Supporting information

Discovery of GLPG2451, a Novel, Once Daily Potentiator for the Treatment of Cystic Fibrosis.

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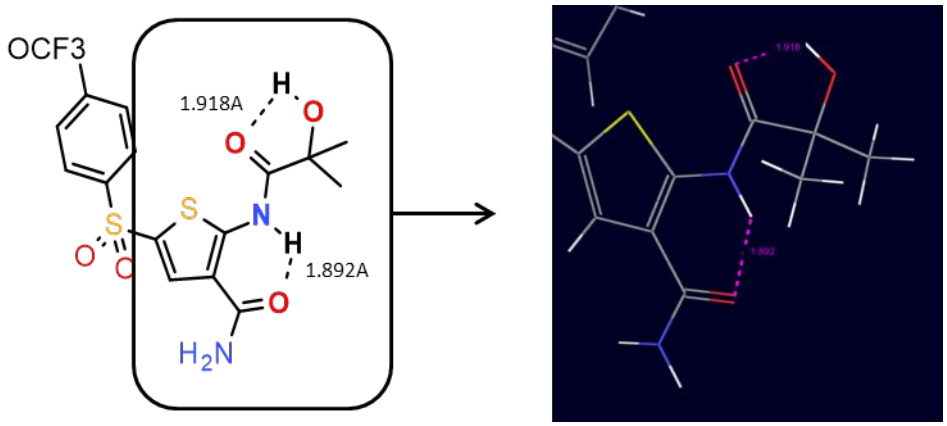
Content of supporting information

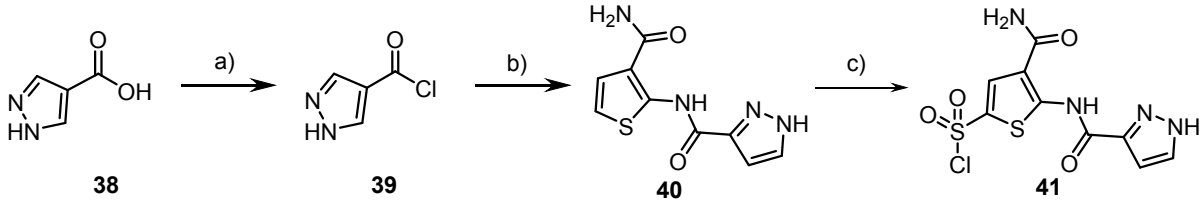
Conformational modelling of 9 in the gas phase.



Internal H bonds are represented with a dotted line. Calculated distances (Angstrom) are depicted in the cartoon.

Experimental procedures and characterisation of representative compounds.

4-Carbamoyl-5-[1H-Pyrazole-3-carbonyl)amino]-thiophene-2-sulfonylchloride (41)



Step a): 1H-Pyrazole-3-carbonyl chloride (**39**)

To a solution of 1H-pyrazole-3-carboxylic acid (**38**, 560 mg, 5 mmol) in DCM (20 mL) were added thionyl chloride (1.80 mL, 25 mmol) and dry DMF (few drops). The reaction mixture was heated at 60°C overnight, cooled to room temperature and concentrated to dryness to afford the title compound **39**.

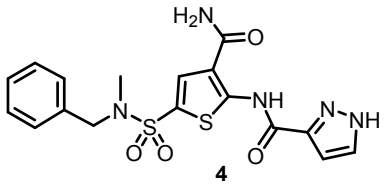
Step b): 1H-Pyrazole-3-carboxylic acid (3-carbamoyl-thiophen-2-yl)-amide (**40**)

To a solution of 2-amino-thiophene-3-carboxylic acid amide (570 mg, 4 mmol), pyridine (0.5 mL, 6 mmol) and DMAP (25 mg, 0.20 mmol) in MeCN (5 mL) was added dropwise solution of compound **39** (650 mg, 5 mmol) in MeCN (5 mL). The reaction mixture was stirred at 60°C for 48h, cooled to room temperature and quenched with saturated solution of $NaHCO_3$. The crude mixture was partially concentrated and filtered. The collected solid was dried in vacuum oven to afford the title compound **40**. LC-MS: m/z = 237 $[M+H]^+$.

Step c): 4-Carbamoyl-5-[1H-Pyrazole-3-carbonyl)amino]-thiophene-2-sulfonylchloride (**41**)

To neat $ClSO_3H$ (1.0 mL, 15.0 mmol) was added portion wise compound **40** (355 mg, 1.5 mmol) at 0 °C. The resulting mixture was stirred at 40 °C until full conversion (monitored by LC-MS). The reaction mixture was cooled to room temperature, diluted with EtOAc and poured into a mixture of ice and water. The crude mixture was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated to dryness to afford the title compound **41** (440 mg, 87% yield). LC-MS: m/z = 335 [M+H]+.

N-(5-(N-benzyl-N-methylsulfamoyl)-3-carbamoylthiophen-2-yl)-1H-pyrazole-3-carboxamide (4)



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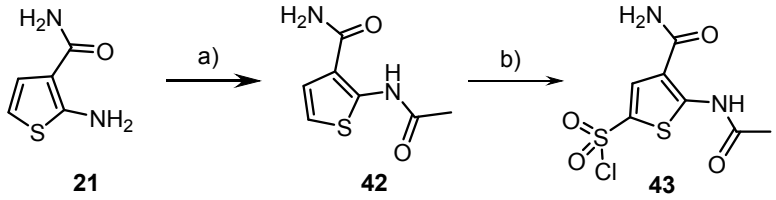
To a suspension of compound **41** (1.3 mmol, 440 mg) in DCM (5 mL) was added a solution of pyridine (1.95 mmol, 0.16 mL) and N-methylbenzylamine (1.6 mmol, 0.2 mL) in DCM (3 mL). The reaction mixture was stirred at room temperature for 2.5 h, diluted with DCM and washed with saturated solution of $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude residue was purified by preparative HPLC to afford the title compound **4** (110 mg, 20 % yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.72 (t, 1H), 13.33 (s, 1H), 8.21 (d, 2H), 8.01 (dd, 1H), 7.78 (s, 1H), 7.44 – 7.28 (m, 6H), 6.90 (dd, 1H), 4.17 (s, 2H), 2.62 (s, 3H). LC-MS: m/z = 420.0 $[M+H]^+$.

