76-4; **6**, 123753-97-9; **6**·HCl, 123753-77-5; **7**, 123753-98-0; **7**·HCl, 103434-08-8; **8**, 103417-89-6; **8**·HCl, 103417-91-0; **9**, 123753-99-1; **9**·HCl, 123753-78-6; **10**, 123754-00-7; **10**·HCl, 103417-94-3; **11**, 123754-01-8; **11**·HCl, 103417-92-1; **12**, 123753-79-7; **12**·$C_4H_6O_4$, 123753-80-0; **13**, 103417-72-7; **13**·maleate, 103417-73-8; **14**, 123753-81-1; **15**, 123753-82-2; **15**·$C_4H_6O_4$, 123753-83-3; **16**, 123753-84-4; **16**·$C_4H_6O_4$, 123753-85-5; **17**, 123753-86-6; **17**·$C_4H_6O_4$, 123753-87-7; **18**, 103417-93-2; **18**·HCl, 123753-88-8; **19**, 123754-02-9; **19**·HCl, 123753-89-9; **20**, 123754-03-0; **20**·HBr, 123753-90-2; **21**, 123754-04-1; **21**·2HCl, 123753-91-3; **22**, 123775-19-9; **22**·2HCl, 123775-17-7; **23**, 123754-05-2; **23**·2HCl, 123775-18-8; **24**, 123754-06-3; **24**·2HCl, 123753-92-4; **25**, 103417-70-5; **25**·oxalate, 103417-90-9; **26**, 103417-77-2; **27**, 123753-93-5; **28**, 123753-94-6; **28**·oxalate, 123754-08-5; **29**, 90444-47-6; $ClCH_2COCH_2CO_2Et$, 638-07-3; *m*-$NO_2C_6H_4CH{=}CHCOCH_2CO_2Me$, 52604-00-9; $MeO_2CCH{=}C(NH_2)Me$, 14205-39-1; $ClCH_2COCH_2CO_2Me$, 32807-28-6; 3-(2-hydroxyethyl)pyridine, 6293-56-7; 3-hydroxypyridine, 109-00-2; 3-(hydroxymethyl)pyridine, 100-55-0; 1-(hydroxyethyl)imidazole, 1615-14-1; imidazole, 288-32-4; 2-chloro-3-(trifluoromethyl)-benzaldehyde, 93118-03-7; 1,4-dihydro-2-methyl-3-[2-(dimethylamino)ethyl]-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid diethyl ester, 103417-71-6; 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)pyridine-3,5-dicarboxylic acid diethyl ester, 21829-26-5; 4-[2-chloro-3-(trifluoromethyl)phenyl]-6-(chloromethyl)-1,4-dihydro-2-methylpyridine-3,5-dicarboxylic acid 3-ethyl-5-methyl diester, 123754-07-4; 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)pyridine-3,5-dicarboxylic acid ethyl methyl diester, 39562-70-4.

(8β)-6-Methylergoline Amide Derivatives as Serotonin Antagonists: N^1-Substituent Effects on Vascular $5HT_2$ Receptor Activity

Jerry W. Misner,* William L. Garbrecht, Gifford Marzoni, Kathleen R. Whitten, and Marlene L. Cohen

Lilly Research Laboratories, Eli Lilly and Co., Lilly Corporate Center, Indianapolis, Indiana 46285. *Received March 16, 1989*

A series of (8β)-6-methylergoline amide derivatives was synthesized with various alkyl substituents in the N^1-position in order to evaluate their effectiveness in blocking vascular $5HT_2$ receptors. The influence of both the N^1 substituent and amide derivative proved to be of great importance on binding affinities to vascular $5HT_2$ receptors. Within each series of amides, however, maximum affinity was achieved with an N^1-isopropyl substituent (**14**, **18**, **26**, **38**, and **41**; all with 2.7–50 times greater affinity than their N^1–H analogues), with the exception of two cases (**22** and **37**) in the cyclohexylamide derivatives wherein N^1-methyl equalled the isopropyl in potency. Other than these exceptions, affinities followed the pattern of H < Me < Et < iPr, with potencies falling off with larger alkyl substituents.

The suggestion has been made that the serotonin antagonist activity of ergot alkaloids may be selectively enhanced by methylation of N^1, the indole nitrogen,[1] but exceptions to this have been reported to exist.[2] Previous papers from these laboratories[2,3] expanded on the structure–activity relationships of the (8β)-ergoline-8-carboxylic acid esters as potent and selective serotonin antagonists as measured specifically by their vascular $5HT_2$ receptor binding affinities. The $5HT_2$ binding affinities of the ergoline esters were shown to be influenced by their ester substituents, alkyl substituents at N^6, but especially by their alkyl substituents at N^1, with isopropyl substitution resulting in maximum affinity. Because the ergoline esters are hydrolyzed in vivo to the less active carboxylic acid moiety,[4] we initiated studies of the structure–activity of a series of amide ergolines. We now report on the effect of N^1 substitution of a variety of ergoline amide derivatives on $5HT_2$ receptor affinity as measured by antagonism of serotonin-induced contractions in the rat jugular vein. This tissue is known to possess $5HT_2$ receptors that are responsible for contractions induced by serotonin.[5]

