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Ethnobotanical-Directed Discovery of the Antihyperglycemic Properties of Cryptolepine: Its Isolation from *Cryptolepis sanguinolenta*, Synthesis, and in Vitro and in Vivo Activities†

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Using an ethnobotanical approach in combination with in vivo-guided fractionation as a means for lead discovery, cryptolepine was isolated as an antihyperglycemic component of *Cryptolepis sanguinolenta*. Two syntheses of cryptolepine, including an unambiguous synthesis, are reported. The hydroiodide, hydrochloride, and hydrotrifluoromethanesulfonate (hydrotriflate) salts of cryptolepine were synthesized, and a comparison of their spectral properties and their in vitro activities in a 3T3-L1 glucose transport assay is made. Cryptolepine and its salt forms lower blood glucose in rodent models of type II diabetes. While a number of bioactivities have been reported for cryptolepine, this is the first report that cryptolepine posesses antihyperglycemic properties.

Introduction

The enormous biodiversity of the tropical rainforest has provided indigenous cultures around the globe with a deep and diversified knowledge about the use of plants for medicinal purposes. As part of our effort to access and record these oral healing traditions before they disappear,[1,2] we were interested in the bioactive constituent present in *Cryptolepis sanguinolenta* responsible for its observed use for treating associated symptoms of diabetes.[3] *Cryptolepis sanguinolenta* (Lindl.) Schlechter, a member of the Asclepiadaceae family and Periplocoideae subfamily, is a shrub indigenous to tropical West Africa with a history of ethnomedical use.[4] In Guinea Bissau, the roots are sold locally, its decoction is used in the treatment of hepatitis, and its leaves have been used for the treatment of malaria or powdered as a cicatrizant of wounds.[9−12] Root decoctions have been used in traditional West African medicine as a remedy for colic and stomach aches,[13,14] and in Ghana aqueous extracts of the roots and stems have been used clinically for over two decades in the therapies of malaria and of urinary and upper respiratory tract infections.[15−17] Our own ethnobotanical field research indicated that the aqueous extract of the root of *C. sanguinolenta* is used by traditional healers in Ghana to treat a variety of symptoms that could occur in a diabetic patient including fungal infections, pain, and inflammation. An alcohol extract of the root is also used in Ghana as a tonic to strengthen the metabolism. On the basis of this information, a 40-kg sample of *C. sanguinolenta* roots was collected. In vivo-guided fractionation using a non-insulin-dependent diabetes mellitus (NIDDM) mouse model led to the discovery of an indoloquinoline alkaloid, cryptolepine (**1**), as the active constituent.[18,19]

Cryptolepine is a rare example of a natural product whose synthesis was reported prior to its isolation from nature. Cryptolepine was first synthesized in 1906 by Fichter and co-workers.[20−22] Its isolation was first reported by Clinquart[23] in 1929 from *Cryptolepis triangularis* N. E. Br. and soon thereafter by Delvaux.[24−26] Cryptolepine was first isolated from *C. sanguinolenta* by Gellért from root samples obtained in Nigeria[27] and again by Dwuma-Badu and co-workers from root samples obtained in Ghana.[28,29] Cryptolepine has also been isolated as the major alkaloid from *Sida* sp. (Malvaceae), medicinal plants used by indigenous practitioners of Sri Lanka.[30] Cryptolepine and its hydrochloride salt possess a number of reported bioactivities, including antimicrobial,[30,31] antibacterial,[32−36] antiinflammatory,[37−39] antihypertensive,[39−41] antipyretic,[41] antimuscarinic,[42] antithrombotic,[43−46] noradrenergic receptor antagonistic,[39,47] and vasodilative[40,48] properties. Cryptolepine also possesses significant antimalarial activity[49−51] and is being pursued in some West African nations for possible therapeutic use.[52,53]

We wish to report a new bioactivity for cryptolepine, namely, its ability to lower blood glucose in NIDDM animal models. We report its isolation from the roots of *C. sanguinolenta* and three syntheses of cryptolepine and its salt forms.

Isolation

For initial screening purposes a dichloromethane extract and a hot water extract obtained from the roots of *C. sanguinolenta* were prepared and tested at 1 g/kg in genetically altered obese diabetic mice (designated C57BL/Ks-*db*/*db*, or *db*/*db*). Both extracts lowered blood glucose levels approximately 100 mg/dL 24 h postdose with no significant effect on food intake relative to the control. Using in vivo testing as a guide for fraction-

†Dedicated to Professor Henry Rapoport on the occasion of his 79th birthday.

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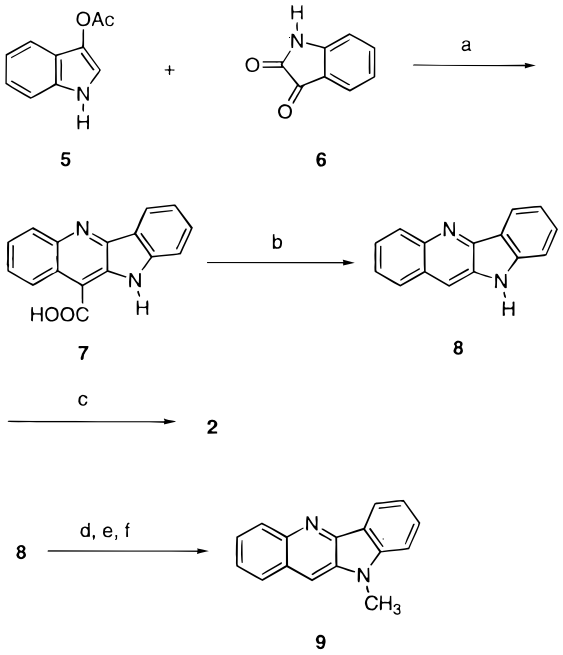
Scheme 1[a]



[a] (a) KOH, H_2O, N_2; (b) Ph_2O, 255 °C, 6 h; (c) CH_3I, MeOH, bomb, 120 °C; (d) KOH, BaO; (e) CH_3I; (f) Na_2CO_3.

