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Synthesis and antimalarial evaluation of a screening library based on a tetrahydroanthraquinone natural product scaffold

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ABSTRACT

As part of a research program aimed at discovering new antimalarial leads from Australian macrofungi a unique fungi-derived prefractionated library was screened against a chloroquine-sensitive *Plasmodium falciparum* line (3D7) using a radiometric growth inhibition assay. A library fraction derived from a *Cortinarius* species displayed promising antimalarial activity. UV-guided fractionation on the CH_2Cl_2/MeOH extract from this fungus resulted in the isolation of four known compounds: (1*S*,3*R*)-austrocortirubin (**1**), (1*S*,3*S*)-austrocortirubin (**2**), 1-deoxyaustrocortirubin (**3**), and austrocortinin (**4**). Compound **2** was used as a natural product scaffold in the parallel solution-phase synthesis of a small library of N-substituted tetrahydroanthraquinones (**5–15**). All compounds (**1**–**15**) were tested in vitro against *P. falciparum* 3D7 parasites and (1*S*,3*S*)-austrocortirubin (**2**), the major fungal constituent, was shown to be the most active compound with an IC_{50} of 1.9 μM. This compound displayed moderate cytotoxicity against neonatal foreskin fibroblast (NFF) cells with an IC_{50} of 15.6 μM.

1. Introduction

Malaria is a significant global infectious disease, resulting in the deaths of 0.8–1.2 million people annually, and predominantly affects children under five years of age.[1] Despite recent preliminary data on a partially effective pre-erythrocytic stage vaccine (RTS, S),[2] malaria prevention and treatment presently relies on vector control and small molecule drugs. It is forseen that even if the RTS, S vaccine is approved for clinical use, it will be implemented as part of a multi-pronged approach that will also include antimalarial drugs in order to eradicate the parasite. Whilst a number of drugs and drug combinations are used therapeutically to treat malaria, all have now succumbed to parasite drug resistance and/or reduced efficacy.[3] This, together with the fact that only five of the ~1700 new drugs registered over the last 30 years were antimalarials,[4] highlights the need for the discovery and development of new antimalarial therapeutics.

Historically, natural products have played a significant role in the development of antimalarial drugs.[5–7] Notable examples include artemisinin from the Chinese medicinal plant *Artemisia annua,* and quinine, which was isolated from the bark of the South American tree *Cinchona succirubra.*[5–7] This research has subsequently led to the development of numerous antimalarial drugs based on these two important plant natural products.[7]

As part of our continuing research into the discovery of new antimalarial leads from nature[8–12] we screened a unique prefractionated fungi-derived library (2035 fractions) against a chloroquine-sensitive *Plasmodium falciparum* line (3D7), using a radiometric growth inhibition assay. A total of 20 fractions derived from 18 fungal species (10 genera, 8 families) showed promising antimalarial activity; this equated to a screening hit-rate of 0.98%. UV spectrum analysis of one antimalarial fraction derived from a *Cortinarius* species identified λ_{max} values of 230 and 305 nm that were predicted to correspond to the compound(s) responsible for the observed antiparasitic activity. Large-scale UV-guided fractionation of the CH_2Cl_2/MeOH extract from this fungus resulted in the isolation of four known compounds: (1*S*,3*R*)-austrocortirubin (**1**),[13] (1*S*,3*S*)-austrocortirubin (**2**),[14,15] 1-deoxyaustrocortirubin (**3**),[14,15] and austrocortinin (**4**)[16] (Fig. 1). Compound **2** was identified as a natural product scaffold that could be used in the generation of a tetrahydroanthraquinone-based compound library. Parallel solution-phase synthesis utilising scaffold **2** with 11 primary amines afforded a small library of N-substituted tetrahydroanthraquinones (**5**–**15**). Herein we report the design and synthesis of **5**–**15**, along with the in vitro antimalarial activity and mammalian cell toxicity for the natural products (**1**–**4**) and semi-synthetic analogues (**5**–**15**).

2. Results and discussion

The air-dried and ground sample of *Cortinarius* sp. was sequentially extracted with CH_2Cl_2 and MeOH. The combined

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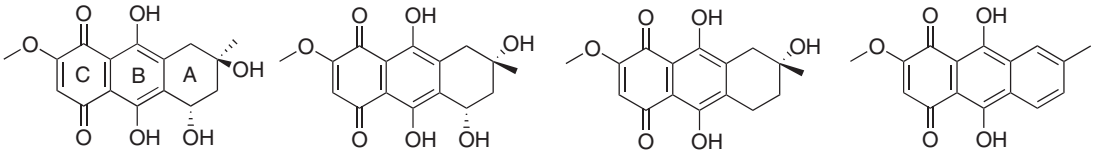


Figure 1. Chemical structures for (1*S*,3*R*)-austrocortirubin (**1**), (1*S*,3*S*)-austrocortirubin (**2**), 1-deoxyaustrocortirubin (**3**) and austrocortinin (**4**).

CH_2Cl_2/MeOH extract was subjected to UV-guided fractionation using semi-preparative C_{18} HPLC. This yielded the previously isolated tetrahydroanthraquinones, (1*S*,3*R*)-austrocortirubin (**1**),[13] (1*S*,3*S*)-austrocortirubin (**2**),[14,15] and 1-deoxyaustrocortirubin (**3**).[14,15] A late eluting fraction from the HPLC fractionation was further purified by gel permeation chromatography to afford the known fungal anthraquinone, austrocortinin (**4**).[16] All compounds were identified on the basis of spectroscopic and spectrometric data and in comparison with the literature values.

The polyketide-derived natural products **1**–**4** were originally isolated from the Australian toadstool, *Dermocybe splendida*, by Gill and co-workers and had their structures tentatively assigned on chemical and spectroscopic grounds, and were subsequently confirmed by single crystal X-ray analyses on several semi-synthetic derivatives.[13–16] The tetrahydroanthraquinone class has been identified as possessing moderate activity towards a variety of bacteria and fungi.[14,17] For example, (1*S*,3*S*)-austrocortirubin (**2**) displayed antibacterial activity against the Gram-positive bacteria *Staphylococcus aureus* with an IC_{50} of 3 μg/mL, and was found to be inactive (IC_{50} >50 μg/mL) towards the Gram-negative bacteria *Pseudomonas aeruginosa*.[17]

Compounds **1**–**4** were tested in vitro against the chloroquine-sensitive 3D7 and chloroquine-resistant Dd2 *P. falciparum* lines and displayed IC_{50} values of 4.7, 1.9, 4.5, and >50 μM (Table 1) and 11.1, 2.2, 5.2, and >50 μM, respectively. Compound **2** was the most potent molecule against both *P. falciparum* lines (IC_{50} 1.9–2.2 μM) while compound **4**, the only anthraquinone screened during these studies, showed no antimalarial activity (IC_{50} >50 μM). While the lack of antiparasitic activity of **4** could possibly be due to the chemical difference in ring A (Fig. 1) of this molecule compared to **1**–**3**, it should be noted that we had difficulty solubilising **4** in the solvent vehicle (DMSO) used for in vitro screening. Cytotoxicity studies with neonatal foreskin fibroblast (NFF) cells showed that compounds **1**–**3** all had approximately 8- to 12-fold better selectivity for the parasite versus the mammalian cell line (Table 1).

