Abstract: The natural product quassin has been modified in order to produce compounds with potential antimalarial action. The modifications include demethylation, reduction of the keto function, esterification with simple organic acids and - to enhance the uptake through the biological barriers and increase the stability of the compounds - with liposomino acids. © 1998 Published by Elsevier Science Ltd. All rights reserved.

INTRODUCTION

The bitter degraded triterpenoid quassinoids have been identified as the active constituents of plants from the Simaroubaceae which are used pantropically to treat malaria, leukaemia, amoebiasis and other ailments.1,2 The work described in this paper is a continuation of our studies on the chemical modification of quassinoids as potential antimalarial agents.3,4

It has been demonstrated that quassinoids are potent inhibitors of protein synthesis and although the active site is not fully known it has been established that the state of oxidation and substitution in ring A, the presence of a C-8 methylene-oxygen bridge to C-11 or to C-13 and the nature of the C-15 substituent have marked effects on antiplasmodial activity.5 It has been suggested that an ester side chain may increase lipophilicity of the compound, thus aiding delivery to the site of action and that the branched or unsaturated nature of the ester moiety may be important for effective interaction or bonding at the site of action.6 The parent quassinoid, quassin 1 which is inactive against tumour cells and against plasmodia differs chemically from potent quassinoids such as brusatol 2. There are three major chemical differences between quassin and brusatol, which contains (i) an extra substituent at C-15, (ii) different substituents in ring A and (iii) a methylene-oxygen bridge in ring C. In order to investigate the effect on biological activity of introducing various substituents on the quassin molecule a series of quassin derivatives has been prepared.
DISCUSSION

To study the structure activity relationship of quassin analogues, different compounds were synthesised systematically varying the substituents at the C-2, C-12 and C-15 positions.

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\begin{align*}
3 & \quad R^1 \\
a & \quad H \\
b & \quad \text{COCH}_3 \\
c & \quad \text{COC}_6\text{H}_5 \\
d & \quad \text{COCH}=\text{C}(\text{CH}_3)_2 \\
e & \quad \text{COCH}_3\text{C}(\text{CH}_3)_3 \\
f & \quad \text{CO}(\text{CH}_3)_4\text{CH}_3 \\
g & \quad \text{CO}(\text{CH}_2)_{16}\text{CH}_3 \\
h & \quad \text{CH}_2\text{O}(\text{CH}_2)_{12}\text{OCH}_3 \\
i & \quad \text{COCH}_3[\text{NHOCOC}(\text{CH}_3)_3] \\
j & \quad \text{COCH}[\text{NHOCOC}(\text{CH}_3)_3]\text{CH}(\text{CH}_3)_3 \\
k & \quad \text{COCH}[\text{NHOCOC}(\text{CH}_3)_3]\text{CH}(\text{CH}_3)\text{C}_6\text{H}_5 \\
l & \quad \text{COCH}[\text{NHOCOC}(\text{CH}_3)_3]\text{CH}_2\text{C}_6\text{H}_5 \\
m & \quad \text{COCH}[\text{NHOCOC}(\text{CH}_3)_3](\text{CH}_2)_{12}\text{CH}_3 \\
n & \quad \text{COCH}[\text{NHOCOC}(\text{CH}_3)_3](\text{CH}_2)_{12}\text{CH}_3 \\
o & \quad \text{COCH}[\text{NHOCOC}(\text{CH}_3)_3](\text{CH}_2)_{17}\text{CH}_3 \\
p & \quad \text{COCH}[\text{NHOCOC}(\text{CH}_3)_3](\text{CH}_2)_{17}\text{CH}_3 \\
r & \quad \text{H} \\
s & \quad \text{COCH}_3 \\
t & \quad \text{COC}_6\text{H}_5 \\
 & \quad \text{R}^2
\end{align*}
\]

Modifications at C-2

For the preparation of C-2 modified quassin analogues, quassin was demethylated with 10% HCl in acetic acid to yield norquassin (3a). Norquassin (3a) was acylated with the corresponding acid, anhydride or acid chloride to yield the series of esters 3b-3g. The presence of methoxyethoxymethylether group in quassinoids was reported to enhance in vitro antimalarial activity. To examine the effect of the presence of methoxyethoxymethylether at C-2, compound 3h was prepared by treatment of compound 3a with methoxyethoxymethyl chloride. Amino acid esters 3i-3l were prepared from N-(tert.butoxycarbonyl)-glycine,-L-valine,-L-isoleucine and -L-phenylalanine respectively.

The lipoamino acids and their oligomers provide an excellent means of enhancing the lipophilicity of different conjugating compounds and also increase the biological stability of the drugs by protecting them from
enzymatic degradation. Lipophilic quassin analogues were prepared from lipoamino acids of increasing lipophilicity. Reaction of compound 3a with 2-tert.butoxycarbonylamino-decanoic, -dodecanoic, tetradecanoic and -eicosanoic acid, using standard solution phase peptide synthetic methods, afforded esters 3m-3p. Since the starting lipoamino acids were racemic, the compounds containing lipoamino acyl substituents were diastereomeric mixtures.

Modification at C-15

The synthesis of C-15 modified quassin analogues commenced with hydroxyquassin 4a, possessing a β-hydroxyl group at C-15, which was prepared and reported previously. Acylation of hydroxyquassin 4a with 3,3-dimethylacryloyl chloride resulted in compound 4b with the same C-15 substituent as brusatol. The C-15 acetylated product 4c was also prepared by reacting compound 4a with acetic anhydride. To enhance the lipophilicity, quassin conjugate 4d was prepared by reacting compound 4a with 2-(tertbutoxycarbonylamino)-decanoic acid.

Acylation of the OH at C-15 resulted approximately in a 0.5 ppm chemical shift movement downfield of the C-15 H signal in the ¹H-NMR of the compounds, clearly indicating the completion of acylation reactions. To examine the effect of the presence of methoxyethoxymethylether at C-15, compound 4e was prepared by treatment of compound 4a with methoxyethoxyethyl chloride.

Modification at C-2 and C-12

Compound 3 was reacted with BBr₃ resulting in production of diol 3r. Demethylation at C-12 of acetate 3b and benzoate 3c with BBr₃ provided C-12 hydroxy esters 3s and 3t respectively. The presence of a free hydroxyl group at C-12 in quassinoids was reported to enhance biological activity.
Modification on the C-2 and C-15

Further analogues of quassin 1 were prepared from the C-15 substituted compounds. Hydrolysis of the C-2 methoxy group in hydroxyquassin 4a was achieved with hydrochloric acid in acetic acid under reflux to provide norhydroxyquassin 4f. Compound 4f served as starting material for the preparation of C-2, and C-15 modified quassin analogues. When norhydroxyquassin 4f was treated with one equivalent of 2-(tert-butoxycarbonylamino)-decanoic acid monoester 4g containing the lipidic substituent on C-2 was obtained as the major product. This was confirmed by 1H NMR examination, the protons of compound 4g on C-3 and C-15 appeared as doublets at δ=6.13 ppm and 4.5 ppm respectively.

Norhydroxyquassin 4f provided diacrylate 4h on esterification with an excess of 3,3-dimethylacryloyl chloride. In the 1H NMR spectrum of compound 4h the vinylic proton at C-3 had moved down field to δ=6.1 ppm from 5.7 ppm of 4f. A similar trend was observed in the case of the proton at C-15, a shift from δ=4.5 ppm to 5.1 ppm was observed (see Experimental).

Modifications at C-2, C-12 and C-15

Exposure of ester 4b to BBr3 in dichloromethane at -78 °C hydrolysed the C-2 and C-12 methoxy groups to provide diol 4i. Compound 4f was reacted with BBr3 resulting in formation of the 2,12,15-triol 4j. Triol 4j generated the C-2 monoester 4k, by treatment with one equivalent of 2-(tert-butoxycarbonylamino)-decanoic acid, indicating that position C-2 was the most reactive. The substituents at C-12 and C-15 remained unreactive. The positive reaction observed with ferric chloride (on thin layer chromatography plate) indicated the presence of a diosphenol group in the molecule and the 1H NMR spectrum showed a doublet at δ=4.5 ppm for H-15. However, when compound 4j was reacted with excess of acetic anhydride, hexanoic anhydride and stearic acid, triacetate 4l, trihexanoate 4m and tristearate 4n were formed respectively.

Further modifications at C-15

Diacrylate 4h and esters 3j and 30 were reduced with sodium borohydride in ethanol at room temperature to yield diastereomeric hemiacetals 5, 6a and 6b respectively.

