Supporting Information

Kinesin spindle protein (KSP) inhibitors. Part 9: The discovery of KSP inhibitor MK-0731 for the treatment of taxane-refractory cancer

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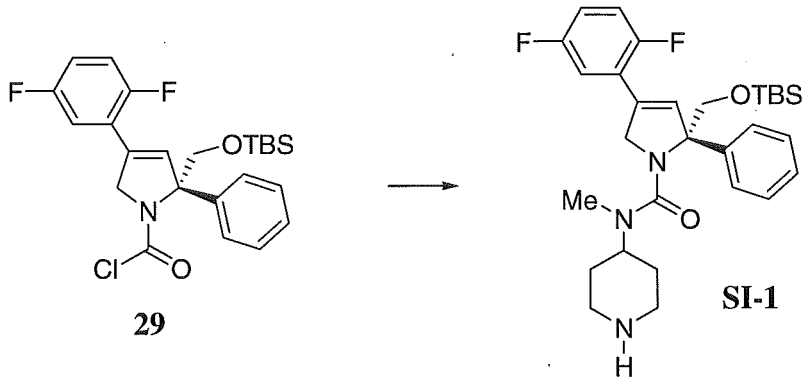
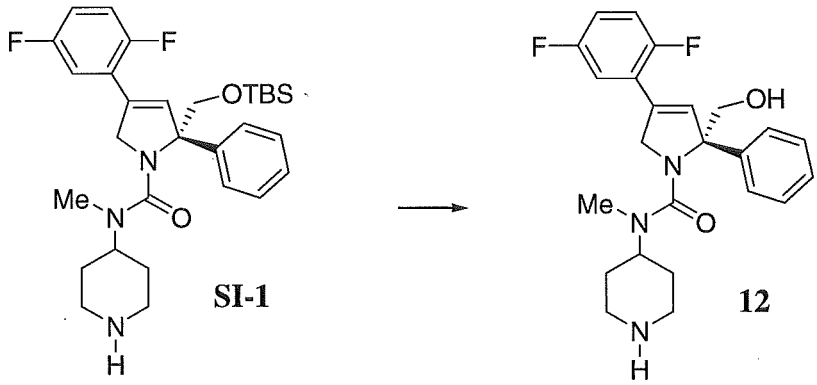
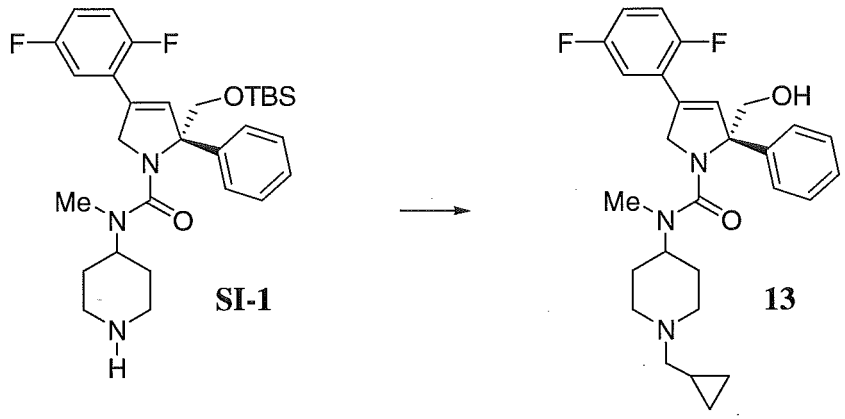


