



## Antiprotozoal, anticancer and antimicrobial activities of dihydroartemisinin acetal dimers and monomers

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### ABSTRACT

Nine dihydroartemisinin acetal dimers (**6–14**) with diversely functionalized linker units were synthesized and tested for in vitro antiprotozoal, anticancer and antimicrobial activity. Compounds **6**, **7** and **11** [IC<sub>50</sub>: 3.0–6.7 nM (D6) and 4.2–5.9 nM (W2)] were appreciably more active than artemisinin (**1**) [IC<sub>50</sub>: 32.9 nM (D6) and 42.5 nM (W2)] against the chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of the malaria parasite, *Plasmodium falciparum*. Compounds **10**, **13** and **14** displayed enhanced anticancer activity in a number of cell lines compared to the control drug, doxorubicin. The antifungal activity of **7** and **12** against *Cryptococcus neoformans* (IC<sub>50</sub>: 0.16 and 0.55 μM, respectively) was also higher compared to the control drug, amphotericin B. The antileishmanial and antibacterial activities were marginal. A number of dihydroartemisinin acetal monomers (**15–17**) and a trimer (**18**) were isolated as byproducts from the dimer synthesis and were also tested for biological activity.

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### 1. Introduction

Malaria, a devastating infectious disease caused by highly adaptable protozoan parasites of the genus *Plasmodium*, has impacted on humans for more than 4000 years, causing illness and an estimated 1.5–2.5 million deaths each year.<sup>1</sup> Malaria is endemic throughout the tropics, especially in sub-Saharan Africa and the developing world, threatening about 40% of the world's population. Although four *Plasmodium* parasite species can infect humans, *Plasmodium falciparum* causes the majority of illnesses and deaths. Severe malaria, defined as acute malaria with major signs of organ dysfunction or high levels of parasitemia, predominantly affects children and pregnant women.<sup>2</sup>

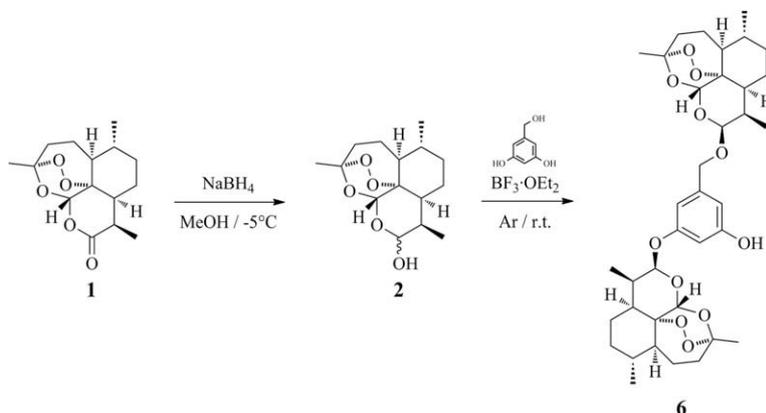
Chemotherapy is still at the forefront in the fight against malaria due to the unavailability of effective vaccines.<sup>3</sup> Numerous drugs have been developed for the treatment of uncomplicated malaria, for example, mefloquine, primaquine, quinidine, proguanil, ato-

vaquone and pyrimethamine. In areas where malaria is endemic, control is limited by drug resistance, cytotoxicity, cost and availability of new drugs. The persistence, and in some cases, resurgence, of malaria, especially in the developing world, is almost exclusively due to multiple drug resistance caused by monotherapy drug treatment with traditional antimalarials.<sup>4</sup> Chloroquine resistance has become unequivocal in *P. falciparum* regions. The WHO recommends the use of combination therapies for the treatment of uncomplicated *P. falciparum* malaria to delay or prevent drug resistance.

The emergence of artemisinin (**1**), derived from the Chinese herb Qing Hao (*Artemisia annua* L., family: Asteraceae), as a fast-acting effective antimalarial agent resulted in the development of numerous derivatives as well as artemisinin-based combination therapies (ACTs) to prevent parasite resistance.<sup>5,6</sup> The advantages of artemisinins over conventional antimalarial drugs include killing parasites more rapidly, toxicity at nanomolar concentrations, short fever clearance time (32 h vs 2–3 days for conventional antimalarials), activity against the sexual and asexual parasite stages, rapid action against all erythrocytic stages of parasite resulting in decreased transmission, limited resistance, safer and simpler oral

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Scheme 1. Synthesis of 6.

and parenteral administration, no significant side effects and efficacy for uncomplicated and severe malaria. Artemisinins have, however, short half-lives (1–4 h), resulting in recrudescence of infecting parasites and recurrent illness within days to weeks when used as a monotherapy over a standard three day course.<sup>5–7</sup> Artemisinin monotherapy was therefore banned by the WHO in 2006, while ACT was adopted as the suggested first-line treatment for uncomplicated *P. falciparum* malaria in all endemic areas. Intravenous artesunate (dihydroartemisinin hemisuccinate) became the treatment of choice for severe malaria, except for children in Africa.<sup>8</sup> ACT in conjunction with a long acting agent is a highly efficacious first-line therapy used in most countries where malaria is endemic. Disadvantages of ACTs are higher cost and a shorter shelf-life compared to traditional antimalarial drugs.<sup>5,9</sup> There is currently no ACT available in the United States.<sup>2a</sup>

The mechanism(s) of action of artemisinins have not been fully elucidated, with parasite specific and non-specific mechanisms hypothesized. The former includes interference with sarcoplasmic/endoplasmic reticulum  $\text{Ca}^{2+}$ -ATPases (SERCA) through homolytic cleavage of the endoperoxide bond, forming free radicals, and subsequent alkylation of heme and *Plasmodium*-specific proteins. Non-specific mechanisms include iron mediated production of reactive species via a reductive scission or an open peroxide model.<sup>4a,10</sup>

Recent reports of artemisinin-resistant malaria warrants the development of new artemisinin based drugs that can be used as ACTs.<sup>11</sup> Artemisinin dimers are known to possess not only significant antimalarial activity, but also remarkable cytotoxicity against tumor cells, with inhibitory activity in the nano- to micromolar range.<sup>12</sup> This manuscript reports the synthesis (Scheme 1, Table 1) of nine dihydroartemisinin acetal dimers (6–14) with diversely functionalized linker units (Fig. 1). A number of monomers (15–17) and a trimer (18) were isolated as byproducts from the dimer synthesis. The antimalarial, antileishmanial, anticancer (Tables 2–

4), antifungal and antibacterial activities of the artemisinins are also presented.

