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Supporting Information

Small molecule induces mitochondrial impairment in colon cancer cells

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Materials:

All the chemical reagents were purchased from either Sigma Aldrich, ChemScene, or TCI. The solvents were purchased from local commercial sources and used without further purification. All reactions were performed in a round bottom flask or Schlenk-tubes and were monitored by thin-layer chromatography using pre-coated silica gel sheets 60 F254 on aluminum support, purchased from Merck. The purifications were performed on column chromatography using silica gel (100-200 mesh or 230-400 mesh). All the ^{1}H NMR (400 MHz) and 13C NMR (100 MHz) spectra were recorded on Bruker 400 MHz spectrometers. The chemical shifts are expressed in parts per million (ppm) using suitable deuterated NMR solvents with the residual solvent signal as an internal reference on the δ scale. UV-Vis spectra were recorded on a V-750 JASCO spectrophotometer. Fluorescence experiments were done on Fluorolog HORIBA JOBIN YVON. Colon cancer cell line HCT116 was procured from National Centre for Cell Science (NCCS), Pune, India. Fetal bovine serum, pen-strep, trypsin-EDTA were purchased from Invitrogen corporation (Carlsbad, CA, USA); DMEM was purchased from LONZA; transparent 96-well plates were purchased from TARSON (Mumbai, India), Nunc Glass bottom dishes and Mito Tracker Green FM were purchased from Thermo Fisher Scientific (Rochester, New York), 2', 7'- Dichlorofluorescin diacetate was purchased from Sigma-Aldrich (Bengaluru, India) and MTT [3-(4, 5-Dimethyl-2-thiazolyl)-2, 5- diphenyl tetrazolium bromide] dye was purchased from HIMEDIA (Mumbai, India), Pro- Caspase 3 monoclonal Antibody (31A893) was procured from Invitrogen (Bengaluru, India), Bcl-2 Monoclonal Antibody (#13-8800) was purchased from Thermo Fisher Scientific (Bengaluru, India) and Anti- Mouse IgG HRP- linked Antibody (7076P2) was procured from Cell signaling Technology (Danvers, Massachusetts, USA).

Methods:

Synthesis of N-((5-bromo-3-methoxy-2H-pyrrol-2-ylidene) methyl)-N-ethylethanamine (2):

To an oven dry two neck 25 mL round bottom flask with teflon-coated magnetic stir bar, *N*,*N*-diethylformamide **2** (0.540 □L, 5.30 mmol) and 2 mL dry DCM was taken under argon. The solution was stirred at 0°C for 10 min. A solution of phosphorous(v)oxybromide (1.27 g, 4.42 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise to the reaction mixture and was kept stirred at 0˚C for 30 min. After complete formation of white colored solid, CH_2Cl_2 was removed to dryness in vacuum. Fresh dry CH_2Cl_2 (20 mL) was added to the solid and cooled to 0 ˚C under argon. A solution of the 4-methoxy-3-pyrrolin-2-one **1** (200 mg, 26.52 mmol) in dry CH_2Cl_2 (3 mL) was added dropwise over 15 minutes under argon atmosphere. After complete addition, the flask was then removed from the ice bath and was warmed to room temperature and refluxed at 40 °C for 4 hours. To quench the reaction, the reaction mixture was poured onto aqueous sodium bicarbonate-ice-ethyl acetate mixture and was stirred for 10 min. The resulting reaction mixture was filtered through Celite and washed with cold ethyl acetate several time. Finally, aqueous layer was extracted with ethyl acetate (3 × 50 mL). The organic layer was washed with brine and dried over anhydrous sodium sulfate, filtered and evaporated under reduced pressure. The resulting residue was dissolved in DCM and adsorbed on silica (100-200 mesh) and was purified using column chromatography (5% ethyl acetate in hexane) gives product as yellow oil (324 mg, 70%).

General procedure for the synthesis of aldehyde (3) by Suzuki coupling:

10 mL solution of dioxane-water (9:1) degassed by freez-thaw method was added to a mixture of N-((5-bromo-3-methoxy-2H-pyrrol-2-ylidene)methyl)-N-ethylethanamine **2** (300 mg, 1.16 mmol), aryl boronic acid (2.32 mmol), sodium carbonate (184 mg, 1.74 mmol), and Tetrakis(triphenylphosphine)palladium(0)

(5-7 mol%) in 20 mL sealed tube under nitrogen atmosphere. The resulting suspension was heated at 100 °C for 10 hours. After the completion of the reaction solvent was evaporated and water was added. The mixture was neutralized with 0.1M HCl to obtain pH 7. The product was extracted with ethyl acetate. Product was purified by silica gel chromatography eluting with 6-10% ethyl acetate in hexane yielding the aldehyde (**3**) as solid in good to excellent yields.

General Procedure for the synthesis of 3-methoxy-pyrrole derivatives (7a-7c, 7e-7j, 7l):

To a solution of aldehyde (**3**) in dry methanol (3 mL) was added the 3-Methylindole in one portion. The resulting suspension was stirred for 5 min. To the above suspension, cat. 4M HCl in methanol (15 □L) was added and resulting suspension was stirred for 5 hours. After completion of reaction, methanol was evaporated on rotary evaporator. The resulting solid was dissolved in sat. $NaHCO_3$ and extracted with CH_2Cl_2 (2 × 10 mL). the combined organic layer dried over Na_2SO_4 a concentrated. Residue was purified by column chromatography over SiO2 (230-400/100-200 mesh) eluting with 2-10% ethyl acetate in hexane to obtain brick-red colored solid. All the products were characterized by NMR and HRMS.

Procedure for synthesis of 7d and 7k:

In a 25 mL round bottom flask, to a mixture of pyrrole-2-carbaldehyde **3** (50mg, 135 mmol) and 3-methyl Indole (18 mg, 135 mmol) was added dry CH_2Cl_2. To the above mixture $POCl_3$ (19 □L, 203 mmol) was added in one portion and reaction mixture was stirred for 2h. After the completion of reaction was quenched by addition of ice pieces followed by addition of Sat. $NaHCO_3$ (15 mL). Reaction mixture was extracted with CH_2Cl_2 (10 mL × 2). Organic layer was separated and dried over Na_2SO_4. Product was purified by column chromatography over SiO2 (100-200 Mesh) to obtain product as orange color floppy solid.

Cell Culture:

HCT116 were procured from NCCS, Pune. The cells were cultured in DMEM with 10% FBS and 1% Pen-Strep in n humidified incubator at 37°C with 5% CO_2.

Cell Viability assay:

Cell viability was performed through MTT assay in HCT116 cells. The cells were seeded at the count of 7000 cells per well in a 96 well transparent plate. The cells were treated with compound SS-36 for 24h. MTT [3-(4, 5-Dimethyl-2-thiazolyl)-2, 5- diphenyl tetrazolium bromide] dye was added after incubation for 4 hours, further the formazan crystals were solubilized in DMSO. The absorbance was recorded at 570nm using PerkinElmer Envision Multilabel Reader (Perkin Elmer, Inc., MA, and USA). The percentage cell viability was calculated using the formula: % cell viability = (Mean OD of treated Cells/ Mean OD of untreated cells) X 100. All the MTT assays were performed in triplicates.

Mitochondrial Localization:

The cells were seeded at the count of 25,000 cells in a Nunc glass bottom well plate and incubated overnight. The compound treatment was given for 3 hours and 6 hours at the final concentration of 2.5 µM along with control. The cells were washed with 1X PBS and Mitochondria were labelled using

Mitotracker Green dye with the concentration of 100 nM followed by incubation of 20 minutes. Imaging and analysis were done by confocal laser scanning microscopy.

TMRM assay:

25000 HCT-116 cells were seeded in 2 live cell plates and incubated overnight for attachment. 2.5 µM concentration of compound 7f in one plate and 0.01% DMSO was added in the control plate, incubated further for 24h. Cells were then gently washed with PBS, 100 nM concentration of TMRM dye in PBS was added and incubated for 30 minutes. Finally, a gentle wash with PBS and images were recorded using Leica confocal instrument. 5% CO_2 and 37°C temperature was maintained during the experiment.

Colocalization with LysoTracker Green DND-26:

25000 cell were seed in 2 live cell plates and incubated overnight for attachment. 2.5 µM concentration of compound 7f was added into the cells and was incubated for 3h. 0.01 % of DMSO was added into the control cells and incubated for 3h. Cells were then gently washed with PBS, 50 nM concentration of LysoTracker Green DND-26 in PBS was added and incubated for 15 minutes. Finally, a gentle wash with PBS and images were recorded using Leica confocal instrument. 5% CO_2 and 37°C temperature was maintained during the experiment.

H2DCFDA assay for ROS generation:

The cells were seeded at the count of 25,000 cells in a Nunc glass bottom well plate and incubated overnight, followed by treatment of 2.5 µM of compound for 24 hours. The cells were washed twice with 1X PBS and 2', 7'- Dichlorofluorescin diacetate dye was used at the concentration of 2 µM to check the ROS generation. Imaging and analysis were done by confocal laser scanning microscopy.

FITC annexin V staining:

25000 cells were seeded in 2 live cell plates and incubated overnight for attachment. 2.5uM of compound 7f in one plate and 0.01% of DMSO were added in another plate, incubated further for 24h. Cells were then gently washed with PBS, 5 µL of FITC Annexin V (Invitrogen, Catalogue # - V13242 A) in PBS is added and incubated for 30 minute. Finally, a gentle wash performed with PBS and images were recorded using Leica confocal instrument. 5% CO2 and 37°C temperature was maintain during the experiment. Intensity is measured using Image J software and plot created by GraphPad Prism.

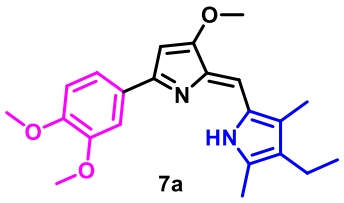
PI staining:

25000 HCT-116 cells were seeded in 2 live cell plates and incubated overnight for attachment. The cells were treated with 2.5 µM concentration of compound 7f and 0.01% DMSO as control for 24h. Cells were then gently washed with PBS and 500 nM concentration of PI in PBS is added and incubated for 30 minutes. Finally, a gentle wash with PBS and the images were recorded using Leica confocal instrument. 5% CO2 and 37°C temperature was maintained during the experiment.

Western Blotting:

After the 24 hours treatment with the compound, the cells were lysed using RIPA- buffer and 1X phosphatase inhibitor cocktail. The quantification was done using Bradford assay and 30 µg of protein was loaded on a SDS-gel and then transferred on a 0.22 micron nitrocellulose membrane. The membrane was blocked with 5% skimmed milk in 1X TBST and probed with primary and secondary antibodies. The blots were developed using Clarity Western ECL substrate. Further the blots were analysed using ImageLab Bio-Rad software, Beta-actin was taken as a loading control.

Characterization:



7a

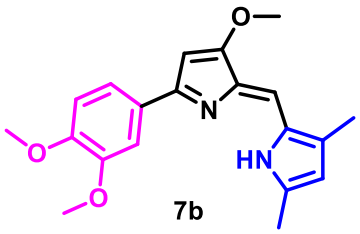
Purification: SiO_2 230-400 mesh, Eluted with 10% EA/PE.

Yield: 64%.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.60 (d, *J* = 1.8 Hz, 1H), 7.49 (dd, *J* = 8.3, 1.8 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.91 (s, 1H), 6.43 (bs, 1H), 6.04 (s, 1H), 4.00 (s, 3H), 3.94 (s, 3H), 3.92 (s, 3H), 2.40 (q, *J* = 7.6 Hz, 2H), 2.31 (s, 3H), 2.17 (s, 3H), 1.07 (t, *J* = 7.6 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 167.6, 162.9, 150.4, 149.1, 138.7, 136.4, 129.8, 128.7, 128.0, 125.4, 120.1, 115.0, 111.1, 109.3, 94.1, 58.3, 56.1, 55.9, 17.6, 15.3, 12.5, 9.5.

HRMS: Calculated for $C_{22}H_{27}N_2O_3$: 367.2022; Observed: 367.2021



7b

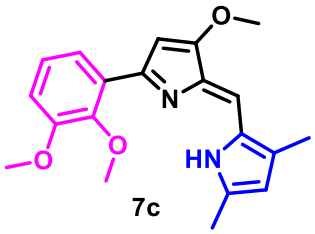
Purification: SiO_2 230-400 mesh, Eluted with 10% EA/PE.

Yield: 65%.

^{1}H NMR (500 MHz, $CDCl_3$): δ 9.07 (bs, 1H), 7.62 (s, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 6.93 (d, *J* = 8.8 Hz, 1H), 6.91 (s, 1H), 6.05 (s, 1H), 5.85 (s, 1H), 4.00 (s, 3H), 3.94 (s, 3H), 3.92 (s, 3H), 2.36 (s, 3H), 2.23 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 167.6, 162.9, 150.4, 149.1, 138.7, 136.4, 129.8, 128.7, 128.0, 125.4, 120.1, 115.0, 111.1, 109.3, 94.1, 58.3, 56.1, 55.9, 17.6, 15.3, 12.5, 9.5.

HRMS: Calculated for $C_{20}H_{23}N_2O_3$: 339.1709; Observed: 339.1710.



7c

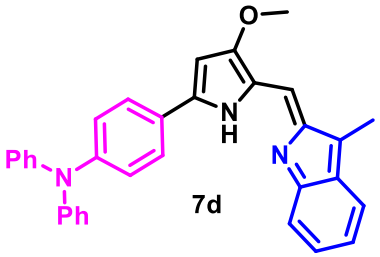
Purification: SiO_2 230-400 mesh, Eluted with 10% EA/PE.

Yield: 69%.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.59 (d, *J* = 7.6 Hz, 1H), 7.12 (t, *J* = 8.0 Hz, 1H), 6.94 (s, 1H), 6.93 (d, *J* = 8.1 Hz, 1H), 6.32 (s, 1H), 5.92 (s, 1H), 3.91 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H), 2.37 (s, 3H), 2.23 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 164.3, 155.4, 153.6, 148.2, 146.5, 135.9, 133.5, 133.3, 128.5, 124.2, 120.7, 115.7, 114.9, 112.8, 97.2, 61.2, 58.2, 56.2, 15.4, 11.5.

HRMS: Calculated for $C_{20}H_{23}N_2O_3$: 339.1709; Observed: 339.1710.



7d

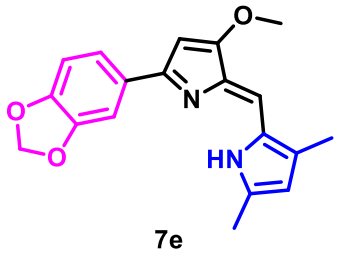
Purification: SiO_2 100-200 mesh, Eluted with 2% EA/PE.

Yield: 95%.

^{1}H NMR (500 MHz, $CDCl_3$): δ 11.41 (bs, 1H), 7.93 (d, *J* = 8.7 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.38 – 7.28 (m, 5H), 7.23 (t, *J* = 7.5 Hz, 1H), 7.18 (d, *J* = 7.7 Hz, 4H), 7.15 – 7.08 (m, 5H), 7.05 (t, *J* = 7.5 Hz, 1H), 6.08 (s, 1H), 3.96 (s, 3H), 2.50 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 168.8, 168.0, 150.0, 147.1, 145.4, 138.7, 133.0, 129.5, 128.6, 128.4, 127.8, 125.4, 125.2, 123.9, 121.8, 120.4, 120.0, 119.3, 113.8, 111.4, 95.6, 58.4, 29.7.

HRMS: Calculated for $C_{33}H_{28}N_3O$: 482.2232; Observed: 482.2233



7e

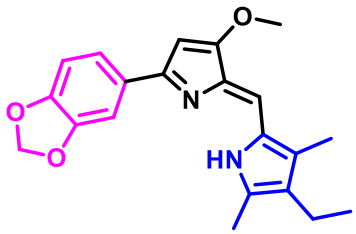
Purification: SiO_2 230-400 mesh, Eluted with 10% EA/PE.

Yield: 85%.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.54 (s, 1H), 7.46 (d, *J* = 8.1 Hz, 1H), 6.91 (s, 1H), 6.87 (d, *J* = 8.1 Hz, 1H), 6.02 (s, 2H), 6.01 (s, 1H), 5.85 (s, 1H), 3.91 (s, 3H), 2.38 (s, 3H), 2.22 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 168.1, 164.1, 148.9, 148.1, 139.5, 138.6, 131.8, 130.2, 128.3, 121.6, 115.2, 111.9, 108.4, 106.7, 101.4, 94.5, 58.3, 14.2, 11.4.

HRMS: Calculated for $C_{19}H_{19}N_2O_3$: 323.1396; Observed: 323.1393.



7f

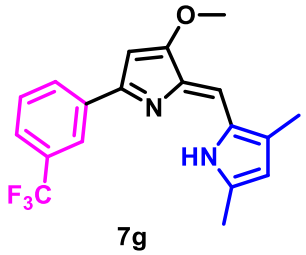
Purification: SiO_2 230-400 mesh, Eluted with 10% EA/PE.

Yield = 67%

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.44 (d, *J* = 8.1 Hz, 1H), 6.90 (s, 1H), 6.87 (d, *J* = 8.1 Hz, 1H), 6.02 (s, 2H), 6.00 (s, 1H), 3.90 (s, 3H), 2.40 (q, *J* = 7.5 Hz, 2H), 2.34 (s, 3H), 2.17 (s, 3H), 1.07 (t, *J* = 7.6 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 167.6, 162.8, 148.7, 148.1, 138.7, 136.5, 130.3, 129.9, 127.8, 125.3, 121.4, 115.2, 108.4, 106.7, 101.4, 94.2, 58.3, 17.6, 15.3, 12.5, 9.5.

HRMS: Calculated for $C_{21}H_{23}N_2O_3$: 351.1709; Observed: 351.1708.



7g

Purification: SiO_2 230-400 mesh, Eluted with 2-3% EA/PE.

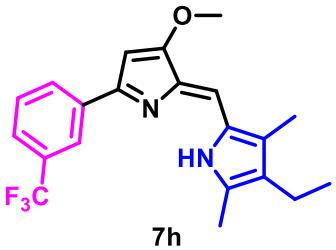
Yield: 62%.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (s, 1H), 8.14 (d, *J* = 7.7 Hz, 1H), 7.61 (d, *J* = 7.7 Hz, 1H), 7.55 (t, *J* = 7.7 Hz, 1H), 7.02 (s, 1H), 6.10 (s, 1H), 5.89 (s, 1H), 3.94 (s, 3H), 2.40 (s, 3H), 2.25 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 168.2, 162.5, 139.8, 139.1, 136.3, 133.6, 131.2, 130.9, 129.7, 129.1, 128.6, 125.6 (q, *J* = 3.4 Hz), 124.3 (d, *J* = 272 Hz), 123.44 (q, *J* = 3.8 Hz), 117.1, 112.5, 94.2, 58.4, 14.2, 11.5.

^{19}F NMR: δ = -62.68.

HRMS: Calculated for $C_{19}H_{18}F_3N_2O$: 347.1371; Observed: 347.1370.



7h

Purification: SiO_2 230-400 mesh, Eluted with 2% EA/PE.

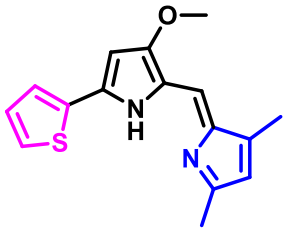
Yield: 64%.

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.50 (bs, 1H), 8.19 (s, 1H), 8.11 (d, *J* = 7.7 Hz, 1H), 7.59 (d, *J* = 7.7 Hz, 1H), 7.53 (t, *J* = 7.7 Hz, 1H), 7.00 (s, 1H), 6.10 (s, 1H), 3.93 (s, 3H), 2.41 (q, *J* = 7.6 Hz, 2H), 2.36 (s, 3H), 2.19 (s, 3H), 1.08 (t, *J* = 7.6 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 167.6, 160.9, 138.7, 138.1, 136.4, 131.6, 131.0 (q, *J* = 32.2 Hz), 129.5, 129.1, 128.4, 126.1, 125.3 (q, *J* = 3.6 Hz), 124.3 (d, *J* = 272 Hz), 123.3 (q, *J* = 3.8 Hz), 116.9, 94.0, 58.4, 17.6, 15.2, 12.7, 9.6.

^{19}F NMR: δ = -62.75.

HRMS: Calculated for $C_{21}H_{22}F_3N_2O$: 375.1684; Observed: 375.1683.



7i

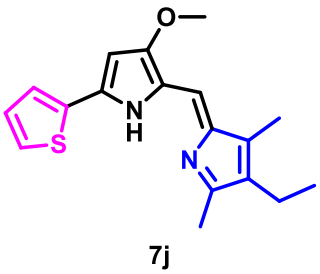
Purification: SiO_2 230-400 mesh, Eluted with 2% EA/PE.

Yield: 56%.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.46 (d, *J* = 3.5 Hz, 1H), 7.37 (d, *J* = 5.0 Hz, 1H), 7.10 (dd, *J* = 5 Hz & 3.5 Hz, 1H), 6.91 (s, 1H), 6.04 – 5.93 (m, 1H), 5.89 – 5.79 (m, 1H), 5.16 (s, 1H), 3.91 (s, 1H), 2.37 (s, 1H), 2.22 (s, 1H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 168.0, 159.1, 141.3, 139.4, 138.3, 132.1, 128.3, 128.0, 127.5, 126.6, 115.4, 111.9, 94.7, 58.4, 14.1, 11.4.

HRMS: Calculated for $C_{16}H_{17}N_2OS$: 285.1062; Observed: 285.1061.



7j

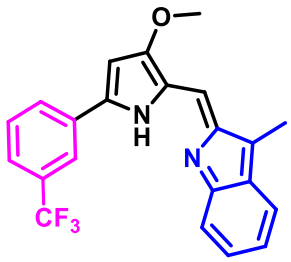
Purification: SiO_2 230-400 mesh, Eluted with 2% EA/PE.

Yield: 39%.