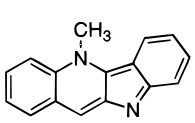
ation, the dichloromethane fraction was extracted further with aqueous Na_2CO_3, resulting in a solid residue. This material was extracted with 90% ethanol and purified over Dowex 50 × 8-400. Following HPLC, a pure alkaloid was obtained and identified as cryptolepine (**1**) on the basis of one-bond and long-range proton-detected heteronuclear correlation experiments (HMQC and HMBC). These assignments are in agreement with those reported in the literature (see Supporting Information).[29,54,55] For further studies, scale up using an alternative procedure led to a yield of 1.5 g/kg (see the Experimental Section).

Synthesis

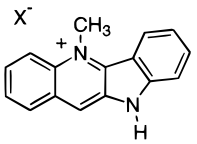
Because our planned biological evaluation using in vivo models required multigram quantities of cryptolepine, we explored a synthetic approach as an alternative to isolation of the natural product. Our first approach utilized the procedures of Holt and Petrow[56] and Deguitis and Ezyarskaite[57] with some modification (Scheme 1). Reaction of 3-indolyl acetate with isatin under an inert atmosphere gave quindoline-11-carboxylic acid (**7**) in 75% yield. Decarboxylation in diphenyl ether gave quindoline (**8**) in 90% yield. Alkylation of the N-5 nitrogen with methyl iodide was accomplished using the method of Fichter and Boehringer.[20] This method required the use of a bomb but gave good yields of the hydroiodide salt of cryptolepine. A more convenient method amenable to larger scale reactions involved the use of methyl triflate as the alkylating agent, which was accomplished at room temperature and afforded near quantitative yields of the hydrotrifluoromethanesulfonate (hydrotriflate) salt of cryptolepine. This procedure was the method of choice for preparing multigram quantities of cryptolepine hydrotriflate and, ultimately, cryptolepine.

Standard basic extraction methods could be used to convert the hydroiodide and hydrotriflate salts of cryptolepine to its free base **1**.[27] We preferred an alternative to this standard method. Small amounts of unreacted quindolines **8** or **23** were generally present as an impurity (1−2%) following alkylation with methyl iodide or methyl triflate, which could not be removed by this extractive method. Conversion to the free base with concomitant removal of the quindoline impurity was conveniently accomplished in the following manner. The cryptolepine salt was adsorbed onto sodium carbonate and loaded onto a short column of basic alumina as a dry powder. Elution with ethanol-free chloroform[58] removed the quindoline impurity. Elution with a 1−2% methanol in chloroform solution eluted the cryptolepine free base **(1)** as a purple band, which could be isolated by simple evaporation of the purple fractions. Alternatively, this purple solution was treated with an ethereal 1 M HCl solution to provide cryptolepine hydrochloride (**4**) as a bright-yellow solid.

Recognizing that the above synthetic route contained a regioisomeric ambiguity, we also synthesized cryptolepine (**1**) by a route which definitively established the location of the methyl substituent at N-5. Full details are described in the Supporting Information section. The *N*-methyl regioisomer of cryptolepine (**9**) was also synthesized (Scheme 1), and the spectral data for this compound are also shown in the Supporting Information section along with the spectral data for salts **2**−**4**. The ^1H and ^{13}C NMR spectral data for synthetic **1** matched the spectral data from the natural product **1**. Co-NMR and co-HPLC experiments on the synthetic and natural cryptolepine provided further structural confirmation.



1 Cryptolepine



2 X = I
3 X = OTf
4 X = Cl

In Vitro Studies

Cryptolepine and its salt forms were tested for their ability to stimulate glucose transport in 3T3-L1 adipocytes, a recognized in vitro model that represents an important mode of action for glucose utilization and disposal in mammals.[59] Cryptolepine (**1**) showed an increasing ability to stimulate glucose transport beginning at 3 μM with increasing concentration. Hydrochloride salt **4** gave a similar result in this assay. Hydroiodide salt **2** showed increasing activity at 3 and 10 μM, but the activity at higher concentrations leveled off, possibly due to iodide toxicity. Regioisomer **9** was inactive in this assay, indicating that the location of the methyl group at the N-5 postion was important for bioactivity. The results are shown in Figure 1.

The ability of cryptolepine hydrochloride (**4**) to enhance the effect that insulin has on 2-deoxyglucose uptake in 3T3-L1 adipocytes was also measured, and the result is shown in Figure 2. Glucose transport stimulation was shown to increase beginning at 10 μM with increasing concentration.

