Ethyl 1-(2-Chloroethyl)-6-fluoro-1,4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylate (22). A mixture containing 1.5 g (4.2 mmol) of **21c**, 1.0 g (8.4 mmol) of $SOCl_2$, and 30 mL of $CHCl_3$ was heated to reflux for 30 min. After the mixture was cooled, ice and 10% NaOH were added. The organic layer was separated and concentrated to dryness in vacuo. The residue was crystallized from CH_3CN to give 1.25 g (79%) of **22**, mp 192-194 °C. Anal. ($C_{18}H_{15}ClFN_3O_3 \cdot 0.5H_2O$) C, H, Cl, F, N.

Ethyl 2-[*N*-Cyclopropyl-*N*-[2-(ethoxycarbonyl)ethyl]-amino]-5-nitro-6-(4-pyridyl)nicotinate (23). A mixture containing 2.0 g (7.7 mmol) of **9** and 20 mL of $POCl_3$ was heated to reflux for 2 h and then concentrated to dryness in vacuo. The residue was taken up in a mixture of $CHCl_3$ and EtOH. The solution was heated to reflux for 1 h and concentrated to dryness in vacuo, and the residue was taken up in a mixture of ice, 10% NaOH, and $CHCl_3$. The organic layer was separated and concentrated to dryness in vacuo. To the residue was added 2.4 g (15.4 mmol) of ethyl 3-(cyclopropylamino)propionate in 30 mL of EtOH. The mixture was heated to reflux for 1.5 h and then concentrated to dryness in vacuo. The residue was taken up in a mixture of water and $CHCl_3$. The organic layer was separated and concentrated to dryness in vacuo. The residue was chromatographed on silica gel with $CHCl_3$ to give 1.5 g (61%) of **23**, which was recrystallized from i-Pr_2O; mp 106-107 °C. Anal. ($C_{21}H_{24}N_4O_6$) C, H, N.

Ethyl 1-Cyclopropyl-1,2,3,4-tetrahydro-6-nitro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylate (24). To a solution of **23**, (2.25 g, 5.3 mmol) in 230 mL of t-BuOH was added portionwise 920 mg of t-BuOK (8.2 mmol) at room temperature and the mixture was stirred at the same temperature for 7 h, and then 200 mL of water was added. The reaction mixture was adjusted to pH 7.0 with AcOH. The precipitate was filtered off, washed with water, and dried to give 1.6 g (83%) of **24**, which was recrystallized from EtOH; mp 195-198 °C. Anal. ($C_{16}H_{18}N_4O_5$) C, H, N.

Ethyl 1-Cyclopropyl-1,4-dihydro-6-nitro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylate (25). A mixture containing 550 mg (1.4 mmol) of **24**, 650 mg (2.6 mmol) of chloranil, and 30 mL of dioxane was heated at 60 °C for 1 h with stirring and then concentrated to dryness in vacuo, and the residue was taken up in a mixture of 1 N NaOH and $CHCl_3$. The organic layer was separated and concentrated to dryness in vacuo and the residue was crystallized from AcOEt to give 500 mg (92%) of **25**, mp 200-201 °C. Anal. ($C_{19}H_{16}N_4O_5$) C, H, N.

Ethyl 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-pyridyl)-1,8-naphthyridine-3-carboxylate (28). A mixture containing 1.85 g (4.9 mmol) of **25**, 100 mg of 5% Pd-C, and 40 mL of AcOH was shaken under H_2 gas until the required volume of hydrogen was absorbed. The reaction mixture was filtered to remove the catalyst and the filtrate was concentrated to dryness in vacuo. The residue was dissolved in 25 mL of 42% HBF_4. To this solution kept at 1-10 °C was gradually added a solution of $NaNO_2$ (500 mg, 7.2 mmol) in 3 mL of water. The mixture was stirred at the same temperature for an additional 20 min and diluted with 90 mL of cold EtOH. The resulting solid was collected, dried in vacuo, and suspended in 70 mL of toluene. The suspension was heated at 100 °C for 1 h with stirring. The solvent was removed by decantation and the residue was taken up in a mixture of 1 N NaOH and $CHCl_3$. The organic layer was separated, concentrated to dryness in vacuo and the residue was crystallized from AcOEt to give 700 mg (42%) of **28**, mp 209-211 °C. Anal. ($C_{19}H_{16}FN_3O_3$) C, H, F, N.

In Vitro Antibacterial Activity. According to the method of Goto et al.,[10] the MIC (in micrograms per milliliter) was determined by the twofold agar dilution method using Mueller-Hinton agar (pH 7.4, Difco); bacterial inocula contained approximately 10^6 colony-forming units and the bacterial growth was observed after 20-h incubation at 37 °C.

In Vivo Efficacy on Systemic Infections. In vivo activity assay was carried out according to the method of Shimizu et al.[11] Groups of 10 or more male mice (Std-ddY, 20 ± 2 g) were infected with *Staphylococcus aureus* 50774 (iv, 5×10^8 cells), *Escherichia coli* P-5101 (ip, 9×10^6 cells), and *Pseudomonas aeruginosa* 12 (ip, 4×10^3 cells). The test compounds were suspended in 0.2% sodium (carboxymethyl)cellulose and administered orally at 0 and 6 h postinfection. Survival rates were evaluated after 2 weeks for the staphylococcal infection and after 1 week for others.

