Synthesis, Cytotoxicity, and Antiplasmodial and Antitrypanosomal Activity of New Neocryptolepine Derivatives

Tim H. M. Jonckers,[†,‡] Sabine van Miert,[§,‡] Kanyanga Cimanga,[§] Christian Bailly,[∥] Pierre Colson,[⊥] Marie-Claire De Pauw-Gillet,[#] Hilde van den Heuvel,[§] Magda Claeys,[§] Filip Lemière,[†] Eddy L. Esmans,[†] Jef Rozenski,[▽] Ludo Quirijnen,[◇] Louis Maes,[◇] Roger Dommisse,[†] Guy L. F. Lemière,[†] Arnold Vlietinck,[§] and Luc Pieters*[,§]

Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, B-2610 Antwerp, Belgium, Department of Chemistry, University of Antwerp, B-2020 Antwerp, Belgium, INSERM U-524, Institut de Recherches sur le Cancer de Lille, F-59045 Lille, France, Institute of Chemistry, University of Liège, Sart-Tilman (B6), B-4000 Liège, Belgium, CRCE, Histology−Cytology, Institute of Anatomy (L3), University of Liège, B-4020 Liège, Belgium, Rega Institute for Medical Research, B-3000 Leuven, Belgium, and Tibotec-Virco NV, B-2800 Mechelen, Belgium

Received November 13, 2001

On the basis of the original lead neocryptolepine or 5-methyl-5*H*-indolo[2,3-*b*]quinoline, an alkaloid from *Cryptolepis sanguinolenta*, derivatives were prepared using a biradical cyclization methodology. Starting from easily accessible educts, this approach allowed the synthesis of hitherto unknown compounds with a varied substitution pattern. As a result of steric hindrance, preferential formation of the 3-substituted isomers over the 1-substituted isomers was observed when cyclizing *N*-(3-substituted-phenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]carbodiimides. All compounds were evaluated for their activity against chloroquine-sensitive as well as chloroquine-resistant *Plasmodium falciparum* strains, for their activity against *Trypanosoma brucei* and *T. cruzi*, and for their cytotoxicity on human MRC-5 cells. Mechanisms of action were investigated by testing heme complexation using ESI-MS, inhibition of β-hematin formation, DNA interactions (DNA−methyl green assay and linear dichroism), and inhibition of human topoisomerase II. Neocryptolepine derivatives with a higher antiplasmodial activity and a lower cytotoxicity than the original lead have been obtained. This selective antiplasmodial activity was associated with inhibition of β-hematin formation. 2-Bromoneocryptolepine was the most selective compound with an IC_{50} value against chloroquine-resistant *P. falciparum* of 4.0 μM in the absence of cytotoxicity ($IC_{50} > 32$ μM). Although cryptolepine, a known lead for antimalarials also originally isolated from *Cryptolepis sanguinolenta*, was more active ($IC_{50} = 2.0$ μM), 2-bromoneocryptolepine showed a low affinity for DNA and no inhibition of human topoisomerase II, in contrast to cryptolepine. Although some neocryptolepine derivatives showed a higher antiplasmodial activity than 2-bromocryptolepine, these compounds also showed a higher affinity for DNA and/or a more pronounced cytotoxicity. Therefore, 2-bromoneocryptolepine is considered as the most promising lead from the present work for new antimalarial agents. In addition, 2-bromo-, 2-nitro-, and 2-methoxy-9-cyanoneocryptolepine exhibited antitrypanosomal activity in the micromolar range in the absence of obvious cytotoxicity.

Introduction

Malaria is one of the most serious parasitic diseases confronting both developing and industrialized nations, causing more than 2.5 million deaths annually and a staggering amount of chronic ill health. The main species of human malaria parasites are *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. Malaria caused by *P. falciparum* is the most severe form. A major problem at the moment in the prevention and treatment of malaria is the growing resistance of the malarial parasite *P. falciparum* to currently available drugs such as chloroquine. The incidence of malaria is increasing, especially in tropical and subtropical areas; in addition, morbidity is rising in the industrialized world. Therefore, the development of new chemotherapeutic treatments for this disease is urgently needed.[1,2]

The first antimalarial compound to be discovered, which also served as the lead compound for synthetic antimalarials of the chloroquine/mefloquine type, was the alkaloid quinine. A lot of other leads for potential new antimalarial agents have been characterized since then, and many of them have been isolated from medicinal plants.[3] One of these is the plant alkaloid cryptolepine (Figure 1) (5-methyl-5*H*-indolo[3,2-*b*]quinoline), the major alkaloid of the African plant *Cryptolepis sanguinolenta*. Infusions of *Cryptolepis* root have a long-standing reputation in the treatment of malaria in Central and West Africa (Ghana, Congo). Indeed, cryptolepine showed potent in vitro antiplasmodial activity and no cross-resistance with chloroquine, and it was also active in vivo (in mice infected with *P. berghei*).[4,5] In addition to its antiplasmodial activity cryptolepine also

* To whom correspondence should be addressed. Phone and fax: (32) 3 820 27 09. E-mail pieters@uia.ua.ac.be.
[†] Department of Chemistry, University of Antwerp.
[‡] These authors contributed equally to this work.
[§] Department of Pharmaceutical Sciences, University of Antwerp.
[∥] INSERM U-524, Lille.
[⊥] Institute of Chemistry, University of Liège.
[#] Institute of Anatomy, University of Liège.
[▽] Rega Institute for Medical Research.
[◇] Tibotec-Virco NV.

10.1021/jm011102i CCC: $22.00
Published on Web 06/29/2002

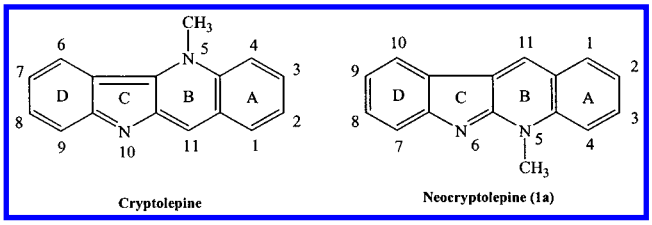


Figure 1. Two constituents of *Cryptolepis sanguinolenta*.

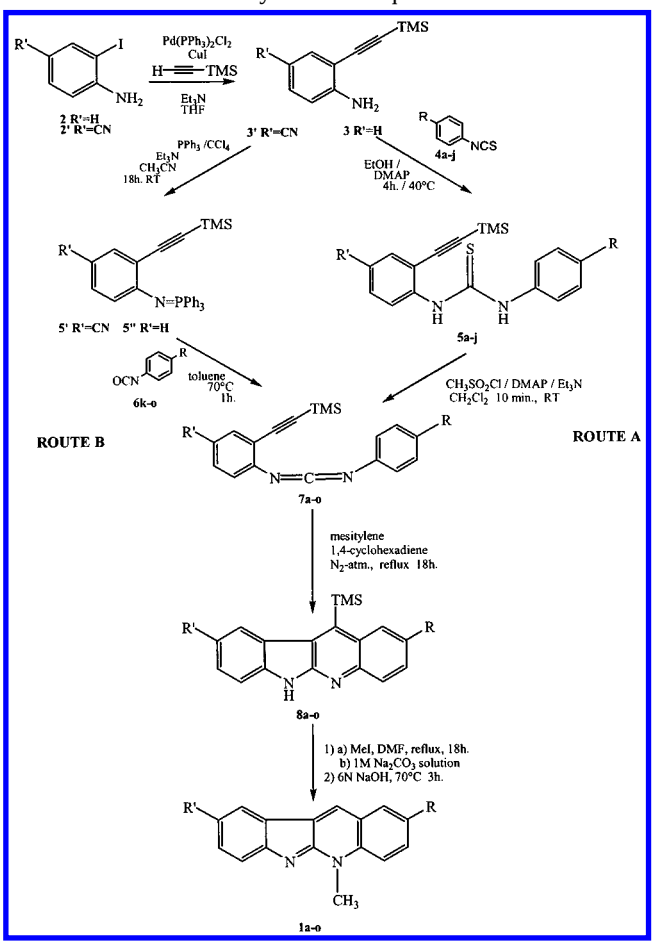
has antibacterial properties[6] and is highly cytotoxic. Its cytotoxicity is due to interaction with DNA and inhibition of topoisomerase II.[7] Also, the minor alkaloid neocryptolepine (**1a**) (Figure 1) (5-methyl-5*H*-indolo[2,3-*b*]quinoline), from the same plant, showed in vitro antiplasmodial activity against chloroquine-resistant strains of *P. falciparum*.[5] However, a direct comparison of the cytotoxicity of cryptolepine and neocryptolepine demonstrated that the latter was much less cytotoxic.[8] Synthetic *δ*-carbolines, benzo-*δ*-carbolines, and cryptolepine derivatives were evaluated for their cytotoxic, antiplasmodial, and antitrypanosomal activities. Most benzo-*δ*-carbolines and cryptolepine derivatives remained relatively cytotoxic; 1-methyl-*δ*-carboline was selected as the most promising antimalarial compound.[9] Synthetic derivatives of cryptolepine have also been evaluated for their antihyperglycaemic properties.[10] Cryptolepine also displays a series of other pharmacological effects, such as antimuscarinic, noradrenergic receptor antagonistic, antihypertensive, vasodilative, antithrombotic, antipyretic, and antiinflammatory properties,[10] some of which may contribute to the relatively high systemic toxicity observed during preliminary in vivo experiments in mice.[11]

Therefore, and because of its reduced cytotoxicity compared to cryptolepine, neocryptolepine was selected in the present work as a lead for the development of new antimalarial agents. The antiplasmodial properties of a series of synthetic neocryptolepine derivatives are discussed, as well as their activity in a series of functional assays relating to possible mechanisms of action, to establish structure−activity relationships. In addition, the antitrypanosomal activity against *T. cruzi* and *T. brucei* was also evaluated.

Chemistry

During the past 8 years, several research groups developed synthetic pathways toward **1a**. In 1994, Peczynska-Czoch et al.[12] reported the coupling between substituted 2-chloroquinolines and benzotriazole. The molecules thus obtained were subjected to a Graebe−Ullmann reaction with polyphosphoric acid (PPA) to give the corresponding 6*H*-indolo[2,3-*b*]quinolines in moderate yield. The subsequent transformation of these molecules into the corresponding neocryptolepine derivatives is straightforward. Molina et al.[13,14] reported the synthesis of **1a** via an iminophosphorane methodology, while Timári et al.[15] synthesized neocryptolepine via a Pd-catalyzed cross-coupling strategy. Although the above-mentioned procedures are straightforward for the synthesis of **1a**, using these methods for the synthesis of derivatives of neocryptolepine with a diverse substitution pattern is not that obvious. A much more promising procedure has been developed by Schmittel et al.[16] who reported a biradical cyclization reaction on *N*-[4-methyl-2-(2-trimethylsilylethynyl)phenyl]-*N*′-phenylcarbodiimide yielding 9-methyl-11-trimethylsilyl-6*H*-indolo-[2,3-*b*]quinoline, which is an obvious precursor of 9-methylneocryptolepine. Similar to this is Shi's thermolysis of *N*-[2-(1-alkynyl)phenyl]-*N*′-phenylcarbodiimides in *γ*-terpinene or *p*-xylene giving 6*H*-indolo[2,3-*b*]quinolines.[17] It is worth noting that in 1992 Saito and co-workers[18] published the Lewis acid induced intramolecular Diels−Alder reaction of conjugated carbodiimides leading to indolo[2,3-*b*] quinolines if the reaction conditions are carefully chosen. From our point of view, the methods of Schmittel and Shi seemed to be the most convenient, since they allow an easy alteration of the substitution pattern of the A-ring of **1a** by starting from appropriately substituted and commercially available phenyl isothiocyanates and phenyl isocyanates. Variation of the D-ring can arise by starting from the appropriately substituted *o*-iodoanilines. Moreover, the possibility of introducing different substituents at position 11 of the C-ring arising from the presence of a suitably substituted alkynyl group[19] in the carbodiimide made this method the most attractive one (Scheme 1).

