Synthesis and anti-plasmodial activity of 8β, 13β-dihydroxypodocarpane derivatives

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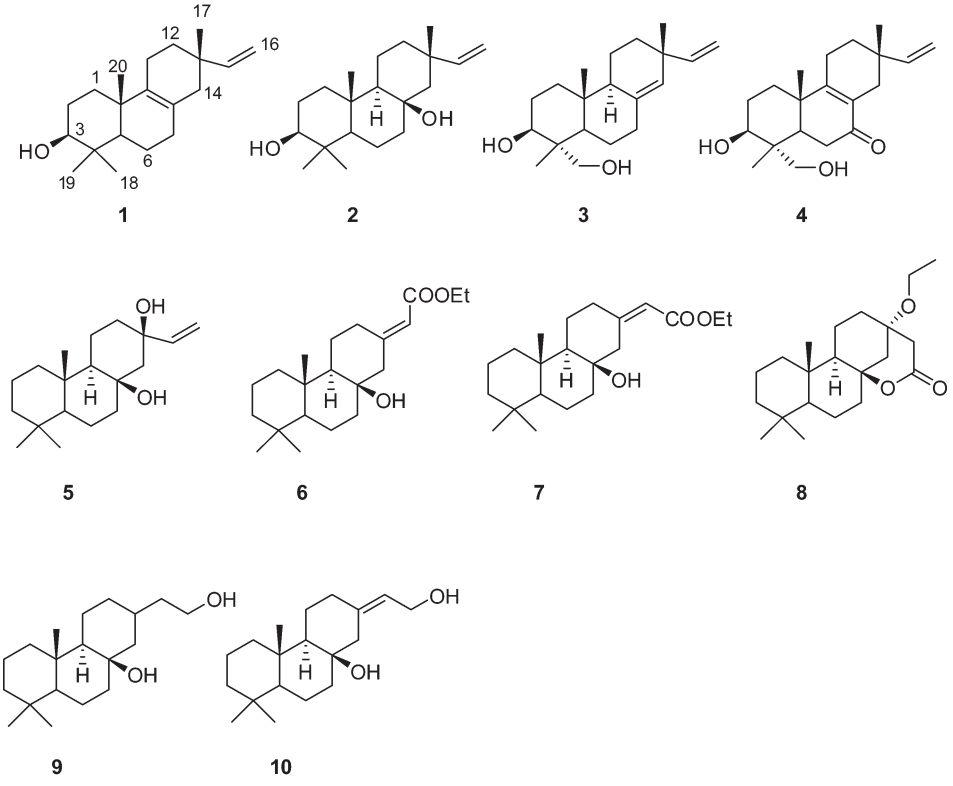
8β,13β-Dihydroxypodocarpane and eight C-13 substituted derivatives were prepared from the precursor 8β-hydroxy-13-podocarpanone synthesised from the naturally occurring diterpene (+)-manool. The synthetic compounds exhibited a range of anti-plasmodial activities (IC_{50} 1–29 μM) and only induced minimal haemolysis of erythrocytes at concentrations 50 and 100 μM. No changes in the morphology of erythrocytes were detected at sub-haemolytic concentrations.

Keywords: manool, podocarpane, antiplasmodial, haemolysis

The ongoing global health burden of malaria is well-documented.[1–3] The malaria pandemic, especially in sub-Saharan Africa where the socio-economic impact of this disease is most pronounced, is further exacerbated by ubiquitous malarial parasite resistance to chloroquine and sulfadoxine-pyrimethamine prophylaxis.[2–5] As attempts to find a malaria vaccine remain elusive, chaemoprophylaxis and chaemotherapeutic interventions in the form of either synthetic or natural product-based drugs appear to be the only current treatment options.[5,6] Two of the most well-established natural product anti-malarials in past and present clinical use are quinine from the South American *Cinchona* tree, which provided the template for the development of chloroquine and related quinolines, and the sesquiterpene peroxide – artemisinin and analogues, originally isolated from the Chinese herb *Artemisia annua*.[3–6]

The erythrocytic stages of the malarial protozoan parasites of the genus *Plasmodium*, predominantly *P. falciparum*, are readily cultured *in vitro* and continue to provide a relatively accessible target for anti-malarial studies. Not surprisingly, therefore, there are numerous reports in the chemistry literature of both natural and synthetic compounds exhibiting anti-plasmodial activity with the vast majority of reported IC_{50} values in the micromolar range while anti-malarial drugs in clinical use *e.g.* mefloquine, atovaquone and artemisinin have anti-plasmodial IC_{50} values in the low nanomolar range.[5] A series of isopimarane diterpenoid natural products (**1**–**4**), isolated from the Iranian tree *Platycladus orientalis* (L.) Franco (Cupressaceae),[7] are typical examples of this trend (IC_{50} 7–25 μg mL^{-1}). Asili *et al.*[7] proposed that the anti-plasmodial activity of **1**–**4** may be linked to observed erythrocyte shape changes which, while in some cases not appearing to result directly in erythrocyte cell lysis, may indirectly reduce host cell viability through the accumulation of these compounds in the cell membrane bilayer; ultimately resulting in the demise of the parasitic *Plasmodium*.