In Vivo Studies (*db*/*db* Mice)

Cryptolepine lowered mean plasma glucose levels in *db*/*db* mice 20% from predose levels 3 h postdose and

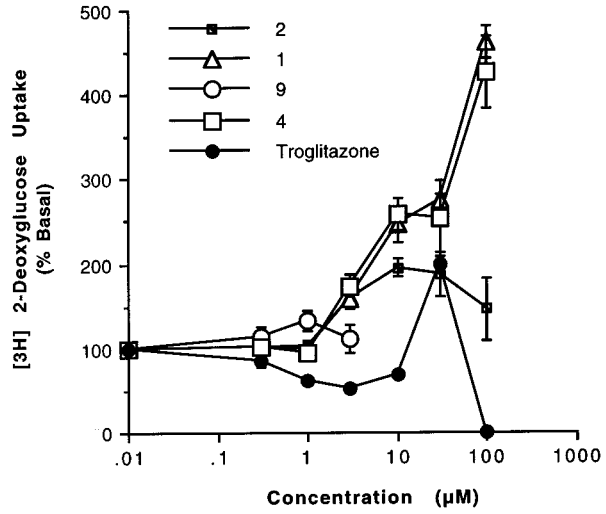


Figure 1. In vitro effect of chronic treatment of cryptolepine (**1**), hydroiodide salt **2**, hydrochloride salt **4**, and regioisomer **9** on glucose transport in 3T3-L1 adipocytes (absence of insulin).

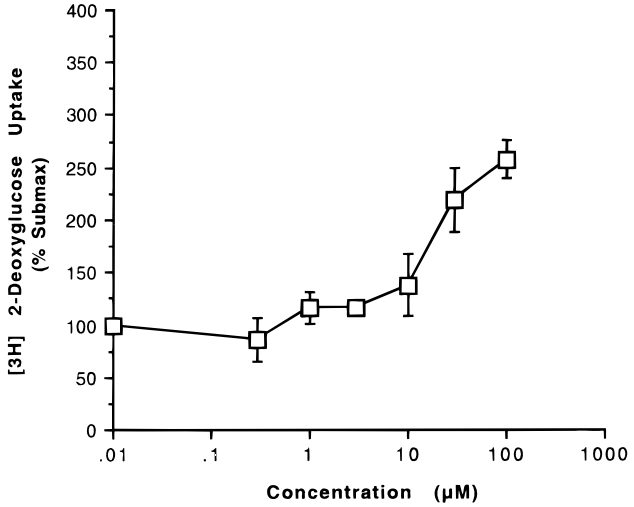


Figure 2. In vitro effect of chronic treatment of cryptolepine hydrochloride (**4**) on glucose transport in 3T3-L1 adipocytes (presence of insulin).

Table 1. Effects of Cryptolepine and Its Salts on Plasma Glucose, Body Weight, and Food Intake in *db*/*db* Mice

	plasma glucose (% change of predose)		mean body weight (g/mouse)		food intake (g/mouse/day)
treatment	3 h	24 h	0 h	24 h	48 h
vehicle	−3.6	8.6	41.3 ± 0.6	41.5 ± 0.8	4.0
1, 100 mg/kg	−19.7	−30.0	39.4 ± 1.5	38.7 ± 1.4	1.0
4, 100 mg/kg	−16.4	−43.1	41.0 ± 0.5	39.8 ± 0.5	0.9
2, 100 mg/kg	−11.5	−22.5	42.7 ± 1.3	41.9 ± 1.6	1.0
9, 100 mg/kg	15.3	14.1	41.4 ± 1.3	42.0 ± 1.5	4.4

reached 30% 24 h postdose at 100 mg/kg. Hydroiodide salt **2** and hydrochloride salt **4** lowered mean plasma glucose levels 3 h postdose and reached 22.5% and 43.1% reductions, respectively, from predose levels 24 h postdose at 100 mg/kg. These effects were accompanied by significant reductions in food consumption and mean body weight (Table 1). In this model, regioisomer **9** was inactive at 100 mg/kg, indicating the importance of the N-5 methyl substituent.

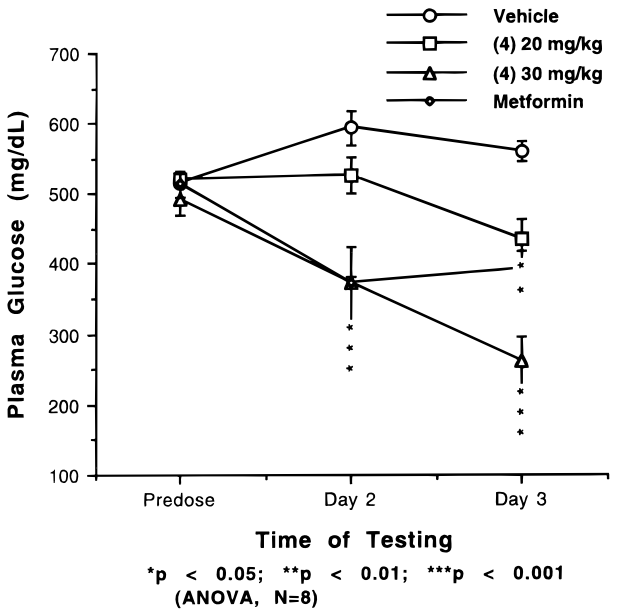


Figure 3. In vivo effects of cryptolepine hydrochloride (**4**) on plasma glucose concentrations in *db*/*db* mice.

