SUPPORTING INFORMATION

Optimization of 3-Pyrimidin-4-yl-oxazolidin-2-ones as Orally Bioavailable and Brain Penetrant Mutant IDH1 Inhibitors

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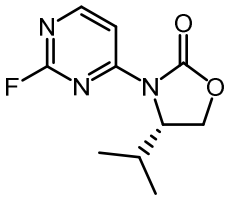
Contents

Experimental details for the synthetic procedures and characterization data. All starting materials were commercially available from Sigma Aldrich, VWR, Oakwood, Chembridge, Ryan Scientific and Anichem. All chiral ethylamines were commercially available as racemates, and were either used as such (with separation of the final compounds), separated prior to the final coupling reactions, or synthesized as pure enantiomers using Ellman chiral sulfinamides as chiral auxiliaries. Commercially available chiral building blocks were purchased as >97% ee, and assumed to have the chiral identity and purity as claimed.

Experimental details for the synthetic procedures and characterization data of 4 – 19

Synthesis of starting materials

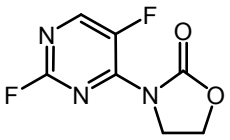
(*S*)-3-(2-Fluoropyrimidin-4-yl)-4-isopropyloxazolidin-2-one (SI-1)



(General procedure A)

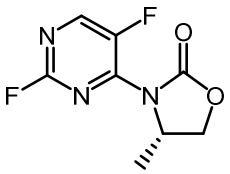
A solution of 2,4-difluoropyrimidine (3.5 mL, 41 mmol) and (*S*)-4-isopropyloxazolidin-2-one (5.3 g, 41 mmol) in DMF (30 mL) was cooled to 0 °C under an atmosphere of nitrogen. Sodium hydride (2.1 g of 60% suspension, 53 mmol) was added in portions. An exothermic reaction with bubbling was observed. The internal temperature was kept below 5 °C. After 5 minutes, the cold bath was removed and the reaction mixture (a sandy suspension) was allowed to warm to rt and stirred for 18 h. The reaction mixture was then diluted with water (100 mL) and extracted with EtOAc (3 x 75 mL). The combined organic layers were washed with water (50mL), brine (50mL), dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/heptane) to give **SI-1** (3.1 g, 33%) as a crystalline white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.50 (dd, *J* = 5.8, 2.2 Hz, 1 H), 8.19 (dd, *J* = 5.8, 3.8 Hz, 1 H), 4.79 (dt, *J* = 8.1, 3.5 Hz, 1 H), 4.48 – 4.34 (m, 2 H), 2.64 (heptd, *J* = 7.0, 3.6 Hz, 1 H), 1.01 (d, *J* = 7.0 Hz, 3 H), 0.90 (d, *J* = 6.9 Hz, 3 H). MS m/z $(M + H)^+$ $C_{10}H_{13}FN_3O_2$: measured 226.1, calcd 226.1.

3-(2,5-Difluoropyrimidin-4-yl)oxazolidin-2-one (SI-2)



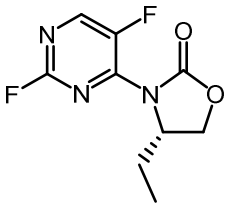
Synthesized by the method of general procedure A, using 2,4,5-trifluoropyrimidine and oxazolidin-2-one. MS m/z $(M + H)^+$ $C_7H_6F_2N_3O_2$: measured 202.4, calcd 202.0. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.42 (dd, J = 2.3, 0.9 Hz, 1H), 4.60 (t, J = 7.6 Hz, 2H), 4.23 (t, J = 7.6 Hz, 2H).

(*S*)-3-(2,5-Difluoropyrimidin-4-yl)-4-methyloxazolidin-2-one (SI-3)



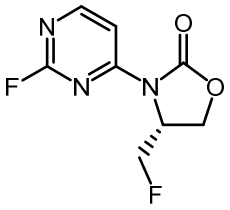
Synthesized by the method of general procedure A, using 2,4,5-trifluoropyrimidine and (*S*)-4-methyloxazolidin-2-one. MS m/z $(M + H)^+$ $C_8H_8F_2N_3O_2$: measured 216.0, calcd 216.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.45 (dd, J = 2.2, 0.9 Hz, 1H), 4.83 (tq, J = 8.2, 6.1 Hz, 1H), 4.67 (dd, J = 8.5, 8.0 Hz, 1H), 4.13 (t, J = 8.5 Hz, 1H), 1.47 (d, J = 6.1 Hz, 3H).

(*S*)-3-(2,5-Difluoropyrimidin-4-yl)-4-ethyloxazolidin-2-one (SI-4)



Synthesized by the method of general procedure A, using 2,4,5-trifluoropyrimidine and (*S*)-4-ethyloxazolidin-2-one. MS m/z $(M + H)^+$ $C_9H_{10}F_2N_3O_2$: measured 230.1, calcd 230.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (dd, J = 2.1, 0.9 Hz, 1H), 4.74 (qd, J = 8.1, 3.2 Hz, 1H), 4.64 (t, J = 8.4 Hz, 1H), 4.24 (dd, J = 8.5, 7.7 Hz, 1H), 1.98 (dqd, J = 13.8, 7.6, 3.2 Hz, 1H), 1.79 – 1.66 (m, 1H), 0.93 (t, J = 7.5 Hz, 3H).

(*R*)-4-(Fluoromethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one (26)



(General procedure B)

Step 1: Imidazole (1.74 g, 25.6 mmol) was added to a solution of (*S*)-4-(hydroxymethyl)oxazolidin-2-one (1.5 g, 12.8 mmol) and *tert*-butylchlorodiphenylsilane (3.95 mL, 15.4 mmol) in DCM (43 mL) at room temperature. A white precipitate formed. The mixture was stirred at room temperature for 16 hours and then diluted with water (50 mL). The layers were separated and the aqueous layer was extracted with DCM (50 mL). The combined organic extracts were washed with saturated aqueous sodium chloride (50 mL), dried over Na_2SO_4, filtered and concentrated. Silica gel chromatography (EtOAc/Heptane) provided (*R*)-4-(((tertbutyldiphenylsilyl)oxy)methyl)oxazolidin-2-one (3.55 g, colorless oil) in 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.64 (m, 4 H), 7.50 – 7.38 (m, 6 H), 5.31 (m, 1 H), 4.44

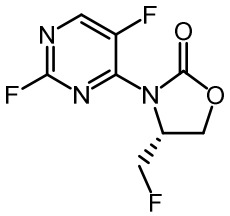
(t, *J* = 8.7 Hz, 1 H), 4.15 (m, 1 H), 4.00 (m, 1 H), 3.65 (d, *J* = 5.5 Hz, 2 H), 1.07 (s, 9 H). MS m/z 356.1 $(M + H)^+$ $C_{20}H_{26}NO_3Si$: measured 356.1, calcd 356.2.

Step 2: Sodium hydride (34 mg, 0.84 mmol) was added to a solution of (*R*)-4-(((tertbutyldiphenylsilyl)oxy)methyl)oxazolidin-2-one (200 mg, 0.563 mmol) in DMF (2.8 mL). The mixture was stirred at room temperature for 20 minutes and then 2,4-difluoropyrimidine (0.072 mL, 0.84 mmol) was added (bubbling). The yellow suspension was stirred for 10 minutes and the reaction was then carefully quenched with saturated aqueous ammonium chloride (5 mL). Water (20 mL) was added and the mixture was extracted with ethyl acetate (2 x 25 mL). The combined organic extracts were washed with saturated aqueous sodium chloride (20 mL), dried over Na_2SO_4, filtered and concentrated. Silica gel chromatography (EtOAc/Heptane) provided (*R*)-4-(((tertbutyldiphenylsilyl)oxy)methyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one (0.18 g, colorless oil) in 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.44 (dd, *J* = 5.8, 2.2 Hz, 1 H), 8.12 (dd, *J* = 5.8, 3.4 Hz, 1 H), 7.58 (m, 2 H), 7.48 – 7.32 (m, 6 H), 7.23 (m, 2 H), 4.80 (m, 1 H), 4.65 (dd, *J* = 8.6, 3.0 Hz, 1 H), 4.55 (m, 1 H), 4.18 (dd, *J* = 11.1, 3.5 Hz, 1 H), 3.83 (dd, *J* = 11.1, 2.1 Hz, 1 H), 1.04 (s, 9 H). MS m/z $(M + H)^+$ $C_{24}H_{27}FN_3O_3Si$: measured 452.3, calcd 452.2.