5-acetylamino-4-carbamoyl-thiophene-2-sulfonylchloride (43)



21 **42** **43**

Step a): 2-acetylamino-thiophene-3-carboxylic acid amide (**42**)

A solution of 2-amino-thiophene-3-carboxylic acid amide **21** (20.3 g, 143 mmol) in dry pyridine (150 mL) was added dropwise acetylchloride (11 mL, 156 mmol) at 0 °C. The reaction mixture was stirred at room temperature overnight, diluted with water and the resulting

suspension was filtered. The collected solid was dried overnight in a vacuum oven at 50°C to afford the compound **42** (19.7 g, 76% yield). LC-MS: m/z = 185 $[M+H]^+$.

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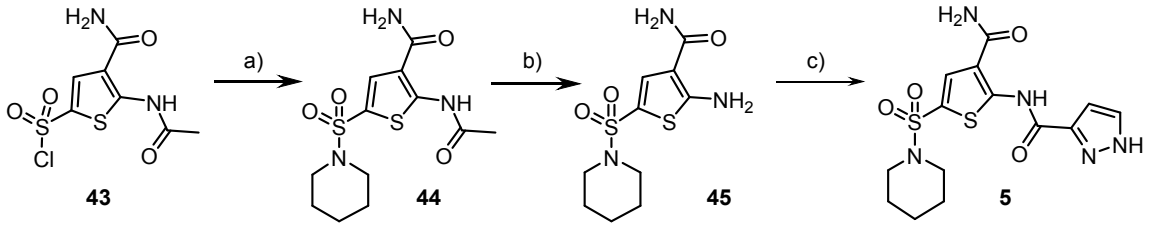
Step b): 5-acetylamino-4-carbamoyl-thiophene-2-sulfonylchloride (**43**)

To neat $ClSO_3H$ (29 mL, 440 mmol) was added portion wise compound **42** (8.1 g, 44 mmol) at 0 °C. The resulting mixture was stirred at 50 °C for 3h. The reaction mixture was cooled to room temperature, diluted with EtOAc and poured into a mixture of ice and water. The crude mixture was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated to dryness to afford the title compound **43** (4.5 g, 42% yield).

N-[3-carbamoyl-5-(1-piperidylsulfonyl)-2-thienyl]-1H-pyrazole-3-carboxamide (5)



Step a): 2-acetamido-5-(1-piperidylsulfonyl)thiophene-3-carboxamide (**44**)

To a suspension of compound **43** (14.1 mmol, 4.0 g) in DCM (50 mL) were added pyridine (28.2 mmol, 2.3 mL) and piperidine (28.2 mmol, 2.8 mL). The reaction mixture was diluted with 1,4-dioxane (6 mL) and stirred at room temperature for 1h. The crude mixture was diluted with DCM and quenched with saturated solution of $NaHCO_3$. The suspension was filtered and the collected solid was washed with water and Et_2O to afford the compound **44** (3.0 g, 64% yield). LC-MS: m/z = 332 $[M+H]^+$.

Step b): 2-amino-5-(1-piperidylsulfonyl)thiophene-3-carboxamide (**45**)

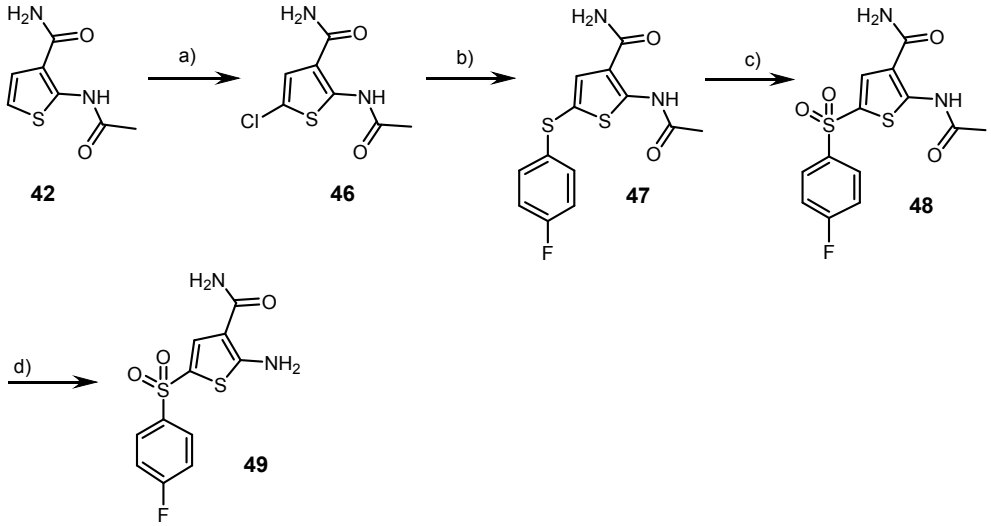
A solution of compound **44** (3.0 g, 9.05 mmol) in 1:1 mixture of 6 N HCl/1,4-dioxane (40 mL) was stirred at 100 °C for 2 h. The reaction mixture was concentrated to dryness. The crude residue was washed with water and dried to afford the title compound **45** (2.4 g, 81% yield). LC-MS: m/z = 290 $[M+H]^+$.

Step c): N-[3-carbamoyl-5-(1-piperidylsulfonyl)-2-thienyl]-1H-pyrazole-3-carboxamide (**5**)

To a solution of compound **45** (50 mg, 0.15 mmol), 1H-pyrazole-3-carboxylic acid (26 mg, 0.23 mmol) and Et_3N (105 µl, 0.75 mmol) in MeCN (1 mL) were added Mukaiyama reagent

(2-chloro-1-methylpyridinium iodide, 59 mg, 0.23 mmol) and DMAP (9 mg, 0.07 mmol). The reaction mixture was stirred at room temperature for 1 h, diluted with DCM and washed with saturated solution of $NaHCO_3$.The organic phase was concentrated and the crude residue was purified by preparative HPLC to afford the title compound **5** (13 mg, 23% yield).
1H NMR (400 MHz, DMSO-d6) δ 13.68 (s, 1H), 13.30 (s, 1H), 8.16 (s, 1H), 8.11 (s, 1H), 8.01-7.97 (m, 1H), 7.70 (s, 1H), 6.90-6.86 (m, 1H), 3.02-2.93 (m, 4H), 1.63-1.52 (m, 4H), 1.46-1.35 (m, 2H). LC-MS: m/z = 384 $[M+H]^+$.

