Synthesis and Biological Evaluation of Analogues of Cryptolepine, an Alkaloid Isolated from the Suriname Rainforest[1]

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Bioassay-guided fractionation of an extract of a mixture of *Microphilis guyanensis* and *Genipa americana* collected in the rainforest of Suriname yielded the known alkaloid cryptolepine (**2**) as the major active compound in a yeast bioassay for potential DNA-damaging agents; the same compound was later reisolated from *M. guyanensis*. The structure of cryptolepine was identified unambiguously by spectral data and by its total synthesis. Several cryptolepine derivatives (**3**−**29**, **32**−**41**) were synthesized based on modifications of the C-2, N-5, N-10, and C-11 positions. Two cryptolepine dimers (**30**, **31**) were also prepared. The structure modifications did not result in compounds with a higher potency than the parent compound cryptolepine in the yeast assay system, although some derivatives did show significant activity. Selected compounds (**6**, **7**, **17**, **22**, **23**, **26**, and **27**) were also tested for cytotoxicity in mammalian cell culture, and two compounds showed significant cytotoxic activity.

In continuation of our search for anticancer and other bioactive agents from the Suriname rainforest,[2] an extract of a mixture of *Microphilis guyanensis* (A. DC) Pierre (Sapotaceae) and *Genipa americana* L. (Rubiaceae) collected on an ethnobotanical basis near Asindopo village in the rain forest of Suriname[3] was found to show strong but nonselective activity in a yeast bioassay utilizing mutants 1138, 1140, and 1353 of the yeast *Saccharomyces cerevisiae*.[4] In this assay system, general DNA-damaging agents and antifungal agents show activity against all three strains.[2] This extract was thus selected for further investigation.

Results and Discussion

Isolation Studies. Bioassay-guided fractionation of the extract was carried out using a combination of column chromatography with Sephadex LH-20 and MCI gel to yield compound **1** as the major active component. Once the salt nature of **1** became clear it was obtained more efficiently by an acid−base partition using methods described in the literature,[5] followed by further purification by chromatography on LH-20.

The structure of compound **1** was assigned as cryptolepine hydrochloride by NMR techniques and HRFABMS, and its anion was determined as chloride using negative-ion FABMS (Cl^- = *m*/*z* 35, 37). The ^1H and ^{13}C NMR resonances for **1** were assigned unambiguously by spectral data including $^1H-^1H$ COSY, HMBC, HMQC, and ROESY spectra, and are listed in the Experimental Section; most NMR data are reported for the free base cryptolepine (**2**) instead of its hydrochloride **1**. The free base form of compound **1** was identified as cryptolepine (**2**) by comparison of its ^1H NMR data with literature data. Compound **1** was active in the 1138, 1140, and 1353 yeast strains, with IC_{12} values of 0.6 μg/mL in each strain. It was also cytotoxic to the M109 cell line, with an IC_{50} of <1 μg/mL.

Following the completion of this work an extract of *M. guyanensis* (A. DC) (Sapotaceae) was reinvestigated and found to contain cryptolepine, thus implicating this plant as the source of cryptolepine in the mixed extract.

Cryptolepine has previously been isolated from *Cryptolepis triangulariz* N. E. Br.,[6] *Cryptolepis sanguinolenta* from Africa,[7] and *Sida* sp. (Malvaceae) from Sri Lanka.[8] Most recently it has been isolated as the antihyperglycemic principle of *C. sanguinolenta*,[9] and the introduction to this paper provides a useful bibliography of previous work on cryptolepine, including its antimicrobial, antibacterial, antiinflammatory, antihypertensive, antipyretic, antimuscarinic, antithrombotic, noradrenergic recepter antagonistic, vasodilative, antimalarial, and antihyperglycemic biological activities. Cryptolepine belongs to the indoloquinoline alkaloid family, a relatively rare group of alkaloids in Nature, and interestingly its total synthesis was accomplished before its isolation from nature.[10] Because of its various interesting biological activities, several synthetic pathways related to cryptolepine and its derivatives have been established.[11] Other examples of natural indoloquinolines include quindoline, hydroxycryptolepine, cryptoheptine, and cryptoquindoline,[12] cryptospirolepine,[13] neocryptolepine,[14] and isocryptolepine.[15]

Synthetic Studies. Because of the promising biological activity of cryptolepine in both our yeast assays and in the M109 cytotoxicity assay, we decided to carry out a limited study of the structure−activity relationships of this class of compound. A similar study was also carried out by Bierer and co-workers independently of this work.[16] The compounds prepared by Bierer et al. are, however, for the most

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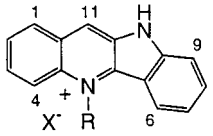
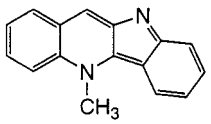
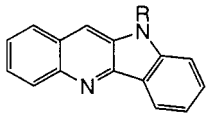


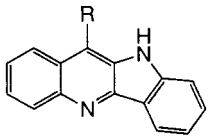
1 R = CH_3; X = Cl
5 R = CH_3; X = I
6 R = CH_3; X = Tf
7 R = CH_2CH_3; X = I
8 R = $CH_2CH_2CH_3$; X = I
9 R = $CH_2CH_2CH_2CH_3$; X = Br



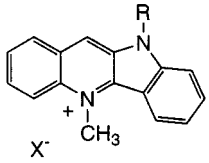
2 Cryptolepine



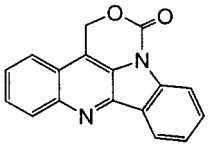
4 R = H
10 R = CH_3
11 R = CH_2CH_3
12 R = $CH_2CH_2CH_3$
13 R = $CH_2CH_2CH_2CH_3$
14 R = $CH_2CH{=}CH_3$
15 R = $CH_2C_6H_5$
16 R = $COCH_3$



3 R = CO_2H
23 R = CO_2CH_3
24 R = CH_2OH
25 R = CHO
26 R = CH_3
28 R = COCl
29 R = $COOCH_2CH_2OH$



17 R = CH_3; X = Tf
18 R = CH_2CH_3; X = Tf
19 R = $CH_2CH_2CH_3$; X = Tf
20 R = $CH_2CH_2CH_2CH_3$; X = Tf
21 R = $CH_2CH{=}CH_3$; X = Tf
22 R = $COCH_3$; X = Cl



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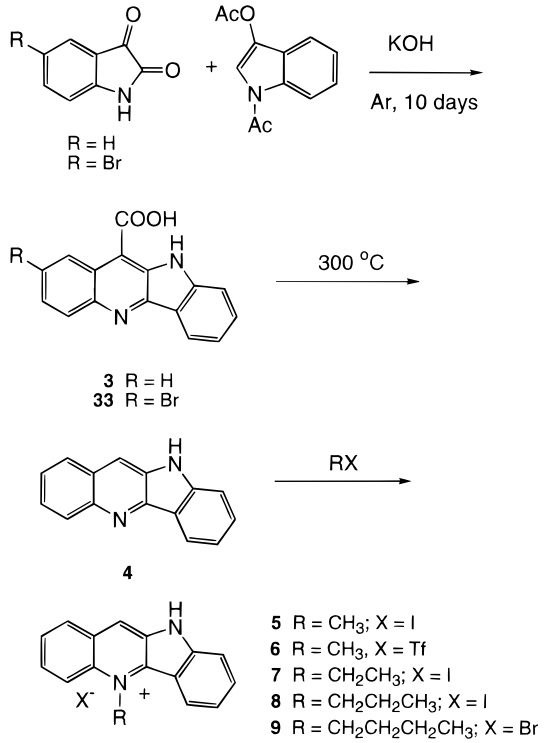
part different than those described in this work, and the bioassays used are also different.

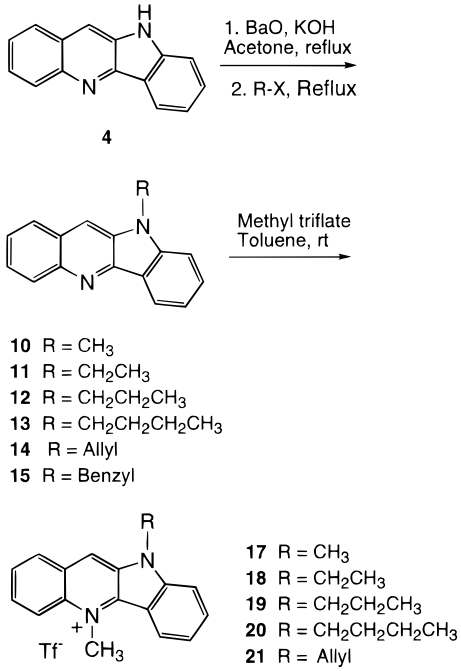
The total synthesis of 5-*N*-methylquindolinium salts was achieved by the methods described in the literature (Scheme 1).[9−11] Thus quindoline 11-carboxylic acid (**3**) was prepared by condensation of isatin and indoxyl-1,3-diacetate in aqueous KOH under argon,[10,11] and decarboxylated by heating at 300 °C to yield quindoline (**4**) in sublimed form. 5-*N*-Methylation of **4** could then be carried out in two ways: treatment of quindoline mixed with iodomethane in methanol in a tightly closed vial at 110 °C for 4 h gave the iodide salt (**5**),[10] while treatment with methyl triflate in toluene at room temperature[9] gave the triflate salt (**6**). The 5-*N*-ethyl-, 5-*N*-propyl-, and 5-*N*-butylquindolines (**7**, **8**, and **9**, respectively) were prepared by alkylation with iodoethane, propyl iodide, and butyl bromide, respectively, at high temperatures in different solvents. Attempted alkylation using isopropyl bromide and 1,4-dibromobutane failed to produce quaternary salts.

