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π-Delocalized β-carbolinium cations as potential antimalarials

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Abstract—Several β-carboline compounds including natural products and their corresponding salts were synthesized and evaluated for antimalarial activity and cytotoxicity levels. Quaternary carbolinium cations showed much higher potencies than neutral β-carbolines and a good correlation was observed between π-delocalized lipophilic cationic structure and antimalarial efficacy.

Malaria, which is caused by plasmodium protozoa, is one of the most serious infectious diseases in tropical and subtropical regions. Plasmodium parasites rapidly develop resistance to new drugs, creating an ongoing need for the development of new antimalarial compounds.[1] Recently we have reported that rhodacyanines, having a π-delocalized lipophilic cationic (DLC) structure, exhibit strong antimalarial efficacies against *Plasmodium falciparum* in vitro.[2] The conceptual term, DLC, was originally proposed by Chen et al. in their anticancer research work.[3] It has subsequently been reported that several DLC compounds exhibit antitumor activity by their selective accumulation in the mitochondria of carcinoma cells.[3,4]

β-Carboline alkaloids are widely found in a number of plant and mammalian species. Some of these alkaloids, such as manzamine A and akagerine (Fig. 1), exhibit a variety of biological and pharmaceutical properties, including antimalarial activity.[5] Pavanand et al. isolated the simple β-carboline, 4-methoxy-1-vinyl-β-carboline; (MVC; Fig. 1, **1a**)[6] and related compounds as antimalarial components from *Picrasma javanica* B1, a medicinal plant used in the treatment of malaria.[7] However, their antimalarial potency is not so strong as alternative compounds that are in current clinical use. We envisaged that β-carbolines could be transformed into DLC's via quaternarization of the pyridine nitrogen atom and that the resulting β-carbolinium salts may have higher antimalarial properties than neutral carbolines. In this communication we report the synthesis of β-carboline compounds and their quaternary salts, and we evaluate their antimalarial and cytotoxic activity.

MVC (**1a**) was synthesized according to Cook's procedure for the synthesis of crenatine (**1b**) with some modifications (Scheme 1).[8] This involved a Pictet–Spengler reaction of tryptamine hydrochloride (**2**) with ethyl glyoxalate in ethanol, followed by acylation with acetyl chloride. This furnished tetrahydro-β-carboline (**3**) with a 44% yield (2 steps). Treatment of tetrahydro-β-carboline (**3**) with 5 equivalents of DDQ produced 4-oxocarboline (**4**), which proved difficult to isolate owing to its instability, although the TLC profile of the reaction

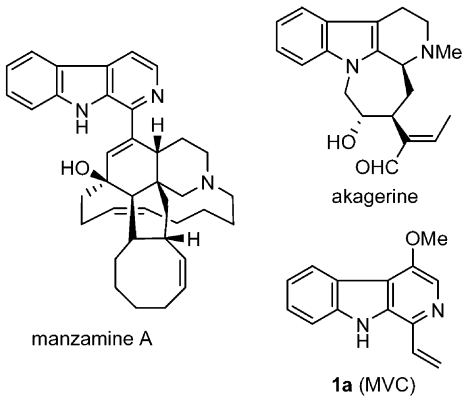

Figure 1. β-Carbolines having antimalarial activity.

Keywords: Antimalarials; DLC's; Total synthesis; β-Carbolines; Selective toxicity.
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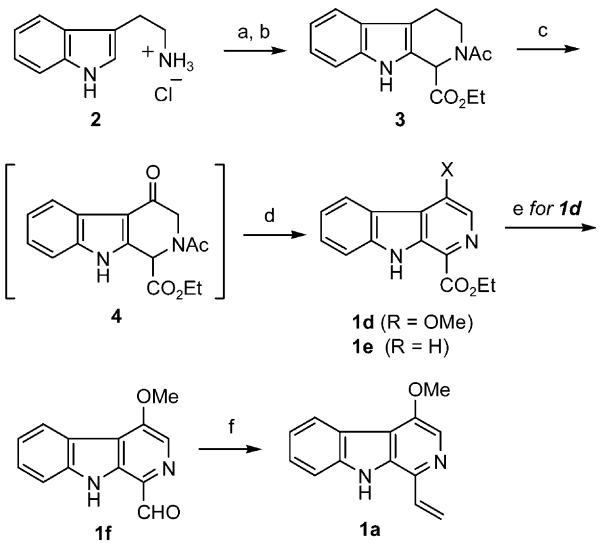