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Registry No. **7a**, 90679-45-1; **7b**, 99108-92-6; **7c**, 109034-04-0; **7d**, 90679-48-4; **7e**, 99108-94-8; **8**, 39883-32-4; **9**, 90679-37-1; **10**, 99108-96-0; **11**, 90679-38-2; **12**, 99108-97-1; **13**, 90679-39-3; **14**, 109034-05-1; **15**, 109034-08-4; **16**, 90679-40-6; **17**, 109034-09-5; **18**, 90679-41-7; **19**, 90679-42-8; **20**, 90679-43-9; **21a**, 90679-44-0; **21b**, 99108-91-5; **21c**, 90679-46-2; **22**, 90679-47-3; **23**, 99108-98-2; **24**, 109034-10-8; **25**, 99109-00-9; **26**, 109034-11-9; **27**, 109034-14-2; **28**, 99108-93-7; $(CO_2Et)_2C{=}CHOEt$, 87-13-8; c-$C_3H_5NH(CH_2)_2CO_2Et$, 76731-85-6; $F(CH_2)_2Br$, 762-49-2; $OH(CH_2)_2Br$, 540-51-2.

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Synthesis and Biological Properties of Actinomycin D Chromophoric Analogues Substituted at the 7-Carbon with Aziridine and Aminopropoxy Functions

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The growing importance of functionalized aziridines in numreous organic biomolecules led us to develop syntheses of novel actinomycin D (AMD) analogues substituted with an aziridine. Reaction of 7-hydroxyactinomycin D with 2-(iodomethyl)aziridine produced the desired 7-(2-aziridinylmethoxy)actinomycin analogue. In an attempt to develop an alternate route to this analogue, 7-(2-azido-3-iodopropoxy)actinomycin was subjected to reduction with dimethylamine-borane complex; the reaction did not produce the three-membered aziridine; instead the reaction product was found to be linear 7-(2-aminopropoxy)actinomycin D. Calf-thymus-DNA binding of these analogues was comparable to that of AMD as examined by UV-visible difference spectral measurements, thermal denaturation of DNA, and CD techniques. The analogues were found to be about $^1/_4$ to $^1/_{30}$ as cytotoxic to human lymphoblastic CCRF-CEM leukemia and B_{16} melanoma cells in vitro as AMD.

Actinomycin D (AMD, **1b**) has been used clinically as a chemotherapeutic agent in the treatment of Wilms' tumor[1] and gestational choriocarcinoma[2] for some time. It is known to bind to double-helical DNA by intercalation

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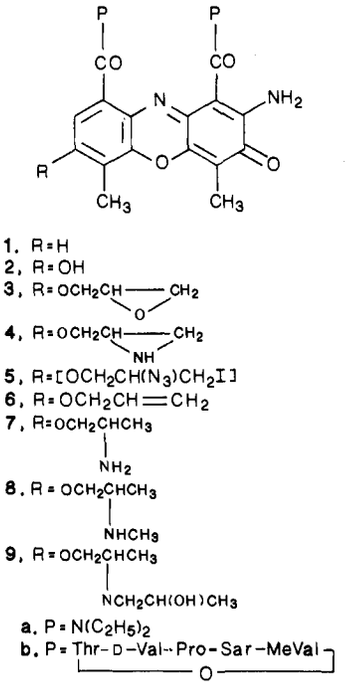
Chart I

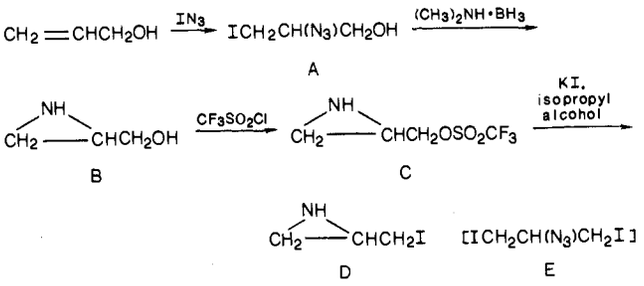


and through hydrogen bonding and by hydrophobic interactions of the peptide lactone moieties.[3-5] The nature of binding of AMD has been established by spectral shift,[3] viscosity changes,[3,6] thermal denaturation of DNA,[6] and other measurements.[3,6,7] The mechanism of action of AMD is proposed to be its binding to intracellular DNA and thus inhibiting DNA-dependent RNA synthesis.[8,9]

In an earlier report we synthesized 7-(2,3-epoxypropoxy)actinomycin D (**3b**), which appeared to retain the DNA-binding property and to enhance the in vivo tumor-inhibiting activity of AMD.[10]

In view of the extreme cytotoxicity of the epoxy compound **3b** to human lymphoblastic leukemia cells (CCRF-CEM) in vitro and its improved antitumor activity against murine leukemias in vivo and simultaneously its lower toxicity to normal CDF_1 mice in vivo, the synthesis of the isoelectronic analogue, i.e., 7-(2-aziridinylmethoxy)actinomycin D (**4b**), appeared to be logical. Other functionalized aziridines like AZQ and mitomycin C are known to act as tumor-inhibiting alkylating agents (refer to Chart I for the structures of AMD and its analogues).[17]

Scheme I



Chemistry

We initially attempted these syntheses with the 7-hydroxy model chromophore **2a** (Chart I).[11] The model 7-(2-aziridinylmethoxy) derivative **4a** was prepared from the reaction of 2-(iodomethyl)aziridine (D) with 7-hydroxy-substituted chromophore **2a**. For synthesis of 2-(iodomethyl)aziridine (D), the intermediate $ICH_2CH(N_3)CH_2OH$ (trans) (A) was prepared from allyl alcohol by reaction with IN_3; the latter was obtained by addition of iodine monochloride to a cold slurry of sodium azide in acetonitrile according to the procedure of Fowler, Hassner, and Levy.[12,13] When the trans iodoazidopropanol (A) was reduced with borane–dimethylamine complex in anhydrous tetrahydrofuran, it gave the cyclized product 2-(hydroxymethyl)aziridine (B). Purified 2-(hydroxymethyl)aziridine (B) was reacted with trifluoromethanesulfonyl chloride in the presence of sodium hydride in tetrahydrofuran to produce the corresponding triflate (C), and the latter was displaced by iodide (potassium iodide in isopropyl alcohol) to the iodo analogue D (Scheme I).

