Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Discovery of Hydroxamate Bioisosteres as KATII Inhibitors with Improved Oral Bioavailability and Pharmacokinetics

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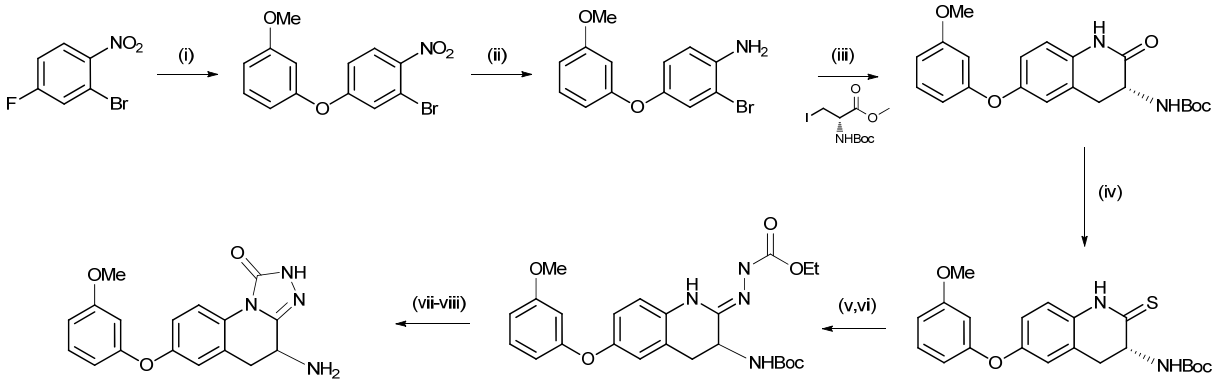
Synthetic protocols and spectral information

Scheme 1. Synthesis of compound ***5***



Step (i). Synthesis of 2-bromo-4-(3-methoxyphenoxy)-1-nitrobenzene.

To a 0 °C solution of 2-bromo-4-fluoro-1-nitrobenzene (20 g, 91 mmol) in acetonitrile (300 mL) was added cesium carbonate (36 g, 110 mmol) followed by 3-methoxyphenol (12.0 ml, 109 mmol), and the reaction mixture was stirred for 12 hours at room temperature. Solvent was removed *in vacuo*, and the residue was diluted with ethyl acetate and washed with water. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure. Purification via silica gel chromatography (Eluent: 1% ethyl acetate in petroleum ether) afforded the product as a pale yellow liquid. Yield: 24.8 g, 76.5 mmol, 84%. GCMS *m/z* 323.1 [M^+]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (d, *J*=9.1 Hz, 1H), 7.44 (d, *J*=2.6 Hz, 1H), 7.40 (dd, *J*=8.4, 8.1 Hz, 1H), 7.11 (dd, *J*=9.1, 2.6 Hz, 1H), 6.89 (br dd, *J*=8.4, 2.3 Hz, 1H), 6.80 (br dd, *J*=2.6, 2.3 Hz, 1H), 6.75 (br dd, *J*=7.9, 2.3 Hz, 1H), 3.77 (s, 3H).

Step (ii). Synthesis of 2-bromo-4-(3-methoxyphenoxy)aniline.

Iron powder (26.2 g, 469 mmol) was added to a solution of 2-bromo-4-(3-methoxyphenoxy)-1-nitrobenzene (36 g, 110 mmol) in a 2:1:1 mixture of tetrahydrofuran, methanol and water (580 mL). Ammonium chloride (23.8 g, 445 mmol) was added and the reaction mixture was heated to 70 °C for 3 hours. After filtration through a pad of Celite, the

reaction mixture was concentrated *in vacuo* to afford an aqueous residue, which was diluted with ethyl acetate and washed with water. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure; trituration with diethyl ether provided the product as a brown solid. Yield: 29 g, 99 mmol, 90%. LCMS *m/z* 294.2 [M+H$^+$]. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.21 (dd, *J*=8.4, 8.0 Hz, 1H), 7.09 (dd, *J*=2.1, 0.7 Hz, 1H), 6.86 (dd, half of ABX pattern, *J*=8.7, 2.1 Hz, 1H), 6.83 (dd, half of ABX pattern, *J*=8.7, 0.7 Hz, 1H), 6.63 (ddd, *J*=8.2, 2.4, 0.9 Hz, 1H), 6.46 (dd, *J*=2.4, 2.1 Hz, 1H), 6.42 (ddd, *J*=8.0, 2.4, 0.7 Hz, 1H), 5.20 (br s, 2H), 3.71 (s, 3H).

