Supporting Information

Discovery of Alogliptin: A Potent, Selective, Bioavailable, and Efficacious Inhibitor of Dipeptidyl Peptidase IV

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X-ray diffraction data: Wild-type human DPP-4, was purified and crystallized as previously reported, utilizing TSD's automated Nanovolume Crystallization TM technology.[1,2] All protein-inhibitor complexes were obtained by soaking preformed DPP-4 crystals in a solution containing compound of interest. Crystals were then cryo-protected with ethylene glycol and flash frozen in liquid nitrogen. X-ray diffraction data were collected at Advanced Light Source (ALS) beam line 5.0.3, and processed using the program HKL2000.[3] The structures of DPP-4 inhibitor complexes were determined by molecular replacement using MOLREP, utilizing the previously determined coordinates of DPP-4 with accession code 1R9M.[1,4] Subsequent structure refinement and model building were performed utilizing REFMAC and XtalView.[4,5] Bound inhibitors were clearly visible in the electron density maps.

	Compound **1a**
Space Group	$P2_1$
Unit cell Lengths (Å)	122.4, 123.7, 145.4
Unit cell angles (°)	90, 114.9, 90
Resolution (Å)	2.55
Observations	432121
Unique	118392
Completeness (%)	92.9 (56.6)
I/σ_I	10.1 (2.1)
R_{sym} (%)	6.3 (38.9)
Model Refinement	
Reflections (work/free)	112425 / 5942
R_{factor} (work/free %)	19.8 / 25.4
Protein molecules per ASU	4
Solvent molecules	761
Mean B value ($Å^2$)	54.1
RMSD ideal bond lengths (Å)	0.008
RMSD ideal bond angles (°)	1.198

$R_{sym}=\Sigma|I-<I>|/\Sigma I$, where I is the integrated intensity for a reflection. $R_{factor}=\Sigma|F_P-F_c|/\Sigma F_P$, where F_p and F_c are the observed and calculated structure factor amplitudes, while R_{free} is calculated on 5% of the data excluded from refinement. Values in parenthesis are for the highest resolution shell.

DPP-4 Assay: Solutions of test compounds in varying concentrations (≤10 mM final concentration) were prepared in Dimethyl Sulfoxide (DMSO) and then diluted into assay buffer comprising: 20 mM Tris, pH 7.4; 20 mM KCl; and 0.1 mg/mL BSA. Human DPP-4 (0.1 nM final concentration) was added to the dilutions and pre-incubated for 10 minutes at ambient temperature before the reaction was initiated with A-P-7-amido-4-trifluoromethylcoumarin (AP-AFC; 10 μM final concentration). The total volume of the reaction mixture was 10-100 μL depending on assay formats used (384 or 96 well plates). The reaction was followed kinetically (excitation λ= 400 nm; emission λ= 505 nm) for 5-10 minutes or an end-point was measured after 10 minutes. Inhibition constants (IC_{50}) were calculated from the enzyme progress curves using standard mathematical models.

Microsomal Stability: The test compounds (1 μM) were incubated at 37 °C in phosphate buffer (50 mM, pH 7.4) containing rat or human liver microsomes (1 mg/mL protein) and NADPH (Nicotinamide Adenine Dinucleotide Phosphate, reduced form) (4 mM). The incubation mixtures were quenched with trichloroacetic acid (0.3 M) over 0, 5, 15, 30 minute time-course. Quenched solutions were centrifuged and supernatants were transferred for LC/MS quantitation. The half-life of test compounds was derived from the compound stability curve over the time course.

General Chemistry Procedures: All references to ether are diethyl ether; brine refers to a saturated aqueous solution of NaCl. Unless otherwise indicated, all temperatures are expressed in °C (degrees Centigrade). All reactions conducted under an inert atmosphere at room temperature unless otherwise noted. ^{1}H NMR spectra were recorded on a Bruker Avance 400. Chemical shifts are expressed in parts per million (ppm). Coupling constants are in units of Hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad). Low-resolution mass spectra (MS) and compound purity data were acquired on a Waters ZQ LC/MS single quadrupole system equipped with electrospray ionization (ESI) source, UV detector (220 and 254 nm), and evaporative light scattering detector (ELSD). Preparative HPLC was conducted on the same system using mixtures of TFA (0.05%) buffered water and acetonitrile. Thin-layer chromatography was performed on 0.25 mm E. Merck silica gel plates (60F-254), visualized with UV light, 5% ethanolic phosphomolybdic acid, Ninhydrin or p-anisaldehyde solution. Flash column chromatography was performed on silica gel (230-400 mesh, Merck).

2,4-Dichloroquinazoline (4a): To 3.2 g of 1*H*-quinazoline-2,4-dione in 20 mL $POCl_3$ was added 0.8 mL *N*,*N*-dimethylaniline. The mixture was then heated at reflux for 16 hours. Excess $POCl_3$ was removed *in vacuo*, providing crude product.

2-Chloro-3*H*-quinazolin-4-one (5a): A mixture of 20 mL of 1N NaOH, 20 mL of THF, and 2 g of 2,4-dichloroquinazoline was stirred at room temperature under N_2 for 4 hours. The solution was chilled and adjusted to pH 5 with AcOH. The solids that precipitated

were filtered to give 1.62 g (90%) of product. MS: (ES) [M+H] calc'd for $C_8H_6ClN_2O$, 181; found 181.