## 2. Results and discussion

### 2.1. Chemistry

Artemisinin (1) was obtained commercially.<sup>13</sup> Dihydroartemisinin (DHA) (2) was prepared by sodium borohydride ( $\text{NaBH}_4$ ) reduction of 1 (Scheme 1).<sup>14</sup> Anhydrodihydroartemisinin (3)<sup>15</sup> was isolated during the purification of 4.<sup>12b</sup> Dimers 4–5 were prepared and purified as previously described.<sup>12b,16</sup> Dimers 6–10 (Fig. 1) were synthesized by reacting DHA (2) with 3,5-dihydroxybenzyl alcohol, 3-aminophenol, *cis*-2-butene-1,4-diol, (*S*)-(-)-1-butene-3,4-diol and (*S*)-(-)-1,2,4-butanetriol, respectively, under mild acidic conditions using borontrifluoride etherate ( $\text{BF}_3\cdot\text{OEt}_2$ ) as a catalyst in dry ether (Scheme 1, Table 1). The reaction mixtures were chromatographed to isolate 6–10, as well as monomers 15 (byproduct from 4<sup>12b</sup>), 16 (byproduct from 6), 17 (byproduct from 8<sup>17</sup>) and trimer 18 (byproduct from 10). The vicinal diols 11 and 12 were prepared by osmium tetroxide *syn*-hydroxylation of 8 and 9, respectively. Prilezhaev stereospecific *cis*-epoxidation of 8 with *m*-chloroperoxybenzoic acid (*m*-CPBA) yielded 13, while methanesulfonyl chloride sulfonation of 5<sup>12b</sup> yielded 14.

### 2.2. Biological activity

#### 2.2.1. Antimalarial activity

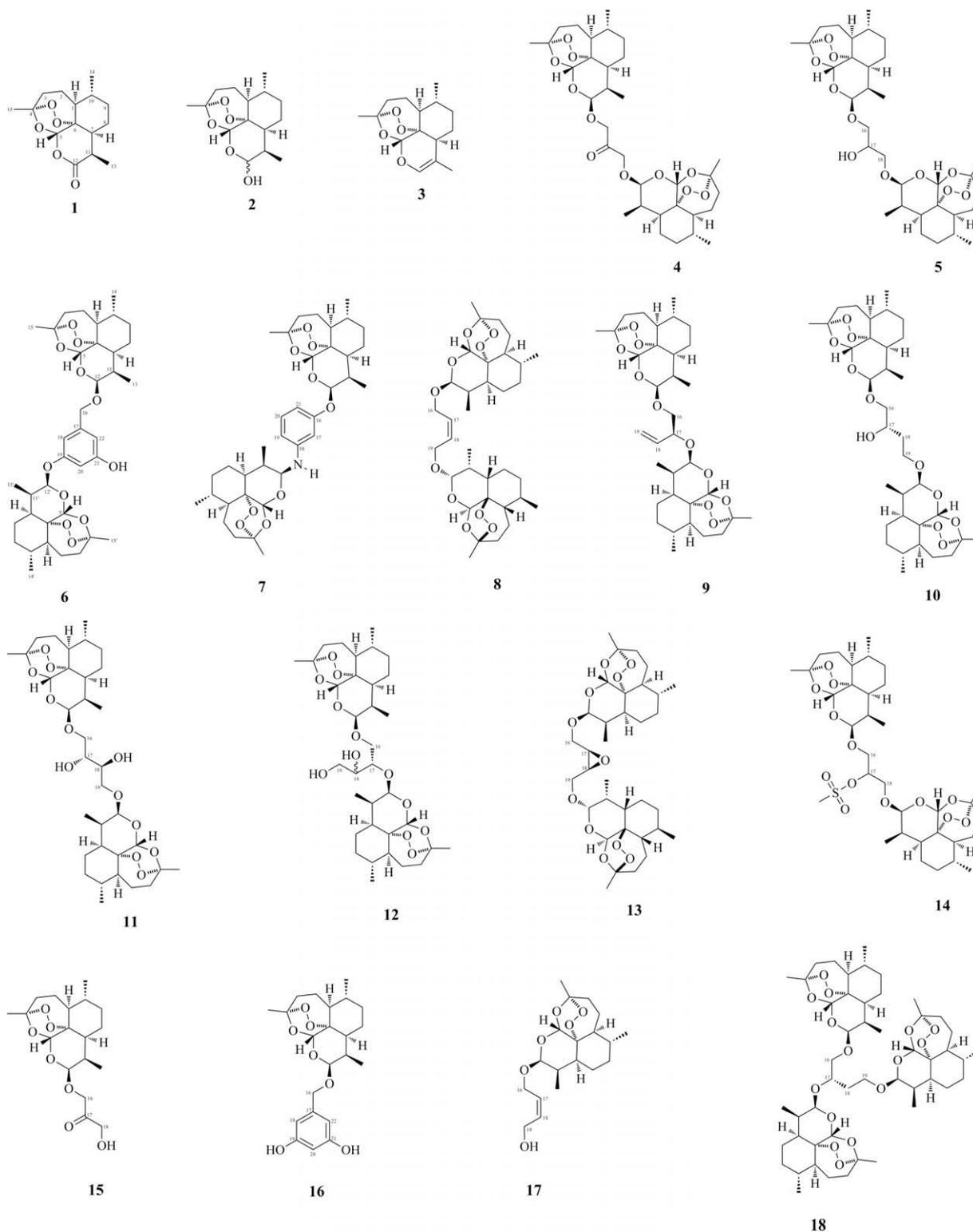
The *in vitro* antimalarial activity of 1–18 against chloroquine-sensitive (D6) and chloroquine-resistant (W2) clones of *P. falciparum* was evaluated based on the determination of plasmodial LDH activity.<sup>18</sup> In order to determine the selectivity index (S.I.) of antimalarial activity, cytotoxicity of 1–18 was also determined against mammalian Vero cells (Table 2).<sup>19</sup> S.I. is calculated as the ratio of  $\text{TC}_{50}$  cytotoxicity and  $\text{IC}_{50}$  antimalarial activity values,

Table 1  
Reaction details for synthesis of 6–10

Compound	DHA (2)			Linker			Ether (mL)	$\text{BF}_3\cdot\text{OEt}_2$ ( $\mu\text{L}$ )	MS <sup>a</sup> (g)	Crude <sup>b</sup> (mg)	Product	
	mg	mmol	equiv	mg	mmol	equiv					mg	%
6	360	1.27	2.11	84.0	0.60	1	120	400	1.7	330	28.0	6.9
7	510	1.79	18.82	10.4	0.10	1	75	700	–	410	44.0	71.9
8	402	1.41	2.22	56.2	0.64	1	75	220	–	335	88.0	22.2
9	480	1.69	3.10	48.0	0.54	1	50	410	–	416	135.0	39.9
10	540	1.90	2.08	97.0	0.91	1	60	450	–	575	55.0	9.4

<sup>a</sup> MS: molecular sieve.