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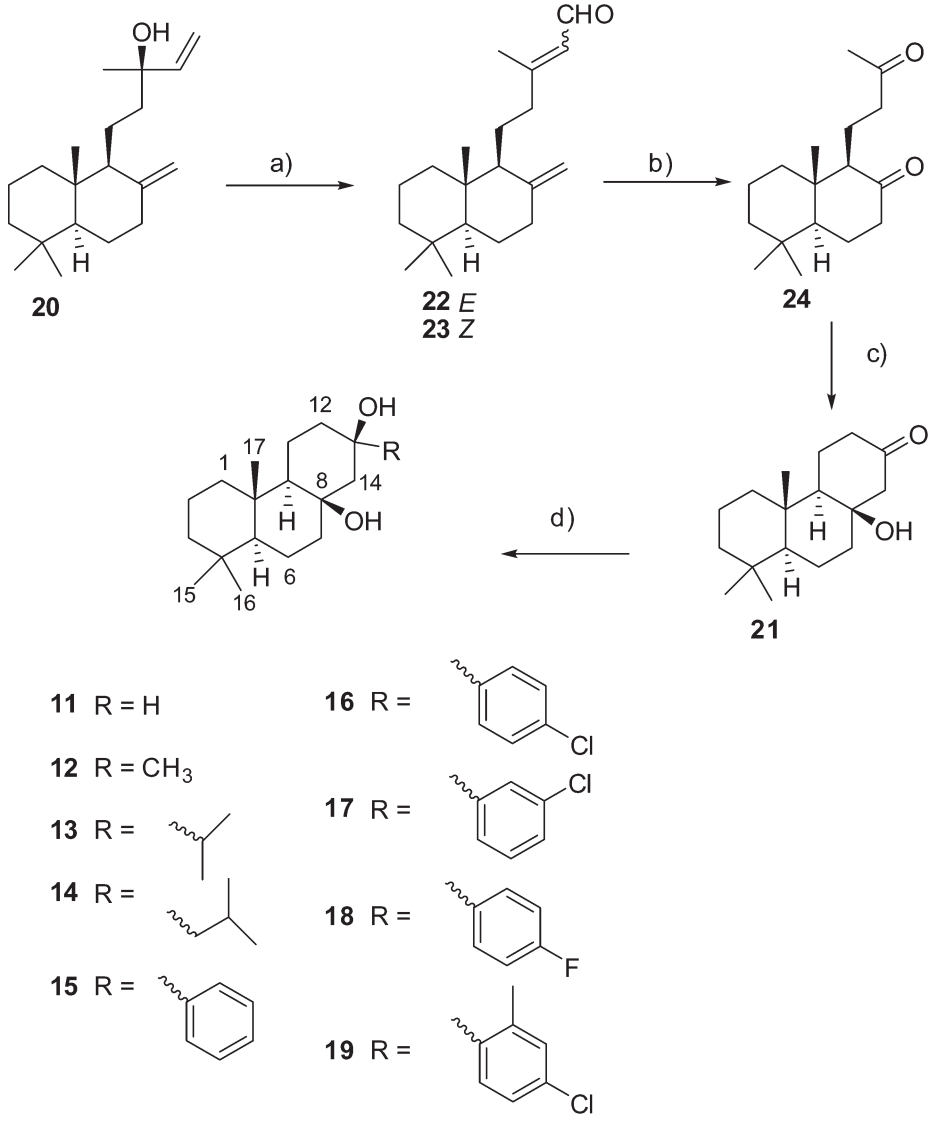
In an attempt to improve on the anti-plasmodial activity and reduce the related haemolysis and possibly deleterious erythrocyte shape altering effects of **1**–**4** at sub-haemolytic concentrations, we recently synthesised six related compounds (**5**–**10**) from (+)-manool.[8] Only the three semi-synthetic compounds (**6**, **7**, **10**) with an exocyclic olefin substituent at C-13 exhibited similar haemolytic activity and shape change effects to those observed for **1–4**. In the absence of any observable haemolytic activity or cell shape changing effects for **5**, **8** and **9** (IC_{50} 11–45 μM), we report here, as part of an ongoing structure activity relationship study, the preparation and anti-plasmodial activity of 8β,13β-dihydroxypodocarpane (**11**) and eight analogues (**12**–**19**) of **5** in which the C-13-α substituent is varied.

Results and discussion

Naturally occurring (+)-manool (**20**) is a commonly used semi-synthetic precursor for the synthesis of both marine and terrestrial natural products.[9–12] The key intermediate in the synthesis of **11**–**19**, 8-hydroxy-13-podocarpanone (**21**), was readily accessed from manool *via* initial pyridinium chlorochromate mediated oxidative rearrangement of **20** to afford a mixture of *E* and *Z* olefins (**22** and **23**) which on subsequent reductive ozonolyis gave the diketone (**24**) in good yield (*ca* 80%).[8] An intramolecular aldol condensation of **24** in the presence of base (NaH) yielded **21** which was eventually crystallised from mixed solvents (dichloromethane/hexane) and thus invalidated the perceived instability of this compound alluded to in our earlier paper.[8] A full assignment of the previously unassigned NMR data for **21** is presented in the experimental section.

A possible relationship between the structure and the activity of compound **5** suggests that varying functionalisation of the ketone moiety in **21**, with retention of the tertiary alcohol moiety at this position, might afford compounds with similar or possibly improved anti-plasmodial activity to that observed for **5**. Also of interest to us was the effect which a variation in the substitution pattern at C-13 might elicit on erythrocyte morphology. Accordingly, the addition of a series of Grignard reagents (methyl-, isopropyl-, isobutyl-, phenyl-, 4-chlorophenyl-, 3-chlorophenyl-, 4-fluorophenyl- and 2-methyl-4-chlorophenyl- magnesium bromide) to **21** afforded compounds **12–19** respectively in variable yields. While addition of anhydrous cerium chloride[13] to the Grignard preparation of **12** and **16**–**18** significantly improved the yields of this compound the same effect was not observed in the preparation of the other compounds where the isolated yields seldom exceeded 30%.



Scheme 1 Synthesis of podocarpane derivatives **11–19** (a) PCC, CH_2Cl_2; (b) O_3, CH_2Cl_2, –78 °C; (c) NaH, THF; (d) $CeCl_3$, THF, RMgBr, 0 °C.

Our earlier synthesis of **5** from **21** suggested that the carbon nucleophiles generated by the Grignard reagents would attack the electrophilic carbon of the carbonyl moiety selectively from the less hindered α-face of **21**. Although the doublet assigned to one of the H_2-14 diastereotopic methylene protons (δ_H *ca.* 1.3, J = 14 Hz, H-14α) and the doublet of doublets assigned to the other methylene proton [δ_H *ca* 1.6–1.8, J = 14 Hz, 2.8 Hz (from W-coupling with H-12β), H-14β] were clearly delineated in each of the ^{1}H NMR spectra of **12–19**, the extensive overlap of signals in the surrounding methylene envelope precluded the unequivocal NOESY assignment of an α-equatorial alkyl or aromatic substituent (**15–19**) at C-13. Suitable crystals of **15** for X-ray analysis were thus obtained *via* slow crystallisation from methanol. The perspective view of **15** presented in Fig. 1 clearly confirmed the presence of a 13α-equatorial phenyl substituent and a similar orientation of the C-13 alkyl and aryl substituents were anticipated for the two series of compounds **12–14** and **15–19** respectively. Careful analysis of gCOSY, gHSQC and gHMBC data enabled a complete assignment of the NMR data for **12–19**.

Interestingly, the diol (**11**) was isolated as a minor product during the HPLC purification of the product mixture obtained from the Grignard addition of isobutylmagnesium bromide to **21**. The structure of this compound was established as 8β, 13β–dihydroxypodocarpane from NMR and MS data while the β-orientation of the C-13 hydroxyl moiety was corroborated by X-ray analysis (Fig. 1). No evidence of the C-13 epimer of **11** was found in the product mixture.