Table 2. Effects of Cryptolepine Hydrochloride (**4**) on Body Weight and Food Intake in *db*/*db* Mice

	mean body weight (g/mouse)			food intake (g/mouse/day)	
treatment	0 h	24 h	48 h	0−24 h	24−48 h
vehicle	38.8 ± 0.2	38.6 ± 0.2	39.3 ± 0.2	5.2	7.0
20 mg/kg	38.0 ± 0.4	37.9 ± 0.4	37.4 ± 0.5	3.3	3.8
30 mg/kg	37. ± 0.8	36.9 ± 0.8	35.5 ± 0.9	2.4	2.4

Cryptolepine hydrochloride (**4**) was chosen for further study and was effective at lowering mean plasma glucose levels 16% and 47% from predose levels, 3 days postdose, respectively, at 20 and 30 mg/kg (Figure 3). At these doses, the reduction in food consumption was not as significant but was still apparent[60] (Table 2). To determine the euglycemic effect that cryptolepine possessed, we tested hydrochloride salt **4** in C57BL/ks lean mice at 30 mg/kg/day. After day 3, there was a 10% drop in mean plasma glucose concentration with a similar reduction in food consumption that was observed in the *db*/*db* mice at 30 mg/kg/day (Table 3).

To further separate the anorexigenic effect from the antihyperglycemic effect of cryptolepine hydrochloride (**4**), we performed two experiments. In the first experiment, *db*/*db* mice were divided into a control group and a treatment group. After the mice were dosed with vehicle or 30 mg/kg hydrochloride salt **4**, respectively, food was removed from both groups and plasma glucose levels were measured over time. From the data shown in Figure 4, a statistically significant difference in plasma glucose levels was observed at 3, 6, and 12 h postdose. In the second experiment, *db*/*db* mice were divided into a control group given free access to food and two pair-fed groups treated with either vehicle or 30 mg/kg hydrochloride salt **4**. Plasma glucose concentrations were measured initially and 3 days postdose. While glucose concentrations were lower than control in both pair-fed groups, mice receiving cryptolepine hydrochloride treatment had significantly lower plasma glucose concentrations than did the vehicle-treated group (Table 4).[60]

Table 3. Effects of Cryptolepine Hydrochloride (**4**) on Plasma Glucose, Body Weight, and Food Intake in Normal (C57BL/ks) Mice

	plasma glucose (mg/dL)		mean body weight (g/mouse)			food intake (g/mouse/day)	
treatment	predose	day 3	0 h	24 h	48 h	0−24 h	24−48 h
vehicle	184.5 ± 3.7	189.1 ± 7.0	24.3 ± 0.5	24.6 ± 0.5	24.6 ± 0.5	4.1	4.2
30 mg/kg	184.5 ± 4.7	170.7 ± 4.4[a]	23.7 ± 0.5	23.3 ± 0.6	22.7 ± 0.5	2.7	2.3

[a] $p < 0.05$ vs vehicle (Student's *t*-test, $n = 8$).

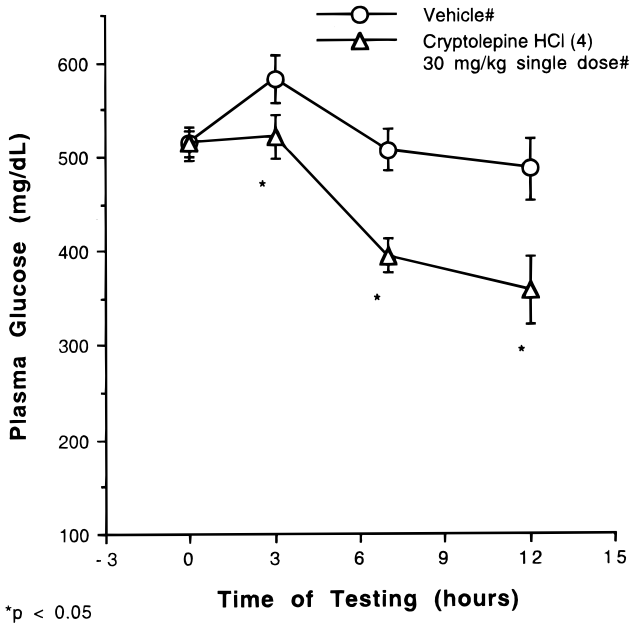


Figure 4. In vivo time course study of cryptolepine hydrochloride (**4**) in *db*/*db* mice in the absence of food.

Table 4. Pair-Fed Experiment of Cryptolepine Hydrochloride (**4**) in *db*/*db* Mice

treatment[a]	plasma glucose concentrations[b] 3 days postdose (mg/dL)
control	520 ± 20
pair-fed control	439 ± 28
pair-fed **4**, 30 mg/kg	358 ± 23[c]

[a] $n = 8$. [b] Initial plasma glucose concentrations on all groups were 495 ± 36 mg/dL. [c] $p < 0.05$.

In Vivo Studies (Fructose-Fed STZ-Treated Rats)

The effect that cryptolepine hydrochloride had on serum glucose levels in a fructose-fed STZ-treated rat model of NIDDM was investigated. The fructose-fed STZ-treated rats used in this study had significantly elevated glucose and triglyceride concentrations compared to chow-fed controls (Figures 5 and 6, $p < 0.05$). Cryptolepine hydrochloride (**4**) (30 mg/kg) lowered serum glucose concentrations in fructose-fed STZ-treated rats by 16%, 34%, and 45% on days 1, 2, and 3 of dosing, respectively (Figure 5, $p < 0.05$ on days 2 and 3). In addition, **4** lowered serum triglyceride concentrations by 30%, 39%, and 69% on days 1, 2, and 3 of dosing, respectively (Figure 6, $p < 0.05$ at all time points). Glucose and triglyceride lowering was accompanied by a significant decline in food consumption (29%, $p < 0.05$), but body weight gain over the course of the experiment was not altered by the cryptolepine hydrochloride treatment (Table 5).

