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Neocryptolepine: A Promising Indoloisoquinoline Alkaloid with Interesting Biological Activity. Evaluation of the Drug and its Most Relevant Analogs

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Abstract: Plants are one of the most important resources for the discovery of new drugs. The potential of natural compounds as new drug leads is clearly illustrated by the discovery and development of many modern medicines. This is an encouraging factor that drives natural products research in the vegetable kingdom. Neocryptolepine is a tetracyclic nitrogen heterocycle isolated from the African climber *Cryptolepis sanguinolenta*, which is widely used in traditional African medicine in many countries of Central and West Africa. The natural product is one of the representative examples of the small family of indolo[2,3-*b*]quinoline alkaloids, being endowed of multiple biological activities, including DNA-binding and inhibition of the enzyme topoisomerase II. It is also cytotoxic, antibacterial, antifungal and molluscicidal, also displaying antiprotozoal activity, particularly as antitrypanosomal, antileishmanial, antischistosomal and antiplasmodial. Some of these activities have been related to the product's ability to bind to DNA and to inhibit topoisomerase II; however, the exact mechanisms behind all of the observed bioactivities have not been comprehensively clarified. Major research activities regarding neocryptolepine have been focused into two seemingly opposite fields, related to its cytotoxic and antimalarial properties. Optimization of the natural product as a cytotoxic agent implied improvements in its bioavailability and activity, while the need of non-cytotoxic compounds guided the design and optimization of antimalarial agents. Therefore, the aim of the present article is to systematically review the current knowledge about the diversity of the biological activities related to neocryptolepine, its analogs and derivatives.

Keywords: Antimalarial activity, Bioactive natural product derivatives, Cytotoxicity, DNA binding compounds, Neocryptolepine (cryptotakieine).

INTRODUCTION

Finding new leads in the low molecular weight dimension of the chemical space poses a formidable challenge for the rational drug design [1]. The abundance and diversity of bioactive natural products offers excellent opportunities for the discovery of drugs, aided by the structural sophistication fuelled by millions of years of evolution [2]. Thus, the screening of natural sources has historically proven to be one of the major and most successful strategies for finding novel structures. For both, lead discovery purposes and scientific validation of a traditional medicinal plant or its phytopharmaceutical and ethnomedical products, the active principles in their complex matrices need to be identified and characterized. The resulting natural products do not necessarily have to be the final therapeutical drug entities; however, they may serve as the key sources of inspiration towards new active pharmaceutical ingredients [3].

Cryptolepis sanguinolenta (Lindl.) Schlecter; syn *Pergularia sanguinolenta* Lindl., (Asclepiadaceae) is a plant indigenous to the west coast of Africa [4], being found in Ghana, Cote d'Ivoire, Guinea, Guinea-Bissau, Mali, Nigeria, Senegal, Sierra Leone, Angola, Congo, Uganda, and Cameroon. The plant grows wild, as a thin-stemmed twining and scrambling shrub up to 8 m long [5]. *Cryptolepis sanguinolenta*, which is part of the traditional African Pharmacopeia [6], is commonly known under the names of *Nibima* (Ashanti language), *delboi* (Fulani language), *gangamau* (Hausa language), *nombon* (Dioule language), *ouidoukoi* (Bambara language), *kpokpo-yangolei* (Mende language), *kadze*, *Ghana quinine*, and *yellow dye root*; in the Yoruba-speaking areas of Nigeria, it is also called *paran pupa* [7]. The plant contains a yellow-orange juice, which becomes red upon drying and has found use in the dyeing of leather and textiles [8].

Regarding to the ethnobotanical uses of *C. sanguinolenta*, it is well known that in traditional Central African medicine, an aqueous macerate and the root bark of the plant are widely used by herbalists and practitioners [9], while in West Africa the entire root is used, as a tisane. Decoctions containing the root and root's bark of the plant have been employed, especially in Ghana, Nigeria, Congo, Zaire and Senegal, to treat different health conditions, particularly fevers and malaria [10]. Interestingly, amebiasis, intestinal conditions, hepatitis, jaundice, colics and spasms [11], as well as hypertension and inflammation [12], have also been treated with this phytomedicine.

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In Congo, *C. sanguinolenta* is employed as stomachic, in Nigeria it is prescribed for rheumatism and urogenital infections, whereas in Ghana it is officially used in clinical therapeutics, to treat upper respiratory tract, and urinary disorders, fevers and malaria, since 1974 [13]. Extracts of the roots are also used as a tonic, often taken daily for years without evidence of toxicity [12a]. Currently, the dried roots of *C. sanguinolenta* are formulated as an herbal preparation useful for the treatment of fever and symptomatic uncomplicated malaria [14] in semi-immune patients, and dispensed as teabags in Ghana, under the trade name of Phyto-Laria [15].

Extracts of the plant and compounds bearing the indoloquinoline ring system have demonstrated to possess antibacterial, antifungal and antiviral activity [16]. They have also shown to exhibit antimuscarinic [11d], anti-inflammatory [17], antihyperglycemic [7,18], cytotoxic and antitumor properties. It has been reported that the root and leaf extracts have hypotensive, antipyretic, anti-inflammatory, antidiarrhoeal, antibacterial and antimalarial effects [19].

Cryptolepis sanguinolenta is the main source of indoloquinoline alkaloids. These heterocycles display a core with indole and quinoline fused rings. The indoloquinolines are a small, relatively rare and unique family of alkaloids. Interestingly, however, some indoloquinolines have also been isolated from *Cryptolepis triangularis* N. E. Br., *Microphilis guyanensis* (A. DC) Pierre (Sapotaceae), *Sida acuta* Burm. (Malvaceae) and *Genipa americana* L. (Rubiaceae) [20].

To date, slightly over a dozen of natural indolequinolines are known. Among them (Fig. **1**) are the monomeric neocryptolepine (cryptotackieine, **1**), the related quinindoline (**1a,** norcryptotackieine) [21], quindoline (**2**) [22], quindolinone (**3**) [23], cryptosanguinolentine (isocryptolepine, **4**) [24], 11-isopropylcryptolepine (**5**) [25], cryptolepine (**6**) [7,26] and 11-hydroxycryptolepine (**7**) [27], as well as the dimeric biscryptolepine (**8**) [28], cryptoquindoline (**9**) [29], cryptospirolepine (**10**) [4a], cryptomisrine (**11**) [30], quindolinocryptotackieine (**12**) [31] and cryptolepicarboline (**13**) [32].

In view of the wide array of biological activities exhibited by *Cryptolepis sanguinolenta*, and the decoction of its root and root bark, some of the indoloquinolines have attracted great interest from the Organic and Medicinal Chemists communities. Cryptolepine is the major constituent of this family of alkaloids. This natural product was found to be active against chloroquine-resistant *Plasmodium falciparum* and to display other valuable bioactivities [33]. Unfortunately, cryptolepine and its analogs also exhibited high cytotoxicity [34], probably resulting from interaction with DNA and inhibition of the enzyme topoisomerase II. The bioactivity profile of cryptolepine and the indoloquinolines as a class of natural products, has been recently reviewed [35].

The observations relating to the unwanted cytotoxicity of cryptolepine suggested that the alkaloid would be an unlikely candidate as a lead towards new antimalarial agents, due to its supposed lack of selectivity. In search of alternatives and because of its lower cytotoxicity, neocryptolepine became the new focus of attention and drug lead, currently being considered as a promising scaffold for drug discovery.

The first isolation of neocryptolepine was carried out independently and simultaneously in 1996, by the groups of Pieters and Schiff. The alkaloid was isolated from the bark of the roots of *C. sanguinolenta*, as an amorphous yellowish powder. In both cases, the structure of **1** was elucidated by NMR spectroscopy; nevertheless, the Schiff group termed cryptotakieine the new alkaloid [14].

The natural product was defined as an *N*-methylated indolo[2,3-*b*]quinoline, isomeric with cryptolepine and isocryptolepine [24]. Interestingly, however, the *N*-methylation of quinindoline (norcryptotackieine, **1a**) with Me_2SO_4 in nitrobenzene at 160°C for 1 h, followed by deprotonation with aqueous NaOH, which affords **1**, was already described long time before the isolation of neocryptolepine from Nature [36]. The natural product and its congeners have been synthesized many times employing different approaches [35a,37].

The molecular basis for the diverse biological effects of neocryptolepine were slowly unveiled during the last 20 years. A wide amount of knowledge on the subject was gained from numerous experiments with the related cryptolepine and thanks to the synthesis and evaluation of structural analogs of the natural product. Therefore, taking into account its interesting bioactivity profile and the immense attention captured by neocryptolepine and its analogs, we review herein the main findings with regards to its multiple biological activities as DNA-binding compounds, inhibitors of the topoisomerase II, cytotoxics, antibacterial, antifungal, antiprotozoal, and molluscicidal agents.

DNA BINDING ACTIVITY

Small molecules that bind genomic DNA can be effective anticancer, antibiotic and antiviral therapeutic agents. This turns DNA into a major target for anticancer drugs because they can be structurally tuned to interfere with DNA transcription and replication [38]. The molecular basis for designing new active pharmaceutical compounds based on DNA binding relates to the Medicinal Chemists' ability to identify the key structural requirements of the lead molecule that are responsible for both, the stabilization of the drug-DNA complex and the specificity of this binding.

Cryptolepine behaves as a typical DNA intercalating agent [39] and stimulates DNA cleavage by topoisomerase II [40], the nuclear ubiquitous enzyme, which serves to regulate the topological states of DNA in cells. Based on these facts both, DNA and topoisomerase II were considered as the primary targets of cryptolepine [41]. However, experiments with analogs of neocryptolepine suggested that topoisomerase II may not be the primary target of these compounds [42]. It was also demonstrated that neocryptolepine interacts with DNA [36c] as an intercalator, but exhibits a reduced affinity for nucleic acids compared with cryptolepine [43]. Both natural products interfere with the catalytic activity of human topoisomerase II, but the poisoning activity is slightly more pronounced with cryptolepine than with neocryptolepine.

The interaction of small molecules with DNA can be inferred from measurements of the increase of the denaturating temperature (ΔT_m) of calf thymus DNA. When the interaction of methylated 5*H*- and 6*H*- indolo[2,3-*b*]quinolines with DNA were studied, it was observed that the ΔT_m values for