The anti-plasmodial and haemolytic activity of compounds **5** and **11–19** are presented in Table 1. Compound **19** possessed the most promising anti-plasmodial activity of the cohort of compounds prepared in this study. Although **19** exhibited a 13-fold increase in activity over **5** this compound was still approximately 10 and 100 fold less active than quinine and chloroquine respectively. However, all the aromatic compounds **15–19** were more active than cycloproguanil ($P < 0.05$), with no haemolysis or changes in erythrocyte morphology being detected after 48 hours of exposure of the healthy red blood cells at 50 and 100 μM concentrations of these compounds. Gelb[5] has provided evidence to suggest that micromolar anti-plasmodial activity may be indicative of multi-target binding in *Plasmodium* and it is probable that **19** acts at more than one, as yet unidentified, target in *P. falciparum*.

Experimental

Melting points were determined using a Reichert hot stage microscope and are uncorrected. Optical rotations were measured using a Perkin-Elmer 141 polarimeter calibrated at the sodium D line (598 nm). Infrared spectra were recorded on a Perkin Elmer Spectum 2000 FT-IR and DIGILAB Excalibur HE Series FTS 3100 FT-IR spectrometer. NMR spectra were acquired on a Bruker 600 MHz Avance II spectrometer using standard pulse sequences. Chemical shifts are reported in ppm, referenced to residual solvent resonances ($CDCl_3$ δ_H 7.25, δ_C 77.0), and coupling constants are reported in Hz. Reactions where exclusion of water was necessary were performed in flame dried glassware under Ar. Immediately prior to their use THF was distilled from sodium metal/benzophenone ketyl and CH_2Cl_2 from CaH_2. General laboratory solvents were distilled before use. Reactions were monitored by thin layer chromatography (DC-Plastikfolien Kieselgel 60 F_{254} plates) and visualised under UV light and developed by spraying with either 10% conc. H_2SO_4 in methanol or iodine. Kieselgel 60 (230–400 mesh) was used for initial flash chromatographic separations. Semi-preparative HPLC was performed using a Whatman's Magnum 9 Partisil 10 column (10 mm i.d., length 50 cm) with RI detection and an eluent flow rate of 4 mL min^{-1}.

Preparation of **21–24**

(E)-labda-8(17)dien-15-al (**22**), *(Z)-labda-8(17)dien-15-al* (**23**), *and 15,16,17-trinorlabdane-8,13-dione* (**21**): The mixture of geometric isomers of (**22** and **23**) and the diketone (**24**) were prepared as described previously.[8]

8β-hydroxy-13-podocarpanone (**21**): The diketone (**24**) 1.12 g (4.2 mmol) was dissolved in dry THF (5 mL), NaH (0.49 g, 12.7 mmol) added and the solution stirred under argon (4 h). The reaction mixture was quenched with sat. aq. NH_4Cl and extracted with Et_2O (3 × 10 mL). The ether extracts were combined, washed with water (2 × 5 mL), sat. brine (1 × 5 mL) and dried over anhyd. $MgSO_4$. The ether was removed *in vacuo* and the resultant yellow oil crystallised from CH_2Cl_2: hexane (1:4) to yield colourless needles of **21** (893 mg, 80%): m.p.

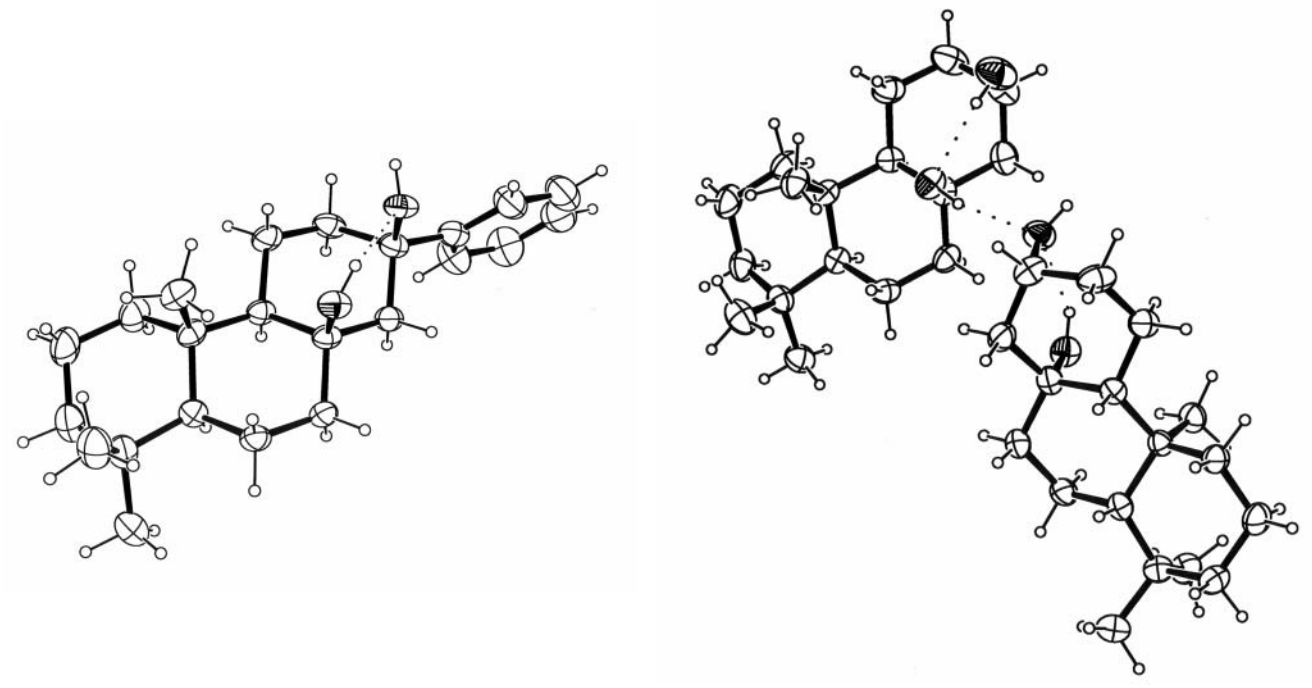


Fig. 1 Perspective view of a molecule of **15** (left) and two independent molecules of **11** in the asymmetric unit (right) from the crystal structures. Thermal ellipsoids for the non hydrogen atoms are shown at the 50% probability level. The intramolecular O–H···O hydrogen bond is shown.

