Supporting Information

for

Optimized Plk1 PBD inhibitors based on Poloxin induce mitotic arrest and apoptosis in tumor cells

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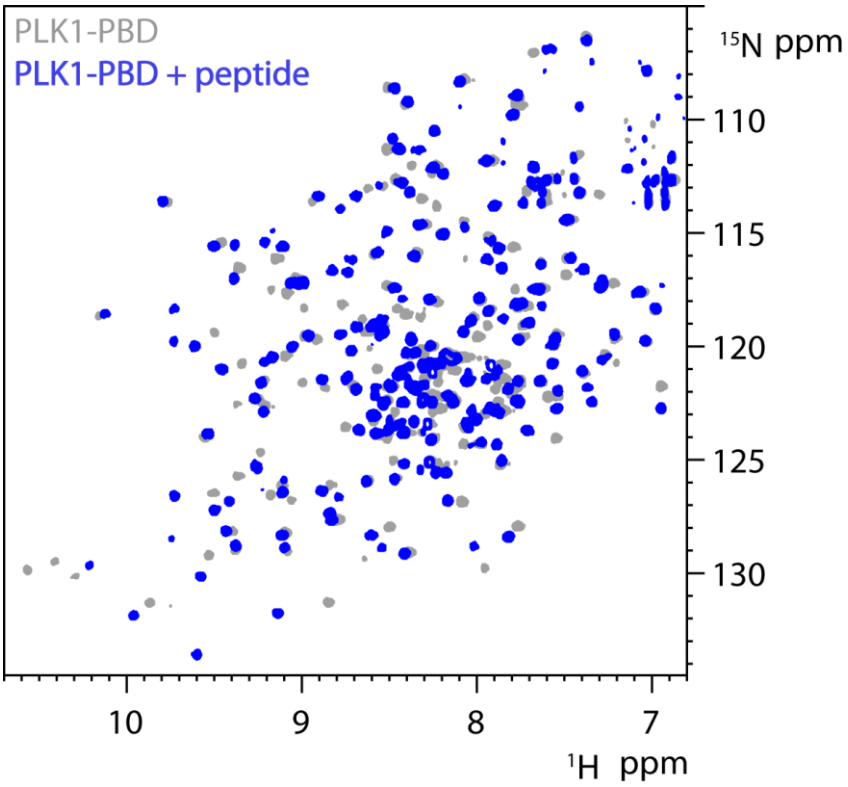
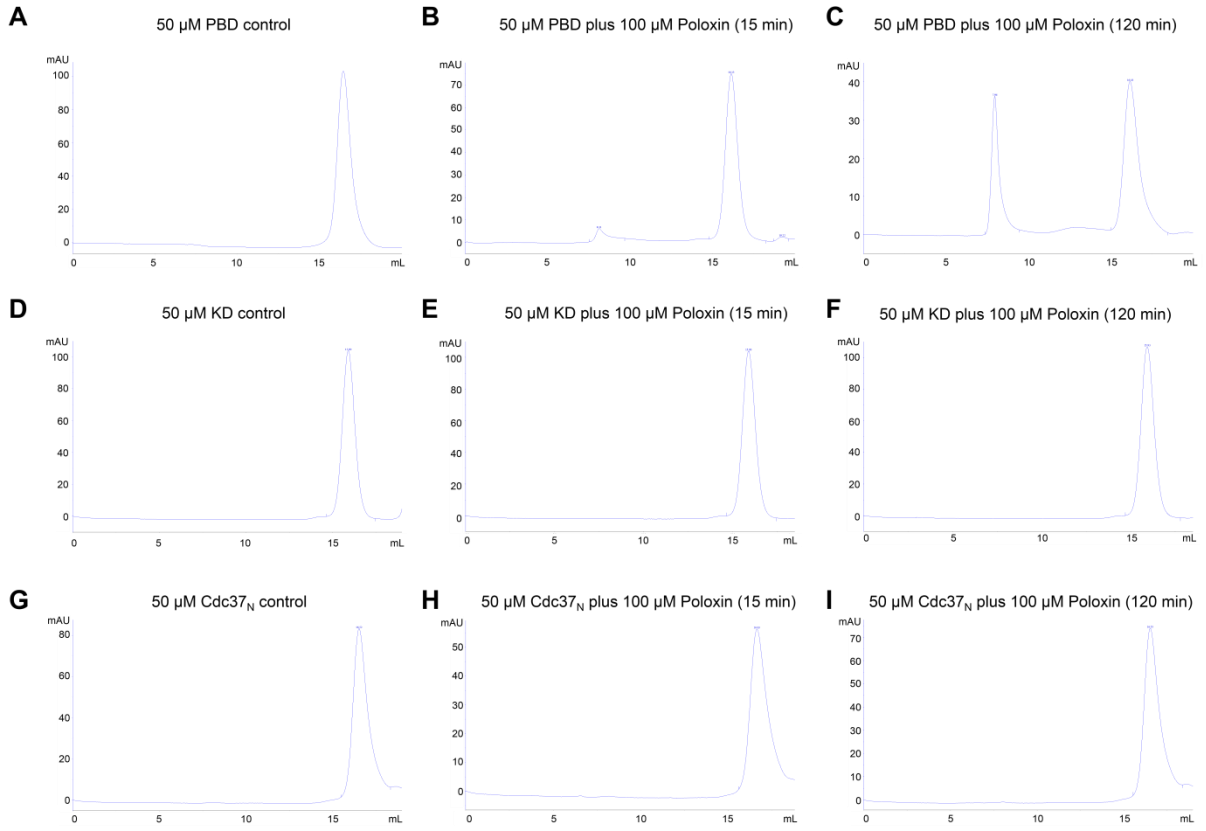
Supplementary Figures and Tables

Supplementary Figure 1



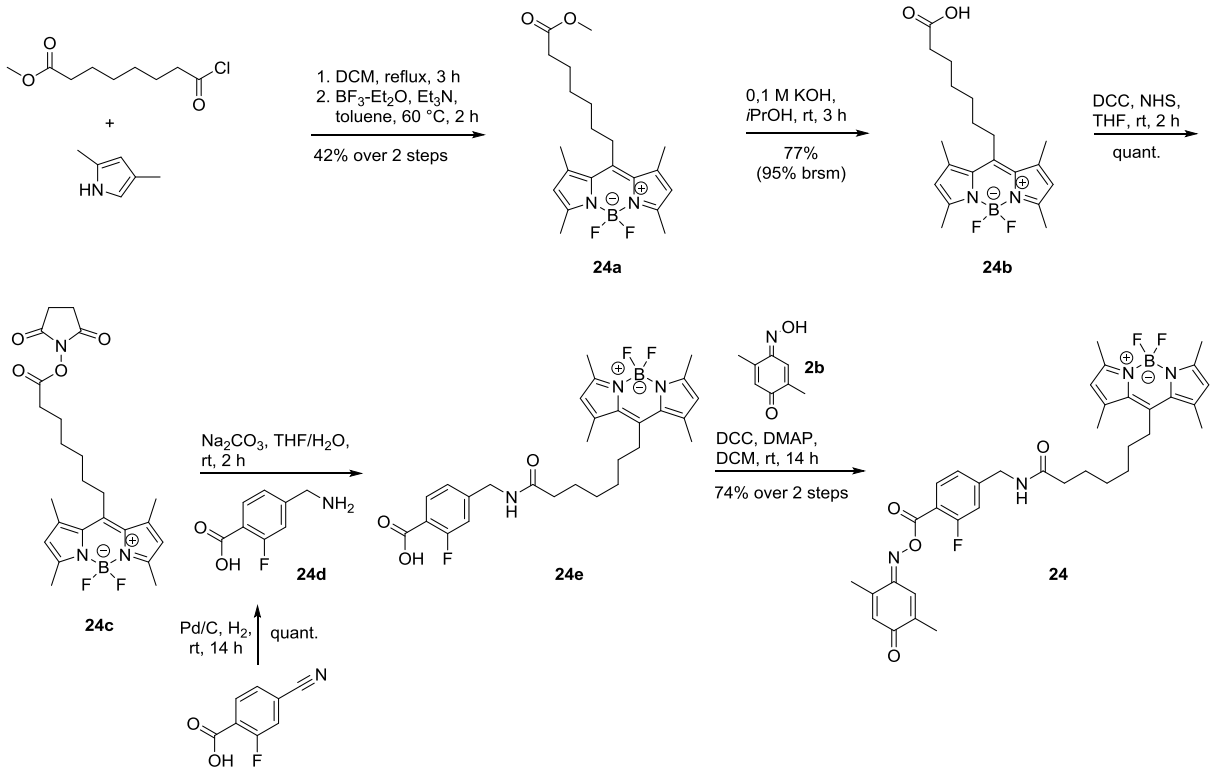
Supplementary Figure 1: Interaction of the Plk1 PBD and the peptide MQSpTPL (PB-tide). Overlay of 2D $^{1}H,^{15}N$-TROSY spectra of Plk1 PBD (250 µM, grey) in the absence or presence of PB-tide (500 µM, blue). All spectra were acquired at 950 MHz (T = 298 K) in the following buffer: 50 mM Tris pH 8, 500 mM NaCl, 2 mM TCEP, 10% D_2O and 1 mM 3-(trimethylsilyl)-2,2',3,3'-tetradeuteropropionic acid (TSP-d4).

Supplementary Figure 2



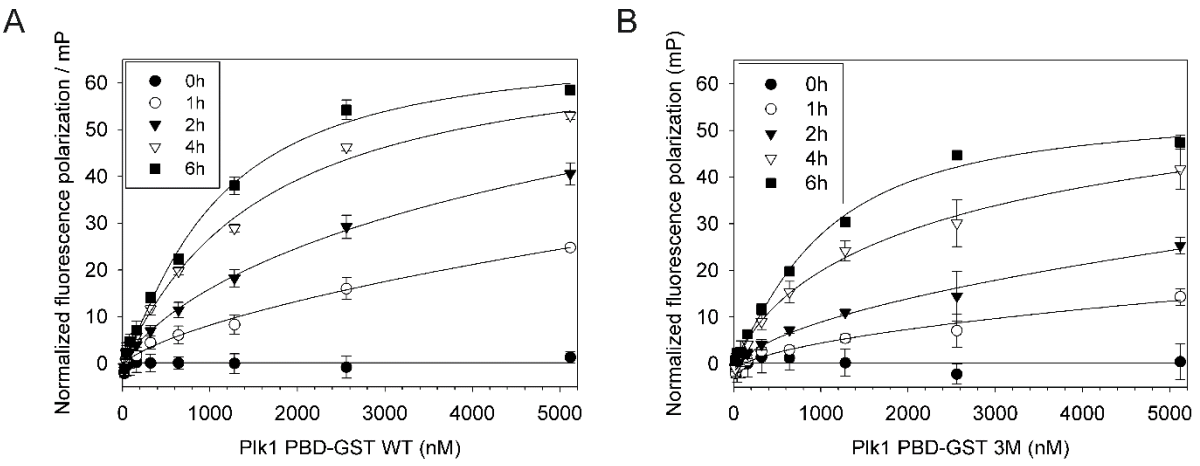
Supplementary Figure 2: Size exclusion chromatography (SEC). Superdex 200 elution profiles (UV_{280} detection) of proteins (50 µM) in the absence of Poloxin and after incubation with 100 µM Poloxin for 15 min and 120 min at room temperature. A) Plk1 PBD without Poloxin, B) after 15 min of incubation with Poloxin, and C) after 120 min of incubation with Poloxin. D) Plk1 kinase domain (KD) without Poloxin, E) after 15 min of incubation with Poloxin, and F) after 120 min of incubation with Poloxin. G) The N-terminal domain of Cdc37 ($Cdc37_N$) without Poloxin, H) after 15 min of incubation with Poloxin, and I) after 120 min of incubation with Poloxin. Typically, monomeric proteins elute at a retention volume of 16.5 mL. Protein multimerization is detected by the occurrence of an earlier elution peak (ca. 8 mL) and decreased intensity of the monomer peak. Assay conditions: 50 mM Tris pH 8.0, 500 mM NaCl, 2 mM TCEP on a Superdex 200 10/300 GL (GE Healthcare) column.

Supplementary Figure 3



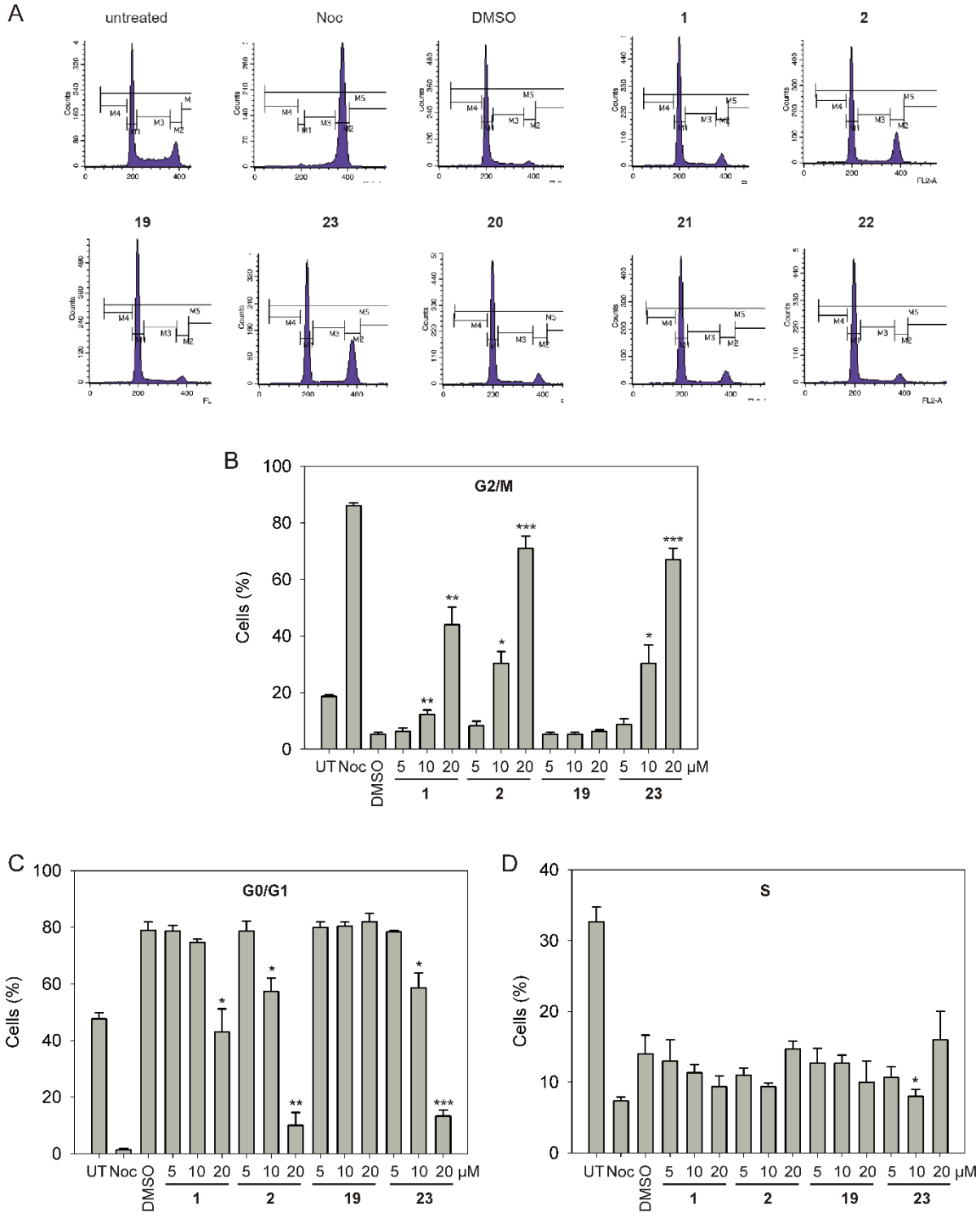
Supplementary Figure 3: Synthesis of BODIPY-FL-labeled Poloxin derivative **24**.

Supplementary Figure 4



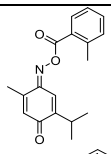
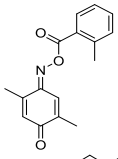
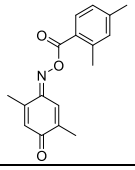
Supplementary Figure 4: Incubation of **24** with the GST-tagged Plk1 PBD leads to a time-dependent increase in fluorescence polarization. A) Wildtyp Plk1 PBD. B) Plk1 PBD W414A, H538A, K540A.

Supplementary Figure 5



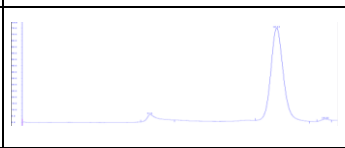
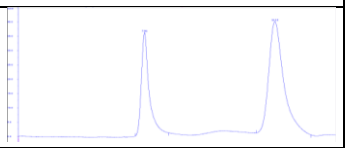
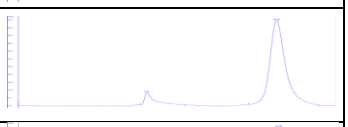
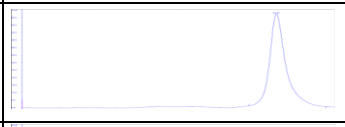
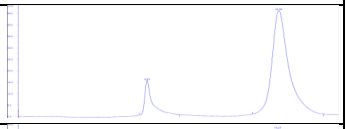
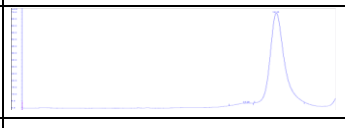
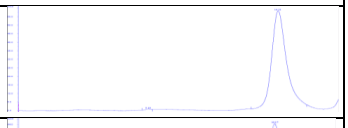
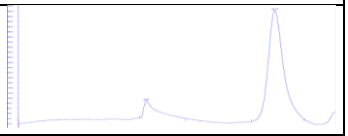
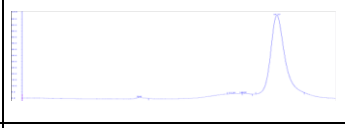
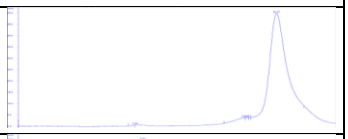
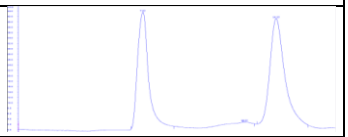
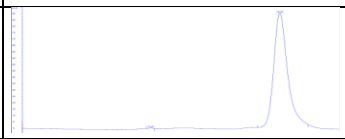
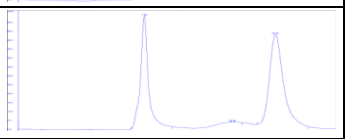
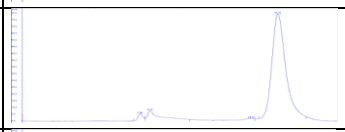
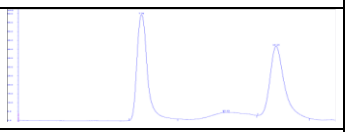
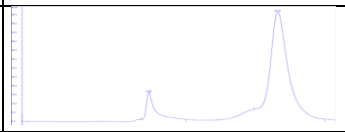
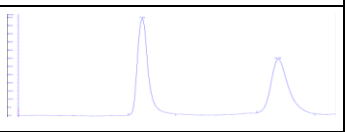
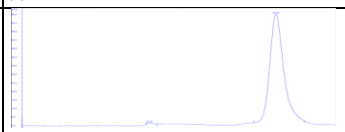
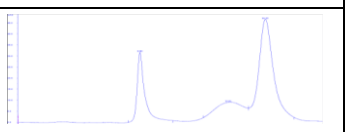
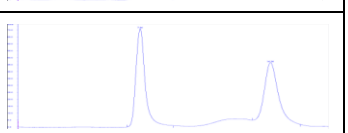
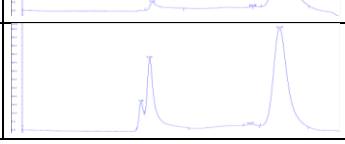
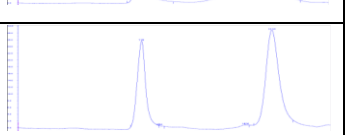
Supplementary Figure 5: Induction of mitotic arrest by Poloxin and analogs in HeLa cells. A) Primary data used for Figure 4B. B-D) Detailed quantitation of three independent experiments used for Figure 4D. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.002$.

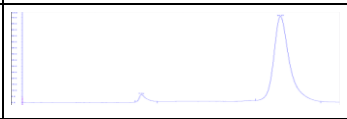
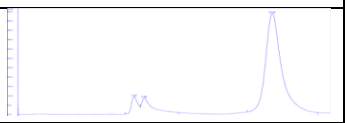
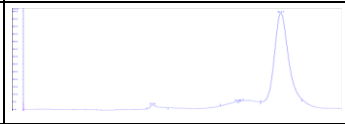
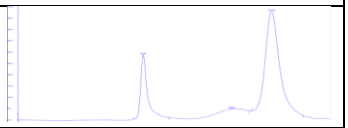
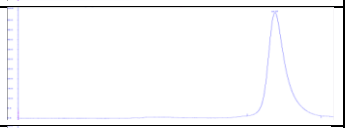
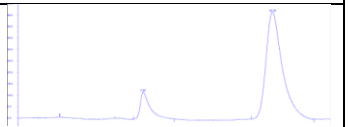
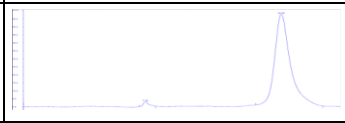
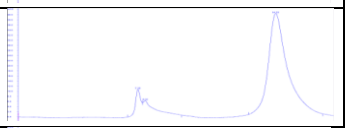
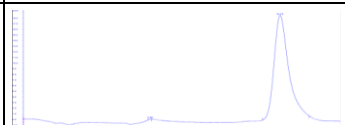
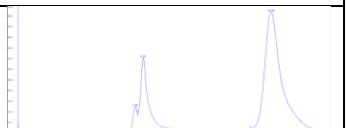
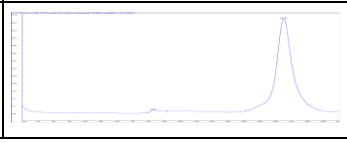
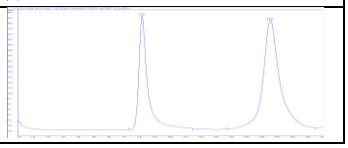
Supplementary Table 1

Compound	Structure	app. IC_{50} [µM] Plk1 PBD 1 mM DTT	app. IC_{50} [µM] Plk2 PBD 1 mM DTT	app. IC_{50} [µM] Plk3 PBD 1 mM DTT
Poloxin (**1**)		58.2 ± 11.3	135 ± 8	36 ± 5% inhibition at 200 µM
2		42.7 ± 1.9	50 ± 3 % inhibition at 200 µM	35 ± 5% inhibition at 200 µM
23		22.8 ± 2.4	43.7 ± 2.1	47 ± 7% inhibition at 200 µM

Supplementary Table 1. Activities of compounds **1**, **2**, and **23** in fluorescence polarization assays against the PBD of Plk1, Plk2, and Plk3. Assays were carried out as described in the Supporting methods, with an additional 1 mM DTT in the assay buffer.

Supplementary Table 2

Cmpd	app. IC_{50} [µM] Plk1 PBD	app. IC_{50} [µM] Plk1 PBD with DTT	Effect in SEC assay	Primary data SEC 15 min	Primary data SEC 120 min
1	6.4 ± 1.2	58.2 ± 11.3	strong		
2	1.36 ± 0.13	42.7 ± 1.9	medium		
3	4.63 ± 0.89	n.d.	medium		
4	2.54 ± 0.28	65.8 ± 4.5	weak		
5	22.0 ± 1.8	n.d.	medium		
6	23.2 ± 2.1	n.d.	n.d.		
7	-4 ± 13% inhibition at 80 µM	n.d.	weak		
8	0.74 ± 0.07	72 ± 3	strong		
9	2.26 ± 0.44	n.d.	strong		
10	1.24 ± 0.38	n.d.	strong		
11	0.81 ± 0.19	n.d.	strong		
12	11.4 ± 1.3	n.d.	strong		
13	0.53 ± 0.04	23.4 ± 0.3 % inhibition at 100 µM	strong		
14	0.60 ± 0.08	n.d.	strong		

Cmpd	app. IC_{50} [µM] Plk1 PBD	app. IC_{50} [µM] Plk1 PBD with DTT	Effect in SEC assay	Primary data SEC 15 min	Primary data SEC 120 min
15	1.02 ± 0.14	n.d.	medium		
16	3.25 ± 1.17	n.d.	n.d.		
17	0.47 ± 0.12	n.d.	n.d.		
18	4.95 ± 0.55	n.d.	strong		
19	70.1 ± 23.1	no inhibition at 100 µM	weak		
20	0.59 ± 0.31	74.9 ± 8.0	medium		
21	0.42 ± 0.24	44 ± 3 % inhibition at 100 µM	medium		
22	0.31 ± 0.02	8 ± 1 % inhibition at 100 µM	strong		
23	11.0 ± 0.8	22.8 ± 2.4	strong		

Supplementary Table 2. Activities of test compounds in fluorescence polarization assays against the Plk1 PBD in the absence or presence of 1 mM DTT, and in size exclusion chromatography. Assays were carried out as described in the Supporting methods. n.d.: not determined

Protein expression and purification

The PBDs of Plk1, Plk2, and Plk3 for use in fluorescence polarization assays were expressed and purified as described.[1] For use in size exclusion chromatography and NMR, the PBD (aa 345-603) and the kinase domain (KD) (aa 37-345) of Plk1 (UniProt ID P25321) were separatly cloned into a pETTEV vector, a modified pET 16b vector (Novagen) with an N-terminal His7-tag followed by a TEV protease cleavage site. The Plk1 PBD and KD were expressed in *E. coli* BL21 (DE3) cells (Invitrogen). Cells were grown at 310 K to an OD600 of 0.6, stored on ice for 20 min, induced with 1 mM IPTG and incubated for 18-20 h at 291 K. Cultures were centrifuged and microfluidized in a buffer containing 50 mM Tris pH 8.0, 5 mM BME, 5 mM imidazole, 500 mM NaCl, and 10 % glycerol. The Plk1 PBD and the KD were purified from the supernatant using immobilized metal ion affinity chromatography (IMAC; Ni-NTA Superflow, Qiagen). The His7-tags were removed from the protein by TEV protease digestion while dialyzing the protein against lysis buffer. Final purification of the PBDs was achieved by size

exclusion chromatography using a Hi Load 26/60 75 pG column (GE Healthcare) equilibrated with 50 mM Tris pH 8.0, 5 mM TCEP, and 500 mM NaCl. Via SDS-PAGE analysis fractions containing pure protein were pooled and concentrated to a final concentration of 100 µM. The N-terminal kinase-binding domain $Cdc37_N$ (1–126) was expressed and purified as described.[2]

Fluorescence polarization assays

Activities of the test compounds against the function of the PBDs of Plk1, Plk2, and Plk3 were carried out as described.[1] In brief, test compounds were incubated with the proteins at the indicated concentrations for 1 h at room temperature, followed by addition of the fluorescent-labeled peptides (final concentration: 10 nM). After another hour of incubation, fluorescence polarization (excitation: 485 nm, emission: 535 nm) was measured in a Tecan Infinite F500 384-well plate reader. Proteins were used at the following concentrations: 1) in the absence of DTT: Plk1 PBD (aa 326-603): 20 nM; Plk2 PBD (aa 355-685): 100 nM; Plk3 PBD PBD (aa 335-646): 320 nM; 2) in the presence of 1 mM DTT: Plk1 PBD: 15 nM; Plk2 PBD: 60 nM; Plk3 PBD: 200 nM. The following peptides were used as fluorescent tracers: Plk1 PBD: 5-carboxyfluorescein-GPMQSpTPLNG-OH; Plk2 PBD: 5-carboxyfluorescein-GPMQTSpTPKNG-OH; Plk3 PBD: 5-carboxyfluorescein-GPLATSpTPKNG-OH. Buffer conditions: 10 mM Tris (pH 8.0), 50 mM NaCl,1 mM EDTA, 0.1 % Nonidet P-40 substitute, final DMSO concentration: 2 %. 1 mM DTT was added to the buffer when indicated. Experiments were carried out in triplicate. Data were analyzed using SigmaPlot 8.

Size exclusion chromatography (SEC)

An Aekta purifier (GE Healthcare) was used to perform all analytical runs (0.5 mL/min, 0.05 mL sample injected) with an equilibrated (50 mM Tris pH 8.0, 500 mM NaCl, 2 mM TCEP) Superdex 200 10/300 GL (GE Healthcare) column. Proteins (50 µM) were incubated in the absence of presence of the compounds (100 µM) for 15 min and 120 min at room temperature prior to column loading. Samples were isocratically eluted by monitoring the absorbance at 280 nm. The column was calibrated according to gel filtration MWGF200 protein calibration kit (Sigma).

NMR spectroscopy

NMR experiments were performed at T = 298 K on Bruker 950-MHz spectrometer equipped with room temperature TXI-HCN probes. NMR samples were prepared with 10% D_2O to lock the spectrometers and 3-(trimethylsilyl)-2,2',3,3'-tetradeuteropropionic acid (TSP-d4; 1 mM) was used as internal standard for spectral referencing. The processing and analysis of NMR spectra were done in Topspin version 3.1 (Bruker Biospin). Plk1-peptide (phospho-peptide: MQSpTPL, PB-tide) interactions were studied by acquiring 2D $^{1}H,^{15}N$-TROSY (Bruker 950 MHz) of PLK1-PBD (250 µM ^{15}N-labeled PLK1-PBD in 50 mM Tris pH 8, 500 mM NaCl, 2 mM TCEP) in the presence or absence of the peptide. The peptide was added to the protein in two fold excess.

Cell culture

HeLa cells were maintained in Dulbecco's Modified Eagle-medium (High Glucose) supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 10% foetal calf serum, 100 U/ml penicillin and 100 µg/ml streptomycin (GIBCO and PAA) at 37°C with 5% CO_2 in a humidified atmosphere.

Western Blot

Hela cells were either treated with Nocodazole (Sigma) for 14 hours or arrested at the G1/S boundary by thymidine block (24 hours), and released into thymidine free medium containing the indicated concentration of compounds at a final DMSO concentration of 0.5%. After 14 hours cells were lysed with RIPA buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1% Triton X-100, 0.5% Na-desoxycholate, 0.1 % SDS, 1 mM Na_3VO_4, 1 mM PMSF, 1 mM DTT NaF and protease inhibitor complete (Roche). Polyvinylidene difluoride (PVDF) membranes were used for Western blotting applications. Blocking of membranes was performed with TBST including 2% BSA. The following antibodies were used at the indicated concentrations: mouse monoclonal anti-Plk1 (1:1000), Cdk1 (1:1000), Cyclin B1 (1:1000), Cdc25C (1:1000), (all Santa Cruz, Biotechnology Heidelberg, Germany), β-actin (1:200.000) (Sigma-Aldrich, Taufkirchen, Germany), rabbit anti-phospho-histone H3(S10) (1:1000) (Millipore, Schwalbach, Germany), HRP-conjugated secondary antibodies (1:5000) (GE Healthcare). The ECL Western Blotting Substrate was used for detection.

