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Borrelidin analogues with antimalarial activity: Design, synthesis and biological evaluation against *Plasmodium falciparum* parasites

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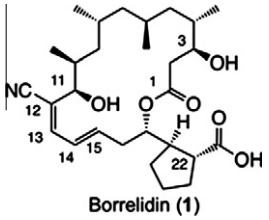
ABSTRACT

Borrelidin, a structurally unique 18-membered macrolide, was found to express antimalarial activity against drug-resistant *Plasmodium falciparum* malaria parasites, with IC_{50} value of 0.93 ng/mL. However, it also displays strong cytotoxicity against human diploid embryonic MRC-5 cells. To investigate the issue of the cytotoxicity of borrelidin, borrelidin-based analogues were synthesized and their anti-Plasmodium properties were evaluated. In this communication, we report that a novel borrelidin analogue, bearing the CH_2SPh moiety via a triazole linkage, was found to retain a potent antimalarial activity, against drug-sensitive and drug-resistant parasite strains, but possess only weak cytotoxicity against human cells.

Malaria, caused by *Plasmodium* species, such as *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale*, has historically been a major threat to public health worldwide. According to the World Malaria Report 2011,[1] malaria caused an estimated 655,000 deaths in 2010, primarily among under 5-year-old children in sub-Saharan Africa. Artemisinins, used for centuries in China for anti-fever treatment, have relatively recent been used as anti-malarial drugs. Artemisinin-based combination therapies are now recommended by the World Health Organization for the treatment of malaria. However, malarial parasites have been used against them and reports of artemisinin-resistant parasites are continuing to spread. Therefore development of new anti-malarial agents, especially those with novel a mode of action, is both an urgent and continuing need.

In 1949, Berger et al. reported the isolation of borrelidin (**1**, Fig. 1), a structurally unique 18-membered macrolide, from a culture broth of *Streptomyces rochei*,[2] of which the planar structure was elucidated by Keller-Schierlein et al. in 1967,[3] and the absolute configuration was determined by X-ray crystallography of a chiral solvent.[4]

Importantly, **1** expresses a variety of biological properties, such as antiangiogenesis activity,[5a,b] antibacterial activity,[2] antiviral activity,[6] insecticidal and herbicidal activity.[7] Additionally, in 2003, as depicted in Table 1, we have found that **1** exhibited more potent antimalarial activity against both the drug-resistant K1 strain[8] and the drug-sensitive FCR3 strain of *Plasmodium falciparum* (IC_{50} = 0.93 ng/mL against the K1 strain, IC_{50} = 0.88 ng/mL against the FCR3 strain). This was better than the clinically used antimalarials, artemisinin (IC_{50} = 6 ng/mL against K1 strain), and chloroquine (IC_{50} = 184 ng/mL against K1 strain).[9–11] Antimalarial activity against both the K1 and FCR3 strains of **1** was almost the same. However, the cytotoxicity[9] of **1** against human dipoid embryonic cell line MRC-5 was high, 201 ng/mL, significantly higher than that of artemisinin (45,170 ng/mL). To evaluate the combined antimalarial activity and cytotoxicities, a Selectivity index (SI) (cytotoxicity (IC_{50} for the MCR-5 cells)/antimalarial activity (IC_{50} for the K1 or FCR3 strains)) was created. Consequently, the SI for K1 strain of **1** (SI = 216) was considerably lower than that of artemisinin (SI = 7528). In addition, our research group reported that **1** showed stage-specific growth inhibition, most



Borrelidin (1)

Figure 1. Structure of borrelidin (**1**).

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Table 1
Biological evaluation of **1**–**4**, artemisinin and chloroquine

Compd	IC_{50} (ng/mL)			Selectivity index[a] (SI)	
	Antimalarial activity		Cytotoxicity[b] (MCR-5)	MCR-5/K1	MCR-5/FCR3
	K1 strain[c]	FCR3 strain[d]			
1	0.93	0.88	201	216	228
2	107	120	55,700	522	464
3	10	10	14,600	1460	1460
4	130	130	13	102	102
Artemisinin[e]	6	6	45,170	7528	7528
Chloroquine[e]	184	15	18,572	101	1238

[a] Selectivity index = cytotoxicity (ng/mL)/IC_{50} (ng/mL).
[b] Cytotoxicity (ng/mL), human dipioid embryonic cell line MRC-5.
[c] K1 strain, drug-resistant *Plasmodium falciparum*.
[d] FCR3, drug-sensitive; *P. falciparum*.
[e] Drugs commonly used to treat malaria.

strongly in the trophozoite stage.[12] However, the detailed mode of action at molecular level has not been revealed yet.

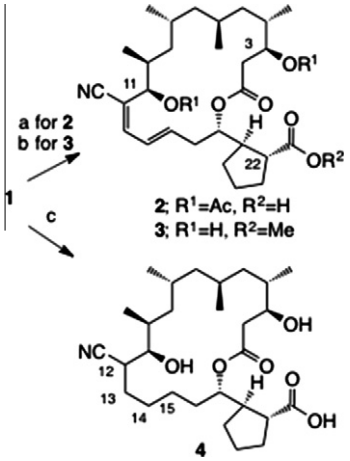
Given these biological activities, since **1** has promising antimalarial activity against *P. falciparum*, even the chloroquine-resistant K1 strain, it would be desirable to develop borrelidin-based analogues possessing weak cytotoxicity against human cells. To the best of our knowledge, no literature describing the chemical synthesis of borrelidin analogues has been reported to date.