^{1}H NMR (500 MHz, $CDCl_3$): δ 7.44 (d, *J* = 3.6 Hz, 1H), 7.35 (d, *J* = 5.0 Hz, 1H), 7.09 (dd, *J* = 4.6, 4.0 Hz, 1H), 6.90 (s, 1H), 4.90 (s, 2H), 3.90 (s, 4H), 2.39 (q, *J* = 7.6 Hz, 2H), 2.33 (s, 3H), 2.17 (d, *J* = 4.7 Hz, 3H), 1.06 (t, *J* = 7.6 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 167.6, 158.3, 141.5, 138.9, 136.3, 130.0, 128.0, 127.6, 127.1, 126.2, 125.2, 115.3, 94.4, 58.4, 17.5, 15.3, 12.4, 9.5.

HRMS: Calculated for $C_{18}H_{21}N_2OS$: 313.1375 Observed: 313.1373.



7k

Purification: SiO_2 230-400 mesh, Eluted with 2% EA/PE.

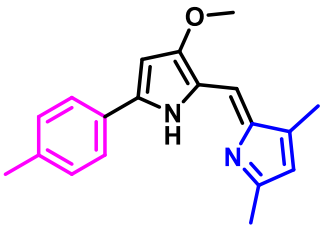
Yield: 89 %.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.26 (d, *J* = 5.5 Hz, 2H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 8.4 Hz, 1H), 7.41 (d, *J* = 8.2 Hz, 1H), 7.30 (t, *J* = 7.5 Hz, 1H), 7.27 (s, 1H), 7.08 (t, *J* = 7.5 Hz, 1H), 6.14 (s, 1H), 3.99 (s, 3H), 2.53 (s, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 169.4, 167.1, 144.7, 139.4, 135.7, 132.8, 131.4, 130.3, 129.3, 128.6, 126.4, 126.2 – 126.1 (m), 124.1, 122.7, 120.5, 119.8, 116.8, 114.81 – 114.66 (m), 111.8, 95.50, 58.7, 9.34.

^{19}F NMR: δ = -62.7.

HRMS: Calculated for $C_{22}H_{18}F_3N_2O$: 383.1371; Observed: 383.1369.



7l

Purification: SiO_2 100-200 mesh, Eluted with 2% EA/PE.

Yield: 51 %.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.1 Hz, 1H), 7.23 (d, *J* = 8.6 Hz, 1H), 7.01 (s, 1H), 6.92 (s, 1H), 6.06 (s, 1H), 5.84 (s, 1H), 3.90 (s, 2H), 2.39 (s, 2H), 2.37 (s, 2H), 2.21 (s, 2H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 167.8, 164.2, 139.5, 139.2, 138.5, 132.6, 131.9, 129.3, 128.4, 126.6, 115.3, 111.9, 94.4, 58.2, 21.5, 14.1, 11.3.

HRMS: Calculated for $C_{19}H_{21}N_2O$: 293.1654; Observed: 293.1648.

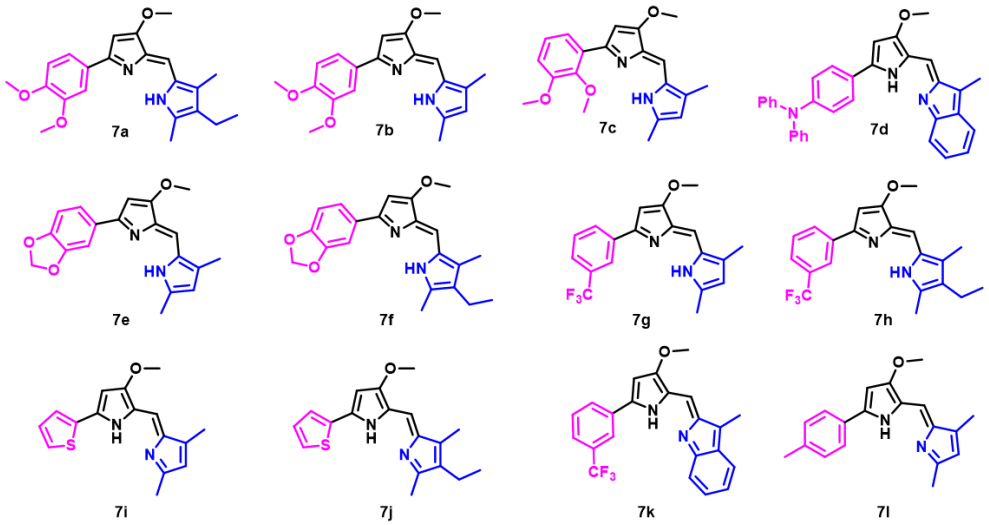


Fig. S1: Chemical structures of the 3-methoxy-pyrrole library members.

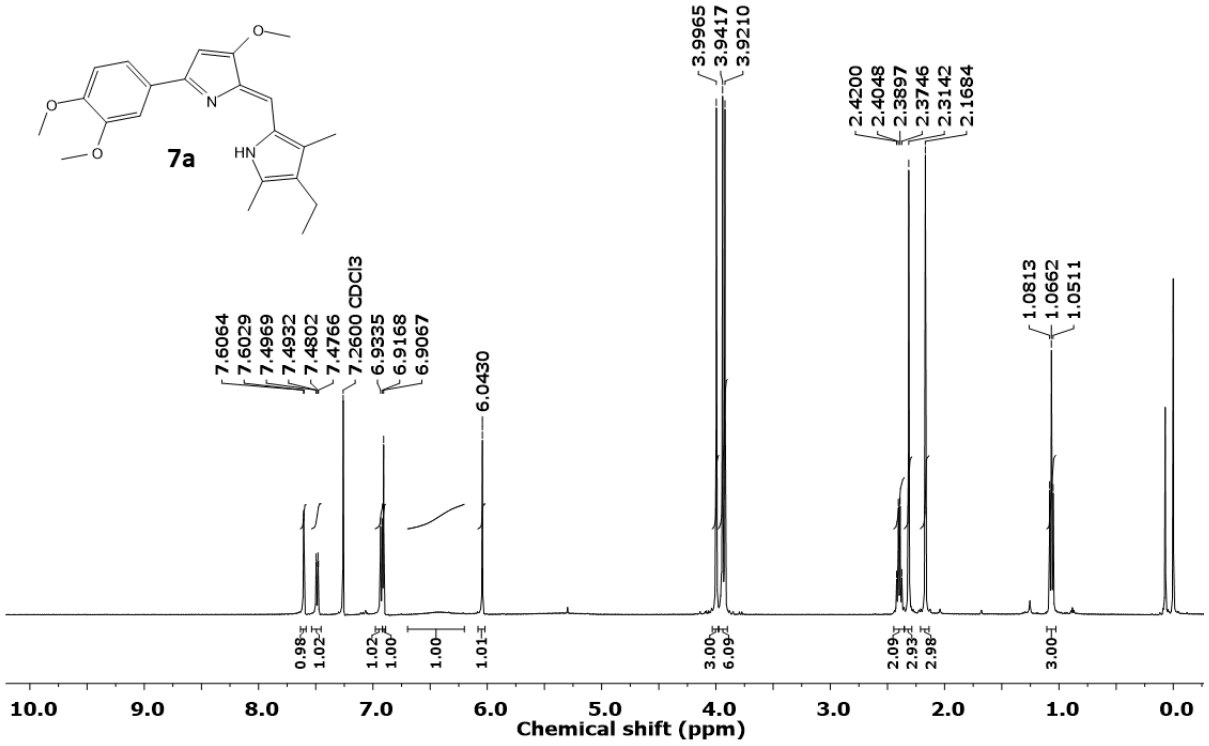


Fig. S2: ^{1}H NMR spectra of compound 7a.

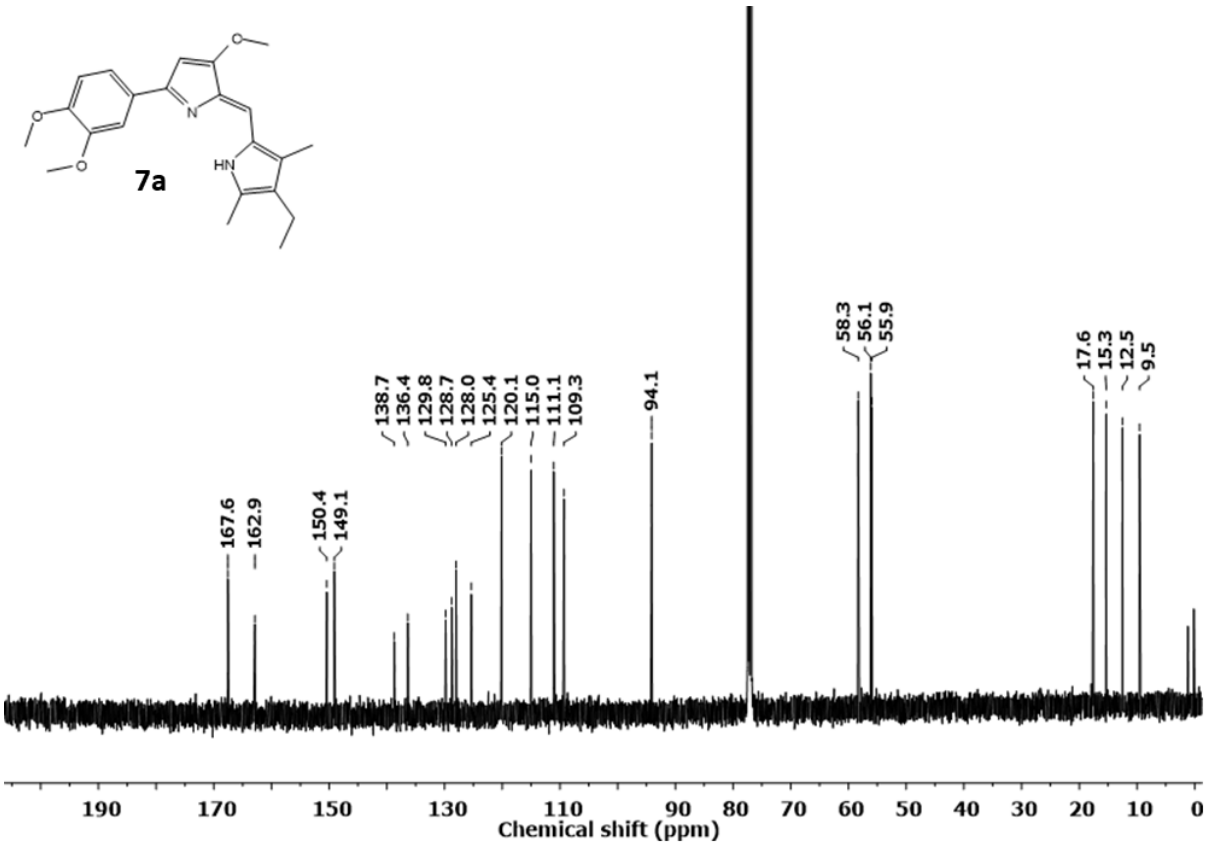


Fig. S3: ^{13}C NMR spectra of compound 7a.

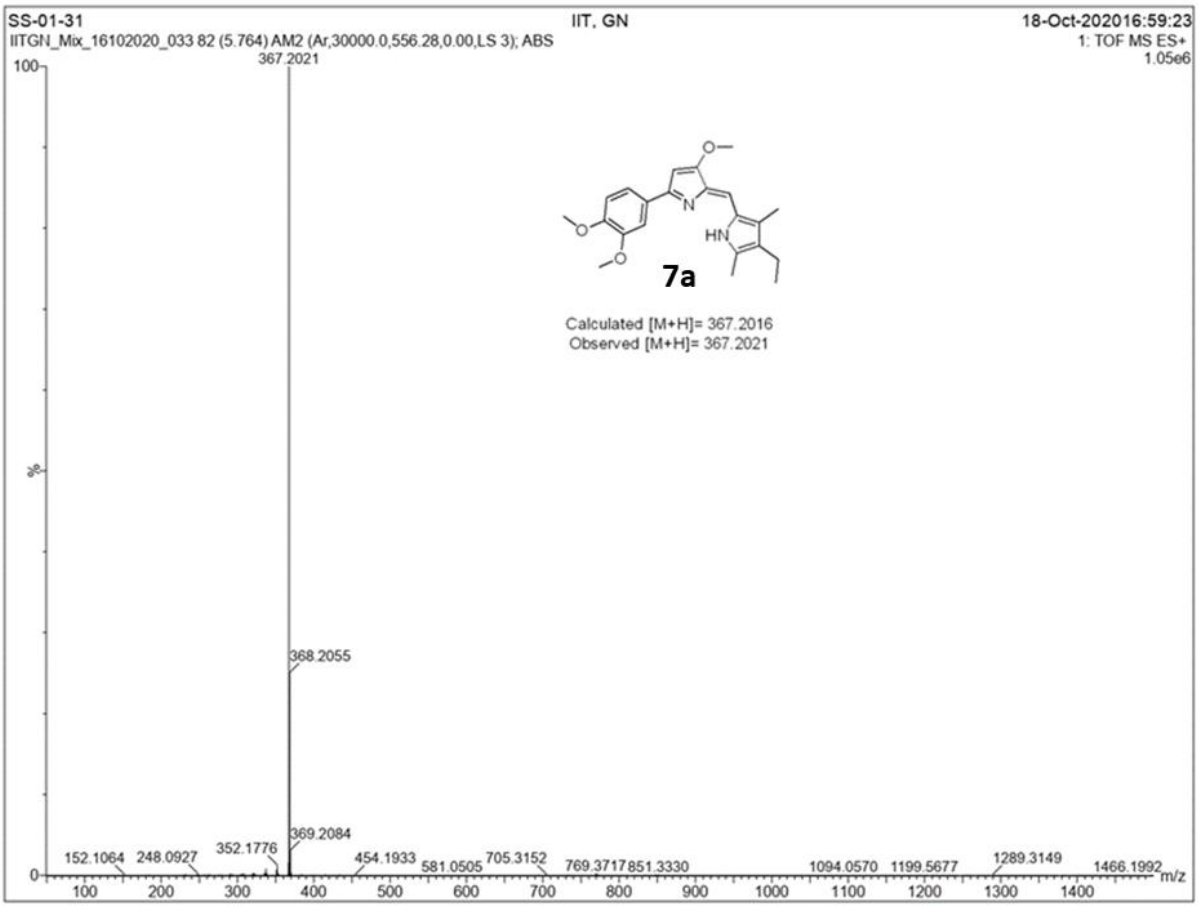


Fig. S4: HR-MS spectra of compound 7a.

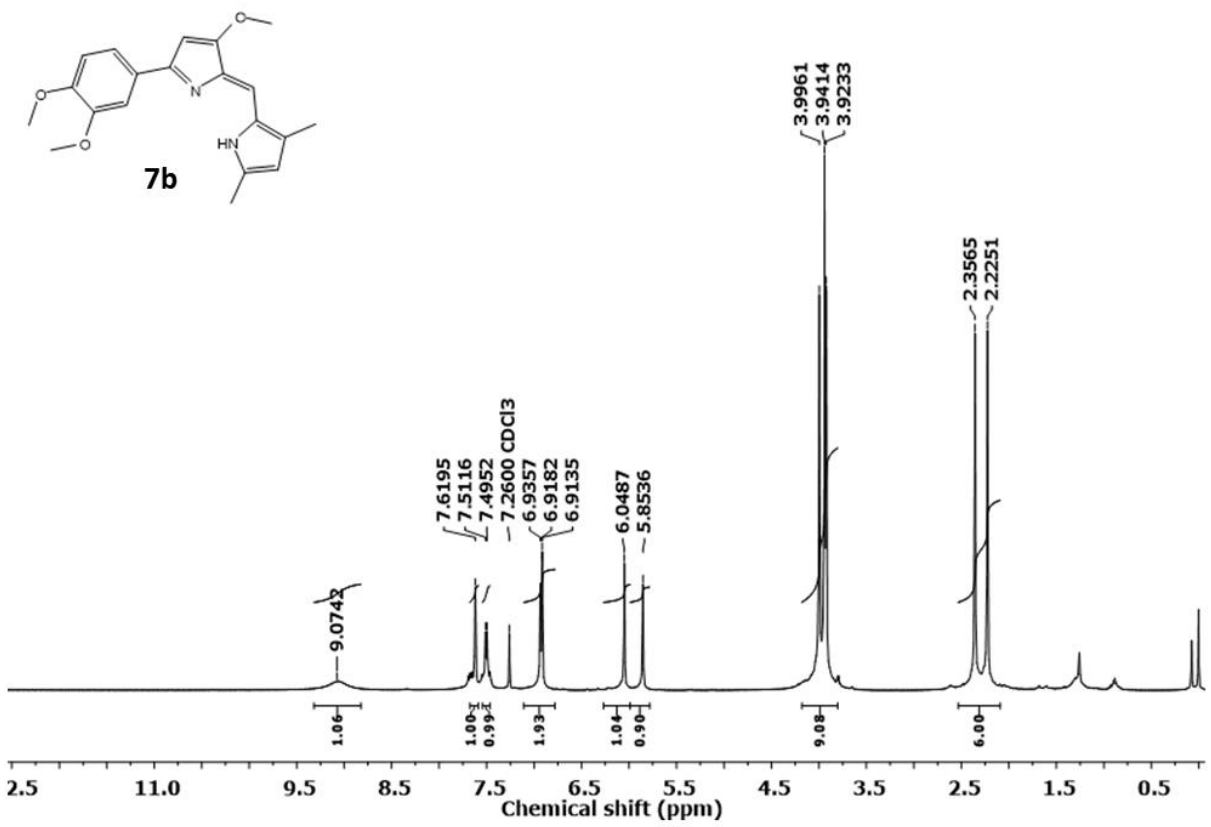


Fig. S5: ^{1}H NMR spectra of compound 7b.

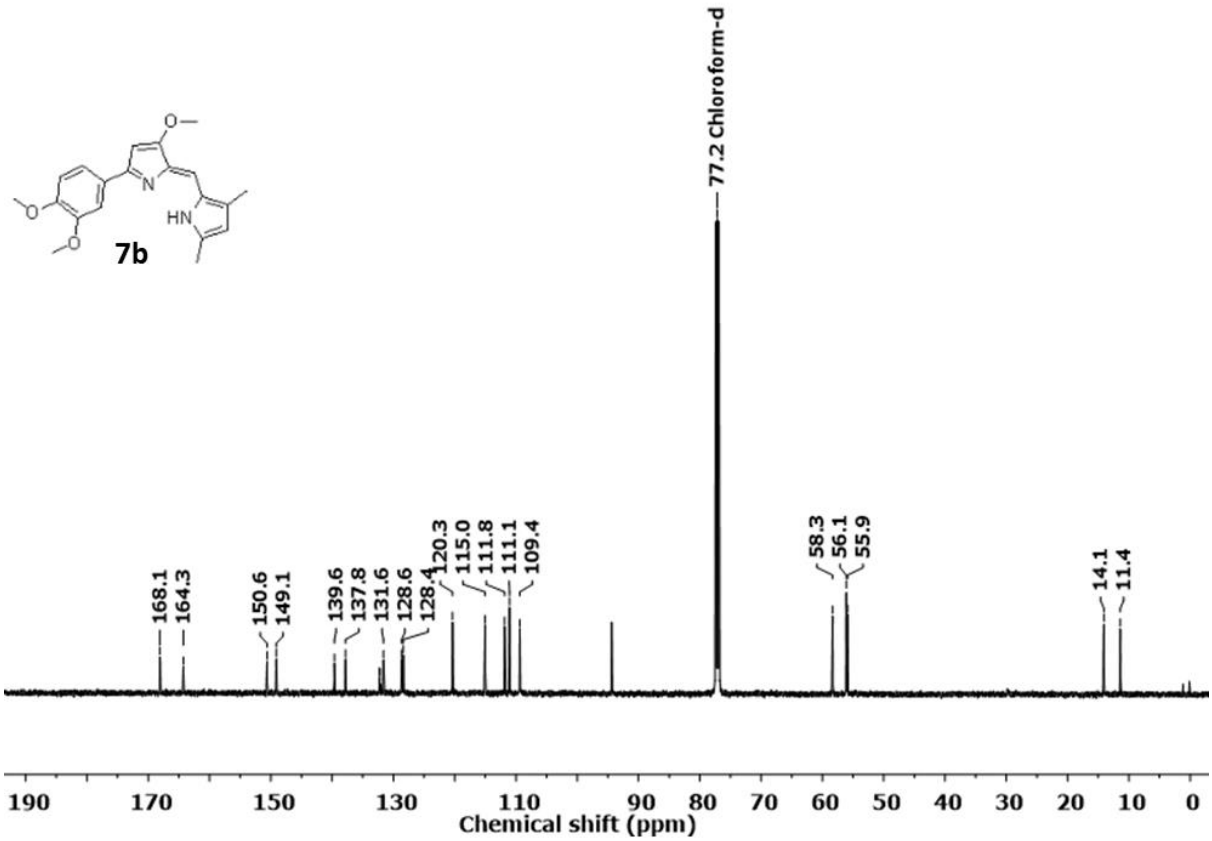


Fig. S6: ^{13}C NMR spectra of compound 7b.