In an attempt to synthesize the aziridines by another route, 2-azido-1,3-diiodopropane intermediate (E) was readily prepared from allyl iodide by reaction with IN_3. 2-Azido-1,3-diiodopropane (E) was then reacted with 7-hydroxy-substituted compound **2a** in dry acetone in the presence of finely powdered anhydrous potassium carbonate at room temperature for 48 h. The resulting products were partially fractionated on a silica gel column, first by elution with chloroform to remove excess 2-azido-1,3-diiodopropane and followed by an elution with 1:1 acetone/chloroform to afford two compounds, an alkylated iodo azide intermediate **5a** and an allyloxy-substituted compound **6a**; the latter originated probably via elimination of an iodo azide from the intermediate **5a**. The products were further separated and purified by preparative thin-layer chromatography. Authentic 7-(allyloxy)-substituted compound **6a** was also independently prepared from **2a** via reaction with allyl iodide in the presence of anhydrous potassium carbonate in dry acetone medium for 4 h. The products were identical by their NMR and TLC behavior.

When the iodo azide intermediate **5a** was reduced with borane–dimethylamine complex in anhydrous tetrahydrofuran, it afforded the 7-(2-aminopropoxy) compound **7a**, instead of the desired aziridine **4a**. Reduction of the intermediate **5a** with lithium aluminum hydride in anhydrous ether also produced the same aminopropoxy derivative **7a**. The probability of iodide hydrogenolysis resulting in the reaction product correlates well with the postulated mechanism for halide reduction; the mechanism

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Table I. Bathochromic Shifts in the Absorption Maxima on Binding to DNA

drugs	drugs λ_{max}, nm	drugs + DNA[a] λ_{max}, nm	concn, mmol
1b	440	476–478	0.015
4b	450	502–504	0.011
6b	442	484	0.011
7b	458–460	502–504	0.013

[a] DNA concentration, 0.26 in mmol.

is believed to proceed by an attack of a nucleophilic hydride complex on the halide-bearing carbon atom.[14] This backside attack appears to be sensitive to steric effects, and only primary iodo functions are displaced in this manner by hydride. On reduction, the trans iodo azide derivative **5a** apparently failed to undergo ring closure and instead yielded only **7a**. Further proof of the structure of **7a** was provided by its conversion into N-alkylated derivatives **8a** and **9a** as follows.

Reaction of the amino group in **7a** with methyl iodide in acetonitrile in the presence of base for 1 h afforded N-methylated derivative **8a**. Similar reaction of the amino function in **7a** with propylene oxide in absolute alcohol in the presence of a catalytic amount of base afforded *N*-hydroxypropyl-substituted derivative **9a**. This reaction would not proceed in a lower boiling solvent, e.g., absolute methanol.

The synthesis of the corresponding AMD analogues, i.e., 7-(2-aminopropoxy)- and 7-(2-aziridinylmethoxy)actinomycin D (**7b** and **4b**, respectively), was accomplished by following similar procedures (see Experimental Section). All IR, NMR, and combustion analytical data were consistent with the structures proposed here.

Biophysical Experiments

DNA-Binding Studies. The DNA-binding property of the analogues was examined by spectral shift of the absorption maxima. On complexing with calf-thymus DNA the long wavelength absorption maximum of the analogues underwent simultaneous bathochromic and hypochromic shift similar to the one observed for AMD (Table I). The difference spectra of free and DNA-bound actinomycin analogues were obtained by subtracting from the spectrum of a solution containing actinomycin analogue bound to DNA, a spectrum of a reference solution containing the same concentration of free actinomycin analogue.

The characteristic hypochromic and bathochromic troughs and peaks in the typical difference UV spectra of AMD and analogues (Figure 1, supplementary material) indicate their binding to DNA; these difference spectra show characteristic positive and negative maxima, and these observations are similar to those found for other intercalative analogues of AMD.[6]

DNA binding to **4b** and **7b** shifts the absorption maxima at 450 and 458–460 nm bathochromically to 502–504 nm (Table I). From the $-\Delta A$ value in the difference spectra (Figure 1), which is a function of binding affinity to DNA, it is clear that the relative strength of association is in the order AMD (**1b**) > **7b** $\simeq$ **4b** $\gg$ **6b**.

Thermal Denaturation of DNA. Thermal denaturation studies were carried out according to the procedure described by us.[15] The ΔT_m values give a measure of stabilization of DNA helical structure as a consequence of drug binding. These values are derived at high temperature (67–85 °C) and may not always correlate with the results of other DNA-binding studies. The ΔT_m data for new analogues show that binding of all of these analogues to the double helices of DNA effect strong to moderate stabilization of the double helix (Table II).

Table II.[a] Elevation of T_m values of DNA (ΔT_m) on Complexation with Actinomycin D Analogues

compd	ΔT_m, °C
1b	8.0 ± 0.1
4b	2.8 ± 1.1
6b	6.8 ± 0.1
7b	4.8 ± 0.1

[a] ΔT_m = T_m of DNA–drug complex minus T_m of DNA. Concentration of drugs, 2.5×10^{-5} M; of DNA, 5.2×10^{-5} M; in 0.01 M phosphate buffer (pH 7).

