dry 200-ml three-necked round-bottomed flask was fitted with a nitrogen inlet, a condenser with drying tube, a dropping funnel, and a magnetic stir bar. The flask was cooled in ice–H_2O and 8.7 g of a 70% solution of sodium bis(2-methoxyethoxy)aluminum hydride in benzene (Red-Al) diluted with 30 ml of dry benzene was added. Dropwise, with stirring and cooling, 1.6 g (0.005 mol) of **13** in 50 ml of dry benzene was added. When addition was complete the mixture was heated to reflux for 1 h. The mixture was then cooled in ice while 10 ml of 8% NaOH was added slowly and carefully. An additional 40 ml of H_2O was added and the layers were separated. The benzene was washed with 40 ml of 1 M Na_2CO_3. The aqueous portions were combined and extracted with Et_2O. The Et_2O and benzene layers were combined, dried ($MgSO_4$), filtered, and roto-evaporated. The product was obtained as a yellow oil: yield 1.26 g (91%); mp 138.5–141 °C; ir (neat) 3385 cm^{-1} (OH). The HCl salt was precipitated from Et_2O and crystallized from 2-propanol–Et_2O; mp 183–186 °C; mass spectrum (70 eV) m/e 279 (M^+). Anal. ($C_{16}H_{26}NO_3Cl$) H, N; C: calcd, 60.84; found, 59.65.

***N*-Methyl-3-(2′,5′-dimethoxy-4′-methylphenyl)-5-cyanomethylpiperidine (2) Hydrochloride.** A solution of 0.45 g (0.0016 mol) of **14** in 5 ml of pyridine was stirred magnetically and cooled in an ice bath while 0.57 g (0.003 mol) of *p*-toluenesulfonyl chloride in 5 ml of pyridine was added. The mixture was stirred overnight at 9 °C. The solvent was evaporated in vacuo at 45–50 °C; toluene was added and then evaporated in vacuo again. The residue was slurried in 20 ml of 1.5 M HCl, made basic with 40 ml of 1 M Na_2CO_3, and extracted with Et_2O. The extracts were washed once with 1 M $NaHCO_3$ and dried ($MgSO_4$), filtered, diluted with toluene, and roto-evaporated. The product was obtained as a yellow oil: yield 0.6 g (86%); ir (neat) 1175 cm^{-1} (S=O). A mixture of 0.35 g (0.0008 mol) of the tosylate derivative and 0.39 g (0.008 mol) of NaCN in 10 ml of Me_2SO was stirred and heated on the steam bath under N_2 for 7 h. The mixture was pipetted into 60 ml of 0.5 M Na_2CO_3 on ice and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were washed repeatedly with H_2O, dried ($MgSO_4$), filtered, and evaporated to give a yellow oil: yield 0.2 g (87%); ir (neat) 2245 cm^{-1} (C=N). The HCl salt was precipitated from Et_2O and crystallized from 2-propanol–Et_2O: mp 237–240 °C; mass spectrum (70 eV) m/e 288 (M^+). Anal. ($C_{17}H_{25}N_2O_2Cl$) H, N; C: calcd, 62.85; found, 61.84.

Pharmacological Evaluation. The target compound, **2**, and several intermediates were evaluated for inhibition of prolactin release in vivo. This assay procedure[22] involves ip administration of the test compound to reserpinized male rats followed by radioimmunoassay of serum prolactin levels 1 h after injection. Prolactin was assayed using the kit distributed by the NIAMDD. The results are expressed as nanograms of NIAMDD prolactin RP-1 per milliliter of serum and have been converted to percent inhibition relative to the control value. Ten rats were used for each drug and significance was determined using Student's t test.

Results and Discussion

The results of the in vivo assay are shown in Table I. As can be seen, most of the congeners did not have a significant effect on prolactin release. The standard, apomorphine, significantly inhibited prolactin release. In light of the inactivity of most members in this series, and since a "false positive" result is occasionally obtained from this assay,[23] the effect observed for **10** may be in question.