Modification at the N-10 position was achieved by treating quindoline **4** with base (BaO and KOH), followed by treatment with the selected alkylating agent (methyl, ethyl, or propyl iodide, butyl bromide, allyl bromide, or benzyl bromide), as described for the synthesis of 10-*N*-methylquindoline (**10**),[9] to yield compounds **10**−**15** (Scheme 2). 10-*N*-Acetylquindoline (**16**) was obtained from treatment of quindoline (**4**) with acetic anhydride in pyridine. Since methyl substitution on N-5 is the most effective substitution for biological activity in our assays, all of the 10-*N*-alkyl quindolines obtained were converted to their 5-*N*-methyl derivatives (**17**−**21**) by treatment with methyl triflate. 5-*N*-Methyl-10-*N*-acetylquindolinium chloride (**22**) was prepared by acetylation of **1** in the usual way.

Scheme 1



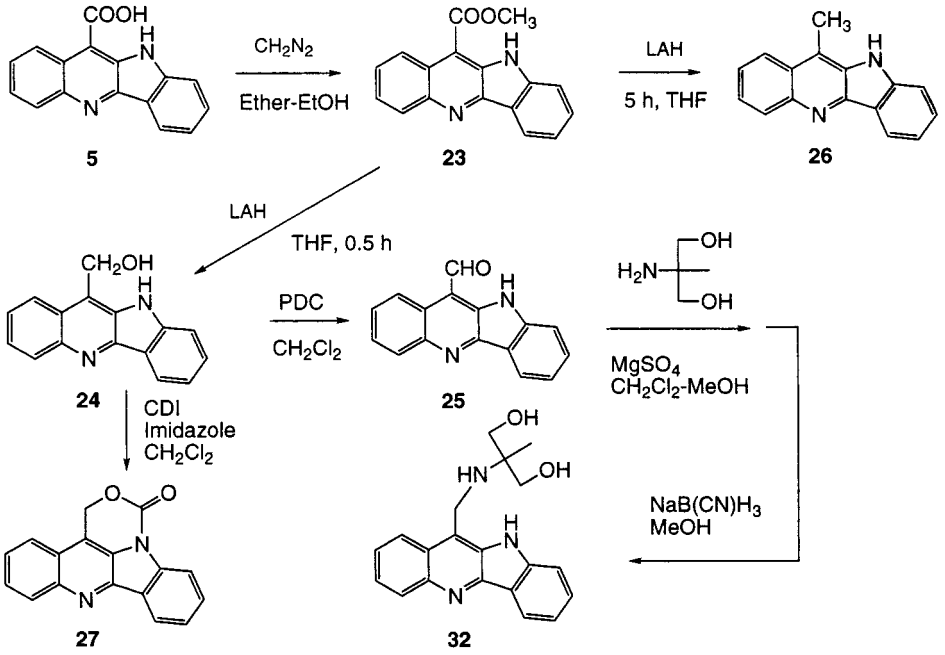
Scheme 2



For modifications at the 11 position (Scheme 3), quindoline 11-carboxylic acid (**3**) was methylated to its methyl ester **23** with diazomethane, and **23** was reduced under moderate conditions to the alcohol **24** with lithium aluminum hydride (LAH). The use of excess LAH and a longer duration of reflux gave 11-methylquindoline (**26**) as the only reduced product, while oxidation of **24** with pyridinium dichromate (PDC) gave aldehyde **25**. The presence of the 11-CH_2OH and 10-NH groups of compound **24** allowed the formation of cyclic carbamate **27** by the use of 1,1′-carbonyl diimidazole (CDI) and imidazole in CH_2Cl_2.

Further transformations of these 11-substituted compounds yielded additional products. Thus two dimers were

Scheme 3

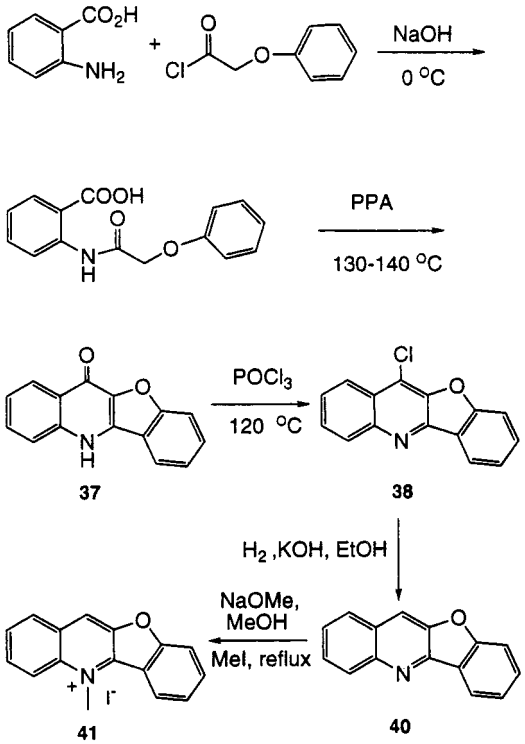


prepared through acid chloride **28**; the dimeric diester **30** and the cyclic dimer **31**. Diester **30** was prepared from mono-ester **29**, which itself was formed by treatment of acid chloride **28** with ethylene glycol. A second acylation of **29** with **3** under more vigorous conditions gave **30**. The dimer **31** was made by self-condensation between two acid chlorides (**28**), as previously described.[11a] Finally in this group an aminopropanediol side chain was attached at the C-11 position of **25** through reductive coupling of 2-amino-2-methyl-1,3-propanediol to give compound **32**; similar derivatives of polycyclic aromatic hydrocarbons have shown improved anticancer activity.[17]

2-Bromoquindoline-11-carboxylic acid (**33**) was prepared through condensation of 5-bromoisatin and indoxyl-1,3-diacetate. The decarboxylation of **33** at 300 °C led to both decarboxylation and debromination, to generate quindoline (**4**) as the major product. 11-Carboxylquindoline was found to be the major product when the temperature was controlled at 270−280 °C for 1 h, indicating that debromination occurs before decarboxylation. 2-Bromoquindoline could thus not be obtained in acceptable yield by this route. Some 2-bromo-11-substituted quindolines were prepared, including the methyl ester **34**, the acid halide **35**, and the amide **36**. Bioassay of compound **34** gave an IC_{12} value of 100 μg/mL in the 1138 strain, which was worse than that of compound **23** lacking the bromine substitution. This indicated that the 2-bromo substitution decreases activity, and the preparation of other derivatives of 2-bromo-substituted quindolines was not pursued.

A final modification was the substitution of an oxygen atom for the nitrogen atom at the 10-position. The ring system of the target compound **40** has been synthesized previously.[18] We modified the route to improve the yield, shown in Scheme 4; a similar strategy has been applied to the synthesis of quindoline (**4**) from quinolone.[11c] Intermediates **37** and **38**[18c] and target compound **40** were synthesized. Finally, 5-*N*-methylation was achieved by refluxing a mixture of compound **40**, MeI and MeOH in the presence of NaOMe. Compound **39**, in which the 11-chloro substituent is converted to an 11-iodo substituent, was obtained as the major product when 5-*N*-methylation of compound **38** was attempted under the same conditions used for

Scheme 4



compound **41**. Presumably iodide ion generated from reaction of MeI with methoxide acts as a nucleophile to displace the 11-chloro substituent, and *N*-methylation is inhibited by the electron-withdrawing nature of the halo substituent.

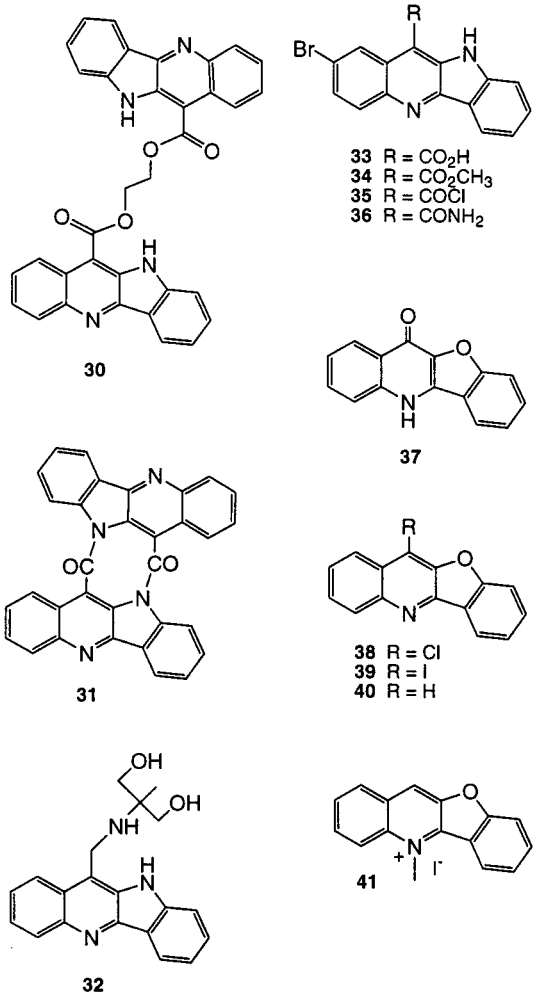
Biological Studies. The bioassay results are shown in Table 1; most of the compounds were tested only in the 1138 yeast strain, so these results will be discussed first.

