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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_{50}: 3.0–6.7 nM (D6) and 4.2–5.9 nM (W2)] were appreciably more active than artemisinin (**1**) [IC_{50}: 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_{50}: 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.

Keywords

Artemisia annua; Artemisinin; Dihydroartemisinin; Acetal monomers; dimers and trimers; Antimalarial activity; Antileishmanial activity; Anticancer activity; Antifungal activity; Antibacterial 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 4,000 years, causing illness and an estimated 1.5–2.5 million deaths each year.[1] 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, *P. 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.[2]

Chemotherapy is still at the forefront in the fight against malaria due to the unavailability of effective vaccines.[3] Numerous drugs have been developed for the treatment of uncomplicated malaria, e.g., mefloquine, primaquine, quinidine, proguanil, atovaquone 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.[4] 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.5–6 The advantages of artemisinins over conventional antimalarial drugs include killing parasites more rapidly, toxicity at nanomolar concentrations, short fever clearance time (32 h versus 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 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.5–7 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.[8] 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.5,9 There is currently no ACT available in the United States.2a

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 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.[10]

Recent reports of artemisinin-resistant malaria warrants the development of new artemisinin based drugs that can be used as ACTs.[11] Artemisinin dimers are known to possess not only significant antimalarial activity, but also remarkable cytotoxicity against tumor cells, with inhibitory activity of artemisinins in the nano- to micromolar range.[12] 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.[13] Dihydroartemisinin (DHA) (**2**) was prepared by sodium borohydride ($NaBH_4$) reduction of **1** (Scheme 1).[14] Anhydrodihydroartemisinin (**3**)[15] was isolated during the purification of **4**.[12b] Dimers **4–5** were prepared and purified as previously described.[12b,16] 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 ($BF_3{\cdot}OEt_2$) 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**12b), **16** (byproduct from **6**), **17** (byproduct from **8**17) 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**[12b] 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.[18] 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).[19] S.I. is calculated as the ratio of TC_{50} cytotoxicity values and IC_{50} antimalarial activity values, 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). A S.I. $\geq$ 10 is generally considered significant, indicating that antimalarial activity is not due to cytotoxicity.[20] 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 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,[12b] 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 latter being potentially lethal. Leishmaniasis is caused by various species of the protozoan parasite *Leishmania*, which is transmitted by female sandflies.[21] Artemisinin (**1**) has been shown to

have promising antileishmanial activity that is mediated by induction of apoptosis and therefore warrants further study as a therapeutic option for the treatment of leishmaniasis.[12b,22] Compounds **1–18** were tested against the protozoan parasite *Leishmania donovani*, using pentamidine and amphotericin B as drug controls (Table 3).[18] The in vitro antileishmanial activity of **6**, **10–13**, **16** and **18** was in the low micromolar range (IC_{50}: 2.5 6.4 μM; IC_{90}: 9.4 47.1 μM), with **10** possessing excellent IC_{50} (2.5 μM) and IC_{90} (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_{50} 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, e.g., colon, breast, lung, leukemia and pancreatic cancer.[23] Artemisinins cause decreased proliferation, apoptosis-induction, angiogenesis-inhibition and increased levels of oxidative stress to cancer cells.[23] The selectivity of artemisinins towards cancer cells coupled with its non-cytotoxicity towards normal cells makes it an ideal candidate for targeted delivery via, e.g., the transferrin receptor mechanism, since artemisinins only become toxic after reacting with iron.[23] 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_{11}) (Table 4).[18–19] 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).[24] 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[25] to estimate cell growth. Anticancer activity, inferred from dose-response curves, is expressed through GI_{50} (drug concentration resulting in a 50% reduction in the net protein increase), TGI (drug concentration of total growth inhibition) and LC_{50} (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_{50}, TGI and LC_{50} 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 *C. neoformans*, with **7**, **12** and **17** displaying enhanced activity compared to the control drug, amphotericin B, confirming previous reports (See Supplementary data).[26]

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

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, i.e., 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_{254} 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 (^{1}H) and 100 MHz (^{13}C) using the solvent peak as internal standard. The spectra were recorded in $CDCl_3$ and pyridine-d_5. 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.[13] DHA (**2**) was prepared by $NaBH_4$ reduction of **1** (Scheme 1).[14] Anhydrodihydroartemisinin (**3**) was isolated during the purification of **4**.[15] Dimers **4**–**5** were prepared and purified as previously described.[12b,16]