2-amino-5-(4-fluorophenyl)sulfonyl-thiophene-3-carboxamide (49)



Step a): 2-acetamido-5-chloro-thiophene-3-carboxamide (**46**)

To a solution of 2-acetamidothiophene-3-carboxamide (2.14 g, 1.16 mmol) in AcOH (23 mL) was added NCS (1.71 g, 12.8 mmol). The reaction mixture was stirred at room temperature for 15h. NCS (0.26 g, 1.92 mmol) was added and the reaction mixture was stirred at 50 °C for 1 h. The reaction mixture was concentrated to dryness. The crude residue was triturated with EtOH and dried to afford the title compound **46** (2.37 g, 83% yield). LC-MS: m/z = 202 $[M-NH_3]^+$.

Step b): 2-acetamido-5-(4-fluorophenyl)sulfanyl-thiophene-3-carboxamide (**47**)

To a solution of compound **46** (437 mg, 2.0 mmol) in NMP (6 mL) was added Cs_2CO_3 (1.30 g, 4.0 mmol) and 4-fluorothiophenol (235 µl, 2.20 mmol). The reaction mixture was stirred in sealed tube at 145 °C for 1h. The mixture was cooled to room temperature, diluted with water and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated to dryness to afford the title compound **47**. LC-MS: m/z = 311 $[M+H]^+$.

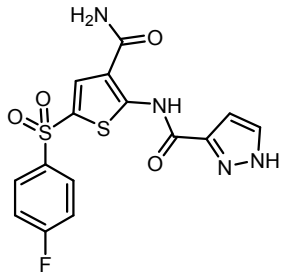
Step c): 2-acetamido-5-(4-fluorophenyl)sulfonyl-thiophene-3-carboxamide (**48**)

To a solution of compound **47** (989 mg, 3.19 mmol) in AcOH (10 mL) was added H_2O_2 (35% in water, 0.9 mL, 10.26 mmol). The reaction mixture was stirred in sealed tube at 60 °C for 4 h. The mixture was cooled to room temperature, diluted with DCM and washed saturated solution of $NaHCO_3$. The organic layer was dried over $MgSO_4$, filtered and concentrated to dryness to afford the title compound **48** (2.6 g, 81% yield). LC-MS: m/z = 343 $[M+H]^+$.

Step d): 2-amino-5-(4-fluorophenyl)sulfonyl-thiophene-3-carboxamide (**49**)

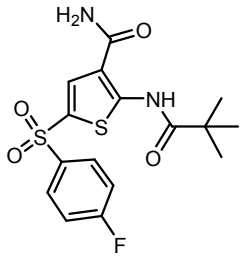
To a solution of compound **48** (883 mg, 2.58 mmol) in a mixture of water (3.9 mL) and 1,4-dioxane (7.7 mL) was added 12N HCl (3.9 mL, 8.70 mol). The reaction mixture was stirred in sealed tube at 100 °C for 2 h. The mixture was cooled to room temperature and concentrated to dryness. The crude residue was suspended in a mixture of water/EtOAc, neutralized to pH 7 and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated to dryness to afford the title compound **49** (444 mg, 57% yield). LC-MS: m/z = 301 $[M+H]^+$.

N-[3-carbamoyl-5-(4-fluorophenyl)sulfonyl-2-thienyl]-1H-pyrazole-3-carboxamide (6)



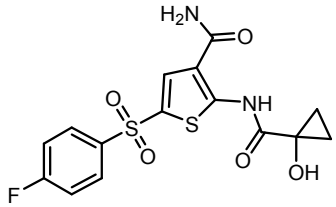
To a solution of compound **49** (100 mg, 0.333 mmol), 1H-pyrazole-3-carboxylic acid (49 mg, 0.433 mmol) and Et_3N (100 mg, 0.333 mmol) in MeCN (4 mL) were added Mukaiyama reagent (2-chloro-1-methylpyridinium iodide, 109 mg, 0.433 mmol) and DMAP (8 mg, 0.07 mmol). The reaction mixture was stirred at 60 °C for 15 h, cooled to room temperature, diluted with DCM and washed with saturated solution of $NaHCO_3$. The organic phase was concentrated and the crude residue was purified by preparative HPLC to afford the title compound **6** (40 mg, 30% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.73 (s, 1H), 13.31 (s, 1H), 8.37 (s, 1H), 8.17 (s, 1H), 8.07 – 7.97 (m, 3H), 7.78 (s, 1H), 7.56 – 7.44 (m, 2H), 6.88 (t, 1H). LC-MS: m/z = 395 $[M+H]^+$.

2-(2,2-dimethylpropanoylamino)-5-(4-fluorophenyl)sulfonyl-thiophene-3-carboxamide (7)



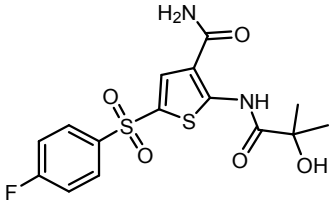
To a solution of compound **49** (100 mg, 0.333 mmol), pivalic acid (44 mg, 0.433 mmol) and Et_3N (186 µl, 1.333 mmol) in MeCN (4 mL) were added Mukaiyama reagent (2-chloro-1-methylpyridinium iodide, 109 mg, 0.433 mmol) and DMAP (8 mg, 0.07 mmol). The reaction mixture was stirred at 60 °C for 15 h, cooled to room temperature, diluted with DCM and washed with saturated solution of $NaHCO_3$. The organic phase was concentrated and the crude residue was purified by preparative HPLC to afford the title compound **7** (32 mg, 25% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 12.91 (s, 1H), 8.34 (s, 1H), 8.22 (s, 1H), 8.04 – 7.94 (m, 2H), 7.84 (s, 1H), 7.55 – 7.44 (m, 2H), 1.23 (s, 9H). LC-MS: m/z = 385 $[M+H]^+$.

