the bath in three to five different concentrations. The drug-induced contraction was checked for atropine antagonism. In antagonistic studies muscle contractions were induced by acetylcholine (0.22 μM). Comparisons were made between the acetylcholine-induced contractions before and 3 min after addition of the test drug in three to five different concentrations. EC_{50} values and IC_{50} values were calculated by log probit analysis.

Inhibition of [^{3}H]PrBCM Binding to Rat Brain Homogenates. Rat brain homogenates were prepared as described earlier in detail.[19] Whole brain minus cerebellum of a rat was homogenized in 10 vol ice-cold 0.32 M sucrose, pH 7.4. The homogenate was centrifuged at 600*g* for 10 min and the supernatant at 25000*g* for 55 min at 4 °C with rehomogenization of the pellet in 0.32 M sucrose. Incubation tubes in triplicate received at 30 °C test substance and tissue suspension. After 10 min of incubation, ligand (New England Nuclear, Boston, MA; 28–44 Ci/mmol) was added (final concentration of [^{3}H]PrBCM: 1.5 nM). After 15 min of incubation, the reaction was stopped by addition of sodium thiosulfate. The samples were filtered through Whatman GF/B filters (25 mm). The tubes and filters were rinsed twice with buffer. Nonspecific binding was determined in the presence of 20 μM atropine. IC_{50} values were calculated by log probit analysis.

Acknowledgment. This work was supported by a grant from the Danish Medical Research council. The assistance of Dr. Eddi Meier in some of the binding studies is gratefully acknowledged. The technical assistance of U. Geneser, T. Lindgreen, and J. S. Johansen and the secretarial assistance of B. Hare are gratefully acknowledged.

Registry No. 1, 53601-98-2; **2a**, 95628-20-9; **2b**, 101697-33-0; **2c**, 101697-34-1; **2d**, 101697-35-2; **2e**, 101697-36-3; **2f**, 101697-37-4; **2g**, 101697-38-5; **2h**, 101697-39-6; **2i**, 101697-40-9; **3a**, 82988-64-5; **3b**, 95597-29-8; **3c**, 95597-30-1; **3d**, 95597-40-3; **3e**, 95597-36-7; **3f**, 95597-42-5; **3g**, 95597-38-9; **3h**, 95597-44-7; **3i**, 95597-31-2; 4, 53601-99-3; **5**, 95579-09-2; **6j**, 95579-10-5; **6k**, 101697-41-0; **6l**, 101697-42-1; **7j**, 101697-43-2; **7k**, 101697-44-3; **7l**, 101697-45-4; **8**, 101697-46-5; **9**, 101697-47-6; **10a**, 82988-65-6; **10a** (free base), 95579-17-2; **10b**, 95597-32-3; **10b** (free base), 95579-18-3; **10c**, 95597-33-4; **10c** (free base), 95579-19-4; **10d**, 95597-41-4; **10d** (free base), 95597-21-0; **10e**, 95597-37-8; **10e** (free base), 95579-20-7; **10f**, 95597-43-6; **10f** (free base), 95597-22-1; **10g**, 95597-39-0; **10g** (free base), 95597-23-2; **10h**, 95597-45-8; **10h** (free base), 95628-19-6; **10i**, 95597-34-5; **10i** (free base), 95579-21-8; **10j**, 95579-11-6; **10j** (free base), 95578-97-5; **10k**, 101697-48-7; **10k** (free base), 101697-50-1; **10l**, 101697-49-8; **10l** (free base), 101697-51-2; 11 (free base) (R^1 = Me, R^2 = Ac), 95597-27-6; **11a**, 95597-35-6; **11b**, 95578-99-7; **11c**, 95597-28-7; **11d**, 95579-08-1.

Supplementary Material Available: ^{1}H NMR spectral data for the 3-alkoxyisoxazoles **3a–i** and **6j-l** (Table Is) and for the isomeric 2-alkyl-4-isoxazolin-3-ones **2a–i** and **7l–l** (Table I's) (2 pages). Ordering information is given on any current masthead page.

Indole–Phenol Bioisosterism. Synthesis and Antihypertensive Activity of a Pyrrolo Analogue of Labetalol

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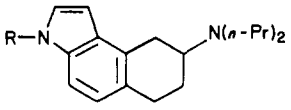
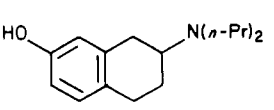
The synthesis of 5-[hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]-1*H*-indole-7-carboxamide, **5**, a pyrrolo analogue of labetalol, is described. Compound **5** was found to reduce blood pressure in spontaneously hypertensive rats with an ED_{50} of 5 mg/kg po, without causing any decrease in heart rate. Isolated tissue studies with **5** shows that it is a nonselective β-adrenoceptor antagonist and that it is a weaker α-adrenoceptor antagonist with a relative selectivity for α_1-receptors. Additionally, the compound displayed significant β-adrenoceptor intrinsic sympathomimetic activity. Evidence is presented that the β-adrenoceptor antagonist and agonist properties of **5** are mediated via hydrogen-bond formation with the receptor.

We have recently demonstrated[1] the bioisosterism of the pyrrolo ring and the phenolic hydroxyl group at the dopamine receptor. Thus, the benz[*e*]indole **1**, a pyrrolo analogue of the dopaminergic agonist **3**,[2–4] was found to display potent dopaminergic properties, to be orally active, and to have a longer duration of action than the phenol **3**. The bioisosterism of the pyrrolo ring and the phenolic hydroxyl group was ascribed to the ability of both groups to function as hydrogen-bond donors to a common acceptor nucleus on the dopamine receptor macromolecule, and evidence supporting this contention derived from the inactivity of the *N*-methylpyrrolo analogue **2**.[1]



1, R = H
2, R = CH_3



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To examine the generality of the "bioisofunctionality" of the pyrrolo ring and the phenolic hydroxyl group, we were attracted to the structure of labetalol (**4**),[5] an agent that is an antagonist at both α- and β-adrenergic receptors[6,7] and thus has a mechanism of action that is unique among clinically used antihypertensive agents. This paper describes the synthesis and pharmacological evaluation of 5-[hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]-1*H*-indole-7-carboxamide, **5** (AY-28,925), the pyrrolo analogue of labetalol, and **6**, the *N*-methylpyrrolo analogue.