Scheme 1. General Synthetic Sequence



The reaction sequence starts with a Sonogashira reaction on *o*-iodoanilines **2** and **2′** giving the 2-trimethylsilylethynylanilines **3** and **3′**, respectively, in good yields. Compound **2′** was synthesized starting from 4-aminobenzonitrile using a combination of ICl and $CaCO_3$ in MeOH as an iodine source.[20] First, we tried to obtain all the carbodiimides **7a**−**o** via the corresponding thioureas, but unfortunately compound **3′** did not react with the phenyl isothiocyanates **4a**−**j**. In these

Table 1. Biradical Cyclization of Carbodiimides **7a**−**o** to 11-Trimethylsilyl-6*H*-indolo[2,3-*b*]quinolines **8a**−**o**

compd type	R′	R	yield,[a] %	compd type	R′	R	yield,[a] %
a	H	H	60	**i**	H	SCH_3	89
b	H	OCH3	92	**j**	H	CN	81
c	H	Br	89	**k**	CN	H	70
d	H	Cl	85	**l**	CN	Cl	71
e	H	F	82	**m**	CN	OCH_3	91
f	H	I	74	**n**	CN	CF_3	74
g	H	CH_3	76	**o**	CN	F	72
h	H	NO_2	62				

[a] Yields given refer to analytically pure compounds.

cases, we synthesized the carbodiimides **7k**−**o** via an aza-Wittig reaction of the iminophoshorane **5′** with phenyl isocyanates[14] **6k**−**o**. Another problem was encountered in synthesizing the thioureas. When the conditions described in the Schmittel paper were used, the formation of compounds **5a**−**j** was rather troublesome. These intermediates could only be obtained in 40−50% yield after 5 days of reaction time and a problematic chromatographic purification. We therefore tried to develop a more practical method to overcome this problem. Replacing the solvent acetone by ethanol had a dramatic effect on the reaction rate as well as on the yield. After a solution of 2-(2-trimethylsilylethynyl)-aniline **3** and the appropriate isothiocyanate in ethanol was stirred in the presence of a catalytic amount of (dimethylamino)pyridine (DMAP) for 1−2 h at 40 °C, a white precipitate is formed, which after filtration and drying gives the corresponding thioureas **5a**−**j** in good yields (60−70%). Transformation of the thioureas into the carbodiimides **7a**−**j** was achieved using the procedure reported by Fell and Coppola.[21] Most of these carbodiimides are quite susceptible to degradation. Therefore, after their purification, these products were immediately used in the cyclization reaction. Such unstable behavior of carbodiimides has been reported before.[22] After biradical cyclization in the presence of 1,4-cyclohexadiene, substituted 11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinolines **8a**−**o** were obtained in good yields (Table 1). These compounds were subsequently transformed into the new neocryptolepine derivatives **1a**−**o** by methylation of the 5-nitrogen atom followed by desilylation.[17] Initially ethanol was used instead of dimethylformamide (DMF) as the solvent for the methylation; the use of the latter, however, greatly enhanced the yield. The desilylation can be performed prior to the methylation, but this in general reduces the overall yield. The outcome of the biradical cyclization does not seem to be influenced by the nature of the R and R′ substituents. This is in contrast to the effect of the substituent at the alkyne terminus, which determines the reaction product of the biradical cyclization.[16] Besides the products listed in Table 1, we also prepared 7-trimethylsilylnaphtho[1,2-*b*]-α-carboline **8p** and 2,4-dichloro-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline **8q** (via route A), but these compounds could not be methylated even after prolonged reaction times (Figure 2). A low electron density in the A-ring caused by an electron-withdrawing substituent may deactivate the 5-nitrogen atom toward nucleophilic attack. However, this possibility can be ruled out by the successful synthesis of 2-cyanoneocryptolepine (**1j**) and 2-nitroneocryptolepine (**1h**). It therefore seems that the presence of a substituent at the 4-position prohibits the introduction of a methyl group at the 5-nitrogen atom because of steric hindrance.

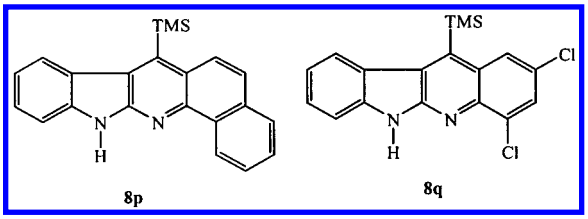
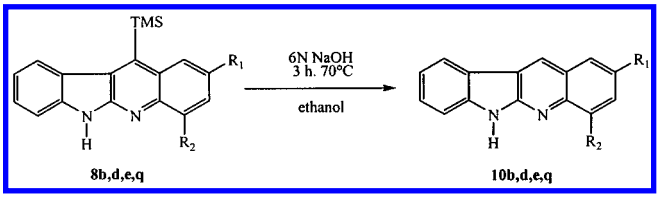


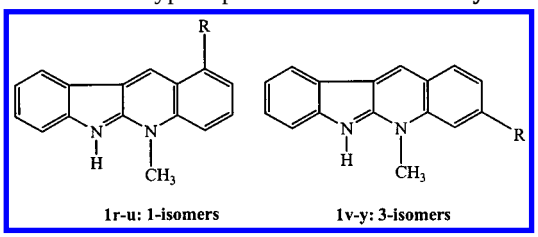
Figure 2. Cyclization products resistant to methylation.

Scheme 2. Synthesis of Some Desilylated Compounds[a]



[a] Compound **b**: $R_1 = OCH_3$, $R_2 = H$. Compound **d**: $R_1 = Cl$, $R_2 = H$. Compound **e**: $R_1 = F$, $R_2 = H$. Compound **q**: $R_1 = Cl$, $R_2 = Cl$.

Scheme 3. Neocryptolepine Derivatives **1r**−**1y**



We were also interested in some non-methylated derivatives of **1a**, and therefore, we prepared 2-methoxy-6*H*-indolo[2,3-*b*]quinoline (**10b**), 2-chloro-6*H*-indolo[2,3-*b*]quinoline (**10d**), 2-fluor-6*H*-indolo[2,3-*b*]quinoline (**10e**), and 2,4-dichloro-6*H*-indolo[2,3-*b*]quinoline (**10q**) by desilylation of the corresponding silylated compounds (**8b, 8d, 8e, 8q**) (Scheme 2).

Besides 2- and 9-substituted neocryptolepine derivatives, we wanted to prepare other isomers of the products synthesized so far for structure−activity relationship (SAR) studies. As a starting point, a further modification of the A-ring was investigated. However, some important remarks have to be made in this context. First of all, from a chemical point of view, the iminophosphorane route (route B, Scheme 1) proved to be more practical than the thiourea pathway (route A, Scheme 1). Therefore, we decided to use this approach for the synthesis of the extra set of neocryptolepine derivatives using the iminophosphorane (**5″**).[17] Second, if one uses the biradical cyclization reaction to construct the tetracyclic core starting from *N*-(3-substituted-phenyl)-*N′*-[2-(2-trimethylsilylethynyl)phenyl]carbodiimides (**7r**−**u**), one can expect a mixture of the 1- and 3-substituted isomers because two different ring-closure reactions can occur (Scheme 3, Table 2).

Both isomers were formed with the 3-isomer predominating in each case. In the case of the chloro-substituted carbodiimide (**7t**), only a very low amount of the 1-isomer (**8t**) was formed (less than 1%). The preferential formation of the 3-isomers is undoubtedly the result of the absence of steric hindrance between the substitu-

Table 2. Synthesis of Isomeric Neocryptolepine Precursors

compd type	carbodiimide **7**	reaction product **8**	substitution	yield,[a] %
r	**7r**	**8r**	1-OCH_3	8
		8v	3-OCH_3	41
s	**7s**	**8v**	1-Br	13
		8w	3-Br	55
t	**7t**	**8t**	1-Cl	<1[b]
		8x	3-Cl	52
u	**7u**	**8u**	1-CF_3	4
		8y	3-CF_3	39

[a] Yields given refer to analytically pure compounds. [b] Product not characterized.

ent and the bulky trimethylsilyl group when the ring-closure reaction takes place.

Results and Discussion

(a) In Vitro Antiplasmodial Activity and Cytotoxicity. All synthesized compounds and cryptolepine from natural origin[5] were evaluated as their hydrochloride salts for in vitro antiplasmodial activity against a chloroquine-sensitive and a chloroquine-resistant *P. falciparum* strain and for cytotoxicity on a human cell line (MRC-5 cells) (Table 3). All compounds were marginally more active against the chloroquine-resistant strain than against the chloroquine-sensitive one, so the discussion will be focused on activity against the first one. It is confirmed that neocryptolepine (**1a**), the original lead in this work, is less cytotoxic than cryptolepine; however, the antiplasmodial activity is also reduced. Many of the 2-substituted neocryptolepine derivatives are more antiplasmodially active than neocryptolepine itself but are also more cytotoxic. The most notable compound in this regard is 2-methylneocryptolepine (**1g**), with a cytotoxicity and antiplasmodial activity comparable to those of cryptolepine. This is in agreement with earlier findings for a series of methyl-substituted indolo[2,3-*b*]quinoline derivatives.[12] Although 2-methylneocryptolepine was not included in this study, it was found that the cytotoxicity (against KB cells) was strongly influenced by the position and the number of methyl substituents, the most cytotoxic one being 2,5,9,11-tetramethyl-5*H*-indolo[2,3-*b*]quinoline. The 2-halo-substituted neocryptolepines, on the other hand (2-bromo-, 2-chloro-, 2-fluoro-, and 2-iodoneocryptolepine) (**1c**, **1d**, **1e**, and **1f**, respectively), are more active against *P. falciparum* than neocryptolepine (**1a**) and less cytotoxic, the most active and selective compound being 2-bromoneocryptolepine (**1c**) with an IC_{50} against cloroquine-resistant *P. falciparum* of 4.0 μM while the IC_{50} on the MRC-5 cells is >32 μM. Removal of the *N*-methyl group, as in compounds **10d** (compared to 2-chloroneocryptolepine **1d**) and **10e** (compared to 2-fluoroneocryptolepine **1e**), leads to a complete loss of biological activity in the concentration range tested. When comparing 1-, 2-, and 3-substituted neocryptolepine isomers, e.g., in the Br-substituted series, 1-bromo substitution (**1s**) leads to a loss of antiplasmodial activity; 3-bromoneocryptolepine (**1w**) has about the same antiplasmodial activity against the chloroquine-resistant strain, but it is more cytotoxic. In the methoxy-substituted series, 2-methoxyneocryptolepine (**1b**) and 3-methoxyneocryptolepine (**1v**) are especially more antiplasmodially active than neocryptolepine (**1a**) but also more cytotoxic. Interestingly, introduction of a 9-cyano substituent, as in compound **1k** (compared to neocryptolepine **1a**), compound **1l** (compared to 2-chloroneocryptolepine **1d**), and compound **1m** (compared to 2-methoxyneocryptolepine **1b**), leads to a loss of cytotoxicity but also to a reduction of the antiplasmodial activity.