The general inactivity that was found in these 3-phenylpiperidine derivatives indicates that not all features of a potent prolactin release inhibitor are present and that the criteria for the design of these congeners should be reconsidered. Cassady et al.[24] have emphasized the importance of the entire tetracyclic ring system in their analysis of prolactin release inhibiting ergoline derivatives. However, we have previously observed a potent prolactin release inhibitory effect following administration of a 2-amino-1,2,3,4-tetrahydronaphthalene fragment of the ergoline structure bearing two hydroxyl substituents on the aromatic ring.[17] The lack of activity in the present 3-phenylpiperidine derivatives may be due to the absense of appropriate hydroxyl substituents on the aromatic ring. On the other hand, the inactivity may be attributable to a lack of planarity in the 3-phenylpiperidine system. The ergoline derivatives, the dopamine agonist apomorphine, and the 2-amino-1,2,3,4-tetrahydronaphthalene derivative

mentioned above have rigid planar structures whereas molecular models suggest that steric factors in our 3-phenylpiperidine derivatives may cause the plane of the aromatic ring to lie nearly perpendicular to the plane of the piperidine ring.

Acknowledgment. The authors wish to thank Dr. R. Crenshaw and Dr. D. L. Vulcano for the 220-MHz NMR interpretation and Dr. James F. Caputo for the computer-generated NMR spectrum. We also wish to acknowledge a Salsbury Foundation Fellowship (D.B.R.) and to thank the NIAMDD Rat Pituitary Hormone Distribution Program for radioimmunoassay materials. This work was supported in part by a grant from the National Institutes of Health, GM 22365.

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Irreversible Enzyme Inhibitors. 200.[1] Active-Site-Directed Inhibitors of Deoxycytidine Kinase

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Received May 13, 1976

Forty-three pyrimidine derivatives, mainly containing the 4-aminopyrimidine system, have been prepared and evaluated as inhibitors of deoxycytidine kinase. The most effective inhibitors were 2-alkylthio-4-aminopyrimidines and 1-alkylcytosines. The best inhibitors in both groups were those with large alkyl substituents, which indicate that hydrophobic bonding is occurring, possibly in the same area adjacent to the active site.

Deoxycytidine kinase is an enzyme which phosphorylates deoxycytidine and, less readily, deoxyguanosine. The enzyme also phosphorylates the synthetic antileukemic drug *ara*-C (1-β-D-arabinofuranosylcytosine), and the development of resistance to this drug is associated with a decreased concentration of the enzyme in resistant cells.[3] It has also been suggested that the deoxycytidine kinase from resistant cells could be due to structural modification of the enzyme in the resistant cells.[3a]

It is known that the affinity of substrates for deoxycytidine kinase decreases in the order deoxycytidine > *ara*-C > deoxyguanosine and it has been shown that deoxycytidine inhibits the phosphorylation of *ara*-C by over 90%.[3a] However, there is a paucity of data on inhibitors of this enzyme. As part of a program investigating active-site-directed irreversible enzyme inhibitors[4a] as possible chemotherapeutic agents, an investigation of inhibitors of deoxycytidine was commenced[4b] and our results obtained from an initial survey of this area form the subject of this paper.

Enzyme Results. Of the 43 compounds tested the ones which caused an inhibition of the enzyme of greater than 20% ($V_0/V_I > 1.25$) are listed in Table I. These compounds can be further divided into several groups. 1-Substituted derivatives of cytosine (**1–8**) show some inhibition of the enzyme with the activity increasing as the length of the side chain attached to N-1 is increased. This inhibition, and the nature of the side chain in these compounds, suggests that hydrophobic bonding of the side chain to the enzyme is occurring. In contrast, the 5- and 6-substituted cytosines, **14–17** and **38–43**, and the related compounds **34–36** showed no significant inhibition of the enzyme when tested at comparable concentrations. Compounds **40–43** all contain a side chain at N-1 which, in the absence of the 5-substituent, can give rise to inhibition of the enzyme. This suggests that the active site of the enzyme cannot tolerate bulky groups at the 5 position of the substrate but that there is a hydrophobic bonding area capable of interaction with a 1-substituent.

The N-4 substituted cytosines were all inactive. This is not surprising in the case of the acyl derivatives **9** and **10** since the ability of the amine group to coordinate to an active site on the enzyme could well be substantially reduced when it is converted to an amide system. The *N*-alkyl derivatives **11–13** are also inactive suggesting that there is little bulk tolerance for an N-4 group. Neither