Table 1 Comparative anti-plasmodial activity and % haemolysis of compounds **5** and **11–19** and five common anti-malarial drugs (SD = standard deviation, n = number of replicates)

Compound	Antiplasmodial activity (μM)			Haemolytic activity at 100 μM			Haemolytic activity at 50 μM		
	IC_{50}	SD	n	% Haemolysis	SD	n	% Haemolysis	SD	n
5	18.6	2.6	4	0.6	0.3	4	0.2	0.2	5
11	21.3	2.9	4	0.6	0.3	3	0.6	0.3	3
12	29.1	1.2	4	1.0	0.4	3	0.5	0.1	3
13	22.3	1.6	4	0.5	0.3	4	0.4	0.2	6
14	26.1	0.8	4	0.8	0.4	5	0.5	0.2	3
15	6.6	0.3	4	0.6	0.3	5	0.7	0.2	4
16	4.1	0.5	4	0.1	0.1	3	0.001	0.001	3
17	8.2	1.7	3	0.5	0.2	3	0.06	0.02	3
18	3.5	0.4	4	0.2	0.1	3	0.02	0.02	3
19	1.4	0.2	3	0.4	0.2	3	0.03	0.003	3
Chloroquine	0.07	0.01	3	0.2	0.1	5	0.06	0.04	4
Quinine	0.1	0.01	5	0.5	0.3	6	0.6	0.1	5
Primaquine	0.6	0.04	5	0.6	0.3	6	0.3	0.2	5
Pyrimethamine	0.1	0.02	4	0.4	0.2	6	0.4	0.3	5
Cycloproguanil	11.3	1.9	7	0.07	0.05	5	0.2	0.2	4

201–203 °C, lit. 204–205 °C,[25] $[\alpha]_D^{23}$ +9.8 (*c* 0.4, $CHCl_3$, lit. +11.6, *c* 2.2, $CHCl_3$[25]); IR (film) ν_{max} 3434, 3019, 2926, 1215, 756, 668. ^{1}H NMR (600 MHz, $CDCl_3$) δ 2.42 (m, H-12a, 1H), 2.35 (d, *J* = 14.4, H-14β, 1H), 2.26 (m, H-12b, 1H), 2.23 (d, *J* = 14.4, H-14α, 1H), 1.92, (m, H_2-11, 2H), 1.76 (m, H-1a, 1H), 1.71 (m, H-7β, 1H), 1.60 (m, H-2a, 1H), 1.56 (m, H_2-6, and H-7α, 3H), 1.41 (m, H-2b and H-3β, 2H), 1.40 (m, H-9, 1H), 1.16 (td, *J* = 13.2, 4.4 Hz, H-3α, 1H), 0.98 (s, H_3-17, 3H), 0.94 (m, H-1a and H-5, 2H), 0.88 (s, H_3-15, 3H), 0.85 (s, H_3-16, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ = 210.7 (C, C-13) 75.5 (C, C-8), 57.3 (CH_2, C-14), 56.1 (CH, C-5), 55.3 (CH, C-9), 42.2 (CH_2, C-7), 42.0 (CH_2, C-3), 41.5 (CH_2, C-12), 39.8 (CH_2, C-1), 37.5 (C, C-10), 33.6 (CH_3, C-15), 33.3 (C, C-4), 21.8 (CH_3, C-16), 21.4 (CH_2, C-11) , 18.4 (CH_2, C-2), 17.9 (CH_2, C-6), 15.3 (CH_3, C-17) ; EIMS (70 eV *m/z* (rel. int.) 264 [M$^+$] (32), 250 (19), 249 (100), 179 (27), 123 (25); HREIMS (70 eV) *m/z* 264.2083 (Calcd for $C_{17}H_{28}O_2$, 264.2089).

Preparation of **12–19**

8β, 13β-dihydroxy-13α-methylpodocarpane (**12**): The following method is representative for the preparation of compounds **12–19**. A suspension of anhyd. $CeCl_3$ (137 mg, 0.57 mmol) in dry THF (5 mL) was stirred under argon at ambient temperature (2 h), cooled to 0 °C and an aliquot (1.89 mL, 0.57 mmol) of a THF solution of methylmagnesium bromide (3.0 M) added. The suspension was stirred (0 °C, 1.5 h) and a solution of **21** (100 mg, 0.37 mmol) in dry THF (2 mL) added. Stirring was continued (0 °C, 1 h) and the reaction mixture gradually allowed to reach room temperature (8 h) before quenching with sat. aq. NH_4Cl (5 mL) and extraction with Et_2O (3 × 5 mL). The combined ether fractions were washed with water (2 × 5 mL), sat. brine (1 × 5 mL), dried over anhyd. $MgSO_4$, filtered and concentrated under reduced pressure to yield a yellow oil. Further purification of the yellow oil via normal phase semi-preparative HPLC (4:1 hexane: EtOAc) afforded a white solid which was recrystallised from MeOH to give colourless prisms of **12** (73 mg, 57%): m.p. 157–159 °C; $[\alpha]_D^{23}$ −3.2 (*c* 0.5, CH_2Cl_2); IR (film) ν_{max} 3311 (br.), 2945, 1462, 1216, 1135, 780. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.77 (ddd, *J* = 13.3, 7.2, 3.1 Hz, H-12a, 1H), 1.73 (dt, *J* = 13.3, 3.3 Hz, H-7β, 1H), 1.71 (m, H-11a and H-1a, 2H), 1.63 (dd, *J* = 14.0, 2.8 Hz, H-14β, 1H), 1.61 (m, H-2a, 1H), 1.51 (m, H-6, 2H), 1.49 (m, H-11b, 1H), 1.39 (m, H-3β, 1H), 1.37 (m, H-2b, 1H), 1.33 (td, *J* = 14.2, 4.4 Hz, H-12b, 1H), 1.30 (m, H-7α, 1H), 1.28 (m, H-14α, 1H), 1.15 (s, H_3-1′, 3H), 1.13 (td, *J* = 12.9, 4,3 Hz, H-3α, 1H), 1.00 (s, H_3-17, 3H), 0.85 (s, H_3-15, 3H), 0.84 (s, H_3-16, 3H), 0.83 (m, H-5 and H-9, 2H), 0.80 (m, H-1b, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 73.2 (C, C-8), 71.0 (C, C-13), 56.4 (CH, C-5), 56.2 (CH, C-9), 51.1 (CH_2, C-14), 42.1 (CH_2, C-7), 42.0 (CH_2, C-3), 39.8 (CH_2, C-12), 39.5 (CH_2, C-1), 37.2 (C, C-10), 33.6 (CH_3, C-15), 33.3 (C, C-4), 30.9 (CH_3, C-1′), 21.7 (CH_3, C-16), 18.4 (CH_2, C-2), 17.7 (CH_2, C-6), 16.7 (CH_2, C-11), 15.4 (CH_3, C-17); EIMS (70 eV) *m/z* (rel. int.) 280 [M$^+$] (13), 262 (82), 244 (38), 179 (75), 126 (54); HREIMS (70 eV) *m/z* 280.2392 (Calcd for $C_{18}H_{32}O_2$, 280.2402).

