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ANTILEISHMANIAL ACTIVITIES OF RHODACYANINE DYES

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Abstract – Rhodacyanine dyes, potent antimalarial agents, was found to possess strong antileishmanial activity against *Leishmania major in vitro*. The efficacies of several compounds are comparable to one of clinically used amphotericin B.

INTRODUCTION

In the tropical and sub-tropical area, parasitic diseases infected by protozoa, such as malaria, trypanosoma, filaria, and leishmania, prevent social and economical development. Leishmaniasis is a major tropical disease that is endemic in 88 countries with affection of 12 million people.[1] The disease is classified into three major types: cutaneous leishmaniasis (oriental sore), visceral leishmaniasis (Kala Azar), and mucocutaneous leishmaniasis (espundia). The leishmaniasis parasite is transmitted to humans by a phlebotomine sand fly. The therapeutic agents in use for the clinical treatment include pentavalent antimony compounds, such as sodium stibogluconate and meglumine antimoniate, as well as pantamidine and amphotericin B. However, these drugs present high toxicity and low availability, and they must be administered by injection over several days. Moreover, widespread of drug-resistance parasites becomes severe worldwide issue. Therefore, development of novel antileishmanial chemotherapeutics is the emergent subject.[2]

Recently, we have reported rhodacyanine dyes exhibit strong antimalarial activity *in vitro* against the *Plasmodium falciparum* parasite and excellent selective toxicity.[3] In general, rhodacyanines consist of three, linearly linked heterocycles, in which two end heteroaromatic rings edge a rhodanine (4-oxothiazolidine) ring (Figure 1). The dyes are double conjugates of two different dye units, having left

[†]Dedicated to Dr. Pierre Potier on the occasion of his 70th birthday.

and right parts comprised of neutral merocyanine and cationic cyanine structures. Our hypothesis for their biological action is that a π-delocalized lipophilic cationic (DLC) structure is important for strong antimalarial efficacies.[4-6] DLC compounds would selectively accumulate in the electronically negative organelle, such as mitochondria, of protozoal cells. We have envisaged that rhodacyanines also display antileishmanial activity by the same biological mechanism (DLC hypothesis[4]).

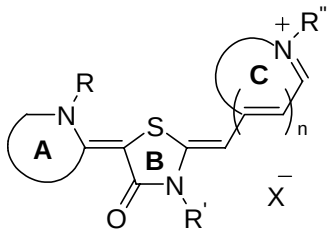
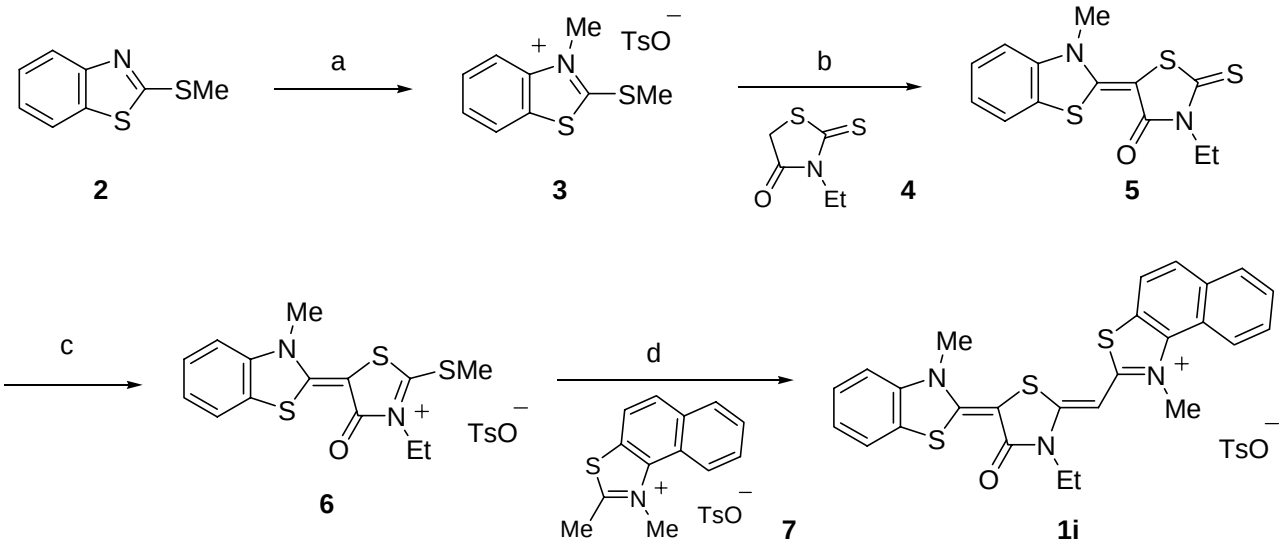