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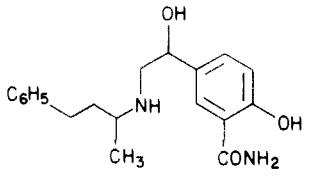

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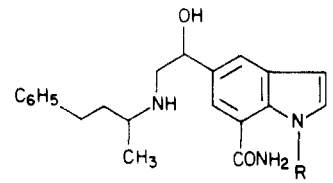


5, R=H
6, R=CH_3

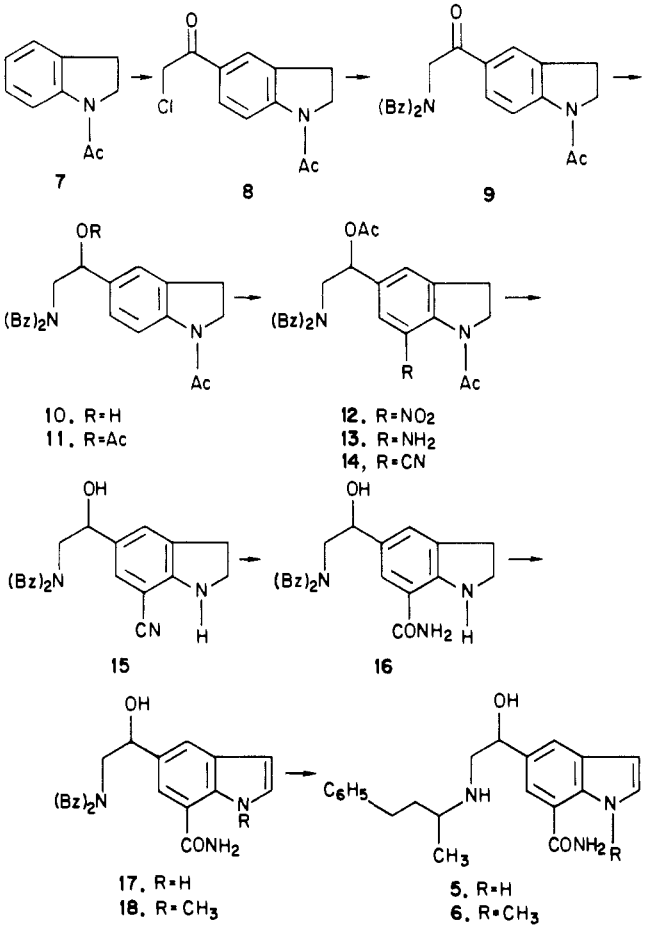
Chemistry. Examination of the structure of the target molecules **5** and **6** suggested that 1-acetylindoline would serve as a convenient starting material as it is the equivalent of an indole with positions 1, 2, and 3 protected. Moreover, it is a good substrate for Friedel–Crafts reactions, and electrophilic aromatic substitutions should occur at the required positions 5 and 7. The strategy adopted for the preparation of the target compounds must also take into account the presence of two assymetric centers. To avoid working with mixtures of diastereoisomers, the introduction of the phenylbutyl side chain bearing the second assymetric center was planned as one of the last steps.

Discrete steps in the synthesis of **5** and **6** are illustrated in Scheme I. Substitution at position 5 of indoline was effected by using a modification of the method described by Terent'ev et al.[8] Thus, the condensation of 1-acetylindoline (**7**) with chloroacetyl chloride in 1,2-dichloroethane, instead of the toxic and flammable carbon disulfide,[8] afforded a 93% yield of the 5-chloroacetyl derivative **8**. Upon reaction with dibenzylamine in dimethylformamide, the dibenzylamino ketone **9** was obtained, and reduction with sodium borohydride in ethanol gave the aminoethanol derivative **10**.

Functionalization of position 7 required protection of the benzylic alcohol as nitration of the aminoethanol derivative **10** afforded a low yield of the desired product, with 1-acetyl-5-nitroindoline being the major side product. When the benzylic hydroxyl of **10** was first acetylated with acetic anhydride in pyridine, the nitration of the diacetyl derivative **11**, with 90% nitric acid in glacial acetic acid, gave a 85.9% yield of the 7-nitro derivative **12**. A clean reduction of the nitro group to amine **13** was achieved by using either the dodecacarbonyltriiron–methanol method[9] or zinc in acetic acid.[10] The conversion of the amine to nitrile **14** via the diazonium chloride salt under a variety of conditions gave the desired product in low yield. Better results were obtained when the more stable diazonium fluoborate salt was used in this reaction. Treatment of the diazonium chloride salt in situ with fluoboric acid precipitated out the fluoborate salt, which could then be isolated and reacted further with a solution of copper(I) cyanide and potassium cyanide in dimethyl sulfoxide[11] to afford a good yield of nitrile **14**.

Removal of the acetyl groups by treatment with sodium hydroxide in ethanol and water at room temperature afforded the 7-cyanoindoline **15**. Hydrolysis of the cyano group to produce the carboxamide derivative **16** was effected by adding sodium hydroxide to a solution of **15** in dimethyl sulfoxide and 30% aqueous hydrogen peroxide. Dehydrogenation of the indoline ring using activated manganese(IV) oxide led to the indole-7-carboxamide **17**. This compound was debenzylated by catalytic hydrogen-

Scheme I



olysis; reductive alkylation of the resulting primary amine with 4-phenyl-2-butanone in the presence of sodium cyanoborohydride yielded the desired product **5**. It was purified by flash chromatography[12] and was obtained as a white foam shown by HPLC to be a mixture of two diastereoisomers. A solid form of this compound was prepared by precipitation of its hydrochloride salt. A 15.4% overall yield of compound **5**·HCl was obtained from commercially available indoline in 12 steps.

The *N*-methylpyrrolo analogue **6** was obtained by methylating the indolic nitrogen of **17** with methyl iodide and sodium hydride in dimethylformamide, followed by treatment in methanol with 4-phenyl-2-butanone in the presence of hydrogen, platinum oxide, and palladium on charcoal.

Results and Discussion

The data in Table I show that compound **5** and labetalol produced dose-related decreases in mean arterial blood pressure (MABP). The maximal effects occurred between 45 and 60 min after drug administration. The ED_{30} (i.e., the dose that produced a 30% decrease in MABP) was calculated to be 5 mg/kg po for **5** and 25 mg/kg po for labetalol, thus making **5** more potent than labetalol by a factor of 5 after oral administration. The change in MABP seen after the administration of compound **6** (at 50 mg/kg po) was not significantly different from that seen after administration of the control vehicle.

