Supporting Information

Modulating ADME Properties by Fluorination: MK2 Inhibitors with Improved Oral Exposure

Juraj Velcicky,* Achim Schlapbach, Richard Heng, Laszlo Revesz, Daniel Pflieger, Ernst Blum, Stuart Hawtin, Christine Huppertz, Roland Feifel, and Rene Hersperger

Novartis Institutes for BioMedical Research, CH-4002 Basel, Switzerland

Table of Contents

In vitro assays

MK2 biochemical assay. MK2 was pre-activated in kinase buffer (25 mM Tris–HCl, pH 7.5, 25 mM β-glycerophosphate, 0.1 mM Na_3VO_4, 25 mM $MgCl_2$, 20 μM DTT) containing 5 μM ATP, 150 μg/mL human MK2, 30 μg/mL active human p38α for 30 min at 22 °C. For MK2 inhibition, reactions contained compound (10 μL; 0.5% DMSO final) or vehicle control, 250 nM hsp27 peptide biotinyl-AYSRALSRQLSSGVSEIRCOOH as substrate (10 μL) and activated MK2 mix (10 μL) containing ATP (5 μM final). Following incubation at 22 °C for 45 min, reactions were terminated with 125 μM EDTA (10 μL). Samples (10 μL) were transferred to black 384-well plates for detection of p-hsp27 by time-resolved fluorescence resonance energy transfer (FRET) using an antibody mix (10 μL) containing a rabbit anti-phospho-hsp27 (Ser^{82}) antibody (2.5 M, Upstate), and anti-rabbit europium-labeled secondary antibody LANCE Eu-W1024 (3.6 nM; Perkin Elmer) as fluorescence donor along with streptavidin SureLight-APC (6.25 nM; Perkin Elmer) as acceptor. Following incubation at 22 °C for 90 min, the FRET ratio 665/620 nm was determined.

p-hsp27 assay. THP-1 cells were stimulated with anisomycin (25 μL) diluted in low-serum RPMI media (150 ng/mL final) for 15 min at 37 °C. Following stimulation, cells were fixed with 10% (w/v) paraformaldehyde (34 μL; 1.8% v/v final), briefly mixed and incubated for 10 min at 37 °C. Plates were briefly centrifuged (720 *g* for 5 min at 4 °C) prior to careful aspiration of media on ice. Cells were permeabilised with the addition of 1 mL ice-cold 90% (v/v) methanol, centrifuged (720 *g* for 5 min at rt) following the addition of wash buffer (0.5 mL) (PBS containing 1% v/v FCS). Cells were washed twice (1.5 mL) with careful aspiration of media and repeated centrifugation steps. The primary antibody anti-phospho (Ser^{78}) hsp27

diluted to 1:125 in wash buffer (25 μL/well) was incubated on cells for 60 min at room temperature. Cells were washed, prior to incubation with secondary goat anti-rabbit IgG ALEXA (fluor) 647-conjugated antibody diluted 1:5000 in wash buffer (50 μL/well) for 60 min at rt in the dark. Cells were washed as described above, prior to careful resuspension in wash buffer (50 μL) for FACS (fluorescence activated cell sorting) analysis. Cells were transferred to 'V' bottom 96-well plates and analysed using a FACSCaliburTM cytometer (Becton Dickinson) equipped with red-diode laser (excitation 635 nm). Gating of cells according to forward and side scatter, mean fluorescent intensity (MFI) was calculated at 653–669 nm (emission).

TNFα release from human peripheral blood mononuclear cells (hPBMCs). hPBMCs were prepared from peripheral blood of healthy volunteers using Ficoll-Plaque Plus (Amersham) density separation. Cells were seeded at a 1 x 10^5 cells/well in 96-well plates in RPMI 1640 medium (Invitrogen) containing 10% (v/v) fetal calf serum. After pre-incubation with serial dilutions of test compound (0.25% v/v DMSO final) for 30 min at 37 °C, cells were stimulated with the addition of IFNγ (10 ng/mL) and lipopolysaccharide (LPS) (5 μg/mL) per well and incubated for 3 h at 37 °C. Following a brief centrifugation, supernatant (10 μL) sample from each well was quantified against TNFα calibration curve using HTRF TNFα kit (CisBio).

TNFα release from whole blood. Human blood was anticoagulated with sodium citrate (10%) and diluted 1:5 with RPMI 1640 medium containing 10% fetal calf serum and 0.05 mM β-mercaptoethanol. Recombinant hirudin was added at a final concentration of 1 μg/ml. After pre-incubation with serial dilutions of test compound for 30 min at 37 °C, blood was stimulated with LPS (5 μg/mL) and IFNγ (10 ng/mL) and incubated for 3 h at 37 °C. Following a brief

centrifugation, a supernatant sample from each well was quantified against TNFα calibration curve using HTRF TNFα kit (CisBio).

Pharmacokinetic studies in rats

***In vivo* part.** First, 96−120 h before administration of the test substance, adult female wild-type Sprague−Dawley rats (Iffa Credo, France) were anesthetized with isoflurane and catheters were surgically implanted under aseptic precautions (use of sterile instruments and surgical material in combination with local antibiotic prophylaxis) into the femoral artery and vein. Then the catheters were exteriorized in the neck region, connected to a Harvard swivel system (Harvard Instruments), and filled with 0.9% saline containing 100 $U \cdot mL^{-1}$ heparin. After recovery from anesthesia, the animals were housed individually in special cages with free access to food and tap water until and throughout the experiment. Analgesic treatment with Temgesic (10 μg/kg sc, application volume 1 mL/kg) was performed the evening following surgery and the next morning. Compound administration in cassette format was in the morning (6−8 AM). Intravenous and oral dosing was typically performed in the same animals after a 48 h wash-out interval between the single dose applications. The test substances were administered intravenously as a solution in 1-methyl-2-pyrrolidone and polyethylene glycol 200 (30:70, v/v) at a dose of 1 mg/kg per compound and orally as a homogeneous aqueous suspension in Tween 80 and carboxymethyl cellulose sodium 0.5/0.5/99 (w/w) at a dose of 3 mg/kg per compound. Blood samples were collected at various time points from the femoral artery catheter into Eppendorf tubes coated with sodium EDTA. Blood samples were immediately frozen at −20 °C until final processing (maximum storage was 8 days).

PK analysis. The concentrations of compounds in whole blood were quantified using a Liquid Chromatography/Mass Spectrometry (LC-MS/MS) assay. To 10 µL of each blood sample, 2 µL (conc 2.5 µg/mL) of an internal standard was added and the samples were precipitated with 120 µL acetonitrile. The samples were vortexed thoroughly then centrifuged (10 min, 4 °C). The supernatant (120 µL) was transferred to a clean 96-well plate and mixed with 50 µL of Milli-Q water. The samples were injected (2 µL) onto suitable analytical columns, using gradient methods at various flow rates. Mobile phases typically consisting of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) were used. Compounds and the internal standards were eluted at various retention times. The HPLC systems were interfaced to mass spectrometers. MS/MS analyses were carried out using Electrospray ionization (ESI) typically in the positive ionization mode. Compounds and the internal standards were monitored using multiple reaction monitoring (MRM). The standard curves employed for sample quantitation ranged over several log units. The lower limit of quantitation (LLOQ) in blood was determined and used as cutoff for the analytical sensitivity. Known amounts of the compounds were spiked into blood to create quality control samples with three to four known concentrations. The accuracy of the in vivo blood sample concentration determination was considered acceptable when the intra-assay accuracies obtained for the quality control samples were within 70% to 130% of the expected concentrations. Subsequently, from the time concentration data, pharmacokinetic parameters were calculated by non-compartmental regression analyses using an in house fitting program.