Scheme 1. Conditions: (a) EtO_2CCHO, EtOH; (b) AcCl, *cat*. DMAP, Et_3N, CH_2Cl_2 (44% for 2 steps); (c) DDQ, THF–H_2O, −78 °C to rt; (d) $Me_2C(OMe)_2$, *p*-TsOH, benzene; then *p*-chloranil, rt (**1d**: 30%, **1e**: 7% for 2 steps); (e) DIBALH, CH_2Cl_2 (96%); (f) $Ph_3P{=}CH_2$, THF (51%).

mixture indicated an almost pure single spot with no remaining starting material. It is known that treatment of tetrahydro-β-carbolines with DDQ promotes C1- and C4-oxidation in a competitive reaction.[8] In this case, the introduction of an electron-withdrawing group as a C-1 substituent may electrochemically inhibit C-1 oxidation. Crude product **4** was used in the subsequent reaction. The reaction of product **4** with dimethoxypropane in the presence of PTSA under azeotropic conditions, followed by oxidative aromatization with *p*-chloranil,[9] produced 1-ethoxycarbonyl-4-methoxy-β-carboline (**1d**) along with demethoxy compound (**1e**; kumujian A)[10] with yields of 30% and 7% (2 steps), respectively. Because the starting material was completely consumed in the reaction of DDQ-oxidation of **3**, the production of **1e** was caused by the successive dehydroxylation–aromatization of **4**. Ester **1d** was reduced by DIBAL-H at −78 °C to produce aldehyde **1f** (kumujancine) with a yield of 96%; Kumujancine (**1f**) is a natural β-carboline isolated from the wood of *Picrasma quassioide*.[11] Finally, Wittig olefination of **1f** afforded **1a** (51% yield). It is noteworthy that we achieved the first total synthesis of the two natural products, MVC (**1a**) and kumujancine (**1f**). Crenatine (**1b**) and the synthetic compound 4-methoxy-1-methyl-β-carboline (**1c**) were also synthesized according to the above procedure.[8] Harmane (**1g**) and harmine (**1i**) were obtained commercially, and compounds **1h** and **1j** were synthesized according to standard procedures (Fig. 2). β-Carbolinium cations (**5**), which correspond to DLC's, were prepared from the corresponding carbolines **1** by simple quaternarization with either alkyl tosylate or alkylhalide, resulting in good yields. Dihydro-β-carbolinium cation **7**, which lacks one conjugation in the aromatic system (thus, non-DLC), was prepared from **6**. *N*-Methyl neutral analogue **8** was quantitatively obtained by the treatment of carbolinium salt **5g** with aqueous NaOH.

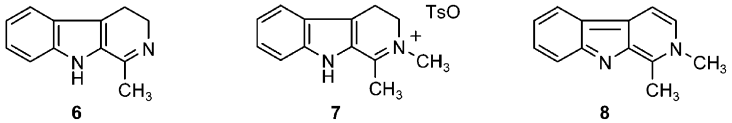
The antimalarial potencies of the β-carbolines, their corresponding salts and related compounds were evaluated in vitro against *P. falciparum* (the antimalarial drug sensitive FCR-3 strain) and their cytotoxicities were determined using mouse mammary tumor FM3A cells. Selective toxicities, defined by the ratio EC_{50} (FM3A)/EC_{50} (*P. falciparum*), were determined.[12] The biological results are summarized in Table 1. Neutral β-carbolines **1a**, **c**, **i** and **j** display weak to medium inhibitory effects against *P. falciparum* (EC_{50} = 0.5 to 3.1×10^{-5} M), and their cytotoxicities are comparable to their antimalarial activity levels (runs 1–4). In contrast, *N*-alkyl carbolinium salts **5** display considerably enhanced antimalarial activities (runs 5–14) except for