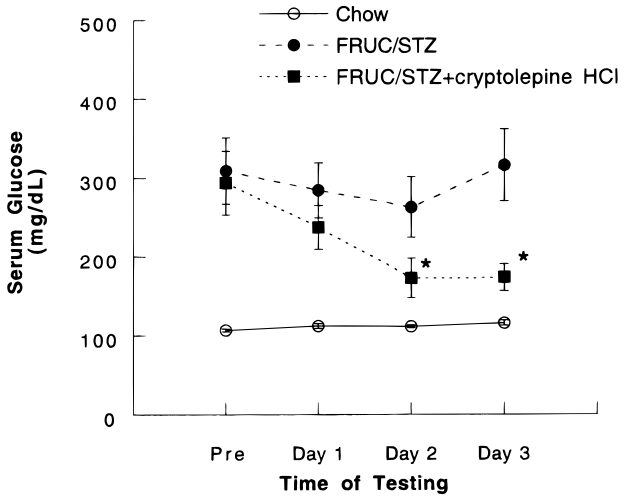


Figure 5. In vivo effects of cryptolepine hydrochloride (**4**) on serum glucose concentrations in fructose-fed STZ-treated rats.

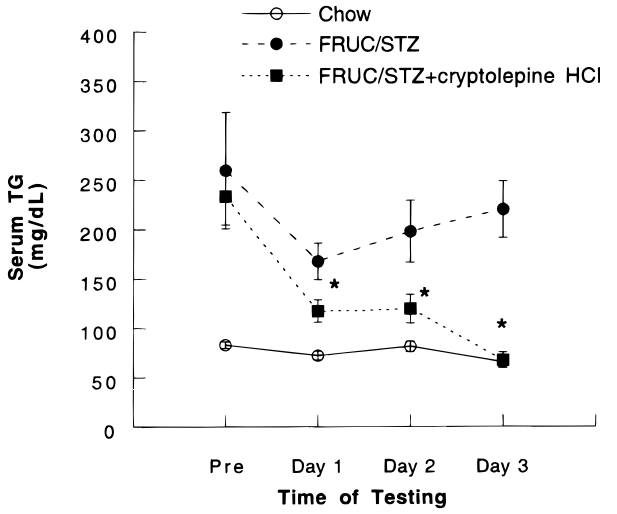


Figure 6. In vivo effects of cryptolepine hydrochloride (**4**) on serum triglyceride concentrations in fructose-fed STZ-treated rats.

Table 5. Effect of Cryptolepine Hydrochloride (**4**) on Food Consumption and Body Weight in Fructose-Fed STZ-Treated Rats

	body weight, (g)			food consumption
treatment group	predose	day 3	Δ	(g/day)
chow	207 ± 6	244 ± 2	37 ± 5	22 ± 1
fructose/STZ	193 ± 5	211 ± 6	18 ± 3	25 ± 1
fructose/STZ + cryptolepine HCl, 30 mg/kg	201 ± 4	223 ± 4	21 ± 2	18 ± 2

Conclusion

Using an ethnobotanical approach and in vivo-guided fractionation, the active component from the roots of *C. sanguinolenta* was found to be cryptolepine (**1**). Two syntheses, including an unambiguous synthesis which

provides structural proof, are reported. Three salt forms of cryptolepine (**2**−**4**) were prepared, and their spectral data were compared to that of the parent free base. The antihyperglycemic properties of cryptolepine and its salt forms are reported and measured in NIDDM animal models and using an in vitro glucose transport assay. Hydrochloride salt **4** demonstrated greater activity in vivo and in vitro than hydroiodide salt **2**. When compared to cryptolepine (**1**), hydrochloride salt **4** showed a similar in vitro profile but displayed greater efficacy in vivo. The presence of the methyl substituent at N-5 appears to be critical for activity, as the N-10 methyl regioisomer **9** was inactive in vitro and in vivo. While cryptolepine hydrochloride (**4**) lowers blood glucose in NIDDM animals models with reduced food intake, the results of a fasting time course study and a pair-fed study in *db*/*db* mice seem to indicate that this antihyperglycemic effect is separate from the anorexigenic effect.[60] The results described herein provided the basis for a cryptolepine analogue program.[61] Linkage of these biological studies on cryptolepine hydrochloride (**4**) and results of a human study involving the aqueous extract of *C. sanguinolenta* will be published elsewhere.[19]

Experimental Section

Isolation of Cryptolepine (1). Dry powdered roots (1.12 kg) of *C. sanguinolenta* were percolated in 1% acetic acid in water (10 L) at room temperature for 48 h. The filtered aqueous extract was extracted with chloroform (3 × 5 L), and the chloroform layer was separated and then discarded. The remaining aqueous extract was basified to pH 9 and extracted three times with chloroform (3 × 5 L). The chloroform layer was separated and concentrated under reduced pressure to dryness to yield 2.24 g of a crude alkaloid extract. The crude alkaloid extract was purified by a chromatotron (2-mm Si gel plates, solvent system: toluene−ethyl acetate−diethylamine−methanol, 60/25/10/5) to yield 1.5 g (0.13%) of cryptolepine as dark-purple needles, mp 178−179 °C (lit.[27] mp 175−178 °C). Spectral and chromatographic data of cryptolepine agree with reported values.[29,54,55] HRMS (EI) calcd for $C_{16}H_{12}N_2$ 232.1000, found 232.0994.

