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Letters

Rhodacyanine Dyes as Antimalarials. 1. Preliminary Evaluation of Their Activity and Toxicity

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Abstract: The rhodacyanine dye MKT-077 (**1**), a potent antitumor agent, was found to possess strong in vitro activity against *Plasmodium falciparum* and a low cytotoxicity. Several new rhodacyanine dyes related to **1**, containing a variety of linked heterocyclic moieties, were synthesized, and their antimalarial potencies were evaluated. The synthetic rhodacyanines were found to have EC_{50} values against *P. falciparum* in vitro in the range of 4−300 nM. Several compounds in this series have remarkable selective toxicity profiles (>100).

Malaria is the most serious and widespread parasitic disease in tropical and subtropical zones. This disease is estimated to cause millions of deaths each year with most occurring in infants and young children. Recently, even inhabitants of temperate zones may be exposed to the danger of malaria infection owing to global warming. In addition, the appearance and rapid spread of drug-resistant malaria parasites has reduced the effectiveness of commonly used chemotherapeutic agents.[1−3] Therefore, the development of new families of antimalarial compounds has become a worldwide issue. New antimalarials, which have novel mechanisms of action, are of great importance in this regard since these substances will likely be effective in the treatment of patients infected with drug-resistant parasites.[4]

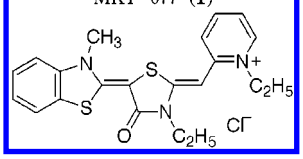
Table 1. Antimalarial Activity and Cytotoxicity

compd	EC_{50} (M) *P. falciparum*[a]	EC_{50} (M) FM3A[b]	selective toxicity[c]
quinine	1.1×10^{-7}	1.0×10^{4}	910
chloroquine	1.8×10^{-8}	3.2×10^{-5}	1800
methylene blue	1.7×10^{-8}	1.1×10^{-6}	65
rhodamine 123	3.0×10^{-7}	1.0×10^{-5}	33
MKT-077 (**1**)	7.0×10^{-8}	1.5×10^{-5}	210

[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*.

Broad screening of a number of heterocycles and carbohydrates in our compound library[5−7] indicated that several substances containing delocalized cationic moieties have good antimalarial activity (Table 1). Particularly significant was the finding that the rhodacyanine dye MKT-077 (**1**)[8] displays strong activity (EC_{50} = 7.0 × 10^{-8} M) in vitro against the erythrocytic stage of *Plasmodium falciparum* and a good selective toxicity (210). These properties make this dye nearly as potent as traditional antimalarial drugs, such as chloroquine and quinine (Table 1). Recently, **1** has been developed as a novel anticancer agent, and it has been subjected to further clinical investigation for the treatment of solid tumors.[9,10] In this communication, we describe preliminary results of our studies concentrating on the synthesis of rhodacyanine dyes related to **1** and an evaluation of the antimalarial activity of these substances in vitro against *P. falciparum*.

MKT-077 (**1**)



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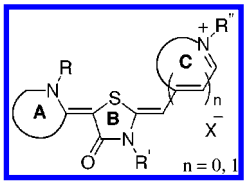
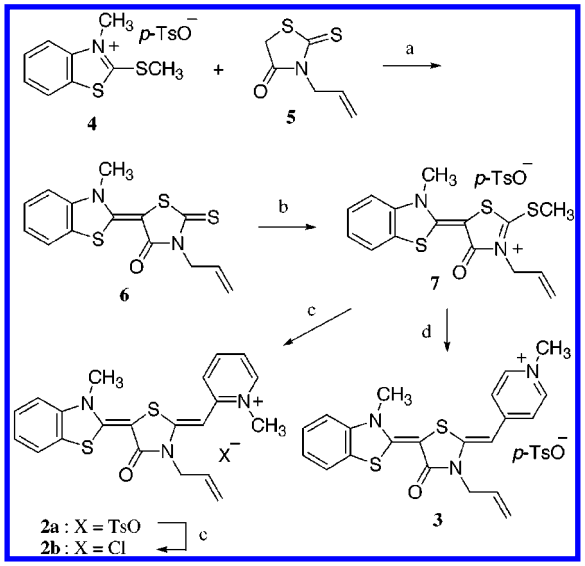


Figure 1. General formula of the rhodacyanine dye.

