

Discovery of 3-Quinazolin-4(3H)-on-3-yl-2,N-dimethylpropanamides as Orally Active and Selective PI3K α Inhibitors

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SUPPORTING INFORMATION

In Vitro Biology

Biochemical assays for PI3K isoforms

KinaseGlo format

1. Compound preparation

Compound dilution: Transfer 50 nL 100× compound / DMSO to assay plate using Echo. Carried out 3-fold serial dilutions from 0.111 mM for 10 points (Final Top=1 μM): PI3K alpha. Carried out 3-fold serial dilutions from 1.11 mM for 10 points (Final Top=10 μM): PI3K beta, delta & gamma.

2. Enzyme Reaction

In a polypropylene plate, PI3Ks lipid kinases, appropriate substrate, ATP (concentration at K_m), and test compound were incubated together in a kinase reaction buffer. The 5 μL assay mixtures contained 50 mM HEPES, 3 mM $MgCl_2$, 50 mM NaCl, 0.01% BSA, 50 μM PIP2:3PS peptide substrate, and PI3Ks enzyme (0.2 nM alpha; 0.6 nM beta; 0.25 nM delta; 0.4 nM gamma). In all cases, each PI3K biochemical assay used ATP at a final concentration equal to its $K_{mapparent}$. Incubations were carried out at 22 °C. Prior to the assay, enzyme, peptide substrate and serially diluted test compounds were pre-incubated together in assay buffer (3 μL) for 15 minutes, and the assay was initiated by the addition of 2 μL assay buffer containing 2.5× final ATP concentration (these were the 2.5× concentrations: 100 μM alpha & beta & delta; 62.5 μM gamma). Following the 120-minutes incubation for beta, delta and gamma, 150-minutes incubation for alpha. Then the activity of the kinase was detected using the ADP-Glo™ Assay in two steps. First, the kinase reaction was terminated, and any ATP remaining after the reaction was depleted, leaving only ADP. Then the Kinase Detection Reagent was added to convert ADP to ATP, which was used in a coupled luciferin/luciferase reaction. The luminescent output was measured and was correlated with kinase activity.

3. Data Analysis

Curves were fit by XLFIT5 as % inhibition vs. log [compound concentration] using a 4 parameter logistic model²⁰⁵.

Cellular assays for PI3K isoforms

MCF-7 cell line

Compounds were dissolved in DMSO and stored in a nitrogen cabinet. The stock concentration was 10 mM. Prepared compound dilution plate, the test compounds starting concentration was 10 μ M, 3-fold dilution, 10 doses. Seeded MCF-7 cells in a full 96 well plate, 100 μ l/well (2.5×10^4 cell per well) in cell culture media. The next day, replaced the culture media with 100 μ l none FBS media, and starved overnight. Compounds were diluted from top concentration 10 μ M with 3-fold serial dilution for 10 points. Added 25 μ L compounds dilution to the cell media, incubated at 37 $^{\circ}$ C for 2 hours. Stimulated the cells with 10 μ g/mL insulin in 96 well plates, incubated for 30min, then centrifuged at the rate of 1000rpm for 5min at room temperature. Washed the cells once with 250 μ l/well HBSS(4 $^{\circ}$ C) with 1 mM/L Na_3VO_4 . Added 100 μ L lysis buffer to the plate, shook for 60min at 4 $^{\circ}$ C. Then centrifuged at the rate of 4000rpm for 10 min at 4 $^{\circ}$ C. Other steps were processed according to the manual of the ELISA Kit.

PI3K α specific BT-474 cell line

Compounds were dissolved in DMSO and stored in a nitrogen cabinet. The stock concentration was 10 mM. Prepared compound dilution plate, the test compounds starting concentration was 10 μ M, 3-fold dilution, 10 doses. Seeded BT-474 cells in a full 96 well plate, 1.0×10^5 cell per well in cell culture media. The next day, replaced the culture media with 100 μ l none FBS media, and starved overnight. Compounds were diluted from top concentration 10 μ M with 3-fold serial dilution for 10 points. Added 25 μ L compounds dilution to the cell media, incubated at 37 $^{\circ}$ C for 2 hours. Stimulated the cells with final concentration of 10 μ g/mL insulin in 96 well plates, incubated for 30 min, then centrifuged at the rate of 1000rpm for 5 min at room temperature. Washed the cells once with 250 μ l/well HBSS (4 $^{\circ}$ C) with 1 mM/L Na_3VO_4 . Added 100 μ L lysis buffer to the plate, shook for 60 min at 4 $^{\circ}$ C. Then centrifuged at the rate of 4000rpm for 10 min at 4 $^{\circ}$ C. Other steps were processed according to the manual of the ELISA Kit.

PI3K β specific MDA-MB-468 cell line

The MDA-MB-468 cells are seeded at 100 μ L/ 1.0×10^5 /well in 96 well plate with

starvation overnight. The following morning, the cells were treated with compound top concentration was 10 μM with 8 points, 4 fold dilution. The stimulation was stopped by addition of cell lysis buffer and left for 30 minutes. Phospho-AKT was detected using 2 different specific antibodies in a sandwich assay format. An antibody with donor conjugate (Eu^{3+} -cryptate) binds to phospho-AKT and the antibody with acceptor conjugate (d2) binds to AKT. After incubation for 4 hours, the plates were read on EnVingen at 620 and 665 nm. The level of phospho-AKT was expressed as the 665 nm / 620 nm ratio.

PI3K γ specific RAW264.7 cell line

The RAW 264.7 cells are seeded at 100 $\mu\text{L}/1.0 \times 10^5/\text{well}$ in 96 well plate with starvation overnight. Prepared compounds with DMSO as 10 mM solution and diluted it with media in 10 μM (1 mL media+1 μL compound) then 4-fold dilution with media (40 μL last dose+120 next dose). The next morning, the cells were treated with compound, whose top concentration was 10 μM with 8 points 4-fold dilution 100 $\mu\text{L}/\text{well}$. Prepared 4 μM mouse C5a with ddH₂O and diluted with media in 25 nM (8 mL media+50 μL C5a), and then added 100 $\mu\text{L}/\text{well}$ 25 nM mouse C5a for 3 minutes. The stimulation was stopped to remove the media and then added cell lysis buffer 50 $\mu\text{L}/\text{well}$ and left for 30 minutes on vibrators. Removed 16 μL lysis buffer from 96-well cell culture plate to reading plate and added 4 $\mu\text{L}/\text{well}$ mixture which contained antibody and acceptor conjugate (d2) (1:1). Phospho-AKT was detected using 2 different specific antibodies in a sandwich assay format. An antibody with donor conjugate (Eu^{3+} -cryptate) bound to phospho-AKT and the antibody with acceptor conjugate (d2) bound to AKT. After incubation for 4 hours, the plates were read on EnVingen at 620 and 665 nm. The level of phospho-AKT was expressed as the 665 nm / 620 nm ratio.

PI3K δ specific Jeko-1 cell line

The Jeko-1 cells were seeded at 40 $\mu\text{L}/1.0 \times 10^5/\text{well}$ in 96 well plate with starvation overnight. The next morning, the cells were treated with compound (top concentration 10 μM , with 8 points, 4 fold dilution) at 37°C for 2 h. Then the cells were stimulated with a final concentration of 0.6 $\mu\text{g}/\text{mL}$ anti-IgM for 10 minutes. The stimulation was stopped by addition of cell lysis buffer and left for 30 minutes. Phospho-AKT was

detected using two different specific antibodies in a sandwich assay format. An antibody with donor conjugate (Eu³⁺-cryptate) bound to phospho-AKT and the antibody with acceptor conjugate (d2) bound to AKT. After incubation for 4 hours, the plates were read on EnVingen at 620 and 665 nm. The level of phospho-AKT was expressed as the 665 nm / 620 nm ratio.

In vivo study

Ovarian Carcinoma SKOV3 mouse xenograft subcutaneous models

Cell Culture: The SKOV-3 tumor cells were maintained in vitro as a monolayer culture in RPMI-1640 medium supplemented with 10% heat inactivated fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin at 37 °C in an atmosphere of 5% CO₂ in air. The tumor cells were routinely subcultured twice weekly by trypsin-EDTA treatment. The cells growing in an exponential growth phase were harvested and counted for tumor inoculation.

Animals: BALB/c nude mice, female, 6-8 weeks, weighing approximately 18-22g. They were purchased from Vital River., LTD or other certified vendors.

Tumor Inoculation: Each mouse was inoculated subcutaneously at the right flank with SKOV-3 tumor cells (10 x 10⁶) with matrigel (1:1 media-to-matrigel) in 0.2 mL of tumor development. The animals were randomized and treatment was started when the average tumor volume reaches approximately 150-200 mm³ for the efficacy study.

Assignment to Groups: Before commencement of treatment, all animals were weighed and the tumor volumes were measured. Since the tumor volume can affect the effectiveness of any given treatment, mice were assigned into groups using an Excel-based randomization software performing stratified randomization based upon their tumor volumes. This ensures that all the groups were comparable at the baseline.

Animal Housing: An acclimation period of approximately one week was allowed between animal arrival and tumor inoculation in order to accustom the animals to the laboratory environment. The mice were maintained in a special pathogen-free environment and in individual ventilation cages (5 mice per cage). All cages, bedding, and water were sterilized before use. When working in the mouse room, the

investigators wore lab coat and latex or vinyl gloves. Each cage was clearly labeled with a cage card indicating number of animals, sex, strain, date received, treatment, study number, group number, and the starting date of the treatment. The cages with food and water were changed twice a week. The targeted conditions for animal room environment and photoperiod were as follows: temperature 20~26 °C, humidity 40~70 %, light cycle 12 hours light and 12 hours dark.

Dietary Materials: All animals had free access to a standard certified commercial laboratory diet. Maximum allowable concentrations of contaminants in the diet were controlled and routinely analyzed by the manufacturers. Autoclaved municipal tap water, suitable for human consumption was available to the animals ad libitum. It was considered that there were no known contaminants in the dietary materials that could influence the tumor growth.

Observations: The protocol and any amendment(s) or procedures involving the care and use of animals in this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of WuXi AppTec prior to conduct. During the study, the care and use of animals were conducted in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). After inoculation, the animals were checked daily for morbidity and mortality. At the time of routine monitoring, the animals were checked for any effects of tumor growth and treatments on normal behavior such as mobility, food and water consumption, body weight gain/loss (body weights will be measured twice weekly or every other day), eye/hair matting and any other abnormal effect. Death and observed clinical signs were recorded on the basis of the numbers of animals within each subset.

Endpoints: The major endpoint was to see if the tumor growth can be delayed or mice could be cured. Tumor sizes were measured twice weekly (or every other day) in two dimensions using a caliper, and the volume was expressed in mm³ using the formula: $V = 0.5 a \times b^2$ where a and b were the long and short diameters of the tumor, respectively. The tumor sizes were then used for the calculations of TGI values. TGI was calculated for each group using the formula: $TGI (\%) = [1 - (T_i - T_0) / (V_i - V_0)] \times 100$; T_i is the average tumor volume of a treatment group on a given day, T_0 is the average tumor

volume of the treatment group on the first day of treatment, V_i is the average tumor volume of the vehicle control group on the same day with T_i , and V_0 is the average tumor volume of the vehicle group on the first day of treatment.

Termination:

1) Bodyweight loss: Any animal exhibiting 20% bodyweight loss at any one day was humanely killed or the veterinary staff was contacted.

2) Tumor burden: Tumor burden should not exceed 10% of the animal's bodyweight. The study was terminated with all animals being sacrificed when the mean tumor volume of the vehicle control group reaches a value of 2,000 mm³.

Ulceration: If tumor ulceration occurs, the following procedures will apply: animals with ulcerated tumors were monitored at least 3 times per week with increasing frequency, up to daily, depending upon clinical signs. Ulcerated tumors, which had not scabbed over, should be cleaned with an appropriate wound cleansing solution (e.g., Novalsan). Antibiotic cream was to be applied to the ulceration/lesion only if directed by the Veterinary staff.

Criteria for euthanasia include if the lesion: did not heal or form a scab within 1 week. Was greater than 5 mm diameter, became cavitated, developed signs of infection (such as presence of pus) or bleeding, or if the animal showed signs of discomfort (e.g. excessive licking and biting directed at the site) or systemic signs of illness (lethargy, decreased activity, decreased food consumption, decreased body condition or weight loss). Contact the veterinary staff to discuss any possible exceptions.

3) Clinical signs: Animals must be euthanized if they found to be moribund (unless special permission is granted by the IACUC based on adequate justification, which must be included in the protocol and increased supportive care provided such as warm SQ fluids, Diet Gel food cup next to animal so they can reach food, cage on a warming pad for supplemental heat, etc. Note: a moribund condition indicates an animal was unlikely to survive.) For questions regarding these endpoints, please contact the Veterinary Staff.

Clinical examples of morbidity may include: hunched, persistent recumbency and lack of response to handling or other stimuli, signs of severe organ or system failure,

emaciation, hypothermia, CNS deficits: convulsions, respiratory: rapid respiratory rate, labored breathing, coughing, rales, GI: diarrhea lasting > 2 days, jaundice. Any animal that exhibited the above clinical issues were humanely sacrificed by CO₂. Necropsy was not performed in the event of an unexpected death.

Statistical Analysis: For comparison between two groups, an independent sample t-test was used. For comparison among three or more groups, a one-way ANOVA was performed. If a significant F -statistics (a ratio of treatment variance to the error variance) was obtained, multiple comparison procedures were applied after ANOVA. The potential synergistic effect between treatments was analyzed by two-way ANOVA. All data was analyzed using SPSS 17.0. $p < 0.05$ was considered to be statistically significant.

Breast Cancer BT-474 mouse xenograft subcutaneous models

Cell Culture: The BT-474 tumor cells (ATCC, Manassas, VA, cat # HTB-20) were maintained in vitro as a monolayer culture in Hybri-care medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 µg/mL streptomycin, at 37°C in an atmosphere of 5% CO₂ in air. The tumor cells will be routinely subcultured twice weekly by trypsin-EDTA treatment. The cells growing in an exponential growth phase will be harvested and counted for tumor inoculation.

Animals: BALB/c nude, female, 6~8 weeks, weighing approximately 18~22g. They were purchased from Shanghai Sino-British SIPPR/BK Laboratory Animal Co., LTD. or other certified vendors.

Tumor Inoculation: Each mouse were inoculated subcutaneously at the right flank with BT-474 tumor cells (10×10^6) in 0.2 mL of PBS with Matrigel (1:1) for tumor development, which has been S.C. implanted with 17-beta Estradiol pellet (0.36mg, 60-day release) 2~3 days before cell implantation. The animals were randomized and treatment was started when the average tumor volume reached approximately 100~200 mm³ for the efficacy study.

Animal Housing: An acclimation period of approximately one week was allowed between animal arrival and tumor inoculation in order to accustom the animals to the

laboratory environment. The mice were maintained in a special pathogen-free environment and in individual ventilation cages (5 mice per cage). All cages, bedding, and water were sterilized before use. When working in the mouse room, the investigators wore lab coat and latex or vinyl gloves. Each cage was clearly labeled with a cage card indicating number of animals, sex, strain, date received, treatment, study number, group number, and the starting date of the treatment. The cages with food and water were changed twice a week. The targeted conditions for animal room environment and photoperiod were as follows: temperature 20~26 °C, humidity 40~70 %, light cycle 12 hours light and 12 hours dark.

Dietary Materials: All animals had free access to a standard certified commercial laboratory diet. Maximum allowable concentrations of contaminants in the diet were controlled and routinely analyzed by the manufacturers. Autoclaved municipal tap water, suitable for human consumption was available to the animals ad libitum. It was considered that there were no known contaminants in the dietary materials that could influence the tumor growth.

Assignment to Groups: Before commencement of treatment, all animals were weighed and the tumor volumes were measured. Since the tumor volume could affect the effectiveness of any given treatment, mice were assigned into groups using an Excel-based randomization software performing stratified randomization based upon their tumor volumes. This ensured that all the groups were comparable at the baseline.

Observations: The protocol and any amendment(s) or procedures involving the care and use of animals in this study will be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of WuXi AppTec prior to conduct. During the study, the care and use of animals were conducted in accordance with the regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). After inoculation, the animals were checked daily for morbidity and mortality. At the time of routine monitoring, the animals were checked for any effects of tumor growth and treatments on normal behavior such as mobility, food and water consumption, body weight gain/loss (body weights were measured twice weekly), eye/hair matting and any other abnormal effect. Death and observed clinical signs were

recorded on the basis of the numbers of animals within each subset. Mice were also monitored for signs of estrogen toxicity. Monitoring included bladder palpation twice per week for the first 1~2 weeks after pellet implantation. After 1~2 weeks the animals were monitored daily, since signs of toxicity developed. Monitoring consisted of bladder palpation to determine if the bladders were enlarged. If enlarged, bladders were expressed. Add Enrofloxacin into the drinking water at the final concentration of 0.25mg/mL since the toxicity of 17beta-estradiol tablets developed. If the mice were not able to urinate normally and blood or precipitates were observed in the urine, the mice were euthanized.

Endpoints: The major endpoint was to see if the tumor growth could be delayed or mice could be cured. Tumor sizes were measured twice weekly in two dimensions using a caliper, and the volume were expressed in mm³ using the formula: $V = 0.5 a \times b^2$ where a and b were the long and short diameters of the tumor, respectively. The tumor sizes were then used for the calculations of both TGI(%) values. TGI is calculated for each group using the formula: $TGI (\%) = [1 - (T_i - T_0) / (V_i - V_0)] \times 100$; T_i is the average tumor volume of a treatment group on a given day, T_0 is the average tumor volume of the treatment group on the first day of treatment, V_i is the average tumor volume of the vehicle control group on the same day with T_i , and V_0 is the average tumor volume of the vehicle group on the first day of treatment.

Termination:

- 1) Bodyweight loss: Any animal exhibiting 20% bodyweight loss at any one day were humanely killed or the veterinary staff will be contacted.
- 2) Tumor burden: The size of a single animal tumor volume exceeded 3,000 mm³, it was sacrificed. The study was terminated with all animals being sacrificed when the mean tumor volume of the vehicle control group reached a value of 2,000 mm³.
- 3) Clinical signs: Animals must be euthanized if they were found to be moribund (unless special permission is granted by the IACUC based on adequate justification, which must be included in the protocol and increased supportive care provided such as warm SQ fluids, Diet Gel food cup next to animal so they can reach food, cage on a warming pad for supplemental heat, etc. Note: a moribund condition indicates an

animal is unlikely to survive.) For questions regarding these endpoints, please contact the Veterinary Staff.

Clinical examples of morbidity may include: hunched, persistent recumbency and lack of response to handling or other stimuli, signs of severe organ or system failure, emaciation, hypothermia, CNS deficits: convulsions, respiratory: rapid respiratory rate, labored breathing, coughing, rales, GI: diarrhea lasting > 2 days, jaundice.

Any animal that exhibited the above clinical issues were humanely sacrificed by CO₂.

Abnormal urinating: If animals cannot urinate on their own or if there was blood or sediment in the urine, they were euthanized.

Statistical Analysis: For comparison between two groups, an independent sample t-test was used. For comparison among three or more groups, a one-way ANOVA was performed. If a significant F -statistics (a ratio of treatment variance to the error variance) was obtained, multiple comparison procedures were applied after ANOVA. All data was analyzed using SPSS 17.0. $p < 0.05$ was considered to be statistically significant.

PD study

1. Protein extraction & protein quantification

Took the snap-frozen tissues out from -80°C freezer or liquid nitrogen tank. Cut up to 30 mg of tissue. Placed the tissues in a 2 mL microcentrifuge tube, and added 300-500 µL of RIPA Buffer (contains 1% Protease Inhibitor Cocktail and 1% Phosphatase Inhibitor Cocktail 2). Grinded tumors with TissueLyser LT at full speed for 5min. Kept the tissue lysates on ice for 30 min. Centrifuged at the rate of 12,000 rpm at 4°C for 10 min; gently transferred the supernatants to a new microcentrifuge tube kept on ice. Measured protein concentrations by Pierce™ BCA Protein Assay Kit. According to the result of BCA protein quantification, diluted all samples to the same final concentration (at 2µg/µL) using RIPA buffer plus 4× LDS Sample Buffer and 10× Sample Reducing Agent. Heat the samples at 100 °C for 10min. Proceeded to western blotting or kept the denatured samples in the -80°C refrigerator for long term storage.