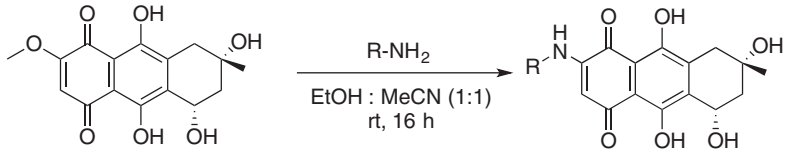
Inspired by the literature reports of high-yielding N-alkylations of quinone motifs, such as those present in **1**–**3**, we decided to exploit this chemistry for tetrahydroanthraquinone analogue generation and potential structure activity relationship (SAR) studies.[18–21] This additional research had the potential to lead to compounds with increased potency, better selectivity and/or an increased stability toward biogenic nucleophiles.[18–21] (1*S*,3*S*)-Austrocortirubin (**2**) was chosen for the semi-synthetic studies since this molecule displayed the best in vitro antimalarial activity (with a similar selectivity to **1** and **3**), and was available in suitable quantities (>50 mg) for library generation due to its moderate yield from the toadstool.[8,22,23] Furthermore, the low Mw (320 Da), favourable *c*Log*P* (0.82), and multiple stereogenic centres (n = **2**) made **2** a suitable natural product scaffold for library synthesis (Table 1).[24–27]

The concept of isolating a natural product scaffold for lead optimisation studies or screening library generation has previously been reported in the literature, although relatively few examples exist.[28] The purification and subsequent use of tambjamine,[22] teicoplanin,[29] yohimbine,[30] fredericamycin A[21] and muurolane[8] scaffolds in the synthesis of biodiscovery and lead optimisation libraries all exemplify this approach. This strategy is dependent on: (1) access to adequate biota supplies for the large-scale isolation of the desired scaffold; (2) the abundance or high-yielding nature of the compound of interest from the biota source; and (3) the presence of chemical handles (e.g. –COOH, –OH, $–NH_2$, Ar–Br) on the scaffold that allow for simple and high-yielding analogue generation. Isolation of a desired scaffold, rather than de novo

Table 1
Physicochemical and biological profiles of compounds **1**–**15**

Compound	Physicochemical parameters[a]				IC_{50} ± SD (μM)		
	MW	*c*Log*P*	HBA	HBD	3D7[b]	NFF[c]	SI[d]
1	320	0.82	7	4	4.7 ± 0.4	43.7 ± 7.3	9.3
2	320	0.82	7	4	1.9 ± 0.4	15.6 ± 4.1	8.2
3	304	2.13	6	3	4.5 ± 1.0	54.6 ± 9.4	12.1
4	286	2.70	5	2	>50	76.0 ± 8.6	>1.5
5	462	1.26	8	6	6.7 ± 1.2	36.5 ± 0.8	5.4
6	347	1.39	7	5	4.3 ± 1.9	78.8 ± 15.5	18.3
7	362	-0.81	8	6	16.5 ± 3.5	89.8 ± 6.2	5.4
8	363	0.46	8	5	10.3 ± 0.4	84.3 ± 0.3	8.2
9	409	2.52	7	5	3.8 ± 0.5	3.5 ± 0.6	0.9
10	425	2.22	8	6	2.4 ± 0.8	23.3 ± 5.1	9.7
11	443	3.12	7	5	4.2 ± 0.6	66.0 ± 0.6	15.7
12	418	0.31	9	5	15.8 ± 1.9	62.0 ± 1.0	3.9
13	395	2.23	7	5	7.4 ± 0.3	10.7 ± 1.5	1.4
14	319	0.51	7	5	6.9 ± 3.9	59.1 ± 6.9	8.6
15	402	0.93	8	5	6.3 ± 1.8	15.9 ± 5.0	2.5
Chloroquine	319	3.93	4	1	0.04 ± 0.004	37.3 ± 3.4	932.5

[a] In silico calculations performed using Instant JChem software,[32] MW, molecular weight (Da); *c*Log*P*, calculated partition coefficient; HBA = H-bond acceptors; HBD = H-bond donors.
[b] 3D7, *P. falciparum* chloroquine sensitive line.
[c] NFF, neonatal foreskin fibroblast cells.
[d] SI, selectivity index = NFF cell-line IC_{50}/*P. falciparum* IC_{50}.



2 5-15

Scheme 1. Reaction conditions for the amino alkylation of (1*S*,3*S*)-austrocortirubin (**2**).

synthesis, can prove to be both a cost- and time-effective mechanism for lead optimisation studies.

In order to obtain larger quantities of (1*S*,3*S*)-austrocortirubin for our semi-synthetic studies additional large-scale extraction and isolation work was conducted on the air-dried and ground *Cortinarius* material, which yielded 91 mg of **2**.

Prior to semi-synthetic studies commencing on scaffold **2**, a virtual library was constructed using a list of commercially available primary amines (30 in total)[31] and Instant JChem software[32] and the data compared to Lipinski's drug-like "Rule of Five"[27] (MW < 500; HBA < 10; HBD < 5; Log*P* < 5) criteria. The physicochemical parameter profiles (MW, HBA, HBD, Log*P*) for all virtual molecules were analysed in an effort to select the most desirable drug-like molecules for subsequent synthesis. On the basis of the in silico data (Table 1), 11 primary amines were initially prioritised and subsequently reacted with scaffold **2** in EtOH/MeCN at room temperature for 16 h (Scheme 1). Purification of all reaction mixtures by gel permeation chromatography gave the desired products **5**–**15** in moderate to good yields (Fig. 2).

The chemical structures and NMR chemical shift assignments of all new tetrahydroanthraquinone analogues (**5**–**15**) were determined following 1D (^{1}H, ^{13}C) and 2D NMR (COSY, HSQC, HMBC and ROESY) and (+)-HRESIMS data analysis.