5-(4-fluorophenyl)sulfonyl-2-[(1-hydroxycyclopropanecarbonyl)amino]thiophene-3-carboxamide (8)



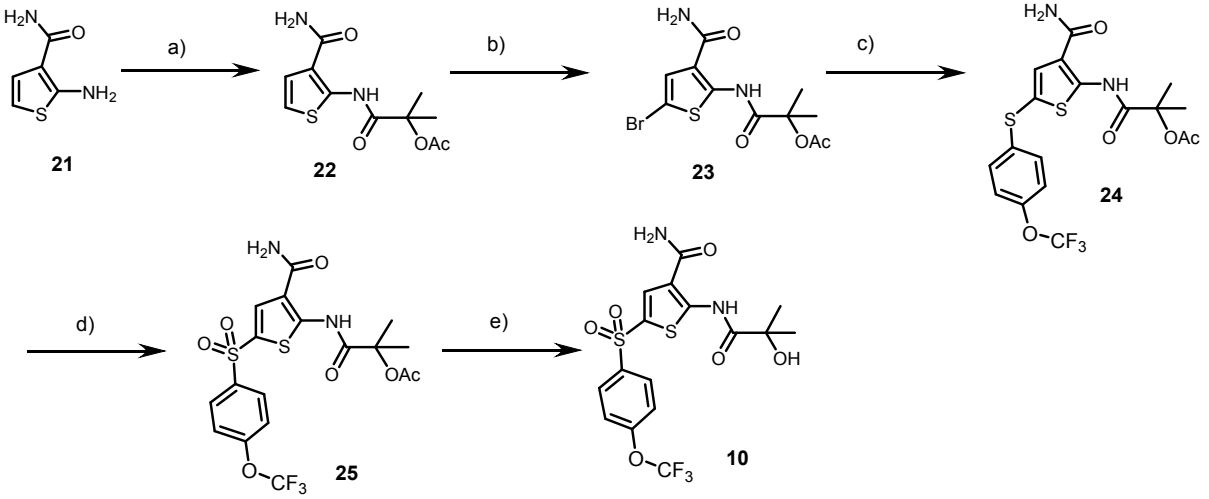
To a solution of compound **49** (100 mg, 0.333 mmol), 1-(acetyloxy)cyclopropane-1-carboxylic acid (60 mg, 0.42 mmol) and Et_3N (186 µl, 1.333 mmol) in MeCN (4 mL) were added Mukaiyama reagent (2-chloro-1-methylpyridinium iodide, 117 mg, 0.46 mmol) and DMAP (12 mg, 0.1 mmol). The reaction mixture was stirred in sealed tube at 50 °C for 1 h. The reaction mixture was cooled to room temperature and partitioned between DCM and water. The organic layer was dried over $MgSO_4$, filtered and concentrated to dryness. The crude residue was stirred in 7N NH MeOH solution (10 mL) at room temperature for 48 h. The mixture was concentrated to dryness and the crude residue was purified by preparative HPLC to afford the title compound **8** (20 mg, 18% yield). ^{1}H NMR δ (ppm)(DMSO-d_6): 13.15 (s, 1 H), 8.34 (s br, 1 H), 8.14 (s, 1 H), 7.99 (dd, 2 H), 7.75 (s, 1 H), 7.49 (t, 2 H), 7.00 (s, 1 H), 1.21 (m, 2 H), 1.11 (m, 2 H). LC-MS: m/z = 385 $[M+H]^+$.

5-(4-fluorophenyl)sulfonyl-2-[(2-hydroxy-2-methyl-propanoyl)amino]thiophene-3-carboxamide (9)



To a solution of compound **49** (150 mg, 0.499 mmol) in DCM (1.5 mL) was added 2-acetoxyisobutyryl chloride (90 mg, 0.549 mmol) and Et_3N (133 µl, 0.749 mmol). The reaction mixture was stirred in sealed vial at 60 °C for 15 h. The reaction mixture was cooled to room temperature and portioned between DCM and water. The organic layer was dried over $MgSO_4$, filtered and concentrated to dryness. The crude residue was dissolved in MeOH (2.6 mL) and K_2CO_3 (177 mg, 1.28 mmol) was added. The mixture was stirred at 65 °C for 15h, cooled to room temperature and portioned between EtOAc and water. The organic layer was dried over $MgSO_4$, filtered and concentrated to dryness. The crude residue was purified by preparative HPLC to afford the title compound **9** (20 mg, 15% yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 12.94 (s, 1H), 8.35 (s, 1H), 8.14 (s, 1H), 8.05 – 7.95 (m, 2H), 7.72 (s, 1H), 7.56 – 7.44 (m, 2H), 6.15 (s, 1H), 1.34 (s, 7H), 1.23 (s, 1H). LC-MS: m/z = 421[M+H]$^+$.

2-[(2-hydroxy-2-methyl-propanoyl)amino]-5-[4-(trifluoromethoxy)phenyl]sulfonyl-thiophene-3-carboxamide (10)



Step a): [2-[(3-carbamoyl-2-thienyl)amino]-1,1-dimethyl-2-oxo-ethyl] acetate (**22**)

To a solution of 2-aminothiophene-3-carboxamide **21** (3.9 g, 27.4 mmol) in DCM (45 mL) was added pyridine (3.77 mL, 45.8 mmol). The mixture was cooled to 0 °C and a solution of 2-acetoxyisobutyryl chloride (5 g, 30.5 mmol) in DCM (25 mL) was added dropwise. The

reaction mixture was stirred at room temperature for 2 h, quenched with water and extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated to dryness. The crude residue was triturated with water and dried to afford the title compound **22** (6.89 g, 93% yield). LC-MS: m/z = 271$[M+H]^+$.

Step b): [2-[(5-bromo-3-carbamoyl-2-thienyl)amino]-1,1-dimethyl-2-oxo-ethyl] acetate (**23**)

To a solution of compound **22** (4.05 g, 15.0 mmol) in AcOH (60 mL) was added dropwise NBS (2.8 g, 15.75 mmol). The reaction mixture was stirred at room temperature for 1h, quenched with water and extracted with EtOAc. The combined organic layers were washed with saturated solution of Na_2CO_3, dried over Na_2SO_4, filtered and concentrated to afford the title compound **23** (5.12 g, 98% yield). LC-MS: m/z = 351$[M+H]^+$.

Step c): [2-[[3-carbamoyl-5-[4-(trifluoromethoxy)phenyl]sulfanyl-2-thienyl]amino]-1,1-dimethyl-2-oxo-ethyl] acetate (**24**)

To a solution of compound **23** (250 mg, 0.72 mmol) in dioxane (3 ml) were added trifluoromethoxybenzenethiol (181 mg, 0.93 mmol), $Pd(OAc)_2$ (8.1 mg, 0.036 mmol), DiPPF (9.7 mg, 0.043 mmol) and NaOtBu (83 mg, 0.86 mmol). The reaction mixture was flushed with nitrogen, the vial was sealed and the mixture was stirred at 160 °C for 16 h. The crude mixture was cooled to room temperature, filtered through a silica plug and washed with EtOAc. The filtrate was concentrated to afford the title compound **24**. LC-MS: m/z = 463$[M+H]^+$.