Table III. In Vitro Antitumor Activity

drug	ID_{50}[a] CCRF-CEM cells[b]	ID_{50}[a] B_{16} melanoma cells[c]
1b	13.0[d]	3.3[d]
2b	600.0	260.0
4b	76.0	110.0
6b	>750.0	>1000.0
7b	60.0	55.0

[a] Concentration of compound in ng/mL required for 50% inhibition at 48 h of cells in suspension culture. Compounds were dissolved in Me_2SO/saline; the final growth medium contained less than 1% Me_2SO. [b] Human leukemic lymphoblastic cells. [c] Murine melanoma cell line. [d] These values are an average of three experiments. The standard deviation for all experiments ranged between 8% and 10% of the mean value.

The UV–visible difference spectral results appear to be in agreement with the thermal denaturation of DNA experiments. The efficiency of binding appears to be in the same order except for the allyloxy analogues **6b**, which shows better binding by ΔT_m than that indicated by $-\Delta A$ values in difference spectra. By difference spectral $-\Delta A$ values, both **4b** and **7b** appear to bind to DNA with some definite affinity but less strongly than actinomycin D. Compound **4b** has an additional mode of binding. These studies provide an indirect evidence of binding to DNA probably via intercalation of their chromophores (Figure 1, supplementary material).[22]

Circular Dichroic Spectral Investigations. The circular dichroic spectra in agreement with UV–visible spectra show that these analogues are similar in the electronic nature of the chromophores and further that their peptide conformations are very nearly the same (Figures 2 and 3, supplementary material).

In Vitro Tumor-Growth-Inhibitory Activity

The analogues were assayed for growth-inhibitory activity against human lymphoblastic leukemic cells (CCRF-CEM) in continuous spinner culture and also monolayer culture of B_{16} melanoma cells, both in the log phase growth (Table III).[16] These assays are sensitive for actinomycin and its analogues and provide their relative cytotoxicity values.

Actinomycin analogues **4b** and **7b** are moderately cytotoxic as compared to AMD, which is extremely cytotoxic to both the CCRF and B_{16} melanoma cells. Compound **6b** is found least active in these systems. In any case, the ID_{50} values of **4b** and **7b** are in the region of concentrations of nanogram/milliliter only and, therefore, should be considered quite high, when compared with other active drug intercalators, for instance, adriamycin and daunomycin. The in vivo activity of these active analogues should be of interest not only due to their potency against tumor cells but also for their possible metabolic conversion in vivo.

Discussion

Compound **4b** shows weaker binding by ΔT_m and is also somewhat less active than AMD against the cell lines

studied (CCRF and B_{16}). Nevertheless, **4b** shows similar conformational and circular dichroic effects on interaction with DNA. It is very likely that this analogue interacts both intercalatively and covalently with DNA as has been demonstrated in the case of another epoxy-substituted analogue, **3b**; **3b** is known to destabilize the DNA helix at higher than 37 °C and, therefore, exhibits a smaller degree of change in ΔT_m value compared to AMD.[10] Moreover, **4b** may not be stable in the biological medium and during the prolonged (>2 days) incubation conditions at 37 °C, and, therefore, the ID_{50} values of this agent are not as high as one would expect. Conversely, **7b** shows stronger binding by ΔT_m. It is also more active than **4b** and the other actinomycin analogue **6b** (Table III). In summary, our present studies demonstrate that, like AMD, **7b** and **4b** are also active against CCRF and B_{16} cell lines in vitro.

For AMD, the interaction with DNA in the cells via intercalation is attributed to its inhibition of transcription of DNA, and this mechanism is presumed to be the primary source of its biological activity. AMD is also known to exhibit topoisomeraase II dependent DNA fragmentation in L1210 cells in vitro, but produces relatively few double-strand breaks.[18] AMD is relatively less effective in L1210 in vivo compared to mitomycin C; mitomycin C, which has an aziridine function, is reported to form covalent binding to DNA and to generate extensive double-strand breaks.[19] In general, alkylating agents show greater efficiency of DNA double-strand and chromosome breaks than intercalating agents and are prone to respond effectively toward tumors that are not responsive to AMD and other intercalating agents.[10]

AMD is highly toxic to hosts including humans because it accumulates for prolonged periods in the cell nuclei, causing acute toxicity, and, consequently, its clinical application is severely restricted.[20] To enhance the in vivo activity, perhaps **4b**, which has a prominent alkylating aziridine function, might act via both intercalation and alkylation and thus broaden the scope of activity, especially against certain AMD-resistant tumors. An evaluation of the in vivo biological activity and a determination of the pharmacokinetic activity of **7b** and **4b** are currently under investigation.

Experimental Section

The IR spectra were taken with a Perkin-Elmer Model 457A grating spectrophotometer in potassium bromide pellets unless otherwise noted, UV spectra were measured with a Gilford Model 250 spectrophotometer, and NMR spectra were determined on a Varian A-60 or 300-MHz spectrometer in deuteriochloroform with tetramethylsilane as internal standard. Analytical TLC's were done on 5 × 20 cm precoated glass plates with a 0.25-mm layer of silica gel 25 (Macherey-Nagel) with chloroform/acetone (4:1) as the developing agent.

Preparative thin-layer chromatography was performed on 20 × 20 cm glass plates coated with a 2-mm layer of silica gel PF 254 (E. Merck, Darmstadt, Germany). The compounds were detected by visual examination under UV light (254 nm). Microanalyses were performed by Multichem Laboratories, Lowell, MA. Where analyses are specified by symbols of the elements, analytical results obtained for those elements were within ±0.4% of theoretical values. Evaporation of solvents was done under diminished pressure at less than 40 °C by using a rotary evaporator.

DNA-binding experiments were carried out in 0.01 M phosphate buffer (pH 7) containing EDTA (10^{-5} M). Calf-thymus DNA was purchased from Sigma Chemical Co., St. Louis, MO. Spectral shift experiments involving shift of the absorption maxima on complexing with DNA were performed in a Gilford 250 spectrophotometer in a quartz cell, which, with the addition of a base-line reference compensator (Analog Multiplexer 6064) and thermoprogrammer, auto four cell programmer and thermoelectric cell holder 2577, was used to obtain thermal denaturation curves. CD spectra were obtained on a Cary 61 spectropolarimeter.