General Experimental. Benzene and toluene were distilled from CaH_2. Anhydrous DMF, dioxane, and methanol were obtained from Aldrich. Methyl triflate was distilled prior to use and stored in a Schlenk flask under nitrogen. All other reagents were used as received. Moisture-sensitive reactions were done under a nitrogen atmosphere, using dry solvents; air-sensitive reactions were done under a nitrogen atmosphere. Thin-layer chromatography for synthetic intermediates was performed on E. Merck 230−400-mesh, 200-μm, silica gel plates. Thin-layer chromatography for cryptolepine was performed on E. Merck neutral alumina plates. Low-pressure liquid chromatography (LPLC) for cryptolepine intermediates was performed on E. Merck 230−400-mesh silica gel using nitrogen pressure unless otherwise noted. Chromatography on cryptolepine and its salts was done on Fisher Activity I basic alumina using *ethanol-free chloroform* (see note).[58] ^{1}H and ^{13}C NMR were recorded at 400 and 100 MHz, respectively, with NMR shifts being expressed in ppm downfield from internal TMS. NMR assignments were determined on the basis of COSY, HMQC, and HMBC experiments, and NMR coupling constants are reported in hertz. Mass spectrometry was performed on a Kratos MS 50 spectrometer. Melting points are uncorrected.

Quindoline-11-carboxylic Acid (7). In a three-necked flask containing indolyl acetate (25 g, 0.143 mol) was added, under nitrogen with shaking, a cooled solution of isatin (21.25 g, 0.144 mol) in aqueous KOH (125.5 g of KOH in 575 mL of water). The reaction mixture was stirred vigorously under nitrogen at room temperature for 3 days. The reaction mixture was diluted with water (250 mL), and then oxygen was bubbled through the mixture while it was heated for 20 min at 75−80 °C. The reaction mixture was filtered hot, the filtrate was diluted with ethanol (750 mL) to dissolve the formed precipitate, and then concentrated HCl was added to adjust the pH of the solution to 4. The precipitate was filtered, washed with hot water and ethanol, and then dried (Na_2SO_4), yielding 28.2 g (75%) of the title compound, mp >250 °C (lit.[56] mp 330−337 °C dec): ^{1}H NMR (DMSO-d_6) δ 11.39 (s, 1H, NH), 9.14 (d, J = 7.6, 1H), 8.35 (d, J = 8.0, 1H), 8.25 (d, J = 7.2, 1H), 7.77 (d, J = 8.4, 1H), 7.67 (m, 4H), 7.32 (dd, J = 8.0, 8.0, 1H); ^{13}C NMR (DMSO-d_6) δ 167.94, 147.15, 144.43, 143.42, 132.12, 130.20, 129.41, 126.37, 125.77, 125.27, 123.82, 121.22, 120.60, 120.09, 112.47; MS (EI, m/z) 262 (M^+). Anal. ($C_{16}H_{10}N_2O_2 \cdot 0.5H_2O$) C, H, N.

Quindoline (8). A mixture of **7** (29.2 g, 0.111 mol) and diphenyl ether (300 mL) was refluxed for 6 h, cooled to 30 °C, and then diluted with petroleum ether (250 mL). The precipitate which formed was filtered and washed with petroleum ether, yielding 22.1 g (91%) of the title compound. A portion of this material was further purified by LPLC (ethyl acetate−hexane, 1:3), mp 249−251 °C (lit.[57] mp 251−252 °C): ^{1}H NMR (DMSO-d_6) δ 11.43 (s, 1H, NH), 8.36 (d, J = 7.8, 1H), 8.29 (s, 1H), 8.19 (d, J = 8.4, 1H), 8.10 (d, J = 8.2, 1H), 7.67−7.53 (m, 4H), 7.27 (dd, J = 7.4, 7.4, 1H); ^{13}C NMR (DMSO-d_6) δ 145.72, 144.03, 143.39, 132.44, 129.68, 128.68, 127.48, 126.71, 126.01, 124.84, 121.34, 120.97, 119.33, 112.99, 111.49; MS (EI, m/z) 218 (M^+).

10-Methylquindolinium Hydrochloride and 10-Methylquindoline (9). A mixture of powdered KOH (1.0 g, 17.8 mmol), BaO (2.8 g, 17.8 mmol), acetone (50 mL), and quindoline (**8**) (1 g, 4.58 mmol) was refluxed for 1 h and cooled to room temperature, and then methyl iodide (1.42 mL, 3.25 g, 22.9 mmol) was added. The mixture was refluxed for 4 h, cooled, and filtered, and the filtrate was evaporated to dryness. The residue was extracted with ether (2 × 50 mL), and the ether solution was washed with water, dried ($MgSO_4$), and then filtered. A stream of HCl gas passed through the solution, and the solid product was collected to give, after drying, 0.9 g (73.2%) of 10-methylquindolinium hydrochloride as a yellow solid.

The 10-methylquindolinium hydrochloride obtained above (250 mg, 0.9 mmol) was shaken with an aqueous 5% Na_2CO_3 solution (50 mL), extracted with ethyl ether (2 × 50 mL), and purified by LPLC (ethyl acetate−hexane, 1:6) to give 100 mg (45.5%) of **9**, mp 115−116 °C: see Supporting Information for NMR and UV data; MS (EI, m/z) 232 (M^+). Anal. ($C_{16}H_{12}N_2$) C, H, N.

5-Methylquindolinium Hydroiodide (2) (Cryptolepine Hydroiodide). A mixture of quindoline (**8**) (2.0 g, 9.2 mmol), methanol (5 mL), and methyl iodide (880 μL, 14.1 mmol) was heated in a Parr bomb at 120 °C for 4 h. After cooling, the brown precipitate (2.5 g, 75.5%) was filtered and washed with ether. Recrystallization from water gave 1.65 g (50%) of **2** as bright-yellow crystals, mp 283.5−284 °C (lit.[27] mp 284−286 °C): see Supporting Information for NMR and UV data; MS (EI, m/z) 232 (M^+). Anal. ($C_{16}H_{13}N_2I$) C, H, N.