<sup>b</sup> Crude: crude reaction mixture after workup.



**Figure 1.** Artemisinin (1), dihydroartemisinin (DHA) (2), anhydrodihydroartemisinin (3), DHA acetal dimers 4–14, monomers 15–17 and trimer 18.

and measures the therapeutic index of the compound under investigation to malaria parasites in comparison to its toxicity to the host cells (if there is any). An S.I.  $\geq 10$  is generally considered significant, indicating that antimalarial activity is not due to cytotoxicity.<sup>20</sup> The synthesized analogs 6–17 exhibited strong in vitro antimalarial activities, with  $IC_{50}$  values in the nanomolar range (3.0–45.5 nM for D6 clone and 4.2–47.1 nM for W2 clone) (Table 2). Dimers 6, 7, 8 and 11, and monomers 15 and 17 displayed excellent

activity against both clones ( $IC_{50} < 10$  nM), while trimer 18 displayed weak activity compared to the monomers and dimers. The S.I. for all compounds was  $>10$ , indicating that there is at least a 10-fold or higher difference in the effective dose for antimalarial activity and cytotoxicity; however, no significant difference was observed in the antimalarial efficacy towards D6 and W2 clones (Table 2). The activities of monomers 15 and 17 were comparable to their dimer analogs 4 and 8; however, monomer 16 was 5–6

**Table 2**  
Antimalarial and cytotoxic activity of **1–18**

Compound	<i>Plasmodium falciparum</i>				Cytotoxicity Vero TC <sub>50</sub> (nM)
	D6 clone		W2 clone		
	IC <sub>50</sub> (nM)	S.I.	IC <sub>50</sub> (nM)	S.I.	
<b>1</b>	32.9	>51	42.5	>40	>1686
<b>2</b>	14.8	95	14.1	100	1407
<b>3</b>	15.8	>113	25.5	>70	>1787
<b>4</b>	5.3	49	5.8	44	257
<b>5</b>	11.2	34	3.8	100	384
<b>6</b>	3.0	>2380	4.2	>1700	>7075
<b>7</b>	5.8	>1287	5.9	>1253	>7417
<b>8</b>	6.3	113	6.1	116	709
<b>9</b>	24.2	32	25.8	30	773
<b>10</b>	43.8	>170	45.4	>164	>7452
<b>11</b>	6.7	>1082	5.2	>1400	>7270
<b>12</b>	11.7	71	14.0	59	825
<b>13</b>	45.5	>164	47.1	>159	>7475
<b>14</b>	11.8	542	11.8	542	6403
<b>15</b>	7.9	>1700	7.0	>1904	>13,355
<b>16</b>	20.9	>560	20.7	>567	>11,711
<b>17</b>	6.2	>2164	7.6	>1763	>13,430
<b>18</b>	298.3	18	243.1	22	5259
Chloroquine <sup>a</sup>	41.6	>357.9	378.3	>39.3	>14,881

IC<sub>50</sub>: concentration causing 50% growth inhibition. TC<sub>50</sub>: concentration toxic to 50% of the cells. Vero: African monkey kidney fibroblast. S.I.: selectivity index [TC<sub>50</sub> (Vero)/IC<sub>50</sub> (*P. falciparum*)].

<sup>a</sup> Control.

times less active than the corresponding dimer **6**. Also, dimer **8** was ca. 4 times more active than its structural isomer **9**. Dihydroxylation of **8** and **9** yielded **11** and **12**, respectively, with **8** and **11** having similar activity, while **12** was twice as active as **9**. Dimer **5** was appreciably more active than its methylene homologue, dimer **10**; however, the hydroxy-analog of **10**, namely **11**, had activity comparable to **5**. These results, taken together with previously published data,<sup>12b</sup> indicate that increasing the chain length between two DHA units decreases antimalarial activity, while hydroxylation of the linker-chain increases the activity.

### 2.2.2. Antileishmanial activity

The three major forms of human leishmaniasis are normally differentiated as cutaneous, mucocutaneous and visceral, with the

**Table 3**  
Antileishmanial activity of **1–18**

Compound	IC <sub>50</sub> (μM)	IC <sub>90</sub> (μM)
<b>1</b>	40.7	NA
<b>2</b>	87.9	NA
<b>3</b>	30.0	NA
<b>4</b>	7.4	38.5
<b>5</b>	12.8	47.9
<b>6</b>	5.9	32.7
<b>7</b>	24.9	62.3
<b>8</b>	NA	NA
<b>9</b>	20.9	62.8
<b>10</b>	2.5	9.4
<b>11</b>	6.0	22.9
<b>12</b>	6.4	29.7
<b>13</b>	3.9	47.1
<b>14</b>	22.8	55.5
<b>15</b>	25.8	112.2
<b>16</b>	5.7	24.6
<b>17</b>	42.3	NA
<b>18</b>	5.0	44.2
Pentamidine <sup>a</sup>	2.8	6.4
Amphotericin B <sup>a</sup>	0.2	0.4

IC<sub>50</sub>: concentration causing 50% growth inhibition. IC<sub>90</sub>: concentration causing 90% growth inhibition. NA: not active.

<sup>a</sup> Control.

latter being potentially lethal. Leishmaniasis is caused by various species of the protozoan parasite *Leishmania*, which is transmitted by female sandflies.<sup>21</sup> Artemisinin (**1**) has been shown to have promising antileishmanial activity that is mediated by induction of apoptosis and therefore warrants further study as a possible therapeutic option for the treatment of leishmaniasis.<sup>12b,22</sup> Compounds **1–18** were tested against the protozoan parasite *Leishmania donovani*, using pentamidine and amphotericin B as drug controls (Table 3).<sup>18</sup> The in vitro antileishmanial activity of **6**, **10–13**, **16** and **18** was in the low micromolar range (IC<sub>50</sub>: 2.5–6.4 μM; IC<sub>90</sub>: 9.4–47.1 μM), with **10** possessing excellent IC<sub>50</sub> (2.5 μM) and IC<sub>90</sub> (9.4 μM) values. Dimers **10** and **13**, which showed relatively weak antimalarial activity (Table 2), were the most active compounds in the antileishmanial assay, with IC<sub>50</sub> values comparable to the control drug, pentamidine.

