Supporting Information

Discovery of 2,4-1*H*-imidazole carboxamides as potent and selective TAK1 inhibitors

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General Experimental Methods. All solvents and reagents were used as purchased from commercial suppliers without further purification. Starting materials which were not commercially available were synthesized by previously reported methods. Flash column chromatography was performed using prepacked silica gel (20–40 mesh) columns. Analytical thin-layer chromatography was performed on Merck 60 F254 glass plates. Visualization was accomplished with UV light, iodine, anisaldehyde or potassium permanganate, followed by heating. Standard ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-*d*6 n a 400 MHz Bruker spectrometer and are reported in ppm using TMS (0.00 ppm) as an internal standard.

LCMS analytical methods:

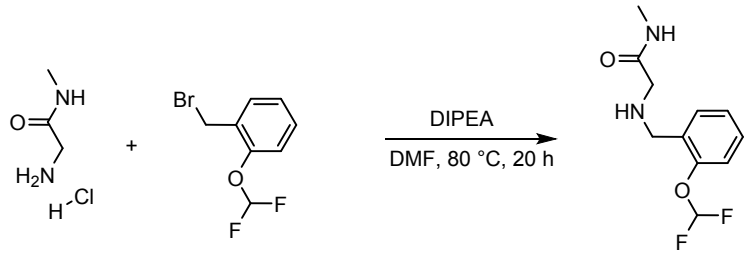
Method A: Apparatus: Agilent 1260 Bin. Pump: G1312B, degasser; autosampler, ColCom, DAD: Agilent G1315D, 220-320 nm, MSD: Agilent LC/MSD G6130B ESI, pos/neg 100-800, ELSD Alltech 3300 gas flow 1.5 ml/min, gas temp: 40°C; column: Waters XSelectTM C18, 30x2.1mm, 3.5µ Temp: 35 ºC, Flow: 1 mL/min, Gradient: t_0 = 5% A, $t_{1.6min}$ = 98% A, t_{3min} = 98% A, Posttime: 1.3 min, Eluent A: 0.1% formic acid in acetonitrile, Eluent B: 0.1% formic acid in water).
Method B: Apparatus: Agilent 1260 Bin. Pump: G1312B, degasser; autosampler, ColCom, DAD: Agilent G1315D, 220-320 nm, MSD: Agilent LC/MSD G6130B ESI, pos/neg 100-800, ELSD Alltech 3300 gas flow 1.5 ml/min, gas temp: 40°C; column: Waters XSelectTM C18, 50x2.1mm, 3.5µ, Temp: 35 ºC, Flow: 0.8 mL/min, Gradient: t_0 = 5% A, $t_{3.5min}$ = 98% A, t_{6min} = 98% A, Posttime: 2 min; Eluent A: 0.1% formic acid in acetonitrile, Eluent B: 0.1% formic acid in water).
Method C: Apparatus: Agilent 1260 Bin. Pump: G1312B, degasser; autosampler, ColCom, DAD: Agilent G1315C, 220-320 nm, MSD: Agilent LC/MSD G6130B ESI, pos/neg 100-800; column: Waters XSelectTM CSH C18, 50x2.1mm, 3.5µ, Temp: 25 ºC, Flow: 0.8 mL/min, Gradient: t_0 = 5% A, $t_{3.5min}$ = 98% A, t_{6min} = 98% A, Posttime: 2 min, Eluent A: 95% acetonitrile + 5% 10mM ammoniumbicarbonate in water in acetonitrile, Eluent B: 10mM ammoniumbicarbonate in water (pH=9.5).
Method D: Apparatus: Agilent 1260 Bin. Pump: G1312B, degasser; autosampler, ColCom, DAD: Agilent G1315C, 220-320 nm, MSD: Agilent LC/MSD G6130B ESI, pos/neg 100-800; column: Waters XSelectTM CSH C18, 30x2.1mm, 3.5µ, Temp: 25 ºC, Flow: 1 mL/min, Gradient: t_0 = 5% A, $t_{1.6min}$ = 98% A, t_{3min} = 98% A, Posttime: 1.3 min, Eluent A: 95% acetonitrile + 5% 10mM ammoniumbicarbonate in water in acetonitrile, Eluent B: 10mM ammoniumbicarbonate in water (pH=9.5).

Reversed phase chromatography:

Method A: Instrument type: Reveleris™ prep MPLC; Column: Phenomenex LUNA C18 (150x25 mm, 10µ); Flow: 40 mL/min; Column temp: room temperature; Eluent A: 0.1% (v/v) Formic acid in water, Eluent B: 0.1% (v/v) Formic acid in acetonitrile; Gradient: t=0 min 5% B, t=1 min 5% B, t=2 min 30% B, t=17 min 70% B, t=18 min 100% B, t=23 min 100% B; Detection UV: 220/254 nm.
Method B: Instrument type: Reveleris™ prep MPLC; Column: Waters XSelect CSH C18 (145x25 mm, 10µ); Flow: 40 mL/min; Column temp: room temperature; Eluent A: 10 mM ammoniumbicarbonate in water pH = 9.0); Eluent B: 99% acetonitrile + 1% 10 mM ammoniumbicarbonate in water; Gradient: t=0 min 5% B, t=1 min 5% B, t=2 min 30% B, t=17 min 70% B, t=18 min 100% B, t=23 min 100% B; Detection UV: 220/254 nm.

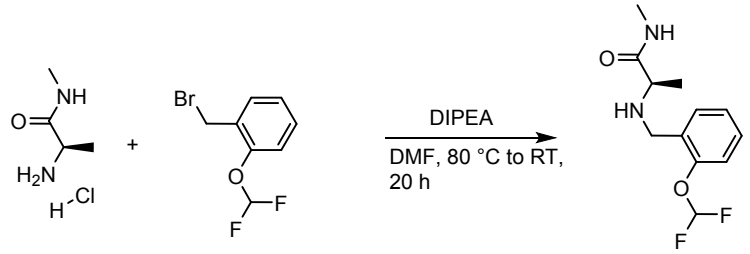
Synthetic procedures for non-commercially available amines and intermediates:

Synthesis of 2-((2-(difluoromethoxy)benzyl)amino)-*N*-methylacetamide



To a solution of 2-amino-*N*-methylacetamide hydrochloride (1.6 g, 12.9 mmol) and DIPEA (9.2 mL, 53 mmol) in *N*,*N*-dimethylformamide (50 mL) 1-(bromomethyl)-2-(difluoromethoxy)benzene (2.5 g, 10.55 mmol) was added and the mixture was stirred at 80 °C for 20 hours. The reaction mixture was partitioned between water and dichloromethane and the aqueous layer was extracted twice more with dichloromethane. The combined organic layers were dried with sodium sulfate, filtered, concentrated and the residue was purified by flash column chromatography (0 to 10% methanol in dichloromethane) to afford 2-((2-(difluoromethoxy)benzyl)amino)-*N*-methylacetamide (1.52 g, 59%) as an oil. ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.37 – 7.28 (m, 3H), 7.19 (t, J = 7.4 Hz, 1H), 7.11 (d, J = 8.2 Hz, 1H), 6.58 (t, J = 74.0 Hz, 1H), 3.79 (s, 2H), 3.29 (s, 2H), 2.84 (d, J = 5.0 Hz, 3H); LCMS (Method D): t_R 1.73 min, LRMS (ESI) calcd. $C_{11}H_{14}F_2N_2O_2$ $(M+H)^+$ 245.1, found 245.0.

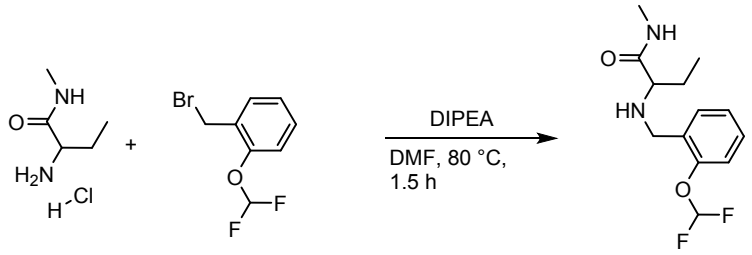
Synthesis of (*R*)-2-((2-(difluoromethoxy)benzyl)amino)-*N*-methylpropanamide



To a suspension of (*R*)-2-amino-*N*-methylpropanamide hydrochloride (1.35 g, 9.7 mmol) in *N*,*N*-dimethylformamide (22 mL) DIPEA (6.53 mL, 37.5 mmol) was added and the mixture was stirred at 80 °C until all the solids had dissolved. Next, 1-(bromomethyl)-2-(difluoromethoxy)benzene (1.78 g, 7.5 mmol) was added dropwise and the reaction mixture was stirred at 80 °C for 30 minutes and then at room temperature for 19 hours. The mixture was partitioned between water and dichloromethane and the aqueous layer was extracted twice more with dichloromethane. The combined organic layers were washed three times with water, brine, dried with sodium sulfate and concentrated. The residue was purified by flash column chromatography (50 to 100% ethyl acetate in heptane) to afford (*R*)-2-((2-(difluoromethoxy)benzyl)amino)-*N*-methylpropanamide (1.3 g, 67%) as an oil. ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.41 – 7.28 (m, 3H), 7.23 – 7.16 (m, 1H), 7.14 – 7.08 (m, 1H), 6.58 (t, J = 74.0 Hz, 1H), 3.82 (d, J = 12.8 Hz, 1H), 3.69 (d, J = 12.9 Hz, 1H), 3.23 (q, J = 6.9 Hz, 1H), 2.83 (d, J = 5.0 Hz, 3H), 1.30 (d, J = 7.0 Hz, 3H); LCMS (Method D): t_R 1.91 min, LRMS (ESI) calcd.

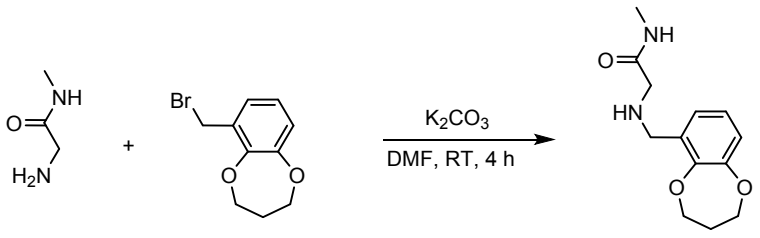
$C_{12}H_{16}F_2N_2O_2$ $(M+H)^+$ 259.2, found 259.2 The corresponding *S*-enantiomer was made using the same procedure starting from (*S*)-2-amino-*N*-methylpropanamide hydrochloride.

Synthesis of 2-((2-(difluoromethoxy)benzyl)amino)-*N*-methylbutanamide



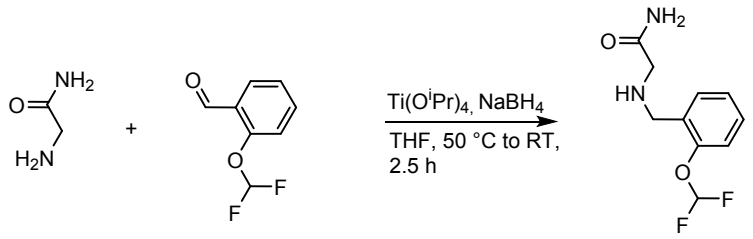
To a solution of 2-amino-*N*-methylbutanamide hydrochloride (1.19 g, 7.8 mmol) and DIPEA (5.66 mL, 32.5 mmol) in *N*,*N*-dimethylformamide (50 mL) 1-(bromomethyl)-2-(difluoromethoxy)benzene (1.54 g, 6.5 mmol) was added dropwise and the reaction mixture was stirred at 80 °C for 1.5 hours. The mixture was partitioned between water and dichloromethane and the aqueous layer was extracted twice more with dichloromethane. The combined organic layers were washed three times with water, brine, dried with sodium sulfate and concentrated. The residue was purified by flash column chromatography (0 to 5% methanol in dichloromethane) to afford 2-((2-(difluoromethoxy)benzyl)amino)-*N*-methylbutanamide (961 mg, 54%) as an oil. ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.41 – 7.28 (m, 3H), 7.19 (t, J = 7.2 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 6.58 (t, J = 74.0 Hz, 1H), 3.82 (d, J = 12.8 Hz, 1H), 3.65 (d, J = 12.8 Hz, 1H), 3.07 (dd, J = 7.6, 4.7 Hz, 1H), 2.84 (d, J = 5.0 Hz, 3H), 1.84 – 1.72 (m, 1H), 1.62 – 1.51 (m, 1H), 0.91 (t, J = 7.5 Hz, 3H); LCMS (Method D): t_R 2.14 min, LRMS (ESI) calcd. $C_{13}H_{18}F_2N_2O_2$ $(M+H)^+$ 273.3, found 273.2.

Synthesis of 2-((3,4-dihydro-2*H*-benzo[b][1,4]dioxepin-6-yl)methylamino)-*N*-methylacetamide



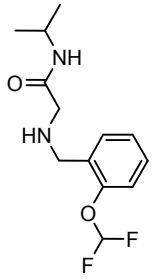
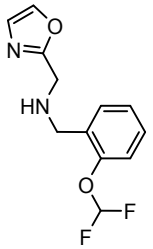
Under nitrogen atmosphere, a solution of 2-amino-*N*-methylacetamide (176 mg, 2.0 mmol), potassium carbonate (828 mg, 6.0 mmol,) and 6-(bromomethyl)-3,4-dihydro-2*H*-benzo[b][1,4]dioxepine (337 mg, 1.4 mmol) in DMF (6 mL) was stirred at room temperature for 4 hours. The mixture was concentrated, the residue was purified by reversed phase chromatography (method B) and the appropriate fractions were concentrated to afford 2-((3,4-dihydro-2*H*-benzo[b][1,4]dioxepin-6-yl)methylamino)-*N*-methylacetamid (138 mg, 39%) as a white solid. LRMS (ESI) calcd. $C_{13}H_{18}N_2O3$ $(M+H)^+$ 251.1, found 251.0.

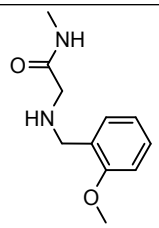
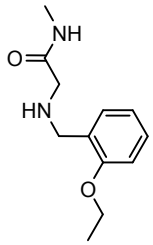
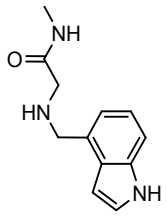
Synthesis of 2-((2-(difluoromethoxy)benzyl)amino)acetamide



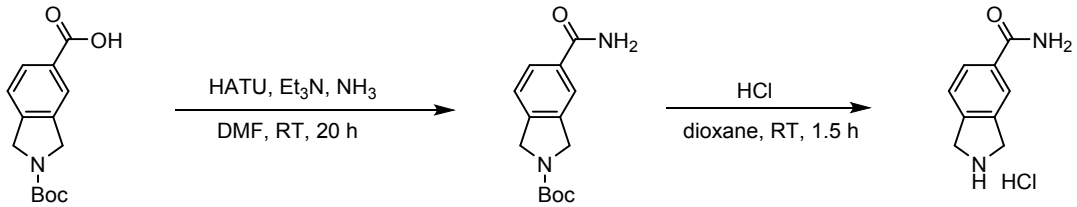
Under nitrogen atmosphere, 2-aminoacetamide (148 mg, 2.0 mmol) and 2-(difluoromethoxy)benzaldehyde (287 mg, 1.7 mmol) were dissolved in tetrahydrofuran (6 mL) followed by addition of $Ti(O^iPr)_4$ (1.42 g, 5.0 mmol). The mixture was heated to 50 °C and stirred for one hour. Next, the mixture was cooled to room temperature, $NaBH_4$ (95 mg, 2.5 mmol) was added and the mixture was heated to 50 °C for 90 minutes. The mixture was cooled to room temperature, quenched with water (2 mL) and concentrated. The residue was purified by reversed phase chromatography (method B) and the appropriate fractions were concentrated to afford 2-((2-(difluoromethoxy)benzyl)amino)acetamide (225 mg, 59%) as a white solid. LRMS (ESI) calcd. $C_{10}H_{12}F_2N_2O_2$ $(M+H)^+$ 231.1, found 231.0.

The following amines were prepared using procedures analogous to the example above.

Structure and compound name	Analytical data
 2-((2-(difluoromethoxy)benzyl)amino)-*N*-isopropylacetamide	Yield 54%, LRMS (ESI) calcd. $C_{13}H_{18}F_2N_2O_2$ $(M+H)^+$ 273.1, found 273.0.
 *N*-(2-(difluoromethoxy)benzyl)-1-(oxazol-2-yl)methanamine	Yield 69%, LRMS (ESI) calcd. $C_{12}H_{12}F_2N_2O_2$ $(M+H)^+$ 255.1, found 255.0.

 2-((2-methoxybenzyl)amino)-*N*-methylacetamide	Yield 59%, LRMS (ESI) calcd. $C_{11}H_{16}N_2O_2$ $(M+H)^+$ 209.2, found 209.2.
 2-((2-ethoxybenzyl)amino)-*N*-methylacetamide	Yield 76%, LRMS (ESI) calcd. $C_{12}H_{18}N_2O_2$ $(M+H)^+$ 223.3, found 223.2.
 2-(((1*H*-indol-4-yl)methyl)amino)-*N*-methylacetamide	Yield 74%, LCMS (ESI) calcd. $C_{12}H_{15}N3O$ $(M+H)^+$ 218.1, found 218.1.

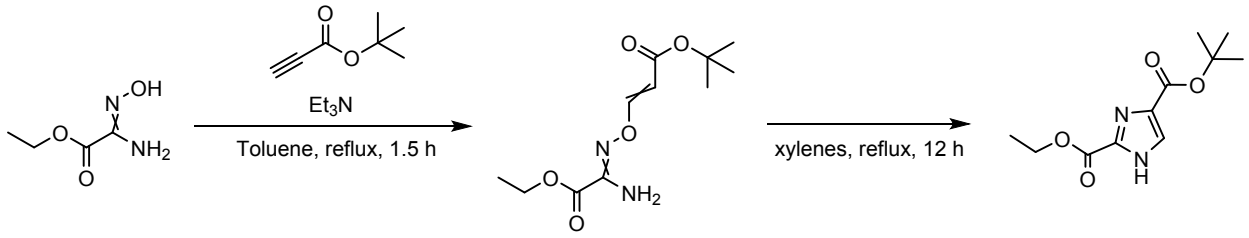
Synthesis of isoindoline-5-carboxamide hydrochloride



A solution of 2-(tert-butoxycarbonyl)isoindoline-5-carboxylic acid (998 mg, 3.79 mmol), HATU (1.44 g, 3.79 mmol) and triethylamine (0.63 mL, 4.5 mmol) in *N*,*N*-dimethylformamide (5 mL) was stirred at room temperature. After 1 hour ammonia (0.5M solution in 1,4-dioxane, 20 mL, 10 mmol) was added and a precipitate formed. After 20 hours, the reaction mixture was diluted with water and extracted three times with EtOAc. The combined organic layers were washed with water, four times with brine, dried over sodium sulfate and concentrated to afford

918 mg of tert-butyl 5-carbamoylisoindoline-2-carboxylate (918 mg, 92%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 – 7.66 (m, 2H), 7.37 – 7.28 (m, 1H), 6.03 (s, 1H), 5.58 (s, 1H), 4.80 – 4.63 (m, 4H), 1.52 (s, 9H). Hydrochloric acid in dioxane (4 M in dioxane, 17.5 mL, 70 mmol) was added to a suspension of tert-butyl 5-carbamoylisoindoline-2-carboxylate (918 mg, 3.50 mmol) in dry 1,4-dioxane (4 mL) and the reaction mixture was stirred at room temperature overnight. The mixture was concentrated to afford isoindoline-5-carboxamide hydrochloride (692 mg, 99%) as a cream coloured solid. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 9.80 (s, 2H), 8.02 (s, 1H), 7.92 – 7.82 (m, 2H), 7.47 (d, *J* = 8.0 Hz, 1H), 7.43 (s, 1H), 4.61 – 4.49 (m, 4H).