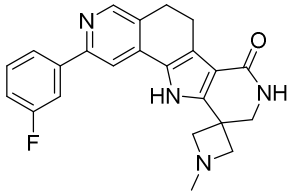
General chemistry

All reagents and solvents were purchased from commercial suppliers and used without further purification. Intermediate **9** was prepared according to the literature procedure,[1] in the meantime being also commercially available (CAS: 1105664-04-7). All reactions were performed under inert conditions (argon) unless otherwise stated. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz or a Bruker 500 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to an internal solvent reference. Peaks are tabulated in the order multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quintet; m, multiplet; br, broad), coupling constants, number of protons. The FTIR spectra were recorded on Bruker Tensor 27 and selected, significant peaks are reported as wavenumber in cm^{-1}. For HRMS, the analyses were performed by using electrospray ionization in positive ion modus after separation by liquid chromatography (Nexera from Shimadzu). The elemental composition was derived from the mass spectra acquired at the high resolution of about 30'000 on an LTQ Orbitrap XL mass spectrometer (Thermo Scientific). The high mass accuracy below 1 ppm was obtained by using a lock mass. Final compounds were purified to ≥95% purity as assessed by analytical liquid chromatography:

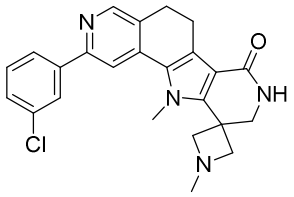
LCMS method a: Waters UPLC Acquity; column: Acquity HSS T3 1.8 μm, 2.1 x 50 mm at 60 °C, Eluent A: water + 0.05% HCOOH + 3.75 mM NH_4OAc, B: MeCN + 0.04% HCOOH, Gradient: 5 to 98% B in 1.4 min, Flow: 1.0 mL/min.

LCMS method b: Waters UPLC Acquity; column: Acquity HSS T3 1.8 μm, 2.1 x 100 mm at 60 °C, Eluent A: water + 0.05% HCOOH + 3.75 mM NH_4OAc, B: MeCN + 0.04% HCOOH, Gradient: 5 to 98% B in 9.4 min, Flow: 0.8 mL/min.

Characterization data of previously described compounds 1-3[1,2]

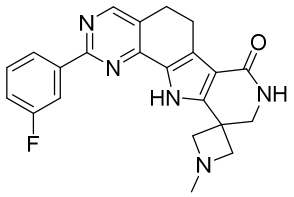


2'-(3-fluorophenyl)-1-methyl-6',8',9',11'-tetrahydrospiro[azetidine-3,10'-pyrido[3',4':4,5]pyrrolo[2,3-f]isoquinolin]-7'(5'H)-one (1). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.84 (brs, 1H), 8.41 (s, 1H), 8.24 (s, 1H), 7.95 (d, J = 7.8 Hz, 1H), 7.86 (d, J = 10.6 Hz, 1H), 7.54 - 7.61 (m, 1H), 7.28 (td, J = 8.4, 2.2 Hz, 1H), 7.16 (brs, 1H), 3.55 (d, J = 1.6 Hz, 2H), 3.33 - 3.40 (m, 4H), 2.82 - 2.98 (m, 4H), 2.37 (s, 3H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 165.0, 162.7 (d, $J_{C,F}$ = 243 Hz), 153.3, 147.9, 141.7 (d, $J_{C,F}$ = 8 Hz), 141.4, 136.5, 130.6 (d, $J_{C,F}$ = 8 Hz), 127.8, 125.8, 122.5, 122.2, 115.4 (d, $J_{C,F}$ = 21 Hz), 112.6 (d, $J_{C,F}$ = 23 Hz), 111.6, 110.5, 63.1, 49.6, 44.8, 34.0, 24.9, 19.8. LCMS (method b) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{22}FN_4O$ 389.2; Found 389.2, t_R = 2.06 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{22}FN_4O$ 389.1778; Found 389.1781.



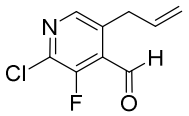
2'-(3-chlorophenyl)-1,11'-dimethyl-6',8',9',11'-tetrahydrospiro[azetidine-3,10'-pyrido[3',4':4,5]pyrrolo[2,3-f]isoquinolin]-7'(5'H)-one (2). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.49 (s, 1H), 8.14 (s, 1H), 8.07 (d, J = 7.2 Hz, 1H), 7.95 (s, 1H), 7.45 - 7.59 (m, 2H), 7.26 (br s, 1H), 4.49 (s, 3H), 3.76 (d, J = 7.8 Hz, 2H), 3.50 (br s, 2H), 3.25 (d, J = 7.9 Hz, 2H), 2.87 - 2.97

(m, 2H), 2.76 - 2.85 (m, 2H), 2.40 (s, 3H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 165.0, 153.4, 148.2, 142.0, 141.3, 136.4, 133.6, 130.5, 129.4, 128.5, 127.8, 126.2, 125.1, 124.3, 111.6, 111.1, 63.4, 52.0, 45.3, 34.4, 34.0, 25.7, 19.9. LCMS (method b) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{24}ClN_4O$ 419.2; Found 419.2, t_R = 2.76 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{24}ClN_4O$ 419.1639; Found 419.1633.



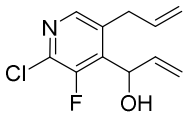
2'-(3-fluorophenyl)-1-methyl-6',8',9',11'-tetrahydrospiro[azetidine-3,10'-pyrido[3',4':4,5]pyrrolo[3,2-h]quinazolin]-7'(5'H)-one (3). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.11 (br s, 1H), 8.53 (s, 1H), 8.27 - 8.44 (m, 2H), 7.52 - 7.64 (m, 1H), 7.36 (t, J = 7.8 Hz, 1H), 7.27 (s, 1H), 3.60 (s, 2H), 3.46 (d, J = 5.6 Hz, 2H), 3.32 - 3.38 (m, 2H), 2.87 - 3.02 (m, 4H), 2.38 (s, 3H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 164.7, 162.5 (d, $J_{C,F}$ = 242 Hz), 160.2 (d, $J_{C,F}$ = 3 Hz), 153.9, 153.8, 143.2, 140.3 (d, $J_{C,F}$ = 8 Hz), 130.4 (d, $J_{C,F}$ = 8 Hz), 127.6, 126.1, 124.5, 123.7, 117.1 (d, $J_{C,F}$ = 21 Hz), 114.2 (d, $J_{C,F}$ = 23 Hz), 112.2, 62.4, 49.7, 44.7, 34.2, 24.3, 19.6. LCMS (method b) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{21}FN_5O$ 390.2; Found 390.2, t_R = 2.80 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{21}FN_5O$ 390.1730; Found 390.1723.