4.2.2. Dimers 6–10—To a solution of DHA (**2**) (360 mg, 1.27 mmol, 2.11 eq.) in dry ether (120 mL) and molecular sieves (1.7 g) was added 3,5-dihydroxybenzyl alcohol (84 mg, 0.60 mmol, 1 eq.) and $BF_3{\cdot}OEt_2$ (400 μL) (Scheme 1, Table 1).[12b] The reaction mixture was stirred (2 h, r.t., Ar) and quenched with 2% aqueous $NaHCO_3$, followed by addition and extraction with ether. The combined organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield a crude product (330 mg) which was purified by column chromatography (hexanes/EtOAc, 1:0 to 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 eq.) was dissolved in dry pyridine (1 mL), osmium tetroxide (47 mg, 184.9 μmol, 3.83 eq.) added and the mixture stirred (105 min., r.t.). The reaction was subsequently quenched by addition of 10% aqueous $Na_2S_2O_5$ and

stirring (30 min), followed by addition and extraction with CH_2Cl_2. The organic layer was sequentially washed with 4% HCl and 2% $NaHCO_3$, dried over anhydrous Na_2SO_4 and concentrated under high vacuum to yield **11** (30 mg, 94.8% yield). Dimer **9** (42 mg, 67.7 μmol, 1 eq.) was similarly oxidized (osmium tetroxide, 60 mg, 236.0 μmol, 3.49 eq.), yielding **12** (44 mg, 99.3 % yield).

4.2.4. Dimer 13—Dimer **8** (22 mg, 35.4 μmol, 1 eq.), dissolved in dry CH_2Cl_2 (2 mL), was epoxidized with *m*-CPBA acid (15 mg, 86.9 μmol, 2.45 eq.) (500 min., r.t.). The reaction mixture was quenched with 2% aqueous $NaHSO_3$, followed by addition and extraction with CH_2Cl_2. 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 eq.), dissolved in dry pyridine (2 mL), was sulfonated with methanesulfonyl chloride (31 mg, 270.6 μmol, 7.04 eq.) (180 min., 0–6°C, N_2). The reaction mixture was quenched by the addition of water, followed by addition and extraction with CH_2Cl_2. The organic layer was sequentially washed with 4% HCl and distilled water, dried over anhydrous Na_2SO_4 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**,[12b,16] **6**, **8** and **10**, respectively.

4.3. Spectroscopic and physical data for 6–18

NMR spectroscopic data are given in Table 5 (^1H) and Table 6 (^{13}C), and HRESIFTMS data are given in Table 7.

4.3.1. Dimer 6—White solid; R_f 0.28 (hexanes/EtOAc, 1:1); $[\alpha]_D$ +181.5 (*c* 0.13, MeOH); IR (neat) ν_{max}: 3393 (OH) cm^{-1}.

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) ν_{max}: 3447 (NH), 3376 (NH) 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) ν_{max}: 3503 (OH) 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) ν_{max}: 1361 (SO_2Me) 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) ν_{max}: 3462 (OH), 1733 (C=O) 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) ν_{max}: 3385 (OH) 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) ν_{max}: 3482 (OH) 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. In vitro antimalarial assay—The assay is based on the determination of plasmodial LDH activity.[18] A suspension of red blood cells infected with D6 or W2 strains of *P. falciparum* [200 μL, 2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and Amikacin (60 μg/mL)] was added to a 96-well plate containing 10 μL of serially diluted test samples. The plate was placed in a modular incubation chamber flushed with $N_2/O_2/CO_2$ (90:5:5) and incubated (37°C, 72 h). Parasitic LDH activity was determined by using Malstat™ reagent (Flow Inc., Portland, OR).[27] The incubation mixture (20 μL) was mixed with the Malstat™ reagent (100 μL) and incubated (30 min). Nitro blue tetrazolium (NBT)/phenazine ethosulfate (PES) (20 μL, 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 μL) and the plate was read at 650 nm on an EL340 BioKinetics Reader (Bio-Tek Instruments, Winooski, VT). 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. In vitro antileishmanial assay—The in vitro 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.[18] A three-day-old culture was diluted to 5×10^5 promastigotes/mL. Drug dilutions (50–3.1 μg/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).[28] 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 IC_{50} and IC_{90} values.

