(m, 5 H, aromatic), and 3.84-3.06 (m, 13 H); ^{31}P NMR (36.23 MHz, $CDCl_3$) δ 9.83.

A suspension of phenyl *N,N,N'*-tris(2-chloroethyl)phosphorodiamidate (0.5 g, 1.4 mmol) and PtO_2 (50 mg) in absolute ethanol (5 mL) was hydrogenated at 50 psi for 2 h with use of a Paar medium-pressure shaker-hydrogenator. The reaction mixture was then diluted with ethanol (100 mL) and was stirred 10 min prior to filtration. Cyclohexylamine (0.64 mL, 5.6 mmol) was added and the solution was stirred 10 min and was then concentrated at reduced pressure. The residue was taken up in minimal $CHCl_3$ and Et_2O was added to cloudiness. Storage of this mixture at -20 °C gave **4c**·CHA as a white solid (140 mg, 0.37 mmol, 26% yield): mp 152-162 °C; ^{1}H NMR (89.55 MHz, $CDCl_3$) δ 5.65 (br s), 3.72-2.62 (m), and 2.18-0.71 (m); ^{13}C NMR (22.49 MHz, $CDCl_3$) δ 50.5 [d, J_{CP} = 4.4 Hz, $N(CH_2CH_2Cl)_2$], 46.08 (d, J_{CP} = 7.3 Hz, $HNCH_2$), 43.71 (CH_2Cl), 43.12 [$N(CH_2CH_2Cl)_2$], and 50.21, 31.93, 24.91, and 24.46 (cyclohexylamine); ^{31}P NMR (36.23 MHz, $CDCl_3$) δ 8.86 [impurity (14%) at δ 4.45].

^{31}P NMR Kinetic Studies. General Procedure. NMR sample solutions were prepared immediately prior to use by first dissolving the compound (0.012-0.034 mmol) in Me_2SO-d_6 (0.34 mL) and then adding 1 M lutidine buffer (1.35 mL). The solution was transferred to a vial containing $Na_2S_2O_3$·$5H_2O$ (3-5 molar equiv) and use of a high-speed vortex mixer effected dissolution in 1-2 min. The solution pH was checked and adjusted, if necessary, with 2-4 M HCl. The sample was then placed in a 10-mm NMR tube and a vortex plug was inserted. The sample was allowed to thermally equilibrate for 2-3 min in the spectrometer probe (37 ± 2 °C) prior to optimization of the magnetic field homogeneity. At time "zero", which was 10-15 min after dissolution of compound, 36.23-MHz ^{31}P NMR data accumulation was initiated, using a 5-kHz spectral window, 8192 data points, a $\pi/2$ pulse of 20 μs, low-power ^{1}H decoupling, and a pulse recycle time of 2 s. The free induction decay (FID) signal that was obtained after 100 pulses was stored on a diskette, and the next spectral acquisition was initiated at time t, relative to the "zero" time. The stored FID signals were exponentially multiplied so as to result in an additional 0.97 Hz of line broadening in the frequency-domain spectra. Possible nuclear Overhauser effects (NOE) were not suppressed by gated decoupling. Signal intensities (peak heights) were used to measure relative concentrations of components as a function of time. Select kinetic runs used samples with added methylphosphonate as a standard. Normalizing the peak heights of the reaction components relative to that of methylphosphonate gave half-lives which were within ±5% of those calculated without normalizing.

Partition Coefficients. A 14 mM solution of cyclophosphamide (**1a**) monohydrate in water (8 mg/2 mL) was vortexed (5 min) with an equal volume (2 mL) of octanol. After the solution was allowed to stand (5-10 min), the layers were separated and each was analyzed by ^{31}P NMR spectroscopy, using an identical set of acquisition and display parameters (0.15 mL of D_2O or Me_2SO-d_6 was added to each layer as an NMR lock signal). The signal intensities [(peak height) × (width at half-height)] given after 1000 pulses were used to determine the relative concentration of **1a** in the octanol and water layers. The partition coefficient ($P = [\mathbf{1a}]_{octanol}/[\mathbf{1a}]_{water}$) was 5.42.[37]

Phenylketophosphamide (**14a**) and phenylketoifosfamide (**14b**) were treated as above, except that each was first dissolved in octanol (10 mg/2 mL; 14 mM solutions) and then extracted with water. Under the conditions of the NMR experiment, **14a** or **14b** could not be detected in the water layers. Control experiments with **14a** indicated that if 2 mg of **14a** had been extracted into water, its signal would have been observed under the NMR conditions used, and the partition coefficient would have been 49. Therefore, the partition coefficients for **14a**, and by analogy **14b**, must be greater than 49.

Acknowledgment. This work was supported in part by Public Health Service Grant CA-21345 to G. Z. (01-04 years) and S.M.L. (04-present) from the National Institutes of Health. We are grateful to Dr. William Egan of the Food and Drug Administration for ^{13}C NMR spectra at 75.47 MHz.

Registry No. *cis*-**2a**, 100993-78-0; *trans*-**2a**, 100993-79-1; **3a**, 35144-64-0; **4a**, 10159-53-2; **4b**, 31645-39-3; **4c**·CHA, 100993-71-3; **14a**, 100993-68-8; **14b**, 100993-69-9; **14c**, 100993-70-2; **16a**, 100993-65-5; **16b**, 100993-66-6; **16c**, 100993-67-7; **18a**, 88802-97-5; **18b**, 100993-80-4; **18c**, 100993-81-5; *cis*-**19**, 100993-82-6; **20**, 100993-83-7; **23**, 100993-75-7; *cis*-**24**, 100993-73-5; *cis*-**26**, 100993-74-6; (*E*)-**27**, 100993-76-8; (*Z*)-**27**, 100993-77-9; $P(O)(Cl)_2N(CH_2CH_2Cl)_2$, 127-88-8; $PhOP(O)(NHCH_2CH_2Cl)_2$, 70772-68-8; $PhOP(O)(Cl)N(CH_2CH_2Cl)_2$, 4798-75-8; $PhOP(O)(NHCH_2CH_2Cl)N(CH_2CH_2Cl)_2$, 100993-72-4; 3-phenyl-3-butenyl acetate, 7306-12-9; paraformaldehyde, 30525-89-4; α-methylstyrene, 98-83-9; 3-phenyl-3-buten-1-ol, 3174-83-2; 2-chloroethylamine hydrochloride, 870-24-6; phenol, 108-95-2; 3-methyl-3-buten-1-ol, 763-32-6; *O*-methylhydroxylamine hydrochloride, 593-56-6.