8β, 13β-dihydroxy-13α-iso-propylpodocarpane (**13**): Colourless needles from MeOH (23 mg, 20%): m.p. 132–136 °C; $[\alpha]_D^{23}$ −16.1 (*c* 0.4, CH_2Cl_2); IR (film) ν_{max} 3303 (br.), 2946, 1702, 1451, 1190, 757. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.76 (m, H-7β, 1H), 1.72 (m, H-12a, 1H), 1.71 (m, H-1a and H-11a, 2H), 1.62 (m, H-2a, 1H), 1.56 (dd, *J* = 14.1, 3.0 Hz, H-14β, 1H), 1.52 (m, H_2-6, H-11b and H-1′, 4H), 1.39 (m, H-2b and H-3β, 2H), 1.33 (m, H-12b, 1H), 1.30 (td, *J* = 13.3, 5.1 Hz, H-7α, 1H), 1.25 (d, *J* = 14.1 Hz, H-14α, 1H), 1.13 (td, *J* = 14.0, 3.8 Hz, H-3α, 1H), 1.00 (s, H_3-17, 3H), 0.88 (d, *J* = 7.0 Hz, H_3-2′ and H_3-3′, 6H), 0.87 (s, H_3-15, 3H), 0.84 (m, H-9, 1H), 0.84 (s, H_3-16, 3H), 0.82 (m, H-1b, 1H), 0.81 (m, H-5, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 75.2 (C, C-13), 73.2 (C, C-8), 56.7 (CH, C-5), 56.6 (CH, C-9), 46.5 (CH_2, C-14), 42.6 (CH_2, C-3) 42.3 (CH_2, C-7), 39.7 (CH_2, C-1), 37.4 (C, C-10), 38.6 (CH, C-1′), 35.0 (CH_2, C-12), 33.7 (CH_3, C-15), 33.4 (C, C-4), 21.8 (CH_3, C-16), 18.4 (CH_2, C-2), 17.9 (CH_2, C-6), 16.9 (CH_3, C-3′), 16.8 (CH_3, C-2′), 16.5 (CH_2, C-11), 15.6 (CH_3, C-17); EIMS (70 eV) *m/z* (rel. int.) 308 [M$^+$] (28), 265 (50), 248, (35), 247 (100), 141 (30); HREIMS (70 eV) *m/z* 308.2717 (Calcd for $C_{20}H_{36}O_2$, 308.2715).

8β, 13β-dihydroxy-13α-iso-butylpodocarpane (**14**): Colourless needles from MeOH (31 mg, 27%): m.p. 138–140 °C; $[\alpha]_D^{33}$ −12.1 (*c* 0.4, CH_2Cl_2); IR (film) ν_{max} 3473 (br.), 1640, 1215, 1034, 750, 667. ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.83 (non, *J* = 6.5 Hz, H-2′ 1H), 1.79 (m, H-12a, 1H), 1.74 (m, H-7β, 1H), 1.71 (m, H-1a and H-11a, 2H), 1.66 (dd, *J* = 14.0, 2.8 Hz, H-14β, 1H), 1.61 (m, H-2a, 1H), 1.51 (m, H_2-6, 2H), 1.50 (m, H-11b, 1H), 1.39 (m, H-2b and H-3β, 2H), 1.31 (m, H-12b, 1H), 1.30 (m, H-7α and H-1′, 2H), 1.24 (d, *J* = 14.0 Hz, H-14α, 1H), 1.13 (td, *J* = 13.8, 4.0, H-3α, 1H), 1.01 (s, H_3-17, 3H), 0.94 (d, *J* = 6.2 Hz, H_3-3′, 3H), 0.93 (d, *J* = 6.2 Hz, H_3-4′, 3H), 0.86 (s, H_3-15, 3H), 0.85 (m, H-5 and H-9, 2H), 0.84 (s, H_3-16, 3H), 0.81 (m, H-1b, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 73.8 (C, C-13), 73.2 (C, C-8), 56.7 (CH, C-9), 56.6 (CH, C-5), 52.9 (CH_2, C-1′), 50.0 (CH_2, C-14), 42.5 (CH_2, C-7), 42.3 (CH_2, C-3), 39.7 (CH_2, C-1), 38.9 (CH_2, C-12), 37.4 (C, C-10), 33.7 (CH_3, C-15), 33.5 (C, C-4), 25.2 (CH_3, C-3′), 25.1 (CH_3, C-4′), 23.5 (CH, C-2′), 21.8 (CH_3, C-16), 18.6 (CH_2, C-2), 17.9 (CH_2, C-6), 16.5 (CH_2, C-11), 15.6 (CH_3, C-17); EIMS (70 eV) *m/z* (rel. int.) 322 [M$^+$] (8), 286 (100), 271, (55), 179 (96), 155 (73); HREIMS (70 eV) *m/z* 322.2865 (Calcd for $C_{21}H_{38}O_2$, 322.2872).

