A Challenge to Synthesis and Development of Novel Antimalaria and Anticancer Agents of Indole-Quinoline Cores

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Introduction

Malaria is a major cause of morbidity and mortality worldwide, especially in developing countries where it has serious economic and social costs. The disease is present in over 100 countries and threatens half of the world's population. The main reason for the recent dramatic increase in deaths from malaria is attributed to the spread of *Plasmodium falciparum* (*Pf*) strains resistant to the mainstay antimalarial chloroquine (CQ, **A**, Figure 1).[1] To date, an effective therapeutic option for the treatment of resistant malaria is represented by the natural endoperoxide artemisinin and its semisynthetic derivatives, although there are concerns that artemisinin tolerant plasmodia are emerging.[2] As a consequence of drug resistance, the present situation is alarming and new drugs are urgently needed. Plants are still 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 artemisinin-inspired endoperoxides as antimalarial drugs. The importance of artemisinins as antimalarials has encouraged research for other plant derived antiparasitic agents. A promising plant appeared to be *Cryptolepis sanguinolenta*.[3] The roots of this climbing shrub are used in Central and West Africa in traditional medicine for the treatment of malaria. Its main alkaloid, cryptolepine (5-methyl-5*H*-indolo[3,2-*b*]quinoline) (**B**) has been shown to have potent antimalarial potential, both against chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum* (Figure 1). Further experiments indicated that cryptolepine inhibits the formation of *β*-hematin, a mechanism also responsible for the antiplasmodial activity of chloroquine (**A**). Unfortunately, cryptolepine is also a DNA intercalating agent and an inhibitor of topoisomerase II, resulting in a high level of non-specific cytotoxicity.

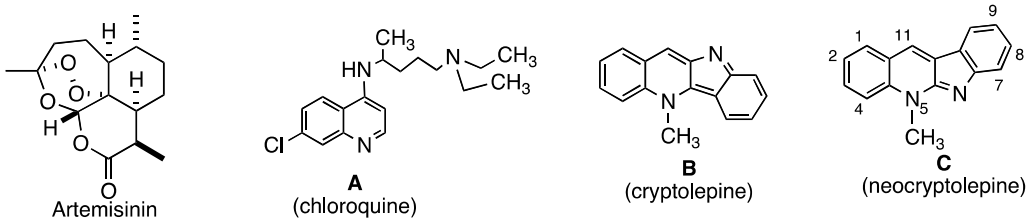


Figure 1. Structures of artemisinin, chloroquine (**A**), cryptolepine (**B**) and its structural isomer neocryptolepine **(C)**.

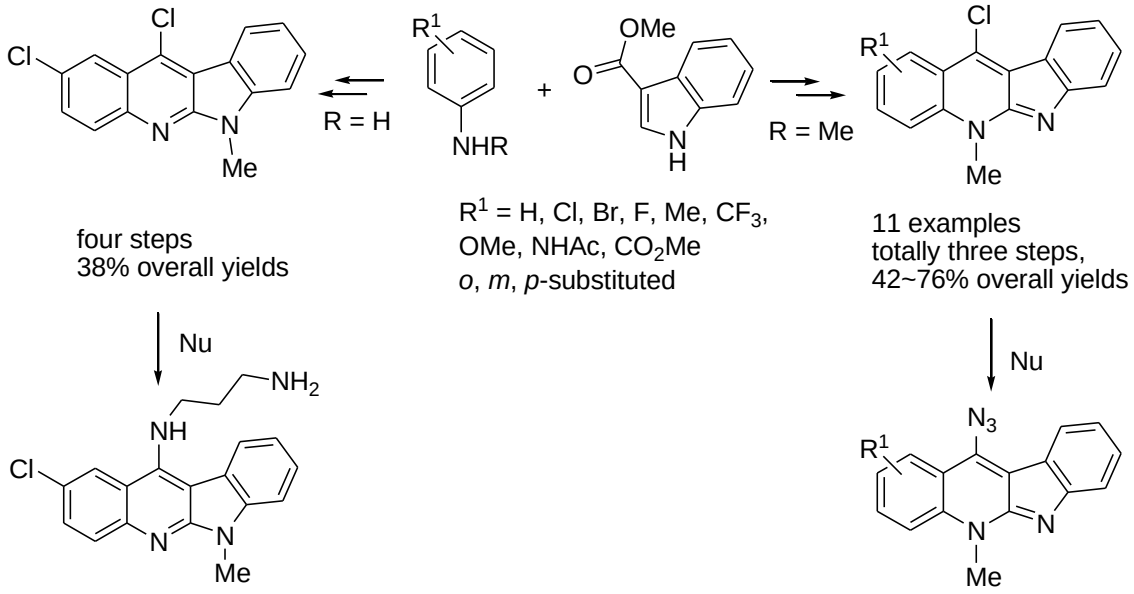
In search for novel antimalarial compounds, we focused on minor alkaloids of *C. sanguinolenta* such as neocryptolepine (5-methyl-5*H*-indolo[2,3-*b*]quinoline, cryptotackieine) (**C**), which also showed antiplasmodial activity against chloroquine-resistant *P. falciparum*. Neocryptolepine appeared to have lower affinity for DNA and topoisomerase II compared to cryptolepine.[3] We tried to reduce the cytotoxicity by introducing halo-substituents in order to reduce the DNA-intercalating properties of the parent compound. In addition we tried to improve the biological activity of the parent compound by substituting it with basic (aminoalkylamino) side chains. A basic side chain is indeed an important feature for the activity of chloroquine and is required for