Step d): [2-[[3-carbamoyl-5-[4-(trifluoromethoxy)phenyl]sulfonyl-2-thienyl]amino]-1,1-dimethyl-2-oxo-ethyl] acetate (**25**)

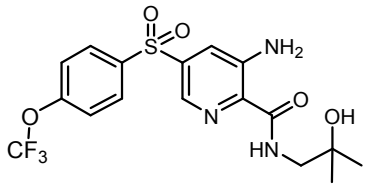
To a solution of compound **24** (330 mg, 0.72 mmol) in AcOH (10 mL) was added H_2O_2 (35% in water, 0.25 mL, 2.88 mmol). The reaction mixture was stirred in sealed tube at 60 °C for 4 h. The mixture was cooled to room temperature, diluted with water and extracted with EtOAc. The combined organic layers were washed with saturated solution of Na_2CO_3, dried over $MgSO_4$, filtered and concentrated to dryness to afford the title compound **25**. LC-MS: m/z = 495$[M+H]^+$.

Step e): 2-[(2-hydroxy-2-methyl-propanoyl)amino]-5-[4-(trifluoromethoxy)phenyl]sulfonyl-thiophene-3-carboxamide (**10**)

To a solution of compound **25** (360 mg, 0.72 mmol) in MeOH (10 mL) was added K_2CO_3 (200 mg, 1.44 mmol). The reaction mixture was stirred at 60 °C for 16 h, cooled to room

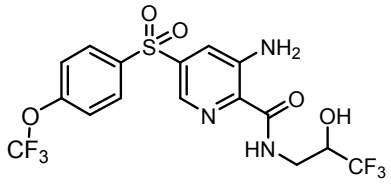
temperature, quenched with water and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered and concentrated to dryness. The crude residue was purified by preparative HPLC to afford the title compound **10** (22 mg, 8% yield). LC-MS: m/z = 453$[M+H]^+$.

3-amino-N-(2-hydroxy-2-methyl-propyl)-5-[4-(trifluoromethoxy)phenyl]sulfonyl-pyridine-2-carboxamide (11)



To a solution of compound **28** (50 mg, 0.14 mmol) in DMA (550 µL) were added 1-amino-2-methyl-propan-2-ol (12 mg, 0.14 mmol), HATU (52 mg, 0.14 mmol) and Et_3N (39 µL, 0.28 mmol) The reaction mixture was stirred at room temperature for 15 h, diluted with water and extracted with EtOAc. The combined organic layers were then dried over $MgSO_4$, filtered and concentrated. The crude residue was purified by preparative HPLC to afford the title compound **11** (10 mg, 17% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 8.39 (t, 1 H), 8.24 (d, 1 H), 8.13 (d, 2 H), 7.74 (d, 1 H), 7.65 (d, 2 H), 7.27 (s br, 5 H), 4.65 (s, 1 H), 3.21 (d, 2 H), 1.07 (s, 6 H). LC-MS: m/z = 434 $[M+H]^+$

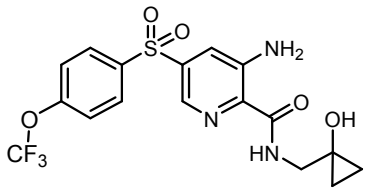
3-amino-N-(3,3,3-trifluoro-2-hydroxy-propyl)-5-[4-(trifluoromethoxy)phenyl]sulfonyl-pyridine-2-carboxamide (12)



To a solution of compound **28** (50 mg, 0.14 mmol) in DMA (550 µL) were added 3-amino-1,1,1-trifluoro-propan-2-ol (18 mg, 0.14 mmol), HATU (52 mg, 0.14 mmol) and Et_3N (39 µL, 0.28 mmol) The reaction mixture was stirred at room temperature for 15 h, diluted with water and extracted with EtOAc. The combined organic layers were then dried over $MgSO_4$, filtered and concentrated. The crude residue was purified by preparative HPLC to afford the title compound **22** (12 mg, 18% yield). 1H NMR (400 MHz, DMSO-d6) *δ* ppm 8.39 (t, 1 H), 8.24

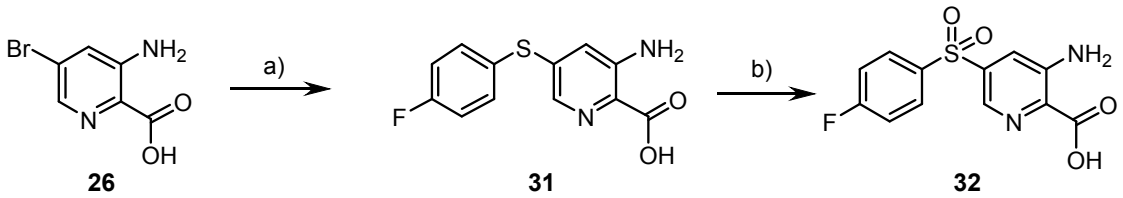
(d, 1 H), 8.13 (d, 2 H), 7.74 (d, 1 H), 7.65 (d, 2 H), 7.27 (s , 2 H), 6.44 (d, 1 H), 4.20 (m, 1 H), 3.57 (m, 1 H), 3.38 (m, 1 H). LC-MS: m/z = 474 $[M+H]^+$

3-amino-N-[(1-hydroxycyclopropyl)methyl]-5-[4-(trifluoromethoxy)phenyl]sulfonyl-pyridine-2-carboxamide (14)



To a solution of compound **28** (50 mg, 0.14 mmol) in DMA (550 µL) were added 1-(aminomethyl)cyclopropanol (12 mg, 0.14 mmol), HATU (52 mg, 0.14 mmol) and Et_3N (39 µL, 0.28 mmol) The reaction mixture was stirred at room temperature for 15 h, diluted with water and extracted with EtOAc. The combined organic layers were then dried over $MgSO_4$, filtered and concentrated. The crude residue was purified by preparative HPLC to afford the title compound **14** (13 mg, 22% yield).^{1}H NMR (400 MHz, DMSO-d6) δ ppm 8.56 (t, 1H), 8.25 (d, 1H), 8.14 (d, 2H), 7.74 (d, 1H), 7.66 (d, 2H), 7.28 (s, 2H), 5.49 (s, 1H), 3.37 (d, 2H), 0.60 – 0.48 (m, 4H)LC-MS: m/z =432 $[M+H]^+$

