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SYNTHESIS AND BIOLOGICAL PROPERTIES OF A RHODACYANINE DERIVATIVE, SSJ-127, HAVING HIGH EFFICACY AGAINST MALARIA PROTOZOA[#]

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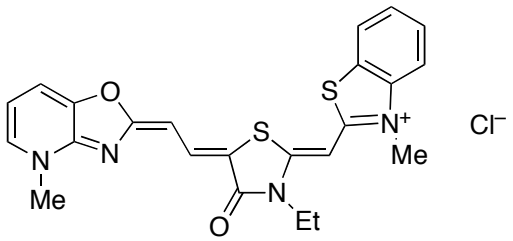
Abstract – One of rhodacyanine derivatives, SSJ-127 (**1**), showed a complete cure of a mouse model infected with *Plasmodium berghei* by subcutaneous administration. The synthesis, in vitro activities against several protozoa and in vivo antimalarial test and PK study of SSJ-127 (**1**) are reported.

Malaria is one of the most perilous infectious diseases caused by protozoan parasites in tropical and subtropical regions. Each year, there are approximately 515 million cases of malaria, killing between one and three million people, the majority of whom are young children.[1] No vaccine is currently available for malaria and the resistance of the protozoa to clinically used chemotherapeutic agents is increasingly common. Therefore, the development of new classes of antimalarial medicines having a novel mechanism of action is highly desired.[2-7] Recently, we recorded that rhocyanine derivatives displayed potent in vitro and in vivo activities against *Plasmodium* parasites.[8-11] Further investigation of antimalarial activities of a number of newly synthesized rhodacyanine derivatives has led us finding SSJ-127 (**1**), which exhibits a complete cure of a mouse model infected with *P. berghei*. Here, we would like to communicate the synthesis and biological properties of the rhodacyanine derivative, SSJ-127 (**1**).

[#]This paper is dedicated to Professor Emeritus Keiichiro Fukumoto as we celebrate his 75th birthday and his significant contributions to the field of heterocyclic chemistry and The International Journal, "*Heterocycles*".



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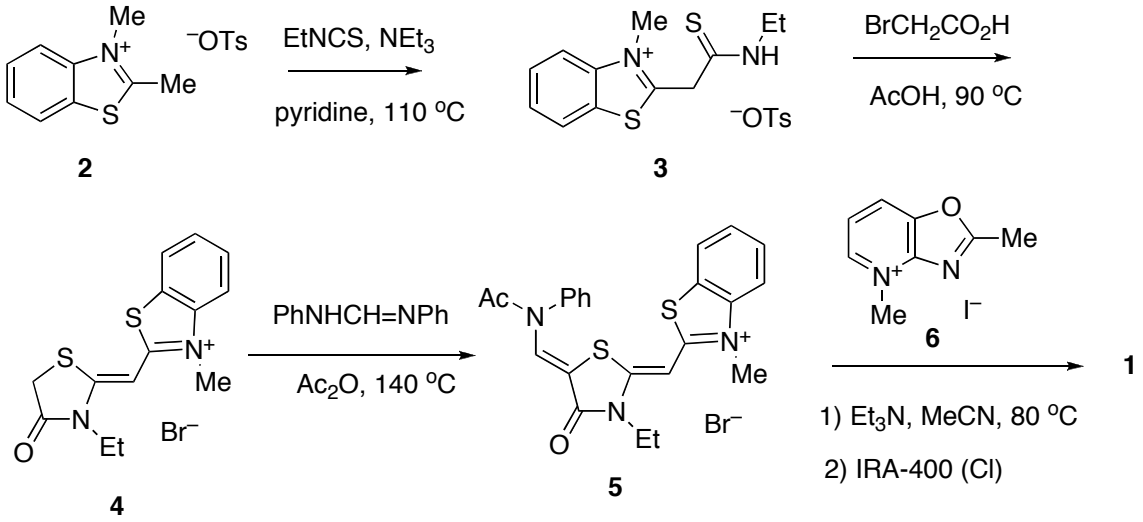
Synthesis: SSJ-127 (**1**) was prepared starting with the right hand segment, benzo[d]thiazol-3-ium part, as shown in **Scheme 1**. Reaction of 2,3-dimethylbenzo[d]thiazol-3-ium tosylate (**2**) and ethyl isothiocyanate in the presence of triethylamine in pyridine at 110 °C for 1 h gave the thioamide **3** in 55% yield. Without purification, **3** was treated with bromoacetic acid in acetic acid at 90 °C for 30 min to afford the 4-oxothiazolidine **4** in 77% yield. Treatment of **4** with *N,N'*-diphenylformimidamide in acetic anhydride at 140 °C for 30 min provided the phenylacetamidomethylenethiazolidine **5** in 97% yield. Reaction of **5** with 2,4-dimethyloxazolo[4,5-b]pyridinium iodide in the presence of triethyamine in acetnitrile at 80 °C for 2.5 h, followed by eluation of the product through IRA-400 (Cl), gave SSJ – 127 (**1**)[12] in 49% overall yield. Thus, SSJ-127 (**1**) was easily synthesized in five steps from known compounds using a standard synthetic procedure.[13,14]



