Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Synthetic quinolone signal analogues inhibiting the virulence factor elastase of *Pseudomonas aeruginosa*

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1. Materials and Methods

Chemicals and solvents for the synthesis were purchased from Sigma-Aldrich, Acros Organics, Carl Roth or VWR Chemicals and were used without further purification. For Silica gel chromatography, distilled technical grade solvents and silica gel 60 Å (Carl Roth) was used. Thin layer chromatography (TLC) was performed using aluminium sheets "TLC Silica gel 60 F_{254}" from Merck Millipore® and analysed with UV-light, permanganate solution or an iodine chamber. NMR spectra were obtained with Bruker Avance-III 400 and Bruker Avance-III 600 NMR spectrometers at ambient temperature. Multiplicities are given as follows: s - singlet, d - doublet, t - triplet, q - quartet, quint. - quintet, m - multiplet. Chemical shifts (δ) are given in parts per million (ppm) relative to the solvent residual signal with $CDCl_3$ δ_H = 7.26 ppm and δ_C = 77.16 ppm, DMSO-d_6 δ_H = 2.50 ppm and δ_C = 39.52 ppm, THF-d_8 δ_H = 1.72 and 3.58 ppm and δ_C = 67.21 and 25.31 ppm.[1] The obtained data were processed and analysed with MestReNova or Bruker Topspin 3.5 software. Mass spectrometry data were obtained by LCMS2020 from Shimadzu (high-pressure pump LC-20 AD, auto sampler SIL-20AT HAT, column oven CTO-20AC, UV-Vis detector SPD-20A, controller CBM-20, ESI detector, software LCMS Solution, column Nucleodur 100-3-C18ec, 125 x 4 mm Machery Nagel). The method used for LCMS was a gradient with a flow rate of 0.4 ml/min. Mobile phase with A = H_2O + 0.1% formic acid, B = acetonitrile + 0.1% formic acid. Gradient over 20 min: T_0: B = 10%; T_{20}: B = 95%; T_{25}: B = 95%. For smaller molecules, EI-MS JMS-Q1500GC from Joel

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Ltd. without coupled GC, the Head Space Auto Sampler (HS) and the analysis software Escrime was used. Absorption and fluorescence data were acquired with a TECAN infinite® M200 PRO plate reader using i-control™ software and 96-well plates from Sarstedt (Microtest plates 96-well, flat-bottom, without lid) or Thermo Scientific (Microfluor® 1, 96-Well Fluorescence Microplates, flat-bottom, without lid).

2. Bioassays and biochemical testing

Pseudomonas aeruginosa PA14 (DSM 19882) was purchased from DSMZ. Overnight cultures were prepared in LB medium (Lennox broth) at 37°C and 200 rpm in a glass culture tube.

The pyocyanin assay was performed according to Essar *et al*[2] but with different media. 10 µL of an overnight culture of *P. aeruginosa* PA14 was added to 3 mL of PTSB medium[3] together with 15 µL of the compounds **1**-**8** dissolved in DMSO to give a final concentration of 500 µM. The cultures were incubated for 24 h at 37 °C and 200 rpm. 1.4 mL of each culture was centrifuged and the supernatant filtered through a sterile filter. Thereafter, 0.5 mL of the sterile flow-through were extracted with 0.6 mL chloroform. The chloroform phase was taken off and added to 0.4 mL 0.2 N HCl solution. After mixing by vortex, 0.2 mL of the aqueous phase was measured at 520 nm in a 96 well plate. The experiments were performed in three independent replicates and the results are given in Figure S1.

The rhamnolipid assay was performed according to Wilhelm *et al*[4] but with different media. 10 µL of an overnight culture of PA14 was added to 3 mL of PTSB medium[3] together with 15 µL of the compounds **1**-**8** dissolved in DMSO to give a final concentration of 500 µM. The cultures were incubated for 24 h at 37 °C and 200 rpm. 1.4 mL of each culture was centrifuged and the supernatant filtered through a sterile filter. Thereafter, 0.5 mL of the sterile flow-through was extracted with 0.6 mL diethyl ether. The organic phase was taken off and evaporated under a nitrogen stream. The dry residue was dissolved in water. 0.1 mL of 1.6% orcinol in water was added together with 0.8 mL 60% H_2SO_4 in water. The mixture was incubated at 80 °C and 700 rpm for 30 minutes. The mixture was cooled on ice and 310 µL aqueous NaOH (9.4 M) added until the pH was neutral. 0.2 mL of the brown solution was measured at 421 nm in a 96 well plate. The experiments were performed in three independent replicates and the results are given in Figure S2.

The elastase assay was performed according to Calfee *et al.*[5] 10 µL of an overnight culture of PA14 was added to 3 mL of PTSB medium together with 15 µL of the compounds **1**-**8** dissolved

in DMSO to give a final concentration of 500 µM. The culture was incubated for 24 h at 37 °C and 200 rpm. 1.4 mL of the culture was centrifuged and the supernatant filtered through a sterile filter. Thereafter, 0.5 mL ECR-buffer (0.1 M Tris HCl, 1 mM $CaCl_2$, pH = 7.2) together with 10 mg Elastin-Congo Red was added to 0.5 ml of the sterile flow-through and the mixture incubated for 3 h at 37 °C and 1600 rpm. After the incubation, 0.1 mL Na_2EDTA (0.12 M) was added to quench the enzymatic reaction. After mixing, the solution was centrifuged (10 min at 6000 rpm) and 0.2 mL of the supernatant measured at 495 nm in a 96 well plate. For concentration dependent experiments, the same method was used with varying concentration of the compounds added (15 µL) to the culture medium from corresponding DMSO stock solutions. All experiments were performed in at least three independent replicates (Figure 2A, 4C, and S9).

The *in-vitro* elastase assay was performed according to Cathcart *et al*.[6] For a typical in vitro elastase assay 82 µL buffer (0.05 M TRIS HCl, 2.5 mM $CaCl_2$, pH = 7.2) and 5 µL of the test compound solved in DMSO were added in a 96 well plate. 10 µL of 3 µg/mL elastase (solved in the same Tris/$CaCl_2$ Buffer) was added and the mixed with the compound. After incubation for 10 min at room temperature, 3 µL fluorogenic substrate Abz-peptide-Nba (2-aminobenzoyl-Ala-Gly-Leu-Ala-4-nitrobenzylamide) in DMF was added and the fluorescence measured for 90 min in one minute steps (excitation at 330 nm, emission at 460 nm). The experiments were performed in three independent replicates and the slope of the resulting graphs were used to calculate the enzymatic activity (Figure 2B and S6). For the elastase inhibition test with **11**, the in-vitro assay was performed as described with a concentration of 50 µM (Figure S7).

3. Zinc-binding studies

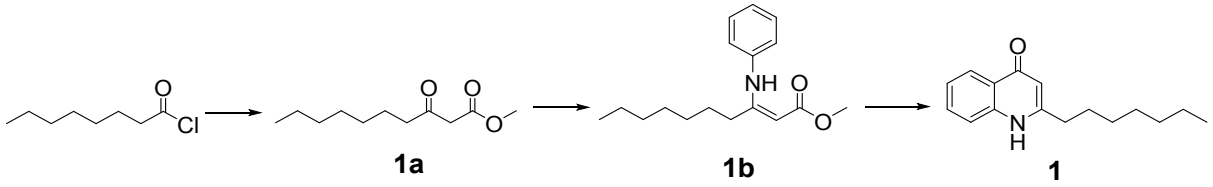
For the NMR-titration of **8** against $ZnCl_2$, 10 mg of **8** (0.0363 mmol) was dissolved in 500 µL DMSO-d_6 and successively 10 µL (0.1 eq.) of a 0.363 M $ZnCl_2$ solution in DMSO-d_6 was added, mixed and the ^1H and ^{13}C-spectra measured (Figure S4 and S5).

For the zinc fluorescence titration, 50 µL **8** (0.5 mM in EtOH) and 50 µl of a $ZnCl_2$ solution (2 mM – 1 µM in EtOH) in an appropriate concentration were mixed and incubated for 5 min at room temperature in a 96 well plate. The fluorescence spectrum of the **8**-Zn-complex was measured from 300 – 600 nm at 26 °C, with an excitation wavelength of 276 nm and 25 flashes/sec.

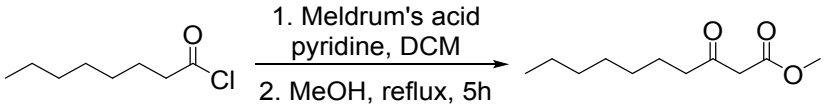
For the competitive titration, 25 µL $ZnCl_2$ solution (0.5 mM in EtOH) was mixed with 25 µl **8** (0.5 mM in EtOH) and incubated for 5 min at room temperature in a 96 well plate. Thereafter, 25 µl of **9** or **10** in EtOH in an appropriate concentration was added and incubated for another 5 min at room temperature. The decrease in fluorescence of the **8**-Zn-complex was measured from 300 – 600 nm at 26°C, with an excitation wavelength of 276 nm and 25 flashes/sec. In Figure S8 the fluorescence is given at maximum emission wavelength of 448 nm.

4. Syntheses

Synthesis of HHQ (1)

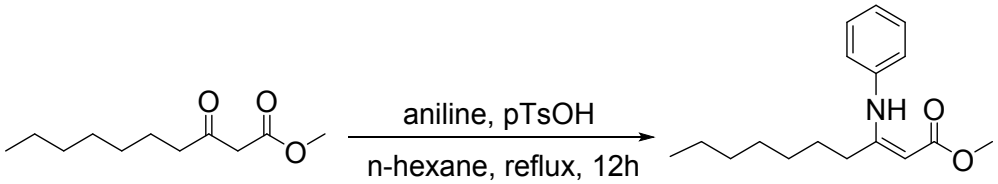


Methyl-3-oxodecanoate (**1a**)



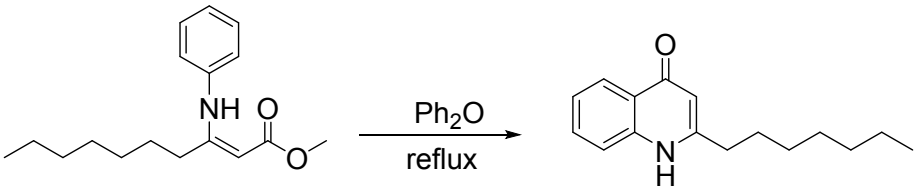
2,2-Dimethyl-1,3-dioxane-4,6-dione (5.04 g, 34.9 mmol) was dissolved in 50 mL DCM and cooled to 0˚ C. 5.5 mL Pyridine (68.1 mmol) was added and after 20 minute at 0 ˚C, 6.0 mL octanoyl chloride (35.15 mmol) was added dropwise. The resulting orange solution was allowed to stir for 1 h at 0 °C and 1 h at room temperature. The reaction mixture was washed with 5% HCl (3 x 60 mL), distilled water (2 x 60 mL) and brine (2 x 60 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The remaining brown oil was dissolved in 100 mL MeOH and refluxed for 5 h. The solvent was evaporated and the residue purified by column chromatography on silica gel using hexane/ethyl acetate (4:1). The product was obtained as a colorless oil (m = 4.5 g, 64%). *Rf* = 0.62 (hexane/ethyl acetate 4:1). ^{1}H-NMR ($CDCl_3$ 600.33 MHz) δ (ppm): 0.87 (m, 3H, $-CH_3$), 1.24-1.32 (m, 8H, $(CH_2)_4$), 1.59 (m, 2H, -C**H**$_2$-CH_2-CO), 2.25 (t, *J* = 7.4 Hz, 2H, $-CH_2$-C**H**$_2$-CO), 3.44 (s, 2H, $-CH_2$-COO), 3.73 (s, 3H, $-OCH_3$). ^{13}C-NMR ($CDCl_3$ 150.95 MHz) δ (ppm): 14.1 ($-CH_3$), 23.5(-**C**H_2-CH_2-CO), 22.6, 28.9, 29.1, 31.7, (4 x $-CH_2$), 43.1 ($-CH_2$-**C**H_2-CO), 49.0 (-**C**H_2-COO), 52.4 ($-OCH_3$) 167.7 (-COO), 202.9 (-CO).

Methyl-3-anilino-2-decanoate (**1b**)



9.7 g β-ketoester **1a** (48.4 mmol) was dissolved in 150 mL n-hexane. 4.0 g aniline (43 mmol) and 0.16 g *p*-toluene sulfonic acid (0.92 mmol) was added. The mixture was refluxed for 12 h. The reaction was allowed to cool to room temperature and the solvent was evaporated. The residue was purified by column chromatography on silica gel using n-hexane/ethyl acetate (9:1). The product was obtained as a yellow oil (m = 9.26 g, 69.5%). *Rf* = 0.66 (hexane/ethyl acetate 9:1). ^{1}H-NMR ($CDCl_3$ 400.13 MHz) δ (ppm): 0.84 (m, 3H, -CH_3), 1.09-1.30 (m, 8H, $(CH_2)_4$), 1.41 (m, 2H, -C**H**$_2$-CH_2-CN), 2.28 (t, *J* = 7.8 Hz, 2H, CH_2-C**H**$_2$-CN), 3.69 (s, 3H, -OCH_3), 4.74 (s, 1H, =CH-COO), 7.09 (d, *J* = 7.6 Hz, 2H, Ar-CH), 7.17 (t, *J* = 7.50 Hz, 1H, Ar-CH), 7.33 (m, 2H, Ar-CH), 10.29 (s, 1H, -NH-). ^{13}C-NMR ($CDCl_3$ 100.61 MHz) δ (ppm): 13.8 (-CH_3), 27.8 (-**C**H_2-CH_2-CO), 22.3, 28.6, 28.8, 31.3 (4 x -CH_2), 32.0 (-CH_2-**C**H_2-CO), 50.1 (-OCH_3), 84.3 (=CH), 124.9 (2C), 125.0, 128.8 (2C), 139.1 (6 x Ar-CH), 163.6 (=C-N), 170.8 (-COO). ESI-MS: m/z = 260.95 $[M\text{-}CH_3+H]^+$, calc. for $C_{16}H_{22}NO + H^+ = 261.17$; m/z = 302.00 $[M\text{-}CH_3+MeCN+H]^+$, calc. for $C_{16}H_{22}NO + C_2H_3N + H^+ = 302.20$.

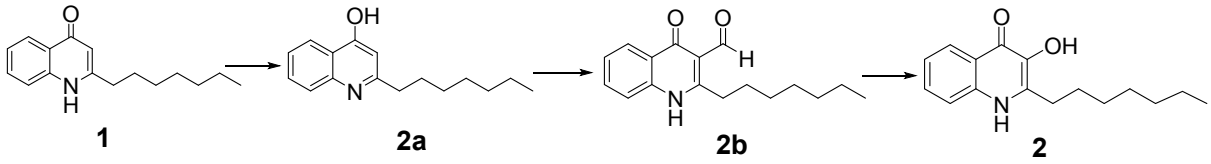
2-Heptyl-4-quinolone, HHQ (**1)**



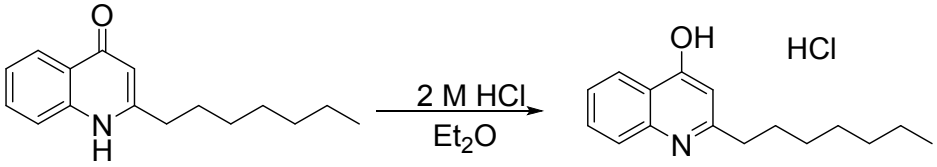
3 g Methyl-3-anilino-2-decenoate **1b** (10.9 mmol) was added dropwise to 15 mL diphenyl ether at reflux temperature and reflux was maintained for 6 h. The reaction mixture was allowed to cool to room temperature and added dropwise to n-hexane. The precipitate was filtered and washed with n-hexane. The product was obtained as a white solid (m = 2.27 g, 81%). ^{1}H-NMR ($CDCl_3$ 600.33 MHz) δ (ppm): 0.82 (m, 3H, H-15), 1.14-1.25 (m, 6H, H-12-14), 1.26-1.33 (m, 2H, H-11), 1.69 (m, 2H, H-10), 2.65 (t, *J* = 7.8 Hz, 2H, H-9), 6.21 (s, 1H, H-3), 7.32 (m, 1H, H-6), 7.57 (m, 1H, H-7), 7.64 (d, *J* = 8.2 Hz, 1H, H-8), 8.35 (d, *J* = 8.2 Hz, 1H, H-5), 11.14 (s,

1H, =NH). ^{13}C-NMR ($CDCl_3$ 150.95 MHz) δ (ppm): 14.2 (C-15), 28.9 (C-10), 29.0 (C-11), 22.6, 28.9, 31.6 (C-12-14), 34.4 (C-9), 108.4 (C-3), 118.0 (C-8), 123.5 (C-6), 125.0 (C-4a), 125.5 (C-5), 131.7 (C-7), 140.4 (C-8a), 154.7 (C-2), 179.1 (C-4). ESI-MS: m/z = 243.95 $[M+H]^+$, calc. for $C_{16}H_{21}NO + H^+ = 244.36$; m/z = 284.95 $[M+MeCN+H]^+$, calc. for $C_{16}H_{21}NO + C_2H_3N + H^+ = 285.20$; m/z = 487.10 $[2M+H]^+$, calc. for $C_{32}H_{42}N_2O_2 + H^+ = 487.33$.

Synthesis of PQS from HHQ

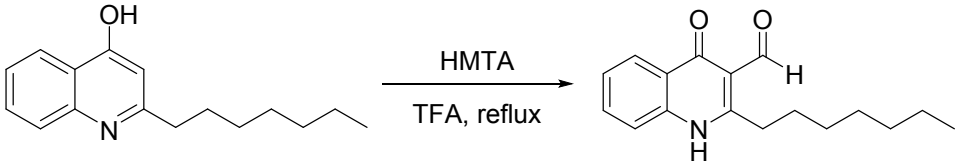


2-Heptyl-quinolin-4-ol · HCl (**2a)**



306 mg 2-heptyl-4-quinolone **1** (1.26 mmol) was dispersed in 15 mL 2 M HCl and 15 mL Et_2O was added. The mixture was treated with sonication for 5 min. The reaction was allowed to proceed for 3 h at 5 ˚C. The solid which formed was filtered and washed with diethyl ether to afford a white solid (m = 0.342 g, 97%). ^{1}H-NMR ($CDCl_3$ 400.13 MHz) δ (ppm): 0.78 (m, 3H, H-15), 1.16 (m, 4H, H-13-14), 1.25 (m, 2H, H-12), 1.36 (m, 2H, H-11), 1.87 (quint, *J* = 7.8 Hz, 2H, H-10), 3.17 (t, *J* = 7.9 Hz, 2H, H-9), 7.62 (m, 1H, H-6), 7.65 (s, 1H, H-3), 7.84 (m, 1H, H-7), 8.33 (d, *J* = 8.4 Hz, 1H, H-5), 8.54 (d, *J* = 8.6 Hz, 1H, H-8), 14.96 (s, 1H). ^{13}C-NMR ($CDCl_3$ 100.61 MHz) δ (ppm): 14.1 (CH_3), 22.7, 31.7 (C-13-14), 29.0, 29.4 (C-12-11), 29.9 (C-10), 34.4 (C-9), 105.6 (C-3), 119.7 (C-4a), 120.1 (C-8), 123.9 (C-5), 127.3 (C-6), 134.1 (C-7), 139.9 (C-8a), 161.2 (C-2), 169.9 (C-4). ESI-MS: m/z = 243.95 $[M+H]^+$, calc. for $C_{16}H_{21}NO + H^+ = 244.36$; m/z = 284.95 $[M+MeCN+H]^+$, calc. for $C_{16}H_{21}NO + C_2H_3N + H^+ = 285.20$.

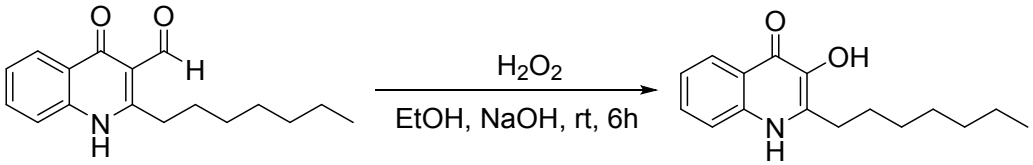
3-Formyl-2-heptyl-4-quinolone (**2b**)



0.3 g HHQ·HCl **2a** (1.07 mmol) and 0.351 g hexamethyltetramine (2.50 mmol) were stirred under argon for 15 minutes. Trifluoroacetic acid (3 mL) was added and the reaction vessel flushed with argon. The reaction was heated at reflux temperature for 16 h. Distilled water (10 ml) and methanol (10 mL) were added and the reaction mixture was allowed to reflux for 2.5 h. 2 M HCl (10 mL) was added and reflux maintained for 1 h. The reaction mixture was allowed to cool and the precipitate was filtered and washed with acetone (30 mL). The product was obtained as a white powder (m = 60 mg, 20.5%). *Rf* = 0.56 (hexane/ethyl acetate 1:1). ^{1}H-NMR (DMSO-d_6 400.13 MHz) δ (ppm): 0.86 (m, 3H, H-15), 1.20-1.34 (m, 6H, H-12-14), 1.39 (m, 2H, H-11), 1.61 (m, 2H, H-10), 3.06 (m, 2H, H-9), 7.43 (m, 1H, H-6), 7.61 (d, *J* = 8.2 Hz, 1H, H-8), 7.74 (m, 1H, H-7), 8.14 (d, *J* = 8.1 Hz, 1H, H-5), 10.39 (s, 1H, -COH), 12.16 (s, br, 1H, -NH-). ^{13}C-NMR (DMSO-d_6 100.61 MHz) δ (ppm): 13.9 (C-15), 22.0, 31.1 (C-13-14), 28.3, 28.8, 28.9 (C-10-12), 31.5 (C-9), 113.4 (C-3), 118.4 (C-8), 124.9 (2C, C-5-6), 126.1 (C-4a), 133.0 (C-7), 139.1 (C-8a), 160.0 (C-2), 178.0 (C-4), 190.8 (-CHO).

^{1}H-NMR (THF-d_8 400.13 MHz) δ (ppm): 0.89 (m, 3H, H-15), 1.28 (m, 4H, H-13-14), 1.37 (m, 2H, H-12), 1.49 (m, 2H, H-11), 1.67 (m, 2H, H-10), 3.07 (m, 2H, H-9), 7.31 (m, 1H, H-6), 7.42 (d, br, *J* = 8.3 Hz, 1H, H-8), 7.59 (m, 1H, H-7), 8.28 (dd, *J* = 8.0, 1.3 Hz, 1H, H-5), 10.51 (s, 1H, -COH), 10.97 (s, br, 1H, =NH). ^{13}C-NMR (THF-d_8 100.61 MHz) δ (ppm): 14.4 (C-15), 23.5, 32.7 (C-13-14), 30.0, 30.3, 30.6 (C-10-12), 33.1 (C-9), 115.1 (C-3), 118.6 (C-8), 125.2 (C-6), 126.7 (C-5), 128.2 (C-4a), 133.2 (C-7), 140.6 (C-8a), 160.3 (C-2), 179.0 (C-4), 191,6 (-CHO). ESI-MS: m/z = 271.95 $[M+H]^+$, calc. for $C_{17}H_{21}NO_2 + H^+$ = 272.17. m/z = 312.95 $[M+MeCN+H]^+$, calc. for $C_{17}H_{21}NO_2 + C_2H_3N + H^+$ = 312.95; m/z = 543.15 $[2M+H]^+$, calc. for $C_{34}H_{42}N_2O_4 + H^+$ = 543.32.

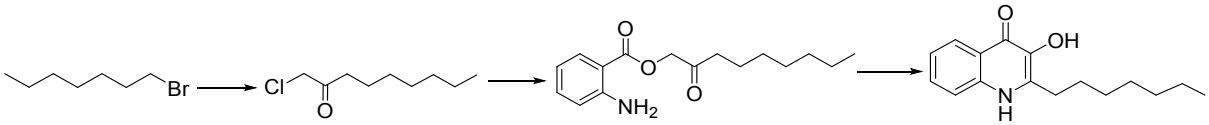
2-Heptyl-3-hydroxy-4-quinolone, PQS from 2b (**2**)



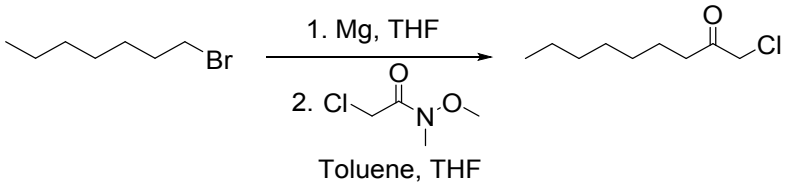
Aqueous hydrogen peroxide (1.05 M, 0.2 mL) was added to a solution of 3-formyl-2-heptylquinolone **2b** (50 mg, 0.18 mmol) in ethanol (1 mL) and aqueous sodium hydroxide (1.08 M, 0.2 mL) under argon. The mixture was stirred at room temperature for 6 h. The precipitate was removed by filtration and dried. NMR spectrum showed a mixture of PQS and **2b**. The reaction was repeated in order to consume all the remaining aldehyde. The final product was obtained as a white powder and was crystallized from ethyl acetate to give PQS of 93.5% purity

(m = 12.3 mg, 24%). ^{1}H-NMR (DMSO-d_6 400.13 MHz) δ (ppm): 0.84 (m, 3H, H-15), 1.20-1.37 (m, 8H, H-11-14), 1.67 (m, 2H, H-10), 2.72 (m, 2H, H-9), 7.21 (m, 1H, H-6), 7.52 (m, 2H, H-7-8), 8.09 (d, *J* = 8.2 Hz, 1H, H-5), 11.40 (s, 1H, =NH). ^{13}C-NMR (DMSO-d_6 100.61 MHz) δ (ppm): 13.9 (C-15), 22.0, 27.8, 28.1, 28.4, 28.7, 31.2 (C-9-14), 117.7 (C-8), 121.5 (C-6), 122.1 (C-4a), 124.5 (C-5), 129.9 (C-7), 135.5 (C-3), 137.4 (C-8a), 137.8 (C-2), 168.9 (C-4). MS (ESI): m/z = 260.0 $[M+H]^+$,calc. for $C_{16}H_{21}NO_2 + H^+ = 260.2$; m/z = 519.2 $[2M+H]^+$, calc. for $C_{32}H_{42}N_2O_4 + H^+ = 519.3$.

Synthesis of PQS from 1-bromoheptane and anthranilic acid (2)

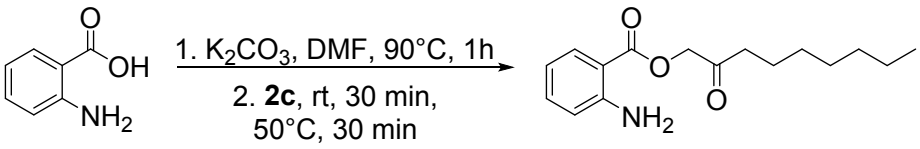


1-Chloro-2-nonanone (**2c**)



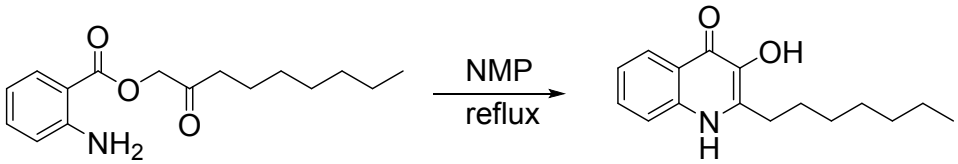
0.134 g Magnesium (5.54 mmol) in THF (5.5 mL) was stirred under an argon atmosphere. 0.87 mL 1-Bromoheptane (4.85 mmol) was added and the reaction vessel was flushed with argon. The reaction was stirred for 1 h at room temperature in which the Grignard reagent formed under heating. Afterwards *N*-methoxy-*N*-methylchloroacetamide (0.500 g, 3.69 mmol) in toluene (5 mL) and THF (10 mL) were added to a solution at 0 ˚C. The mixture was stirred at room temperature for 1.5 h. To this solution was added cold 2 M HCl (5 mL) and the layers were separated. The aqueous phase was extracted with n-hexane (3x50 mL). The combined organic layers were washed with brine solution (3x50 mL), dried over anhydrous $MgSO_4$ and filtered. The solvent was evaporated and the product was purified by column chromatography on silica gel using hexane/ethyl acetate (9:1). The product was obtained as a yellow oil (m = 0.480 g, 74%). *Rf* = 0.65 (hexane/ethyl acetate 9:1). ^{1}H-NMR ($CDCl_3$ 400.13 MHz) δ (ppm): 0.87 (m, 3H, -CH_3), 1.23-1.32 (m, 8H, (-C**H**$_2$)$_4$-CH_3), 1.61 (m, 2H, -C**H**$_2$-CH_2-CO-), 2.57 (m, 2H, -CH_2-C**H**$_2$-CO-), 4.06 (s, 2H, -CH_2-Cl). ^{13}C-NMR ($CDCl_3$ 100.61 MHz) δ (ppm): 14.1 (-CH_3), 22.7, 23.7, 29.1, 29.2, 31.7, 39.8 (6 x CH_2), 48.3 (-CH_2-Cl), 202.9 (-CO-).

2-Oxononyl-2-aminobenzoate (**2d**)



3.26 g (23.8 mmol) of anthranilic acid were dissolved in 44 mL dry DMF under argon. Potassium carbonate powder (2.56 g, 18.5 mmol) was added. The reaction was stirred at 90 °C for 1 h. During the reaction time, the solution became brown and finally turbid and white. At room temperature, 3.5 g (19.8 mmol) 1-chlorononan-2-one **2c** was added to the reaction and the mixture was stirred for 30 minutes at 25 °C. Afterwards the temperature was raised to 50 °C and the reaction was stirred for further 30 minutes at this temperature before it was allowed to cool to room temperature. The mixture was poured on ice and the white precipitate was filtered and washed with water. The product was obtained as a white solid (5.15 g, 94%). ^{1}H-NMR ($CDCl_3$ 400.13 MHz) δ (ppm): 0.88 (t, *J* = 7.0 Hz, 3H, -CH_3), 1.21 – 1.35 (m, 8H, -$(CH_2)_4$-), 1.63 (m, 2H, -CO-CH_2-C**H**$_2$-), 2.50 (t, *J* = 7.4 Hz, 2H, -CO-C**H**$_2$-CH_2-), 4.83 (s, 2H, -COO-CH_2-CO-), 5.68 (s, br, 2H, -NH_2), 6.65 – 6.67 (m, 2H, H-5´, H-3´), 7.29 (m, 1H, H-4´), 7.93 (dd, *J* = 8.5, 1.7 Hz, 1H, H-6`). ^{13}C-NMR ($CDCl_3$ 100.61 MHz) δ (ppm): 14.1 (-CH_3), 22.7, 23.4, 29.1, 29.2, 31.7 (-(**C**H_2)$_5$-CH_3), 39.0 (-CO-**C**H_2-), 68.1 (-COO-**C**H_2-CO-), 110.0 (C-1´), 116.7 (C-5´, C-3´), 131.5 (C-6´), 134.7 (C-4´), 150.9 (C-2´), 167.3 (-COO-), 204.9 (-CO-). MS (ESI): m/z = 277.9 $[M+H]^+$,calc. for $C_{16}H_{23}NO_3 + H^+ = 278.2$; m/z = 555.1 $[2M+H]^+$, calc. for $C_{32}H_{46}N_2O_6 + H^+ = 555.3$.

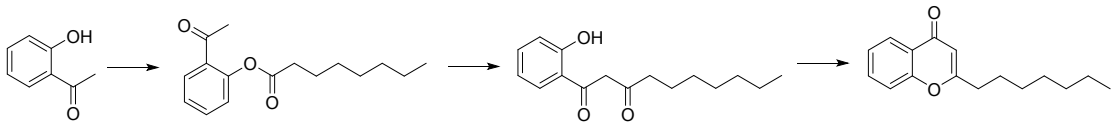
Synthesis of 2-heptyl-3-hydroxy-4-quinolone, PQS from 8 (**2**)



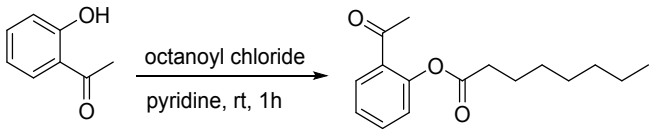
1.91 g (6.9 mmol) of **2d** was dissolved in 19.2 mL NMP and heated up to reflux temperature and kept at this temperature for 2 h. After cooling to room temperature the mixture was poured on ice and the brown precipitate was filtered. The solid was recrystallized in ethanol and the product was obtained as slightly brown crystals (1.09 g, 61%). ^{1}H-NMR (DMSO-d_6 400.13 MHz) δ (ppm): 0.84 (t, *J* = 6.9 Hz, 3H, H-15), 1.20 – 1.37 (m, 8H, H-11-14), 1.67 (m, 2H, H-10), 2.72 (t, *J* = 7.9 Hz, 2H, H-9), 7.21 (ddd, *J* = 2.6, 5.4, 8.1 Hz, 1H, H-6), 7.52 (m, 2H, H-7, H-8), 8.09 (d, *J* = 8.1 Hz, 1H, H-5), 11.40 (s, 1H, NH-). ^{13}C-NMR (DMSO-d_6 100.61 MHz) δ (ppm): 13.9 (C-15), 22.0 (C-14), 27.8 (C-10), 28.1 (C-9), 28.4 (C-11), 28.7 (C-12), 31.2 (C-

13), 117.7 (C-8), 121.5 (C-6), 122.1 (C-4a), 124.5 (C-5), 139.9 (C-7), 135.5 (C-2), 137.4 (C-8a), 137.8 (C-3), 168.9 (C-4). MS (ESI): m/z = 260.0 $[M+H]^+$,calc. for $C_{16}H_{21}NO_2 + H^+ = 260.2$; m/z = 519.2 $[2M+H]^+$, calc. for $C_{32}H_{42}N_2O_4 + H^+ = 519.3$.

