3-(4-Piperidinylalkyl)indoles, Selective Inhibitors of Neuronal 5-Hydroxytryptamine Uptake

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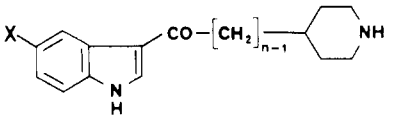
A series of 3-(4-piperidinylalkyl)indoles was synthesized and tested as uptake inhibitors of biogenic amines. Some of these compounds are potent and very selective in blocking the 5-hydroxytryptamine (5-HT) uptake, as evidenced by biochemical data and behavioral tests. A discussion on structure–activity relationships is given. The most interesting member of the series, indalpine, 3-[2-(4-piperidinyl)ethyl]indole (1), was selected for clinical studies.

Uptake into the presynaptic neuron is the principal mechanism for the rapid inactivation of released biogenic amines in the neuronal synapse.[1] The tricyclic antidepressant agents inhibit to a varying extent the uptake of noradrenaline (NA) and 5-hydroxytryptamine (5-HT).[2] Until recently, clomipramine was the most selective inhibitor of 5-HT uptake in vitro,[3-5] but the selectivity was greatly reduced in vivo due to the biotransformation into chlordesipramine, a NA uptake inhibitor.[6]

In search for more specific inhibitors of 5-HT uptake, attention was mainly focused, during the last few years, on nontricyclic compounds. Recently, a number of compounds with various structures have been reported to inhibit selectively the uptake of 5-HT.[7-17] It should be noted that all these compounds are aliphatic or cycloaliphatic amines possessing a benzene ring separated from the nitrogen atom by a chain of two to five atoms. Suprisingly, none of these inhibitors were indole derivatives, except β-carbolines[17] which are rather weak uptake inhibitors and potent monoamine oxidase inhibitors.

In connection with our previous work in the cinchona alkaloids field, we have prepared indolic analogues of viquidil. Some of these compounds, having a 4-piperidinylalkyl group, were shown to be strong inhibitors of 5-HT uptake by rat brain synaptosomes and human blood platelets and to possess a weak inhibitor activity on the uptake of catecholamines.[18-21]

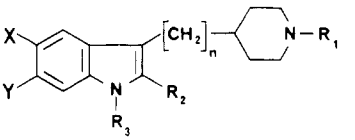
Table I. 3-Indolyl 4-Piperidinylalkyl Ketones[a]



no.	X	n	formula	mp, °C	recrystn solvent	yield,[b] %
19	H	1	$C_{14}H_{16}N_2O\cdot HCl$	260[c]	MeOH	35
20	CH_3O	1	$C_{15}H_{18}N_2O_2\cdot HCl$	>260	*d*	31
21	Br	1	$C_{14}H_{15}N_2BrO\cdot HCl$	>260	*d*	42
22	F	1	$C_{14}H_{15}N_2FO\cdot HCl$	>260	*d*	55
23	Cl	1	$C_{14}H_{15}N_2ClO\cdot HCl$	>260	*d*	54
24	H	3	$C_{16}H_{20}N_2O\cdot CH_4O_3S$[e]	198	EtOH	27

[a] All these compounds were prepared by method A. [b] Yields have not been optimized and are based on starting indoles. [c] Lit.[38] (base) mp 221–224 °C. [d] Not recrystallized, washed with Me_2CO. [e] C: calcd, 57.94; found, 57.40.

In the present report we describe the synthesis of these compounds and a number of analogues (1–18) and test



1-18

their inhibitory activity on the NA, dopamine (DA), and 5-HT uptake in rat brain synaptosomes and on 5-HT uptake in human blood platelets. Potentiation of 5-hydroxytryptophan (5-HTP) in mouse and antagonism of tetrabenazine ptosis in rat have also been determined.