the accumulation into the food vacuole and, hence, for inhibition of hemozoin formation. We prepared a series of neocryptolepine analogues with chlorosubstituents, an N^1,N^1-diethylpentane-1,4-diamine group (the side chain of chloroquine), various aminoalkylamino groups, and a combination of both, in various positions.

Results and discussion

I. Chemistry

Various 11-chloro-5-methyl-5*H*-indolo[2,3-b]quinolines (neocryptolepines) with different substituents on the quinoline ring, key intermediates for antimalaria agents, are prepared from the substituted *N*-methylanilines, easily accessible by the *N*-methylation of anilines, and indole-3-carboxylate as a counterpart. This protocol is benign in terms of the reduced number of steps to reach the target, compared with the known method using anilines, and easy product purification. Alternatively, their 6-methyl congener is prepared by *N*-methylation of the indole moiety of 2-arylaminoindole-3-carboxylate followed by successive cyclization and chlorination. 11-Chloroneocryptolepines are found more reactive than their 6-methyl congener in the nucleophilic substitution at the C11 position, as depicted in Scheme 1.[9]

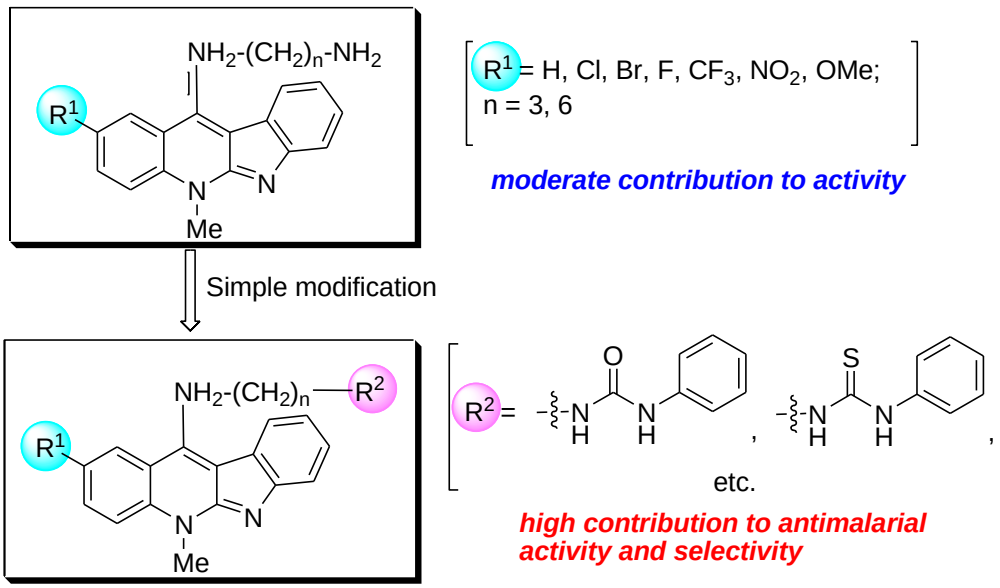


Scheme 1. General synthetic sequence of *2*-substituted neocryptolepine derivatives

