5 μg/mL, penicillin 5 units/mL and kanamycin at 5 μg/mL.

Effect of the Compounds on Cell Multiplication. Promastigotes at 0.5×10^6 cells in 500 μL of medium were seeded in 24-well Nunclon multiwell plates. Compounds to be tested were added a few hours later at various concentrations in 25 μL of H_2O. Each test was performed in duplicate, and untreated cultures were run in parallel. After 3 days of culture, promastigotes were counted in a hemacytometer. Cells in control wells grew to a final density of 3.5×10^{-7} organisms mL^{-1}.

Cell Fractionation. Cells were disrupted by five cycles of freezing in dry ice–ethanol and quick thawing at 37 °C in Tris-HCl 10 mM at pH 7.0 containing 2-mercaptoethanol 10 mM and PMSF 0.1 mM. Centrifugation of the homogenate was performed in a Beckman J 2/21 ME centrifuge equipped with a J 20 rotor at 4 °C for 20 min. The supernatant was taken up whereas the 12000*g* pellet was resuspended in 1 mL of the same buffer. The proteins were extracted with 0.2% Triton X 100 in 1.7 M NaCl. The DNA was then precipitated at 4 °C for 1 h upon addition of 10% PEG (w/w) in 1.3 M NaCl (final concentrations). The suspension was centrifuged at 12000*g* for 15 min, and the supernatant was dialyzed and assayed for enzyme activity.

Protein Methylase Activity. Protein methylase II (EC 2.1.1.77) activity was assayed at 37 °C by measuring the incorporation of radiolabeled methyl groups into α-globulin after separation from unreacted AdoMet by precipitation of the methylated protein with TCA, as described by Kim and Paik.[18]

The apparent kinetic constants were calculated from Lineweaver–Burk plots.[19] Protein concentration was measured by the dye adsorption method of Bradford,[20] using bovine serum albumin as the standard.

(18) Kim, S.; Paik, W. K. Purification and Properties of Protein Methylase II. *J. Biol. Chem.* **1970**, *245*, 1806–1813.
(19) Lineweaver, M. L.; Burk, D. Determination of Enzyme Dissociation Constants. *J. Am. Chem. Soc.* **1934**, *56*, 658–666.
(20) Bradford, M. M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254.
(21) Samadi, M. Nouvelle Voie d'Acces Aux Homologue Supérieurs de Sucres et de Nucléosides par Décarboxylation Radicalaire, Ph. D. Thesis, Université de Paris-Sud (Orsay), 1989.

(±)-3-Allyl-7-halo-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepines as Selective High Affinity D1 Dopamine Receptor Antagonists: Synthesis and Structure–Activity Relationship

Nandkishore Baindur,[†] Mai Tran,[†] Hyman B. Niznik,[‡] H. C. Guan,[‡] Phillip Seeman,[‡] and John L. Neumeyer*[,†,§]

Section of Medicinal Chemistry, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, Massachusetts 02115, Departments of Psychiatry and Pharmacology, University of Toronto, Toronto, Ontario, Canada M5S 1A8, and Research Biochemicals, Inc., 1 Strathmore Road, Natick, Massachusetts 01760. *Received June 17, 1991*

Substituted 1-phenyl-3-benzazepines form a class of compounds possessing potent and selective affinity for the D1 DA receptor. 7,8-Dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SKF 38393) and its 6-halo analogues are potent and selective D1 receptor agonists. Recently, the 3-allyl derivatives of SKF 38393 and its analogues were described as selective D1 agonists with higher D1 efficacy and CNS potency. In order to extend these results to compounds in the 7-halo-8-hydroxy-substituted antagonist series, we have synthesized and pharmacologically characterized 3-allyl analogues of 7-substituted (Cl, Br, H) 8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepines. These 3-allyl derivatives were compared with their 3-methyl and 3-unsubstituted analogues in terms of their D1 receptor affinity and selectivity. The results have been used to generate structure–affinity relationships. The D1 receptor affinity, for 3-substitution, is found to be in the order: methyl > allyl > H. For 7-substitution, the affinity is in the order: Cl = Br > H. The 3-allyl compounds show affinity close to that of the parent (3-methyl) compounds while exhibiting a slightly diminished D1 selectivity. However, the greater lipophilicity of the 3-allyl compounds may enable them to cross the blood–brain barrier more readily and thereby exhibit higher in vivo CNS potency. Thus 3-allylbenzazepines have potential as high affinity selective D1 antagonists.

Dopamine (DA) receptors have been implicated in neuropsychiatric diseases, and consequently there is considerable continuing interest in the search for suitable selective and potent DA receptor agonists and antagonists which can be useful both as agents for therapeutic purposes and as tools for pharmacological and biochemical research.[1,2] Until recently, the DA receptors were classified into D1 and D2 subtypes on the basis of available biochemical and pharmacological evidence.[3-5] The recent discovery of the existence of new DA receptor subtypes (D3, D4, and D5), has stimulated renewed interest in the chemistry and pharmacology of agents acting selectively at these receptor subtypes.[6-8] While the physiological and pathophysiological roles of these new receptor subtypes are as yet unknown, it is known that D1 and D2 receptor antagonists have clinical applications as potential antipsychotics in the treatment and management of schizo-

* To whom correspondence should be addressed at: Research Biochemicals Inc., 1 Strathmore Road, Natick, MA 01760.
† Section of Medicinal Chemistry.
‡ Departments of Psychiatry and Pharmacology.
§ Research Biochemicals Inc.

(1) Hornykiewicz, O. In *Biogenic amines and physiological membranes in drug therapy*; Biel, J. H., Abood, L. G., Eds.; Med. Chem. Res. Ser. 5, Part B; Dekker: New York, 1971; pp 173–258.
(2) Waddington, J. L.; O'Boyle, K. M. Drugs acting on brain dopamine receptors: a conceptual re-evaluation five years after the first selective D1 antagonist. *Pharmacol. Ther.* **1989**, *43*, 1–52.
(3) Kebabian, J. W.; Calne, D. B. Multiple receptors for dopamine. *Nature (London)* **1979**, *277*, 93–96.
(4) Seeman, P.; Grigoriadis, D. E. Dopamine receptors in the brain and periphery. *Neurochem. Int.* **1987**, *10*, 1–25.
(5) Anderson, P. H.; Gingrich, J. A.; Bates, M. D.; Dearry, A.; Falardeau, P.; Senogles, S. E.; Caron, M. G. Dopamine receptor subtypes beyond the D1/D2 classification. *Trends Pharmacol. Sci.* **1990**, *11*, 231–236.
(6) Sokoloff, P.; Martres, M.-P.; Bouthenot, M. L.; Schwartz, J. C. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature (London)* **1990**, *347*, 146–151.