Synthesis of compounds 19-21

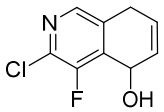


5-allyl-2-chloro-3-fluoroisonicotinaldehyde (11). *t*-BuLi (1.7 M in heptane, 14.1 mL, 24 mmol) was added over 15 min to a solution of 2-chloro-3-fluoropyridine (**10**) (3.0 g, 22.8 mmol) in THF (70 mL) at -78 °C. After stirring at -78 °C for 1 h, *N*-formyl-l-*N*,*N*′,*N*′-trimethylethylene-1,2-diamine (3.21 g, 24 mmol) was added slowly and the reaction mixture was left to warm to -40 °C, followed by addition of *n*-BuLi (1.6 M in hexanes, 21.4 mL, 34.2 mmol). The red-brown solution was stirred at -30 °C for 3 h before CuBr (4.25 g, 29.6 mmol) was added. The reaction mixture was then allowed to reach 0 °C and was stirred at this temperature for 1 h. After cooling back to -30 °C, a solution of allylbromide (3.1 mL, 36.5 mmol) in anhydrous THF (50 mL) was added. The reaction mixture was stirred for 1 h at -10 °C; then quenched by addition of sat. aq. NH_4Cl, filtered over Hyflo® and washed with diethyl ether. The organic layer was then washed with sat. aq NH_4Cl and brine, dried (Na_2SO_4) and concentrated. The crude product was further purified by chromatography (5-15% ethyl acetate in cyclohexane) followed by a second chromatography (10-15% of diethyl ether in cyclohexane). The product **11** was obtained in 1.85 g (41%) yield as an yellow oil. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 10.31 (s, 1H), 8.32 (s, 1H), 5.97 (m, 1H), 5.07 (dd, J = 10.2, 1.5 Hz, 1H), 5.00 (dd, J = 17.2, 1.6 Hz, 1H), 3.72 (d, J = 6.3 Hz, 2H); ^{13}C NMR (DMSO-d_6, 101 MHz): δ 187.6, 153.0 (d, $J_{C,F}$ = 268 Hz), 146.3 (d, $J_{C,F}$ = 7 Hz), 136.4 (d, $J_{C,F}$ = 20 Hz), 135.2, 128.8 (d, $J_{C,F}$ = 6 Hz), 116.6, 32.2; LCMS (method a) *m/z*: $[M - H]^-$ Calcd for C_9H_6ClFNO 198.0; Found 198.0, t_R = 0.87 min. HRMS (ESI) *m/z*: [M +

H]$^+$ Calcd for C_9H_8ClFNO 200.0278; Found 200.0274. FTIR: 3083, 2880, 2778, 1715, 1639, 1392, 1219, 1199, 1179, 995, 923.

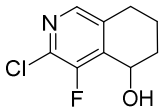


1-(5-allyl-2-chloro-3-fluoropyridin-4-yl)prop-2-en-1-ol (12). Vinylmagnesium bromide (1 M in THF, 9.8 mL, 9.8 mmol) was added at 0 °C to a mixture of **11** (1.3 g, 6.5 mmol) in THF (40 mL). After stirring at 0 °C for 1 h the mixture was quenched with sat. aq. NH_4Cl and extracted with ethyl acetate. The organic layers were washed with brine, dried (Na_2SO_4) and concentrated. The crude material was purified by chromatography (10-25% ethyl acetate in cyclohexane) to provide the compound **12** in 1.12 g (76%) yield as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.04 (s, 1H), 6.13 (dddd, *J* = 17.2, 10.5, 5.3, 1.4 Hz, 1H), 5.96 (ddt, *J* = 16.9, 10.4, 6.1, 6.1 Hz, 1H), 5.56 (dd, *J* = 5.4, 1.1 Hz, 1H), 5.26 - 5.37 (m, 2H), 5.17 (dd, *J* = 10.2, 1.4 Hz, 1H), 5.02 (dd, *J* = 17.2, 1.5 Hz, 1H), 3.46 - 3.61 (m, 2H), 2.17 (brs, 1H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ . 153.0 (d, $J_{C,F}$ = 260.9 Hz), 146.1 (d, $J_{C,F}$ = 6.1 Hz), 137.9 (d, $J_{C,F}$ = 9.9 Hz), 136.6, 135.4, 134.1, 117.5, 117.1, 69.0 (d, $J_{C,F}$ = 2.7 Hz), 33.8. LCMS (method a) *m/z*: [M + H]$^+$ Calcd for $C_{11}H_{12}ClFNO$ 228.1; Found 228.1, t_R = 0.93 min. HRMS (ESI) *m/z*: [M + H]$^+$ Calcd for $C_{11}H_{12}ClFNO$ 228.0591; Found 228.0587. FTIR: 3357, 3084, 2983, 2918, 1639, 1591, 1401, 1222, 1181, 1045, 992, 925.

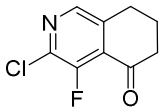


3-chloro-4-fluoro-5,8-dihydroisoquinolin-5-ol (13). A solution of **12** (14.0 g, 61.5 mmol) in CH_2Cl_2 (615 mL) was degassed with argon and treated at rt with Grubbs II catalyst (2.61 g, 3.1

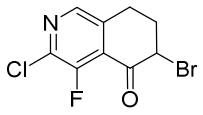
mmol). After stirring at rt for 2 h, the reaction mixture was concentrated and the crude product was purified by chromatography (20-33% ethyl acetate in cyclohexane) to give the product **13** in 11.85 g (97%) yield as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.13 (s, 1H), 6.15 - 6.28 (m, 1H), 6.08 - 6.15 (m, 1H), 5.49 (brs, 1H), 3.33 - 3.59 (m, 2H), 2.41 (br s, 1H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ 153.6 (d, $J_{C,F}$ = 262 Hz), 144.4 (d, $J_{C,F}$ = 7 Hz), 134.1, 131.1, 127.2, 125.6, 59.5, 25.8. LCMS (method a) *m/z*: $[M + H]^+$ Calcd for C_9H_8ClFNO 200.0; Found 200.0, t_R = 0.66 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for C_9H_8ClFNO 200.0278; Found 200.0274. FTIR: 3281, 3040, 2883, 1665, 1599, 1418, 1233, 1216, 1029, 995.



3-chloro-4-fluoro-5,6,7,8-tetrahydroisoquinolin-5-ol (14). A solution of **13** (3.7 g, 18.4 mmol) in MeOH (50 mL) was added at rt to a suspension of PtO_2 (370 mg) in MeOH (110 mL). The mixture was flushed with H_2 and then hydrogenated with H_2 (1 atm) at rt for 1 h. The mixture was then filtered over Hyflo®, washed with MeOH and the filtrate was concentrated. The crude material was purified by chromatography (20-35% ethyl acetate in cyclohexane) to provide the product **14** in 2.7 g (73%) yield as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.03 (s, 1H), 5.10 (q, J = 3.8 Hz, 1H), 2.86 (dt, J = 17.3, 4.5 Hz, 1H), 2.60 - 2.70 (m, 1H), 2.33 (dd, J = 4.0, 1.9 Hz, 1H), 2.02 - 2.14 (m, 1H), 1.78 - 2.02 (m, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ .154.0 (d, $J_{C,F}$ = 259.4 Hz), 145.0 (d, $J_{C,F}$ = 6.1 Hz), 134.3, 131.2, 61.3, 30.4, 25.6, 17.2. LCMS (method a) *m/z*: $[M + H]^+$ Calcd for $C_9H_{10}ClFNO$ 202.0; Found 202.0, t_R = 0.71 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_9H_{10}ClFNO$ 202.0435; Found 202.0431.