For example, the ^{1}H NMR spectrum for compound **5** in $CDCl_3$ showed signals at δ_H 1.43 (3H, s), 1.90 (1H, dd, *J* = 14.2, 4.9 Hz), 2.32 (1H, br d, *J* = 14.2 Hz), 2.51 (1H, d, *J* = 18.5 Hz), 3.16 (1H, dd,

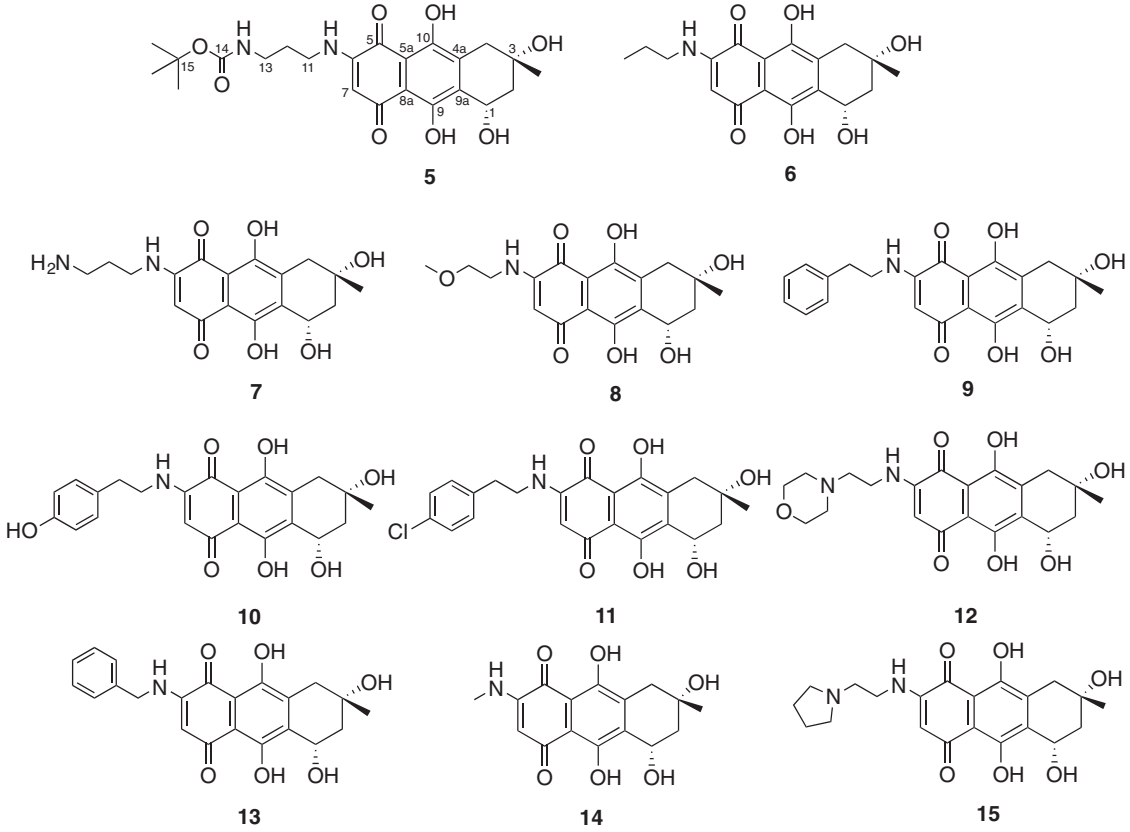


Figure 2. Chemical structures of the semi-synthetic tetrahydroanthraquinones **5**–**15**.

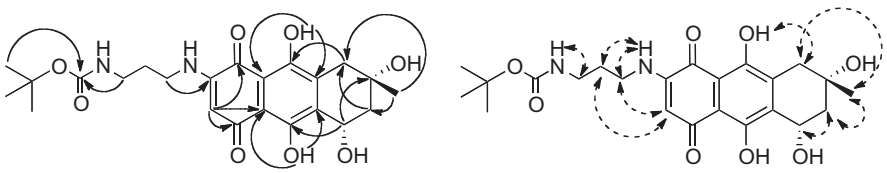


Figure 3. Key HMBC (→) and ROESY (↔) correlations for **5**.

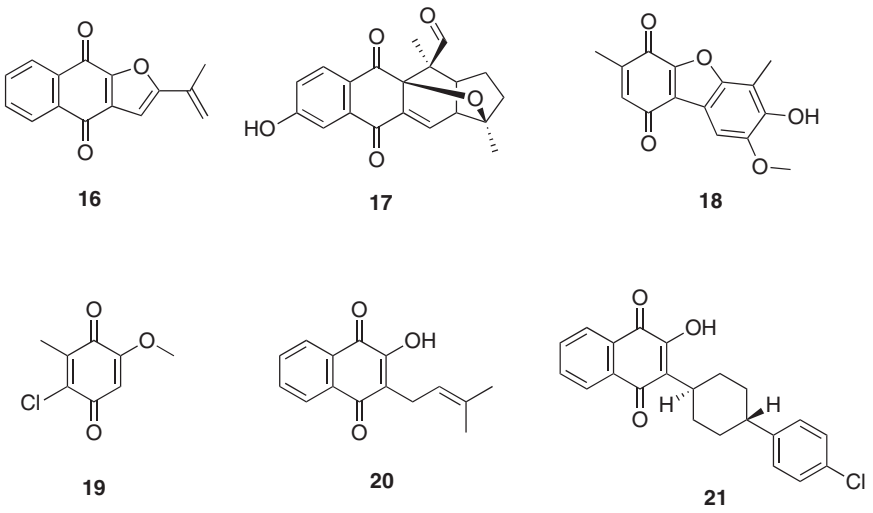


Figure 4. Quinone-based antimalarial compounds.

J = 18.5, 1.7 Hz), 5.21 (1H, br d, *J* = 4.9 Hz), and 12.24 (1H, s), 14.10 (1H, s), which were essentially identical, in respect to magnitude and multiplicities, to the proton resonances corresponding to the 3-methyl-1,2,3,4-tetrahydronaphthalene-1,3,5,8-tetraol system (rings A and B) present in the known natural product, (1*S*,3*S*)-austrocortirubin (**2**).[14,15] Additional proton signals for **5** were identified at δ_H 1.46 (9H, s), 1.84 (2H, q, *J* = 6.7 Hz), 3.25 (2H, m), 3.29 (2H, m), 4.77 (1H, br s) and 6.61 (1H, br s), and were assigned to a mono-Boc protected 1,3-diaminepropyl moiety following interpretation of the gCOSY, gHSQC and gHMBC data. The N-alkylation of the tetrahydroanthraquinone core was deemed to have taken place at C-6, on the basis of a literature report on the related mono-methoxylated quinone, fredericamycin A.[21] Semi-synthetic studies on this *Streptomyces*-derived natural product had shown that the methoxy group was readily replaced by nucleophiles such as primary amines.[21] Confirmation of the N-alkylation position for **5** was also supported by the absence of methoxy NMR signals in conjunction with gHMBC and ROESY correlations (Fig. 3). These data enabled the chemical structure of **5** to be unequivocally assigned.

The relative configuration and absolute stereochemistry of **5**–**15** were all shown to be identical to that of the starting material, (1*S*,3*S*)-austrocortirubin (**2**), based on ^1H–^1H coupling constants, the magnitude of the ^1H NMR chemical shifts, and specific rotation comparisons.[14,15] It is noteworthy that the magnitude of the specific rotation values for the semi-synthetic analogues **5**–**15** all showed larger than expected values (+725 to +1520) in MeOH compared to the natural product scaffold and starting material, (1*S*,3*S*)-austrocortirubin {$[\alpha]_D^{20}$ +34 (*c* 0.543, $CHCl_3$),[14,15] $[\alpha]_D^{25}$ +172 (*c* 0.2, MeOH)}. Subsequent literature searching for related optically active N-alkylated quinones identified examples such as awamycin {$[\alpha]_D^{20}$ +1010 (*c*, 0.1, $CHCl_3$)},[33] and rubradirin {$[\alpha]_D^{25}$ +777 (*c* 0.028, $(CH_3)_2CO$)},[34] which also displayed large specific rotations. Furthermore, compounds **5**–**15**, awamycin and rubradirin are all red, and have UV chromophores that typically extend past 500 nm.[33,34] We postulate that the UV absorption characteristics of these molecules may be the cause for the large specific rotations.

