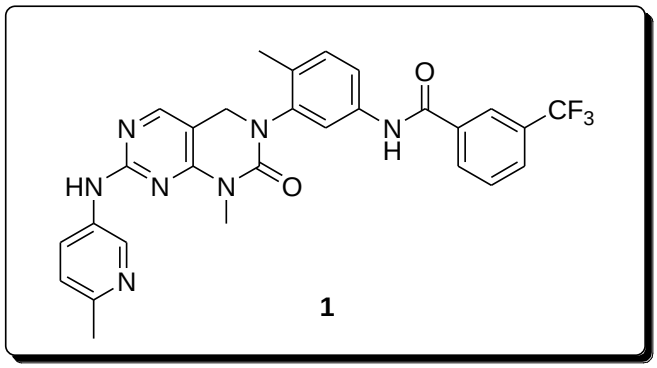
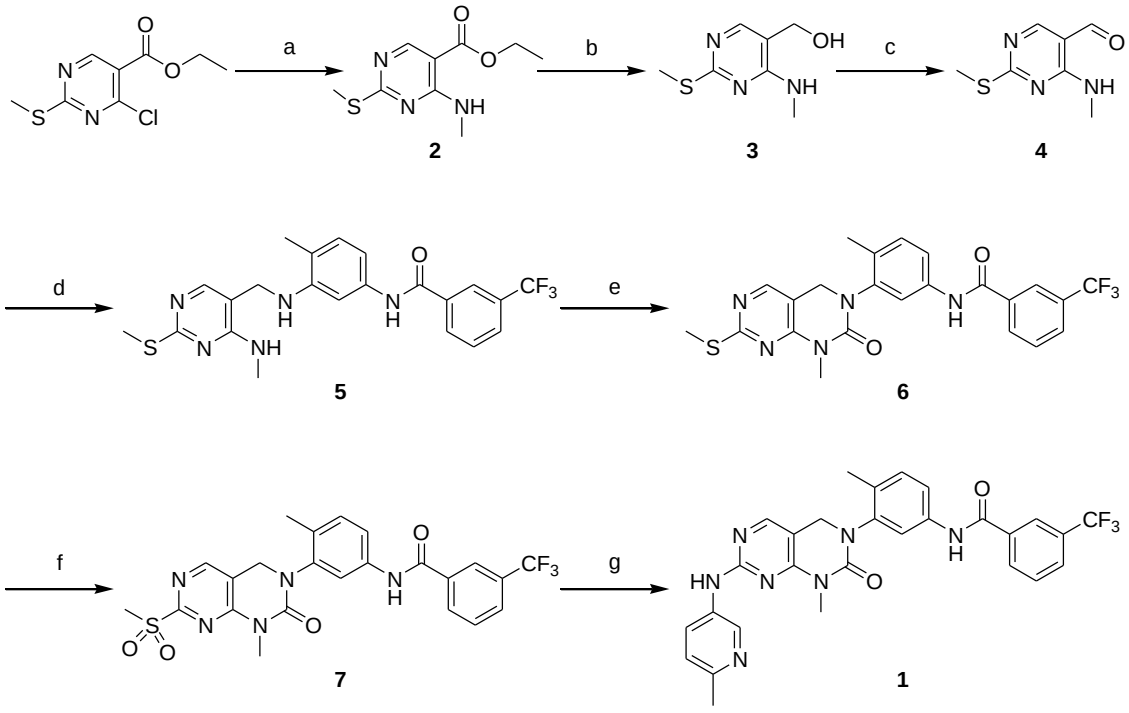
SUPPLEMENTAL DATA

1. Synthesis of GNF-7



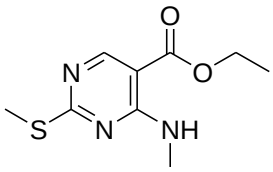
1



2 **3** **4** **5** **6** **7** **1**

a. methyl amine, THF, b. $LiAlH_4$, THF, c. MnO_2, DCM, d. aniline, $Na(CN)BH_3$, AcOH, MeOH, e. triphosgene, DIEA, THF, f. *m*CPBA, DCM, g. aniline, TFA, 1,4-dioxane

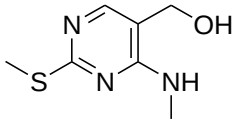
ethyl 4-(methylamino)-2-(methylthio)pyrimidine-5-carboxylate



To a solution of ethyl 4-chloro-2-(methylthio)pyrimidine-5-carboxylate (1.0 g, 4.3 mmol) in THF (14.3 mL) was added 2.0 M methylamine solution in THF (5.37 mL, 10.7 mmol) at 0 ^{o}C. After stirring at room temperature for 1 h, the reaction mixture was partitioned between ethyl acetate (50 mL) and sat. $NaHCO_3$ (30 mL). The organic phase was washed with brine, dried over $MgSO_4$ and concentrated. The resulting white solid 950 mg (97% yield) was used for the next step without further purification.