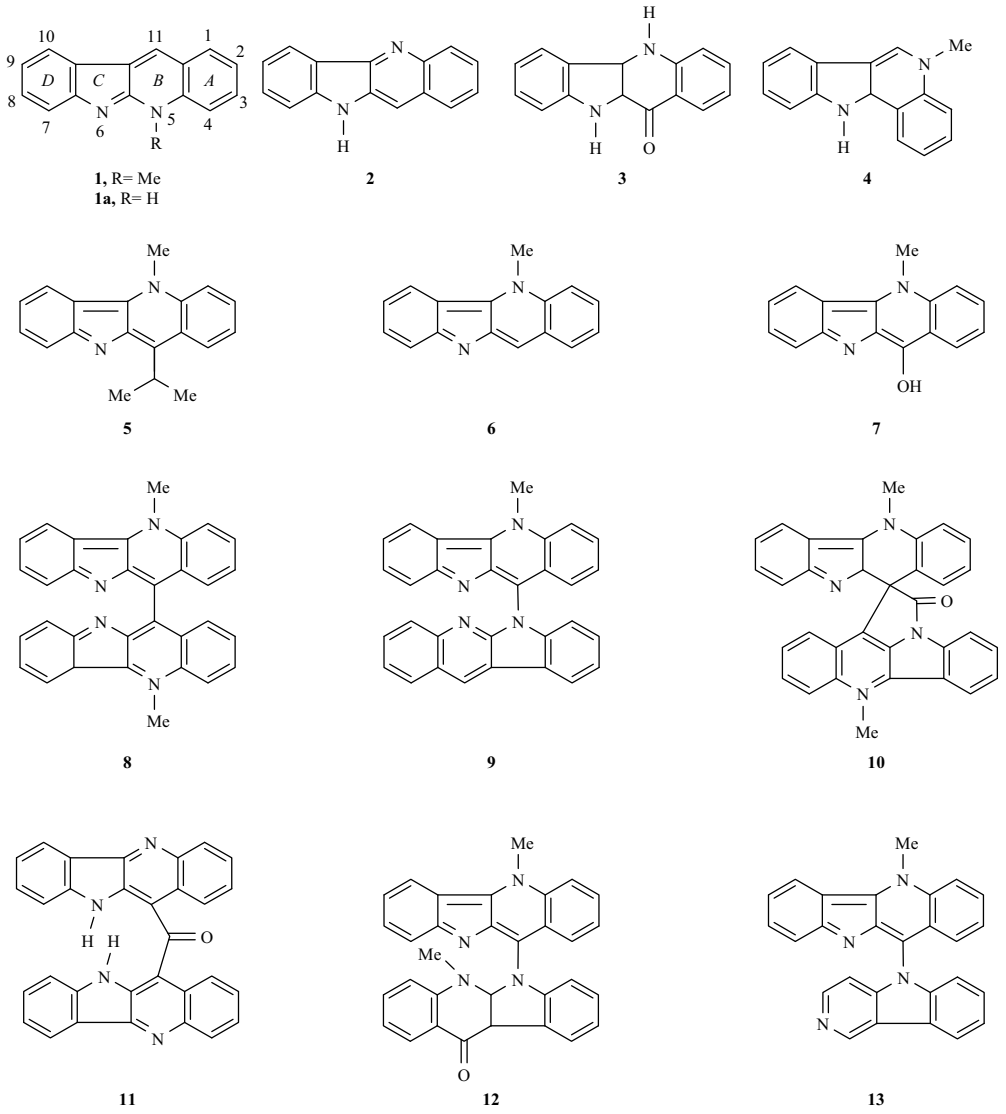


Fig. (1). Chemical structures of relevant naturally-occurring indoloquinoline alkaloids.

the 5*H* series were about 10 times higher than the related 6*H*- compounds. In addition, the indolo[2,3-*b*]quinolines with the highest number of methyl groups exhibited the greatest contribution to ΔT_m of calf thymus DNA. For the most substituted compounds of both series, ΔT_m= reached values as high as 19°C and 1.6°C, respectively [36c].

Neocryptolepine exhibits an ultraviolet absorption band centered at 340 nm. Upon binding of the alkaloid to DNA, the intensity of this absorption band decreases considerably, with a hypochromic effect that exceeds 40% at high DNA/drug ratios, and it suffers a bathochromic effect to 352 nm. During titration with DNA, a well-defined isosbestic point is observed at 353 nm. This indicates that the free drug and a single type of complex exist in equilibrium [36c,44].

Employing a two-site model that assumes the existence of two independent non-cooperative binding sites, the apparent association constants (K_1 and K_2) of neocryptolepine to DNA were determined, being 4.5×10^6 and 3.5×10^4 M^{-1}, with 0.3 and 0.37 sites per nucleotide, respectively. The non-cooperative ligand binding model of McGhee and von Hippel provided an association constant of 1.2 μM and 2.2 nucleotides per binding site, revealing that the binding affinity of neocryptolepine for DNA is approximately three times lower than that of cryptolepine. The latter provides more thermal protection to DNA than the former (ΔT_m= 16ºC vs. 5ºC) [36c].

The orientation of cryptolepine and neocryptolepine with respect to the DNA helix was inferred from Circular Dichroism (CD) and Electric linear dichroism **(**ELD) experiments. The CD spectrum of the complex between neocryptolepine and calf thymus DNA revealed a weakly negative CD band around 330-370 nm. This weak negative CD effect has been observed for conventional intercalating drugs, such as the ellipticines. On the other side, ELD measurements provided strong evidence suggesting that neocryptolepine is a DNA intercalating agent, like cryptolepine, and that the drug is oriented parallel to the DNA base pairs, as expected for an intercalation binding mode [36c,43].

Energy calculations for different 5,11-dimethylindolo[2,3-*b*]quinoline (DiMIQ, **21**) orientations inside the

DNA duplex have shown that the most preferred intercalation position, characterized by the lowest complex energy, was the parallel orientation of the long DiMIQ axis and the neighbor base-pair axes [45].

Listgartent and coworkers have shown that cryptolepine interacts with DNA at CC and GC-rich places [39b]; however, X-ray crystallographic studies demonstrated that it has a previously unknown mode of intercalation. The crystal structure of a cryptolepine-DNA complex revealed that it intercalates preferentially between non-alternating G-C sequences. The group of Guittat demonstrated that neocryptolepine binds to DNA at GC-rich sequences, also preferring GC over AT-rich duplex sequences. However, these dialysis competition and MS experiments revealed that the drug also recognizes triplex and quadruplex structures, exhibiting significant preference for triplexes over quadruplexes or duplexes. Additionaly, the natural product is a weak telomerase inhibitor [46]. The formation of reversible complexes with DNA takes place by insertion of the heterocycle in GC-rich sequences of the minor groove. This event induces conformational changes in the DNA structure; the observed cytotoxicity results when this interaction takes place in genomic regions involved in key biological activities, like DNA replication or transcription.

It was noted that the affinity of neocryptolepine towards DNA is lower than that of cryptolepine. This can be understood in terms of the reduced stability of the molecule within the complex. Neocryptolepine is unable to fit as well as cryptolepine on both sides of the DNA in the major and minor grooves. This is related to steric and electrostatic effects and was explained considering that in cryptolepine the NH and the *N*-Me groups are on opposite sides of the molecule; therefore, each one can interact with a C=O group of the base pair. However, neocryptolepine lacks one of these interactions; in addition, there is a steric hindrance interaction between the *N*-Me group of the small molecule and one of the NH_2 groups of the base pairs [48a,47]. This conclusion is in agreement with the fact that analogs of neocryptolepine carrying electron-withdrawing or electron-donating groups have poor affinity to DNA, as demonstrated by the methyl green assay (IC_{50} > 150 μM), slightly stimulating the topoisomerase II-mediated DNA cleavage.

ESI-MS and ESI-MS/MS experiments in the negative and positive ion modes were employed for the systematic investigation of complexes between intercalators, including neocryptolepine, with different duplex DNA [48]. Neocryptolepine behaves as a neutral, non-polar stacking ligand for which the complex dissociates mainly via the loss of the neutral drug. In the negative mode, the complex with neocryptolepine dissociated faster than the complex with cryptolepine, reflecting their relative solution-phase binding affinities, due to specific intermolecular interactions. However, when the ESI-MS experiment was performed in the positive ion detection mode, no complex could be observed, whatever the sequence of the double-stranded DNA used.

In the methyl green test, 2-methoxyneocryptolepine (77.9 ± 4.4 μM) and neocryptolepine (92.8 ± 9.7 μM) were highly active, whilst for 3-methoxyneocryptolepine 37% displacement of the dye was observed at a 150 μM concentration level [49]. In contrast, 2-bromoneocryptolepine, as well as various mono- and di-chlorinated derivatives, especially those having C-1 and C-2 halogens, exhibited low DNA affinity. In addition, the DNA-binding ability was also lost upon removal of the *N*-methyl group [50]. Reduction of the DNA-binding properties was not always associated to a similar loss of cytotoxicity, suggesting that more than one mechanism should be modulating the cytotoxic activity. Another structural feature responsible for the stability of the neocryptolepine-DNA complex seems to be the charged *N*-6 moiety [51].

In fact, the presence of a methyl group attached to the pyridine nitrogen in the 5*H*-indolo[2,3-*b*]quinolines increased their basic properties. The 6*H*-indolo[2,3-*b*]quinolines have pK_a values in the range 5.55-5.98, while the pK_a values of their 5*H*- congeners range between 7.24 and 7.68, being partially protonated under physiological conditions.

A series of 11-methyl 6*H*-indolo[2,3-*b*]quinolines derivatives, substituted at C-2 and C-9 with dialkyl(alkylamino)alkyl chains differing in the number of methylene groups, was synthesized and evaluated for their calf thymus DNA-binding properties. The synthesized compounds were compared with **21**, a known DNA-intercalator [36c] (Table **1**). Their DNA-binding properties and inhibition of topoisomerase II activity were studied [52]. It was concluded that all 6*H*-indolo[2,3-*b*]quinolines substituted with a (dialkylamino)alkyl chain can bind to DNA. The combined introduction of halosubstituents and basic side chains (alkylaminoalkyl), resulted in compounds with reduced DNA-intercalating properties and improved antiplasmodial activity.

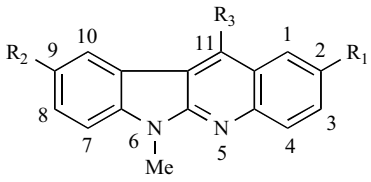
The binding constant of compounds **20**, **22** and **23** to salmon fish sperm DNA were also evaluated in a different assay, using UV-Vis absorption spectroscopy. After processing the data with a double reciprocal equation [53], the observed binding constants were 3.28×10^5, 2.93×10^5 and 2.27×10^5 L mol^{-1}, respectively [54]. These binding constants were one order of magnitude higher than substituted chromeno[2,3-*b*]indoles ($K = 2.79 \times 10^4$ L mol^{-1}) [44]. Interestingly, these kind of compounds have been suggested as candidates for modulating RNA-binding proteins [55].

Interestingly, when the mutagenic activity of a series of linear, methyl-substituted derivatives of 5*H*-indolo[2,3-*b*]quinolines was tested with the battery of Ames test strains, it was observed that the position and number of methyl groups strongly affected the activity. The tested compounds behaved as DNA frame-shift mutagens [56].

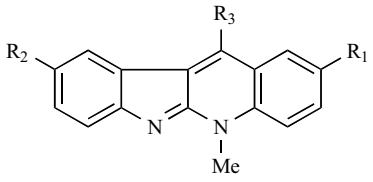
INHIBITION OF TOPOISOMERASE II

Neocryptolepine stimulates DNA cleavage by the human DNA relaxing enzyme topoisomerase II. However, it does not stabilize topoisomerase I-DNA cleavable complexes. The inhibition results from the intercalation of the heterocycle into the DNA which, in turn, also prevents its relaxation by the enzyme. The alkaloid has a slightly less pronounced poisoning activity than cryptolepine, which may provide the molecular grounds to explain the reduced cytotoxicity of neocryptolepine compared with cryptolepine [43]. In addition, unlike some of its derivatives [42], the topoisomerase II poisoning activity of neocryptolepine was considerably lower than etoposide.

Table 1. Interaction of 6*H*-indolo[2,3-*b*]quinoline derivatives with calf thymus DNA.



A



B

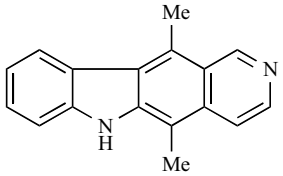
Compound	R_1	R_2	R_3	Skeleton	ΔT_m (°C)	$K_{app} \times 10^6$ (M^{-1})	n
14	$-N(CH_2)_2NMe_2$	H	Me	A	16.0	0.58	1.05
15	H	$-N(CH_2)_2NMe_2$	Me	A	14.0	0.77	4.83
16	$-N(CH_2)_3NMe_2$	H	Me	A	14.0	2.86	3.53
17	H	$-N(CH_2)_3NMe_2$	Me	A	16.0	2.51	1.8
18	$-N(CH_2)_2NEt_2$	H	Me	A	13.0	1.12	0.72
19	H	$-N(CH_2)_2NEt_2$	Me	A	11.0	1.30	0.92
20	Cl	H	$NH(CH_2)_3NH_2$	A	-	32.8	-
21	H	H	Me	B	11.0	1.43	3.36
22	Br	H	$NH(CH_2)_3NH_2$	B	-	29.3	-
23	H	CO_2Me	$NH(CH_2)_3NH_2$	B	-	27.7	-

The group of Peczyriska-Czoch prepared a series of methylated 5*H*-indolo[2,3-*b*]quinolines analogues to neocryptolepine, and found that only the cytotoxic compounds stimulated efficiently DNA cleavage [36c]. When purified calf thymus topoisomerase II and circular pSP65 DNA were used as substrates, the maxima of the cleavable complexes were produced in the range 0.4-10 μM (Fig. **2**).

