SUPPORTING INFORMATION

Discovery and Evaluation of Clinical Candidate IDH305, a Brain Penetrant Mutant IDH1 Inhibitor

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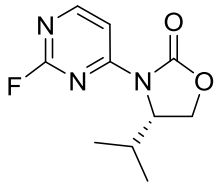
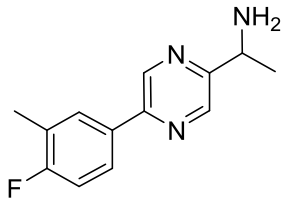


(S)-3-(2-fluoropyrimidin-4-yl)-4-isopropyloxazolidin-2-one (reference 20)

A solution of 2,4-difluoropyrimidine (3.5 mL, 41 mmol) and (S)-4-isopropyloxazolidin-2-one (5.3 g, 41 mmol) in 30 mL DMF was cooled to 0 °C under N_2 atmosphere. NaH (2.1 g of 60% suspension, 53 mmol) was slowly added. A bubbling exotherm was observed. Internal temp was kept below 5 °C. After 5 minutes, the cold bath was removed. Reaction mixture (a sandy suspension) was allowed to warm to room temp and stir for 18 h. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (3 x 75 mL). Combind organic layers were washed with water (50mL), brine (50mL), dried over Na_2SO_4, and concentrated on silica gel in vacuo. Column chromatography (EtOAc/heptane 10 to 100% gradient) gave 3.1 g (S)-3-(2-fluoropyrimidin-4-yl)-4-isopropyloxazolidin-2-one as a crystalline white solid (33%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (dd, J = 5.8, 2.2 Hz, 1H), 8.19 (dd, J = 5.8, 3.8 Hz, 1H), 4.79 (dt, J = 8.1, 3.5 Hz, 1H), 4.48 – 4.34 (m, 2H), 2.64 (heptd, J = 7.0, 3.6 Hz, 1H), 1.01 (d, J = 7.0 Hz, 3H), 0.90 (d, J = 6.9 Hz, 3H). MS m/z $(M + H)^+$ C10H13FN3O2: measured 226.1, calcd 226.2.

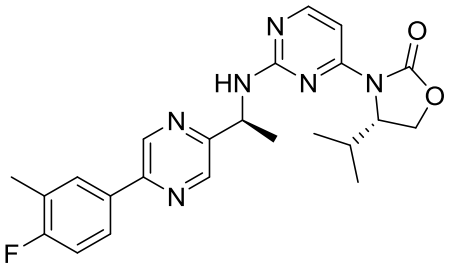


1-(5-(4-fluoro-3-methylphenyl)pyrazin-2-yl)ethanamine

Step 1: **(General procedure A for suzuki coupling)** N2 was bubbled through a solution of 1-(5-chloropyrazin-2-yl)ethanone (380 mg, 2.43 mmol), (4-fluoro-3-methylphenyl)boronic acid (448 mg, 2.91 mmol) and Na_2CO_3 (2.0 M, 2.43 ml, 4.85 mmol) in dioxane for 5 min. Cl2Pd(dppf) (CH2Cl2 adduct) (198 mg, 0.24 mmol) was added. The reaction mixture was stirred at 100 °C for 3 hr. The mixture was diluted with EtOAc, washed with water, brine, dried over Na_2SO_4, filtered, concentrated and purified through flash column chromatography (0-100% EtOAc/Heptane) to give 326 mg 1-(5-(4-fluoro-3-methylphenyl)pyrazin-2-yl)ethanone as a light yellow solid (58%). ^{1}H NMR (400 MHz, CDCl3) δ 9.24 (d, J = 1.5 Hz, 1H), 9.01 (d, J = 1.5 Hz, 1H), 7.97 (dd, J = 7.3, 1.9 Hz, 1H), 7.89 (ddd, J = 7.6, 4.8, 2.3 Hz, 1H), 7.17 (t, J = 8.9 Hz, 1H), 2.74 (s, 3H), 2.39 (d, J = 2.0 Hz, 3H). MS m/z $(M + H)^+$ C13H12FN2O: measured 230.9 , calcd 231.3.

Step 2: A mixture of 1-(5-(4-fluoro-3-methylphenyl)pyrazin-2-yl)ethanone (326 mg, 1.42 mmol), NH4OAc (1.64 g, 21.2 mmol), and NaBH3CN (356 mg, 5.66 mmol) in 200 proof EtOH (7mL) was heated at 120ºC for 5 min in a microwave apparatus. The mixture was concentrated to remove the EtOH. Crude mixture was dissolved in water (30mL) + EtOAc (25mL). 6N NaOH was added until aqueous pH

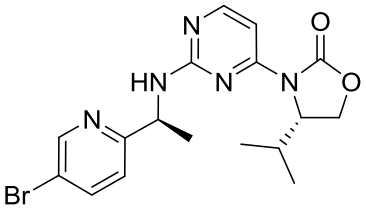
was ~10. The layers were separated and the aqueous layer was extracted with EtOAc (25 mL). The combined organic layers were washed with brine (25mL), dried with Na_2SO_4, filtered, concentrated and and purified through flash column chromatography (0-10% MeOH/DCM) to give 200 mg 1-(5-(4-fluoro-3-methylphenyl)pyrazin-2-yl)ethanamine as a light brown oil (61%). MS m/z $(M+H)^+$ C13H15FN3: measured 231.9, calcd 232.3.



Compound 2

(**General procedure B for SnAr reaction**) A solution of 1-(5-(4-fluoro-3-methylphenyl)pyrazin-2-yl)ethanamine (66 mg, 0.285 mmol), (S)-3-(2-fluoropyrimidin-4-yl)-4-isopropyloxazolidin-2-one (64.3 mg, 0.285 mmol) and DIPEA (150 µl, 0.856 mmol) in DMSO (Volume: 1.43 ml) was heated at 110 °C for 1 hr. The solution was then cooled to rt, diluted with EtOAc, washed with water, brine, dried over Na_2SO_4, filtered, concentrated and purified through flash column chromatography (0-100% EtOAc/Heptane) to give 40 mg of (S)-3-(2-(((S)-1-(5-(4-fluoro-3-methylphenyl)pyrazin-2-yl)ethyl)amino)pyrimidin-4-yl)-4-isopropyloxazolidin-2-one as a white solid (32%) and another diastereomer. 1H NMR (400 MHz, CDCl3) δ 8.89 (d, J = 1.5 Hz, 1H), 8.61 (s, 1H), 8.21 (d, J = 5.7 Hz, 1H), 7.83 (dd, J = 7.4, 2.2 Hz, 1H), 7.76 (ddd, J = 7.6, 5.0, 2.4 Hz, 1H), 7.49 (d, J = 5.8 Hz, 1H), 7.12 (t, J = 8.9 Hz, 1H), 5.84 (br s, 1H), 5.21 (br s, 1H), 4.62 (br s, 1H), 4.33 - 4.17 (m, 2H), 2.36 (d, J = 2.0 Hz, 3H), 1.71 (br s, 1H), 1.64 (d, J = 7.0 Hz, 3H), 0.69 (br s, 6H). HRMS *m/z* $(M + H)^+$ C23H26FN6O2: measured 437.2083, calcd 437.4994.

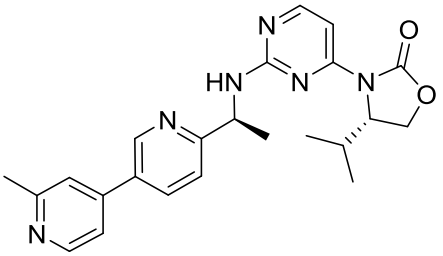
The following intermediate prepared using general procedure B for SnAr reaction.



(S)-3-(2-((S)-1-(5-bromopyridin-2-yl)ethylamino)pyrimidin-4-yl)-4-isopropyloxazolidin-2-one

^{1}H NMR (400 MHz, Chloroform-d) δ 8.61 (d, J = 2.1 Hz, 1H), 8.19 (d, J = 5.8 Hz, 1H), 7.74 (dd, J = 8.3, 2.3 Hz, 1H), 7.47 (d, J = 5.8 Hz, 1H), 7.21 (d, J = 8.3 Hz, 1H), 5.73 (br s, 1H), 5.03 (br s, 1H), 4.57 (br s, 1H), 4.29 - 4.20 (m, 2H), 1.60 (br s, 1H), 1.56 (d, J = 7.0 Hz, 3H), 0.68 (br s, 6H). MS *m/z* $(M+H)^+$ C17H21BrN5O2: measured 407.2, calcd 407.3.

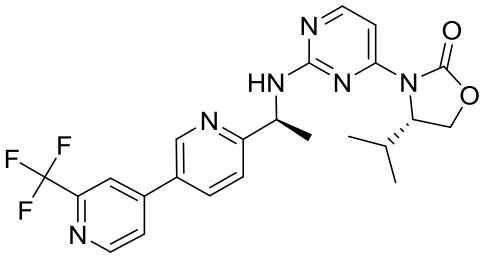
The following compounds **3** and **5** were prepared using general procedure A for suzuki coupling.



Compound 3

(S)-4-isopropyl-3-(2-(((S)-1-(2'-methyl-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one

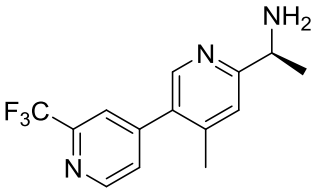
^{1}H NMR (400 MHz, MeOD) δ 8.89 (d, J = 2.3 Hz, 1H), 8.50 (d, J = 5.4 Hz, 1H), 8.18 - 8.13 (m,, 2H), 7.64 (s, 1H), 7.56 - 7.52 (m, 2H), 7.40 (d, J = 5.8 Hz, 1H), 5.11 (q, J = 6.8 Hz, 1H), 4.59 (s, 1H), 4.33 - 4.25 (m, 2H), 2.61 (s, 3H), 1.60 (d, J = 7.1 Hz, 3H), 1.47 (br s, 1H), 0.57 (br s, 3H), 0.50 (br s, 3H). HRMS *m/z* (M + H)$^+$ C23H27N6O2: measured 419.2179, calcd 419.5090.