Synthesis of Glaucarubinone analogues

Conversion of quassin 1 into the corresponding lactol with sodium borohydride followed by treatment with concentrated HCl in methanol at room temperature provided the methyl acetal 7 (scheme 1). Transformation of compound 7 and 9 was carried out according to the method of Nakamura et al. The carbonyl at C-1 was reduced with sodium borohydride in the presence of CeCl3 in methanol at -10 °C to give enol ether 8. Hydrolysis of the C-2 methoxy group in compound 8 proceeded smoothly with pyridinium p-toluene sulphonate in aqueous acetone to afford α-ketol 9. Compound 9 was acetylated with acetic anhydride and dimethylaminopyridine (DMAP) in CH₂Cl₂ affording acetate 10. The lactone in ring D was generated in two steps. Deprotection of acetate 10 with 10 % HCl
Scheme 1. Synthesis of glaucarubinone analogues. i) NaBH₄, ii) HCl, iii) NaBH₄, CeCl₃, MeOH, iv) pyridinium p-toluene sulphonate in acetone, v) Ac₂O, DMAP, vi) 10 % HCl, THF, vii) PPC, CH₂Cl₂

in THF furnished lactol 11 which was transformed into lactone 12 with pyridinium chlorochromate (PCC) in CH₂Cl₂. The double bond between C-3 and C-4 was introduced by the following procedure. The lactol in compound 11 was protected by treatment with concentrated HCl in ethanol resulting in compound 13. Bromination of compound 13 with phenyltrimethylammonium tribromide (PTAT) in THF at 0 °C gave rise to the C-3 brominated product 14. Dehydrobromination of compound 14 with LiCO₃-LiBr in dimethylformamide furnished the glaucarubinone analogue 15 (scheme 2). Compound 15 was fully characterised by ¹H NMR (see Experimental). The in vitro antimalarial activities of these compounds will be reported elsewhere.
EXPERIMENTAL

UV Spectra were recorded on a Perkin-Elmer 402 Ultraviolet-Visible Spectrophotometer using spectroscopic grade methanol. ¹H NMR spectra (CDCl₃) were recorded on a Bruker WM 250 spectrometer, or Bruker AMX 400, or Bruker AM 500 spectrometer. Electron Impact (EI) mass spectra were recorded on a VG Analytical LTD ZAB IF Spectrophotometer. Fast atom bombardment (FAB) spectra were recorded on a VG analytical ZAB-SE spectrometer; samples were dissolved in a 2-nitrobenzyl alcohol plus sodium iodide matrix (MNOBA + NaI) unless otherwise stated. High resolution MS (M+H or M+Na). Thin layer chromatography analysis were performed on Merck aluminium backed precoated thin layer Kiesel gel 60 F₂₅₄ plates (0.25mm thick). Chromatograms were visualised by spraying with p-anisaldehyde solution (135 ml of ethanol, 5 ml of concentrated H₂SO₄, 1.5 ml acetic acid, 4 ml of p-anisaldehyde) and heating at 110°C. Column chromatography was carried out by flash technique using silica gel Sorbsil C 60-H (40-60µm) Rhone-Poulenc. The solvents were evaporated in vacuo.

Norquassin (3a): A mixture of quassin (1) (25 mg, 6.44 mmol), 10% HCl (15 ml), and glacial acetic acid (4 ml) was refluxed at 115°C for 2 h. The mixture was poured into water (15 ml), neutralised with 2 M NaOH, extracted with CHCl₃ (3x15 ml) and washed with brine. The organic layer was dried (MgSO₄), concentrated and the residue recrystallised from ethanol to yield 16 mg (69%) of norquassin (3a); grey-black with ferric chloride. MS (EI) m/z (%): 375 [M+H]+ (100), 360 (12), 331 (14), 313 (7), 181 (6). ¹H NMR: δ=5.75 (d, 1H, J=2.3 Hz, C-3 H), 5.6 (s, 1H, C-2 OH, exchangeable with D₂O), 4.3 (m, 1H, C-7 H), 3.7 (s, 3H, C-12 OCH₃), 3.0 (dd, J=6Hz and 18Hz 1H, C-15 H), 2.9 (s, 1H, C-9 H), 2.55 (m, 1H, C-4 H), 2.4 (dd, J=6Hz and 18Hz 1H, C-14 H), 2.2-1.5 (m, 3H, C-5 H, C-6 Hs, C-15 H), 1.85(s, 3H, C-13 CH₃), 1.6 (s, 3H, C-10 CH₃), 1.25 (s, 3H, C-8 CH₃), 1.1 (d, 3H, J=6.1 Hz, C-4 CH₃). RF=0.77; CHCl₃:MeOH 95:5 (v/v).

2-O-desmethyl-2-O-acetylnorquassin (3b): To a cooled solution of norquassin (3a) (93 mg, 0.249 mmol) and DMAP
(45 mg, 0.367 mmol) in CH₂Cl₂ (5 ml) acetyl chloride (0.1 ml) was added and the reaction mixture stirred at room temperature for 15 hours. The mixture was diluted with CH₂Cl₂ (10 ml) washed with saturated aqueous NaHCO₃ (10 ml) and brine. The organic layer was dried (MgSO₄) and concentrated. The residue was purified by tlc (CHCl₃:MeOH 95:5) to obtain 49 mg (41 %) of 3b as a white solid. MS (FAB) m/z(%): 438 [M+23]+ (100), 351 (30), 172 (14), 107 (14). ¹H NMR (B): δ= 6.22 (d, 1H J=2.3 Hz, C-3H), 4.3 (m, 1H, C-7H), 3.63 (s, 3H, C-12 OCH₃), 3.1-2.95 (m, 1H, C-15), 3.0 (s, 1H, C-9 H), 2.6 (m, 1H, C-4), 2.5 (m, 1H, C-15 H), 2.35 (m, 1H, C-14 H), 2.2 (3H, s, CH₃CO), 2.2-1.95 (m, 3H, C-6 H, C-5 H), 1.85 (s, 3H, C-13 CH₃), 1.72 (s, 3H, C-10 CH₃) 1.23 (s, 3H, C-8 CH₃), 1.14 (d, 3H, J=6.1 Hz, C-4 CH₃).

2-O-desmethyl-2-O-benzoylquassin (3c): Compound 3c (46 mg, 39%) was prepared from norquassin (3a) and benzoylchloride using the method described for 3b. MS (FAB) m/z(%): 501 [M+23]+ (100), 413 (19), 393 (7), 301 (2), 176 (16), 149 (16), 105 (44), 91 (12). High resolution MS: Calculated for C₂₉H₃₀O₇Na(501.1889), Found=501.2029. ¹H NMR (B): δ=8.1-7.4 (m, 5H, aromatic-H's), 6.25 (d,1H J=2.3 Hz, C-3H), 4.3 (m, 1H, C-7H), 3.65 (s, 3H, C-12 OCH₃), 3.1-2.95 (m, 1H, C-15), 3.0 (s, 1H, C-9 H), 2.6 (m, 1H, C-4), 2.5 (m,1H, C-15 H), 2.4 (m, 1H, C-14 H), 2.2-1.95 (m, 3H, C-6 H, C-5 H), 1.85 (s, 3H, C-13 CH₃), 1.75 (s, 3H, C-10 CH₃) 1.25 (s, 3H, C-8 CH₃), 1.15 (d, 3H, J=6.1 Hz, C-4 CH₃).

2-O-desmethyl-2-O-(3,3-dimethylacryloyl)quassin (3d): Compound 3d (16.3 mg, 67%) was prepared from norquassin (3a) and 3,3-dimethylacryloyl-cWoride using the method described for 3b. Purification: CHCl₃-MeOH 98:2 (v/v). MS (EI) m/z(%): 456[M]+ (30), 374 (62), 359 (14), 346 (13), 331 (14), 315 (11), 303 (9), 287 (15), 271 (14), 243 (16), 223 (21), 205 (26), 189 (29), 165 (45), 149 (97), 137 (47), 123 (53), 105 (77), 91 (100). ¹H NMR:

2-O-desmethyl-2-O-tert-butylacetylquassin (3e): To a mixture of norquassin (3a) (30 mg, 0.0802 mmol), DMAP (14.3 mg, 0.098 mmol), EDC (22 mg, 0.115 mmol) in CH₂Cl₂ (5 ml) tert-butylacetic acid (0.1 ml) was added and the reaction mixture stirred for 1.5 hours at room temperature. The mixture was diluted with CH₂Cl₂ (15 ml), poured into saturated aqueous NaHCO₃ (10 ml), washed with brine, the organic phase dried (MgSO₄) and concentrated. The crude product was purified by tlc (CHCl₃:MeOH 95:5 v/v, RF=0.82) to obtain 16.5 mg, (44 %) of compound. MS (EI) m/z(%): 472[M]+ (12), 457 (6), 374 (43), 359 (12), 346 (13), 331 (19), 314 (8), 287 (6), 262 (6), 223 (7), 165 (9), 151 (15), 100 (42), 91 (41), 83 (24), 69 (78), 57 (100). High resolution MS: Calculated for C₂₇H₃₆O₇Na (495.2359), Found=495.2886. ¹H NMR (B): δ=6.05 (d, 1H J=2.3 Hz, C-3 H), 4.25 (m, 1H, C-7 H), 3.65 (s, 3H, C-12 OCH₃), 3.05 (m, 1H, C-9 H), 2.95 (s, 1H, C-9 H), 2.6 (m, 2H, C-4 H, C-14 H), 2.45-2.4 (m,3H, C-14 H, α-CH₃), 2.05-1.90 (m, 3H, C-6 H, C-5 H), 1.85 (s, 3H, C-13 CH₃), 1.6 (s, 3H, C-10 CH₃), 1.2 (s, 1H, C-8 CH₃), 1.1 (d, 3H, J=6.1 Hz, C-4 CH₃).

