Steroid–Quinoline Hybrids for Disruption and Reversion of Protein Aggregation Processes

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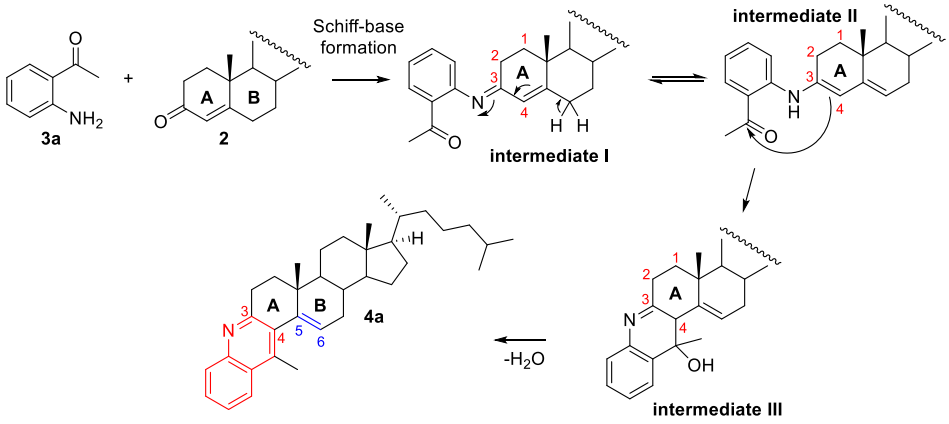
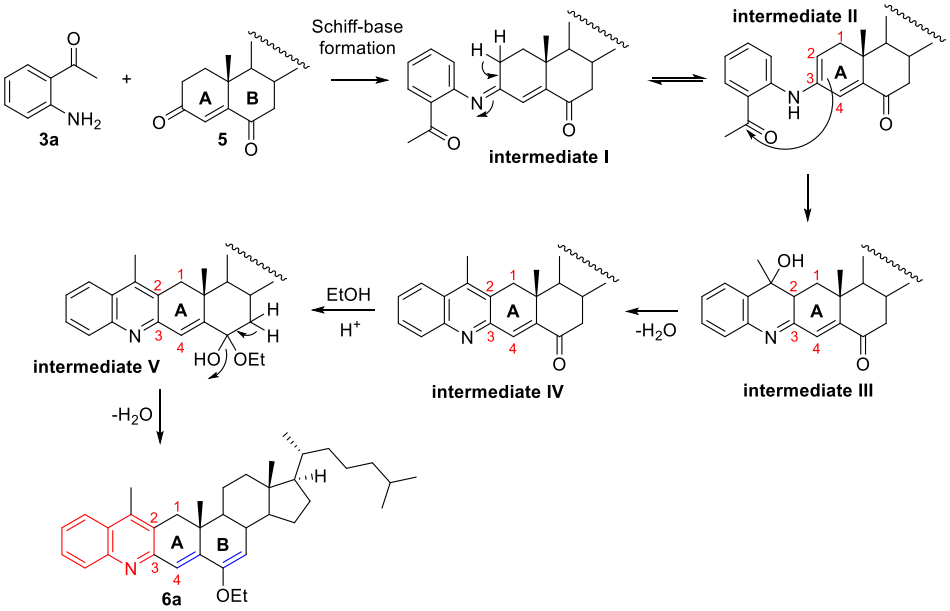
1. Reaction mechanisms



Scheme S1: Plausible mechanism for the formation of the angular compounds **4a-d** (**4a** as model compound).



Scheme S2: Plausible mechanism for the formation of the linear compounds **6a-d** (**6a** as model compound).

2. Materials, synthetic methods, and structural characterization data

General:

Melting points were measured in a Büchi B-540 apparatus fitted with a microscope and are uncorrected. NMR spectra were recorded with Bruker DRX 300 spectrometers (300 for ^{1}H and 75 MHz or 125 MHz for ^{13}C), in $CDCl_3$ as solvent, if not stated otherwise. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz; internal standard was residual peak of the solvent. Unequivocal ^{13}C assignments were made with the aid of 2D *g*HSQC and *g*HMBC (delays for one-bond and long-range *J* C/H couplings were optimised for 145 and 7 Hz, respectively) experiments. High resolution mass spectra analysis (HRMS-ESI.) were performed on a microTOF (focus) mass spectrometer. Ions were generated using an ApolloII (ESI) source. Ionization was achieved by electrospray, using a voltage of 4500 V applied to the needle, and a counter voltage between 100 and 150 V applied to the capillary. Cholesterol, 2'-aminoketones (**3a-d**) and aluminum isopropoxide were purchased from Alfa Aesar and Sigma-Aldrich, respectively, and used without any further purification. Solvents of commercial grade were used as received unless otherwise stated.

Synthesis – general procedures and structural characterization

*Experimental procedure for the synthesis of cholest-4-en-3-one (**2**):*

To a solution of cholesterol (1 g, 2.59 mmol) in a mixture of toluene/cyclohexanone (1:1) (30 mL) was added aluminum isopropoxide (0.79 g, 3.88 mmol). The reaction mixture was refluxed for 12 h, after cooling to room temperature, it was treated with water (5 mL). The precipitated aluminum

salt was filtered off the mixture, the filtrate was removed under reduced pressure and the crude product was purified by column chromatography, using hexane/EtOAc (10:2) as eluent.

(10*R*,13*R*,17*R*)-10,13-dimethyl-17-[(*R*)-6-methylheptan-2-yl]-1,2,6,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3*H*-cyclopenta[*a*]phenanthren-3-one (2): Yield 845.6 mg (85%), white solid, mp = 81-82 °C. **^{1}H-NMR (300 MHz, $CDCl_3$):** δ = 0.71 (s, 3H, 18-C*H*$_3$), 0.85 (d, 3H, 26 or 27-C*H*$_3$, *J* 1.4 Hz), 0.88 (d, 3H, 26 or 27-C*H*$_3$, *J* 1.4 Hz), 0.90-1.16 (m, 10H), 1.18 (s, 3H, 19-C*H*$_3$), 1.33-2.43 (m, 21H), 5.72 (br s, 1H, H-4) ppm. **^{13}C-NMR (75 MHz, $CDCl_3$):** δ = 12.0 (18-*C*H$_3$), 17.4 (19-*C*H$_3$), 18.6, 21.0, 22.6, 22.8, 23.8, 24.2, 28.0, 28.2, 32.0, 33.0, 34.0, 35.6, 35.68, 35.75, 36.1, 38.6, 39.5, 39.6, 42.4, 53.8, 55.9, 56.1, 123.7 (C-4), 171.7 (C-5), 199.7 (C-3) ppm. **HRMS (ESI$^+$):** *m/z* [M+H]$^+$ calcd for $C_{27}H_{45}O$: 385.3470; found 385.3475.

*General procedure for the synthesis of cholesterol-quinoline hybrids (**4a-d**):*

Cholest-4-en-3-one (**2**) (60 mg, 0.156 mmol), the appropriate 2'-aminoketone (**3a-d**) (0.156 mmol), *p*-toluenesulfonic acid monohydrate (29.7 mg, 0.156 mmol) and EtOH (2 mL) were mixed up in a sealed glass vessel. The reaction mixture was heated at 100 °C under MW irradiation for 20 min. The resulting mixture was treated with 10% NaOH (5 mL), extracted with CH_2Cl_2 (3 x 8 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The resulting crude mixture was purified through preparative thin-layer chromatography using hexane/EtOAc (10:1) as eluent.

(3*R*,3a*R*,5b*R*)-3a,5b,13-trimethyl-3-[(*R*)-6-methylheptan-2-yl]-2,3,3a,4,5,5a,5b,6,7,15,15a,15b-dodecahydro-1*H*-cyclopenta[5,6]naphtho[2,1-*a*]acridine (4a): Yield 45.3 mg (60%), mp = 126-128 °C. **^{1}H-NMR (300 MHz, $CDCl_3$):** δ = 0.76 (s, 3H, 18-C*H*$_3$), 0.87 (d, 3H, 26 or 27-C*H*$_3$, *J* 1.4 Hz), 0.89 (d, 3H, 26 or 27-C*H*$_3$, *J* 1.4 Hz), 0.92 (s, 3H, 19-C*H*$_3$), 0.94-2.34 (m, 27H), 2.70 (s, 3H, 4'-C*H*$_3$), 2.81-2.86 (m, 2H, H-2), 5.61 (dd, 1H, H-6, *J* 5.2, 2.1 Hz), 7.50 (ddd, 1H, H-6', *J* 8.3, 6.8, 1.4 Hz), 7.62 (ddd, 1H, H-7', *J* 8.4, 6.8, 1.4 Hz), 7.96-8.01 (m, 2H, H-5' and H-8') ppm. **^{13}C-NMR (75 MHz, $CDCl_3$):** δ = 12.1 (18-*C*H$_3$), 15.5 (4-*C*H$_3$), 18.7, 22.1 (*C*H$_2$), 22.6, 22.9, 23.7, 23.9 (*C*H$_2$), 24.2 (*C*H$_2$), 28.0, 28.4 (*C*H$_2$), 32.0 (*C*H$_2$), 32.3, 34.6 (*C*H$_2$), 35.8, 36.2 (*C*H$_2$), 38.0, 39.5 (*C*H$_2$), 40.0 (*C*H$_2$), 42.6, 47.3, 56.2, 56.9, 124.3 (C-5' or C-8'), 125.3 (C-6'), 128.1 (C-4'a), 128.2 (C-7'), 128.6 (C-6), 128.9 (C-5' or C-8'), 132.4 (C-4), 139.2 (C-4'), 139.7 (C-5), 145.6 (C-8'a), 161.3 (C-3) ppm. **HRMS (ESI$^+$):** *m/z* [M+H]$^+$ calcd for $C_{35}H_{50}N$: 484.3943; found 484.3938.

(3*R*,3a*R*,5b*R*)-3a,5b-dimethyl-3-[(*R*)-6-methylheptan-2-yl]-13-phenyl-2,3,3a,4,5,5a,5b,6,7,15,15a,15b-dodecahydro-1*H*-cyclopenta[5,6]naphtho[2,1-*a*]acridine (4b): Yield 72.4 mg (85%), mp = 117-119 °C. **^{1}H-NMR (300 MHz, $CDCl_3$):** δ = 0.69 (s, 3H, 18-C*H*$_3$), 0.85 (d, 3H, 26 or 27-C*H*$_3$, *J* 1.2 Hz), 0.87 (d, 3H, 26 or 27-C*H*$_3$, *J* 1.2 Hz), 0.87-2.13 (m, 27H), 1.02 (s, 3H, 19-C*H*$_3$), 2.95-3.00 (m, 2H, H-2), 5.27 (dd, 1H, H-6, *J* 5.2, 2.4 Hz), 7.12-7.16 (m, 1H, H-4''), 7.33 (ddd, 1H, H-6', *J* 8.3, 6.7, 1.2 Hz), 7.39-7.49 (m, 5H, H-5', H-2'', H-3'', H-5'' and H-6''), 7.60 (ddd, 1H, H-7', *J* 8.5, 6.7, 1.5 Hz), 8.03 (dd, 1H, H-8', *J* 8.5, 1.2 Hz) ppm. **^{13}C-NMR**

(75 MHz, $CDCl_3$): δ = 12.0 (18-*C*H_3), 18.7 (21-*C*H_3), 21.9 (*C*H_2), 22.6, 22.8, 23.0, 23.8 (*C*H_2), 24.1 (*C*H_2), 28.0, 28.3 (*C*H_2), 31.7, 32.0 (*C*H_2), 32.2 (*C*H_2), 34.8 (*C*H_2), 35.8, 36.2 (*C*H_2), 37.3, 39.5 (*C*H_2), 39.9 (*C*H_2), 42.5, 47.8, 56.1, 56.9, 125.3 (C-5'), 126.6, 127.2 (C-4'a), 127.5, 128.38 (C-7' or C-8'), 128.44 (C-7' or C-8'), 128.6 (C-6'), 128.7 (C-4''), 130.7 (C-4), 131.0 (C-6), 131.6, 137.8 (C-4'), 138.1 (C-5), 144.5 (C-1''), 146.3 (C-8'a), 160.8 (C-3) ppm. **HRMS (ESI^+):** *m/z* $[M+H]^+$ calcd for $C_{40}H_{52}N$: 546.4099; found 546.4094.

(3*R*,3a*R*,5b*R*)-11-chloro-3a,5b-dimethyl-3-[(*R*)-6-methylheptan-2-yl]-13-phenyl-2,3,3a,4,5,5a,5b,6,7,15,15a,15b-dodecahydro-1*H*-cyclopenta[5,6]naphtho[2,1-*a*]acridine (4c): Yield 73.3 mg (81%), mp = 156-158 °C (Crystal obtained from slow evaporation in MeOH). **^{1}H-NMR (300 MHz, $CDCl_3$):** δ = 0.69 (s, 3H, 18-C*H*$_3$), 0.85 (d, 3H, 26 or 27-C*H*$_3$, *J* 1.2 Hz), 0.87 (d, 3H, 26 or 27-C*H*$_3$, *J* 1.2 Hz), 0.92 (d, 3H, 21-C*H*$_3$, *J* 6.5 Hz), 1.01 (s, 3H, 19-C*H*$_3$), 1.06-1.82 (m, 22H), 2.02-2.10 (m, 2H), 2.92-2.97 (m, 2H), 5.27 (dd, 1H, H-6, *J* 5.3, 2.4 Hz), 7.09-7.13 (m, 1H, H-4''), 7.36-7.50 (m, 5H, H-5', H-2'', H-3'', H-5'' and H-6''), 7.53 (dd, 1H, H-7', *J* 8.9, 2.3 Hz), 7.95 (d, 1H, H-8', *J* 8.9 Hz) ppm. **^{13}C-NMR (75 MHz, $CDCl_3$):** δ = 12.0 (18-*C*H_3), 18.7 (21-*C*H_3), 21.9 (*C*H_2), 22.6, 22.8, 23.1, 23.8 (*C*H_2), 24.1 (*C*H_2), 28.0, 28.3 (*C*H_2), 31.6, 32.0 (*C*H_2), 32.2 (*C*H_2), 34.7 (*C*H_2), 35.8, 36.2 (*C*H_2), 37.2, 39.5 (*C*H_2), 39.9 (*C*H_2), 42.4, 47.8, 56.1, 56.8, 125.4 (C-5'), 127.5, 127.8, 128.4 (C-4'a), 128.5 (C-4''), 128.9, 129.2 (C-7'), 130.1 (C-8'), 131.2 (C-6'), 131.4, 131.5 (C-4), 131.6 (C-6), 137.1 (C-4'), 137.8 (C-5), 143.6 (C-1''), 144.7 (C-8'a), 161.2 (C-3) ppm. **HRMS (ESI^+):** *m/z* $[M+H]^+$ calcd for $C_{40}H_{51}NCl$: 580.3710; found 580.3705.