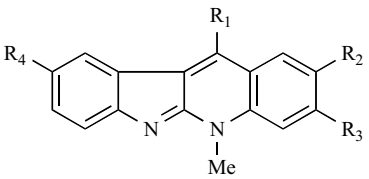
The introduction of methyl substituents on carbons greatly affected 5*H*-indolo[2,3-*b*]quinolines action on topoisomerase II. The induction of the calf thymus topoisomerase II-mediated complexes cleavable of pSP65 DNA depended on both, the position and number of methyl groups at carbons. The tetramethyl compound **28**, was the most active derivative, exerting the most efficient induction of calf thymus topoisomerase II-mediated DNA cleavage at 0.4 μM, being 25 times more potent than the less active species, the unsubstituted compound **1**. Remarkably, neither analogous 6*H*-indolo[2,3-*b*]quinolines nor ellipticine (**24**) produced detectable amounts of pSP65 DNA breaks mediated by calf thymus topoisomerase II at concentrations in the range 1-100 μM.

However, after a series of toxicity experiments using different cell lines with altered levels of topoisomerase II catalytic activity, and employing different topoisomerase poisons, it was suggested that topoisomerase II inhibition would play a relatively minor role in the cytotoxicity of neocryptolepine [57].

Godlewska *et al.* synthesized different ϖ-(dialkylamino)alkyl derivatives of neocryptolepine and observed that the 6*H*-indolo[2,3-*b*]quinolines substituted at C-2, C-9 or *N*-6 were able to bind to DNA and interfere with the human topoisomerase II catalytic activity *in vitro* (IC_{100}= 0.025-0.25 mM), being more active than DiMIQ (**21**) itself (IC_{100}= 0.5 mM) [52]. On the other hand, other researchers observed that



24



1, $R_1=R_2=R_3=R_4=$ H
21, $R_1=$ Me, $R_2=R_3=R_4=$ H
25, $R_1=R_2$ Me, $R_3=R_4=$ H
26, $R_1=R_3=$ Me, $R_2=R_4=$ H
27, $R_1=R_4=$ Me, $R_2=R_3=$ H
28, $R_1=R_2=R_4=$ Me, $R_3=$ H

Comp. N°	Conc. (μM)[a]
1	10
21	5.0
25	0.6
26	1.0
27	0.8
28	0.4

[a]Concentration of the compound producing the maximum of cleavable complexes

Fig. (2). Methyl substituted 5*H*-indolo[2,3-*b*]quinolines and their effect on topoisomerase II.

at a 20 μM concentration level, compounds such as 2-methoxy-, 2-bromo-, or 2-chloro-neocryptolepine were found to slightly stimulate the topoisomerase II mediated DNA cleavage; however, in all cases the DNA cleavage never exceeded 15% of the DNA products [49]. Additional derivatives have also been tested and exhibited limited activity [58].

The 11-anilinoindolo[2,3-*b*]quinolines were synthesized as bioisosteric analogues of acridine derivatives. Their anticancer activity were evaluated, due to their capability of intercalating DNA and inhibiting mammalian topoisomerase II [59]. In addition, 11-methyl-6-(2-dimethylamino)ethyl-6*H*-indolo[2,3-*b*]quinolines bearing an extra ω-(dimethylamino)-alkyl chain attached at C-2 or C-9 through ether, amine or amide linkers (Fig. **3**) have also been synthesized and evaluated. These compounds were found to inhibit the human topoisomerase II activity, being as potent as the antineoplastic aminoacridine derivative m-AMSA and much more active than anthracycline aminoglycoside antibiotic daunorubicin. However, these compounds were not able to overcome the topoisomerase II-dependent resistance barrier in HL-60/MX2 cells. This supports the hypothesis that their mechanism of action is more complex than the inhibition of topoisomerase II activity [42,60].

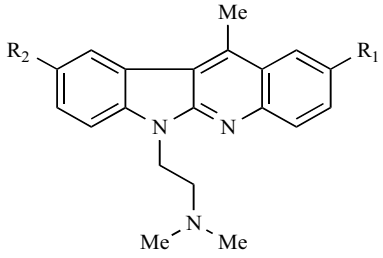
CYTOTOXIC ACTIVITY

The molecular modes of action of cytotoxic alkaloids have been discussed [61]. The group of Kaczmarek was among the first to observe structural analogies of ellipticine (**24**) with 9*H*-pyrido[2,3-*b*]indoles (α-carbolines, **35**) and 1*H*-pyrido[2,3-*b*]indoles (iso-α-carbolines, **36**), postulating the ability of the latter to act as DNA intercalants [36b]. Accordingly, they prepared a series of heterocycles (Fig. **4**) and tested their *in vitro* cytotoxicity against the mammary carcinoma KB cells. Some of the synthetic compounds (**37**, **38** and **21**) proved to be more potent than ellipticine and neocryptolepine.

These early investigations also found that DiMIQ (**21**), an 11-methylated analog of neocryptolepine, was a promising lead compound for potential anticancer agents among different indoloquinoline derivatives [36b]. DiMIQ bears interesting similarity to **24**, not only from the structural point of view, but also regarding to its physicochemical properties [62]. Contrary to its 6,11-dimethyl-6*H*-analog, DiMIQ has potent antiproliferative activity *in vitro*, apparently resulting from its ability to intercalate the DNA and create a drug-DNA-topoisomerase II complex [36c,36e].

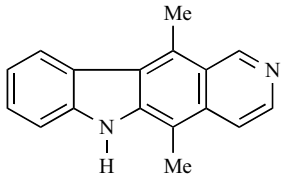
When the cytotoxicity of a series of analogues of neocryptolepine was studied, it was found that the activity was strongly influenced by the position and number of the methyl substituents. The trimethyl analog **28** was the most potent compound, and the presence of a methyl group on the *N*-5 pyridine nitrogen was essential for good levels of cytotoxicity against KB cells (ID_{50}), which was in the range 2-9 μM [36c].

However, DiMIQ which carries two methyl groups, has low aqueous solubility. Therefore, its practical application for cancer treatments was considered rather limited. Consequently, a liposomal formulation was designed to overcome this situation [63].



29, R_1= $OCH_2CH_2NMe_2$, R_2= H
30, R_1= H, R_2= $OCH_2CH_2NMe_2$
31, R_1= $NHC(O)CH_2NMe_2$, R_2= H
32, R_1= H, R_2= $NHC(O)CH_2NMe_2$
33, R_1= $NHCH_2CH_2NMe_2$, R_2= H
34, R_1= H, R_2= $NHCH_2CH_2NMe_2$

Fig. (3). 1-Methyl-6-(2-dimethylamino)ethyl-6*H*-indolo[2,3-*b*]quinolines carrying ω-(dimethylamino)alkyl chain at C-2 or C-9.



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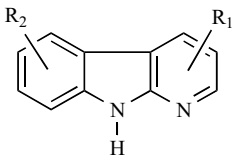
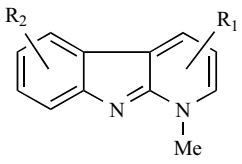
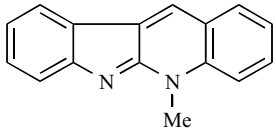


35, R_1= H, 4-Me;
R_2= H, 6-F, 6-Cl, 6-Me



36, R_1= 2-Cl, 2-Ph, 4-Me, 2,4-di-Me
R_2= H, 8-MeO



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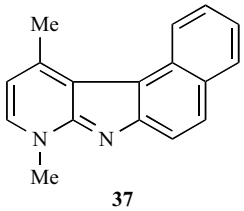

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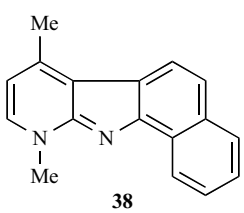

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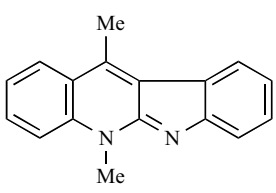


21

Cytotoxicity (KB cells)

Comp. N°	IC_{50} (μM)
24	1×10^{-2}
1	1×10^{-2}
37	2×10^{-3}
38	4×10^{-3}
21	1×10^{-3}

Fig. (4). Ellipticine, neocryptolepine and structurally similar heterocycles.

The group of Peczynska-Czoch also prepared cytotoxic derivatives bearing methyl groups at N-5, C-11, C-2 and/or C-9, as well as methoxy-groups al C-2 and/or C-9, employing the modified Graebe-Ullmann reaction and subjected the compounds to biotransformation with *Cunninghamella elegans* ATCC 9245 and a microsomal fraction of rat liver, in an effort to elucidate their metabolic pathways. They observed three forms of conversion, including *O*-demethylation and hydroxylation of the aromatic ring or the methyl group. The reactions took place only at C-2 and C-9, and the metabolites were as cytotoxic *in vitro* against colon adenocarcinoma SW-707 and lung carcinoma A-549 (ID_{50}= 0.27-3.04 μM) as the corresponding substrates. The metabolites with a hydroxyl group on the aromatic ring were transformed into non-cytotoxic products by human ceruloplasmin and fungal laccase [64].

Therapeutic and toxic effects of drugs are strongly influenced by their lipid affinity. Therefore, a study of the interaction with membranes of DIMIQ as well as C-2 and C-9 substituted analogs bearing different (dialkylamino)alkyl side chains was carried out [62]. It was noted that all the tested compounds caused hemolysis in isotonic conditions. The HC_{50} values (0.12-0.88 mM) were above the cytotoxic range.

Since the bioavailability limitation precludes the development of analogs with a higher number of alkyl substituents, series of substituted 5*H*- and 6*H*-indolo[2,3-*b*]quinolines were synthesized in order to improve the bioavailability of the indoloquinoline system, including methoxy, 6-dimethylaminoalkyl [65], glycosyl [66], aminoglycosyl [58], and alkylaminoalkyl [52] derivatives.

The different experiments confirmed that the 5*H*- derivatives carrying *N*-Me substituents on *N*-5 are two orders of magnitude more cytotoxic than their analog 6*H*-, 6-methyl counterparts [36c,54a]. They also revealed that the installation of hydrophilic side chains decreased the hemolytic activity of the resulting heterocycles, while simultaneously improving their hydrophilic properties.

Murine and human leukemia cells were employed to evaluate the cytotoxicity of neocryptolepine and its effects on the cell cycle. The natural product caused a massive accumulation of P388 murine leukemia cells in the G2/M phase. In addition, western blotting analysis revealed that neocryptolepine is unable to induce cleavage of poly-ADP-ribose polymerase but it is capable of inducing the release of cytochrome *c* from the mitochondria [57].

When the HL-60/MX2 cell line was used, which has reduced expression of topoisomerase II and is resistant to the anticancer drug mitoxantrone, neocryptolepine was only two times less toxic compared to the parental cells, suggesting that topoisomerase II may not represent the main cellular target for the alkaloids [57]. Neocryptolepine proved to be less cytotoxic than the related cryptolepine. Accordingly, it was observed that the proteolytic activity of the Asp-Glu-Val-Asp- and Ile-Glu-Thr-Asp-caspases was less pronounced with neocryptolepine than with its isomer.

In the conventional tetrazolium-based colorimetric cell proliferation assay, neocryptolepine exhibited IC_{50}= 6.5 μM against B-16 melanoma cells (for ellipticine, IC_{50}= 1.63 μM), resulting five times less toxic than cryptolepine [43]. Employing KB cells, these authors also confirmed previous findings. The IC_{50} values for cryptolepine, ellipticine and neocryptolepine were 1.3, 2.0 and 4.8 μM, respectively.

It was also shown that tetracycles lacking the Me group attached to *N*-5 were not cytotoxic and that 2-bromo and 2-nitro derivatives, as well as 9-cyano substituted compounds carrying different C-2 functionalities (Cl, CF_3, OMe or F) were not cytotoxic (IC_{50} > 32 μM) against the MRC-5 human lung cell line [49].