Compounds **5**–**15** were all tested for in vitro growth inhibitory activity against *P. falciparum* 3D7 parasites and mammalian cell toxicity using the NFF cell line (Table 1). Eight of the 11 semi-synthetic analogues (**5**, **6**, **9**–**11**, **13**–**15**) displayed IC_{50} values <7.4 μM against the malaria parasite that were comparable to the natural products **1**–**3**. While none of the synthons displayed improved potency compared to **2**, compounds **6** and **11** showed 2.2- and 1.9-fold better selectivity. The lack of significant SAR for analogues **5**–**15** indicates that ring A plays a more important role for bioactivity than ring C (Fig. 1) and thus any future medicinal chemistry on this unique tetrahydroanthraquinone core should focus on generating analogues that would probe ring A for SAR.

A variety of quinone-based antimalarial natural products have been isolated from fungi, lichens, plants and marine invertebrates.[35] Plant-derived examples include the napthoquinoids, 2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione (**16**) and isopinnatal (**17**) from *Kigelia pinnata*, which both inhibited the *P. falciparum* K1 line with IC_{50} values of 0.63 and 0.76 μM, respectively (Fig. 4).[36] The benzoquinone metabolites, xylariaquinone A (**18**) and 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (**19**), were isolated from an endophytic fungus *Xylaria* sp. and were reported to display IC_{50} values towards *P. falciparum* K1 line of 1.84 and 6.68 μM, respectively.[37] It is noteworthy that lapachol (**20**), a hydroxynapthoquinone isolated from several South American species belonging to the plant family Bignoniaceae,[38] served as a drug lead for the synthetic antimalarial drug atovaquone (**21**), which is typically co-administered with proguanil in the treatment of *P. falciparum* infections. The mechanism of action for atovaquone has been determined and involves the inhibition of the cytochrome *bc*1 complex in *Plasmodium* parasites.[39–41] On the basis of this data it has been hypothesized that many of the previously reported quinone-based antimalarial natural products may also interfere with the mitochondrial electron transport system in the malaria parasite.[42–44]

3. Conclusion

In summary, UV-guided fractionation on the bioactive CH_2Cl_2/MeOH extract from the Australian toadstool *Cortinarius* sp. resulted in the isolation of four known compounds: (1*S*,3*R*)-austrocortirubin (**1**), (1*S*,3*S*)-austrocortirubin (**2**), 1-deoxyaustrocortirubin (**3**), and austrocortinin (**4**). Compound **2** was isolated in quantities that allowed its use as a natural product scaffold in the generation of a small tetrahydroanthraquinone-based compound library (**5**–**15**). All compounds were tested in vitro against a chloroquine-sensitive *P. falciparum* (3D7) malaria parasite line and (1*S*,3*S*)-austrocortirubin (**2**), the major fungal constituent, was shown to be the most active compound with an IC_{50} of 1.9 μM. Compound **2** displayed moderate cytotoxicity against neonatal foreskin fibroblast (NFF) cells with an IC_{50} of 15.6 μM. This is the first report of antimalarial activity for the tetrahydroanthraquinone structure class.

4. Experimental section

4.1. Materials and methods

Optical rotations were recorded on a JASCO P-1020 polarimeter. NMR spectra were recorded at 30 °C on either a Varian 500 MHz or a 600 MHz Unity INOVA spectrometer. The latter spectrometer was equipped with a triple resonance cold probe. The ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peak for CD_3OD at δ_H 3.31 and δ_C 49.0, and for $CDCl_3$ at δ_H 7.26 and δ_C 77.0, respectively. LRESIMS spectra were recorded on a Waters ZQ mass spectrometer. HRESIMS data were recorded on a Bruker Daltronics Apex III 4.7e Fourier-transform mass spectrometer. A BIOLINE orbital shaker was used for the large-scale extractions of the fungal material. Alltech Davisil 40–60 μm 60 Å C_{18} bonded silica was used for pre-adsorption of extracts or fractions and was packed into an open glass column (100 mm × 200 mm) for C_{18} flash chromatography. A Waters 600 pump equipped with a Waters 996 PDA detector, and either a Waters 717 autosampler or Gilson 215 liquid handler were used for HPLC. An Alltech stainless steel guard cartridge (10 mm × 30 mm) was used for loading pre-adsorbed extracts or fractions onto the semi-preparative HPLC columns. A Phenomenex C_{18} ONYX column (4.6 mm × 100 mm) was used for analytical HPLC associated with the library generation. A Thermo Electron C_{18} Betasil column (5 μm, 143 Å, 21.2 mm × 150 mm) and a Phenomenex Luna C_{18} column (5 μm, 21.2 mm × 250 mm) were used for semi-preparative HPLC separation. Gel permeation chromatography was used for natural product purification (Sephadex LH20, 50 mm × 120 mm, open column) and synthetic reaction products purification (Sephadex LH20, 25 mm × 290 mm, open column). All solvents used for chromatography, $[\alpha]_D$, UV, and MS were Lab-Scan HPLC grade, and the H_2O was Millipore Milli-Q PF filtered. All amine reagents were purchased from Sigma-Aldrich.

4.2. Fungal material

The toadstool *Cortinarius* sp. (Family: Cortinariaceae) was collected in South-East Queensland, Australia, on the 1st May 2003. A voucher sample (0801050310001075) has been lodged at the Queensland Herbarium, Brisbane, Australia.