Isohelical Analysis of DNA Groove-Binding Drugs

David Goodsell and Richard E. Dickerson*

Molecular Biology Institute, University of California at Los Angeles, Los Angeles, California 90024. *Received July 26, 1985*

Many antitumor drugs, and many carcinogens, act by binding within the minor groove of double-helical DNA, interfering with both replication and transcription. Several of these, including netropsin and distamycin, are quite base specific, recognizing and binding only to certain base sequences. The repeating pyrrole-amide unit of netropsin, and the repeated benzimidazole unit of the DNA stain and carcinogen Hoechst 33258, both are approximately 20% too long for synchronous meshing with base pairs along the floor of the minor groove in B DNA. We have carried out a systematic computer search for possible repeating drug backbones that are isohelical with DNA and that also provide chemical groups capable of reading and differentiating between A·T and G·C base pairs. These isohelical sequence-reading drug polymers or "isolexins" should offer the possibility of targeting synthetic drug analogues specifically against one region of a genome rather than another, or against neoplastic cells in preference to normal cells.

Some DNA-binding antitumor antibiotics such as actinomycin D or daunomycin bind by intercalation; that is, the DNA helix is expanded so that a flat polycyclic group can be inserted between two adjacent base pairs as though it was itself another base pair.[1,2] Other drugs such as netropsin and distamycin bind within the minor groove of the double helix without gross helical distortion.[3-6]

(1) Sobell, H. M.; Jain, S. C. *J. Mol. Biol.* **1972**, *68*, 21-34.
(2) Quigley, G. J.; Wang, A. H.-J.; Ughetto, G.; van der Marel, G.; Van Boom, J. H.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A* **1980**, *77*, 7204-7208.
(3) Patel, D. J. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6424-6428.
(4) Kopka, M. L.; Pjura, P.; Yoon, C.; Goodsell, D.; Dickerson, R. E. In *Structure and Motion: Membranes, Nucleic Acids and Proteins*; Clementi, E., Corongiu, G., Sarma, M. H., Sarma, R. H., Eds.; Adenine Press: Guilderland, NY, 1985; pp 461-483.
(5) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376-1380.

0022-2623/86/1829-0727$01.50/0

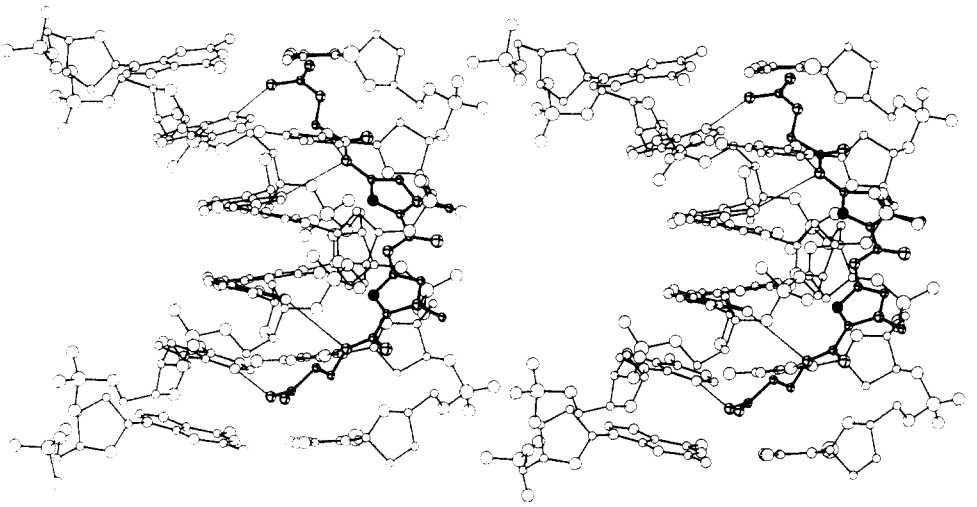


Figure 1. Stereopair drawing of the complex of netropsin (dark bonds) with B-helical C-G-C-G-A-A-T-T-^{Br}C-C-C-G. Only the central six base pairs of the DNA are shown for clarity. From top to bottom, the components of a netropsin molecule are guanidinium–amide–methylpyrrole–amide–methylpyrrole–amide–propylamidinium. Thin lines from DNA to drug reperesent hydrogen bonds between adenine N3 or thymine O2 of the DNA and amide NH or charged ends of the drug. The two black atoms on the pyrrole rings are the CH that, by being in van der Waals packing contact with an adenine at its C2 position, prevent the substitution of guanine, which would have a bulky $-NH_2$ group attached to the C2 position.

Netropsin and distamycin also exhibit strong base specificity; they demand binding sites of four or five successive A·T base pairs and avoid regions with G·C pairs.[7,8]

The recent X-ray crystal structure analysis of the complex of netropsin with a B DNA double-helical oligomer of sequence C-G-C-G-A-A-T-T-^{Br}C-G-C-G,[4-6] depicted in Figure 1, has shown the reason for this base specificity. The drug molecule sits with its two pyrrole rings wedged into the narrow minor groove, each with a CH pointing down toward the floor of the groove. These two pyrrole CH, and the methylene $-CH_2-$ groups flanking the outer two amides, are packed so closely against the adenine C2 hydrogen atoms of the four A·T base pairs that it would be impossible to substitute a guanine, with its N2 amine group. Hence the "reading" of base sequence is carried out by van der Waals nonbonded packing contacts, and the hydrogen bonds serve only to position the drug along the groove, placing it in the correct reading frame.

Examination of binding in netropsin has led to a proposal for a new class of synthetic netropsin analogues: longer polymers with pyrrole–amide as a repeating unit. Longer netropsins of this type should be capable of recognizing longer regions of A·T base pairs. If, at certain positions, methylpyrrole were to be replaced by imidazole or by furan, then room would be created for accommodation of the amine group of a guanine at that position. The new hydrogen bond from that $-NH_2$ to the N of imidazole or O of furan would provide increased stability for the DNA/drug complex. (See Figure 5 of ref 5.) It should be possible in principle to synthesize long polymers on the netropsin pattern, with pyrrole–amide where A·T base pairs are to be read and imidazole–amide where G·C pairs are to be permitted. Such a class of sequence-reading netropsin analogues or "lexitropsins" should be useful in laboratory work and should also have potential clinical applications as site-directed antitumor drugs.