Cell cycle, apoptosis and cell viability assays

For cell cycle analysis, cells were harvested, washed with PBS, fixed in chilled 70% ethanol at 4 ºC for 30 min, treated with 1 mg/ml RNase A (Sigma-Aldrich) for 30 min, and stained with 100 µg/ml propidium iodine. Cell cycle quantification was performed using a FACS Calibur instrument and Cellquest Pro software (both BD Biosciences). Apoptotic loss of membrane asymmetry was analyzed by staining for PE Annexin V and 7-AAD (BD Biosciences) and quantified on a FACS Calibur instrument. For apoptosis assays, non-synchronized cells were treated with the test compounds for 24 h. Cell viability assays were conducted using the Cell Titer-Blue® Cell Viability Assay (Promega) according to the manufacturer's instructions using fluorescence as a read-out (excitation/emission wavelengths: 562 nm / 615 nm). Test compounds were used at final concentrations of 20 µM.

Statistical methods

All experiments were performed at least in triplicate. Statistics were analyzed by Student's t-test (two-sided, paired). Significant differences are indicated with an asterisk (*$p<0.05$, **$p<0.01$, ***$p<0.002$).

Chemistry

Unless otherwise noted, reactions were carried out under an atmosphere of argon in air-dried glassware with a magnetic stirrer. Air- and/or moisture sensitive liquids were transferred *via* syringe. Organic solutions were concentrated under reduced pressure at 40 °C. Analytical thin layer chromatography (TLC) was performed using plates cut from aluminium sheets (silica gel 60 F-254 from Merck). Visualization was achieved under a 254 nm UV light and by immersion in solutions of cerium sulfate, vanillin or potassium permanganate followed by treatment with a heat gun. Column chromatography was carried out as flash chromatography using silica gel (silica gel 60, 40-63 µM from Merck). All reagents were obtained from commercial sources and used without further purifications. Dry MeOH and DMF were obtained from Aldrich. DCM was dried by refluxing over calcium hydride. ^{1}H and ^{13}C NMR spectra were recorded at 26°C on: Varian (Mercury plus) 400 MHz, Varian (Mercury plus) 300 MHz, Bruker (DRX-400) 400 MHz or Bruker (Fourier 300) 300 MHz spectrometers. Recorded shifts are reported in parts per million (δ) and calibrated using residual undeuterated solvent. Infrared Spectra were recorded in KBr using a JASCO FT/IR-4100. UV/Vis spectra were recorded using on a JASCO V-630. High-resolution electrospray ionization (ESI) mass spectra were obtained on a Bruker

Daltonics APEX™ II Fourier Transform mass spectrometer (ESI-FT-ICR-MS, 7 T). Low-resolution electrospray ionization (ESI) mass spectra were obtained on a Bruker Daltonics ESQUIRE 3000 Plus mass spectrometer (ESI-Ion Trap LC MSMS). Melting points were recorded on a Rapido PHMK apparatus from VEB Wägetechnik and are uncorrected. Optical rotations were measured on a Schmidt+Haensch Polartronic MHZ-8 Polarimeter with a path length of 5 cm at 22 °C.

General chemical procedures

Method 1: Synthesis of quinones from phenols

Phenol was dissolved in DMF and oxygen was bubbled into the reaction mixture for a few minutes, with an oxygen atmosphere subsequently maintained using a balloon. [Co(II)(salen)] (6% mol) was added, and stirred at room temperature for 3 h. Further [Co(II)(salen)] (6% mol) was added and the reaction mixture stirred for another 3 h at room temperature. The process was repeated once more, for a total addition of 18% mol catalyst, and a total reaction time of 24 h. Ether was added and the black mixture washed with 0.1 M HCl, water and brine. The organic phase was dried over anhydrous $MgSO_4$ and the solvent evaporated under reduced pressure. The residue was purified by flash column chromatography (eluent: hexane/ethyl acetate, 8:1) to give the product.

Method 2: Synthesis of oximes from quinones[3]

Unless stated otherwise, a solution of the hydroxylamine hydrochloride (2 equiv.) and sodium acetate (2 equiv.) was added to the quinone (1 equiv.) in ethanol, and the resulting mixture was stirred and refluxed until the reaction was completed. After evaporation of the solvent under reduced pressure the solid residue was poured into water and extracted with ethyl acetate. The organic layer was washed with aq. $NaHCO_3$, brine, dried over sodium sulfate and evaporated under reduced pressure. The solid residue was purified using column chromatography (eluent: hexane/ethyl acetate) to provide the desired product.

Method 3: Synthesis of Poloxin derivatives from acid chlorides

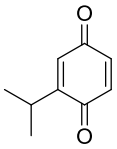
The oxime (1 equiv.) and triethylamine (3 equiv.) were dissolved in THF. The resulting solution was stirred for 10 min at room temperature. Acid chloride (1.3 equiv.) was added drop wise and stirred at room temperature over night. After evaporation of the solvent under reduced pressure, the solid residue was poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and evaporated under reduced pressure. The solid residue was purified using column chromatography (eluent: hexane/ethyl acetate) to provide the desired product. Highly pure samples can be obtained by additional recrystallization from hexane/ethyl acetate.

Method 4: Synthesis of Poloxin derivatives from carboxylic acids

The oxime intermediate (1 equiv.), carboxylic acid (1 equiv.) and DMAP (0.1 equiv.) were suspended in DCM. Upon addition of DCC (1.3 equiv. dissolved in DCM) the reaction mixture became clear and was stirred over night at room temperature. The resulting urea derivative was filtered off, and the solvent was evaporated under reduced pressure. The solid residue was purified using column chromatography (eluent: hexane/ethyl acetate) to provide the desired product. Highly pure samples can be obtained by recrystallization from hexane/ethyl acetate.

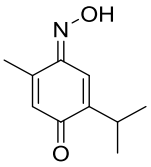
Synthesis and spectroscopic characterization of compounds

2-Isopropylcyclohexa-2,5-diene-1,4-dione (**4a**)[3]



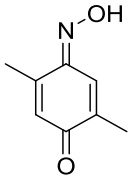
2.5 g (18.36 mmol) 2-isopropylphenol was reacted with oxygen and a total amount of 1.075 g (3.3 mmol) [Co^{II}(salen)] in 120 mL DMF according to Method 1. Yield: 1.9 g (69%). Melting point: 62°C.^{1}H-NMR (300 MHz, $CDCl_3$): δ = 6.77-6.66 (m, 2H), 6.53 (m, 1H), 3.03 (sept, *J* = 6.9 Hz, *J* = 1.2 Hz, 1H), 1.13 (d, *J* = 6.9Hz, 6H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 188.3, 187.3, 155.1, 137.2, 136.1, 130.5, 26.9, 21.5 ppm; UV/Vis (MeOH): $\lambda_{max}(\varepsilon)$ = 310, 247 nm; IR (KBr): $\tilde{\nu}$ = 3434, 3327, 3308, 3271, 3054, 2967, 2934, 2875, 2359, 2342, 1657, 1633, 1617, 1595, 1467, 1455, 1386, 1368, 1312, 1281, 1224, 1203, 1121, 1082, 1036, 930, 908, 837, 783, 675, 577, 483, 473, 465, 448, 439, 430, 413 cm^{-1}; HR-ESI-MS $C_9H_{10}O_2$ calcd: 323.1254 [2M+Na^+], found: 323.1258.
CAS Registry Number: 15232-10-7

(E)-4-(Hydroxyimino)-2-isopropyl-5-methylcyclohexa-2,5-dien-1-one (**1b**)



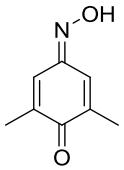
5 g (30.45 mmol) thymoquinone was reacted with 4.23 g (60.9 mmol) hydroxylamine hydrochloride in 450 mL ethanol for 2.5 h according to Method 2. Yield: 2.46 g (45%). Melting point: 164°C (Lit.[4]: 155 °C under decomposition). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 7.46 (s, 1H), 6.29 (s, 1H), 2.94 (sept, *J* = 6.9 Hz, 1H), 2.15 (s, 3H), 1.06 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-APT-NMR (75 MHz, DMSO-d_6): δ = 149.4, 147.4, 146.1, 128.0, 117.7, 26.1, 21.4, 16.6 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 292, 228 nm; IR (KBr): $\tilde{\nu}$ = 3435, 3182, 3073, 2961, 2924, 2848, 2794, 1639, 1605, 1573, 1466, 1441, 1380, 1311, 1271, 1242, 1147, 1056, 1015, 1003, 907, 884, 852, 775, 631, 571 cm^{-1}; HR-ESI-MS $C_{10}H_{13}NO_2$ calcd: 202.0839 [M+Na^+], found: 202.0836.
CAS Registry Number: 17302-61-3

(E)-4-(Hydroxyimino)-2,5-dimethylcyclohexa-2,5-dien-1-one (**2b**)



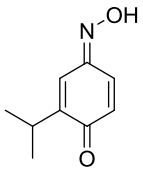
5 g (36.72 mmol) 2,5-dimethyl-1,4-benzoquinone was reacted with 4.594 g (66.1 mmol) hydroxylamine hydrochloride in 300 mL ethanol for 2 h according to Method 2. Yield: 1.96 g (35%). Melting point: 175 °C (Lit.[4]: 173 °C under decomposition). ^{1}H-NMR (400 MHz, DMSO-d_6): δ = 7.56 (m, 1H), 6.28 (m, 1H), 2.14 (m, 3H), 1.90 (d, *J* = 1.5 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 186.3, 149.3, 146.6, 138.1, 127.5, 121.1, 16.7, 15.5 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 284 nm; IR (KBr): $\tilde{\nu}$ = 3434, 3197, 3090, 2950, 2925, 2843, 2801, 1641, 1612, 1595, 1572, 1434, 1426, 1380, 1355, 1272, 1237, 1165, 1110, 1026, 1003, 903, 705, 617, 553 cm^{-1}; HR-ESI-MS $C_8H_9NO_2$ calcd: 174.0526 [M+Na$^+$], found: 174.0526.
CAS Registry Number: 2593-53-5

4-(Hydroxyimino)-2,6-dimethylcyclohexa-2,5-dien-1-one (**3b**)



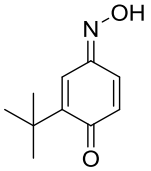
600 mg (4.4 mmol) 2,6-dimethyl-1,4-benzoquinone was reacted with 568 mg (8.8 mmol) hydroxylamine hydrochloride and 723 mg (8.8 mmol) sodium acetate in 140 mL ethanol for 2 h according to Method 2. Yield: 400 mg (60%). Melting point: 173°C (Lit.[4]: 173 °C under decomposition). ^{1}H-NMR (400 MHz, DMSO-d_6) δ 13.20 (s, 1H), 7.58 (s, 1H), 7.16 (s, 1H), 1.98 (d, *J* = 1.3 Hz, 3H), 1.95 (d, *J* = 1.1 Hz, 3H) ppm; ^{13}C-APT-NMR (101 MHz, DMSO-d_6): δ = 187.0, 148.8, 138.8, 136.1, 133.9, 120.4, 16.2, 15.6 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 301 nm; IR (KBr): $\tilde{\nu}$ = 3191, 3095, 3034, 2962, 2923, 2860, 2771, 2652, 2631, 1632, 1604, 1557, 1464, 1424, 1380, 1342, 1311, 1223, 1184, 1049, 1003, 942, 918, 873, 809, 791, 780, 690, 682, 583, 521, 469, 421 cm^{-1}; HR-ESI-MS $C_8H_9NO_2$ calcd: 174.0526 [M+Na$^+$], found: 174.0527.
CAS Registry Number: 4965-29-1

(E)-4-(Hydroxyimino)-2-isopropylcyclohexa-2,5-dien-1-one (**4b**)



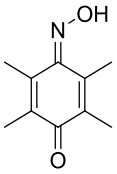
950 mg (3.325 mmol) 2-isopropyl-1,4-benzoquinone (**4a**) was reacted with 489 mg (7.59 mmol) hydroxylamine hydrochloride and 623 mg (7.59 mmol) sodium acetate in 200 mL ethanol for 2 h at room temperature according to Method 2. Yield: 178 mg (17%, as a mixture of E/Z isomers). Melting point: 99 °C (Lit:[5] 102-104 °C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 13.49 (s, br, 1H), 7.83 – 6.85 (m, 2H), 6.43 (d, *J* = 9.5 Hz, 1H), 3.09 – 2.72 (m, 1H), 1.06 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 185.9, 148.3, 137.4, 131.5, 131.1, 129.2, 123.2, 117.3, 26.4, 26.0, 21.4 ppm; UV/Vis (MeOH): $\lambda_{max}(\varepsilon)$ = 304, 228 nm; IR (KBr): $\tilde{\nu}$ = 3420, 3229, 3095, 2964, 2871, 2854, 2360, 2342, 1635, 1614, 1581, 1558, 1466, 1440, 1384, 1366, 1322, 1298, 1232, 1132, 1092, 1051, 1018, 905, 860, 838, 794, 684, 607, 553, 441, 418 cm^{-1}; HR-ESI-MS $C_9H_{11}NO_2$ calcd: 188.0682 [M+Na$^+$], found: 188.0683.
CAS Registry Number: 107244-57-5

(E)-2-(tert-Butyl)-4-(hydroxyimino)cyclohexa-2,5-dien-1-one (**5b**)



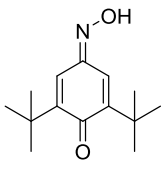
800 mg (4.87 mmol) 2-*tert*-butyl-1,4-benzoquinone was reacted with 628 mg (9.74 mmol) hydroxylamine hydrochloride and 799 mg (9.74 mmol) sodium acetate in 200 mL ethanol for 1.5 h at room temperature according to Method 2. Yield: 230 mg (26%, as a mixture of E/Z isomers). Melting point: 123°C (Lit:[5] 132-134 °C). ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 7.71 – 6.97 (m, 2H), 6.35 (dd, *J* = 9.9, 4.3 Hz, 1H), 1.23 (s, 9H) ppm; ^{13}C-NMR (101 MHz, DMSO-d_6): δ = 149.3, 136.4, 133.0, 132.2, 130.7, 122.5, 118.2, 34.9, 34.4 28.8 ppm; UV/Vis (MeOH): $\lambda_{max}(\varepsilon)$ = 302, 226, 202 nm; IR (KBr): $\tilde{\nu}$ = 3428, 3234, 3090, 3000, 2961, 2870, 2360, 2342, 1635, 1606, 1574, 1559, 1484, 1456, 1444, 1410 ,1392, 1362, 1337, 1319, 1254, 1233, 1200, 1123, 1054, 1032, 1007, 905, 886, 839, 817, 808, 608, 568 cm^{-1}; HR-ESI-MS $C_{10}H_{13}NO_2$ calcd: 202.0839 [M+Na$^+$], found: 202.0840. CAS Registry Number: 37405-22-4.

4-(Hydroxyimino)-2,3,5,6-tetramethylcyclohexa-2,5-dien-1-one (**6b**)



124 mg (0.76 mmol) duroquinone was reacted with 87.6 mg (1.36 mmol) hydroxylamine hydrochloride in 5 mL pyridine for 36 h at 105 °C according to Method 2. Yield: 52 mg (39%). Melting point: 175 °C (Lit.[4]: 89 °C under decomposition). ^{1}H-NMR (300 MHz, DMSO-d_6) δ = 13.02 (s, 1H), 2.32 (d, *J* = 1.0 Hz, 3H), 2.12 (d, *J* = 0.9 Hz, 3H), 1.87 (s, 6H). ppm; ^{13}C-NMR (75 MHz, DMSO-d_6) δ = 184.4, 148.0, 142.3, 135.4, 134.9, 131.3, 19.4, 14.6, 11.7, 11.4 ppm; UV/Vis ($CHCl_3$): $\lambda_{max}(\varepsilon)$ = 299 nm; IR (KBr): $\tilde{\nu}$ = 3565, 3544, 3521, 3510, 3500, 3240, 3096, 2932, 2802, 2360, 1631, 1596, 1557, 1403, 1380, 1361, 1310, 1273, 1155, 1123, 1094, 1045, 1018, 1008, 969, 876, 818, 793, 778, 666, 615, 575, 484, 435, 417 cm^{-1}; HR-ESI-MS $C_{10}H_{13}NO_2$ calcd: 202.0839 [M+Na$^+$], found: 202.0839. CAS Registry Number: 6120-24-7

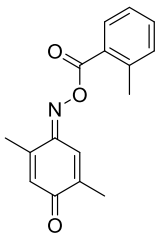
2,6-Di-tert-butyl-4-(hydroxyimino)cyclohexa-2,5-dien-1-one (**7b**)



600 mg (2.72 mmol) 2,6-di-*tert*-butyl-1,4-benzoquinone was reacted with 351 mg (5.45 mmol) hydroxylamine hydrochloride and 447 mg (5.45 mmol) sodium acetate in 140 mL ethanol for 1.5 h according to Method 2. Yield: 590 mg (92%). Melting point: 219 °C (Lit.[6]: 218.5-220 °C).

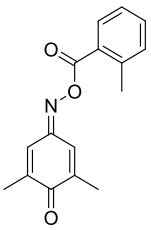
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 7.46 (d, *J* = 2.7 Hz, 1H), 6.95 (d, *J* = 2.7 Hz, 1H), 1.30 (d, *J* = 8.4 Hz, 18H) ppm; ^{13}C-NMR (75 MHz, DMSO-d_6): δ = 186.5, 150.5, 148.9, 148.1, 130.6, 117.0, 35.2, 34.6, 29.1 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 301, 227 nm; IR (KBr): $\tilde{\nu}$ = 3323, 3103, 3000, 2956, 2915, 2884, 2867, 1646, 1635, 1610, 1585, 1557, 1487, 1454, 1417, 1387, 1363, 1338, 1302, 1250, 1176, 1041, 1009, 933, 920, 884, 822, 815, 746, 731, 691 cm^{-1}; HR-ESI-MS $C_{14}H_{21}NO_2$ calcd: 258.1465 [M+Na$^+$], found: 258.1461. CAS Registry Number: 15052-28-5

(E)-2,5-Dimethyl-4-(((2-methylbenzoyl)oxy)imino)cyclohexa-2,5-dien-1-one (**2**)



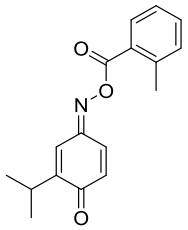
255 mg (1.687 mmol) **2b** was reacted with 339 mg (2.193 mmol) *o*-toluoyl chloride and 512 mg (5.061 mmol) Et_3N in 25 mL THF according to Method 3. Yield: 264 mg (58%). Melting point: 135°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.95 (dd, *J* = 8.2, 1.5 Hz, 1H), 7.53 (q, *J* = 1.6 Hz, 1H), 7.49 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.39 – 7.28 (m, 2H), 6.42 (q, *J* = 1.4 Hz, 1H), 2.67 (s, 3H), 2.36 (d, *J* = 1.4 Hz, 3H), 2.07 (d, *J* = 1.6 Hz, 3H) ppm; ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 187.0, 163.6, 154.5, 146.5, 142.3, 141.4, 133.1, 132.2, 131.0, 130.4, 127.6, 126.1, 122.2, 21.7, 17.5, 16.4 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 291, 232 nm; IR (KBr): $\tilde{\nu}$ = 3504, 3442, 3255, 2986, 2968, 2926, 1766, 1652, 1634, 1601, 1571, 1482, 1459, 1443, 1428, 1383, 1374, 1358, 1300, 1285, 1260, 1230, 1163, 1137, 1055, 1021, 1011, 946, 893, 818, 799, 785, 741, 706, 688, 635, 536, 488 cm^{-1}; HR-ESI-MS $C_{16}H_{15}NO_3$ calcd: 292.0944 [M+Na$^+$], found: 292.0941.

2,6-Dimethyl-4-(((2-methylbenzoyl)oxy)imino)cyclohexa-2,5-dien-1-one (**3**)



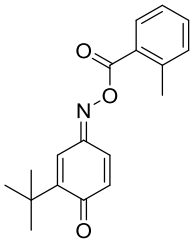
300 mg (1.985 mmol) **3b** was reacted with 399 mg (2.581 mmol) *o*-toluoyl chloride and 602 mg (5.955 mmol) Et_3N in 28 mL THF according to Method 3. Yield: 430 mg (80%). Melting point: 129°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.94 (dd, *J* = 8.3, 1.3 Hz, 1H), 7.55 – 7.47 (m, 2H), 7.37 – 7.30 (m, 2H), 7.21 (dd, *J* = 2.6, 1.5 Hz, 1H), 2.67 (s, 3H), 2.11 (d, *J* = 1.5 Hz, 3H), 2.08 (d, *J* = 1.4 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 187.3, 163.8, 153.7, 143.0, 141.3, 140.9, 133.0, 132.5, 132.2, 130.4, 127.6, 126.1, 121.5, 21.7, 17.0, 16.3 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 294, 233 nm; IR (KBr): $\tilde{\nu}$ = 3490, 3442, 3246, 2985, 2976, 2958, 2932, 1761, 1633, 1604, 1575, 1490, 1458, 1442, 1383, 1375, 1346, 1314, 1304, 1286, 1229, 1205, 1183, 1168, 1139, 1020, 958, 915, 874, 790, 781, 732, 693, 672 cm^{-1}; HR-ESI-MS $C_{18}H_{19}NO_3$ calcd: 292.0944 [M+Na$^+$], found: 292.0947.

(E)-2-Isopropyl-4-(((2-methylbenzoyl)oxy)imino)cyclohexa-2,5-dien-1-one (**4**)



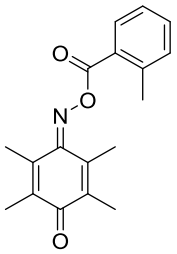
116 mg (0.702 mmol) **4b** was reacted with 141 mg (0.913 mmol) *o*-toluoyl chloride and 213 mg (2.106 mmol) Et_3N in 20 mL THF according to Method 3. Yield: 77 mg (39%). Melting point: 103°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.96 – 7.91 (m, 1H), 7.70 (dd, *J* = 10.1, 2.6 Hz, 1H), 7.50 (td, *J* = 7.8, 1.4 Hz, 1H), 7.36 – 7.28 (m, 2H), 7.25 (dd, *J* = 2.6, 1.1 Hz, 1H), 6.57 (d, *J* = 10.1 Hz, 1H), 3.09 (pd, *J* = 6.8, 1.0 Hz, 1H), 2.66 (s, 3H), 1.15 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 186.3, 163.5, 154.1, 150.7, 141.4, 134.7, 133.2, 132.2, 130.4, 129.9, 127.2, 126.1, 124.3, 26.9, 21.7, 21.6 ppm; UV/Vis (MeOH): $\lambda_{max}(\varepsilon)$ = 284, 233, 204 nm; IR (KBr): $\tilde{\nu}$ = 3435, 3061, 2975, 2962, 2934, 2871, 1767, 1644, 1629, 1604, 1587, 1489, 1465, 1383, 1365, 1339, 1287, 1231, 1203, 1130, 1059, 1028, 954, 944, 912, 892, 839, 736, 695, 661, 476 cm^{-1}; HR-ESI-MS $C_{18}H_{19}NO_3$ calcd: 306.1101 [M+Na$^+$], found: 306.1103.

(E)-2-(tert-Butyl)-4-(((2-methylbenzoyl)oxy)imino)cyclohexa-2,5-dien-1-one (**5**)



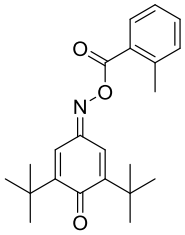
125 mg (0.697 mmol) **5b** was reacted with 140 mg (0.907 mmol) *o*-toluoyl chloride and 212 mg (2.091 mmol) Et_3N in 20 mL THF according to Method 3. Yield: 135 mg (65%). Melting point: 149°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.96 – 7.90 (m, 1H), 7.64 (dd, *J* = 10.1, 2.6 Hz, 1H), 7.50 (td, *J* = 7.7, 1.4 Hz, 1H), 7.37 – 7.28 (m, 3H), 6.49 (d, *J* = 10.1 Hz, 1H), 2.66 (s, 3H), 1.31 (s, 6H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 186.7, 163.6, 154.4, 152.0, 141.4, 136.1, 133.2, 132.2, 131.1, 130.4, 127.3, 126.1, 123.4, 35.6, 29.2, 21.7 ppm; UV/Vis (MeOH): $\lambda_{max}(\varepsilon)$ = 283, 233 nm; IR (KBr): $\tilde{\nu}$ = 3501, 3442, 3056, 2991, 2972, 2955, 2936, 2914, 2869, 1763, 1638, 1624, 1603, 1581, 1486, 1458, 1383, 1364, 1356, 1339, 1286, 1259, 1236, 1228, 1205, 1168, 1138, 1123, 1061, 1023, 997, 955, 947, 931, 919, 913, 868, 847, 812, 791, 768, 735, 682, 661, 490, 479 cm^{-1}; HR-ESI-MS $C_{18}H_{19}NO_3$ calcd: 320.1257 [M+Na$^+$], found: 320.1258.

2,3,5,6-Tetramethyl-4-(((2-methylbenzoyl)oxy)imino)cyclohexa-2,5-dien-1-one (**6**)



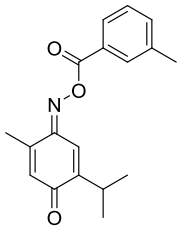
42 mg (0.234 mmol) **6b** was reacted with 47 mg (0.304 mmol) *o*-toluoyl chloride and 71 mg (0.702 mmol) Et_3N in 5 mL THF according to Method 3. Yield: 69 mg (99%). Melting point: 117°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.88 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.47 (td, *J* = 7.5, 1.5 Hz, 1H), 7.35 – 7.27 (m, 2H), 2.65 (s, 3H), 2.37 (d, *J* = 1.2 Hz, 3H), 2.34 (d, *J* = 1.1 Hz, 3H), 2.02 (d, *J* = 1.1 Hz, 6H) ppm; ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 185.5, 164.0, 155.1, 142.3, 141.5, 139.4, 135.5, 135.3, 132.9, 132.2, 130.1, 127.8, 126.0, 21.6, 19.8, 15.1, 12.3, 12.2 ppm; UV/Vis ($CHCl_3$): λ_{max}(***ε***) = 295, 241 nm; IR (KBr): $\tilde{\nu}$ = 3483, 3446, 3229, 2976, 2933, 2359, 1754, 1644, 1623, 1600, 1574, 1558, 1531, 1487, 1456, 1375, 1353, 1307, 1283, 1266, 1223, 1163, 1135, 1054, 1018, 984, 944, 897, 849, 821, 797, 779, 735, 688, 672, 619, 485, 467 cm^{-1}; HR-ESI-MS $C_{18}H_{19}NO_3$ calcd: 320.1257 [M+Na^+], found: 320.1257.

2,6-Di-tert-butyl-4-(((2-methylbenzoyl)oxy)imino)cyclohexa-2,5-dien-1-one (**7**)



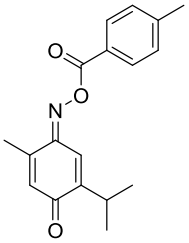
266 mg (1.130 mmol) **7b** was reacted with 262 mg (1.696 mmol) *o*-toluoyl chloride and 343 mg (3.391 mmol) Et_3N in 20 mL THF according to Method 3. Yield: 360 mg (77%). Melting point: 68°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.98 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.56 – 7.50 (m, 2H), 7.40 – 7.34 (m, 2H), 7.24 (d, *J* = 2.6 Hz, 1H), 2.71 (s, 3H), 1.35 (d, *J* = 6.1 Hz, 18H) ppm; ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 186.6, 164.1, 155.1, 154.4, 153.2, 141.0, 133.0, 132.2, 130.5, 129.4, 127.8, 126.2, 118.3, 36.2, 35.8, 29.6, 29.5, 21.9 ppm; UV/Vis (MeOH): λ_{max}(***ε***) = 294, 229 nm; IR (KBr): $\tilde{\nu}$ = 3648, 3453, 3062, 3003, 2993, 2956, 2913, 2871, 1741, 1650, 1642, 1632, 1603, 1591, 1484, 1467, 1457, 1440, 1389, 1363, 1356, 1343, 1311, 1284, 1249, 1228, 1200, 1169, 1128, 1116, 1070, 1039, 1025, 957, 930, 902, 879, 859, 822, 812, 792, 785, 737, 688, 659, 604, 507, 492 cm^{-1}; HR-ESI-MS $C_{18}H_{19}NO_3$ calcd: 376.1883 [M+Na^+], found: 376.1885.

(E)-2-Isopropyl-5-methyl-4-(((3-methylbenzoyl)oxy)imino)cyclohexa-2,5-dien-1-one (**8**)



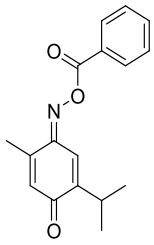
200 mg (1.116 mmol) **1b** was reacted with 224 mg (1.451 mmol) *m*-toluoyl chloride and 339 mg (3.348 mmol) Et_3N in 25 mL THF according to Method 3. Yield: 232 mg (70%). Melting point: 124°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.97 – 7.88 (m, 2H), 7.53 (d, *J* = 1.2 Hz, 1H), 7.50 – 7.39 (m, 2H), 6.41 (q, *J* = 1.4 Hz, 1H), 3.12 (pd, *J* = 6.9, 1.1 Hz, 1H), 2.46 (s, 3H), 2.35 (d, *J* = 1.4 Hz, 3H), 1.18 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 186.2, 163.1, 155.0, 151.5, 145.8, 138.9, 134.9, 131.5, 130.6, 128.9, 128.3, 127.0, 118.9, 27.2, 21.7, 21.5, 17.3 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 290, 236 nm; IR (KBr): $\tilde{\nu}$ = 3481, 3443, 3238, 2967, 2928, 2873, 1753, 1646, 1626, 1603, 1586, 1469, 1445, 1434, 1383, 1269, 1257, 1247, 1175, 1145, 1110, 1081, 1046, 1030, 995, 926, 903, 876, 853, 811, 793, 734, 704, 678, 531, 494, 412 cm^{-1}; HR-ESI-MS $C_{18}H_{19}NO_3$ calcd: 320.1257 [M+Na^+], found: 320.1255.

(E)-2-Isopropyl-5-methyl-4-(((4-methylbenzoyl)oxy)imino)cyclohexa-2,5-dien-1-one (**9**)



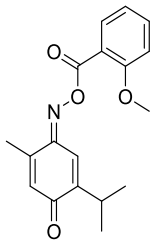
200 mg (1.116 mmol) **1b** was reacted with 224 mg (1.451 mmol) *p*-toluoyl chloride and 339 mg (3.348 mmol) Et_3N in 25 mL THF according to Method 3. Yield: 142 mg (43%). Melting point: 129°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 8.04 – 7.98 (m, 2H), 7.52 (d, *J* = 1.2 Hz, 1H), 7.37 – 7.31 (m, 2H), 6.41 (q, *J* = 1.4 Hz, 1H), 3.10 (sept, *J* = 6.9, 1.1 Hz, 1H), 2.46 (s, 3H), 2.35 (d, *J* = 1.4 Hz, 3H), 1.18 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 186.2, 163.0, 154.8, 151.5, 145.8, 145.1, 131.5, 130.0, 129.7, 125.6, 118.9, 27.2, 22.0, 21.8, 17.3 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 295, 241 nm; IR (KBr): $\tilde{\nu}$ = 3486, 3442, 3248, 2967, 2951, 2930, 2870, 1758, 1646, 1631, 1610, 1602, 1577, 1542, 1508, 1471, 1443, 1426, 1410, 1378, 1361, 1312, 1268, 1239, 1216, 1208, 1178, 1142, 1055, 1033, 1017, 993, 934, 907, 896, 868, 852, 825, 811, 786, 741, 701, 687, 675, 656, 637, 628, 606, 554, 507, 497, 484, 470, 405 cm^{-1}; HR-ESI-MS $C_{18}H_{19}NO_3$ calcd: 320.1257 [M+Na^+], found: 320.1254.