2. Western blotting

Added protein samples to NuPAGE® Novex 4-12% Bis-Tris gel, 10 µL per slot. Electrophoresis. 80V for 30 min and then 120V for 90 min. Transferred protein to nitrocellulose membranes with iBlot®2 Gel Transfer Device, 7min. Blocked the non-specific protein by 1×TBST with 5% nonfat milk at room temperature, 1h. Washed the membrane with 1×TBST three times for 5min each. Incubated membrane with 5-10 mL diluted primary antibody in 1×TBST with 5% nonfat milk or BSA at 4°C, with gentle agitation overnight. Washed the membrane with 1×TBST three times for 10min each. Incubated membrane with HRP-conjugated secondary antibody (at the appropriate dilution, according to antibody vendor, generally 1:2000) with gentle agitation for 1h at room temperature. Washed the membrane with 1×TBST three times for 10min each. Added HRP substrate from West Femto Maximum Sensitivity kit to the NC membranes. Detected chemiluminescence by Tanon 5200 multi.

3. Quantification of band intensity

Intensity of individual bands was quantified using Tanon 5200 multi densitometry software.

Pharmacokinetic studies

Microsome Metabolic Stability Assay

Liver microsomes of human, SD rat, Beagle dog and CD-1 mouse were purchased from Xenotech, (Cat. No. H2610, R1000, D1000 and M1000). Cynomolgus monkey liver microsomes were prepared by WuXiAppTec. 10 µM test and control compound working solutions were prepared from 10 mM dimethyl sulfoxide (DMSO) stock solutions *via* a 100× dilution with methanol (MeOH) followed by a 100× dilution with potassium phosphate buffer (PB, 100 mM, pH 7.4±0.1). 10 µL/well of these working solutions were added to all 96-well time-point plates (Apricot) except for the blank plate (T0, T5, T10, T20, T30, T60, and NCF60). 300 µL/well of acetonitrile (ACN) containing 100 ng/mL tolbutamide and 100 ng/mL labetalol as internal standards (stop solution) were added to the T0 plate. 80 µL/well of microsomes diluted with 100 mM PB such that the protein concentration in the final incubation medium would be 0.5 mg/mL were then added to all plates, and the plates were pre-incubated in a 37°C water bath for 10 minutes. After pre-incubation, the

addition of 10 μL /well NADPH (β -nicotinamide adenine dinucleotide phosphate powder (Sigma, Cat. No. N0505) dissolved in 10 mM MgCl_2) initiated the reactions in each time-point plate except for the NCF60 plate. At each time-point, the corresponding plate was removed from the water bath for the addition of 300 μL /well stop solution. The plates were then sealed and shaken for 10 minutes prior to centrifugation at 4000 rpm and 4°C for 20 minutes. 100 μL /well of the resulting supernatant were diluted with 300 μL /well of pure water and sealed and shaken for 10 minutes prior to LC-MS/MS (API 4000, AB Sciex, MA, USA) analysis.

Hepatocyte Metabolic Stability Assay

Cryopreserved CD-1 mouse, SD rat, beagle dog and human hepatocytes were purchased from In Vitro Technologies, Cat. No. M005052, M00005, M00205 and X008001; cryopreserved cynomolgus monkey hepatocytes were purchased from RILD, Cat. No. HP-SXH-02M. Control and test compound dosing solutions were prepared from 30 mM and 10 mM dimethyl sulfoxide (DMSO) stock solutions, respectively, *via* a 50 \times dilution with 45% methanol (MeOH)/water, followed by a 5 \times dilution with pre-incubated Williams' Medium E (Sigma, Cat. No. W1878). Cryopreserved hepatocytes were thawed, isolated, and suspended in Williams' Medium E, then diluted with pre-incubated Williams' Medium E to a final concentration of 0.625×10^6 cells/mL. 10 μL /well of control or test compound dosing solutions were added to all 96-well time-point plates (Greiner, Cat. No. 655201) except for the blank plate (T0, T15, T30, T60, and T90), followed by the addition of 40 μL /well of cell suspensions to initiate the reactions. For the T0 plate, 150 μL /well of ACN containing 200 ng/mL tolbutamide and 200 ng/mL labetalol as internal standards (stop solution) were added prior to the addition of cell suspensions. The remaining time-point plates were placed in a 95% humidified, 5% CO_2 incubator (Thermo, Waltham, MA, USA) at 37°C and 800 rpm for incubation. At each time-point, the corresponding plate was removed from the incubator for the addition of 150 μL /well stop solution. Medium control (MC) plates (T0-MC and T90-MC) were prepared by adding everything except for Williams' Medium E at the corresponding time-points. The plates were then sealed and shaken for 10 minutes prior to centrifugation at 4000 rpm and 4°C for 20

minutes. The resulting supernatant was diluted 1:3 with pure water and sealed and shaken for 10 minutes prior to LC-MS/MS analysis (API 4000, AB Sciex, MA, USA).

Plasma Protein Binding Assay

The test compound or positive control warfarin (Struchem CO., LTD., Cat. No. SC-16139) was spiked into frozen CD-1 mouse plasma (BioreclamationIVT, Cat. No. MSEPLEDTA2-M) and human plasma (BioreclamationIVT, Cat. No. HMPLEDTA2, pooled from multiple individuals) at the final concentration of 2 μ M. An aliquot of 150 μ L of the compound-spiked plasma sample was added to one side of the chamber in a 96-well equilibrium dialysis plate (HTDialysis LLC, Cat. No. 1006) and an equal volume of dialysis buffer (Phosphate Buffered Saline, 100mM, pH 7.4 \pm 0.1) was added to the other side of the chamber. An aliquot of plasma sample was harvested before the incubation and used as T0 samples for recovery calculation. Triplicate incubations were performed. The plate was then incubated in the humidified incubator (HERAcell 240i, Thermo Fisher) with 5% CO₂ at 37°C for 4 hours. After incubation, 50 μ L of samples were transferred from the plasma side as well as the buffer side into new 96-well plates (Apricot, Cat. No. DWP-22-96-SQ-U-C-L). Each sample was added with equal volume of opposite blank matrix (buffer or plasma) to reach a final volume of 100 μ L with volume ratio of plasma: Dialysis Buffer at 1:1 (v:v) in each well. All samples were further processed by adding 300 μ L stop solution (Acetonitrile/Methanol (50:50, v:v)) containing internal standards (tolbutamide at 200 ng/mL). The mixture was vortexed and centrifuged at 4000 rpm for about 20 min. Then an aliquot of 100 μ L of supernatant of all the samples was removed and mixed with 100 μ L of water. The samples were vortexed for 10 min, and then injected onto LC/MS/MS (API 4000, AB Sciex, MA, USA).

The following formula for calculation was used:

$$\% \text{ Unbound} = 100 \times [F] / [T],$$

$$\% \text{ Bound} = 100 - \% \text{ Unbound},$$

$$\% \text{ Recovery} = 100 \times ([F] + [T]) / [T_0]$$

$$F_u \text{ (apparent)} = [F] / [T]$$

$$\% \text{ Undiluted Unbound} = 100 * (1/D) / [(1/F_u \text{ (apparent)} - 1) + 1/D]$$

where

[F] = Concentration of compound in free fraction (from receiver chamber)

[T] = Concentration of compound in total plasma fraction (from donor chamber)

[T₀] = Concentration of compound in T₀ samples (before dialysis)

MDR1-MDCK II study

MDR1-MDCKII cells were grown in α -MEM (Gibco, Cat. No. 11900-024) supplemented with 10% heat-inactivated FBS (Invitrogen, Cat. No. 10099-141), 100mg/mL penicillin-G, 100mg/mL streptomycin (HyClone, Cat. No. SV30010) and 1% non-essential amino acids. The cells were incubated at 37°C, 5% CO₂/95% air, and saturated humidity. After reaching 80-90% confluency, the cells were gently detached with a 0.25% (w/v) trypsin (Invitrogen, Cat. No. 27250-018) / 0.53 mM EDTA solution. Cells (passage 15) were seeded on the 96-well BD insert system (Becton Dickinson, Cat. No. 359274 and 359205) at a density of 2.5×10^5 cells/mL and cultured for 4 days before being used for this transport study.

Bidirectional permeability in MDR1-MDCK II cells:

Test compounds were diluted with the transport buffer (HBSS with 10 mM HEPES, pH 7.4 \pm 1) from DMSO stock solution to a concentration of 2 μ M (0.4%DMSO) and applied to the apical (A) or basolateral (B) side of the cell monolayer. Permeation of the test compounds from A to B and B to A directions was determined in duplicate. Digoxin (Sigma, Cat. No. 20830-75-5), the known P-gp substrate, was tested at 2 μ M in A to B and B to A directions. Fenoterol (Sigma, Cat. No. F1016) and propranolol (Sigma, Cat. No. P0884) were used as low and high permeability markers, and tested at 2 μ M in the A to B direction in duplicate.

Transport buffer (TB) and dosing solutions were pre-warmed to 37 °C before the transport assay. For A to B directional transport assay, 75 μ L dosing solution was added to the apical well. Fill each basolateral well with 250 μ L TB containing 0.4% DMSO. For B to A directional transport assay, 250 μ L of dosing solution was added to the basolateral well in duplicate after filling each apical well with 75 μ L TB containing 0.4% DMSO. The plates were incubated for 150 minutes at 37 °C with 5% CO₂/95% air, and saturated humidity. The time zero samples were generated by mixing 50 μ L dosing solution of test compounds or reference compounds with 100 μ L TB containing 0.4% DMSO and 250 μ L stop solution (acetonitrile (ACN) containing 250 ng/mL

Tolbutamine). At 150 minutes, 150 μL of solution was collected from each A to B receiver well followed by addition of 250 μL quench solution to get A to B receiver sample. And for other samples (A to B donor, B to A donor and receiver), 50 μL of solution was collected from each corresponding well followed by addition of 250 μL quench solution and 100 μL TB containing 0.4% DMSO. All samples were vortex-mixed and centrifuged at 3220 g for 10 minutes. Subsequently, supernatant was diluted with ultra pure water for LC-MS/MS analysis (API 4000, AB Sciex, MA, USA).

Cell Monolayer Integrity Measurement:

After transport assay, Lucifer yellow (Sigma, Cat. No. L0144-100 mg) rejection assay were applied to determine the cell monolayer integrity. Buffers were removed from both apical and basolateral chambers, followed by the addition of 75 μL of 100 μM lucifer yellow in transport buffer and 250 μL transport buffer in apical and basolateral chambers, respectively. The plate was incubated for 30 minutes at 37°C with 5% CO₂ and 95% relative humidity without shaking. After 30 minutes incubation, 20 μL of lucifer yellow samples were taken from the apical sides, followed by the addition of 60 μL of TB. And then 80 μL of lucifer yellow samples were taken from the basolateral sides. The relative fluorescence unit (RFU) of lucifer yellow was measured at 425/528 nm (excitation/emission) with a Molecular Device M2e plate reader.

The following formula for calculation was used:

Equation of apparent permeability coefficients (P_{app}):

$$P_{app} = (dC_r/dt) \times V_r / (A \times C_0)$$

Where dC_r/dt is the cumulative concentration of compound in the receiver chamber as a function of time ($\mu\text{M/s}$); V_r is the solution volume in the receiver chamber (0.050 mL on the apical side, 0.25 mL on the basolateral side); A is the surface area for the transport, i.e. 0.0804 cm^2 for the area of the monolayer; C_0 is the initial concentration in the donor chamber (μM).

Equation of efflux ratio:

$$\text{Efflux Ratio} = P_{app}(\text{B to A}) / P_{app}(\text{A to B})$$

Equation of Solution Recovery:

$$\% \text{ Solution Recovery} = 100 \times [(V_r \times C_r) + (V_d \times C_d)] / (V_d \times C_0)$$

Where V_d is the volume in the donor chambers (0.05 mL on the apical side, 0.25 mL on the basolateral side); C_d and C_r are the final concentrations of transport compound in donor and receiver chambers, respectively.

CYP Inhibition assay

The inhibitory potency of the test compound to the five human live Cytochrome P450 (CYP) isoforms including CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4, were determined using human liver microsomes (Coning, Cat. No. 452117). The reaction mixtures were prepared in 100 mM potassium phosphate buffer (pH7.4) containing the human liver microsomes at 0.200 mg protein/mL, test compound at 0.0500, 0.150, 0.500, 1.50, 5.00, 15.0 and 50.0 μ M in singlet or the positive control inhibitors at a particular concentration in duplicate, and a cocktail of five marker substrates, including phenacetin, diclofenac, *s*-mephenytoin, dextromethorphan and midazolam at concentrations of 10.0, 5.00, 30.0, 5.00 and 2.00 μ M, respectively. After warm up at 37°C for 10.0-minute, the cofactor NADPH (Chem-impex international, Cat. No. 00616) was added into the mixture at 1.00 mM to initiate the reaction. The 200 μ l reaction mixture was continued to incubate at 37°C for 10.0 minutes, followed by addition of 0.400 ml stop solution containing internal standard to terminate the reaction. Samples were centrifuged at 3220 \times g at 4°C for 20.0 minutes. The 200 μ l resulting supernatant was taken and diluted with 100 μ l ultrapure water. Samples plates were shaken at 1000 rpm for 10.0 min to make the mixture homogeneous prior to the analysis using LC-MS/MS to measure the metabolites of substrates.

The detailed information on CYP enzyme isoforms, marker substrates, substrate concentrations, positive control inhibitors, inhibitor concentrations, and analytes is listed in the following table.

CYP Isoform	Marker Substrate	Substrate Concentration (μ M)	Positive Control Inhibitor	Inhibitor Concentration (μ M)	Analyte for LC/MS/MS
1A2	Phenacetin	10.0	α -Naphthoflavone	3.00	Acetaminophen
2C9	Diclofenac	5.00	Sulfaphenazole	3.00	4'-Hydroxydiclofenac
2C19	<i>S</i> -Mephenytoin	30.0	<i>S</i> -(+)-N-3-Benzylrivanol	3.00	4'-Hydroxymephenytoin
2D6	Dextromethorphan	5.00	Quinidine	3.00	Dextrorphan

3A4	Midazolam	2.00	Ketoconazole	3.00	1'- Hydroxymidazolam
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Pharmacokinetic studies in mice

Twelve Female Balb/c mice (Beijing Vitalriver Laboratory Animal Technology Co., LTD) were divided into 2 groups (iv and po group) with 6 animals/group. The animal room environment was controlled (target conditions: temperature 18 to 26°C, relative humidity 30 to 70%, 12 hours artificial light and 12 hours dark). Animals were fasted at least 12 hours prior to the administration and had access to Certified Rodent Diet (Catalog # M01-F, SLAC Laboratory Animal Cl. Ltd., Shanghai, China) *ad libitum* 4 hours post dosing. Blood collection was performed in rotation sampling manner, 3 animals/time point, from submandibular vein of each animal into polypropylene tubes with potassium EDTA as anti-coagulant. Each collected blood was put in the wet ice until processed for plasma by centrifugation at approximately 4°C, 3,200 g for 10min. Then plasma was collected respectively and transferred into pre-labeled 96 well plate in the wet ice. After terminal collection, all plasmas were stored at approximately -80°C Freezer until delivered to BA group. For iv group, the test substance was administered intravenously as a clear solution in 10% HP-beta-CD:10% solutol=1:1(pH=8) at a dose of 1 mg/kg, while for po group the test substance was dosed orally as a homogenous opaque suspension in 0.5% Methyl cellulose/0.2% Tween 80 at a dose of 10 mg/kg. HP-beta-CD was purchased from Roquette. Solutol, Methyl cellulose (4000 cps), and Tween 80 were purchased from Sigma.

Pharmacokinetic studies in rats

Six male Sprague-Dawley rats (Beijing Vitalriver Laboratory Animal Technology Co., LTD) were divided into 2 groups (iv and po group) with 3 animals/group. The animal room environment was controlled (target conditions: temperature 20 to 26°C, relative humidity 40 to 70%, 12 hours artificial light and 12 hours dark). Animals were fasted at least 12 hours prior to the administration and had access to Certified Rodent Diet (Beijing Keaoxieli Feed Co., Ltd. Beijing, China) *ad libitum* 4 hours post dosing. Each blood collection was performed at each

timepoint from jugular vein into polypropylene tubes with potassium EDTA as anti-coagulant. Each collected blood was put in the wet ice until processed for plasma by centrifugation at approximately 4°C, 3200 g for 10min. Then plasma was collected respectively and transferred into pre-labeled 96 well plate in the wet ice. After terminal collection, all plasmas were stored at approximately -80°C Freezer until delivered to BA group. For iv group, the test substance was administered intravenously as a clear solution in 10% HP-beta-CD:10% solutol=1:1 (pH=8) at a dose of 1 mg/kg, while for po group the test substance was dosed orally as a homogenous opaque suspension in 0.5% Methyl cellulose /0.2% Tween 80 at a dose of 3 mg/kg.

Bioanalytical method for pharmacokinetics

Chemicals and reagents: HPLC grade water was purified by PURELAB OPTION S15 System. HPLC grade acetonitrile (MeCN) was purchased from Merck (MERCK-1.00030.400). Ammonium formate and formic acid were purchased from Honeywell (Fluka-17836, Fluka-94318). ACQUITY UPLC BEH C18 1.7 μ m 2.1 \times 50 mm column was purchased from Waters (186002350).

An aliquot of 10 μ L mouse plasma sample was protein precipitated with 200 μ L internal standards (100 ng/mL Labetalol & 100 ng/mL dexamethasone & 100 ng/mL tolbutamide & 100 ng/mL Verapamil & 100 ng/mL Glyburide & 100 ng/mL Celecoxib in MeCN), the mixture was vortex-mixed well and centrifuged at 4000 rpm for 20 min, 4°C. 2 μ L supernatant was injected for LC-MS/MS analysis. The solution was separated on a ACQUITY UPLC BEH C18 1.7 μ m 2.1 \times 50 mm column using a linear gradient (0 min, 80% A/20% B; 1 min, 10% A/90% B; 1.2 min, 10% A/90% B; 1.21 min, 80% A/20% B; 1.5 min, 80% A/20% B at a flow rate of 0.5 mL/min). Mobile phase A consisted of 0.3% formic acid in HPLC grade water. Mobile phase B consisted of 0.3% formic acid in HPLC grade acetonitrile. The flow from the UPLC system was directly introduced into the ion source of the Triple Quad 6500 mass spectrometer (AB Sciex, MA, USA) and subjected to electrospray ionization (positive ion mode).

An aliquot of 30 μ L rat plasma sample was protein precipitated with 300 μ L internal standards (100 ng/mL Labetalol & 100 ng/mL dexamethasone & 100 ng/mL tolbutamide & 100 ng/mL

Verapamil & 100 ng/mL Glyburide & 100 ng/mL Celecoxib in MeCN), the mixture was vortex-mixed well and centrifuged at 4000 rpm for 20 min, 4°C. 4 µL supernatant was injected for LC-MS/MS analysis. The solution was separated on a ACQUITY UPLC BEH C18 1.7 µm 2.1 × 50 mm column using a linear gradient (0 min, 90% A/10% B; 1.2 min, 10% A/90% B; 1.4 min, 10% A/90% B; 1.41 min, 90% A/10% B; 1.5 min, 90% A/10% B at a flow rate of 0.6 mL/min). Mobile phase A consisted of 0.025% formic acid and 1mM ammonium formate in HPLC grade water and HPLC grade acetonitrile (v: v, 95:5). Mobile phase B consisted of 0.025% formic acid and 1mM ammonium formate in HPLC grade acetonitrile and HPLC grade water (v: v, 95:5). The flow from the UPLC system was directly introduced into the ion source of the API 4000 mass spectrometer (AB Sciex, MA, USA) and subjected to electrospray ionization (positive ion mode).

The test article was detected via a specific daughter ion of its protonated quasi-molecular ion. A calibration curve with 8 non-zero calibration standards was applied for the quantitation of plasma drug concentration using blank rat or mouse plasma samples spiked with stock solutions of internal standards.

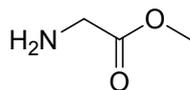
Experimental part chemistry

General

Unless otherwise stated, all reagents and solvents were purchased from commercial suppliers and used without further purification. ¹H NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer. Chemical shifts are reported in parts per million (ppm) relative to an internal solvent reference. Recorded peaks are listed in the order multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; qt, quintet; m, multiplet; br, broad), coupling constants, and number of protons. Final compounds were purified to ≥95% purity as assessed by analytical liquid chromatography.