(b) DNA Interactions and Inhibition of β-Hematin Formation. All compounds were also evaluated in functional assays related to possible mechanisms of action. The first mechanism of action to be proposed for cryptolepine as an antimalarial agent was intercalation into DNA, like 9-aminoacridine,[4] but conflicting results have been published.[23] For chloroquine, an aminoquinoline, the hypothesis that interaction with DNA is responsible for the antiplasmodial effect has been abandoned. Recent hypotheses propose that quinoline antimalarials, such as quinine and chloroquine, active against the intraerythrocytic stages of the *Plasmodium* parasite act by interfering with the digestion of hemoglobin in the acid food vacuole of the *Plasmodium* parasite. While the globin part is used as a source of amino acids, the heme part is toxic to the parasite and is therefore converted to an insoluble crystalline substance known as hemozoin or malaria pigment. The latter process is thought to be inhibited by the quinoline antimalarials.[1] It has been shown that the corresponding in vitro process, the spontaneous polymerization of hematin to β-hematin in cell-free systems, is also inhibited by quinoline antimalarials such as chloroquine and quinine.[24] For chloroquine, it has also been claimed that depolymerization of malarial hemozoin, and/or inhibition of the glutathione-dependent degradation of heme in the cytosol of the parasite, may be responsible in part for its activity.[25,26] Cryptolepine, similarly to chloroquine, was able to inhibit the formation of β-hematin in vitro, and it was proposed that this mechanism is likely to be responsible, at least in part, for its antiplasmodial activity.[11,27]

Taking this into account, all compounds synthesized were evaluated not only for their inhibiting effect on the polymerization of hematin to β-hematin in cell-free systems but also in the DNA−methyl green assay, a colorimetric microassay for the detection of agents that interact with DNA.[28] Compounds interacting with DNA are able to displace methyl green from a methyl green−DNA complex, leading to a loss of color and a decrease in absorbance. IC_{50} values obtained in this assay (i.e., the concentration of test compound leading to a 50% decrease of absorbance, corresponding to 50% displacement of the dye from the DNA complex), together with the effect on the formation of polymerized β-hematin, are displayed in Table 3. Cryptolepine, neocryptolepine (**1a**), and many of the substituted neocryptolepine derivatives are able to inhibit the formation of β-hematin in cell-free systems. For some compounds, most notably those lacking the *N*-methyl group (compounds **10b**, **10d**, **10e**, and **10q**), this is not accompanied by an antiplasmodial activity, so obviously the ability to inhibit β-hematin formation is not enough to show in vitro antiplasmodial activity. This may be related to the presence of the compound at the site of action, i.e., the heme detoxification process in the plasmodial food vacuole. Only four compounds show affinity for DNA in the concentration range tested: cryptolepine, being

Table 3. In Vitro Cytotoxic, Antiplasmodial, and Antitrypanosomal Activity, DNA Interaction (DNA Methyl Green Assay), and Inhibition of β-Hematin Formation of Synthetic Neocryptolepine Derivatives

compd	cytotoxicity (MRC-5 cells) IC_{50} (μM)	*Plasmodium falciparum* (chloroquine sensitive) (Ghana strain) IC_{50} (μM)	*Plasmodium falciparum* (chloroquine resistant) (W2 strain) IC_{50} (μM)	*Trypanosoma brucei* IC_{50} (μM)	*Trypanosoma cruzi* IC_{50} (μM)	DNA interaction IC_{50} (μM)	inhibition of β-hematin formation[a]
1a	11.0 ± 1.4	27.3 ± 5.7	14.0 ± 1.7	4.0 ± 0.1	4.0 ± 0.1	92.8 ± 9.7	+
1b	4.0 ± 0.1	4.3 ± 0.6	4.7 ± 0.6	0.3 ± 0.1	1.7 ± 0.6	77.9 ± 4.4	−
10b	>32	>32	>32	>32	*b*	>1000	+
1c	>32	6.0 ± 6.1	4.0 ± 0.1	1.0 ± 0.1	>32	>400	+
1d	16.5 ± 0.7	21.0 ± 8.9	5.0 ± 0.1	1.3 ± 0.6	12.0 ± 1.7	>500	+
10d	>32	>32	>32	>32	>32	>1000	+
1e	15.0 ± 0.1	19.3 ± 3.8	4.7 ± 0.6	14.3 ± 0.6	6.0 ± 1.0	>500	+
10e	>32	>32	>32	>32	>32	>1000	−
1f	16.0 ± 0.1	17.7 ± 5.1	6.3 ± 0.6	1.0 ± 0.1	18.3 ± 0.6	>400	+
1g	0.95 ± 0.07	2.7 ± 2.1	2.3 ± 0.6	0.1 ± 0.1	*b*	*b*	*b*
1h	>32	29.0 ± 1.7	>32	0.7 ± 0.1	*b*	>1000	*b*
1i	5.0 ± 0.1	4.0 ± 1.0	3.7 ± 0.6	0.8 ± 0.1	4.0 ± 0.1	>600	+
1j	16.0 ± 0.1	17.0 ± 1.0	15.3 ± 0.6	4.0 ± 1.0	16.3 ± 0.6	>600	*b*
1k	>32	>32	>32	>32	*b*	>1000	+
1l	>32	>32	>32	>32	*b*	*b*	*b*
1m	>32	28.3 ± 3.5	17.0 ± 6.2	1.0 ± 0.1	1.0 ± 0.1	>500	−
1n	>32	14.0 ± 2.6	6.7 ± 1.1	>32	>32	>200	*b*
1o	11.0 ± 5.7	16.3 ± 1.2	14.7 ± 4.9	4.0 ± 0.1	>32	>600	+
10q	>32	>32	>32	>32	*b*	>1000	+
1r	5.0 ± 0.1	17.7 ± 0.6	12.3 ± 4.2	1.3 ± 0.6	4.0 ± 0.1	>1000	−
1s	16.0 ± 0.1	>32	>32	16.7 ± 0.6	15.7 ± 0.6	>600	−
1u	16.0 ± 0.1	>32	>32	>32	16.3 ± 0.6	>400	*b*
1v	3.5 ± 0.7	3.3 ± 0.6	1.7 ± 0.6	0.4 ± 0.1	1.0 ± 0.1	150.3 ± 4.0 (37% inhibition)	−
1w	18.5 ± 0.7	30.0 ± 3.5	4.7 ± 0.6	27.3 ± 4.5	>32	>600	+
1x	15.5 ± 0.7	21.7 ± 4.0	4.7 ± 0.6	9.3 ± 1.5	17.0 ± 2.0	>700	+
1y	18.0 ± 0.1	16.3 ± 0.6	15.7 ± 1.5	16.7 ± 1.5	>32	>500	+
cryptolepine	1.5 ± 0.7	2.3 ± 0.6	2.0 ± 0.1	3.0 ± 0.1	*b*	65.7 ± 3.0	+

[a] +, inhibition of β-hematin formation; −, no inhibition of β-hematin formation. [b] Not tested.

the most potent one (IC_{50} 65.7 ± 3.0 μM), followed by 2-methoxyneocryptolepine (**1b**) (77.9 ± 4.4 μM) and neocryptolepine (**1a**) (92.8 ± 9.7 μM); for 3-methoxyneocryptolepine (**1v**) 37% displacement was observed at a concentration of 150 μM. Cryptolepine and neocryptolepine (**1a**), but not 2-methoxy- (**1b**) or 3-methoxyneocryptolepine (**1v**), are capable of inhibiting the formation of β-hematin. Nevertheless, the last two compounds also show a higher antiplasmodial activity than neocryptolepine (**1a**) itself.

From these data, it is obvious that there are at least two different targets for the antiplasmodial activity of cryptolepine, neocryptolepine, and the neocryptolepine derivatives synthesized, and some typical compounds can be selected. Neocryptolepine derivatives, such as 2-bromoneocryptolepine (**1c**), are more antiplasmodially active than neocryptolepine (**1a**) itself, are able to inhibit the formation of β-hematin, and show a reduced or no cytotoxicity and no DNA interactions in the concentration range tested. Their antiplasmodial activity is likely to be due to a specific mechanism of action (inhibition of the heme detoxification process in the parasite). On the other hand, a compound such as 2-methoxyneocryptolepine (**1b**) is also more antiplasmodially active than neocryptolepine (**1a**) but is not able to inhibit formation of β-hematin. However, it shows affinity for DNA, and hence, it is also cytotoxic. The antiplasmodial activity is not due to a selective mechanism of action. Other compounds, and most notably cryptolepine itself, show inhibition of β-hematin formation as well as DNA interactions and most probably have at least two targets in the *Plasmodium* parasite: the heme detoxification process and DNA-containing structures. To support this hypothesis, more detailed experiments were performed for some selected compounds, i.e., cryptolepine, neocryptolepine (**1a**), 2-methoxy- (**1b**), 2-bromo- (**1c**), and 2-chloroneocryptolepine (**1d**) (see below).

Earlier work on synthetic indoloquinolines has already led to compounds with a higher antiplasmodial activity than cryptolepine, such as 11-methylcryptolepine[9] and 2,7-dibromocryptolepine.[11] However, 11-methylcryptolepine was also more cytotoxic than cryptolepine. Although the antiplasmodial activity of 2,7-dibromocryptolepine was confirmed in in vivo experiments in mice infected with *Plasmodium berghei* without apparent toxicity to the host, this compound also showed slightly higher in vitro cytotoxicity than cryptolepine, which may limit its clinical usefulness. δ-Carbolines such as 1-methyl-δ-carboline showed a selective antiplasmodial activity and no cytotoxicity on L6 cells.[9] This compound was found to be specifically localized in a parasite structure that could correspond to the parasite nucleus. Inhibition of β-hematin formation was not evaluated. Although in the present work we have not been able to design neocryptolepine derivatives with a higher antiplasmodial activity than cryptolepine, it has been possible to improve the antiplasmodial activity of neocryptolepine while at the same time reducing its cytotoxicity, i.e., to obtain more selective antiplasmodial compounds.

