

HETEROCYCLES, Vol. 66, 2005, pp. 161 – 166. © The Japan Institute of Heterocyclic Chemistry
Received, 3rd October, 2005, Accepted, 7th November, 2005, Published online, 11th November, 2005. COM-05-S(K)67

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.

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*.

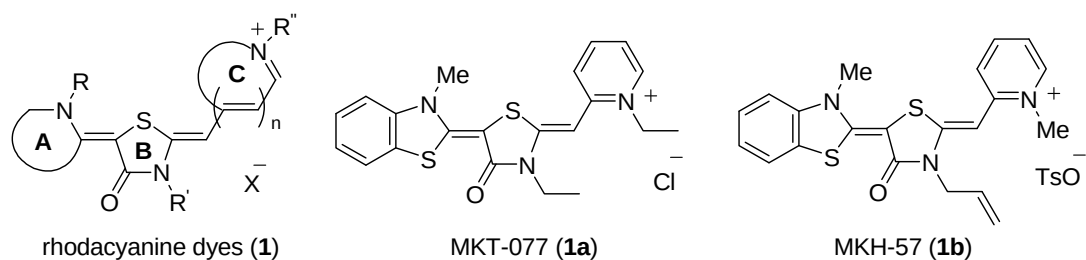


Figure 1. General and representative structures of rhodacyanine dyes

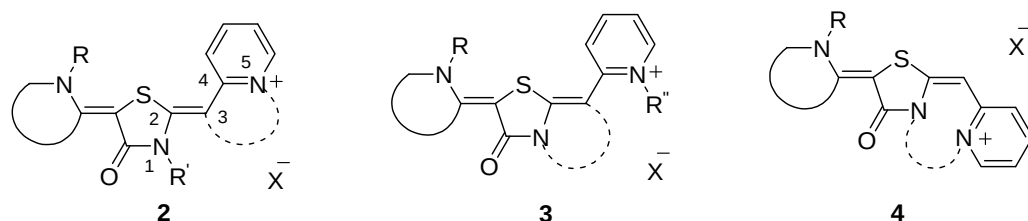
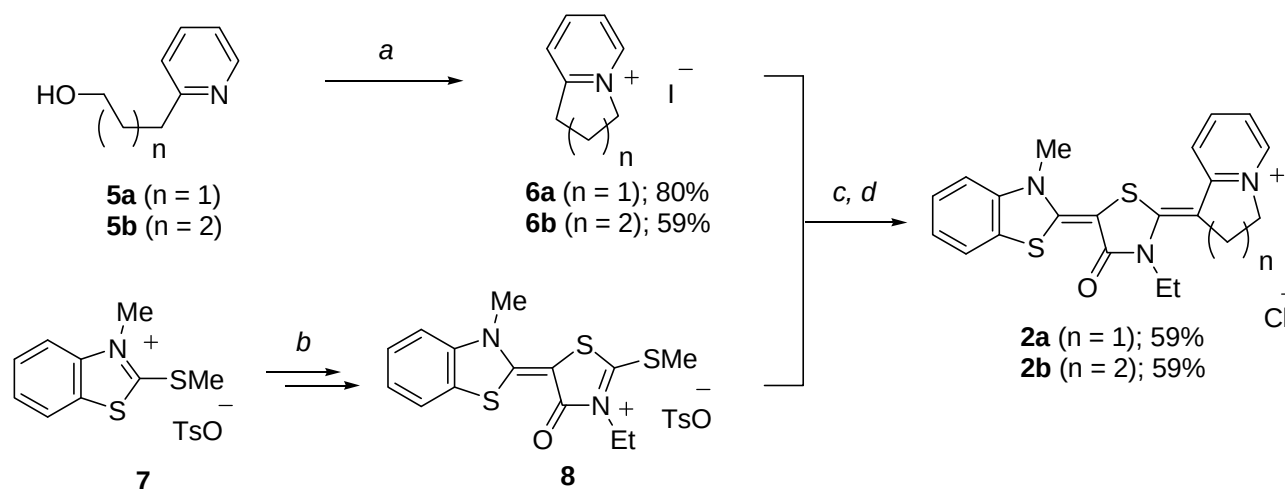