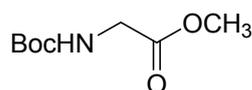
Synthesis and full characterization of (S)-N¹-(7-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)pyrrolidine-1,2-dicarboxamide (1)

Methyl 2-aminoacetate



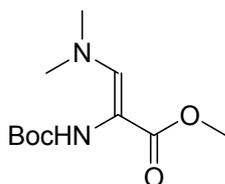
To a solution of 2-aminoacetic acid (5.00 g, 66.60 mmol, 1.00 *eq*) in anhydrous MeOH (60.00 mL) was added a solution of SOCl₂ (9.51 g, 79.92 mmol, 5.80 mL, 1.20 *eq*) drop-wise at 0°C over a period of 3 min and stirred at 0°C for 15 min. Then the reaction mixture was warmed to 65°C and stirred at 65°C for 4.5 hours. The solution was concentrated under reduce pressure to yield the title compound (11.24 g, crude) and it was used directly. ¹H-NMR (400 MHz, CDCl₃): δ 3.75 (s, 2 H), 3.80 (s, 3 H).

Methyl 2-(tert-butoxycarbonylamino)acetate



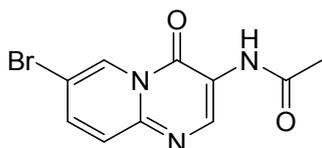
To a solution of methyl 2-aminoacetate (5.94 g, 66.67 mmol, 1.00 *eq*) in THF (48.00 mL) and MeOH (48.00 mL) at 0°C was added NaHCO₃ (16.80 g, 200.01 mmol, 7.78 mL, 3.00 *eq*). Then Boc₂O (15.28 g, 70.00 mmol, 16.08 mL, 1.05 *eq*) was added in one portion, then remove the ice bath and the mixture was stirred at 28°C for 20 h. The mixture was filtered and the solid was washed with EA (3×10 mL). The filtrate was washed with water (2×20 mL) and saturated NH₄Cl aqueous (2×20 mL). The organic layer was then dried over anhydrous Na₂SO₄ and then evaporated in vacu to to yield the title compound (11.24 g, crude) used directly. ¹H NMR (400 MHz, CDCl₃): δ 1.47 (s, 9 H), 3.77 (s, 3 H), 3.91 - 3.96 (m, 2 H), 4.94 - 5.15 (m, 1 H).

(Z)-methyl 2-((tert-butoxycarbonyl)amino)-3-(dimethylamino)acrylate



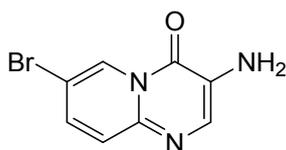
A mixture of methyl 2-((tert-butoxycarbonyl)amino)acetate (3.58 g, 19 mmol), anhydrous toluene (16 mL), and bis(dimethylamino)-tert-butoxymethane (3.58 g, 20 mmol) was stirred at the reflux temperature (oil bath) for 3.5 h. The reaction mixture was concentrated under reduced pressure to yield the title compound (4.07 g, crude) used directly. ¹H NMR (400 MHz, CDCl₃): δ 1.46 (d, *J*=2.51 Hz, 9 H), 3.03 - 3.05 (m, 6 H), 3.67 (s, 3 H), 5.27 - 5.37 (m, 1 H), 7.28 - 7.33 (m, 1 H).

N-(7-bromo-4-oxo-4H-pyrido[1,2-*a*]pyrimidin-3-yl)acetamide



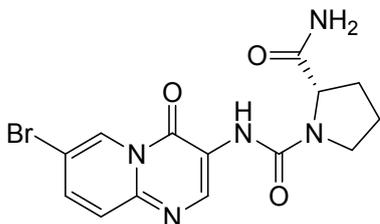
A mixture of (Z)-methyl 2-((tert-butoxycarbonyl)amino)-3-(dimethylamino)acrylate (4.07 g, 16.66 mmol, 1.00 eq) and 5-bromopyridin-2-amine (2.88 g, 16.66 mmol, 1.00 eq) in AcOH (30.00 mL) was stirred and heated to reflux. After the reaction had been reacted for 23 h. The mixture was cooled to 28 °C and diluted in water (100 mL). Then saturated aqueous NaOH was added to adjust the pH of the mixture to 9. The solution was again extracted with EA (3×300 mL). The organic layer was dried over Na₂SO₄. The EA was evaporated under reduced pressure, and then the residue was purified by column chromatography (SiO₂, Petroleum ether/Ethyl acetate=10:1 to 1:3) to yield the title compound. ¹H NMR (400 MHz, DMSO-*d*₆): δ 2.17 (s, 3 H), 7.60 - 7.68 (m, 1 H), 7.88 - 7.98 (m, 1 H), 8.96 - 9.01 (m, 1 H), 9.12 - 9.21 (m, 1 H), 9.69 - 9.77 (m, 1 H). MS: 281.9/283.9(M+1)⁺.

3-amino-7-bromo-4H-pyrido[1,2-*a*]pyrimidin-4-one



To a mixture of N-(7-bromo-4-oxo-pyrido[1,2-a]pyrimidin-3-yl)acetamide (2.50 g, 8.86 mmol, 1.00 *eq*) in MeOH (40.00 mL) was added 6N HCl (6 M, 5.02 mL, 3.40 *eq*), and then the mixture was stirred at reflux temperature for 18 h. The mixture was cooled to 28 °C and some solid precipitated. Then the solid was filtered and washed with water (2×10 mL). The solid (2.12 g, 8.83 mmol, 99.67% yield) was used directly without further purification. ¹H NMR (400 MHz, DMSO-*d*₆): δ 3.15 (s, 2 H), 7.57 - 7.61 (m, 1 H), 7.75 - 7.80 (m, 1 H), 7.92 - 7.95 (m, 1 H), 8.88 - 8.91 (m, 1 H). MS:239.9/241.9(M+1)⁺.

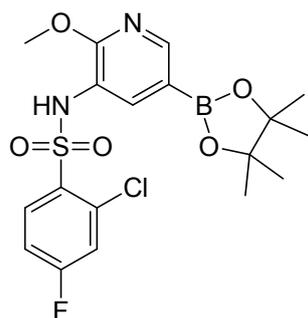
(*S*)-N1-(7-bromo-4-oxo-4H-pyrido[1,2-a]pyrimidin-3-yl)pyrrolidine-1,2-dicarboxamide



To a stirring solution of triphosgene (48.96 mg, 165.00 μmol, 0.33 *eq*) in 25 mL DCM at 0 °C was added dropwise a mixed solution of 3-amino-7-bromo-pyrido[1,2-a]pyrimidin-4-one (120.03 mg, 500.00 μmol, 1.00 *eq*) and DIEA (193.36 mg, 1.50 mmol, 261.87 μL, 3.00 *eq*) in DCM (6.6 mL). The reaction mixture was stirred at 0 °C for 30 min. Then to the mixture at 0 °C was added a solution of L-prolinamide (57.08 mg, 500.00 μmol, 1.00 *eq*) and DIEA (193.36 mg, 1.50 mmol, 261.87 μL, 3.00 *eq*) in DCM (6.6 mL) slowly. The resulting mixture was stirred at 0 °C for 30 min, then at room temperature for 17 h. The reaction mixture was partitioned between H₂O 10 mL and DCM 10 mL. The organic phase was separated, aqueous phase was washed with DCM (5 mL×3). The combined organic phase was washed with saturated NaHCO₃ (20 mL) and brine (20 mL), dried over Na₂SO₄, filtered and concentrated under reduced pressure to yield the title compound (110.00 mg, 289.32 μmol, 57.86% yield) and it

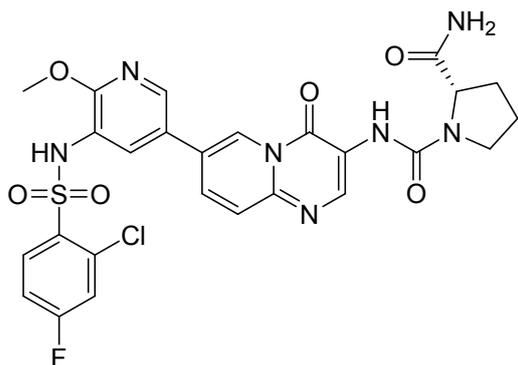
was used directly. $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 1.87 - 1.92 (m, 2 H), 2.37 - 2.44 (m, 2 H), 3.39 - 3.68 (m, 1 H), 4.19 - 4.31 (m, 1 H), 6.99 - 7.06 (m, 1 H), 7.40 - 7.53 (m, 2 H), 7.56 - 7.65 (m, 1 H), 7.80 - 7.91 (m, 1 H), 8.89 - 8.94 (m, 1 H), 8.96 - 9.03 (m, 1 H). MS:380.0/382.0(M+1) $^+$.

2-chloro-4-fluoro-N-(2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)benzenesulfonamide



To a solution of 2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-amine (5.00 g, 19.99 mmol, 1.00 *eq*) in pyridine (10.00 mL) was added 2-chloro-4-fluoro-benzenesulfonyl chloride (4.58 g, 19.99 mmol, 2.92 mL, 1.00 *eq*) drop-wise at room temperature (28°C) over a period of 10 min. The reaction mixture was stirred at room temperature for 18 hours. Water (5 mL) was poured into the reaction mixture, large amount of orange solid was precipitated. And then the mixture was filtered and the solid was washed with water (3 \times 10 mL) and PE (3 \times 10 mL) to yield the title compound (8.01 g, 18.09 mmol, 90.50% yield) and it was used directly. $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 1.31 (s, 12 H), 3.89 (s, 3 H), 6.99 - 7.05 (m, 1 H), 7.21 - 7.25 (m, 1 H), 7.34 - 7.38 (m, 1 H), 7.97 - 7.99 (m, 1 H), 8.02 - 8.07 (m, 1 H), 8.20 - 8.22 (m, 1 H). MS:443.0(M+1) $^+$.

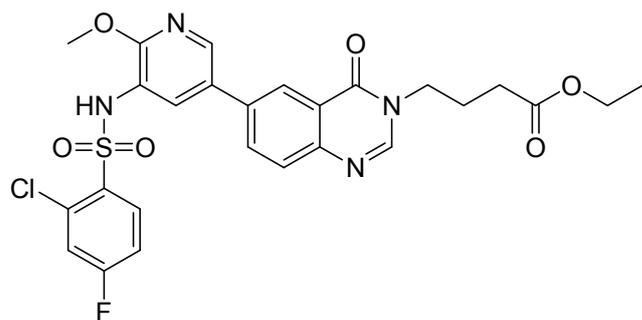
(*S*)-*N* 1 -(7-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxo-4H-pyrido[1,2-*a*]pyrimidin-3-yl)pyrrolidine-1,2-dicarboxamide (**1**)



A mixture of (2*S*)-*N*'-(7-bromo-4-oxo-pyrido[1,2-*a*]pyrimidin-3-yl)pyrrolidine-1,2-dicarboxamide (200.00 mg, 526.04 μmol , 1.00 *eq*), 2-chloro-4-fluoro-*N*-[2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-pyridyl]benzenesulfonamide (256.17 mg, 578.64 μmol , 1.10 *eq*), KOAc (206.50 mg, 2.10 mmol, 4.00 *eq*), cyclopentyl(diphenyl)phosphane;dichloropalladium;iron (38.49 mg, 52.60 μmol , 0.10 *eq*) in dioxane (3.74 mL) and H₂O (700.00 μL) was degassed and purged with N₂ for 3 times, and then the mixture was stirred at 105 °C for 1.5 h under nitrogen atmosphere. The mixture was cooled to 25°C and the volatile component was evaporated under reduced pressure. Then the residue was washed with water (30 mL), and extracted by EA (3×30 mL). The organic layer was dried over anhydrous Na₂SO₄. The residue was purified by flash silica gel chromatography. (SiO₂, Petroleum ether/Ethyl acetate=10:1 to 1:3) to yield the title compound (132.00 mg, 214.28 μmol , 30.63% yield). ¹H NMR (400 MHz, DMSO-*d*₆): δ 1.83 - 1.96 (m, 3 H), 2.05 - 2.17 (m, 1 H), 3.44 - 3.53 (m, 1 H), 3.58 - 3.65 (m, 1 H), 3.69 (s, 3 H), 4.22 - 4.31 (m, 1 H), 6.97 - 7.08 (m, 1 H), 7.31 - 7.40 (m, 1 H), 7.43 - 7.52 (m, 2 H), 7.69 - 7.77 (m, 2 H), 7.90 - 7.99 (m, 2 H), 8.01 - 8.09 (m, 1 H), 8.35 - 8.42 (m, 1 H), 8.88 - 8.95 (m, 1 H), 8.96 - 9.03 (m, 1 H). MS:616.1(M+1)⁺.

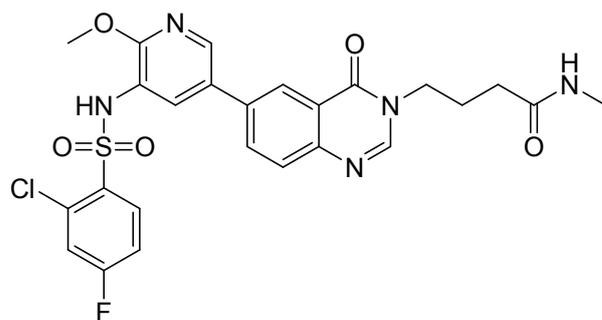
Synthesis and full characterization of 4-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*-methylbutanamide (2)

Ethyl 4-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)butanoate



The title compound refers to the synthesis method of methyl 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxy-pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-methylpropanoate. MS: 575.1(M+1)⁺.

4-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxy-pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*-methylbutanamide (**2**)

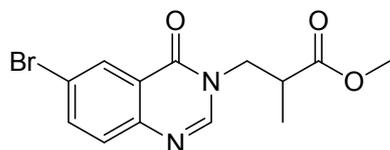


To a solution of ethyl 4-[6-[5-[(2-chloro-4-fluoro-phenyl)sulfonylamino]-6-methoxy-3-pyridyl]-4-oxo-quinazolin-3-yl]butanoate (300.00 mg, 521.73 μmol , 1.00 *eq*) in EtOH (20.00 mL) was added methanamine (5.00 mL, 25~30% in MeOH). The mixture was stirred at 80 °C for 16 h. The solvents was removed in vacuum. The residue was purified by flash silica gel chromatography to yield the title compound (39.00 mg, 69.23 μmol , 13.27% yield). ¹H NMR (400MHz, CDCl₃): δ 8.27 (d, *J*=2.0 Hz, 1H), 8.13 - 7.99 (m, 3H), 7.91 (d, *J*=2.3 Hz, 1H), 7.83 - 7.76 (m, 1H), 7.75 - 7.67 (m, 1H), 7.21 (br s, 1H), 7.07 - 6.98 (m, 1H), 5.80 (br s, 1H), 4.07 (t, *J*=6.8 Hz, 2H), 3.91 (s, 3H), 2.76 (d, *J*=4.8 Hz, 3H), 2.33 - 2.18 (m, 1H), 2.27 - 2.00 (m, 1H), 2.16 - 1.93 (m, 1H), 2.16 - 1.93 (m, 1H). MS: 560.1(M+1)⁺.

Synthesis and full characterization of (S)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxy-pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-

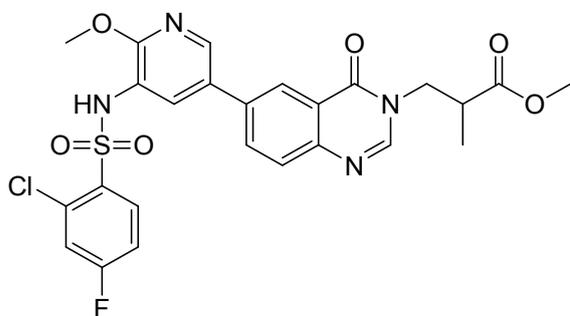
methylpropanamide (3)

Methyl 3-(6-bromo-4-oxoquinazolin-3(4*H*)-yl)-2-methylpropanoate



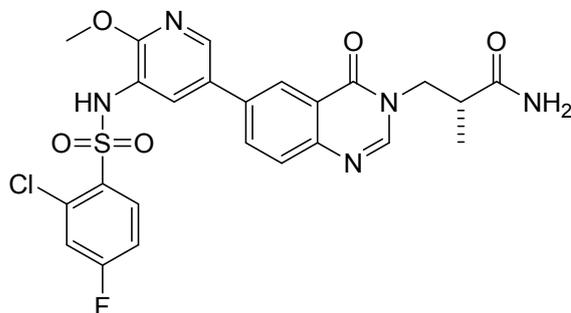
6-bromo-3*H*-quinazolin-4-one (50.00 mg, 222.18 μmol , 1.00 *eq*), methyl (2*R*)-3-bromo-2-methyl-propanoate (80.44 mg, 444.36 μmol , 2.00 *eq*) and Cs_2CO_3 (144.78 mg, 444.36 μmol , 2.00 *eq*) were taken up into a microwave tube in DMF (2.00 mL). The sealed tube was heated at 100°C for 2 h under microwave. The solvent was concentrated in vacuum. The residue was purified by prep-TLC (Petroleum ether: Ethyl acetate = 3:1) to yield the title compound (180.00 mg, 424.26 μmol , 31.83% yield). MS: 325.0/327.0 ($\text{M}+1$)⁺.

Methyl 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-methylpropanoate



To a solution of methyl methyl 3-(6-bromo-4-oxoquinazolin-3(4*H*)-yl)-2-methylpropanoate (60.02 mg, 141.47 μmol , 1.00 *eq*) in dioxane (2.00 mL) and H_2O (200.00 μL) was added 2-chloro-4-fluoro-*N*-[2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-pyridyl]benzenesulfonamide (65.44 mg, 141.47 μmol , 1.00 *eq*), KOAc (55.53 mg, 565.88 μmol , 4.00 *eq*) and $\text{Pd}(\text{dppf})\text{Cl}_2$ (2.07 mg, 2.83 μmol , 0.02 *eq*) under nitrogen atmosphere. The mixture was stirred at 95°C for 2 h. The solvent was removed in vacuum. The residue was purified by prep-TLC (Dichloromethane: Methanol: TEA = 20: 1: 0.2) to yield the title compound (160.00 mg, 239.21 μmol , 84.54% yield, 83.87% purity). MS: 561.0 ($\text{M}+1$)⁺.

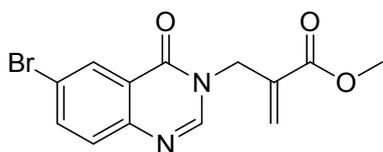
(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-methylpropanamide (**3**)



Ammonia gas (15 psi) was bubbled in to MeOH (30.00 mL) at 0°C for 30 minutes. Methyl (2*S*)-3-[6-[5-[(2-chloro-4-fluoro-phenyl)sulfonylamino]-6-methoxy-3-pyridyl]-4-oxo-quinazolin-3-yl]-2-methyl-propanoate (190.00 mg, 284.06 μ mol, 1.00 eq) was added in to above solution, the mixture was stirred at 80°C for 16 h in a sealed tube. The solvent was removed in vacuum. The residue was purified by prep-TLC (Dichloromethane: Methanol: TEA=20: 1: 0.2) and was purified by SFC (column: Chiralpak AD (250mm*30mm, 10 μ m); mobile phase: [0.1%NH₃H₂O ETOH];B%: 35%-35%,min) to yield the title compound (9.00 mg, 5.75% yield). ¹H NMR (400 MHz, CDCl₃): δ 8.31 (d, *J*=2.0 Hz, 1H), 8.15 - 8.05 (m, 3H), 7.98 (d, *J*=2.3 Hz, 1H), 7.90 - 7.69 (m, 2H), 7.28 (br s, 1H), 7.19 - 7.00 (m, 1H), 5.58 (br s, 1H), 5.40 - 5.23 (m, 1H), 4.28 - 4.12 (m, 1H), 4.12 - 4.01 (m, 1H), 3.98 (s, 3H), 3.10 (br dd, *J*=7.4, 14.4 Hz, 1H), 1.33 (d, *J*=7.0 Hz, 3H), MS:546.1 (M+1)⁺.

Synthesis and full characterization of 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2,2-difluoropropanamide (4**)**

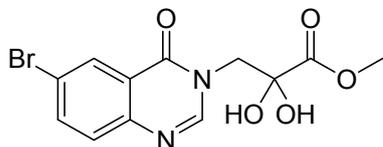
Methyl 2-((6-bromo-4-oxoquinazolin-3(4*H*)-yl)methyl)acrylate



The title compound refers to the synthesis method of methyl 3-(6-bromo-4-

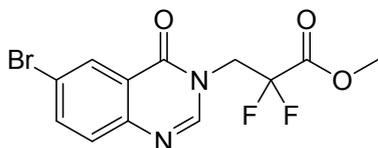
oxoquinazolin-3(4*H*)-yl)-2-methylpropanoate in 34.8% yield. MS: 323.0 (M+1)⁺.

