Synthesis and Evaluation of β-Carbolinium Cations as New Antimalarial Agents Based on π-Delocalized Lipophilic Cation (DLC) Hypothesis

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Several β-carbolines including naturally occurring substances and their corresponding cationic derivatives were synthesized and evaluated for antimalarial (antiplasmodial) activity *in vitro* and *in vivo*. A tetracyclic carbolinium salt was elucidated for antileishmanial and antitrypanosomal activities *in vitro* as well as antiplasmodial activity. Quarternary carbolinium cations showed much higher potencies *in vitro* than electronically neutral β-carbolines and a good correlation was observed between π-delocalized lipophilic cationic (DLC) structure and antimalarial efficacy. β-Carbolinium compounds exhibit medium suppressive activity *in vivo* against rodent malaria.

Key words antimalarial agent; β-carbolinium salt; total synthesis; π-delocalized lipophilic cationic (DLC) hypothesis; tropical disease; structure–activity relationship

Malaria, which is caused by plasmodium protozoa, is one of the most serious infectious disease as well as HIV and tuberculosis. The disease exists in 100 countries but is mainly confined to tropical areas of Africa, Asia and South America. More than one million people, most occurring in infants and young children, die annually from malaria, and disease burden is estimated at 42 million DALYs (disable adjusted life years).[1)] Another tropical parasitic diseases, such as leishmaniasis (Kara azar, oriental sore), African trypanosomasis (African sleeping sickness) and American trypanosomasis (Chagas disease), which are called as "neglected diseases", also prevent social and economical development in those countries.[2)] Despite of the tremendous toll, effective treatments and prevention for the diseases are quite difficult; no vaccine is available in clinically use: limited chemotherapeutics, even including some drawbacks, are available: parasites develop resistance against clinically used drug: and so on.[3)] So that, development of new class of antiprotozoal including antimalarial agents are required and the efforts are steadily increasing in non-profit institutions.[4)]

Recently we have reported the rhodacyanines, having a π-delocalized lipophilic cationic (DLC) structure, exhibit *in vitro* strong antiplasmodial and antileishmanial activities against *Plasmodium falciparum*[5,6)] and against *Leishmania major*,[7)] respectively, with low cytotoxicity against mammalian cell. The conceptual term, DLC, was originally proposed by Chen in their anticancer research work.[8)] It has subsequently been reported several DLC compounds exhibit selective antitumor activity by their selective accumulation in the mitochondria of carcinoma cells.[9—11)] Namely, lipophilic cations possessing a delocalized positive charge (DLCs) penetrate the hydrophobic barriers of the plasma and mitochondria membrane in response to the negative inside transmembrane potentials. The higher mitochondrial membrane potentials of carcinoma cells compared to normal cells induce the selective transfer of DLCs into carcinoma mitochondria. Since most DLCs are toxic to mitochondria at high concentrations, their selective accumulation in carcinoma mitochondria and consequent mitochondrial toxicity provide a basis for selective carcinoma cell killing. It has been reported by Vaidya *et al.* that collapse of mitochondrial membrane potential in a malarial parasites is observed by the treatment with antimalarial drugs.[12)] Consequently, we envisaged that DLCs would be a new candidate for the chemotherapeutics of malaria as well as another neglected diseases.

β-Carboline alkaloids are widely found in a number of plants and mammalian species. Some of these alkaloids, such as manzamine A, 10-hydroxycanthin-6-one and akagerine (Chart 1), exhibit a variety of biological and pharmaceutical potencies including antiplasmodial activity.[13—15)] Pavanand *et al.* isolated the simple β-carboline, 4-methoxy-1-vinyl-β-carboline; (MVC, **1a**; Chart 1)[16)] and related compounds as antiplasmodial components from *Picrasma javanica* B1, a medicinal plant used for the treatment of malaria.[17)] However their antiplasmodial activities are not so strong as alternatives for a clinical use. We considered that β-carbolines could be easily transformed into DLCs by means of quarternarization of the pyridine nitrogen atom and the resulting β-carbolinium salts may have higher antiplasmodial properties than neutral carbolines. Herein, we report the synthesis of β-carboline compounds and their quarternary salts, and we evaluate their *in vitro* antiprotozoal activities and cell cytotoxicity. Moreover, antimalarial efficacy *in vivo* was evaluated using malaria-infected mice.[18)]

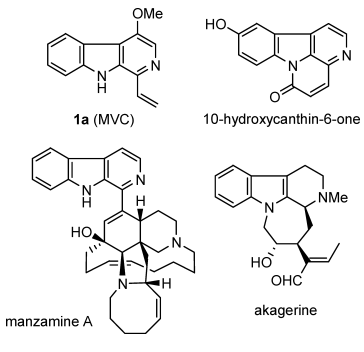

Chart 1

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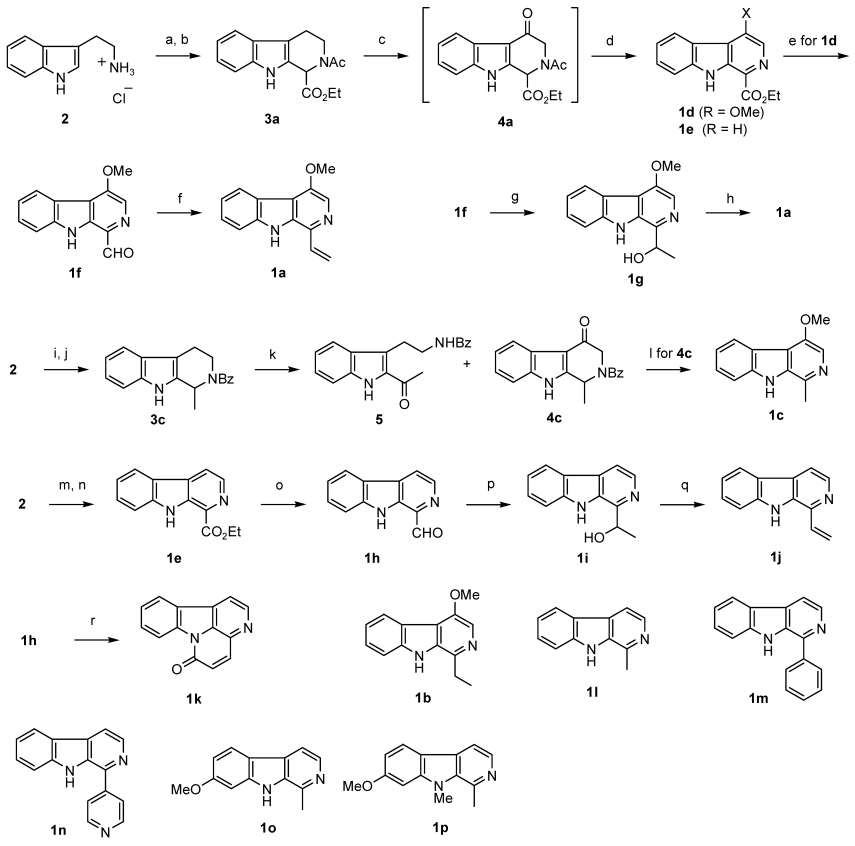
Results and Discussion

Synthesis of β-Carbolines and β-Carbolinium Salts MVC (**1a**) was synthesized according as Cook's procedure for the synthesis of crenatine (**1b**)[19] with some modifications (Chart 2).[20,21] Namely, Pictet-Spengler reaction of tryptamine hydrochloride (**2**) with ethyl glyoxylate in ethanol, followed by acylation with acetyl chloride, furnished tetrahydro-β-carboline **3a** in 44% yield (2 steps). Treatment of **3a** with 5 equivalent of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) led to 4-oxocarboline **4a**, which was difficult to be isolated owing to its instability, although the TLC profile of the reaction mixture indicated nearly single spot and no remaining of the starting material **3a**. It is known that treatment of tetrahydro-β-carbolines with DDQ promote C1- and C4- oxidation competitively.[20] In this case, the introduction of electron-withdrawing group as a C-1 substituent might inhibit C-1 oxidation owing to the electrochemical reason (see below; for the synthesis of **1c**). The crude product **4a** was used in the following reaction without further purification. The reaction of **4a** with dimethoxypropane in the presence of *p*-TsOH under azeotropic conditions, followed by oxidative aromatization with *p*-chloranil,[22] furnished 1-ethoxycarbonyl-4-methoxy-β-carboline (**1d**) along with demethoxy compound (**1e**; kumujian A)[23] in 30% and 7% yield (2 steps), respectively. Ester **1d** was reduced by diisobutylaluminum hydride (DIBAL-H) at −78 °C to produce aldehyde **1f** (kumujancine) in 96% yield; Kumujancine is a natural β-carboline isolated from the wood of *Picrasma quassioide*.[24] Finally, Wittig olefination of **1f** afforded **1a** in 51% yield. It is noteworthy that we first achieved the total synthesis of the two natural products, MVC (**1a**) and kumujancine (**1f**). Transformation of **1f** into **1a** was also achieved by stepwise synthesis *via* alcohol **1g** by methylation, followed by dehydration.