Synthesis of 5-(tert-butyl) 2-ethyl 1*H*-imidazole-2,5-dicarboxylate (compound 23)



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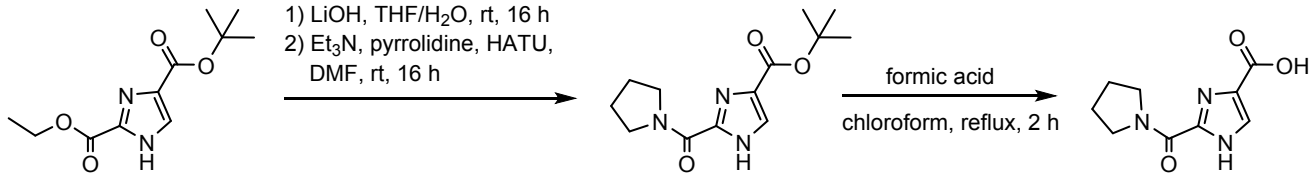
To a solution of ethyl 2-amino-2-(hydroxyimino)acetate (5.0 g, 37.8 mmol) and tert-butyl propiolate (4.77 g, 37.8 mmol) in dry toluene (100 mL) triethylamine (3.83 g, 37.8 mmol) was added and the resulting solution was heated at reflux for 1.5 hours. The mixture was concentrated and taken up in dichloromethane. The solution was washed with water, dried with sodium sulfate and concentrated. The crude product was purified by silica column chromatography (0% to 50% EtOAc in heptane) to afford tert-butyl 3-((((E)-1-amino-2-ethoxy-2oxoethylidene)amino)oxy)acrylate (9.65 g, 99%) as an orange oil. LCMS (Method D): t_R 2.05 min, LRMS (ESI) calcd. $C_7H_{10}N_2O_5$ $(M\text{-}tBu+H)^+$ 203.1, found 203.0. A solution of tert-butyl 3-((((E)-1-amino-2-ethoxy-2-oxoethylidene)amino)oxy)acrylate (9.77 g, 37.8 mmol) was dissolved in xylenes (400 mL) and heated to reflux. Water was removed *via* azeotropic distillation with a Dean-Stark apparatus for 12 hours. The mixture was concentrated and purified by silica column chromatography (50% to 100% ethyl acetate in heptane) to afford 5-(tert-butyl) 2-ethyl 1*H*-imidazole-2,5-dicarboxylate (3.2 g, 35%) as an orange solid. ^{1}HNMR (400 MHz, $CDCl_3$) 2:3 mixture of tautomers δ 11.16 (s, 0.4H), 10.64 (s, 0.6H), 7.79 (s, 0.4H), 7.69 (s, 0.6H), 4.50 – 4.36 (m, 2H), 1.56 (s, 9H), 1.45 – 135 (m, 3H); LCMS (Method D): t_R 1.79 min, LRMS (ESI) calcd. $C_{11}H_{16}N_2O_4$ $(M+H)^+$ 241.1, found 241.0.

Synthesis of 5-(tert-butyl) 2-ethyl 1*H*-imidazole-2,5-dicarboxylate (compound 24)



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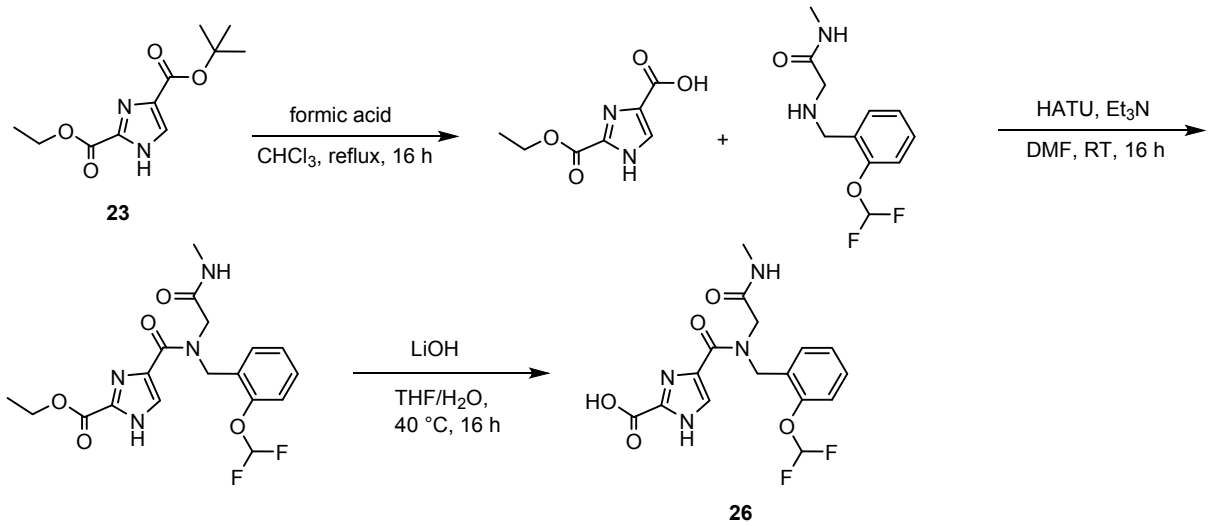
To a solution of 5-(tert-butyl) 2-ethyl 1*H*-imidazole-2,5-dicarboxylate (1.8 g, 7.5 mmol) in tetrahydrofuran (40 mL) a 2M solution of lithium hydroxide in water 2M (15 mL, 30 mmol) was added and the mixture was stirred at room temperature for 16 hours. The reaction mixture was acidified to ~pH 5 using HCl, concentrated, taken up in water/acetonitrile, frozen and lyophilized to afford 5-(tert-butoxycarbonyl)-1*H*-imidazole-2-carboxylic acid as a beige solid. LCMS (Method A): t_R 1.43 min, LRMS (ESI) calcd. $C_9H_{12}N_2O_4$ $(M+H)^+$ 213.1, found 213.0. The product from the previous step (7.5 mmol) and triethylamine (1.25 mL, 9 mmol) were dissolved in *N*, *N*-dimethylformamide (30 mL) and HATU (2.9 g, 7.5 mmol) was added. After stirring at room temperature for 15 minutes, pyrrolidine (0.74 mL, 9 mmol) was added and the mixture was stirred at room temperature for 16 hours. The mixture was poured into water and extracted with ethyl actetate (3x). The combined organic layers were washed with brine (2x), dried with sodium sulfate and concentrated to afford an orange oil. This was purified by silica column chromatography (50% ethyl actetate in heptane) to afford tert-butyl 2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-5carboxylate (717 mg, 36% over two steps) as a white solid. LCMS (Method A): t_R 1.78 min, LRMS (ESI) calcd. $C_{13}H_{19}N_3O_3$ $(M+H)^+$ 266.1, found 266.1. As solution of tert-butyl 2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-5-carboxylate (500 mg, 1.89 mmol) was dissolved in chloroform (10 mL) and formic acid (10 mL) was refluxed for 2 hours. The mixture was concentrated and co-evaporated with chloroform to 2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-5-carboxylic acid (375 mg, 95%) as a beige solid. ^{1}H NMR (400 MHz, DMSO-*d6*) mixture of rotamers δ 13.37 (br. s, 1H), 12.57 (br. s, 1H), 7.80 (s, 1H), 4.04 – 4.01 (m, 2H), 3.52 – 3.46 (m, 2H), 1.95 – 1.85 (m, 4H); LCMS (Method A): t_R 1.14 min, LRMS (ESI) calcd. $C_9H_{11}N_3O_3$ $(M+H)^+$ 210.1, found 210.1.

Synthesis of 5-(tert-butyl) 2-ethyl 1*H*-imidazole-2,5-dicarboxylate (compound 26)

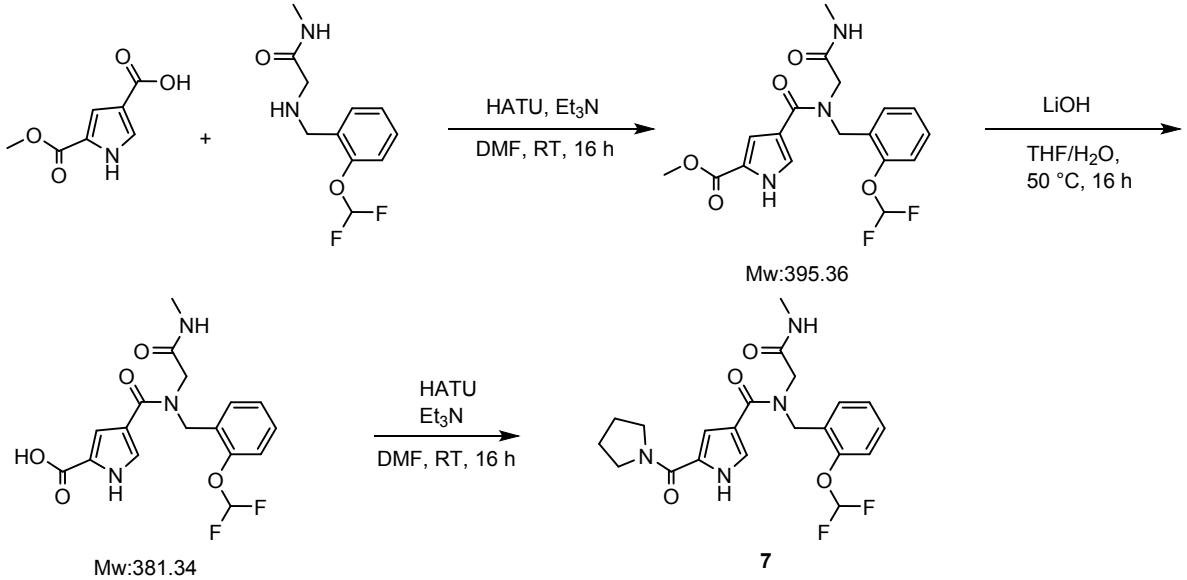


A mixture of 4-(tert-butyl) 2-ethyl 1*H*-imidazole-2,4-dicarboxylate (870 mg, 3.62 mmol) in chloroform (10 mL) and formic acid (10 mL) was refluxed for 16 hours. The mixture was cooled to room temperature, concentrated, taken up in water and lyophilized to afford 2-(ethoxycarbonyl)-1*H*-imidazole-4-carboxylic acid (590 mg, 88%) as a white solid. ^{1}HNMR (400 MHz, DMSO-d6) δ 13.80 (s, 1H), 12.59 (s, 1H), 7.93 (s, 1H), 4.33 (q, *J* = 7.1 Hz, 2H), 1.32 (t, *J* = 7.1 Hz, 3H). To a solution of 2-(ethoxycarbonyl)-1*H*-imidazole-4-carboxylic acid (1.0 g, 5.4 mmol) in *N*,*N*-dimethylformamide (11 mL), HATU (2.07 g, 5.4 mmol) and

triethylamine (1.9 mL, 13.6 mmol) were added. After 1 hour a solution of 2-((2-(difluoromethoxy)benzyl)amino)-*N*-methylacetamide (1.6, 6.5 mmol) in *N*,*N*-dimethylformamide (4 mL) was added and the reaction mixture was stirred at room temperature for 15 hours. The crude mixture was poured into water and extracted with EtOAc (2x). The combined organic layers were washed with water, brine (2x), dried with sodium sulfate and concentrated. The crude product was purified by silica column chromatography (60% to 100% ethyl acetate in heptane) to afford ethyl 4-((2-(difluoromethoxy)benzyl)(2-(methylamino)-2-oxoethyl)carbamoyl)-1*H*-imidazole-2-carboxylate (1.71 g, 77%) as a white solid. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.02 – 7.91 (m, 1H), 7.83 (s, 1H), 7.45 – 6.94 (m, 5H), 5.41 (br s, 1H), 4.67 – 4.58 (m, 2H), 4.39 – 4.22 (m, 2H), 3.89 (s, 1H), 2.59 (d, *J* = 4.5 Hz, 3H), 1.40 – 1.21 (m, 3H). LCMS (Method D): t_R 1.82 min, LRMS (ESI) calcd. $C_{18}H_{20}F_2N_4O_5$ $(M+H)^+$ 411.1, found 411.2.
To a solution of 4-((2-(difluoromethoxy)benzyl)(2-(methylamino)-2-oxoethyl)carbamoyl)-1*H*-imidazole-2-carboxylate (1.7 g, 4.1 mmol) in tetrahydrofuran (50 mL) and water (5 mL) lithium hydroxide monohydrate (354 mg, 8.45 mmol) was added and the mixture was stirred at 40 °C for 16 hours. The mixture was concentrated to remove most of the THF, water (10 mL) was added and it was extracted with ethyl acetate. The water layer was separated, acidified to ~pH 3 using 1M HCl and extracted with ethyl acetate (5x). The combined organic layers were washed with brine, dried with sodium sulfate and concentrated to afford 4-((2-(difluoromethoxy)benzyl)(2-(methylamino)-2-oxoethyl)carbamoyl)-1*H*-imidazole-2-carboxylic acid (1.54 g, 98%) as a yellow solid. ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.01 – 7.72 (m, 2H), 7.43 – 6.99 (m, 5H), 5.41 (br s, 1H), 4.66 (br s, 1H), 4.60 (br s, 1H), 3.87 (br s, 1H), 2.58 (d, *J* = 4.0 Hz, 3H). LCMS (Method A): t_R 1.54 min, LRMS (ESI) calcd. $C_{16}H_{16}F_2N_4O_5$ $(M+H)^+$ 383.1, found 383.1.

Synthesis of 2,4-1*H*-pyrrole carboxamides:

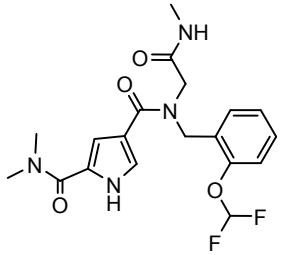
Representative example 1: *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxamide (compound 7).

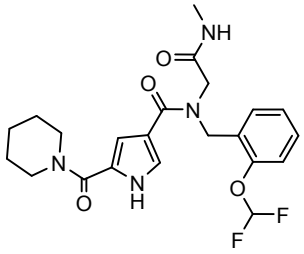
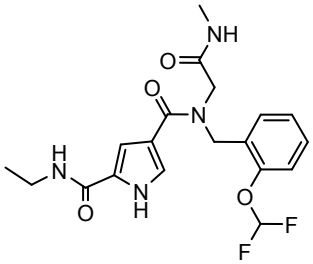


To a solution of 5-(methoxycarbonyl)-1*H*-pyrrole-3-carboxylic acid (53 mg, 0.31 mmol) in *N*,*N*-dimethylformamide (3 mL), HATU (106 mg, 0.8 mmol) and triethylamine (0.11 mL, 0.78

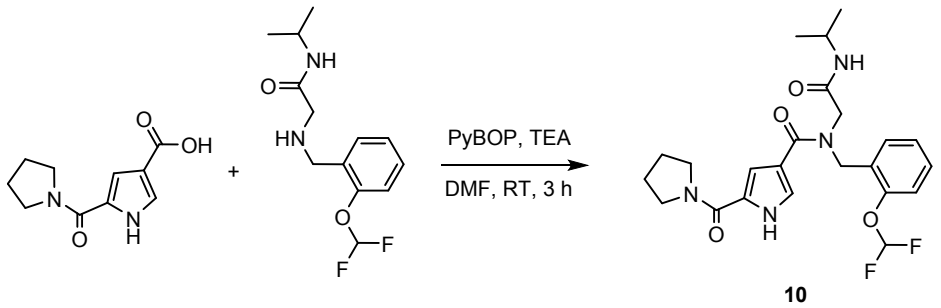
mmol) were added. After 30 minutes, a solution of 2-((2-(difluoromethoxy)benzyl)amino)-*N*-methylacetamide (93 mg, 0.38 mmol) in *N*,*N*-dimethylformamide (1 mL) was added and the reaction mixture was stirred at room temperature for 16 hours. The crude mixture was poured into water and extracted with EtOAc (2x). The combined organic layers were washed with water, brine (2x), dried with sodium sulfate and concentrated. The crude product was purified reversed phase chromatography (method B) and the appropriate fractions were lyophilized to afford methyl 4-((2-(difluoromethoxy)benzyl)(2-(methylamino)2-oxoethyl)carbamoyl)-1*H*-pyrrole-2-carboxylate (102 mg, 82%) as a white solid. LCMS (Method A): t_R 1.73 min, LRMS (ESI) calcd. $C_{18}H_{19}F_2N_3O_5$ $(M+H)^+$ 396.1, found 396.1. To a solution of 4-((2-(difluoromethoxy)benzyl)(2-(methylamino)2-oxoethyl)carbamoyl)-1*H*-pyrrole-2-carboxylate (100 mg, 0.25 mmol) in tetrahydrofuran (6 mL) 2M lithium hydroxide in water (0.4 mL, 0.8 mmol) was added and the mixture was stirred at 50 °C for 16 hours. The mixture was concentrated to remove most of the THF, acidified to ~pH 3 using 1M HCl and concentrated to afford 4-((2-(difluoromethoxy)benzyl)(2-(methylamino)-2-oxoethyl)carbamoyl)-1*H*-pyrrole-2-carboxylic acid. The product was used as such in the next step. LCMS (Method A): t_R 1.64 min, LRMS (ESI) calcd. $C_{17}H_{17}F_2N_3O_5$ $(M+H)^+$ 382.1, found 382.1. To a solution of 4-((2-(difluoromethoxy)benzyl)(2-(methylamino)-2-oxoethyl)carbamoyl)-1*H*-pyrrole-2-carboxylic acid (50 mg, 0.25 mmol) in *N*,*N*-dimethylformamide (3 mL), HATU (81 mg, 0.29 mmol) and triethylamine (50 µL, 0.36 mmol) were added. After 30 minutes, pyrrolidine (25 µL, 0.3 mmol) was added and the reaction mixture was stirred at room temperature for 16 hours. The reaction mixture was purified by reversed phase chromatography (method A) and the appropriate fractions were lyophilized to afford *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxamide (compound **7**, 53 mg, 49%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 7.42 – 7.25 (m, 4H), 6.90 – 6.72 (m, 2H), 4.97 (s, 2H), 4.14 (s, 2H), 3.75 – 3.45 (m, 4H), 2.78 (s, 3H), 2.01 – 1.93 (m, 4H). LCMS (Method C): t_R 1.77 min, LRMS (ESI) calcd. $C_{21}H_{24}F_2N_4O_4$ $(M+H)^+$ 435.2, found 435.2.

The following compounds were prepared using procedures analogous to representative Example **1**.

Compound #	Structure and compound name	Analytical data
17	 *N*⁴-(2-(difluoromethoxy)benzyl)-*N*²,*N*²-dimethyl-N⁴-(2-(methylamino)-2-oxoethyl)-1*H*-pyrrole-2,4-dicarboxamide	Yield 20%, ^{1}HNMR (400 MHz, DMSO-d6) δ 11.82 (s, 1H), 8.04 (s, 1H), 6.88 – 7.74 (m, 6H), 6.74 (s, 1H), 4.73 (d, 2H), 4.02 (s, 2H), 2.95 (d, 6H), 2.62 (d, 3H). LRMS (ESI) calcd. $C_{19}H_{22}F_2N_4O_4$ $(M+H)^+$ 409.2, found 409.2.

18	 2-(*N*-[[2-(difluoromethoxy)phenyl]methyl]-1-[5-[(piperidin-1-yl)carbonyl]-1*H*-pyrrol-3-yl]formamido)-*N*-methylacetamide	Yield 21%, ^{1}HNMR (300 MHz, DMSO-d6) δ 11.84 (s, 1H), 8.06 (s, 1H), 7.68 – 6.81 (m, 6H), 6.60 (s, 1H), 4.72 (d, 2H), 4.03 (s, 2H), 3.56 (d, 4H), 2.62 (d, 3H), 1.95 – 1.07 (m, 6H). LRMS (ESI) calcd. $C_{22}H_{26}F_2N_4O_4$ (M+H)+ 449.2, found 449.2
19	 N^4-(2-(difluoromethoxy)benzyl)-N^2-ethyl-N^4-(2-(methylamino)-2-oxoethyl)-1*H*-pyrrole-2,4-dicarboxamide	Yield 20%, ^{1}HNMR (400 MHz, DMSO-d6) δ 11.81 (s, 1H), 8.18 (s, 2H), 7.51 – 7.17 (m , 5H), 7.02 (d, 2H), 4.62 (s, 2H), 4.03 (s, 2H), 3.23 (s, 2H), 2.62 (s, 3H), 1.09 (t, 3H). LRMS (ESI) calcd. $C_{19}H_{22}F_2N_4O_4$ $(M+H)^+$ 409.2, found 409.2.