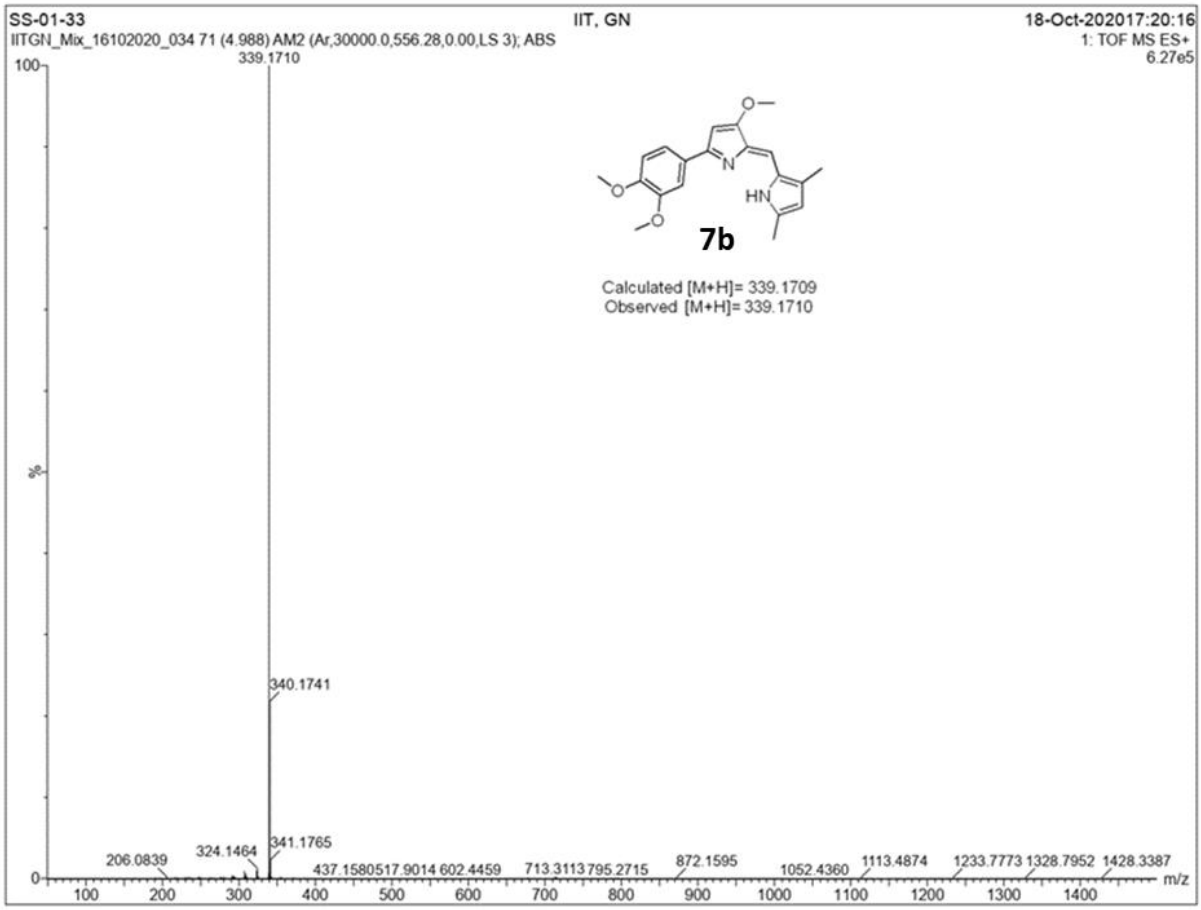


Fig. S7: HR-MS spectra of compound 7b.

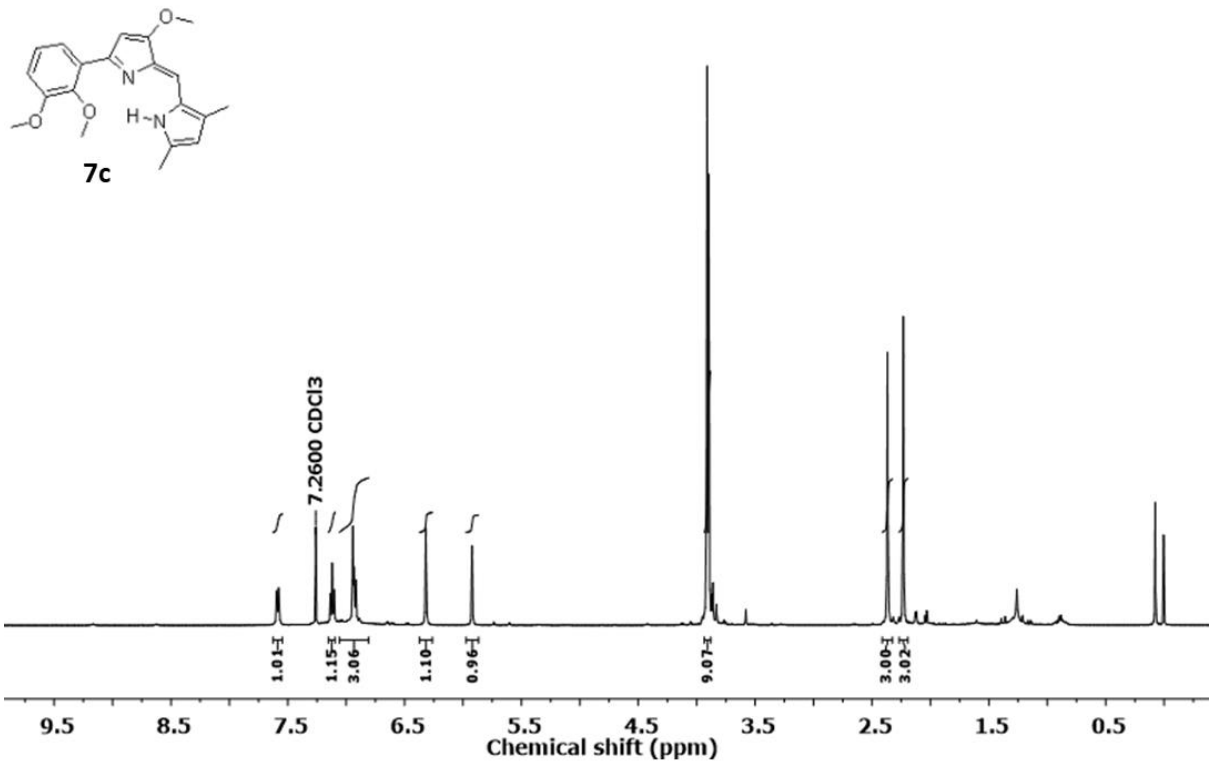


Fig. S8: ^{1}H NMR spectra of compound 7c.

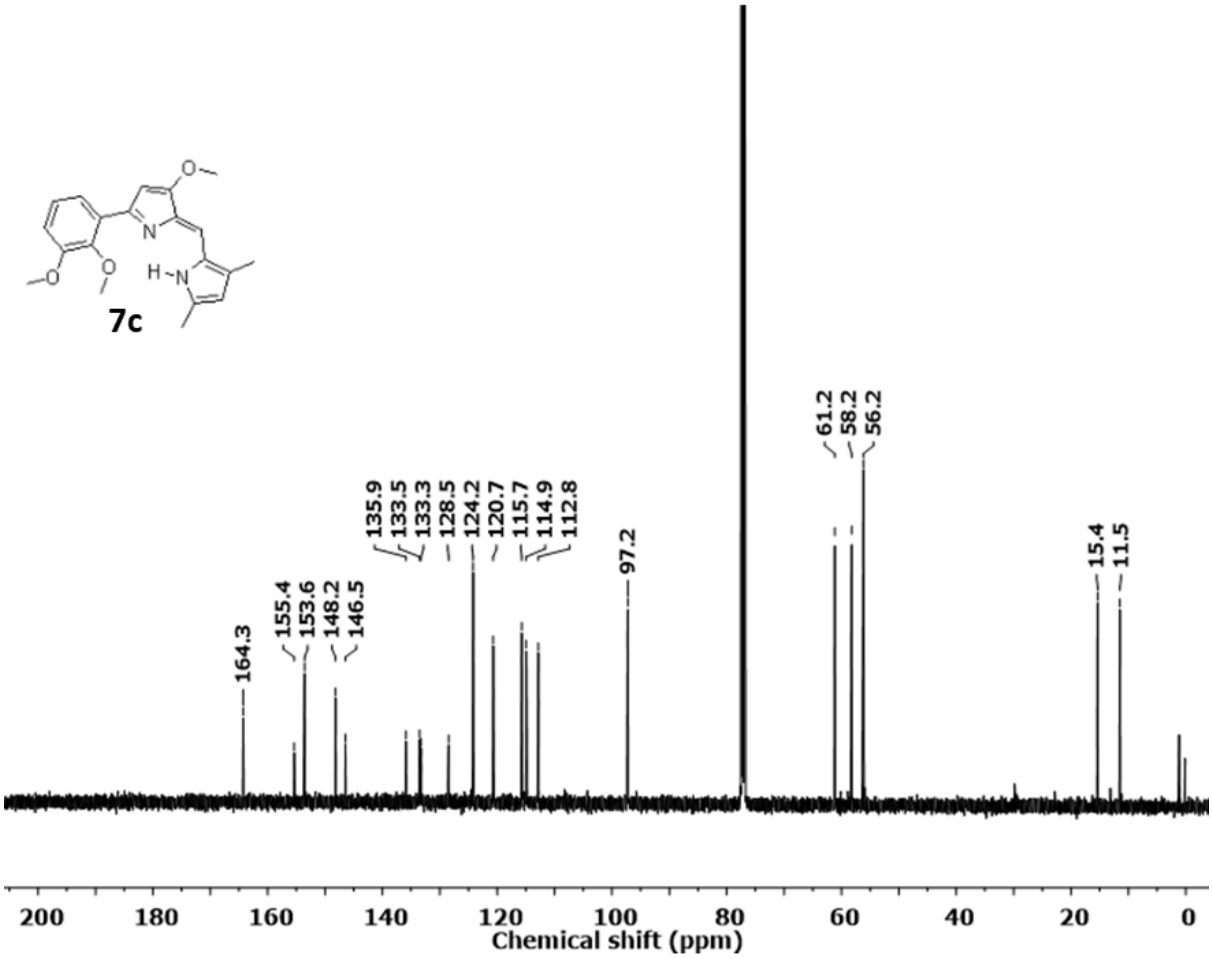


Fig. S9: ^{13}C NMR spectra of compound 7c.

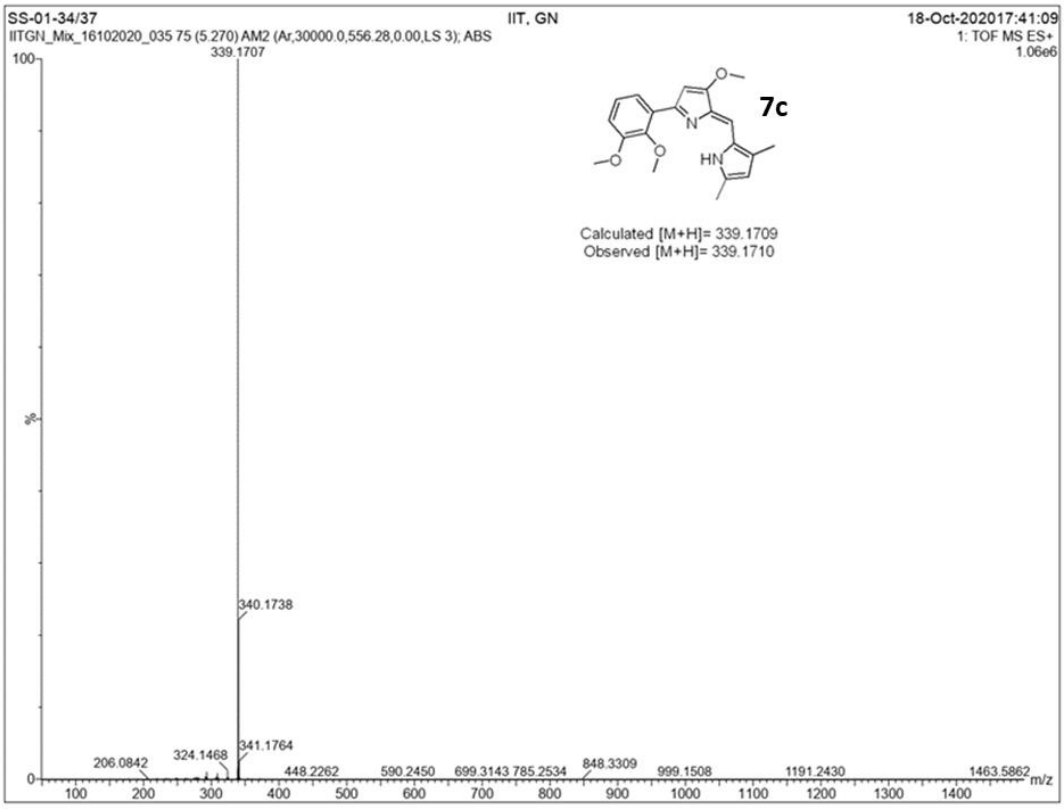


Fig. S10: HR-MS spectra of compound 7c.

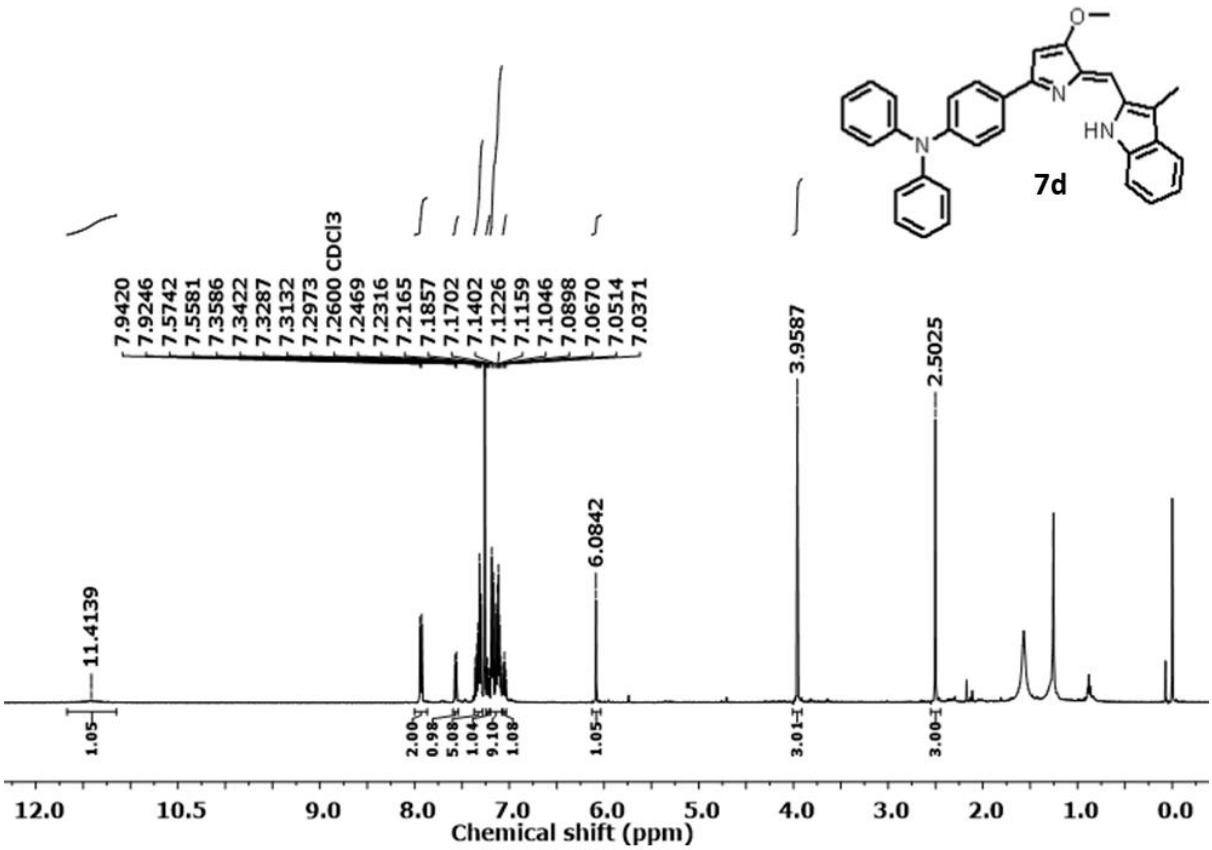


Fig. S11: 1H NMR spectra of compound 7d.

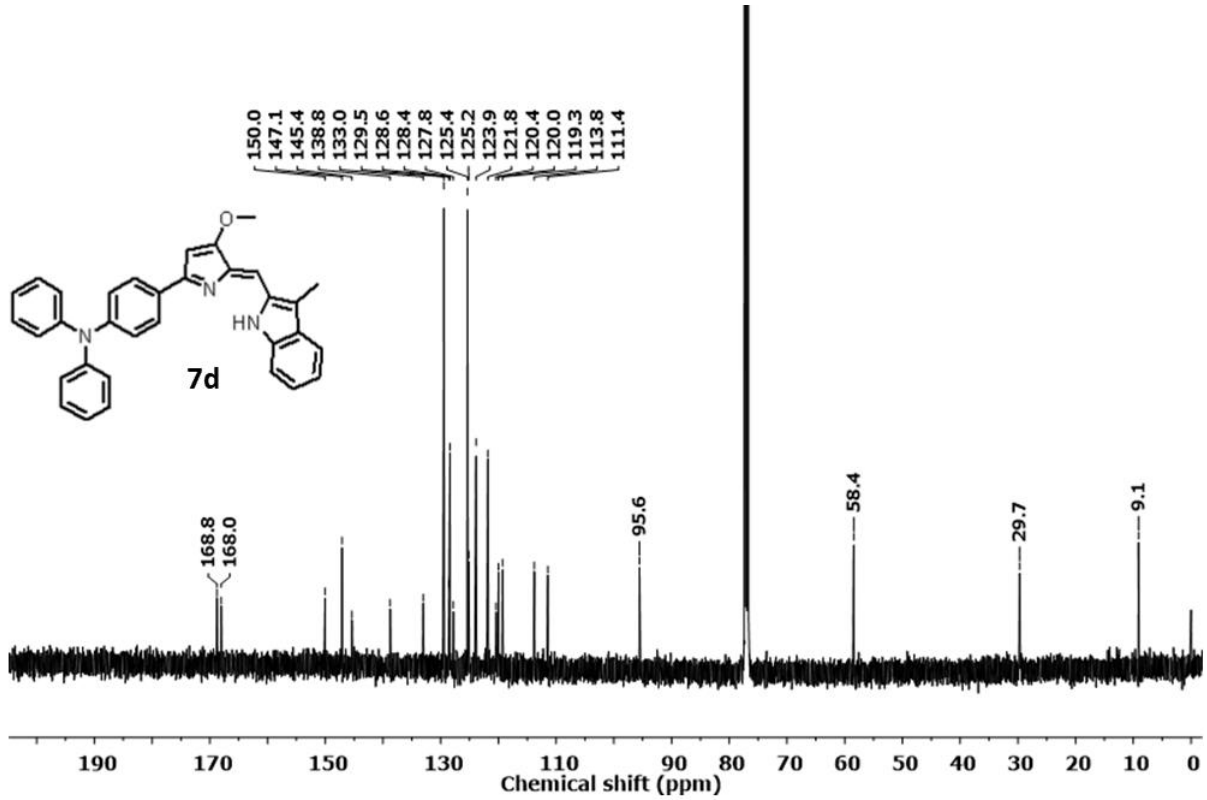


Fig. S12: ^{13}C NMR spectra of compound 7d.

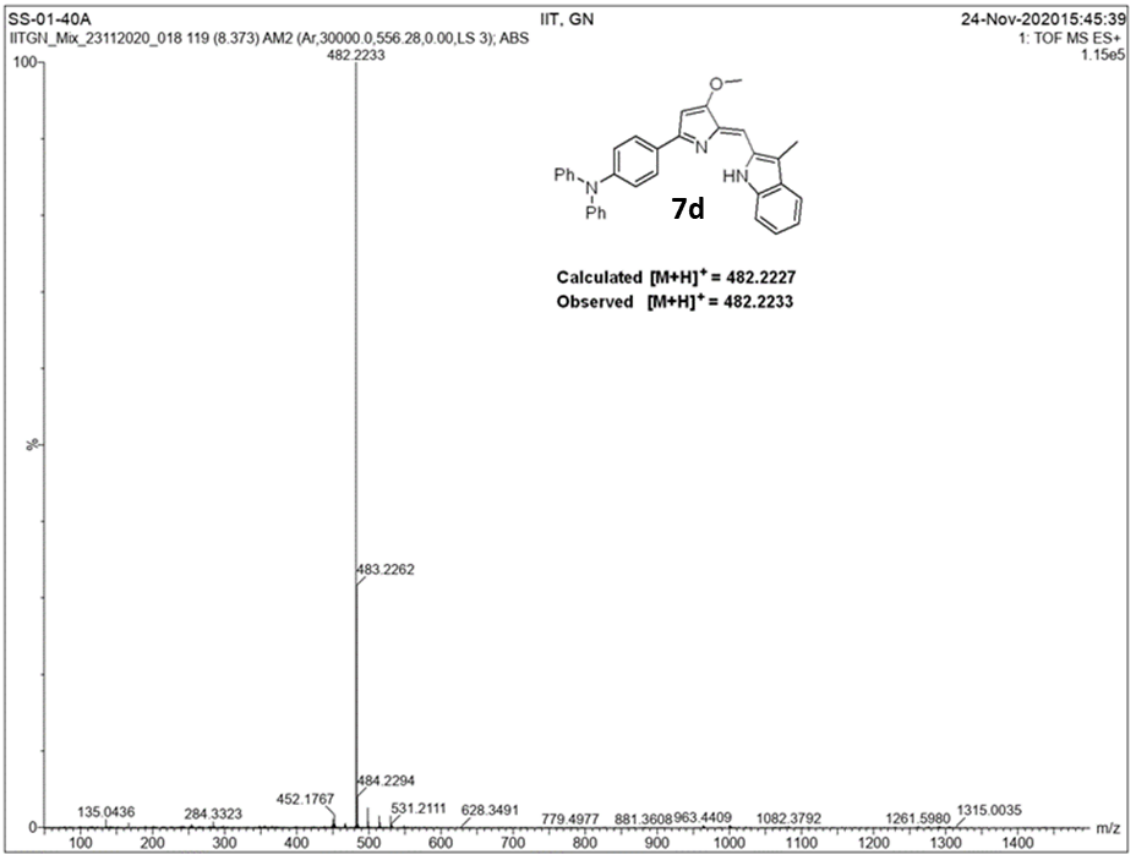


Fig. S13: HR-MS spectra of compound 7d.