The initial conclusion is that methylation at the 5-*N*-position is critical for activity. Thus quindoline (**4**) has an IC_{12} of 150 μg/mL, but its 5-*N*-methyl derivative **1** has an IC_{12} of 0.6 μg/mL. Replacement of the methyl group at the 5-*N* position with an ethyl group decreases the activity significantly, and the activity decreases further as the length of the N-5 alkyl chain increases. Thus the methyl,

Table 1. Bioactivity of Cryptolepine Analogs[a]

compound	IC_{12} (1138), μg/mL	M109 cytotoxicity, μM
1	0.6	
2	0.5, 1.0, 5.0	
4	150	
5	1.5	
6		<1
7	40	<50
8	400	
16	80	
17	5	<10
18	80	
19	125	
20	90	
21	200	
22	5	<1
23	40	<10
24	125	
26	50	<10
27	50	<10
34	100	
36	400	
41	400	

[a] Compounds **3**, **9**, **15**, **25**, **29**−**32**, and **37**−**40** had IC_{12} values >400 μg/mL in the 1138 yeast strain.



ethyl, propyl, and butyl analogues **1**, **7**−**9** gave IC_{12} values of 0.6, 40, 400, and >400 μg/mL in the 1138 assay.

Methylation at the N-10 position also causes a slight decrease in activity (compare **1** with IC_{12} 0.6 μg/mL and **17** with IC_{12} 5 μg/mL). Increasing the size of the 10-substituent causes further loss of activity, as indicated by a comparison of **18**, **19**, and **20** (IC_{12} 80, 125, and 90 μg/mL, respectively). On the other hand, *N*-acetylation (compound **22**) gives a product which is as active as the *N*-methyl analogue **17** (IC_{12} 5 μg/mL), suggesting that the *N*-acetyl analogue **22** may be partially hydrolyzed to **1**.

Substitution at C-11 with the polar electron-withdrawing groups COOH and CHO, compounds **3** and **25**, leads to greatly reduced activity (IC_{12} > 400 μg/mL in each case). However, the activity is reduced by a lesser amount when the C-11 substituent is carbomethoxy, hydroxymethyl, or methyl (compounds **23**, **24**, and **26**, with IC_{12} values of 40, 125, and 50 μg/mL, respectively). These results indicate that the polarity and electron withdrawing characteristics of C-11-substituted groups may affect the activity.

Replacement of N-10 by an oxygen atom (compounds **38**−**41**) caused the loss of activity, with IC_{12} values ≥400 μg/mL. Finally, both dimers **30** and **31** were inactive at 400 μg/mL, as was compound **32** with the 2-amino-2-methyl-1,3-propanediol side chain.

Selected analogues **6**, **7**, **17**, **22**, **23**, **26**, and **27** were tested for cytotoxicity to the M109 Madison lung carcinoma cell culture. The most active compounds were cryptolepine hydrotriflate (**6**) and the *N*-acetyl derivative **22**. As noted above, the activity of **22** may be due to a ready hydrolysis to the free base form. Compounds **6** and **22** were also tested in a panel of 25 cell lines; both compounds gave similar patterns, with the cell lines OVCAR-3 (IC_{50} 0.5 μM) and A431 (IC_{50} 0.34 μM) being the most sensitive and M109 (IC_{50} 5.6 μM) and MLF (IC_{50} 4.9 μM) being the least sensitive.

Compounds **6** and **22** were also subjected to analysis of their effect on cell cycle dependent growth disturbances. The results showed a G1 cell cycle arrest and apoptosis in A2780/p53wt cells at 5 × IC_{50}. Higher concentrations showed a dose-dependent increase in apoptosis. This effect appears to be p53 dependent since A2780/mutp53 cells showed no arrest in G1 and at 10 × IC_{50} showed a slow cell cycle traverse with some arrest at G2/M. Apoptosis was present only at 20 × IC_{50}. These characteristics are typical of DNA-damaging agents.

In conclusion, cryptolepine was isolated by bioassay-guided fractionation of a mixture of two Surinamese plants, and its source was subsequently identified as *M. guyanensis*. Structure modification studies have shown that the N-5 methyl substitution is critical for activity, and that alkyl substitution on N-10 and replacement of 5-*N*-methyl with larger alkyl groups causes reduction of activity. Substitution on C-11 also led to decreased activity. The rest of the modifications, including dimers, 2-bromo substitutions, replacement of 10-NH with an oxygen, and addition of a side chain at the C-11 position all cause total loss of the yeast inhibitory activity. Besides cryptolepine hydrochloride itself (**1**), the most active compounds were **17** and **22**.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in various deuterated solvents ($CDCl_3$, DMSO-d_6, and CD_3OD) on a Varian Unity 400 NMR instrument at 399.951 MHz for ^1H and 100.578 MHz for ^{13}C, using standard Varian pulse sequence programs (*J* values are in Hz). LRMS were taken on a VG 7070E-HF at VPI&SU. Exact mass measurements were obtained at the Nebraska Center for Mass Spectrometry. Other conditions were as previously described.[2] All chemicals were purchased from Sigma or Aldrich and were used without further purification.

Plant Material. The plants *M. guyanensis* (A. DC) Pierre (Sapotaceae) and *Genipa americana* L. (Rubiaceae) were collected near Asindopo Village, Suriname, as a part of an effort to investigate plant mixtures as used by the tribal

healers; *M. guyanensis* was also collected as a single sample. Voucher specimens of the plants are located in the National Herbarium of Suriname, Paramaribo, Suriname. The mixed plants were dried, ground, and extracted with EtOAc followed by MeOH for 24 h each at BGVS, followed by separate evaporation of each solvent in vacuo to give EtOAc and MeOH-soluble fractions. The MeOH-soluble extract, coded as BGVS M950167, was active in the yeast bioassays described earlier.[2c]

Isolation and Purification. Dried extract (500 mg) was dissolved in MeOH (2 mL) and passed through a Sephadex LH-20 column with elution with MeOH−EtOH (1:1). The active fractions 2 and 3 were combined and dried (155 mg) and then passed through a column of MCI gel eluted with H_2O, H_2O−MeOH (1:1), and MeOH. The active and pure fractions 4−7, eluted with H_2O−MeOH (1:1), were combined to give cryptolepine hydrochloride (**1**) (3 mg). Cryptolepine hydrochloride was also isolated by an acid−base partition as previously described.[19]

Subsequent investigation of an extract of *M. guyanensis* (BGVS M950147) using the same methods described above led to the isolation of cryptolepine from this plant alone.

Cryptolepine Hydrochloride (5-*N*-Methylquindolinium Chloride) (1). ^{1}H NMR (CD_3OD) δ 9.14 (s, 1H, H-11), 8.76 (d, J = 8.4, 1H, H-6), 8.67 (d, J = 9.6, 1H, H-4), 8.49 (d, J = 8.3, 1H, H-1), 8.18 (t, J = 7.9, 1H, H-3), 7.93−7.95 (m, 2H, H-2 and H-8), 7.83 (d, J = 8.6, 1H, H-9), 7.56 (td, J = 8.0, 0.8, 1H, H-7);^{13}C NMR (CD_3OD) δ 147.5 (s, C-9a), 139.7 (s, C-5a), 137.2 (s, C-4a), 135.4 (d, C-8), 134.0 (d, C-3), 131.2 (d, C-1), 128.5 (d, C-2), 128.5 (s, C-11a), 128.1 (s, C-10a), 126.9 (d, C-6), 126.3 (d, C-11), 123.0 (d, C-7), 118.4 (d, C-4), 115.4 (s, C-5b), 114.4 (d, C-9), 40.4 (5-*N*-CH_3); ROESY correlations, H-1 and H-11, H-4 and 5-*N*-CH_3, and H-6 and 5-*N*-CH_3. NMR assignments were based on ^{1}H−^{1}H COSY, HMQC, HMBC, and ROESY; EIMS m/z 233 (22%, $[M - Cl]^+$), 232 (53%, $[M - HCl]^{\bullet+}$), HRFABMS m/z 233.1075 $[M - Cl]^+$ (calcd. for $C_{16}H_{13}N_2$, 233.1079).