One potentially serious flaw in this lexitropsin concept is the observation by Dervan and co-workers (ref 9 and personal communication) that binding of longer and longer distamycin analogues begins to become less favorable as the length of the polymer increases. (Distamycins are similar to netropsins, but with the charged guanidinium end replaced by an uncharged formamide.) The pyrrole–amide backbone appears to be longer than optimal for a fit from one base pair to the next along the bottom of the minor groove of B DNA. In the crystal structure the netropsin molecule is humped slightly at its central amide, producing longer than normal hydrogen bonds from the amide NH to the two central thymine O2 of the DNA. (See Figure 4 of ref 5.) Indeed, in Figure 1 the vertical distance between the two black pyrrole carbon atoms is noticeably longer than the rise along the helix axis from one base pair to the next. Longer netropsin or distamycin analogues may bind less well to B DNA simply because they get out of phase with the base pair steps along the floor of the groove. We have examined the geometry of a repeating pyrrole–amide polymer to see how compatible it might be with the B DNA helix and have searched for other polycyclic repeating units with a more favorable geometry, as the basis for synthesis of extended lexitropsin-like molecules.

Methods

Minor-groove-binding drugs have a characteristic structure, usually containing from two to five aromatic heterocycles linked by amide or vinyl groups and flanked at either end by cationic groups. Work on the bis(quaternary ammonium)heterocycles,[10] yielded several structural requirements for groove-binding antitumor agents: (1) a short distance between cationic ends, (2) close approach to overall planarity, (3) a certain degree of stiffness or rigidity to the molecule, and (4) a radius of curvature of 20–25 Å if the drug molecule is flattened down onto a plane by adjusting freely rotating torsion angles. Among the DNA-binding drugs that meet these requirements are netropsin and distamycin, Hoechst 33258[11,12] berenil,[13] and possibly also bleomycin.[14]

(6) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *J. Mol. Biol.* **1985**, *183*, 553–563.
(7) Wartell, R. M.; Larson, J. E.; Wells, R. E. *J. Biol. Chem.* **1974**, *249*, 6719–6731.
(8) Van Dyke, M. W.; Hertzberg, R. P.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5470–5474.
(9) Youngquist, R. S.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2565–2569.
(10) Braithwaite, A. W.; Baguley, B. C. *Biochemistry* **1980**, *19*, 1101–1106.
(11) Mikhailov, M. V.; Zasedatelev, A. S.; Krylov, A. S.; Gurskii, G. V. *Mol. Biol. (Engl. Transl.)* **1981**, *15*, 541–553.
(12) Martin, R. F.; Holmes, N. *Nature* **1983**, *302*, 452–454.
(13) Newton, B. A. In *Antibiotics*; Corcoran, J. W., Hahen, F. E., Eds.; Springer Verlag: New York, 1975; Vol. 3, pp 34–47.

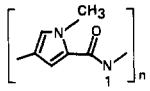
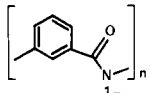
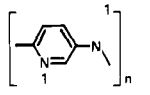
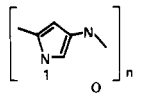
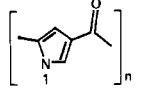
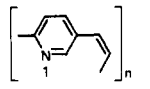
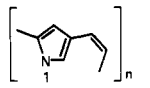
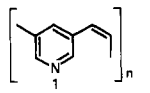
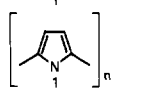
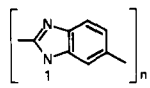
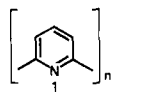
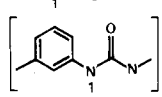
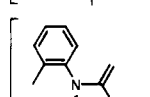
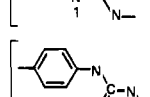
	T(°)		Δ(°)
R =	4.5Å	5.0Å	
	46.1	43.8	1.5
	46.4	44.3	8.0
	39.2	37.3	1.25
	36.2	34.5	-3.75
	35.1	33.5	-1.0
	–	–	>10
	–	–	>10
	36.2	34.4	3.0
	27.0	25.7	4.0
	45.2	43.1	1.0
	–	–	>10
	–	–	>10
	–	–	>10
	56.4	53.3	0.6

Figure 2. Some of the drug polymer backbone units that were tested in this study: t = helical twist angle at a radius of 4.5 or 5.0 Å from the helix axis; Δ = required uniform deformation of bond angles along the main chain.

Figure 2 depicts most of the possible backbone monomer units that were tested systematically for dimensional compatibility with the B DNA double helix. In screening the suitablility of these polymers, the following procedure was used:

1. Generate the polymer from the selected monomer, using standard bond lengths and angles derived from analogous small-molecule crystal structures. Vary main-chain torsion angles about single bonds along the polymer in a uniform manner, and calculate the parameters of the resulting helix.[15,16]

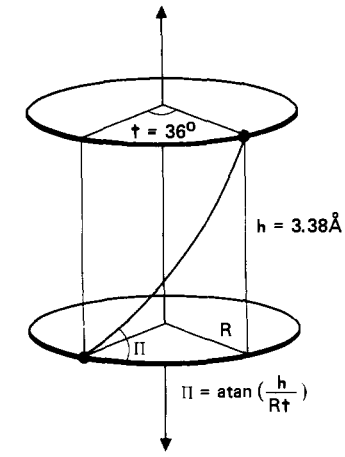


Figure 3. Definitions of helical twist angle, t, rise per repeat unit, h, radius, R, and pitch angle, Π. Values of t and h are given for an idealized B DNA helix.

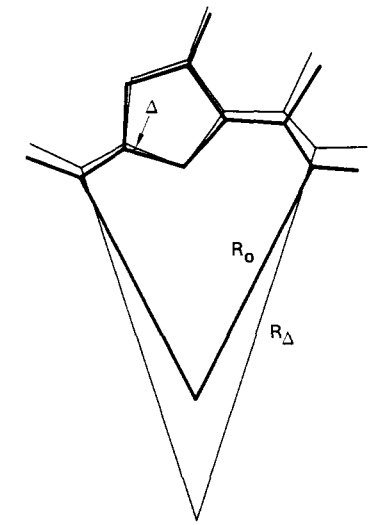


Figure 4. Effect on helical radius, R, of altering main-chain bond angles by an increment, Δ. Heavy lines represent the standard structure, and thin lines, the modified structure.

