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# SYNTHESES AND BIOLOGICAL ACTIVITIES OF STRUCTURALLY STIFF RHODACYANINES AS NOVEL ANTIMALARIAL CANDIDATES

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**Abstract** – New classes of rhodacyanine as structurally stiff derivatives were designed and synthesized. The synthetic compounds were evaluated the antimalarial activity and cytotoxicity *in vitro*.

We continue our studies on the π-delocalized lipophilic cations (DLCs)[1] as a novel drug candidate for tropical diseases, such as malaria, leishmaniasis, Chagas disease, and sleeping sickness.[2-5] We have reported that rhodacyanine dyes (**1**) exhibit strong antimalarial and antileishmanial activity *in vitro* against the *Plasmodium falciparum* and *Leishmania major*, respectively, parasites and excellent selective toxicity (Figure 1).[2-4] For example, MKT-077 (**1a**) and MKH-57 (**1b**) were found to display strong inhibitory effect against drug-sensitive malaria parasites (*P. falciparum* FCR−3 strain) *in vitro*. In general, rhodacyanines (**1**)[2-4,6] have two different conjugated systems, neutral merocyanine and cationic cyanine moiety, and consist of three, linearly linked heterocycles, in which two end heteroaromatic rings edge a rhodanine (4-oxothiazolidine) ring. Although the *trans*-configuration for each isomerizable double bond of **1** is usually thermodynamic stable, there should be several possible geometrical isomers (conformers). From a medicinal point of view, rigidification of a molecular structure of a drug candidate has been a popular tactic used to increase its activity or to reduce its side effects.[7] We have envisaged that the fixation of flexible bonds of rhodacyanine dyes would show improved biological actions, such as enhanced antimalarial activity and/or decreased cytotoxicity against mammary cells. In this regards, we designed three new classes of rhodacyanine (**2-4**), which have fused ring skeleton, as shown in Figure 2.

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This paper is dedicated to the memory of the Emeritus Professor Kenji Koga of Tokyo University.

Thus, the free rotation of the C(3)-C(4) single bond and the *trans*/*cis* isomerization of the C(2)-C(3) double bond are restricted in **2** and **3**, respectively. Both of the flexibilities of the C(2)-C(3) and C(3)-C(4) bonds are suppressed in compound (**4**) and its geometry of C(2)-C(3) double bond should be fixed (*E*)-configuration. In this communication, we report the synthesis of structurally stiff rhodacyanines (**2-4**) and their antimalarial activities against chloroquine-resistant *P. falciparum* K1 *in vitro*.