Compound 5

(S)-4-isopropyl-3-(2-(((S)-1-(2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one

1H NMR (400 MHz, MeOD) δ 8.98 (d, J = 2.4 Hz, 1H), 8.80 (d, J = 5.1 Hz, 1H), 8.23 (dd, J = 8.3, 2.4 Hz, 1H), 8.17 (s, 1H), 8.14 (s, 1H), 7.99 (dd, J = 5.1, 1.7 Hz, 1H), 7.58 (d, J = 8.2 Hz, 1H), 7.40 (d, J = 5.8 Hz, 1H), 5.13 (q, J = 6.8 Hz, 1H), 4.60 (s, 1H), 4.30 (m, 2H), 1.61 (d, J = 7.1 Hz, 3H), 1.52 (br s, 1H), 0.58 (br s, 3H), 0.52 (br s, 3H). HRMS *m/z* (M + H)$^+$ C23H24F3N6O2: measured 473.1896, calcd 473.4802.



Compound 20 (S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethanamine

(**General procedure C**)

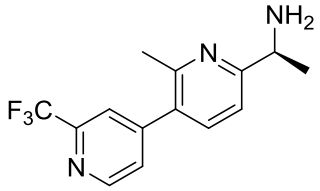
Step 1: To a solution of (S)-1-(5-bromo-4-methylpyridin-2-yl)ethanamine hydrochloride (**19**, 750 mg, 2.98 mmol) in DCM (30 mL) was added di-tert-butyl dicarbonate (0.761 mL, 3.28 mmol) and triethylamine (1.25 mL, 8.94 mmol). The resulting solution was stirred at r.t. overnight. Mixture was concentrated under reduced pressure and diluted with 40 mL EtOAc, washed with water, brine, dried over Na2SO4 and concentrated to give 940 mg of (S)-tert-butyl (1-(5-bromo-4-methylpyridin-2-yl)ethyl)carbamate as a light brown oil. MS m/z (M + H)$^+$ C13H20BrN2O2: measured 317.2, calcd 316.2.

1H NMR (400 MHz, CDCl3) δ 8.57 (s, 1H), 7.14 (s, 1H), 5.57 – 5.50 (m, 1H), 4.79 (p, J = 7.0 Hz, 1H), 2.40 (s, 3H), 1.47-1.43 (m, 12H).

Step 2: N2 was bubbled through a solution of (S)-tert-butyl (1-(5-bromo-4-methylpyridin-2-yl)ethyl)carbamate (200 mg, 0.635 mmol), (2-(trifluoromethyl)pyridin-4-yl)boronic acid (145 mg, 0.761 mmol) and Na2CO3 (2.0 M, 635 µl, 1.269 mmol) in dioxane for 5 min. Cl2Pd(dppf) (CH2Cl2 adduct) (51.8 mg, 0.063 mmol) was added. The reaction mixture was stirred at 90 °C for 16 hr. The mixture was diluted with EtOAc, washed with water, brine, dried over Na2SO4, filtered, concentrated and purified through flash column chromatography (0-100% EtOAc/Heptane) to give 200 mg (S)-tert-butyl (1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)carbamate as a white solid (83%). 1H NMR (400 MHz, CDCl3) δ 8.83 (d, J = 4.9 Hz, 1H), 8.36 (s, 1H), 7.66 (s, 1H), 7.51 - 7.42 (m, 1H), 7.21 (s, 1H), 5.60 (d, J = 7.7 Hz, 1H), 4.87 (p, J = 6.9 Hz, 1H), 2.30 (s, 3H), 1.48 (d, J = 6.9 Hz, 3H), 1.45 (s, 9H). MS m/z $(M + H)^+$ C19H23F3N3O2: measured 382.3, calcd 382.4.

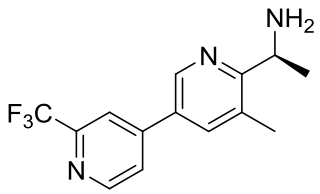
Step 3: To a solution of (S)-tert-butyl (1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)carbamate (200 mg, 0.524 mmol) in DCM (2 ml) at -78 °C was added trifluoroacetic acid (2 mL, 12.98 mmol). The solution was stirred at r.t. for 1 hr. The mixture was concentrated, diluted with 10 mL DCM and stirred with solid MP-carbonate to remove TFA. Filtered and concentrated to give 147 mg (S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethanamine as a light brown sticky oil. The crude product was used to next step without further purification. MS m/z $(M + H)^+$ C14H15F3N3: measured 282.1, calcd 282.3; Rt = 0.85 min.

The following compounds were synthesized by the similar methods as described for (S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethanamine (general procedure C).



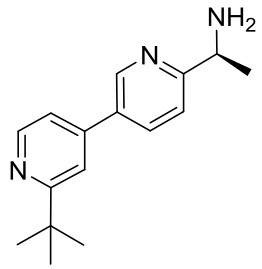
(S)-1-(2-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethanamine.

MS *m/z* $(M + H)^+$ C14H15F3N3: measured 282.1, calcd 282.3; RT.: 0.86 min.



(S)-1-(5-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethanamine.

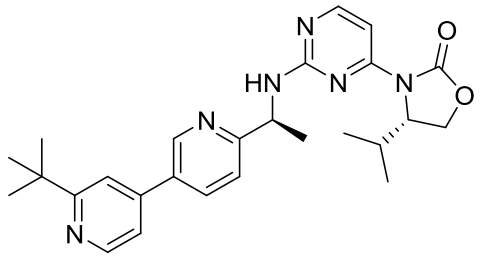
MS *m/z* $(M + H)^+$ C14H15F3N3: measured 282.1, calcd 282.3; RT.: 0.89 min.



(S)-1-(2'-(tert-butyl)-[3,4'-bipyridin]-6-yl)ethan-1-amine

MS m/z $(M + H)^+$ C16H22N3: measured 256.2, calcd 256.4.

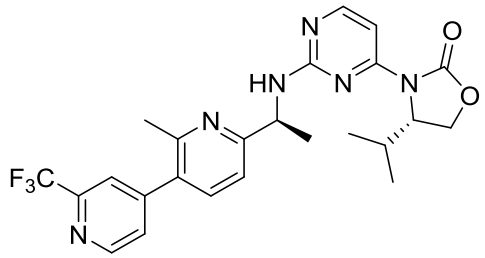
The following compounds (**4**, **6**, **7** and **8**) were prepared using general procedure B for SnAr reaction.



Compound 4

(S)-3-(2-(((S)-1-(2'-(tert-butyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)-4-isopropyloxazolidin-2-one

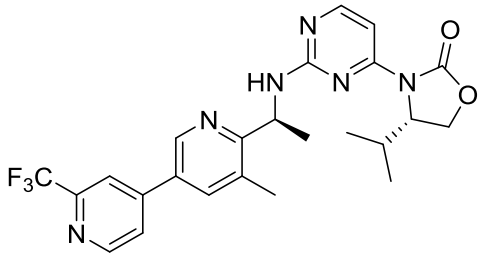
1H NMR (400 MHz, MeOD) δ 8.86 (d, J = 1.8 Hz, 1H), 8.57 (dd, J = 5.2, 0.8 Hz, 1H), 8.18 (d, J = 4.9 Hz, 1H), 8.13 (dd, J = 8.2, 2.3 Hz, 1H), 7.71 - 7.68 (m, 1H), 7.57 - 7.50 (m, 2H), 7.40 (d, J = 5.8 Hz, 1H), 5.16 - 5.06 (m, 1H), 4.59 (br s, 1H), 4.34 - 4.24 (m, 2H), 1.61 (d, J = 7.1 Hz, 3H), 1.42 (s, 9H), 0.58 (br s, 3H), 0.51 (br s, 3H). HRMS *m/z* $(M + H)^+$ C26H33N6O2: measured 461.2649, calcd 461.5900.



Compound 6

(S)-4-isopropyl-3-(2-(((S)-1-(2-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one

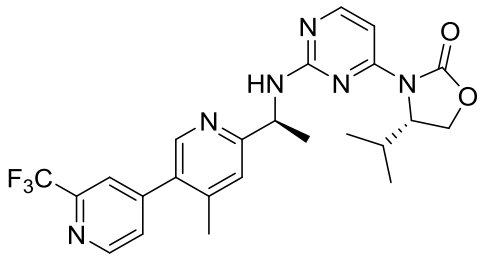
1H NMR (400 MHz, MeOD) δ 8.80 (d, J = 5.0 Hz, 1H), 8.17 (d, J = 5.4 Hz, 1H), 7.82 (s, 1H), 7.74 - 7.65 (m, 2H), 7.40 (d, J = 5.8 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 5.10 (q, J = 6.9 Hz, 1H), 4.65 (br s, 1H), 4.40 - 4.26 (m, 2H), 2.53 (s, 3H), 1.70 (br s, 1H), 1.59 (d, J = 7.1 Hz, 3H), 0.63 (br s, 6H). HRMS *m/z* $(M + H)^+$ C24H26F3N6O2: measured 487.2039, calcd 487.5072; RT.: 2.57 min.



Compound 7

(S)-4-isopropyl-3-(2-(((S)-1-(5-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one

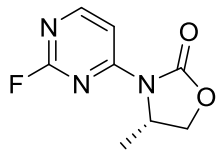
1H NMR (400 MHz, MeOD) δ 8.80 (d, J = 2.3 Hz, 1H), 8.77 (d, J = 5.1 Hz, 1H), 8.13 (s, 2H), 8.07 (s, 1H), 7.97 (dd, J = 5.2, 1.7 Hz, 1H), 7.37 (d, J = 5.7 Hz, 1H), 5.41 (br s, 1H), 4.73 (br s, 1H), 4.43 - 4.30 (m, 2H), 2.55 (s, 3H), 2.12 (br s, 1H), 1.52 (d, J = 6.8 Hz, 3H), 0.86 (br s, 3H), 0.71 (br s, 3H). HRMS *m/z* $(M + H)^+$ C24H26F3N6O2: measured 487.2041, calcd 487.5072; RT: 2.78 min.