On the other hand, 6*H*-indolo[2,3-*b*]quinoline derivatives, carrying dialkyl-(alkylamino)-alkyl chains with different number of methylene groups at C-2, C-9 or *N*-6, exhibited cytotoxic activity against KB cells (IC_{50}= 5.0-9.0 μM). They were also able to overcome multidrug resistance in LoVo/DX (colorectal adenocarcinoma), MES-SA/DX5 (uterine sarcoma) and HL-60/MX2 (promyelocytic leukemia) cells, with resistance index values in the range of 0.54-2.4 [52]. In Jurkat T-cell leukemia cells, these compounds induced G2M-phase cell cycle arrest; they also revealed DNA-binding properties and inhibited topoisomerase II activity.

Aminoacid derivatives of 6*H*-indolo[2,3-*b*]quinoline have a higher activity than the parent tetracycle (Fig. **5**). For the C-9 substituted compounds, the order was: Gly > L-His > L-Ser >> L-Met ~ L-Pro > L-Lys > L-Ala > L-Leu (Table **2**), while in the case of C-2 substituted derivatives, the activity decreased in order: L-Pro > L-His > Gly >> L-Leu > L-Lys ~ L-Met. The highest cytotoxic activities attained were comparable to that of DiMIQ (IC_{50}= 3.32 ± 0.65 μM and 4.39± 0.81 μM for 9-Gly and 2-Pro, respectively) [60b].

Furthermore, the 9-glycylglycine conjugate (**45**) inhibited tumor growth in mice and was less toxic than DiMIQ, confirming that they are promising lead compounds for the development of new, highly potent, and selective agents against cancer [67].

Some indolo[2,3-*b*]quinoline derivatives induced apoptosis in HL-60 and HL-60/MX2 cells, but only in concentrations close to IC_{50} determined in cytotoxic assays [42]; however, at sub-cytotoxic concentrations the cell cycle progression of HL-60 cells was not affected. Interestingly, cell lines resistant to etoposide, a topoisomerase II poison, were still sensitive to methyl- and methoxy-substituted neocryptolepine derivatives.

Some 2- and 3-substituted neocryptolepine derivatives exhibited some degree of cytotoxicity (IC_{50}= 15-18.5 μM against MRC-5 cells) in the absence of DNA interactions, suggesting that their cytotoxic effect may be due to a different mechanism [68]. Seemingly, the apoptotic pathway would involve caspase-3 activation; in addition, cryptolepine, but not neocryptolepine, caused death by inhibition of poly(ADP ribose) polymerase (PARP) [57].

Indolo[2,3-*b*]quinoline derivatives carrying substituents at *N*-6 and C-2 or C-9 were prepared, with (dimethylamino)ethyl chains linked to heteroaromatic core by ether, amide or amine bonds (Fig. **3**). These were tested *in vitro* for their activity as cytotoxics against various cell lines, including multidrug resistant sublines. It was found that all compounds exhibited cytotoxic activity, inducing the G2M phase cell cycle arrest in Jurkat cells [60a].

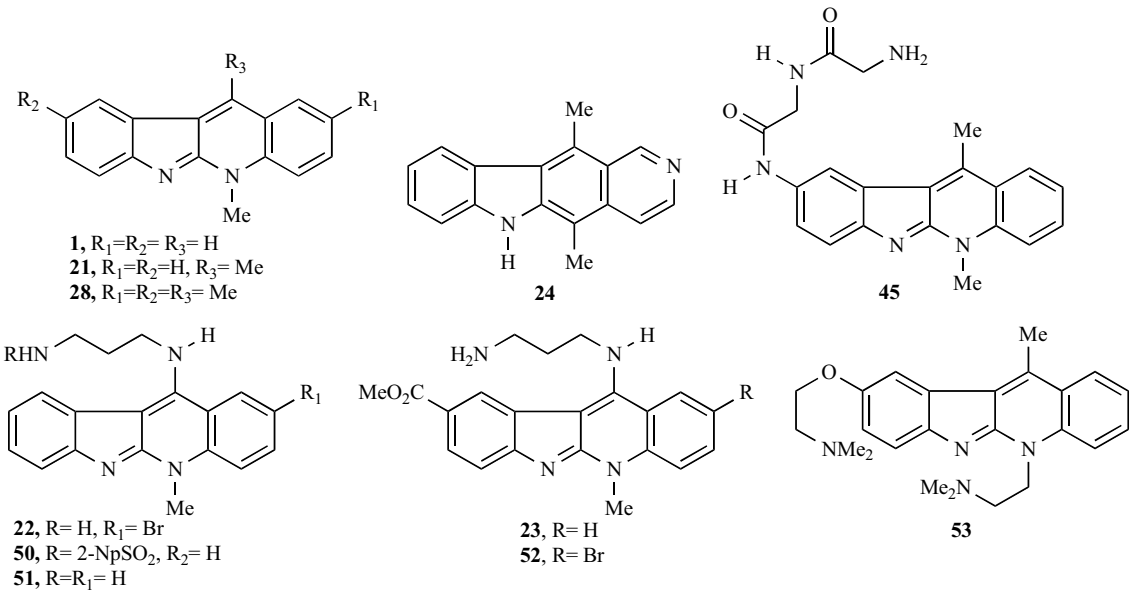
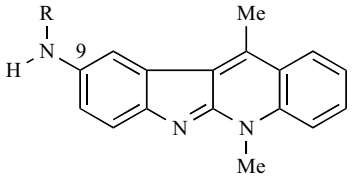


Fig. (5). Chemical structures of DiMIQ (**21**), ellipticine (**24**) and C-9 derivatives.

Table 2. ***In vitro*** **cytotoxic activities of the hydrochloride amino acid and peptide derivatives of DiMIQ against human cancer cell lines and normal mice fibroblasts.**



Nº	R	KB IC_{50}, μM	BALB/3T3 IC_{50}, μM	A549 IC_{50}, μM	MCF-7 IC_{50}, μM	LOVO IC_{50}, μM
21	DiMIQ	1.14 ± 0.61	5.77 ± 0.93	2.19 ± 0.48	1.54 ± 0.52	0.20 ± 0.40
39	Doxorubicin	0.84 ± 0.03	1.08 ± 0.03	0.33 ± 0.10	0.44 ± 0.16	0.11 ± 0.03
40	Gly	0.67 ± 0.93	0.55 ± 0.01	1.31 ± 0.11	1.45 ± 0.39	0.88 ± 0.14
41	L-Pro	0.42 ± 0.16	0.56 ± 0.07	0.48 ± 0.32	0.82 ± 0.05	0.81 ± 0.12
42	D-Pro	0.73 ± 0.24	0.36 ± 0.04	0.32 ± 0.10	0.60 ± 0.07	0.62 ± 0.21
43	L-His	3.46 ± 0.68	-	-	-	-
44	D-His	4.85 ± 0.93	-	-	-	-
45	Gly-Gly	4.65 ± 0.75	1.71 ± 0.34	0.90 ± 0.03	2.70 ± 0.54	0.73 ± 0.13
46	L-His-Gly	3.64 ± 1.4	-	-	-	-
47	L-Pro-Gly	3.73 ± 1.0	3.96 ± 0.51	1.15 ± 0.20	1.43 ± 0.65	0.98 ± 0.02
48	L-Pro-L-Pro	0.56 ± 0.24	0.48 ± 0.11	0.54 ± 0.13	0.96 ± 0.01	0.36 ± 0.05
49	Gly-L-Pro	0.7 ± 0.21	0.45 ± 0.12	0.86 ± 0.08	1.13 ± 0.13	0.44 ± 0.12

The C-11 amino-functionalization of neocryptolepine was carried out, observing that it improves potency and selectivity. Thus, bromoderivative **22** (Fig. **5**) exhibited an IC_{50}= 0.12 μM against the human leukemia MV4-11 cell line, being also selectively cytotoxic against A549 (lung cancer) and HCT116 (colon cancer) cell lines, and BALB/3T3 (normal fibroblast) cells, with IC_{50} values of 0.543, 0.274 and 0.869 μM, respectively [54a]. Furthermore, sulfonylated compound **50** exhibited high potency against various L6 cell lines (18.2 nM) and against NF54 cells (malaria parasites, 14.2 nM), but poor selectivity (SI= 1.3; SI= cytotoxicity/antiplasmodial activity ratio). Compound **50**

was approximately 200 times more potent than the parent neocryptolepine, 16 times more potent than the non-sulfonylated precursor **51** [69], and with a potency comparable to podophylotoxin (14.5 nM against L6 cells) [70]. Furthermore, the synthesis and evaluation of the isosteric 11-aminoalkylamino-substituted chromeno[2,3-*b*]indoles revealed that the N5-Me group is highly relevant for optimum activity [44]. The 3-propylamino derivatives carrying 2-OMe or 2-Cl substituents were among the most active compounds, underscoring both, the need of the C-2 substituent and the C-11 aminoalkylamino functionality.

Copper(II) complexes of indolo[2,3-*b*]quinolines were prepared [71] and tested against human colon carcinoma (HT-29) cells. The complexes exhibited higher activity than their free ligands, with the complex derived from **51** exhibiting the best results (IC_{50}= 0.58 μM).

Introduction of an ester moiety on C-9 improved the antiproliferative activity and selectivity [54a]. Compound **23** exhibited IC_{50}= 0.044 μM against MV4-11 cells, while **52** was 28 times more potent against the human colon cancer cell line HCT116 than against BALB/3T3, a normal mice fibroblast cell line [54].

The 11-methyl, *N*-5-substituted neocryptolepine derivatives functionalized at C-2 or C-9 with dimethylaminoethyl chains linked to heteroaromatic cores by ether, amide or amine bonds like **53**, were recently prepared as more soluble analogs of DiMIQ [60a,b]. They proved to be cytotoxic against various cell lines (LoVo/DX, MES-SA/DX5 and HL-60) including multidrug resistant sublines.

The 6*H*-indolo[2,3-*b*]quinoline substituted at C-2, C-9 or *N*-6 positions with *O*-(L-daunosamine) or (L-acosamine) *via* an alkoxy or alkyl linker exhibited cytotoxic activity against A549, MCF-7 and Hs294T cells. They were also able to overcome multidrug resistance in LoVo/DX (colorectal adenocarcinoma), MES-SA/DX5 (uterine sarcoma) and HL-60/MX2 (promyelocytic leukemia) cell lines. The compounds were able to induce the G2M or G0/G1 phase cell cycle arrest in Jurkat T-cells [66].

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

The roots of *C. sanguinolenta* have traditionally been used in African folk medicine for treating infectious diseases. Following the initial report of Boakye-Yiadom [16a] on the antibacterial activity of cryptolepine against *Staphylococcus aureus*, Cimanga *et al.* studied the 80% EtOH extract of the plant root bark [16d,72]. They isolated neocryptolepine, biscryptolepine and cryptoquindoline, and tested the extract fractions as well as these compounds for antibacterial and antifungal activities. Neocryptolepine was found to possess antibacterial activity against Gram-positive bacteria (MIC <100 μg/mL), inhibiting *B. cereus, M. fortuitum, P. vulgaris* and *S. aureus*, among others, but was less active against Gram-negative bacteria, such as *K. pneumoniae,* P. *aeruginosa* and *E. coli*. Analyzing additional evidence about the behavior of this substance revealed that the antibacterial activity of neocryptolepine is bacteriostatic rather than bactericidal. On the other side, no antifungal activity could be observed for the alkaloid against *E. floccosum,* T. *rubrum* and *A. fumigatus* at the 100 μg/mL level, but the authors reported a weak inhibition on the growth of the yeast *Candida albicans* (MIC= 125 μg/mL).

The group of Peczyriska-Czoch synthesized a series of alkyl-substituted analogs of neocryptolepine and studied their antibacterial and antifungal activities [36c]. It was observed that the 6*H*-indolo[2,3-*b*]quinolines were inactive up to 5 mM, whereas their 5*H*-indolo[2,3-*b*]quinoline congeners exhibited significant activity against Gram-positive bacteria (*Staphylococcus aureus, Micrococcus luteus*) and fungi (*Candida albicans* and *Trichophyton mentagrophytes*) with MIC values in the ranges 1.5-25 × 10^{-2} mM and 1.5-12 × 10^{-2} mM, respectively. All compounds were found to be inactive against Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*).