### 2.2.3. Anticancer activity

Artemisinins have shown promising anticancer properties in various cell lines and animal models, displaying activity against a variety of unrelated tumor cells lines, for example, colon, breast, lung, leukemia and pancreatic cancer.<sup>23</sup> Artemisinins cause decreased proliferation, apoptosis-induction, angiogenesis-inhibition and increased levels of oxidative stress to cancer cells.<sup>23</sup> The selectivity of artemisinins towards cancer cells coupled with its non-cytotoxicity towards normal cells makes it an ideal candidate for targeted delivery via, for example, the transferrin receptor mechanism, since artemisinins only become toxic after reacting with iron.<sup>23</sup> The in vitro cytotoxicity of **4–18** was determined against four human solid tumor (SK-MEL, KB, BT-549 and SK-OV-3) and two noncancerous mammalian cell lines (Vero and LLC-PK<sub>11</sub>) (Table 4).<sup>18,19</sup> The anti-cell proliferative activity of **8–10**, **13–14** and **18** was significantly enhanced compared to the control drug, doxorubicin, with **14** showing selectivity towards epidermal carcinoma (KB). The observation that **10**, **13** and **18** exhibited relatively weak antimalarial activity, strong antileishmanial activity and higher toxicity to all six cell lines indicates that the observed antileishmanial activity for these compounds could be related to their cytotoxic effect, while potential antimalarial activity of the other analogs is more specific.

The antitumor activity of **6** was evaluated against the National Cancer Institute's 60 cultured human tumor cell lines (leukemia, non-small cell lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer).<sup>24</sup> The cytostatic activity of **6** was evaluated at five concentrations (100, 10, 1.0, 0.1 and 0.01 μM) via the sulforhodamine B (SRB) protein assay<sup>25</sup> to estimate cell growth. Anticancer activity, inferred from dose–response curves, is expressed through GI<sub>50</sub> (drug concentration resulting in a 50% reduction in the net protein increase), TGI (drug concentration of total growth inhibition) and LC<sub>50</sub> (concentration of drug resulting in a 50% reduction in the measured protein at the end of the drug treatment as compared to that at the beginning) values for each cell line. The mean value for all tested cancer cell lines [MG\_MID (mean graph midpoint)] was also calculated. Dimer **6** displayed MG\_MID GI<sub>50</sub>, TGI and LC<sub>50</sub> values of 24.5 nM, 2.82 μM and 15.1 μM, respectively.

### 2.2.4. Antifungal activity

The in vitro antifungal activity of **1–18** indicated selective activity against *Cryptococcus neoformans*, with **7**, **12** and **17** displaying enhanced activity compared to the control drug, amphotericin B, confirming previous reports (see Supplementary data).<sup>26</sup>

### 2.2.5. Antibacterial activity

The in vitro antibacterial assay of **1–18** showed almost complete inactivity to a panel of bacteria (see Supplementary data). A

**Table 4**  
Anticancer and cytotoxic activity of **4–18**

Compound	Cancer cell lines				Noncancerous cell lines		
	SK-MEL	KB	BT-549	SK-OV-3	Vero	LLC-PK <sub>11</sub>	
	IC <sub>50</sub> (μM)				TC <sub>50</sub> (μM)		
<b>4</b>	0.56	0.24	0.24	NA	>8.03	0.10	
<b>5</b>	8.00	0.26	0.48	NA	5.60	0.15	
<b>6</b>	NA	NA	NA	NA	NA	NA	
<b>7</b>	NA	NA	NA	NA	NA	NA	
<b>8</b>	NA	0.32	1.53	NA	0.18	NC	
<b>9</b>	12.93	0.27	2.58	NA	0.43	>16.11	
<b>10</b>	0.24	0.16	0.08	NA	>1.57	0.10	
<b>11</b>	NA	NA	NA	NA	NA	NA	
<b>12</b>	NA	NA	NA	NA	NA	NA	
<b>13</b>	0.22	0.47	0.30	NA	>1.57	0.68	
<b>14</b>	NA	0.28	NA	NA	2.13	NC	
<b>15</b>	NA	NA	NA	NA	NA	20.76	
<b>16</b>	NA	NA	NA	NA	NA	2.46	
<b>17</b>	NA	NA	NA	NA	NA	NA	
<b>18</b>	0.24	0.11	0.06	NA	>1.10	0.07	
Doxorubicin <sup>a</sup>	1.73	2.04	1.96	2.04	NC	1.29	
Paclitaxel <sup>a</sup>	4.10	0.02	0.02	0.53	0.53	4.39	

IC<sub>50</sub>: concentration causing 50% growth inhibition. TC<sub>50</sub>: concentration toxic to 50% of the cells. SK-MEL: human malignant melanoma. KB: human epidermal carcinoma. BT-549: human breast carcinoma (ductal). SK-OV-3: human ovary carcinoma. Vero: African monkey kidney fibroblast. LLC-PK<sub>11</sub>: pig kidney epithelial. NA: not active. NC: not cytotoxic.

<sup>a</sup> Control.

cursory electronic literature search did not reveal any reports of significant antibacterial activity for artemisinin.

### 3. Conclusion

The remarkable chemistry and biological activity of artemisinin (**1**) and its derivatives, especially towards malaria and cancerous tumors, have been demonstrated in numerous reports, including the current communication. However, the disconcerting accounts of parasite resistance to artemisinins indicate that this valuable natural product might follow the same fate as numerous other drugs, that is, worldwide clinical resistance. Careful management of the current artemisinins and newly developed derivatives, such as the dimers reported herein, by health officials is critical in ensuring the longevity of these drugs in the global fight against disease. The excellent *in vitro* antimalarial and anticancer activity of the selected analogs presented in this study warrants further study as potential drug leads through *in vivo* assays and pharmacokinetic and pharmacodynamic analysis.

## 4. Experimental

### 4.1. General experimental conditions

All reactions were carried out in oven dried glassware. Diethyl ether (anhydrous) was dried over molecular sieves prior to use. All chemicals were purchased from Sigma–Aldrich or Acros Organics and used without further purification. Flash column chromatography was conducted with silica gel (particle size 230–400 mesh; SiliCycle). Analytical thin-layer chromatography (TLC) was performed with Silica Gel 60 F<sub>254</sub> plates (250 μm thickness; SiliCycle) using hexanes/EtOAc mixtures as solvent systems. Visualization was accomplished by spraying with *p*-anisaldehyde spray reagent followed by heating with a hot-air gun. IR spectra were obtained using an AATI Mattson Genesis Series FTIR. Optical rotations were recorded at ambient temperature using a Rudolph Research Analytical Autopol IV automatic polarimeter. 1D and 2D NMR spectra were obtained on Varian AS400 and Bruker DRX 400 spectrometers at 400 MHz (<sup>1</sup>H) and 100 MHz (<sup>13</sup>C) using the solvent peak as internal standard. The spectra were recorded in CDCl<sub>3</sub> and pyridine-*d*<sub>5</sub>.

The following abbreviations are used for NMR multiplicities: singlet (s), broad singlet (br s), doublet (d), broad doublet (br d), doublet of doublet (dd), triplet (t), broad triplet (br t) and multiplet (m). Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in hertz. HRESIFTMS were obtained on an Agilent Series 1100 SL mass spectrometer.