Dose-related decreases in heart rate (HR) were also produced by both **5** and labetalol (Table I). However, the change in HR induced by **5** at the antihypertensive ED_{30} (5 mg/kg) was not significantly different from that of the

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Table I. Maximal Change in Mean Arterial Blood Pressure (MABP) and Heart Rate (HR) Induced by Oral Administration of 5·HCl, 6, Labetalol Hydrochloride, or the Control Vehicle (0.5% Methylcellulose) to SHR

			mean arterial blood pressure		heart rate	
compound	dose, mg/kg po	n	pretreat.,[a] mmHg	max % change from pretreat.	pretreat.,[a] beats/min	max % change from pretreat.
5	1	8	171 ± 2	−9	442 ± 5	−3
	2.5	8	176 ± 7	−25[b]	419 ± 11	−10
	5	8	176 ± 3	−32[b]	439 ± 9	−12
	10	7	166 ± 4	−40[b]	430 ± 7	−15
	25	8	183 ± 6	−47[b]	426 ± 6	−18[b]
	100	8	178 ± 5	−45[b]	425 ± 6	−26[b]
6	50	4	166 ± 1	−13	411 ± 17	−15
labetalol	5	8	161 ± 3	−14	424 ± 12	−15[c]
	10	8	158 ± 3	−25[b]	457 ± 6	−23[b]
	25	8	163 ± 2	−31[b]	437 ± 6	−27[b]
control	0	8	162 ± 3	−8	465 ± 7	−4

[a] Pretreatment values are means ± SEM. [b] $p < 0.01$ when compared to control group using Dunnetts "t" test for multiple comparisons. [c] $p < 0.05$.

Table II. In Vitro Antagonist Activity of 5, Labetalol, and 6

	receptor pA_2 values[a]			
compound	β_1	β_2	α_1	α_2[d]
5	7.4 ± 0.1[b] (18)	7.3 ± 0.2 (4)	6.6 ± 0.1 (5)	5.4 ± 0.2 (6)
labetalol	7.5 ± 0.1[c] (6)	7.4 ± 0.1[c] (4)	6.8 ± 0.1[c] (3)	<5.0 (4)
6	<6.0[e] (3)	<5.0[e] (3)	6.2 ± 0.04[e] (3)	<5.0 (3)

[a] Data are expressed as pA_2 ± SEM from multiple observations from the number of tissues indicated in parentheses. [b] $pA_2 = -\log K_p$. K_p was derived by using the method of Kaumann and Blinks.[18] [c] pA_2 values ($-\log K_B$) derived from studies using single concentrations of labetalol, β_1, 1×10^{-7} M, β_2, 1×10^{-6} M, α_1, 3×10^{-6} M. [d] All α_2 pA_2 values ($-\log K_B$) derived from studies using two concentrations of test compound, 1×10^{-6} and 1×10^{-5} M. [e] pA_2 values ($-\log K_B$) derived from studies using the following concentrations of 6: β_1, 1×10^{-6} M; β_2, 1×10^{-6} and 1×10^{-5} M; α_1, 1×10^{-5} M.

Table III. Intrinsic Sympathomimetic Activity of 5, Labetalol, and 6

	β_1-ISA[a]			β_2-ISA[b]		
compound	% change[c]	n	concn M	% relaxation[d]	n	concn, M
5	17.0 ± 2.4[e]	5	1×10^{-6}	89.0 ± 6.1[f]	5	3×10^{-7}
labetalol	0[g]	3	1×10^{-7}	62.0 ± 5.1[f]	4	1×10^{-5}
6	2.7 ± 1.4	3	1×10^{-6}	34.6 ± 5.3[h]	4	1×10^{-5}

[a] Positive chronotropic effect on guinea pig right atria. [b] Relaxant effect against a $PGF_{2\alpha}$-contracted guinea pig trachea. [c] Percentage increase from basal spontaneous beating rate. [d] Relative to maximum relaxation induced by salbutamol (1×10^{-6} M). [e] Equivalent to 18.6% of maximum response to isoproterenol (1×10^{-6} M). Inhibited by propranolol (1×10^{-7} M). [f] Inhibited by ICI-118,551 (3×10^{-6} M). [g] Actual negative chronotropic effect of −7.1 ± 2.6%. [h] Not inhibited by ICI-118,551 (3×10^{-6} M).

control group. Therefore, at equivalent antihypertensive doses labetalol displayed more negative chronotropic activity than 5. In contrast, when compared to the control group, compound 6 produced a nonsignificant change in HR.

In isolated tissue studies designed to quantify α- and β-adrenoceptor antagonist activity, 5 produced a parallel shift of the appropriate specific agonist concentration–response curves with no depression of the maximum response. The derived pA_2 values (Table II) indicate that 5 like labetalol, possesses nonselective β-adrenoceptor antagonist activity, and in addition a lesser degree of selective α_1-adrenoceptor antagonist activity. In contrast, compound 6, the methyl analogue of 5, was essentially inactive at β-adrenoceptors and displayed only a low degree of α_1-antagonist activity (Table II). Studies to determine β-adrenoceptor ISA (intrinsic sympathomimetic activity) using the guinea pig right atrial and tracheal preparations indicated that 5 displayed significant β_1- and β_2-ISA (Table III). In contrast, labetalol had no β_1-ISA and displayed a degree of apparent β_2-ISA that was significantly less than that seen with 5. Unlike 5 or labetalol, compound 6 displayed no ISA at either β_1- or β_2-adrenoceptors.

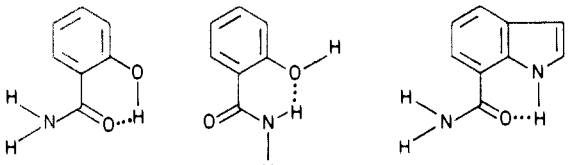
The present finding, that 5, like labetalol, is an antihypertensive agent that apparently acts by blocking both α- and β-adrenergic receptors, provides further support for the concept of indole–phenol bioisosterism. Previously, we demonstrated indole–phenol bioisosterism in the field of dopamine receptor agonists,[1] where this property was ascribed to the ability of the phenolic hydroxyl group and the pyrrole NH group to participate in hydrogen-bond formation with a common acceptor nucleus on the dopamine receptor macromolecule. In the present study we invoke a similar rationalization for the like antihypertensive activities of 5 and labetalol. Support for this contention derives from the virtual lack of activity of 6, the analogue of 5 wherein the indolic nitrogen is methylated, thereby rendering it incapable of participating in hydrogen bond formation.

A comparison of the pA_2 values for 5 and 6 (Tables II and III) indicates that while hydrogen-bond formation appears to be a critical factor in eliciting both antagonist and agonist effects at β-adrenergic receptors, the blockade of α-receptors does not appear to be dependent on hydrogen-bond formation. These observations suggest that the phenolic hydroxyl group of labetalol may similarly, and selectively, mediate the blockade of β-receptors.

While 5 and labetalol are both antihypertensive agents with similar pharmacological profiles, there are some differences between the compounds. Compound 5 is more potent than labetalol after po administration and has less negative chronotropic activity than labetalol at equieffective antihypertensive doses (i.e., ED_{30} doses). In contrast to labetalol, 5 displays significant β_1-ISA, and it is this property that is likely responsible for the relative lack of negative chronotropic activity seen with 5.