2-((2-Chloro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6a): A mixture of 0.36 g of 2-chloro-3*H*-quinazolin-4-one, 0.47 g (2.4 mmol) of 2-cyanobenzylbromide and 0.35 g (2.54 mmol) of K_2CO_3 in 10 mL of DMF was stirred overnight. The reaction mixture was diluted with water, extracted with ethyl acetate, and dried over $MgSO_4$. Removal of the solvent gave crude product (containing O-alkylated product).

2-((2-(3-Aminopiperidin-1-yl)-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (1a): A mixture of 200 mg ($\leq$ 0.68 mmol) of crude 2-(2-chloro-4-oxo-4*H*-quinazolin-3-ylmethyl)benzonitrile, 3 eq. of 3-aminopiperidne dihydrochloride (350 mg), 5 eq. of $NaHCO_3$ (286 mg) and 3 mL of ethanol in a sealed tube was heated to 150 °C for 6 hours. After cooling to room temperature and filtering out the inorganic salts, purification via preparative HPLC afforded 108 mg (45% yield) of product. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (d, J = 7.60 Hz, 1H), 7.69-7.79 (m, 2H), 7.56-7.62 (m, 2H), 7.37-7.46 (m, 2H), 7.30 (d, J = 8.0 Hz, 1H), 5.52 (AB q, J = 15.2 Hz, 2H), 3.64-3.71 (m, 1H), 3.55 (br s, 1H), 3.19-3.32 (m, 2H), 2.98-3.08 (m, 1H), 2.10-2.18 (m, 1H), 1.62-1.94 (m, 3H). MS: (ES) [M+H] calc'd for $C_{21}H_{21}N_5O$, 360; found 360. HRMS for $C_{21}H_{22}N_5O$, calcd: 360.1824, found: 360.1808.

2-Chloro-5-fluoroquinazolin-4(*3H*)-one (5b): The title compound was prepared from 5-fluoro-1*H*-quinazoline-2,4-dione[6] in 11% yield according to the procedures of examples **4a** and **5a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.31 (br s, 1H), 7.77-7.83 (m, 1H), 7.41 (d, 1H, J = 7.6 Hz), 7.26-7.32 (m, 1H). MS (ES) [M+H] calc'd for $C_8H_4FClN_2O$ 199, 201; found 199, 201.

2-((2-Chloro-5-fluoro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6b): The title compound was prepared from 2-chloro-5-fluoro-3*H*-quinazolin-4-one in 70% yield

according to the procedure for example **6k**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.70-7.79 (m, 2H), 7.40-7.58 (m, 3H), 7.17-7.24 (m, 2H), 5.72 (s, 2H). MS (ES) [M+H] calc'd for $C_{16}H_9FClN_3O$ 314, 316; found 314, 316.

(R)-2-((2-(3-aminopiperidin-1-yl)-5-fluoro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile, TFA salt (1b): The title compound was prepared from 2-(2-chloro-5-fluoro-4-oxo-4*H*-quinazolin-3-ylmethyl)benzonitrile in 53% yield according to the procedure for example **1a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.81 (dd, 1H, J = 7.6, 1.2 Hz), 7.68-7.73 (m, 1H), 7.61 (dt, 1H, J = 7.6, 1.2 Hz), 7.44 (t, 1H, J = 6.8 Hz), 7.32 (d, 1H, J = 7.6 Hz), 7.26 (d, 1H, J = 7.6 Hz), 7.08-7.13 (m, 1H), 6.97 (br s, 2H), 5.33 (AB q, 2H, J = 35.6, 15.2 Hz), 3.49-3.55 (m, 1H), 3.17-3.36 (m, 2H), 2.81-2.99 (m, 2H), 1.90-1.99 (m, 1H), 1.78-1.86 (m, 1H), 1.41-1.66 (m, 2H). MS (ES) [M+H] calc'd for $C_{21}H_{20}FN_5O$ 378; found 378. HRMS for $C_{21}H_{21}FN_5O$, calcd: 378.1730, found: 378.1734.

6-Fluoroquinazoline-2,4(*1H,3H*)-dione (3c): 2-Amino-6-fluorobenzoic acid was converted to the title compound by the method used for **3a** (yield not determined). MS: (ES) [M+H] calc'd for $C_8H_6FN_2O_2$, 181; found 181.

2,4-Dichloro-6-fluoroquinazoline (4c): **3c** was converted to the title compound by the method used for **4a** (yield: 70%). MS: (ES) [M+H] calc'd for $C_8H_4Cl_2FN_2$, 217; found 217.

2-Chloro-6-fluoroquinazolin-4(*3H*)-one (5c): **4c** was converted to the title compound by the method used for **5a** (yield: 95%). MS: (ES) [M+H] calc'd for $C_8H_5ClFN_2O$, 199; found 199.

2-((2-Chloro-6-fluoro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6c): **5c** was converted to the title compound by the method used for **6a** (yield: 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ7.93 (dd, J = 2.8, 8.0 Hz, 1H) 7.68-7.75 (m, 2H), 7.50-7.60 (m, 2H), 7.42

(dd, J = 7.2, 7.6 Hz, 1H), 7.15 (d, J = 8.0 Hz, 1H), 5.74 (s, 2H). MS (ES) [M+H] calculated for $C_{16}H_9ClFN_3O$, 314; found 314.