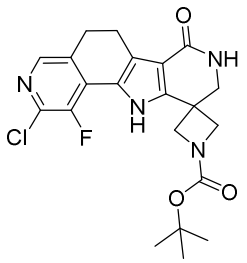


3-chloro-4-fluoro-7,8-dihydroisoquinolin-5(6H)-one (15). A solution of DMSO (1.94 mL, 27.3 mmol) in CH_2Cl_2 (15 mL) was added at -60 °C to a solution of oxalyl chloride (1.17 mL, 0.19 mmol) in CH_2Cl_2 (35 mL). After stirring at -60 °C for 5 min, a solution of **14** (2.5 g, 12.4 mmol) in CH_2Cl_2 (15 mL) was added over 3 min. The mixture was stirred at -60 °C for 15 min before adding TEA (8.6 mL, 62 mmol). After stirring at -60 °C for 5 min, the solution was allowed to warm to rt and was stirred at rt for 4 h. It was then poured onto ice-water and extracted with CH_2Cl_2. The organic layers were washed with sat. aq. $NaHCO_3$, water and brine, dried (Na_2SO_4) and concentrated. The crude material was triturated with cold *n*-heptane, the precipitate was filtered off, washed with *n*-heptane and dried in vacuum to provide the product **15** in 2.2 g (89%) yield as beige crystals. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.25 (s, 1H), 2.98 (t, J = 6.1 Hz, 2H), 2.71 (t, J = 6.3 Hz, 2H), 2.18 (quin, J = 6.4 Hz, 2H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ = 193.6, 150.5 (d, $J_{C,F}$ = 273 Hz), 145.1 (d, $J_{C,F}$ = 8 Hz), 139.5, 135.8 (d, $J_{C,F}$ = 20 Hz), 126.9, 39.3, 25.0, 21.5. LCMS (method a) *m/z*: $[M + H]^+$ Calcd for C_9H_8ClFNO 200.0; Found 200.0, t_R = 0.81 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for C_9H_8ClFNO 200.0278; Found 200.0274. FTIR: 3388, 3074, 2961, 2879, 1703, 1585, 1435, 1400, 1272, 1222, 1200, 1065, 1013, 945.



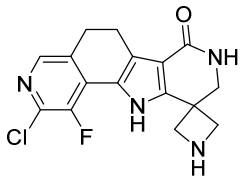
6-bromo-3-chloro-4-fluoro-7,8-dihydroisoquinolin-5(6*H*)-one (8). A solution of bromine (0.28 mL, 5.5 mmol) in AcOH (10 mL) was added dropwise at rt over 15 min to a mixture of **15**

(1.1 g, 5.5 mmol) and 48% aq. HBr (10 mL) and the solution was stirred at 35 °C for 15 min. The reaction mixture was then carefully poured into a vigorously stirring mixture of $NaHCO_3$ (25 g), H_2O (50 mL) and diethyl ether (50 mL). After stirring at rt for 15 min, the aqueous layer was extracted with diethyl ether and the combined organic phases were washed with brine, dried (Na_2SO_4) and concentrated to give product **8** in 1.5 g (98%) yield as a colorless solid. The compound was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.30 (s, 1H), 4.69 (t, J = 3.7 Hz, 1H), 3.27 - 3.38 (m, 1H), 3.01 (dt, J = 17.6, 3.9 Hz, 1H), 2.46 - 2.61 (m, 2H). ^{13}C NMR ($CDCl_3$, 101 MHz): δ 186.3, 153.2 (d, $J_{C,F}$ = 281 Hz), 145.0 (d, $J_{C,F}$ = 8.0 Hz), 136.5 (d, $J_{C,F}$ = 3.1 Hz), 125.1, 49.2, 30.3, 22.2. LCMS (method a) *m/z*: $[M + H]^+$ Calcd for $C_9H_7BrClFNO$ 280.0; Found 280.0, t_R = 0.97 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_9H_7BrClFNO$ 277.9384; Found 277.9375.

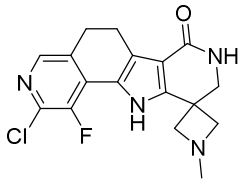


***tert*-Butyl 2'-chloro-1'-fluoro-7'-oxo-5',6',7',8',9',11'-hexahydrospiro[azetidine-3,10'-pyrido[3',4':4,5]pyrrolo[2,3-*f*]isoquinoline]-1-carboxylate (16)**. NH_4OAc (1.52 g, 19.8 mmol) was added at rt to a mixture of **8** (1.1 g, 4 mmol) and **9** (1.03 g, 4 mmol) in MeOH (40 mL). The mixture was then stirred at 60 °C for 3 h. After cooling to rt the reaction mixture was treated with water (100 mL) and the precipitate was stirred in an ice bath for 30 min. The solid was filtered off and then dried on high vacuum to provide product **16** in 1.45 g (85%) yield as a beige solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.91 (s, 1H), 8.08 (s, 1H), 7.33 (s, 1H), 4.24 (d, J = 8.2 Hz, 2H), 3.68 – 3.85 (m, 2H), 3.57 (d, J = 2.2 Hz, 2H), 2.83 –2.96 (m, 4H), 1.42 (s, 9H). ^{13}C NMR

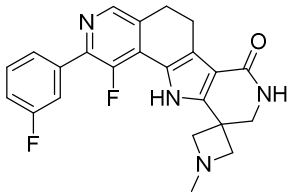
(DMSO-d_6, 101 MHz): δ 164.6, 143.0, 142.9, 141.6, 131.2, 124.9, 121.0, 112.0, 78.8, 57.2, 50.1, 32.8, 28.1, 24.6, 19.4. LCMS (method a) *m/z*: $[M + H]^+$ Calcd for $C_{21}H_{23}ClFN_4O_3$ 433.1; Found 433.2, t_R = 0.99 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{21}H_{23}ClFN_4O_3$ 433.1443; Found 433.1441. FTIR: 3250, 3084, 2971, 2882, 1696, 1663, 1604, 1514, 1398, 1367, 1326, 1166, 1117, 1056, 798, 759.



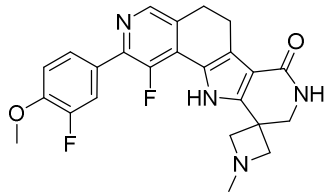
2'-Chloro-1'-fluoro-6',8',9',11'-tetrahydrospiro[azetidine-3,10'-pyrido[3',4':4,5]pyrrolo[2,3-f]isoquinolin]-7'(5'H)-one (17). 4M HCl in dioxane (1.1 mL, 4.4 mmol) was added at rt to a suspension of **16** (190 mg, 0.44 mmol) in dioxane (2.2 mL) and the mixture was stirred at rt for 3 h. As the reaction did not go to completion, few drops of conc. HCl were added and the mixture was stirred at rt for additional 1.5 h. The suspension was concentrated and triturated with diethyl ether. The solid was filtered off, washed with diethyl ether and dried in high vacuum. Product **17** was obtained as an HCl salt (yellow solid) in 174 mg (quant.) yield. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 12.07 (s, 1H), 9.94 – 10.13 (m, 1H), 8.90 – 9.07 (m, 1H), 8.09 (s, 1H), 7.48 (brs, 1H), 4.47 – 4.52 (m, 2H), 3.84 – 3.97 (m, 2H), 3.78 (s, 2H), 2.81 – 3.00 (m, 4H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 164.4, 146.6 (d, $J_{C,F}$ = 258 Hz), 143.1 (d, $J_{C,F}$ = 5 Hz), 139.7, 135.7 (d, $J_{C,F}$ = 18 Hz), 131.3, 125.3 (d, $J_{C,F}$ = 13 Hz), 124.7, 121.3, 112.1, 66.3, 52.5, 48.5, 35.8, 24.5, 19.3. LCMS (method a) *m/z*: $[M + H]^+$ Calcd for $C_{16}H_{15}ClFN_4O$ 333.1; Found 333.1, t_R = 0.48 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{16}H_{15}ClFN_4O$ 333.0918; Found 333.0914. FTIR: 3347, 3162, 3073, 2970, 2663, 1644, 1601, 1518, 1398, 1328, 1198, 1066, 925, 808, 785.