Chemistry. The compounds 1–18 are listed in Table III. A few of them have been previously described: **1**, 2,[22] **3**,[23] **6**,[24] **15**,[25] **17**.[26] To synthesize the compounds bearing

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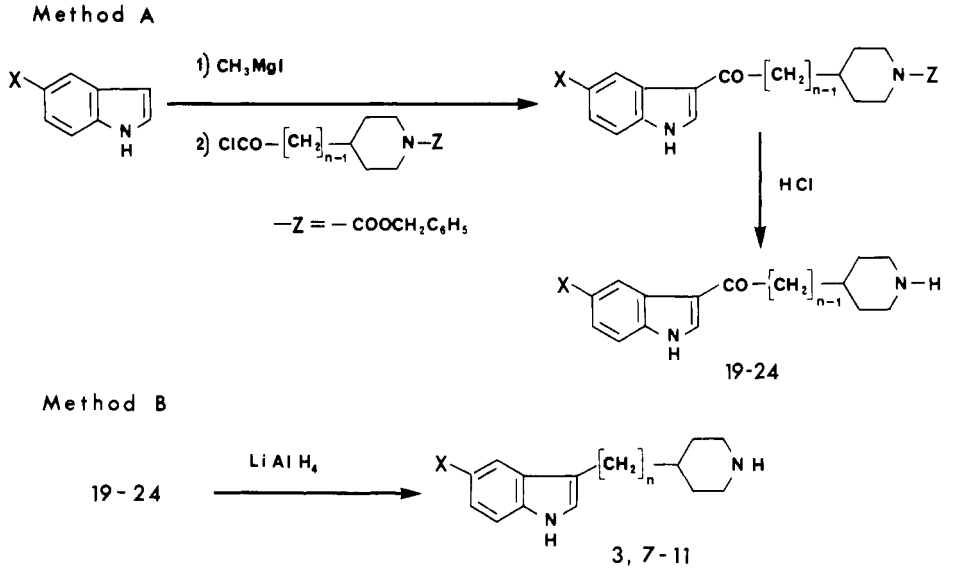
Scheme I



no substituent on the indolic and piperidinic nitrogen, the most general method, outlined in the Scheme I, is inspired by the De Graw procedure.[27] This method was preferred to obtain compounds with one or three carbon atoms in the alkyl chain: **3** and **7–11**. In the first step (method A), indolylmagnesium halides, from indoles, were condensed with *N*-(carbobenzoxy)-4-piperidinylalkylcarboxylic acid chlorides. Acidic hydrolysis of the carbobenzoxy group gave 3-indolyl-4-piperidinylalkyl ketones **19–24** (Table I). In a second step (method B), $LiAlH_4$ reduction of the ketones gave the required derivatives.

The compounds **1**, **2**, **4–6**, and **16–18**, with two carbon atoms in the alkyl chain, were prepared by the Gray procedure.[22] Treatment of indoles with 4-vinylpyridine in acetic acid (method C) gave 3-(4-pyridylethyl)indole derivatives **25–34** (Table II). The desired products were obtained by catalytic hydrogenation of these pyridyl derivatives in acetic acid (method D). When (benzyloxy)-indoles were used as the starting products, the benzyl group was removed during the catalytic hydrogenation, and hydroxy compounds **12** and **13** were obtained. Compounds **14** and **15**, bearing a methyl group at the piperidinic nitrogen, were conveniently prepared from nonalkylated products **2** and **1**, respectively, via the *N*-formyl derivatives (method E): the methyl group was generated by $LiAlH_4$ reduction of formyl-substituted derivatives obtained by treatment of **2** and **1** with ethyl formate.

Biological Results. The biological test results are summarized in Table IV. From the results of rat brain synaptosomes experiments it is evident that most of the compounds examined were much more potent in inhibiting the uptake of 5-HT than that of catecholamines. **1**, **3**, and **7** are the most potent selective inhibitors of 5-HT uptake into rat brain synaptosomes.

The inhibition of 5-HT uptake into human platelets was also measured because of the possible significance of platelets as a model of the nerve ending with respect to its amine uptake system.[28,29] A highly significant correlation between the IC_{50} for the inhibition of 5-HT uptake by various drugs into the two models has previously been shown.[30] In the present series, discordance can be observed for **3** and **12**.

Potentiation by 5-HT uptake inhibitors of behavioral syndrome induced by 5-HTP in mice has been shown to correlate with the ability of drugs to inhibit 5-HT uptake.[31] As shown by the results in Table IV, **1**, **3**, and **7**, like clomipramine, potentiated 5-HTP induced tremors. These data confirm the findings of the uptake experiments: the most active 5-HTP potentiators were the most active 5-HT uptake inhibitors.