2-O-desmethyl-2-O-hexylquassin (3f)

To a cooled (-5 °C) solution of norquassin (3a) (20 mg, 0.053 mmol) and DMAP (22 mg, 0.115 mmol) in CH₂Cl₂ (5 ml) tert-butylacetic acid (0.1 ml) was added and the reaction mixture stirred for 1.5 hours at room temperature. The mixture was diluted with CH₂Cl₂ (15 ml), washed with brine, the organic phase dried (Na₂SO₄) and concentrated in vacuo. Chromatography of the crude product on a preparative TLC plate (SiO₂, CHCl₃:MeOH 95:5) yielded 8.6 mg (33%)
of hexanoate 3f. MS (FAB) m/z (%) = 472 [M+Na]+ (41), 458 (10), 388 (11), 374 (100) 359 (58), 346 (68), 331 (50), 315 (39), 287 (29), 262 (40), 245 (18), 223 (47), 203 (20), 179 (26), 152 (88), 137 (32), 99 (98). 1H NMR (B): δ=6.1 (d, 1H, J=2.3 Hz, C-3 H), 4.3 (m, 1H, C-7 H), 3.67 (s, 3H, C-12 OCH3), 3.05 (m, 1H, C-15 H), 2.96 (s, 1H, C-9 H), 2.60-2.40 (m, 4H, α-CH2, C-4 H, C-14 H), 1.87 (s, 3H, C-13 CH3), 1.67 (s, 3H, C-10 CH3), 1.33 (m, 6H, 3 CH2), 1.17 (s, 3H, C-8 CH3), 1.14 (d, 3H, J=6.1 Hz, C-4 CH3), 0.89 (m, 3H, CH3). Rf=0.66 (CHCl3:MeOH 95:5 v/v).

2-O-desmethyl-2-O-stearoylnorquassin (3g) Compound 3g (17.6 mg, 32 %) was prepared from norquassin (3a) and stearic acid using the method described for 3e. MS (FAB) m/z (%)= 663 [M+Na]+ (88), 635 (100), 605 (5), 451 (6). 1H NMR: δ=6.10 (d, 1H, J=2.3 Hz, C-3 H), 4.3 (m, 1H, C-7 H), 3.65 (s, 3H, C-12 OCH3), 3.05 (m, 1H, C-15 H), 2.95 (s, 1H, C-9 H), 2.6-2.35 (m, 5H, α-CH2, C-4 H, C-14 H, C-15 H), 2.1 (m, 2H), 1.85 (s, 3H, C-13 CH3), 1.65 (s, 3H, C-10 CH3), 1.3 (32H, m, 16 CH2), 1.2 (s, 3H, C-8 CH3), 1.15 (d, 3H, J=6.1 Hz, C-4 CH3), 0.85 (m, 3H, CH3). Rf=0.73; CHCl3:MeOH; 95:5 v/v.

2-O-desmethyl-2-O-(methoxyethoxymethyl)quassin (3h) Compound 3h (27.4 mg, 44 %) was prepared from norquassin (3a) and MEMCl using the method described for 3b. MS (FAB) m/z (%)= 485 [M+23]+ (28), 413 (40), 385 (9), 353 (8), 329 (7), 301 (10), 277 (10), 247 (24), 217 (17), 199 (11), 176 (100), 149 (56), 107 (30), 95 (48). 1H NMR (A): δ=5.85 (d, 1H, J=2.4 Hz, C-3 H), 4.8 (m, 2H, αCH), 4.4 (m, 1H, C-7 H), 3.55 (s, 3H, C-12 OCH3), 3.4 (s, 3H, OCH3), 3.1 (m, 1H, C-15 H), 2.6-2.4 (m, 2H, C-4 H, C-14 H), 2.25-1.5 (m, 2H, C-6 H, C-5 H), 1.85 (s, 3H, C-13 CH3), 1.55 (s, 3H, C-10 CH3), 1.25 (s, 3H, C-8 CH3), 1.15 (d, 3H, J=6.1 Hz, C-4 CH3).

2-O-desmethyl-2-O-[2-(tert-butoxycarbonylamino)acetyl]quassin (3i) Compound 3i (37 mg, 33 %) was prepared from norquassin (3a) and N-(tert-butoxycarbonyl)glycine using the method described for 3e. MS (FAB) m/z (%)= 554 [M+23]+ (100), 498 (69), 454 (5), 419 (6), 395 (6), 176 (16). High resolution MS: Calculated for C30H46O9NNa (554.2366), Found=554.3176 1H NMR (A): δ=6.15 (d, 1H, J=2.4 Hz, C-3 H), 5.15 (m, 1H, NH), 4.3 (m, 1H, C-7 H), 4.15-4.0 (m, 2H, αCH2), 3.65 (s, 3H, C-12 OCH3), 3.1 (m, 1H, C-15 H), 2.95 (s, 1H, C-9 H), 2.6-2.4 (m, 2H, C-4 H, C-14 H), 2.1-2.0 (m, 3H, C-6 H, C-5 H), 1.9 (s, 3H, C-13 CH3), 1.65 (s, 3H, C-10 CH3), 1.45 (s, 9H, C(CH3)3), 1.25 (s, 3H, C-8 CH3), 1.15 (d, 3H, J=6.1 Hz, C-4 CH3).

2-O-desmethyl-2-O-[2-(tert-butoxycarbonylamo)-3-methylbutyl] quassin (3j) Compound 3j (27.2 mg, 44 %) was prepared from norquassin (3a) and N-(tert-butoxycarbonyl)-L-valine using the method described for 3e. MS (FAB) m/z (%)= 596 [M+23]+ (64), 580 (53), 540 (17), 524 (41), 502 (11), 485 (13), 440 (18), 413 (20), 379 (14), 358 (15), 329 (23), 289 (11), 223 (8). High resolution MS: Calculated for C31H41O9NNa (596.2836), Found=596.3767. 1H NMR (A): δ=6.13 (d, 1H, J=2.3 Hz, C-3 H), 5.05 (m, N-H), 4.4 (m, α-CH), 3.65 (s, C-12 OCH3), 2.96 (s, 1H, C-9 H), 1.87 (s, 3H, C-13 CH3), 1.65 (s, 3H, C-10 CH3), 1.45 [s, 9H, C(CH3)3], 1.22 (s, 3H, C-8 CH3), 1.15 (d, 3H, J=6.1 Hz, C-4 CH3), 1.04 (d, CH3), 0.96 (m, 3H, CH3). Rf=0.88; CHCl3:MeOH; 95:5 v/v.

2-O-desmethyl-2-O-[2-(tert-butoxycarbonylamo)-3-methylpentyllox] quassin (3k) Compound 3k (37 mg, 32 mg, 42 %) was prepared from norquassin (3a) and N-(tert-butoxycarbonyl)-L-isoleucine using the method described for 3e. MS (FAB) m/z (%)= 610 [M+23]+ (10), 554 (29), 488 (4), 451 (3), 397 (23), 276 (7), 202 (7), 176 (24), 136 (11). 1H NMR: δ=6.1 (d, 1H, J=2.3 Hz, C-3 H), 5.28 (m, 1H, N-H), 4.25 (m, 2H, αCH2, C-7 H), 3.60 (s, 3H, C-12 OCH3), 2.95 (m, 1H, C-15 H), 2.94 (s, 1H, C-9 H), 2.49-2.45 (m, 2H, C-4 H, C-14H), 1.85 (s, 3H, C-13 CH3), 1.52
(s, 3H, C-10 CH$_3$), 1.16 (s, 3H, C-8 CH$_3$), 1.12 [s, 9H, C(CH$_3$)$_3$], 1.08 (d, 3H, C-4 CH$_3$), 1.1 (d, 3H, J=6.1 Hz, CH$_3$), 0.96 (m, 3H, CH$_3$).

2-O-desmethyl-2-O-[2-(tert-butoxycarbonylamino)-3-benzyl propyloxy]quassin (3i) : Compound 3i (21 mg, 25 %) was prepared from norquassin (3a) and N-(tert-butoxycarbonyl)-L-phenylalanine using the method described for 3e. MS (FAB) m/z (%)=644 [M+Na] (96), 588 (24), 561 (9), 522 (3), 439 (23), 411 (100), 381 (9), 316 (13), 236 (24), 176 (59), 120 (87). $^1$H NMR (B): δ=7.4-7.2 (m, 5H, aromatic-H's), 6.0 (d, 1H, J=2.3 Hz, C-3 H), 5.35 (m, 1H, N-H), 4.25 (m, 2H, aCH$_2$), 3.75 (s, 3H, C-12 OCH$_3$), 3.0 (s, 1H, C-9 H), 2.6-2.35 (m, 3H, C-15 H, C-4 H, C-14 H), 2.2-1.5 (m, 3H, C-6 H, C-15 H), 1.88 [s, 9H, C(CH$_3$)$_3$], 1.56 (s, 3H, C-10 CH$_3$), 1.2 (s, 3H, C-8 CH$_3$), 1.11 (d, 3H, J=6.1 Hz, C-4 CH$_3$).