Compound 8

(S)-4-isopropyl-3-(2-(((S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one

1H NMR (400 MHz, MeOD) δ 8.82 (d, J = 5.0 Hz, 1H), 8.40 (s, 1H), 8.17 (s, 1H), 7.83 (s, 1H), 7.69 (dd, J = 5.0, 1.5 Hz, 1H), 7.47 - 7.34 (m, 2H), 5.10 (q, J = 7.1 Hz, 1H), 4.65 (br s, 1H), 4.42 - 4.24 (m, 2H), 2.32 (s, 3H), 1.69 (br s, 1H), 1.59 (d, J = 7.1 Hz, 3H), 0.63 (br s, 6H). HRMS *m/z* $(M + H)^+$ C24H26F3N6O2: measured 487.2042, calcd 487.5072; RT.: 2.53 min.



(S)-3-(2-fluoropyrimidin-4-yl)-4-methyloxazolidin-2-one

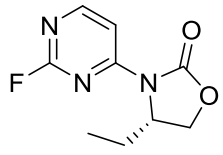
(**General procedure D**)

Step 1: To a solution of (S)-2-aminopropan-1-ol (17.05 g, 227 mmol) in DCM (200 mL) at 0 ^{o}C was added portionwise di-tert-butyl dicarbonate (49.5 g, 227 mmol) over 3 min. The solution was stirred for 16 h at room temperature then washed with 0.1% HCl squeous soluiton. The organic solution was dried over $MgSO_4$, filtered and concentrated to give tert-butyl (S)-(1-hydroxypropan-2-yl)carbamate as a clear oil.

Step 2: The above tert-butyl (S)-(1-hydroxypropan-2-yl)carbamate was dissoved in THF (500 mL) and to the solution was added sodium hydride (11 g, 275 mmol) over 5 min at room temperature. The solution was stirred at 65 ^{o}C for 2 hr then additional sodium hydride (4 g, 100 mmol) was added and stirred for another 1 hr at 65 ^{o}C. The soluiton then cooled to room temperature and quenched with sat. NH_4Cl solution and brine. The organic phase was seprated and the aqueous phase was extracted with EtOAc. Combined orgainc layers was dried over $MgSO_4$, filtered, concentrated and purified through flash column chromatography (75% EtOAc/Heptane) to give 18.9 g (S)-4-methyloxazolidin-2-one as a white solid (82%). ^{1}H NMR (400 MHz, CDCl3) δ 6.23 (br s, 1H), 4.50 (t, J = 7.83 Hz, 1H), 4.12 - 3.74 (m, 1H), 1.30 (d, J = 5.87 Hz, 3H).

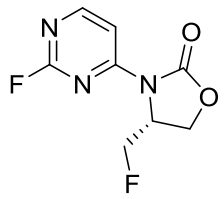
Step 3: Sodium hydride (2.57 g, 64.3 mmol) was carefully added to a solution of (S)-4-methyloxazolidin-2-one (5.0 g, 49.5 mmol) and 2,4-difluoropyrimidine (5.74 g, 49.5 mmol) in DMF (50 mL) at 0 ^{o}C. The reaction mixture was stirred at room temperature for 2 hr was then quenched with 10% NaHCO3 aqueous solution. The mixture was extracted with ethyl acetate (2 x 125 mL). The combined organic extracts were washed with water, brine, dried over Na_2SO_4, filtered and concentrated. Silica gel column chromatography (10-100% EtOAc/Heptane) provided 6.06 g of (S)-3-(2-fluoropyrimidin-4-yl)-4-methyloxazolidin-2-one as a white solid (61%). 1H NMR (400 MHz, CDCl3) δ 8.46 (dd, J = 5.8, 2.2 Hz, 1H), 8.10 (dd, J = 5.8, 3.8 Hz, 1H), 4.87 (dqd, J = 8.1, 6.3, 3.1 Hz, 1H), 4.56 (t, J = 8.3 Hz, 1H), 4.14 (dd, J = 8.7, 3.1 Hz, 1H), 1.53 (d, J = 6.3 Hz, 3H). MS *m/z* $(M + H)^+$ C8H9FN3O2: measured 198.1, calcd 198.2.

The following compound was synthesized by the similar methods as described for (S)-3-(2-fluoropyrimidin-4-yl)-4-methyloxazolidin-2-one (general procedure D)



(S)-4-ethyl-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one

MS *m/z* $(M + H)^+$ C9H11FN3O2: measured 212.1, calcd 212.2.



(R)-4-(fluoromethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one

Step 1

Imidazole (1.74 g, 25.6 mmol, 2.0 equiv) 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, 1.2 equiv) 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 Na2SO4, filtered and concentrated. Silica gel column chromatography (EtOAc/Heptane 20 to 65%) provided (R)-4-(((tert-butyldiphenylsilyl)oxy)methyl)oxazolidin-2-one (3.55 g, sticky colorless oil) in 78% yield. 1H NMR (400 MHz, CDCl3) δ 7.64 (m, 4H), 7.50 – 7.38 (m, 6H), 5.31 (m, 1H), 4.44 (t, J = 8.7 Hz, 1H), 4.15 (m, 1H), 4.00 (m, 1H), 3.65 (d, J = 5.5 Hz, 2H), 1.07 (s, 9H). MS m/z 356.1 $(M + H)^+$ C20H25NO3Si: measured 356.1, calcd 356.5; Rt-1.00 min.

Step 2

Sodium hydride (34 mg, 0.84 mmol, 1.5 equiv) was carefully added to a solution of (R)-4-(((tert-butyldiphenylsilyl)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, 1.5 equiv) 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 Na2SO4, filtered and concentrated. Silica gel column chromatography (EtOAc/Heptane 30%) provided (R)-4-(((tert-butyldiphenylsilyl)oxy)methyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one (0.185 g, sticky colorless oil) in 73% yield. 1H NMR (400 MHz, CDCl3) δ 8.44 (dd, J = 5.8, 2.2 Hz, 1H), 8.12 (dd, J = 5.8, 3.4 Hz, 1H), 7.58 (m, 2H), 7.48 – 7.32 (m, 6H), 7.23 (m, 2H), 4.80 (m, 1H), 4.65 (dd, J = 8.6, 3.0 Hz, 1H), 4.55 (m, 1H), 4.18 (dd, J = 11.1, 3.5 Hz, 1H), 3.83 (dd, J = 11.1, 2.1 Hz, 1H), 1.04 (s, 9H). MS m/z $(M + H)^+$ C24H27FN3O3Si: measured 452.3, calcd 452.6; Rt-1.15 min.

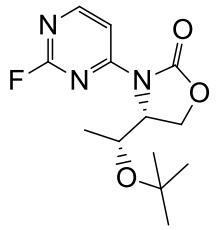
Step 3

A solution of tetrabutylammonium fluoride (1.0 M in THF, 0.44 mL, 0.44 mmol, 1.1 equiv) 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 Na2SO4, filtered and concentrated. Silica gel column chromatography (EtOAc/Heptane) provided (S)-3-(2-fluoropyrimidin-4-yl)-4-(hydroxymethyl)oxazolidin-2-one (0.051 g,white solid) in 60% yield. 1H NMR (400 MHz, CDCl3) δ 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 $(M + H)^+$ C8H9FN3O3: measured 214.0, calcd 214.2; Rt-0.37 min.

Step 4

Perfluorobutanesulfonyl fluoride (0.18 mL, 0.98 mmol, 4 equiv) 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, 4 equiv) and triethylamine (0.41 mL, 3.0 mmol, 12 equiv) 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 Na2SO4, filtered and concentrated. Silica gel column chromatography (EtOAc/Heptane) provided (R)-4-(fluoromethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one (0.021 g,colorless oil) in 40% yield. 1H NMR (400 MHz, CDCl3) δ 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 $(M + H)^+$ C8H8F2N3O2: measured 215.9, calcd 216.2; Rt-0.57 min.



(R)-4-((R)-1-(tert-butoxy)ethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one

Step 1

A solution of (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-(tert-butoxy)butanoic acid (500 mg, 1.0 mmol) and isobutyl chloroformate (167 mg, 1.2 mmol, 1.2 equiv) in 10 ml of THF at -25 ^oC was added N-methylmorpholine (124 mg, 1.2 mmol, 1.2 equiv). The resulting mixture was stirred for 10 min and filtered. The filtrate was cooled to -20 ^oC and was treated with NaBH4 (58 mg) followed by addition of 2 ml of water immediately afrerwards. The reaction mixture was stirred for 5 min and then graduately warmed to room temperature over 25 min. The mixture was poured into water (10ml) and extracted with EtOAc (2x20ml). The combined organic phases were washed with water, brine and dried over Na2SO4. The solvent was removed to yield the desired product as the clear oil in quantitative yield. No further purification was required for next step. 1H NMR (400 MHz, CDCl3) δ 7.37 – 7.16 (m, 5H), 5.25 (d, J = 8.0 Hz, 1H), 5.02 (s, 1H), 4.04 (ddd, J = 12.0, 8.9, 2.8 Hz, 1H), 3.92 – 3.75 (m, 1H), 3.59 (dddd, J = 32.3, 14.6, 8.2, 4.3 Hz, 2H), 1.10 (s, 9H), 1.09 – 1.06 (m, 3H).