Methyl 3-(6-bromo-4-oxoquinazolin-3(4*H*)-yl)-2,2-dihydroxypropanoate



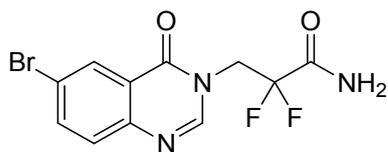
To a solution of methyl 2-((6-bromo-4-oxoquinazolin-3(4*H*)-yl)methyl)acrylate (1 g, 3.09 mmol, 1.00 eq) in DCM (50 mL) was pumped O₃ until the solution turns blue. Immediately nitrogen was pumped until the solution turns colorless, then add Me₂S (961.41 mg, 15.47 mmol, 1.14 mL, 5 eq). The mixture was stirred at 25°C for 1 h. The solvent was concentrated under reduced pressure to yield the title compound (750 mg, 70.63% yield). ¹H NMR (400MHz, CDCl₃): δ 8.41 (d, *J*=2.0 Hz, 1H), 7.97 - 7.83 (m, 2H), 7.63 (d, *J*=8.8 Hz, 1H), 5.24 (s, 2H), 3.97 (s, 3H), MS: 342.9/344.9 (M+1)⁺.

Methyl 3-(6-bromo-4-oxoquinazolin-3(4*H*)-yl)-2,2-difluoropropanoate



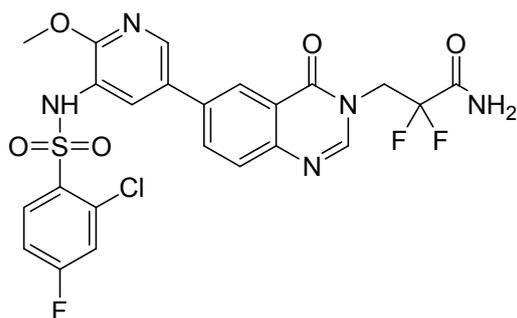
To a solution of methyl 3-(6-bromo-4-oxoquinazolin-3(4*H*)-yl)-2,2-dihydroxypropanoate (630 mg, 1.84 mmol, 1.00 eq) in DCM (1.00 mL) was added DAST (1.48 g, 9.18 mmol, 1.21 mL, 5.00 eq). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 25°C for 12 h. The reaction mixture was partitioned between ice water 10 mL and DCM 10 mL. The organic phase was separated, aqueous phase washed with DCM (5 mL * 3). The combined organic phase dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (Petroleum ether: Ethyl acetate =1:1) to yield the title compound (140 mg, 19.77% yield). ¹H NMR (400MHz, CDCl₃): δ 8.41 (d, *J*=2.4 Hz, 1H), 8.04 (s, 1H), 7.87 (dd, *J*=2.0, 8.7 Hz, 1H), 7.61 (d, *J*=8.8 Hz, 1H), 4.62 (t, *J*=13.0 Hz, 2H), 3.90 (s, 3H), MS: 346.9/348.9 (M+1)⁺.

3-(6-bromo-4-oxoquinazolin-3(4H)-yl)-2,2-difluoropropanamide



To a solution of methyl 3-(6-bromo-4-oxoquinazolin-3(4H)-yl)-2,2-difluoropropanoate in MeOH (1 mL) was added a solution of NH₃ in MeOH (7N, 1.15 mL), then the mixture was stirred at 25°C for 12 h. The solution was concentrated under reduce pressure to yield the title compound (100 mg, 301.11 umol, 74.66% yield). ¹H NMR (400MHz, CDCl₃): δ 8.44 (d, *J*=2.2 Hz, 1H), 8.08 (s, 1H), 7.89 - 7.85 (m, 1H), 7.61 (d, *J*=8.4 Hz, 1H), 4.72 (t, *J*=14.6 Hz, 2H), MS: 331.9/333.9 (M+1)⁺.

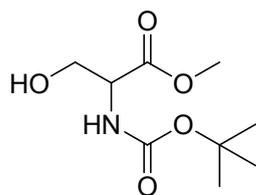
3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)-2,2-difluoropropanamide (**4**)



The title compound refers to the synthesis method of methyl 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)-2-methylpropanoate in 16% yield. ¹H NMR (400 MHz, METHANOL-d₄): δ 8.45 - 8.21 (m, 3H), 8.15 - 7.98 (m, 3H), 7.82 (d, *J*=8.2 Hz, 1H), 7.49 (br d, *J*=8.2 Hz, 1H), 7.25 (br t, *J*=7.8 Hz, 1H), 3.89 (d, *J*=1.0 Hz, 3H), MS: 568.0 (M+1)⁺

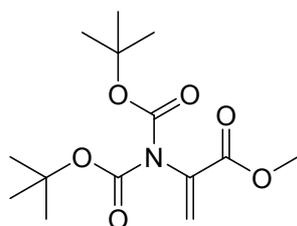
Synthesis and full characterization of 2-amino-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)propanamide (5)

Methyl 2-((tert-butoxycarbonyl)amino)-3-hydroxypropanoate



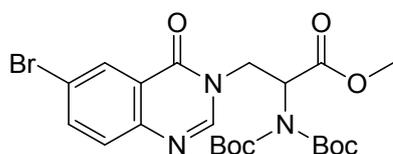
To a solution of methyl 2-amino-3-hydroxy-propanoate; hydrochloride (5.00 g, 32.14 mmol, 1.00 eq) in dioxane (120.00 mL) was added NaHCO₃ (172.80 g, 80.00 mL, 8% purity) and tert-butoxycarbonyl tert-butyl carbonate (21.04 g, 96.42 mmol, 22.15 mL, 3.00 eq). The reaction was degassed and purged with N₂ for 3 times, and then the mixture was stirred at 25°C for 14 h. The solution was concentrated under reduced pressure to obtain the title compound (17.83 g, crude) and it was used directly.

Methyl 2-[bis(tert-butoxycarbonyl)amino]prop-2-enoate



To a solution of methyl 2-(tert-butoxycarbonylamino)-3-hydroxy-propanoate (7.00 g, 31.93 mmol, 1.00 eq) in MeCN (150.00 mL) was added DMAP (780.18 mg, 6.39 mmol, 0.20 eq) and tert-butoxycarbonyl tert-butyl carbonate (34.84 g, 159.65 mmol, 36.68 mL, 5.00 eq). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 60°C for 12 h. The mixture was concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (Petroleum ether : Ethyl acetate = 7:1) to yield the title compound (6.55 g, 21.74 mmol, 68.08% yield). ¹H NMR (400 MHz, CDCl₃): δ 6.32(s, 1H), 5.62(s, 1H), 3.77(s, 3H), 1.44(s, 18H).

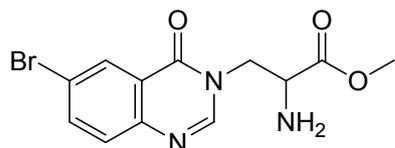
Methyl 2-[bis(tert-butoxycarbonyl)amino]-3-(6-bromo-4-oxo-quinazolin-3-yl)propanoate



The title compound refers to the synthesis method of methyl 3-(6-bromo-4-

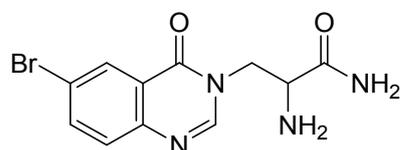
oxoquinazolin-3(4*H*)-yl)-2-methylpropanoat in 38.9% yield. ¹H NMR (400MHz, DMSO-*d*₆): δ 8.28(s, 1H), 8.21-8.22(d, *J*=2.4Hz, 1H), 7.97-8.00(dd, *J*= 8.8, 1H), 7.63-7.65(d, *J*=8.8Hz, 1H), 5.35-5.39(m, 1H), 4.51-4.60(m, 1H), 3.73 (s, 3H), 1.26 (s, 18H). MS: 526.1 (M+1)⁺.

Methyl 2-amino-3-(6-bromo-4-oxoquinazolin-3(4*H*)-yl)propanoate



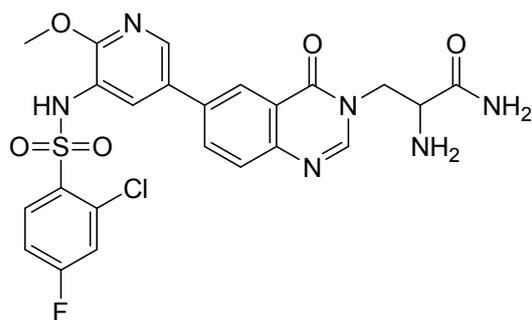
To a solution of HCl/EtOAc (4 M, 30.00 mL, 31.58 *eq*) was added methyl 2-[bis(tert-butoxycarbonyl)amino]-3-(6-bromo-4-oxo-quinazolin-3-yl)propanoate (2.00 g, 3.80 mmol, 1.00 *eq*). Then the mixture was stirred at 20°C for 4 h. The mixture was filtered and the filter cake was collected to yield the title compound (1.35 g, 3.61 mmol, 95.03% yield), MS: 326/328 (M+1)⁺.

2-amino-3-(6-bromo-4-oxoquinazolin-3(4*H*)-yl)propanamide



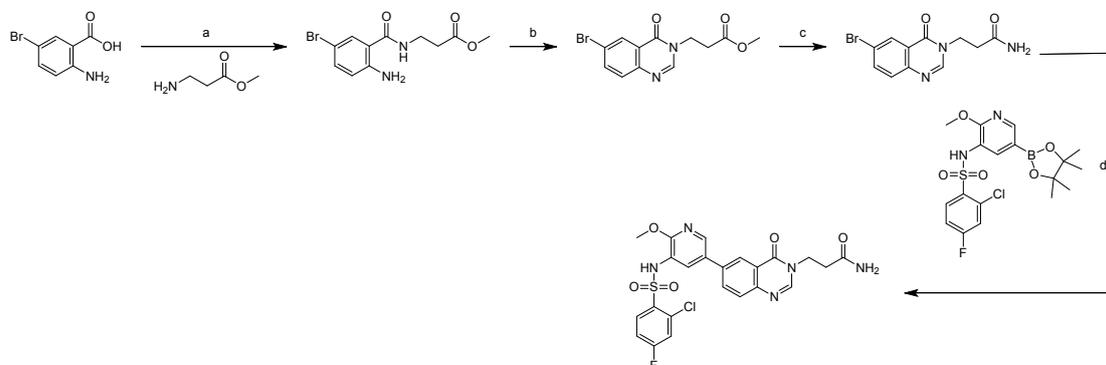
The title compound refers to the synthesis method of 3-(6-bromo-4-oxoquinazolin-3(4*H*)-yl)-2,2-difluoropropanamide in 58.04% yield. ¹H NMR (400MHz, DMSO-*d*₆): δ 8.31(s, 1H), 8.23-8.24(d, *J*=2Hz, 1H), 7.97-7.99(m, 1H), 7.63-7.65(m, 1H), 7.52-7.54(m, 1H), 7.17(s, 1H), 4.26-4.31(m, 1H), 3.77-3.83(m, 1H), 3.17(s, 2H), MS: 311.0/313.0 (M+1)⁺.

2-amino-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)propanamide (**5**)



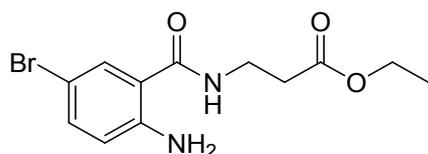
The title compound refers to the synthesis method of methyl 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)-2-methylpropanoate in 6.2% yield. ^1H NMR (400MHz, DMSO- d_6): δ 8.31(s, 1H), 8.26(s, 1H), 8.24-8.25(d, $J=2\text{Hz}$, 1H), 8.06-8.08(m, 1H), 7.95-7.99(m, 1H), 7.87(s, 1H), 7.75-7.77(d, $J=8.4\text{Hz}$, 1H), 7.69-7.71(m, 2H), 7.36-7.37(m, 2H), 4.31-4.35(m, 1H), 3.97-4.01(m, 1H), 3.73-3.76(m, 1H), 3.71(s,3H), MS: 547.0 (M+1) $^+$.

Synthesis and full characterization of 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)propanamide (6)



Supplementary Scheme 1: a) TEA, EDCI, HOPO, DCM, 50°C, 16h; b) formic acid, 100°C, 16h; c) NH_3 (7N MeOH solution), 25°C, 12h; d) KOAc, Pd(dppf)Cl $_2$, dioxane/ H_2O , 95°C, 2h

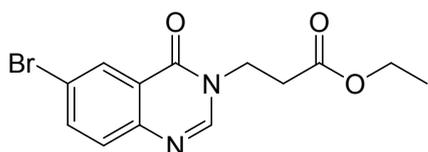
Ethyl 3-(2-amino-5-bromobenzamido)propanoate



To a solution of 2-amino-5-bromobenzoic acid (500.00 mg, 2.31 mmol, 1.00 *eq*) in DCM (50.00 mL) was added ethyl 3-aminopropanoate (350.00 mg, 2.28 mmol, 0.99

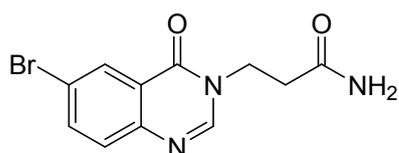
eq), TEA (730.00 mg, 7.21 mmol, 1.00 mL, 3.12 eq), 2-Hydroxypyridine-N-Oxide (260.00 mg, 2.34 mmol, 1.01 eq) and EDCI (450.00 mg, 2.35 mmol, 1.02 eq). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 50 °C for 16 h. The reaction mixture was partitioned between H₂O 10 mL and DCM 10 mL. The organic phase was separated, aqueous phase washed with DCM (5 mL × 3). The combined organic phase dried over Na₂SO₄, filtered and concentrated under reduced pressure to yield the title compound (900.00 mg, crude) and it was used directly. MS: 315.0/317.0 (M+1)⁺.

Ethyl 3-(6-bromo-4-oxoquinazolin-3(4H)-yl)propanoate



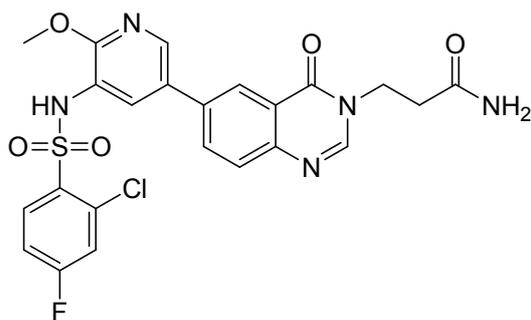
The mixture of ethyl 3-(2-amino-5-bromobenzamido)propanoate (900.00 mg, 2.52 mmol, 1.00 eq) in formic acid (20.00 mL) was stirred at 100 °C for 16 h. The solution was concentrated under reduced pressure to yield the title compound (950.00 mg, crude) and it was used directly. MS: 325.0/327.0 (M+1)⁺.

3-(6-bromo-4-oxoquinazolin-3(4H)-yl)propanamide



The title compound refers to the synthesis method of 3-(6-bromo-4-oxoquinazolin-3(4H)-yl)-2,2-difluoropropanamide. MS: 295.9/297.9 (M+1)⁺.

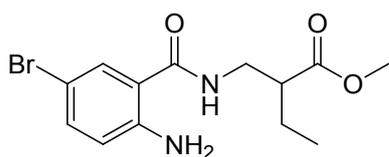
3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)propanamide (**6**)



The title compound refers to the synthesis method of methyl 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)-2-methylpropanoate in 41.75% yield. ^1H NMR (400 MHz, CDCl_3): δ 8.34 (d, $J=2.26$ Hz, 1 H) 8.28 (s, 1 H) 8.11 - 8.21 (m, 2 H) 7.99 (d, $J=2.26$ Hz, 1 H) 7.77 - 7.91 (m, 2 H) 7.56 (s, 1 H) 7.27 (d, $J=2.51$ Hz, 1 H) 7.09 - 7.18 (m, 1 H) 5.33 - 5.63 (m, 2 H) 4.35 (t, $J=6.02$ Hz, 2 H) 4.00 (s, 3 H) 2.86 (t, $J=6.02$ Hz, 2 H), MS: 532.0 ($\text{M}+1$) $^+$.

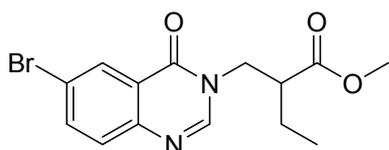
Synthesis and full characterization of 2-((6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)methyl)butanamide (7)

Methyl 2-((2-amino-5-bromobenzamido)methyl)butanoate



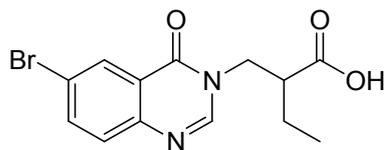
The title compound refers to the synthesis method of ethyl 3-(2-amino-5-bromobenzamido)propanoate in 24.99% yield. MS: 359.0/361.0 ($\text{M}+1$) $^+$.

Methyl 2-((6-bromo-4-oxoquinazolin-3(4H)-yl)methyl)butanoate



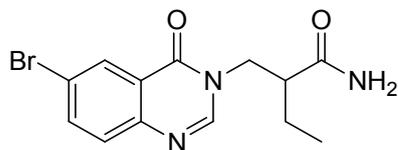
The title compound refers to the synthesis method of ethyl 3-(6-bromo-4-oxoquinazolin-3(4H)-yl)propanoate. MS: 339.0/341.0 ($\text{M}+1$) $^+$.

2-((6-bromo-4-oxoquinazolin-3(4*H*)-yl)methyl)butanoic acid



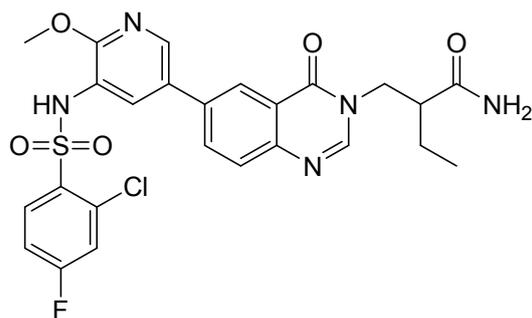
To a solution of methyl 2-((6-bromo-4-oxoquinazolin-3(4*H*)-yl)methyl)butanoate (1.0 g, 3.10 mmol) in MeOH (30.00 mL) and H₂O (40.00 mL) was added lithium hydroxide monohydrate (1.30 g, 31.00 mmol). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 25 °C for 16 h. The mixture was concentrated to remove MeOH. The mixture was acidified with 1.0 M aqueous solution of HCl to pH 5-6 and then extracted with EA (20 mL×2). The combined organic layers were dried with Na₂SO₄, filtered and concentrated to afford the crude title compound (600.00 mg) and it was used directly. MS: 325.0/327.0 (M+1)⁺.

2-((6-bromo-4-oxoquinazolin-3(4*H*)-yl)methyl)butanamide



To a solution of 2-((6-bromo-4-oxoquinazolin-3(4*H*)-yl)methyl)butanoic acid (180.00 mg, 553.57 μmol, 1.00 eq) in DCM (20.00 mL) was added (COCl)₂ (139.83 mg, 1.10 mmol, 96.43 μL, 1.99 eq). The mixture was stirred at 20 °C for 1 hour. And then ammonia gas (9.43 mg, 553.57 μmol, 1.00 eq) was bubbled to the mixture for 10 min. The mixture was stirred at 20 °C for 14 hours and 50 mins. The mixture was washed with water (15 mL) and the organic layer was concentrated to afford the title compound (100.00 mg, crude) and it was used directly. MS: 324.0/326.0 (M+1)⁺.

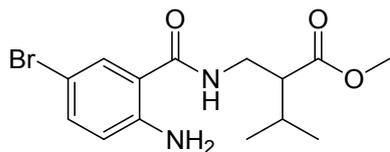
2-((6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)methyl)butanamide (7)



The title compound refers to the synthesis method of methyl 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-methylpropanoate in 14.50% yield. ¹H NMR (400 MHz, DMSO-*d*₆) δ : 10.27 (s, 1 H) 8.40 (s, 1 H) 8.15 - 8.30 (m, 2 H) 8.04 - 8.13 (m, 1 H) 7.88 - 7.99 (m, 2 H) 7.76 (d, *J*=8.53 Hz, 2 H) 7.30 - 7.52 (m, 2 H) 6.95 (br s, 1 H) 4.15 (dd, *J*=12.92, 4.14 Hz, 1 H) 3.85 - 4.00 (m, 1 H) 3.70 (s, 3 H) 2.74 (br d, *J*=3.76 Hz, 1 H) 1.37 - 1.68 (m, 1 H) 1.35 - 1.64 (m, 1 H) 0.91 (t, *J*=7.40 Hz, 3 H). MS: 560.1 (M+1)⁺.