Figure 1. General structure of rhodacyanine dyes

RESULTS AND DISCUSSION

According to the reported procedure,[5b] rhodacyanines (**1a-o**) and their analogs (**8**, **9**) were prepared; typical synthetic procedure of **1i** was outlined in Scheme 1. Namely, condensation of thiazolium salt (**3**), which was prepared from 2-methylthiobenzothiazole (**2**), with 3-ethylrhodanine (**4**) in the presence of triethylamine afforded merocyanine (**5**) in 89% yield (2 steps). After *S*-methylation of **5**, treatment of the resulting thioiminium (**6**) with 2,3-dimethylnaphtho[1,2-*d*]thiazolium *p*-toluenesulfonate (**7**) in the presence of triethylamine provided the desired rhodacyanines (**1i**) in 56% yield (2 steps) as purple crystals.[7]

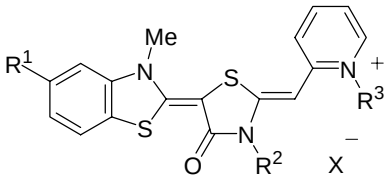


Scheme 1. Reagents and Conditions: (a) TsOMe, anisole, 120 °C; (b) **4**, NEt_3, MeCN, 10 °C (89% for 2 steps); (c) TsOMe, DMF, 130 °C; (d) **7**, NEt_3, MeCN, 70 °C (56% for 2 steps).

Tabele 1. Antileishmanial activity of synthetic rhodacyanines

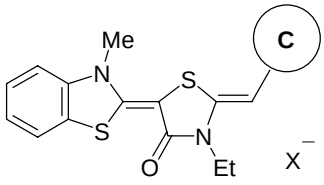
entry	compound	EC_{50} (M) against *L. major*	entry	compound	EC_{50} (M) against *L. major*
1	**1a** (MKT-077)	4.6×10^{-7}	10	**1j**	1.0×10^{-5}
2	**1b** (MKH-57)	3.2×10^{-7}	11	**1k**	1.8×10^{-6}
3	**1c**	4.1×10^{-7}	12	**1l**	1.7×10^{-4}
4	**1d**	1.0×10^{-5}	13	**1m**	1.3×10^{-6}
5	**1e**	5.4×10^{-7}	14	**1n**	1.8×10^{-7}
6	**1f**	2.7×10^{-7}	15	**1o**	6.1×10^{-8}
7	**1g**	4.2×10^{-5}	16	**8**	4.9×10^{-7}
8	**1h**	8.2×10^{-7}	17	**9**	1.2×10^{-6}
9	**1i**	1.2×10^{-8}	18	amphotericin B	1.4×10^{-7}

structures of evaluated compounds



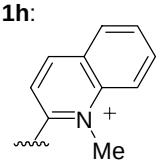
1a-g

	R^1	R^2	R^3	X
1a	H	Et	Et	Cl
1b	H	allyl	Me	TsO
1c	H	Bn	Me	TsO
1d	H	Et	$(CH_2)_4CO_2H$	Cl
1e	Cl	Et	Me	TsO
1f	Cl	allyl	Me	TsO
1g	OMe	Et	$(CH_2)_2OH$	Br



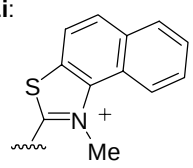
1h-k

1h:



X = I

1i:



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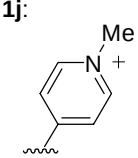
1j:



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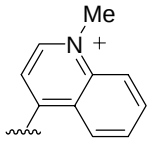
1k:



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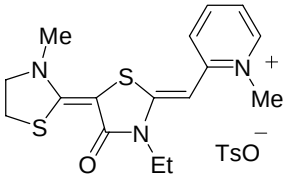
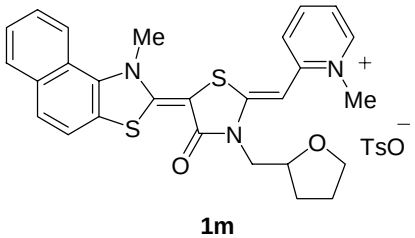
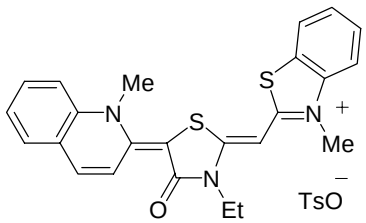