(3*R*,3a*R*,5b*R*)-11-chloro-13-(2-chlorophenyl)-3a,5b-dimethyl-3-[(*R*)-6-methylheptan-2-yl]-2,3,3a,4,5,5a,5b,6,7,15,15a,15b-dodecahydro-1*H*-cyclopenta[5,6]naphtho[2,1-*a*]acridine (4d): Yield 79.6 mg (83%), mp = 167-169 °C. **^{1}H NMR (500 MHz, $CDCl_3$):** δ = 0.68 (s, 3H, 18-C*H*$_3$), 0.85 (d, 3H, 26 or 27-C*H*$_3$, *J* 1.3 Hz), 0.87 (d, 3H, 26 or 27-C*H*$_3$, *J* 1.3 Hz), 0.92 (d, 3H, 21-C*H*$_3$, *J* 6.5 Hz), 0.95 (s, 3H, 19-C*H*$_3$), 0.98-1.87 (m, 22H), 2.03-2.13 (m, 2H), 2.97-3.02 (m, 2H), 5.45 (dd, 1H, H-6, *J* 5.3, 2.6 Hz), 7.04 (dd, 1H, H-2'', *J* 7.5, 1.8 Hz), 7.18 (d, 1H, H-5', *J* 2.3 Hz), 7.35 (dt, 1H, H-3'', *J* 7.6, 1.4 Hz), 7.40 (dt, 1H, H-4'', *J* 7.6, 1.8 Hz), 7.53-7.57 (m, 2H, H-7', H-5''), 7.98 (d, 1H, H-8', *J* 8.9 Hz) ppm. **^{13}C NMR (125 MHz, $CDCl_3$):** δ = 12.0 (18-*C*H_3), 18.7 (21-*C*H_3), 21.9 (*C*H_2), 22.6, 22.9, 23.8 (*C*H_2), 24.1 (*C*H_2), 28.0, 28.3 (*C*H_2), 31.7, 31.8, 32.4 (*C*H_2), 34.6 (*C*H_2), 35.8, 36.2 (*C*H_2), 37.2, 39.5 (*C*H_2), 39.9 (*C*H_2), 42.4, 47.9, 56.1, 56.8, 124.9 (C-5'), 127.4 (C-3''), 127.5 (C-4'a), 129.39 and 129.41 (C-7', C-5''and C-4''), 130.1, 130.16 and 130.18 (C-6, C-8' and C-2''), 131.6 (C-6'), 131.8 (C-4), 135.3 (C-6''), 136.8 (C-1''), 138.1 (C-5), 140.9 (C-4'), 144.4 (C-8'a), 161.3 (C-3) ppm. **HRMS (ESI^+):** *m/z* $[M+H]^+$ calcd for $C_{40}H_{50}NCl_2$: 614.3320; found 614.3315.

*Experimental procedure for the synthesis of cholest-4-ene-3,6-dione (**5**):*

To a solution of cholesterol (1 g, 2.59 mmol) in CH_2Cl_2 (30 mL) was added pyridinium chlorochromate (PCC) (5.57 g, 25.9 mmol). The reaction mixture was stirred at room temperature for 24 h, The crude residue was filtered through celite, the solvent was removed under reduced

pressure and the crude product was purified by column chromatography, using hexane/EtOAc (10:3) as eluent. The desired cholest-4-ene-3,6-dione (**5**) was obtained in 70% yield.

(10*R*,13*R*,17*R*)-10,13-dimethyl-17-[(*R*)-6-methylheptan-2-yl]-1,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3*H*-cyclopenta[*a*]phenanthrene-3,6(2*H*)-dione (5): Yield 722 mg (70%), white solid, mp = 112-114 °C. **^{1}H-NMR (300 MHz, $CDCl_3$):** δ = 0.72 (s, 3H, 18-CH_3), 0.85-0.88 (m, 6H), 0.93 (d, 3H, *J* 6.5 Hz), 0.99-2.55 (m, 25H), 1.17 (s, 3H, 19-CH_3), 2.68 (dd, 1H, *J* 15.7, 3.8 Hz), 6.17 (s, 1H, H-4) ppm. **^{13}C-NMR (75 MHz, $CDCl_3$):** δ = 11.9 (18-*C*H_3), 17.5 (19-*C*H_3), 18.6, 20.9, 22.6, 22.8, 23.8, 24.0, 28.0, 34.0, 34.2, 35.5, 35.7, 36.1, 39.1, 39.5, 39.8, 42.5, 46.8, 51.0, 55.9, 56.5, 125.4 (C-4), 161.1 (C-5), 199.5 (C-3), 202.4 (C-6) ppm. **HRMS (ESI$^+$):** *m/z* $[M+H]^+$ calcd for $C_{27}H_{43}O_2$: 399.3263; found 399.3266.

*General procedure for the synthesis of cholesterol-quinoline hybrids (**6a-d**):*

Cholest-4-ene-3,6-dione (**5**) (60 mg, 0.150 mmol), the appropriate 2'-aminoketone (**3a-d**) (0.150 mmol), *p*-toluenesulfonic acid monohydrate (28.6 mg, 0.150 mmol) and EtOH (2 mL) were mixed up in a sealed glass vessel. The reaction mixture was heated at 100 °C under MW irradiation for 20 min. The resulting mixture was treated with 10% NaOH (5 mL), extracted with CH_2Cl_2 (3 x 8 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The resulting crude mixture was purified through preparative thin-layer chromatography using hexane/EtOAc (10:2) as eluent.

(1*R*,13a*R*,15a*R*)-5-ethoxy-12,13a,15a-trimethyl-1-[(*R*)-6-methylheptan-2-yl]-2,3,3a,3b,13,13a,13b,14,15,15a-decahydro-1*H*-cyclopenta[5,6]naphtho[1,2-*b*]acridine (6a): Yield 47.5 mg (60%), mp = 101-102 °C. **^{1}H-NMR (300 MHz, $CDCl_3$):** δ = 0.77 (s, 3H, 18-CH_3), 0.87 (d, 3H, CH_3, *J* 1.4 Hz), 0.89 (d, 3H, CH_3, *J* 1.4 Hz), 0.92 (s, 3H, 19-CH_3), 0.92-1.93 (m, 21H), 1.38 (t, 3H, 6-OCH$_2$CH_3, *J* 6.9 Hz), 2.09-2.13 (m, 1H), 2.22-2.28 (m, 1H), 2.99 (s, 3H, 4'-CH_3), 2.55-2.61 (m, 1H), 3.23 (d, 1H, H-1, *J* 15.6 Hz), 3.80 (q, 2H, 6-OCH_2CH$_3$, *J* 6.9 Hz), 5.01 (d, 1H, H-7, *J* 2.1 Hz), 7.14 (s, 1H, H-4), 7.43 (ddd, 1H, H-6', *J* 8.3, 6.8, 1.3 Hz), 7.58 (ddd, 1H, H-7', *J* 8.3, 6.8, 1.3 Hz), 7.91 (dd, 1H, H-5', *J* 8.3, 1.3 Hz), 7.98 (dd, 1H, H-8', *J* 8.3, 1.3 Hz) ppm. **^{13}C-NMR (75 MHz, $CDCl_3$):** δ = 12.0 (18-*C*H_3), 13.6 (4'-*C*H_3), 14.8 (6-OCH$_2$*C*H$_3$), 17.5 (19-*C*H_3), 18.7, 21.2 (*C*H_2), 22.6, 22.8, 23.9 (*C*H_2), 24.0 (*C*H_2), 28.0, 28.2 (*C*H_2), 35.3, 35.8, 36.1 (*C*H_2), 37.1, 37.8 (*C*H_2), 39.5 (*C*H_2), 39.8 (*C*H_2), 43.1, 50.7, 55.1, 56.2, 62.6 (6-O*C*H$_2$CH$_3$), 105.6 (C-7), 120.7 (C-4), 123.5 (C-5'), 125.4 (C-6'), 126.4 (C-2), 127.7 (C-4'a), 128.1 (C-7'), 129.4 (C-8'), 139.4 (C-4'), 146.7 and 146.8 (C-5 and C-8'a), 149.9 (C-6), 153.7 (C-3) ppm. **HRMS (ESI$^+$):** *m/z* $[M+H]^+$ calcd for $C_{37}H_{52}NO$: 526.4048; found 526.4043.

(3b*S*,13a*R*,15a*R*)-5-ethoxy-13a,15a-dimethyl-1-[(*R*)-6-methylheptan-2-yl]-12-phenyl-2,3,3a,3b,13,13a,13b,14,15,15a-decahydro-1*H*-cyclopenta[5,6]naphtho[1,2-*b*]acridine (6b): Yield 54.9 mg (62%), mp = 120-122 °C. **^{1}H-NMR (300 MHz, $CDCl_3$):** δ = 0.69 (s, 3H, 18-CH_3), 0.85 (d, 3H, CH_3, *J* 1.5 Hz), 0.87 (d, 3H, CH_3, *J* 1.5 Hz), 0.89 (s, 3H, 19-CH_3), 1.07-1.41 (m, 15H), 1.39 (t, 3H, 6-OCH$_2$CH_3, *J* 6.9 Hz), 1.47-1.99 (m, 6H), 2.15-2.22 (m, 1H), 2.48 (d, 1H, H-1, *J* 15.4 Hz),

2.79 (d, 1H, H-1, *J* 15.4 Hz), 3.80 (q, 2H, 6-OC*H*$_2$CH$_3$, *J* 6.9 Hz), 5.01 (d, 1H, H-7, *J* 2.1 Hz), 7.23 (s, 1H, H-4), 7.23-7.36 (m, 5H), 7.44-7.54 (m, 3H), 7.58 (ddd, 1H, H-7', *J* 8.4, 6.3, 2.0 Hz), 8.05 (d, 1H, H-8', *J* 8.4 Hz) ppm. **^{13}C-NMR (125 MHz, CDCl$_3$):** δ = 12.0 (18-*C*H$_3$), 14.8 (6-OCH$_2$*C*H$_3$), 16.9 (19-*C*H$_3$), 18.6, 20.9 (*C*H$_2$), 22.6, 22.8, 23.9 (*C*H$_2$), 24.0 (*C*H$_2$), 28.0, 28.1 (*C*H$_2$), 35.3, 35.8, 36.1, 37.3, 38.5 (*C*H$_2$), 39.5 (*C*H$_2$), 39.6 (*C*H$_2$), 43.1, 50.5, 55.0, 56.1, 62.7 (6-O*C*H$_2$CH$_3$), 106.1 (C-7), 120.3 (C-4), 125.6 (C-2), 126.0 (C-6'), 126.1 (C-4'), 127.3, 127.8, 128.4, 128.56 (C-7'), 128.59 (C-8'), 128.8, 129.2, 129.5, 129.7, 136.7 (C-1''), 145.2 (C-4'), 147.2 (C-8'a), 147.5 (C-5), 149.9 (C-6), 154.2 (C-3) ppm. **HRMS (ESI$^+$):** *m/z* [M+H]$^+$ calcd for $C_{42}H_{54}NO$: 588.4205; found 588.4200.

(1*R*,13a*R*,15a*R*)-10-chloro-5-ethoxy-13a,15a-dimethyl-1-[(*R*)-6-methylheptan-2-yl]-12-phenyl-2,3,3a,3b,13,13a,13b,14,15,15a-decahydro-1*H*-cyclopenta[5,6]naphtho[1,2-*b*]acridine (6c): Yield 59.9 mg (64%), mp = 130-132 °C. **^{1}H NMR (300 MHz, CDCl$_3$):** δ = 0.69 (s, 3H, 18-C*H*$_3$), 0.85 (d, 3H, C*H*$_3$, *J* 1.5 Hz), 0.86-1.99 (m, 27H), 1.39 (t, 3H, 6-OCH$_2$C*H*$_3$, *J* 6.9 Hz), 2.15-2.21 (m, 1H), 2.46 (d, 1H, H-1, *J* 15.4 Hz), 2.77 (d, 1H, H-1, *J* 15.4 Hz), 3.80 (q, 2H, 6-OC*H*$_2$CH$_3$, *J* 6.9 Hz), 5.03 (d, 1H, H-7, *J* 2.1 Hz); 7.20 (s, 1H, H-4), 7.20-7.27 (m, 3H), 7.30 (d, 1H, H-5', *J* 2.3 Hz), 7.46-7.60 (m, 4H), 7.97 (d, 1H, H-8', *J* 8.9 Hz) ppm. **^{13}C NMR (125 MHz, CDCl$_3$):** δ = 11.9 (18-*C*H$_3$), 14.8 (6-OCH$_2$*C*H$_3$), 17.0, 18.6, 20.9 (*C*H$_2$), 22.6, 22.8, 23.8 (*C*H$_2$), 23.9 (*C*H$_2$), 28.0, 28.1 (*C*H$_2$), 35.3, 35.7, 36.1 (*C*H$_2$), 37.3, 38.5 (C-1), 39.5 (*C*H$_2$), 39.6 (*C*H$_2$), 43.1, 50.5, 55.0, 56.1, 62.7 (6-O*C*H$_2$CH$_3$), 106.4 (C-7), 119.9 (C-4), 124.8 (C-5'), 127.1 (C-2), 128.0 (C-4'a), 128.1, 128.76, 128.79, 129.0, 129.2 (C-7'), 129.6, 130.3 (C-8'), 131.3 (C-6'), 136.0 (C-1''), 144.4 (C-4'), 145.6 (C-8'a), 147.9 (C-5), 149.8 (C-6), 154.5 (C-3) ppm. **HRMS (ESI$^+$):** *m/z* [M+H]$^+$ calcd for $C_{42}H_{53}ClNO$: 622.3815; found 621.3740.

(1*R*,13a*R*,15a*R*)-10-chloro-12-(2-chlorophenyl)-5-ethoxy-13a,15a-dimethyl-1-[(*R*)-6-methylheptan-2-yl]-2,3,3a,3b,13,13a,13b,14,15,15a-decahydro-1*H*-cyclopenta[5,6]naphtho[1,2-*b*]acridine (6d): Yield 54.2 mg (55%), mp = 133-135 °C. **^{1}H-NMR (500 MHz, CDCl$_3$):** δ = 0.69 (s, 3H, 18-C*H*$_3$), 0.85 (d, 3H, C*H*$_3$, *J* 1.5 Hz), 0.86-1.99 (m, 29H), 1.39 (t, 3H, 6-OCH$_2$C*H*$_3$, *J* 6.9 Hz), 2.58 (s, 2H, H-1), 3.80 (q, 2H, 6-OC*H*$_2$CH$_3$, *J* 6.9 Hz), 5.03 (d, 1H, H-7, *J* 2.1 Hz), 7.12 (d, 1H, H-5', *J* 2.3 Hz), 7.21 (s, 1H, H-4), 7.21-7.24 (m, 1H), 7.44-7.60 (m, 4H), 8.00 (d, 1H, H-8', *J* 8.8 Hz) ppm. **^{13}C-NMR (125 MHz, CDCl$_3$):** δ = 11.9 (18-*C*H$_3$), 14.8 (6-OCH$_2$*C*H$_3$), 17.2, 18.6, 20.9 (*C*H$_2$), 22.6, 22.8, 23.8 (*C*H$_2$), 23.9 (*C*H$_2$), 28.0, 28.1 (*C*H$_2$), 35.3, 35.7, 36.1 (*C*H$_2$), 37.2, 37.9 (C-1), 39.6 (*C*H$_2$), 43.1, 50.3, 54.9, 56.1, 62.8 (6-O*C*H$_2$CH$_3$), 106.5 (C-7), 119.9 (C-4), 124.2 (C-5'), 127.2 (C-2), 127.4, 127.9, 128.1 (C-4'a), 129.5 (C-7'), 129.9, 130.6 (C-8'), 131.2 (C-6'), 131.6, 133.0, 134.1, 134.9 (C-1''), 141.6 (C-4'), 145.6 (C-8'a), 148.1 (C-5), 149.8 (C-6), 154.5 (C-3) ppm. **HRMS (ESI$^+$):** *m/z* [M+H]$^+$ calcd for $C_{42}H_{52}Cl_2NO$: 656.3426; found 656.3420.

