Supporting Information

Synthesis of fusidic acid bioisosteres as antiplasmodial agents and molecular docking studies in the binding site of Elongation Factor-G

Gurminder Kaur,[a,b] Kawaljit Singh,[a,b] Elumalai Pavadai,[a,b] Mathew Njoroge,[a,b] Marlene Espinoza-Moraga,[a,b] Carmen De Kock,[c] Peter J. Smith,[c] Sergio Wittlin[d,e] and Kelly Chibale[a,b,f,*]

[a]Department of Chemistry, University of Cape Town, Rondebosch 7701, South Africa. e-mail: Kelly.Chibale@uct.ac.za; Fax: +27-21-6505195; Phone: +27-21-6502553.

[b]South African Medical Research Council Drug Discovery and Development Research Unit, University of Cape Town, Rondebosch 7701, South Africa.

[c]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Groote Schuur Hospital, Observatory, 7925, South Africa.

[d]University of Basel, 4002 Basel, Switzerland.

[e]Swiss Tropical and Public Health Institute, Socinstrasse 57, 4002 Basel, Switzerland.

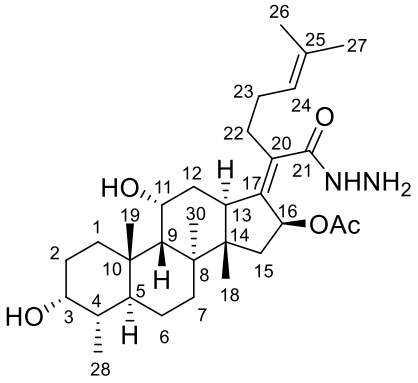
[f]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Rondebosch 7701, South Africa.

Experimental Section

Fusidic acid was purchased from Ava Chem Scientific while all other reagents were purchased from Sigma Aldrich. All target compounds and intermediates were characterised by ^{1}HNMR, ^{13}CNMR and MS. NMR spectra were recorded on a Bruker 400 spectrometer. The ^{1}H NMR data are reported as follows: chemical shift in parts per million (δ) downfield of tetramethylsilane (TMS), multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, dd =doublets of doublets and m = multiplet), coupling constant (Hz), and integrated value. The ^{13}C NMR spectra were measured with complete proton decoupling. Purities were determined on Waters' HPLC using X-bridge C18 5µm column (4.6 x 150 mm); organic phase: 10 mM Ammonium acetate (pH 3.7) in HPLC grade methanol, aqueous phase: 10 mM Ammonium acetate (pH 3.7) in HPLC grade water; flow rate = 1.20 mL/min; detector: photodiode array (PDA) (Method A). Peak purities of some target compounds were also determined using a liquid chromatograph equipped with a UV-Diode Array Detector. Chromatographic conditions were: stationary phase: Agilent Poroshell 120 C18 reversed phase column, internal diameter 3.0 mm×50 mm length containing 2.7 µm particles. Mobile phase A: 10 mM CH_3COONH_4 pH 3.7 in H_2O and B: 10 mM CH_3COONH_4 pH 3.7 in MeOH was delivered at a flow–rate of 1.0 ml/min in gradient mode set to initially run at 10% B for

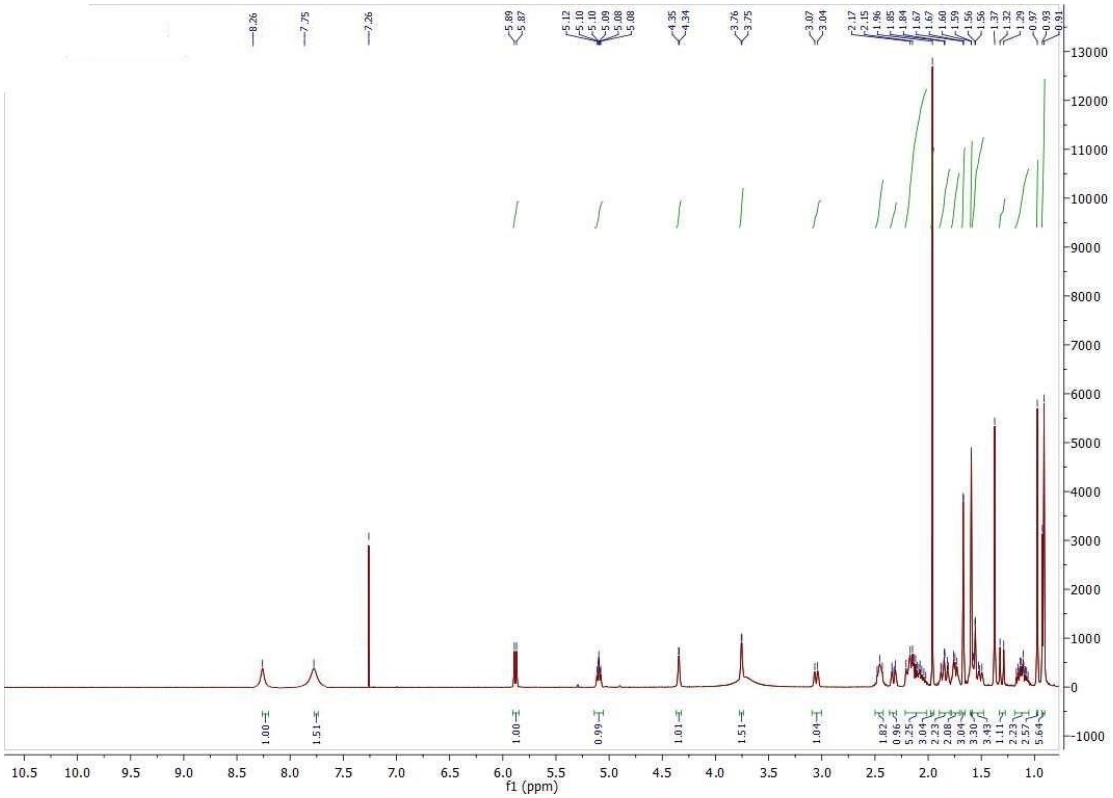
0.5 min, then linearly increased to 90% B over 2 min and held constant for another 3.5 min. The column was subsequently re-equilibrated to starting mobile phase composition for 2 min to give total run time of 8 min. Column temperature was maintained at 40^{o}C. The diode array detector was programmed to monitor absorption over the wavelength range of 190-600 nm (Method B). The purities of all compounds were found to be >95%. Melting points were obtained from a Reichert-Jung Thermovar hot-stage microscope apparatus and are uncorrected.

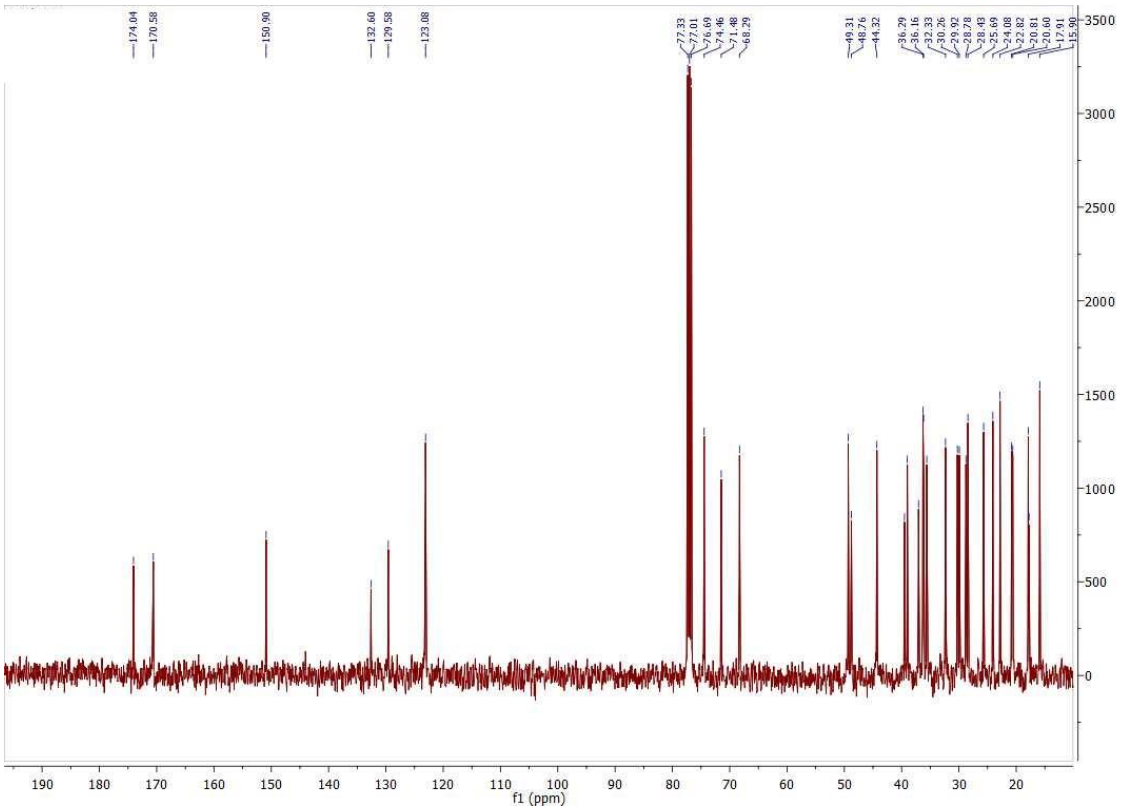
Compound 2: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-17-(1-hydrazinyl-6-methyl-1-oxohept-5-en-2-ylidene)-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



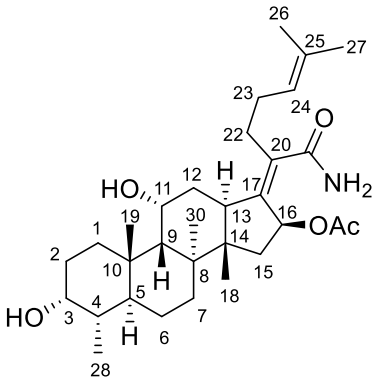
To a solution of Fusidic acid **1** (0.2g, 0.38 mmol) in acetonitrile (10 ml), N-hydroxybenzotriazole (HOBt) (0.062g, 0.44 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (0.085g, 0.44 mmol) were added and reaction was stirred at room temperature for 3h. Hydrazine hydrate (0.07 ml, 1.52 mmol) was then added and resulting reaction mixture was further stirred at room temperature for 16 h. After completion of reaction (TLC), solvent was removed *in vacuo* and the residue was taken in EtOAc (25 ml). The organic phase was washed with water (3×15 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography on silica gel using the EtOAc-hexane mixture as eluent afforded product **2** as a colourless solid (0.2g, 98%); R_f 0.2 (90% EtOAc:hexane); M. pt. 128-130^{o}C; ^{1}H NMR (400MHz, $CDCl_3$) δ 8.26 (br s, 1H, CON*H*), 7.75 (br s, 2H, NH_2), 5.88 (d, *J* = 8.25 Hz, 1H, C16-*H*), 5.10 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.34 (m, 1H, C11-*H*), 3.76 (m, 1H, C3-*H*), 3.07-3.04 (m, 1H, C13-*H*), 2.48-2.43 (m, 2H, C22-*H*), 2.34-2.31 (m, 1H, C12-*H*), 2.21-2.02 (m, 5H, C1-*H*, C5-*H*, C15-*H* and 2×C23-*H*), 1.96 (s, 3H, OCOCH_3), 1.88-1.81 (m, 2H, C2-*H* and C12-*H*), 1.76-1.73 (m, 2H, C2-*H* and C7-*H*), 1.67 (s, 3H, C27-CH_3), 1.60 (s, 3H, C26-CH_3), 1.56-1.49 (m, 4H, C1-*H*, C4-*H*, C6-*H* and C9-*H*), 1.37 (s, 3H, C30-CH_3), 1.32-1.29 (m, 1H, C15-*H*), 1.17-1.06 (m, 2H, C6-*H*

and C7-*H*), 0.97 (s, 3H, C19-CH_3), 0.93-0.91 (m, 6H, C18-CH_3 and C28-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 170.5, 150.9, 132.6, 129.5, 123.0, 74.4 (2C), 71.4, 68.2, 49.3, 48.7, 44.3, 39.5, 39.0, 37.0, 36.2, 36.1, 35.6, 32.3, 30.2, 29.9, 28.7, 28.4, 25.6, 22.8, 20.8, 20.6, 17.9, 17.7 and 15.9; MS: *m/z* 531 $[M+H]^+$, 471 $[M-OAc]^+$; HPLC purity (Method A) 97% (t_r = 18.44).

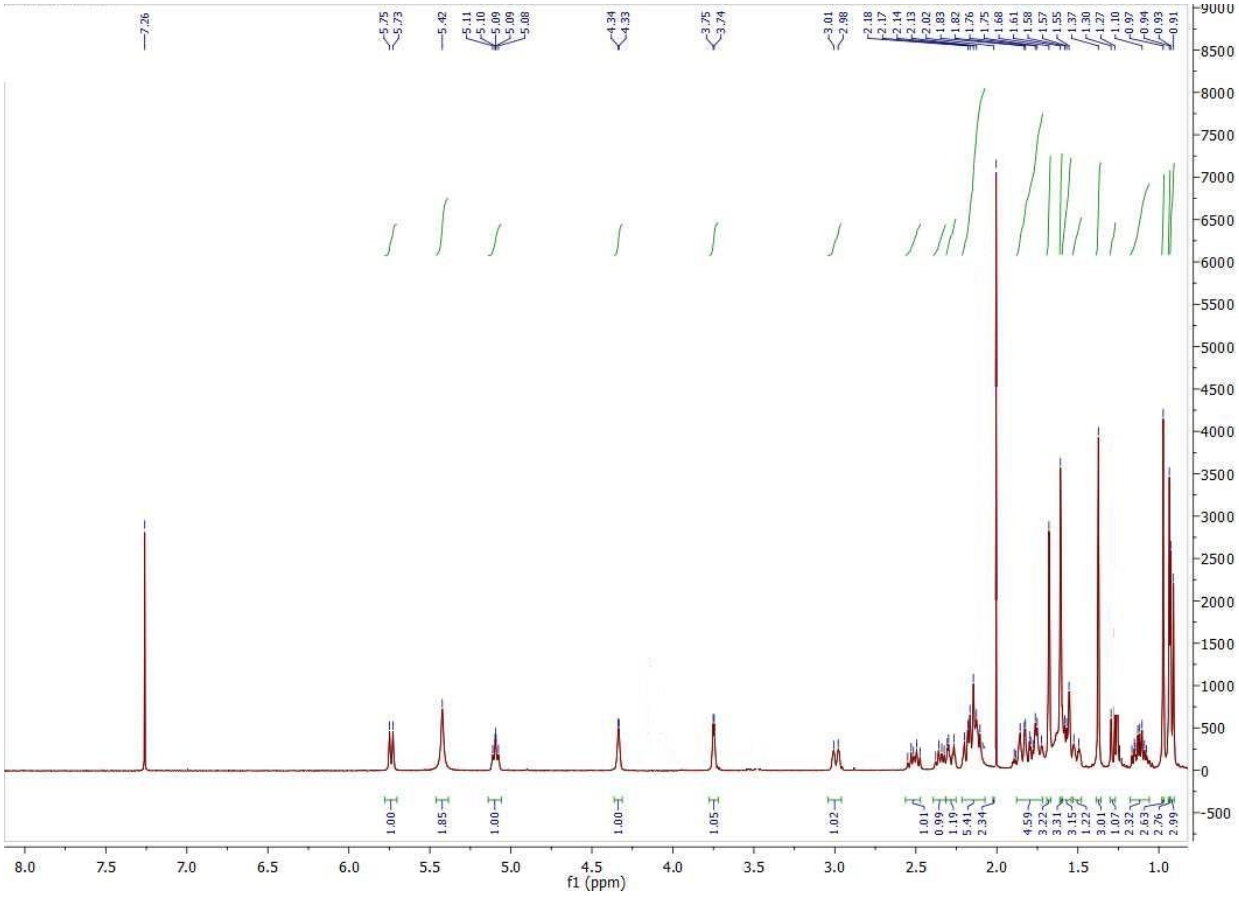




Compound 3: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-17-(1-amino-6-methyl-1-oxohept-5-en-2-ylidene)-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



To a solution of Fusidic acid **1** (3g, 0.0058 mol) in DMF (10 ml), ammonium chloride (1.9g, 0.035 mol), DIPEA (6 ml, 0.035 mol) and HOBt (1.2g, 0.0087 mol) were added and stirred at room temperature for 15 min. followed by addition of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (4.53g, 0.0087 mol) and resulting reaction mixture was stirred at room temperature for 16 h. After completion of reaction (TLC), reaction mixture was diluted with EtOAc (15 ml) and washed with water (3×15 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography on silica gel using EtOAc:hexane as eluent afforded products **3** as a colourless solid (0.93g, 31%); R_f 0.5 (90% EtOAc:hexane); M. pt. 223-225^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 5.74 (d, *J* = 8.44 Hz, 1H, C16-*H*), 5.42 (br s, 2H, CONH_2), 5.09 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.34 (m, 1H, C11-*H*), 3.75 (m, 1H, C3-*H*), 3.01-2.98 (m, 1H, C13-*H*), 2.55-2.47 (m, 1H, C22-*H*), 2.38-2.33 (m, 1H, C22-*H*), 2.31-2.26 (m, 1H, C12-*H*), 2.20-2.08 (m, 5H, C1-*H*, C5-*H*, C15-*H* and 2×C23-*H*), 2.02 (s, 3H, OCOCH_3), 1.89-1.73 (m, 4H, 2×C2-*H*, C7-*H* and C12-*H*), 1.68 (s, 3H, C27-CH_3), 1.61 (s, 3H, C26-CH_3), 1.58-1.55 (m, 3H, C1-*H*, C6-*H* and C9-*H*), 1.52-1.49 (m, 1H, C4-*H*), 1.37 (s, 3H, C30-CH_3), 1.30-1.27 (m, 1H, C15-*H*), 1.17-1.08 (m, 2H, C6-*H* and C7-*H*), 0.97 (s, 3H, C19-CH_3), 0.94 (s, 3H, C18-CH_3), 0.92 (d, *J* = 6.79 Hz, C28-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.6, 171.0, 142.5, 133.9, 132.6, 123.2, 73.7, 71.3, 68.3, 60.3, 49.3, 48.7, 43.2, 39.5, 39.2, 37.1, 36.2, 35.6, 32.4, 30.3, 29.9, 29.3, 27.9, 25.6, 24.1, 22.7, 20.7 (C2), 17.8, 17.7 and 15.9; MS: *m/z* 516 $[M+H]^+$, 456 $[M-OAc]^+$; HPLC purity (Method A) 96% (t_r = 18.53).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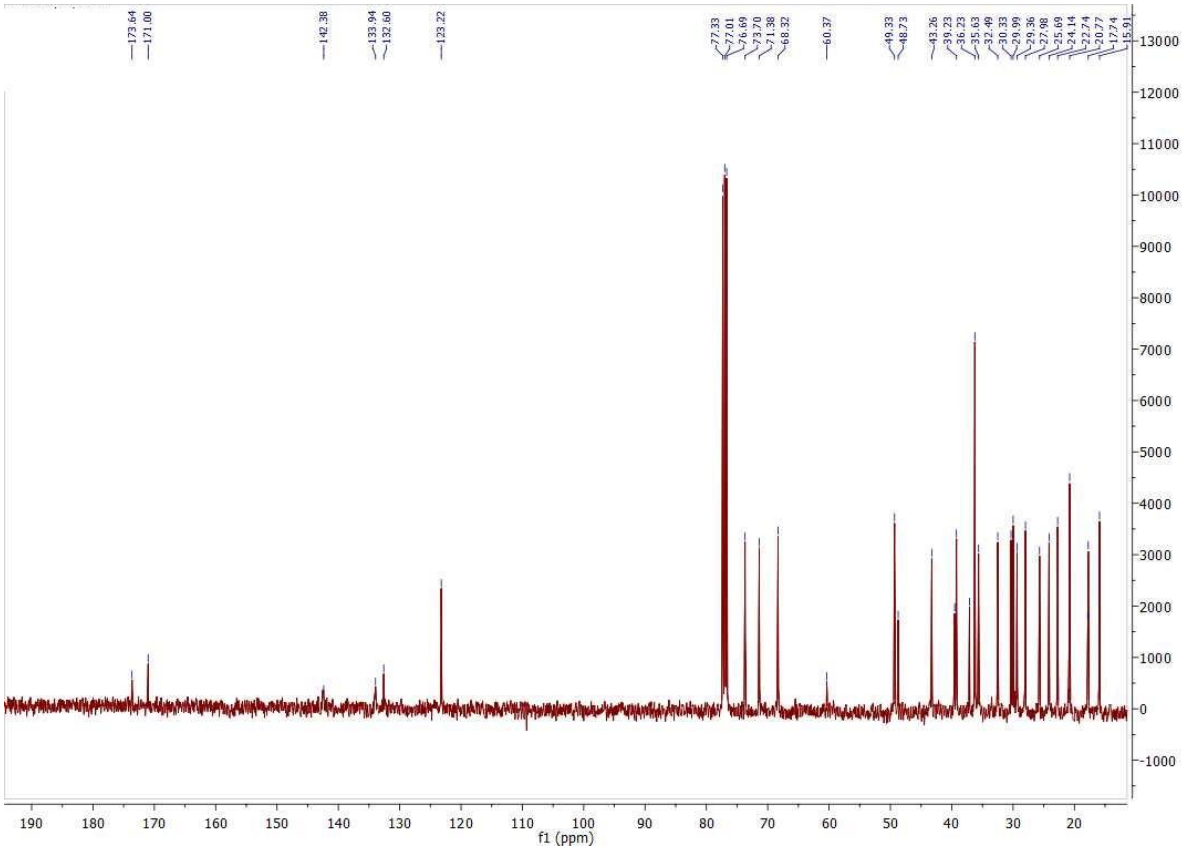
f1 (ppm)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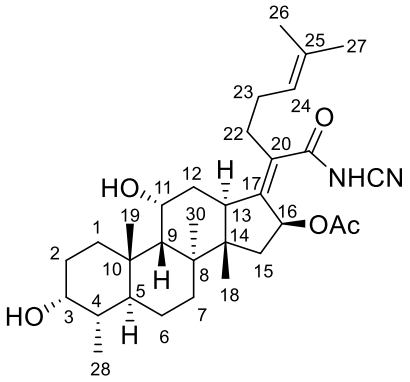
29.99
29.36
27.98
25.69
24.14
22.74
20.77
17.74
15.91
f1 (ppm)

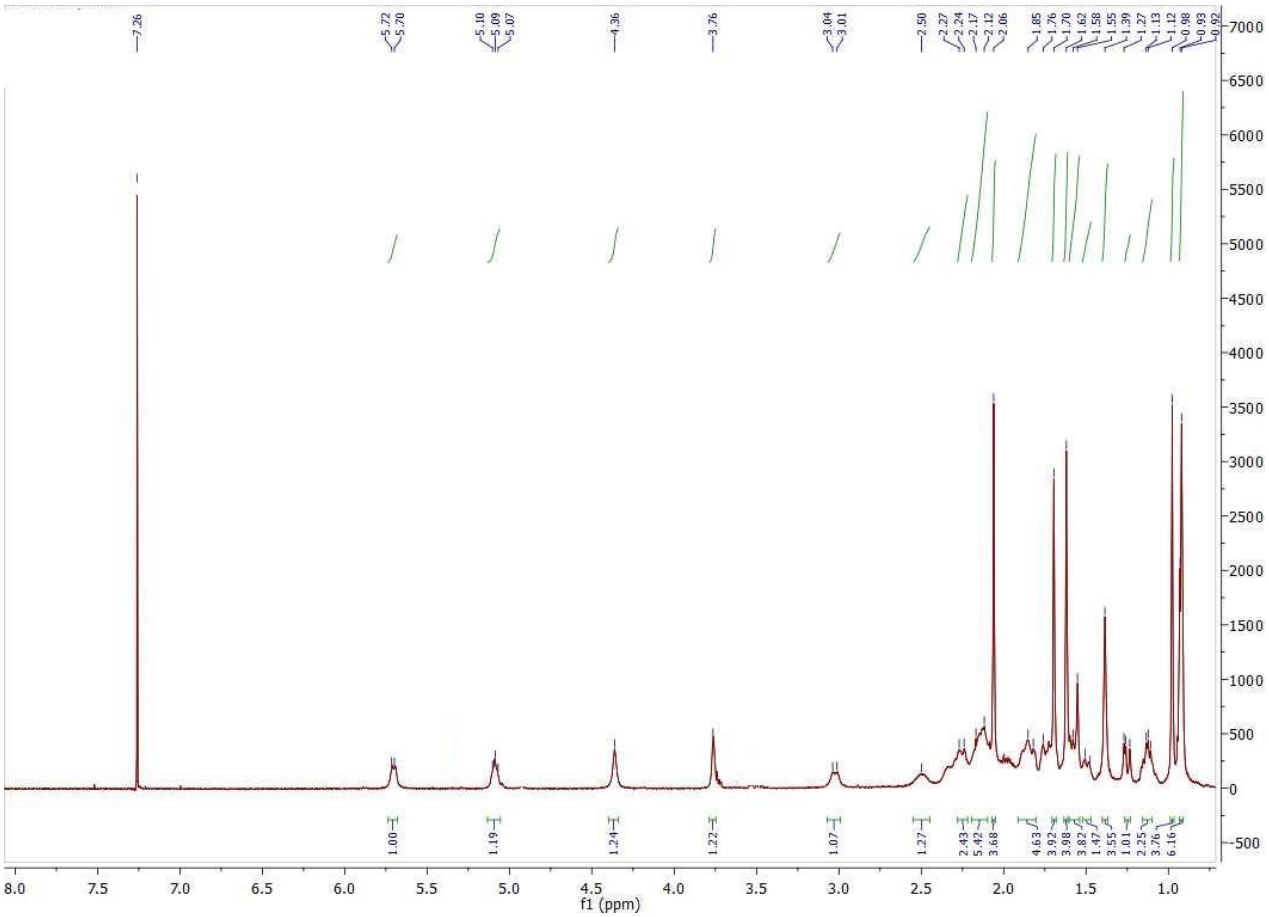
General synthetic procedure for compounds 4 and 5

To a solution of Fusidic acid **1** (1.0 equiv.) in DCM (20 ml), cyanamide (in case of **4**) or aminotetrazole (in case of **5**) (2.0 equiv.), EDCI (1.20 equiv.), HOBt (1.20 equiv.) and DIPEA (3.0 equiv.) were added and resulting reaction mixture was stirred at room temperature for 24 h. After completion of reaction (TLC), reaction mixture was diluted with DCM (15 ml) and washed with water (3×15 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography on silica gel using MeOH:DCM as eluent afforded product.