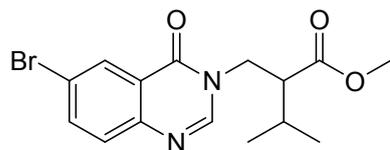
Synthesis and full characterization of 2-((6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)methyl)-3-methylbutanoate (8)

Methyl 2-((2-amino-5-bromobenzamido)methyl)-3-methylbutanoate



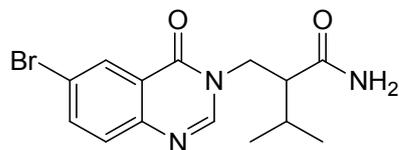
The title compound refers to the synthesis method of ethyl 3-(6-bromo-4-oxoquinazolin-3(4*H*)-yl)propanoate. MS: 343.0/345.0 (M+1)⁺.

Methyl 2-((6-bromo-4-oxoquinazolin-3(4*H*)-yl)methyl)-3-methylbutanoate



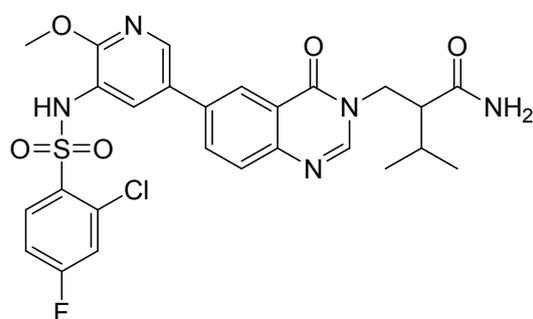
The title compound refers to the synthesis method of ethyl 3-(6-bromo-4-oxoquinazolin-3(4*H*)-yl)propanoate, MS: 353.0/355.0 (M+1)⁺.

2-((6-bromo-4-oxoquinazolin-3(4H)-yl)methyl)-3-methylbutanamide



The title compound refers to the synthesis method of 3-(6-bromo-4-oxoquinazolin-3(4H)-yl)-2,2-difluoropropanamide. MS: 338.0/340.0 (M+1)⁺.

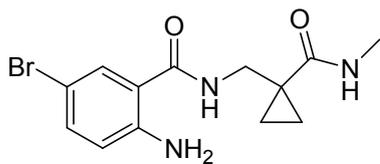
2-((6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)methyl)-3-methylbutanamide (**8**)



The title compound refers to the synthesis method of methyl 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)-2-methylpropanoate. ¹H NMR (400 MHz, CDCl₃): δ 8.09 - 8.19 (m, 3 H) 8.06 (d, *J*=2.20 Hz, 1 H) 7.90 (d, *J*=2.20 Hz, 1 H) 7.70 - 7.78 (m, 1 H) 7.60 - 7.68 (m, 2 H) 7.21 - 7.35 (m, 1 H) 7.11 (ddd, *J*=8.89, 7.51, 2.42 Hz, 1 H) 5.81 - 6.24 (m, 2 H) 4.49 (dd, *J*=12.98, 3.52 Hz, 1 H) 3.81 - 4.05 (m, 4 H) 2.85 (ddd, *J*=10.78, 7.43, 3.58 Hz, 1 H) 1.91 - 2.17 (m, 1 H) 1.04 - 1.23 (m, 6 H), MS: 574.1 (M+1)⁺.

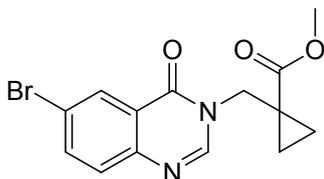
Synthesis and full characterization of 1-((6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)methyl)cyclopropanecarboxamide (9**)**

2-amino-5-bromo-N-((1-(methylcarbamoyl)cyclopropyl)methyl)benzamide



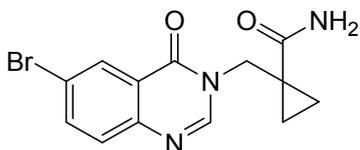
The title compound refers to the synthesis method of ethyl 3-(6-bromo-4-oxoquinazolin-3(4H)-yl)propanoate. MS: 326.0/328.0 (M+1)⁺.

methyl 1-((6-bromo-4-oxoquinazolin-3(4H)-yl)methyl)cyclopropanecarboxylate



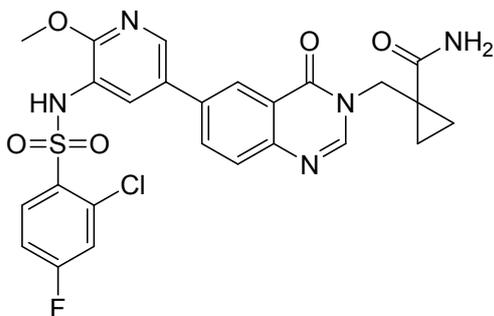
The title compound refers to the synthesis method of ethyl 3-(6-bromo-4-oxoquinazolin-3(4H)-yl)propanoate, MS: 337.0/339.0 (M+1)⁺.

1-((6-bromo-4-oxoquinazolin-3(4H)-yl)methyl)cyclopropanecarboxamide



The title compound refers to the synthesis method of 3-(6-bromo-4-oxoquinazolin-3(4H)-yl)-2,2-difluoropropanamide. MS: 322.0/324.0 (M+1)⁺.

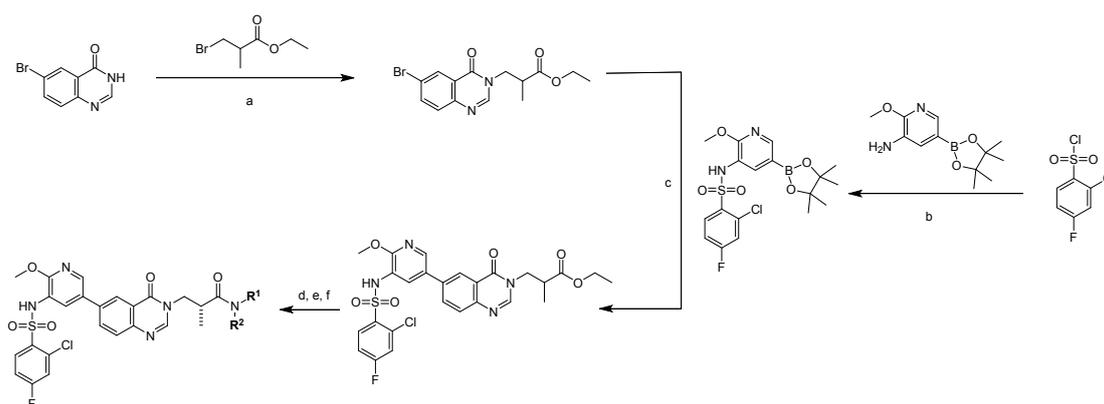
1-((6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)methyl)cyclopropanecarboxamide (**9**)



The title compound refers to the synthesis method of methyl 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4H)-yl)-2-

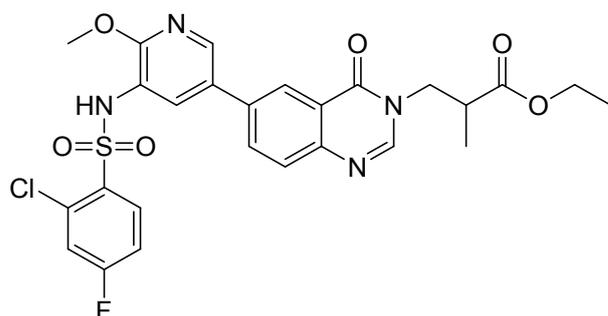
methylpropanoate. $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$): δ 9.52 - 10.62 (m, 1 H) 8.52 (s, 1 H) 8.40 (d, $J=2.26$ Hz, 1 H) 8.25 (d, $J=2.26$ Hz, 1 H) 8.04 - 8.12 (m, 1 H) 7.88 - 7.99 (m, 2 H) 7.71 - 7.79 (m, 2 H) 7.36 (td, $J=8.53, 2.51$ Hz, 1 H) 6.89 - 7.09 (m, 2 H) 4.24 (s, 2 H) 3.70 (s, 3 H) 1.14 (s, 4 H), MS: 558.0 ($\text{M}+1$) $^+$.

Synthesis and full characterization of compound 10-26



Supplementary Scheme 2: a) Cs_2CO_3 , DMF, 100°C , 2h; b) Pyridine; c) KOAc, $\text{Pd}(\text{dppf})\text{Cl}_2$, dioxane/ H_2O , 95°C , 2h; d) LiOH, THF/ H_2O , 0°C , 1h; e) T_3P , DIEA, DCM, 20°C , 16h; f) SCF split

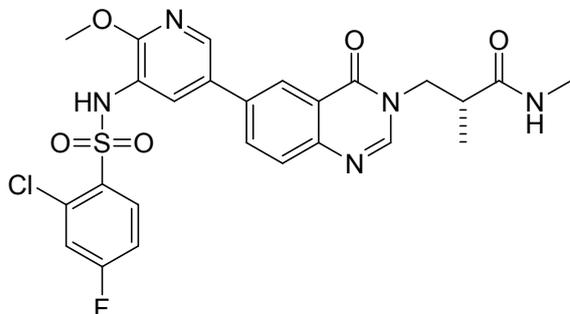
Ethyl 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-methylpropanoate



The title compound refers to the synthesis method of methyl 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-methylpropanoate in 92.78% yield. $^1\text{H NMR}$ (400MHz, CDCl_3): δ 8.35 (d, $J=2.0$ Hz, 1H), 8.25 - 8.10 (m, 2H), 7.99 (d, $J=2.3$ Hz, 1H), 7.90 - 7.71 (m, 2H), 7.71 - 7.52 (m, 2H), 7.30 - 7.28 (m, 1H), 7.20 - 7.06 (m, 1H), 4.21 - 4.10 (m, 4H), 4.03 (s, 3H), 3.26 -

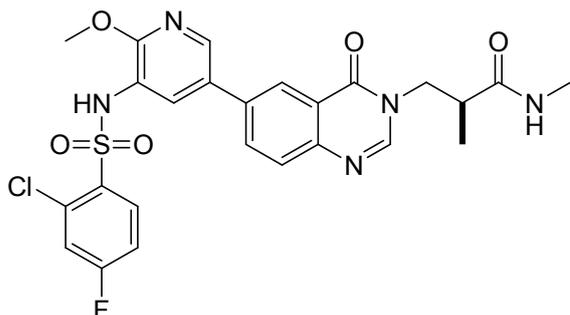
3.12 (m, 1H), 1.34 (d, $J=7.0$ Hz, 3H), 1.24 - 1.18 (m, 3H). MS: 575.1 (M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**10**)



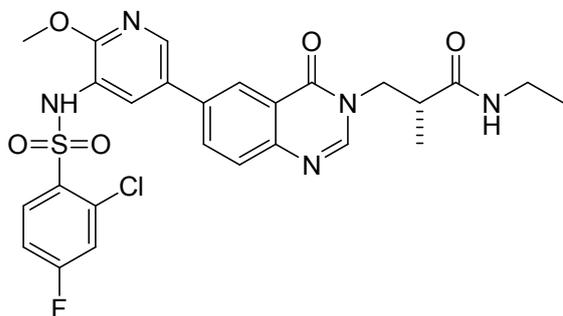
Methyl-3-[6-[5-[(2-chloro-4-fluoro-phenyl)sulfonylamino]-6-methoxy-3-pyridyl]-4-oxo-quinazolin-3-yl]-2-methyl-propanoate (300.00 mg, 534.78 umol, 1.00 eq) was added in to methanamine (16.61 mg, 534.78 umol, 20.00 mL, 1.00 eq), the mixture was stirred at 80 °C for 16 h in sealed tube. The solvent was removed in vacuum. The residue was purified by prep-TLC (Dichloromethane: Methanol: TEA = 20:1:0.2) and was purified by SFC (column: Chiralpak AD (250mm*30mm, 10um); mobile phase: [0.1%NH₃H₂O ETOH];B%: 35%-35%,min) to yield the title compound (20.00 mg, 35.50 umol, 6.64% yield). ¹H NMR (400MHz, CDCl₃): δ 8.24 (d, $J=2.0$ Hz, 1H), 8.14 - 8.04 (m, 3H), 7.91 (d, $J=2.3$ Hz, 1H), 7.83 - 7.65 (m, 2H), 7.20 (d, $J=2.5$ Hz, 1H), 7.11 - 6.93 (m, 1H), 5.49 (br d, $J=4.5$ Hz, 1H), 4.17 - 4.05 (m, 1H), 4.04 - 3.93 (m, 1H), 3.91 (s, 3H), 2.93 - 2.81 (m, 1H), 2.67 (d, $J=5.0$ Hz, 3H), 1.22 (d, $J=7.0$ Hz, 3H). MS:560.1 (M+1)⁺.

(*S*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**11**)



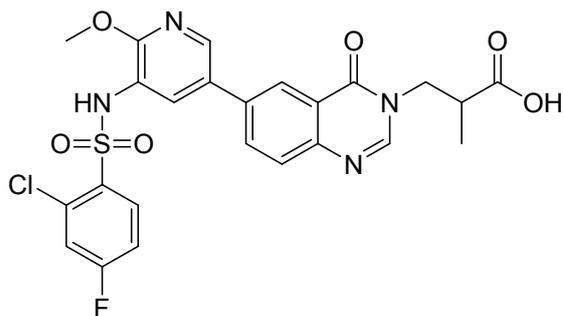
The title compound refers to the synthesis method of compound **10** in 6.64% yield. ¹H NMR (400MHz, CDCl₃): δ 8.24 (d, *J*=2.0 Hz, 1H), 8.14 - 8.04 (m, 3H), 7.91 (d, *J*=2.3 Hz, 1H), 7.83 - 7.65 (m, 2H), 7.20 (d, *J*=2.5 Hz, 1H), 7.11 - 6.93 (m, 1H), 5.49 (br d, *J*=4.5 Hz, 1H), 4.17 - 4.05 (m, 1H), 4.04 - 3.93 (m, 1H), 3.91 (s, 3H), 2.93 - 2.81 (m, 1H), 2.67 (d, *J*=5.0 Hz, 3H), 1.22 (d, *J*=7.0 Hz, 3H). MS: 560.1 (M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*-ethyl-2-methylpropanamide (**12**)



The title compound refers to the synthesis method of compound **13**, and was purified by SFC (column: AD(250mm*30mm,5um); mobile phase: [0.1%NH₃H₂O ETOH];B%: 35%-35%,min). ¹H NMR (400MHz, METHANOL-d₄): δ 7.78 (s, 1H), 7.64 (d, *J*=2.0 Hz, 1H), 7.62 (s, 1H), 7.54 (dd, *J*=6.0, 8.8 Hz, 1H), 7.47 - 7.41 (m, 2H), 7.20 (d, *J*=8.4 Hz, 1H), 6.90 (dd, *J*=2.3, 8.5 Hz, 1H), 6.68 (br t, *J*=8.5 Hz, 1H), 3.69 (dd, *J*=4.9, 13.5 Hz, 1H), 3.45 (dd, *J*=9.9, 13.2 Hz, 1H), 3.31 (s, 3H), 2.60 - 2.47 (m, 2H), 2.42 (s, 1H), 0.68 (d, *J*=7.1 Hz, 3H), 0.37 (t, *J*=7.3 Hz, 3H), MS: 574.1 (M+1)⁺.

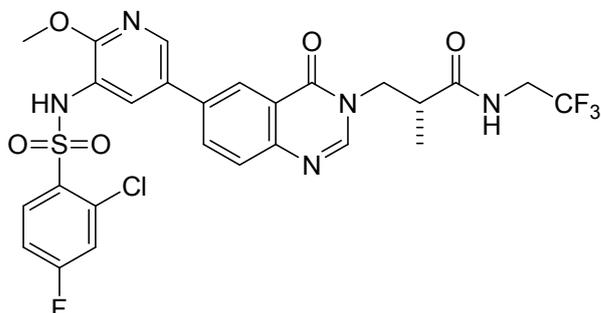
3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-methylpropanoic acid



To a solution of ethyl 3-[6-[5-[(2-chloro-4-fluoro-phenyl)sulfonylamino]-6-methoxy-

3-pyridyl]-4-oxo-quinazolin-3-yl]-2-methyl-propanoate (1.30 g, 2.26 mmol, 1 *eq*) in THF (10 mL) and H₂O(10 mL) was added lithium hydroxide monohydrate (271.30 mg, 6.78 mmol, 3 *eq*). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 0°C for 1 h. The mixture was acidified with 1.0 M aqueous solution of HCl to pH 5-6 and then extracted with EA (20 mL×2). The combined organic layers were dried with Na₂SO₄, filtered and concentrated to afford the crude title compound (180.00 mg) and it was used directly. ¹H NMR (400MHz, METHANOL-d₄): δ 8.31 (s, 2H), 8.21 (d, *J*=2.0 Hz, 1H), 8.08 (dd, *J*=6.0, 8.8 Hz, 1H), 8.02 - 7.96 (m, 2H), 7.75 (d, *J*=8.6 Hz, 1H), 7.44 (br d, *J*=8.6 Hz, 1H), 7.21 (br t, *J*=8.6 Hz, 1H), 4.21 - 4.15 (m, 2H), 3.85 (s, 3H), 3.11 (br d, *J*=6.4 Hz, 1H), 1.27 (d, *J*=7.3 Hz, 3H), MS: 547.0 (M+1)⁺.

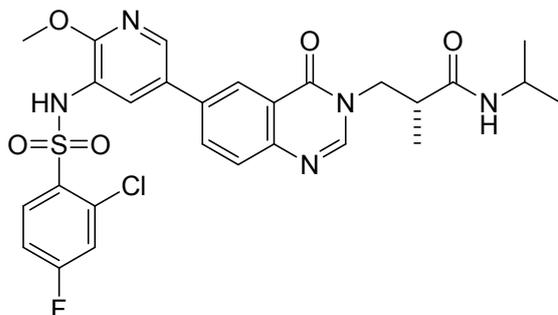
(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-methyl-*N*-(2,2,2-trifluoroethyl)propanamide (**13**)



To a solution of 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-methylpropanoic acid (850 mg, 1.55 mmol, 1 *eq*) in DCM (10 mL) was added 2,2,2-trifluoroethanamine (307.87 mg, 3.11 mmol, 244.34 uL, 2 *eq*), T₃P (741.71 mg, 2.33 mmol, 693.18 uL, 1.5 *eq*) and DIEA (602.54 mg, 4.66 mmol, 812.05 uL, 3 *eq*). The reaction was degassed and purged with N₂ for 3 times, and then the mixture was stirred at 20°C for 16 h. The reaction mixture was partitioned between H₂O 10 mL and EA 10 mL. The organic phase was separated, aqueous phase washed with EA (5 mL * 3). The combined organic phase dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (Dichloromethane: Methanol =20: 1), and was purified by SFC (column:

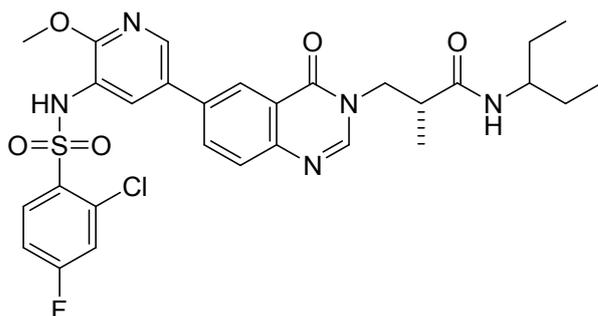
AD(250mm*30mm,5um); mobile phase: A for CO₂ and B for EtOH(0.1%NH₃H₂O); Gradient: B%=42%; Flow rate:70 g/min ;) to yield the title compound (223.1 mg, 355.26 umol, 22.86% yield). ¹H NMR (400MHz, CDCl₃): δ 8.24 (s, 1H), 8.16 - 8.07 (m, 2H), 8.06 (s, 1H), 7.93 (d, *J*=2.2 Hz, 1H), 7.84 - 7.78 (m, 1H), 7.77 - 7.71 (m, 1H), 7.52 (br s, 1H), 7.26 - 7.23 (m, 1H), 7.09 (ddd, *J*=2.4, 7.5, 8.8 Hz, 1H), 6.12 (br s, 1H), 4.22 - 4.11 (m, 1H), 4.11 - 4.01 (m, 1H), 3.96 (s, 3H), 3.94 - 3.84 (m, 1H), 3.83 - 3.71 (m, 1H), 3.16 - 3.03 (m, 1H), 1.31 (d, *J*=7.1 Hz, 2H), 1.35 - 1.27 (m, 1H), MS: 628.0 (M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*-isopropyl-2-methylpropanamide (**14**)