Tricyclic antidepressants generally antagonize the tetrabenazine-induced ptosis in rats; among them, clomipramine has the weakest activity. Compound **9** was relatively active but very toxic (LD_{50} = 25 mg/kg po); therefore, this result must be interpreted with caution. The other compounds, including the most potent inhibitors of 5-HT uptake, had no or very poor antagonizing effect. This observation confirms the fact that central 5-HT neurons are not principally involved in the tetrabenazine-induced ptosis in rats.[32]

In more detailed in vivo studies, indalpine (**1**) was shown to be the most potent and the most selective inhibitor of the series. Indalpine was selected for clinical trials.

Structure–Activity Relationship. It is obvious, from the results obtained, that the introduction of substituents is very critical for the activity, the most active compounds (**1**, **3**, and **7**) being those where all the substituents are hydrogen atoms. Starting from **1**, methylation of the piperidinic nitrogen or methylation or phenylation of the indole ring in the 2 position gave inactive compounds, **15**, **17**, and **18**, respectively. 5-Cl (**4**) and 5-MeO (**5**) derivatives were ten times less potent in vitro than the parent compound **1**. Substitution of the indolic nitrogen of **1** by a methyl (**2**), benzyl (**6**), or phenyl group (**16**) led to a substantial decrease in activity. The substituted analogues of **3**, **8–11**, are poorly or not active, the most active being the 5-fluoro derivative. 5-Hydroxy and 6-hydroxy derivatives (**12** and **13**, respectively) were considerably less potent than the parent compound in the synaptosomes

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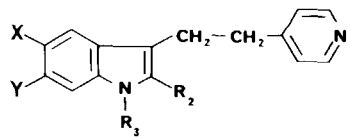
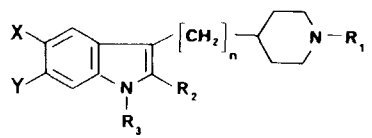
Table II. 3-(4-Pyridylethyl)indoles[a]



no.	X	Y	R_2	R_3	formula	mp, °C	recrystn solvent	yield,[b] %
25	H	H	H	H	$C_{15}H_{14}N_2$	150[c]	EtOH-H_2O	53
26	Cl	H	H	H	$C_{15}H_{13}ClN_2$	210	EtOH	63
27	OCH_3	H	H	H	$C_{16}H_{16}N_2O$	135[d]	AcOEt	46
28	$OCH_2C_6H_5$	H	H	H	$C_{22}H_{20}N_2O$	159[e]	EtOH	72
29	H	$OCH_2C_6H_5$	H	H	$C_{22}H_{20}N_2O$	201	EtOH	70
30	H	H	H	CH_3	$C_{16}H_{16}N_2$	97[f]	EtOH	45
31	H	H	H	$CH_2C_6H_5$	$C_{22}H_{21}ClN_2 \cdot HCl$	200[g]	EtOH	18
32	H	H	H	C_6H_5	$C_{21}H_{18}N_2$	190	AcOEt	20
33	H	H	CH_3	H	$C_{16}H_{16}N_2 \cdot HCl$	219-222[h]	EtOH	73
34	H	H	C_6H_5	H	$C_{21}H_{18}N_2$	201[i]	MeOH-$CHCl_3$	38

[a] All these compounds were prepared by method C. [b] Yields have not been optimized and are based on starting indoles. [c] Lit.[25] mp 149-151 °C. [d] Lit.[39] mp 136-137 °C. [e] Lit.[39] mp 160-163 °C. [f] Lit.[25] mp 96-98 °C. [g] Lit.[25] mp 199-200 °C. [h] Lit.[40] (base) mp 153-154 °C. [j] Lit.[39] mp 199-201 °C.