Step 3: A solution of tetrabutylammonium fluoride (1.0 M in THF, 0.44 mL, 0.44 mmol) was added to a solution of (R)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one (180 mg, 0.40 mmol) in THF (4 mL) at room temperature. The solution was stirred for 1 hour and then diluted with saturated aqueous sodium chloride (30 mL). The mixture was extracted with ethyl acetate (2 x 30 mL) and the combined extracts were dried over Na_2SO_4, filtered and concentrated. Silica gel chromatography (EtOAc/Heptane) provided (*S*)-3-(2-fluoropyrimidin-4-yl)-4-(hydroxymethyl)oxazolidin-2-one (0.051 g, white solid) in 60% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.51 (dd, *J* = 5.8, 2.1 Hz, 1H), 8.19 (dd, *J* = 5.8, 3.8 Hz, 1H), 4.86 (dq, *J* = 8.1, 4.0 Hz, 1H), 4.55 (m, 2H), 4.09 (m, 1H), 3.96 (m, 1H), 2.18 (t, *J* = 5.4 Hz, 1H). MS m/z $(M + H)^+$ $C_8H_9FN_3O_3$: measured 214.0, calcd 214.1.

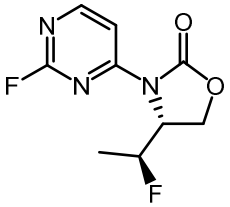
Step 4: Perfluorobutanesulfonyl fluoride (0.18 mL, 0.98 mmol) was added to a solution of (*S*)-3-(2-fluoropyrimidin-4-yl)-4-(hydroxymethyl)oxazolidin-2-one (52 mg, 0.24 mmol) in THF (1.2 mL) at room temperature. Triethylamine trihydrofluoride (0.16 mL, 0.98 mmol) and triethylamine (0.41 mL, 3.0 mmol) were then added and the solution was stirred at 40 °C for 18 hours. The reaction was then cooled to room temperature and diluted with water (25 mL). The mixture was extracted with ethyl acetate (2 x 25 mL). The combined organic extracts were washed with saturated aqueous sodium chloride (10 mL), dried over Na_2SO_4, filtered and concentrated. Silica gel chromatography (EtOAc/Heptane) provided (*R*)-4-(fluoromethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin- 2-one (**26**) (0.021 g,colorless oil) in 40% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.53 (dd, *J* = 5.7, 2.1 Hz, 1H), 8.18 (dd, *J* = 5.7, 3.7 Hz, 1H), 5.08 – 4.93 (m, 2H), 4.91 – 4.72 (m, 1H), 4.66 – 4.55 (m, 2H). MS m/z $(M + H)^+$ $C_8H_8F_2N_3O_2$: measured 215.9, calcd 216.1.

(R)-3-(2,5-Difluoropyrimidin-4-yl)-4-(fluoromethyl)oxazolidin-2-one (SI-5)



Synthesized by the method of general procedure B, using 2,4,5-trifluoropyrimidine in step 2. MS m/z $(M + H)^+$ $C_8H_7F_3N_3O_2$: measured 234.2, calcd 234.0. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.49 (s, 1 H), 4.84 - 5.01 (m, 2 H), 4.54 - 4.80 (m, 3 H).

(*R*)-4-((S)-1-Fluoroethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one (SI-6)



(General Procedure C)

Step 1: *N*-Methylmorpholine (124 mg, 1.2 mmol) was added to a solution of dicyclohexylamine (2*S*,3*R*)-2-(((benzyloxy)carbonyl)amino)-3-(tert-butoxy)butanoate (500 mg, 1.0 mmol) in 10 ml of THF and isobutyl chloroformate (167 mg, 1.2 mmol, 1.2 equiv) at -25 °C. The mixture was stirred at the same temperature for 10 min and then filtered. The filtrate was cooled to -20 ^{o}C and $NaBH_4$ was added, followed immediately by 2 ml of water. The reaction mixture was stirred at the same temperature for 5 min. then gradually warmed to room temperature over 25 min. The reaction was poured into water (10 mL) and extracted with ethyl acetate (2x20ml). The combined organic phases were washed with water, brine and dried over Na_2SO_4. The solvent was removed in vacuo to yield benzyl ((2*R*)-(3*R*)-3-(tert-butoxy)-1-hydroxybutan-2-yl)carbamate as a colorless oil. The material was used in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.37 – 7.16 (m, 5 H), 5.25 (d, *J* = 8.0 Hz, 1 H), 5.02 (s, 1 H), 4.04 (ddd, *J* = 12.0, 8.9, 2.8 Hz, 1 H), 3.92 – 3.75 (m, 1 H), 3.59 (dddd, *J* = 32.3, 14.6, 8.2, 4.3 Hz, 2 H), 1.10 (s, 9 H), 1.09 – 1.06 (m, 3 H).

Step 2: To a solution of benzyl ((2*R*,3*R*)-3-(tert-butoxy)-1-hydroxybutan-2-yl)carbamate (5.88 g, 19.9 mmol) in DMF (100 mL) was added NaH (60% in mineral oil, 1.62 g, 40.6 mmol) at 0 °C. The reaction mixture was stirred for 30 min at 0 °C. To the reaction mixture were added 4-methoxybenzyl chloride (4.07 mL, 29.9 mmol) and tetrabutylammonium iodide (0.74 g, 1.99 mmol) and the resulting mixture was warmed to room temperature and stirred for 15.5 h. The reaction mixture was poured into ice water (200 mL) forming a white suspension. Ethyl acetate (100 mL) was added and the resulting mixture was stirred for 5 min to form a clear two-layered solution. After separation, the aqueous phase was extracted with EtOAc (100 mL x 3). The combined organic layers were washed with brine (80 mL), dried over Na_2SO_4, filtered and concentrated. Silica gel chromatography (EtOAc/Heptane) gave (*R*)-4-((*R*)-1-(*tert*-butoxy)ethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (5.90 g, 96%) containing minor impurities. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.38 - 7.29 (m, 2 H), 6.99 - 6.93 (m, 2 H), 4.68 - 4.58 (m, 1

H), 4.33 (dd, J = 9.3, 4.5 Hz, 1 H), 4.27 - 4.17 (m, 2 H), 3.89 (dd, J = 6.4, 4.8 Hz, 1 H), 3.81 (s, 3 H), 3.65 (dd, J = 9.0, 4.6 Hz, 1 H), 1.09 (s, 9 H), 1.02 (d, J = 6.3 Hz, 3 H). MS m/z $(M + H)^+$ $C_{17}H_{26}NO_4$: measured 308.2, calcd 308.2.

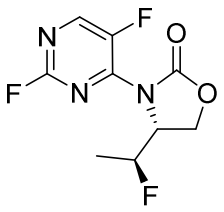
Step 3: A solution of (*R*)-4-((*R*)-1-(tert-butoxy)ethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (5.90 g, 19.2 mmol) in DCM (40 mL) was treated with TFA (40 mL) at room temperature for 20 min. The reaction mixture was then concentrated in vacuo. The residue was taken up in DCM and concentrated (3 times) to remove excess TFA. The residue was then purified by silica gel chromatography (EtOAc/Heptane) to give (*R*)-4-((*R*)-1-hydroxyethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (3.81 g, 79%). ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.31 - 7.21 (m, 2 H), 7.00 - 6.87 (m, 2 H), 4.67 (d, *J* = 15.0 Hz, 1 H), 4.34 - 4.18 (m, 3 H), 3.95 (q, *J* = 6.2 Hz, 1 H), 3.80 (s, 3 H), 3.68 (dt, *J* = 8.7, 5.5 Hz, 1 H), 1.09 (d, *J* = 6.4 Hz, 3 H). MS m/z $(M + H)^+$ $C_{13}H_{18}NO_4$: measured 252.2, calcd 252.1.