(c) DNA Binding. Electric Linear Dichroism (ELD) Measurements. It is now well established that cryptolepine and related indoloquinolines (in particular ellipticines) interact with DNA as intercalators.[7,29] Neocryptolepine (**1a**) also intercalates into DNA but exhibits a reduced affinity for DNA compared to cryptolepine.[8] Electric linear dichroism (ELD) experiments provide firm evidence that the newly designed neocryptolepine analogues also behave as typical DNA intercalating agents. Figure 3a shows the ELD spectra for

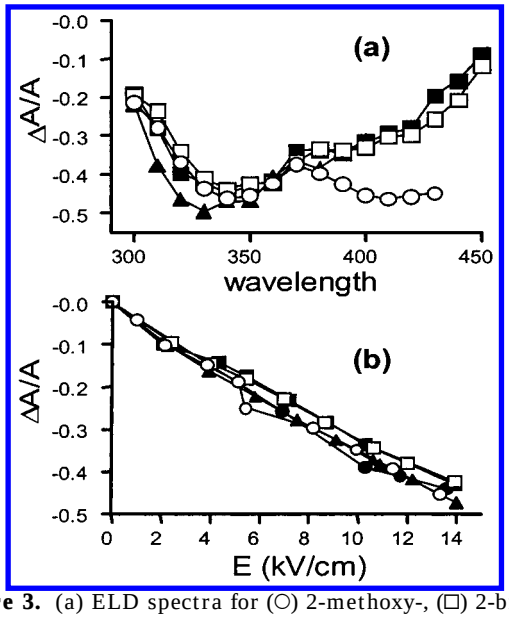


Figure 3. (a) ELD spectra for (○) 2-methoxy-, (□) 2-bromo-, (■) 2-chloro-, and (▲) neocryptolepine bound to calf thymus DNA. (b) Dependence of the reduced dichroism $\Delta A/A$ on the electric field strength for the different drug−DNA complexes at 350 nm and for DNA alone at 260 nm (●). ELD measurements were performed at a P/D ratio of 20 (200 μM DNA, 10 μM drug) in 1 mM sodium cacodylate buffer, pH 7.0.

2-methoxy- (**1b**), 2-bromo- (**1c**), and 2-chloroneocryptolepine (**1d**) bound to calf thymus DNA. Their mode of binding to DNA was analyzed on the basis of the highest ELD values obtained when the drug molecules are fully bound to DNA, i.e., for P/D ratios of 20. In each case, the reduced dichroism is negative in sign in the 300−400 nm region corresponding to the absorption band of the indoloquinoline unit. The reduced dichroism measured for the drug−DNA complexes at 320−360 nm is almost identical to that obtained with DNA alone at 260 nm (Figure 3b). This implies that the drug is oriented parallel to the DNA base pairs, as expected for an intercalation binding mode. ELD defines the orientation of drug bound to DNA, whereas the DNA−methyl green assay defines the relative affinity for DNA. 2-Chloro- (**1d**) and especially 2-bromoneocryptolepine (**1c**) have a low affinity for DNA, which is in agreement with their reduced cytotoxicity, whereas 2-methoxyneocryptolepine (**1b**) has a higher affinity for DNA.

(d) Inhibition of Topoisomerase II. In contrast to cryptolepine, which is a DNA intercalator and a potent poison for topoisomerase II, neocryptolepine poorly inhibits the DNA relaxing enzyme. We have shown recently that cryptolepine is about 5 times more potent than neocryptolepine at inhibiting the unwinding of closed circular duplex DNA by human topoisomerase II.[8] Both DNA relaxation and cleavage assays with plasmid DNA and ^{32}P-labeled DNA restriction fragments, respectively, were performed to evaluate the effects of the 2-substituted neocryptolepine analogues on topoisomerase II activity (human p170 isoform), but none of them showed an effect superior to that of neocryptolepine. In the presence of 2-methoxy (**1b**), 2-bromo- (**1c**), or 2-chloroneocryptolepine (**1d**) (20 μM each), topoisomerase II mediated DNA cleavage was found to be slightly stimulated, but in all cases, the extent of DNA cleavage never exceeded 15% of the DNA products (data not shown). As discussed previously, we consider that topoisomerase II may not represent the essential target for the cytotoxicity of neocryptolepine.[29] However, it is important to bear in mind that these experiments were performed with commercially available human topoisomerase II and it remains possible that these compounds selectively interfere with the activity of *Plasmodium* topoisomerase II. For some compounds known as inhibitors of topoisomerase II, antiplasmodial activity has been reported, e.g., the fluoroquinolone antibiotics, which are gyrase (bacterial, prokaryotic topoisomerase II) inhibitors.[30] For *P. falciparum*, two distinct topoisomerase II activities have been demonstrated: a nuclear eukaryotic type II and a bacterial (prokaryotic) type II topoisomerase activity associated with plastid DNA replication.[31] This plastid is an atypical organelle essential for parasite survival and represents an effective target for parasitic drug design.[32] Therefore, it would be quite useful to compare the effects of the test drugs on mammalian versus plasmodial topoisomerase II. This hypothesis will be explored in our laboratories.

(e) Characterization of Heme: Gas-Phase Ion Drug Complexes by Electrospray Ionization (ESI) Mass Spectrometry. Recently, Wright et al.[33] have described a mass spectrometric method to detect complexes formed between a test compound and heme, which was adapted in our laboratory and applied to cryptolepine, neocryptolepine (**1a**), and its 2-methoxy- (**1b**), 2-bromo- (**1c**), and 2-chloro- (**1d**) derivatives. Figure 4a illustrates the ESI mass spectrum obtained for a mixture containing 40 μM heme and 100 μM cryptolepine in MeOH/H_2O (1:1; v/v; pH 7). Under these conditions, stable gas-phase cryptolepine complexes were detected at *m/z* 848, *m/z* 1463, and *m/z* 1481, corresponding to the complex of the drug with the heme monomer $[FP:C]^+$, the heme dimer $[(2FP - H):C]^+$, and the dehydrated dimer of hematin $[(FP-O-FP):C]^+$ respectively. For neocryptolepine and its derivatives, only the complex with the heme monomer $[FP:C]^+$ and the dimer $[(2PF - H):C]^+$ could be detected (data not shown). The relative strength of binding between the drugs and the monomeric and dimeric heme forms was assessed using low-energy collision-induced dissociation. Figure 4b illustrates the collision-induced dissociation (CID) spectra obtained for the complexes between the heme monomer and cryptolepine, neocryptolepine (**1a**), and 2-bromoneocryptolepine (**1c**). Ions corresponding to the drug complexes with the heme monomer $[FP:C]^+$ were selected and collided with helium. In each case, an FP^+ peak (*m/z* 616) was produced because of loss of the drug that, relative to the precursor $[FP:C]^+$ peak, was about 12-fold weaker with cryptolepine than with neocryptolepine, indicating a higher binding capacity of cryptolepine. On the basis of the CID spectral data, the order of stability for $[FP:C]^+$ complexes of neocryptolepine and its derivatives was 2-methoxy > 2-Cl > 2-Br > neocryptolepine (the ion intensity ratios $FP^+/[FP:C]^+$ were 0.81, 0.88, 0.98, and 1.22, respectively; relative standard deviation (RSD) = 0.02). The order of stability for the complexes with the heme dimer $[(2FP - H):C]^+$ of neocryptolepine and its derivatives inferred from the CID spectral data was 2-methoxy > 2-Br > neocryptolepine > 2-Cl (the ion intensity ratios $[2FP - H]^+/[(2FP - H):C]^+$ were 0.12, 0.24, 0.25, and 0.27, respectively; RSD = 0.02). These data demonstrate that

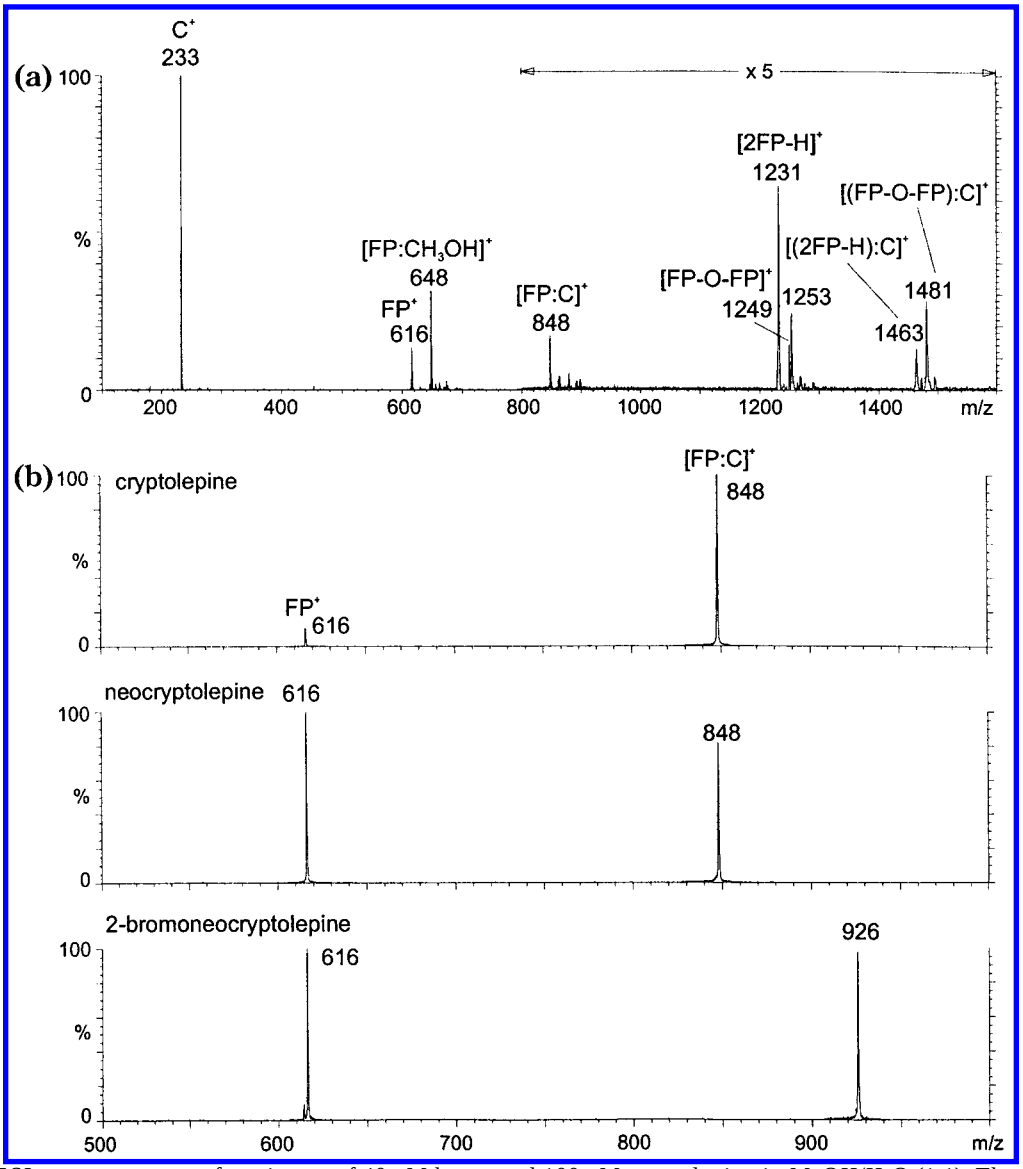


Figure 4. (a) ESI mass spectrum of a mixture of 40 μM heme and 100 μM cryptolepine in MeOH/H_2O (1:1). The ion at *m*/*z* 1253 corresponds to the complex consisting of the heme dimer and methanol $[(2FP-H):MeOH]^+$. (b) CID spectra obtained for the complex consisting of the heme monomer with cryptolepine, neocryptolepine (**1a**), and 2-bromoneocryptolepine (**1c**).

neocryptolepine and its derivatives show a stronger binding to the dimeric heme form than to the monomeric one. Apparently all selected compounds were able to form complexes with the monomeric and dimeric forms of heme, but 2-methoxyneocryptolepine (**1b**) was not able to inhibit the polymerization of hematin to β-hematin in cell-free systems as described above. Whereas complex formation as detected by MS may be a prerequisite for inhibition of β-hematin formation, it does not necessarily indicate that the test compound is actually capable of inhibiting this polymerization reaction.