Compound 4: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-17-(1-cyanamido-6-methyl-1-oxohept-5-en-2-ylidene)-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



Colourless solid (0.10g, 53%); R_f 0.3 (8% MeOH:DCM); M. pt. 142-144^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 5.71 (d, *J* = 8.44 Hz, 1H, C16-*H*), 5.09 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.36 (m, 1H, C11-*H*), 3.76 (m, 1H, C3-*H*), 3.04-3.01 (m, 1H, C13-*H*), 2.50 (m, 1H, C22-*H*), 2.27-2.24 (m, 2H, C12-*H* and C22-*H*), 2.17-2.12 (m, 5H, C1-*H*, C5-*H*, C15-*H* and 2×C23-*H*), 2.06 (s, 3H, OCOCH_3), 1.85-1.82 (m, 4H, 2×C2-*H*, C7-*H* and C12-*H*), 1.70 (s, 3H, C27-CH_3), 1.62 (s, 3H, C26-CH_3), 1.58-1.55 (m, 3H, C1-*H*, C6-*H* and C9-*H*), 1.50-1.48 (m, 1H, C4-*H*), 1.39 (s, 3H, C30-CH_3), 1.27-1.23 (m, 1H, C15-*H*), 1.13-1.11 (m, 2H, C6-*H* and C7-*H*), 0.98 (s, 3H, C19-CH_3), 0.93-0.92 (m, 6H, C18-CH_3 and C28-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8 (2C), 147.4, 133.5, 129.8, 122.5, 106.7, 73.8, 71.5, 68.3, 49.5, 48.7, 43.6, 39.5, 39.0, 36.8, 36.5, 35.8, 35.1, 31.9, 30.1, 29.7, 29.1, 28.1, 25.7, 23.8, 23.2, 20.8, 20.7, 17.8, 17.7 and 15.8; MS: *m/z* 563 $[M+23]^+$, 481 $[M-OAc]^+$; HPLC purity (Method B) 96% (t_r = 3.85).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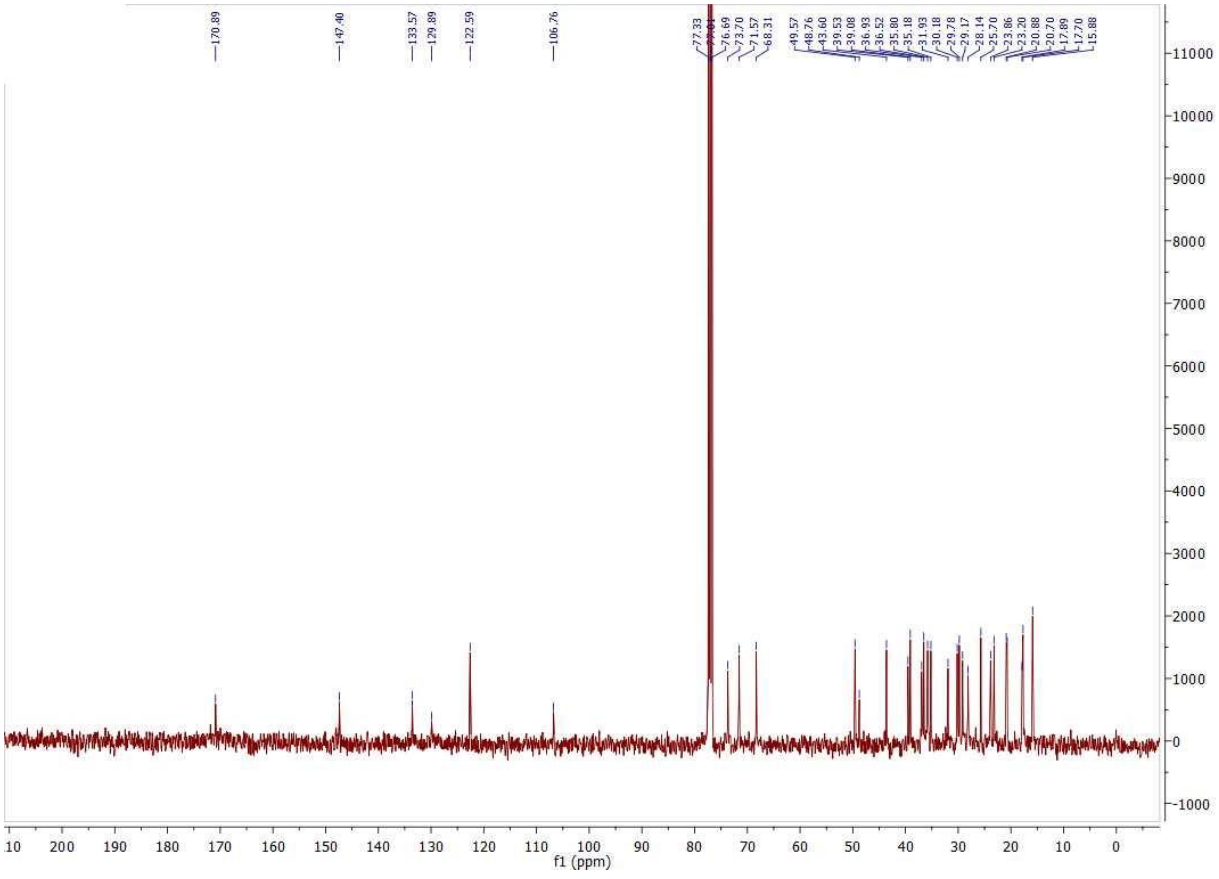
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39.08
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31.93
30.18
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20.88
20.70
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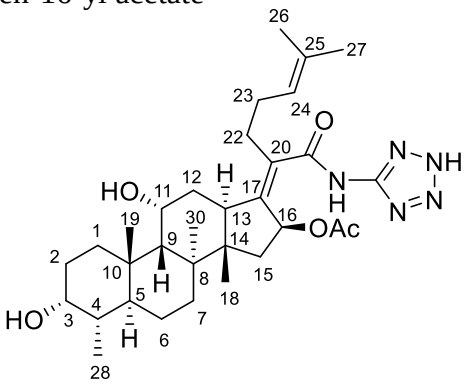
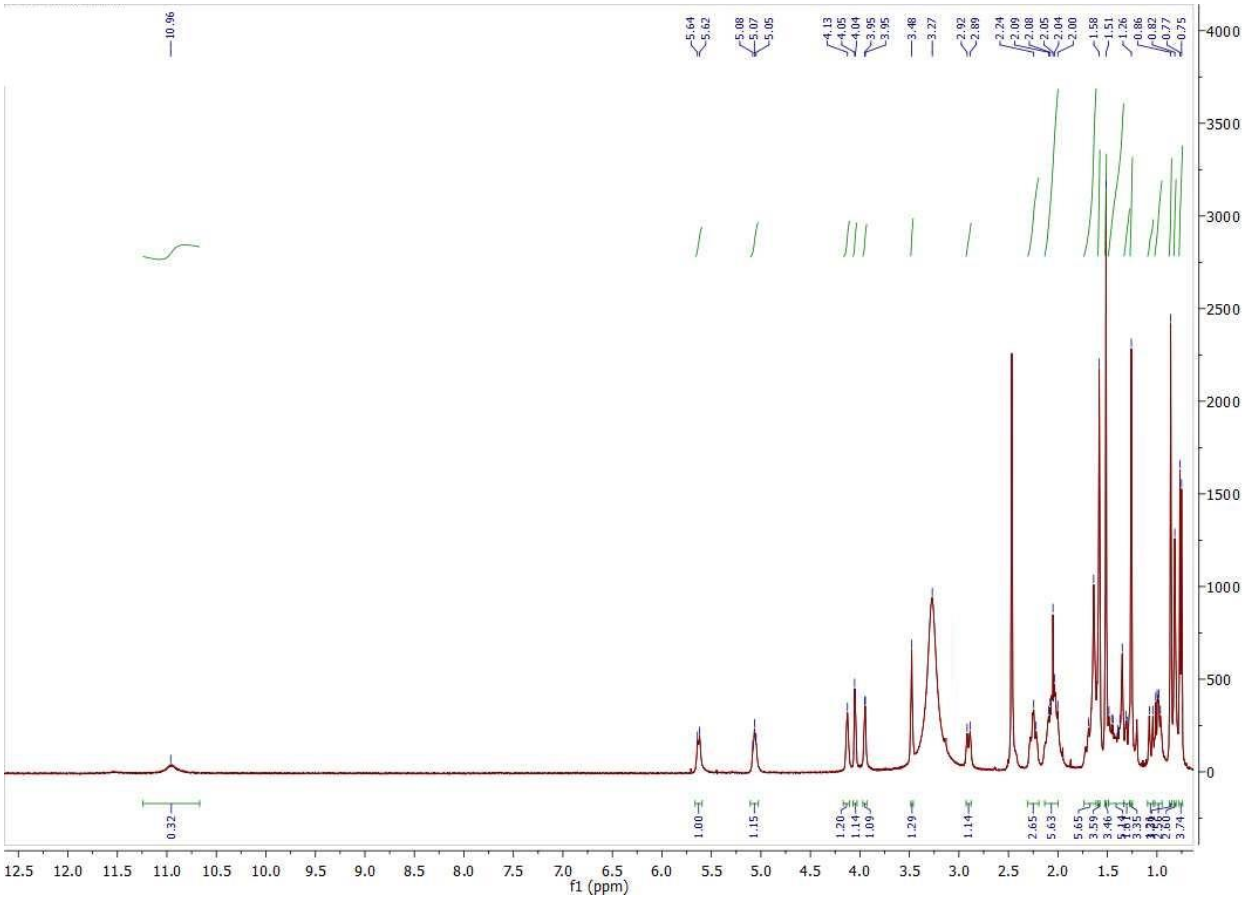
17.70
15.88
f1 (ppm)

Compound 5: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-17-(1-((2H-tetrazol-5-yl)amino)-6-methyl-1-oxohept-5-en-2-ylidene)-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



Colourless solid (0.10g, 29%); R_f 0.2 (8% MeOH:DCM); M. pt. >280^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.96 (br s, 1H, CON*H*), 5.63 (d, *J* = 8.80 Hz, 1H, C16-*H*), 5.07 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.13 (m, 1H, C11-*H*), 4.05 (d, *J* = 3.48 Hz, 1H, C11-O*H*), 3.95 (d, *J* = 3.12 Hz, 1H, C3-O*H*), 3.48 (m, 1H, C3-*H*), 2.92-2.89 (m, 1H, C13-*H*), 2.24-2.22 (m, 3H, C12-*H* and 2×C22-*H*), 2.09-2.00 (m, 5H, C1-*H*, C5-*H*, C15-*H* and 2×C23-*H*), 1.69-1.64 (m, 5H, C2-*H*, C12-*H* and OCOCH_3), 1.58 (s, 3H, C27-CH_3), 1.51 (s, 3H, C26-CH_3), 1.48-1.35 (m, 5H, C1-*H*, C2-*H*, C6-*H*, C7-*H* and C9-*H*), 1.31-1.30 (m, 1H, C4-*H*), 1.26 (s, 3H, C30-CH_3), 1.08-1.04 (m, 1H, C15-*H*), 1.00-0.97 (m, 2H, C6-*H* and C7-*H*), 0.86 (s, 3H, C19-CH_3), 0.82 (s, 3H, C18-CH_3), 0.76 (d, *J* = 6.60 Hz, 3H, C28-CH_3); ^{13}C NMR (100 MHz, CD3OD) δ 170.6 (2C), 152.0, 145.0, 132.4, 132.2, 122.8, 73.9, 71.03, 67.1, 54.1, 49.4, 48.5, 43.3, 39.3, 38.8, 36.8, 36.4, 35.8, 35.4, 31.4, 29.6, 28.9, 27.4, 24.4, 22.4 (2C), 20.9, 19.1, 16.5 (2C) and 15.0; MS: *m/z* 606 $[M+23]^+$, 524 $[M\text{-}OAc]^+$; HPLC purity (Method B) 99% (t_r = 3.92).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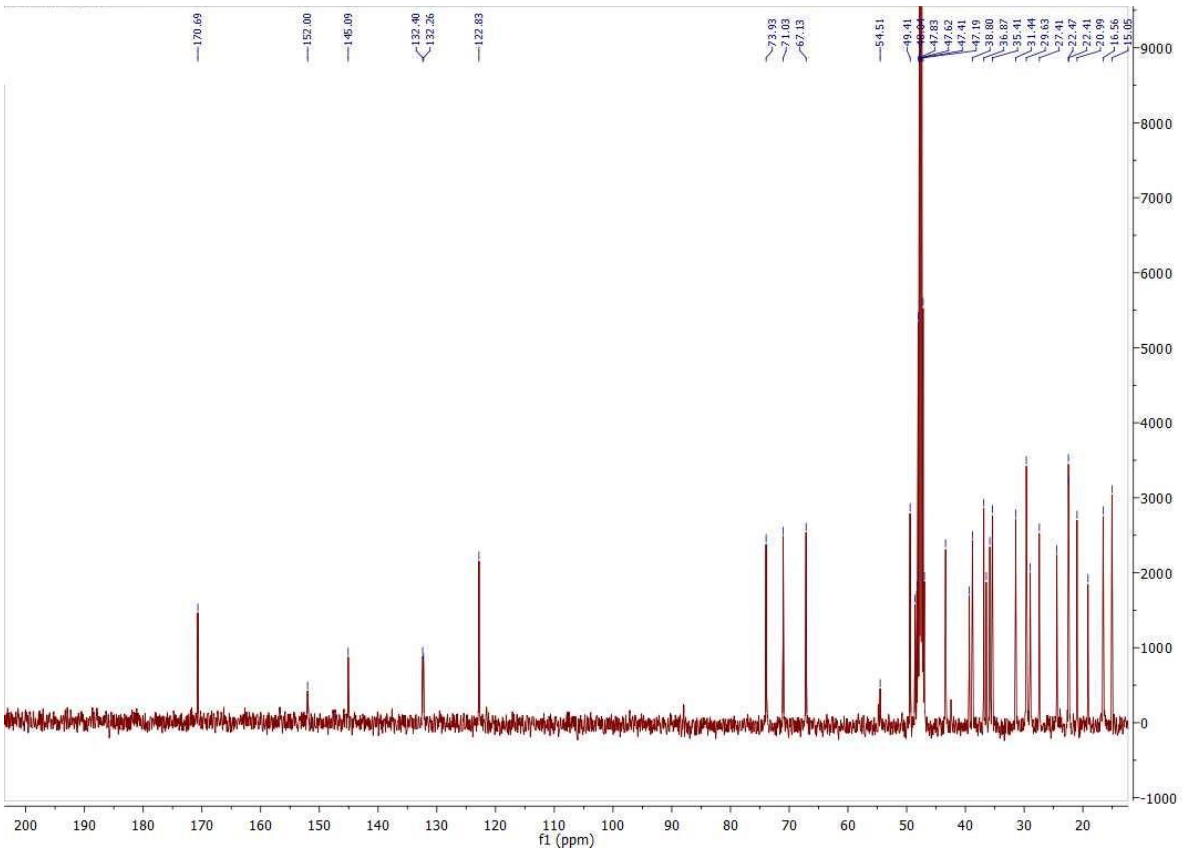
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47.41
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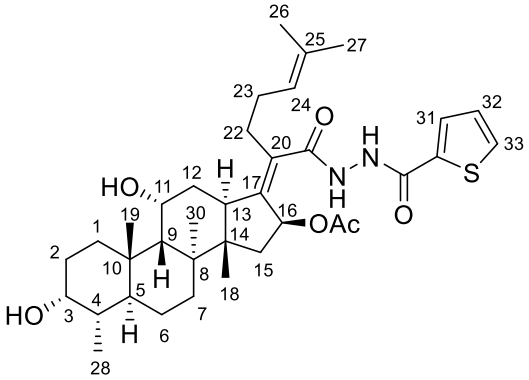
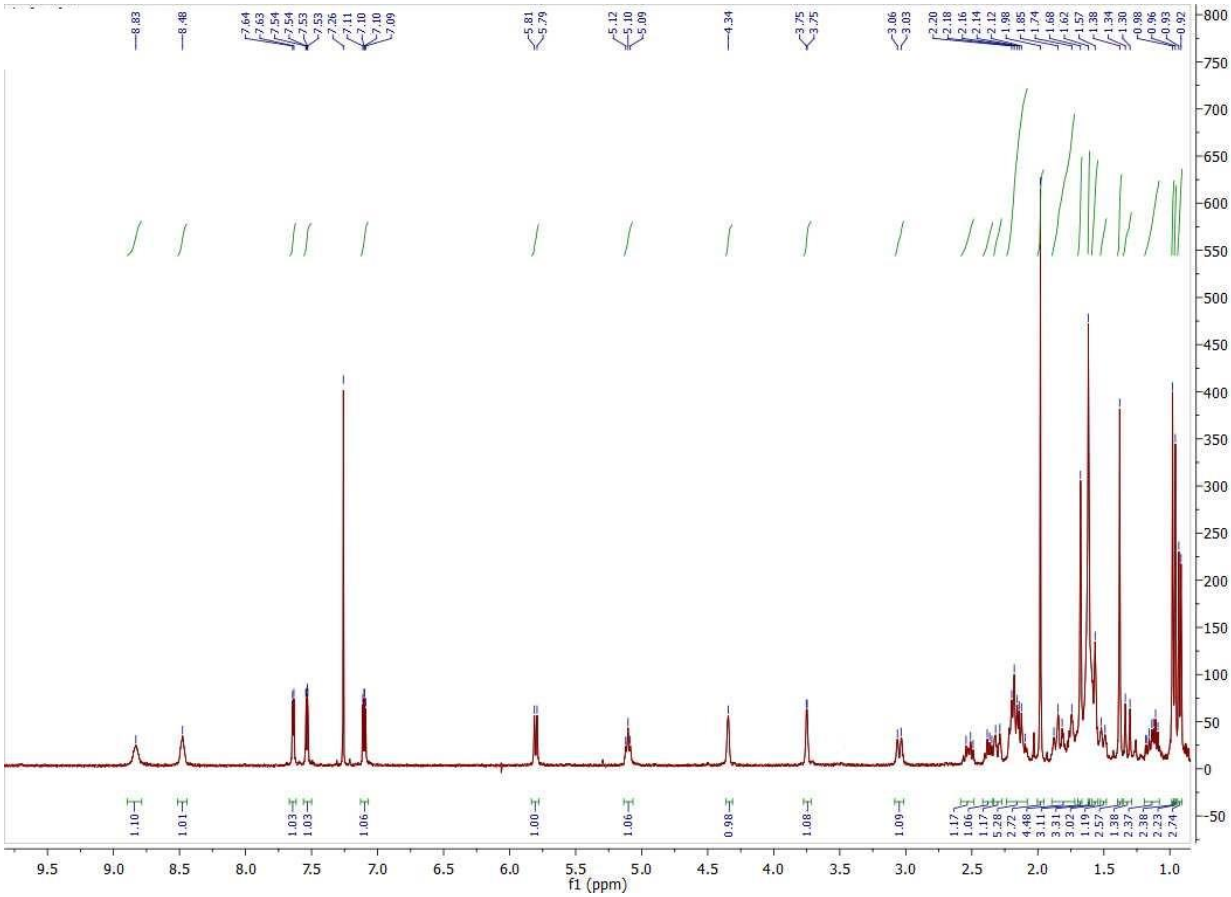
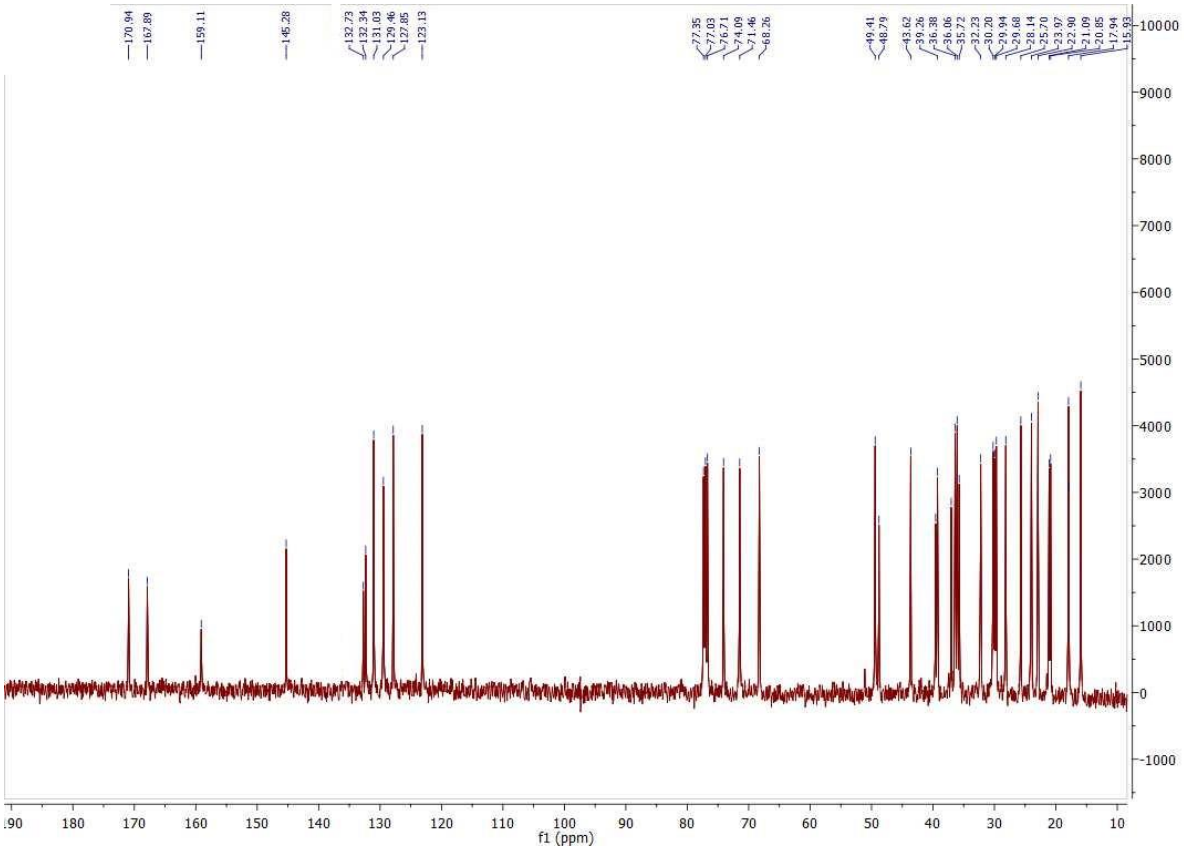
38.80
36.87
35.41
31.44
29.63
27.41
22.47
22.41
20.99
16.56
15.05
f1 (ppm)

Compound 6: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-3,11-dihydroxy-4,8,10,14-tetramethyl-17-(6-methyl-1-oxo-1-(2-(thiophene-2-carbonyl)hydrazinyl)hept-5-en-2-ylidene)hexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



To a solution of Fusidic acid **1** (0.1g, 0.19 mmol) in DMF (3 ml), thiophene-2-carbohydrazide (0.03g, 0.23 mmol), TBTU (0.07g, 0.23 mmol) and DIPEA (0.07g, 0.57 mmol) were added and resulting reaction mixture was stirred at room temperature for 16 h. After completion of reaction (TLC), reaction mixture was diluted with EtOAc (15 ml) and washed with water (3×15 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography on silica gel using EtOAc:hexane as eluent afforded product **6** as a colourless solid (0.062g, 50%); R_f 0.5 (60% EtOAc:hexane); M. pt. 144-146^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (br s, 1H, CON*H*), 8.48 (br s, 1H, N*H*), 7.64 (d, *J* = 4.0 Hz, 1H, ArH^{31}), 7.55 (d, *J* = 4.94 Hz, 1H, ArH^{33}), 7.10 (t, *J* = 4.76 Hz, 1H, ArH^{32}), 5.80 (d, *J* = 8.06 Hz, 1H, C16-*H*), 5.10 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.34 (m, 1H, C11-*H*), 3.75 (m, 1H, C3-*H*), 3.06-3.03 (m, 1H, C13-*H*), 2.54-2.49 (m, 1H, C22-*H*), 2.38-2.35 (m, 1H, C22-*H*), 2.32-2.29 (m, 1H, C12-*H*), 2.20-2.10 (m, 5H, C1-*H*, C5-*H*, C15-*H* and 2×C23-*H*), 1.98 (s, 3H, OCOCH_3), 1.88-1.74 (m, 4H, C2-*H*, C12-*H*, C2-*H* and C7-*H*), 1.68 (s, 3H, C27-CH_3), 1.62 (s, 3H, C26-CH_3), 1.62-1.57 (m, 3H, C1-*H*, C6-*H* and C9-*H*), 1.52-1.49 (m, 1H, C4-*H*), 1.38 (s, 3H, C30-CH_3), 1.34-1.30 (m, 1H, C15-*H*), 1.18-1.09 (m, 2H, C6-*H* and C7-*H*), 0.98 (s, 3H, C19-CH_3), 0.96 (s, 3H, C18-CH_3), 0.92 (d, *J* = 6.96 Hz, 3H, C28-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 167.8, 159.1, 145.2, 132.7, 132.3, 131.0, 129.4, 127.8, 123.1, 74.0, 71.4, 68.2, 51.1, 49.4, 48.7, 43.6, 39.5, 39.2, 37.0, 36.3, 36.0, 35.7, 32.2, 30.1, 29.9, 29.6, 28.1, 25.6, 23.9, 22.9, 21.0, 20.8, 17.9, 17.8 and 15.9; MS: *m/z* 641 $[M+H]^+$, 581 $[M-OAc]^+$; HPLC purity (Method A) 98% (t_r = 11.39).

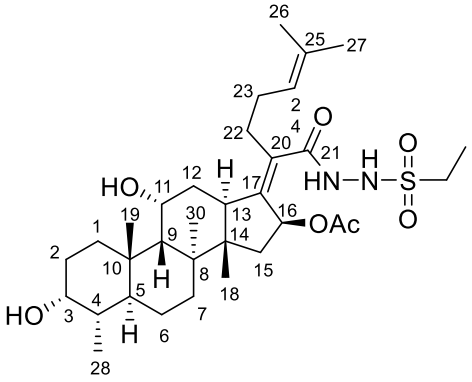

f1 (ppm)


f1 (ppm)

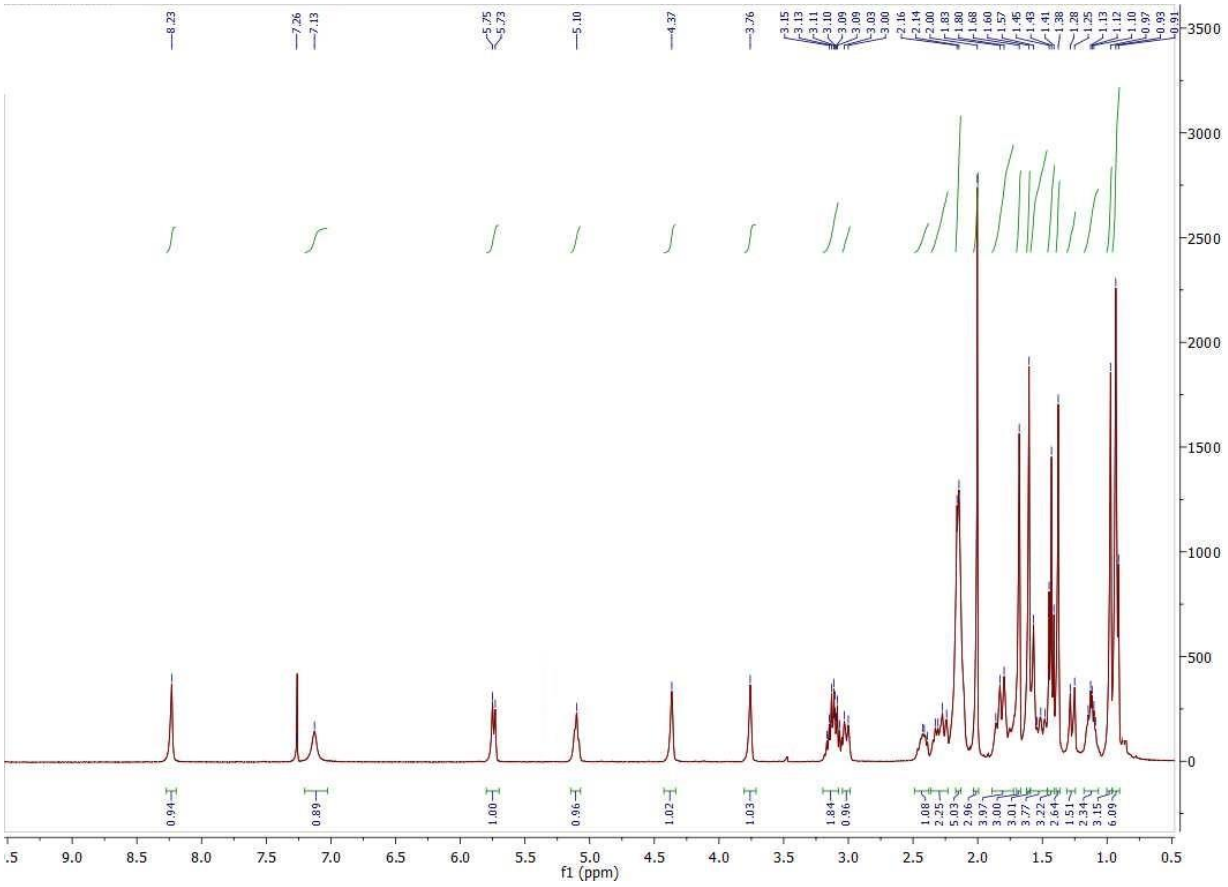
General synthetic procedure for compounds 7-11

To a solution of compound **2** (1.0 equiv.) in pyridine (5ml), respective sulfonyl chloride (1.0 equiv.) was added and reaction mixture was stirred at room temperature for 1-3 h. After completion of reaction (TLC), reaction mixture was diluted with EtOAc (25 ml) and washed with water (3×15 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography on silica gel using EtOAc:hexane as eluent afforded products **7-11**.