Table III. 3-(4-Piperidinylalkyl)indoles



no.	X	Y	R_1	R_2	R_3	n	starting material	method	formula	mp, °C	recrystn solvent	yield,[a] %
1	H	H	H	H	H	2	25	D	$C_{15}H_{20}N_2$	159[b]	AcOEt	74
2	H	H	H	H	CH_3	2	30	D	$C_{16}H_{22}N_2 \cdot HCl$	198[c]	EtOH	50
3	H	H	H	H	H	1	19	B	$C_{14}H_{18}N_2$	172[d]	Me_2CO	52
4	Cl	H	H	H	H	2	26	D	$C_{15}H_{19}ClN_2$	160	Me_2CO	60
5	OCH_3	H	H	H	H	2	27	D	$C_{16}H_{22}N_2O$	119	AcOEt-*i*-Pr_2O	48
6	H	H	H	H	$CH_2C_6H_5$	2	31	D	$C_{22}H_{26}N_2 \cdot HCl$	177[e]	EtOH	33
7	H	H	H	H	H	3	24	B	$C_{16}H_{22}N_2$	110	AcOEt-*i*-Pr_2O	44
8	OCH_3	H	H	H	H	1	20	B	$C_{15}H_{20}N_2O$	153	Me_2CO	38
9	Br	H	H	H	H	1	21	B	$C_{14}H_{17}BrN_2$	145	AcOEt[f]	34
10	F	H	H	H	H	1	22	B	$C_{14}H_{17}FN_2$	160	AcOEt[g]	50
11	Cl	H	H	H	H	1	23	B	$C_{14}H_{17}ClN_2 \cdot 0.5C_4H_4O_4$[h]	260	EtOH[i]	27
12	OH	H	H	H	H	2	28	D	$C_{15}H_{20}N_2O \cdot C_4H_4O_4$[h]	200	H_2O[j]	5
13	H	OH	H	H	H	2	29	D	$C_{15}H_{20}N_2O \cdot C_4H_4O_4$[h]	235	H_2O	32
14	H	H	CH_3	H	CH_3	2	2	E	$C_{17}H_{24}N_2 \cdot HCl$	100	CH_3CN	71
15	H	H	CH_3	H	H	2	1	E	$C_{16}H_{22}N_2$	171[k]	MeOH-H_2O	76
16	H	H	H	H	C_6H_5	2	32	D	$C_{21}H_{24}N_2 \cdot {}^2/_3C_4H_4O_4$[i,l]	162	EtOH	25
17	H	H	H	CH_3	H	2	33	D	$C_{16}H_{22}N_2$	172[m]	AcOEt	41
18	H	H	H	C_6H_5	H	2	34	D	$C_{21}H_{24}N_2$	183	EtOH	75

[a] Yields have not been optimized. [b] Lit.[22] mp 162-163 °C. [c] Lit.[22] mp 200-201 °C. [d] Lit.[23] mp 171 °C. [e] Lit. (base) mp 51-53 °C. [f] Before recrystallization crude product was chromatographed on 70-230 mesh silica gel [EtOH-Et_2NH (90:10)]. [g] Crude base purified as in footnote *f* [MeOH-Me_2CO-Et_2NH (45:50:5)]. [h] Fumarate. [i] Crude base purified as in footnote *f* [toluene-EtOH-Et_2NH (45:50:5)]. [j] Crude base purified as in footnote *f* [MeOH-Et_2NH (90:10)]. [k] Lit.[25] mp 171-173 °C. [l] C: calcd, 74.44; found, 73.41. [m] Lit.[26] (HCl salt) mp 212-215 °C.

Table IV. Biological Activity of 3-(4-Piperidinylalkyl)indoles

compd	inhibn of uptake: IC_{50}, μM: rat brain synaptosomes[a] 5-HT	NA	DA	human platelets: 5 HT	potentiation of 5-HTP (mouse): ED_{50}, mg/kg sc	antagonism of tetrabenazine ptosis (rat): AD_{50}, mg/kg po
1	0.01	1.6	18	0.03	4	inactive[b]
2	0.4	1.3	30	0.4	inactive[c]	inactive
3	0.05	1.5	20	0.55	10	inactive
4	0.13	0.65	1.5	0.22	inactive	inactive
5	0.14	3	20	>1	inactive	inactive
6	1.9	5	0.3	>1	inactive	inactive
7	0.04	0.5	3	0.06	8	56
8	1	9	>100	>1	inactive	inactive
9	0.7	0.6	8	1	inactive[d]	10
10	0.1	1	7	0.17	32	100
11	0.7	1.8	12	>1	inactive[d]	inactive[e]
12	0.6	1	2.7	0.02	inactive	inactive
13	1.5	0.6	0.15	>1	inactive	inactive
14	1.7	>10	>100	>1	inactive	inactive
15	0.45	40	6.5	>1	inactive	inactive
16	1	0.4	20	>1	inactive	>100
17	2.5	6.5	23	>1	inactive	>100
18	2.2	1.5	3.5	>1	inactive	inactive
clomipramine	0.02	0.7	1	0.07	6.5	45