4.4.3. Anticancer assay—The in vitro 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_{11}) (American Type Culture Collection, Rockville, MD). The assay was performed in 96-well tissue culture-treated microplates.18–19 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.29 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), *C. glabrata* (ATCC 90030), *C. krusei* (ATCC 6258), *Cryptococcus neoformans* (ATCC 90113) and *Aspergillus fumigatus* (ATCC 90906); bacteria: *Staphylococcus aureus* (ATCC 29213), methicillin-resistant *S. aureus* (MR*Sa*) (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.[30] *M. intracellulare* susceptibility was tested using a modified

Franzblau-method.[31] 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 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 IC_{50}. Minimum fungicidal or bactericidal concentrations were determined by removing 5 μL from each clear well, transferring to agar, and incubating until growth was seen. The MFC/MBC is defined as the lowest test concentration that kills the organism (allows no growth on agar).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References and notes

1. Pierce SK, Miller LH. J Immunol 2009;182:5171. [PubMed: 19380759]
2. (a) Rosenthal PJ. N Engl J Med 2008;358:1829. [PubMed: 18434652] (b) White NJ. Science 2008;320:330. [PubMed: 18420924]
3. (a) Genton B. Expert Rev Vaccines 2008;7:597. [PubMed: 18564015] (b) Vekemans J, Ballou WR. Expert Rev Vaccines 2008;7:223. [PubMed: 18324891]
4. (a) Turschner S, Efferth T. Mini-Rev Med Chem 2009;9:206. [PubMed: 19200025] (b) Edwards G, Biagini GA. Br J Clin Pharmacol 2006;61:690. [PubMed: 16722830]
5. (a) Aweeka FT, German PI. Clin Pharmacokin 2008;47:91. (b) Plowe CV. Clin Infect Dis 2007;44:1075. [PubMed: 17366452] (c) Kindermans J-M, Pilloy J, Olliaro P, Gomes M. Malar J 2007:6. [PubMed: 17233887]
6. (a) Efferth T. Planta Med 2007;73:299. [PubMed: 17354163] (b) Namdeo AG, Mahadik KR, Kadam SS. Phcog Mag 2006;2:106.
7. (a) Gomes M, Ribeiro I, Warsame M, Karunajeewa H, Petzold M. BMC Infect Dis 2008;8:1. [PubMed: 18171470] (b) Rydén, A-M.; Kayser, O. Chemistry, Biosynthesis and Biological Activity of Artemisinin and Related Natural Peroxides. In: Gupta, RR.; Khan, MTH., editors. Bioactive Heterocycles III (Topics in Heterocyclic Chemistry). Vol. 9. Springer-Verlag; Berlin, Heidelberg: 2007. p. 1-31. (c) Sharma VP. Current Science 2006;90:1323. (d) Pasvol G. Br Med Bull 2005;75–76:29.
8. Global Malaria Programme. World Health Organization; 2006. Guidelines for the treatment of malaria. (www.who.int/malaria/docs/TreatmentGuidelines2006.pdf)
9. Li Q, Weina PJ, Milhous WK. Curr Drug Ther 2007;2:210.
10. (a) Turschner S, Efferth T. Mini-Rev Med Chem 2009;9:206. [PubMed: 19200025] (b) Lelievre J, Berry A, Benoit-Vical F. Curr Opin Investig Drugs 2007;8:117. (c) Golenser J, Waknine JH, Krugliak M, Hunt NH, Grau GE. Int J Parasitol 2006;36:1427. [PubMed: 17005183] (c) Stockwin LH, Han B, Yu SX, Hollingshead MG, ElSohly MA, Gul W, Slade D, Galal AM, Newton DL. Int J Cancer 2009;125:1266. [PubMed: 19533749]
11. (a) Maude RJ, Pontavornpinyo W, Saralamba S, Aguas R, Yeung S, Dondorp AM, Day NPJ, White NJ, White LJ. Malar J 2009:8. [PubMed: 19134197] (b) Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM. N Engl J Med 2008;359:2619. [PubMed: 19064625]