2-O-desmethyl-2-O-[2-(tert-butoxycarbonylamino)-decanoyloxy]quassin (3m) Compound 3m (33 mg, 36%) was prepared from norquassin (3a) and 2-(tert-butoxycarbonylamino)-decanoic acid using the method described for 3e. MS (FAB) m/z (%)=666 [M+23] (100), 610 (23), 545 (8), 142 (51). $^1$H NMR: δ=6.15 (d, 1H, J=2.3 Hz, C-3 H), 5.05 (m, 1H, N-H), 4.4 (m, 1H, a-CH), 3.65 (s, 3H, C-12 OCH$_3$), 2.95 (s, 1H, C-9 H), 2.6-2.55 (m, 2H, C-15 H, C-14 H), 2.1-1.6 (m, 3H, C-6 H, C-5 H), 1.85 (s, 3H, C-13 CH$_3$), 1.65 (s, 3H, C-10 CH$_3$), 1.45 [s, 9H, C(CH$_3$)$_3$], 1.25 (m, 14 H, 7 CH$_2$), 1.20 (s, 3H, C-8 CH$_3$), 1.15 (d, 3H, J=6.1 Hz, C-4 CH$_3$), 0.85 (m, CH$_3$). IR: $3300, 2900, 2800, 1700, 1660, 1620$ cm$^{-1}$.

2-O-desmethyl-2-O-[2-(tert-butoxycarbonylamino)-tetradecanoyloxy]quassin (3n): Compound 3n (24.1 mg, 45%) was prepared from norquassin (3a) and 2-(tert-butoxycarbonylamino)-tetradecanoic acid using the method described for 3e. MS (FAB) m/z (%)=695 [M+23$^+$Na] (100), 639 (47), 595 (4), 567 (2), 452 (3), 395 (10), 353 (4), 286 (5), 260 (10), 215 (8), 170 (49), 136 (7), 107 (6). High resolution MS: Calculated for C$_{38}$H$_{57}$O$_7$NNa (694.3931), Found=694.3758. $^1$H NMR (B): δ=6.15 (1H, J=2.3 Hz, C-3 H), 5.05 (m, 1H, N-H), 4.4 (m, 1H, a-CH), 4.25 (m, 1H, C-7 H), 3.15 (s, 3H, C-12 OCH$_3$), 2.95 (s, 1H, C-9 H), 2.55-2.35 (m, 3H, C-15 H, C-14 H, C-13 H), 2.1-1.6 (m, 3H, C-6 H, C-5 H), 1.85 (s, 3H, C-13 CH$_3$), 1.65 (s, 3H, C-10 CH$_3$), 1.45 [s, 9H, C(CH$_3$)$_3$], 1.25 (m, 18H, 9 CH$_2$), 1.20 (s, 3H, C-8 CH$_3$), 1.15 (d, 3H, J=6.1 Hz, C-4 CH$_3$), 0.85 (m, 3H, CH$_3$). R$_f$=0.91 (CHCl$_3$:MeOH 95:5 v/v).

2-O-desmethyl-2-O-[2-(tert-butoxycarbonylamino)-eicosanoyloxy]quassin (3p): Compound 3p (29.3 mg, 35%) was prepared from norquassin (3a) and 2-(tert-butoxycarbonylamino)-eicosanoic acid using the method described for 3e. MS (FAB) m/z (%)=807 [M+23$^+$H] (35), 667 (8), 623 (3), 558 (3), 452 (2), 395 (37), 316 (19), 273 (76), 243 (33), 199 (100), 137 (7), 95 (9). $^1$H NMR (B): δ=6.15 (d, 1H, J=2.3 Hz, C-3 H), 5.05 (m, 1H, N-H), 4.4 (m, 1H, a CH), 4.25 (m, 1H, C-7 H), 3.67 (s, 3H, C-12 OCH$_3$), 2.95 (s, 1H, C-9 H), 2.6-2.55 (m, 2H, C-15 H, C-4 H), 2.40 (dd, 1H, C-14 H, J=6Hz and 18 Hz), 2.1-1.5 (m, 2H, C-6 H, C-5 H), 1.85 (s, 3H, C-13 CH$_3$), 1.6 (s, 3H, C-10 CH$_3$), 1.45 [s, 9H, C(CH$_3$)$_3$], 1.25 (m, 22H, 11 CH$_2$), 1.2 (s, 3H, C-8 CH$_3$), 1.15 (d, 3H, J=6.1 Hz, C-4 CH$_3$), 0.85 (m, 3H, CH$_3$). IR: $3300, 2900, 2800, 1700, 1660, 1620$ cm$^{-1}$. 

2-O-desmethyl-2-O-[2-(tert-butoxycarbonylamino)-eicosanoyloxy]quassin (3p): Compound 3p (29.3 mg, 35%) was prepared from norquassin (3a) and 2-(tert-butoxycarbonylamino)-eicosanoic acid using the method described for 3e. MS (FAB) m/z (%)=807 [M+23$^+$H] (100), 751 (44), 707 (5), 608 (3), 510 (4), 451 (6), 395 (7), 283 (30), 223 (5), 176 (13). $^1$H NMR (B): δ=6.15 (d, 1H, J=2.3 Hz, C-3 H), 5.05 (t, 1H, N-H), 4.4 (m, 1H, aCH), 3.65 (s, 3H, C-12 OCH$_3$), 2.95 (s, 1H, C-9 H), 2.6-3.5 (m, C-4 H, C-14 H, C-15 H), 2.1-1.5 (m, 2H, C-6 H, C-5 H), 1.85 (s, 3H, C-13 CH$_3$), 1.65 (s, 3H, C-10 CH$_3$), 1.45 [s, 9H, C(CH$_3$)$_3$], 1.25 (m, 34H, 17 CH$_2$), 1.1 (s, 3H, C-8 CH$_3$), 1.1 (d, 3H, J=6.1 Hz, C-4 CH$_3$).
2,12-dihydroxypicrasa-2,12-diene-1,11,16-trione (3r): To a solution of quassin (1) (39 mg, 1.01 mg) in dry CH₂Cl₂ (15 ml) a solution of 1 M BBr₃ in CH₂Cl₂ (4 ml) was added at -78 °C and the reaction mixture stirred for 20 min. The mixture was poured into water (20 ml) and the organic layer separated. The aqueous layer was extracted with CH₂Cl₂ (2x10 ml) and the combined organic extract washed with brine, dried (MgSO₄), and concentrated. Recrystallisation from ethanol afforded 15 mg, (41%) of compound 3r as colourless prisms, which gave a grey-black colour reaction with ferric chloride. MS (EI) m/z (%) = 360 [M]+ (100), 345 (86), 327 (10), 317 (27), 299 (24), 271 (17), 257 (16), 231 (16), 203 (19), 193 (26), 165 (35), 151 (35), 138 (39), 121 (22), 105 (24), 91 (42), 83 (57), 77 (25). ¹H NMR: δ=6.15 (s, 1H, C-2 OH), 5.75 (d, 1H, J=2.3 Hz, C-3 H), 4.35 (m, 1H, C-7 H), 2.95 (dd, J=6 Hz and 18 Hz, 1H, C-15 H), 2.55 (m, 1H, C-4 CH₃), 2.4 (dd, J=6 Hz and 18 Hz, 1H, C-14 H), 2.5-1.5 (m, 4H, C-5 H, C-6 Hs, C-15 H), 1.85 (s, 3H, C-13 CH₃), 1.55 (s, 3H, C-10 CH₃), 1.2 (s, 3H, C-8 CH₃), 1.1 (d, 3H, J=6.1 Hz, C-4 CH₃). Rf=0.42 (CHCl₃:MeOH 95:5 v/v).

2-Acetyloxy-12-hydroxypicrasa-2,12-diene-1,11,16-trione (3s): 2-acetyloxyquassin (3b) was reacted as described for 3r to yield 14.6 mg (50%) 3s. MS (EI) m/z (%) = 402 [M]+ (22), 387 (11), 374 (12), 360 (100), 345 (87), 317 (34), 271 (15), 255 (13), 231 (16), 215 (10), 203 (12), 193 (20), 151 (21), 138 (25), 105 (17), 91 (37), 77 (27). ¹H NMR: δ=6.15 (d, 1H, J=2.4 Hz, C-3 H), 4.3 (m, 1H, C-7 H), 3.0 (s, 1H, C-9 H), 3.1 (m, 1H, C-15 H), 2.5 (m, 1H, C-4 H), 2.2 (s, COCH₃), 1.9 (s, 3H, C-13 CH₃), 1.65 (s, 3H, C-10 CH₃), 1.3 (s, 3H, C-8 CH₃), 1.1 (d, 3H, J=6.1 Hz, C-4 CH₃).

2-benzoyl-12-hydroxypicrasa-2,12-diene-1,11,16-trione (3t): Compound 3c was reacted as described for 3r to yield 14.8 mg (38%) 3t. MS (EI) m/z (%) = 464 [M]+ (100), 449 (5), 378 (5), 360 (100), 345 (97), 327 (15), 317 (53), 299 (47), 271 (28), 255 (26), 239 (33), 215 (28), 203 (33), 195 (74), 175 (35). ¹H NMR: δ=8.13-7.48 (m, 5H, aromatic-H's), 6.25 (d, 1H, J=2.3 Hz, C-3 H), 5.74 (m, 1H, C-12 OH), 4.2 (m, 1H, C-7 H), 3.03 (s, 1H, C-9 H), 2.50-2.40 (m, 2H, C-4/C-14 H), 1.88 (s, 3H, C-13 CH₃), 1.70 (s, 3H, C-10 CH₃), 1.25 (s, 3H, C-8 CH₃), 1.1 (d, 3H, J=6.1 Hz, C-4 CH₃).