Step (iii). Synthesis of *tert*-butyl [(3R)-6-(3-methoxyphenoxy)-2-oxo-1,2,3,4-tetrahydroquinolin-3-yl]carbamate

Zinc (1.38 g, 21.1 mmol) was dried for 30 minutes under vacuum using a heat gun and then suspended in *N,N*-dimethylformamide (10 mL). Crystals of iodine (0.267 g, 1.05 mmol) were added, and the resulting deep red solution was stirred until the color disappeared. To this solution was added methyl *N*-(*tert*-butoxycarbonyl)-3-iodo-D-alaninate (6.26 g, 19.0 mmol) and stirring was continued for 30 minutes. In a separate flask, a mixture of palladium(II) acetate (47 mg, 0.21 mmol) and 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (X-Phos, 0.252 g, 0.529 mmol) in *N,N*-dimethylformamide (15 mL) was stirred for 5 minutes before addition of 2-bromo-4-(3-methoxyphenoxy)aniline (3.1 g, 11 mmol). The zincate solution was added to this flask, and the reaction mixture was heated at 60 °C for 12 hours. After dilution with ethyl acetate, the reaction mixture was washed with ice-cold water. The organic layer was dried over sodium sulfate, filtered, and concentrated *in vacuo*; purification via chromatography on silica gel (Eluent: 20% ethyl acetate in petroleum ether) afforded the product as a brown solid. Yield: 2.3 g, 6.0 mmol, 55%. LCMS *m/z* 329.0 {[M - (2-methylprop-1-ene)]+H$^+$}. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.23 (br s, 1H), 7.23 (dd, *J*=8.3, 8.1 Hz, 1H), 6.93-7.00 (br m, 2H), 6.85-6.88 (m, 2H), 6.66 (ddd, *J*=8.2, 2.3, 0.7 Hz, 1H), 6.52 (dd, *J*=2.4, 2.2 Hz, 1H), 6.48 (ddd, *J*=8.1, 2.4, 0.8 Hz, 1H), 4.10-4.21 (m, 1H), 3.72 (s, 3H), 2.86-3.01 (m, 2H), 1.40 (s, 9H).

*Step (iv). Synthesis of tert-butyl [(3*R*)-6-(3-methoxyphenoxy)-2-thioxo-1,2,3,4-tetrahydroquinolin-3-yl]carbamate.*

Sodium carbonate (1.46 g 13.8 mmol) and phosphorus pentasulfide (3.06 g, 13.8 mmol) were combined in tetrahydrofuran (50 mL) and stirred for 30 minutes at room temperature. *tert*-Butyl [(3*R*)-6-(3-methoxyphenoxy)-2-oxo-1,2,3,4-tetrahydroquinolin-3-yl]carbamate (2.3 g, 6.0 mmol) was added and the reaction mixture was heated at reflux for 12 hours, then poured into ice water and extracted with ethyl acetate. The combined organic layers were washed with water

and with saturated aqueous sodium chloride solution, dried over sodium sulfate, filtered, and concentrated *in vacuo*. Purification using silica gel chromatography (Eluent: 5% ethyl acetate in petroleum ether) provided the product as a yellow solid. Yield: 1.8 g, 4.5 mmol, 75%. LCMS *m/z* 401.1 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (br s, 1H), 7.26 (dd, *J*=8.3, 8.1 Hz, 1H), 7.11 (d, *J*=8.6 Hz, 1H), 7.04 (v br d, *J*=7.8 Hz, 1H), 6.97 (br d, *J*=2.7 Hz, 1H), 6.92 (dd, *J*=8.3, 2.7 Hz, 1H), 6.69 (ddd, *J*=8.3, 2.4, 0.8 Hz, 1H), 6.55 (dd, *J*=2.4, 2.2 Hz, 1H), 6.52 (ddd, *J*=8.1, 2.2, 0.7 Hz, 1H), 4.20-4.32 (m, 1H), 3.73 (s, 3H), 3.01 (dd, *J*=15.9, 5.9 Hz, 1H), 2.80 (br dd, *J*=15, 14 Hz, 1H), 1.41 (s, 9H).

Step (v). Synthesis of *tert*-butyl [6-(3-methoxyphenoxy)-2-(methylsulfanyl)-3,4-dihydroquinolin-3-yl]carbamate .

