ANTIMALARIAL ACTIVITIES *IN VITRO* OF HOMOPROTOBERBERINE DERIVATIVES. DESIGN OF NOVEL ANTIMALARIALS AND STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS†

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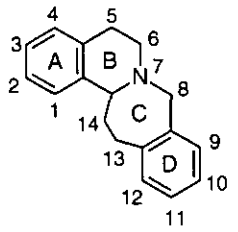
Abstract — Antimalarial activities *in vitro* of homoprotoberberine derivatives against *Plasmodium falciparum* were examined. It has shown that 9-hydroxy-2,3,10-trimethoxyhomoprotoberberine (**10**) was the most potent compound and the hydroxyl group at the C-9 position would play a significant role in exhibiting antimalarial activity and selective toxicity.

Malaria is one of the most important parasitic problems spreading in tropical areas. *Plasmodium falciparum* is the most serious human malaria parasite causing about two billion suffers per year. The rapidly acquiring resistance of *P. falciparum* to chloroquine is now a significant problem throughout the world.[1] New antimalarial agents are needed and various compounds have been studied to exploit efficient chemotherapy. Moderate antimalarial activity was reported for protoberberine alkaloids.[2,3] We have studied homoprotoberberine derivatives as candidates of novel antimalarial agents and examined their antimalarial activities and the structure-activity relationship. Homoprotoberberines have the 1-phenethyl-1,2,3,4-tetrahydroisoquinoline unit as shown in Figure 1.

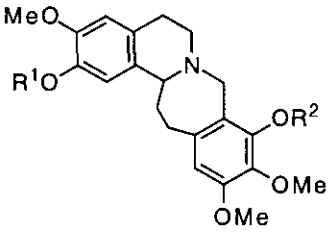
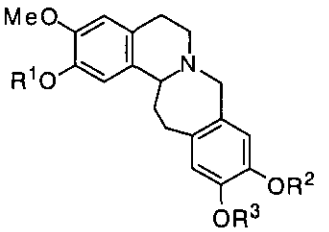
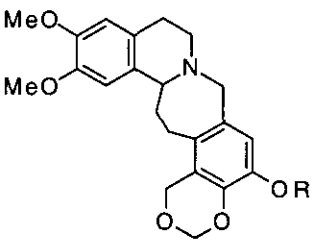
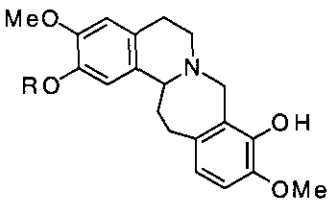
Figure 1 The Basic Structure of Homoprotoberberine



† This paper is dedicated to Dr. Bernhard Witkop on the occasion of his 80th birthday.

The homoprotoberberines were synthesized *via* various ring closure procedures of C-ring by ways of 1) Pictet-Spengler reaction,[4a-c] 2) phenolic cyclization method,[4d] and 3) cyclization of *N*-methylisoquinoline-*N*-oxides with $FeSO_4$.[4e]

Table 1 Antimalarial Activities *in vitro* of Homoprotoberberine Derivatives

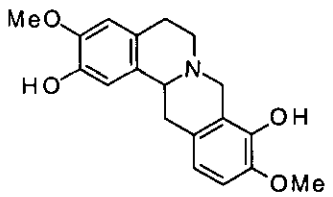
Compounds		Antimalarial Activity[a] IC_{50} (M) (*P. falciparum*)	Cytotoxicity[b] IC_{50} (M) (FM3A)	Selective Toxicity[c]
	**1**; $R^1 = R^2 = Me$	2.9×10^{-5}	—	—
	2; $R^1 = H$, $R^2 = Me$	5.6×10^{-6}	3.4×10^{-5} (81%)[d]	> 6
	3; $R^1 = R^2 = H$	1.2×10^{-6}	1.0×10^{-5}	8
	**4**; $R^1 = R^2 = R^3 = Me$	3.4×10^{-5} (61%)	—	—
	5; $R^1 = H$, $R^2 = R^3 = Me$	1.2×10^{-5}	—	—
	6; $R^1 = R^2 = Me$, $R^3 = H$	3.4×10^{-5} (100%)	3.4×10^{-5} (75%)	—
	7; $R^1 = Me$, R^2, $R^3 = CHMe_2$	2.8×10^{-6}	3.2×10^{-6}	—
	**8**; R = H	3.0×10^{-5} (90%)	—	—
	9; R = Ac	2.0×10^{-5} (69%)	—	—
	**10**; R = Me	4.0×10^{-7}	3.7×10^{-5}	93
	11; R = H	6.8×10^{-7}	1.0×10^{-6} (100%)	> 1.5