Synthesis of unnatural 4-methoxy-1-methyl-β-carboline (**1c**) was started from tryptamine hydrochloride (**2**) and aqueous acetaldehyde. *N*-Benzoyl-tetrahydro-β-carboline **3c** was synthesized by Pictet-Spengler reaction, followed by benzoylation. Oxidation of **3c** with DDQ afforded desired 4-oxotetrahydrocarboline **4c** and 2-acetylindole **5** in 43% and 20% yield, respectively. Compound **4c** was isolable by silica gel chromatography and showed better stability than **4a**. Byproduct **5** would be produced by oxidation of **3c** at C-1 position and subsequent hydration. Oxidation of 1-methyltetrahydrocarboline **3c** would occur competitively at C-1 and C-4 position because of the small electrochemical difference. Further oxidation of **4c** by *p*-chloranil, followed by methylation, gave **1c**. 4-Unsubstituted analogs **1e** and **1h**—**j** were prepared by the similar procedure as the synthesis of **1a**. Pictet-Spengler reaction of tryptamine hydrochloride (**2**) with ethyl glyoxylate, followed by direct oxidative aromatization by Pd/C catalyst, yielded kumujian A (**1e**). Kumujian C (**1h**),[23] alcohol



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) for **1d**, DIBALH, CH_2Cl_2, –50 °C (96%); (f) $Ph_3P{=}CH_2$, THF (51%); (g) MeLi, CH_2Cl_2 (95%); (h) MsCl, NEt_3, CH_2Cl_2 (19%); (i) MeCHO aq., cat. H_2SO_4, 100 °C; (j) BzCl, pyridine, benzene (81% for 2 steps); (k) DDQ, THF–H_2O, –78 °C to rt (**4c**: 43%); (l) for **4c**, $Me_2C(OMe)_2$, *p*-TsOH, benzene; then *p*-chloranil, rt (43%); (m) EtO_2CCHO, EtOH; (n) Pd/C, *p*-xylene, 140 °C (41% for 2 steps); (o) DIBALH, CH_2Cl_2, –50 °C (89%); (p) MeLi, CH_2Cl_2 (79%); (q) MsCl, NEt_3, CH_2Cl_2 (18%); (r) NaH, AcCl (31%).

Chart 2

Table 1. β-Carbolinium Salts **6**

Run	Carbolinium	Carboline	R^1	R^2	R^3	R^4	R^5	X	Yield (%)
1	**6aa**	**1a**	H	OCH_3	$CH=CH_2$	H	CH_3	TsO	60
2	**6ab**	**1a**	H	OCH_3	$CH=CH_2$	H	C_2H_5	TsO	30
3	**6b**	**1b**	H	OCH_3	C_2H_5	H	CH_3	I	74
4	**6ca**	**1c**	H	OCH_3	CH_3	H	CH_3	I	68
5	**6cb**	**1c**	H	OCH_3	CH_3	H	CH_3	TsO	75
6	**6cc**	**1c**	H	OCH_3	CH_3	H	CH_3	Cl	88
7	**6cd**	**1c**	H	OCH_3	CH_3	H	C_2H_5	TsO	94
8	**6ce**	**1c**	H	OCH_3	CH_3	H	C_2H_5	Cl	77
9	**6cf**	**1c**	H	OCH_3	CH_3	H	$(CH_2)_2OH$	Cl	19
10	**6cg**	**1c**	H	OCH_3	CH_3	H	$(CH_2)_2OCH_3$	Cl	76
11	**6ch**	**1c**	H	OCH_3	CH_3	H	$[(CH_2)_2O]_2CH_3$	Cl	92
12	**6ci**	**1c**	H	OCH_3	CH_3	H	$[(CH_2)_2O]_3CH_3$	Cl	12
13	**6cj**	**1c**	H	OCH_3	CH_3	CH_3	C_2H_5	Cl	76
14	**6d**	**1d**	H	OCH_3	$CO_2C_2H_5$	H	CH_3	TsO	72
15	**6e**	**1e**	H	H	$CO_2C_2H_5$	H	CH_3	TsO	72
16	**6i**	**1i**	H	H	$CH(OH)CH_3$	H	CH_3	TsO	72
17	**6k**	**1k**	H	H	–CH=CHC(=O)–		CH_3	TsO	41
18	**6l**	**1l**	H	H	CH_3	H	CH_3	TsO	87
19	**6m**	**1m**	H	H	C_6H_5	H	CH_3	TsO	78
20	**6o**	**1o**	OCH_3	H	CH_3	H	CH_3	TsO	91
21	**6pa**	**1p**	OCH_3	H	CH_3	CH_3	CH_3	TsO	45
22	**6pb**	**1p**	OCH_3	H	CH_3	CH_3	$(CH_2)_2OH$	Br	38

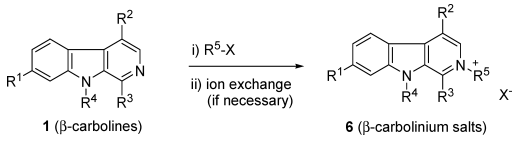


Chart 3

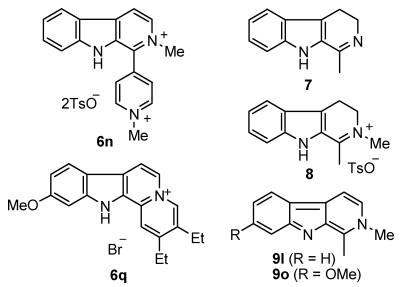


Chart 4

1i,[25] and pavettine (**1j**),[26] which all are naturally occurring products, were prepared in due course. Canthin-6-one (**1k**)[27] was synthesized from aldehyde **1h** in a single operation.[28] Thus, acetylation and consequent intramolecular aldol condensation occurred by the reaction of **1h** with acetyl chloride in the presence of triethylamine. Harmane (**1l**) and harmine (**1o**) were purchased, and crenatine (**1b**), **1m**, **1n** and **1p** were synthesized according as known procedures.[20,21,29]

β-Carbolinium salts **6**, which correspond to DLCs, were synthesized from the corresponding β-carbolines **1** by the simple quarternarization with alkyl tosylate or alkylhalide (Chart 3). Preparation of chloride salts **6cc** and **6ce**—**cj** was achieved by ion exchange operation from the corresponding tosylate or iodide salts (Table 1).[30] Dicationic salt **6n** was prepared from **1n** by the treatment of excess amount of methyl tosylate, and tetracyclic compound **6q** was synthesized from harmine (**1o**) according as the reported procedure (Chart 4).[31,32] Dihydro-β-carbolinium salt **8**, which lacks of one conjugation in the aromatic system (thus, non-DLC), was prepared from **7**. *N*-Methyl deprotonated analogs **9l** and **9o** were quantitatively obtained by the treatment of the corresponding β-carbolinium salts **6l** and **6o**, respectively, with aqueous NaOH.[33]

Evaluation of Antiplasmodial Activity *in Vitro* The antiplasmodial potencies of the β-carbolines **1**, β-carbolinium salts **6** and related compounds **7**—**9** were evaluated *in vitro* against *P. falciparum* (chloroquine 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. The biological results are summarized in Table 2. Electronically neutral β-carbolines **1a**—**c**, **1o** and **1p** displayed weak to medium inhibitory effects against *P. falciparum* (EC_{50}=0.5—3.1×10^{-5} M), and their cytotoxicities were comparable to their antiplasmodial activity levels (runs 1—5). In contrast, *N*-alkyl carbolinium salts **6** displayed considerably enhanced antiplasmodial activities (runs 6—23) except for **6d**, **6m** and **6n**. Introduction of a methyl and ethyl group on the pyridine nitrogen atom of **1a** resulted in a 5-fold and a 39-fold increase in antiplasmodial activity, respectively (**1a** *versus* **6aa** or **6ab**). In addition, the cytotoxicity levels of these substances were decreased 2—3-fold by quarternarization. A similar enhancement of antiplasmodial effectiveness was observed by the transformation of carbolines **1** into the corresponding carbolinium salts **6** (**1b** *versus* **6b**, **1c** *versus* **6c**, **1o** *versus* **6o**, and **1p** *versus* **6pa** or **6pb**). In particular, **6pa** showed a 67-fold increase in an antiplasmodial activity compared to the corresponding **1p**. These results indicate that DLC compounds have increased antiplasmodial potency within the class of β-carbolines by simple transformation. In contrast, quarternarization of dihydro-β-carboline had no effect in biological activity; both the electronically neutral molecule **7** and cationic salt **8** displayed similarly low antiplasmodial activities (runs 24, 25). Deprotonated analogs **9** having no charge were less active than the corresponding cations