Cryptolepine (2). Treatment of **1** with ammonium hydroxide gave compound **2** as a purple solid. The ^{1}H NMR data of **2** were consistent with literature data.[19]

Quindoline-11-carboxylic Acid (3). Compound **3** (990 mg) was prepared by treatment of isatin (685 mg) with indoxyl-1,3-diacetate (1 g) in aqueous KOH (4.15 g/18.5 mL) under argon at room temperature for 10 days and workup as previously described.[11a] ^{1}H NMR (DMSO-d_6) δ 11.5 (s, 1H, 10-NH), 9.07 (dd, J = 7.6, 2.0, 1H), 8.37 (d, J = 7.6, 1H), 8.27 (dd, J = 8.4, 2.0, 1H), 7.77 (d, J = 8.4, 1H), 7.75−7.66 (m, 3H), 7.34 (t, J = 7.6, 1H); EIMS m/z 218 $[M - CO_2]^+$ (calcd for $C_{15}H_{10}N_2$, 218).

Quindoline (4). Decarboxylation of compound **3** (1 g) was carried out by heating a vial containing compound **3** in an oil bath at 300 °C for 1.5 h.[11a] Quindoline (**4**, 790 mg) was obtained as yellow crystals through sublimation on the vial walls (95%). ^{1}H and ^{13}C NMR data were consistent with the data in the literature.[9,20] EIMS: 218 $[M]^+$, 190, 89; $C_{15}H_{10}N_2$.

5-*N*-Methylquindolinium Hydroiodide (5). Compound **5** was synthesized by treatment of quindoline (**4**) with iodomethane in anhydrous MeOH at 110 °C.[11] The ^{1}H NMR spectral data for **5** in CD_3OD were indistinguishable from those of compound **1**.

5-*N*-Methylquindolinium Triflate (6). Compound **6** was obtained by treatment of quindoline (**4**, 19 mg) with excess methyl triflate (30 μL) in anhydrous toluene (3 mL) under argon for 24 h.[9] Toluene was removed in vacuo, and the residue was washed with ether. The dry yellow solid was identified as compound **6** (18 mg). The ^{1}H NMR spectral data for **5** in CD_3OD were indistinguishable from those of compound **1**.

5-*N*-Ethylquindolinium Iodide (7). Compound **7** (10 mg) was synthesized from the *N*-ethylation of quindoline (**4**, 20 mg) using iodoethane (30 μL) in anhydrous EtOH (2 mL) in a tightly closed vial at 140 °C for 4 h.[10a] The product **7** and starting material **4** were obtained in a 1:1 ratio and were separated by chromatography on an alumina column eluted with CH_2Cl_2, then CH_2Cl_2−MeOH (10:1). Compound **7** was obtained in the CH_2Cl_2−MeOH fraction, and quindoline (**4**) was recovered in the CH_2Cl_2 fraction. ^{1}H NMR ($CDCl_3$) δ 9.50 (s, 1H, H-11), 8.36 (d, J = 7.6, 1H), 8.31 (d, J = 8.8, 1H), 8.26 (d, J = 8.8, 1H), 8.11 (t, J = 8.0, 1H), 8.04 (t, J = 8.0, 1H), 7.86 (t, J = 7.6, 1H), 7.84 (d, J = 8.8, 1H, H-9), 7.48 (t, J = 8.0, 1H, H-7), 5.50 (q, J = 7.6, 2H, $-CH_2-$), 2.01 (t, J = 7.6, 3H, $-CH_3$); ^{1}H NMR (CD_3OD) δ 9.18 (s, 1H, H-11), 8.70 (d, J = 8.8, 1H, H-4), 8.62 (d, J = 8.8, 1H, H-6), 8.51 (d, J = 8.4, 1H, H-1), 8.20 (td, J = 8.0, 1.2, 1H, H-3), 7.95 (m, 2H, H-2 and H-8), 7.84 (d, J = 8.4, 1H, H-9), 7.59 (td, J = 8.0, 1.2, 1H, H-7), 5.62 (q, J = 7.6, 2H, $-CH_2-$), 1.91 (t, J = 7.6, 3H, $-CH_3$); EIMS m/z 218 $[M - I - Et]^+$; FABMS 247 $[M - I]^+$, 219.

5-*N*-Propylquindolinium Iodide (8). Quindoline (**4**, 15 mg) was mixed with excess propyl iodide (100 μL) and DMF (200 μL) and heated at 150 °C for 24 h in a closed vial. The solvent was removed under high vacuum, and the product isolated using an alumina column as described for compound **7** to give compound **8** (5 mg) in the CH_2Cl_2−MeOH eluent. ^{1}H NMR (CD_3OD) δ 9.18 (s, 1H, H-11), 8.69 (d, J = 9.2, 1H, H-4), 8.50 (d, J = 8.4, 1H, H-6), 8.49 (d, J = 8.4, 1H, H-1), 8.19 (td, J = 8.4, 1.2, 1H, H-3), 7.96−7.91 (m, 2H, H-2 and H-8), 7.82 (d, J = 8.4, 1H, H-9), 7.59 (td, J = 8.4, 1.2, 1H, H-7), 5.51 (t, J = 7.6, 2H, $-CH_2-$), 2.30 (sextet, J = 7.6, 2H, $-CH_2-$), 1.34 (t, J = 7.6, 3H, $-CH_3$); EIMS m/z 260 $[M - HI]^+$, 218 $[M - PrI]^+$; HREIMS m/z 260.1299 (calcd for $C_{18}H_{17}N_2$, 260.1313).

5-*N*-Butylquindolinium Hydrobromide (9). Treatment of quindoline (**4**, 23 mg) with butyl bromide as described for compound **8** gave compound **9** (7 mg). ^{1}H NMR (CD_3OD) δ 9.09 (s, 1H, H-11), 8.58 (d, J = 9.2, 1H, H-4), 8.42 (d, J = 8.4, 2H, H-1 and H-6), 8.14 (td, J = 8.0, 1.6, 1H, H-3), 7.89 (td, J = 8.4, 1.6, 2H, H-2 and H-8), 7.77 (d, J = 8.4, 1H, H-9), 7.54 (t, J = 8.4, 1H, H-7), 5.45 (t, J = 7.6, 2H, $-CH_2-$), 2.15 (quintet, J = 7.6, 2H, $-CH_2-$), 1.75 (sextet, J = 7.6, 2H, $-CH_2-$), 1.11 (t, J = 7.6, 3H, $-CH_3$); ^{13}C NMR (MeOD) δ 147.6 (s), 138.5 (s), 136.3 (s), 135.3 (d), 135.0 (s), 134.2 (d), 131.3 (d), 128.4 (d), 128.1 (s), 126.4 (d), 125.9 (d), 123.3 (d), 118.1 (d), 114.5 (d), 114.3 (s), 52.8 (t), 31.4 (t), 20.9 (t), 14.2 (q); EIMS m/z 274 $[M - HBr]^+$, 218 $[M - BuBr]^+$, 190, 88; HREIMS m/z 274.1472 (calcd for $C_{19}H_{19}N_2$, 274.1470).

10-*N*-Methylquindoline (10). The method described in the literature was used and summarized as below.[9] Quindoline (**4**, 15 mg) mixed with BaO (45 mg) and KOH (15 mg) in acetone (5 mL) was refluxed for 1 h. Excess iodomethane (60 μL) was added to the cooled residue and the mixture refluxed for an additional 4 h; the excess reagent was then removed in a vacuum. The organic material was extracted with ether, dried over $MgSO_4$, and the solution evaporated to obtain compound **10** (15 mg). ^{1}H NMR ($CDCl_3$) δ 8.58 (d, J = 8.4, 1H), 8.36 (d, J = 9.2, 1H), 7.95 (d, J = 8.4, 1H), 7.92 (s, 1H, H-11), 7.65 (m, 2H), 7.54 (t, J = 8.4, 1H), 7.40 (d, J = 8.8, 1H), 7.34 (t, J = 8.4, 1H), 3.86 (s, 3H, 10-*N*-CH_3); EIMS m/z 232 $[M]^+$, 217 $[M - CH_3]^+$, 190, 89; HREIMS m/z 232.0993 (calcd for $C_{16}H_{12}N_2$, 232.1000).

10-*N*-Ethylquindoline (11). Using iodoethane as alkylating agent (30 μL), compound **11** (5 mg, 95% yield) was synthesized using the methods described for compound **10**. ^{1}H NMR ($CDCl_3$) δ 8.65 (d, J = 8.0, 1H), 8.40 (d, J = 7.9, 1H), 8.01 (s, 1H, H-11), 7.98 (d, J = 8.3, 1H), 7.67 (m, 2H), 7.56 (t, J = 7.9, 1H), 7.44 (d, J = 8.3, 1H), 7.35 (t, J = 7.9, 1H), 4.42 (q, J = 7.2, 2H, $-CH_2-$), 1.49 (t, J = 7.2, 3H, $-CH_3$); EIMS m/z 246 $[M]^+$, 231 $[M - CH_3]^+$, 217 $[M - Et]^+$, 190, 89; HREIMS m/z 246.1150 (calcd for $C_{17}H_{14}N_2$, 246.1157).