(f) Antitrypanosomal Activity. Because of the antitrypanosomal activity of cryptolepine,[9] all compounds synthesized were also tested against *T. brucei* and *T. cruzi* (Table 3). Cryptolepine showed an IC_{50} against *T. brucei* of 3.0 μM, but this is obviously not due to a selective mechanism of action, in view of its antiplasmodial and cytotoxic activity. The same is true for a series of other compounds, most notably 2-methylneocryptolepine (**1g**), with an IC_{50} value against *T. brucei* of 0.1 μM and also a significant cytotoxicity (IC_{50} on MRC-5 cells of 0.95 μM). More specific antitrypanosomal activity against at least one of the two *Trypanosoma* species tested, combined with absence of obvious cytotoxicity in the concentration range tested, is observed for 2-bromo- (**1c**), 2-nitro- (**1h**), and 2-methoxy-9-cyanoneocryptolepine (**1m**). The last compound showed an IC_{50} of 1.0 μM against *T. brucei* as well as *T. cruzi*, whereas the IC_{50} on the MRC-5 cells was >32 μM. Therefore, this neocryptolepine derivative could be considered as a new lead structure with antitrypanosomal potential. Additional exploration in this area is warranted.

Conclusion

In conclusion, some new neocryptolepine (indolo[2,3-*b*]quinoline) derivatives were synthesized with a more selective antiplasmodial activity than the original lead. This activity is most probably due to inhibition of the heme detoxification process (formation of hemozoin), as demonstrated by the investigation of heme complexation and the polymerization of hematin to β-hematin in cell-free systems. 2-Bromoneocryptolepine (**1c**) was the most selective compound with an IC_{50} value against

chloroquine-resistant *P. falciparum* of 4.0 μM, in the absence of obvious cytotoxicity ($IC_{50} > 32$ μM). Although this was higher than the IC_{50} value of cryptolepine (2.0 μM), 2-bromoneocryptolepine showed a low affinity for DNA, and no inhibition of human topoisomerase II. Although some neocryptolepine derivatives with an in vitro antiplasmodial activity higher than that of 2-bromoneocryptolepine were obtained, these compounds also showed a higher affinity for DNA and/or a more pronounced cytotoxicity on a human cell line, as observed for cryptolepine as well. From the present work, 2-bromoneocryptolepine is therefore considered as the most promising lead for potentially new antimalarial agents. In addition, 2-bromo-, 2-nitro-, and 2-methoxy-9-cyanoneocryptolepine derivatives exhibited some antitrypanosomal activity and could be considered as new lead structures for antitrypanosomal agents.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on Varian Unity and Bruker DRX-400 spectrometers, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C and with $CDCl_3$ as the solvent unless otherwise stated. In every case tetramethylsilane was used as the internal standard. Chemical shifts are given in ppm and *J* values in Hz. Multiplicity is indicated using the following abbreviations: d for a doublet, t for a triplet, m for a multiplet, etc. In those cases where standard ^{1}H and ^{13}C measurements were insufficient, additional 2D measurements (i.e., COSY, HETCOR or HSQC, LR-HETCOR or HMBC) were performed for complete structure elucidation. IR spectra were recorded on a Bruker Vector 22 spectrometer. Low-resolution mass spectra were recorded on a triple quadrupole mass spectrometer (Quattro II, Micromass, Manchester, U.K.) using electrospray ionization (ESI). Samples were dissolved in CH_3CN and infused at 5 μL/min into the mass spectrometer. Fragmentation was induced by low-energy collisional activation using an Ar gas pressure of approximately 10^{-3} mbar and a collision energy between 25 and 35 eV depending on the compound. Exact mass measurements were performed on a quadrupole/orthogonal-acceleration time-of-flight (Q/oaTOF) tandem mass spectrometer (qTof 2, Micromass, Manchester, U.K.) equipped with a standard electrospray ionization (ESI) interface. Samples were diluted in an appropriate solvent (MeOH/0.1% formic acid 90/10), and 1 μL aliquots were injected at a flow rate of 5 μL/min using the CapLC system (Waters, Brussels, Belgium). The mass scale was corrected by an internal lock-mass provided by the protonated molecule of 2′-deoxyadenosine (*m*/*z* 252.1096), which was added into the LC stream at a flow rate of 2.5 μL/min (concentrated 10^{-5} M using the same solvent composition) using a low dead volume tee union. Combined spectra of the compound were processed using the Masslynx software suite (Micromass, Manchester, U.K.). Melting points were determined on a Büchi B-545 apparatus and are uncorrected. All reagents were purchased from commercial sources (Acros, Aldrich, Lancaster) and were used as such. THF was distilled from sodium benzophenone. Column chromatography was performed on Kieselgel 60 (Merck), 0.040−0.063 mm. Thin-layer chromatography was performed on precoated ALUGRAM SIL G/UV$_{254}$ Kieselgel 60 0.25 mm TLC plates. For the sake of brevity, only the data for complete characterization of the final products (i.e., the new neocryptolepine derivatives) are given. Characterization data for the intermediate compounds are supplied in the Supporting Information. Analytical HPLC using two independent systems was performed to check the purity of the products: (column A) Merck, Lichrospher Si 600 (4 mm × 250 mm); (column B) Merck, Lichrocart-Lichrospher 100 RP 18 (4 mm × 250 mm). Also, two different solvent systems were used: (system A) 1-propanol−hexane 5%/water 95:5 or 1-propanol−hexane 5%/water 90:10; (system B) gradient from 80% to 100% acetonitrile (ACN) in 20 min. Experimental details of the HPLC analysis are reported for all test compounds in the following form: column, mobile phase, retention time. The flow rate in system A was 0.65 mL/min, and the flow rate was 1.0 mL/min in system B.

4-Amino-3-iodobenzenecarbonitrile (2′). 4-Aminobenzonitrile (1.0 g, 8.47 mmol) is dissolved in 20 mL of dry methanol while stirring. The flask is cooled to 0 °C, after which $CaCO_3$ (2.5 g, 25 mmol) is added. Then a solution of ICl in HCl (4 mL, 5 M ICl in concentrated HCl) is added dropwise in 10 min while maintaining the temperature at 0−5 °C. After complete addition, the cooling bath is removed and the mixture is stirred for 30 min at room temperature and further for 2 days at 35 °C. After completion of the reaction, 60 mL of a 0.5 M $Na_2S_2O_3$ solution is added followed by 100 mL of water. The mixture is extracted with CH_2Cl_2 (2 × 80 mL), and after removal of the solvent the crude product was purified by column chromatography with CH_2Cl_2 as the eluent.

2-(2-Trimethylsilylethynyl)aniline (3). $Pd(PPh_3)_2Cl_2$ (280 mg, 0.40 mmol) and CuI (64 mg, 0.33 mmol) are added to a stirred solution of 2-iodoaniline (8.76 g, 0.04 mol) in Et_3N (100 mL). To this suspension, trimethylsilylacetylene (6 mL, 0.43 mol) is added, and the resulting mixture is stirred for 18 h at room temperature. Then 100 mL of water is added and the mixture is extracted with CH_2Cl_2 (3 × 50 mL). The solvent is removed in vacuo, and the product is purified by column chromatography using CH_2Cl_2 as the eluent.

4-Amino-3-(2-trimethylsilylethynyl)benzenecarbonitrile (3′). To a stirred solution of 4-amino-3-iodobenzenecarbonitrile (0.60 g, 2.5 mmol) (**2′**) in THF (10 mL), Et_3N (5 mL), $Pd(PPh_3)_2Cl_2$ (17.2 mg, 0.024 mmol), and CuI (4.2 mg, 0.022 mmol) are added. To this solution trimethylsilylacetylene (0.36 mL, 2.6 mmol) is added, and the resulting mixture is stirred for 24 h at room temperature. Then 20 mL of water is added and the mixture is extracted with CH_2Cl_2 (3 × 30 mL). The solvent is removed in vacuo, and the product is purified by column chromatography using heptane/ether (1:3) as the eluent.

3-(2-Trimethylsilylethynyl)-4-triphenylphosphoranylideneaminobenzenecarbonitrile (5′). A flask is charged with 4-amino-3-(2-trimethylsilylethynyl)benzenecarbonitrile (**3′**) (1.30 g, 6.07 mmol), triphenylphosphine (3.28 g, 12.5 mmol), Et_3N (12 mL), CCl_4 (7 mL), and CH_3CN (15 mL). The resulting mixture is stirred at room temperature for 24 h. Then, 20 mL of MeOH is added and stirring is continued for 30 min. After the addition of 100 mL of water, the mixture is extracted with CH_2Cl_2 (3 × 50 mL), after which the solvent is removed. The product is then purified by column chromatography using EtOAc/MeOH (98:2) as the eluent.

General Procedure for the Preparation of Thioureas 5a−j, 5p, and 5q. 2-(2-Trimethylsilylethynyl)aniline (**3**) (1.2 g, 6.35 mmol) and 6.5 mmol of the appropriate 4-substituted phenyl isothiocyanate (**4a−j**) are dissolved in EtOH (25 mL). A few crystals of DMAP (*N*,*N*-(dimethylamino)pyridine) are added, and the resulting solution is stirred at 40 °C in an oil bath. After 1−2 h, a white precipitate is formed. The solid is filtered off and washed with 20 mL of cold EtOH and subsequently dried in vacuo to yield the corresponding thiourea. The filtrate is stirred further at 40 °C, giving another amount of precipitate after a few hours. The following products were prepared in this manner:

N-phenyl-*N*′-[2-(2-trimethylsilylethynyl)phenyl]thiourea (**5a**)

N-(4-methoxyphenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-thiourea (**5b**)

N-(4-bromophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-thiourea (**5c**)

N-(4-chlorophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-thiourea (**5d**)

N-(4-Fluorophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-thiourea (**5e**)

N-(4-iodophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]thiourea (**5f**)

N-(4-methylphenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-thiourea (**5g**)

N-(4-isothiocyanatophenyl)-*N*′-[2-(2-trimethylsilylethynyl)-phenyl]thiourea (**5h**)

N-(4-methylthiophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]thiourea (**5i**)

N-(4-cyanophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-thiourea (**5j**)

N-(1-naphthyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]thiourea (**5p**)

N-(2,4-dichlorophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-thiourea (**5q**)

General Procedure for the Synthesis of Carbodiimides 7a−j, 7p, and 7q from Thioureas 5a−j, 5p, and 5q. A two-necked flask equipped with a drying tube is charged with the appropriate thiourea (2.13 mmol). Dry CH_2Cl_2 (25 mL) was added, followed by Et_3N (0.59 mL, 4.25 mmol) and a catalytic amount of DMAP. The solution is stirred, and CH_3SO_2Cl (0.5 mL, 6.40 mmol) is added dropwise. It is important that the rate of addition is kept low because of the very exothermic nature of the reaction. After complete addition, the solution is stirred for 15 min at room temperature, after which 50 mL of water is added. The mixture is extracted with CH_2Cl_2 (3 × 30 mL), and the solvent is removed on a rotavapor. Purification of the product using column chromatography (CH_2Cl_2 as eluent) yields the carbodiimide as a viscous oil. The product is used immediately in the next step to prevent degradation. The following products were prepared in this manner:

N-phenyl-*N*′-[2-(2-trimethylsilylethynyl)phenyl]carbodiimide (**7a**)

N-(4-methoxyphenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-carbodiimide (**7b**)

N-(4-bromophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-carbodiimide (**7c**)

N-(4-chlorophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-carbodiimide (**7d**)

N-(4-fluorophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-carbodiimide (**7e**)

N-(4-iodophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]carbodiimide (**7f**)

N-(4-methylphenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-carbodiimide (**7g**)

N-(4-isothiocyanatophenyl)-*N*′-[2-(2-trimethylsilylethynyl)-phenyl]carbodiimide (**7h**)

N-(4-methylthiophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]carbodiimide (**7i**)

N-(4-cyanophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-carbodiimide (**7j**)

N-(1-naphthyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]carbodiimide (**7p**)

N-(2,4-dichlorophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-carbodiimide (**7q**)

General Procedure for the Synthesis of Carbodiimides 7k−o from Iminophosphorane 5′ and of 7r−u from Iminophosphorane 5″. Iminophosphorane **5′** or **5″** (2.1 mmol) is dissolved in 20 mL of dry toluene. To this solution the appropriate substituted phenyl isocyanate (2.2 mmol) is added, and the resulting solution is stirred at 70 °C for 1 h. The solvent is removed in vacuo, and the product is purified by column chromatography using CH_2Cl_2 as the eluent. The product is used immediately in the next step. The following products were prepared in this manner:

N-[4-cyano-2-(2-trimethylsilylethynyl)phenyl]-*N*′-phenylcarbodiimide (**7k**)

N-(4-chlorophenyl)-*N*′-[4-cyano-2-(2-trimethylsilylethynyl)-phenyl]carbodiimide (**7l**)

N-[4-cyano-2-(2-trimethylsilylethynyl)phenyl]-*N*′-(4-methoxyphenyl)carbodiimide (**7m**)

N-[4-cyano-2-(2-trimethylsilylethynyl)phenyl]-*N*′-(4-trifluoromethylphenyl)carbodiimide (**7n**)

N-[4-cyano-2-(2-trimethylsilylethynyl)phenyl]-*N*′-(4-fluorophenyl)carbodiimide (**7o**)

N-(3-methoxyphenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-carbodiimide (**7r**)

N-(3-bromophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-carbodiimide (**7s**)

N-(3-chlorophenyl)-*N*′-[2-(2-trimethylsilylethynyl)phenyl]-carbodiimide (**7t**)

N-(3-trifluoromethylphenyl)-*N*′-[2-(2-trimethylsilylethynyl)-phenyl]carbodiimide (**7u**)

General Procedure for the Conversion of Carbodiimides 7a−u into the Substituted 11-Trimethylsilyl-6*H*-indolo[2,3-*b*]quinolines (8a−y). This reaction has to be performed using freshly prepared carbodiimides. The carbodiimide (approximately 2.0 mmol) is dissolved in 30 mL of mesitylene. Helium gas is bubbled through the solution for 10 min. After the solution was degassed, 1,4-cyclohexadiene (3 mL, 31.7 mmol) is added and the solution is refluxed for 18 h under a nitrogen atmosphere. The solution is cooled to room temperature, and the solvent is removed under high vacuum. Purification of the product is performed by column chromatography using CH_2Cl_2/EtOAc (7:3) as the solvent. The following products were prepared in this manner:

11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8a**)

2-methoxy-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8b**)

2-bromo-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8c**)

2-chloro-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8d**)

2-fluoro-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8e**)

2-iodo-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8f**)

2-methyl-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8g**)

2-isothiocyanato-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8h**)

2-methylthio-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8i**)

2-cyano-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8j**)

9-cyano-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8k**)

2-chloro-9-cyano-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8l**)

9-cyano-2-methoxy-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8m**)

9-cyano-2-trifluoromethyl-11-trimethylsilyl-6*H*-indolo[2,3-*b*]-quinoline (**8n**)

9-cyano-2-fluoro-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8o**)

7-trimethylsilylnaphtho[1,2-*b*]-α-carboline (**8p**)

2,4-dichloro-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8q**)

1-methoxy-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8r**)

1-bromo-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8s**)

1-trifluoromethyl-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8u**)

3-methoxy-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8v**)

3-bromo-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8w**)

3-chloro-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8x**)

3-trifluoromethyl-11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (**8y**)

General Procedure for the Conversion of the Substituted 11-Trimethylsilyl-6*H*-indolo[2,3-*b*]quinolines 8b, 8d, 8e, 8q into the Desilylated 6*H*-Indolo[2,3-*b*]quinolines 10b, 10d, 10e, 10q. The appropriate 11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinoline (0.35 mmol) is dissolved in ethanol (30 mL), after which NaOH (10 mL, 6 N) is added. The mixture is stirred for 3 h at 70 °C and cooled to room temperature. Water (50 mL) is added, and the mixture is extracted with CH_2Cl_2 (3 × 50 mL). Purification of the product is performed by column chromatography using CH_2Cl_2/EtOAc (7:3) as the eluent. The following products were prepared in this manner:

2-methoxy-6*H*-indolo[2,3-*b*]quinoline (**10b**)

2-chloro-6*H*-indolo[2,3-*b*]quinoline (**10d**)

2-fluoro-6*H*-indolo[2,3-*b*]quinoline (**10e**)

2,4-dichloro-6*H*-indolo[2,3-*b*]quinoline (**10q**)

General Procedure for the Conversion of the Substituted 11-Trimethylsilyl-6*H*-indolo[2,3-*b*]quinolines 8a−y into the Substituted 11-Trimethylsilylneocryptolepines 9a−y. The appropriate 11-trimethylsilyl-6*H*-indolo[2,3-*b*]-quinoline (0.625 mmol) is dissolved in DMF (30 mL). To this solution, CH_3I (3 mL, 48 mmol) is added, and the solution is refluxed overnight. The mixture is then cooled to room temperature, after which it is poured into 50 mL of a 1 M Na_2CO_3 solution followed by extraction with CH_2Cl_2 (3 × 50 mL). After removal of the solvent, the product is purified by column chromatography using CH_2Cl_2/EtOAc (7:3) as the solvent. In two cases, namely, the synthesis of 9-cyano-2-trifluoromethyl-11-trimethylsilylneocryptolepine (**9n**) and 9-cyano-2-fluoro-11-

trimethylsilylneocryptolepine (**9o**), formation of the compounds was accompanied by the presence of a large amount of desilylated product. Unfortunately, these products could not be separated from each other. Therefore, the mixture of the two was used in the desilylation reaction (see below). As a consequence, no spectral data were recorded for these two compounds. After the reaction in which substituted 11-trimethylsilyl-6*H*-indolo[2,3-*b*]quinolines were formed, the 1-substituted compound is separated from the 3-substituted one and other impurities by using a silica column and CH_2Cl_2/EtOAc (7:3) as the eluent. The following products were prepared in this manner:

11-trimethylsilylneocryptolepine (**9a**)
2-methoxy-11-trimethylsilylneocryptolepine (**9b**)
2-bromo-11-trimethylsilylneocryptolepine (**9c**)
2-chloro-11-trimethylsilylneocryptolepine (**9d**)
2-fluoro-11-trimethylsilylneocryptolepine (**9e**)
2-iodo-11-trimethylsilylneocryptolepine (**9f**)
2-methyl-11-trimethylsilylneocryptolepine (**9g**)
2-nitro-11-trimethylsilylneocryptolepine (**9h**)
2-methylthio-11-trimethylsilylneocryptolepine (**9i**)
2-cyano-11-trimethylsilylneocryptolepine (**9j**)
9-cyano-11-trimethylsilylneocryptolepine (**9k**)
2-chloro-9-cyano-11-trimethylsilylneocryptolepine (**9l**)
9-cyano-2-methoxy-11-trimethylsilylneocryptolepine (**9m**)
9-cyano-2-trifluoromethyl-11-trimethylsilylneocryptolepine (**9n**)
9-cyano-2-fluoro-11-trimethylsilylneocryptolepine (**9o**)
1-methoxy-11-trimethylsilylneocryptolepine (**9r**)
1-bromo-11-trimethylsilylneocryptolepine (**9s**)
1-trifluoromethyl-11-trimethylsilylneocryptolepine (**9u**)
3-methoxy-11-trimethylsilylneocryptolepine (**9v**)
3-bromo-11-trimethylsilylneocryptolepine (**9w**)
3-chloro-11-trimethylsilylneocryptolepine (**9x**)
3-trifluoromethyl-11-trimethylsilylneocryptolepine (**9y**)

General Procedure for the Conversion of the Substituted 11-Trimethylsilylneocryptolepines 9a−y into the Neocryptolepine Derivatives 1a−y. The appropriate 11-trimethylsilylneocryptolepine (0.35 mmol) is dissolved in 30 mL of ethanol, after which NaOH (10 mL, 6 N) is added. The mixture is stirred for 3 h at 70 °C and cooled to room temperature. Water (50 mL) is added, and the mixture is extracted with CH_2Cl_2 (3 × 50 mL). Purification of the product is performed by column chromatography using acetone as the eluent. 3-Methoxyneocryptolepine (**1v**) is purified by column chromatography using toluene/diethylamine (9.5:0.5) as the eluent. The following products were prepared in this manner:

neocryptolepine (**1a**)
2-methoxyneocryptolepine (**1b**)
2-bromoneocryptolepine (**1c**)
2-chloroneocryptolepine (**1d**)
2-fluoroneocryptolepine (**1e**)
2-iodoneocryptolepine (**1f**)
2-methylneocryptolepine (**1g**)
2-nitroneocryptolepine (**1h**)
2-methylthioneocryptolepine (**1i**)
2-cyanoneocryptolepine (**1j**)
9-cyanoneocryptolepine (**1k**)
2-chloro-9-cyanoneocryptolepine (**1l**)
9-cyano-2-methoxyneocryptolepine (**1m**)
9-cyano-2-trifluoromethylneocryptolepine (**1n**)
9-cyano-2-fluoroneocryptolepine (**1o**)
1-methoxyneocryptolepine (**1r**)
1-bromoneocryptolepine (**1s**)
1-trifluoromethylneocryptolepine (**1u**)
3-methoxyneocryptolepine (**1v**)
3-bromoneocryptolepine (**1w**)
3-chloroneocryptolepine (**1x**)
3-trifluoromethylneocryptolepine (**1y**)

Conversion to Hydrochloride Salts. All compounds were biologically evaluated as their hydrochloride salts. Compounds were dissolved in 0.1 N HCl in MeOH, and the solutions were evaporated to dryness after 30 min.