0022-2623/92/1835-0067$03.00/0

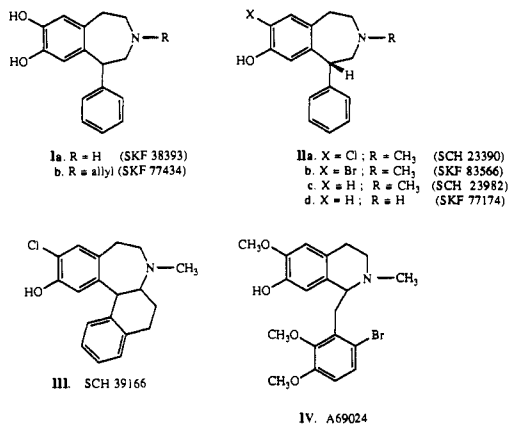
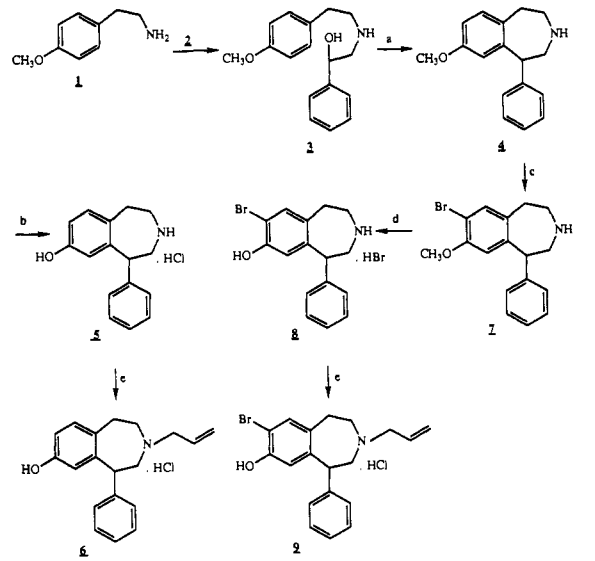


Figure 1. Structures of selective D1 receptor ligands.

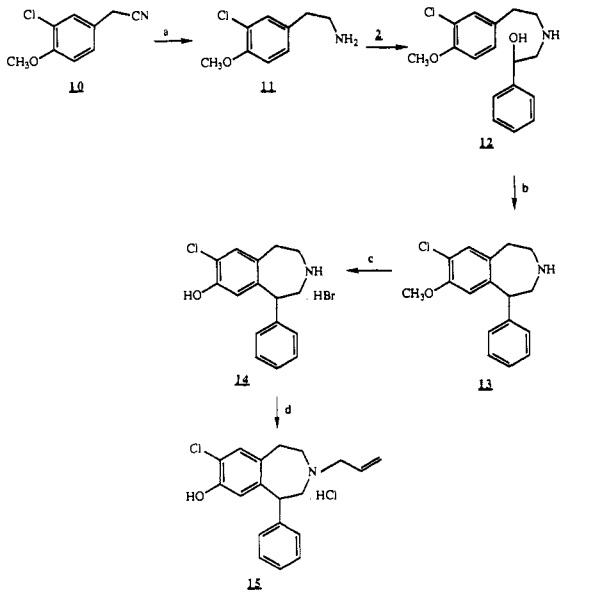
phrenia and related mental disorders.[1,2] The first selective D1 receptor antagonists belonged to the 1-phenyl-3-benzazepine class [IIa (SCH 23390) and IIb (SKF 83566) Figure 1].[9-13] While selective D1 affinity and antagonist activity has been demonstrated in other chemical classes of compounds [III (SCH 39166) and IV (A69024) Figure 1],[14-17] it is clear that the 7-halo-8-hydroxy-1-phenyl-3-benzazepines (IIa and IIb) still prove to be the ligands with the highest affinity and D1/D2 selectivity. The structure–activity relationship (SAR) of this class of compounds

Scheme I[a]



[a] (a) TFA/H_2SO_4. (b) HBr. (c) $Br_2/AcOH$. (d) BBr_3/CH_2Cl_2, −70 °C. (e) Allyl bromide/K_2CO_3.

Scheme II[a]



[a] (a) BH_3–THF. (b) PPA. (c) BBr_3/CH_2Cl_2, −70 °C. (d) Allyl bromide/K_2CO_3.

has been studied previously, and several generalizations have been established which have been extended to other chemical classes of D1 ligands.[9,10,18] A brief mention of the generalizations is made here in order to provide a rationale for our present work which further extends the SAR. For the substituted 1-phenyl-3-benzazepines, the 7,8-dihydroxy compounds are potent, selective agonists (Figure 1).[10,18] When the 7-OH group is removed, the agonist activity is lost but the resulting compounds can still bind the D1 receptor with high affinity. These monohydroxy compounds are D1 antagonists.[9] When the 3-substituent is a methyl and the 7-substituent a halogen (Cl, Br), the resulting compounds are the most potent, selective D1 antagonists known.[9] Lengthening of the alkyl chain at the 3-position to ethyl, propyl, butyl, etc. results