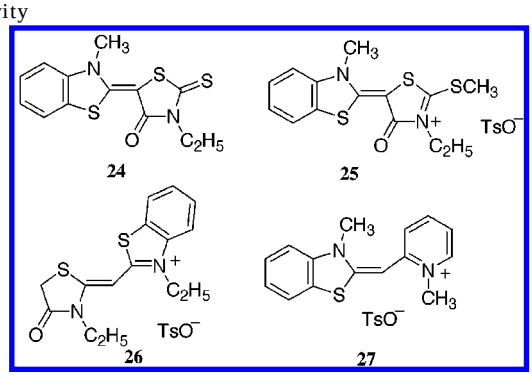
Scheme 1[a]



[a] Conditions: (a) NEt_3, CH_3CN, 0 °C; (b) *p*-TsOMe, DMF, 120 °C; (c) 1,2-dimethylpyridinium *p*-toluenesulfonate, CH_3CN, NEt_3 70 °C; (d) 1,4-dimethylpyridinium *p*-toluenesulfonate, CH_3CN, NEt_3 70 °C; (e) Amberlite IRA-400 (Cl).

In general, rhodacyanine dyes consist of three, linearly linked heterocyclic groups, in which two end heteroaromatic rings (**A** and **C**, Figure 1) flank a rhodanine (4-oxothiazolidine) **B**-ring. The dyes are double conjugates of two different dye units, having left and right parts comprised of neutral merocyanine and cationic cyanine structures. MKT-077 (**1**), a member of this family, was previously synthesized by means of sequential condensation of its **A** and **B** components followed by addition of the **C**-ring moiety. Following this general strategy (with some modifications),[8,11] rhodacyanines **2**, **3**, **8**−**23** were prepared. The sequence, shown in Scheme 1 to prepare **2** and **3**, typifies the procedures used. Accordingly, condensation of thiazolium salt **4**, prepared from 2-(methylthio)benzothiazole and methyl *p*-toluenesulfonate, with 3-allylrhodanine (**5**) in the presence of triethylamine at 0 °C affords the neutral merocyanine **6** (1 h) in 78% yield (from **5** for two steps). S-Methylation of **6** is accomplished by reaction with methyl *p*-toluenesulfonate at 120 °C for 1.5 h and gives **7** in 99% yield. Treatment of **7** with 1,2-dimethylpyridinium *p*-toluenesulfonate in the presence of triethylamine at 70 °C for 1.5 h then provides the desired rhodacyanine **2a** as bright orange crystals in 41% yield. Treatment of **2a** with the chloride ion form of ion-exchange resins, such as Amberlite IRA-400, gives the chloride salt **2b** quantitatively. Rhodacyanine **3** was obtained by use of this methodology starting with **7** and 1,4-dimethylpyridinium *p*-toluenesulfonate. To see if smaller structural components of these substances are responsible for their biological activity, we prepared merocyanine dyes **24** and **25** and cyanine **26** by using

Table 2. Effect of Skeletal Composition on Antimalarial Activity



compd	framework	*P. falciparum* EC_{50} (M)
1	**A-B-C**	7.0×10^{-8}
24	**A-B**	$>2.8 \times 10^{-5}$ [a]
25	**A-B**	3.7×10^{-6}
26	**B-C**	5.2×10^{-7}
27	**A-C**	2.4×10^{-7}

[a] EC_{32} value (68% growth of *P. falciparum* was observed).

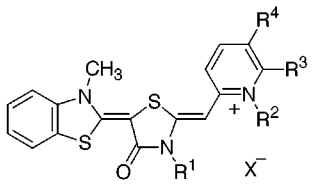
reported procedures (structures see Table 2).[11] Each of these substances lack one of the three ring systems found in **1**. Cyanine **27**, which does not contain a rhodanine (**B**) ring, was also prepared by the condensation of **4** and 1,2-dimethylpyridinium *p*-toluenesulfonate in the presence of triethylamine (44% yield).