In Vitro Activity against *P. falciparum*. For the determination of the antiplasmodial activity, the parasite lactate dehydrogenase assay was used, with slight modifications, as previously described by Makler et al.[34] Briefly, the assay is based on the observation that the lactate dehydrogenase (LDH) enzyme of *Plasmodium falciparum* has the ability to rapidly use 3-acetylpyridine NAD (APAD) as a coenzyme in the reaction leading to the formation of pyruvate from lactate. Test compounds were added in 2-fold serial dilutions to *Plasmodium falciparum* (chloroquine-sensitive Ghana strain or chloroquine-resistant W2 strain) cultures (1% parasitaemia, 2% HCT) in 96-well plates. After 48 h at 37 °C and a gas mixture of 93% N_2, 4% CO_2, and 3% O_2, the parasite cultures were frozen at −20 °C to lyse the erythrocytes and to store the plates until further processing. After thawing, 20 μL of the lysed culture was added to 100 μL of Malstat reagent (Flow Inc.), and the formation of APADH was determined. Adding 40 μg of nitroblue tetrazolium (NBT) and 2 μg of phenazine ethosulfate (PES) to the Malstat reagent promoted the spectrophotometric assessment of LDH activity. As APADH is formed, the NBT is reduced and forms a blue formazan product that can be detected visually and measured spectrophotometrically at 650 nm. Determination of the IC_{50} values was performed in triplicate (mean ± SD). Cryptolepine[9,11] (Table 3) and chloroquine were used as positive controls. IC_{50} values of chloroquine were 0.01 μM (Ghana) and 0.09 μM (W2).

In Vitro Acitivity against *T. brucei* Trypomastigotes. IC_{50} values against *Trypanosoma brucei* were determined in triplicate (mean ± SD) as described before.[35] Briefly, bloodstream forms of *T. brucei* were cultivated in HMI-9 medium. In a 96-well microplate, 10 000 hemoflagellates were incubated at different concentrations of the test compound for 4 days. Parasite multiplication was measured colorimetrically (490 nm) following addition of MTT, which converts to an water-soluble formazan product. Suramin was used as positive control (IC_{50} = 0.3 ± 0.1 μM).

In Vitro Acitivity against Intracellular *T. cruzi* Amastigotes. IC_{50} values against *Trypanosoma cruzi* were determined in triplicate (mean ± SD) as described before.[35] Briefly, primary mouse peritoneal macrophages were seeded in 96-well microplates at 30 000 cells per well. After 24 h, about 10 000 trypomastigotes of *T. cruzi* were added per well together with 2-fold dilutions of the drug. The cultures were incubated at 37 °C in5% CO_2/95% air for 4 days. Following fixation in MeOH and Giemsa staining, the drug activity was semiquantitatively scored as a percent reduction of the total parasite load (free trypomastigotes and intracellular amastigotes) compared with untreated control cultures. Scoring was performed microscopically. Nifurtimox was used as a positive control (IC_{50} = 0.4 ± 0.1 μM).

Cytotoxicty on MRC-5 Cells. A human diploid embryonic lung cell line (MRC-5) was used to assess the cytotoxicity of the test compounds as described before.[35] Briefly, MRC-5 cells were seeded at 5000 cells per well in 96-well microtiter plates. After 24 h, the cells were washed and 2-fold dilutions of the drug in 200 μL of standard culture medium (RPMI + 5% FCS) were added. The final DMSO concentration of the culture remained below 0.5%. The cultures were incubated with different concentrations of test compounds at 37 °C in 5% CO_2/95% air for 7 days. Untreated cultures were included as controls. Cytotoxicity was determined using the colorimetric MTT assay and scored as a percent reduction of absorption at 540 nm of treated cultures versus untreated control cultures. Determination of the IC_{50} values was performed in triplicate (mean ± SD). Vinblastine was used as a positive control (IC_{50} < 10 nM).

Inhibition of β-Hematin Formation. An in vitro method was used to measure the inhibition of β-hematin formation, based on the original method described by Egan et al.[24] and modified by Wright et al.[11] Heme refers to Fe(II)protoporphyrin IX, hemin is Fe(II)protoporphyrin IX chloride, and hematin is Fe(II)protoporphyrin IX hydroxide. Briefly, 0.1 M NaOH was added to Fe(II)hemin (Fluka; HPLC purity, >98%) (typically 7.5 mg), which forms hematin. Polymerization to β-hematin,

which has been characterized as $[Fe(III)protoporphyrin\ IX]_2$,[36] proceeds after addition of a 9.9 M acetate buffer, pH 5. Test compounds (3 equiv with respect to hemin) were added to the hematin solutions prior to acidification. After incubation for 40 min at 60 °C, cooling and filtration, the precipitate was washed with water and dried, and FT-IR (Perkin-Elmer FT-IR 1760) was used to check the presence of β-hematin, which shows sharp bands at 1660 and 1207 cm^{-1}. The spectra are examined on the basis of the presence or absence of the two typical peaks. When they are absent the compound has inhibited the formation of β-hematin. A sample containing hemin but without the test compound, which was not incubated, was used as a negative control (no formation of β-hematin). A sample containing hemin without the test compound, which was incubated for 40 min at 60 °C, was used as a positive control (formation of β-hematin).

Characterization of Heme: Gas-Phase Ion Drug Complexes by Electrospray Ionization (ESI) Mass Spectrometry. Abbreviations are the following: C, cationic form of the drug; FP, heme = ferriprotoporphyrin (depending on the conditions, FP may be present in an unliganded form $[FP]^+$, in a dimeric form $[2FP - H]^+$, as a dehydrated dimer of hematin $[FP-O-FP]^+$, or as the CH_3OH complex of the latter dimeric form $[FP-O-FP + MeOH]^+$). Mixtures of the drugs (hydrochloride salts) with FP were prepared in $MeOH/H_2O$ (1:1; v/v; pH 7). Positive-ion mass spectra were recorded on an Autospec-oa TOF mass spectrometer (Micromass, Manchester, U.K.) employing electrospray ionization. Low-energy collision-induced dissociation (CID) spectra were acquired at a collision energy (E_{lab}) of 400 eV using helium as the collision gas. Helium was introduced into the collision cell until the signal of the very weak FP^+:CH_3OH complex (*m*/*z* 648) reached 60% of its original value. Under these conditions, the CID spectrum of the FP^+:MeOH complex revealed the FP^+ ion (*m*/*z* 616) as the base peak, and the residual FP^+:MeOH ion had a relative abundance (RA) of 3%. To allow comparisons between binding strengths of the different $[FP:C]^+$ complexes, the CID experiments were performed on the same day using the same collision gas pressure.

DNA−Methyl Green Assay. The DNA−methyl green assay is a simple microtiter assay for the detection of compounds that bind DNA. Agents that displace methyl green from a DNA−methyl green complex (deoxyribonucleic acid methyl green; Sigma) are detected spectrophotometrically (Labsystems Multiscan MCC/340) by a decrease in absorbance at 620 nm.[28] DNA−methyl green was suspended in 100 mL of 0.05 M Tris-HCl buffer, pH 7.5, containing 7.5 mM $MgSO_4$ and was stirred at 37 °C for 24 h. The dissolved samples were dispensed into wells of a 96-well microtiter plate. Solvent was removed under vacuum, and 200 μL of the DNA−methyl green solution was added. The initial absorbance was compared with the final absorbance (after 24 h) in order to calculate the IC_{50} value (50% displacement of methyl green from DNA). Determination of the IC_{50} values was performed in triplicate (mean ± SD). The decrease in absorbance observed represents the initial rapid displacement of methyl green from DNA by the drug, followed by the slower reaction with water that yields the colorless carbinol.

Electric Linear Dichroism (ELD) Measurements. ELD measurements were performed with a computerized optical measurement system using the procedures previously outlined.[37] All experiments were conducted with a 10 mm path length Kerr cell having a 1.5 mm electrode separation. The samples were oriented under an electric field strength varying from 1 to 13 kV/cm. The concentration of the test compound was 10 μM in the presence of DNA at 200 μM unless stated otherwise. This electrooptical method has proved to be most useful for determining the orientation of the drugs bound to DNA. It has the additional advantage that it senses only the orientation of the polymer-bound ligand; free ligand is isotropic and does not contribute to the signal.

Topoisomerase II Mediated DNA Cleavage Assay. Supercoiled pKMp27 DNA (0.5 μg) was incubated with 4 units of human topoisomerase II (TopoGen Inc.) at 37 °C for 30 min in relaxation buffer (50 mM Tris, pH 7.8, 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA, and 0.5 mM ATP) in the presence of varying concentrations of the test compound. Reactions were terminated by adding SDS to 0.25% and proteinase K to 250 μg/mL. DNA samples were then added to the electrophoresis dye mixture (3 μL) and electrophoresed in a 1% agarose gel containing ethidium bromide (1 μg/mL) at room temperature for 2 h at 120 V. Gels were washed and photographed under UV light.

Acknowledgment. We thank J. Verreydt, J. Schrooten, N. Verhaert, V. Van Heurck, W. Van Lierde, and W. Van Dongen for their technical assistance. T. Jonckers thanks the "Vlaams Instituut voor de Bevordering van het Wetenschappelijk Technologisch Onderzoek in de Industrie (IWT)" for a scholarship. He is also indebted to the foundation "Rosa Blanckaert" for a research grant. Financial support from the Fund for Scientific Research (FWO-Vlaanderen) (Project Nos. G.0119.96, G.0082.98, G.0334.00, and 1.5.139.00) and from the Special Research Fund of the University of Antwerp (Concerted Research Project No. 99/3/34, fellowship for S. van Miert) is gratefully acknowledged. Dr. Colin Wright (University of Bradford, U.K.) is kindly acknowledged for assistance with the assay on inhibition of β-hematin formation.

Appendix

Table 4. Degree of Purity for All Compounds

compd	% purity, system A[a]	% purity, system B[b]	compd	% purity, system A[a]	% purity, system B[b]
1a	100.0	100.0	**1k**	100.0	95.7
1b	97.1	99.2	**1l**	99.0	96.4
10b	98.3	95.3	**1m**	100.0	96.8
1c	100.0	95.6	**1n**	96.2	94.5
1d	100.0	100.0	**1o**	97.6	95.0
10d	100.0	95.7	**10q**	100.0	93.2
1e	100.0	100.0	**1r**	100.0	97.1
10e	100.0	95.9	**1s**	100.0	97.1
1f	100.0	95.5	**1u**	98.4	95.2
1g	98.0	96.1	**1v**	92.8	99.5
1h	95.4	95.1	**1w**	99.3	96.8
1i	99.4	95.5	**1x**	100.0	95.8
1j	100.0	95.0	**1y**	99.5	95.2

[a] System A: column, MERCK, Lichrospher Si 600 (4 mm × 250 mm); solvent system, 1-propanol−hexane 5%/water 95:5 or 1-propanol−hexane 5%/water 90:10. [b] System B: column, MERCK, Lichrocart-Lichrospher 100 RP 18 (4 mm × 250 mm); solvent system, gradient from 80% to 100% acetonitrile in 20 min.

Supporting Information Available: Characterization of intermediate compounds and target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) O'Neill, P. M.; Bray, P. G.; Hawley, S. R.; Ward, S. A.; Park, B. K. 4-Aminoquinolines−Past, Present, and Future: A Chemical Perspective. *Pharmacol. Ther.* **1998**, *77*, 29−58.
(2) Rang, H. P.; Dale, M. M.; Ritter, J. M. *Pharmacology*, 4th ed.; Churchill Livingstone: Edinburgh, U.K., 1999; Chapter 46 (Antiprotozoal Drugs).
(3) Newman, D. J.; Cragg, G. M.; Snader, K. M. The Influence of Natural Products upon Drug Discovery. *Nat. Prod. Rep.* **2000**, *17*, 215−234.
(4) Kirby, G. C.; Paine, A.; Warhurst, D. C.; Noamese, B. K.; Phillipson, J. D. In Vitro and in Vivo Antimalarial Activity of Cryptolepine, a Plant-Derived Indoloquinoline. *Phytother. Res.* **1995**, *9*, 359−363.
(5) Cimanga, K.; De Bruyne, T.; Pieters, L.; Vlietinck, A.; Turger, C. A. In Vitro and in Vivo Antiplasmodial Activity of Cryptolepine and Related Alkaloids from *Cryptolepis sanguinolenta*. *J. Nat. Prod.* **1997**, *60*, 688−691.