5-Methylquindoline (1) (Cryptolepine). Hydroiodide **2** (1.5 g, 4.2 mmol) was shaken with a 5% solution of Na_2CO_3 (100 mL) and then extracted with chloroform (2 × 200 mL). Sodium carbonate was added to the solution, the mixture was concentrated, and the adsorbed product was loaded onto a short column of basic alumina. Elution with chloroform to elute the quindoline impurity followed by further elution with 1−2% methanol in chloroform gave 0.50 g (51.2%) of **1** as a purple solid, mp 178−180 °C (lit.[27] mp 175−178 °C): see Supporting Information for NMR and UV data. Anal. ($C_{16}H_{12}N_2 \cdot 1.5H_2O$) C, H, N.

Cryptolepine Hydrotrifluoromethanesulfonate (3). A suspension of **8** (11 g, 50.4 mmol) in anhydrous toluene (100 mL) was treated with methyl triflate (11.0 g, 97.2 mmol, 16 mL) at room temperature for 24 h. The mixture was diluted with ether (200 mL) and filtered, and then the solid product

was washed with ether and dried, yielding 18.3 g (94.8%) of **3** as a yellow solid, mp 241−243 °C: see Supporting Information for NMR and UV data; MS (EI, *m/z*) 232 (M^+). Anal. ($C_{17}H_{13}N_2F_3O_3S$) C, H, N.

5-Methylquindolinium Hydrochloride (4) (Cryptolepine Hydrochloride). Cryptolepine salt **3** (18.3 g) was shaken with 5% Na_2CO_3 solution (500 mL) and extracted with chloroform (3 L). The chloroform extract was dried, concentrated to a small volume (~150 mL), and then acidified with a 1 M solution of HCl in ether to give, after filtration and drying, 11.4 g (84.4%) of **4**, mp 268 °C (lit.[27] mp 263−265 °C): see Supporting Information for NMR and UV data; MS (EI, *m/z*) 232 (M^+). Anal. ($C_{16}H_{13}N_2Cl{\cdot}1.5H_2O$) C, H; N: calcd, 5.45; found, 4.89.

In Vitro Studies. Preparation of the 3T3-L1 Adipocytes. Murine 3T3-L1 preadipocytes (American Type Culture Collection CL 173) were maintained in Dulbecco's modified Eagles medium (DMEM) containing 10% (v/v) supplemented calf serum, antibiotics, and 25 mM glucose. Cells were seeded in 24-well cluster plates (10 000 cells/well), grown to confluence (typically 5 days), and induced to differentiate 2 days post-confluence (day 0) according to the standard protocol of Frost and Lane.[59] Following differentiation, adipocytes were maintained in DMEM containing 10% fetal bovine serum and provided with fresh medium every 2−3 days. Adipocytes employed in this study were used on days 7−10 postdifferentiation. On the day before the experiment, adipocytes were washed with phosphate-buffered saline and switched to serum-free DMEM medium. Concentrated stock solutions of the test compounds were freshly prepared in DMSO and diluted into culture medium. The final concentration of DMSO was 0.5% (v/v) which was also included in basal and insulin controls.

In Vitro Glucose Transport Assay (without exogenously added insulin): [^{3}H]-2-Deoxy-D-glucose Uptake in Differentiated 3T3-L1 Adipocytes. Adipocytes were treated (in triplicate) for 18 h with a test compound at 0.1, 0.3, 1, 3, 10, 30, and 100 μM final concentrations. Following overnight (18 h) treatment, the cell monolayers were washed, and the medium was switched to Krebs-Ringer Hepes (KRH) buffer. Compound-treated adipocytes were given the insulin vehicle KRH/1% bovine serum albumin (KRH/1% BSA) (containing no insulin). The final concentration of KRH/1% BSA was 4%, which was also included in the basal control. For the insulin control adipocytes, a concentrated porcine insulin stock was freshly diluted into KRH/1% BSA. The final concentration of insulin in the insulin control was 0.5 nM in 4% KRH/1% BSA. The plates were then incubated for 30 min at 37 °C. To assess the effects of the compounds on glucose transport, 2-deoxy-D-glucose uptake (a nonmetabolizable analogue of glucose) was measured in the absence of insulin stimulation. Glucose transport assays were initiated by the addition of 2-deoxy-D-[^{3}H]glucose (0.5 mCi/mL, 100 mM final concentrations) to each well followed by incubation for 10 min at 22 °C. Assays were terminated by aspirating the media and rapidly washing the monolayer two times with ice-cold phosphate-buffered saline solution. Cell monolayers were solubilized in 0.1 N NaOH and transferred to scintillation vials, and radioactivity was determined by liquid scintillation counting. All data were corrected for nonspecific hexose uptake determined in parallel samples treated for 5 min with 200 mM cytochalasin B.