Chemistry

The N^1-alkylated (8β)-6-methylergoline-8-carboxylic acids were prepared from dihydrolysergic acid as reported previously.[6] As depicted in Scheme I, ergoline acetic acid (**2**) was synthesized from dihydrolysergol (**1**) by the procedure of Semonsky and Kucharcyzk.[7] The N^1-alkylation of **2** by the above procedure yielded the homologated ergoline carboxylic acids necessary to prepare the amides of Table III. The amides of both Tables I and III were synthesized by several methods, in part to evaluate the synthetic utility of these methods for the preparation of ergoline derivatives. Method A, using 1,1′-carbonyldiimidazole, and method E, using the acid chloride produced via $POCl_3$/DMF, proved to be the most convenient to use and gave excellent yields. Method B, acylation via the mixed anhydride with isobutyl chloroformate, also gave excellent yields, but required the use of less convenient (−45 °C) temperatures. Method C, condensation with EEDQ, and method D, oxidative amidation of the ergoline carboxylic acid hydrazide, proved to be least useful because of low and variable yields. Chromatographic procedures were not required to obtain pure products. Instead, crystallization of either the free base or maleate salt from various solvents proved sufficient.

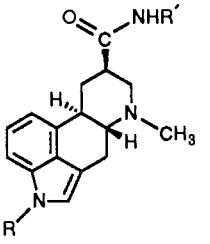
The ergoline tetrazoles of Table II were produced from their respective parent amides by method F, which is derived from the standard method of making tetrazoles from N-substituted amides.[8,9] These were all isolated as maleate or mesylate salts.

The (8β)-aminoergolines (Scheme II) required as intermediates for the synthesis of the "reverse amides" in Table IV were made via the Curtius degradation of the parent acids.[10] The previously reported[11,12] syntheses of

(1) Floss, H. G. *Tetrahedron* **1976**, *32*, 873.
(2) Marzoni, G.; Garbrecht, W. L.; Fludzinski, P.; Cohen, M. L. *J. Med. Chem.* **1987**, *30*, 1823.
(3) Garbrecht, W. L.; Marzoni, G.; Whitten, K. R.; Cohen, M. L. *J. Med. Chem.* **1988**, *31*, 444.
(4) Cohen, M. L.; Fuller, R. W.; Kurz, K. D.; Parli, J.; Mason, N. R.; Meyers, D. B.; Smallwood, J. K.; Toomey, R. E. *J. Pharmacol. Exp. Ther.* **1988**, *244*, 106.
(5) Cohen, M. L.; Fuller, R. W.; Wiley, K. S. *J. Pharmacol. Exp. Ther.* **1981**, *218*, 421.
(6) Marzoni, G.; Garbrecht, W. L. *Synthesis* **1987**, 651.
(7) Semonsky, M.; Kucharczyk, N. *Collect. Czech. Chem. Commun.* **1968**, *33*, 577.
(8) Benson, F. R. In *Heterocyclic Compounds*; Elderfield, R. C., Ed.; Wiley: New York, 1967; Vol. 8, p 20.
(9) Harvill, E. K.; Herbst, R. M.; Schreiner, E. C. *J. Org. Chem.* **1950**, *15*, 662.

0022-2623/90/1833-0652$02.50/0

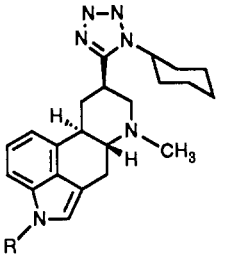
Table I. $5HT_2$ Receptor Affinities for Ergoline-8-carboxamides