(6) Cimanga, K.; De Bruyne, T.; Lasure, A.; Van Poel, B.; Pieters, L.; Claeys, M.; Vanden Berghe, D.; Kambu, K.; Tona, L.; Vlietinck, A. In Vitro Biological Activities of Alkaloids from *Cryptolepis sanguinolenta*. *Planta Med*. **1996**, *62*, 22−27.
(7) Bonjean, K.; De Pauw-Gillet, M.-C.; Defresne, M.-P.; Colson, P.; Houssier, C.; Dassonneville, L.; Bailly, C.; Greimers, R.; Wright, C.; Quetin-Leclercq, J.; Tits, M.; Angenot, L. The DNA Intercalating Alkaloid Cryptolepine Interferes with Topoisomerase II and Inhibits Primarily DNA Synthesis in B16 Melanoma Cells. *Biochemistry* **1998**, *37*, 5136−5146.
(8) Bailly, C.; Laine, W.; Baldeyrou, B.; De Pauw-Gillet, M.-C.; Colson, P.; Houssier, C.; Cimanga, K.; Van Miert, S.; Vlietinck, A. J.; Pieters, L. DNA Intercalation, Topoisomerase II Inhibition and Cytotoxic Activity of the Plant Alkaloid Neocryptolepine. *Anti-Cancer Drug Des.* **2000**, *15*, 191−201.
(9) Arzel, E.; Rocca, P.; Grellier, P.; Labaeïd, M.; Frappier, F.; Guéritte, F.; Gaspard, C.; Marsais, F.; Godard, A.; Quéguiner, G. New Synthesis of Benzo-*δ*-carbolines, Cryptolepines, and Their Salts: In Vitro Cytotoxic, Antiplasmodial, and Antitrypanosomal Activities of *δ*-Carbolines, Benzo-*δ*-carbolines, and Cryptolepines. *J. Med. Chem.* **2001**, *44*, 949−960.
(10) Bierer, D. E.; Fort, D. M.; Mendez, C. D.; Luo, J.; Imbach, P. A.; Dubenko, L. G.; Jolad, S. D.; Gerber, R. E.; Litvak, J.; Lu, Q.; Zhang, P.; Reed, M. R.; Waldeck, N.; Bruening, R. C.; Noamesi, B. K.; Hector, R. F.; Carlson, T. J.; King, S. R. Ethnobotanical-Directed Discovery of the Antihyperglycemic Properties of Cryptolepine: Its Isolation from *Cryptolepis sanguinolenta*, Synthesis, and in Vitro and in Vivo Activities. *J. Med. Chem.* **1998**, *41*, 894−901.
(11) Wright, C. W.; Addae-Kyereme, J.; Breen, A. G.; Brown, J. E.; Cox, M. F.; Croft, S. L.; Gökçek, Y.; Kendrick, H.; Phillips, R. M.; Pollet, P. L. Synthesis and Evaluation of Cryptolepine Analogues for Their Potential as New Antimalarial Agents. *J. Med. Chem.* **2001**, *44*, 3187−3194.
(12) Peczynska-Czoch, W.; Pognan, F.; Kaczmarek, L.; Boratynski, J. Synthesis and Structure−Activity Relationship of Methyl-Substituted Indolo[2,3-*b*]quinolines: Novel Cytotoxic, DNA Topoisomerase II Inhibitors. *J. Med. Chem.* **1994**, *37*, 3503. Kaczmarek, L.; Balicki, R.; Nantka-Namirski, P. Synthesis and Antineoplastic Properties of some Benzo-iso-α-carbolines. *Arch. Pharm.* **1988**, *321*, 463−467.
(13) Alajarín, M.; Molina, P.; Vidal, A. Formal Total Synthesis of the Alkaloid Cryptotackieine (Neocryptolepine). *J. Nat. Prod.* **1997**, *60*, 747−748.
(14) Molina, P.; Fresneda, P. M.; Delgado, S. Iminophosphorane-Mediated Synthesis of the Alkaloid Cryptotackieine. *Synthesis* **1999**, 326−329.
(15) Timári, G.; Soós, T.; Hajós G. A Convenient Synthesis of Two New Indoloquinoline Alkaloids. *Synlett* **1997**, 1067−1068.
(16) Schmittel, M.; Steffen, J. P.; Engels, B.; Lennartz, C. Two Novel Thermal Biradical Cyclizations in Theory and Experiment: New Synthetic Routes to 6*H*-Indolo[2,3-*b*]quinolines and 2-Aminoquinolines from Enyne-Carbodiimides. *Angew. Chem., Int. Ed.* **1998**, *37*, 2371−2373.
(17) Shi, C.; Zhang, Q.; Wang K. K. Biradicals from Thermolysis of *N*-[2-(1-Alkynyl)phenyl]-*N*′-phenylcarbodiimides and Their Subsequent Transformations to 6*H*-Indolo[2,3-*b*]quinolines. *J. Org. Chem.* **1999**, *64*, 925−932.
(18) Saito, T.; Ohmori, H.; Ohkubo, T.; Motoki, S. Periselective, Lewis Acid-Induced Intramolecular Diels−Alder Reaction of Conjugated Carbodiimides: Efficient Synthesis of Nitrogen Heterocycles, Indolo[2,3-*b*]quinolines and Pyrido[2,3-*b*]indole. *J. Chem. Soc., Chem. Commun.* **1993**, 1802−1803.
(19) A trimethylsilyl (TMS) group can be transformed into other substituents. (a) Jacob, L. A.; Chen, B. L.; Stec, D. Further Studies on the Iodination of Aryltrimethylsilanes. *Synthesis* **1993**, 611−614. (b) Bennetau, B.; Dunogues, J. Unusual Electrophilic Substitution in the Aromatic Series via Organosilicon Intermediates. *Synlett* **1993**, 171−176.
(20) Chen, C.; Lieberman, D. R.; Larsen, R. D.; Reamer, R. A.; Verhoeven, T. R.; Reider, P. J. Synthesis of the 5-HT_{1D} Receptor Agonist MK-0462 via a Pd-Catalyzed Coupling Reaction. *Tetrahedron Lett.* **1994**, *35*, 6981−6984.
(21) Fell, J. B.; Coppola, G. M. A Mild and Efficient Preparation of Carbodiimides. *Synth. Commun.* **1995**, *25* (1), 43−47.
(22) Palomo, C.; Mestres, R. Convenient and Improved Synthesis of Unstable Carbodiimides. *Synthesis* **1981**, 373.
(23) Wright, C. W.; Phillipson, J. D.; Awe, S. O.; Kirby, G. C.; Warhurst, D. C.; Leclercq, J.; Angenot, L. The Antimalarial Action of Cryptolepine Is Unlikely To Be Due to Intercalation with Parasite DNA. Presented at The U.K. Association of Pharmaceutical Scientists: Natural Products as Drugs and Medicines, November 18, 1994; Abstract p 23.
(24) Egan, T. J.; Ross, D. C.; Adams, P. A. Quinoline Anti-Malarial Drugs Inhibit Spontaneous Formation of *β*-Haematin (Malaria Pigment). *FEBS Lett.* **1994**, *352*, 54−57.
(25) Pandey, A. V.; Tekwani, B. L. Depolymerization of Malarial Hemozoin: A Novel Reaction Initiated by Blood Schizonticidal Antimalarials. *FEBS Lett.* **1997**, *402*, 236−240.
(26) Ginsburg, H.; Famin, O.; Zhang, J.; Krugliak, M. Inhibition of Glutathione-Dependent Degradation of Heme by Chloroquine and Amodiaquine as a Possible Basis of Their Antimalarial Mode of Action. *Biochem. Pharmacol.* **1998**, *56*, 1305−1313.
(27) Wright, C. W.; Addae-Kyereme, J.; Brown, J. E.; Tranter, D. A. Evaluation of Cryptolepine and Some Analogues as Leads to Selective Antimalarial Agents. *J. Pharm. Pharmacol.* **1997**, *49* (Suppl. 4), 36.
(28) Burres, N. L.; Frigo, A.; Rasmussen, R. R.; McAlpine, J. B. A Colorimetric Microassay for the Detection of Agents That Interact with DNA. *J. Nat. Prod.* **1992**, *55*, 1582−1587.
(29) Dassonneville, L.; Lansiaux, A.; Wattelet, A.; Wattez, N.; Mahieu, C.; Van Miert, S.; Pieters, L.; Bailly, C. Cytotoxicity and Cell Cycle Effects of the Plant Alkaloids Cryptolepine and Neocryptolepine: Relation to Drug-Induced Apoptosis. *Eur. J. Pharmacol.* **2000**, *409*, 9−18.
(30) Nenortas, E. C.; Bodley, A. L.; Shapiro, T. A. DNA Topoisomerases: A New Twist for Antiparasitic Chemotherapy? *Biochim. Biophys. Acta* **1998**, *1400*, 349−354.
(31) Weissig, V.; Vetro-Widenhouse, T. S.; Rowe, T. C. Topoisomerase II Inhibitors Induce Cleavage of Nuclear and 35-kb Plastid DNAs in the Malarial Parasite *Plasmodium falciparum*. *DNA Cell Biol.* **1997**, *16*, 1483−1492.
(32) Fichera, M. E.; Roos, D. S. A Plastid Organelle as a Drug Target in Apicomplexan Parasites. *Nature* **1997**, *390*, 407−409.
(33) Wright, A.; Wang, H.; Gurrath, M.; Konig, G.; Kocak, G.; Neumann, G.; Loria, P.; Foley, M.; Tilley, L. Inhibition of Heme Detoxification Processes Underlies the Antimalarial Activity of Terpene Isonitrile Compounds from Marine Sponges. *J. Med. Chem.* **2001**, *44*, 873−885.
(34) Makler, M. T.; Hinrichs, D. J. Measurement of the Lactate Dehydrogenase Activity of *Plasmodium falciparum* as an Assessment of Parasitemia. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 205−210.
(35) Girault, S.; Grellier, P.; Berecibar, A.; Maes, L.; Mouray, E.; Lemière, P.; Debreu, M.-A.; Davioud-Charvet, E.; Sergheraert, C. Antimalarial, Antitrypanosomal, and Antileishmanial Activities and Cytotoxicity of Bis(9-amino-6-chloro-2-methoxyacridines): Influence of the Linker. *J. Med. Chem.* **2000**, *43*, 2646−2654.
(36) Pagola, S.; Stephens, P. W.; Bohle, D. S.; Kosar, A. D.; Madsen, S. K. The Structure of Malaria Pigment *β*-Haematin. *Nature* **2000**, *404*, 307−310.
(37) Colson, P.; Bailly, C.; Houssier, C. Electric Linear Dichroism as a New Tool To Study Sequence Preference in Drug Binding to DNA. *Biophys. Chem.* **1996**, *58*, 125−140.

JM011102I