(R)-2-((2-(3-aminopiperidin-1-yl)-6-fluoro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile, TFA salt (1c): **6c** was converted to the title compound by the method used for **1a** (yield: 90%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.52-7.9 (m, 5H), 7.41-7.51 (m, 1H), 7.35 (d, J = 8.0 Hz, 1H), 5.44-5.66 (AB q, J = 16.0 Hz, 2H), 3.62-3.71 (m, 1H), 3.55-3.60 (m, 1H), 3.19-3.33 (m, 2H), 2.94-3.05 (m, 1H), 2.11-2.20 (m, 1H), 1.60-1.95 (m, 3H). MS (ES) [M+H] calculated for $C_{21}H_{20}FN_5O$, 378; found 378. HRMS for $C_{21}H_{21}FN_5O$, calcd: 378.1730, found: 378.1740.

2,6-Dichloroquinazolin-4(*3H*)-one (5d): The title compound was prepared from 6-chloro-1*H*-quinazoline-2,4-dione[7] in 59% yield according to the procedures of examples **4a** and **5a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.44 (br s, 1H), 8.01 (d, 1H, J = 2.4 Hz), 7.85 (dd, 1H, J = 8.4, 2.4 Hz), 7.63 (d, 1H, J = 8.4 Hz). MS (ES) [M+H] calc'd for $C_8H_4Cl_2N_2O$ 215, 217; found 215, 217.

2-((2,6-Dichloro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6d): The title compound was prepared from 2,6-dichloro-3*H*-quinazolin-4-one in 63% yield according to the procedure for example **6k**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (s, 1H), 7.72-7.77 (m, 2H), 7.63 (d, 1H, J = 8.8 Hz), 7.54 (dt, 1H, J = 7.6, 1.2 Hz), 7.43 (t, 1H, J = 7.6 Hz), 7.15 (d, 1H, J = 7.6 Hz), 5.74 (s, 2H). MS (ES) [M+H] calc'd for $C_{16}H_9Cl_2N_3O$ 330, 332; found 330, 332.

(R)-2-((2-(3-aminopiperidin-1-yl)-6-chloro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile, TFA salt (1d): The title compound was prepared from 2-(2,6-dichloro-4-oxo-4*H*-quinazolin-3-ylmethyl)benzonitrile in 70% yield according to the procedure for example **1a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.99 (br s, 3H), 7.88 (d, 1H, J = 1.2 Hz), 7.76-7.83 (m, 2H), 7.54-7.63 (m, 2H), 7.44 (t, 1H, J = 7.6 Hz), 7.25 (d, 1H, J = 7.6 Hz), 5.38 (AB q, 2H, J = 48.0, 15.2 Hz), 3.51-3.59 (m, 1H), 3.38-3.45 (m, 1H), 3.02-3.21 (m, 2H), 2.84-2.93 (m, 1H), 1.91-2.00 (m, 1H), 1.79-1.88 (m, 1H), 1.50-

1.69 (m, 2H). MS (ES) [M+H] calc'd for $C_{21}H_{20}ClN_5O$ 394, 396; found 394, 396. HRMS for $C_{21}H_{21}ClN_5O$, calcd: 394.1435, found: 394.1443.

2,7-Dichloroquinazolin-4(*3H*)-one (5e): The title compound was prepared from 7-chloro-1*H*-quinazoline-2,4-dione[8] in 58% yield according to the procedures of examples **4a** and **5a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.41 (br s, 1H), 8.07 (d, 1H, J = 6.3 Hz), 7.70 (d, 1H, J = 1.5 Hz), 7.57 (dd, 1H, J = 6.3, 1.5 Hz). MS (ES) [M+H] calculated for $C_8H_5Cl_2N_2O$ 214, 216; found 215, 217.

2-((2,7-Dichloro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6e): The title compound was prepared from 2,7-dichloro-3*H*-quinazolin-4-one in 70% yield according to the procedure for **6k**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (d, 1H, J = 6.3 Hz), 7.74 (dd, 1H, J = 5.7, 0.9 Hz), 7.68 (d, 1H, J = 0.3 Hz), 7.49-7.57 (m, 2H), 7.43 (t, 1H, J = 5.7 Hz), 7.15 (d, 1H, J = 5.7 Hz), 5.73 (s, 2H). MS (ES) [M+H] calculated for $C_{16}H_{10}Cl_2N_3O$ 330, 332; found 330, 332.

(R)-2-((2-(3-aminopiperidin-1-yl)-7-chloro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile, TFA salt (1e): The title compound was prepared from 2-(2,7-dichloro-4-oxo-4*H*-quinazolin-3-ylmethyl)benzonitrile in 80% yield according to the procedure for compound **1a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.90-8.01 (m, 4H), 7.81 (d, 1H, J = 5.7 Hz), 7.56-7.64 (m, 2H), 7.38-7.48 (m, 2H), 7.26 (d, 1H, J = 5.7 Hz), 5.36 (dd, 2H, J = 34.8, 11.4 Hz), 3.52-3.58 (m, 1H), 3.36-3.46 (m, 1H), 3.03-3.24 (m, 2H), 2.87-2.94 (m, 1H), 1.92-1.99 (m, 1H), 1.78-1.85 (m, 1H), 1.50-1.69 (m, 2H). MS (ES) [M+H] calculated for $C_{21}H_{21}ClN_5O$ 394, 396; found 394, 396. HRMS for $C_{21}H_{21}ClN_5O$, calcd: 394.1435, found: 394.1446.

2,8-Dichloroquinazolin-4(*3H*)-one (5f): The title compound was prepared from 8-chloro-1*H*-quinazoline-2,4-dione[9] in 37% yield according to the procedures of examples **4a** and **5a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.50 (br s, 1H), 8.04 (dd, 1H, J = 6.0, 1.2

Hz), 7.98 (dd, 1H, J = 6.0, 1.2 Hz), 7.51 (t, 1H, J = 6.0 Hz). MS (ES) [M+H] calculated for $C_8H_5Cl_2N_2O$ 215, 217; found 215, 217.