Compound 7: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-17-(1-(2-(ethylsulfonyl)hydrazinyl)-6-methyl-1-oxohept-5-en-2-ylidene)-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



Colourless solid (0.16g, 45%); R_f 0.5 (70% EtOAc:hexane); M. pt: 127-129^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (br s, 1H, CON*H*), 7.13 (br s, 1H, N*H*), 5.74 (d, *J* = 8.25 Hz, 1H, C16-*H*), 5.10 (t, *J* = 6.60 Hz, 1H, C24-*H*), 4.37 (m, 1H, C11-*H*), 3.76 (m, 1H, C3-*H*), 3.17-3.07 (m, 2H, SO_2CH_2), 3.03-3.00 (m, 1H, C13-*H*), 2.43-2.39 (m, 1H, C22-*H*), 2.33-2.24 (m, 2H, C12-*H* and C22-*H*), 2.16-2.14 (m, 5H, C1-*H*, C5-*H*, C15-H and 2×C23-*H*), 2.00 (s, 3H, $OCOCH_3$), 1.86-1.80 (m, 4H, 2×C2-*H*, C7-*H* and C12-*H*), 1.68 (s, 3H, C27-CH_3), 1.60 (s, 3H, C26-CH_3), 1.57-1.51 (m, 4H, C1-*H*, C4-*H*, C6-*H* and C9-*H*), 1.43 (t, *J* = 7.34 Hz, 3H, $SO_2CH_2CH_3$), 1.38 (s, 3H, C30-CH_3), 1.28-1.25 (m, 1H, C15-*H*), 1.15-1.09 (m, 2H, C6-*H* and C7-*H*), 0.97 (s, 3H, C19-CH_3), 0.93-0.91 (m, 6H, C18-CH_3 and C28-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.4, 145.3, 132.9, 131.9, 122.8, 73.8, 71.6, 68.3, 49.4, 48.7, 46.4, 43.6, 39.6, 39.2, 36.9, 36.5, 35.8, 35.5, 31.8, 30.1, 29.7, 29.7, 28.2, 25.6, 23.7, 23.1, 21.1, 20.9, 17.7, 15.8 and 7.8; MS: *m/z* 645 $[M+23]^+$, 563 $[M-OAc]^+$; HPLC purity (Method B) 96% (t_r = 4.15).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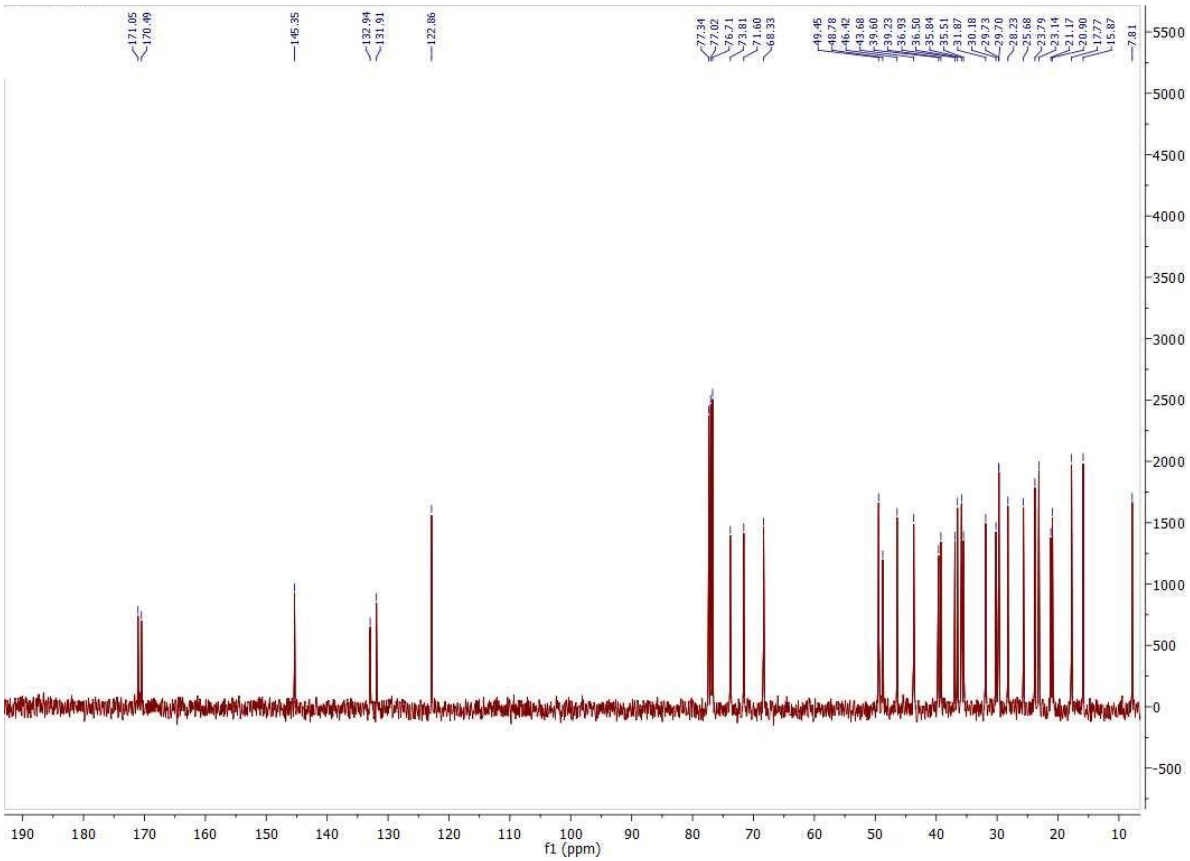
5.10
4.37
3.76
f1 (ppm)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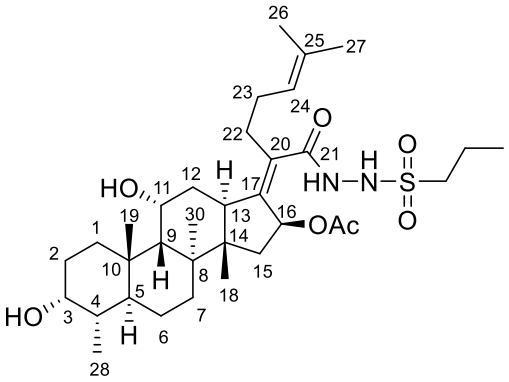
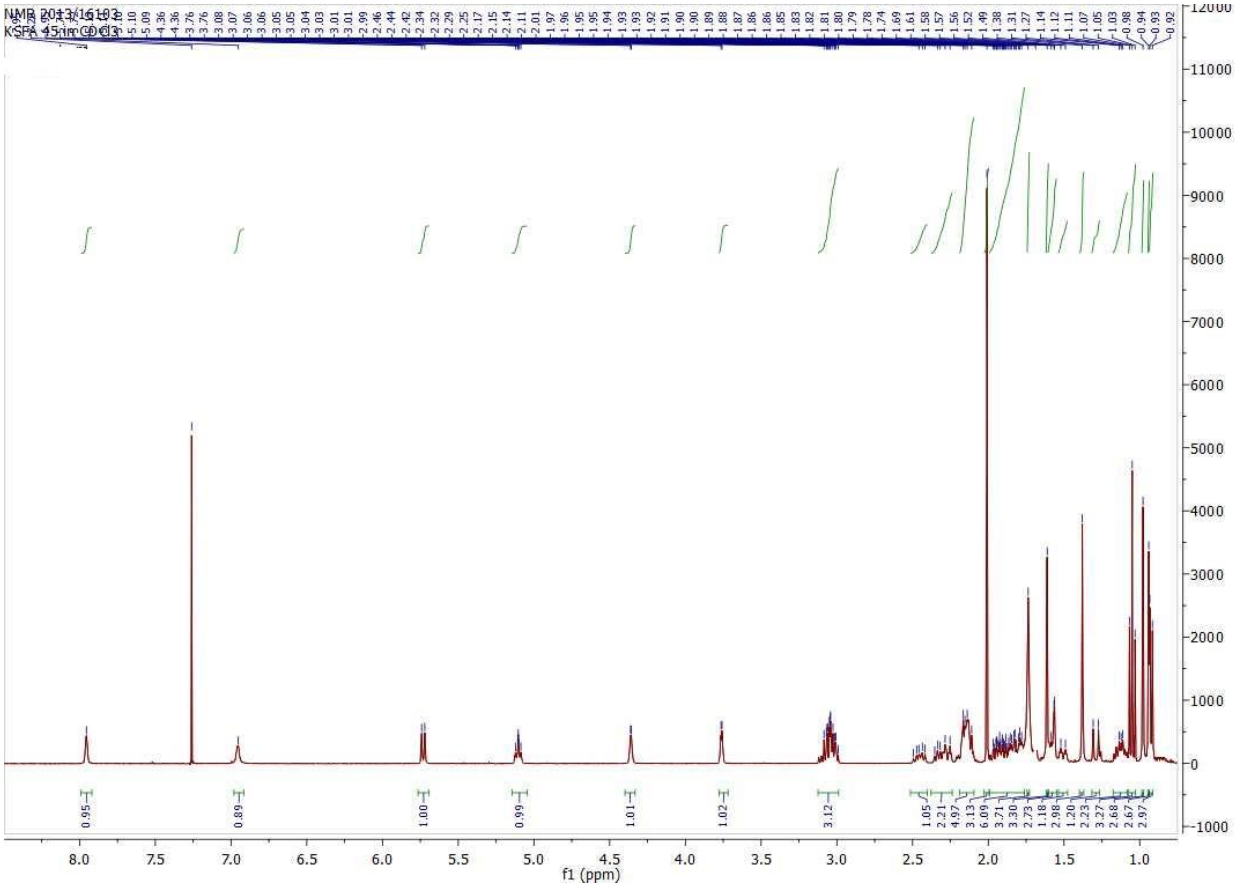
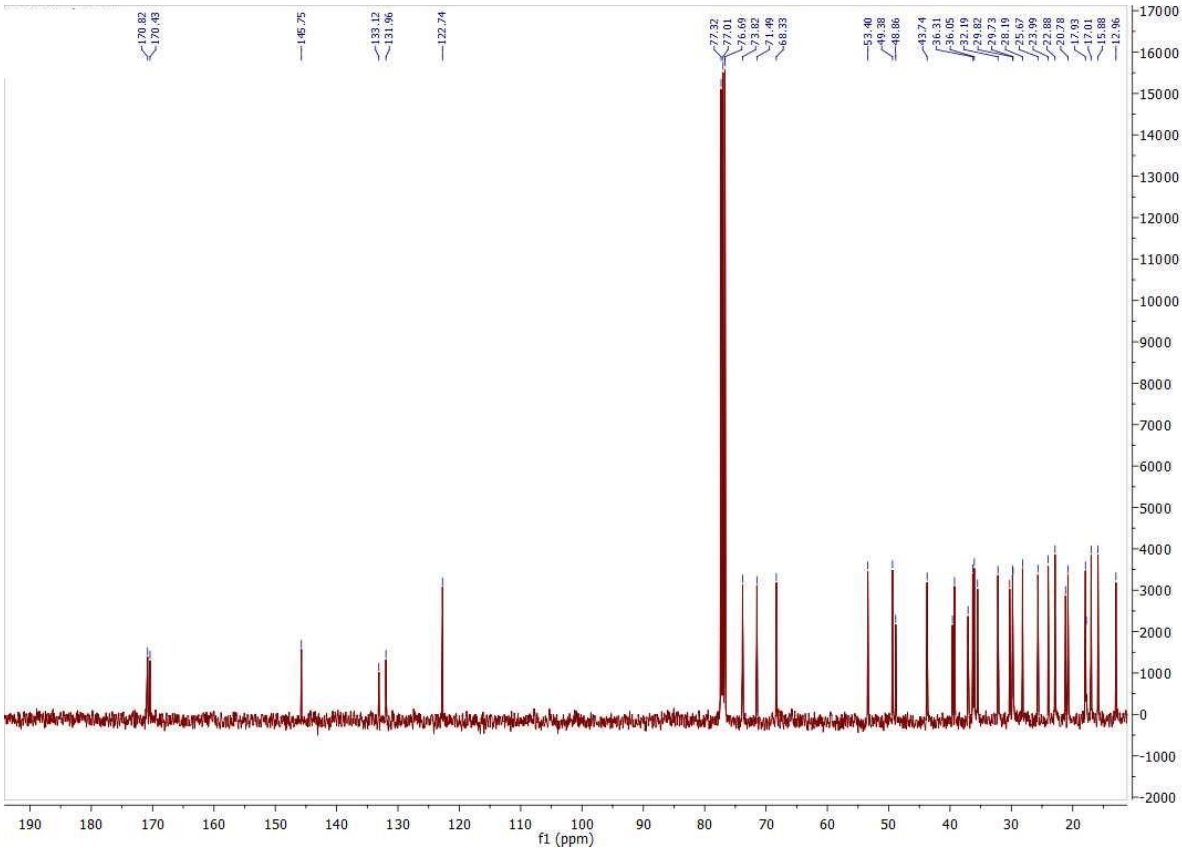
170.49
145.35
132.94
131.91
122.86
77.34
77.02
76.71
73.81
71.60
68.33
f1 (ppm)

Compound 8: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-3,11-dihydroxy-4,8,10,14-tetramethyl-17-(6-methyl-1-oxo-1-(2-(propylsulfonyl)hydrazinyl)hept-5-en-2-ylidene)hexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate

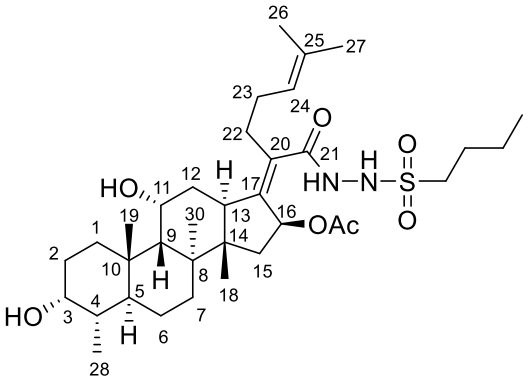


Colourless solid (0.15g, 25%); R_f 0.4 (5% MeOH:DCM); M. pt. 125-126^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (br s, 1H, CON*H*), 6.95 (br s, 1H, N*H*), 5.73 (d, *J* = 8.25 Hz, 1H, C16-*H*), 5.10 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.36 (m, 1H, C11-*H*), 3.76 (m, 1H, C3-*H*), 3.08-2.99 (m, 3H, SO_2CH_2 and C13-*H*), 2.49-2.42 (m, 1H, C22-*H*), 2.35-2.25 (m, 2H, C12-*H* and C22-*H*), 2.17-2.11 (m, 5H, C1-*H*, C5-*H*, C15-H and 2×C23-*H*), 2.01 (s, 3H, $OCOCH_3$), 1.97-1.78 (m, 6H, 2×C2-*H*, C7-*H*, C12-*H* and $SO_2CH_2CH_2$), 1.73 (s, 3H, C27-CH_3), 1.61 (s, 3H, C26-CH_3), 1.58-1.56 (m, 3H, C1-*H*, C6-*H* and C9-*H*), 1.52-1.49 (m, 1H, C4-*H*), 1.38 (s, 3H, C30-CH_3), 1.31-1.27 (m, 1H, C15-*H*), 1.14-1.11 (m, 2H, C6-*H* and C7-*H*), 1.05 (t, *J* = 7.34 Hz, 3H, $SO_2CH_2CH_2CH_3$), 0.98 (s, 3H, C19-CH_3), 0.94 (s, 3H, C18-CH_3), 0.93 (d, *J* = 6.97 Hz, 3H, C28-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.4, 145.7, 133.1, 131.9, 122.7, 73.8, 71.3, 68.3, 53.4, 49.3, 48.8, 43.7, 39.6, 39.2, 37.0, 36.3, 36.1, 35.5, 32.1, 30.2, 29.8, 29.7, 28.1, 25.5, 23.9, 22.8, 21.1, 20.7, 17.9, 17.7, 17.0, 15.8 and 12.9; MS: *m/z* 659 $[M+23]^+$, 577 $[M\text{-}OAc]^+$; HPLC purity (Method B) 99% (t_r = 4.20).

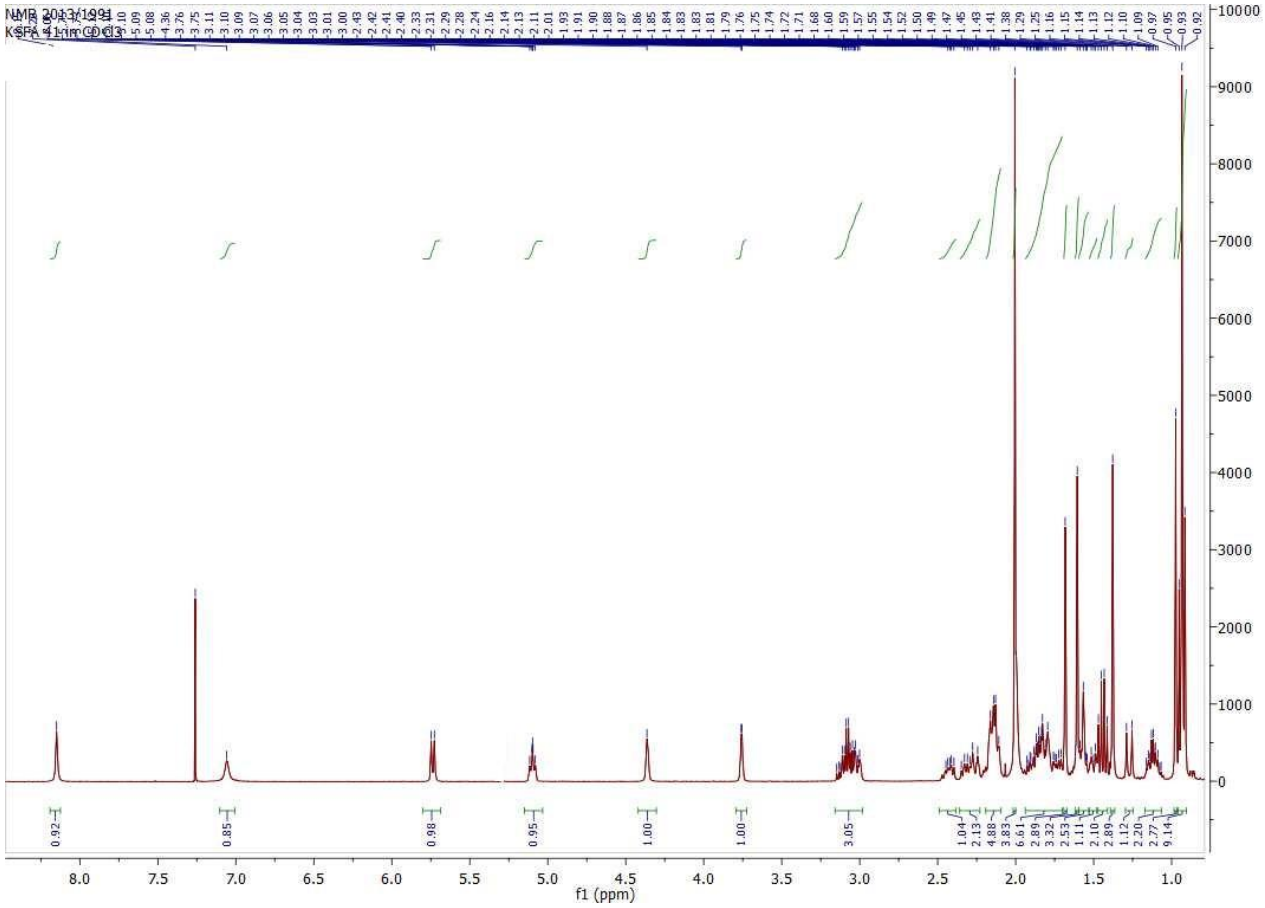

f1 (ppm)

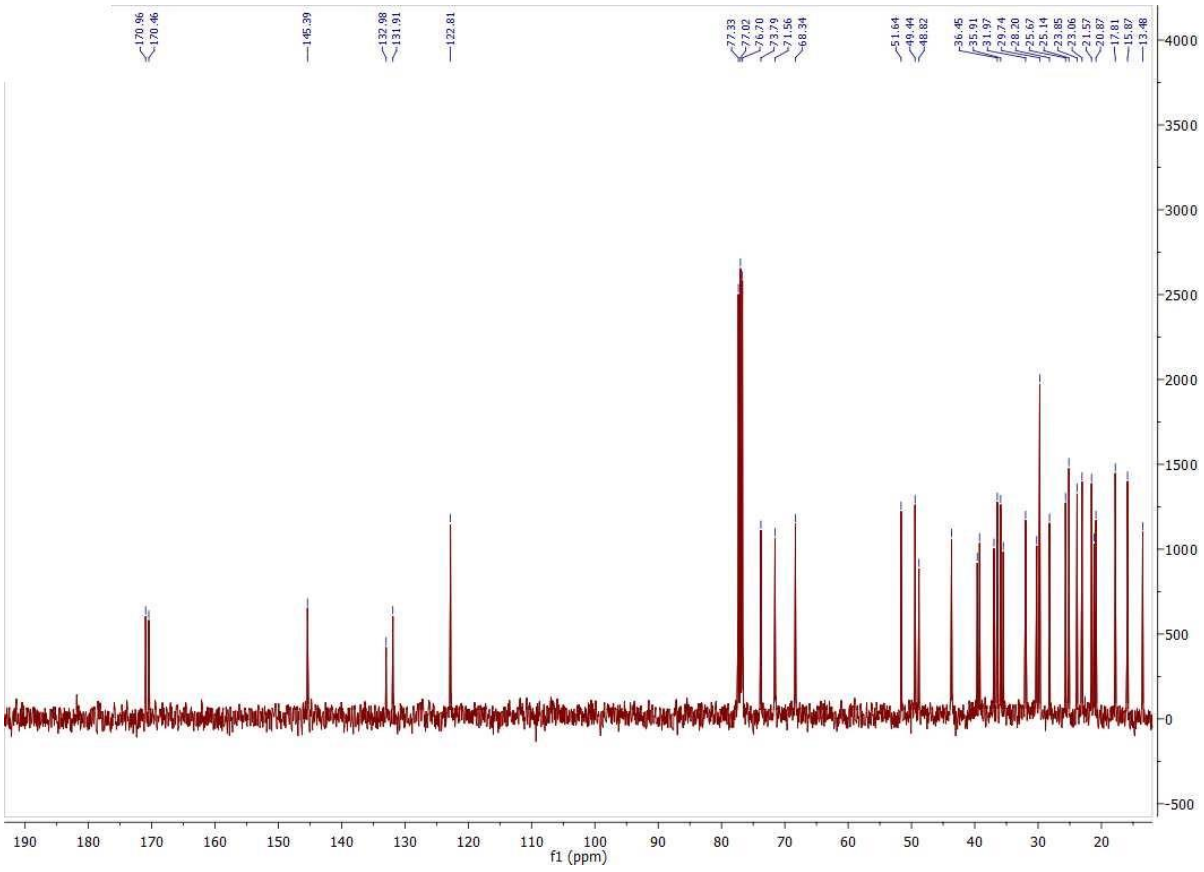

f1 (ppm)

Compound 9: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-17-(1-(2-(butylsulfonyl)hydrazinyl)-6-methyl-1-oxohept-5-en-2-ylidene)-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate

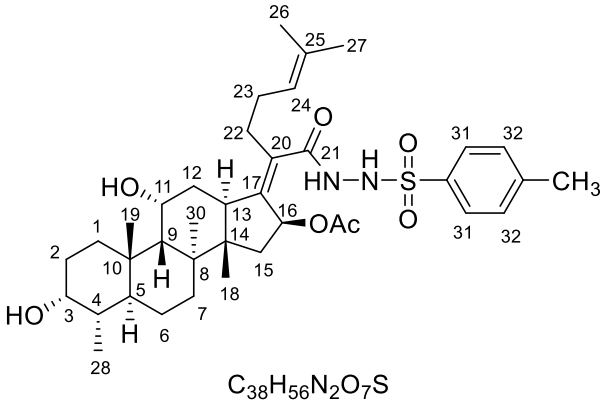


Colourless solid (0.35g, 71%); R_f 0.6 (70% EtOAc:hexane); M. pt. 121-123^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (br s, 1H, CON*H*), 7.06 (br s, 1H, N*H*), 5.74 (d, *J* = 8.25 Hz, 1H, C16-*H*), 5.10 (t, *J* = 6.65 Hz, 1H, C24-*H*), 4.36 (m, 1H, C11-*H*), 3.76 (m, 1H, C3-*H*), 3.15-3.00 (m, 3H, SO_2CH_2 and C13-*H*), 2.45-2.40 (m, 1H, C22-*H*), 2.35-2.24 (m, 2H, C12-*H* and C22-*H*), 2.16-2.11 (m, 5H, C1-*H*, C5-*H*, C15-H and 2×C23-*H*), 2.01 (s, 3H, $OCOCH_3$), 1.93-1.71 (m, 6H, 2×C2-*H*, C7-*H*, C12-*H* and $SO_2CH_2CH_2$), 1.68 (s, 3H, C27-CH_3), 1.60 (s, 3H, C26-CH_3), 1.59-1.54 (m, 3H, C1-*H*, C6-*H* and C9-*H*), 1.52-1.49 (m, 1H, C4-*H*), 1.47-1.41 (m, 2H, $SO_2CH_2CH_2CH_2$), 1.38 (s, 3H, C30-CH_3), 1.29-1.25 (m, 1H, C15-*H*), 1.16-1.07 (m, 2H, C6-*H* and C7-*H*), 0.97 (s, 3H, C19-CH_3), 0.95-0.92 (m, 9H, C18-CH_3, C28-CH_3 and $SO_2CH_2CH_2CH_2CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.4, 145.3, 132.9, 131.7, 122.8, 73.7, 71.5, 68.3, 51.6, 49.4, 48.7, 43.6, 39.6, 39.2, 36.9, 36.4, 35.9, 35.5, 31.9, 30.2, 29.7, 28.2, 25.6, 25.1, 23.8, 23.0, 21.5, 21.1, 20.8, 17.8 (C3), 15.8 and 13.4; MS: *m/z* 673 $[M+23]^+$, 591 $[M\text{-}OAc]^+$; HPLC purity (Method B) 97% (t_r = 4.47).


f1 (ppm)


f1 (ppm)