12. (a) Chadwick J, Mercer AE, Park BK, Cosstick R, O'Neill PM. Bioorg Med Chem 2009;17:1325. [PubMed: 19136263] (b) Galal AM, Gul W, Slade D, Ross SA, Feng S, Hollingshead MG, Alley MC, Kaur G, ElSohly MA. Bioorg Med Chem 2009;17:741. [PubMed: 19084416] (c) Posner GH, D'Angelo J, O'Neill PM, Mercer A. Expert Opin Ther Pat 2006;16:1665.
13. Holley Pharmaceutical Company, Inc., 1440 N. Harbor Blvd., # 900, Fullerton, CA 92835, USA (www.holleypharma.com).
14. Lin AJ, Klayman DL, Milhous WK. J Med Chem 1987;30:2147. [PubMed: 3669021]
15. Lin AJ, Lee M, Klayman DL. J Med Chem 1989;32:1249. [PubMed: 2657065]
16. ElSohly, M.A.; Gul, W. WO2006002105A1, US20080275106A1, 2006.
17. Grellepois F, Crousse B, Bonnet-Delpon D, Begue JP. Org Lett 2005;7:5219. [PubMed: 16268542]
18. (a) Jain M, Khan SI, Tekwani BL, Jacob MR, Singh S, Singh PP, Jain R. Bioorg Med Chem 2005;13:4458. [PubMed: 15878667] (b) Bharate SB, Khan SI, Tekwani BL, Jacob M, Khan IA, Singh IP. Bioorg Med Chem 2008;16:1328. [PubMed: 17976995]
19. Mustafa J, Khan SI, Ma G, Walker LA, Khan IA. Lipids 2004;39:167. [PubMed: 15134144]
20. Vonthron-Senecheau C, Weniger B, Ouattara M, Bi Fezan T, Kamenan A, Lobstein A, Brun R, Anton RJ. Ethnopharmacol 2003;87:221.
21. (a) Singh IP, Lal UR, Bodiwala HS, Mahajan RC, Bhutani KK. Recent Prog Med Plants 2006;13:115. (b) Maltezou HC. Recent Patents on Anti-Infective Drug Discovery 2008;3:192. [PubMed: 18991801]
22. (a) Sen R, Bandyopadhyay S, Dutta A, Mandal G, Ganguly S, Saha P, Chatterjee MJ. Med Microbiol 2007;56:1213. (b) Menon RB, Kannoth MM, Tekwani BL, Gut J, Rosenthal PJ, Avery MA. Comb Chem High Throughput Screening 2006;9:729.
23. Krishna S, Bustamante L, Haynes RK, Staines HM. Trends Pharmacol Sci 2008;29:520. [PubMed: 18752857]
24. (a) Wang H, Klinginsmith J, Dong X, Lee AC, Guha R, Wu Y, Crippen GM, Wild DJ. Journal of Chemical Information and Modeling 2007;47:2063. [PubMed: 17915856] (b) Huang R, Wallqvist A, Covell DG. Journal of Medicinal Chemistry 2006;49:1964. [PubMed: 16539384] (c) Boyd MR, Paull KD. Drug Development Research 1995;34:91.
25. Vichai V, Kirtikara K. Nature Protocols 2006;1:1112.
26. (a) Galal AM, Ross SA, Jacob M, ElSohly MA. J Nat Prod 2005;68:1274. [PubMed: 16124777] (b) Lee S. Mini-Rev Med Chem 2007;7:411. [PubMed: 17430226]
27. (a) Makler MT, Hinrichs DJ. Am J Trop Med Hyg 1993;48:205. [PubMed: 8447524] (b) Nkhoma S, Molyneux M, Ward S. Am J Trop Med Hyg 2007;76:1107. [PubMed: 17556619]
28. (a) Ma G, Khan SI, Jacob MR, Tekwani BL, Li Z, Pasco DS, Walker LA, Khan IA. Antimicrob Agents Chemother 2004;48:4450. [PubMed: 15504880] (b) Mikus J, Steverding D. Parasitol Int 2000;48:265. [PubMed: 11227767] (c) Hamid R, Rotshteyn Y, Rabadi L, Parikh R, Bullock P. Toxicol in Vitro 2004;18:703. [PubMed: 15251189]
29. Borenfreund E, Babich H, Martin-Alguacil N. In Vitro Cell Dev Biol: J Tissue Culture Assoc 1990;26:1030.
30. (a) National Committee for Clinical Laboratory Standards (NCCLS) (Wayne, Pa.): Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved Standard – Second Edition. Document M27-A2, 2002, *22*. (b) NCCLS: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard – Seventh Edition. Document M7-A7, 2006, *26*. (c) NCCLS: Susceptibility testing of mycobacteria, nocardia, and other aerobic actinomycetes; Tentative Standard – Approved Standard. Document M24-A, 2003, *23*. (d) NCCLS: Reference method for broth dilution antifungal susceptibility testing of filamentous fungi; Approved Standard. Document M38-A, 2002, *22*.
31. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, Degnan MT, Cook MB, Quenzer VK, Ferguson RM, Gilman RH. J Clin Microbiol 1998;36:362. [PubMed: 9466742]