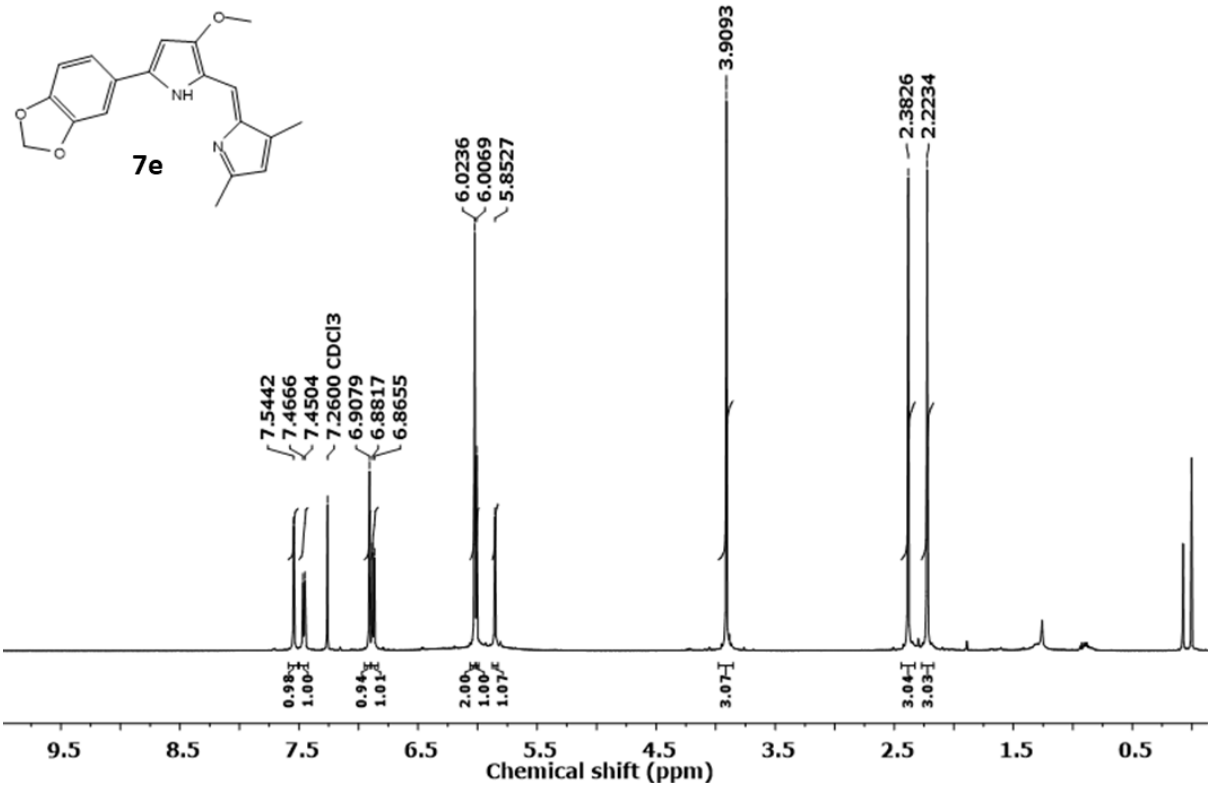


Fig. S14: ^{1}H NMR spectra of compound 7e.

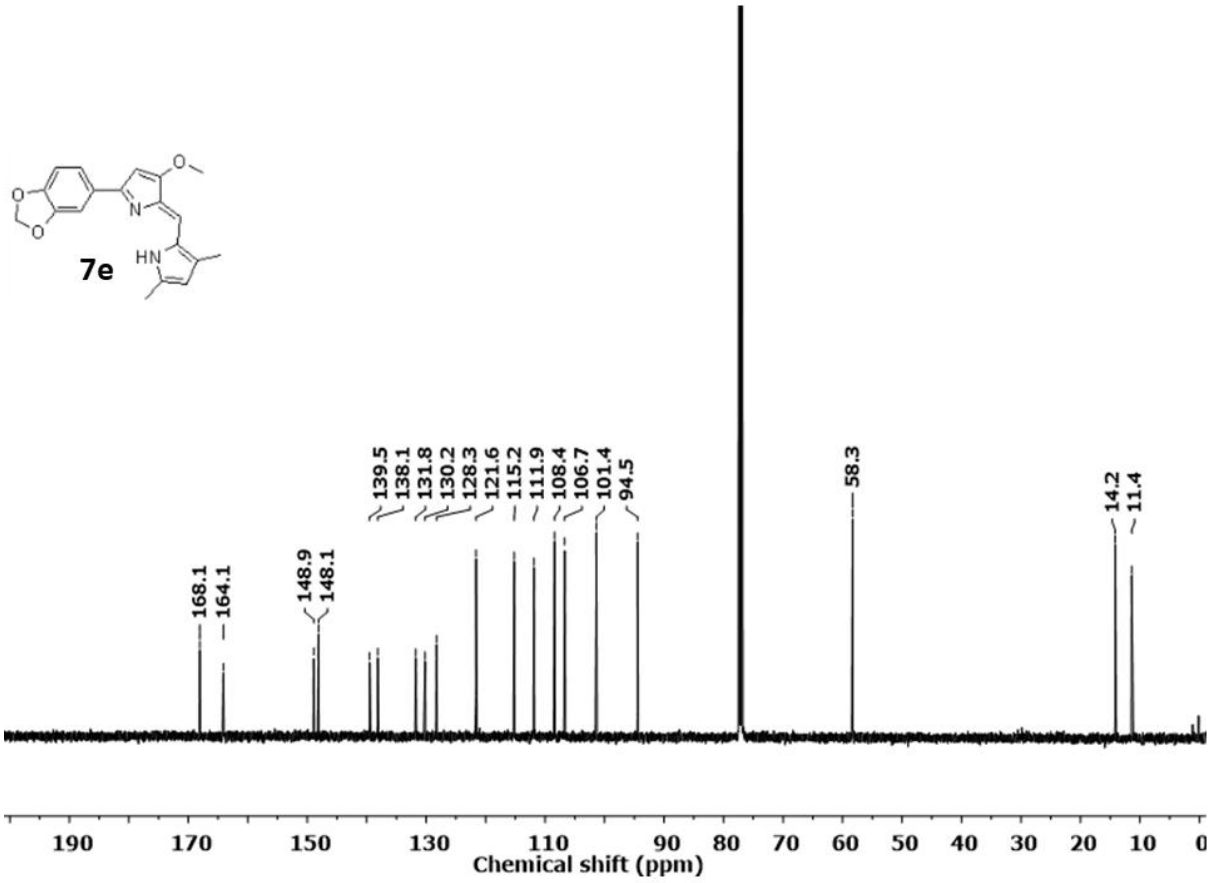


Fig. S15: ^{13}C NMR spectra of compound 7e.

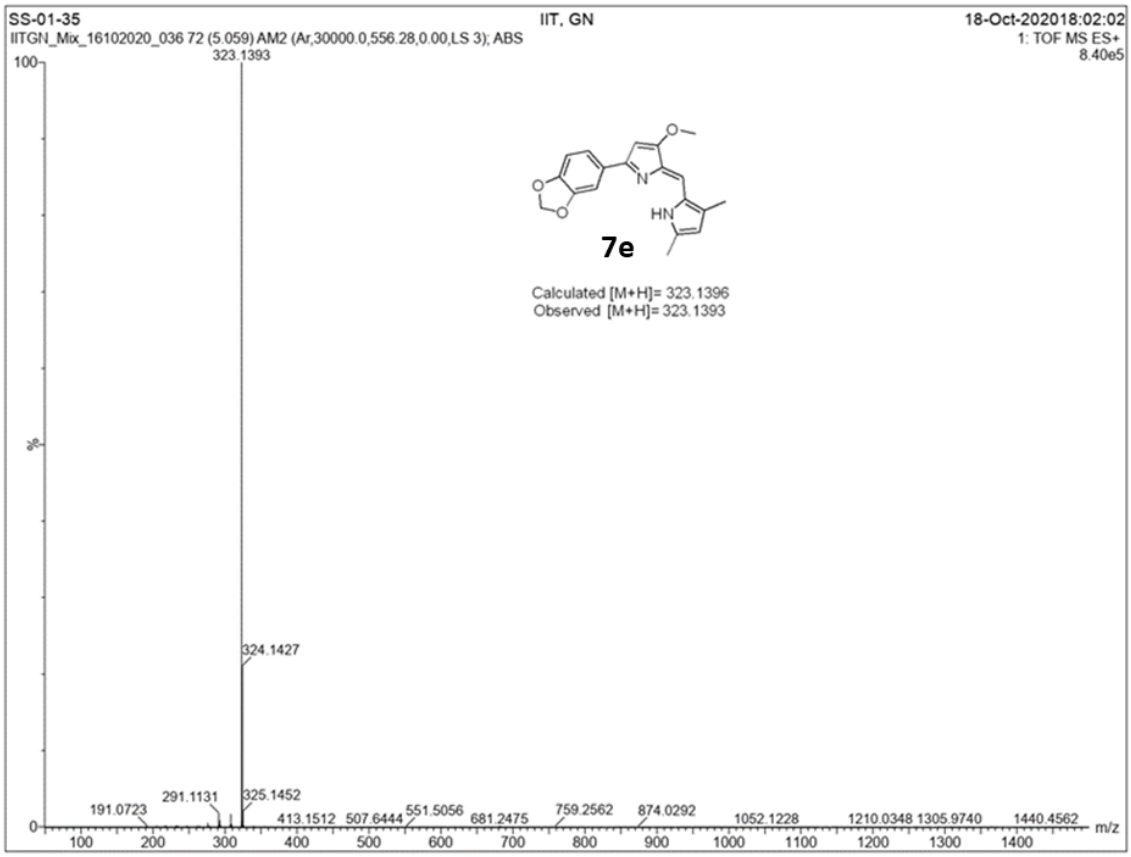


Fig. S16: HR-MS spectra of compound 7e.

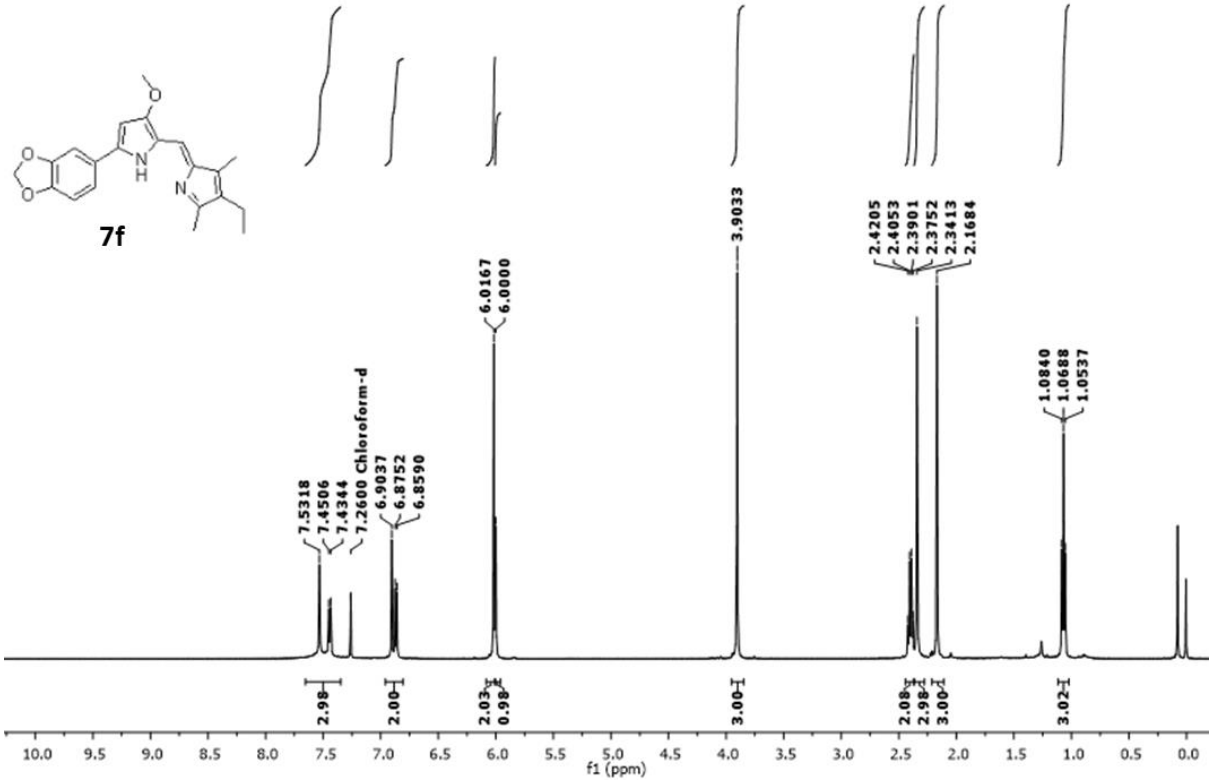


Fig. S17: ^{1}H NMR spectra of compound 7f.

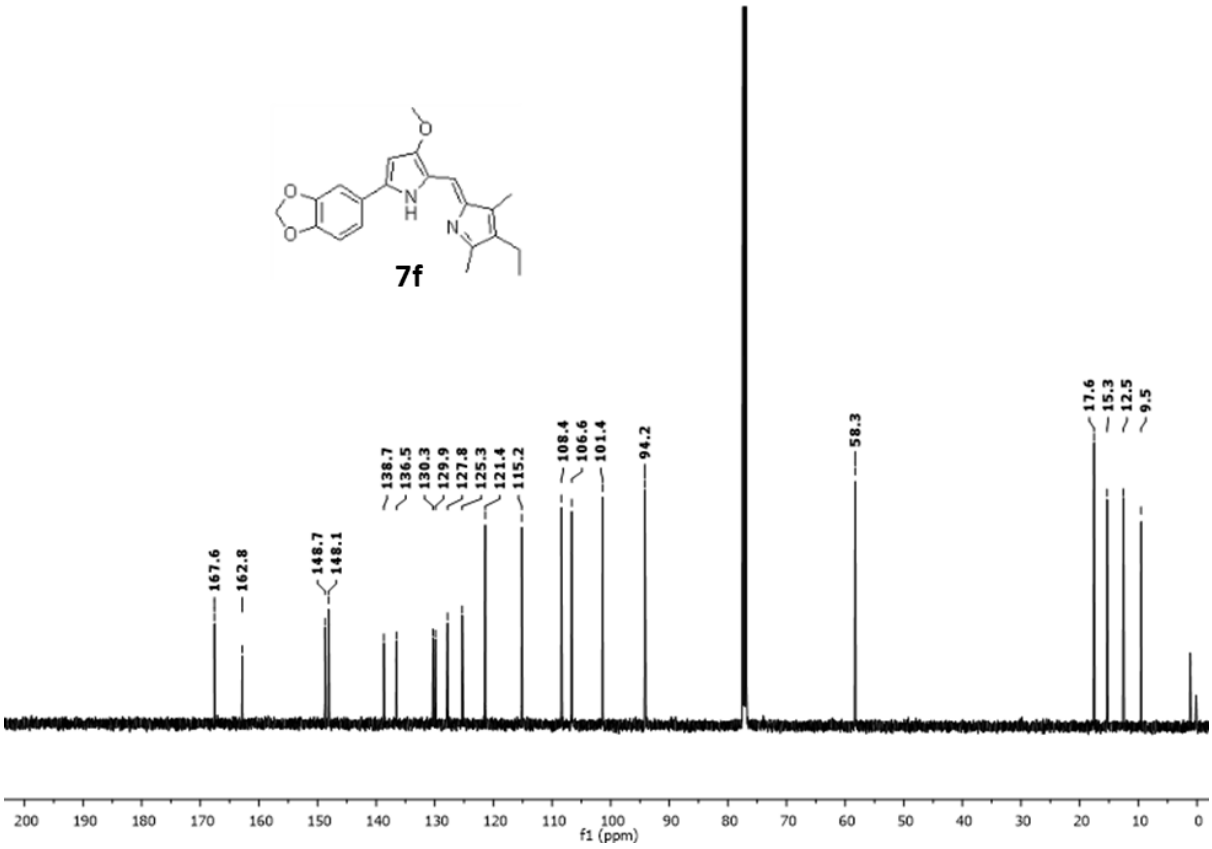


Fig. S18: ^{13}C NMR spectra of compound 7f.

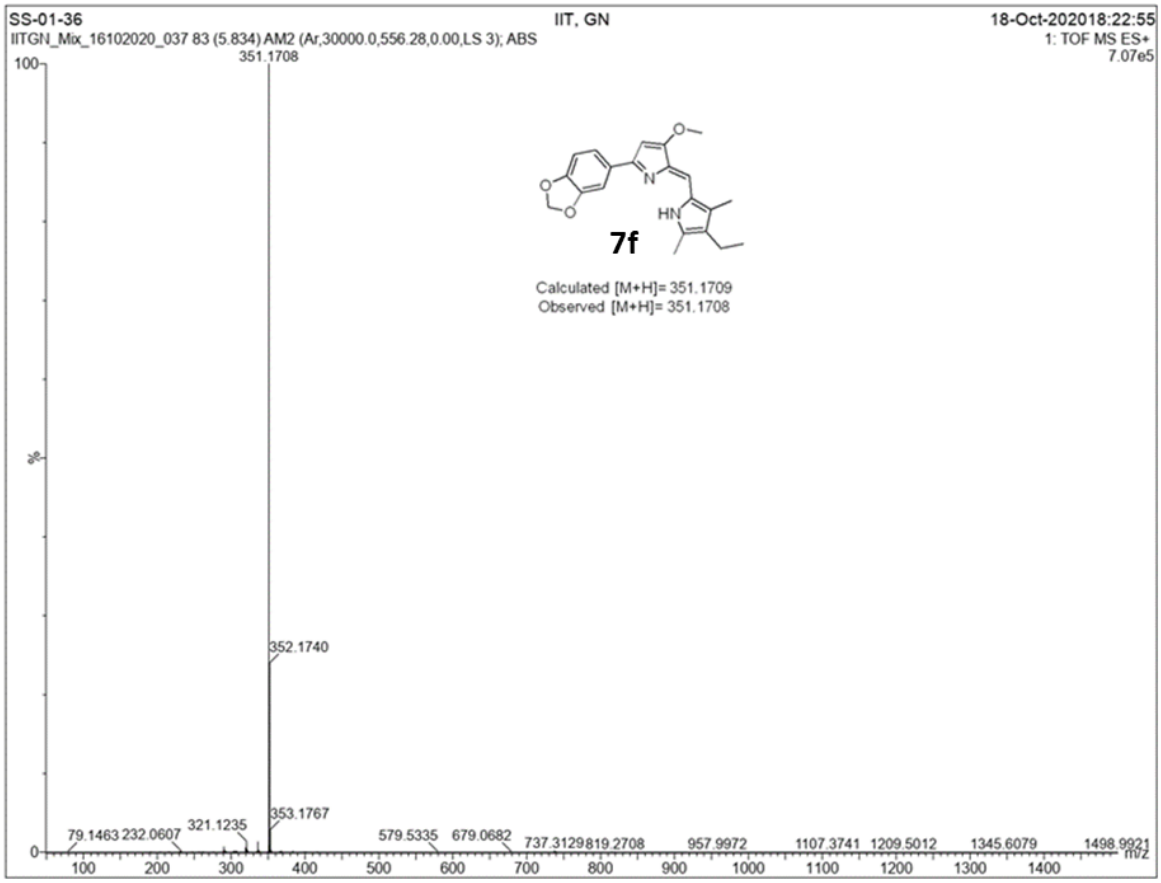


Fig. S19: HR-MS spectra of compound 7f.

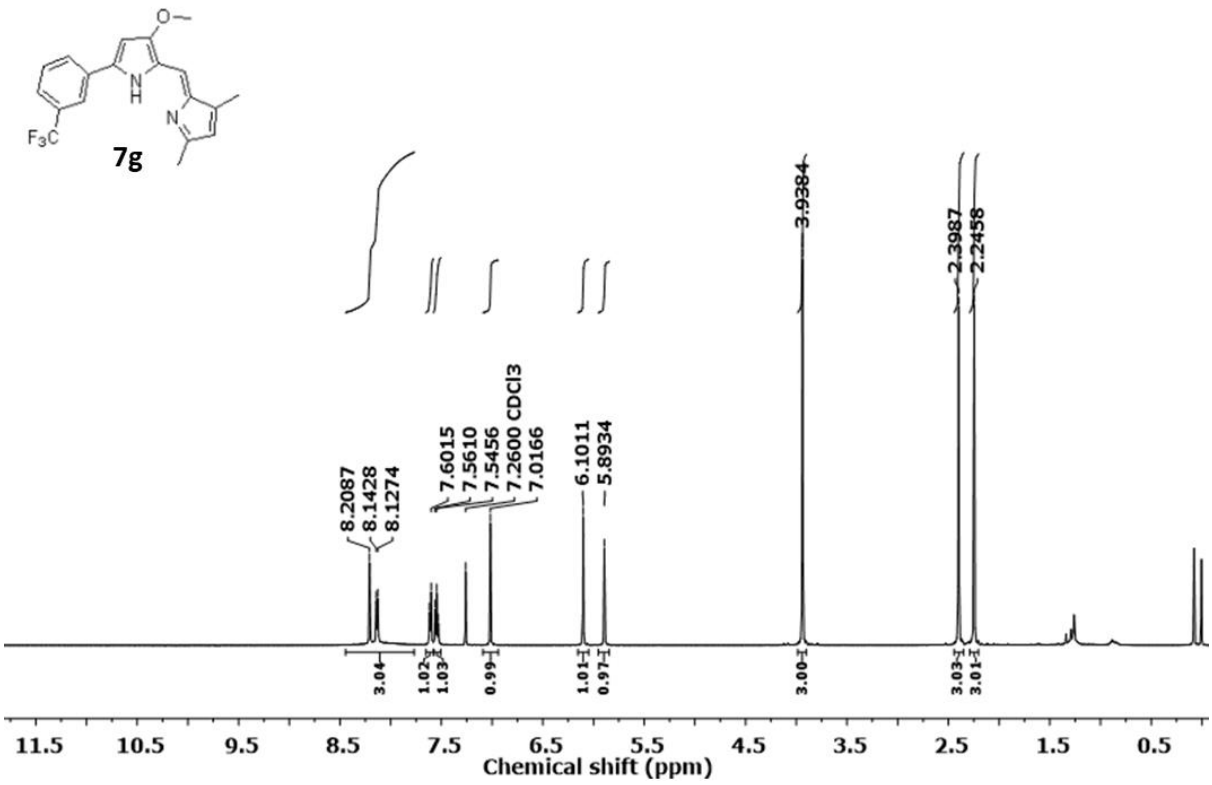


Fig. S20: ^{1}H NMR spectra of compound 7g.