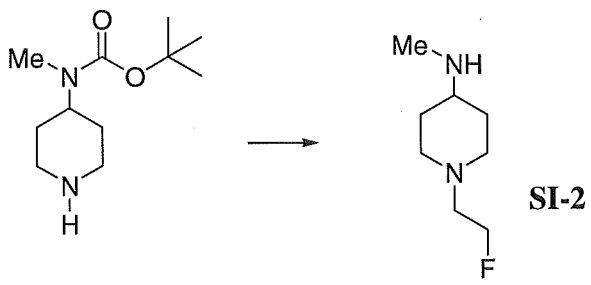
(2*S*)-2-({[*tert*-Butyl(dimethyl)silyl]oxy}methyl)-4-(2,5-difluorophenyl)-*N*-methyl-2-phenyl-*N*-piperidin-4-yl-2,5-dihydro-1*H*-pyrrole-1-carboxamide (SI-1). To a solution of **29** (4.6 g, 9.96 mmol) in 100 mL of 1,4-dioxane was added triethylamine (4.2 mL, 29.9 mmol), 4-methylamino-piperidine-1-carboxylic acid benzyl ester (1-*N*-CBz-4-methylamino-piperidine; 1.85 g, 7.5 mmol), and a catalytic amount of DMAP. After stirring for 3 hours at 60°C, the reaction was cooled to room temperature and methylamine in THF was added to consume excess, unreacted **29**. After stirring for 30 minutes, the reaction was added to a separatory funnel containing EtOAc and 10% aqueous citric acid, and the layers were cut. The aqueous phase was extracted again with EtOAc, the combined organic layers were washed with 10% aqueous citric acid, saturated brine, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography with a gradient of 2 to 75% EtOAc in hexanes to provide 3.5 g of a white taffy. LRMS = 676.1 (M + H). This material was dissolved in 150 mL EtOH, degassed for 5 minutes with a stream of nitrogen, and to the solution was added 1,4-cyclohexadiene (9.8 mL, 104 mmol) and 10% palladium on carbon (~ 300mg). After stirring for 3 hours at room temperature, the reaction was filtered through celite and concentrated to provide 2.65 g (66%) of **SI-1** as a colorless oil. LC/MS (3.7 minute run): R_t = 2.30 min, (92% pure). LRMS = 542.2 (M + H).



(2*S*)-4-(2,5-Difluorophenyl)-2-(hydroxymethyl)-*N*-methyl-2-phenyl-*N*-piperidin-4-yl-2,5-dihydro-1*H*-pyrrole-1-carboxamide (12). To a solution of **SI-1** (900mg, 1.6 mmol) in 100 mL of THF at 0°C was added 4M HCl in dioxane (1.8 mL, 7.2 mmol) dropwise. The reaction was allowed to warm to room temperature and stir for 3 hours before being added to a separatory funnel containing 5% aqueous Na_2CO_3. The mixture was extracted twice with EtOAc, washed with saturated brine, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography by eluting the column first with 1:1 EtOAc/hexanes, followed by a gradient of 0 to 75% 20:1:1 EtOH/H_2O/NH_4OH in EtOAc to provide 600 mg of **12** (85%) as a white solid of ~ 95% purity. Analytically pure material was obtained by reverse phase HPLC purification of a portion of this material, and free-basing the fractions with $NaHCO_3$. The basic fractions were extracted with EtOAc, dried over Na_2SO_4, and concentrated to provide **12** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (m, 4H), 7.25 (m, 1H), 7.05 (m, 1H), 6.95 (m, 2H), 6.28 (s, 1H), 5.4 (bs, 2H), 4.82 (d, J = 13.7 Hz, 1H), 4.58 (d, J = 13.7 Hz, 1H), 4.42 (d, J = 12.2 Hz, 1H), 4.00 (d, J = 12.2 Hz, 1H), 3.88 (m, 1H), 3.37 (m, 2H), 2.95 (s, 3H), 2.82 (m, 2H), 2.0 (m, 3H), 1.82 (m, 1H) ppm (N-H and O-H appear exchange broadened together at 5.4 ppm). HPLC Purity: Method A = 100%; Method B = 100%. HRMS calcd for $C_{24}H_{27}F_2N_3O_2$ (M + H): 428.2144; found 428.2155. $[\alpha]_D$ = - 37.9 (c = 1.0, chloroform).

