

## COMMUNICATION TO THE EDITOR

# *In vitro* and *in vivo* antiprotozoal activities of bispolides and their derivatives

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During the course of our screening program to discover new antiprotozoal (antimalarial and antitrypanosomal) chemicals, we have evaluated isolates from soil microorganisms, as well as compounds from the antibiotic libraries of the Kitasato Institute for Life Sciences and Nimura Genetic Solutions. We have previously reported various microbial metabolites exhibiting potent antimalarial<sup>1–4</sup> and antitrypanosomal properties.<sup>5–7</sup> We have recently found that the known 20-membered ring macrodiolide antibiotics, the bispolides,<sup>8</sup> together with new derivatives, exhibit selective antitrypanosomal and potent antimalarial activities, both *in vitro* and *in vivo*. Here, we report the antitrypanosomal and antimalarial profiles of bispolides, their derivatives (Figure 1) and the related 16-membered ring macrodiolide antibiotic as elaiophylin (azalomycin B)<sup>9,10</sup> (Figure 1) in comparison with those of clinically used antitrypanosomal drugs, such as suramin and eflornithine, and two clinically used antimalarial drugs, artemisinin and chloroquine. We also present some conclusions on structure–activity relationships.

Bispolides A1, A3, B1 and B3 were purified from the culture broth of *Microbispora* sp. A34030.<sup>8</sup> Derivatives of bispolide A1 (derivatives I and II) were prepared as follows. The 13,13'-dimethoxy compound, bispolide A3, prepared from bispolide A1 by reaction with 0.04%-HCl in MeOH, was reduced by NaBH<sub>3</sub>CN in EtOH to give the 13,13'-dideoxy compound (derivative I (ESI-MS *m/z* 1123.69114; <sup>12</sup>C<sub>62</sub><sup>1</sup>H<sub>100</sub><sup>23</sup>Na<sub>1</sub><sup>16</sup>O<sub>16</sub>)). Partial hydrolysis of derivative I in the presence of *p*-toluenesulfonic acid in aqueous acetonitrile gave derivative II (FAB-MS *m/z* 841 (M<sup>+</sup>)). Elaiophylin was obtained from the antibiotic library of the Kitasato Institute for Life Sciences.

*In vitro* antiprotozoal activities against *Trypanosoma brucei brucei* strain GUTat 3.1, *Plasmodium falciparum* strains K1 (drug-resistant) and FCR3 (drug-sensitive), and

cytotoxicity against human diploid embryonic cell line MRC-5 were measured as described previously.<sup>1,5</sup> *In vivo* antitrypanosomal activity for *T. b. brucei* strain S427 was measured as described previously.<sup>6</sup> Test compounds were solubilized in an aqueous mixture of 10% DMSO-Tween 80 and EtOH (7:3) and administered i.p. to mice on the next day (day 1) following infection with parasites (day 0). Subsequently, the compounds were successively administered (i.p.) to the infected mice once a day for 3 days (days 2–4). Efficacies of compounds were determined by the parasitemia levels and the mean of survival days (MSD), compared with that of the untreated control mice.