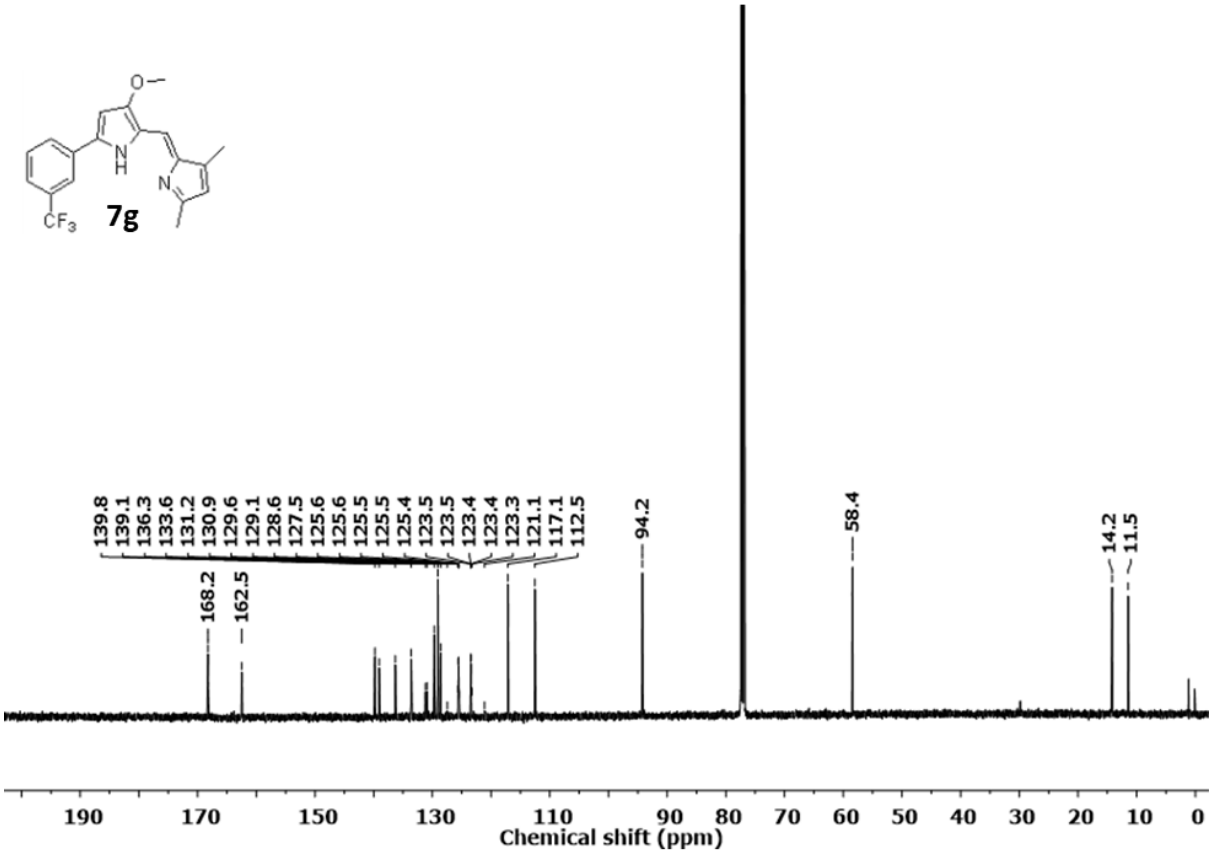


Fig. S21: ^{13}C NMR spectra of compound 7g.

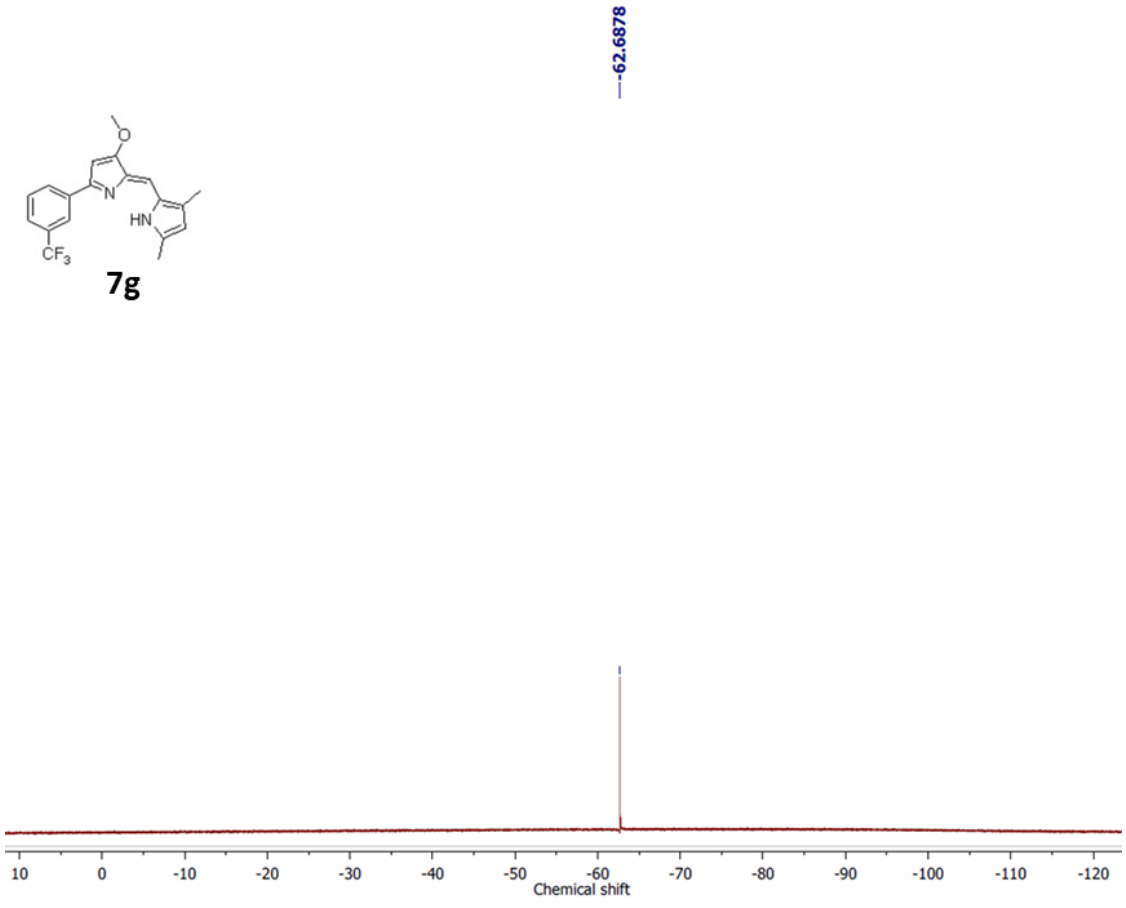


Fig. S22: ^{19}F NMR spectra of compound 7g.

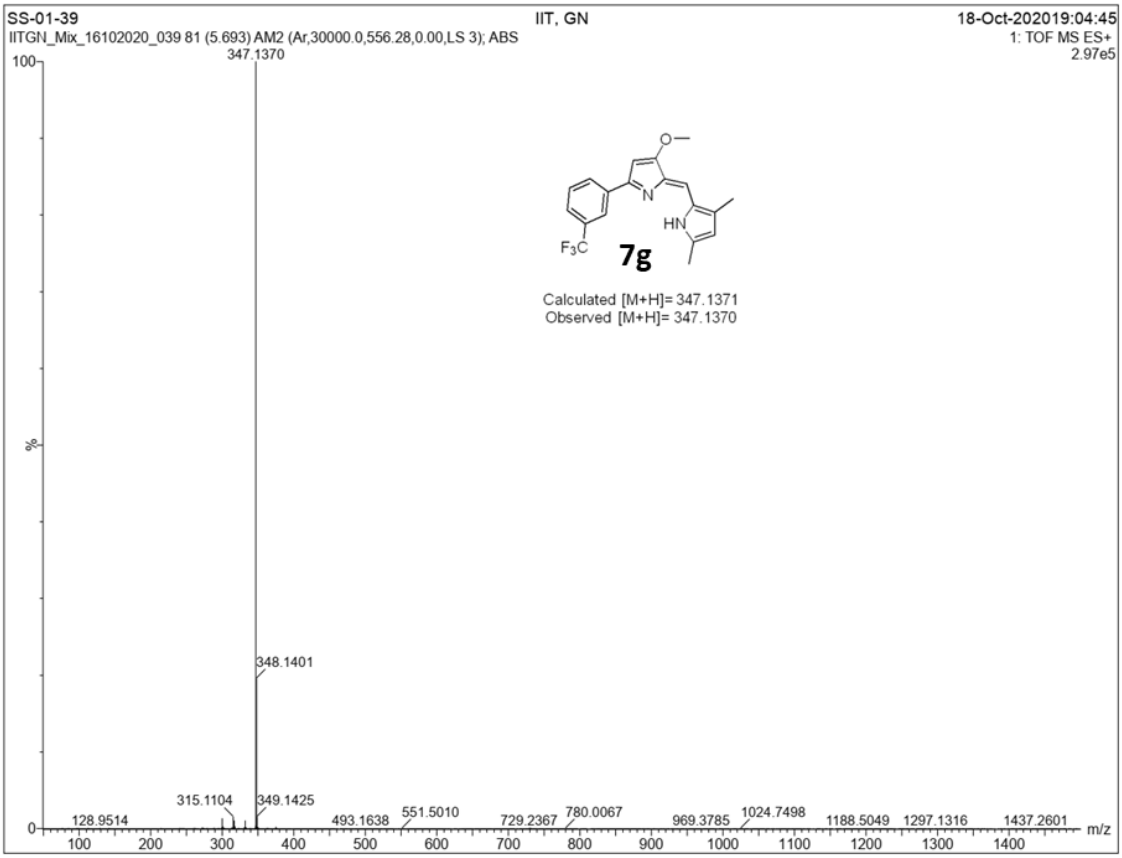


Fig S23: HHR-MS spectra of compound 7g.

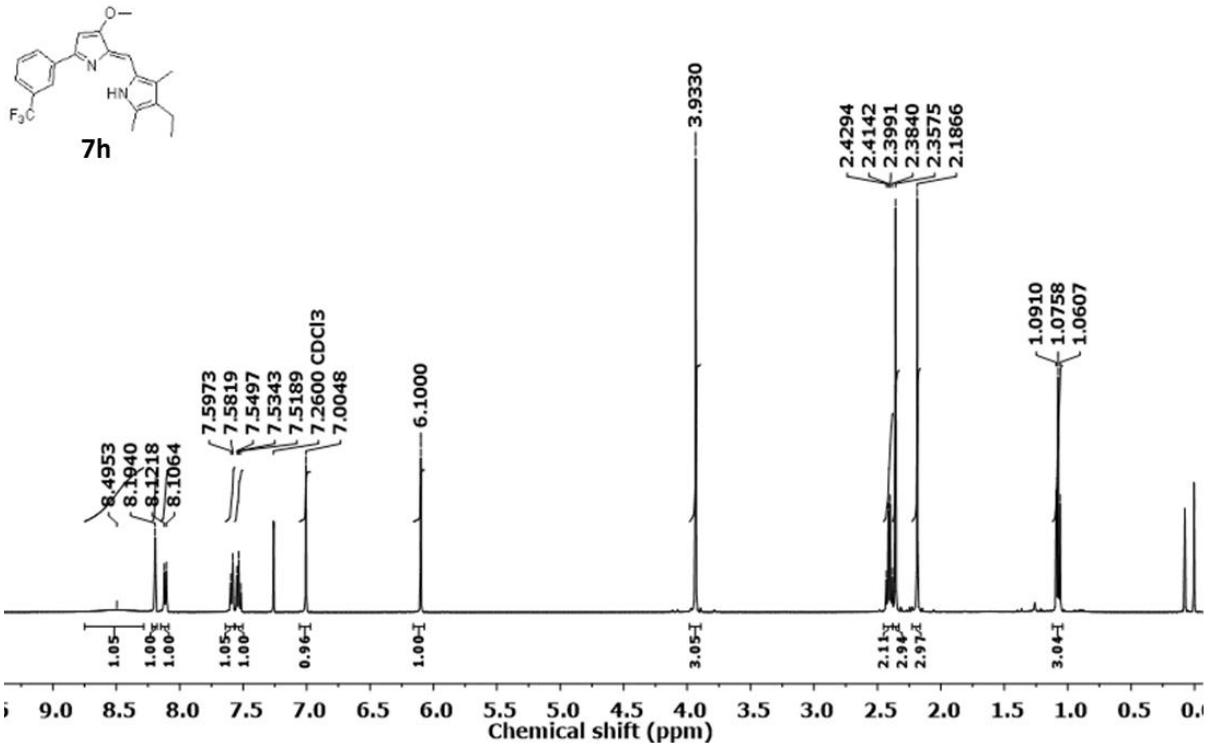


Fig. S24: ^{1}H NMR spectra of compound 7h.

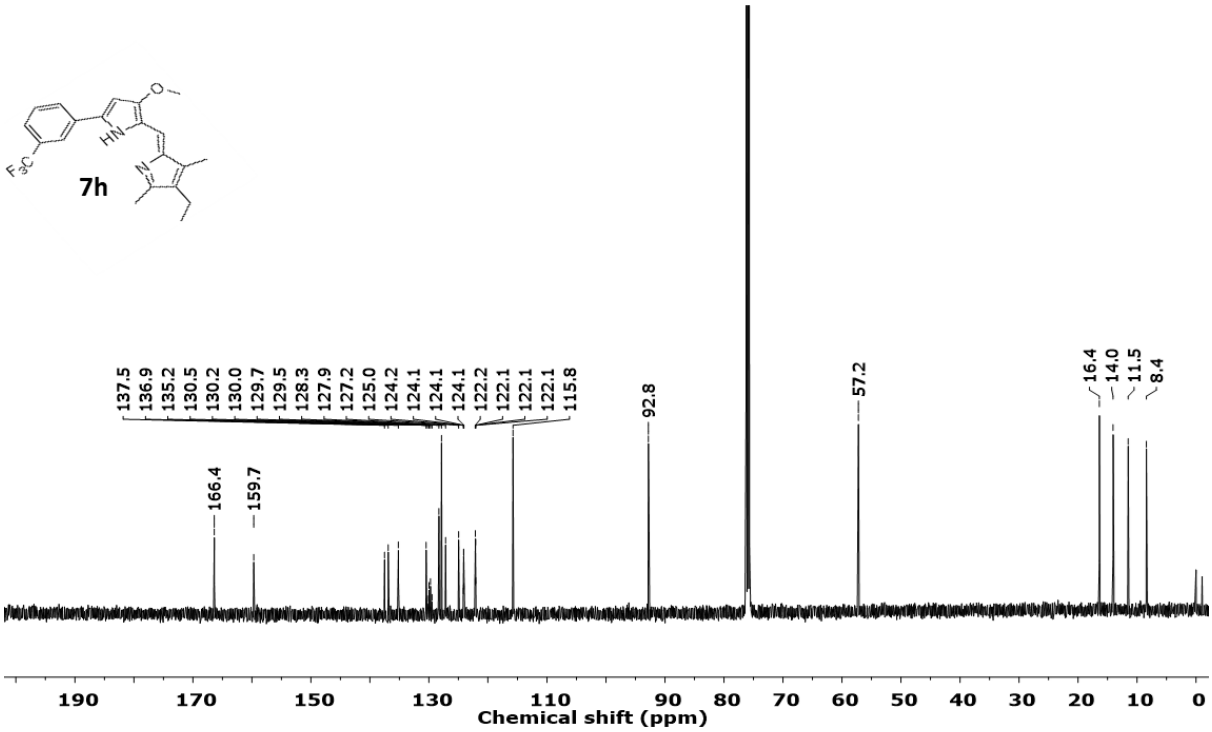


Fig. S25: ^{13}C NMR spectra of compound 7h.

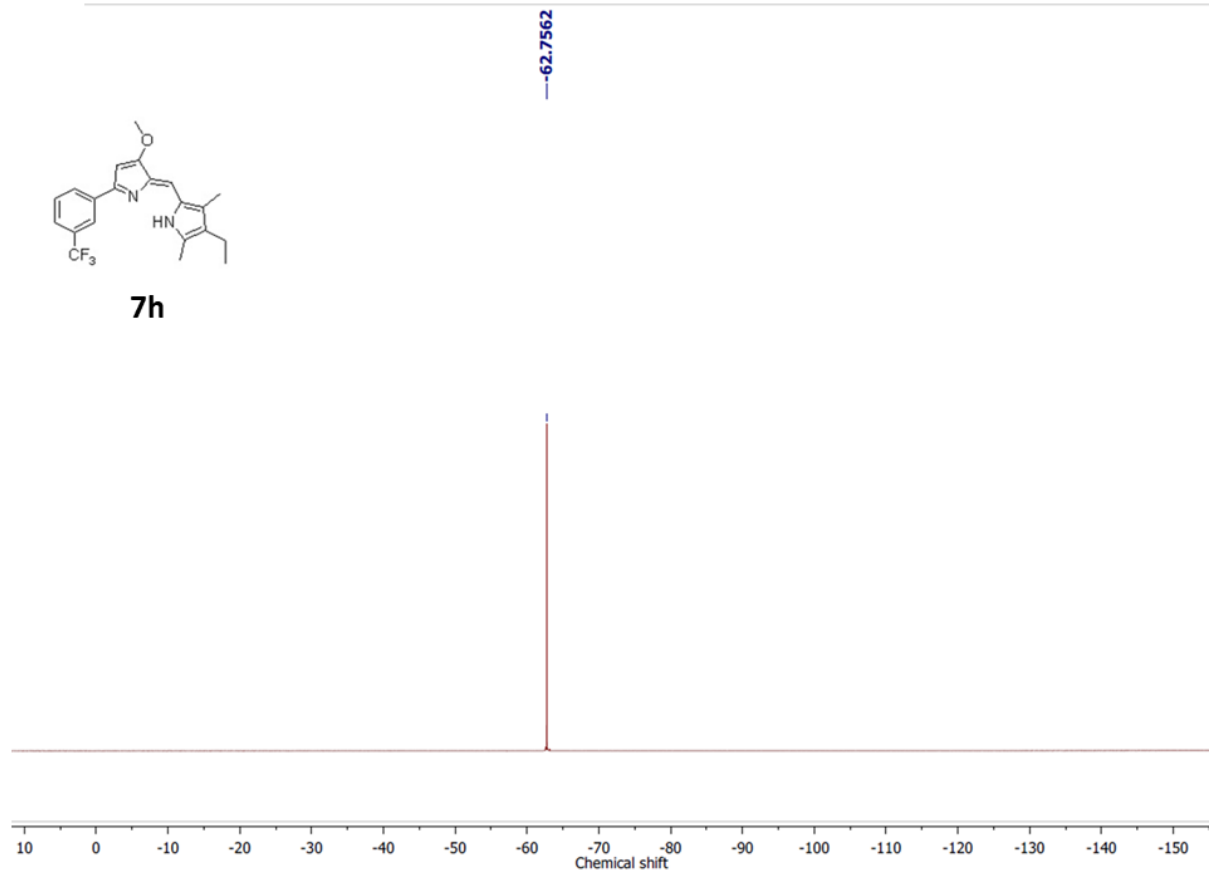


Fig. S26: ^{19}F NMR spectra of compound 7h.

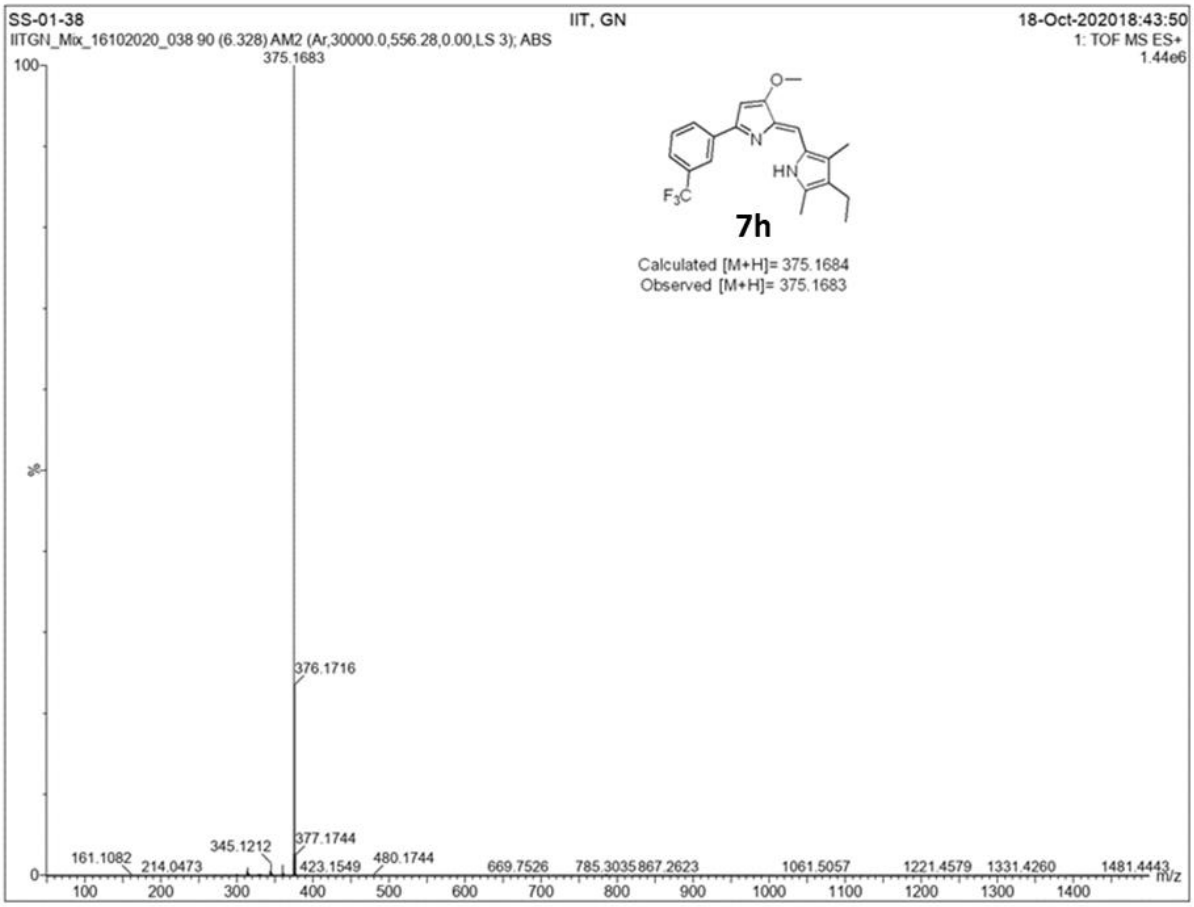


Fig. S27: HR-MS spectra of compound 7h.