ISP-(3,4-dimethylacryloxy)quassin (4b): Hydroxyquassin (4a) and 3,3-dimethylacryloyl chloride was reacted as described for 3b to afford 14 mg (58%) of 4b. High resolution MS: Calculated for C₁₇H₂₃O₇S (486.2254), Found=486.2250. ¹H NMR (B): δ=5.79 (s, 1H, C-2' H), 5.3 (d, 1H, J=2.4 Hz, C-3 H), 5.16 (d, 1H, J=10.2 Hz, C-15 H), 4.5 (m, 1H, C-7 H), 3.69 (s, 3H, C-12 OCH₃), 3.59 (s, 3H, C-2 OCH₃), 3.05 (s, 1H, C-9 H), 2.39 (d, 1H, J=10.2 Hz, C-14 H), 2.48 (m, 1H, C-4 H), 2.21 (s, 3H, C-5'CH₃), 1.96 (s, 3H, C-4' CH₃), 1.92 (s, 3H, C-13 CH₃), 1.54 (s, 3H, C-10 CH₃), 1.21 (s, 3H, C-8 CH₃), 1.12 (d, 3H, J=6.1 Hz, C-4 CH₃). Rf=0.89 (CHCl₃:MeOH 95:5 v/v).

ISP-acetyloxyquassin (4c): Hydroxyquassin (4a) and acetic anhydride was reacted as described for 3b to afford 14 mg (61%) of 4c. High resolution MS: Calculated for C₂₄H₃₀O₁₀ (466.1941), Found=466.1947. MS (EI) m/z (%) = 446 [M]+ (8), 404 (4), 386 (94), 371 (86), 353 (25), 343 (31), 329 (19), 315 (17), 301 (18), 283 (15), 269 (15), 255 (14), 205 (25), 165 (46), 151 (28), 121 (29), 105 (34), 94 (100), 77 (54). ¹H NMR: δ=5.35 (d, 1H, J=2.5 Hz, C-3 H), 5.25 (d, 1H, J=10.5 Hz, C-15 H), 4.5 (m, 1H, C-7 H), 3.7 (s, 3H, C-12 OCH₃), 3.6 (s, 3H, C-2 OCH₃), 3.1 (s, 1H, C-9 H), 2.6 (d, 1H, J=10.5 Hz, C-14 H), 2.45 (m, 1H, C-4 H), 2.2 (s, 3H, COCH₃), 2.15-1.6 (m, 3 H,
C-5 H, C-6 Hs), 1.95 (s, 3H, C-13 CH), 1.5 (s, 3H, C-10 CH), 1.2 (s, 3H, C-8 CH), 1.1 (d, 3H, J=6.1 Hz, C-4 CH). Rf=0.62 (CHCl₃:MeOH 95:5 v/v).

15β-[2-tert butoxycarbonylamino]-decanoxy]quassin (4d): Compound 4d (22 mg, (33%) was prepared from hydroxyquassin (4a) and N-(tert-butoxycarbonyl-arnino)-decanoic acid- using the method described for 3e. MS (FAB) m/z (%)=696 [M+Na]⁺ (45), 640 (50), 425 (11), 395 (20), 332 (14), 186 (21), 142 (100). ¹H NMR (A): δ=5.3 (d, 1H, J=2.4 Hz, C-3 H), 5.0 (m, 1H, N-H), 4.95 (d, 1H, J=10.6 Hz, C-15 H), 4.55 (m, 1H, α-CH), 4.35 (m, 1H, C-7 H), 3.65 (s, 3H, C-12 OCH₃), 3.55 (s, 3H, C-2 OCH₃), 2.95 (s, 1H, C-9 H), 2.6 (d, 1H, J=10.6 Hz, C-14 H), 2.35 (m, 1H, C-4 H), 2.10-1.5 (m, 3H, C-5 H, C-6 H), 1.95 (s, 3H, C-13 CH), 1.5 (s, 3H, C-10 CH), 1.2 (s, 9H, CH(CH₃)₃), 1.2 (s, 14H, C-8 CH₂), 1.1 (s, 3H, C-8 CH₂), 1.25 (s, 3H, C-8 CH₂), 1.13 (d, 3H, J=6.1 Hz, C-4 CH₂). Rf=0.62 (CHCl₃:MeOH 95:5 v/v).

15β-[methoxyethoxymethoxy]quassin (4e): Hydroxyquassin (4a) and methoxyethoxymethyl chloride was reacted as described for 3b to afford 11.3 mg (46%) of 4e as a colourless oil. MS (EI) m/z (%)=493 [M]⁺ (26), 416 (26), 403 (75), 386 (37), 371 (18), 329 (100), 315 (17), 297 (17), 269 (32), 255 (24), 203 (31), 185 (35), 165 (76), 151 (39). ¹H NMR: δ=5.25 (d, 1H, J=2.4 Hz, C-3 H), 4.6 (m, 2H, CH₂), 4.5 (d, 1H, J=10.6 Hz, C-15 H), 3.8 (m, 2H, CH₂), 3.7 (m, 2H, CH₂), 3.65 (s, 3H, C-12 OCH₃), 3.59 (s, 3H, C-2 OCH₃), 3.4 (s, 3H, OCH₃), 3.06 (s, 1H, C-13 CH), 2.50 (m, 1H, C-4 H), 2.35 (d, 1H, J=10.6 Hz, C-14), 2.08 (s, 3H, C-13 CH), 1.54 (s, 3H, C-10 CH), 1.25 (s, 3H, C-8 CH), 1.13 (d, 3H, J=6.1 Hz, C-4 CH). Rf=0.76 (CHCl₃:MeOH 95:5 v/v).

2,15β-dihydroxy-12-methoxypicrasa-2,12-diene-1,11,16-trione (4f): A mixture of hydroxyquassin 4a (50 mg, 1.24 mmol), 10% HCl (15 ml), and AcOH (4 ml) was refluxed for 2 hours. The mixture was neutralised with 2M NaOH at room temperature, extracted with CH₂Cl₂ (3x15 ml), washed with brine, dried (MgSO₄), and concentrated. The residue was purified by (CH₂Cl₂:MeOH 95:5 v/v) and recrystallised from ethanol to afford 35 mg (73%) of 4f as colourless prisms which gave a grey-black colour with ferric chloride. MS (EI) m/z (%)= 390 [M]⁺ (100), 376 (7), 361 (9), 316 (69), 301 (32), 283 (9), 273 (14), 245 (6), 231 (13), 203 (7), 165 (12), 151 (7), 137 (7), 83 (33), 69 (31). ¹H NMR (A): δ=5.7 (d, 1H, J=2.4 Hz, C-3 H), 5.5 (s, 1H, C-2 OH), 4.5 (d, 1H, J=10.6 Hz, C-15 H), 4.4 (m, 1H, C-7 H), 3.7 (s, 3H, C-12 OCH₃), 3.2 (s, 1H, C-9 H), 2.5 (m, 1H, C-4 H), 2.4 (d, 1H, J=10.6 Hz, C14 H), 2.1-1.5 (m, 3H, C-5, C-6 Hs), 2.1 (s, 3H, C-13 CH), 1.5 (s, 3H, C-10 CH₂), 1.2 (s, 3H, C-8 CH₂), 1.1 (d, 3H, J=6.1 Hz, C-4 CH₂). Rf=0.76 (CHCl₃:MeOH 95:5 v/v).

2-O-desmethyl-2-O-[2-tert butoxycarbonylamino]-decanoxy]hydroxyquassin (4g): Compound 4g (17 mg,21%) was prepared from 4f and N-(tert-butoxycarbonyl-arnino)-decanoic acid using the method described for 3e. The compound gave negative ferric chloride reaction. MS (FAB) m/z (%)=682 [M+23-1]⁺ (100), 626 (39), 582 (3), 413 (14), 258 (6), 232 (8), 176 (45), 142 (35). High resolution MS: Calculated for C₉₆H₂₃O₁₃Na (682.3567), Found=682.2808. ¹H NMR (B): δ=6.13 (d, 1H, J=2.3 Hz, C-3 H), 5.02 (m, 1H, N-H), 4.50 (d, 1H, J=10.7 Hz, C15H), 4.48 (m, 1H, C-2'H), 4.44 (m, 1H, C-7 H), 3.68 (s, 3H, C-12 OCH₃), 3.29 (bs, 1H, C-15 OH), 3.02 (s, 1H, C-9 H), 2.56 (m, 1H, C-4 H), 2.36 (d, 1H, J=10.7 Hz, C-14 H), 2.08 (s, C-13 CH₂), 1.92 (m, 3H, C-6 H, C-5 H), 1.43 [s, 9H, (CH₂)₉], 1.43 (s, 3H, C-10 CH₂), 1.25 (m, 14 H, 7 CH₂), 1.15 (d, 3H, J=6.1 Hz, C-4 CH₂), 0.86 (m, 3H, CH₃). Rf=0.75 (CHCl₃:MeOH 95:5 v/v).