Synthesis of 1-O-HHQ (4)

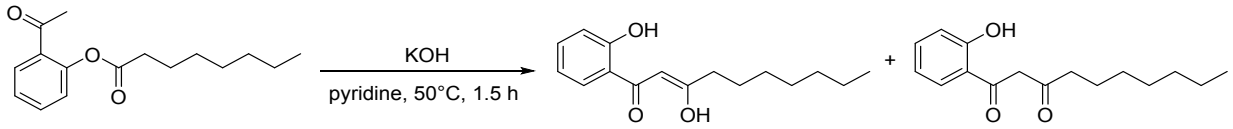


Synthesis of 2-acetylphenyl octanoate (**3a**)



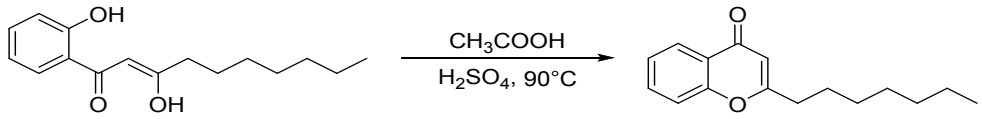
2-Hydroxyacetophenone (5 mL, 36.7 mmol) and octanoyl chloride (7.16 mL, 35.2 mmol) in dry pyridine (7.34 mL) were stirred for 1 h at room temperature. The mixture was then poured into a separatory funnel containing crushed ice (70 g) and 1 M HCl (180 mL). Ether was added and the organic layer was collected. The water layer was washed twice with ether and the combined organic layers were dried over $MgSO_4$ and evaporated. The product was purified by column chromatography on silica gel using hexane/ethyl acetate 9:1. The product was obtained as a colorless oil (m = 9 g, 93%). *Rf* = 0.33 (hexane/ethyl acetate 9:1). ^{1}H-NMR ($CDCl_3$ 400.13 MHz) δ (ppm): 0.90 (m, 3H, -$(CH_2)_4$-C**H**$_3$), 1.27-1.45 (m, 8H, -$(CH_2)_4$-CH_3), 1.77 (m, 2H, -COO-CH_2-C**H**$_2$-), 2.55 (s, 3H, -CO-CH_3), 2.61 (t, *J* = 7.6 Hz, 2H, -COO-C**H**$_2$-CH_2-), 7.10 (d, *J* = 8.1 Hz, 1H, Ar-CH), 7.31 (t, *J* = 7.7 Hz, 1H, Ar-CH), 7.52 (ddd, *J* = 7.8, 7.7, 1.5 Hz, 1H, Ar-CH), 7.79 (dd, *J* = 7.8, 1.5, 1H, Ar-CH). ^{13}C-NMR ($CDCl_3$ 100.61 MHz) δ (ppm): 14.2 (-$(CH_2)_4$-**C**H_3), 22.7, 29.1, 29.2, 29.6, 31.8, (-(C**H**$_2$)$_4$-**C**H_3 and -CO-**C**H_3), 24.7 (-COO-CH_2-**C**H_2-), 34.5 (-COO-**C**H_2-CH_2-), 124.0, 126.0, 130.3, 133.4 (4 x Ar-CH), 131.2 (=**C**-CO-CH_3), 149.3 (=**C**-O-) 172.4 (-COO), 197.8 (-**C**O-CH_3). ESI-MS: m/z = 263.00 $[M+H]^+$, calc. for $C_{16}H_{22}O_3 + H^+ = 263.16$.

Synthesis of 3-hydroxy-1-(2-hydroxyphenyl)-dec-2-en-1-one (**3b**)



Ester **3a** (5 g, 19.1 mmol) was dissolved in dry pyridine (20 mL) and the solution was heated at 50 ˚C. KOH (1.71 g, 30.5 mmol) was quickly powdered and added in one portion to the solution. The mixture was stirred at 50 ˚C for 1.5 h and allowed to cool down to room temperature. A 10% solution of acetic acid (30 mL) was added and the mixture was extracted with ether. The combined organic phases were dried over $MgSO_4$, filtered and the solvent evaporated. The product was purified by column chromatography on silica gel using hexane/ethyl acetate 9:1. The red solid product was obtained as a mixture of isomers (m = 3.295 g, 66%). *Rf* = 0.45 (hexane/ethyl acetate 9:1). The main product was identified as 3-hydroxy-1-(2-hydroxyphenyl)-dec-2-en-1-one. ^{1}H-NMR ($CDCl_3$ 400.13 MHz) δ (ppm): 0.89 (m, 3H, -CH_3), 1.20-1.44 (m, 8H, -(C**H**$_2$)$_4$-CH_3), 1.68 (m, 2H, =C(OH)-CH_2-C**H**$_2$-), 2.36 (m, 2H, =C(OH)-C**H**$_2$-CH_2-), 6.16 (s, 1H, -CO-C**H**=C(OH)-), 6.87 (m, 1H, Ar-CH), 6.97 (m, 1H, Ar-CH), 7.43 (m, 1H, Ar-CH), 7.64 (m, 1H, Ar-CH), 12.07 (s, 1H, =C-OH), 15.02 (s, 1H, -CO-CH=C(O**H**)-). ^{13}C-NMR ($CDCl_3$ 100.61 MHz) δ (ppm): 14.2 (-CH_3), 22.7, 29.1, 29.3, 31.8 (-(C**H**$_2$)$_4$-CH_3), 26.6 (=C(OH)-CH_2-C**H**$_2$-), 36.7 (=C(OH)-C**H**$_2$-CH_2-), 94.8 (-CO-**C**H=COH-), 118.7 (=**C**-CO-), 118.8, 119.1, 128.6, 135.7 (4 x Ar-CH), 162.6 (=C-OH), 186.8 (-CO-CH=**C**OH-), 195.6 (-**C**O-CH=C(OH)-). ESI-MS: m/z = 285.90 $[M+Na]^+$, calc. for $C_{16}H_{22}O_3$ + Na^+ = 285.15.

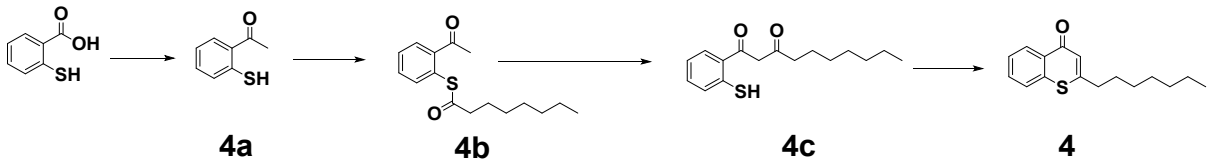
Synthesis of 2-heptyl-chromen-4-one, 1-O-HHQ (**3**)



1.00 g of **3b** (1g, 3.8 mmol) was dissolved in glacial acetic acid (5 mL). To the solution was added concentrated H_2SO_4 (0.2 mL) and the mixture was heated at 90˚C for 1.5 h. The hot solution was then poured into 25 g crushed ice. Stirring was continued until complete melting of the ice. The mixture was extracted four times with ether and the combined organic layers were dried over $MgSO_4$, filtered and the solvent evaporated. The product was purified by column chromatography on silica gel using hexane/ethyl acetate 9:1. The product was obtained as a yellow crystalline solid (m = 0.699 g, 75%). *Rf* = 0.22 (hexane/ethyl acetate 9:1). ^{1}H-NMR ($CDCl_3$ 400.13 MHz) δ (ppm): 0.88 (m, 3H, H-15), 1.22-1.45 (m, 8H, H-11-14), 1.74 (m, 2H, H-10), 2.61 (t, *J* = 7.6 Hz, 2H, H-9), 6.18 (s, 1H, H-3), 7.37 (m, 1H, H-6), 7.42 (d, *J* = 8.4 Hz, 1H, H-8), 7.63 (m, 1H, H-7), 8.18 (dd, *J* = 8.0, 1.6 Hz, 1H, H-5). ^{13}C-NMR ($CDCl_3$ 100.61

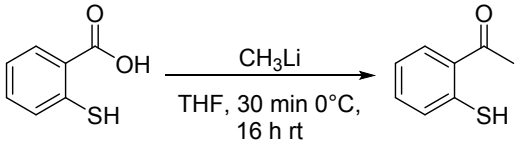
MHz) δ (ppm): 14.2 (C-15), 22.7, 29.06, 29.09, 31.8, (C-11-14), 26.9 (C-10), 34.5 (C-9), 109.9 (C-3), 118.0 (C-8), 123.9 (C-4a), 125.0 (C-6), 125.8 (C-5), 133.5 (C-7), 156.7 (C-8a), 170.0 (C-2), 178.6 (C-4). ESI-MS: m/z = 244.90 $[M+H]^+$, calc. for $C_{16}H_{20}O_2 + H^+ = 245.15$; m/z = 489.15 $[2M+H]^+$, calc. for $C_{32}H_{40}O_4 + H^+ = 489.29$.

Synthesis of 1-S-HHQ (**3**)



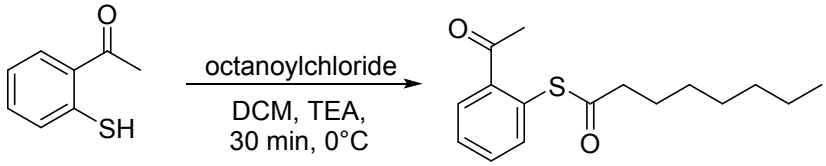
4a **4b** **4c** **4**

Synthesis of 2'-thio-acetophenone (**4a**)



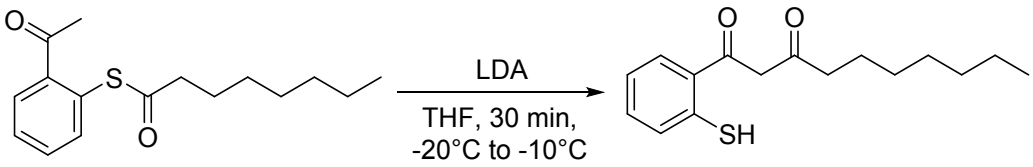
Under nitrogen atmosphere 1.5 g of thiosalicylic acid (9.7 mmol) was dissolved in 48 mL of dry THF and 26 mL (38.9 mmol) of a 5% lithium methyl solution in diethyl ether were added at 0 °C. After 30 min at 0 °C the solution was allowed to warm to room temperature and stirred for 16 h overnight. The reaction was quenched with water and the clear mixture was brought to pH 7 with saturated NH_4Cl solution and extracted three times with ethyl acetate. The organic phases were combined and washed with 5% $NaHCO_3$ solution and brine, dried over $MgSO_4$, evaporated and the product was purified over silica gel chromatography in n-hexane/ethyl acetate 5:1. The product was obtained as an orange liquid (1.15 g, 78%). *Rf* = 0.57 (n-hexane/ethyl acetate 5:1). ^{1}H-NMR ($CDCl_3$ 399.79 MHz) δ (ppm): 2.62 (s, 3H, H-2), 4.49 (s, 1H, -SH), 7.21 (m, 1H, H-5'), 7.31 (dd, *J* = 1.1, 3.7 Hz, 2H, H-3', H-4'), 7.88 (d, *J* = 7.9 Hz, 1H, H-6'). ^{13}C-NMR ($CDCl_3$ 100.53 MHz) δ (ppm): 27.2 (C-2), 124.7 (C-5'), 131.7 (C-6'), 131.8, 132.3 (C-3' or C-4'), 132.7 (C-1'), 137.5 (C-2'), 198.8 (C-1). ESI-MS: m/z = 152.8 $[M+H]^+$, calc. for $C_8H_8OS + H^+ = 153.0$, m/z = 326.8 $[M+Na]^+$, calc. for $C_{16}H_{16}O_2S_2 + Na^+ = 327.0$.

Synthesis of 2'-acetylphenyl-octanoic-acid-thioester (**4b**)



Under argon atmosphere 0.455 g (3 mmol) of **4a** were dissolved in 12 mL dry DCM and cooled to 0 °C. Triethylamine (0.42 mL, 3 mmol) and octanoylchloride (0.51 mL, 2.9 mmol) was added. The clear solution became white and the reaction was stirred for 0.5 h at 0 °C. Then the mixture was quenched with saturated NH_4Cl solution and extracted three times with DCM. The organic phases were combined, dried over $MgSO_4$ and evaporated. The product was purified by column chromatography on silica gel using n-hexane/ethyl acetate 5:1 and obtained as a yellow liquid (0.766 g, 92%). *Rf* = 0.69 (n-hexane/ethyl acetate 5:1). ^{1}H-NMR ($CDCl_3$ 399.79 MHz) δ (ppm): 0.88 (t, *J* = 7.0 Hz, 3H, H-8), 1.20 – 1.40 (m, 8H, H-4-7), 1.70 (quint., *J* = 7.5 Hz, 2H, H-3), 2.57 (s, 3H, COC**H**$_3$), 2.64 (t, *J* = 7.5 Hz, 2H, H-2), 7.43 – 7.55 (m, 3H, H-4', H-5', H-6'), 7.65 (m, 1H, H-3'). ^{13}C-NMR ($CDCl_3$ 100.53 MHz) δ (ppm): 14.2 (C-8), 22.7, 29.0, 29.0, 31.7 (C-4, C-5, C-6, C-7), 25.7 (C-3), 29.5 (CO**C**H3), 43.9 (C-2), 126.2 (C-1'), 128.5 (C-3'), 129.4 (C-6'), 131.2 (C-5'), 136.7 (C-4'), 143.2 (C-2'), 197.0 (C-1), 201.0 (**C**OCH3). ESI-MS: m/z = 278.9 $[M+H]^+$, calc. for $C_{16}H_{22}O_2S + H^+ = 279.1$.

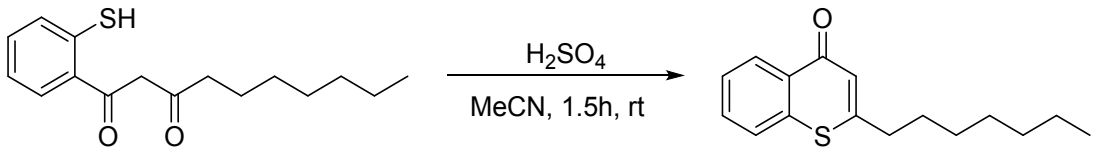
Synthesis of 1-(2-mercaptophenyl)decane-1,3-dione (**4c**)



Under argon atmosphere 0.47 g (1.69 mmol) of **4b** were dissolved in 7 mL dry THF and cooled to – 20 °C. A solution of 1.8 M lithium diisopropylamide in THF (1.9 mL, 3.38 mmol) was added and stirred for 0.5 h at -20°C to -10°C. The dark red colored mixture was quenched with 1 M HCl. Then the THF was evaporated and afterwards the aqueous residue was extracted three times with DCM. The combined organic phases were washed three times in brine and dried over $MgSO_4$. The product was purified by column chromatography on silica gel using n-hexane/ethyl acetate 5:1. The product was received as a mixture of isomers (0.167 g, 36%). *Rf* = 0.56 (n-hexane/ethyl acetate 5:1). ^{1}H-NMR ($CDCl_3$ 399.79 MHz) δ (ppm): 0.90 (t, *J* = 6.8 Hz, 3H, H-16), 1.24 – 1.40 (m, 8H, H-12-15), 1.56 (m, 2H, H-11), 1.96 (m, 2H, H-10), 3.11 (m, 2H, H-8), 7.21 (m, 2H, H-3, H-5), 7.42 (ddd, *J* = 1.6 5.7, 7.3 Hz, 1H, H-4), 8.12 (dd, *J* = 1.1, 8.0 Hz, 1H, H-6). ^{13}C-NMR ($CDCl_3$ 100.53 MHz) δ (ppm): 14.2 (C-16), 22.7, 31.8 (C-14,

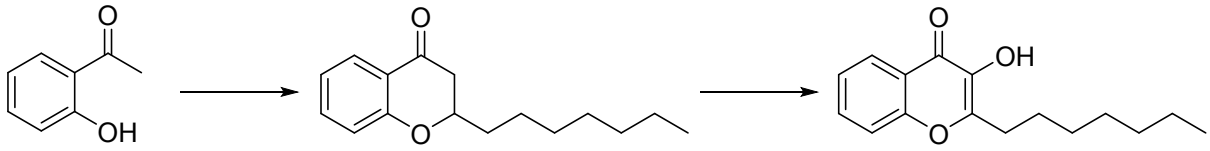
C-15), 23.7 (C-11), 29.2 (C-12), 29.6 (C-13), 43.3 (C-10), 52.0 (C-8), 85.0 (C-9), 125.2 (C-5), 127.9 (C-3), 128.8 (C-6), 130.0 (C-1), 133.8 (C-4), 139.3 (C-2), 194.4 (C-7).

Synthesis of 2-heptyl-4*H*-thiochromen-4-one, 1-S-HHQ (**4**)

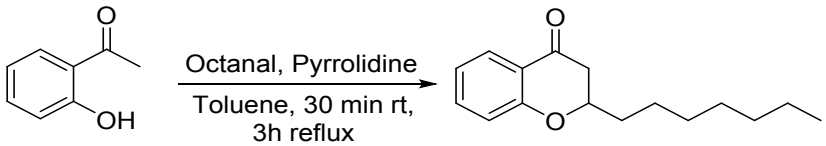


0.157 g (0.56 mmol) of **4c** were dissolved in 4.7 mL acetonitrile and 30 µL H_2SO_4 conc. (0.56 mmol) were added. The brown mixture was stirred for 1.5 h at room temperature before it was poured into saturated $NaHCO_3$ solution and extracted three times with DCM. The combined organic phases were washed with brine and dried over $MgSO_4$. The reaction mixture was evaporated and without further purification the product was obtained as a brown oil (0.139 g, 94%). *Rf* = 0.51 (n-hexane/ ethyl acetate 5:1). ^{1}H-NMR ($CDCl_3$ 400.13 MHz) δ (ppm): 0.87 (dd, *J* = 5.9, 6.9 Hz, 3H, H-15), 1.19 - 1.43 (m, 8H, H-11-14), 1.73 (quint., *J* = 7.3 Hz, 2H, H-10), 2.66 (t, *J* = 7.6 Hz, 2H, H-9), 6.86 (s, 1H, H-3), 7.49 (m, 1H, H-6), 7.56 (m, 2H, H-7, H-8), 8.49 (d, *J* = 8.1 Hz, 1H, H-5). ^{13}C-NMR ($CDCl_3$ 100.61 MHz) δ (ppm): 14.2 (C-15), 28.9 (C-11), 29.9 (C-10), 22.7, 29.9, 31.8 (C-12-14), 37.6 (C-9), 124.2 (C-3), 126.3 (C-8), 127.5 (C-6), 128.7 (C-5), 131.1 (C-4a), 131.4 (C-7), 137.9 (C-8a), 156.7 (C-2), 180.9 (C-4). ESI-MS: m/z = 260.9 $[M+H]^+$, calc. for $C_{16}H_{20}OS + H^+ = 261.1$; m/z = 521.1 $[2M+H]^+$, calc. for $C_{32}H_{40}O_2S_2 + H^+ = 521.3$.

Synthesis of 2-heptyl-3-hydroxy-Chromen-4-one, 1-O-PQS (5)



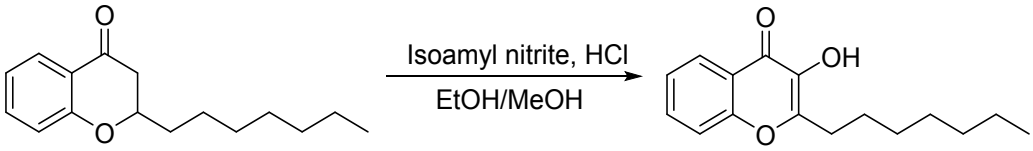
Synthesis of 2-heptylchroman-4-one (**5a**)



Octanal (0.26 mL, 1.66 mmol) and 0.067 mL (0.82 mmol) pyrrolidine were dissolved in 5 mL dry toluene under nitrogen atmosphere. For additional dryness molecular sieve was used. After 10 min 2'-hydroxyacetophenone (0.226 g, 1.66 mmol) was added and the white solution was

stirred for 0.5 h at room temperature. The solution became clear and was heated to 110 °C and refluxed for 3 h. The mixture was allowed to cool to room temperature and diluted with diethyl ether. Afterwards it was washed with a solution of saturated $NaHCO_3$, a solution of saturated NH_4Cl, water and brine. The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The product was purified by column chromatography on silica gel using n-hexane/DCM 3:1. The product was obtained as a colorless oil (0.109 g, 27%). *Rf* = 0.65 (DCM/n-hexane 1:1). ^{1}H-NMR ($CDCl_3$ 399.79 MHz) δ (ppm): 0.89 (t, *J* = 7.1 Hz, 3H, H-15), 1.24 – 1.39 (m, 8H, H-11-14), 1.46 (m, 1H, H-10), 1.53 (m, 1H, H-10), 1.70 (m, 1H, H-9), 1.88 (m, 1H, H-9), 2.68 (d, *J* = 7.7 Hz, 2H, H-3), 4.44 (m, 1H, H-2), 6.96 – 6.99 (m, 2H, H-6, H-8), 7.46 (ddd, *J* = 1.9, 7.2, 8.4 Hz, 1H, H-7), 7.87 (dd, *J* = 1.7, 7.8 Hz, 1H, H-5). ^{13}C-NMR ($CDCl_3$ 100.53 MHz) δ (ppm): 14.2 (C-15), 22.8 (C-14), 25.0 (C-10), 29.3 (C-11), 29.5 (C-12), 31.9 (C-13), 35.1 (C-9), 43.1 (C-3), 78.1 (C-2), 118.1 (C-8), 121.2 (C-4a), 121.3 (C-6), 127.1 (C-5), 136.1 (C-7), 161.9 (C-8a), 192.8 (C-4). ESI-MS: m/z = 269.1 $[M+H]^+$, calc. for $C_{16}H_{22}O_2 + H^+ = 269.2$.

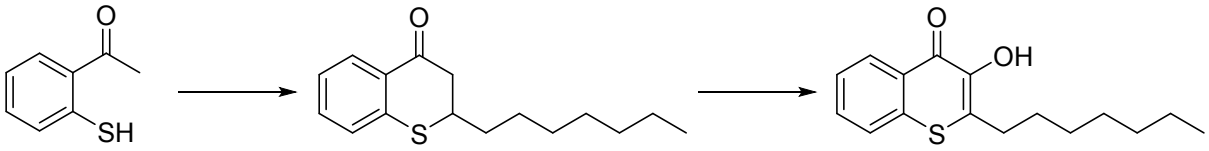
Synthesis of 2-heptyl-3-hydroxy-chromen-4-one, 1-O-PQS (**5**)



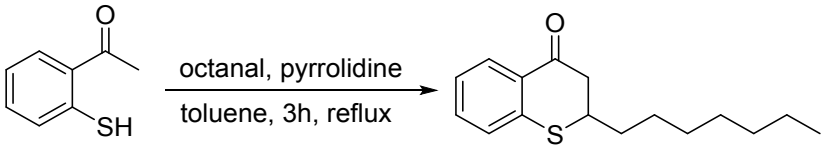
Under nitrogen atmosphere 95 mg (0.386 mmol) of **5a** were dissolved in a mixture of ethanol/methanol 5:4 and 0.15 mL (1.10 mmol) isoamyl nitrite were added carefully. The yellow solution was cooled to 0 °C and 0.23 mL of concentrated HCl was dropped slowly to the reaction mixture. After 10 minutes on ice the solution became orange and the mixture was stirred at room temperature for 1 h. Afterwards the temperature was raised to 80 °C and the reaction was stirred for further 45 minutes. The mixture was allowed to cool to room temperature and poured into H_2O. Then it was extracted three times with diethyl ether and the unified organic phases were washed with brine and dried over $MgSO_4$. The product was separated from the majority of byproducts by column chromatography on silica gel using n-hexane/ethyl acetate 5:1 and purified by column chromatography on silica gel using DCM/MeOH 20:1. The product was obtained as grey solid (m = 15 mg, 15%). *Rf* = 0.71 (DCM). Unreacted starting material was recovered during the first chromatography in n-hexane/ethyl acetate 5:1 (22 mg, 23%). ^{1}H-NMR ($CDCl_3$ 400.13 MHz) δ (ppm): 0.88 (t, *J* = 7.0 Hz, 3H, H-15), 1.22 – 1.47 (m, 8H, H-11-14), 1.77 (quint., *J* = 7.5 Hz, 2H, H-10), 2.84 (t, *J* = 7.7 Hz, 2H, H-9), 6.23 (s, 1H, -OH), 7.37 (t, *J* = 7.3 Hz, 1H, H-6), 7.47 (d, *J* = 8.5 Hz, 1H, H-8), 7.64 (t, *J*

= 7.7 Hz, 1H, H-7), 8.22 (d, *J* = 7.8 Hz, 1H, H-5). ^{13}C-NMR ($CDCl_3$ 100.61 MHz) δ (ppm): 14.2 (C-15), 22.7 (C-14), 26.8 (C-10), 29.1 (C-9), 29.1 (C-12), 29.3 (C-11), 31.8 (C-13), 118.3 (C-8), 121.6 (C-4a), 124.4 (C-6), 125.6 (C-5), 133.1 (C-7), 138.3 (C-3), 152.6 (C-2), 155.8 (C-8a), 172.6 (C-4). ESI-MS: m/z = 260.9 $[M+H]^+$, calc. for $C_{16}H_{20}O_3 + H^+ = 261.2$.

Synthesis of 2-heptyl-3-hydroxy-4H-thiochromen-4-one, 1-S-PQS (6)



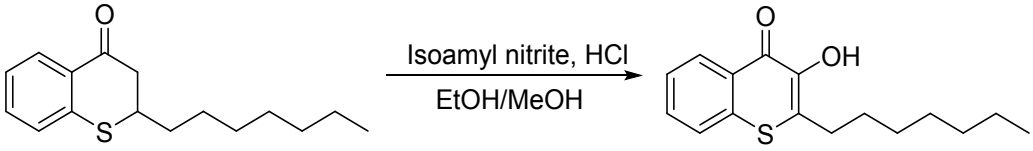
Synthesis of 2-heptylthiochroman-4-one (**6a**)



Under nitrogen atmosphere octanal (0.84 g, 6.57 mmol) was dissolved in 33.33 mL dry toluene and pyrrolidine (0.27 mL, 3.3 mmol) was added. The solution becomes turbid and after 10 minutes 1.00 g (6.57 mmol) of 2-thioacetophenone were added carefully. After it was stirred for 30 min at room temperature the solution became clear and yellow and the temperature was raised to 110 °C. The reaction was refluxed for 3 h and after it was cooled to room temperature the mixture was diluted with diethyl ether, washed with saturated $NaHCO_3$ solution, water and brine. The organic solution was dried over $MgSO_4$, evaporated and the pure product was obtained over silica gel chromatography in n-hexane/ethyl acetate 9:1 as a yellow oil (0.7 g, 41%). *Rf* = 0.51 (n-hexane/ethyl acetate 12:1). ^{1}H-NMR ($CDCl_3$ 399.79 MHz) δ (ppm): 0.88 (t, *J* = 7.1 Hz, 3H, H-15), 1.20 – 1.36 (m, 8H, H-11-14), 1.47 (m, 2H, H-10), 1.72 (m, 2H, H-9), 2.79 (dd, *J* = 11.2 Hz, 16.3 Hz, 1H, H-3), 3.04 (dd, *J* = 3.1, 16.3 Hz, 1H, H-3), 3.50 (m, 1H, H-2), 7.16 (ddd, *J* = 1.2, 7.3, 8.1 Hz, 1H, H-6), 7.27 (dd, *J* = 1.0, 8.0 Hz, 1H, H-8), 7.38 (ddd, *J* = 1.6, 7.3, 8.0 Hz, 1H, H-7), 8.08 (dd, *J* = 1.5, 8.0 Hz, 1H, H-5). ^{13}C-NMR ($CDCl_3$ 100.53 MHz) δ (ppm): 14.2 (C-15), 22.8 (C-14), 26.8 (C-10), 29.2 (C-12), 29.3 (C-11), 31.9 (C-13), 34.7 (C-9), 41.8 (C-2), 46.4 (C-3), 125.0 (C-6), 127.8 (C-8), 129.0 (C-5), 130.8 (C-4a), 133.6

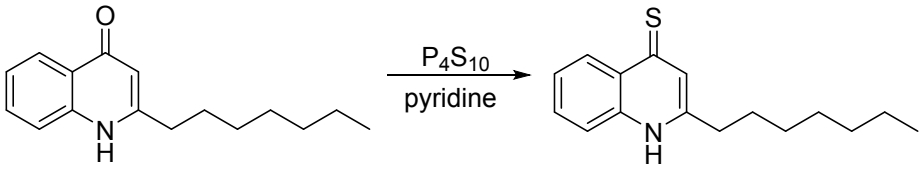
(C-7), 141.9 (C-8a), 194.9 (C-4). EI-MS: m/z = 262 [M], calc. for $C_{16}H_{22}OS^{\bullet}$ = 262; m/z = 136 [M - C_9H_{18}], calc. for $C_7H_4OS^{\bullet}$ = 136; m/z = 163 [M - C_7H_{15}], calc. for $C_9H_7OS^{\bullet}$ = 163.

Synthesis of 2-heptyl-3-hydroxy-4*H*-thiochromen-4-one, 1-S-PQS (**6**)



Under nitrogen atmosphere **6a** (79 mg, 0.3 mmol) was dissolved in a mixture of ethanol (1.66 mL) and methanol (1.33 mL). Isoamyl nitrite (117 µL, 0.87 mmol) was added to the reaction solution dropwise and the mixture was cooled to 0 °C. At this temperature HCl conc. (0.18 mL) was added and it was stirred for 10 minutes. The orange reaction solution was allowed to warm up to room temperature and after 1 h at 25 °C the mixture was refluxed for 3 h at 80 °C. The reaction was brought to room temperature again and poured into water. Then it was extracted three times with diethyl ether and the combined organic phases were washed with brine and dried over $MgSO_4$. The solution was evaporated and the product was purified using silica gel chromatography in n-hexane/ethyl acetate 4:1. The product was obtained as a brown solid (0.032 g, 38%). *Rf* = 0.67 (n-hexane/ethyl acetate 4:1). ^{1}H-NMR ($CDCl_3$ 600.17 MHz) δ (ppm): 0.88 (t, *J* = 7.1 Hz, 3H, H-15), 1.25-1.32 (m, 6H, H-12-14), 1.35-1.42 (m, 2H, H-11), 1.75 (quint., *J* = 7.6 Hz, 2H, H-10), 2.83 (t, *J* = 7.8 Hz, 2H, H-9), 7.51 (t, *J* = 7.3 Hz, 1H, H-6), 7.58 (t, *J* = 7.8 Hz, 1H, H-7), 7.63 (d, *J* = 8.1 Hz, 1H, H-8), 8.53 (d, *J* = 8.0 Hz, 1H, H-5). ^{13}C-NMR ($CDCl_3$ 150.91 MHz) δ (ppm): 14.2 (C-15), 22.8 (C-14), 29.1 (C-10), 29.1, 29.3 (C-11, C-12), 31.2 (C-9), 31.8 (C-13), 126.1 (C-8), 126.7 (C-6), 128.9 (C-5), 129.0 (C-2), 129.2 (C-4a), 130.8 (C-7), 137.9 (C-8a), 144.1 (C-3), 174.2 (C-4). ESI-MS: m/z = 276.9 $[M+H]^+$, calc. for $C_{16}H_{20}O_2S + H^+$ = 277.1.

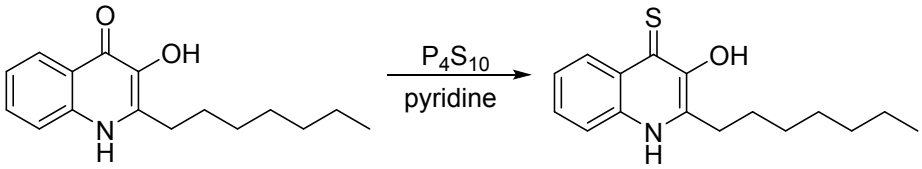
Synthesis of 2-heptylquinoline-4(1*H*)-thione, 4-S-HHQ (**7**)



0.25 g (1.03 mmol) of 2-heptylquinolin-4(1*H*)-one (HHQ) were dissolved in 12.5 mL pyridine, P_4S_{10} (0.342 g, 1.54 mmol) was added and the reaction was refluxed for 4 h. The amber mixture was allowed to cool to room temperature and poured into ice cold water. With a 6 M HCl solution the pH was brought to 7 before it was extracted two times with 60 mL DCM. The

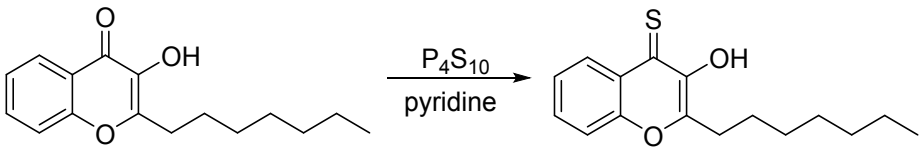
combined organic phases were dried over $MgSO_4$ and evaporated. The brown oil was purified using silica gel chromatography in DCM/MeOH (20:1) and the pure product was obtained as an orange-red solid (0.207 g, 78%). *Rf* = 0.5 (DCM/MeOH 20:1). ^{1}H-NMR (DMSO-d_6 399.79 MHz) δ (ppm): 0.84 (t, *J* = 6.9 Hz, 3H, H-15), 1.17 – 1.34 (m, 8H, H-11-14), 1.68 (quint., *J* = 7.5 Hz, 2H, H-10), 2.65 (t, *J* = 7.7 Hz, 2H, H-9), 7.24 (s, 1H, H-3), 7.42 (ddd, *J* = 1.4, 6.6 Hz, *J* = 8.2 Hz, 1H, H-6), 7.66-7.70 (m, 2H, H-7, H-8), 8.64 (dd, *J* = 0.9, 8.2 Hz, 1H, H-5), 12.61 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6 100.53 MHz) δ (ppm): 13.9 (C-15), 22.1 (C-14), 22.1, 28.4, 31.1 (C-11-13), 28.5 (C-10), 32.6 (C-9), 119.0 (C-8), 123.7 (C-3), 124.9 (C-6), 128.5 (C-5), 131.0 (C-4a), 132.0 (C-7), 136.1 (C-8a), 148.2 (C-2), 191.6 (C-4). ESI-MS: m/z = 259.9 $[M+H]^+$, calc. for $C_{16}H_{21}NS+ H^+ = 260.2$.