10-*N*-Propylquindoline (12). Using propyl iodide as alkylating agent (30 μL), compound **12** (5 mg, 95% yield) was synthesized using the methods described for compound **10**. ^{1}H NMR ($CDCl_3$) δ 8.62 (d, J = 8.3, 1H), 8.38 (d, J = 7.9, 1H), 7.99 (s, 1H, H-11), 7.97 (d, J = 8.4, 1H), 7.66 (m, 2H), 7.56 (t, J = 7.9, 1H), 7.44 (d, J = 8.3, 1H), 7.34 (t, J = 7.9, 1H), 4.32 (t, J = 7.2, 2H, $-CH_2-$), 1.98 (sextet, J = 7.2, 2H, $-CH_2-$), 1.02 (t, J = 7.2, 3H, $-CH_3$); EIMS m/z 260 $[M]^+$, 231 $[M - Et]^+$, 190; HREIMS m/z 260.1311 (calcd for $C_{18}H_{16}N_2$, 260.1313).

10-*N*-Butylquindoline (13). Using butyl bromide as alkylating agent (30 μL), compound **13** (5 mg, 95% yield) was synthesized using the methods described for compound **10**. ^{1}H NMR ($CDCl_3$) δ 8.65 (d, J = 8.3, 1H), 8.41 (d, J = 7.9, 1H), 8.00 (s, 1H, H-11), 7.98 (d, J = 8.4, 1H), 7.67 (m, 2H), 7.56 (t, J = 7.9, 1H), 7.44 (d, J = 8.3, 1H), 7.35 (t, J = 7.9, 1H), 4.36

(t, J = 7.2, 2H, $-CH_2-$), 1.92 (quintet, J = 7.2, 2H, $-CH_2-$), 1.44 (sextet, J = 7.2, 2H, $-CH_2-$), 0.97 (t, J = 7.2, 3H, $-CH_3$); EIMS m/z 274 $[M]^+$, 231 $[M - propyl]^+$, 217, 190, 89; HREIMS m/z 274.1471 (calcd for $C_{19}H_{18}N_2$, 274.1470).

10-*N*-Allylquindoline (14). Using allyl bromide as alkylating agent (30 μL), compound **14** (2 mg, 95% yield) was synthesized using the methods described for compound **10**. ^{1}H NMR ($CDCl_3$) δ 8.64 (d, J = 8.3, 1H), 8.42 (d, J = 7.9, 1H), 7.98 (s, 1H, H-11), 7.97 (d, J = 8.4, 1H), 7.67 (m, 2H), 7.56 (t, J = 7.6, 1H), 7.41 (d, J = 8.3, 1H), 7.36 (t, J = 7.6, 1H), 6.04 (dddd, J = 17, 10, 5.0, 5.0, 1H, $=CH-$), 5.23 (brd, J = 10, 1H, $=CH_2$), 5.10 (brd, J = 17, 1H, $=CH_2$), 4.97 (brd, J = 5.0, 2H, $-CH_2-$); EIMS m/z 258 $[M]^+$, 231 $[M - CH=CH_2]^+$, 217 $[M - CH_2CH=CH_2]^+$, 190.

10-*N*-Benzylquindoline (15). Using benzyl bromide as alkylating agent (30 μL), compound **15** (4 mg, 95% yield) was synthesized using the methods described for compound **10**. ^{1}H NMR ($CDCl_3$) δ 8.70 (d, J = 8.3, 1H), 8.43 (d, J = 7.9, 1H), 7.97 (s, 1H, H-11), 7.92 (d, J = 8.0, 1H), 7.69 (t, J = 7.6, 1H), 7.64 (t, J = 8.3, 1H), 7.55 (t, J = 7.6, 1H), 7.41 (d, J = 8.2, 1H), 7.38−7.18 (m, 6H), 5.58 (s, 2H); EIMS m/z 308 $[M]^+$, 216 $[M - C_7H_8]^+$.

10-*N*-Acetylquindoline (16). Quindoline (**4**, 8 mg) was stirred with acetic anhydride (0.5 mL) and pyridine (0.5 mL) at room temperature for 48 h. The product **16** (9 mg) was obtained after removing excess reagent in vacuo, and passing through an alumina column with elution with CH_2Cl_2. ^{1}H NMR (CD_3OD) δ 9.00 (s, 1H, H-11), 8.50 (d, J = 8.0, 1H), 8.27 (d, J = 8.4, 1H), 8.13 (d, J = 8.0, 1H), 7.99 (d, J = 8.4, 1H), 7.75 (t, J = 7.6, 1H), 7.66 (t, J = 7.6, 1H), 7.59 (t, J = 7.6, 1H), 7.51 (t, J = 7.6, 1H), 2.95 (s, 3H, -*N*-CO-CH_3); ^{13}C NMR (CD_3OD) δ 169.7 (s), 147.3 (s), 145.9 (s), 141.4 (s), 131.3 (s), 130.6 (d), 128.8 (d), 128.6 (d), 128.5 (d), 127.2 (s), 126.1 (d), 125.6 (s), 124.4 (d), 122.0 (d), 121.5 (d), 115.7 (d), 27.6 (q); FABMS m/z 261 $[M + 1]^+$, 219; HREIMS m/z 260.0941 (calcd for $C_{17}H_{12}N_2O$, 260.0950).

5,10-*N*,*N*-Dimethylquindolinium Triflate (17). Compound **10** (7.5 mg) in anhydrous toluene was treated with excess methyl triflate under argon at room temperature for 24 h. The excess reagent was removed in a vacuum, and compound **17** (7 mg) was obtained after washing with ether and drying. ^{1}H NMR (CD_3OD) δ 9.36 (s, 1H, H-11), 8.81 (d, J = 8.4, 1H, H-6), 8.69 (d, J = 8.4, 1H, H-4), 8.53 (d, J = 8.4, 1H, H-1), 8.21 (td, J = 8.4, 0.8, 1H, H-3), 8.04 (t, J = 8.0, 1H, H-8), 7.97 (m, 2H, H-2 and H-9), 7.62 (t, J = 8.4, 1H, H-7), 5.13 (s, 3H, 5-*N*-CH_3), 4.21 (s, 3H, 10-*N*-CH_3); ^{13}C NMR (CD_3OD) δ 148.3 (s), 135.8 (s), 135.6 (d), 134.0 (d), 133.3 (s), 131.2 (d), 130.2 (s), 128.7 (d), 127.9(s), 127.2 (d), 124.7 (d), 123.1 (d), 118.4 (d), 115.3 (s), 112.3 (d), 40.8 (q), 30.2 (q); EIMS m/z 247 $[M - Tf]^+$, 233 $[M - Tf - CH_2]^+$ (calcd for $C_{17}H_{15}N_2$, 247).

5-*N*-Methyl-10-*N*-ethylquindolinium Triflate (18). Using compound **11** (2.5 mg) as starting material, compound **18** (2.3 mg) was synthesized using the methods described for compound **17**. ^{1}H NMR (CD_3OD) δ 9.39 (s, 1H, H-11), 8.81 (d, J = 8.0, 1H, H-6), 8.68 (d, J = 8.8, 1H, H-4), 8.52 (d, J = 8.4, 1H, H-1), 8.20 (td, J = 8.0, 1.2, 1H, H-3), 8.03 (t, J = 8.0, 1H, H-8), 7.98 (m, 2H, H-2 and H-9), 7.61 (td, J = 8.0, 0.8, 1H, H-7), 5.12 (s, 3H, 5-*N*-CH_3), 4.78 (q, J = 7.2, 2H, $-CH_2-$), 1.56 (t, J = 7.2, 3H, $-CH_3$); EIMS m/z 261 $[M - Tf]^+$, 246 $[M - Tf - CH_3]^+$ (calcd for $C_{18}H_{17}N_2$, 261).

5-*N*-Methyl-10-*N*-propylquindolinium Triflate (19). Compound **19** (2.1 mg) was synthesized from **12** (2.5 mg) using the methods described for compound **17**. ^{1}H NMR (CD_3OD) δ 9.40 (s, 1H, H-11), 8.81 (d, J = 8.4, 1H, H-6), 8.69 (d, J = 8.8, 1H, H-4), 8.53 (d, J = 8.4, 1H, H-1), 8.20 (td, J = 8.0, 1.6, 1H, H-3), 8.03 (td, J = 8, 1.2, 1H, H-8), 7.98 (m, 2H, H-2 and H-9), 7.61 (td, J = 8.0, 1.6, 1H, H-7), 5.13 (s, 3H, $N-CH_3$), 4.70 (t, J = 7.2, 2H, $-CH_2-$), 2.04 (sextet, J = 7.2, 7.2, 2H, $-CH_2-$), 1.04 (t, J = 7.2, 3H, $-CH_3$); EIMS m/z 275 $[M - Tf]^+$, 260 $[M - Tf - Me]^+$, 231 $[M - Tf - Et]^+$ (calcd for $C_{19}H_{19}N_2$, 275).