3. Structural characterization – ^{1}H and ^{13}C-NMR spectra

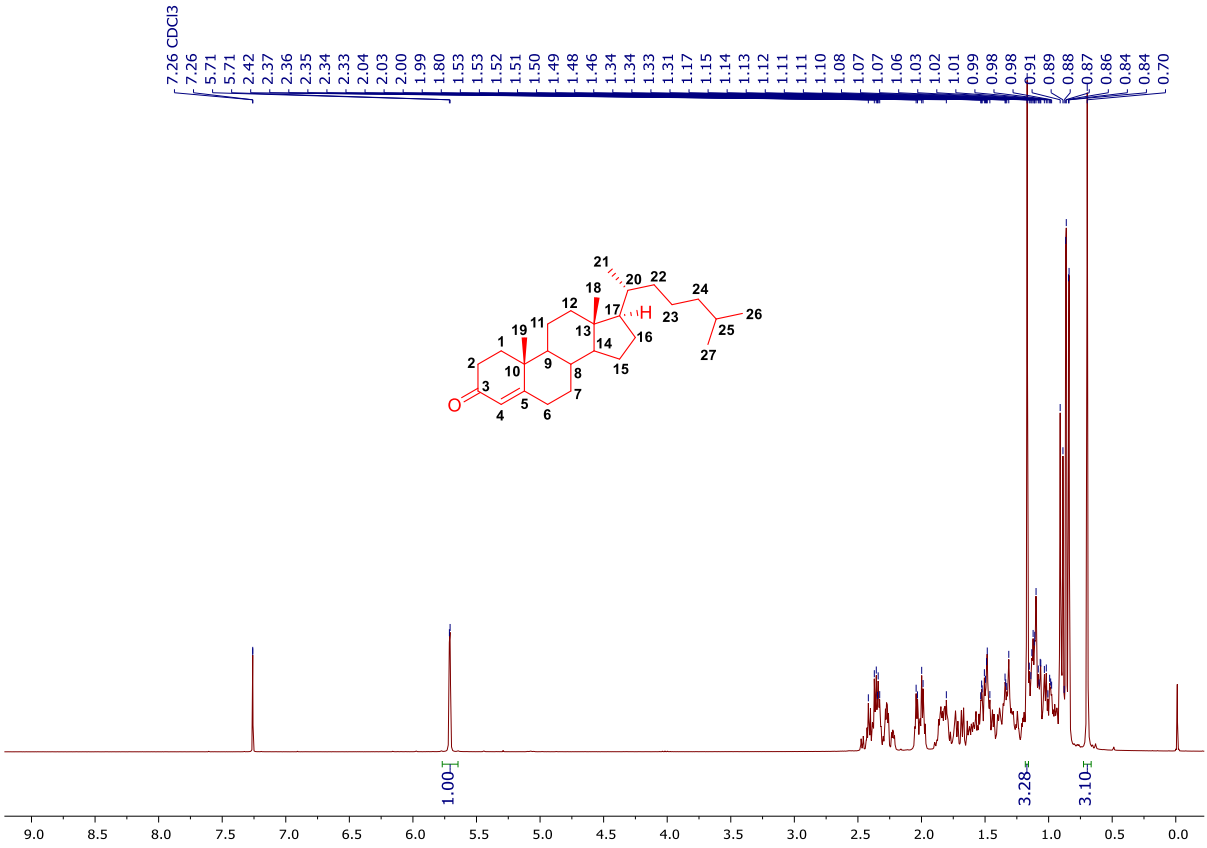


Figure S1: ^{1}H-NMR (300 MHz, $CDCl_3$) of 4-cholesten-3-one (**2**).

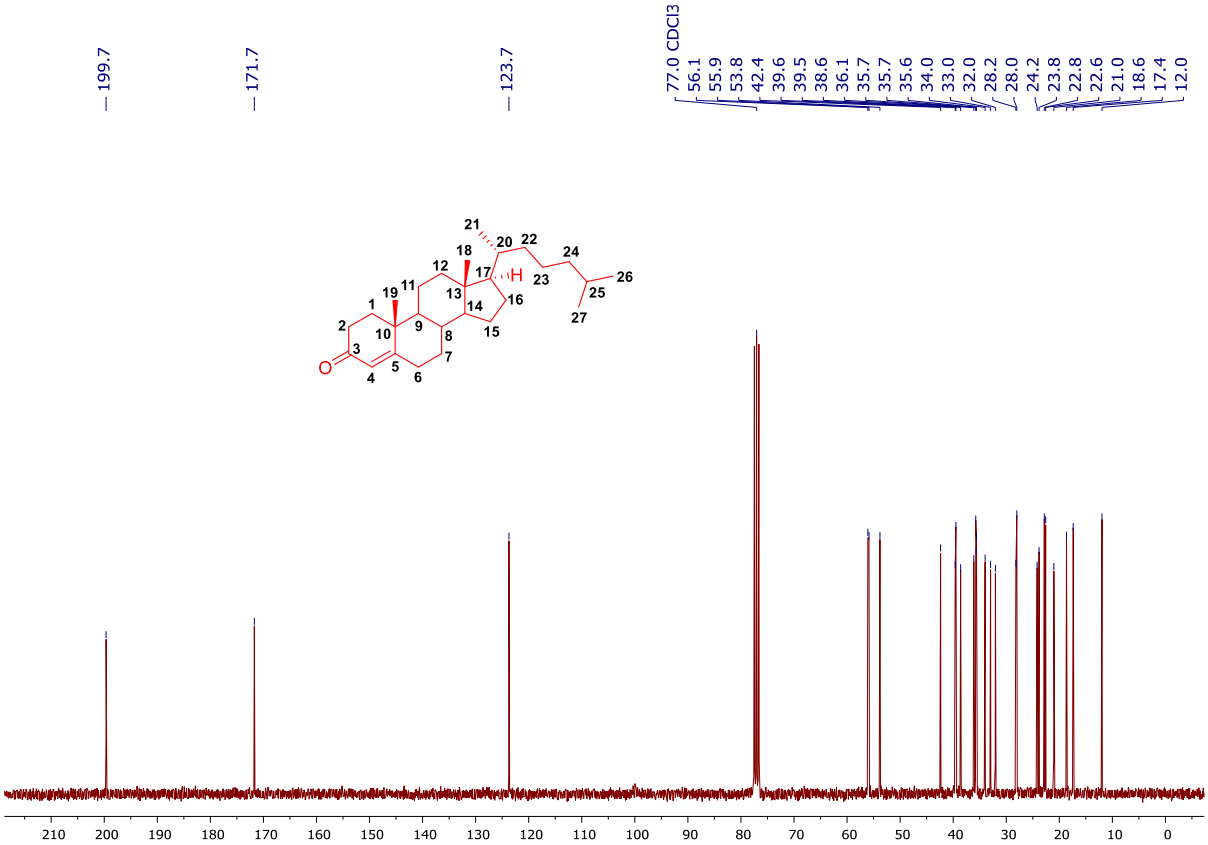


Figure S2: ^{13}C-NMR (75 MHz, $CDCl_3$) of 4-cholesten-3-one (**2**).

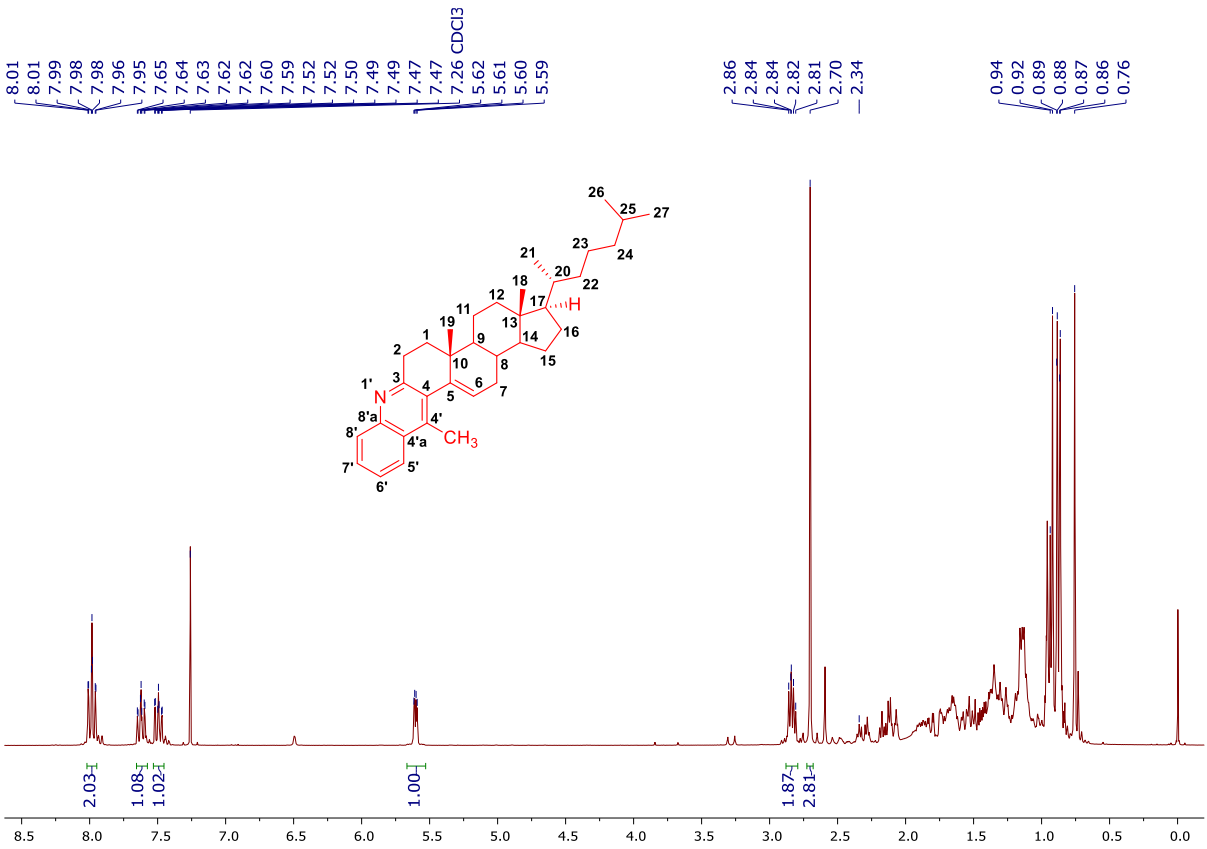


Figure S3: ^{1}H-NMR (300 MHz, $CDCl_3$) of compound **4a**.

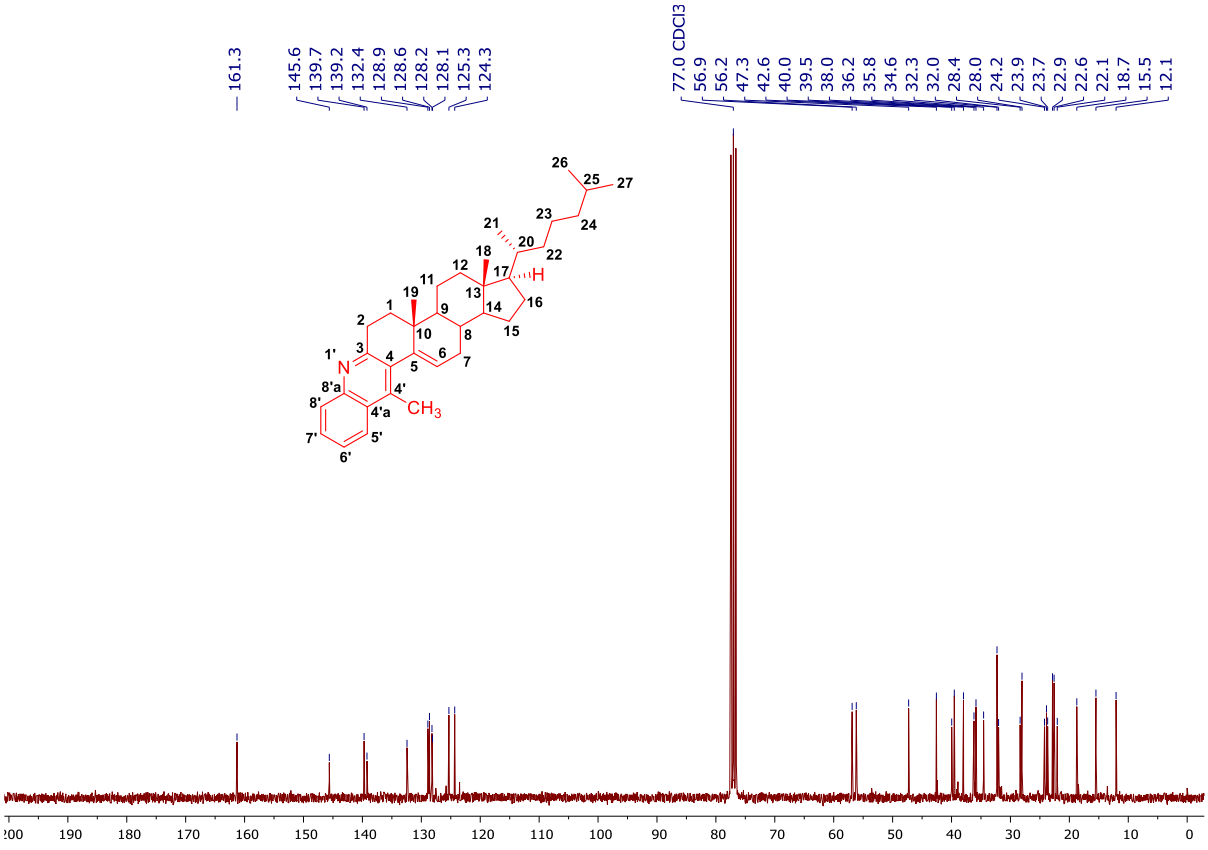


Figure S4: ^{13}C-NMR (75 MHz, $CDCl_3$) of compound **4a**.