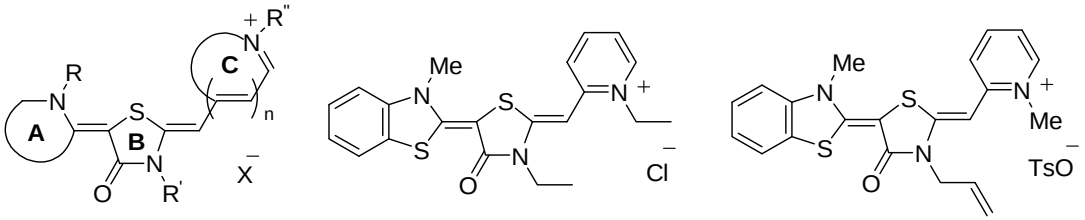



**Figure 1.** General and representative structures of rhodacyanine dyes

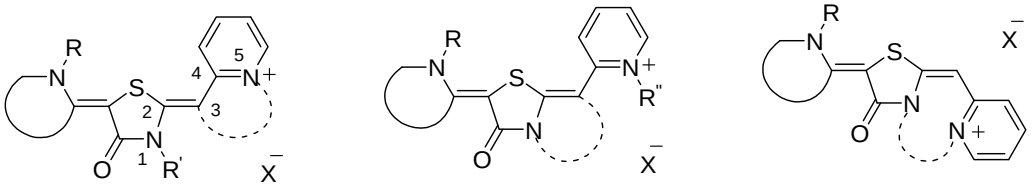
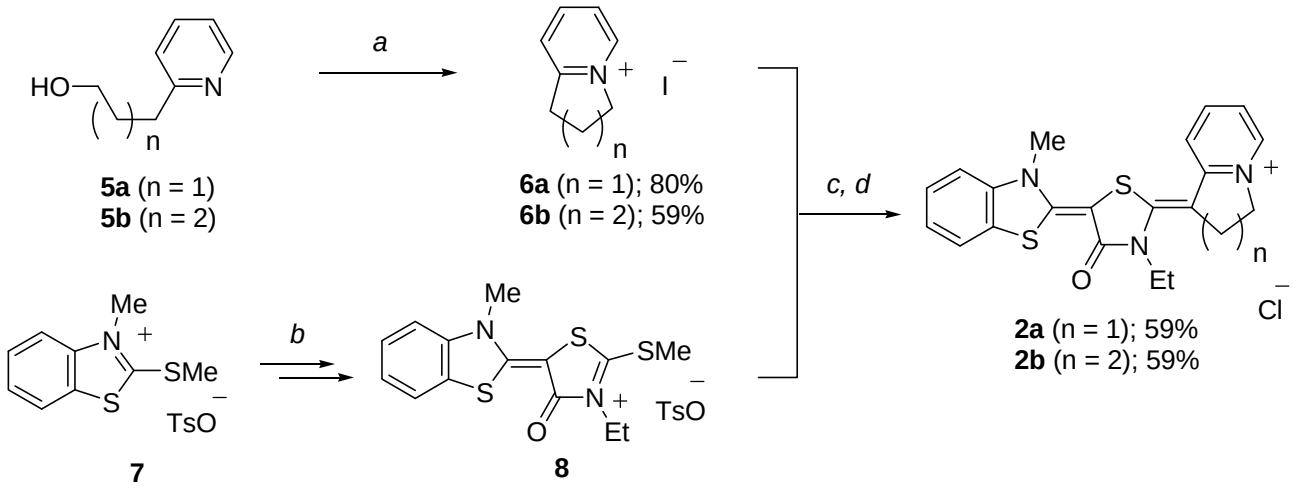