6 (runs 26 *versus* 18, and 27 *versus* 21). On the other hand, dicationic salt **6n** showed no inhibitory effect against *P. falciparum* even in high concentration ($>2\times10^{-6}$ M) although its cytotoxicity level was pretty high (run 20). Accordingly, these findings further indicate that a broad delocalized system and the cationic charge on the delocalized system, that is a DLC structure, are critical factors in the biological properties of this family of compounds.[34—37)]

A structure–activity relationship (SAR) analysis concerning R^1—R^5 substituents was also undertaken. The introduction of a methoxyl group as an R^1 or R^2 substituent caused a 2-fold (**6l** *versus* **6o**) or 3-fold (**6l** *versus* **6cb**) enhancement in activity against *P. falciparum*, whereas the cytotoxicities of these substances were decreased by these substitutions. In contrast, substitution of R^3 with phenyl or ethoxycarbonyl groups resulted in a remarkable decrease in antiplasmodial activity (runs 14, 19), and introduction of an α-hydroxyethyl substituent lead slightly loss in the activity (run 16). The introduction of a substituent at the indole-NH by a methyl group caused an increase in activity (**6o** *versus* **6pa**). The protection of indole nitrogen atom might inhibit conversion into an electronically neutral molecule under cellular conditions (Chart 5).[38)] Probably, according as the same reason, tetracyclic canthin-6-one analog **6k** displayed good antiplasmodial property. Replacement of methyl group as an R^5 substituent by an ethyl group resulted in a 10-fold increase in antiplasmodial potency for **6ab** (*versus* **6aa**), but a 2-fold decrease in activity was observed for **6ce** (*versus* **6cc**). β-Hydroxyethyl substituent for R^5 group also lead decrease in activity (**6pa** *versus* **6pb**). It was observed that counter anion (X^-) affected antiplasmodial properties to some extent (runs 9, 11, 12). The difference might be caused by the solubility of the substances in aqueous media. Among our tested compounds, 2-ethyl-4-methoxy-1-vinyl-β-carbolinium *p*-toluenesulfonate (**6ab**) was found to display good antiplasmodial efficacy. Its selective toxicity was less than that of quinine, which is an antimalarial medicine in current clinical use, but its antiplasmodial activity against *P. falciparum* is comparable. It is additionally noteworthy that **6ca** is effective even against chloroquine resistant parasites (*P. falciparum* K1 strain) with EC_{50} values of 3.6×10^{-7} M (run 10).

Table 2. *In Vitro* Antimalarial Activity and Cytotoxicity for β-Carbolines, β-Carbolinium Salts, and Their Related Compounds

Run	Compound	EC_{50} (M)		Selective toxicity[c)]
		P. falciparum[a)]	FM3A[b)]	
1	**1a**	5.0×10^{-6}	3.8×10^{-6}	0.76
2	**1b**	1.6×10^{-5}	1.8×10^{-5}	1.1
3	**1c**	2.2×10^{-5}	1.8×10^{-5}	0.82
4	**1o**	2.2×10^{-5}	1.8×10^{-5}	0.82
5	**1p**	3.1×10^{-5}	3.2×10^{-5}	1.0
6	**6aa**	1.1×10^{-6}	8.4×10^{-6}	7.6
7	**6ab**	1.3×10^{-7}	1.0×10^{-5}	77
8	**6b**	9.5×10^{-7}	$>3.0\times10^{-5}$ [f)]	>32
9	**6ca**	3.7×10^{-7}	3.0×10^{-5}	81
10	**6ca**	3.6×10^{-7} [d)]	3.0×10^{-5}	83
11	**6cb**	7.1×10^{-7}	$>2.9\times10^{-5}$ [g)]	>41
12	**6cc**	1.1×10^{-6}	4.1×10^{-5}	37
13	**6ce**	2.1×10^{-6}	$>4.3\times10^{-6}$ [h)]	>2.0
14	**6d**	1.9×10^{-5}	2.2×10^{-5}	1.2
15	**6e**	8.8×10^{-6}	1.5×10^{-5}	1.7
16	**6i**	8.8×10^{-6}	$>2.7\times10^{-5}$ [i)]	>3.1
17	**6k**	8.2×10^{-7}	$>2.6\times10^{-5}$ [j)]	>32
18	**6l**	2.0×10^{-6}	3.5×10^{-5}	18
19	**6m**	1.3×10^{-5}	$>2.3\times10^{-5}$ [k)]	>1.8
20	**6n**	NA[e)]	5.2×10^{-7}	—
21	**6o**	1.1×10^{-6}	1.9×10^{-5}	17
22	**6pa**	4.6×10^{-7}	2.2×10^{-5}	48
23	**6pb**	1.1×10^{-6}	$>7.1\times10^{-5}$ [l)]	>65
24	**7**	1.8×10^{-5}	4.5×10^{-5}	2.1
25	**8**	1.6×10^{-5}	$>3.8\times10^{-5}$ [m)]	>2.4
26	**9l**	4.8×10^{-6}	2.9×10^{-5}	6.0
27	**9o**	2.6×10^{-6}	1.9×10^{-5}	7.3
28	Quinine	1.1×10^{-7}	1.0×10^{-4}	910

a) Chloroquine sensitive strain (FCR-3) except for run 10. *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*) Chloroquine resistant *P. falciparum* K-1 was used. *e*) NA means no activity (99% growth of *P. falciparum* was observed in 2.2×10^{-6} M treatment). *f*) EC_{37} value (63% growth of FM3A was observed). *g*) EC_{13} value (87% growth). *h*) EC_{36} value (64% growth). *i*) EC_{15} value (85% growth). *j*) EC_{16} value (84% growth). *k*) EC_{10} value (90% growth). *l*) EC_{14} value (86% growth). *m*) EC_{16} value (84% growth).

Antiprotozoal Activity of Tetracyclic Cation 6q *in Vitro*

For tetracyclic compound **6q**, various antiprotozoal properties including antimalarial activity were examined *in vitro* (Table 3). Antileishmanial (Kara azar), anti-African-trypanosomal (African sleeping sickness), anti-American-trypanosomal (Chagas disease) and antigiargial (diarrhoeal giardiasis) activities were determined as EC_{50} values against the following parasites; *L. donovani*, *T. brucei rhodesiense*, *T. cruzi* and *Giardia lamblia*, respectively.[33)] *G. lamblia* is categorized as a class of diplonadida parasites, which have no mitochondrial organelle in its cell. The other tested parasites possess parasitic mitochondria in their cells. Cell cytotoxicity was tested using L-6 (a rat skeletal myoblast cell line). Interestingly, compound **6q** showed good inhibitory effect within a range of 2.6—10×10^{-7} M against all parasites except for *G. lamblia*. The efficacies of **6q** against the parasites were com-

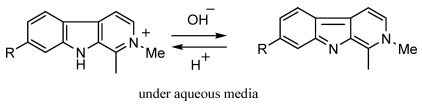


Chart 5

Table 3. *In Vitro* Antiprotozoal Properties of **6q**

Run	Parasites	EC_{50} of **6q** (M)	EC_{50} of std. (M)
1	*Plasmodium falciparum* K-1	3.5×10^{-7}	1.5×10^{-6} (chloroquine)
2	*Leishmania donovani* axenic	2.6×10^{-7}	2.9×10^{-7} (miltefosine)
3	*Leishmania donovani* inf, macrophages	1.0×10^{-6}	1.2×10^{-6} (miltefosine)
4	*Trypanosoma brucei rhodesiense*	6.3×10^{-7}	6.0×10^{-9} (meralsporol)
5	*Trypanosoma cruzi*	4.5×10^{-7}	8.7×10^{-7} (benznidazole)
6	*Giargia lamblia*	Inactive	—
7	Cytotoxicity L-6	4.9×10^{-6}	2.4×10^{-8} (podophyllotoxin)

Table 4. *In Vivo* Antimalarial Properties of β-Carbolinium Salts

Run	Compound	Dose schedule (mg kg^{-1} for 4 d)	Suppression of parasitemia (%)
1	**6ab**	10 (ip)	25.9
2	**6ab**	20 (ip)	28.2
3	**6ab**	2.5 (iv)	22.7
4	**6ab**	5.0 (iv)	23.9
5	**6ce**	10 (ip)	21.3
6	**6ce**	3.0 (iv)	12.4
7	**6ce**	10 (iv)	—[a]
8	**6cf**	10 (ip)	12.7
9	**6cg**	10 (ip)	2.1
10	**6ch**	10 (ip)	8.6
11	**6ci**	10 (ip)	0.6
12	**6cj**	10 (ip)	13.1
13	**6g**	10 (ip)	33.5
14	**6q**	10 (ip)	38.0

a) All mice died in 24 h due to an acute toxicity.

parable to the corresponding chemotherapeutics in clinically use, and the cytotoxicity level of **6q** was about 10-fold less than its antiprotozoal activity. Antileishmanial activity of **6q** was observed against not only axenic leishmania parasites but also ones infected to macrophages (runs 2, 3).