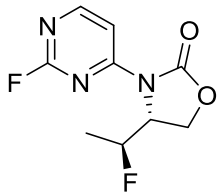
Step 2

A solution of benzyl ((2R,3R)-3-(tert-butoxy)-1-hydroxybutan-2-yl)carbamate (134 mg, 0.45 mmol in 5 mL of THF) was pre-cooled to 0 ^oC under nitrogen was treated with potassium tert-butoxide (153 mg, 1.4 mmol, 3.0 equiv) and the resulting mixture was stirred at 0 ^oC for 2 hours. The reaction mixture was treated with 5 mL of water and extracted with EtOAc (2x20mL). The combined organic layers were washed with water, brine, dried over Na2SO4 and concentrated to yield the desired product as a

yellow oil, no further purification was required for next step. 1H NMR (400 MHz, CDCl3) δ 4.33 (t, J = 8.7 Hz, 1H), 4.07 (dd, J = 8.9, 5.5 Hz, 1H), 3.67 – 3.58 (m, 1H), 3.58 – 3.49 (m, 1H), 1.13 (s, 9H), 1.02 (d, J = 6.0 Hz, 3H).

Step 3

A solution of (R)-4-((R)-1-(tert-butoxy)ethyl)oxazolidin-2-one (86mg, 0.46 mmol) and 2,4-difluoropyrimidine (79 mg, 0.55 mmol, 1.2 equiv) in 3 ml of DMF was cooled to 0 ^{o}C under N_2 before adding NaH (60%, 28 mg, 0.69 mmol, 1.5 equiv) slowly. The reaction mixture was stirred at 0 ^{o}C for 45 min, then gradually warmed to room temperature, and stirred at room temperature overnight. The reaction mixture was treated with 5 ml of water, and extracted with EtOAc (2x10 ml). The solvent was removed to yield the crude product. Silica gel column chromatography (ethyl acetate in heptane 10 to 50%) provided the desired product as a white solid. 1H NMR (400 MHz, CDCl3) δ 8.50 (dd, J = 5.8, 2.1 Hz, 1H), 8.14 (dd, J = 5.8, 3.8 Hz, 1H), 4.85 – 4.63 (m, 2H), 4.56 – 4.30 (m, 2H), 1.26 (s, 9H), 1.05 (d, J = 6.5 Hz, 3H); MS m/z $(M + H)^+$ C13H19FN3O3: measured 284.1, calcd 284.3.



Compound 18 (R)-4-((S)-1-fluoroethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one

Step 1: A solution of (2S,3R)-2-(((benzyloxy)carbonyl)amino)-3-(tert-butoxy)butanoic acid dicyclohexylammonium salt (500 mg, 1.0 mmol) in 10 ml of THF and isobutyl chloroformate (167 mg, 1.2 mmol, 1.2 equiv) at -25^{o}C was added N-methylmorpholine (124 mg, 1.2 mmol, 1.2 equiv), the mixture was stirred at same temperature for 10 min and filtered. The filtrate was cooled to -20^{o}C and to it was added $NaBH_4$**,** followed by 2 ml of water immediately afterwards. The reaction mixture was stirred at same temperature for 5 min. then gradually warmed to room temperature for 25 min, poured into water(10ml) 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 to yield benzyl ((2R)-(3R)-3-(tert-butoxy)-1-hydroxybutan-2-yl)carbamate as a clear oil. No further purification was required for next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.16 (m, 5H), 5.25 (d, *J* = 8.0 Hz, 1H), 5.02 (s, 1H), 4.04 (ddd, *J* = 12.0, 8.9, 2.8 Hz, 1H), 3.92 – 3.75 (m, 1H), 3.59 (dddd, *J* = 32.3, 14.6, 8.2, 4.3 Hz, 2H), 1.10 (s, 9H), 1.09 – 1.06 (m, 3H).

Step 2: To a solution of benzyl ((2R,3R)-3-(tert-butoxy)-1-hydroxybutan-2-yl)carbamate (5.88 g, 19.9 mmol) in 100 mL DMF 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. EtOAc (100 mL) was added and the resulting mixture was stirred for 5 min to form a clear two layer solution. After separation, the aqueous phase was extracted with EtOAc (100 mL x 3). The combined organic solution was washed with brine (80 mL), dried over Na2SO4, filtered and concentrated. Flash column chromatography (EtOAc/Heptane 0 to 70%) gave 5.90 g of (R)-4-((R)-1-(tert-butoxy)ethyl)-3-(4-methoxybenzyl)oxazolidin-2-one with minor impurities (96%). Major product 1H NMR (400 MHz, MeOD) δ 7.38 - 7.29 (m, 2H), 6.99 - 6.93 (m, 2H), 4.68 - 4.58

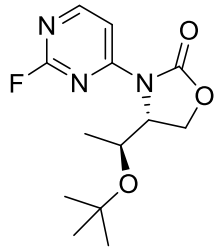
(m, 1H), 4.33 (dd, J = 9.3, 4.5 Hz, 1H), 4.27 - 4.17 (m, 2H), 3.89 (dd, J = 6.4, 4.8 Hz, 1H), 3.81 (s, 3H), 3.65 (dd, J = 9.0, 4.6 Hz, 1H), 1.09 (s, 9H), 1.02 (d, J = 6.3 Hz, 3H). MS m/z $(M + H)^+$ C17H26NO4: measured 308.2, calcd 308.4.

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 CH2Cl2 (40 mL) was treated with TFA (40 mL) at room temperature for 20 min. The reaction mixture was concentrated in vacuo, then diluted with CH2Cl2 (~50 mL), and again concentrated. This procedure was repeated three times to remove TFA. Flash column chromatography (EtOAc/Heptane 30 ~ 100%) gave 3.81 g (R)-4-((R)-1-hydroxyethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (79%). 1H NMR (400 MHz, MeOD) δ 7.31 - 7.21 (m, 2H), 7.00 - 6.87 (m, 2H), 4.67 (d, J = 15.0 Hz, 1H), 4.34 - 4.18 (m, 3H), 3.95 (q, J = 6.2 Hz, 1H), 3.80 (s, 3H), 3.68 (dt, J = 8.7, 5.5 Hz, 1H), 1.09 (d, J = 6.4 Hz, 3H). MS m/z (M + H)+ C13H18NO4: measured 252.2, calcd 252.3.

Step 4: To a cooled (0 °C) solution of (R)-4-((R)-1-hydroxyethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (2.27 g, 9.04 mmol) in 30 mL MeCN were added triethylamine (11.4 mL, 82 mmol) followed by perfluoro-1-butanesulfonyl fluoride (4.9 mL, 27.3 mmol) and NEt3(HF)3 (4.5 mL, 27.6 mmol) and the resulting mixture was stirred at 0 °C for 70 min. The reaction mixture was diluted with water (60 mL) and extracted with EtOAc (3 x 60 mL). Combined organics were washed with water (70 mL), brine (70 mL), dried over Na2SO4, filtered and concentrated. Flash column chromatography (EtOAc/heptane 5 to 70%) gave 2.19 g (R)-4-((S)-1-fluoroethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (96%). 1H NMR (400 MHz, CDCl3) δ 7.25 - 7.20 (m, 2H), 6.92 - 6.83 (m, 2H), 4.87 (d, J = 15.1 Hz, 1H), 4.75 (dqd, J = 47.6, 6.6, 2.1 Hz, 1H), 4.26 (td, J = 9.2, 1.4 Hz, 1H), 4.17 - 4.05 (m, 2H), 3.81 (s, 3H), 3.71 (dddd, J = 19.8, 9.5, 5.8, 2.1 Hz, 1H), 1.29 (dd, J = 23.1, 6.2 Hz, 3H). MS m/z $(M + H)^+$ C13H17FNO3: measured 254.5, calcd 254.3.

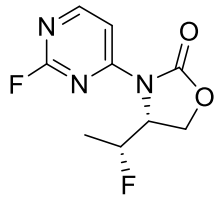
Step 5: A solution of (R)-4-((S)-1-fluoroethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (1.98 g 7.8 mmol) in 40 mL TFA was heated at 65 °C for 16 h. The reaction mixture was concentrated to remove TFA. Flash column chromatography (EtOAc/CH2Cl2, 0 to 100%) gave 0.91 g (R)-4-((S)-1-fluoroethyl)-oxazolidin-2-one as a pale brown solid (88%). TLC (1:2 heptane:EtOAc) Rf = 0.25. 1H NMR (400 MHz, CDCl3) δ 5.60 (br s, 1H), 4.72 - 4.54 (m, 1H), 4.51 (td, J = 8.9, 0.9 Hz, 1H), 4.32 (dd, J = 9.2, 4.8 Hz, 1H), 4.02 - 3.88 (m, 1H), 1.38 (dd, J = 24.0, 6.3 Hz, 3H).

Step 6: To a cooled (0 °C) solution of 2,4-difluoropyrimidine (160 mg, 1.375 mmol) and (R)-4-((S)-1-fluoroethyl)oxazolidin-2-one (183 mg, 1.375 mmol) in DMF (Volume: 4.6 mL) was added NaH (60% in mineral oil, 66.0 mg, 1.650 mmol). The resulting mixture was stirred at 0 °C for 30 min and at room temperature for 2 hr. Desired product was observed from LC-MS. The mixture was quenched with brine (1 ml). Diluted w/ EtOAc (20 ml) and water (10 ml), and separated layers. The organic was extracted with an additional 20 mL EtOAc. The combined organics were washed with brine, dried, and concentrated. Crude was purified through flash column chromatography (10-100% EtOAc/Heptane) to give 210 mg (R)-4-((S)-1-fluoroethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one as a white solid (67%). 1H NMR (400 MHz, CDCl3) δ 8.51 (dd, J = 5.8, 2.1 Hz, 1H), 8.20 (dd, J = 5.7, 3.7 Hz, 1H), 5.33 (dqd, J = 49.5, 6.6, 1.3 Hz, 1H), 4.77 (dddd, J = 26.5, 9.1, 3.4, 1.4 Hz, 1H), 4.65 (dd, J = 9.0, 3.4 Hz, 1H), 4.50 (td, J = 9.0, 1.3 Hz, 1H), 1.43 (dd, J = 23.1, 6.6 Hz, 3H). MS m/z $(M + H)^+$ C9H10F2N3O2: measured 230.1, calcd 230.2.