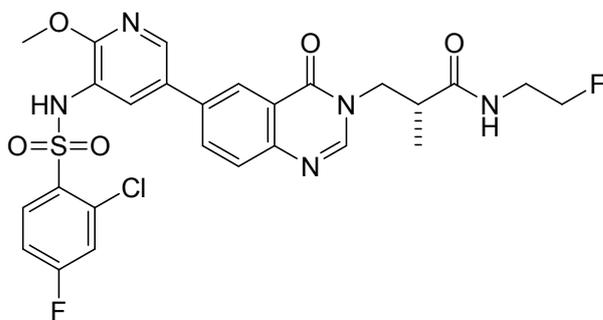
The title compound refers to the synthesis method of compound **13**, and was purified by SFC (column: Chiralpak AD-H 250*30mm i.d. 5u; mobile phase: A for CO₂ and B for EtOH(0.1%NH₃H₂O); B%: B%=42%; Flow rate:70 g/min;). ¹H NMR (400MHz, METHANOL-d₄): δ 8.34 (d, *J*=2.1 Hz, 1H), 8.22 (d, *J*=2.3 Hz, 1H), 8.17 (s, 1H), 8.10 (dd, *J*=5.8, 8.9 Hz, 1H), 8.04 - 7.98 (m, 2H), 7.76 (d, *J*=8.5 Hz, 1H), 7.47 (dd, *J*=2.6, 8.5 Hz, 1H), 7.28 - 7.20 (m, 1H), 4.26 (dd, *J*=4.8, 13.3 Hz, 1H), 3.99 (dd, *J*=10.3, 13.2 Hz, 1H), 3.92 - 3.83 (m, 4H), 3.02 - 2.91 (m, 1H), 1.23 (d, *J*=6.9 Hz, 3H), 1.04 (d, *J*=6.5 Hz, 3H), 0.85 (d, *J*=6.7 Hz, 3H), MS: 588.1 (M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-methyl-*N*-(pentan-3-yl)propanamide (**15**)



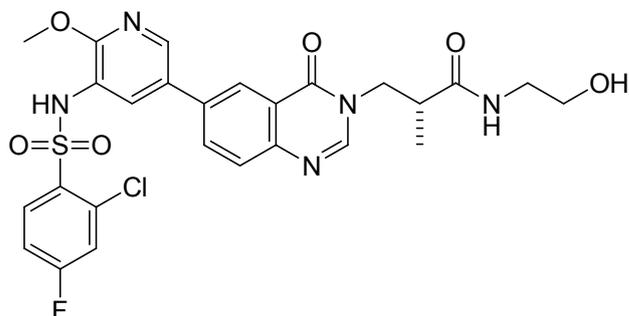
The title compound refers to the synthesis method of compound **13**, and was purified by SFC (column: Luna C18 100*30 5u; mobile phase: [water(0.1%TFA)-ACN]; B%: 40%-65%,10min). ¹H NMR (400MHz, CDCl₃): δ 8.32 (s, 1H), 8.21 - 8.08 (m, 3H), 7.98 (d, *J*=2.0 Hz, 1H), 7.91 - 7.80 (m, 1H), 7.80 - 7.73 (m, 1H), 7.51 (br s, 1H), 7.18 - 7.06 (m, 1H), 5.17 (br d, *J*=9.3 Hz, 1H), 4.20 (dd, *J*=4.7, 13.1 Hz, 1H), 4.08 - 3.99 (m, 1H), 3.98 (s, 3H), 3.72 (br d, *J*=8.6 Hz, 1H), 3.02 - 2.91 (m, 1H), 1.49 (br dd, *J*=6.2, 13.7 Hz, 1H), 1.38 - 1.24 (m, 5H), 1.15 (tt, *J*=7.4, 14.5 Hz, 1H), 0.85 (t, *J*=7.4 Hz, 3H), 0.51 (t, *J*=7.4 Hz, 3H), MS: 616.1 (M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*-(2-fluoroethyl)-2-methylpropanamide (**16**)



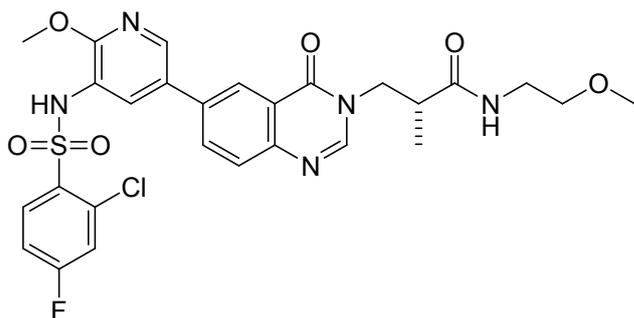
The title compound refers to the synthesis method of compound **13**, and was purified by SFC (column: Chiralcel OJ-H 250*30mm i.d. 5u; mobile phase: A for CO₂ and B for MeOH; B%: B%=35%; Flow rate:60 g/min;). ¹H NMR (400MHz, CDCl₃): δ 8.27 (d, *J*=1.8 Hz, 1H), 8.16 - 8.12 (m, 1H), 8.11 - 8.09 (m, 2H), 7.96 (d, *J*=2.2 Hz, 1H), 7.84 - 7.80 (m, 1H), 7.77 - 7.73 (m, 1H), 7.25 (d, *J*=2.4 Hz, 1H), 7.15 - 7.07 (m, 1H), 6.13 (br t, *J*=5.6 Hz, 1H), 4.50 - 4.38 (m, 1H), 4.37 - 4.26 (m, 1H), 4.21 (dd, *J*=5.0, 13.3 Hz, 1H), 4.03 (dd, *J*=9.5, 13.2 Hz, 1H), 3.98 (s, 3H), 3.54 (q, *J*=5.1 Hz, 1H), 3.47 (q, *J*=5.1 Hz, 1H), 3.08 - 2.99 (m, 1H), 1.31 (d, *J*=7.1 Hz, 3H), MS: 592.1 (M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*-(2-hydroxyethyl)-2-methylpropanamide (**17**)



The title compound refers to the synthesis method of compound **13**, and was purified by SFC (column: Agela Durashell C18 150*25 5u; mobile phase: [water(10mM NH₄HCO₃)-ACN];B%: 14%-44%,10.5min). ¹H NMR (400MHz, CDCl₃): δ 8.26 (d, *J*=2.0 Hz, 1H), 8.15 (dd, *J*=5.7, 8.8 Hz, 1H), 8.11 - 8.09 (m, 2H), 7.95 (d, *J*=2.2 Hz, 1H), 7.82 - 7.78 (m, 1H), 7.76 - 7.72 (m, 1H), 7.48 (br s, 1H), 7.25 (d, *J*=2.4 Hz, 1H), 7.17 - 7.11 (m, 1H), 6.24 (br t, *J*=5.4 Hz, 1H), 4.22 (dd, *J*=4.9, 13.5 Hz, 1H), 4.03 (dd, *J*=9.5, 13.2 Hz, 1H), 3.98 (s, 3H), 3.63 (t, *J*=5.0 Hz, 2H), 3.45 - 3.37 (m, 1H), 3.35 - 3.27 (m, 1H), 3.05 - 2.96 (m, 1H), 2.37 (br s, 1H), 1.31 (d, *J*=7.1 Hz, 3H), 590.1 (M+1)⁺.

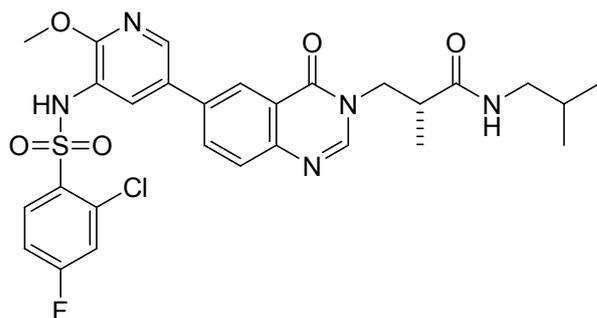
(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*-(2-methoxyethyl)-2-methylpropanamide (**18**)



The title compound refers to the synthesis method of compound **13**, and was purified by SFC (column: Chiralcel OJ-H 250*30mm i.d. 5u; mobile phase: A for CO₂ and B for MeOH;B%: B%=30%; Flow rate:60 g/min;). ¹H NMR (400MHz, CDCl₃): δ 8.31 (s, 1H), 8.15 - 8.15 (m, 1H), 8.15 - 8.11 (m, 2H), 8.10 (s, 1H), 7.97 (d, *J*=2.0 Hz, 1H), 7.85 - 7.79 (m, 1H), 7.79 - 7.74 (m, 1H), 7.25 (d, *J*=2.2 Hz, 1H), 7.15 - 7.07 (m, 1H),

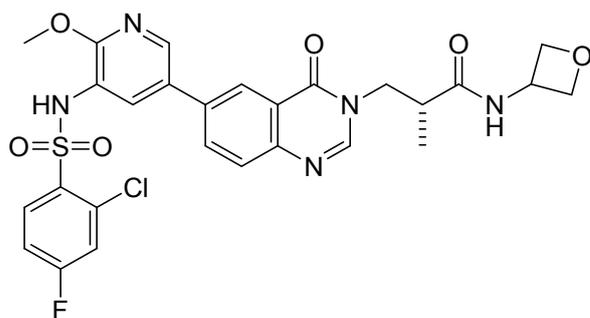
5.99 (br s, 1H), 4.27 - 4.15 (m, 1H), 4.04 - 3.99 (m, 1H), 3.97 (s, 3H), 3.46 - 3.37 (m, 1H), 3.37 - 3.27 (m, 2H), 3.26 - 3.19 (m, 1H), 3.12 (s, 3H), 3.02 - 2.93 (m, 1H), 1.33 - 1.33 (m, 1H), 1.29 (d, $J=7.1$ Hz, 2H), 1.30 - 1.22 (m, 1H), 504.1 (M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*-isobutyl-2-methylpropanamide (**19**)



The title compound refers to the synthesis method of compound **13**, and was purified by SFC (column: Chiralpak AD-H 250*30mm i.d. 5 μ ; mobile phase: A for CO₂ and B for IPA(0.1%NH₃H₂O); B%: B%=42%; Flow rate:70 g/min;). ¹H NMR (400MHz, METHANOL-d₄): δ 8.21 (d, $J=2.1$ Hz, 1H), 8.10 (d, $J=2.3$ Hz, 1H), 8.07 (s, 1H), 8.00 (dd, $J=5.9, 8.9$ Hz, 1H), 7.93 - 7.85 (m, 2H), 7.64 (d, $J=8.5$ Hz, 1H), 7.37 (dd, $J=2.5, 8.5$ Hz, 1H), 7.19 - 7.08 (m, 1H), 4.16 (dd, $J=4.8, 13.3$ Hz, 1H), 3.97 - 3.84 (m, 1H), 3.05 - 2.94 (m, 1H), 2.89 (dd, $J=6.7, 13.2$ Hz, 1H), 2.66 (dd, $J=7.2, 13.2$ Hz, 1H), 1.46 (quind, $J=6.8, 13.6$ Hz, 1H), 1.15 (d, $J=7.0$ Hz, 3H), 0.59 (d, $J=6.7$ Hz, 3H), 0.54 (d, $J=6.8$ Hz, 3H), MS: 602.2(M+1)⁺.

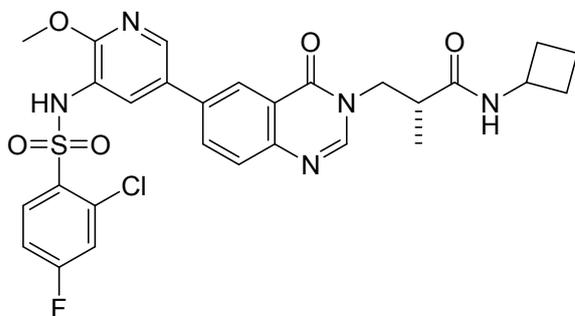
(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-methyl-*N*-(oxetan-3-yl)propanamide (**20**)



The title compound refers to the synthesis method of compound **13**, and was purified

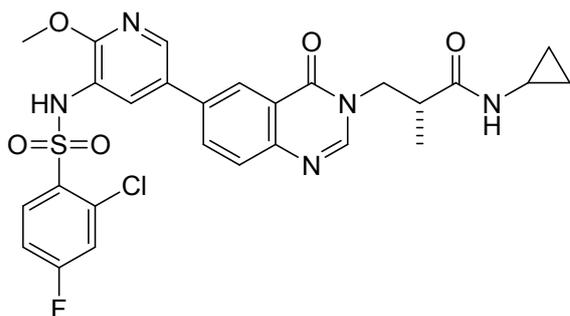
by SFC (column: Chiralpak AD-H 250*30mm i.d. 5u; mobile phase: A for CO₂ and B for IPA(0.1%NH₃H₂O); Gradient: B%=40%; Flow rate:60 g/min;). ¹H NMR (400MHz, CDCl₃): δ 8.28 (d, *J*=1.8 Hz, 1H), 8.15 - 8.08 (m, 2H), 8.04 (s, 1H), 7.96 (d, *J*=2.2 Hz, 1H), 7.85 - 7.80 (m, 1H), 7.77 - 7.72 (m, 1H), 7.53 (s, 1H), 7.26 - 7.24 (m, 1H), 7.14 - 7.07 (m, 1H), 6.19 (br d, *J*=7.5 Hz, 1H), 5.03 - 4.90 (m, 1H), 4.88 - 4.83 (m, 1H), 4.79 (t, *J*=7.1 Hz, 1H), 4.40 (t, *J*=6.4 Hz, 1H), 4.21 - 4.13 (m, 2H), 4.00 (dd, *J*=9.3, 13.5 Hz, 1H), 3.96 (s, 3H), 3.08 - 2.93 (m, 1H), 1.29 (d, *J*=6.8 Hz, 3H), MS: 602.1(M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*-cyclobutyl-2-methylpropanamide (**21**)



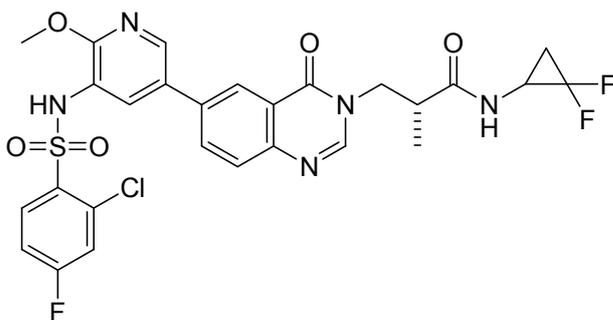
The title compound refers to the synthesis method of compound **13**, and was purified by SFC (column: Chiralpak AD-H 250*30mm i.d. 5u; mobile phase: A for CO₂ and B for IPA(0.1%NH₃H₂O); Gradient: B%=42%; Flow rate:70 g/min;). ¹H NMR (400MHz, CDCl₃): δ 8.25 (d, *J*=2.0 Hz, 1H), 8.12 - 8.03 (m, 2H), 8.01 (s, 1H), 7.91 (d, *J*=2.3 Hz, 1H), 7.80 - 7.74 (m, 1H), 7.73 - 7.67 (m, 1H), 7.47 (s, 1H), 7.21 (d, *J*=2.5 Hz, 1H), 7.09 - 6.98 (m, 1H), 5.58 (br d, *J*=8.0 Hz, 1H), 4.32 - 4.18 (m, 1H), 4.10 (dd, *J*=5.0, 13.2 Hz, 1H), 3.98 - 3.85 (m, 4H), 2.91 - 2.67 (m, 1H), 2.30 - 2.16 (m, 1H), 2.15 - 2.03 (m, 1H), 1.77 - 1.62 (m, 1H), 1.54 - 1.50 (m, 3H), 1.20 (d, *J*=7.0 Hz, 3H), MS: 600.1(M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*-cyclopropyl-2-methylpropanamide (**22**)



The title compound refers to the synthesis method of compound **13**, and was purified by SFC (column: Chiralpak AD-H 250*30mm i.d. 5u; mobile phase: A for CO₂ and B for MeOH(0.1%NH₃H₂O); Gradient: B%=42%; Flow rate:70 g/min;). ¹H NMR (400MHz, METHANOL-d₄): δ 8.10 (d, *J*=2.1 Hz, 1H), 7.99 (d, *J*=2.1 Hz, 1H), 7.97 (s, 1H), 7.88 (dd, *J*=5.8, 8.9 Hz, 1H), 7.83 - 7.76 (m, 2H), 7.55 (d, *J*=8.4 Hz, 1H), 7.25 (dd, *J*=2.6, 8.5 Hz, 1H), 7.02 (ddd, *J*=2.6, 7.9, 8.8 Hz, 1H), 4.02 (dd, *J*=4.8, 13.4 Hz, 1H), 3.79 (dd, *J*=10.0, 13.4 Hz, 1H), 3.65 (s, 3H), 2.78 - 2.65 (m, 1H), 2.33 (tt, *J*=3.8, 7.3 Hz, 1H), 1.01 (d, *J*=6.9 Hz, 3H), 0.39 (dd, *J*=1.7, 7.2 Hz, 2H), 0.08 - -0.02 (m, 2H), MS: 586.1(M+1)⁺.

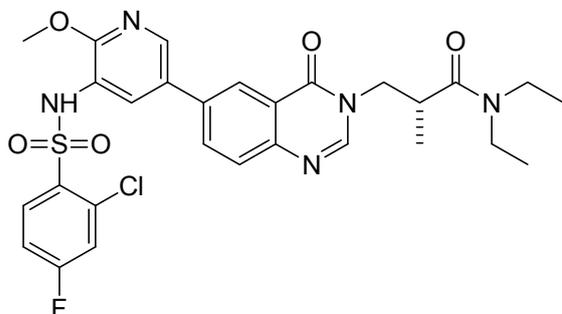
(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*-(2,2-difluorocyclopropyl)-2-methylpropanamide (**23**)



The title compound refers to the synthesis method of compound **13**, and was purified by SFC (column: OJ(250mm*30mm,5um); mobile phase: [0.1%NH₃H₂O MEOH]; B%: 35%-35%,4min). ¹H NMR (400MHz, CDCl₃): δ 8.29 (s, 1H), 8.17 - 8.08 (m, 3H), 7.98 (d, *J*=2.0 Hz, 1H), 7.87 - 7.83 (m, 1H), 7.82 - 7.75 (m, 1H), 7.58 (s, 1H), 7.29 - 7.25 (m, 1H), 7.17 - 7.08 (m, 1H), 6.00 (br s, 1H), 4.21 (br dd, *J*=4.8, 13.2 Hz, 1H), 4.10 - 4.03 (m, 1H), 4.00 (s, 3H), 3.30 (br s, 1H), 3.12 - 3.01 (m, 1H), 1.83 - 1.74 (m, 1H), 1.34 (br d, *J*=6.9 Hz, 3H), 1.23 - 1.13 (m, 1H), 1.17 (ddd, *J*=4.8, 9.3, 14.0 Hz, 1H),

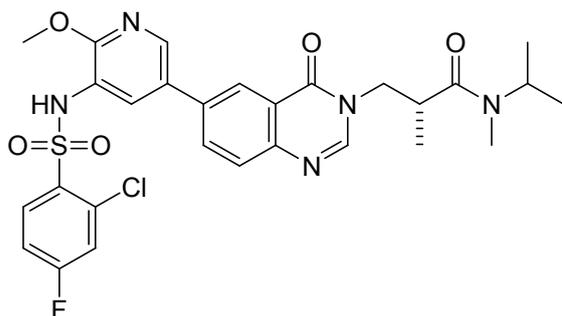
MS: 622.1(M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N,N*-diethyl-2-methylpropanamide (**24**)



The title compound refers to the synthesis method of compound 13, and was purified by SFC (column: AD(250mm*30mm,5um); mobile phase: [0.1%NH₃H₂O ETOH];B%: 35%-35%,10min). ¹H NMR (400MHz, CDCl₃): δ 8.33 (d, *J*=1.9 Hz, 1H), 8.18 - 8.11 (m, 3H), 7.99 (d, *J*=2.3 Hz, 1H), 7.86 - 7.81 (m, 1H), 7.80 - 7.75 (m, 1H), 7.53 (s, 1H), 7.16 - 7.10 (m, 1H), 4.24 - 4.15 (m, 1H), 4.12 - 4.05 (m, 1H), 4.15 - 4.01 (m, 1H), 3.98 (s, 3H), 3.54 - 3.43 (m, 1H), 3.35 - 3.27 (m, 2H), 3.26 - 3.20 (m, 2H), 1.26 (d, *J*=6.9 Hz, 3H), 1.07 - 0.98 (m, 6H), MS: 602.1(M+1)⁺.