no.	R	R′	−log K_B ± SE (n)	formula	mp, °C	recrystn solvent	analyses[a]	method
11	H	H	~7 (3)	$C_{16}H_{19}N_{30}$	>260	MeOH/H_2O	C, H, N	A
12	methyl	H	7.81 ± 0.12 (3)	$C_{17}H_{21}N_3O$	255–8	MeOH/H_2O/NH_4OH	C, H, N	A
13	ethyl	H	8.44 ± 0.23 (3)	$C_{18}H_{23}N_3O$	254.5–5.5	MeOH/H_2O/NH_4OH	C, H, N	A
14	*i*-propyl	H	8.70 ± 0.06 (3)	$C_{19}H_{25}N_3O$	221–3	EtOAc	C, H, N	B
15	H	cyclopentyl	8.87 ± 0.22 (3)	$C_{21}H_{27}N_3O$	225–5.5	CH_2Cl_2/Et_2O/hexanes	C, H, N	B
16	methyl	cyclopentyl	9.23 ± 0.35 (3)	$C_{22}H_{29}N_3O$	233.5–4.5	MeOH/H_2O	C, H, N	B
17	ethyl	cyclopentyl	9.80 ± 0.40 (3)	$C_{23}H_{31}N_3O$	251.5–3	MeCN	C, H, N	C
18	isopropyl	cyclopentyl	10.33 ± 0.28 (3)	$C_{24}H_{33}N_3O$	249.–5.50–5	MeCN	C, H, N	B
19	isobutyl	cyclopentyl	≤7 (4)	$C_{25}H_{35}N_3O$	238.5–9	MeOH/H_2O/NH_4OH	C, H, N	B
20	3-pentyl	cyclopentyl	7.3 ± 0.06 (4)	$C_{26}H_{37}N_3O$·maleate	220–1 dec	EtOAc	C, H, N	B
21	H	cyclohexyl	9.24 ± 0.24 (3)	$C_{22}H_{29}N_3O$·maleate	241–2 dec	EtOH	C, H, N	B
22	methyl	cyclohexyl	9.70 ± 0.11 (13)	$C_{23}H_{31}N_3O$	260–1.5	MeOH/H_2O	C, H, N	B
23	ethyl	cyclohexyl	9.25 ± 0.14 (3)	$C_{24}H_{33}N_3O$	253–5	MeCN	C, H, N	D
24	allyl	cyclohexyl	8.15 ± 0.05 (3)	$C_{25}H_{33}N_3O$	254–5	CH_2Cl_2/EtOH/H_2O	C, H, N	E
25	*n*-propyl	cyclohexyl	8.00 ± 0.15 (3)	$C_{25}H_{35}N_3O$	258–9	CH_2Cl_2/EtOH/H_2O	C, H, N	E
26	isopropyl	cyclohexyl	9.67 ± 0.10 (3)	$C_{25}H_{35}N_3O$	262.5–3.5	EtOH/H_2O	C, H, N	B
27	*n*-butyl	cyclohexyl	7.5 ± 0.14 (4)	$C_{26}H_{37}N_3O$	238–9	CH_2Cl_2/EtOH/H_2O	C, H, N	E
28	isobutyl	cyclohexyl	<7 (4)	$C_{26}H_{37}N_3O$	238.5–9	MeOH/H_2O/NH_4OH	C, H, N	B
29	3-pentyl	cyclohexyl	7.80 ± 0.24 (4)	$C_{27}H_{39}N_3O$·maleate	197–8	EtOAc	C, H, N	E
30	cyclopentyl	cyclohexyl	<7 (3)	$C_{27}H_{37}N_3O$·maleate	184–5	EtOAc	C, H, N	E
31	*n*-octyl	cyclohexyl	<7 (4)	$C_{30}H_{45}N_3O$	187.5–8	CH_2Cl_2/EtOH/H_2O	C, H, N	E
32	benzyl	cyclohexyl	<7 (3)	$C_{29}H_{35}N_3O$	259.5–60.5	CH_2Cl_2/EtOH/H_2O	C, H, N	E

[a] All compounds gave satisfactory analyses.

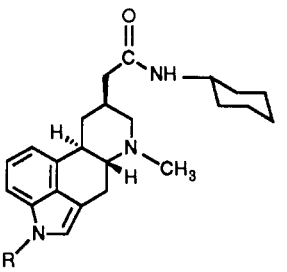
Table II. $5HT_2$ Receptor Affinities for Ergoline Cyclohexyltetrazoles



no.	R	−log K_B ± SE (n)	formula	mp, °C	recrystn solvent	analyses[a]	method
33	H	<6.52 (3)	$C_{22}H_{28}N_6$·maleate	226–7 dec	MeOH/Et_2O	C, H, N	F
34	methyl	<7.0 (3)	$C_{23}H_{30}N_6$·mesylate	>300	MeCN	C, H, N, S	F
35	*i*-propyl	<8 (3)	$C_{25}H_{34}N_6$·maleate	220.5–1.5 dec	MeCN/Et_2O	C, H, N	F

[a] All compounds gave satisfactory analyses.

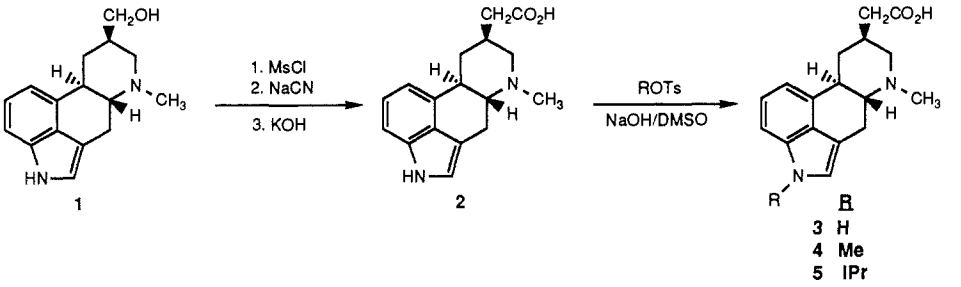
Table III. $5HT_2$ Receptor Affinities for Homologated Ergoline Cyclohexylcarboxamides