CAUTION: Both iodo and azido functions are labile in the iodo azido intermediates. These intermediate iodo azides and the aziridines are prone to decompose on heating *and may explode*, so care must be exercised when handling these intermediates.

2-Azido-3-iodo-1-propanol (A). Iodine monochloride (18.3 g; 0.113 mol) was slowly added to a stirred slurry of sodium azide (15 g; 0.25 mol) in acetonitrile (100 mL) in a methanol/ice cold bath over a period of 20 min. The reaction mixture was stirred for an additional 10 min, and allyl alcohol (5.8 g; 0.1 mol) was then added. The mixture was allowed to attain room temperature (20 °C) and then stirred for 24 h. The red-brown slurry was poured into water (250 mL), and the mixture was extracted with ether (3 × 80 mL). The extract was washed with sodium thiosulfate solution (20%, 150 mL), and the colorless ether extract was washed with water (4 × 200 mL) and dried ($MgSO_4$). Removal of the solvent in vacuo at room temperature afforded the desired iodo azide compound A, which was slightly yellow in color (17.1 g; 75.3%): IR (neat) 2110 cm^{-1} (N_3), 3440 (OH); NMR δ 3.21 (d, CH_2I, 2 H), 3.76 (m, OCH_2, 2 H), 4.17 [m, $CH(N_3)$, 1 H], 2.73 (br s, OH, 1 H).

2-(Hydroxymethyl)aziridine (B). A solution of 2-azido-3-iodo-1-propanol (A) (8.5 g; 0.037 mol) in anhydrous THF (50 mL) was allowed to react with borane–dimethylamine complex (4.42 g; 0.075 mol), and the mixture was stirred at 40 °C for 48 h. The mixture was diluted with chloroform (250 mL) and filtered. The filtrate was then washed with saturated aqueous $NaHCO_3$ (20 mL) and with saturated NaCl solution (10 mL). After removal of solvent under vacuum, the residue was purified by silica gel column chromatography (120 g) by elution with 1:8 acetone/chloroform to afford 2-(hydroxymethyl)aziridine (B) (2.1 g; 77%): IR (neat) 3425 cm^{-1} (OH); NMR δ 3.88 (m, CH_2OH, 2 H), 2.56 (m, aziridine ring protons, 3 H).

2-[[[(Trifluoromethyl)sulfonyl]oxy]methyl]aziridine (C). Sodium hydride (0.8 g; 80% dispersion in mineral oil) in small portions was added to a solution of 2-(hydroxymethyl)aziridine (B) (2.19 g; 0.03 mol) in anhydrous THF (35 mL) in an atmosphere of nitrogen cooled to −80 °C in a methanol/dry ice bath over a period of 30 min. Trifluoromethanesulfonyl chloride (7 g; 0.0417 mol) was then added in small portions and the mixture stirred in the cold for 4 h and then at room temperature overnight. The mixture was decomposed with $NaHCO_3$ solution (2%, 50 mL) and the aqueous layer extracted with ethyl acetate (6 × 30 mL). The ethyl acetate layer was dried (Na_2SO_4), and after evaporation of the solvent, the residue was purified by column chromatography (silica gel, 60 g). Elution by chloroform gave relatively pure triflate derivative C (0.63 g; 10%): NMR δ 5.2 (d, OCH_2, 2 H), 2.0 (m, aziridine ring protons, 3 H).

2-Amino-1,9-bis(*N*,*N*-diethylcarbamoyl)-4,6-dimethyl-7-(2-aziridinylmethoxy)-3*H*-phenoxazin-3-one (4a). To a solution of the above compounds, namely 2-[[[(trifluoromethyl)-sulfonyl]oxy]methyl]aziridine (C) (0.61 g; 3 mmol) in isopropyl alcohol (25 mL) was added powdered potassium iodide (0.6 g; 3.6 mmol), and the mixture was heated in an atmosphere of nitrogen

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(22) Note Added in Proof: The analogue **4b**, like **3b**,[10] shows a broad ΔT_m value which is characteristic of a DNA alkylating agent. Our recent studies using agarose gel electrophoresis on the analogue–cccSv40 DNA complex give additional evidence that all these compounds **3b**, **4b**, **6b**, and **7b** intercalate into DNA in the same manner as found for actinomycin D and ethium bromide. The new analogues **3b** and **4b** appear to have a dual mode of binding to DNA and thus they are distinctly different from the other reported analogues of AMD and also from the known DNA intercalating and alkylating agents we know about.

for 8 h at a bath temperature of 64 °C. Isopropyl alcohol was distilled under diminished pressure, and the residue was treated with water (15 mL), the product extracted with ethyl acetate (6 × 25 mL), the extract dried (Na_2SO_4), and solvent removed under reduced pressure. The residue was added to a solution of the 7-hydroxy compound **2a** (56.75 mg; 0.125 mmol) in dry acetone (30 mL), and the mixture, containing powdered anhydrous potassium carbonate (50 mg), was stirred in a nitrogen atmosphere at room temperature for 48 h. The reaction mixture was filtered to remove inorganic salts, and the filtrate was evaporated. The residue was purified from excess of the iodo compound by passing through a short column of silica gel and eluting with chloroform. The column was then extracted with 1:1 acetone/chloroform and the residue purified by preparative-layer chromatography (silica gel; eluant 1:8 acetone/chloroform) to afford **4a** (16 mg; 25%): R_f 0.55; UV λ_{max} ($CHCl_3$) inf (inflexion) 290, 454 nm (ϵ 34 114); mass spectrum calcd for $C_{27}H_{35}N_5O_5$ M^+ (509), found M^+ (509); NMR δ 6.61 (s, 8-H, 1 H), 5.35 (br s, NH, 1 H), 4.67 (s, NH_2, 2 H), 3.76 (m, OCH_2, 2 H), 2.22 (s, 4-CH_3, 3 H), 2.43 (s, 6-CH_3, 3 H), 2.17 (d, aziridine, 2 H); 2.32 (m, aziridine, 1 H), 3.03–3.8 [m, $N(CH_2CH_3)_2$, 8 H], 0.86–1.73 [m, $N(CH_2CH_3)_2$, 12 H]. Anal. ($C_{27}H_{35}N_5O_5 \cdot CH_3COCH_3 \cdot H_2O$), C, H, N.