2. Take note of torsion angle combinations for which the extended polymer is isohelical with the DNA—that is, for which the pitch angles of the drug polymer and DNA helix are identical and for which the polymer has an appropriate helical radius for making hydrogen bonds to N and O atoms on the floor of the minor groove. As shown in Figure 3, pitch angle Π is given by $\tan \Pi = h/Rt$, where h is the rise per helix step, t is the rotation per step, and R is the radius or distance of the polymer backbone from the helix axis.

3. When torsion angles have been adjusted so that the drug polymer is comfortably nested within the minor groove of the DNA helix, compare the natural helix rotation and rise per polymer unit repeat with that of the DNA to see whether an infinite polymer would remain in phase with the base pairs or not.

One further degree of freedom was invoked as shown in Figure 4: Main-chain bond angles were allowed a uniform deformation angle of ±Δ, to permit better fitting of the polymer into the minor groove. Since Berman et al.[17] observed main-chain bond angles in crystalline netropsin that deviated as much as 4.5° from "standard" values, solutions were judged acceptable where Δ was not greater than this value.

Results

The analysis for a repeating pyrrole–amide backbone unit as found in netropsin and distamycin is illustrated in Figure 5. Main-chain atoms are numbered as shown

(14) Kuroda, R.; Neidle, S.; Riordan, J. M.; Sakai, T. T. *Nucleic Acids Res.* **1982**, *10*, 4753–4763.
(15) Shimanouchi, T.; Mizushima, S. *J. Chem. Phys.* **1955**, *23*, 707–711.
(16) Miyazawa, T. *J. Polym. Sci.* **1961**, *55*, 215–231.
(17) Berman, H. M.; Neidle, S.; Zimmer, C.; Thrum, H. *Biochim. Biophys. Acta* **1979**, *561*, 124–131.

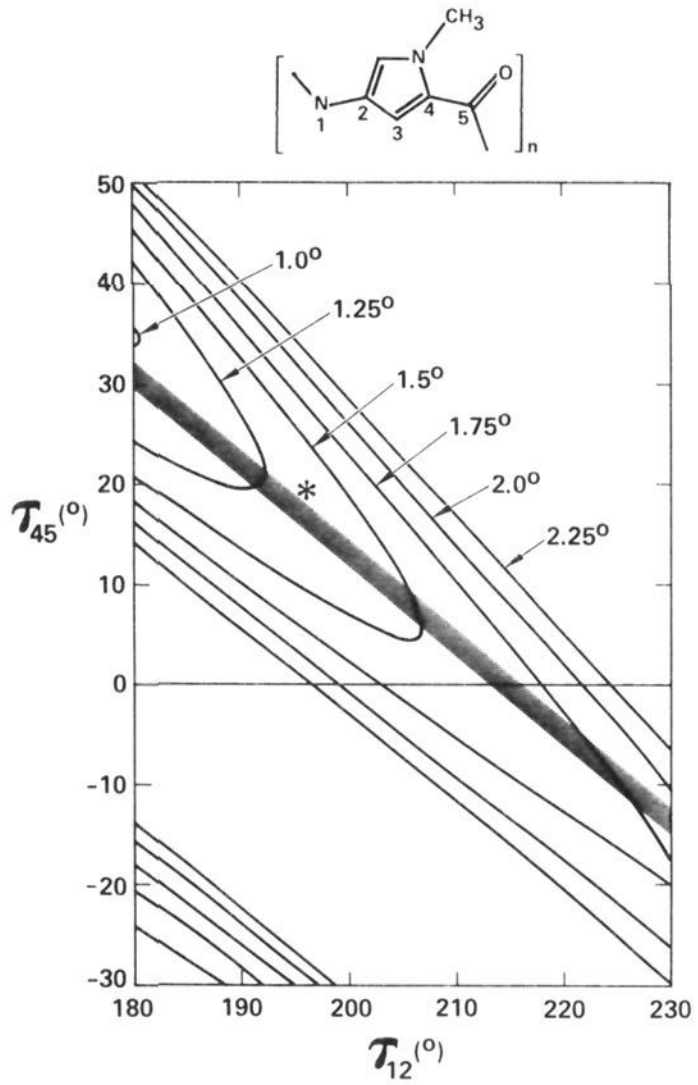


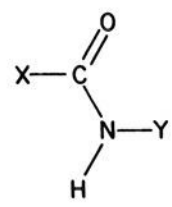
Figure 5. Conformation plot for the repeating pyrrole–amide unit of netropsin or distamycin. Torsion angles τ_{12} and τ_{45} represent rotations about single bonds between atoms 1 and 2 or atoms 4 and 5, respectively, as shown at the top. Curves mark isohelical conditions, where the drug polymer fits smoothly into the B DNA minor groove, for various mean bond angle deformations from 1.0 to 2.25°. Diagonal shaded zone indicates conformations for which the distance of nitrogen atom 1 from the helix axis is 4.5 Å (upper edge) to 5.0 Å (lower edge), suitable for hydrogen bonding to the floor of the groove. Asterisk marks conformation about the central amide of netropsin in the netropsin–DNA crystal structure analysis.

at the top, assigning number 1 to that atom that makes a hydrogen bond with the DNA. Single-bond torsion angles τ_{12} (the rotation about the bond connecting atoms 1 and 2) and τ_{45} are varied systematically in intervals of 5° about their fully extended cis or trans values, over a range of ±50°. For each combination of torsion angles the following helix parameters are calculated: h, the rise per repeat along the helix axis; t, the helical twist angle per repeat; R, the helical radius of hydrogen-bonding atom number 1. The pitch angle for the drug polymer, Π_{dr}, is calculated and compared with the pitch angle of the DNA helix, Π_{DNA}, at that same radius R. The drug and DNA are defined as being isohelical when $\Pi_{dr} = \Pi_{DNA}$.

The black curves in Figure 5 map out isohelical combinations of main-chain torsion angles for various degrees of bond angle deformation, Δ. For the repeating pyrrole–amide chain, no isohelical conditions are encountered without at least a 1° opening up of main-chain angles. The nested set of curves in Figure 5 plots isohelical conditions for deformations between 1 and 2.25°. At each value of Δ, following an isohelical curve leads one through a large variation in helical radius, R. Conformations near torsion angles $\tau_{12} = 180°$ and $\tau_{45} = 0°$ produce nearly flat helices of large radius, whereas those with greater rotation about main-chain single bonds have smaller radii and wind closer to the helix axis.