Representative example 2: Synthesis of (*N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(isopropylamino)-2-oxoethyl)-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxamide (compound 10).

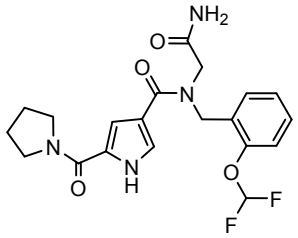
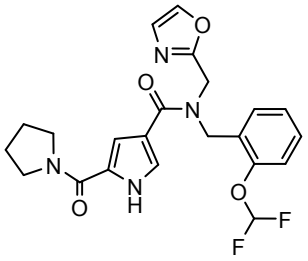
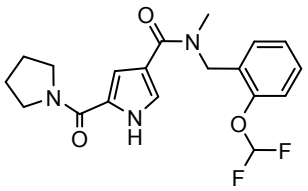


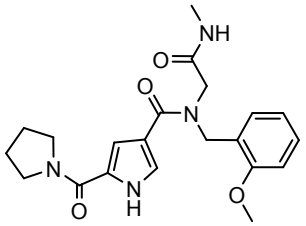
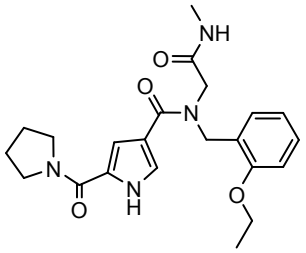
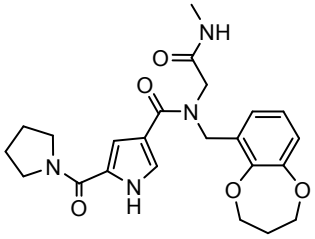
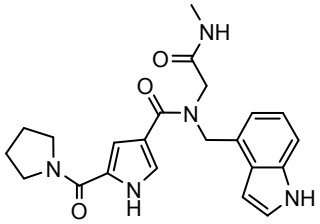
10

Under nitrogen atmosphere, 5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxylic acid (47 mg, 0.23 mmol), triethylamine (45 mg, 0.5 mmol) and PyBOP (115 mg, 0.2 mmol) were dissolved in *N*,*N*-dimethylformamide (2 mL) and the mixture was stirred at room temperature for 30 minutes. Next, 2-(2-(difluoromethoxy)benzylamino)-*N*-isopropylacetamide (41 mg, 0.2 mmol) was added and the mixture was stirred for 2.5 hours. The mixture was concentrated, the residue was purified by reversed phase chromatography (method A) and lyophilized to afford *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(isopropylamino)-2-oxoethyl)-5-(pyrrolidine-1-carbonyl)-1*H*-

pyrrole-3-carboxamide (**compound 10**, 13 mg, 20%) as a white solid. ^{1}HNMR (300 MHz, DMSO-d6) δ 11.83 (s, 1H), 7.92 – 7.63 (m, 1H), 7.46 – 6.97 (m, 6H), 6.80 (d, 1H), 4.78 – 4.61 (m, 2H), 3.96 – 3.45 (m, 7H), 1.82 – 1.73 (m, 4H), 1.03 (d, 6H). LRMS (ESI) calcd. $C_{23}H_{28}F_2N_4O_4$ $(M+H)^+$ 463.2, found 463.2.

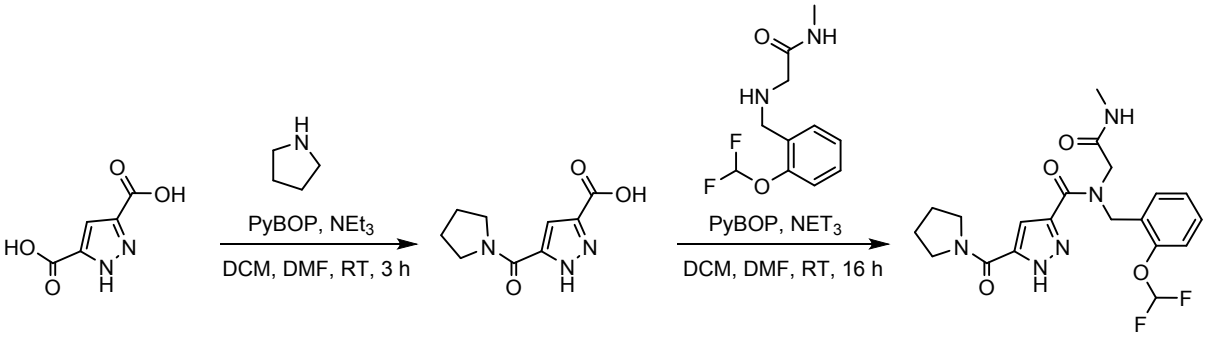
The following compounds were prepared using procedures analogous to representative Example **2**.

Compound #	Structure and compound name	Analytical data
9	 *N*-(2-amino-2-oxoethyl)-*N*-(2-(difluoromethoxy)benzyl)-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxamide	Yield 14%, ^{1}HNMR (300 MHz, DMSO-d6) δ 11.88 (s, 1H), 7.47 – 6.98 (m, 8H), 6.82 (d, 1H), 4.79 (d, 2H), 3.99 – 3.38 (m, 6H), 1.91 (d, 4H). LCMS (ESI) calcd. $C_{20}H_{22}F_2N_4O_4$ $(M+H)^+$ 421.2, found 421.2.
11	 *N*-(2-(difluoromethoxy)benzyl)-*N*-(oxazol-2-ylmethyl)-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxamide	Yield 4%, ^{1}HNMR (300 MHz, DMSO-d6) complex mixture of rotamers δ 11.89 (s, 1H), 8.07 (s, 1H), 7.47 – 6.97 (m, 7H), 6.75 (s, 1H),4.77 (s, 4H), 3.44 (t, 4H), 1.80 (d, 4H). LRMS (ESI) calcd. $C_{22}H_{22}F_2N_4O_4$ $(M+H)^+$ 445.2, found 445.2.
12	 *N*-(2-(difluoromethoxy)benzyl)-*N*-methyl-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxamide	Yield 7%, ^{1}HNMR (300 MHz, DMSO-d6) δ 11.84 (s, 1H), 7.47 – 7.06 (m, 6H), 6.79 (s, 1H), 4.70 (s, 2H), 3.59 – 3.46 (m, 4H), 3.07 (s, 3H), 1.80 (d, 4H). LRMS (ESI) calcd. $C_{19}H_{21}F_2N_3O_3$ $(M+H)^+$ 378.1, found 378.1.

13	 N-(2-methoxybenzyl)-N-(2-(methylamino)-2-oxoethyl)-5-(pyrrolidine-1-carbonyl)-1H-pyrrole-3-carboxamide	Yield 5%, ^{1}HNMR (300 MHz, DMSO-d6) δ 11.78 (s, 1H), 7.82 (d, 1H), 7.23 – 6.53 (m, 6H), 4.71 (s, 1H), 4.53 (s, 1H), 3.96 – 3.60 (m, 6H), 3.42 (s, 2H), 3.26 (s, 1H), 2.67 (d, 3H), 1.85 – 1.77 (m, 4H). LRMS (ESI) calcd. $C_{21}H_{26}N_4O_4$ $(M+H)^+$ 399.2, found 399.2.
14	 *N*-(2-ethoxybenzyl)-*N*-(2-(methylamino)-2-oxoethyl)-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxamide	Yield 9%, ^{1}HNMR (300 MHz, DMSO-d6) δ 11.82 (s, 1H), 7.85 (s, 1H), 7.64 – 6.85 (m, 5H), 6.56 (s, 1H), 4.65 (d, 2H), 4.22 – 3.88 (m, 4H), 3.55 (d, 4H), 2.63 (s, 3H), 1.82 (s, 4H), 1.33 (s, 3H). LRMS (ESI) calcd. $C_{22}H_{28}N_4O_4$ $(M+H)^+$ 413.2, found 413.2.
15	 *N*-((3,4-dihydro-2*H*-benzo[b][1,4]dioxepin-6-yl)methyl)-*N*-(2-(methylamino)-2-oxoethyl)-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxamide	Yield 8%, ^{1}HNMR (300 MHz, DMSO-d6) δ 11.79 (s, 1H), 7.83 (d, 1H), 7.13 – 6.58 (m, 5H), 4.74 – 4.56 (d, 2H), 4.11 – 3.76 (m, 6H), 3.61 – 3.43 (m, 4H), 2.61 (s, 3H), 2.09 (t, 2H), 1.79 (d, 4H). LRMS (ESI) calcd. $C_{23}H_{28}N_4O_5$ $(M+H)^+$ 441.2, found 441.2.
16		Yield 6%, ^{1}HNMR (300 MHz, DMSO-d6) δ 11.75 (s, 1H), 11.27 (s, 1H), 789 (d, 1H), 7.36 (d, 2H), 7.09 (s, 2H), 6.82 (d, 1H), 6.46 (d, 2H), 4.98 (d, 2H),

	N-((1*H*-indol-4-yl)methyl)-*N*-(2-(methylamino)-2-oxoethyl)-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxamide	4.00 (s, 2H), 3.66 (s, 2H), 3.46 (s, 1H), 2.92 (d, 1H), 2.63 (d, 3H), 1.40 (s, 4H). LRMS (ESI) calcd. $C_{22}H_{25}N_5O_3$ $(M+H)^+$ 408.2, found 408.2.

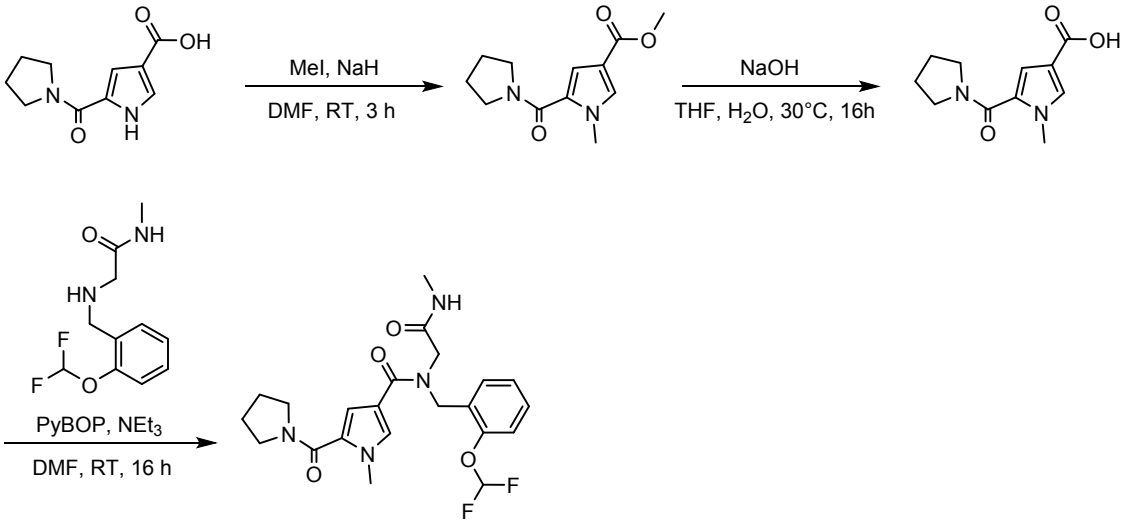
Representative example 3: Synthesis of *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide (compound 20).



20

To a solution of 1*H*-pyrazole-3,5-dicarboxylic acid (1 g, 6.4 mmol) in a mixture of dichloromethane (6 mL) and *N*,*N*-dimethylformamide (6 mL) was added PyBOP (3.33 g, 6.4 mmol) and triethylamine (647 mg, 6.4 mmol). The mixture was stirred for 15 minutes, pyrrolidine (455 mg, 6.4 mmol) was added and the mixture was stirred at room temperature for 3 hours. The mixture was partitioned between ethyl acetate and water and the layers were separated. The aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried with sodium sulfate and concentrated. The crude product was purified by reversed phase chromatography (method A) and lyophilized to afford 5-(pyrrolidine-1-carbonyl)-1*H*-pyrazole-3-carboxylic acid (683 mg, 51%) as a white solid. LRMS (ESI) calcd. $C_9H_{11}N_3O_3$ $(M+H)^+$ 209.1, found 209.1. To a solution of 5-(pyrrolidine-1-carbonyl)-1*H*-pyrazole-3-carboxylic acid (51 mg, 0.2 mmol) in a mixture of dichloromethane (1 mL) and *N*,*N*-dimethylformamide (1 mL) was added PyBOP (128 mg, 0.2 mmol) and triethylamine (62 mg, 0.6 mmol). The mixture was stirred for 15 minutes, 2-(2-(difluoromethoxy)benzylamino)-*N*-methylacetamide (50 mg, 0.20 mmol) was added and the mixture was stirred at room temperature for 16 hours. The mixture was partitioned between ethyl acetate and water and the layers were separated. The aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried with sodium sulfate and concentrated. The crude product was purified by reversed phase chromatography (method A) and lyophilized to afford *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-5-(pyrrolidine-1-carbonyl)-1H-pyrazole-3-carboxamide (compound **20**, 7.3 mg, 8%) as a white solid. ^{1}HNMR (300 MHz, DMSO-d6) δ 11.39 (s, 1H), 7.34 (d, 2H), 7.20 (dd, 3H), 7.02 – 6.16 (m, 2H), 5.31 (s, 1H), 4.91 (s, 1H), 4.47 (d, 1H), 4.11 (s, 1H), 3.80 (s, 2H), 3.71 (d, 2H), 2.79 (s, 3H), 2.20 – 2.03 (m, 2H), 2.03 – 1.92 (m, 2H). LRMS (ESI) calcd. $C_{20}H_{23}F_2N_5O_4$ $(M+H)^+$ 436.2, found 436.2.

Representative example 4: Synthesis of *N*-(2-(difluoromethoxy)benzyl)-1-methyl-*N*-(2-(methylamino)-2-oxoethyl)-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxamide (compound 21).

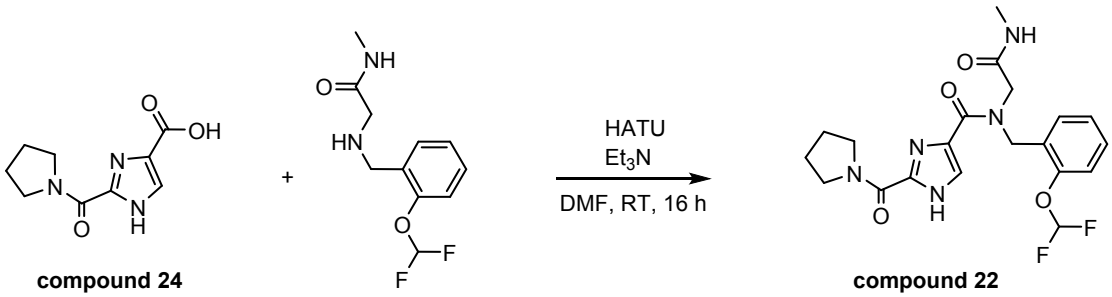


21

To a solution of 5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxylic acid (200 mg, 0.9 mmol) in *N*,*N*-dimethylformamide (5 mL) was added sodium hydride (163 mg, 4.0 mmol, followed by iodomethane (579 mg, 4.0 mmol) and the mixture stirred at room temperature for 3 hours. The mixture was quenched with water and extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate and concentrated to afford methyl 1-methyl-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxylate (258 mg, 92%) as a white solid. LRMS (ESI) calcd. $C_{12}H_{16}N_2O_3$ $(M+H)^+$ 237.1, found 237.1. To a solution of methyl 1-methyl-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxylate (258 mg, 1.1 mmol) in a mixture of tetrahydrofuran (3.6 mL) and water (0.4 mL) was added sodium hydroxide (88 mg, 2.2 mmol) and the mixture was stirred at 30 °C for 16 hours. The mixture was concentrated, redissolved in water and acidified with 1M hydrochloric acid solution until pH 4-5 at 0 °C. The precipitate was filtered off to afford 1-methyl-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxylic acid (206 mg, 85%) as a white solid. LRMS (ESI) calcd. $C_{11}H_{14}N_2O_3$ $(M+H)^+$ 223.1, found 223.1. To a solution of 1-methyl-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxylic acid (91 mg, 0.4 mmol) in *N*,*N*-dimethylformamide (5 mL) was added PyBOP (255 mg, 0.5 mmol) followed by triethylamine (124 mg,1.2 mmol) and the mixture was stirred for 15 minutes. Next, 2-(2-(difluoromethoxy)benzylamino)-*N*-methylacetamide (100 mg, 0.7 mmol) was added and the mixture was stirred at room temperature for 16 hours . The mixture was partitioned between ethyl acetate and water and the layers were separated. The aqueous layer was extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried with sodium sulfate and concentrated to afford the crude product that was purified by reversed phase chromatography (method A) and lyophilized to afford *N*-(2-(difluoromethoxy)benzyl)-1-methyl-*N*-(2-(methylamino)-2-oxoethyl)-5-(pyrrolidine-1-carbonyl)-1*H*-pyrrole-3-carboxamide (compound **21**, 28 mg, 15%) as a white solid. ^{1}HNMR (300 MHz, DMSO-d6) δ 7.93 (d, 1H), 7.51 – 6.94 (m, 6H), 6.81 – 6.29 (m ,1H), 4.71 (d, 2H), 4.03 (s, 2H), 3.74 (s, 3H), 3.33 (s, 4H), 2.62 (d, 3H), 1.83 (s, 4H). LRMS (ESI) calcd. $C_{22}H_{26}F_2N_4O_4$ $(M+H)^+$ 449.2, found 449.2.

Synthesis of 2,4-1*H*-imidazole carboxamides:

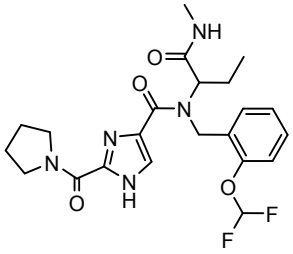
Representative example 5 (route A): Synthesis of *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide (compound 22).

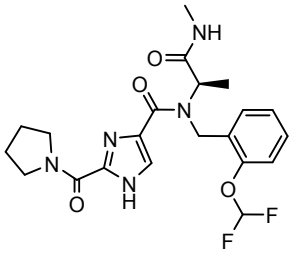
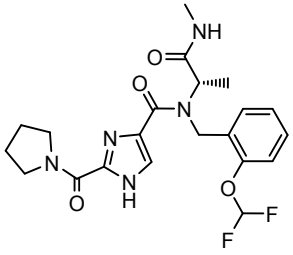


compound 24 **compound 22**

To a solution of 2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-5-carboxylic acid (50 mg, 0.24 mmol) in *N*,*N*-dimethylformamide (3 mL), HATU (81 mg, 0.29 mmol) and triethylamine (50 µL, 0.36 mmol) were added. After 30 minutes 2-((2-(difluoromethoxy)benzyl)amino)-*N*-methylacetamide (71 mg, 0.29 mmol) was added and the reaction mixture was stirred at room temperature for 16 hours. The reaction mixture was purified by reversed phase chromatography (method A) and the appropriate fractions were lyophilized to afford *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1H-imidazole-4-carboxamide (compound **22**, 70 mg, 67%) as a white solid. ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.35 (s, 1H), 7.93 – 7.82 (m, 1H), 7.74 (s, 1H), 7.44 – 7.01 (m, 5H), 5.25 (s, 1H), 4.62 (s, 1H), 4.56 (s, 1H), 3.95 – 3.84 (m, 2H), 3.50 (t, *J* = 6.7 Hz, 1H), 3.39 (t, *J* = 6.7 Hz, 1H), 3.27 (t, *J* = 6.6 Hz, 1H), 2.64 – 2.55 (m, 3H), 1.89 (q, *J* = 6.5 Hz, 1H), 1.82 (q, *J* = 6.5 Hz, 1H), 1.66 (q, *J* = 6.5 Hz, 1H), 1.59 (q, *J* = 6.5 Hz, 1H). LCMS (Method B): t_R 2.87 min, 100%, LRMS (ESI) calcd. $C_{20}H_{23}F_2N_5O_4$ $(M+H)^+$ 436.4, found 436.2.