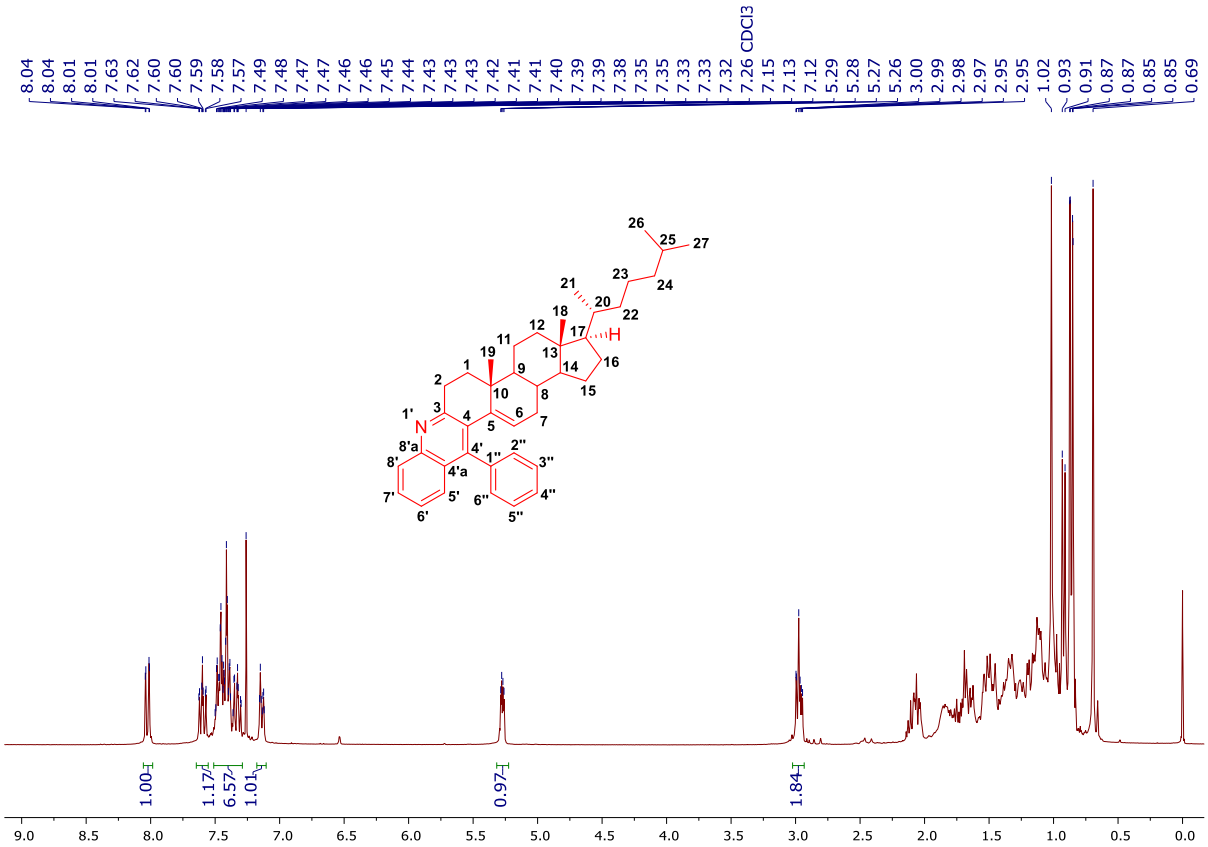


Figure S5: ^{1}H-NMR (300 MHz, $CDCl_3$) of compound **4b**.

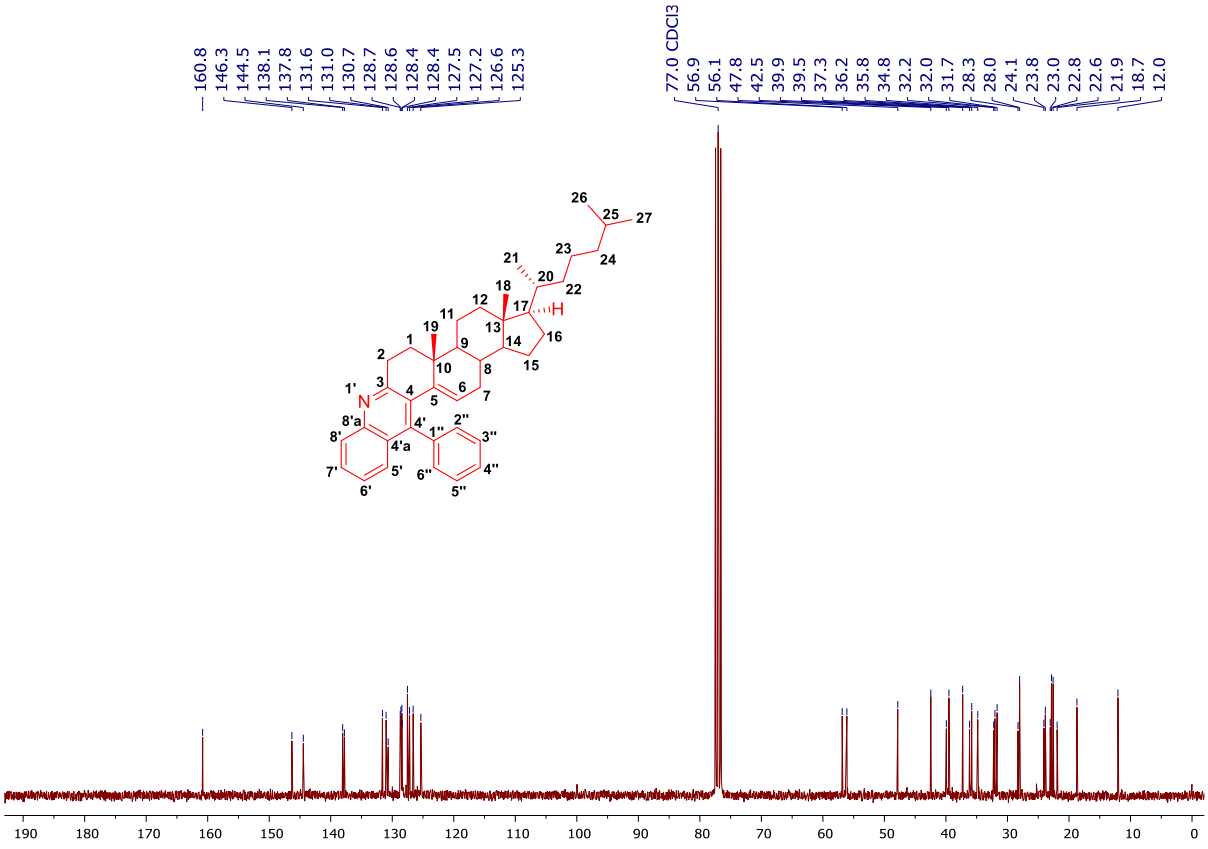


Figure S6: ^{13}C-NMR (75 MHz, $CDCl_3$) of compound **4b**.

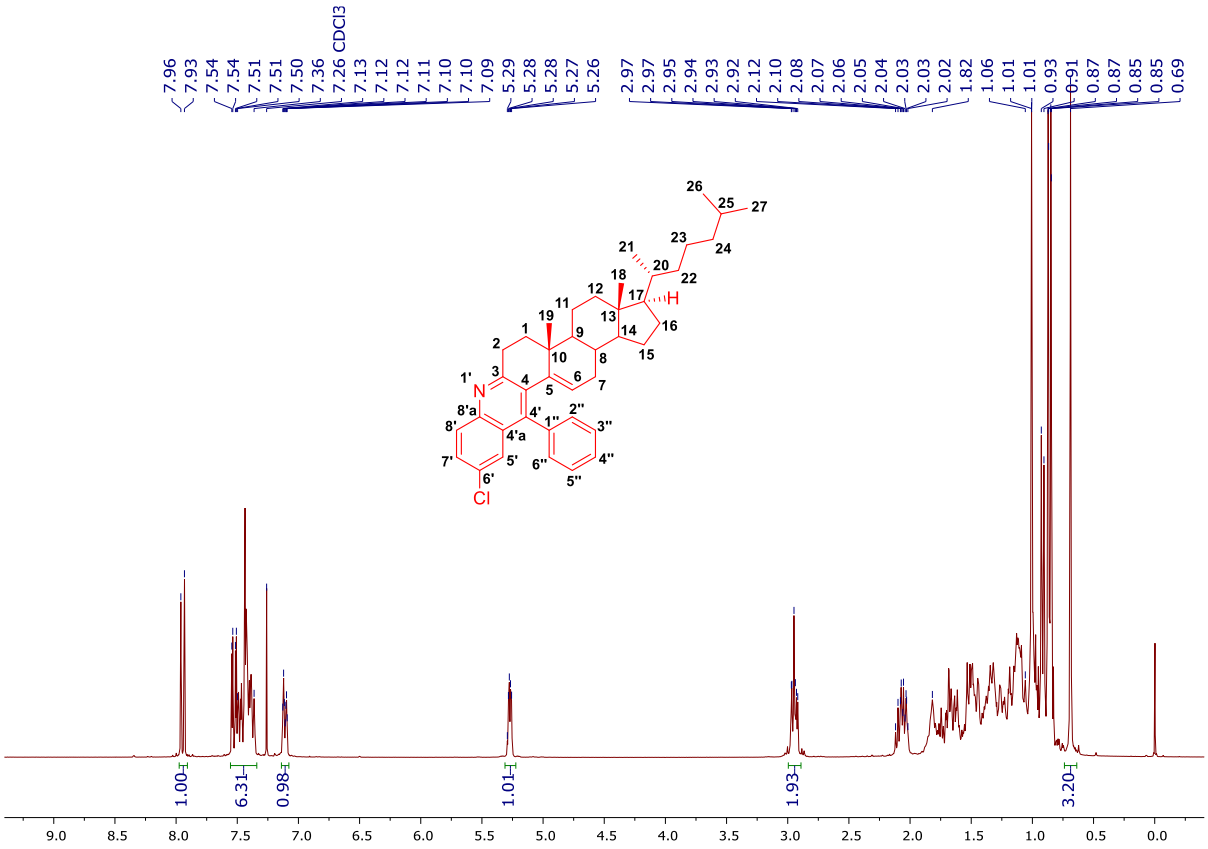


Figure S7: ^{1}H-NMR (300 MHz, $CDCl_3$) of compound **4c**.

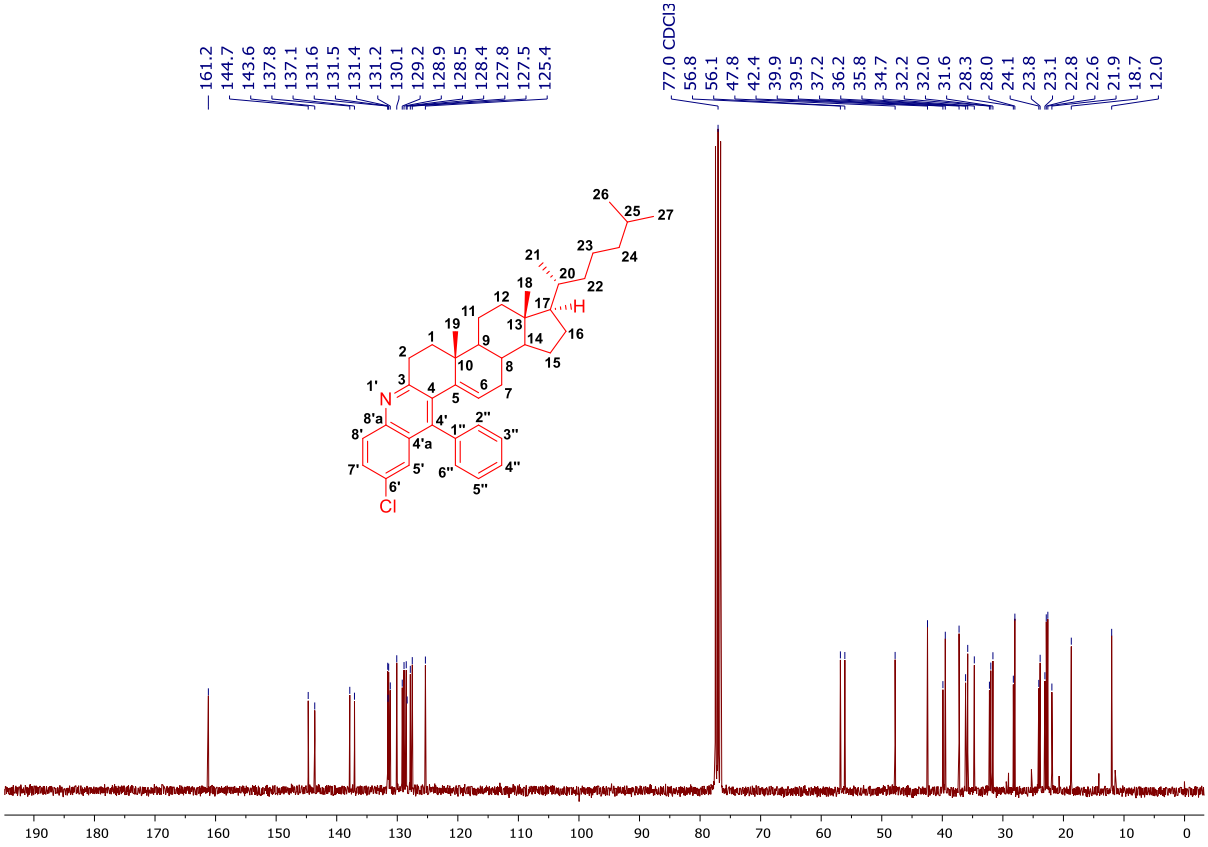


Figure S8: ^{13}C-NMR (75 MHz, $CDCl_3$) of compound **4c**.

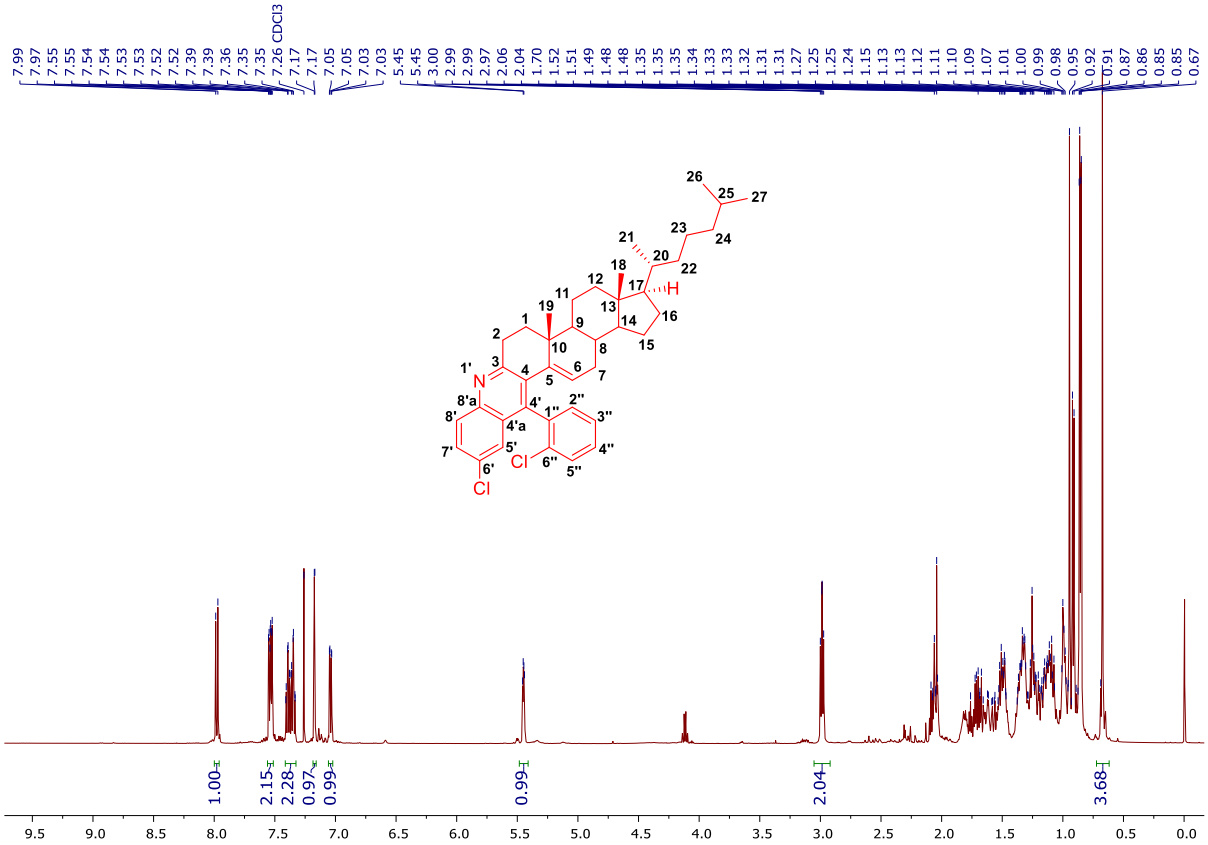


Figure S9: ^{1}H-NMR (500 MHz, $CDCl_3$) of compound **4d**.

