Synthesis of novel substituted α-methylamino derivatives of α-santonin as potential anticancer agents-Part 1: Eudesmanolide derivatives

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ABSTRACT

A series of eudesmanolide sesquiterpenoid structures incorporating the α-methylene-γ-lactone moiety or its amino conjugates was synthesised from α-santonin and screened against lymphoblastic leukemia, promyelocytic leukemia and colorectal cancer cell lines. The α-methylene group or the amino conjugate and at least one of the double bonds of the dienone of the santonin parent are prerequisites for activity. Most of the amino adducts showed activity equal to or poorer than the parent α-methylene-γ-lactones against the colorectal cancer cell line, but several examples exhibited improved or similar activity against the promyelocytic leukemia cell line with improved toxicity profiles against non-cancerous, rapidly dividing cells (as measured by activity against WI38 fibroblasts). Enhanced activity was observed against the lymphoblastic leukaemia cell line with improved toxicity profiles (WI38 fibroblasts) when compared to the parent α-methylene-γ-lactone. The 2-fluorobenzyl adduct (8p), with IC_{50} = 7.4µM, 5.6µM and 56µM against promyelocytic, lymphoblastic leukaemia and WI38 fibroblasts respectively, showed both improved potency and leukemia selectivity compared with the α-methylene-γ-lactone parent (7). Analogues with small aliphatic amino substitution such as dimethylamino (8a) showed selectivity towards promyelocytic leukemia over all other cell lines examined and useful toxicity profiles [IC_{50}(HL60) = 6.3µM, IC_{50}(WI38) = 66µM].

Keywords: α-Santonin, Sesquiterpene lactones, α-Methylene-γ-lactone, Eudesmanolides, Exocyclic amines, Cytotoxicity
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INTRODUCTION

Sesquiterpene lactones are a rich source of chemical diversity for the investigation of natural product-derived biological activity, with a vast array of known effects on living systems [1]. These secondary metabolites, products of the isoprenoid pathway, tend to be highly lipophilic and, as a result, suffer from poor bioavailability due to their generally low aqueous solubility. Strategies to partially address this situation involve, among others, adding a hydrophilic group to the parent structure, usually in the form of an amino moiety (**Figure 1**) [2,3]. This strategy has been successfully applied, for example, to parthenolide (**1**), a germacranolide sesquiterpenoid active against human B-chronic lymphocytic leukaemia and acute myeloid leukaemia (AML) [2,4]. This initial amino conjugate series of (**1**), derived from a range of (hetero)aryl- and alkylamines, yielded a tyramine adduct as a selective nanomolar inhibitor of human Caucasian acute lymphoblastic leukaemia [GI_{50}, TGI and IC_{50} all under 0.01µM against CCRF-CEM cells, although no other adducts were reported within 1000 fold of that potency in the study] [2]. Further

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refinement and optimisation led to the dimethylamino adduct (**2**) at the exocyclic double bond of parthenolide as the compound with the best bioavailability and potency characteristics [IC_{50}(AML) = 1.7µM] [5,6]. It has been suggested that elimination of the Michael adduct under acidic conditions and consequent regeneration of the exocyclic α-methylene-γ-lactone group, affords the retained activity of the amino adducts [7].

Figure 1: Sesquiterpene lactones and conjugate adducts
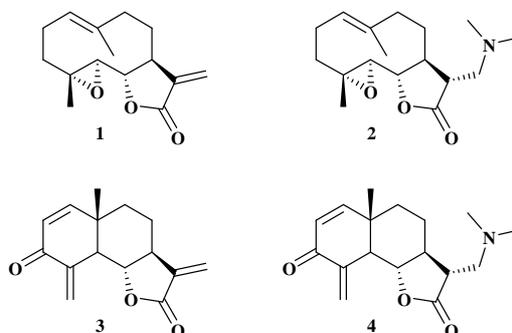
Our interest in this area stems from the isolation of eudesmanolide (**3**), having two exocyclic double bonds, from the plant *Dicoma anomala* [8]. When tested against various cancer cell-lines, compound (**3**) showed appreciable total growth inhibition effects, being superior to parthenolide (**1**) in our studies (Table 1). Conversion into the simple dimethylamine adduct (**4**) resulted in a compound of similar cytostatic activity to (**3**) against HCT-116 cells. Due to the limited availability of (**3**), we set about investigating the role the amino substituent and the additional exocyclic bond may play in the anticancer activity of more readily accessible compounds related to (**3**) but derived from the readily available α-santonin, using parthenolide (**1**) as the positive control.

Table 1: Cytotoxicity data for compounds 1, 3 and 4 (µM) [8]

Cell lines[a]	1		3		4	
	GI_{50}	TGI	GI_{50}	TGI	GI_{50}	TGI
HCT-116	10	25.1	0.79	28	0.84	>10
CCRF-CEM	7.94	63.1	0.63	>100	nd[b]	nd
MOLT-4	15.9	63.1	0.5	3.16	nd	nd
RPMI-A226	7.94	50.1	0.32	6.31	nd	nd

[a] HCT-116: human colon cancer cell line; CCRF-CEM: human Caucasian acute lymphoblastic leukaemia; MOLT-4: human acute T lymphoblastic leukaemia; RPMI-A226: human B-lymphocyte cell line; [b] nd = not determined

EXPERIMENTAL SECTION

Synthesis
NMR spectra were run on a 400MHz Varian INOVA instrument. Samples were referenced against chloroform at 77.00ppm for ^{13}C and against tetramethylsilane at 0.00 ppm for ^{1}H. High resolution mass spectra were recorded on a Waters SYNAPT G1 HDMS mass spectrometer operated in electrospray mode. Leucine enkephalin (50 pg/ml) was used as reference calibrant to obtain typical mass accuracies between 1 and 3 mDa. Melting points were determined using a Mettler FP62 capillary melting point apparatus and are uncorrected. All reagents were of reagent grade purchased from Sigma-Aldrich (Schnelldorf, Germany) and were used without any further purification. Solvents used for chromatography or extractions were distilled prior to use. Thin-layer chromatography was carried out using pre-coated aluminium-backed plates (Merck Silica Gel 60 F$_{254}$). Column chromatography was performed on Fluka Silica Gel 60 (70–230 mesh). Dry solvents were purified as described by Perrin and Armarego [16]. All starting materials were obtained commercially and used without further purification.

Synthesis of (3S,3aR,5aS,9bS)-3,5a,9-trimethyl-3-(phenylselenyl)-3a,4,5,5a-tetrahydronaphtho[1,2-b]furan-2,8(3H, 9bH)-dione **6**
A solution of LDA [generated from n-butyllithium (1.6M in hexanes, 26.7ml, 42.71mmol) and diisopropylamine (6.2ml, 44.158mmol) in dry THF (73.7ml)] was cooled to –78ºC. A solution of α-santonin **5** (3.598g, 14.727mmol) in dry THF (73.7ml) was added dropwise to the LDA solution over 30 minutes, affording a deep red mixture. After stirring for 1h, phenylselenyl chloride (8.185g, 42.737mmol) in dry THF (122.7ml) was added dropwise over 1h,

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giving a pale orange solution. This was stirred a further 1h at –78ºC, then warmed to 0ºC and stirred for 2h. Saturated aqueous ammonium chloride (150ml) was then added, and the mixture stirred for 30 minutes, then partitioned and washed with ethyl acetate. Drying (MgSO$_4$) and concentration to an orange oil, followed by column chromatography (30 – 50% ethyl acetate:hexane as eluent) afforded a yellow malodorous solid. Recrystallisation (dichloromethane/hexane at 0ºC) yielded a white powder (2.062g, 35%); R_f 0.39 (30% ethyl acetate:hexane); mp. 170 - 174ºC (ethyl acetate/hexane); δ_H (400MHz, CDCl$_3$) 7.68 – 7.65 (1H, m), 7.65 – 7.62 (1H, m), 7.49 – 7.41 (1H, m), 7.39 – 7.33 (2H, m), 6.73 (1H, d, *J* 9.9), 6.24 (1H, d, *J* 9.9), 5.23 (1H, dd, *J* 1.3 and 10.9), 2.12 (3H, d, *J* 1.4), 2.08 – 1.91 (4H, m), 1.60 (3H, s), 1.59 – 1.46 (1H, m) and 1.34 (3H, s); δ_C (101MHz, CDCl$_3$) 185.80, 174.43, 154.67, 150.89, 137.83, 129.63, 128.87, 128.42, 125.42, 123.52, 78.98, 57.01, 48.47, 41.02, 36.98, 24.65, 21.88, 20.12 and 10.63; HRMS (ESI) calculated C$_{21}$H$_{22}$O$_3$NaSe 425.0632, found 425.0618 (M$^+$ + Na$^+$).

Synthesis of 3-oxo-7αH,6βH-eudesma-1,4,11-trien-6,12-olide **7**
Selenide **2** (2.012g, 5.013mmol) in THF (50ml) at 0ºC was treated with 50% hydrogen peroxide (14.7M, 0.85ml, 12.533mmol) and the mixture stirred vigorously for 1h. Brine (100ml) was added, and the organic components were extracted with ethyl acetate. Drying (MgSO$_4$) and concentration to an orange oil, followed by column chromatography (30 – 50% ethyl acetate:hexane as eluent) afforded a bright yellow solid (0.694g, 57%); R_f 0.50 (50% ethyl acetate:hexane); δ_H (400MHz, CDCl3) 6.73 (1H, d, *J* 9.9), 6.27 (1H, d, *J* 9.9), 6.25 (1H, d, *J* 3.3), 5.58 (1H, d, *J* 3.1), 4.79 (1H, dq, *J* 1.2 and 11.6), 2.72 (1H, tq, *J* 3.3 and 11.7), 2.27 – 2.19 (1H, m), 2.16 (3H, d, *J* 1.4), 1.95 (1H, ddd, *J* 2.2, 3.8 and 13.4), 1.80 (1H, tdd, *J* 3.9, 11.9 and 13.1), 1.60 (1H, td, *J* 4.6 and 13.2) and 1.33 (3H, s); δ_C (101MHz, CDCl3) 186.23, 169.15, 154.84, 150.81, 137.43, 128.84, 125.89, 119.74, 81.41, 50.26, 41.34, 37.59, 25.15, 21.61 and 10.82; HRMS (ESI) calculated C$_{15}$H$_{17}$O$_3$ 245.1178, found 245.1161 (MH$^+$), and C$_{15}$H$_{16}$O$_3$Na 267.0997, found 267.0994 (M$^+$ + Na$^+$).