Step 4: Triethylamine (11.4 mL, 82 mmol), perfluoro-1-butanesulfonyl fluoride (4.9 mL, 27.3 mmol), and triethylamine trihydrofluoride (4.5 mL, 27.6 mmol) were added sequentially to a solution of (*R*)-4-((*R*)-1-hydroxyethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (2.27 g, 9.04 mmol) in MeCN (30 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 70 min. The reaction mixture was then diluted with water (60 mL) and extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with water (70 mL), brine (70 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/heptane) to give (*R*)-4-((*S*)-1-fluoroethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (2.19 g, 96%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.25 - 7.20 (m, 2 H), 6.92 - 6.83 (m, 2 H), 4.87 (d, *J* = 15.1 Hz, 1 H), 4.75 (dqd, *J* = 47.6, 6.6, 2.1 Hz, 1 H), 4.26 (td, *J* = 9.2, 1.4 Hz, 1 H), 4.17 - 4.05 (m, 2 H), 3.81 (s, 3 H), 3.71 (dddd, *J* = 19.8, 9.5, 5.8, 2.1 Hz, 1 H), 1.29 (dd, *J* = 23.1, 6.2 Hz, 3 H). MS m/z $(M + H)^+$ $C_{13}H_{17}FNO_3$: measured 254.5, calcd 254.1.

Step 5: A solution of (*R*)-4-((*S*)-1-fluoroethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (1.98 g 7.8 mmol) in TFA (40 mL) was heated at 65 °C for 16 h. The reaction mixture was then concentrated in vacuo to remove TFA. Silica gel chromatography (EtOAc/DCM) gave (R)-4-((S)-1-fluoroethyl)-oxazolidin-2- one as a pale brown solid (0.91 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 5.60 (br s, 1 H), 4.72 - 4.54 (m, 1 H), 4.51 (td, *J* = 8.9, 0.9 Hz, 1 H), 4.32 (dd, *J* = 9.2, 4.8 Hz, 1 H), 4.02 - 3.88 (m, 1 H), 1.38 (dd, *J* = 24.0, 6.3 Hz, 3 H).

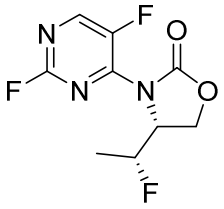
Step 6: Sodium hydride (60% in mineral oil, 66 mg, 1.7 mmol) was added to a solution of 2,4-difluoropyrimidine (160 mg, 1.375 mmol) and (*R*)-4-((*S*)-1- fluoroethyl)oxazolidin-2-one (183 mg, 1.38 mmol) in DMF (4.6 mL) at 0 °C. The resulting mixture was stirred at 0 °C for 30 min and at room temperature for 2 hr. The reaction was then quenched with brine (1 ml) and diluted with EtOAc (20 ml) and water (10 ml). The layers were separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/Heptane) to give (*R*)-4-((*S*)-1-fluoroethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one (**SI-6**) as a white solid (210 mg, 67%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.51 (dd, J = 5.8, 2.1 Hz, 1 H), 8.20 (dd, J = 5.7, 3.7 Hz, 1 H), 5.33 (dqd, J = 49.5, 6.6, 1.3 Hz, 1 H), 4.77 (dddd, J = 26.5, 9.1, 3.4, 1.4 Hz, 1 H), 4.65 (dd, J = 9.0, 3.4 Hz, 1 H), 4.50 (td, J = 9.0, 1.3 Hz, 1 H), 1.43 (dd, J = 23.1, 6.6 Hz, 3 H). MS m/z $(M + H)^+$ $C_9H_{10}F_2N_3O_2$: measured 230.1, calcd 230.1.

(*R*)-3-(2,5-Difluoropyrimidin-4-yl)-4-((*S*)-1-fluoroethyl)oxazolidin-2-one (SI-7)



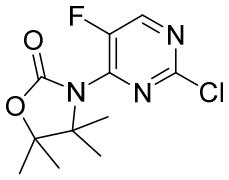
Synthesized by the method of general procedure C, using 2,4,5-trifluoropyrimidine in step 6. MS m/z $(M + H)^+$ $C_9H_9F_3N_3O_2$: measured 248.0, calcd 248.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.50 (s, 1 H), 5.00 - 5.25 (m, 1 H), 4.75 - 4.92 (m, 1 H), 4.54 - 4.68 (m, 2 H), 1.39 (dd, J=23.1, 7.0 Hz, 3 H).

(*R*)-3-(2,5-Difluoropyrimidin-4-yl)-4-((*R*)-1-fluoroethyl)oxazolidin-2-one (SI-8)



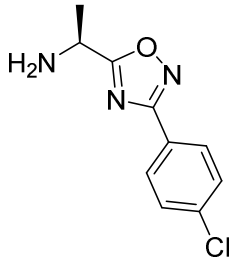
Synthesized by the method of general procedure C, using *N*-((benzyloxy)carbonyl)-O-(*tert*-butyl)-*L*-allothreonine in step 1 and 2,4,5-trifluoropyrimidine in step 6. MS m/z $(M + H)^+$ $C_9H_9F_3N_3O_2$: measured 248.0, calcd 248.1. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.50 (s, 1 H), 5.02 - 5.25 (m, 1 H), 4.90 - 5.02 (m, 1 H), 4.63 (t, J=8.80 Hz, 1 H), 4.48 (dd, J=9.39, 4.70 Hz, 1 H), 1.37 (dd, J=24.26, 6.26 Hz, 3 H).

3-(2-Chloro-5-fluoropyrimidin-4-yl)-4,4,5,5-tetramethyloxazolidin-2-one (SI-9)



Synthesized by the method of general procedure A using 4,4,5,5-tetramethyloxazolidin-2-one and 2,4-dichloro-5-fluoropyrimidine. MS m/z $(M + H)^+$ $C_{11}H_{14}ClFN_3O_2$: measured 274.2, calcd 274.1.

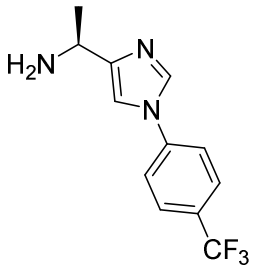
(*S*)-1-(3-(4-Chlorophenyl)-1,2,4-oxadiazol-5-yl)ethan-1-amine (SI-10)



Step 1: A solution of 4-chloro-N'-hydroxybenzimidamide (1.24 g, 7.27 mmol), (*S*)-2-(tert-butoxycarbonylamino)propanoic acid (1.38 g, 7.27 mmol), and DCC (1.65 g, 8.00 mmol) in 1,4-dioxane (73 mL) was heated at 100 °C for 18 hours. The reaction was then cooled to room temperature and concentrated in vacuo. Silica gel chromatography (EtOAc/Heptane) provided (*S*)-tert-butyl 1-(3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl)ethylcarbamate (1.13 g, white solid) in 48% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 8.8 Hz, 2 H), 7.47 (d, J = 8.8 Hz, 2 H), 5.18 (m, 1 H), 1.64 (d, J = 6.8 Hz, 3 H), 1.47 (s, 9 H).

Step 2: 2,2,2-Trifluoroacetic acid (4 mL, 52 mmol) was added to a solution of (*S*)-tert-butyl 1-(3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl)ethylcarbamate (0.613 g, 1.89 mmol) in DCM (10 mL) at room temperature. The solution was stirred at room temperature for 1 hour and then concentrated in vacuo. The residue was dissolved in chloroform (100 mL) and washed with saturated aqueous sodium bicarbonate (100 mL). The layers were separated and the aqueous layer was extracted with chloroform (3 x 30 mL) and the combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated in vacuo to give (*S*)-1-(3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl)ethanamine (500 mg, yellow oil). The material was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.7 Hz, 2 H), 7.47 (d, *J* = 8.6 Hz, 2 H), 4.37 (q, *J* = 6.9 Hz, 1 H), 1.62 (d, *J* = 6.9 Hz, 3 H). MS m/z $(M + H)^+$ $C_{10}H_{11}ClN_3O$: measured 224.0, calcd 224.1.