3-Amino-5-(4-fluoro-benzenesulfonyl)-pyridine-2-carboxylic acid (32)



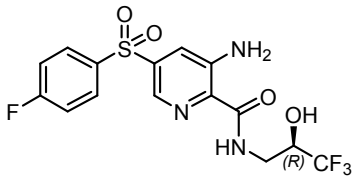
Step a): 3-Amino-5-(4-fluoro-phenylsulfanyl)-pyridine-2-carboxylic acid (**31**):

To a solution of 3-amino-5-bromo-pyridine-2-carboxylic acid (**26**) (13 g, 60 mmol) in DMA (90 mL) were added 4-fluoro-benzenethiol (7.68 g, 60 mmol) and DBU (9.0 mL, 60 mmol). The reaction mixture was stirred at 140 °C for 50 min in microwave reactor. The crude mixture was cooled to room temperature and diluted with a 1% AcOH solution in water. The resulting precipitate was collected by filtration, washed with petroleum ether and dried in a vacuum oven to afford the tittle compound **31** (10.6 g, 67% yield). LC-MS: m/z = 265 $[M+H]^+$

Step b): 3-Amino-5-(4-fluoro-benzenesulfonyl)-pyridine-2-carboxylic acid (**32**):

To a solution of compound **31** (10.6 g, 40.2 mmol) in TFA (110 mL) was added H_2O_2 (13.7 mL, 160 mmol) at 0 °C. The reaction mixture was stirred at 0°C for 30 min and then at room temperature until full conversion (monitored by LC-MS). The crude mixture was diluted with water while stirring at 0°C. The resulting precipitate was collected by filtration, washed with water and dried in a vacuum oven to afford the tittle compound **32** (9.52 g, 80% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 12.98 (s, 1H), 8.25 (d, 1H), 8.08 (dd, 2H), 7.78 (d, 1H), 7.53 (dd, 2H), 7.07 (s, 1H). LC-MS: m/z = 297 $[M+H]^+$

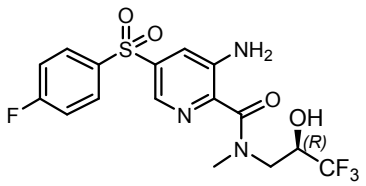
3-Amino-5-(4-fluorophenyl)sulfonyl-N-(3,3,3-trifluoro-2-hydroxy-propyl)pyridine-2-carboxamide (15)



To a solution of compound **32** (4.44 g, 15 mmol) in NMP (75 mL) were added **(**2R**)**-3-amino-1,1,1-trifluoro-propan-2-ol (2.48 g, 15 mmol), HATU (5.70 g, 15 mmol) and Et_3N (4.18mL, 30 mmol) The reaction mixture was stirred for 30 min, diluted with water and extracted with EtOAc. The combined organic layers were then dried over Na_2SO_4, filtered and concentrated. The crude residue was purified by flash chromatography, using a mixture of EtOAc/petroleum ether (ratio 1/3) to afford the title compound **15** (700 mg, 12% yield).

^{1}H NMR (400 MHz, DMSO-d6) *δ* 8.74 (1 H, t), 8.21 (1 H, d), 8.07 (2 H, m), 7.73 (1 H, d), 7.50 (2 H, m), 7.26 (2 H, s, br), 6.45 (1 H, d), 4.21 (1 H, m), 3.56 (1 H, m), 3.38 (1 H, m). LC-MS: m/z = 408 $[M+H]^+$

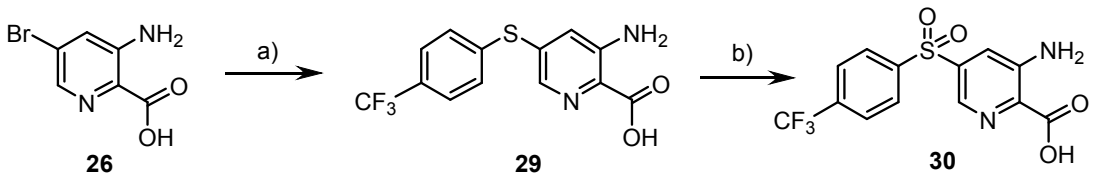
3-Amino-5-(4-fluorophenyl)sulfonyl-N-methyl-N-[(2R)-3,3,3-trifluoro-2-hydroxy-propyl]pyridine-2-carboxamide (16)



To a solution of compound **32** (50 mg, 0.17 mmol) in DMA (675 µL) were added (2*R*)-1,1,1-trifluoro-3-(methylamino)propan-2-ol (24 mg, 0.17 mmol), HATU (64 mg, 0.17 mmol) and Et_3N (47 µL, 0.34 mmol). The reaction mixture was stirred at room temperature for 15 h, diluted with water and extracted with EtOAc. The combined organic layers were then dried

over $MgSO_4$, filtered and concentrated. The crude residue was purified by preparative HPLC to afford the title compound **16** (12 mg, 17% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 8.23 (dd, 1H), 8.06 (dt, , 2H), 7.61 (dd, 1H), 7.56 – 7.47 (m, 2H), 6.62 (dd, 1H), 6.04 (s, 1H), 5.97 (s, 1H), 4.33 (dd, 1H), 3.93 – 3.46 (m, 1H), 3.47 – 3.35 (m, 1H), 3.00 (d, 3H). LCM-MS: m/z = 422 $[M+H]^+$

3-Amino-5-(4-trifluoromethyl-benzenesulfonyl)-pyridine-2-carboxylic acid (30)



26 **29** **30**

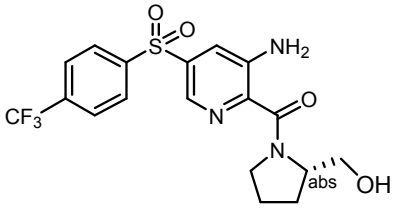
Step a): 3-Amino-5-(4-trifluoromethyl-phenylsulfanyl)-pyridine-2-carboxylic acid (**29**):

To a solution of 3-amino-5-bromo-pyridine-2-carboxylic acid (**26**) (3.79 g, 17.4 mmol) in DMA (15 mL) were added trifluoromethyl-benzenethiol (4.1 g, 21 mmol) and DBU (2.6 mL, 17.4 mmol). The reaction mixture was stirred at 140 °C for 45 min in microwave reactor. The crude mixture was cooled to room temperature and diluted with a 1% AcOH solution in water. The resulting precipitate was collected by filtration, washed with a 1% AcOH/water mixture followed by with petroleum ether. Obtained solid was dried in vacuum oven to afford the tittle compound **29** (3.6 g, 65% yield). LC-MS: m/z = 315$[M+H]^+$.