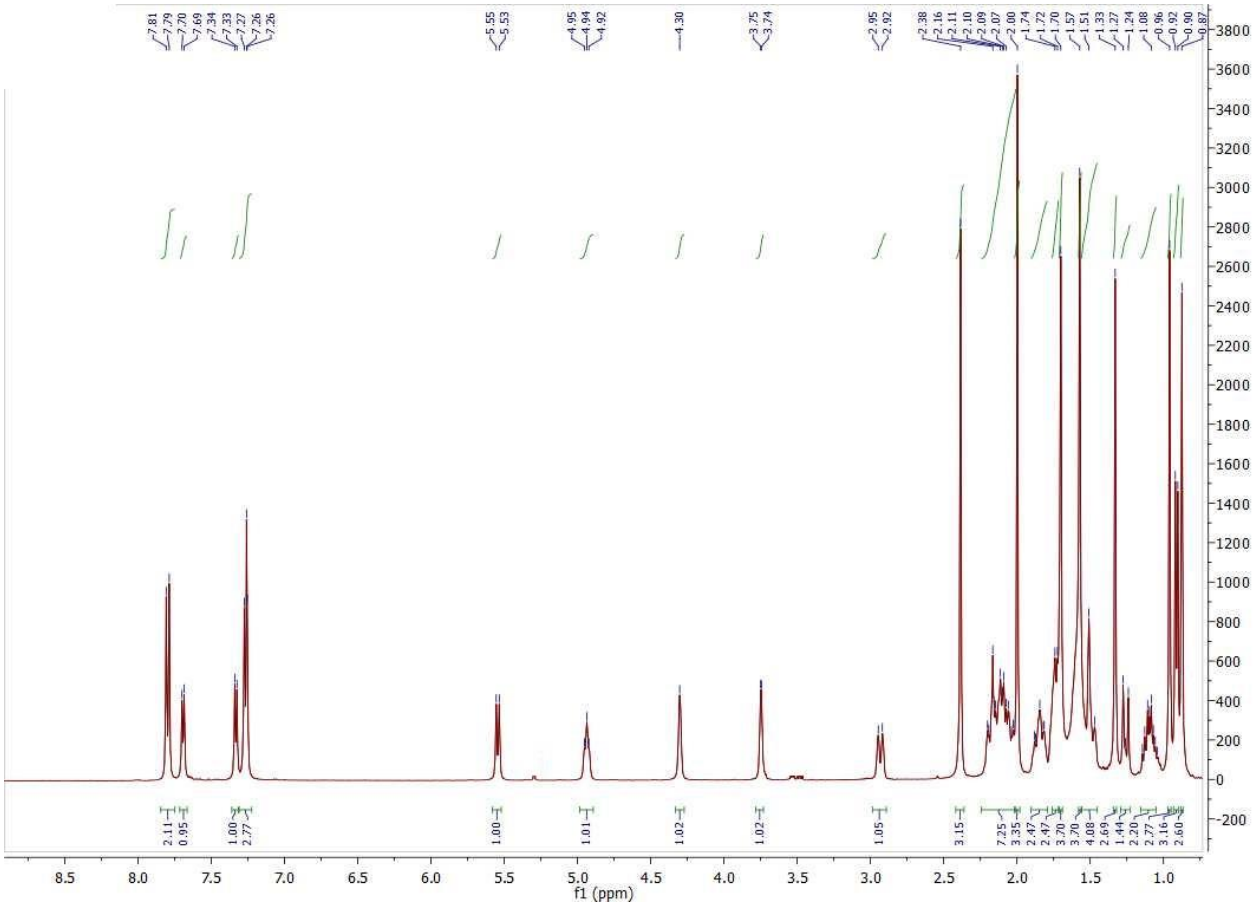
Compound 10: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-3,11-dihydroxy-4,8,10,14-tetramethyl-17-(6-methyl-1-oxo-1-(2-tosylhydrazinyl)hept-5-en-2-ylidene)hexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



$C_{38}H_{56}N_2O_7S$

Mol. Wt: 684.38

Colourless solid (0.03g, 46%); R_f 0.3 (60% EtOAc:hexane); M. pt. 220-222^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 8.44 Hz, 2H, ArH^{31}), 7.70 (d, *J* = 6.05 Hz, 1H, CON*H*), 7.32 (d, *J* = 6.05 Hz, 1H, N*H*), 7.26 (d, *J* = 7.89 Hz, 2H, ArH^{32}), 5.54 (d, *J* = 8.25 Hz, 1H, C16-*H*), 4.94 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.30 (m, 1H, C11-*H*), 3.75 (m, 1H, C3-*H*), 2.95-2.92 (m, 1H, C13-*H*), 2.38 (s, 3H, CH_3), 2.20-2.02 (m, 8H, C1-*H*, C5-*H*, C12-*H*, C15-*H*, 2×C22-*H* and 2×C23-*H*), 2.00 (s, 3H, OCOCH_3), 1.88-1.81 (m, 2H, C2-*H* and C12-*H*), 1.74-1.72 (m, 2H, C2-*H* and C7-*H*), 1.70 (s, 3H, C27-CH_3), 1.57 (s, 3H, C26-CH_3), 1.51-1.47 (m, 4H, C1-*H*, C4-*H*, C6-*H* and C9-*H*), 1.33 (s, 3H, C30-CH_3), 1.27-1.24 (m, 1H, C15-*H*), 1.14-1.04 (m, 2H, C6-*H* and C7-*H*), 0.96 (s, 3H, C19-CH_3), 0.91 (d, *J* = 6.25 Hz, 3H, C28-CH_3), 0.87 (s, 3H, C18-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 169.6, 145.9, 144.8, 133.3 (C2), 132.7, 131.4, 129.5 (C2), 128.8 (C2), 122.9, 73.9, 71.3, 68.2, 49.3, 48.8, 43.5, 39.5, 39.1, 37.1, 36.2, 36.1, 35.3, 32.4, 30.3, 29.9, 29.5, 28.0, 25.7, 24.1, 22.6, 21.6, 21.0, 20.6, 17.9 and 15.8; MS: *m/z* 685 $[M+H]^+$, 625 $[M\text{-}OAc]^+$; HPLC purity (Method A) 97% (t_r = 12.56).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2.00
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1.70
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1.27
1.24
1.08
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0.92
0.90
0.87
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0.95
1.00
2.77
1.00
1.01
1.02
1.02
1.05
3.15
7.25
3.35
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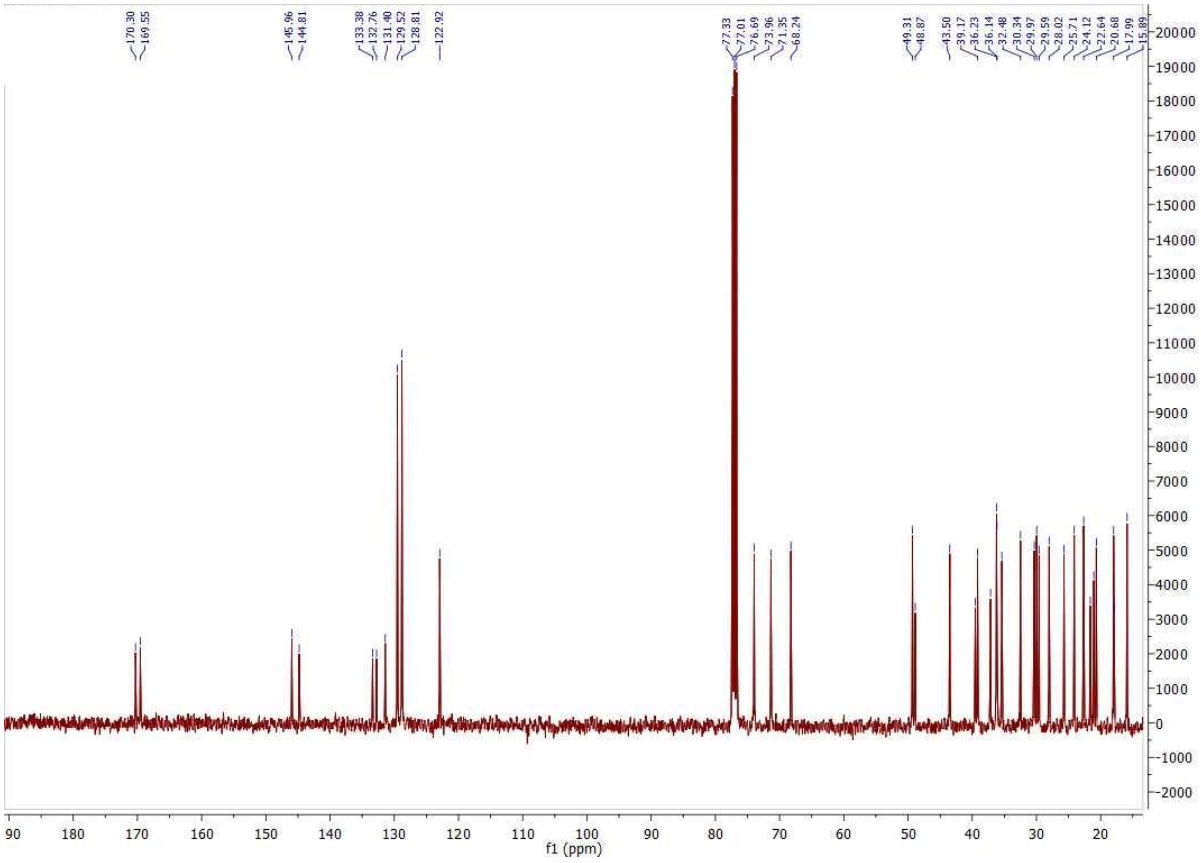
f1 (ppm)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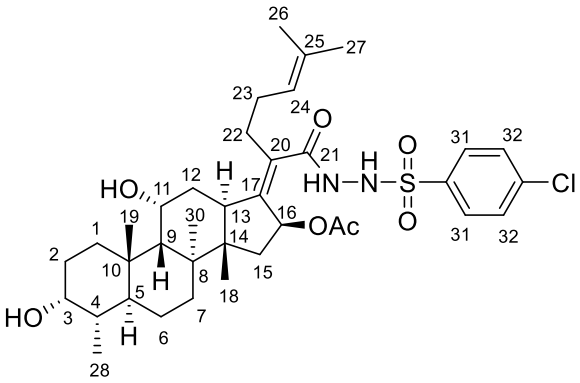
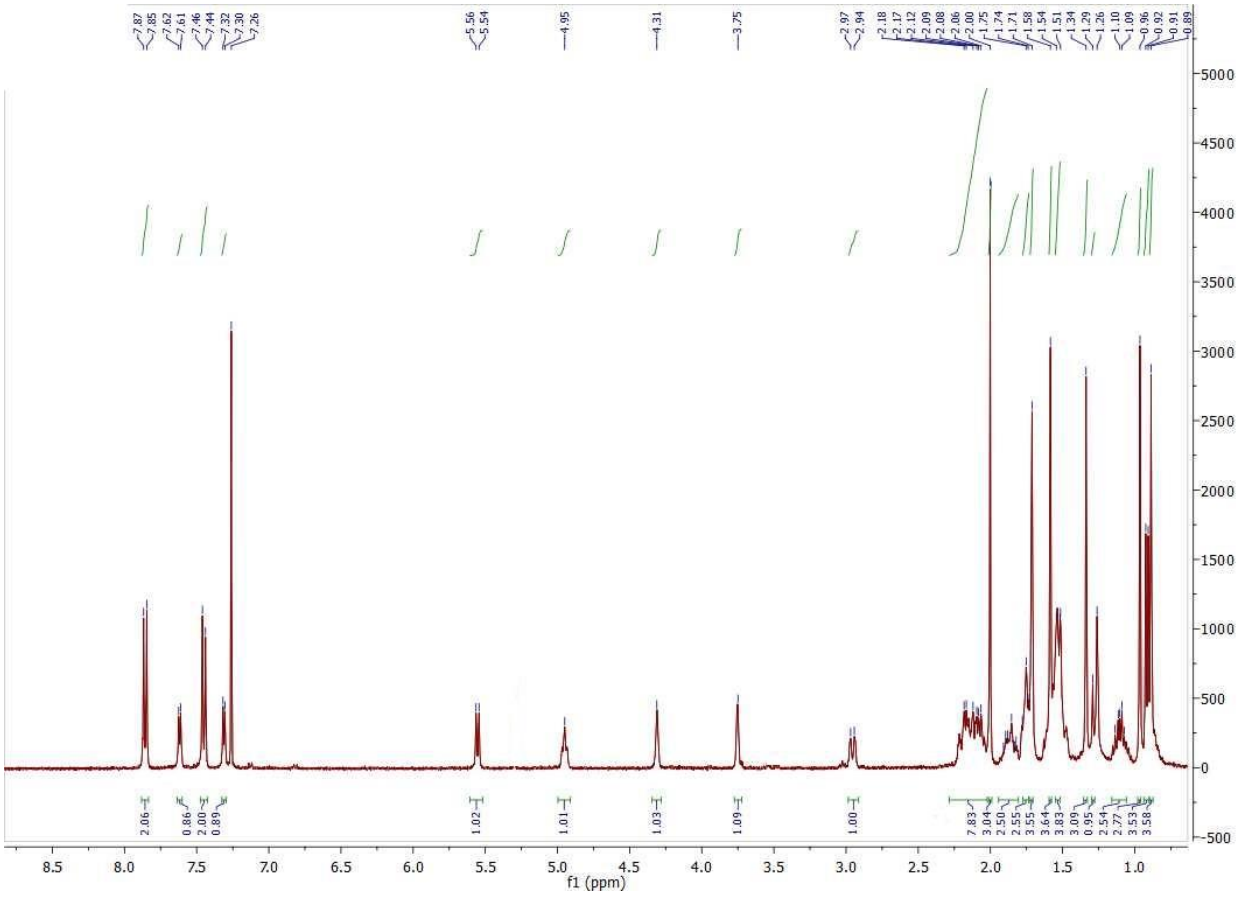
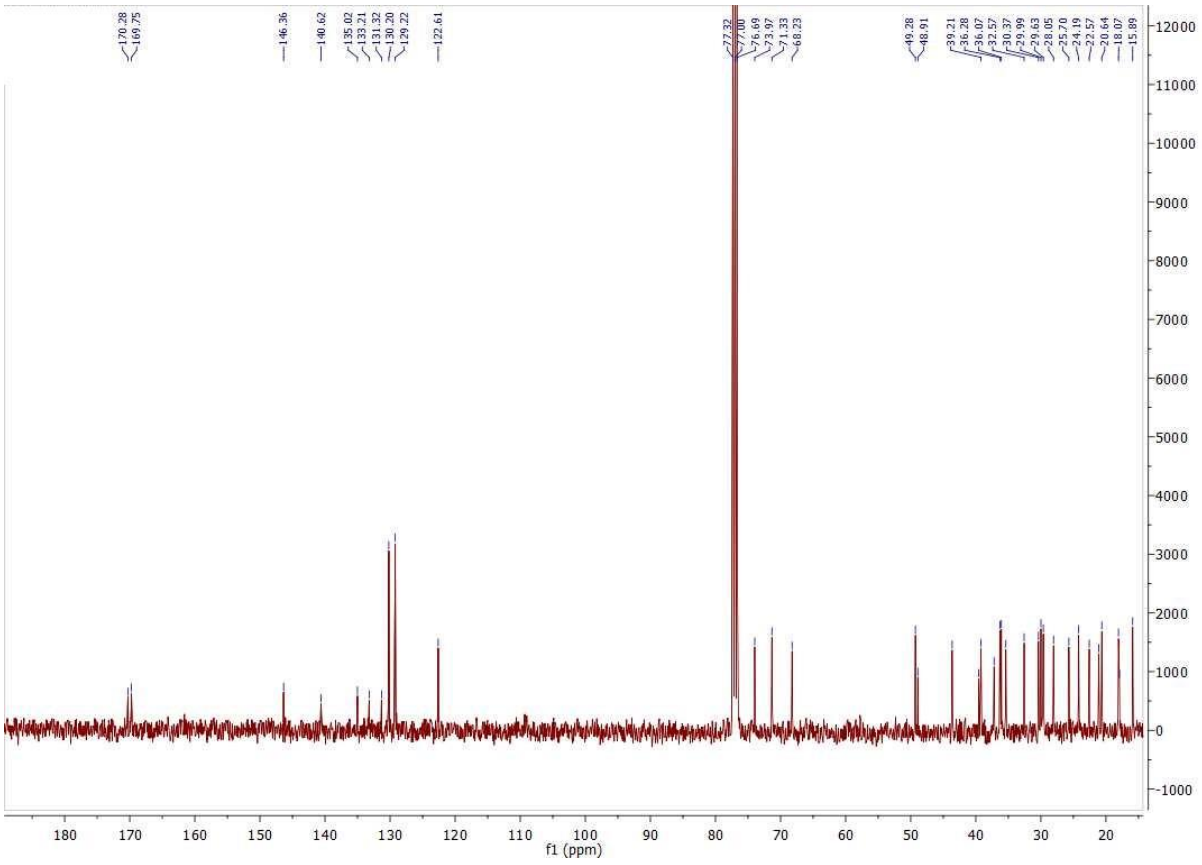
48.87
43.50
39.17
36.23
36.14
32.48
30.34
29.97
29.59
28.02
25.71
24.12
22.64
20.68
17.99
15.89
f1 (ppm)

Compound 11: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-17-(1-(2-((4-chlorophenyl)sulfonyl)hydrazinyl)-6-methyl-1-oxohept-5-en-2-ylidene)-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



Colourless solid (0.093g, 65%); R_f 0.5 (60% EtOAc:hexane); M. pt. 230-232^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.62 Hz, 2H, ArH^{32}), 7.62 (d, *J* = 5.32 Hz, 1H, CON*H*), 7.45 (d, *J* = 8.44 Hz, 2H, ArH^{31}), 7.31 (d, *J* = 5.50 Hz, 1H, N*H*), 5.55 (d, *J* = 8.44 Hz, 1H, C16-*H*), 4.95 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.31 (m, 1H, C11-*H*), 3.75 (m, 1H, C3-*H*), 2.97-2.94 (m, 1H, C13-*H*), 2.18-2.06 (m, 8H, C1-*H*, C5-*H*, C12-*H*, C15-*H*, 2×C22-*H* and 2×C23-*H*), 2.00 (s, 3H, OCOCH_3), 1.91-1.82 (m, 2H, C2-*H* and C12-*H*), 1.78-1.74 (m, 2H, C2-*H* and C7-*H*), 1.71 (s, 3H, C27-CH_3), 1.58 (s, 3H, C26-CH_3), 1.54-1.51 (m, 4H, C1-*H*, C4-*H*, C6-*H* and C9-*H*), 1.34 (s, 3H, C30-CH_3), 1.29-1.26 (m, 1H, C15-*H*), 1.14-1.07 (m, 2H, C6-*H* and C7-*H*), 0.96 (s, 3H, C19-CH_3), 0.92 (d, *J* = 6.79 Hz, 3H, C28-CH_3), 0.89 (s, 3H, C18-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 169.7, 146.3, 140.6, 134.9, 133.0, 131.3, 130.2 (C2), 129.2 (C2), 122.6, 73.8, 71.3, 68.1, 49.2, 48.9, 43.6, 39.5, 39.2, 37.1, 36.3, 36.0, 35.3, 32.5, 30.3, 29.9, 29.6, 28.0, 25.7, 24.1, 22.4, 21.1, 20.6, 18.0, 17.9 and 15.8; MS: *m/z* 722 $[M+NH_4]^+$, 645 $[M\text{-}OAc]^+$; HPLC purity (Method A) 98% (t_r = 11.92).

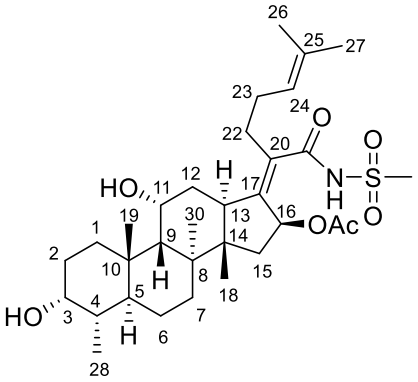

7.87
7.85
7.62
7.61
7.46
7.44
7.32
7.30
7.26
5.56
5.54
4.95
4.31
3.75
2.97
2.94
2.18
2.17
2.12
2.09
2.08
2.06
2.00
1.75
1.74
1.71
1.58
1.54
1.51
1.34
1.29
1.26
1.10
1.09
0.96
0.92
0.91
0.89
2.06
0.86
2.00
0.89
1.02
1.01
1.03
1.09
1.00
7.83
3.04
2.50
2.55
3.55
3.64
3.83
3.09
0.95
2.54
2.77
3.53
3.58
f1 (ppm)


170.28
169.75
146.36
140.62
135.02
133.21
131.32
130.20
129.22
122.61
77.32
77.00
76.69
73.97
71.33
68.23
49.28
48.91
39.21
36.28
36.07
32.57
30.37
29.99
29.63
28.05
25.70
24.19
22.57
20.64
18.07
15.89
f1 (ppm)

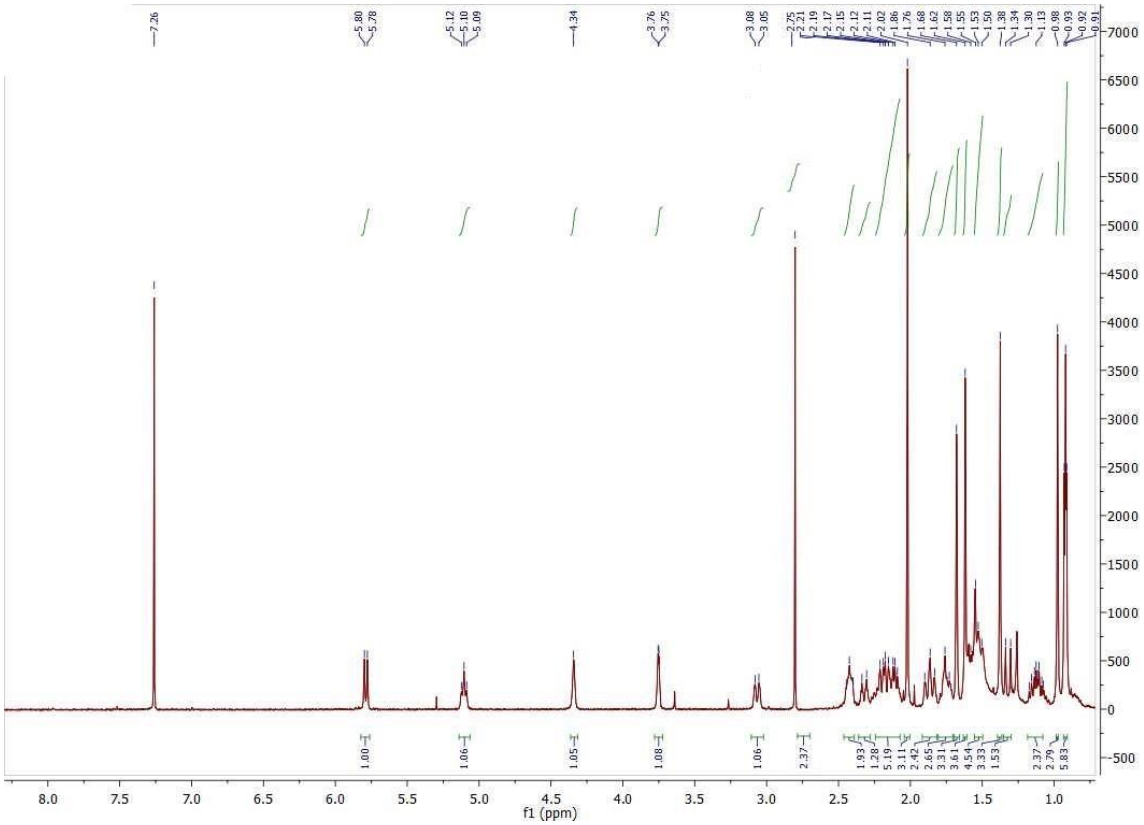
General synthetic procedure for compounds 12-14

To a solution of Fusidic acid **1** (1.0 equiv.) in DCM (10 ml), respective sulphonamide (1.50 equiv.), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (1.50 equiv.) and DMAP (0.4 equiv.) were added and reaction mixture was stirred at room temperature for 48 h. After completion of reaction (TLC), solvent was removed *in vacuo* and the residue was taken in EtOAc (25 ml). The organic phase was washed with water (3×15 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography on silica gel using MeOH:DCM as eluent afforded products **12-14**.

Compound 12: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-3,11-dihydroxy-4,8,10,14-tetramethyl-17-(6-methyl-1-(methylsulfonamido)-1-oxohept-5-en-2-ylidene)hexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



Colourless solid (0.108g, 31%); R_f 0.4 (5% MeOH:DCM); M. pt. 214-216^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.79 (d, *J* = 8.44 Hz, 1H, C16-*H*), 5.10 (t, *J* = 6.76 Hz, 1H, C24-*H*), 4.34 (m, 1H, C11-*H*), 3.76 (m, 1H, C3-*H*), 3.08-3.05 (m, 1H, C13-*H*), 2.75 (s, 3H, SO_2CH_3), 2.42-2.40 (m, 2H, C12-*H* and C22-*H*), 2.34-2.30 (m, 1H, C22-*H*), 2.21-2.09 (m, 5H, C1-*H*, C5-*H*, C15-*H* and 2×C23-*H*), 2.02 (s, 3H, $OCOCH_3$), 1.90-1.93 (m, 2H, C2-*H* and C12-*H*), 1.76-1.73 (m, 2H, C2-*H* and C7-*H*), 1.68 (s, 3H, C27-CH_3), 1.62 (s, 3H, C26-CH_3), 1.58-1.50 (m, 4H, C1-*H*, C4-*H*, C6-*H* and C9-*H*), 1.38 (s, 3H, C30-CH_3), 1.34-1.30 (m, 1H, C15-*H*), 1.17-1.07 (m, 2H, C6-*H* and C7-*H*), 0.98 (s, 3H, C19-CH_3), 0.93-0.91 (m, 6H, C28-CH_3 and C18-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 164.7, 153.1, 132.5, 129.1, 123.0, 74.1, 71.3, 68.2, 49.2, 48.8, 44.6, 43.3, 39.5, 39.1, 37.1, 36.3, 36.1, 35.4, 32.5, 30.3, 30.0, 29.0, 28.8, 25.7, 24.2, 22.6, 21.1, 20.7, 18.0, 17.8 and 15.9; MS: *m/z* 594 $[M+H]^+$, 534 $[M-OAc]^+$; HPLC purity (Method B) 98% (t_r = 4.11).