The antimalarial activities of the synthetic compounds were evaluated in vitro against *P. falciparum* (chloroquine sensitive FCR-3 strain), and their cytotoxicities were determined against mouse mammary tumor FM3A cells.[12] Selective toxicities, defined by the ratio EC_{50}(FM3A)/EC_{50}(*P. falciparum*), were determined.

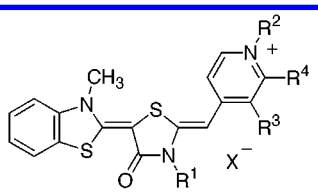
The requirement for each of the heterocyclic components of the rhodacyanine dyes (Table 2) was evidenced by the very low (EC_{50} values of less than 10^{-6} M) antimalarial activities of merocyanines **24** and **25**. Cyanines **26** and **27**, which have no merocyanine conjugation, display moderate activity against *P. falciparum*, but their potency is lower than that of MKT-077 (**1**). Thus, tricyclic-rhodacyanine structures, containing both merocyanine and cyanine conjugation, are required for high antimalarial activity.

The antimalarial potencies of rhodacyanines, which contain an **A**-ring 2-benzothiazole moiety, are summarized in Table 3. Compound **8a**, which is the *N*-methylpyridinium analogue of **1**, displays enhanced antimalarial activity (EC_{50} value of 2.6×10^{-8} M), although its cytotoxicity is the same as that of **1**. Four rhodacyanine dyes (**8a**−**d**) with different counteranions were found to have similar activities. Cytotoxicities against FM3A cells in vitro are significantly altered by changing the central oxothiazolidine ring N-substituent. Replacement of ethyl substituents by methyl or benzyl leads to 3-fold (**8d** vs **11**) or 14-fold (**8d** vs **12**) increases in toxicity, respectively, although the antimalarial activities of these substances are only slightly decreased by this substitution. In contrast, the *N*-allylrhodacyanine **2a** has low toxicity (EC_{50} value of 1.2×10^{-5} M) and a high inhibitory effect on malaria parasites ($EC_{50} = 1.2 \times 10^{-8}$ M). Its selective toxicity is estimated to be 1000.

Table 3. Effect of Substituents and Counteranion on Antimalarial Activity and Cytotoxicity



2, 8-15

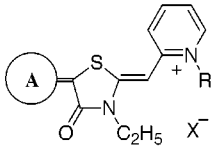


3, 16-18

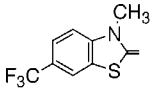
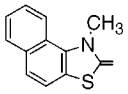
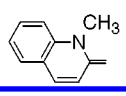
compound	R^1	R^2	R^3	R^4	X	EC_{50} (M) *P. falciparum* [a]	EC_{50} (M) FM3A [b]	selective toxicity [c]
8a	C_2H_5	CH_3	H	H	Cl	2.6×10^{-8}	1.6×10^{-5}	620
8b	C_2H_5	CH_3	H	H	Br	2.8×10^{-8}	1.4×10^{-5}	500
8c	C_2H_5	CH_3	H	H	OH	2.2×10^{-8}	1.3×10^{-5}	590
8d	C_2H_5	CH_3	H	H	OTs	2.3×10^{-8}	1.1×10^{-5}	480
9	C_2H_5	$(CH_2)_2OH$	H	H	Br	2.1×10^{-7}	$> 1.7 \times 10^{-5}$ [e]	> 81
10	C_2H_5	$(CH_2)_4CO_2H$	H	H	Cl	6.8×10^{-7}	$> 1.8 \times 10^{-5}$ [f]	> 26
11	CH_3	CH_3	H	H	OTs	4.5×10^{-8}	3.8×10^{-6}	84
12	CH_2Ph	CH_3	H	H	OTs	3.1×10^{-8}	8.0×10^{-7}	26
2a	$CH_2CH{=}CH_2$	CH_3	H	H	OTs	1.2×10^{-8}	1.2×10^{-5}	1000
2b	$CH_2CH{=}CH_2$	CH_3	H	H	Cl	$< 3.8 \times 10^{-8}$ [d]	NT [g]	—
13	$(CH_2)_4CO_2H$	C_2H_5	H	H	OTs	1.5×10^{-6}	$> 1.7 \times 10^{-5}$ [h]	> 11
14	C_2H_5	CH_3	Ph	H	I	5.6×10^{-8}	1.9×10^{-6}	34
15	C_2H_5	CH_3			I	4.6×10^{-9}	5.8×10^{-7}	130
16	C_2H_5	CH_3	H	H	OTs	5.6×10^{-8}	$> 2.4 \times 10^{-5}$ [i]	> 430
3	$CH_2CH{=}CH_2$	CH_3	H	H	OTs	1.4×10^{-8}	6.7×10^{-6}	480
17	CH_2Ph	CH_3	H	H	OTs	5.0×10^{-8}	8.1×10^{-7}	16
18	C_2H_5	CH_3			OTs	7.8×10^{-9}	6.8×10^{-7}	87