Our efforts have been concentrated on not only reducing cytotoxicity, while retaining potent antimalarial activity, but also on revealing structure–activity and/or –cytotoxicity maps. Herein we report the design and synthesis of borrelidin analogues as well as observations on some structure–activity and/or –cytotoxicity relationships.

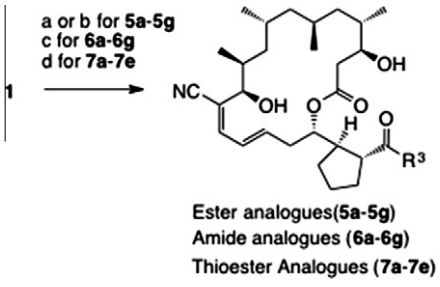
At the outset, our efforts focused on making brief SAR maps by analogue synthesis (1) 3,11-diacetylation, (2) methylation of the carboxylic acid at C22 position and (3) reduction of the double bonds at C12–15 position. As outlined in Scheme 1 and (1) treatment of **1** with Ac_2O, Et_3N, DMAP in DCM provided the corresponding diacetylated borrelidin (**2**) in 58% yield, (2) methylation with $TMSCHN_2$ afforded the corresponding methyl ester borrelidin (**3**) in 82% yield, and (3) hydrogenation of **1** provided C12–15-tetrahydro borrelidin (**4**) as a mixture of diastereomers at C12 position in 74% yield (Scheme 1).

We tested analogues **2**–**4** for in vitro activity against *P. falciparum* strains K1 (drug-resistant) and FCR3 (drug-sensitive), as well as for cytotoxicity against the human diploid embryonic cell line MRC-5. The biological evaluation revealed that the diacetylated analogue (**2**) exhibited approximately 100-fold lower antimalarial activity against K1 strain than that of **1** (IC_{50} = 107 ng/mL, compound **2** as opposed to IC_{50}=0.93 ng/mL) (Table 1). Likewise, the C12,13-14,15-tetrahydro derivative (**4**) exhibited a decreased IC_{50} against K1 (IC_{50} = 130 ng/mL) together with strong cytotoxicity (IC_{50} = 13 ng/mL). Although the methyl ester (**3**) showed approximately 14-fold weaker IC_{50} against K1 (IC_{50} = 10 ng/mL), the SI for K1 (SI = 1460) was approximately sevenfold better than that of natural **1** (SI = 216), suggesting that modifying the carboxyl group at C22 position to various functional groups could produce a more effective and usable molecule which possesses strong antiparasitic activity and weak cytotoxicity (Table 1). Based on these findings, our strategy focused on modification of the carboxyl group at C22 position to the ester-, amide-, and thioester-type analogues.

From our synthetic strategy, the ester analogues (**5a** (R^3 = OEt), **5b** (R^3 = O*n*-Pr), **5c** (R^3 = OBn), **5d** (R^3 = Oallyl), **5e** (R^3 = Opropargyl), **5f** (R^3 = $O(CH_2)_2NMe_2$), **5g** (R^3 = *O*-morpholylethyl)) were prepared by the following procedures, method (A) alcohols (EtOH, *n*-PrOH, BnOH), *p*-TsOH, reflux for **5a**–**5c**, or (B) alcohols, PyBOP, Et_3N, DMAP, DCM for **5d**–**5g**. To introduce the amino acid moiety (Scheme 2), treatment of **1** with chloroisobutyl formate, Et_3N followed by addition of several amino acids (Gly, Ala, Ser, Asn, Ile, D-Ala, and Tyr) provided the corresponding amide analogues **6a**–**6g** (**6a** (R^3 = Gly); 85%, **6b** (R^3 = Ala); 82%, **6c** (R^3 = Ser); 70%, **6d** (R^3 = Asn); 44%, **6e** (R^3 = Ile); 84%, **6f** (R^3 = D-Ala); 70%, **6g** (R^3 = Tyr); 70%). Whereas, thioester analogues (**7a** (R^3 = SEt), **7b** (R^3 = S*n*-Pr), **7c** (R^3 = S*i*-Pr), **7d** (R^3 = S*n*-Bu), **7e** (R^3 = SPh)) were



Scheme 1. Reagents and conditions: (a) Ac_2O, Et_3N, DMAP, DCM, rt, 58%; (b) $TMSCHN_2$, PhH/MeOH (v/v 10/1), rt, 82%; (c) H_2, Pd/C, EtOH, rt, 74%.



Scheme 2. Reagents and conditions: For synthesis of ester analogues (**5a**–**5g**); (a) alcohols (EtOH, *n*-PrOH, BnOH), *p*-TsOH, reflux (**5a**;85%, **5b**; 51%, **5c**; 29%); (b) alcohols, PyBOP, Et_3N, DMAP, DCM (**5d**; 91%, **5e**; 81%, **5f**; 83%, **5g**; 73%), for synthesis of amide analogues (**6a**–**6g**); (c) chloro*iso*butylformate, Et_3N, THF, then amino acids, rt (**6a**; 85%, **6b**; 82%, **6c**; 70%, **6d**; 44%, **6e**; 84%, **6f**; 70%, **6g**; 70%), for synthesis of thioester analogues (**7a**–**7e**); (d) thiols, PyBOP, Et_3N, DMAP, DCM, rt (**7a**; 88%, **7b**; 59%, **7c**; 93%, **7d**; 89%, **7e**; 96%).