no.	R	−log K_B ± SE (n)	formula	mp, °C	recrystn solvent	analyses[a]	method
36	H	8.05 ± 0.07 (3)	$C_{23}H_{31}N_{30}$	182.5–3.5	MeOH/H_2O	C, H, N	B
37	methyl	8.97 ± 0.14 (4)	$C_{24}H_{33}N_3O$	211–12	MeOH/H_2O	C, H, N	B
38	isopropyl	8.88 ± 0.19 (8)	$C_{26}H_{37}N_3O$	215–16	MeOH/H_2O	C, H, N	B

[a] All compounds gave satisfactory analyses.

Scheme I



Scheme II

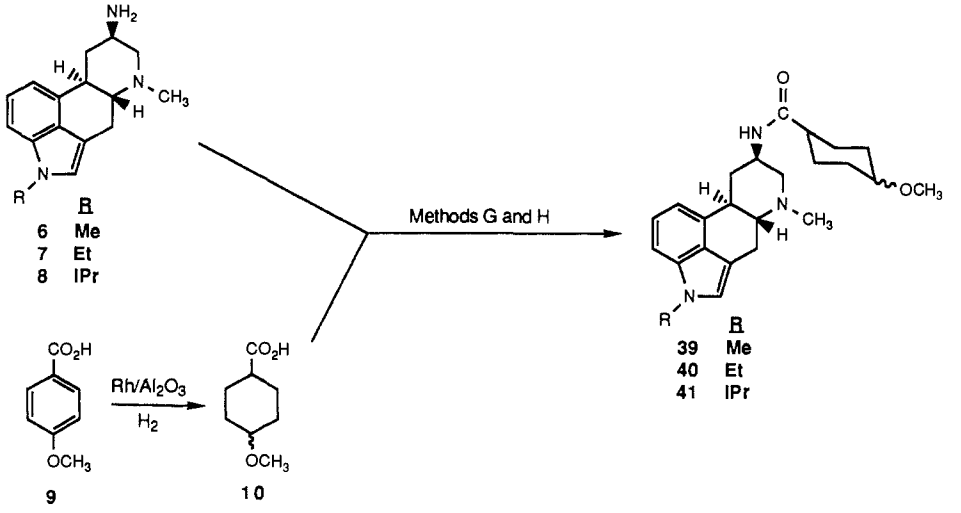
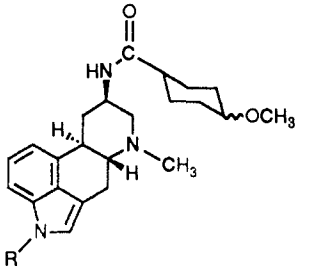


Table IV. $5HT_2$ Receptor Affinities of Ergoline "Reverse Amides"



no.	R	$-\log K_B \pm SE$ (n)	formula	mp, °C	recrystn solvent	analyses[a]	method
39	methyl	8.71 ± 0.07 (3)	$C_{24}H_{33}N_3O_2$·maleate	149–51	EtOH/Et_2O	C, H, N	G
40	ethyl	9.43 ± 0.20 (3)	$C_{25}H_{35}N_3O_2$·maleate	168–69.5	EtOH/Et_2O	C, H, N	G
41	*i*-propyl	9.64 ± 0.07 (4)	$C_{26}H_{37}N_3O_2$	272–7 dec	DMF/H_2O/NH_4OH	C, H, N	H

[a] All compounds gave satisfactory analyses.

4-methoxycyclohexanecarboxylic acid (**10**) involved the catalytic reduction of methyl 4-methoxybenzoate (methyl anisate) followed by hydrolysis to the acid. We now report the direct catalytic hydrogenation of 4-methoxybenzoic acid (**9**) to a 3:1 cis:trans mixture of **10**. The acylations to the reverse amides were then completed through the acid chlorides (method G) or mixed anhydrides with isobutyl chloroformate (method H).

Results and Discussion

Cyclopentyl and cyclohexyl amides were chosen for the study of the N^1 substituent effect, because in their analogous ergoline ester series, these groups had proven to impart high affinities as $5HT_2$ antagonists.[3] For comparison, several N^1-alkylated unsubstituted amides were also prepared. Indeed, as the data in Table I show, the cycloalkyl amides possessed considerably higher affinities as $5HT_2$ receptor antagonists than their unsubstituted amide counterparts. Interestingly, the relative differential in affinities decreased appreciably as the N^1 substituents increased from hydrogen to isopropyl, thereby emphasizing the importance of this substituent in enhancing $5HT_2$ receptor binding. Alkyl substituents larger than isopropyl effected an immediate and steep drop in affinity in all cases, indicating a probable steric effect. Also paralleling the ester series,[2] compounds with allyl and *n*-propyl N^1 substituents (**24** and **25**) appeared to constitute the alkyl chain length at which optimum affinity had been surpassed. Indeed, the only anomalous result was compound **22**, which had essentially the same binding affinity as **26**, thereby conferring equivalency of potency for the N^1-methyl and N^1-isopropyl in the cylohexyl amide series. This would also seem to suggest that the N^1 substituent