7.26
5.80
5.78
5.12
5.10
5.09
4.34
3.76
3.75
3.08
3.05
2.75
2.21
2.19
2.17
2.15
2.12
2.11
2.02
1.86
1.76
1.68
1.62
1.58
1.55
1.53
1.50
1.38
1.34
1.30
1.13
0.98
0.93
0.92
0.91
1.00
1.06
1.05
1.08
1.06
2.37
1.93
1.28
5.19
3.11
2.42
2.65
3.31
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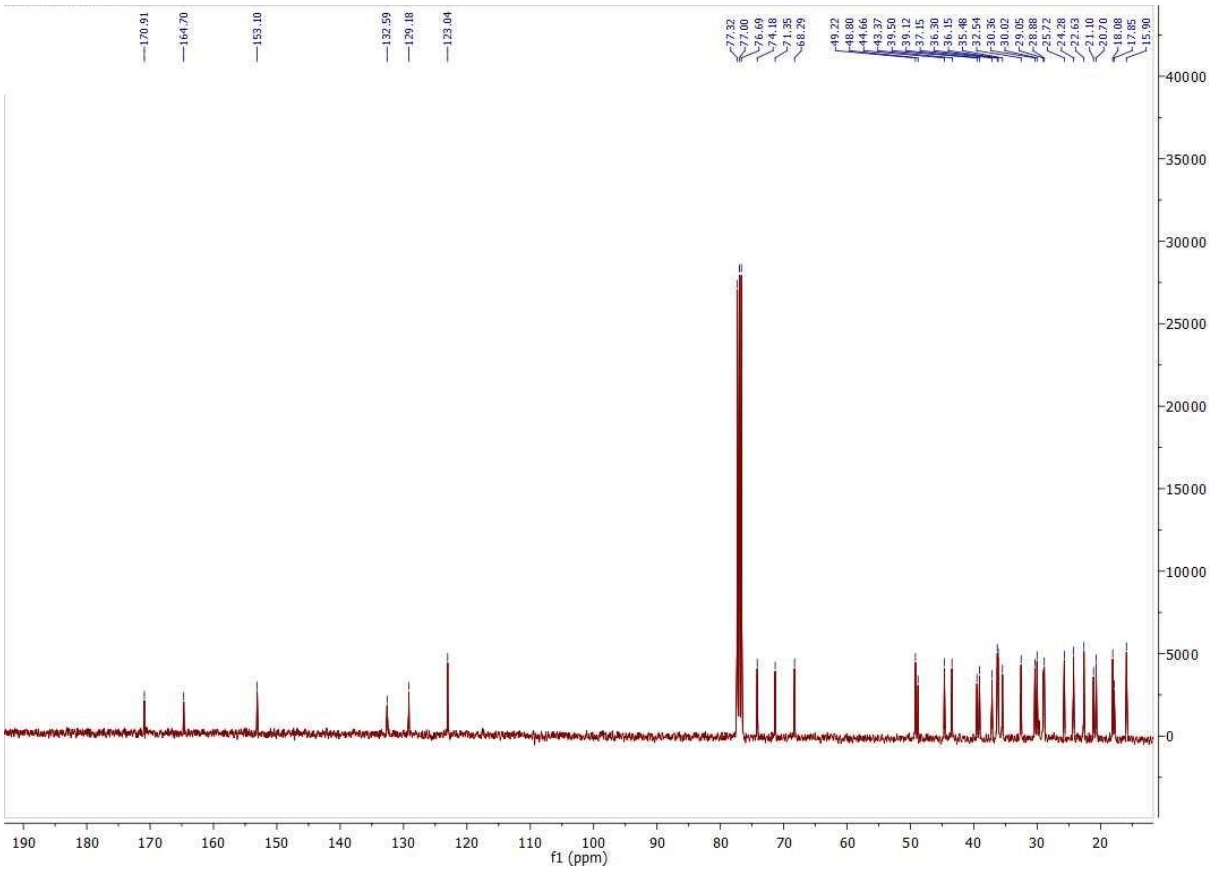
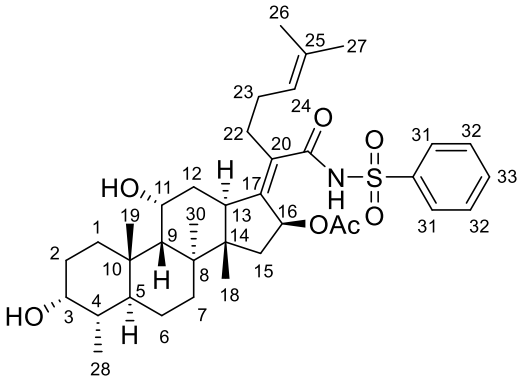
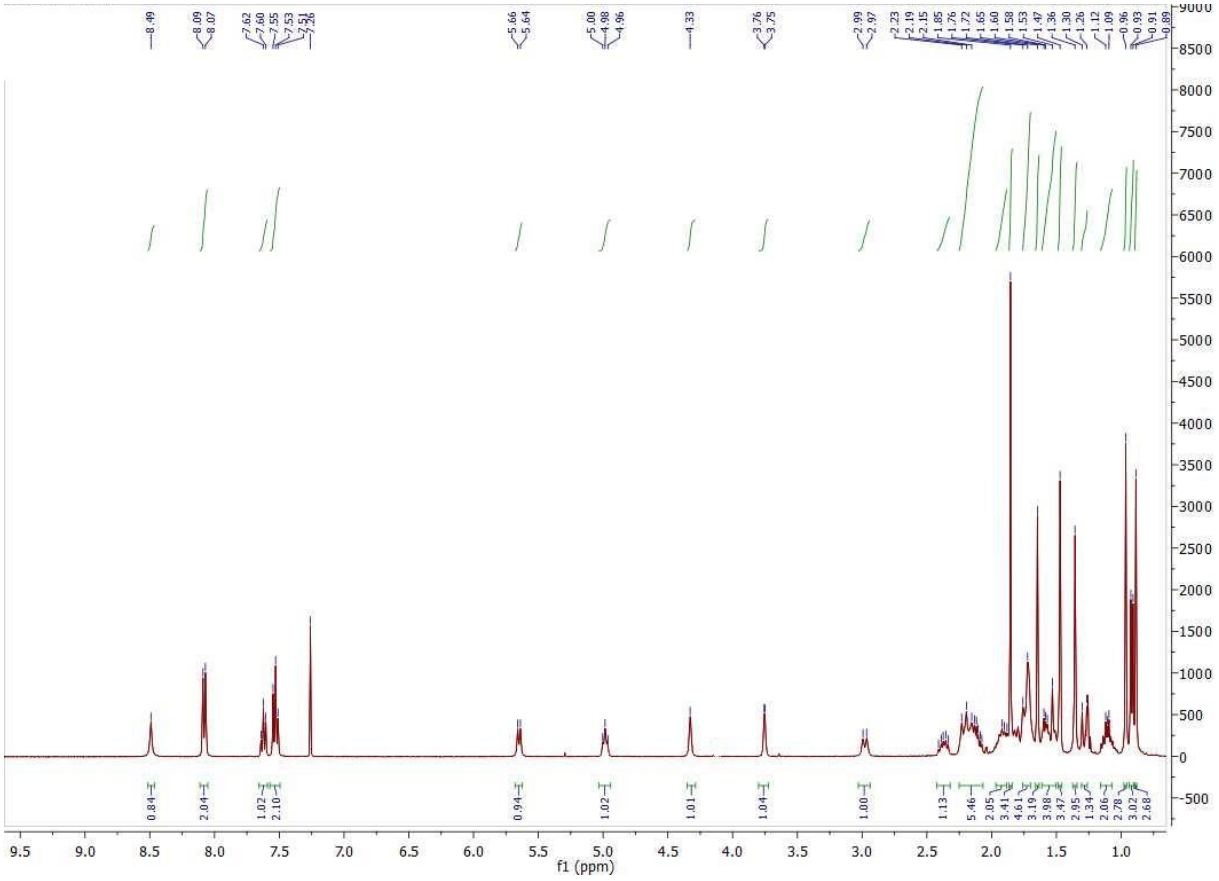
1.53
2.37
2.79
5.83
f1 (ppm)


170.91
164.70
153.10
132.59
129.18
123.04
77.32
77.00
76.69
74.18
71.35
68.29
49.22
48.80
44.66
43.37
39.50
39.12
37.15
36.30
36.15
35.48
32.54
30.36
30.02
29.05
28.88
25.72
24.28
22.63
21.10
20.70
18.08
17.85
15.90
f1 (ppm)

Compound 13: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-3,11-dihydroxy-4,8,10,14-tetramethyl-17-(6-methyl-1-oxo-1-(phenylsulfonamido)hept-5-en-2-ylidene)hexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



Colourless solid (0.10g, 38%); R_f 0.4 (5% MeOH:DCM); M. pt. 229-231^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (br s, 1H, NH), 8.08 (d, *J* = 7.52 Hz, 2H, ArH^{31}), 7.62 (t, *J* = 7.34 Hz, 1H, ArH^{33}), 7.53 (t, *J* = 7.34 Hz, 2H, ArH^{32}), 5.65 (d, *J* = 8.44 Hz, 1H, C16-*H*), 4.98 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.33 (m, 1H, C11-*H*), 3.76 (m, 1H, C3-*H*), 2.99-2.97 (m, 1H, C13-*H*), 2.41-2.34 (m, 1H, C22-*H*), 2.23-2.08 (m, 5H, C1-*H*, C5-*H*, C12-*H*, C22-*H* and C23-*H*), 1.92-1.88 (m, 2H, C15-*H* and C23-*H*), 1.85 (s, 3H, OCOCH_3), 1.76-1.72 (m, 4H, 2×C2-*H*, C7-*H* and C12-*H*), 1.65 (s, 3H, C27-CH_3), 1.60-1.53 (m, 4H, C1-*H*, C4-*H*, C6-*H* and C9-*H*), 1.47 (s, 3H, C26-CH_3), 1.36 (s, 3H, C30-CH_3), 1.30-1.26 (m, 1H, C15-*H*), 1.12-1.09 (m, 2H, C6-*H* and C7-*H*), 0.96 (s, 3H, C19-CH_3), 0.92 (d, *J* = 6.79 Hz, 3H, C28-CH_3), 0.89 (s, 3H, C18-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 167.7, 146.7, 138.8, 133.8, 133.6, 132.0, 128.8 (C2), 128.5 (C2), 122.6, 73.7, 71.4, 68.2, 49.2, 48.6, 43.7, 39.5, 39.1, 37.0, 36.2, 36.0, 35.5, 32.3, 30.2, 29.9, 29.1, 27.9, 25.6, 24.0, 22.8, 20.7 (C2), 17.9, 17.7 and 15.9; MS: *m/z* 678 $[M+23]^+$, 596 $[M\text{-}OAc]^+$; HPLC purity (Method B) 99% (t_r = 4.32).


8.49
8.09
8.07
7.62
7.60
7.55
7.53
7.51
7.50
5.66
5.64
5.00
4.98
4.96
4.33
3.76
3.75
2.99
2.97
2.23
2.19
2.15
1.85
1.76
1.72
1.65
1.60
1.58
1.53
1.47
1.36
1.30
1.26
1.12
1.09
0.96
0.93
0.91
0.89
0.84
2.04
1.02
2.10
0.94
1.02
1.01
1.04
1.00
1.13
5.46
2.05
3.41
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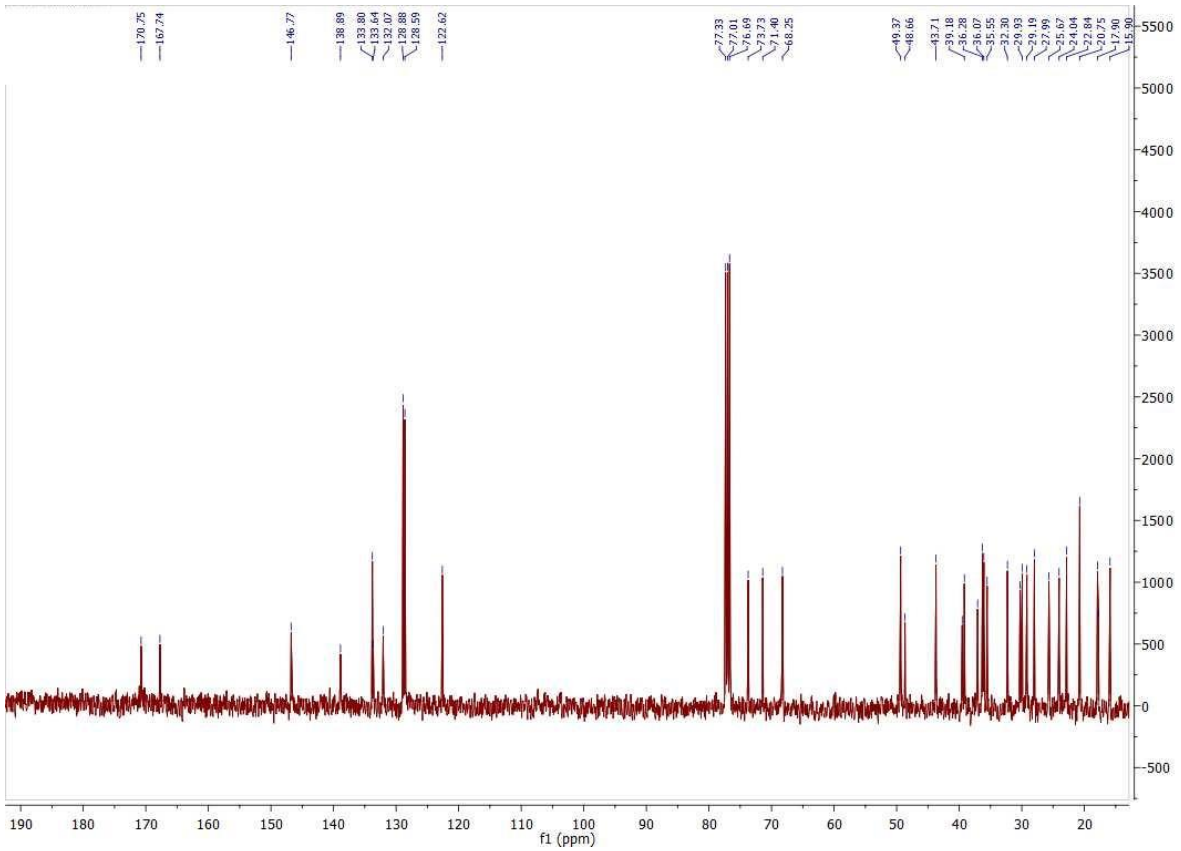
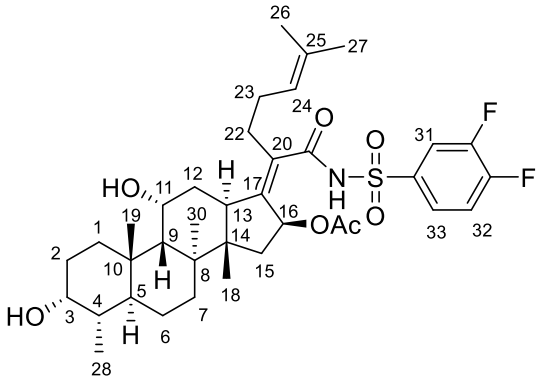
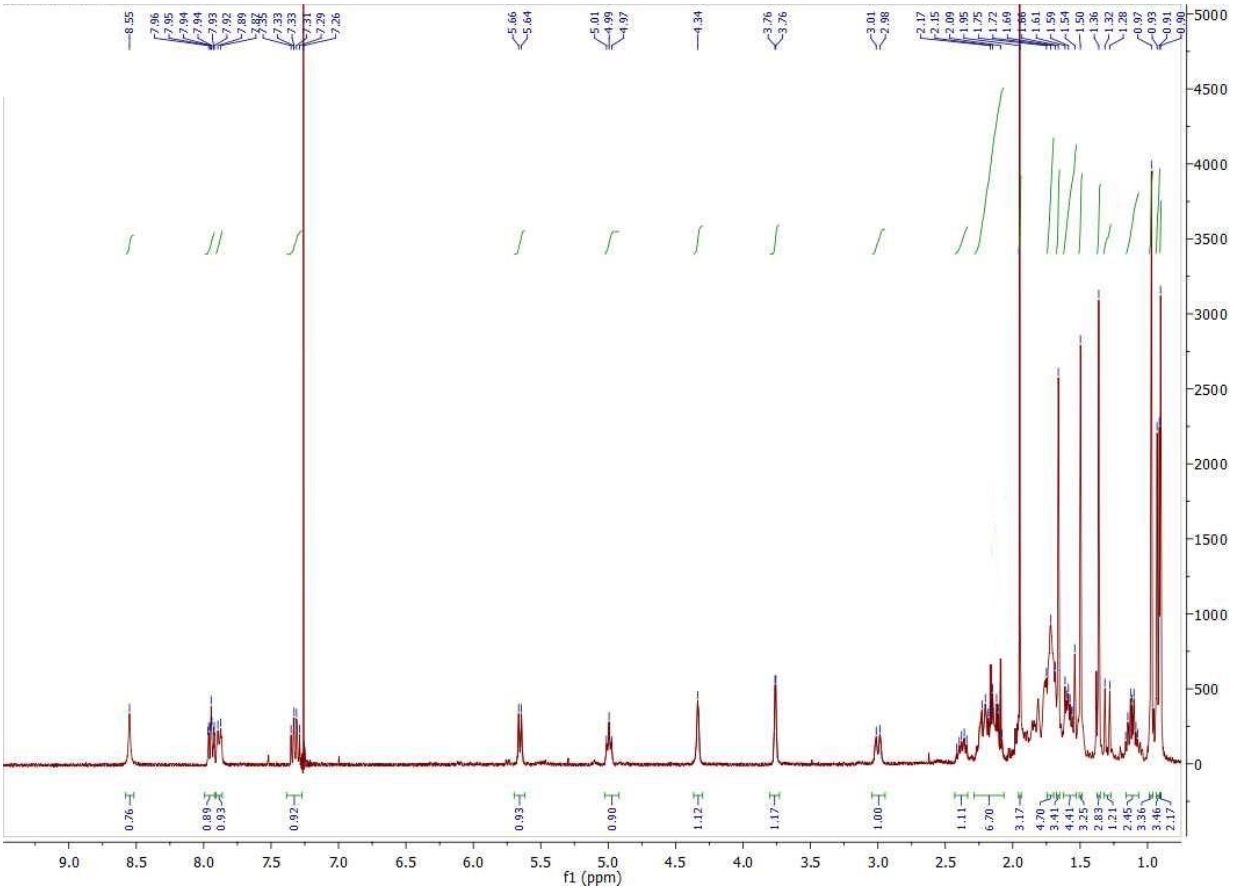
1.34
2.06
2.78
3.02
2.68
f1 (ppm)

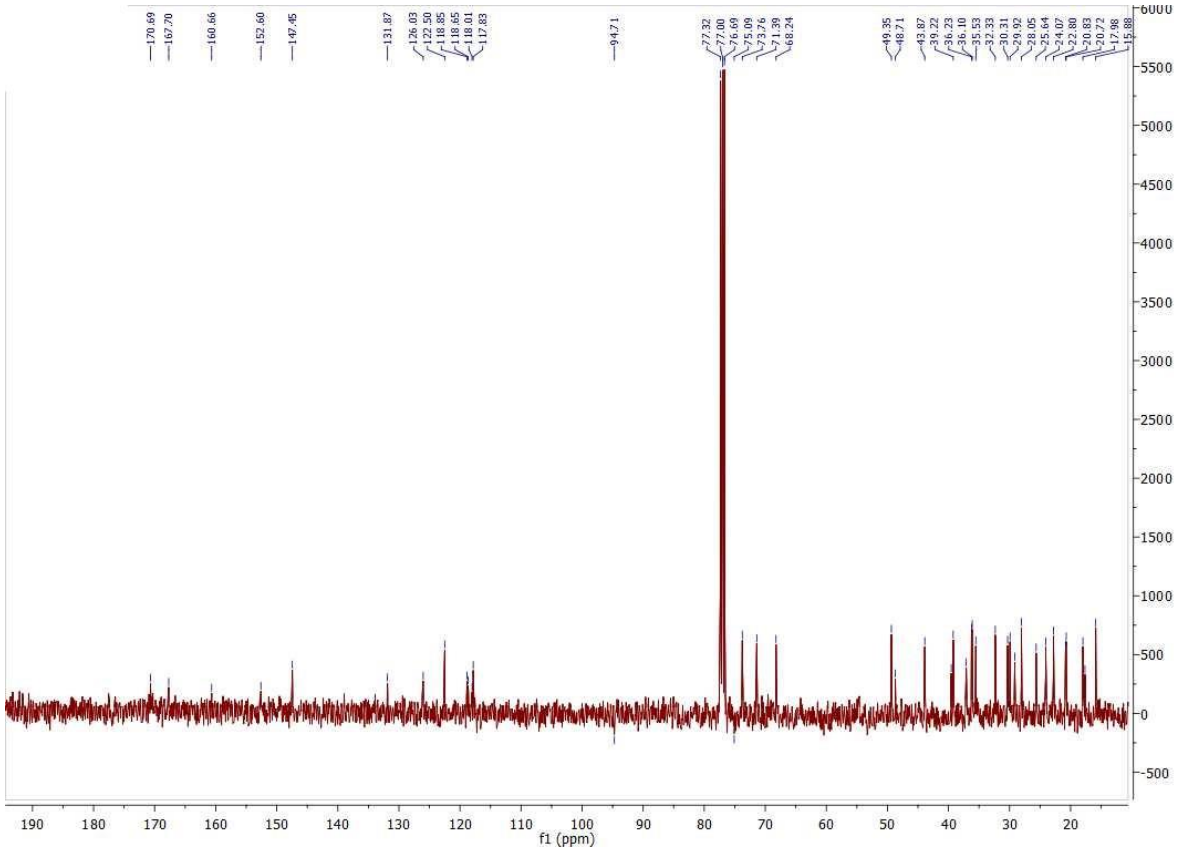

170.75
167.74
146.77
138.89
133.80
133.64
132.07
128.88
128.59
122.62
77.33
77.01
76.69
73.73
71.40
68.25
49.37
48.66
43.71
39.18
36.28
36.07
35.55
32.30
29.93
29.19
27.99
25.67
24.04
22.84
20.75
17.90
15.90
f1 (ppm)

Compound 14: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-17-(1-(3,4-difluorophenylsulfonamido)-6-methyl-1-oxohept-5-en-2-ylidene)-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



Colourless solid (0.12g, 30%); R_f 0.3 (4% MeOH:DCM); M. pt. 232-234^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (br s, 1H, NH), 7.97-7.92 (m, 1H, ArH^{31}), 7.89-7.87 (m, 1H, ArH^{33}), 7.35-7.29 (m, 1H, ArH^{32}), 5.65 (d, *J* = 8.07 Hz, 1H, C16-*H*), 4.99 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.34 (m, 1H, C11-*H*), 3.76 (m, 1H, C3-*H*), 3.01-2.98 (m, 1H, C13-*H*), 2.42-2.34 (m, 1H, C22-*H*), 2.23-2.09 (m, 7H, C1-*H*, C5-*H*, C12-*H*, C15-*H*, C22-*H* and 2×C23-*H*), 1.95 (s, 3H, OCOCH_3), 1.75-1.69 (m, 4H, 2×C2-*H*, C7-*H* and C12-*H*), 1.66 (s, 3H, C27-CH_3), 1.61-1.54 (m, 4H, C1-*H*, C4-*H*, C6-*H* and C9-*H*), 1.50 (s, 3H, C26-CH_3), 1.36 (s, 3H, C30-CH_3), 1.32-1.28 (m, 1H, C15-*H*), 1.16-1.07 (m, 2H, C6-*H* and C7-*H*), 0.97 (s, 3H, C19-CH_3), 0.92 (d, *J* = 6.97 Hz, 3H, C28-CH_3), 0.90 (s, 3H, C18-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 167.8, 167.7, 152.6, 147.4, 131.8, 126.0, 122.5, 118.8, 118.6, 118.0, 117.8, 73.7, 71.4, 68.2, 49.3, 48.7, 43.8, 39.5, 39.2, 37.0, 36.2, 36.1, 35.5, 32.3, 30.3, 29.9, 29.1, 28.0, 25.6, 24.0, 22.8, 20.8, 20.7, 17.9, 17.6 and 15.8; MS: *m/z* 714 $[M+23]^+$, 632 $[M\text{-}OAc]^+$; HPLC purity (Metod B) 94% (t_r = 4.36).

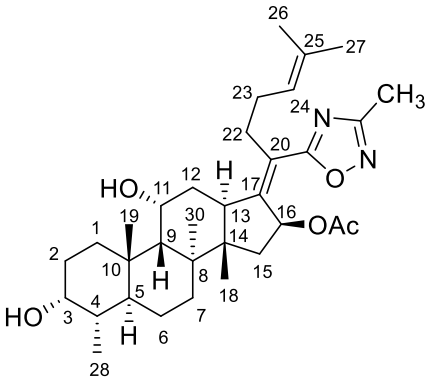

f1 (ppm)


f1 (ppm)

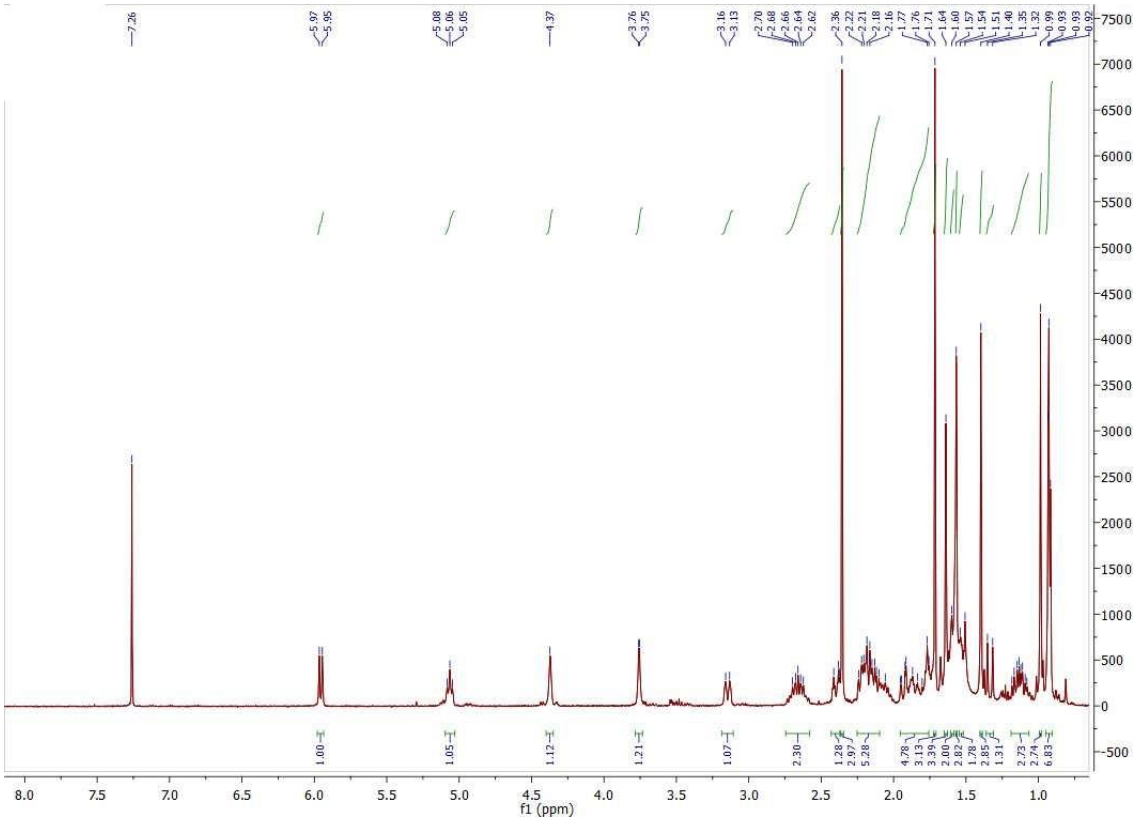
General synthetic procedure for compounds 15-18

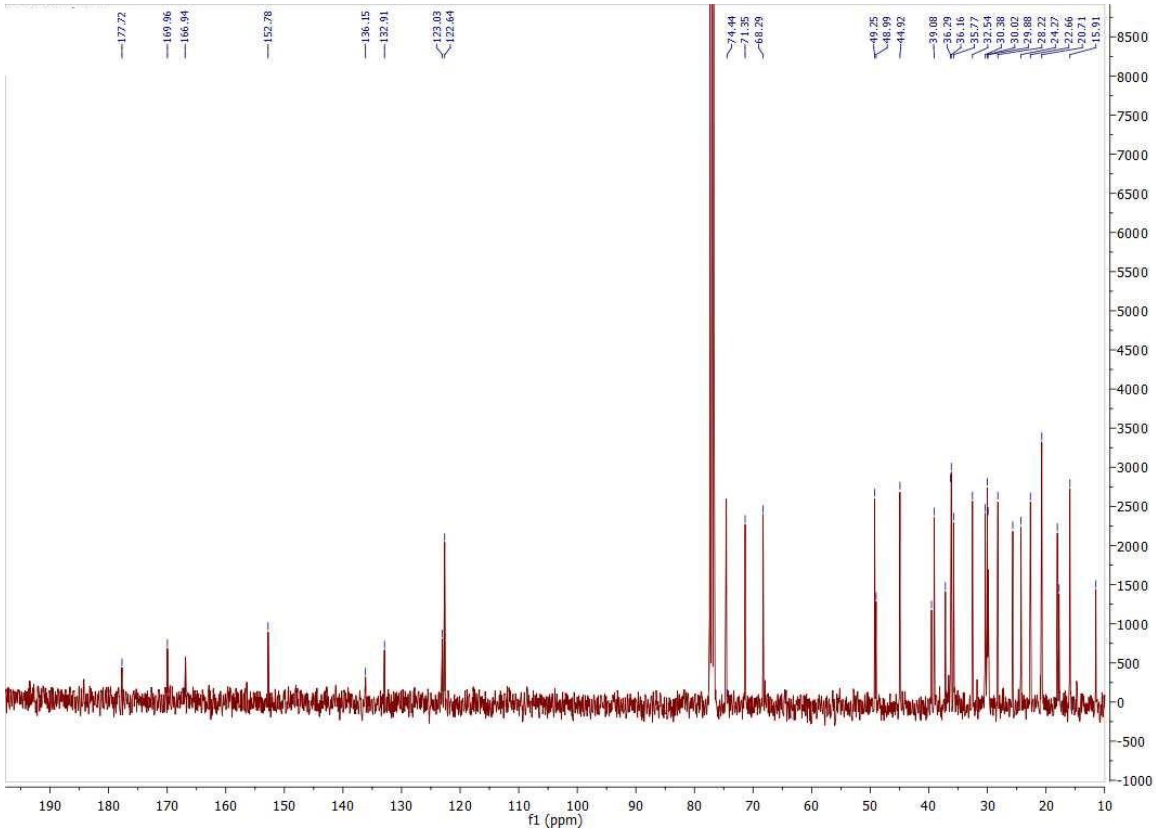
EDCI (1.15 equiv.) and HOBt (1.20 equiv.) were added to a solution of Fusidic acid **1** (1.0 equiv.) in acetonitrile (20 ml) and resulting reaction mixture was stirred at room temperature for 3 h followed by the addition of respective amidoxime (1.10 equiv.) and then reaction mixture was heated at 80^{o}C for 12 h. After completion of reaction (TLC), solvent was removed under reduced pressure and residue was washed with water (3×15 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Without further purification this intermediate and NaOAc (5.0 equiv.) were taken in EtOH (20 ml) and irradiated in microwave at 100^{o}C for 2h. After completion of reaction (TLC), solvent was removed under reduced pressure and reaction mixture was washed with water (2×15 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography on silica gel using EtOAc:hexane as eluent afforded products **15-18**.