Figure 2. Our designed structurally rigidified rhodacyanine derivatives

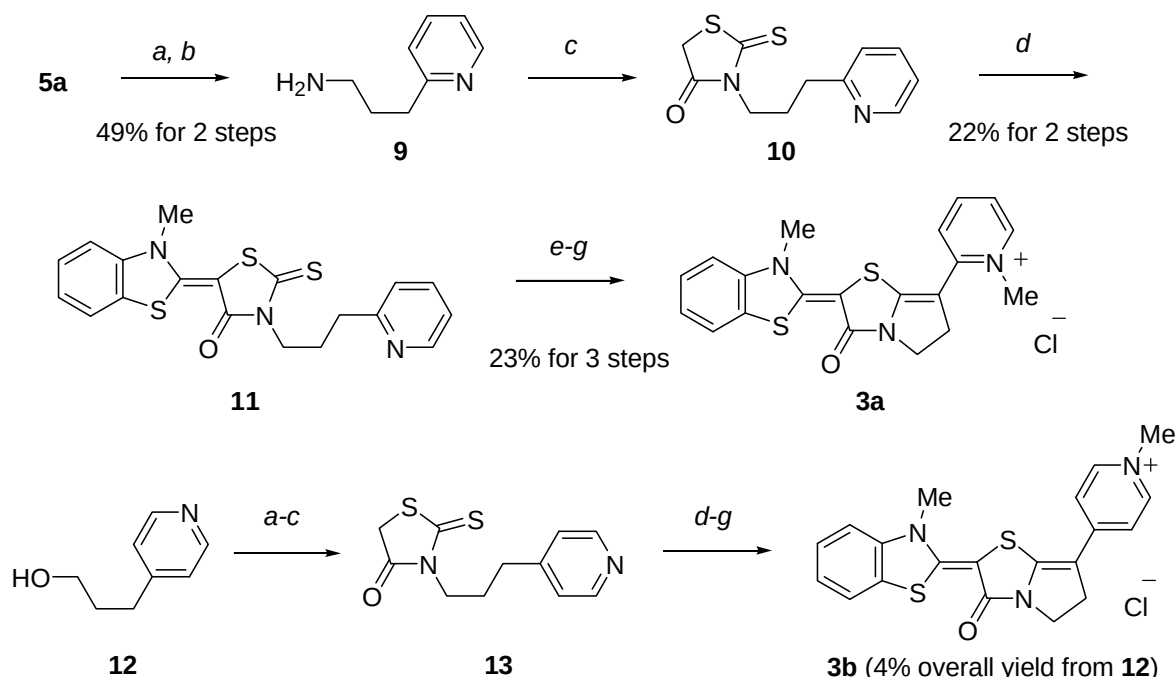
The synthetic route to afford a series of rhodacyanine (**2**) is shown in Scheme 1. Dihydroindolizinium salt (**6a**) and dihydroquinolizinium salt (**6b**) were prepared from 2-pyridinepropanol (**5a**) and 2-pyridinebutanol (**5b**),⁷ respectively, by the treatment with iodine in the presence of triphenylphosphine 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 disulfide⁸ 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-pyridinepropanol (**12**) via **13** by the same reaction sequence as above.