4.3. Extraction and isolation

The air-dried and ground fruit-bodies of *Cortinarius* sp. (10 g) were transferred to a conical flask (1 L) and extracted with CH_2Cl_2 (250 mL), the mixture was shaken at 200 rpm for 2 h. The resulting extract was filtered under gravity, and set aside. MeOH (250 mL) was added to the fungal sample, and the flask shaken at 200 rpm for 2 h before filtration. Another volume of MeOH (250 mL) was then added and the MeOH/fungal mixture shaken for a further 16 h at 200 rpm, followed by gravity filtration. Finally, all CH_2Cl_2 and MeOH extracts were combined and dried under reduced pressure to yield a red gum (3.57 g). A portion of the CH_2Cl_2/MeOH extract (0.5 g) was re-suspended in MeOH/CH_2Cl_2, pre-adsorbed to C_{18} silica and packed into a stainless steel guard cartridge that was subsequently attached to a semi-preparative C_{18} Betasil HPLC column. Isocratic conditions of 10% MeOH/90% H_2O were initially performed for the first 10 min, followed by a linear gradient to MeOH in 40 min, then isocratic conditions of MeOH were run for 10 min all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected from the start of the HPLC run and fractions containing UV absorbing material at λ 230 and 305 nm were analysed by (±)-LRESIMS and ^{1}H NMR spectroscopy. Three pure compounds were obtained after the first C_{18} HPLC fractionation; (1*S*,3*R*)-austrocortirubin (**1**, 3.0 mg, 0.12% dry wt), (1*S*,3*S*)-austrocortirubin (**2**, 6.0 mg, 0.24% dry wt) and 1-deoxyaustrocortirubin (**3**, 4.5 mg, 0.18% dry wt). In addition, fraction 55 obtained from the first isolation step, was further purified by gel permeation chromatography [Sephadex LH20, MeOH/CH_2Cl_2 (1:1)] at a flow rate of 1.0 mL/min to yield austrocortinin (**4**, 12.0 mg, 0.48% dry wt). Identical extraction and isolation conditions were employed on 50 g of *Cortinarius* sp. in order to obtain larger quantities of scaffold **2** (91.0 mg, 1.68% dry wt.) for the semi-synthetic studies.

4.3.1. (1*S*,3*R*)-Austrocortirubin (1)

Red amorphous solid; NMR, MS, and $[\alpha]_D$ data were consistent with the literature values.[13]

4.3.2. (1*S*,3*S*)-Austrocortirubin (2)

Red amorphous solid; NMR, MS, and $[\alpha]_D$ data were consistent with the literature values.[14,15]

4.3.2.1. 1-Deoxyaustrocortirubin (3)

Red amorphous solid; NMR, MS, and $[\alpha]_D$ data were consistent with the literature values.[14,15]

4.3.3. Austrocortinin (4)

Orange amorphous solid; NMR and MS were consistent with the literature values.[16]

4.4. General procedure for the amino alkylation reaction[18]

Scaffold **2** (5.0 mg, 0.016 mmol), EtOH (0.5 mL), MeCN (0.5 mL) and the relevant primary amine (0.08 mmol, 5 mol excess) were stirred for 16 h at room temperature under Ar. The solution was evaporated to dryness and redissolved in CH_2Cl_2 (20 mL), before being extracted with H_2O (20 mL). The CH_2Cl_2 extract was purified by gel permeation chromatography [Sephadex LH20, CH_2Cl_2:MeOH (1:1)] at a flow rate of 1.0 mL/min. Nine fractions (1 min each) were collected after 20 min, evaporated to dryness and analysed by analytical HPLC to determine purity.

4.4.1. Compound 5

Dark red powder, (4.4 mg, 77%); $[\alpha]_D^{29}$ +725 (*c* 0.004, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.82), 266 (3.83), 325 (3.33), 511 (3.45) nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 1.45 (3H, s, 3-Me), 1.46 (9H, s, H-16), 1.84 (2H, tt, *J* = 6.7, 6.7 Hz, H-12), 1.90 (1H, dd, *J* = 14.2, 4.9 Hz, H-2_{ax}), 2.32 (1H, ddd, *J* = 14.2, 1.7, 1.7 Hz, H-2_{eq}), 2.51 (1H, d, *J* = 18.5 Hz, H-4_{ax}), 3.16 (1H, dd, *J* = 18.5, 1.7 Hz, H-4_{eq}), 3.25 (2H, m, H-13), 3.29 (2H, m, H-11), 4.77 (1H, br s, 13-NH), 5.21 (1H, dd, *J* = 4.9, 1.7 Hz, H-1), 5.62 (1H, s, H-7), 6.61 (1H, br s, 6-NH), 12.24 (1H, s, 10-OH), 14.10 (1H, s, 9-OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 28.4 (C-16), 28.7 (3-Me), 28.7 (C-12), 37.3 (C-4), 37.6 (C-13), 39.8 (C-11), 40.0 (C-2), 63.1 (C-1), 68.1 (C-3), 80.0 (C-15), 108.9 (C-8a), 100.1 (C-7), 110.0 (C-5a), 133.1 (C-4a), 138.6 (C-9a), 149.2 (C-6), 155.2 (C-9), 156.7 (C-14), 157.3 (C-10), 183.3 (C-5), 186.9 (C-8); (−)-LRESIMS *m*/*z* (rel. int.) 461 (100) $[M-H]^-$; (+)-LRESIMS *m*/*z* (rel. int.) 445 (20) $[M-OH]^+$, 463 (100) $[M+H]^+$; (+)-HRESIMS *m*/*z* 485.1899 ($C_{23}H_{30}N_2O_8Na$ $[M+Na]^+$ requires 485.1894).

4.4.2. Compound 6

Red powder, (4.0 mg, 59%); $[\alpha]_D^{29}$ +1450 (*c* 0.001, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.08), 265 (3.99), 325 (3.49), 510 (3.61) nm; ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 1.06 (3H, t, *J* = 7.5 Hz, H-13), 1.48 (3H, s, 3-Me), 1.78 (2H, qt, *J* = 7.5, 7.0 Hz, H-12), 1.93 (1H, dd, *J* = 14.8, 5.0 Hz, H-2_{ax}), 2.35 (1H, ddd, *J* = 14.8, 1.7, 1.7 Hz, H-2_{eq}), 2.54 (1H, d, *J* = 18.4 Hz, H-4_{ax}), 3.17 (1H, d, *J* = 18.4 Hz, H-4_{eq}), 3.20 (2H, dt, *J* = 7.0, 5.8 Hz, H-11), 5.25 (1H, dd, *J* = 5.0, 1.7 Hz, H-1), 5.68 (1H, br s, H-7), 6.13 (1H, t, *J* = 5.8 Hz, 6-NH), 12.28 (1H, s, 10-OH), 14.18 (1H, s, 9-OH); ^{13}C NMR (125 MHz,

$CDCl_3$) δ_C 11.7 (C-13), 21.7 (C-12), 30.2 (3-Me), 37.6 (C-4), 40.3 (C-2), 44.6 (C-11), 63.4 (C-1), 68.0 (C-3), 100.6 (C-7), 109.0 (C-8a), 110.4 (C-5a), 133.2 (C-4a), 138.7 (C-9a), 149.1 (C-6), 155.2 (C-9), 157.3 (C-10), 183.5 (C-5), 187.1 (C-8); (−)-LRESIMS *m/z* (rel. int.) 346 (100) $[M-H]^-$; (+)-LRESIMS *m/z* (rel. int.) 330 (100) $[M-OH]^+$, 348 (80) $[M+H]^+$, 717 (80) $[2M+Na]^+$; (+)-HRESIMS *m/z* 370.1266 ($C_{18}H_{21}NO_6Na$ $[M+Na]^+$ requires 370.1261).