Step b): 3-Amino-5-(4-trifluoromethyl-benzenesulfonyl)-pyridine-2-carboxylic acid (**30**):

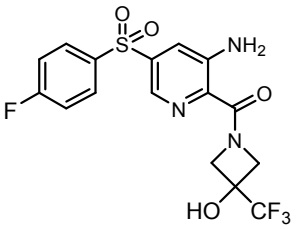
To a solution of compound **29** (5.5 g, 17.5 mmol) in TFA (35 mL) was added H_2O_2 (6 mL, 70 mmol) at 0 °C. The reaction mixture was stirred at 0°C until full conversion (monitored by LC-MS). The crude mixture was diluted with 1% AcOH solution in water while stirring at 0°C. The resulting precipitate was collected by filtration, washed with 1% AcOH solution in water and dried in a vacuum oven to afford the tittle compound **30** (4.84 g, 80% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 13.10 (s, 1H), 8.28 (d, 1H), 8.22 (d, 2H), 8.06 (d, 2H), 7.82 (d, 1H), 7.14 (s, 2H). LC-MS: m/z = 347 $[M+H]^+$

[3-Amino-5-[4-(trifluoromethyl)phenyl]sulfonyl-2-pyridyl]-[(2S)-2-(hydroxymethyl)pyrrolidin-1-yl]methanone (17)



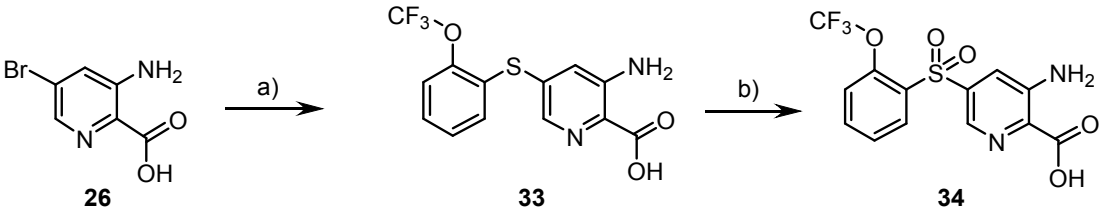
To a solution of compound **30** (50 mg, 0.14 mmol) in DMA (578 µL) were added [(2S)-pyrrolidin-2-yl]methanol (16 mg, 0.16 mmol), HATU (60 mg, 0.16 mmol) and Et_3N (44 µL, 0.32 mmol). The reaction mixture was stirred at room temperature for 15 h, diluted with water and extracted with EtOAc. The combined organic layers were then dried over $MgSO_4$, filtered and concentrated. The crude residue was purified by preparative HPLC to afford the title compound **17** (9 mg, 15% yield).

[3-Amino-5-(4-fluorophenyl)sulfonyl-2-pyridyl]-[3-hydroxy-3-(trifluoromethyl)azetidin-1-yl]methanone (18)



To a solution of compound **32** (50 mg, 0.17 mmol) in DMA (680 µL) were added 3-(trifluoromethyl)azetidin-3-ol (24 mg, 0.17 mmol), HATU (64mg, 0.17 mmol) and Et_3N (47 µL, 0.34 mmol). The reaction mixture was stirred at room temperature for 15 h, diluted with water and extracted with EtOAc. The combined organic layers were then dried over $MgSO_4$, filtered and concentrated. The crude residue was purified by preparative HPLC to afford the title compound **18** (10 mg, 14% yield). 1H NMR (400 MHz, DMSO-d6) δ ppm 8.20 (d, 1 H), 8.06 (m, 2 H), 7.72 (d, 1 H), 7.51 (m, 2 H), 7.44 (s, 1 H), 7.16 (s br, 2 H), 4.75 (m, 1 H), 4.50 (m, 1 H), 4.24 (m, 1 H), 4.00 (m,1 H). LCM-MS: m/z = 420 $[M+H]^+$

3-Amino-5-[2-(trifluoromethoxy)phenyl]sulfonyl-pyridine-2-carboxylic acid (34)



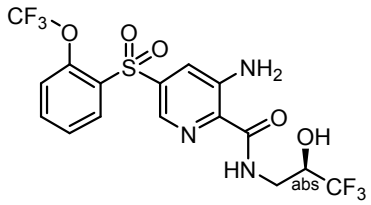
Step a): 3-Amino-5-(2-trifluoromethoxy-phenylsulfanyl)-pyridine-2-carboxylic acid (**33**)

To a solution of 3-amino-5-bromo-pyridine-2-carboxylic acid (**26**) (868 mg, 4.0 mmol) in DMA (15 mL) were added 2-trifluoromethoxy-thiophenol (776 mg, 4.0 mmol) and DBU (600 µL, 4.0 mmol). The reaction mixture was stirred at 140 °C for 60 min in microwave reactor. The crude mixture was cooled to 0 °C, diluted with water and acidified to pH = 4 with AcOH. The resulting precipitate was collected by filtration, washed with water followed by petroleum ether. Obtained solid was dried in vacuum oven to afford the tittle compound **33** (3830 mg, 83% yield). LC-MS: m/z = 331$[M+H]^+$

Step b): 3-Amino-5-(2-trifluoromethoxy-benzenesulfonyl)-pyridine-2-carboxylic acid (**34**)