(E)-4-((Benzoyloxy)imino)-2-isopropyl-5-methylcyclohexa-2,5-dien-1-one (**10**)



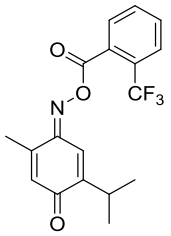
200 mg (1.116 mmol) **1b** was reacted with 204 mg (1.451 mmol) benzoyl chloride and 339 mg (3.348 mmol) Et_3N in 25 mL THF according to Method 3. Yield: 305 mg (96%). Melting point: 108°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 8.16 – 8.09 (m, 2H), 7.71 – 7.63 (m, 1H), 7.59 – 7.50 (m, 3H), 6.42 (q, *J* = 1.4 Hz, 1H), 3.12 (sept, *J* = 6.9, 1.1 Hz, 1H), 2.35 (d, *J* = 1.4 Hz, 3H), 1.18 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 186.2, 163.0, 155.0, 151.6, 145.8, 134.1, 131.6, 129.9, 129.0, 128.4, 118.9, 27.2, 21.8, 17.3 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 292, 232 nm; IR (KBr): $\tilde{\nu}$ = 3435, 3246, 2971, 2954, 2940, 2930, 2872, 1759, 1648, 1631, 1600, 1583, 1490, 1473, 1463, 1450, 1429, 1385, 1375, 1314, 1264, 1234, 1177, 1157, 1144, 1076, 1049, 1032, 1023, 932, 905, 896, 817, 801, 710, 692, 616, 554, 412 cm^{-1}; HR-ESI-MS $C_{18}H_{19}NO_3$ calcd: 306.1101 [M+Na^+], found: 306.1105.

(E)-2-Isopropyl-4-(((2-methoxybenzoyl)oxy)imino)-5-methylcyclohexa-2,5-dien-1-one (**11**)



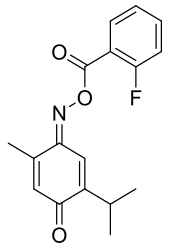
125 mg (0.698 mmol) **1b** was reacted with 155 mg (0.907 mmol) 2-methoxybenzoyl chloride and 212 mg (2.093 mmol) Et_3N in 20 mL THF according to Method 3. Yield: 212 mg (96%). Melting point: 81°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.94 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.62 – 7.52 (m, 2H), 7.12 – 7.02 (m, 2H), 6.40 (q, *J* = 1.3 Hz, 1H), 3.96 (s, 3H), 3.11 (sept, *J* = 6.9, 1.1 Hz, 1H), 2.34 (d, *J* = 1.4 Hz, 3H), 1.16 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 186.3, 163.5, 159.5, 154.6, 151.2, 145.9, 134.8, 132.7, 131.3, 120.7, 119.8, 118.0, 112.3, 56.2, 27.0, 21.9, 17.3 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 289, 228 nm; IR (KBr): $\tilde{\nu}$ = 3443, 3074, 2964, 2929, 2872, 2838, 1737, 1646, 1628, 1601, 1581, 1490, 1462, 1437, 1383, 1375, 1296, 1257, 1219, 1182, 1165, 1144, 1117, 1068, 1039, 1019, 931, 911, 870, 825, 792, 752, 692, 555 cm^{-1}; HR-ESI-MS $C_{18}H_{19}NO_4$ calcd: 336.1206 [M+Na^+], found: 336.1205.

(E)-2-Isopropyl-5-methyl-4-(((2-(trifluoromethyl)benzoyl)oxy)imino)cyclohexa-2,5-dien-1-one (**12**)



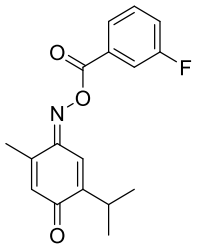
125 mg (0.698 mmol) **1b** was reacted with 189 mg (0.907 mmol) 2-(trifluormethyl)benzoyl chloride and 212 mg (2.093 mmol) Et_3N in 20 mL THF according to Method 3. Yield: 214 mg (87%). Melting point: 65°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.95 – 7.78 (m, 2H), 7.77 – 7.68 (m, 2H), 7.41 (d, *J* = 1.2 Hz, 1H), 6.41 (q, *J* = 1.4 Hz, 1H), 3.07 (sept, *J* = 6.9, 1.1 Hz, 1H), 2.32 (d, *J* = 1.4 Hz, 3H), 1.10 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR-APT (75 MHz, $CDCl_3$) δ = 186.1, 163.8, 155.0, 151.9, 145.5, 132.3 (m), 132.2, 131.8, 131.0, 129.0 (m), 128.9 (q, *J* = 32.7 Hz), 127.0 (q, *J* = 5.2 Hz), 123.4 (q, *J* = 273.4 Hz), 119.2 (m), 27.0, 21.7, 17.3 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 288, 228 nm; IR (KBr): $\tilde{\nu}$ = 3541, 3440, 2979, 2968, 2940, 2880, 1781, 1649, 1631, 1600, 1584, 1468, 1445, 1434, 1388, 1377, 1310, 1276, 1226, 1165, 1146, 1119, 1074, 1043, 1017, 964, 929, 904, 888, 821, 792, 766, 698, 645, 594, 555 cm^{-1}; HR-ESI-MS $C_{18}H_{16}F_3NO_3$ calcd: 374.0975 [M+Na$^+$], found: 374.0970.

(E)-4-(((2-Fluorobenzoyl)oxy)imino)-2-isopropyl-5-methylcyclohexa-2,5-dien-1-one (**13**)



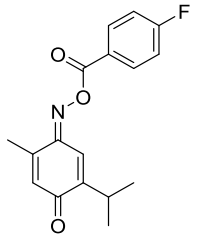
250 mg (1.395 mmol) **1b** was reacted with 354 mg (2.232 mmol) 2-fluorobenzoyl chloride and 423 mg (4.185 mmol) Et_3N in 25 mL THF according to Method 3. Yield: 386 mg (92%). Melting point: 133°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 8.12 (td, *J* = 7.6, 1.9 Hz, 1H), 7.69 – 7.58 (m, 2H), 7.37 – 7.16 (m, 2H), 6.41 (q, *J* = 1.3 Hz, 1H), 3.19 – 3.03 (m, 1H), 2.34 (d, *J* = 1.4 Hz, 3H), 1.17 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 186.3, 162.0 (d, *J* = 258.0 Hz), 161.5 (d, *J* = 3.8 Hz), 155.24, 151.73, 145.47, 135.8 (d, *J* = 9.0 Hz), 133.1, 131.7, 124.8 (d, *J* = 3.8 Hz), 119.8 (d, *J* = 3.0 Hz), 117.4 (d, *J* = 23.3 Hz), 116.7 (d, *J* = 11.3 Hz), 27.1, 21.7, 17.3 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 289, 230 nm; IR (KBr): $\tilde{\nu}$ = 3436, 3243, 2963, 2939, 2919, 2874, 1750, 1651, 1631, 1610, 1583, 1486, 1455, 1433, 1380, 1284, 1261, 1244, 1229, 1156, 1108, 1065, 1049, 1029, 930, 905, 879, 802, 787, 750, 686, 659, 532, 525 cm^{-1}; HR-ESI-MS $C_{17}H_{16}FNO_3$ calcd: 324.1006 [M+Na$^+$], found: 324.1010.

(E)-4-(((3-Fluorobenzoyl)oxy)imino)-2-isopropyl-5-methylcyclohexa-2,5-dien-1-one (**14**)



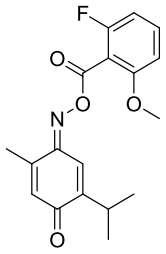
250 mg (1.395 mmol) **1b** was reacted with 288 mg (1.814 mmol) 3-fluorobenzoyl chloride and 423 mg (4.185 mmol) Et_3N in 25 mL THF according to Method 3. Yield: 345 mg (82%). Melting point: 101°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.92 (dt, *J* = 7.7, 1.3 Hz, 1H), 7.79 (ddd, *J* = 9.0, 2.6, 1.6 Hz, 1H), 7.54 (td, *J* = 8.0, 5.5 Hz, 1H), 7.48 (d, *J* = 1.1 Hz, 1H), 7.37 (tdd, *J* = 8.3, 2.6, 1.0 Hz, 1H), 6.42 (q, *J* = 1.3 Hz, 1H), 3.12 (heptd, *J* = 6.9, 1.0 Hz, 1H), 2.35 (d, *J* = 1.4 Hz, 3H), 1.18 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 186.1, 162.8 (d, *J* = 246.8 Hz), 161.9 (d, *J* = 3 Hz), 155.2, 151.9, 145.6, 131.7, 130.8 (d, *J* = 8.3 Hz), 130.5 (d, *J* = 7.5 Hz), 125.7 (d, *J* = 3.0 Hz), 121.3 (d, *J* = 21.0 Hz), 118.7, 116.9 (d, *J* = 23.3 Hz), 27.3, 21.7, 17.3 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 287, 232 nm; IR (KBr): $\tilde{\nu}$ = 3443, 3233, 3070, 3015, 2988, 2964, 2932, 2918, 2875, 1778, 1736, 1715, 1645, 1625, 1599, 1591, 1539, 1484, 1469, 1442, 1425, 1409, 1385, 1353, 1309, 1269, 1259, 1244, 1174, 1153, 1112, 1052, 1030, 1001, 927, 905, 896, 862, 799, 795, 741, 700, 687, 671, 663, 643, 558, 533, 506, 499, 405 cm^{-1}; HR-ESI-MS $C_{17}H_{16}FNO_3$ calcd: 324.1006 [M+Na$^+$], found: 324.1004.

(E)-4-(((4-Fluorobenzoyl)oxy)imino)-2-isopropyl-5-methylcyclohexa-2,5-dien-1-one (**15**)



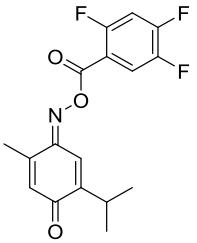
250 mg (1.395 mmol) **1b** was reacted with 288 mg (1.814 mmol) 4-fluorobenzoyl chloride and 423 mg (4.185 mmol) Et_3N in 25 mL THF according to Method 3. Yield: 371 mg (88%). Melting point: 119°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 8.19 – 8.10 (m, 2H), 7.48 (d, *J* = 1.1 Hz, 1H), 7.27 – 7.17 (m, 2H), 6.42 (q, *J* = 1.3 Hz, 1H), 3.12 (sept, *J* = 6.9, 1.1 Hz, 1H), 2.34 (d, *J* = 1.4 Hz, 3H), 1.18 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 186.1, 166.4 (d, *J* = 254.3 Hz), 162.0, 155.04, 151.74, 145.69, 132.6 (d, *J* = 9.8 Hz), 131.62, 124.6 (d, *J* = 3.0 Hz), 118.7, 116.4 (d, *J* = 21.8 Hz), 27.3, 21.8, 17.3 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 293, 233 nm; IR (KBr): $\tilde{\nu}$ = 3500, 3443, 3247, 3109, 3077, 3066, 2978, 2956, 2931, 2872, 1765, 1717, 1646, 1631, 1600, 1540, 1506, 1486, 1473, 1463, 1445, 1412, 1384, 1376, 1361, 1308, 1297, 1267, 1239, 1178, 1156, 1142, 1107, 1096, 1047, 1032, 1012, 931, 905, 896, 862, 840, 825, 797, 755, 744, 701, 686, 676, 658, 634, 625, 605, 553, 509, 497 cm^{-1}; HR-ESI-MS $C_{17}H_{16}FNO_3$ calcd: 324.1006 [M+Na$^+$], found: 324.1004.

(E)-4-(((2-Fluoro-6-methoxybenzoyl)oxy)imino)-2-isopropyl-5-methylcyclohexa-2,5-dien-1-one (**16**)



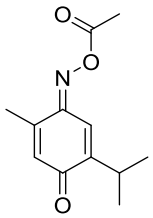
79 mg (0.442 mmol) **1b** was reacted with 75 mg (0.442 mmol) 2-fluoro-6-methoxybenzoic acid, 119 mg (0.575 mmol) DCC and a catalytic amount of DMAP in 10 mL DCM according to Method 4. Yield: 105 mg (72%). Melting point: 88°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.50 – 7.40 (m, 2H), 6.85 – 6.77 (m, 2H), 6.39 (q, *J* = 1.3 Hz, 1H), 3.91 (s, 3H), 3.08 (heptd, *J* = 6.9, 1.1 Hz, 1H), 2.27 (d, *J* = 1.4 Hz, 3H), 1.12 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 186.25, 161.1 (d, *J* = 252.7 Hz), 160.73, 159.1 (d, *J* = 6.3 Hz), 154.96, 151.54, 145.60, 133.3 (d, *J* = 10.7 Hz), 131.56, 119.46, 109.5 (d, *J* = 18.3 Hz) 108.5 (d, *J* = 21.8 Hz), 107.3 (d, *J* = 3.1 Hz), 56.61, 27.09, 21.76, 17.22 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 283, 206 nm; IR (KBr): $\tilde{\nu}$ = 3493, 3445, 3249, 3097, 2971, 2938, 2917, 2897, 2877, 2847, 1760, 1650, 1631, 1613, 1302, 1582, 1485, 1475, 1444, 1428, 1403, 1387, 1374, 1358, 1307, 1289, 1259, 1245, 1231, 1142, 1091, 1078, 1036, 946, 911, 901, 889, 866, 824, 795, 764, 703, 647, 609, 539, 523, 490, 480 cm^{-1}; HR-ESI-MS $C_{18}H_{18}FNO_4$ calcd: 354.1112 [M+Na^+], found: 354.1111.

(E)-2-Isopropyl-5-methyl-4-(((2,4,5-trifluorobenzoyl)oxy)imino)cyclohexa-2,5-dien-1-one (**17**)



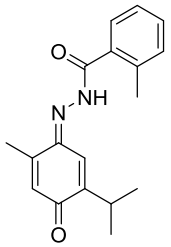
200 mg (1.116 mmol) **1b** was reacted with 282 mg (1.451 mmol) 2,4,5-trifluorobenzoyl chloride and 339 mg (3.348 mmol) Et_3N in 20 mL THF according to Method 3. Yield: 310 mg (82%). Melting point: 120°C. ^{1}H-NMR (300 MHz, $CDCl_3$) 7.96 (ddd, *J* = 10.0, 8.7, 6.3 Hz, 1H), 7.58 (s, 1H), 7.12 (td, *J* = 9.8, 6.1 Hz, 1H), 6.42 (d, *J* = 1.2 Hz 1H), 3.11 (sept, *J* = 6.8, 0.9 Hz, 1H), 2.33 (d, *J* = 1.1 Hz, 3H), 1.16 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR-APT (101 MHz, $CDCl_3$) δ = 186.2, 159.8 (m), 158.0 (ddd, *J* = 259.2, 10.1, 2.6 Hz), 155.6, 154.1 (ddd, *J* = 261.3, 14.5, 12.7 Hz), 152.1, 147.2 (ddd, *J* = 248.3, 12.7, 3.5), 145.3, 131.9, 121.0 (dt, *J* = 20.9, 2.6 Hz), 119.7, 113.4 (m), 107.7 (dd, *J* = 29.2, 21.2 Hz), 27.2, 21.8, 17.3 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 290, 229 nm; IR (KBr): $\tilde{\nu}$ = 3488, 3444, 3247, 3067, 2973, 2960, 2941, 2914, 2877, 1755, 1651, 1632, 1601, 1522, 1508, 1469, 1449, 1432, 1410, 1383, 1362, 1345, 1308, 1295, 1261, 1227, 1208, 1181, 1152, 1037, 925, 904, 875, 834, 791, 766, 731, 679, 653, 638, 610, 545, 476 cm^{-1}; HR-ESI-MS $C_{17}H_{14}F_3NO_3$ calcd: 360.0818 [M+Na^+], found: 360.0816.

(E)-4-(Acetoxyimino)-2-isopropyl-5-methylcyclohexa-2,5-dien-1-one (**18**)



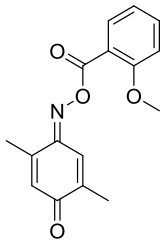
150 mg (0.837 mmol) **1b** was reacted with 103 mg (1.005 mmol) acetic anhydride and 254 mg (2.511 mmol) Et_3N in 20 mL THF in analogy to Method 3. Yield: 168 mg (91%). Melting point: 75°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.38 (d, *J* = 1.1 Hz, 1H), 6.38 (q, *J* = 1.4 Hz, 1H), 3.07 (sept, *J* = 6.9, 1.1 Hz, 1H), 2.35 (s, 3H), 2.26 (d, *J* = 1.4 Hz, 3H), 1.14 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 186.2, 168.3, 153.7, 151.5, 145.5, 131.5, 118.8, 27.1, 21.8, 19.9, 17.2 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 286 nm; IR (KBr): $\tilde{\nu}$ = 3538, 3442, 3241, 3034, 2992, 2965, 2935, 2927, 2875, 1785, 1738, 1650, 1631, 1602, 1466, 1442, 1428, 1413, 1388, 1368, 1312, 1266, 1249, 1178, 1143, 1100, 1040, 1028, 999, 945, 916, 890, 836, 702, 661, 605, 587, 546, 475 cm^{-1}; HR-ESI-MS $C_{12}H_{15}NO_3$ calcd: 244.0944 [M+Na^+], found: 244.0941.

(E)-N'-(5-Isopropyl-2-methyl-4-oxocyclohexa-2,5-dien-1-ylidene)-2-methylbenzohydrazide (**19**)



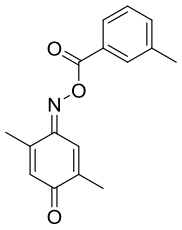
548 mg (3.654 mmol) 2-methylbenzohydrazide was dissolved in 100 mL water/ethanol (95:5). After the addition of 600 mg (3.654 mmol) thymoquinone and a catalytic amount of glacial acetic acid the reaction mixture was refluxed for 36 hours. The solvent was removed and the solid residue purified using column chromatography (eluent: hexane/ethyl acetate, (8:1) to provide the desired product. Yield: 400 mg (37%). Melting point: 189°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.58 – 7.32 (m, 3H), 7.31 – 7.21 (m, 2 H), 6.37 – 6.26 (m, 1H), 3.09 (sept, *J* = 6.9 Hz, 1H), 2.41 (s, 3H), 1.08 (d, *J* = 6.9 Hz, 6H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 186.5, 150.6, 148.2, 133.9, 130.4, 130.4, 129.6, 128.0, 125.3, 117.1, 117.0, 27.1, 21.9, 20.0, 17.1 ppm; UV/Vis (MeOH): $\lambda_{max}(\varepsilon)$ = 352, 203 nm; IR (KBr): $\tilde{\nu}$ = 3443, 3220, 3177, 2963, 2927, 2872, 2359, 2341, 1667, 1638, 1622, 1531, 1490, 1465, 1435, 1407, 1399, 1324, 1291, 1265, 1248, 1205, 1147, 1119, 1107, 1025, 932, 897, 866, 781, 741, 647, 632, 612, 587, 545, 469 cm^{-1}; HR-ESI-MS $C_{18}H_{20}N_2O_2$ calcd: 319.1417 [M+Na^+], found: 319.1419.

(E)-4-(((2-Methoxybenzoyl)oxy)imino)-2,5-dimethylcyclohexa-2,5-dien-1-one (**20**)



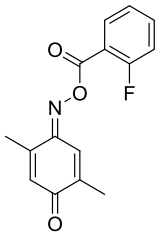
100 mg (0.662 mmol) **2b** was reacted with 147 mg (0.860 mmol) 2-methoxybenzoyl chloride and 200 mg (1.986 mmol) Et_3N in 25 mL THF according to Method 3. Yield: 171 mg (91%). Melting point: 126°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 7.93 (dd, *J* = 7.7, 1.9 Hz, 1H), 7.68 – 7.63 (m, 1H), 7.63 – 7.51 (m, 1H), 7.12 – 6.99 (m, 2H), 6.43 – 6.37 (m, 1H), 3.96 (s, 3H), 2.34 (d, *J* = 1.4 Hz, 3H), 2.06 (d, *J* = 1.6 Hz, 3H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 187.2, 163.3, 159.6, 154.7, 146.6, 142.0, 134.9, 132.6, 131.0, 123.1, 120.8, 118.1, 112.4, 56.2, 17.5, 16.5 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 289, 231 nm; IR (KBr): $\tilde{\nu}$ = 3445, 3240, 3087, 3002, 1769, 1737, 1652, 1629, 1601, 1576, 1492, 1467, 1455, 1437, 1378, 1287, 1260, 1220, 1172, 1140, 1054, 1042, 1026, 1006, 945, 900, 890, 815, 791, 764, 709, 689 cm^{-1}; HR-ESI-MS $C_{16}H_{15}NO_4$ calcd: 308.0893 [M+Na$^+$], found: 308.0893.

(E)-2,5-Dimethyl-4-(((3-methylbenzoyl)oxy)imino)cyclohexa-2,5-dien-1-one (**21**)



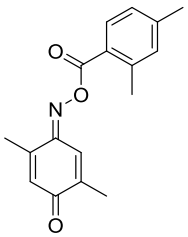
100 mg (0.662 mmol) **2b** was reacted with 130 mg (0.860 mmol) *m*-toluoyl chloride and 200 mg (1.986 mmol) Et_3N in 25 mL THF according to Method 3. Yield: 164 mg (92%). Melting point: 144°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.00 – 7.85 (m, 2H), 7.63 – 7.55 (m, 1H), 7.52 – 7.37 (m, 2H), 6.48 – 6.37 (m, 1H), 2.46 (s, 3H), 2.36 (d, *J* = 1.3 Hz, 3H), 2.10 (d, *J* = 1.5 Hz, 3H) ppm; ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 186.9, 163.2, 154.7, 146.5, 142.3, 138.9, 135.0, 131.0, 130.6, 128.8, 128.2, 127.1, 122.1, 21.5, 17.5, 16.4 ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 289, 236 nm; IR (KBr): $\tilde{\nu}$ = 3490, 3443, 3063, 2977, 2960, 2946, 2921, 1757, 1714, 1653, 1627, 1606, 1587, 1487, 1444, 1427, 1378, 1362, 1270, 1449, 1231, 1178, 1166, 1076, 1042, 1015, 994, 929, 921, 897, 846, 807, 790, 734, 706, 689, 681, 664, 635, 530, 408 cm^{-1}; HR-ESI-MS $C_{16}H_{15}NO_3$ calcd: 292.0944 [M+Na$^+$], found: 292.0943.

(E)-4-(((2-Fluorobenzoyl)oxy)imino)-2,5-dimethylcyclohexa-2,5-dien-1-one (**22**)



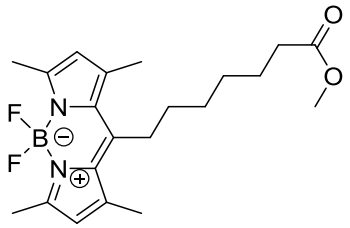
100 mg (0.662 mmol) **2b** was reacted with 136 mg (0.860 mmol) *o*-fluorobenzoyl chloride and 200 mg (1.986 mmol) Et_3N in 25 mL THF according to Method 3. Yield: 155 mg (86%). Melting point: 128°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.14 – 8.03 (m, 1H), 7.71 – 7.56 (m, 2H), 7.37 – 7.17 (m, 2H), 6.42 (s, 1H), 2.35 (s, 3H), 2.08 (s, 3H) ppm; ^{13}C-NMR (101 MHz, $CDCl_3$) δ = 187.0, 162.0 (d, *J* = 258.0 Hz), 161.4 (d, *J* = 4.1 Hz), 154.9, 146.2, 142.4, 135.8 (d, *J* = 9.2 Hz), 133.0, 131.2, 124.7 (d, *J* = 3.7 Hz), 122.7 (d, *J* = 2.2 Hz), 117.4 (d, *J* = 22.8 Hz), 116.8 (d, *J* = 11.0 Hz), 17.4, 16.4 ppm*;* UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 286, 231 nm; IR (KBr): $\tilde{\nu}$ = 3625, 3487, 3444, 3243, 3081, 3066, 1759, 1655, 1631, 1608, 1485, 1456, 1446, 1425, 1382, 1361, 1288, 1262, 1232, 1166, 1108, 1070, 1032, 1015, 928, 903, 804, 785, 755, 707, 687, 648, 637, 528, 522 cm^{-1}; HR-ESI-MS $C_{15}H_{12}FNO_3$ calcd: 296.0693 [M+Na^+], found: 296.0696.

(E)-4-(((2,4-Dimethylbenzoyl)oxy)imino)-2,5-dimethylcyclohexa-2,5-dien-1-one (**23**)



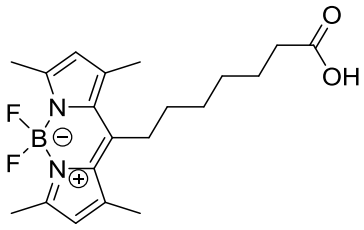
100 mg (0.662 mmol) **2b** was reacted with 99 mg (0.662 mmol) 2,4-dimethylbenzoic acid, 163 mg (0.792 mmol) DCC and catalytic amount of DMAP in 5 mL DCM according to Method 4. Yield: 188 mg (quant.). Melting point: 137°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 7.87 (d, *J* = 7.8 Hz, 1H), 7.53 (q, *J* = 1.5 Hz, 1H), 7.18 – 7.12 (m, 2H), 6.46 – 6.37 (m, 1H), 2.64 (s, 3H), 2.40 (s, 3H), 2.36 (d, *J* = 1.4 Hz, 3H), 2.07 (d, *J* = 1.6 Hz, 3H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 187.0, 163.5, 154.3, 146.6, 144.0, 142.1, 141.7, 133.1, 130.9, 130.6, 126.9, 124.5, 122.3, 21.8, 21.7, 17.5, 16.3 ppm; UV/Vis ($CHCl_3$): $\lambda_{max}(\varepsilon)$ = 289, 244 nm; IR (KBr): $\tilde{\nu}$ = 3649, 3445, 3246, 3017, 2962, 2925, 2857, 2360, 2342, 1758, 1714, 1651, 1629, 1613, 1605, 1570, 1539, 1496, 1446, 1406, 1380, 1354, 1304, 1287, 1265, 1231, 1166, 1143, 1097, 1029, 1006, 976, 937, 898, 876, 836, 805, 761, 725, 703, 685, 635, 609, 560, 535, 505, 437, 414 cm^{-1}; HR-ESI-MS $C_{17}H_{17}NO_3$ calcd: 306.1101 [M+Na^+], found: 306.1102.

Methyl 7-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)heptanoate (**24a**)



The synthesis was carried out essentially as previously described,[7, 8] in a variation of the original procedure.[9] To a solution of 2,4-dimethyl-1*H*-pyrrole (460 mg, 4.84 mmol) in DCM (15 mL), methyl 8-chloro-8-oxooctanoate (500 mg, 2.42 mmol) was added dropwise and the resulting deep red solution was refluxed for 2 h. Volatiles were evaporated in vacuo, the solid residue was dissolved in DCM (1 mL) and toluene (18 mL), triethylamine was added (1.68 mL, 12.1 mmol) and the resulting mixture stirred at room temperature for 30 min. Subsequently, BF_3-Et_2O (2.13 mL, 19.94 mmol) was added dropwise, and the mixture was stirred for 2 h at 60°C. The deep red solution was washed with water, followed by brine, and the organic layer was dried over Na_2SO_4. After removal of the solvent under reduced pressure and column chromatography (hexane/DCM, 1:1), **24a** was obtained as an orange-green fluorescing solid. Yield: 397 mg (42%). Melting point: 129°C. ^{1}H-NMR (300 MHz, $CDCl_3$) δ = 1.32 – 1.56 (m, 4H), 1.64 (tt, *J* = 14.3, 7.1, 4H), 2.31 (t, *J* = 7.4, 2H), 2.40 (s, 6H), 2.51 (s, 6H), 2.84 – 3.01 (m, 2H), 3.66 (s, 3H), 6.04 (s, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 14.57, 16.51, 24.99, 28.50, 29.08, 30.17, 31.86, 34.06, 51.64, 121.72, 131.54, 140.38, 146.51, 153.91, 165.97, 174.14; ^{19}F-NMR (282 MHz, $CDCl_3$) δ = -147.10 (dd, *J* = 65.2, 31.6) ppm; UV/Vis (DCM) $\lambda_{max}(\varepsilon)$ = 495, 358, 304, 241 nm; IR (KBr): $\tilde{\nu}$ = 3448, 2931, 2867, 2857, 1735, 1640, 1552, 1535, 1510, 1474, 1464, 1447, 1433, 1408, 1370, 1324, 1307, 1276, 1250, 1225, 1205, 1162, 1112, 1082, 1060, 1023, 988, 838, 810, 730, 716, 581, 514, 482 cm^{-1}; ESI-MS $C_{21}H_{29}BF_2N_2O_2$ calcd: 413.2 [M+Na$^+$], found: 413.2.

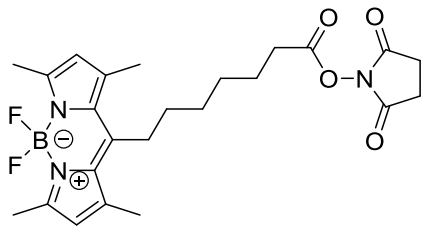
7-(5,5-Difluoro-1,3,7,9-tetramethyl-5H-4λ⁴,5λ⁴-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)heptanoic acid (**24b**)



The synthesis was carried out essentially as previously described,[7, 8] in a variation of the original procedure.[9] To the solution of **24a** (308 mg, 0.79 mmol) in isopropanol (65 mL), an aqueous solution of 0.1 M KOH (30 mL) was added dropwise and the mixture was stirred for 3.5 h at room temperature. The solution was diluted with water and acidified with 1 M HCl. The solution was extracted with DCM and the combined organic phases were dried over Na_2SO_4. After removal of the solvent under reduced pressure and column chromatography (2% to 4% methanol in DCM), **24b** was obtained as an orange solid. Yield: 233 mg (77%, 95% brsm).

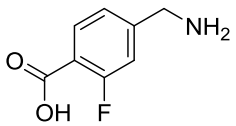
Melting point: 199°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.38 – 1.56 (m, 4H), 1.59 – 1.72 (m, 4H), 2.36 (t, *J* = 7.4, 2H), 2.40 (s, 6H), 2.51 (s, 6H), 2.83 – 3.02 (m, 2H), 6.05 (s, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 14.58, 16.53, 24.72, 28.48, 28.98, 30.13, 31.83, 33.94, 121.75, 131.56, 140.38, 146.46, 153.97, 165.97, 179.26; ^{19}F-NMR (376 MHz, cdcl$_3$) δ = -147.18 – -146.80 (m) ppm; UV/Vis (DCM) $\lambda_{max}(\varepsilon)$ = 495, 355, 305, 241 nm; IR (KBr): $\tilde{\nu}$ = 3651, 3434, 2929, 2864, 1737, 1709, 1631, 1551, 1510, 1475, 1440, 1409, 1372, 1308, 1225, 1203, 1161, 1114, 1081, 1028, 986, 824, 715, 503, 482 cm^{-1}; HR-ESI-MS $C_{20}H_{27}BF_2N_2O_2$ calcd: 399.2026 [M+Na$^+$], found: 399.2023.