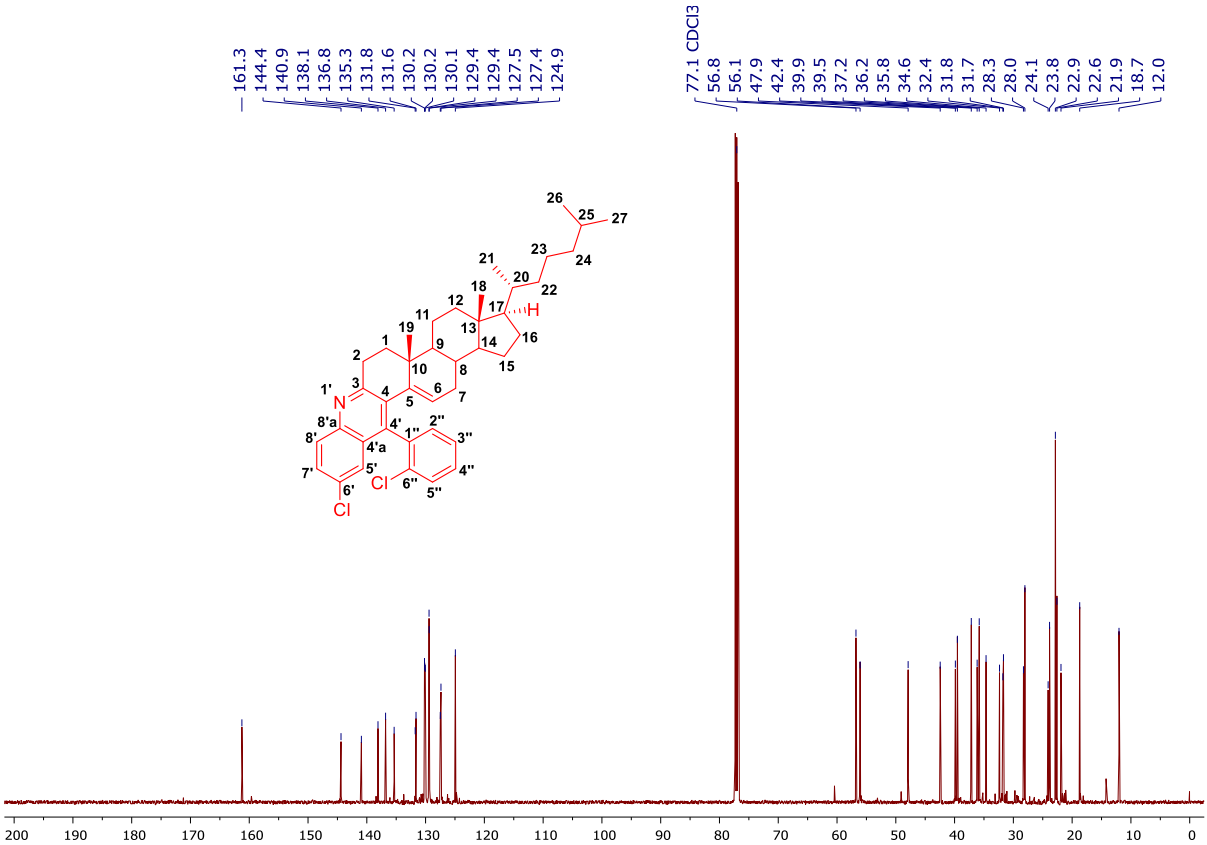


Figure S10: ^{13}C-NMR (125 MHz, $CDCl_3$) of compound **4d**.

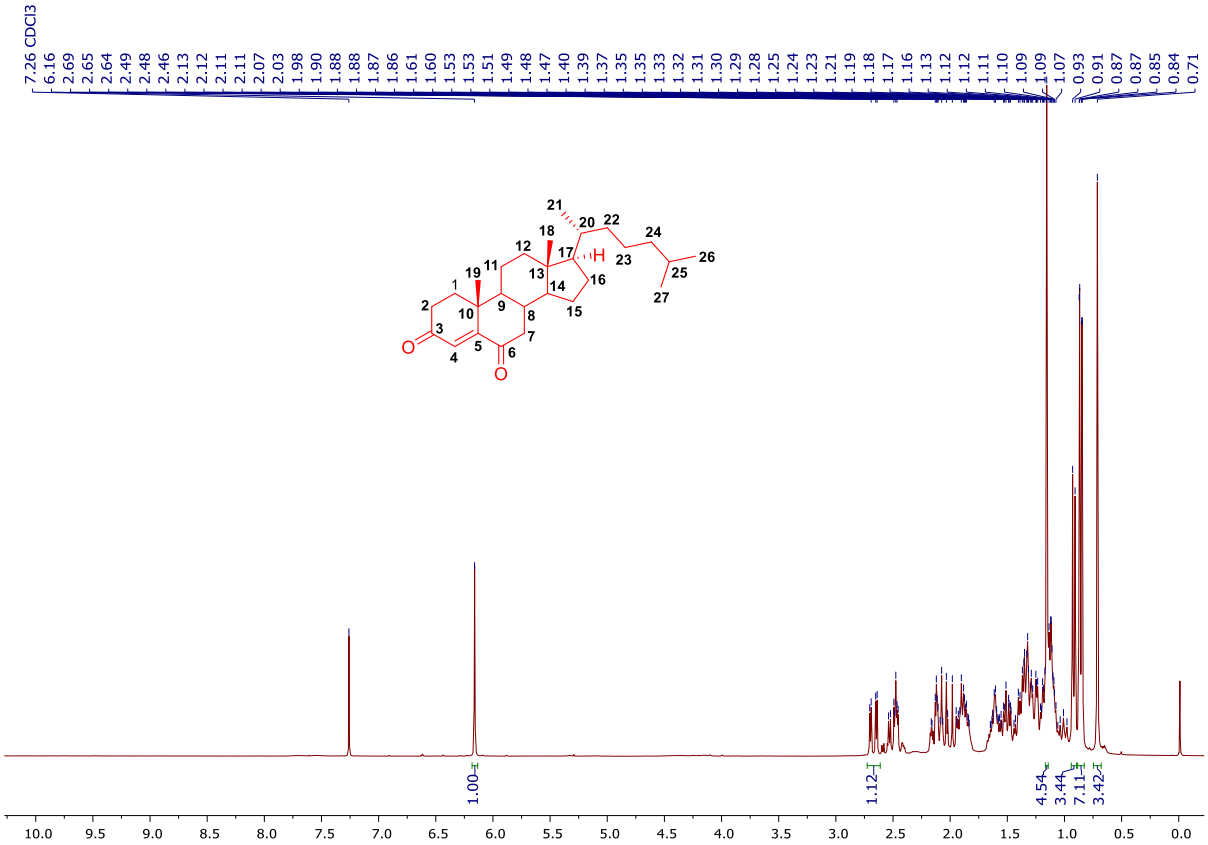


Figure S11: ^{1}H-NMR (300 MHz, $CDCl_3$) of cholest-4-en-3,6-dione (**5**).

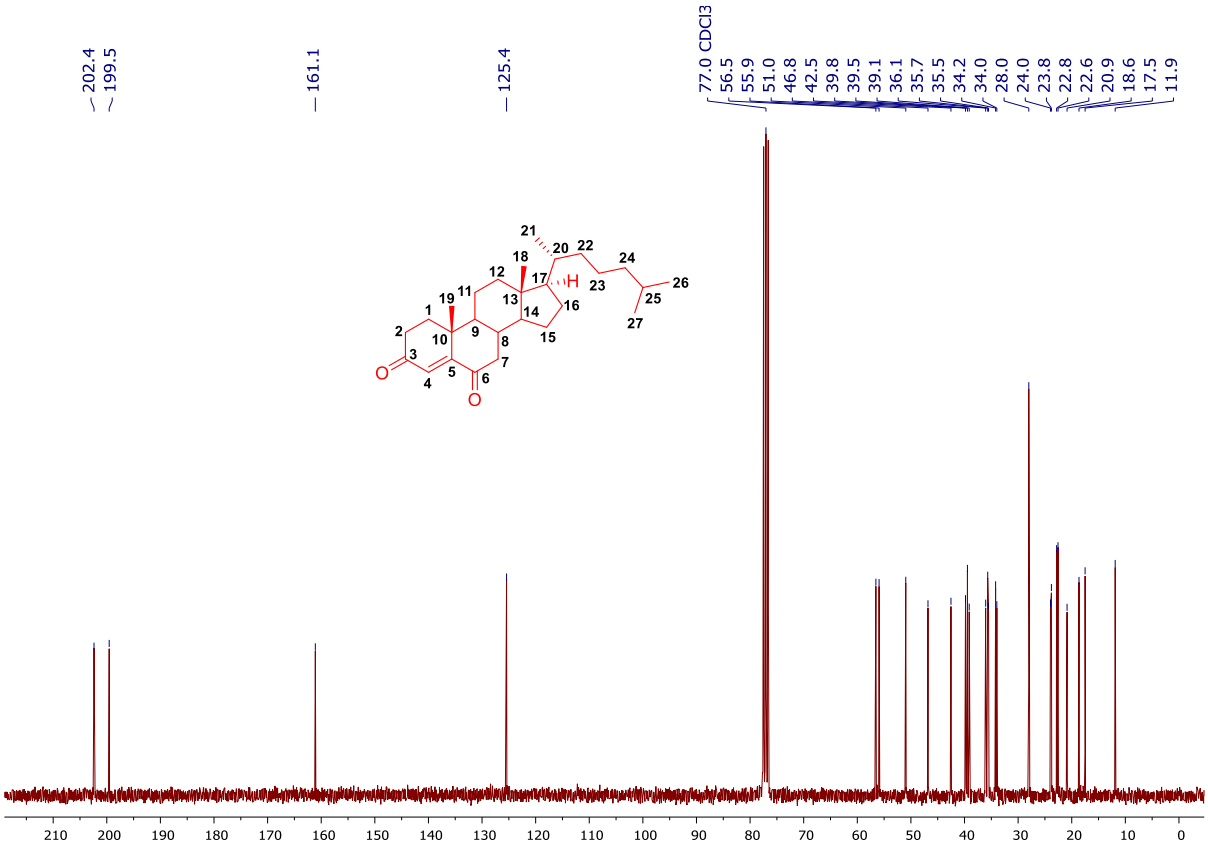


Figure S12: ^{13}C-NMR (75 MHz, $CDCl_3$) of cholest-4-en-3,6-dione (**5**).

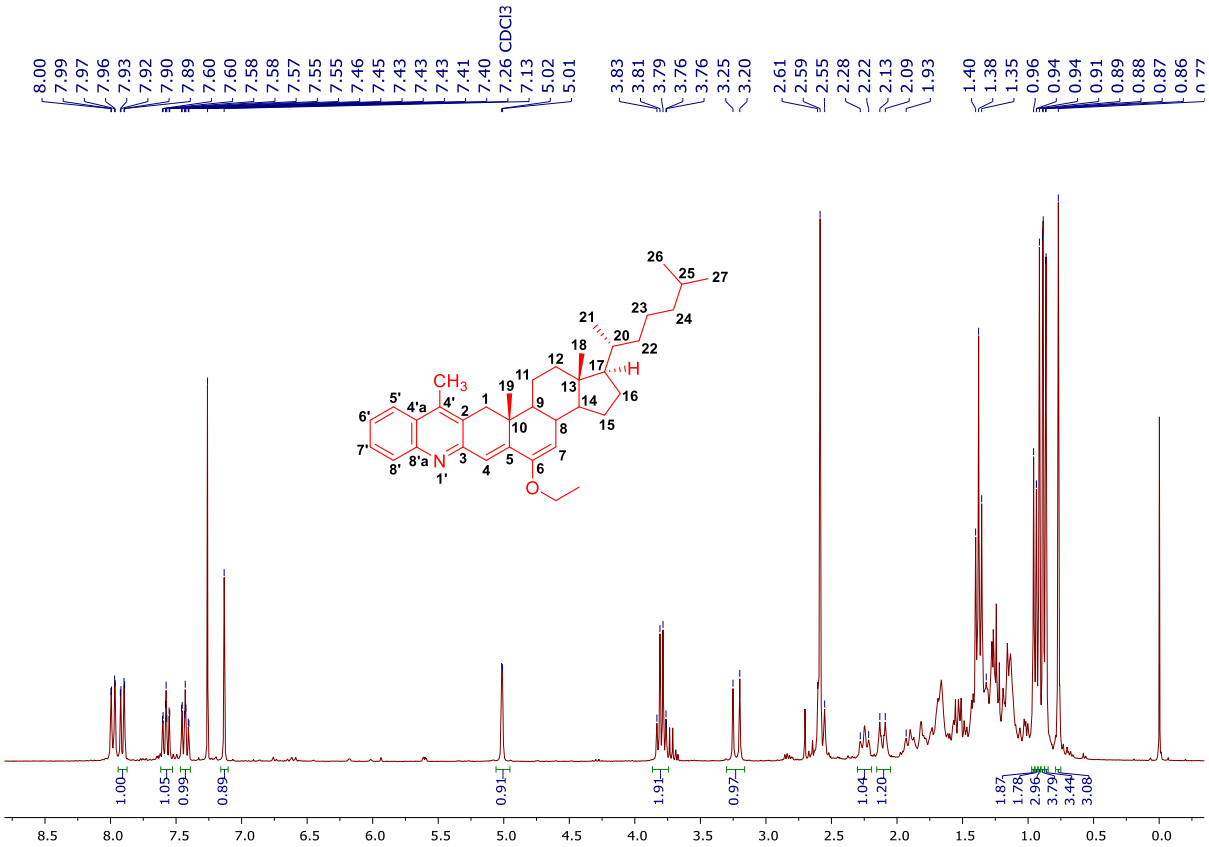


Figure S13: ^{1}H-NMR (300 MHz, $CDCl_3$) of compound **6a**.