**Figure 2**. Our designed structurally rigidified rhodacyanine derivatives

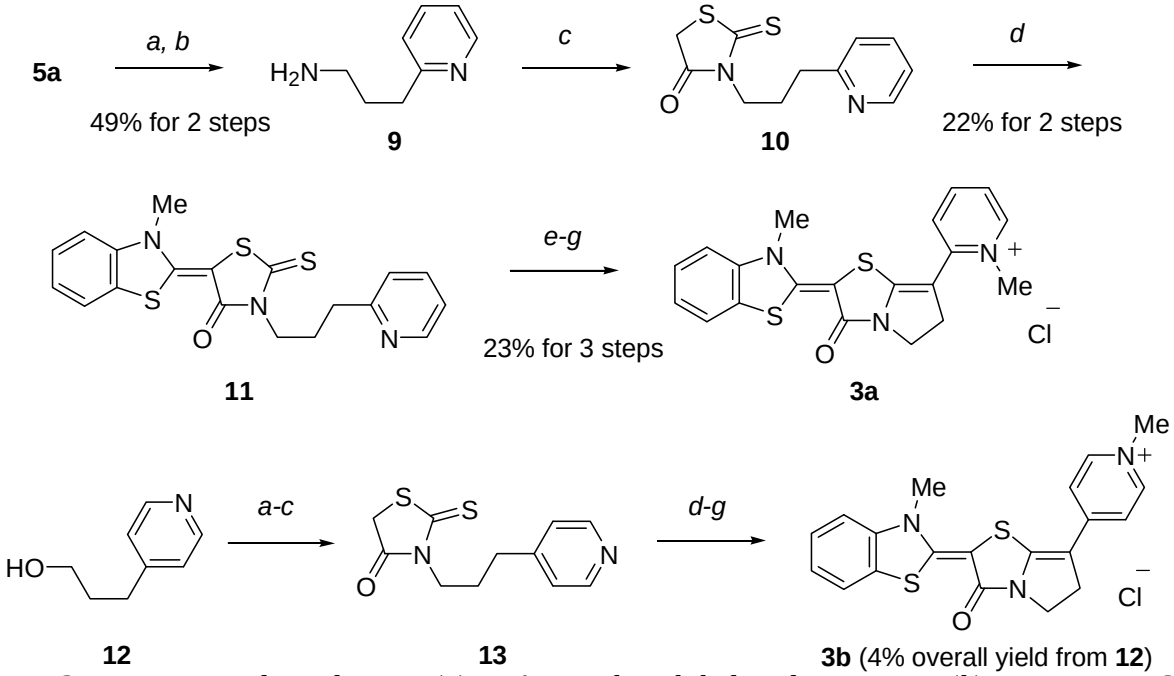
The synthetic route to afford a series of rhodacyanine (**2**) is shown in Scheme 1. Dihydroindolizinium salt (**6a**) and dihydroquinolizinium salt (**6b**) were prepared from 2-pyridinepropanol (**5a**) and 2-pyridinebutanol (**5b**),[7] respectively, by the treatment with iodine in the presence of triphenylphosphine and imidazole. According to the reported procedure,[2,6] reaction of **6a** and **6b** with thioiminium salt (**8**), which was easily prepared from benzothiazolium (**7**), and subsequent ion exchange process using Amberlyte® IRA-400 furnished rhodacyanines (**2a** and **2b**), respectively, in moderate yields.

Preparation of rhodacyanines (**3**) was achieved by the following procedure as shown in Scheme 2. First we synthesized rhodanine (**10**) containing 2-pyridine side chain from **5a** in 3 steps. Namely, Mitsunobu reaction of **5a** with phthalimide and deprotection by hydrazine afforded 2-pyridylpropylamine (**9**). Rhodanine (**10**) was obtained by three-component reaction of **9** with methyl bromoacetate and carbon disulfide[8] in good yield. Next, condensation of **10** with **7** smoothly proceeded to give merocyanin (**11**), which was then transformed into rhodacyanine (**3a**)[9] by methylation, intramolecular condensation to construct pyrrolo[2,1-*b*]thiazolone skeleton, and anion exchange. Rhodacyanine (**3b**) was also synthesized from 4-pyryidinepropanol (**12**) *via* **13** by the same reaction sequence as above.