(*S*)-1-(1-(4-(Trifluoromethyl)phenyl)-1*H*-imidazol-4-yl)ethan-1-amine (22)



(General Procedure D)

Step 1: (*S*)-2-methylpropane-2-sulfinamide (32.1 g, 264 mmol) was added to a solution of tetraethoxytitanium (65.8 g, 288 mmol) and *tert*-butyl 4-formyl-1*H*-imidazole-1-carboxylate (47 g, 240 mmol) in THF (500 mL). The reaction was heated to 60 °C for 4 hours. The reaction mixture was then cooled to room temperature and diluted with EtOAc (400 mL) and brine (400 mL). The phases were separated and the aqueous layer was extracted with EtOAc (3 x 300 mL). The organic layers were

combined, dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography (heptane/EtOAc) to give *tert*-butyl (*S,E*)-4-(((tert-butylsulfinyl)imino)methyl)-1*H*-imidazole-1-carboxylate (53 g, 73.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.52 (s, 1 H), 8.13 (s, *1* H), 7.85(s, 1 H), 1.64 (s, 9 H), 1.25 (s, 9 H). MS m/z $(M + H)^+$ $C_{13}H_{22}N_3O_3S$: measured 300.2, calcd 300.1.

Step 2: To (*S,E*)-tert-butyl 4-(((tert-butylsulfinyl)imino)methyl)-1*H*-imidazole-1-carboxylate (30 g, 100 mmol) in DCM (300 mL) at -70 °C (IPA with dry ice) was added dropwise methylmagnesium bromide (50.1 mL, 150 mmol) in Et_2O. The reaction was stirred at -70 °C for 4 hours. The reaction mixture was then warmed to -40 °C and stirred for one hour. The reaction was then quenched by careful addition of HCl (1N). The cold bath was removed and with stirring the aqueous layer was adjusted to pH=8. The aqueous layer was separated and extracted with DCM (3 x 300 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to give crude product as a mixture of the desired and the de-Boc product (about 1:1), taken to the next step without further purification.

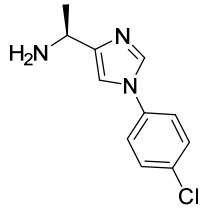
Step 3: To the above crude material in DCM (300 mL) at 0 °C was added formic acid (300 mL). The cold bath was then removed and the reaction was stirred for 3 hours. The reaction mixture was then concentrated under reduced pressure to remove DCM and formic acid. The residue was diluted with DCM (500 mL) and washed with saturated Na_2CO_3 aqueous solution (2 x 300 mL). The combined aqueous layers were extracted with DCM (3 x 500 mL). The combined organic layers were then dried (Na_2SO_4) and concentrated in vacuo to give crude (*S*)-*N*-((*S*)-1-(1*H*-imidazol-4-yl)ethyl)-2-methylpropane-2-sulfinamide (18.5 g, 86%). MS m/z $(M + H)^+$ $C_9H_{18}N_3OS$: measured 216.2, calcd 216.1.

Step 4: To a round bottomed flask was added toluene/dioxane (100mL/25mL). The flask was cooled to 0 °C and the mixture of solvents was evacuated under high vacuum for 2 minutes and then recharged with argon. The process was repeated three more times. This solvent was then used for the reaction.
A vial containing di-*tert*-butyl(2',4',6'-triisopropyl-3,4,5,6-tetramethyl-[1,1'-biphenyl]-2-yl)phosphine (1.34 g, 2.79 mmol) and $Pd_2(dba)_3$ (1.021 g, 1.115 mmol) was evacuated under high vacuum for 1 minute and then recharged with argon. The process was repeated three more times. To the vial under argon was added the toluene/dioxane solvent (10 mL) prepared as above. The reaction mixture was sealed and heated to 120 °C and stirred for 5 minutes. The reaction was cooled to room temperature.
A separate 250 mL flask was charged with (*S*)-*N*-((*S*)-1-(1*H*-imidazol-4-yl)ethyl)-2-methylpropane-2-sulfinamide (6 g, 27.9 mmol), 1-bromo-4-(trifluoromethyl)benzene (8.15 g, 36.2 mmol) and K_3PO_4 (13.0 g, 61.3 mmol). The vial was evacuated under high vacuum for 1 minute and then recharged with argon. The process was repeated three more times and the toluene/dioxane solvent (110 mL) prepared as above was added followed by the palladium/ligand complex prepared as above. The reaction was heated at 120 °C with a condenser under argon for 18 hours. The reaction mixture was then cooled to room temperature and filtered through a pad of celite. The solid was rinsed with EtOAc (200 mL). The combined filtrate was then concentrated in vacuo. The residue was purified by silica gel chromatography (EtOAc/Heptane 70%-100% with 5% MeOH) to give intermediate product.

To the above intermediate product was added MeOH (100 mL) and HCl (4M in dioxane, 14 mL). The reaction mixture was stirred at rt for one hour. The mixture was then concentrated in vacuo to give (*S*)-1-(1-(4-(trifluoromethyl)phenyl)-1H-imidazol-4-yl)ethan-1-amine (6.56 g, 81%). ^{1}H NMR (400 MHz,

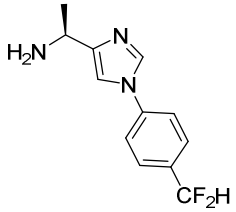
CD_3OD) δ ppm 9.43 - 9.52 (m, 1 H), 8.23 - 8.30 (m, 1 H), 7.98 (d, J=2.0 Hz, 4 H), 4.78 (q, J=6.9 Hz, 1 H), 1.80 (d, J=7.0 Hz, 3 H), missing protons attributed to deuterium exchange with solvent. MS m/z $(M + H)^+$ $C_{12}H_{13}F_3N_3$: measured 256.2, calcd 256.1.

(*S*)-1-(1-(4-Chlorophenyl)-1H-imidazol-4-yl)ethan-1-amine (SI-11)



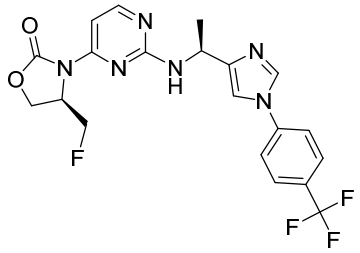
Synthesized by the method of general procedure D. ^1H NMR (400 MHz, D_2O) δ ppm 9.04 (s, 1 H), 8.00 (s, 1 H), 7.61 (q, *J* = 9.00 Hz, 4 H), 4.76-4.85 (m, 1 H), 1.74 (d, *J* = 6.65 Hz, 3 H), missing protons attributed to deuterium exchange with solvent. MS m/z $(M + H)^+$ $C_{11}H_{13}ClN_3$: measured 222.2, calcd 222.1.

(*S*)-1-(1-(4-(Difluoromethyl)phenyl)-1H-imidazol-4-yl)ethan-1-amine (SI-12)



Synthesized by the method of general procedure D. MS m/z $(M + H)^+$ $C_{12}H_{14}F_2N_3$: measured 238.2, calcd 238.1.

(*R*)-4-(Fluoromethyl)-3-(2-(((*S*)-1-(1-(4-(trifluoromethyl)phenyl)-1*H*-imidazol-4-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one (19)



(General Procedure E)

To a solution (*S*)-1-(1-(4-(trifluoromethyl)phenyl)-1*H*-imidazol-4-yl)ethan-1-amine (4.00 g, 13.70 mmol) and (*R*)-4-(fluoromethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one (2.68 g, 12.5 mmol) in DMSO (20 mL) was added Huenig's Base (6.5 mL, 37.4 mmol). The reaction was heated at

90 °C for 4 hours. The reaction mixture was then cooled to room temperature and DMSO was removed under reduced pressure with an 80 °C bath temperature. The residue was purified by silica gel chromatography (EtOAc/heptane with 5% MeOH) to give **19**. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.22 (d, *J* = 5.8 Hz, 1 H), 7.86 (d, *J* = 1.4 Hz, 1 H), 7.75 (d, *J* = 8.4 Hz, 2 H), 7.45 - 7.53 (m, 3 H), 7.19 (s, 1 H), 5.16 (br s, 1 H), 4.87 - 5.02 (m, 1 H), 4.42 - 4.67 (m, 4 H), 1.64 (d, *J* = 6.8 Hz, 3 H), missing proton attributed to deuterium exchange with solvent. HRMS m/z $(M + H)^+$ $C_{20}H_{19}N_6O_2F_4$: measured 451.1511, calcd 451.1506.

