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Potent and Selective Antiplasmodial Activity of the Cyanobacterial Alkaloid Nostocarboline and its Dimers

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Abstract—The quaternary β-carbolinium alkaloid nostocarboline from the cyanobacterium *Nostoc* 78-12A and ten bis-cationic dimeric derivatives were evaluated against four protozoan parasites and low micromolar values against *Trypanosoma brucei*, submicromolar values against *Leishmania donovani* and low nanomolar values against *Plasmodium falciparum* K1 were determined. Selectivity against rat myoblasts (L6 cells) was found to be up to > 2500 fold.

Malaria remains a public health problem in large areas of the developing world, with 40% of the earth's population living in malaria-endangered areas.[1] As a direct consequence, over 1 million humans die annually of this disease, with the number of clinical cases estimated to be a hundred times higher.[2] These public health problems are accentuated by the rise of malaria cases in yet non-endemic areas, and increasing resistance of *Plasmodium* to current lines of therapy.[3] These facts combined with the absence of a vaccine and the lack of systematic vector control strategies provides the rationale for the development of novel drugs against this disease.[4]

Natural products are extremely successful in providing mankind with substances to combat diseases, and today, roughly 50% of all small molecule drugs on the market addressing infectious diseases are natural products or derivatives thereof.[5] With respect to malaria, quinine and its derivatives are still in use today, and new therapies based on artemisinin were recently introduced in the clinic.[4] Cyanobacteria have been shown to be an important source for novel bioactive natural products, as these organisms face large pressure from grazers or competing organisms requiring them to develop chemical defense strategies.[6] Several cyanobacterial metabolites have been shown to possess antiplasmodial activity: Calothrixins A and B inhibited the FAF6 strain of *Plasmodium falciparum* with IC_{50} values of roughly 60 and 180 nM, albeit with no or very little selectivity against HeLa human tumor cell lines.[7a] Venturamide B displayed a low micromolar value against *Plasmodium falciparum* W2 with a selectivity > 10 against green monkey Vero kidney cells reported.[7b] Symplocamide A displayed an IC_{50} value of 0.95 μM against the same strain, but rather strong cytotoxicity.[7c] We have recently isolated nostocarboline (**1**), an acetyl- and butyryl-cholinesterase, and trypsin inhibitor from *Nostoc* 78-12A.[8] Moreover, this compound class has been shown to possess strong algicidal activity against both eukaryotic and prokaryotic phototrophs.[9] The chemical ecology rationale for evaluating nostocarboline against *Plasmodium* was based on a large body of research demonstrating that *Plasmodium* contains a plastid-like organelle, which was suggested to be a relic of a photoautotrophic (cyanobacterial) endosymbiont.[10] Targeting this apicoplast with algicidal compounds has been suggested as a strategy for effective antiplasmodial

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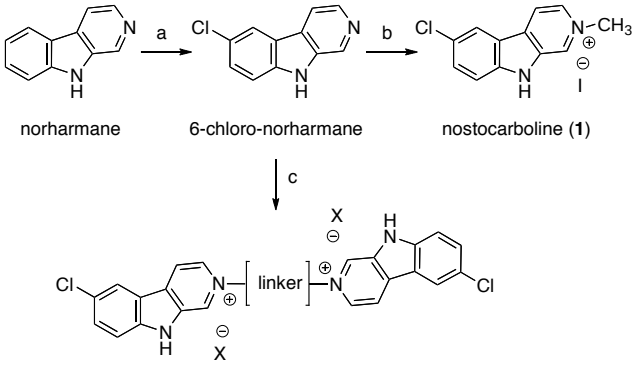
agents.[11] In this communication, we demonstrate that nostocarboline (**1**) inhibits *Plasmodium* with high selectivity in nanomolar concentrations. In addition, we demonstrate that dimerization of nostocarboline leads to very potent and selective antiplasmodial agents. This latter route presents a strategic option for the chemical diversification of natural products[12] and provides an entry into bis-cationic compounds, a class of compounds known for strong antiplasmodial activity.[13]