2-((2,8-Dichloro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6f): The title compound was prepared from 2,8-dichloro-3*H*-quinazolin-4-one in 72% yield according to the procedure for **6k**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.22 (dd, 1H, J = 6.0, 1.2 Hz), 7.89 (dd, 1H, J = 6.0, 1.2 Hz), 7.74 (dd, 1H, J = 6.0, 0.9 Hz), 7.42-7.76 (m, 3H), 7.14 (d, 1H, J = 6.0 Hz), 5.75 (s, 2H). MS (ES) [M+H] calculated for $C_{16}H_{10}Cl_2N_3O$ 330, 332; found 330, 332.

2-((2-(3-Aminopiperidin-1-yl)-8-chloro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile, TFA salt (1f): The title compound was prepared from 2-(2,8-dichloro-4-oxo-4*H*-quinazolin-3-ylmethyl)benzonitrile in 76% yield according to the procedure for compound **1a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.88-8.02 (m, 5H), 7.81 (dd, 1H, J = 5.7, 0.6 Hz), 7.60 (dt, 1H, J = 5.7, 0.9 Hz), 7.44 (t, 1H, J = 5.7 Hz), 7.27-7.36 (m, 2H), 5.37 (dd, 2H, J = 33.3, 11.4 Hz), 3.60-3.66 (m, 1H), 3.41-3.50 (m, 1H), 3.15-3.25 (m, 2H), 2.90-2.99 (m, 1H), 1.92-1.99 (m, 1H), 1.79-1.87 (m, 1H), 1.51-1.69 (m, 2H). MS (ES) [M+H] calculated for $C_{21}H_{21}ClN_5O$ 394, 396; found 394, 396. HRMS for $C_{21}H_{21}ClN_5O$, calcd: 394.1435, found: 394.1434.

6,8-Dichloroquinazoline-2,4(*1H,3H*)-dione (3g): 2-Amino-3,5-dichlorobenzoic acid (1g, 4.85 mmol) and urea (1 g, 16.7 mmol) were heated together at 200 °C for 1 hour. The mixture was cooled and triturated with water. The solid was filtered and dried to give **3g** (0.9 mg, green solid, 80%). This material was used in the next step without further purification. MS: (ES) [M+H] calc'd for $C_8H_4Cl_2N_2O_2$, 230; found 230.

2,6,8-Trichloroquinazolin-4(*3H*)-one (5g): The title compound was prepared from 6,8-dichloro-1*H*-quinazoline-2,4-dione in 69% yield according to the procedures of examples **4a** and **5a**. MS: (ES) [M+H] calc'd for $C_8H_3Cl_3N_2O$, 250; found 250.

2-((2,6,8-Trichloro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6g): To a stirred solution of **5g** (400 mg, 1.6 mmol) in DME (4 mL) and DMF (1 mL) at 0 °C was added NaH (43 mg, 1.8 mmol, 95%). After ten minutes, LiBr (280 mg, 3.2 mmol) was added and the mixture was allowed to warm to room temperature. After 15 minutes, α-bromo-*o*-tolunitrile (350 mg, 1.8 mmol) was added and the mixture was heated at 65 °C overnight. After cooling, water (10 mL) was added. A precipitate formed. This precipitate was filtered and dried to give **6g** which was not further purified. MS (ES) [m+H] calculated for $C_{16}H_8Cl_3N_3O$, 363; found 363.

(R)-2-((2-(3-aminopiperidin-1-yl)-6,8-dichloro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile, TFA salt (1g): A mixture of **6g** (92 mg, 0.25 mmol), 3-(*R*)-aminopiperidine dihydrochloride (66 mg, 0.38 mmol), $NaHCO_3$ (63 mg, 0.75 mmol) and 2 mL of ethanol in a sealed tube was heated to 150 °C for 6 hours. After cooling to room temperature and filtering the inorganic salts, purification via preparative HPLC afforded 55 mg (51% yield) of product **1g.** ^{1}H NMR (400 MHz, MeOD): δ 7.93 (d, J = 2.53 Hz, 1H), 7.88 (d, J = 2.53 Hz, 1H), 7.71 (dd, J = 7.58, 1.01 Hz, 1H), 7.61 (ddd, J = 7.58, 7.58, 1.26 Hz, 1H), 7.44 (dd, J = 7.58, 2.4 Hz, 1H), 7.39 (d, J = 7.83 Hz, 1H), 5.47 (AB q, J = 34.86, 15.16 Hz, 2H), 3.61 - 3.80 (m, 2H), 3.34 - 3.42 (m, 1H), 3.24 - 3.27 (m, 1H), 3.10 - 3.19 (m, 1H), 2.10 - 2.20 (m, 1H), 1.64 - 1.90 (m, 3H). MS: (ES) [M+H] calculated for $C_{21}H_{19}Cl_2N_5O$, 428; found 428. HRMS for $C_{21}H_{20}Cl_2N_5O$, calcd: 428.1045, found: 428.1049.

6-Bromoquinazoline-2,4(*1H,3H*)-dione (3h): The title compound was prepared from methyl 2-amino-5-bromobenzoate in 90% yield according to the procedure for example **3l**. MS: (ES) [M+H] calc'd for $C_8H_5BrN_2O_2$, 240, 242; found 240, 242.