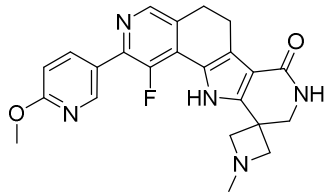
2'-Chloro-1'-fluoro-1-methyl-6',8',9',11'-tetrahydrospiro[azetidine-3,10'-pyrido[3',4':4,5]pyrrolo[2,3-f]isoquinolin]-7'(5'H)-one (18). A mixture of **17** (1.4 g, 3.5 mmol) and DIPEA (1.8 mL, 10.4 mmol) in CH_2Cl_2 (40 mL) was stirred at rt for 5 min. After addition of paraformaldehyde (355 mg, 11.8 mmol) and $NaBH(OAc)_3$ (4.2 g, 19.7 mmol) the mixture was stirred at rt for 16 h. The reaction was then treated with 2 M aq. HCl to destroy residual $NaBH(OAc)_3$. The pH was adjusted to >7 by addition of conc. aq. NH_3 and the mixture was extracted with ethylacetate. The collected organic layers were washed with water and brine, dried (Na_2SO_4) and concentrated. The residue was further purified by chromatography (10-20% MeOH in CH_2Cl_2) to produce the title compound in 400 mg (39%) yield as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.62 (br s, 1H), 8.07 (s, 1H), 7.27 (s, 1H), 3.59 (s, 2H), 3.37 - 3.48 (m, 2H), 3.25 - 3.29 (m, 2H), 2.81 - 2.97 (m, 4H), 2.35 (s, 3H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 164.6, 146.4 (d, $J_{C,F}$ = 258 Hz), 142.9 (d, $J_{C,F}$ = 4 Hz), 135.7 (d, $J_{C,F}$ = 18 Hz), 131.2, 125.6 (d, $J_{C,F}$ = 13 Hz), 125.1, 120.7, 111.6, 62.1, 49.6, 44.3, 34.1, 24.6, 19.4. LCMS *m/z*: $[M + H]^+$ Calcd for $C_{17}H_{17}ClFN_4O$ 347.1; Found 347.1, t_R = 0.49 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{17}H_{17}ClFN_4O$ 347.1075; Found 347.1073. FTIR: 3430, 3201, 3084, 2942, 2841, 1674, 1602, 1511, 1398, 1326, 1190, 1061, 907, 839, 807, 788.



1'-Fluoro-2'-(3-fluorophenyl)-1-methyl-6',8',9',11'-tetrahydrospiro[azetidine-3,10'-pyrido[3',4':4,5]pyrrolo[2,3-f]isoquinolin]-7'(5'H)-one (19). A mixture of **18** (100 mg, 0.29 mmol), 3-fluorophenylboronic acid (41 mg, 0.29 mmol), PPh_3 (227 mg, 0.87 mmol) and Na_2CO_3 (1 M in H_2O, 0.87 mL, 0.87 mmol) in *n*-propanol (2.9 mL) was degassed with argon for 5 min. After addition of $Pd(PPh_3)_2Cl_2$ (41 mg, 0.06 mmol) the mixture was heated in a microwave oven at 150 °C for 15 min. After cooling to rt, the mixture was filtered through Hyflo and rinsed with ethyl acetate. The water phase was then extracted with ethyl acetate and the collected organic layers were washed with sat. aq. $NaHCO_3$, water and brine, dried (Na_2SO_4) and concentrated. The residue was further purified by chromatography (10-20% MeOH in CH_2Cl_2) to produce product **19** in 78 mg (66%) yield as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.44 (br s, 1H), 8.35 (s, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.72 (d, J = 10.9 Hz, 1H), 7.59 (q, J = 7.3 Hz, 1H), 7.33 (t, J = 8.5 Hz, 1H), 7.24 (s, 1H), 3.60 (s, 2H), 3.45 (d, J = 6.1 Hz, 2H), 3.25 - 3.30 (m, 2H), 2.86 - 3.01 (m, 4H), 2.35 (s, 3H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 164.8, 162.1 (d, $J_{C,F}$ = 243 Hz), 150.1 (d, $J_{C,F}$ = 258 Hz), 144.0 (d, $J_{C,F}$ = 3.8 Hz), 142.2, 137.6, 131.1, 130.4 (d, $J_{C,F}$ = 8 Hz), 124.8, 124.7, 124.4 (d, $J_{C,F}$ = 4 Hz), 124.2 (d, $J_{C,F}$ = 23 Hz), 121.1, 115.8 (d, $J_{C,F}$ = 21 Hz), 114.9 (dd, $J_{C,F}$ = 23, 6 Hz), 111.5, 62.1, 49.7, 44.3, 34.2, 24.9, 19.5. LCMS (method b) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{21}F_2N_4O$ 407.2; Found 407.2, t_R = 2.83 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{21}F_2N_4O$ 407.1683; Found 407.1675. FTIR: 3161, 3069, 2944, 2842, 2784, 1649, 1608, 1572, 1541, 1506, 1453, 1402, 1323, 1261, 1202, 1190, 904, 834, 790, 770, 698.