Of course only those solutions are physically acceptable that bring the drug polymer backbone close enough to the floor of the minor groove to form hydrogen bonds with adenine N and thymine O atoms. Examination of pyrimidine–purine, purine–pyrimidine, and pyrimidine–pyrimidine steps of an idealized B DNA double helix[18] suggests that the hydrogen-bonding drug atoms numbered 1 should be located at helical radius of 4.5–5.0 Å. In the actual netropsin/DNA structure,[4-6] helical radii of the outer two amide nitrogen atoms are 5.00 and 5.24 Å. (The central amide N is 6.24 Å away from the helix axis, but this is because the netropsin molecule is bowed up in the center for reasons that will emerge from this analysis.) The diagonal shaded band across Figure 5 shows the zone with R values from 4.5 Å (upper edge) to 5.0 Å (lower edge). Conformations within this diagonal band are at satisfactory distances from the helix axis for proper hydrogen bonding and also are isohelical with the DNA for the indicated mean bond angle deformations, Δ. The experimental conformation observed for the central amide of the netropsin/DNA complex as determined by X-ray diffraction, with $\tau_{45} = +19°$ and $\tau_{12} = +196°$, is marked by an asterisk.

It is interesting to note that even though individual torsion angle combinations change considerably along the shaded diagonal in Figure 5, this corresponds only to a small change in either helical twist angle, t, or vertical rise per polymer step, h. The "isotwist" curves of constant t, if plotted in Figure 5, would form a set of lines parallel to the shaded band, representing conformations in which the *sum* of torsion angles, $\tau_{12} + \tau_{45}$, is constant. To a very good approximation, both t and R depend only on the sum of the two torsion angles and not on their separate values. This can be understood by regarding the amide group



as a crankshaft linkage. Rotation by an arbitrary angle ϕ about the X–C bond and by $-\phi$ about the parallel N–Y bond does not alter the orientation of these two bonds but only their relative displacement. Hence, the continuous helix of which this amide is a part is not grossly altered.

With a main-chain bond angle deformation of $\Delta = 1.5°$, the mean helical twist angle of a pyrrole–amide chain for $R = 4.5$ Å is $t = 46.1°$, and that for $R = 5.0$ Å is $t = 43.8°$. The rise per residue for the latter case is $h = 4.11$ Å. Both t and h are 22% greater than the standard values for B DNA: $t = 36°$ and $h = 3.38$ Å. The pyrrole–amide repeating unit clearly is a misfit to the B DNA helix. This probably is the origin of the bowing up of the netropsin molecule at its central amide when it binds to DNA[5] and the reason why longer distamycin analogues begin to bind less well to DNA (ref 9 and personal communication).

Hoechst 33258 is a polycyclic polymer containing two successive benzimidazole rings (10th entry in Figure 2), which binds tightly to double-helical DNA. This binding makes it both a useful DNA stain for microscopy and a potent carcinogen. Its structure of repeating polycycles suggests that it might bind within the minor groove of B DNA in a manner analogous to netropsin. An X-ray crystal structure analysis of the complex of Hoechst 33258 with CGCGAATTCGCG (ref 19; Pjura, P., personal com-

(18) Arnott, S.; Hukins, D. W. L. *J. Mol. Biol.* **1973**, *81*, 93–105.
(19) Dickerson, R. E.; Pjura, P.; Kopka, M. L.; Goodsell, D.; Yoon, C. In *Crystallography in Molecular Biology*, Proceedings of the NATO Advanced Study Institute and EMBO Lecture Course, Bischenberg, Alsace, France, 12–21 Sep 1985; Moras, D., Ed. Plenum: New York, 1985; NATO ASI Series.

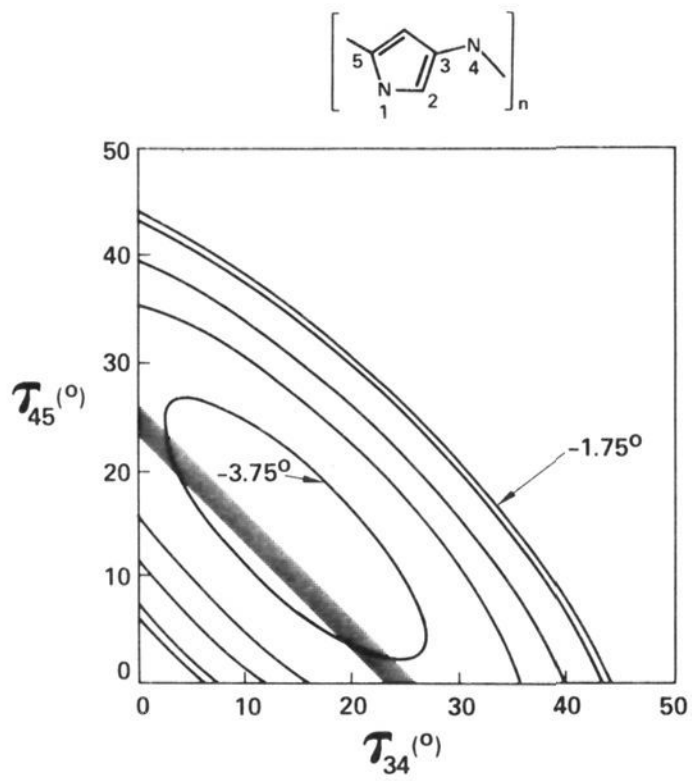


Figure 6. Conformation plot for a repeating pyrrole–amine unit, similar to that of netropsin but with deletion of the carbonyl groups. Same conventions as in Figure 5.