^{1}H NMR 600 MHz ($CDCl_3$) δ 8.60 (*s,* 1H), 8.16 (*bs*, 1H), 4.32 (*q*, *J* = 7.2 Hz, *J* = 13.8 Hz, 2H), 3.07 (*d*, *J* = 5.4 Hz, 3H), 2.54 (*s*, 3H), 1.36 (*t*, *J* = 7.2 Hz, 3H), MS *m/z*:228.2 (M + 1).

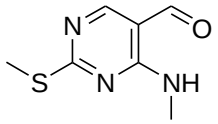
(4-(methylamino)-2-(methylthio)pyrimidin-5-yl)ethanol



To a solution of ethyl 4-(methylamino)-2-(methylthio)pyrimidine-5-carboxylate (300 mg, 1.32 mmol) in THF (6.6 mL) was added 2.0 M lithium aluminum hydride solution in THF (0.79 mL, 1.58 mmol) at 0 ^{o}C. The reaction mixture was stirred at room temperature for 2 h and treated with sat. NH_4Cl solution (2 mL). After stirring at room temperature for 30 min, the reaction mixture was filtered. The filtrate was partitioned between ethyl acetate (30 mL) and water (20 mL) and the organic phase was washed with brine, dried over $MgSO_4$ and concentrated. The resulting white solid 210 mg (86% yield) was used for the next step without further purification.

^{1}H NMR 600 MHz ($CDCl_3$) δ 7.61 (*s,* 1H), 5.90 (*bs*, 1H), 4.76 (*s*, 2H), 3.03 (*d*, *J* = 5.4 Hz, 3H), 2.51 (*s*, 3H), MS *m/z*:186.2 (M + 1).

4-(methylamino)-2-(methylthio)pyrimidine-5-carbaldehyde

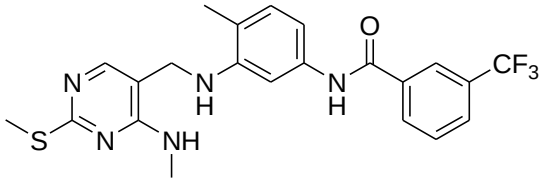


To a solution of (4-(methylamino)-2-(methylthio)pyrimidin-5-yl)methanol (210 mg, 1.14 mmol) in dichloromethane (3.8 mL) was added manganese(IV) oxide (980 mg, 11.4

mmol) at room temperature. After stirring at room temperature overnight, the reaction mixture was filtered and concentrated. The resulting crude product was chromatographed on silica gel with ethyl acetate/Hexane (1/9 to 1/4) to give 190 mg (89% yield) of the title product as a white solid.

^{1}H NMR 600 MHz ($CDCl_3$) δ 9.67 (*s*, 1H), 8.52 (*bs,* 1H), 8.27 (*s*, 1H), 3.10 (*d*, *J* = 5.4 Hz, 3H), 2.54 (*s*, 3H), MS *m/z*:184.2 (M + 1).

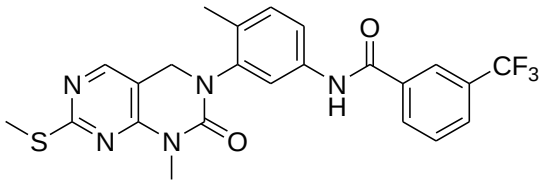
_N_-(4-methyl-3-((4-(methylamino)-2-(methylthio)pyrimidin-5-yl)methylamino)phenyl)-3-(trifluoromethyl)benzamide



To a solution of (4-(methylamino)-2-(methylthio)pyrimidin-5-yl)methanol (190 mg, 1.03 mmol) in methanol (5.2 mL) were added acetic acid (0.12 mL, 2.06 mmol), *N*-(3-amino-4-methylphenyl)-3-(trifluoromethyl)benzamide (304 mg, 1.03 mmol) and $Na(CN)BH_3$ (323 mg, 5.15 mmol) at room temperature. After stirring at room temperature overnight, the reaction mixture was partitioned between ethyl acetate (30 mL) and sat. $NaHCO_3$ (20 mL) solution. The organic phase was washed with brine and dried over $MgSO_4$ and concentrated. The resulting crude product was chromatographed on silica gel with ethyl acetate/Hexane (1/4 to 2/3) to give 334 mg (63% yield) of the title product as a white solid.