2,5-Dioxopyrrolidin-1-yl 7-(5,5-difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)heptanoate (**24c**)[8]



To the solution of **24b** (27.3 mg, 0.073 mmol) and *N*-hydroxysuccinimide (16.8 mg, 0.146 mmol) in THF (3 mL), a solution of DCC (30.2 mg, 0.146 mmol) in THF (1 mL) was added dropwise, and the mixture was stirred at room temperature overnight. Volatiles were removed under reduced pressure, the residue taken up in DCM and dicyclohexylurea was filtered off. After removal of the solvent under reduced pressure and column chromatography (10% acetone in DCM), **24c** was obtained as an orange solid. Yield: 35 mg (quant.). Melting point: 128°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 6.1 (s, 2H), 3.0 - 2.9 (m, 2H), 2.8 (s, 4H), 2.6 (t, *J* = 7.3 Hz, 2H), 2.5 (s, 6H), 2.4 (s, 6H), 1.8 (p, *J* = 7.2 Hz, 2H), 1.7 - 1.6 (m, 2H), 1.6 - 1.4 (m, 4H) ppm; ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 169.3, 168.7, 154.0, 146.5, 140.5, 131.6, 121.8, 31.8, 31.0, 30.0, 28.7, 28.5, 25.8, 24.8, 16.6, 14.7 ppm; ^{19}F-NMR (376 MHz, $CDCl_3$) δ = -152.4 – -152.1 (m) ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 499, 276 nm; IR (KBr): $\tilde{\nu}$ = 3434, 2934, 2866, 1811, 1784, 1739, 1653, 1628, 1550, 1509, 1474, 1466, 1437, 1407, 1371, 1307, 1270, 1203, 1160, 1098, 1078, 1024, 986, 975, 895, 836, 823, 813, 805, 715, 647, 579, 482, 420 cm^{-1}; HR-ESI-MS $C_{24}H_{30}BF_2N_3O_4$ calcd: 496.2190 [M+Na$^+$], found: 496.2189.

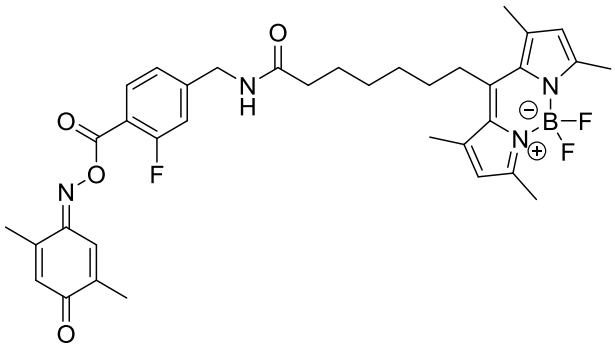
4-(Aminomethyl)-2-fluorobenzoic acid (**24d**)



2 g (12.11 mmol) 4-cyano-2-fluorobenzoic acid was stirred at room temperature overnight under a hydrogen atmosphere in the presence of 300 mg Pd/C (10 wt-%). The reaction mixture was filtered and concentrated under reduced pressure to provide **24d** as a white solid. Yield: 2.045 g (quant.). Melting point: 241°C. ^{1}H-NMR (300 MHz, D_2O) δ = 7.96 – 7.85 (m, 1H), 7.57 – 7.42 (m, 2H), 4.48 (s, 2H) ppm; ^{13}C-NMR (75 MHz, D_2O) δ = 172.6, 160.3 (d, *J* = 249.0 Hz), 136.7 (d, *J* = 8.2 Hz), 131.4 (d, *J* = 3.6 Hz), 124.9 (d, *J* = 3.5 Hz), 118.1 (d, *J* = 24.2 Hz), 117.0

(d, *J* = 24.3 Hz), 43.0 ppm; ^{19}F-NMR (377 MHz, D_2O) δ = -114.81 (dd, *J* = 10.8, 7.7 Hz) ppm; UV/Vis (water): $\lambda_{max}(\varepsilon)$ = 271, 223 nm; IR (KBr): $\tilde{\nu}$ = 3440, 3238, 3046, 2928, 2904, 2841, 2643, 2446, 2360, 2230, 2132, 1928, 1628, 1587, 1541, 1500, 1463, 1426, 1391, 1372, 1301, 1268, 1241, 1171, 1162, 1121, 1093, 988, 947, 905, 885, 854, 823, 794, 735, 699, 645, 617, 594, 585, 540, 522, 508, 498, 486, 469, 421 cm^{-1}; HR-ESI-MS $C_8H_8FNO_2$ calcd: 361.0970 [2M+Na^+], found: 361.0971.

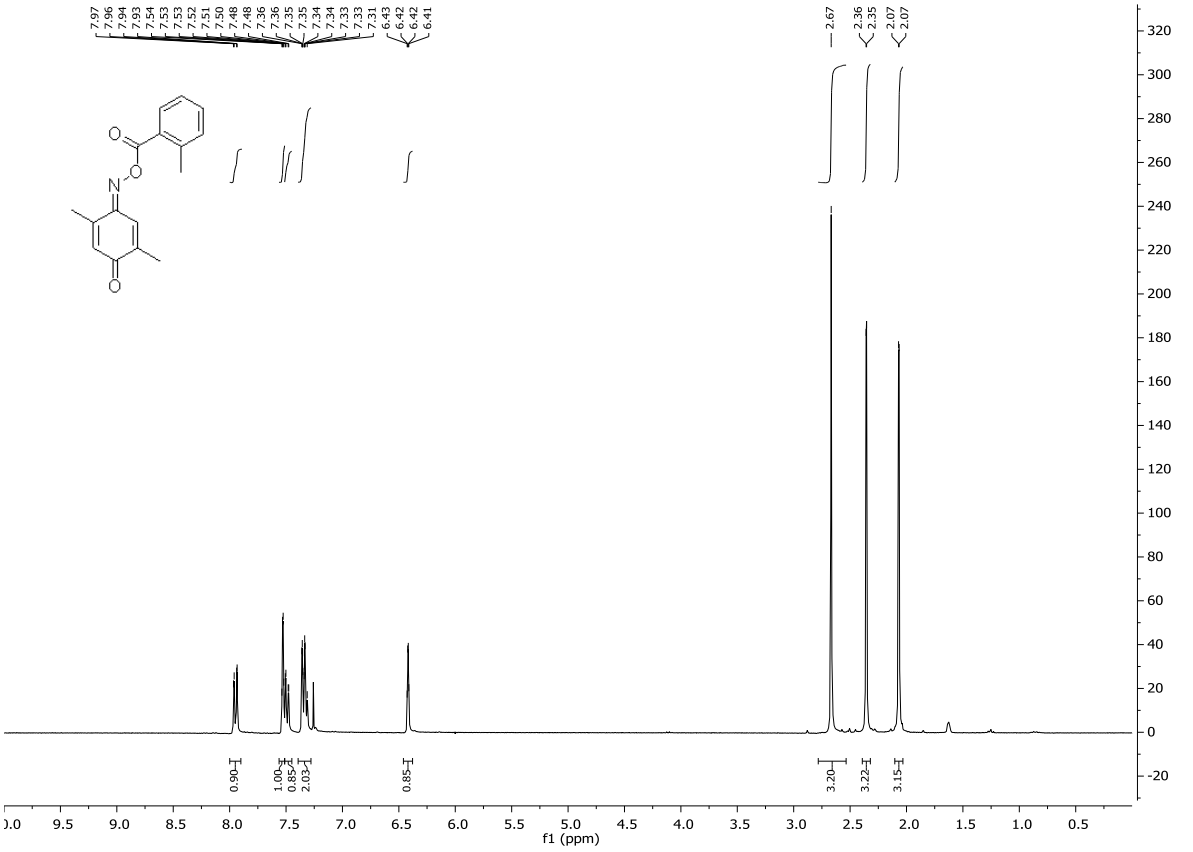
(E)-7-(5,5-Difluoro-1,3,7,9-tetramethyl-5H-4l4,5l4-dipyrrolo[1,2-c:2',1'-f][1,3,2]diazaborinin-10-yl)-N-(4-((((2,5-dimethyl-4-oxocyclohexa-2,5-dien-1-ylidene)amino)oxy)carbonyl)-3-fluorobenzyl)heptanamide (**24**)

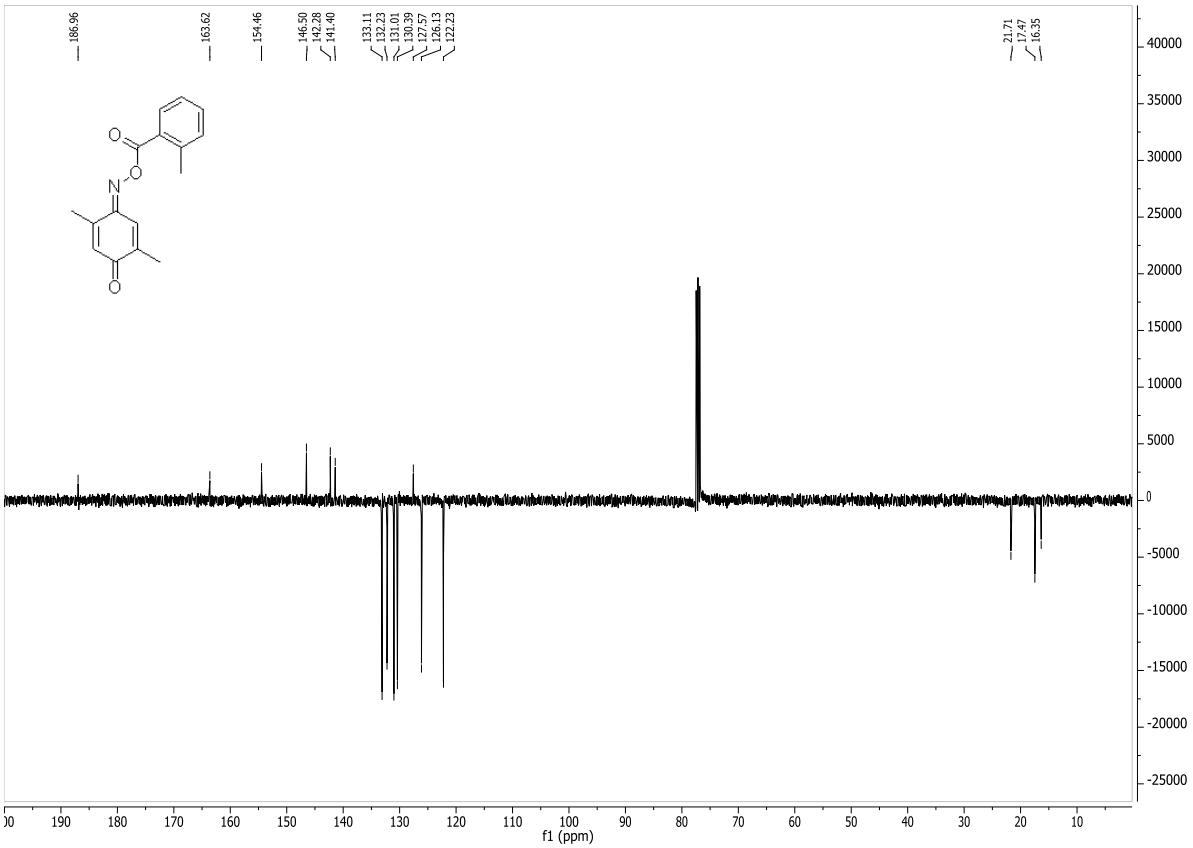


12.4 mg (0.073 mmol) **24d** and Na_2CO_3 (23.2 mg, 0.219 mmol) were suspended in 10 mL of an H_2O/THF mixture (1:1). 35 mg (0.073 mmol) **24c** was added in one portion and the mixture was stirred for 30 min at room temperature. 2 mL sat. $NaHCO_3$ were added and the aqueous phase was washed twice with ethyl acetate. The aqueous phase was acidified with 1 M HCl until precipitation set in, and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, and the solvent was removed under reduced pressure to give amide **24e** as a raw product. 21 mg (0.04 mmol) of raw **24e** were reacted with **2b** (6 mg, 0.04 mmol) in DCM (4 mL) according to the general synthetic method 4 to give **24** as a deep orange solid. Yield: 19.4 mg (74%). Melting point: 162°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 8.05 – 7.99 (m, 1H), 7.59 (q, *J* = 1.5 Hz, 1H), 7.18 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.15 – 7.09 (m, 1H), 6.41 (q, *J* = 1.3 Hz, 1H), 6.04 (s, 2H), 5.99 (t, *J* = 6.0 Hz, 1H), 4.49 (d, *J* = 6.1 Hz, 2H), 3.00 – 2.88 (m, 2H), 2.49 (s, 6H), 2.39 (s, 6H), 2.34 (d, *J* = 1.4 Hz, 3H), 2.25 (t, *J* = 7.5 Hz, 2H), 2.06 (d, *J* = 1.6 Hz, 3H), 1.76 – 1.58 (m, 4H), 1.56 – 1.46 (m, 2H), 1.46 – 1.35 (m, 2H) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$) δ = 187.01, 173.15, 162.17 (d, *J* = 260.4 Hz), 161.16, 154.99, 154.02, 148.04, 147.93, 146.59, 146.27, 142.55, 140.46, 133.33 (d, *J* = 1.3 Hz), 131.68, 131.27, 123.59 (d, *J* = 3.3 Hz), 122.74 (d, *J* = 2.0 Hz), 121.86, 116.19 (d, *J* = 23.5 Hz), 115.62 (d, *J* = 11.3 Hz), 104.99, 42.90, 36.49, 31.93, 30.05, 29.27, 28.50, 25.60, 17.51, 16.64, 16.45, 14.65 ppm. ^{19}F-NMR (377 MHz, $CDCl_3$) δ = -105.26 – -106.68 (m), -145.51 – -148.20 (m) ppm; UV/Vis (DCM): $\lambda_{max}(\varepsilon)$ = 499, 293, 242 nm; IR (KBr): $\tilde{\nu}$ = 3444, 2926, 2856, 2360, 2338, 1758, 1650, 1630, 1550, 1511, 1470, 1431, 1413, 1371, 1308, 1279, 1255, 1227, 1201, 1161, 1124, 1078, 1056, 1013, 985, 927, 842, 810, 761, 482 cm^{-1}; HR-ESI-MS $C_{36}H_{40}BF_3N_4O_4$ calcd: 683.2987 [M+Na^+], found: 683.2985.

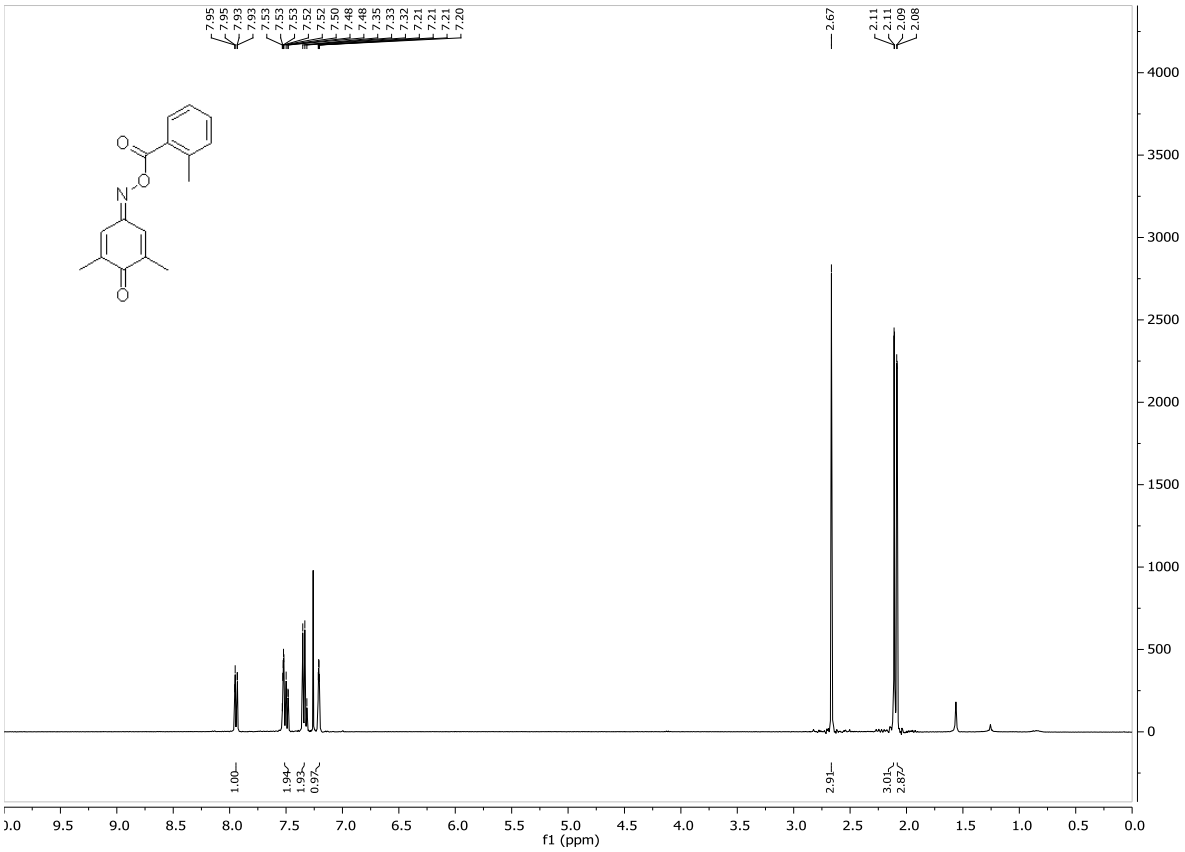
NMR spectra

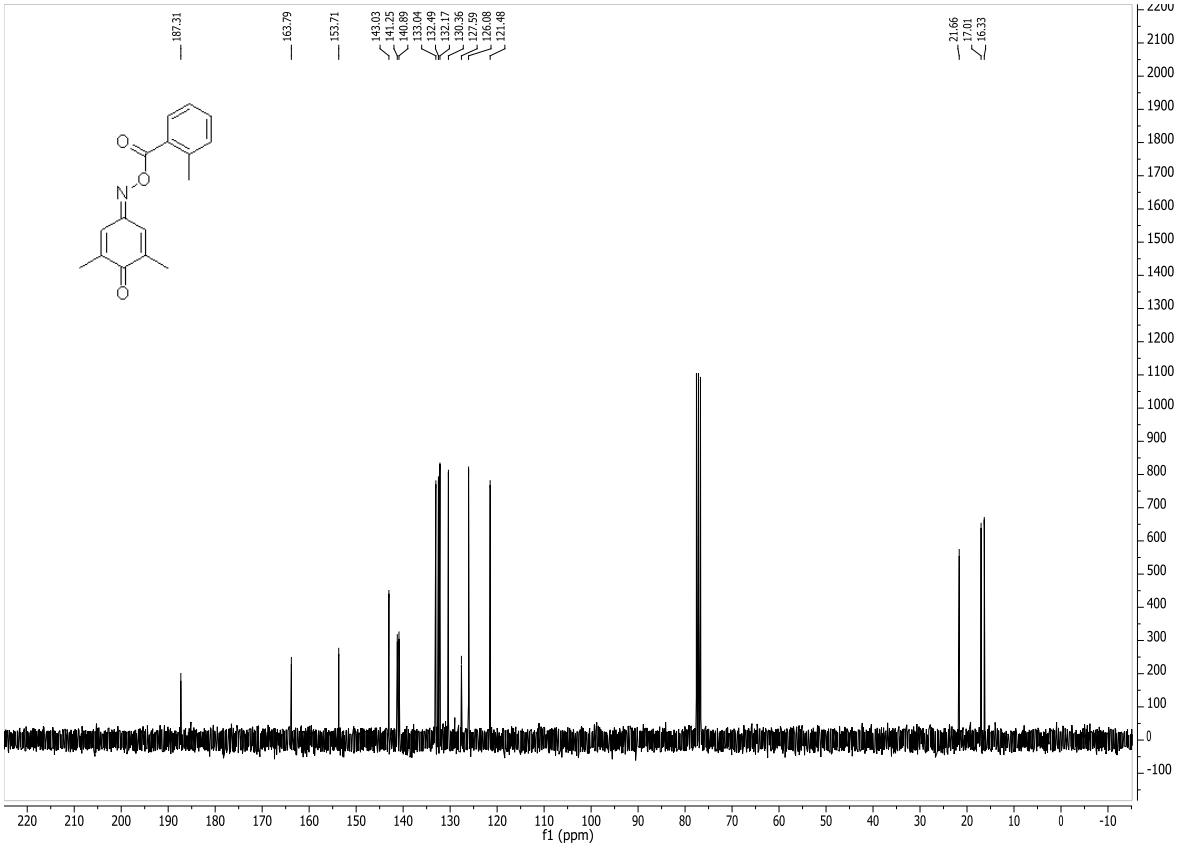
<u>^{1}H- and ^{13}C-NMR spectra of compound **2**</u>



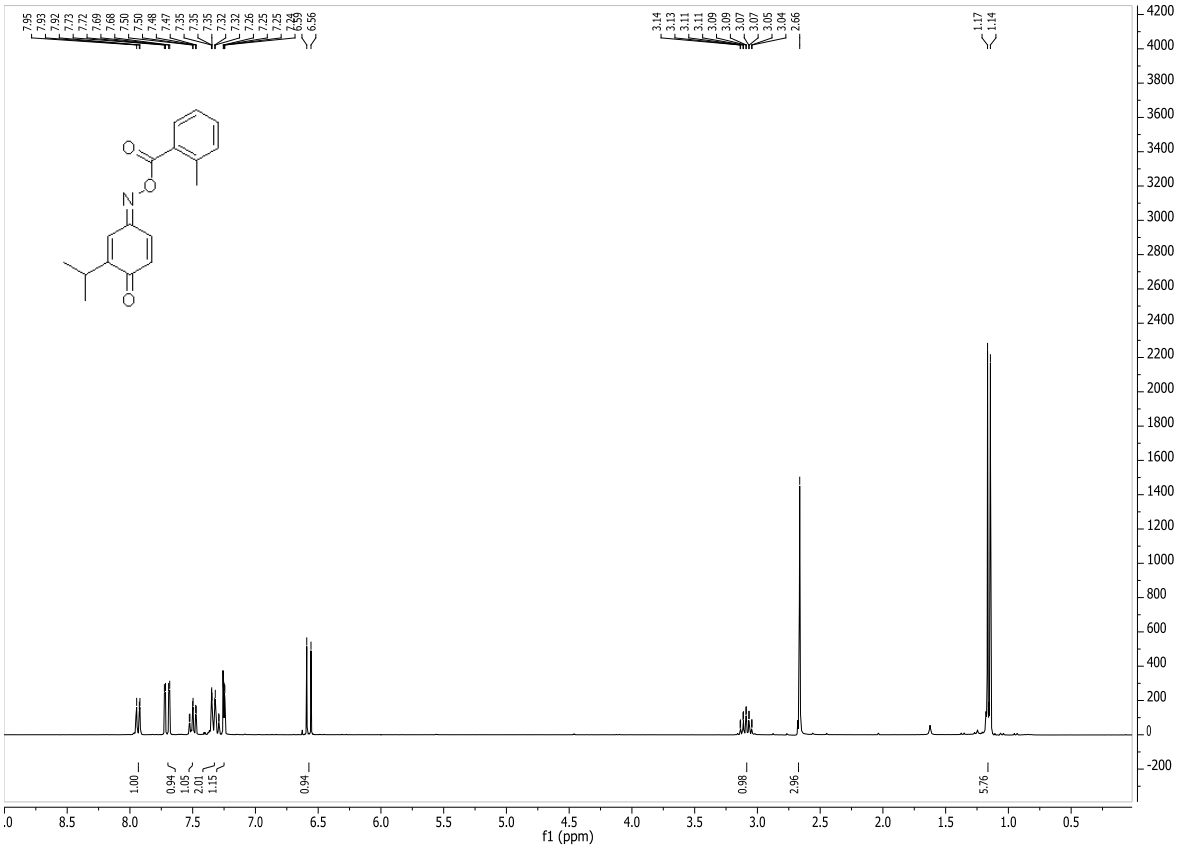


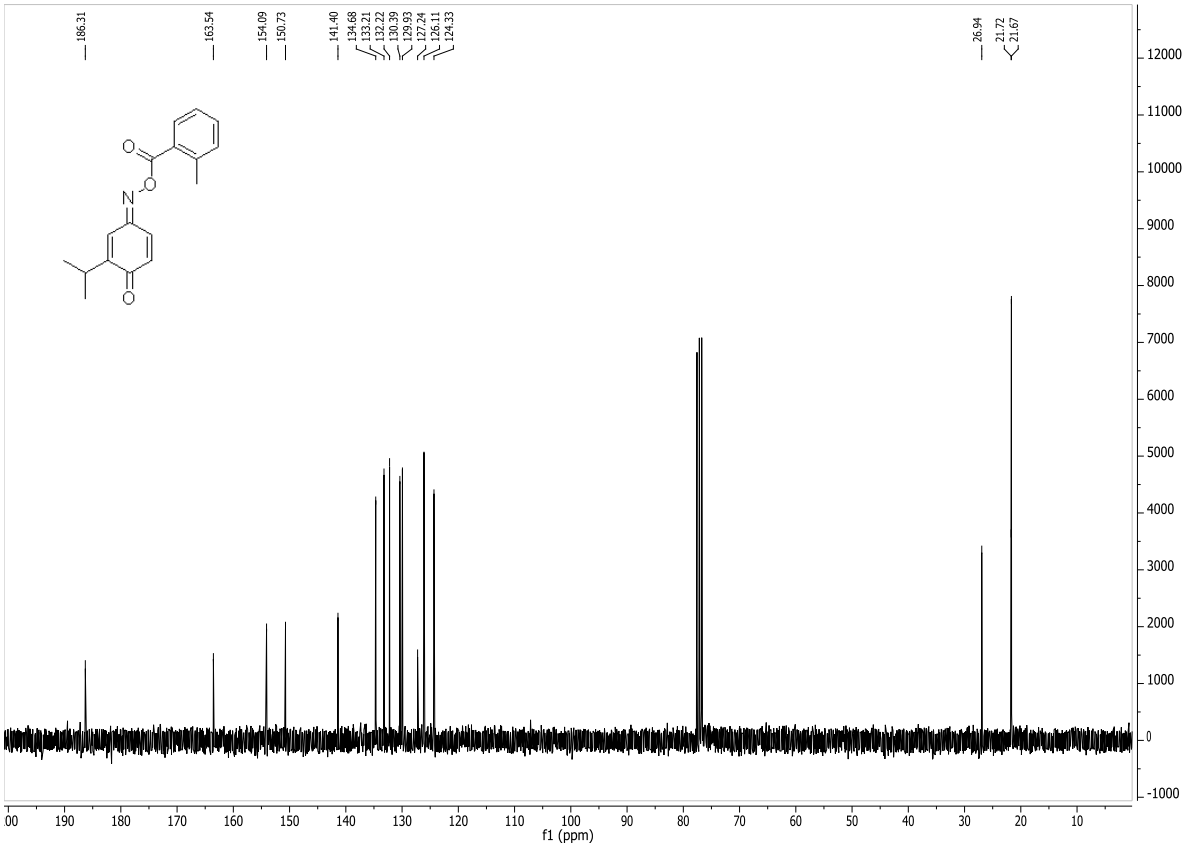
^{1}H- and ^{13}C-NMR spectra of compound **3**



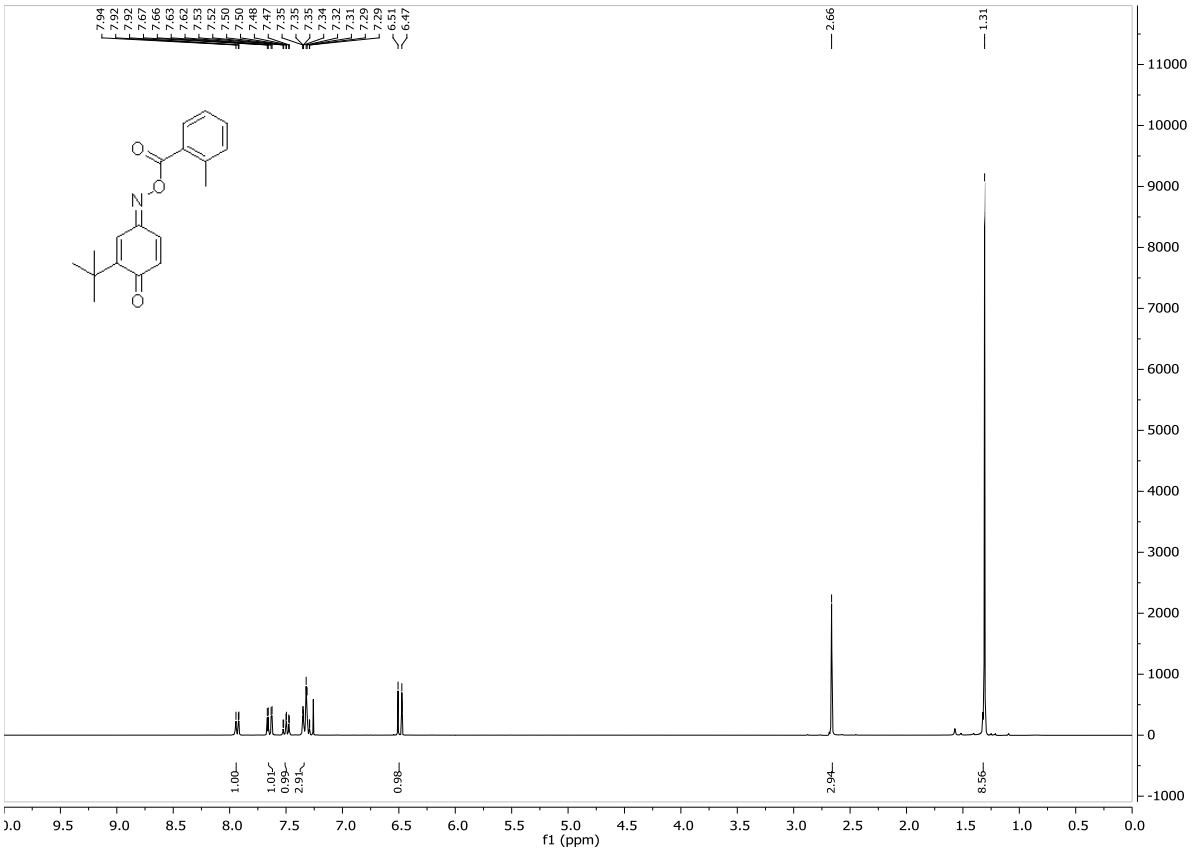


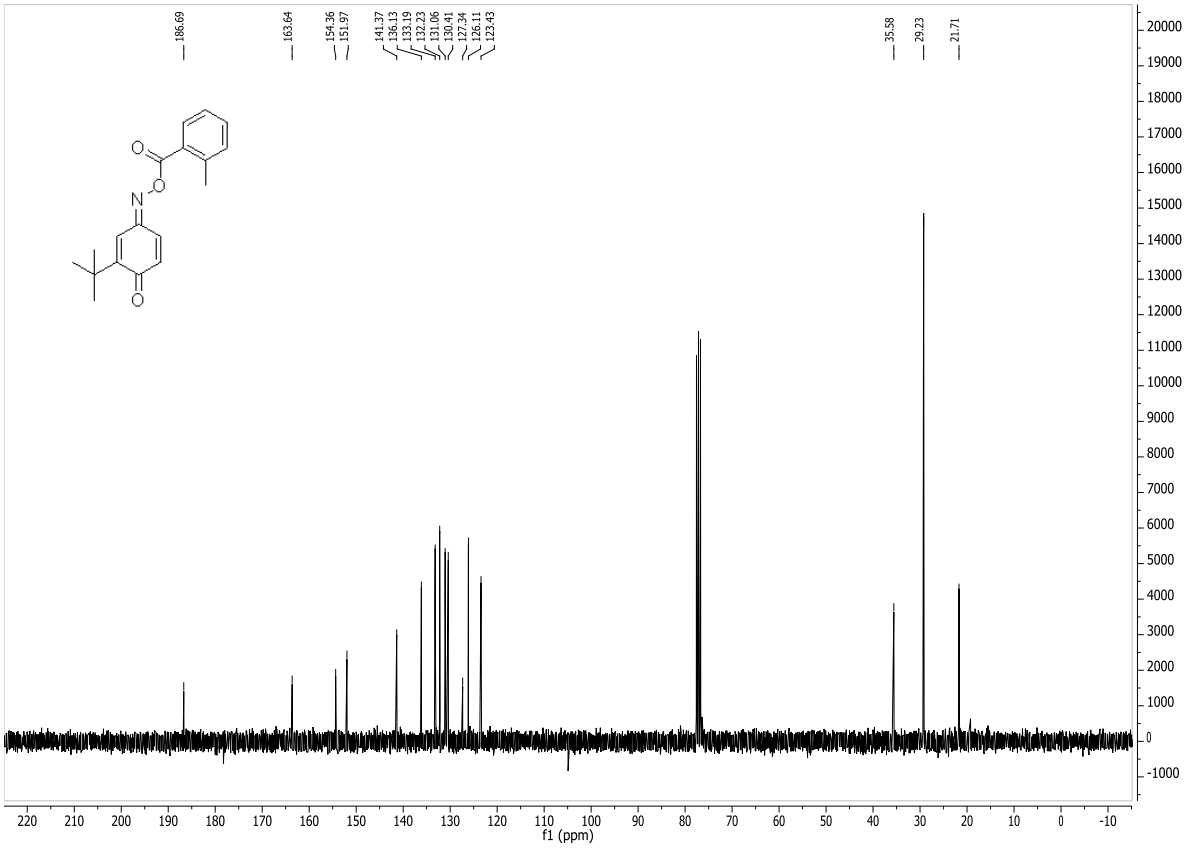
^{1}H- and ^{13}C-NMR spectra of compound **4**