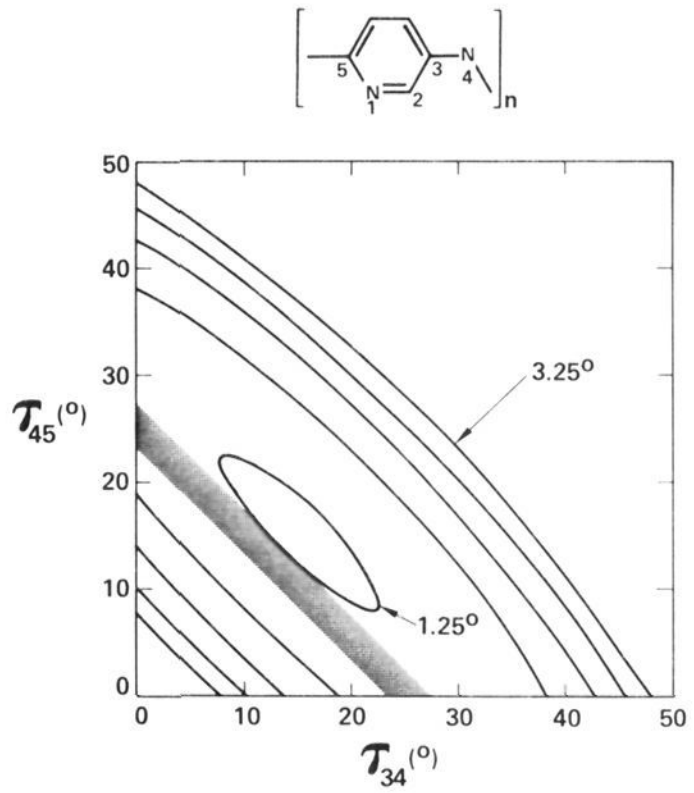


Figure 7. Conformation plot for a repeating pyridyl–amine unit. Same conventions as in Figure 5.

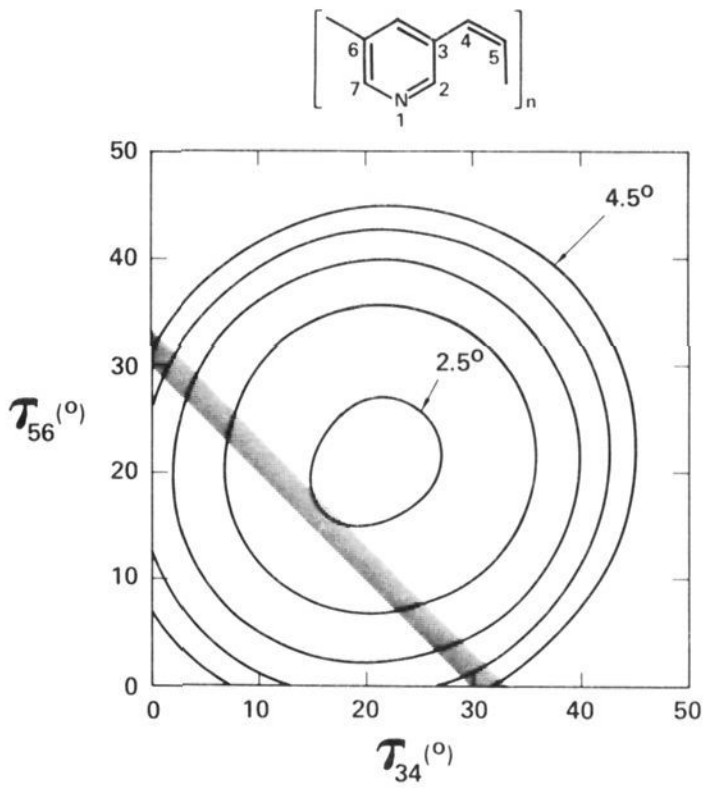


Figure 8. Conformation plot for a repeating pyridyl–vinyl unit. This chain is sterically impossible because of clashes between C2 and C7 hydrogens on adjacent six-membered rings.

munication) reveals that this is indeed the case. Although a polymer constructed from benzimidazole units has only one torsional degree of freedom, it can be coiled up the minor groove of B DNA in an isohelical manner. When this is done, however, the helix parameters of the polybenzimidazole chain once again are seen to be mismatched to B DNA: $t = 43.1°$ and $h = 4.05$ Å (for $R = 5.0$ Å), both 20% longer than the corresponding DNA values.

Both the pyrrole–amide repeat in netropsin and the benzimidazole repeat in Hoechst 33258 are intrinsically too long for the DNA. Netropsin manages to deform the DNA double helix slightly when it binds;[4-6] it forces open the narrow minor groove somewhat, and bends the helix axis backward by 8° at the site of bending. But it does not seem capable of unwinding the helix or otherwise deforming it sufficiently to produce a better match between DNA and drug. Similar observations can be made with the DNA–Hoechst complex.[19] If the drug polymer is too long for the DNA, and if the DNA is incapable of stretching to fit it, then one would expect that an even tighter and more specific binding could be produced by shortening the repeat of the drug to match the natural parameters of DNA.

Accordingly, helix calculations were made in which the pyrrole–amide chain of netropsin was shortened by deletion of either the amine –NH– or the carbonyl C=O to form (2,4-disubstituted pyrrole)–ketone and (2,4-disubstituted pyrrole)–amine, respectively. These and other monomers are listed in Figure 2. Alongside each are given the helical twist angles for isohelical conditions at $R = 4.5$ and 5.0 Å, along with the required main-chain deformation, Δ. In each case the helical rise, h can be calculated from

$$R \tan \Pi = h_{DNA}/t_{DNA} = h_{drug}/t_{drug}$$

or more simply

$$h_{drug} = 0.0939 t_{drug}$$

Four monomers were observed to conform to ideal DNA helix geometry with Δ less than 4° and with t in the range of 24–38°. These are (3,5-disubstituted pyrrole)–amine, (3,5-disubstituted pyrrole)–ketone, (3,5-disubstituted pyridiyl)–amine, and (3,6-disubstituted pyridyl)–vinyl. (The unorthodox ring numbering has been chosen to agree with the diagrams in Figures 6–8.) Conformation plots for three of these appear in Figures 6–8; that for pyrrole–ketone is virtually identical with that of pyrrole–amine. Isohelical conformations in each case occur with the sum of the two torsion angles around 25–30°.

The structures in Figures 6–8 were tested for too close steric contacts by using energy idealization program MM2,[20] giving the same rotation to both torsion angles. (This corresponds to a 45° trajectory from lower left to uper right in each conformation plot.) The pseudoenergy calculated from van der Waals contacts is plotted in Figure 9 as a function of the sum of torsion angles, $\sum\tau$. The two amine-containing polymers remain energetically allowable for all values of torsion angles, with the pyrrole polymer being somewhat favored. The van der Waals energy remains low and changes little, for all values of $\sum\tau$. But the pyridyl–vinyl polymer exhibits too close approach to hydrogen atoms at positions 2 and 7 as numbered at the top

(20) Allinger, N. L. *J. Am. Chem. Soc.* **1977**, *99*, 8127–8134.