5-*N*-Methyl-10-*N*-butylquindolinium Triflate (20). Compound **20** (2.0 mg) was synthesized from **13** (2.5 mg) using the methods described for compound **17**. ^{1}H NMR (CD_3OD) δ 9.19 (s, 1H, H-11), 8.59 (d, J = 8.4, 1H, H-6), 8.42 (d, J = 8.8, 1H, H-4), 8.39 (d, J = 8.8, 1H, H-1), 7.96 (t, J = 8.0, 1H, H-3), 7.87 (t, J = 8.0, 1H, H-8), 7.66 (t, J = 8.0, 1H, H-2), 7.60 (d, J = 8.4, 1H, H-9), 7.25 (t, J = 8.0, 1H, H-7), 5.00 (s, 3H, 5-*N*-CH_3), 4.58 (t, J = 7.6, 2H, $-CH_2-$), 1.88 (quintet, J = 7.6, 2H, $-CH_2-$), 1.40 (sextet, J = 7.6, 2H, $-CH_2-$), 0.93 (t, 3H, $-CH_3$); EIMS m/z 289 $[M - Tf]^+$, 275 $[M - Tf - CH_2]^+$, 245, 232 $[M - Tf - CH_2 - propyl]^+$ (calcd for $C_{20}H_{21}N_2$, 289).

5-*N*-Methyl-10-*N*-allylquindolinium Triflate (21). Compound **21** (0.6 mg) was synthesized from **14** (1.0 mg) using the methods described for compound **17**. ^{1}H NMR (CD_3OD) δ 9.33 (s, 1H, H-11), 8.82 (d, J = 8.4, 1H, H-6), 8.69 (d, J = 8.8, 1H, H-4), 8.51 (d, J = 8.0, 1H, H-1), 8.21 (td, J = 8.8, 1.2, 1H, H-3), 8.02 (td, J = 8.0, 1.2, 1H, H-8), 7.97 (t, J = 8.0, 1H, H-2), 7.92 (d, J = 8.4, 1H, H-9), 7.62 (t, J = 8.0, 1H, H-7), 6.15 (dddd, J = 17, 10, 5, 5, 1H, $=CH-$), 5.36 (brd, 5.0, 2H, $-CH_2-$), 5.26 (d, J = 10, 1H, $=CH_2$), 5.11 (d, J = 17, 1H, $=CH_2$), (s, 3H, 5-*N*-CH_3); EIMS m/z 273 $[M - Tf]^+$, 258 $[M - Tf - CH_3]^+$, 231 $[M - Tf - C_3H_6]^+$, 217 $[M - Tf - C_4H_8]^+$ (calcd for $C_{19}H_{17}N_2$, 273).

5-*N*-Methyl-10-*N*-acetylquindolinium Chloride (22). Compound **1** (1 mg) was stirred with acetic anhydride (0.3 mL) and pyridine (0.3 mL) for 24 h. The product **22** (1 mg) was obtained after removing excess reagent in a vacuum, extraction with 1 mL of MeOH−H_2O (1:1), and drying. ^{1}H NMR ($CDCl_3$) δ 10.02 (s, 1H, H-11), 8.77 (d, J = 8.2, 1H), 8.59 (d, J = 9.0, 1H), 8.40 (d, J = 8.5, 1H), 8.27 (d, J = 8.5, 1H), 8.20 (t, J = 8.5, 1H), 7.97 (t, J = 8.2, 1H), 7.91 (t, J = 7.6, 1H), 7.71 (t, J = 8.2, 1H), 5.26 (s, 3H, 5-*N*-CH_3), 3.16 (s, 3H, $COCH_3$); FABMS m/z 275 $[M - Cl]^+$, 233 $[M - Cl - COCH_2]^+$, 218 $[M - Cl - Ac - CH_3]^+$ (calcd for $C_{18}H_{15}N_2O$, 275).

Methyl Quindoline-11-carboxylate (23). Compound **3** (30 mg) was treated with excess diazomethane in ether, and the mixture was stirred at room temperature for 3 h until the starting material had dissolved. The solvent was removed, and the product purified by chromatography on alumina with elution by CH_2Cl_2 to give **23** (31 mg). ^{1}H NMR ($CDCl_3$) δ 9.73 (bs, 1H, 10-*N*H), 9.04 (dd, J = 7.6, 2.4, 1H), 8.49 (d, J = 7.6, 1H), 8.37 (dd, J = 7.6, 2.0, 1H), 7.67 (m, 2H), 7.60 (td, J = 7.9, 1.2, 1H,), 7.46 (d, J = 8, 1H,), 7.35 (td, J = 8, 1.2, 1H,), 4.16 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 168.1 (s), 147.7 (s), 144.3 (s), 143.5 (s), 133.8 (s), 130.5 (d), 130.1 (d), 127.4 (d), 126.2 (d), 125.0 (d), 123.6 (s), 122.2 (d), 121.5 (s), 121.0 (d), 111.2 (d), 109.1 (s), 52.5 (q); HREIMS m/z 276.0895 (calcd for $C_{17}H_{12}N_2O_2$, 276.0899).

11-Hydroxymethylquindoline (24). Methylquindoline-11-carboxylate (**23**, 23 mg) was treated with LAH (30 mg) in THF (5 mL) under reflux for 30 min. The reaction residue was cooled, excess LAH allowed to settle out, and the supernatant transferred to another flask and treated with EtOAc (25 mL) and MeOH (1 mL). After filtration, the filtrate was evaporated to give compound **24** (21 mg). ^{1}H NMR ($CDCl_3$) δ 8.45 (ddd, J = 8.0, 0.8, 0.8, 1H), 8.27 (ddd, J = 8.4, 0.8, 0.8, 1H), 8.20 (ddd, J = 8.0, 0.8, 0.8, 1H), 7.67 (td, J = 8.0, 1.2, 1H), 7.63−7.54 (m, 3H), 7.28 (td, J = 8.0, 1.2, 1H), 5.49 (s, 2H, CH_2OH); ^{1}H NMR (CD_3OD) δ 8.56 (d, J = 8.0, 1H), 8.52 (d, J = 8.4, 1H), 8.35 (d, J = 8.4, 1H), 8.05 (td, J = 8.0, 1.2, 1H), 7.87 (t, J = 8.4, 2H), 7.81 (d, J = 8.4, 1H), 7.48 (td, J = 8.0, 1.2, 1H), 5.74 (s, 2H, $-CH_2OH$); ^{13}C NMR (CD_3OD) δ 147.3 (s), 145.8 (s), 144.7 (s), 132.8 (s), 131.3 (d), 129.1 (d), 127.5 (d), 126.3 (d), 126.1 (s), 125.5 (s), 124.3 (d), 122.8 (d), 121.8 (s), 120.7 (d), 112.5 (d), 58.7 (t); EIMS m/z 248 $[M]^+$, 230 $[M - H_2O]^+$, 218 $[M - CH_2O]^+$, 190 (calcd for $C_{16}H_{12}N_2O$, 248).

11-Formylquindoline (25). Compound **24** (10 mg) was treated with PDC (30 mg) in CH_2Cl_2 at room temperature for 12 h. The reaction mixture was passed through an alumina column eluted with *n*-hexanes−EtOAc (3:1), and fractions containing the desired compound were combined and dried to give **25** (5 mg). Continued elution with MeOH gave recovered starting material **24** (4 mg). ^{1}H NMR ($CDCl_3$−CD_3OD, 10:1) δ 11.19 (s, 1H, COH), 8.67 (m, 1H), 8.44 (d, J = 7.6, 1H, H-6), 8.32 (m, 1H), 7.68 (m, 2H, H-2 and H-3), 7.59 (t, J = 8.0, 1H, H-8), 7.50 (d, J = 8.0, 1H, H-9), 7.34 (t, J = 8.0, 1H, H-7); ^{13}C NMR ($CDCl_3$−CD_3OD, 10:1) δ 191.6 (d), 148.3 (s), 144.4 (s), 143.3 (s), 133.1 (s), 131.0 (s), 130.8 (d), 129.8 (d), 127.8 (d), 126.6 (d), 124.1 (s), 122.2 (d), 121.6 (d), 120.4 (s), 119.9 (d),

111.7 (d); EIMS *m/z* 246 $[M]^+$, 217 $[M - COH]^+$; HREIMS *m/z* 246.0794 (calcd for $C_{16}H_{10}N_2O$, 246.0793).

11-Methylquindoline (26). Treatment of methylquindoline-11-carboxylate (**23**, 15 mg) with LAH (60 mg) in THF (5 mL) under reflux for 5 h, followed by chromatography on alumina with CH_2Cl_2−MeOH (50:1) as eluent gave **26** (8 mg). ^1H NMR ($CDCl_3$−CD_3OD, 10:1) δ 8.4 (d, J = 8.8, 1H), 8.22 (d, J = 8.8, 1H), 7.98 (dd, J = 9.2, 0.8, 1H), 7.613 (td, J = 8.0, 1.2, 1H), 7.49 (td, J = 8.0, 1.2, 1H), 7.33 (d, J = 9.2, 1H), 7.16 (td, J = 8.8, 1.2, 1H), 2.78 (s, 3H, $-CH_3$); ^{13}C NMR ($CDCl_3$−CD_3OD, 10:1) δ 143.7 (s), 142.2 (s), 132.0 (s), 130.0 (d), 127.4 (d), 126.7 (d), 125.8 (s), 124.8 (d), 123.5 (s), 122.9 (d and s, 2C), 122.2 (d), 120.3 (s), 119.8 (d), 111.1 (d), 12.4 (q); EIMS *m/z* 233 $[MH]^+$, 232 $[M]^+$; HREIMS *m/z* 232.1001 (calcd for $C_{16}H_{12}N_2$, 232.1000).