There are fundamental differences between a phenol and an indole that could be responsible for the different activities cited above between **5** and labetalol. For example, **5** was found to have a partition coefficient of 46.6 measured in a 1-octanol–buffer system at pH 7.4, while labetalol had a value of 18.2. The markedly different lipophilicities could result in different tissue/organ distributions for the two compounds. Other physicochemical differences could also play a role in eliciting the different activity profiles. For example, phenol is acidic with a pK_a of ~10 whereas indole is neutral or slightly basic.

The identity of the acceptor nucleus with which the indolic nitrogen of **5** and the phenolic hydroxyl group of labetalol would form a hydrogen bond is uncertain. Salicylamides can theoretically exist in two intramolecular hydrogen-bonded states, **19** and **20**. A search of the



19 **20** **21**

Cambridge Crystallographic data base was conducted, and of the 13 salicylamides found, all have hydrogen-bonded structures of type **19** in which the average distance between the carbonyl oxygen and the phenolic oxygen was 2.433 Å (2.377–2.719 Å). This distance is within the range typical for O–H···O hydrogen bonds.[13] It is thus likely that labetalol has an intramolecular hydrogen-bonded structure of type **19** rather than **20** and that **5** has a structure of type **21**. On interaction of these compounds with the adrenergic receptor, these hydrogen-bonded structures may be maintained or, in a concerted process involving recognition of other features of the molecules by the receptor, they may be replaced by intermolecular hydrogen bonds with an acceptor nucleus in the receptor. The intramolecular hydrogen bonds in salicylamides deviate significantly from linearity; the average of the angles formed by O–H···O (see structure **19**) in nine of the salicylamides obtained from the Cambridge Crystallographic data base is 153.09° (130.72–171.00°). If a more stable linear hydrogen bond could be formed with an acceptor nucleus in the receptor, this could be the driving force for the formation of an intermolecular hydrogen bond with a receptor-based acceptor nucleus.

In conclusion, the results described above suggest that indole–phenol bioisosterism is not restricted to events involving only the dopamine receptor but suggest that the β-adrenergic receptor is also unable to distinguish between the hydrogen-bond donor capacities of these groups. Hydrogen bonding is one of the more important forces contributing to drug–receptor interactions, and the concept of indole–phenol bioisosterism may lead to the design of novel receptor ligands with unique pharmacokinetic and pharmacodynamic properties.

Experimental Section

Pharmacology. In Vitro Studies. Tissues that were appropriate to each pharmacological test were isolated and suspended in 10-mL organ baths containing Krebs–Henseleit solution maintained at 37 °C and aerated with 95% O_2/5% CO_2. Antagonist activity was assessed by observing the ability of various concentrations of test compounds to modify a cumulative concentration–response curve to a specific agonist. β_1-Antagonist activity was assessed against the positive chronotropic response to isoproterenol in the guinea pig right atrium;[14] β_2-antagonist activity against the vasorelaxant response to isoproterenol in the $PGF_{2\alpha}$-contracted canine saphenous vein;[15] α_1-antagonist activity against the vasoconstrictor response to phenylephrine in the rabbit aorta[16] and α_2-antagonist activity against the inhibitory effect of clonidine on the electrically stimulated rat vas deferens.[17] Antagonist activity was quantified by using the methods of Kaumann and Blinks,[18] Arunlakshana and Schild,[19] and Furchgott.[20] β-Intrinsic sympathomimetic activity (ISA) was demonstrated by cumulative addition of test compounds to isolated guinea pig right atria (for determination of β_1-ISA) and assessing resultant positive chronotropic activity and $PGF_{2\alpha}$-contracted guinea pig trachea (for determination of β_2-ISA) and assessing tracheal relaxant activity. Confirmation of specific ISA was achieved by subsequent blockade by either propranolol or ICI-118,551.

Assessment of Antihypertensive Activity in SHR. Male SHR (240–400 g) obtained from Charles River (Lakeview, MA) or Taconic Farms (Germantown, NY) were used in these studies. Each rat was anesthetized with halothane and the left femoral artery cannulated with polyethylene tubing (o.d., 0.038 in.; i.d., 0.023 in.). The animals were wrapped in rubber mesh jackets, suspended from horizontal bars with towel clamps, and allowed to recover from the anesthesia. The arterial cannula was connected to a Gould Statham pressure transducer (Model P23), which in turn was attached to a polygraph to record mean arterial blood pressure (MABP) and heart rate (HR). All test compounds were dissolved or dispersed in a solution of 0.5% methylcellulose and prepared for oral injection in a volume of 5 mL/kg of body wt. Doses tested ranged from 1 to 100 mg/kg and at each dose tested a minimum of four and a maximum of eight animals was used. After a 1-h equilibration period the test compound was given by gastric gavage and then MABP and HR were monitored for 4 h. The maximal changes seen after drug treatment were compared to those seen after the control vehicle by use of Dunnett's t test for multiple comparisons.

Chemistry. Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. IR spectra were taken on a Perkin-Elmer 225 spectrophotometer. ^1H NMR spectra were determined in the indicated solvent on a Varian CFT-20 instrument with tetramethylsilane as internal standard. Chemical shifts are given in δ units, and coupling constants are in hertz. Splitting patterns are designated as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Mass spectra were obtained on an LKB-9000 S spectrometer and ultraviolet spectra on a Zeiss DMR-21 spectrophotometer. CHN were measured on a Perkin-Elmer 240 analyzer. Silica gel 60 F-254 (Merck) was used for thin-layer chromatography (TLC). For flash column chromatography,[12] silica gel 60 (400–230 mesh, E. Merck No. 9384) was used.

1-Acetyl-5-(chloroacetyl)indoline (8). In a 2-L, three-necked flask equipped with a mechanical stirrer, a dropping funnel, and a thermometer are placed 200 mL of 1,2-dichloroethane and 93.3 g (0.70 mol) of aluminum chloride. To the stirred mixture, which is under N_2 and cooled to 15–20 °C, is added 23 mL (33.5 g, 0.29 mol) of chloroacetyl chloride in one lot and stirring continues for 20 min. To the above solution is added a solution of 39 g (0.24 mol) of 1-acetylindoline in 100 mL of 1,2-dichloroethane over a period of 20 min; the reaction temperature is in the range of 20–30 °C during the addition. When the addition is complete, the mixture is stirred at 50 °C for 1 h. TLC of a workup sample indicates no starting material (Et_3N/EtOAc/hexane = 15:30:55).