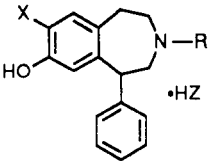
(7) Van Tol, H. H. M.; Bunzow, J. R.; Guan, H. C.; Sunahara, R. K.; Seeman, P.; Niznik, H. B.; Civelli, O. Cloning of the gene for a human dopamine D4 receptor with a high affinity for the antipsychotic clozapine. *Nature (London)* **1991**, *350*, 610–614.
(8) Sunahara, R. K.; Guan, H. C.; O'Dowd, B.; Seeman, P.; Laurier, L. G.; Ng, G.; George, S.; Torchia, J.; Van Tol, H. H. M.; Niznik, H. B. Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature (London)* **1991**, *350*, 614–619.
(9) Iorio, L. C.; Barnett, A.; Billard, W.; Gold, E. H. Benzazepines: structure–activity relationships between D1 receptor blockade and selected pharmacological effects. In *Neurobiology of central D1 dopamine receptors*; Breese, G. R., Creese, I., Eds.; Plenum Press: New York, 1986; pp 1–14.
(10) Kaiser, C.; Jain, T. Dopamine receptors: functions, subtypes and emerging concepts. *Med. Res. Rev.* **1985**, *5*, 145.
(11) Iorio, L. C.; Barnett, A.; Leitz, F. H.; Houser, B. P.; Korduba, C. A. SCH 23390, a potential benzazepine antipsychotic with unique interactions on dopaminergic systems. *J. Pharmacol. Exp. Ther.* **1983**, *226*, 462–468.
(12) Hyttel, J. SCH 23390: the first selective dopamine D1 antagonist. *Eur. J. Pharmacol.* **1983**, *91*, 153–154.
(13) Billard, W.; Ruperto, V.; Crosby, G.; Iorio, L. C.; Barnett, A. Characterization of the binding of 3H-SCH 23390, a selective D1 receptor antagonist ligand, in rat striatum. *Life Sci.* **1984**, *35*, 1885–1893.
(14) Chipkin, R. E.; Iorio, L. C.; Coffin, V. L.; McQuade, R. D.; Berger, J. G.; Barnett, A. Pharmacological profile of SCH 39166: a dopamine D1 selective benzonaphthazepine with potential antipsychotic activity. *J. Pharmacol. Exp. Ther.* **1988**, *247*, 1093–1102.
(15) Berger, J. G.; Chang, W.-K.; Clader, J. W.; Hou, D.; Chipkin, R. E.; McPhail, A. T. Synthesis and receptor affinities of some conformationally restricted analogues of the dopamine D1 selective ligand (5R)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol. *J. Med. Chem.* **1989**, *32*, 1913–1921.
(16) Kerkman, D. J.; Ackerman, M.; Artman, L. D.; MacKenzie, R. G.; Johnson, M. C.; Bednarz, L.; Montana, W.; Asin, K. E.; Stampfli, H.; Kebebian, J. W. A-69024: a non-benzazepine antagonist with selectivity for the dopamine D1 receptor. *Eur. J. Pharmacol.* **1989**, *166*, 481–491.
(17) Michaelides, M. R.; Schoenleber, R.; Thomas, S.; Yamamoto, D. M.; Britton, D. R.; MacKenzie, R.; Kebabian, J. W. Synthesis and pharmacological evaluation of 1-aminomethyl-3,4-dihydro-5-hydroxy-1H-2-benzopyrans as dopamine D1 selective ligands. *J. Med. Chem.* **1991**, *34*, 2946–2953.
(18) Weinstock, J.; Heible, J.; Wilson, J. W. The chemistry and pharmacology of 3-benzazepines. *Drugs Future* **1985**, *10*, 645–696.

in considerable successive loss of affinity.[9] Similarly, any substituent other than a halogen at the 7-position leads to considerable loss of affinity and D1 selectivity.[9] Recently, several 3-allyl-7,8-dihydroxy-1-phenyl-3-benzazepines have been described as potent D1 agonists similar to the prototype 7,8-dihydroxy-1-phenyl-3-benzazepine (Ia, Figure 1).[19,20] 3-Allyl substitution (Ib, SKF 77434) not only retains the high affinity of the parent ligand Ia but also possesses higher agonist efficacy as shown by its ability to stimulate adenyl cyclase.[19-22] Furthermore, the 3-allyl analogues also have been found to possess superior in vivo biological activity.[21,22] This is particularly true where central nervous system (CNS) activity is concerned, probably owing to increased penetration of the blood–brain barrier by these more lipophilic 3-allyl derivatives compared to the relatively more hydrophilic 3-unsubstituted compounds.[21,22] Recent investigations in our laboratory were focused on the effects of concurrent substitution of a halogen, more specifically a bromine in place of a chlorine, at the 6-position and an allyl group at the 3-position of the 7,8-dihydroxy-substituted compounds.[23] This study has shown that these substitutions result in the retention of high D1 affinity and selectivity.[23] Furthermore, the 6-bromo- and the 6-chloro-substituted analogues had virtually identical pharmacological activity. While 3-allyl-6-halo-7,8-dihydroxy-1-phenyl-3-benzazepines have been developed as D1 agonists with the 3-allyl group providing an increase in the lipophilicity and thus CNS potency, there has been no extrapolation of these results to the D1 antagonist 7-halo-8-hydroxy-1-phenyl-3-benzazepines. It was therefore of interest to examine the activity of these novel 3-allyl benzazepines in terms of D1 receptor affinity, selectivity, and in vivo CNS activity. The development of these 3-allyl compounds in the D1 antagonist series should also further extend the SAR for this class of benzazepines. In this report, we describe the synthesis and pharmacological characterization of 3-allyl-7-halo-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepines and a comparison of their D1 affinity and selectivity with that of the related 3-methyl and 3-unsubstituted analogues.

Chemistry

The 1-phenyl-3-benzazepine skeleton was constructed by the method of Walter and Chang[24] (Schemes I and II).

Table I.[a] Affinity of 1-Phenyl-3-benzazepine Derivatives at Dopamine Receptors



compound[b]	X	R	HZ	K_i (nM) D1	K_i (nM) D2
IId (SKF 77174)	H	H	HCl	702	4408
6	H	allyl	HCl	48	NT[d]
IIc ((*R*)-SCH 23982)	H	CH_3	HCl	6[c]	NT
8	Br	H	HBr	4.1	5760
IIb ((*R*)-SKF 83566)	Br	CH_3	HCl	0.58	NT
9	Br	allyl	HCl	0.9	629
14	Cl	H	HBr	4.9	1374
IIa ((*R*)-SCH 23390)	Cl	CH_3	HCl	0.17	NT
15	Cl	allyl	HCl	1.2	NT

[a] All compounds were tested in the presence of NaCl (see Experimental Section), and all inhibited the binding of [^{3}H]SCH 23390 at D1 with a single dissociation constant. The dissociation constants (K_i values) of all compounds were determined from inhibition of the binding of 1 nM [^{3}H]SCH 23390. The K_i values were calculated using the computer program LIGAND or using the Cheng–Prusoff equation (see ref 4 and 23). K_i values indicate an average of two separate experiments, the individual values agreeing to within 10% of the average. [b] All compounds are racemates unless otherwise noted. [c] K_i value taken from ref 9. [d] NT = not tested.

