Electronic Supplementary Information

Cu-catalyzed ligand-free synthesis of rosuvastatin based novel indole derivatives as potential anticancer agents

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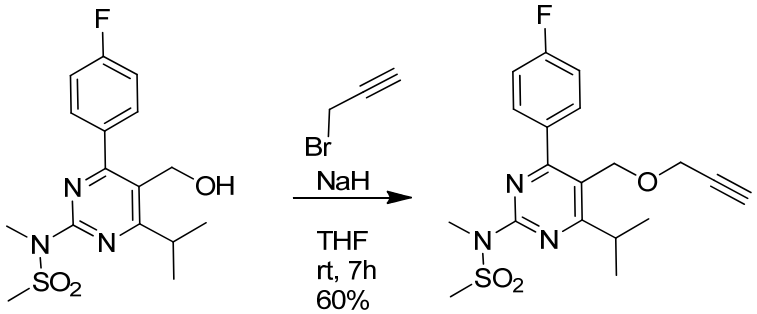
Chemistry

General methods: Unless stated otherwise, solvents and chemicals were obtained from commercial sources and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using hexane and ethyl acetate. ^{1}H and ^{13}C NMR spectra were determined in $CDCl_3$ or DMSO-d_6 solutions by using 400 or 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (*J*) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer. Melting points were determined using a melting point apparatus and are uncorrected. MS spectra were obtained on a mass spectrometer. Chromatographic purity by HPLC (Agilent 1200 series Chem Station software) was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, detection wavelength, and retention times.

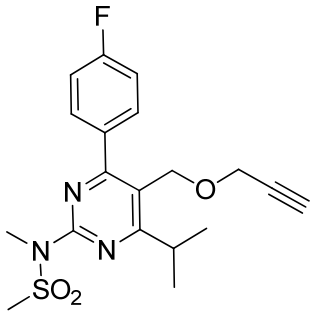
Preparation of *N*-(4-(4-fluorophenyl)-6-isopropyl-5-((prop-2-yn-1-yloxy)methyl)pyrimidin-2-yl)-*N*-methylmethanesulfonamide (1)



4 **1**

Propargyl bromide (10.6 mmol) was added to a solution of *N*-(4-(4-fluorophenyl)-5-(hydroxymethyl)-6-isopropylpyrimidin-2-yl)-*N*-methylmethanesulfonamide (**4**) (9.6 mmol) and sodium hydride (14.4 mmol) in DMF (10 mL) under a nitrogen atmosphere. The mixture was stirred at room temperature for 12 h. After completion of the reaction (confirmed by TLC), the mixture was diluted with ice water (30 mL) and extracted with ethyl acetate (3 × 15 mL). The

organic layers were collected, combined, dried over anhydrous Na_2SO_4, filtered and concentrated under low vacuum. The residue was purified by column chromatography using hexane-ethyl acetate as an eluent to afford the title compound.

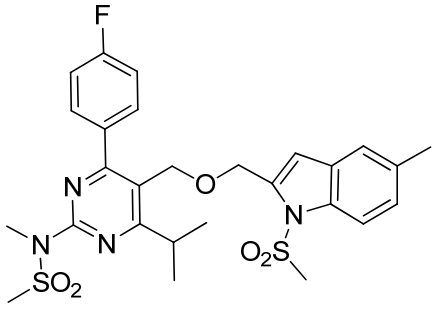


Off white solid; Yield: 60 %; mp: 72-75 °C; IR : 2929, 1550, 1151, 771 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.80-7.77 (m, 2H), 7.26-7.13 (m, 2H), 4.45 (s, 2H), 4.26 (s, 2H), 3.57 (s, 3H), 3.50 (s, 3H), 3.46-3.48 (m, 1H), 2.47 (s, 1H), 1.33 (s, 3H), 1.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.3, 166.5, 165.0, (d, C–F J = 248.4 Hz), 158.1, 133.9 (d, C–F J = 28.0 Hz), 133.8, 131.7, (d, C–F J = 8.4 Hz), 118.2, 115.4, 115.2, 78.9, 75.3, 64.7, 57.9, 42.4, 33.1, 31.5, 22.2 (2C); m/z (CI) 392.3 (M + 1, 100%).

General procedure for the preparation of 3:

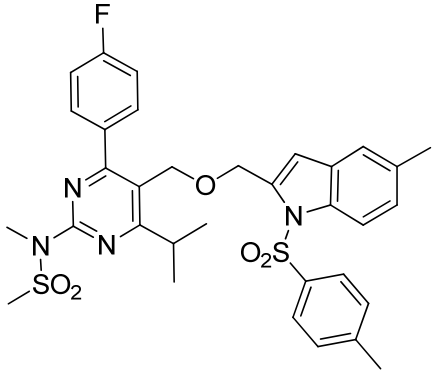
A mixture of alkyne (**1**) (1.0 mmol), *o*-iodoanilide (1.0 mmol), K_2CO_3 (2.0 mmol), and CuI (20 mol%) in PEG-400 (5.0 mL) was stirred at 60 ^oC for 8 h under nitrogen. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was diluted with EtOAc (10 mL) and filtered through Celite. The filtrate was collected and washed with water (20 mL). The EtOAc layer was collected and concentrated. The residue was purified by column chromatography using EtOAc-petroleum ether.

***N*-(4-(4-Fluorophenyl)-6-isopropyl-5-(((5-methyl-1-(methylsulfonyl)-1*H*-indol-2-yl)methoxy)methyl)pyrimidin-2-yl)-*N*-methylmethanesulfonamide (3a)**



Brown solid; Yield: 82%; mp: 75-80 °C; IR : 2925, 1551, 1364, 1157, 771 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.92 (d, J = 8.4 Hz, 1H), 7.81-7.77 (m, 2H), 7.37 (s, 1H), 7.20-7.12 (m, 3H), 6.64 (s, 1H), 4.81 (s, 2H), 4.49 (s, 2H), 3.56 (s, 3H), 3.51 (s, 3H), 3.49-3.48 (m, 1H), 3.08 (s, 3H), 2.45 (s, 3H), 1.31 (s, 3H) 1.39 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.2, 166.6, 165.1 (d, C–F J = 248.7 Hz), 158.2, 136.2, 135.3, 133.9 (d, C–F J = 29.0 Hz), 131.5 (d, C–F J = 8.4 Hz), 130.2, 128.9, 126.8, 121.2, 118.3, 115.6, 115.3, 113.8, 112.0, 65.7, 65.5, 42.5, 40.7, 33.1, 31.4, 29.7, 22.2 (2C), 21.2; LCMS : 97.4%, column: AQUITY UPLC BEH C-18 1.7 µm, 2.1 x 50mm mobile phase B: 0.1% FA in H_2O, mobile phase A: 0.1 % FA in ACN T/%B: 0/97, 0.3/97, 3.2/2, 4/2, 4.01/97; flow rate: 0.6 mL/min, Temp : 35 ºC, retention time 2.97 min ; m/z (CI) 575.3 (M + 1, 100%).

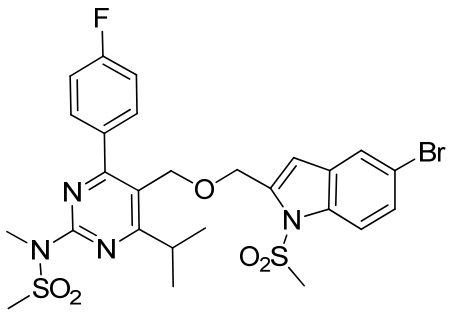
_N_-(4-(4-Fluorophenyl)-6-isopropyl-5-(((5-methyl-1-tosyl-1_H_-indol-2-yl)methoxy)methyl)pyrimidin-2-yl)-_N_-methylmethanesulfonamide (3b)



Dark brown solid; Yield: 80%; mp: 55-60 °C; IR : 2925, 1510, 1217, 745 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 8.4 Hz, 1H), 7.70-7.66 (m, 4H), 7.24 (s, 1H), 7.15-7.09 (m, 3H), 7.01 (t, J = 8.4 Hz, 2H), 6.56 (s, 1H), 4.93 (s, 2H), 4.47 (s, 2H), 3.57 (s, 3H), 3.52 (s, 3H), 3.50-

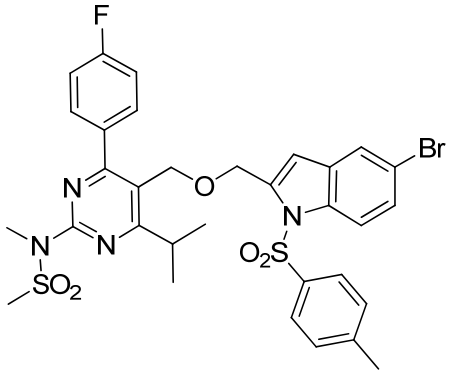
3.42 (m, 1H), 2.40 (s, 3H), 2.29 (s, 3H), 1.29 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.3, 166.4, 164.9 (d, C–F J = 247.4 Hz), 158.1, 144.9, 136.7, 135.7 (d, C–F J = 31.7 Hz), 133.8, 133.4, 131.5 (d, C–F J = 8.5 Hz), 129.6 (2C), 129.3, 126.6 (2C), 126.4, 120.9, 118.6, 115.4, 115.2, 114.4, 111.8, 111.7, 66.0, 65.4, 42.5, 33.1, 31.6, 29.7, 22.2 (2C), 21.5, 21.2; LCMS : 95.5%, column: AQUITY UPLC BEH C-18 1.7 µm, 2.1 x 50 mm mobile phase B: 0.1% FA in H_2O, mobile phase A: 0.1 % FA in ACN T/%B: 0/97, 0.3/97, 3.2/2, 4/2, 4.01/97; flow rate: 0.6 mL/min, Temp: 35 ºC, retention time 3.22 min; m/z (CI) 651.4 (M + 1, 100%).

_N_-(5-(((5-Bromo-1-(methylsulfonyl)-1H-indol-2-yl)methoxy)methyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-_N_-methylmethanesulfonamide (3c)



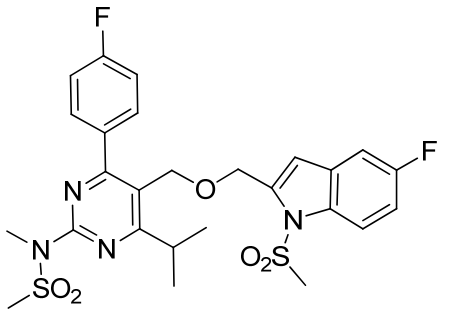
Light brown color solid; Yield: 80%; mp: 100-105 °C; IR : 2929, 1551, 1169, 747 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.93 (d, J = 8.8 Hz, 1H), 7.80-7.76 (m, 2H), 7.72 (d, J = 1.6 Hz, 1H), 7.46 (dd, J = 1.6 & 2.0 Hz, 1H), 7.16 (t, J = 8.8 Hz, 2H), 6.66 (s, 1H), 4.81 (s, 2H), 4.51 (s, 2H), 3.56 (s, 3H), 3.51 (s, 3H), 3.50-3.45 (m, 1H), 3.12 (s, 3H), 1.31 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.1, 166.6, 165.1 (d, C–F J = 248.9 Hz), 158.2, 137.4, 135.7 (d, C–F J = 27.4 Hz), 133.8 (2C), 131.5 (d, C–F J = 8.5 Hz), 130.3, 128.2, 123.9, 118.1, 117.3, 115.6, 115.5, 115.4, 111.0, 65.9, 65.4, 42.5, 41.1, 33.1, 31.5, 22.2 (2C); ^{19}F NMR (400 MHz, $CDCl_3$): δ -110.7; LCMS : 97.6%, column: AQUITY UPLC BEH C-18 1.7 µm, 2.1 x 50 mm mobile phase B: 0.1% FA in H_2O, mobile phase A: 0.1 % FA in ACN T/%B: 0/97, 0.3/97, 3.2/2, 4/2, 4.01/97; flow rate: 0.6 mL/min, Temp : 35 ºC; retention time 3.03 min; m/z (CI) 639.2 (M + 1, 100%).

_N_-(5-(((5-Bromo-1-(methylsulfonyl)-1_H_-indol-2-yl)methoxy)methyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-_N_-methylmethanesulfonamide (3d)



White solid; Yield: 80%; mp: 70-75 °C; IR : 2925, 1551, 1170, 770, 665 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.99 (d, J = 8.0 Hz, 1H), 7.70-7.66 (m, 4H), 7.59 (d, J = 2 Hz, 1H), 7.40-7.43 (m, 1H), 7.14 (d, J = 8.0 Hz, 2H), 7.04 (t, J = 8 Hz, 2H), 6.58 (s, 1H), 4.92 (s, 2H), 4.50 (s, 2H), 3.57 (s, 3H), 3.52 (s, 3H), 3.46-3.42 (m, 1H), 2.32 (s, 3H), 1.29 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 178.2, 166.5, 164.9 (d, C–F J = 248.6 Hz), 158.2, 145.4, 138.2, 135.8 (d, C–F J = 27.4 Hz), 135.3, 133.9, 133.8, 131.4 (d, C–F J = 8.4 Hz), 130.8, 129.9 (2C), 127.8, 126.6 (2C), 123.6, 118.4, 117.1, 116.0, 115.5, 115.2, 110.6, 65.8, 65.7, 42.5, 33.1, 31.6, 22.2 (2C), 21.5 ; LCMS : 98.9%, column: AQUITY UPLC BEH C-18 1.7 µm, 2.1 x 50 mm mobile phase B: 0.1% FA in H_2O, mobile phase A: 0.1 % FA in ACN T/%B: 0/97, 0.3/97, 3.2/2, 4/2, 4.01/97; flow rate: 0.6 mL/min, retention time 3.30 min; Temp: 35 ºC ; m/z (CI) 715 (M + 1, 100%).

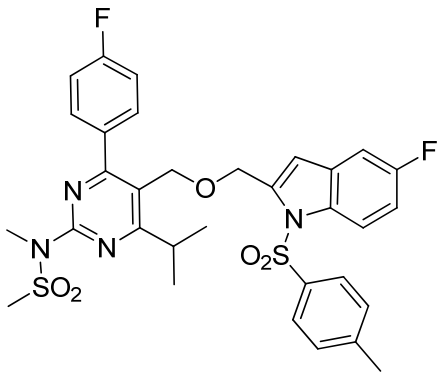
_N_-(5-(((5-Fluoro-1-(methylsulfonyl)-1_H_-indol-2-yl)methoxy)methyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-_N_-methylmethanesulfonamide (3e)



Brown solid; Yield: 78%; mp: 105-110 °C; IR : 2929, 1550, 1335, 1154, 750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (dd, J = 4.4 & 4.8 Hz, 1H), 7.80-7.77 (m, 2H), 7.24 (dd, J = 2.4 & 2.8

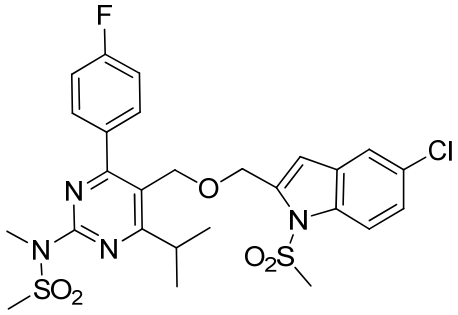
Hz, 1H), 7.18-7.07 (m, 3H), 6.68 (s, 1H), 4.81 (s, 2H), 4.51 (s, 2H), 3.56 (s, 3H), 3.51 (s, 3H), 3.48-3.45 (m, 1H), 3.11 (s, 3H), 1.31 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.1, 166.6, 165.0 (d, C–F J = 249.2 Hz), 160.1 (d, C–F J = 277.7 Hz), 137.7, 133.8, 133.7 (d, C–F J = 48.7 Hz), 131.5 (d, C–F J = 8.4 Hz), 129.6, 129.5, 118.1, 115.5 (d, C–F J = 21.5 Hz), 115.2, 113.4 (d, C–F J = 25.3 Hz), 111.6 (2C), 106.8, 106.6, 65.8, 65.4, 42.4, 40.1, 33.0, 31.4, 22.2 (2C); LCMS : 96.2%, column: AQUITY UPLC BEH C-18 1.7 µm, 2.1 x 50 mm mobile phase B: 0.1% FA in H_2O, mobile phase A: 0.1 % FA in ACN T/%B: 0/97, 0.3/97, 3.2/2, 4/2, 4.01/97; flow rate: 0.6 mL/min, Temp: 35 ºC; retention time 2.86 min m/z (CI) 579 (M + 1, 100%).

_N_-(5-(((5-Fluoro-1-tosyl-1_H_-indol-2-yl)methoxy)methyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-_N_-methylmethanesulfonamide (3f)



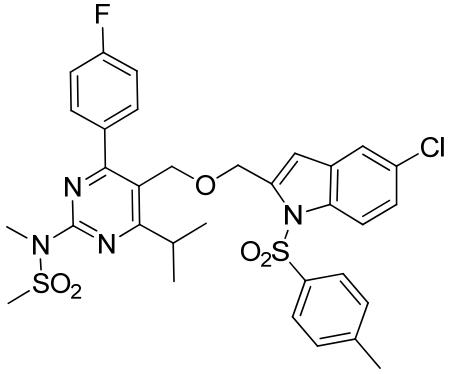
Brown solid; Yield: 80%; mp: 95-100 °C; IR : 2924, 1551, 1339, 1163, 771 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (dd, J = 4.4 & 4.8 Hz, 1H), 7.70-7.66 (m, 4H), 7.12 (dd, J = 8.8 & 6.4 Hz, 3H), 7.07-7.01 (m, 3H), 6.60 (s, 1H), 4.92 (s, 2H), 4.50 (s, 2H), 3.57 (s, 3H), 3.52 (s, 3H), 3.48-3.41 (m, 1H), 2.32 (s, 3H), 1.30 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.2, 172.0, 169.0 (d, C–F J = 239.9 Hz), 158.1, 145.2, 138.5, 135.3, 133.8 (d, C–F J = 49.1 Hz), 131.4, 131.3, 130.1, 129.8 (2C), 126.5 (2C), 118.4, 115.7 (d, C–F J = 9.0 Hz), 115.4, 115.2, 113.0, 112.8, 111.3 (2C), 106.6, 65.9, 65.7, 42.5, 33.1, 31.6, 29.7, 22.1 (2C); ^{19}F NMR (400 MHz, $CDCl_3$): δ -111.1, 119.2: LCMS : 98.9%, column: AQUITY UPLC BEH C-18 1.7 µm, 2.1 x 50 mm mobile phase B: 0.1% FA in H_2O, mobile phase A: 0.1 % FA in ACN T/%A: 0/97, 0.3/97, 3.2/2, 54.0/2, 4.01/97; flow rate: 0.6 mL/min, Temp: 35 ºC, retention time 3.17 min; m/z (CI) 655 (M + 1, 100%).