[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_{70} value (30% growth of *P. falciparum* was observed). [e] EC_9 value (91% growth of FM3A cells was observed). [f] EC_{17} value (83% growth). [g] NT means not tested. [h] EC_{19} value (81% growth). [i] EC_{36} value (64% growth).

Table 4. Effect of Ring **A** on Antimalarial Activity and Cytotoxicity



19-23

compound	A	R	X	EC_{50} (M) *P. falciparum*	EC_{50} (M) FM3A	selective toxicity
19		C_2H_5	I	6.6×10^{-8}	4.2×10^{-6}	64
20		$(CH_2)_2OH$	Cl	4.3×10^{-8}	5.0×10^{-6}	120
21		CH_3	OTs	2.3×10^{-7}	$> 3.4 \times 10^{-5}$ [a]	>150
22		CH_3	OTs	3.5×10^{-7}	NT [b]	—
23		CH_3	OTs	2.4×10^{-7}	NT [b]	—

[a] EC_{14} value (86% growth of FM3A cells was observed). [b] NT means not tested.

The introduction of hydrophilic groups, such as hydroxyl and carboxyl, as R^1 or R^2 substituent, results in a 1−2 order of magnitude decrease in activity (e.g., **9**, **10**, **13**). This result points out that hydrophobicity is a critical factor determining the antimalarial activity of compounds in this family. The quinolinium ring containing dye **15** displays the most potent inhibitory activity ($EC_{50} = 4.6 \times 10^{-9}$ M), but the cytotoxicity of this substance is very high. Hydrophobic aromatic residues on the dye skeleton (e.g., **12**, **14**, **15**) cause increases in cytotoxicity but not in antimalarial activity. Thus, it appears that an appropriate balance needs to be struck between hydrophilicity and hydrophobicity in order to maximize the efficacy of the rhodacyanines. Dyes containing a 1,4-pyridinium **C**-ring moiety (e.g., **3**, **16**−**18**) have nearly the same activities as the corresponding dyes containing the 1,2-pyridinium grouping (e.g., **2a**, **8d**, **12**, and **15**).

The effect of the merocyanine component was also examined (Table 4). Compounds **19** and **20**, which contain substituted benzothiazole **A**-rings, have strong antimalarial activities with EC_{50} values of 10^{-8} M. However, other analogues with thiazoline, pyridine, and quinoline **A**-rings (e.g., **21**−**23**) are ca.10-fold less active against *P. falciparum* than **8d**. Thus, a benzothiazole merocyanine unit is required for high antimalarial activity.

The preliminary studies described above have uncovered new types of rhodacyanine dyes, which display high levels of antimalarial activity. Structure−activity studies indicate that the rhodacyanine skeleton is essential for strong activity and that a balance between molecular hydrophilicity and hydrophobicity is important for efficacy. Finally, compound **2a** (named MKH-57) was found to display high antimalarial activity and a significantly good selective toxicity.

Currently, we are attempting to optimize the antimaliarial properties of the rhodacyanine dyes based on structure−activity relationships uncovered thus far and we are carrying out studies to gain information about the mechanism of antimalarial action of these substances.

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Supporting Information Available: Synthetic procedures and characterization data for compounds **2**, **3**, **6**−**9**, **11**, **12**, **14**−**18**, **21**−**23**, and **27**. This material is available free of charge via the Internet at http://pubs.acs.org.

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