a) Expressed as IC_{50} against malaria parasite *Plasmodium falciparum*. b) Evaluated as IC_{50} inhibiting growth of mouse mammary cells FM3A. c) Calculated as the ratio of IC_{50} (*P. falciparum*) to IC_{50} (FM3A). d) Numbers in the parentheses are growth (%) at the concentration.

Antimalarial activity against *Plasmodium falciparum* are shown in Table 1, and **10** was the most potent antimalarial compound possessing selective index, 93. Obviously, a hydroxyl group, especially on D-ring has an important role to show high antimalarial activity and selective toxicity. Compounds (**3**, **10**, and **11**) possessing the hydroxyl group at the C-9 position exhibited higher activity and selectivity, while **6** and **8** carrying the hydroxyl group at the C-10 or C-11 position did not show any apparent effect. **3**, possessing a methoxy group at the C-11 position, was considerably less potent antimalarial than **10** which is not substituted at this position. This fact implies that the substituent at the C-11 position could decrease the activity. Interestingly, **2** showed a moderate antimalarial activity and a little selective toxicity without the hydroxyl substituent at the C-9 position. In comparison with **1**, antimalarial activity of **2** was improved by the presence of the hydroxyl group on A-ring. The hydroxyl group at the C-2 position, however, would not be necessary because significant improvement was not found in **5** and **11** compared with **4** and **10**. Compounds (**1**, **4**, **9**) having no hydroxyl group were less effective. In addition, methiodides of **1** and **4** did not show any activity against *P. falciparum*.

We also studied the activity of tetrahydroprotoberberines, but most of them did not show significant activities. Exceptionally, (±)-scoulerine (**12**)[4a] had moderate antimalarial effect as shown in Figure 2, although **12** showed weaker activity than that of homoscoulerine (**11**).

Figure 2 Antimalarial Activity of Scoulerine (**12**)



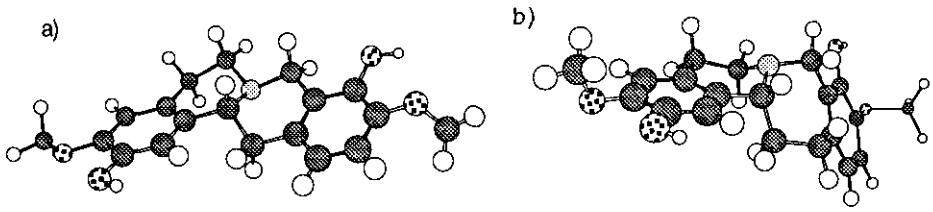
12

	P. falciparum IC_{50} (M)	FM3A IC_{50} (M)	Selective Toxicity
	1.2×10^{-6}	11×10^{-5}	9

The difference might result from conformations due to the 6 or 7 membered C-ring. MMX calculation demonstrated that scoulerine (**12**) prefers the *trans* conformation rather than *cis* one by 1.61 kcal/mol, and homoscoulerine (**11**) favors the *cis* conformation[5] by 1.58 kcal/mol (Figure 3).

These results imply that the antimalarial activities should depend on the hydroxyl group at the C-9 position in homoprotoberberines. Further evaluation of related compounds would disclose effective design of antimalarial agents with improvement.

Figure 3 MMX Calcultation Showing the Most Stable Conformers of a) Scoulerine (**12**) and b) Homoscoulerine (**11**)



ACKNOWLEDGMENT

This work was partly supported by Grant-in-Aid for Scientific Research on Priority Areas (09270202 and 08281105) from the Ministry of Education, Science, Culture and Sports of Japan.

We thank Emeritus Professor Keiichiro Fukumoto of Tohoku University for his helpful discussion.

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Received, 11th June, 1998