***N*-(5-(((5-Chloro-1-(methylsulfonyl)-1*H*-indol-2-yl)methoxy)methyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-*N*-methylmethanesulfonamide (3g)**



Light brown color solid; Yield: 78%; mp: 50-60 °C; IR : 2928, 1550, 1151, 960, 751 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.98 (d, J = 8.8 Hz, 1H), 7.82-7.77 (m, 2H), 7.56 (t, J = 8.0 Hz, 1H), 7.33 (d, J = 7.2 Hz, 1H), 7.16 (t, J = 8.8 Hz, 2H), 6.66 (s, 1H), 4.81 (s, 2H), 4.51 (s, 2H), 3.56 (s, 3H), 3.51 (s, 3H), 3.50-3.45 (m, 1H), 3.12 (s, 3H), 1.31 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.1, 166.6, 165.1 (d, C–F J = 248.9 Hz), 158.2, 138.5, 137.6, 135.4 (d, C–F J = 27.4 Hz), 133.9, 131.5 (d, C–F J = 8.4 Hz), 129.6, 125.6, 123.1, 120.8, 118.2, 115.6, 115.4, 115.1, 111.1, 65.9, 65.4, 42.4, 41.1, 33.0, 31.4, 22.2 (2C); LCMS : 98.8%, column: AQUITY UPLC BEH C-18 1.7 µm, 2.1 x 50 mm mobile phase B: 0.1% FA in H_2O, mobile phase A: 0.1 % FA in ACN T/%B: 0/97, 0.3/97, 3.2/2, 4/2, 4.01/97; flow rate: 0.6 mL/min, Temp: 35 ºC, retention time 3.00 min; m/z (CI) 595 (M + 1, 100%).

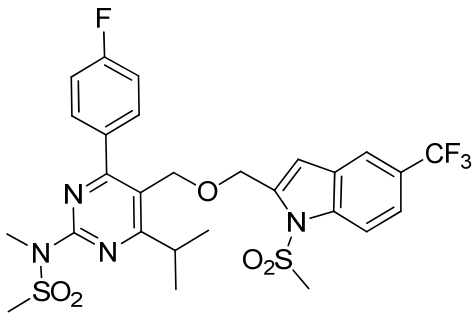
***N*-(5-(((5-Chloro-1-tosyl-1*H*-indol-2-yl)methoxy)methyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-*N*-methylmethanesulfonamide (3h)**



Off white solid; Yield: 75%; mp: 70-75 °C; IR : 2927, 1551, 1371, 1152, 771 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, J = 7.6 Hz, 1H), 7.66-7.62 (m, 4H), 7.42 (s, 1H), 7.31-7.35 (m,

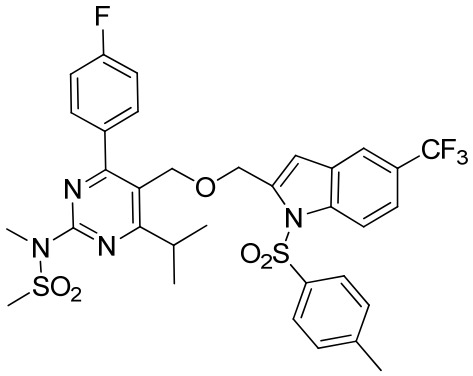
1H), 7.18 (d, *J* = 7.6 Hz, 2H), 7.20 (t, *J* = 8 Hz, 2H), 4.61 (s, 1H), 4.85 (s, 2H), 4.46 (s, 2H), 2.60 (s, 3H), 2.51 (s, 3H), 2.53-2.42 (m, 1H), 2.32 (s, 3H), 1.32 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.2, 166.5, 164.9 (d, C–F *J* = 248.6 Hz), 162.4, 158.1, 145.3, 138.3, 135.3 (2C) (d, C–F *J* = 26.4 Hz), 133.8 (d, *J*= 3 Hz, 1C), 131.4, 131.3 (d, C–F *J* = 8.6 Hz), 130.3, 129.8, 129.5, 126.5, 125.1, 120.5, 118.4, 115.6, 115.4, 115.2, 110.7, 65.8, 65.7, 42.4, 33.0, 31.6, 22.1, 21.5; LCMS : 96.6%, column: AQUITY UPLC BEH C-18 1.7 µm, 2.1 x 50 mm mobile phase B: 0.1% FA in H_2O, mobile phase A: 0.1 % FA in ACN T%B: 0/97, 0.3/97, 3.2/2, 4/2, 4.01/97; flow rate: 0.6 mL/min, Temp: 35 ºC, retention time 3.2 min; m/z (CI) 593 ((M-SO_2Me) + 1, 100%).

_N_-(4-(4-Fluorophenyl)-6-isopropyl-5-(((1-(methylsulfonyl)-5-(trifluoromethyl)-1H-indol-2-yl)methoxy)methyl)pyrimidin-2-yl)-_N_-methylmethanesulfonamide (3i)



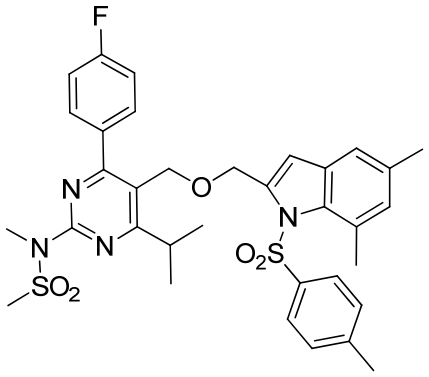
Brown solid; Yield: 79%; mp: 90-95 °C; IR : 3020, 1552, 1214, 742 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, *J* = 8.8 Hz, 1H), 7.88 (s, 1H), 7.75-79 (m, 2H), 7.61 (d, *J* = 7.6 Hz, 1H), 7.15 (t, *J* = 8.8 Hz, 2H), 6.78 (s, 1H), 4.85 (s, 2H), 4.54 (s, 2H), 3.57 (s, 3H), 3.51 (s, 3H), 3.50-3.45 (m, 1H), 3.17 (s, 3H), 1.32 (s, 3H), 1.30 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.1, 166.7, 165.1 (d, C–F *J* = 248.9 Hz), 158.2, 138.4, 138.0, 133.8 (2C), 131.5 (d, C–F *J* = 8.4 Hz), 128.3, 122.1, 122.0, 118.8 (2C), 118.1, 115.6, 115.4, 114.4, 111.5, 66.0, 65.3, 42.5, 41.5, 33.1, 31.5, 22.2 (2C); LCMS : 98.6%, column: AQUITY UPLC BEH C-18 1.7 µm, 2.1 x 50 mm mobile phase A: 0.1% FA in H_2O, mobile phase B: 0.1 % FA in ACN T%A: 0/97, 0.3/97, 3.2/2, 54.0/2, 4.01/97; flow rate: 0.6 mL/min, Temp: 35 ºC, retention time 3.04 min ; m/z (CI) 629 (M + 1, 100%).

N-(4-(4-Fluorophenyl)-6-isopropyl-5-(((1-tosyl-5-(trifluoromethyl)-1*H*-indol-2-yl)methoxy)methyl)pyrimidin-2-yl)-*N*-methylmethanesulfonamide (3j)



Brown semi solid; Yield: 81%; mp: 75-80 °C; IR : 2926, 1552, 1335, 1151, 771 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.21(d, J = 8.8 Hz, 1H), 7.75 (s, 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.67 (m, 2H), 7.56 (d, J = 7.2 Hz, 1H), 7.15 (d, J = 8.4 Hz, 2H), 7.02 (t, J = 8.4 Hz, 2H), 6.70 (s, 1H), 4.94 (s, 2H), 4.52 (s, 2H), 3.57 (s, 3H), 3.52 (s, 3H), 3.48-3.42 (m, 1H), 2.33 (s, 3H), 1.30 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.2, 165.5, 163.9 (d, C–F J = 248.6 Hz), 157.1, 144.6, 137.7, 137.4, 134.3, 132.8 (2C), 130.4 (d, C–F J = 8.4 Hz), 128.9 (2C), 127.7, 125.6, 120.6 (2C), 117.5, 117.4, 117.3, 114.4, 114.2, 113.8, 109.9, 64.8 (2C), 41.5, 32.1, 30.6, 28.6, 21.1 (2C), 20.5; LCMS : 97.5%, column: AQUITY UPLC BEH C-18 1.7 µm, 2.1 x 50 mm mobile phase A: 0.1% FA in H_2O, mobile phase B: 0.1 % FA in ACN T%A: 0/97, 0.3/97, 3.2/2, 54.0/2, 4.01/97; flow rate: 0.6 mL/min, Temp: 35 ºC, retention time 3.27 min; m/z (CI) 705 (M + 1, 100%).

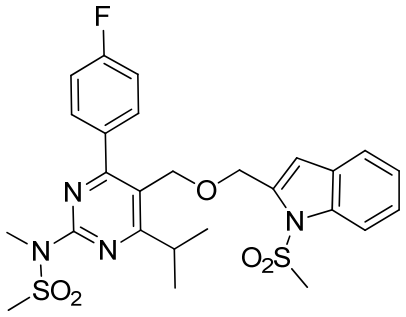
_N_-(5-(((5,7-Dimethyl-1-tosyl-1H-indol-2-yl)methoxy)methyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-_N_-methylmethanesulfonamide (3k)



Yellow colour solid; Yield: 80%; mp: °C; IR : 2925, 1551, 1154, 751 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.73 (q, J = 5.2 3.2 & 5.6 Hz, 2H), 7.33 (d, J = 8.4 Hz, 2H), 7.07-6.95 (m, 6H), 6.54

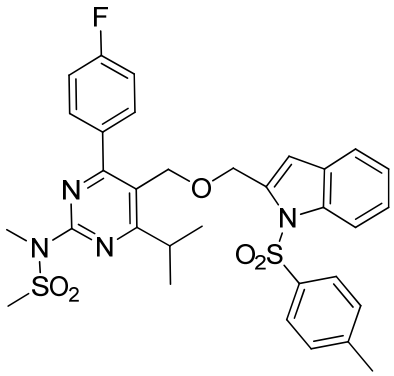
(s, 1H), 4.89 (s, 2H), 4.44 (s, 2H), 3.56 (s, 3H), 3.50 (s, 3H), 3.44-3.37 (m, 1H), 2.61 (s, 3H), 2.35 (s, 3H), 2.31 (s, 3H), 1.27 (s, 3H), 1.26 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.2, 166.4, 164.9 (d, C–F J = 248.3 Hz), 158.1, 144.4, 140.4, 137.5, 134.7 (2C), 133.9 (2C), 132.8, 131.5 (d, C–F J = 8.4 Hz), 130.6, 129.2 (2C), 128.1, 126.2 (2C), 118.9, 118.6, 116.5, 115.4, 115.2, 67.5, 65.4, 42.4, 33.1, 31.5, 22.2 (2C), 22.0, 21.5, 21.0; LCMS: 99.5%, column: AQUITY UPLC BEH C-18 1.7 µm, 2.1 x 50 mm mobile phase B: 0.1% FA in H_2O, mobile phase A: 0.1 % FA in ACN T%B: 0/97, 0.3/97, 3.2/2, 4/2, 4.01/97; flow rate: 0.6 mL/min, Temp: 35 ºC, retention time 3.33 min ; m/z (CI) 665 (M + 1, 100%).

_N_-(4-(4-Fluorophenyl)-6-isopropyl-5-(((1-(methylsulfonyl)-1_H_-indol-2-yl)methoxy)methyl)pyrimidin-2-yl)-_N_-methylmethanesulfonamide (3l)



Yellow solid; Yield: 82%; mp: °C; IR : 2929, 1551, 1331, 1152, 747 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (d, J=8.4 Hz, 1H), 7.82-7.77 (m, 2H), 7.60 (d, J= 7.2 Hz, 1H), 7.38-7.22 (m, 2H), 7.14 (t, J=8.4 Hz, 2H), 6.72 (s, 1H), 4.84 (s, 2H), 4.50 (s, 2H), 3.53 (s, 3H), 3.51 (s, 3H), 3.49-3.44 (m, 1H), 3.12 (s, 3H), 1.31 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.2, 166.6, 165.1 (d, C–F J = 248.8 Hz), 158.2, 137.1, 136.2, (d, C–F J = 27.4 Hz) 133.9, 133.8, 131.5 (d, C–F J = 8.5 Hz), 128.6, 125.4, 123.9, 121.3, 118.3, 115.6, 115.3, 114.1, 112.1, 65.7, 65.5, 42.5, 40.9, 33.1, 31.4, 22.2 (2C); ^{19}F NMR (400 MHz, $CDCl_3$): δ -110.9: LCMS : 99.2%, column: AQUITY UPLC BEH C18 1.7 µm, 2.1 x 50 mm mobile phase B: 0.1% FA in H_2O, mobile phase A: 0.1 % FA in ACN T%B: 0/97, 0.3/97, 3.2/2, 4/2, 4.01/97; flow rate: 0.6 mL/min, Temp: 35 ºC, retention time 2.86 min; m/z (CI) 561 (M + 1, 100%).

_N_-(4-(4-Fluorophenyl)-6-isopropyl-5-(((1-tosyl-1_H_-indol-2-yl)methoxy)methyl)pyrimidin-2-yl)-_N_-methylmethanesulfonamide (3m)

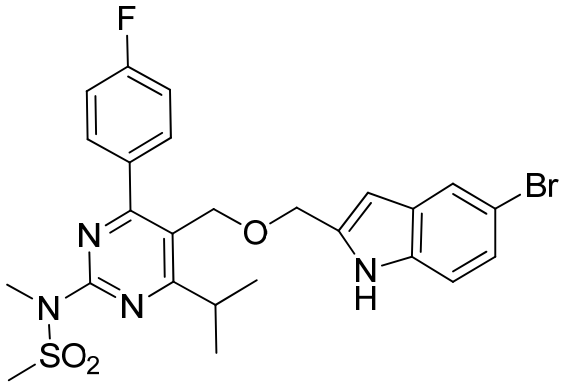


Pale yellow solid; Yield: 82%; mp: 260-270 °C; IR : 2924, 1551, 1151, 750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, *J*=8.4 Hz, 1H), 7.71-7.66 (m, 4H), 7.46 (d, *J*= 7.6 Hz, 1H), 7.33 (t, *J*=8.4 Hz, 1H), 7.23 (d, *J*=7.2 Hz, 1H), 7.10 (d, *J*= 8.0 Hz, 2H), 7.00 (t, *J*= 8.4 Hz, 2H), 6.64 (s, 1H), 4.95 (s, 2H), 4.49 (s, 2H), 3.57 (s, 3H), 3.52 (s, 3H), 3.49-3.42 (m, 1H), 2.29 (s, 3H), 1.29 (s, 3H), 1.28 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.2, 166.5, 164.9 (d, C–F *J* = 248.4 Hz), 158.1, 145.0, 137.1 (d, C–F *J* = 34.1 Hz), 135.6, 133.9, 133.8, 131.5 (d, *J* = 8.4 Hz), 129.7 (2C), 129.0, 126.6 (2C), 125.3 125.0, 123.7, 121.0, 118.5, 115.4, 115.2, 114.6, 111.8, 66.0, 65.5, 42.5, 33.1, 31.6, 22.2 (2C), 21.5; LCMS : 98.6%, column: AQUITY UPLC BEH C18 1.7 µm, 2.1 x 50 mm mobile phase B: 0.1% FA in H_2O, mobile phase A: 0.1 % FA in ACN T%B: 0/97, 0.3/97, 3.2/2, 4/2, 4.01/97; flow rate: 0.6 mL/min, Temp: 35 ºC, retention time 3.13 min; m/z (CI) 637 (M + 1, 100%).

General procedure for the desulfonylation of *N*-tosylated indoles (4)

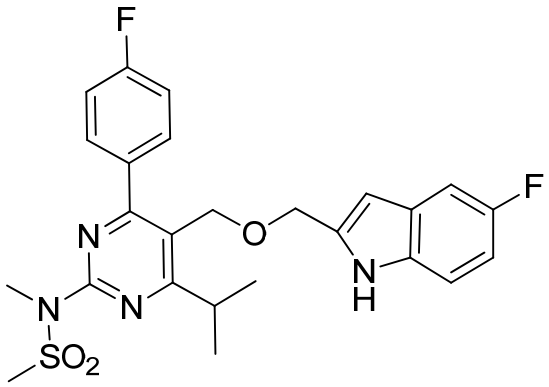
To a solution of *N*-tosyl indole derivative (**3**) (0.5 mmol) in 2:1 THF–MeOH (3 mL) was added cesium carbonate (1.5 mmol) and the mixture was stirred at room temperature for 8-12 h. After completion of the reaction (indicated by TLC) the mixture was concentrated under vacuum, poured into cold water (15 mL), stirred for 10 min and then extracted with ethylacetate (3 × 20 mL). The organic layers were collected, combined, washed with cold water (2 × 20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The residue obtained was purified by column chromatography on silica gel (230–400 mesh) using ethylacetate–hexene to give the desired product.

***N*-(5-(((5-Bromo-1*H*-indol-2-yl)methoxy)methyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-*N*-methylmethanesulfonamide (4a)**



Off white solid; Yield: 85%; mp: 152-154 °C; IR : 3402, 2972, 2931, 2893, 1552,771 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 88.20 (bs, 1H), 7.70 (s, 1H), 7.64 (t, *J*= 7.8 Hz, 2H), 7.30-7.28 (m, 1H), 7.23-7.21 (m, 1H), 7.02 (t, *J*= 7.8 Hz, 2H), 6.35 (s, 1H), 4.70 (s, 2H), 4.42 (s, 2H), 3.55 (s, 3H), 3.50 (s, 3H), 3.30-3.22 (m, 1H), 1.28 (s, 3H), 1.26 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): 177.9, 166.4, 164.8 (d, C–F *J* = 248.9), 158.1, 135.4 (d, C–F *J* = 41.0), 133.9, 133.8, 131.2 (d, C–F *J* = 10.8), 129.7, 125.2, 123.1, 118.4, 115.4, 115.2, 115.2, 113.1, 112.2, 101.9, 65.6, 64.8, 42.4, 33.0, 31.5, 22.1 (2C); m/z (CI) 561 (M + 1, 100%).

