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Synthesis and antimalarial testing of neocryptolepine analogues: Addition of ester function in SAR study of 2,11-disubstituted indolo [2,3-*b*]quinolines

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ABSTRACT

This report describes the synthesis, and *in vitro* and *in vivo* antimalarial evaluations of certain ester-modified neocryptolepine (5-methyl-5*H*-indolo[2,3-*b*]quinoline) derivatives. The modifications were carried out by introducing ester groups at the C2 and/or C9 position on the neocryptolepine core and the terminal amino group of the 3-aminopropylamine substituents at the C11 position with a urea/thiourea unit. The antiplasmodial activities of our derivative agents against two different strains (CQS: NF54, and CQR: K1) and the cytotoxic activity against normal L6 cells were evaluated. The test results showed that the ester modified neocryptolepine derivatives have higher antiplasmodial activities against both strains and a low cytotoxic activity against normal cells. The best results were achieved by compounds **9c** and **12b** against the NF54 strain with the IC_{50}/SI value as 2.27 nM/361 and 1.81 nM/321, respectively. While against K1 strain, all the tested compounds showed higher activity than the well-known antimalarial drug chloroquine. Furthermore, the compounds were tested for β-haematin inhibition and 12 were found to be more active than chloroquine (IC_{50} = 18 μM). Structure activity relationship studies exposed an interesting linear correlation between polar surface area of the molecule and β-haematin inhibition for this series. *In vivo* testing of compounds **7** and **8a** against NF54 strain on *Plasmodium berghei* female mice showed that the introduction of the ester group increased the antiplasmodial activity of the neocryptolepine core substantially.

1. Introduction

Malaria is a potentially deadly tropical disease caused by parasites of the genus *Plasmodium*, which is transmitted via the bites of infected mosquitoes [1]. According to the World Health Organization (WHO), this disease led to about 216 million malarial infected cases in 2010, and approximately 0.7 million died due to the non-availability of proper treatment, mostly involving children under 5 years old [2]. The *Plasmodium falciparum* species, which is the most virulent and deadly of the malaria parasites, is responsible in more than 90% of the cases. In spite of the intensive efforts to combat malaria, the incidence of malaria has not decreased, especially in the tropical and subtropical areas [3].

As one of the most effective and cheapest therapeutic agents, chloroquine (CQ) has been used for more than half a century as a specific drug for the treatment of malaria patients [4]. The unique endoperoxide structure of artemisinin emerged in the 1970s as the results of efforts to find a new drug from plants [5]. These drugs are still important regarding medical treatment and making efforts to find more active derivatives from the leads are still continued [6,7]. However, the *P. falciparum* strains resistant to CQ are still spreading in the endemic areas [8] and *in vitro* and *in vivo* resistances even against the most recently introduced artemisinin-based combination therapy (ACT) have also been demonstrated as therapy for uncomplicated *P. falciparum* infections [9]. Therefore, the development

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of new chemotherapeutic treatments for this disease is urgently needed.

The isolation of new lead compounds from plants is one of the strategies in the search for new drugs against infectious diseases [10–12]. The present work has been carried out as part of our ongoing program to develop novel antimalarial drugs based on the natural product neocryptolepine isolated from the roots of the climbing shrub *Cryptolepis sanguinolenta* [13] that is used in Central and West Africa in traditional medicine for the treatment of malaria [14].

The indoloquinoline alkaloids [15,16] such as cryptolepine (5-methyl-5*H*-indolo[2,3-*c*]quinoline) (**I**), neocryptolepine (5-methyl-5*H*-indolo[2,3-*b*]quinoline, cryptotackieine) (**II**), isocryptolepine (5-methyl-5*H*-indolo[3,2-*c*]quinoline, cryptosanguinolentine) (**III**), and the non-natural isoneocryptolepine (5-methyl-5*H*-indolo[2,3-*c*]quinoline) (**IV**) were reported to show potent antiplasmodial activity against chloroquine-resistant *P. falciparum* [17], as shown in Fig. 1. Due to the lower affinity of neocryptolepine for DNA and topoisomerase II inhibition compared to cryptolepine and isocryptolepine, the neocryptolepine core has been further used as the lead for developing an antimalarial agent [18].

In 2002, Pieters and the co-authors reported that the 2-halo-substituted neocryptolepines (2-bromo-, 2-chloro-, 2-fluoro-, and 2-iodo) are more active against *P. falciparum* than neocryptolepine and less cytotoxic, the most active and selective compound being 2-bromoneocryptolepine with an IC_{50} against the chloroquine-resistant *P. falciparum* of 4.0 μM while the IC_{50} on the MRC-5 cells is >32 μM. Furthermore, the 2,9-disubstituted derivatives with a cyano group at C9 and various substituents at C2 exhibited an antitrypanosomal activity in the micromole range in the absence of an obvious cytotoxicity [19]. Recently, El-Sayed et al. introduced aminoalkylamino groups at C8 or C11 of neocryptolepine skeleton by varying the substituents at C2, which showed an increased antiplasmodial activity of the neocryptolepine core with the *in vitro* selectivity index >500. The most active compounds bearing 5-methyl and 8-(N^1,N^1-diethylpentane-1,4-diamine) side chains showed an IC_{50} of 10 nM, which has the same potency as chloroquine and a selectivity index of 1800 [20].