Condensation of 4-methoxyphenethylamine (**1**) with styrene oxide (**2**) gave the benzyl alcohol **3** (Scheme I).[25] Cyclization of **3** was accomplished by refluxing in TFA–H_2SO_4 solution to yield the benzazepine **4**, which was O-demethylated by refluxing in 48% HBr to obtain the 8-hydroxybenzazepine **5**. Alkylation of **5** with allyl bromide in the presence of anhydrous K_2CO_3 yielded the corresponding 3-allyl analogue **6**. Treatment of **4** with bromine in acetic acid gave the 7-bromo derivative **7**,[25] which on O-demethylation with BBr_3 yielded **8**. Alkylation of **8** with allyl bromide as before yielded the corresponding *N*-allyl derivative **9**. The 7-chloro analogue was prepared in a similar way[26] (Scheme II) from 3-chloro-4-methoxyphenylacetonitrile (**10**). Reduction of **10** gave the phenethylamine **11**, which was condensed with styrene oxide to obtain the benzyl alcohol **12**. Cyclization of **12** with PPA yielded the benzazepine **13**,[26] which was subsequently O-demethylated to **14** with BBr_3. Alkylation of **14** with allyl bromide yielded **15**.

Pharmacology

The affinity of all the new compounds is tabulated in Table I together with the corresponding data for structurally related analogues which were evaluated concurrently. The D1 and D2 receptors were labeled with [^{3}H]SCH 23390 and [^{3}H]spiperone, respectively. All the ligands were evaluated for their ability to competitively displace the aforementioned radioligands from their respective receptor sites as described in our previous report.[23]

Discussion

The present study evaluates the effects of the substi-

(19) Ross, S. T.; Franz, R. G.; Wilson, J. W.; Brenner, M.; DeMarinis, R. M.; Hieble, J. P.; Sarau, H. M. Dopamine receptor agonists: 3-allyl-6-chloro-2,3,4,5-tetrahydro-1-(4-hydroxyphenyl)-1H-3-benzazepine-7,8-diol and a series of related 3-benzazepines. *J. Med. Chem.* **1986**, *29*, 733–740.

(20) Pfeiffer, F. R.; Wilson, J. W.; Weinstock, J.; Kuo, G. Y.; Chambers, P. A.; Holden, K. G.; Hahn, R. A.; Wardell, J. R.; Tobia, A. J.; Setler, P. E.; Sarau, H. M. Dopaminergic activity of substituted 6-chloro-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepines. *J. Med. Chem.* **1982**, *25*, 352–360.

(21) O'Boyle, K. M.; Waddington, J. L. New substituted 1-phenyl-3-benzazepine analogues of SKF 38393 and N-methyl-thienopyridine analogues of dihydroxynomifensine with selective affinity for the D1 dopamine receptor in human post-mortem brain. *Neuropharmacology* **1987**, *26*, 1807–1810.

(22) Arnt, J.; Hyttel, J.; Meier, E. Inactivation of dopamine D1 or D2 receptors differentially inhibits stereotypes induced by dopamine agonists in rats. *Eur. J. Pharmacol.* **1988**, *155*, 37–47.

(23) Neumeyer, J. L.; Baindur, N.; Niznik, H. B.; Guan, H. C.; Seeman, P. (±)-3-Allyl-6-bromo-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine, a new high affinity D1 dopamine receptor ligand: synthesis and structure–activity relationship. *J. Med. Chem.* **1991**, *34*, 3366–3371.

(24) Walter, L. F.; Chang, W.-K. Benzazepines. U.S. Patent 3393192, 1968.

(25) Weinstock, J. Trisubstituted 1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepines and their salts. SKF Co. Inc., U.S. Patent 4352754, 1982.

(26) Gold, E. H.; Chang, W.-K. 7-Chloro-8(substituted amino carbonyloxy)-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepines. Schering Co. Inc., U.S. Patent 4284555, 1981.

tutions at the 7-position and the 3-position of the 8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepines which possess binding affinity at DA D1 and D2 receptors with a high degree of selectivity for the D1 receptor. In particular, the present study examined the effect of a 3-allyl group vs a 3-CH_3 or 3-H group of various 7-substituted benzazepines. 3-Allyl-substituted benzazepine D1 agonists have been shown to retain high D1 receptor affinity, selectivity, and agonist activity. Extrapolation of these results to the benzazepine D1 antagonists suggests that 3-allyl substitution should also result in similar retention of D1 affinity, selectivity, and potency. Earlier work by the Schering group, however, had pointed out that lengthening of the 3-substituent to ethyl, propyl, butyl, or branched-chain analogues all lead to considerable loss of D1 affinity and selectivity. The present study shows that all the 3-allyl benzazepines possess activity comparable to their 3-methyl and 3-unsubstituted analogues. Actually, the affinity order for 3-substituted compounds is CH_3 > allyl > H. When substitution at the 7-position was examined, it was found that the 7-halogenated analogues possess affinity considerably higher than the corresponding 7-unsubstituted analogues. Thus, for 7-substitution, the affinity order is Cl = Br $\gg$ H. While the 3-methyl compounds have the highest affinity in this series, it must be remembered that the affinity values considered here are for the more active *R*-(+) isomers [i.e. IIa (*R*)-SCH 23390 and IIb (*R*)-SKF 83566]. On the other hand, the 3-allyl compounds examined in this study are all racemates. Thus, 3-allyl-7-bromo derivative **9** with an affinity of 0.9 nM may possess affinity even closer to that of the 7-chloro-3-methyl analogue IIa had its *R*-(+) enantiomer been synthesized and evaluated.

The 7-unsubstituted compounds, whatever the 3-substituent may be, show considerably lower affinity than the corresponding 7-halogenated compounds. It can thus be inferred that the 7-substituent plays an important role in conferring higher affinity on the parent compound.