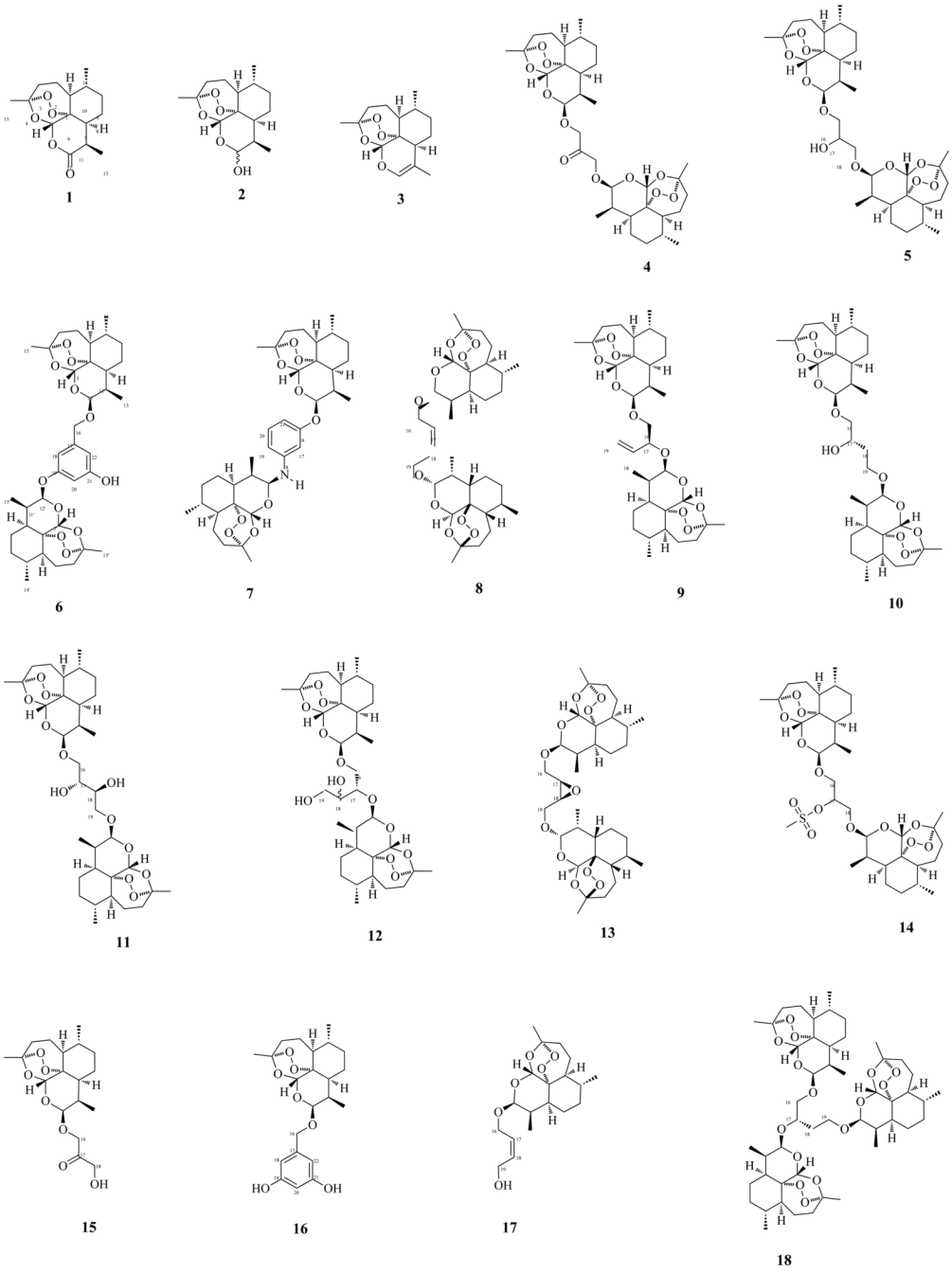
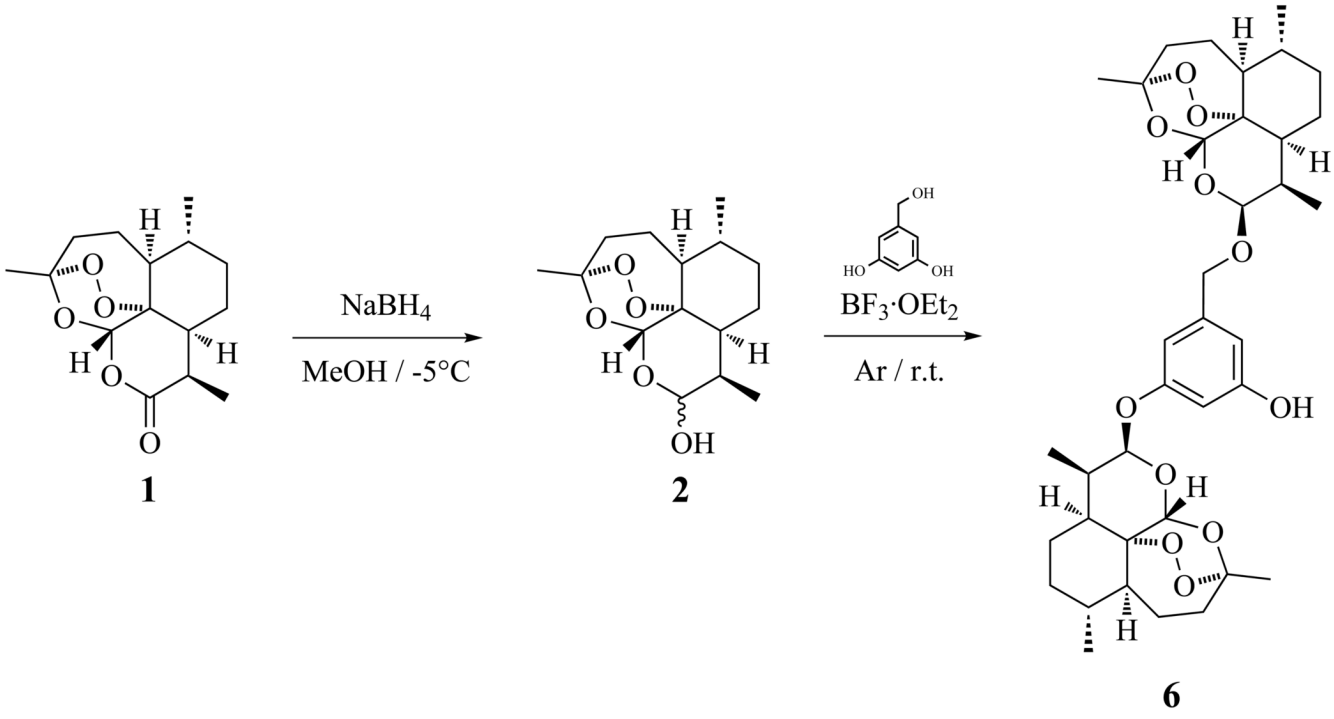