(10) Hofmann, A. *Helv. Chim. Acta* **1947**, *30*, 44.
(11) Noyce, D. S.; Weingarten, H. I. *J. Am. Chem. Soc.* **1957**, *79*, 3093.
(12) Krapcho, A. P.; Dundulis, E. A. *J. Org. Chem.* **1980**, *45*, 3236.

effect involves more than steric interactions alone, though steric considerations appear to predominate.

Since, in other chemical series, tetrazoles have been reported to be pharmacologically equivalent to carboxyl moieties,[13] compounds **21**, **22**, and **26** were elaborated to their ergoline tetrazole counterparts. From the data in Table II it is obvious that pharmacological equivalence was not preserved in this series. The affinity data in Table III show that the homologation of compounds **21**, **22**, and **26** resulted in a 10-fold decrease in $5HT_2$ receptor affinities. As with parent compounds **22** and **26**, the virtual parity of the methyl and isopropyl N^1 substituent effect of the parent compounds is preserved in homologues **37** and **38**.

Unlike the tetrazole and homologue derivatives, the "reverse amides" of Table IV all maintained very high affinity, roughly equal to that of the corresponding amides (see Table I). Maximum affinity was again achieved by isopropyl compound **41**.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Identities of all compounds were confirmed by ^1H NMR, mass spectra, and elemental analysis after block drying at 120 °C. Elemental analyses were provided by the Physical Chemistry Department of the Lilly Research Laboratories and are indicated by the symbols of the elements and are within ±0.4% of theoretical values. Reactions were monitored and the purity of products was determined by HPLC (Zorbax CN, mobile phase 4:1 CH_3OH/0.1 M NH_4OAc, flow rate = 1 mL/min, UV detector set at 290 nm; or Altex ODS, mobile phase 4:1 CH_3OH/0.1 M NH_4OAc, flow rate = 1 mL/min, UV detector fixed at 254 nm). The experimental procedures described below are representative of the procedures used to prepare the ergolines listed in Tables I–IV.

Method A. (8β)-1,6-**Dimethylergoline-8-carboxamide** (**12**). A mixture of 3.06 g (10 mmol) of 1-methyldihydrolysergic acid,[6] 2.43 g (15 mmol) of 1,1′-carbonyldiimidazole, and 80 mL of CH_2Cl_2 were combined under a nitrogen atmosphere and stirred for 1 h to give a turbid brown solution. Anhydrous NH_3 was bubbled in below the surface with cooling until a heavy precipitate developed. After a half-hour, the solvent was removed in vacuo and the resulting solid was heated in 50 mL of refluxing MeOH. The insoluble matter was removed by filtration. The filtrate was diluted with 90 mL of H_2O and 10 mL of NH_4OH. The light brown precipitate produced was collected by suction filtration, reheated to reflux in 75 mL of MeOH, stirred with 1 g of Calgon ADP carbon, and filtered hot over Hyflo Super Cel. The pale yellow filtrate was diluted with an equal volume of 2 N NH_4OH and cooled for 4 h. The off-white product was collected and dried in vacuo to give 1.36 g of **2** (48%).

Method B. (8β)-*N*-**Cyclopentyl-6-methyl-1-(1-methylethyl)ergoline-8-carboxamide** (**18**). A mixture of 6.25 g (20 mmol) of 1-isopropyldihydrolysergic acid,[6] 2.76 g (20 mmol) of anhydrous K_2CO_3, and 150 mL of dry DMF was heated to 140 °C under a nitrogen atmosphere. The resulting potassium salt was cooled to −52 °C. The addition of 3.0 g (22 mmol) of isobutyl chloroformate to the hazy brown solution was controlled so as to maintain the temperature at <−45 °C. Following a 10 min stir, 2.55 g (30 mmol) of cyclopentylamine was added dropwise so as to maintain the temperature at <−45 °C. The mixture was allowed to warm to ambient temperature while stirring overnight under nitrogen. It was then quenched by pouring into 20 mL of NH_4OH/300 mL of ice H_2O. The precipitate was collected by filtration, dried, and heated to reflux in 350 mL of CH_3CN. The small amount of insoluble material was removed by filtration, giving a clear golden filtrate. The white crystals which formed on cooling were filtered and dried in vacuo to give 3.49 g of **8** (46%).