Evaluation of Antimalarial Activity *in Vivo* Several β-carbolinium salts **6** were subjected to *in vivo* assay for their effects on the suppression of parasitemia in malaria (*P. berghei*)-infected mice. Preliminary toxic study has revealed that compounds **6ab** and **6q** show acute toxicity to normal mice by intraperitoneal (i.p.) injection at a dose of more than 50 and 200 mg kg^{-1} d^{-1}, respectively. *In vivo* antimalarial assay was carried out using Peters' 4-d suppressive test.[39,40] Compound **6ab** displayed an antimalarial activity at a dose of 10 mg kg^{-1} d^{-1} of body weight by i.p. injection (26% suppression of parasitemia; Table 4, run 1). However, no significant increase in efficacy was observed even at a dose of 20 mg kg^{-1} d^{-1} (run 2). The antimalarial properties were displayed by intravenous (i.v.) injection even at smaller doses (2.5—5.0 mg kg^{-1} d^{-1}) (runs 3, 4). However, treatment by i.v. injection at a higher dose produced side effects (acute toxicity).

As the results, all compounds were tested at doses of 10 mg kg^{-1} of body weight by i.p. injection. The results are summarized in Table 4. The similar toxic effect was observed for compound **6ce**; it showed 21% suppression by i.p. injection, whereas deaths of all mice by acute toxicity were observed by i.v injection at the same dose (run 5 *versus* run 7). Some of the tested compounds **6g** and **6q** were found to display a greater *in vivo* activity than compound **6ab** (runs 13, 14). These *in vivo* results indicate that further studies for biological effects in host animals, such as bioavailability and clearance, are indispensable.

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 antiplasmodial potency and better selective toxicity than non-DLC compounds. Some of the β-carbolinium salts have *in vivo* antimalarial potency to a certain extent. Thus, this study indicates that transformation into a DLC structure may prove to be a highly effective modification methodology in the antimalarial medicinal chemistry. Tetracyclic β-carbolinium analog **6q** also displays good antileishmanial and antitrypanosomal properties as well as antimalarial activity.

Experimental

Melting points (mp) were measured with a Yanaco micro melting point apparatus and are uncorrected. UV and fluorescence spectra were recorded with a BECKMAN DU-640 and a Hitachi F 2000 spectrometers, respectively. IR spectra were recorded with a SHIMADZU FTIR-8300. ^{1}H- and ^{13}C-NMR spectra (400, 100 MHz, respectively) were recorded on a JEOL AL400 spectrometer. Chemical shifts were relative to tetramethylsilane (TMS). Fast atom bombardment (FAB) mass spectra were determined with a JEOL JMS DX-303 mass spectrometer. Electron ionization (EI) mass spectra were recorded on a JEOL JMS AX-500 instrument. Elemental analyses were performed on Yanagimoto MT-3, and the results (C, H, N) were within $\pm$0.4% of theoretical values. All reactions were carried out under an argon atmosphere. Unless otherwise described, the materials and the solvent were obtained by reaction of commercial suppliers and used without further purification.

Ethyl 2-Acetyl-1,2,3,4-tetrahydro-β-carboline-1-carboxylate (3a) To a suspension of tryptamine hydrochloride (**2**; 17.1 g, 86.9 mmol) in ethanol (200 ml) was added a solution of glyoxylic acid ethyl ester (21.4 g, 105 mmol) in toluene (50% v/v) at 0 °C. After the reaction mixture was stirred over night at ambient temperature, solvent was removed under reduced pressure. The resulting residue was treated with sat. $NaHCO_3$, and was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was used in the next step without further purification. To a solution of the above residue and DMAP (0.131 g, 0.925 mmol) in CH_2Cl_2 (200 ml) at 0 °C were added Et_3N (15.5 ml, 111 mmol) and AcCl (7.89 ml, 111 mmol). The mixture was stirred over night at ambient temperature. After concentration under reduced pressure, the residue was treated with sat. $NaHCO_3$, and was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was chromatographed on silica gel with AcOEt–hexane (1 : 1) to give compound **3a** (11.0 g, 44% for 2 steps) as colorless solids, mp 193—195 °C. IR (KBr) cm^{-1}: 3277, 1744, 1643. ^{1}H-NMR ($CDCl_3$) δ: 8.39 (1H, br s), 7.49 (1H, d, J=7.8 Hz), 7.37 (1H, d, J=8.1 Hz), 7.20 (1H, t, J=8.1 Hz), 7.11 (1H, t, J=7.8 Hz), 6.16 (1H, s), 4.24 (2H, q, J=7.1 Hz) , 4.30—4.14 (1H, m), 3.67—3.60 (1H, m), 2.88—2.85 (2H, m), 2.29 (3H, s), 1.30 (3H, t, J=7.1 Hz). ^{13}C-NMR ($CDCl_3$) δ: 170.4, 168.7, 136.2, 126.7, 126.1, 122.4, 119.6, 118.2, 111.1, 109.4, 62.0, 52.3, 43.1, 21.7, 14.3. LR-MS (EI) *m/z*: 286 (M^+). *Anal.* Calcd for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.44; N, 9.78. Found: C, 67.06; H, 6.44; N, 9.84.

Ethyl 4-Methoxy-β-carboline-1-carboxylate (1d) Compound **3a** (3.00 g, 10.5 mmol) and DDQ (11.9 g, 52.4 mmol) were mixed together until a uniform color was observed. The mixture of powders was cooled on a dry ice–acetone bath. The aqueous THF (THF–H_2O=9 : 1 v/v; 70 ml) was added slowly to the mixture of powders. The reaction mixture was stirred at −78 °C for 1 h, and then allowed to warm to ambient temperature. After the reaction mixture was stirred over night at ambient temperature, solvent was removed under reduced pressure. The residue was treated with sat. $NaHCO_3$, and extracted with $CHCl_3$. The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product of **4a** was used in the next step without further purification. A solution of *p*-toluenesulfonic acid monohydrate (2.40 g, 12.6 mmol) in benzene (70 ml) was heated under azeotropic conditions for 2 h, and then cooled to ambient temperature. To the mixture were added 2,2-dimethoxypropane (3.87 ml, 31.5 mmol), followed by the above the crude product of **4a**, and the resulting mixture was stirred at ambient temperature for 30 min. Then, to the mixture was added *p*-chloranil (5.16 g, 21.0 mmol), and was further stirred over night at the same temperature. After concentration of the solution *in vacuo*, the residue was treated with $CHCl_3$ and sat. $NaHCO_3$. The aqueous phase was extracted with $CHCl_3$ and the combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel with AcOEt–hexane (1 : 1) to give **1d** (884 mg, 30%) as colorless solids and **1e** (195 mg, 7%) as colorless solids. For **1d**, recrystallization from CH_2Cl_2–diisopropylether gave colorless needles, mp 168—170 °C. IR (KBr) cm^{-1}: 3404, 3018, 2980, 2947, 2905, 1663. ^{1}H-NMR ($CDCl_3$) δ: 9.88 (1H, br s), 8.30 (1H, d, J=8.1 Hz), 8.22 (1H, 1H), 7.57—7.52 (2H, m), 7.31 (1H, t, J=8.1 Hz), 4.57 (2H, q, J=7.2 Hz), 4.23 (3H, s), 1.52 (3H, t, J=7.2 Hz). ^{13}C-NMR ($CDCl_3$) δ: 166.4, 154.1, 139.4, 138.5, 128.0, 124.0, 123.9, 122.4, 120.7, 120.3, 118.6, 111.2, 61.6, 56.4,

14.6. LR-MS (EI) *m/z*: 270 (M^+). *Anal.* Calcd for $C_{15}H_{14}N_2O_3$: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.43; H, 5.30; N, 10.34. For **1e**, recrystallization from acetone gave colorless solids, whose spectral data were identical with reported ones.[23]

4-Methoxy-β-carboline-1-carbaldehyde (Kumujancine; 1f) To a stirred solution of **1d** (468 mg, 1.73 mmol) in CH_2Cl_2 (30 ml) was added DIBAL-H solution (1.0 M in hexane; 12.1 ml, 12.1 mmol) at −50 °C. The mixture was stirred at −50 °C for 10 min and quenched by sequential addition of MeOH (14.0 ml) and 10% NaOH (9.53 ml) at −50 °C. Then the mixture was stirred at ambient temperature for an additional 1 h. The precipitates were removed through a Celite® and washed with $CHCl_3$–MeOH (10 : 1). The combined filtrate was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. Recrystallization from CH_2Cl_2–hexane gave compound **1f** (376 mg, 96%) as yellow solids. The spectral data were identical with reported ones.[24]

1-(1-Hydroxyethyl)-4-methoxy-β-carboline (1g) To a stirred solution of **1f** (100 mg, 0.442 mmol) in THF (2.0 ml) was added MeLi (1.2 M in Et_2O; 1.33 ml, 1.60 mmol) at −78 °C. The mixture was stirred at the sam temperature for 10 min, quenched by addition of MeOH, and concentrated *in vacuo*. The residue was treated with sat. $NaHCO_3$, and extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. Recrystallization from CH_2Cl_2–hexane gave compound **1g** (102 mg, 95%) as colorless solids, mp 190 °C. IR (KBr) cm^{-1}: 3263. ^{1}H-NMR ($CDCl_3$) δ: 8.98 (1H, br s), 8.32 (1H, d, J=8.3 Hz), 7.92 (1H, s), 7.50 (2H, m), 7.28 (1H, m), 5.34 (1H, q, J= 6.5 Hz), 4.12 (3H, s), 1.69 (3H, d, J=6.5 Hz). ^{13}C-NMR (CD_3OD) δ: 152.7, 142.6, 141.5, 134.9, 128.3, 124.7, 121.4, 120.6, 120.0, 119.3, 112.4, 71.0, 56.5, 23.3. LR-MS (EI) *m/z*: 242 (M^+). HR-MS Calcd for $C_{14}H_{14}N_2O_2$, 242.1055; found 242.1042.