Table SI-1. Chemical name, NMR chemical shifts and LCMS signal for each compound synthesized by the same method as described for compound **19**.

Name	**^{1}H NMR**	**HRMS**
4: (*S*)-3-(2-(((*S*)-1-(3-(2-chlorophenyl)-1,2,4-oxadiazol-5-yl)ethyl)amino)pyrimidin-4-yl)-4-isopropyloxazolidin-2-one	(400 MHz, CD_3OD) δ ppm 8.28-8.18 (m, 1 H), 7.88 (d, *J* = 7.0 Hz, 1 H), 7.71-7.41 (m, 4 H), 5.46-5.32 (m, 1 H), 4.81-4.62 (m, 1 H), 4.42-4.22 (m, 2 H), 1.75 (d, *J* = 7 Hz, 3 H), 0.92-0.55 (m, 6 H)	m/z $(M + H)^+$ $C_{20}H_{22}ClN_6O_3$: measured 429.1450, calcd 429.1442.
5: (*S*)-3-(2-(((*S*)-1-(3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl)ethyl)amino)pyrimidin-4-yl)-4-isopropyloxazolidin-2-one	(400 MHz, CD_3OD) δ ppm 8.26-8.16 (m, 1 H), 8.02 (d, *J* = 7.0 Hz, 2 H), 7.61-7.45 (m, 3 H), 5.46-5.32 (m, 1 H), 4.81-4.62 (m, 1 H), 4.41-4.20 (m, 2 H), 1.77 (d, *J* = 7 Hz, 3 H), 0.95-0.51 (m, 6 H)	m/z $(M + H)^+$ $C_{20}H_{22}ClN_6O_3$: measured 429.1448, calcd 429.1442.
6: (*S*)-3-(2-((1-(3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl)ethyl)amino)-5-fluoropyrimidin-4-yl)-4,4,5,5-tetramethyloxazolidin-2-one	(400 MHz, CD_3OD) δ ppm 8.22 (s, 1 H), 8.06-7.96 (m, 2 H), 7.62-7.42 (m, 2 H), 5.4-5.2 (m, 1 H), 1.72 (d, *J* = 7 Hz, 3 H), 1.41 (s, 3 H), 1.38 (s, 3 H), 1.35 (s, 3 H), 1.25 (s, 3 H)	m/z $(M + H)^+$ $C_{21}H_{23}ClFN_6O_3$: measured 461.1511, calcd 461.1504.
7: 3-(2-((1 fluorophenoxy)pyrimidin-2-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one	(400 MHz, $CDCl_3$) δ ppm 8.52-8.35 (m, 2 H), 8.21 (d, *J* = 7.0 Hz, 1 H), 7.51 (d, *J* = 7.0 Hz, 1 H), 7.23-7.0 (m, 4 H), 6.2 (s, 1 H), 5.49-5.32 (m, 1 H), 4.61-4.42 (m, 2 H), 4.35-4.02 (m, 2 H), 1.62 (d, *J* = 6.7 Hz, 3 H)	m/z $(M + H)^+$ $C_{19}H_{18}FN_6O_3$: measured 397.1424, calcd 397.1424.
8: (*S*)-3-(2-((*S*)-1 fluorophenoxy)pyrimidin-2-yl)ethylamino)pyrimidin-4-isopropyloxazolidin-2-one	(400 MHz, $CDCl_3$) δ ppm 8.42 (s, 2 H), 8.21 (d, *J* = 5.8 Hz, 1 H), 7.49 (d, *J* = 5.7 Hz, 1 H), 7.18 – 6.99 (m, 4 H), 6.18 (br s, 1 H), 5.28 (br s, 1 H), 4.75 (dt, *J* = 8.2, 3.4 Hz, 1 H), 4.39 – 4.25 (m, 2 H), 2.34 (br s, 1 H), 1.65 – 1.59 (m, 3 H), 0.95 – 0.86 (d, *J* = 6.9 Hz, 3	m/z $(M + H)^+$ $C_{22}H_{24}FN_6O_3$: measured 439.1897, calcd 439.1894.

	H), 0.82 (d, *J* = 6.9 Hz, 3 H).	
9: (*S*)-3-(2-((1-(1-(4-chlorophenyl)-1*H*-imidazol-4-yl)ethyl)amino)-5-fluoropyrimidin-4-yl)oxazolidin-2-one	(400 MHz, CD_3OD) δ ppm 9.36 (d, *J* = 1.56 Hz, 1 H), 8.25 (d, *J* = 3.52 Hz, 1 H), 7.83 - 8.08 (m, 1 H), 7.57 - 7.78 (m, 4 H), 5.24 (q, *J*=6.78 Hz, 1 H), 4.43 - 4.64 (m, 2 H), 4.03 - 4.29 (m, 2 H), 1.70 (d, *J* = 7.04 Hz, 3 H)	m/z $(M + H)^+$ $C_{18}H_{17}ClFN_6O_2$: measured 403.1087, calcd 403.1086.
10: (*S*)-3-(2-(((*S*)-1-(1-(4-chlorophenyl)-1*H*-imidazol-4-yl)ethyl)amino)-5-fluoropyrimidin-4-yl)-4-methyloxazolidin-2-one	(400 MHz, CD_3OD) δ ppm 9.38 (d, *J* = 1.5 Hz, 1 H), 8.31 (d, *J* = 3.5 Hz, 1 H), 7.8 - 8.0 (m, 1 H), 7.6 - 7.8 (m, 4 H), 5.22 (m, 1 H), 4.61 - 4.82 (m, 2 H), 4.03 - 4.2 (m, 1 H), 1.70 (d, *J* = 7.0 Hz, 3 H), 1.26 (d, *J* = 7.0 Hz, 3 H)	m/z $(M + H)^+$ $C_{19}H_{19}ClFN_6O_2$: measured 417.1245, calcd 417.1242.
11: (*S*)-3-(2-(((*S*)-1-(1-(4-chlorophenyl)-1*H*-imidazol-4-yl)ethyl)amino)-5-fluoropyrimidin-4-yl)-4-ethyloxazolidin-2-one	(400 MHz, CD_3OD) δ ppm 9.40 (s, 1 H) 8.34 (br s, 1 H), 8.02 (s, 1 H), 7.73 (br d, *J* = 8.75 Hz, 2 H), 7.64 - 7.70 (m, 2 H), 5.06 - 5.28 (m, 1 H), 4.65 - 4.81 (m, 2 H), 4.28 (br t, *J* = 7.63 Hz, 1 H), 1.73 (br d, *J* = 6.98 Hz, 3 H), 1.53 - 1.68 (m, 2 H), 0.81 - 0.93 (m, 3 H)	m/z $(M + H)^+$ $C_{20}H_{21}ClFN_6O_2$: measured 431.1402, calcd 431.1399.
12: (*R*)-3-(2-(((*S*)-1-(1-(4-chlorophenyl)-1*H*-imidazol-4-yl)ethyl) amino)-5-fluoropyrimidin-4-yl)-4-((*R*)-1-fluoroethyl) oxazolidin-2-one	(400 MHz, $CDCl_3$) δ ppm 8.62 (br. s., 1 H), 8.19 (br. s., 1 H), 7.56 (d, *J* = 7.83 Hz, 2 H), 7.42 (d, *J* = 7.83 Hz, 3 H), 5.32 (br. s., 1 H), 4.93 (br. s., 1 H), 4.58 (t, *J* = 8.80 Hz, 1 H), 4.38 (d, *J* = 6.26 Hz, 1 H), 1.75 (d, *J* = 6.65 Hz, 3 H), 1.29 (dd, *J* = 23.9, 5.09 Hz, 3 H)	m/z $(M + H)^+$ $C_{20}H_{20}ClF_2N_6O_2$: measured 449.1313, calcd 449.1304.
13: (*R*)-3-(2-(((*S*)-1-(1-(4-chlorophenyl)-1*H*-imidazol-4-yl)ethyl)amino)-5-fluoropyrimidin-4-yl)-4-((S)-1-fluoroethyl)oxazolidin-2-one	(400 MHz, $CDCl_3$) δ ppm 8.64 (s, 1 H), 8.18 (d, *J* = 3.1 Hz, 1 H), 7.57 (d, *J* = 8.6 Hz, 2 H), 7.51 (br. s., 1 H), 7.44 (d, *J* = 8.6 Hz, 2 H), 5.35 (d, *J* = 6.7 Hz, 1 H), 5.01 (br. s., 1 H), 4.89 (br. s., 1 H), 4.71 - 4.86 (m, 1 H), 4.54 - 4.64 (m, 1 H), 4.44 - 4.53 (m, 1 H), 1.74 (d, *J* = 7.0 Hz, 3 H), 1.33 (dd, *J* = 23.5, 6.7 Hz, 3 H)	m/z $(M + H)^+$ $C_{20}H_{20}ClF_2N_6O_2$: measured 449.1299, calcd 449.1304.
14: (*R*)-3-(2-(((*S*)-1-(1-(4-chlorophenyl)-1*H*-imidazol-4-yl)ethyl)amino)-5-fluoropyrimidin-4-yl)-4-(fluoromethyl)oxazolidin-2-one	(400 MHz, $CDCl_3$) δ ppm 8.22 (d, *J* = 2.9 Hz, 1 H), 7.75 (d, *J* = 1.2 Hz, 1 H), 7.44 (d, *J* = 8.9 Hz, 2 H), 7.30 (d, *J* = 8.9 Hz, 2 H), 7.11 (s, 1H), 5.57 (d, *J* = 7.7 Hz, 1 H), 5.02 - 5.14 (m, 1 H), 4.75 - 4.89 (m, 1 H), 4.56 - 4.64 (m, 2 H), 4.43 - 4.53 (m, 2 H), 1.61 (d, *J* = 6.8 Hz, 3 H)	m/z $(M + H)^+$ $C_{19}H_{18}ClF_2N_6O_2$: measured 435.1147, calcd 435.1148.
15: (*S*)-3-(2-(((*S*)-1-(1-(4-chlorophenyl)-1*H*-	(400 MHz, CD_3OD) δ ppm 9.19 (d, *J* = 1.57 Hz, 1 H), 8.22 (d, *J* = 7.04 Hz, 1 H), 7.96 (s, 1	m/z $(M + H)^+$ $C_{21}H_{24}ClN_6O_2$:

imidazol-4-yl)ethyl)amino)pyrimidin-4-yl)-4-isopropyloxazolidin-2-one	H), 7.74 - 7.92 (m, 1 H), 7.57 - 7.74 (m, 4 H), 5.36 (q, *J* = 6.26 Hz, 1 H), 4.68 - 4.80 (m, 1 H), 4.33 - 4.52 (m, 2 H), 1.75 (d, *J* = 6.65 Hz, 3 H), 0.71 - 0.85 (m, 6 H)	measured 427.1654, calcd 427.1649.
16: (*R*)-3-(2-(((*S*)-1-(1-(4-chlorophenyl)-1*H*-imidazol-4-yl)ethyl) amino)pyrimidin-4-yl)-4-((S)-1-fluoroethyl) oxazolidin-2-one	(400 MHz, $CDCl_3$) δ ppm 11.02 (d, *J* = 7.04 Hz, 1 H), 8.41 (br. s., 1 H), 7.99 (d, *J* = 7.04 Hz, 1 H), 7.90 (d, *J* = 6.65 Hz, 1 H), 7.57 (d, *J* = 9.00 Hz, 3 H), 7.42 (d, *J* = 8.61 Hz, 2 H), 5.59 (br. s., 1 H), 4.86 - 5.12 (m, 2 H), 4.59 - 4.69 (m, 1 H), 4.46 - 4.58 (m, 1 H), 1.71 (d, *J* = 6.26 Hz, 3 H), 1.36 (dd, *J* = 23.87, 6.26 Hz, 3 H)	m/z (M + H)$^+$ $C_{20}H_{21}ClFN_6O_2$: measured 431.1400, calcd 431.1399.
17: (*R*)-3-(2-(((*S*)-1-(1-(4-chlorophenyl)-1*H*-imidazol-4-yl)ethyl)amino)pyrimidin-4-yl)-4-(fluoromethyl)oxazolidin-2-one	(400 MHz, $CDCl_3$) δ ppm 8.22 (d, *J* = 5.5 Hz, 1 H), 7.77 (s, 1 H), 7.45 (m, 3 H), 7.30 (d, *J* = 8.7 Hz, 2 H), 7.11 (s, 1 H), 5.14 (m, 1 H), 4.93 (m, 1 H), 4.48 (m, 4 H), 1.63 (d, *J* = 6.8 Hz, 3 H)	m/z (M + H)$^+$ $C_{19}H_{19}ClFN_6O_2$: measured 417.1243, calcd 417.1242.
18: (*R*)-3-(2-(((*S*)-1-(1-(4-(difluoromethyl)phenyl)-1*H*-imidazol-4-yl)ethyl)amino)pyrimidin-4-yl)-4-((*S*)-1-fluoroethyl)oxazolidin-2-one	(400 MHz, $CDCl_3$) δ ppm 11.00 (d, *J* = 7.0 Hz, 1 H), 8.46 (s, 1 H), 7.99 (d, *J* = 7.0 Hz, 1 H), 7.90 (d, *J* = 7.0 Hz, 1 H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.66 (s, 1 H), 7.59 (d, *J* = 8.2 Hz, 2 H), 6.74 (t, *J* = 55.6 Hz, 1 H), 5.58 (t, *J* = 6.8 Hz, 1 H), 4.91 - 5.14 (m, 2 H), 4.60 - 4.68 (m, 1 H), 4.49 - 4.59(m, 1 H), 1.72 (d, *J* = 6.7 Hz, 3 H), 1.36 (dd, *J* = 23.5, 6.3 Hz, 3 H)	m/z (M + H)$^+$ $C_{21}H_{22}F_3N_6O_2$: measured 447.1760, calcd 447.1756.

Biochemical IDH1^{R132H} 2-HG LCMS assay protocol

IDH enzyme assays were run in the following buffer: 50 mM HEPES, pH 7.3, 10 mM $MgCl_2$, 50 mM KCl, 0.02% BSA, and 1mM DTT. Compounds were diluted in DMSO. IDH^{R132H} was added to a final concentration of 500 pM. NADPH (5 µM final concentration) and αKG (200 µM final concentration) were added as a premixed solution to start the reaction. Reactions were quenched using formic acid (4.4% final concentration). 20 µL of quenched sample was added to 100 µL of acetonitrile, and samples were centrifuged before injection onto the LC/MS/MS system. Quantitation of 2-HG was performed using Agilent 1260 LC systems coupled to an Applied Biosystems API 4000 mass spectrometer.

Biochemical mutant (R132H and R132C) and wild type IDH1 fluorescence assay protocol

Biochemical assays were run in the following reaction buffer: 50mM HEPES pH7.5, 50mM KCl, 1mM dithiothreitol (DTT), 10mM MgCl2, and 0.02% bovine serum albumin (BSA). IDH1 WT assays used 30

μM isocitrate and 30 μM NADP. IDH1 mutant assays used 100μM α-ketoglutarate and 10 μM NADPH. Reactions were initiated through addition IDH1 protein, and monitored for the production (WT) or consumption (MUT) of NADPH through measuring the fluorescence of NADPH (excitation wavelength=355 nm, emission wavelength=520 nm).