Synthesis of 2-heptyl-3-hydroxyquinoline-4(1*H*)-thione, 4-S-PQS (**8**)



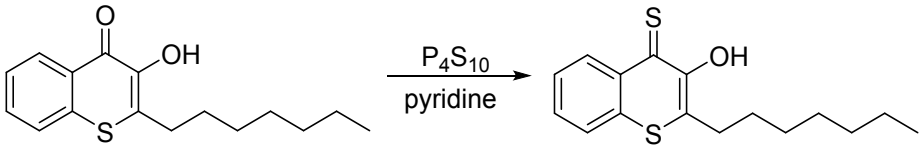
0.16 g (0.617 mmol) of 2-heptyl-3-hydroxyquinoline-4(1*H*)-one (PQS) were dissolved in 7.4 mL pyridine, P_4S_{10} (0.206 g, 0.925 mmol) was added and the reaction was refluxed for 4 h. The mixture was allowed to cool to room temperature and poured into ice cold water. With a 6 M HCl solution the pH was brought to 7 before it was extracted three times with DCM. The combined organic phases were washed three times with 1 M HCl, dried over $MgSO_4$ and evaporated. The pure product was obtained after recrystallization in MeOH as yellow acicular crystals (0.104 g, 61%). *Rf* = 0.27 (DCM/MeOH 10:1). ^{1}H-NMR (DMSO-d_6 600.33 MHz) δ (ppm): 0.84 (t, *J* = 7.1 Hz, 3H, H-15), 1.20 – 1.39 (m, 8H, H-11-14), 1.74 (quint., *J* = 7.6 Hz, 2H, H-10), 2.92 (t, *J* = 7.9 Hz, 2H, H-9), 7.49 (ddd, *J* = 1.1, 6.9, 8.2 Hz, 1H, H-6), 7.66 (ddd, *J* = 1.4, 6.9, 8.3 Hz, 1H, H-7), 7.77 (d, *J* = 8.3 Hz, 1H, H-8), 8.51 (dd, *J* = 1.1, 8.4 Hz, 1H, H-5), 8.67 (s, 1H, OH), 13.16 (s, 1H, NH). ^{13}C-NMR (DMSO-d_6 150.95 MHz) δ (ppm): 13.9 (C-15), 22.0 (C-14), 27.6 (C-10), 28.4, 28.8 (C-11, C-12), 29.1 (C-9), 31.1 (C-14), 119.2 (C-8), 125.0 (C-6), 127.8 (C-5), 129.2 (C-4a), 129.8 (C-7), 132.8 (C-8a), 134.7 (C-2), 147.2 (C-3), 169.3 (C-4). ESI-MS: m/z = 275.9 $[M+H]^+$, calc. for $C_{16}H_{21}ONS + H^+ = 276.1$; m/z = 549.2 $[2M-H]^-$, calc. for $C_{32}H_{42}O_2N_2S_2 - H^+ = 549.3$.

Synthesis of 2-heptyl-3-hydroxy-4*H*-chromene-4-thione, 1-O-4-S-PQS (**9**)



Under nitrogen atmosphere 33.2 mg (0.127 mmol) 1-O-PQS (**5**) and 42.2 mg (0.190 mmol) phosphorus P_4S_{10} were dissolved in 2 mL dry pyridine and the solution was refluxed for 4 h at 170 °C. During the time the color of the reaction changed from orange to brown. The mixture was brought to room temperature and poured on ice. Then it was quenched with 6 M HCl and the aqueous solution was extracted three times with DCM. The combined organic phases were washed three times with 1 M HCl and dried over $MgSO_4$. The solvent was evaporated and purified by silica gel chromatography in petroleum ether/ ethyl acetate 5:1. The product was obtained as a brown crystalline solid (23.6 mg, 67%). *Rf* = 0.85 (petroleum ether/ ethyl acetate 5:1). ^{1}H-NMR ($CDCl_3$ 399.79 MHz) δ (ppm): 0.89 (t, *J* = 6.8 Hz, 3H, H-15), 1.22 – 1.50 (m, 8H, H-11-14), 1.82 (quint., *J* = 7.5 Hz, 2H, H-10), 2.93 (t, *J* = 7.7 Hz, 2H, H-9), 7.45 (ddd, *J* = 0.85 Hz, *J* = 7.1 Hz, *J* = 8.0 Hz, 1H, H-6), 7.54 (d, *J* = 8.4 Hz, 1H, H-8), 7.67 (ddd, *J* = 1.5 , 7.1, 8.5 Hz, 1H, H-7), 7.85 (s, 1H, OH), 8.56 (dd, *J* = 1.3, 8.3 Hz, 1H, H-5).^{13}C-NMR ($CDCl_3$ 100.53 MHz) δ (ppm): 14.2 (C-15), 22.7 (C-14), 26.8 (C-10), 29.1 (C-12), 29.4 (C-11), 29.9 (C-9), 31.8 (C-13), 118.5 (C-8), 125.8 (C-6), 128.3 (C-4a), 128.8 (C-5), 132.7 (C-7), 146.8 (C-3), 148.8 (C-2), 150.9 (C-8a), 186.9 (C-4). ESI-MS: m/z = 276.9 $[M+H]^+$, calc. for $C_{16}H_{20}O_2S + H^+ = 277.1$; m/z = 298.9 $[M+Na]^+$, calc. for $C_{16}H_{20}O_2S + Na^+ = 299.1$

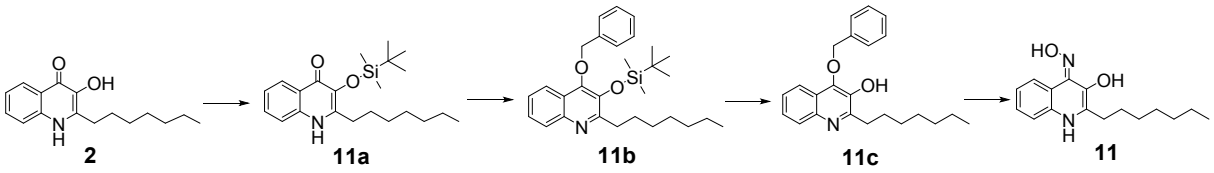
Synthesis of 2-heptyl-3-hydroxy-4*H*-thiochromene-4-thione, 1,4-S-PQS (**10**)



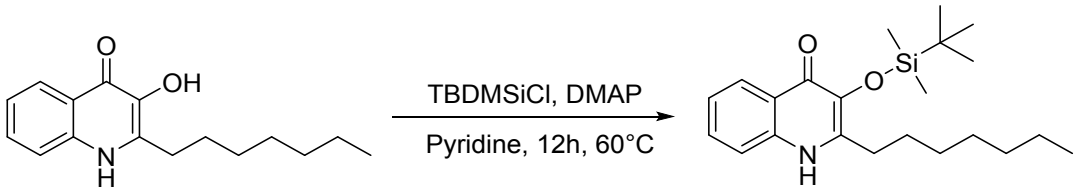
Under nitrogen atmosphere 54.4 mg (0.197 mmol) 1-S-PQS (**6**) and 65.6 mg (0.295 mmol) phosphorus P_4S_{10} were dissolved in 2.4 mL pyridine and the solution was refluxed for 4 h at 170 °C. The orange colored mixture was cooled to room temperature and poured into ice. Then it was brought to pH 7 using 6 M HCl solution. The turbid ochre colored suspension was extracted with DCM three times and the red colored organic layers were combined and washed with 1 M HCl solution three times. The dissolved crude product was dried over $MgSO_4$, the solvent was evaporated and the red residue was recrystallized in methanol. The red precipitate was purified by silica gel chromatography (petroleum ether/ethyl acetate 1:4) and the product was obtained as dark violet/black crystalline solid (41.4 mg, 72%), *Rf* = 0.19 (petroleum ether/ ethyl acetate 2:1). ^{1}H-NMR (DMSO-d_6 399.79 MHz) δ (ppm): 0.85 (t, *J* = 7.0 Hz, 3H, H-15),

1.20 – 1.38 (m, 8H, H-11-14), 1.75 (quint., J = 7.5 Hz, 2H, H-10), 2.95 (t, J = 7.8 Hz, 2H, H-9), 7.74 (ddd, J = 1.7, 7.0, 8.4 Hz, 1H, H-6), 7.79 (ddd, J = 1.6, 7.0, 8.5 Hz, 1H, H-7), 8.10 (dd, J = 1.3, 7.5 Hz, 1H, H-8), 8.88 (dd, J = 1.5, 8.1 Hz, 1H, H-5), 9.38 (s, 1H, OH). ^{13}C-NMR (DMSO-d_6 100.53 MHz) δ (ppm): 13.9 (C-15), 22.0, 31.1, 28.6 (C12-C14), 28.3 (C-11), 28.4 (C-10), 31.9 (C-9), 127.1 (C-8), 128.7 (C-6), 130.4 (C-7), 131.1 (C-2), 132.1 (C-5), 132.7 (C-8a), 135.4 (C-4a), 152.2 (C-3), 185.8 (C-4). ESI-MS: m/z = 292.8 $[M+H]^+$, calc. for $C_{16}H_{20}OS_2$ + H^+ = 293.

Synthesis of PQS-Oxime (**11**)



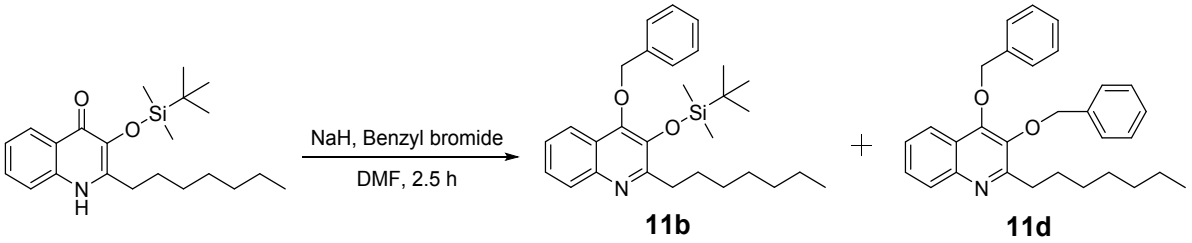
Synthesis of 3-((tert-butyldimethylsilyl)oxy)-2-heptylquinolin-4(1*H*)-one (**11a**)



0.230 g (0.89 mmol) PQS and 22 mg DMAP (0.18 mmol) were dissolved in 10 mL dry pyridine under nitrogen atmosphere and warmed up to 60 °C. 267 mg (1.77 mmol) TBDMSiCl was added in one portion and the reaction was stirred at 60 °C for 12 h. The solvent was evaporated under reduced pressure at 60 °C. The residue was dissolved in DCM and washed twice with water and once with brine, dried with $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography on silica gel using DCM/MeOH (97:3). The product was received as a white crystalline solid (293.5 mg, 88%). *Rf* = 0.37 (DCM/MeOH (97:3). ^{1}H-NMR ($CDCl_3$ 399.79 MHz) δ (ppm): 0.35 (s, 6H, -Si-$(CH_3)_2$), 0.83 (m, 3H, H-15), 1.00 (s, 9H, -C-$(CH_3)_3$), 1.10-1.36 (m, 8H, H-11-14), 1.71 (m, 2H, H-10), 2.85 (m, 2H, H-9), 7.18 (ddd, J = 8.7, 8.3, 0.7 Hz, 1H, H-6), 7.46 (ddd, J = 8.7, 8.4, 1.3 Hz, 1H, H-7), 7.59 (d, J = 8.4 Hz, 1H, H-8), 8.28 (dd, J = 8.3, 1.3 Hz, 1H, H-5), 10.67 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$ 100.53 MHz) δ (ppm): -2.9

(2C, -Si-(CH_3)$_2$), 14.4 (C-15), 19.1 (-**C**-(CH_3)$_3$), 22.7, 29.2, 29.7, 31.8 (C-11-14), 26.4 (3C, -C-(**C**H_3)$_3$), 28.7 (C-10), 30.0 (C-9), 117.8 (C-8), 122.5 (C-6), 124.7 (C-4a), 125.6 (C-5), 130.5 (C-7), 137.5 (C-3), 137.8 (C-8a), 142.5 (C-2), 171.5 (C-4). ESI-MS: m/z = 371.95 $[M-H]^-$, calc. for $C_{22}H_{34}NO_2Si^-$ = 372.24.

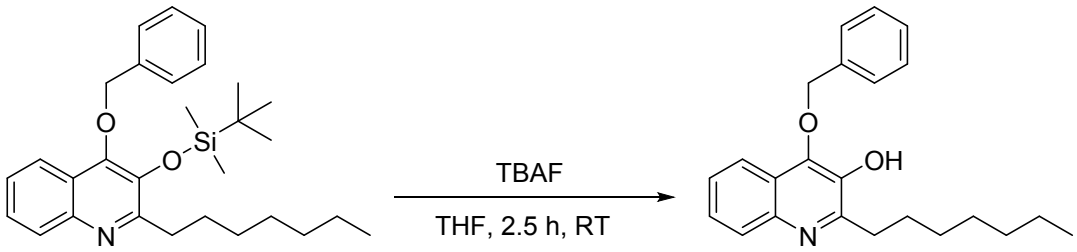
Synthesis of 4-(benzyloxy)-3-((tert-butyldimethylsilyl)oxy)-2-heptylquinoline (**11b**)



0.229 g (0.613 mmol) of **11a** and 40 mg NaH (1.0 mmol, 60% in mineral oil) were dissolved in 6 mL dry DMF under nitrogen atmosphere and were stirred for 30 min at room temperature. 175 mg (1.0 mmol) benzyl bromide was dissolved in 4 mL DMF and added to the reaction dropwise. A black precipitate appeared by the addition of benzyl bromide. After the reaction of 2h time at room temperature, the now clear solution was quenched by the addition of 5 mL water. Water and DMF were evaporated with reduced pressure at 60°C. The residue was dissolved in DCM and washed twice with water and once with brine, dried with $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography on silica gel using ethyl acetate/ petrol ether (1:5). The product was received as off-white solid (48 mg, 17%). ^{1}H-NMR ($CDCl_3$ 399.79 MHz) δ (ppm): 0.21 (s, 6H,-Si-(C**H**$_3$)$_2$), 0.90 (m, 3H, H-15), 1.09 (s, 9H, -C-(C**H**$_3$)$_3$), 1.26-1.50 (m, 8H, H-11-14), 1.82 (m, 2H, H-10), 3.03 (m, 2H, H-9), 5.18 (s, 2H, -O-C**H**$_2$-Ar), 7.29-7.41 (m, 4H, H-6, Ar-CH), 7.46 (m, 2H, Ar-CH), 7.54 (ddd, *J* = 8.9, 8.4, 1.5 Hz, 1H, H-7), 7.95 (m, 1H, H-5), 7.98 (d, *J* = 8.4 Hz, 1H, H-8). ^{13}C-NMR ($CDCl_3$ 100.53 MHz) δ (ppm): -3.9 (2C, -Si-(**C**H_3)$_2$), 14.2 (C-15), 18.6 (-**C**-(CH_3)$_3$), 22.8, 29.5, 29.9, 32.0 (C-11-14), 26.1 (3C, -C-(**C**H_3)$_3$), 28.9 (C-10), 34.4 (C-9), 74.8 (-O-**C**H_2-Ar), 121.5 (C-5), 124.4 (C-4a), 125.5 (C-6), 127.6 (C-7), 127.9 (2C, Ar-CH), 128.2 (Ar-CH), 128.5 (C-8), 128.6 (2C, Ar-CH), 137.0 (Ar-C=), 139.4 (C-3), 144.6 (C-8a), 149.7 (C-4), 160.4 (C-2).

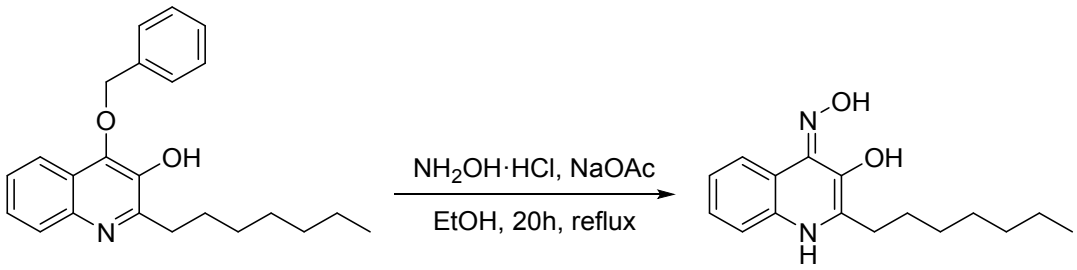
The main product isolated was the 3,4-bis(benzyloxy)-2-heptylquinoline (**11d**) (140 mg, 52%). *Rf* = 0.5 (DCM/MeOH 99:1). ^{1}H-NMR ($CDCl_3$ 399.79 MHz) δ (ppm): 0.95 (m, 3H, H-15), 1.25-1.55 (m, 8H, H-11-14), 1.87 (m, 2H, H-10), 3.05 (m, 2H, H-9), 5.19 (s, 2H, -O-C**H**$_2$-Ar), 5.37 (s, 2H, -O-C**H**$_2$-Ar), 7.34-7.54 (m, 11H, H-6, Ar-CH), 7.63 (ddd, *J* = 8.9, 8.4, 1.5 Hz, 1H, H-7), 8.07 (d, *J* = 8.5 Hz, 1H, H-5), 8.14 (d, *J* = 8.4 Hz, 1H, H-8).

Synthesis of 4-(benzyloxy)-2-heptylquinolin-3-ol (**11c**)



45 mg (0.097 mmol) of **11b** was dissolved in 5 mL dry THF under nitrogen atmosphere and 200 µL TBAF (1 M in THF) was added at 0 °C. After the reaction time of 2.5 h at room temperature, water was added and the mixture extracted with ethyl acetate. The organic phase was washed with brine, dried with $MgSO_4$, filtered and evaporated. The residue was purified by column chromatography on silica gel using ethyl acetate/ petrol ether (1:5). The product was received as off-white solid (30 mg, 88.5%). ^{1}H-NMR ($CDCl_3$ 399.79 MHz) δ (ppm): 0.87 (m, 3H, H-15), 1.20-1.42 (m, 8H, H-11-14), 1.74 (m, 2H, H-10), 2.98 (m, 2H, H-9), 5.18 (s, 2H, -O-C**H**$_2$-Ar), 7.38 (m, 5H, Ar-CH), 7.47 (ddd, *J* = 8.7, 8.3, 1.2 Hz, 1H, H-6), 7.54 (ddd, *J* = 8.7, 8.3, 1.5 Hz, 1H, H-7), 7.93 (dd, *J* = 8.3, 1.2 Hz, 1H, H-5), 8.05 (d, *J* = 8.3 Hz, 1H, H-8). ^{13}C-NMR ($CDCl_3$ 100.53 MHz) δ (ppm): 14.2 (C-15), 22.8, 29.3, 29.8, 31.9 (C-11-14), 28.6 (C-10), 34.1 (C-9), 76.5 (-O-**C**H_2-Ar), 120.6 (C-5), 123.0 (C-4a), 126.0 (C-6), 126.9 (C-7), 128.3 (2C, Ar-CH), 129.1 (2C, C-8, Ar-CH), 136.5 (Ar-C≡), 140.8 (C-3), 143.8 (C-8a), 144.7 (C-4), 156.3 (C-2).

Synthesis of 2-heptyl-3-hydroxyquinolin-4(1*H*)-one oxime, PQS-Oxime (**11**)



30 mg (0.086 mmol) of **11c** was dissolved in 10 mL EtOH together with 0.5 g (7.2 mmol) $NH_2OH{\cdot}HCl$ and 0.6 g (7.3 mmol) sodium acetate. The reaction was stirred at reflux conditions for 20 h. After the reaction cooled to room temperature the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate and washed once with water and sat. NH_4Cl solution. The organic phase was tried with $MgSO_4$, filtered and evaporated leaving a yellow residue which was purified by column chromatography on silica gel using DCM/MeOH (4:1). The product was received as a yellow solid (15 mg, 70.5%). *Rf* = 0.8 (DCM/MeOH 4:1). ^{1}H-NMR (DMSO-d_6 399.79 MHz) δ (ppm): 0.83 (m, 3H, H-15), 1.15-1.40 (m, 8H, H-11-14), 1.69 (m, 2H, H-10), 2.97 (m, 2H, H-9), 7.47 (m, 2H, H-6), 7.67 (m, 2H, H-

7), 7.79 (s, br, 1H, =N-OH), 7.91 (d, *J* = 8.5 Hz, 1H, H-8), 8.32 (d, *J* = 8.7 Hz, 1H, H-5). ^{13}C-NMR (DMSO-d_6 100.53 MHz) δ (ppm): 13.9 (C-15), 22.0, 28.5, 28.9, 31.1 (C-11-14), 28.0 (C-10), 29.8 (C-9), 116.0 (C-4a), 121.4 (C-8), 122.8 (C-5), 124.4 (C-6), 129.8 (C-7), 131.9 (C-3), 137.1 (C-8a), 145.8 (C-4), 147.5 (C-2). ^{1}H-NMR (MeOD-d_4 400.13 MHz) δ (ppm): 0.90 (m, 3H, H-15), 1.25-1.50 (m, 8H, H-11-14), 1.80 (m, 2H, H-10), 3.02 (m, 2H, H-9), 7.56 (m, 1H, H-6), 7.77 (m, 2H, H-7-8), 8.24 (d, J=8.4 Hz, 1H, H-5). ESI-MS: m/z = 274.95 $[M+H]^+$, calc. for $C_{16}H_{22}N_2O_2 + H^+ = 275.18$; m/z = 315.90 $[M+MeCN+H]^+$, calc. for $C_{16}H_{22}N_2O_2 + C_2H_3N + H^+ = 316.20$.

5. References

[1] G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, K. I. Goldberg, *Organometallics* **2010**, *29*, 2176-2179.

[2] D. W. Essar, L. Eberly, A. Hadero, I. P. Crawford, *Journal of bacteriology* **1990**, *172*, 884-900.

[3] D. E. Ohman, S. J. Cryz, B. H. Iglewski, *Journal of bacteriology* **1980**, *142*, 836-842.

[4] S. Wilhelm, A. Gdynia, P. Tielen, F. Rosenau, K. E. Jaeger, *Journal of bacteriology* **2007**, *189*, 6695-6703.

[5] M. W. Calfee, J. P. Coleman, E. C. Pesci, *Proceedings of the National Academy of Sciences of the United States of America* **2001**, *98*, 11633-11637.

[6] G. R. Cathcart, B. F. Gilmore, B. Greer, P. Harriott, B. Walker, *Bioorganic & medicinal chemistry letters* **2009**, *19*, 6230-6232.

6. Figures

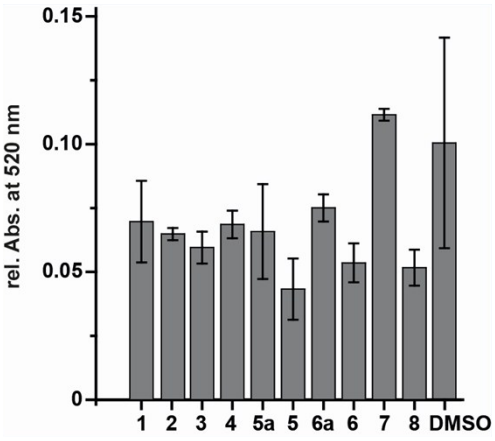


Figure S1. Pyocyanin production of *P. aeruginosa* with compounds **1**-**8** and DMSO as control.

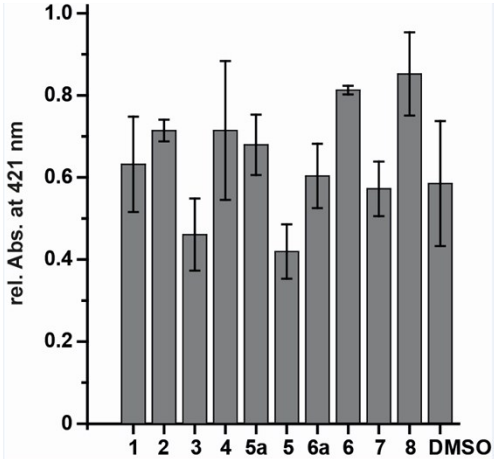


Figure S2. Rhamnolipid production of *P. aeruginosa* with compounds **1**-**8** and DMSO as control.

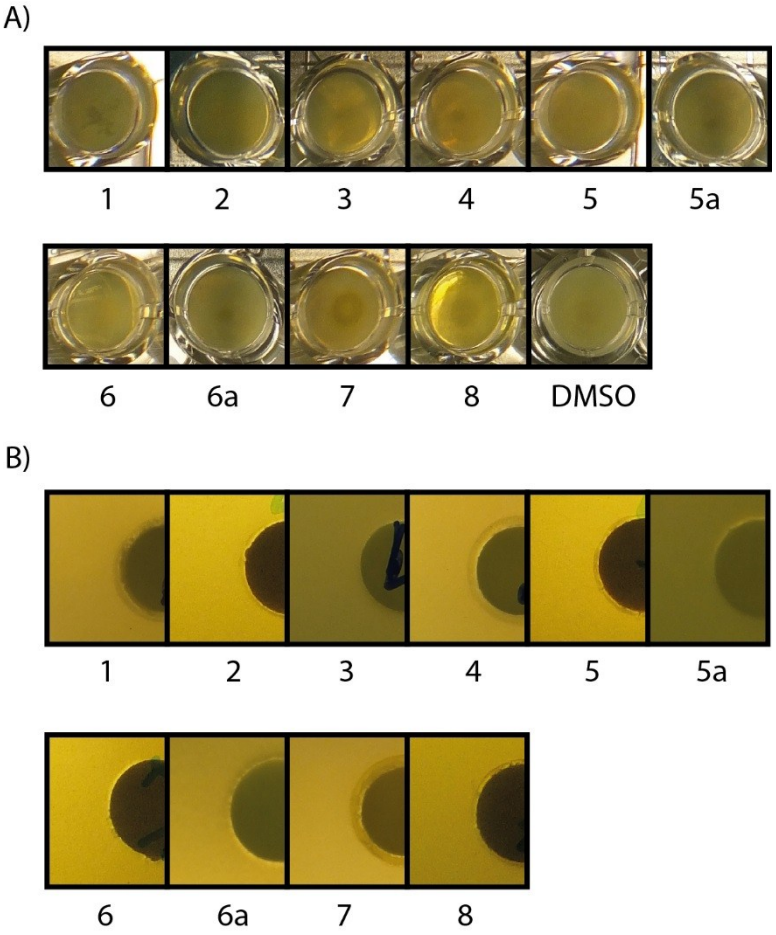


Figure S3. Growth of *Pseudomonas aeruginosa* PAO1 cultured in presence of HHQ and PQS derivatives. A) No growth inhibition of *P. aeruginosa* with compounds **1-9** at 1 mM concentration in liquid cultures. B) An LB disc-diffusion assay with a dose of 500 nmol of compounds **1-9** resulted in no inhibition zones.

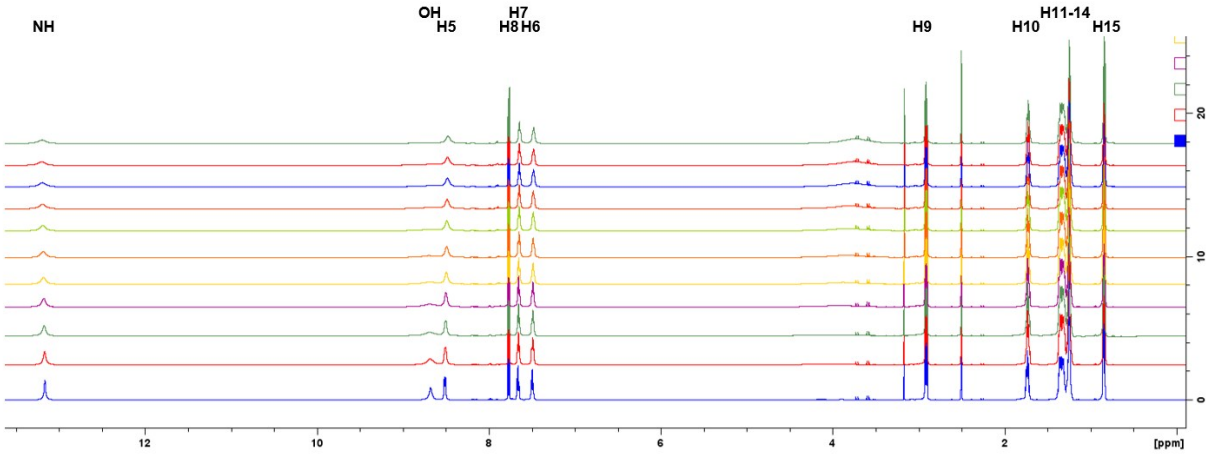


Figure S4. ^{1}H-NMR titration of **8** (73 µM) in DMSO-d_6 with increasing concentrations of Zn^{2+} from bottom to top (0 - 1 equivalent, in 0.1 eq. steps).

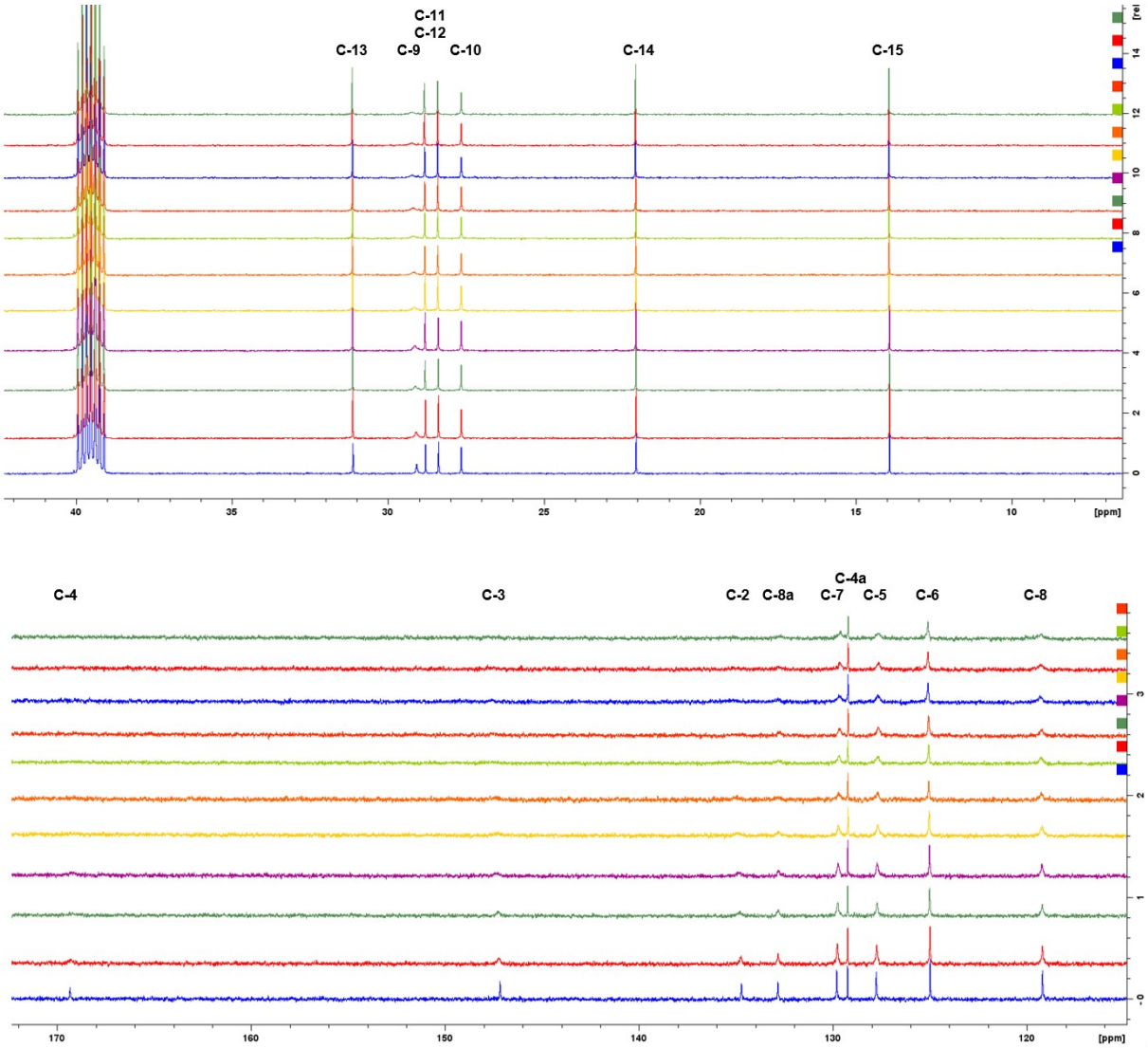


Figure S5. ^{13}C-NMR titration of **8** (73 µM) in DMSO-d_6 with increasing concentrations of Zn^{2+} from bottom to top (0 - 1 equivalent, in 0.1 eq. steps).

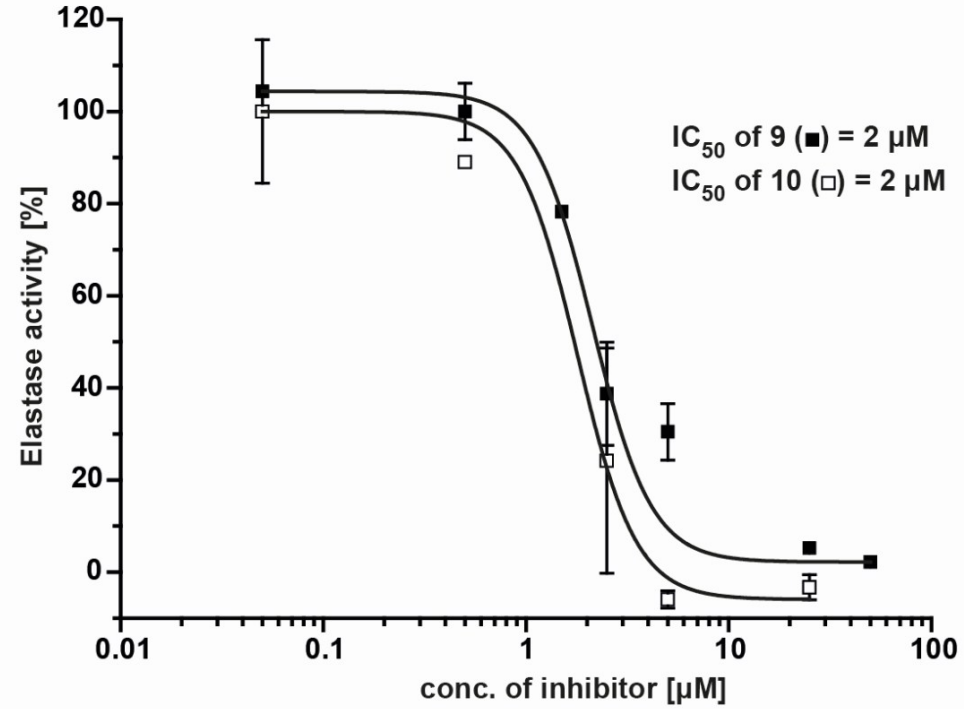


Figure S6. *In vitro* elastase (LasB) inhibition with compounds **9** and **10**.