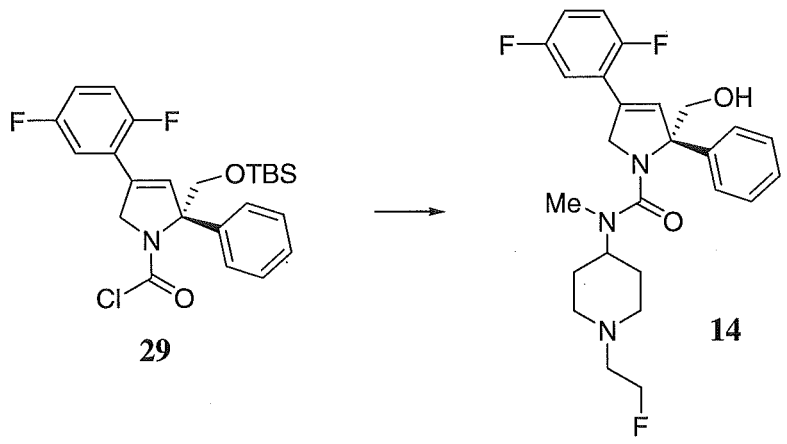


(2*S*)-*N*-[1-(Cyclopropylmethyl)piperidin-4-yl]-4-(2,5-difluorophenyl)-2-(hydroxymethyl)-*N*-methyl-2-phenyl-2,5-dihydro-1*H*-pyrrole-1-carboxamide (13). To a solution of **SI-1** (270 mg, 0.5 mmol) in 2.5 mL of 1,2-dichloroethane was added cyclopropane carboxaldehyde (112 μL, 1.5 mmol), acetic acid (86 μL, 1.5 mmol), and sodium triacetoxyborohydride (211 mg, 1 mmol). After stirring overnight at room temperature, 2 mL of trifluoroacetic acid was added and stirring was continued for 1.5 hours before being added to a separatory funnel containing 15% aqueous Na_2CO_3. The mixture was extracted with EtOAc, washed with saturated aqueous $NaHCO_3$, water, and saturated brine, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography with a gradient of 0 to 75% 20:1:1 EtOH/H_2O/NH_4OH in EtOAc to provide 204 mg of **13** (85%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (m, 4H), 7.25 (m, 1H), 7.05 (m, 1H), 6.95 (m, 2H), 6.29 (s, 1H), 5.40 (bs, 1H), 4.82 (d, J = 13.4 Hz, 1H), 4.58 (d, J = 13.4 Hz, 1H), 4.42 (d, J = 12.2 Hz, 1H), 3.98 (d, J = 12.2 Hz, 1H), 3.76 (m, 1H), 3.23 (m, 2H), 2.92 (s, 3H), 2.32 (m, 2H), 2.2 – 1.8 (m, 5H), 1.70 (m, 1H), 0.90 (m, 1H), 0.55 (m, 2H), 0.13 (m, 2H) ppm. HPLC Purity: Method A = 100%; Method B = 98%. HRMS calcd for $C_{28}H_{33}F_2N_3O_2$ (M + H): 482.2614; found 482.2610. $[\alpha]_D$ = - 35.3 (c = 1.0, chloroform).



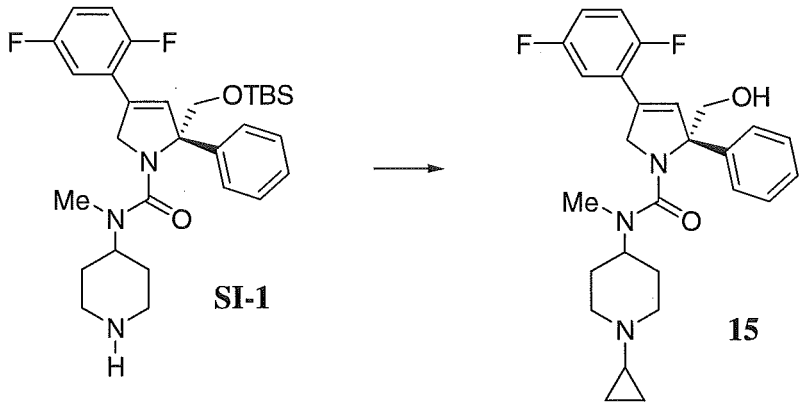
SI-2

1-(2-Fluoroethyl)-*N*-methylpiperidin-4-amine (SI-2). To a solution of 4-*N*-Boc-4-*N*-methyl-aminopiperidine (1.0 g, 4.67 mmol) in 10 mL DMF at 0°C was added 1-bromo-2-fluoroethane (520 μL, 7.0 mmol), NaI (350 mg, 2.33 mmol), and NaH (60% suspension in oil; 140 mg, 5.8 mmol). After warming to room temperature and stirring for several hours, more reagents were added and stirring was continued for an additional 24 hours. The reaction was added to a separatory funnel containing saturated aqueous NH_4Cl and was extracted with EtOAc, washed with saturated brine, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography with a gradient of 0 to 10% MeOH in CH_2Cl_2 (containing ~ 0.1% triethylamine) to provide 850 mg of a colorless oil. This material was dissolved in EtOAc and HCl gas was bubbled through the solution until warm to the touch, the flask was capped and stirred for 1 hour. The procedure was repeated once more, and then the solvents were removed by rotary evaporation. Trituration of the residue with Et_2O provided 575 mg (53%) of the bis-HCl salt of **SI-2** as a white solid. LRMS = 161 (M + H).