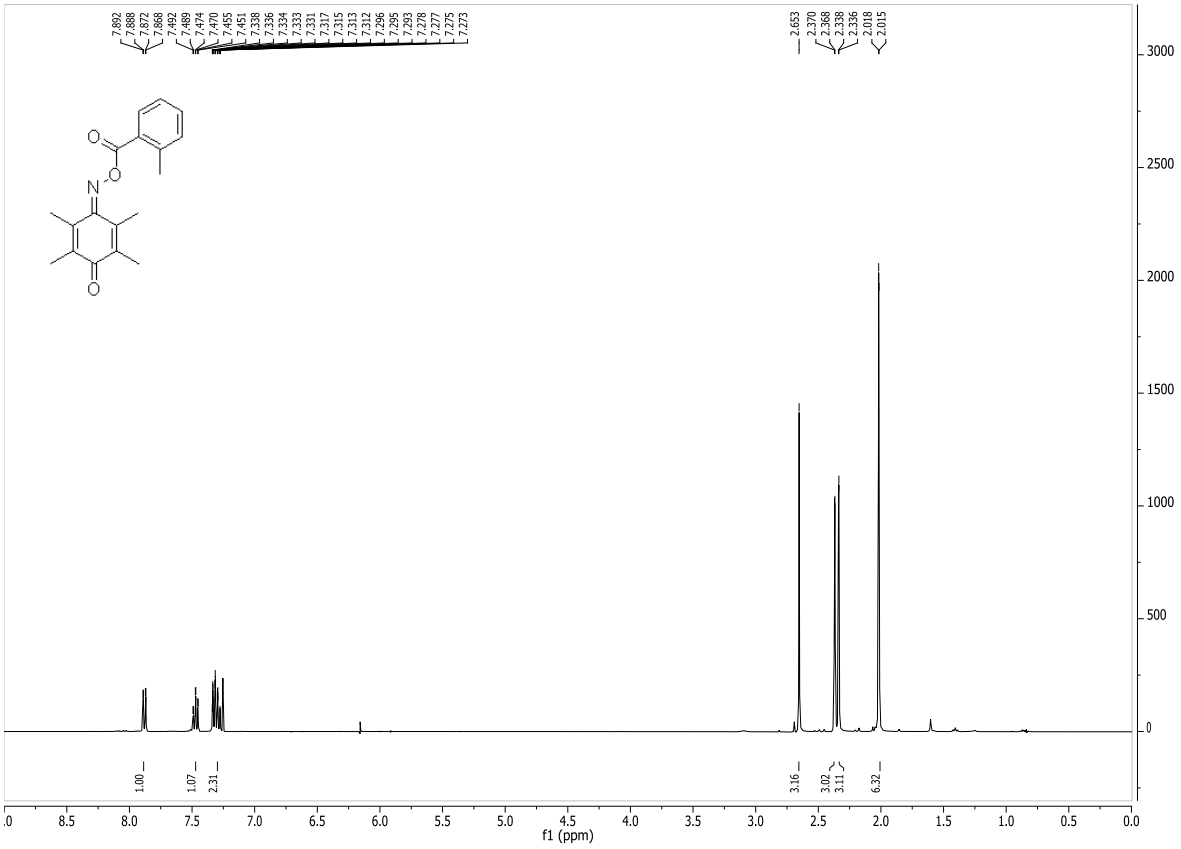


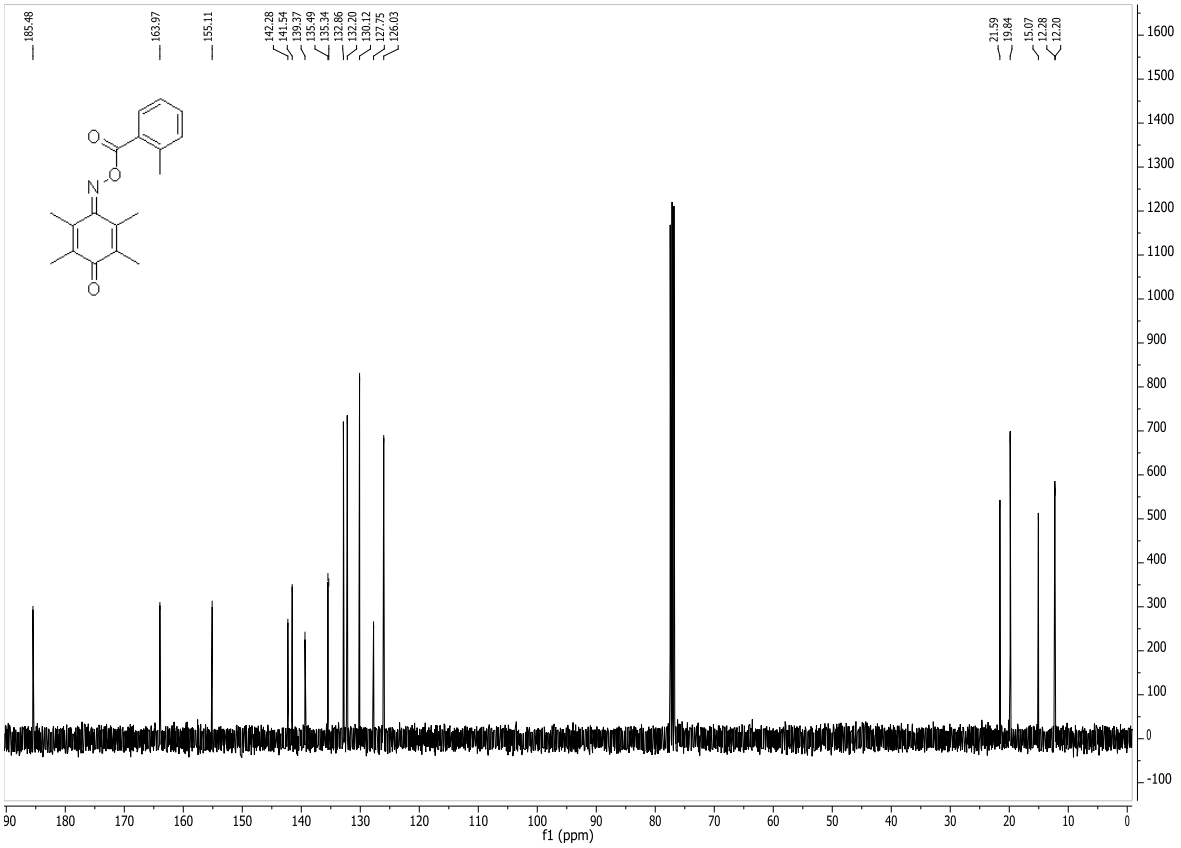
^{1}H- and ^{13}C-NMR spectra of compound **5**



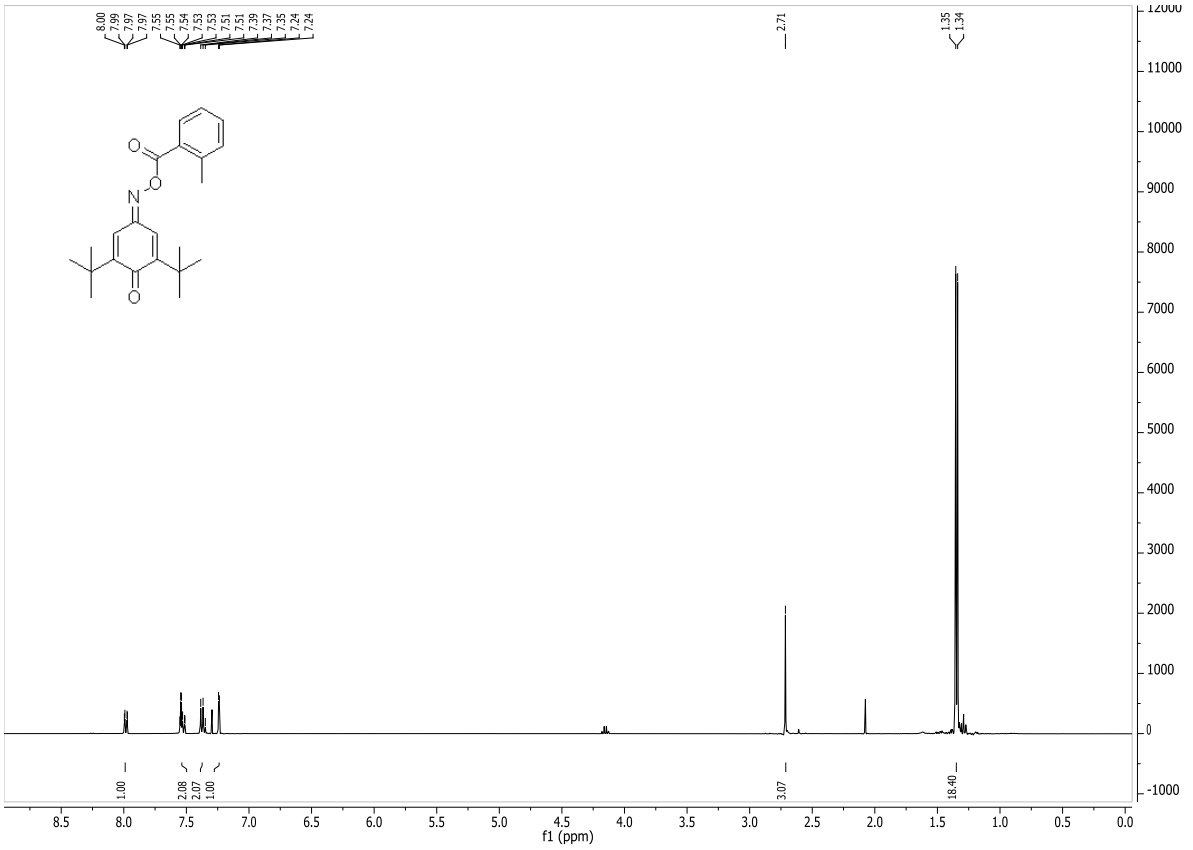


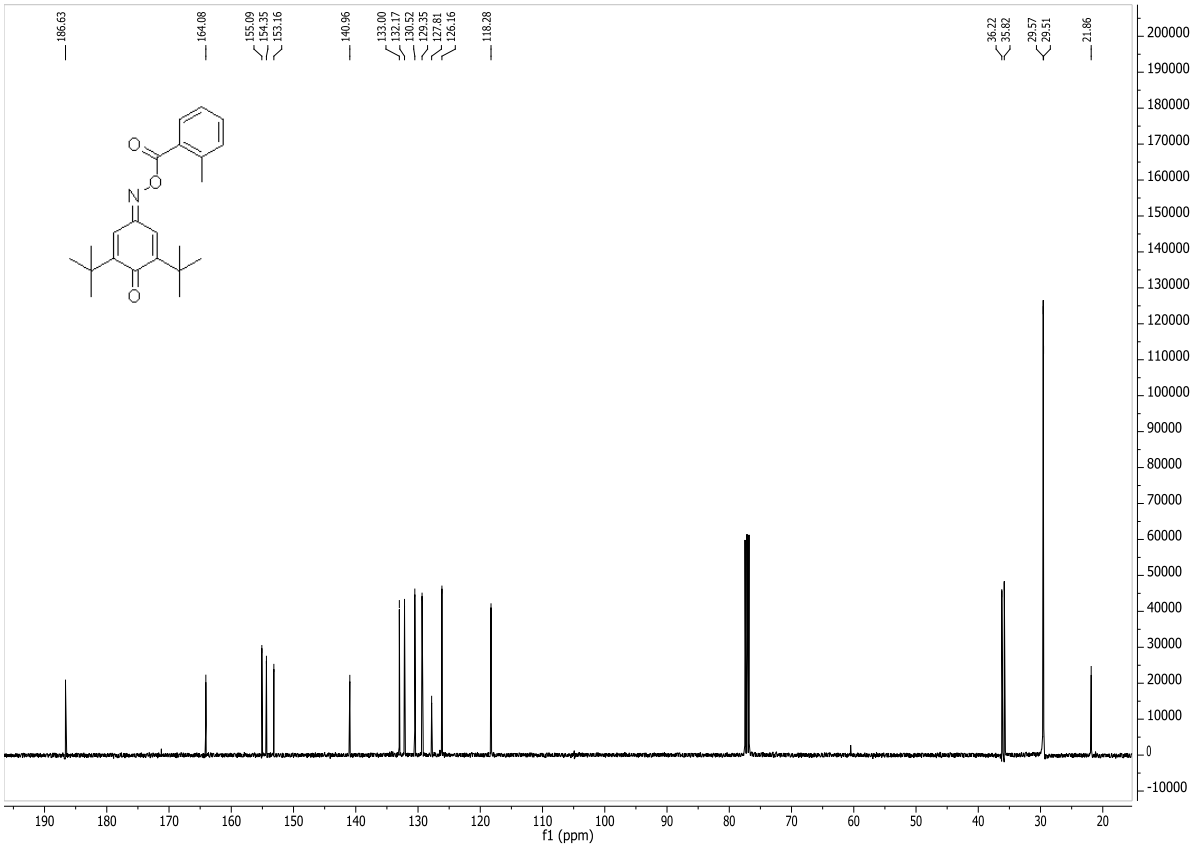
^{1}H- and ^{13}C-NMR spectra of compound **6**



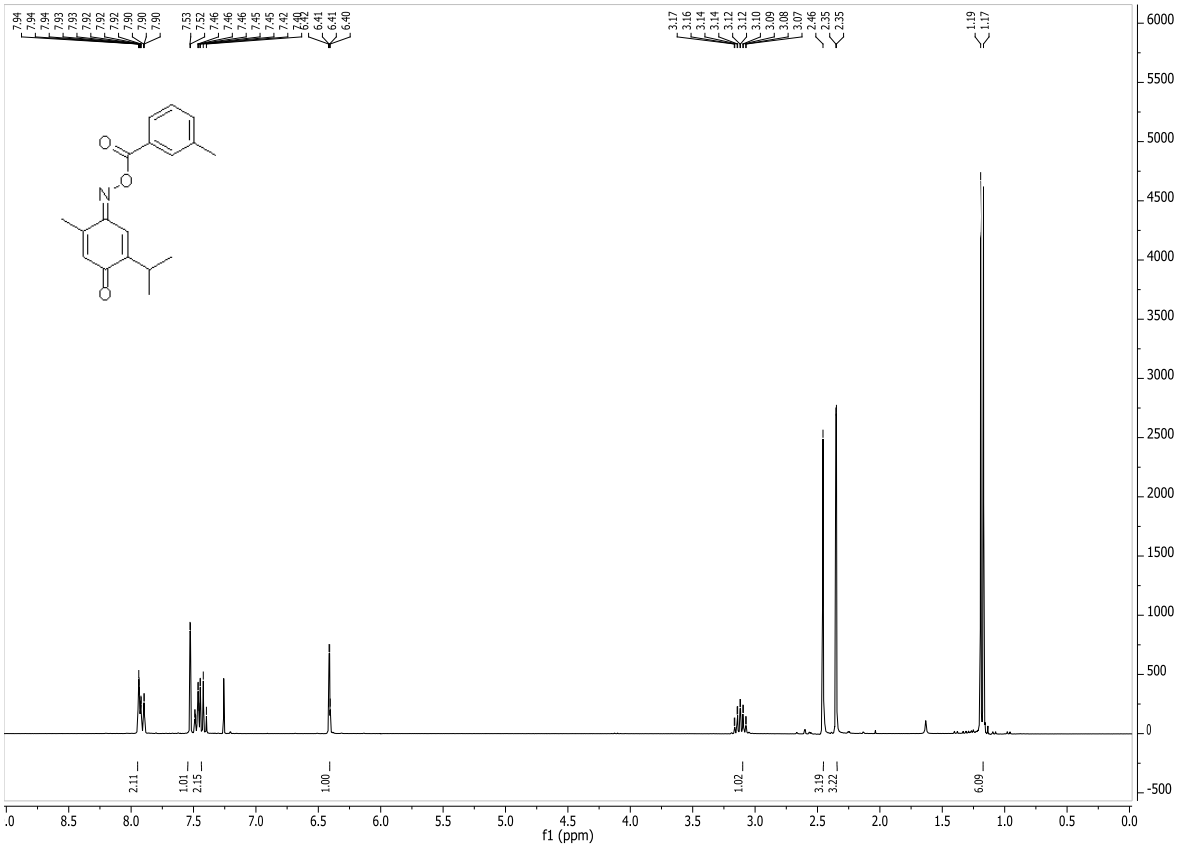


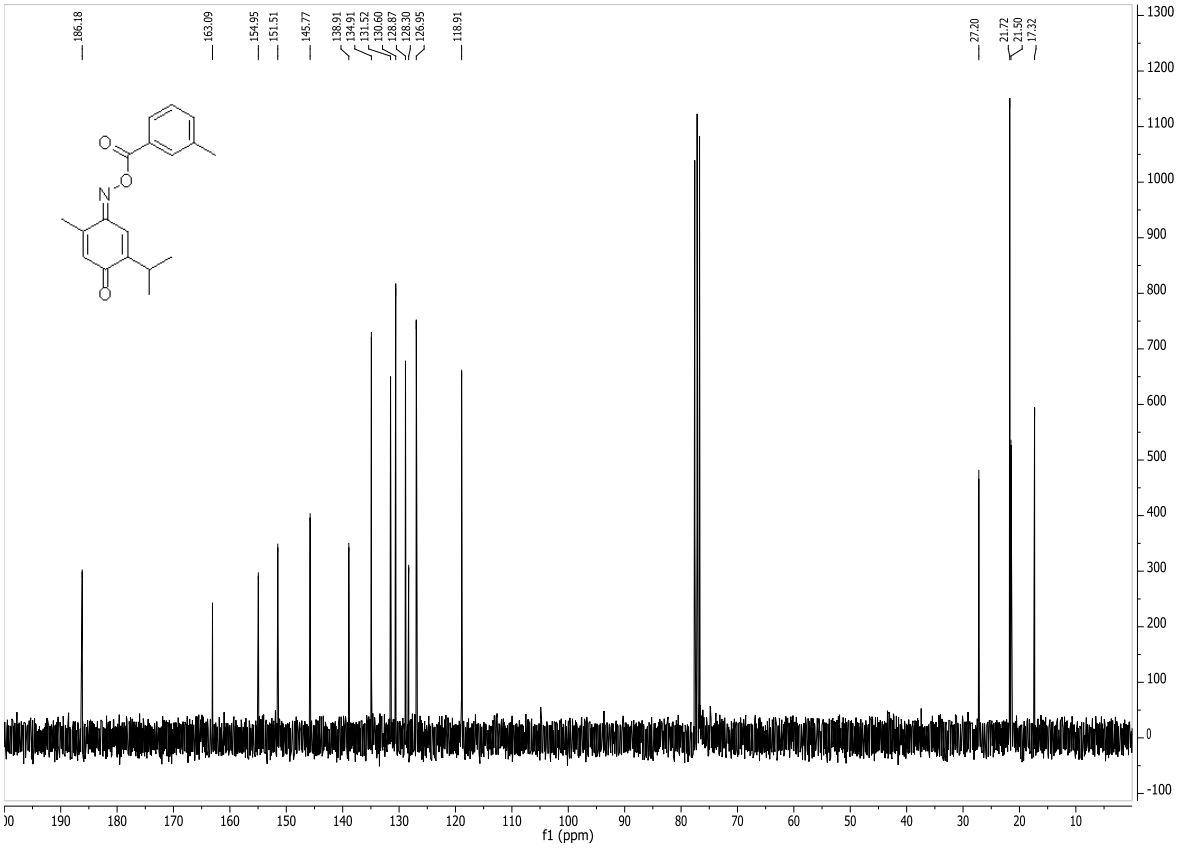
^{1}H- and ^{13}C-NMR spectra of compound **7**



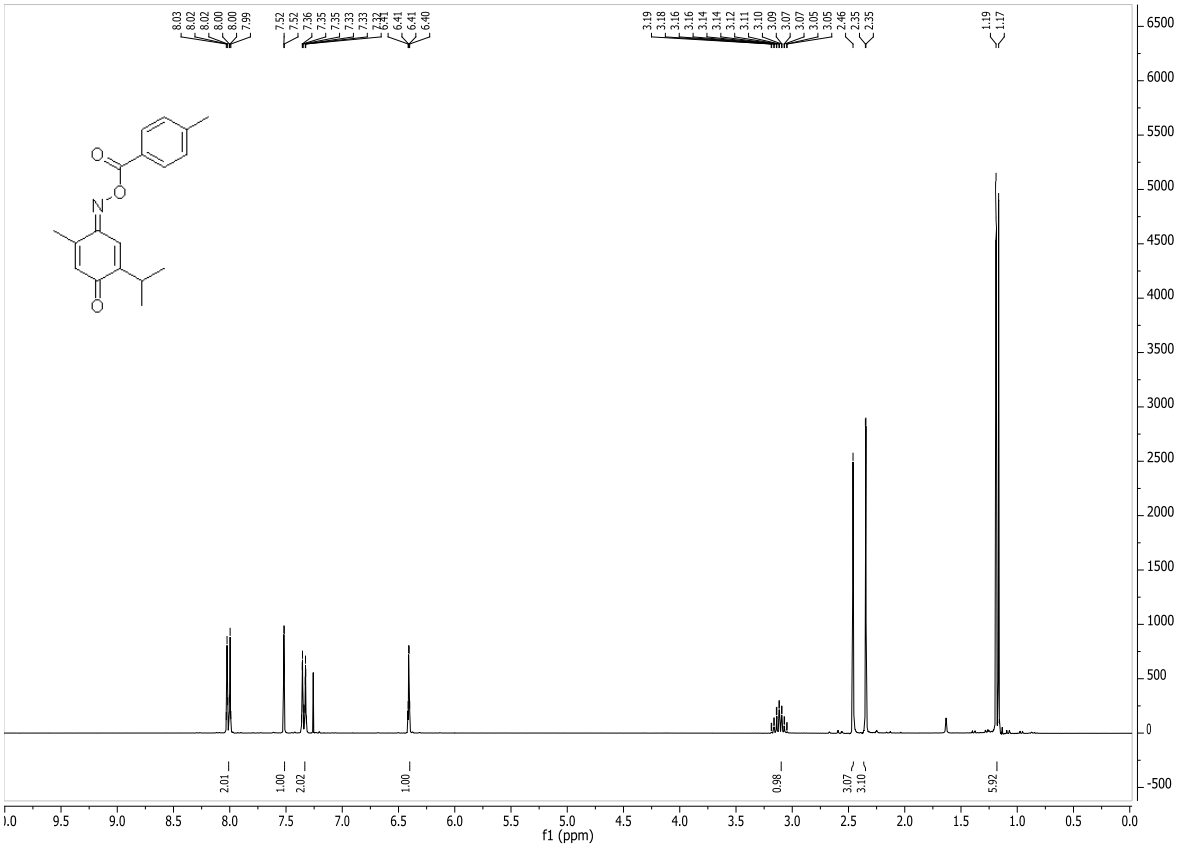


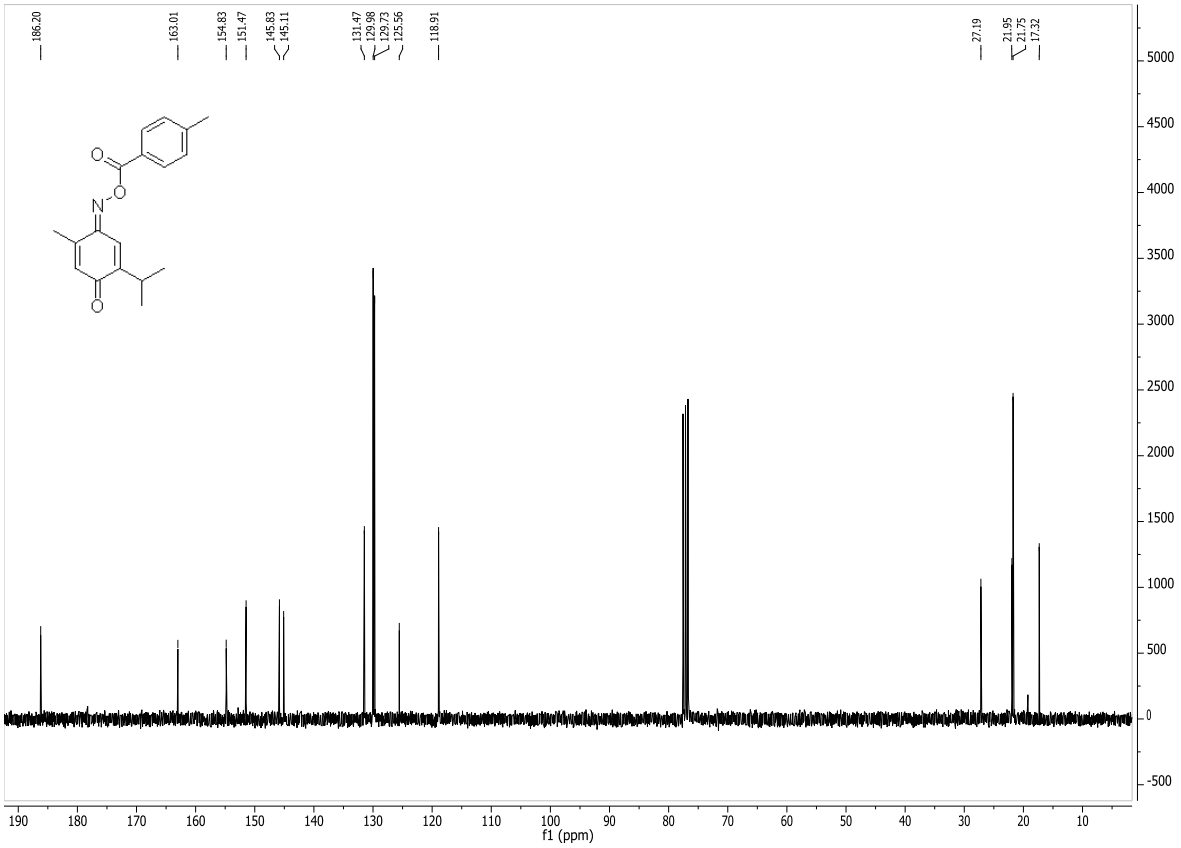
^{1}H- and ^{13}C-NMR spectra of compound **8**