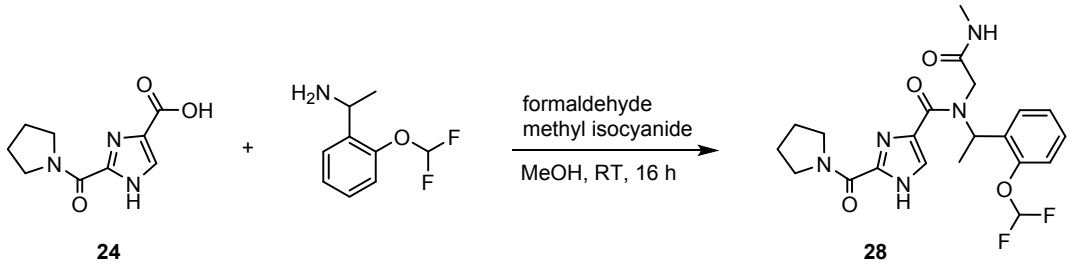
The following compounds were prepared using procedures analogous to representative example **5**.

Compound #	Structure and compound name	Analytical data
38	 *N*-(2-(difluoromethoxy)benzyl)-*N*-(1-(methylamino)-1-oxobutan-2-yl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	Yield: 20%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.65 – 13.19 (m, 1H), 8.08 – 7.82 (m, 1H), 7.75 (s, 1H), 7.50 – 6.99 (m, 5H), 5.84 – 5.70 (m, 0.4H), 5.38 – 5.18 (m, 1H), 4.93 – 4.78 (m, 0.5H), 4.72 – 4.54 (m, 0.7H), 4.13 – 3.93 (m, 0.8H), 3.61 – 3.46 (m, 0.9H), 3.44 – 3.33 (m, 1.1H), 3.25 – 3.05 (m,

		1H), 2.48 – 2.34 (m, 3H), 2.01 – 1.42 (m, 6H), 0.90 – 0.67 (m, 3H). LCMS (Method B): t_R 3.03 min, LRMS (ESI) calcd. $C_{22}H_{27}F_2N_5O_4$ $(M+H)^+$ 464.2, found 464.2.
40	 (*R*)-*N*-(2-(difluoromethoxy)benzyl)-*N*-(1-(methylamino)-1-oxopropan-2-yl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	Yield: 74%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.57 – 13.15 (m, 1H), 7.94 – 7.79 (m, 1H), 7.74 (s, 1H), 7.46 – 7.03 (m, 5H), 5.97 (d, *J* = 6.3 Hz, 0.3H), 5.49 (d, *J* = 18.1 Hz, 0.6H), 5.09 – 4.76 (m, 1.5H), 4.36 – 4.19 (m, 0.3H), 4.11 – 3.89 (m, 0.7H), 3.60 – 3.47 (m, 0.7H), 3.42 – 3.34 (m, 1.7H), 3.23 – 3.12 (m, 0.6H), 3.06 – 2.94 (m, 0.6H), 2.56 – 2.51 (m, 3H), 2.01 – 1.76 (m, 1.5H), 1.72 – 1.47 (m, 2.5H), 1.36 – 1.09 (m, 3H). LCMS (Method B): t_R 2.91 min, 100%, LRMS (ESI) calcd. $C_{21}H_{25}F_2N_5O_4$ $(M+H)^+$ 450.2, found 450.2.
41	 (*S*)-*N*-(2-(difluoromethoxy)benzyl)-*N*-(1-(methylamino)-1-oxopropan-2-yl)-2-	Yield 47%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.57 – 13.15 (m, 1H), 7.94 – 7.79 (m, 1H), 7.74 (s, 1H), 7.46 – 7.03 (m, 5H), 5.97 (d, *J* = 6.3 Hz, 0.3H), 5.49 (d, *J* = 18.1 Hz, 0.6H), 5.09 –

	(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	4.76 (m, 1.5H), 4.36 – 4.19 (m, 0.3H), 4.11 – 3.89 (m, 0.7H), 3.60 – 3.47 (m, 0.7H), 3.42 – 3.34 (m, 1.7H), 3.23 – 3.12 (m, 0.6H), 3.06 – 2.94 (m, 0.6H), 2.56 – 2.51 (m, 3H), 2.01 – 1.76 (m, 1.5H), 1.72 – 1.47 (m, 2.5H), 1.36 – 1.09 (m, 3H). LCMS (Method B): t_R 2.92 min, 100%, LRMS (ESI) calcd. $C_{21}H_{25}F_2N_5O_4$ $(M+H)^+$ 450.2, found 450.2

Representative example 6 (route A): synthesis of *N*-(1-(2-(difluoromethoxy)phenyl)ethyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide (compound 28).

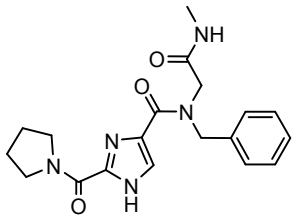
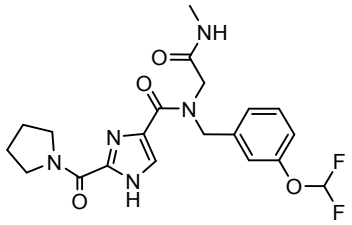


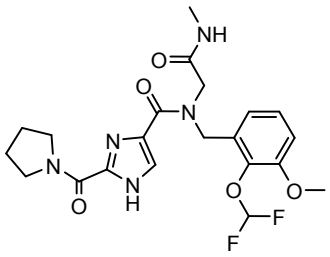
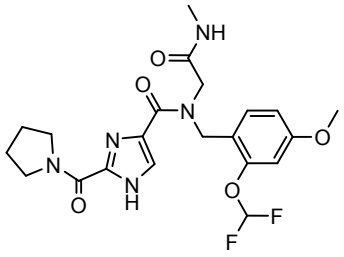
24 **28**

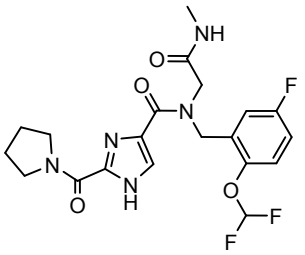
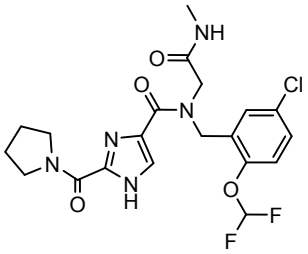
To a solution of 1-[2-(difluoromethoxy)phenyl]ethanamine (23.4 mg, 0.125 mmol) and formaldehyde (9.4 µL, 0.125 mmol, 37%) in methanol (1 mL), 2-(pyrrolidine-1-carbonyl))-1*H*-imidazole-4-carboxylic acid (25 mg, 0.12 mmol) and methyl isocyanide (14 µL, 0.24 mmol) were added and the mixture was stirred at room temperature for 16 hours. The reaction mixture was purified by reversed phase chromatography (method A) and the appropriate fractions were lyophilized to afford *N*-(1-(2-(difluoromethoxy)phenyl)ethyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide (compound **28**, 25 mg, 44%) as a white solid. ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.30 (s, 1H), 7.72 – 6.95 (m, 7H), 6.95 – 6.73 (m, 0.5H), 6.04 – 5.78 (m, 0.4H), 5.08 – 4.83 (m, 0.4H), 4.25 – 4.07 (m, 0.4H), 4.07 – 3.66 (m, 2.9H), 3.61 – 3.40 (m, 2.4H), 3.11 (q, J = 13.9 Hz, 0.4H), 2.70 (s, 0.7H), 2.46 (d, J = 4.5 Hz, 3H), 1.96 – 1.74 (m, 3.5H), 1.74 – 1.57 (m, 1.3H), 1.53 – 1.30 (m, 1.4H), 1.25 (d, J = 6.5 Hz, 0.7H); LCMS (Method B): t_R 2.90 min, 100%, LRMS (ESI) calcd. $C_{21}H_{25}F_2N_5O_4$ $(M+H)^+$ 450.2, found 450.2.

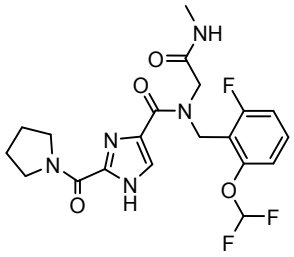
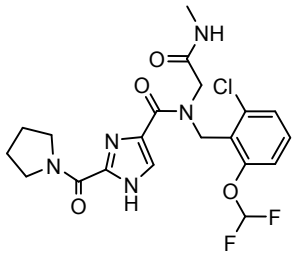
The following compounds were prepared using procedures analogous to representative Example **6**.

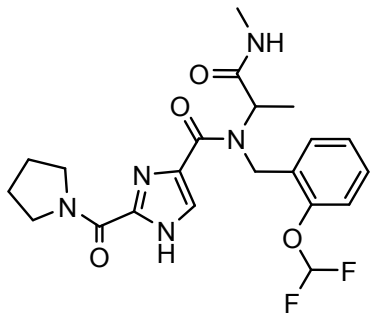
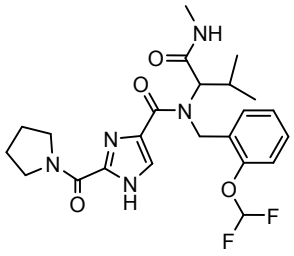
Compound #	Structure and compound name	Analytical data

29	 *N*-benzyl-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	Yield 53%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.38 (s, 1H), 7.93 – 7.79 (m, 1H), 7.75 (d, J = 5.3 Hz, 1H), 7.40 – 7.21 (m, 5H), 5.26 (s, 1H), 4.60 (s, 1H), 4.47 (s, 1H), 3.88 (t, *J* = 6.7 Hz, 1H), 3.83 (s, 1H), 3.49 (t, *J* = 6.7 Hz, 1H), 3.46 – 3.38 (m, 2H), 2.59 (d, *J* = 4.5 Hz, 3H), 1.96 – 1.75 (m, 2H), 1.74 – 1.54 (m, 2H). LCMS (Method B): t_R 2.73 min, 100%, LRMS (ESI) calcd. $C_{19}H_{23}N_5O_3$ $(M+H)^+$ 370.2, found 370.2.
30	 *N*-(3-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	Yield 38%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.39 (s, 1H), 7.95 – 7.80 (m, 1H), 7.75 (d, *J* = 4.4 Hz, 1H), 7.42 – 7.37 (m, 1H), 7.21 (t, *J* = 60.2 Hz, 1H), 7.19 – 7.04 (m, 3H), 5.24 (s, 1H), 4.61 (s, 1H), 4.50 (s, 1H), 3.94 – 3.82 (m, 2H), 3.50 (t, *J* = 6.8 Hz, 1H), 3.45 – 3.37 (m, 2H), 2.59 (d, *J* = 4.4 Hz, 3H), 1.95 – 1.76 (m, 2H), 1.75 – 1.55 (m, 2H). LCMS (Method B): t_R 2.92 min, 100%, LRMS (ESI) calcd. $C_{20}H_{23}F_2N_5O_4$ $(M+H)^+$ 436.2, found 436.2.

31	 *N*-(2-(difluoromethoxy)-3-methoxybenzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	Yield 42%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.36 (s, 1H), 7.97 – 7.67 (m, 2H), 7.29 – 7.18 (m, 1H), 7.18 – 7.01 (m, 1.3H), 7.00 – 6.89 (m, 0.5H), 6.88 – 6.72 (m, 1.2H), 5.28 (s, 1H), 4.65 (s, 1H), 4.51 (s, 1H), 4.04 – 3.73 (m, 5H), 3.58 – 3.38 (m, 3H), 2.59 (s, 3H), 1.98 – 1.52 (m, 4H). (Method C): t_R 2.95 min, 97%, LRMS (ESI) calcd. $C_{21}H_{25}F_2N_5O_5$ $(M+H)^+$ 466.2, found 466.2.
32	 *N*-(2-(difluoromethoxy)-4-methoxybenzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	Yield 64%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.36 (s, 1H), 7.91 – 7.80 (m, 1H), 7.72 (s, 1H), 7.45 – 7.01 (m, 2H), 6.87 – 6.73 (m, 2H), 5.17 (s, 1H), 4.64 – 4.44 (m, 2H), 3.93 – 3.82 (m, 2H), 3.76 (s, 3H), 3.50 (t, J = 6.7 Hz, 1H), 3.46 – 3.35 (m, 2H), 2.64 – 2.55 (m, 3H), 1.96 – 1.56 (m, 4H). LCMS (Method B): t_R 2.83 min, 99%, LRMS (ESI) calcd. $C_{21}H_{25}F_2N_5O_5$ $(M+H)^+$ 466.2, found 466.2.

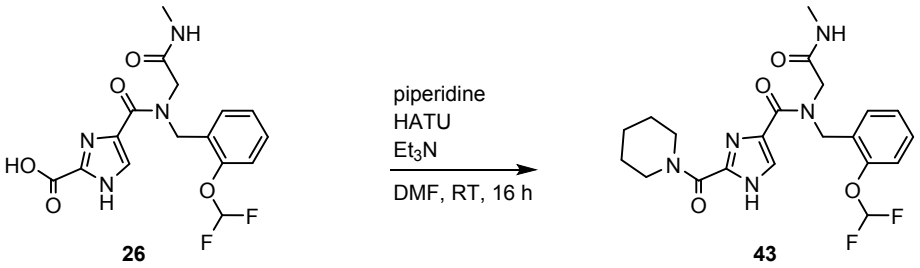
33	 *N*-(2-(difluoromethoxy)-5-fluorobenzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	Yield 32%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.38 (s, 1H), 7.97 – 7.83 (m, 1H), 7.75 (s, 1H), 7.37 – 6.92 (m, 4H), 5.25 (s, 1H), 4.62 (s, 2H), 4.01 – 3.84 (m, 2H), 3.50 (t, *J* = 6.7 Hz, 1H), 3.46 – 3.35 (m, 2H), 2.59 (s, 3H), 1.97 – 1.77 (m, 2H), 1.76 – 1.57 (m, 2H). LCMS (Method B): t_R 2.86 min, 100%, LRMS (ESI) calcd. $C_{20}H_{22}F_3N_5O_4$ $(M+H)^+$ 454.2, found 454.2.
34	 *N*-(5-chloro-2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	Yield 52%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.40 (s, 1H), 8.02 – 7.81 (m, 1H), 7.75 (s, 1H), 7.48 – 7.03 (m, 4H), 5.16 (s, 1H), 4.71 – 4.50 (m, 2H), 3.97 – 3.83 (m, 2H), 3.50 (t, *J* = 6.7 Hz, 1H), 3.40 (t, *J* = 6.8 Hz, 1H), 3.29 – 3.23 (m, 1H), 2.65 – 2.55 (m, 3H), 1.97 – 1.86 (m, 1H), 1.86 – 1.77 (m, 1H), 1.74 – 1.56 (m, 2H). LCMS (Method B): t_R 3.01 min, 100%, LRMS (ESI) calcd. $C_{20}H_{22}ClF_2N_5O_4$ $(M+H)^+$ 470.2, found 470.2.

35	 *N*-(2-(difluoromethoxy)-6-fluorobenzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	Yield 58%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.32 (s, 1H), 7.82 – 7.63 (m, 2H), 7.46 – 7.37 (m, 1H), 7.25 – 6.96 (m, 3H), 5.60 (s, 0.4H), 4.87 – 4.45 (m, 2.8H), 4.04 – 3.68 (m, 2.7H), 3.48 (t, *J* = 6.7 Hz, 2H), 2.55 (d, J = 4.5 Hz, 3H), 1.95 – 1.69 (m, 4H). LCMS (Method B): t_R 2.83 min, 95%, LRMS (ESI) calcd. $C_{20}H_{22}F_3N_5O_4$ $(M+H)^+$ 454.2, found 454.2.
36	 *N*-(2-chloro-6-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	Yield 9%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.27 (s, 1H), 7.77 – 7.61 (m, 2H), 7.46 – 7.33 (m, 2.3H), 7.26 – 6.95 (m, 1.7H), 5.71 (s, 0.4H), 4.81 (s, 1.4H), 4.56 (s, 1.3H), 3.96 – 3.80 (m, 2H), 3.78 – 3.64 (m, 0.7H), 3.48 (t, *J* = 6.5 Hz, 2.6H), 2.53 (d, *J* = 4.6 Hz, 3H), 1.93 – 1.74 (m, 4H). LCMS (Method B): t_R 2.83 min, 95%, LRMS (ESI) calcd. $C_{20}H_{22}ClF_2N_5O_4$ $(M+H)^+$ 470.2, found 470.2.

37	 (*R*)-*N*-(2-(difluoromethoxy)benzyl)-*N*-(1-(methylamino)-1-oxopropan-2-yl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	Yield 31%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.57 – 13.15 (m, 1H), 7.94 – 7.79 (m, 1H), 7.74 (s, 1H), 7.46 – 7.03 (m, 5H), 5.97 (d, *J* = 6.3 Hz, 0.3H), 5.49 (d, *J* = 18.1 Hz, 0.6H), 5.09 – 4.76 (m, 1.5H), 4.36 – 4.19 (m, 0.3H), 4.11 – 3.89 (m, 0.7H), 3.60 – 3.47 (m, 0.7H), 3.42 – 3.34 (m, 1.7H), 3.23 – 3.12 (m, 0.6H), 3.06 – 2.94 (m, 0.6H), 2.56 – 2.51 (m, 3H), 2.01 – 1.76 (m, 1.5H), 1.72 – 1.47 (m, 2.5H), 1.36 – 1.09 (m, 3H). LCMS (Method B): t_R 2.91 min, 98%, LRMS (ESI) calcd. $C_{21}H_{25}F_2N_5O_4$ $(M+H)^+$ 450.2, found 450.2
39	 *N*-(2-(difluoromethoxy)benzyl)-*N*-(3-methyl-1-(methylamino)-1-oxobutan-2-yl)-2-(pyrrolidine-1-carbonyl)-1*H*-imidazole-4-carboxamide	Yield 58%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.34 (s, 1H), 8.13 – 7.80 (m, 1H), 7.74 (s, 1H), 7.45 – 6.95 (m, 5H), 5.88 – 5.61 (m, 1H), 4.99 – 4.83 (m, 1H), 4.78 (d, *J* = 17.7 Hz, 0.5H), 4.34 (d, *J* = 16.1 Hz, 0.5H), 4.19 – 4.08 (m, 0.5H), 4.06 – 3.93 (m, 0.5H), 3.55 (t, *J* = 6.6 Hz, 1H), 3.47 – 3.35 (m, 1.5H), 3.21 – 3.08 (m, 0.5H), 2.45 – 2.17 (m, 4H), 2.02 – 1.79 (m, 2H), 1.77 – 1.50 (m, 2H), 0.94 – 0.74 (m,

		6H). LCMS (Method C): t_R 3.34 min, 99%, LRMS (ESI) calcd. $C_{23}H_{29}F_2N_5O_4$ $(M+H)^+$ 478.2, found 478.2.

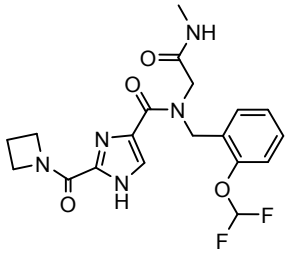
Representative example 7 (route B): Synthesis of *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(piperidine-1-carbonyl)-1*H*-imidazole-4-carboxamide (compound 43).