[a] Regional uptake inhibition: 5-HT, cortex; NA, hypothalamus; DA, striatum. [b] Unless otherwise indicated, inactive = inactive at 100 mg/kg po. [c] Unless otherwise indicated, inactive = inactive at 25 mg/kg sc. [d] Inactive at 3 mg/kg sc. [e] Inactive at 10 mg/kg po.

model. The unexpected inhibitory activity of **12** in the platelets model suggests that the human platelet 5-HT carrier differs somewhat from the rat neuronal 5-HT carrier with regard to the structural requirements for inhibition. The most interesting result is that the unsubstituted compounds **1**, **3**, and **7**, possessing two, one, and three carbon atoms in the alkyl chain, respectively, were approximatively equipotent in all the tests. The significance of this result is now under investigation. Conformational studies of **1** and its analogues will be the subject of a future report.

Experimental Section

Chemistry. Melting points were determined on a Kofler hot stage. ^1H NMR spectra were recorded on a Varian T-60 spectrophotometer. Infrared spectra were recorded on a Beckmann IR-20 spectrophotometer. TLC was performed on silica gel GF plates and components were visualized by UV fluorescence properties and by spraying with potassium iodoplatinate reagent. All compounds exhibited proper spectral characteristics and were homogeneous by TLC analysis. Microanalytical results on new compounds are within ±0.4% of the theoretical values unless otherwise indicated. Yields were not optimized and refer to the amount of the analytical sample. The general procedures listed in the tables and the preparation of the requisite starting materials are described by the following examples.

Indoles. Indole, 1-methylindole, 2-methylindole, 2-phenylindole, 5-methoxyindole, 5-chloroindole, 5-bromoindole, and 5-(benzyloxy)indole were commercially available. The following substituted indoles were synthesized by literature procedures: *N*-benzylindole,[33] *N*-phenylindole,[34] 5-fluoroindole,[35] and 6-(benzyloxy)indole.[36]

4-Vinylpyridine was obtained from a commercial source.

β-[1-(Carbobenzoxy)-4-piperidine]propionyl chloride was prepared using a slight modification of the reported procedure.[27]

[1-(Carbobenzoxy)-4-piperidine]carboxylic Acid (35). To a stirred solution of 6.5 g (0.05 mol) of 4-piperidinecarboxylic acid in 50 mL (0.1 mol) of 2 N NaOH was added dropwise, at 10 °C, 8,5 g (0.05 mol) of benzyl chloroformate. After the addition was completed, the reaction mixture was stirred at room temperature for 15 h and then diluted with 50 mL of H_2O and extracted with 2 × 50 mL of Et_2O. The aqueous layer was acidified with 37% HCl. The resulting oily precipitate was extracted with 3 × 50 mL of CH_2Cl_2, and the organic extract was dried ($MgSO_4$) and concentrated to a viscous, colorless oil (8.7 g, 66%).

[1-(Carbobenzoxy)-4-piperidine]carbonyl Chloride (36). A mixture of 8.7 g (0.033 mol) of **35**, 7 mL (0.066 mol) of thionyl chloride, and 100 mL of $CHCl_3$ was refluxed for 2 h and then evaporated in vacuo to give a yellow oil (8.8 g, 94%) suitable for use in the following step.