Carbamate 27. Compound **24** (3 mg) was treated with excess 1,1′-carbonyldiimidazole (15 mg) and imidazole (1 mg) in CH_2Cl_2 (3 mL) at room temperature for 24 h. The reaction residue was purified by PTLC on alumina PTLC with development by CH_2Cl_2. To give carbamate **27** (1.5 mg). ^1H NMR ($CDCl_3$) δ 8.46 (d, J = 8.0, 1H), 8.40 (d, J = 8.4, 1H), 8.38 (d, J = 8.4, 1H), 7.80 (t, J = 8.0, 1H), 7.75 (m, 2H), 7.69 (t, J = 8.0, 1H), 7.57 (t, J = 8.0, 1H), 6.24 (s, 2H, $-CH_2O-$); EIMS *m/z* 274 $[M]^+$, 230 $[M - CO_2]^+$ (calcd for $C_{17}H_{10}N_2O_2$, 274).

Quindoline-11-carbonyl Chloride (28). Quindoline-11-carboxylic acid (**5**) was refluxed with $SOCl_2$ for 2 h, and then excess $SOCl_2$ was removed in vacuo. The dried material was used directly without further purification.

2-Hydroxyethyl Quindoline-11-carboxylate (29). Quindoline-11-carbonyl chloride (**28**, 10 mg) was heated in ethylene glycol (0.5 mL) at 110 °C for 1 h. The cooled reaction mixture was filtered and the residue was washed with cold acetone to give **29** (9 mg).^1H NMR (CD_3OD) δ 9.06 (d, J = 8.5, 1H), 8.57 (d, J = 8.1, 1H), 8.38 (d, J = 8.6, 1H), 8.04 (t, J = 7.4, 1H), 7.90 (t, J = 8.3, 2H), 7.76 (d, J = 8.3, 1H), 7.51 (t, J = 7.6, 1H), 4.80 (t, J = 4.7, 2H, $-CO_2CH_2-$), 4.08 (t, J = 4.7, 2H, $-CH_2OH$); ^{13}C NMR δ 165.3 (s), 147.8 (s), 141.5 (s), 136.3 (d), 136.2 (s), 134.9 (s), 132.3 (d), 129.7 (d), 127.4 (d), 124.7 (d), 123.4 (d), 122.2 (s), 121.8 (d), 115.2 (s), 114.3 (d), 69.2 (t), 60.7 (t); FABMS *m/z* 307 $[MH]^+$ (calcd for $C_{18}H_{15}N_2O_3$, 307).

1,2-Ethanediol 1,2-Bis(quindoline-11-carboxylate) (30). A mixture of compound **29** (1.2 mg), compound **3** (3 mg), DCC (15 mg), 4-pyrrolidinylpyridine (trace), and CH_2Cl_2 (2 mL) was stirred at room temperature for 12 h. The excess of compound **3** was sparingly soluble in CH_2Cl_2, and was separated easily by filtration. The reaction residue was purified by PTLC on alumina with development by CH_2Cl_2 to give compound **30** (2 mg). ^1H NMR ($CDCl_3$) δ 9.87 (s, 2H, NH-10 and −10′), 9.12 (dd, J = 8.8, 0.8, 2H), 8.44 (d, J = 8.0, 2H), 8.33 (dd, J = 8.0, 0.4, 2H), 7.62 (td, J = 8.4, 1.2, 2H), 7.48−7.58 (m, 4H), 7.323 (t, J = 8.0, 2H), 7.21 (d, J = 8.0, 2H), 5.17 (s, 4H, $-O-CH_2-CH_2-O-$); ^{13}C NMR δ 167.4 (s), 147.9 (s), 144.3 (s), 143.6 (s), 139.8 (s), 133.7 (s), 130.6 (d), 130.3 (d), 127.6 (d), 126.2 (d), 124.7 (d), 123.6 (s), 122.1 (d), 121.5 (s), 121.1 (d), 111.2 (d), 63.5 (t); FABMS *m/z* 551 $[MH]^+$, 307 (calcd for $C_{34}H_{23}N_4O_4$, 551).

Bisquindoline (31). Compound **31** was prepared as previously described.[11a] ^1H NMR ($CDCl_3$) δ 9.05 (d, J = 8.8, 2H), 8.65 (d, J = 8.0, 2H), 8.48 (d, J = 8.4, 2H), 7.87−7.74 (m, 8H), 7.38 (t, J = 8.0, 2H); ^{13}C NMR ($CDCl_3$) δ 166.9 (s), 145.3 (s), 135.3 (s), 132.6 (d), 132.3 (d), 129.9 (s), 128.0 (d), 127.4 (d), 125.5 (s), 125.4 (d), 123.4 (s), 123.3 (s), 123.0 (d), 120.9 (d), 117.5 (s), 113.0 (d).

2-Methyl-2-[11-(aminomethyl)quindoline]-1,3-propanediol (32). A mixture of aldehyde **25** (2 mg), anhydrous $MgSO_4$ (40 mg), and 2-amino-2-methyl-1,3-propanediol (10 mg) was dissolved in CH_2Cl_2−anhydrous MeOH (5:1, 2 mL) and the mixture stirred at room temperature for 24 h. The reaction residue was treated with an additional 4 mL of solvent (CH_2Cl_2−MeOH, 5:1), then filtered to remove $MgSO_4$ and excess amine. The filtrate was evaporated in vacuo, and the dried residue was dissolved in 1.5 mL of MeOH. $NaB(CN)H_3$ (15 mg) was added, and the solution was stirred at room temperature for 1 h. The MeOH in the reaction mixture was then removed in vacuo, and the product was partitioned between EtOAc and H_2O (3 mL each). The EtOAc layer was dried (Na_2SO_4), and the EtOAc was removed in a vacuum. The residue was purified by PTLC on alumina and developed by $CHCl_3$−MeOH (10:1). The product band gave compound **32** (1.2 mg). ^1H NMR (CD_3OD) δ 8.45 (dd, J = 7.6, 0.8, 1H), 8.38 (d, J = 8.4, 1H), 8.21 (d, J = 8.4, 1H), 7.67 (td, J = 8.0, 1.2, 1H), 7.64−7.58 (m, 2H), 7.54 (dd, J = 8.0, 0.8, 1H), 7.29 (td, J = 8.0, 1.2, 1H), 4.57 (s, 2H, $-CH_2-N$), 3.68 (s, 4H, 2 × $-CH_2OH$), 1.21 (s, 3H, $-CH_3$); ^{13}C NMR (CD_3OD) δ 147.0 (s), 145.7 (s), 144.8 (s), 133.6 (s), 131.2 (d), 129.3 (d), 127.5 (d), 126.6 (s), 126.4 (d), 125.6 (s), 124.2 (d), 122.8 (d), 122.0 (s), 120.8 (d), 112.5 (d), 66.2 (t, 2C), 59.1 (s), 39.2 (t), 18.7 (q); FABMS *m/z* 336 $[MH]^+$, 231 $[M -$ side chain$]^+$ (calcd for $C_{20}H_{22}N_3O_2$, 336).

Methyl 2-Bromoquindoline-11-carboxylate (34). 2-Bromoquindoline-11-carboxylic acid (**33**) (850 mg) was synthesized in crude form as described for compound **3** from 5-bromoisatin (685 mg) and indoxyl-1,3-diacetate (1 g) in aqueous KOH (4.15 g/18.5 mL) under argon at room temperature for 10 days. ^{13}C NMR (DMSO-d_6) δ 168.2, 145.0, 143.5, 132.7, 132.0, 131.3, 130.9, 129.7, 129.3, 127.2, 126.5, 125.4, 124.1, 121.8, 120.8, 113.0; EIMS *m/z* 342, 340 $[M]^+$, 324, 322 $[M - H_2O]^+$, 296, 294, 262, 244, 216, 215, 188 (calcd for $C_{16}H_9N_2O_2Br$, 342, 340). Treatment of crude **33** (15 mg) with excess CH_2N_2 as described for **23** gave **34** (16 mg). ^1H NMR ($CDCl_3$) δ 9.75 (bs, 1H, 10-NH), 9.23 (d, J = 2, 1H), 8.45 (d, J = 8, 1H), 8.20 (d, J = 8.8, 1H), 7.73 (dd, J = 8.8, 2.4, 1H), 7.63 (td, J = 7.6, 1.2, 1H), 7.47 (d, J = 8, 1H), 7.37 (td, J = 8, 0.8, 1H), 4.19 (s, 3H, $COOCH_3$); EIMS *m/z* 356, 354 $[MH]^+$, 324, 322 $[M - HOCH_3]^+$, 296, 294 $[M - C_2H_4O_2]^+$, 276, 244, 215 $[M - HCO_2CH_3 - Br]^+$, 216, 188 (calcd for $C_{17}H_{12}N_2O_2Br$, 356, 354).