2-O-desmethyl-2-O-(3,3-dimethylacryloyl)-15β-(3,3-dimethylacryloxy)quassin (4h): Norhydroxyquassin 4f and 3,3-dimethylacryloyl chloride were reacted as described for compound 3b to afford 30 mg (53%) of 4h. MS (CI)
2,12-dihydroxy-15β-(3,3-dimethylacryloxy)-picrasa-2,12-diene-1,11,16-trione (4i): Hydroxyquassin 4a and 3,3-dimethylacryloyl chloride were reacted as described for compound 3b to afford 5.6 mg (27%) of compound 4i, which gave a grey-black colour ferric chloride reaction. MS (FAB) m/z (%) = 481 [M+23+] (36), 413 (33), 383 (6), 357 (3), 326 (6), 301 (9), 287 (18), 273 (14), 259 (8), 231 (8), 215 (9), 192 (12), 165 (12), 151 (19), 137 (10), 105 (7), 91 (12), 77 (9), 69 (59), 55 (8). ^1H NMR (A): δ = 5.79 (s, 1H, C-2'H), 5.74 (m, 1H, C-3 H), 5.16 (d, 1H, J = 10.2 Hz, C-15 H), 4.52 (m, 1H, C-7 H), 3.11 (s, 1H, C-9 H), 2.70 (1H, m, C-4 H), 2.65 (d, 1H, J = 10.6 Hz, C-14H), 2.21 (s, 3H, C-5' CH3), 1.96 (s, 3H, C-4' CH3), 1.96 (s, 3H, C-13 CH3), 1.52 (s, 3H, C-10 CH3), 1.24 (s, 3H, C-8 CH3), 1.12 (d, 3H, J = 6.1 Hz, C-4 H).

2,12-di-O-desmethyl-15-hydroxyquassin (4j): Hydroxyquassin 4a was reacted as described for compound to give 30 mg (50%) of ferric chloride positive compound 4j. Recrystallisation from ethanol gave colourless prisms. MS (EI) m/z (%) = 376 [M+] (44), 361 (3), 330 (7), 315 (13), 301 (100), 287 (18), 273 (14), 259 (8), 231 (8), 215 (9), 192 (12), 165 (12), 151 (19), 137 (10), 105 (7), 91 (12), 77 (9), 69 (59), 55 (8). ^1H NMR (A): δ = 6.2 (s, 1H, C-12 OH), 5.7 (d, 1H, J = 2.4 Hz, C-3 H), 5.6 (s, 1H, C-2 OH), 4.5 (d, 1H, J = 10.2 Hz, C-15 H), 4.4 (m, 1H, C-7 H), 3.1 (s, 1H, C-9 H), 2.5 (m, 1H, C-4 H), 2.4 (d, 1H, J = 10.2 Hz, C-14 H), 2.2-1.8 (m, 3H, C-6 H, C-5 H), 2.1 (s, 3H, C-13 CH3), 1.5 (s, 3H, C-10 CH3), 1.2 (s, 3H, C-8 CH3), 1.1 (d, 3H, J = 6.1 Hz, C-4 CH3). U.V. (MeOH) λ = 274 nm.

2,12-di-O-desmethyl-2-O-[(2-tert-butoxycarbonylamino)-decanoyl]-15-hydroxyquassin (4k): 2,12,15-Trihydroxyquassin 4j and 2-(tert-butoxycarbonylamino)-decanoic acid were reacted as described for compound 3e to yield 12.6 mg (21%) of 4k. MS (FAB) m/z (%) = [668+23+] (37), 613 (13), 186 (10), 142 (100), 95 (36). High resolution MS: Calculated for C31H30O11Na (668.3411), Found = 668.3766. ^1H NMR (B): δ = 6.5 (d, 1H, J = 2.4 Hz, C-3 H), 6.15 (s, 1H, C-12 OH), 5.0 (m, 1H, N-H), 4.45 (d, 1-H, J = 10.7 Hz, C-15 H), 4.4 (m, 1H, C-2' H), 4.35 (m, 1H, C-7 H), 3.2 (s, 1H, C-15 OH), 3.1 (s, 1H, C-9 H), 2.6 (m, 1H, C-4 H), 2.38 (d, 1H, J = 10.7 Hz), 2.05 (s, 3H, C-13 CH3), 1.55 (s, 3H, C-10 CH3), 1.45 (m, 9H, C(CH3)2), 1.25 (m, 14H, CCH3), 1.15 (d, 3H, J = 6.1 Hz, C-4 CH3), 0.85 (m, 3H, CH3). Rf = 0.80 (CHCl3:MeOH 95:5 v/v).

2,12-di-O-desmethyl-2,12-di-O-acetyl-15-acetyloxyquassin (4l): Compound 4j was reacted with acetic anhydride as described for compound 3f to yield 21 mg (45%) of 4l. MS (Cl) m/z (%) = 525 [M+23+] (100), 503 [M'] (28), 478 (8), 460 (25), 443 (15), 418 (6), 391 (11), 343 (6), 279 (6), 198 (4), 151 (3), 120 (4). High resolution MS: Calculated for C33H30O11Na (525.1737), Found = 525.2489. ^1H NMR (B): δ = 6.1 (d, 1H, J = 2.4 Hz, C-3 H), 5.25 (d, 1H, J = 10.8 Hz, C-15 H), 4.5 (m, 1H, C-7 H), 3.1 (s, C-9 H), 2.7 (d, 1H, J = 10.8 Hz, C-14 H), 2.55 (t, 1H, C-4 H), 2.25 (s, 3H, C-12 COCH3), 2.2 (s, 3H, C-2 COCH3), 2.15 (s, 3H, C-15 COCH3), 2.1-1.85 (m, 3H, C-6 Hs, C-5 H), 1.85 (s, C-13 CH3), 1.6 (s, 3H, C-10 CH3), 1.4 (s, 3H, C-8 CH3), 1.15 (d, 3H, J = 6.1 Hz, C-CH3). Rf = 0.84 (CHCl3:MeOH 95:5 v/v).

2,12-di-O-desmethyl-2,12-di-O-hexanoyl-15-hexanoyloxyquassin (4m): Compound 4j was reacted with
hexanoic anhydride as described for compound 3f to afford 9.3 mg (21%) 4m. MS (FAB) m/z (%)=671 [M+1]+
(100), 646 (9), 605 (13), 573 (33), 559 (10), 527 (13), 489 (8), 463 (10), 391 (24), 359 (13), 302 (16), 279 (36), 206 (9). 1H NMR (A): δ=6.07 (d, 1H, J=2.4 Hz, C-3 H), 5.15 (d, 1H, J=10.7 Hz, C-15 H), 4.55 (m, 1H, C-7 H), 3.05 (s, 1H, C-9 H), 2.7 (d, 1H, J=10.7 Hz, C-14 H), 2.55-2.49 (m, 7H, C-4 H, CH2), 2.2-1.6 (m, 3H, C-6 H, C-5 H), 1.85 (s, 3H, C-13 CH3), 1.57 (s, 3H, C-10 CH3), 1.37 (s, 3H, C-8 CH3), 1.16 (d, 3H, J=6.1 Hz, C-4 CH3), 0.91 (m, 9H, 3 CH3).

2,12-di-O-desmethyl-2,12-di-O-stearoyl-15 stearoyloxyquassin (4n) Compound 4j was reacted with stearic acid as described for compound 3e to yield 18 mg (28%) of tristearate 4n). MS: (FAB) m/z (%)=1197 [M+23]+ (20), 1181 (14), 1169 (100), 1156 (18), 143 (98), 1128 (18), 1114 (63), 1097 (13), 1084 (13), 1068 (11), 1056 (17), 1042 (15), 1028 (14), 1014 (14). 1H NMR (B) 0=6.1 (d, 1H, J=2.3 Hz, C-3 H), 5.2 (d, 1H, J=10.6 Hz, C-15 H), 4.6 (m, 1H, C-16 H), 3.15 (s, 1H, C-9 H), 2.75 (d, 1H, J=10.6 Hz, C-14 H), 2.65-2.4 (m, 7H, C-4 H, CH2), 2.2-1.5 (m, 3H, C-6 H, C-5 H), 1.89 (s, C-13 CH3), 1.62 (s, 3H, C-10 CH3), 1.41 (s, C-8 CH3), 1.20 (d, 3H, J=6.1 Hz, C-4 CH3), 0.90 (m, 9H, 3 CH3). Rf=0.93 (CHCl3:MeOH 95:5 v/v).

2,15β-di(3,3-acryloxy)-16-hydroxy-12-methoxypiercasa-2,12-diene-1,11-dione (5): To a solution of 4h (8.6 mg, 0.015 mmol) in ethanol/ chloroform (2 ml, 11ml) NaBH4 (10 mg) was added and the reaction mixture stirred at room temperature for 30 min. The mixture was diluted with CHCl3 (5 ml), washed with brine, the organic phase dried (Na2SO4) and concentrated to yield 7.5 mg (87%) of 5 as a diastereomeric mixture. MS (FAB) m/z (%)=557 [M+1]+ (27), 541 (47), 499 (100), 484 (20), 457 (21), 415 (48), 391 (94), 373 (8), 355 (13), 331 (14), 289 (40), 279 (19), 269 (14), 241 (20), 219 (33). High resolution MS: Calculated for C31H40O9Na (579.2570), Found=579.3464. 1H NMR (B): δ=6.08 (d, 1H, J=2.4 Hz, C-3 H), 5.81 (s, 1H, C-2'H), 5.7 (s, 1H, C-2'H), 5.42 (m, 0.5H, C-16 H), 5.23 (dd, 0.5H, J=11.3 and 3.4 Hz, C-15 H), 5.07 (m, 0.5H, C-15 H), 4.6 (m, 0.5H, C-16 H), 4.04 (m, 0.5H, C-7 H), 3.65 (s, 3H, C-12 OCH3), 3.27 (s, 1H, C-9 H), 2.53 (d, 1H, J=11.3 Hz, C-14 H), 2.51 (m, 1H, C-4 H), 2.20 (s, 3H, C-5' CH3), 2.19 (s, 3H, C-5'CH3), 1.94 (s, 3H, C-4' CH3), 1.93 (s, 3H, C-4' CH3), 1.93 (s, 3H, C-13 H), 1.60 (s, 3H, C-10 CH3), 1.25 (s, 3H, C-8 CH3), 1.11 (d, 3H, J=6.1 Hz, C-4 CH3).