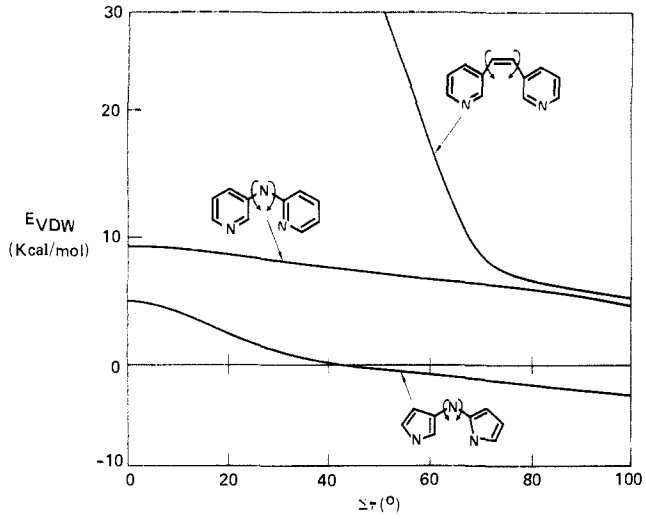


Figure 9. Plot of van der Waals contact pseudoenergy of three repeating polymers, as a function of the sum of the two central torsion angles (marked by curved arrows in the molecular drawings). Both torsion angles are turned by the same amount for this calculation. The vinyl-linked polymer (upper right) is unstable for all torsion angle combinations that are isohelical with B DNA. (See Figure 8).

of Figure 8, for all values of torsion angle sum below 70°. This is reflected in the steeply rising curve in Figure 9. All regions of the isohelical zone marked by the shaded stripe in Figure 8 are completely forbidden, and the pyridyl–vinyl polymer therefore must be rejected.

Discussion

An approximate but rapid calculation could have shown from the outset that the pyrrole–amide repeat of netropsin was inherently too long for perfect registration with B DNA. In ideal B DNA, a rise of h = 3.38 Å and helical twist angle of t = 36° yield, at a radius of R = 5.0 Å a pitch angle of II = 47.1°. The hypotenuse of this right triangle wrapped around the surface of the helix cylinder, or the distance from one repeating monomer to the next along the chain, is

$$Rt/\cos \text{II} = 4.61 \text{ Å}$$

For a fully extended chain with bond lengths 1.54 Å and bond angles 120°, the foreshortened distance along the overall chain direction is $1.54 \times 3^{1/2}/2$ Å = 1.33 Å. The ideal DNA repeat of 4.61 Å therefore could only accommodate three and one-half bonds, yet the netropsin monomer has five bonds. There is no way in which a fully extended netropsin chain could match the DNA groove and no way in which the chain could be "folded" to reduce the five bonds to an effective length of three and one-half. The true distance along a netropsin chain when it is wound isohelically with DNA is $Rt/\cos$ II = 5.61 Å. The actual contortions of the netropsin backbone have folded five bonds down into a running length corresponding to 4.2 bonds in a fully extended chain, but the drug polymer still is out of phase with DNA by 1.0 Å per monomer step.

The pyrrole–amine and pyrrole–ketone repeats that proved to be compatible with B DNA geometry amount to two different ways of shortening the netropsin backbone, by eliminating either C=O or N—H. The pyridyl–amine polymer is similar, but with six-membered rings rather than five. Physical evidence for the reality of all these calculations is provided by a groove-binding drug with related backbone, berenil.[13] Berenil has two six-membered rings connected by a triazo group: -NHN=N-. If this is shortened to diazo. -N=N-, then the compound no

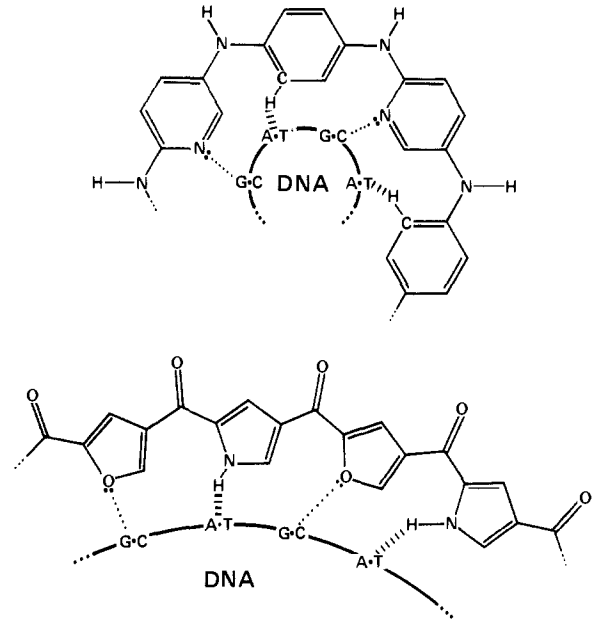


Figure 10. Two possible "isolexins", or sequence-reading polymers that are geometrically isohelical with B DNA: (top) pyrrole–ketone polymer, with pyrroles in tight van der Waals contact with adenines at A·T base pairs and furans hydrogen bonded to guanine $-NH_2$ at G·C base pairs; (bottom) pyrimidyl–ketone polymer, with benzyls in tight van der Waals contact with adenines at A·T base pairs and pyrimidyls hydrogen bonded to guanine $-NH_2$ at G·C base pairs. An arbitrary arrangement of A·T and G·C base pairs along the DNA could be read and recognized by synthesizing polymers with the corresponding sequence of the two kinds of monomer unit depicted in each drawing.

longer binds to DNA in vitro, and 20 times as much drug is required for an equivalent trypanocidal activity in vivo. In contrast, shortening the chain by still another nitrogen to produce –NH–, as in our pyridyl–amine, restores DNA binding in the berenil analogue and requires only 40% as much drug to produce equivalent trypanocidal activity as with berenil itself. Hence, experimental evidence exists to suggest that a polymer based on repeating pyridyl–amine units would bind to DNA and exhibit useful pharmacological activity.

Figure 10 depicts two possible sequence-specific polymers, isohelical with B DNA, that should be capable of reading any desired base sequence. Strictly speaking they no longer are lexitropsins, since the netropsin-like backbone has been abandoned. If another neologism may be forgiven, the term "isolexin" is a convenient abbreviation for "isohelical base sequence reading polymer". In both the isolexins illustrated in Figure 10, a small hydrogen-bond acceptor (imidazole or furan) is incorporated wherever a G·C base pair is to be recognized, and a ring with a more bulky group is used at prospective A·T loci. These isolexins in Figure 10 should be superior to the lexitropsins in having an intrinsic structural match between their dimensions and those of the DNA double helix, and no tendency to go out of phase.