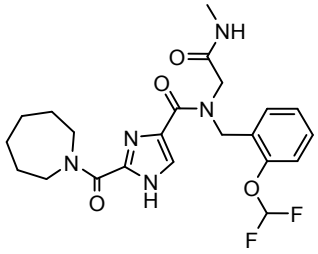
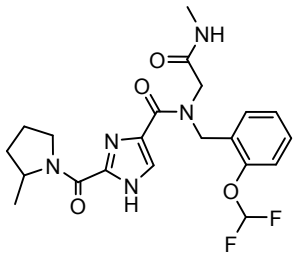


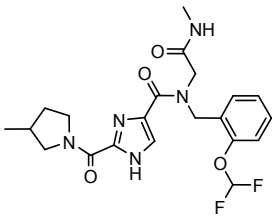
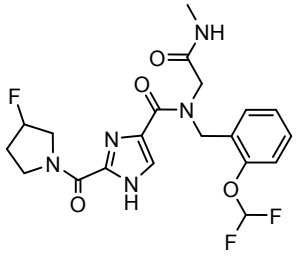
26 43

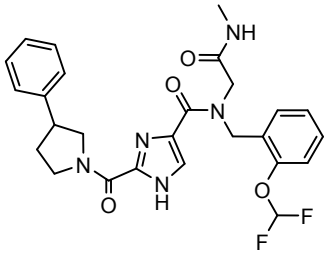
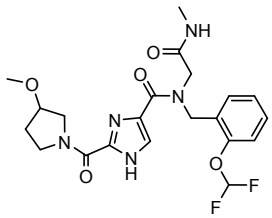
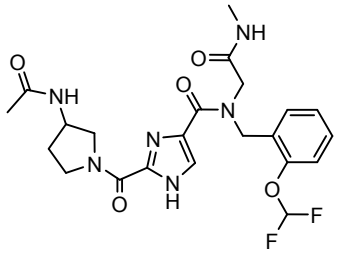
To a solution of 4-((2-(difluoromethoxy)benzyl)(2-(methylamino)-2-oxoethyl)carbamoyl)-1*H*-imidazole-2-carboxylic acid (25 mg, 0.065 mmol) in *N*,*N*-dimethylformamide (2 mL), HATU (25 mg, 0.065 mmol) and triethylamine (11 μL, 0.078 mmol) were added. After 10 minutes piperidine (5.6 mg, 0.065 mmol) was added and the reaction mixture was stirred at room temperature for 16 hours. The reaction mixture was purified by reversed phase chromatography (method B) and the appropriate fractions were lyophilized to afford *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(piperidine-1-carbonyl)-1*H*-imidazole-4-carboxamide (compound **43**, 16 mg, 55%) as a white solid. ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.15 (s, 1H), 7.92 – 7.80 (m, 1H), 7.72 (s, 1H), 7.43 – 7.01 (m, 5H), 5.25 (s, 1H), 4.69 – 4.45 (m, 2H), 4.16 (s, 1H), 3.85 (s, 1H), 3.80 (s, 1H), 3.64 – 3.45 (m, 2H), 2.59 (d, *J* = 4.3 Hz, 3H), 1.73 – 1.58 (m, 1H), 1.57 – 1.37 (m, 4H), 1.13 – 0.99 (m, 1H). LCMS (Method C): t_R 3.18 min, 100%, LRMS (ESI) calcd. $C_{21}H_{25}F_2N_5O_4$ $(M+H)^+$ 450.2, found 450.2.

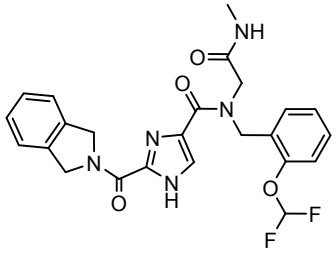
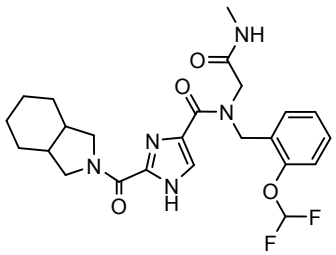
The following compounds were prepared using procedures analogous to representative Example **7**.

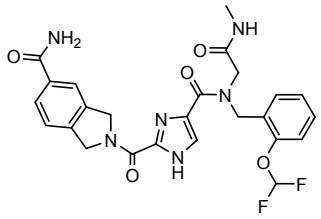
Compound #	Structure and compound name	Analytical data
42		Yield 72%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.49 (s, 1H), 7.92 – 7.80 (m, 1H), 7.74 (s, 1H), 7.44 – 7.01 (m, 5H), 5.31 (s, 1H), 4.61 (s, 2H), 4.48 (t, *J* = 7.6 Hz,

	2-(azetidine-1-carbonyl)-*N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-1*H*-imidazole-5-carboxamide	1H), 4.06 (t, *J* = 7.6 Hz, 1H), 3.93 (dt, *J* = 14.9, 7.6 Hz, 2H), 3.86 (s, 1H), 2.64 – 2.55 (m, 3H), 2.36 – 2.22 (m, 1H), 2.13 – 2.01 (m, 1H). LCMS (Method B): t_R 2.76 min, 100%, LRMS (ESI) calcd. $C_{19}H_{21}F_2N_5O_4$ $(M+H)^+$ 422.2, found 422.2.
44	 2-(azepane-1-carbonyl)-*N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-1*H*-imidazole-4-carboxamide	Yield 52%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.32 (s, 1H), 7.96 – 7.66 (m, 2H), 7.43 – 6.93 (m, 5H), 5.30 (s, 1H), 4.60 (s, 2H), 4.14 – 4.01 (m, 1H), 3.84 (s, 1H), 3.78 – 3.67 (m, 1H), 3.63 – 3.54 (m, 1H), 3.53 – 3.42 (m, 1H), 2.65 – 2.54 (m, 3H), 1.78 – 1.16 (m, 8H). LCMS (Method C): t_R 3.51 min, 99%, LRMS (ESI) calcd. $C_{22}H_{27}F_2N_5O_4$ $(M+H)^+$ 464.2, found 464.2.
45	 *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(2-methylpyrrolidine-1-carbonyl)-1*H*-imidazole-5-carboxamide	Yield 52%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.35 (s, 1H), 7.94 – 7.82 (m, 1H), 7.78 – 7.70 (m, 1H), 7.43 – 7.00 (m, 5H), 5.42 – 5.01 (m, 1.2H), 4.77 – 4.43 (m, 1.9H), 4.29 – 4.04 (m, 0.6H), 4.03 – 3.81 (m, 1.6H), 3.34 (s, 1.7H), 2.66 – 2.55 (m, 3H), 2.06 – 1.30 (m, 4H), 1.27 – 0.60 (m, 3H). LCMS (Method

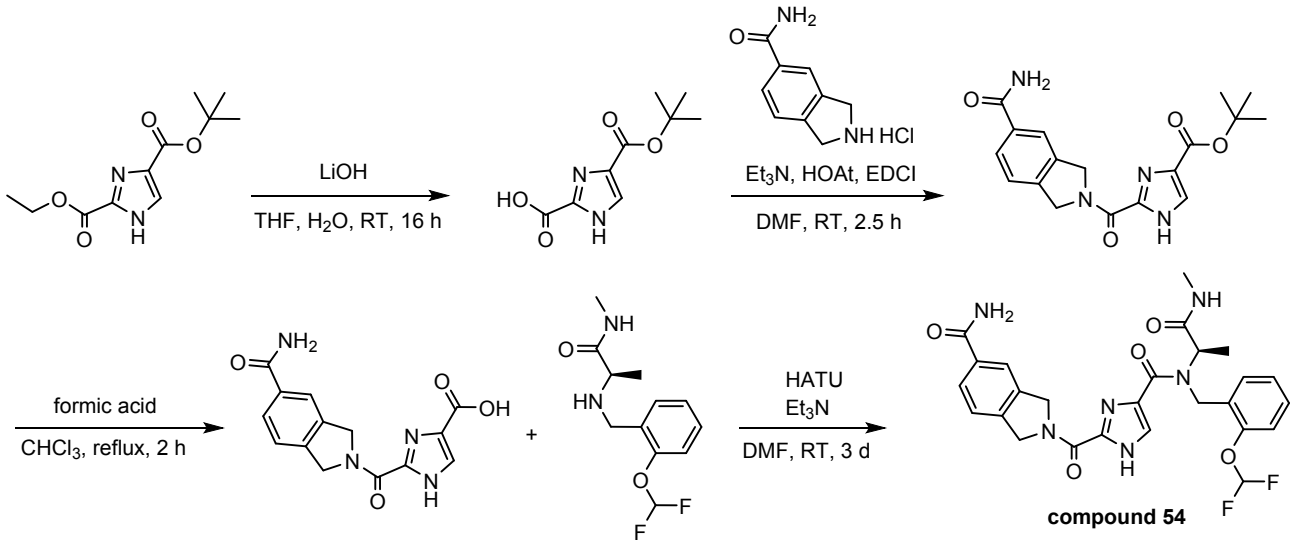
		B): t_R 3.00 min, 100%, LRMS (ESI) calcd. $C_{21}H_{25}F_2N_5O_4$ $(M+H)^+$ 450.2, found 450.2.
46	 *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(3-methylpyrrolidine-1-carbonyl)-1*H*-imidazole-5-carboxamide	Yield 54%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.34 (s, 1H), 8.05 – 7.82 (m, 1H), 7.74 (s, 1H), 7.50 – 6.94 (m, 5H), 5.40 – 5.16 (m, 1H), 4.70 – 4.47 (m, 1.7H), 4.21 – 4.02 (m, 0.4H), 3.96 – 3.39 (m, 3.4H), 3.20 – 2.81 (m, 1.2H), 2.66 – 2.54 (m, 3H), 2.36 – 1.16 (m, 3.1H), 1.04 (d, *J* = 6.5 Hz, 1.3H), 0.94 (d, *J* = 6.6 Hz, 0.9H), 0.74 (d, *J* = 6.6 Hz, 0.8H). LCMS (Method B): t_R 3.00 min, 100%, LRMS (ESI) calcd. $C_{21}H_{25}F_2N_5O_4$ $(M+H)^+$ 450.2, found 450.2.
47	 *N*-(2-(difluoromethoxy)benzyl)-2-(3-fluoropyrrolidine-1-carbonyl)-*N*-(2-(methylamino)-2-oxoethyl)-1*H*-imidazole-5-carboxamide	Yield 21%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.48 (s, 1H), 7.98 – 7.72 (m, 2H), 7.44 – 7.01 (m, 5H), 5.51 – 4.85 (m, 2H), 4.74 – 4.20 (m, 2H), 4.01 – 3.12 (m, 5H), 2.65 – 2.55 (m, 3H), 2.29 – 1.74 (m, 2H). LCMS (Method B): t_R 2.84 min, 100%, LRMS (ESI) calcd. $C_{20}H_{22}F_3N_5O_4$ $(M+H)^+$ 454.2, found 454.2.

48	 *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(3-phenylpyrrolidine-1-carbonyl)-1*H*-imidazole-5-carboxamide	Yield 36%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.44 (s, 1H), 7.94 – 7.61 (m, 2H), 7.43 – 6.65 (m, 10H), 5.43 – 5.09 (m, 1H), 4.74 – 4.42 (m, 2H), 4.31 – 3.39 (m, 5H), 3.29 – 3.15 (m, 1H), 2.65 – 2.54 (m, 2.3H), 2.40 – 1.69 (m, 2.7H). LCMS (Method B): t_R 3.29 min, 99%, LCMS (ESI) calcd. $C_{26}H_{27}F_2N_5O_4$ $(M+H)^+$ 512.2, found 512.2.
49	 *N*-(2-(difluoromethoxy)benzyl)-2-(3-methoxypyrrolidine-1-carbonyl)-*N*-(2-(methylamino)-2-oxoethyl)-1*H*-imidazole-4-carboxamide	Yield 80%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.33 (s, 1H), 7.99 – 7.82 (m, 1H), 7.80 – 7.70 (m, 1H), 7.47 – 6.95 (m, 5H), 5.43 – 5.15 (m, 1H), 4.77 – 4.39 (m, 2H), 4.19 – 3.36 (m, 6H), 3.27 – 2.96 (m, 3H), 2.65 – 2.55 (m, 3H), 2.14 – 1.58 (m, 2H). LCMS (Method B): t_R 2.80 min, 100%, LRMS (ESI) calcd. $C_{21}H_{25}F_2N_5O_5$ $(M+H)^+$ 466.2, found 466.2.
50	 2-(3-acetamidopyrrolidine-1-carbonyl)-*N*-(2-(difluoromethoxy)benzyl)-*N*-(2-	Yield 18%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.42 (s, 1H), 8.21 – 7.69 (m, 3H), 7.44 – 6.94 (m, 5H), 5.36 – 5.15 (m, 1H), 4.73 – 4.48 (m, 2H), 4.36 – 3.80 (m, 3H), 3.73 – 3.23 (m, 3H), 2.64

	(methylamino)-2-oxoethyl)-1*H*-imidazole-5-carboxamide	– 2.56 (m, 3H), 2.21 – 1.46 (m, 5H). (Method B): t_R 2.54 min, 99%, LRMS (ESI) calcd. $C_{22}H_{26}F_2N_6O_5$ $(M+H)^+$ 493.2, found 493.2.
51	 *N*-(2-(difluoromethoxy)benzyl)-2-(isoindoline-2-carbonyl)-*N*-(2-(methylamino)-2-oxoethyl)-1*H*-imidazole-5-carboxamide	Yield 55%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.56 (s, 1H), 8.14 – 8.03 (m, 0.5H), 7.95 – 7.87 (m, 0.5H), 7.84 (s, 1H), 7.51 – 6.85 (m, 9H), 5.35 (s, 1H), 5.27 (s, 1H), 4.90 (s, 1H), 4.80 (s, 1H), 4.76 – 4.59 (m, 3H), 3.87 (s, 1H), 2.72 – 2.57 (m, 3H). LCMS (Method B): t_R 3.18 min, 100%, LRMS (ESI) calcd. $C_{24}H_{23}F_2N_5O_4$ $(M+H)^+$ 484.2, found 484.2.
52	 *N*-(2-(difluoromethoxy)benzyl)-*N*-(2-(methylamino)-2-oxoethyl)-2-(octahydro-1*H*-isoindole-2-carbonyl)-1H-imidazole-4-carboxamide	Yield 52%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.39 (s, 1H), 7.94 – 7.82 (m, 1H), 7.75 (s, 1H), 7.43 – 7.00 (m, 5H), 5.41 – 5.18 (m, 1H), 4.74 – 4.44 (m, 2H), 3.95 – 3.75 (m, 2H), 3.56 – 3.19 (m, 3H), 2.59 (d, J = 4.5 Hz, 3H), 2.30 – 1.88 (m, 2H), 1.63 – 0.94 (m, 8H). LCMS (Method B): t_R 3.18 min, 100%, , LRMS (ESI) calcd. $C_{24}H_{29}F_2N_5O_4$ $(M+H)^+$ 490.2, found 490.2.

53	 2-(4-((2-(difluoromethoxy)benzyl)(2-(methylamino)-2-oxoethyl)carbamoyl)-1*H*-imidazole-2-carbonyl)isoindoline-5-carboxamide	Yield 64%, ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.47 (s, 1H), 8.10 (s, 0.5H), 8.04 – 7.73 (m, 4.3H), 7.56 – 7.16 (m, 6.7H), 7.11 – 6.93 (m, 0.5H), 5.54 – 5.19 (m, 2H), 5.00 – 4.53 (m, 5H), 3.99 – 3.79 (m, 1H), 2.71 – 2.58 (m, 3H). LCMS (Method B): t_R 2.62 min, 100%, LRMS (ESI) calcd. $C_{25}H_{24}F_2N_6O_5$ $(M+H)^+$ 527.2, found 527.2.

Representative example 8 (route A): Synthesis of (*R*)-2-(4-((2-(difluoromethoxy)benzyl)(1-(methylamino)-1-oxopropan-2-yl)carbamoyl)-1*H*-imidazole-2-carbonyl)isoindoline-5-carboxamide (compound 54).



To a solution of 4-(tert-butyl)-2-ethyl-1*H*-imidazole-2,4-dicarboxylate (3.0 g, 12.5 mmol) in tetrahydrofuran (30 mL) and water (15 mL), lithium hydroxide mono hydrate (2.62 g, 62.4 mmol) was added and the mixture was stirred at room temperature for 16 hours. The mixture was neutralized to pH = 7 with formic acid and partially concentrated (~30 mL). The residue was purified by reversed phase chromatography (method A) and the appropriate fractions were lyophilized to afford 4-(tert-butoxycarbonyl)-1*H*-imidazole-2-carboxylic acid (2.63 g, 99%) as a white solid. ^{1}HNMR (400 MHz, DMSO-d6): δ 7.80 (s, 1H), 1.51 (s, 9H); LCMS (Method A): t_R 1.42 min, LRMS (ESI) calcd. $C_9H_{12}N_2O_4$ $(M+H)^+$ 213.2, found 213.1. To a solution of 4-(tert-butoxycarbonyl)-1*H*-imidazole-2-carboxylic acid (53.4 mg, 0.25 mmol) in dry *N*,*N*-dimethylformamide (3 mL) at room temperature, EDCI (53.1 mg, 0.28 mmol) and HOAt (37.7

mg, 0.28 mmol) were added and the mixture was stirred for 30 minutes. Next, triethylamine (70 μL, 0.50 mmol) and isoindoline-5-carboxamide hydrochloride (50 mg, 0.25 mmol) were added and the mixture was stirred for 2 hours. The reaction mixture was purified by reversed phase chromatography (method A) and lyophilized to afford tert-butyl 2-(5-carbamoylisoindoline-2-carbonyl)-1*H*-imidazole-4-carboxylate (51 mg, 57%) as a white solid. ^{1}HNMR (400 MHz, DMSO-d6): δ 13.55 (s, 1H), 7.99 (d, *J* = 8.1 Hz, 1H), 7.90 (d, *J* = 8.7 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.80 (d, *J* = 2.9 Hz, 1H), 7.53 – 7.45 (m, 1H), 7.39 (d, *J* = 7.6 Hz, 1H), 5.42 (s, 2H), 4.93 (s, 2H), 1.53 (s, 9H); LRMS (Method A): t_R 1.72 min, LCMS (ESI) calcd. $C_{14}H_{12}N_4O_4$ $(M\text{-}tBu+H)^+$ 301.1, found 301.1. A suspension of tert-butyl-2-(5-carbamoylisoindoline-2-carbonyl)-1*H*-imidazole-4-carboxylate (249 mg, 0.70 mmol) in formic acid (10 mL) and chloroform (10 mL) was refluxed for 2 hours. The mixture was cooled to room temperature, concentrated, taken up in water and lyophilized to afford 2-(5-carbamoylisoindoline-2-carbonyl)-1*H*-imidazole-4-carboxylic acid (182 mg, 87%) as a white solid. ^{1}HNMR (400 MHz, DMSO-d6) δ 13.57 (s, 1H), 12.55 (s, 1H), 8.04 – 7.78 (m, 4H), 7.50 (t, *J* = 8.7 Hz, 1H), 7.39 (d, *J* = 6.4 Hz, 1H), 5.44 (s, 2H), 4.93 (s, 2H); LCMS (Method A): t_R 1.35 min, 99%, MS (ESI) 301.1 $(M+H)^+$. LRMS (Method A): t_R 1.35 min, LCMS (ESI) calcd. $C_{14}H_{12}N_4O_4$ $(M+H)^+$ 301.1, found 301.1. To a solution of 2-(5-carbamoylisoindoline-2-carbonyl)-1*H*-imidazole-4-arboxylic acid (17.4 mg, 0.058 mmol) in *N*,*N*-dimethylformamide (3 mL), HATU (22 mg, 0.058 mmol) and triethylamine (16 μL, 0.12 mmol) were added. After 45 minutes (*R*)-2-((2-(difluoromethoxy)benzyl)amino)-*N*-methylpropanamide (16.5 mg, 0.064 mmol) was added and the reaction mixture was stirred at room temperature for 3 days. The reaction mixture was purified by reversed phase chromatography (method A) and the appropriate fractions were lyophilized to afford (*R*)-2-(4-((2-(difluoromethoxy)benzyl)(1-(methylamino)-1-oxopropan-2-yl)carbamoyl)-1*H*-imidazole-2-carbonyl)isoindoline-5-carboxamide (compound **54**, 17 mg, 54%) as a white solid. ^{1}HNMR (400 MHz, DMSO-d6) complex mixture of rotamers δ 13.23 (s, 1H), 8.24 – 7.65 (m, 4.6H), 7.60 – 6.90 (m, 7.4H), 6.19 – 5.91 (m, 0.3H), 5.65 – 5.31 (m, 1.3H), 5.19 – 4.24 (m, 5H), 2.65 – 2.55 (m, 3H), 1.49 – 1.04 (m, 3H); LCMS (Method B): t_R 1.73 min, LRMS (ESI) calcd. $C_{26}H_{26}F_2N_6O_5$ $(M+H)^+$ 541.5, found 541.2.