2-desmethyl-2-[2-(tert-butoxycarbonylamino)-3-methylbutyloxy]neoquassin (6a): Compound 3j was reduced using the procedure described for compound 5 to yield 10 mg (66%) of 6a as a diastereomeric mixture. MS (FAB) m/z (%)=599 [M+Na+1]+ (100), 581 (14), 542 (18), 526 (7), 458 (8), 377 (6), 329 (12), 272 (9), 207 (7), 176 (54), 154 (49), 136 (30), 116 (21). High resolution MS: Calculated for C31H45O9NNa (598.2992), Found=598.3852. 1H NMR (B): δ=6.03 (d, 1H, J=2.4 Hz, C-3 H), 5.81 (s, 1H, C-2'H), 5.7 (s, 1H, C-2'H), 5.42 (m, 0.5H, C-16 H), 5.23 (dd, 0.5H, J=11.3 and 3.4 Hz, C-15 H), 5.07 (m, 0.5H, C-15 H), 4.6 (m, 0.5H, C-16 H), 4.04 (m, 0.5H, C-7 H), 3.65 (s, 3H, C-12 OCH3), 3.27 (s, 1H, C-9 H), 2.53 (d, 1H, J=11.3 Hz, C-14 H), 2.51 (m, 1H, C-4 H), 2.20 (s, 3H, C-5' CH3), 2.19 (s, 3H, C-5'CH3), 1.94 (s, 3H, C-4' CH3), 1.93 (s, 3H, C-4' CH3), 1.93 (s, 3H, C-13 H), 1.60 (s, 3H, C-10 CH3), 1.25 (s, 3H, C-8 CH3), 1.11 (d, 3H, J=6.1 Hz, C-4 CH3). Rf=0.77 (CHCl3:MeOH 95:5 v/v).

2-desmethyl-2-[2-(tert-butoxycarbonylamino)tetradecanoyloxy]neoquassin (6b): Compound 30 was reduced using the procedure described for compound 5 to yield 11 mg (73%) of 6b. MS (FAB) m/z (%)=725 [M+23]+ (54), 707 (11), 669 (8), 651 (7), 599 (5), 594 (82), 316 (36), 272 (94), 242 (38), 198 (100), 176 (32), 154 (26), 154 (26), 136 (17). 1H NMR (B): δ=6.1 (d, 1H, J=2.4 Hz, C-3 H), 5.39 (m, 1H, C-16 H), 5.0 (m, 1H, NH), 4.4 (m, 1H, CH2), 3.9 (m, 1H, C-7 H), 3.7 (s, 3 H, C-12 OCH3), 3.13 (s, 1H, C-9 H), 2.51 (m, 1H, C-4 H), 1.85 (s, 3H, C-13 CH3),
2,12,16-trimethoxypicrasa-2,12-diene-1,11-dione (7): To a solution of quassin 1 (2.8 g) in abs. ethanol (400 ml) 1 equivalent of NaBH₄ was added and the mixture was stirred for 2.5 h at room temperature. A few drops of acetic acid was added to destroy NaB₄ and the solvent was evaporated to give a white residue. The residue was placed in water, extracted with CH₂Cl₂ (4x35 ml) and the combined organic extracts washed with brine (50 ml), dried (MgSO₄), and concentrated in vacuo to yield the hemiacetal, neoquassin which was used without further purification. To a solution of neoquassin (2.4 g, 6.15 mmol), in methanol (30 ml), cone. HCl (2 ml) was added and the reaction mixture stirred at room temperature for 5 h. The mixture was poured into a solution of saturated aqueous NaHCO₃ (30 ml) and extracted with CHCl₃ (3x25 ml). The combined organic extracts were washed with brine (20 ml), dried (Na₂SO₄) and concentrated. The residue was purified by tlc (ethyl acetate 100%) to afford 2.15g (86%) of 7. MS (El) m/z (%)=404 [M⁺] (100%), 389 (22), 372 (18), 357 (13), 329 (23), 302 (37), 212 (32), 152 (80), 127 (32), 105 (24), 91 (38), 69 (82), 55 (37). 'H NMR (A): δ=5.28 (d, 1H, J=2.4 Hz, C-3 H), 4.8 (m, 1H, C-16 H), 3.62 (m, 1H, C-7 H), 3.6 (s, 3H, OCH₃), 3.6 (s, 3H, C-2 OCH₃), 3.35 (s, C-16 OCH₃), 3.2 (s, 1H, C-9 H), 2.4 (m, 1H, C-4 H), 2.1-1.2 (m, 3H, C-6 Hs, C-5 H), 1.85 (s, 3H, C-13 CH₃), 1.55 (s, 3H, C-10 CH₃), 1.2 (d, 3H, J=6.1 Hz, C-4 CH₃), 1.15 (s, C-8 CH₃).

1β-hydroxy-2,12,16-trimethoxypicrasa-2,12-diene-11-one (8): To a solution of 7 (2.0 g, 4.95 mmol), in abs. ethanol, CeCl₃·7H₂O (1.9 mg, 5.09 mmol) was added and the reaction mixture stirred for 15 min at room temperature. The mixture was cooled (-5°C) and a solution of NaBH₄ (200 mg) in abs. ethanol (15 ml) was added and the mixture stirred for 1 h. The reaction was quenched with acetone and the solvents evaporated. The residue was dissolved in CHCl₃ (50 ml), washed with saturated NaHCO₃ (20 ml), brine (20 ml), dried (Na₂SO₄) and concentrated. The crude compound was purified by tlc (ethyl acetate: hexane 3:1) to yield 1.62 g (81%) of 8. MS (El) m/z (%)=406 [M⁺] (9), 391 (8), 375 (81), 359 (14), 343 (13), 327 (6), 315 (6), 299 (7), 212 (23), 179 (14), 165 (16), 152 (32), 94 (89), 69 (35), 55 (54), 43 (100). 'H NMR (500 MHz): δ=6.71 (s, 1H, OH), 4.83 (m, 1H, C-16 H), 4.51 (s, 1H, C-1 H), 4.03 (m, 1H, C-3 H), 3.65 (m, 1H, C-7 H), 3.63 (s, 3H, C-12 OCH₃), 3.35 (s, C-16 OCH₃), 3.38 (s, 3H, C-16 OCH₃), 2.89 (s, 1H, C-9 H), 2.36-2.15 (m, 3H,C-15 H, C-4 H, C-14 H), 2.04 (s, 3H, C-13 CH₃), 1.15 (s, 3H, C-10 OCH₃), 1.1 (s, 3H, C-8 CH₃), 1.01 (d, 3H, J=6.1 Hz, C-4 CH₃).

1β-hydroxy-12,16-dimethoxypicras-12-ene-2,11-dione (9): A mixture of 8 (800 mg, 1.97 mmol), and pyridinium toluene sulphonate (240 mg, 0.95 mmol) in actone (20 ml), and water (2.6 ml) was refluxed for 3 hours. The mixture was cooled and concentrated. The residue was dissolved in CHCl₃ (50 ml), washed with saturated NaHCO₃ (20 ml), brine (20 ml), dried (Na₂SO₄) and concentrated. Purification of the crude product by tlc (ethyl acetate-hexane 3:1) afforded 550 mg, (71%) of 9. MS (El) m/z (%)=392 [M⁺] (12), 360 (9), 331 (6), 317 (7), 287 (9), 245 (11), 212 (58), 179 (28), 165 (32), 152 (100), 137 (27), 105 (27), 91 (47), 77 (40), 69 (65), 55 (72), 43 (85), 29 (24). 'H NMR (500 MHz): δ=4.9 (m, 1H, C-16 H), 4.01 (s, 1H, C-1 H), 3.65 (m, 1H, C-7 H), 3.58 (s, 3H, C-12 OCH₃), 3.36 (s, 3H, C-16 OCH₃), 3.11 (s, 1H, C-9 H), 2.3-1.5 (m, 6H, C-3 H, C-4 H, C-5 H, C-6 H, C-14 H, C-15 H), 1.85 (s, C-13 CH₃), 1.04 (s, 3H, C-10 CH₃), 1.03 (s, 3H, C-8 CH₃), 1.01 (d, 3H, J=6.1 Hz, C-4 CH₃). IR: ν max =3400, 1730, 1700, 1670 cm⁻¹.