A solution of *tert*-butyl [(3*R*)-6-(3-methoxyphenoxy)-2-thioxo-1,2,3,4-tetrahydroquinolin-3-yl]carbamate (1.8 g, 4.5 mmol) in tetrahydrofuran (50 mL) was cooled to 15 °C. Potassium carbonate (3.1 g, 22 mmol) was added, followed by methyl iodide (3.34 mL, 54.0 mmol), and the reaction mixture was stirred for 20 hours at 15 °C. The reaction mixture was then diluted with ethyl acetate and washed with ice-cold water. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure; the residue was purified using chromatography on silica gel (Eluent: 6% ethyl acetate in petroleum ether) to afford the product as a solid. Chiral analysis via HPLC [Column: Chiral Technologies Chiralpak IA, 5 µm; Eluent: 20% 2-propanol in (0.1% diethylamine in hexanes)] revealed that racemization occurred during this transformation. Yield: 0.60 g, 1.4 mmol, 31%. LCMS *m/z* 415.1 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.48 (br d, *J*=9.3 Hz, 1H), 7.27 (dd, *J*=8.3, 8.1 Hz, 1H), 7.18-7.21 (m, 1H), 6.84-6.89 (m, 2H), 6.70 (ddd, *J*=8.3, 2.3, 0.9 Hz, 1H), 6.57 (dd, *J*=2.4, 2.2 Hz, 1H), 6.54 (ddd, *J*=8.1, 2.2, 0.7 Hz, 1H), 4.25-4.34 (m, 1H), 3.73 (s, 3H), 2.81 (d, *J*=10.5 Hz, 2H), 2.35 (s, 3H), 1.42 (s, 9H).

Step (vi). Synthesis of ethyl 2-{3-[(*tert*-butoxycarbonyl)amino]-6-(3-methoxyphenoxy)-3,4-dihydroquinolin-2(1H)-ylidene}hydrazinecarboxylate.

Ethyl hydrazinecarboxylate (0.15 g, 1.4 mmol) was added to a solution of *tert*-butyl [6-(3-methoxyphenoxy)-2-(methylsulfanyl)-3,4-dihydroquinolin-3-yl]carbamate (0.60 g, 1.4 mmol) in ethanol (12 mL) and the reaction mixture was heated to reflux for 4 hours. After removal of solvent *in vacuo*, the crude residue was taken up in ethyl acetate and washed with ice-cold water. The organic layer was dried over sodium sulfate, filtered, and concentrated under reduced pressure; purification via silica gel chromatography (Eluent: 25% ethyl acetate in petroleum ether) provided the product as a solid. Yield: 0.29 g, 0.62 mmol, 44%. LCMS *m/z* 471.1 [M+H$^+$]. ^{1}H NMR (400

MHz, DMSO-d_6) δ 8.96 (s, 1H), 7.23 (dd, *J*=8.3, 8.1 Hz, 1H), 6.83-6.90 (m, 3H), 6.65 (ddd, *J*=8.3, 2.4, 0.7 Hz, 1H), 6.50 (dd, *J*=2.4, 2.2 Hz, 1H), 6.47 (ddd, *J*=8.1, 2.2, 0.7 Hz, 1H), 4.19-4.28 (m, 1H), 4.10 (q, *J*=7.1 Hz, 2H), 3.72 (s, 3H), 2.97 (dd, *J*=15.6, 4.4 Hz, 1H), 2.77 (dd, *J*=15.6, 9.5 Hz, 1H), 1.39 (s, 9H), 1.22 (t, *J*=7.0 Hz, 3H).

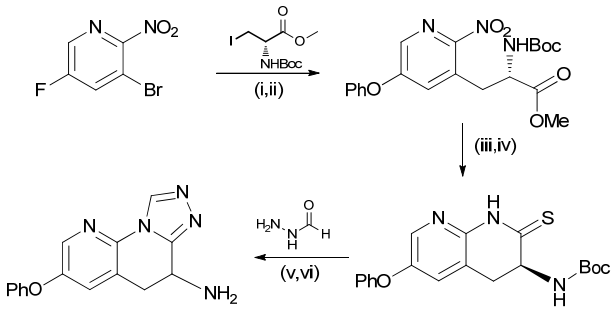
Step (vii). Synthesis of *tert*-butyl [7-(3-methoxyphenoxy)-1-oxo-1,2,4,5-tetrahydro[1,2,4]triazolo[4,3-*a*]quinolin-4-yl]carbamate