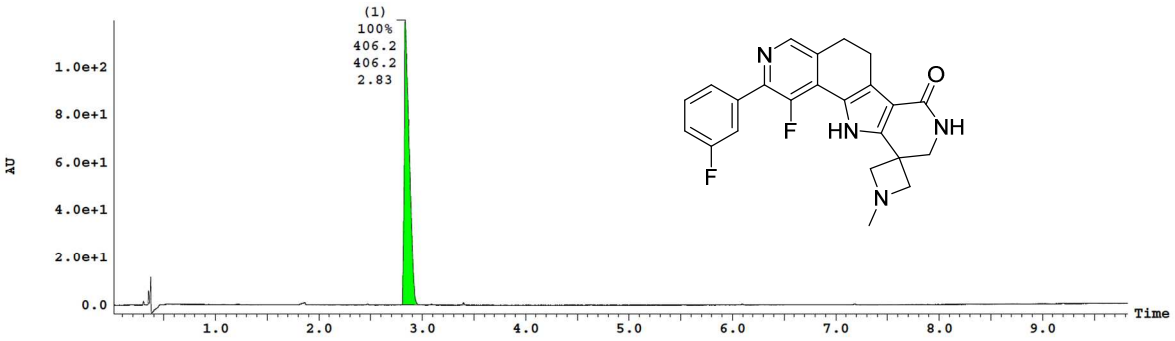
1'-Fluoro-2'-(3-fluoro-4-methoxyphenyl)-1-methyl-6',8',9',11'-tetrahydrospiro[azetidine-3,10'-pyrido[3',4':4,5]pyrrolo[2,3-f]isoquinolin]-7'(5'H)-one (20). A mixture of **18** (100 mg, 0.29 mmol), 3-fluoro-4-methoxy-phenylboronic acid (59 mg, 0.35 mmol), PPh_3 (227 mg, 0.87 mmol) and Na_2CO_3 (1 M in H_2O, 0.87 mL, 0.87 mmol) in *n*-propanol (2.9 mL) was degassed with argon for 5 min. After addition of $Pd(PPh_3)_2Cl_2$ (61 mg, 0.087 mmol) the mixture was heated in a microwave oven at 150 °C for 15 min. After cooling to rt, the mixture was filtered through Hyflo and rinsed with ethyl acetate. The water phase was then extracted with ethyl acetate and the collected organic layers were washed with sat. aq. $NaHCO_3$, water and brine, dried (Na_2SO_4) and concentrated. The residue was further purified by chromatography (10-20% MeOH in CH_2Cl_2) to produce the title compound in 67 mg (53%) yield as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.46 (br s, 1H), 8.31 (s, 1H), 7.74 - 7.84 (m, 2H), 7.33 (t, J = 9.0 Hz, 1H), 7.22 - 7.29 (m, 1H), 3.92 (s, 3H), 3.61 (s, 2H), 3.50 (br s, 2H), 3.32 - 3.38 (m, 2H), 2.84 - 3.00 (m, 4H), 2.39 (br s, 3H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 164.8, 151.2 (d, $J_{C,F}$ = 243 Hz), 145.0 (d, $J_{C,F}$ = 258 Hz), 147.7 (d, $J_{C,F}$ = 11 Hz), 143.7 (d, $J_{C,F}$ = 4 Hz), 142.0, 141.8 (d, $J_{C,F}$ = 9 Hz), 130.3, 128.1, 124.8 (d, $J_{C,F}$ = 4 Hz), 124.6 (d, $J_{C,F}$ = 14 Hz), 124.0, 121.2, 115.5 (dd, $J_{C,F}$ = 20, 6 Hz), 113.6, 111.4, 62.1, 56.1, 49.7, 44.3, 34.2, 24.8, 19.6. LCMS (method b) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{23}F_2N_4O_2$ 437.2; Found 437.2, t_R = 2.86 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{23}F_2N_4O_2$ 437.1789; Found 437.1783. FTIR: 3223, 3087, 2940, 2844, 1653, 1610, 1519, 1456, 1398, 1324, 1295, 1273, 1139, 1124, 1023, 793, 763.

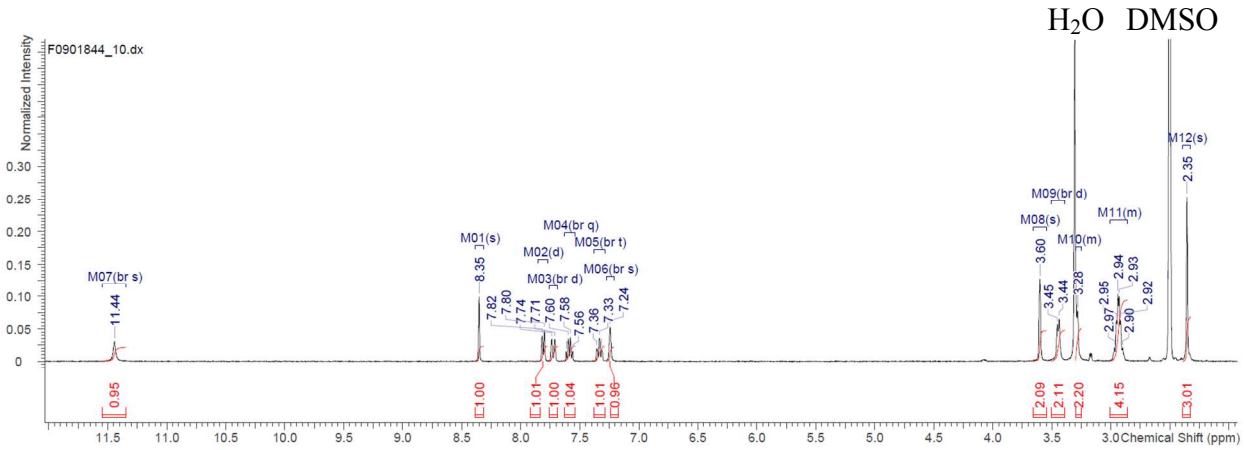


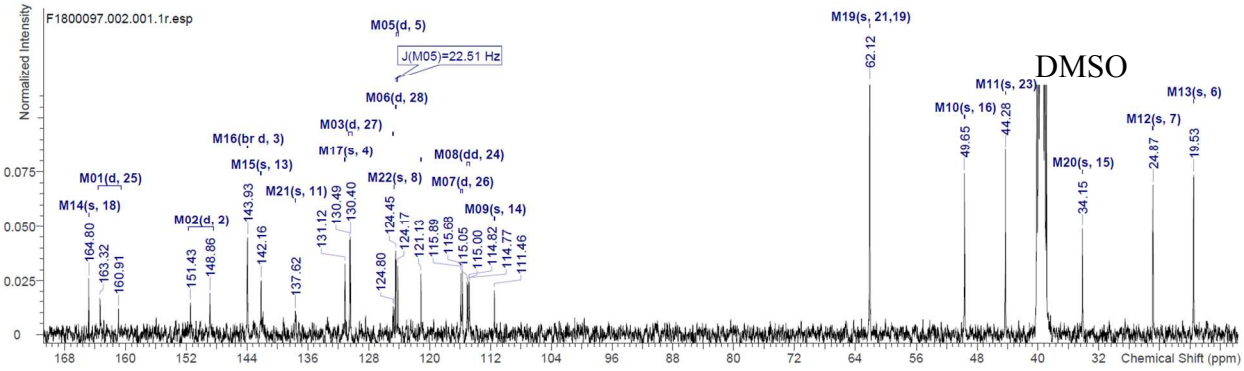
1'-Fluoro-2'-(6-methoxypyridin-3-yl)-1-methyl-6',8',9',11'-tetrahydrospiro[azetidine-3,10'-pyrido[3',4':4,5]pyrrolo[2,3-f]isoquinolin]-7'(5'H)-one (21). A mixture of **18** (100 mg, 0.29 mmol), 2-methoxy-5-pyridineboronic acid (44 mg, 0.29 mmol), PPh_3 (227 mg, 0.87 mmol) and Na_2CO_3 (1 M in H_2O, 0.87 mL, 0.87 mmol) in *n*-propanol (2.9 mL) was degassed with argon for 5 min. After addition of $Pd(PPh_3)_2Cl_2$ (41 mg, 0.06 mmol) the mixture was heated in a microwave oven at 150 °C for 15 min. After cooling to rt, the mixture was filtered through Hyflo and rinsed with ethyl acetate. The water phase was then extracted with ethyl acetate and the collected organic layers were washed with sat. aq. $NaHCO_3$, water and brine, dried (Na_2SO_4) and concentrated. The residue was further purified by chromatography (10-20% MeOH in CH_2Cl_2) to produce the title compound in 71 mg (59%) yield as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.48 (s, 1H), 8.73 (s, 1H), 8.33 (s, 1H), 8.24 (d, J = 8.8 Hz, 1H), 7.24 (s, 1H), 6.99 (d, J = 8.6 Hz, 1H), 3.94 (s, 3H), 3.60 (s, 2H), 3.44 (d, J = 5.8 Hz, 2H), 3.23 - 3.28 (m, 2H), 2.85 - 3.02 (m, 4H), 2.35 (s, 3H). ^{13}C NMR (DMSO-d_6, 101 MHz): δ 164.8, 163.7, 149.9 (d, $J_{C,F}$ = 258 Hz), 146.7 (d, $J_{C,F}$ = 8 Hz), 143.9 (d, $J_{C,F}$ = 4 Hz), 142.1, 141.1 (d, $J_{C,F}$ = 11 Hz), 138.9 (d, $J_{C,F}$ = 5 Hz), 130.4, 124.9 (d, $J_{C,F}$ = 6 Hz), 124.5 (d, $J_{C,F}$ = 14 Hz), 124.1, 121.1, 111.4, 110.3, 62.1, 53.4, 49.7, 44.3, 34.2, 24.8, 19.6. LCMS *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{23}FN_5O_2$ 420.2; Found 420.3, t_R = 2.56 min. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{23}FN_5O_2$ 420.1836; Found 420.1833. FTIR: 3443, 3225, 3164, 2968, 2942, 2840, 2786, 1646, 1604, 1504, 1462, 1402, 1307, 1285, 1029, 844, 833, 790.