8β, 13β-dihydroxypodocarpane (**11**): Purification of the product mixture afforded from the preparation of **14** via normal phase semi-preparative HPLC (2:1 hexane:EtOAc), also yielded **11** as colourless prisms from MeOH (18 mg, 19%): m.p. 157–160 °C; $[\alpha]_D^{33}$ −16.9 (*c* 0.5, CH_2Cl_2); IR (film) ν_{max} 3430 (br.), 3020, 1637, 1214, 754, 670. ^{1}H NMR (600 MHz, $CDCl_3$) δ 4.07 (br. s, H-13, 1H), 3.09 (br. s, O<u>H</u>), 2.70 (br. s, O<u>H</u>), 1.94 (dquin, *J* = 13.1, 2.6 Hz, H-12β, 1H), 1.81 (ddd, *J* = 14.3, 3.5, 2.6 Hz, H-14β, 1H), 1.76 (m, H-11a, 1H), 1.74 (m, H-7β, 1H), 1.71 (m, H-1a, 1H), 1.62 (ddd, *J* = 13.8, 4.2, 3.45 Hz, H-2a, 1H), 1.49 (m, H-6a, 1H), 1.48 (m, H-11b, 1H), 1.46 (m, H-12α, 1H), 1.39 (m, H-14α, 1H), 1.38 (m, H-2b and H-3β, 2H), 1.33 (m, H-6b, 1H), 1.31 (m, H-7α, 1H), 1,13 (td, *J* = 13.5, 4.1 Hz, H-3α, 1H), 1.00 (s, H_3-17, 3H), 0.94 (dd, *J* = 12.6, 2.8 Hz, H-9, 1H), 0.87 (s, H_3-15, 3H), 0.83 (m, H-5, 1H), 0.83 (s, H_3-16, 3H), 0.82 (td, *J* = 13.1, 3.6 Hz, H-1b, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 73.4 (C, C-8), 67.7 (CH, C-13), 56.3 (CH, C-5), 56.5 (CH, C-9), 45.5 (CH_2, C-14), 42.2 (CH_2,

C-3) 42.1 (CH_2, C-7), 39.4 (CH_2, C-1), 37.3 (C, C-10), 33.9 (CH_2, C-12), 33.5 (CH_3, C-15), 33.3 (C, C-4), 21.7 (CH_3, C-16), 18.4 (CH_2, C-2), 17.6 (CH_2, C-6), 15.4 (CH_3, C-17), 14.9 (CH_2, C-11); EIMS (70 eV) *m/z* (rel. int.) 266 [M$^+$] (49), 251 (41), 233, (75), 180 (17), 179 (100); HREIMS (70 eV) *m/z* 266.2239 (Calcd for $C_{17}H_{30}O_2$, 266.2246).

8β, 13β-dihydroxy-13α-phenylpodocarpane (**15**): Colourless prisms from MeOH (38 mg, 34%): m.p. 161–164 °C; $[\alpha]_D^{23}$ −14.4 (*c* 0.6, CH_2Cl_2); IR (film) ν_{max} 3317 (br.), 2401, 1661, 1216, 757, 668. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46 (d, *J* = 7.8 Hz, H-2′ and H-6′, 2H), 7.33 (t, *J* = 7.4, H-3′ and H-5′, 2H), 7.23 (t, *J* = 7.3, H-4′, 1H), 1.95 (m, H-12a, 1H), 1.86 (m, H-12b, 1H) 1.81 (dd, *J* = 14.3, 2.7 Hz, H-14β, 1H), 1.76 (m, H-1a, 1H), 1.74 (m, H-7β, 1H), 1.68 (d, *J* = 14.2 Hz, H-14α, 1H), 1.65 (m, H-11b, 1H), 1.64 (m, H-2a, 1H), 1.55 (m, H_2-6, 2H), 1.43 (m, H-2b, 1H), 1.41 (m, H-3β, 1H), 1.36 (td, *J* = 13.3, 4.6 Hz, H-7α, 1H), 1.16 (td, *J* = 13.6, 3.9 Hz, H-3α, 1H), 1.06 (s, H_3-17, 3H), 1.04 (m, H-9, 1H) 0.90 (dd, *J* = 11.3, 3.4 Hz, H-5, 1H), 0.88 (m, H-1b, 1H), 0.88 (s, H_3-15, 3H), 0.86 (s, H_3-16, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 148.3 (C, C-1′), 128.2 (2 × CH, C-3′ and C-5′), 126.7 (CH, C-4′), 124.4 (2 × CH, C-2′ and C-6′), 74.5 (C, C-13), 73.5 (C, C-8), 56.4 (CH, C-5), 56.2 (CH, C-9), 51.4 (CH_2, C-14), 42.2 (CH_2, C-3) 42.1 (CH_2, C-7), 39.8 (CH_2, C-12), 39.5 (CH_2, C-1), 37.3 (C, C-10), 33.6 (CH_3, C-15), 33.3 (C, C-4), 21.7 (CH_3, C-16), 18.4 (CH_2, C-2), 17.7 (CH_2, C-6), 16.9 (CH_2, C-11), 15.5 (CH_3, C-17); EIMS (70 eV) *m/z* (rel. int.) 324 [M$^+$] (50), 306 (24), 179, (22), 175 (100), 105 (83); HREIMS (70 eV) *m/z* 324.2579 (Calcd for $C_{23}H_{34}O_2$, 324.2559).

8β, 13β-dihydroxy-13α-4′-chlorophenylpodocarpane (**16**): White needles from MeOH (64% yield), m.p. 182–184 °C; $[\alpha]_D^{23}$ −94.7 (*c* 1.1, $CHCl_3$); IR (film) ν_{max} 3272 (br.), 2945, 1441, 1093, 812, 758; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.6 Hz, H-2′ and H-6′, 2H), 7.28 (d, *J* = 8.5, H-3′ and H-5′, 2H), 1.90 (m, H-12a, 1H), 1.89 (m, 11a, 1H), 1.83 (m, H-12b, 1H) 1.76 (m, H-14β, 1H), 1.75 (m, H-1a, 1H), 1.74 (m, H-7β, 1H), 1.66 (m, H-14α, 1H), 1.65 (m, H-11b, 1H), 1.63 (m, H-2a, 1H), 1.55 (m, H_2-6, 2H), 1.42 (m, H-7α, 1H), 1.41 (m, H-2b and H-3β, 2H), 1.14 (td, *J* = 13.6, 4.5 Hz, H-3α, 1H), 1.04 (s, H_3-17, 3H), 1.04 (m, H-9, 1H) 0.89 (m, H-5, 1H), 0.87 (m, H-1b, 1H), 0.87 (s, H_3-15, 3H), 0.85 (s, H_3-16, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 147.1 (C, C-1′), 132.6 (C, C-4′), 128.4 (2 × CH, C-3′ and C-5′), 126.3 (2 × CH, C-2′ and C-6′), 74.2 (C, C-13), 74.1 (C, C-8), 56.6 (CH, C-5), 56.3 (CH, C-9), 51.7 (CH_2, C-14), 42.4 (CH_2, C-3) 42.2 (CH_2, C-7), 40.1 (CH_2, C-12), 39.6 (CH_2, C-1), 37.5 (C, C-10), 33.7 (CH_3, C-15), 33.5 (C, C-4), 21.9 (CH_3, C-16), 18.6 (CH_2, C-2), 17.8 (CH_2, C-6), 17.0 (CH_2, C-11), 15.7 (CH_3, C-17); EIMS *m/z* (rel. int.) 376 [M$^+$] (14), 358 (32), 340, (51), 211 (40), 209 (100); HREIMS (70 eV) *m/z* 376.2158 (Calcd for $C_{23}H_{33}O_2Cl$ 376.2169).