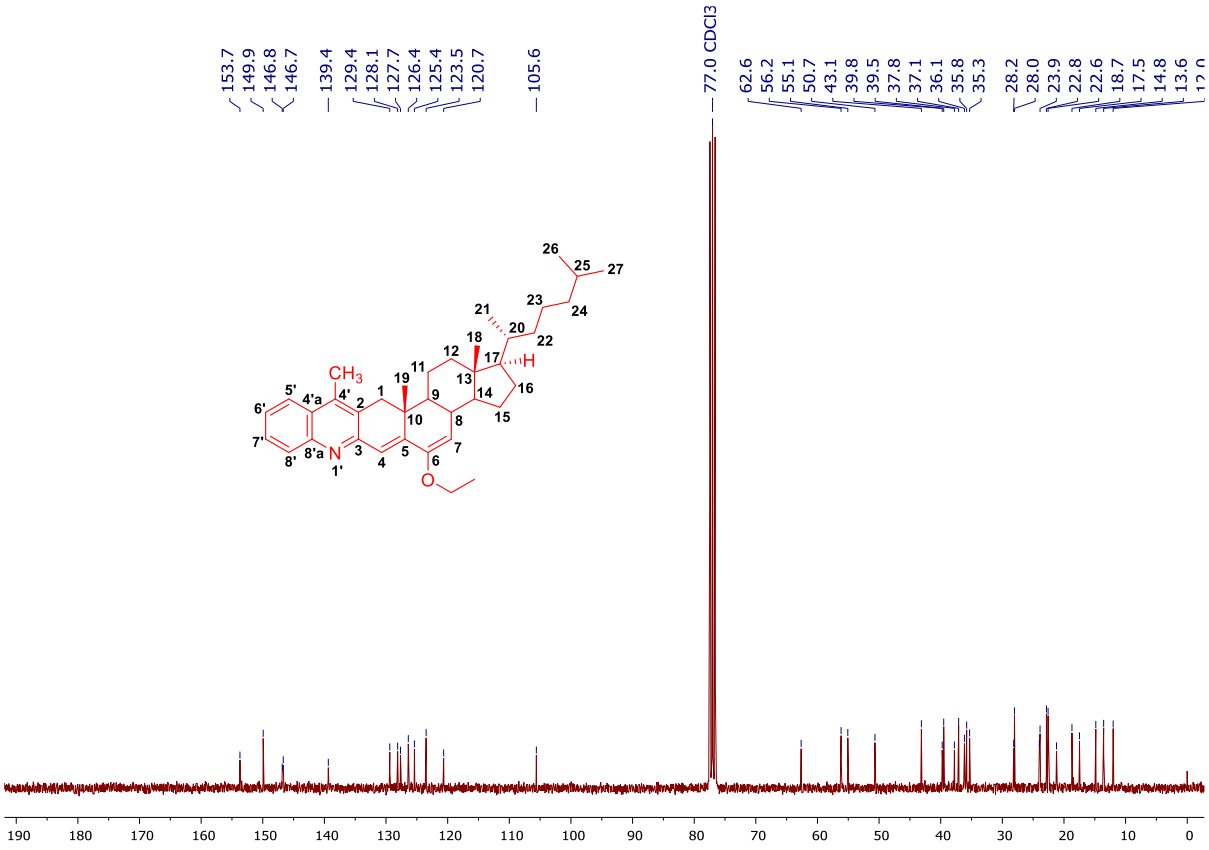


Figure S14: ^{13}C-NMR (75 MHz, $CDCl_3$) of compound **6a**.

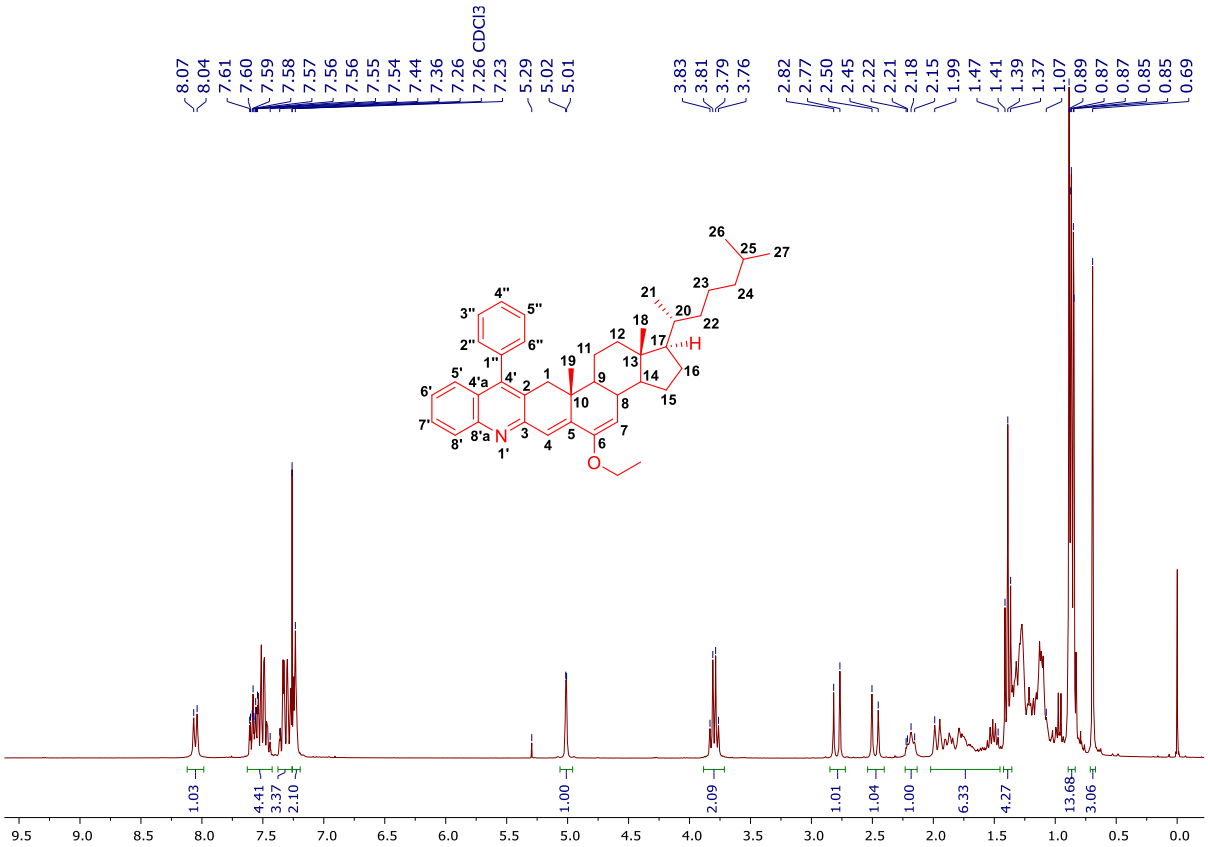


Figure S15: ^{1}H-NMR (300 MHz, $CDCl_3$) of compound **6b**.

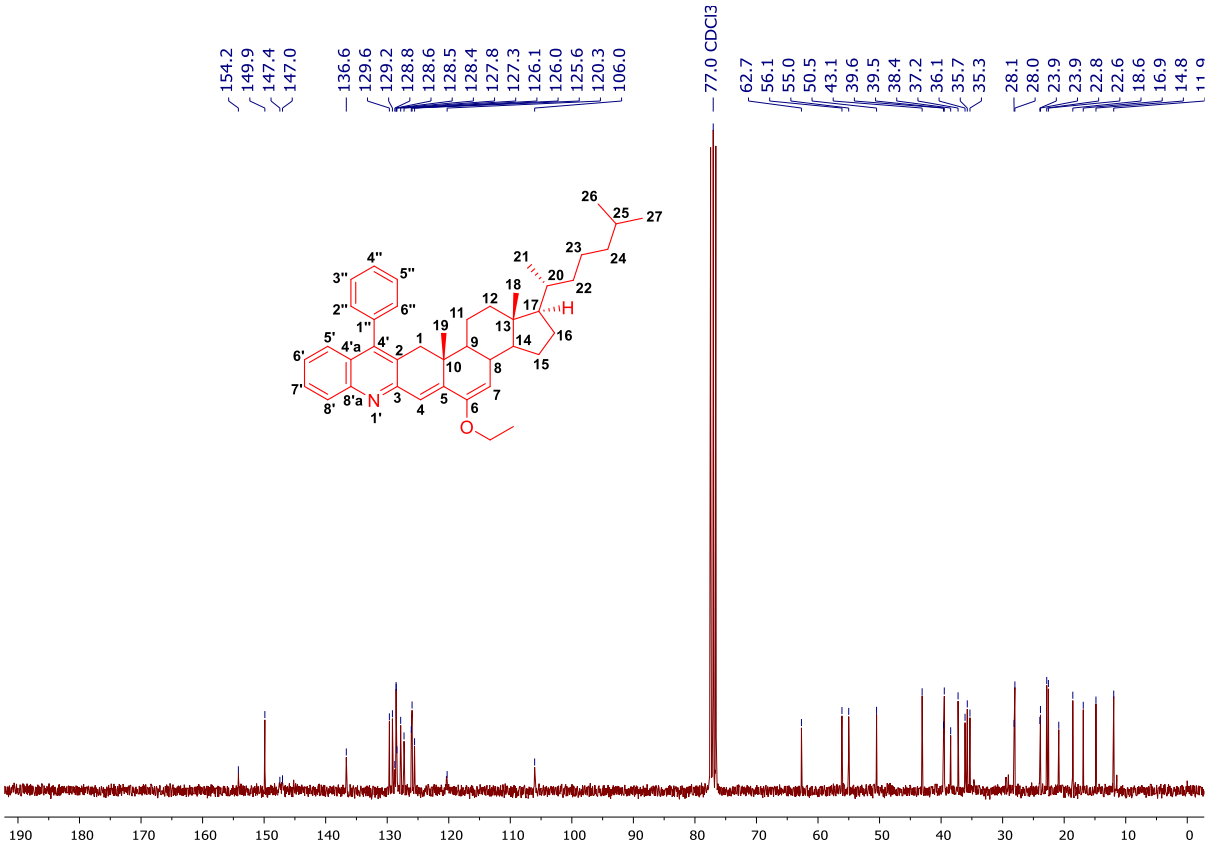


Figure S16: ^{13}C-NMR (75 MHz, $CDCl_3$) of compound **6b**.

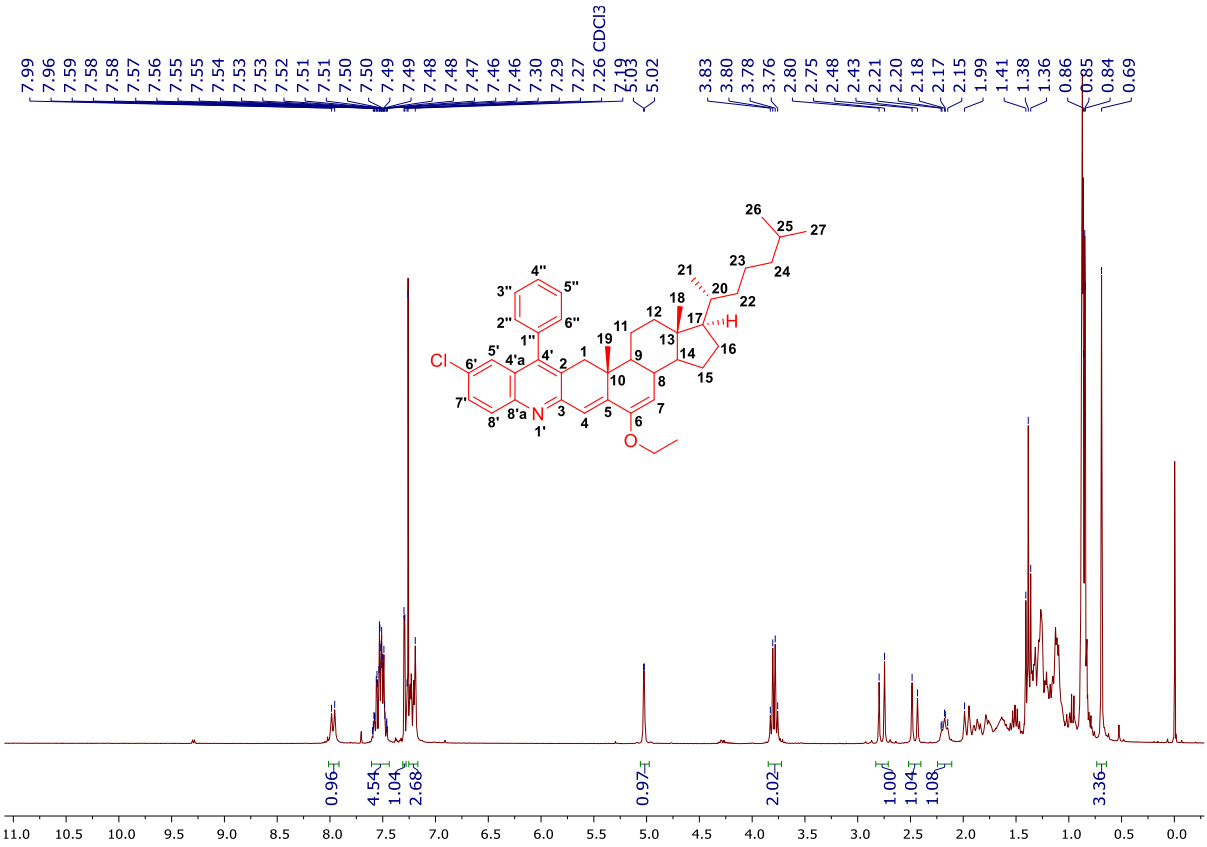


Figure S17: ^{1}H-NMR (300 MHz, $CDCl_3$) of compound **6c**.

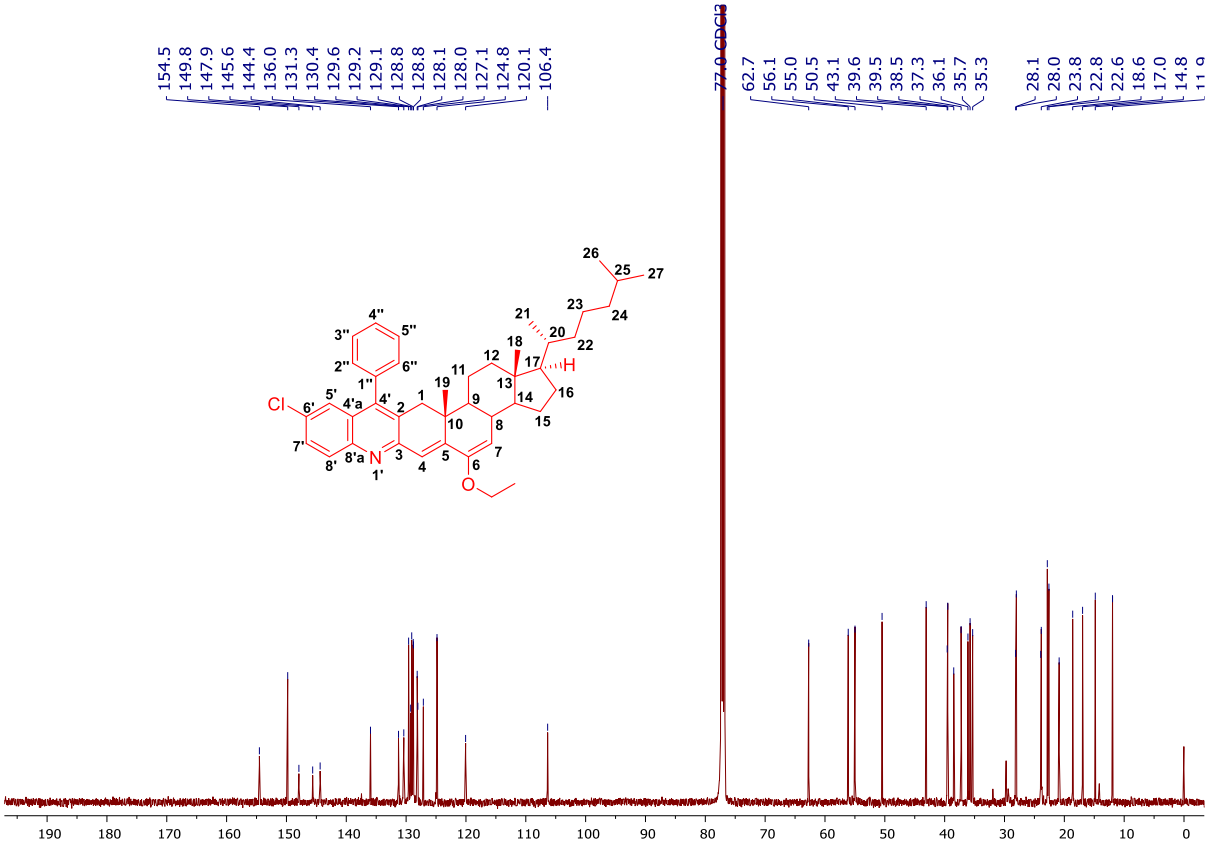


Figure S18: ^{13}C-NMR (125 MHz, $CDCl_3$) of compound **6c**.