General procedure for conjugate addition of amines to enoate 3
A solution of the enoate (1 eq.) in ethanol (0.1M) containing the required volatile amine (2.5 eq.) or non-volatile amine (0.6 eq.) and triethylamine (1.1 – 2.5eq., for the appropriate hydrochloride salt) was heated at 85ºC under microwave irradiation set at 30W for 30 minutes to 1h, depending on the amine. All were prepared on a sufficiently small scale that the solutions could simply be concentrated and purified by column chromatography. The following compounds were produced this way:

Synthesis of (3R,3aS,5aS,9bS)-3-[(dimethylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydro-naphtho[1,2-b]furan-2,8(3H,9bH)-dione **8a**
Enoate **7** (49.5mg, 0.204mmol), dimethylamine hydrochloride (42.6mg, 0.522mmol), triethylamine (71.2ml, 0.51mmol) and ethanol (2ml) afforded, after column chromatography (2 – 4% methanol:chloroform as eluent), a yellow solid (24.6mg, 42%); R_f 0.12 (4% methanol: chloroform). Recrystallisation yielded yellow needles, sublimed > 130ºC, mp. 147 - 149ºC (ethyl acetate/hexane); δ_H (400MHz, CDCl$_3$) 6.70 (1H, d, *J* 9.9), 6.26 (1H, d, *J* 9.9), 4.80 (1H, dd, *J* 1.4 and 11.5), 2.80 – 2.69 (1H, m), 2.64 – 2.52 (2H, m), 2.32 – 2.25 (1H, m), 2.24 (6H, s), 2.13 (3H, d, *J* 1.3), 2.04 (1H, qd, *J* 3.4 and 11.7), 1.86 (1H, ddd, *J* 2.3, 3.8 and 13.4), 1.79 (1H, m), 1.55 (1H, td, *J* 4.5 and 13.2) and 1.33 (3H, s); δ_C (101MHz, CDCl$_3$) 186.31, 176.33, 154.99, 151.07, 128.56, 125.77, 81.40, 58.55, 51.67, 45.73, 44.33, 41.19, 37.97, 25.08, 23.77 and 10.84; HRMS (ESI) calculated C$_{17}$H$_{24}$NO$_3$ 290.1756, found 290.1730 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(diethylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydro-naphtho[1,2-b]furan-2,8(3H,9bH)-dione **8b**
Enoate **7** (53.1mg, 0.219mmol), diethylamine (56.7μl, 0.55mmol) and ethanol (2ml) afforded, after column chromatography (2 – 4% methanol:chloroform as eluent), a yellow wax (59.5mg, 86%); R_f 0.24 (4% methanol:chloroform); δ_H (400MHz, CDCl$_3$) 6.71 (1H, d, *J* 9.9), 6.25 (1H, d, *J* 9.9), 4.80 (1H, dd, *J* 1.3 and 11.5), 2.94 (1H, dd, *J* 3.8 and 12.8), 2.70 – 2.51 (4H, m), 2.44 (2H, dq, *J* 7.0 and 13.9), 2.37 – 2.27 (1H, m), 2.13 (3H, d, *J* 1.3), 2.00 (1H, ddd, *J* 3.5, 11.7 and 23.3), 1.87 (1H, ddd, *J* 2.2, 3.7 and 13.4), 1.72 (1H, m), 1.52 (1H, td, *J* 4.3 and 13.1), 1.37 (3H, s) and 1.00 (6H, s); δ_C (101MHz, CDCl$_3$) 186.34, 176.66, 155.05, 151.29, 128.41, 125.70, 81.38, 52.58, 52.09, 46.86, 44.63, 41.17, 38.01, 25.02, 23.93, 11.59 and 10.80; HRMS (ESI) calculated C$_{19}$H$_{28}$NO$_3$ 318.2069, found 318.2056 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(tert-butylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydro-naphtho[1,2-b]furan -2,8(3H,9bH)-dione **8c**
Enoate **7** (50.4mg, 0.208mmol), *tert*-butylamine (54.7μl, 0.52mmol) and ethanol (2ml) afforded, after column chromatography (2 – 4% methanol:chloroform as eluent), a yellow solid (26.5mg, 38%).

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Recrystallisation yielded yellow needles, mp. 118 - 120ºC (ethyl acetate/hexane); R_f 0.15 (4% methanol:chloroform); δ_H (400MHz, CDCl$_3$) 6.71 (1H, d, *J* 9.9), 6.25 (1H, d, *J* 9.9), 4.85 (1H, dd, *J* 1.2 and 11.4), 2.91 (1H, dd, *J* 4.9 and 11.8), 2.85 (1H, dd, *J* 6.6 and 11.8), 2.79 (1H, br s), 2.62 (1H, ddd, *J* 5.2, 6.6 and 11.8), 2.21 – 2.14 (1H, m), 2.13 (3H, s), 2.11 – 2.03 (1H, m), 1.89 (1H, ddd, *J* 2.2, 3.5 and 13.3), 1.76 (1H, m), 1.52 (1H, td, *J* 4.6 and 13.2), 1.33 (3H, s) and 1.12 (9H, s); δ_C (101MHz, CDCl$_3$) 186.30, 176.86, 154.97, 151.01, 128.55, 125.72, 81.53, 50.70, 49.39, 46.61, 41.19, 40.27, 37.71, 28.56, 25.04, 23.16 and 10.84; HRMS (ESI) calculated C$_{19}$H$_{28}$NO$_3$ 318.2069, found 318.2043 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(cyclopropylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydro-naphtho[1,2-b] furan-2,8(3H,9bH)-dione **8d**
Enoate **7** (0.184g, 0.752mmol), cyclopropylamine (78μl, 1.13mmol) and ethanol (2ml) afforded, after column chromatography (50% ethyl acetate:hexane - 5% methanol:ethyl acetate gradient elution), a pale yellow oil which solidified on standing (0.126g, 55%); R_f 0.28 (5% methanol:ethyl acetate); δ_H (400MHz, CDCl$_3$) 6.68 (1H, d, *J* 9.9), 6.24 (1H, d, *J* 9.9), 4.80 (1H, d, *J* 11.4), 3.01 (2H, ddd, *J* 5.7, 12.5 and 18.9), 2.66 – 2.56 (1H, m), 2.49 (1H, br s), 2.19 – 2.00 (6H, m), 1.92 – 1.84 (1H, m), 1.77 – 1.64 (1H, m), 1.50 (1H, td, *J* 4.3 and 13.2), 1.31 (3H, s) and 0.51 – 0.25 (4H, m); δ_C (101MHz, CDCl$_3$) 186.61, 176.96, 155.19, 151.15, 129.08, 126.21, 81.90, 49.89, 47.47, 46.41, 41.55, 38.14, 30.95, 25.48, 23.67, 11.26, 6.69; HRMS (ESI) calculated C$_{18}$H$_{24}$NO$_3$ 302.1756, found 302.1727 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(cyclopentylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydro-naphtho[1,2-b] furan-2,8(3H,9bH)-dione **8e**
Enoate **7** (50.0mg, 0.206mmol), cyclopentylamine (49μl, 0.52mmol) and ethanol (2ml) afforded, after column chromatography (2 – 4% methanol:chloroform as eluent), a yellow-orange wax (20.5mg, 30%); R_f 0.19 (4% methanol:chloroform); δ_H (400MHz, CDCl$_3$) 6.70 (1H, d, *J* 9.9), 6.26 (1H, d, *J* 9.9), 4.81 (1H, dd, *J* 1.2 and 11.4), 3.06 (1H, p, *J* 6.6), 2.89 (2H, qd, *J* 5.8 and 12.2), 2.67 – 2.49 (1H, m), 2.13 (3H, d, *J* 1.1), 2.12 – 2.00 (1H, m), 1.96 – 1.76 (5H, m), 1.68 (3H, tdd, *J* 2.9, 12.1 and 13.8), 1.60 – 1.45 (3H, m), 1.40 – 1.19 (2H, m) and 1.33 (3H, s); δ_C (101MHz, CDCl$_3$) 186.27, 176.74, 154.85, 150.84, 128.71, 125.84, 81.57, 59.91, 49.59, 46.51, 46.15, 41.19, 37.77, 32.96, 32.92, 25.13, 23.91, 23.32 (2C) and 10.91; HRMS (ESI) calculated C$_{20}$H$_{28}$NO$_3$ 330.2069, found 330.2047 (MH$^+$).

4.1.3.5. Synthesis of (3R,3aS,5aS,9bS)-3-{[(1R)-1,2,3,42-tetrahydro-1-naphthylamino]methyl}-5a,9-dimethyl-3a,4, 5,5a-tetrahydronaphtho[1,2-b]furan-2,8(3H,9bH)-dione **8f**
Enoate **7** (0.035g, 0.014mmol), (1*R*)-1,2,3,4-tetrahydro-1-naphthylamine (4.5μl, 0.03mmol) and ethanol (1ml) afforded, after preparative layer chromatography (50% ethyl acetate:hexane), a pale yellow oil (49.0mg, 87%); R_f 0.81 (10% methanol:ethyl acetate); δ_H (400MHz, CDCl3) 7.37 (1H, dq, *J* 3.7 and 7.3), 7.19 – 7.11 (2H, m), 7.11 – 7.04 (1H, m), 6.69 (1H, d, *J* 9.9), 6.26 (1H, d, *J* 9.9), 4.81 (1H, dd, *J* 1.3 and 11.5), 3.82 (1H, t, *J* 5.1), 3.04 (1H, dd, *J* 4.9 and 12.3), 2.97 (1H, dd, *J* 5.5 and 12.4), 2.89 – 2.63 (2H, m), 2.57 (1H, dt, *J* 5.2 and 12.2), 2.34 – 2.19 (2H, m), 2.13 (3H, d, *J* 1.2), 2.10 – 1.99 (1H, m), 1.99 – 1.77 (3H, m), 1.77 – 1.62 (2H, m), 1.52 (1H, td, *J* 4.5 and 13.2) and 1.32 (3H, s); δ_C (101MHz, CDCl3) 186.32, 176.60, 154.87, 150.94, 138.53, 137.53, 129.02, 128.76, 128.74, 126.74, 125.88, 125.73, 81.54, 55.64, 49.13, 47.10, 43.79, 41.24, 37.87, 29.38, 28.33, 25.13, 23.33, 19.19 and 10.94; HRMS (ESI) calculated C$_{25}$H$_{30}$NO$_3$ 392.2226, found 392.2240 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-5a,9-dimethyl-3-(pyrrolidin-1-ylmethyl)-3a,4,5,5a-tetrahydro-naphtho[1,2-b]furan-2,8(3H,9bH)-dione **8g**
Enoate **7** (53.0mg, 0.219mmol), pyrrolidine (45.7μl, 0.55mmol) and ethanol (2ml) afforded, after column chromatography (2 – 4% methanol:chloroform as eluent), a yellow-orange wax (26.5mg, 38%); R_f 0.36 (4% methanol:chloroform); δ_H (400MHz, CDCl$_3$) 6.70 (1H, d, *J* 9.9), 6.25 (1H, d, *J* 9.9), 4.81 (1H, dd, *J* 1.4 and 11.5), 2.90 (1H, dd, *J* 5.2 and 12.5), 2.87 (1H, dd, *J* 5.1 and 12.4), 2.68 – 2.54 (3H, m), 2.54 – 2.41 (2H, m), 2.32 – 2.22 (1H, m), 2.13 (3H, d, *J* 1.3), 2.07 (1H, m), 1.90 – 1.83 (1H, m), 1.83 – 1.68 (5H, m), 1.54 (1H, td, *J* 4.5 and 13.2) and 1.33 (3H, s); δ_C (101MHz, CDCl$_3$) 186.36, 176.32, 155.05, 151.17, 128.52, 125.76, 81.43, 54.61, 54.29, 51.50, 45.26, 41.21, 37.97, 25.08, 23.71, 23.50 and 10.84; HRMS (ESI) calculated C$_{19}$H$_{26}$NO$_3$ 316.1913, found 316.1893 (MH$^+$).