8β, 13β-dihydroxy-13α-3′-chlorophenylpodocarpane (**17**): White needles from MeOH (54% yield), m.p. 199–201 °C; $[\alpha]_D^{23}$ −6.3 (*c* 0.6, $CHCl_3$); IR (film) ν_{max} 3267 (br.), 2919, 1435, 1204, 772, 692; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.48 (t, *J* = 1.9 Hz, H-2′, 1H), 7.34 (dt, *J* = 7.9, 1.1 Hz, H-4′, 1H), 7.24 (t, *J* = 7.9, H-5′, 1H), 7.19 (ddd, *J* = 8.0, 1.9, 1.0 Hz, H-6′, 1H) 1.91 (m, H-12a, 1H), 1.89 (m, H-11a, 1H) 1.81 (m, H-12b, 1H), 1.77 (m, H-14a, 1H), 1.76 (m, H-1a, 1H), 1.74 (m,H-7a, 1H), 1.66 (m, H-14b, H-11b, 2H), 1.64 (s, H-2a, 1H), 1.54 (m, H_2-6, H-7b 3H), 1.44 (m, H-2b, 1H), 1.41 (m, H-3β, 1H), 1.16 (td, *J* = 14.4, 4.5 Hz, H-3α, 1H), 1.04 (s, H_3-17, 3H), 1.04 (m, H-9, 1H) 0.90 (m, H-5, 1H), 0.87 (m, H-1b, 1H), 0.87 (s, H_3-15, 3H), 0.85 (s, H_3-16, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 150.5 (C, C-1′), 134.1 (C, C-3′), 129.4 (CH, 5′), 126.7 (CH, 6′), 125.1 (CH, 2′), 122.7 (CH, 4′), 74.0 (C, C-13), 73.9 (C, C-8), 56.3 (CH, C-5), 56.1 (CH, C-9), 51.4 (CH_2, C-14), 42.1 (CH_2, C-3) 42.0 (CH_2, C-7), 39.8 (CH_2, C-12), 39.4 (CH_2, C-1), 37.2 (C, C-10), 33.5 (CH_3, C-15), 33.3 (C, C-4), 21.6 (CH_3, C-16), 18.3 (CH_2, C-2), 17.5 (CH_2, C-6), 16.8 (CH_2, C-11), 15.5 (CH_3, C-17); EIMS *m/z* (rel. int.) 376 [M$^+$] (9), 340 (100), 209, (79), 204 (68), 137 (60); HREIMS (70 eV) *m/z* 376.2148 (Calcd for $C_{23}H_{33}O_2Cl$ 376.2169).

8β, 13β-dihydroxy-13α-4′-fluorophenylpodocarpane (**18**): White needles from MeOH, (52% yield) m.p. 176–178 °C; $[\alpha]_D^{23}$ −105.6 (*c* 0.9, $CHCl_3$); IR (film) ν_{max} 3267 (br.), 2946, 1509, 1220, 824, 757; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43 (dd, $^3J_{H,H}$ = 9.2, $^4J_{F,H}$ = 5.5, H-2′ and H-6′, 2H), 7.00 (dd, $^3J_{H,H}$ = 9.2, $^3J_{F,H}$ = 8.8, H-3′ and H-5′, 2H), 1.94 (m, H-12a, 1H), 1.90 (m, H-11a, 1H), 1.84 (m, H-12b, 1H) 1.81 (m, H-14β, 1H), 1.77 (m, H-1a, 1H), 1.76 (m, H-7β (beta), 1H), 1.66 (m, 11b, 1H), 1.65 (m, H-14α, 1H), 1.64 (m, H-2a, 1H), 1.55 (m, H_2-6, H-3α, 3H), 1.43 (m, H-2b, 1H), 1.41 (m, H-7α, 1H), 1.16 (td, *J* = 13.6, 4.4 Hz, H-3β, 1H), 1.05 (s, H_3-17, 3H), 1.05 (m, H-9, 1H) 0.91 (m, H-5, 1H), 0.88 (m, H-1b, 1H), 0.88 (s, H_3-15, 3H), 0.85 (s, H_3-16, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 161.6 (C, d, $^1J_{F,C}$ = 245.4, C-4′), 144.2 (C, d, $^4J_{F,C}$ = 3.3, C-1′), 126.2 (CH, d, $^3J_{F,C}$ = 7.6, C-2′, C-6′), 114.1 (CH, d, $^2J_{F,C}$ = 20.8, C-3′, C-5), 74.0 (C, C-13), 73.8 (C, C-8), 56.4 (CH, C-5), 56.7 (CH, C-9), 51.7 (CH_2, C-14), 42.3 (CH_2, C-3) 42.1 (CH_2, C-7), 40.0 (CH_2, C-12), 39.5 (CH_2, C-1), 37.3 (C, C-10), 33.5 (CH_3, C-15), 33.3 (C, C-4), 21.7 (CH_3, C-16), 18.4 (CH_2, C-2), 17.6 (CH_2, C-6), 16.9 (CH_2, C-11), 15.5 (CH_3, C-17); EIMS *m/z* (rel. int.) 360 [M$^+$] (17), 342 (26), 324, (26), 193 (100), 123 (48); HREIMS (70 eV) *m/z* 360.2465 (Calcd for $C_{23}H_{34}O_2F$ 360.2462).