Figure 1.
Artemisinin (**1**), dihydroartemisinin (DHA) (**2**), anhydrodihydroartemisinin (**3**), DHA acetal dimers **4–14**, monomers **15–17** and trimer **18**.



Scheme 1.
Synthesis of **6**.

Table 1

Reaction details for synthesis of **6–10**

	DHA (2)			Linker			Ether	$BF_3{\cdot}OEt_2$	MS[a]	Crude[b]	Product	
Compound	**mg**	**mmol**	**eq.**	**mg**	**mmol**	**eq.**	**mL**	**μL**	**g**	**mg**	**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

[a] MS: molecular sieve.

[b] Crude: crude reaction mixture after workup.

Table 2

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

Compound	*Plasmodium falciparum*				Cytotoxicity
	D6 clone		W2 clone		Vero
	IC_{50} (nM)	S.I.	IC_{50} (nM)	S.I.	TC_{50} (nM)
1	32.9	> 51	42.5	> 40	> 1,686
2	14.8	95	14.1	100	1,407
3	15.8	> 113	25.5	> 70	> 1,787
4	5.3	49	5.8	44	257
5	11.2	34	3.8	100	384
6	3.0	> 2,380	4.2	> 1,700	> 7,075
7	5.8	> 1,287	5.9	> 1,253	> 7,417
8	6.3	113	6.1	116	709
9	24.2	32	25.8	30	773
10	43.8	> 170	45.4	> 164	> 7,452
11	6.7	> 1,082	5.2	> 1,400	> 7,270
12	11.7	71	14.0	59	825
13	45.5	> 164	47.1	> 159	> 7,475
14	11.8	542	11.8	542	6,403
15	7.9	> 1,700	7.0	> 1,904	> 13,355
16	20.9	> 560	20.7	> 567	> 11,711
17	6.2	> 2,164	7.6	> 1,763	> 13,430
18	298.3	18	243.1	22	5,259
chloroquine[a]	**41.6**	**> 357.9**	**378.3**	**> 39.3**	**> 14,881**

IC_{50}: concentration causing 50% growth inhibition. TC_{50}: concentration toxic to 50% of the cells. S.I.: selectivity index [TC_{50} (Vero)/IC_{50} (*P. falciparum*)].

[a]Control.