1 (β-carbolines)

5 (β-carbolinium salts)

compound	R^1	R^2	R^3	R^4
1a (MVC)	H	OCH_3	$CH{=}CH_2$	H
1b (crenatine)	H	OCH_3	C_2H_5	H
1c	H	OCH_3	CH_3	H
1d	H	OCH_3	$CO_2C_2H_5$	H
1e (kumujian A)	H	H	$CO_2C_2H_5$	H
1f (kumujancine)	H	OCH_3	CHO	H
1g (harmane)	H	H	CH_3	H
1h	H	H	C_6H_5	H
1i (harmine)	OCH_3	H	CH_3	H
1j	OCH_3	H	CH_3	CH_3

compound	R^1	R^2	R^3	R^4	R^5	X^-
5a	H	OCH_3	$CH{=}CH_2$	H	CH_3	TsO^-
5a'	H	OCH_3	$CH{=}CH_2$	H	C_2H_5	TsO^-
5b	H	OCH_3	C_2H_5	H	CH_3	I^-
5c	H	OCH_3	CH_3	H	CH_3	I^-
5d	H	OCH_3	$CO_2C_2H_5$	H	CH_3	TsO^-
5g	H	H	CH_3	H	CH_3	TsO^-
5h	H	H	C_6H_5	H	CH_3	TsO^-
5i	OCH_3	H	CH_3	H	CH_3	TsO^-
5j	OCH_3	H	CH_3	CH_3	CH_3	TsO^-
5j'	OCH_3	H	CH_3	CH_3	C_2H_4OH	Br^-



6 **7** **8**

Figure 2. β-Carbolines and β-carbolinium salts.

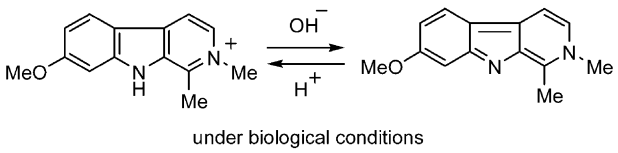
Table 1. Antimalarial activity and cytotoxicity

Run	Compd	EC_{50}		Selective toxicity[c]
		P. falciparum[a]	FM3A[b]	
1	**1a**	5.0×10^{-6}	3.8×10^{-6}	0.76
2	**1c**	2.2×10^{-5}	1.8×10^{-5}	0.82
3	**1i**	2.2×10^{-5}	1.8×10^{-5}	0.82
4	**1j**	3.1×10^{-5}	3.2×10^{-5}	1.0
5	**5a**	1.1×10^{-6}	8.4×10^{-6}	7.6
6	**5a′**	1.3×10^{-7}	1.0×10^{-5}	77
7	**5b**	9.5×10^{-7}	$>3.0\times10^{-5}$ [d]	>32
8	**5c**	3.7×10^{-7}	3.0×10^{-5}	81
9	**5d**	1.9×10^{-5}	2.2×10^{-5}	1.2
10	**5g**	2.0×10^{-6}	3.5×10^{-5}	18
11	**5h**	1.3×10^{-5}	$>2.3\times10^{-5}$ [e]	>1.8
12	**5i**	1.1×10^{-6}	1.9×10^{-5}	17
13	**5j**	4.6×10^{-7}	2.2×10^{-5}	48
14	**5j′**	1.1×10^{-6}	$>7.1\times10^{-5}$ [f]	>65
15	**6**	1.8×10^{-5}	4.5×10^{-5}	2.1
16	**7**	1.6×10^{-5}	$>3.8\times10^{-5}$ [g]	>2.4
17	**8**	4.8×10^{-6}	2.9×10^{-5}	6.0
18	Quinine	1.1×10^{-7}	1.0×10^{-4}	910