Method C. (8β)-*N*-**Cyclopentyl-1-ethyl-6-methylergoline-8-carboxamide** (**17**). A solution of 3.41 g (40 mmol) of cyclohexylamine in 30 mL of $ClCH_2CH_2Cl$ was added to a mixture of 2.98 g (10 mmol) of 1-ethyldihydrolysergic acid,[6] 2.97 g (12 mmol) of 2-ethoxy-1-(ethoxycarbonyl)-1,2-dihydroquinoline (EEDQ), and 50 mL of $ClCH_2CH_2Cl$. After stirring at 70 °C for 5 h, it was cooled to 25 °C and extracted with 80 mL of 2 N NH_4OH. The organic layer was concentrated in vacuo to a brown oil and crystallized twice from CH_3CN to give, after drying in vacuo, 0.53 g of **7** (14.5%) as white needles.

Method D. (8β)-*N*-**Cyclohexyl-1-ethyl-6-methylergoline-8-carboxamide** (**23**). A mixture of 1.56 g (5 mmol) of (8β)-6-methyl-1-ethylergoline-8-carboxylic acid hydrazide,[6,14] 3.97 g (40 mmol) of cyclohexylamine, 2.14 g (10 mmol) of $NaIO_4$, and 100 mL of H_2O were stirred at 25 °C for 1 h. The excess cyclohexylamine was extracted with toluene, and then the product was extracted with $ClCH_2CH_2Cl$. After concentration in vacuo, the crude product was crystallized from CH_3CN to give 0.51 g of **13** (26.9%) as an off-white solid.

Method E. (8β)-*N*-**Cyclohexyl-1-cyclopentyl-6-methylergoline-8-carboxamide, (*Z*)-2-Butenedioate** (1:1) (**30**). A solution of 3.38 g (10 mmol) of 1-cyclopentyldihydrolysergic acid[6] in 60 mL of DMF under a nitrogen atmosphere was chilled to −10 °C. $POCl_3$ (25 mmol) was added dropwise at <0 °C. After 30 min, 4.96 g (50 mmol) of cyclohexylamine was added dropwise, also at <0 °C. After 1 h, the solution was poured into 170 mL of H_2O/10 mL of concentrated NH_4OH. The resulting precipitate was collected by filtration, and dissolved in 50 mL of CH_2Cl_2/50 mL of EtOH. The CH_2Cl_2 was distilled off until precipitate appeared. The mixture was then cooled to 20 °C and filtered. The dried filter cake (2.5 g, 6 mmol) was refluxed in 30 mL of EtOAc. The addition of a solution of 0.69 g (6 mmol) of maleic acid in 20 mL of hot EtOAc rapidly produced a clear dark yellow solution, followed promptly by the formation of a heavy crystalline precipitate. After cooling to 20 °C, the product was collected by filtration and dried in vacuo to give 3.13 g of **30** (58.4%) as a dull white solid.

Method F. (8β)-8-(1-**Cyclohexyl-1*H*-tetrazol-5-yl**)-1,6-**dimethylergoline, Methanesulfonate** (1:1) (**34**). A mixture of 1.83 g (5 mmol) of **22**, 0.6 mL (7.6 mmol) of pyridine, and 50 mL of $ClCH_2CH_2Cl$ was stirred under a nitrogen atmosphere followed by the addition of 1.56 g (7.5 mmol) of PCl_5 in one portion. This was stirred for 4 h. Meanwhile, sodium azide (6.5 g, 100 mol) was stirred in a mixture of 25 mL each of water and $ClCH_2CH_2Cl$ as 8.0 mL (96 mmol) of concentrated HCl was added dropwise with cooling. The layers were then separated and the organic layer was dried ($MgSO_4$). This hydrazoic acid solution was then added to the above mixture and allowed to react for 16 h. After extraction with 50 mL of concentrated NH_4OH, the solution was decolorized with 5 g of Calgon ADP carbon, filtered through Hyflo Super Cel, and concentrated in vacuo to 1.83 g of yellow foam. This was dissolved in 25 mL of $CHCl_3$ along with 0.32 mL (5 mmol) of 98% methanesulfonic acid. The dropwise addition of an equal volume of Et_2O produced crystals. The beige product was collected by filtration, partitioned between 25 mL each of $CHCl_3$ and concentrated NH_4OH, dried over Na_2SO_4, and concentrated in vacuo to give 1.28 g of a gummy yellow-green solid. This was dissolved in 30 mL of refluxing CH_3CN and treated with 0.21 mL (3.3 mmol) of methanesulfonic acid. The resulting thick precipitate was collected by filtration and dried in vacuo to give 1.36 g of **34** (56%) as a white solid.

4-Methoxycyclohexanecarboxylic Acid (**10**). A mixture of 76.0 (0.5 mol) of **9**, 400 mL of THF, and 15 g of 5% Rh/Al_2O_3 was hydrogenated at 50 psi and 50 °C for 16 h. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo to 77.8 g of **10** (98.3%) as an oil (3:1 cis:trans), bp 135–137 °C (6 mmHg) [lit.[11] bp 142 °C (6 mmHg)]. Anal. ($C_8H_{14}O_3$) C, H.