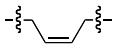
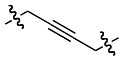
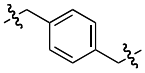
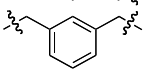
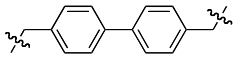
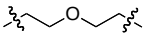
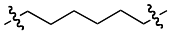
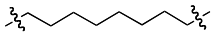
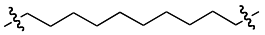
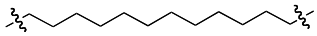
Nostocarboline (**1**) was synthesized according to published procedures starting from norharmane via chlorination at C-6 and methylation (Scheme 1).[8a] 6-Cl-norharmane was also the starting material for the synthesis of nostocarboline homo-dimers, which were prepared using symmetrical dihalogeno-linkers to afford the desired bis-β-carbolinium homo-dimers **2-11** (Table 1). We were able to employ alkenyl, alkynyl, aryl and biaryl, as well as alkyl linkers of various lengths for the corresponding dimers, which were obtained in a two-step process and isolated in good to excellent yields. It should be pointed out that cheap, short and high-yielding syntheses should be regarded as a requirement for successful antimalarial agents, given the prevalence of this disease in developing countries.



Scheme 1. Reagents and conditions: (a) NaOCl, EtOH, 0 °C, 30 min, then r.t., 5 h, 75%; b) CH_3I, *i*-PrOH, reflux, 4 h, 94%; c) dihalogeno-linkers, *i*-PrOH, reflux, 12-48 h, 60-95%.

Nostocarboline (**1**) as well as its dimers **2-11** were evaluated against four parasites, *Trypanosoma brucei rhodesiense* STIB 900, *Trypanosoma cruzi* Tulahuen C2C4, *Leishmania donovani* MHOM-ET-67/L82 axenic amastigotes, and *Plasmodium falciparum* K1 and the corresponding IC_{50} values are given in Table 2. Nostocarboline (**1**) showed a pronounced activity against *Plasmodium* (IC_{50} = 194 nM), while being inactive against the other parasites tested. In addition, nostocarboline (**1**) showed very weak cytotoxicity (> 0.1 mM), giving rise to a 600 fold selectivity of *Plasmodium* over L6 cells. The results for the dimers **2-11** display a consistent pattern of activity: While against *T. brucei* IC_{50} values around 1 μM were determined, the dimers **2-11** were roughly by a factor of 5 to 50 less active against *T. cruzi*. Interesting submicromolar activity against *L. donovani* could be determined for some dimers, in particular those incorporating a long, flexible linker such as **7-11**. In this series, higher activity nicely correlates with longer linkers. Interestingly, the most active compound was the dimer **5** with the *meta* substituted aryl linker (0.2 μM), which was roughly 100 fold more active than dimer **4** with the corresponding *para* substituted linker. This suggests that relative orientation of the two nostocarboline units has an impact on activity against *L. donovani*.

Table 1. Structure of bis-β-carbolinium homodimers **2-11**.

Compound[a]	Linker	Time (h)	Yield (%)
2		12	72
3		12	65
4		12	95
5		12	89
6		12	86
7		24	60
8		48	93
9		48	87
10		48	78
11		48	71

[a] For **2-7**: X=Cl; for **8-11**: X=Br.

The best results were obtained against *Plasmodium falciparum*, where submicromolar IC_{50} values were determined for all dimers **2-11**. In this series and against this parasite, long and flexible linkers are preferred, with the corresponding compounds **6-11** all displaying IC_{50} values below 100 nM. The most active compound **10** containing a $(CH_2)_{10}$ linker displayed an IC_{50} value of 14 nM against *P. falciparum* K1.

We also determined cytotoxicity against the L6 rat myoblast cell line, and the values were generally dispersed in the 5-60 μM range. While the effect of linker length on antiplasmodial activity residues for **7-11** was very small, its effect on cytotoxicity was more pronounced. Clearly, cytotoxicity increased with linker length, thus leading to decreased selectivity for longer linkers. Compounds **7** and **8** with 5 and 6 atom linkers can thus be considered optimal in this series, as *e.g.* **7** displayed high potency (18 nM) and an excellent selectivity of > 2500 fold against the L6 cell line.