Aqueous solubility assay

Test compounds were dissolved in DMSO to a concentration of 10 mM and further diluted to 100 µM in buffer (10 mM PBS, pH 7.4) in a 96 well plate. This results in a final DMSO concentration of 1%. The plates were shaken for 24 h at room temperature in an Eppendorf Thermomixer. After 24 h incubation, the plates were centrifuged for 20 minutes at 4680 rpm. From the supernatant 150 µl was transferred to a new 96 well plate and 50 µl DMSO was added to ensure continued dissolution. Samples were measured on LC-UV at injection volumes of 1 and 8 µl. Peak areas were determined, and compared to peak areas obtained using calibration curves of the test compounds in DMSO.

Caco-2 assay

The assay was ran at Pharmaron. Briefly, 50 µL and 25 mL of cell culture medium (DMEM (Corning, 10-017-CVR) supplemented with 10% fetal bovine serum (Corning, 35-076-CVR), and Penicillin, Streptomycin mixture (Solarbio, P1400-100)) were added to each well of the Transwell insert and reservoir, respectively and then the HTS transwell plates (Corning, 3391) were incubated at 37 °C, 5% CO_2 for 1 hour before cell seeding. The Caco-2 plate was removed from the incubator and washed twice with pre-warmed HBSS (10 mM HEPES, pH 7.4), and then incubated at 37 °C for 30 minutes. The stock solutions of control compounds were diluted in DMSO to get 1 mM solutions and then diluted with HBSS (10 mM HEPES, pH 7.4) to get 5 µM working solutions. The stock solutions of the test compounds were diluted in DMSO to get 400 µM solutions and then diluted with HBSS (10 mM HEPES, pH 7.4) to get 2 µM working solutions. The final concentration of DMSO in the incubation system was 0.5%. To determine the rate of drug transport in the apical to basolateral direction, 75 µL of 2 µM working solution of test compound was added to the Transwell insert (apical compartment) and the wells in the receiver plate (basolateral compartment) were filled with 235 µL of HBSS (10 mM HEPES, pH 7.4). To determine the rate of drug transport in the basolateral to apical direction. 235 µL of 2 µM working solution of test compound was to the receiver plate wells (basolateral compartment) and then the Transwell inserts (apical compartment) were filled with 75 µL of HBSS (10 mM HEPES, pH 7.4). Time 0 samples were prepared by transferring 50 µL of 2 µM working solution to wells of the 96-deepwell plate, followed by the addition of 200 µL cold acetonitrile containing appropriate internal standards (IS). The plates were incubated at 37 °C for 2 hours. At the end of the incubation, 50 µL samples from donor sides (apical compartment for Ap→Bl flux, and basolateral compartment for Bl→Ap) and receiver sides (basolateral compartment for Ap→Bl flux, and apical compartment for Bl→Ap) were transferred to wells of a new 96-well plate, followed by the addition of 4 volume of cold acetonitrile containing appropriate internal standards (IS). Samples were Vortexed for 5 minutes and then centrifuged at 3,220 g for 40 minutes. An aliquot of 100 µL of the supernatant was mixed with an appropriate volume of ultra-pure water before LC-MS/MS analysis. Cell monolayer integrity was assessed by measuring TEER. The apparent permeability coefficient (Papp), in units of centimeter per second, can be calculated for Caco-2 drug transport assays using the following equation:

$$Papp = (VA \times [drug]_{acceptor})/(Area \times Time \times [drug]_{initial,donor})$$

Where VA is the volume (in mL) in the acceptor well, Area is the surface area of the membrane (0.143 cm^2 for Transwell-96 Well Permeable Supports), and time is the total transport time in seconds. Propranolol and digoxin were used as controls in this assay.

S9 metabolic stability assay

The assay was ran at BioDuro. Briefly, liver S9 fractions (final concentration 1 mg/mL) from mouse (CD-1 male, Xenotech, 1310026), rat (male, Xenotech, 1310212) and human (pooled, Xenotech, 1210091) were incubated with 1 μM test compounds at 37°C for 0, 5, 15, 30, and 60 minutes using NADPH (1 mM) in a phosphate buffer (0.05M, pH7.4). The reaction was stopped by the addition of cold acetonitrile, precipitated protein was removed, and the supernatants were analyzed using LCMS. The percent parent remaining was calculated using area ratio obtained from analyte peak area to the internal standard peak area in comparison to time 0 min. Phenacetin, diclofenac sodium salt, omeprazole, dextromethorphan hydrobromide and midazolam maleate were used as controls.

Biochemical assay

Assays were conducted in 384 well-plates. MAP3K7/MAP3K7IP1 (TAK1-TAB1), all reagents and buffer were obtained from Life Technologies. The assay was performed according to the manufacturers protocol: http://tools.thermofisher.com/content/sfs/manuals/MAP3K7-MAP3K7IP1_(TAK1-TAB1)_LanthaScreen_Activity.pdf.
In short: compounds were diluted and transferred to assay plate by echo, to get 10 concentration points in duplicate (98910 nM, 9891nM or 2968 top concentration, about 3 folds dilution). The compounds and protein were incubated for 30 minutes, before the reaction was started for 60 minutes at 25 °C. The final concentration of DMSO was 1%, enzyme was 700ng/ml, ATP was 10 µM and substrate was 0.1 µM. Next, 10µl EDTA and antibody detection reagent mixture were added, the mixture was incubated for 30 minutes, and the TR-FRET 520/495 ratio signal was read using a TECAN plate reader. The final concentration of EDTA was 10 mM and antibody was 2 nM. For the more potent compounds **53** and **54**, the assay was repeated using 989 nM top concentration and a final enzyme concentration of 140 ng/mL.

Single IC_{50} values per compound:

Cpd	TAK1 IC_{50} in µM	Cpd	TAK1 IC_{50} in µM
Staurosporine	0.026; 0.036; 0.036; 0.039; 0.041; 0.048[c]	**33**	>10; >10[b]
2	0.009; 0.011[b]	**34**	>10; >10[b]
3	0.024; 0.036[b]	**35**	>10; >10[b]
7	1.2; 1.4[a]	**36**	>10; >10[b]
9	0.9; 0.9[a]	**37**	0.2; 0.8[b]
10	1.6; 1.8[a]	**38**	0.8; 1.2[b]
11	3.1; 3.2[a]	**39**	>10; >10[b]
12	3.1; 3.9[a]	**40**	0.2; 0.3[b]
13	2.8; 2.9[a]	**41**	3.2; 5.8[b]
14	1.9; 3.3[a]	**42**	1.9; 3.3[b]
15	3.4; 3.4[a]	**43**	0.8; 1.5[b]
16	1.7; 1.9[a]	**44**	1.2; 4.6[b]
17	10; 42[a]	**45**	3.4; 8.0[a]
18	1.8; 1.8[a]	**46**	0.6; 1.1[b]
19	>100; >100[a]	**47**	1.1; 1.3[b]
20	51; 67[a]	**48**	2.2; 2.4[b]
21	>100; >100[a]	**49**	>10; >10[b]
22	0.5; 0.7[b]	**50**	>10; >10[b]
28	>10; >10[b]	**51**	0.2; 0.4[b]
29	>10; >10[b]	**52**	2.4; 4.4[b]
30	>10; >10[b]	**53**	0.004; 0.006[d]
31	>10; >10[b]	**54**	0.001; 0.002[d]
32	>10; >10[b]		

[a]Top concentration: 98910 nM; [b]Top concentration 9891nM; [c]Top concentration 2968nM; [d]Top concentration 989 nM.

Cell assay

The effect of test compounds on cell proliferation/viability was determined using the CellTiter-Glo® Assay (Promega). The human colorectal cancer cell line HCT-15 (ATCC CCL-25) was cultured in RPMI 1640 medium containing 10% FBS and maintained at 37°C and 5% CO_2.

Eight hundred cells/well in 37 µl were seeded in 384 well plates in complete culture medium containing 10% FBS. Plates were incubated overnight at 37°C and 5% CO_2. Test compounds were serially diluted 3-fold in 100% DMSO. The final DMSO concentration in each dilution was 0.5%. Forty nL of diluted compound was added to the test plate containing cells in triplicate determinations. TNF-α was added to the plates 2 hrs later at a final concentration of 10 ng/mL. Plates then were covered and incubated for 72 hrs at 37°C and 5% CO_2.

Viability was assessed using the CellTiter-Glo® homogeneous luminescent assay kit according to the manufacturer's instructions. The assay determines the number of viable cells in culture by quantifying ATP, which indicates the presence of metabolically active cells. Briefly, 30 µl of room temperature CellTiter-Glo® reagent was added to each well, the plate was incubated at room temperature for 30 minutes followed by luminescence recording on a Spark 10M multimodal plate reader (Tecan).

Single EC_{50} values per compound:

Cpd	EC_{50} in µM
1	0.001, 0.002, 0.002[a]
54	>10, >10, >10[b]

[a]Top concentration: 100 nM; [b]Top concentration 10000 nM

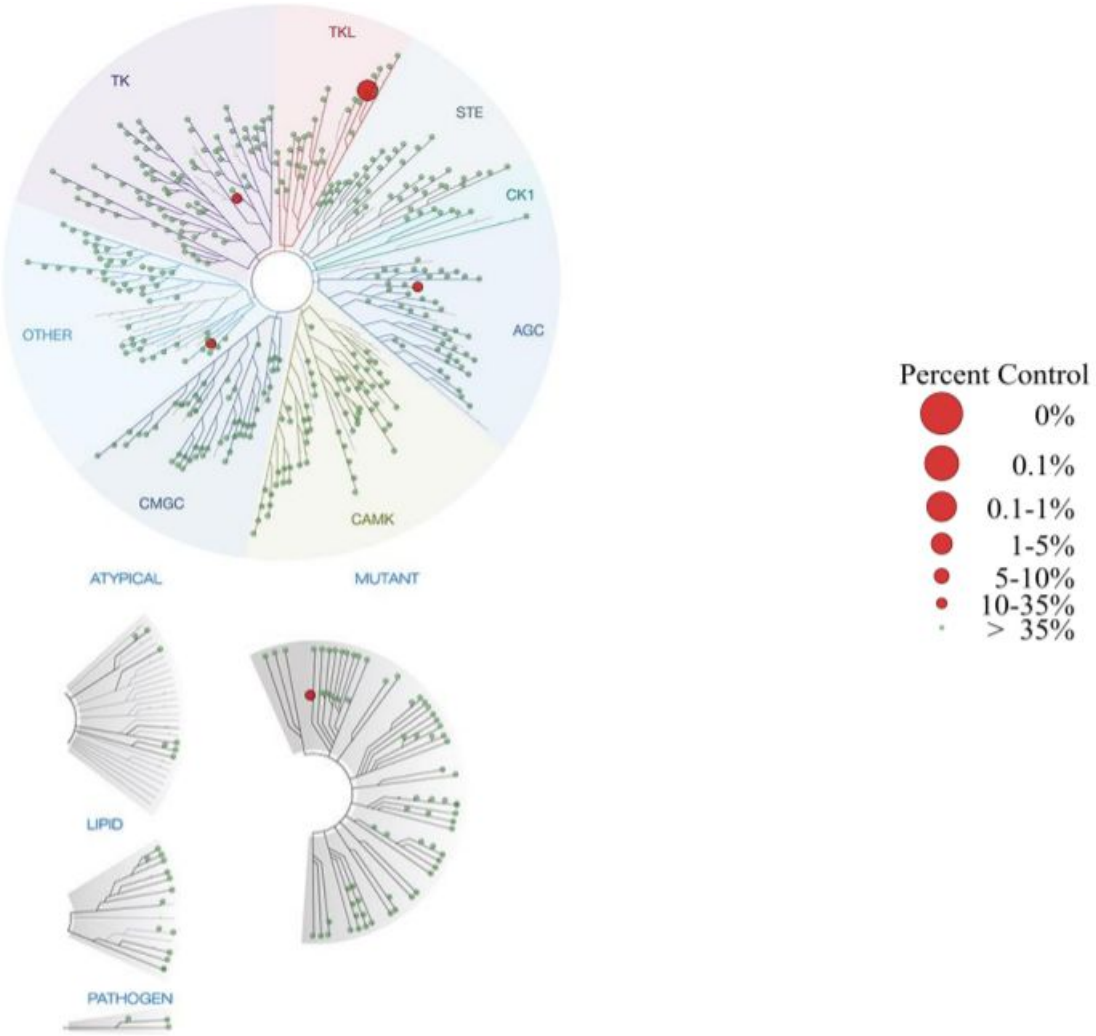
Affinity-Mediated Selection of DNA-Encoded Chemical Libraries

Hexahistidine tagged TAK1-TAB1 fusion protein was sourced from Eurofins (Luxembourg, catalog number 14-600) and an ADP-Glo™ assay (Promega, Madison, WI, catalog number V6930) was established in which the protein was shown to have dose-dependent autophosphorylation activity. The protein was also demonstrated to be a monomer by SEC and to undergo a thermal transition at 54°C. The protein was shown to be efficiently captured on the IMAC matrix His-Select® (Sigma-Aldrich, St. Louis, MO, catalog number P6611). Four parallel affinity-mediated selections were then performed as previously described.[1,2] Briefly, twenty-one different DNA-encoded chemical libraries were combined and this mixture was incubated with TAK1-TAB1 fusion protein each in a 60 µl volume in Cytosolic Selection Buffer (20 mM HEPES, 134 mM Potassium Acetate, 8 mM Sodium Acetate, 4 mM Sodium Chloride, 0.8 mM Magnesium Acetate, 1 mg/ml Sheared Salmon Sperm DNA, 1 mM DTT, 0.02% Tween-20, pH 7.2). Separate incubations were set up with no target, TAK1-TAB1 at 7.8 µM, TAK1-TAB1 at 1.6 µM and with TAK1-TAB1 at 7.8 µM and oxozeaenol at 40 µM (pre-incubated for one hour) with 50 pmoles of each library. After a one-hour incubation the mixtures were captured on 5 µl of His-Select IMAC matrix constrained within a 20 µl Phytip mounted on a Phynexus ME-200 automated 12-tip pipettor. After capture the matrices were washed eight times with changes of buffer and then heat-eluted at 85°C into the same buffer. A second round of selection was performed using the round-one eluate as input and fresh proteins and oxozeaenol repeating the conditions of the first round. The encoding DNA in the output of the second round of selection was amplified using PCR with primers that introduce the Illumina READ1 and READ2 sequences and the amplified sample was submitted for sequencing using an Illumina 2500 instrument in high-output mode. Ninety-seven million single-end reads were generated across all twenty-one libraries for these four selections.

KINOMEscan data

Compound **22** was screened at 10 μM against 468 kinases using DiscoverX's KINOMEscan™ screening technology (scanMAX).

Treespot interaction map for compound **22**, S-Score (35) = 0.01.
S(35) = (number of non-mutant kinases with %Ctrl <35)/(number of non-mutant kinases tested) = (4/403).



DiscoveRx Gene Symbol	Entrez Gene Symbol	Percent Control
AAK1	AAK1	95
ABL1(E255K)-phosphorylated	ABL1	73
ABL1(F317I)-nonphosphorylated	ABL1	85
ABL1(F317I)-phosphorylated	ABL1	100
ABL1(F317L)-nonphosphorylated	ABL1	90
ABL1(F317L)-phosphorylated	ABL1	100
ABL1(H396P)-nonphosphorylated	ABL1	30
ABL1(H396P)-phosphorylated	ABL1	90

ABL1(M351T)-phosphorylated	ABL1	100
ABL1(Q252H)-nonphosphorylated	ABL1	70
ABL1(Q252H)-phosphorylated	ABL1	98
ABL1(T315I)-nonphosphorylated	ABL1	90
ABL1(T315I)-phosphorylated	ABL1	96
ABL1(Y253F)-phosphorylated	ABL1	100
ABL1-nonphosphorylated	ABL1	77
ABL1-phosphorylated	ABL1	92
ABL2	ABL2	92
ACVR1	ACVR1	100
ACVR1B	ACVR1B	94
ACVR2A	ACVR2A	100
ACVR2B	ACVR2B	100
ACVRL1	ACVRL1	91
ADCK3	CABC1	100
ADCK4	ADCK4	93
AKT1	AKT1	97
AKT2	AKT2	100
AKT3	AKT3	94
ALK	ALK	100
ALK(C1156Y)	ALK	100
ALK(L1196M)	ALK	100
AMPK-alpha1	PRKAA1	100
AMPK-alpha2	PRKAA2	70
ANKK1	ANKK1	100
ARK5	NUAK1	83
ASK1	MAP3K5	100
ASK2	MAP3K6	100
AURKA	AURKA	86
AURKB	AURKB	100
AURKC	AURKC	87
AXL	AXL	82
BIKE	BMP2K	81
BLK	BLK	100
BMPR1A	BMPR1A	93
BMPR1B	BMPR1B	91
BMPR2	BMPR2	100
BMX	BMX	100
BRAF	BRAF	100
BRAF(V600E)	BRAF	100
BRK	PTK6	100
BRSK1	BRSK1	100
BRSK2	BRSK2	100
BTK	BTK	84
BUB1	BUB1	88
CAMK1	CAMK1	100
CAMK1B	PNCK	88

CAMK1D	CAMK1D	100
CAMK1G	CAMK1G	100
CAMK2A	CAMK2A	88
CAMK2B	CAMK2B	84
CAMK2D	CAMK2D	90
CAMK2G	CAMK2G	96
CAMK4	CAMK4	70
CAMKK1	CAMKK1	100
CAMKK2	CAMKK2	95
CASK	CASK	100
CDC2L1	CDK11B	100
CDC2L2	CDC2L2	100
CDC2L5	CDK13	88
CDK11	CDK19	99
CDK2	CDK2	93
CDK3	CDK3	94
CDK4	CDK4	99
CDK4-cyclinD1	CDK4	100
CDK4-cyclinD3	CDK4	100
CDK5	CDK5	100
CDK7	CDK7	100
CDK8	CDK8	94
CDK9	CDK9	100
CDKL1	CDKL1	95
CDKL2	CDKL2	100
CDKL3	CDKL3	98
CDKL5	CDKL5	100
CHEK1	CHEK1	81
CHEK2	CHEK2	95
CIT	CIT	100
CLK1	CLK1	88
CLK2	CLK2	87
CLK3	CLK3	79
CLK4	CLK4	74
CSF1R	CSF1R	78
CSF1R-autoinhibited	CSF1R	100
CSK	CSK	100
CSNK1A1	CSNK1A1	85
CSNK1A1L	CSNK1A1L	100
CSNK1D	CSNK1D	100
CSNK1E	CSNK1E	90
CSNK1G1	CSNK1G1	100
CSNK1G2	CSNK1G2	100
CSNK1G3	CSNK1G3	100
CSNK2A1	CSNK2A1	97
CSNK2A2	CSNK2A2	100
CTK	MATK	98
DAPK1	DAPK1	95

DAPK2	DAPK2	100
DAPK3	DAPK3	91
DCAMKL1	DCLK1	100
DCAMKL2	DCLK2	96
DCAMKL3	DCLK3	53
DDR1	DDR1	90
DDR2	DDR2	100
DLK	MAP3K12	100
DMPK	DMPK	95
DMPK2	CDC42BPG	100
DRAK1	STK17A	92
DRAK2	STK17B	90
DYRK1A	DYRK1A	100
DYRK1B	DYRK1B	100
DYRK2	DYRK2	100
EGFR	EGFR	67
EGFR(E746-A750del)	EGFR	98
EGFR(G719C)	EGFR	100
EGFR(G719S)	EGFR	100
EGFR(L747-E749del, A750P)	EGFR	88
EGFR(L747-S752del, P753S)	EGFR	98
EGFR(L747-T751del,Sins)	EGFR	98
EGFR(L858R)	EGFR	94
EGFR(L858R,T790M)	EGFR	100
EGFR(L861Q)	EGFR	100
EGFR(S752-I759del)	EGFR	98
EGFR(T790M)	EGFR	100
EIF2AK1	EIF2AK1	14
EPHA1	EPHA1	95
EPHA2	EPHA2	100
EPHA3	EPHA3	90
EPHA4	EPHA4	100
EPHA5	EPHA5	97
EPHA6	EPHA6	100
EPHA7	EPHA7	100
EPHA8	EPHA8	100
EPHB1	EPHB1	100
EPHB2	EPHB2	100
EPHB3	EPHB3	100
EPHB4	EPHB4	100
EPHB6	EPHB6	85
ERBB2	ERBB2	100
ERBB3	ERBB3	100
ERBB4	ERBB4	100
ERK1	MAPK3	100
ERK2	MAPK1	94
ERK3	MAPK6	97
ERK4	MAPK4	100