4-Methoxy-1-vinyl-β-carboline (MVC; 1a) From **1f**: To a stirred solution of methyltriphenylphosphonium iodide (413 mg, 1.02 mmol) in THF (5 ml) was added *n*-BuLi (1.59 M in hexane; 0.611 ml, 0.971 mmol) at 0 °C. The mixture was then stirred at ambient temperature for 2 h. A solution of **1f** (55 mg, 0.243 mmol) in THF (5 ml) was added to the above mixture at 0 °C, and then the whole were heated at 70 °C for 2 h. The reaction mixture was quenched by addition of NH_4Cl at 0 °C, followed by $NaHCO_3$, and extracted with $CHCl_3$. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated. The residue was chromatographed on silica gel with AcOEt–hexane (1 : 1) to **1a** (27.5 mg, 51%) as yellow solids. The spectral data were identical with reported ones.[16]

From **1g**: To a solution of **1g** (50 mg, 0.206 mmol) in CH_2Cl_2 (0.50 ml) at 0 °C were added Et_3N (144 μl, 1.03 mmol) and MsCl (24.0 μl, 0.310 mmol). The reaction mixture was stirred at ambient temperature for 8 h, and then quenched by addition of H_2O at 0 °C. After extraction with $CHCl_3$, the combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel with toluene–acetone (7 : 1) to give compound **1a** (8.6 mg, 19%) as yellow solids, whose spectral data were identical with reported ones.[16]

2-Benzoyl-1-methyl-1,2,3,4-tetrahydro-β-carboline (3c) Compound **3c** was obtained in a similar manner to the synthesis of **3a**. Yield 81% for 2 steps, white amorphous. IR (KBr) cm^{-1}: 3200, 1609. ^{1}H-NMR ($CDCl_3$) δ: 8.22 (1H, br s), 7.45—7.08 (9H, m), 5.87 (1H, m), 3.91 (1H, m), 3.45 (1H, m), 2.88 (1H, m), 2.74 (1H, m), 1.62 (3H, m). LR-MS (EI) *m/z*: 290 (M^+). HR-MS Calcd for $C_{19}H_{18}N_2O$, 290.1419; found 290.1408.

2-Benzoyl-1-methyl-4-oxo-1,2,3,4-tetrahydro-β-carboline (4c) Compound **4c** was obtained in a similar manner to the synthesis of **4a**. Recrystallization from AcOEt gave compound **4c** (4.32 g, 43%) as yellow solids, mp 240 °C. IR (KBr) cm^{-1}: 3061, 1666, 1609. ^{1}H-NMR ($CDCl_3$) δ: 11.0 (1H, br s), 8.12 (1H, m), 7.61—7.07 (8H, m), 6.50 (1H, m), 4.41 (1H, d, J=17.4 Hz), 4.20 (1H, d, J=17.4 Hz), 1.73 (3H, d, J=6.4 Hz). LR-MS (EI) *m/z*: 304 (M^+). *Anal.* Calcd for $C_{19}H_{16}N_2O_2$: C, 74.98; H, 5.30; N, 9.20. Found: C, 75.14; H, 5.41; N, 9.19.

4-Methoxy-1-methyl-β-carboline (1c) Compound **1c** was obtained in a similar manner to the synthesis of **1d**. Yield 43%, as colorless solids, mp 179 °C. IR (KBr) cm^{-1}: 3072, 1624, 1589. ^{1}H-NMR ($CDCl_3$) δ: 9.64 (1H, br s), 8.33 (1H, d, J=8.0 Hz), 7.98 (1H, 2), 7.48—7.46 (2H, m), 7.29—7.24 (1H, m), 4.10 (3H, s), 2.75 (3H, s). ^{13}C-NMR ($CDCl_3$) δ: 150.7, 139.5, 135.5, 135.0, 127.1, 124.1, 121.4, 120.3, 120.1, 117.8, 111.0, 56.1, 19.7. LR-MS (EI) *m/z*: 212 (M^+). *Anal.* Calcd for $C_{13}H_{12}NO_2$: C, 73.56; H, 5.70; N, 13.20. Found: C, 73.19; H, 5.72; N, 12.90.

Ethyl β-Carboline-1-carboxylate (Kumujian A; 1e) A suspension of tryptamine hydrochloride (**2**; 8.00 g, 40.7 mmol) in ethanol (150 ml) was added a solution of glyoxylic acid ethyl ester (12.5 g, 61.2 mmol) in toluene (50% v/v) at 0 °C. After the reaction mixture was stirred over night at ambient temperature, solvent was removed under reduced pressure. The resulting residue was treated with sat. $NaHCO_3$, and was extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was used in the next step without further purification. To a solution of the resulting crude residue in *p*-xylene (100 ml) at 0 °C was added 10% Pd/C (2.17 g), and the mixture was stirred over night at 140 °C under an air atmosphere. The resulting solids were removed through Celite® and washed with $CHCl_3$. After concentration of the filtrate, the resulting residue was chromatographed on silica gel with toluene–acetone (7 : 1) to give compound **1e** (4.01 g, 41%). Recrystallization from acetone gave colorless solids, whose spectral data were identical with reported ones.[23]

β-Carboline-1-carbaldehyde (Kumujian C; 1h) Compound **1h** was obtained in a similar manner to the synthesis of **1f** (89% yield). The spectral data were identical with reported ones.[23]

1-(1-Hydroxyethyl)-β-carboline (1i) Compound **1i** was obtained in a similar manner to the synthesis of **1h** (79% yield). The spectral data were identical with reported ones.[25]

1-Vinyl-β-carboline (Pavettine; 1j) Compound **1j** was obtained from **1i** in a similar manner to the synthesis of **1a** from **1g** (18% yield). The spectral data were identical with reported ones.[26]

Canthin-6-one (1k) To a stirred suspension of sodium hydride (5.0 mg, 0.125 mmol) in THF (0.5 ml) were added **1h** (20 mg, 0.102 mmol) and, then, AcCl (9.0 μl, 0.127 mmol) at 0 °C. The mixture was stirred for 5 h at ambient temperature. After removal of the solvent under reduced pressure, the resulting residue was treated with sat. $NaHCO_3$ and extracted with ethyl acetate. The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel with AcOEt–hexane (1 : 1) to give compound **1k** (6.9 mg, 31%) as yellow solids. The spectral data were identical with reported ones.[27]

General Procedure for Quarternarization of β-Carbolines (Synthesis of β-Carbolinium Salts) To a stirred solution of **1** in CH_3CN was added alkylating agent (2 eq). The mixture was then stirred over night at 80 °C under an open atmosphere. After concentration *in vacuo*, the crude residue was purified by recrystallization from appropriate solvent.

General Procedure for Ion Exchange (Preparation of Chloride Salts) Compound **6** was dissolved in 10% KOH aq. (*ca.* 10 ml), and the resulting mixture was extracted with AcOEt. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. To a solution of the resulting residue in $CHCl_3$ was added anhydrous HCl (1.0 M Et_2O solution). The resulting precipitates were corrected by filtration.

4-Methoxy-2-methyl-1-vinyl-β-carbolin-2-ium *p*-Toluenesulfonate (6aa) Compound **6aa** was obtained from **1a** in 60% yield by recrystallization from CH_3CN–Et_2O as yellow solids, mp 208—220 °C. IR (KBr) cm^{-1}: 3422, 3038, 1622, 1541. UV-vis λ_{max} (MeOH) nm (log ε): 380 (3.80), 317 (4.04). ^{1}H-NMR (CD_3OD) δ: 8.35 (1H, d, J=8.3 Hz), 8.24 (1H, s), 7.73 (2H, d, J=3.7 Hz), 7.65 (2H, d, J=7.9 Hz), 7.45—7.41 (1H, m), 7.18 (1H, dd, J=17.8, 11.7 Hz), 7.15 (2H, d, J=7.9 Hz), 6.34 (1H, d, J=11.7 Hz), 6.28 (1H, d, J=17.8 Hz), 4.35 (3H, s), 4.25 (3H, s), 2.30 (3H, s). ^{13}C-NMR (CD_3OD) δ: 187.5, 153.2, 144.4, 143.5, 141.5, 135.0, 131.9, 130.2, 129.6, 126.8, 126.1, 125.3, 123.3, 123.1, 120.6, 119.0, 113.5, 58.1, 46.4, 21.3. LR-MS (FAB) *m/z*: 239 (M^+). HR-MS Calcd for $C_{15}H_{15}N_2O^+$, 239.1179; found 239.1176.