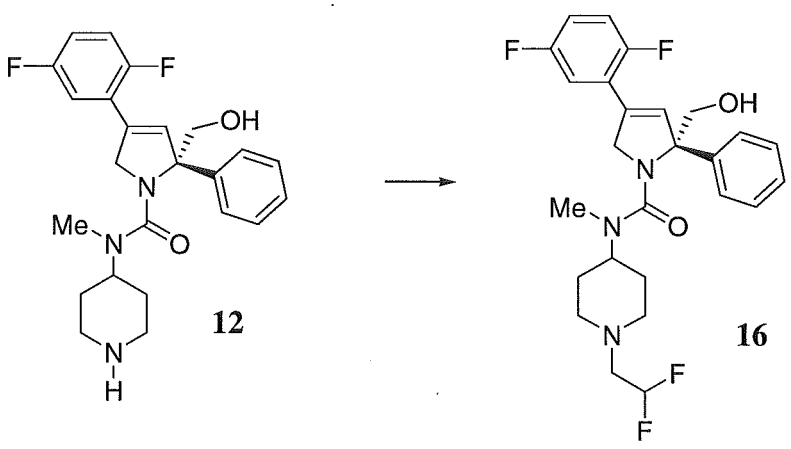


29 **14**

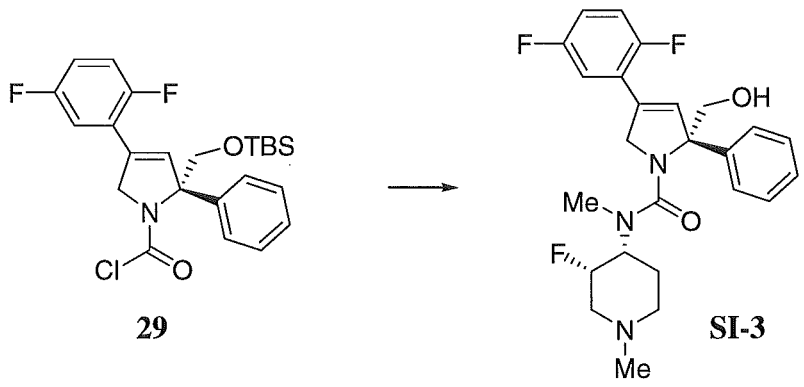
(2*S*)-4-(2,5-Difluorophenyl)-*N*-[1-(2-fluoroethyl)piperidin-4-yl]-2-(hydroxymethyl)-*N*-methyl-2-phenyl-2,5-dihydro-1*H*-pyrrole-1-carboxamide (14). To a solution of **29** (250 mg, 0.54 mmol) in 3 mL of THF was added triethylamine (300 μL, 2.2 mmol), the bis-HCl salt of **SI-2** (151 mg, 0.65 mmol), and a catalytic amount of DMAP. After stirring 48 hours at room temperature, the reaction was added to a separatory funnel containing EtOAc and saturated aqueous $NaHCO_3$. The layers were cut, the organic phase was washed with water, saturated brine, dried over Na_2SO_4 and concentrated. This residue was dissolved in 3 mL of CH_2Cl_2 and 2 mL of trifluoroacetic acid was added. After stirring for 2 hours at room temperature, most of the solvent was removed by rotary evaporation and the residue was added to a separatory funnel containing EtOAc and saturated aqueous $NaHCO_3$. The layers were cut, the organic phase was washed with saturated aqueous $NaHCO_3$, saturated brine, dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography with a gradient of 0 to 90% 20:1:1 EtOH/H_2O/NH_4OH in EtOAc to provide 232 mg (91%) of **14** with ~ 90% purity. This material was purified by reverse phase HPLC, the fractions were free-based with $NaHCO_3$, extracted with EtOAc, dried over Na_2SO_4, and concentrated to provide 128 mg (50%) of **14** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (m, 4H), 7.25 (m, 1H), 7.05 (m, 1H), 6.95 (m, 2H), 6.29 (s, 1H), 5.40 (m, 1H), 4.82 (d, J = 13.7 Hz, 1H), 4.6 (m, 2H), 4.51 (t, J = 4.9 Hz, 1H), 4.45 (m, 1H), 4.00 (dd, J = 12.2, 2.9 Hz, 1H), 3.75 (m, 1H), 3.05 (m, 2H), 2.91 (s, 3H), 2.70 (dt, J = 28.3, 4.9 Hz, 2H), 2.25 – 2.10 (m, 2H), 2.0 – 1.8 (m, 3H), 1.67 (m, 1H) ppm. HPLC Purity: Method A = 100%; Method B = 100%. HRMS calcd for $C_{26}H_{30}F_3N_3O_2$ (M + H): 474.2363; found 474.2352. $[\alpha]_D$ = - 38.0 (c = 0.5, chloroform).



