BOROMYCIN DERIVATIVES: SYNTHESIS AND ANTIMALARIAL ACTIVITY *IN VITRO* AND *IN VIVO*

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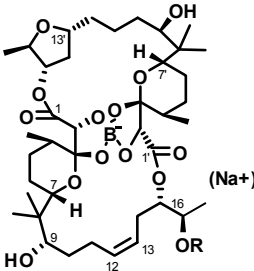
Abstract – Derivatives of boromycin, which has significant antimalarial properties both *in vitro* and *in vivo*, were examined for similar the efficacy against both drug-resistance strain and drug-sensitive strain of malaria and as a means to elucidate structure-activity relationships. More potent *in vitro* compounds tended to lose their properties *in vivo* when administered orally. Some novel 16-*O*-acyl derivatives were found to be more potent and selective antimalarial compounds compared to boromycin. Furthermore, alkyne-bearing 16-*O*-alkylacyl compounds retained reasonably good efficacy *in vivo* when given *per os*.
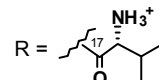
Malaria is a serious infectious disease worldwide, especially in sub-Saharan Africa and Asia. Malaria, caused by *Plasmodium* species, occurs in more than 90 countries, with more than 243 million cases in 2008 causing an estimated 863,000.[1] Resistance to commonly-used antimalarial drugs develops quickly

Table 1. Antimalarial activities of boromycin and the antimalarial drugs against K1 and FCR3 strains of *Plasmodium falciparum*

Compounds	IC$_{50}$ (ng/ml)				
	Antimalarial activity		Cytotoxicity	Selectivity Index	
	K1 strain*	FCR3 strain**	MRC-5***	MRC-5/K1	MRC-5/FCR3
boromycin	35	36	450	12.9	12.5
devalinylboromycin (BRM-01)	9.5	3.5	3510	369.5	1002.9
artemisinin	6	6	45170	7528	7528
chloroquine	184	15	18572	101	1238

boromycin R =

devalinyl boromycin R = H (with Na$^+$ as a counter cation)

* drug-resistant strain   ** drug-sensitive strain   *** against human diploid ambryonic cell

and spreads rapidly, and has rendered most available antimalarials of very restricted use, apart from the newly-introduced artemisinin compounds which currently maintain their effectiveness extensively. New drugs, particularly those with novel modes of action, are urgently required. In addition, due to the impoverished conditions in which malarial prevails, drugs that are easily and cheaply administered are of greatest use. Oral medicines are therefore of significant interest. In our institute, we have a long-standing program of activity focusing on the search for natural products and their derivatives, which may be useful to combat neglected tropical diseases.[2,3] Recently, we have further investigated, boromycin as a potential antimalarial agent. Boromycin was previously reported to show diverse bioactivity such as anti-Gram positive bacterial, anticoccidial and anti-HIV properties.[4,5] It was independently isolated from a culture broth of *Streptomyces* sp. OH-1034 and found to be a G2 checkpoint inhibitor in 2004.[6] In an early study, Hütter and co-workers demonstrated the antimalarial property of boromycin against *Plasmodium berghei*. (ED$_{50}$ 15 mg/kg *p.o.* oder s.c.).[7] In this report, we describe the synthesis, antimalarial activitiy and cytotoxicity of boromycin and several derivatives.