1β-Acetyloxy-12,16-dimethoxy-12-ene-11-dione (10): To a solution of 9 (500 mg, 1.27 mmol) and
DMAP (200 mg, 1.63 mmol) in CHCl₃ (5 ml), acetic anhydride (3 ml) was added and and the mixture stirred for 1 h at room temperature. The mixture was diluted with CH₂Cl₂ (10 ml), washed with saturated aqueous NaHCO₃ (10 ml), dried (Na₂SO₄) and concentrated. The residue was purified by tlc (ethyl acetate-hexane 3:1) to yield 306 mg (55%) of 10. MS (El) m/z (%)=434 [Mr (16), 404 (29), 392 (42), 374 (7), 302 (12), 271 (14), 212 (35), 179 (25), 165 (28), 152 (100), 137 (28), 121 (84), 105 (28), 91 (47), 83 (62), 69 (56), 55 (51). ¹H NMR (B): δ=5.1 (s, 1H, C-1 H), 4.9 (m, 1H, C-16 H), 3.65 (m, 1H, C-7 H), 3.5 (s, 3H, C-12 OCH), 3.0 (s, 1H, C-9 H), 2.5 (m, 1H, C-15 H), 2.3-1.5 (m, 6H, C-15 H, C-14 H, C-6 Hs, C-5 H, C-4 H), 2.2 (s, 3H, C-1 COCH₃), 1.75 (s, 3H, C-13 CH₃), 1.4 (s, 3H, C-10 CH₃), 1.1 (s, 3H, C-8 CH₃), 1.05 (d, 3H, J=6.1 Hz, C-4 CH₃).

1β-acetyloxy-16-hydroxy-12-methoxypicras-12-ene-2,11-dione (11): A solution of 10 (100 mg, 0.23 mmol) in THF (2 ml) and 10% HCl (2 ml) was stirred at room temperature for 10 h. The mixture was poured into a cool (0°C) solution of saturated NaHCO₃ (5 ml), and solid NaHCO₃ added until no bubbles were seen. The mixture was extracted with diethyl ether (3 x 10 ml), dried (Na₂SO₄), and concentrated. Purification of the residue by tlc (ethyl acetate-hexane 75:25) yielded 73 mg (75%) of 11. MS (FAB) m/z (%)=443 [M+N⁺]⁺ (83), 429 (58), 413 (75), 401 (27), 379 (33), 361 (7), 329 (87), 307 (21), 289 (26), 273 (7), 219 (50). ¹H NMR (B): δ=5.0 (s, 1H, C-1 H), 4.8 (m, 1H, C-16 H), 3.90 (m, 1H, C-7 H), 3.55 (s, 3H, C-12 OCH), 3.01 (dd, J=6 Hz and 18 Hz, 1H, C-15 H), 2.85 (s, 1H, C-9 H), 2.6-2.4 (m, 3H, C-4 H, C-14 H, C-15 H), 2.2-1.6 (m, 4H, C-6 Hs, C-15 H, C-3 H), 2.1 (s, 3H, C-1 COCH₃), 1.84 (s, 3H, C-13 CH₃), 1.43 (s, 3H, C-10 CH₃), 1.3 (s, 3H, C-8 CH₃), 1.0 (d, 3H, J=6.1 Hz, C-4 CH₃).

1β-acetyloxy-12-methoxypicras-12-ene-2,11,16-trione (12): To a solution of lactol 11 (73 mg, 0.174 mmol) in CH₂Cl₂ (4 ml) pyridinium chlorochromate (260 mg, 1.2 mmol) was added and mixture stirred at room temperature for 2 h. The reaction mixture was diluted with diethyl ether and filtered through a pad of flash silica gel. The filtrate was concentrated and the residue purified by column chromatography (100% CHCl₃) to give 32.2 mg (44%) of lactone 12. MS (FAB) m/z (%)=441 [M+23]+ (27), 413 (69), 391 (14), 329 (6), 259 (4), 219 (3), 176 (35), 149 (100). ¹H NMR (B): δ=5.1 (s, 1H, C-1 H), 4.3 (m, 1H, C-7 H), 3.55 (s, 3H, C-12 OCH), 3.01 (dd, J=6 Hz and 18 Hz, 1H, C-15 H), 2.85 (s, 1H, C-9 H), 2.6-2.4 (m, 3H, C-4 H, C-14 H, C-15 H), 2.2-1.6 (m, 4H, C-6 Hs, C-5 H, C-3 H), 2.1 (s, 3H, C-1 COCH₃), 1.84 (s, 3H, C-13 CH₃), 1.43 (s, 3H, C-10 CH₃), 1.17 (s, 3H, C-8 CH₃), 1.05 (d, 3H, J=6.1 Hz, C-4 CH₃).

1β-acetyloxy-16-ethoxy-12-methoxypicras-12-ene-2,11-dione (13): The compound was prepared by an identical way as compound 10, using ethanol for the introduction of C-16 ethoxy group. MS (El) m/z (%)=449 [M+H]+ (21), 435 (11), 421 (9), 407 (100), 393 (23), 375 (10), 361 (14), 345 (13), 317 (11), 287 (13), 274 (11), 245 (14), 217 (15), 207 (18). High resolution MS Calculated for C₂₅H₂₇O₇ (449.2539), Found=449.2535. ¹H NMR (B): δ=5.11 (s, 1H, C-1 H), 4.98 (m, 1H, C-16 H), 3.74 (m, 1H, C-16 OCH₂), 3.71 (m, 1H, C-7 H), 3.51 (s, 3H, C-12 OCH₂), 3.50 (m, 1H, C-16 OCH₂), 3.0 (s, 1H, C-9 H), 2.54 (m, 1H, C-14 H), 2.4-2.15 (m, 3H, C-15, C-6 Hs, C-5 H, C-3 H), 2.13 (s, 3H, C-1 COCH₃), 1.79 (s, 3H, C-13 H), 1.32 (s, 3H, C-10 CH₃), 1.25 (m, 3H, C-16 CH₃), 1.02 (s, 3H, C-8 CH₃), 1.01 (d, 3H, J=6.1 Hz, C-4 CH₃).

1β-acetyloxy-16-bromo-12-methoxypicras-12-ene-2,11-dione (14): To a cooled (-5°C) solution of 13 (34 mg, 0.076 mmol) in dry THF (5 ml) phenyl-trimethylammonium tribromide (PTAT, 30 mg, 0.0798) was added and the mixture was stirred for 1.5 h at this temperature. White crystals of phenyl trimethylammonium bromide precipitated at the bottom of the flask from the orange solution. The reaction mixture was poured into a mixture
of 0.1N Na₂S₂O₅, 5H₂O and saturated aqueous NaHCO₃ (10 ml 1:1) and extracted with ether (3x10 ml). The organic phase was washed with water (3x15 ml), brine (10 ml), dried (Na₂SO₄) and concentrated. Column chromatography of the residue (ethyl acetate-hexane 3:1) yielded 17 mg (42%) of 14. MS (EI) m/z (%)=528 [M+H]+ (23), 484 (42), 440 (100), 404 (35), 388 (17), 361 (67), 347 (17), 331 (13), 313 (7), 279 (9), 219 (8), 179 (18), 107 (4). 1H NMR: δ=5.90 (s, 1H, C-3 H), 5.0 (m, IH, C-1 H), 4.4 (m, 1H, C-16), 3.7 (m, 1H, C-7 H), 3.56 (m, 2H, C-16 OCH₂), 3.55 (s, 3H, C-12 OCH₃), 3.1 (s, IH, C-9 H), 2.55 (m, 1H, C-14 H), 2.2-1.5 (m, 6H, C-15 H₂, C-6 H₅, C-5 H), 2.15 (s, 3H, C-1 COCH₃), 1.8 (s, 3H, C-13 CH₃), 1.35 (s, 3H, C-10 CH₃), 1.1 (m, 3H, C-16 CH₃), 1.10 (d, 3H, J=6.1 Hz, C-4 CH₃), 1.05 (s, 3H, C-8 CH₃).

1β-acetyloxy-16-ethoxy-12-methoxypicrasa-3,12-diene-2,11-dione (15): To a solution of 14 (12 mg, 0.0227 mmol) in anhydrous DMF (5 ml), LiCO₃ (43 mg, 0.581 mmol) and LiBr (42 mg, 0.484 mmol) were added and the mixture refluxed at 115°C for 2 h. The mixture was cooled, poured into water (10 ml), extracted with ether (3x10 ml), washed with brine (10 ml), dried (Na₂SO₄) and concentrated. The residue was purified by column chromatography (ethylacetate:hexane 3:1) to afford 5.3 mg (52%) of 15. MS (EI) m/z (%)=447 [M+H]+ (24), 406 (100), 392 (17), 378 (6), 360 (7), 325 (5), 257 (3), 217 (4), 183 (13). High resulution MS calculated for C₂₅H₃₄O₇ (446.2305), Found= 446.2301. 1H NMR (B): δ=6.04 (m, 1H, C-3 H), 5.26 (s, 1H, C-1 H), 5.0 (m, 1H, C-16 H), 3.76 (m, 1H, C-7 H), 3.75-3.54 (m, 2H, C-16 OCH₂), 3.52 (s, 3H, C-12 OCH₃), 3.23 (m, 1H, C-5 H), 3.14 (s, 1H, C-9 H), 2.15 (s, 3H, C-1 COCH₃), 2.3-1.80 (m, 5H, C-15H₅s, C-14 H, C-6H₅s), 1.96 (s, 3H, C-4 CH₃), 1.81 (s, 3H, C-13 CH₃), 1.36 (s, 3H, C-10 CH₃), 1.25 (m, 3H, C-16 CH₃).

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REFERENCES