Compound 15: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-3,11-dihydroxy-4,8,10,14-tetramethyl-17-(5-methyl-1-(3-methyl-1,2,4-oxadiazol-5-yl)hex-4-en-1-ylidene)hexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate

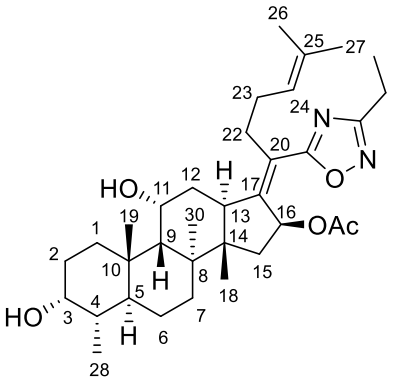


Colourless solid (0.090g, 24%); R_f 0.8 (80% EtOAc:hexane); M. pt. 152-154^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.96 (d, *J* = 8.25 Hz, 1H, C16-*H*), 5.06 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.37 (m, 1H, C11-*H*), 3.76 (m, 1H, C3-*H*), 3.16-3.13 (m, 1H, C13-*H*), 2.70-2.62 (m, 2H, 2×C22-*H*), 2.41-2.37 (m, 1H, C12-*H*), 2.36 (s, 3H, CH_3), 2.24-2.06 (m, 5H, C1-*H*, C5-*H*, C15-*H* and 2×C23-*H*), 1.95-1.76 (m, 4H, 2×C2-*H*, C7-*H* and C12-*H*), 1.71 (s, 3H, OCOCH_3), 1.64 (s, 3H, C27-CH_3), 1.60-1.50 (m, 4H, C1-*H*, C4-*H*, C6-*H* and C9-*H*), 1.57 (s, 3H, C26-CH_3), 1.40 (s, 3H, C30-CH_3), 1.35-1.32 (m, 1H, C15-*H*), 1.18-1.08 (m, 2H, C6-*H* and C7-*H*), 0.99 (s, 3H, C19-CH_3), 0.93-0.92 (m, 6H, C18-C*H*3 and C28-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.7, 170.0, 166.8, 152.7, 136.0, 132.8, 123.0, 122.6, 74.6, 71.3, 68.2, 49.2, 48.9, 44.8, 39.5, 39.0, 37.0, 36.2, 36.1, 35.7, 32.5, 30.3, 30.0, 29.8, 28.2, 25.6, 24.2, 22.6, 20.7, 18.0, 17.7, 15.9 and 11.4; MS: *m/z* 555 $[M+H]^+$; HPLC purity (Method A) 96% (t_r = 13.91).

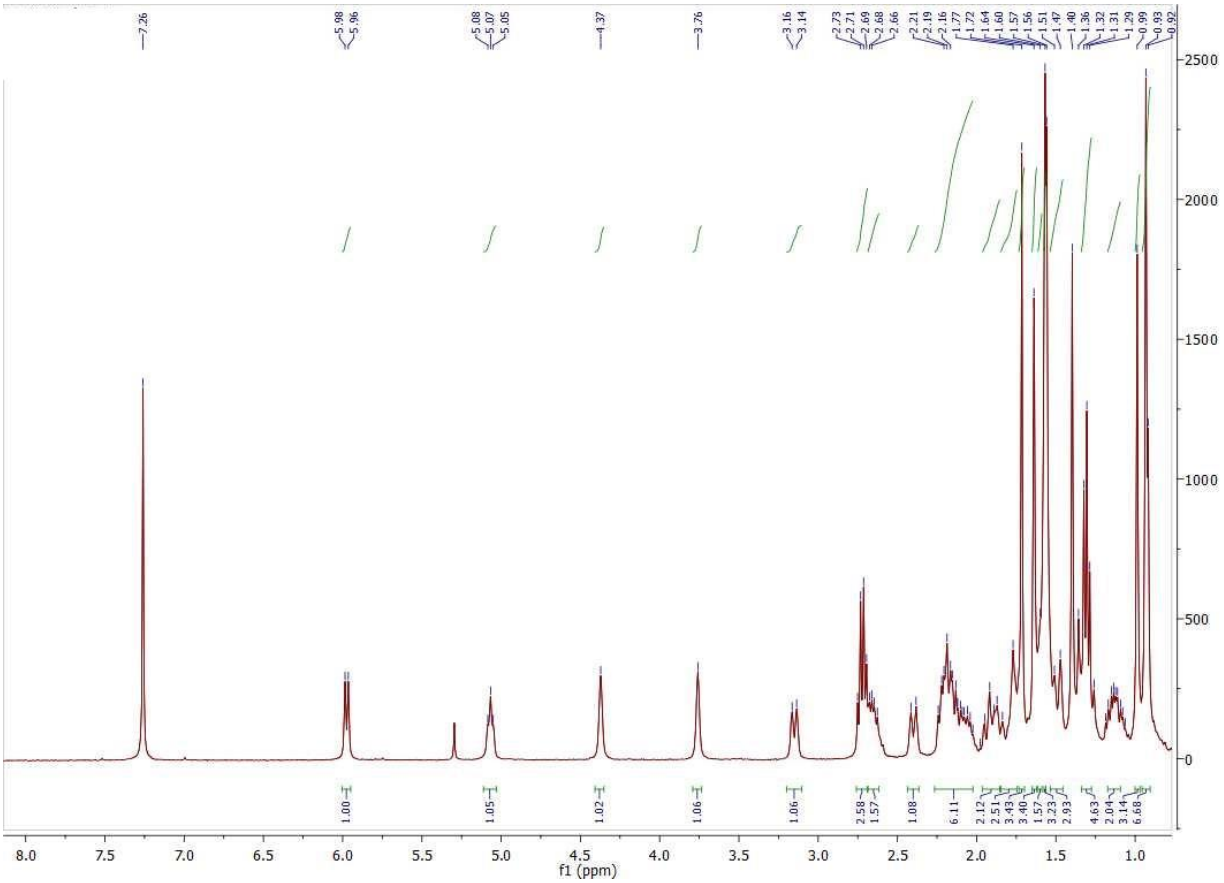

f1 (ppm)


f1 (ppm)

Compound 16: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-17-(1-(3-ethyl-1,2,4-oxadiazol-5-yl)-5-methylhex-4-en-1-ylidene)-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



Colourless solid (0.050g, 23%); R_f 0.6 (80% EtOAc:hexane); M. pt. 126-128^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.97 (d, *J* = 8.44 Hz, 1H, C16-*H*), 5.07 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.37 (m, 1H, C11-*H*), 3.76 (m, 1H, C3-*H*), 3.16-3.14 (m, 1H, C13-*H*), 2.72 (q, *J* = 7.50 Hz, 2H, CH_2CH$_3$), 2.68-2.63 (m, 2H, 2×C22-*H*), 2.41-2.38 (m, 1H, C12-*H*), 2.24-2.02 (m, 5H, C1-*H*, C5-*H*, C15-*H* and 2×C23-*H*), 1.98-1.87 (m, 2H, C2-*H* and C12-*H*), 1.84-1.77 (m, 2H, C2-*H* and C7-*H*), 1.72 (s, 3H, OCOCH_3), 1.64 (s, 3H, C27-CH_3), 1.64-1.47 (m, 4H, C1-*H*, C4-*H*, C6-*H* and C9-*H*), 1.57 (s, 3H, C26-CH_3), 1.40 (s, 3H, C30-CH_3), 1.32-1.29 (m, 4H, C15-*H* and CH$_2$CH_3), 1.19-1.06 (m, 2H, C6-*H* and C7-*H*), 0.99 (s, 3H, C19-CH_3), 0.93-0.92 (m, 6H, C18-CH_3 and C28-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.6, 171.3, 169.9, 152.7, 132.8, 123.0, 122.6, 74.5, 71.3, 68.3, 49.2, 48.9, 44.8, 39.5, 39.0, 37.1, 36.3, 36.1, 35.7, 32.5, 30.3, 30.0, 29.9, 28.2, 25.6, 24.2, 22.6, 20.7 (C2), 19.6, 18.0, 17.7, 15.9 and 11.4; MS: *m/z* 569 $[M+H]^+$, 509 $[M\text{-}OAc]^+$; HPLC purity (Method A) 98% (t_r = 15.38).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5.08
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4.37
3.76
3.16
3.14
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1.72
1.64
1.60
1.57
1.56
1.51
1.47
1.40
1.36
1.32
1.31
1.29
0.99
0.93
0.92
1.00
1.05
1.02
1.06
1.06
2.58
1.57
1.08
6.11
2.12
2.51
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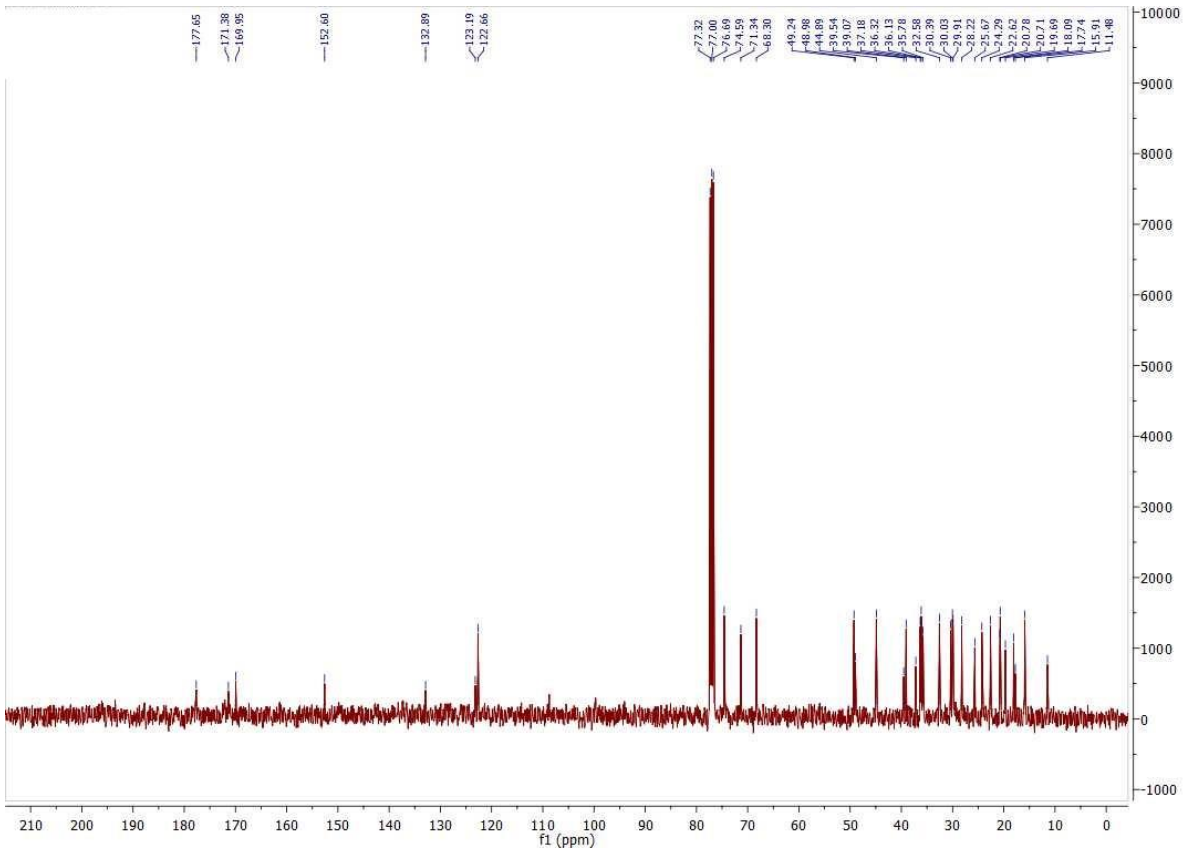
4.63
2.04
3.14
6.68
f1 (ppm)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171.38
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123.19
122.66
77.32
77.00
76.69
74.59
71.34
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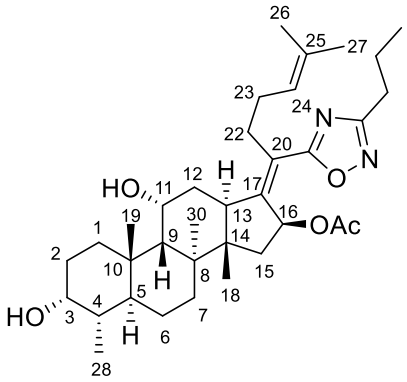
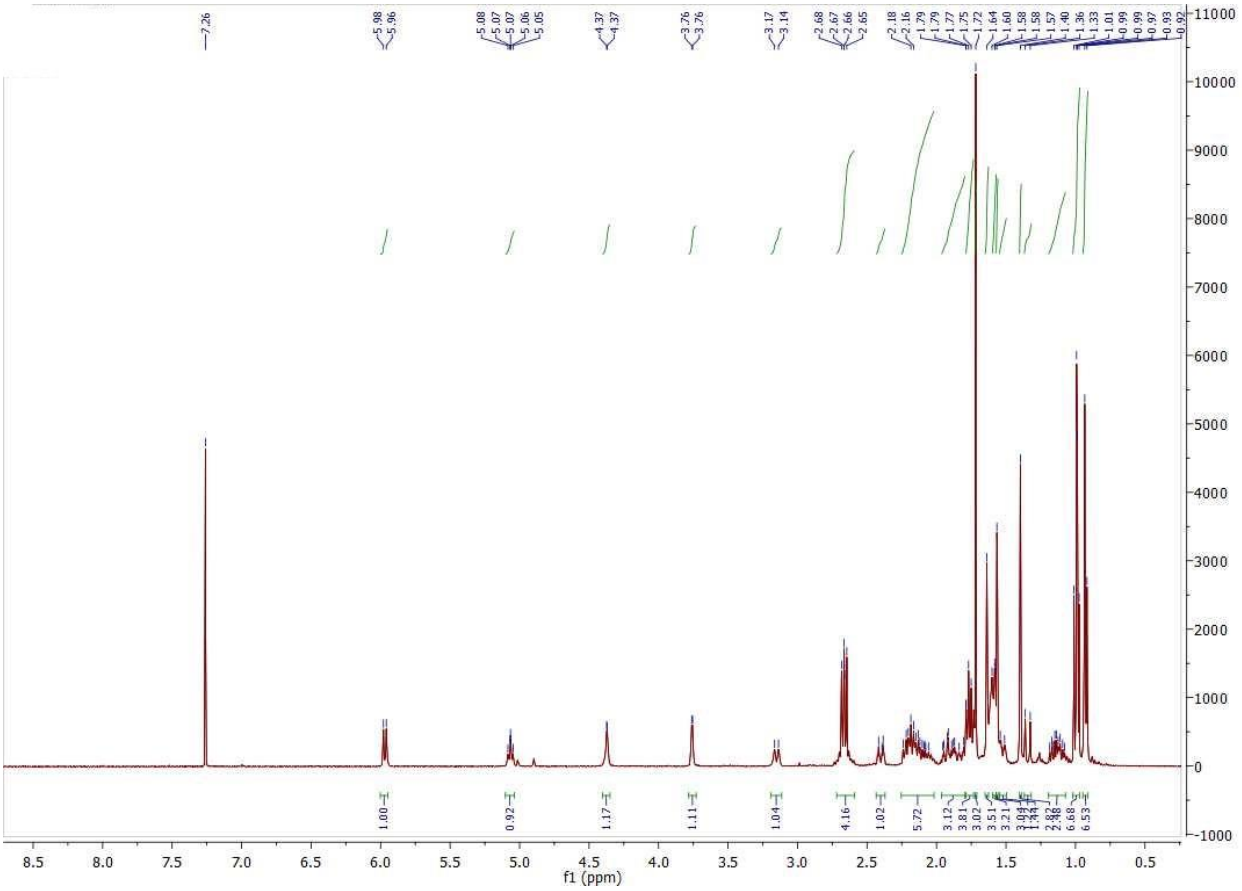
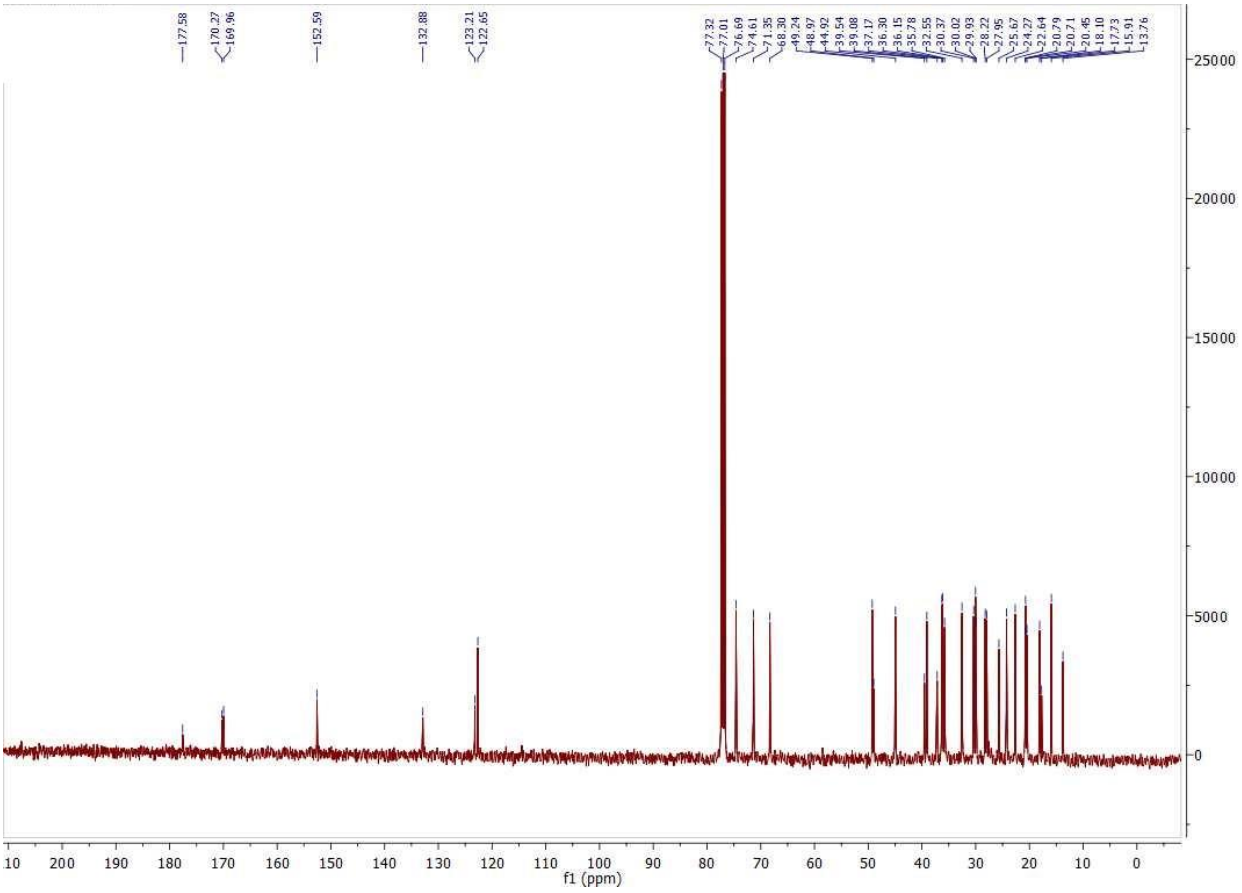
49.24
48.98
44.89
39.54
39.07
37.18
36.32
36.13
35.78
32.58
30.39
30.03
29.91
28.22
25.67
24.29
22.62
20.78
20.71
19.69
18.09
17.74
15.91
11.48
f1 (ppm)

Compound 17: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-3,11-dihydroxy-4,8,10,14-tetramethyl-17-(5-methyl-1-(3-propyl-1,2,4-oxadiazol-5-yl)hex-4-en-1-ylidene)hexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate

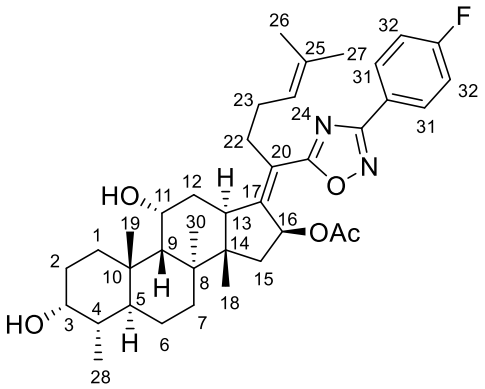


Colourless solid (0.080g, 14%); R_f 0.6 (70% EtOAc:hexane); M. pt. 110-112^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.97 (d, *J* = 8.25 Hz, 1H, C16-*H*), 5.07 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.37 (m, 1H, C11-*H*), 3.76 (m, 1H, C3-*H*), 3.17-3.14 (m, 1H, C13-*H*), 2.68-2.65 (m, 4H, 2×C22-*H* and $CH_2CH_2CH_3$), 2.42-2.38 (m, 1H, C12-*H*), 2.24-2.06 (m, 5H, C1-*H*, C5-*H*, C15-*H* and 2×C23-*H*), 1.95-1.87 (m, 3H, C2-*H*, C7-*H* and C12-*H*), 1.80-1.75 (m, 3H, C2-*H* and $CH_2CH_2CH_3$), 1.77 (s, 3H, $OCOCH_3$), 1.64 (s, 3H, C27-CH_3), 1.60-1.58 (m, 3H, C1-*H*, C6-*H* and C9-*H*), 1.57 (s, 3H, C26-CH_3), 1.54-1.51 (m, 1H, C4-*H*), 1.40 (s, 3H, C30-CH_3), 1.36-1.33 (m, 1H, C15-*H*), 1.19-1.08 (m, 2H, C6-*H* and C7-*H*), 1.01-0.97 (m, 6H, C19-CH_3 and $CH_2CH_2CH_3$), 0.93-0.92 (m, 6H, C28-CH_3 and C18-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.4, 170.2, 169.9, 152.5, 132.7, 123.2, 122.6, 74.6, 71.3, 68.3, 49.2, 48.9, 44.9, 39.5, 39.0, 37.1, 36.3, 36.1, 35.7, 32.5, 30.3, 30.0, 29.9, 28.2, 27.9, 25.6, 24.2, 22.6, 20.7 (C2), 20.4, 18.1, 17.7, 15.9 and 13.7; MS: *m/z* 583 $[M+H]^+$, 523 $[M-OAc]^+$; HPLC purity (Method B) 98% (t_r = 4.33).

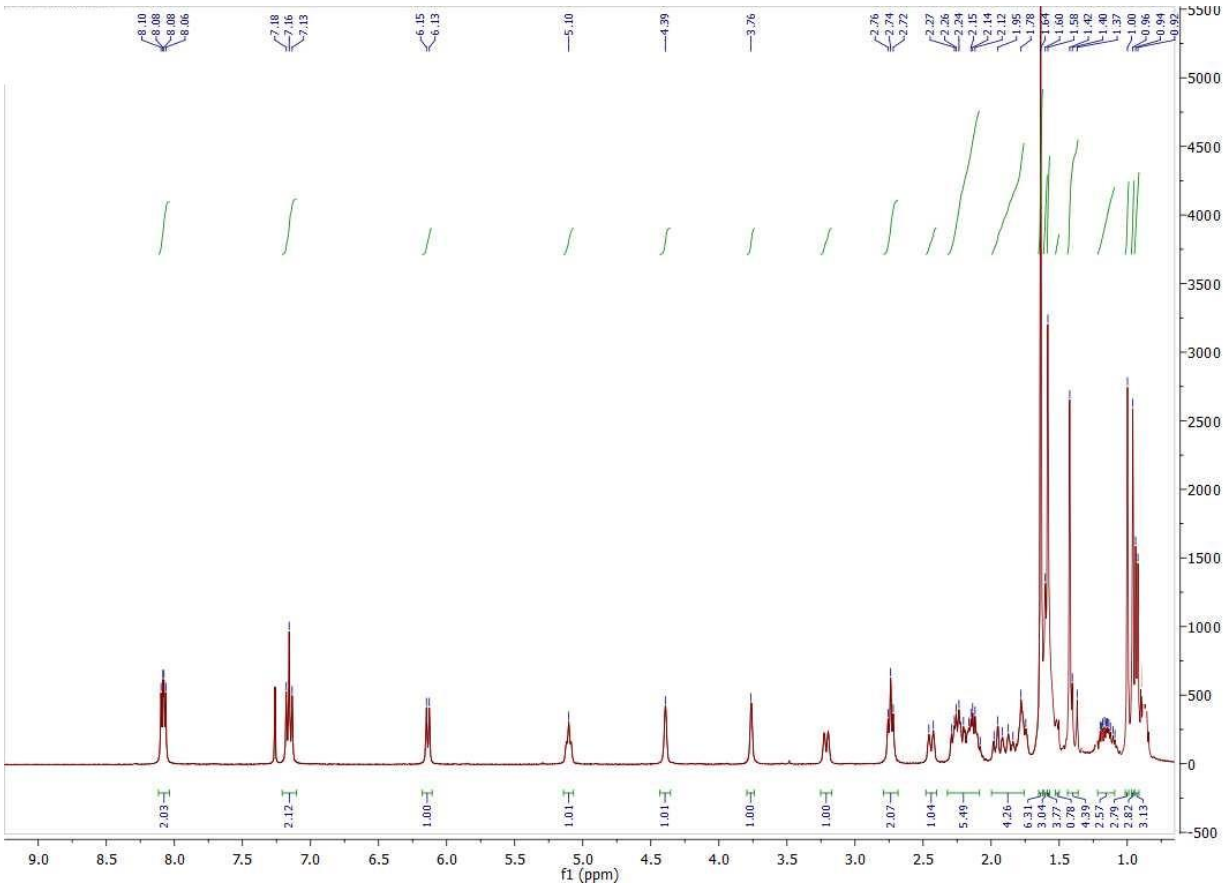

f1 (ppm)