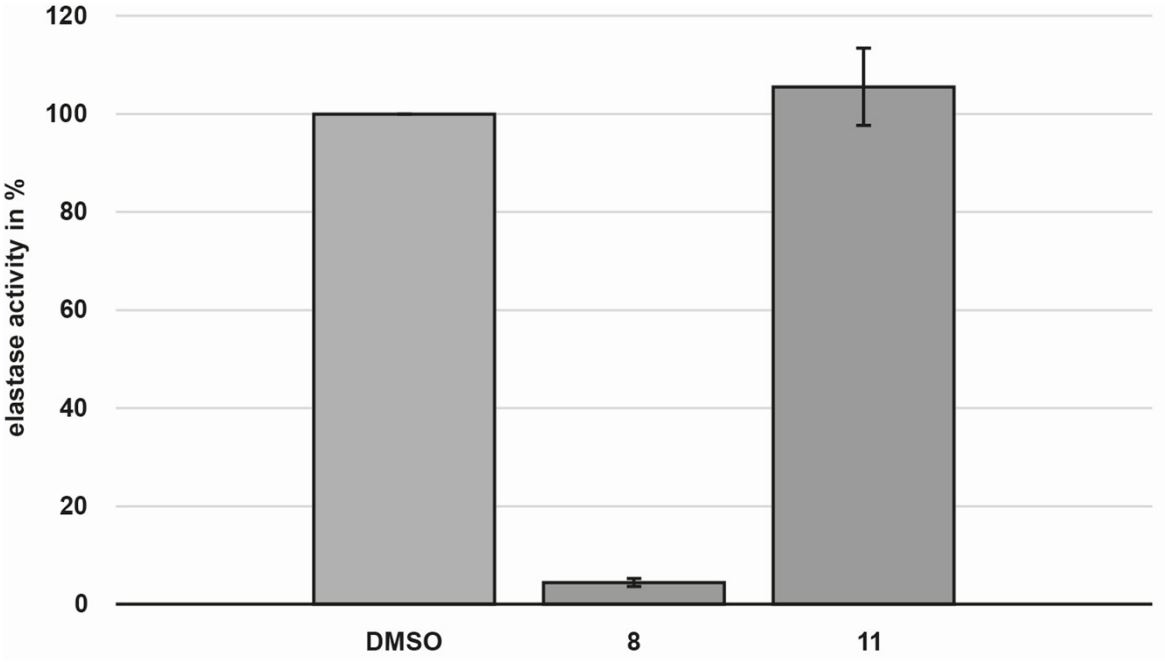


Figure S7. *In vitro* activity of elastase (LasB) at 50 µM of compound **11** in comparison to **8** (50 µM) and DMSO as control.

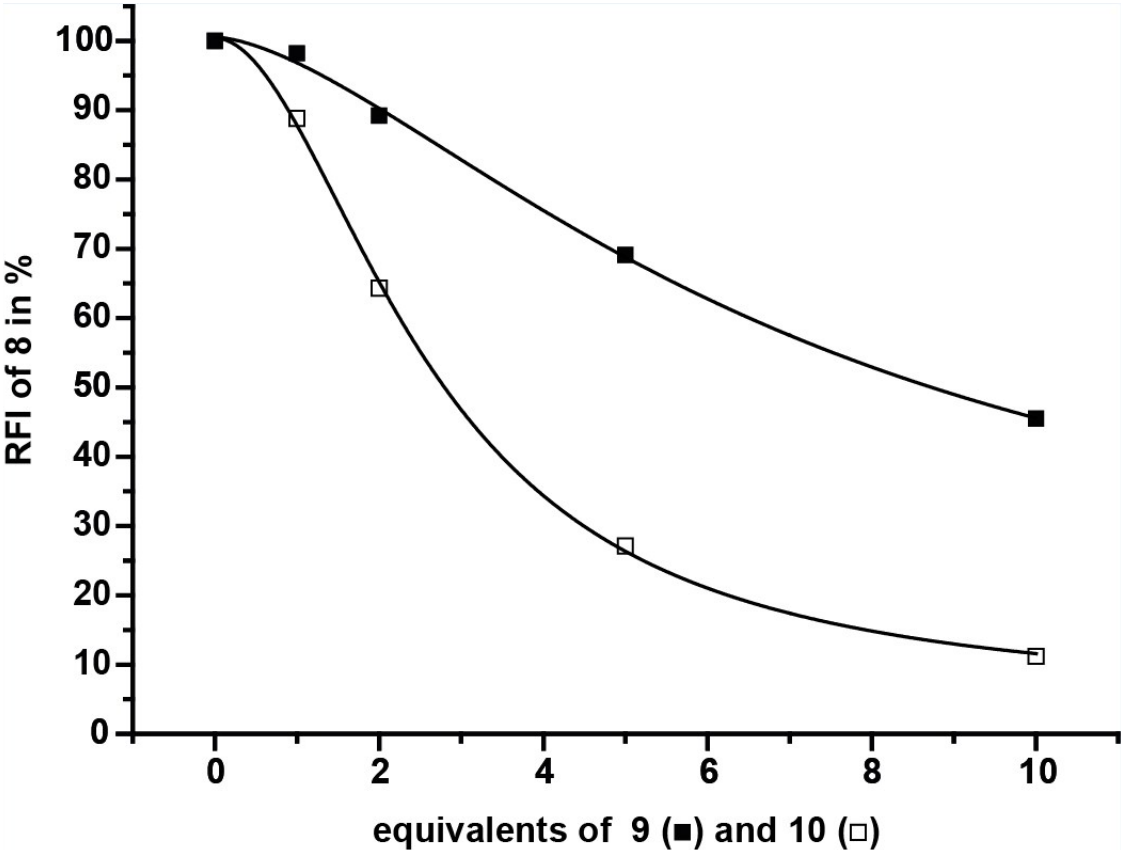


Figure S8. Compounds **9** and **10** quench the zinc dependent fluorescence of compound **8**. The number of equivalents of **9** and **10** are relative to the concentration of **8** (250 μM).

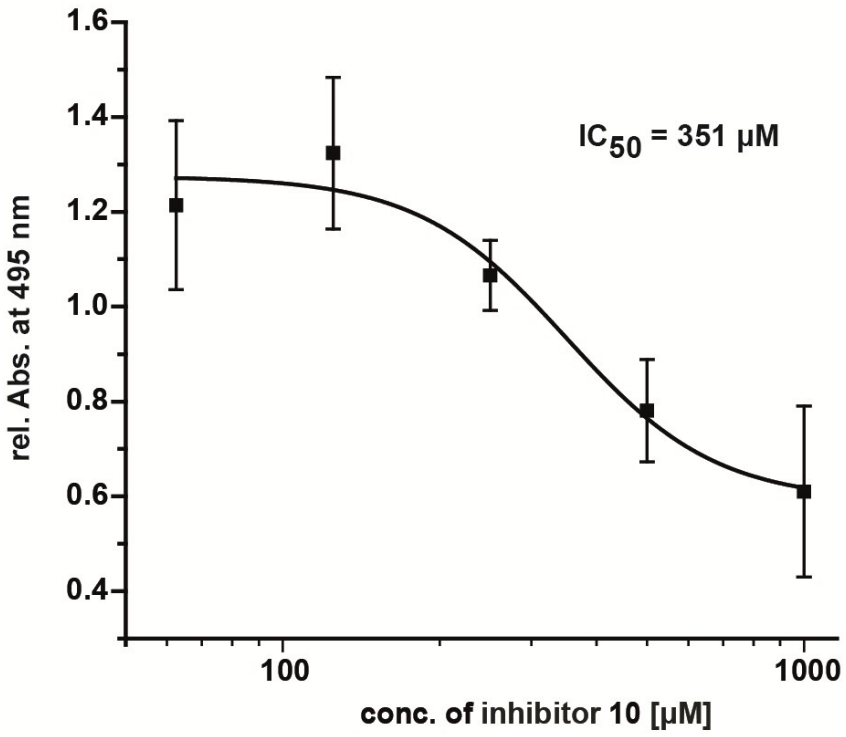
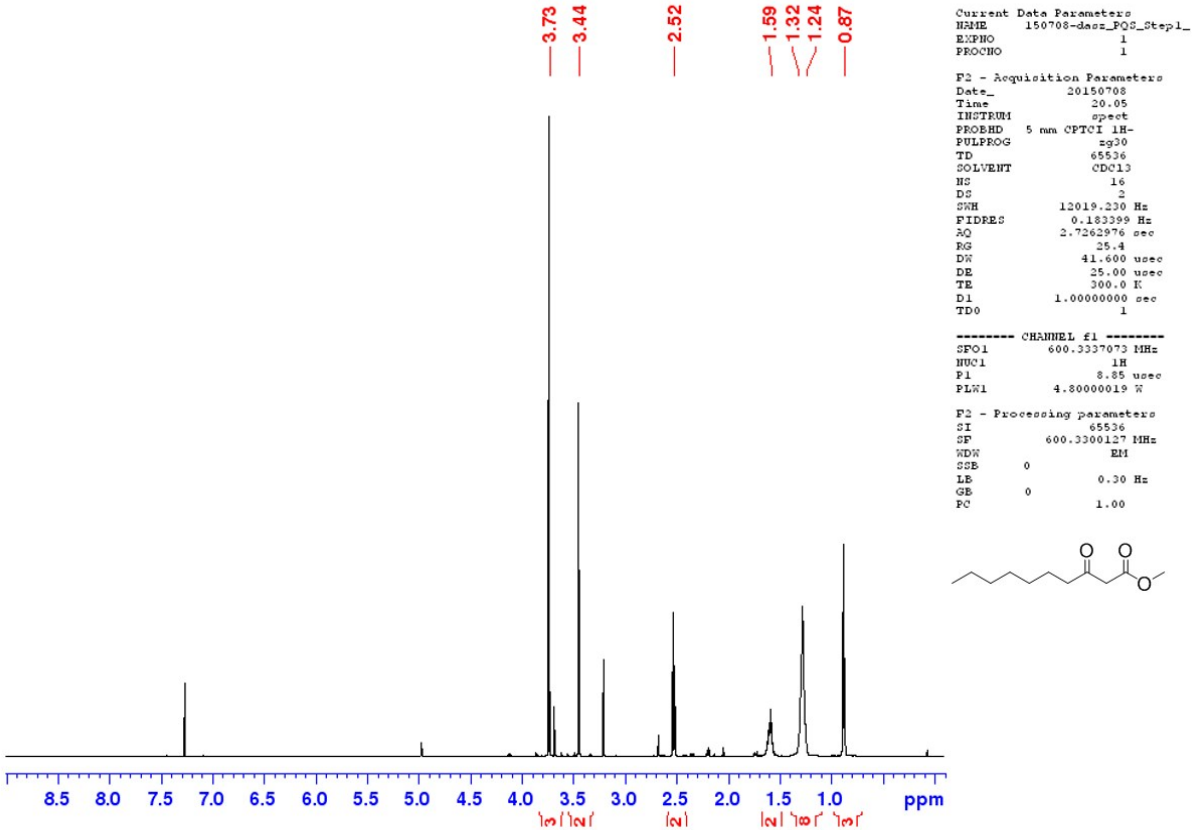
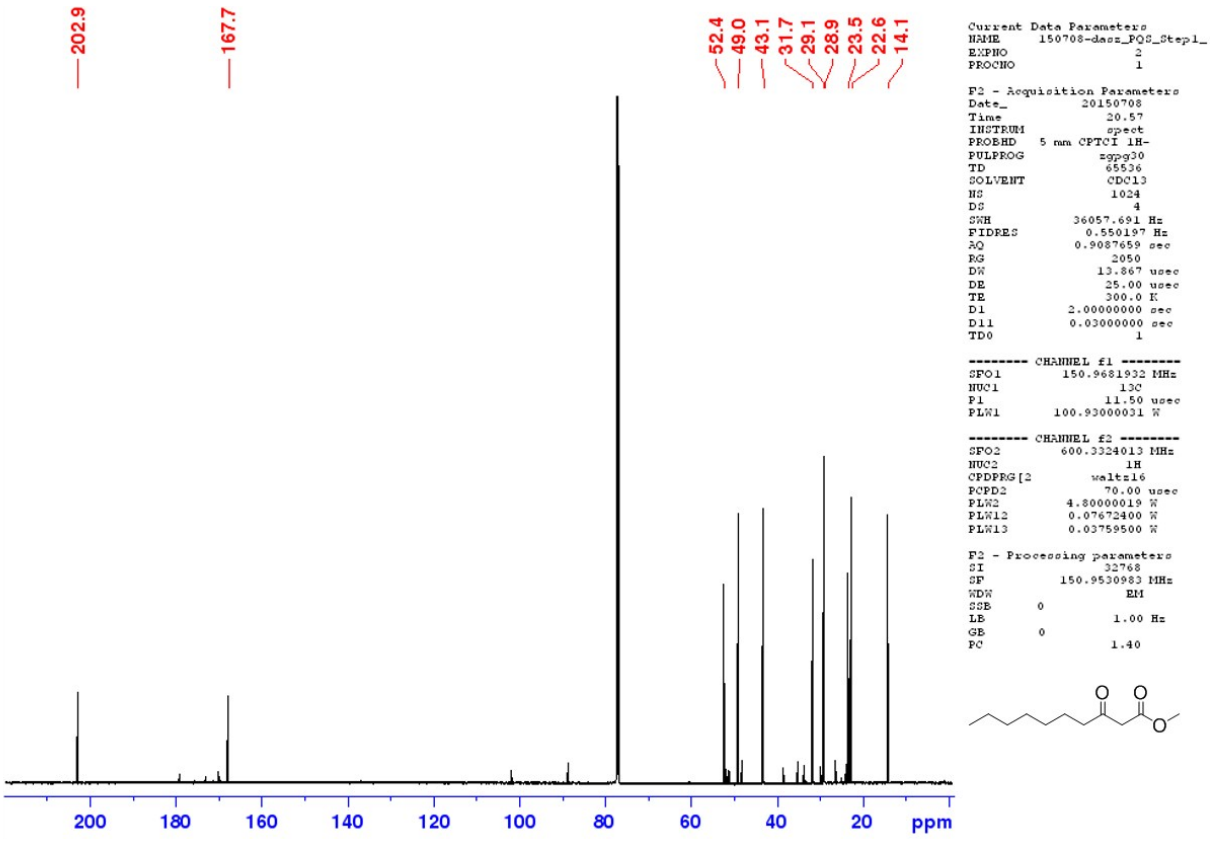


Figure S9. *In situ* inhibition of elastolytic activity by compound **10** with life cells of *P. aeruginosa*.

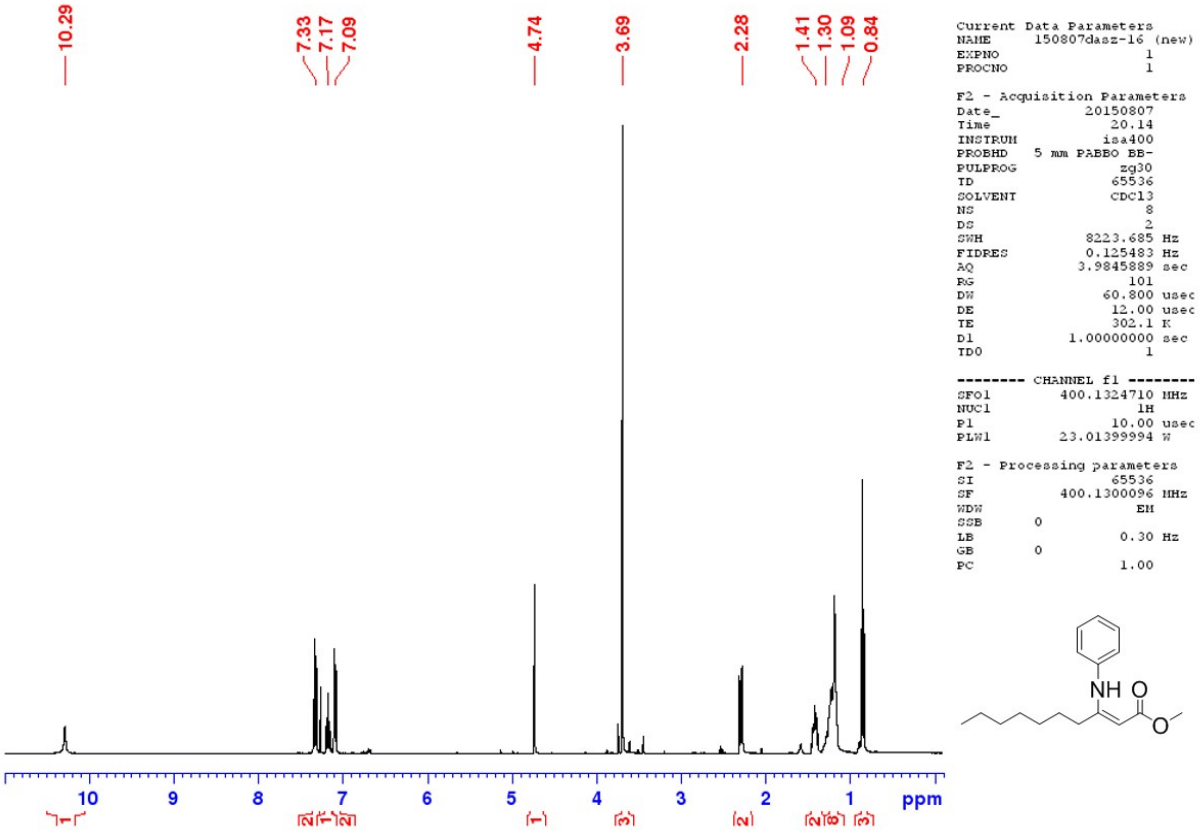
Appendix: NMR spectra

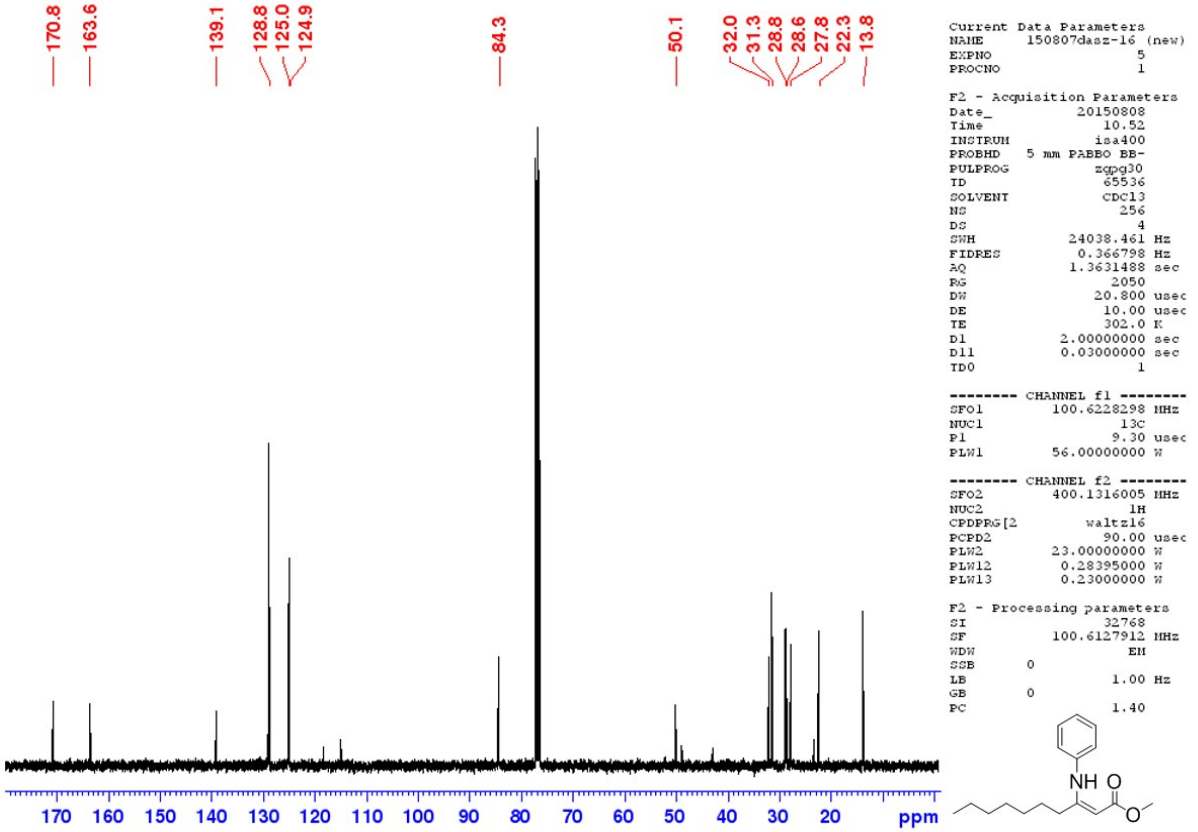
^{1}H- and ^{13}C-NMR spectra of methyl-3-oxodecanoate in $CDCl_3$ (**1a**)



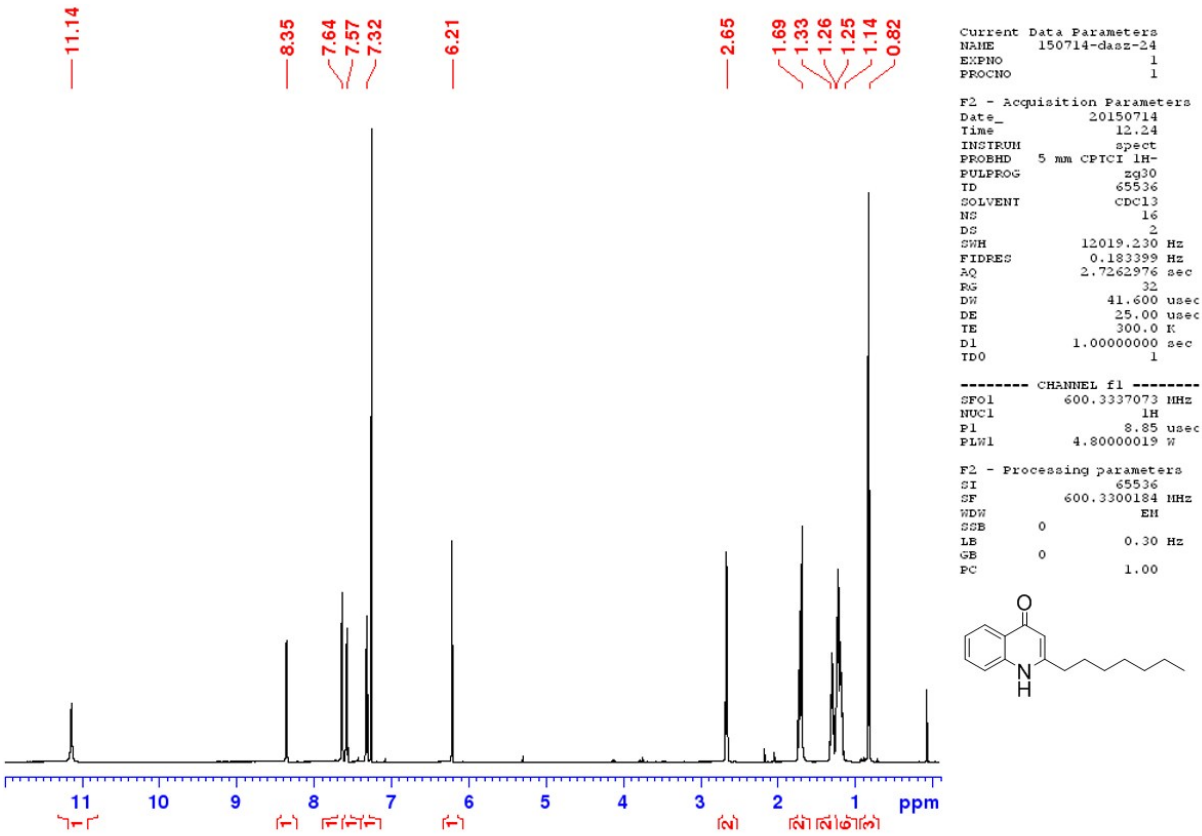


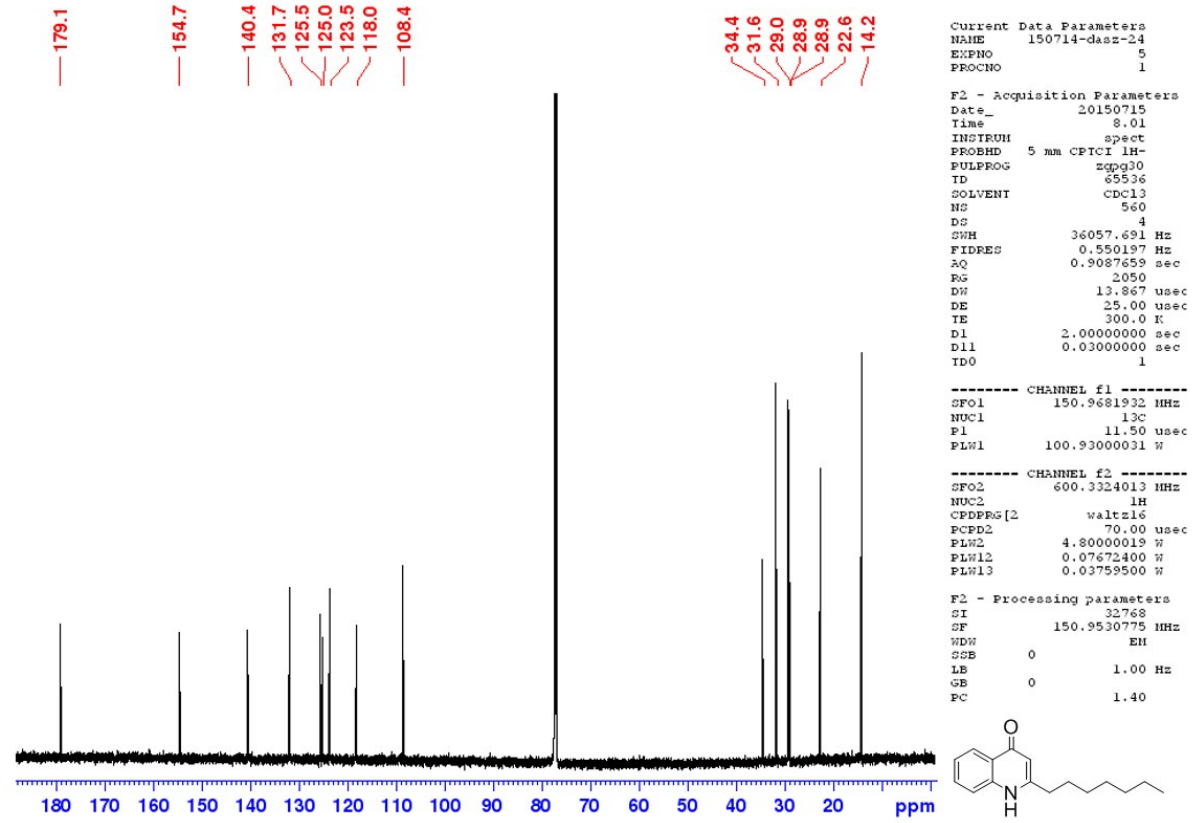
^{1}H- and ^{13}C-NMR spectra of methyl-3-anilino-2-decanoate in $CDCl_3$ (**1b**)



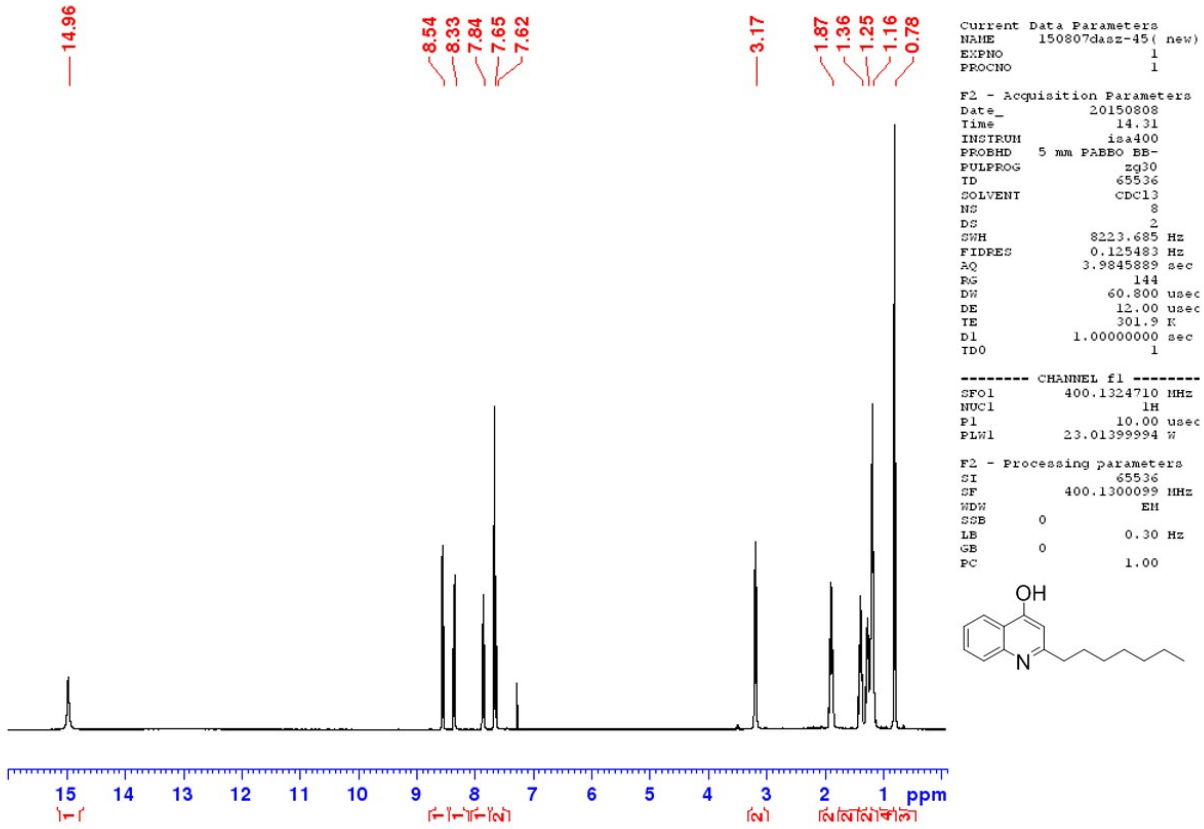


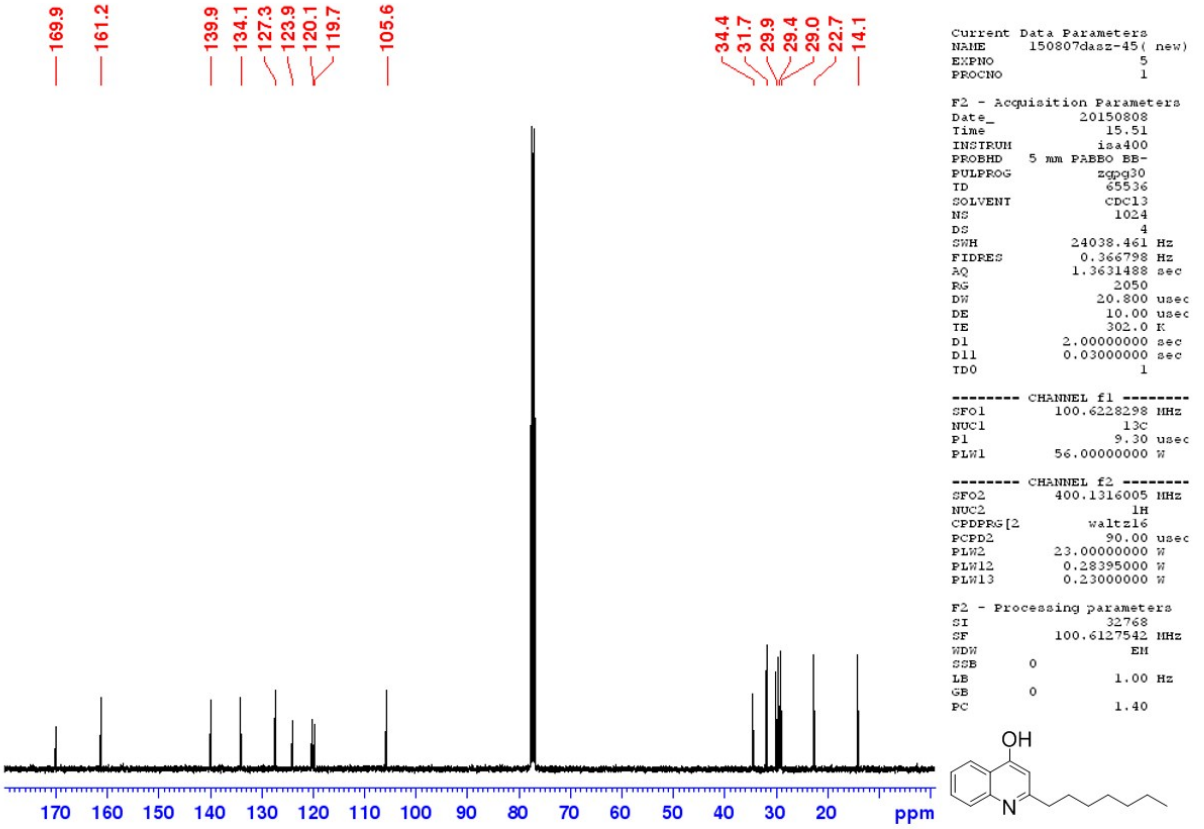
^{1}H- and ^{13}C-NMR spectra of 2-heptyl-4-quinolone, HHQ in $CDCl_3$ (**1**)