UPLC and NMR Charts

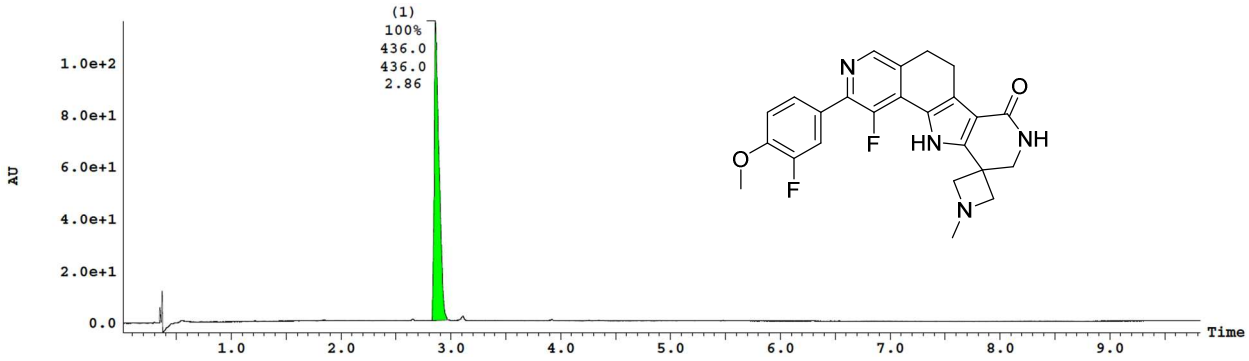
Compound **19**

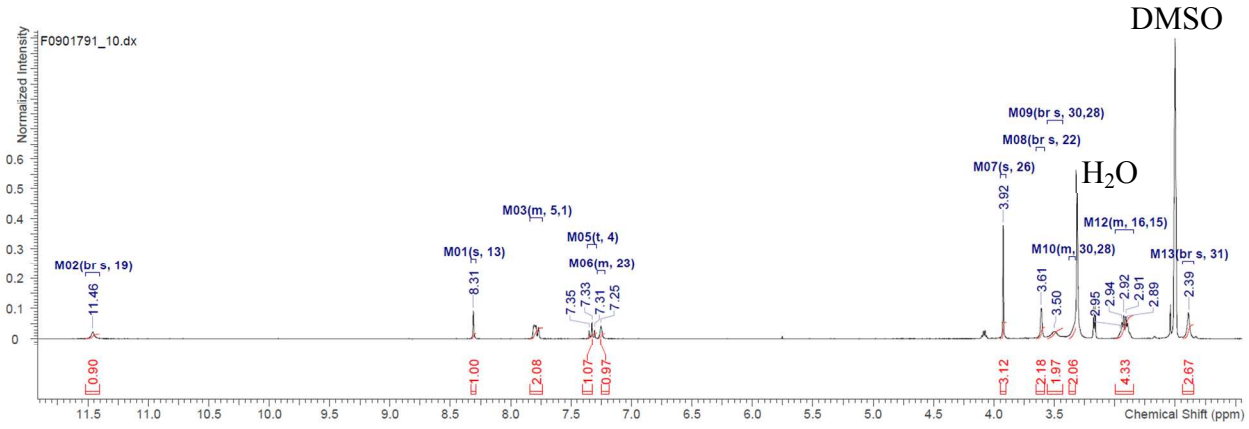


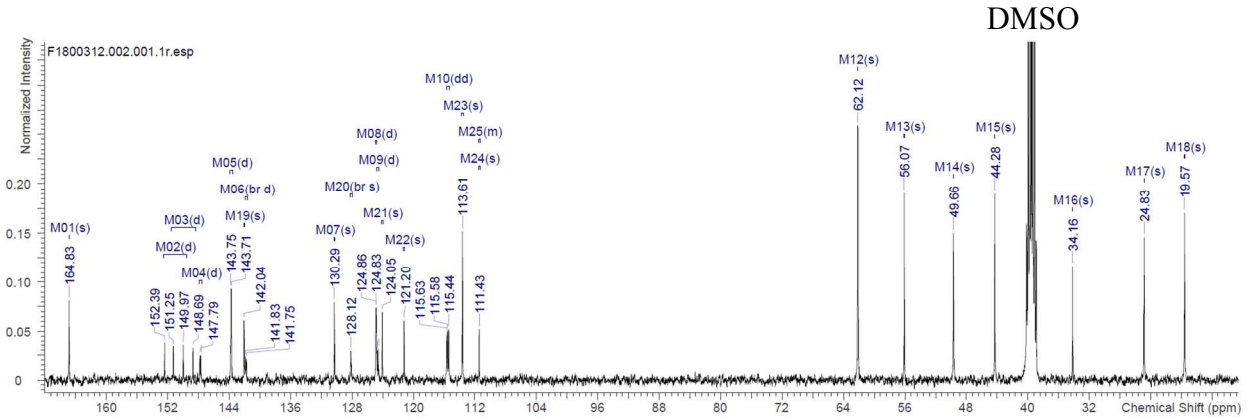




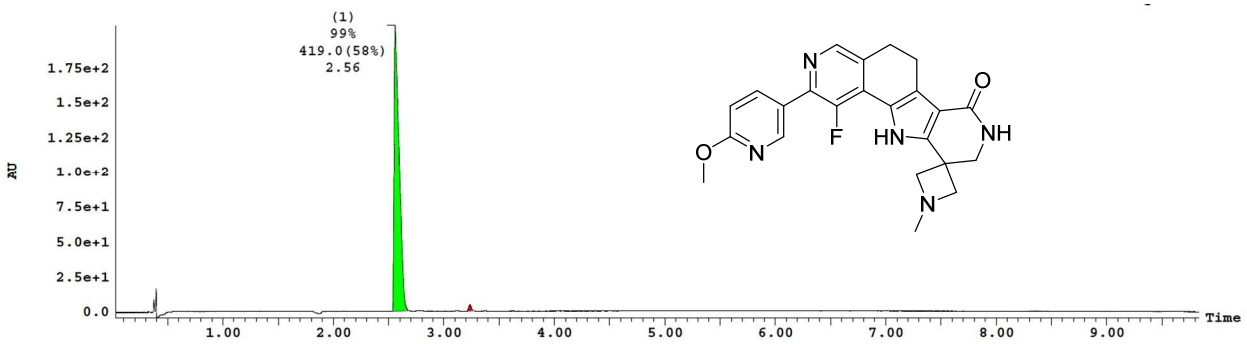
Compound **20**

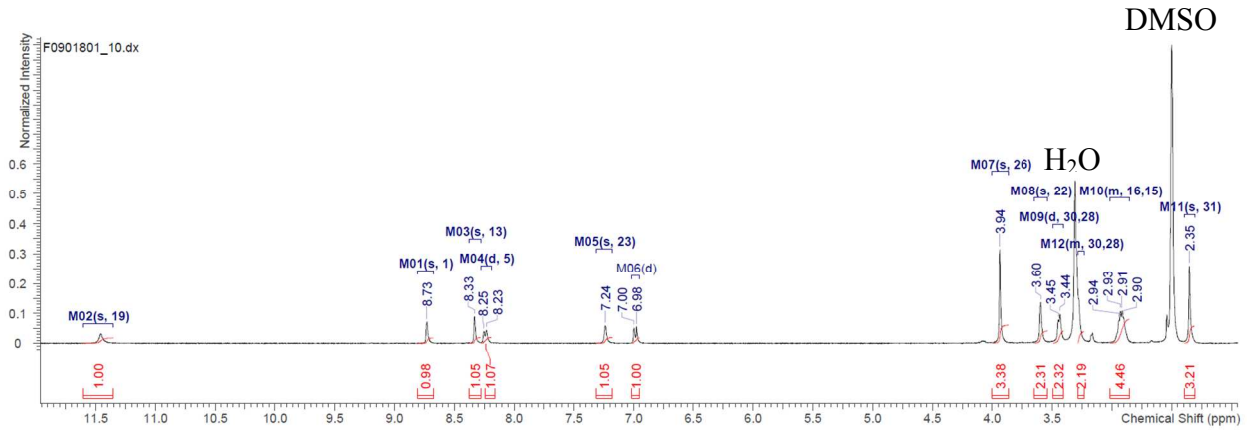

(1)
100%
436.0
436.0
2.86
AU
1.0e+2
8.0e+1
6.0e+1
4.0e+1
2.0e+1
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
Time
N
O
NH
HN
F
O
F
N

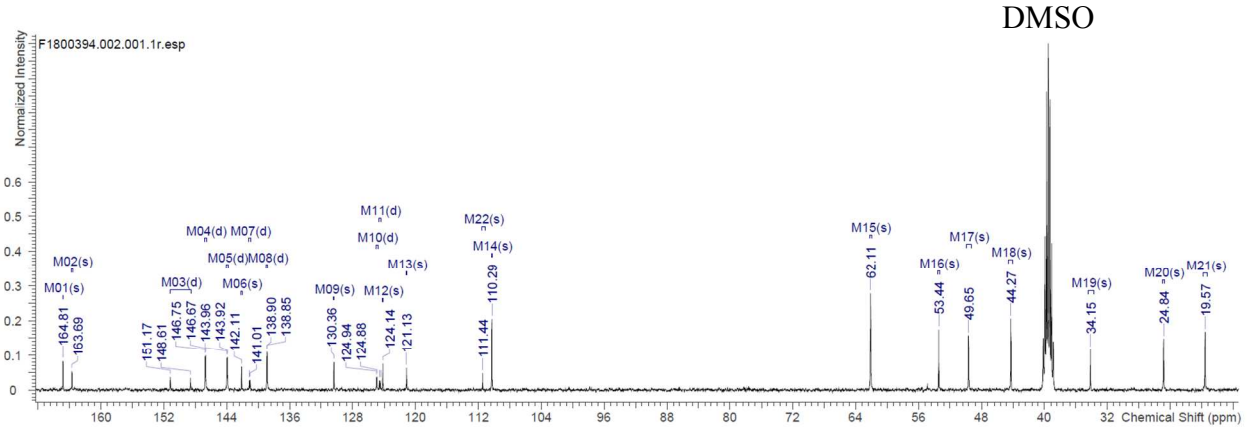

DMSO
F0901791_10.dx
Normalized Intensity
M09(br s, 30,28)
M08(br s, 22)
M07(s, 26)
H2O
M12(m, 16,15)
M10(m, 30,28)
M13(br s, 31)
M03(m, 5,1)
M05(t, 4)
M01(s, 13)
M06(m, 23)
M02(br s, 19)
11.46
8.31
7.35
7.33
7.31
7.25
3.92
3.61
3.50
2.95
2.94
2.92
2.91
2.89
2.39
0.90
1.00
2.08
1.07
0.97
3.12
2.18
1.97
2.06
4.33
2.67
Chemical Shift (ppm)


DMSO
F1800312.002.001.1r.esp
Normalized Intensity
M10(dd)
M23(s)
M25(m)
M08(d)
M09(d)
M24(s)
M05(d)
M06(br d)
M20(br s)
M21(s)
M03(d)
M19(s)
M07(s)
M22(s)
M01(s)
M02(d)
M04(d)
164.83
152.39
151.25
149.97
148.69
147.79
143.75
143.71
142.04
141.83
141.75
130.29
128.12
124.86
124.83
124.05