Concomitantly, Kazmareck *et al.* [47] also prepared substituted 5*H*-indolo[2,3-*b*]quinoline derivatives, carrying methoxy and methyl groups at C-2 and C-9. Evaluation of their antibacterial profile revealed that these derivatives were active against Gram-positive bacteria, whereas no antimicrobial activity was observed for Gram-negative bacteria. This confirmed previous observations regarding the behavior of neocryptolepine. In addition, antimicrobial activity against Gram-positive bacteria was also found in the series of ϖ-(dialkylamino)alkyl derivatives of neocryptolepine synthesized by Godlewska *et al.* [52].

Interestingly, it was recently reported that among a series of novel amino acid and dipeptide derivatives of 11-methyl neocryptolepine, those carrying glycinamido substituents at C-2 or C-9 were observed to display the best profile of antibacterial activity (MIC down to 6.25 μg/mL). These compounds were active against *Candida albicans* biofilms at doses significantly lower than those required against free-floating planktonic fungal cells [67b]. In addition, neocryptolepine, as well as its congeners cryptolepine and biscryptolepine, exhibited significant activity against *Mycobacterium fortuitum*. The MIC values were 25 and 5.25 μg/mL for neocryptolepine and cryptolepine, respectively, whereas the corresponding MBC levels were 31 and 25 μg/mL [16d].

A more recent report informed the synthesis of 11-substituted derivatives of neocryptolepine bearing α-aminophosphonate moieties and their activity as antibacterials against *E. coli*, *S. aureus*, *B. subtilis* and *K. spp.* [73]. The compounds were prepared from neocryptolepine analogs carrying 11-(1-ω)-diaminoalkyl side chains. The activity of the phosphonates and their precursor amino compounds was tested, resulting quite similar to tetracycline, employed as comparator (Fig. **6**).

ANTIPROTOZOAL ACTIVITY

Among the parasite-borne diseases, protozoa are responsible of causing African trypanosomiasis, amebiasis, Chagas disease, giardiasis, leishmaniasis, malaria and schistosomiasis. Trypanosomiasis and leishmaniasis cause severe infections in both, humans and domestic animals, especially in the tropics. These infections represent a serious health problem in terms of the human suffering and economic loses.

Chagas disease, the largest human parasitic condition of the American Continent, is caused by *Trypanosoma cruzi*. The available drugs are poorly effective and the number of

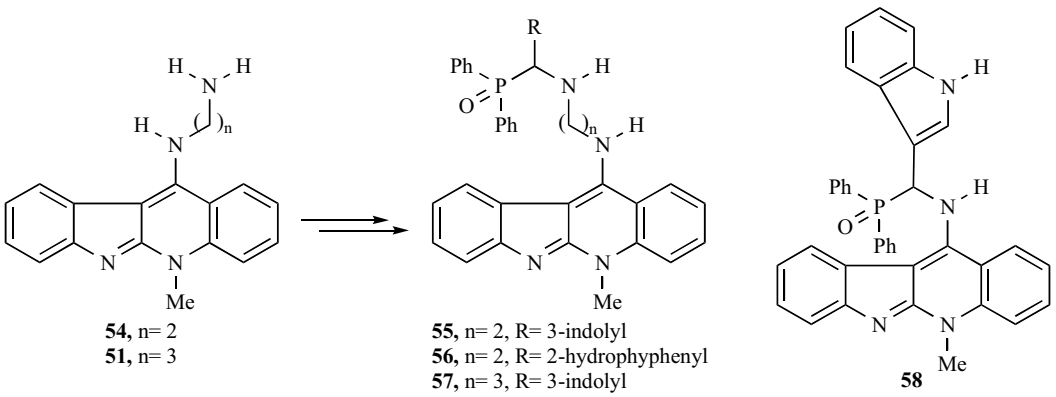


Fig. (6). α-Aminophosphonate derivatives of 11-aminosubstituted neocryptolepine.

deaths caused by the disease demand urgent pharmaceutical solutions. Currently, this is one of the most serious health problems of parasitic origin; the magnitude of the disease follows malaria and schistosomiasis, taking the third place in number of annual deaths.

Following earlier bioactivity studies with cryptolepine, which exhibited a comparatively high toxicity profile [74], neocryptolepine was tested and demonstrated to have antiprotozoal activity against *T. cruzi*, (IC_{50}= 2.01 ± 1.30 μM) and *T. brucei rhod* (IC_{50}= 2.23 ± 0.82 μM). However, the natural product is clearly less active than the corresponding comparators, benznidazole (IC_{50}= 1.50 ± 0.58 μM) and melarsoprol (IC_{50}= 0.004 ± 0.002 μM) [75]. It was observed that 5*H*-indolo[2,3-*b*]quinolines display antiprotozoal activity, whereas the related 6*H*-indolo[2,3-*b*]quinolines are essentially inactive. In addition, some 2-substituted neocryptolepine analogs such as the 2-bromo, 2-nitro-, and 2-methoxy-9-cyano derivatives also exhibited good antitrypanosomal activity against *T. brucei* and *T. cruzi*, without being cytotoxic to MRC-5 cells [49].

Furthermore, several 11-amino derivatives of neocryptolepine were synthesized and tested against *T. cruzi* and *T. brucei* (Table **3**) [69]. Data on neocryptolepine are also available [74b,76]. The activity of these compounds was up to two orders of magnitude lower than the corresponding comparators. In addition, all of these neocryptolepine derivatives showed cytotoxicity; thus, they were not selective.

Therefore, neocryptolepine was studied in docking experiments into validated drug targets of *T. brucei*, which included trypanothione reductase, rhodesain, farnesyl diphosphate synthase, and triose-phosphate isomerase [77]. The natural product was also incorporated in different sets of compounds used for developing computational models for the classification and rational design of new antitrypanosomal drugs [78].

On the other hand, leishmaniasis is a vector-transmitted protozoal disease that currently affects 12 million people in 88 countries. Interestingly, neocryptolepine also exhibited weak activity against *Leishmania donovani*, with IC_{50}= 49.5 ± 3.7 μM (Table **3**) [69], almost two orders of magnitude higher than the standard miltefosine (**67**, IC_{50}= 0.56 ± 0.07 μM) [74b]. Neocryptolepine and other quinoline alkaloids have shown notable docking to *Lmaj*MetRS, a protein from *Leishmania major* [79].

Schistosomiasis is the second most prevalent parasitic disease in the world after malaria. In 74 countries, the disease accumulates around 200 million infected people, 10% of them carrying the serious forms of the disease or affected with disease-related disabilities. The disease takes every year the lives of as much as 200000 human beings. There is a real need for discovery of newer active principles to fight schistosomiasis. Therefore, some neocryptolepine derivatives were studied, demonstrating *in vitro* schistosomicidal activity (Table **4**). The most effective compound had IC_{50}= 1.26 and 4.05 μM against the Egyptian and Puerto Rican strains of *Schistosoma mansoni*, respectively [80,81].

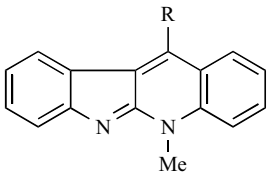
ANTIMALARIAL ACTIVITY

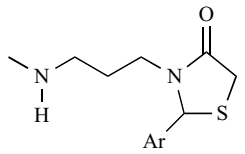
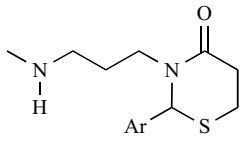
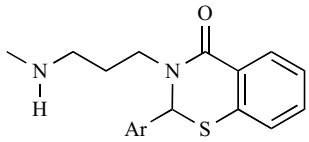
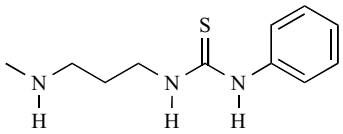
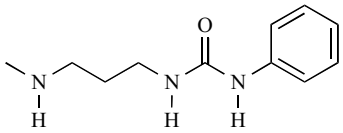
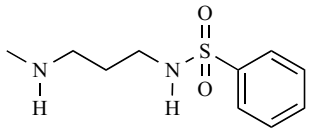
Malaria is a tropical disease caused by parasitic protozoa of the genus Plasmodium. The main species of human malaria parasites are *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*; however, its most serious form is caused by *P. falciparum*. According to the WHO, in 2013 there were approximately 200 million cases of malaria worldwide, and the disease caused near 600.000 deaths; three-quarters being children under the age of five. Malaria remains as the most important of the parasitic diseases worldwide, being considered by the WHO as a major infectious disease and a concerning health issue [82], like tuberculosis and AIDS.

Malaria is endemic in wide areas between the tropics; however, it can also be found in temperate regions and, favored by the global climate change, it is expected to increase its prevalence. Parasites are becoming increasingly resistant to antimalarial drugs; therefore there is a continuous and unavoidable need for finding new therapeutic agents against this scourge [83].

The effort to develop new antimalarial drugs from traditional medicines has been a widely explored and is a time-validated strategy [84]. Decoctions of the roots of *C. sanguinolenta* are used by herbalists and practitioners in traditional medicine of West Africa to treat malaria as well as a variety of other infectious diseases, including respiratory and sexually transmitted infections [10b]. In 1989, the effectiveness of the aqueous extract of the roots of *C. sanguinolenta*, was compared with chloroquine (**80**) in a small, open and randomized clinical trial in adult patients, in the treatment of uncomplicated falciparum malaria [5]. It was concluded that the decoction had efficacy comparable to chloroquine.

Table 3. Neocryptolepine analogs. Activity against *Trypanosoma* (*cruzi*, *brucei*) and *Leishmania* donovani.

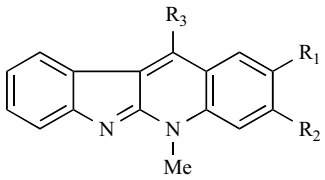


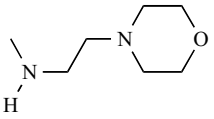
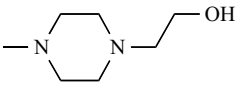
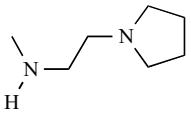
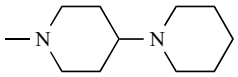
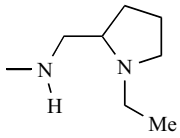
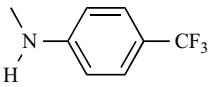
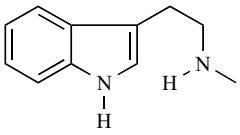
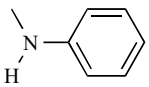
R (Ar= 4Cl-C_6H_4)	Compound	*T. brucei rhod.* IC_{50}, nM	*T. cruzi* IC_{50}, nM	*L. donovani* (Anexic Amastigotes), IC_{50}, nM
	**59**	1373	2727	26095
	**60**	547.5	3184	12950
	**61**	868.4	2362	9749
	**62**	589.2	6120	79167
	**63**	606.8	10602	97518
	**64**	164.2	1597	58036
H	**1**	2230	2010	49500
Melarsoprolol	**65**	5.0	-	-
Benznidazole	**66**	-	1606	-
Miltefosine	**67**	-	-	360.7

Natural products bearing the indole ring have been tested as antimalarials [85]. The activity of the extracts has been assigned to the abundant alkaloid cryptolepine (>1% of the dried root). Tests against several cancer cell lines, revealed that cryptolepine is moderately toxic; subsequent investigations revealed that this is due to its properties as DNA intercalating and topoisomerase II inhibiting agent [40]. In addition, in mice infected with *P. berghei*, intraperitoneal administration of cryptolepine (20 mg/Kg.day) was also found to be toxic [86].