A solution of ethyl 2-{3-[(*tert*-butoxycarbonyl)amino]-6-(3-methoxyphenoxy)-3,4-dihydroquinolin-2(1*H*)-ylidene}hydrazinecarboxylate (0.25 g, 0.53 mmol) in *N,N*-dimethylformamide (5 mL) was heated to 150 °C for 2 hours. The reaction mixture was poured into ice-cold water and extracted with ethyl acetate; the organic layer was then dried over sodium sulfate, filtered, and concentrated *in vacuo*. Purification via silica gel chromatography (Eluent: 25% ethyl acetate in petroleum ether) provided the racemic product *tert*-butyl [7-(3-methoxyphenoxy)-1-oxo-1,2,4,5-tetrahydro[1,2,4]triazolo[4,3-*a*]quinolin-4-yl]carbamate. Yield: 0.12 g, 0.28 mmol, 53%. LCMS *m/z* 425.0 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.91 (s, 1H), 8.21 (d, *J*=8.8 Hz, 1H), 7.41-7.48 (m, 1H), 7.28 (dd, *J*=8.3, 7.8 Hz, 1H), 7.09 (d, *J*=2.9 Hz, 1H), 7.03 (dd, *J*=8.8, 2.4 Hz, 1H), 6.72 (dd, *J*=7.8, 2.4 Hz, 1H), 6.58 (dd, *J*=2.4, 2.0 Hz, 1H), 6.55 (dd, *J*=8, 2 Hz, 1H), 4.75-4.85 (m, 1H), 3.74 (s, 3H), 3.11 (dd, half of ABX pattern, *J*=15.6, 5.4 Hz, 1H), 2.98 (dd, half of ABX pattern, *J*=15.6, 9.8 Hz, 1H), 1.40 (s, 9H).

Step (viii). Synthesis of 4-amino-7-(3-methoxyphenoxy)-4,5-dihydro[1,2,4]triazolo[4,3-a]quinolin-1(2H)-one (**5**)

tert-Butyl [7-(3-methoxyphenoxy)-1-oxo-1,2,4,5-tetrahydro[1,2,4]triazolo[4,3-*a*]quinolin-4-yl]carbamate (50 mg, 0.12 mmol) was dissolved in diethyl ether (2 mL), cooled to 0 °C, and treated with a solution of hydrogen chloride in diethyl ether (4 M, 5 mL). After the reaction mixture had been stirred at room temperature for 30 minutes, it was concentrated *in vacuo*, and the residue was triturated with pentane to provide the product as a solid. Yield: 30 mg, 0.083 mmol, 69%. LCMS *m/z* 325.1 [M+H$^+$]. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.32 (s, 1H), 8.84 (br s, 3H), 8.24 (d, *J*=8.8 Hz, 1H), 7.30 (dd, *J*=8.3, 8.1 Hz, 1H), 7.17 (br d, *J*=2.7 Hz, 1H), 7.10 (dd, *J*=8.8, 2.9 Hz, 1H), 6.74 (ddd, *J*=8.3, 2.4, 1.0 Hz, 1H), 6.58 (dd, *J*=2.4, 2.2 Hz, 1H), 6.56 (ddd, *J*=8.1, 2.2, 0.7 Hz, 1H), 4.78 (dd, *J*=9.3, 5.9 Hz, 1H), 3.74 (s, 3H), 3.3-3.40 (m, 1H, assumed; partially obscured by water peak), 3.15 (dd, *J*=16.0, 9.4 Hz, 1H).

Scheme 2.Synthesis of compound ***7***



Step (i). Synthesis of 3-bromo-2-nitro-5-phenoxypyridine

To a solution of 3-bromo-5-fluoro-2-nitropyridine (1.0 g, 4.5 mmol) in MeCN (80 mL) was added phenol (478 mg, 5.08 mmol) and Cs_2CO_3 (326 mg, 5.43 mmol). The resulting mixture was stirred at 60 °C for 3 hours. The reaction was diluted with EtOAc and washed with water. The organic layer was dried, filtered and concentrated under reduced pressure, and the residue was purified using silica gel chromatography (Gradient: 10% EtOAc in heptane) to provide the product as an oil (1.3 g, 99%). GCMS *m/z* 294 (M^+). ^{1}H NMR (400 MHz, $CDCl_3$): 8.18 (d, *J=2.4* Hz, 1H), 7.58 (d, *J=2.4* Hz, 1H), 7.49 (dd, *J=8.5,* 7.5 Hz, 2H), 7.33 (brt, *J*=7.5 Hz, 1H), δ 7.10-7.14 (m, 2H).