For 3-substitution, while a 3-methyl group leads to a considerable enhancement of the D1 affinity when compared to the 3-unsubstituted compound, the deleterious effects observed upon substitution with groups larger than methyl probably arise due to steric occlusion in this region of the receptor. This explanation, however, fails to explain why the 3-allyl substitution leads to a retention of high D1 affinity. It is likely that any deleterious steric effect due to the allyl group is counterbalanced by some other favorable effect on the D1 binding affinity of the ligand. It is conceivable that the higher lipophilicity of the 3-allyl compound, e.g., compound **9**, may result in its in vivo potency by inducing CNS effects due to a greater capacity to cross the lipophilic blood–brain barrier. Hence **9** with an affinity close to that of IIa, but with higher lipophilicity, has the potential to be a potent in vivo CNS-active D1 antagonist.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were determined using a Varian XL-300 (300 MHz) NMR spectrometer, and the observed peaks were listed in ppm downfield from TMS (standard). The IR spectra were determined using a Perkin-Elmer 599B IR spectrophotometer, and the bands were listed in cm^{-1} using polystyrene film for calibration. The KBr pellet method was used for recording the IR spectra for all the compounds. Mass spectra were recorded by the electron-impact (EI) method using a Nuclide 12-90-G mass spectrometer at Boston College. The elemental analyses were carried out by Atlantic Microlab Co., Atlanta, GA, and were within ±0.4% of the theoretical values. TLC was carried out with 0.25-mm silica gel F-254 (E. Merck) plastic plates. Flash column chromatography was carried out with flash silica gel 7024-R (40-μm particle diameter, Baker).

α-[[*N*-(4-Methoxyphenethyl)amino]methyl]benzyl Alcohol (3). 4-Methoxyphenethylamine (1, 37 g, 0.245 mol) and styrene oxide (2, 29.5 g, 0.245 mol) were mixed and stirred at 90–95 °C for 16 h under N_2 atmosphere. The hot mixture was poured into 150 mL of a 3:1 mixture of hexane–EtOAc. The precipitated solid was filtered, washed with a further 150 mL of the 3:1 hexane–EtOAc mixture, washed with hexane, and finally dried in vacuo to yield 23 g of a white fluffy powder (35%): mp 94–96 °C (lit.[25] mp 95–96 °C). TLC: silica gel, CH_2Cl_2–MeOH 9:1, homogeneous spot at R_f 0.55.

8-Methoxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (4). α-[[*N*-(4-Methoxyphenethyl)amino]methyl]benzyl alcohol (3, 21.2 g, 0.08 mol) was dissolved in 240 mL of trifluoroacetic acid (TFA) under N_2 atmosphere was added dropwise 6 mL (0.12 mol) of concentrated H_2SO_4. The reaction mixture was stirred under reflux for 3 h. The reaction mixture was then cooled and concentrated in vacuo to remove most of the TFA. The residual mixture was poured onto 300 mL of ice–water. This solution was then made alkaline with cautious addition of a 40% aqueous NaOH solution. The alkaline mixture was extracted with EtOAc. The extract was washed with water and brine and then dried over anhydrous $MgSO_4$. The extract was filtered and concentrated in vacuo to a pale yellow oil. It was converted to the hydrochloride salt with EtOH·HCl and crystallized from EtOH–Et_2O to yield 16.8 g of a off-white crystalline powder (80%): mp 227–230 °C (lit.[25] mp 230–233 °C).

8-Hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine Hydrobromide (5). 8-Methoxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (4, 8.25 g, 0.03 mol) was mixed with 100 mL of 48% HBr. The solution was refluxed under N_2 atmosphere for 4 h. It was then allowed to stand at room temperature. The crystalline solid which separated out was filtered, washed with a little cold water, and dried in vacuo to yield 4.0 g of a tan crystalline solid (50%): mp 225–228 °C (lit.[25] mp 227–229 °C).

3-Allyl-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine Hydrochloride (6). 8-Hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (5, 0.96 g, 3 mmol) was dissolved in 50 mL of 90% EtOH solution. To this solution was added 0.42 g (3 mmol) of finely powdered anhydrous K_2CO_3. To the stirred mixture was then added 0.4 g (33 mmol) of allyl bromide dissolved in 5 mL of absolute EtOH. The mixture was stirred overnight at room temperature under N_2 atmosphere. The reaction mixture was then filtered, and the filtrate was concentrated in vacuo to a dark oil. The oil was purified by flash column chromatography (silica gel; CH_2Cl_2–MeOH, 5%). Fractions containing the pure product were pooled together and concentrated in vacuo to a pale yellow oil. The oily free base was converted to the hydrochloride salt with Et_2O–Et_2O·HCl. The hydrochloride salt was recrystallized from EtOH/Et_2O to obtain colorless crystalline powder. Yield: 0.32 g (40%), mp 245 °C. TLC: R_f = 0.35 (silica gel, CH_2Cl_2–MeOH 9.5:0.5). IR (KBr): 3350 (br, O–H stretch), 3100 (sh, intense aromatic C–H stretch), 2900 (sh, aliphatic C–H stretch), 1600 and 1570 cm^{-1} (allylic double bond stretch). ^{1}H NMR (CD_3OD): δ 7.45 (3 H, m, aryl *H*), 7.4 (1 H, d, 6-*H*), 7.25 (2 H, dd, aryl *H*), 7.1 (1 H, d, 7-*H*), 6.65 (1 H, s, 9-*H*), 6.0 (1 H, m, C*H*=CH_2), 5.6 (2 H, dd, =CH_2), 4.65 (1 H, d, 1-*H*), 3.9 (2 H, d, NCH_2). MS: m/z = 275 (M^+). Calc for $C_{19}H_{22}ClNO$: C, H, N.

7-Bromo-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine Hydrobromide (8). 8-Methoxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (6, 6.6 g, 0.023 mol) was dissolved in 30 mL of glacial AcOH. To the stirred solution at room temperature was added dropwise bromine (4.3 g, 0.027 mol). The reaction mixture was heated to 95 °C for 1 h. It was then cooled, and the precipitate was filtered. The precipitate was suspended in EtOAc and washed with a 10% aqueous $NaHCO_3$ solution. The organic layer was separated, washed with water and brine, dried over anhydrous $CaCl_2$, filtered, and concentrated in vacuo to a dark oil. Purification by flash chromatography (CH_2Cl_2–MeOH 2.5%) gave a brown oil which was dried in vacuo, yielding 4.3 g (57%) of the product 7-bromo-8-methoxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (7)[25] which was used as such for the subsequent step. ^{1}H NMR ($CDCl_3$): δ 7.35–7.05 (6 H, m, phenyl *H*, 6-*H*), 6.4 (1 H, s 9-*H*), 4.2 (1 H, d, 1-*H*), 3.65 (3 H, s, OCH_3).