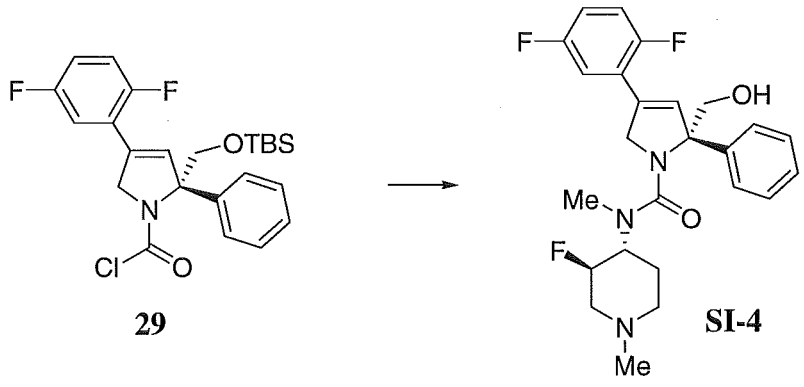
(2*S*)-*N*-(1-Cyclopropylpiperidin-4-yl)-4-(2,5-difluorophenyl)-2-(hydroxymethyl)-*N*-methyl-2-phenyl-2,5-dihydro-1*H*-pyrrole-1-carboxamide (15). To a solution of **SI-1** (400 mg, 0.74 mmol) in 2.2 mL of MeOH in a microwave vial was added powdered 4 Å molecular sieves (300 mg), acetic acid (422 μL, 7.4 mmol), (1-ethoxycyclopropoxy) trimethylsilane (445 μL, 2.2 mmol), and sodium cyanoborohydride (139 mg, 2.2 mmol). After heating in the microwave at 100°C for 10 minutes, the reaction was cooled to 0°C and 1.5 mL of trifluoroacetic acid was added. After stirring for 90 minutes at room temperature, the reaction was filtered through a pad of Celite and added to a separatory funnel containing EtOAc and saturated aqueous $NaHCO_3$. The layers were cut, the organic was washed with saturated aqueous $NaHCO_3$, water, and saturated brine, dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography with a gradient of 0 to 85% 20:1:1 EtOH/H_2O/NH_4OH in EtOAc to provide 250 mg (73%) of **15** as an off-white solid. ^1H NMR (500 MHz, $CDCl_3$) δ 7.35 (m, 4H), 7.25 (m, 1H), 7.05 (m, 1H), 6.95 (m, 2H), 6.29 (s, 1H), 5.40 (bs, 1H), 4.82 (d, J = 13.4 Hz, 1H), 4.57 (d, J = 13.6 Hz, 1H)), 4.42 (m, 1H), 3.98 (d, J = 12.0 Hz, 1H), 3.75 (m, 1H), 3.12 (m, 2H), 2.89 (s, 3H), 2.30 (m, 2H), 1.83 (m, 3H), 1.65 (m, 2H), 0.45 (m, 4H) ppm. HPLC Purity: Method A = 100%; Method B = 97.5%. HRMS calcd for $C_{27}H_{31}F_2N_3O_2$ (M + H): 468.2457; found 468.2447. $[\alpha]_D$ = - 40.2 (c = 0.5, chloroform).



(2*S*)-*N*-[1-(2,2-Difluoroethyl)piperidin-4-yl]-4-(2,5-difluorophenyl)-2-(hydroxymethyl)-*N*-methyl-2-phenyl-2,5-dihydro-1*H*-pyrrole-1-carboxamide (16). To a solution of **12** (114 mg, 0.27 mmol) in 2 mL THF was added triethylamine (82 μL, 0.59 mmol) and 2,2-difluoroethyl trifluoromethanesulfonate (63 mg, 0.29 mmol). After stirring for 3 hours at room temperature, additional reagents were added and the reaction was heated to 50°C overnight. An additional portion of reagents were added and the reaction was heated 7 hours at 80°C, at which time the reaction was complete by LC/MS. The reaction was added to a separatory funnel containing saturated aqueous $NaHCO_3$, and was extracted with EtOAc, washed with saturated brine, dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography with a gradient of 0 to 100% EtOAc in hexanes to provide 119 mg (91%) of **16** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (m, 4H), 7.25 (m, 1H), 7.05 (m, 1H), 6.95 (m, 2H), 6.28 (s, 1H), 5.86 (dt, J = 55.9, 3.7 Hz, 1H), 5.35 (m, 1H), 4.82 (d, J = 13.7 Hz, 1H), 4.57 (d, J = 13.7 Hz, 1H), 4.43 (t, J = 12.0 Hz, 1H), 4.00 (dd, J = 12.0, 3.5 Hz, 1H), 3.73 (m, 1H), 3.02 (m, 2H), 2.91 (s, 3H), 2.74 (m, 2H), 2.34 (m, 2H), 2.0 – 1.8 (m, 3H), 1.64 (m, 1H) ppm. HPLC Purity: Method A = 96%; Method B = 97%. HRMS calcd for $C_{26}H_{29}F_4N_3O_2$ (M + H): 492.2269; found 492.2265. $[\alpha]_D$ = - 38.4 (c = 1.0, chloroform).