The cryptolepine-induced cell death seems to be the result of cytotoxic effects. Therefore, efforts have been made to separate the DNA-intercalating effects of the drug from its antiplasmodial activity. Currently, the antimalarial activity of cryptolepine does not seem to be so actively pursued, favoring instead research on neocryptolepine, which has a more acceptable biological profile [10g,87]. Due to the lower affinity of neocryptolepine for DNA and topoisomerase II inhibition compared with cryptolepine and isocryptolepine, the neocryptolepine core has been further used as the lead for developing an antimalarial agent [74b].

Table 4. Schistosomicidal activity of neocryptolepine derivatives on adult *Schistosoma mansoni* worms.



Substitution Pattern			Compound	Primary Test Mortality (%)	Secondary Bioassay			
					IC_{50}, μM		IC_{90}, μM	
R_1	R_2	R_3		R_2	A	B	A	B
Cl	H	$NHCH(Me)(CH_2)_2NEt_2$	**68**	100	11.74	14.98	16.78	21.65
H	Cl	$NHCH(Me)(CH_2)_3NEt_2$	**69**	100	19.26	29.33	24.32	32.83
Cl	H		**70**	100	**1.26**	**3.54**	**4.05**	**6.83**
Cl	H		**71**	100	1.77	3.29	4.55	5.57
Cl	H	$NH(CH_2)_2NMe_2$	**72**	100	3.68	5.95	7.65	13.03
Cl	H		**73**	91.7	-	-	-	-
Cl	H		**74**	100	3.46	7.85	8.31	13.39
Cl	H		**75**	58.7	-	-	-	-
H	H		**76**	100	3.73	-	4.81	-
H	H		**77**	100	2.70	-	3.95	-
Br	H		**78**	100	3.07	-	4.30	-
Praziquantel (comparator)			**79**	100	0.6	0.89	1.08	1.5

Schistosoma mansoni tested strains: **A**, Egyptian strain; **B**, Puerto Rican strain.

Cimanga *et al.* carried out one of the first series of studies on the antimalarial activity of neocryptolepine as part of their evaluation of cryptolepine [88]. They demonstrated that neocryptolepine displayed valuable antiplasmodial activity against chloroquine-resistant strains of *P. falciparum* (IC_{50}= 35 ± 0.7 ng/mL, 51 ± 0.1 ng/mL and 65 ± 1.3 ng/mL against D-6, K-1 and W-2 strains, respectively), being comparable to chloroquine (IC_{50}= 72 ± 0.1 and 68 ± 0.1 ng/L against K-1 and W-2 strains, respectively).

In order to separate the antimalarial mode of action of neocryptolepine from its cytotoxic activity, it was decided to prepare analogues, attempting to retain or enhance the antiplasmodial activity and reduce cytotoxicity by attaching

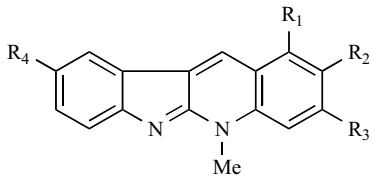
atoms or groups that may attenuate the intercalating ability of the resulting product.

The basis of the antiplasmodial mode of action of chloroquine and its derivatives resides in their activity against the malaria parasites, where they act as blockers of the transformation of hematin into hemozoin, which is part of the haem detoxification process [89]. These drugs accomplish this objective through a π–π stacking interaction between their 4-aminoquinoline core with the heme ring system, or by docking into grooves on the hemozoin crystals, this preventing their further growth. Then the hematin, which has proven toxicity, is left to cross from the digestive vacuole into the cytosol of the parasite, where it induces oxidative membrane damage [90].

In vitro, the inhibition of the transformation of hematin into hemozoin can be evaluated by measuring the conversion of hemin to β-hematin (synthetic hemozoin). Accordingly, this test was used to obtain additional information on the mechanism of action of neocryptolepine and its derivatives.

In this direction, various substituted derivatives of neocryptolepine were prepared by the group of Pieters [49], employing a biradical cyclization approach. The compounds were evaluated against chloroquine-resistant (W2) and chloroquine-sensitive (Ghana) strains of *P. falciparum* (Table **5**). Neocryptolepine showed antiplasmodial effect and inhibited β-hematin formation. Its antiplasmodial activity was ascribed, at least in part, to a selective mechanism involving inhibition of the process of haem detoxification [91],

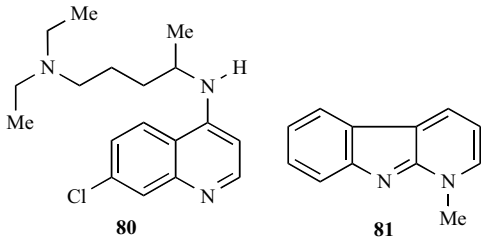
Table 5. Antiplasmodial activity and Inhibition of β-hematin formation of synthetic neocryptolepine derivatives.



R_1	R_2	R_3	R_4	Compound	*P. falciparum* Ghana Strain IC_{50}, μM	*P. falciparum* W2 Strain IC_{50}, μM	Inhibition of β-Hematin Formation
H	H	H	H	**1**	27.3±5.7	14.0 ± 1.7	+
H	OMe	H	H	**82**	4.3 ± 0.6	4.7 ± 0.6	-
H	**Br**	**H**	**H**	**83**	**6.0 ± 6.1**	**4.0 ± 0.1**	**+**
H	Cl	H	H	**84**	21.0 ± 8.9	5.0 ± 0.1	+
H	F	H	H	**85**	19.3 ± 3.8	4.7 ± 0.6	+
H	I	H	H	**86**	17.7 ± 5.1	6.3 ± 0.6	+
H	Me	H	H	**87**	2.7 ± 2.1	2.3 ± 0.6	ND
H	NO_2	H	H	**88**	29.0 ± 1.7	>32	ND
H	SMe	H	H	**89**	4.0 ± 1.0	3.7 ± 0.6	+
H	CN	H	H	**90**	17.0 ± 1.0	15.3 ± 0.6	ND
H	H	H	CN	**91**	>32	>32	+
H	Cl	H	CN	**92**	>32	>32	ND
H	OMe	H	CN	**93**	28.3 ± 3.5	17.0 ± 6.2	-
H	CF_3	H	CN	**94**	14.0 ± 2.6	6.7 ± 1.1	ND
H	F	H	CN	**95**	16.3 ± 1.2	14.7 ± 4.9	+
OMe	H	H	H	**96**	17.7 ± 0.6	12.3 ± 4.2	-
CF_3	H	H	H	**97**	>32	>32	ND
Br	H	OMe	H	**98**	3.3 ± 0.6	1.7 ± 0.6	ND
H	H	Br	H	**99**	30.0 ± 3.5	4.7 ± 0.6	+
H	H	Cl	H	**100**	21.7 ± 4.0	4.7 ± 0.6	+
H	H	CF_3	H	**101**	16.3 ± 0.6	15.7 ± 1.5	+

ND= Not determined.

and a non-selective mechanism related to interaction with DNA [68]. Interestingly, it was also observed that 1-methyl-1*H*-pyrido[2,3-*b*]indole (**81**) (Fig. **7**), which lacks one of the benzenoid rings of neocryptolepine, proved to be much less active and less cytotoxic than neocryptolepin and its derivatives [76b].



80 **81**

Fig. (7). Chemical structures of chloroquine (**80**) and a pyridoindole (**81**).

It was demonstrated that, despite their *in vitro* antiplasmodial activity being lower than that of chloroquine [68], some 2- or 3-substituted neocryptolepine derivatives inhibit the formation of β-hematin with about the same potency of chloroquine. These findings suggest that inhibition of haemozoin formation makes is at least an important contribution to their antiplasmodial activity [68].

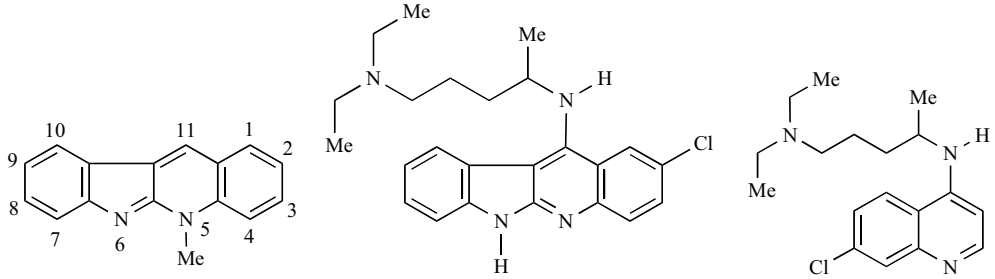
The 2-bromo analog (**83**) was 3.5 more potent than neocryptolepine and exhibited one of the best profiles [92], with good activity against the chloroquine-resistant strain, good potency for inhibiting β-hematin formation and low toxicity. Because of its low affinity for DNA and lack of inhibition of topoisomerase II, this was considered a promising lead for new antimalarial agents [93]. Interestingly, some of the analogs of this series exhibited enhanced cytotoxicity and were studied for their potential as anticancer agents [58a,b]. Mathematical models for the classification of neocryptolepine analogs as inhibitors of β-hematin formation have also been developed [94].

The 4-aminoquinoline core is a useful scaffold for rational antimalarial drug research; many 4-aminoquinoline derivatives are useful antimalarial compounds [90]. The basic side chain found in chloroquine is an important feature for its activity. This is required for accumulation of the drug into the food vacuole and, hence, for inhibition of hemozoin formation.

Taking into account the above details and considering some precedents with the isomeric cryptolepine [95], El Sayed *et al.* prepared a series of neocryptolepine analogues carrying N_1,N_1-diethylpentane-1,4-diamine (the side chain of chloroquine) and chloro-substituents, as well as a combination of both in several positions [50].

The mono- and dichlorosubstituted neocryptolepines exhibited lower activity than neocryptolepine itself and most of them were less cytotoxic than the parent compound (Table **6**). However, introduction of the N_1,N_1-diethylpentane-1,4-

Table 6. Antiplasmodial activity of neocryptolepine derivatives substituted with halogens and ω-dialkyl aminoalkylamino side chains.



178 **Chloroquine**

Substitution Pattern (Compound)	*P. falciparum* IC_{50}, μM	β-hematin Formation IC_{50} (meq)	Substitution Pattern (Compound)	*P. falciparum* IC_{50}, μM	β-hematin Formation IC_{50} (meq)
2-Cl (**84**)	52	1.31	3-NHCH(Me)$(CH_2)_3NEt_2$ (**109**)	0.015	ND
3-Cl (**100**)	41.11	ND	**8-NHCH(Me)$(CH_2)_3NEt_2$ (110)**	**0.01**	**4.40**
8-Cl (**102**)	> 64	1.55	9-NHCH(Me)$(CH_2)_3NEt_2$ (**111**)	0.14	< 20%/5 meq
9-Cl (**103**)	29	1.23	11-NHCH(Me)$(CH_2)_3NEt_2$ (**112**)	0.62	4.11
11-Cl (**104**)	> 64	ND	1-Cl, 11-NHCH(Me)$(CH_2)_3NEt_2$ (**113**)	0.35	< 20%/5 meq
1-Cl, 11-Cl (**105**)	39	No	**2-Cl,11-NHCH(Me)$(CH_2)_3NEt_2$ (114)**	**0.043**	**< 20%/5 meq**
2-Cl, 11-Cl (**106**)	> 64	No	3-Cl, 11-NHCH(Me)$(CH_2)_3NEt_2$ (**115**)	0.13	5.25
3-Cl, 11-Cl (**107**)	> 64	1.14	2-Cl, 11-NH$(CH_2)_2NMe_2$ (**116**)	< 0.25	No
2-NHCH(Me)$(CH_2)_3NEt_2$ (**108**)	0.03	1.45	Neocryptolepine (**1**)	27	5.97

ND= Not determined.

diamine side chain resulted in a substantial increase of the antiplasmodial activity not being accompanied by increase in toxicity.

The 8-substituted compound appeared to be the most potent (IC_{50}= 0.01 μM) derivative, with potency comparable to chloroquine, used as reference. The 11-substituted neocryptolepine was less potent, but its activity and toxicity seemed to increase by adding a chlorine substituent on the A-ring. Furthermore, although the presence of an *N*-methyl group has been considered as essential for antiplasmodial activity, removal of the 5-methyl group in compounds bearing basic side chains attached to C-11 afforded chloroquine-like compounds, which resulted in up to 3-fold loss in potency [9,49].