II. Antimalarial activity

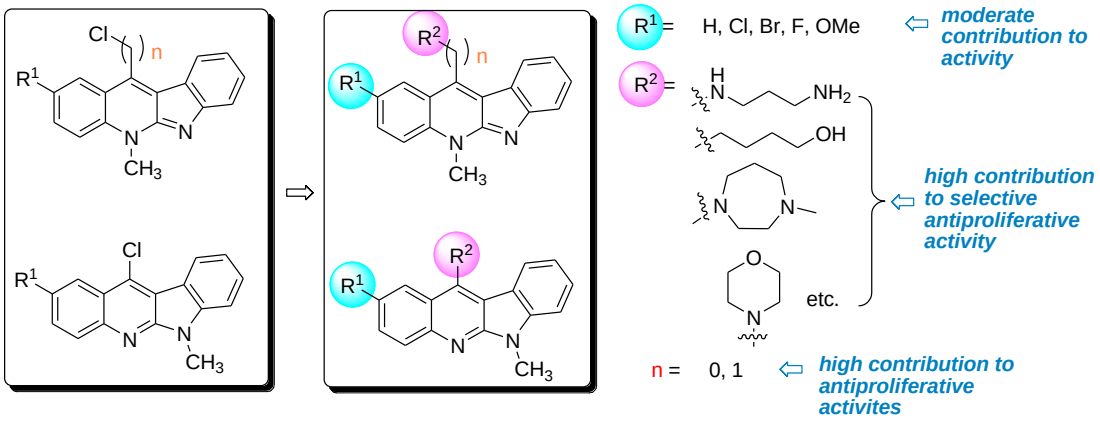
In order to obtain a high antimalarial activity with neocrytolepine derivatives, modifying and changing the side chains at the C11 position with varying the substituents of an electron-withdrawing or electron-donating nature at the C2 position for a SAR study were executed. Installation of alkylamino and □-aminoalkylamino groups at the C11 position of the neocrytolepine core was achieved by the reaction of the 11-chloroneocryptolepines and the appropriate amines. For further variation, the aminoalkylamino subsituents were transformed into the corresponding acyclic or cyclic carbamides or thiocarbamides. These side-chain modified neocryptolepine derivatives were tested for antimalarial activity against CQR (K1) and CQS (NF54) of *Plasmodium falciparum* in vitro. The evaluation also included cytotoxicity toward mammalian L6 cells. In particular, among the tested compounds, the compound **17f**, bearing an additional Cl at the C2 position, showed antimalarial activity 4 times more potent

than chloroquine (CQ) for CQS (NF54) with an IC_{50} of 2.2 nM and a selectivity index of 1410.00, and **17c** without substituents at the C2 position, showed a 22 times greater potency than CQ for CQR (K1) with an IC_{50} of 9.4 nM, a selectivity index of 131.75 and a resistance index of 0.44 by K1/NF54.[4,6]



III. Antiproliferative activity

The synthesis and antiproliferative evaluation of certain 11-aminoalkylamino-substituted 5*H*- and 6*H*-indolo[2,3-*b*]quinolines and their methylated derivatives was examined. These 5-Me- and 6-Me-indolo[2,3-*b*]quinoline derivatives **10**–**14**, **20** were prepared by amination at the C-11 position of the 11-chloro-5-methyl-5*H*- and 11-chloro-6-methyl-6*H*-indolo[2,3-*b*]quinolines with different substituents on the quinoline ring. The 11-aminoalkylaminomethylated **23**, the homologue of **11**, was prepared from the same intermediate for a further SAR study. These intermediates are accessible from 4-substituted anilines or their *N*-methylated analogues and methyl indole-3-carboxylate as a counterpart. The *in vitro* antiproliferative assay indicated that the 5-methylated derivatives **10–14** are more cytotoxic than their respective 6-methylated 6*H*-indolo[2,3-b]quinoline derivatives **20**. Among them, *N*-(3-aminopropyl)-2-bromo-5-methyl-5*H*-indolo[2,3-*b*]quinolin-11-amine **12f** was the most cytotoxic with a mean IC_{50} value of 0.12 μM against human leukemia MV4-11 cell line, and also exhibited selective cytotoxicities against A549 (lung cancer), HCT116 (colon cancer) cell lines and normal fibroblast BALB/3T3 with IC_{50} values of 0.543, 0.274 and 0.869 μM, respectively. The binding constant of products **12f** and **20f** to salmon fish sperm DNA were also evaluated using UV-Vis absorption spectroscopy, indicating intercalation binding with a constant of 2.93×10^5 and 3.28×10^5 $L \cdot mol^{-1}$ respectively.[5,7,8]



Conclusion

A series of chloro- and aminoalkylamino-substituted neocryptolepine (5-methyl-5*H*-indolo[2,3-*b*]quinoline) derivatives were synthesized and evaluated as antimalarial agents. Introduction of aminoalkylamino chains increased the antiplasmodial activity of the neocryptolepine core substantially. The most active compounds showed antiplasmodial activities in the nM range. We have also described the synthesis of a series of 11-amino-substituted 5*H*- and 6*H*-indolo[2,3-*b*]quinolines, whose antiproliferative activities were evaluated using the MV4-11 (human leukemia), A549 (human lung cancer), HCT116 (human colon cancer), and normal mice fibroblast (BALB/3T3) cell lines. A few of the tested compounds showed very high antiproliferative activities against the MV4-11 leukemia cell line (IC_{50}: 0.012–0.450 μM). Some drug candidate compounds showed selective antiproliferative activity against cancer cell lines A549 or HCT116, but have lower cytotoxicities against the normal fibroblast BALB/3T3. These results indicated that the 11-amino group is important for their activity, especially the 3-aminopropylamino group, which could increase the activity against MV4-11 about 67 times compared to its precursor **7**. The antiproliferaive test indicated that the 5-methylated derivatives are usually more cytotoxic than their respective 6-methylated counterparts**.**

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