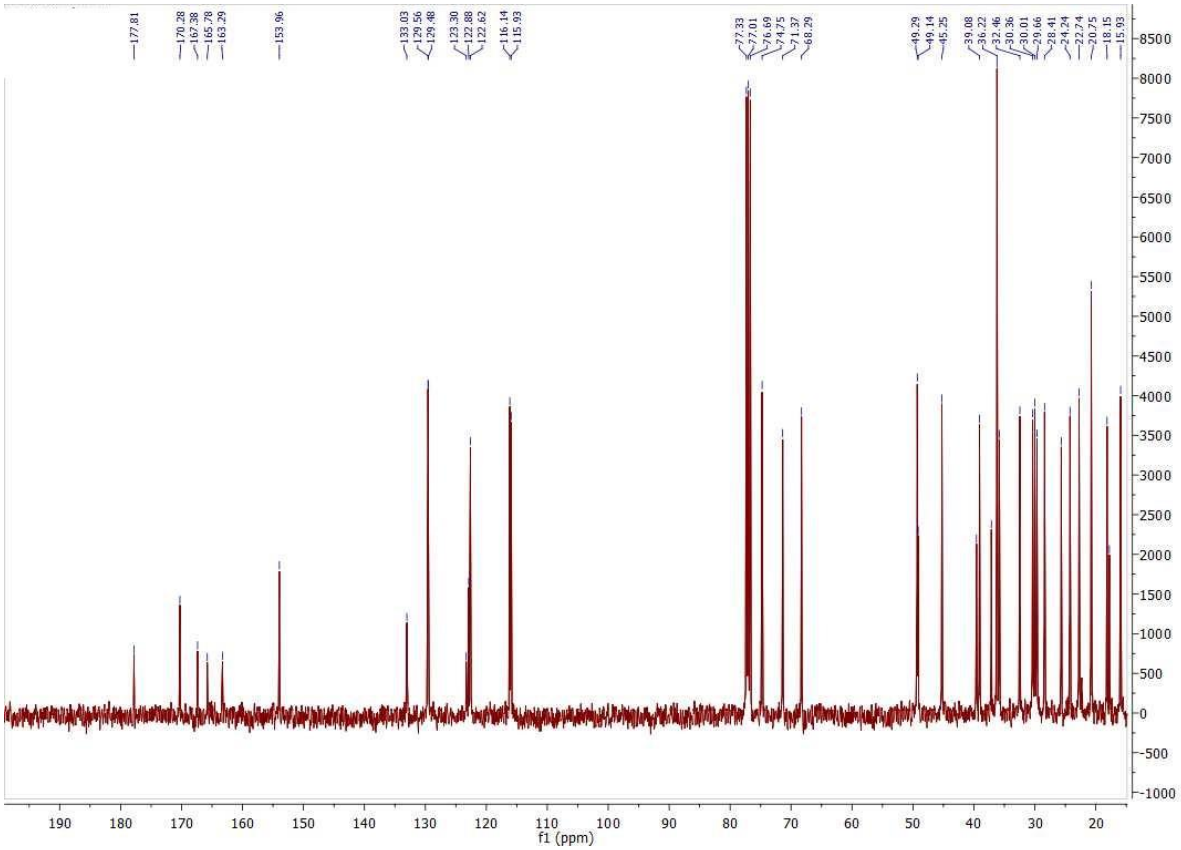

f1 (ppm)

Compound 18: **(**3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-17-(1-(3-(4-fluorophenyl)-1,2,4-oxadiazol-5-yl)-5-methylhex-4-en-1-ylidene)-3,11-dihydroxy-4,8,10,14-tetramethylhexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate

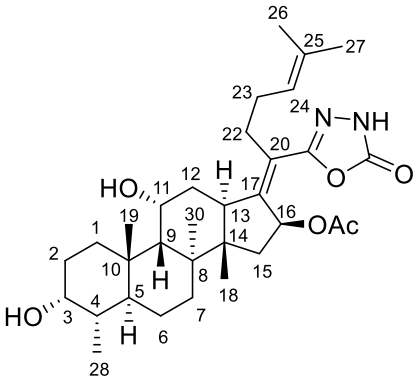


Colourless solid (0.10g, 20%); R_f 0.7 (50% EtOAc:hexane); M. pt. 86-88^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, *J* = 8.80 and 5.32 Hz, 2H, ArH^{32}), 7.16 (dd, *J* = 8.60 and 5.50Hz, 2H, ArH^{31}), 6.14 (d, *J* = 8.07 Hz, 1H, C16-*H*), 5.10 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.39 (m, 1H, C11-*H*), 3.76 (m, 1H, C3-*H*), 3.23-3.20 (m, 1H, C13-*H*), 2.74 (t, *J* = 7.70 Hz, 2H, 2×C22-*H*), 2.46-2.42 (m, 1H, C12-*H*), 2.29-2.08 (m, 5H, C1-*H*, C5-*H*, C15-*H* and 2×C23-*H*), 1.98-1.75 (m, 4H, 2×C2-*H*, C7-*H* and C12-*H*), 1.64 (s, 6H, OCOCH_3 and C27-CH_3), 1.58 (s, 3H, C26-CH_3), 1.60-1.50 (m, 4H, C1-*H*, C4-*H*, C6-*H* and C9-*H*), 1.42 (s, 3H, C30-CH_3), 1.40-1.37 (m, 1H, C15-*H*), 1.22-1.09 (m, 2H, C6-*H* and C7-*H*), 1.00 (s, 3H, C19-CH_3), 0.96 (s, 3H, C18-CH_3), 0.93 (d, *J* = 6.79 Hz, 3H, C28-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 170.2, 167.3, 165.7, 163.2, 153.9, 133.0, 129.5, 129.4, 123.2, 122.8, 122.6, 116.1, 115.9, 74.7, 71.3, 68.2, 49.2, 49.1, 45.2, 39.5, 39.0, 37.1, 36.2, 35.8, 32.4, 30.3, 30.0, 29.6, 28.4, 25.6, 24.2, 22.7, 20.7 (C2), 18.1, 17.7 and 15.9; MS: *m/z* 635 $[M+H]^+$, 575 $[M\text{-}OAc]^+$; HPLC purity (Method A) 97% (t_r = 16.80).

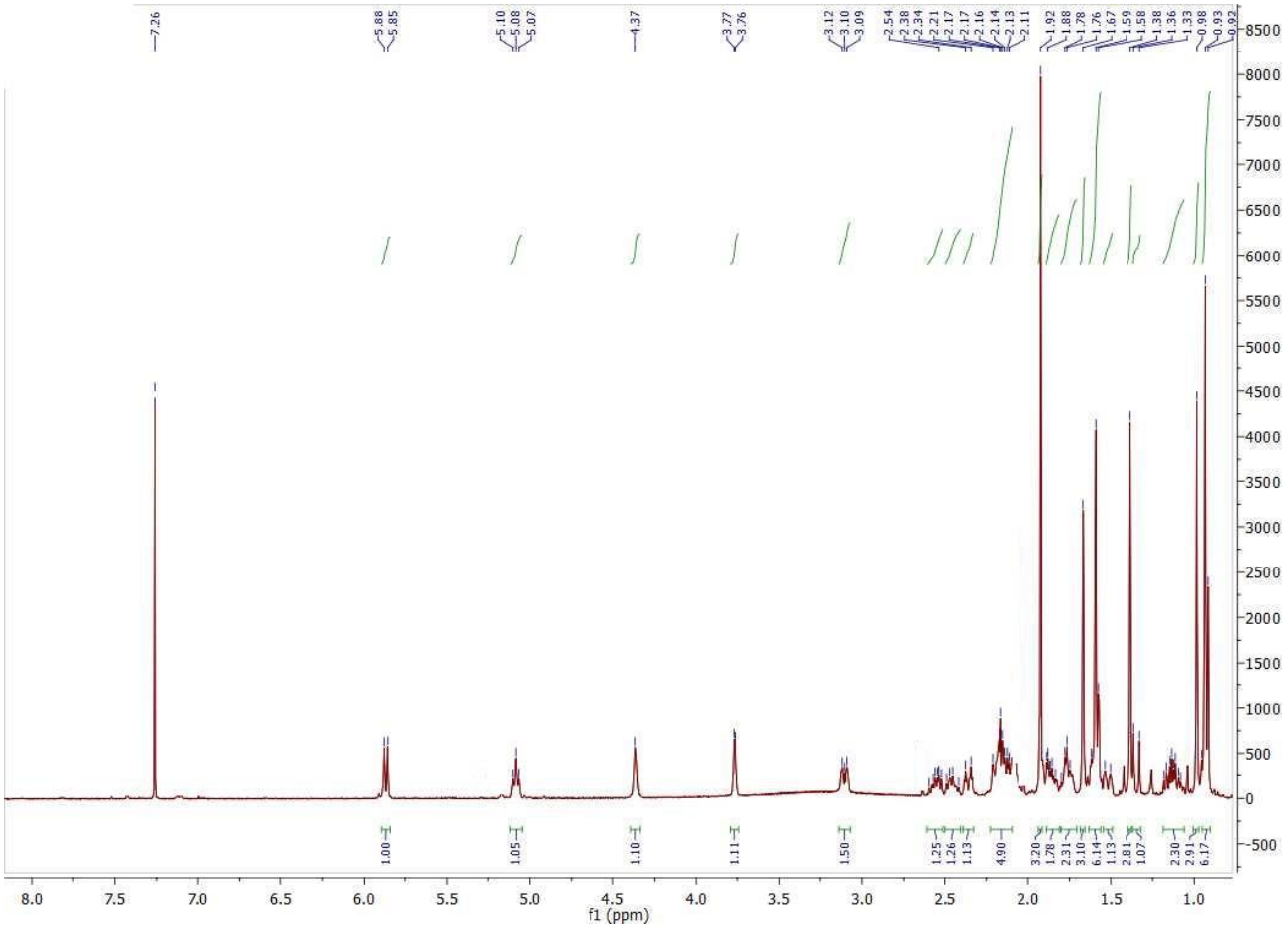

f1 (ppm)


f1 (ppm)

Compound 19: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-3,11-dihydroxy-17-(1-(5-hydroxy-1,3,4-oxadiazol-2-yl)-5-methylhex-4-en-1-ylidene)-4,8,10,14-tetramethylhexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



Et_3N (0.1 ml, 0.72 mmol) was added to a solution of compound **2** (0.2g, 0.38 mmol) in THF (10 ml) and reaction mixture was cooled to 0^{o}C followed by addition of 1,1' carbonyldiimidazole (CDI) (0.092g, 0.56 mmol). Reaction mixture was stirred at 0^{o}C for 5 h followed by further addition of Et_3N (0.1 ml, 0.72 mmol) and CDI (0.092g, 0.56 mmol). Reaction mixture was stirred at room temperature for 11 h and solvent was removed under reduced pressure and residue was taken in EtOAc (15 ml) and washed with water (3×15 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography on silica gel using EtOAc:hexane as eluent afforded products **19** as colourless solid (0.052g, 25%); R_f 0.5 (70% EtOAc: hexane); M. pt. 137-139^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 (d, *J* = 8.25 Hz, 1H, C16-*H*), 5.08 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.37 (m, 1H, C11-*H*), 3.77 (m, 1H, C3-*H*), 3.12-3.09 (m, 1H, C13-*H*), 2.59-2.52 (m, 1H, C22-*H*), 2.49-2.42 (m, 1H, C22-*H*), 2.38-2.34 (m, 1H, C12-*H*), 2.21-2.11 (m, 5H, C1-*H*, C5-*H*, C15-*H* and 2×C23-*H*), 1.92 (s, 3H, OCOCH_3), 1.89-1.83 (m, 2H, C2-*H* and C12-*H*), 1.78-1.75 (m, 2H, C2-*H* and C7-*H*), 1.67 (s, 3H, C27-CH_3), 1.62-1.58 (m, 6H, C26-CH_3, C1-*H*, C6-*H* and C9-*H*), 1.54-1.50 (m, 1H, C4-*H*), 1.38 (s, 3H, C30-CH_3), 1.36-1.33 (m, 1H, C15-*H*), 1.18-1.08 (m, 2H, C6-*H* and C7-*H*), 0.98 (s, 3H, C19-CH_3), 0.93-0.92 (m, 6H, C18-CH_3 and C28-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.6, 170.3, 156.6, 154.4, 151.5, 132.9, 122.5, 122.2, 74.5, 71.4, 68.2, 49.2, 48.8, 44.8, 39.5, 39.1, 37.1, 36.1, 35.7, 32.4, 30.3, 29.9, 28.5, 28.1, 25.6, 24.2, 22.6, 20.9, 20.7, 18.1, 17.8 and 15.8; MS: *m/z* 557 $[M+H]^+$; HPLC purity (Method A) 96% (t_r = 12.91).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5.10
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5.07
4.37
3.77
3.76
3.12
3.10
3.09
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2.34
2.21
2.17
2.17
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2.13
2.11
1.92
1.88
1.78
1.76
1.67
1.59
1.58
1.38
1.36
1.33
0.98
0.93
0.92
1.00
1.05
1.10
1.11
1.50
1.25
1.26
1.13
4.90
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2.30
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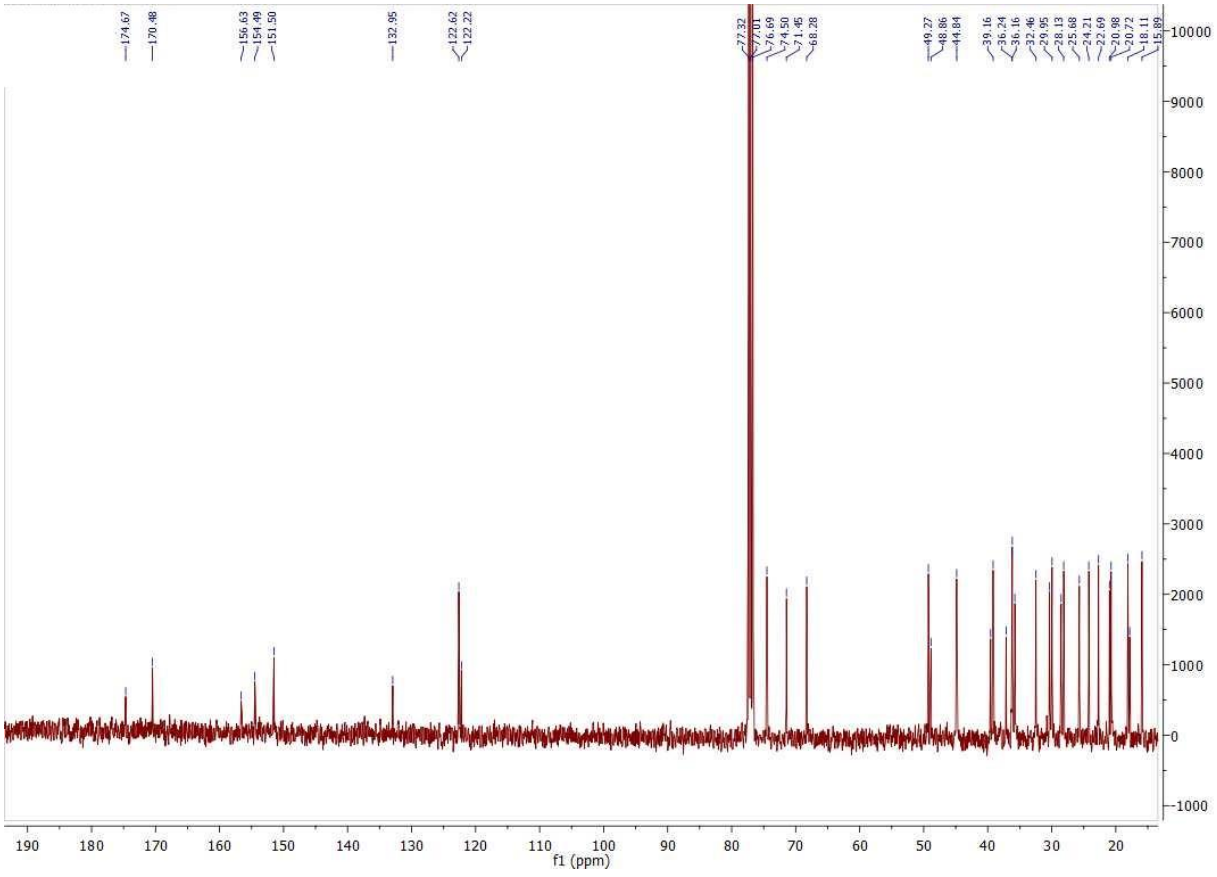
6.17
f1 (ppm)


174.67
170.48
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154.49
151.50
132.95
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122.22
77.32
77.01
76.69
74.50
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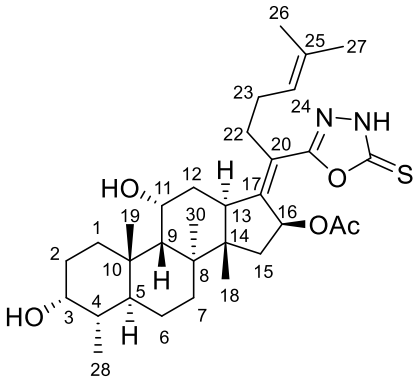
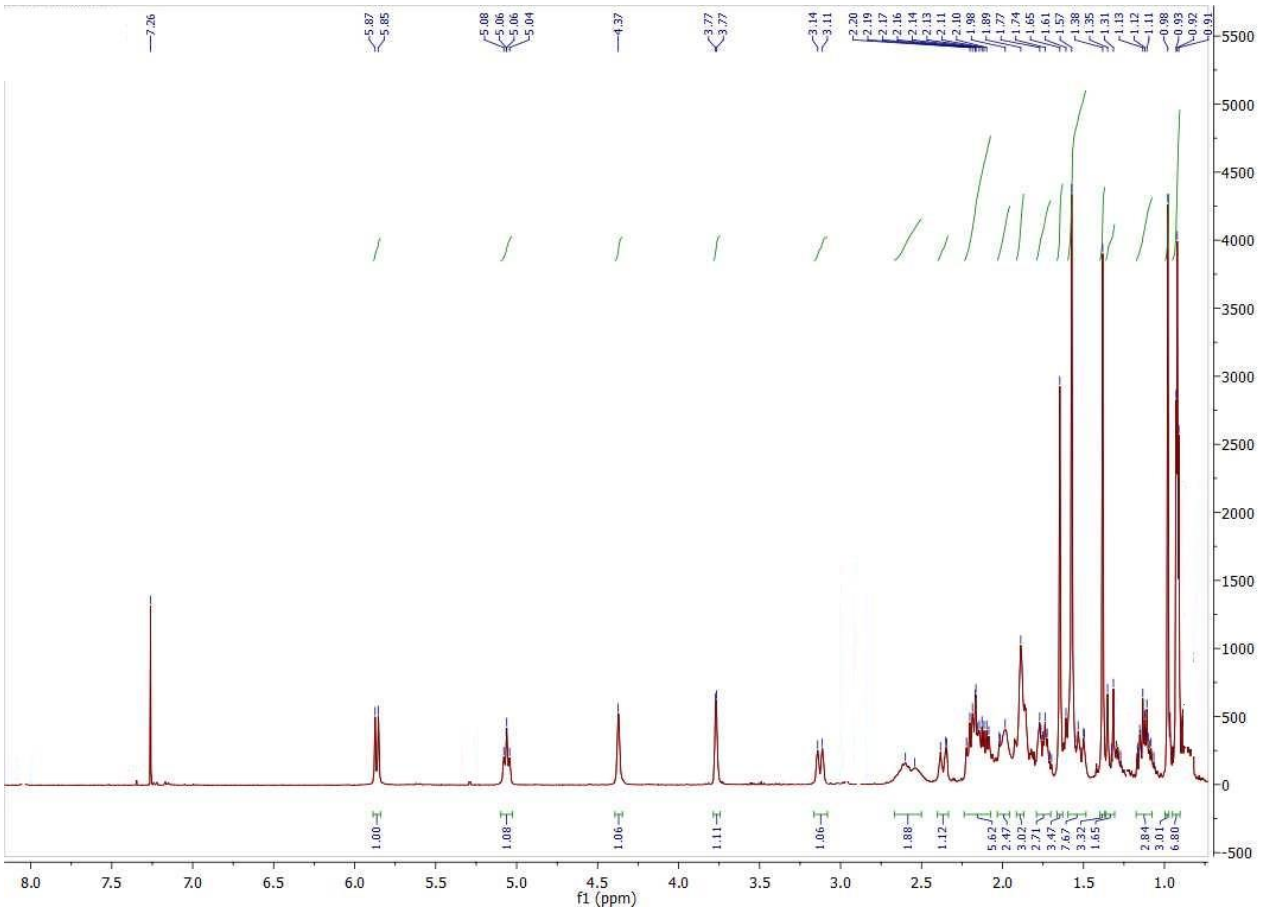
68.28
49.27
48.86
44.84
39.16
36.24
36.16
32.46
29.95
28.13
25.68
24.21
22.69
20.98
20.72
16.11
15.99
f1 (ppm)

Compound 20: (3R,4S,5S,8S,9S,10S,11R,13R,14S,16S,Z)-3,11-dihydroxy-4,8,10,14-tetramethyl-17-(5-methyl-1-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)hex-4-en-1-ylidene)hexadecahydro-1H-cyclopenta[a]phenanthren-16-yl acetate



Carbon disulfide (0.03 ml, 0.52 mmol) and KOH (0.026g, 0.47 mmol) were added to a solution of compound **2** (0.25g, 0.47 mmol) in ethanol (5 ml) at 0^{o}C and then reaction mixture was shifted to heating at 80^{o}C until reaction proceeds to completion. After completion of reaction (TLC, 16 h), solvent was removed under reduced pressure and diluted with EtOAc (15 ml) and washed with water (3×15 ml), dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. Purification by flash chromatography on silica gel using EtOAc:hexane as eluent afforded products **20** as a colourless solid (0.15g, 56%); R_f 0.4 (90% EtOAc:hexane); M. pt. 130-132^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 (d, *J* = 8.25 Hz, 1H, C16-*H*), 5.06 (t, *J* = 6.79 Hz, 1H, C24-*H*), 4.37 (m, 1H, C11-*H*), 3.77 (m, 1H, C3-*H*), 3.14-3.11 (m, 1H, C13-*H*), 2.60-2.54 (m, 2H, 2×C22-*H*), 2.38-2.34 (m, 1H, C12-*H*), 2.20-2.08 (m, 5H, C1-*H*, C5-*H*, C15-*H* and 2×C23-*H*), 2.02-1.98 (m, 2H, C2-*H* and C12-*H*), 1.89 (s, 3H, OCOCH_3), 1.77-1.70 (m, 2H, C2-*H* and C7-*H*), 1.65 (s, 3H, C27-CH_3), 1.61-1.50 (m, 7H, C26-CH_3, C1-*H*, C4-*H*, C6-*H* and C9-*H*), 1.38 (s, 3H, C30-CH_3), 1.35-1.31 (m, 1H, C15-*H*), 1.17-1.07 (m, 2H, C6-*H* and C7-*H*), 0.98 (s, 3H, C19-CH_3), 0.93-0.91 (m, 6H, C18-CH_3 and C28-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (C2), 153.0 (C2), 133.0, 122.4, 121.0, 74.5, 71.5, 68.1, 49.3, 48.9, 45.1, 39.5, 39.1, 37.0, 36.3, 36.0, 35.7, 32.1, 30.2, 29.8, 29.0, 28.1, 25.6, 24.0, 22.9, 20.9, 20.8, 18.0, 17.8 and 15.9; MS: *m/z* 595 $[M+23]^+$, 513 $[M-OAc]^+$; HPLC purity (Method B) 98% (t_r = 4.55).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5.06
5.04
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3.77
3.14
3.11
2.20
2.19
2.17
2.16
2.14
2.13
2.11
2.10
1.98
1.89
1.77
1.74
1.65
1.61
1.57
1.38
1.35
1.31
1.13
1.12
1.11
0.98
0.93
0.92
0.91
1.00
1.08
1.06
1.11
1.06
1.88
1.12
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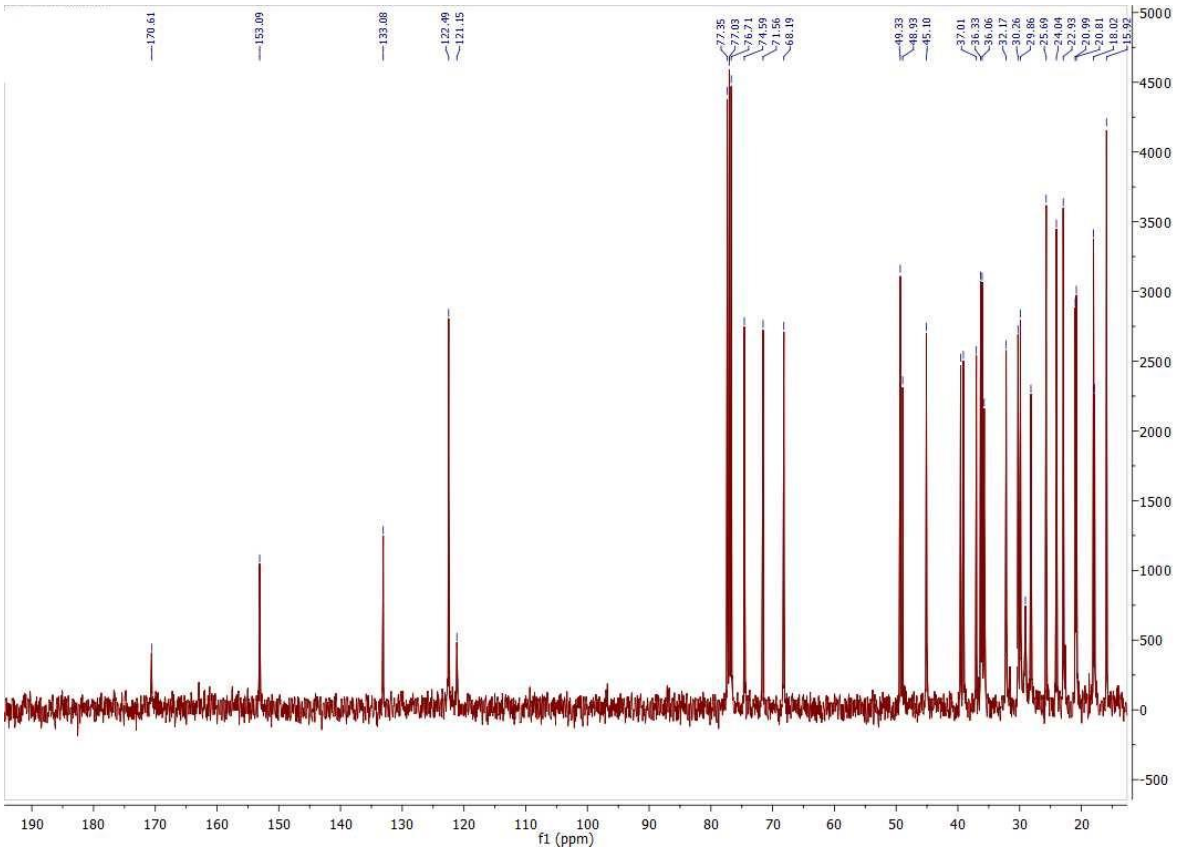
6.80
f1 (ppm)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74.59
71.56
68.19
49.33
48.93
45.10
37.01
36.33
36.06
32.17
30.26
29.86
25.69
25.04
24.93
20.99
20.81
18.01
15.92
f1 (ppm)