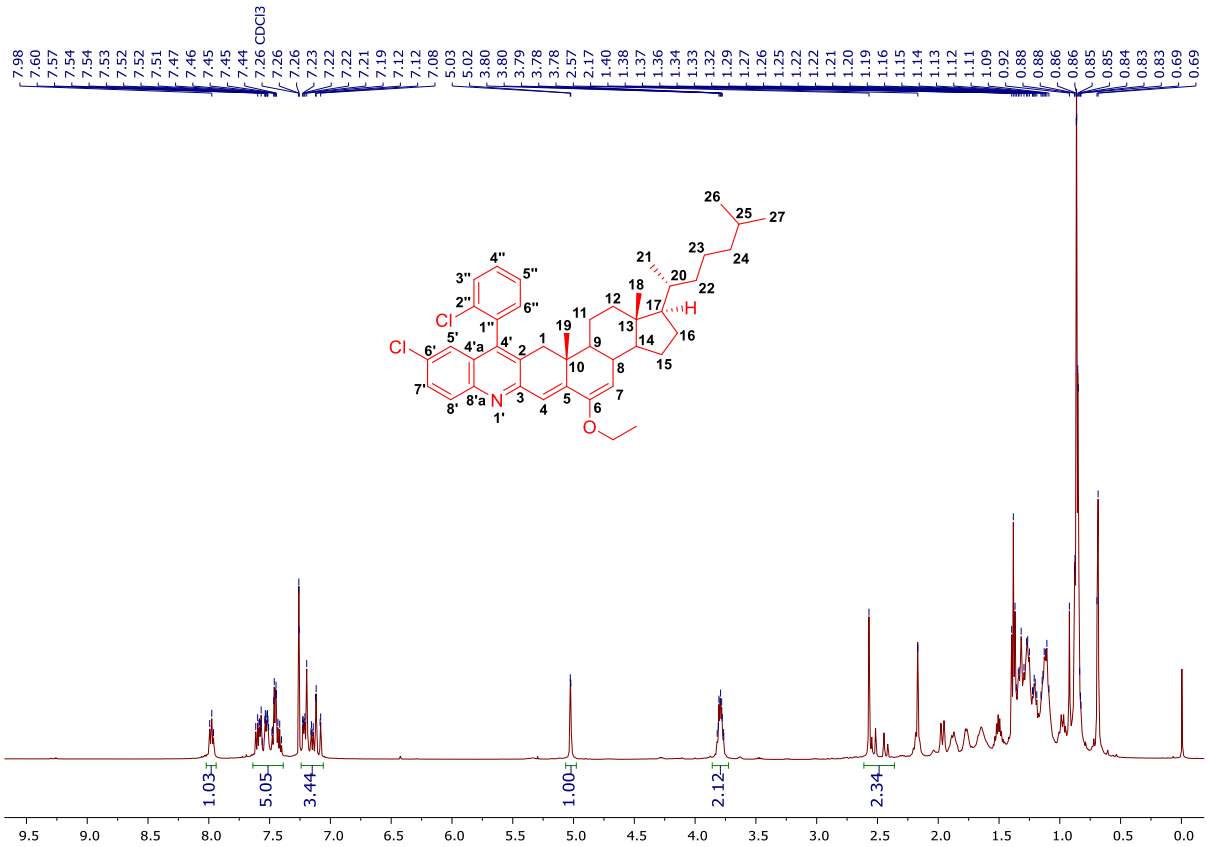


Figure S19: ^{1}H-NMR (500 MHz, $CDCl_3$) of compound **6d**.

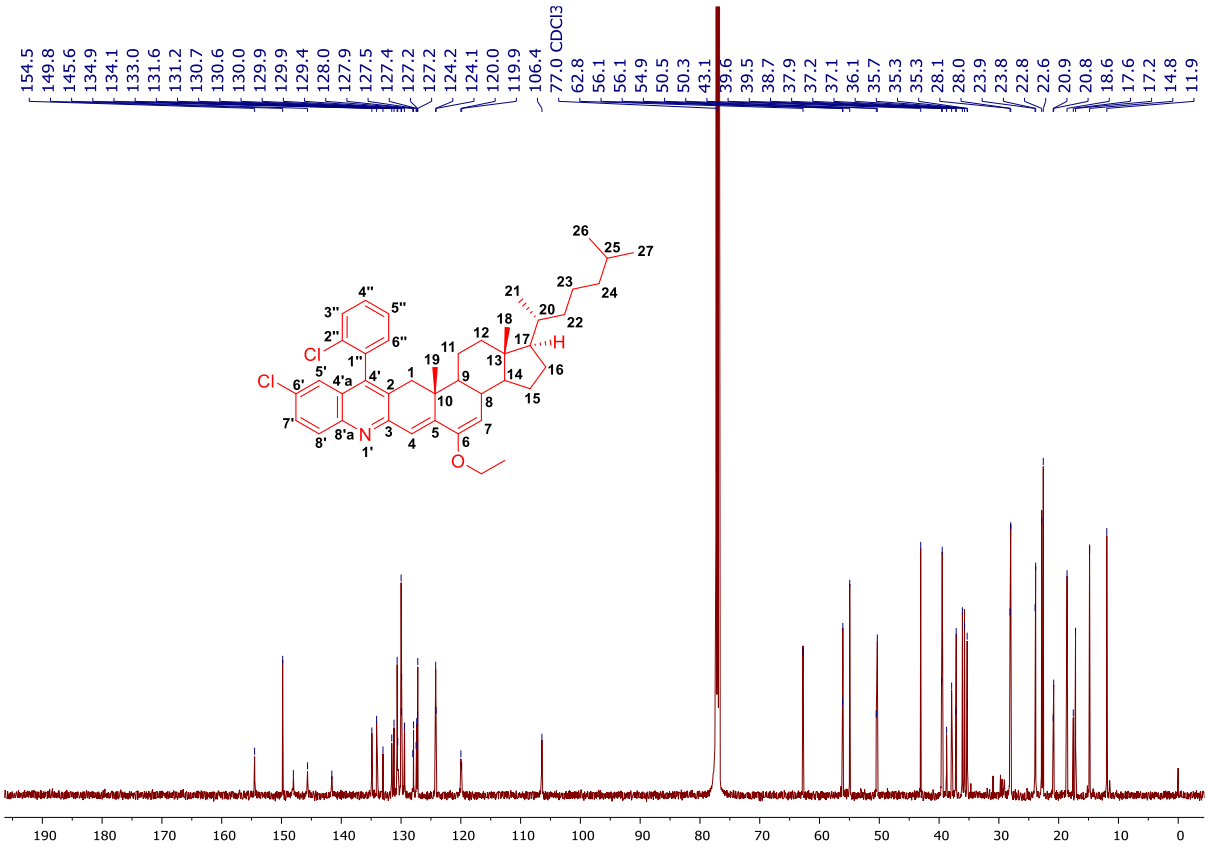


Figure S20: ^{13}C-NMR (125 MHz, $CDCl_3$) of compound **6d**.

4. Structural characterization – Single crystal X-ray diffraction

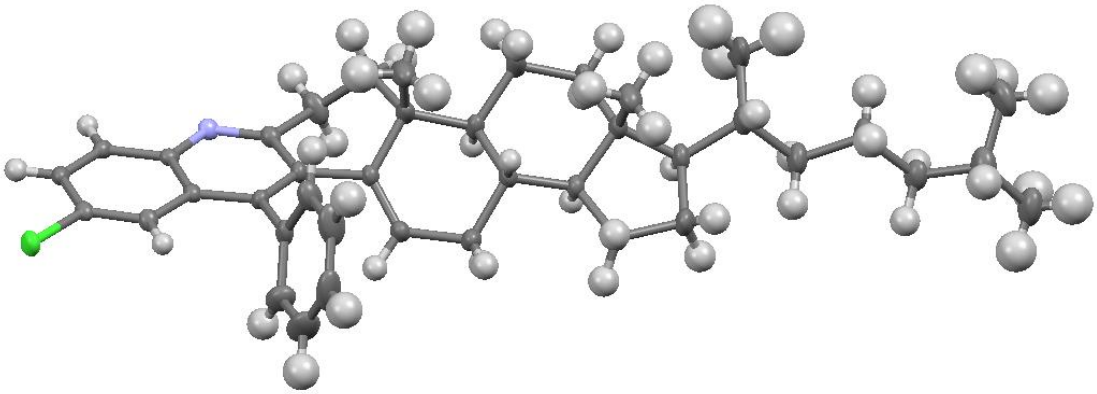


Figure S21: Crystal structure of compound **4c**, asymmetric unit. Ellipsoids are drawn at the 50% level, hydrogen are shown as sphere of arbitrary radius of 0.30 Å. C, grey; H, white; Cl, green; N, blue.

Single crystals with needle shape of compound **4c** were manually selected from the crystallization vial. A suitable single crystal was mounted on a glass fiber with the help of silicon grease. Data were collected at 180(2) K on a Bruker X8 Kappa APEX II charge-coupled device (CCD) area-detector diffractometer (Mo K_a graphite-monochromated radiation, λ = 0.71073 Å) controlled by the APEX2 software package,[1] and equipped with an Oxford Cryosystems Series 700 cryostream monitored remotely using the software interface Cryopad.[2] Images were processed using the software package SAINT+,[3] and data were corrected for absorption by the multi-scan semi-empirical method implemented in SADABS.[4] The structure was solved using the direct methods algorithm implemented in SHELXS-97,[5,6] which allowed the immediate location of the majority of the atoms. All remaining non-hydrogen atoms were located from difference Fourier maps calculated from successive full-matrix least squares refinement cycles on F^2 using SHELXL-97.[5,7] All non-hydrogen atoms were successfully refined using anisotropic displacement parameters.
Hydrogen atoms bound to carbon were located at their idealized positions using appropriate *HFIX* instructions in SHELXL (*43* for the aromatic and vinylic, *23* for the $–CH_2–$ moieties and *13* for the chiral tertiary carbon atoms) and included in subsequent refinement cycles in riding-motion approximation with isotropic thermal displacements parameters (U_{iso}) fixed at 1.2 times U_{eq} of the atom to which they are attached.
Crystallographic data (including structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC-1958452. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 2EZ, U.K. FAX: (+44) 1223 336033. E-mail: deposit@ccdc.cam.ac.uk.

5. Biocompatibility with mammalian cells

The human immortalized HeLa cell line, derived from cervical cancer, was cultured in Dulbeco's Minimal Essential Media (DMEM) supplemented with 1% Non-Essential amino acids (Gibco, Invitrogen), 10% heat inactivated Fetal Bovine Serum (FBS; Gibco, Invitrogen) and 1% antibiotic/antimycotic mix (AA; Gibco, Invitrogen). Cultures were maintained at 37 °C under 5% CO_2 in a humidified incubator. Live cells at 60% confluence were incubated for 24 h and 48 h with 0 (negative control, NC), 0.1, 1.0, 10, 50 and 100 μM media solutions of the compounds **4a-d** and **6a-d** in 0.5% ethanol, the vehicle used in control conditions. The resazurin metabolic assay was used to assess cytotoxicity.[8] Briefly, in the last 4 h of the 24 h and 48 h incubation period, a resazurin (Sigma-Aldrich) solution (0.1 $mg.mL^{-1}$ resazurin in phosphate buffer saline (PBS; Thermo Scientific) was added to the cells' media at 10%. Reduction of resazurin to resofurin in the cells media was measured spectrophotometrically (Infinite M200 PRO, Tecan) at 570 and 600 nm, and the final O.D. calculated.[8] Data was plotted as percentage of the NC cells' final O.D.

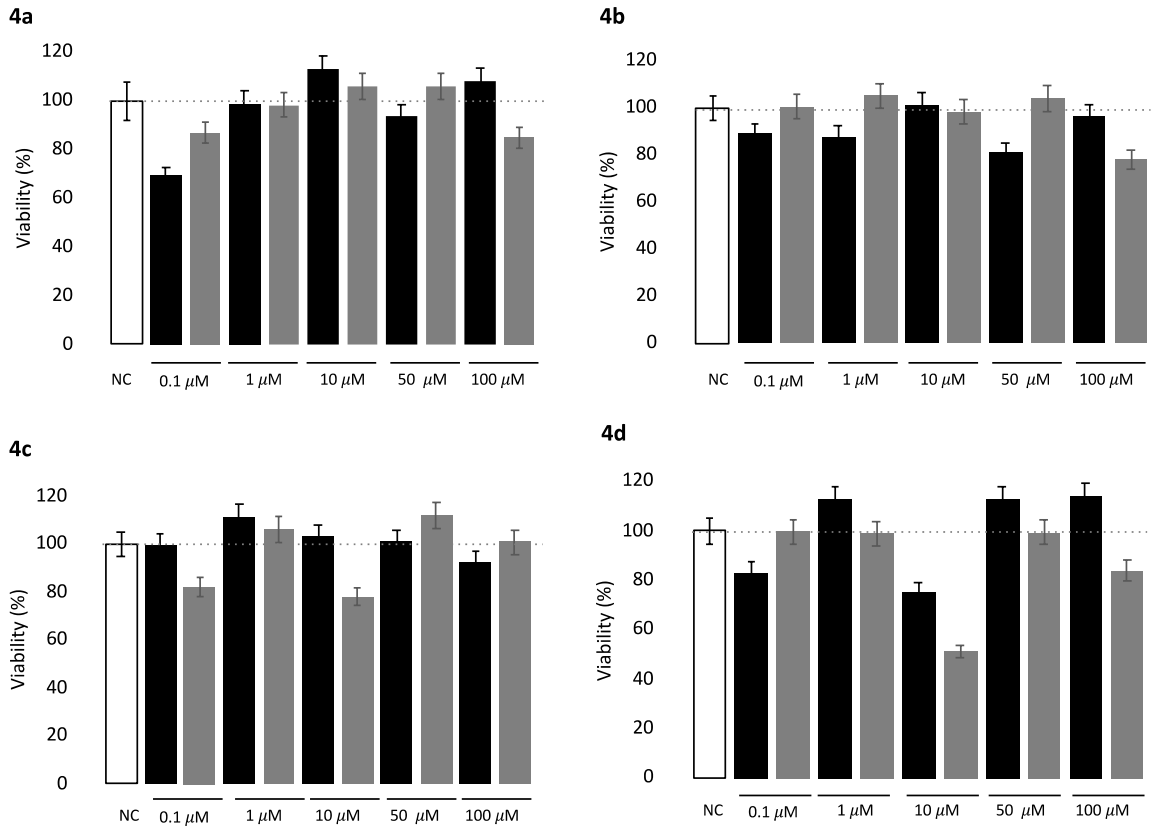


Figure S22: Cell viability assays of human HeLa cells exposed to 0, 0.1, 1, 10, 50 and 100 μM of compounds **4a-d** for 24 h (black solid fill) and 48 h (grey solid fill). NC, negative control (white solid fill). N = 4.