_N_-(5-(((5-Fluoro-1_H_-indol-2-yl)methoxy)methyl)-4-(4-fluorophenyl)-6-isopropylpyrimidin-2-yl)-_N_-methylmethanesulfonamide (4b)



Off white solid; Yield: 79%; mp: 165-167 °C; IR : 3390, 3084, 2976, 2926, 2873, 1548, 954 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (bs, 1H), 7.66-7.63 (m, 2H), 7.28-7.27 (m, 1H), 7.23-7.20 (m, 1H), 7.03-6.93 (m, 3H), 6.38 (s, 1H), 4.70 (s, 2H), 4.42 (s, 2H), 3.55 (s, 3H), 3.49 (s, 3H), 3.28-3.25 (m, 1H), 1.28 (s, 3H), 1.26 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.9, 166.4, 164.8 (d, C–F *J* = 248.9), 159.1, 158.1, 135.8, 133.8 (d, C–F *J* = 98.0), 131.2 (d, C–F *J* = 10.8),

128.3, 121.3, 120.5, 118.4, 115.4, 115.1, 111.4, 111.2, 105.5, 105.3, 65.7, 64.8, 42.4, 33.0, 31.5, 22.1 (2C); m/z (CI) 501 (M + 1, 100%).

Cell proliferation Assay

The anti-proliferative activity and cancer cell selectivity of the synthesized compounds on normal and cancer cells was evaluated using the SRB (Sulforhodamine B) cell proliferation assay. This assay was chosen because of its sensitivity, large dynamic range and the ability to measure cell proliferation over three days with normalization to initial cell number as well as to vehicle-treated cells. Further, this assay is the standardized assay of choice for anticancer compound screening at the National Cancer Institute (NIH). The SRB assay provides a colorimetric readout which can be spectrophotometrically measured and does not involve antibodies or toxic reagents. The assay is based on detection of total protein content of cells, which increases or decreases in proportion with cell number.

In brief, the assay was performed as follows: TZM-BL (Human cervical carcinoma cells) and A549 (human lung carcinoma cell) were seeded in 96-well plates and incubated overnight. The optimum cell numbers to be seeded were determined by a growth curve analysis for each cell line. In the initial (single dose) screen, compounds (dissolved in 100% DMSO to a stock concentration of 100 mM) were added to the adhered cells at a final concentration of 10 μM. After 72 h of treatment, the cells were washed with phosphate-buffered saline and ice-cold 10% trichloroacetic acid added to the cells to precipitate all proteins for 1h at 4 ^{o}C. The cells were then washed with water and air-dried. Cellular proteins were then stained using 0.4% SRB solution in 1% acetic acid for 10 min at room temperature. The unbound dye was washed away by destaining with 1% acetic acid and bound dye solubilized with 10 μM Tris solution. Absorbance of solubilized dye was measured at a wavelength of 590 nm. Percentage growth was determined by the formula [(At-A0/Ac-A0)] X 100, where At=absorbance after 72h of test compound treatment, A0=Absorbance at time 0, Ac=Absorbance after 72h without treatment.

References

1. Rubinstein, L.V., et al., *Comparison of in vitro anticancer-drug-screening data generated with a tetrazolium assay versus a protein assay against a diverse panel of human tumor cell lines.* J Natl Cancer Inst, 1990. **82**(13): p. 1113-8.

2. Skehan, P., et al., *New colorimetric cytotoxicity assay for anticancer-drug screening.* J Natl Cancer Inst, 1990. **82**(13): p. 1107-12.
3. NCI. *http://dtp.nci.nih.gov/branches/btb/ivclsp.html*. In vitro Compound Screening at the National Cancer Institute.

Zebrafish studies:

***Animal husbandry*:** Wild-type zebrafish of the Turku line were maintained and raised as described previously[1] at the zebrafish core facility (Biomedicum, University of Helsinki, Finland). Embryos were staged according to hours post fertilization (hpf) or days post fertilization (dpf). The embryos were raised in E3 medium. Animal experiments were performed in compliance with the national ethical guidelines, following the regulations set by the European Union, and were approved by the National Animal Experiment Board.

Toxicity screening for zebrafish embryos and larvae: Test compound stock solutions were prepared by dissolving the compounds in 100% DMSO. 1-dpf embryos were manually dechorionated and distributed into 24-well plates (n=10 embryos/well). Compounds were added to wells to obtain final 3, 6, 12 and 24 µM concentrations, and additionally, 36 µM concentration for **3d**. The plates were incubated at 28°C until the zebrafish reached the age of 5dpf. The larvae were washed with PBS and anesthetized using tricaine (0.03%). We also tested the toxic effects of the compounds at a stage when organogenesis is already completed. For this, 4-dpf larvae were exposed to various concentrations of test compounds prepared from stock solutions as described above. The larvae were distributed into 24-well plates, 10 larvae / well. The plates were incubated at 28 °C until the zebrafish reached the age of 7 dpf, when the larvae were washed and morphological scoring was performed as described above.

Gene expression analysis for apoptosis study: Starting at 4dpf, zebrafish larvae were treated with the compounds for 48 h (n=20) in 24-well plates. The larvae were collected and total RNA was isolated using the RNeasy mini Kit (Qiagen, Hilden, Germany) and reverse transcribed with random hexamer primers and Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA) as previously described.[3] qRT-PCR was performed using the Power SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA) in an iCycleriQTM Real-Time PCR Detection System (Bio-

Rad Laboratories, Hercules, CA). Data were normalized to β-actin. Primers used were *p21*forward *5´*-CGGAATAAACGGTGTCGTCT-3`, p21reverse 5´-CGCAAACAGACCAACATCAC -3`[4]; actin forward-*5´*-CATCACCAACGTAGCTGTCT -3` and actin reverse 5´-CACTGGTTGTTGACAACGGA-3`[5].

References

1. Westerfield, M. *The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish* (Daniorerio); 4th Edition, Eugene, University of Oregon Press, 2000.
2. Panzica-Kelly, J. M.; Zhang, C. X.; Danberry, T. L.; Flood, A.; DeLan, J. W.; Brannen, K. C.; Augustine-Rauch, K. A. 2010, *Birth Defects Res. B*, 89, 382–395.
3. Dash SN, Lehtonen E, Wasik AA, Schepis A, Paavola J, Panula P, Nelson WJ, Lehtonen S. Sept7b is essential for pronephric function and development of left-right asymmetry in zebrafish embryogenesis. J Cell Sci. 2014 Apr 1;127(Pt 7):1476-86.
4. Robu ME, Larson JD, Nasevicius A, Beiraghi S, Brenner C, Farber SA, Ekker SC. p53 activation by knockdown technologies. PLoS Genet. 2007 May 25;3(5):e78. Epub 2007 Apr 10
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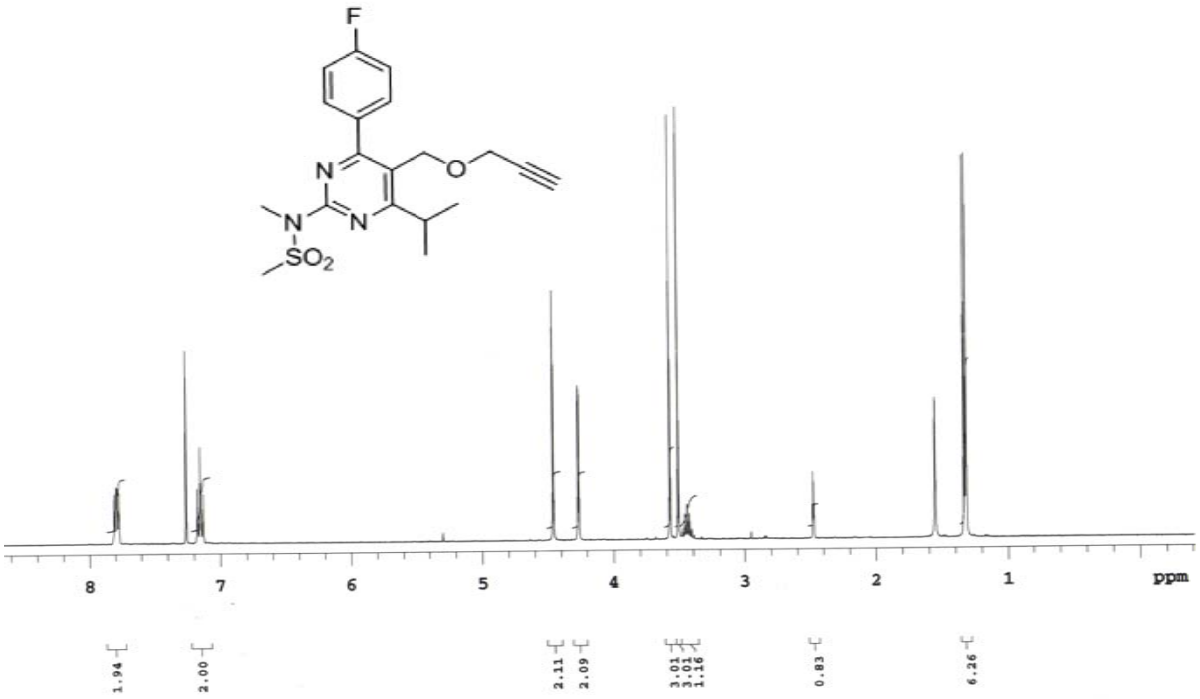
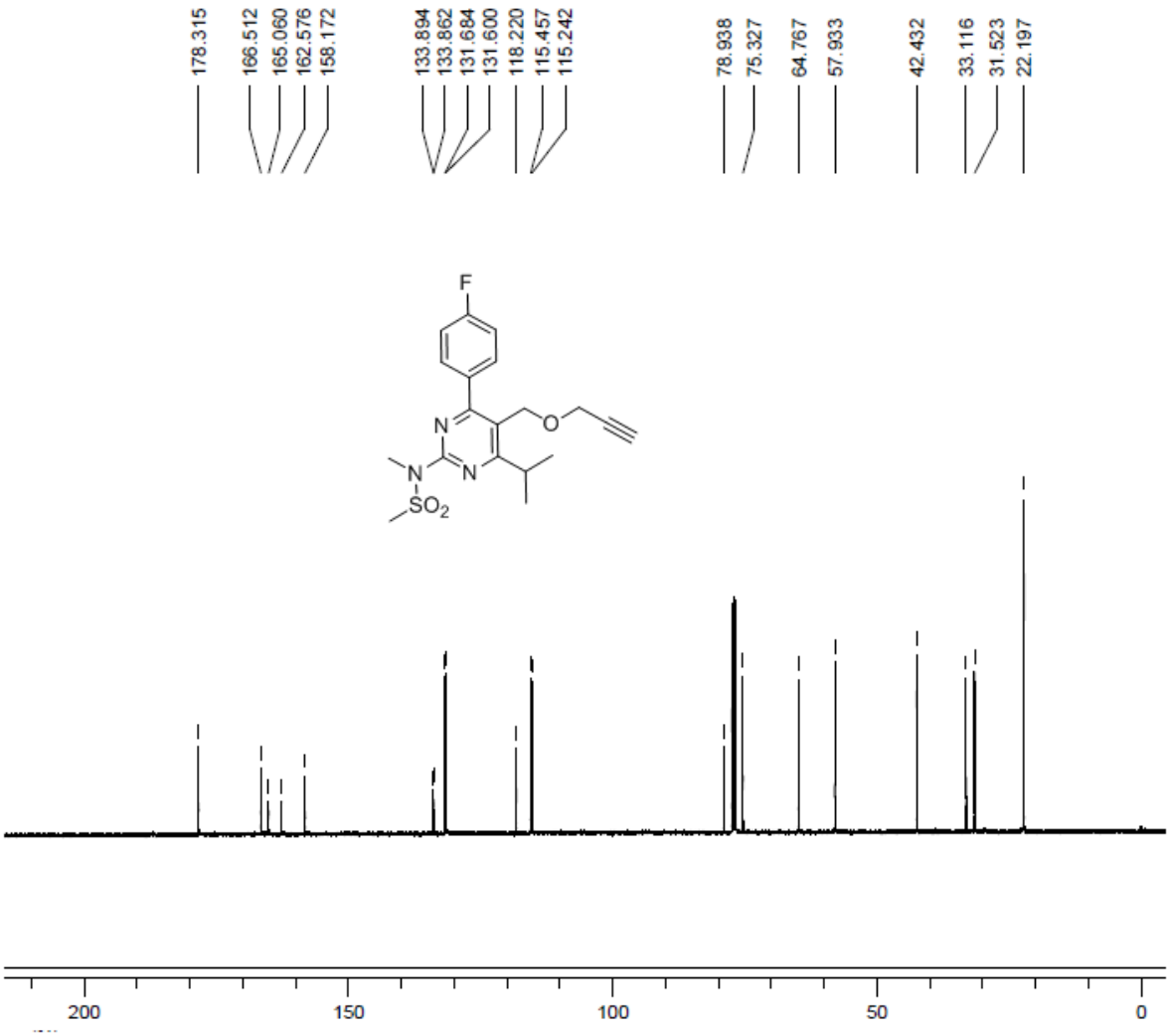
Copies of spectra

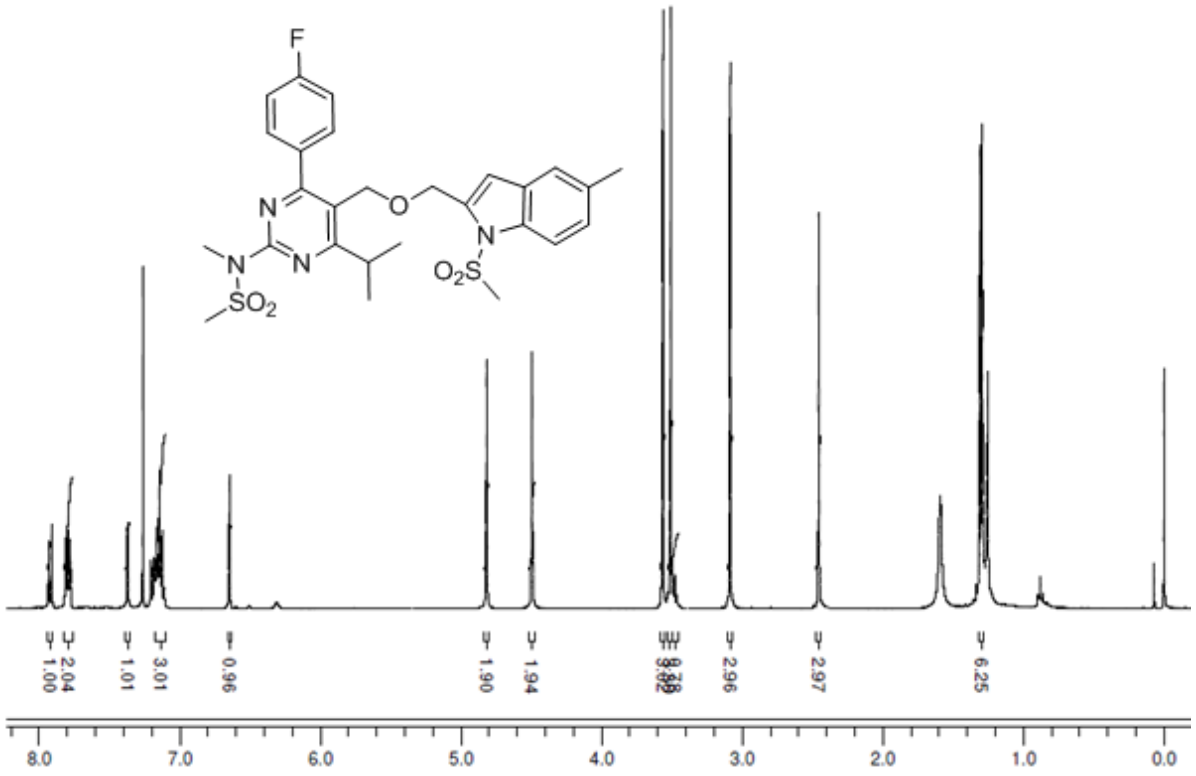
1 ^{1}H NMR (400 MHz, $CDCl_3$)



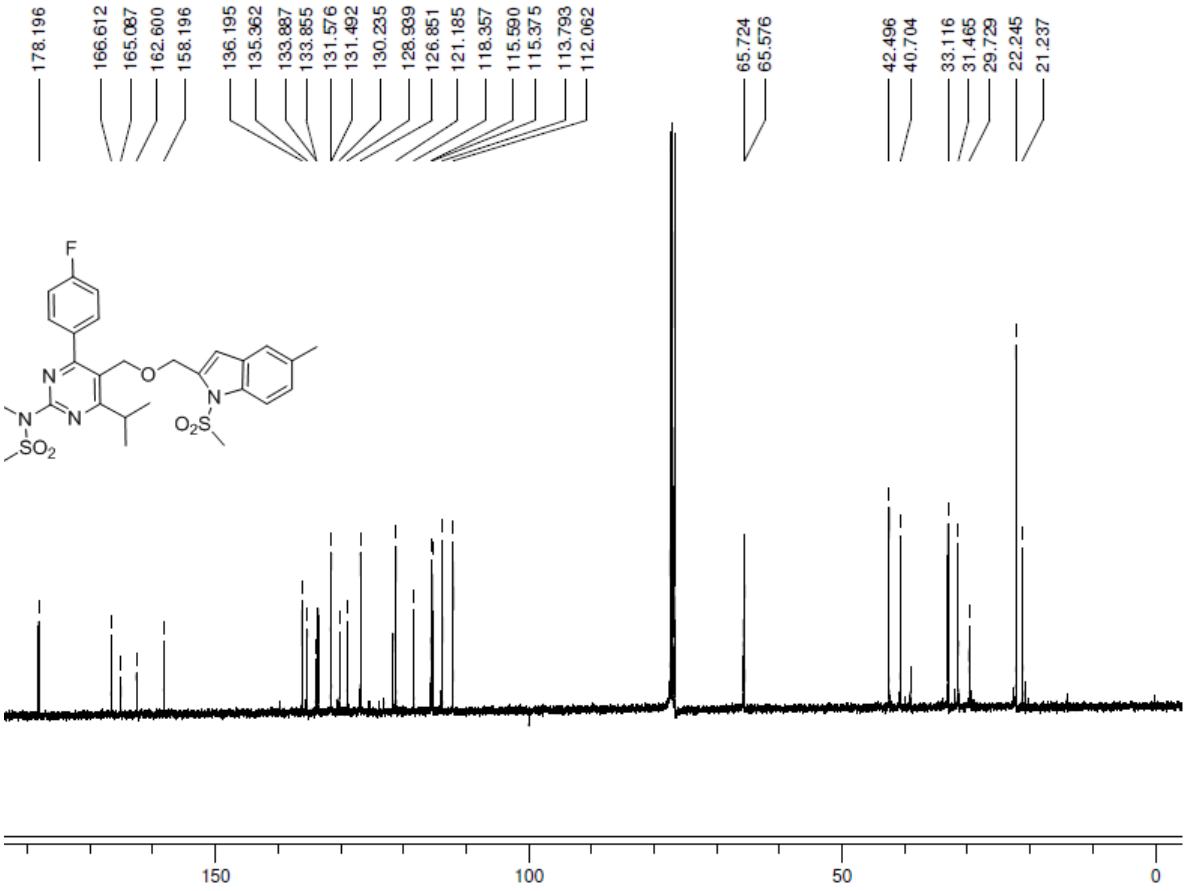
1 ^{13}C NMR (400 MHz, $CDCl_3$)