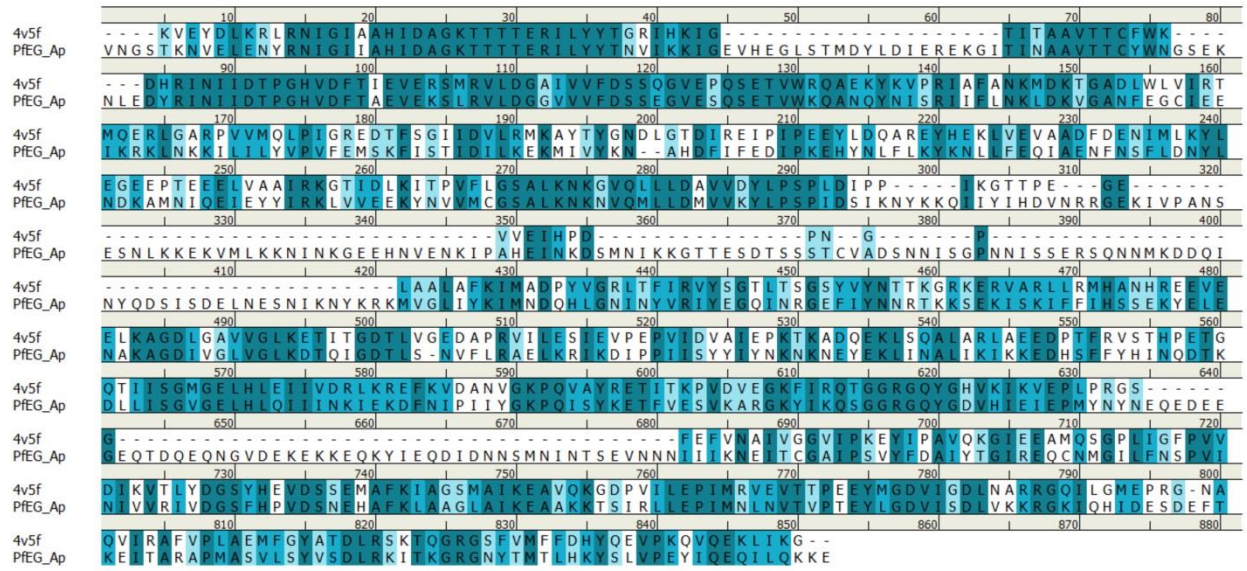
Homology modelling

The primary sequence of target proteins, apicoplast (*Pf*EF-G_{Ap}) and mitochondrial (*Pf*EF-G_{Mit}) elongation factor G of *Plasmodium falciparum* were obtained from PlasmoDB (http://plasmodb.org/plasmo/) (*Pf*EF-G_{Ap} Gene ID: PF3D7_0602400 and *Pf*EF-G_{Mit} Gene ID: PF3D7_1233000). The matured form of *Pf*EF-G_{Ap} (89-937) and *Pf*EF-G_{Mit} (35-803) were considered for this modeling study[1,2]. The EF-G of *Thermus thermophiles* (*Tt*EF-G) (PDB code: 4V5F, Y chain) was selected as a template for building of both *Pf*EF-Gs models.[3] Multiple sequence alignment of *Pf*EF-G_{Ap} and *Pf*EF-G_{Mit} with this template sequence was separately performed using Align123algorithm[4] which calculates multiple sequence alignments using sequence and predicted structural information. The homology models for *Pf*EF-G_{Ap} and *Pf*EF-G_{Mit} were constructed using *Modeler 9v7*, implemented in Discovery Studio 4 (Accelrys, San Diego, CA, USA). The *Modeler* is an automated homology modeling program that performs automated protein homology modeling and loop modeling for efflux protein by satisfaction of spatial restraints.[5] The quality of selected models were evaluated by a test for its internal consistency and reliability such as stereochemical quality and non-bonded atom interactions using PROCHECK.[6]

A similar fold protein of *Pf*EF-Gs, the crystal structure of *Tt*EF-G (PDB code: 4V5F, Y chain), was used as a template to build both *Pf*EF-G_{Ap} and *Pf*EF-G_{Mit} models, and the Y chain of template is a ternary complex (EF-G:GDP:Fusidic Acid) that bound to the post translocation state of the ribosome. The correct sequence alignment between the *Tt*EF-G and *Pf*EF-G_{Ap} and *Pf*EF-G_{Mit} was performed separately using the Align123 and the resulted alignment of *Pf*EF-G_{Ap} and *Pf*EF-G_{Mit} with *Tt*EF-G are shown in Figure S1A and S1B, respectively. The sequence identity and similarity between the *Tt*EF-G and *Pf*EF-G_{Ap} were 34.5% and 55.9%, respectively, whereas the sequence identity and similarity between the *Tt*EF-G and *Pf*EF-G_{Mit} were 34.5% and 52.4%, respectively. The predicted structural information of *Pf*EF-G_{Ap} and *Pf*EF-G_{Mit} and PDB structural assignments of *Tt*EF-G were also used for the alignment. The 3D models for the *Pf*EF-G_{Ap} and *Pf*EF-G_{Mit} proteins were built using *Modeler* 9v7 program and the input parameters of *Modeler* were set to generate 25 models with the level of optimization set to high. The best model was selected based on the lowest PDF energy for the docking studies and the model's quality assessed with structural evaluation program such as PROCHECK. Ramachandran plot analysis of *Pf*EF-G_{Ap} and *Pf*EF-G_{Mit} from PROCHECK is shown in Figure S2 and S3, respectively, which showed that the models are within the range of a reasonable quality. The structure of the *Pf*EF-G_{Ap} and *Pf*EF-G_{Mit} models, as shown in Figure S4A and S4C, are similar in overall fold with the

template protein *Tt*EF-G. The superimposition of the *Pf*EF-G_{Ap} and *Pf*EF-G_{Mit} models with that of *Tt*EF-G showed a 0.61 A˚ RMSD (Figure S4B) for 658 C_{α}-pair and a 0.48 A˚ RMSD (Figure S4D) for 663 C_{α}-pair, respectively. These low RMSDs signify that the overall tertiary structures of the models are similar with the template structure. In addition, the superimpositions reveal that the most part of the secondary structures are almost conserved with *Tt*EF-G. However, there are insertions in both models which appear as loops (Figure S4 (B) and (D)).

(A)



(B)

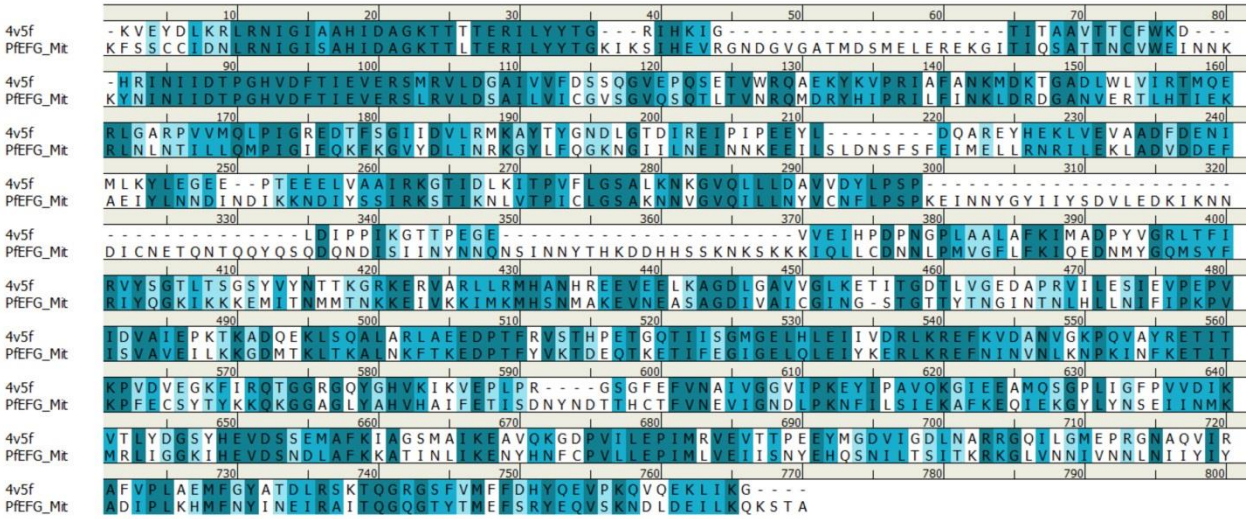


Figure S1. Sequence alignment of *Pf*EF-G_{Ap} **(A)** and *Pf*EF-G_{Mit} **(B)** with the crystal structure of *Tt*EF-G (PDB ID: 4V5F). Colouring scheme indicates the degree of similarity at each alignment column. Identical (strong blue background), strongly similar (light blue

background), weakly similar (very light blue background) and non-matching residues (white background) are highlighted.

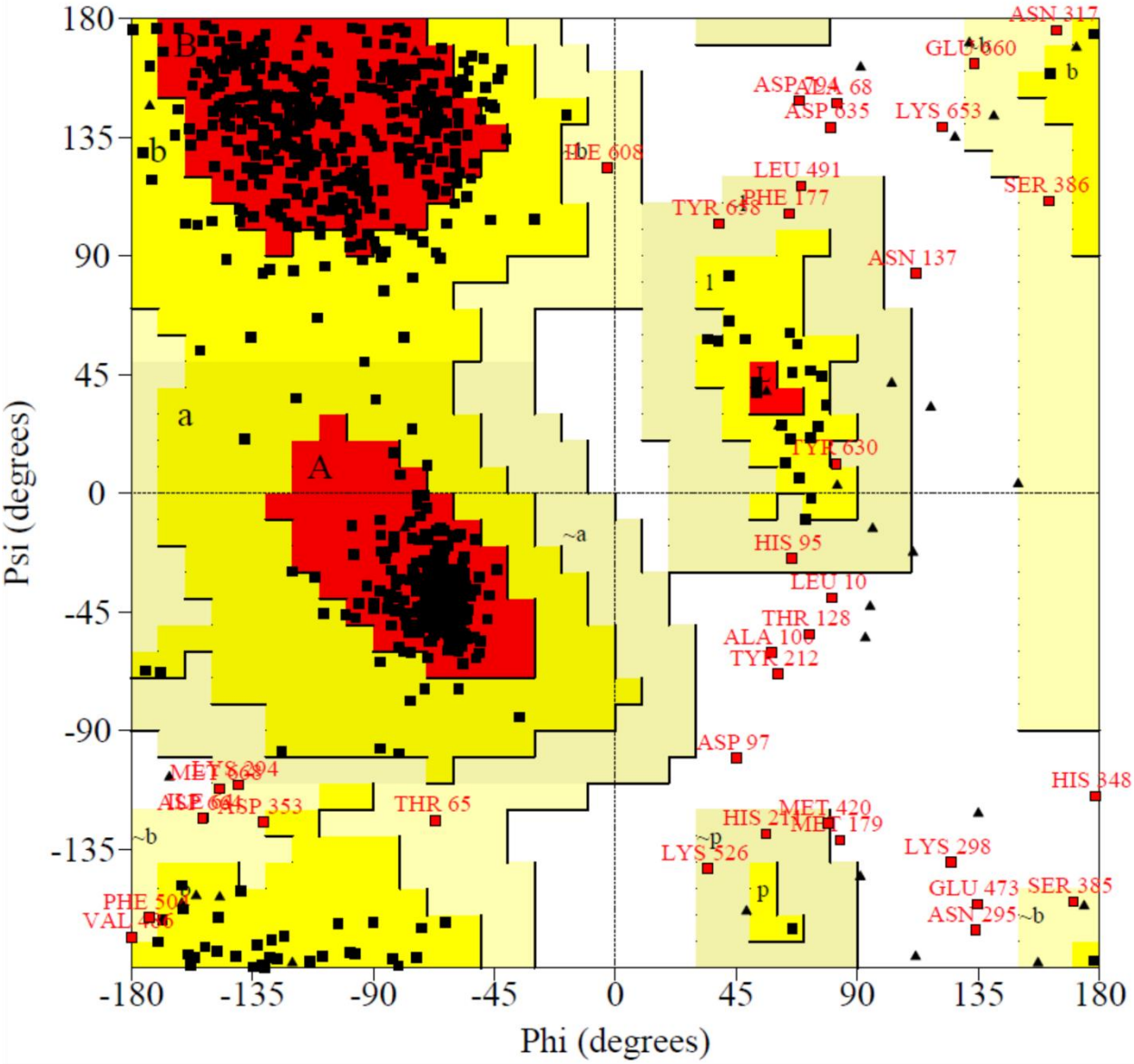


Figure S2. Ramachandran plot of the *Pf*EF-G_{Ap} model. Of the model residues, 79% occupy the core regions (red), 16.3% occupy allowed regions (yellow), 2.7% occupy generously allowed regions (light yellow), and 1.9% occupy disallowed regions (white). Over 90% of residues in the allowed regions indicate that the model is within a range of reasonable quality.

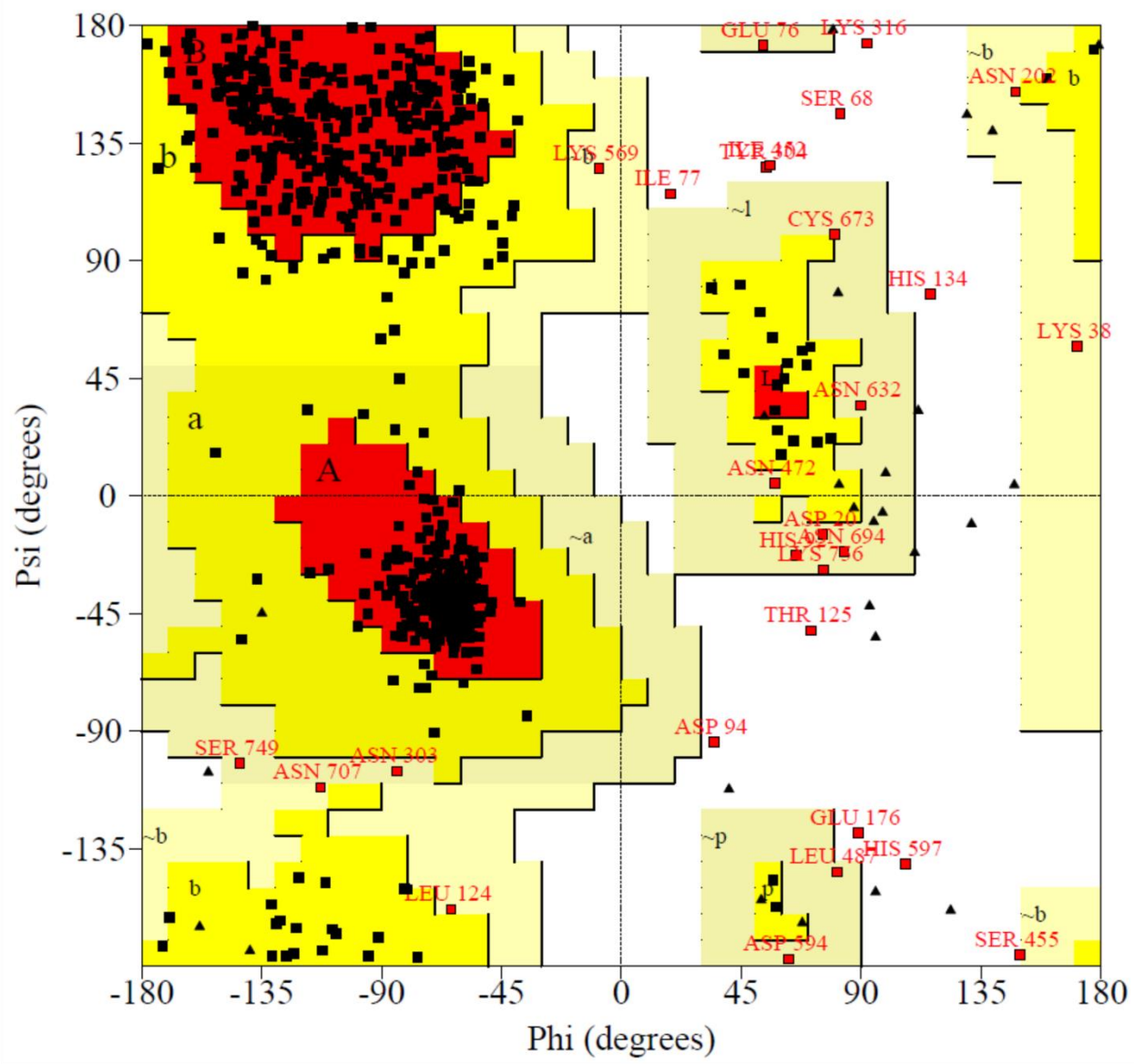


Figure S3. Ramachandran plot of the *Pf*EF-G_{Mit} model. Of the model residues, 82% occupy the core regions (red), 14 % occupy allowed regions (yellow), 2.4% occupy generously allowed regions (light yellow), and 1.6% occupy disallowed regions (white). Over 90% of residues in the allowed regions indicate that the model is within a range of reasonable quality.

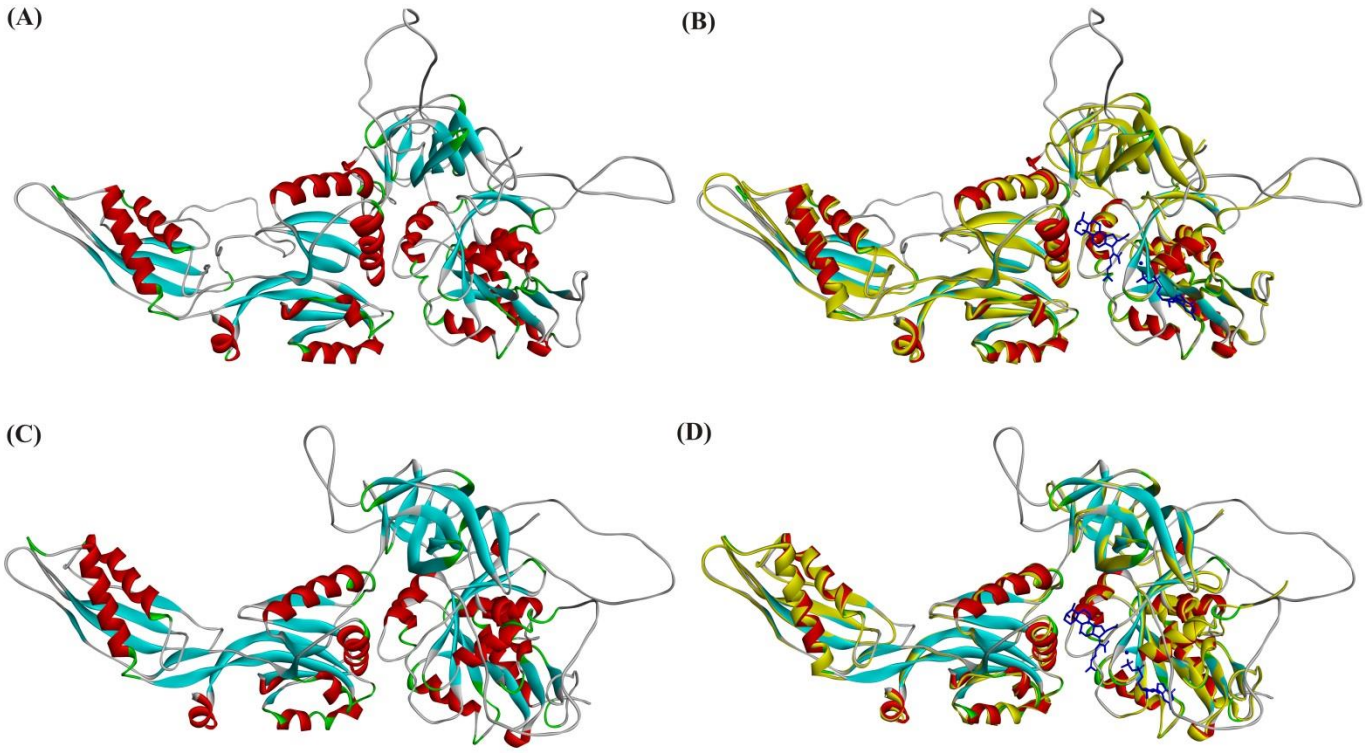


Figure S4 The final 3D structure of *Pf*EF-G_{Ap} **(A)** and *Pf*EF-G_{Mit} (**C**). The comparison of *Pf*EF-G_{Ap} (**B**) and *Pf*EF-G_{Mit} **(D)** (in cyan color) models with its template structure *Tt*EF-G (in yellow color). Proteins are shown as ribbon diagram. The bound fusidic acid, GDP and Mg^{2+} are shown in stick representation in blue colour in (**B**) and **(D).**

Docking studies

The validated models of *Pf*EF-G_{Ap} and *Pf*EF-G_{Mit} were prepared for docking studies using *Protein Preparation Wizard* of Schrödinger suite (Schrödinger, Inc., New York, NY, USA). This wizard corrects bond orders, adds hydrogen atoms, optimizes the orientations of added hydrogens for optimal hydrogen bond formation, and finally minimizes heavy atoms to RMSD threshold of 0.3 Å using OPLS_2005 (optimized potential for liquid simulations_2005) force fields. The 2D structures of fusidic acid 1 and its most active derivative **18**, reported in this study, were sketched using *ChemDraw* and saved as *mol* file format. The ligand structures were imported to *Maestro* and added hydrogens and saved as PDB files for the docking studies. The fusidic acid binding site on the validated models of *Pf*EF-G_{Ap} and *Pf*EF-G_{Mit} was defined based on the bound fusidic acid of template by sequence alignment and match selection procedures.

To check the suitability of the docking programs for fusidic acid and **18**, preliminary docking calculations were carried out using *Glide 5.7* (Glide, version 5.7, Schrödinger, LLC, New York, NY, 2014), *Autodock 4.2*[7] and *Autodock Vina*.[8] For this, the template structure,

*Tt*EF-G in complex with fusidic acid (PDB code: 4V5F, Y chain), was used and the bound fusidic acid was sketched and re-docked to the fusidic acid binding site of *Tt*EF-G. Glide and Autodock could not able to regenerate the reasonable bound conformation of fusidic acid, which may be due to the high flexibility of ligand and the absence of strong interaction between receptor and ligand such as hydrogen bond. However, the *AutoDock Vina*, improved the speed and accuracy of docking, is able to regenerate the experimentally observed binding conformation of fusidic acid as shown in Figure S5. The heavy atom root-mean square deviation (RMSD) between the bound and the scored pose of the docked fusidic acid was found to be 1.56 A˚. So *Autodock Vina* was used for docking of fusidic acid and **18** in this study. In the Autodock Vina docking calculations, the search space size of grid points 20 x 20 x 20 was centred at xyz coordinates -55.881, -81.584 and 50.564 with a spacing of 1 Å as shown in Figure S6, which was achieved, based on the center of bound fusidic acid in the template, using *AutoDock Tools 1.5.6*. The running of the *AutoDock Vina* program for fusidic acid resulted in 9 scored different poses and the pose corresponding to the more negative score of binding affinity was selected as the most probable binding pose. As fusidic acid binding site of *Tt*EF-G is conserved with the *Pf*EF-Gs, the same docking parameters of *Autodock Vina* was used to dock the fusidic acid and **18** to *Pf*EF-G_{Ap} and *Pf*EF-$G_{Mit,}$ and the reasonable scored binding poses for the ligands were selected as done in docking validation.

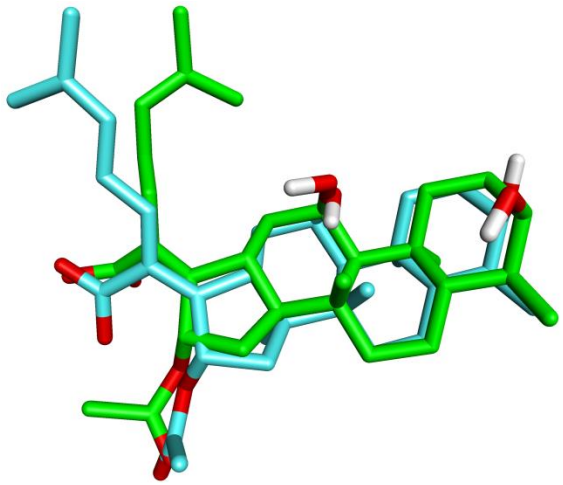


Figure S5. Superimposition of docked conformation (green colour) with the crystal bound conformation (cyan colour) of Fusidic acid of *Tt*EF-G. Both conformations are shown in the stick representation.

```
receptor=prep_1.pdbqt
ligand=cry_FUA_dock.pdbqt

out=FUA.pdbqt

center_x=-55.881
center_y=-81.584
center_z=50.564

size_x=20
size_y=20
size_z=20

exhaustiveness=25
```

Figure S6. Docking parameters of *Autodock Vina* program, which was used to dock fusidic acid and **18** to the *Pf*EF-G_{Ap} and *Pf*EF-$G_{Mit.}$

In vitro Assays

Antiplasmodial assay

Compounds were screened against drug sensitive NF54 strain of *Plasmodium falciparum in vitro* using the modified [^{3}H]hypoxanthine incorporation assay.[9] *Plasmodium falciparum*, NF54 (sensitive to all known drugs) was used and IC_{50} values of the tested compounds were determined. Chloroquine and artesunate are used for each assay as positive controls.

Materials: Screening Medium: RPMI 1640 (10.44g/l) (no hypoxanthine) supplemented with HEPES (5.94g/l), $NaHCO_3$ (2.1g/l), Neomycin (100microg/ml)+ Albumax II (5g/l). Human red blood cells: 50% hematocrit (all blood groups are fine). [3H]-hypoxanthine: dilute stock (5mCi/5ml) ½ with 50% EtOH. 1ml aliquots are then diluted 1/50 with screening medium ready to use.

Plates: Falcon 96-well microtiter plates (No. 353072)

Gas: 37°C; 93% N2, 4% CO2, 3% O2

Preparation of stock solution:

Compounds were dissolved in DMSO at 10mg/ml. The stocks can be kept at 4°C for usually at least 2 weeks. For the assays, fresh 4X dilutions of all drugs in screening medium were prepared.

Assay procedure:

Blood smears of stock cultures of the NF54 strain were prepared and parasitemia is determined (parasitemia < 3% should not be used). Stock solutions were diluted with screening medium to the right start concentrations. Infected red cells solution was prepared

(parasitemia (p) of 0.3%, and hematocrit (h) of 2.5%), therefore the final concentration of p and h in the assay are 0.3 and 1.25%, respectively.

Calculation example for one 96-well plate:

Goal: 10ml of 0.3% parasitemia / 2.5% hematocrit

Start solutions:

- Human erythrocytes (50% hematocrit)

- Continuous culture with 5% parasitemia (with 5% hematocrit)

1. 0.3% parasitemia = (x ml * 5% p)/ (10 ml /2); x= 0.3 ml of infected culture

2. 2.5% hematocrit = [(0.3ml*5% hematocrit) + (x ml*50% hematocrit)]/10ml x=0.47ml of blood

3. 10ml – 0.3ml – 0.47ml = 9.23ml of screening medium

Mix these three volumes 10ml of 0.3% parasitemia / 2.5% hematocrit solution

- 1ml of un-infected red cells solution was prepared (no parasites, 2.5% hematocrit): 50µl of washed human erythrocytes (50% hematocrit) were mixed with 950µl screening medium.

- 100 µl of screening medium were added to each well of the microtiter plate (multipette).

- 100 µl of screening medium, containing 4x the highest compound concentration, were added to wells in row B. Six drugs can be tested this way on each plate. For each assay a reference substance is tested as well.

Serial drug dilutions were prepared with a multichannel pipette. 100 µl were taken from wells of row B and transferred, after gentle mixing, to wells of row C. After mixing, 100 µl were transferred from wells of row C to wells of row D and so forth to row H. The 100 µl removed from wells of row H were discarded. A two-fold serial dilution of drugs was thus obtained. For too active compounds the highest concentration was appropriately lowered. Wells of rows A served as controls without drug. 100 µl of infected blood (parasitemia of 0.3%, 2.5% hematocrit) were added to all wells with a multipette. Only the control wells (A9-12) got uninfected blood of 2.5% hematocrit. The plates were incubated in an incubation chamber at 37°C in an atmosphere containing the special gas mixture (93% N2, 4% CO2, 3% O2). After 48 h 50 µl [3H]-hypoxanthine (= 0.5 µCi) solution were added to each well of the plate. The

plates were incubated for another 24 h. The plates then were harvested with a BetaplateTM cell harvester (Wallac, Zurich, Switzerland), which transfers the red blood cells onto a glass fiber filter and washes with distilled water. The dried filters were inserted into a plastic foil with 10 ml of scintillation fluid and counted in a BetaplateTM liquid scintillation counter (Wallac, Zurich, Switzerland). The results were recorded as counts per minute (cpm) per well at each drug concentration.

Finally, Data were transferred into a graphic programme and expressed as percentage of the untreated controls. The 50% inhibitory concentration (IC_{50}) value is evaluated by Logit regression analysis.

Cytotoxicity assay

Title compounds were screened for *in vitro* cytotoxicity against a mammalian cell-line, Chinese Hamster Ovarian (CHO) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT)-assay. The MTT-assay is used as a colorimetric assay for cellular growth and survival, and compares well with other available assays[10,11]. The tetrazolium salt MTT was used to measure all growth and chemosensitivity. Compounds were tested in triplicate on one occasion.

Test compounds were stored at -20ºC until use. Dilutions were prepared on the day of the experiment. Emetine was used as the reference drug in all experiments. The initial concentration of emetine was 100 μg/ml, which was serially diluted in complete medium with 10-fold dilutions to give 6 concentrations, the lowest being 0.001 μg/ml. The same dilution technique was applied to the all test samples. The highest concentration of solvent to which the cells were exposed to had no measurable effect on the cell viability (data not shown). The 50% inhibitory concentration (IC_{50}) values were obtained from full dose-response curves, using a non-linear dose-response curve fitting analysis via GraphPad Prism v.4 software.

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