[a] Chloroquine sensitive strain (FCR-3).
[b] Mouse mammary tumor FM3A cells representing a model of host.
[c] Selective toxicity = EC_{50} value for FM3A/EC_{50} for *P. falciparum*.
[d] EC_{37} value (63% growth of FM3A was observed).
[e] EC_{10} value (90% growth).
[f] EC_{14} value (86% growth).
[g] EC_{16} value (84% growth).

5d and **h**. Introduction of a methyl or ethyl group on the pyridine nitrogen atom of **1a** results in a 5-fold and a 39-fold increase in antimalarial activity, respectively (**1a** versus **5a** or **5a′**). In addition, the cytotoxicity levels of these substances are decreased 2–3-fold by quarternarization. A similar enhancement of antimalarial effectiveness was observed by the transformation of carboline **1** into carbolinium salts **5** (**1c** versus **5c**, **1i** versus **5i**, and **1j** versus **5j** or **5j′**). In particular, **5j** shows a 67-fold increase in antimalarial activity compared to the corresponding **1j**. These results indicate that DLC compounds have increased antimalarial potency within the class of β-carbolines. In contrast, quarternarization of dihydro-β-carboline has no effect on its biological activity; both the neutral molecule **6** and cationic salt **7** display similarly low antimalarial activities (runs 15 and 16). The neutral *N*-methylharmane analogue **8** is less active than the corresponding cation **5g** (run 17). Accordingly, this further indicates that a broad delocalized system and the cationic charge of the compound, that is a DLC structure, are critical factors in the biological properties of this family of compounds.[13]

A preliminary structure–activity relationship analysis concerning R^1–R^5 substituents was also undertaken.[14] The introduction of a methoxyl group as an R^1 or R^2 substituent causes a 2-fold (**5g** versus **5i**) or 5-fold (**5g** versus **5c**) enhancement in activity against *P. falciparum*, whereas the cytotoxicities of these substances are decreased by these substitutions. In contrast, substitution of R^3 with phenyl or ethoxycarbonyl groups results in a remarkable decrease in antimalarial activity (runs 9 and 11). The introduction of a substituent at the indole-NH by a methyl group causes an increase in activity (**5i** versus **5j**), and this protection might inhibit conversion into an electronically neutral molecule under biological conditions (Scheme 2). Replacement of methyl group as an R^5 substituent by an ethyl or hydroxyethyl group results in both an increase in antimalarial potency (**5a** versus **5a′**) and a decrease in cytotoxicity (**5j** versus **5j′**), respectively. Among our tested compounds, 2-ethyl-4-methoxy-1-vinyl-β-carbolinium tosylate (**5a′**) was found to display good antimalarial efficacy. Its selective toxicity is less than that of quinine, which is an antimalarial medicine in current clinical use, but its antimalarial activity against *P. falciparum* is comparable. It is additionally noteworthy that **5c** is effective even against multidrug resistant parasites (*P. falciparum* K1 strain) with an EC_{50} value of 3.6×10^{-7} M.



Scheme 2.

In summary, we have synthesized β-carbolines and their corresponding salts, including several naturally occurring compounds, and evaluated them for their antimalarial potency in vitro. The β-carbolinium salts, which have a DLC (π-delocalized lipophilic cationic) structure, show a higher antimalarial potency and better selective toxicity than non-DLC compounds. Thus, this study indicates that transformation of specific compounds into a DLC structure may prove to be a highly effective modification methodology in antimalarial medicinal chemistry. Currently, we are carrying out further structural optimization of β-carbolinium salts based on structure–activity relationships uncovered thus far.

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