3a ^{1}H NMR (100 MHz, $CDCl_3$)



3a ^{13}C NMR (100 MHz, $CDCl_3$)



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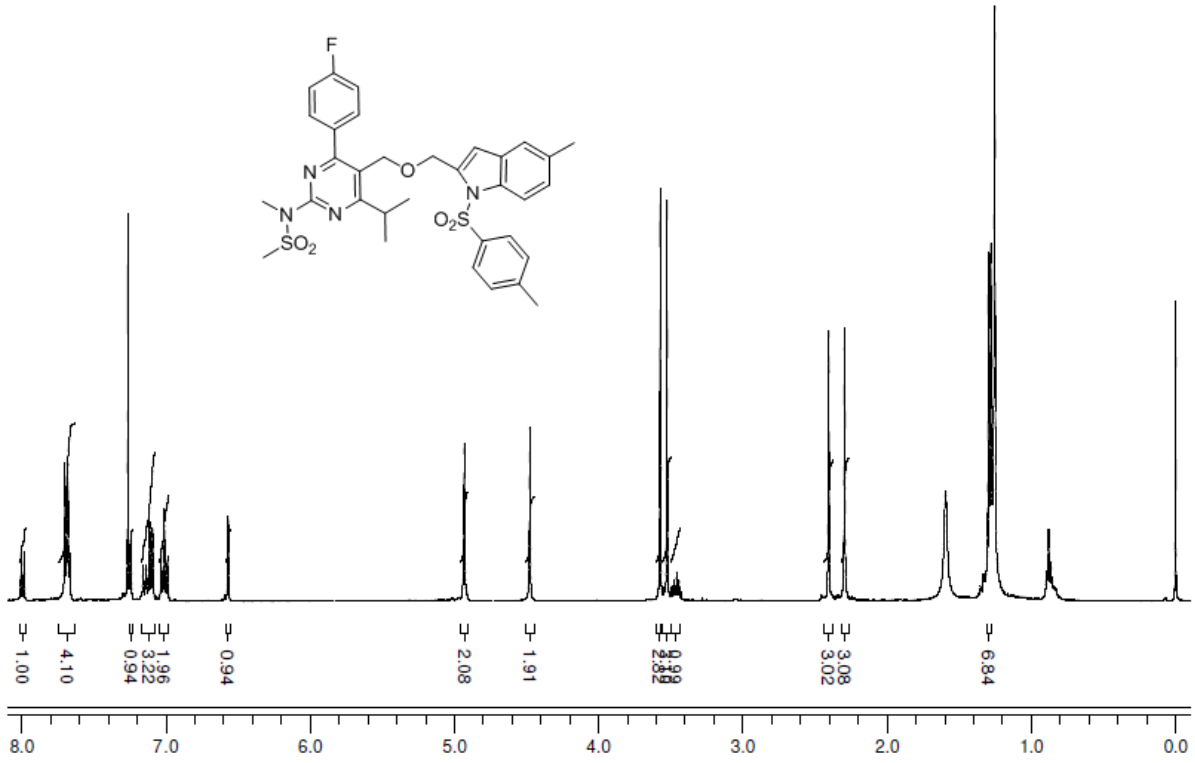

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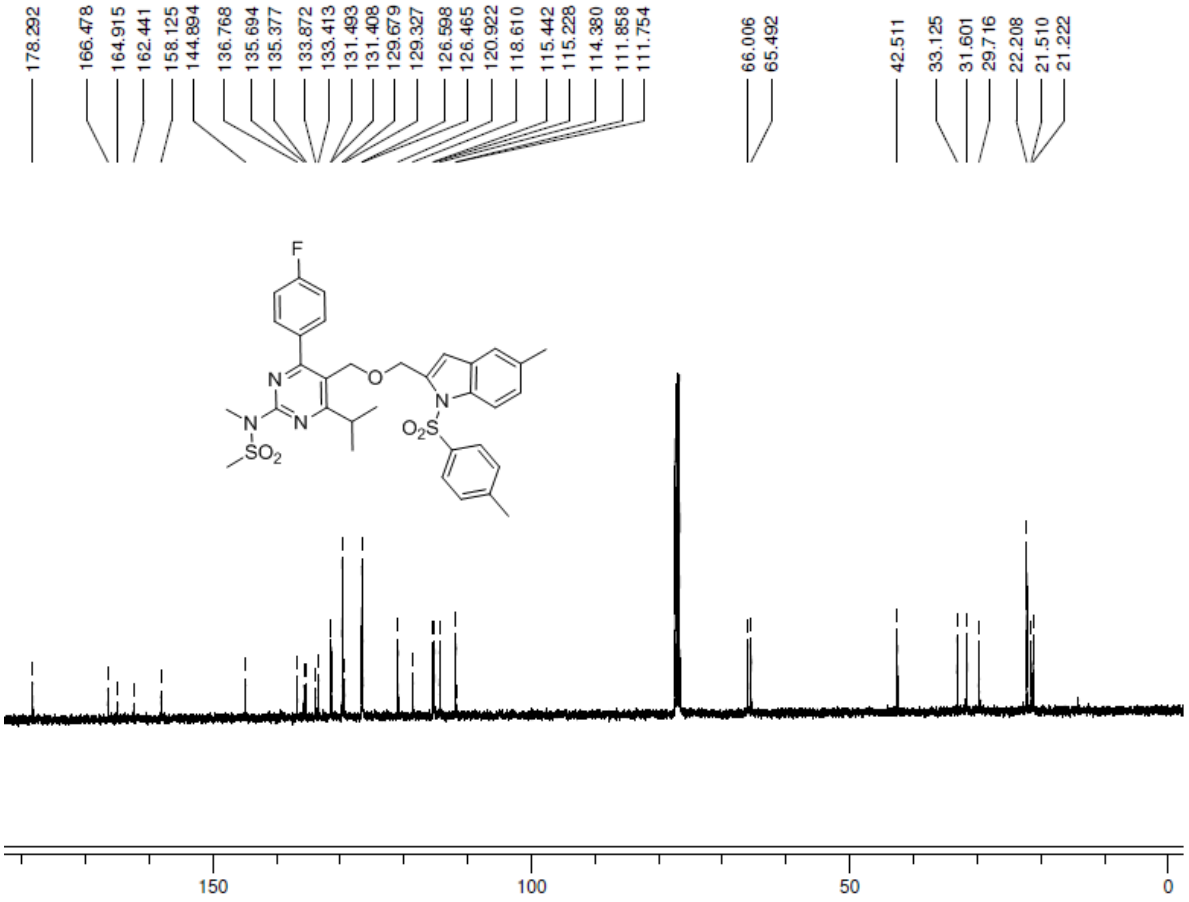

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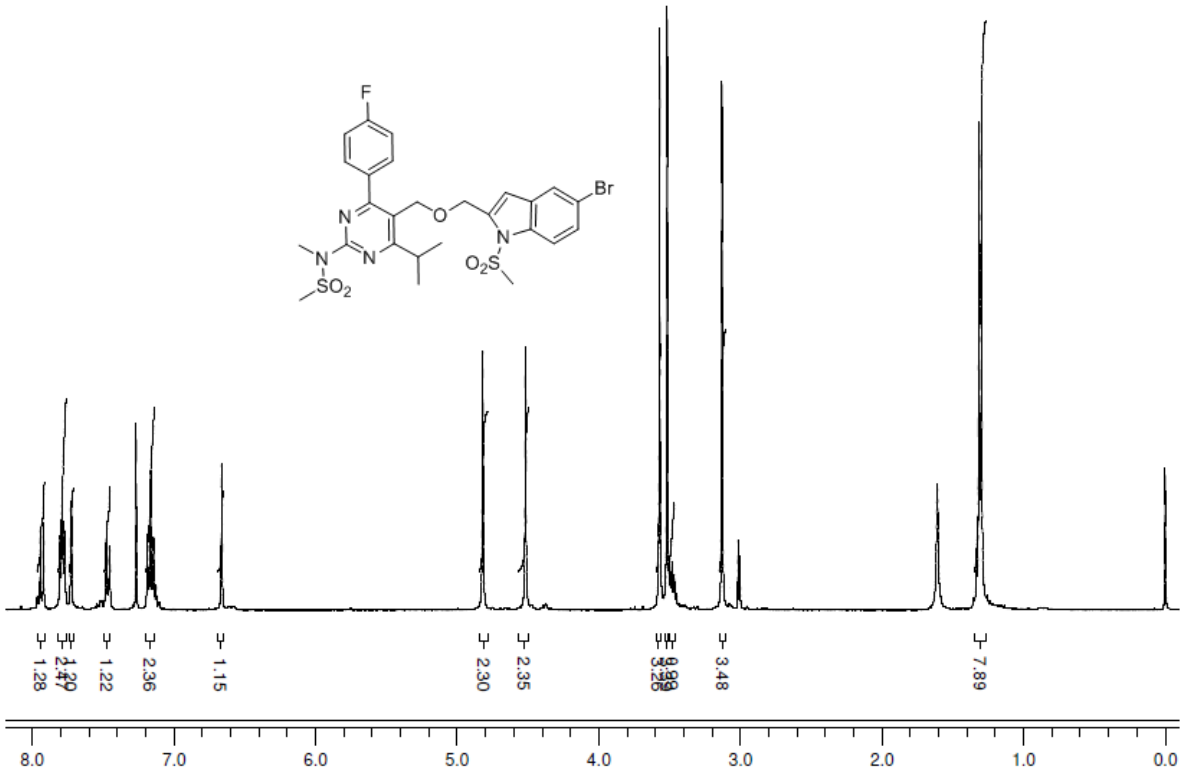
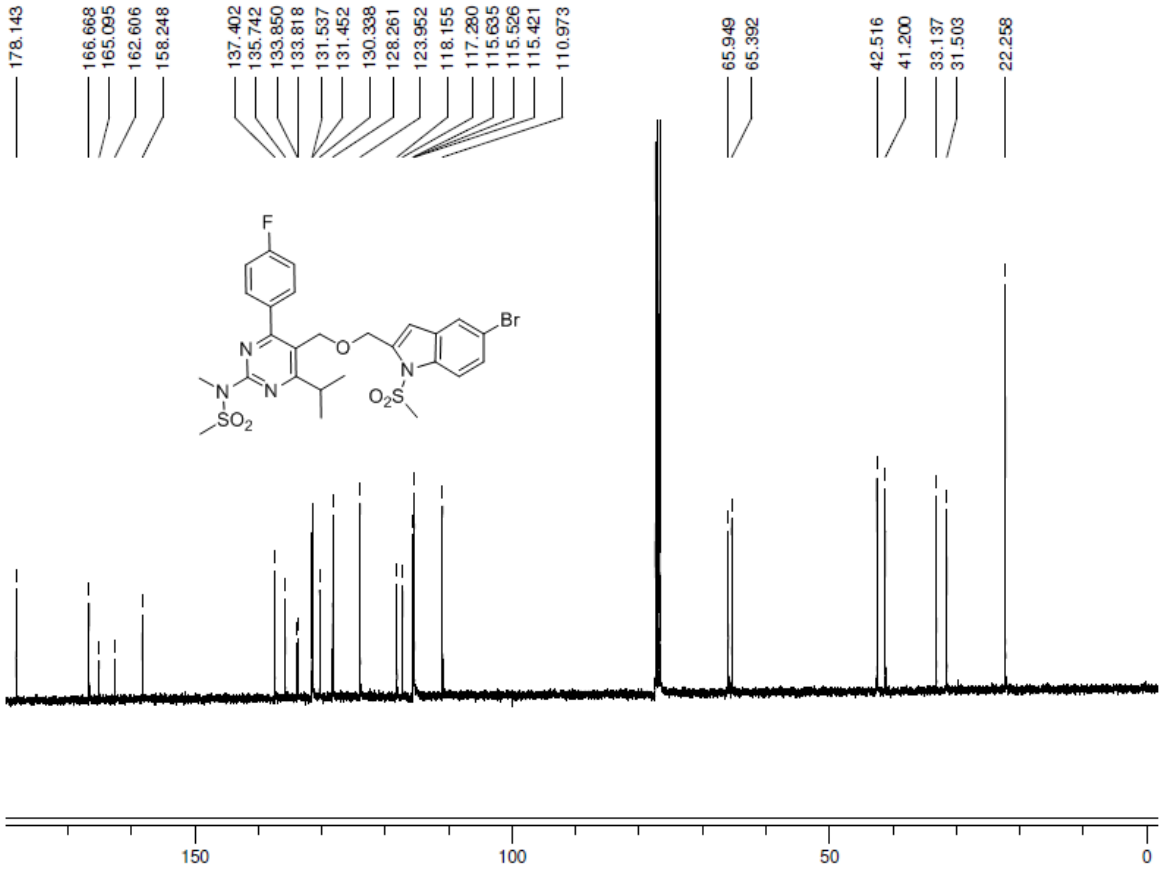
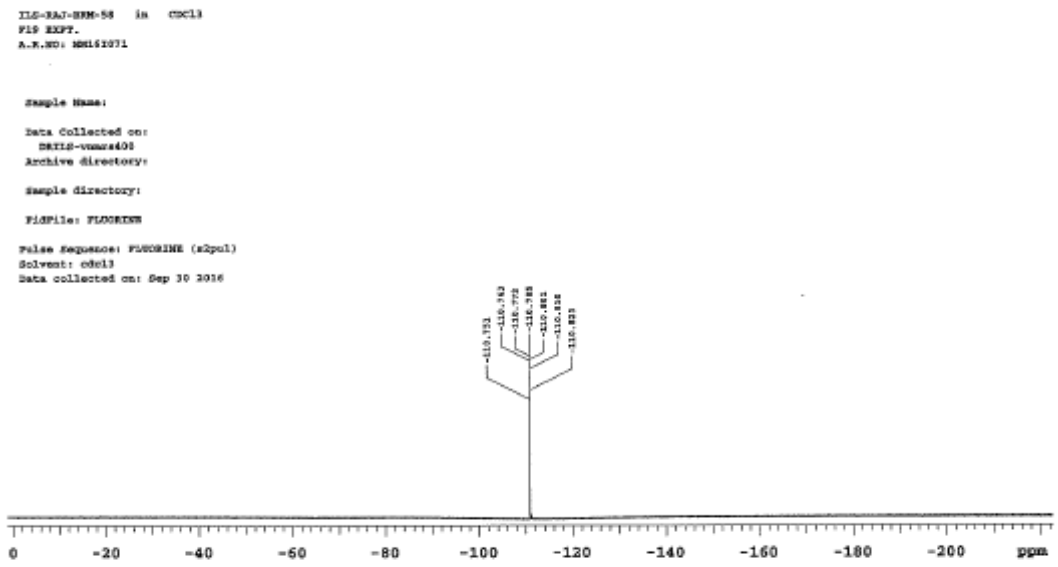


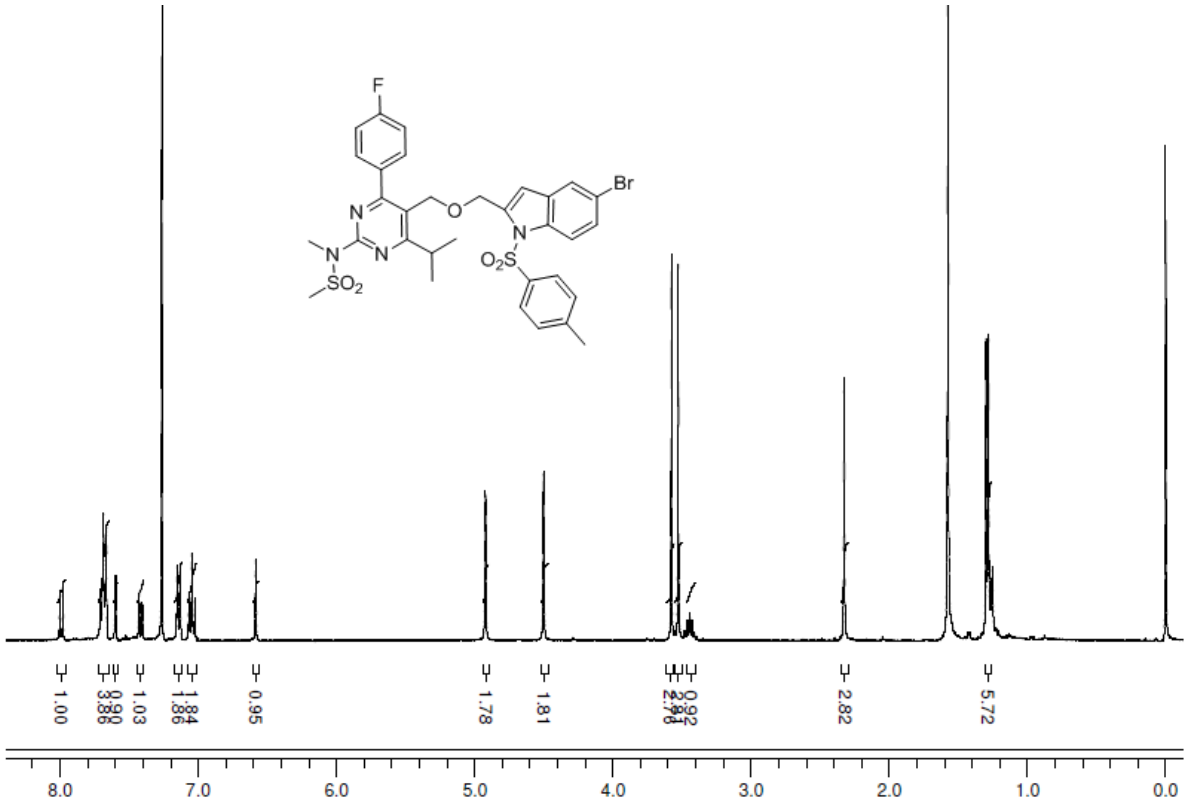
3c ^{13}C NMR (100 MHz, $CDCl_3$)



3c ^{19}F NMR (400 MHz, $CDCl_3$)



3d ^{1}H NMR (400 MHz, $CDCl_3$)