### 4.2. Synthesis

See [Supplementary data](#) for IUPAC nomenclature of **1–18**.

#### 4.2.1. Artemisinin (**1**), DHA (**2**), anhydrodihydroartemisinin (**3**) and dimers **4–5**

Artemisinin (**1**) was obtained commercially.<sup>13</sup> DHA (**2**) was prepared by NaBH<sub>4</sub> reduction of **1** (Scheme 1).<sup>14</sup> Anhydrodihydroartemisinin (**3**) was isolated during the purification of **4**.<sup>15</sup> Dimers **4–5** were prepared and purified as previously described.<sup>12b,16</sup>

#### 4.2.2. Dimers **6–10**

To a solution of DHA (**2**) (360 mg, 1.27 mmol, 2.11 equiv) in dry ether (120 mL) and molecular sieves (1.7 g) was added 3,5-dihydroxybenzyl alcohol (84 mg, 0.60 mmol, 1 equiv) and BF<sub>3</sub>·OEt<sub>2</sub> (400 μL) (Scheme 1, Table 1).<sup>12b</sup> The reaction mixture was stirred (2 h, rt, Ar) and quenched with 2% aqueous NaHCO<sub>3</sub>, followed by addition and extraction with ether. The combined organic layer was washed with water, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure to yield a crude product (330 mg) which was purified by column chromatography (hexanes/EtOAc, 1:0–1:1, stepwise) to yield **6** (28 mg, 6.9% yield).

Dimers **7–10** were similarly prepared as described for **6** using DHA (**2**) and 3-aminophenol, *cis*-2-butene-1,4-diol, (*S*)-(–)-1-butene-3,4-diol and (*S*)-(–)-1,2,4-butanetriol, respectively, as linkers (Table 1). Molecular sieves were, however, not utilized in these reactions.

#### 4.2.3. Dimers **11–12**

Dimer **8** (30 mg, 48.3 μmol, 1 equiv) was dissolved in dry pyridine (1 mL), osmium tetroxide (47 mg, 184.9 μmol, 3.83 equiv) added and the mixture stirred (105 min, rt). The reaction was subsequently quenched by addition of 10% aqueous Na<sub>2</sub>S<sub>2</sub>O<sub>5</sub> and

stirring (30 min), followed by addition and extraction with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was sequentially washed with 4% HCl and 2% NaHCO<sub>3</sub>, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under high vacuum to yield **11** (30 mg, 94.8% yield). Dimer **9** (42 mg, 67.7 μmol, 1 equiv) was similarly oxidized (osmium tetroxide, 60 mg, 236.0 μmol, 3.49 equiv), yielding **12** (44 mg, 99.3% yield).

#### 4.2.4. Dimer 13

Dimer **8** (22 mg, 35.4 μmol, 1 equiv), dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (2 mL), was epoxidized with *m*-CPBA (15 mg, 86.9 μmol, 2.45 equiv) (500 min, rt). The reaction mixture was quenched with 2% aqueous NaHSO<sub>3</sub>, followed by addition and extraction with CH<sub>2</sub>Cl<sub>2</sub>. The crude product was purified via preparative TLC (hexanes/EtOAc, 75:25) to afford **13** (15 mg, 66.5% yield).

#### 4.2.5. Dimer 14

Dimer **5** (24 mg, 38.4 μmol, 1 equiv), dissolved in dry pyridine (2 mL), was sulfonated with methanesulfonyl chloride (31 mg, 270.6 μmol, 7.04 equiv) (180 min, 0–6 °C, N<sub>2</sub>). The reaction mixture

was quenched by the addition of water, followed by addition and extraction with CH<sub>2</sub>Cl<sub>2</sub>. The organic layer was sequentially washed with 4% HCl and distilled water, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under high vacuum to yield **14** (21 mg, 77.8% yield).

#### 4.2.6. Monomers 15–17 and trimer 18

Monomers **15** (80 mg), **16** (99 mg) and **17** (46 mg), and trimer **18** (155 mg) were isolated during the purification of **4**,<sup>12b,16</sup> **6**, **8** and **10**, respectively

#### 4.3. Spectroscopic and physical data for 6–18

NMR spectroscopic data are given in Table 5 (<sup>1</sup>H) and Table 6 (<sup>13</sup>C), and HRESIFTMS data are given in Table 7.

##### 4.3.1. Dimer 6

White solid; *R*<sub>f</sub> 0.28 (hexanes/EtOAc, 1:1); [ $\alpha$ ]<sub>D</sub> +181.5 (c 0.13, MeOH); IR (neat)  $\nu_{\max}$ : 3393 (OH) cm<sup>-1</sup>.