The 2-chloro heterocycle bearing a C-11 N^1,N^1-diethylpentane-1,4-diamine side chain (**114**) was found to be potent (IC_{50}= 0.043 μM); therefore, it was tested in a Swiss mice model against *Plasmodium berghei*. After daily injection of 50 mg/kg (intraperitoneal), it showed 100% reduction in parasitaemia on day 4; however, it demonstrated to be too toxic and half of the animals died by day 7. Furthermore, an intraperitoneal dose of 20 mg/kg proved to be ineffective. On the other side, the related nor-compound **117** was less potent (IC_{50}= 0.12 μM), and unable to attain 100% reduction in parasitaemia, emphasizing the role of methyl groups as nitrogen substituents. These experiments suggest that the *N*-methyl group is not the critical moiety, needed for biological activity, but rather the presence of a basic nitrogen atom.

Introduction of the aminoalkylamino side chains did not result in a marked loss of the DNA-interacting properties, and all compounds showing interaction with DNA were also cytotoxic to various degrees. Many of the compounds in this series attained selectivities above 500. However, compounds that were inactive in both functional assays did not show a pronounced antiplasmodial activity.

In 2013, the effects simultaneously of changing the side chains at the C-11 position and modifying the nature of the C-2 substituent were examined [69]. The C-11 basic side chains examined included alkylamino and ω-aminoalkylamino versions, as well as acyclic or cyclic carbamides or thiocarbamides (Table **7**). The resulting neocryptolepine derivatives were tested *in vitro* for antimalarial activity against chloroquine-resistant (K1) and -sensitive (NF54) strains of *P. falciparum.* Among the tested compounds, the heterocycle **125** showed an IC_{50} of 2.2 nM against the NF54 strain and a selectivity index of 1400, whereas **128** showed an IC_{50} of 2.2 nM for the K1 strain, a selectivity index of 1243, and a resistance index of 0.5.

In vivo testing against *Plasmodium berghei* in Swiss mice in the "4 Day Test" revealed that compounds **123** and **63** reduced parasitaemia in 15.4% and 22.1%, respectively. However, derivative **125** showed no activity and all mice lost weight.

More recently, the effects of introducing ester groups on C-2 or C-9 and changing the end-capping substituent at the terminal amino group of 3-aminopropylamine substituents at C-11 position with urea or thiourea units were evaluated [54b,96] (Table **8**). The rationale behind this effort is that enhancement of the antiplasmodial and antitrypanosomal activities have been observed with bicyclic amides and esters of dialkylamino acids. These improvements are believed to result from increasing drug lipophilicity and bioavailability [97].

Values of the resistence index (RI) were as high as 12.3. This index, which provides a quantitative measurement of the antiplasmodial activity against the chloroquine-resistant (K1) strain relative to the chloroquine-sensitive strain (NF54), is useful to unveil promising drug leads [98].

It was observed that the neocryptolepine derivatives modified with ester groups displayed higher antiplasmodial activities against *P. falciparum* (NF54 and K1) and low cytotoxicity activity against normal cells [96]. The related carboxylic acids exhibited lower potency. The IC_{50} values of the most potent examples were approximately 2 nM, with selectivity indexes above 320. Furthermore, the compounds showed higher activity than chloroquine against the K1 strain, and many were found to be more active than chloroquine (IC_{50}= 18 μM) in the β-hematin inhibition test.

Factors governing the uptake of these compounds into the parasite seem to be more predictive of the biological activity [99], as stemmed from the weak correlation between the antiplasmodial and β-hematin inhibiting activities. However, a linear correlation between polar surface area of the molecule and β-hematin inhibition was found. The introduction of the ester group also increased the *in vivo* antiplasmodial activity of the compounds, being of low cytotoxicity against the normal cell line.

The synthesis and *in vitro* antimalarial activity of analogues carrying linear and branched dibasic side chains at C-11 revealed that a branched structural motif is not superior for antimalarial activity over a linear side chain in the antimalarial test against the chloroquine-sensitive *P. falciparum* strain NF54 [69]. It was also observed that a three-carbon atom chain separating both nitrogen atoms was the most appropriate. In addition, it was detected that thioureido derivatives such as **150** (Fig. **8**) showed lower cytotoxicity but higher selectivity than the linear precursor, and stronger β-hematin inhibition than the corresponding free amines [69].

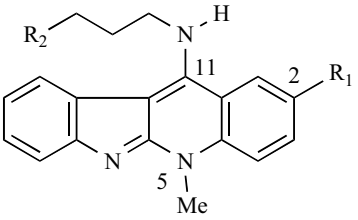
Combinations of ureido derivatives carrying 2-Cl and 2-OMe functions also exhibited good antimalarial potency [69]. Testing of compounds **151** and **152** against the *Plasmodium* strain NF54 on female mice also demonstrated that the introduction of the ester group substantially increased the *in vivo* antiplasmodial activity of the neocryptolepine core.

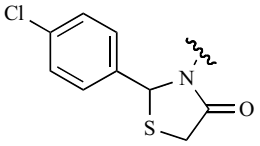
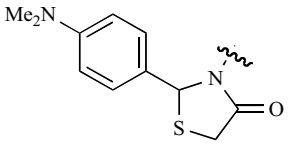
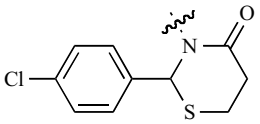
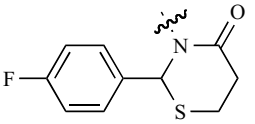
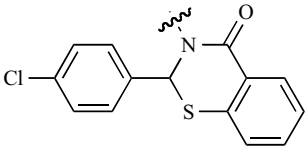
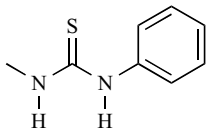
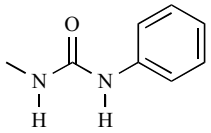
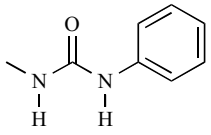
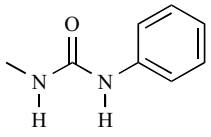
Recently, derivatives of 6-methyl-6*H*-indolo[2,3-*b*]quinolines carrying alkylamino and ω–aminoalkylamino substituents at C-11, and Cl at C-2, or a CO_2Me group at C-9 were prepared for SAR studies [99]. It was observed that their anti-malarial activities were significantly increased compared to the 11-nonsubstituted derivatives.

Again, among the various ω-aminoalkylamino side chains tested, the 3-aminopropylamino group at C-11 was the most efficacious one. Improved cytotoxicity against normal cells was observed when the terminal amino moiety was end-capped as urea or thiourea derivatives.

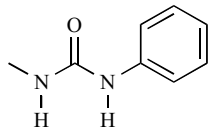
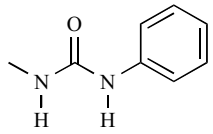
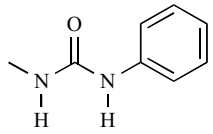
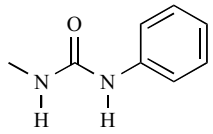
In its series, the best results were achieved with compounds **153** and **134** against the NF54 strain, with IC_{50}= 317 and 86 nM, and SI= 369.5 and 20, respectively. The correlations of β-hematin inhibition suggest that inhibition of

Table 7. Antiplasmodial activity against *P. falciparum* of C-2 and C-11 substituted neocryptolepine derivatives.



R_1	R_2	Compound	NF54 IC_{50}, nM	SI L6/NF54	K1 IC_{50}, nM	SI L6/K1	RI K1/NF54
H	NH_2	**51**	78.8	3.5	29.6	9.4	0.4
H		**118**	63.9	54.4	143.7	24.2	2.2
H		**119**	54.9	64.7	143.2	24.8	2.6
H		**120**	52.4	64.1	38.8	86.6	0.7
H		**121**	38.1	71.6	112.3	24.3	2.9
H		**122**	26.6	41.3	21.3	51.6	0.8
H		**123**	9.1	134.9	111.5	11.0	12.3
H		**63**	21.3	58.4	9.4	132.3	0.4
Br		**124**	4.0	706.5	11.9	237.5	3.0
Cl		**125**	2.2	1400	21.8	141.2	9.9

(Table 7) contd….

R_1	R_2	Compound	NF54 IC_{50}, nM	SI L6/NF54	K1 IC_{50}, nM	SI L6/K1	RI K1/NF54
F		**126**	24.9	142.8	312.6	11.4	12.6
CF_3		**127**	4.1	312.7	48.8	26.3	11.9
OMe		**128**	4.4	621.4	2.2	1243	0.5
NO_2		**129**	2.1	1301	29.9	91.4	14.2
Neocryptolepine		**1**	1580	2.0	1696	1.9	1.1
Chloroquine		**80**	9.4	-	209.5	-	22.3
Artemisinin		**130**	4.3	-	2.8	-	0.7

haemozoin may play a role in the activity of the compounds. It is currently considered that the mode of action of the neocryptolepine derivatives is analogous to that of chloroquine [100]. However, the physicochemical factors related to the solvation and polarity, are also important factors that determine the activities of these compounds [99].

Based on bleomycine as hybrid drug model, Meunier proposed a new design and strategy for hybrid trioxaquine molecules as antimalarial drugs bearing a dual mode of action to increase activity; an antimalarial mechanism of these hybrid trioxaquine molecules was also proposed [101]. Guided by this paradigm, very recently artesunate-indolo[2,3-*b*]quinoline hybrid molecules were prepared [100]. These were screened for their antiplasmodial activity against NF54 and K1 malaria strains (Table **9**).

The synthesized hybrids were less cytotoxic, exhibited increased antimalarial activity, and stronger β-hematin inhibition than their corresponding parent molecules. The most effective hybrid (**160**), with a linear unsubstituted 1,3-diamino side chain, displayed IC_{50}=0.45 and 0.42 nM against the NF54 and K1 strains, respectively. The compound was tested in mice at a dose of 10 mg/Kg.day during four consecutive days. It was observed that the parasitemia was significantly reduced on day 4 (antiparasitic activity= 89.6%); however, the hybrid is still too toxic, leading to a mean mouse survival time of 7.7 days.

MOLLUSCICIDAL ACTIVITY

Several 2-chloro neocryptolepine derivatives have been found to cause 100% snail mortality of the snail *Biomphalaria alexandrina* (Ehrenberg) (Planorbidae), the intermediate host of *S. mansoni* in Egypt, in a primary test at a 5 ppm level (Table **10**).

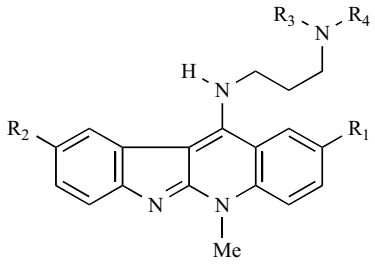
In the secondary test, their LC_{50} values were found to be between 1.3-3.9 ppm after 24 h, which parallels the potency of niclosamide (LC_{50}= 0.2 ppm), employed as comparator. On the other hand, their LC_{90} ranged from 2.12 to 4.8 ppm, whereas for niclosamide was 0.6 ppm [80].

CONCLUSION

During the last decade, neocryptolepine has emerged as a valuable lead for the development of new chemical entities, active against multiple relevant targets and conditions. In addition to the astonishing great number of total syntheses of the natural product, complex sets of analogs and derivatives of the naturally-occurring heterocyle have been prepared and tested. From this complex research, neocryptolepine has emerged as a privileged core for the design of new cytotoxic and antimalarial drugs.

The various research groups that have unveiled the properties of neocryptolepine as a drug lead, have found that 5*H*-indolo[2,3-*b*]quinolines are more potent both, as cytotoxics and antimalarials than the congener 6*H*-indolo[2,3-*b*]quinolines. In addition, it has been shown that the presence of the 5-methyl group among the neocryptolepine derivatives is crucial for an optimum pKa of the products, hence relevant to promote their interaction with DNA and inhibition of topoisomerase, as well as influential to the antiplasmodial action.