Table 3

Antileishmanial activity of **1–18**

Compound	IC_{50} (μM)	IC_{90} (μM)
1	40.7	NA
2	87.9	NA
3	30.0	NA
4	7.4	38.5
5	12.8	47.9
6	5.9	32.7
7	24.9	62.3
8	NA	NA
9	20.9	62.8
10	2.5	9.4
11	6.0	22.9
12	6.4	29.7
13	3.9	47.1
14	22.8	55.5
15	25.8	112.2
16	5.7	24.6
17	42.3	NA
18	5.0	44.2
pentamidine[a]	**2.8**	**6.4**
amphotericin B[a]	**0.2**	**0.4**

IC_{50}: concentration causing 50% growth inhibition. IC_{90}: concentration causing 90% growth inhibition.

NA: not active.

[a]Control.

Bioorg Med Chem. Author manuscript; available in PMC 2010 December 1.

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$_{11}$
	IC_{50}(μM)				TC_{50}(μM)	
4	0.56	0.24	0.24	NA	> 8.03	0.10
5	8.00	0.26	0.48	NA	5.60	0.15
6	NA	NA	NA	NA	NA	NA
7	NA	NA	NA	NA	NA	NA
8	NA	0.32	1.53	NA	0.18	NC
9	12.93	0.27	2.58	NA	0.43	> 16.11
10	0.24	0.16	0.08	NA	> 1.57	0.10
11	NA	NA	NA	NA	NA	NA
12	NA	NA	NA	NA	NA	NA
13	0.22	0.47	0.30	NA	> 1.57	0.68
14	NA	0.28	NA	NA	2.13	NC
15	NA	NA	NA	NA	NA	20.76
16	NA	NA	NA	NA	NA	2.46
17	NA	NA	NA	NA	NA	NA
18	0.24	0.11	0.06	NA	> 1.10	0.07
doxorubicin[a]	**1.73**	**2.04**	**1.96**	**2.04**	**NC**	**1.29**
paclitaxel[a]	**4.10**	**0.02**	**0.02**	**0.53**	**0.53**	**4.39**

IC_{50}: concentration causing 50% growth inhibition. TC_{50}: 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$_{11}$: pig kidney epithelial. NA: not active. NC: not cytotoxic.

[a] Control.

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Table 5

^{1}H NMR data for **5** and **6–18** (δ_H in ppm, *J* values in Hz)

Position	5[a]	6[a]	7[a]	8[a]	9[a]	10[a]	11[a]	12[a]	13[a]	14[b]	15[a]	16[b]	17[a]	18[a]
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*)	5.39 (*s*)	5.65 (*s*) 5.72 (*s*)	5.42 (*s*)	5.69 (*s*)	5.35 (*s*)	5.33 (*s*) 5.40 (*s*)
		2.64 (*m*)												
11/11′	2.61 (*m*)	2.76 (*m*)	2.78 (*m*)	2.54 (*br s*)	2.58 (*m*)	2.60 (*m*)	2.65 (*m*)	2.66 (*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)	4.87 (*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)	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)	0.97 (*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)	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)	0.93 (*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)	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*)	1.41 (*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*)	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*)	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*)	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*)	-	6.48 (*d*, 1.2)	5.59 (*br s*)	4.33 (*m*)	3.88	3.73 (*m*)	3.80 (*m*)	3.19 (*m*)	5.30 (*m*)	-	-	5.59 (*m*)	3.86 (*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*)	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	-	-	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*)	3.83 (*m*) 3.57 (*m*)	-	-	-	5.59 (*m*) 5.75 (*m*)	3.44 (*m*) 3.86 (*m*)
20	-	6.57 (*s*)	7.03 (*d*, 8.0)	-	-	-	-	-	-	-	-	6.96 (*s*)	-	-
21	-	-	6.50 (*br d*)	-	-	-	-	-	-	-	-	-	-	-
22	-	6.45 (*s*) 6.56 (*s*)	-	-	-	-	-	-	-	-	-	6.90 (*s*)	-	-

[a] $CDCl_3$.

[b] pyridine-d_5.

Table 6

^{13}C NMR data for **5** and **6–18** (δ_C in ppm)