ERK5	MAPK7	100
ERK8	MAPK15	94
ERN1	ERN1	91
FAK	PTK2	99
FER	FER	100
FES	FES	100
FGFR1	FGFR1	98
FGFR2	FGFR2	83
FGFR3	FGFR3	96
FGFR3(G697C)	FGFR3	90
FGFR4	FGFR4	98
FGR	FGR	100
FLT1	FLT1	100
FLT3	FLT3	85
FLT3(D835H)	FLT3	90
FLT3(D835V)	FLT3	69
FLT3(D835Y)	FLT3	84
FLT3(ITD)	FLT3	100
FLT3(ITD,D835V)	FLT3	100
FLT3(ITD,F691L)	FLT3	82
FLT3(K663Q)	FLT3	89
FLT3(N841I)	FLT3	80
FLT3(R834Q)	FLT3	88
FLT3-autoinhibited	FLT3	100
FLT4	FLT4	100
FRK	FRK	100
FYN	FYN	100
GAK	GAK	100
GCN2(Kin.Dom.2,S808G)	EIF2AK4	87
GRK1	GRK1	73
GRK2	ADRBK1	100
GRK3	ADRBK2	98
GRK4	GRK4	99
GRK7	GRK7	100
GSK3A	GSK3A	77
GSK3B	GSK3B	100
HASPIN	GSG2	89
HCK	HCK	100
HIPK1	HIPK1	100
HIPK2	HIPK2	90
HIPK3	HIPK3	100
HIPK4	HIPK4	100
HPK1	MAP4K1	92
HUNK	HUNK	98
ICK	ICK	93
IGF1R	IGF1R	90
IKK-alpha	CHUK	99
IKK-beta	IKBKB	100

IKK-epsilon	IKBKE	100
INSR	INSR	100
INSRR	INSRR	88
IRAK1	IRAK1	100
IRAK3	IRAK3	98
IRAK4	IRAK4	97
ITK	ITK	100
JAK1(JH1domain-catalytic)	JAK1	76
JAK1(JH2domain-pseudokinase)	JAK1	100
JAK2(JH1domain-catalytic)	JAK2	50
JAK3(JH1domain-catalytic)	JAK3	62
JNK1	MAPK8	87
JNK2	MAPK9	89
JNK3	MAPK10	96
KIT	KIT	100
KIT(A829P)	KIT	89
KIT(D816H)	KIT	74
KIT(D816V)	KIT	100
KIT(L576P)	KIT	97
KIT(V559D)	KIT	100
KIT(V559D,T670I)	KIT	98
KIT(V559D,V654A)	KIT	100
KIT-autoinhibited	KIT	98
LATS1	LATS1	97
LATS2	LATS2	100
LCK	LCK	100
LIMK1	LIMK1	98
LIMK2	LIMK2	100
LKB1	STK11	88
LOK	STK10	99
LRRK2	LRRK2	100
LRRK2(G2019S)	LRRK2	83
LTK	LTK	97
LYN	LYN	100
LZK	MAP3K13	83
MAK	MAK	86
MAP3K1	MAP3K1	100
MAP3K15	MAP3K15	97
MAP3K2	MAP3K2	89
MAP3K3	MAP3K3	100
MAP3K4	MAP3K4	97
MAP4K2	MAP4K2	100
MAP4K3	MAP4K3	100
MAP4K4	MAP4K4	91
MAP4K5	MAP4K5	100
MAPKAPK2	MAPKAPK2	100
MAPKAPK5	MAPKAPK5	100
MARK1	MARK1	89

MARK2	MARK2	90
MARK3	MARK3	100
MARK4	MARK4	99
MAST1	MAST1	100
MEK1	MAP2K1	86
MEK2	MAP2K2	90
MEK3	MAP2K3	100
MEK4	MAP2K4	100
MEK5	MAP2K5	98
MEK6	MAP2K6	78
MELK	MELK	97
MERTK	MERTK	83
MET	MET	97
MET(M1250T)	MET	96
MET(Y1235D)	MET	94
MINK	MINK1	91
MKK7	MAP2K7	100
MKNK1	MKNK1	100
MKNK2	MKNK2	76
MLCK	MYLK3	96
MLK1	MAP3K9	100
MLK2	MAP3K10	89
MLK3	MAP3K11	100
MRCKA	CDC42BPA	100
MRCKB	CDC42BPB	93
MST1	STK4	100
MST1R	MST1R	94
MST2	STK3	86
MST3	STK24	98
MST4	MST4	100
MTOR	MTOR	92
MUSK	MUSK	100
MYLK	MYLK	94
MYLK2	MYLK2	100
MYLK4	MYLK4	97
MYO3A	MYO3A	100
MYO3B	MYO3B	91
NDR1	STK38	88
NDR2	STK38L	97
NEK1	NEK1	87
NEK10	NEK10	100
NEK11	NEK11	100
NEK2	NEK2	100
NEK3	NEK3	83
NEK4	NEK4	100
NEK5	NEK5	100
NEK6	NEK6	98
NEK7	NEK7	100

NEK9	NEK9	97
NIK	MAP3K14	100
NIM1	MGC42105	94
NLK	NLK	99
OSR1	OXSR1	100
p38-alpha	MAPK14	97
p38-beta	MAPK11	96
p38-delta	MAPK13	95
p38-gamma	MAPK12	100
PAK1	PAK1	94
PAK2	PAK2	69
PAK3	PAK3	88
PAK4	PAK4	100
PAK6	PAK6	100
PAK7	PAK7	89
PCTK1	CDK16	100
PCTK2	CDK17	100
PCTK3	CDK18	93
PDGFRA	PDGFRA	97
PDGFRB	PDGFRB	82
PDPK1	PDPK1	96
PFCDPK1(P.falciparum)	CDPK1	83
PFPK5(P.falciparum)	MAL13P1.279	100
PFTAIRE2	CDK15	100
PFTK1	CDK14	100
PHKG1	PHKG1	100
PHKG2	PHKG2	100
PIK3C2B	PIK3C2B	100
PIK3C2G	PIK3C2G	100
PIK3CA	PIK3CA	100
PIK3CA(C420R)	PIK3CA	100
PIK3CA(E542K)	PIK3CA	93
PIK3CA(E545A)	PIK3CA	98
PIK3CA(E545K)	PIK3CA	97
PIK3CA(H1047L)	PIK3CA	100
PIK3CA(H1047Y)	PIK3CA	89
PIK3CA(I800L)	PIK3CA	100
PIK3CA(M1043I)	PIK3CA	100
PIK3CA(Q546K)	PIK3CA	90
PIK3CB	PIK3CB	84
PIK3CD	PIK3CD	88
PIK3CG	PIK3CG	92
PIK4CB	PI4KB	100
PIKFYVE	PIKFYVE	100
PIM1	PIM1	100
PIM2	PIM2	96
PIM3	PIM3	89
PIP5K1A	PIP5K1A	100

PIP5K1C	PIP5K1C	62
PIP5K2B	PIP4K2B	97
PIP5K2C	PIP4K2C	93
PKAC-alpha	PRKACA	99
PKAC-beta	PRKACB	92
PKMYT1	PKMYT1	100
PKN1	PKN1	82
PKN2	PKN2	98
PKNB(M.tuberculosis)	pknB	100
PLK1	PLK1	100
PLK2	PLK2	100
PLK3	PLK3	100
PLK4	PLK4	92
PRKCD	PRKCD	100
PRKCE	PRKCE	100
PRKCH	PRKCH	78
PRKCI	PRKCI	87
PRKCQ	PRKCQ	88
PRKD1	PRKD1	85
PRKD2	PRKD2	95
PRKD3	PRKD3	87
PRKG1	PRKG1	92
PRKG2	PRKG2	98
PRKR	EIF2AK2	78
PRKX	PRKX	100
PRP4	PRPF4B	100
PYK2	PTK2B	88
QSK	KIAA0999	100
RAF1	RAF1	100
RET	RET	92
RET(M918T)	RET	100
RET(V804L)	RET	100
RET(V804M)	RET	100
RIOK1	RIOK1	84
RIOK2	RIOK2	98
RIOK3	RIOK3	92
RIPK1	RIPK1	100
RIPK2	RIPK2	99
RIPK4	RIPK4	89
RIPK5	DSTYK	100
ROCK1	ROCK1	100
ROCK2	ROCK2	97
ROS1	ROS1	84
RPS6KA4(Kin.Dom.1-N-terminal)	RPS6KA4	89
RPS6KA4(Kin.Dom.2-C-terminal)	RPS6KA4	100
RPS6KA5(Kin.Dom.1-N-terminal)	RPS6KA5	77
RPS6KA5(Kin.Dom.2-C-terminal)	RPS6KA5	86
RSK1(Kin.Dom.1-N-terminal)	RPS6KA1	100

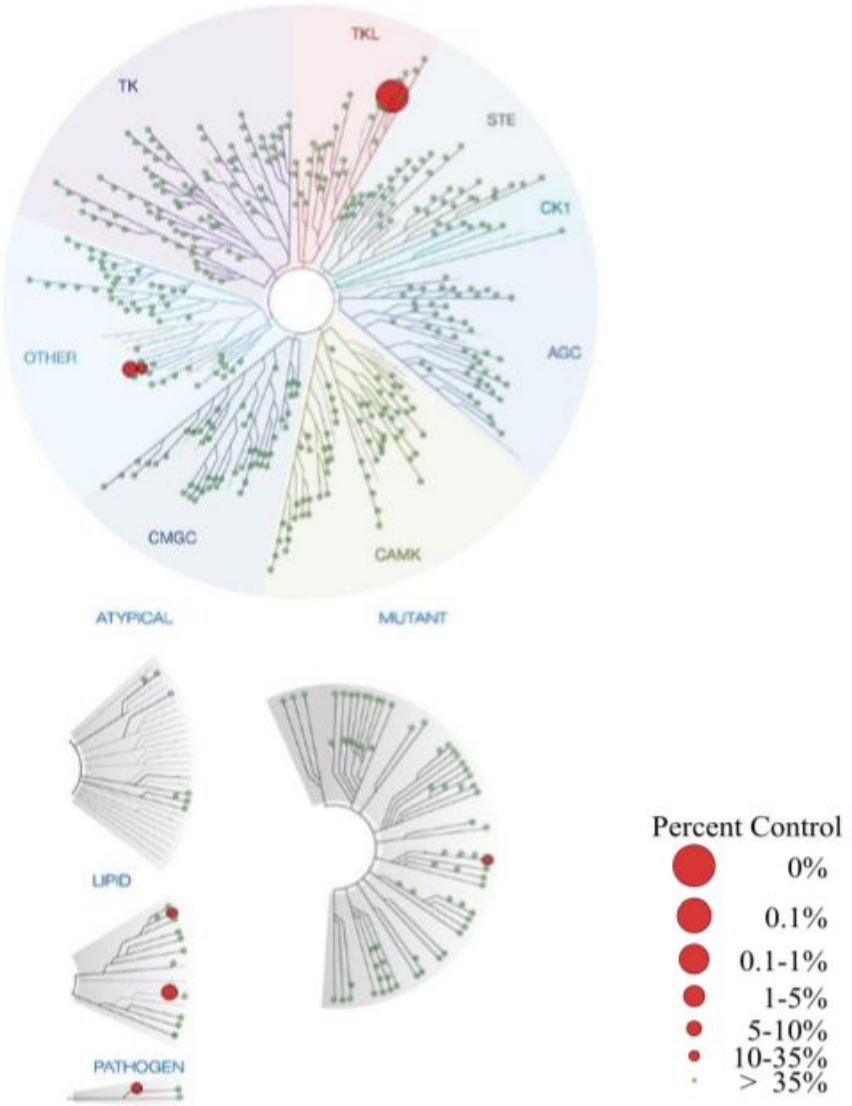
RSK1(Kin.Dom.2-C-terminal)	RPS6KA1	85
RSK2(Kin.Dom.1-N-terminal)	RPS6KA3	100
RSK2(Kin.Dom.2-C-terminal)	RPS6KA3	100
RSK3(Kin.Dom.1-N-terminal)	RPS6KA2	100
RSK3(Kin.Dom.2-C-terminal)	RPS6KA2	95
RSK4(Kin.Dom.1-N-terminal)	RPS6KA6	100
RSK4(Kin.Dom.2-C-terminal)	RPS6KA6	95
S6K1	RPS6KB1	100
SBK1	SBK1	100
SGK	SGK1	100
SgK110	SgK110	93
SGK2	SGK2	100
SGK3	SGK3	100
SIK	SIK1	100
SIK2	SIK2	89
SLK	SLK	97
SNARK	NUAK2	88
SNRK	SNRK	94
SRC	SRC	100
SRMS	SRMS	100
SRPK1	SRPK1	96
SRPK2	SRPK2	94
SRPK3	SRPK3	93
STK16	STK16	91
STK33	STK33	100
STK35	STK35	99
STK36	STK36	100
STK39	STK39	100
SYK	SYK	69
TAK1	MAP3K7	1
TAOK1	TAOK1	100
TAOK2	TAOK2	98
TAOK3	TAOK3	100
TBK1	TBK1	100
TEC	TEC	97
TESK1	TESK1	97
TGFBR1	TGFBR1	96
TGFBR2	TGFBR2	88
TIE1	TIE1	90
TIE2	TEK	100
TLK1	TLK1	57
TLK2	TLK2	43
TNIK	TNIK	98
TNK1	TNK1	59
TNK2	TNK2	20
TNNI3K	TNNI3K	90
TRKA	NTRK1	98
TRKB	NTRK2	100

TRKC	NTRK3	95
TRPM6	TRPM6	100
TSSK1B	TSSK1B	90
TSSK3	TSSK3	100
TTK	TTK	94
TXK	TXK	91
TYK2(JH1domain-catalytic)	TYK2	100
TYK2(JH2domain-pseudokinase)	TYK2	70
TYRO3	TYRO3	100
ULK1	ULK1	100
ULK2	ULK2	100
ULK3	ULK3	100
VEGFR2	KDR	100
VPS34	PIK3C3	85
VRK2	VRK2	100
WEE1	WEE1	100
WEE2	WEE2	96
WNK1	WNK1	100
WNK2	WNK2	100
WNK3	WNK3	100
WNK4	WNK4	100
YANK1	STK32A	16
YANK2	STK32B	75
YANK3	STK32C	87
YES	YES1	64
YSK1	STK25	91
YSK4	MAP3K19	99
ZAK	ZAK	97
ZAP70	ZAP70	100

KINOMEscan data

Compound **54** was screened at 1 μM against 468 kinases using DiscoverX's KINOMEscan™ screening technology (scanMAX).

Treespot interaction map for compound **54**, S-Score (35) = 0.015.
S(35) = (number of non-mutant kinases with %Ctrl <35)/(number of non-mutant kinases tested) = (6/403).



DiscoveRx Gene Symbol	Entrez Gene Symbol	Percent Control
AAK1	AAK1	84
ABL1(E255K)-phosphorylated	ABL1	65
ABL1(F317I)-nonphosphorylated	ABL1	80
ABL1(F317I)-phosphorylated	ABL1	61
ABL1(F317L)-nonphosphorylated	ABL1	76
ABL1(F317L)-phosphorylated	ABL1	82

ABL1(H396P)-nonphosphorylated	ABL1	56
ABL1(H396P)-phosphorylated	ABL1	68
ABL1(M351T)-phosphorylated	ABL1	63
ABL1(Q252H)-nonphosphorylated	ABL1	64
ABL1(Q252H)-phosphorylated	ABL1	53
ABL1(T315I)-nonphosphorylated	ABL1	91
ABL1(T315I)-phosphorylated	ABL1	81
ABL1(Y253F)-phosphorylated	ABL1	62
ABL1-nonphosphorylated	ABL1	82
ABL1-phosphorylated	ABL1	80
ABL2	ABL2	100
ACVR1	ACVR1	100
ACVR1B	ACVR1B	96
ACVR2A	ACVR2A	82
ACVR2B	ACVR2B	87
ACVRL1	ACVRL1	95
ADCK3	CABC1	92
ADCK4	ADCK4	87
AKT1	AKT1	95
AKT2	AKT2	98
AKT3	AKT3	96
ALK	ALK	67
ALK(C1156Y)	ALK	84
ALK(L1196M)	ALK	83
AMPK-alpha1	PRKAA1	94
AMPK-alpha2	PRKAA2	88
ANKK1	ANKK1	74
ARK5	NUAK1	100
ASK1	MAP3K5	75
ASK2	MAP3K6	74
AURKA	AURKA	82
AURKB	AURKB	80
AURKC	AURKC	83
AXL	AXL	90
BIKE	BMP2K	84
BLK	BLK	77
BMPR1A	BMPR1A	95
BMPR1B	BMPR1B	83
BMPR2	BMPR2	89
BMX	BMX	100
BRAF	BRAF	80
BRAF(V600E)	BRAF	82
BRK	PTK6	99
BRSK1	BRSK1	98
BRSK2	BRSK2	97
BTK	BTK	80

BUB1	BUB1	72
CAMK1	CAMK1	92
CAMK1B	PNCK	77
CAMK1D	CAMK1D	89
CAMK1G	CAMK1G	100
CAMK2A	CAMK2A	85
CAMK2B	CAMK2B	95
CAMK2D	CAMK2D	97
CAMK2G	CAMK2G	83
CAMK4	CAMK4	100
CAMKK1	CAMKK1	86
CAMKK2	CAMKK2	82
CASK	CASK	90
CDC2L1	CDK11B	95
CDC2L2	CDC2L2	99
CDC2L5	CDK13	89
CDK11	CDK19	97
CDK2	CDK2	96
CDK3	CDK3	95
CDK4	CDK4	60
CDK4-cyclinD1	CDK4	76
CDK4-cyclinD3	CDK4	70
CDK5	CDK5	98
CDK7	CDK7	56
CDK8	CDK8	99
CDK9	CDK9	92
CDKL1	CDKL1	77
CDKL2	CDKL2	91
CDKL3	CDKL3	100
CDKL5	CDKL5	95
CHEK1	CHEK1	100
CHEK2	CHEK2	54
CIT	CIT	74
CLK1	CLK1	87
CLK2	CLK2	82
CLK3	CLK3	93
CLK4	CLK4	66
CSF1R	CSF1R	90
CSF1R-autoinhibited	CSF1R	93
CSK	CSK	85
CSNK1A1	CSNK1A1	74
CSNK1A1L	CSNK1A1L	86
CSNK1D	CSNK1D	92
CSNK1E	CSNK1E	98
CSNK1G1	CSNK1G1	91
CSNK1G2	CSNK1G2	92
CSNK1G3	CSNK1G3	98
CSNK2A1	CSNK2A1	25