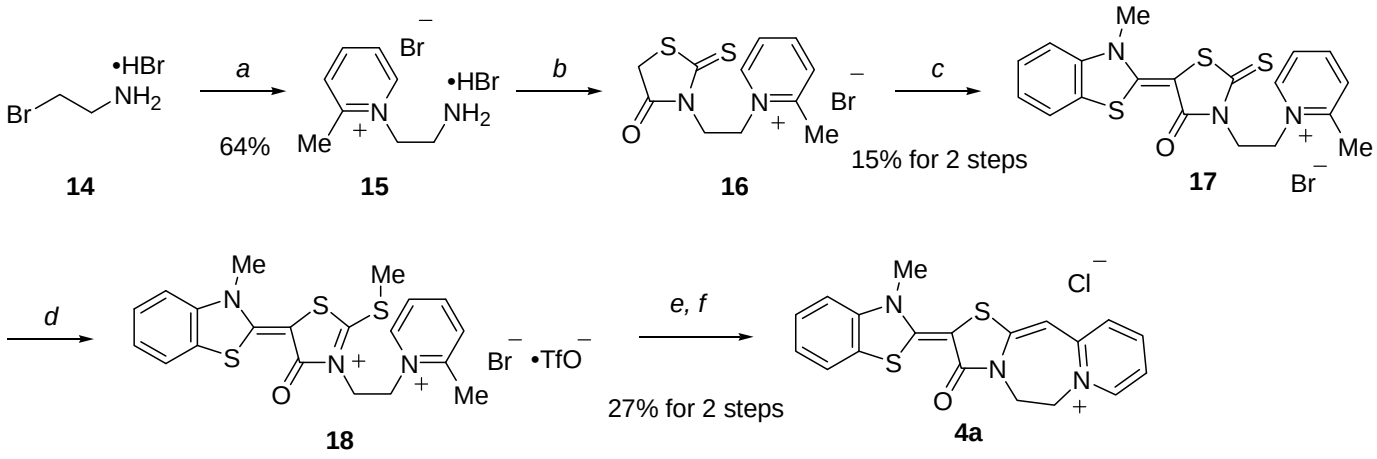
Scheme 3 summarizes the synthetic route affording rhodacyanine (**4a**), in which rhodanine and pyridine rings were interlocked by ethylene tether. According as the aforementioned strategy, we started with the preparation of rhodanine (**16**) from bromoethylamine hydrobromide (**14**) *via* picolinium salt (**15**). Although the reaction of **16** with **7** smoothly occurred to furnish merocyanine (**17**), its isolation yield was resulted in poor (15% yield) due to the difficulty of its purification from the crude mixture. Next critical




**Scheme 1.** Reagents and conditions; (*a*) $PPh_3$, $I_2$, imidazole, toluene, rt, (*b*) *see* ref. 2. (*c*) $NEt_3$, MeCN, 70 °C. (*d*) Amberlyte® IRA-400.




**Scheme 2.** Reagents and conditions; (*a*) DIAD, $PPh_3$, phthalimide, THF, rt. (*b*) $H_2NNH_2$, EtOH, reflux. (*c*) $BrCH_2CO_2Me$, $CS_2$, $NEt_3$, MeOH, rt. (*d*) **7**, $NEt_3$, MeCN, rt. (*e*) TsOMe, DMF, 80 °C. (*f*) $NEt_3$, MeCN, 70 °C. (*g*) Amberlyte® IRA-400.




**Scheme 3**. Reagents and conditions; (*a*) 2-picoline, 30 °C. (*b*) $BrCH_2CO_2Me$, $CS_2$, $NEt_3$, MeOH, rt. (*c*) **7**, $NEt_3$, MeCN, rt. (*d*) TfOMe, DMF, 100 °C. (*e*) $NEt_3$, MeCN, 70 °C. (*f*) Amberlyte® IRA-400.

issue is the activation of thioimide moiety of **17** to transform into the corresponding thioiminium cation. However, the reaction of **17** with methyl *p*-toluenesulfonate, which is the general activating method for the synthesis of rhodacyanine, was unsuccessful under any conditions. Finally, we found more harsh conditions using methyl trifluoromethanesulfonate at 100 °C in DMF to provide **18**. Subsequent intramolecular condensation reaction of **18** in the presence of triethylamine, followed by ion exchange, furnished the desired rhodacyanine (**4a**).[10]

Next, we examined *in vitro* antimalarial activity against *P. falciparum* K1 (chloroquine-resistant) of the selected compounds according to published methods.[11] Their cytotoxicity was also examined against L-6 (a rat skeletal myoblast cell line). The selective index, defined by the ratio $EC_{50}$ (L-6) / $EC_{50}$ (*P. falciparum*), was determined. There has been no information of the antimalarial efficacy of rhodacyanine dyes against drug-resistant strain, so that it is first time to report their activity against chloroquine-resistant parasites. The biological profiles of the tested compounds are shown in Table 1. Compounds (**1a** and **1b**) showed 10-fold stronger inhibitory effect against *P. falciparum* K1 than chloroquine. Their $EC_{50}$ values against chloroquine-resistant strains are comparable with ones against drug-sensitive strains.[2,3] Structurally stiff rhodacyanines (**3a** and **4a**) also displayed strong antimalarial activity with $EC_{50}$ values of $6.2 \times 10^{-8}$ and $4.6 \times 10^{-8}$ M, but, contrary to our expectations, their $EC_{50}$ values against *P. falciparum* K1 are slightly less than those of structurally flexible compounds (**1a** and **1b**). Moreover, cytotoxicity of **3a** and **4a** is not so different from one of **1a** and **1b**. These results indicate that the conformational rigidity concerning the cyanine moiety of rhodacyanines would almost not affect on the antimalarial efficacy.