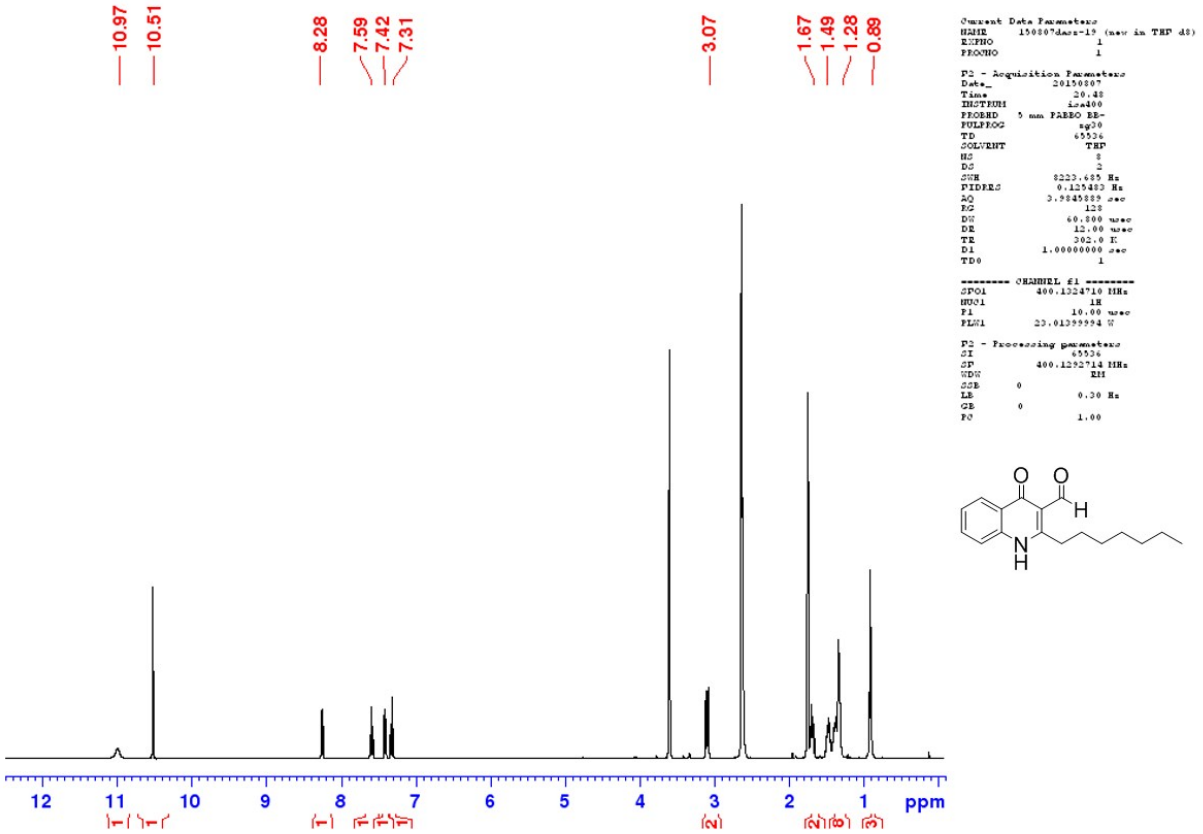


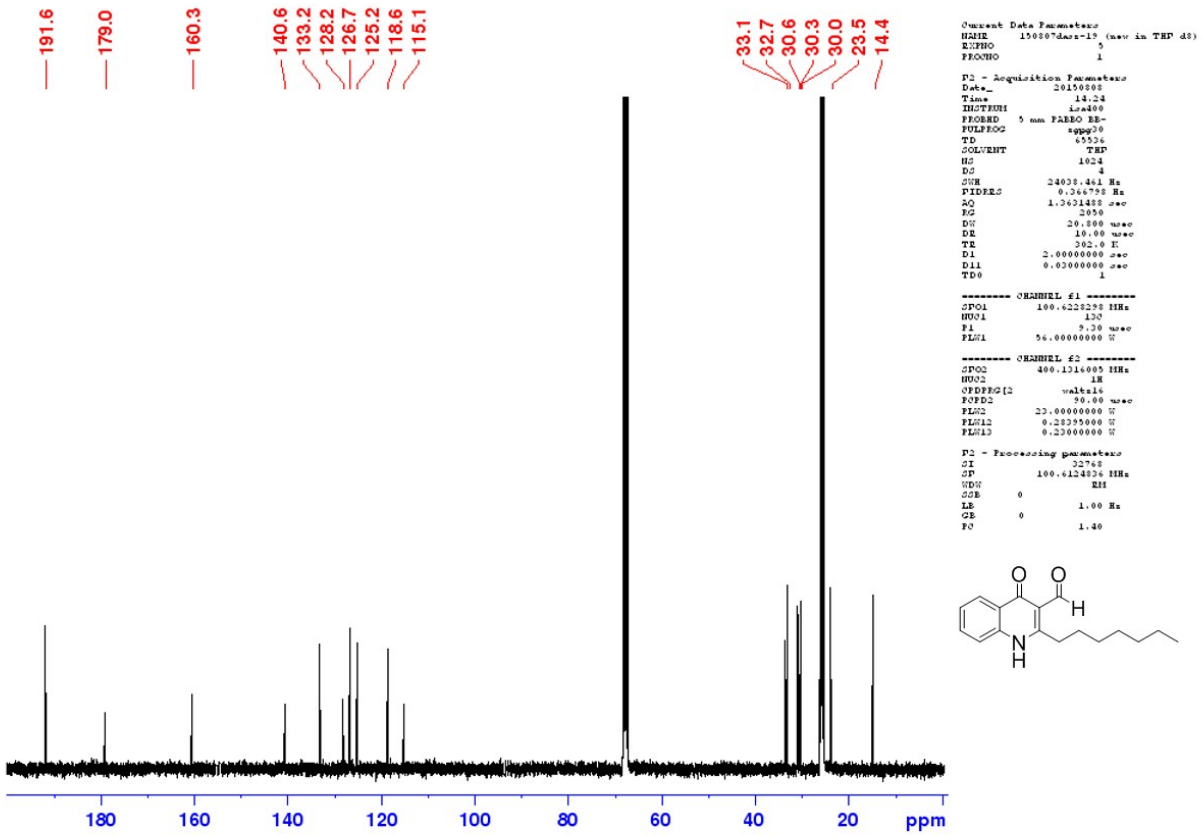
^{1}H- and ^{13}C-NMR spectra of 2-heptyl-quinolin-4-ol · HCl in $CDCl_3$ (**2a)**



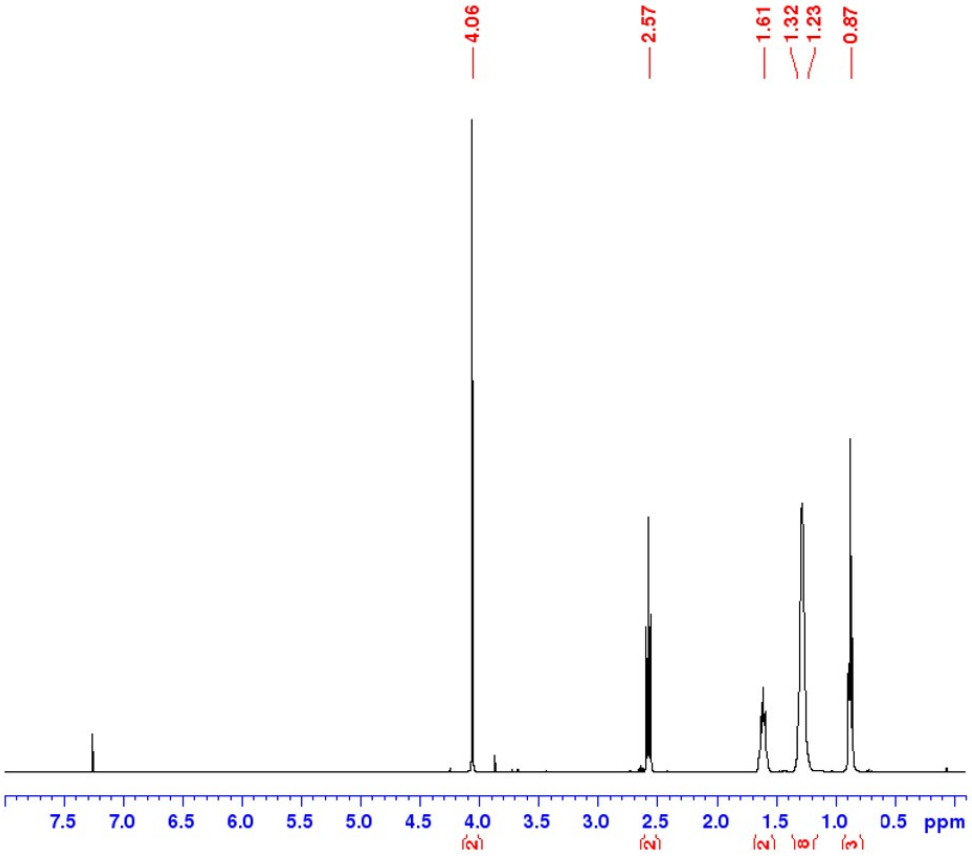


^{1}H- and ^{13}C-NMR spectra of 3-formyl-2-heptyl-4-quinolone in THF-d_8 (**2b**)





^{1}H- and ^{13}C-NMR spectra of 1-chloro-2-nonanone in $CDCl_3$ (**2c**)

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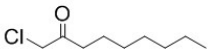

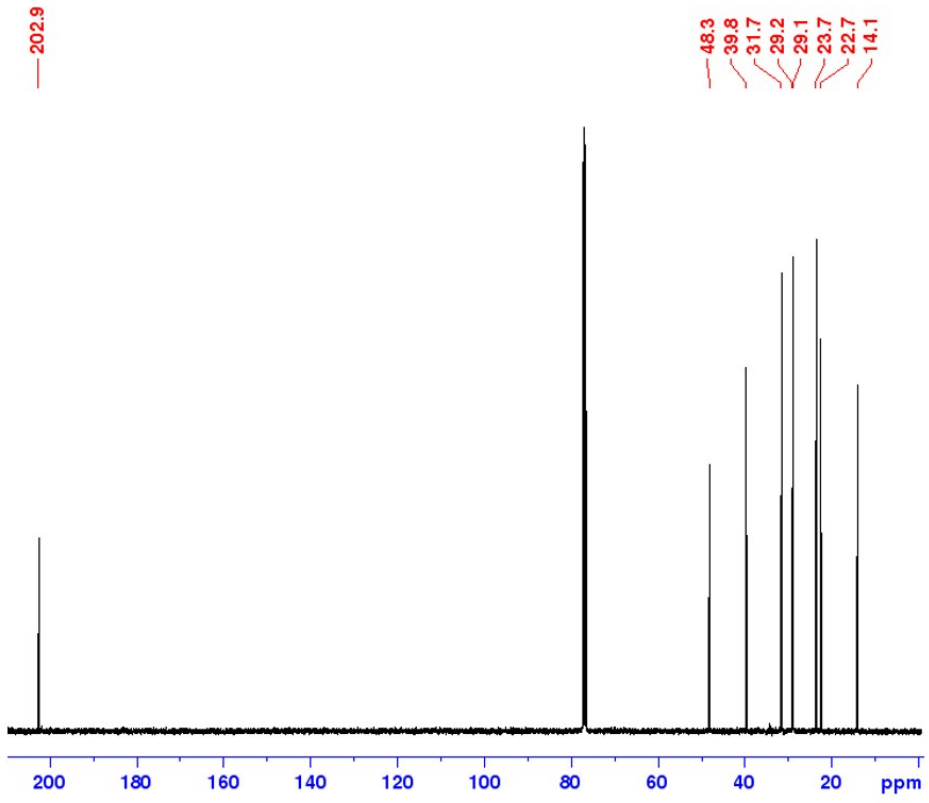


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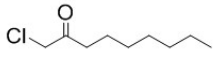
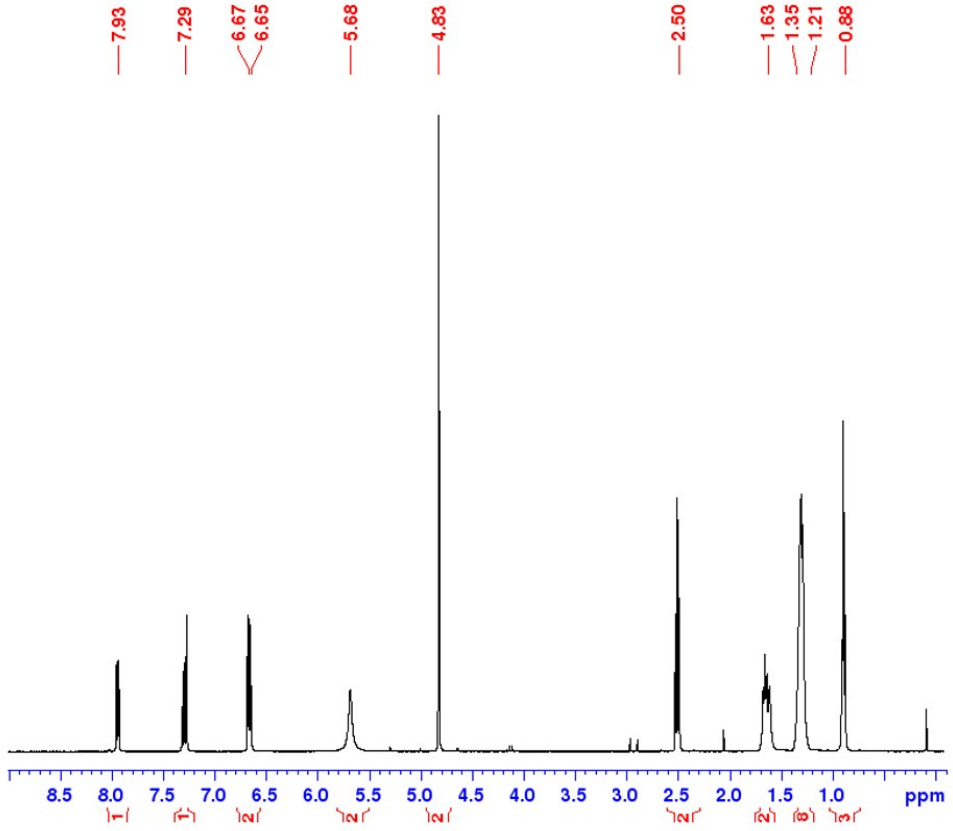


^{1}H- and ^{13}C-NMR spectra of 2-oxononyl-2-aminobenzoate in $CDCl_3$ (**2d**)

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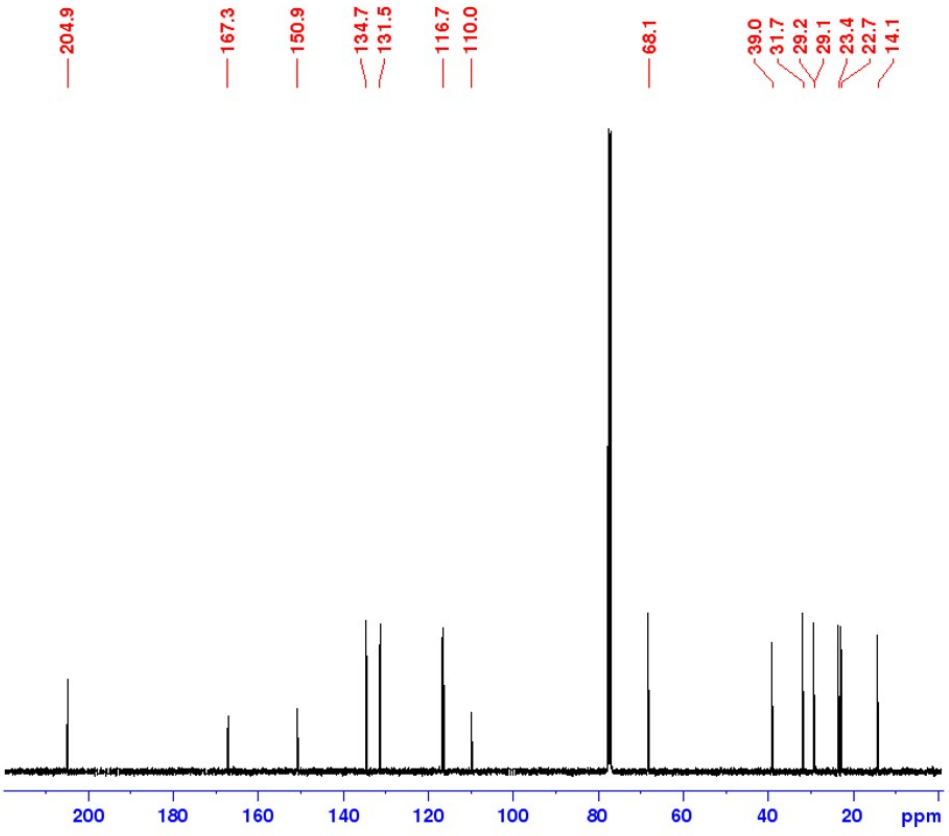


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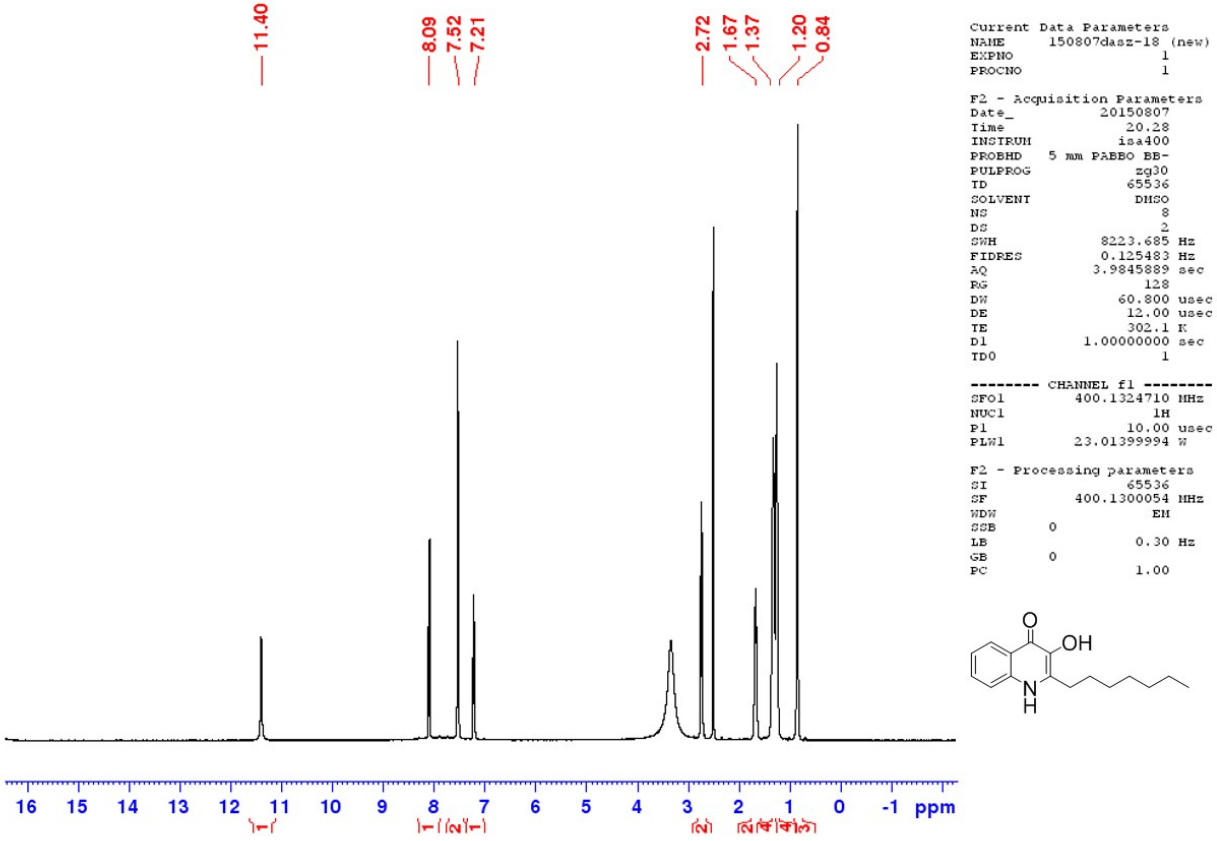
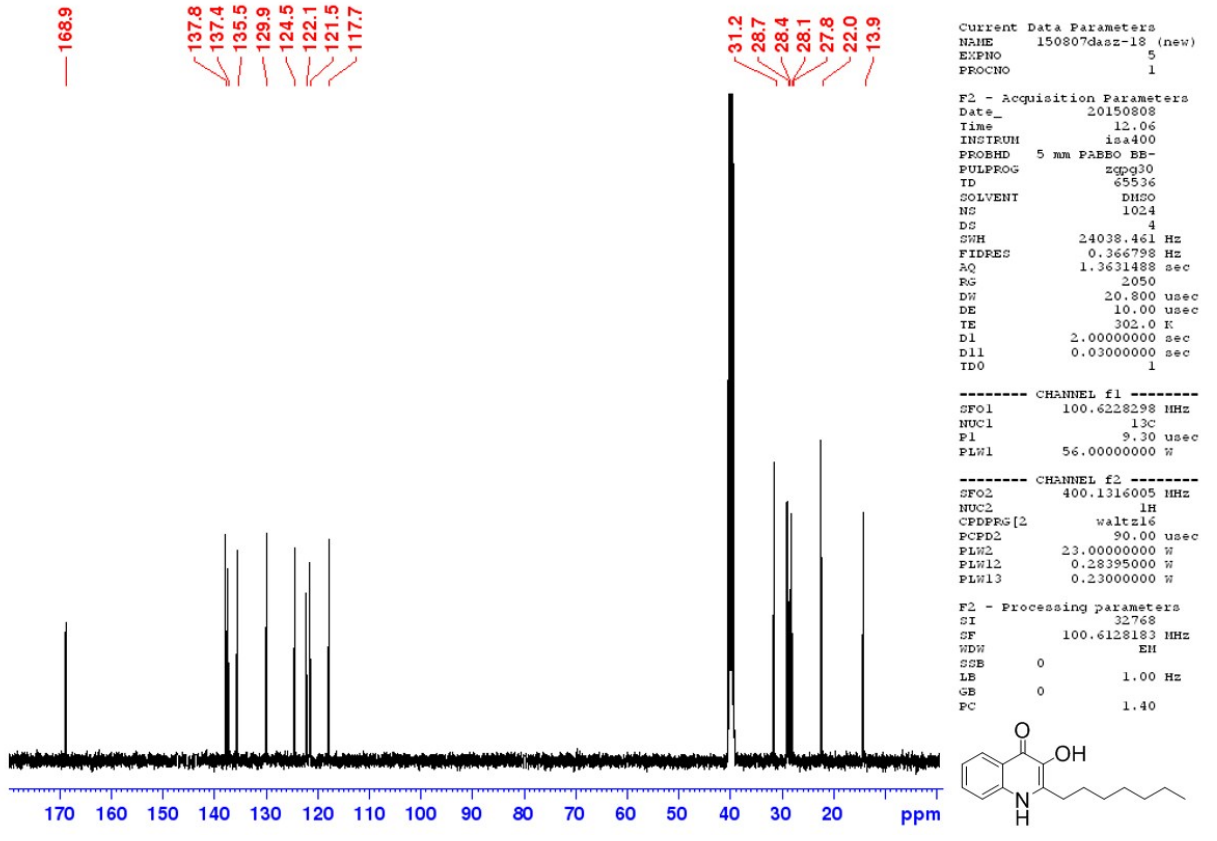
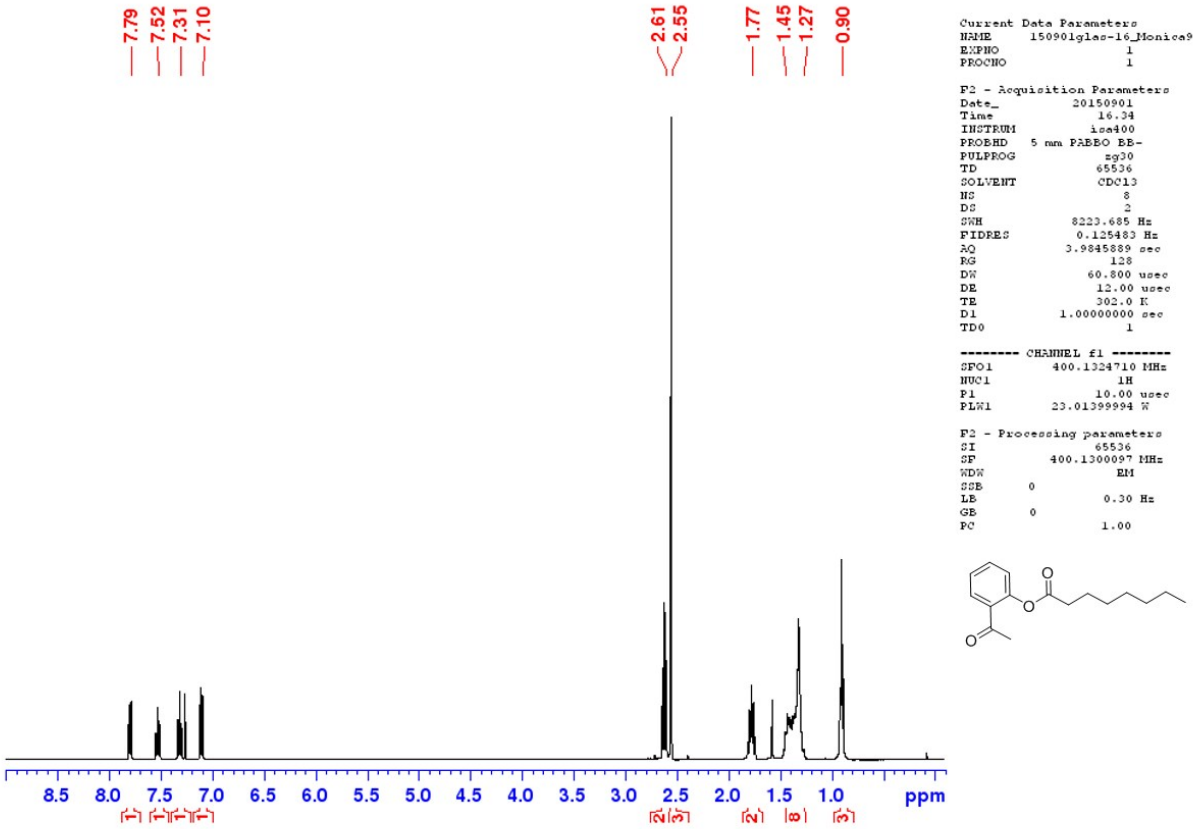
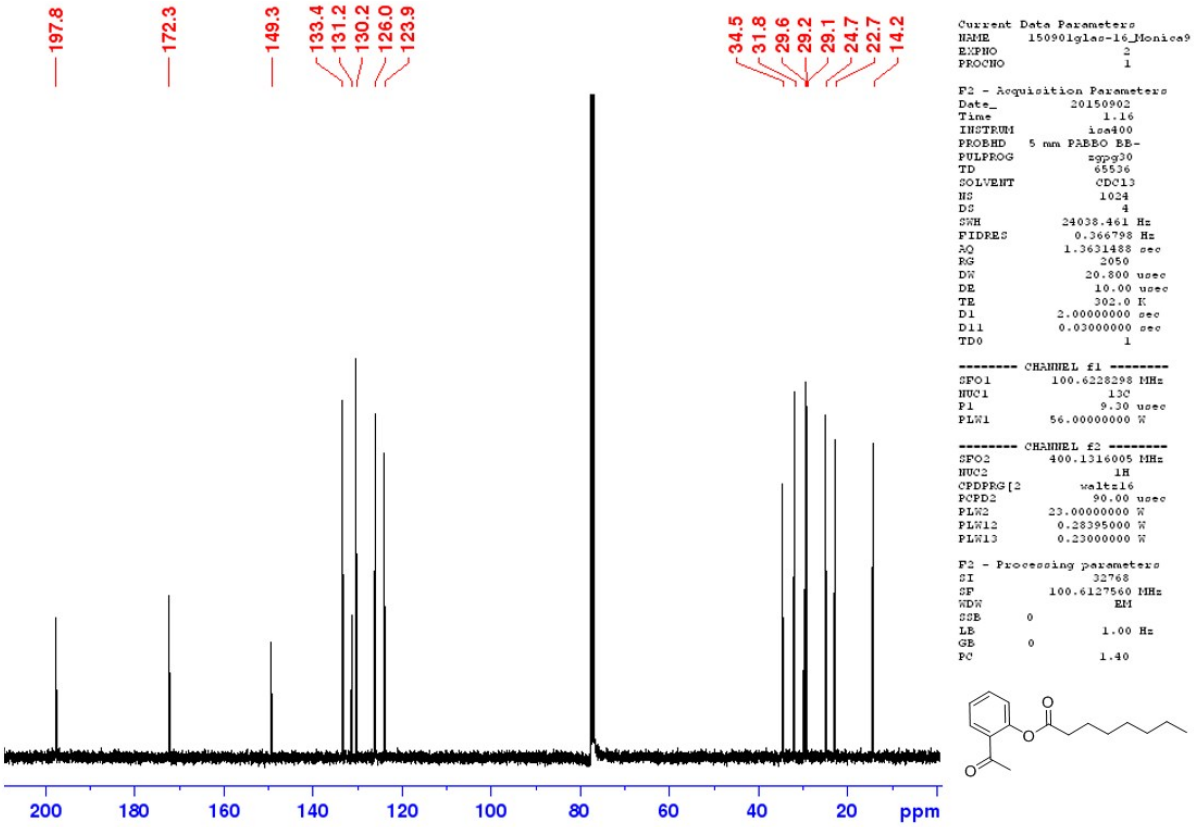
^{1}H- and ^{13}C-NMR spectra of 2-heptyl-3-hydroxy-4-quinolone, PQS in DMSO-d_6 (**2**)



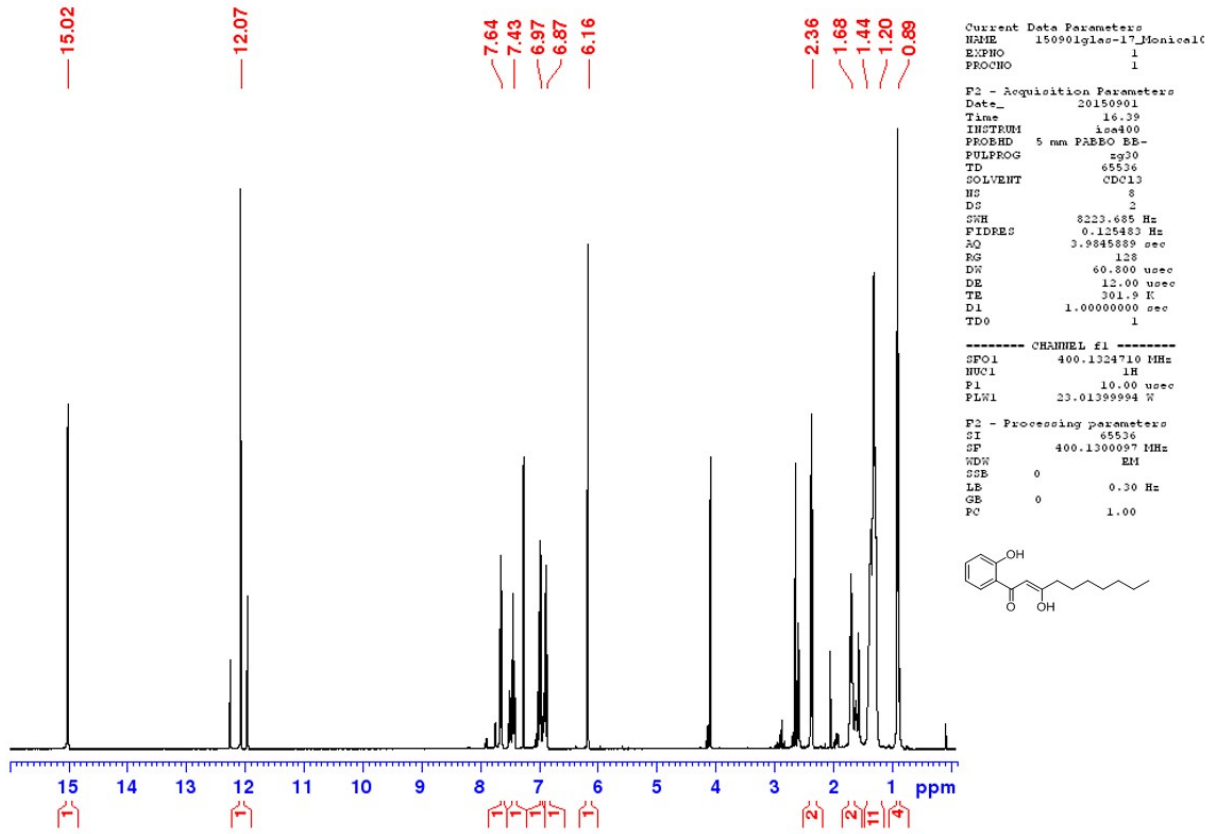


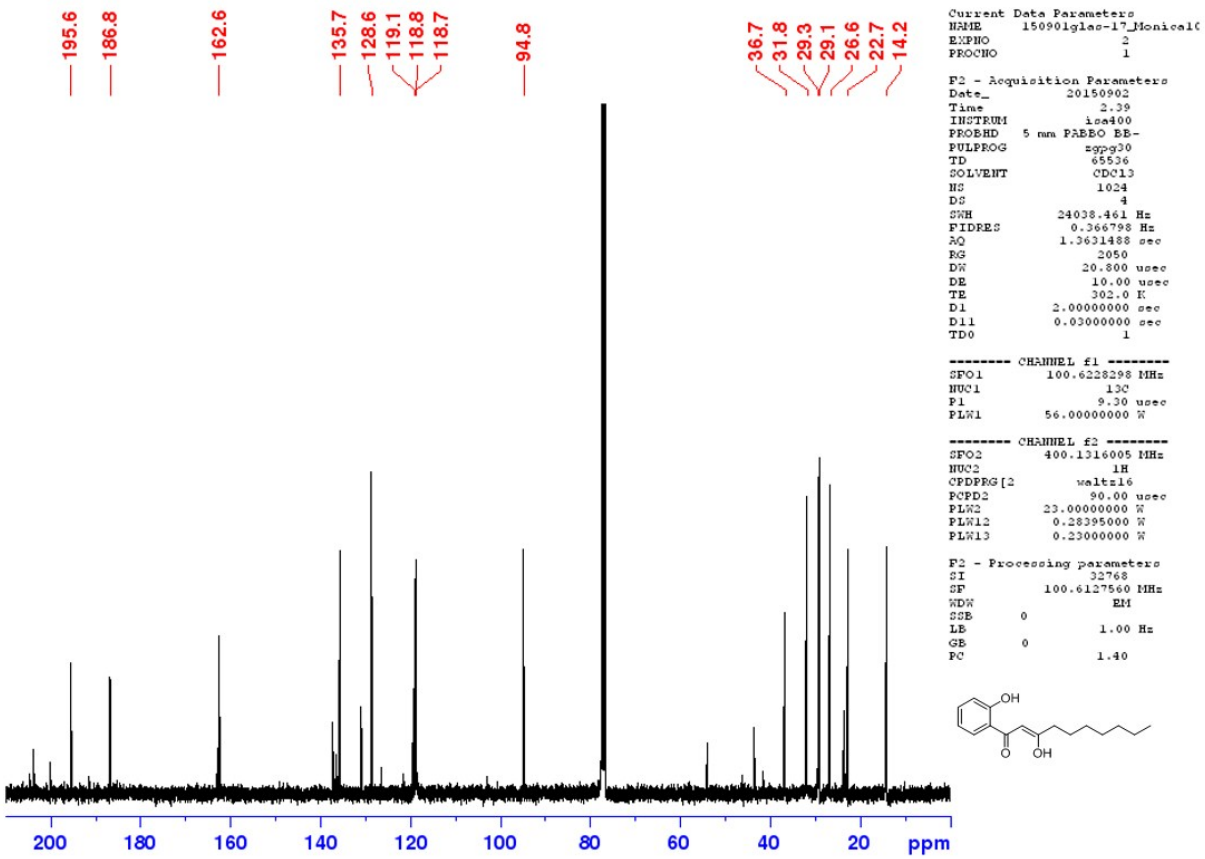
^{1}H- and ^{13}C-NMR spectra of 2-acetylphenyl octanoate in $CDCl_3$ (**3a**)