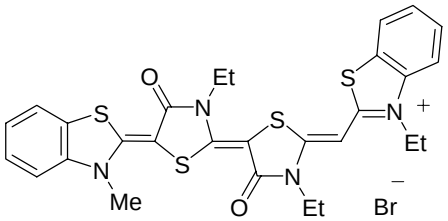
1l



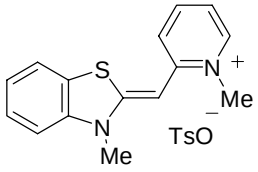
1m



1n



1o



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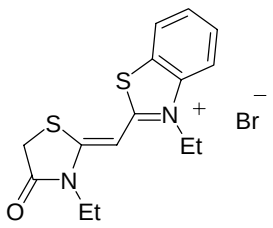

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The *in vitro* inhibitory efficacies of synthetic compounds (**1a-n**, **8** and **9**) against *Leishmania major*, which causes cutaneous leushmaniasis, were evaluated according to published methods.[2c] The results are shown in Table 1. The results were compared with amphotericin B as a positive control.

Rhodacyanine (**1a**; MKT-077), which was originally developed as an antitumor agent by Fuji Photo Film Company's group,[5b] shows strong inhibitory effect against *L. major* (EC_{50} value of 4.6×10^{-7} M) (entry 1, Table 1). Compound (**1b**; MKH-57), which was developed as a potent antimalarial candidate by us,[3a] also exhibits good antileishmanial efficacies (entry 2). Upon modification of substituents R^1–R^3, significant decrease was observed in activity by the introduction of hydrophilic group, such as carboxyl and hydroxyl functions (entries 4 and 7), whereas substitution by alkyl group or halogen atom results in no considerable difference in activity (entries 3, 5 and 6). Structure of two end heteroaromatic rings (A- and C-rings) intensely affects on antileishmanial activity. Generally rhodacyanines having many aromatic regions, such as **1i** and **1n**, displays stronger inhibitory effects rather than less aromatic compounds (entries 8–14). It has been found that rhodacyanine (**1i**) (EC_{50} value of 1.2×10^{-8} M) is 12-fold more active against *L. major* than amphotericin B (entry 9 versus 18). On the contrary, 4-pyridinium and 4-quinolinium compounds (**1j** and **1k**), result in a 1-2 order of magnitude decrease in activity (entries 10 and 11).

Investigation on the effect of the skeletal components gives an interesting result. The installation of another rhodanine unit, such as compound (**1o**), results in enhancement of inhibitory effect (entry 15) compared to **1a**, but no significant difference was observed in antileishmanial activity by the removal of the central rhodanine core (entry 16). On the other hand, compound (**9**), which lacks of a merocyanine conjugation, exhibits weaker activity (entry 17).

In summary, we have found that rhodacyainine dyes show a potent leishmanial activity. Among them, **1i** and **1o** exhibit stronger inhibitory effects against *L. major* than clinically used amphotericin B. Currently, we are attempting to optimize the antileishmanial properties of the rhodacyanine dyes based on structure-activity relationships uncovered thus far and we are carrying out *in vivo* assay.

EXPERIMENTAL

All melting points were determined on Yanaco micro melting point apparatus and are uncorrected. The ^{1}H NMR (300 MHz) spectra were recorded on a Varian Gemini 2000 spectrometer with tetramethylsilane as internal standard. Chemical shifts are given in ppm. IR spectra were measured on Shimadzu FTIR-8300 spectrometer. The UV-VIS spectra were recorded on a Beckman DU 640 spectrophotometer. MS spectra were determined with a JEOL JMS DX-303 or JMS AX-500 or JMS-700 mass spectrometer. Elemental analyses were performed on Yanagimoto MT-3. Because of deliquescence and hygroscopicity, correct elemental analyses for most of the compounds could only be obtained by factoring in partial hydration of these organic salts.

Typical procedure for the synthesis of rhodacyanine (1i).

3-Ethyl-5-(3-methyl-2(3*H*)-benzothiazolylidene)-2-thioxo-4-thiazolidinone (5). A mixture of 2-methylthiobenzothiazole (**2**) (2.98 g, 16.4 mmol), methyl *p*-toluenesulfonate (3.7 mL, 24.6 mmol), and anisole (4.1 mL) was stirred at 120 ˚C for 4 h. After the mixture including thiazolium salt (**3**) was cooled to rt, acetonitrile (60 mL) was poured onto the mixture. To the resulting mixture were added 3-ethylrhodanine (**4**) (2.67 g, 16.6 mmol) and acetonitrile (3.6 mL). To this mixture was added triethylamine (3.6 mL, 25.8 mmol) dropwise under 10 ˚C with constant stirring and cooling, and the resulting mixture was stirred at 10 ˚C for 4 h. The yellow precipitate was collected and washed with acetonitrile to give **5** (4.52 g, 89% yield) as yellow crystals. The spectral data were identical with reported ones.[5b]