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Synthesis of (3R,3aS,5aS,9bS)-5a,9-dimethyl-3-(morpholinomethyl)-3a,4,5,5a-tetrahydronaphtho[1,2-b]furan-2,8 (3H,9bH)-dione **8h**

Enoate **7** (51.7mg, 0.213mmol), morpholine (46.5 µl, 0.53mmol) and ethanol (2ml) afforded, after column chromatography (2 – 4% methanol:chloroform as eluent), a yellow solid (45.7mg, 65%); R_f 0.24 (4% methanol:chloroform). Recrystallisation yielded orange needles, mp. 165 - 167ºC (ethyl acetate/hexane); δ_H (400MHz, CDCl$_3$) 6.71 (1H, d, *J* 9.9), 6.26 (1H, d, *J* 9.9), 4.82 (1H, dd, *J* 1.3 and 11.5), 3.89 – 3.46 (4H, m), 2.95 – 2.81 (1H, m), 2.71 – 2.57 (2H, m), 2.57 – 2.47 (2H, m), 2.47 – 2.35 (2H, m), 2.34 – 2.25 (1H, m), 2.12 (3H, d, *J* 1.2), 2.05 (1H, qd, *J* 3.5 and 11.6), 1.89 (1H, ddd, *J* 2.2, 3.6 and 13.4), 1.74 (1H, m), 1.55 (1H, td, *J* 4.5 and 13.2) and 1.33 (3H, s); δ_C (101MHz, CDCl$_3$) 186.22, 176.14, 154.93, 150.92, 128.52, 125.73, 81.35, 66.73, 57.70, 53.72, 51.82, 43.46, 41.12, 37.89, 25.00, 23.75 and 10.80; HRMS (ESI) calculated C$_{19}$H$_{26}$NO$_4$ 332.1862, found 332.1835 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(4-acetylpiperazin-1-yl)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydro-naphtho[1,2-b]furan-2,8(3H,9bH)-dione **8i**

Enoate **7** (51.6mg, 0.213mmol), 1-acetylpiperazine (69.9mg, 0.543mmol) and ethanol (2ml) afforded, after column chromatography (2 – 4% methanol: δ_H (400MHz, CDCl$_3$) 6.72 (1H, d, *J* 9.9), 6.26 (1H, d, *J* 9.9), 4.84 (1H, dd, *J* 1.3 and 11.5), 3.71 (1H, ddd, *J* 3.0, 6.2 and 13.0), 3.56 – 3.32 (3H, m), 2.93 – 2.81 (1H, m), 2.73 – 2.59 (2H, m), 2.58 – 2.41 (3H, m), 2.34 (1H, ddd, *J* 3.1, 7.6 and 11.0), 2.31 – 2.20 (2H, m), 2.12 (3H, d, *J* 1.1), 2.09 (3H, s), 2.08 – 2.01 (1H, m), 1.90 (1H, ddd, *J* 2.1, 3.5 and 13.4), 1.77 (1H, m), 1.55 (1H, td, *J* 4.5 and 13.2) and 1.34 (3H, s); δ_C (101 MHz, CDCl$_3$) 186.18, 176.00, 168.87, 154.93, 150.86, 128.48, 125.67, 81.30, 56.99, 53.49, 52.71, 51.64, 45.99, 43.72, 41.10 (2C), 37.79, 24.95, 23.66, 21.19 and 10.77; HRMS (ESI) calculated C$_{21}$H$_{29}$N$_2$O$_4$Na 395.1947, found 395.1887 (M$^+$ + Na$^+$), and calculated C$_{21}$H$_{29}$N$_2$O$_4$ 373.2127, found 373.2065 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(4-cyclohexylpiperazin-1-yl)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydro-naphtho [1,2-b]furan-2,8(3H,9bH)-dione **8j**

Enoate **7** (0.186g, 0.760mmol), 1-cyclohexylpiperazine (102mg, 0.608mmol) and ethanol (2ml) afforded, after column chromatography (50% ethyl acetate:hexane – 5% methanol:ethyl acetate gradient elution), an off-white foam (0.175g, 70%); R_f 0.18 (ethyl acetate); δ_H (400MHz, CDCl$_3$) 6.67 (1H, d, *J* 9.9), 6.22 (1H, d, *J* 9.9), 4.77 (1H, d, *J* 11.5), 2.83 (1H, dd, *J* 3.2 and 11.6), 2.70 – 2.14 (11H, m), 2.09 (3H, d, *J* 1.3), 2.05 – 1.92 (1H, m), 1.91 – 1.42 (9H, m) and 1.32 – 1.00 (8H, m); δ_C (101MHz, CDCl$_3$) 186.71, 176.77, 155.32, 151.37, 129.01, 126.21, 81.82, 63.78, 57.79, 54.10, 52.37, 49.18, 44.04, 41.54, 38.39, 29.26, 26.59, 26.15, 25.45, 24.23 and 11.23; HRMS (ESI) calculated C$_{25}$H$_{37}$N$_2$O$_3$ 413.2804, found 413.2764 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-{[4-(2-chlorophenyl)piperazin-1-yl]methyl}-5a,9-dimethyl-3a,4,5,5a-tetrahydro naphtho[1,2-b]furan-2,8(3H,9bH)-dione **8k**

Enoate **7** (49.0mg, 0.202mmol), 1-(2-chlorophenyl)piperazine (0.120g, 0.516mmol) and ethanol (2ml) afforded, after column chromatography (2 – 4% methanol:chloroform as eluent), a beige solid (65.3mg, 73%); R_f 0.41 (4% methanol:chloroform). Recrystallisation yielded a white powder, mp. >190ºC (ethyl acetate/hexane); δ_H (400MHz, CDCl$_3$) 7.35 (1H, dd, *J* 1.2 and 7.9), 7.27 – 7.15 (1H, m), 7.06 – 7.01 (1H, m), 7.01 –6.92 (1H, m), 6.71 (1H, d, *J* 9.9), 6.26 (1H, d, *J* 9.9), 4.83 (1H, d, *J* 11.4), 2.99 (4H, s), 2.95 (1H, t, *J* 8.4), 2.81 – 2.64 (4H, m), 2.60 (2H, d, *J* 5.5), 2.33 (1H, d, *J* 12.8), 2.14 (3H, s), 2.05 (1H, m), 1.89 (1H, d, *J* 13.4), 1.76 (1H, m), 1.56 (1H, td, *J* 4.3 and 13.1) and 1.34 (3H, s); δ_C (101MHz, CDCl$_3$) 186.24, 176.26, 154.97, 151.03, 148.93, 130.53, 128.58, 128.49, 127.46, 125.72, 123.65, 120.19, 81.36, 57.26, 53.40, 51.87, 51.00, 43.68, 41.14, 37.92, 25.00, 23.77 and 10.82; HRMS (ESI) calculated C$_{25}$H$_{29}$ClN$_2$O$_3$Na 463.1764, found 463.1759 (M$^+$ + Na$^+$), and calculated C$_{25}$H$_{30}$ClN$_2$O$_3$ 441.1945, found 441.1870 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(4-benzylpiperidin-1-yl)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydro-naphtho[1,2-b]furan-2,8(3H,9bH)-dione **8l**

Enoate **7** (0.201g, 0.821mmol), 4-benzylpiperidine (117µl, 0.61mmol) and ethanol (2ml) afforded, after column chromatography (20-80% ethyl acetate:hexane gradient elution), a pale yellow oil (0.264g, 76%); R_f 0.51 (ethyl acetate); δ_H (400MHz, CDCl$_3$) 7.32 – 7.24 (2H, m), 7.21 – 7.10 (3H, m), 6.70 (1H, d, *J* 9.9), 6.25 (1H, d, *J* 9.9), 4.79 (1H, d, *J* 11.5), 2.91 – 2.68 (3H, m), 2.69 – 2.59 (1H, m), 2.59 – 2.45 (3H, m), 2.34 – 2.23 (1H, m), 2.12 (3H, d, *J* 1.2), 2.08 – 1.95 (3H, m), 1.93 – 1.77 (2H, m), 1.77 – 1.47 (6H, m), 1.31 (3H, s) and 1.29 – 1.11 (2H, m); δ_C (101MHz, CDCl$_3$) 186.44, 176.68, 155.19, 151.35, 140.61, 129.08, 128.53, 128.20, 125.84, 125.82, 81.51, 57.84, 55.62, 52.63, 52.15, 43.90, 43.18, 41.29, 38.11, 37.83, 32.42, 32.01, 25.12, 23.87 and 10.94; HRMS (ESI) calculated C$_{27}$H$_{34}$NO$_3$ 420.2539, found 420.2502 (MH$^+$).

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Synthesis of (3R,3aS,5aS,9bS)-3-[(benzylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydro-naphtho[1,2-b]furan-2,8(3H,9bH)-dione **8m**

Enoate **7** (0.201g, 0.823mmol), benzylamine (72µl, 0.66mmol) and ethanol (2ml) afforded, after column chromatography (50% ethyl acetate:hexane - 5% methanol:ethyl acetate gradient elution), a pale yellow oil which solidified on standing (0.129g, 56%); R_f 0.22 (5% methanol:ethyl acetate); δ_H (400MHz, CDCl$_3$) 7.37 – 7.21 (5H, m), 6.69 (1H, d, *J* 9.7), 6.26 (1, d, *J* 9.9), 4.80 (1H, dd, *J* 1.3 and 11.5), 3.81 (2H, q, *J* 13.4), 2.97 (1H, dd, *J* 5.0 and 12.3), 2.85 (1H, dd, *J* 6.1 and 12.3), 2.64 – 2.52 (1H, m), 2.23 – 2.06 (4H, m), 2.02 – 1.92 (1H, m), 1.91 – 1.82 (1H, m), 1.79 (1H, br s), 1.67 (1H, m), 1.49 (1H, td, *J* 4.5 and 13.2) and 1.31 (3H, s); δ_C (101MHz, CDCl$_3$) 186.66, 176.93, 155.20, 151.17, 140.15, 129.13, 128.78, 128.40, 127.43, 126.24, 81.94, 54.33, 49.70, 46.97, 46.72, 41.57, 38.16, 25.50, 23.63 and 11.30; HRMS (ESI) calculated C$_{22}$H$_{26}$NO$_3$ 352.1913, found 352.1884 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(4-chlorobenzylamino)methyl]-5a,9-di-methyl-3a,4,5,5a-tetrahydro-naphtho[1,2-b]furan-2,8(3H,9bH)-dione **8n**

Enoate **7** (0.218g, 0.902mmol), 4-chlorobenzylamine (72.0µl, 0.59mmol) and ethanol (2ml) afforded, after column chromatography (10 – 20% ethanol:ethyl acetate as eluent), a yellow oil (0.156g, 69%); R_f 0.52 (20% ethanol:ethyl acetate); δ_H (400MHz, CDCl$_3$) 7.28 (2H, d, *J* 8.7), 7.24 (2H, d, *J* 8.7), 6.71 (1H, d, *J* 9.9), 6.24 (1H, d, *J* 9.9), 4.83 (1H, dd, *J* 1.4 and 11.5), 3.79 (1H, d, *J* 13.6), 3.74 (1H, d, *J* 13.6), 2.94 (1H, dd, *J* 4.9 and 12.3), 2.82 (1H, dd, *J* 6.1 and 12.3), 2.60 (1H, ddd, *J* 5.0, 6.0 and 12.2), 2.17 (1H, dd, *J* 3.5 and 12.1), 2.14 – 2.06 (1H, m), 2.12 (3H, d, *J* 1.3), 2.02 – 1.93 (1H, m), 1.88 (1H, ddd, *J* 2.2, 3.6 and 13.4), 1.70 (1H, m), 1.48 (1H, td, *J* 4.5 and 13.2) and 1.32 (3H, s); δ_C (101MHz, CDCl$_3$) 186.15, 176.47, 154.92, 150.91, 138.22, 132.46, 129.23, 128.39, 128.31, 125.59, 81.41, 53.00, 49.16, 46.32, 46.09, 41.10, 37.58, 24.93, 23.02 and 10.79; HRMS (ESI) calculated C$_{22}$H$_{25}$ClNO$_3$ 386.1523, found 386.1474 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(2-fluorobenzylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydro-naphtho[1,2-b]furan-2,8(3H,9bH)-dione **8o**

Enoate **7** (0.035g, 0.014mmol), 2-fluorobenzylamine (3.5µl, 0.03mmol) and ethanol (1ml) afforded, after preparative layer chromatography (50% ethyl acetate:hexane elution), a pale yellow oil (14.1mg, 27%); R_f 0.73 (10% methanol:ethyl acetate); δ_H (400MHz, CDCl3) 6.68 (1H, d, *J* 9.9), 6.24 (1H, d, *J* 9.9), 4.80 (1H, d, *J* 11.4), 3.01 (2H, m), 2.66 – 2.56 (1H, m), 2.49 (1H, br s), 2.19 – 2.00 (6H, m), 1.92 – 1.84 (1H, m), 1.77 – 1.64 (1H, m), 1.50 (1H, td, *J* 4.3, 13.2), 1.31 (3H, s), 0.51 – 0.25 (4H, m); δ_C (101MHz, CDCl3) 187.36, 173.96, 161.16 (d, J_{CF} 246.1), 157.96, 154.71, 130.39 (d, J_{CF} 31.9), 129.84, 129.26 (d, J_{CF} 8.3), 125.63, 129.26 (d, J_{CF} 8.3), 124.21 (d, J_{CF} 3.7), 115.27 (d, J_{CF} 13.4), 72.93, 53.00, 48.50, 47.44, 46.84, 41.75, 37.98, 23.43 (2C), 11.34; HRMS (ESI) calculated C$_{22}$H$_{25}$FNO$_3$ 370.1818, found 370.1838 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(4-fluorobenzylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydro-naphtho[1,2-b]furan-2,8(3H,9bH)-dione **8p**