Step (ii). Synthesis of methyl {(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(2-nitro-5-phenoxypyridin-3-yl)}propanoate

Trimethylsilyl chloride (0.77 m!, 6.09 mmol) was added to a stirring suspension of zinc dust (1.99 g, 30.5 mmol) in dry DMF (2 mL) and the mixture was stirred for 30 min. The stirring was stopped, and the solids were allowed to settle for 10 min, at which time the supernatant was removed via syringe. The activated zinc was washed with DMF and the solvent was again removed with a syringe; the zinc was then dried under vacuum using a heat gun. A solution of methyl *N-(tert*-butoxycarbonyl)-3-iodo-L-alaninate (4.02 g, 12.2 mmol) in DMF (1.0 M) was added to the dry activated zinc, and the resulting suspension was stirred for 30 min at RT. In a separate flask, a mixture of palladium(II) acetate (114 mg, 0.51 mmol) and 2-dicyclohexylphosphino-2',4',6'-triisopropylbiphenyl (X-Phos, 485 mg, 1.02 mmol) in *N,N*-dimethylformamide (2 mL) was stirred for 5 minutes before addition of 3-bromo-2-nitro-5-phenoxypyridine (3.0 g, 10 mmol). The zincate solution was added to this flask, and the reaction mixture was stirred at room temperature for 12 hours. After dilution with ethyl acetate, the reaction mixture was washed with water and brine. The organic layer was dried over sodium

sulfate, filtered, and concentrated *in vacuo*; purification via chromatography on silica gel (Eluent: 10% ethyl acetate in heptane) afforded the product as a yellow oil. Yield: 1.2 g, 2.8 mmol, 28%. LCMS *mlz* 418.1 [M+H$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (br s, 1H), 7.47 (brdd, *J*=8, 8 Hz, 2H), 7,27-7.32 (m, 2H), 7.09-7.13 (m, 2H), 5.19 (br d, *J=8* Hz, 1H), 4.62-4.68 (m, 1H), 3.74 (8, 3H), 3.5 (m, 1H), 3.14-3.21 (m,. 1H), 1.38 (br s, 9H),

Step (iii). Synthesis of *tert*-butyl [(3S)-2-oxo-6-phenoxy-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl]carbamate

Zinc (1.88 g, 28.7 mmol) and ammonium chloride (3.08 g, 57.6 mmol) were added to a solution of methyl {(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(2-nitro-5-phenoxypyridin-3-yl)}propanoate (1.20 g, 2.87 mmol) in tetrahydrofuran (4 mL) and methanol (8 mL), and the resulting slurry was heated at 60 °C for 48 hours. The reaction mixture was then treated with saturated aqueous sodium carbonate solution (15 mL) and ethyl acetate (100 mL), and allowed to stir for 10 minutes. The mixture was filtered, and the organic layer was washed with water (2 x 100 mL) and with saturated aqueous sodium chloride solution (100 mL), dried over sodium sulfate, filtered, and concentrated *in vacuo*. Purification via silica gel chromatography (Gradient: 0% to 70% ethyl acetate in heptane) afforded the product as a white foam. Yield: 750 mg, 2.11 mmol, 73%. LCMS *m/z* 356.2 [M+H$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.66 (br s, 1H), 8.08 (br d, *J*=2.5 Hz, 1H), 7.37 (br dd, *J*=8, 8 Hz, 2H), 7.22 (br d, *J*=2 Hz, 1H), 7.15 (br dd, *J*=8, 8 Hz, 1H), 7.00 (br d, *J*=8 Hz, 2H), 5.70 (br s, 1H) 4.32-4.42 (m, 1H), 3.45-3.54 (m, 1H), 2.75-2.85 (m, 1H), 1.47 (s, 9H).

Step (iv). Synthesis of *tert*-butyl [(3S)-6-phenoxy-2-thioxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl]carbamate.