(2*S*)-4-(2,5-Difluorophenyl)-*N*-[(3*S*,4*R*)-3-fluoro-1-methylpiperidin-4-yl]-2-(hydroxymethyl)-*N*-methyl-2-phenyl-2,5-dihydro-1*H*-pyrrole-1-carboxamide (SI-3). A procedure analogous to that used to prepare **30** was employed beginning with **23** to provide **SI-3** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (m, 4H), 7.25 (m, 1H), 7.05 (m, 1H), 6.95 (m, 2H), 6.29 (s, 1H), 5.35 (bs, 1H), 5.15 (d, J = 50.3 Hz, 1H), 4.89 (d, J = 13.4 Hz, 1H), 4.53 (d, J = 13.4 Hz, 1H), 4.40 (m, 1H), 3.98 (d, J = 12.2 Hz, 1H), 3.78 (m 1H), 3.21 (s, 3H), 3.13 (m, 1H), 3.03 (m, 1H), 2.4 – 2.1 (m, 3H), 2.33 (s, 3H), 1.67 (m, 1H) ppm. HPLC Purity: Method A = 100%; Method B = 100%. HRMS calcd for $C_{25}H_{28}F_3N_3O_2$ (M + H): 460.2206. Found: 460.2191. $[\alpha]_D$ = - 13.0 (c = 1.0, chloroform).



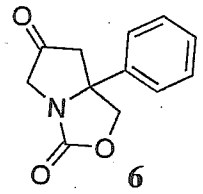
(2*S*)-4-(2,5-Difluorophenyl)-*N*-[(3*R*,4*R*)-3-fluoro-1-methylpiperidin-4-yl]-2-(hydroxymethyl)-*N*-methyl-2-phenyl-2,5-dihydro-1*H*-pyrrole-1-carboxamide (SI-4). A procedure analogous to that used to prepare **30** was employed beginning with **22** to provide **SI-4** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (m, 4H), 7.25 (m, 1H),

7.05 (m, 1H), 6.95 (m, 2H), 6.33 (s, 1H), 5.31 (bs, 1H), 4.82 (d, J = 13.7 Hz, 1H), 4.8 – 4.6 (m, 1H), 4.59 (d, J = 13.7 Hz, 1H), 4.41 (d, J = 12.2 Hz, 1H), 3.97 (d, J = 12.2 Hz, 1H), 3.85 (m, 1H), 3.24 (m, 1H), 3.01(s, 3H), 2.84 (m, 1H), 2.35 (s, 3H), 2.10 (m, 2H), 2.0 – 1.8 (m, 2H) ppm. HPLC Purity: Method A = 100%; Method B = 100%. HRMS calcd for $C_{25}H_{28}F_3N_3O_2$ (M + H): 460.2206. Found: 460.2199. $[\alpha]_D$ = - 18.3 (c = 1.0, chloroform).

Table SI-1. Profiles of the Four Epimeric 3-Fluoro-4-Aminomethylpiperidine Ureas.[a]

Compound	Fluorine disposition	KSP IC_{50} (nM)	Cell IC_{50} (nM)	MDR ratio
30	axial	2.2 ± 1.2	5.3 ± 2.3	4.5
SI-3	axial	13.3 ± 7.0	13.8[b]	4.8
31	equitorial	11.5 ± 6.1	16.5[b]	2.4
SI-4	equitorial	11.9 ± 3.5	37.6[b]	2.9

[a] Average from at least n = 3 experiments, unless otherwise noted. [b] Value is from n = 1 determination.