^{1}H NMR 600 MHz ($CDCl_3$) δ 8.69 (*s*, 1H), 8.09 (*s,* 1H), 8.00 (*d*, *J* = 8.4 Hz, 1H), 7.74 (*s*, 1H), 7.69 (*d*, *J* = 7.8 Hz, 1H), 7.49 (*t*, *J* = 7.8 Hz, 1H), 7.19 (*s*, 1H), 6.97 (*d*, *J* = 7.8 Hz, 1H), 6.93 (*d*, *J* = 8.4 Hz, 1H), 5.83 (*m*, 1H), 3.92 (*s*, 2H), 3.40 (*bs*, 1H), 2.97 (*d*, *J* = 5.4 Hz, 3H), 2.48 (*s*, 3H), 2.03 (*s*, 3H), MS *m/z*:462.5 (M + 1).

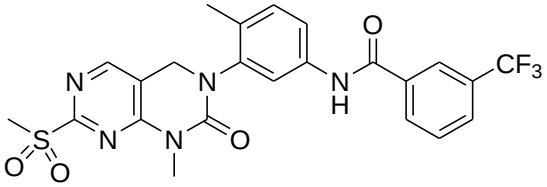
_N_-(4-methyl-3-(1-methyl-7-(methylthio)-2-oxo-1,2-dihydropyrimido[4,5-*d*]pyrimidin-3(4*H*)-yl)phenyl)-3-(trifluoromethyl)benzamide



To a solution *N*-(4-methyl-3-((4-(methylamino)-2-(methylthio)pyrimidin-5-yl)methylamino)phenyl)-3-(trifluoromethyl)benzamide (334 mg, 0.73 mmol) in dried THF (3.65 mL) were added diisopropylethylamine (0.36 mL, 2.19 mmol) and triphosgene (70 mg, 0.24 mmol) at 0 ^{o}C. After stirring at room temperature first for 1 h and at 80 ^{o}C for additional 3 h, the reaction mixture was cooled to room temperature and was partitioned between ethyl acetate (20 mL) and sat. $NaHCO_3$ (20 mL) solution. The organic phase was washed with brine and dried over $MgSO_4$ and concentrated. The resulting crude product was chromatographed on silica gel with ethyl acetate/hexane (1/4 to 2/3) to give 230 mg (65% yield) of the title product.

^{1}H NMR 600 MHz ($CDCl_3$) δ 8.98 (*s*, 1H), 8.13 (*s,* 1H), 8.08 (*d*, *J* = 7.8 Hz, 1H), 8.03 (*s*, 1H), 7.73 (*m*, 2H), 7.56 (*t*, *J* = 7.8 Hz, 1H), 7.29 (*d*, *J* = 8.4 Hz, 1H), 7.03 (*d*, *J* = 8.4 Hz, 1H), 4.68 (*d*, *J* = 14.4 Hz, 1H), 4.42 (*d*, *J* = 14.4 Hz, 1H), 3.51 (*s*, 3H), 2.59 (*s*, 3H), 1.72 (*s*, 3H), MS *m/z*:488.5 (M + 1).

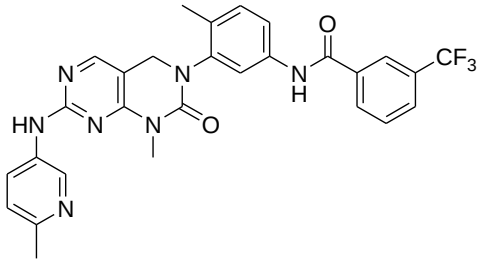
_N_-(4-methyl-3-(1-methyl-7-(methylsulfonyl)-2-oxo-1,2-dihydropyrimido[4,5-*d*]pyrimidin-3(4*H*)-yl)phenyl)-3-(trifluoromethyl)benzamide



To a solution of *N*-(4-methyl-3-(1-methyl-7-(methylthio)-2-oxo-1,2-dihydropyrimido[4,5-*d*]pyrimidin-3(4*H*)-yl)phenyl)-3-(trifluoromethyl)benzamide (230 mg, 0.47 mmol) in dichloromethane (2 mL) was added *meta*-chloroperbenzoic acid (245 mg, 1.42 mmol) at 0 ^{o}C. The reaction mixture was stirred for 30 min at 0 ^{o}C and then stirred for 3 h at room temperature. The reaction mixture was partitioned between dichloromethane (20 mL) and sat. $NaHCO_3$ (20 mL) and then organic phase was washed with brine and dried over $MgSO_4$ and concentrated. The resulting white solid 200 mg (81% yield) was used for the next step without further purification.