121.20
115.63
115.58
115.44
113.61
111.43
M12(s)
62.12
M13(s)
56.07
M14(s)
49.66
M15(s)
44.28
M16(s)
34.16
M17(s)
24.83
M18(s)
19.57
Chemical Shift (ppm)

Compound **21**


(1)
99%
419.0(58%)
2.56
AU
Time
N
O
F
HN
NH
O
N
N


DMSO
F0901801_10.dx
Normalized Intensity
H2O
M02(s, 19)
M01(s, 1)
M03(s, 13)
M04(d, 5)
M05(s, 23)
M06(d)
M07(s, 26)
M08(s, 22)
M10(m, 16,15)
M09(d, 30,28)
M12(m, 30,28)
M11(s, 31)
Chemical Shift (ppm)


DMSO
F1800394.002.001.1r.esp
Normalized Intensity
Chemical Shift (ppm)

REFERENCES

[1]Schlapbach, A.; Revesz, L.; Koch, G. Heterocyclic compounds useful as MK2 inhibitors and their preparation, pharmaceutical compositions and use in the treatment of diseases. WO 2009010488, **2009**; *Chem. Abstr.* **2009**, *150*, 168327.

[2]Revesz, L.; Schlapbach, A.; Aichholz, R.; Dawson, J.; Feifel, R.; Hawtin, S.; Littlewood-Evans, A.; Koch, G.; Kroemer, M.; Möbitz, H.; Scheufler, C.; Velcicky, J.; Huppertz, C. In vivo and in vitro SAR of tetracyclic MAPKAP-K2 (MK2) inhibitors. Part II. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4719–4723.