2 **3** **4** **5** **6** **1**

Scheme 1. Synthesis of SSJ – 127 (**1**)

Antiprotozoal Activities: IC_{50} values of SSJ-127 (**1**) against *P. falciparm* K1, *Trypanosoma cruzi*, *T. brucei rhodesiense*, *Leishmania donovani* and L-6 rat skeletal myoblast cell line together with the selective indexes, determined at Swiss Tropical Institute (STI), are shown in **Table 1**. SSJ-127 (**1**) showed activities at ten ng/mL concentrations against *P. falciparm* K1 and *T. brucei rhodesiense* with good selectivity.

Table 1. Antiprotozoal and Cytotoxic Activities (IC_{50} values, μg/mL) of SSJ-127 (**1**).[a]

P. falc.K_1		*T. cruzi*		*T. b. rhod.*		*L. don.*, axenic		Cytotox. L6
IC_{50}	SI [b]	IC_{50}	SI [b]	IC_{50}	SI [b]	IC_{50}	SI [b]	IC_{50}
0.029	420	2.41	5.1	0.022	554	0.304	40.1	12.2

[a]Values indicate the inhibitory concentration of SSJ – 127 in μg/mL that is necessary to achieve 50% growth inhibition (IC_{50}). Data shown are values from two replicate experiments.
[b]Selectivity index = (IC_{50} value for L6)/(IC_{50} value for *P. falciparum, T. cruzi, T. b. rhodesiense,* or *L. donovani*).

Next, in vivo experiments of SSJ-127 (**1**) were studied using *P. berghei*, GFP ANKA strain at STI and using *P. berghei*, NK 65 strain at Hoshi University, respectively. Single subcutaneous (sc) administration of 100 mg/kg of SSJ-127 (**1**) to NMRI mice (femeles) infected with *P. berghei*, GFP ANAK exhibited 95% suppression after 4 days. A positive result, 40 % suppression, was also obtained by single po administration of 100 mg/kg of **1** to the infected mice. It was very pleased for us to observe the complete cure on in vivo test dosing SSJ-127 (**1**). Namely, tree time sc administrations of 40 mg/kg/d of **1** to ICR mice (males) infected with *P. berghei*, NK 65 strain provided 100% suppression and all treated mice have survived after 18 months. They have grown well and are now very active at Hoshi University.

PK Study: A preliminary pharmacokinetic study using male rats, Crl:CD(SD), was carried out by intravenous (iv) and sc administrations. The results, analyzed by two-compartmental methods using the computer program 3P87, are summarized in **Table 2**. The sc bioavailability of **1** was determined to be excellent.

Table 2. Pharmacokinetic Parameters of ID-127 (**1**)

route	$T_{1/2\alpha}$ (h)	$T_{1/2\beta}$ (h)	CL (L/h·kg)	V (L/kg)	T(peak) (h)	$AUC_{0\text{-}24h}$ (ng×h/mL)
iv[a]	0.055	4.17	11.35	4.45		88.08
sc[b]	0.79	5.22	4.34	66.09	5.36	4606.3

[a]dosage: 1 mg/kg, n=2
[b]dosage: 20 mg/kg, n=2

SSJ-127 (**1**) showed characteristic features such as the accumulation into a specific organelle of malaria parasites with fluorescence and further results of investigation will be published in a future.

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12. Characteristic data of SSJ-127 (**1**): mp 250.3-251.0 ℃; UV-vis (H_2O): λ (nm) (log ε/L $mol^{-1}cm^{-1}$): 613 (3.88), 382 (1.13); IR ν (neat, cm^{-1}): 2922, 2865, 1525, 1474, 1382, 1346, 1317, 1275, 1225, 1185, 1128, 1056, 1033, 1013, 889, 824, 753; ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.22 (d, J = 7.7 Hz, 2H), 8.12 (m, 1H), 7.93 (d, J = 7.7 Hz, 2H), 7.70 (t, J = 7.7 Hz, 1H), 7.53 (t, J = 7.7 Hz, 1H), 7.28-7.08 (m, 1H), 6.72 (s, 1H), 5.52 (d, J = 13.7 Hz, 1H), 4.17 (q, J = 7.0 Hz, 2H), 4.08 (s, 6H), 1.25 (t, J = 7.0, Hz, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ ppm 163.1, 162.1, 160.4, 157.9, 156.7, 154.2, 146.2, 140.2, 135.2, 128.5, 125.9, 125.3, 123.4, 116.8, 114.1, 113.6, 85.4, 84.3, 69.7, 38.8, 34.4, 34.0, 12.5; MS (ESI$^+$): *m/z*: 449.1 [M^+]; Anal. Calcd. for $C_{23}H_{21}ClN_4O_2S_2 \cdot 5.5H_2O$: C, 47.29; H, 5.52; N, 9.59. Found: C, 47.03; H, 5.19; N, 9.41.
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