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Then it is cooled to 15 °C and added portionwise over a period of 10 min to a stirred mixture of ice (500 g) and water (500 mL); the temperature of the quenched mixture is below 20 °C during the addition. Stirring continues for 30 min, and the mixture is filtered. The wet solid is washed with heptane (200 mL) and then thoroughly with water (200 mL × 4). The pH of the aqueous phase of the last washing is 6.6. The solid is dried at 70 °C under reduced pressure until constant weight. It affords 54.31 g (93%) of the product 8 as an off-white solid, mp 232.5–234 °C dec (lit. mp 230–231 °C dec).[8]

1-(1-Acetyl-2,3-dihydro-1*H*-indol-5-yl)-2-[bis(phenylmethyl)amino]ethanone (9). To a solution of 1-acetyl-5-(chloroacetyl)indoline (8; 209 g, 0.871 mol) in *N,N*-dimethylformamide (1045 mL) is added 514 g (2.61 mol) of dibenzylamine in one lot. The mixture is stirred at 60 °C under N_2 for 2 h and then cooled to 20–25 °C. Cold water (1050 mL) is added over a period of 10 min, and the mixture is stirred at room temperature for 1 h. The aqueous layer is discarded and the residual solid is stirred twice with water (1.5 L × 2) at room temperature for 30 min each time and then the aqueous phases are discarded.

The residual light brown solid is then stirred with 1.5 L of heptane at 80 °C for 30 min, and the heptane layer is decanted while hot and discarded. The product is restirred once with heptane (1.5 L) at 80 °C for 30 min, the mixture is filtered, and the solid is washed with an additional 500 mL of warm heptane and dried under reduced pressure overnight. This solid (452.3 g), which still contains water, was used as such for the subsequent reaction. A sample was recrystallized from hot ethyl acetate to give a white solid: mp 130–130.5 °C; IR ($CHCl_3$) 1660 cm^{-1} (carbonyls); ^1H NMR ($CDCl_3$) δ 2.2 (s, 3 H), 3.1 (t, 2 H, J = 8), 3.74 (s, 4 H), 3.76 (s, 2 H), 4.05 (t, 2 H, J = 8). Anal. ($C_{28}H_{26}N_2O_2$) C, H, N.

1-Acetyl-2,3-dihydro-α-[[bis(phenylmethyl)amino]methyl]-1*H*-indole-5-methanol (10). The above ketone 9 (452.3 g) is suspended in 2712 mL of ethanol and cooled to 5 °C. To this cold suspension is added portionwise $NaBH_4$ (38 g, 1 mol) over a period of 20 min; the reaction temperature is at 5–10 °C during the addition. When the addition is complete, stirring continues at room temperature for 4.5 h and then at 50 °C for 1 h. TLC (Et_3N/EtOAc/hexane = 1:3:6) of a workup sample indicates the completion of the reaction. The mixture is cooled to 5 °C and cold water (2.7 L) is added while the mixture is stirred over a period of 10 min. When the addition is complete, the mixture is stirred at 5–10 °C for 1 h. The solid is filtered and washed thoroughly with water (1.5 L × 4); the pH of the last wash is 8.30. The solid product is dried at 25 °C in vacuo to yield 292.89 g of product 10 as beige fine crystals: mp 117–118.5 °C (the overall yield from chloromethyl ketone 8 to 10 is 84%); IR ($CHCl_3$) 3440 (OH), 1650 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.15 (s, 3 H), 2.57 (d, J = 7, 2 H), 3.05 (t, J = 8, 2 H), 3.42 (d, J = 13, 2 H), 3.85 (d, J = 13, 2 H), 3.95 (t, J = 8, 2 H), 4.6 (t, J = 7, 1 H). Anal. ($C_{26}H_{28}N_2O_2$) C, H, N.

1-Acetyl-α-[[bis[(phenylmethyl)amino]methyl]-2,3-dihydro-1*H*-indole-5-methanol, Acetate Ester (11). A mixture of hydroxyl compound 10 (225 g, 0.56 mol) in pyridine (225 mL) and acetic anhydride (337.5 mL) is stirred at room temperature overnight. The resultant mixture is evaporated under reduced pressure, and the residual liquid is dissolved in 1 L of ethyl acetate. After the mixture is stirred with 500 mL of cold water for 30 min, the organic phase is separated and washed four times with cold water (500 mL × 4) and then with saturated $NaHCO_3$ solution (500 mL). The organic layer is further washed with water (500 mL × 3), dried over $MgSO_4$, and evaporated to afford 234.93 g of product 11 as a brown syrup (94.5% yield. A sample was crystallized from ether to give an off-white solid: mp 91–92 °C; IR ($CHCl_3$) 1728, 1655 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 2.01 (s, 3 H), 2.18 (s, 3 H), 2.77 (m, 2 H), 3.06 (t, J = 9, 2 H), 3.59 (s, 4 H), 4.00 (t, J = 9, 2 H), 5.81 (t, J = 6.5, 1 H). Anal. ($C_{28}H_{30}N_2O_3$) C, H, N.

1-Acetyl-α-[[bis(phenylmethyl)amino]methyl]-2,3-dihydro-7-nitro-1*H*-indole-5-methanol, Acetate Ester (12). In a 3-L, three-necked, round-bottomed flask equipped with a mechanical stirrer, a thermometer, and a dropping funnel is placed a solution of compound 11 (234.93 g from the previous reaction) in acetic acid (1175 mL), and the solution is stirred and cooled to 15 °C under nitrogen. To this cold solution is added dropwise 258 mL of 90% nitric acid over a period of 35 min; the reaction temperature during the addition is in the range of 14–16 °C. When the addition is complete, the mixture is stirred at room temperature for 6 h, then cooled to 10 °C, and poured slowly with stirring into a cold mixture (precooled to 5 °C) of water (2350 mL) and methylene chloride (1500 mL) over a period of 20 min. When the addition is complete, stirring continues an additional 30 min.

The organic phase is separated and washed three times with water (3 × 2 L) and then with 1 L of saturated $NaHCO_3$ solution. The organic layer is further washed with water (1 L × 3), dried over $MgSO_4$, and filtered. The brown filtrate is stirred with 23 g of Darco KB at refluxing temperature for 30 min and the mixture is cooled to room temperature and filtered through Celite. The brown filtrate is evaporated to afford 241.81 g of crude product as a light brown gum. It is dissolved in 482 mL of warm acetone (50 °C), and 1.1 L of heptane is added portionwise. The mixture is warmed to 60 °C for 10 min, then gradually cooled to 5 °C, and stirred for 1 h. The crystalline solid is filtered and dried at 25 °C in vacuo overnight. It affords 206.35 g of product as yellow crystals, mp 109.5–110.5 °C.