6-Bromo-2-chloroquinazolin-4(*3H*)-one (5h): The title compound was prepared from **3h** according to the procedures of examples **4a** and **5a**. MS: (ES) [M+H] calc'd for $C_8H_4BrClN_2O$, 260; found 260.

2-((6-Bromo-2-chloro-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6h): The title compound was prepared from **5h** as a mixture of N- and O- alkylation products according to the procedure for **6a**. MS: (ES) [M+H] calc'd for $C_{16}H_9BrClN_3O$, 375; found 375.

(R)-2-((2-(3-aminopiperidin-1-yl)-6-bromo-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile, TFA salt (1h): The title compound was prepared from **6h** according to the procedure for compound **1a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23 (d, J = 1.77 Hz, 1H), 7.77 (d, J = 6.82 Hz, 1H), 7.61 (d, J = 7.33 Hz, 1H), 7.55 (dd, J = 7.58, 7.07 Hz, 1H), 7.43 - 7.49 (m, 1H), 7.31 - 7.41 (m, 2H), 5.44 (AB q, J = 137.18, 14.91 Hz, 2H), 3.48 - 3.81 (m, 3H), 3.18 - 3.34 (m, 2H), 1.83 - 2.14 (m, 3H), 1.64 - 1.76 (m, 1H). MS: (ES) [M+H] calc'd for $C_{21}H_{20}BrN_5O$, 438; found 438. HRMS for $C_{21}H_{21}BrN_5O$, calcd: 438.0929, found: 438.0945.

6-Methoxyquinazoline-2,4(*1H,3H*)-dione (3i): 2-Amino-5-methoxybenzoic acid (2 g, 12 mmol) and urea (2.2 g, 36 mmol) were heated together at 200 °C for 1 hour. The mixture was cooled and triturated with water. The solid was filtered and dried to give **3i** (2.1 g, green solid, 90%). This material was used in the next step without further purification. MS: (ES) [M+H] calculated for $C_9H_8N_2O_3$ 193; found 193.

6-Methoxyquinazoline-2,4(*1H,3H*)-dione (4i): To 2.1 g of **3i** in 10 mL $POCl_3$ was added 0.5 mL *N*,*N*-dimethylaniline. The mixture was then heated at reflux for 16 hours. Excess $POCl_3$ was removed *in vacuo* and the residue was purified by column chromatography (hexane: ethyl acetate = 4:1), providing 1.8 g of product **4i**. MS (ES) [M+H] calculated for $C_9H_6Cl_2N_2O$ 230; found 230.

2-Chloro-6-methoxyquinazolin-4(*3H*)-one (5i): The title compound was prepared from **4i** in 80% yield according to the procedure for example **5a**. MS: (ES) [M+H] calculated for $C_9H_7ClN_2O_2$ 211; found 211.

2-((2-Chloro-6-methoxy-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6i): The title compound was prepared from **5i** in 91% yield according to the procedure for example **6k**. MS (ES) [M+H] calculated for $C_{17}H_{12}ClN_3O_2$ 326; found 326.

(R)-2-((2-(3-aminopiperidin-1-yl)-6-methoxy-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile, TFA salt (1i): A mixture of **6i** (99 mg, 0.3 mmol), 3-(*R*)-aminopiperidine dihydrochloride (80 mg, 0.46 mmol), $NaHCO_3$ (76 mg, 0.9 mmol) and 2 mL of ethanol in a sealed tube was heated to 120 °C for 6 hours. After cooling to room temperature and filtering the inorganic salts, purification via preparative HPLC afforded 38 mg (44% yield) of product **1i.** ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53 - 7.68 (m, 3H), 7.32 - 7.47 (m, 3H), 7.25 - 7.30 (m, 1H), 5.42 (AB q, J = 72.76, 14.65 Hz, 2H), 3.84 - 3.94 (m, 1H), 3.65 - 3.79 (m, 2H), 3.33 - 3.50 (m, 2H), 2.10 - 2.23 (m, 1H), 1.91 - 2.05 (m, 2H), 1.70 - 1.82 (m, 1H). MS: (ES) [M+H] calculated for $C_{22}H_{23}N_5O_2$ 390; found 390. HRMS for $C_{22}H_{24}N_5O_2$, calcd: 390.1930, found: 390.1933.

7-Fluoro-6-methoxyquinazoline-2,4(*1H,3H*)-dione (3j): The title compound was prepared from 2-amino-4-fluoro-5-methoxybenzoic acid methyl ester (see EP602851) in 90% yield according to the procedure for **3l**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.05 (br s, 2H), 7.50 (d, 1H, J = 9.2 Hz), 6.98 (d, 1H, J = 12.0 Hz), 3.88 (s, 3H). MS (ES) [M+H] calc'd for $C_9H_7N_2O_3F$ 211; found 211.

2-Chloro-7-fluoro-6-methoxyquinazolin-4(*3H*)-one (5j): The title compound was prepared from 7-fluoro-6-methoxy-1*H*-quinazoline-2,4-dione in 80% yield according to the procedures of examples **4a** and **5a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 13.29 (br s, 1H), 7.62 (d, 1H, J = 9.2 Hz), 7.51 (d, 1H, J = 12.0 Hz), 3.95 (s, 3H). MS (ES) [M+H] calc'd for $C_9H_6N_2O_2FCl$ 229, 231; found 229, 231.