4.4.3. Compound 7

Red powder, (5.0 mg, 76%); $[\alpha]_D^{29}$ +1050 (*c* 0.003, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.09), 265 (3.92), 325 (3.50), 507 (3.46) nm; ^1H NMR (500 MHz, CD_3OD) δ_H 1.39 (3H, s, 3-Me), 1.86 (2H, tt, *J* = 7.1, 7.1 Hz, H-12), 1.92 (1H, br dd, *J* = 14.4, 4.3 Hz, H-2_{ax}), 2.16 (1H, br d, *J* = 14.4 Hz, H-2_{eq}), 2.55 (1H, br d, *J* = 18.0 Hz, H-4_{ax}), 2.78 (2H, br t, *J* = 7.1 Hz, H-13), 3.29 (2H, br t, *J* = 7.1 Hz, H-11), 3.02 (1H, br d, *J* = 18.0 Hz, H-4_{eq}), 5.07 (1H, br s, H-1), 5.59 (1H, br s, H-7); ^{13}C NMR (150 MHz, CD_3OD) δ_C 30.1 (3-Me), 31.3 (C-12), 38.3 (C-4), 39.9 (C-13), 41.3 (C-11), 41.6 (C-2), 63.9 (C-1), 69.5 (C-3), 100.2 (C-7), 110.1 (C-8a), 111.6 (C-5a), 134.6 (C-4a), 139.7 (C-9a), 151.4 (C-6), 156.2 (C-9), 157.8 (C-10), 185.8 (C-5), 188.0 (C-8); (−)-LRESIMS *m/z* (rel. int.) 361 (100) $[M-H]^-$; (+)-LRESIMS *m/z* (rel. int.) 363 (100) $[M+H]^+$; (+)-HRESIMS *m/z* 363.1561 ($C_{18}H_{23}N_2O_6$ $[M+H]^+$ requires 363.1551).

4.4.4. Compound 8

Red powder, (5.1 mg, 75%); $[\alpha]_D^{29}$ +1500 (*c* 0.002, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.13), 264 (4.03), 325 (3.54), 508 (3.63) nm; ^1H NMR (500 MHz, CD_3OD) δ_H 1.40 (3H, s, 3-Me), 1.93 (1H, dd, *J* = 15.0, 4.8 Hz, H-2_{ax}), 2.18 (1H, ddd, *J* = 15.0, 2.2, 2.2 Hz, H-2_{eq}), 2.58 (1H, bd, *J* = 19.9 Hz, H-4_{ax}), 3.05 (1H, br d, *J* = 19.9 Hz, H-4_{eq}), 3.41 (3H, s, H-13), 3.43 (2H, t, *J* = 6.3 Hz, H-11), 3.64 (2H, t, *J* = 6.3 Hz, H-12), 5.09 (1H, dd *J* = 4.8, 2.2 Hz, H-1), 5.68 (1H, br s, H-7); ^{13}C NMR (125 MHz, CD_3OD) δ_C 30.0 (3-Me), 38.2 (C-4), 41.6 (C-2), 43.3 (C-11), 59.1 (C-13), 63.9 (C-1), 69.5 (C-3), 70.8 (C-12), 100.7 (C-7), 109.9 (C-8a), 111.4 (C-5a), 134.2 (C-4a), 139.9 (C-9a), 151.2 (C-6), 156.2 (C-9), 157.9 (C-10), 184.6 (C-5), 188.3 (C-8); (−)-LRESIMS *m/z* (rel. int.) 362 (100) $[M-H]^-$; (+)-LRESIMS *m/z* (rel. int.) 364 (100) $[M+H]^+$; (+)-HRESIMS *m/z* 386.1203 ($C_{18}H_{21}NO_7Na$ $[M+Na]^+$ requires 386.1210).

4.4.5. Compound 9

Red powder, (5.9 mg, 70%); $[\alpha]_D^{29}$ +1450 (*c* 0.001, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.92), 264 (3.74), 325 (3.23), 506 (3.34) nm; ^1H NMR (500 MHz, $CDCl_3$) δ_H 1.45 (3H, s, 3-Me), 1.89 (1H, dd, *J* = 14.8, 5.0 Hz, H-2_{ax}), 2.32 (1H, ddd, *J* = 14.8, 1.7, 1.7 Hz, H-2_{eq}), 2.51 (1H, d, *J* = 18.5 Hz, H-4_{ax}), 3.00 (2H, t, *J* = 7.0 Hz, H-12), 3.14 (1H, dd, *J* = 18.5, 1.5 Hz, H-4_{eq}), 3.48 (2H, dt, *J* = 7.0, 6.5 Hz, H-11), 5.22 (1H, dd, *J* = 5.0, 1.7 Hz, H-1), 5.69 (1H, br s, H-7), 6.12 (1H, br t, *J* = 6.5 Hz, 6-NH), 7.23 (2H, br d, *J* = 7.5 Hz, H-14, H-18), 7.29 (1H, br d, *J* = 7.5 Hz, H-16), 7.35 (2H, br d, *J* = 7.5 Hz, H-15, H-17); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 30.1 (3-Me), 34.5 (C-12), 37.6 (C-4), 40.3 (C-2), 44.0 (C-11), 63.3 (C-1), 68.0 (C-3), 100.8 (C-7), 108.9 (C-8a), 110.4 (C-5a), 127.3 (C-16), 128.8 (C-14, C-18), 129.1 (C-15, C-17), 133.4 (C-4a), 137.7 (C-13), 138.8 (C-9a), 148.9 (C-6), 155.2 (C-9), 157.3 (C-10), 183.3 (C-5), 187.0 (C-8); (−)-LRESIMS *m/z* (rel. int.) 408 (100) [M-H]$^-$; (+)-LRESIMS *m/z* (rel. int.) 410 (80) $[M+H]^+$, 432 (70) $[M+Na]^+$, 841 (100) [2 M+Na]$^+$; (+)-HRESIMS *m/z* 432.1430 ($C_{23}H_{23}NO_6Na$ $[M+Na]^+$ requires 432.1418).

4.4.6. Compound 10

Red powder, (7.2 mg, 85%); $[\alpha]_D^{29}$ +1120 (*c* 0.0025, MeOH); UV (MeOH) λ_{max} (log ε) 215 (3.53), 277 (3.53), 264 (3.32), 311 (2.95), 504 (2.99) nm; ^1H NMR (500 MHz, CD_3OD) δ_H 1.39 (3H, s, 3-Me), 1.92 (1H, dd, *J* = 14.0, 4.8 Hz, H-2_{ax}), 2.17 (1H, d, *J* = 14.0 Hz, H-2_{eq}), 2.57 (1H, d, *J* = 18.5 Hz, H-4_{ax}), 2.87 (2H, t, *J* = 7.3 Hz, H-12), 3.04 (1H, br d, *J* = 18.5 Hz, H-4_{eq}), 3.43 (2H, t, *J* = 7.3 Hz, H-11), 5.08 (1H, br s, H-1), 5.61 (1H, s, H-7), 6.74 (2H, br d, *J* = 8.0 Hz, H-15, H-17), 7.10 (2H, br d, *J* = 8.0 Hz, H-14, H-18); ^{13}C NMR (125 MHz, CD_3OD) δ_C 30.1 (3-Me), 34.3 (C-12), 38.2 (C-4), 41.6 (C-2), 45.4 (C-11), 63.8 (C-1), 69.5 (C-3), 100.4 (C-7), 109.9 (C-8a), 111.4 (C-5a), 116.6 (2C, C-15, C-17), 130.5 (C-13), 130.8 (C-14, C-18), 134.0 (C-4a), 139.9 (C-9a), 150.9 (C-6), 155.9 (C-16), 156.0 (C-9), 157.2 (C-10), 184.8 (C-5), 188.3 (C-8); (−)-LRESIMS *m/z* (rel. int.) 424 (100) $[M-H]^-$; (+)-HRESIMS *m/z* 448.1388 ($C_{23}H_{23}NO_7Na$ $[M+Na]^+$ requires 448.1367).