Sodium carbonate (99.5%, 673 mg, 6.32 mmol) and phosphorus pentasulfide (99%, 1.42 g, 6.32 mmol) were added to tetrahydrofuran (4.2 mL), and the suspension was vigorously stirred for 15 minutes at room temperature. To the resulting yellow solution was added a solution of *tert*-butyl [(3*S*)-2-oxo-6-phenoxy-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl]carbamate (748 mg, 2.10 mmol) in tetrahydrofuran (3 mL), and the reaction mixture was heated at 70 °C for 1 hour. After cooling to room temperature, the reaction mixture was poured into water and extracted with ethyl acetate. The combined organic layers were washed with water and with saturated aqueous sodium chloride solution, dried over magnesium sulfate, filtered, and concentrated *in vacuo*. Purification via silica gel chromatography (Gradient: 0% to 70% ethyl acetate in heptane) afforded the product as a yellow solid. Yield: 590 mg, 1.59 mmol, 76%. This material contained a

contaminant identified by NMR and MS as *tert*-butyl [(3*S*)-6-fluoro-2-thioxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl]carbamate. LCMS *m/z* 372.2 [M+H$^+$]. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.57 (br s, 1H), 8.33 (dd, *J*=2.7, 0.8 Hz, 1H), 7.40 (br dd, *J*=8.5, 7.5 Hz, 2H), 7.17-7.23 (m, 2H), 7.02-7.06 (m, 2H), 6.21 (br s, 1H), 4.34-4.42 (m, 1H), 3.45-3.55 (m, 1H), 2.68-2.79 (m, 1H), 1.49 (s, 9H).

Step (v). Synthesis of tert-butyl (3-phenoxy-5,6-dihydro-[1,2,4]triazolo[4,3-a][1,8]naphthyridin-6-yl)carbamate

tert-butyl [(3S)-6-phenoxy-2-thioxo-1,2,3,4-tetrahydro-1,8-naphthyridin-3-yl]carbamate (100 mg, 0.27 mmol), formic hydrazide (34 mg, 0.54 mmol) and acetic acid (100 µL, 1.69 mmol) were combined in cyclohexanol (1 mL) and heated to 160 °C for 1 hour. The reaction mixture was allowed to cool, and cyclohexanol was removed by heating under high vacuum. The residue was purified via silica gel chromatography (Eluents: 10% MeOH in DCM), affording the product as a light brown foam. Yield: 67 mg, 0.177 mmol, 67%. LCMS *m/z* 380.2 [M+H$^+$].

Step (vi). Synthesis of 3-phenoxy-5,6-dihydro-[1,2,4]triazolo[4,3-a][1,8]naphthyridin-6-amine (**7**)

A solution of HCl in 2-propanol (5M) was added to (3-phenoxy-5,6-dihydro-[1,2,4]triazolo[4,3-a][1,8]naphthyridin-6-yl)carbamate (67 mg, 0.18 mmol) at room temperature, and the reaction stirred for 2 h. Reaction was concentrated and triturated to yield a white solid as the HCl salt. Yield: 36 mg, 68%. LCMS *m/z* 280.1 [M+H$^+$]. ^{1}H NMR (400 MHz, MeOD): δ 9.70 (s, 1H), 8.19 (d, *J*=2.7 Hz, 1H), 7.59 (m, 1H), 7.45 – 7.40 (m, 2H), 7.22 (tt, *J*=7.4, 1.1 Hz, 1H), 7.12 (m, 1H), 7.10 (m, 1H), 5.18, (dd, *J*=9.2, 6.4 Hz, 1H), 3.6 (dd, *J*=16.4, 6.6 Hz, 1H), 3.32 (dd, *J*=16.2, 9.2 Hz).

4-amino-7-phenoxy-4,5-dihydro[1,2,4]triazolo[4,3-a]quinolin-1(2H)-one (**4**) was prepared in a similar manner to compound **5**. . LCMS *m/z* 295.0 [M+H$^+$]. ^{1}H NMR (300 MHz, DMSO-d_6), δ 12.32 (s, 1H), 8.85 (br s, 3H), 8.24 (d, *J*=8.7 Hz, 1H), 7.37-7.45 (m, 2H), 7.13-7.20 (m, 2H), 7.08 (dd, *J*=8.7, 2.8 Hz, 1H), 6.99-7.05 (m, 2H), 4.73-4.82 (m, 1H), 3.3-3.41 (m, 1H), 3.15 (dd, *J*=16.2, 9.6 Hz, 1H);