We re-evaluated boromycin against the *Plasmodium falciparum* strain K1 (chloroquine-resistant) and FCR3 (drug-sensitive) strain *in vitro*, in comparison with clinically-used antimalarial drugs (Artemisinin and Chloroquine), and found inhibitory activities with IC$_{50}$ values of 35 ng/mL and 36 ng/mL against the K1 and FCR3 strains, respectively (Table 1). Furthermore, *in vivo* antimalarial activity of boromycin *per os* (*p.o.*) at 10 mg/kg was determined using a rodent malaria-derived *Plasmodium berghei* strain according to the 4-days suppressive test of Peters and co-workers.[5,8,9] Parasite growth was inhibited by 97.9%. The cytotoxicity of boromycin was measured by colorimetric MTT assay[5,10,11] with human MRC-5 cells, producing an IC$_{50}$ value of 450 ng/mL. We devised Selectivity Indexes (cytotoxicity [IC$_{50}$
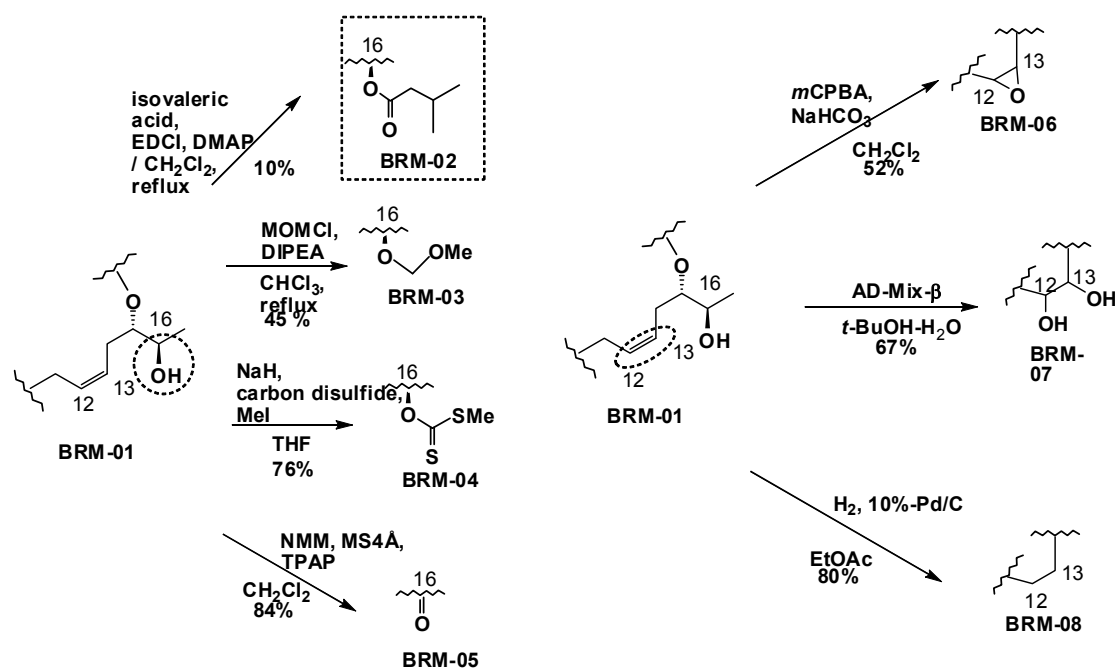
Scheme 1. A variety of boromycin derivatives

for the MRC-5 cell]/antimalarial activity [IC_{50} for the K1 strain or the FCR3 strain], to allow better comparisons (Table 1).

Our results, indicated that the selectivity between cytotoxicity and antimalarial activity for boromycin is relatively low and that the compound is thus deemed unsuitable for development as a human medicine. We subsequently produced a variety of boromycin derivatives in order to test their efficacy and suitability for human use, as well as to help gain a better understanding of structure-activity relationships.

Table 2. Antimalaria activities of boromycin derivatives against K1 and FCR3 strains of *Plasmodium falciparum*

| Compounds | IC_{50} (ng/ml) | | | Selectivity Index | |
| | Antimalarial activity | | Cytotoxicity | | |
	K1 strain[*]	FCR3 strain[**]	MRC-5[***]	MRC-5/K1	MRC-5/FCR3
BRM-02	75	74	1660	22.1	22.4
BRM-03	280	160	10270	36.7	64.2
BRM-04	520	150	3610	6.9	24.1
BRM-05	100	50	4740	47.4	94.8
BRM-06	7350	n.d.	>100000	>13.6	——
BRM-07	6750	n.d.	>100000	>14.8	——
BRM-08	330	260	94	0.3	0.4
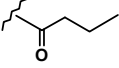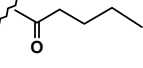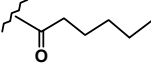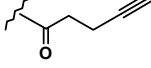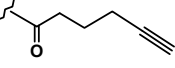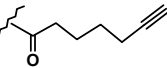
[*] drug-resistant strain [**] drug-sensitive strain [***] against human diploid ambryonic cell line

One particular derivative, devalinylboromycin (**BRM-01**), which is easy to prepare by hydrolysis of boromycin according to the protocol of previous report,[12] showed extremely good *in vitro* efficacy against both K1 and FCR3 strains and had lower cytotoxicity than the parent compound (Table 1). Unfortunately, when tested *in vivo* **BRM-01** showed no parasite growth inhibition at 10 mg/kg when administered orally. Consequently, to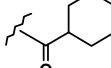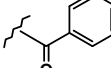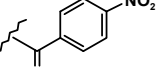 elucidate the antimalarial property and structure-activity relationships, a variety of derivatives were synthesized from the highly-promising devalinylboromycin (**BRM-01**). Modifications of the sterically less hindered alcohol (16-OH) 16-hydroxy group were carried out to prepare acetal, dithiocarbonate and ketone derivatives. Isovaleric acid was selected to prepare an acyl derivative (**BRM-02**), because isovaleryl moiety can imitate the valinyl function. Actually, isovalerylation of 16-OH hardly proceeded under reflux conditions with EDCI and DMAP (10% yield) due to sterically hindrance of the substrate. MOMCl was reacted with 16-OH of **BRM-01** to give the acetal derivative in moderate isolated yield (45%, **BRM-03**). Other derivatives, dithiocarbonate (**BRM-04**) and ketone (**BRM-05**), were obtained under standard conditions in 76% and 84% yields, respectively. Subsequently, conversion of the *cis*- substituted olefin at C12-13 within **BRM-01** produced the epoxide (**BRM-06**), diol (**BRM-07**) and saturated derivative (**BRM-08**). *m*CPBA together with NaHCO$_3$ were added to a solution of **BRM-01** in CH$_2$Cl$_2$ to give 12, 13-epoxide **BRM-06**, in 52% yield, as a nearly 1:1 diasteromeric mixture.