Compound 7 (4.0 g, 12 mmol) was dissolved in 100 mL of dry CH_2Cl_2 and stirred under N_2 at −70 °C. To the stirred solution was added dropwise with vigorous stirring a solution of boron tribromide in hexane (1.0 M solution, 15.0 g, 60 mL, 60 mmol). After the addition was complete, the reaction mixture was stirred at −70 °C for 1 h and then at room temperature for 2 h. It was then quenched by again cooling to −70 °C under N_2 followed by the dropwise addition of 50 mL of anhydrous MeOH with vigorous stirring. The reaction mixture was concentrated in vacuo, and the residue was treated with an additional 50 mL of methanol and again concentrated in vacuo. This procedure was repeated twice more. Finally the residue was dried in vacuo over P_2O_5 overnight. Recrystallization from EtOH–Et_2O yielded 2.5 g of tan crystalline solid (60%): mp 235–236 °C. ^{1}H NMR (CD_3OD): δ 7.5–7.2 (6 H, m, phenyl *H*, 6-H), 6.35 (1 H, s, 9-*H*), 4.55 (1 H, dd, 1-*H*). MS: m/z = 317 (M^+). Calc for $C_{16}H_{17}Br_2NO$: C, H, N.

3-Allyl-7-bromo-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine Hydrochloride (9). 7-Bromo-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrobromide (8, 0.12 g, 0.3 mmol) was dissolved in 25 mL of a 90% EtOH solution. Anhydrous K_2CO_3 (63 mg, 0.45 mmol) was added to the above solution, and the mixture was stirred at room temperature. Allyl bromide (50 mg, 0.4 mmol) was added dropwise to the stirred mixture, and stirring was continued overnight at room temperature. The mixture was then filtered and concentrated in vacuo to a dark oil. Purification by flash chromatography (CH_2Cl_2–MeOH, 2.5%) gave the pure product as a yellow oil. The oily free base was converted to the hydrochloride salt with EtOH·HCl/Et_2O. Recrystallization from EtOH–Et_2O gave 50 mg of a colorless crystalline powder (42%): mp 155–157 °C. ^{1}H NMR (CD_3OD): δ 7.5–7.2 (6 H, m, phenyl *H*, 6-*H*), 6.2 (1 H, br s, 9-*H*), 6.05–5.9 (1 H, br, C*H*=), 5.65–5.55 (2 H, m =CH_2), 4.6 (1 H, dd, 1-*H*), 3.85 (2 H, d, NCH_2). MS: m/z = 357 (M^+). Calc for $C_{19}H_{21}BrClNO{\cdot}1.25H_2O$: C, H, N.

3-Chloro-4-methoxyphenethylamine (11). 3-Chloro-4-methoxyphenylacetonitrile (27 g, 0.15 mol) was dissolved in dry THF, and the solution was added dropwise with vigorous stirring under N_2 atmosphere to a solution of 1.0 M BH_3–THF (300 mL, 0.3 mol). The mixture was refluxed under N_2 for 2 h, and 50 mL of MeOH were slowly added. The mixture was again refluxed for 0.5 h and then concentrated in vacuo. The residue was dissolved in dilute HCl, and the solution was washed with Et_2O. The aqueous layer was made basic with a 40% aqueous NaOH solution and extracted with CH_2Cl_2. The extracts were washed with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo to a yellow oil which was distilled (105 °C, 0.45 mmHg) to obtain a colorless oil (18.5 g) (lit.[27] bp 140 °C, 0.6 mmHg).

2-[*N*-(3-Chloro-4-methoxyphenethyl)amino]-1-phenylethanol (12). 3-Chloro-4-methoxyphenethylamine (11, 21.0 g, 0.113 mol) and styrene oxide (2, 13.6 g, 0.113 mol) were dissolved in 75 mL of anhydrous CH_3CN. The mixture was stirred under reflux for 16 h in an atmosphere of N_2. It was then cooled and concentrated in vacuo, and the residual oil was triturated with anhydrous Et_2O. The precipitate was filtered, washed with anhydrous Et_2O until colorless, and dried in vacuo to yield 11 g of a white crystalline solid (32%): mp 94–96 °C (lit.[26] mp 95–96 °C). ^{1}H NMR ($CDCl_3$): δ 7.4–7.15 (5 H, m, phenyl *H*), 7.0 (1 H, dd, 6′-*H*), 6.85 (1 H, s, 2′-*H*), 6.85 (1 H, s, 2′-*H*), 4.7 (1 H, dd, 1-*H*), 3.85 (3 H, s, OCH_3).

7-Chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine Hydrobromide (14). 2-[*N*-(3-Chloro-4-methoxyphenethyl)amino]-1-phenylethanol (12, 5.0 g, 0.017 mol) was triturated with 50 g of PPA. The mixture was heated at 100 °C, and trituration was continued until all the solid dissolved into PPA. The mixture was heated for an additional 30 min. It was then diluted with water and poured on to ice–water. The resulting solution was basified with aqueous NH_4OH solution to a pH >8 and extracted with CH_2Cl_2. The extracts were washed with water and brine and dried over anhydrous $CaCl_2$. The dried extract was filtered and concentrated in vacuo to 3.0 g of the product 7-chloro-8-methoxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (13) as a pale yellow oil (65%). ^{1}H NMR ($CDCl_3$): δ 7.4–7.1 (6 H, m, phenyl *H*, 6-*H*), 6.45 (1 H, s, 9-*H*), 4.3 (1 H, dd, 1-*H*), 3.7 (3 H, s, NCH_3).