The mother liquid is concentrated to give 30.49 g of a brown gum, which is dissolved in 150 mL of ethanol at 80 °C. This solution is gradually cooled to room temperature and stirred at this temperature overnight. The solid is filtered, washed with ethanol, and dried at 25 °C in vacuo overnight to afford an additional 16.27 g of product as yellow crystals, mp 107.5–108.5 °C. The overall yield is 85.9%: IR ($CHCl_3$) 1735, 1680 (C=O), 1540, 1380 cm^{-1} (NO_2); ^1H NMR ($CDCl_3$) δ 2.0 (s, 3 H), 2.2 (s, 3 H), 2.75 (d, J = 6.5, 1 H), 2.80 (d, J = 6.5, 1 H), 3.06 (t, J = 8, 2 H), 3.5 (d, J = 13.8, 2 H), 3.7 (d, J = 13, 2 H), 4.2 (t, J = 8, 2 H), 5.75 (t, J = 6.5, 1 H). Anal. ($C_{28}H_{29}N_3O_5$) C, H, N.

1-Acetyl-5-[1-(acetyloxy)-2-[bis(phenylmethyl)amino]ethyl]-2,3-dihydro-1*H*-indole-7-amine (13). Method A. The 7-nitroindoline 12 (10.21 g, 20.9 mmol) is refluxed for 4 h under nitrogen atmosphere in dry benzene (167 mL) containing triiron dodecacarbonyl (13.70 g, 27 mmol) and methanol (4.6 mL). Then, the solution is cooled to room temperature, filtered, washed with water, dried, and evaporated to afford an oil (9.24 g). Purification through a flash column using petroleum ether–ethyl acetate (35:65) as the eluant afforded the pure product 13 (7.81 g) as a foam (82% yield): IR ($CHCl_3$) 3420, 3270 (NH_2), 1733, 1650 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 2.01 (s, 3 H), 2.25 (s, 3 H), 2.75 (m, 2 H), 2.93 (t, J = 8, 2 H), 3.61 (s, 4 H), 3.99 (t, J = 8, 2 H), 5.81 (m, 1 H), 6.30 (d, J = 2, 1 H), 6.38 (d, J = 2, 1 H), 7.22 (s, 10 H).

Method B. The 7-nitroindoline 12 (23 g, 47 mmol) is dissolved in warm ethanol (470 mL). Water (91 mL), acetic acid (51 mL), and zinc dust (portionwise, 95 g) are successively added to the solution. The whole is vigorously stirred at room temperature for 30 min. The sludge of zinc dust and zinc oxide is then filtered from the solution and extracted with warm ethanol. The combined filtrates are poured into saturated sodium chloride solution, and the product is extracted in methylene chloride (three times). The organic phase is successively washed with water, saturated sodium bicarbonate solution, and water, dried over magnesium sulfate, and evaporated to dryness. An off-white foam (20.0 g, 93%) is obtained under high vacuum. The compound is identical with the product obtained by method A.

1-Acetyl-5-[1-(acetyloxy)-2-[bis(phenylmethyl)amino]ethyl]-2,3-dihydro-1*H*-indole-7-carbonitrile (14). The 7-aminoindoline 13 (24.80 g, 54.26 mmol) and 200 mL of 6 N hydrochloric acid are stirred at 5 °C to form a solution and cooled to −5 °C. To this cold solution is added dropwise a solution of sodium nitrite (8.23 g, 119.2 mmol) in water (24 mL) over a period of 20 min. When the addition is complete, the mixture is stirred at −5 °C for an additional 20 min and then further cooled to −20 °C. The mixture is then treated dropwise with a cold solution of 24% fluoboric acid (190 mL, precooled to −10 °C) over a 20-min period. A precipitate forms during the addition. After the addition, stirring continues at −20 °C for 20 min and then the reaction temperature is allowed to rise to 0–5 °C for an additional 20 min. The yellow diazonium salt is collected by filtration, washed with cold 24% fluoboric acid (60 mL, precooled to 5 °C), and ether (150 mL). Upon drying at 25 °C in vacuo overnight, the product weighs 37.71 g and is used immediately in the following way.

In a 1-L, three-necked flask equipped with a mechanical stirrer and a thermometer are placed potassium cyanide (21.16 g), copper(I) cyanide (24.149 g), and 200 mL of dimethyl sulfoxide. This mixture is stirred at 50 °C for 30 min and then the solution is cooled to 20 °C. To this viscous solution is added portionwise the diazonium fluoborate (37.71 g) prepared above; gas evolution is observed during the period of addition. When the addition is complete (15 min), stirring continues at 20 °C for 1.5 h. After the mixtures cools to 10 °C, 200 mL of methylene chloride is added, followed by 300 mL of cold water, and the mixture is stirred for 20 min. The organic phase is separated, washed with water (300 mL × 3), and dried over $MgSO_4$. Removal of the solvent gives 18.43 g of crude product as a brown foam, which is used for the subsequent reaction without further purification. A sample was crystallized from ethanol and petroleum ether and had mp 108–109 °C: IR ($CHCl_3$) 2220 (CN), 1733, 1678 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 2.02 (s, 3 H), 2.29 (s, 3 H), 2.76 (m, 2 H), 3.01 (t, J = 8.5, 2 H), 3.61 (s, 4 H), 4.1 (t, J = 8.5, 2 H), 5.73 (t, J = 7, 1 H), 7.2 (m, 12 H). Anal. ($C_{29}H_{29}N_3O_3$) C, H, N.

2,3-Dihydro-5-[1-hydroxy-2-[bis(phenylmethyl)amino]ethyl]-1*H*-indole-7-carbonitrile (15). The crude 7-cyanoindoline diacetate 14 (18.43 g, from previous step) is dissolved in 150 mL of warm ethanol (50 °C) and then the solution is cooled to 15–20 °C. To this solution is added dropwise 50 mL of 5 N aqueous sodium hydroxide over a period of 20 min and the reaction mixture is further stirred at 20 °C for 1.5 h. Then it is cooled to 10 °C, and 200 mL of cold water is added portionwise. After the mixture is stirred at 5–10 °C for 20 min, the aqueous phase is decanted and discarded. The residual solid is stirred once with cold water (100 mL) for 20 min, and the aqueous phase is discarded. This solid is used as such in the next step. A sample was dried and purified through a flash column using ethyl acetate–petroleum ether (25:75) as the eluant. It was crystallized from ethyl acetate and petroleum ether and had mp 139–140 °C: IR ($CHCl_3$) 3420 (OH, NH), 2210 cm^{-1} (CN); ^1H NMR ($CDCl_3$) δ 2.54 (d, J = 7, 2 H), 2.97 (t, J = 8, 2 H), 3.43 (d, J = 13, 2 H), 3.85 (d, J = 13, 2 H), 3.60 (t, J = 8, 2 H), 4.47 (t, J = 7, 1 H). Anal. ($C_{25}H_{25}ON_3$) C, H, N.