(R)-4-((S)-1-(tert-butoxy)ethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one was synthesized by the similar methods as described for (R)-4-((R)-1-(tert-butoxy)ethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one.

^{1}H NMR (400 MHz, CDCl3) δ 8.47 (dd, J=5.87, 2.35 Hz, 1 H), 8.18 (dd, J=5.87, 3.91 Hz, 1 H), 4.70 (dd, J=8.41, 2.93 Hz, 1 H), 4.54 - 4.63 (m, 1 H), 4.33 - 4.41 (m, 1 H), 4.20 - 4.31 (m, 1 H), 1.19 (d, J=6.65 Hz, 3 H), 0.99 (s, 9 H). MS m/z $(M + H)^+$ C13H19FN3O3: measured 284.2, calcd 284.3



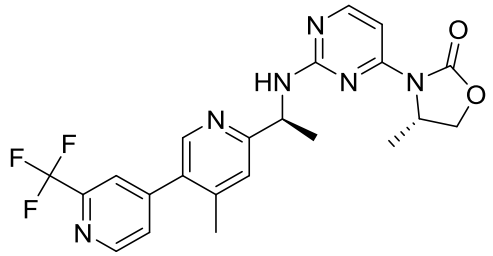
(R)-4-((R)-1-fluoroethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one

Step 1: To a solution of (R)-4-((S)-1-(tert-butoxy)ethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one (780 mg, 2.75 mmol) in DCM (3 ml) was added trifluoroacetic acid (3.18 mL, 42 mmol) at rt. The solution was stirred at rt for 1.5 hr. The mixture was concentrated, diluted with EtOAc (20 ml), washed with saturated NaHCO3 solution, brine, dried over NaSO4, and concentrated. Crude material was purified through flash column chromatography (10-100% EtOAc/Heptane) to provide 468 mg of (R)-3-(2-fluoropyrimidin-4-yl)-4-((S)-1-hydroxyethyl)oxazolidin-2-one a white solid (75%)

^{1}H NMR (400 MHz, MeOD) δ 8.52 (dd, J = 5.8, 2.3 Hz, 1H), 8.20 (dd, J = 5.9, 3.9 Hz, 1H), 4.71 (ddd, J = 8.8, 3.3, 1.6 Hz, 1H), 4.61 (dd, J = 8.6, 3.3 Hz, 1H), 4.51 - 4.43 (m, 2H), 1.19 (d, J = 6.7 Hz, 3H). MS m/z $(M+H)^+$ C9H11FN3O3: measured 228.1, calcd 228.2.

Step 2: To a solution of (R)-3-(2-fluoropyrimidin-4-yl)-4-((S)-1-hydroxyethyl)oxazolidin-2-one (221 mg, 0.973 mmol) in MeCN (Volume: 0.21 ml) were added triethylamine (1220 µl, 8.75 mmol) followed by perfluorobutanesulfonyl fluoride (525 µl, 2.92 mmol) and triethylamine trihydrofluoride (475 µl, 2.92 mmol). The resulting mixture was stirred at 0 °C for 16 hr. The reaction mixture was filtered through a pad of celite, rinsed with EtOAc and concentrated. The crude material with impurities was used to next step without further purification. MS m/z $(M + H)^+$ C9H10F2N3O2: measured 230.4, calcd 230.2.

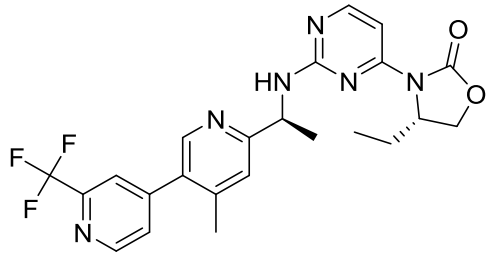
The following compounds (**9**, **10**, **11**, **12**, **13**, **14**) were prepared using general procedure B for SnAr reaction.



Compound 9

(S)-4-methyl-3-(2-(((S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one.

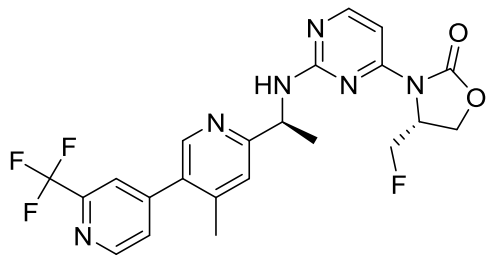
1H NMR (400 MHz, MeOD) δ 8.80 (d, J = 5.0 Hz, 1H), 8.38 (s, 1H), 8.14 (d, J = 6.0 Hz, 1H), 7.84 (s, 1H), 7.67 (dd, J = 5.0, 1.7 Hz, 1H), 7.47 (s, 1H), 7.34 (d, J = 5.8 Hz, 1H), 5.07 (q, J = 7.4 Hz, 1H), 4.75 (br s, 1H), 4.48 (d, J = 8.4 Hz, 1H), 4.04 (d, J = 7.1 Hz, 1H), 2.31 (s, 3H), 1.58 (d, J = 7.1 Hz, 3H), 0.81 (br s, 3H). HRMS *m/z* $(M + H)^+$ C22H22F3N6O2: measured 459.1754, calcd 459.4532



Compound 10

(S)-4-ethyl-3-(2-(((S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one

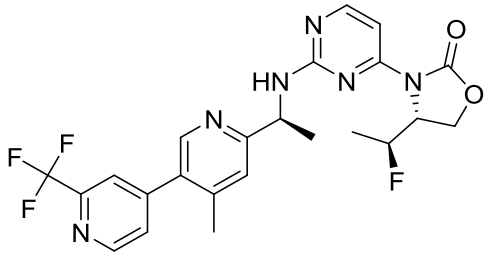
1H NMR (400 MHz, MeOD) δ 8.81 (d, J = 5.0 Hz, 1H), 8.39 (s, 1H), 8.15 (d, J = 6.1 Hz, 1H), 7.84 (s, 1H), 7.68 (dd, J = 5.0, 1.6 Hz, 1H), 7.44 (s, 1H), 7.37 (d, J = 5.8 Hz, 1H), 5.09 (q, J = 7.6, 7.2 Hz, 1H), 4.66 (br s, 1H), 4.44 (t, J = 8.5 Hz, 1H), 4.20 (d, J = 5.9 Hz, 1H), 2.32 (s, 3H), 1.59 (d, J = 7.1 Hz, 3H), 1.22 (br s, 2H), 0.64 (br s, 3H). HRMS *m/z* $(M + H)^+$ C23H24F3N6O2: measured 473.1926, calcd 473.4802.



Compound 12

(R)-4-(fluoromethyl)-3-(2-(((S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one

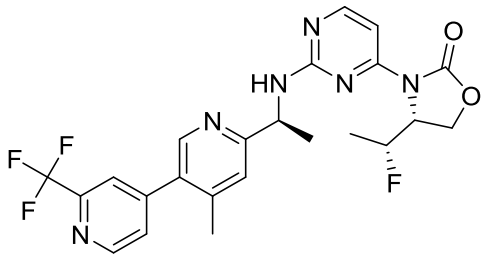
1H NMR (400 MHz, MeOD) δ 8.81 (d, J = 5.0 Hz, 1H), 8.38 (s, 1H), 8.17 (d, J = 5.3 Hz, 1H), 7.85 (s, 1H), 7.69 (dd, J = 5.0, 1.6 Hz, 1H), 7.47 (s, 1H), 7.38 (d, J = 5.8 Hz, 1H), 5.05 - 4.96 (m, 1H), 4.84 (br s, 1H), 4.52 (t, J = 8.9 Hz, 1H), 4.46 - 4.38 (m, 1H),4.10- 3.74 (m, 2H), 2.32 (s, 3H), 1.57 (d, J = 7.1 Hz, 3H). HRMS *m/z* $(M + H)^+$ C22H21F4N6O2: measured 477.1631, calcd 477.4436.



Compound 13 (IDH305)

(R)-4-((S)-1-fluoroethyl)-3-(2-(((S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one

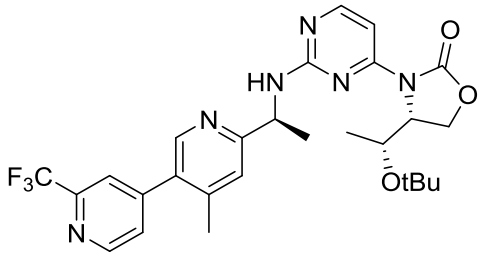
1H NMR (400 MHz, MeOD) δ 8.81 (d, J = 5.0 Hz, 1H), 8.41 (s, 1H), 8.18 (s, 1H), 7.86 (s, 1H), 7.69 (dd, J = 5.0, 1.6 Hz, 1H), 7.44 (s, 1H), 7.41 (d, J = 5.8 Hz, 1H), 5.08 (br s, 1H), 4.73 (br d, J = 26.0 Hz, 1H), 4.56 - 4.27 (m, 3H), 2.32 (s, 3H), 1.58 (d, J = 7.1 Hz, 3H), 1.12 (br s, 3H). HRMS *m/z* $(M + H)^+$ C23H23F4N6O2: measured 491.1782, calcd 491.4706.



Compound 14

(R)-4-((R)-1-fluoroethyl)-3-(2-(((S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one.

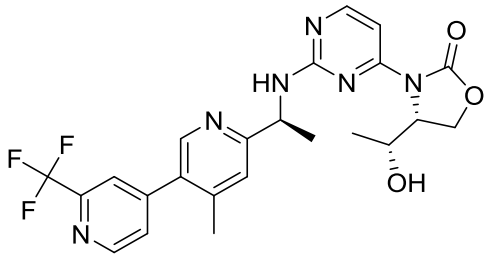
^{1}H NMR (400 MHz, MeOD) δ 8.82 (d, J = 5.0 Hz, 1H), 8.37 (s, 1H), 8.18 (s, 1H), 7.78 - 7.65 (m, 1H), 7.46 (s, 1H), 7.37 (d, J = 5.8 Hz, 1H), 4.99 (br s, 2H), 4.57 - 4.30 (m, 2H), 4.06 (br d, J = 41.6 Hz, 1H), 2.32 (s, 3H), 1.59 (d, J = 7.1 Hz, 3H), 0.90 (br s, 3H). HRMS m/z $(M + H)^+$ C23H23F4N6O2: measured 491.1839, calcd 491.4706.