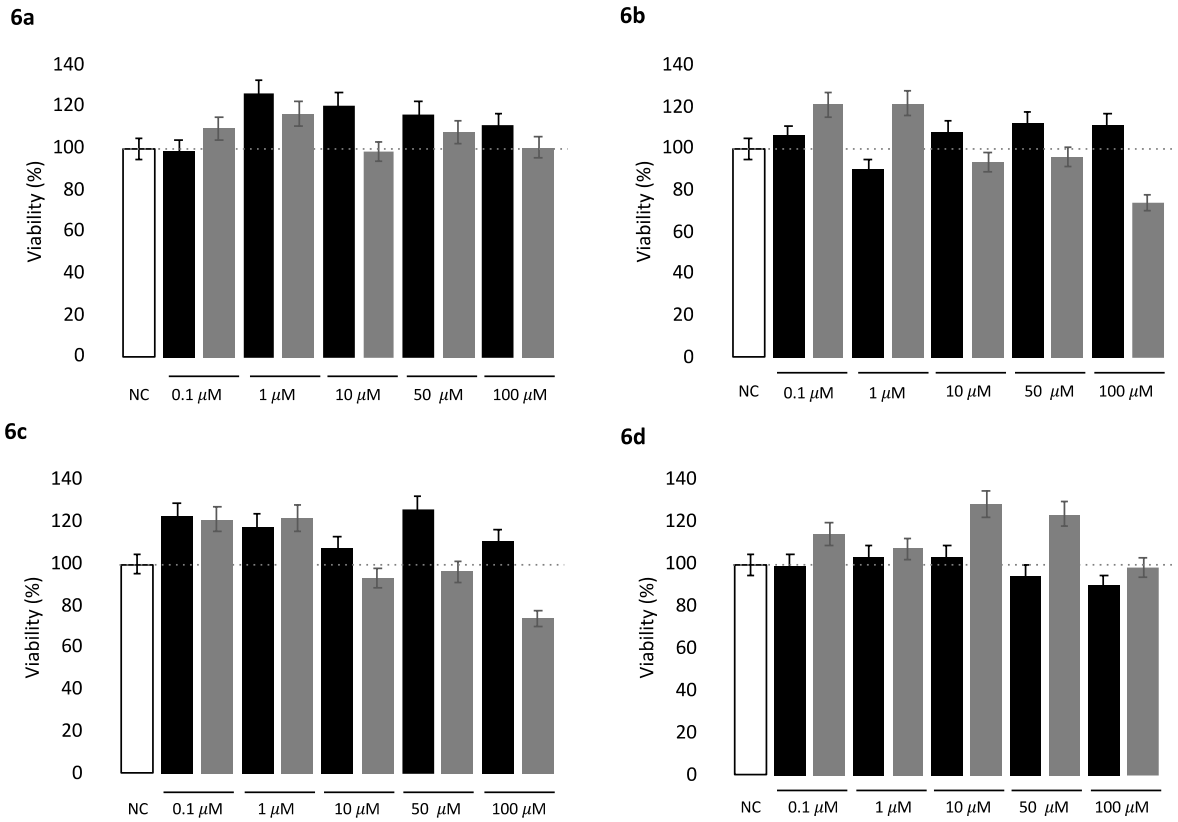


Figure S23: Cell viability assays of human HeLa cells exposed to 0, 0.1, 1, 10, 50 and 100 μM of compounds **6a-d** for 24 h (black solid fill) and 48 h (grey solid fill). NC, negative control (white solid fill). N = 4.

6. Thioflavin-T Aβ1-42 *in vitro* fibrillation assay

Aβ1-42 pre-treatment: Aβ1-42 (AnaSpec Inc.) was first dissolved in 1,1,1,3,3,3,-Hexafluoro-2-propanol (HFIP) at 1 mg/mL before incubating at room temperature for 30 min. The resultant solution was sonicated for 5 min, and then dispensed in desired volumes. The solution was left at room temperature overnight to volatilize the HFIP, followed by vacuum concentration to obtain HFIP-treated Aβ1-42 (HFIP-Aβ1-42).

The inhibition of Aβ1-42 aggregation was determined through a ThT-based fluorimetric assay. HFIP treated Aβ1-42 stock solution (111 μM) was prepared in 0.1% NH_4OH. Stock solutions of compounds **4a-d** and **6a-d** (400 μM) were prepared in EtOH. ThT stock solution (400 μM) was prepared in distilled water. Assay buffer (PBS), Aβ1-42 peptide (final concentration 10 μM), ThT (final concentration 20 μM) and test compounds (final concentration 20 μM) were aliquoted to a 96 well black clear bottom microplate, to a final volume of 200 μL. The fluorescence intensity (430 nm excitation and 485 nm emission) was then monitored for 30 h, at 23 °C with agitation at 10 min intervals using a microplate reader (Synergy/HTX multi-mode reader, Biotek).

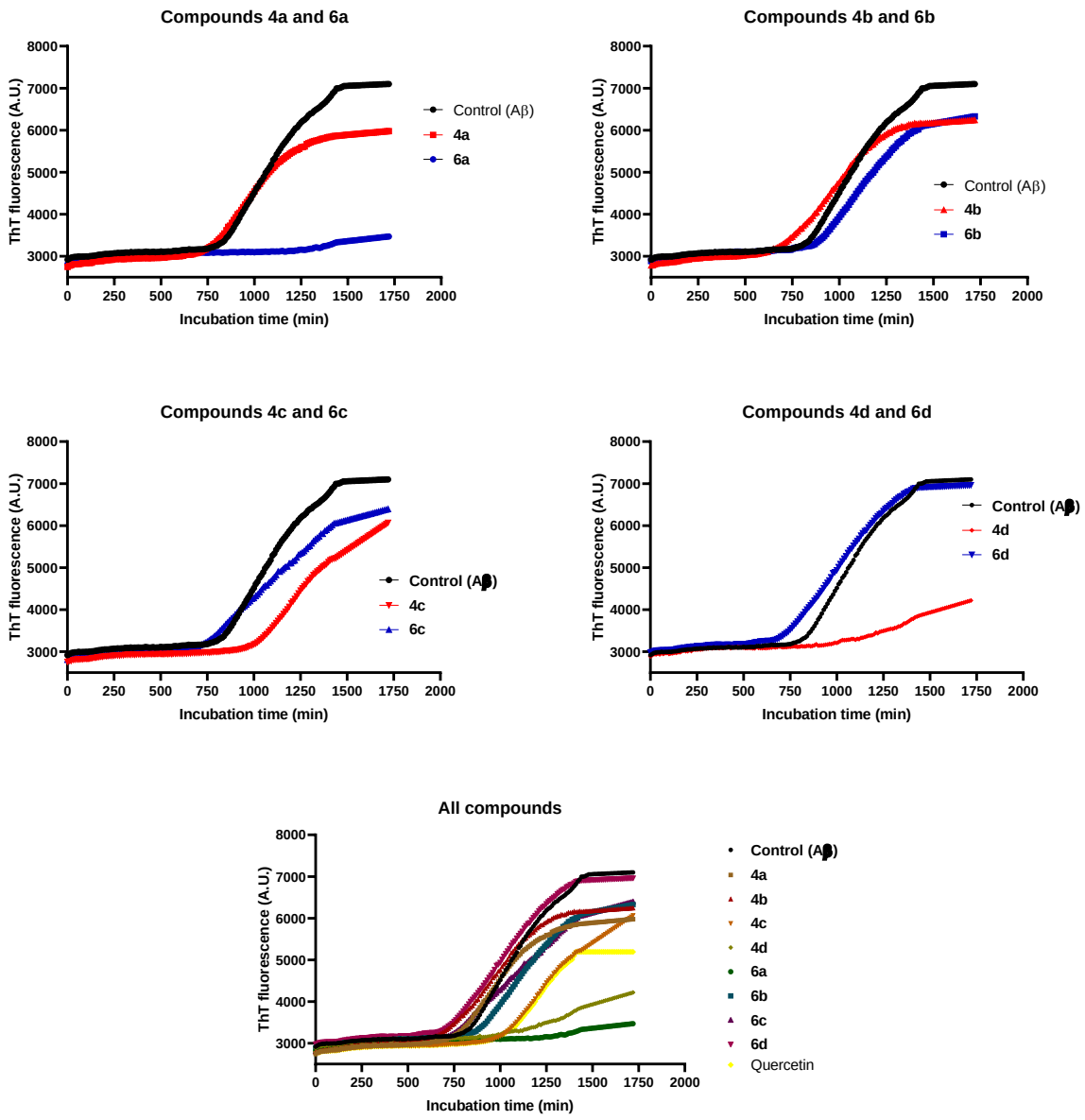


Figure S24: Effect of quinoline orientation on Aβ1−42 peptides aggregation by ThT fluorescence. Comparison between derivatives **4a**/**6a**, **4b**/**6b**, **4c**/**6c** and **4d**/**6d**. Compounds **4a-d** and **6a-d** plotted together the positive control (quercetin at 20 μM). Data are presented as the mean of three experiments (n = 3).

7. Disaggregation assays – protein aggregation cell models

Evaluation of the number and average size of aggregates foci per cell, detected with Proteostat®, in SH-SY5Y cells incubated with Aβ1-42 amyloid aggregates

Aβ1-42 amyloid (AnaSpec Inc.) was diluted in miliQ H_2O to a 1 mM concentration. A final solution of 100 μM Aβ1-42 amyloid in PBS was incubated for 48 h at 37 ºC prior to its addition to cells (pre-aggregated Aβ1-42). The SH-SY5Y human neuroblastoma cell line, a well-known *in vitro* neuronal model,[10] was maintained in Minimum Essential Media (MEM):F12 (1:1; Gibco, Invitrogen) supplemented with 10% FBS and 1% AA solution, and incubated in a 5% CO_2 humidified incubator

at 37 ºC. Cells were sub-cultured when a cell density of 90% was achieved. $1.5x10^5$ SH-SY5Y cells were plated in 12 well plates and incubated for 24 h. Cells were then incubated with 10 μM of the pre-aggregated Aβ1-42 amyloid in DMEM (without FBS) for 16 h (internalization of Aβ1-42). After this period, the medium was changed to fresh DMEM medium without FBS, and cells incubated with compounds **4a-d** and **6a-d** at 50 μM for 24 h. Cells without Aβ1-42 amyloid and incubated only with the compounds' vehicle (0.5% EtOH) were used as non-aggregation controls, and cells incubated with Aβ1-42 amyloid and 0.5% EtOH were used as positive controls. After the incubation period, cells were washed with PBS, fixed in 4% formaldehyde, permeabilized with Triton 0.2% in PBS, washed with PBS again, and protein aggregates stained with Proteostat®, as detailed explained above. Coverslips with cells were mounted with DAPI-containing Vectashield (Vector) and z-stacks of 10-20 randomly selected Fields of View (FOV) were acquired with a Zeiss LSM 880 AiryScan confocal microscope equipped with the Zen Black software (Carl Zeiss, Jena, Germany), using 405 nm and 561 nm laser lines for DAPI and Proteostat® excitation, respectively, in a minimum of 200 cells analyzed per condition (average of 370 cells/condition; n = 2). The total number of cells of the imaged population (DAPI-stained), and the number of cytoplasmic Proteostat-positive foci were manually scored in each z-stack maximum projection, with the support of Fiji/ImageJ. This software was also used to retrieve the total foci area per cell, by determination of the area occupied by the Proteostat red channel staining. Both the number of foci and their area were divided by the total number of all cells in each FOV and data presented as mean ± SD.

Disaggregation ability of general protein aggregation

HeLa cells expressing the protein aggregation sensor HSP27:GFP[9] were used to evaluate the ability of the compounds to disaggregate general protein aggregates. Cells were cultured in DMEM, supplemented with 10% heat inactivated and 1% AA solution, at 37 °C in an atmosphere containing 5% CO_2 in a humidified incubator. Briefly, HeLa HSP27:GFP cells were seeded in black bottom 96 well plates at a $0.6x10^5$ cells/well density, and incubated for 24 h at 37 ºC. Nilotinib (Sigma-Aldrich) was added to cells at a 5 μM and incubated for 48 h. In the last 12 h of incubation, the compounds (**4a-d** and **6a-d**) were added at 50 μM and their vehicle 0.5% ETOH added to the NTB condition (positive aggregation control). As negative controls, cells incubated with the compound's vehicle (0.5% EtOH) were used. Upon washing with PBS, cells were fixed with 4% formaldehyde (Sigma-Aldrich) and washed three times with PBS. Fluorescent images of the different conditions tested were acquired in an In Cell Analyzer 2000 (GE Healthcare) high content screening microscope (serial number=B30182 and software version=14311), using a Nikon 20X/0.45 Plan Fluor ELWD Corr Collar 0-2.0 and CFI/60 objective, equipped with a Photometrics CoolSNAP K4 CCD camera, with a pixel array of 2048x2048 (7.4 μm^2 Pixel). A 1000 cells per well detection threshold was set, using the DAPI (excitation: 350 nm and emission: 455 nm) filter to

detect the cell nucleus and the FITC (excitation: 490 nm and emission: 525 nm) filter to detect HSP27:GFP foci.

The data was analysed using Ilastik ([10] https://doi.org/10.1038/s41592-019-0582-9) 1.3.0 and Cell Profiler ([11] https://doi.org/10.1186/gb-2006-7-10-r100) (2.2.0) softwares, to determine the number and average size of green fluorescing *foci* (aggresomes). Briefly, TIFF images were imported into Ilastik (1.2.2) software to perform the nuclei and foci segmentation based on pixel classification. The Otsu thresholding method was applied to better distinguish the foreground (area of interest) from the background. The software creates probability maps that can be then loaded into CellProfiler (2.2.0). The pipeline generated identifies the nuclei outlines in blue, the cell boundary in red (Mask created), and the foci in green (2-34 pixel units). The foci parameters (min, max) were defined according to the positive control (NTB). Output files were generated for the total number of cells, the number of foci per cell, and the foci area. Pixel area data was converted to μm2 using a conversion factor of 0.1369 (square of the 0.37 μm pixel size). Data was presented as mean ± SD of 3-4 independent experiments and the statistical analysis was conducted at GraphPad Prism (7.00).

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