**Table 1.** Antimalarial activity and cytotoxicity of rhodacyanines.

| compound | $EC_{50}$ (M) [*P. falciparum*] | $EC_{50}$ (M) [L-6 cells] | selective index |
|---|---|---|---|
| **1a** | $2.1 \times 10^{-8}$ [a] | $1.1 \times 10^{-4}$ | $5.2 \times 10^{3}$ |
| **1a** | $7.0 \times 10^{-8}$ [b] | $1.1 \times 10^{-4}$ | $1.6 \times 10^{3}$ |
| **1b** | $1.9 \times 10^{-8}$ [a] | $1.0 \times 10^{-4}$ | $5.3 \times 10^{3}$ |
| **1b** | $1.2 \times 10^{-8}$ [b] | $1.0 \times 10^{-4}$ | $8.3 \times 10^{3}$ |
| **3a** | $6.2 \times 10^{-8}$ [a] | $1.6 \times 10^{-4}$ | $2.6 \times 10^{3}$ |
| **4a** | $4.6 \times 10^{-8}$ [a] | $1.5 \times 10^{-4}$ | $3.3 \times 10^{3}$ |
| chloroquine | $1.5 \times 10^{-7}$ [a] | $4.7 \times 10^{-5}$ | $3.2 \times 10^{2}$ |

[a] chloroquine-resistant *P. falciparum* K1. [b] drug-sensitive *P. falciparum* FCR-3 (*see* ref. 2).

In summary, we synthesized novel rhodacyanines (**2-4**), whose conformations are stiffened by the ring fusion, and evaluated their antimalarial efficacy. It is first time to report the antimalarial activity of rhodacyanines against chloroquine-resistant *P. falciparum*. Although no improvement on the biological properties is observed by the structural rigidification, we found the novel rhodacyanines (**3a** and **4a**) also displayed strong antimalarial activity with high selective index. We believe these new rhodacyanines would be alternative candidates for antimalarial lead compounds.

**ACKNOWLEDGEMENTS**

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9. Spectral data for **3a**; $^1$H-NMR (300 MHz, DMSO-$d_6$) δ 8.57 (1H, d, $J$ = 6.1 Hz), 8.16 (1H, t, $J$ = 7.6 Hz), 7.75 (2H, m), 7.51 (4H, m), 4.13 (3H, s), 4.04 (2H, t, $J$ = 8.2 Hz), 3.93 (3H, s), 3.59 (2H, d, $J$ = 8.2 Hz); IR (KBr) ν 3566, 2355, 1697, 1508, 1473, 1418 cm$^{-1}$; FABMS (*m*/*z*) 380 ($M^+$).
10. Spectral data for **4a**; $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 8.28 (1H, d, $J$ = 6.4 Hz), 8.01 (1H, t, $J$ = 8.0 Hz), 7.88 (1H, d, $J$ = 7.8 Hz), 7.56 (2H, m), 7.49 (1H, t, $J$ = 7.3 Hz), 7.30 (2H, m), 6.23 (1H, s), 5.16 (1H, br dd), 5.05 (1H, br dd), 4.24 (1H, br dd), 4.00 (3H, s), 3.69 (1H, br dd); IR (KBr) ν 3363, 2314, 1651, 1624, 1581, 1489, 1419, 1357, 1344, 1302 cm$^{-1}$; FABMS (*m*/*z*) 382 ($M^+$).
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