3-Ethyl-4,5-dihydro-5-(3-methyl-2(3*H*)-benzothiazolylidene)-2-methylthioxo-4-thiazolium *p*-toluenesulfonate (6). A mixture of **5** (2.10 g, 6.8 mmol), methyl *p*-toluenesulfonate (3.1 mL, 20.7 mmol), and *N*, *N*-dimethylformamide (2.3 mL) was stirred for 2.5 h at 130 ˚C. After the mixture was cooled to rt, acetone was added. The precipitate was collected and washed with acetone to give **6** (3.0 g, 90% yield) as orange crystals. The spectral data were identical with reported ones.[5b]

2-[3-Ethyl-{5-(3-methyl-2(3*H*)-benzothiazolylidene)-4-oxo-2-thiazolydinylidene}methyl]-1-methyl-1-naphtho[1,2-*d*]thiazolium *p*-toluenesulfonate (1i). To a mixture of **6** (341 mg, 0.57 mmol) and 1,2-dimethylnaphtho[1,2-*d*]thiazolium *p*-toluenesulfonate (224 mg, 0.58 mmol) in acetonitrile (2.8 mL) was dropwise added triethylamine (0.11 mL, 0.79 mmol) at 70 ˚C, and the mixture was stirred for 1 h at the same temperature. After the mixture was cooled to rt, ethyl acetate was poured onto the resulting mixture. The purple precipitate was collected and washed with ethyl acetate to give **1i** (280 mg, 62% yield) as purple crystals. mp >300 ˚C; IR (KBr) ν 1200, 1371, 1467, 1503, 1539, 1649, 3422 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.30 (3H, t, *J* = 7.1 Hz), 2.27 (3H, s), 4.12 (3H, s), 4.28 (2H, q, *J* = 7.1 Hz), 4.49 (3H, s), 6.78 (1H, s), 7.09 (2H, d, *J* = 8.0 Hz), 7.30 (1H, dd, *J* = 7.4, 7.7 Hz), 7.45-7.52 (3H, m), 7.45-7.79 (3H, m), 7.92 (1H, d, *J* = 7.7 Hz), 8.04 (1H, d, *J* = 8.8 Hz), 8.14 (1H, d, *J* = 8.2 Hz), 8.24 (1H, d, *J* = 8.8 Hz), 8.81 (1H, d, *J* = 8.5 Hz); *Anal. Calcd for* $C_{33}H_{29}N_3O_4S_4 \cdot 1.4H_2O$: C, 57.86; H, 4.68; N, 6.13. Found: C, 58.05, H, 4.80, N, 5.84.

2-[[3-Ethyl-5-(1-methyl-2(1*H*)-quinolinylidene)-4-oxo-2-thiazolidinylidene]methyl]-3-methylbenzothiazolium *p*-toluenesulfonate (1n). Purple needles (from MeOH), mp 277–280 °C; UV-VIS (MeOH): λ_{max} 543.5 nm (ε 7.57 x 10^4); ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.29 (3H, t, *J* = 7.0 Hz), 2.28 (3H, s), 4.05 (3H, s), 4.13 (3H, s), 4.26 (2H, q, *J* = 7.0 Hz), 6.71 (1H, s), 7.11 (2H, d, *J* = 7.7 Hz), 7.46 (2H, d, *J* =

7.7 Hz), 7.50–7.61 (2H, m), 7.72 (1H, dd, *J* = 6.8, 9.4 Hz), 7.85–7.99 (4H, m), 8.21 (2H, dd, *J* = 6.8, 8.8 Hz), 8.25–8.29 (1H, m); MS (FAB^+) *m/z*: 432 (M^+).