Enoate **7** (0.207g, 0.856mmol), 4-fluorobenzylamine (67.3µl, 0.59mmol) and ethanol (2ml) afforded, after column (10 – 20% ethanol:ethyl acetate as eluent), a yellow oil (0.118g, 54%); R_f 0.45 (20% ethanol:ethyl acetate); δ_H (400MHz, CDCl$_3$) 7.27 (2H, dd, *J* 5.5 and 8.4), 6.99 (2H, t, *J* 8.7), 6.71 (1H, d, *J* 9.9), 6.24 (1H, d, *J* 9.9), 4.83 (1H, dd, *J* 1.2 and 11.5), 3.77 (2H, q, *J* 13.3), 2.95 (1H, dd, *J* 4.9 and 12.3), 2.83 (1H, dd, *J* 6.1 and 12.3), 2.66 – 2.55 (1H, m), 2.20 – 2.12 (1H, m), 2.12 (3H, d, *J* 1.0), 2.11 – 2.06 (1H, m), 2.02 – 1.92 (1H, m), 1.92 – 1.83 (1H, m), 1.70 (1H, qd, *J* 3.8 and 12.9), 1.49 (1H, td, *J* 4.5 and 13.2) and 1.32 (3H, s); δ_C (101MHz, CDCl$_3$) 186.20, 176.52, 161.72 (d, *J* 244.7), 154.95, 150.95, 135.42 (d, *J* 3.1), 129.44 (d, *J* 7.9), 128.42, 125.62, 115.01 (d, *J* 21.2), 81.43, 53.02, 49.19, 46.35, 46.09, 41.13, 37.61, 24.95, 23.04 and 10.81; HRMS (ESI) calculated C$_{22}$H$_{25}$FNO$_3$ 370.1818, found 370.1780 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(2,4-dimethoxybenzylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydro-naphtho [1,2-b]furan-2,8(3H,9bH)-dione **8q**

Enoate **7** (0.192g, 0.786mmol), 2,4-dimethoxybenzylamine (95µl, 0.63mmol) and ethanol (2ml) afforded, after column chromatography (50% ethyl acetate:hexane - 5% methanol:ethyl acetate gradient elution), a pale yellow oil which solidified on standing (0.90g, 35%); R_f 0.15 (5% methanol:ethyl acetate); δ_H (400MHz, CDCl$_3$) 7.10 (1H, d, *J* 8.1), 6.67 (1H, d, *J* 9.9), 6.45 (1H, d, *J* 2.3), 6.42 (1H, dd, *J* 2.4 and 8.1), 6.24 (1H, d, *J* 9.9), 4.80 (1H, dd, *J* 1.3 and 11.4), 3.81 (3H, s), 3.79 (3H, s), 3.78 – 3.67 (2H, m), 2.94 – 2.78 (3H, m), 2.72 – 2.62 (1H, m), 2.13 – 2.01 (5H, m), 1.91 – 1.81 (1H, m), 1.71 (1H, qd, *J* 3.7 and 12.8), 1.48 (1H, td, *J* 4.3 and 13.1) and 1.30 (3H, s); δ_C (101MHz, CDCl$_3$) 186.61, 176.94, 160.74, 158.94, 155.21, 151.12, 130.80, 129.09, 126.20, 119.47, 104.10, 98.88, 81.96,

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55.70, 55.64, 50.01, 49.41, 46.76, 46.35, 41.55, 38.12, 25.48, 23.63 and 11.28; HRMS (ESI) calculated $C_{24}H_{30}NO_5$ 412.2124, found 412.2079 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(pyridin-2-ylmethylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydronaphtho [1,2-b]furan-2,8(3H,9bH)-dione **8r**

Enoate **7** (0.201g, 0.825mmol), 2-picolylamine (70μl, 0.65mmol) and ethanol (2ml) afforded, after column chromatography (ethyl acetate - 5% methanol:ethyl acetate gradient elution), an orange oil (0.138g, 60%); R_f 0.20 (5% methanol:ethyl acetate); $δ_H$ (400MHz, CDCl$_3$) 8.51 (1H, d, *J* 4.4), 7.62 (1H, t, *J* 7.7), 7.28 (1H, d, *J* 7.8), 7.14 (1H, t, *J* 6.1), 6.67 (1H, d, *J* 9.9), 6.22 (1H, d, *J* 9.8), 4.80 (1H, d, *J* 11.4), 3.90 (2H, d, *J* 4.0), 2.94 (2H, t, *J* 5.0), 2.74 (1H, br s), 2.63 (1H, dt, *J* 5.7 and 11.5), 2.17 – 1.97 (5H, m), 1.84 (1H, d, *J* 13.5), 1.77 – 1.62 (m, 1H), 1.47 (1H, t, *J* 13.3) and 1.28 (s, 3H); $δ_C$ (101MHz, CDCl$_3$) 186.58, 176.75, 159.35, 155.26, 151.20, 149.51, 136.88, 128.91, 126.06, 122.45, 122.39, 81.81, 55.46, 49.91, 47.18, 46.74, 41.49, 38.01, 25.39, 23.61 and 11.21; HRMS (ESI) calculated $C_{21}H_{25}N_2O_3$ 353.1865, found 353.1827 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(pyridin-3-ylmethylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydronaphtho [1,2-b]furan-2,8(3H,9bH)-dione **8s**

Enoate **7** (0.198g, 0.809mmol), 3-picolylamine (66μl, 0.65mmol) and ethanol (2ml) afforded, after column chromatography (ethyl acetate - 5% methanol:ethyl acetate gradient elution), a pale yellow oil (0.138g, 48%); R_f 0.11 (5% methanol:ethyl acetate; $δ_H$ (400MHz, CDCl$_3$) 8.55 (1H, d, *J* 1.5), 8.51 (1H, dd, *J* 1.5 and 4.8), 7.67 (1H, ddd, *J* 1.9 and 7.8), 7.27 (1H, ddd, *J* 0.8, 4.8 and 7.8), 6.70 (1H, d, *J* 9.9), 6.26 (1H, d, *J* 9.9), 4.83 (1H, dd, *J* 1.4 and 11.5), 3.83 (2H, d, *J* 4.0), 2.97 (1H, dd, *J* 4.9 and 12.2), 2.85 (1H, dd, *J* 6.2 and 12.3), 2.66 – 2.54 (1H, m), 2.40 – 2.07 (5H, m), 2.02 – 1.92 (1H, m), 1.92 – 1.82 (1H, m), 1.70 (1H, m), 1.50 (1H, td, *J* 4.5 and 13.2) and 1.32 (3H, s); $δ_C$ (101MHz, CDCl$_3$) 186.38, 176.63, 154.99, 150.86, 149.68, 148.68, 135.88, 135.24, 128.84, 125.95, 123.56, 81.70, 51.37, 49.38, 46.60, 46.43, 41.32, 37.83, 25.23, 23.31 and 11.05; HRMS (ESI) calculated $C_{21}H_{25}N_2O_3$ 353.1865, found 353.1836 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-5a,9-dimethyl-3-[(pyridin-4-ylmethylamino)-methyl]-3a,4,5,5a-tetrahydronaphtho [1,2-b]furan-2,8(3H,9bH)-dione **8t**

Enoate **7** (0.249g, 1.029mmol), 4-(aminomethyl)pyridine (59.3μl, 0.58mmol) and ethanol (2ml) afforded, after column chromatography (10 – 20% ethanol:ethyl acetate as eluent), a yellow oil (78.7mg, 39%); R_f 0.13 (20% ethanol:ethyl acetate); $δ_H$ (400MHz, CDCl$_3$) 8.53 (2H, d, *J* 5.6), 7.27 (2H, d, *J* 5.9), 6.72 (1H, d, *J* 9.9), 6.25 (1H, d, *J* 9.9), 4.87 (1H, dd, *J* 1.3 and 11.5), 3.86 (1H, d, *J* 14.8), 3.82 (1H, d, *J* 14.8), 2.97 (1H, dd, *J* 4.8 and 12.2), 2.84 (1H, dd, *J* 6.2 and 12.2), 2.70 – 2.61 (1H, m), 2.57 (1H, s), 2.24 – 2.14 (1H, m), 2.12 (3H, s), 2.01 (1H, ddd, *J* 4.3, 6.9 and 10.0), 1.94 – 1.85 (1H, m), 1.73 (1H, m), 1.51 (1H, td, *J* 4.5 and 13.2) and 1.33 (3H, s); $δ_C$ (101MHz, CDCl$_3$) 186.12, 176.41, 154.89, 150.80, 149.49, 148.90, 128.41, 125.58, 122.74, 81.42, 52.44, 49.09, 46.29, 46.22, 41.08, 37.53, 24.91, 22.98 and 10.76; HRMS (ESI) calculated $C_{21}H_{25}N_2O_3$ 353.1865, found 353.1821 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(4-chlorophenethylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydronaphtho [1,2-b]furan-2,8(3H,9bH)-dione **8u**

Enoate **7** (0.190g, 0.779mmol), 4-chlorophenethylamine (87μl, 0.62mmol) and ethanol (2ml) afforded, after column chromatography (50% ethyl acetate:hexane - 5% methanol:ethyl acetate gradient elution), a pale yellow oil (0.171g, 69%); R_f 0.25 (5% methanol:ethyl acetate); $δ_H$ (400MHz, CDCl$_3$) 7.24 (2H, d, *J* 8.5), 7.12 (2H, d, *J* 8.5), 6.68 (1H, d, *J* 9.9), 6.26 (1H, d, *J* 9.9), 4.79 (1H, dd, *J* 1.4 and 11.4), 2.98 – 2.82 (4H, m), 2.75 (2H, t, *J* 6.8), 2.61 – 2.51 (1H, m), 2.12 (3H, d, *J* 1.3), 2.11 – 2.02 (1H, m), 2.02 – 1.93 (1H, m), 1.89 – 1.81 (1H, m), 1.67 (1H, m), 1.47 (1H, td, *J* 4.5 and 13.3) and 1.31 (s, 3H); $δ_C$ (101MHz, CDCl$_3$) 186.60, 176.89, 155.19, 151.12, 138.54, 132.25, 130.35, 129.03, 128.84, 126.19, 81.89, 51.57, 49.87, 47.60, 46.66, 41.53, 38.08, 35.91, 25.45, 23.63 and 11.25; HRMS (ESI) calculated $C_{23}H_{27}NO_3Cl$ 400.1679, found 400.1674 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-{[2-(2-fluorophenyl)ethylamino]methyl}-5a,9-dimethyl-3a,4,5,5a-tetrahydro naphtho [1,2-b]furan-2,8(3H,9bH)-dione **8v**

Enoate **7** (0.035g, 0.014mmol), 2-fluorophenethylamine (4.0μl, 0.03mmol) and ethanol (1ml) afforded, after preparative layer chromatography (50% ethyl acetate:hexane elution), a pale yellow oil (26.9mg, 49%); R_f 0.65 (10% methanol:ethyl acetate); $δ_H$ (400MHz, CDCl3) 7.23 (2H, dt, *J* 4.0 and 9.7), 7.14 – 6.99 (2H, m), 6.67 (1H, d, *J* 9.8), 6.23 (1H, d, *J* 9.8), 4.66 (1H, d, *J* 10.7), 4.27 (1H, br s), 3.74 – 3.49 (3H, m), 2.94 (2H, t, *J* 6.8), 2.53 (1H, ddd, *J* 3.9, 10.9 and 12.8), 2.28 (3H, d, *J* 1.1), 1.97 (1H, m), 1.78 (1H, ddd, *J* 2.4, 4.1 and 13.3), 1.68 (1H, dtd, *J* 2.4,