**Table 5**  
<sup>1</sup>H NMR data for **5** and **6–18** ( $\delta_{\text{H}}$  in ppm, *J* values in Hz)

Position	<b>5</b> <sup>a</sup>	<b>6</b> <sup>a</sup>	<b>7</b> <sup>a</sup>	<b>8</b> <sup>a</sup>	<b>9</b> <sup>a</sup>	<b>10</b> <sup>a</sup>	<b>11</b> <sup>a</sup>	<b>12</b> <sup>a</sup>
5/5'	5.35 (s)	5.45 (s) 5.40 (s)	5.49 (s)	5.40 (s)	5.36 (s) 5.41 (s)	5.37 (s) 5.43 (s)	5.8 (s) 5.9 (s)	5.44 (s) 5.43 (s)
11/11'	2.61 (m)	2.64 (m) 2.76 (m)	2.78 (m)	2.54 (br s)	2.58 (m)	2.60 (m)	2.65 (m)	2.66 (m)
12/12'	4.773 (d, 3.8) 4.766 (d, 3.8)	4.87 (d, 3.6) 5.42 (d, 3.2)	5.45 (d, 4.0) 6.48 (d, 1.2)	4.80 (d, 3.2)	4.74 (d, 2.8) 4.84 (d, 3.6)	4.78 (d, 3.2) 4.76 (d, 3.2)	4.80 (d, 3.6) 4.82 (d, 3.6)	4.85 (d, 3.2) 4.94 (d, 3.2)
13/13'	0.88 (d, 7.4)	0.97 (d, 7.6) 0.99 (d, 7.2)	0.99 (d, 7.2)	0.90 (d, 7.6)	0.86 (d, 7.2) 0.83 (d, 7.2)	0.88 (d, 7.2)	0.89 (d, 7.2)	0.93 (d, 7.2)
14/14'	0.91 (d, 6.3)	0.93 (d, 6.0)	0.95 (d, 6.0)	0.94 (d, 6.4)	0.92 (d, 6.4)	0.91 (d, 6.0)	0.93 (d, 6.8)	0.95 (d, 6.4)
15/15'	1.38 (s)	1.41 (s) 1.44 (s)	1.43 (s)	1.43 (s)	1.39 (s)	1.38 (s)	1.40 (s)	1.40 (s) 1.43 (s)
16	3.47 (dd, 10.3, 4.3) 3.84 (m)	4.40 (d, 12.4) 4.74 (d, 12.4)	—	4.01 (d, 10.8) 4.26 (d, 10.8)	3.43 (m) 3.89 (m)	3.48 (m) 3.68 (m)	4.01 (dd, 10.8, 3.2) 3.91 (m)	3.80 (m)
17	3.84 (m)	—	6.48 (d, 1.2)	5.59 (br s)	4.33 (m)	3.88	3.73 (m)	3.80 (m)
18	3.40 (dd, 9.8, 5.4) 3.84 (m)	6.45 (s) 6.56 (s)	—	5.59 (br s)	5.61 (m)	1.72 (m)	3.73 (m)	3.80 (m)
19	—	—	6.32 (d, 7.6)	4.01 (d, 10.8) 4.26 (d, 10.8)	5.23 (m)	3.52 (m) 3.93 (m)	3.66 (dd, 11.2, 3.6) 3.56 (m)	4.14 (br d, 8.8) 3.66 (m)
20	—	6.57 (s)	7.03 (d, 8.0)	—	—	—	—	—
21	—	—	6.50 (br d)	—	—	—	—	—
22	—	6.45 (s) 6.56 (s)	—	—	—	—	—	—
Position	<b>5</b> <sup>a</sup>	<b>13</b> <sup>a</sup>	<b>14</b> <sup>b</sup>	<b>15</b> <sup>a</sup>	<b>16</b> <sup>b</sup>	<b>17</b> <sup>a</sup>	<b>18</b> <sup>a</sup>	
5/5'	5.35 (s)	5.39 (s)	5.65 (s) 5.72 (s)	5.42 (s)	5.69 (s)	5.35 (s)	5.33 (s) 5.40 (s)	
11/11'	2.61 (m)	2.62 (br s)	2.78 (br s)	2.70 (m)	2.81 (br m)	2.54 (br s)	2.62 (m)	
12/12'	4.773 (d, 3.8) 4.766 (d, 3.8)	4.77 (d, 3.2) 4.84 (d, 3.2)	4.92 (d, 2.8) 4.98 (d, 3.2)	4.83 (d, 3.6)	5.06 (br s)	4.80 (d, 3.6)	4.72 (d, 3.2) 4.79 (d, 2.8) 4.81 (d, 3.2)	
13/13'	0.88 (d, 7.4)	0.91 (d, 7.2)	0.90 (d, 7.2)	0.94 (d, 6.0)	0.87 (d, 7.2)	0.85 (d, 7.6)	0.86 (d, 7.2) 0.90 (d, 7.6)	
14/14'	0.91 (d, 6.3)	0.92 (d, 6.4)	0.85 (d, 6.8)	0.99 (d, 6.4)	0.80 (d, 6.0)	0.89 (d, 6.4)	0.925 (d, 6.4) 0.932 (d, 6.0)	
15/15'	1.38 (s)	1.40 (s)	1.48 (s)	1.43 (s)	1.51 (s)	1.37 (s)	1.36 (s) 1.39 (s) 1.40 (s)	
16	3.47 (dd, 10.3, 4.3) 3.84 (m)	3.96 (dd, 3.2, 2.8) 3.57 (m)	3.76 (dd, 5.6, 10.8) 4.37 (dd, 3.6, 10.8)	4.23 (d, 17.6) 4.42 (s)	4.62 (d, 12.0) 5.04 (d, 13.6)	5.59 (m) 5.75 (m)	3.71 (dd, 3.6, 6.8) 3.86 (m)	
17	3.84 (m)	3.19 (m)	5.30 (m)	—	—	5.59 (m)	3.86 (m)	
18	3.40 (dd, 9.8, 5.4) 3.84 (m)	3.19 (m)	3.80 (dd, 4.4, 11.2) 4.37 (dd, 3.6, 10.8)	4.53 (d, 17.2) 4.42 (s)	6.90 (s)	5.75 (m)	1.73 (m) 1.86 (m)	
19	—	3.83 (m) 3.57 (m)	—	—	—	5.59 (m) 5.75 (m)	3.44 (m) 3.86 (m)	
20	—	—	—	—	6.96 (s)	—	—	
21	—	—	—	—	—	—	—	
22	—	—	—	—	6.90 (s)	—	—	

<sup>a</sup> CDCl<sub>3</sub>.

<sup>b</sup> Pyridine-*d*<sub>5</sub>.

**Table 6**  
<sup>13</sup>C NMR data for **5** and **6–18** ( $\delta_c$  in ppm)

Position	5 <sup>a</sup>	6 <sup>a</sup>	7 <sup>a</sup>	8 <sup>a</sup>	9 <sup>a</sup>	10 <sup>a</sup>	11 <sup>a</sup>	12 <sup>a</sup>
1/1'	52.9	52.6	52.8	52.7	52.8	52.7/52.8	52.7	52.65/52.71
2/2'	25.0	24.4/24.5/24.67/24.71	24.7/24.9	24.6/24.8	24.5/24.7/24.9	24.69/24.85/24.87	24.8	24.8/24.9
3/3'	36.9	36.4/36.5	36.6	36.6	36.62/36.64	36.59/36.63	36.6	36.6
4/4'	104.5	104.2/104.3	104.4	104.2	104.3	104.27/104.32	104.4	104.3/104.5
5/5'	88.3	88.1/88.3	88.5	88.1	88.27/88.30	88.07/88.12	88.1/88.2	88.3/88.2
6/6'	81.4	81.0/81.2	81.3	81.3	81.3/81.4	81.1/81.2	81.1/81.2	81.2
7/7'	44.8	44.42/44.44	44.7	44.6	44.59/44.64	44.5/44.6	44.4/44.5	44.5
8/8'	25.0	24.4/24.5/24.67/24.71	24.7/24.9	24.6/24.8	24.5/24.7/24.9	24.69/24.85/24.87	24.8	24.8/24.9
9/9'	35.0	34.66/34.69	34.9	34.8	34.9	33.9/34.2	34.8	34.8
10/10'	37.8	37.41/37.46	37.6	37.5	37.56/37.64	37.66/37.67	37.7	37.6/37.7
11/11'	31.3	31.0	31.3	31.0	30.9/31.2	31.1	31.1	31.0/31.4
12/12'	103.2/103.1	100.6/101.4	100.6/103.8	101.7	98.9/103.0	102.4/102.6	102.8/103.0	103.2/103.3
13/13'	13.5	13.0/13.2	13.2	13.2	13.2/13.3	13.19/13.21	13.3	13.2/13.5
14/14'	20.8	20.36/20.39	20.6	20.6	20.58/20.62	20.50/20.53	20.6	20.5/20.6
15/15'	26.5	26.0/26.2	26.3	26.3	26.4	26.29/26.34	26.3	26.4
16	70.2	69.4	158.9	64.1	71.8	73.3	70.6	63.7
17	70.1	141.2	103.8	129.2	76.4	68.7	71.4	78.5
18	70.3	108.0/108.2	148.0	129.2	135.9	33.9	71.4	71.6
19	—	156.9/158.5	109.2	64.1	119.3	65.7	70.2	69.5
20	—	103.3	130.0	—	—	—	—	—
21	—	156.9/158.5	107.2	—	—	—	—	—
22	—	108.0/108.2	—	—	—	—	—	—