Method G. (8β)-*N*-(1-**Ethyl-6-methylergolin-8-yl**)-4-**methoxycyclohexanecarboxamide** (*Z*)-**2-Butenedioate** (1:1) (**40**). A solution of 1.00 g (3.71 mmol) of (8β)-1-ethyl-6-methylergolin-8-amine (**7**) in 25 mL of dry pyridine was placed under a nitrogen atmosphere and chilled to 5 °C. The addition of 0.85 g (4.8 mmol) the acid chloride of **10** made via oxalyl chloride in toluene[15] produced an exotherm to 11 °C. After 2 h, the reaction was quenched into 150 mL of cold H_2O. The crystals that formed were collected by filtration and dried to give 1.37 g of solid. This

(13) Lipinski, C. A. *Annu. Rep. Med. Chem.* **1986**, *21*, 283.

(14) Stoll, A.; Hofmann, A. *Helv. Chim. Acta* **1943**, *26*, 2070.

(15) Adams, R.; Ulich, L. H. *J. Am. Chem. Soc.* **1920**, *42*, 599.

was combined with 0.44 g (3.79 mmol) of maleic acid in 25 mL of MeOH to give a solution. The slow addition of 250 mL of Et_2O produced crystals which were collected and dried in vacuo to yield 1.70 g of **40** (87.2%) as an off-white solid.

Method H. (8β)-***N*-[6-Methyl-1-(1-methylethyl)ergolin-8-yl]-4-methoxycyclohexanecarboxamide** (41). A solution of 1.67 g (10.54 mmol) of **10**, 1.07 g (10.54 mmol) of Et_3N, and 30 mL of DMF under a nitrogen atmosphere was chilled to −20 °C. The addition of 1.44 g (10.54 mmol) of isobutyl chloroformate produced an immediate precipitate. After 10 min, (8β)-1-(methylethyl)-6-methylergoline-8-amine (**8**, 2.00 g, 7.06 mmol) was added and the mixture was stirred at −20 °C for 30 min, and then allowed to warm to ambient temperature. It was then added to a cold solution of 7 mL of concentrated NH_4OH in 150 mL of H_2O. The resulting precipitate was collected by filtration. The filter cake was slurried in refluxing methanol, cooled to 0 °C, filtered, and dried in vacuo to give 1.62 g of 41 (54.2%).

Isolation of Tissue for Receptor Antagonist Studies. Male Wistar rats (150–300 g) (Harlan Sprague–Dawley, Inc.) were killed by cervical dislocation. External jugular veins from the rats were dissected free of connective tissue, cannulated in situ with polyethylene tubing (PE-50, o.d. = 0.97 mm), and placed in petri dishes containing Krebs' bicarbonate buffer (see below). The tips of two 30-gauge stainless steel hypodermic needles bent into an L shape were slipped into the polyethylene tubing. Vessels were gently pushed from the cannula onto the needles. The needles were then separated so that the lower one was tied with thread to a transducer. This procedure for ring preparations (circular smooth muscle) of blood vessels has been described previously.[16]

Tissues were mounted in organ baths containing 10 mL of modified Krebs solution of the following composition (millimolar concentrations): NaCl, 118.2; KCl, 4.6; $CaCl_2 \cdot H_2O$, 1.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; dextrose, 10.0; and $NaHCO_3$, 24.8. Tissue bath solutions were maintained at 37 °C and aerated with 95% O_2/5% CO_2. An initial optimum resting force of 1 g was applied to the jugular vein. Isometric contractions were recorded as changes in grams of force on a Beckman Dynograph with Statham UC-3 transducers and a microscale accessory attachment. Tissues were allowed to equilibrate for 1–2 h before exposure to drugs.

Determination of Apparent $5HT_2$ Receptor Antagonist Dissociation Constants. After control cumulative contractile responses to serotonin in the jugular vein were obtained, vessels were incubated with an appropriate concentration of antagonist for 1 h. Contractile responses to serotonin were then repeated in the presence of the antagonist. Contraction to serotonin was evaluated in the jugular vein as this tissue produced marked responses to serotonin in the absence of α receptors.[17] Only one antagonist concentration was examined in each tissue. Apparent antagonist dissociation constants (K_B) were determined for each concentration of antagonist according to the following equation:

$$K_B = [B]/(\text{dose ratio} - 1)$$

where [B] is the concentration of the antagonist and dose ratio is the ED_{50} of the agonist in the presence of the antagonist divided by the control ED_{50}. These results were then expressed as the negative logarithm of the K_B (i.e., $-\log K_B$).