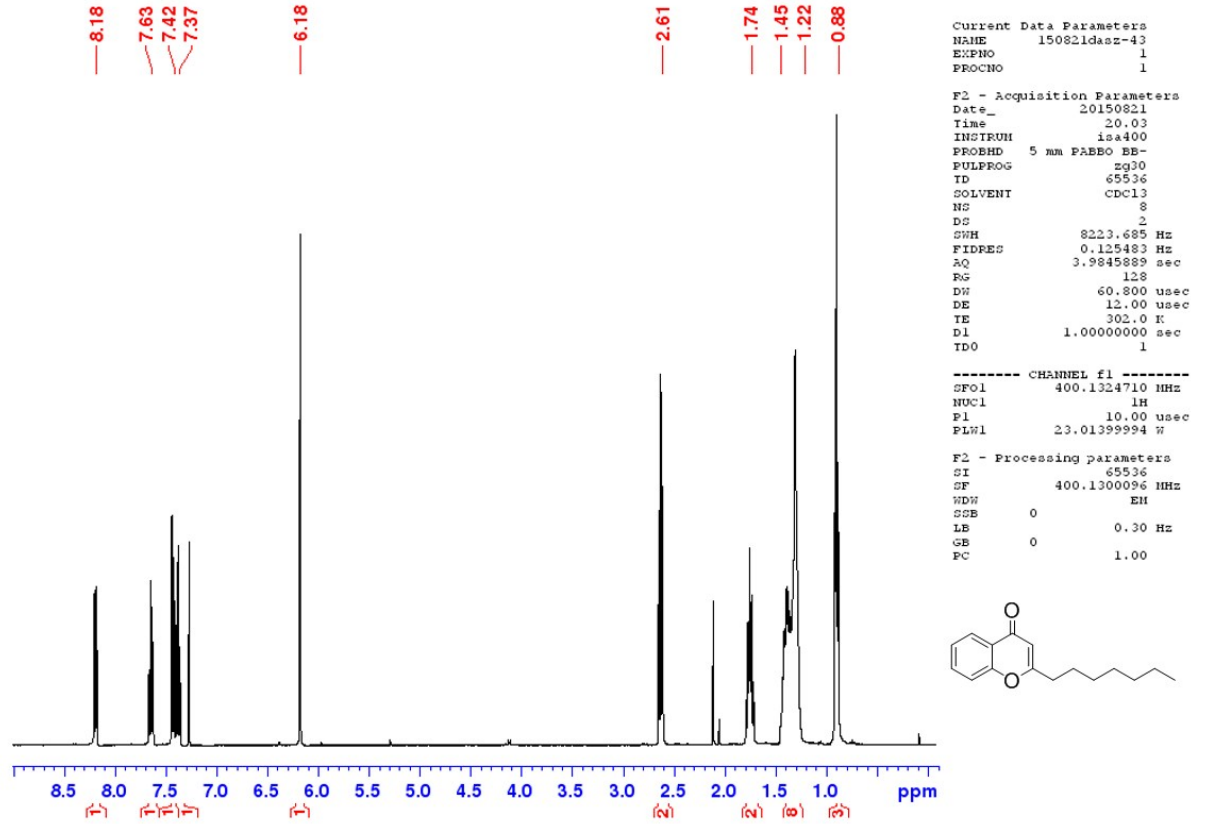


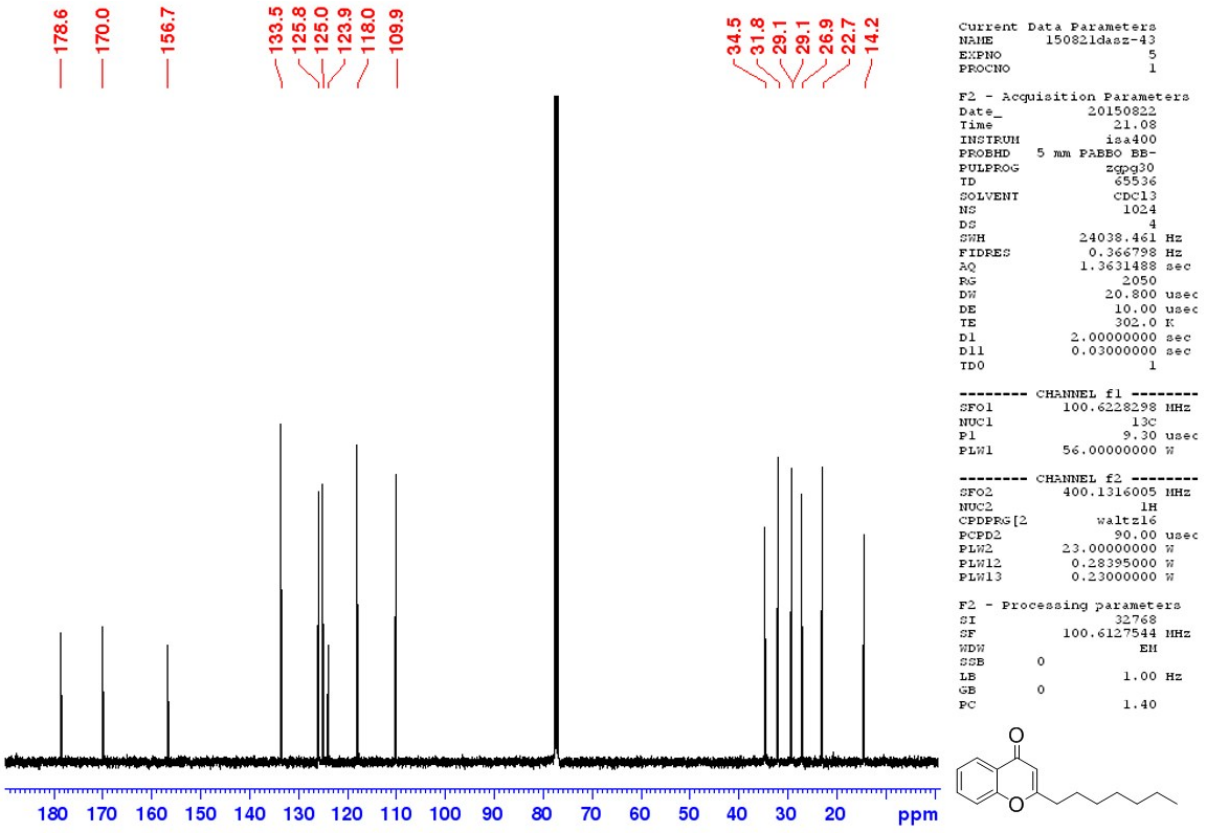
^{1}H- and ^{13}C-NMR spectra of 3-hydroxy-1-(2-hydroxyphenyl)-dec-2-en-1-one in $CDCl_3$ (**3b**)



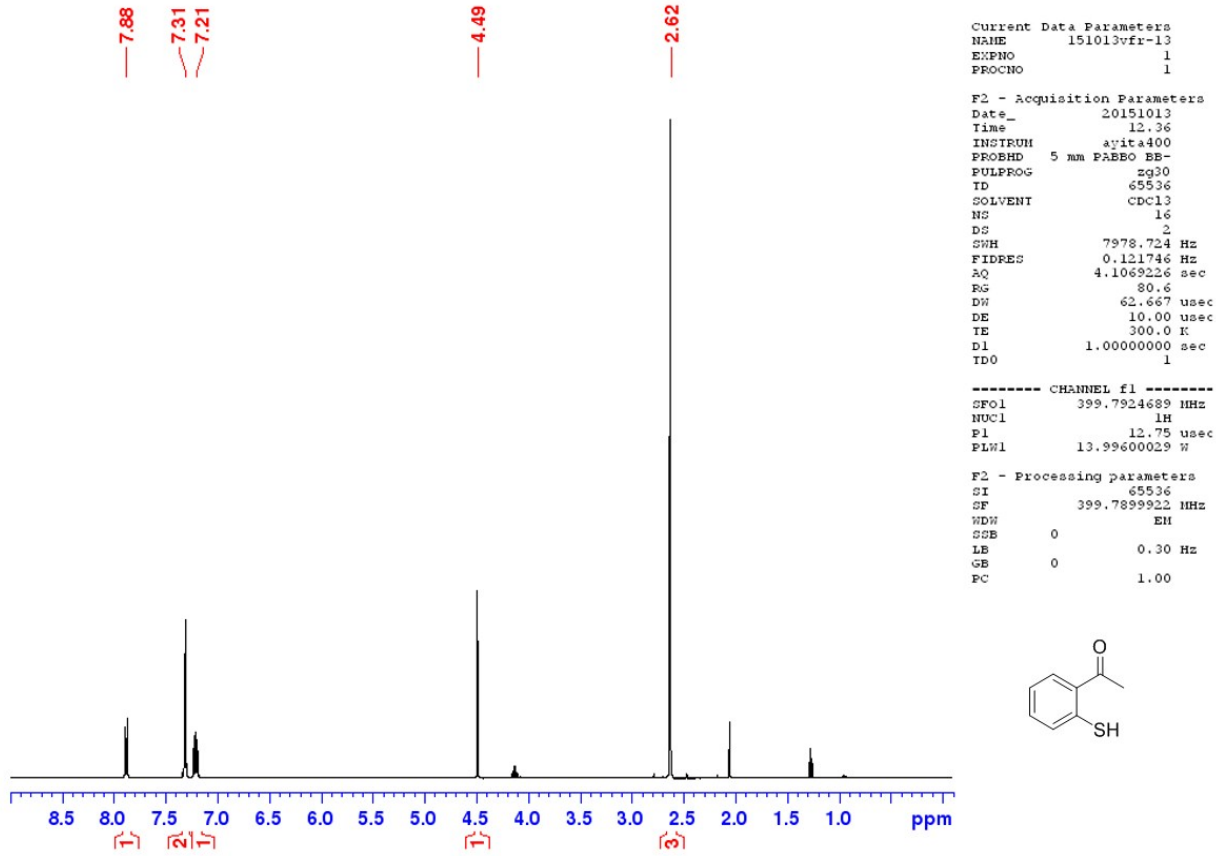


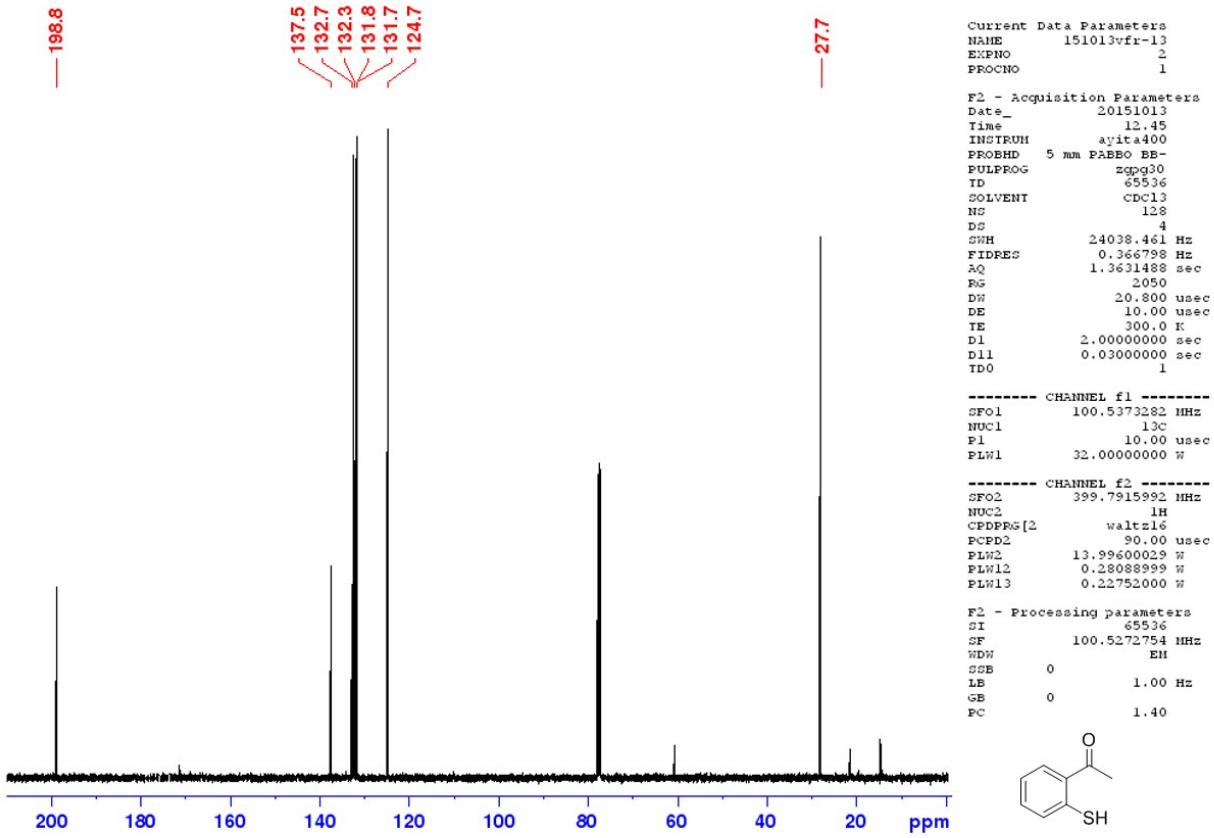
^{1}H- and ^{13}C-NMR spectra of 2-heptyl-chromen-4-one, 1-O-HHQ in $CDCl_3$ (**3**)



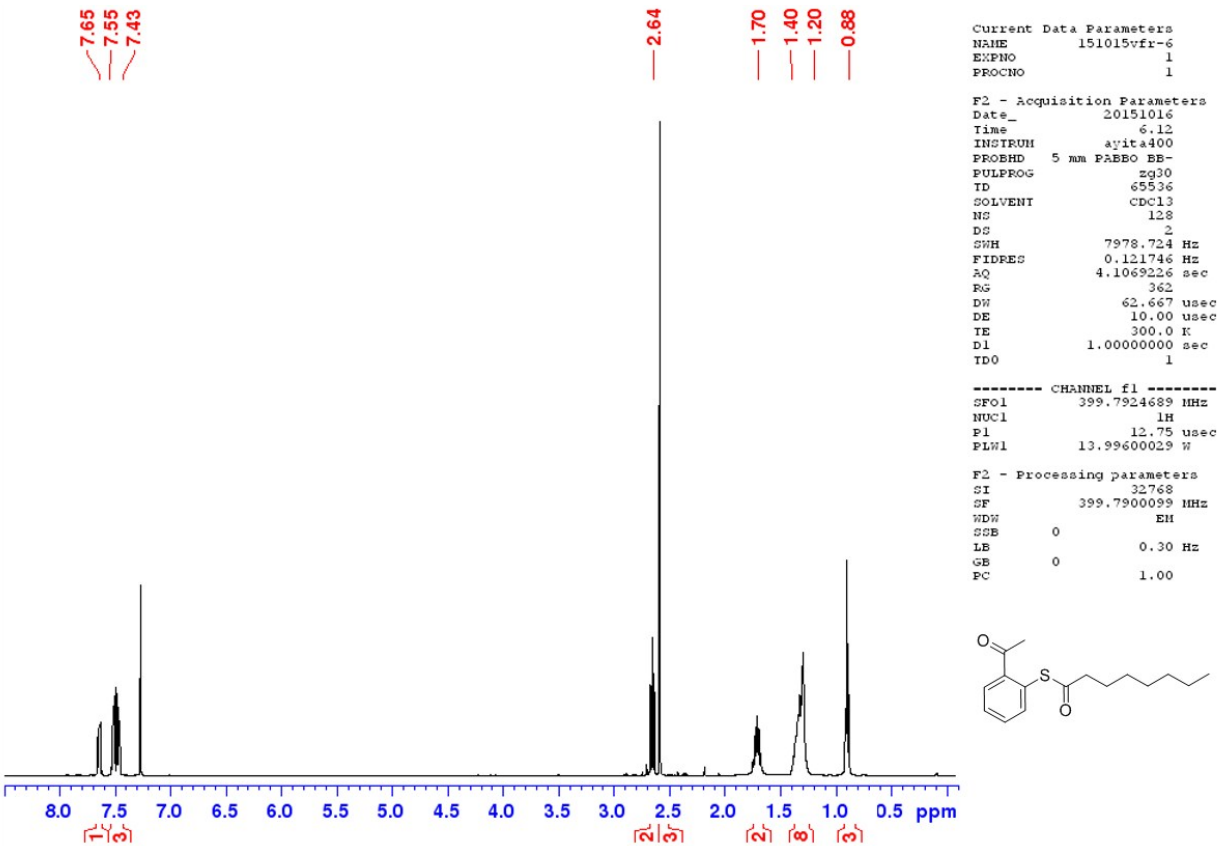


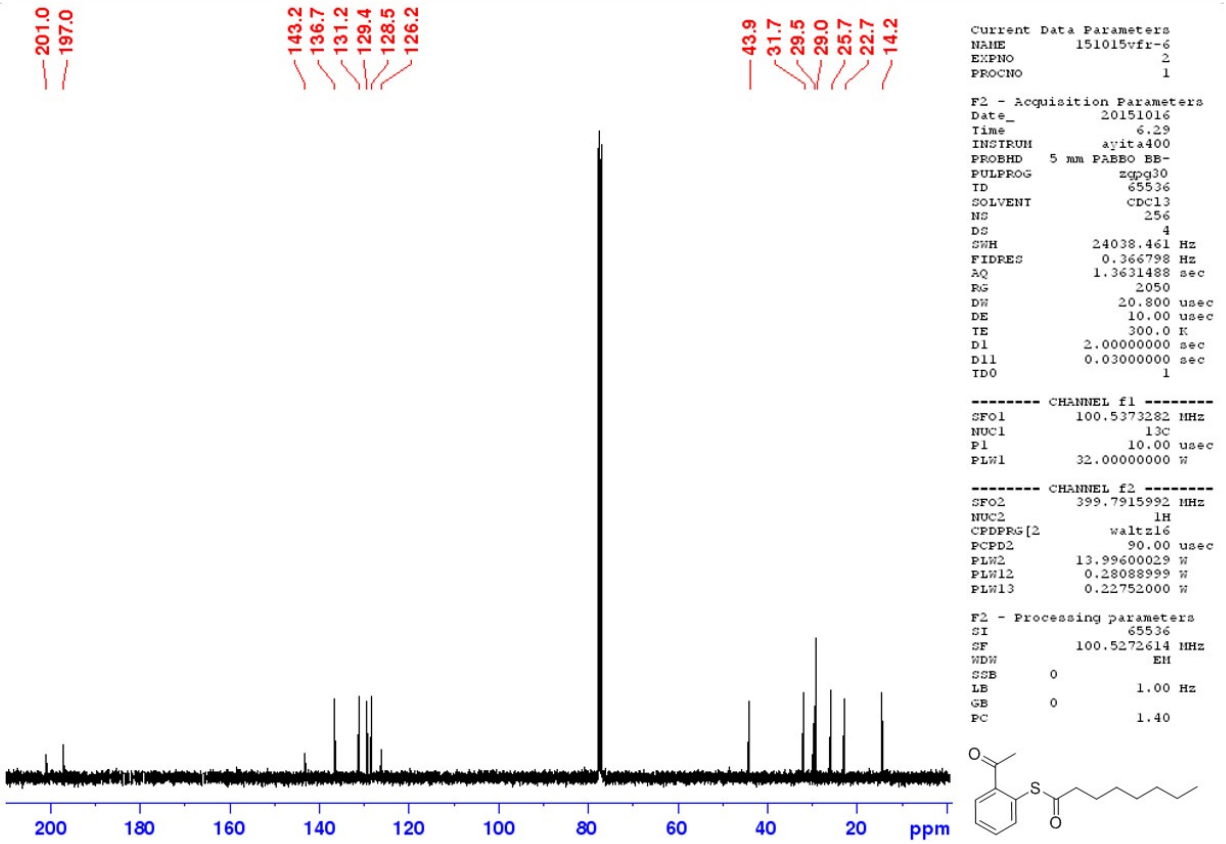
^{1}H- and ^{13}C-NMR spectra of 2-mercaptoacetophenone in $CDCl_3$ (**4a**)



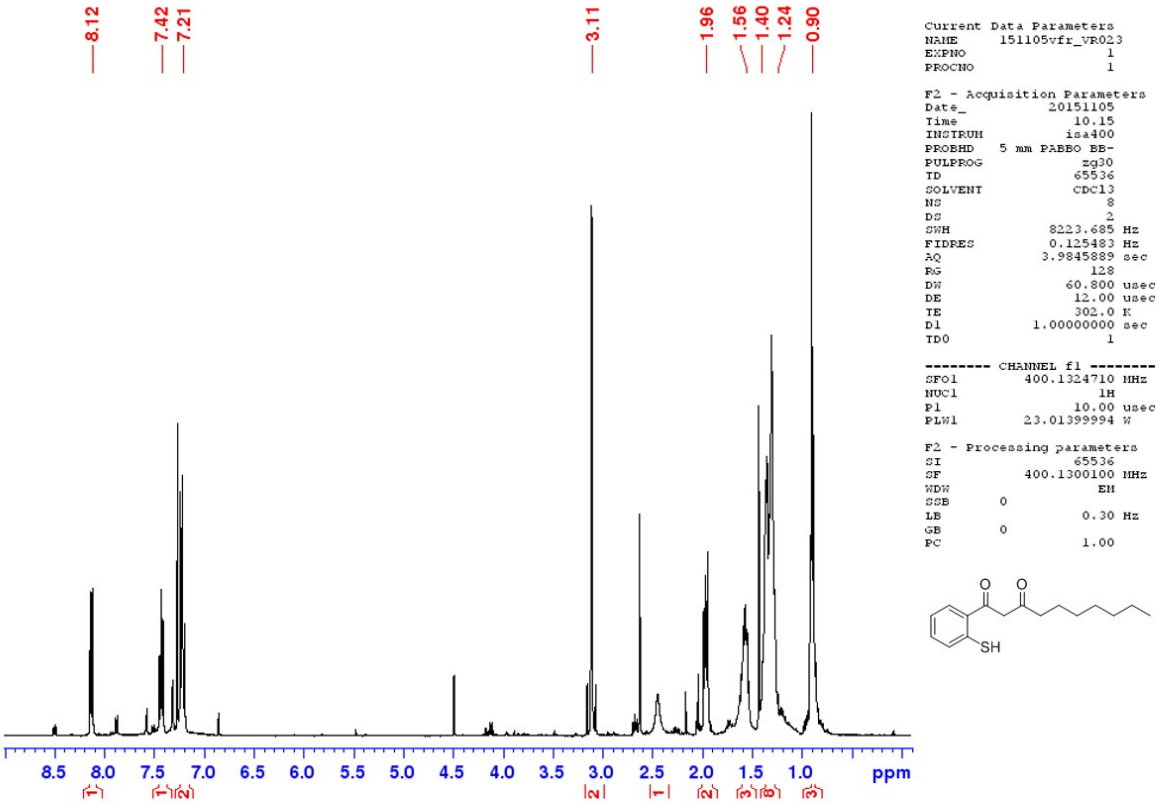


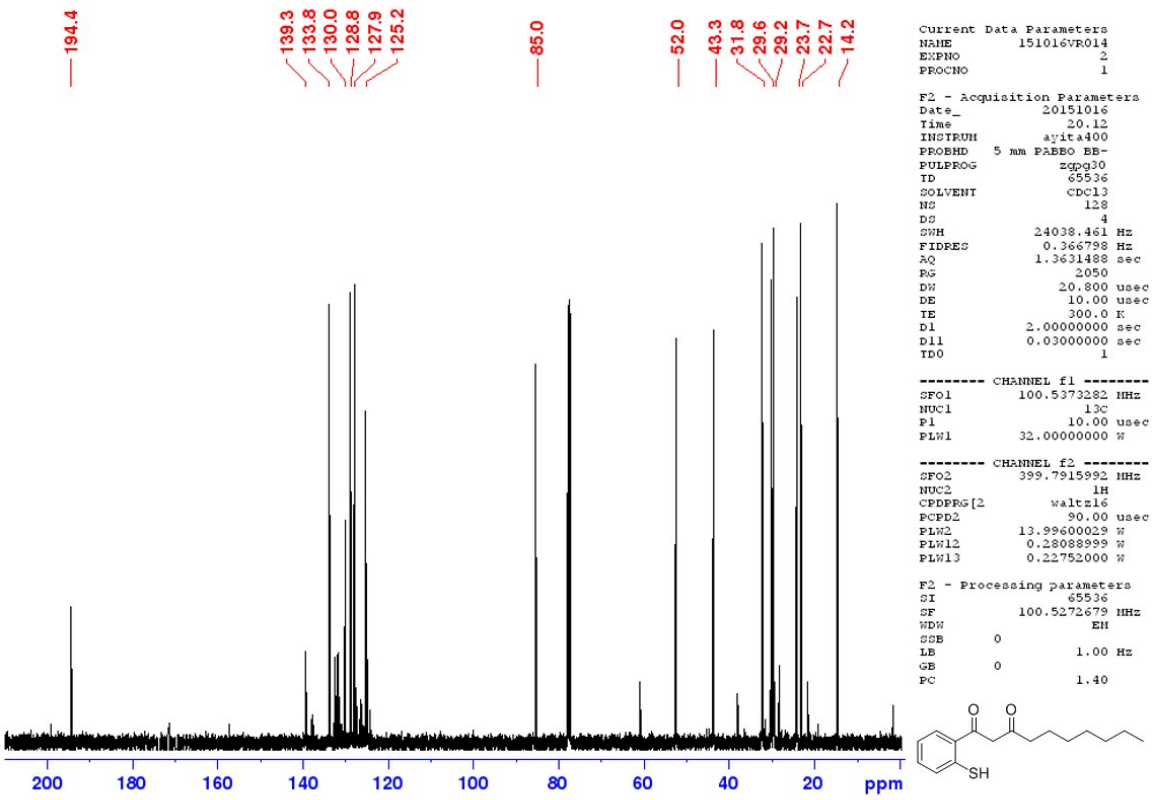
^{1}H- and ^{13}C-NMR spectra of 2-acetylphenyl-octanoic-acid-thioester in $CDCl_3$ (**4b**)



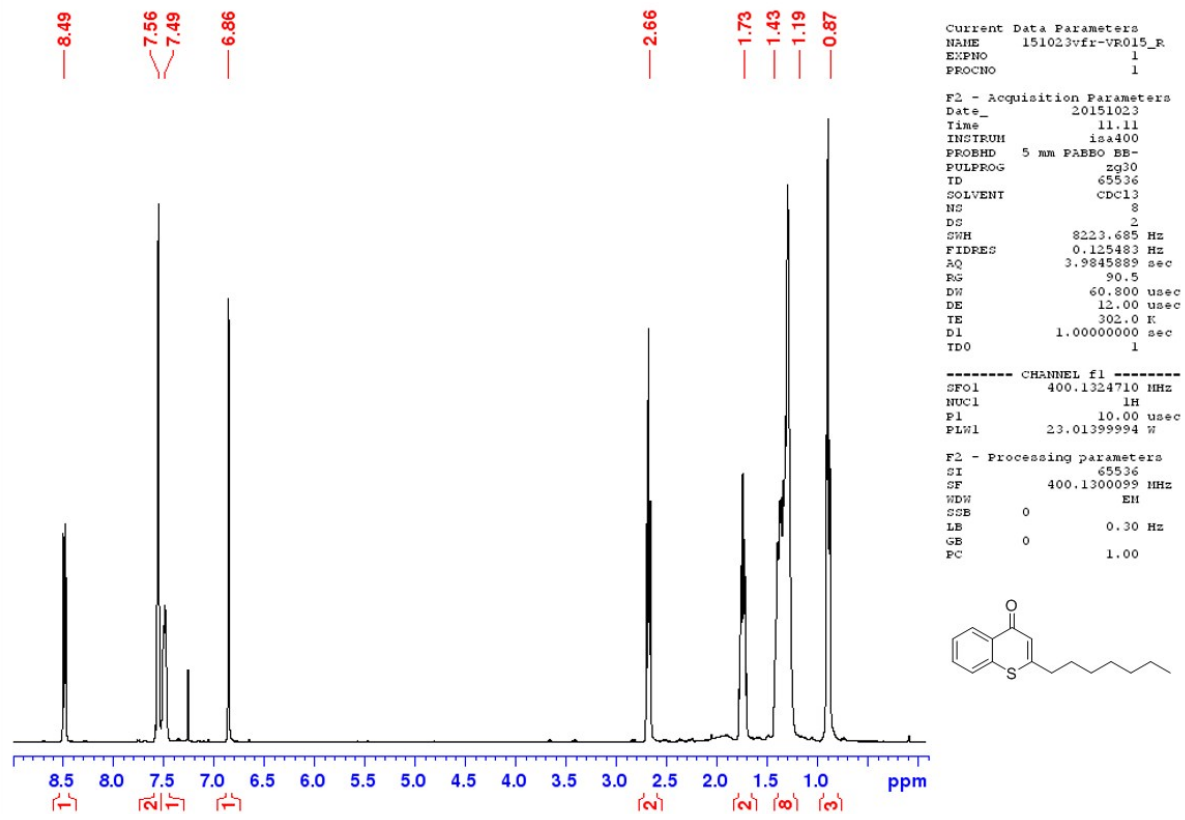


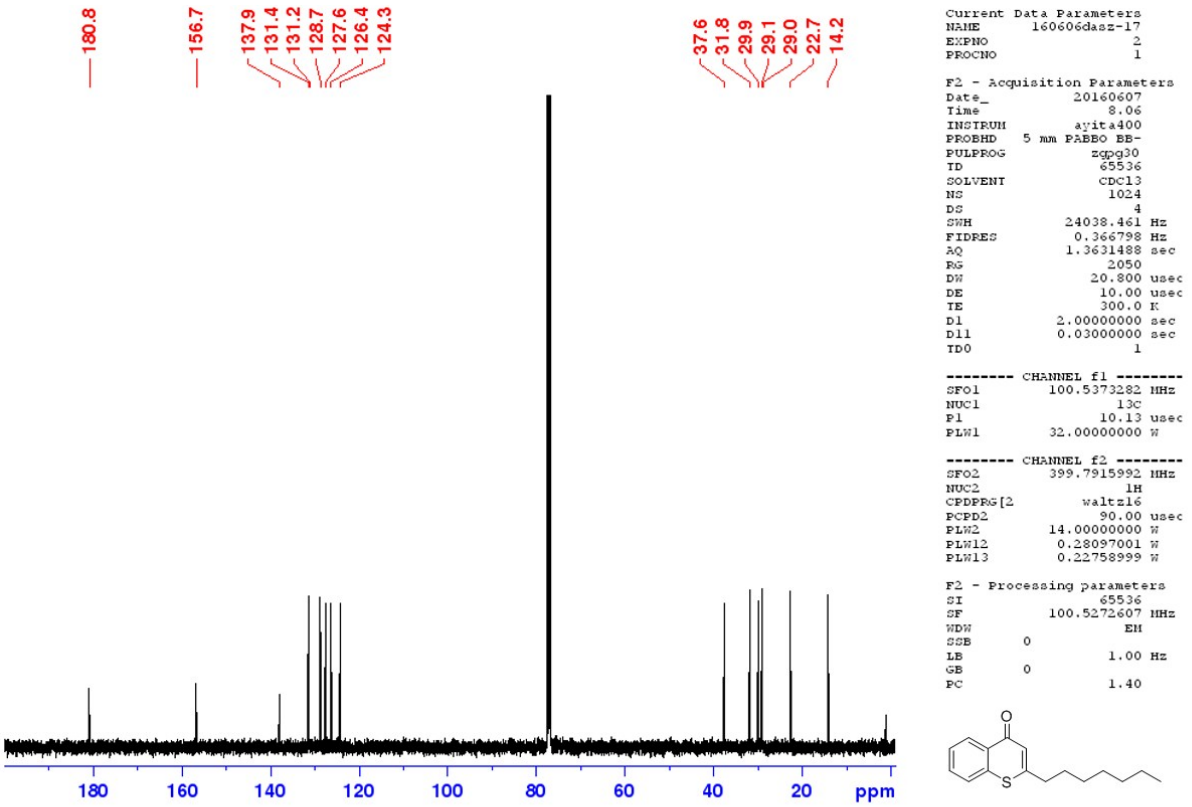
^{1}H- and ^{13}C-NMR spectra of 1-(2-mercaptophenyl)decane-1,3-dione in $CDCl_3$ (**4c**)