Cellular HCT116-IDH1$^{R132H/+}$ 2-HG LCMS assay protocol

HCT116: parental and IDH1^{R132H} heterozygous mutant cells (Horizon Discovery) were cultured in McCoy's 5A Modified medium with 10% fetal bovine serum unless otherwise noted. For assessing cellular 2-HG inhibition, cells were plated at 3,500 cells/well in 384-well plates (Corning) and incubated overnight at 37 ^{o}C prior to compound addition. Compounds were added to wells in order to allow 10-point 3-fold dilutions in triplicate, starting at 10 μM. Cells were incubated with compounds for an additional 48 hours. To extract 2-HG, media was removed and 70 μL of 90% methanol was added to each well. Plates were then covered with foil seals and shaken for 30 seconds at high frequency, then incubated on dry ice for 15 minutes, spun at 2000 RPM for 15 minutes, and 30 μL of supernatant was used to measure 2-HG. 2-HG quantification was performed by LC-MS/MS analysis using an AB Sciex 4000 triple quadrupole mass spectrometer equipped with an Agilent 1200 series HPLC system, as previously described (Grassian, A.R. et al. (2012) Isocitrate dehydrogenase (IDH) mutations promote a reversible ZEB1/microRNA (miR)-200-dependent epithelial-mesenchymal transition (EMT). J Biol Chem 287, 42180-94).

Liver Microsomal Stability. The *in vitro* intrinsic clearance (CLint) in rat liver microsomes (RLM) (BD Gentest, Woburn, MA) was determined based on published method (Ref: Obach, R. S. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. Drug Metab. Dispos. 1999, 27, 1350−1359).

In brief, 1 μM test compound was incubated at 37 °C in pooled RLM (containing 0.5 mg of microsomal protein per mL) in the presence of 25 μg of alamethacin per mg protein, 1.0 mM NADPH, 1.0 mM UDPGA, and 2 mM MgCl2. The half-life (t1/2) was derived by monitoring the disappearance of test compound over a short period of time (e.g., 30 min) by LC/MS/MS. The CLint was calculated according to the following equation:

$$Clint = \frac{0.693}{t1/2} * \frac{Incubation\ volume,\mu L}{Total\ microsomal\ protein,mg}$$

Plasma protein binding assay. *In vitro* plasma protein binding was assessed in triplicate using an equilibrium dialysis method (Rapid Equilibrium Dialysis (RED) System (Thermo Fisher Scientific, Inc., Waltham MA). Compound was added to mouse, rat, dog, or human plasma at a final concentration of 5 μM (in 1% DMSO). The plasma was incubated at 37 ^{o}C under 5% CO2 for 4 h in the RED Device. Parent compound concentrations in the plasma and phosphate-buffered saline (PBS) compartments were measured at time 0 and 4 h by LC/MS/MS. A fraction unbound (fu) of compound in plasma was calculated as:

fu = $[PBS]_{4h}/[Plasma]_{4h}$

Brain homogenate protein binding assay. *In vitro* brain homogenate protein binding was assessed in triplicate using an equilibrium dialysis method (Rapid Equilibrium Dialysis (RED) System (Thermo Fisher Scientific, Inc., Waltham MA). Brain tissue was homogenized in 4 volumes (w/v) of phosphate-buffered saline (PBS) (dilution factor D = 5). Compound was then added to brain homogenate at a final concentration of 5 µM (in 1% DMSO). The brain homogenate was incubated at 37ºC under 5% CO2 for 4 h in the RED Device. Parent compound concentrations in the brain homogenate and PBS compartments were measured at time 0 and 4 h by LC/MS/MS. A fraction unbound (fu) of compound in diluted brain tissue was calculated as:

fu = [PBS]4 h/[Plasma]4 h

A fraction unbound (fu_{brain}) of compound in brain tissue (undiluted) was calculated as:

fu_{brain} = (1/D) / (((1/fu)-1)+1/D)

Pharmacokinetics Studies in rodents and non-rodents. Male C57BL/6 mice (25-30 g) (Harlan Laboratories Inc., Indianapolis, IN, USA), male Sprague Dawley rats (200−300 g) (Harlan Laboratories Inc., Indianapolis, IN, USA), and male Beagle dogs (9-11 kg) (Marshall (IT), Montichiari, Italy) were used in the experiments. All animal experiments were performed in accordance with IACUC protocol or the regulations effective in the Canton Basel-City, Switzerland. Two to three animals received 1-5 mg/kg by slow intravenous injection or 5-10 mg/kg orally. Blood was collected at multiple time points postdose and transferred to a EDTA tube. The blood was centrifuged at 3000 rpm, and the plasma was transferred to a polypropylene tube, capped, and stored frozen (−20 °C) for parent compound analysis. In the rodent studies, brain samples were collected at multiple time points to assess brain penetration. Brain tissues were homogenized in 10% Acetonitrile/90% PBS (4 times dilution). Protein precipitation was employed for sample preparation. A 25 µL aliquot of sample (plasma or brain homogenate) was subjected to protein precipitation using 150 µL of acetonitrile containing 100 ng/mL of internal standard (Glyburide). After vortex and centrifugation for 5 min at 4000 rpm, the supernatant (125 µL) was transferred to a 1 mL 96-well plate, followed by the addition of 50 µL of water. The analysis was conducted by using HPLC separation coupled with mass spectrometric detection. All pharmacokinetic (PK) parameters were derived from concentration− time data by noncompartmental analyses. All pharmacokinetic parameters were calculated with the computer program Watson (Version 7.4.2) (Thermo Fisher Scientific, Inc.)

$HCT116^{IDH1\ R132H+/-}$ xenograft model

Cell line culture

HCT116 cells expressing the R132H mutant form of IDH1 ($HCT116^{IDH1\ R132H/+}$ Clone 2H1, HCHZ2H1) were purchased from Horizon (Cat#: HD 115-002, clone 2H1), expanded for 5 passages, then stored in liquid nitrogen (reference 21). Cells were tested free of mycoplasma and viral contamination (MAP Impact panel VIII testing, Radil). Cells used for subcutaneous implantation were cultured in McCoy's 5A medium (Corning, Cat #: 10-050-CV) supplemented with 10% FBS (High Clone cat#sh30071) and split 1:3 twice weekly. Cells were cultured for 7-10 passages prior to implantation.

Generation of HCHZ2H1 xenografts

Outbred athymic (*nu/nu*) female mice ("HSD: Athymic Nude-nu") weighing 19-32 grams (Harlan, Indianapolis) were allowed to acclimate in the Novartis NIBRI animal facility with access to food and water ad libitum for minimum of 3 days prior to manipulation. All studies were performed in accordance with Novartis Institutes for Biomedical Research Animal Care and Use Committee. HCHZ2H1 cells were harvested at 80-90% confluency, washed once with Hank's Balanced Salt Solutions (HBSS, Cat No. 14175, Invitrogen Corporation, Gibco, Grand Island, NY), and suspended in 100% HBSS at 5×10^7 cells/mL for implantation. For tumor cell implantation, mice were anesthetized with continuous flow of 2-4% isoflurane/oxygen mixture using the Integrated Multi Patient Anesthesia Center (IMPAC6) and induction chamber (Vetequip, Inc., Pleasanton, CA). Female nude mice were injected subcutaneously (dorsal right axillary region) with 5×10^6 HCHZ2H1 tumor cells suspended in 100% HBSS in a total volume of 100 µL. Mice were monitored for tumor growth, and once palpable, tumors were measured by caliper. 14-22 days post implant mice with tumors ranging from 100-300 mm^3 were selected for enrollment on study and randomized to experimental groups.

PK/PD experiment

Female nude mice bearing HCHZ2H1 tumors were treated with a compound, followed by blood and tumor tissue collections at various time points post treatment. The plasma concentration of the compound and concentration of 2-HG in tumor tissue were determined using sensitive LC/MS/MS methods. The percent inhibition in tumor tissue of treated mice relative to vehicle treate mice was determined.