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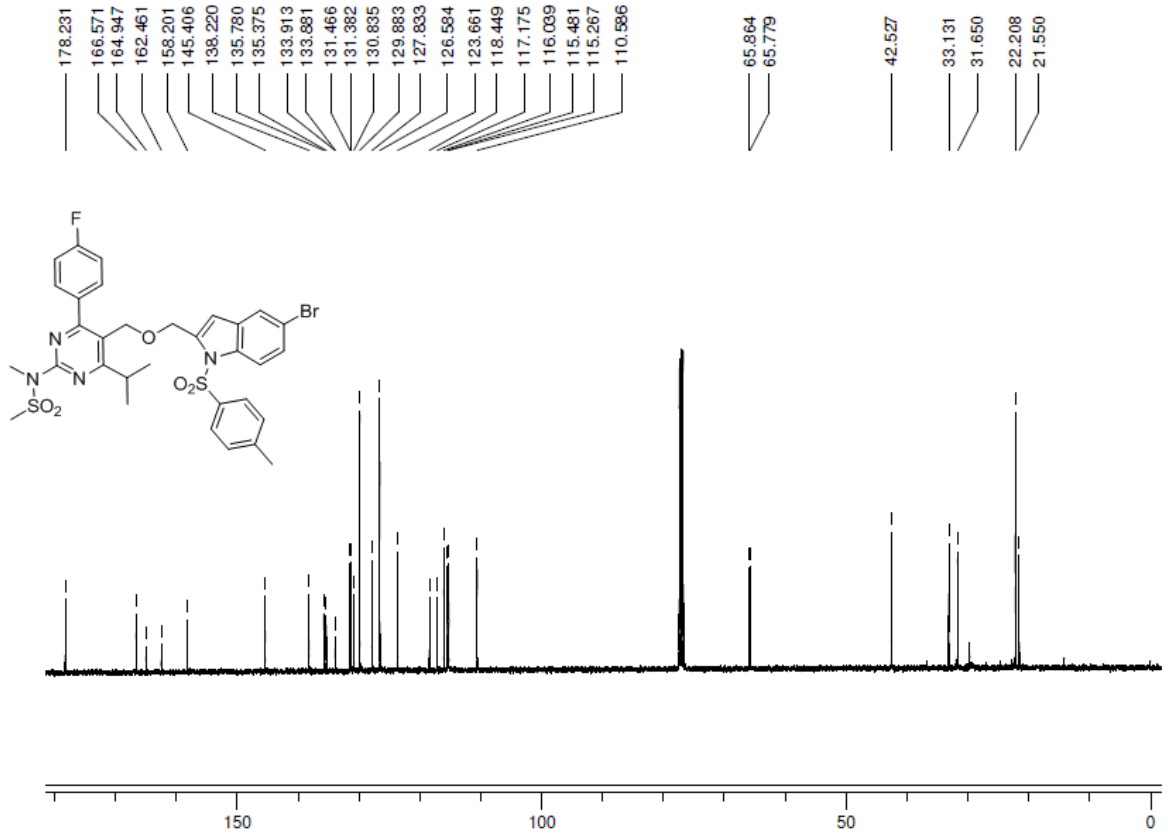

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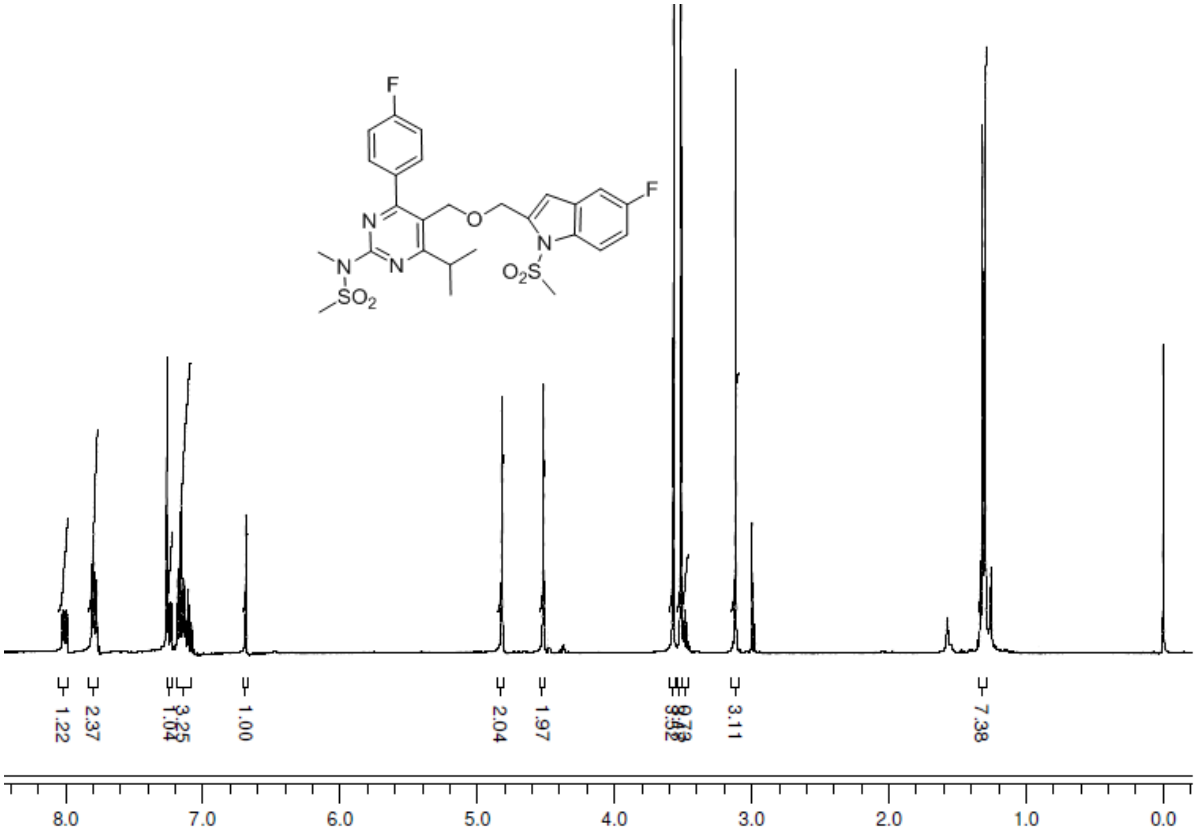
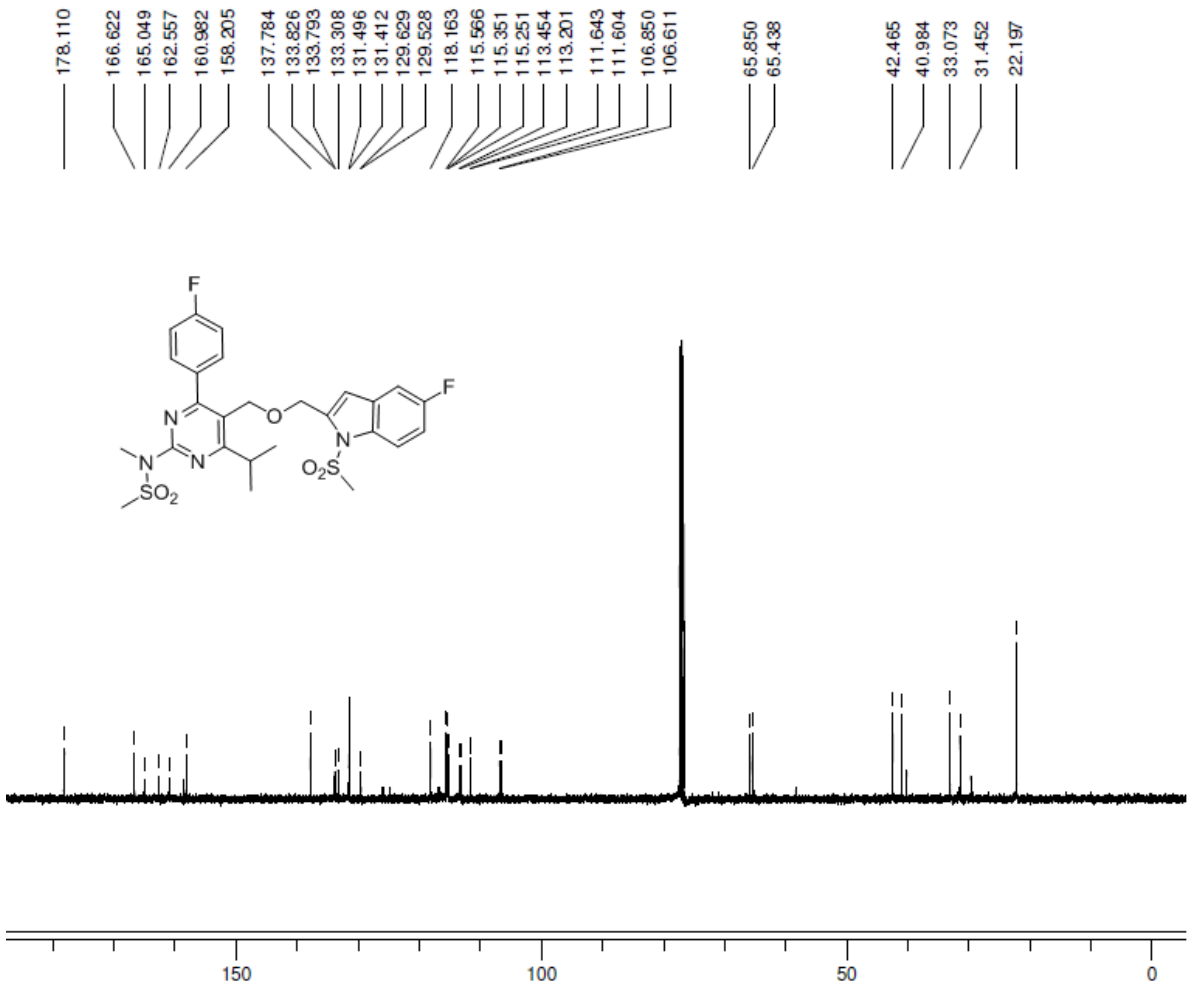


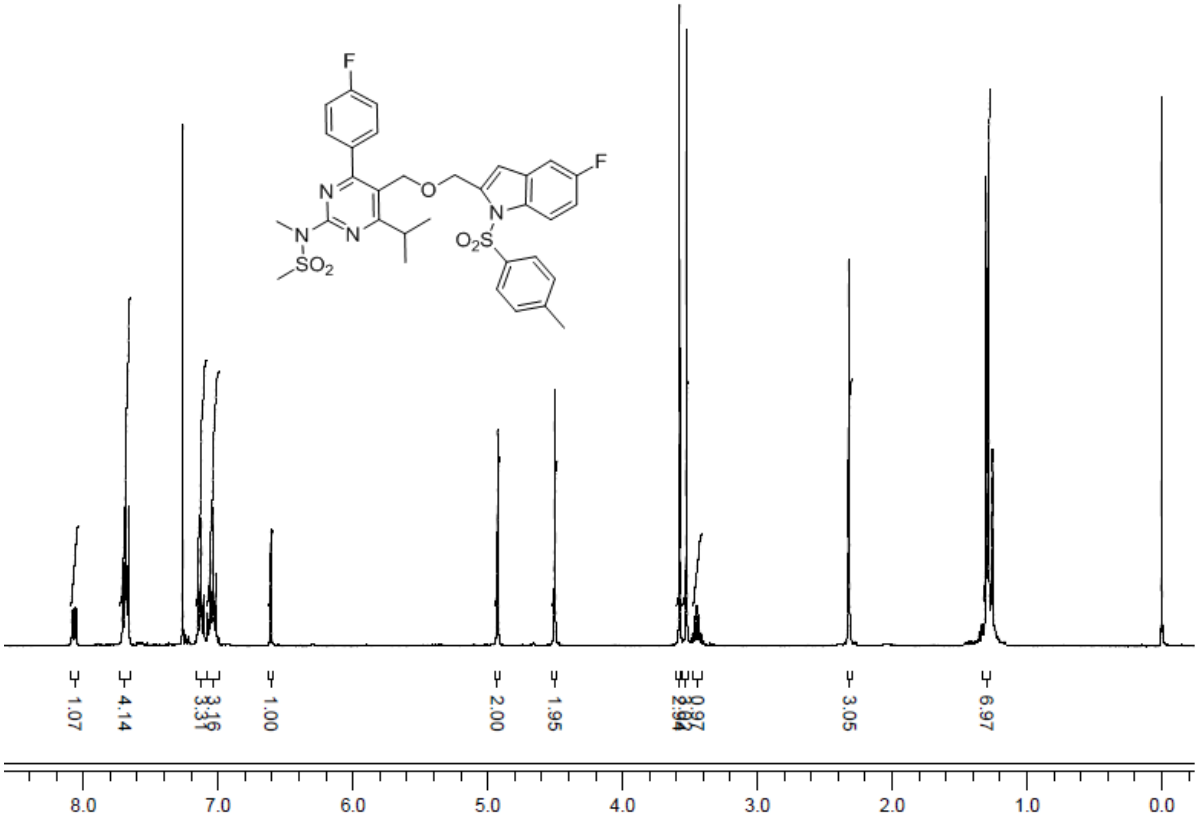
3e

1

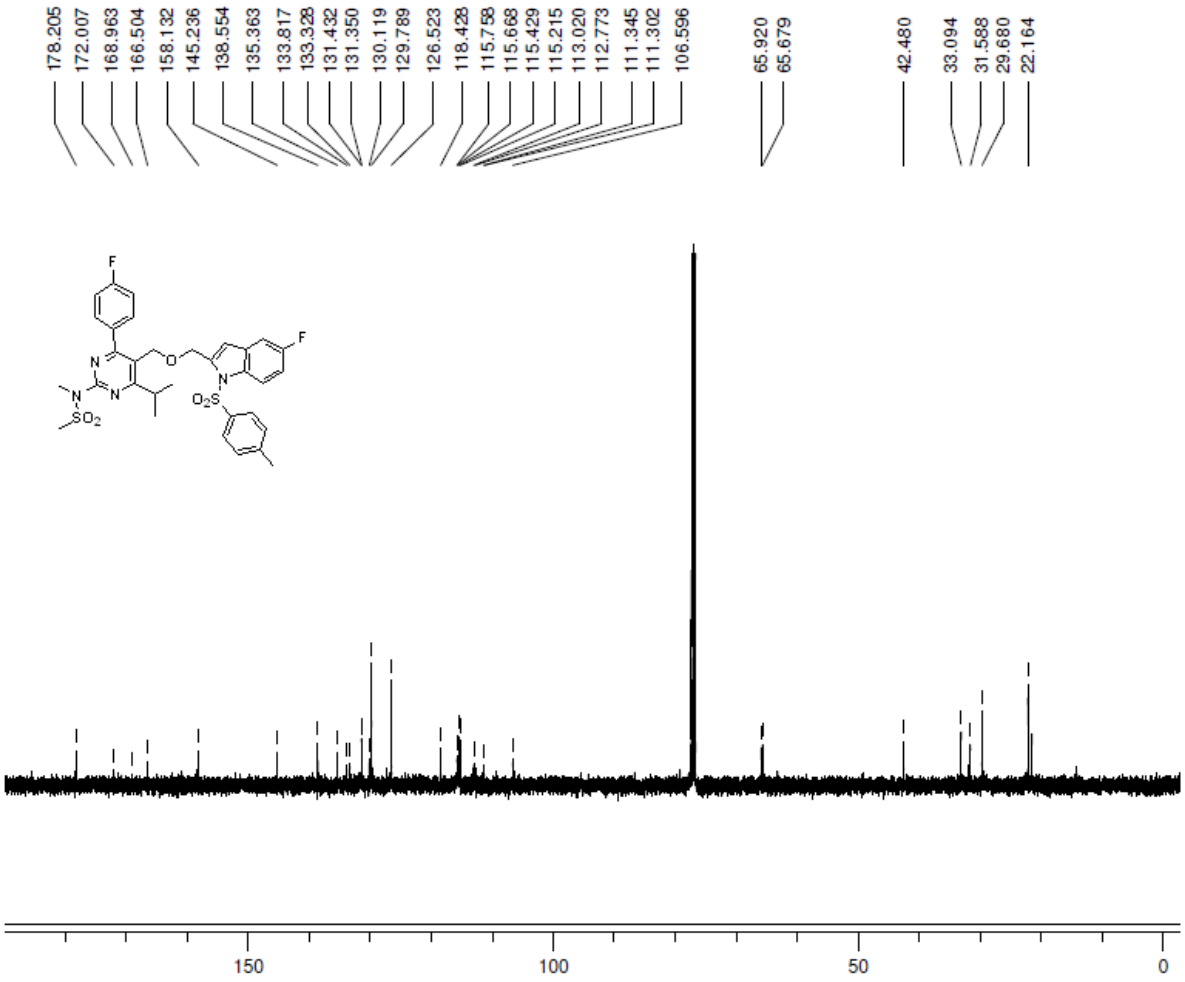
3e ^{13}C NMR (100 MHz, $CDCl_3$)



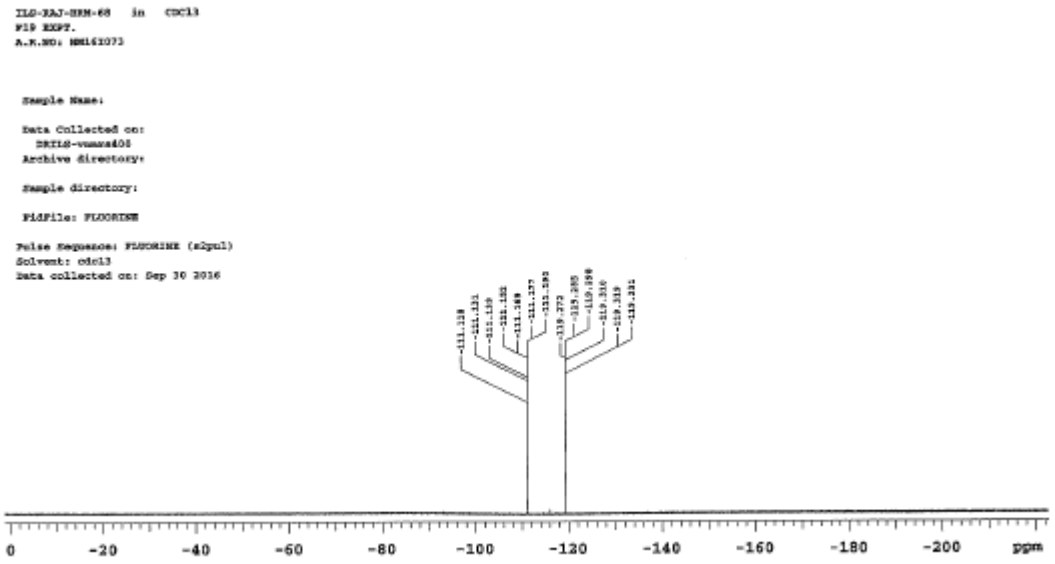
3f ^{1}H NMR (400 MHz, $CDCl_3$)