Utilization of AD-mix-β to prepare the 12, 13-diol derivative resulted in relatively good conversion yield (67% without decomposed substrate) due to the gentle reactivity compared with standard dihydroxylation conditions, such as OsO$_4$/NMO. Additionally, **BRM-01** was quickly reduced to a saturated carbon skeleton by hydrogenation of the olefin with Pd/C under a hydrogen atmosphere to give 80% yield. The IC$_{50}$ values of each compound were then determined, as summarized in Table 2. In comparison with **BRM-01**, the activity of **BRM-02,** which contains a 16-*O*-vareloyl residue, was halved, whereas the other compounds possessed relatively little antimalarial activity.

Significantly, the importance of the 12,13-olefin of **BRM-01** was highlighted from the IC$_{50}$ value of

Table 3. Antimalarial activities of boromycin acyl derivatives against K1 and FCR3 strains of *Plasmodium falciparum*

Compounds		Isolated yields (%)	IC$_{50}$ (ng/ml)			Selectivity Index	
			Antimalaria activity		Cytotoxicity		
			K1 strain[*]	FCR3 strain[**]	MRC-5[***]	MRC-5/K1	MRC-5/FCR3
BRM-09		22	250	280	5490	22.0	19.6
BRM-10		86	48	60	2060	42.9	34.3
BRM-11		89	39	38	1370	35.1	36.1
BRM-12		90	61	110	1740	28.5	15.8
BRM-13		84	37	40	1760	47.6	44.0
BRM-14		92	17	35	3950	232.4	112.9
BRM-15		83	68	38	1860	27.4	48.9
BRM-16		92	57	560	2430	42.6	4.3
BRM-17		35	650	910	4370	6.7	4.8
BRM-18		45	100	130	4370	43.7	33.6
BRM-19		93	1200	1380	2810	2.3	2.0
BRM-20		quant.	20	8.0	1020	51.0	127.5

* drug-resistant strain ** drug-sensitive strain *** against human diploid ambryonic cell line

BRM-06, **BRM-07** and **BRM-08**. These results intimated that 16-*O*-acyl derivatives (such as **BRM-02**) would be good lead antimalarial compounds. Subsequently, several 16-*O*-alkylacyl derivatives of **BRM-01** were synthesized to try and obtain more potent antimalarial compounds. The 16-*O*-acetyl **BRM-09** was synthesized with Ac_2O in pyridine (22% yield). The other esters, **BRM-10** to **BRM-20**, were synthesized via EDCI or Yamaguchi esterification reaction[13,14] in 35~100% yields. The IC_{50} values of these compounds were subsequently evaluated, as shown in Table 3. Generally, the 16-*O*-acyl compounds exhibited some activity against both K1 and FCR3 strains. Among them, **BRM-11**, **13**, **14** and **20** demonstrated relatively good impact, with IC_{50} values of 39 ng/mL, 37 ng/mL, 17 ng/mL and 20 ng/mL against the *P. falciparum* K1 strain, respectively. In addition, **BRM-14**, surprisingly, proved twice more potent against the drug-resistant strain than against drug-sensitive parasites. Subsequently, *in vivo* tests were carried out using **BRM-11**, **14** and **20** in the *P. berghei* mouse model. **BRM-11** and **14**, at 10 mg/kg were administered intraperitoneally and both curtailed parasite growth by more than 70% (**BRM-11**; 77.9%, **BRM-14**; 72.3%). However, the inhibitory activity of **BRM-20** was low (59.0%). Consequently, the former two compounds (**BRM-11** and **14**) were examined using oral at 10 mg/kg. **BRM-14** showed an inhibition 46.2% while **BRM-11** was rendered almost ineffective at 9.6% inhibition. The cytotoxicity of **BRM-14** against MRC-5 cells indicated an IC_{50} value of 3.95 μg/mL creating high Selectivity Indexes, with values of 232.4 for the MRC-5 cell/K1 strain and 112.9 for MRC-5 cell/FCR3 strain, respectively, which were superior to boromycin and most other compounds but well bellow that of **BRM-01**. As the alkyne-bearing analogue (**BRM-14**) expressed moderate efficacy *in vivo* and showed relatively good Selectivity Index values *in vitro*, similar compounds, **BRM-13** and **15**, were also examined *in vivo*, administered *i.p.* and *p.o.* at 10 mg/kg, to identify the effect of the terminal alkynes. Administered intraperitoneally, **BRM-13** and **15** exhibited good parasite growth inhibition, with values of 89.5% and 93.1%, respectivily. However, the impact of **BRM-13** administered orally resulted in parasite growth inhibition of a moderate 59.2%, while **BRM-15** administered *p.o.* produced a very weak result (24.7% inhibition).