Position	5[a]	6[a]	7[a]	8[a]	9[a]	10[a]	11[a]	12[a]	13[a]	14[b]	15[a]	16[b]	17[a]	18[a]
1/1′	52.9	52.6	52.8	52.7	52.8	52.7/52.8	52.7	52.65/52.71	52.7/52.8	53.00/53.03	52.4	53.0	52.6	52.8
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	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	36.4/36.5	36.6	36.6	36.62/36.64	36.59/36.63	36.6	36.6	36.6	37.1	36.3	37.1	36.5	36.7
4/4′	104.5	104.2/104.3	104.4	104.2	104.3	104.27/104.32	104.4	104.3/104.5	104.3/104.4	104.0	104.3	104.5	104.5	104.17/104.27/104.30
5/5′	88.3	88.1/88.3	88.5	88.1	88.27/88.30	88.07/88.12	88.1/88.2	88.3/88.2	88.1/88.2	88.5/88.6	88.1	88.5	88.1	88.11/88.15/88.17
6/6′	81.4	81.0/81.2	81.3	81.3	81.3/81.4	81.1/81.2	81.1/81.2	81.2	81.3	80.2/81.4	80.9	81.5	81.3	81.2/81.3
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	44.55/44.56	44.9/45.0	44.1	45.1	44.5	44.64/44.69/44.74
8/8′	25.0	24.4/24.5/24.67/24.71	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	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	34.66/34.69	34.9	34.8	34.9	33.9/34.2	34.8	34.8	34.8	35.1	34.5	35.0	34.7	34.8/34.9
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	37.6/37.7	37.5/37.7	37.5	37.6	37.6	37.65/37.69
11/11′	31.3	31.0	31.3	31.0	30.9/31.2	31.1	31.1	31.0/31.4	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	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	102.4/103.0	102.5/103.2	102.7	101.7	101.6	102.3/102.6/103.1
13/13′	13.5	13.0/13.2	13.2	13.2	13.2/13.3	13.19/13.21	13.3	13.2/13.5	13.2	13.39/13.45	13.0	13.6	13.14	13.2/13.4/13.7
14/14′	20.8	20.36/20.39	20.6	20.6	20.58/20.62	20.50/20.53	20.6	20.5/20.6	20.5	20.77/20.81	20.3	20.7	20.54	20.49/20.53
15/15′	26.5	26.0/26.2	26.3	26.3	26.4	26.29/26.34	26.3	26.4	26.3	26.3/26.5	26.0	26.5	26.2	26.4
16	70.2	69.4	158.9	64.1	71.8	73.3	70.6	63.7	67.1	67.3/67.8	71.5	70.4	58.3	71.7
17	70.1	141.2	103.8	129.2	76.4	68.7	71.4	78.5	54.4	80.2	207.8	142.0	132.7	75.6
18	70.3	108.0/108.2	148.0	129.2	135.9	33.9	71.4	71.6	55.1	67.3/67.8	66.7	106.6	128.0	33.0
19	-	156.9/158.5	109.2	64.1	119.3	65.7	70.2	69.5	66.5	-	-	160.8	63.6	64.6
20	-	103.3	130.0	-	-	-	-	-	-	-	-	103.4	-	-
21	-	156.9/158.5	107.2	-	-	-	-	-	-	-	-	160.8	-	-
22	-	108.0/108.2	-	-	-	-	-	-	-	-	-	106.6	-	-

[a] $CDCl_3$.

[b] pyridine-d_5.

Table 7

HRESIFTMS (direct injection, positive mode) data for **6–18**

Compound	Molecular formula	$[M+Na]^+$			$[M+K]^+$		
		Exact	Exp	Error	Exact	Exp	Error
6	$C_{37}H_{52}O_{11}$	695.3408	695.3284	17.8	-	-	-
7	$C_{36}H_{51}NO_9$	664.3462	664.3330	19.9	680.3201	680.3117	12.3
8	$C_{34}H_{52}O_{10}$	643.3458	643.3391	10.4	659.3197	659.3090	16.2
9	$C_{34}H_{52}O_{10}$	643.3458	643.3369	13.8	659.3197	659.3101	14.6
10	$C_{34}H_{54}O_{11}$	661.3564	661.3624	9.1	-	-	-
11	$C_{34}H_{54}O_{12}$	677.3513	677.3413	14.8	693.3252	693.3115	19.8
12	$C_{34}H_{54}O_{12}$	677.3513	677.3416	14.3	693.3252	693.3172	11.5
13	$C_{34}H_{52}O_{11}$	659.3408	659.3630	33.7	-	-	-
14	$C_{34}H_{54}O_{13}S$	725.3183	725.3140	5.9	741.2922	741.2840	11.1
15[a]	$C_{18}H_{28}O_7$	355.1757	355.1762	1.5	-	-	-
16	$C_{22}H_{30}O_7$	429.1890	429.1888	0.5	445.1629	445.1613	3.6
17	$C_{19}H_{30}O_6$	377.1940	377.1913	7.2	393.1679	393.1680	0.3
18	$C_{49}H_{76}O_{15}$	927.5082	927.5106	2.6	943.4821	943.5021	21.2

Exp: experimental. Error: error in ppm.

[a] $[M-H]^-$ data.