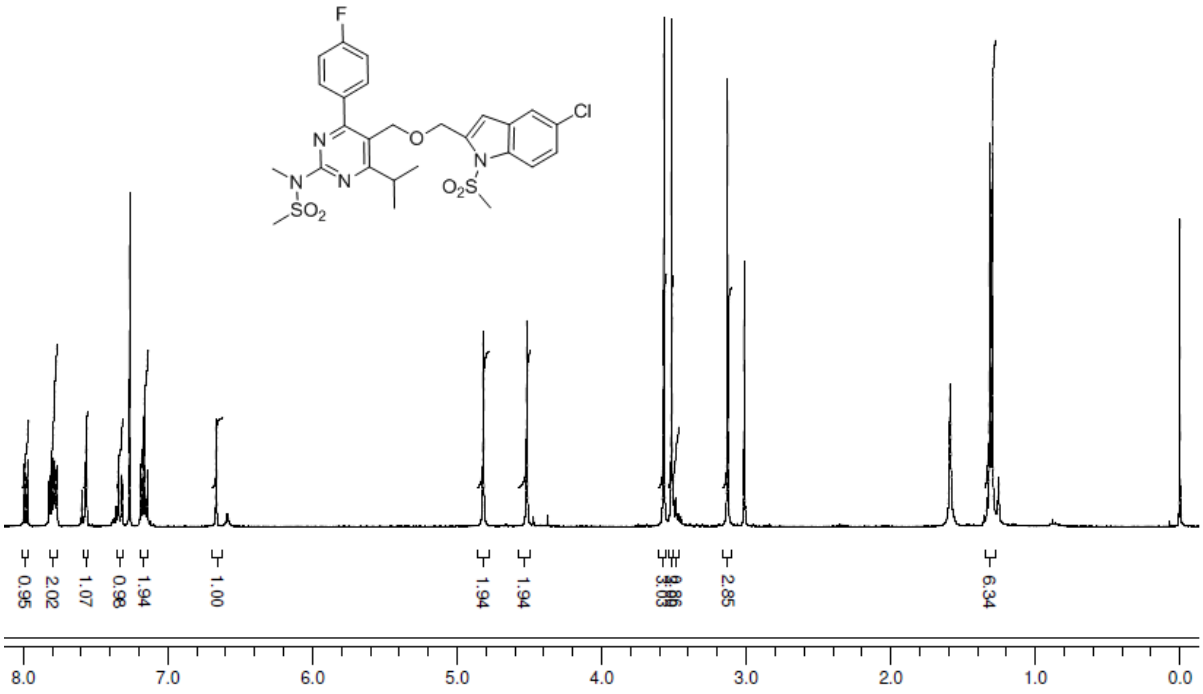
3f ^{13}C NMR (100 MHz, $CDCl_3$)



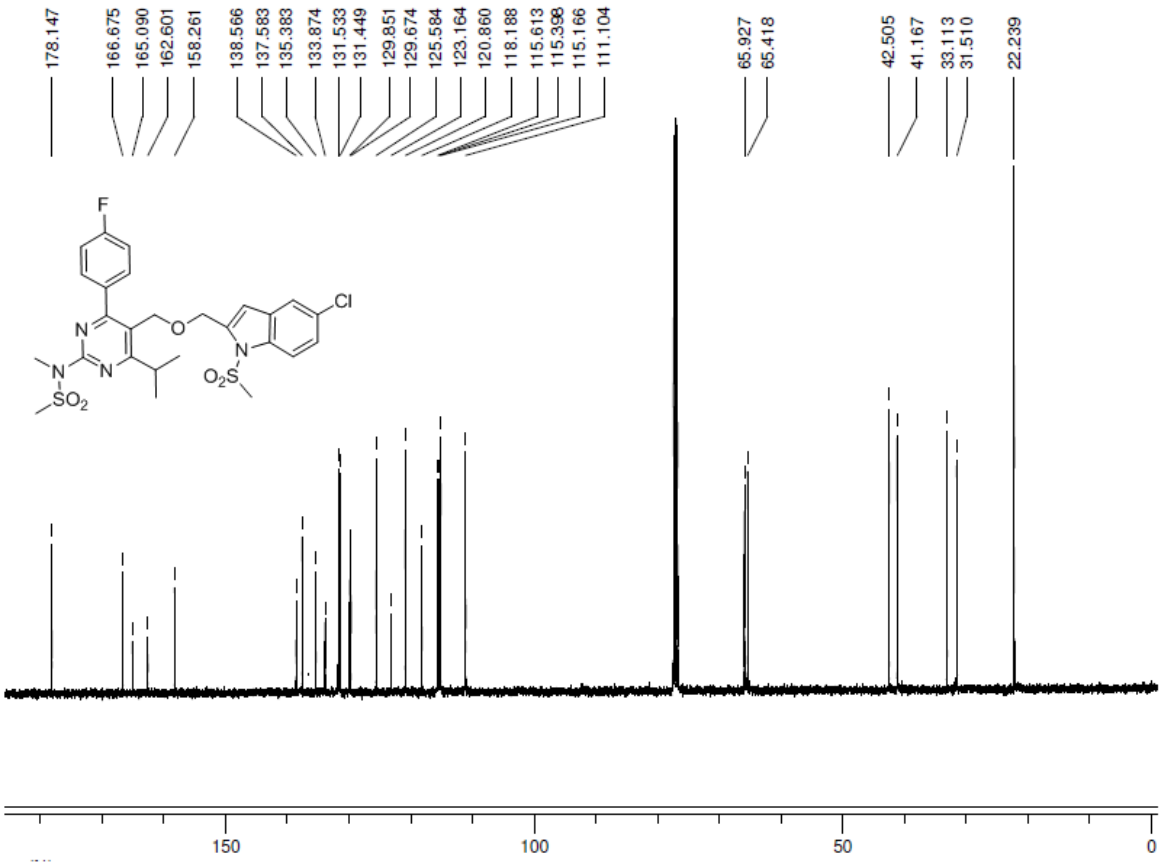
3f ^{19}F NMR (400 MHz, $CDCl_3$)



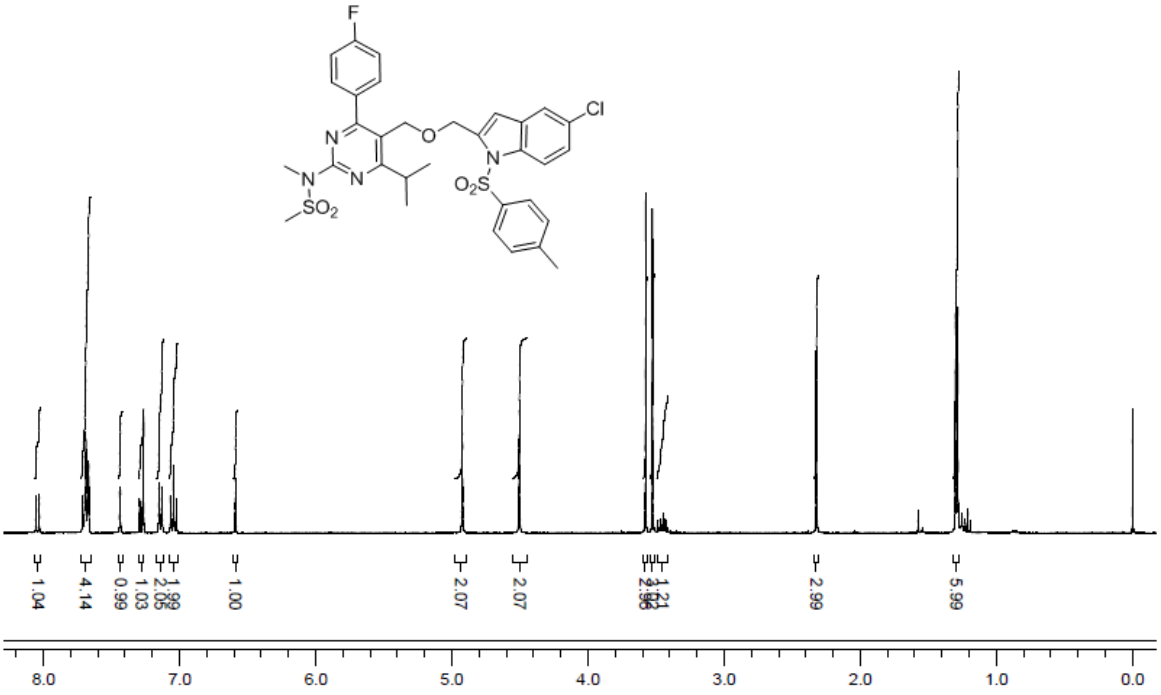
3g ^{1}H NMR (400 MHz, $CDCl_3$)



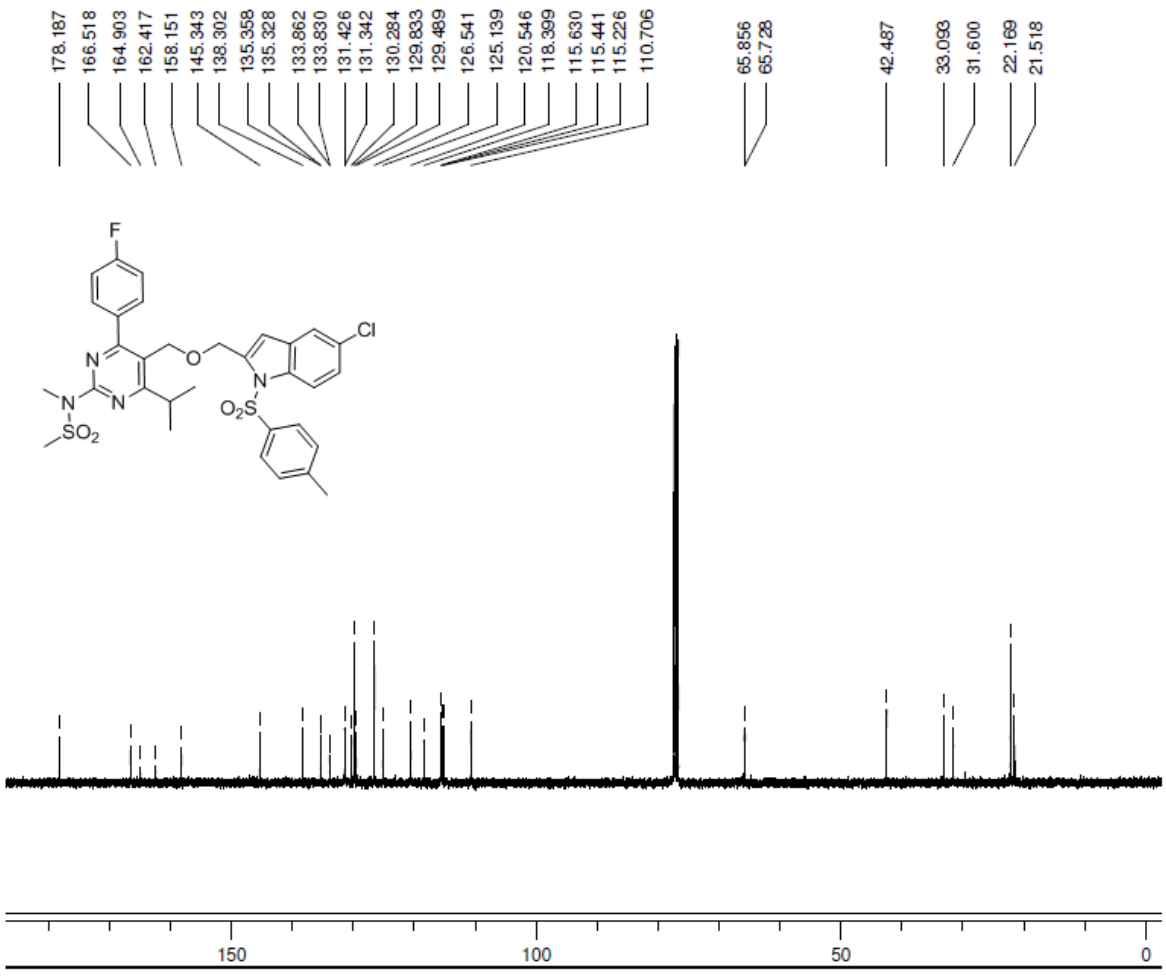
3g ^{13}C NMR (100 MHz, $CDCl_3$)



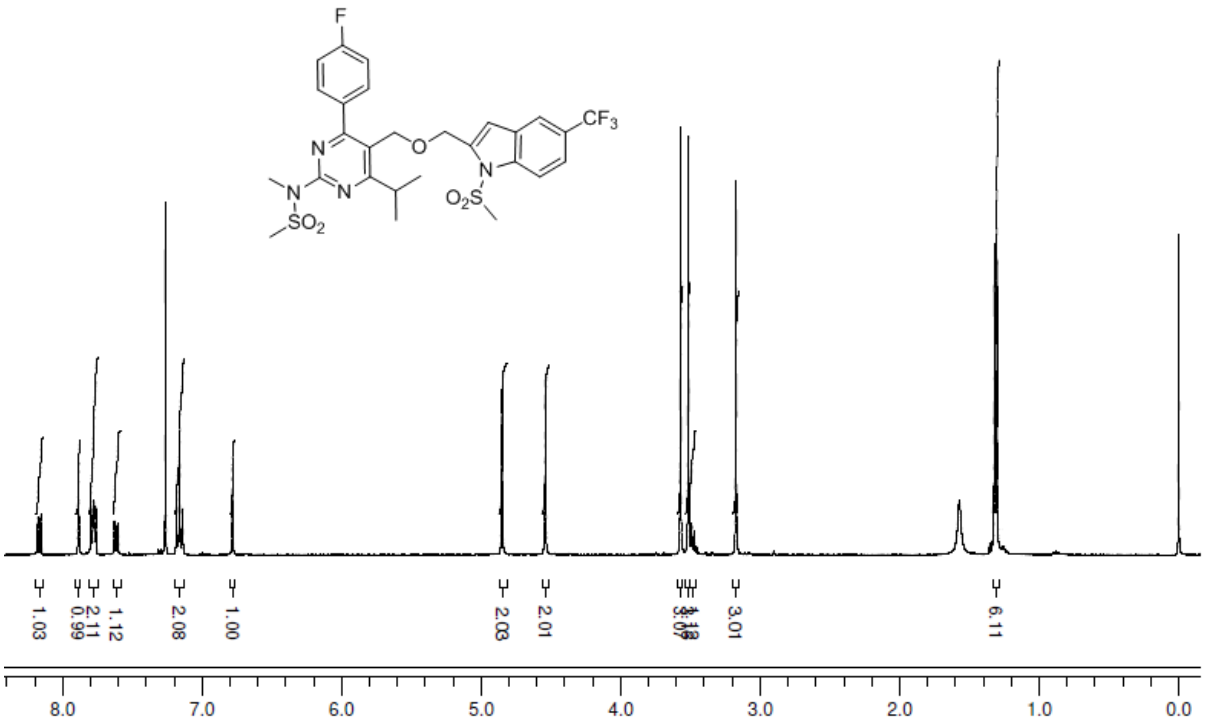
3h ^{1}H NMR (400 MHz, $CDCl_3$)



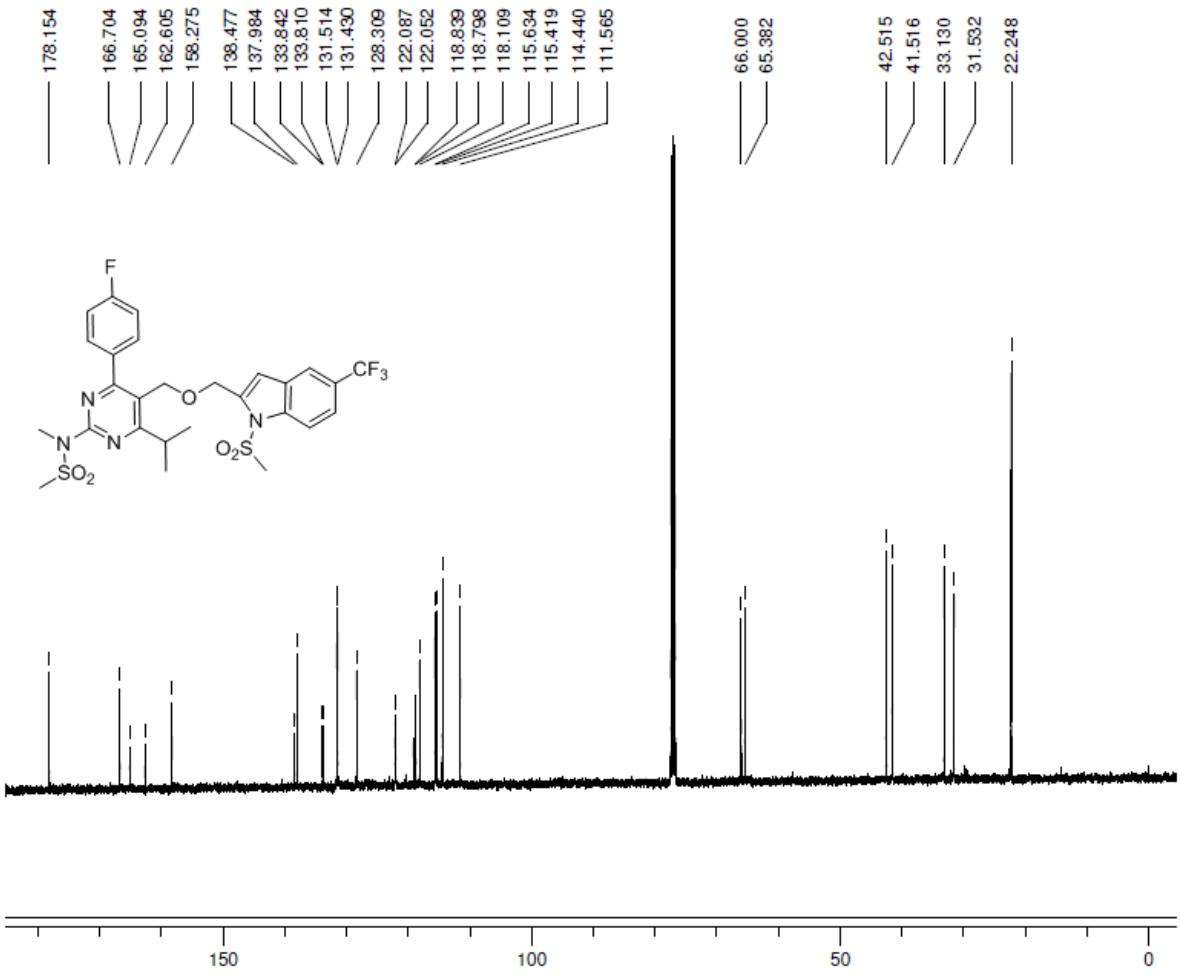
3h ^{13}C NMR (100 MHz, $CDCl_3$)