2-Azido-1,3-diiodopropane (E). To a stirred slurry of sodium azide (15 g; 0.25 mol) in acetonitrile (100 mL) in a methanol/ice cold bath was added slowly iodine monochloride (18.3 g; 0.113 mol) over a period of 20 min. The reaction mixture was stirred for an additional 10 min, and allyl iodide (16.8 g; 0.1 mol) was then added; then the mixture was allowed to attain room temperature and stirred for 24 h. The red brown slurry was poured into water (250 mL) the mixture was extracted with ether (3 × 80 mL), and the colorless extract was washed with water (4 × 200 mL) and dried ($MgSO_4$). Removal of the solvent in vacuo at room temperature afforded the iodo azide intermediate E (15 g; 50%) as a colorless liquid: IR (neat) 2080 cm^{-1} (N_3); NMR δ 3.5 (m, CH_2, 4 H), 4.05 [(m, $CH(N_3)$, 1 H].

2-Amino-1,9-bis(*N*,*N*-diethylcarbamoyl)-4,6-dimethyl-7-(allyloxy)-3*H*-phenoxazin-3-one (6a). A solution of the 7-hydroxy compound **2a** (113.5 mg; 0.25 mmol) in dry acetone (60 mL) was allowed to react with 2-azido-1,3-diiodopropane (1 mL) and powdered anhydrous potassium carbonate (100 mg) in a nitrogen atmosphere at ambient temperature for 48 h. The bluish color of the reaction mixture turned to red-brown when the reaction was completed. The reaction mixture was filtered to remove inorganic salts, and the filtrate was evaporated. The residue was partially purified by silica gel (60 g) column chromatography. Initial elution with chloroform (150 mL) afforded unreacted 2-azido-1,3-diiodopropane (E) along with impurities; subsequent elution with 1:1 acetone/chloroform (175 mL) gave a mixture of the intermediate **5a** and its iodo- and azide-eliminated side product **6a**. After removal of the solvent, the residue was separated into components by preparative-layer chromatography (silica gel, 1:8 acetone/chloroform), yielding the intermediate **5a** (72 mg; 43%), R_f 0.56, and **6a** (32 mg; 26%), R_f 0.76.

The structure assignment for faster moving **6a** was achieved by NMR comparison and by comparison with authentic compound prepared via direct coupling of allyl iodide with **2a** according to the conditions described above except that the reaction mixture was stirred for 4 h. Compound **6a**: UV λ_{max} ($CHCl_3$) inf 290, 443–444 nm (ϵ 22 666); NMR ($CDCl_3$) δ 6.86 (s, 8-H, 1 H), 5.43 (s, NH_2, 2 H), 6.1 (m, CH_2=CH, 1 H), 5.06 (m, CH_2=CH, 2 H), 4.24 (m, OCH_2, 2 H), 3.06–3.93 (m, $N(CH_2CH_3)_2$, 8 H), 0.83–1.83 [m, $N(CH_2CH_3)_2$, 12 H], 2.28 (s, 4-CH_3, 3 H), 2.45 (s, 6-CH_3).

2-Amino-1,9-bis(*N*,*N*-diethylcarbamoyl)-4,6-dimethyl-7-(2-aminopropoxy)-3*H*-phenoxazin-3-one (7a). A solution of the above 2-azido-3-iodopropoxy-substituted intermediate product **5a** (66.3 mg; 0.1 mmol) in anhydrous tetrahydrofuran (30 mL) was allowed to react with borane–dimethylamine complex (117.8 mg; 2 mmol) at room temperature for 48 h. TLC indicated completion of reaction. The mixture was diluted with chloroform (50 mL) and filtered. The filtrate was then washed with saturated aqueous $NaHCO_3$ (10 mL) and with brine (10 mL). After removal of solvent in vacuo, the residue was separated from impurities by preparative layer chromatography and the major band was extracted with 1:1 acetone/chloroform to afford **7a** (42 mg; 82%): R_f 0.36; UV λ_{max} ($CHCl_3$) inf 290, 451 nm (ϵ 36 111); mass spectrum calcd for $C_{27}H_{37}N_5O_5$ M^+ (511), found M^+ (511); NMR ($CDCl_3$) δ 6.83 (s, 8-H, 1 H), 5.37 (s, NH_2, 2 H), 4.22 (m, OCH_2, 2 H), 2.22 (s, 4-CH_3, 3 H), 2.37 (s, 6-CH_3, 3 H), 3.06–4.06 [m, $N(CH_2CH_3)_2$, 8 H], 0.83–1.76 [m, $N(CH_2CH_3)_2$, 12 H]. Anal. ($C_{27}H_{37}N_5O_5 \cdot 3CH_3COCH_3$) C, H, N.