In our previous paper describing the SAR study of the 5-methyl-5*H*-indolo[2,3-*b*]quinoline core, we modified and changed the ω-aminoalkylamino side chains at C11 by varying the electron-withdrawing or electron-donating nature of the C2 substituent. The compound bearing (3-(3-phenylureido)propyl)amino at C11 and Cl at C2 showed an 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 1400, and the compound substituted with ((3-phenylthioureido)pentyl)amino at C11 and MeO at C2 showed a much greater potency than CQ for CQR (K1) with an IC_{50} of 2.2 nM, a selectivity index of 1243 and a resistance index of 0.5 by K1/NF54 [21].

However, in spite of the significantly improved antimalarial activities of some compounds against the NF54 strain, testing of the *in vivo* drug screening model against *Plasmodium berghei* in Swiss mice were not satisfactory, as the neocryptolepine derivatives showed only some reduction in parasitaemia on day 4, with activities of 15.4% and 22.1%, respectively. Until now, the SAR studies of neocryptolepines (indolo[2,3-*b*]quinoline) have been conducted by changing the halogen, alkyl, nitro, alkoxy or alkylthio, amino, cyano groups as the substitution at the C2, C8, C9, and C11 positions [15]. Therefore, a search for a more effective group amenable to inducing a higher activity and less toxicity are urgently needed to improve the *in vivo* potency.

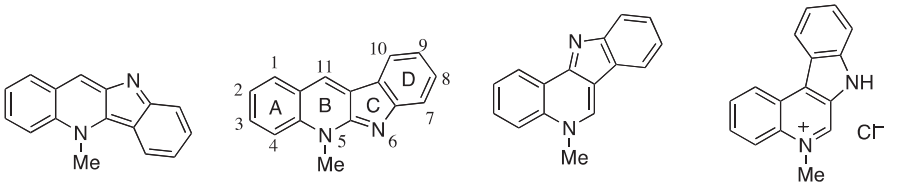
In our previous paper concerning the antiproliferative activity of neocryptolepine derivatives [22], we demonstrated that the introduction of an ester function on the indolo[2,3-*b*]quinoline core improved both the antiproliferative activity against several cell lines and the cytotoxicity index against the usual cell line [23]. A similar effect was also demonstrated on the activity of other plant-derived anticancer agents such as taxol [24], camptothecin [25], bryostatin [26], and 5-fluorouracil [27]. In addition, similar enhancement of the activities is demonstrated in the antiplasmodial and antitrypanosomal activities with bicyclic amides and esters of dialkylamino acids [28]. These improvements in the anticancer and antimalarial activity are believed to be due to increasing both the lipophilicity [29] and bioavailability of the drug by introducing an ester group into the core structure. Encouraged by these significant examples, we examined the introduction of an ester group to the neocryptolepine core and studied its influence on the antiplasmodial activity. In this study, we report the further investigation of the antimalarial potential of the neocryptolepine core. An ester group, which may act as a potential lipophilic site, was introduced at the C-2 position of the A-ring and/or the C-9 position of the D-ring in order to establish or extend the structure activity relationship (SAR) study for these regions.

2. Results and discussion

2.1. Chemistry

The C2- and C9-substituted 11-chloro-5-methyl-5*H*-indolo[2,3-*b*]quinolines **6** were prepared using indole-3,5-dicaboxylate **2b** or 5-bromo-indole-3-carboxylate **2c** as the starting material. According to the method previously described by us [30], *N*-methylanilines were used for the three-step synthesis of **6**. The dimethyl 1*H*-indole-3,5-dicarboxylate (**2b**), a key starting compound, was first prepared by installation of ester group at the C-3 position of methyl 1*H*-indole-5-carboxylate (**1b**) using the reaction with trichloroacetyl chloride in the presence of pyridine, followed by alkaline treatment with KOH at reflux in MeOH.

The obtained 5-substituted methyl indole-3-carboxylate **2** was oxidatively combined with *N*-methylanilines **3** via chlorination with *N*-chlorosuccinimide (NCS) in the presence of 1,4-dimethylpiperazine, giving 2-(methyl(aryl)amino)-1*H*-indole-3,5-dicarboxylate **4**. The heating of **4** at 250 °C in diphenyl ether induced intramolecular acylation at the C-2 position of the aniline core, forming the tetracyclic indolo[2,3-*b*]quinolinone **5**. The treatment of **5** with $POCl_3$ afforded the desired chlorides **6** as a result of dehydrative chlorination. Addition-substitution at the C11 position smoothly proceeded by



Cryptolepine (**I**) Neocryptolepine (**II**) Isocryptolepine (**III**) Isoneocryptolepine (**IV**)

Fig. 1. Structures of indoloquinolines from *Cryptolepis sanguinolenta*.