2-((2-Chloro-7-fluoro-6-methoxy-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6j): The title compound was prepared from 2-chloro-7-fluoro-6-methoxy-3*H*-quinazolin-4-one in 67% yield according to the procedure for example **6k**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71-7.74 (m, 2H), 7.54 (dt, 1H, J = 7.6, 1.2 Hz), 7.36-7.44 (m, 2H), 7.14 (d,

1H, J = 7.6 Hz), 5.74 (s, 2H), 4.01 (s, 3H). MS (ES) [M+H] calc'd for $C_{17}H_{11}N_3O_2FCl$ 344, 346; found 344, 346.

(R)-2-((2-(3-aminopiperidin-1-yl)-7-fluoro-6-methoxy-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile, TFA salt (1j): The title compound was prepared from 2-(2-chloro-7-fluoro-6-methoxy-4-oxo-4*H*-quinazolin-3-ylmethyl)benzonitrile in 85% yield according to the procedure for compound **1a**. ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.93 (br s, 3H), 7.82 (d, 1H, J = 7.6 Hz), 7.60 (dt, 1H, J = 7.6, 1.2 Hz), 7.52 (d, 1H, J = 9.2 Hz), 7.38-7.46 (m, 2H), 7.21 (d, 1H, J = 7.6 Hz), 5.39 (AB q, 2H, J = 51.2, 15.2 Hz), 3.89 (s, 3H), 3.46-3.53 (m, 1H), 3.34-3.42 (m, 1H), 3.01-3.18 (m, 2H), 2.81-2.89 (m, 1H), 1.91-1.99 (m, 1H), 1.78-1.86 (m, 1H), 1.49-1.70 (m, 2H). MS (ES) [M+H] calc'd for $C_{22}H_{22}N_5O_2F$ 408; found 408. HRMS for $C_{22}H_{23}N_5O_2F$, calcd: 408.1836, found: 408.1819.

2-Chloro-6,7-dimethoxyquinazolin-4(*3H*)-one (5k): 2,4-Dichloro-6,7-dimethoxyquinazoline (1.02 g, 3.95 mmol) was converted to the title compound (664 mg, 70%) by the method used for **5a**. ^{1}H NMR (400 MHz, DMSO): δ 13.1 (s, 1H), 7.41 (s, 1H), 7.13 (s, 1H), 3.90 (s, 3H), 3.87 (s, 3H). MS: (ES) [M+H] calc'd for $C_{10}H_9ClN_2O_3$ 241; found 241.

2-((2-Chloro-6,7-dimethoxy-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6k): To a stirred solution of **5k** (280 mg, 1.17 mmol) in DME (2 mL) and DMF (0.5 mL) at 0 °C was added NaH (30 mg, 1.23 mmol). After ten minutes, LiBr (203 mg, 2.33 mmol) was added and the mixture was allowed to warm to room temperature. After 15 minutes, α-bromo-*o*-tolunitrile (457 mg, 2.33 mmol) was added and the mixture was heated at 65 °C overnight. After cooling, water (10 mL) was added. A precipitate formed. This precipitate was filtered and dried to give **6k**, which was not further purified.

(R)-2-((2-(3-aminopiperidin-1-yl)-6,7-dimethoxy-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (1k): **6k** (215 mg, 0.6 mmol) was converted to **1k** by the method used for **1a**. The product was recrystallized to give the title compound (95 mg). ^{1}H

NMR (400 MHz, DMSO): δ 7.84 (dd, J = 0.89, 7.7 Hz, 1H), 7.60 (ddd, J = 1.0, 1.1, 7.7 Hz, 1H), 7.43 (t, J = 7.6 Hz, 1H), 7.32 (s, 1H), 7.05 (d, J = 7.9 Hz), 7.01 (s, 1H), 5.41 (s, 2H), 3.90 (s, 3H), 3.81 (s, 3H), 3.25 (m, 1H), 3.17 (m, 1H), 2.72 (m, 2H), 1.80 (m, 1H), 1.67 (m, 2H), 1.52 (m, 1H), 1.11 (m, 1H). MS: (ES) [M+H] calc'd for $C_{23}H_{25}N_5O_3$ 420; found 420. HRMS for $C_{23}H_{26}N_5O_3$, calcd: 420.2036, found: 420.2033.

8-Methoxyquinazoline-2,4(*1H,3H*)-dione (3l): 2-amino-3-methoxybenzoic acid (842 mg, 5 mmol) and urea (1.5 g, 25 mmol) were heated together at 200 °C for 1.5 hours. The mixture was cooled and triturated with water. The solid was filtered and dried to give **3l** (843 mg, yellow solid, 88%). MS: (ES) [M+H] calc'd for $C_9H_8N_2O_3$ 193; found 193.

2,4-Dichloro-8-methoxyquinazoline (4l): **3l** (843 mg, 4.39 mmol) was converted to crude **4l** by the method used for **4a**.

2-Chloro-8-methoxyquinazolin-4(*3H*)-one (5l): Crude **4l** was converted to **5l** (388 mg) by the method used for **5a**. MS: (ES) [M+H] calc'd for $C_9H_7ClN_2O_2$ 211; found 211.

2-((2-Chloro-8-methoxy-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6l): **5l** (210 mg, 1 mmol) was converted to **6l** by the procedure used for **6k**. MS: (ES) M+H calc'd for $C_{17}H_{12}ClN_3O_2$ 326; found 326.