3-Ethyl-2-[[5-{5-(3-methyl-2(3*H*)-benzothiazolylidene)-4-oxo-3-ethyl-2-thiazolydinilidene}-4-oxo-3-ethyl-2-thiazolydinilidene]methyl]benzothiazolium bromide (1o). Deep purple solids, mp >300 ˚C; IR (KBr) ν 1003, 1065, 1154, 1200, 1275, 1318, 1478, 1539, 1641, 1664 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.28 (3H, t, *J* = 7.1 Hz), 1.34 (3H, t, *J* = 6.9 Hz), 1.42 (3H, t, *J* = 6.9 Hz), 4.10 (3H, s), 4.27 (4H, m), 4.65 (2H, m), 6.66 (1H, s), 7.26 (1H, dd, *J* = 7.4, 7.7 Hz), 7.35 (1H, dd, *J* = 7.4, 8.2 Hz), 7.49 (1H, d, *J* = 8.2 Hz), 7.57 (1H, dd, *J* = 7.6, 8.0 Hz), 7.72 (1H, dd, *J* = 7.6, 8.4 Hz), 7.87 (1H, d, *J* =7.7 Hz), 7.93 (1H, d, *J* = 8.4 Hz), 8.29 (1H, d, *J* = 8.0 Hz); *Anal. Calcd for* $C_{28}H_{27}N_4O_2BrS_4 \cdot 0.5H_2O$: C, 50.29; H, 4.22; N, 8.38. Found: C, 50.39; H, 4.35; N, 8.32.

Synthesis and characterization data for compounds (**1a-h, 1j-m** and **2-9**) were previously reported.[3a,3b,5b]

Cultivation of leishmania promastigotes

Medium 199 (Life Technologies Co., Ltd.) was used for cultigvation of promastigotes of *Leishmania major* (MHOM/SU/73/5ASKH). Promastigotes were cultured in medium at 27 °C, 5% CO_2 in an incubator.

***In vitro* antileishmanial activity assay**[2c]

The antileishmanial activities of synthetic compounds were assessed by an improved MTT method as follows. Cultured promastigotes were centrifuged at 600 *g* for 5 min at 4 °C. The parasites were resuspended in each culture and diluted to a density of 1 x 10^5/mL. *L. major* promastigotes were seeded at 0.5 x 10^4/50 μL in medium/well in a 96 well microplate. Then, further 50 μL medium with different concentrations of test compounds dissolved in DMSO were added into each well. Each concentration was tested in triplicate. As a positive control amphotericin B was investigated. The microplate was incubated at 27 °C in 5% CO_2 for 72 h. TetraColor ONE (Seikagaku Kogyo Co., Ltd.) was added into each well and the plates were incubated at 27 °C for 6 h. Optical density values (test wavelength 450 nm; reference wavelength 639 nm) were measured using a microplate reader (Molecular Devices Co., Ltd.).

ACKNOWLEDGEMENTS

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REFERENCES AND NOTES

1. World Health Organization. **2004**, http://www.who.int/tdr/diseases/leish/.
2. Several investigations towards antileishmanial agents have been reported. a) B. del Rey, A. C. Ramos, E. Caballero, A. Inchaustti, G. Yaluff, M. Medarde, A. R. de Arias, and A. S. Feliciano, *Bioorg. Med. Chem. Lett.*, 1999, **9**, 2711; b) M. C. A. Costa, L. C. G. Freitas, L. E. S. Barata, and Y. Takahata, *J. Mol. Struct.*, 2001, **543**, 147; c) H. Fuchino, T. Koide, M. Takahashi, S. Sekita, and M. Satake, *Planta Med.*, 2001, **67**, 647; d) G. Bhattacharya, M. M. Salem, and K. A. Werbovetz, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2395.
3. a) K. Takasu, H. Inoue, H.-S. Kim, M. Suzuki, T. Shishido, Y. Wataya, and M. Ihara, *J. Med. Chem.*, 2002, **45**, 995; b) K. Takasu, H. Terauchi, H. Inoue, H.-S. Kim, Y. Wataya, and M. Ihara, *J. Combi. Chem.*, 2003, **5**, 211.
4. The conceptual term, DLC, was originally proposed by Chen in their anticancer research work. L. B. Chen, *Ann. Rev. Cell. Biol.*, 1988, **4**, 155.
5. a) M. Kawakami, K. Koya, T. Ukai, N. Tatsuta, A. Ikegawa, K. Ogawa, T. Shishido, and L. B. Chen, *J. Med. Chem.*, 1997, **40**, 3151; b) M. Kawakami, K. Koya, T. Ukai, N. Tatsuta, A. Ikegawa, K. Ogawa, T. Shishido, and L. B. Chen, *J. Med. Chem.*, 1998, **41**, 130.
6. K. Takasu, T. Shimogama, C. Saiin, H.-S. Kim, Y. Wataya, and M. Ihara, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1689.
7. Although there are several possible geometrical isomers of rhodacyanines (**1**), the structures shown in Scheme 1 and Table 1 are depicted as a single geometrical isomer. It is well know that the conjugated double bonds can be easily isomerized in the solution and the *trans* geometrical isomers are usually thermodynamically stable products.