Intermediate of compound 11

(R)-4-((R)-1-(tert-butoxy)ethyl)-3-(2-(((S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one

1H NMR (400 MHz, CDCl3) δ 8.85 (d, J = 5.0 Hz, 1H), 8.41 (s, 1H), 8.23 (d, J = 5.8 Hz, 1H), 7.68 (s, 1H), 7.49 (dd, J = 5.2, 2.7 Hz, 2H), 7.26 (s, 1H), 5.29 (p, J = 6.9 Hz, 1H), 4.77 (ddd, J = 8.3, 4.4, 3.0 Hz, 1H), 4.64 (dd, J = 9.3, 3.0 Hz, 1H), 4.46 (qd, J = 6.4, 4.5 Hz, 1H), 4.36 (t, J = 9.0 Hz, 1H), 2.33 (s, 3H), 1.63 (d, J = 6.8 Hz, 3H), 1.28 (s, 9H), 1.05 (d, J = 6.5 Hz, 3H). HRMS m/z $(M + H)^+$ C27H32F3N6O3: measured 545.2493, calcd 545.5872.



Compound 11

(R)-4-((R)-1-hydroxyethyl)-3-(2-(((S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one

To a solution of (R)-4-((R)-1-(tert-butoxy)ethyl)-3-(2-(((S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one (121 mg, 0.222 mmol) in DCM (2 ml) at -78 °C was added trifluoroacetic acid (1 mL, 12.98 mmol). The solution was stirred at 0 ^oC for 5 hr. The mixture was concentrated, diluted with 10 mL DCM and stirred with solid MP-carbonate to remove TFA. Filtered, concentrated and purified through RP-HPLC to give 60 mg of (R)-4-((R)-1-hydroxyethyl)-3-(2-(((S)-1-(4-methyl-2'-(trifluoromethyl)-[3,4'-bipyridin]-6-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one as a white solid (55%). 1H NMR (400 MHz, MeOD) δ 8.81 (d, J = 5.0 Hz, 1H), 8.38 (s, 1H), 8.15 (d, J = 5.1 Hz, 1H), 7.90 (s, 1H), 7.72 (dd, J = 5.0, 1.6 Hz, 1H), 7.45 (s, 1H), 7.37 (d, J = 5.8 Hz, 1H), 5.14 (q, J = 7.0 Hz, 1H), 4.79 (br s, 1H), 4.52 (dd, J = 9.3, 2.7 Hz, 1H), 4.38 (t, J = 8.9 Hz, 1H), 3.65 (br s, 1H), 2.32 (s, 3H), 1.58 (d, J = 7.1 Hz, 3H), 0.77 (br s, 3H). HRMS *m/z* $(M + H)^+$ C23H24F3N6O3: measured 489.1881, calcd 489.4792.

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 $MgCl_2$, 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).

MCF10A-IDH1$^{R132H/+}$ Proliferation assay [21]

MCF10A: parental, IDH1R132H heterozygous mutant, and PIK3CAE545K heterozygous mutant cells (Horizon Discovery) were cultured in DMEM/F12 media with hydrocortisone (0.5 mg/mL), cholera toxin (100 ng/mLO), insulin (10 µg/mL), horse serum (4%), and recombinant EGF (20 ng/mL); for growth factor depleted conditions, serum was reduced to 2% and EGF was omitted.

IDH305 (13) – IDH1$^{R132H+/+}$ crystallography

Protein expression and purification

The R132H mutant IDH1 was expressed in *E. coli* cells using a construct expressing human IDH1 bearing the R132H mutation with N-terminal 6xHis tag and PreScission protease cleavage site. ***E. coli*** strain Rosetta™2(DE3) (Novagen) transformed with the IDH1^{R132H} expression construct was grown at 37˚C in shaker flasks to an OD_{600} of 0.8 in Terrific Broth (Teknova) with 50µg/ml of Kanamycin and 34µg/mL of chloramphenicol, then cooled down to below 18˚C. IDH1^{R132H} protein expression was induced by addition of Isopropyl-**β**-D-thiogalactopyranoside (IPTG) to 0.2 mM for 18 hours at 18˚C. The harvested cells were resuspended in lysis buffer (50mM Tris pH=7.4, 500mM NaCl, 20mM Imidazole, 0.5mM DTT) containing DNAse I and protease inhibitors (cOmplete EDTA-free protease inhibitor tablets (1 tablet per 50mL of buffer) and 200uM PMSF), and lysed on ice using a microfluidizer (M-110L, Microfluidics). After lysis, Trition X-100 was added to 0.1% and stirred at 4˚C for 30 minutes. The cleared lysate containing His-tagged IDH1^{R132H} fusion protein was then loaded onto 2x 5mL HisTrap FF crude columns (GE Healthcare), and the His-tagged protein eluted with Ni Elution Buffer (50mM Tris pH=7.4, 150mM NaCl, 200mM Imidazole, 0.5mM DTT). Peak eluted fractions were concentrated to 30mL, EDTA was added to 1mM and GST-PreScission protease (*in house*) was added to 3U/100µg of protein. The sample was dialyzed against 2L Dialysis Buffer I (20mM Tris pH=7.4, 150mM NaCl, 0.5mM DTT, 50mM Imidazole) for 6 hours at 4˚C then dialyzed against 2L of Dialysis Buffer II (20mM Tris pH=7.4, 150mM NaCl, 0.5mM DTT) 4°C for at least 6 more hours. GST-PreScission cleaved sample was rocked with Glutathione Agarose Beads, spun down and then the supernatant was loaded through a 5mL HisTrap HP column (GE Healthcare) and the flow through was collected. The collected flow through was then diluted with 20mM Tris pH 7.4 and 0.5mM DTT until the conductivity dropped to less than 5 mS/cm and loaded onto a HiTrap Q column (GE Healthcare). The tag-free IDH1^{R132H} protein was then collected from the flow through of HiTrapQ column (GE Healthcare) and further purified by size exclusion chromatography (HiLoad 26/60 Superdex 200, GE Healthcare). The purified IDH1^{R132H} was concentration to 5 mg/mL in buffer (20 mM Tris pH7.5, 150mM NaCl), and frozen in liquid N_2 for storage at -80°C.

Crystallization, data collection and structure determination

To obtained crystals of IDH1^{R132H}:IDH305 complex, IDH1^{R132H} was diluted to 1mG/mL in storage buffer then incubated with IDH305 at 2-5x molar excess of protein concentration. The mixture was concentrated 10 fold prior to crystallization. The complexes were crystallized using sitting drop vapor diffusion method at 20 °C by mixing equal volumes (2 µL + 2 µL) was diluted to 1mg/mL in storage buffer then incubated with IDH305 at 2-5x molar excess of protein concentration. The mixture was concentrated 10 fold prior to crystallization. The complexes were crystallized using sitting frozen in liquid nitrogen for diffraction experiment.

Crystal of IDH1^{R132H}: IDH305 complex was determined to have the orthorhombic space group $P2_12_12_1$ with two complexes in the asymmetric unit. Each complex comprises a dimer of IDH1^{R132H}, with each protein in complex with one NADPH molecule (co-purified with protein) and one compound molecule. A citrate molecule is modeled at the substrate binding site for each protein, as a result of presence of high concentration of trisodium citrate dihydrate in the crystallization solution. An additional IDH305 molecule is modeled at the protein dimer interface, which likely resulted from high concentration of IDH305 present in the crystallization sample.

All diffraction data were collected at the X-ray Operations and Research beamline 17-ID at the Advanced Photon Source, Argonne National Laboratory, with the crystal kept at 100K and wavelength of X-ray beam at 1.0 Å. The diffraction data from all crystals were integrated and scaled using autoPROC (6). The structures were solved by molecular replacement with Phaser (1) using another $IDH1^{R132H}$ structure (in-house data) as a starting model. Model building and refinement was performed using COOT (5) and PHENIX (2). Statistics for the collected data and refined model are summarized in Table 1. PDB coordinates and accompanying structure factors was deposited to protein data bank (PDB 6B0Z).

Reference Citation (PHENIX and COOT)

1. McCoy AJ, Grosse-Kunstleve RW, Adams PD, Winn MD, Storoni LC, et al. (2007) **Phaser** crystallographic software. J Appl Crystallogr 40: 658–674. doi: 10.1107/s0021889807021206.
2. Adams PD, Afonine PV, Bunkóczi G, Chen VB, Davis W, Echols N, Headd JJ, Hung L-W, Kapral GJ, Grosse-Kunstleve RW, McCoy AJ, Moriarty NW, Oeffner R, Read RJ, Richardson DC, Richardson JS, Terwilliger TC, and Zwart PH. (2010) **PHENIX**: a comprehensive Python-based system for macromolecular structure solution. Acta Cryst. D66, 213-221.
3. Afonine PV, Grosse-Kunstleve RW, Echols N, Headd JJ, Moriarty NW, Mustyakimov M, Terwilliger TC, Urzhumtsev A, Zwart PH, and Adams PD. (2012) Towards automated crystallographic structure refinement with **phenix.refine**. Acta Cryst. D68, 352-367.
4. Chen VB, Arendall WB, Headd JJ, Keedy DA, Kapral GL, Murry LW, Richardson JS, and Richardson DC. (2010) **MolProbity**: all-atom structure validation for macromolecular crystallography. Acta Cryst. D66, 16-21 .
5. P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan. (2010) Features and development of **Coot**. Acta Crystallogr. D Biol. Crystallogr. 66, 486.
6. Vonrhein, C., Flensburg, C., Keller, P., Sharff, A., Smart, O., Paciorek, W., Womack, T. & Bricogne, G. (2011) Data processing and analysis with the **autoPROC** toolbox. Acta Cryst. D67, 293-302.