2-Amino-1,9-bis(*N*,*N*-diethylcarbamoyl)-4,6-dimethyl-7-[2-(*N*-methylamino)propoxy]-3*H*-phenoxazin-3-one (8a). A solution of 7-(2-aminopropoxy)phenoxazinone **7a** (34.25 mg% 0.05 mmol) in acetonitrile (20 mL) was allowed to react with methyl iodide (1 mL) in the presence of potassium hydroxide (5 mg) under reflux (40 °C) for 2 h. On completion of the reaction as indicated by TLC, the orange reaction mixture was filtered and the filtrate was evaporated. The residue was treated with water (20 mL) and extracted with ethyl acetate (3 × 20 mL) and the extract dried (Na_2SO_4). After removal of the solvent, the residue was purified by preparative-layer chromatography. The major band was extracted (1:1 acetone/chloroform) to yield **8a** (20 mg; 57%): R_f 0.44; UV λ_{max} ($CHCl_3$) inf 290, 453 nm (ϵ 40 138); NMR ($CDCl_3$) δ 6.84 (s, 8-H, 1 H), 4.08 (m, OCH_2, 2 H), 2.04 (s, 4-CH_3, 3 H), 2.17 (s, 6-CH_3, 3 H), 3.14 (s, NCH_3, 3 H), 3.03–3.96 [m, $N(CH_2CH_3)_2$, 8 H], 0.83–1.57 [m, $N(CH_2CH_3)_2$, 12 H]. Anal. ($C_{28}H_{39}N_5O_5 \cdot 3CH_3COCH_3$) C, H, N.

2-Amino-1,9-bis(*N*,*N*-diethylcarbamoyl)-4,6-dimethyl-7-[2-[*N*-(2-hydroxypropyl)amino]propoxy]-3*H*-phenoxazin-3-one (9a). A solution of 7-(2-aminopropoxy)phenoxazinone **7a** (34.25 mg; 0.05 mmol) in absolute ethanol (25 mL) was allowed to react with propylene oxide (5 mL; added at regular intervals) in the presence of a catalytic amount of potassium hydroxide (5 mg) under reflux (40 °C) for 48 h. The mixture was filtered, and the filtrate was evaporated. The residue was extracted with chloroform (3 × 25 mL) and the extract dried (Na_2SO_4). After removal of the solvent, the residue was separated from unreacted **7a** by preparative-layer chromatography. The major orange band was extracted with 1:1 acetone/chloroform to afford **9a** (16 mg; 43%): R_f 0.16; UV λ_{max} ($CHCl_3$) inf 290, 456 nm (ϵ 18 000); NMR ($CDCl_3$) δ 6.81 (s, 8-H, 1 H) 4.23 (m, OCH_2, 2 H), 2.21 (s, 4-CH_3, 3 H), 2.36 (s, 6-CH_3, 3 H), 3.10–3.98 [m, $N(CH_2CH_3)_2$, 8 H], 0.86–1.70 [m, $N(CH_2CH_3)_2$, 12 H]. Anal. ($C_{30}H_{43}N_5O_6 \cdot 3CH_3COCH_3$) C, H, N.

7-(2-Aziridinylmethoxy)actinomycin D (4b). A solution of the 7-hydroxyactinomycin D (**2b**) (200 mg; 0.15 mmol) in dry acetone (75 mL) was allowed to react with 2-(iodomethyl)aziridine (2 mL) and finely powdered anhydrous K_2CO_3 (50 mg) in a nitrogen atmosphere at room temperature in the dark for 48 h. The brown reaction mixture was filtered to remove inorganic salts, and the filtrate was evaporated and chromatographed on a silica gel column (60 g). The column was eluted with chloroform (150 mL) to remove excess of the iodo compound. Elution with 1:1 acetone/chloroform (175 mL) afforded **4b** along with impurities. The residue was purified by preparative-layer chromatography (silica gel, 1:8 acetone/chloroform eluant), and the major band was extracted with 1:1 acetone/chloroform to afford **4b** (19 mg; 9%): R_f 0.36; UV λ_{max} ($CHCl_3$) 454 nm (ϵ 11 170); NMR ($CDCl_3$) δ (*J*) 8.15 (5.7), 8.05 (6.3), [NHC=O(Val)], 7.71 (6.2), 7.63 (6.4), [NHC=O(Thr)], 7.3–7.35 (br, Ar NH_2), 7.1 (s, Ar C8-H), 5.96 (7.0), 5.90 (7.0), [α-CH(MeVal)], 5.22 (2.5), 5.18 (5.0), [β-CH(Thr)], 4.85 (6.0), 4.75 (2.0), [α-CH(Thr)], 2.56 (s, 6-CH_3), 2.24 (s, 4-CH_3), 3.7–3.75 [m, 7-(OCH_2)], 5.88 (7-aziridine NH).

7-(2-Aminopropoxy)actinomycin D (7b). A solution of 7-hydroxyactinomycin D (**2b**) (200 mg; 0.15 mmol) in dry acetone (75 mL) was allowed to react with 2-azido-1,3-diiodopropane (2 mL) and finely powdered anhydrous potassium carbonate (50 mg) in a nitrogen atmosphere at room temperature in the dark for 48 h. The red-brown reaction mixture was filtered to remove inorganic salts, and the filtrate was evaporated and chromatographed on a silica gel column (60 g). The column was eluted with chloroform (150 mL) to remove excess of the diiodo azide. Elution with 1:1 acetone/chloroform (175 mL) afforded a mixture of **5b** and **6b** (Chart I). This mixture of **6b** and **5b** was treated with borane–dimethylamine complex (117.8 mg; 2 mmol) in THF (50 mL) at room temperature for 48 h. The mixture was diluted with chloroform (50 mL) and filtered. The filtrate was then washed with saturated aqueous $NaHCO_3$ (10 mL) and with brine (10 mL). After removal of the solvent in vacuo, the residue was separated from impurities by preparative-layer chromatography and the two bands were extracted with 1:1 acetone/chloroform to afford **6b** (9 mg, 4%), R_f 0.48, and **7b** (21 mg, 10%): R_f 0.33;