2,3-Dihydro-5-[1-hydroxy-2-[bis(phenylmethyl)amino]ethyl]-1*H*-indole-7-carboxamide (16). Compound 15 (from previous step) is dissolved in 180 mL of warm dimethyl sulfoxide and then cooled to 15 °C. Forty milliliters of 35% hydrogen peroxide is added over a period of 20 min. After the addition, stirring continues at 15 °C for 15 min and then the mixture is cooled to 5 °C. To this cold solution is added dropwise 5 N aqueous sodium hydroxide (40 mL) at such a rate to maintain the reaction temperature at 10–15 °C. When the addition is complete (20 min), stirring continues at this temperature for 2 h. Then the mixture is cooled to 5 °C, 360 mL of cold water is added, and the mixture is stirred at 15 °C for 30 min. The aqueous phase is decanted and discarded. The residual solid is stirred with water (150 mL × 5). The aqueous layers are discarded. The residual solid is then stirred with 50 mL of 2-propanol at 50 °C for 30 min and then cooled to 5 °C. The solid is filtered, washed with 2-propanol (25 mL × 2), and dried at 25 °C in vacuo. It affords 10.31 g of product 16 as a beige fine solid, mp 169–170 °C. A sample was recrystallized once from a mixture of chloroform and ether to give a white solid. The overall yield from the primary amine 13 to amide 16 is 47%: IR ($CHCl_3$) 3520, 3410 (OH, NH), 1655 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 2.57 (d, J = 7, 2 H), 2.85 (m, 2 H), 3.45 (d, J = 13, 2 H), 3.85 (d, J = 13, 2 H), 3.60 (m, 2 H), 4.5 (t, J = 6.5, 1 H), 5.6 (br s, 2 H), 6.25 (br s, 1 H), 6.9 (s, 2 H), 7.25 (s, 10 H). Anal. ($C_{25}H_{27}N_3O_2$) C, H, N.

5-[1-Hydroxy-2-[bis(phenylmethyl)amino]ethyl]-1*H*-indole-7-carboxamide (17). To a solution of 16 (20 g, 50 mmol) in 500 mL of tetrahydrofuran is added 40 g of activated manganese(IV) oxide.[21] The mixture is stirred at room temperature until all the starting material is consumed (9 h). The reaction mixture is filtered through Celite, and the solid is washed with tetrahydrofuran (150 mL). The filtrate is evaporated to give crude product as a foam (23.0 g), which is triturated with ether (100 mL) and forms a mass of crystals immediately. The crystals are filtered and washed with 50 mL of ether. After the mixture is stirred with toluene (50 mL) at room temperature for 20 min, the product is filtered and dried at 25 °C in vacuo. It affords 18.14 g (91% yield) of compound 17 as beige crystals: mp 153–154.5 °C; IR (KBr) 3440, 3370, 3220 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 2.67 (d, J = 7, 2 H), 3.5 (d, J = 13, 2 H), 3.9 (d, J = 13, 2 H), 4.80 (t, J = 7, 1 H), 5.9 (br s, 2 H), 7.3 (m, 14 H), 10.2 (br s, 1 H). Anal. ($C_{25}H_{25}N_3O_2$) C, H, N.

5-[1-Hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]-1*H*-indole-7-carboxamide (5). 5-[1-Hydroxy-2-[bis(phenylmethyl)amino]ethyl]-1*H*-indole-7-carboxamide (17; 3.99 g, 10 mmol) is hydrogenated for 28 h at atmospheric pressure and room temperature in dry methanol (500 mL) containing 10% palladium on carbon (1 g). The catalyst is then filtered and the solvent removed under vacuum to afford the primary amine intermediate as an oil (2.58 g). Part of this product (2.3 g) is solubilized in methanol (64 mL) and benzene (662 mL) and heated at reflux in the presence of benzylacetone (1.6 mL) for 6 h with use of a Dean–Stark apparatus. The volume of the solution is then reduced down to 30 mL and the residue taken back in methanol (330 mL). The solution is treated with sodium cyanoborohydride (0.67 g, 10 mmol) and stirred 24 h at room temperature. Reaction is followed by TLC. Solvent is removed after completion. Purification through a flash column using chloroform–methanol (85:15) + 0.5% NH_3 as eluant gives the pure product 5 as a white foam (2.49 g, 71%). This product shows two peaks at a ratio of 36 (t_r 14.14 min):44 (t_r 14.70 min) by HPLC (column, silica Si-60 5 μm, length 25 cm, i.d. 4.60 mm, solvent system $CHCl_3$/MeOH, 85:15, plus 0.5% concentrated NH_4OH; flow rate 0.6 mL/min; UV at 312 μm/aufs). IR (Nujol) 3420–3180 (OH, NH), 1650 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 1.07 (d, J = 7, 3 H), 1.65 (m, 2 H), 2.65 (m, 5 H), 4.72 (dd, J_1 = 8, J_2 = 4, 1 H), 6.23 (br s, 2 H), 6.46 (m, 1 H), 7.25 (m, 6 H), 7.48 (s, 1 H), 7.67 (s, 1 H), 10.20 (br, s, 1 H). Anal. ($C_{21}H_{25}N_3O_2 \cdot H_2O$) C, H, N.

5-[Hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]-1*H*-indole-7-carboxamide Hydrochloride (5·HCl). 5-[1-Hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]-1*H*-indole-7-carboxamide (5; 1.404 g, 4 mmol) is dissolved in ethyl acetate (20 mL) and treated at 0 °C with magnetic stirring with a solution of hydrogen chloride in ethyl acetate (9 mL) prepared from 4.34 N ethereal HCl (1 mL) completed to a volume of 10 mL with ethyl acetate. After the mixture is allowed to stand overnight in the cold, the white solid is collected by filtration, washed with ethyl acetate and ether, and then dried at 25 °C in vacuo to give the title product (1.37 g, 89%): mp 185–188 °C; IR (Nujol) 3460, 3440, 3200 (OH, NH); 1680 cm^{-1} (C=O); ^1H NMR (Me_2SO) δ 1.35 (d, J = 7, 3 H), 1.5–3.5 (m, 7 H), 5.05 (m, 1 H), 6.45 (br s, 1 H), 7.2 (m, 6 H), 7.75 (s, 2 H), 11.3 (br s, 1 H). Anal. ($C_{21}H_{25}N_3O_2 \cdot HCl$) C, H, N.