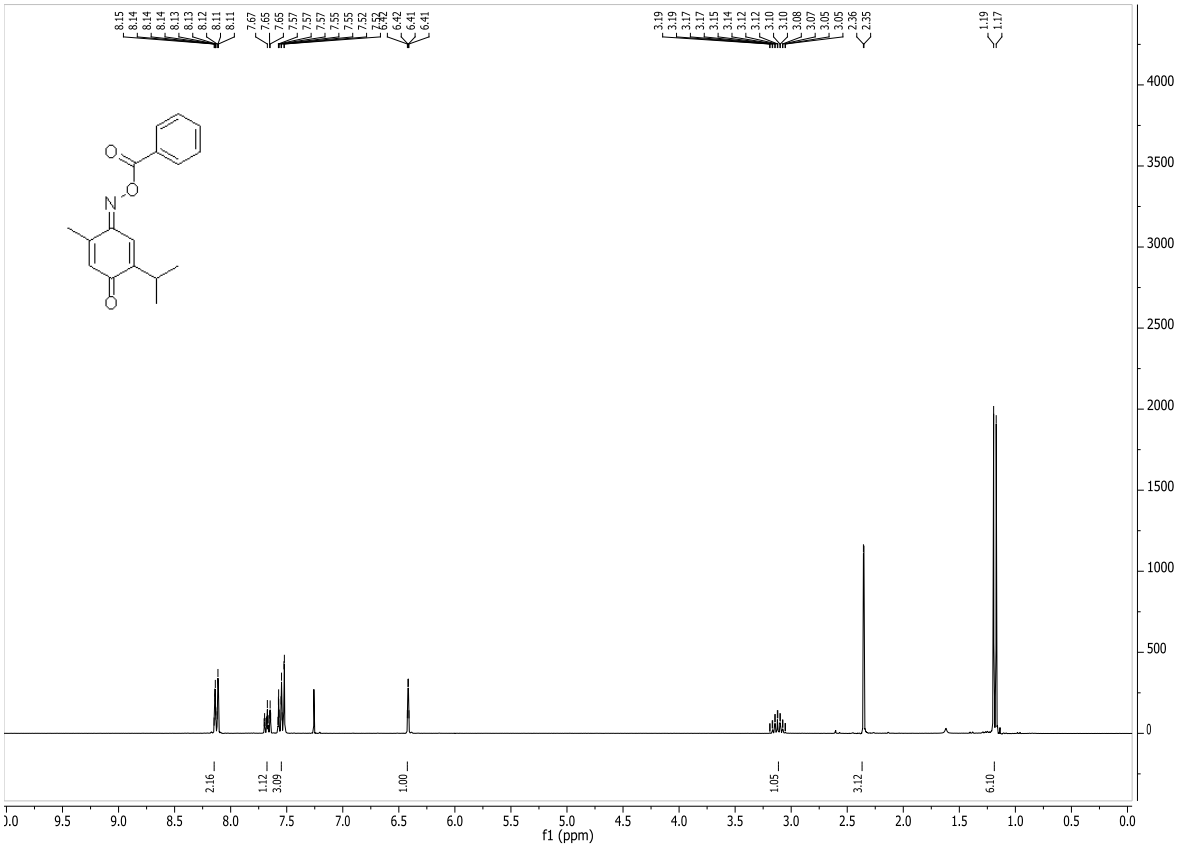


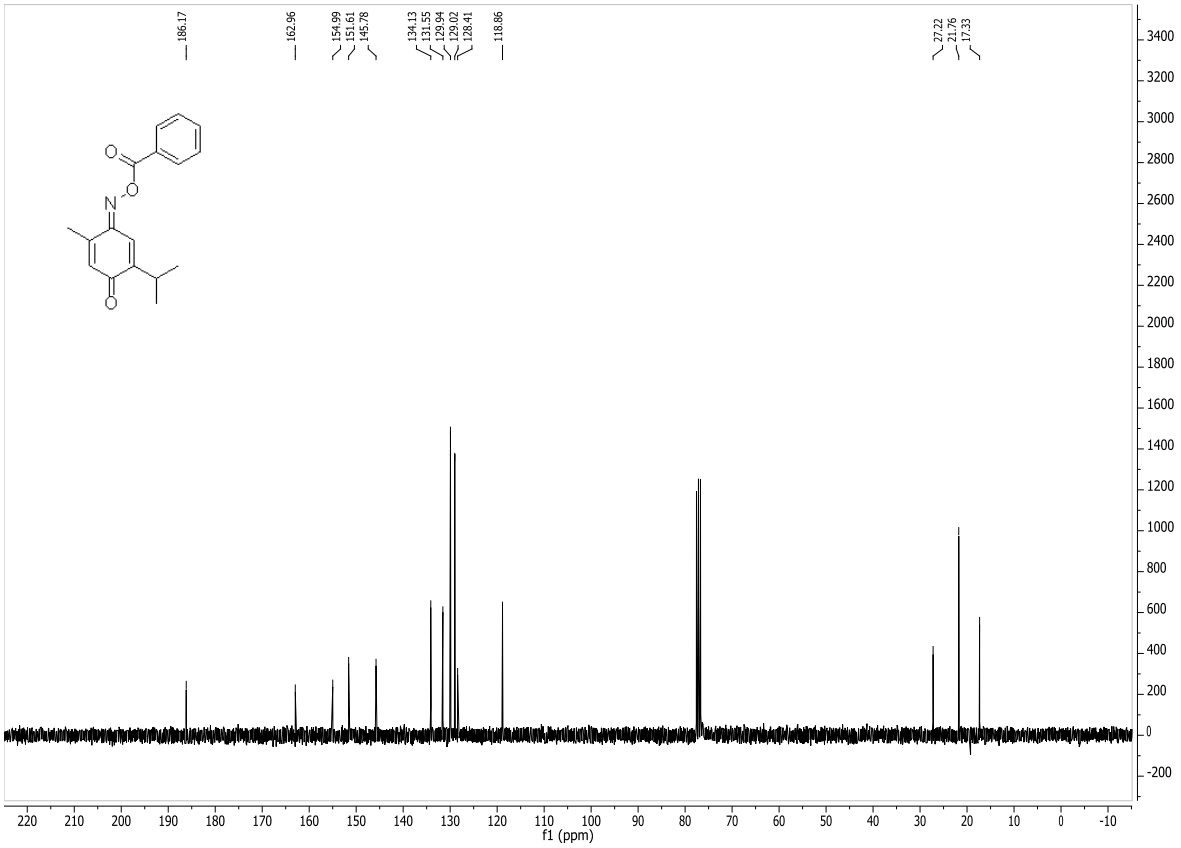
^{1}H- and ^{13}C-NMR spectra of compound **9**



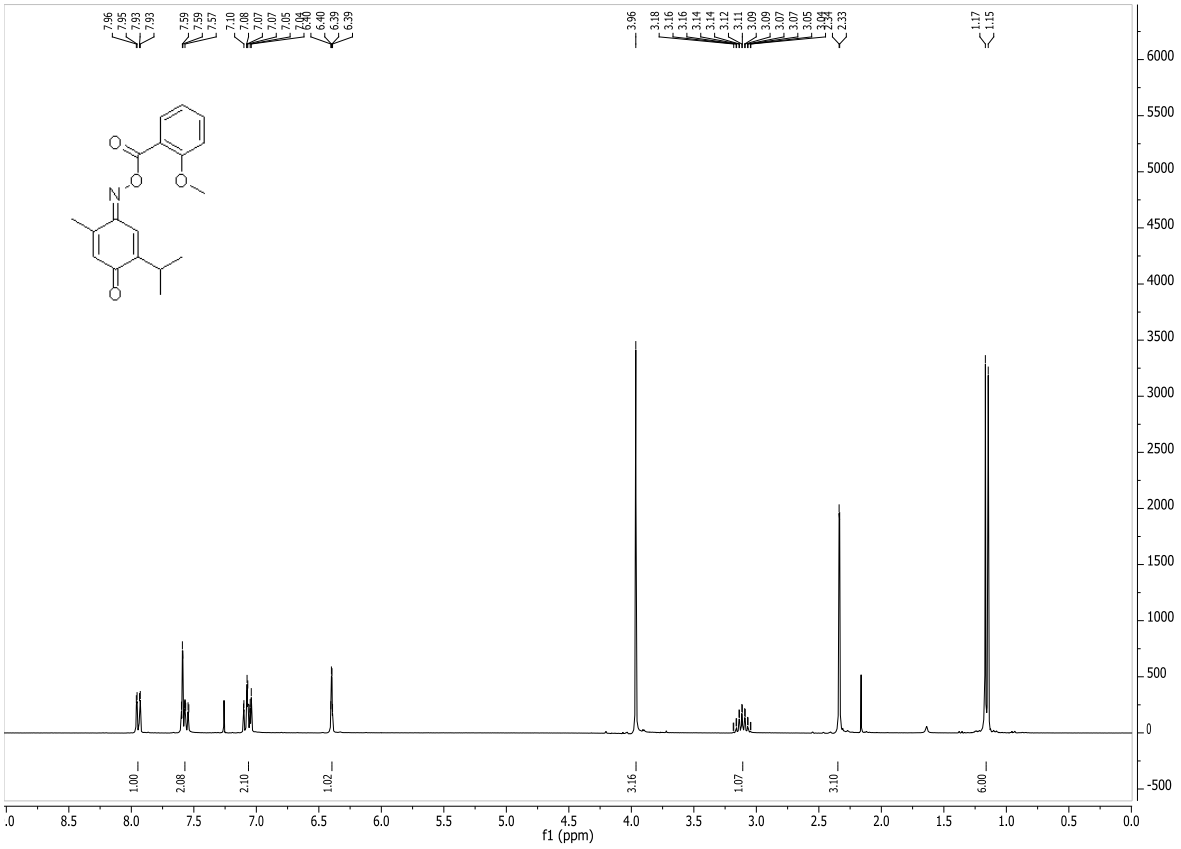


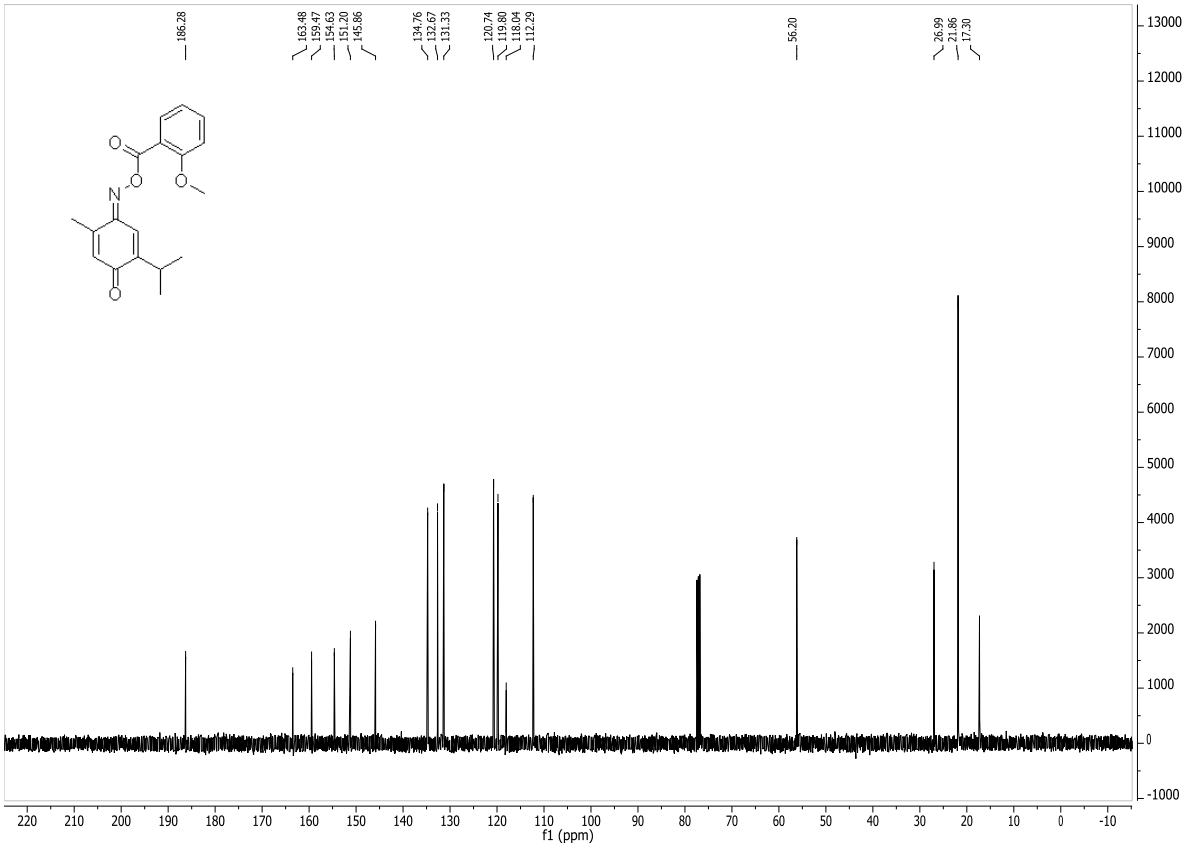
^{1}H- and ^{13}C-NMR spectra of compound **10**



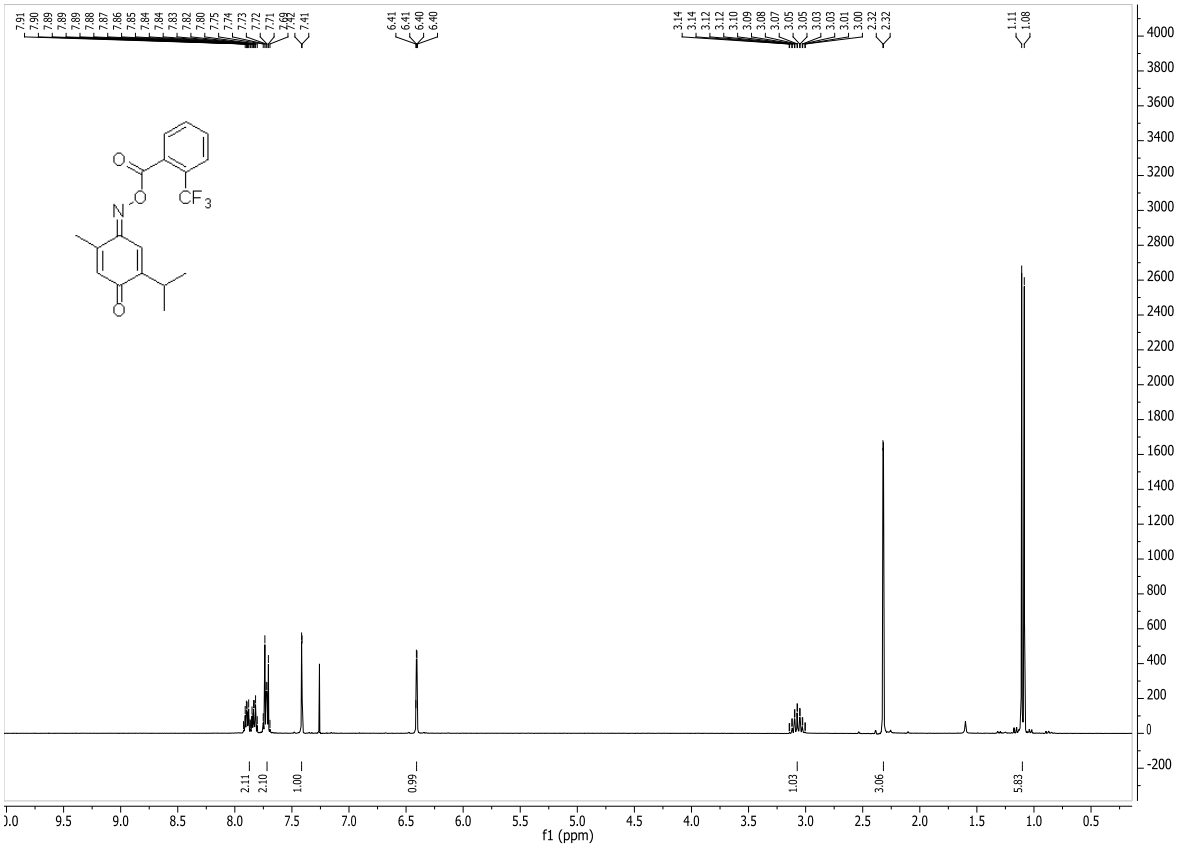


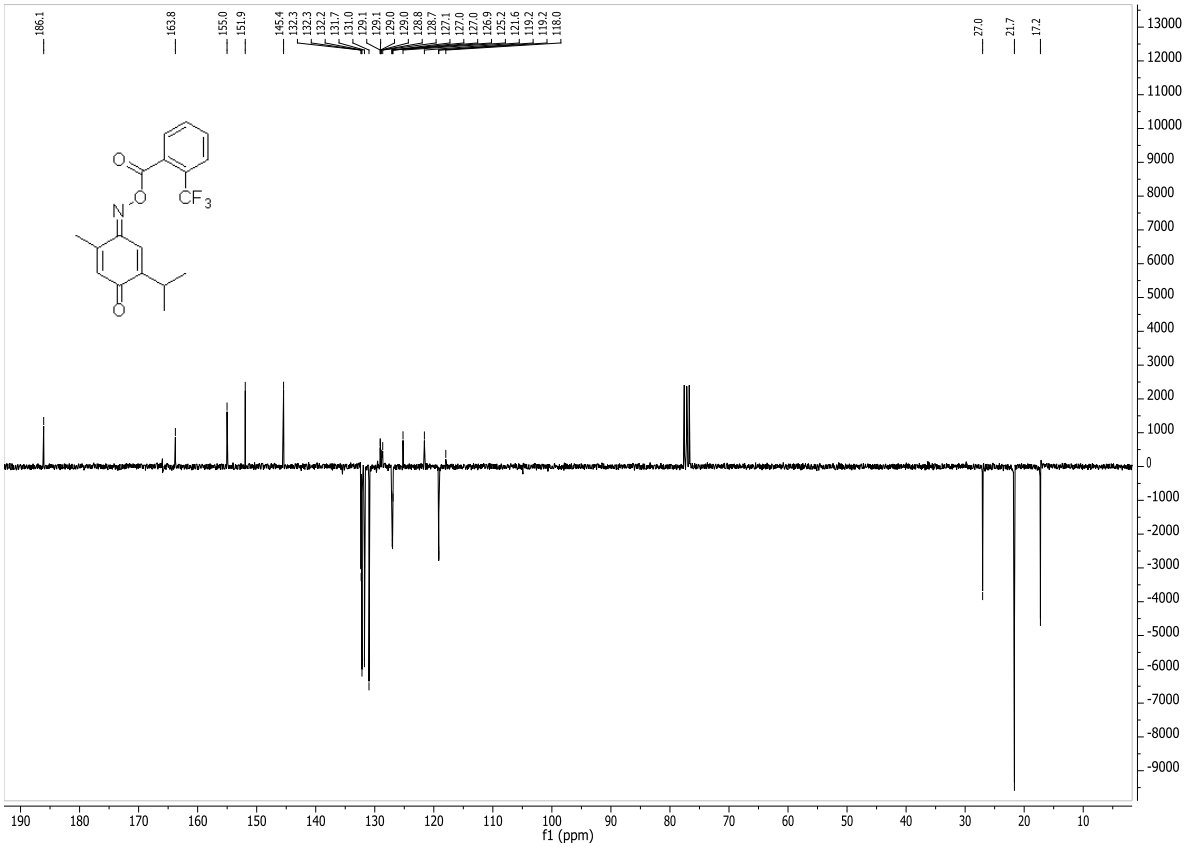
^{1}H- and ^{13}C-NMR spectra of compound **11**



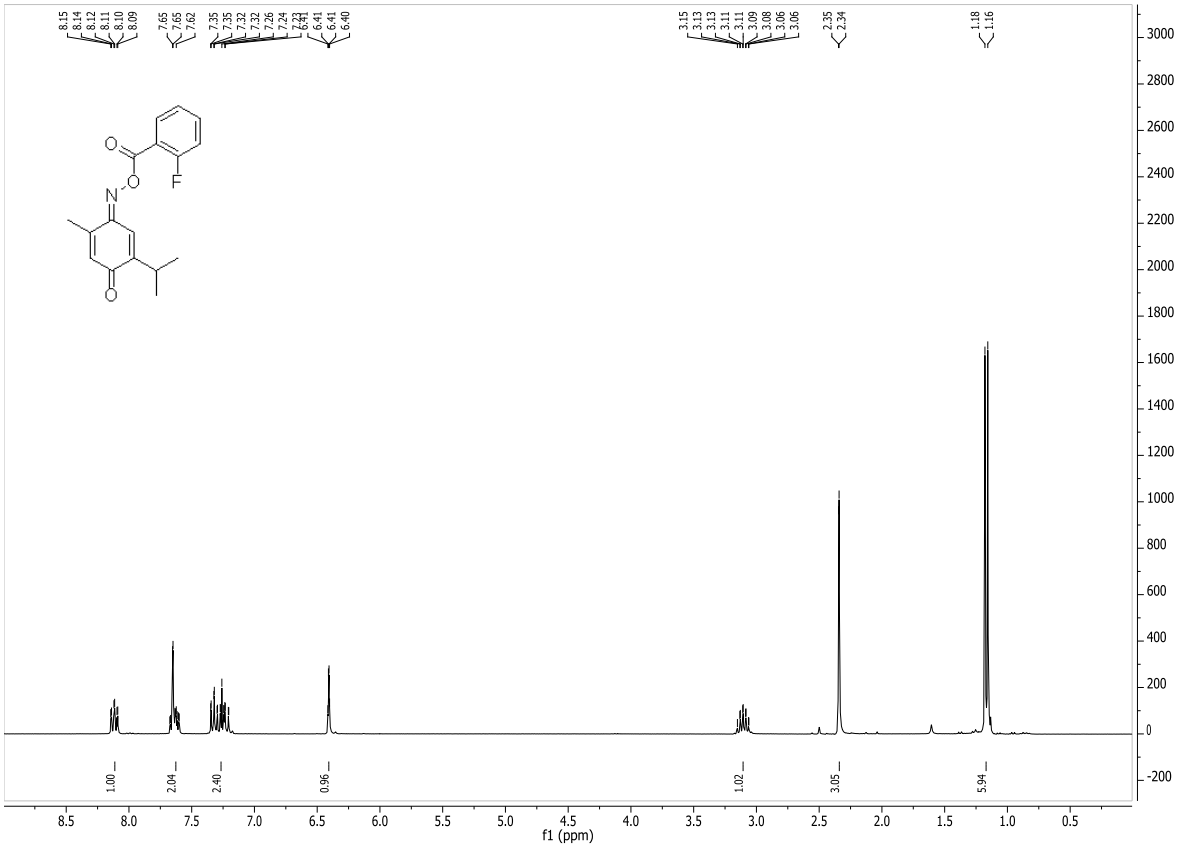


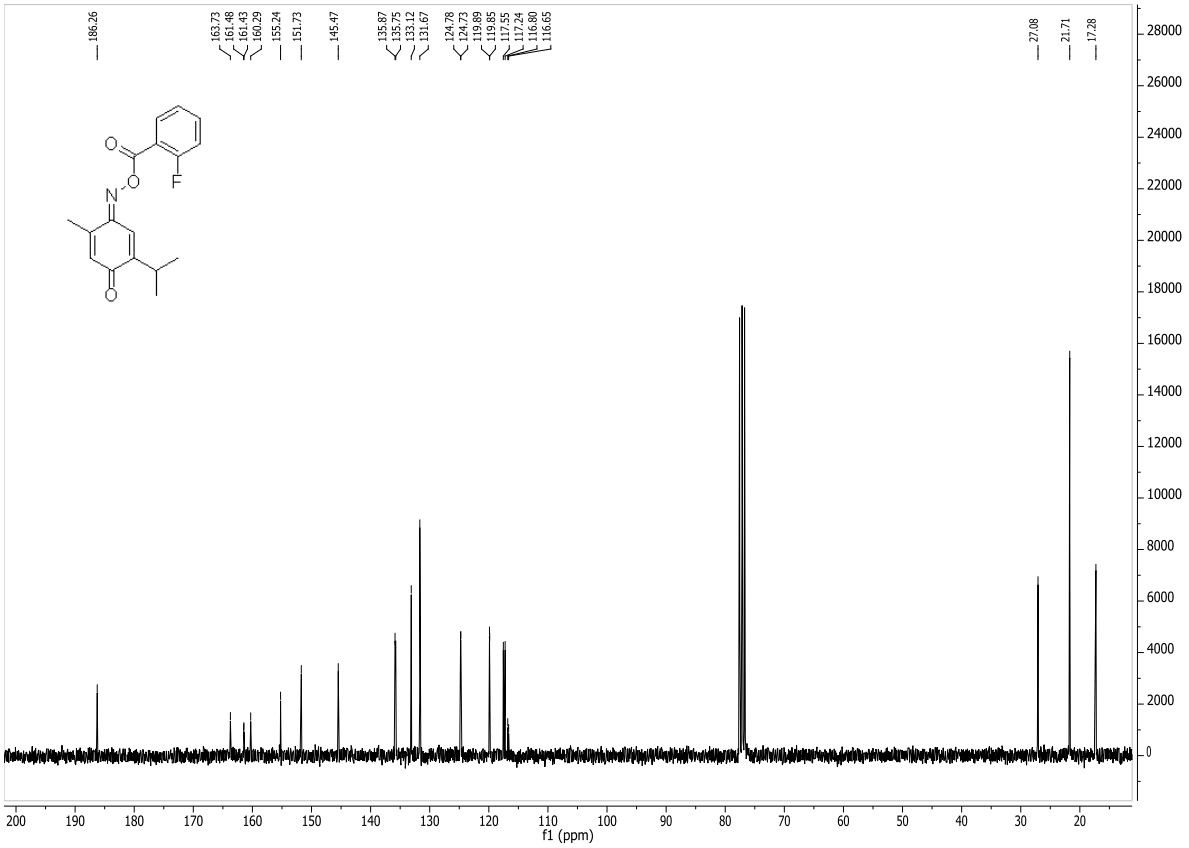
^{1}H- and ^{13}C-NMR spectra of compound **12**



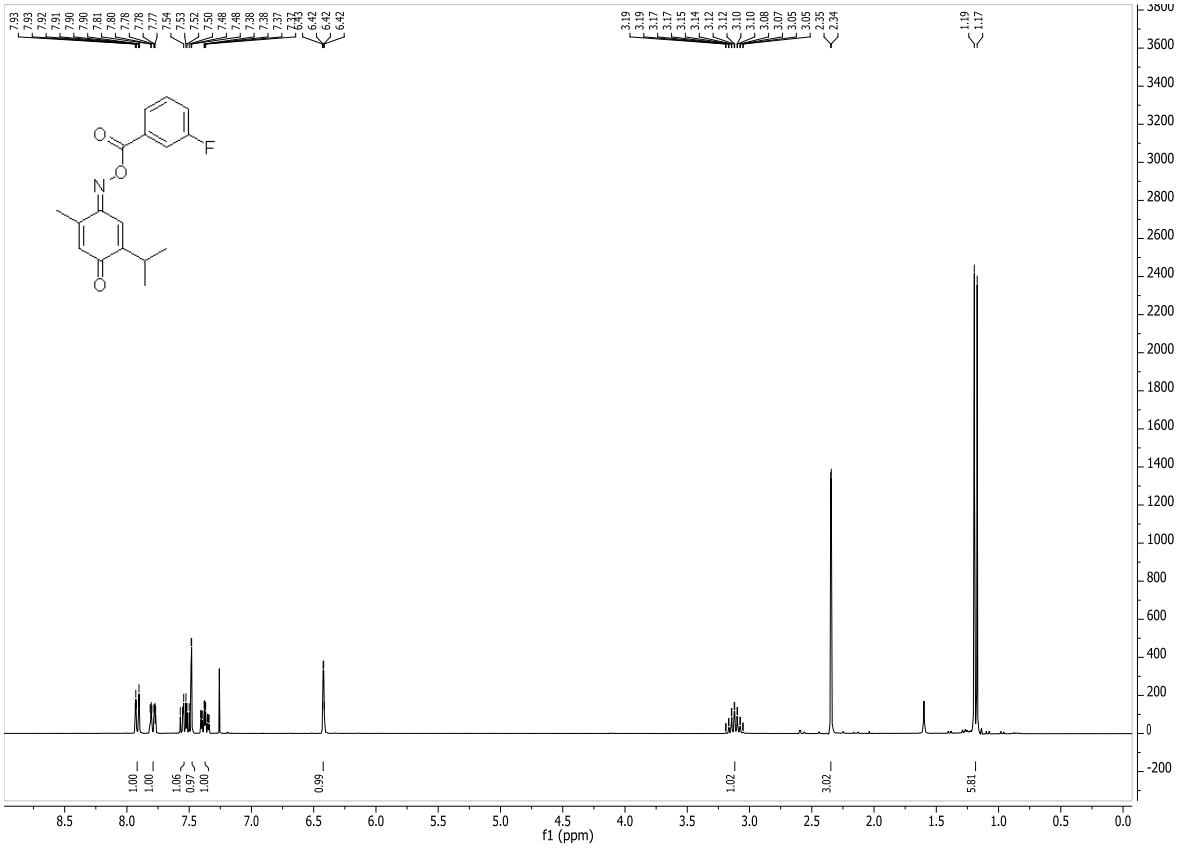


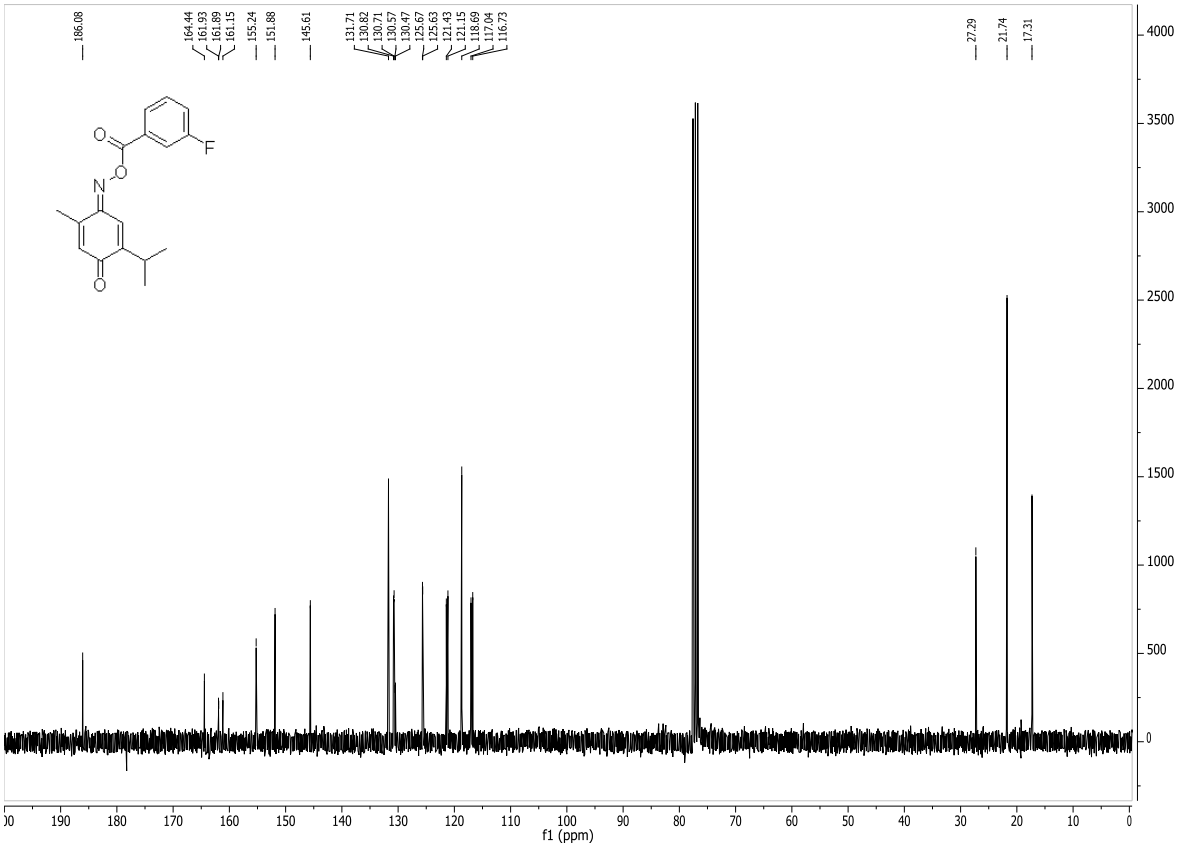
^{1}H- and ^{13}C-NMR spectra of compound **13**