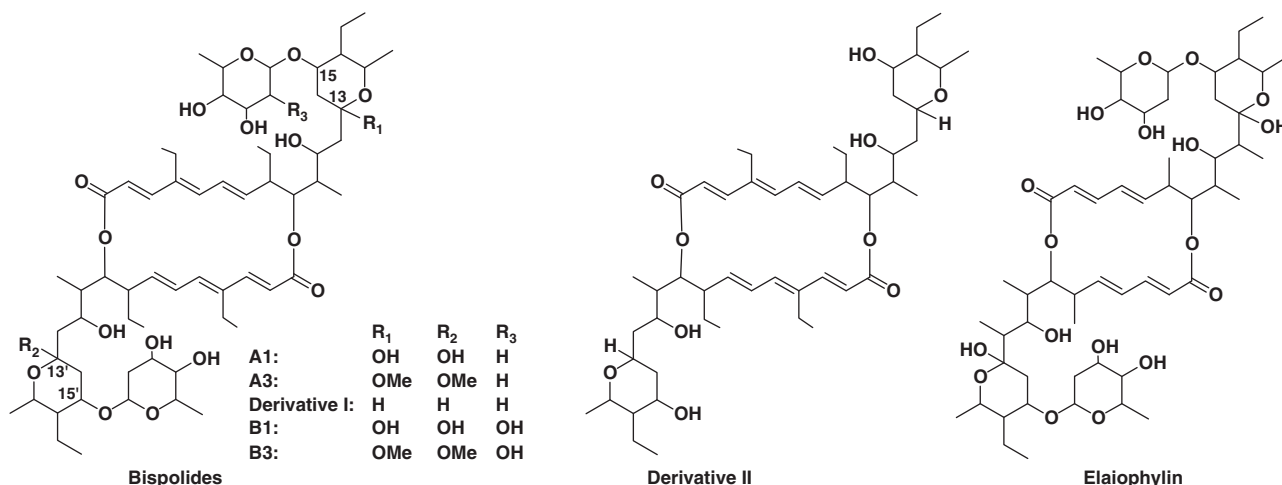
Table 1 shows the *in vitro* antiprotozoal activities of bispolides, their derivatives, elaiophylin and some standard antiprotozoal drugs. Bispolides, derivative I (13,13'-dideoxybispolide A1) and elaiophylin showed the more potent antimalarial activity against the drug-resistant K1 strain of *P. falciparum*, in the 260–620 ng ml<sup>-1</sup> range. The half-maximal inhibitory concentration (IC<sub>50</sub>) values against the drug-sensitive FCR3 strain of *P. falciparum* were similar to those against the K1 strain of *P. falciparum* (data not shown). The antimalarial activity was similar to that of chloroquine, but 43–103-fold less than that of artemisinin. However, the antimalarial activity of derivative II was 14-fold less than that of bispolide A3.

With respect to antitrypanosomal activity, bispolides and derivative I showed the most potency against the GUTat 3.1 strain of *T. b. brucei*, in the 57–150 ng ml<sup>-1</sup> range. The antitrypanosomal activities were 10–40-fold more potent than that of the standard drugs, suramin and eflornithine. However, the antitrypanosomal activities of derivative II and elaiophylin were 3–6-fold less than that of bispolide A3.

The *in vitro* cytotoxicities of bispolides, their derivatives, elaiophylin and some stan-

dard antiprotozoal drugs are presented in Table 1. Among them, the IC<sub>50</sub> values of bispolide B1 and elaiophylin were 0.96 and 0.87 μg ml<sup>-1</sup>, respectively. The IC<sub>50</sub> values of bispolide A1, A3 and B3, and derivative I were in the range of 1.4–3.9 μg ml<sup>-1</sup>, whereas that of derivative II was 15.6 μg ml<sup>-1</sup>. To compare the antiprotozoal activities and cytotoxicities, we introduced selectivity indexes (SIs: cytotoxicity (IC<sub>50</sub> for the MRC-5 cells)/antimalarial or antitrypanosomal activity (IC<sub>50</sub> for the K1 strain or the GUTat 3.1 strain), as presented in Table 1. In the case of the MRC-5 cells/K1 strain, bispolide A1 showed a medium SI, with a ratio of 15. Other bispolides and elaiophylin showed a low SI, with ratios of 2–9. In the case of the MRC-5 cells/GUTat 3.1 strain, bispolides A1 and B3 showed a moderate SI, with ratios of 57–62. Other bispolides and elaiophylin showed a medium or low SI (ratios of 2–26). Among the bispolides, we were interested in bispolides A3 and B3, which had an SI of 26 and 62, respectively. Among the tested bispolides, B3 showed the more potent antitrypanosomal activity and highest SI, whereas bispolide A3 exhibited potent antitrypanosomal activity but with medium SI.

The preliminary *in vivo* antitrypanosomal activities of bispolides A3 and B3 were measured in the *T. b. brucei* S-427 acute mouse model. At a dose of 25 mg kg<sup>-1</sup>, bispolide B3 did not achieve cure but did extend the MSD to 9.5 days, representing a 1.4-fold increase over control MSD (7.0 days). The same dose of bispolide A3 did not achieve cure but also extended the MSD. Under the same conditions, suramin showed a curative effect (MSD: >30 days) at a dose of 1 mg kg<sup>-1</sup>. The bispolide B3 data suggest that it is possibly a new candidate compound for discovering new antitrypanosomal drugs with more potent activity. These efficacy tests, including *in vivo* antimalarial activity, are being investigated further.