UV λ_{max} ($CHCl_3$) 456 nm (ϵ 12000); NMR ($CDCl_3$) δ (J) 7.74 (6.0), 7.69 (6.0), [NHC=O(Thr)], 7.3-7.4 (br, Ar NH_2), 7.28 (s, Ar C8-H), 6.30 (7.0), 6.22 (7.0), [α-CH(MeVal)], 4.90 (2.0), 4.86 (5.0), [β-CH(Thr)], 4.72 (6.0), 4.64 (2.0), [α-CH(Thr)], 2.15 (s, 6-CH_3), 2.10 (s, 4-CH_3), 4.23 [m, 7-(OCH_2)], 4.67 [br, 7-CH(NH_2)CH_3].

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Registry No. **1b**, 50-76-0; **2a**, 57270-61-8; **2b**, 21478-73-9; **4a**, 109122-95-4; **4b**, 109122-96-5; **5a**, 109150-57-4; **5b**, 109122-97-6; **6a**, 109122-98-7; **6b**, 109122-99-8; **7a**, 109123-00-4; **7b**, 109123-01-5; **8a**, 109123-02-6; **9a**, 109123-03-7; A, 109122-91-0; B, 88419-36-7; C, 109122-92-1; D, 109122-93-2; E, 109122-94-3; H_2C=$CHCH_2OH$, 107-18-6; CF_3SO_2Cl, 421-83-0; H_2C=$CHCH_2I$, 556-56-9; propylene oxide, 75-56-9.

Supplementary Material Available: A discussion of the CD spectra and Figures 1-3 detailing UV-visible difference absorption spectra on binding to DNA, CD spectra of free drugs, and CD spectra of drug-DNA complexes, respectively (6 pages). Ordering information is given on any current masthead page.

Charged Analogues of Chlorpromazine as Dopamine Antagonists

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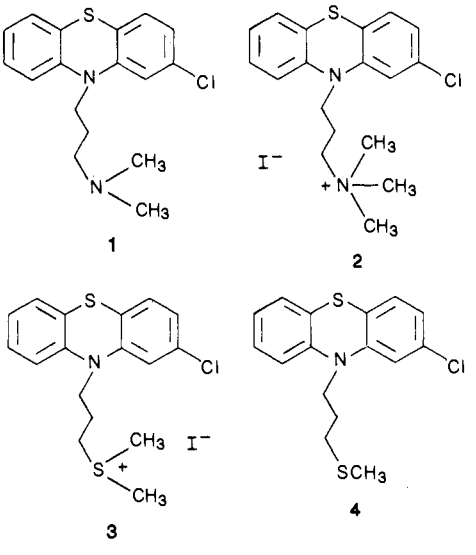
Chlorpromazine (1, CPZ) is a potent dopamine antagonist that has been used widely as an antipsychotic agent. Since dopaminergic antagonists, like dopaminergic agonists, exist in solution as the charged and uncharged molecular species, it is not clear which form of the amine is most important for interaction with the dopamine receptor. Previous work from our laboratory has indicated that a variety of permanently charged species could replace the amine/ammonium moiety of dopamine and retain dopamine agonist activity. This paper describes the synthesis and dopamine antagonist activity of both the trimethylammonium iodide (2) and the dimethylsulfonium iodide (3) analogues of chlorpromazine. The permanently uncharged methyl sulfide analogue (4) was also synthesized; however, due to its lack of aqueous solubility, its pharmacological activity could not be evaluated. Binding of both the dimethylsulfonium iodide and the trimethylammonium iodide analogues to D-2 dopamine receptors of rat striatal tissue was observed. The observed relative order of binding was CPZ > CPZ sulfonium analogue > CPZ ammonium analogue. These compounds had a similar order of activity in antagonizing the apomorphine-induced inhibition of potassium-induced release of [^{3}H]acetylcholine from mouse striatal slices.

Chlorpromazine (1) is a potent antipsychotic agent and is widely used in the treatment of schizophrenia and other mental disorders. The antipsychotic activity of chlorpromazine has been associated with its ability to act as a dopamine-receptor antagonist.[1] Present evidence suggests that there are at least two dopaminergic receptor subtypes.[2] The D-1 dopaminergic receptor when activated by an agonist is associated with the stimulation of adenylate cyclase activity, while the D-2 dopaminergic receptor mediates dopaminergic effects that either do not involve the stimulation of adenylate cyclase or may be related to the inhibition of this enzyme. Chlorpromazine is a nonselective dopamine antagonist, interacting with both D-1 and D-2 receptors; however, work by Seeman[3,4] indicates that most of the effects of dopaminergic drugs are mediated through their interaction with D-2 receptors.

An early issue that was addressed was the manner in which chlorpromazine, with its phenothiazine ring structure, interacted with a receptor for dopamine. Analysis of the X-ray structures of chlorpromazine and dopamine showed that the two structures can be partially superimposed.[5] When the aromatic ring of dopamine is positioned over the chlorine-containing aromatic ring of chlorpromazine, it is found that the amine nitrogens of both



compounds align. This model, therefore, has been used to support the concept that chlorpromazine, as well as other dopamine antagonists, bind to the same receptor as dopamine. In contrast, findings from other laboratories support the hypothesis that dopamine antagonists may not bind to the same receptors as dopamine.[6]

Since dopamine, at physiological pH, may exist as either an uncharged amine or a charged ammonium species, our laboratory has recently completed experiments aimed at determining which of these two species is most important for binding to the dopamine receptor.[7,8] In these studies,

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