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4.4 and 13.6) and 1.29 (3 H, s); δ_C (101MHz, CDCl3) 187.53, 171.03, 161.21 (d, J_{CF} 244.7), 157.93, 155.95, 131.15 (d, J_{CF} 4.8), 129.94, 128.54 (d, J_{CF} 8.1), 125.71, 125.44 (d, J_{CF} 15.9), 124.30 (d, J_{CF} 3.6), 115.39 (d, J_{CF} 22.1), 75.44, 50.98, 41.94, 39.84, 39.82, 37.90, 28.94, 28.92, 26.07, 23.76, 11.29.; HRMS (ESI) calculated $C_{23}H_{27}FNO_3$ 384.1975, found 384.2003 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(4-hydroxyphenethylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydronaphtho [1,2-b]furan-2,8(3H,9bH)-dione **8w**
Enoate **7** (0.220g, 0.910mmol), tyramine (77.1mg, 0.560mmol) and ethanol (2ml) afforded, after column chromatography (10 – 20% ethanol:ethyl acetate as eluent), a yellow foam (89.7mg, 44%); R_f 0.26 (20% ethanol:ethyl acetate); δ_H (400MHz, CDCl$_3$) 7.00 (2H, d, *J* 8.5), 6.74 (2H, d, *J* 8.5), 6.69 (1H, d, *J* 9.9), 6.24 (1H, d, *J* 9.9), 4.78 (1H, dd, *J* 1.1 and 11.4), 4.72 (2H, br s), 2.98 – 2.88 (2H, m), 2.88 – 2.80 (2H, m), 2.72 (2H, t, *J* 7.0), 2.63 (1H, dt, *J* 6.0 and 12.2), 2.08 (3H, d, *J* 0.9), 1.98 (2H, m), 1.88 – 1.76 (1H, m), 1.66 (1H, qd, *J* 3.4 and 12.8), 1.40 (1H, td, *J* 4.2 and 13.1) and 1.27 (3H, s); δ_C (101MHz, CDCl$_3$) 186.57, 176.76, 155.42, 155.07, 151.32, 130.17, 129.60, 128.39, 125.49, 115.52, 81.52, 51.30, 49.55, 47.17, 45.61, 41.22, 37.51, 34.73, 24.86, 22.92, and 10.81; HRMS (ESI) calculated $C_{23}H_{28}NO_4$ 382.2018, found 382.1970 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(3-methoxyphenethylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydronaphtho [1,2-b]furan-2,8(3H,9bH)-dione **8x**
Enoate **7** (0.174g, 0.713mmol), 2-(3-methoxyphenyl)ethylamine (83.0µl, 0.57mmol) and ethanol (2ml) afforded, after column chromatography (50% ethyl acetate:hexane – 5% methanol:ethyl acetate gradient elution), a yellow gum (0.138g, 61%); R_f 0.22 (ethyl acetate); δ_H (400MHz, CDCl$_3$) 7.21 (1H, dd, *J* 7.5 and 9.0), 6.81 – 6.71 (3H, m), 6.69 (1H, d, *J* 9.8), 6.26 (1H, d, *J* 9.9), 4.80 (1H, dd, *J* 1.2 and 11.4), 3.86 – 3.76 (3H, m), 2.99 – 2.86 (m, 4H), 2.77 (t, *J* 7.0, 2H), 2.58 (dt, *J* 5.8, 11.8, 1H), 2.12 (3H, d, *J* 1.1), 2.09 – 1.97 (4H, m), 1.89 – 1.81 (2H, m), 1.68 (1H, qd, *J* 3.8 and 12.8), 1.48 (1H, td, *J* 4.4 and 13.2) and 1.31 (s, 3H); δ_C (101MHz, CDCl$_3$) 186.61, 176.85, 159.94, 155.21, 151.16, 141.72, 129.74, 129.03, 126.17, 121.34, 114.80, 111.69, 81.86, 55.44, 51.63, 49.94, 47.67, 46.73, 41.52, 38.08, 36.63, 25.45, 23.69 and 11.24; HRMS (ESI) calculated $C_{24}H_{30}NO_4$ 396.2175, found 396.2145 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-[(4-methoxyphenethylamino)methyl]-5a,9-dimethyl-3a,4,5,5a-tetrahydronaphtho [1,2-b]furan-2,8(3H,9bH)-dione **8y**
Enoate **7** (0.192g, 0.784mmol), 2-(4-methoxyphenyl)ethylamine (126.0µl, 0.86mmol) and ethanol (2ml) afforded, after column chromatography (50% ethyl acetate:hexane – 5% methanol:ethyl acetate gradient elution), a yellow gum (0.091g, 30%); R_f 0.21 (ethyl acetate); δ_H (400MHz, CDCl$_3$) 7.11 (2H, d, *J* 8.5), 6.83 (2H, d, *J* 8.5), 6.69 (1H, d, *J* 9.8), 6.26 (1H, d, *J* 9.9), 4.79 (1H, d, *J* 11.4), 3.78 (3H, s), 2.93 (2H, dd, *J* 3.0 and 5.8), 2.88 – 2.79 (2H, m), 2.73 (2H, t, *J* 7.0), 2.56 (1H, dt, *J* 5.8 and 11.8), 2.12 (3H, s), 2.09 – 1.94 (2H, m), 1.90 – 1.81 (1H, m), 1.68 (2H, qd, *J* 3.9 and 12.8), 1.48 (1H, td, *J* 4.5 and 13.2) and 1.31 (3H, s); δ_C (101MHz, CDCl$_3$) δ 186.64, 176.91, 158.34, 155.19, 151.14, 132.09, 129.92, 129.09, 126.23, 114.17, 81.89, 55.58, 52.01, 49.99, 47.75, 46.75, 41.54, 38.13, 35.68, 25.48, 23.73 and 11.27; HRMS (ESI) calculated $C_{24}H_{30}NO_4$ 396.2175, found 396.2132 (MH$^+$).

Synthesis of (3R,3aS,5aS,9bS)-3-{[2-(pyridin-2-yl)ethylamino]methyl}-5a,9-dimethyl-3a,4,5,5a-tetrahydronaphtho [1,2-b]furan-2,8(3H,9bH)-dione **8z**
Enoate **7** (0.185g, 0.756mmol), 2-(pyridin-2-yl)ethylamine (73µl, 0.61mmol) and ethanol (2ml) afforded, after column chromatography (ethyl acetate - 5% methanol:ethyl acetate gradient elution), an orange gum (0.097g, 37%); R_f 0.06 (5% methanol:ethyl acetate; δ_H (400MHz, CDCl$_3$) 8.47 (1H, d, *J* 4.8), 7.57 (1H, dd, *J* 6.8 and 8.5), 7.19 – 6.99 (2H, m), 6.66 (1H, d, *J* 9.9), 6.22 (1H, d, *J* 9.9), 4.77 (1H, d, *J* 11.3), 3.11 – 2.82 (6H, m), 2.60 (1H, dt, *J* 5.7 and 11.6), 2.12 – 1.92 (5H, m), 1.83 (1H, dd, *J* 2.5 and 12.4), 1.73 – 1.61 (1H, m), 1.53 – 1.35 (1H, m) and 1.28 (3H, s); δ_C (101MHz, CDCl$_3$) 186.61, 176.84, 160.13, 155.26, 151.23, 149.46, 136.77, 128.94, 126.11, 123.65, 121.70, 81.84, 49.87, 49.67, 47.38, 46.44, 41.52, 38.07, 38.01, 25.41, 23.57 and 11.21; HRMS (ESI) calculated $C_{22}H_{27}N_2O_3$ 367.2022, found 367.1997 (MH$^+$).

General procedure for reduction of amine conjugates 8
A solution of the dienoate (1 eq.) in ethanol (0.1M) containing the 32% hydrochloric acid (0.5ml) and 5% palladium on carbon (1 mass eq.) were reduced under 1atm. of hydrogen gas for 18 – 72h, until complete. Solids were filtered off, the filtrate concentrated and the residue purified as indicated. The following compounds were produced this way:

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Synthesis of (3R,3aS,5aS,9S,9aS,9bS)-5a,9-dimethyl-3-(morpholinomethyl)-octahydronaphtho[1,2-b]furan-2,8(3H, 9bH)-dione as a 2:1 mixture of cis isomers **9a**

Dienone **8h** (0.434g, 1.334mmol), 5% Pd-C (0.470g), 32% hydrochloric acid (0.5cm^3) and ethanol (10cm^3) afforded, after column chromatography (30 – 50% acetone:hexane as eluent), an orange foam (0.209g, 48%); R_f 0.68 (20% ethanol:ethyl acetate); δ_H (400MHz, CDCl$_3$) 3.91 (1H, t, *J* 10.6), 3.76 – 3.60 (4H, m), 2.82 (1H, dd, *J* 4.3 and 12.7), 2.65 – 2.44 (6H, m), 2.44 – 2.32 (3H, m), 2.19 – 2.08 (2H, m), 1.92 – 1.53 (5H, m), 1.42 – 1.28 (1H, m), 1.25 (3H, d, *J* 6.6) and 1.18 (3H, s); δ_C (101MHz, CDCl$_3$) 211.44, 177.55, 83.11, 66.85, 57.72, 53.88, 53.47, 51.11, 44.93, 43.36, 40.65, 40.28, 37.29, 36.32, 23.77, 18.39 and 13.91; HRMS (ESI) calculated C$_{19}$H$_{29}$NaNO$_4$ 358.1994, found 358.2048 (M$^+$ + Na$^+$) and calculated C$_{19}$H$_{30}$NO$_4$ 336.2175, found 336.2152 (MH$^+$).

Synthesis of (3R,3aS,5aS,9aS,9bS)-3-[(4-hydroxyphenethyl-amino)methyl]-5a,9-dimethyl-octahydronaphtho[1,2-b] furan-2,8(3H,9bH)-dione as a 2:1 mixture of 9R/9S isomers **9b**

Dienone **8w** (0.355g, 0.945mmol), 5% Pd-C (0.266g), 32% hydrochloric acid (0.5ml) and ethanol (10ml) afforded, after column chromatography (30 – 50% acetone:hexane as eluent), a pale orange foam (0.144g, 40%); R_f 0.44 (20% ethanol:ethyl acetate); δ_H (400MHz, CDCl$_3$) 7.03 (2H, d, *J* 8.1), 6.72 (2H, d, *J* 8.3), 3.97 (2H, br s), 3.91 (1H, t, *J* 10.4), 3.66 (1H, td, *J* 0.7 and 6.6), 3.03 – 2.80 (4H, m), 2.74 (2H, t, *J* 6.9), 2.58 – 2.37 (3H, m), 1.86 – 1.69 (3H, m), 1.69 – 1.46 (3H, m), 1.33 – 1.23 (2H, m), 1.21 (3H, d, *J* 6.5) and 1.15 (3H, s); δ_C (101MHz, CDCl$_3$) 211.56, 178.11, 154.76, 130.65, 129.75, 115.57, 83.44, 53.37, 51.48, 48.84, 47.26, 45.41, 44.85, 40.62, 39.98, 37.32, 36.39, 34.79, 23.07, 18.34 and 13.81; HRMS (ESI) calculated C$_{23}$H$_{31}$NaNO$_4$ 408.2151, found 408.2169 (M$^+$ + Na$^+$) and calculated C$_{23}$H$_{32}$NO$_4$ 386.2331, found 386.2299 (MH$^+$).