2-((2-(3-Aminopiperidin-1-yl)-8-methoxy-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (1l): **6l** (230 mg, 0.7 mmol) was converted to **1l** (100 mg, 37%) by the method used for **1a**. ^{1}H NMR (400 MHz, DMSO): δ 7.79 (dd, J = 1.2, 7.9 Hz, 1H), 7.68 (dd, J = 0.98, 7.6 Hz, 1H), 7.47 (ddd, J = 1.2, 1.3, 7.8 Hz, 1H), 7.31 (m, 2H), 7.18 (dd, J = 1.1, 8.0 Hz), 7.03 (d, J = 7.8 Hz, 1H), 5.57 (s, 2H), 4.01 (s, 3H), 3.35 (m, 1H), 3.22 (m, 1H), 2.96 (m, 2H), 2.76 (dd, J = 9.1, 11.9 Hz, 1H), 1.96 (m, 1H), 1.80 (m, 1H), 1.67 (m, 1H), 1.25 (m, 1H). MS: (ES) [M+H] calc'd for $C_{22}H_{23}N_5O_2$ 390; found 390. HRMS for $C_{22}H_{24}N_5O_2$, calcd: 390.1930, found: 390.1942.

6,7-Difluoroquinazoline-2,4(*1H,3H*)-dione: 2-Amino-4,5-difluorobenzoic acid (4 g, 23 mmol) and urea (4.2 g, 69 mmol) were heated together at 200 °C for 1 hour. The mixture was cooled and triturated with water. The solid was filtered and dried to give the title compound (4.1 g, green solid, 90%). This material was used in the next step without further purification. MS: (ES) [M+H] calc'd for $C_8H_5F_2N_2O_2$, 199; found 199.

6-Fluoro-7-morpholinoquinazoline-2,4(*1H,3H*)-dione (3m): A mixture of 6,7-difluoro-1*H*-quinazoline-2,4-dione (1 g, 5.1 mmol) and 2 mL of morpholine in 5 mL of DMSO was stirred at 90 °C for 2 hours. The mixture was diluted with water and acidified with concentrated HCl. The solid product was filtered and dried under vacuum to give 1 g (74%) of product. MS: (ES) [M+H] calculated for $C_{12}H_{12}FN_3O_3$ 266; found 266.

4-(2,4-Dichloro-6-fluoroquinazolin-7-yl)morpholine (4m): To 1 g of **3m** in 10 mL $POCl_3$ was added 0.5 mL *N,N*-dimethylaniline. The mixture was then heated at reflux for 16 hours. Excess $POCl_3$ was removed *in vacuo*, and the residue was purified by column chromatography (hexane: ethyl acetate = 4:1), providing 0.38 g of product **4m**. MS: (ES) [M+H] calculated for $C_{12}H_{10}Cl_2FN_3O$ 302; found 302.

2-Chloro-6-fluoro-7-morpholinoquinazolin-4(*3H*)-one (5m): A mixture of 5 mL of 1N NaOH, 10 mL of THF, and 0.38 g of **4m** was stirred at room temperature under N_2 overnight. The solution was acidified with HCl. The solids that precipitated were filtered to give 0.1 g (27%) of product **5m**. MS: (ES) [M+H] calculated for $C_{12}H_{11}ClFN_3O_2$ 384; found 384.

2-((2-Chloro-6-fluoro-7-morpholino-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile (6m): To a stirred solution of **5m** (100 mg, 0.35 mmol) in DME (2 mL) and DMF (0.5 mL) at 0 °C was added NaH (9.6 mg, 0.4 mmol). After ten minutes, LiBr (61 mg, 0.7 mmol) was added and the mixture was allowed to warm to room temperature. After 15 minutes, α-bromo-*o*-tolunitrile (76.4 mg, 0.39 mmol) was added and the mixture was heated at 65 °C overnight. After cooling, water (10 mL) was added. A precipitate

formed. This precipitate was filtered and dried to give **6m** (70 mg), which was not further purified. MS: (ES) [M+H] calculated for $C_{20}H_{16}ClFN_4O_2$ 399; found 399.

(R)-2-((2-(3-aminopiperidin-1-yl)-6-fluoro-7-morpholino-4-oxoquinazolin-3(*4H*)-yl)methyl)benzonitrile, TFA salt (1m): A mixture of 50 mg ($\leq$ 0.126 mmol) of crude **6m**, 2 eq. of 3-(R)-aminopiperidine dihydrochloride (43 mg, 0.25 mmol), 5 eq. of $NaHCO_3$ (53 mg), and 2 mL of ethanol in a sealed tube was heated to 150 °C for 6 hours. After cooling to room temperature and filtering the inorganic salts, purification via preparative HPLC afforded 28 mg (47% yield) of product **1m**. ^{1}H NMR (400 MHz, MeOD): δ 7.71 (d, J = 8.0 Hz, 1H), 7.55-7.62 (m, 2H), 7.43 (dd, J = 8.0, 7.2 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 7.07 (d. J = 8.0Hz, 1H), 5.49 (AB q, J = 15.2, 34.8 Hz, 2H), 3.82-3.90 (m, 4H), 3.49-3.65 (m, 2H), 3.15-3.27 (m, 6H), 2.92-3.02 (m, 1H), 2.09-2.17 (m, 1H), 1.60-1.90 (m, 3H). MS: (ES) [M+H] calculated for $C_{25}H_{27}FN_6O_2$ 463; found 463. HRMS for $C_{25}H_{28}FN_6O_2$, calcd: 463.2258, found: 463.2281.