2-Ethyl-4-methoxy-1-vinyl-β-carbolin-2-ium *p*-Toluenesulfonate (6ab) Compound **6ab** was obtained from **1a** in 30% yield, yellow solids from CH_3CN–Et_2O, mp 108 °C. IR (KBr) cm^{-1}: 3431, 3059, 1624, 1537. ^{1}H-NMR (CD_3OD) δ: 8.40 (1H, d, J=8.1 Hz), 8.33 (1H, s), 7.75—7.74 (2H, m), 7.66 (2H, d, J=8.1 Hz), 7.46—7.42 (1H, m), 7.26 (1H, dd, J=17.8, 11.7 Hz), 7.17 (2H, d, J=8.1 Hz), 6.37 (1H, d, J=11.7 Hz), 6.32 (1H, d, J=17.8 Hz), 4.74 (2H, q, J=7.3 Hz), 4.28 (3H, s), 2.32 (3H, s), 1.63 (3H, t, J=7.3 Hz). ^{13}C-NMR (CD_3OD) δ: 153.8, 144.6, 143.4, 141.4, 132.0, 130.7, 129.7, 126.8, 125.8, 125.7, 123.4, 123.2, 120.7, 117.5, 113.5, 58.1, 54.9, 21.3, 16.4. LR-MS (FAB) *m/z*: 253 (M^+). HR-MS Calcd for $C_{16}H_{17}N_2O^+$, 253.1335; found 253.1328.

4-Methoxy-1,2-dimethyl-β-carbolin-2-ium *p*-Toluenesulfonate (6cb) Compound **6cb** was obtained from **1c** in 75% yield, white solids from CH_3CN, mp 249—250 °C. IR (KBr) cm^{-1}: 3437, 3068, 1632, 1549. ^{1}H-NMR (CD_3OD) δ: 8.32 (1H, d, J=8.0 Hz), 8.17 (1H, s), 7.71 (2H, m), 7.65 (2H, d, J=8.0 Hz), 7.43—7.39 (1H, m), 7.14 (2H, d, J=8.0 Hz), 4.35 (3H, s), 4.21 (3H, s), 2.97 (3H, s), 2.30 (3H, s). LR-MS (FAB) *m/z*: 227 (M^+). HR-MS Calcd for $C_{14}H_{15}N_2O^+$, 227.1179; found 227.1190.

4-Methoxy-1,2-dimethyl-β-carbolin-2-ium Chloride (6cc) Compound **6cc** was prepared from **6cb** in 88% yield, yellow solids from $CHCl_3$, mp 276—278 °C. IR (KBr) cm^{-1}: 3385, 3053, 1630, 1545. ^{1}H-NMR (CD_3OD)

δ: 8.33 (1H, d, J=8.4 Hz), 8.21 (1H, s), 7.72 (2H, m), 7.42 (1H, m), 4.38 (3H, s), 4.23 (3H, s), 3.00 (3H, s). LR-MS (FAB) *m/z*: 227 (M^+). HR-MS Calcd for $C_{14}H_{15}N_2O^+$, 227.1179; found 227.1175.

2-Ethyl-4-methoxy-1-methyl-β-carbolin-2-ium *p*-Toluenesulfonate (6cd) Compound **6cd** was obtained from **1c** in 94% yield, white solids from CH_3CN, mp 171—172 °C. IR (KBr) cm^{-1}: 3445, 3061, 1628, 1545. ^{1}H-NMR (CD_3OD) δ: 8.30 (1H, d, J=8.0 Hz), 8.23 (1H, s), 7.77—7.69 (2H, m), 7.60 (2H, d, J=8.2 Hz), 7.40 (1H, dd, J=8.0, 8.0 Hz), 7.09 (2H, d, J=8.2 Hz), 4.74 (2H, q, J=7.2 Hz), 4.21 (3H, s), 3.19 (3H, s), 2.27 (3H, s), 1.63 (3H, t, J=7.2 Hz). ^{13}C-NMR (CD_3OD) δ: 152.8, 145.6, 143.5, 141.4, 137.3, 136.3, 131.9, 129.6, 126.7, 125.9, 123.0, 119.5, 118.2, 111.5, 58.0, 55.2, 34.0, 21.3, 16.3, 16.1. LR-MS (FAB) *m/z*: 241 (M^+). HR-MS Calcd for $C_{15}H_{17}N_2O^+$, 241.1335; found 241.1340.

2-Ethyl-4-methoxy-1-methyl-β-carbolin-2-ium Chloride (6ce) Compound **6ce** was prepared from **6cd** in 77% yield, white yellow from $CHCl_3$, mp 262—263 °C. IR (KBr) cm^{-1}: 3396, 3061, 1628, 1541. ^{1}H-NMR (CD_3OD) δ: 12.4 (1H, br s), 8.34 (1H, d, J=8.0 Hz), 8.26 (1H, s), 7.73—7.72 (2H, m), 7.43—7.39 (1H, m), 4.74 (2H, q, J=7.2 Hz), 4.24 (3H, s), 3.05 (3H, s), 1.65 (3H, t, J=7.2 Hz). ^{13}C-NMR (CD_3OD) δ: 153.0, 144.0, 136.8, 135.4, 131.6, 125.6, 122.9, 122.0, 120.5, 117.4, 113.3, 58.0, 54.4, 16.3, 14.6. LR-MS (FAB) *m/z*: 241 (M^+). HR-MS Calcd for $C_{15}H_{17}N_2O^+$, 241.1335; found 241.1341.

2-(2-Hydroxyethyl)-4-methoxy-1-methyl-β-carbolin-2-ium Chloride (6cf) Compound **6cf** was synthesized from **1c** and 2-iodoethenol, followed by ion exchange, 19% overall yield, yellow solids from $CHCl_3$, mp 259—261 °C. IR (KBr) cm^{-1}: 3250, 3061, 1632, 1543. ^{1}H-NMR (CD_3OD) δ: 12.4 (1H, br s), 8.36 (1H, d, J=8.0 Hz), 8.22 (1H, s), 7.73—7.72 (2H, m), 7.44—7.40 (1H, m), 4.83 (2H, t, J=5.2 Hz), 4.24 (3H, s), 4.11 (2H, t, J=5.2 Hz), 3.10 (3H, s). LR-MS (FAB) *m/z*: 257 (M^+). HR-MS Calcd for $C_{15}H_{17}N_2O_2^+$, 257.1285; found 257.1296.

4-Methoxy-2-(2-methoxyethyl)-1-methyl-β-carbolin-2-ium Chloride (6cg) Compound **6cg** was synthesized from **1c** and 2-methoxyethyl *p*-toluenesulfonate, followed by ion exchange, 76% overall yield, yellow solids from $CHCl_3$, mp 238—239 °C. IR (KBr) cm^{-1}: 3385, 3040, 1630, 1543. ^{1}H-NMR (CD_3OD) δ: 12.1 (1H, br s), 8.01 (1H, s), 7.89 (1H, d, J=8.0 Hz), 7.57 (1H, dd, J=8.0, 8.0 Hz), 7.49 (1H, d, J=8.0 Hz), 7.21 (1H, dd, J=8.0, 8.0 Hz), 4.77 (2H, t, J=4.8 Hz), 4.13 (3H, s), 3.93 (2H, t, J=4.8 Hz), 3.35 (3H, s), 2.90 (3H, s). ^{13}C-NMR (CD_3OD) δ: 155.2, 143.5, 136.4, 136.0, 131.4, 125.2, 122.6, 121.6, 120.0, 118.0, 113.0, 72.4, 59.4, 58.1, 58.0, 15.5. LR-MS (FAB) *m/z*: 271 (M^+). HR-MS Calcd for $C_{16}H_{19}N_2O_2^+$, 271.1441; found 271.1453.

4-Methoxy-2-[2-(2-methoxyethoxy)ethyl]-1-methyl-β-carbolin-2-ium Chloride (6ch) Compound **6ch** was synthesized from **1c** and 2-(2-methoxyethoxy)ethyl iodide, followed by ion exchange, 92% overall yield, yellow solids from $CHCl_3$, mp 131—132 °C. IR (KBr) cm^{-1}: 3348, 2874, 1628, 1541. ^{1}H-NMR (CD_3OD) δ: 8.08 (2H, m), 7.64—7.60 (2H, m), 7.29 (1H, m), 4.80 (2H, m), 4.18 (3H, s), 4.02 (2H, t, J=4.8 Hz), 3.59—3.57 (2H, m), 3.43—3.41 (2H, m), 3.17 (3H, s), 2.96 (3H, s). ^{13}C-NMR (CD_3OD) δ: 152.3, 143.7, 136.6, 131.6, 125.4, 122.8, 121.8, 120.2, 118.3, 113.2, 72.8, 71.5, 70.9, 59.0, 58.2, 58.0, 15.5. LR-MS (FAB) *m/z*: 315 (M^+). HR-MS Calcd for $C_{18}H_{23}N_2O_3^+$, 315.1703; found 315.1713.