In Vitro Glucose Transport Assay (with exogenously added insulin): [^{3}H]-2-Deoxy-D-glucose Uptake in Differentiated 3T3-L1 Adipocytes. Adipocytes were treated (in triplicate) for 18 h with a test compound at 0.1, 0.3, 1, 3, 10, 30, and 100 μM final concentrations. Following overnight (18 h) treatment, the cell monolayers were washed, and the medium was switched to KRH buffer. Basal control adipocytes were given the insulin vehicle KRH/1% BSA. The final concentration of KRH/1% BSA was 4%. A concentrated porcine insulin stock was freshly diluted into KRH/1% BSA and given to the compound-treated adipocytes and to the insulin control adipocytes. The final concentration of insulin was 0.5 nM in 4% KRH/1% BSA. The plates were then incubated for 30 min at 37 °C. To assess the effects of the compounds on glucose transport, 2-deoxy-D-glucose uptake (a nonmetabolizable analogue of glucose) was measured in the presence of insulin stimulation. Glucose transport assays were initiated by the addition of 2-deoxy-D-[^{3}H]glucose (0.5 mCi/mL, 100 mM final concentrations) to each well followed by incubation for 10 min at 22 °C. Assays were terminated by aspirating the media and rapidly washing the monolayer two times with ice-cold phosphate-buffered saline solution. Cell monolayers were solubilized in 0.1 N NaOH and transferred to scintillation vials, and radioactivity was determined by liquid scintillation counting. All data were corrected for nonspecific hexose uptake determined in parallel samples treated for 5 min with 200 mM cytochalasin B.

In Vivo Studies Using *db/db* Mice. Genetically altered obese diabetic mice (designated C57BL/Ks-*db*/*db*) were purchased from the Jackson Laboratory (Bar Harbor, ME) and served as experimental animals. Male animals between the ages of 8 and 9 weeks were employed in the studies described here. Animals were housed (4 mice/cage) under standard laboratory conditions at 22 °C and given Purina rodent chow and water ad libitum. Prior to treatment, blood was collected from the tail vein of each animal. Mice that had plasma glucose levels between 350 and 600 mg/dL were used. Each treatment group consisted of eight mice that were distributed so that mean glucose levels were equivalent in each group at the start of the study. Diabetic designated C57BL/Ks-*db*/*db* mice were dosed orally by gavage once daily for 1 or 2 days with either vehicle, the experimental compound administered at 30 or 100 mg/kg qd, or metformin at 250 mg (1510 mmol)/kg qd (positive control). Metformin (1,1-dimethylbiguanide) was purchased from Sigma Chemical Co. (St. Louis, MO). Compounds were delivered in a vehicle formulation appropriate for each compound, including components such as 0.25% (w/v) carboxymethylcellulose, 1% (v/v) Tween 60, and up to 10% (v/v) dimethyl sulfoxide (DMSO) in a volume of 10 mL/kg. Blood was sampled from the tail vein 3 h postdosing and analyzed for plasma glucose levels. Individual body weights and mean food consumption (each cage) were also measured after 24−27 h. Plasma glucose levels were determined colorimetrically using a glucose oxidase assay (Sigma Chemical Co.). Significant differences between groups (comparing compound-treated to vehicle-treated) were evaluated using analysis of variance and Fisher's post-hoc test.

In Vivo Studies Using Fructose-Fed STZ-Treated Rats. Male Sprague−Dawley rats at 8 weeks of age were purchased from Charles River Laboratories (Hollister, CA) and served as the experimental animals. Animals were housed (4 rats/cage) under standard laboratory conditions at 22 °C and provided with Purina rodent chow and water ad libitum. Animals were injected with streptozotocin (STZ; 55 mg/kg iv; Sigma Chemical Co.) and placed on a diet containing 60% fructose (Harlan Teklad, Madison, WI) for 1 week. Noninjected animals maintained on normal chow diet ($n = 19$) served as nondiabetic controls. Prior to treatment, blood was collected from the tail vein of each animal. Rats that had serum glucose levels between 250 and 350 mg/dL were considered diabetic and were sorted into two groups ($n = 14$) with equivalent glucose concentrations. Diabetic rats were dosed orally by gavage once daily for 3 days with either vehicle or cryptolepine hydrochloride (**4**) (30 mg/kg). Control nondiabetic animals were dosed with vehicle alone. Cryptolepine hydrochloride was administered in 2% (v/v) DMSO in a volume of 10 mL/kg. Blood was sampled from the tail vein 3 h postdosing on each dosing day and analyzed for glucose and triglyceride levels. Individual body weights and food consumption (each cage) were also measured each day. Serum glucose (glucose oxidase method) and triglyceride (GPO-trinder method) levels were determined colorimetrically using Sigma diagnostic kits (Sigma Chemical Co.). Significant differences between groups (comparing compound-treated to vehicle-treated) were evaluated using analysis of variance and Fisher's post-hoc test. A p value less than 0.05 was considered significant.

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Supporting Information Available: Experimental procedures for *N*-(2-carboxyphenyl)glycine (**12**), 1-acetyl-3-acetoxyindole (**13**), 1-acetyl-3-oxoindole (**14**), oxoindole **16**, 10-acetylquindoline (**17**), and quindolinium salt **18**; an experimental procedure for quindoline (**8**) from **17**; alternative experimental procedures for cryptolepine hydroiodide (**2**), cryptolepine hydrotrifluoromethanesulfonate (**3**), and cryptolepine hydrochloride (**4**); a scheme describing the unambiguous synthesis of cryptolepine (**1**); tables of ^{1}H and ^{13}C NMR data for cryptolepine (**1**), its salts **2**−**4**, and regioisomer **9** (Tables 6 and 7); a table of melting points and UV data (Table 8) for cryptolepine (**1**), its salts **2**−**4**, and regioisomer **9**; and elemental analyses for compounds **1**−**4**, **7**, and **9** (10 pages). Ordering information is given on any current masthead page.

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