7-(3-methoxyphenoxy)-4,5-dihydro[1,2,4]triazolo[4,3-a]quinolin-4-amine (**6**) was prepared using the methods described above. LCMS *m/z* 309.2 [M+H$^+$]. ^{1}H NMR (400 MHz, CD_3OD) δ 9.58 (s, 1H), 7.80 (d, *J*=8.8 Hz, 1H), 7.28-7.33 (m, 1H), 7.17 (br d, *J*=2.5 Hz, 1H), 7.13 (br dd, *J*=8.7, 2.6 Hz, 1H), 6.78 (ddd, *J*=8.4, 2.2, 0.9 Hz, 1H), 6.61-6.64 (m, 2H), 5.10 (dd, *J*=9.7, 6.1 Hz, 1H), 3.79 (s, 3H), 3.52 (dd, *J*=16.1, 6.1 Hz, 1H), 3.23-3.31 (m, 1H).

Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

KAT II X-ray Crystallography Experimental Protocols

Expression and Purification:

Based on our first crystal structure of human KAT II (Dounay et al, ACS Med Chem Lett, 2012, **3**, 187-192), we systematically tested the effect of surface mutations in the protein, with the goal of forcing the protein molecules to pack differently in the crystal so as to improve diffraction resolution. Of many different mutants examined, one (K240S/F241G), consistently gave better crystals, and was the one picked. Full length human KAT II (residues 1-425, Uniprot Q8N5Z0, K240S/F241G), with a C-terminal thrombin cleavage site (-LVPRGSLE-) followed by a hexa-histidine tag was expressed in Sf21 insect cells. The protein was expressed and purified as described before (Dounay et al, ACS Med Chem Lett, 2012, **3**, 187-192), Its specific activity, and Ki for standard inhibitors was no different from the WT protein (data not shown), but replacement of the surface lysine and phenylalanine by smaller side chains led to different packing interactions that gave crystals in many different conditions, including some that diffracted to higher resolution than the original 3.2Å structure.

Crystallization:

Crystals were grown by vapour diffusion as hanging drops. Protein at 10 mg/ml was incubated at room temperature for 2 h with compound (1 mM) and pyridoxal phosphate (2 mM). 2µl drops, containing equal volumes of protein solution and reservoir solution (250 mM NaCl, 0.1M NaCitrate pH 5.6, 24% PEG4K), were equilibrated against 1mL of reservoir solution. Plate-like crystals appeared after a few days.

Structure determination and refinement:

Diffraction data were collected from a single crystal flash frozen in a stream of dry nitrogen at 100 K, using 25% glycerol as a cryoprotectant. Data were collected at the IMCA-CAT beamline 17-ID at the Advanced Photon Source. Use of the IMCA-CAT beamline 17-ID at the Advanced Photon Source was supported by the companies of the Industrial Macromolecular Crystallography Association through a contract with Hauptman-Woodward Medical Research Institute. Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. DE-AC02-06CH11357.