Position	5 <sup>a</sup>	13 <sup>a</sup>	14 <sup>b</sup>	15 <sup>a</sup>	16 <sup>b</sup>	17 <sup>a</sup>	18 <sup>a</sup>
1/1'	52.9	52.7/52.8	53.00/53.03	52.4	53.0	52.6	52.8
2/2'	25.0	24.7/24.9	25.1/25.3	24.4/24.6	25.11/25.38	24.6/24.8	24.6/24.7/24.8/24.90/24.91
3/3'	36.9	36.6	37.1	36.3	37.1	36.5	36.7
4/4'	104.5	104.3/104.4	104.0	104.3	104.5	104.5	104.17/104.27/104.30
5/5'	88.3	88.1/88.2	88.5/88.6	88.1	88.5	88.1	88.11/88.15/88.17
6/6'	81.4	81.4	80.2/81.4	80.9	81.5	81.3	81.2/81.3
7/7'	44.8	44.55/44.56	44.9/45.0	44.1	45.1	44.5	44.64/44.69/44.74
8/8'	25.0	24.7/24.9	25.1/25.3	24.4/24.6	25.11/25.38	24.6/24.8	24.6/24.7/24.8/24.87/24.91
9/9'	35.0	34.8	35.1	34.5	35.0	34.7	34.8/34.9
10/10'	37.8	37.6/37.7	37.5/37.7	37.5	37.6	37.6	37.65/37.69
11/11'	31.3	31.0/31.1	31.56/31.64	30.7	31.7	31.0	31.1/31.2/31.6
12/12'	103.2/103.1	102.4/103.0	102.5/103.2	102.7	101.7	101.6	102.3/102.6/103.1
13/13'	13.5	13.2	13.39/13.45	13.0	13.6	13.14	13.2/13.4/13.7
14/14'	20.8	20.5	20.77/20.81	20.3	20.7	20.54	20.49/20.53
15/15'	26.5	26.3	26.3/26.5	26.0	26.5	26.2	26.4
16	70.2	67.1	67.3/67.8	71.5	70.4	58.3	71.7
17	70.1	54.4	80.2	207.8	142.0	132.7	75.6
18	70.3	55.1	67.3/67.8	66.7	106.6	128.0	33.0
19	—	66.5	—	—	160.8	63.6	64.6
20	—	—	—	—	103.4	—	—
21	—	—	—	—	160.8	—	—
22	—	—	—	—	106.6	—	—

<sup>a</sup> CDCl<sub>3</sub>.<sup>b</sup> Pyridine-*d*<sub>5</sub>.**Table 7**  
HRESIFTMS (direct injection, positive mode) data for **6–18**

Compound	Molecular formula	[M+Na] <sup>+</sup>			[M+K] <sup>+</sup>		
		Exact	Exp	Error	Exact	Exp	Error
<b>6</b>	C <sub>37</sub> H <sub>52</sub> O <sub>11</sub>	695.3408	695.3284	17.8	—	—	—
<b>7</b>	C <sub>36</sub> H <sub>51</sub> NO <sub>9</sub>	664.3462	664.3330	19.9	680.3201	680.3117	12.3
<b>8</b>	C <sub>34</sub> H <sub>52</sub> O <sub>10</sub>	643.3458	643.3391	10.4	659.3197	659.3090	16.2
<b>9</b>	C <sub>34</sub> H <sub>52</sub> O <sub>10</sub>	643.3458	643.3369	13.8	659.3197	659.3101	14.6
<b>10</b>	C <sub>34</sub> H <sub>54</sub> O <sub>11</sub>	661.3564	661.3624	9.1	—	—	—
<b>11</b>	C <sub>34</sub> H <sub>54</sub> O <sub>12</sub>	677.3513	677.3413	14.8	693.3252	693.3115	19.8
<b>12</b>	C <sub>34</sub> H <sub>54</sub> O <sub>12</sub>	677.3513	677.3416	14.3	693.3252	693.3172	11.5
<b>13</b>	C <sub>34</sub> H <sub>52</sub> O <sub>11</sub>	659.3408	659.3630	33.7	—	—	—
<b>14</b>	C <sub>34</sub> H <sub>54</sub> O <sub>13</sub> S	725.3183	725.3140	5.9	741.2922	741.2840	11.1
<b>15<sup>a</sup></b>	C <sub>18</sub> H <sub>28</sub> O <sub>7</sub>	355.1757	355.1762	1.5	—	—	—
<b>16</b>	C <sub>22</sub> H <sub>30</sub> O <sub>7</sub>	429.1890	429.1888	0.5	445.1629	445.1613	3.6
<b>17</b>	C <sub>19</sub> H <sub>30</sub> O <sub>6</sub>	377.1940	377.1913	7.2	393.1679	393.1680	0.3
<b>18</b>	C <sub>49</sub> H <sub>76</sub> O <sub>15</sub>	927.5082	927.5106	2.6	943.4821	943.5021	21.2

Exp: experimental. Error: error in ppm.

<sup>a</sup> [M–H]<sup>–</sup> data.

**4.3.2. Dimer 7**

Yellowish solid;  $R_f$  0.43 (hexanes/EtOAc, 7:3);  $[\alpha]_D +176.0$  (c 0.10, MeOH); IR (neat)  $\nu_{\max}$ : 3447 (NH), 3376 (NH)  $\text{cm}^{-1}$ .

**4.3.3. Dimer 8**

White solid;  $R_f$  0.59 (hexanes/EtOAc, 7:3);  $[\alpha]_D +160.0$  (c 0.10, MeOH).

**4.3.4. Dimer 9**

White solid;  $R_f$  0.67 (hexanes/EtOAc, 7:3);  $[\alpha]_D +131.8$  (c 0.10, MeOH).

**4.3.5. Dimer 10**

White solid;  $R_f$  0.29 (hexanes/EtOAc, 7:3);  $[\alpha]_D +134.8$  (c 0.095, MeOH); IR (neat)  $\nu_{\max}$ : 3503 (OH)  $\text{cm}^{-1}$ .