Determination of plasma drug level

For determination of **19** plasma concentration, blood was collected via tail nick (non-terminal) or cardiac puncture (terminal), collected into EDTA-lined microtainers (BD Microtainer®, Cat No. 365973), centrifuged at 13,200 rpm for 5 minutes and the plasma supernatant will be placed in a 1 mL 96 well collection plate and stored at -20 °C until analysis. Plasma concentrations of **19** were determined by LC-MS/MS. Acetonitrile protein precipitation was employed to extract plasma samples, which were processed using a Freedom EVO® 150 and a Freedom EVO® from TECAN.Test samples were diluted with blank mouse plasma 2 to 10 fold and 25 µL of each undiluted or diluted test sample were transferred to a 96-well plate. A 150 µL volume of acetonitrile with 100 ng/mL glyburide (internal standard) was added to each well containing test sample, or calibration standard, vortexed, then centrifuged at 4,000 rpm for 10 minutes. 125 µL of each supernatant were transferred to a clean 1 mL 96-well plate, followed by the addition of 50 µL of water. For each sample, a 10 µL aliquot was injected into the LC-MS/MS system. Chromatographic separation was achieved with an ACE C18 column (3 µm, 2.1 × 30 mm) from MAC-MOD Analytical, Inc. (Chadds Ford, PA), using 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) as solvents in a multistep gradient. Data were acquired and processed using Sciex Analyst 1.4.2 software. All pharmacokinetic (PK) parameters were derived from concentration-time data by noncompartmental analyses (Thermo Watson 7, Thermo Fisher Scientific, Inc.,Philadelphia, PA). The peak concentrations (C_{max}) and times they occurred (T_{max}) were recorded. The area under the concentration-time curve (AUC_{last}) was calculated using the linear trapezoidal rule. The area under the concentration-time curve from time 0 to infinity (AUC_{inf}) was calculated using the following equation, $AUC_{\inf} = AUC_{last} + \frac{C_{last}}{\lambda_z}$ Where the terminal elimination rate constant (λ_z) for the

unchanged compound was the slope of the log linear line from at least the last three data points. Results are expressed as mean ± SD when are applicable. No further statistical analysis was performed.

Determination of tumor 2-HG level

Tumor fragments of approximately 50 mg were placed into 15 ml geno/grinder tube (Pre-Cleaned 5 mL Polycarbonate Vial Set, SPEX SamplePrep LLC, 15 Liberty Street, Metuchen, NJ, USA, Catalog# 2240-PC), snap frozen in liquid nitrogen, then transferred to -80°C until extraction. For extraction, geno/grinder tubes with tumor were placed on dry ice. The geno/grinder adaptor and cover was chilled on dry ice, then adaptor was loaded with sample tubes, placed in the geno/grinder (SPEX SamplePrep LLC, Catalog# 2010-geno/grinder) and homogenized for 30 seconds. Samples were then removed from the adaptor and set on dry ice. 2-10 mg of pulverized tumor sample was weighed out and transferred to a fresh 2 ml screw-top tube on dry ice. 200ul of 90% cold methanol for every mg of tumor powder was added, then tubes vortexed until sample fully resuspended. Samples were sonicated for 10 minutes in an ice-water bath, then incubated on dry ice for a minimum of 30 minutes. Samples were then centrifuged for 10 minutes at 13,200 rpm at 4 ^{o}C and supernatants were transferred to a 96-well plate and stored at -20 °C until processing. Samples were diluted 1:10 in 90% methanol prior to analysis. Dilution plates were sealed using LC-MS/MS plate sealer and store at -80 °C until LC-MS/MS analysis, as described.[18]

HCT116-IDH1R$^{132H/+}$ single dose PK/PD study data

Table SI-2. Plasma concentration, normalized tumor 2-HG concentration and percent tumor 2-HG inhibition by **19** following a single oral dose at 150 mg/kg dose in HCHZ2H1 xenograft model.

Time post dose (h)	Total Plasma IDH305 Conc. (nM) (Mean ± SD)	Estimated Free Plasma IDH305 Conc. (nM) (Mean ± SD)	Normalized 2-HG (ng/mg sampl weight) (Mean ± SEM)	% 2-HG Inhibition (Mean ± SEM)
0	(Vehicle)	-	301.5 ± 31.2	0
0.25	59435 ± 10019	357 ± 60	ND	ND
0.5	80079 ± 27326	480 ± 164	ND	ND
1	96020 ± 27224	576 ± 163	ND	ND
4	85155 ± 28564	511 ± 171	212.2 ± 29.8	29.6 ± 9.9
8	57006± 11168	342 ± 67	56.2 ± 13.7	81.4 ± 4.6
16	17982± 463	108 ± 3	18.9 ± 15.6	93.7 ± 5.2
20	11184 ± 3071	67 ± 18	28.4 ± 4.8	90.6 ± 1.6
24	14813 ± 6352	89 ± 38	24.8 ±7.3	91.8 ± 2.4
48	29 ± 3	0.2 ± 0.02	328.4 ± 62.1	-8.9 ± 20.6

Table SI-2 notes: The LLOQ for **19** was 10 nM; ND, not determined, fu (fraction unbound) in mouse plasma: 0.006.

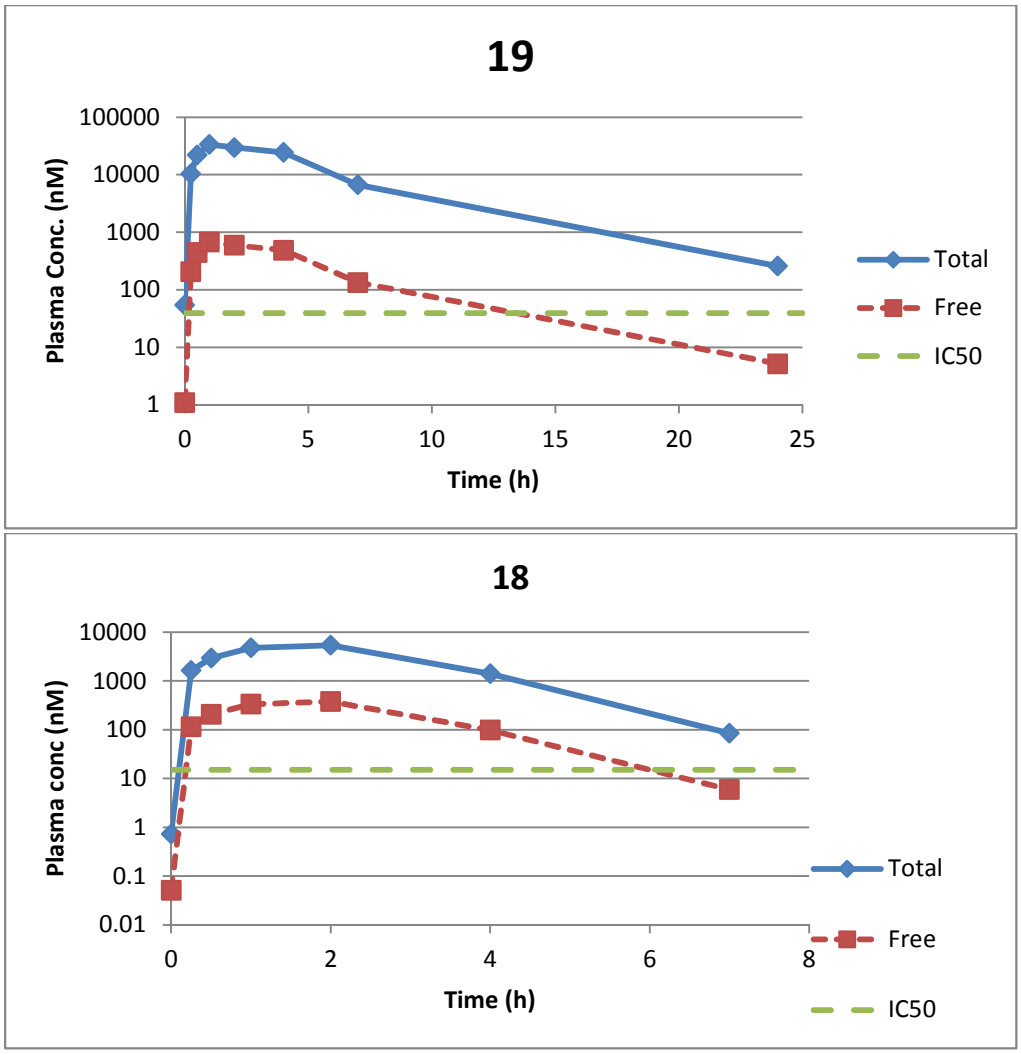


Figure SI-1. Plasma Conc. vs Time Profile After Oral Administration of **19** and **18** to SD Rats.