Synthesis of (3R,3aS,5aS,9aS,9bS)-3-[(3-methoxyphenethylamino)methyl]-5a,9-dimethyl-octahydronaphtho[1,2-b] furan-2,8(3H,9bH)-dione as a 2:1 mixture of 9R/9S isomers **9c**

Dienone **8x** (0.111g, 0.282mmol), 5% Pd-C (0.122g), 32% hydrochloric acid (38.9 µl, 0.34mmol) and ethanol (5ml) afforded, after column chromatography (30 – 50% acetone:hexane as eluent), a pale yellow oil (51.3mg, 46%); R_f 0.27 (20% acetone:hexane); δ_H (400MHz, CDCl$_3$) 7.19 – 7.08 (1H, m), 6.72 (1H, d, *J* 7.6), 6.70 – 6.60 (2H, m), 3.83 (1H, t, *J* 10.4), 3.72 (3H, s), 2.92 – 2.75 (3H, m), 2.70 (2H, t, *J* 7.2), 2.52 – 2.30 (3H, m), 2.30 – 2.12 (1H, m), 2.09 – 1.96 (1H, m), 1.86 (1H, dd, *J* 8.8 and 13.6), 1.81 – 1.64 (2H, m), 1.64 – 1.41 (3H, m), 1.28 – 1.18 (1H, m), 1.17 (3H, d, *J* 6.7) and 1.12 (3H, s); δ_C (101MHz, CDCl$_3$) 211.40, 177.92, 159.59, 141.35, 129.40, 121.02, 114.39, 111.43, 83.27, 55.09, 53.35, 51.37, 48.78, 47.27, 45.89, 44.85, 40.61, 40.00, 37.30, 36.37, 36.19, 23.20, 18.34 and 13.81; HRMS (ESI) calculated C$_{24}$H$_{34}$NO$_4$ 400.2488, found 400.2450 (MH$^+$).

Synthesis of (3S,3aS,5aS,9R,9aS,9bS)-3,5a,9-trimethyl-octahydronaphtho[1,2-b]furan-2,8(3H,9bH)-dione **10**

Santonin **5** (5.026g, 20.406mmol), 5% Pd-C (4.536g, ~1 mass eq.) and ethanol (50ml) were mixed under hydrogen atmosphere (1 atm.) for 48h. The catalyst was filtered off over celite, washed with ethanol and acetone, and concentrated to a white solid. Column chromatography (12% ethyl acetate:hexane – ethyl acetate gradient) afforded a pure white solid, a mixture of two isomers. Recrystallisation (ethyl acetate:hexane) afforded an isomerically pure waxy white solid (1.342g, 26%); R_f 0.66 (50% ethyl acetate: hexane); δ_H (400MHz, CDCl$_3$) 6.72 (1H, d, *J* 9.9), 6.12 (1H, d, *J* 3.2), 5.92 (1H, d, *J* 9.9), 5.45 (1H, d, *J* 3.1), 3.99 (1H, t, *J* 10.9), 2.60 (1H, dq, *J* 6.8, 12.5), 2.68 – 2.52 (1H, m), 2.16 – 2.05 (2H, m), 1.83 – 1.58 (4H, m), 1.41 (3H, d, *J* 6.9), 1.17 (3H, s); δ_C (101MHz, CDCl$_3$) 200.38, 170.08, 157.95, 138.13, 126.52, 117.41, 81.88, 52.04, 50.02, 41.88, 38.39, 36.98, 21.03, 19.13, 14.48; HRMS (ESI) calculated C$_{15}$H$_{22}$NaO$_3$ 273.1467, found 273.1479 (M$^+$ + Na$^+$).

Synthesis of (3S,3aR,5aS,7R,9R,9aS,9bR)-3,5a,9-trimethyl-3,7-bis(phenylselanyl)-octahydro-naphtho[1,2-b]furan-2,8(3H,9bH)-dione **11** *and (3S,3aS,5aS,9R,9aS,9bS)-3,5a,9-trimethyloctahydro-naphtho[1,2-b]furan-2,8(3H,9bH)-dione* **12**

A solution of LiHMDS [generated from n-butyllithium (1.6M in hexanes, 4.02ml, 6.43mmol) and hexamethyldisilazane (1.47ml, 6.97mmol) in dry THF (54ml)] was cooled to –78ºC. A solution of ketone **10** (1.342g, 5.362mmol) in dry THF (10ml) was added drop wise to the LiHMDS solution over 10 minutes, affording a pale yellow mixture. After stirring for 1h, phenylselenyl chloride (1.218g, 6.359mmol) in dry THF (10ml) was added drop wise over ten minutes, giving a yellow solution. This was stirred a further 1h at –78ºC, then warmed to room temperature over 18h. Saturated aqueous ammonium chloride (50ml) was then added, and the mixture stirred for 30 minutes, then partitioned and washed with ethyl acetate. Drying (MgSO$_4$) and concentration to a yellow oil, followed by column chromatography (30% ethyl acetate:hexane as eluent) afforded a pale beige foam, the diselenide **11** (0.925g, 43%); R_f 0.35 (30% ethyl acetate:hexane); δ_H (400MHz, CDCl$_3$) 7.65 – 7.59 (2H, m), 7.58 – 7.52 (2H, m), 7.42 (1H, tdd, *J* 0.8, 1.9 and 6.8), 7.38 – 7.23 (5H, m), 4.39 – 4.31 (1H, m), 4.27 (1H, ddd, *J* 1.0, 6.8 and 12.5),

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2.71 – 2.61 (1H, m), 2.02 (1H, dd, *J* 6.9 and 13.4), 1.90 – 1.71 (2H, m), 1.69 – 1.55 (1H, m), 1.52 (3H, s), 1.41 (3H, d, *J* 6.7) and 1.16 (3H, s); δ_C (101MHz, CDCl$_3$) 207.36, 176.05, 138.16, 135.11, 129.80, 129.15, 129.14, 129.12, 129.09, 128.03, 127.93, 123.93, 80.69, 56.88, 53.68, 49.36, 48.39, 48.28, 45.38, 39.45, 37.90, 22.20, 20.67, 18.61 and 14.92 (note non-equivalence of carbons of selenide attached to lactone); HRMS (ESI) calculated C$_{27}$H$_{30}$O$_3$NaSe$_2$ 585.0423, found 585.0385 (M$^+$ + Na$^+$).

The selenide **12** was also isolated as a beige foam (0.316g, 13%); R_f 0.61 (30% ethyl acetate:hexane); δ_H (400MHz, CDCl$_3$) 7.55 (2H, ddd, *J* 1.3, 3.2 and 5.1), 7.33 – 7.25 (3H, m), 4.26 (1H, ddd, *J* 1.2, 6.9 and 12.4), 3.86 (1H, t, *J* 10.3), 2.64 (1H, dqd, *J* 1.2, 6.6, 13.2), 2.25 (1H, dq, *J* 6.7, 13.7), 2.02 (1H, dd, *J* 6.9 and 13.5), 1.89 – 1.76 (3H, m), 1.69 – 1.44 (5H, m), 1.34 (3H, d, *J* 6.7), 1.31 – 1.22 (3H, m), 1.21 (3H, d, *J* 6.9) and 1.11 (3H, s); δ_C (101MHz, CDCl$_3$) 207.43, 178.84, 138.16, 135.07, 129.11, 128.00, 82.71, 53.54, 52.65, 49.42, 48.34, 45.34, 40.53, 39.58, 37.89, 22.84, 18.65, 15.02 and 12.42; HRMS (ESI) calculated C$_{21}$H$_{26}$O$_3$NaSe 429.0945, found 429.0931 (M$^+$ + Na$^+$).

Synthesis of (3aS,5aS,9R,9aS,9bS)-5a,9-dimethyl-3-methylene-3a,4,5,5a,9,9a-hexahydronaphtho[1,2-b]furan-2,8(3H,9bH)-dione **13**

A solution of diselenide **11** (0.903g, 2.227mmol) in THF (22ml) at 0ºC was treated with 50% hydrogen peroxide (14.7M, 0.38ml, 5.57mmol) and the mixture stirred vigorously for 1h. Brine (100cm^3) was added, and the organic components were extracted with ethyl acetate. Drying (MgSO$_4$) and concentration to an orange oil, followed by column chromatography (30% ethyl acetate:hexane as eluent) afforded a bright yellow solid (0.377g, 66%); R_f 0.50 (30% ethyl acetate:hexane); Recrystallisation yielded pale brown needles, mp. 125 - 127ºC (ethyl acetate:hexane); δ_H (400MHz, CDCl$_3$) 6.70 (1H, d, *J* 9.9), 5.90 (1H, d, *J* 9.9), 3.99 (1H, dd, *J* 9.9 and 10.9), 2.58 (1H, dq, *J* 6.9 and 12.3), 2.30 (1H, dq, *J* 6.9 and 12.2), 1.98 (1H, dd, *J* 11.1 and 12.2), 1.94 – 1.90 (1H, m), 1.79 – 1.73 (1H, m), 1.70 – 1.63 (1H, m), 1.62 – 1.56 (1H, m), 1.37 (3H, d, *J* 6.9), 1.24 (3H, d, *J* 6.9) and 1.18 (3H, s); δ_C (101MHz, CDCl$_3$) 200.66, 178.86, 158.13, 126.63, 81.77, 52.81, 51.67, 42.17, 40.52, 38.43, 37.41, 22.74, 19.23, 14.53 and 12.39; HRMS (ESI) calculated C$_{15}$H$_{20}$NaO$_3$ 271.1310, found 271.1272 (M$^+$ + Na$^+$) and calculated C$_{15}$H$_{21}$O$_3$ 249.1491, found 249.1461 (MH$^+$).

Synthesis of (3S,3aS,5aS,9R,9aS,9bS)-3,5a,9-trimethyl-3a,4,5,5a,9,9a-hexahydronaphtho[1,2-b]furan-2,8(3H,9bH)-dione **14**

The selenide **12** (0.294g, 0.524mmol) in THF (6ml) at 0ºC was treated with 50% hydrogen peroxide (14.7M, 0.089ml, 1.31mmol) and the mixture stirred vigorously for 1h. Similar workup, followed by column chromatography (30% ethyl acetate:hexane as eluent) afforded a white solid (65.1mg, 50%); R_f 0.50 (30% ethyl acetate:hexane); δ_H (400MHz, CDCl$_3$) 6.70 (1H, d, *J* 9.9), 5.90 (1H, d, *J* 9.9), 3.99 (1H, dd, *J* 9.9 and 10.9), 2.58 (1H, dq, *J* 6.9 and 12.3), 2.30 (1H, dq, *J* 6.9 and 12.2), 1.98 (1H, dd, *J* 11.1 and 12.2), 1.94 – 1.90 (1H, m), 1.79 – 1.73 (1H, m), 1.70 – 1.63 (1H, m), 1.62 – 1.56 (1H, m), 1.37 (3H, d, *J* 6.9), 1.24 (3H, d, *J* 6.9) and 1.18 (3H, s); δ_C (101MHz, CDCl$_3$) 200.66, 178.86, 158.13, 126.63, 81.77, 52.81, 51.67, 42.17, 40.52, 38.43, 37.41, 22.74, 19.23, 14.53 and 12.39; HRMS (ESI) calculated C$_{15}$H$_{18}$NaO$_3$ 269.1154, found 269.1107 (M$^+$ + Na$^+$) and calculated C$_{15}$H$_{19}$O$_3$ 247.1334, found 247.1297 (MH$^+$).