2-((6-Chloro-2,4-dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)methyl)benzonitrile (8): To a solution of 6-chlorouracil (20 g, 122 mmol) in a mixture of DMF-DMSO (6:1 600 mL) under nitrogen at 0 °C, was added sodium hydride (60%, 5.5 g, 137 mmol) in portions. After 0.5h, lithium bromide (8 g, 96 mmol) was added into the mixture and stirred for 15 min at 0 °C. A solution of *α*-bromo-*o*-tolunitrile (25.1 g, 128 mmol) in DMF (30 mL) was added dropwise, and stirred at this temperature for 1 h, and then at room temperature overnight. The mixture was evaporated and azeotroped with water *in vacuo* to remove most of the DMF, and then poured into ice-water (1L). Solid product was collected by filtration. The crude product was suspended in hot ethyl acetate-chloroform and sonicated for 5 min, then allowed to stand at 0 °C for 1h. The mixture was filtered to give a white solid of the title compound (19 g) in 54% yield. ^{1}H NMR (400 MHz, DMSO): δ 11.82 (s, 1H), 7.87 (d, 1H, J = 7.6 Hz), 7.71 (t, 1H, J = 7.6 Hz), 7.51 (t, 1H, J = 7.6 Hz), 7.37 (d, 1H, J = 8 Hz), 6.06 (s, 1H). 5.31 (s, 2H). MS (ES) [m+H] calc'd for $C_{12}H_8ClN_3O_2$, 262.03; found 262.03.

2-((6-Chloro-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)methyl)benzonitrile (9): To a cold (0 °C) solution of **8** (10 g, 38 mmol) in DMF-THF (1:1, 300 mL) under nitrogen, was added NaH (60%, 1.6 g, 39.9 mmol) in portions, followed by adding LiBr (2g). The mixture was stirred at room temperature for 20 min. After adding iodomethane (5.4 mL, 76 mmol), the flask was sealed and stirred at 0 °C for 10 min, room temperature for 2h, and 35 °C overnight, and then was concentrated *in vacuo.* The residue was dissolved in $CHCl_3$ and washed with water and brine, dried (Na_2SO_4) and filtered, and then concentrated *in vacuo.* Crude product was crystallized from THF-hexanes to give 7.6 g (72%) of the title compound. ^{1}H NMR (400 MHz, DMSO): δ 7.87 (d, 1H, J = 7.6 Hz), 7.70 (t, 1H, J = 7.6 Hz), 7.51 (t, 1H, J = 7.6 Hz), 7.40 (d, 1H, J = 8 Hz), 6.21 (s, 1H). 5.38 (s, 2H). 3.28 (s, 3H). MS (ES) [m+H] calc'd for $C_{13}H_{10}ClN_3O_2$, 275.05; found 275.05.

(R)-2-((6-(3-aminopiperidin-1-yl)-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(*2H*)-yl)methyl)benzonitrile (10): **9** (3.0 g, 10.8 mmol), (*R*)-3-aminopiperidine dihydrochloride (2.24 g, 10.8 mmol) and sodium bicarbonate (5.5 g, 54 mmol) were stirred with 1 g activated MS (4A) in dry ethanol (30 mL) at 100 °C for 2 h. The reaction was filtered through Celite, concentrated *in vacuo*, and then diluted with $CHCl_3$ and washed with water. The aqueous phase was extracted with $CHCl_3$ and the combined organic phases were washed with water, dried (Na_2SO_4) and filtered. TFA (1mL) was added into the solution and the mixture was concentrated *in vacuo*. The residue was dissolved in a small amount of MeOH, and Et_2O was added to force precipitation. The solvents were decanted and the residue washed with Et_2O two times to give 2.7 g product as an off-white powder. ^{1}H NMR (400 MHz, $CDCl_3$-CD_3OD 10:1): δ 7.82 (d, 1H, J = 7.6 Hz), 7.65 (t, 1H, J = 7.6 Hz), 7.46 (t, 1H, J = 7.6 Hz), 7.23 (d, 1H, J = 8.0 Hz), 5.42 (s, 1H), 5.50-5.00 (ABq, 2H, J = 41.6, 15.2 Hz), 3.30 (m, 2H), 3.16 (s, 3H), 2.91 (m, 1H), 2.76 (m, 2H), 1.93 (m, 1H), 1.79 (m, 1H), 1.51 (m, 2H). MS (ES) [m+H] calc'd for $C_{18}H_{21}N_5O_2$, 339.17; found, 339.17. HRMS for $C_{18}H_{22}N_5O_2$, calcd: 340.1774, found: 340.1769.

Purity data: Elemental analyses were conducted at Robertson Microlit Laboratories:

Compound	Formula	Calculated	Found	Notes
1k	$C_{23}H_{25}N_5O_3$	C, 65.86; H, 6.01; N, 16.70	C, 65.57; H, 6.24; N, 16.59	free base
1l	$C_{22}H_{23}N_5O_2$	C, 67.85; H, 5.95; N, 17.98	C, 67.62; H, 5.76; N, 17.69	free base
10	$C_{25}H_{27}N_5O_4$	C, 65.06; H, 5.90; N, 15.17	C, 65.00; H, 6.04; N, 15.15	benzoate salt

HPLC analyses were conducted using the following conditions:

- Column: Gemini (5um, 4.6x50mm)
- Mobile Phase:
  - A: 0.05%TFA H2O
  - B: 0.035%TFA Acetonitrile
- Flow Rate: 3.5ml/min
- Gradient: 5-95%B in 4.2 min
- Run Time: 6min
- Injection volume: 5 uL
- Sample Concentration: 10mM in DMSO

Compound	HPLC purity by ELSD	Salt form
1a	100	TFA
1b	100	TFA
1c	97	HCl
1d	99	TFA
1e	100	TFA
1f	100	TFA
1g	100	TFA
1h	97	TFA
1i	100	TFA
1j	100	TFA
1m	99	TFA

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