Compound 13 (3.0 g, 0.01 mol) was dissolved in 100 mL of dry CH_2Cl_2 and stirred under N_2 at −70 °C. To the stirred solution was added dropwise with vigorous stirring a solution of boron tribromide in hexane (1.0 M solution, 13.0 g, 52 mL, 0.054 mol). After the addition was complete, the reaction mixture was stirred at −70 °C for 1 h and then at room temperature for 2 h. The reaction was then quenched by again cooling to −70 °C under N_2 followed by the dropwise addition of 50 mL of anhydrous CH_3OH with vigorous stirring. The reaction mixture was concentrated in vacuo, and the residue was treated with an additional 50 mL of MeOH and again concentrated in vacuo. This procedure was repeated twice more. Finally the residue was dried in vacuo over P_2O_5 overnight. Recrystallization from EtOH–Et_2O yielded 1.4 g of a tan crystalline powder (40%): mp 215–217 °C. ^{1}H NMR (CD_3OD): δ 7.5–7.2 (6 H, m, phenyl *H*, 6-*H*), 6.3 (1 H, s, 9-H), 4.6 (1 H, dd, 1-*H*). MS: m/z = 273 (M^+). Calc for $C_{16}H_{17}BrClNO$: H, N, C: calcd, 54.18; found 54.75.

3-Allyl-7-chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (15). 7-Chloro-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (14, 0.11 g, 0.4 mmol) was dissolved in 50 mL of a 90% EtOH solution. To this solution was added 28 mg (0.20 mmol) of finely powdered anhydrous K_2CO_3. To the stirred mixture was then added 48 mg (0.40 mmol) of allyl bromide dissolved in 5 mL of absolute EtOH. The mixture was stirred overnight at room temperature under N_2 atmosphere. The reaction mixture was then filtered, and the filtrate was concentrated in vacuo to a dark oil. The oil was purified by flash column chromatography (silica gel; CH_2Cl_2–MeOH, 5%). Fractions containing the pure product were pooled together and concentrated in vacuo to a pale yellow oil. The oily free base was converted to the hydrochloride salt with Et_2O–Et_2O·HCl. The hydrochloride salt was recrystallized from EtOH–Et_2O to obtain a white crystalline powder, yielding 25 mg (20%), mp 140–142 °C dec. IR (KBr): 3400 (br, O–H stretch), 1600 cm^{-1} (sh, allylic double bond stretch). ^{1}H NMR ($CDCl_3$): δ 7.3 (3 H, m, aryl *H*), 7.15 (2 H, d, aryl *H*), 7.1 (1 H, s, 6-*H*), 6.2 (1 H, s, 9-*H*), 5.9 (1 H, m, C*H*=CH_2), 5.2 (2 H, m, =CH_2), 4.3 (1 H, d, 1-*H*). MS: m/z = 313 (M^+). Calc for $C_{19}H_{21}Cl_2NO$: C, H, N.

Pharmacology Methods. The compounds were tested for their potency at D1 receptors by their ability to inhibit the binding of [^{3}H]SCH 23390 to canine striatum in vitro. The canine striata were purchased from Pel-Freez (Rogers, AR) and kept at −70 °C until used. After thawing, the tissues were suspended at 3 mg of original wet weight per mL of buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$, 4 mM $MgCl_2$, 120 mM NaCl) and were homogenized in a glass homogenizer using a Teflon piston rotating at 650 rpm (8 up–down strokes). The homogenate was washed twice by centrifugation (28000*g* for 15 min) and resuspension. The tissue was finally resuspended at 3 mg wet weight per mL. Aliquots were added to the final incubation tubes (12 × 75 mm) as follows: 0.5 mL of buffer (containing 0.1% ascorbic acid and 10 μM nialamide; Research Biochemicals, Inc., Natick, MA), 0.5 mL of [^{3}H]SCH 23390 (75 Ci/mmol; New England Nuclear, du Pont de Nemours & Co., Boston, MA) (the final concentration of which was between 0.9 and 1 nM), and 0.5 mL of tissue suspension. After a 2-h incubation at room temperature (21 °C), the suspensions were filtered by a Titertek cell harvester (Skatron, Lier, Norway) through a Skatron filter 11734. The filters were rinsed with 7 mL over 15 s. Nonspecific binding was that observed in the presence of 1 μM (+)-butaclamol (RBI, Natick, MA). The radioactivity was monitored by a liquid scintillation spectrometer (Packard Instrument Co., Chicago).

The potencies of the compounds at D2 receptors were determined in the same way as for those at D1, except that pig anterior pituitary tissues were used (Bocknek Co., Mississauga, Ontario). The tissues were homogenized by a Polytron PT-10 (Brinkmann Co.) at setting 6 for 20 s. [^{3}H]Spiperone (87 Ci/mol) was used at a final concentration of 0.15 mM. The final tissue concentration during incubation was 4 mg original weight per mL, and non-

(27) Charifson, P. S.; Wyrick, S. D.; Hoffman, A. J.; Simmons, R. M.; Bowen, J. P.; McDougald, D. L.; Mailman, R. B. Synthesis and pharmacological characterization of 1-phenyl-, 4-phenyl-, and 1-benzyl-1,2,3,4-tetrahydroisoquinolines as dopamine receptor ligands. *J. Med. Chem.* 1988, *31*, 1941–1946.

specific binding was defined as that in the presence of either 1 μM (+)-butaclamol or 10 M S-sulpiride (RBI).

The dissociation constants, K_i values, were obtained by the program LIGAND (see refs 4 and 23) using a value of 0.17 nM as the dissociation constant for [^{3}H]SCH 23390 at D1 and a value of 0.06 nM as the dissociation constant for [^{3}H]spiperone at D2.

Registry No. 1, 55-81-2; 2, 96-09-3; 3, 137464-85-8; 4, 137566-51-9; 4·HCl, 137566-52-0; 5, 137464-86-9; 6, 137464-87-0; 6 free base, 137464-88-1; 7, 137566-53-1; 8, 137566-54-2; 9, 137464-89-2; 9 free base, 137464-90-5; 10, 7569-58-6; 11, 7569-87-1; 12, 84384-03-2; 13, 73445-60-0; 14, 137464-91-6; 15, 137464-92-7; 15 free base, 137464-93-8; allyl bromide, 106-95-6.