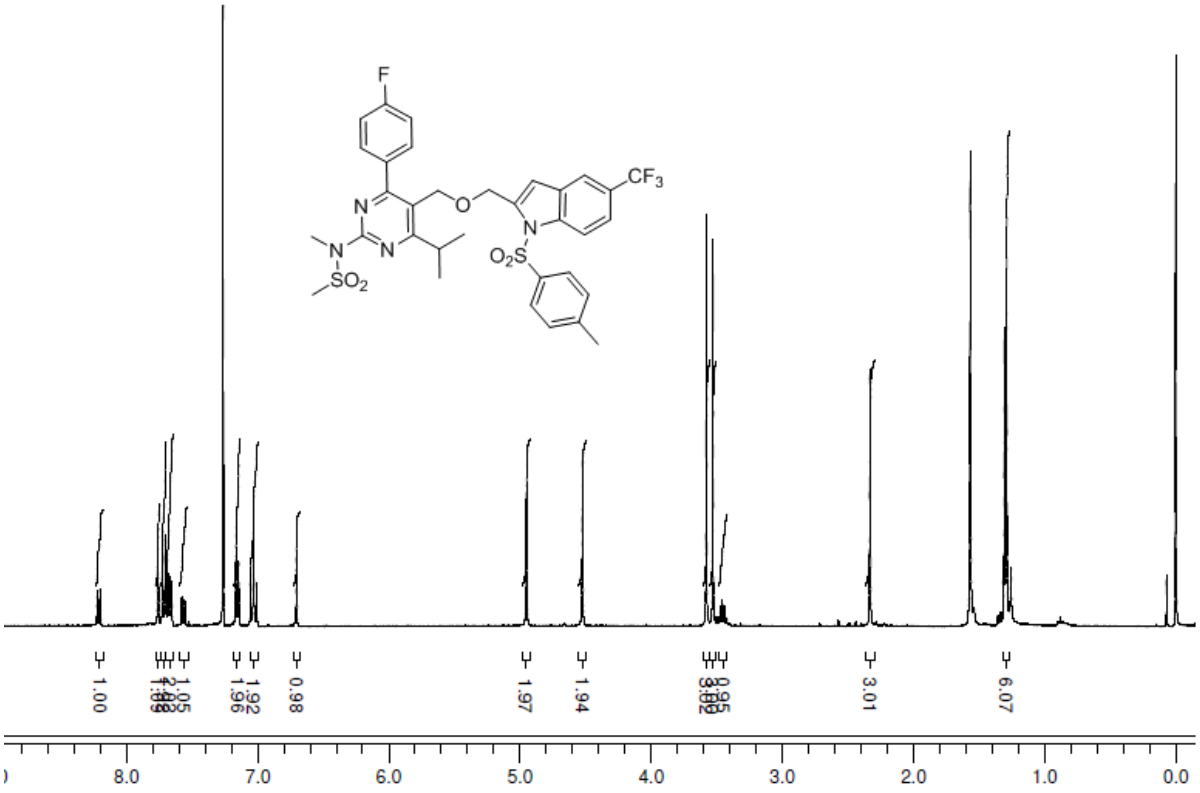
3i ^{1}H NMR (400 MHz, $CDCl_3$)



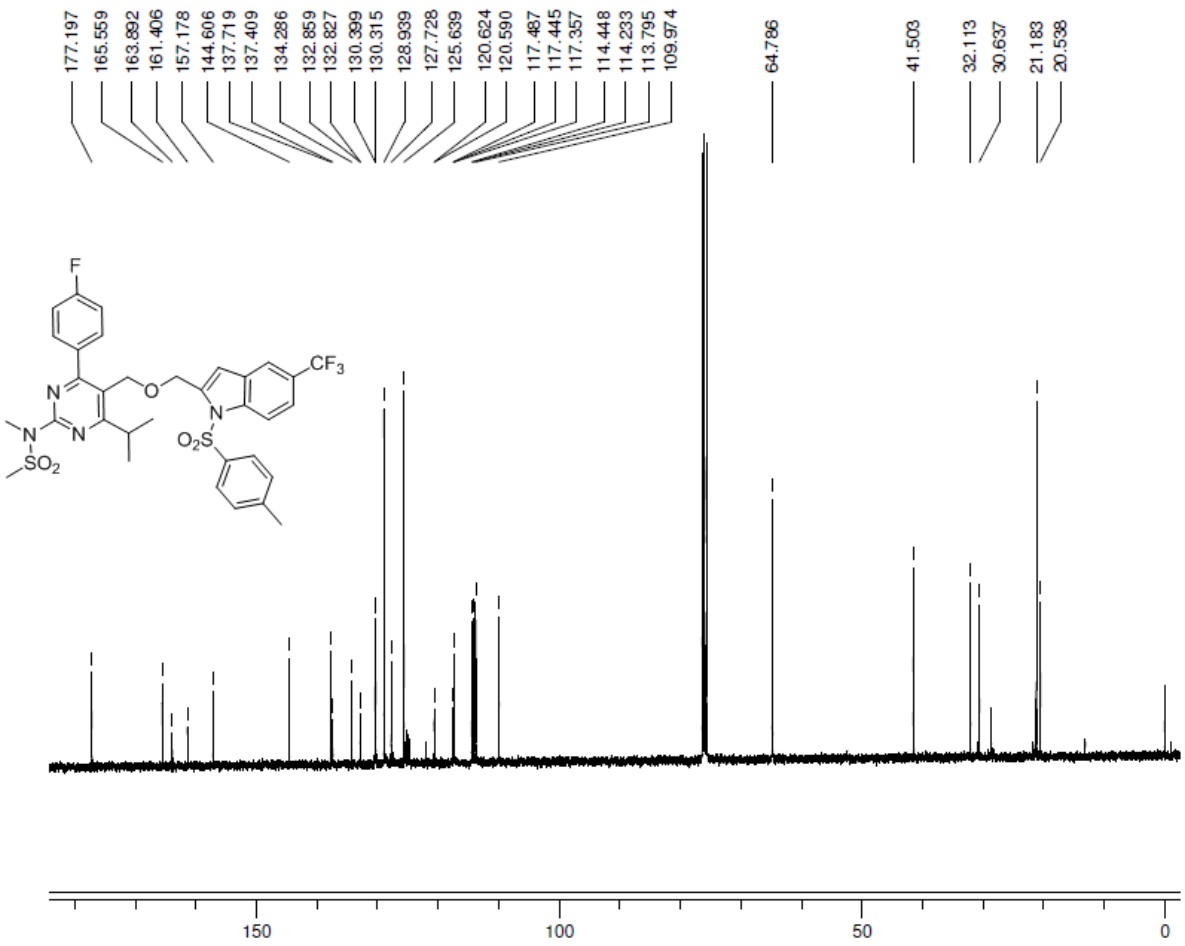
3i ^{13}C NMR (100 MHz, $CDCl_3$)



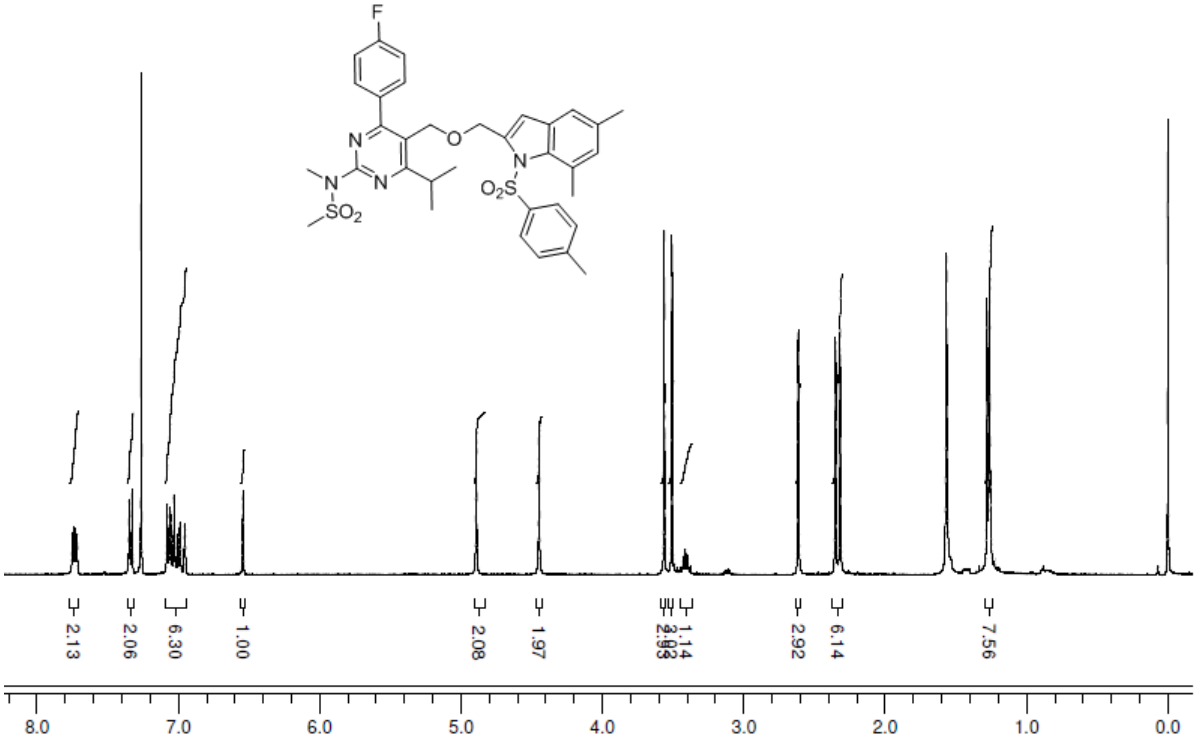
3j ^{1}H NMR (400 MHz, $CDCl_3$)



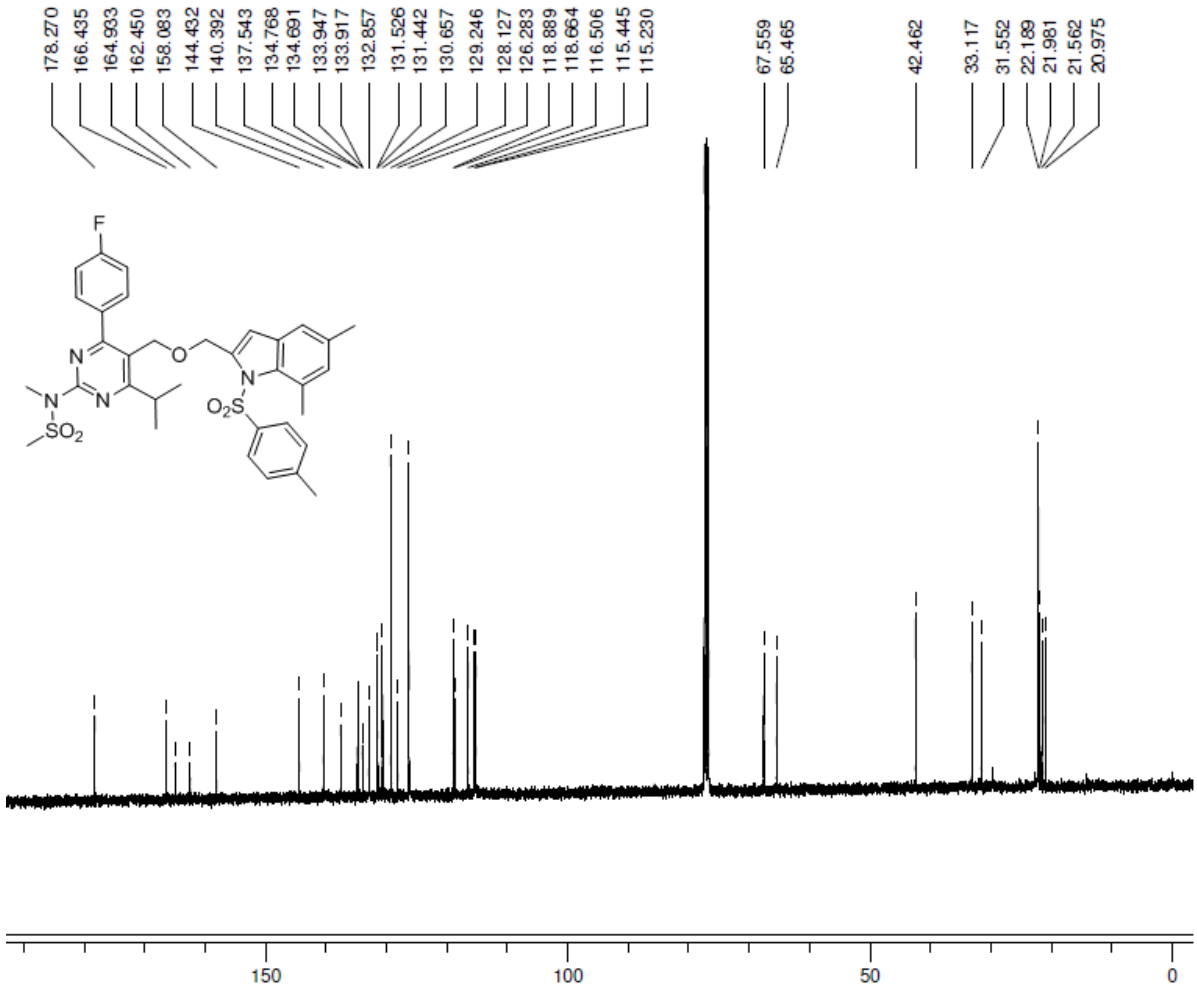
3j ^{13}C NMR (100 MHz, $CDCl_3$)



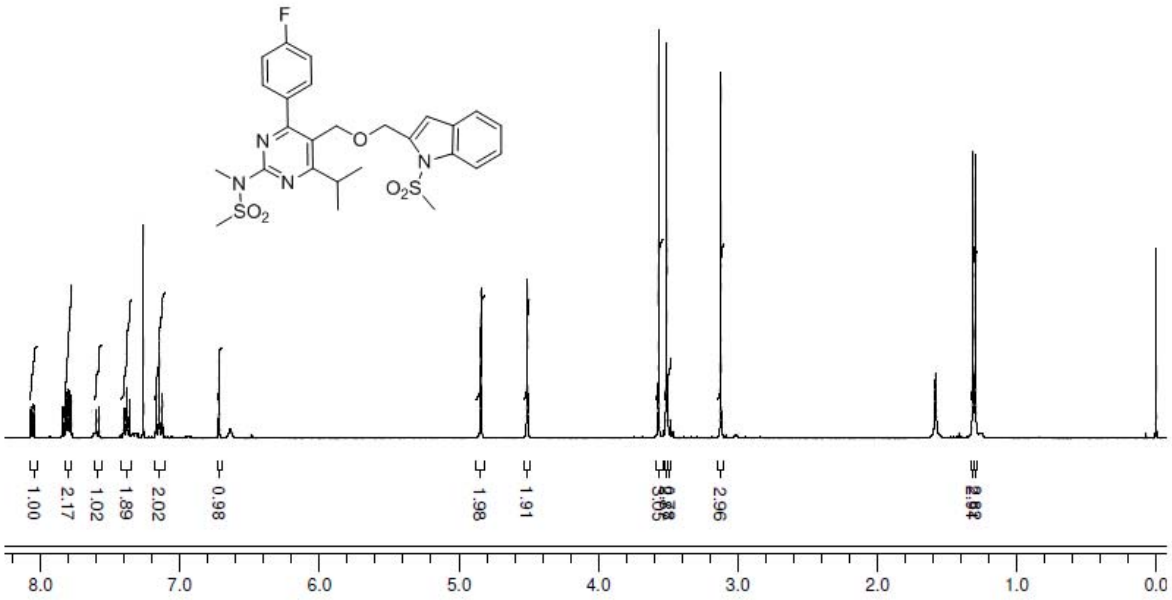
3k ^{1}H NMR (400 MHz, $CDCl_3$)



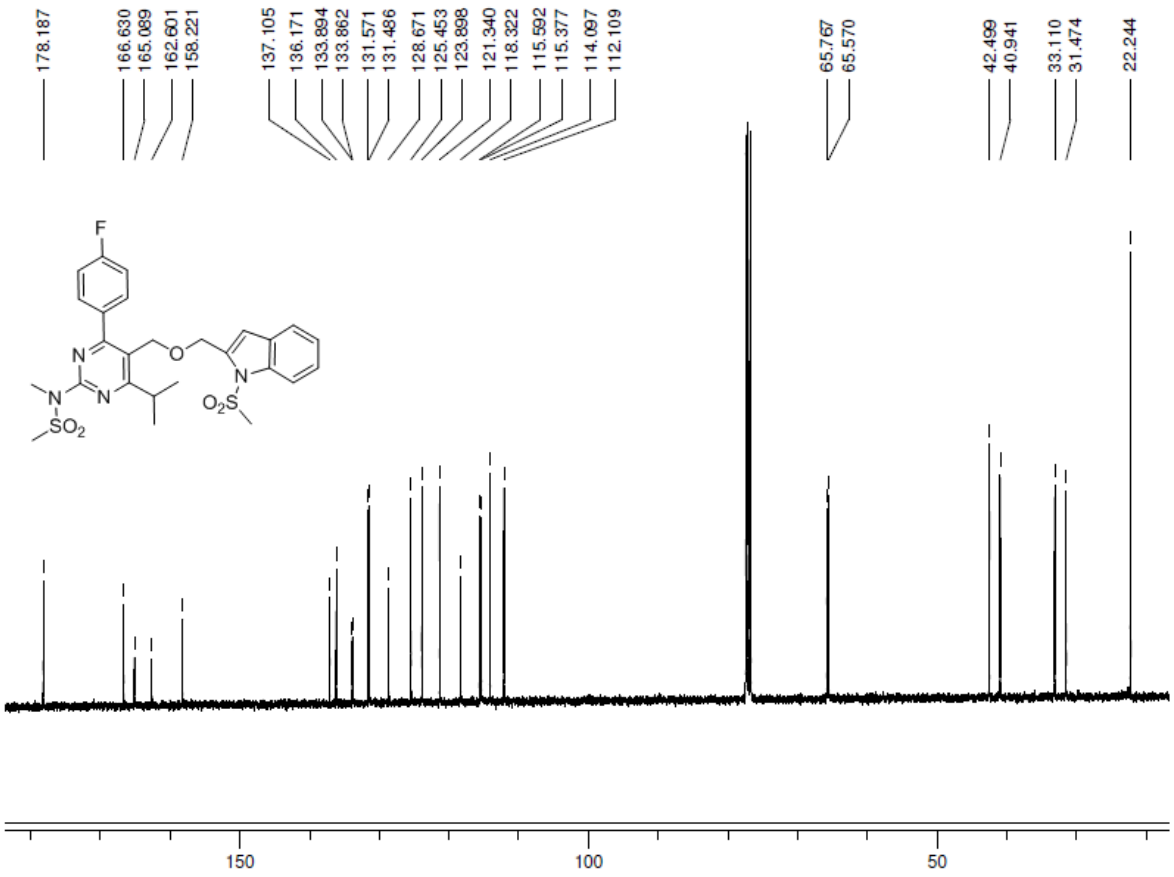
3k ^{13}C NMR (100 MHz, $CDCl_3$)