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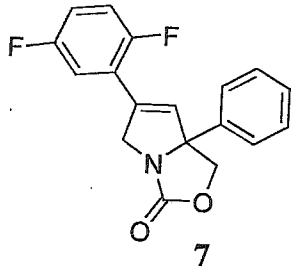

7

9 8 7 6 5 4 3 2 1 ppm

3.71 0.88 0.91
0.97 1.74

1.02 1.02
1.01 1.00

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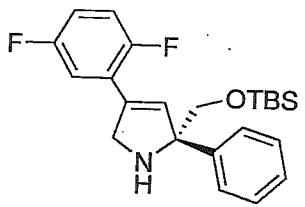

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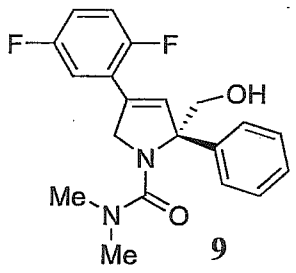

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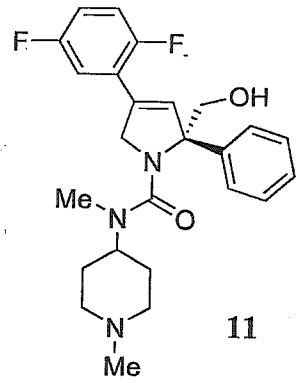

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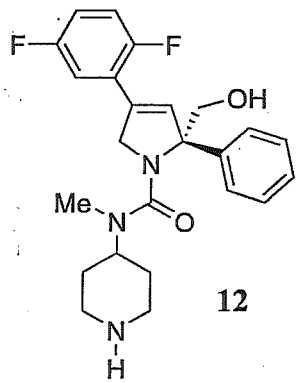

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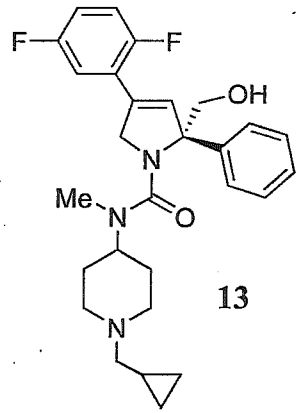

13

9 8 7 6 5 4 3 2 1 ppm

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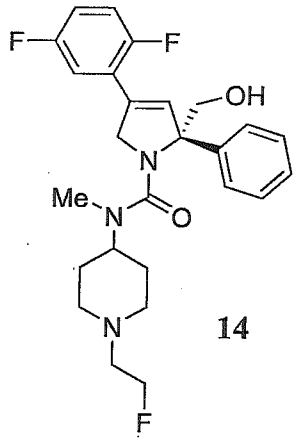

14

9 8 7 6 5 4 3 2 1 ppm

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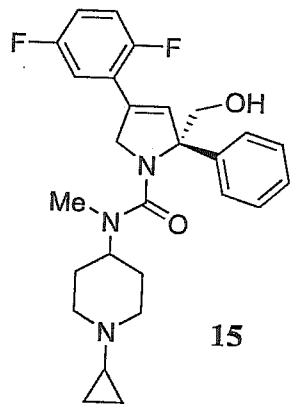

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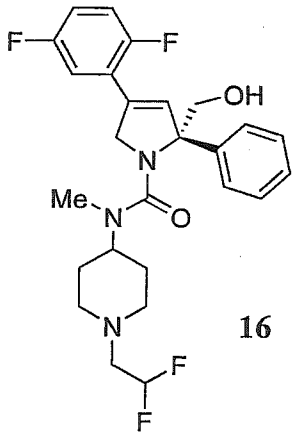

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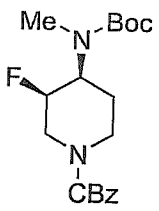

24

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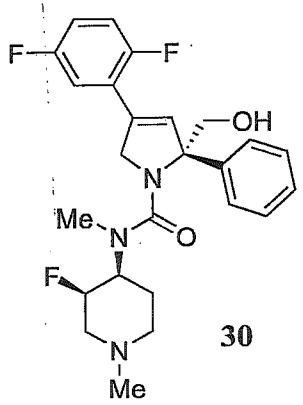

30

9 8 7 6 5 4 3 2 1 ppm

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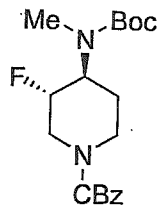
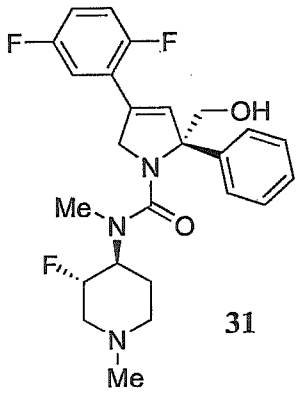