In summary, we discovered boromycin, despite a relatively poor selectivity as an antimalarial *in vitro*, exhibited good impact against *P. berghei in vivo*. Conversely, a derivative, devalinilboromycin, possessed extremely promising antimalarial impact *in vitro* but the effect did not materialize *in vivo* when the compound was given orally. Moreover, we discovered that several boromycin derivatives that exhibited promising antimalarial properties *in vitro* lost this activity *in vivo* when administered orally. We hydpothesized that the 12,13-olefin of boromycin appeared to play a major role in the antimalarial activity. Subsequently, alkyne-bearing 16-*O*-alkylacyl derivatives (**BRM-13** and **14**) showed acceptable inhibitory activity against both chloroquine-resistant and drug-sensitive strains of *P. falciparum in vitro*, and were recognized as being good lead compounds, especially as they maintained a degree of

antimalarial activity when given by mouth *in vivo*. Further studies of these analogues are in progress.

ACKNOWLEDGMENTS

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14. Experimental data of **BRM-14**; ^{1}H NMR (600MHz, CDCl$_3$) δ 6.01 (s, 1H), 5,49 (d, 1H, *J* = 11.3,

10.8 Hz), 5.39 (dd, 1H, J = 12.7, 6.15 Hz), 5.26 (dd, 1H, J= 12.7, 3.5), 5.18 (dt, 1H, J = 11.3, 12.2), 5.05 (d, 1H, J=7.4), 4.98 (d, 1H, J = 3.9), 4.80 (dd, 1H, J = 3.9, 6.7 Hz), 4.43 (s, 1H), 4.42 (s, 1H), 4.09 (m, 2H), 3.87 (dd, 1H, J = 1.7, 11.7 Hz), 3.71 (dd, 1H, J = 2.6, 12.1, Hz), 3.02 (dd, 1H, J = 7.6, 10.4, Hz), 2.60 (t, 1H, J = 13.0 Hz), 2.57 (m, 2H), 2.43 (m, 2H), 2.36 (m, 2H), 1.99 (m, 5H), 1.87 (m, 2H), 1.81 (m, 3H), 1.59 (m, 5H), 1.48 (m, 2H), 1.35 (d, 3H, J = 6.15 Hz), 1.34 (m, 4H), 1.18 (m, 4H), 1.10 (d, 3H, J = 6.7 Hz), 0.99 (d, 3H, J = 6.6 Hz), 0.96 (d, 3H, J = 6.7 Hz), 0.95 (s, 3H, 8-CH$_3$), 0.71 (s, 6H, 8-CH$_3$), 0.70 (s, 3H, 8'-CH$_3$), 0.61 (s, 3H, 8'-CH$_3$); ^{13}C NMR (150MHz, CDCl$_3$) δ 173.4 (C-17), 170.8 (C-1), 170.6 (C-1'), 135.2 (C-12), 122.3 (C-13), 106.0 (C-3'), 105.4 (C-3), 84.6 (C-9), 83.4 (C-21), 79.6 (C-15'), 79.5 (C-7'), 78.7 (C-2), 77.4 (C-2'), 77.2 (C-16'), 76.0 (C-13'), 75.8 (C-9'), 74.1 (C-15), 73.7 (C-16), 73.2 (C-7), 69.2 (C-22), 39.3 (C-8), 39.0 (C-8'), 36.8 (C-14'), 35.8 (C-12'), 33.4 (C-4), 33.0 (C-4'), 32.4 (C-18), 30.0 (C-10), 28.6 (C-5'), 28.2 (C-5), 27.8 (C-14), 27.2 (C-10'), 26.2 (C-11), 25.4 (C-6'), 25.1 (C-6), 23.5 (8-CH$_3$), 23.0 (C-19), 20.9 (8'-CH$_3$), 20.3 (C-11'), 20.0 (8-CH$_3$), 19.5 (C-20), 18.8 (16'-CH$_3$), 17.5 (C-17'), 16.5 (4'-CH$_3$), 14.2 (16-CH$_3$), 12.6 (8'-CH$_3$); ESI-MS: m/z calcd for C$_{46}$H$_{70}$BO$_{15}$Na$_2$ [(M+2Na)$^+$]: 919.46031. Found: m/z 919.46056.