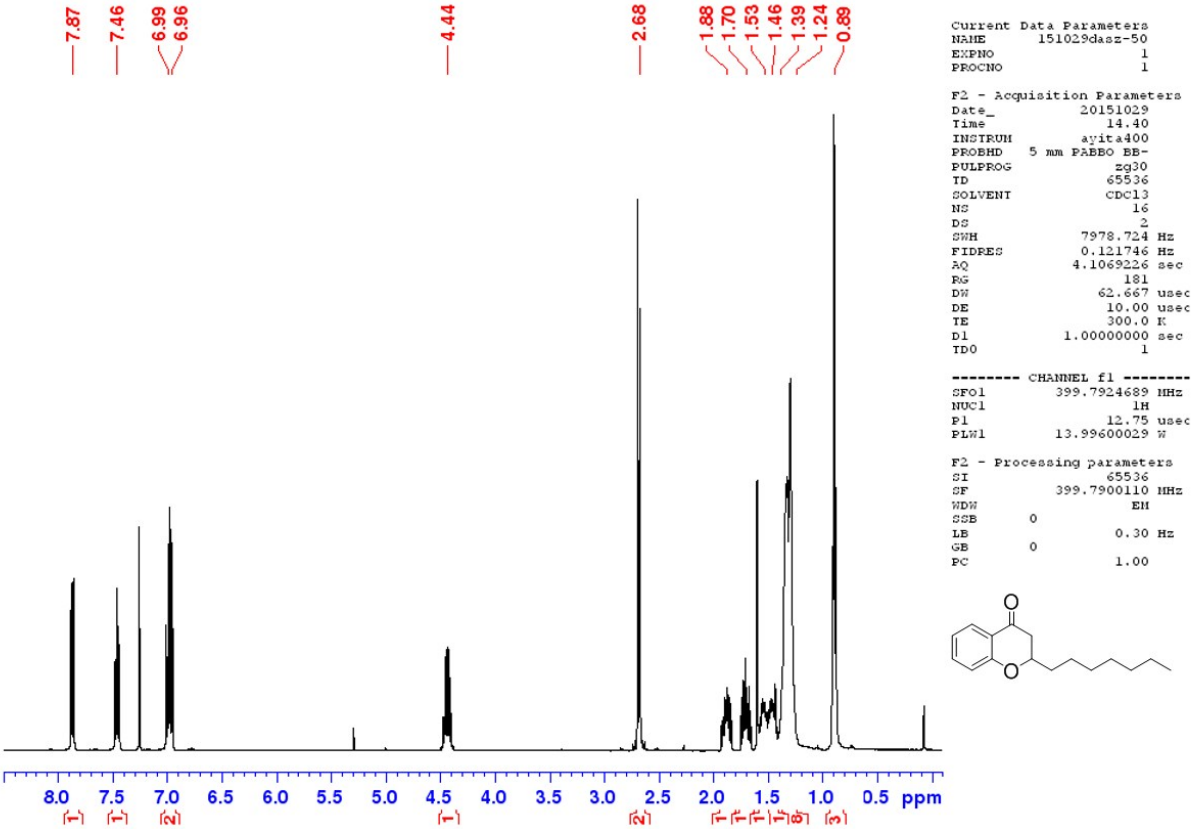


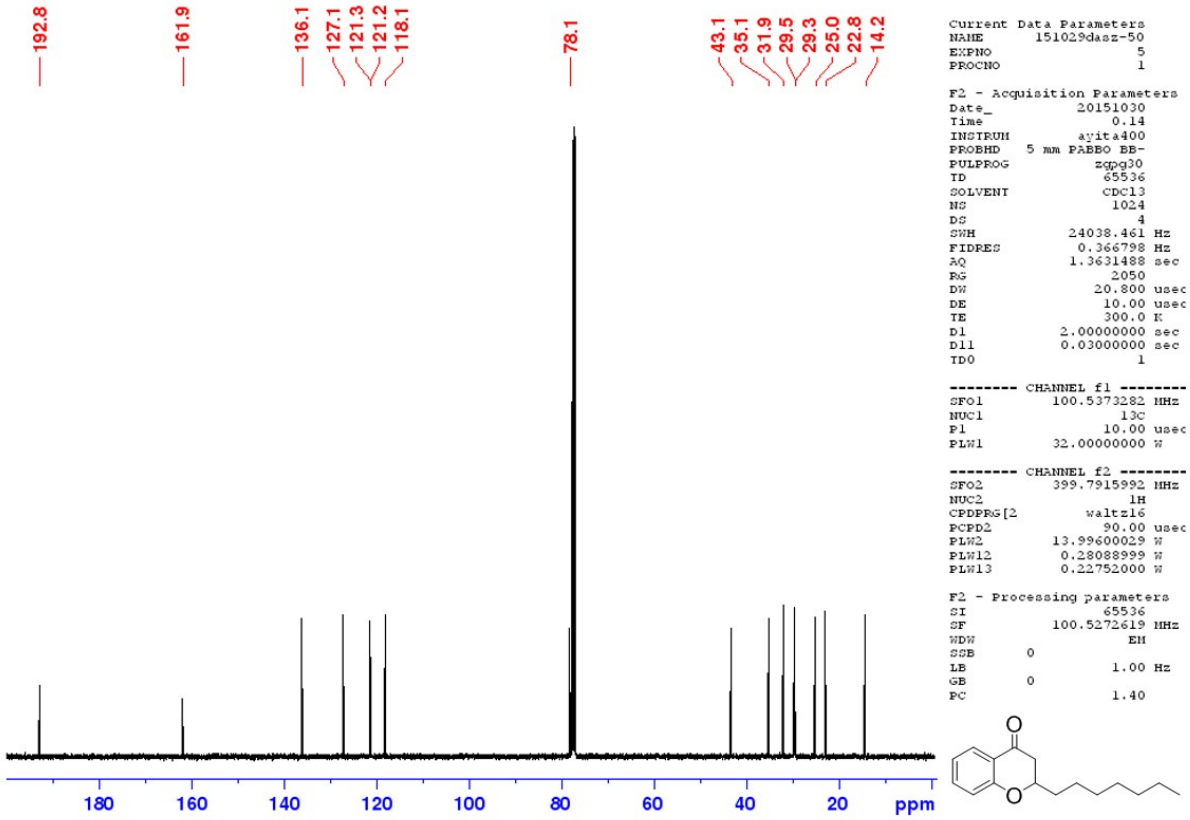
^{1}H- and ^{13}C-NMR spectra of 2-heptyl-4*H*-thiochromen-4-one in $CDCl_3$ (**4**)



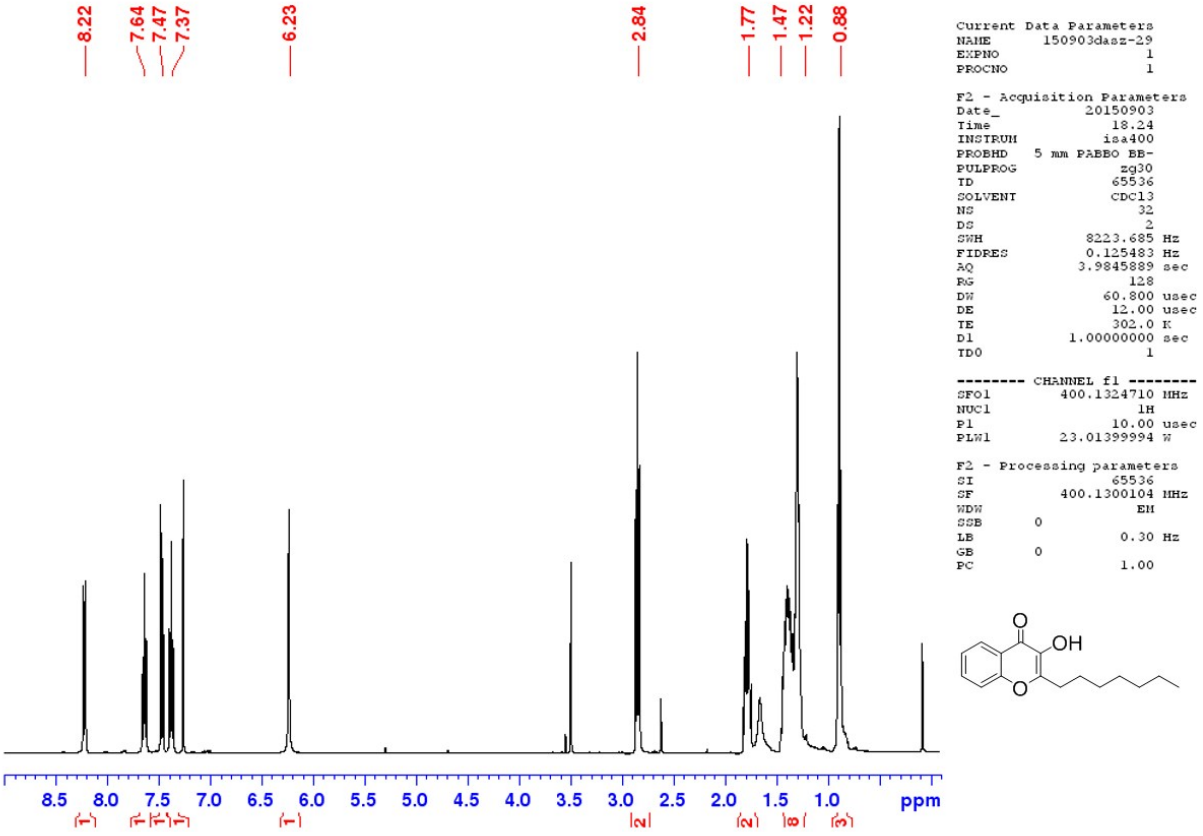


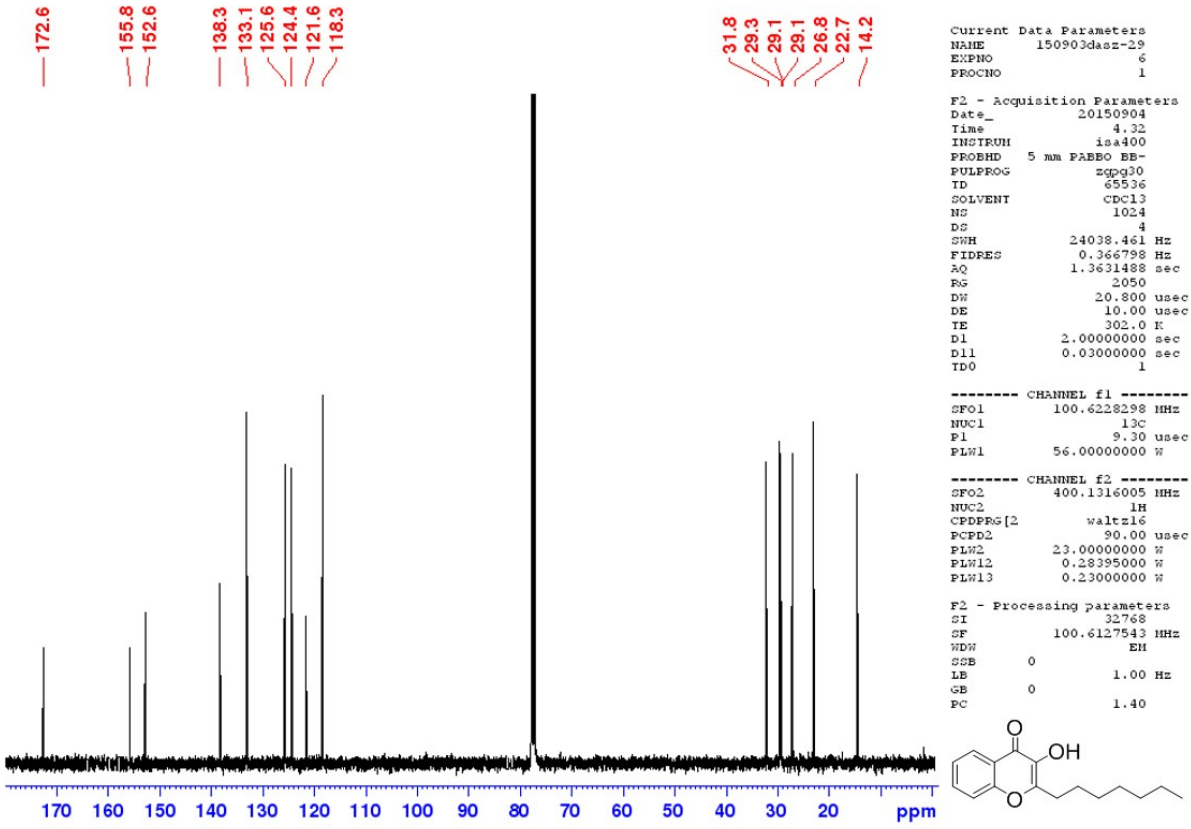
^{1}H- and ^{13}C-NMR spectra of 2-heptylchroman-4-one in $CDCl_3$ (**5a**)



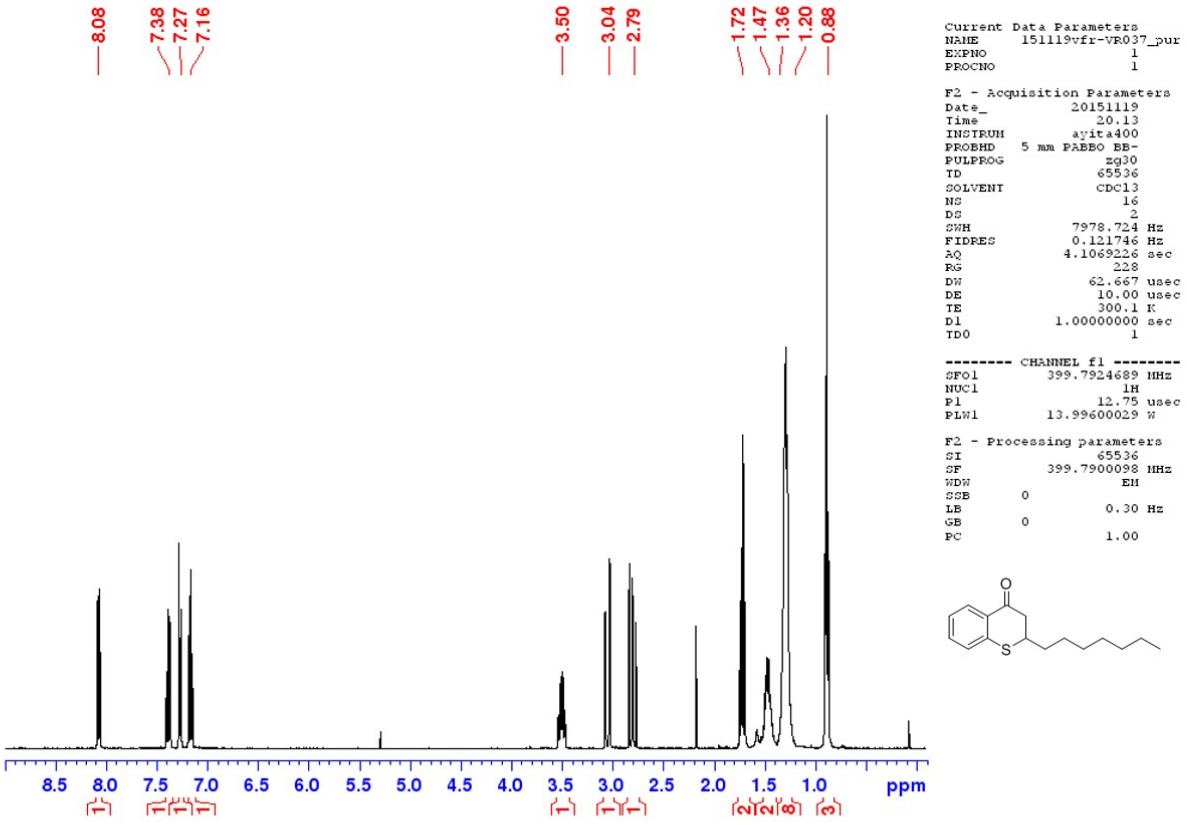


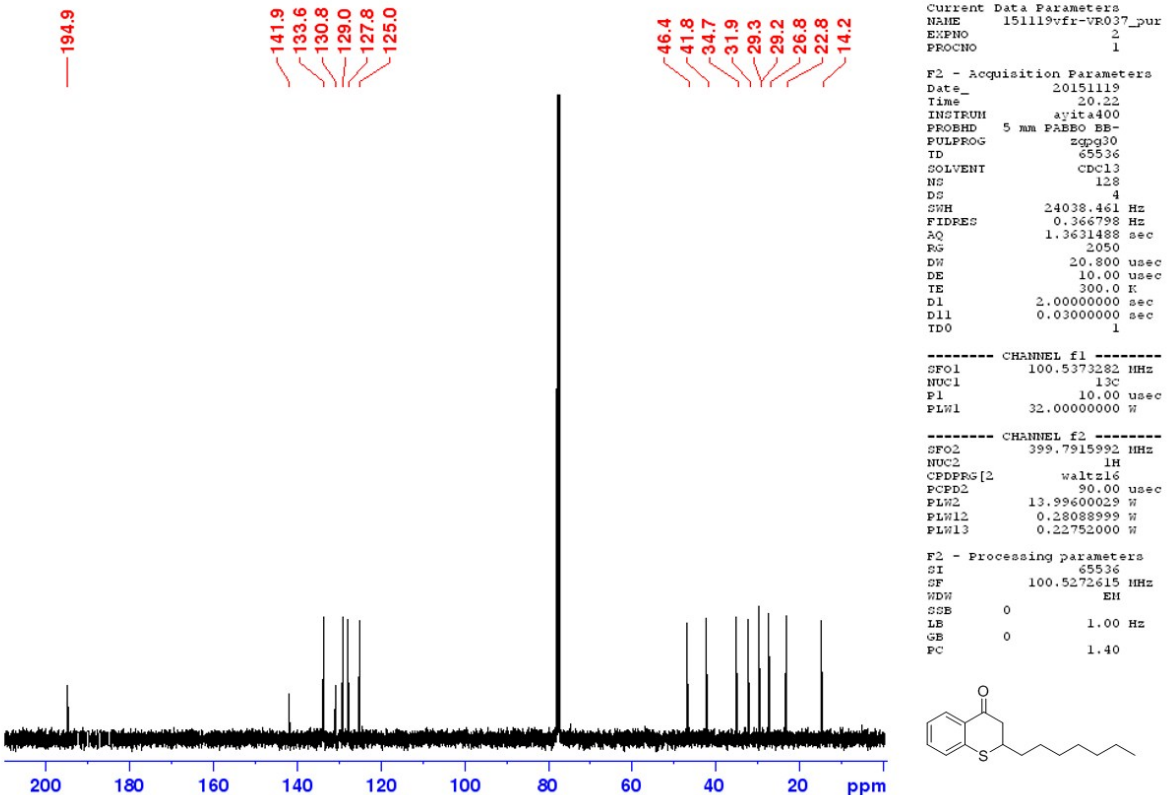
^{1}H- and ^{13}C-NMR spectra of 2-heptyl-3-hydroxy-chromen-4-one, 1-O-PQS in $CDCl_3$ (**5**)



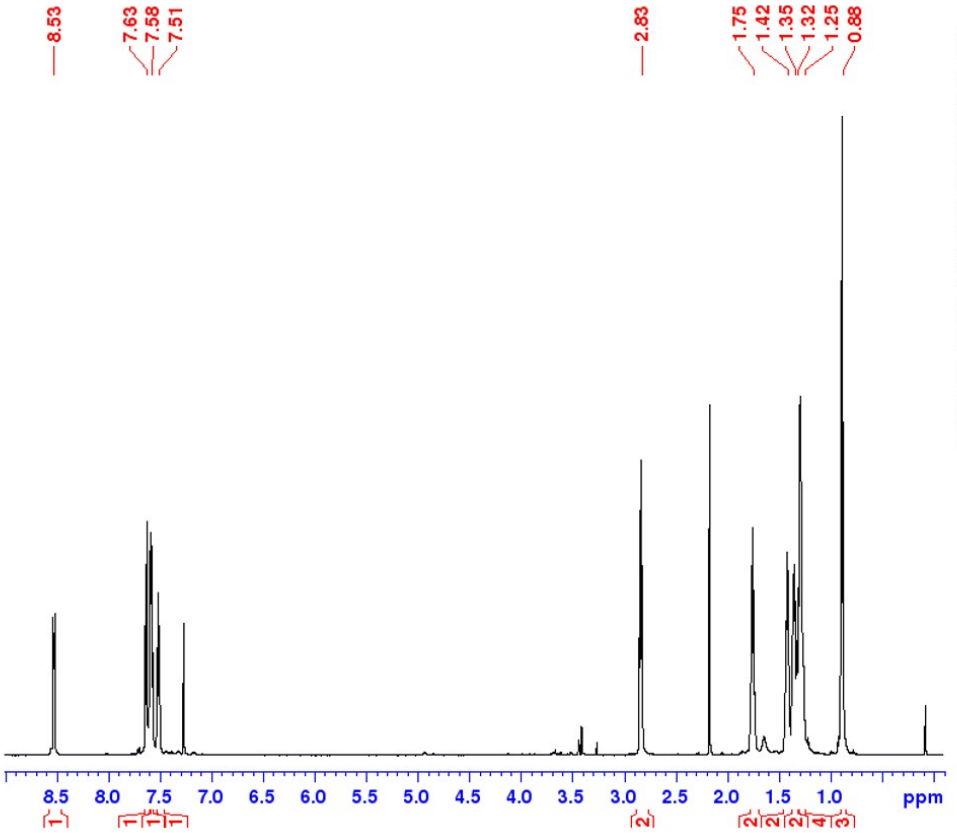


^{1}H- and ^{13}C-NMR spectra of 2-heptylthiochroman-4-one in $CDCl_3$ (**6a**)





^{1}H- and ^{13}C-NMR spectra of 2-heptyl-3-hydroxy-4*H*-thiochromen-4-one in $CDCl_3$ (**6**)

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DE                11.00 usec
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TD0                    1

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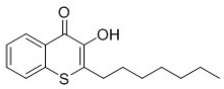

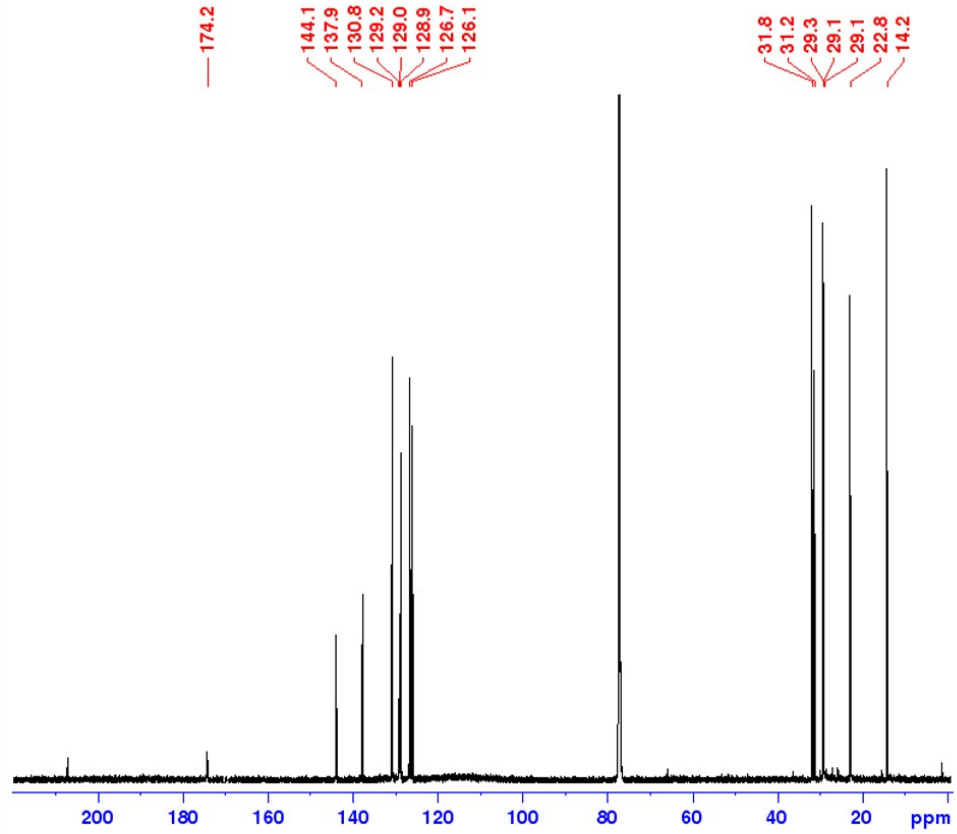


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RG                  1298
DW                13.867 usec
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TE                300.0 K
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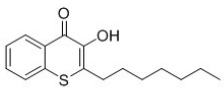
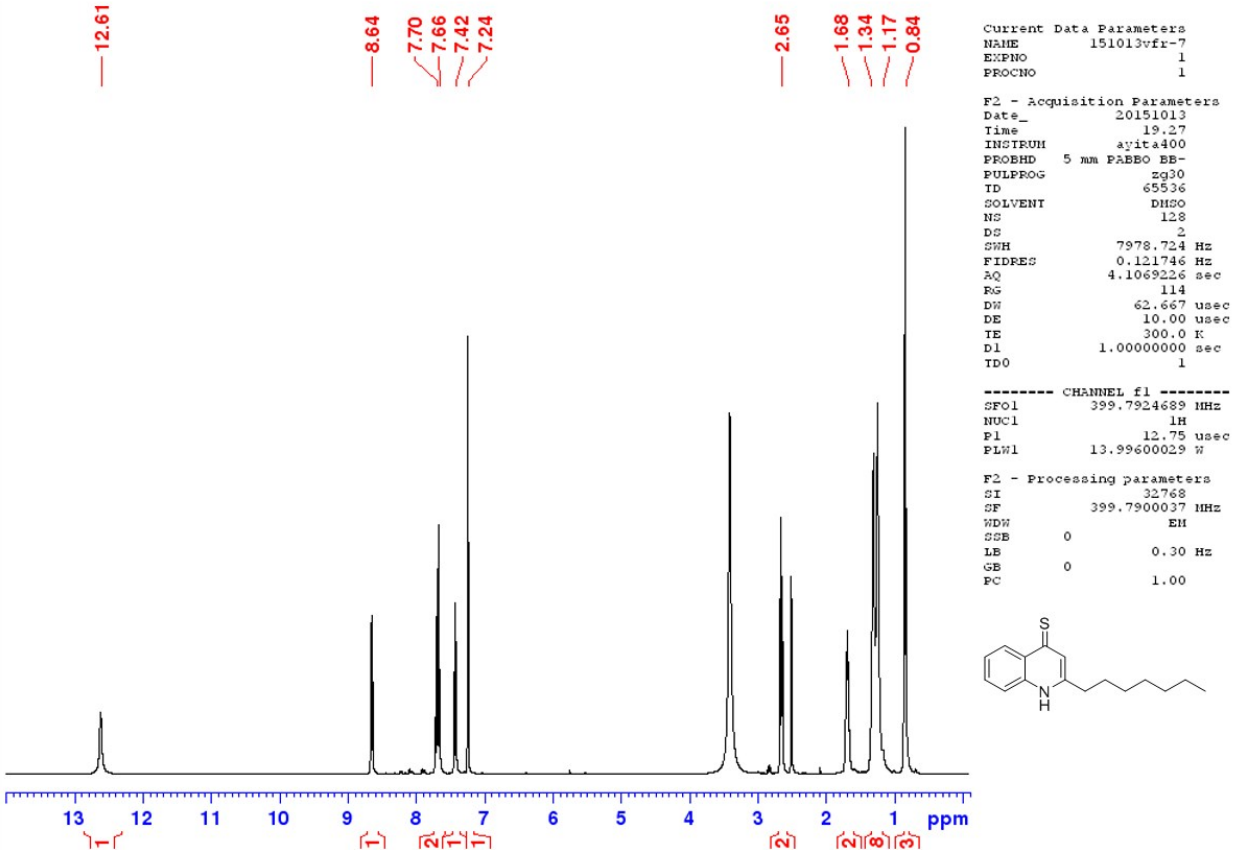
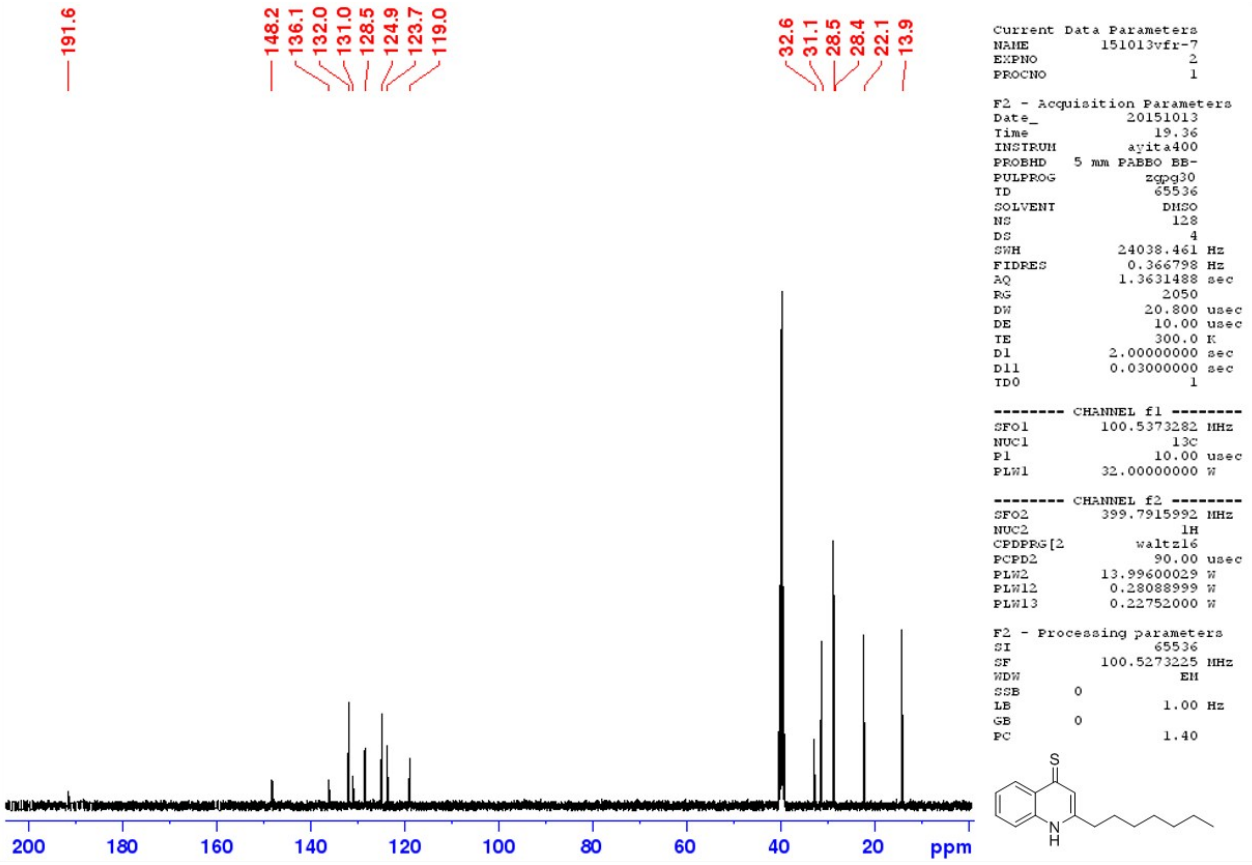
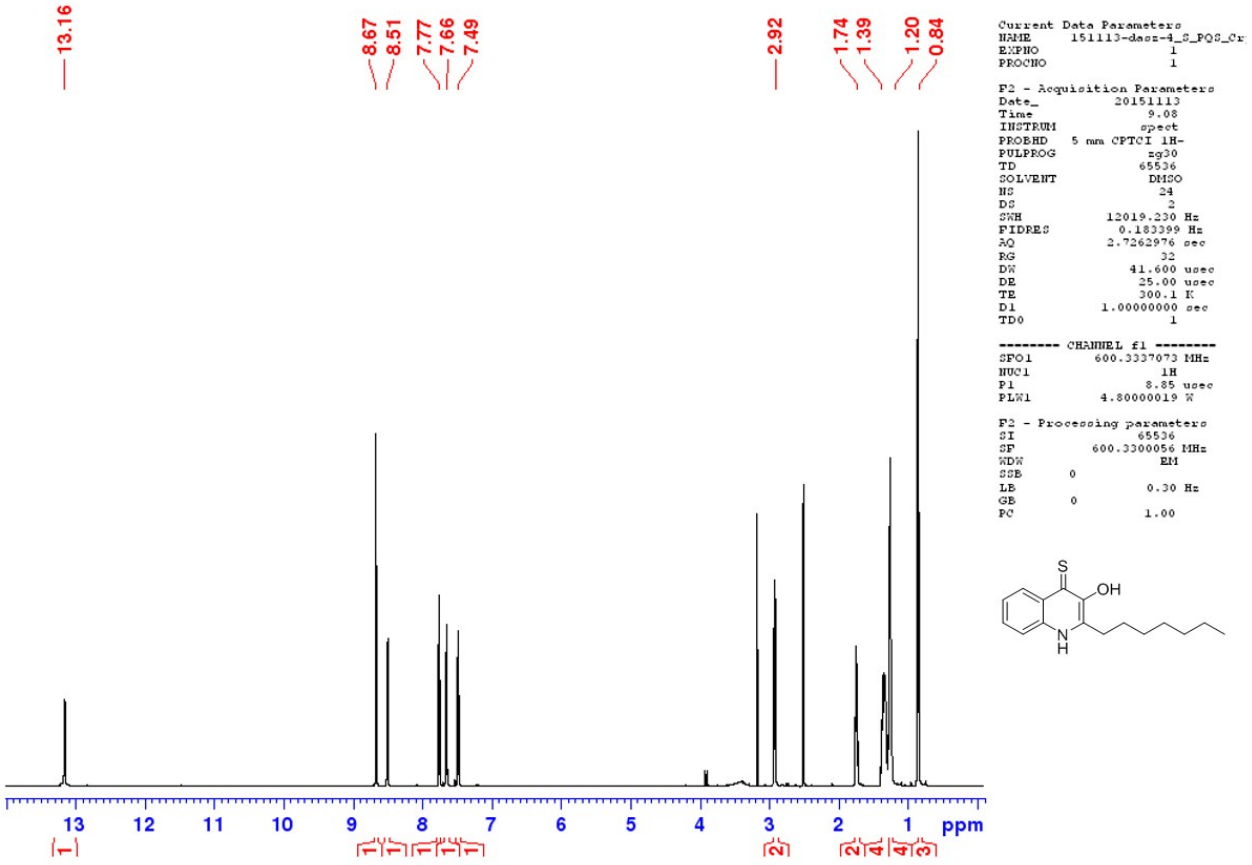