One might ask why, if these isolexins are so efficient in binding to DNA, some microorganism has not already preempted them as the basis for antibiotics. One answer could be that of intrinsic difficulty of synthesis, but there seems nothing unusual about the polymers in Figure 10. A second reason might be that it binds to DNA entirely too well and is too toxic for its host, as well as the surrounding organisms against which it is a defense mechanism. As nations too often forget, suicide is not a rational defense strategy. This may be a warning to the synthetic

organic chemist that polymers of the type of Figure 10 could be potent toxins and carcinogens, as well as potential DNA-binding antitumor drugs. Synthetic investigations of this class of molecule (T. Smith, private communication) are under way in this laboratory.

Acknowledgment. We thank Mary Kopka, Phil Pjura, Chun Yoon, and Gil Privé for stimulating discussions on groove-binding by netropsin, Hoechst 33258, bleomycin, and other antibiotics and for suggestions and criticisms of the DNA groove-fitting concept. We also thank Peter Dervan for information about distamycin binding in advance of publication. This work was supported by NIH Grant GM-31299. D.G. is the holder of a predoctoral traineeship under USPHS National Research Service Award GM-07104.

Dopamine Receptor Agonists: 3-Allyl-6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1*H*-3-benzazepine-7,8-diol and a Series of Related 3-Benzazepines

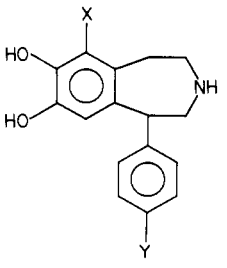
Stephen T. Ross,*,† Robert G. Franz,† James W. Wilson,† Martin Brenner,† Robert M. DeMarinis,† J. Paul Hieble,‡ and Henry M. Sarau§

Research and Development Division, Smith Kline & French Laboratories, Philadelphia, Pennsylvania 19101.
Received August 26, 1985

The *N*-allyl derivative (SK&F 85174) of 6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1*H*-3-benzazepine-7,8-diol (SK&F 82526) not only retains the exceptional D-1 agonist potency of its parent but also displays reasonably potent D-2 agonist activity, as measured by a dopamine-sensitive adenylate cyclase test and a rabbit ear artery assay, respectively. Several additional N-substituted compounds were prepared to explore the D-2/D-1 agonist relationship. The *N*-methyl analogue retained good D-2 agonist potency, but this substitution converted D-1 agonist activity into antagonist activity. Most other N-substituents sharply decreased D-2 agonist potency including the *N*-*n*-propyl group. This observation was surprising since the introduction of mono- or di-*N*-*n*-propyl substituent(s) is commonly linked with retention or enhancement of D-2 agonist potency in other series of dopamine agonists. The *N*-(2-hydroxyethyl) analogue retains about one-fourth the D-2 potency of SK&F 85174. Several synthetic methods were used to prepare these compounds. N-Allylation of a trimethoxybenzazepine followed by cleavage of the methyl ethers with boron tribromide was the preferred method. Other methods used were direct alkylation of the trihydroxy secondary amine, i.e., SK&F 82526, and an acylation-amide reduction-cleavage method.

Research into the nature of dopamine receptors has provided substantial understanding of the role they play in various physiological states.[1,2] In particular, the classification of dopamine receptors into D-1 and D-2 subtypes by Kebabian and Calne[3] has stimulated development of specific biological tests for agents acting selectively at these receptor subtypes and has enabled subsequent development of pharmacological methodology to show the whole animal effects of selectively stimulating the D-1 and D-2 receptors, centrally and peripherally.[4]

Setler et al.[5] have reported on the central effects of SK&F 38393 (1), a D-1 agonist with some mixed antagonist effects, and identified it as a potential antiparkinsonism agent.



1. X = Y = H
2. X = Cl; Y = OH

In more recent research, attention has been focused on the effects of D-1 and D-2 agonists on the cardiovascular and the renal systems, particularly the renal vasculature. SK&F 82526 (**2**) has been shown by Weinstock et al.[6] and Hahn et al.[7] to be a potent and selective D-1 agonist acting primarily peripherally and exerting its effects largely on the kidney vasculature by causing potent vasodilation and increases in renal blood flow. Stote et al.[8] have confirmed these pharmacological findings by clinical experimentation.

Stimulation of D-2 receptors located presynaptically on postganglionic sympathetic neurons would inhibit the amount of neuronally released NE per nerve impulse. Agents acting by this mechanism could be expected to be of therapeutic utility in disease states such as angina pectoris and hypertension where increased sympathetic activity leading to elevated NE levels is believed to play a key role.[1,4,9,10]

† Medicinal Chemistry Department.
‡ Pharmacology Department.
§ Molecular Pharmacology Department.

(1) Cavero, I.; Massingham, R.; Lefevre-Borg, F. *Life Sci.* **1982**, *31*, 939-948.
(2) Lehman, J.; Briley, M.; Langer, S. Z. *Eur. J. Pharmacol.* **1983**, *88*, 11-26.
(3) Kebabian, J. W.; Calne, D. B. *Nature (London)* **1979**, *277*, 93.
(4) Goldberg, L. I.; Volkman, P. H.; Kohli, J. D. *Annu. Rev. Pharmacol. Toxicol.* **1978**, *18*, 57-79.
(5) Setler, P. E.; Sarau, H. M.; Zirkle, C. L.; Saunders, H. L. *Eur. J. Pharmacol.* **1978**, *50*, 419-430.
(6) Weinstock, J.; Wilson, J. W.; Ladd, D. L.; Brush, C. K.; Pfeiffer, F. R.; Kuo, G. Y.; Holden, K. G.; Yim, N. C. F.; Hahn, R. A.; Wardell, J. R., Jr.; Tobia, A. J.; Setler, P. E.; Sarau, H. M.; Ridley, P. T. *J. Med. Chem.* **1980**, *23*, 973-975.
(7) Hahn, R. A.; Wardell, J. R., Jr.; Sarau, H. M.; Ridley, P. T. *J. Pharmacol. Exp. Ther.* **1982**, *223*, 303-313.
(8) Stote, R. M.; Dubb, J. W.; Familiar, R. G.; Erb, B. B.; Alexander, F. *Clin. Pharmacol. Ther.* **1983**, *34*, 309-315.

0022-2623/86/1829-0733$01.50/0