8β, 13β-dihydroxy-13α-4′-chloro-2′-methylphenylpodocarpane (**19**): White needles from MeOH, (36% yield) m.p. 207–209 °C; $[\alpha]_D^{23}$ −23.6 (*c* 0.4, $CHCl_3$); IR (film) ν_{max} 3260 (br.), 2924, 1481, 1201, 862, 759; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.29 (d, *J* = 8.3 Hz, H-6′, 1H), 7.12 (d, *J* = 2.2, H-3′, 1H), 7.08 (dd, *J* = 8.5, 2.2, H-5′, 1H), 2.57 (s, H-7′, 3H), 2.08 (dt, *J* = 9.6, 3.1 Hz, H-12a, 1H), 2.02 (dd, *J* = 14.2, 4.0 Hz, H-14β, 1H) 1.90 (m, H-11a and H-12b, 2H), 1.76 (m, H-1a and H-7a, 2H), 1.66 (m, H-11b and H-14α, 2H) 1.65 (m, H-2a, 1H), 1.55 (m, H-6, 2H), 1.44 (t, *J* = 3.3 Hz, H-2b, 1H), 1.40 (m, H-3α, 1H), 1.15 (td, *J* = 13.5, 4.6 Hz, H-3β, 1H), 1.05 (s, H_3-17, 3H), 1.00 (m, H-9, 1H) 0.89 (m, H-5, 1H), 0.87 (s, H_3-15, 3H), 0.86 (m, H-1b, 1H), 0.85 (s, H_3-16, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 143.4 (C, C-1′), 138.6 (C, C-2′), 132.5 (CH, C-3′), 132.4 (C, C-4′), 126.5 (CH, C-6′), 125.4 (CH, C-5′), 75.4 (C, C-13), 73.9 (C, C-8), 56.4 (CH, C-5), 56.3 (CH, C-9), 49.0 (CH_2, C-14), 42.3 (CH_2, C-7), 42.1 (CH_2, C-3), 39.5 (CH_2, C-1), 38.5 (CH_2, C-12), 37.3 (C, C-10), 33.5 (CH_3, C-15), 33.3 (C, C-4), 22.2 (CH_3, C-7′), 21.7 (CH_3, C-16), 18.4 (CH_2, C-2), 17.6 (CH_2, C-6), 16.8 (CH_2, C-11), 15.5 (CH_3, C-17); EIMS *m/z* (rel. int.) 390 [M$^+$] (16), 354 (52), 223, (100), 179 (32), 153 (46); HREIMS (70 eV) *m/z* 390.2336 (Calcd for $C_{24}H_{35}O_2Cl$ 390.2326).

X-ray analysis of **11:** Diffraction intensities were collected as described in ref. 14 from a specimen of dimensions 0.11 × 0.22 × 0.24 mm^3 using ϕ- and ω-scans of 1.00° on a Nonius Kappa CCD diffractometer, with the crystal cooled to 173(2) K in a nitrogen stream. Crystal data for **11**: $C_{17}H_{30}O_2$, *M* = 266.41, monoclinic, space group *I2* (alternative setting of *C2*, no. 5), *a* = 22.8374(7) Å, *b* = 5.9919(2) Å, *c* = 23.3653(9) Å, β = 104.124(2)°, *V* = 3100.6(2) Å^3, *Z* = 8, D_c = 1.141 Mg m^{-3}, μ(MoKα) = 0.072 mm^{-1}, *F*(000) = 1184. Of the 22594 reflections collected, 6341 were unique (R_{int} = 0.0356). The structure was solved by direct methods and refined on F^2 using all data, with non-hydrogen atoms treated anisotropically. All H atoms were located and then added in idealised positions in a riding model. The absolute structure was not reliably determined by the Flack parameter and the Friedel opposites were merged to yield 3492 reflections (2567 observed data, $I > 2\sigma(I)$). Final *R* factors were R_1 = 0.0705 (all data), 0.0411 (observed data), wR_2 = 0.0972 (all data), 0.0859 (observed data) for 353 parameters. Crystallographic data (excluding structure factors) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 756359).

X-ray analysis of **15**: Intensity data were collected on a Nonius Kappa CCD diffractometer with MoK$_\alpha$-radiation from a crystal of dimensions 0.18 × 0.20 × 0.23 mm^3 using ϕ- and ω-scans of 1.00°, with the crystal cooled to 173(2) K in a nitrogen stream. Crystal data for **15**: $C_{23}H_{34}O_2$, *M* = 342.50, orthorhombic, space group $P2_12_12_1$ (no. 19), *a* = 7.8239(3) Å, *b* = 10.2191(4) Å, *c* = 25.1312(6) Å, *V* = 2009.3(1) Å^3, *Z* = 4, D_c = 1.132 Mg/m^3, μ(MoKα) = 0.070 mm^{-1}, *F*(000) = 752. A total of 13133 reflections were collected, yielding 4083 data after merging equivalent reflections (R_{int} = 0.0302). The structure was solved by direct methods and refined on F^2 with non-hydrogen atoms treated anisotropically. All H atoms were located in difference electron density maps and added in idealised positions in a riding model. Since the Flack parameter did not indicate absolute structure reliably, further merging of the Friedel opposites followed and final refinement was based on the resulting 2349 reflections. Final *R* factors were R_1 = 0.0644 (all data), 0.0399 (1763 observed data, [$I > 2\sigma(I)$]), wR_2 = 0.1010 (all data), 0.0901 (observed data) for 231 parameters. Crystallographic data (excluding structure factors) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC 756358).

Anti-plasmodial screening: The chloroquine-resistant Gambian FCR-3 strain was cultured *in vitro* according to the method described by Jensen and Trager.[14] For experimental purposes the cultures were synchronized with 5% D-sorbitol when the parasites were in the ring.[15] The antiplasmodial activity of compounds **5** and **11**–**19** was

determined using the tritiated hypoxanthine incorporation assay.[16] The parasite suspension, consisting of predominately the ring stage, was adjusted to a 0.5% parasitaemia and 1% haematocrit and exposed to the various concentrations of compounds for a single cycle of parasite growth. All assays were carried out using untreated parasites and uninfected red blood cells as controls. Labelled ^{3}H-hypoxanthine (0.5 μCi/well) was added after 24 h and the cells harvested after a further 24 h incubation period. The concentration that inhibited 50% of parasite growth (IC_{50} value) was determined from the log sigmoid dose response curve generated by the Enzfitter® software. Chloroquine, quinine, primaquine, pyrimethamine and cycloproguanil were used as the reference antimalarial agents. All compounds were tested in at least three independent experiments and statistical analysis using the student t-test performed, where a p value of less than 0.05 was regarded as significant.

Haemolytic assay: Freshly obtained human whole blood was washed three times by centrifugation (1500 rpm for 5 min) in isotonic phosphate buffer saline (pH 7.2) and the buffy coat and plasma discarded. The pellet was resuspended in RPMI-1640 culture media, HEPES and glucose supplemented with 10% human plasma and $NaHCO_3$ (pH 7.4) to a final hematocrit of 1% (v/v). The compounds (50 and 100 μM) were incubated with the erythrocytes for 48 h at 37 °C. Thereafter, the suspension was centrifuged at 1500 rpm for 5 min at room temperature and the absorbance of the supernatant read at 412 nm. The observed haemolysis was expressed as a percentage of the positive control, 0.2% (v/v) Triton X-100.[17]

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