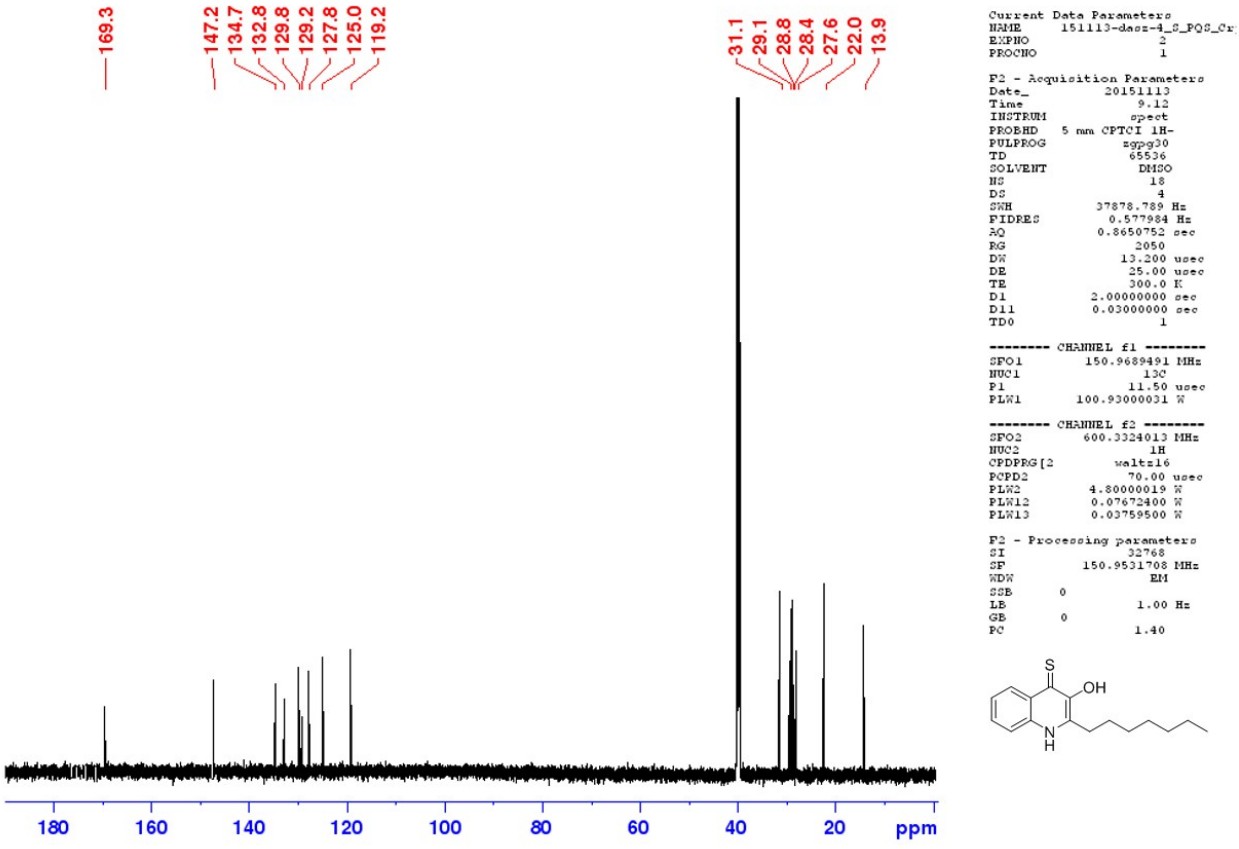
^{1}H- and ^{13}C-NMR spectra of 2-heptylquinoline-4(1*H*)-thione in DMSO-d_6 (**7**)



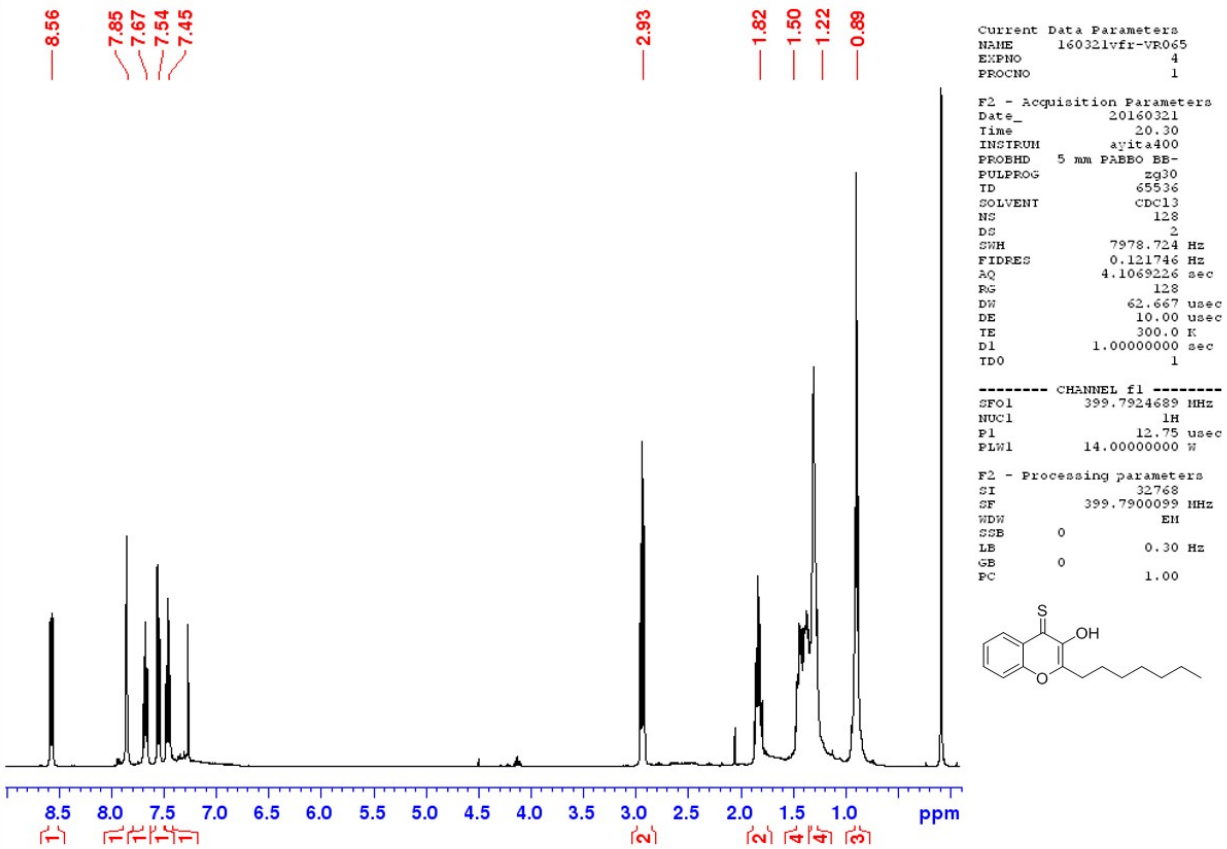


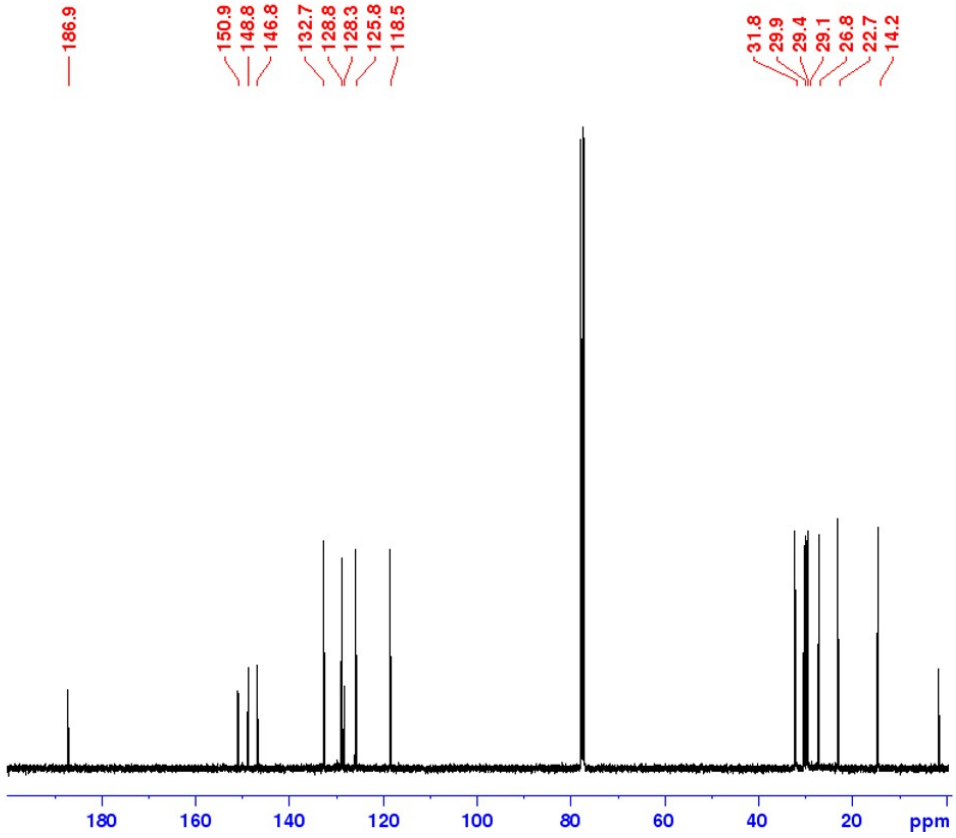
^{1}H- and ^{13}C-NMR spectra of 2-heptyl-3-hydroxyquinoline-4(1*H*)-thione in DMSO-d_6 (**8**)





^{1}H- and ^{13}C-NMR spectra of 2-heptyl-3-hydroxy-4*H*-chromene-4-thione in $CDCl_3$ (**9**)



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TE                300.0 K
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D11          0.03000000 sec
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PLW2        14.00000000 W
PLW12        0.28097001 W
PLW13        0.22758999 W

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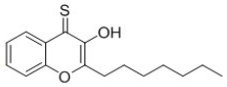
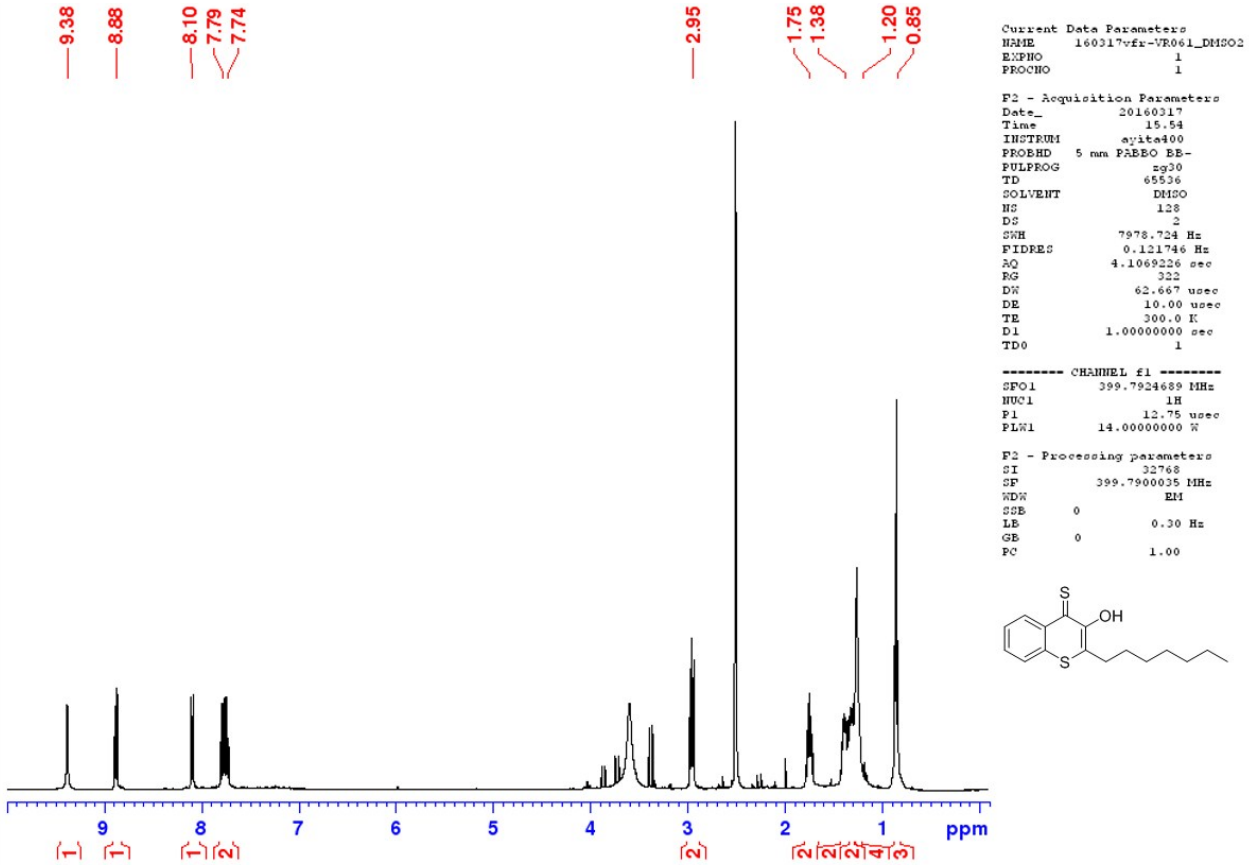
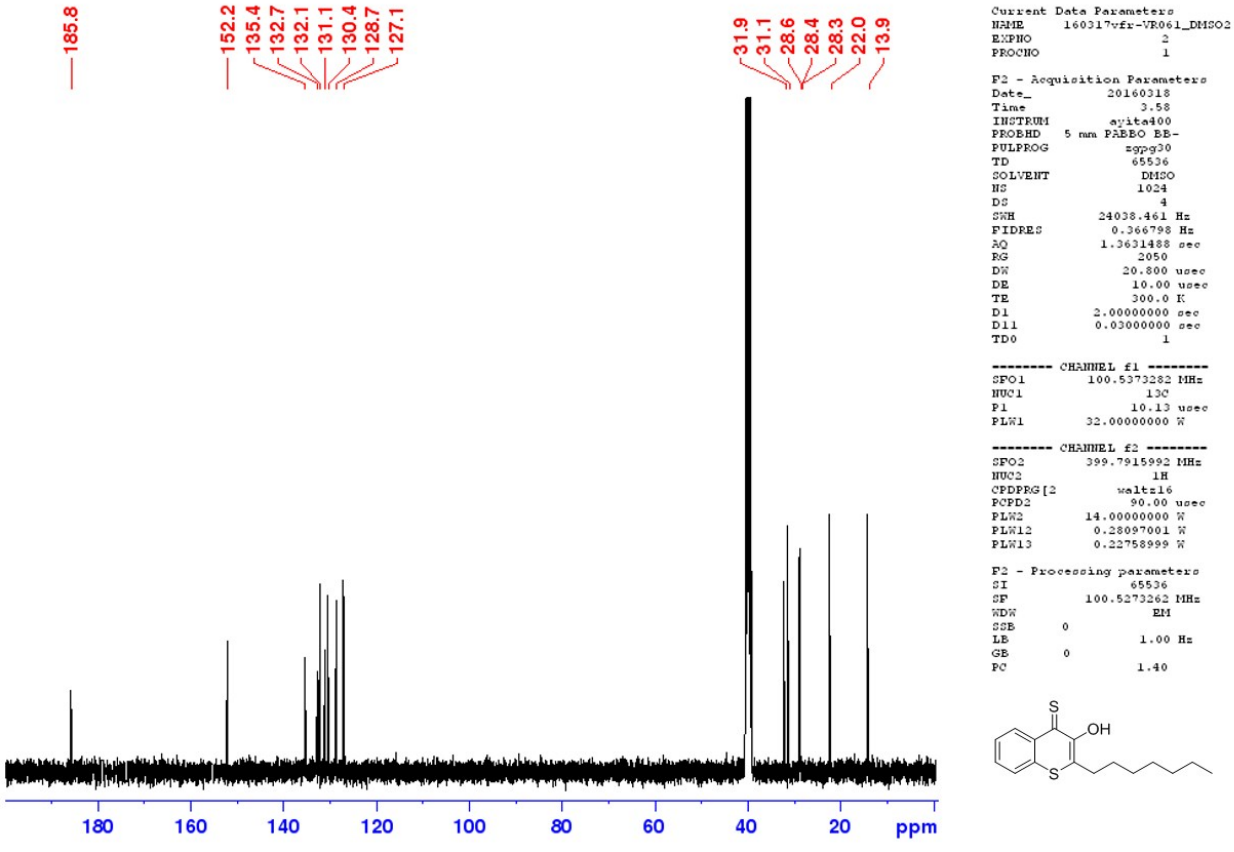
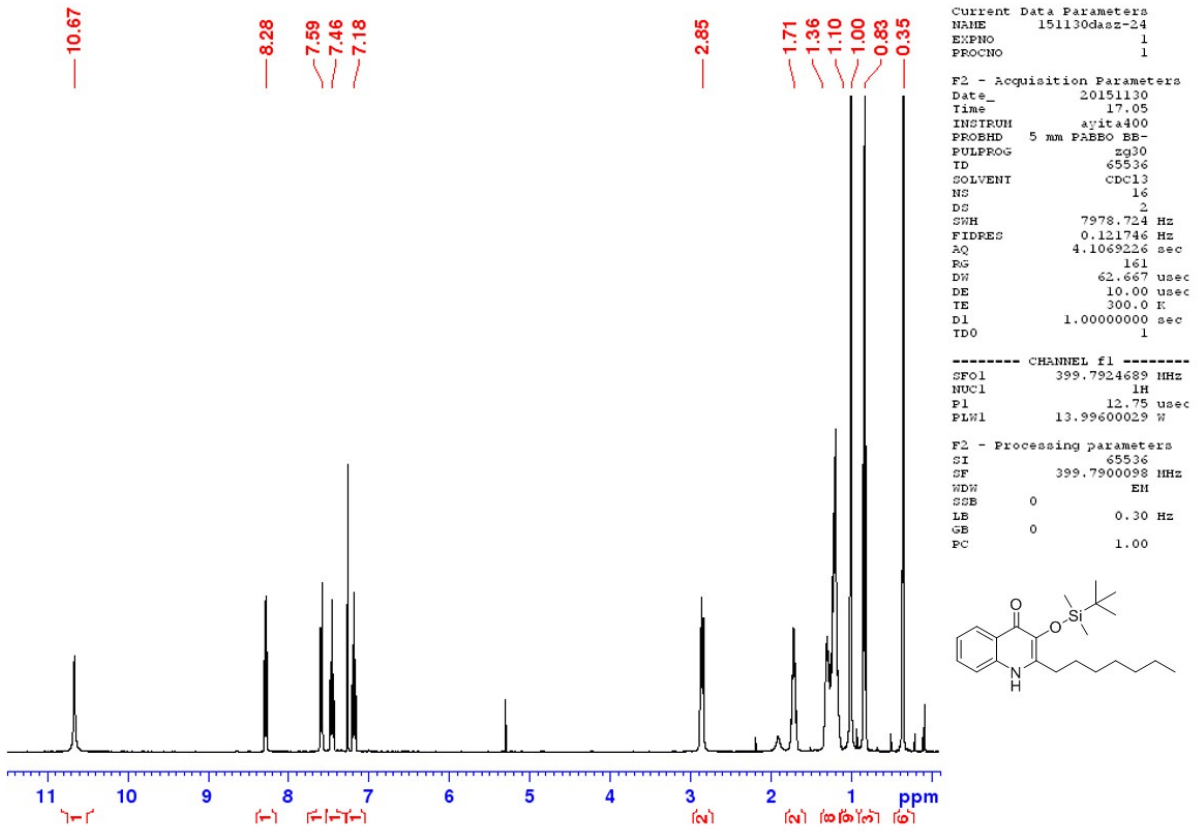


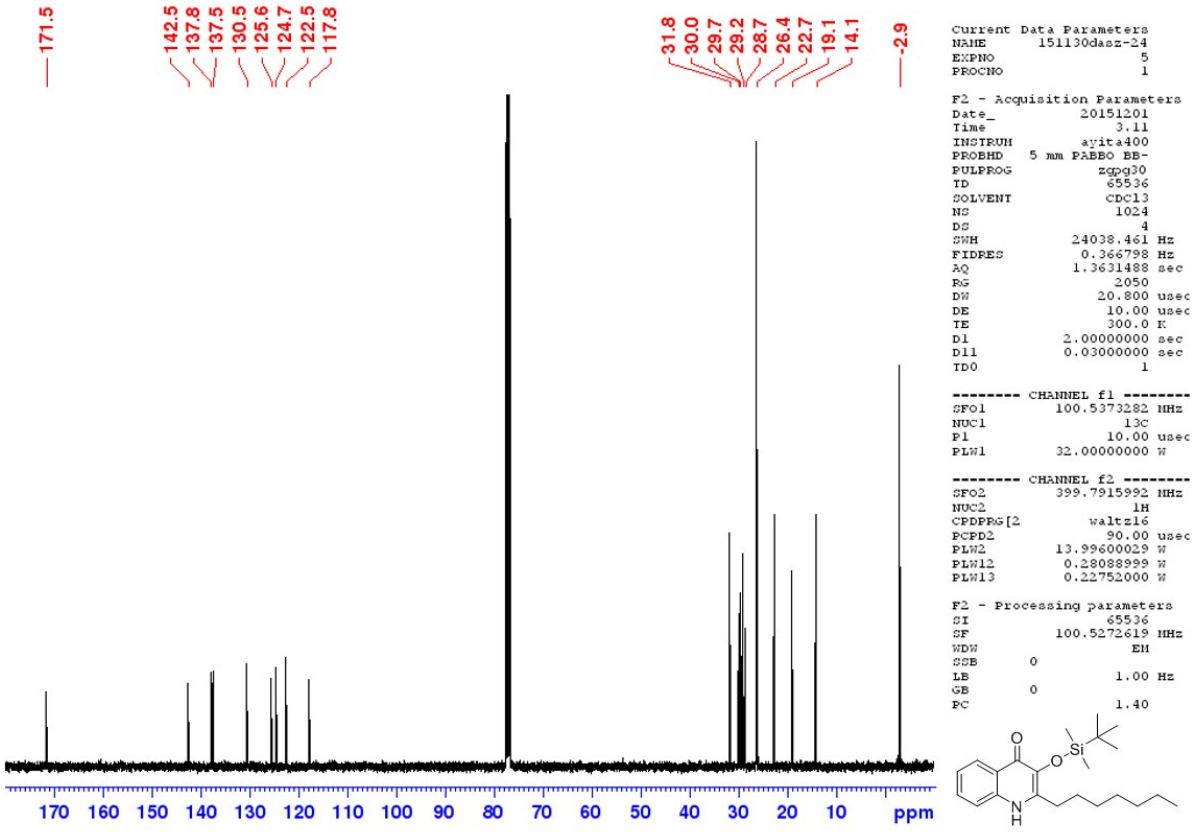
^{1}H-and ^{13}C-NMR spectra of 2-Heptyl-3-hydroxy-4*H*-thiochromene-4-thione in DMSO-d_6 (**10**)



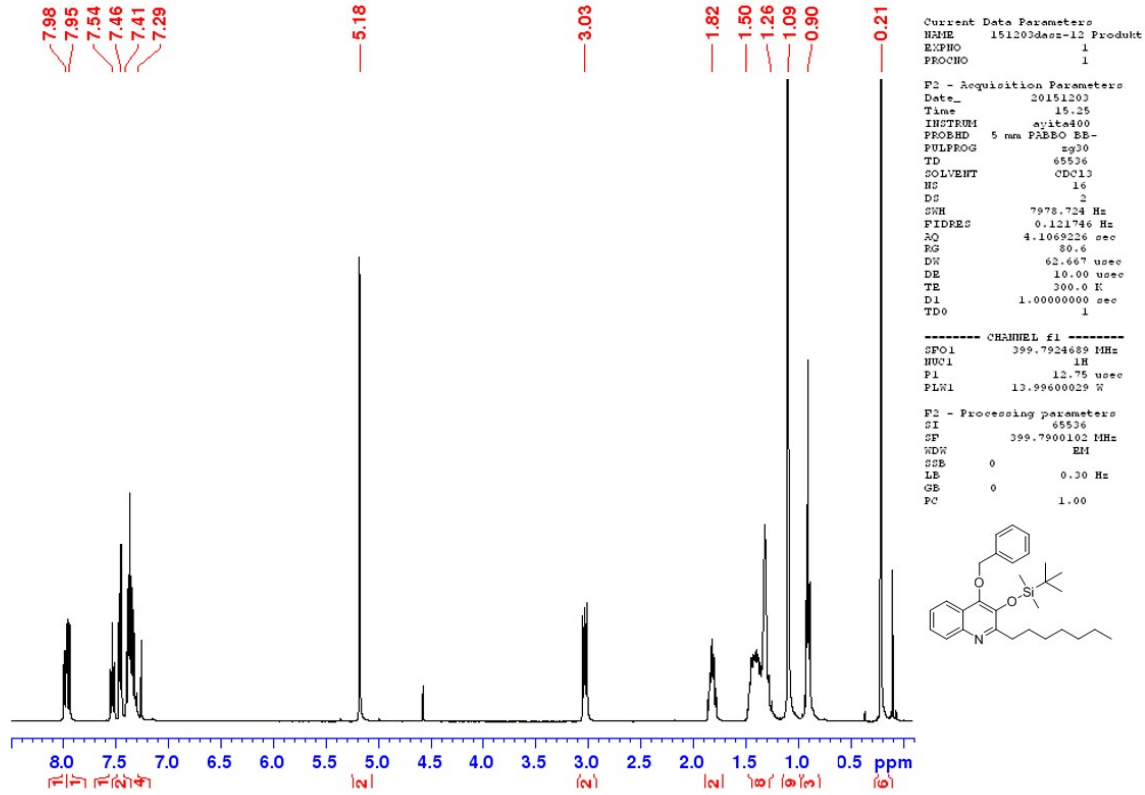


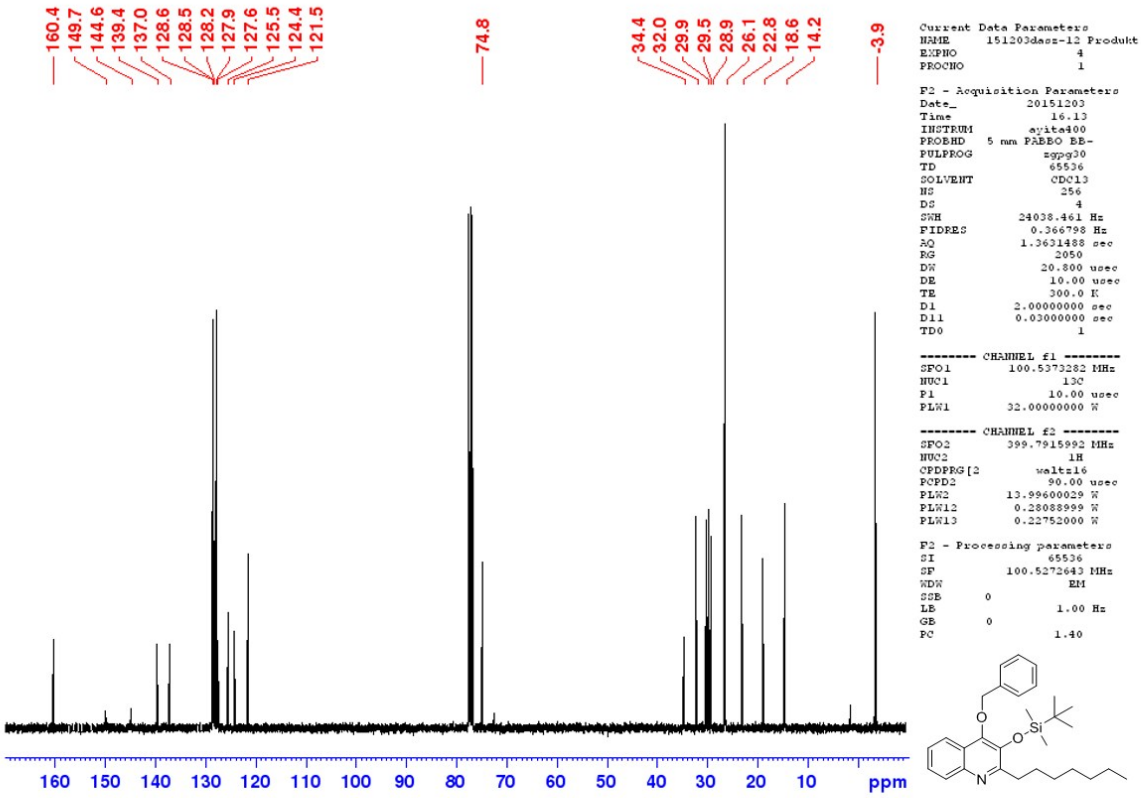
^{1}H- and ^{13}C-NMR spectra of 3-((tert-butyldimethylsilyl)oxy)-2-heptylquinolin-4-one in $CDCl_3$ (**11a**)



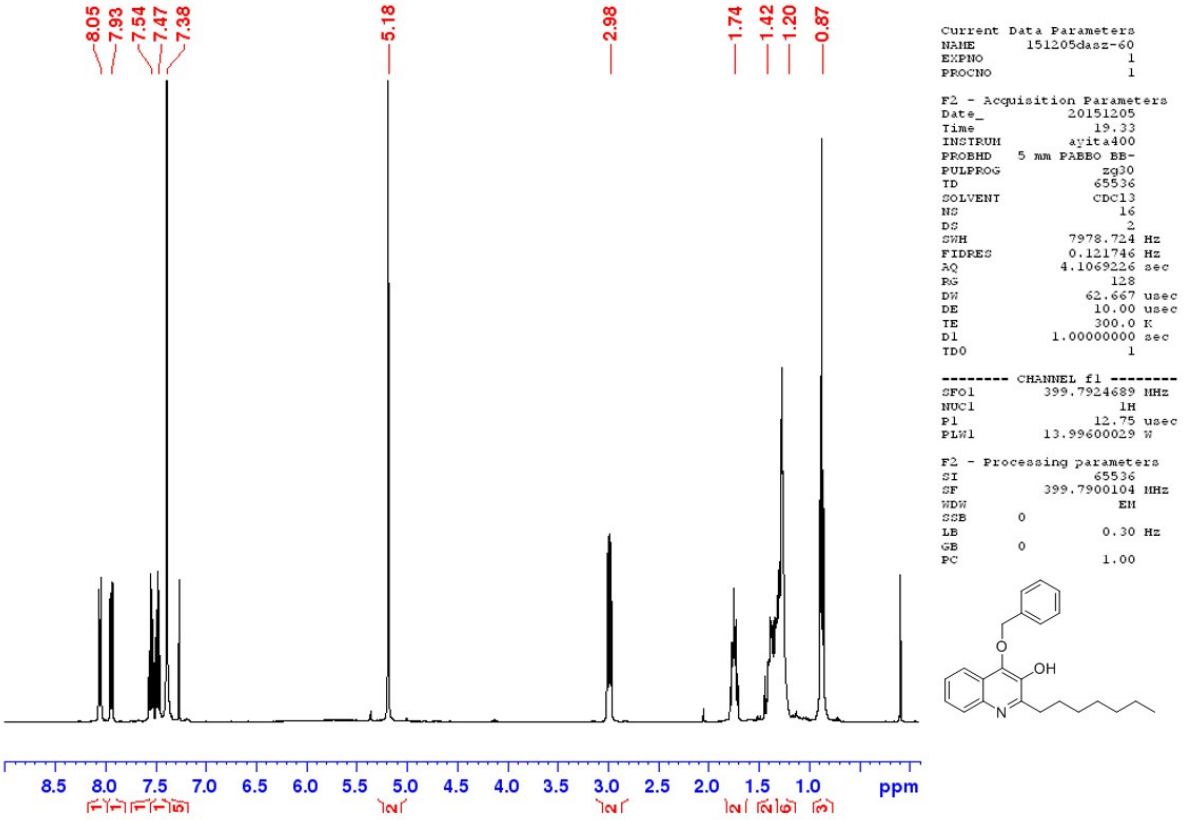


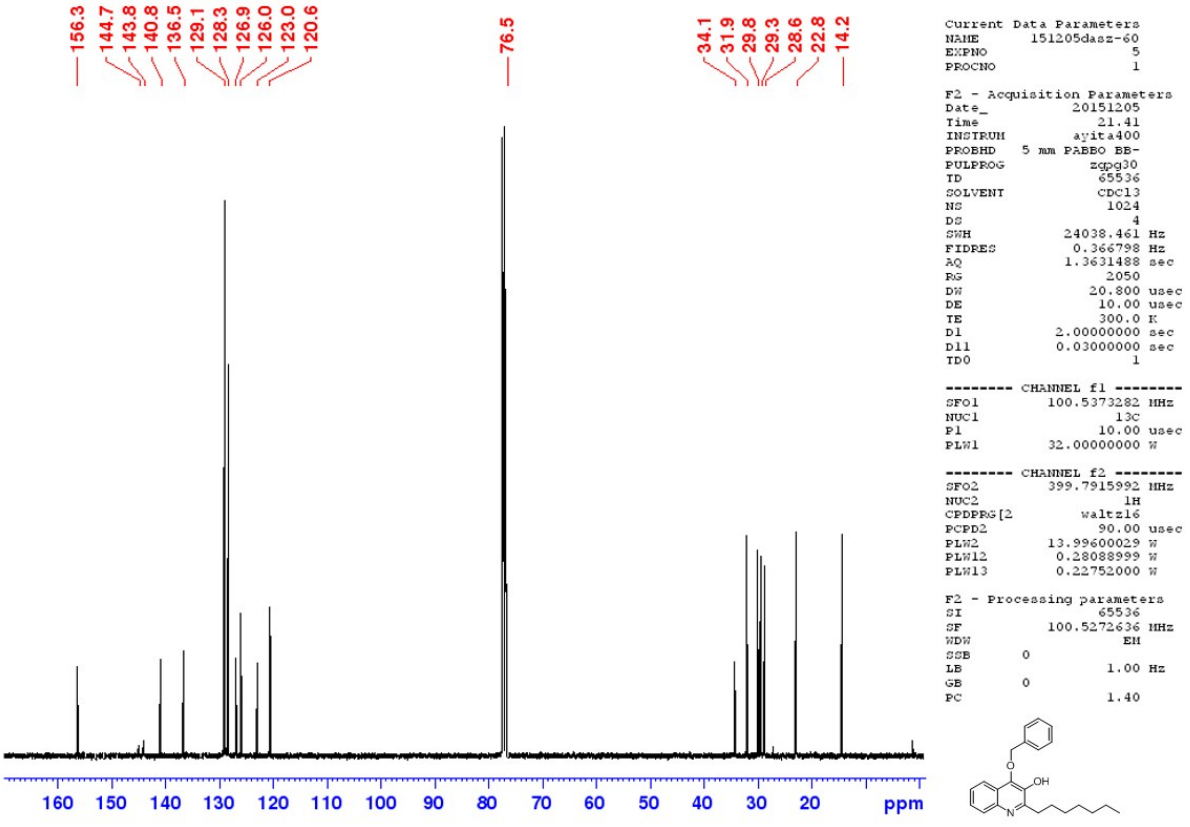
^{1}H- and ^{13}C-NMR spectra of 4-(benzyloxy)-3-((tert-butyldimethylsilyl)oxy)-2-heptylquinoline in $CDCl_3$ (**11b**)





^{1}H- and ^{13}C-NMR spectra of 4-(benzyloxy)-2-heptylquinolin-3-ol in $CDCl_3$ (**11c**)





^{1}H- and ^{13}C-NMR spectra of 2-heptyl-3-hydroxyquinolin-4(1*H*)-one oxime, PQS-oxime in $CDCl_3$ (**11**)