2-Bromoquindoline-11-carbonyl Chloride (35). Compound **33** (10 mg) was mixed with $SOCl_2$ (1 mL) and heated at 70 °C for 2 h. After cooling to room temperature, excess $SOCl_2$ was quickly removed at room temperature in vacuo. The residue was used directly without further purification.

2-Bromoquindoline-11-carboxamide (36). Freshly prepared **35** (12 mg) was treated with aqueous ammonia (37%, 0.5 mL). The dried residue containing compound **36** (8 mg) was obtained after removal of excess ammonia and water in vacuo, and washed with $CHCl_3$ and MeOH. ^1H NMR (DMSO-d_6) δ 11.54 (s, 1H, 10-*N*H), 8.50 and 8.26 (2 × bs, 2H, $-CONH_2$), 8.33 (m, 2H, H-1 and H-6), 8.15 (d, J = 8.8, 1H, H-4), 7.76 (dd, J = 8.8, 2.3, 1H, H-3), 7.62 (m, 2H, H-8 and H-9), 7.31 (t, J = 7.2, 1H, H-7); ^{13}C NMR (DMSO-d_6) δ 166.6 (s), 146.9 (s), 144.7 (s), 141.6 (s), 135.3 (s), 131.3 (d), 130.5 (d), 129.0 (d), 126.3 (d), 123.7 (s), 121.4 (d), 120.4 (s), 120.0 (d), 119.6 (s), 118.7 (s), 112.1(d); EIMS *m/z* 341, 339 $[M]^+$, 324, 322 $[M - NH_3]$, 296, 294 $[M - CONH_3]^+$, 215 $[M - Br - CONH_3]^+$ (calcd for $C_{16}H_{10}N_3OBr$, 341, 339).

Benzofuro[3,2-*b*]quinolin-6(11*H*)one (37). Compound **37** (220 mg) was synthesized by a literature procedure.[18c] ^1H NMR (DMSO-d_6) δ 8.30 (d, J = 8.0, 1H), 8.16 (d, J = 8.0, 1H), 7.73 (d, J = 8.4, 1H), 7.61 (d, J = 8.0, 1H), 7.52 (t, J = 8.4, 1H), 7.45 (t, J = 8.4, 1H), 7.33 (t, J = 8.4, 1H), 7.12 (t, J = 8.0, 1H); ^{13}C NMR (DMSO-d_6) δ 162.0 (s), 156.3 (s), 147.2 (s), 138.9 (s), 128.8 (d), 128.3 (d), 127.9 (s), 125.2 (d), 125.1 (d), 124.2 (s), 122.6 (d), 121.9 (d), 119.8 (d), 117.5 (s), 112.5 (d); EIMS *m/z* 235 $[M]^+$ (calcd for $C_{15}H_9NO_2$, 235).

11-Chlorobenzofuro[3,2-*b*]quinoline (38). Compound **38** (64 mg) was synthesized as previously described.[18c] ^1H NMR ($CDCl_3$) δ 8.82 (dd, J = 8.8, 1.2, 1H), 8.08 (dd, J = 8.0, 1.2, 1H), 7.73 (dd, J = 8.0, 1.6, 1H), 7.59 (td, J = 8.0, 1.6, 1H), 7.26 (td, J = 8.0, 1.6, 1H), 7.11 (td, J = 8.0, 1.2, 1H), 6.78 (td, J = 8.0, 1.2, 1H), 6.71 (dd, J = 8.0, 1.2, 1H); EIMS *m/z* 255, 253 $[M]^+$, 218 $[M - Cl]^+$, 190 (calcd for $C_{15}H_8NOCl$, 255, 253).

11-Iodobenzofuro[3,2-*b*]quinoline (39). A mixture of compound **38** (12 mg), iodomethane (0.7 mL), NaOMe (26 mg), and anhydrous MeOH (2 mL) was refluxed for 24 h. Methanol was removed in vacuo, and the residue was partitioned between H_2O and EtOAc. The EtOAc layer was dried, and the resulting solid (14 mg) was collected as **39**. ^1H NMR (CD_3OD) δ 8.29 (d, J = 8.0, 1H), 8.19 (d, J = 8.4, 1H), 8.14 (d, J = 8.0, 1H), 7.89 (d, J = 8.4, 1H), 7.83 (t, J = 8.0, 1H), 7.77 (m, 2H), 7.55 (t, J = 8.0, 1H); EIMS: 345 $[M]^+$, 218 $[M - I]^+$, 180 (calcd for $C_{15}H_8NOI$, 345).

Benzofuro[3,2-*b*]quinoline (40). A mixture of compound **38** (30 mg) and 10% Pd/C (60 mg) in 4 mL of alcoholic KOH (1N) under H_2 was left at room temperature for 12 h. The Pd/C was removed by filtration through Celite, and EtOH was removed in vacuo. Acetone (1 mL) and distilled water (1 mL) were added to the residue. The insoluble precipitate (20 mg) was collected and identified as product **40** (78%). ^{1}H NMR ($CDCl_3$) δ 8.39 (d, J = 8.0), 8.30 (d, J = 8.5), 8.16 (s), 7.97 (d, J = 8.0), 7.73 (ddd, J = 8.0, 8.5, 1.0), 7.6 (m, 3H), 7.46 (ddd, J = 8.0, 8.5, 1.0); ^{13}C NMR ($CDCl_3$) δ 159.6 (s), 147.9 (s), 147.5 (s), 146.1 (s), 130.9 (d), 129.3 (d), 128.0 (d), 127.9 (d), 127.4 (s), 126.1 (d), 123.6 (d), 122.9 (s), 122.2 (d), 114.5 (d), 112.2 (d); EIMS m/z 219 $[M]^+$, 190 (calcd for $C_{15}H_9NO$, 219).

5-*N*-Methylbenzofuro[3,2-*b*]quinolinium iodide (41). A mixture of compound **40** (16 mg), iodomethane (0.5 mL), NaOMe (20 mg), and anhydrous MeOH (2 mL) was refluxed for 24 h.[21] Ether was added, and the precipitate was obtained through filtration. The solid was washed with CH_2Cl_2, collected, and identified as compound **41** (12 mg). ^{1}H NMR (CD_3OD) δ 9.41 (s, 1H, H-11), 8.80 (dd, J = 8.0, 1.2, 1H, H-6), 8.74 (d, J = 8.8, 1H, H-4), 8.55 (dd, J = 8.4, 1.6, 1H, H-1), 8.30 (td, J = 8.4, 1.6, 1H, H-3), 8.11 (td, J = 8.4, 1.2, 1H, H-8), 8.03 (m, 2H, H-2 and H-9), 7.81 (td, J = 8.4, 1.2, 1H, H-7), 5.10 (s, 3H, 5-*N*-CH_3);^{13}C NMR (CD_3OD) δ 162.3 (s, C-9a), 149.5 (s, C-10a), 144.5 (s, C-5a), 138.9 (s, C-4a), 137.5 (d, C-8), 135.6 (d, C-3), 131.9 (d, C-1), 130.1 (d, C-2), 129.1 (s, C-11a), 127.5 (d, C-6), 127.2 (d, C-11), 126.9 (d, C-7), 119.1 (d, C-4), 117.8 (s, C-5b), 114.8 (d, C-5b), 41.4 (q, 5-*N*-CH_3), assigned by ^{1}H−^{1}H COSY and HMBC; FABMS m/z 234 $[M - I]^+$; HRFABMS m/z 234.0914 $[M - I]^+$ (calcd for $C_{16}H_{12}NO$, 234.0919).

Yeast Bioassay. The manipulated yeast (1138, 1140, and 1353) assay was performed on a 9-well agar plate as previously described.[2c]

Cytotoxicity Assays. Cytotoxicity bioassays were carried out in the M109 cell line at Bristol-Myers Squibb Pharmaceutical Research Institute by standard methods, with the results indicated in Table 1. Compounds **6** and **22** were also tested in a panel of 25 cell lines; both compounds gave similar patterns, with the cell lines OVCAR-3 (IC_{50} 0.5 μM) and A431 (IC_{50} 0.34 μM) being the most sensitive and M109 (IC_{50} 5.6 μM) and MLF (IC_{50} 4.9 μM) being the least sensitive.

Induction of Cell Cycle Dependent Growth Disturbances. Compounds **6** and **22** were subjected to analysis of their effect on cell cycle dependent growth disturbances. FACS analysis was done after 24 h exposure at increasing IC_{50} concentrations of these drugs. Results showed a G1 cell cycle arrest and apoptosis in A2780/p53wt cells at 5 × IC50. Higher concentrations showed a dose dependent increase in apoptosis. This effect appears to be p53 dependent since A2780/mutp53 cells showed no arrest in G1 and at 10 × IC_{50} showed a slow cell cycle traverse with some arrest at G2/M. Apoptosis was present only at 20 × IC_{50}. These characteristics are typical of DNA damaging agents.

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