These results complement the interesting biological profile of nostocarboline: While this compound strongly

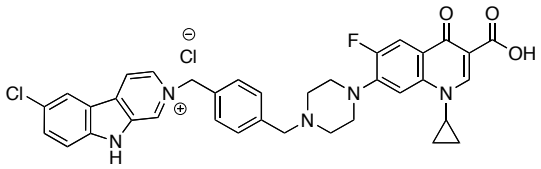
inhibits the growth of cyanobacteria and eukaryotic chlorophytes,[9] it is inactive against bacterial pathogens and fungi,[9] and very weakly toxic against mammalian eukaryotic cells (Table 2) and crustaceans.[8b] At the same time, activity against *Plasmodium* was observed. It is tempting to speculate that this activity might be correlated with the presence of plastids of cyanobacterial origin in *Plasmodium*, where the actual targets of nostocarboline might be present. Whether these targets are related to the known inhibitory action of nostocarboline on hydrolytic enzymes such as esterases and proteases must be validated. It is also unclear at present, whether nostocarboline and its dimers **2-11** act via the same mode of action on *Plasmodium*: The increased cytotoxicity (and decreased selectivity) of some of the dimers suggests a competing non-selective pathway for some dimers (*e.g.* **4** and **5**).

Table 2. Antiparasitic *in vitro* activities of **1-12** (values in μM).

	T. b.[a] IC_{50}	*T. c.*[b] IC_{50}	*L. d.*[c] IC_{50}	*P. f.*[d] IC_{50}	Cytotoxicity[e] IC_{50}	Selectivity *P. f.*[f]
1	70.5	> 87.1	34.3	0.194	120.9	622
2	1.1	> 56.6	9.6	0.113	61.1	540
3	6.4	10.6	34.7	0.738	28.5	39
4	1	> 51.7	19.9	0.223	17.4	78
5	1.2	5.9	0.2	0.121	3.8	32
6	2.5	> 45.7	68.4	0.056	23	408
7	1.2	51.1	8.6	0.018	47.9	2625
8	1.2	36.2	6.6	0.020	36.2	1810
9	0.9	31.4	2.3	0.018	7.5	423
10	1.1	36.6	0.9	0.014	8.2	575
11	1.2	10	0.6	0.023	4.3	186
12	92.5	> 44.6	61.6	> 7.4	121.3	n.d.[g]

[a] *Trypanosoma brucei rhodesiense* STIB 900
[b] *Trypanosoma cruzi* Tulahuen C2C4
[c] *Leishmania donovani* MHOM-ET-67/L82
[d] *Plasmodium falciparum* K1
[e] Rat myoblast L6 cells
[f] The selectivity index is calculated by IC_{50}(L6)/IC_{50}(*P.f.*)
[g] n.d.: not determined

An interesting compound to evaluate the mode of action, as well as potential resistance mechanisms, is the hybrid **12** of nostocarboline and ciprofloxacin (Figure 1).[9] Such dual mode of action hybrids combine the distinct bioactivity of two different fragments leading to synergistic mode of action.[14] We have shown that **12** retains the activity of nostocarboline, with increased properties against some bacteria.[9] Interestingly, against the parasites evaluated, compound **12** was found to be inactive (Table 2).



12

Figure 1. Structure of the quinolone β-carbolinium hybrid **12**.

The results obtained in this study also favorably compare to other carbolinium natural products with antiplasmodial activity, such as *e.g.* normelinonine F,[15a] fascaplysin,[15b] and cryptolepine[15a,15c]. While the activities of these natural products against *Plasmodium falciparum* are comparable, higher selectivity was observed for nostocarboline (**1**). The tetra- and penta-cyclic framework of cryptolepine and fascaplysine appear to increase DNA intercalation properties resulting in increased unspecific cell toxicity[15]. In addition, while natural dimeric cryptolepine alkaloids were inactive against *Plasmodium*,[15c] synthetic nostocarboline dimers such as **7** resulted in significantly higher activity.

In conclusion we have shown that nostocarboline (**1**) and its symetrical homodimers **2-11** are potent and selective inhibitors of *Plasmodium falciparum* and display also interesting activity against *Leishmania donovani*. The compound with the best profile was dimer **7**, with an IC_{50} against *Plasmodium falciparum* of 18 nM and a > 2500 fold selectivity against L6 cells. The benefits of the presented compounds thus include (1) ease of synthesis in two steps from norharmane; (2) potent activity down to 14 nM against *Plasmodium falciparum* and (3) weak cytotoxicity resulting in selectivities of up to > 2500 fold. The elucidation of the mode of action as well as evaluation in animal models is now being carried out in our laboratories.

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