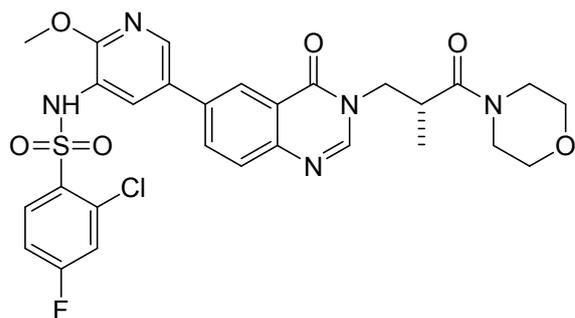
(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*-isopropyl-*N*,2-dimethylpropanamide (**25**)



The title compound refers to the synthesis method of compound 13, and was purified by SFC (column: Chiralcel OD-H 250*30mm i.d. 5u; mobile phase: A for CO₂ and B for MeOH(0.1%NH₃H₂O); Gradient: B%=45%; Flow rate:70 g/min;). ¹H NMR (400MHz, METHANOL-d₄): δ 8.30 (s, 1H), 8.23 (s, 1H), 8.21 (d, *J*=1.9 Hz, 1H), 8.10 (dd, *J*=5.8, 8.8 Hz, 1H), 8.02 - 7.95 (m, 2H), 7.74 (d, *J*=8.4 Hz, 1H), 7.47 (dd, *J*=2.4,

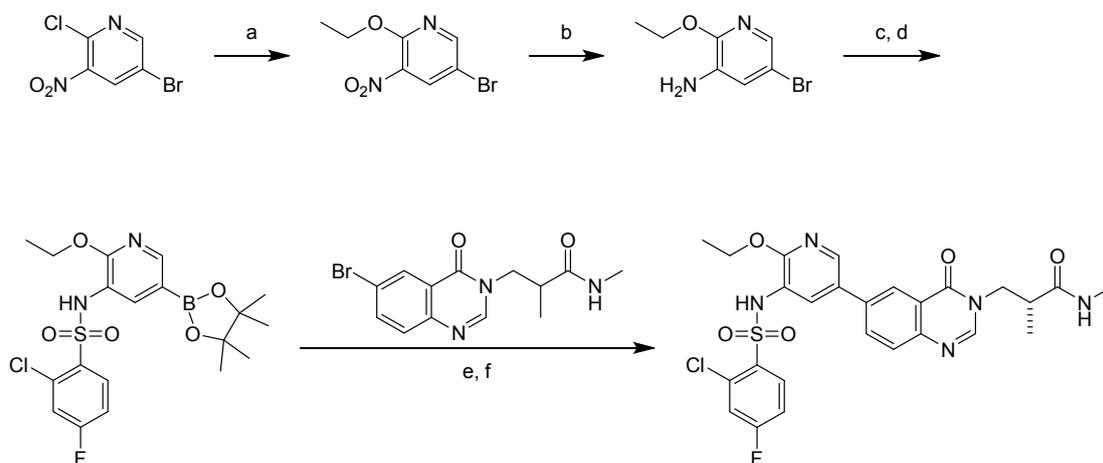
8.5 Hz, 1H), 7.26 - 7.20 (m, 1H), 4.70 (td, $J=6.7, 13.5$ Hz, 1H), 4.28 - 4.02 (m, 3H), 3.86 (s, 3H), 3.35 - 3.34 (m, 1H), 2.80 (s, 1H), 2.70 (s, 1H), 1.26 - 1.16 (m, 4H), 1.05 (d, $J=6.9$ Hz, 1H), 0.93 (d, $J=6.7$ Hz, 1H), 0.88 (d, $J=6.8$ Hz, 1H), MS: 602.1(M+1)⁺.

(*R*)-2-chloro-4-fluoro-*N*-(2-methoxy-5-(3-(2-methyl-3-morpholino-3-oxopropyl)-4-oxo-3,4-dihydroquinazolin-6-yl)pyridin-3-yl)benzenesulfonamide (**26**)



The title compound refers to the synthesis method of compound **13**, and was purified by SFC (column: AD(250mm*30mm,5um); mobile phase: [0.1%NH₃H₂O ETOH];B%: 35%-35%,10min). ¹H NMR (400MHz, METHANOL-d₄): δ 8.36 (s, 1H), 8.31 (d, $J=2.0$ Hz, 1H), 8.21 (d, $J=2.2$ Hz, 1H), 8.09 (dd, $J=5.6, 8.8$ Hz, 1H), 8.02 (dd, $J=2.2, 8.4$ Hz, 1H), 7.98 (d, $J=2.2$ Hz, 1H), 7.76 (d, $J=8.4$ Hz, 1H), 7.46 (dd, $J=2.4, 8.5$ Hz, 1H), 7.22 (dt, $J=2.4, 8.4$ Hz, 1H), 4.28 - 4.09 (m, 2H), 3.86 (s, 3H), 3.68 - 3.49 (m, 7H), 3.47 - 3.38 (m, 2H), 1.23 (d, $J=6.8$ Hz, 3H), MS: 616.1(M+1)⁺.

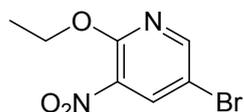
Synthesis and full characterization of compound 27-37



Supplementary Scheme 3: a) EtONa, EtOH, 75°C, 16h; b) Zn, NH₄Cl, MeOH, DCM,

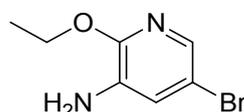
75°C, 4h; c) Pd(dppf)Cl₂, KOAc, dioxane, 105°C, 16h; d) Pyridine, 2-chloro-4-fluorobenzene-1-sulfonyl chloride, r.t. 18h; e) KOAc, Pd(dppf)Cl₂, dioxane/H₂O, 95°C, 2h; f, SCF split

5-bromo-2-ethoxy-3-nitropyridine



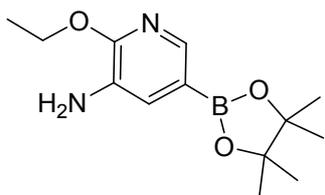
To a solution of 5-bromo-2-chloro-3-nitro-pyridine (2 g, 8.42 mmol, 1 eq) in EtOH (100 mL) was added sodium ethoxide (5 g, 73.48 mmol, 8.72 eq). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 75°C for 16 h. The reaction mixture was partitioned between H₂O 100 mL and EA 100 mL. The organic phase was separated, aqueous phase washed with EA (50 mL×3). The combined organic phase dried over Na₂SO₄, filtered and concentrated under reduced pressure to yield the crude title compound (1.4 g) used directly.

5-bromo-2-ethoxypyridin-3-amine



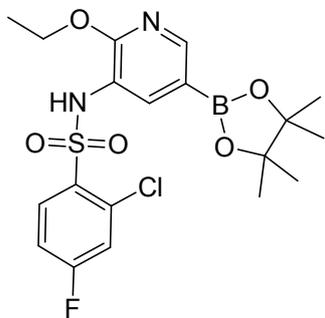
To a solution of 5-bromo-2-ethoxy-3-nitro-pyridine (1.3 g, 5.26 mmol, 1 eq) in MeOH (100 mL) and DCM (10 mL) was added Zn (1.90 g, 29.03 mmol, 5.52 eq) and NH₄Cl (1.43 g, 26.73 mmol, 934.64 uL, 5.08 eq). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 75°C for 4 h. The reaction mixture was partitioned between H₂O 50 mL and EA 50 mL. The organic phase was separated, aqueous phase washed with EA (25 mL×3). The combined organic phase dried over Na₂SO₄, filtered and concentrated under reduced pressure to yield the crude title compound (1 g) and it was used directly. MS: 215.9/516.9(M+1)⁺.

2-ethoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-amine



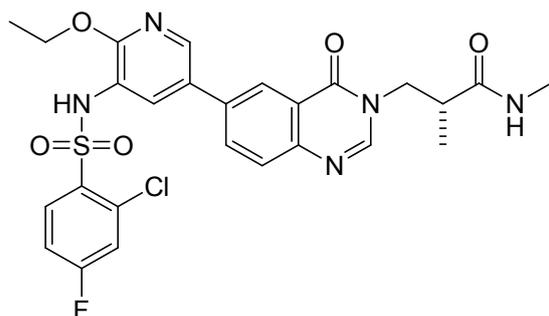
To a solution of 5-bromo-2-ethoxy-pyridin-3-amine (1 g, 4.61 mmol, 1 eq) in dioxane (50 mL) was added 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.20 g, 4.73 mmol, 1.03 eq), Pd(dppf)Cl₂ (400.00 mg, 546.67 μmol, 1.19e-1 eq) and KOAc (1.40 g, 14.27 mmol, 3.10 eq). The reaction was degassed and purged with N₂ for 3 times, and then the mixture was stirred at 105°C for 16 h. The reaction mixture was partitioned between H₂O 10 mL and EA 10 mL. The organic phase was separated, aqueous phase washed with EA (5 mL * 3). The combined organic phase dried over Na₂SO₄, filtered and concentrated under reduced pressure to yield the crude title compound (1.2 g) and it was used directly. MS: 265.1 (M+1)⁺.

2-chloro-*N*-(2-ethoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)-4-fluorobenzenesulfonamide



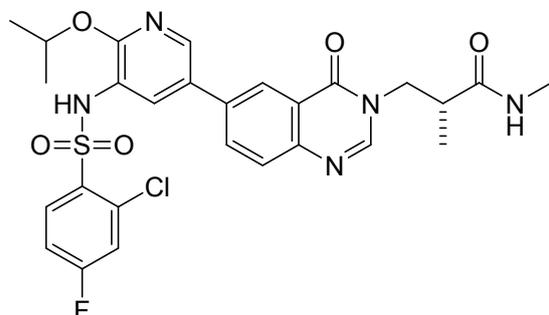
The title compound refers to the synthesis method of 2-chloro-4-fluoro-*N*-(2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)benzenesulfonamide. MS: 457.1(M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-ethoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**27**)



The title compound refers to the synthesis method of compound **13** in 21.4% yield, and was purified by SFC (column: Chiralpak AS-H 250*30 5 μ ;mobile phase: [0.1%NH₃H₂O ETOH];B%: 35%-35%,min). ¹H NMR (400 MHz, CDCl₃): δ 1.21 (br d, $J=7.03$ Hz, 3 H) 1.33 (t, $J=7.03$ Hz, 3 H) 2.67 (d, $J=4.77$ Hz, 3 H) 2.84 - 2.94 (m, 1 H) 3.89 - 4.02 (m, 1 H) 4.04 - 4.16 (m, 1 H) 4.25 - 4.43 (m, 2 H) 5.68 (br s, 1 H) 6.91 - 7.11 (m, 1 H) 7.11 - 7.25 (m, 2 H) 7.60 - 7.83 (m, 2 H) 7.91 (s, 1 H) 7.98 - 8.09 (m, 3 H) 8.20 (s, 1 H), MS: 574.1(M+1)⁺.

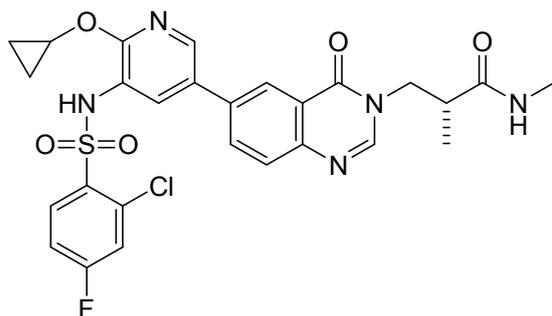
(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-isopropoxy)pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**28**)



The title compound refers to the synthesis method of compound **27**, and was purified by SFC (column: Chiralpak AS-H 250*30 5 μ ;mobile phase: [0.1%NH₃H₂O MeOH];B%: 42%-42%,min).. ¹H NMR (400MHz, CDCl₃): δ 8.32 (d, $J=2.2$ Hz, 1H), 8.15 - 8.08 (m, 3H), 8.01 (d, $J=2.2$ Hz, 1H), 7.87 - 7.82 (m, 1H), 7.80 - 7.76 (m, 1H), 7.58 (s, 1H), 7.28 (s, 1H), 7.14 - 7.09 (m, 1H), 5.60 (br d, $J=4.8$ Hz, 1H), 5.37 (quin, $J=6.1$ Hz, 1H), 4.22 - 4.14 (m, 1H), 4.09 - 4.01 (m, 1H), 3.03 - 2.90 (m, 1H), 2.75 (d, $J=4.8$ Hz, 3H), 1.34 (d, $J=6.1$ Hz, 6H), 1.29 (d, $J=7.0$ Hz, 3H), MS: 588.1(M+1)⁺.

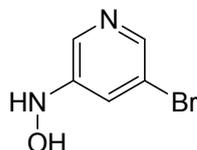
(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-cyclopropoxy)pyridin-3-yl)-4-

oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**29**)



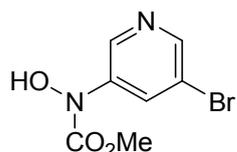
The title compound refers to the synthesis method of compound **27**, and was purified by SFC (column: AD(250mm*30mm,5um);mobile phase: [Neu-IPA];B%: 40%-40%,5.5min). ¹H NMR (400MHz, METHANOL-d₄): δ 8.36 (br s, 1H), 8.27 (d, *J*=2.0 Hz, 1H), 8.19 (s, 1H), 8.06 - 7.99 (m, 3H), 7.77 (d, *J*=8.6 Hz, 1H), 7.79 - 7.74 (m, 1H), 7.48 (dd, *J*=2.4, 8.6 Hz, 1H), 7.21 (dt, *J*=2.4, 8.4 Hz, 1H), 4.58 (br s, 1H), 4.29 - 4.17 (m, 2H), 4.03 (dd, *J*=9.8, 13.4 Hz, 1H), 3.06 - 2.90 (m, 1H), 2.64 - 2.60 (m, 2H), 2.64 - 2.60 (m, 1H), 1.24 (d, *J*=7.0 Hz, 3H), 0.75 - 0.69 (m, 2H), 0.61 - 0.54 (m, 2H), MS: 586.1(M+1)⁺.

N-(5-bromopyridin-3-yl)hydroxylamine



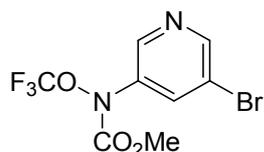
To a solution of 3-bromo-5-nitropyridine (13.7 g, 67.49 mmol, 1 *eq*) in THF (140 mL) was added hydrazine hydrate (4.14 g, 80.99 mmol, 4.02 mL, 98% purity, 1.2 *eq*) and Pt/C (1.71 g, 8.15 mmol, 1.21e-1 *eq*). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 25°C for 3 h. The solution was filtered off and dried under reduced pressure to yield the title compound (12 g, crude) and it was used directly. ¹H NMR (400MHz, METHANOL-d₄): δ 8.09 (d, *J*=2.3 Hz, 1H), 8.03 (d, *J*=1.9 Hz, 1H), 7.52 (t, *J*=2.1 Hz, 1H). MS: 188.9/190.9 (M+1)⁺.

Methyl (5-bromopyridin-3-yl)(hydroxy)carbamate



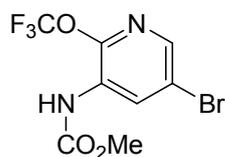
To a solution of *N*-(5-bromopyridin-3-yl)hydroxylamine (5 g, 26.45 mmol, 1 *eq*) in THF (50 mL) was added NaHCO₃ (2.67 g, 31.74 mmol, 1.23 mL, 1.2 *eq*), DMAP (3.23 g, 26.45 mmol, 1 *eq*) and methyl carbonchloridate (3.00 g, 31.74 mmol, 2.46 mL, 1.2 *eq*). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 20°C for 5 h. The solution was concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography to yield the title compound (0.8 g, 3.24 mmol, 11.13% yield). ¹H NMR (400 MHz, CDCl₃): δ 8.72 (d, *J*=2.0 Hz, 1H), 8.33 (d, *J*=1.8 Hz, 1H), 8.29 (s, 1H), 3.94 (s, 3H), MS: 246.9/248.9(M+1)⁺.

Methyl (5-bromopyridin-3-yl)(trifluoromethoxy)carbamate



To a solution of methyl (5-bromopyridin-3-yl)(hydroxy)carbamate (3 g, 12.14 mmol, 1 *eq*) in DCM (121 mL) was added 3,3-dimethyl-1-(trifluoromethyl)-1,2-benziodoxole (4.01 g, 12.14 mmol, 1 *eq*). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 20°C for 10 h. The solution was concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography to yield the title compound (0.76 g, 2.41 mmol, 18.57% yield). ¹H NMR (400MHz, CDCl₃): δ 8.66 (d, *J*=1.9 Hz, 1H), 8.64 (d, *J*=2.3 Hz, 1H), 7.92 (t, *J*=2.1 Hz, 1H), 3.93 (s, 3H), MS: 314.9/316.9 (M+1)⁺.

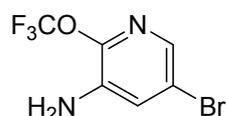
Methyl (5-bromo-2-(trifluoromethoxy)pyridin-3-yl)carbamate



Methyl (5-bromopyridin-3-yl)(trifluoromethoxy)carbamate (0.35 g, 1.11 mmol, 1 *eq*)

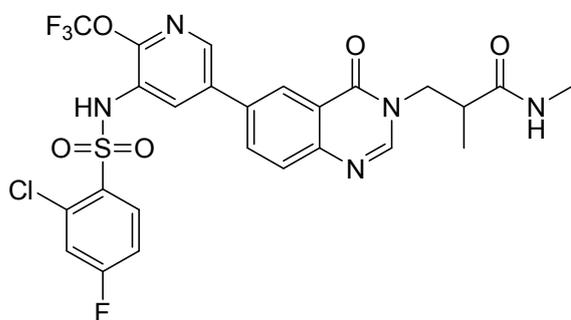
was added nitromethane (11 mL). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 160°C microwave for 10 h. The solution was concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (PE:EA=5: 1) to yield the title compound (0.15 g, 476.12 μmol , 20.03% yield). ^1H NMR (400MHz, CDCl_3): δ 8.75 (br s, 1H), 7.99 (d, $J=2.3$ Hz, 1H), 6.96 - 6.86 (m, 1H), 3.85 (s, 3H), MS: 314.9/316.9 ($\text{M}+1$) $^+$.

5-bromo-2-(trifluoromethoxy)pyridin-3-amine



To a solution of methyl (5-bromo-2-(trifluoromethoxy)pyridin-3-yl)carbamate (0.16 g, 507.87 μmol , 1 *eq*) in MeOH (2 mL), H_2O (0.4 mL) was added lithium hydroxide monohydrate (85.24 mg, 2.03 mmol, 4 *eq*). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 50°C for 24 h. The reaction mixture was partitioned between H_2O 10 mL and DCM 10 mL. The organic phase was separated, aqueous phase washed with DCM (5 mL \times 3). The combined organic phase dried over Na_2SO_4 , filtered and concentrated under reduced pressure to yield the title compound (120 mg, crude) and it was used directly. MS: 256.9/258.9 ($\text{M}+1$) $^+$.

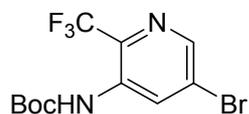
3-(6-(5-(2-chloro-4-fluorophenyl)sulfonamido)-6-(trifluoromethoxy)pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**30**)



The title compound refers to the synthesis method of compound **27**. ^1H NMR (400 MHz, METHANOL- d_4): δ 8.34 (d, $J=2.3$ Hz, 1H), 8.19 - 8.14 (m, 2H), 8.06 (dd, $J=5.8$, 8.9 Hz, 1H), 7.87 (dd, $J=2.3$, 8.5 Hz, 1H), 7.71 (d, $J=8.5$ Hz, 1H), 7.57 - 7.50 (m, 2H), 7.31

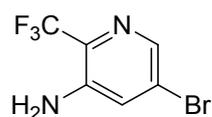
- 7.24 (m, 1H), 4.23 (dd, $J=5.0, 13.4$ Hz, 1H), 4.02 (dd, $J=9.8, 13.4$ Hz, 1H), 3.02 - 2.91 (m, 7H), 2.65 - 2.59 (m, 3H), 1.24 (d, $J=7.0$ Hz, 3H), MS: 614.0(M+1)⁺.