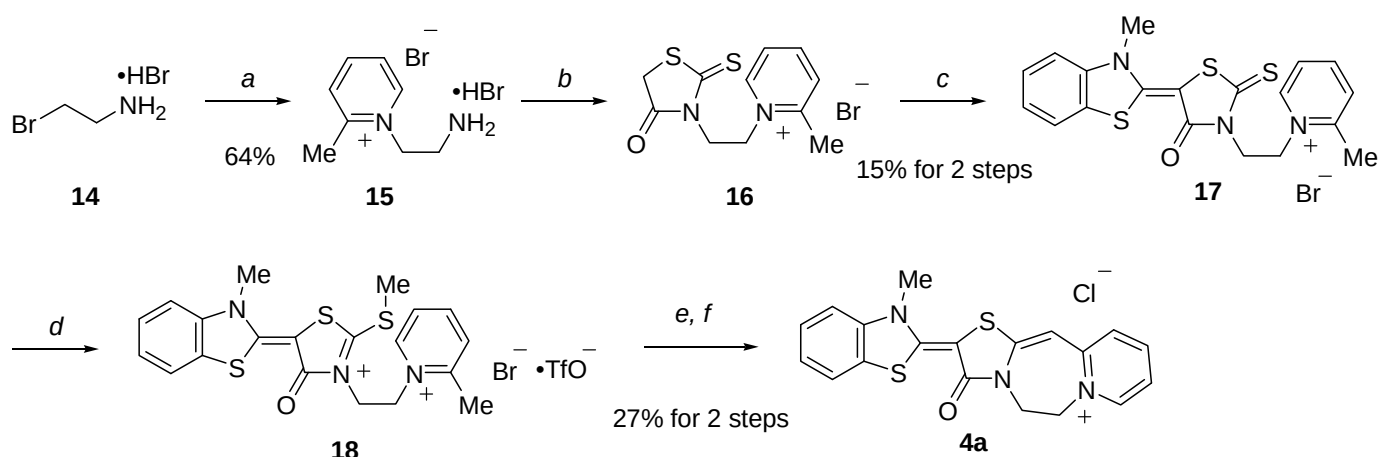
Scheme 3 summarizes the synthetic route affording rhodacyanine (**4a**), in which rhodanine and pyridine rings were interlocked by ethylene tether. According as the aforementioned strategy, we started with the preparation of rhodanine (**16**) from bromoethylamine hydrobromide (**14**) via picolinium 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) $\text{BrCH}_2\text{CO}_2\text{Me}$, 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) BrCH₂CO₂Me, CS₂, NEt₃, MeOH, rt. (c) **7**, NEt₃, MeCN, rt. (d) TfOMe, DMF, 100 °C. (e) NEt₃, 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 EC₅₀ (L-6) / EC₅₀ (*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 EC₅₀ 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 EC₅₀ values of 6.2 × 10⁻⁸ and 4.6 × 10⁻⁸ M, but, contrary to our expectations, their EC₅₀ 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) [<i>P. falciparum</i>]	EC ₅₀ (M) [L-6 cells]	selective index
1a	2.1 x 10 ⁻⁸ ^a	1.1 x 10 ⁻⁴	5.2 x 10 ³
1a	7.0 x 10 ⁻⁸ ^b	1.1 x 10 ⁻⁴	1.6 x 10 ³
1b	1.9 x 10 ⁻⁸ ^a	1.0 x 10 ⁻⁴	5.3 x 10 ³
1b	1.2 x 10 ⁻⁸ ^b	1.0 x 10 ⁻⁴	8.3 x 10 ³
3a	6.2 x 10 ⁻⁸ ^a	1.6 x 10 ⁻⁴	2.6 x 10 ³
4a	4.6 x 10 ⁻⁸ ^a	1.5 x 10 ⁻⁴	3.3 x 10 ³
chloroquine	1.5 x 10 ⁻⁷ ^a	4.7 x 10 ⁻⁵	3.2 x 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

This work was financially supported by Grant-in-Aids for Technology Transfer Facilitation Program from Japan Science and Technology Agency (JST) and the Takeda Science Foundation.

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 9. Spectral data for **3a**; $^1\text{H-NMR}$ (300 MHz, $\text{DMSO-}d_6$) 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**; $^1\text{H-NMR}$ (400 MHz, $\text{DMSO-}d_6$) 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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