CSNK2A2	CSNK2A2	7.3
CTK	MATK	90
DAPK1	DAPK1	99
DAPK2	DAPK2	93
DAPK3	DAPK3	95
DCAMKL1	DCLK1	85
DCAMKL2	DCLK2	100
DCAMKL3	DCLK3	46
DDR1	DDR1	100
DDR2	DDR2	100
DLK	MAP3K12	97
DMPK	DMPK	93
DMPK2	CDC42BPG	95
DRAK1	STK17A	92
DRAK2	STK17B	94
DYRK1A	DYRK1A	71
DYRK1B	DYRK1B	87
DYRK2	DYRK2	97
EGFR	EGFR	94
EGFR(E746-A750del)	EGFR	100
EGFR(G719C)	EGFR	89
EGFR(G719S)	EGFR	78
EGFR(L747-E749del, A750P)	EGFR	96
EGFR(L747-S752del, P753S)	EGFR	100
EGFR(L747-T751del,Sins)	EGFR	78
EGFR(L858R)	EGFR	98
EGFR(L858R,T790M)	EGFR	82
EGFR(L861Q)	EGFR	85
EGFR(S752-I759del)	EGFR	95
EGFR(T790M)	EGFR	62
EIF2AK1	EIF2AK1	69
EPHA1	EPHA1	83
EPHA2	EPHA2	92
EPHA3	EPHA3	91
EPHA4	EPHA4	96
EPHA5	EPHA5	92
EPHA6	EPHA6	100
EPHA7	EPHA7	100
EPHA8	EPHA8	100
EPHB1	EPHB1	100
EPHB2	EPHB2	100
EPHB3	EPHB3	97
EPHB4	EPHB4	95
EPHB6	EPHB6	66
ERBB2	ERBB2	96
ERBB3	ERBB3	93
ERBB4	ERBB4	92
ERK1	MAPK3	98

ERK2	MAPK1	93
ERK3	MAPK6	100
ERK4	MAPK4	100
ERK5	MAPK7	96
ERK8	MAPK15	41
ERN1	ERN1	73
FAK	PTK2	93
FER	FER	95
FES	FES	100
FGFR1	FGFR1	96
FGFR2	FGFR2	88
FGFR3	FGFR3	99
FGFR3(G697C)	FGFR3	83
FGFR4	FGFR4	100
FGR	FGR	88
FLT1	FLT1	93
FLT3	FLT3	93
FLT3(D835H)	FLT3	72
FLT3(D835V)	FLT3	20
FLT3(D835Y)	FLT3	50
FLT3(ITD)	FLT3	88
FLT3(ITD,D835V)	FLT3	67
FLT3(ITD,F691L)	FLT3	88
FLT3(K663Q)	FLT3	68
FLT3(N841I)	FLT3	70
FLT3(R834Q)	FLT3	97
FLT3-autoinhibited	FLT3	97
FLT4	FLT4	93
FRK	FRK	96
FYN	FYN	97
GAK	GAK	78
GCN2(Kin.Dom.2,S808G)	EIF2AK4	100
GRK1	GRK1	74
GRK2	ADRBK1	100
GRK3	ADRBK2	95
GRK4	GRK4	100
GRK7	GRK7	76
GSK3A	GSK3A	43
GSK3B	GSK3B	66
HASPIN	GSG2	81
HCK	HCK	82
HIPK1	HIPK1	61
HIPK2	HIPK2	71
HIPK3	HIPK3	62
HIPK4	HIPK4	67
HPK1	MAP4K1	100
HUNK	HUNK	92
ICK	ICK	67

IGF1R	IGF1R	90
IKK-alpha	CHUK	76
IKK-beta	IKBKB	79
IKK-epsilon	IKBKE	73
INSR	INSR	77
INSRR	INSRR	100
IRAK1	IRAK1	68
IRAK3	IRAK3	94
IRAK4	IRAK4	68
ITK	ITK	100
JAK1(JH1domain-catalytic)	JAK1	86
JAK1(JH2domain-pseudokinase)	JAK1	90
JAK2(JH1domain-catalytic)	JAK2	75
JAK3(JH1domain-catalytic)	JAK3	62
JNK1	MAPK8	61
JNK2	MAPK9	78
JNK3	MAPK10	76
KIT	KIT	93
KIT(A829P)	KIT	96
KIT(D816H)	KIT	88
KIT(D816V)	KIT	92
KIT(L576P)	KIT	89
KIT(V559D)	KIT	92
KIT(V559D,T670I)	KIT	95
KIT(V559D,V654A)	KIT	96
KIT-autoinhibited	KIT	91
LATS1	LATS1	97
LATS2	LATS2	60
LCK	LCK	78
LIMK1	LIMK1	100
LIMK2	LIMK2	97
LKB1	STK11	88
LOK	STK10	96
LRRK2	LRRK2	45
LRRK2(G2019S)	LRRK2	100
LTK	LTK	99
LYN	LYN	100
LZK	MAP3K13	74
MAK	MAK	100
MAP3K1	MAP3K1	80
MAP3K15	MAP3K15	47
MAP3K2	MAP3K2	82
MAP3K3	MAP3K3	72
MAP3K4	MAP3K4	85
MAP4K2	MAP4K2	93
MAP4K3	MAP4K3	100
MAP4K4	MAP4K4	90
MAP4K5	MAP4K5	90

MAPKAPK2	MAPKAPK2	91
MAPKAPK5	MAPKAPK5	82
MARK1	MARK1	92
MARK2	MARK2	89
MARK3	MARK3	96
MARK4	MARK4	92
MAST1	MAST1	72
MEK1	MAP2K1	78
MEK2	MAP2K2	82
MEK3	MAP2K3	88
MEK4	MAP2K4	83
MEK5	MAP2K5	71
MEK6	MAP2K6	72
MELK	MELK	85
MERTK	MERTK	82
MET	MET	97
MET(M1250T)	MET	91
MET(Y1235D)	MET	99
MINK	MINK1	89
MKK7	MAP2K7	88
MKNK1	MKNK1	98
MKNK2	MKNK2	76
MLCK	MYLK3	84
MLK1	MAP3K9	92
MLK2	MAP3K10	66
MLK3	MAP3K11	92
MRCKA	CDC42BPA	90
MRCKB	CDC42BPB	100
MST1	STK4	87
MST1R	MST1R	97
MST2	STK3	63
MST3	STK24	100
MST4	MST4	73
MTOR	MTOR	66
MUSK	MUSK	100
MYLK	MYLK	95
MYLK2	MYLK2	96
MYLK4	MYLK4	85
MYO3A	MYO3A	84
MYO3B	MYO3B	99
NDR1	STK38	91
NDR2	STK38L	89
NEK1	NEK1	84
NEK10	NEK10	80
NEK11	NEK11	86
NEK2	NEK2	87
NEK3	NEK3	87
NEK4	NEK4	87

NEK5	NEK5	100
NEK6	NEK6	94
NEK7	NEK7	92
NEK9	NEK9	89
NIK	MAP3K14	68
NIM1	MGC42105	65
NLK	NLK	96
OSR1	OXSR1	82
p38-alpha	MAPK14	93
p38-beta	MAPK11	95
p38-delta	MAPK13	100
p38-gamma	MAPK12	70
PAK1	PAK1	59
PAK2	PAK2	82
PAK3	PAK3	84
PAK4	PAK4	82
PAK6	PAK6	95
PAK7	PAK7	90
PCTK1	CDK16	90
PCTK2	CDK17	96
PCTK3	CDK18	97
PDGFRA	PDGFRA	76
PDGFRB	PDGFRB	80
PDPK1	PDPK1	83
PFCDPK1(P.falciparum)	CDPK1	28
PFPK5(P.falciparum)	MAL13P1.279	97
PFTAIRE2	CDK15	86
PFTK1	CDK14	90
PHKG1	PHKG1	100
PHKG2	PHKG2	96
PIK3C2B	PIK3C2B	76
PIK3C2G	PIK3C2G	55
PIK3CA	PIK3CA	67
PIK3CA(C420R)	PIK3CA	81
PIK3CA(E542K)	PIK3CA	70
PIK3CA(E545A)	PIK3CA	83
PIK3CA(E545K)	PIK3CA	72
PIK3CA(H1047L)	PIK3CA	78
PIK3CA(H1047Y)	PIK3CA	87
PIK3CA(I800L)	PIK3CA	46
PIK3CA(M1043I)	PIK3CA	97
PIK3CA(Q546K)	PIK3CA	81
PIK3CB	PIK3CB	80
PIK3CD	PIK3CD	32
PIK3CG	PIK3CG	37
PIK4CB	PI4KB	66
PIKFYVE	PIKFYVE	91
PIM1	PIM1	84

PIM2	PIM2	100
PIM3	PIM3	87
PIP5K1A	PIP5K1A	97
PIP5K1C	PIP5K1C	7.3
PIP5K2B	PIP4K2B	100
PIP5K2C	PIP4K2C	50
PKAC-alpha	PRKACA	100
PKAC-beta	PRKACB	91
PKMYT1	PKMYT1	93
PKN1	PKN1	77
PKN2	PKN2	87
PKNB(M.tuberculosis)	pknB	67
PLK1	PLK1	44
PLK2	PLK2	65
PLK3	PLK3	58
PLK4	PLK4	80
PRKCD	PRKCD	96
PRKCE	PRKCE	100
PRKCH	PRKCH	91
PRKCI	PRKCI	61
PRKCQ	PRKCQ	78
PRKD1	PRKD1	97
PRKD2	PRKD2	100
PRKD3	PRKD3	97
PRKG1	PRKG1	88
PRKG2	PRKG2	76
PRKR	EIF2AK2	64
PRKX	PRKX	94
PRP4	PRPF4B	100
PYK2	PTK2B	75
QSK	KIAA0999	82
RAF1	RAF1	91
RET	RET	100
RET(M918T)	RET	93
RET(V804L)	RET	90
RET(V804M)	RET	84
RIOK1	RIOK1	92
RIOK2	RIOK2	68
RIOK3	RIOK3	69
RIPK1	RIPK1	98
RIPK2	RIPK2	96
RIPK4	RIPK4	79
RIPK5	DSTYK	77
ROCK1	ROCK1	61
ROCK2	ROCK2	78
ROS1	ROS1	98
RPS6KA4(Kin.Dom.1-N-terminal)	RPS6KA4	95
RPS6KA4(Kin.Dom.2-C-terminal)	RPS6KA4	84

RPS6KA5(Kin.Dom.1-N-terminal)	RPS6KA5	90
RPS6KA5(Kin.Dom.2-C-terminal)	RPS6KA5	90
RSK1(Kin.Dom.1-N-terminal)	RPS6KA1	92
RSK1(Kin.Dom.2-C-terminal)	RPS6KA1	100
RSK2(Kin.Dom.1-N-terminal)	RPS6KA3	74
RSK2(Kin.Dom.2-C-terminal)	RPS6KA3	83
RSK3(Kin.Dom.1-N-terminal)	RPS6KA2	93
RSK3(Kin.Dom.2-C-terminal)	RPS6KA2	100
RSK4(Kin.Dom.1-N-terminal)	RPS6KA6	70
RSK4(Kin.Dom.2-C-terminal)	RPS6KA6	100
S6K1	RPS6KB1	88
SBK1	SBK1	73
SGK	SGK1	90
SgK110	SgK110	94
SGK2	SGK2	82
SGK3	SGK3	85
SIK	SIK1	94
SIK2	SIK2	100
SLK	SLK	78
SNARK	NUAK2	73
SNRK	SNRK	97
SRC	SRC	90
SRMS	SRMS	100
SRPK1	SRPK1	91
SRPK2	SRPK2	94
SRPK3	SRPK3	93
STK16	STK16	76
STK33	STK33	88
STK35	STK35	100
STK36	STK36	100
STK39	STK39	66
SYK	SYK	84
TAK1	MAP3K7	0.85
TAOK1	TAOK1	94
TAOK2	TAOK2	92
TAOK3	TAOK3	89
TBK1	TBK1	76
TEC	TEC	100
TESK1	TESK1	77
TGFBR1	TGFBR1	95
TGFBR2	TGFBR2	93
TIE1	TIE1	100
TIE2	TEK	93
TLK1	TLK1	96
TLK2	TLK2	57
TNIK	TNIK	88
TNK1	TNK1	100
TNK2	TNK2	87

TNNI3K	TNNI3K	86
TRKA	NTRK1	75
TRKB	NTRK2	79
TRKC	NTRK3	88
TRPM6	TRPM6	96
TSSK1B	TSSK1B	92
TSSK3	TSSK3	94
TTK	TTK	86
TXK	TXK	82
TYK2(JH1domain-catalytic)	TYK2	84
TYK2(JH2domain-pseudokinase)	TYK2	62
TYRO3	TYRO3	68
ULK1	ULK1	68
ULK2	ULK2	72
ULK3	ULK3	76
VEGFR2	KDR	99
VPS34	PIK3C3	81
VRK2	VRK2	92
WEE1	WEE1	81
WEE2	WEE2	87
WNK1	WNK1	72
WNK2	WNK2	89
WNK3	WNK3	77
WNK4	WNK4	98
YANK1	STK32A	54
YANK2	STK32B	95
YANK3	STK32C	99
YES	YES1	88
YSK1	STK25	78
YSK4	MAP3K19	62
ZAK	ZAK	77
ZAP70	ZAP70	89

Crystallographic Data

Crystallization. Crystals of TAK1 in complex with **22** were obtained using sitting drop vapor diffusion set-ups. TAK1 at a concentration of 9.9 mg/ml (20 mM Hepes/sodium hydroxide, 200 mM sodium chloride, 10% (*v/v*) glycerol, 5 mM DTT, pH 8.0) was pre-incubated with 2 mM (7.2-fold molar excess) of adenosine (100 mM in DMSO) for 1 h at 4°C. 0.9 µL of the protein solution was then mixed with 0.9 µL of reservoir solution (0.10 M Tris/HCl pH 7.9, 0.60 M sodium citrate, 0.60 M sodium chloride) and equilibrated at 20°C over 60 µL of reservoir solution. Well diffracting crystals grew to full size over 6 days. Crystals were soaked for 1 day with **22** (150 mM in DMSO, 2-fold diluted with reservoir solution: 1 µL of the diluted solution was directly added to the drop).

Crystals of TAK1 in complex with **54** were obtained using sitting drop vapor diffusion set-ups. TAK1 at a concentration of 9.9 mg/ml (20 mM Hepes/sodium hydroxide, 200 mM sodium chloride, 10% (*v/v*) glycerol, 5 mM DTT, pH 8.0) was pre-incubated with 2 mM (7.2-fold molar excess) of adenosine (100 mM in DMSO) for 1 h at 4°C. 1.0 µL of the protein solution was then mixed with 1.0 µL of reservoir solution (0.10 M Tris/HCl pH 7.6, 0.60 M sodium citrate, 0.30 M sodium chloride) and equilibrated at 20°C over 60 µL of reservoir solution. Well diffracting crystals grew to full size over 7 days. Crystals were soaked for 5 h with 20 nM **54** (150 mM in DMSO, diluted with reservoir solution to 40 mM. 1 µL of the diluted solution was then added to the drop containing suitable crystals).

Data Collection. Complete data sets of TAK1-**22** and TAK1-**54** crystals were collected at the ESRF synchrotron radiation source (Grenoble, FR, beamline ID30a1) (Table S1).

Table S1: Data collection statistics

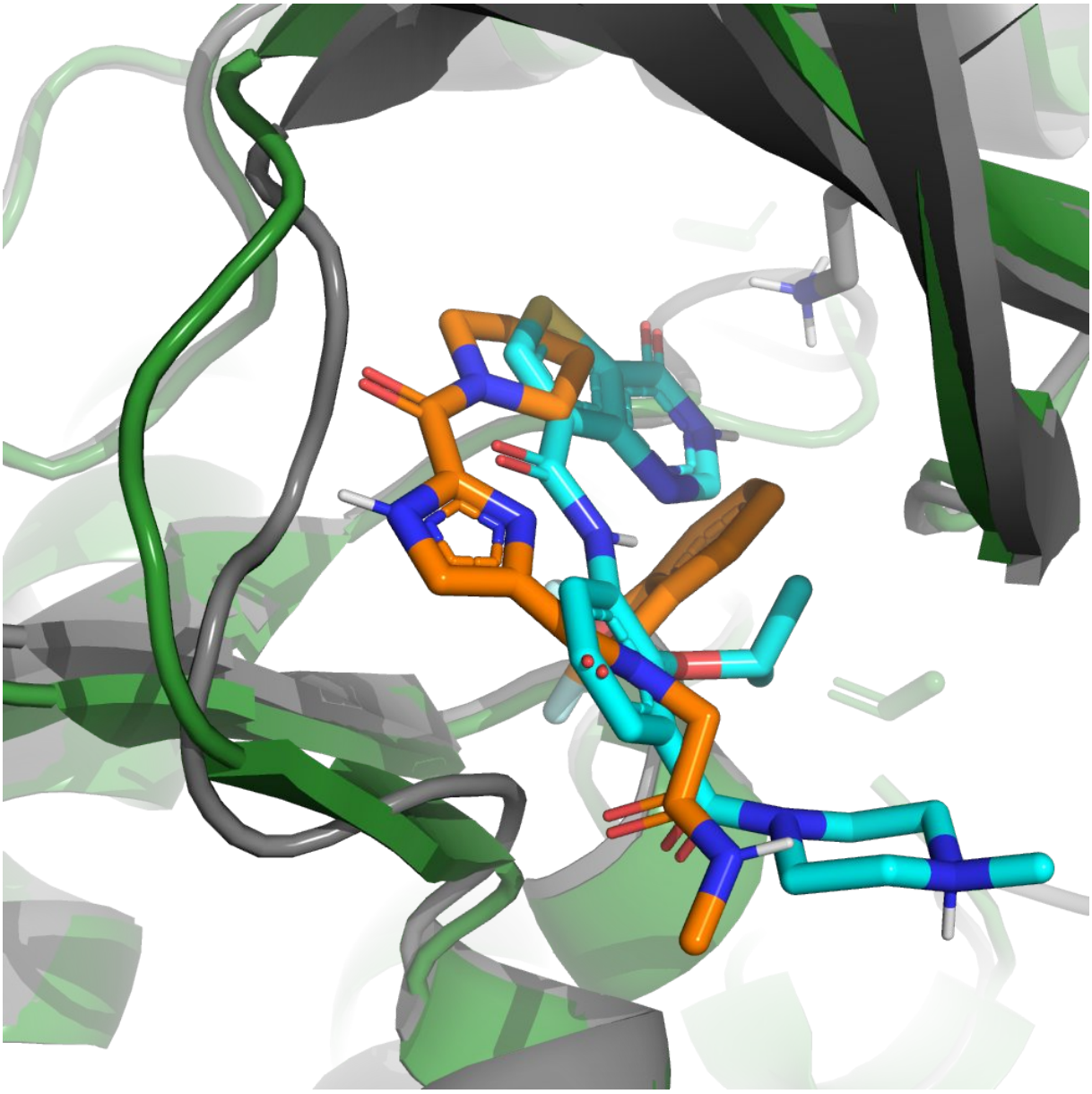
Inhibitor	**22**	**54**
Space group	I222	I222
Unit cell parameters (Å)	a=58.4, b=134.8, c=145.5 α=ß=γ=90°	a=58.4, b=134.8, c=145.5 α=ß=γ=90°
Resolution (Å)	28.6-1.98 (2.09-1.98)	29.21-1.97 (2.08-1.97)
# Unique reflections	37946 (6025)	40707 (5958)
I/σ(I)	10.9 (1.9)	7.1 (1.4)
Completeness (%)	90.5 (99.2)	99.3 (99.9)
Multiplicity	3.0 (3.1)	3.3 (3.3)
R_{meas}	0.072 (0.693)	0.100 (0.530)

Structure determination and refinement. Molecular replacement was done using a published structure of MAP3K7 (PDB accession code 5JGA) as starting model. Several rounds of alternating manual rebuilding and refinement with REFMAC5 resulted in the final model[3] (Table S2).

Table S2: Refinement statistics		
Inhibitor	**22**	**54**
Resolution (Å)	30.00-1.98 (2.03-1.98)	29.21-1.97 (2.02-1.97)
R_{work}	0.221 (0.459)	0.180 (0.329)
R_{free}	0.249 (0.439)	0.225 (0.322)
Completeness (%)	89.9 (99.0)	97.1 (99.8)
r.m.s.d. bonds (Å)	0.014	0.013
r.m.s.d. angles (°)	1.7	1.582

The coordinates of the structures **22** and **54** will be deposited in the PDB upon publication.

Supplementary Figure S-1.



Supplementary Figure S-1: Overlay of the structures of compound **22** (orange and green) with **3** (grey and cyan, PDB ID code: 5JGD). Key lysine residue depicted.

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