**4.3.6. Dimer 11**

White solid;  $R_f$  0.53 (hexanes/EtOAc, 3:7);  $[\alpha]_D +147.8$  (c 0.10, MeOH).

**4.3.7. Dimer 12**

White solid;  $R_f$  0.12 (hexanes/EtOAc, 3:7);  $[\alpha]_D +135.8$  (c 0.10, MeOH).

**4.3.8. Dimer 13**

White solid;  $R_f$  0.51 (hexanes/EtOAc, 75:25);  $[\alpha]_D +141.1$  (c 0.095, MeOH).

**4.3.9. Dimer 14**

Yellowish solid;  $R_f$  0.40 (hexanes/EtOAc, 7:3);  $[\alpha]_D +104.0$  (c 0.10, MeOH); IR (neat)  $\nu_{\max}$ : 1361 ( $\text{SO}_2\text{Me}$ )  $\text{cm}^{-1}$ .

**4.3.10. Monomer 15**

White solid;  $R_f$  0.47 (hexanes/EtOAc, 1:1);  $[\alpha]_D +105.5$  (c 0.165, MeOH); IR (neat)  $\nu_{\max}$ : 3462 (OH), 1733 (C=O)  $\text{cm}^{-1}$ .

**4.3.11. Monomer 16**

White solid;  $R_f$  0.06 (hexanes/EtOAc, 1:1);  $[\alpha]_D +134.2$  (c 0.155, MeOH); IR (neat)  $\nu_{\max}$ : 3385 (OH)  $\text{cm}^{-1}$ .

**4.3.12. Monomer 17**

Oil;  $R_f$  0.18 (hexanes/EtOAc, 7:3);  $[\alpha]_D +125.8$  (c 0.10, MeOH); IR (neat)  $\nu_{\max}$ : 3482 (OH)  $\text{cm}^{-1}$ .

**4.3.13. Trimer 18**

White solid;  $R_f$  0.41 (hexanes/EtOAc, 7:3);  $[\alpha]_D +157.3$  (c 0.15, MeOH).

**4.4. Biological assays****4.4.1. Antimalarial assay**

The assay is based on the determination of plasmodial LDH activity.<sup>18</sup> A suspension of red blood cells infected with D6 or W2 strains of *P. falciparum* [200  $\mu\text{L}$ , 2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and Amikacin (60  $\mu\text{g}/\text{mL}$ )] was added to a 96-well plate containing 10  $\mu\text{L}$  of serially diluted test samples. The plate was placed in a modular incubation chamber flushed with  $\text{N}_2/\text{O}_2/\text{CO}_2$  (90:5:5) and incubated (37 °C, 72 h). Parasitic LDH activity was determined by using Malstat™ reagent (Flow Inc., Portland, OR).<sup>27</sup> The incubation mixture (20  $\mu\text{L}$ ) was mixed with the Malstat™ reagent (100  $\mu\text{L}$ ) and incubated (30 min). Nitro blue tetrazolium (NBT)/phenazine ethosulfate (PES) (20  $\mu\text{L}$ , 1:1) (Sigma, St. Louis, MO) was added and the plate incubated in the dark (60 min). The reaction was stopped by the addition of 5% acetic acid (100  $\mu\text{L}$ ) and the plate was read at 650 nm on an EL340 BioKinetics Reader (Bio-Tek

Instruments, Winooski, VT).  $\text{IC}_{50}$  values were computed from the dose–response curves by plotting percent growth versus test concentration. Artemisinin (**1**) and chloroquine were included in each assay as drug controls.

**4.4.2. Antileishmanial assay**

The antileishmanial activity was evaluated against a culture of *L. donovani* promastigotes grown in RPMI 1640 medium supplemented with 10% GIBCO fetal calf serum at 26 °C.<sup>18</sup> A three-day-old culture was diluted to  $5 \times 10^5$  promastigotes/mL. Drug dilutions (50–3.1  $\mu\text{g}/\text{mL}$ ) were prepared directly in cell suspension in a 96-well plate, followed by incubation (26 °C, 48 h). Growth of leishmanial promastigotes was determined by the Alamar Blue assay (BioSource International, Camarillo, CA).<sup>28</sup> Standard fluorescence was measured by a Fluostar Galaxy plate reader (excitation wavelength, 544 nm; emission wavelength, 590 nm). Pentamidine and amphotericin B were used as the drug controls. Percent growth was calculated and plotted against the tested concentrations in order to determine the  $\text{IC}_{50}$  and  $\text{IC}_{90}$  values.

**4.4.3. Anticancer assay**

The cytotoxicity was determined against a panel of cancer (SK-MEL, KB, BT-549 and SK-OV-3) and noncancerous cell lines (Vero and LLC-PK<sub>11</sub>) (American Type Culture Collection, Rockville, MD). The assay was performed in 96-well tissue culture-treated microplates.<sup>18,19</sup> Cells (25,000 cells/well) were seeded in the wells of the plate and incubated (24 h), followed by addition of the samples and further incubation (48 h). The number of viable cells was determined using Neutral Red.<sup>29</sup>  $\text{IC}_{50}$  values were determined from logarithmic graphs of growth inhibition versus concentration. Doxorubicin was used as a positive control drug, while DMSO was used as the negative (vehicle) control.

**4.4.4. Antimicrobial assay**

All organisms were obtained from the American Type Culture Collection (Manassas, VA) [fungi: *Candida albicans* (ATCC 90028), *Candida glabrata* (ATCC 90030), *Candida krusei* (ATCC 6258), *C. neoformans* (ATCC 90113) and *Aspergillus fumigatus* (ATCC 90906); bacteria: *Staphylococcus aureus* (ATCC 29213), methicillin-resistant *S. aureus* (MRSA) (ATCC 43300), *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853) and *Mycobacterium intracellulare* (ATCC 23068)]. Susceptibility testing was performed for all organisms, except *M. intracellulare*, using modified versions of the CLSI/NCCLS methods.<sup>30</sup> *M. intracellulare* susceptibility was tested using a modified Franzblau-method.<sup>31</sup> Samples dissolved in DMSO were serially diluted in DMSO/saline (20%/0.9%) and transferred in duplicate to 96-well flat bottom microplates. Microbial inocula were prepared by correcting the  $\text{OD}_{630}$  of microbe suspensions in incubation broth to afford final target inocula. Controls [fungi: amphotericin B; bacteria: ciprofloxacin (ICN Biomedicals, OH)] were included in each assay. All organisms were read at 630 or 544(ex)/590(em) nm (*M. intracellulare* and *A. fumigatus*) prior to and after incubation. Percent growth was plotted versus test concentration to afford the  $\text{IC}_{50}$ .

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.10.019.

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