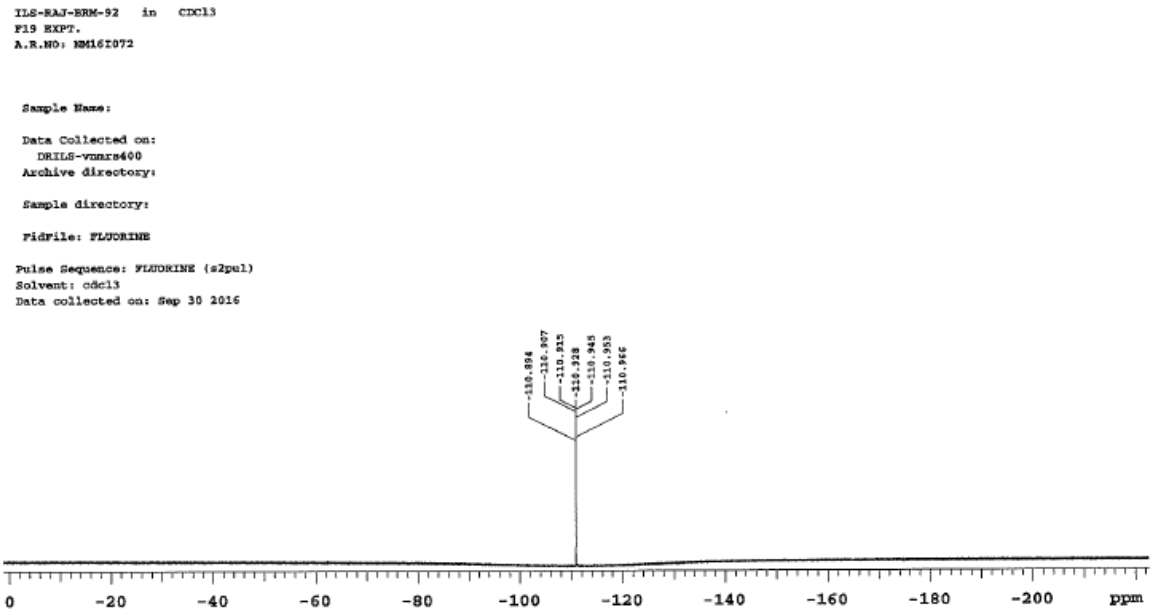
3l ^{1}H NMR (400 MHz, $CDCl_3$)



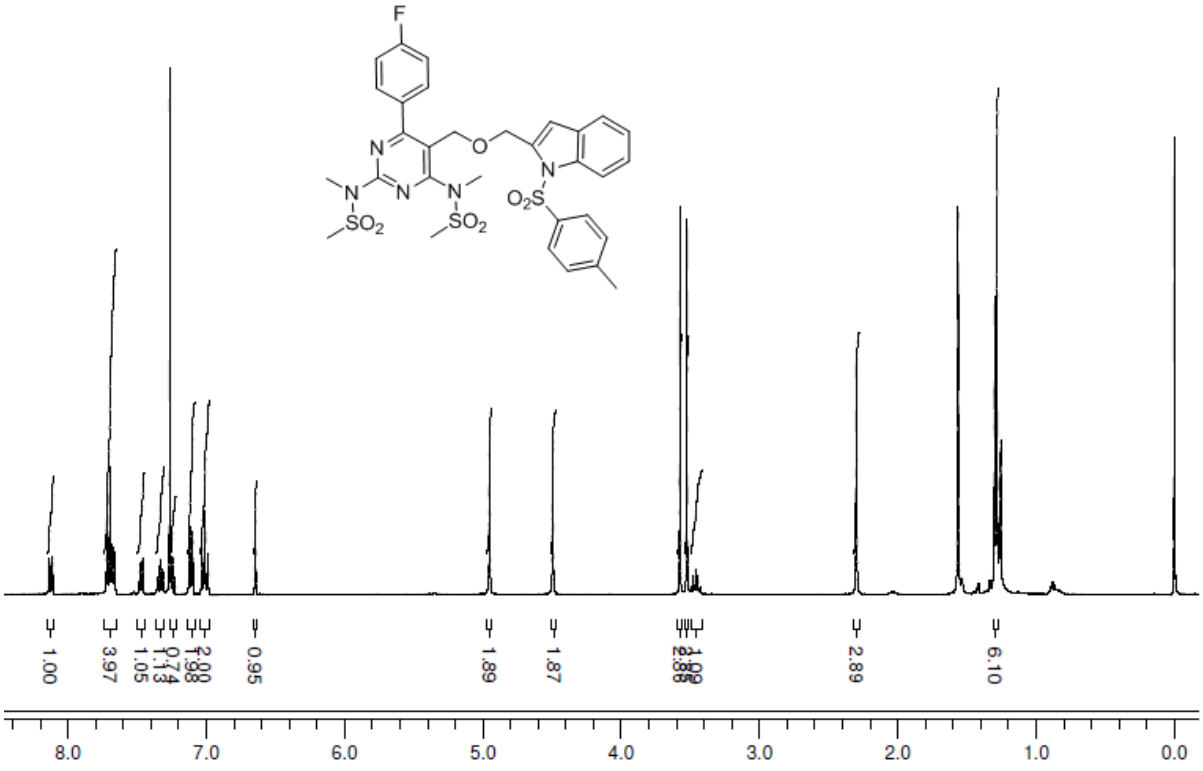
3l ^{13}C NMR (100 MHz, $CDCl_3$)



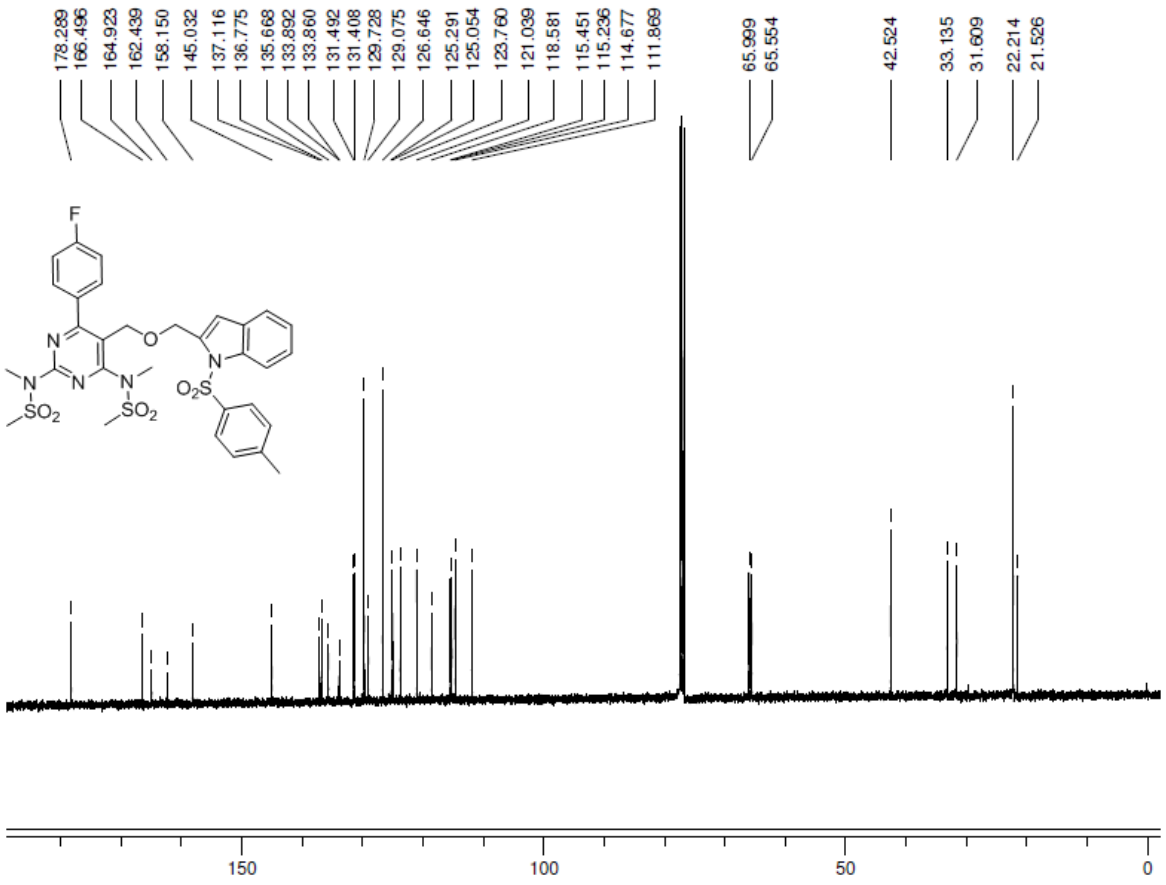
3l ^{19}F NMR (400 MHz, $CDCl_3$)



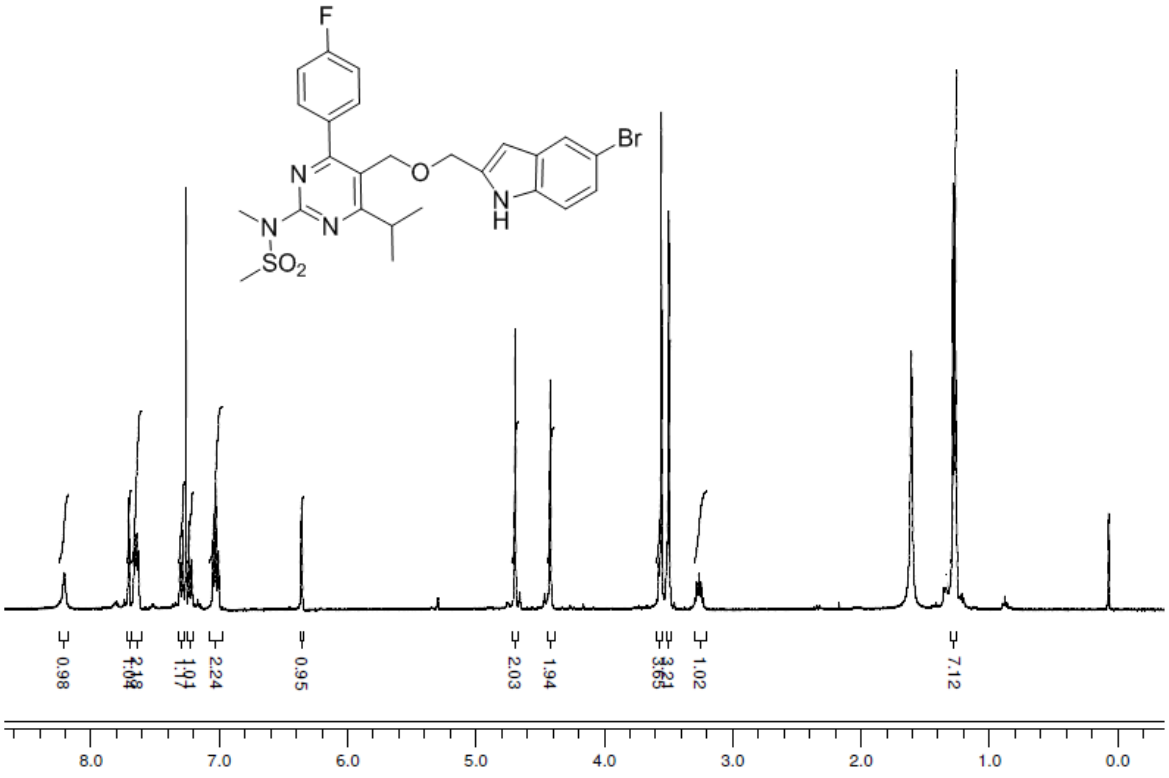
3m ^{1}H NMR (400 MHz, $CDCl_3$)



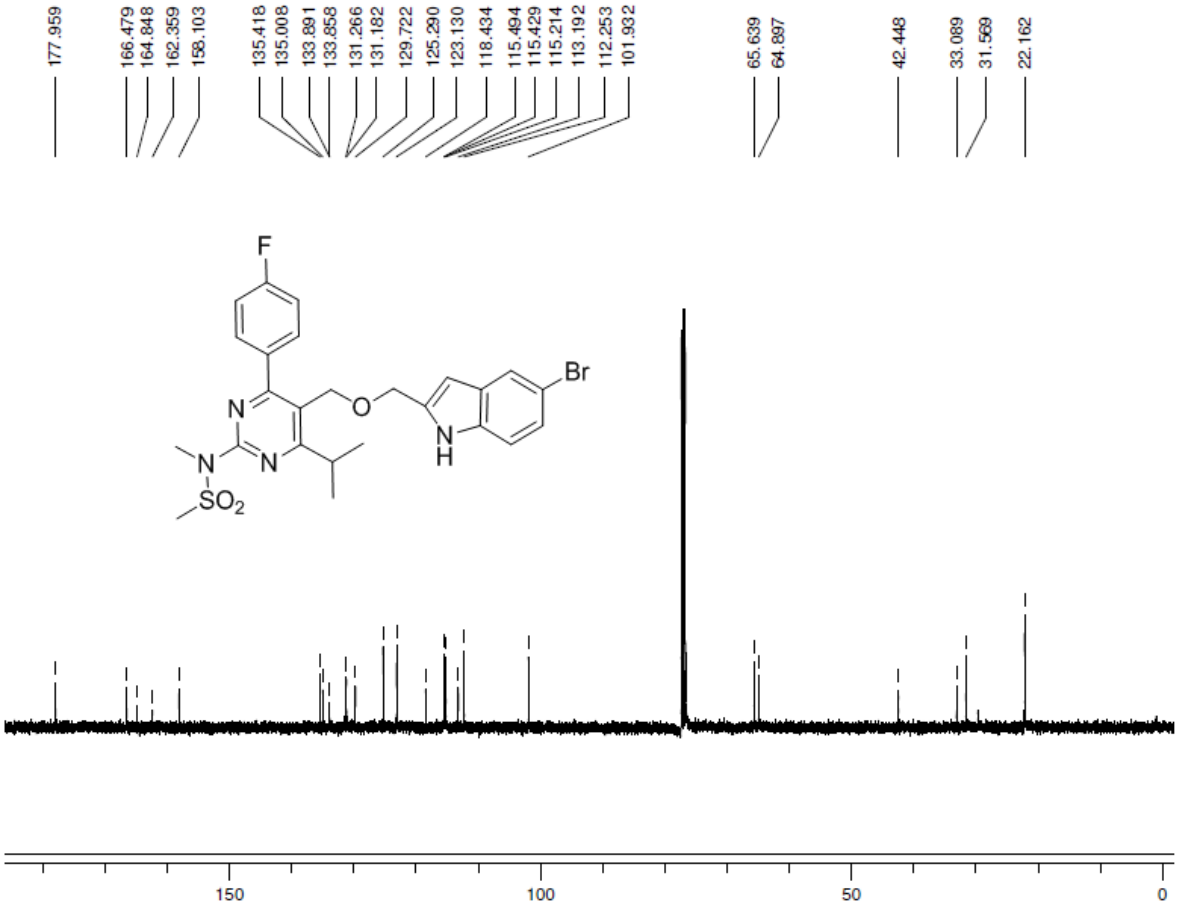
3m ^{13}C NMR (100 MHz, $CDCl_3$)



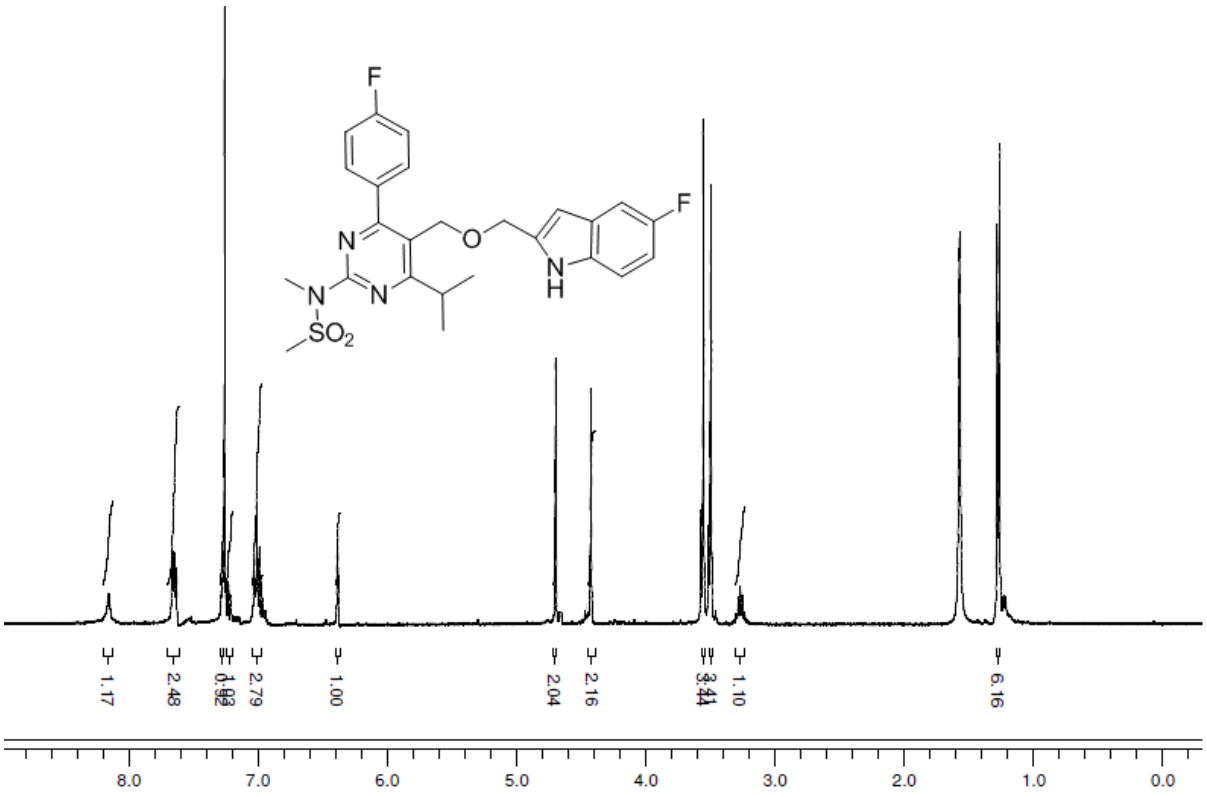
4a ^{1}H NMR (400 MHz, $CDCl_3$)



4a ^{13}C NMR (100 MHz, $CDCl_3$)



4b ^{1}H NMR (400 MHz, $CDCl_3$)



4b ^{13}C NMR (100 MHz, $CDCl_3$)