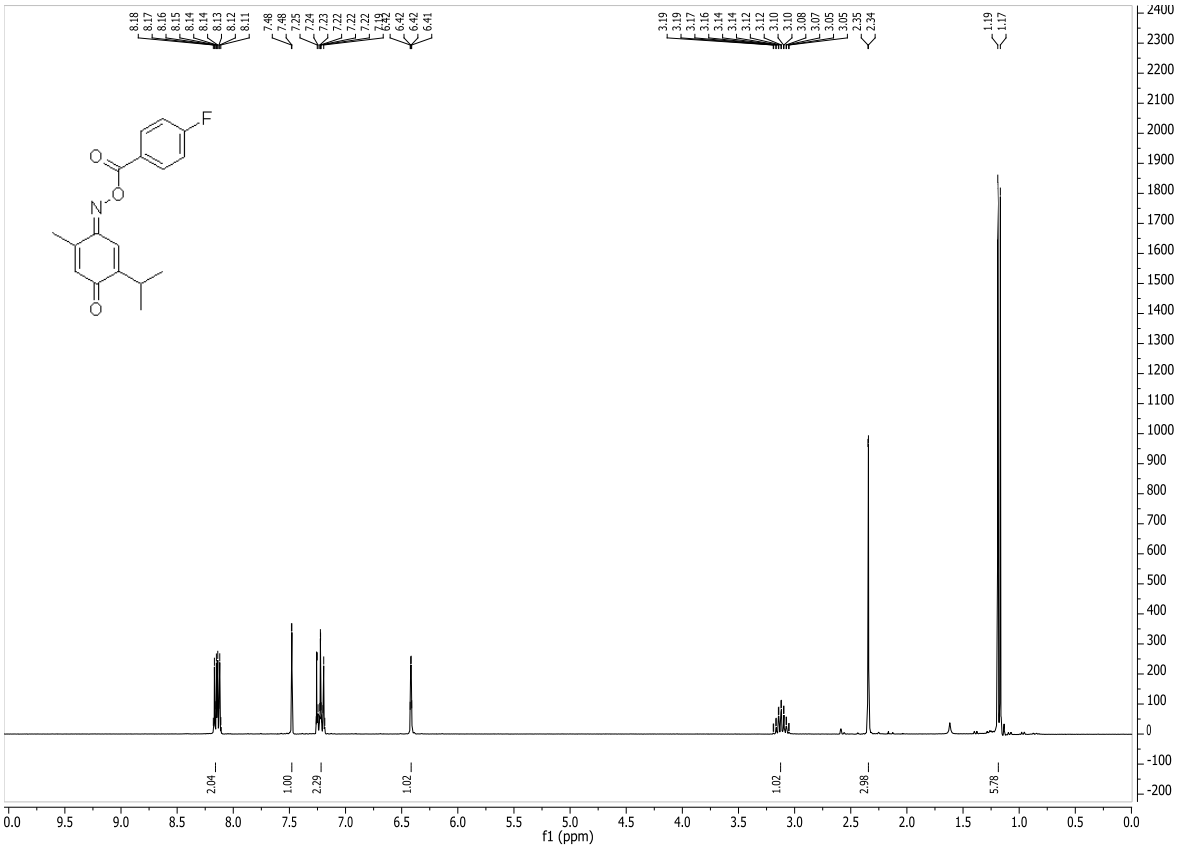


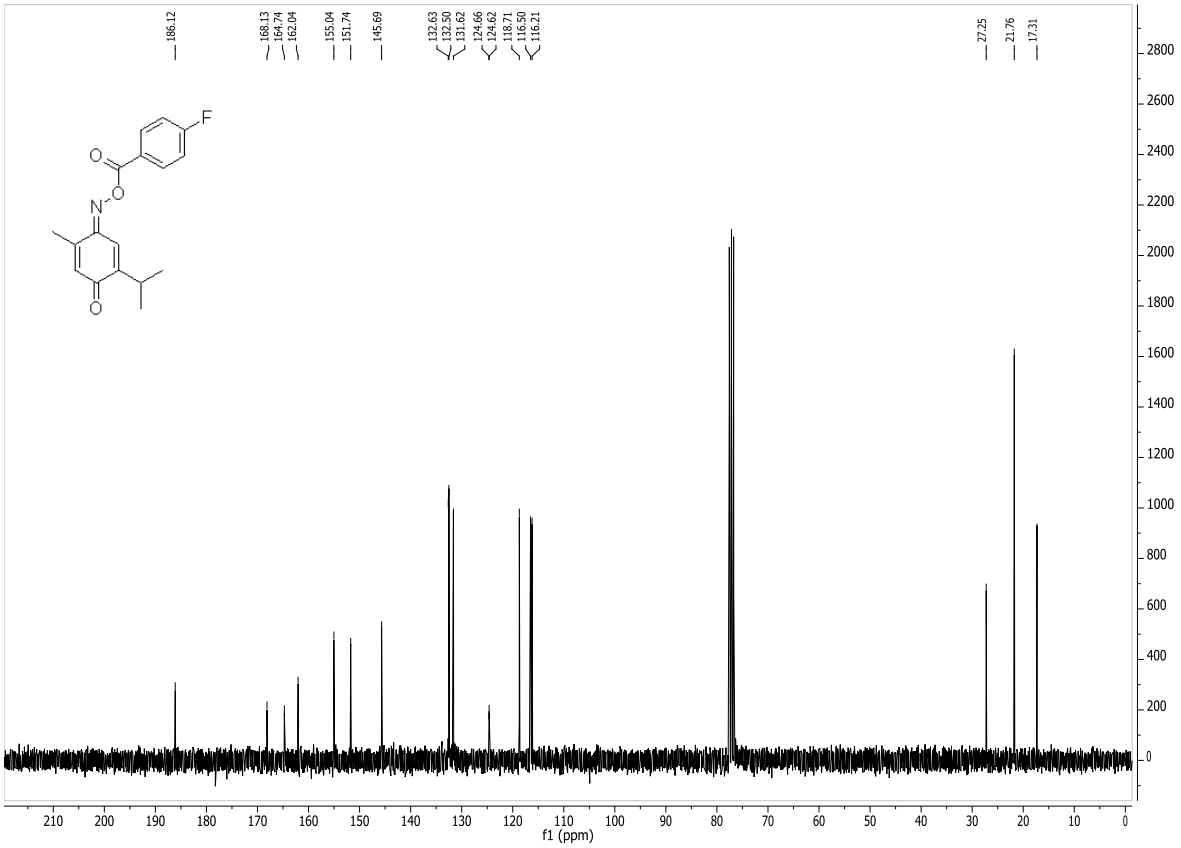
^{1}H- and ^{13}C-NMR spectra of compound **14**



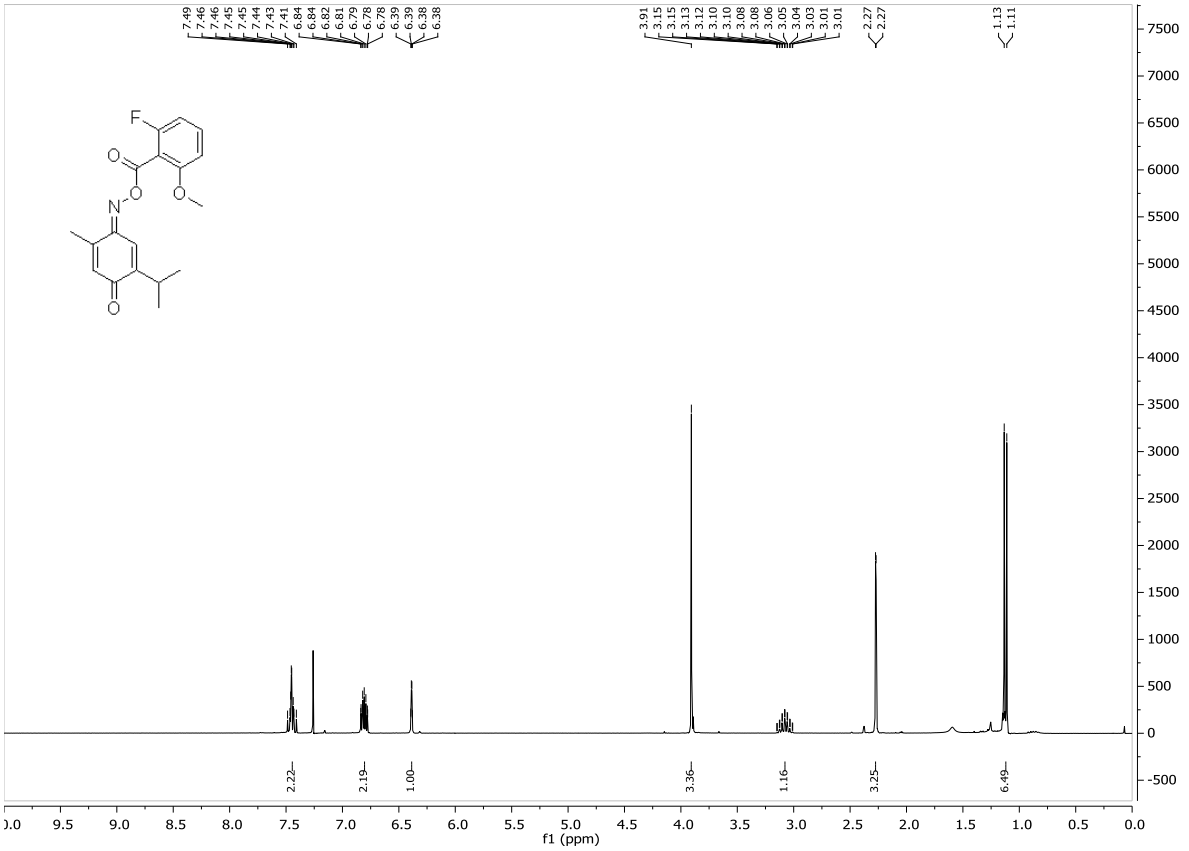


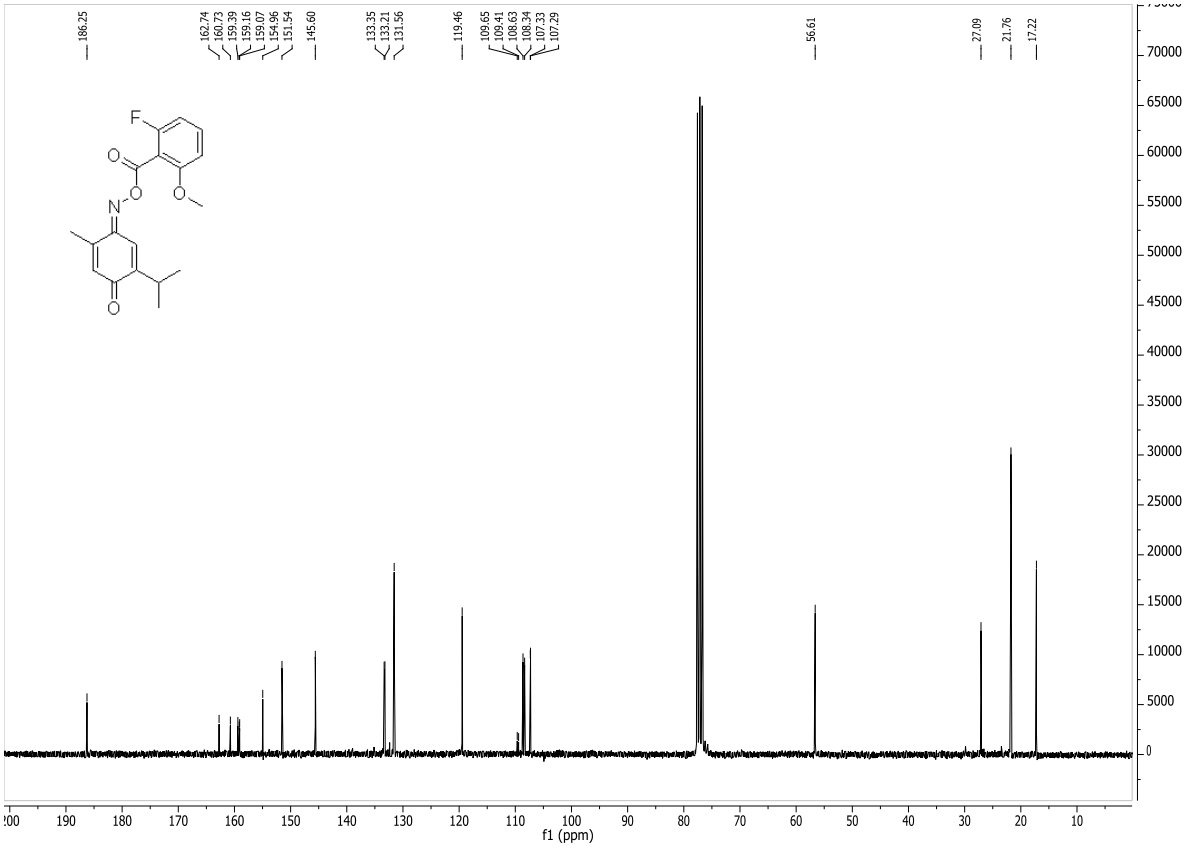
^{1}H- and ^{13}C-NMR spectra of compound **15**



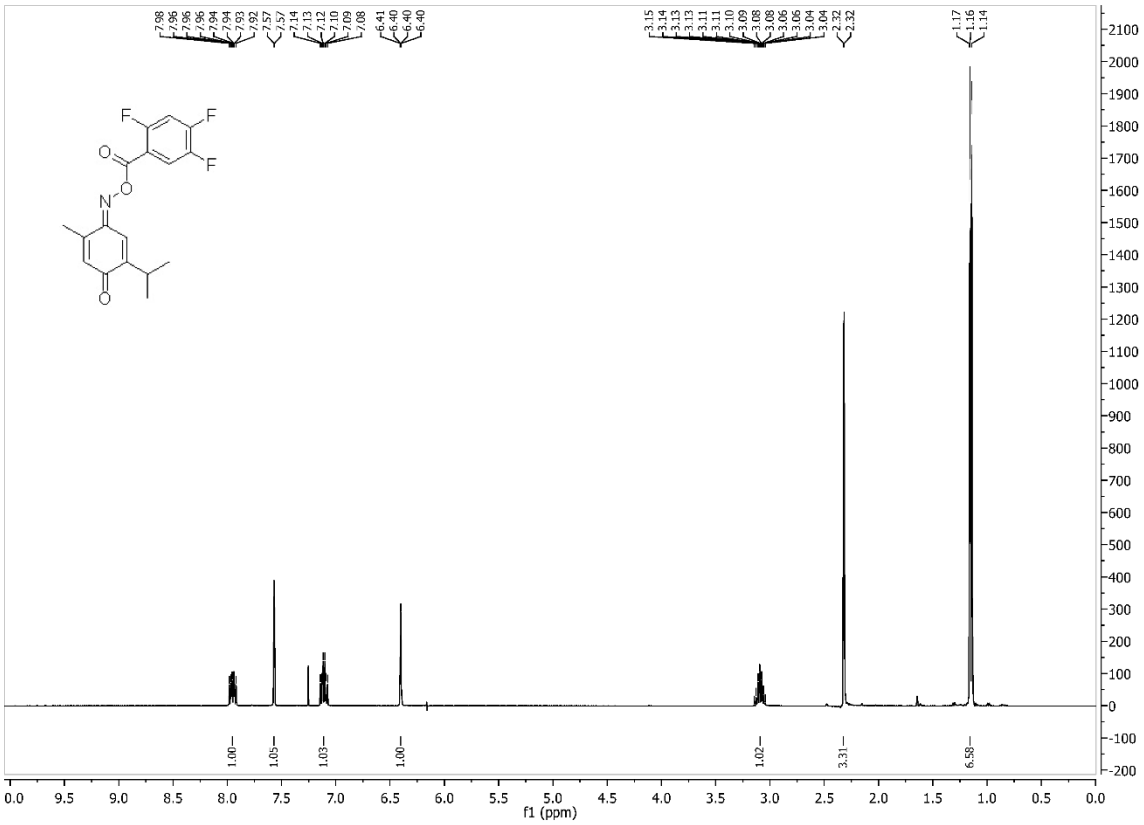


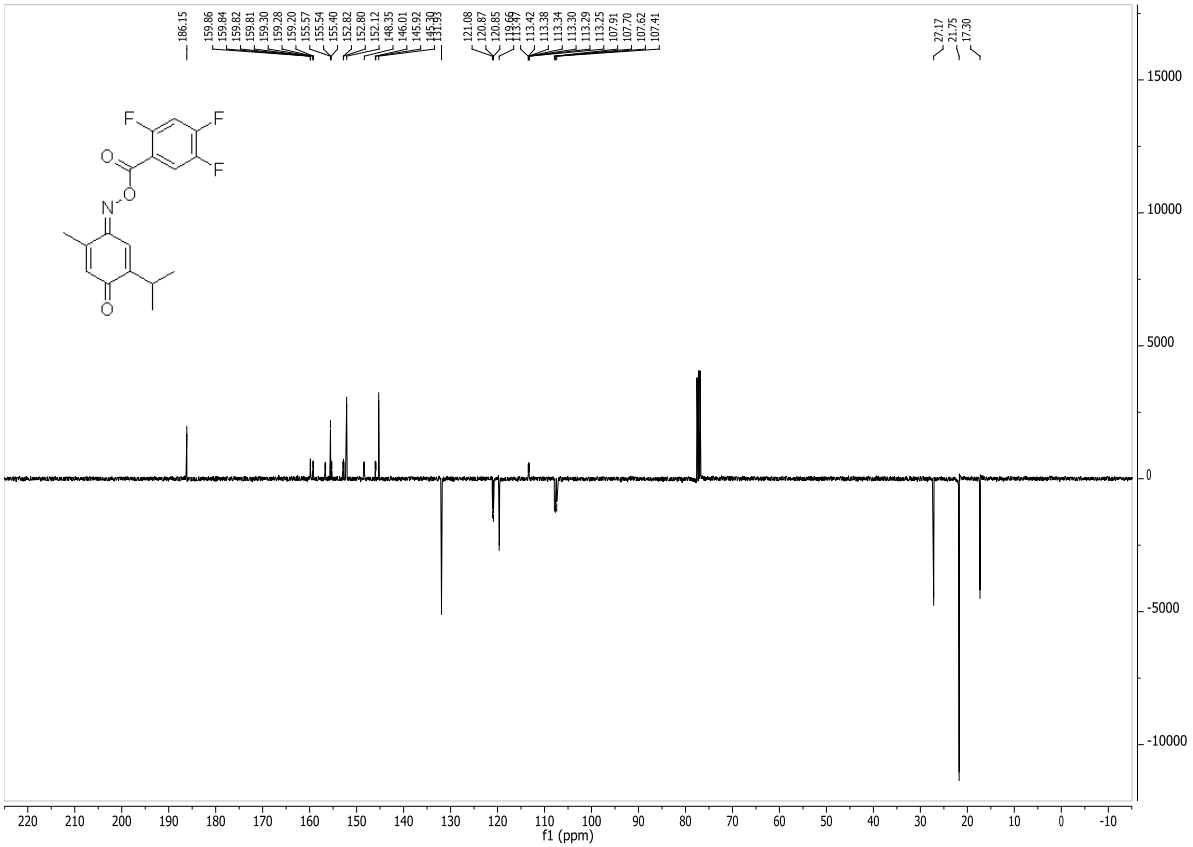
^{1}H- and ^{13}C-NMR spectra of compound **16**



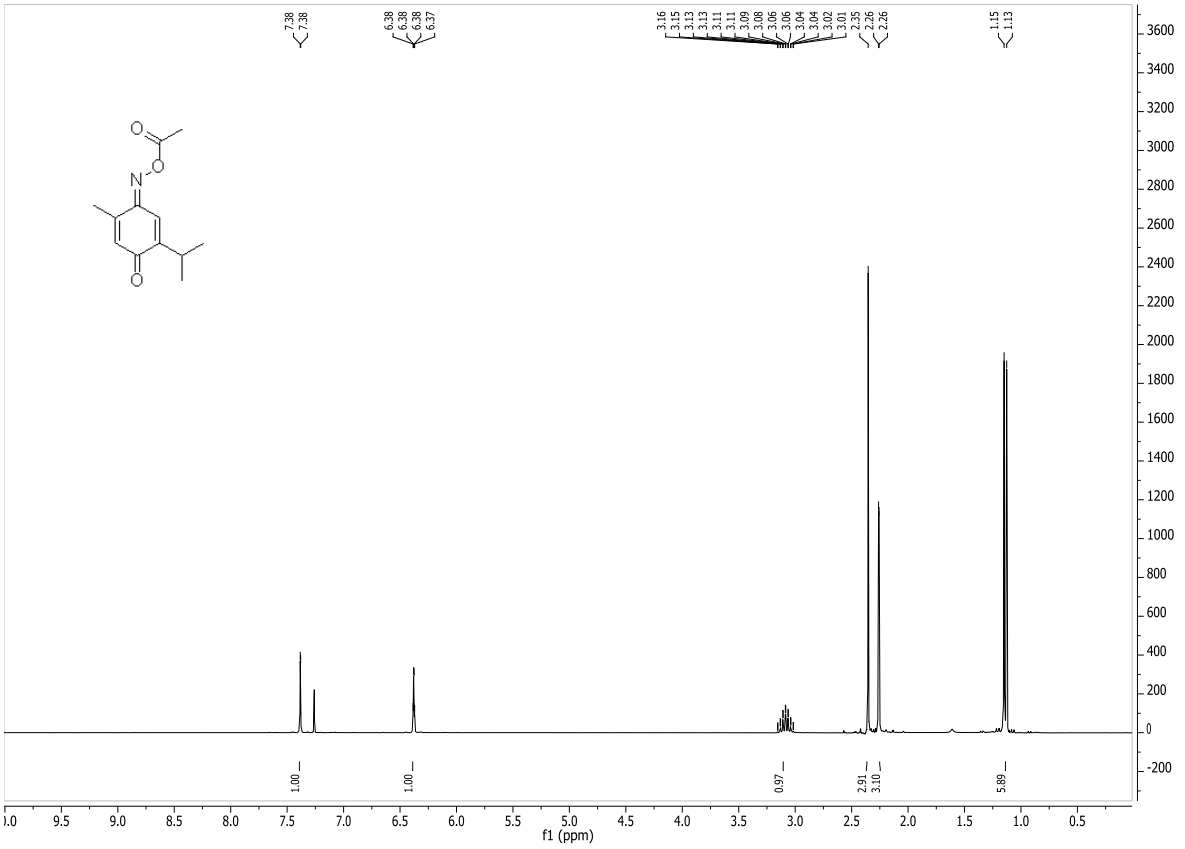


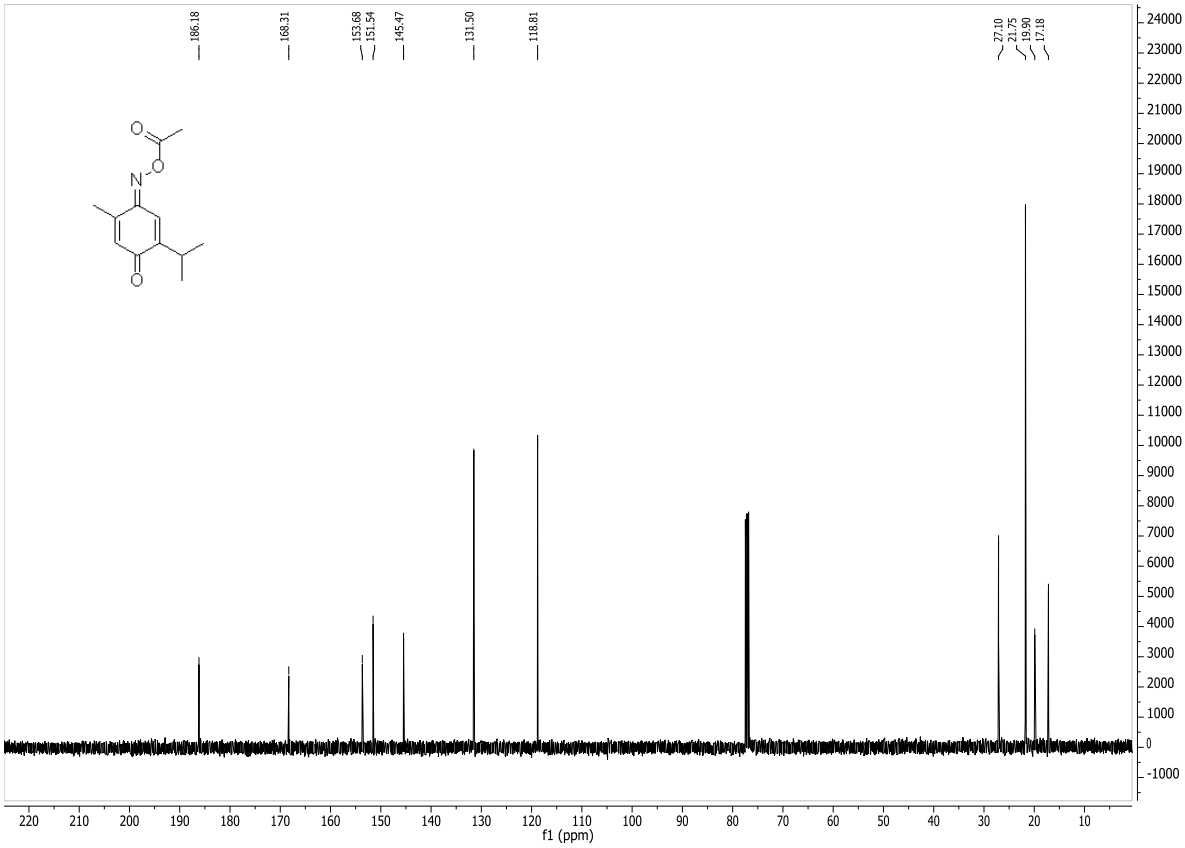
^{1}H- and ^{13}C-NMR spectra of compound **17**



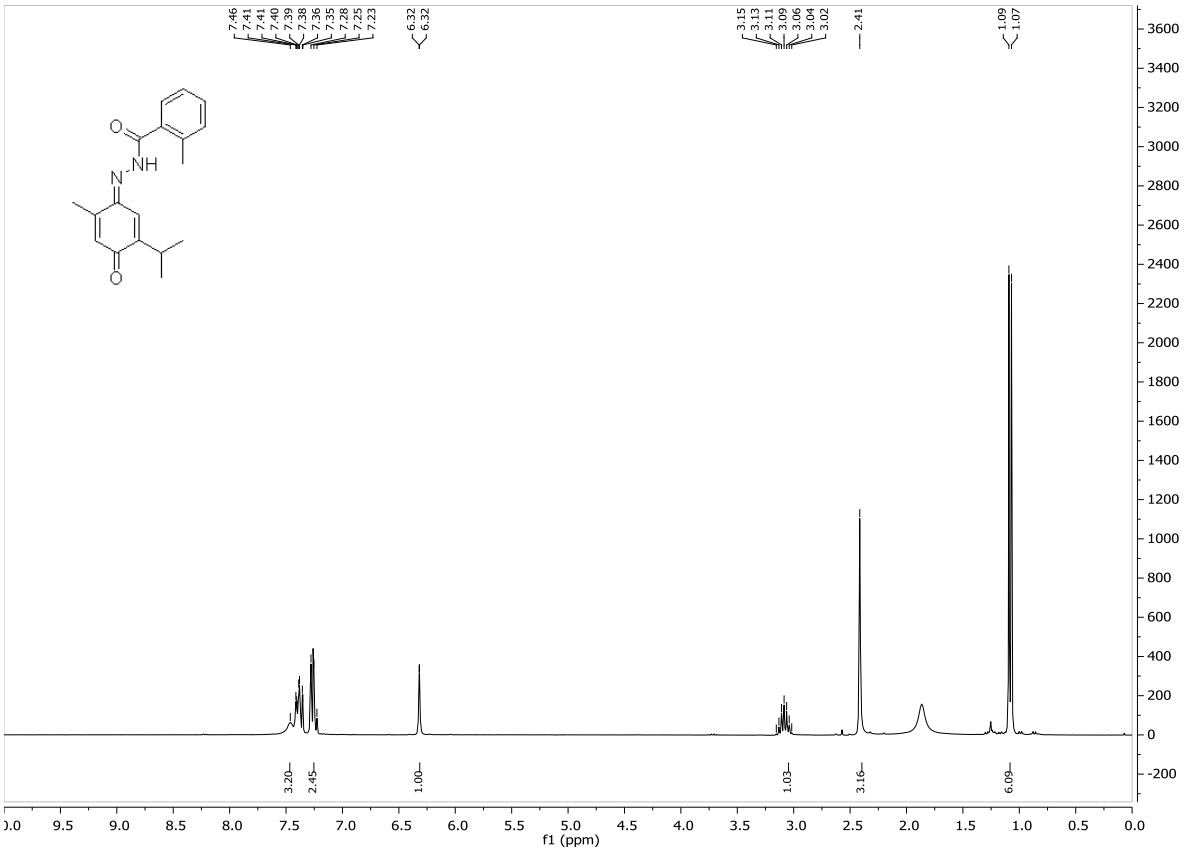


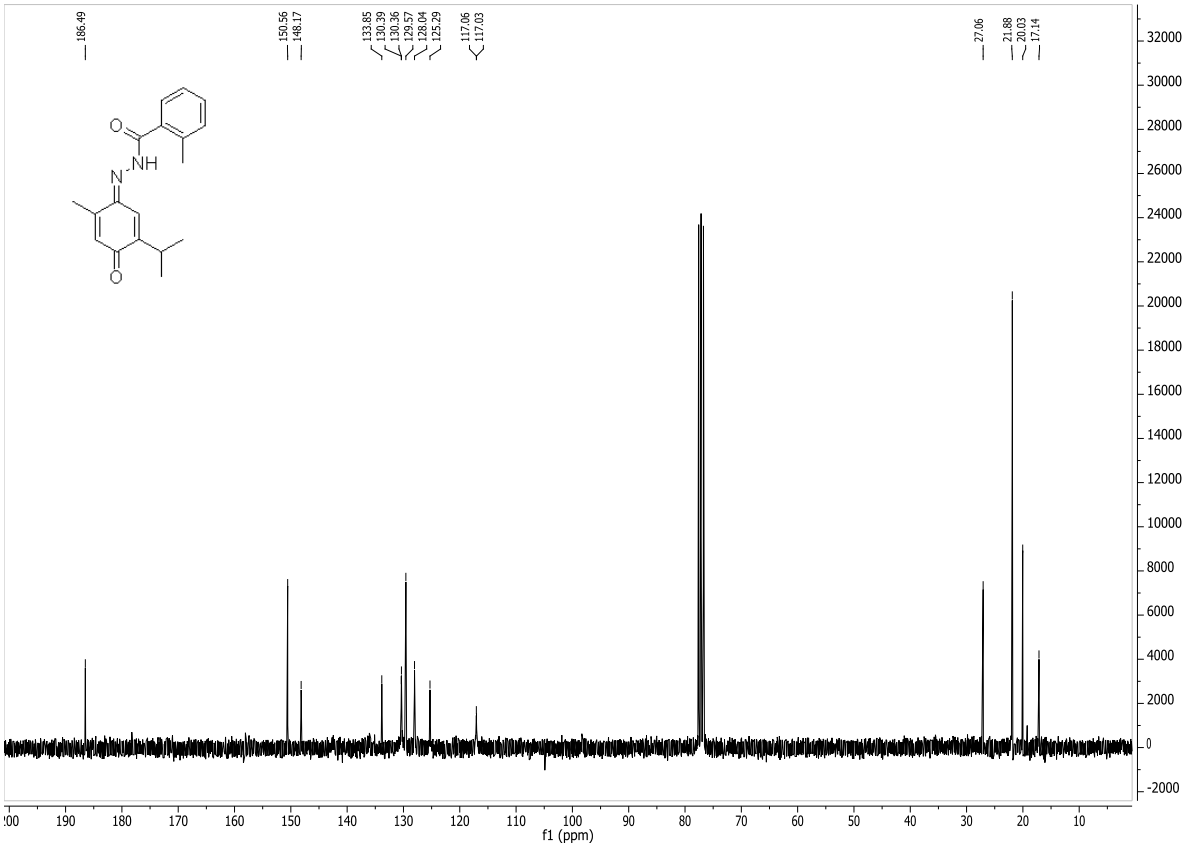
^{1}H- and ^{13}C-NMR spectra of compound **18**