Synthesis of (3R,3aS,5aS,9R,9aS,9bS)-5a,9-dimethyl-3-(morpholinomethyl)-3a,4,5,5a,9,9a-hexahydro-naphtho[1,2-b]furan-2,8(3H,9bH)-dione **15a**

Enoate **13** (53.8mg, 0.218mmol), morpholine (17.7µl, 0.20mmol) and ethanol (2ml) were mixed at 0ºC, then left to warm to room temperature for 72h. The mixture was concentrated to afforded, after column chromatography (30% acetone:hexane as eluent), a pale yellow oil (66.0mg, 98%); R_f 0.25 (30% acetone:hexane); δ_H (400MHz, CDCl$_3$) 6.65 (1H, d, *J* 9.9), 5.83 (1H, d, *J* 9.9), 3.94 (1H, t, *J* 10.7), 3.73 – 3.46 (4H, m), 2.76 (1H, dd, *J* 3.6 and 12.1), 2.62 – 2.39 (4H, m), 2.39 – 2.26 (2H, m), 2.14 (1H, dt, *J* 5.7 and 8.6), 2.13 – 2.05 (1H, m), 1.97 – 1.88 (1H, m), 1.80 (1H, td, *J* 5.8 and 11.5), 1.70 – 1.46 (3H, m), 1.29 (3H, d, *J* 6.9) and 1.11 (3H, s); δ_C (101MHz, CDCl$_3$) 200.54, 177.32, 158.13, 126.55, 81.77, 66.70, 57.61, 53.77, 51.62, 51.08, 43.14, 42.09, 38.14, 37.45, 23.38, 19.18 and 14.53; HRMS (ESI) calculated C$_{19}$H$_{28}$NO$_4$ 334.2018, found 334.1974 (MH$^+$).

Synthesis of (3R,3aS,5aS,9R,9aS,9bS)-3-[(4-hydroxyphenethylamino)methyl]-5a,9-dimethyl-3a,4,5,5a,9,9a-hexa hydronaphtho[1,2-b]furan-2,8-(3H,9bH)-dione **15b**

Enoate **13** (49.7mg, 0.202mmol), tyramine (27.9mg, 0.203mmol) and ethanol (2ml) were mixed at 0ºC, then left to warm to room temperature for 72h. The mixture was concentrated to afforded, after column chromatography (70% acetone:hexane as eluent), a beige foam (65.5mg, 85%); R_f 0.40 (70% acetone:hexane); δ_H (400MHz, CDCl$_3$) 6.95 (2H, d, *J* 8.4), 6.65 (2H, d, *J* 8.4), 6.60 (1H, d, *J* 9.9), 5.81 (1H, d, *J* 9.9), 4.93 (1H, s), 3.91 (1H, t, *J* 10.5), 2.91 –

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2.72 (4H, m), 2.67 (2H, t, *J* 7.0), 2.55 – 2.40 (2H, m), 1.91 – 1.69 (3H, m), 1.67 – 1.57 (1H, m), 1.57 – 1.36 (2H, m), 1.24 (3H, d, *J* 6.8) and 1.06 (3H, s); δ_C (101MHz, CDCl$_3$) 200.76, 177.96, 158.26, 155.00, 130.33, 129.72, 126.54, 115.65, 82.15, 51.55, 51.47, 48.88, 47.26, 45.29, 42.07, 38.26, 37.22, 34.73, 22.75, 19.16 and 14.49; HRMS (ESI) calculated $C_{23}H_{30}NO_4$ 384.2175, found 384.2130 (MH$^+$).

MTS assay
The cytotoxicity of the synthesized compounds was evaluated against HL60 (Human Caucasian promyelocytic leukaemia), CCRF-CEM (Human Caucasian acute lymphoblastic leukaemia), HCT116 (Human colorectal cancer), and WI38 (Human Caucasian foetal lung fibroblast) cell lines, all obtained from the European Collection of Animal Cell Cultures (ECACC, Salisbury, UK).

Cell lines was routinely maintained as a monolayer cell culture (HCT116 and WI38) and suspension cell culture (HL60 and CCRF-CEM) at 37ºC, 5% CO$_2$, 95% air and 100% relative humidity in medium supplemented with 15% foetal bovine serum(Sigma Aldrich), 2mM L-glutamine(Sigma Aldrich) and 50µg/ml gentamicin (Sigma Aldrich). For HL60 and CCRF-CEM cell lines was used RPMI-1640 medium (Lonza), for HCT116–McCoy's medium (Lonza) and for WI38 – EMEM (Lonza). To determine cell viability the colorimetric MTS metabolic activity assay was used (CellTiter 96 Aqueous One Solution, Promega).

One day prior to screening, the cells were seeded in 96-well microtitre plates (100µL per well) at plating densities of 7000 – 10000 cells per well and incubated for 24h. On the day of screening, 10 x 3-fold serial dilutions of compounds were prepared in medium to achieve a final concentration range of 100 – 0.005µM. 100µL of each dilution was added to the cells (duplicate wells) and plates were incubated for a further 48h. The MTS reagent was added directly to the cells (20µL/well) and incubated for 4h, after which colour development was measured at 490nm in a multiwell plate reader. Abs$_{490}$ values obtained from wells without cells (background control) were subtracted from the Abs$_{490}$ obtained for test and untreated control wells. The net Abs$_{490}$ values were used to calculate % cell viability relative to untreated control wells.

Statistical analysis
To derive IC$_{50}$ values for the test compounds, % cell viability compared to that observed in the untreated control was plotted against log(compound concentration) and non-linear regression analysis was performed using GraphPad Prism 5.

To derive GI$_{50}$ and TGI values for the compounds, after the initial 24h incubation (i.e. before compound addition) a plate of each cell line was treated with MTS to represent a measurement of the viable cell population for each cell line at the time of compound addition (T$_0$). Abs$_{490}$ of the test wells after 48h period of exposure to test compound is T$_i$, Abs$_{490}$ at time zero is T$_0$, and the control (untreated cells) Abs$_{490}$ after 48h is C.

Percentage cell growth is calculated as:
[(T$_i$ - T$_0$)/(C - T$_0$)] x 100 for concentrations at which T$_i \geq$ T$_0$
[(T$_i$ - T$_0$)/T$_0$] x 100 for concentrations at which T$_i <$ T$_0$.

Thus: 100% growth – compound has no effect on growth compared to untreated controls;
0% growth – compound completely blocked growth, i.e. number of cells at end of 48h incubation is the same as at the start;

GI$_{50}$ - 50% growth inhibition and signifies the growth inhibitory power of the test agent
TGI - drug concentration resulting in total growth inhibition and signifies the cytostatic effect of the test compound.

## RESULTS AND DISCUSSION

Synthesis
The syntheses all compounds herein began from readily-available α-santonin (**5**), using two related approaches. Initially, the 3-methyl group of the starting material was converted into the exocyclic α-methylene moiety through the well-documented phenylselenylation/oxidative elimination route described previously, affording (**7**) (**Scheme 1**) [9,10].

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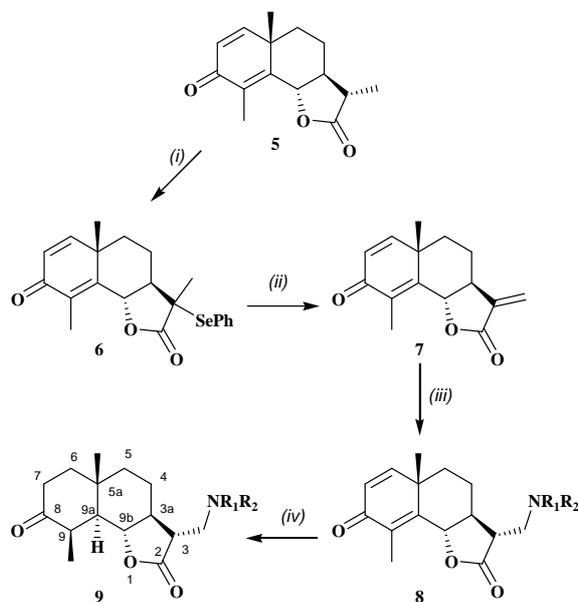
Scheme 1: *Key:* **(i) LDA, PhSeCl; (ii) H_2O_2; (iii) NHR_1R_2, Et_3N; (iv) 5% Pd/C, 32% HCl (cat.), $H_{2(g)}$**
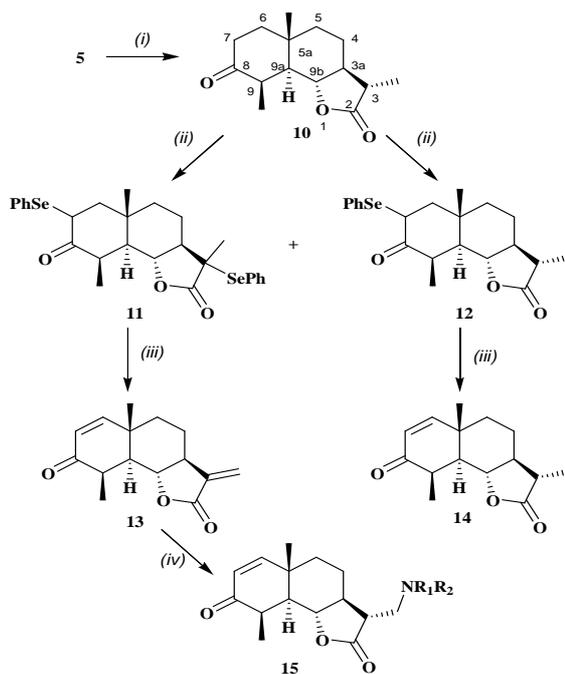
Scheme 2: *Key:* **(i) 5% Pd/C, $H_{2(g)}$, recrystallisation; (ii) LDA, PhSeCl; (iii) H_2O_2; (iv) NHR_1R_2, Et_3N**

A diversified collection of simple aliphatic-, alicyclic-, benzylic-, and phenylethyl-amines (selected based on structure as well as the calculated LogP of the resulting adduct) were added to (**3**) in hot ethanol under microwave irradiation to afford the Michael adducts (**8**) in 30 – 86% isolated yields (**Table 2**) in a modification of the protocol employed by Klochkov for a related series of compounds [11]. Samples of a subset of these adducts were then treated with 5% palladium on carbon under a hydrogen atmosphere in the presence of catalytic hydrochloric acid to reduce the dienone of each system to the corresponding saturated ketone systems (**9**) in modest (40 – 48%) yields.

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These were obtained as an approximately 2:1 ratio of isomers. This is seen by the presence of two signals, a triplet at 3.91ppm (indicative of a doublet of doublets with approximate equivalence of coupling values), and a triplet of doublets at 3.66ppm in the ^{1}H spectrum of (**9b**), in a 2:1 ratio of integrated areas, respectively. This set of signals corresponds to the proton at C9b for each isomer. The triplet at 3.91ppm has coupling constants to the adjacent protons at C9a and C3a of 10.4Hz – indicating only trans-diaxial disposition – derived from hydrogen delivery at the lower (α-face) of the dienone (**8**). The minor isomer with the 9b proton at 3.66ppm (6.6 Hz coupling to proton at 9a) is indicative of hydrogen delivery from the upper face of the dienone. The stereochemical implications of amination and hydrogenation of related systems has been previously examined [12,13].

In a related approach, α-santonin (**5**) was reduced using 5% palladium on carbon in hydrogen atmosphere in the absence of acid. This afforded a mixture of H9/H9a *cis*-isomers, with a pure (9*R*,9a*S*) isomer (**10**) isolable by recrystallisation (**Scheme 2**). This was apparent from the ^{1}H proton spectrum, which had a triplet at 3.99ppm with uniformly large coupling constants of 10.9Hz, indicating trans-diaxial disposition. This was selenylated to afford a mixture of 3,7-bis(phenylselenyl)- (**11**) and 7-phenylselenyl- (**12**) adducts, which were chromatographically separable. Oxidative elimination thereof afforded exocyclic α-methylene enone (**13**) and enone (**14**). Treating (**13**) with amines as before afforded Michael adducts (**15**), in modest yields (**Table 2**).

All the amines (except those represented by (**9**)) were isolated as single isomers, as confirmed by ^{1}H NMR spectroscopy. They were assigned the (3*S*) configuration, consistent with the literature [11].

Cytotoxicity Results
The parent structures (**7**), (**10**), (**13**) and (**14**), as well as amine conjugates (**8**), (**9**) and (**15**) were screened in cell-based MTS cancer assays against three cancer cell lines: CCRF-CEM (human lymphoblastic leukaemia), HL60 (human promyelocytic leukaemia) and HCT116 (human colorectal carcinoma), initially screening at 50μM in a single-point viability study using parthenolide as a control (% viability, **Table 2**). Human lung fibroblasts (WI38) were chosen to determine general toxicity, as an early indication of how similar rapidly-dividing non-cancerous cells may react to the test compounds. Compounds resulting in a cellular viability under 50% in the cancerous cell lines were then subjected to separate IC_{50} determinations as a measure of cytotoxic activity (Table 3).