Summary of X-ray diffraction data collection and structure refinement for IDH1-R132H:NADPH:IDH305

	IDH1-R132H :NADPH: IDH305 **
Data collection	
Space group	P212121
Cell dimensions	
a, *b*, *c* (Å)	81.50, 155.31, 163.05
α, β, γ (°)	90, 90, 90
Resolution (Å)	163.054 – 2.334 (2.341-2.334) *
R_{sym} or R_{merge}	0.082 (0.639)
$I / \sigma I$	15.0 (2.8)
Completeness (%)	100.0 (100.0)
Redundancy	6.6 (6.8)
Refinement	
Resolution (Å)	43.700 - 2.334 (2.417 – 2.334)
No. reflections used in refinement	88573 (8773)
No. reflections used for R_{free}	4441 (459)
R_{work} / R_{free}	0.186 (0.237) / 0.235 (0.293)
No. atoms	
Protein	12831
Ligand/ion	454
Water	511
B-factors	
Protein	47.5
Ligand/ion	51.6
Water	41.8
RMS deviations	
Bond lengths (Å)	0.07
Bond angles (°)	1.47

* Values in parentheses are for highest-resolution shell.
**A single crystal is used for the structure determination

Liver Microsomal Stability. The in vitro intrinsic clearance (Cl_{int}) in rat liver microsomes (RLM) (BD Gentest, Woburn, MA) was determined based on published method (Obach, R. S. (1999) 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. 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 $MgCl_2$. The half-life ($t_{1/2}$) 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˚C under 5% CO_2 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 37˚C under 5% CO_2 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[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 **IDH305** 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 was placed in a 1 mL 96 well collection plate and stored at -20°C until analysis. Plasma concentrations of **IDH305** 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˚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[21].

HCT116-IDH1$^{R132H/+}$ single dose PK/PD study data

Table SI-1. Plasma concentration, normalized tumor 2-HG concentration and percent tumor 2-HG inhibition by **IDH305** following a single oral dose at 200 mg/kg dose in HCHZ2H1 xenograft model. Data graphed in Figure 3A,B.

Time post dose (h)	Total Plasma IDH305 Conc. (nM) (Mean ± SD)	Estimated Free Plasma IDH305 Conc. (nM) (Mean ± SD)	Normalized 2-HG (ng/mg sample weight) (Mean ± SEM)	% 2-HG Inhibition (Mean ± SEM)
0	(Vehicle)	-	301.5 ± 81.4	0
0.25	4797 ± 1228	556 ± 142	ND	ND
0.5	7297 ± 2129	846 ± 247	ND	ND
1	11959 ± 2021	1387 ± 234	ND	ND
4	14185 ± 2974	1645 ± 345	61.0 ± 42.1	79.8 ± 14
8	11830 ± 4794	1372 ± 556	38.7 ± 4.6	87.2 ± 1.5
16	487± 185	57 ± 21	70.2 ± 28.6	76.7 ± 9.5
20	148 ± 137	17 ± 16	102.5 ± 8.5	66.0 ± 2.8
24	10 ± 5	1.2 ± 0.6	183.1 ± 31	39.3 ± 10.3
48	3 ± 1	0.3 ± 0.1	443.1 ± 34.2	-47.0 ± 11.3

Table SI-1 notes: The LLOQ for **IDH305** was 10 nM; ND, not determined, fu (fraction unbound) in mouse plasma: 0.116.

Table SI-2. Plasma concentration, normalized tumor 2-HG concentration and percent tumor 2-HG inhibition by **IDH305** following a single oral dose at 30, 100 or 300 mg/kg in HCHZ2H1 xenograft model. Data graphed in Figure 3-C.

Dose (mg/kg)	Time (h)	Total Plasma IDH305 Conc. (nM) (Mean ± SD)	Estimated Free Plasma IDH305 Conc. (nM) (Mean ± SD)	Normalized 2-HG (ng/mg sample weight) (Mean ± SEM)	% Inhibition (Mean ± SEM)
0 (vehicle)	0	-	-	79.4 ± 38	
30	0.25	1387 ± 207	161 ± 24	ND	ND
	0.5	2068 ± 416	240 ± 48	ND	ND
	1	3652 ± 96	424 ± 11	ND	ND
	12	39 ± 24	4 ± 3	42.7 ± 21.3	46.2 ± 26.9
	16	BQL	BQL	68.3 ± 5.9	13.9 ± 7.5
	20	BQL	BQL	75.5 ± 26.1	4.9 ± 32.9
	24	BQL	BQL	73.1 ± 13.1	7.9 ± 16.5
100	0.25	3196 ± 2312	371 ± 268	ND	ND
	0.5	3812 ± 507	442 ± 59	ND	ND
	1	6377 ± 2312	740 ± 268	ND	ND
	12	304 ± 284	35 ± 33	13.1 ± 1.8	83.6 ± 2.2
	16	19 ± 0	2 ± 0	25.6 ± 22	67.7 ± 27.7
	20	26 ± 0	3 ± 0	36.5 ± 7.2	54.0 ± 9.0
	24	BQL	BQL	29.2 ± 18.4	63.2 ± 23.1
300	0.25	6738 ± 891	782 ± 103	ND	ND
	0.5	9726 ± 3677	1128 ± 427	ND	ND
	1	12384 ± 1144	1436 ± 133	ND	ND
	12	3230 ± 812	375 ± 94	2.5 ± 2.2	96.8 ± 2.8
	16	1451 ± 718	168 ± 83	4.2 ± 2.9	94.7 ± 3.6
	20	877 ± 1129	102 ± 131	11 ± 6.5	86.1 ± 8.2
	24	17 ± 0	2 ±	20.7 ± 5.3	73.9 ± 6.7

Table SI-2 notes: *BQL, below quantitation limit: The LLOQ for **IDH305** was 10 nM; ND, not determined

Patient-derived tumor xenograft (PDX) Tumor Models

HMEX2838-IDH1$^{R132C/+}$ xenograft model

The HMEX2838 model originated from a 59 year old male with IDH1^{R132C}, NF1 mutant metastatic melanoma cancer. The HMEX2700 model originated from a 50 year old male with IDH1^{R132G} mutant, metastatic melanoma cancer. The model was implanted and expanded into nu/nu mice within NIBR Oncology at the Cambridge site as described[24]. Tumors at passage P9-P13 were used for studies.

PDX Tumor passage

PDX tumors were propagated by serial passage of tumor fragments in nu/nu mice. Briefly, 3x3x3 mm fragments of fresh or frozen tumor were implanted subcutaneously into nu/nu female mice with the use of a trocar. The incision wound was sealed with Vetbond tissue glue (3M Cat#1469SB).

Tumor volume and efficacy assessment

Tumor volume was determined by measurement with calipers and calculated using a modified ellipsoid formula, where tumor volume (TV) (mm^3) = [((l x w^2) x 3.14159)) / 6], where l is the longest axis of the tumor and w is perpendicular to l. Mice were monitored for tumor growth, body weight and body condition. Anti-tumor activity was determined by percent treatment/control (%T/C) values, calculated using the following formula: %T/C = 100 $\times$ $\Delta T/\Delta C$ if $\Delta T \geq 0$; *or* % Regression = (-1 $\times$ (100 $\times$ $\Delta T/T_{initial}$)) if $\Delta T < 0$.

where: T = mean tumor volume (mTV) of the drug-treated group on day *F* (Final day); ΔT = mTV of the drug-treated group on day *F* – mTV of the drug-treated group on day *I* (initial day of dosing); $T_{initial}$ = mTV of the drug-treated group on day *I*; C = mTV of the control group on day *F*; and ΔC = mTV of the control group on day *F* – mTV of the control group on day *I*. %T/C values in the range of 100 to 42% are interpreted to have no anti-tumor activity. T/C values that are ≤ 42% and >10% are interpreted to have anti-tumor activity. %T/C values ≤ 10% or % regression values ≤ 10% are interpreted to be tumor stasis. %Regression values > 10% are interpreted as regressions. Tumor Volumes were expressed as mean ± standard error of the mean (SEM). Delta tumor volumes on day *F* were compared by Kruskal-Wallis Analysis of Variance on Ranks, followed by post-hoc Tukey test (SigmaPlot 12.1.0.15, Systat Software, Inc.). The P values for comparisons to vehicle group are reported.

Compound dose and administration

IDH305 was administered by oral gavage at a dosing volume of 10 mL/kg or 3 mL/kg at either 10, 30, 100 or 300 mg/kg p.o. once, or twice daily (bid) as indicated. Bid dosing was performed q12. Animals were euthanized and excluded from analysis if tumor volume equal or exceeded 10% body weight, if body weight loss exceeded 15% for three consecutive days or if body weight loss achieved 20% or more for one day. There were no animals excluded in these studies.

HMEX2838-IDH1$^{R132C/+}$ PK/PD study data

Table SI-3. Plasma concentration, normalized tumor 2-HG concentration and percent tumor 2-HG inhibition by **IDH305** following a single oral dose at 30, 100 or 300 mg/kg in HMEX2838 tumor model. Data graphed in Figure 4-A.