Method A. 3-(5-Fluoroindolyl) 4-Piperidinylmethyl Ketone (22). 5-Fluoroindole (20.2 g, 0.15 mol) in 120 mL of anhydrous Et_2O was added dropwise at room temperature to methylmagnesium iodide [prepared from 9.7 g (0.40 mol) of Mg and 57.5 g (0.40 mol) of ICH_3] in 240 mL of anhydrous Et_2O under N_2 and stirring. The mixture was refluxed for 2 h and then cooled to 0 °C. [1-(Carbobenzoxy)-4 piperidine]carbonyl chloride (42.2 g, 0.15 mol) in 300 mL of toluene was added dropwise and the mixture was stirred at room temperature overnight. After cooling the mixture at 0 °C, 550 mL of 2 N HCl was added dropwise, and the organic layer was separated, dried over $MgSO_4$, and evaporated to dryness. The residue was dissolved in 350 mL of 5 N HCl in EtOH and the solution was refluxed for 4 h. After the solution cooled, the insoluble solid was collected, taken up in 200 mL of Me_2CO, and filtered to give 23.3 g (55%), mp >260 °C.

Method B. 5-Fluoro-3-(4-piperidinylmethyl)indole (10). To 6 g (0.16 mol) of $LiAlH_4$ in 100 mL of anhydrous tetrahydrofuran (THF) was added dropwise under nitrogen 15 g (0.053 mol) of **22** in 150 mL of anhydrous THF and stirred at room temperature. After 3 h at 20 °C, the mixture was refluxed for 15 min. The excess of $LiAlH_4$ was decomposed with 7.1 mL of H_2O, 5.2 mL of 5 N NaOH, and an additional 23 mL of H_2O. The resulting mixture was filtered and the precipitate was thoroughly washed with boiling CH_2Cl_2. The filtrate was evaporated to dryness and the residue (15.3 g) was purified by chromatography on 70–230 mesh silica gel (600 g), eluting with MeOH–Me_2CO–Et_2NH, 45:50:5. The fractions containing pure **10** were combined to give 9.8 g. Recrystallization from AcOEt gave 6 g (50%), mp 160 °C.

Method C. 5-Chloro-3-[2-(4-pyridyl)ethyl]indole (26). 5-Chloroindole (25 g, 0.165 mol), 4-vinylpyridine (25 g, 0.24 mol), and acetic acid (125 mL) were refluxed for 5 h. AcOH was then removed on a rotary evaporator at 80 °C. The solid residue was ground with 250 mL of boiling water and filtered, after cooling, to give 36.1 g. Recrystallization from EtOH gave 26.8 g (63%), mp 210 °C.

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Method D. 5-Chloro-3-[2-(4-piperidinyl)ethyl]indole (4). A mixture of 36 g (0.14 mol) of **26** and 1.8 g of PtO_2 in 360 mL of AcOH was hydrogenated at room temperature and atmospheric pressure until TLC control [MeOH–Me_2CO–Et_2NH, 45:50:5, silica gel GF] indicated that **26** had completely reacted. The catalyst was filtered off and washed with AcOH. The combined filtrate and washings were evaporated to a residue, which was dissolved in 300 mL of H_2O. The solution was alkalinized at pH 9 and the resultant precipitate was extracted with CH_2Cl_2. The organic extract was dried ($MgSO_4$) and evaporated to give 31.3 g of a brown crystalline solid. Recrystallization from Me_2CO gave 22.5 g (60%), mp 160 °C.

Method E. 1-Methyl-3-[2-(4-*N*-methylpiperidinyl)-ethyl]indole (14). To 12.1 g (0.05 mol) of 1-methyl-3-[2-(4-piperidinyl)ethyl]indole (**2**) in 25 mL of toluene was added 6.1 mL (0.075 mol) of ethyl formate in 20 mL of toluene. After 4 h at 75 °C, the mixture was evaporated to dryness. The oily residue was added dropwise under N_2 to 2.85 g (0.075 mol) of $LiAlH_4$ in 100 mL of anhydrous Et_2O. After stirring and refluxing for 3 h, the mixture was cooled to 0 °C and decomposed with 3.4 mL of H_2O, 2.5 mL of 5 N NaOH, and 11 mL of H_2O. The solid material was filtered off and washed thoroughly with Et_2O, and the filtrates were concentrated to dryness to give an oil, which was turned into its hydrochloride and crystallized from 80 mL of CH_3CN: yield 10.35 g (71%); mp 100 °C.