5-[1-Hydroxy-2-[bis(phenylmethyl)amino]ethyl]-1-methyl-1*H*-indole-7-carboxamide (18). 5-[1-Hydroxy-2-[bis(phenylmethyl)amino]ethyl]-1*H*-indole-7-carboxamide (17; 3.0 g, 0.0075 mol) is dissolved in dimethylformamide (50 mL) and stirred in the presence of sodium hydride (0.7 g, 0.029 mol) at room temperature for 1 h. Methyl iodide (0.93 mL, 2.12 g, 0.015 mol) is added and the mixture is stirred for 2 h. Excess methyl iodide is then removed under vacuum. The reaction mixture is poured into water and the product is extracted with dichloromethane. The organic phase is washed with water, dried over magnesium sulfate, and evaporated to dryness. The residue is purified by column chromatography on silica gel with use of a mixture of petroleum ether and ethyl acetate (4:6). Crystallization from dichloromethane and petroleum ether yields a white product (1 g, 32%): mp 163–165 °C; IR (Nujol) 3420 (OH), 3300 (NH_2), 1655 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.65 (d, 2 H, J = 7), 3.8 (s, 3 H), 3.45 (d, 1 H, J = 14), 3.90 (d, 1 H, J = 14), 4.75 (t, 1 H, J = 7). Anal. ($C_{26}H_{27}N_3O_2$) C, H, N.

5-[1-Hydroxy-2-[(1-methyl-3-phenylpropyl)amino]ethyl]-1-methyl-1*H*-indole-7-carboxamide (6) Hydrochloride. A solution of 5-[1-hydroxy-2-[bis(phenylmethyl)amino]ethyl]-1-methyl-1*H*-indole-7-carboxamide (18, 1.0 g, 2.4 mmol), benzylacetone (0.75 g, 5.0 mmol), and acetic acid (0.1 mL) in methanol (125 mL) is hydrogenated in the presence of 10% Pd/C (250 mg) and PtO_2 (250 mg) catalysts. Reaction is followed by TLC. Catalysts and solvent are removed after completion. Purification through a flash column using chloroform–methanol (85:15) + 0.5% NH_3 as the eluant affords the pure product (800 mg). The hydrochloride salt is prepared by adding ethereal HCl (0.9 mL, 2.8 N diluted to 10 mL with ethyl acetate) to a solution of the product

in chloroform (50 mL) at 0 °C. The hydrochloride salt (0.77 g, 75%) is collected by filtration and dried at room temperature under high vacuum: mp 118–121 °C; IR (KBr) 3320, 3200, 3100 (OH, NH_2), 2800 (N^+H_2), 1650 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, free base) δ 1.1 (d, 3 H, J = 6.5), 1.7 (m, 2 H), 3.8 (s, 3 H), 4.67 (m, 1 H). Anal. ($C_{22}H_{27}N_3O_2$·HCl) H, N; C: calcd, 65.74; found, 63.92.

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Registry No. 4, 36894-69-6; 5, 101565-18-8; 5·HCl, 101544-35-8; 6, 101544-37-0; 6-HCl, 101544-36-9; 7, 16078-30-1; 8, 76139-03-2; 9, 101544-38-1; 10, 101544-39-2; 11, 101544-40-5; 12, 101629-42-9; 13, 101544-41-6; 13 (diazonium fluoroborate salt), 101544-48-3; 14, 101544-42-7; 15, 101544-43-8; 16, 101544-44-9; 17, 101544-45-0; 17 (amine), 101544-49-4; 18, 101544-46-1; $ClCH_2COCl$, 79-04-9; $(Bz)_2NH$, 103-49-1; $C_6H_5(CH_2)_2COCH_3$, 2550-26-7.

Synthesis and Biological Activities of Oligo(8-bromoadenylates) as Analogues of 5′-*O*-Triphosphoadenylyl(2′→5′)adenylyl(2′→5′)adenosine

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The 8-bromoadenosine analogue of 5′-*O*-triphosphoadenylyl(2′→5′)adenylyl(2′→5′)adenosine (2-5A) and its derivatives were synthesized, and their biological activity was evaluated in mouse L cell extracts. All compounds, except 5′-dephosphorylated "cores" bound to the 2-5A-dependent endonuclease with a relative activity, depending on the derivative, of 1 to 0.035 of that of 2-5A trimer. 8-Bromoadenylate trimer 5′-mono-, -di-, and -triphosphates inhibited protein synthesis with a relative activity of 0.0023, 0.050, and 0.015 compared to 2-5A. Tetramer 5′-monophosphate also inhibited protein synthesis (relative activity 0.0033). The corresponding pentamer 5′-monophosphate did not; however, the pentamer 5′-diphosphate was able to inhibit translation (relative activity 0.0092). All compounds that possessed inhibitory activity in the protein synthesis inhibition assay gave ribosomal RNA cleavage patterns characteristic of the action of 2-5A-dependent endonuclease. Thus, 8-bromination of the all of the adenine rings of 2-5A leads to 20- to 70-fold reduction in the biological activity of the corresponding 5′-di- and -triphosphate, respectively; however, this same alteration of the three adenine moieties gives rise to a 5′-monophosphate with much enhanced translational inhibitory activity compared to the parent 2-5A trimer 5′-monophosphate.

One of the events that takes place after interferon interaction with cell surface receptors is induction of an enzyme named 2-5A synthetase, which in the presence of dsRNA synthesizes from ATP a mixture of 2′,5′-linked oligoadenylates, ppp5′A(2′p5′A)$_n$ ($n > 2$ to about 10), often referred to as 2-5A.[1-4]

2-5A is a very potent inhibitor of translation in cell-free systems[5] or in intact cells after introduction by cell permeabilization methods[6-8] or microinjection.[9] This protein synthesis inhibitory action is due to the action of ribonuclease L, which is activated by 2-5A to degrade viral and/or cellular RNAs with a preference for cleavage after UNp sequences.[10,11] 2-5A at a concentration sufficient to inhibit protein synthesis has been found in EMC virus-infected, interferon-treated mouse L cells or HeLa cells.[12-14] In addition, ribosomal RNA cleavage patterns characteristic of 2-5A-dependent ribonuclease L activity has been observed in interferon-treated mouse L cells infected with EMC virus[15] and reovirus-infected HeLa cells.[16]

The likely role of 2-5A in some of the antiviral actions of interferon and its potential role in cell regulation processes[17-19] has led to considerable activity in an attempt to develop analogues of 2-5A that might be active in the intact cell or animals.[20-24] Only a limited amount of in-

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