Not surprisingly, the α-methylene lactone appeared to be the most significant feature for cytotoxicity against all cell lines subject to examination [14,15]. Compounds possessing this feature [(**7**) and (**13**)] exhibited substantially higher levels of toxicity to all lines than the respective saturated lactones [(**10**) and (**14**), see **table 2**]. Similar toxicity patterns were observed against the WI38 fibroblast. The IC_{50} values observed for the α-methylene lactone (**7**) have been reported against several cell lines including the HL60 leukemia line (IC_{50} = 1.14 μM) and the reported data differs only slightly from the current work (being a 72 hour drug exposure in the reported data compared to a 48 hour exposure herein) [10]. The α-methylene lactones derived from santonin showed slightly lower potency than the related parthenolide against promyelocytic leukemia and colorectal cancer lines (see **table 3**, IC_{50} comparisons) but drastically reduced potency against lymphoblastic leukemia.

As a general observation, conversion of the α-methylene group of (**7**) into a substituted aminomethyl group maintained or decreased the cytotoxic activity of the parent compound against promyelocytic leukemia and colon cancer lines, but often enhanced activity against lymphoblastic leukemia (Table 3). Amino derivatives, consequently, often exhibited enhanced selectivity against specific cell-lines. Simple dimethyl- and diethylamino adducts (**8a** and **8b**) showed enhanced selectivity for promyelocytic leukaemia, having inhibitory concentrations [IC_{50}(HL60)] of 6.3μM and 14.2μM respectively [parthenolide IC_{50}(HL60) = 3.7μM], but much poorer activity against the colon cancer line subject to this study (**Table 3**). Significantly, the potential toxicity (as illustrated by activity against the WI38 fibroblast) was suppressed by a greater factor than the activity against HL60 when compared to the parent (**7**). The dimethylamino adduct (**8a**), in particular, showed selective cytotoxicity (being 6-7 fold more potent against the myelocytic leukemia line than the lymphoblastic leukemia or colon cancer lines), having IC_{50}(HL60) = 6.3μM and a safety index [defined as IC_{50} (HL60) / IC_{50} (WI38)] of 10 compared to a safety index of 2 for the parent (**7**). Other simple aliphatic substituents on the amino group (**8c**, **8h**) resulted in similar activity profiles.

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Table 2 Cytotoxicity of α-santonin derivatives[a]

Number	NR₁R₂	CCRF-CEM % Viability (Variance)	HL60 % Viability (Variance)	HCT116 % Viability (Variance)	WI38 % Viability (Variance)
7		22.6 (20.6)	22.5 (5.3)	7.5 (0.2)	21.6 (0.4)
10		89.1 (12.1)	78.0 (10.4)	102.1 (5.4)	88.9 (12.1)
13		4.5 (0.3)	3.1 (0.1)	12.0 (2.2)	28.3 (4.2)
14		54.1 (8.5)	77.7 (1.5)	95.3 (3.4)	104.1 (0.6)
Aliphatic amino					
8a	N(Me)₂	4.1 (0.1)	4.9 (0.6)	56.5 (5.9)	26.8 (5.3)
8b	N(Et)₂	4.7 (0.1)	4.0 (0.5)	27.5 (2.1)	21.2 (1.8)
8c	*tert*-butylamine	45.1 (1.4)	44.3 (5.1)	18.5 (1.6)	47.2 (3.0)
8d	cyclopropylamino	113.2 (13.0)	113.1 (2.8)	89.2 (12.0)	103.4 (3.7)
8e	cyclopentylamino	86.9 (4.5)	70.7 (27.6)	84.7 (7.9)	95.6 (13.9)
8f	(1*R*)-1,2,3,4-tetrahydro-1-naphthylamine	14.6 (5.5)	79.7 (10.3)	101.7 (5.3)	98.2 (0.4)
Alicyclic / heterocyclic amino					
8g	pyrrolidino	23.7 (0.7)	50.2 (0.6)	41.2 (0.1)	45.2 (0.4)
8h	morpholino	4.9 (0.1)	22.3 (4.0)	94.4 (9.6)	80.3 (6.5)
9a	morpholino	118.4 (15.6)	75.9 (14.6)	88.8 (11.8)	113.3 (21.8)
15a	morpholino	18.9 (4.3)	63.6 (12.3)	78.8 (1.8)	100.7 (21.7)
8i	4-acetylpiperazin-1-yl	13.5 (10.1)	68.8 (33.5)	91.4 (3.1)	116.7 (17.7)
8j	4-cyclohexylpiperazin-1-yl	68.8 (8.0)	78.9 (4.3)	90.3 (1.0)	91.3 (12.8)
8k	4-(2-chlorophenyl)piperazin-1-yl	6.0 (0.2)	40.2 (9.5)	79.7 (4.3)	67.5 (18.0)
8l	4-benzylpiperidin-1-yl	53.3 (5.5)	72.0 (4.0)	103.2 (8.1)	106.3 (4.1)
Benzyl / heteroarylmethylamino					
8m	benzylamino	105.2 (5.9)	108.8 (26.4)	102.7 (7.3)	107.0 (23.0)
8n	4-chlorobenzylamino	19.6 (4.9)	39.8 (1.4)	104.4 (10.5)	86.9 (17.8)
8o	2-fluorobenzylamino	5.5 (1.3)	6.2 (0.4)	64.7 (6.5)	36.1 (14.1)
8p	4-fluorobenzylamino	86.2 (16.2)	63.7 (11.6)	103.8 (1.2)	100.0 (3.0)
8q	2,4-dimethoxybenzylamino	72.7 (2.9)	96.8 (7.9)	90.5 (14.6)	101.3 (12.5)
8r	pyridin-2-ylmethylamino	94.8 (0.9)	89.7 (1.5)	84.0 (1.9)	122.9 (9.4)
8s	pyridin-3-ylmethylamino	127.6 (6.3)	77.3 (4.3)	102.9 (5.9)	105.9 (7.8)
8t	pyridin-4-ylmethylamino	82.4 (17.7)	56.7 (5.6)	102.1 (10.9)	94.5 (10.9)
(Hetero)arylethylamino					
8u	2-(4-chlorophenyl)ethylamino	35.5 (2.5)	45.4 (2.9)	68.7 (7.6)	110.5 (7.2)
8v	2-(2-fluorophenyl)ethylamino	25.1 (9.5)	45.7 (2.8)	97.6 (10.9)	116.1 (34.6)
8w	2-(4-hydroxyphenyl)ethylamino	82.4 (17.7)	56.7 (5.6)	102.1 (10.9)	94.5 (10.9)
9b	2-(4-hydroxyphenyl)ethylamino	90.7 (3.2)	64.6 (3.2)	74.3 (11.0)	104.3 (36.1)
15b	2-(4-hydroxyphenyl)ethylamino	7.4 (0.8)	23.5 (2.6)	71.5 (2.2)	113.4 (34.1)
8x	2-(3-methoxyphenyl)ethylamino	87.1 (1.8)	77.6 (8.4)	91.9 (5.6)	120.0 (1.3)
9c	2-(3methoxyphenyl)ethylamino	96.5 (24.0)	63.2 (1.0)	84.4 (10.7)	123.5 (29.7)
8y	2-(4-methoxyphenyl)ethylamino	85.0 (3.2)	61.9 (0.6)	94.5 (0.4)	113.8 (7.3)
8z	2-(pyridin-2-yl)ethylamino	96.3 (0.4)	79.6 (1.8)	80.7 (6.8)	122.7 (11.1)
Emetine		7.0 (0.1)	4.2 (0.1)	11.9 (0.2)	7.6 (0.2)
Parthenolide		6.7 (3.0)	3.4 (2.6)	4.8 (0.2)	7.7 (0.4)

[a] All determinations were performed on duplicate samples; [b] Residual cell viability at 50μM concentration

Due to the highly lipophilic nature of the terpenoid scaffold, introduction of the piperazino residue was examined. Only derivatives containing highly lipophilic residues in the 4-position of the piperazine such as 2-chlorophenyl (**8k**) exhibited useful activity, the activity profile showing selectivity towards the lymphoblastic leukemia [IC₅₀(CCRF-CEM) = 13μM]. Consequently, we set out to evaluate adducts containing aromatic residues associated with the amino group.

Our rationale in selection of aryl-substituted amino analogues for evaluation was that the basicity of the amino residue should be retained. Consequently, we set out to avoid poorly basic aniline derivatives. A series of aryl-ethylamino adducts and benzylamine adducts was generated for evaluation. Lipophilic aryl residues appeared to be the prime determinant of activity, substitution of the aromatic residue with a lipophilic group in the *ortho-* position being particularly favoured (**8f**, **8k**, **8o** and **8v, table 3**). Halide substitution of the aromatic ring proved to be optimal for activity, with fluorine substitution preferred (**8o** and **8v**). Unlike the series lacking aryl substituents, the series with lipophilic aryl substituents displayed an enhanced activity against lymphoblastic leukemia (CCRF-CEM), along with activity against promyelocytic leukemia (HL60). Once again these adducts were inactive against the colon cancer line (HCT116) and displayed reduced toxicity against the fibroblast (WI38).

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From a structural point of view, it is interesting to note the trend in biological activity of the morpholino series (**8h** → **9a** → **15a**), particularly against CCRF-CEM cells (Table 2). While (**8h**) proved significantly more cytotoxic than the parent (**7**) [IC_{50}(CCRF-CEM) = 24.3µM and >100µM respectively], all activity was lost on reducing the dienone to (**9a**). This activity is restored with the addition of an endocyclic double bond in (**15a**), itself more active than its parent (**13**) against CCRF cells [IC_{50}(CCRF-CEM) = 15µM and >100µM respectively]. Unsaturation in the ring bearing the ketone was also required for activity against promyelocytic leukaemia. There appears to be no absolute requirement for this ring to contain a second double bond.

Table 3 Cytotoxic activity (IC_{50} / µM) of derivatives of α-santonin[a]

	CCRF	HL60	HCT116	WI38
7	>100	9.6	14	19.9
13	>100	9.4	14	48
8a	35	6.3	42	66
8b	32	14.2	79	86
8c	>100	19.6	51	65
8f	14.2	14.6	>100	>100
8g	>100	54	>100	>100
8h	24.3	14.4	>100	>100
15a	15	54	>100	70
8i	49	68	>100	>100
8k	13	25.7	94	>100
8n	25.5	53	>100	>100
8o	5.6	7.4	51	56
8u	53	43	>100	>100
8v	8.7	16.7	>100	90
15b	29.1	15.8	65	>100
Parthenolide	4.5	5.1	7.2	57

[a] All determinations were performed on duplicate samples

CONCLUSION

In this paper we have described the synthesis and evaluation of three series of aminated eudesmanolide sesquiterpenoids derived from four parent structures (**7**, **10**, **13** and **14**) having different degrees of unsaturation. The general requirement for activity was the presence of the α-methylene-γ-lactone group or an amine conjugate thereof. Amine conjugates generally lacked activity against the HCT116 colon cancer cell line, but small aliphatic amine conjugates such as dimethylamino (**8a**) showed selectivity towards promyelocytic leukemia over all other cell lines examined and useful toxicity profiles [IC_{50} (HL60) = 6.3µM, IC_{50} (WI38) = 66µM]. Highly lipophilic benzylamino adducts [(**8p**), IC_{50} = 7.4µM, 5.6µM and 56µM against promyelocytic, lymphoblastic leukaemia and WI38 fibroblasts respectively] and the related phenethylamino adducts displayed useful activity and toxicity profiles against both lymphoblastic and promyelocytic leukemia cell lines. The present study forms the basis of further investigations into the structural modification of naturally-occurring, bioactive sesquiterpenes.

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