Biological Methods. Uptake of NA, DA, and 5-HT into Rat Brain Synaptosomes. DL-[*methylene*-^{14}C]Noradrenaline bitartrate (53 mCi/mmol), [1-*ethylamine*-^{14}C]dopamine hydrochloride (52 mCi/mmol), 5-hydroxy[side chain 2-^{14}C]tryptamine creatinine sulfate (58 mCi/mmol) were purchased from the Radiochemical Centre, Amersham. Experiments were carried out according to a previously published method[21] using immature female rats (19–21 days). Brain synaptosome preparations were incubated for 5 min at 37 °C with the labelled biogenic amines at a concentation of 10^{-7} M. Four to six concentrations of the drugs were used in duplicate to determine the IC_{50} values (concentrations of drug that inhibit the uptake by 50%).

Uptake of 5-HT into Human Blood Platelets. This was studied by the method described elsewere.[18] Platelet-rich plasma (PRP) was prepared and aliquots were incubated at 37 °C. The drugs and ^{14}C-labeled 5-HT (10^{-7} M) were added after 2 min, and incubation was continued for an additional 1 min. After cooling in an ice bath, the samples were centrifuged. Radioactivity was counted on PRP and supernatant, and the uptake of ^{14}C-labeled 5-HT was calculated by the formula of Buczko et al.[37]

Potentiation of 5-HTP-Induced Tremor in Mice. Groups of eight mice were used. The animals were injected intraperitoneally with a 100 mg/kg dose of L-5-HTP (in 0.9% NaCl solution). The test substances were administered subcutaneously 30 min after 5-HTP injection.

Tremor was evaluated at 75 min in each animal using the following rating scale: 0 = no tremor; 1 = moderate tremor; 2 = severe tremor. The ED_{50} (doses that produced 50% of the maximum effect, i.e., a mean score of 1) were determined from the global scores obtained for each group.

Antagonism of Tetrabenazine-Induced Ptosis in Rats. The test substances were administered orally to rats 0.5 h before tetrabenazine (10 mg/kg, sc). Assessment was made 1 h later, based on the absence or presence of ptosis. ED_{50} values were the doses that prevented ptosis in 50% of animals.

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Cytotoxic and Antitumor Properties of Bleomycin and Several of Its Metal Complexes[1]

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This study examines the concentration-dependent cytotoxicity and antitumor activity of bleomycin (Blm) and Cu-, Zn-, Fe(III)-, and CoBlm using Ehrlich cells in culture and the Ehrlich ascites tumor. The order of activity in culture under several conditions is CuBlm ≈ Blm ≈ ZnBlm > Fe(III)Blm ≫ CoBlm ≈ control. Short exposures of cells to drugs in the presence or absence of serum produced effects on cell proliferation similar to 48-h incubations. With Blm and CuBlm there was no obvious relationship between cytotoxicity and the modest short-term inhibition of DNA synthesis by the drugs. The antitumor experiments produced qualitatively similar results with the order of antitumor potency being CuBlm > Blm > ZnBlm ≈ FeBlm ≫ CoBlm ≈ control tumor. The host toxicity produced by these drugs as measured by weight loss had the opposite ordering: CoBlm ≪ FeBlm ≪ ZnBlm < Blm < CuBlm. At therapeutically effective concentrations, FeBlm was significantly less toxic relative to the other active agents.

The antitumor glycopeptide bleomycin (Blm) is isolated from *Streptomyces verticillus* as a 1:1 copper complex.[2a] The early studies by Umezawa and co-workers demonstrating the antitumor properties of the antibiotic utilized this metal complex.[2b] Later, the metal-free bleomycin was shown to be equally active and to be less toxic to the host.[3] For a number of years thereafter, the interest in metal ions and bleomycin was confined to the demonstration that Cu^{2+}, Zn^{2+}, and Co^{2+} can inhibit the DNA-strand scission reaction carried out by bleomycin in the presence of thiols.[4] The resurgence of the study of metal bleomycins followed the demonstration of Horwitz and co-workers that the addition of Fe^{2+} to the strand-scission assay in the presence or absence of thiols led to a remarkable enhancement of cleavage of the DNA backbone.[5,6] Although biochemical

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