21



31

9 8 7 6 5 4 3 2 1 ppm

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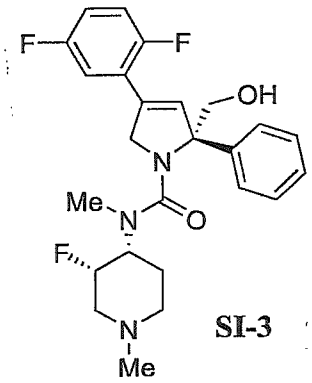
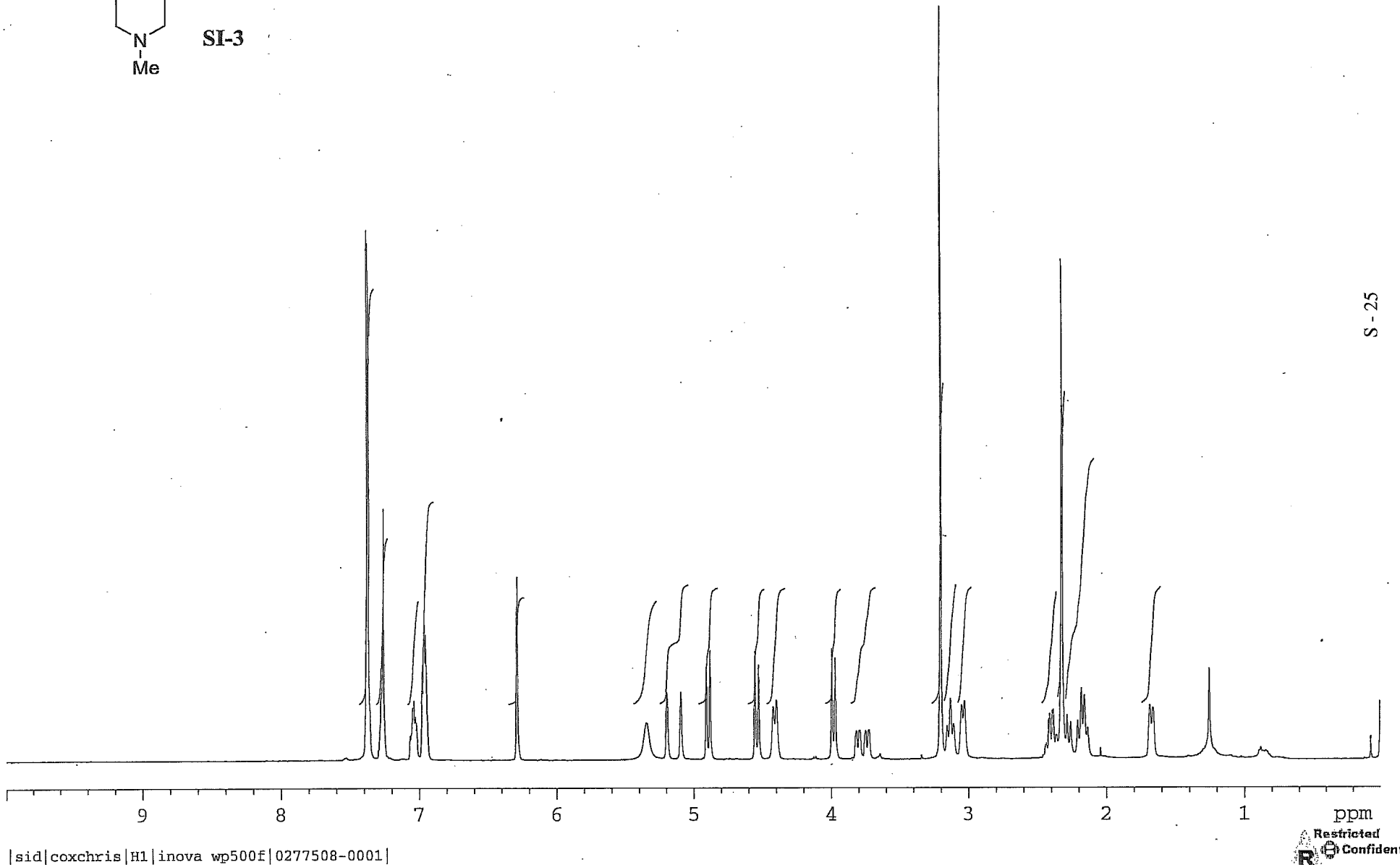


SI-3



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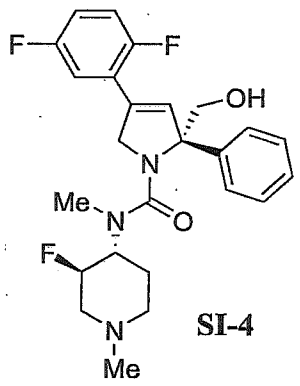

SI-4

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