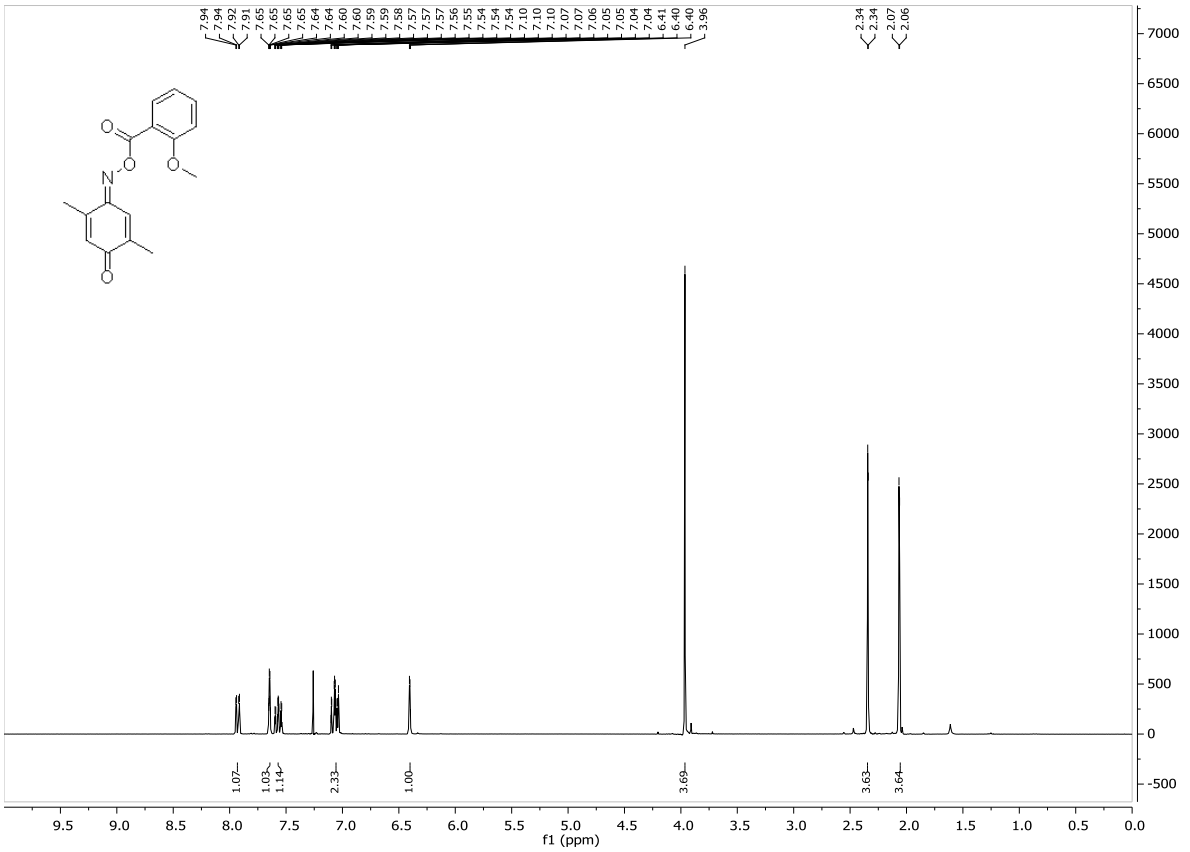


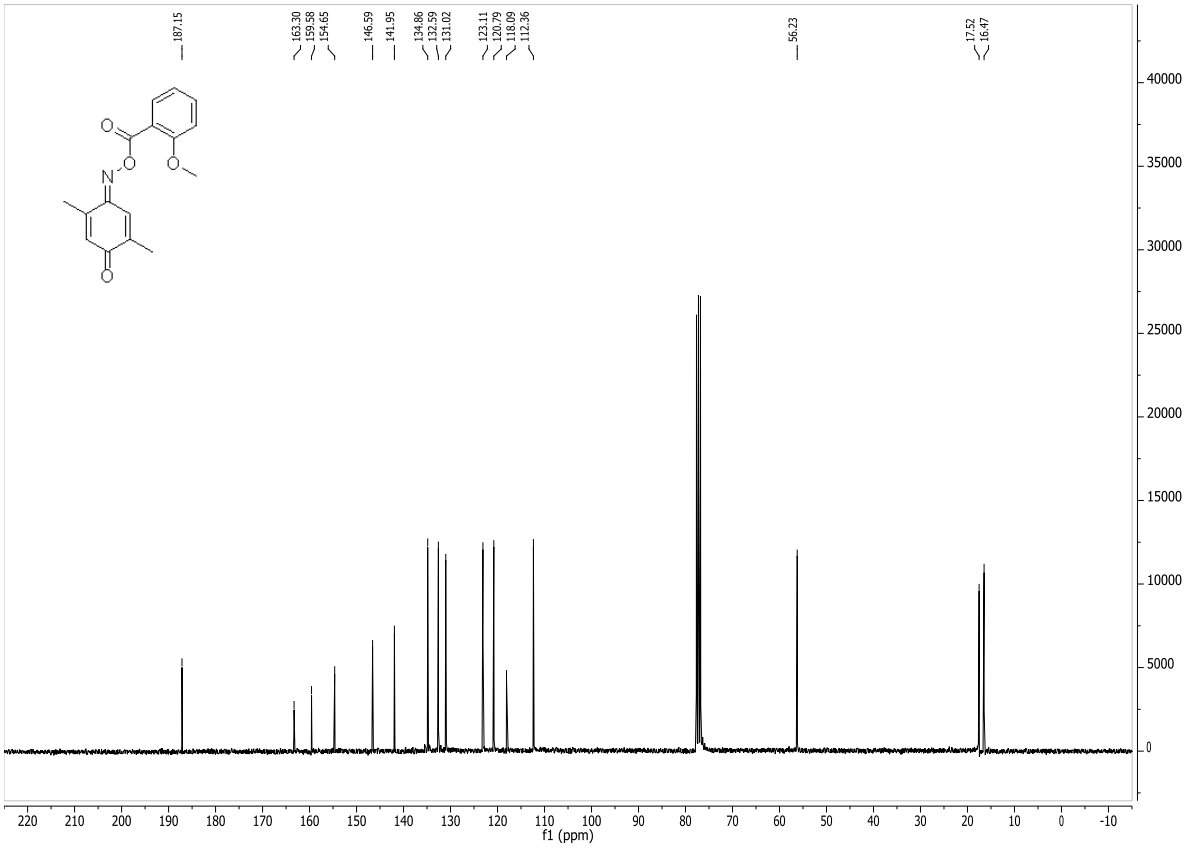
^{1}H- and ^{13}C-NMR spectra of compound **19**



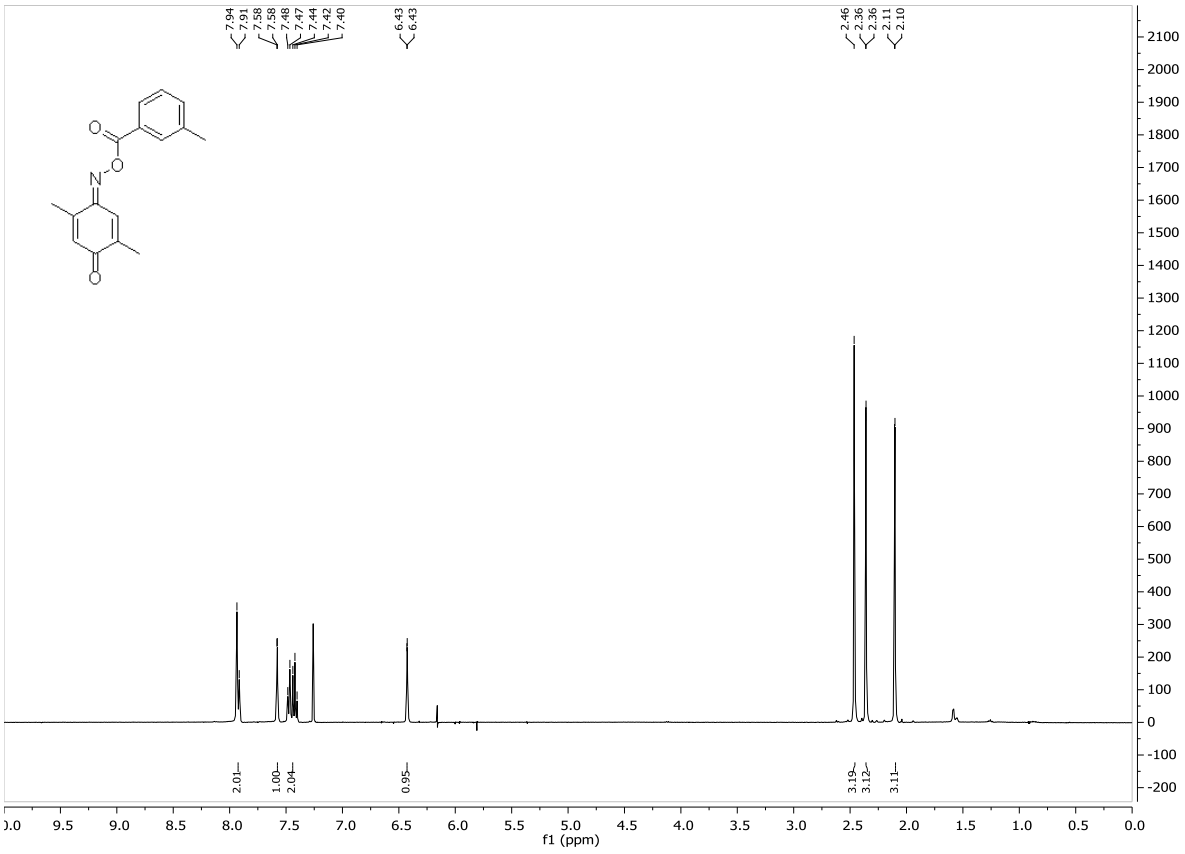


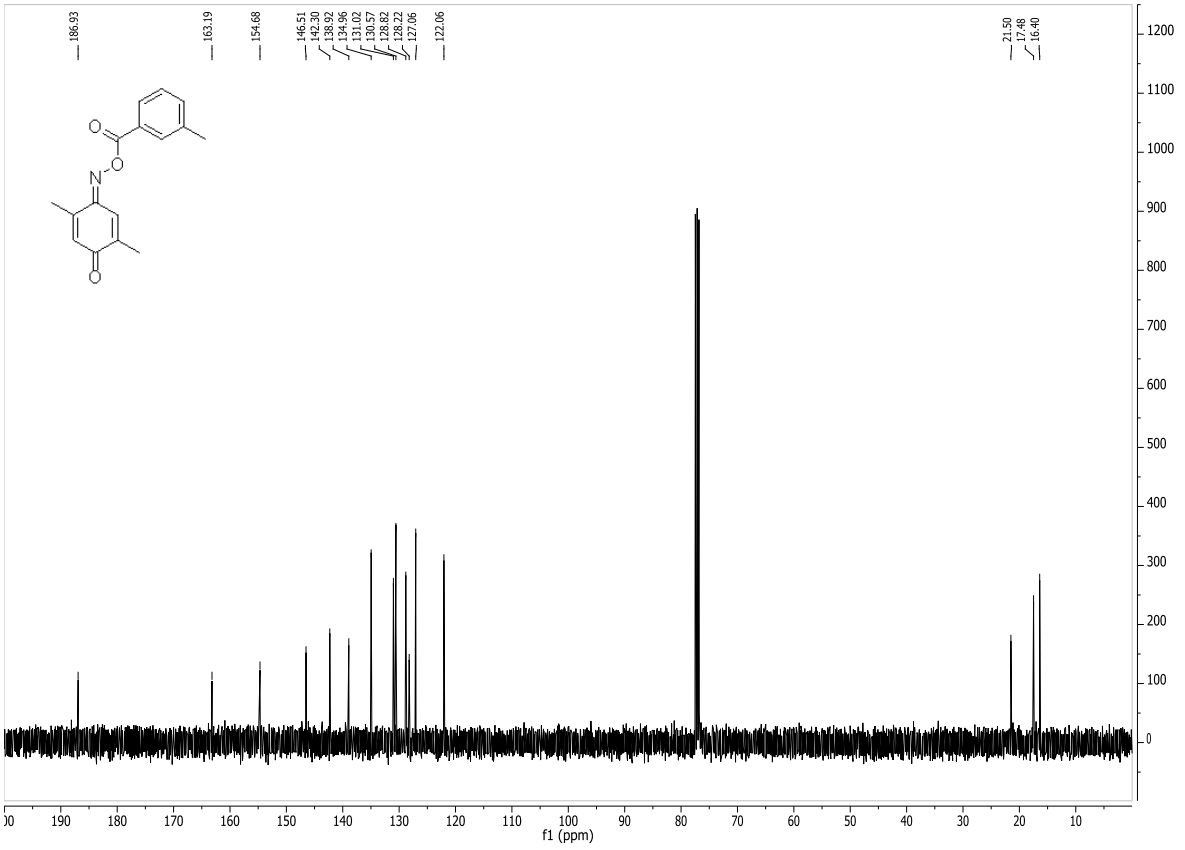
^{1}H- and ^{13}C-NMR spectra of compound **20**



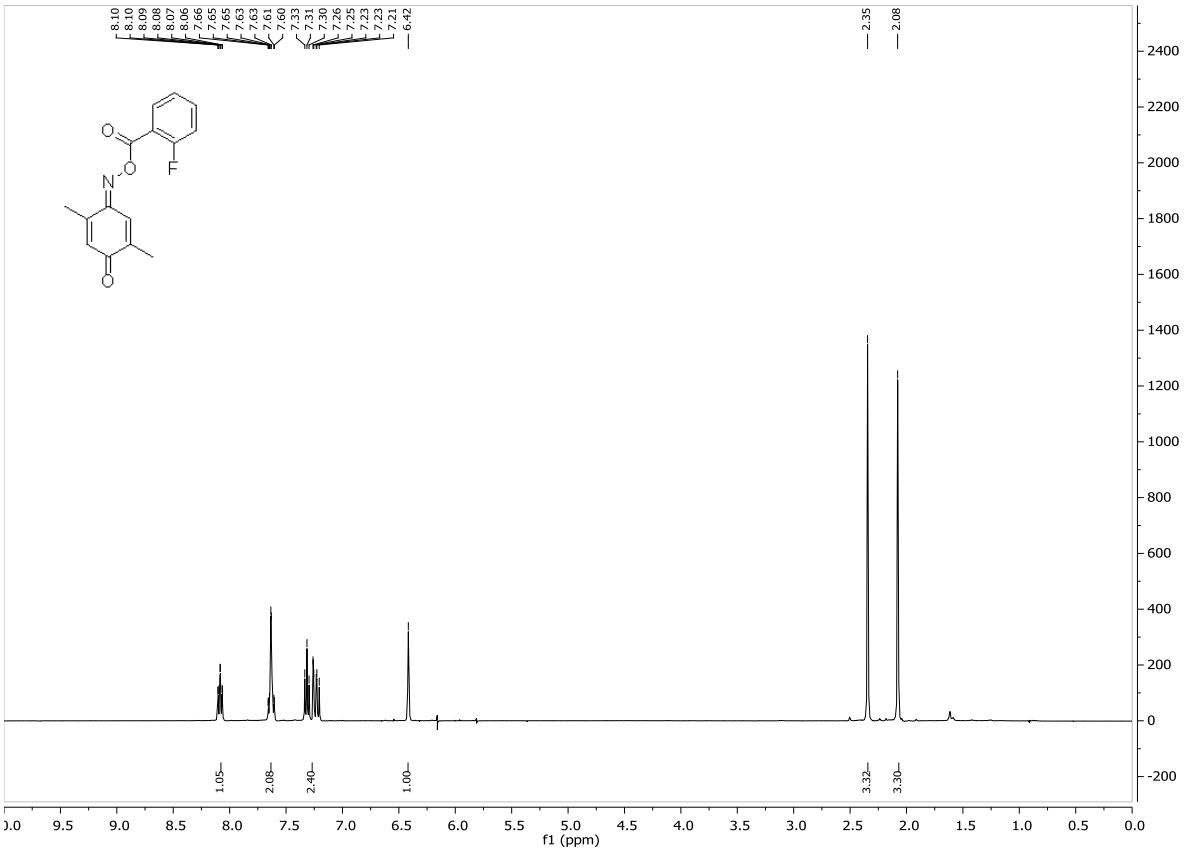


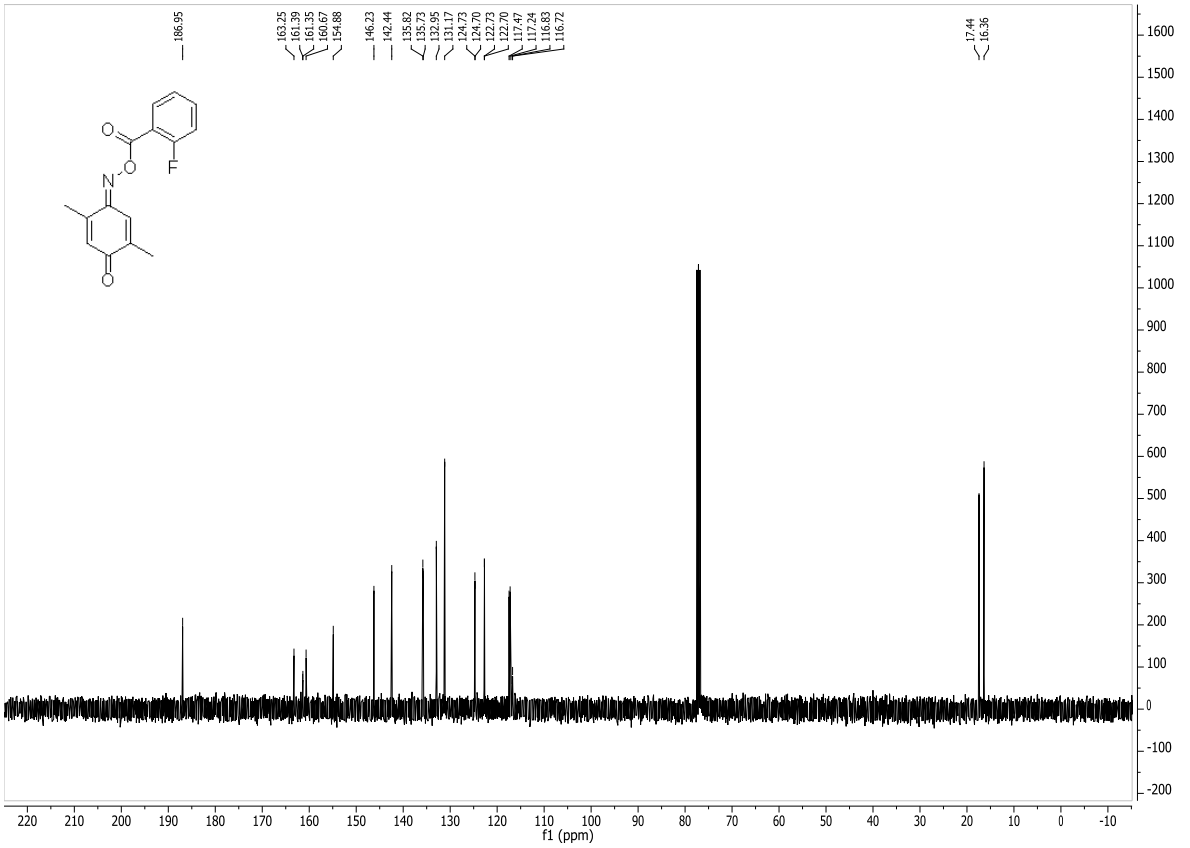
^{1}H- and ^{13}C-NMR spectra of compound **21**



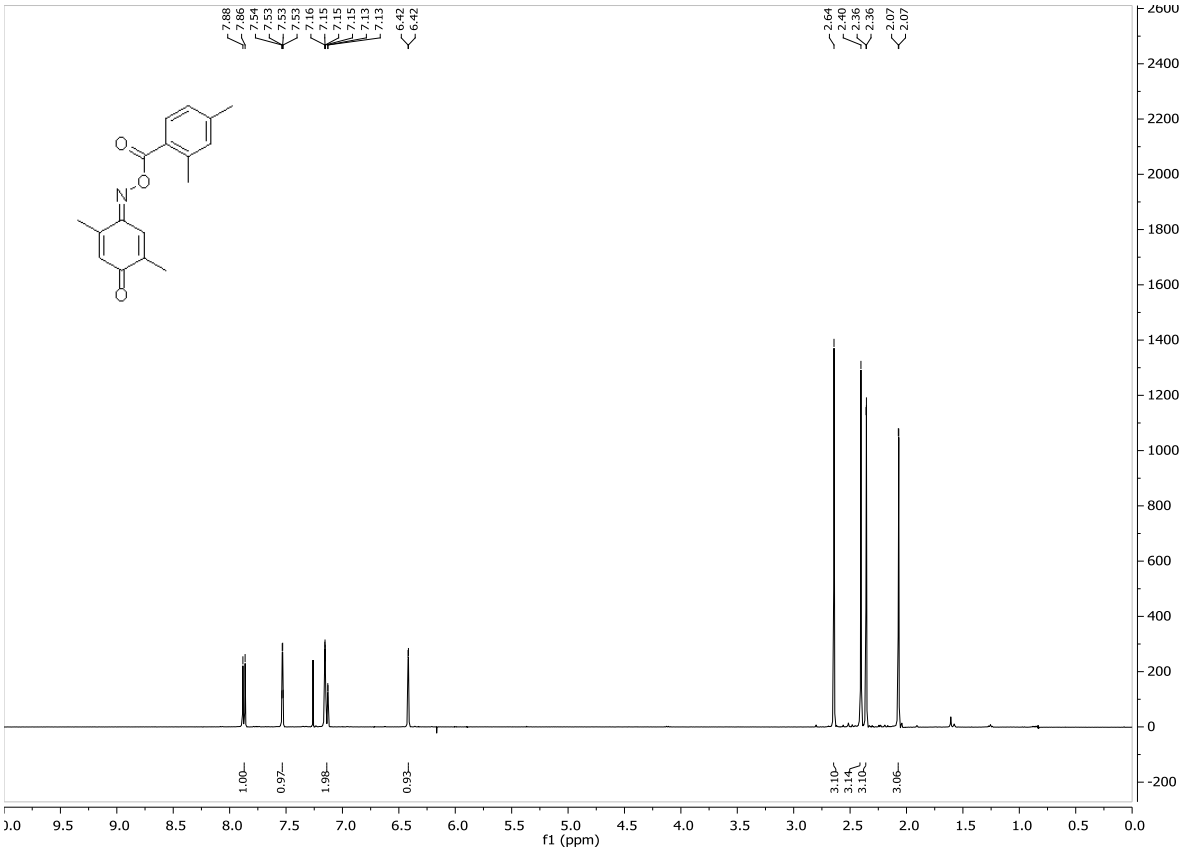


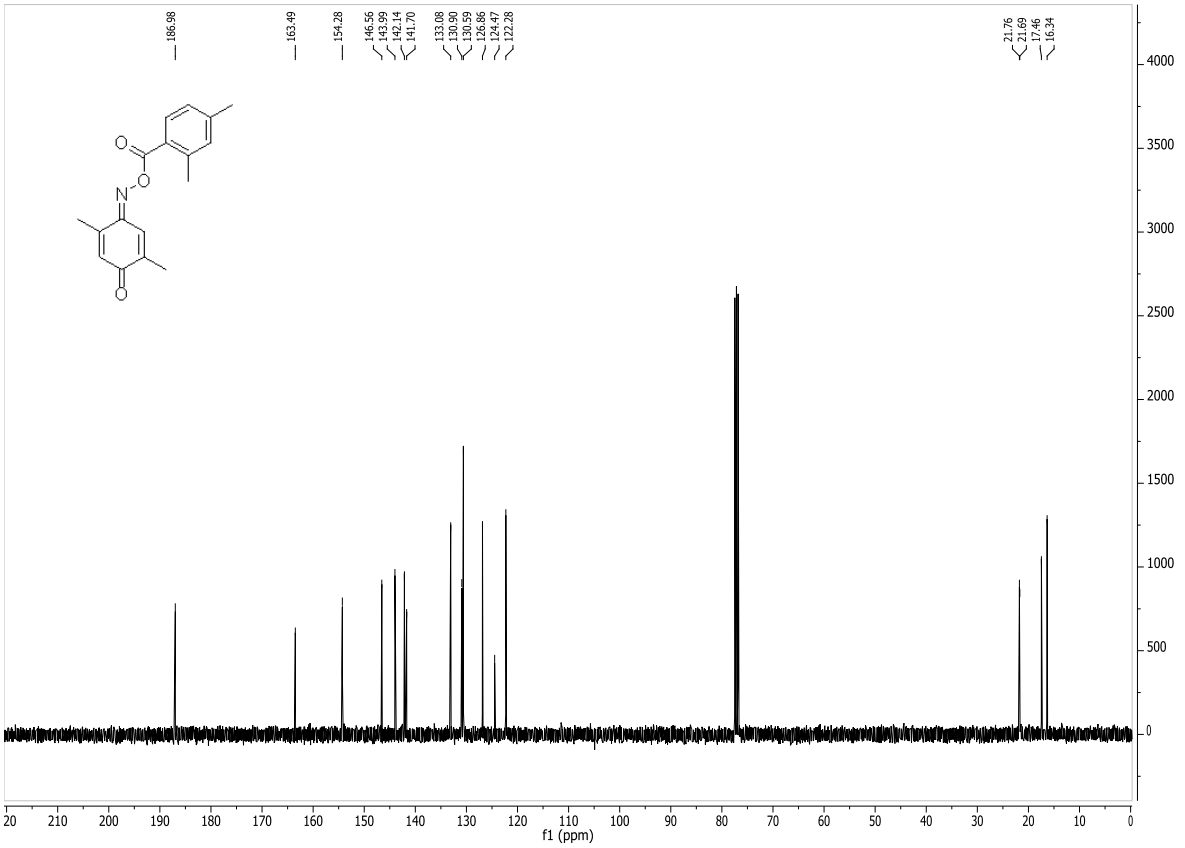
^{1}H- and ^{13}C-NMR spectra of compound **22**



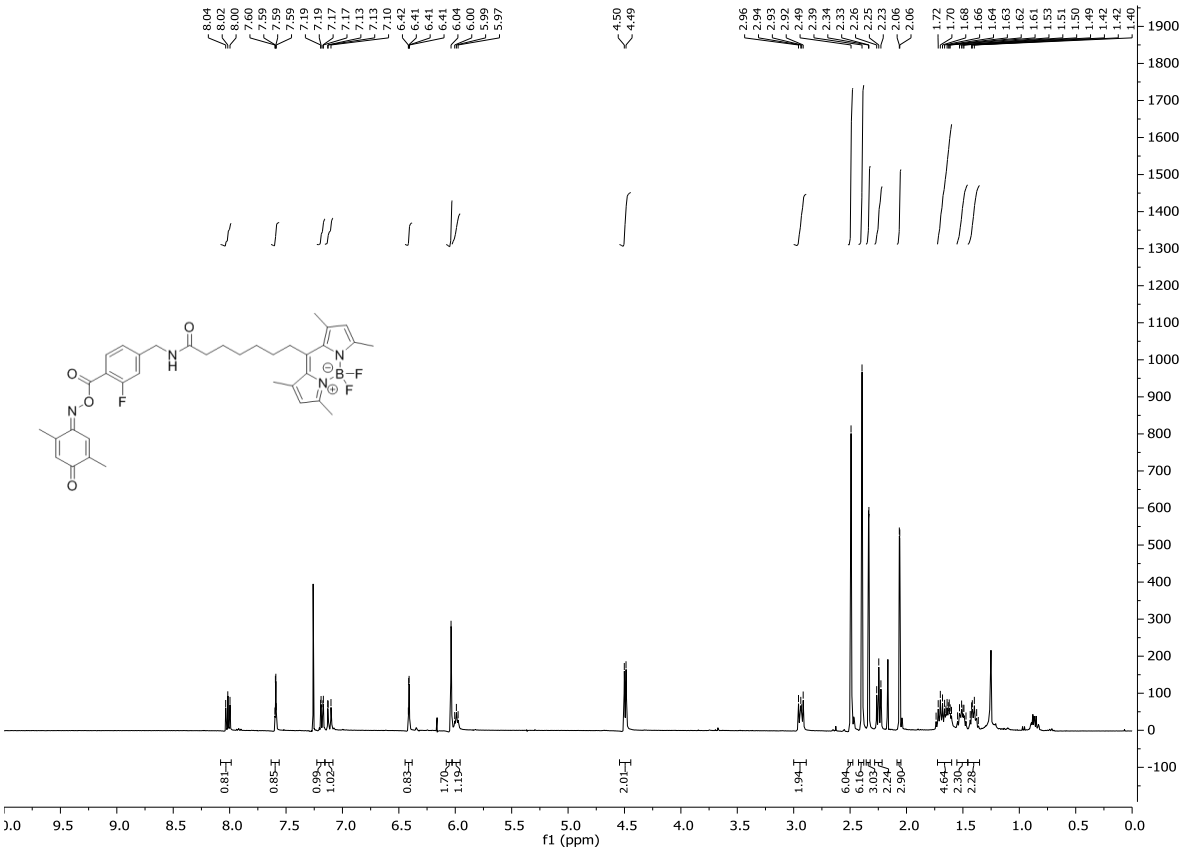


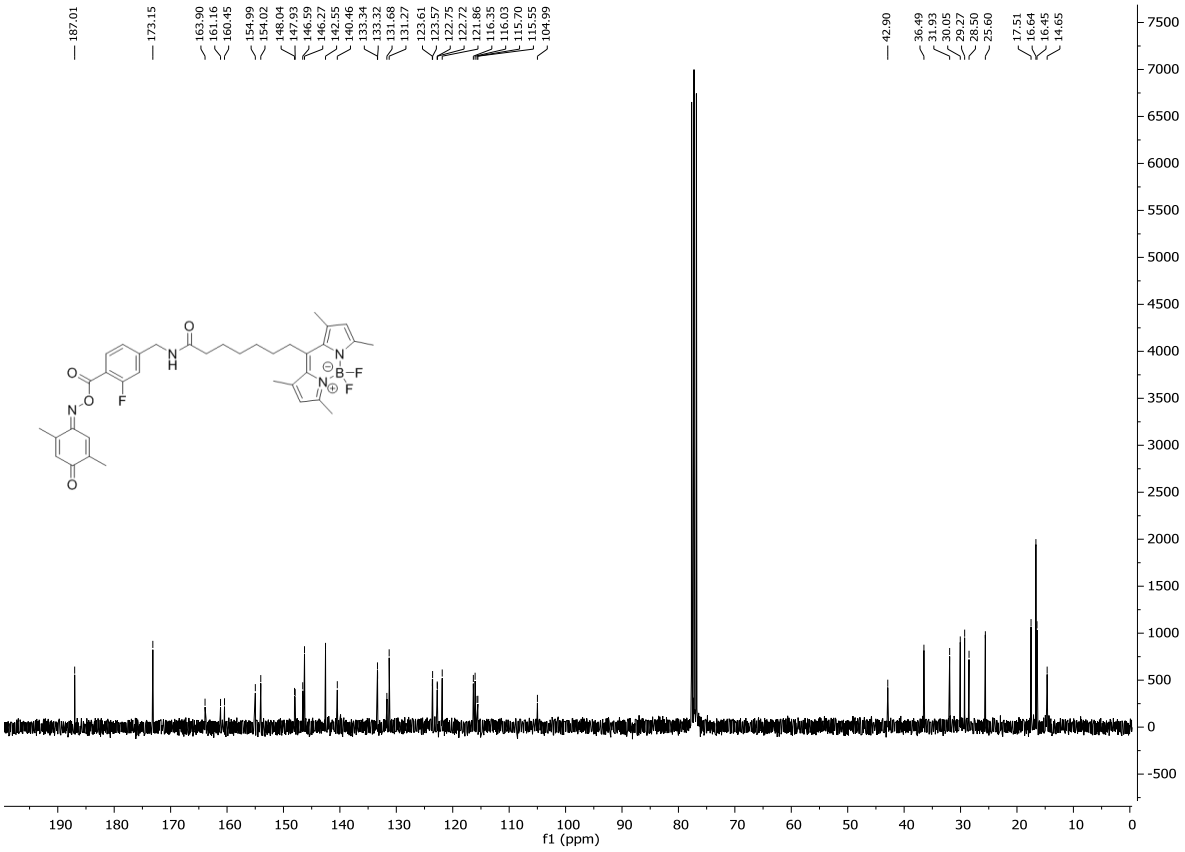
^{1}H- and ^{13}C-NMR spectra of compound **23**





^{1}H- and ^{13}C-NMR spectra of compound **24**





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