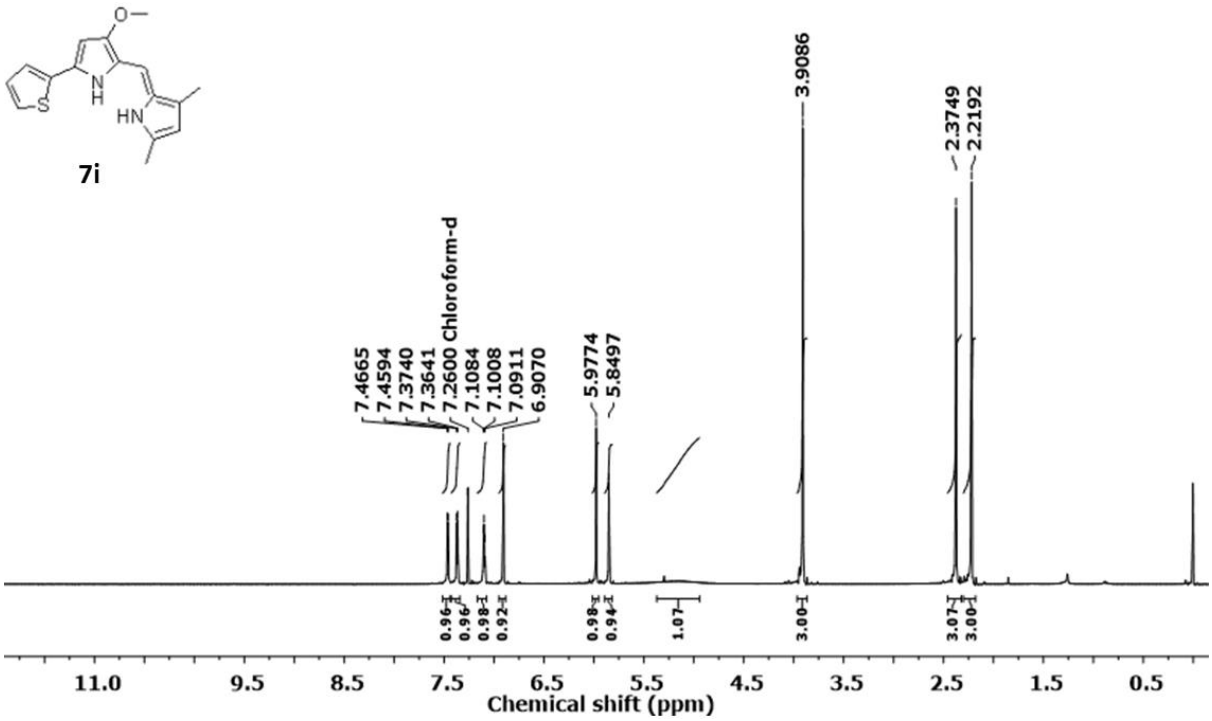


Fig. S28: ^{1}H NMR spectra of compound 7i.

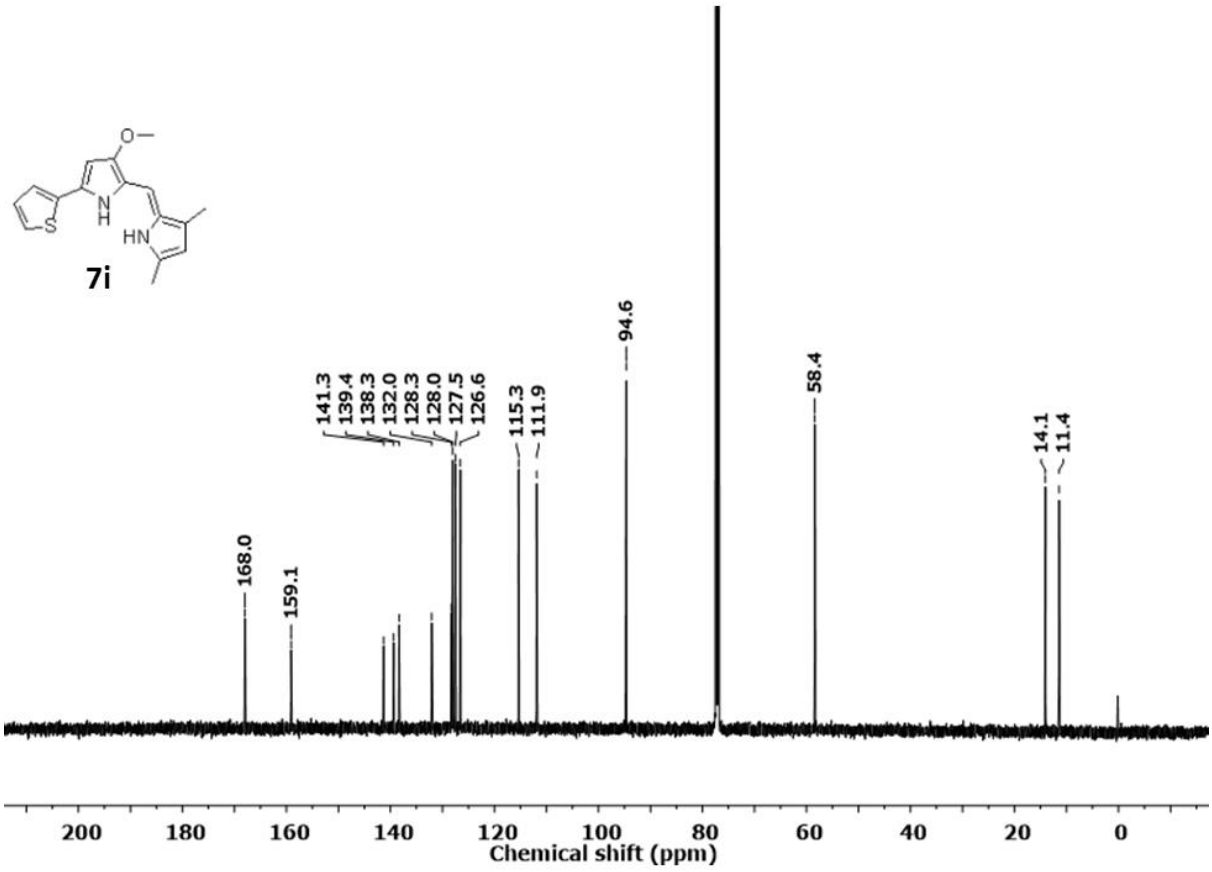


Fig. S29: ^{13}C NMR spectra of compound 7i.

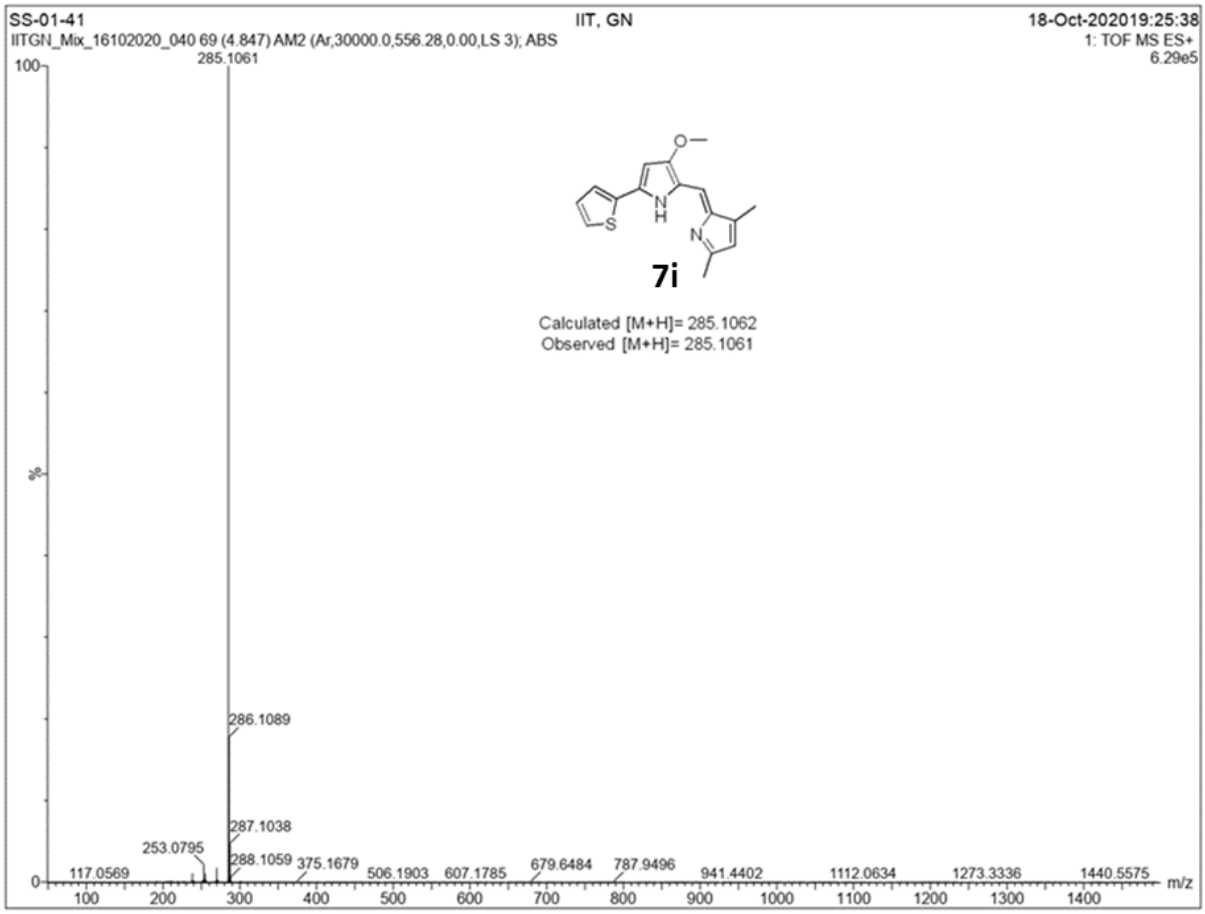


Fig. S30: HR-MS spectra of compound 7i.

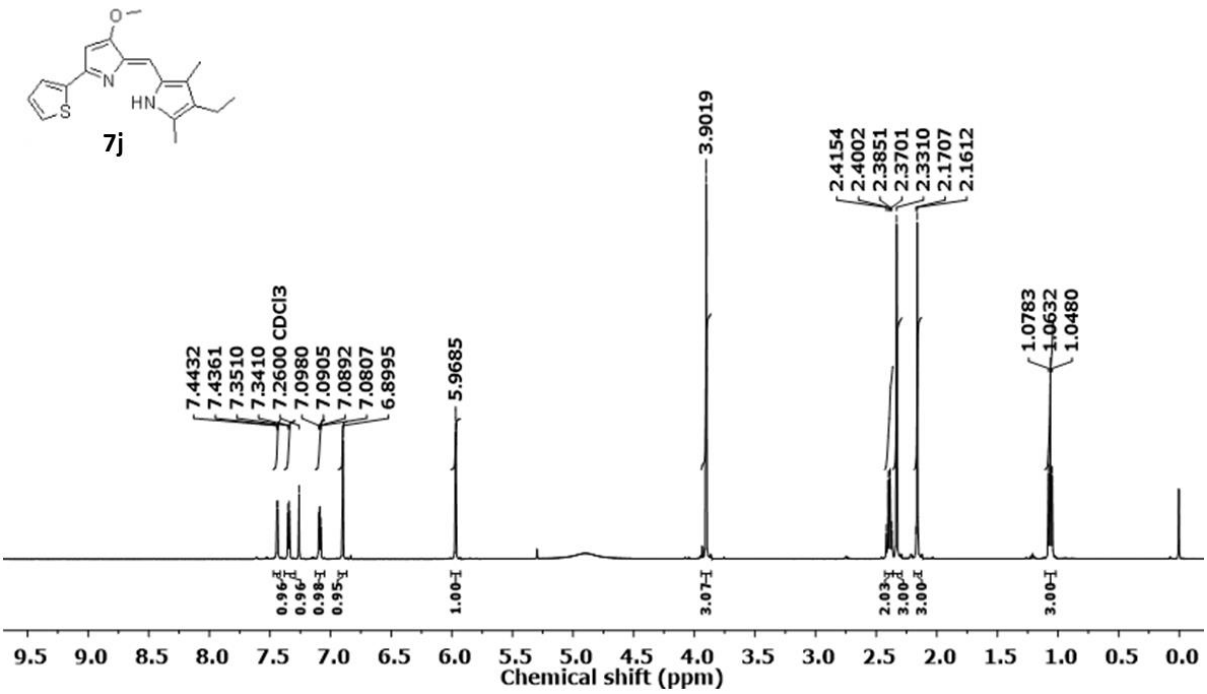


Fig. S31: ^{1}H NMR spectra of compound 7j.

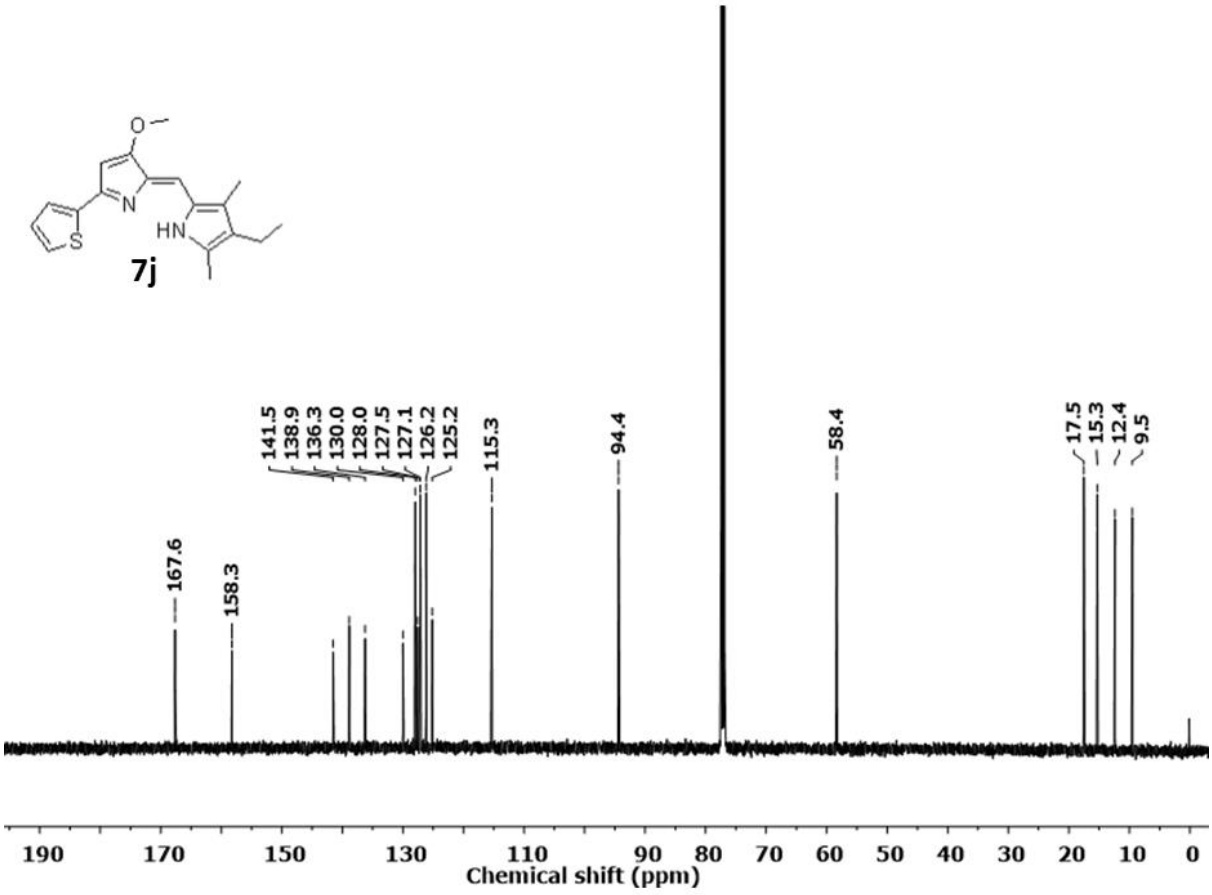


Fig. S32: ^{13}C NMR spectra of compound 7j.

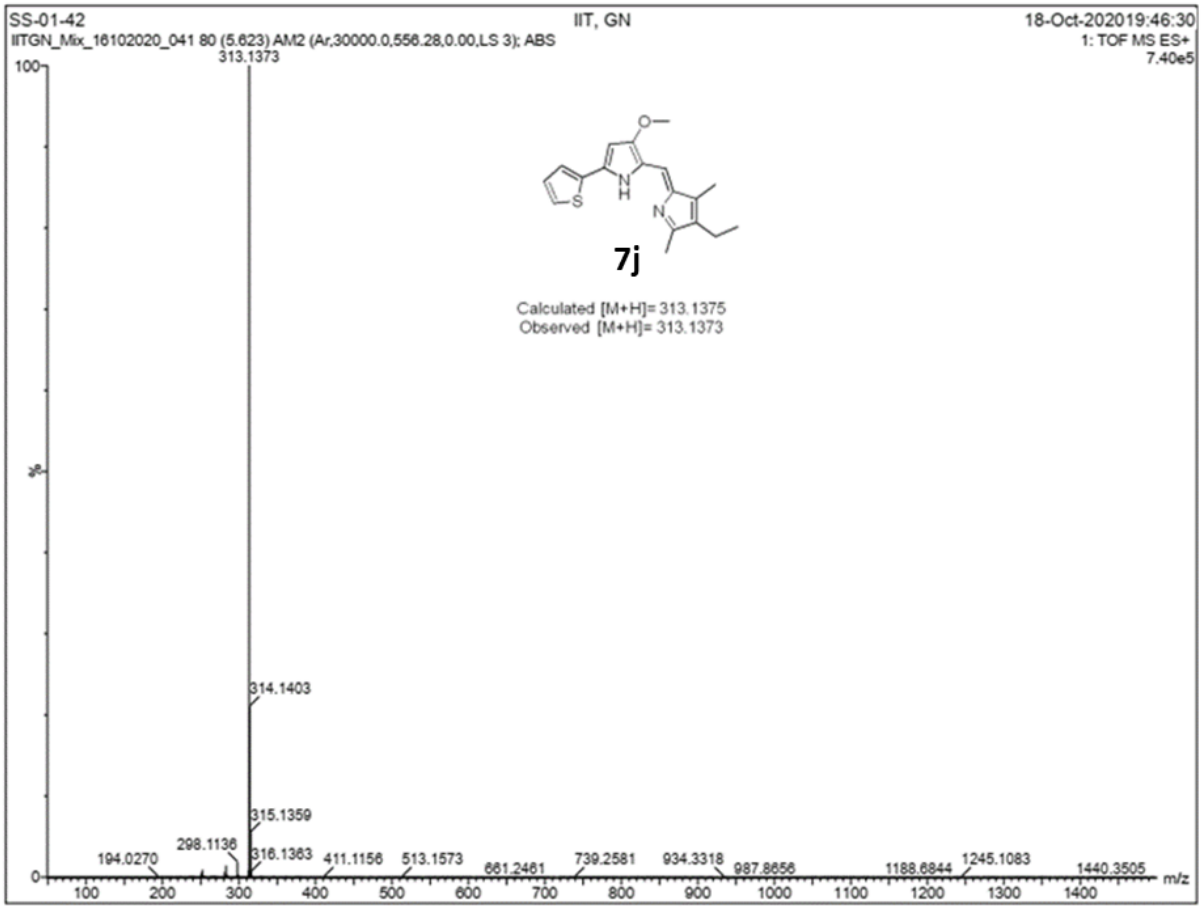


Fig. S33: HR-MS spectra of compound 7j.

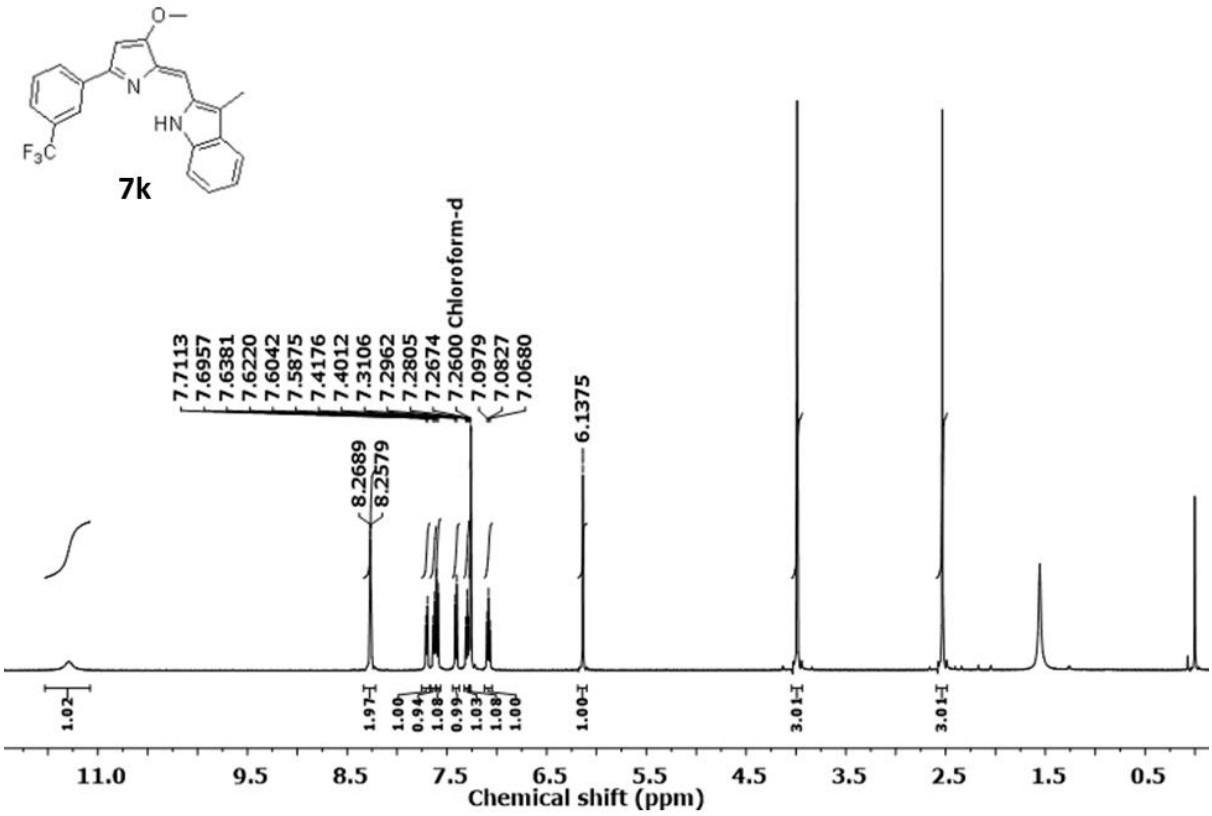


Fig. S34: ^{1}H NMR spectra of compound 7k.