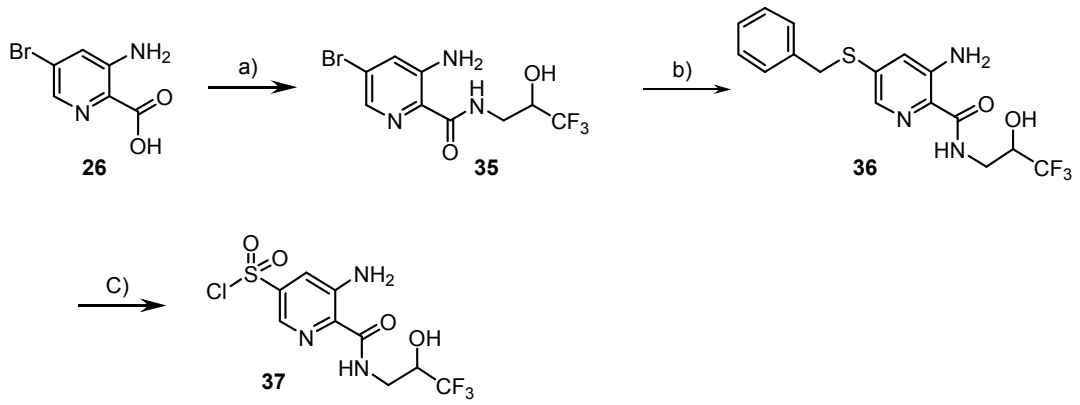
To a solution of compound **33** (830 mg, 2.5 mmol) in TFA (5 mL) was added H_2O_2 (0.86 mL, 10 mmol) at 0 °C. The reaction mixture was stirred at room temperature overnight, poured into water and acidified to pH = 4 with AcOH. The resulting precipitate was collected by filtration, washed with water and dried to afford the tittle compound **34** (700 mg, 77% yield). LC-MS: m/z = 363 $[M+H]^+$

3-Amino-N-[(2R)-3,3,3-trifluoro-2-hydroxy-propyl]-5-[2-(trifluoromethoxy)phenyl]sulfonyl-pyridine-2-carboxamide (19)



To a solution of compound **34** (50 mg, 0.14 mmol) in DMA (550 µL) were added **(**2R**)**-3-amino-1,1,1-trifluoro-propan-2-ol (18 mg, 0.14 mmol), HATU (52 mg, 0.14 mmol) and Et_3N (39 µL, 0.28 mmol). The reaction mixture was stirred at room temperature for 15 h, diluted with water and extracted with EtOAc. The combined organic layers were then dried over $MgSO_4$, filtered and concentrated. The crude residue was purified by preparative HPLC to afford the title compound **19** (9 mg, 14% yield). ^{1}H NMR (400 MHz, DMSO-d6) δ ppm 8.79 (t, 1 H), 8.25 (m, 1 H), 8.12 (d, 1 H), 7.92 (m, 1 H), 7.72 (m, 2 H), 7.60 (m, 1 H), 7.30 (s br, 2 H), 6.44 (s br, 1 H), 4.22 (m, 1 H), 3.57 (m, 1 H), 3.39 (m, 1 H). LCM-MS: m/z = 474 $[M+H]^+$

5-Amino-6-(3,3,3-trifluoro-2-hydroxy-propylcarbamoyl)-pyridine-3-sulfonyl chloride (37):



Step a): 33-amino-5-bromo-N-(3,3,3-trifluoro-2-hydroxy-propyl)pyridine-2-carboxamide (**35**)

To a solution of 3-amino-5-bromopyridine-2-carboxylic acid **26** (5.1 g, 23.5 mmol) in NMP (170 mL) were added of HATU (13.4 g, 35.3 mmol), Et_3N (9.8 mL, 71 mmol) and 3-amino-1,1,1-trifluoro-propan-2-ol (HCl salt, 5.82 g, 35.3 mmol). The reaction mixture was stirred at room temperature until full conversion (monitored by LC-MS). The crude mixture was diluted with water and extracted with EtOAc. The combined organic layers were washed with water, dried over Na_2SO_4, filtered and concentrated to afford the title compound **35** (6.17 g, 80%). LC-MS: m/z = 329 [M+H]+

Step b): 3-Amino-5-benzylsulfanyl-N-(3,3,3-trifluoro-2-hydroxy-propyl)pyridine-2-carboxamide (**36**)

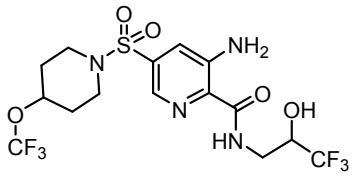
To a solution of compound **35** (2.9 g, 8.87 mmol) in toluene (5 ml) were added benzyl mercaptan (1.25 mL, 10.6 mmol), $Pd_2(dba)_3$ (247 mg, 0.27 mmol), Xantphos (311 mg, 0.54 mmol) and N,N-Diisopropylethylamine (3.1 mL, 17.7 mmol). The reaction mixture was flushed with nitrogen and stirred at 120 °C overnight. The crude mixture was concentrated to dryness and the crude residue was purified by flash chromatography on SiO_2 to afford the title compound **36** (2.5 g, 76% yield). LC-MS: m/z = 371 [M+H]+

Step c): 5-Amino-6-(3,3,3-trifluoro-2-hydroxy-propylcarbamoyl)-pyridine-3-sulfonyl chloride (**37**):

To a solution of compound **36** (371 mg, 1 mmol) in a mixture of AcOH (150 µL), H_2O (250 µL) and CH_3CN (3.5 mL) was added portion wise 1,3-dichloro-5,5-dimethylhydantoin (394

mg, 2 mmol) at 0 °C. The reaction mixture was stirred for 10 min and used as such in next step (sulphonamide formation).

3-Amino-N-(3,3,3-trifluoro-2-hydroxy-propyl)-5-[[4-(trifluoromethoxy)-1-piperidyl]sulfonyl]pyridine-2-carboxamide (20):



Compound **37** (50 mg, 0.14 mmol) was taken up in acetonitrile (0.57 mL). 4,4-difluoropiperidine (26 mg, 0.22 mmol) and Et_3N (80 µL, 0.575 mmol) were then added. The reaction mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic layers were then dried over $MgSO_4$, concentrated and the crude residue was purified by preparative HPLC to afford the title compound **20** (12 mg, 19% yield). 1H NMR (400 MHz, DMSO-d6) δ ppm 8.81 (t, 1 H), 8.01 (d, 1 H, d), 7.56 (d, 1 H), 7.26 (s br, 2 H), 6.49 (d, 1 H), 4.57 (m, 1 H), 4.23 (m, 1 H), 3.59 (m,1 H), 3.42 (m, 2 H), 3.28 (m, 1 H), 2.90 (m, 2 H), 2.00 (m, 2 H), 1.75 (m, 2 H). LC-MS: m/z = 481 $[M+H]^+$.

HPLC traces of lead compounds 10 and 13