^{1}H NMR 600 MHz (DMSO-d_6) δ 10.52 (*s*, 1H), 8.57 (*s,* 1H), 8.28 (*s*, 1H), 8.24 (*d*, *J* = 8.4 Hz, 1H), 7.96 (*d*, *J* = 7.8 Hz, 1H), 7.84 (*dd*, *J* = 1.8 Hz, *J* = 9.6 Hz, 1H), 7.78 (*t*, *J* = 7.8 Hz, 1H), 7.62 (*d*, *J* = 8.4 Hz, 1H), 7.31 (*d*, *J* = 8.4 Hz, 1H), 4.93 (*d*, *J* = 15.6 Hz, 1H), 4.75 (*d*, *J* = 15.6 Hz, 1H), 3.39 (*s*, 3H), 2.47 (*s*, 3H), 2.12 (*s*, 3H), MS *m/z*:520.5 (M + 1).

***N*-(4-methyl-3-(1-methyl-7-(6-methylpyridin-3-ylamino)-2-oxo-1,2-dihydropyrimido[4,5-*d*]pyrimidin-3(4*H*)-yl)phenyl)-3-(trifluoromethyl)benzamide**



To a solution of *N*-(4-methyl-3-(1-methyl-7-(methylsulfonyl)-2-oxo-1,2-dihydropyrimido[4,5-*d*]pyrimidin-3(4*H*)-yl)phenyl)-3-(trifluoromethyl)benzamide (100 mg, 019 mmol) in dioxane (1.0 mL) were added 6-methylpyridin-3-amine (104 mg, 0.96 mmol) and trifluoroacetic acid (73 uL, 0.96 mmol). The reaction mixture was stirred for 48 h at 120 ^{o}C. The reaction mixture was cooled to room temperature and concentrated. The crude reaction mixture was partitioned between ethyl acetate (20 mL) and sat. $NaHCO_3$ (20 mL) solution and then organic phase was washed with brine and dried over $MgSO_4$ and concentrated. The resulting crude product was chromatographed on silica gel with Ethyl acetate/Hexane (1/9 to 1/1) to give 34 mg (32% yield) of the title product as a pale yellow solid.

^{1}H NMR 600 MHz (DMSO-d_6) δ 10.50 (*s*, 1H), 9.61 (*s,* 1H), 8.77 (*d, J* = 3.0 Hz, 1H), 8.28 (*s*, 1H), 8.25 (*d*, *J* = 8.4 Hz, 1H), 8.13 (*s*, 1H), 8.04 (*dd*, *J* = 2.4 Hz, *J* = 8.4 Hz, 1H), 7.95 (*d*, *J* = 7.8 Hz, 1H), 7.78 (*m*, 2H), 7.63 (*dd*, *J* = 1.8 Hz, *J* = 8.4 Hz, 1H), 7.30 (*d*, *J* = 8.4 Hz, 1H), 7.16 (*d*, *J* = 9.0 Hz, 1H), 4.69 (*d*, *J* = 13.8 Hz, 1H), 4.52 (*d*, *J* = 13.8 Hz, 1H), 3.32 (*s*, 3H), 2.40 (*s*, 3H), 2.12 (*s*, 3H), MS *m/z*:548.5 (M + 1).

2. *In vivo* efficacy evaluation using T315I-Bcr-Abl-Ba/F3 orthotopic xenograft model

6-8 weeks old SCID beige female mice (n=5 for each GNF-7-treated or vehicle control group) were injected via tail vein with $1x10^6$ Ba/F3 cells co-expressing Bcr-abl/T315I mutant and luciferase. Three days post-injection, mice were orally dosed once daily with 10 or 20 mg/kg GNF-7 for seven days. At day 5 and 7, bioluminescence was quantified using a Xenogen IVIS living imaging system (Caliper LifeSciences, Hopkinton, MA).

3. Experimental section of PK study

Five to six week old male Balb/c mice (20-25 g) were obtained from Jackson Laboratory (Bar Harbor, Maine, USA). GNF-7 was dissolved in a 100% PEG300 solution formulation (2.5 mg/mL) and dosed at 5 mg/kg intravenously via the lateral vein (n=3). The oral dose was prepared in a 1:1 formulation of PEG300 and distilled water and administered at 20 mg/kg via oral gavage (n=3). Five blood samples (50 µL) were serially drawn via retro orbital sinus within 24 hours after dosing. Plasma concentrations of GNF-7 were quantified utilizing a Liquid Chromatography/Mass Spectrometry (LC/MS/MS) assay. Pharmacokinetic parameters were calculated by non-compartmental regression analysis using Winnonlin 4.0 software (Pharsight, Mountain View, CA, USA).