Dose (mg/kg)	Time (h)	Total Plasma IDH305 Conc. (nM) (Mean ± SD)	Estimated Free Plasma IDH305 Conc. (nM) (Mean ± SD)	Normalized 2-HG (ng/mg sample weight) (Mean ± SEM)	% Inhibition (Mean ± SEM)
0 (vehicle)	0	-	-	2511.9 ± 21.8	0
30	0.5	7966	8027.5 ± 296.8	ND	ND
	1	3241	3635.2 ± 363.6	ND	ND
	2	800	1059.2 ± 550	2084.6 ± 183.1	16.5 ± 7.3
	4	832	530.3 ± 355.4	1392.5 ± 133.3	44.2 ± 5.3
	8	90	91.5	1665.6 ± 183.5	33.3 ± 7.3
	16	BQL*	BQL*	3113.2 ± 337.3	-24.7 ± 13.5
	20	BQL*	BQL*	2328.7 ± 574.1	6.7 ± 23.0
	24	BQL*	BQL*	3053.8 ± 229.4	-22.3 ± 9.2
	48	BQL*	BQL*	2842.6 ± 737.2	-13.9 ± 29.5
100	0.5	20976	17124.0 ± 3545.9	ND	ND
	1	17389	13562.4 ± 3432.7	ND	ND
	2	5463	5400.4 ± 446.4	1436.0 ± 104.1	42.5 ± 4.2
	4	8746	13620.6 ± 4980.5	1068.5 ± 150.4	57.2 ± 6
	8	1131	2096.8 ± 1983.5	442.4 ± 24.2	82.3 ± 1
	16	BQL*	BQL*	2499.6 ± 200.2	-0.1 ± 8.0
	20	BQL*	BQL*	2334.6 ± 309.4	6.5 ± 12.4
	24	BQL*	BQL*	3060.4 ± 129.8	-22.6 ± 5.2
	48	BQL*	BQL*	2558.9 ± 475.2	-2.5 ± 19
300	0.5	16261	22058.5 ± 5044.5	ND	ND
	1	13445	17273.6 ± 3404.5	ND	ND
	2	7155	8945.9 ± 1720	1459.7 ± 438.2	41.5 ± 17.6
	4	10506	10853.9 ± 1509.9	1083.7 ± 242.3	56.6 ± 9.7
	8	13358	12227.2 ± 2543.4	316.3 ± 40.7	87.3 ± 1.6
	16	11782	2854	49.4 ± 22.6	98.0 ± 0.9
	20	1664	8194	51.0 ± 61.2	98.0 ± 2.5
	24	121	BQL*	402.6 ± 424.2	83.9 ± 17
	48	BQL*	BQL*	2822.9 ± 258.8	-13.1 ± 10.4

Table SI-3 notes: *BQL, below quantitation limit: The LLOQ for **IDH305** was 10.2 nM ND, not determined.

HMEX2700- IDH1$^{R132G/+}$ xenograft model PK/PD data

Figure SI-1 HMEX2700- IDH1$^{R132G/+}$ PK/PD

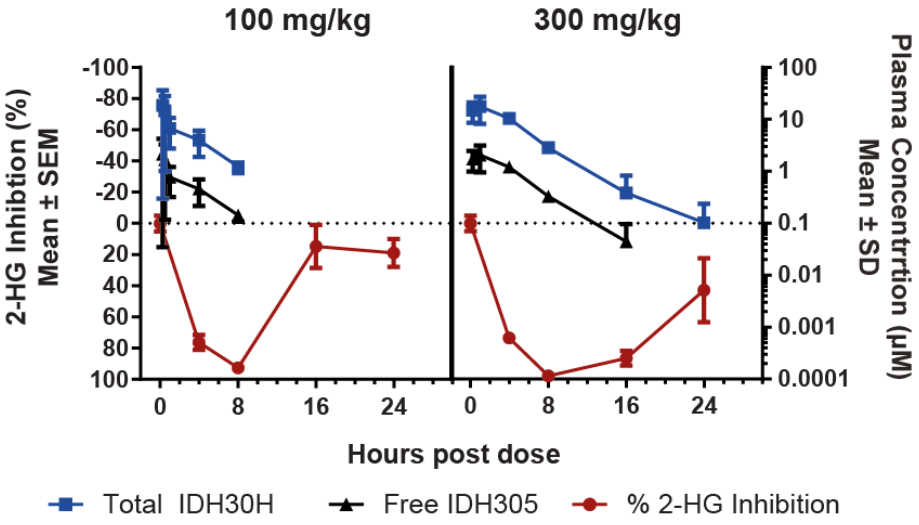


Figure SI-1: 2-HG reduction in IDH1$^{R132G/+}$ mutant HMEX2700 melanoma PDX model relative to vehicle treated tumors in correlation with total and calculated free **5** plasma concentration following a single 100 mg/kg or 300 mg/kg dose of **5**.

Table SI-4. Plasma concentration, normalized tumor 2-HG concentration and percent tumor 2-HG inhibition by **IDH305** following a single oral dose at 100 or 300 mg/kg dose in HMEX2700 xenograft model. Data graphed in Figure SI-1.

Dose (mg/kg)	Time (h)	Total Plasma IDH305 Conc. (nM) (Mean ± SD)	Estimated Free Plasma IDH305 Conc. (nM) (Mean ± SD)	Normalized 2-HG (ng/mg sample weight) (Mean ± SEM)	% Inhibition (Mean ± SEM)
0 (vehicle)	0	-	-	3594 ± 298	0 ± 4.79
	0.25	18430 ± 18129	2138 ± 2103	ND	ND
	0.5	14622 ± 13608	1696 ± 1579	ND	ND
	1	6684 ± 3914	775 ± 454	ND	ND
	4	3982 ± 2095	462 ± 243	854 ± 286	76.24 ± 4.59
	8	1236 ± 309	144 ± 36	267 ± 95	92.56 ± 1.53
	16	BQL		3061 ± 859	14.83 ± 13.8
	24	BQL		2912 ± 554	18.98 ± 8.91
	0.25	14930 ± 6383	1732 ± 741	ND	ND
	0.5	16826 ± 2993	1952 ± 348	ND	ND
	1	17653 ± 9504	2048 ± 1102	ND	ND
	4	10443 ± 900	1212 ± 104	952 ± 162	73.507 ± 2.6
	8	2842 ± 407	330 ± 47	78 ± 14	97.844 ± 0.22
	16	384 ± 443	45 ± 52	485 ± 277	86.504 ± 4.46
	24	103 ± 136	1732 ± 741	2050 ± 1265	42.954 ± 20.33

Table SI-4 notes: The LLOQ for **IDH305** was 10 nM; ND, not determined, fu (fraction unbound) in mouse plasma: 0.116.

HMEX2838-IDH1$^{R132C/+}$ multi-dose PK/PD/efficacy study data

Body Weight Change data

Figure SI-2 Percent body weight change in HMEX2838 xenograft bearing nude mice

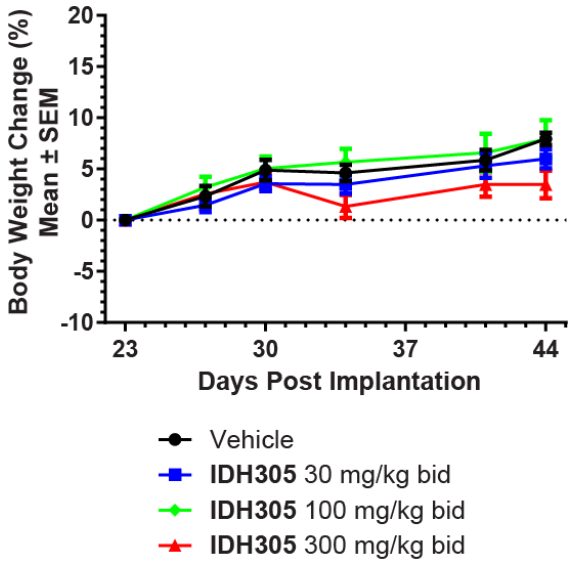


Figure SI-2. Percent body weight change relative to starting body weight in IDH1^{R132C} mutant HMEX2838 xenograft model treated with **5** bid at indicated doses for 21 days.

Table SI-5. Plasma concentration, normalized tumor 2-HG concentration and percent tumor 2-HG inhibition by **IDH305** following 21 days of treatment at 30, 100 or 300 mg/kg p.o. bid in HMEX2838 tumor model. Data graphed in Figure 4-B.

Dose (mg/kg)	Time (h)	Total Plasma IDH305 Conc. (nM) (Mean ± SD)	Estimated Free Plasma IDH305 Conc. (nM) (Mean ± SD)	Normalized 2-HG (ng/mg sample weight) (Mean ± SEM)	% Inhibition (Mean ± SEM)
0 vehicle	0	-	-	8102.1 + 482.4	0
	0 (Cmin)	2.7 ± 1.1	0 ± 0	ND	ND
	0.5	3069 ± 835.2	356 ± 96.7		
	1	2152.6 ± 603.3	250 ± 70.1	ND	ND
30	2	739 ± 206.9	85.6 ± 24.3	ND	ND
	4	356.6 ± 182.5	41 ± 21.2	6243.8 + 146.5	22.9 + 1.8
	8	82 ± 54	9.6 ± 6.5	5878.5 + 436.7	27.4 + 5.4
	12	22 ± 19.9	2.3 ± 2.5	6050.1 + 921.9	25.3 + 11.4
	0 (Cmin)	12 ± 0	1 ± 0	ND	ND
	0.5	8070 ± 986	936.3 ± 114.4		
	1	6066.3 ± 1966	703.3 ± 228.1	ND	ND
100	2	3080.6 ± 1186.2	357.3 ± 137.4	ND	ND
	4	1064.3 ± 83.7	123.6 ± 9.8	2656.5 + 159.7	67.2 + 2.0
	8	715.3 ± 103.1	83 ± 11.7	2718.0 + 895.1	66.5 + 11.0
	12	326 ± 233	38 ± 27.2	3066.2 + 282.5	62.2 + 3.5
	0 (Cmin)	1630.3 ± 759.3	189 ± 87.8	ND	ND
	0.5	14864 ± 3244.3	1724.3 ± 376.5		
	1	15018 ± 8808.3	1741.6 ± 1021.6	ND	ND
300	2	7791 ± 3931.9	903.6 ± 456.2	ND	ND
	4	7193.6 ± 1339.6	834.3 ± 155.6	78.3 + 54.1	99.0 + 0.7
	8	7205.6 ± 5285.6	836 ± 613.3	170.3 + 82.3	97.9 + 1.0
	48	4321.3 ± 2901.2	501 ± 336.3	53.3 + 14.1	99.3 + 0.2

Table SI-5 notes : ND: not determined. * LLOQ = 2.0 nM