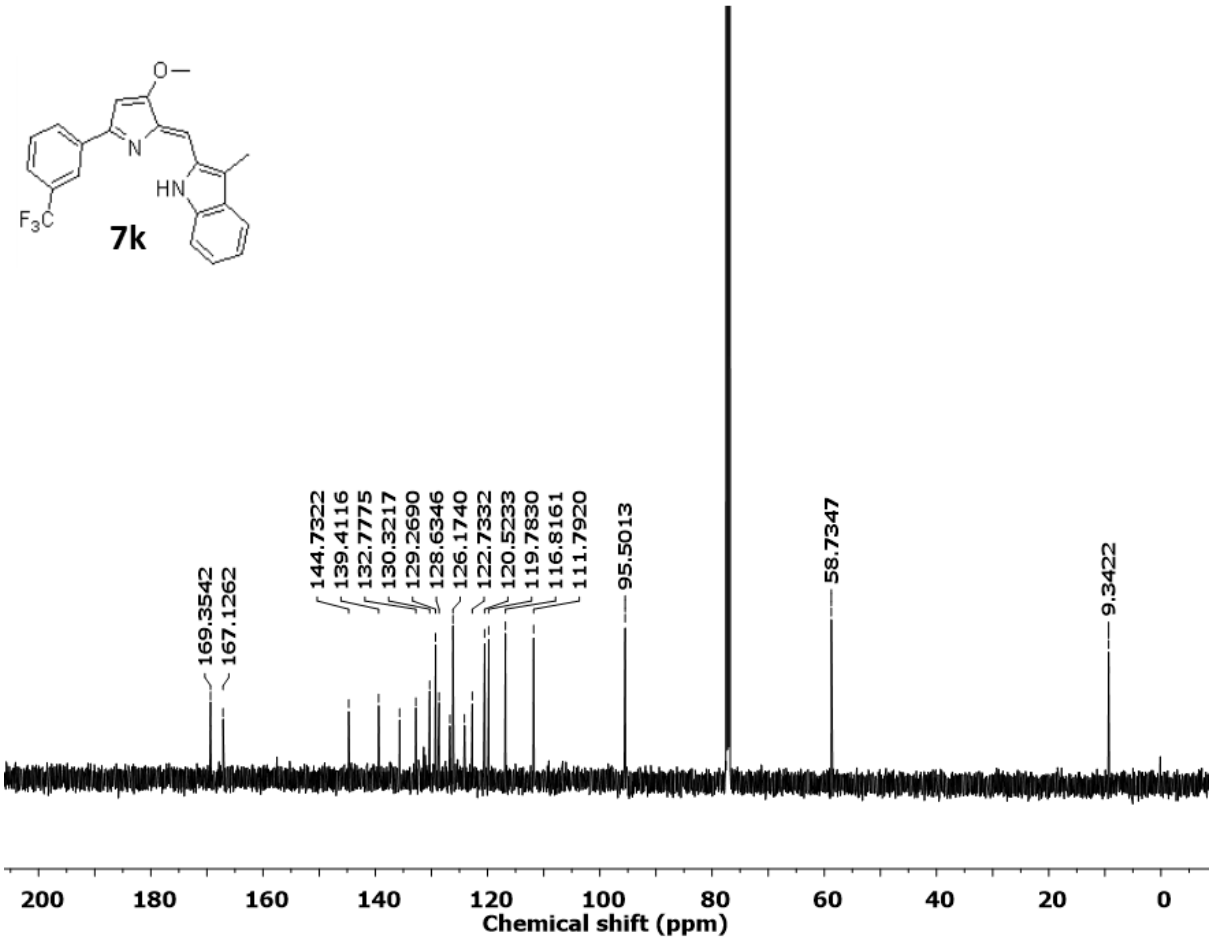


Fig. S35: ^{13}C NMR spectra of compound 7k.

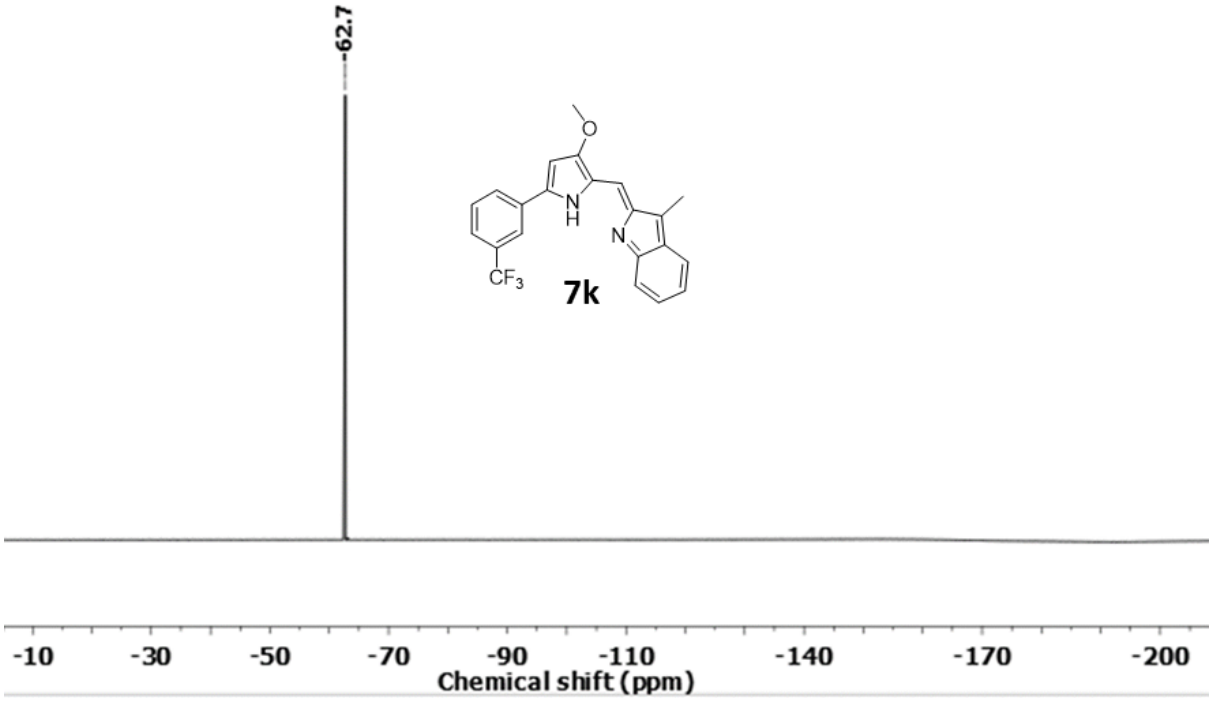


Fig. S36: ^{19}F NMR spectra of compound 7k.

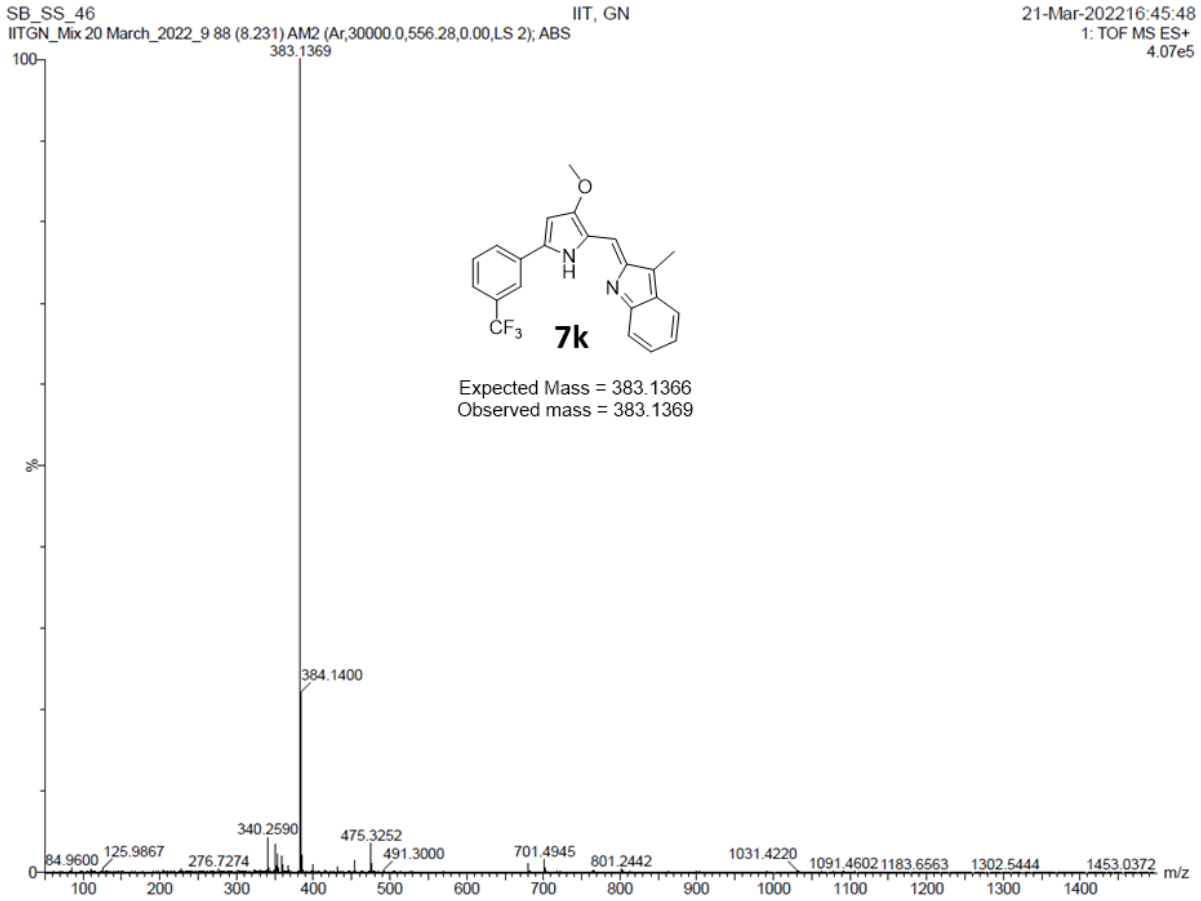


Fig. S37: HR-MS spectra of compound 7k.

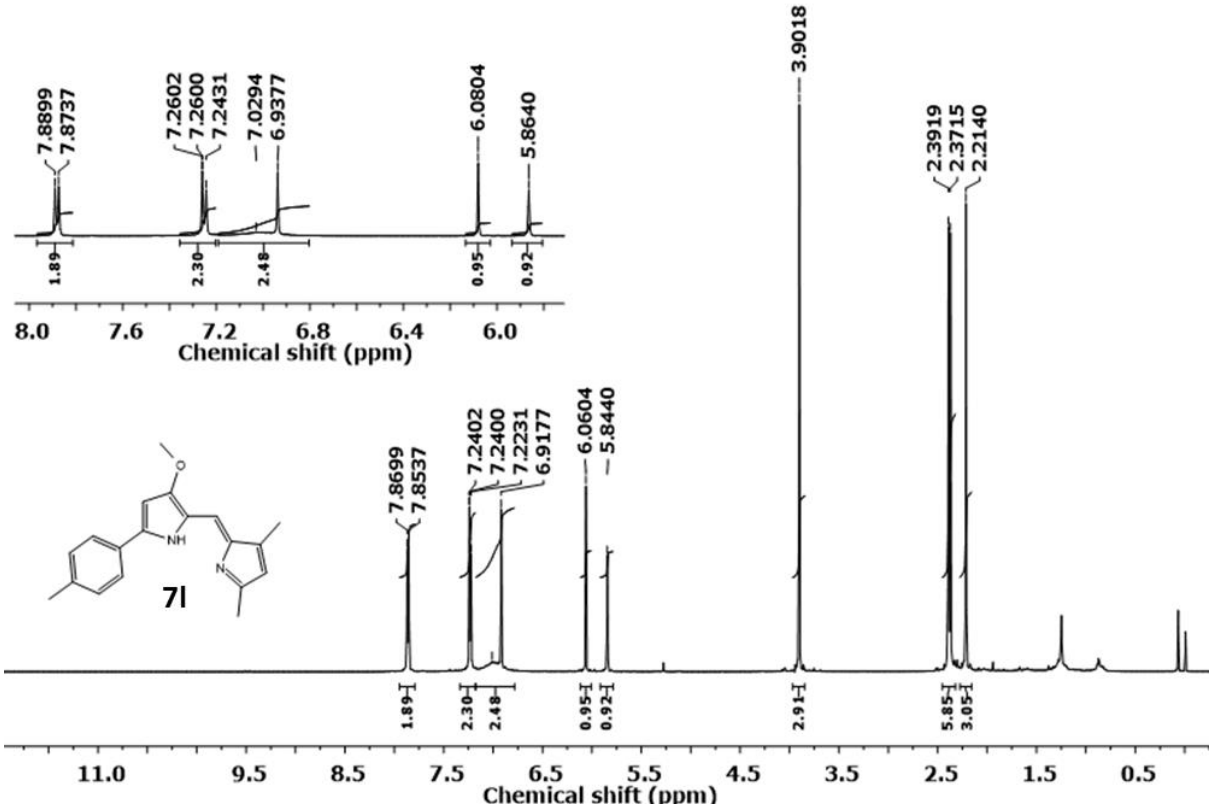


Fig. S38: ^{1}H NMR spectra of compound 7l.

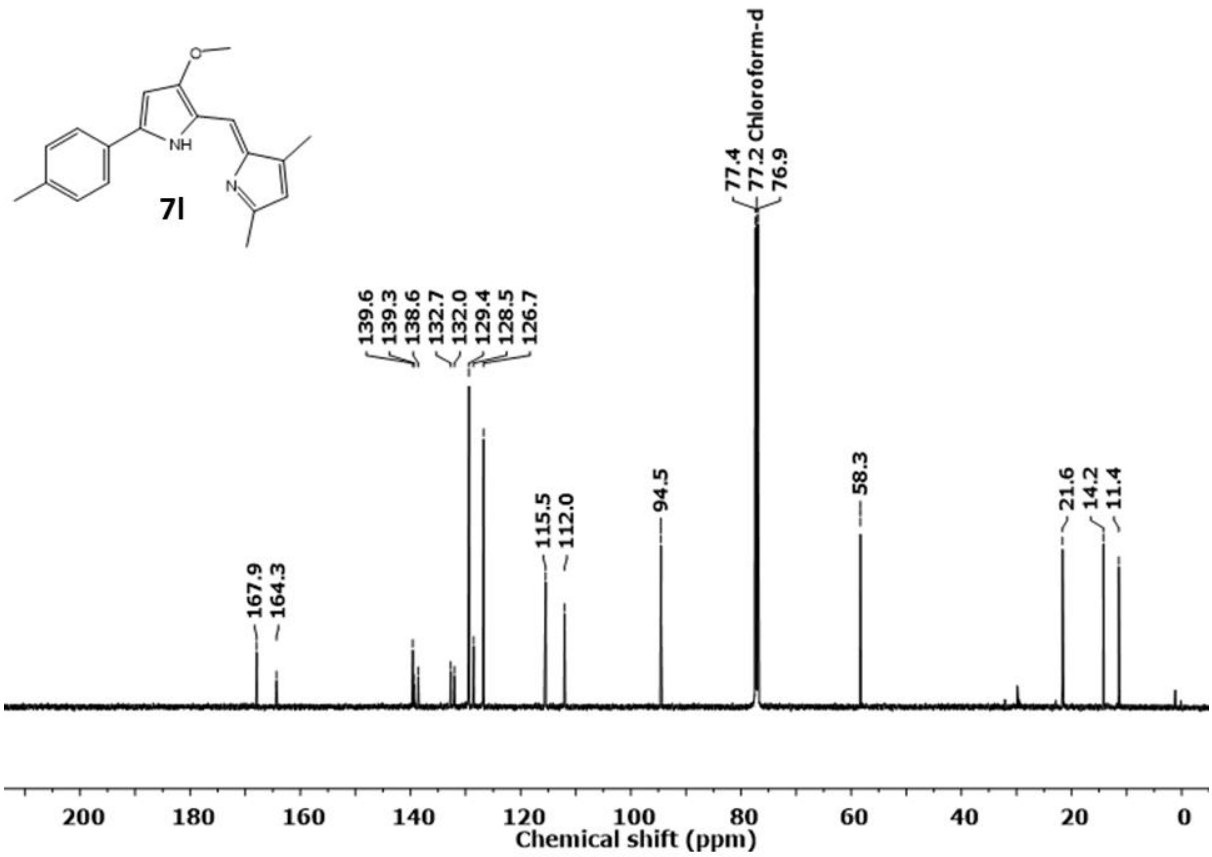


Fig. S39: ^{13}C NMR spectra of compound 7l.

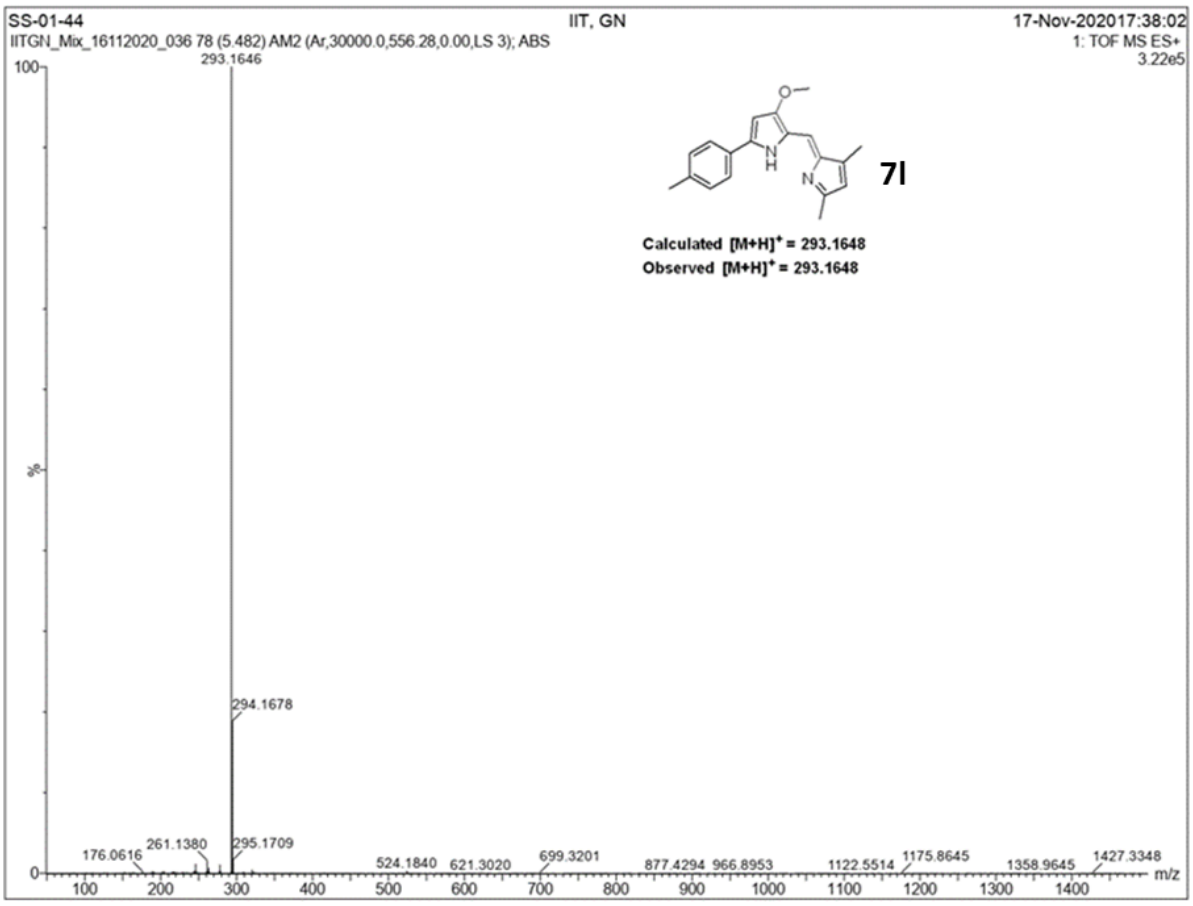


Fig. S40: HR-MS spectra of compound 7l.