4.4.7. Compound 11

Red powder, (8.0 mg, 90%); $[\alpha]_D^{29}$ +1520 (*c* 0.001, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.95), 266 (3.74), 325 (3.23), 512 (3.33) nm; ^1H NMR (500 MHz, CD_3OD) δ_H 1.39 (3H, s, 3-Me), 1.91 (1H, dd, *J* = 14.5, 4.0 Hz, H-2_{ax}), 2.17 (1H, br d, *J* =14.5 Hz, H-2_{eq}), 2.56 (1H, br d, *J* = 18.5 Hz, H-4_{ax}), 2.77 (2H, br t, *J* = 7.0 Hz, H-12), 3.04 (1H, br d, *J* = 18.5 Hz, H-4_{eq}), 3.48 (2H, br t, *J* = 7.0 Hz, H-11), 5.08 (1H, br s, H-1), 5.61 (1H, s, H-7), 7.28 (2H, br dd, *J* = 7.6, 2.5 Hz, H-14, H-18), 7.32 (2H, br d, *J* = 7.6 Hz, H-15, H-17); ^{13}C NMR (125 MHz, CD_3OD) δ_C 30.1 (3-Me), 34.3 (C-12), 38.2 (C-4), 41.6 (C-2), 44.8 (C-11), 63.9 (C-1), 69.5 (C-3), 100.4 (C-7), 109.8 (C-8a), 111.4 (C-5a), 129.6 (2C, C-15, C-17), 131.4 (2C, C-14, C-18), 133.5 (C-16), 134.4 (C-4a), 138.7 (C-13), 139.9 (C-9a), 151.0 (C-6), 156.1 (C-9), 158.5 (C-10), 184.5 (C-5), 188.1 (C-8); (−)-LRESIMS *m/z* (rel. int.) 442 (100) $[M-H]^-$; (+)-HRESIMS *m/z* 466.1047 ($C_{23}H_{22}ClNO_6Na$ $[M+Na]^+$ requires 466.1027).

4.4.8. Compound 12

Red powder, (6.9 mg, 97%); $[\alpha]_D^{29}$ +1450 (*c* 0.002, MeOH); UV (MeOH) λ_{max} (log ε) 214 (3.53), 264 (3.38), 323 (2.93), 510 (2.95) nm; ^1H NMR (500 MHz, CD_3OD) δ_H 1.40 (3H, s, 3-Me), 1.92 (1H, br dd, *J* = 15.5, 5.0 Hz, H-2_{ax}), 2.17 (1H, br d, *J* =15.5 Hz, H-2_{eq}), 2.52 (4H, m, H-13, H-16), 2.58 (1H, br d, *J* = 18.5 Hz, H-4_{ax}), 2.69 (1H, br t, *J* = 6.5 Hz, H-12), 3.04 (1H, br d, *J* = 18.5 Hz, H-4_{eq}), 3.34 (2H, br t, *J* = 6.5 Hz, H-11), 3.70 (4H, m, H-14, H-15), 5.09 (1H, br s, H-1), 5.64 (1H, s, H-7); ^{13}C NMR (125 MHz, CD_3OD) δ_C 30.1 (3-Me), 38.3 (C-4), 40.0 (C-2), 41.6 (C-11), 54.6 (C-13, C-16), 54.8 (C-12), 63.9 (C-1), 67.8 (C-14, C-15), 69.5 (C-3), 100.6 (C-7), 109.9 (C-8a), 111.5 (C-5a), 134.3 (C-4a), 140.0 (C-9a), 151.2 (C-6), 156.3 (C-9), 158.2 (C-10), 185.9 (C-5), 188.3 (C-8); (−)-LRESIMS *m/z* (rel. int.) 417 (100) $[M-H]^-$; (+)-LRESIMS *m/z* (rel. int.) 401 (20) $[M-OH]^+$; 419 (100) $[M+H]^+$; (+)-HRESIMS *m/z* 441.1639 ($C_{21}H_{26}N_2O_7Na$ $[M+Na]^+$ requires 441.1632).

4.4.9. Compound 13

Red powder, (3.3 mg, 80%); $[\alpha]_D^{29}$ +1440 (*c* 0.002, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.64), 264 (3.50), 329 (2.95), 509 (3.10) nm; ^1H NMR (500 MHz, CD_3OD) δ_H 1.38 (3H, s, 3-Me), 1.92 (1H, br dd, *J* = 15.5, 5.0 Hz, H-2_{ax}), 2.17 (1H, d, *J* = 15.5 Hz, H-2_{eq}), 2.59 (1H, d, *J* = 18.5 Hz, H-4_{ax}), 3.06 (1H, d, *J* = 18.5 Hz, H-4_{eq}), 4.49 (2H, s, H-11), 5.09 (1H, dd, *J* = 5.0, 3.0 Hz, H-1), 5.59 (1H, s, H-7), 7.28 (1H, dd, *J* = 8.3, 4.4 Hz, H-15), 7.36 (4H, br d, *J* = 4.4 Hz, H-13, H-14, H-16, H-17); ^{13}C NMR (125 MHz, CD_3OD) δ_C 30.1 (3-Me), 38.2 (C-4), 41.6 (C-2), 47.2 (C-11), 63.9 (C-1), 69.5 (C-3), 101.6 (C-7), 109.9 (C-8a), 111.5 (C-5a), 128.4 (2C, C-13, C-17), 128.7 (C-15), 129.9 (C-14, C-16), 134.2 (C-4a), 139.9 (C-9a), 151.2 (C-6), 156.2 (C-9), 157.8 (C-10), 185.0 (C-5), 188.3 (C-8); (−)-LRESIMS *m/z* (rel. int.) 394 (100) $[M-H]^-$; (+)-LRESIMS *m/z* (rel. int.) 396 (70) $[M+H]^+$, 418 (40) $[M+Na]^+$, 813 (80) $[2M+Na]^+$; (+)-HRESIMS *m/z* 418.1268 ($C_{22}H_{21}NO_6Na$ $[M+Na]^+$ requires 418.1261).