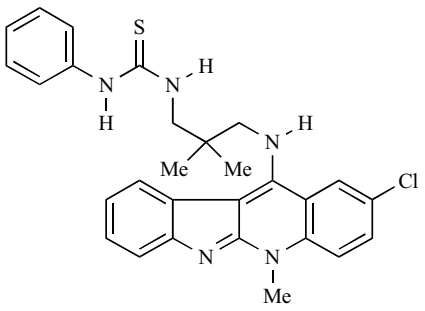
Table 8. Antiplasmodial activity against *P. falciparum* strain NF54 of neocryptolepine acids and esters.



R_1	R_2	R_3	R_4	Compound	NF54 IC_{50}, nM	SI (L6 cells)	β-Hematin Inhibition IC_{50}, μM
H	H	H	H	**51**	78.8	3.50	–
H	CO_2Me	H	H	**23**	24.8	8.79	48.9
H	CO_2Me	Me	Me	**131**	33.3	8.05	62.5
CO_2Me	H	H	H	**132**	8.28	40.7	104.4
CO_2Me	CO_2Me	H	H	**133**	9.52	146	13.0
Cl	CO_2Me	H	H	**134**	7.57	56.4	25.4
Br	CO_2Me	H	H	**52**	2.27	361	11.5
CO_2Me	Br	H	H	**135**	4.54	232	10.2
Cl	Br	H	H	**136**	2.16	186	30.1
CO_2Me	H	PhNHC(O)	H	**137**	4.16	181	23.0
CO_2Me	H	PhNHC(S)	H	**138**	1.81	321	15.7
H	CO_2Me	PhNHC(O)	H	**139**	14.5	178	12.8
H	CO_2Me	PhNHC(S)	H	**140**	6.03	467	10.8
CO_2Me	CO_2Me	PhNHC(O)	H	**141**	16.7	1590	4.6
CO_2Me	CO_2Me	PhNHC(S)	H	**142**	14.4	43.5	7.5
Cl	CO_2Me	PhNHC(O)	H	**143**	13.6	246	14.9
Br	CO_2Me	PhNHC(O)	H	**144**	8.05	837	16.0
CO_2Me	Br	PhNHC(O)	H	**145**	7.15	157	10.1
Cl	Br	PhNHC(O)	H	**146**	5.59	73	17.8
H	CO_2H	H	H	**147**	113	1690	66.8
H	CO_2H	Me	Me	**148**	20	2550	7.84
H	CO_2H	Me	Me	**149**	66.2	2160	30.6
Chloroquine				**80**	9.40	-	18.4

Successive studies have shown that substitution of C-2 or C-9 with halogen atoms, particularly Br and Cl affords compounds with reduced cytotoxicity. Other investigations have placed their emphasis in the beneficial effects of attaching ester moieties to C-2 or C-9, for the antimalarial activity. The effects of a dibasic ω-aminoalkylamino-type side chain at C-11 have been also exhaustively researched. It was established its proper length at three carbon atoms, and it was discovered that there is no need of branched chains.

It was also found that the strategy of end-capping of the amino terminus improves some desirable characteristics of the resulting products and that the synthesis of hybrid neocryptolepine-artesunate derivatives paved the way to new, more complex and highly potent heterocycles. Interestingly, whereas the substitution of C-2 provided a moderate contribution to the antiproliferative and antiplasmodial activities of the resulting heterocycles, the installation of a C-11 ω-aminoalkylamino side chain made a substantial contribution



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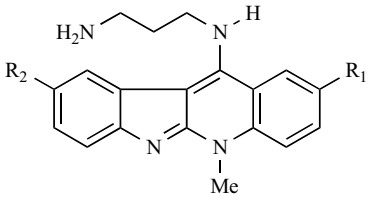
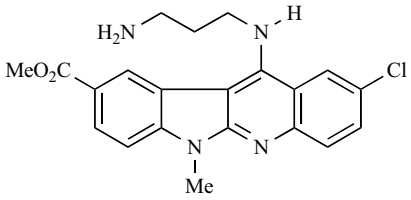
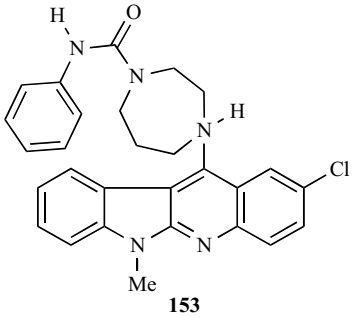


151, R_1= H, R_2= CO_2Et
152, R_1= CO_2Et, R_2= H



134

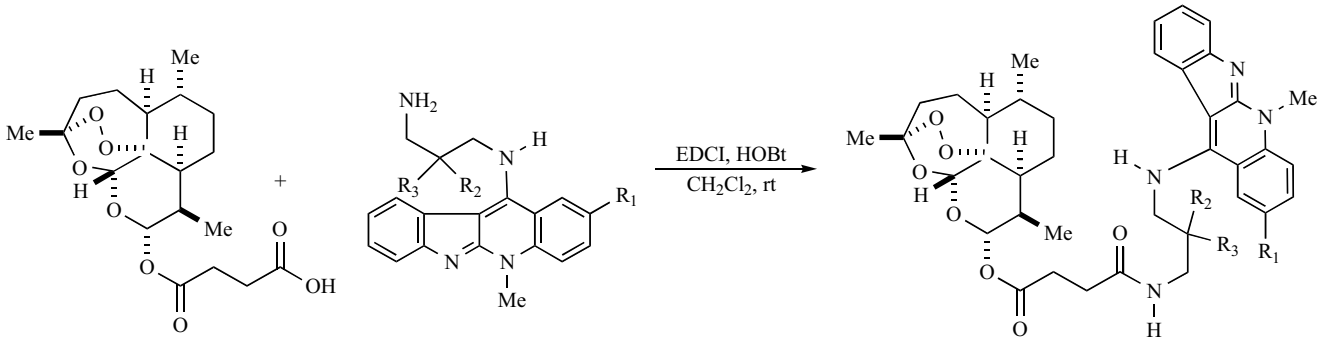


153

Comp. N°	*P. falciparum* NF54, IC_{50} (μM)	Sel. Index (L6/NF54)	Hemozoin Inhibition (%)
150	19.9	164.1	34.1
151	24.8	8.79	48.9
152	8.28	40.7	104.4
134	86	20.2	18.4
153	317	369.5	5.46

Fig. (8). Activity of ureido and thioureido ester derivatives of neocryptolepine and 6-methyl-6*H*-indolo[2,3-*b*]quinolines.

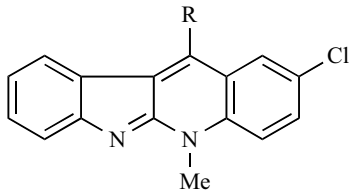
Table 9. Cytotoxicity, antiplasmodial activity against *P. falciparum* and β-hematin inhibition of artesunate-neocryptolepine hybrids.

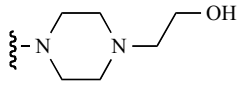
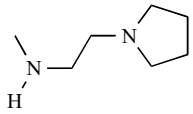
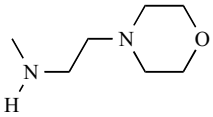
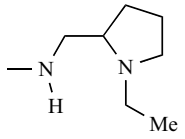
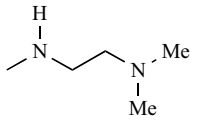
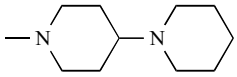


159 **51, 132, 154- 158** **160- 166**

R_1	R_2	R_3	L6 cells IC_{50}, nM	Compound	NF54 IC_{50}, nM	SI L6/NF54	K1 IC_{50}, nM	SI L6/K1	RI K1/NF54	β-Hematin inhibition IC_{50}, μM
H	H	H	1530	**160**	0.45	3399	0.42	3642	0.93	222.5
H	Me	Me	2962	**161**	1.14	2598	0.93	3185	0.82	70.16
Cl	Me	Me	1285	**162**	1.50	856	0.41	3133	0.27	37.78
Br	Me	Me	991	**163**	3.21	3088	0.95	1044	0.30	28.88
CO_2Me	H	H	3718	**164**	2.88	1291	1.00	3718	0.35	29.24
CO_2Me	Me	Me	695	**165**	3.17	219	1.15	604	0.36	25.79
CO_2Me	H	OH	1223	**166**	1.48	826	0.40	3058	0.27	41.66
Artemisinin (comparator)				**130**	4.3	-	2.8	-	-	0.7
Chloroquine (comparator)				**80**	9.4	-	109.5	-	22.3	30-33

Table 10. Molluscicidal activity of substituted neocryptolepines on *Biomphalaria alexandrina* snails.[a]



R	Compound	LC_{50} (ppm)	LC_{90} (ppm)	R	Compound	LC_{50} (ppm)	LC_{90} (ppm)
	**71**	3.9	4.8		**73**	1.74	2.64
	**70**	1.3	2.2		**75**	2.95	4.1
	**167**	1.47	2.12		**74**	2.6	3.5
Niclosamide (comparator)	**169**	0.2	0.6				

[a]Results of the second test (after 24 h) are shown. All compounds exhibited 100% snail mortality at a 5 ppm level (primary test).

to both, the antimalarial and cytotoxic activity, also improving drug selectivity in these cases.

Scattered investigations have also found that neocryptolepine and its analogs have valuable properties against other relevant diseases and conditions. Despite not having the optimum profile, they are antibacterial against Gram positive germs and have some selective antifungal activity, including against *C. albicans* biofilms. Neocryptolepine and its derivatives also behave as antiprotozoal against *Trypanosoma cruzi* and *T. brucei*, as well as against *Leishmania donovani* and *Schistosoma mansoni*, being additionally molluscicidal against *Biomphalaria alexandrina*, which hosts *S. mansoni* in Egypt.

As new research is evolving and its results keep unlocking the secrets of the targeted biomolecules, new and more powerful designed compounds see the light with the aim of finding the perfect one. The successive enhancements of the potency and optimization of the 'drug-like' properties of neocryptolepine as a drug lead, have demonstrated during the last decade that research scientists are following a promising trend. These properties are being taken care at an equal pace as other important design aspects, such as selectivity, host toxicity and the presence of undesirable effects, all of which need to be carefully optimized towards ideality.

It is encouraging to see the amount of significant advances in the Medicinal Chemistry of neocryptolepine that have been accomplished during the past two decades. The currently available analogs and derivatives are still far from being satisfactory; however, since research in the field is progressing at a steady pace and on solid clues, it will not take too long before more useful compounds are developed, and perhaps one of them is approved for marketing.

LIST OF ABBREVIATIONS

ADP = Adenosine Diphosphate

AIDS = Acquired Immunodeficiency Syndrome

CD = Circular Dichroism

DiMIQ = 5,11-Dimethyl 5*H*-indolo[2,3-*b*]quinoline

DNA = Deoxyribonucleic Acid

ELD = Electric Linear Dichroism

ESI-MS = Electrospray Ionization mode Mass Spectrometry

HC_{50} = Hemolytic Concentration for 50% effect

ID_{50} = Inhibition Dose for 50% effect

K_{app} = Apparent binding constant

LC_{50} = Lethal Concentration for 50% effect

m-AMSA = *N*-[4-(Acridin-9-yl)amino])-3-methoxyphenyl]methanesulfonamide

MBC = Minimal Bactericidal Concentration

meq = Miliequivalent(s)

MIC = Minimal Inhibitory Concentration

mM = Micromolar

mM = Milimolar

ND = Not Determined

nM = Nanomolar

NMR = Nuclear Magnetic Resonance

ppm = Parts per Million

RI = Relative Index

SAR = Structure-Activity Relationships

SI = Selectivity Index

UV-vis = Ultraviolet-Visible Spectroscopy

Vs. = Versus

WHO = World Health Organization

ΔT_m = Difference in Denaturating Temperature

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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