**Figure 1** Structures of bispolides and elaiophylin.

The weak antiprotozoal activities of derivative II in comparison with bispolide A3 (the parent compound) and derivative I provide very interesting information on the structure–activity relationships. In contrast to bispolide A3 and derivative I, which possess hexose moieties at 15-OH and 15'-OH of the aglycone macrodiolide, derivative II lacks the hexose moieties and is 5.8–14.3-fold less active for antiprotozoa than bispolide A3 and derivative I. Furthermore, bispolides A1, A3 and B3, together with derivative I, possess two conjugated trienes in the 20-membered ring. Elaiophylin possesses two conjugated dienes in the 16-membered ring and has 3–8-fold less antitrypanosomal activity and 1.6–4.5-fold less potent cytotoxicity than bispolides A1, A3, B3 and derivative I. Our data therefore suggest that the hexose moieties at 15-OH and 15'-OH and the two conjugated trienes in the 20-membered ring present in bispolides have a significant antiprotozoal activity and cytotoxicity.

Further studies are necessary for an extensive structure–*in vitro* antiprotozoal activity evaluation of bispolide-related compounds.

We previously reported that bispolides have inhibitory activity against Gram-positive bacteria, including MRSA.<sup>8</sup> Related 16-membered ring macrodiolide antibiotics, such as elaiophylin and its asymmetric deglycosidation products, have been reported to show both antibacterial and nematocidal traits.<sup>10</sup> However, discovery of the selective antitrypanosomal activity and the potent antimalarial activity of bispolides is novel and our data constitute the first report of such properties.

The above results reveal that bispolides are promising lead compounds for development of novel antiprotozoal (antitrypanosomal and antimalarial) drugs. Further study of

**Table 1** *In vitro* antiprotozoal activity against *Plasmodium falciparum* K1<sup>a</sup> and *Trypanosoma brucei brucei* GUTat 3.1 strains, plus cytotoxicity in MRC-5 cells, of bispolides and their derivatives

Compound	<i>IC</i> <sub>50</sub> (ng ml <sup>-1</sup> )				
	Antiprotozoal activity		Cytotoxicity MRC-5	Selectivity index (SI)	
	P. f. K1	T. b. b. GUTat 3.1		MRC-5/K1	MRC-5/GUTat 3.1
Bispolide A1	260	67	3820	15	57
Bispolide A3	480	150	3910	8	26
Bispolide B1	390	57	960	3	17
Bispolide B3	450	63	3910	9	62
Derivative I	620	120	1390	2	11
Derivative II	6850	870	15630	2	18
Elaiophylin	370	460	870	2	2
Artemisinin	6	ND	45 170	7528	—
Chloroquine	184	ND	18 572	101	—
Suramin	ND	1580	>100 000	—	>63
Eflornithine	ND	2270	>100 000	—	>44

Abbreviations: *IC*<sub>50</sub>, half-maximal inhibitory concentration; ND, not determined.  
<sup>a</sup>Drug-resistant strain.

the antiprotozoal and other biological activities of bispolides is in progress.

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Kazuhiko Otoguro<sup>1</sup>, Masato Iwatsuki<sup>1</sup>,  
 Aki Ishiyama<sup>1</sup>, Miyuki Namatame<sup>1</sup>,  
 Aki Nishihara-Tsukashima<sup>1</sup>, Seigo Sato<sup>2</sup>,  
 Masahiro Hatsu<sup>2</sup>, Hironobu Iinuma<sup>2</sup>,

Seiji Shibahara<sup>2</sup>, Satoshi Nimura<sup>2</sup>,  
 Shinichi Kondo<sup>2</sup>, Haruki Yamada<sup>3</sup> and  
 Satoshi Ōmura<sup>3</sup>

<sup>1</sup>Research Center for Tropical Diseases, Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan; <sup>2</sup>Nimura Genetic Solutions, Tokyo, Japan and <sup>3</sup>Kitasato Institute for Life Sciences, Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan  
 E-mail: otoguro@lisci.kitasato-u.ac.jp or omuras@insti.kitasato-u.ac.jp

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