4.4.10. Compound 14

Red powder, (3.3 mg, 82%); $[\alpha]_D^{29}$ +1510 (*c* 0.001, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.54), 264 (2.82), 322 (2.96), 510 (3.05) nm; ^1H NMR (500 MHz, CD_3OD) δ_H 1.40 (3H, s, 3-Me), 1.94 (1H, br dd, *J* = 14.4, 4.2 Hz, H-2_{ax}), 2.18 (1H, br d, *J* =14.4 Hz, H-2_{eq}),

2.59 (1H, br d, J = 18.6 Hz, H-4_{ax}), 2.91 (3H, s, H-11), 3.05 (1H, br d, J = 18.6 Hz, H-4_{eq}), 5.10 (1H, br s, H-1), 5.56 (1H, s, H-7); ^{13}C NMR (150 MHz, CD_3OD) δ_C 29.4 (C-11), 30.1 (3-Me), 38.2 (C-4), 41.6 (C-2), 64.0 (C-1), 69.5 (C-3), 99.9 (C-7), 110.0 (C-8a), 111.4 (C-5a), 134.1 (C-4a), 139.8 (C-9a), 152.2 (C-6), 156.0 (C-9), 157.7 (C-10), 184.9 (C-5), 188.2 (C-8); (−)-LRESIMS m/z (rel. int.) 318 (100) $[M-H]^-$, 637 (30) $[2M-H]^-$; (+)-LRESIMS m/z (rel. int.) 302 (100) $[M-OH]^+$, 320 (90) $[M+H]^+$; (+)-HRESIMS m/z 342.0946 ($C_{16}H_{17}NO_6Na$ $[M+Na]^+$ requires 342.0948).

4.4.11. Compound 15

Red powder, (5.5 mg, 98%); $[\alpha]_D^{29}$ +1450 (c 0.001, MeOH); UV (MeOH) λ_{max} (log ε) 213 (3.74), 264 (3.62), 323 (3.18), 509 (3.85) nm; ^{1}H NMR (500 MHz, CD_3OD) δ_H 1.40 (3H, s, 3-Me), 1.86 (4H, br s, 1.94, H-14, H-15), 1.92 (1H, br dd, J = 14.4, 3.9 Hz, H-2_{ax}), 2.17 (1H, d, J = 14.4 Hz, H-2_{eq}), 2.56 (1H, d, J = 18.0 Hz, H-4_{ax}), 2.67 (4H, br s, H-13, H-16), 2.82 (2H, br s, H-12), 3.03 (1H, br d, J = 18.0 Hz, H-4_{eq}), 3.39 (2H, br s, H-11), 5.07 (1H, br s, H-1), 5.61 (1H, s, H-7); ^{13}C NMR (150 MHz, CD_3OD) δ_C 24.6 (2C, C-14, C-15), 30.1 (3-Me), 38.1 (C-4), 41.7 (C-2), 42.3 (C-11), 54.4 (C-12), 55.2 (C-13, C-16), 63.9 (C-1), 69.6 (C-3), 100.4 (C-7), 109.8 (C-8a), 111.5 (C-5a), 134.2 (C-4a), 139.8 (C-9a), 151.3 (C-6), 156.0 (C-9), 157.9 (C-10), 184.4 (C-5), 188.1 (C-8); (−)-LRESIMS m/z (rel. int.) 401 (100) $[M-H]^-$; (+)-LRESIMS m/z (rel. int.) 403 (100) $[M+H]^+$; (+)-HRESIMS m/z 425.1673 ($C_{21}H_{26}N_2O_6Na$ $[M+Na]^+$ requires 425.1683).

4.5. Compound purity analysis

Compounds **1**–**15** were all prepared at a concentration of 0.2 mg/200 μL in DMSO and injected (100 μL) onto an analytical Phenomenex C_{18} ONYX monolithic column. HPLC fractionation conditions consisted of a linear gradient (curve #6) from 90% H_2O (0.1% TFA)/10% MeOH (0.1% TFA) to 50% H_2O (0.1% TFA)/50% MeOH (0.1% TFA) in 3 min at a flow rate of 4 mL/min, a convex gradient (curve #5) to MeOH (0.1% TFA) in 3.50 min at a flow rate of 3 mL/min, held at MeOH (0.1% TFA) for 0.50 min at a flow rate of 3 mL/min, held at MeOH (0.1% TFA) for a further 1.0 min at a flow rate of 4 mL/min, then a linear gradient (curve #6) back to 90% H_2O (0.1% TFA)/10% MeOH (0.1% TFA) in 1 min at a flow rate of 4 mL/min, then held at 90% H_2O (0.1% TFA)/10% MeOH (0.1% TFA) for 2 min at a flow rate of 4 mL/min. Total run time for each analytical injection was 11 min. Compound purity was determined by extracting each chromatogram at 210 nm and integrating all UV peaks. The % purity and retention time for **1**–**15** were as follows: **1** (89%, 4.36 min), **2** (98%, 5.43 min), **3** (96%, 5.93 min), **4** (99%, 7.20 min), **5** (99%, 6.22 min), **6** (92%, 6.05 min), **7** (96%, 5.60 min), **8** (96%, 5.68 min), **9** (97%, 6.46 min), **10** (97%, 6.0 min), **11** (98%, 6.65 min), **12** (96%, 4.07 min), **13** (99%, 6.27 min), **14** (99%, 5.52 min), **15** (87%, 4.23 min).

4.6. Antimalarial assays

P. falciparum growth inhibition assays were carried out using an isotopic microtest, as previously described.[45] Briefly, ring-stage *P. falciparum* infected erythrocytes (0.5% parasitemia and 2.5% hematocrit) were seeded into 96 well tissue culture plates containing serial dilutions of control (chloroquine) or test compounds. Following 48 h incubation under standard *P. falciparum* culture conditions, 0.5 μCi ^{3}H-hypoxanthine was added to each well after which the plates were cultured for a further 24 h. Cells were harvested onto 1450 MicroBeta filter mats (Wallac) and ^{3}H incorporation was determined using a 1450 MicroBeta liquid scintillation counter. *P. falciparum* growth inhibition was compared to DMSO controls (0.5%), and IC_{50} values were calculated using linear interpolation of inhibition curves.[45] The mean IC_{50} (±SD) is shown for three independent experiments, each carried out in triplicate.

4.7. Cytotoxicity assay[46]

Neonatal foreskin fibroblast (NFF) cells were cultured in RPMI 1640 (Life Technologies, Inc., Rockville, MD) supplemented with 10% FCS (CSL Biosciences, Parkville, Victoria, Australia), 1% streptomycin (Life Technologies, Inc., Rockville, MD; complete medium) at 37 °C and 5% CO_2. Cells were maintained in log phase growth and seeded (3000/well) into 96-well tissue culture plates (Corning, USA) then cultivated for 24 h before treatment. Compounds were dissolved in DMSO and diluted in complete medium; the DMSO concentration in the medium did not exceed 1%. Control cells were treated with the equivalent dose of DMSO. Three days after treatment initiation, the cells were washed with PBS and fixed in methylated spirits, total protein was determined using sulforhodamine B as described previously.[46] Compounds were tested in triplicate in three independent experiments.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.09.052.

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