4-Methoxy-2-[2-{(2-methoxyethoxy)ethoxy}ethyl]-1-methyl-β-carbolin-2-ium Chloride (6ci) Compound **6ci** was synthesized from **1c** and 2-[(2-methoxyethoxy)ethoxy]ethyl *p*-toluenesulfonate, followed by ion exchange, 12% overall yield, yellow solids from $CHCl_3$, mp 200—201 °C. IR (KBr) cm^{-1}: 2868, 1630, 1543. ^{1}H-NMR (CD_3OD) δ: 8.25 (1H, d, J=8.0 Hz), 8.20 (1H, s), 7.72—7.70 (2H, m), 7.41—7.38 (1H, m), 4.88 (2H, t, J=4.8 Hz), 4.23 (3H, s), 4.05 (2H, t, J=4.8 Hz), 3.60—3.58 (2H, m), 3.53—3.51 (2H, m), 3.42—3.40 (2H, m), 3.33—3.28 (2H, m), 3.15 (3H, s), 3.05 (3H, s). ^{13}C-NMR (CD_3OD) δ: 152.5, 144.0, 136.9, 136.5, 131.8, 125.7, 123.0, 122.2, 120.5, 118.4, 113.3, 72.7, 71.7, 71.4, 71.2, 70.9, 58.9, 58.3, 58.0, 15.5. LR-MS (FAB) *m/z*: 359 (M^+). HR-MS Calcd for $C_{20}H_{27}N_2O_4^+$, 359.1965; found 359.1967.

2-Ethyl-4-methoxy-1,9-dimethyl-β-carbolin-2-ium Chloride (6cj) Compound **6cd** (100 mg, 0.242 mmol) was treated with 10% KOH aq. (10 ml), and extracted with AcOEt. The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated. Mixture of the resulting residue and methyl *p*-toluenesulfonate (90.2 mg, 0.484 mmol) was stirred for 18 h at 80 °C. After cooling to ambient temperature, the resulting precipitate was collected by filtration, which was subjected to ion exchange polymer (Amberlyte IRA-400) to give **6cj** (53.5 mg, 76%) as yellow solids, mp 214—215 °C. IR (KBr) cm^{-1}: 3404, 1624, 1603, 1531. ^{1}H-NMR (CD_3OD) δ: 8.23 (1H, s), 8.08 (1H, m), 7.66 (1H, m), 7.59 (1H, m), 7.29 (1H, m), 4.75 (2H, q, J=7.2 Hz), 4.21 (3H, s), 4.08 (3H, s), 3.13 (3H, s), 1.66 (3H, t, J=7.2 Hz). ^{13}C-NMR (CD_3OD) δ: 152.5, 145.1, 136.8, 136.0, 131.7, 125.5, 122.9, 122.4, 119.0, 118.3, 111.3, 58.2, 55.2, 34.0, 16.4, 16.1. LR-MS (FAB) *m/z*: 255 (M^+). HR-MS Calcd for $C_{16}H_{19}N_2O^+$, 255.1492; found 255.1499.

1-Ethoxycarbonyl-4-methoxy-2-methyl-β-carbolin-2-ium *p*-Toluenesulfonate (6d) Compound **6d** was obtained from **1d** in 72% yield as yellow solids (from CH_3CN–Et_2O), mp 210—212 °C. IR (KBr) cm^{-1}: 3425, 2924, 2855, 1728, 1624, 1601. ^{1}H-NMR ($CDCl_3$) δ: 12.11 (1H, br s), 8.59 (1H, s), 8.15 (1H, d, J=8.2 Hz), 7.91 (2H, d, J=7.9 Hz), 7.70 (1H, d, J=7.6 Hz), 7.62 (1H, dd, J=8.2, 8.2 Hz), 7.29—7.26 (1H, m), 7.21 (2H, d, J=7.9 Hz), 4.77 (2H, q, J=7.0 Hz), 4.66 (3H, s), 4.08 (3H, s), 2.38 (3H, s), 1.41 (3H, t, J=7.0 Hz). ^{13}C-NMR ($CDCl_3$) δ: 192.8, 159.6, 152.3, 143.4, 139.6, 134.9, 131.6, 128.7, 125.8, 124.4, 122.5, 122.4, 122.1, 120.3, 117.7, 114.1, 65.0, 58.3, 48.7, 21.4, 14.2. LR-MS (FAB) *m/z*: 285 (M^+). *Anal.* Calcd for $C_{23}H_{24}O_6N_2S$: C, 60.51; H, 5.30; N, 6.14. Found: C, 60.16; H, 5.53; N, 5.98.

1-(1-Hydroxyethyl)-2-methyl-β-carbolin-2-ium *p*-Toluenesulfonate (6i) Compound **6i** was obtained from **1i** in 72% yield as colorless solids (from $CHCl_3$), mp 183—185 °C. IR (KBr) cm^{-1}: 3207, 1634, 1522. ^{1}H-NMR (CD_3OD) δ: 12.0 (1H, br s), 8.47 (1H, d, J=6.6 Hz), 8.38—8.36 (2H, m), 7.83—7.74 (2H, m), 7.63 (2H, d, J=7.8 Hz), 7.43 (1H, t, J=6.6 Hz), 7.14 (2H, d, J=7.8 Hz), 5.81 (1H, q, J=6.6 Hz), 4.41 (3H, s), 2.31 (3H, s), 1.74 (3H, d, J=6.6 Hz). ^{13}C-NMR (CD_3OD) δ: 146.9, 145.3, 143.3, 141.5, 135.4, 135.0, 134.4, 133.0, 129.6, 126.7, 123.7, 122.8, 120.4, 117.0, 114.1, 66.8, 45.7, 21.4. LR-MS (FAB) *m/z*: 227 (M^+). HR-MS Calcd for $C_{14}H_{15}N_2O^+$, 227.1149; found 227.1175.

3-Methylcanthin-6-on-3-ium *p*-Toluenesulfonate (6k) Compound **6k** was obtained from **1k** in 41% yield, colorless needles from CH_3CN, mp 231—233 °C. IR (KBr) cm^{-1}: 3435, 1693, 1661. ^{1}H-NMR (CD_3OD) δ: 8.98 (1H, d, J=6.4 Hz), 8.70 (1H, d, J=6.4 Hz), 8.65 (1H, d, J=8.3 Hz), 8.47 (1H, d, J=8.3 Hz), 8.45 (1H, d, J=10.2 Hz), 7.96 (1H, t, J=8.3 Hz), 7.72 (1H, t, J=8.3 Hz), 7.62 (2H, d, J=8.1 Hz), 7.30 (1H, d, J=10.2 Hz), 7.16 (1H, d, J=8.1 Hz), 4.66 (3H, s), 2.32 (3H, s). LR-MS (FAB) *m/z*: 235 (M^+). HR-MS Calcd for $C_{15}H_{11}N_2O^+$, 235.0866; found 235.0874.

7-Methoxy-1,2-dimethyl-β-carbolin-2-ium *p*-Toluenesulfonate (6o) Compound **6o** was obtained from **1o** in 91% yield, colorless needles from MeOH–diisopropylether, mp 191—192 °C. IR (KBr) cm^{-1}: 3427, 3074, 1628. UV-vis λ_{max} (MeOH) nm (log ε): 329 (4.29), 201 (5.30). ^{1}H-NMR (CD_3OD) δ: 8.28 (1H, m), 8.15 (1H, dd, J=6.2, 6.2 Hz), 8.10—8.06 (1H, m), 7.66 (2H, d, J=8.3 Hz), 7.14 (2H, d, J=8.3 Hz), 7.07 (1H, m), 6.98 (1H, m), 4.28 (3H, s), 3.93 (3H, s), 2.97 (3H, s), 2.29 (3H, s). ^{13}C-NMR (CD_3OD) δ: 164.9, 147.4, 143.4, 141.5, 140.1, 136.2, 135.4, 133.2, 129.6, 126.8, 124.9, 115.0, 114.3, 95.1, 56.3, 45.0, 21.3, 15.4. LR-MS (FAB) *m/z*: 227 (M^+). HR-MS Calcd for $C_{14}H_{15}N_2O^+$, 227.1179; found 227.1192.