Synthesis and Biological Activity of New Dimers in the 7*H*-Pyrido[4,3-*c*]carbazole Antitumor Series

C. Garbay-Jaureguiberry, M. C. Barsi, A. Jacquemin-Sablon,† J. B. Le Pecq,‡ and B. P. Roques*

Département de Chimie Organique, U266 INSERM—UA498 CNRS, 4, avenue de l'Observatoire, 75270 Paris Cedex 06, France, Unité de Physicochimie Macromoléculaire, UA158 CNRS—U140 INSERM, Institut G. Roussy-Villejuif, France, and Rhône Poulenc Rorer 13, quai Jules Guesdes-94400 Vitry/Seine. *Received April 8, 1991*

Ditercalinium (NSC 366241) is a 7*H*-pyrido[4,3-*c*]carbazole dimer with a diethylbipiperidine rigid chain linking the two heterocyclic rings. Ditercalinium is characterized by a high DNA affinity and bisintercalating ability, associated with potent antitumor properties, involving an original mechanism of action. Unfortunately as ditercalinium is hepatotoxic, its clinical evaluation has been interrupted. In order to eliminate or at least minimize the serious drawbacks related to its toxic effects, several chemical modifications have been made to the structure of ditercalinium, and their influence has been evaluated by measuring the DNA affinities, intercalation properties, and toxicity toward leukemia cells of the newly synthesized dimers. Reduction of the pyridinic moieties of ditercalinium, in order to suppress the permanent charges provided by the quaternizing chain, led to an almost complete loss of activity, although the DNA bisintercalating property of the dimer was preserved. Dimerization of the 7*H*-pyrido[4,3-*c*]carbazole rings by introduction of the rigid spacer on the N_7- or C_6-positions corresponding to the convex face of the pyridocarbazole, instead of the N_2-position in ditercalinium, led to DNA bisintercalating dimers practically devoid of antitumor properties. However after quaternarization of the N_2 atoms, the dimer linked by the N_7 atoms exhibited a very high DNA affinity ($>10^9$ M^{-1}) and recovered antitumor activity, supporting the requirement of positive charges for the emergence of antitumor activity in these dimers. Introduction on the C_6 of the 7*H*-pyridocarbazole ring of an aminomethyl or carboxyl group, a sugar residue, or C or N free amino acids such as Lys or Glu has also been carried out, in order to increase the hydrophilic properties of the molecules or to enable them to use amino acid transport systems. Although some of these compounds were active, none of them exhibited the pharmacological potency of ditercalinium.

Introduction

Ditercalinium (NSC 366 241), a DNA bisintercalating 7*H*-pyrido[4,3-*c*]carbazole dimer,[1,2] behaves as a new type of antitumor drug characterized by an original mechanism of action.[3-9] It binds to DNA with high affinity and elicits antitumor activity on a variety of animal tumors. Ditercalinium has also been shown to be cytotoxic on *Escherichia coli pol*A mutants and not on *pol*A *uvr*A double mutants.[6] Since the dimer binds reversibly, it has been suggested that it could induce, in vivo, DNA conformational changes recognized by the *uvr*ABC repair system in *E. coli*. This feature could lead to a futile and abortive DNA repair process with subsequent cytotoxic effects.[6-8] Extensive NMR studies of the complexes formed between ditercalinium and autocomplementary oligonucleotides have shown that it bisintercalates into the major groove of the DNA helix.[10-12] This situation is rarely encountered for mono- or even bisintercalators, as illustrated with quinoxaline antibiotic dimers which were shown to have their spacer located in the minor groove.[13] Because of the rigidity of the linking chain of ditercalinium, the DNA conformation has to be altered to permit the intercalation of the two rings. NMR studies and theoretical calculations have shown that this can be achieved by a slight bending of the DNA toward the minor groove,[14] a result recently confirmed by X-ray analysis.[15]

As the nature of the complex formed between ditercalinium and DNA appears to play a crucial role in its biological activity, modifications of the structure of the dimer have been extensively investigated.[16-20] Thus, displacement of the nitrogen from position 2 in the 7*H*-

* To whom reprint request should be addressed.
† Unité de Physicochimie Macromoléculaire.
‡ Rhône Poulenc Rorer.

(1) Roques, B. P.; Le Guen, L.; Porcher, G.; Goss, C.; Le Pecq, J. B. DNA bifunctional intercalators: Antileukemic activity of new pyridocarbazole dimers. *Biochem. Pharmacol.* **1979**, *28*, 1811-1815.
(2) Pelaprat, D.; Delbarre, A.; Le Guen, I.; Roques, B. P.; Le Pecq, J. B. DNA intercalating compounds as potential antitumor agents: II. Preparation and properties of 7*H*-pyridocarbazole dimers. *J. Med. Chem.* **1980**, *23*, 1336-1343.
(3) Esnault, C.; Roques, B. P.; Jacquemin-Sablon, A.; Le Pecq, J. B. Effects of new antitumor bifunctional intercalators derived from 7*H*-pyridocarbazole on sensitive and resistant L1210 cells. *Cancer Res.* **1984**, *44*, 4335-4360.
(4) Markovits, J.; Pommier, Y.; Mattern, M. R.; Roques, B. P.; Le Pecq, J. B.; Kohn, K. W. Effects of the bifunctional antitumor intercalator ditercalinium on DNA in mouse leukemia L1210 cells and DNA topoisomerase II. *Cancer Res.* **1986**, *46*, 5821-5826.
(5) Esnault, C.; Lambert, B.; Markovits, J.; Segal-Bendirdjian, E.; Muzard, G.; Garbay-Jaureguiberry, C.; Roques, B. P.; Le Pecq, J. B. Mechanisms of the antitumor action of ellipticines, isoellipticines and analogues: Comparison of mono and bifunctional intercalators. In *Molecular Mechanisms of Carcinogenic and Antitumor Activity*; Chagas, C., Pullman, B., Eds.; Adenine Press: Schenectady, NY, 1987; pp 295-316.
(6) Lambert, B.; Roques, B. P.; Le Pecq, J. B. Induction of an abortive and futile DNA repair process in *E. coli* by the antitumor DNA bifunctional intercalator ditercalinium: Role of *pol*A in death induction. *Nucleic Acid Res.* **1988**, *16*, 1063-1078.
(7) Lambert, B.; Roques, B. P.; Jones, A.; Le Pecq, J. B.; Yeung, T. The non-covalent complex between DNA and the bifunctional intercalator ditercalinium is a substrate for the *uvr*ABC endonuclease of *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S.A.* **1989**, *86*, 6557-6561.

0022-2623/92/1835-0072$03.00/0