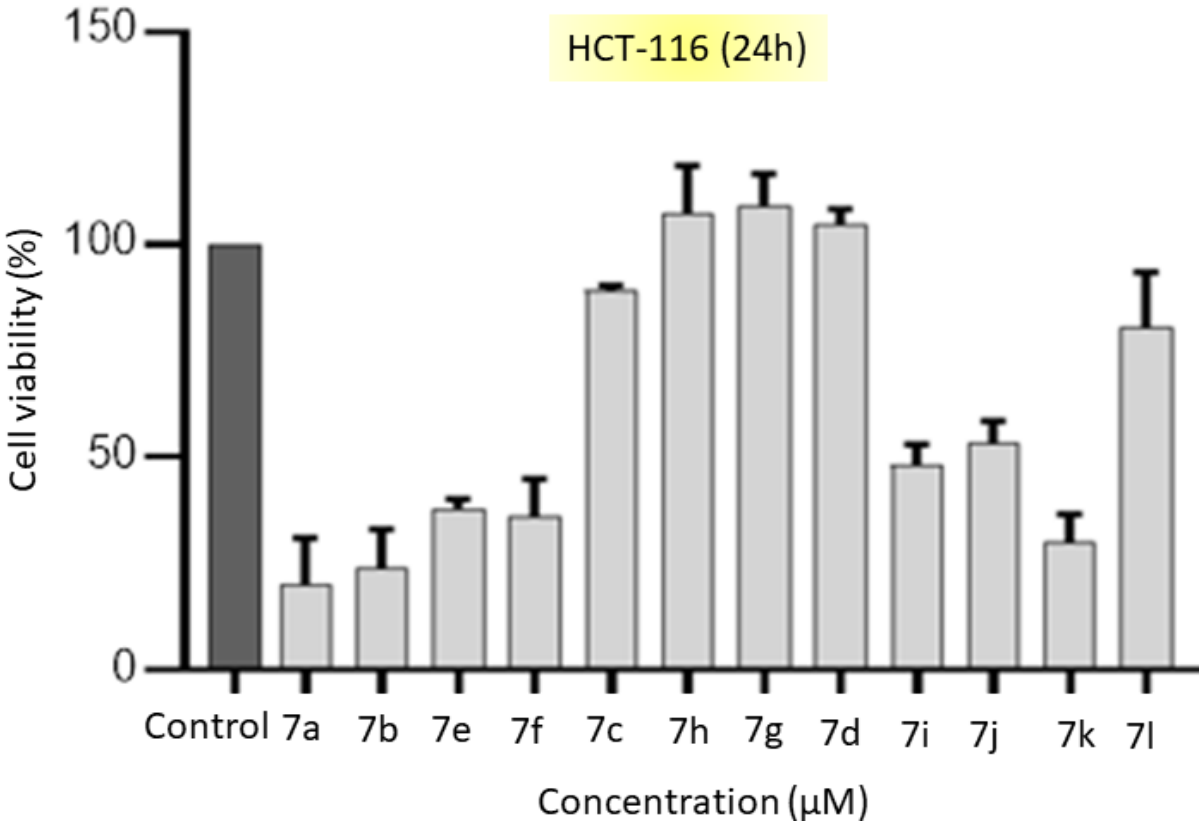


Fig. S41: Viability of HCT-116 cells determined by the MTT assay after incubating with the library members at 10µM concentration for 24h.

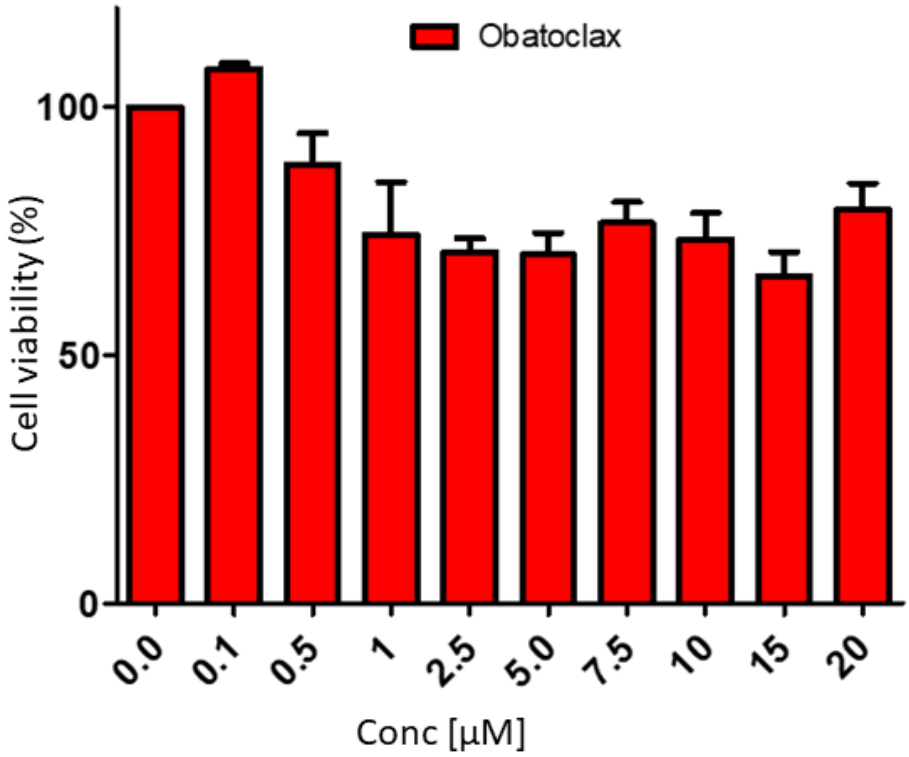


Fig. S42: Viability of HCT-116 cells determined by MTT assay after incubating with obatoclax in a dose dependent manner for 24h.

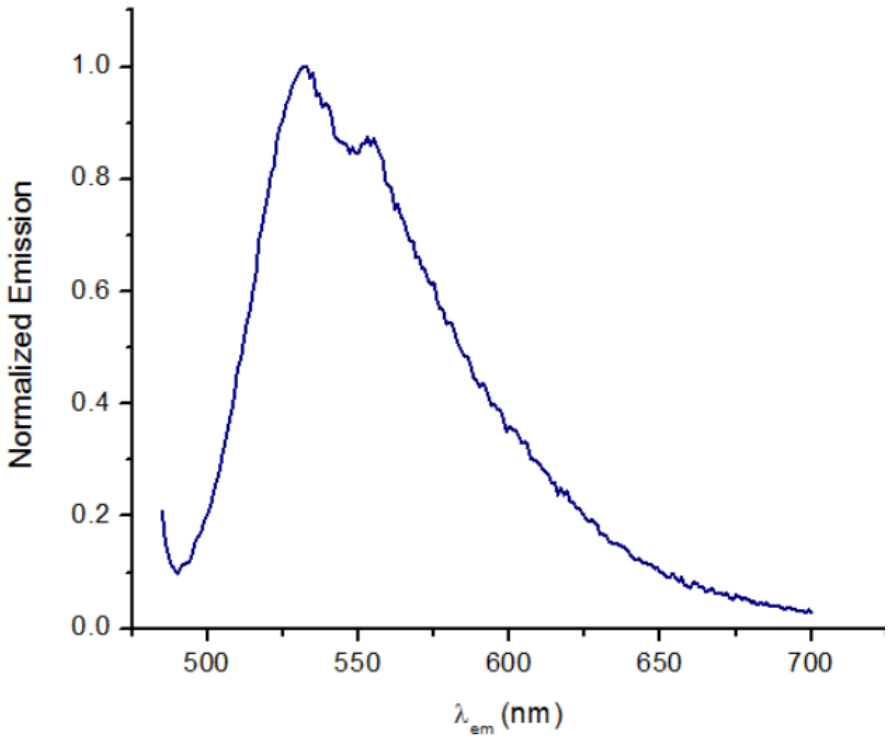


Fig. S43: Fluorescence emission spectra of compound 7f.

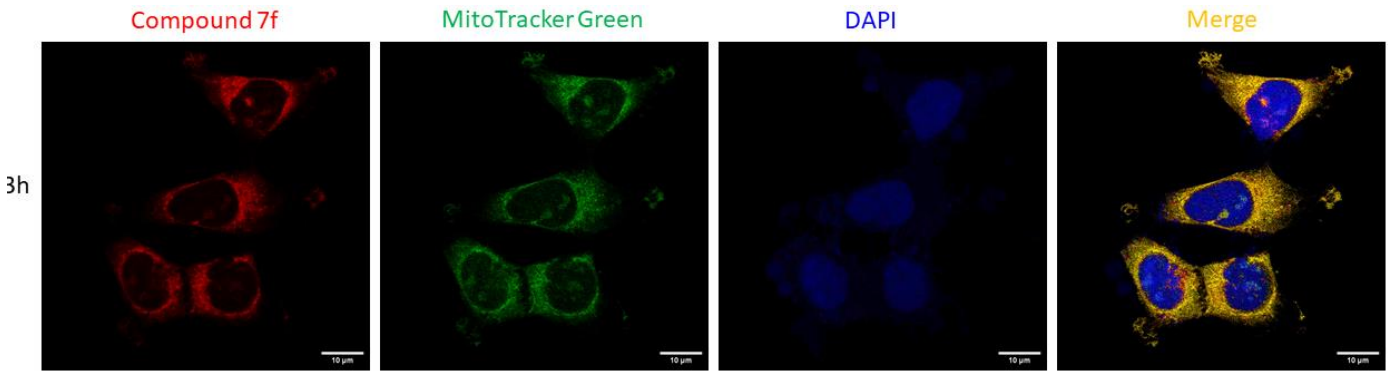


Fig. S44: Confocal microscopy images of HCT-116 cells after treatment with compound 7f (2.5 µM) for 3h followed by staining the mitochondria and nuclei by MitoTracker Green (100 nM) and DAPI (300 nM) respectively. Scale bar = 10 µM.

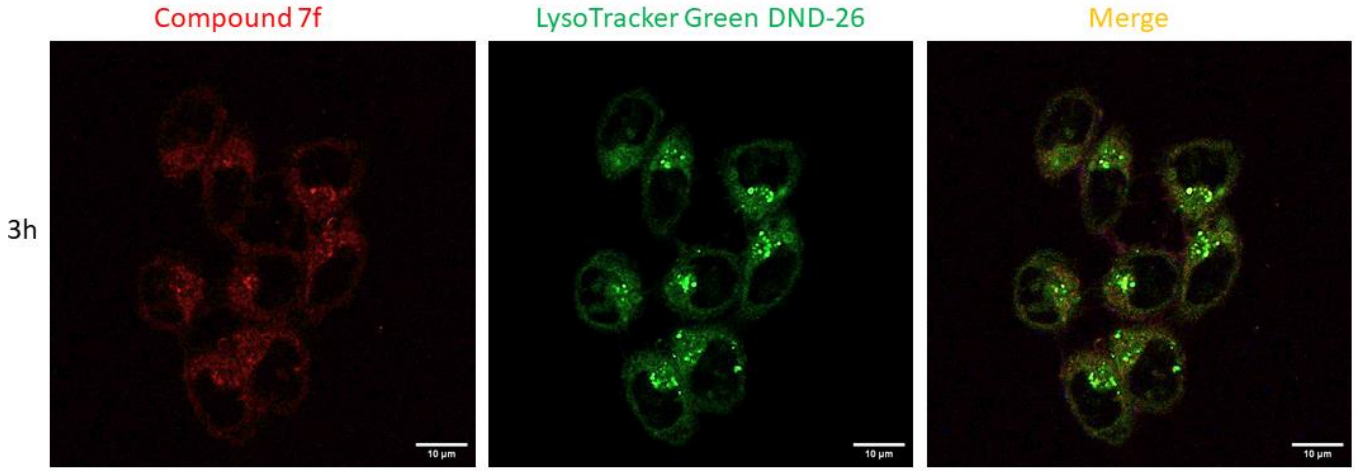


Fig. S45: Confocal microscopy images of HCT-116 cells after treatment with compound 7f (2.5 µM) for 3h followed by staining the lysosomal compartments with LysoTracker Green DND-26 (50 nM). Scale bar = 10 µM.

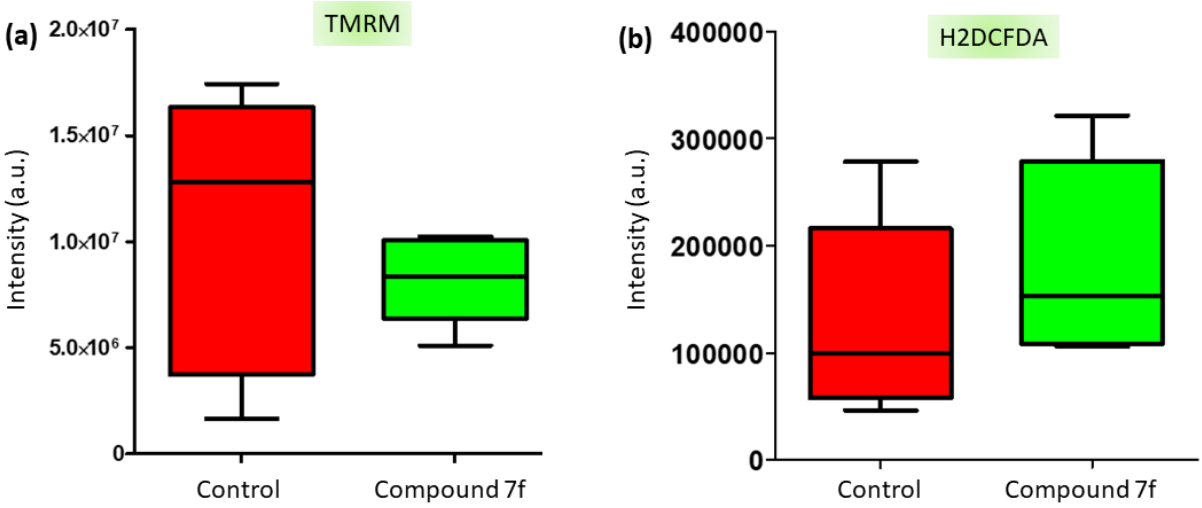


Fig. S46: (a,b) Quantification of TMRM and H2DCFDA from the red and green fluorescence intensity under the confocal microscopy in HCT-116 cells after treatment with compound 7f for 24h followed by staining with TMRM and H2DCFDA dyes respectively. For statistical analysis, t-test was performed. For TMRM and H2DCFDA, P = 0.4714 and 0.3657.

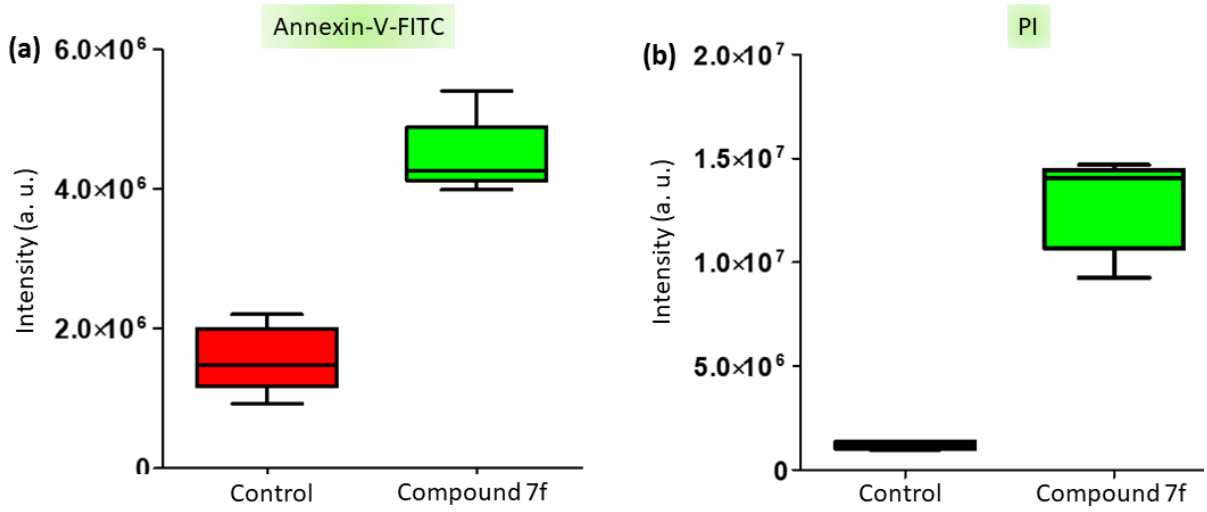


Fig. S47: (a,b) Quantification of FITC-Annexin-V and PI from the green and red fluorescence intensity under the confocal microscopy in HCT-116 cells after treatment with compound 7f for 24h followed by staining with Annexin-V-FITC and PI dyes respectively. For statistical analysis, t-test was performed. For Annexin V and PI, $P < 0.0001$ for both which was significant difference.

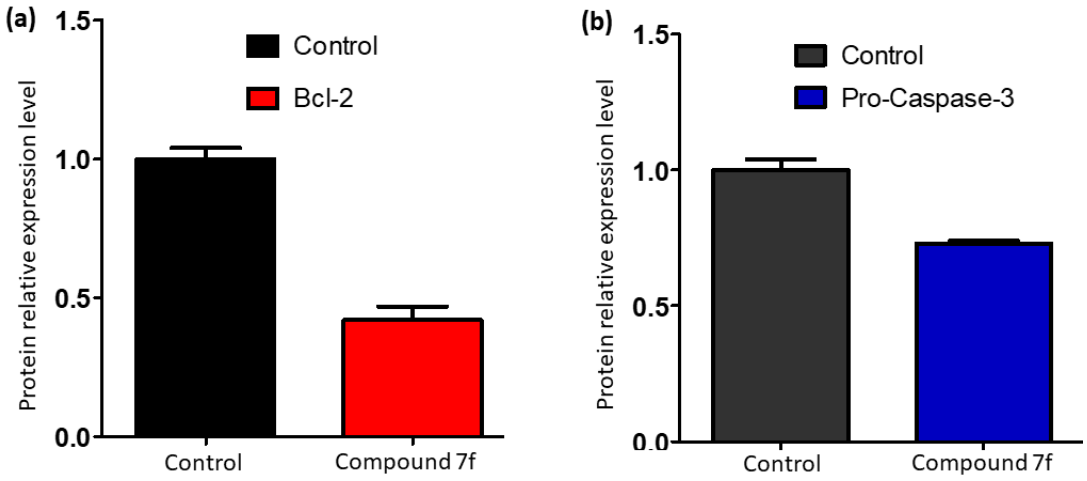


Fig. S48: (a, b) Quantification of the Bcl-2 and Pro-caspase-3 proteins in HCT-116 cells after treatment with compound 7f for 24h respectively. For statistical analysis, t-test was performed. $P < 0.0001$ for Bcl-2 and $P = 0.0003$ for Pro-caspase-3 which are significant difference.

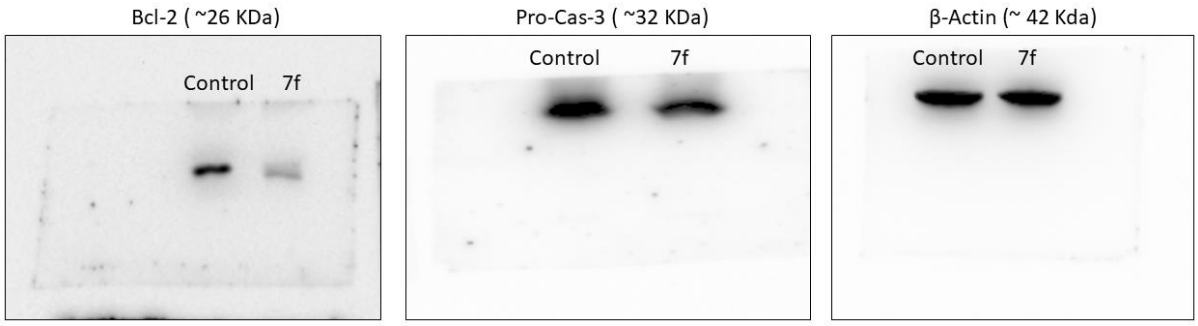


Fig. S49: Uncropped images of blots of Bcl-2, Pro-Caspase 3 and Beta-actin. According to the results of blots, the protein bands can be seen at the respective molecular weight of the targeted proteins, i.e., Bcl-2 at ~26 kDa, Pro- Caspase 3 at ~32 and Beta-actin at ~42 kDa.

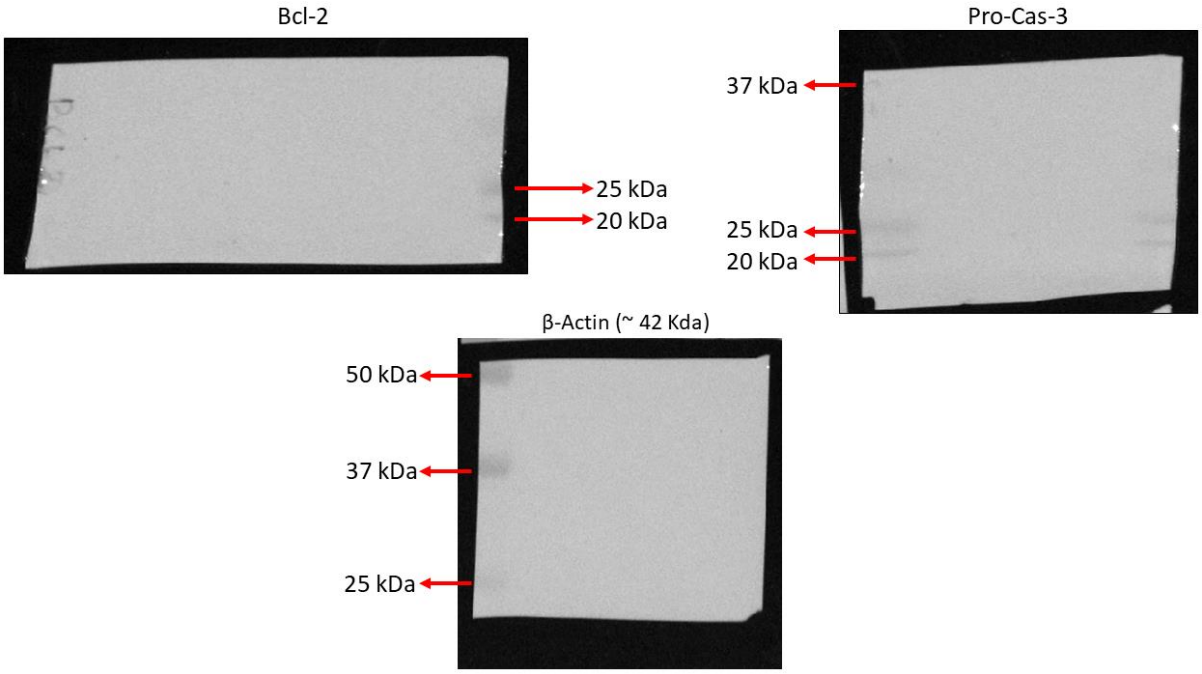


Fig. S50: Colorimetric western blot images of Bcl-2, Pro-Caspase 3 and Beta-actin along with the protein markers.

Compound	IC_{50} (µM)
7a	5.9
7b	2.6
7c	> 10
7d	> 10
7e	3.2
7f	**2.3**
7g	> 10
7h	> 10
7i	> 10
7j	> 10
7k	> 10
7l	5.7
Obatoclax	> 10

Table S1: IC_{50} values of the compounds (7a-7l) and obatoclax in HCT-116 cells.

Compound	IC_{50} (µM)
7f	> 20
Obatoclax	7.5

Table S2: IC_{50} values of the compound 7f and obatoclax in HEK-293 cells.