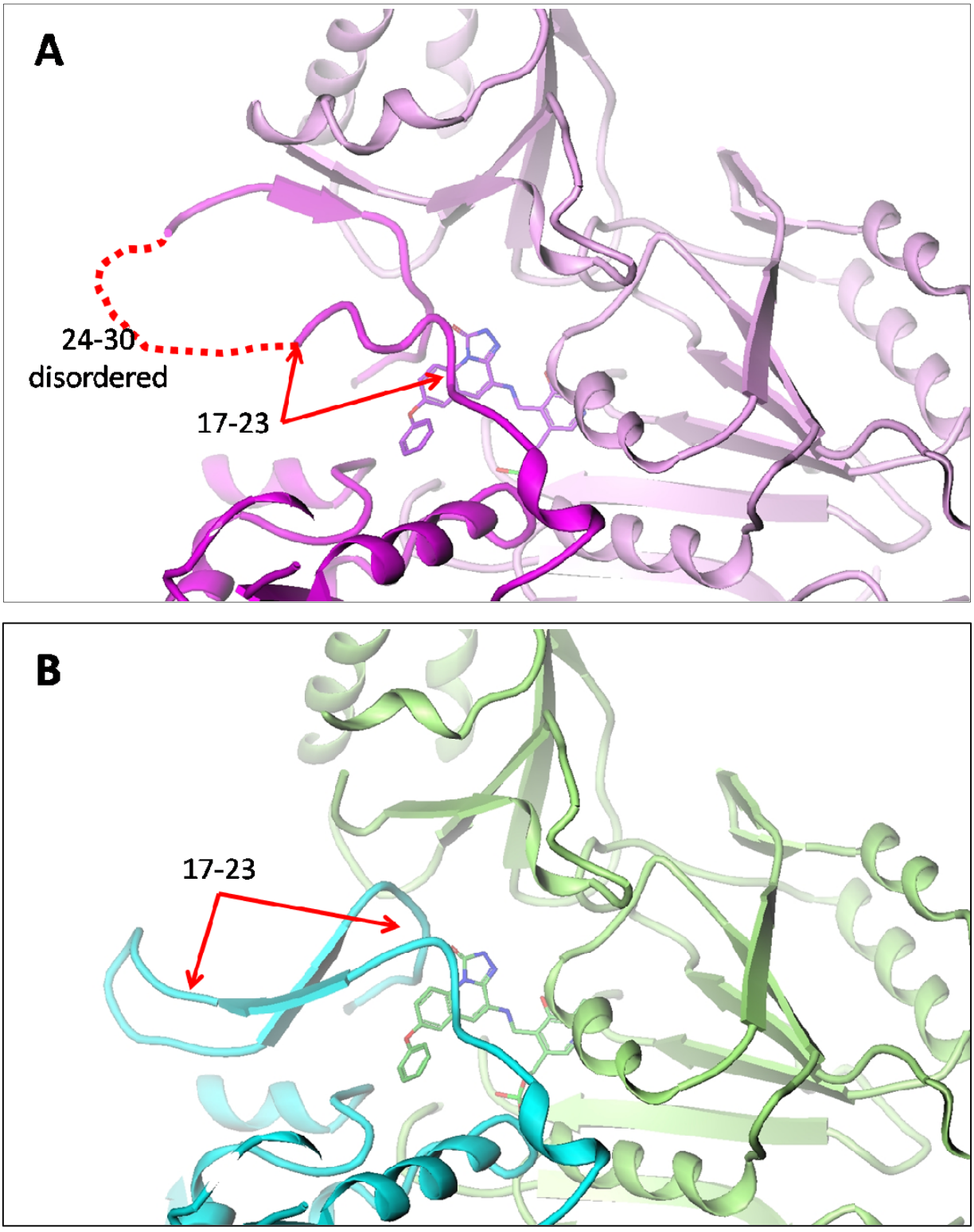
The structures were solved by molecular replacement, using the previously reported structure of human KAT II as a starting model. (Dounay et al, ACS Med Chem Lett, 2012, **3**, 187-192). Refinement was carried out using autoBUSTER[1] using NCS restraints implemented as Local Structure Similarity Restraints[2], while fitting was done in COOT as distributed with the CCP4 package [3]. Complete data collection and refinement statistics are given in Table S1.The final refined model contains two protein molecules, two PLP-inhibitor complexes and 217 water molecules. The entire polypeptide chain of human KAT II is modeled, except for residues 24-30 and 372-377 in molecule A, which were too disordered to be modeled with confidence at this resolution. Because of different crystal packing contacts at molecules A and B, the conformation of residues 18-37 in molecule B is dramatically different from what has been seen before in other KAT II structures. Residues 19-25, which are either disordered, or form an α-helix in all other KATII structures form a β-strand in this structure, making an anti-parallel beta sheet structure with another β-strand at residues 32-36 (Fig S1). Complete data collection and refinement statistics are given in Table S1.

Figure S1:

In these figures, Molecules A and B were first superimposed and then shown separately in Figures A and B to more clearly highlight the change in conformation in the N-terminal section of the protein between residues 17 to 36. In each figure the two molecules of the dimer are shown in two different shades of color. The coordinates of the bound ligand in the two molecules superimpose exactly (r.m.s.d for all atoms 0.1Å).

Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Figure S1



References:

1. Bricogne G., Blanc E., Brandl M., Flensburg C., Keller P., Paciorek W., Roversi P, Sharff A., Smart O.S., Vonrhein C., Womack T.O. (2011). BUSTER version 2.9.3. Cambridge, United Kingdom: Global Phasing Ltd.
2. O. S. Smart, T. O. Womack, C. Flensburg, P. Keller, W. Paciorek, A. Sharff, C. Vonrhein and G. Bricogne (2012) *Acta Cryst.* **D68**, 368-380. Exploiting structure similarity in refinement: automated NCS and target-structure restraints in BUSTER.
3. M. D. Winn *et al.* Acta. Cryst. D67 , 235-242 (2011) Overview of the CCP4 suite and current developments