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Registry No. **3**, 18051-15-5; **4**, 18051-22-4; **5**, 123700-80-1; **6**, 123700-81-2; **7**, 123700-82-3; **8**, 119154-51-7; **9**, 100-09-4; *cis*-**10**, 73873-59-3; *trans*-**10**, 73873-61-7; 11, 2410-19-7; 11 acid, 5878-43-3; **12**, 2312-51-8; 12 acid, 35470-52-1; **13**, 123700-83-4; **13** acid, 41710-25-2; **13** acid hydrazide, 1752-43-8; **14**, 123700-84-5; 14 acid, 41710-27-4; **15**, 101655-79-2; **16**, 123805-55-0; **17**, 123700-85-6; **18**, 121588-81-6; **19**, 123700-86-7; **19** acid, 109839-85-2; **20**, 123700-87-8; **20**·maleate, 123701-06-4; **20** acid, 109839-86-3; **21**, 2300-80-3; **21**·maleate, 50798-40-8; **22**, 121588-80-5; **23**, 121588-82-7; **24**, 123700-88-9; **24** acid, 41710-28-5; **25**, 123700-89-0; **25** acid, 41710-26-3; **26**, 121588-75-8; **27**, 123700-90-3; **27** acid, 123701-05-3; **28**, 123700-91-4; **29**, 123700-92-5; **30**, 123700-93-6; **30**·maleate, 123701-10-0; **30** acid, 109839-87-4; **31**, 123700-94-7; **31**·maleate, 123701-11-1; **31** acid, 109839-89-6; **32**, 123700-95-8; **32** acid, 2618-03-3; **33**, 123700-96-9; **33**·maleate, 123701-07-5; **34**, 123700-97-0; **34**·mesylate, 123701-08-6; **35**, 123700-98-1; **35**·maleate, 123701-09-7; **36**, 123700-99-2; **37**, 123701-00-8; **38**, 123701-01-9; *cis*-**39**, 123701-02-0; *trans*-**39**, 123805-57-2; *cis*-**39**·maleate, 123805-60-7; *trans*-**39**·maleate, 123877-28-1; *cis*-**40**, 123701-03-1; *trans*-**40**, 123805-58-3; *cis*-**40**·maleate, 123805-56-1; *trans*-**40**·maleate, 123877-29-2; *cis*-41, 123701-04-2; *trans*-41, 123805-59-4; cyclopentylamine, 1003-03-8; cyclohexylamine, 108-91-8.

(16) Hooker, C. W.; Calkins, P. J.; Fleisch, J. H. *Blood Vessels* **1977**, *14*, 1.

(17) Cohen, M. L.; Wiley, K. S. *J. Pharmacol. Exp. Ther.* **1978**, *205*, 400.

Antimitotic Agents: Synthesis of Imidazo[4,5-*c*]pyridin-6-ylcarbamates and Imidazo[4,5-*b*]pyridin-5-ylcarbamates

Carroll Temple, Jr.

Southern Research Institute, Birmingham, Alabama 35255-5305. *Received May 30, 1989*

Cyclization of ethyl 5,6-diamino-4-hydrazinopyridin-2-ylcarbamate (**10**) with a mixture of CS_2 and Et_3N in dimethylacetamide gave mainly ethyl 1,4-diamino-2(3*H*)-thioxoimidazo[4,5-*c*]pyridin-6-ylcarbamate (**15**), whereas, in the absence of dimethylacetamide, a double cyclization gave mainly ethyl 5-amino-2(1*H*)-4-dithioxodiimidazo-[4,5-*b*:5,4-*c*]pyridin-7-ylcarbamate (16). Cyclization of the benzylidenehydrazino derivative (**6**) of **10** with either CS_2–Et_3N or $(EtO)_3CH$–HCl gave 1-(benzylideneamino)imidazo[4,5-*c*]pyridines **11** and **7** as major products and 7-(benzylidenehydrazino)imidazo[4,5-*b*]pyridines **12** and **8** as minor products. Dethiolation of **11** to give **7** and of **12** to give **8** was effected with excess Raney nickel in refluxing ethanol. The benzylidene group of **11** was removed with hydrazine in ethanolic HCl to give **15**. This key compound was condensed with benzaldehydes to give 1-benzylideneamino derivatives (**20**, **21**) and alkylated with benzyl halides to give 2-benzylthio derivatives (**24**–**26**). In addition, cyclization of ethyl 5,6-diamino-4-(benzylidene-1-methylhydrazino)pyridin-2-ylcarbamate (**30**) with $(EtO)_3CH$ provided a method for the synthesis of an imidazo[4,5-*b*]pyridine (**23**) uncontaminated with isomeric products. The presence of an aryl group in both the imidazo[4,5-*c*]- and -[4,5-*b*]pyridines gave compounds that inhibited proliferation of growth and caused mitotic arrest against lymphoid leukemia L1210 at micromolar concentrations. However, the more active in vitro compounds (**7**, **8**, **24**–**26**) gave only borderline activity in mice against lymphocytic leukemia P388.

The *Vinca* alkaloids (e.g., vincristine), clinically used anticancer agents, prevent the polymerization of tubulin to give microtubules and as a result inhibit cell division.[1] A new class of antimitotic agents, 1,2-dihydropyrido[3,4-

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