7-Methoxy-1,2,9-trimethyl-β-carbolin-2-ium *p*-Toluenesulfonate (6pa) Compound **6pa** was obtained from **1p** in 45% yield, colorless needles from MeOH–diisopropylether, mp 244—245 °C. IR (KBr) cm^{-1}: 3462, 1628, 1521. UV-vis λ_{max} (MeOH) nm (log ε): 332 (4.22), 201 (5.60). ^{1}H-NMR (CD_3OD) δ: 8.32 (1H, d, J=6.6 Hz), 8.18 (1H, d, J=6.6 Hz), 8.11 (1H, d, J=8.8 Hz), 7.60 (2H, d, J=8.3 Hz), 7.10 (1H, s), 7.09 (2H, d, J=8.3 Hz), 7.01 (1H, d, J=8.8 Hz), 4.31 (3H, s), 4.15 (3H, s), 3.97 (3H, s), 3.17 (3H, s), 2.27 (3H, s). LR-MS (FAB) *m/z*: 240 (M^+). HR-MS Calcd for $C_{15}H_{17}N_2O^+$, 241.1335; found 241.1350.

2-(2-Hydroxyethyl)-7-methoxy-1,9-dimethyl-β-carboli-2-ium Bromide (6pb) Compound **6pb** was obtained from **1p** in 38% yield, colorless needles from MeOH–Et_2O, mp 234—235 °C. IR (KBr) cm^{-1}: 3319, 1626. UV-vis λ_{max} (MeOH) nm (log ε): 366 (3.88), (4.32). ^{1}H-NMR (CD_3OD) δ: 8.40 (1H, d, J=6.4 Hz), 8.29 (1H, d, J=8.7 Hz), 8.17 (1H, d, J=8.7 Hz), 7.19 (1H, s), 7.04 (1H, d, J=8.7 Hz), 4.83 (2H, m), 4.24 (3H, s), 4.07 (2H, m), 4.01 (3H, s), 3.33 (3H, s). ^{13}C-NMR (CD_3OD) δ: 165.6, 149.6, 141.0, 136.1, 134.1, 125.0, 114.7, 114.5, 114.1, 94.0, 61.9, 60.0, 56.6, 34.3, 17.0. LR-MS (FAB) *m/z*: 271 (M^+). HR-MS Calcd for $C_{15}H_{17}N_2O^+$, 271.1441; found 271.1459.

2-Methyl-1-(1-methyl-4-pyridinio)-β-carbolin-2-ium Di-(*p*-toluenesulfonate) (6n) Compound **6n** was obtained from **1n** in 38% yield, yellow solids from CH_3CN, mp 239—241 °C. IR (KBr) cm^{-1}: 3462, 3061, 1636. ^{1}H-NMR (CD_3OD) δ: 9.30 (2H, d, J=6.4 Hz), 8.77—8.71 (2H, m), 8.55 (2H, d, J=6.4 Hz), 8.45 (1H, d, J=8.0 Hz), 7.79 (1H, t, J=8.0 Hz), 7.61 (5H, m), 7.51 (1H, t, J=8.0 Hz), 7.16 (4H, d, J=7.6 Hz), 4.61 (3H, s), 4.29 (3H, s), 2.32 (6H, s). LR-MS (FAB) *m/z*: 275 (M^+). HR-MS Calcd for $C_{18}H_{17}N_3^+$, 275.1411; found 275.1405.

Biological Experiment. Malaria Parasites Chloroquine-sensitive *Plasmodium falciparum* (ATCC 30932, FCR-3 strain) and chloroquine-resistant *P. falciparum* (K1; a clone originating from Thailand) were used in

our study. *P. falciparum* was cultivated by a modification of the method of Trager and Jensen[41] using a 5% hematocrit of type A^+ human red blood cells suspended in RPMI 1640 medium (Gibco, NY, U.S.A.) supplemented with heat-inactivated 10% type A^+ human serum. The plates were placed in a CO_2–O_2–N_2 incubator (5% CO_2, 5% O_2, and 90% N_2 atmosphere) at 37 °C, and the medium was changed daily until 5% parasitemia (which means that 5 parasite-infected erythrocytes in every 100 erythrocytes were existing).

Mammalian Cells Mouse mammary tumor FM3A cells (wild-type, subclone F28-7)[42] were supplied by the Japanese Cancer Research Resources Bank (JCRB). FM3A cells were maintained in suspension culture at 37 °C in a 5% CO_2 atmosphere in plastic bottles containing ES medium (Nissui Pharmaceuticals, Tokyo, Japan) supplemented with 2% heat-inactivated fetal bovine serum (Gibco, NY, U.S.A.).

Evaluation of *in Vitro* Antiplasmodial Activity The following procedures were used for assay of antimalarial activity (except for run 24 in Table 2)[43,44] Asynchronously cultivated *P. falciparum* were used. Various concentrations of compounds in dimethylsulfoxide (DMSO) were prepared. 5 μl of each solution was added to individual wells of a multidish, 24 wells. Erythrocytes with 0.3% parasitemia were added to each well containing 995 μl of culture medium to give a final hematocrit level of 3%. The plates were incubated at 37 °C for 72 h in a CO_2–O_2–N_2 incubator (5% CO_2, 5% O_2, and 90% N_2 atmosphere). To evaluate the antimalarial activity of test compound, we prepared thin blood films from each culture and stained them with Giemsa (E. Merck, Germany). Total 1×10^4 erythrocytes/1 thin blood film were examined under microscopy. All of the test compounds were assayed in duplicate at each concentration. Drug-free control cultures were run simultaneously. All data points represent the mean of three experiments. Parasitemia in control reached between 4% and 5% at 72 h. The EC_{50} value refers to the concentration of the compound necessary to inhibit the increase in parasite density at 72 h by 50% of control.

In vitro antiplasmodial activity against for compound **6q** was assessed using an adaptation of the procedures (^{3}H-hypoxanthine incorporation assay).[45,46]

Evaluation of Cytotoxicity against Mammalian Cell Line FM3A cells grew with a doubling time of about 12 h. Prior to exposure to drugs, cell density was adjusted to 5×10^4 cells/ml. A cell suspension of 995 μl was dispensed to the test plate, and compound at various concentrations suspended in DMSO (5 μl) was added to individual wells of a multidish, 24 wells. The plates were incubated at 27 °C in a 5% CO_2 atmosphere for 48 h. All of the test compounds were assayed in duplicate at each concentration. Cell numbers were measured using a microcell counter CC-130 (Toa Medical Electric Co., Japan). All data points represent the mean of three experiments. The EC_{50} value refers to the concentration of the compound necessary to inhibit the increase in cell density at 48 h by 50% of control. Selectivity refers to the mean of EC_{50} value for FM3A cells per the mean of EC_{50} value for *P. falciparum*.

Evaluation of Antileishmanial, Antitrypanosomal Activities *in Vitro* Evaluation against the protozoa *L. donovani* (axenic and infected amastigotes), *T. brucei rhodesiense* (STIB900) and *T. cruzi*, *G. lamblia* and for cytotoxicity on L6 cells was performed according to experimental procedures described elswhere.[46—48]

Evaluation of *in Vivo* Antiplasmodial Activity *In vivo* antimalarial activities of β-carbolinium salts were determined in mice infected with *P. berghei* (NK 65 strain).[39,40] Five-week-old ICR male mice obtained in sterile containers from Japan SLC, Inc. (Hamamatsu, Japan) weighing 22—25 g were used. They were housed under a natural day-night cycle at 25 °C. The mice were randomly assigned to treated groups and housed in cages each containing five individuals. Parasites were collected by cardiac puncture in a heparinized syringe from a donor mouse harboring about 15% parasitemia. The blood was diluted with 0.9% NaCl solution to final concentrations of 1×10^6 infected erythrocytes/0.2 ml of infecting suspension. Test compounds were prepared at doses of 2.5—20 mg/kg in 0.9% NaCl solution (Otsuka Pharmaceutical Co., Ltd.) or olive oil. Five animals were treated with each dose. Initially, each mouse was inoculated intravenously in the tail vein with 1×10^6 parasitized erythrocytes (infecting suspension in 0.2 ml of 0.9% NaCl solution). The compounds were administrated once a day starting on day 0 and continued on day 1, day 2, and day 3. The first administration of test compound intraperitoneally started 2 h after parasite inoculation. Parasitemia levels were determined on the day following the last treatment (on day 4). To evaluate the antimalarial activity of the compounds, we prepared tail blood smears and stained them with Giemsa (E. Merck, Germany). Total 1×10^4 erythrocytes/1 thin blood film were examined under microscopy. On day 4, parasitemia of control mice were between 20% and 50%. The suppression of parasitemia for the tested compounds was calculated by the formula: [(average % parasitemia in controls (sham-treated)−average % parasitemia, in treated mice)/average % parasitemia in controls (sham-treated)]×100. Five infected, 0.9% NaCl solution (or olive oil)-dosed mice were used as a control. The care and treatment of mice were in accordance with the guidelines (No. 141, 1987) issued by the Science and International Affairs Bureau of the Japanese Ministry of Education, Culture, Science and Technology.

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