Tert-butyl (5-bromo-2-(trifluoromethyl)pyridin-3-yl)carbamate



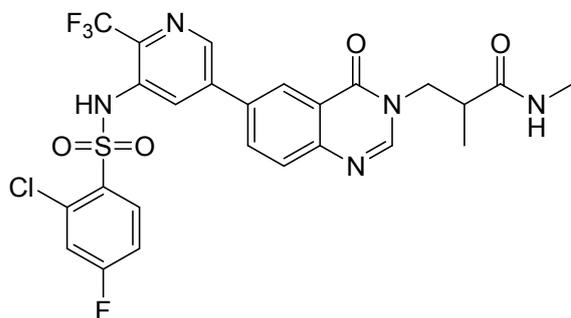
To a solution of 5-bromo-2-(trifluoromethyl)nicotinic acid (500 mg, 1.85 mmol, 1 *eq*) in t-BuOH (10 mL) was added DPPA (560.59 mg, 2.04 mmol, 441.41 μ L, 1.1 *eq*) and TEA (224.86 mg, 2.22 mmol, 309.30 μ L, 1.2 *eq*). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 80°C for 2 h. The reaction mixture was partitioned between saturated NaHCO₃ 10 mL and EA 10 mL. The organic phase was separated, aqueous phase washed with EA (5 mL \times 3). The combined organic phase dried over Na₂SO₄, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-TLC (PE:EA=10:1) to yield the title compound (400 mg, 1.17 mmol, 63.32% yield). ¹H NMR (400MHz, CDCl₃): δ 8.85 (s, 1H), 8.31 (d, $J=1.8$ Hz, 1H), 6.85 (br s, 1H), 1.54 - 1.39 (m, 9H), MS: 341.0/343.0(M+1)⁺.

5-bromo-2-(trifluoromethyl)pyridin-3-amine



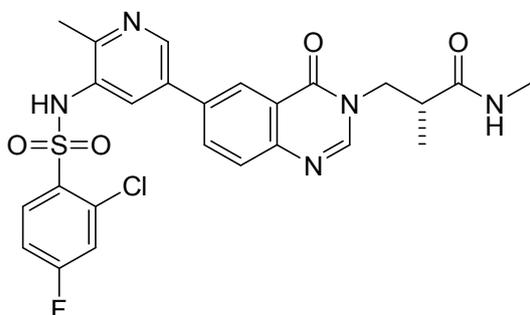
Tert-butyl (5-bromo-2-(trifluoromethyl)pyridin-3-yl)carbamate (350 mg, 1.03 mmol, 1 *eq*) was added HCl/EtOAc (3 mL, 4N), and then the mixture was stirred at 20°C for 16 h. The reaction mixture was partitioned between H₂O 10 mL and EA 10 mL. The organic phase was separated, aqueous phase washed with EA (5 mL * 3). The combined organic phase dried over Na₂SO₄, filtered and concentrated under reduced pressure to yield the title compound (180 mg, crude) and it was used directly. ¹H NMR (400MHz, METHANOL-d₄): δ 7.89 (d, $J=1.8$ Hz, 1H), 7.49 (d, $J=1.3$ Hz, 1H), MS: 241.0/243.0(M+1)⁺.

3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-(trifluoromethyl)pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**31**)



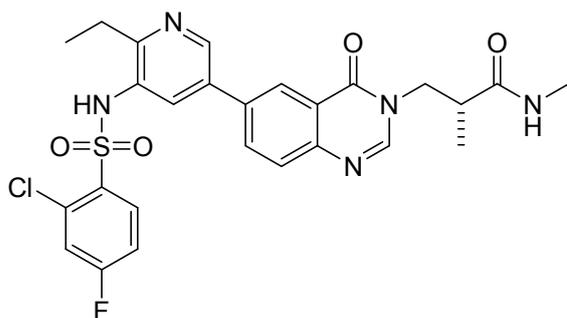
The title compound refers to the synthesis method of compound **27**. ¹H NMR (400MHz, METHANOL-d₄): δ 8.77 (s, 1H), 8.45 (d, *J*=2.2 Hz, 1H), 8.13 - 8.05 (m, 2H), 7.81 (d, *J*=8.6 Hz, 1H), 7.45 (dd, *J*=2.5, 8.5 Hz, 1H), 7.25 (ddd, *J*=2.6, 7.9, 8.9 Hz, 1H), 4.23 (dd, *J*=4.9, 13.5 Hz, 1H), 4.02 (dd, *J*=9.7, 13.5 Hz, 1H), 3.04 - 2.91 (m, 1H), 2.62 - 2.55 (m, 3H), 1.22 (d, *J*=7.1 Hz, 3H), MS: 598.0(M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methylpyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**32**)



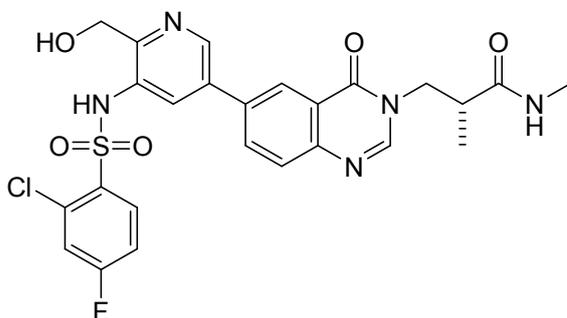
The title compound refers to the synthesis method of compound **27**, and was purified by SFC (column: OD(250mm*30mm,5um);mobile phase: [0.1%NH₃H₂O ETOH];B%: 35%-35%,min). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 8.74 (s, 1 H) 8.20 - 8.39 (m, 2 H) 8.11 (dd, *J*=8.53, 2.01 Hz, 1 H) 8.03 (dd, *J*=8.91, 5.90 Hz, 1 H) 7.95 (br d, *J*=4.77 Hz, 1 H) 7.73 - 7.88 (m, 3 H) 7.35 - 7.50 (m, 1 H) 3.94 - 4.19 (m, 2 H) 2.84 - 2.96 (m, 1 H) 2.54 (br s, 3 H) 2.43 (s, 3 H) 1.14 (d, *J*=7.03 Hz, 3 H),MS: 544.1(M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-ethylpyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**33**)



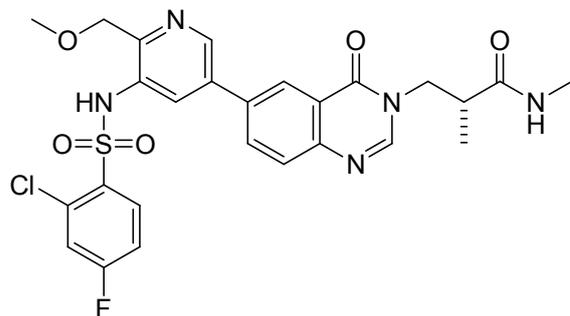
The title compound refers to the synthesis method of compound **27**, and was purified by SFC (column: Chiralpak AS-H 250*30 5u;mobile phase: [0.1%NH₃H₂O ETOH];B%: 35%-35%,min). ¹H NMR (400MHz, METHANOL-d₄): δ 8.56 (d, *J*=2.0 Hz, 1H), 8.23 (d, *J*=2.0 Hz, 1H), 8.10 (s, 1H), 8.04 - 7.82 (m, 2H), 7.80 - 7.59 (m, 2H), 7.45 (dd, *J*=2.4, 8.4 Hz, 1H), 7.15 (dt, *J*=2.5, 8.4 Hz, 1H), 4.14 (dd, *J*=4.8, 13.3 Hz, 1H), 3.93 (dd, *J*=9.8, 13.3 Hz, 1H), 2.99 - 2.81 (m, 1H), 2.76 (q, *J*=7.5 Hz, 2H), 2.64 - 2.42 (m, 3H), 1.14 (d, *J*=7.0 Hz, 3H), 1.04 (t, *J*=7.5 Hz, 3H), MS: 558.1(M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-(hydroxymethyl)pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**34**)



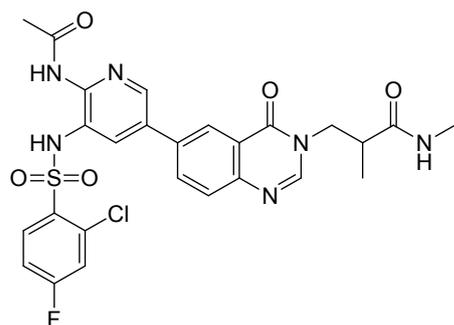
The title compound refers to the synthesis method of compound **27**, and was purified by SFC (column: ChiralcelOD-H 250*30mm i.d. 5u;mobile phase: A for CO₂ and B for MeOH(0.1%NH₃H₂O); B%: 45%-45%;Flow rate:70 g/min). ¹H NMR (400MHz, CDCl₃): δ 8.44 (br s, 1H), 8.26 (br s, 1H), 8.20 - 8.13 (m, 2H), 7.98 (s, 1H), 7.83 - 7.74 (m, 2H), 7.30 (br d, *J*=8.3 Hz, 1H), 7.17 (br t, *J*=7.2 Hz, 1H), 5.79 - 5.79 (m, 1H), 5.79 - 5.79 (m, 1H), 5.79 - 5.79 (m, 1H), 5.79 (br s, 1H), 4.91 (br s, 2H), 4.23 - 4.15 (m, 1H), 4.11 - 4.10 (m, 1H), 4.11 - 4.10 (m, 1H), 4.06 - 4.06 (m, 1H), 4.11 - 3.99 (m, 1H), 2.98 (br d, *J*=8.8 Hz, 1H), 2.75 (br d, *J*=4.5 Hz, 3H), 1.34 - 1.34 (m, 1H), 1.34 - 1.33 (m, 1H), 1.31 (br d, *J*=6.8 Hz, 3H), MS: 560.1(M+1)⁺.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-(methoxymethyl)pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**35**)



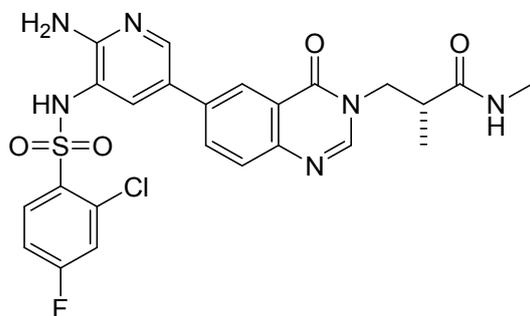
The title compound refers to the synthesis method of compound **27**, and was purified by SFC (column: ChiralcelOD-H 250*30mm i.d. 5u; mobile phase: A for CO₂ and B for MeOH (0.1%NH₃H₂O); B%: 45%-45%,min). ¹H NMR (400MHz, CDCl₃): δ 8.87 (d, *J*=2.0 Hz, 1H), 8.37 - 8.30 (m, 1H), 8.16 (s, 1H), 7.95 (dd, *J*=5.8, 8.9 Hz, 1H), 7.86 - 7.81 (m, 2H), 7.68 (d, *J*=2.0 Hz, 1H), 7.40 (dd, *J*=2.6, 8.2 Hz, 1H), 7.11 (ddd, *J*=2.5, 7.3, 8.9 Hz, 1H), 5.55 (br s, 1H), 4.79 (d, *J*=5.1 Hz, 2H), 4.23 - 4.15 (m, 1H), 4.11 - 4.04 (m, 1H), 3.95 (t, *J*=5.2 Hz, 1H), 3.40 (s, 3H), 3.01 - 2.88 (m, 1H), 2.75 (d, *J*=4.9 Hz, 3H), 1.30 (d, *J*=7.1 Hz, 3H), MS: 574.1(M+1)⁺.

3-(6-(6-acetamido-5-(2-chloro-4-fluorophenylsulfonamido)pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide



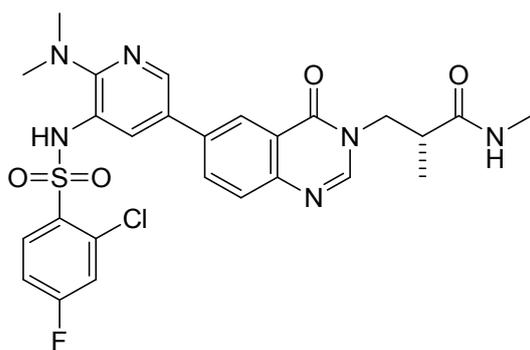
The title compound refers to the synthesis method of compound **27**. ¹H NMR (400MHz, METHANOL-*d*₄): δ 8.55 (s, 1H), 8.43 (d, *J*=2.0 Hz, 1H), 8.27 - 8.15 (m, 3H), 8.09 (dd, *J*=2.1, 8.5 Hz, 1H), 7.92 (dd, *J*=5.8, 8.8 Hz, 1H), 7.80 (d, *J*=8.5 Hz, 1H), 7.62 - 7.47 (m, 6H), 7.18 (dt, *J*=2.4, 8.4 Hz, 1H), 4.34 - 4.17 (m, 2H), 4.09 - 3.95 (m, 2H), 3.60 (s, 1H), 3.05 - 2.92 (m, 2H), 2.63 - 2.61 (m, 3H), 2.22 (s, 3H), 1.24 (d, *J*=7.0 Hz, 3H), MS: 587.1(M+1)⁺.

(*R*)-3-(6-(6-amino-5-(2-chloro-4-fluorophenylsulfonamido)pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**36**)



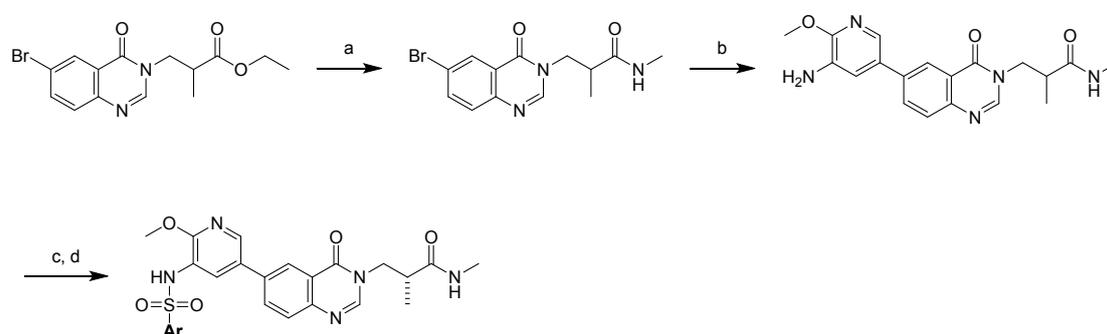
To a solution of 3-(6-(6-acetamido-5-(2-chloro-4-fluorophenylsulfonamido)pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (0.28 g, 476.98 μmol , 1 *eq*) in MeOH (10 mL) was added K_2CO_3 (197.77 mg, 1.43 mmol, 3 *eq*). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 80°C for 10 h. The solution was concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (column: Agela Durashell C18 150*25 5 μ ; mobile phase: [water(10mM NH_4HCO_3)-ACN]; B%: 12%-42%, 10.5min) to yield the title compound (0.1 g, 183.49 μmol , 38.47% yield). ^1H NMR (400MHz, METHANOL- d_4): δ 8.18 - 8.13 (m, 2H), 8.10 (dd, $J=5.9, 8.9$ Hz, 1H), 8.01 (br s, 1H), 7.85 (dd, $J=2.1, 8.5$ Hz, 1H), 7.69 (d, $J=8.5$ Hz, 1H), 7.54 - 7.47 (m, 2H), 7.29 - 7.21 (m, 1H), 4.23 (dd, $J=4.9, 13.3$ Hz, 1H), 4.01 (dd, $J=9.8, 13.4$ Hz, 1H), 3.05 - 2.92 (m, 1H), 2.65 - 2.58 (m, 3H), 1.23 (d, $J=7.0$ Hz, 3H), MS: 545.1(M+1) $^+$.

(*R*)-3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-(dimethylamino)pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**37**)



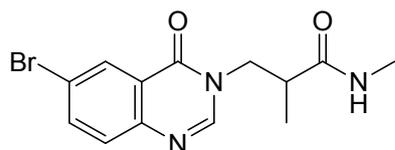
The title compound refers to the synthesis method of compound **27**, and was purified by SFC (column: Chiralpak AS-H 250*30 5 μ ; mobile phase: A for CO₂ and B for IPA; B%: 30%-30%, Flow rate: 65 g/min). ¹H NMR (400MHz, METHANOL-d₄): δ 8.34 (d, J =2.4 Hz, 1H), 8.20 - 8.15 (m, 2H), 8.06 (dd, J =5.8, 9.0 Hz, 1H), 7.88 (dd, J =2.3, 8.5 Hz, 1H), 7.72 (d, J =8.7 Hz, 1H), 7.57 - 7.49 (m, 2H), 7.27 (dt, J =2.6, 8.3 Hz, 1H), 4.24 (dd, J =5.0, 13.3 Hz, 1H), 4.02 (dd, J =9.9, 13.5 Hz, 1H), 2.95 (s, 7H), 2.62 (s, 3H), 1.24 (d, J =6.9 Hz, 3H), MS: 573.1(M+1)⁺.

Synthesis and full characterization of compound 38-47



Supplementary Scheme 4: a) MeNH₂(2N), 20°C, 12h; b) KOAc, Pd(dppf)Cl₂, dioxane/H₂O, 95°C, 2h; c) Pyridine, r.t. 18h; d) SCF split

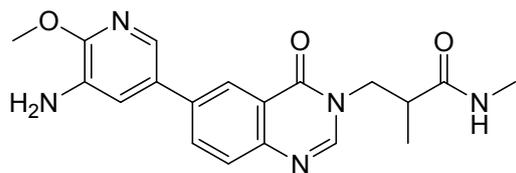
3-(6-bromo-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide



Ethyl 3-(6-bromo-4-oxoquinazolin-3-yl)-2-methylpropanoate (3 g, 8.84 mmol, 1 *eq*) was added 100 mL single bottle, then added methylamine (50 mL, 2N methanol solution), the mixture was stirred at 20°C for 12h. The solution was concentrated under reduce pressure to yield the title compound (2.9 g, crude) used directly. ¹H NMR (400MHz, CDCl₃) δ = 8.30 (d, J =2.4 Hz, 1H), 8.04 (s, 1H), 7.80 - 7.69 (m, 1H), 7.56 - 7.46 (m, 1H), 5.56 (br s, 1H), 4.14 - 4.01 (m, 1H), 3.99 - 3.89 (m, 1H), 2.92 - 2.78 (m, 2H), 2.66 (d, J =4.9 Hz, 3H), 1.20 (d, J =6.8 Hz, 3H).

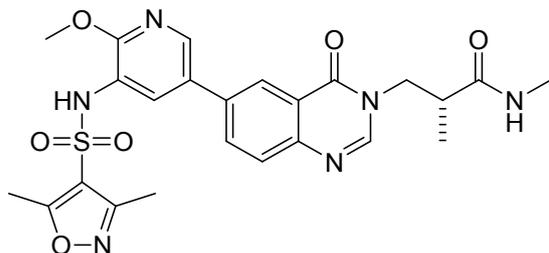
3-(6-(5-amino-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-

dimethylpropanamide



The title compound refers to the synthesis method of methyl 3-(6-(5-(2-chloro-4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-2-methylpropanoate in 89.92% yield. ¹H NMR (400MHz, METHANOL-*d*₄) δ = 8.39 (d, *J*=2.1 Hz, 1H), 8.18 (s, 1H), 8.07 - 8.02 (m, 1H), 7.81 - 7.77 (m, 1H), 7.74 (d, *J*=8.6 Hz, 1H), 7.33 (d, *J*=2.2 Hz, 1H), 4.24 (dd, *J*=4.9, 13.4 Hz, 1H), 4.03 (s, 4H), 3.05 - 2.93 (m, 1H), 2.66 - 2.59 (m, 3H), 1.25 (d, *J*=7.0 Hz, 3H), MS: 368.1(M+1)⁺.

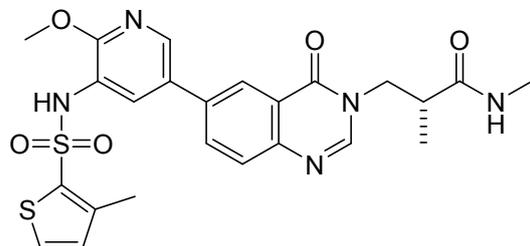
(*R*)-3-(6-(5-(3,5-dimethylisoxazole-4-sulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**38**)



To a solution of 3-[6-(5-amino-6-methoxy-3-pyridyl)-4-oxo-quinazolin-3-yl]-*N*,2-dimethyl-propanamide (0.3 g, 816.55 umol, 1 *eq*) in pyridine (5 mL) was added 3,5-dimethylisoxazole-4-sulfonyl chloride (159.74 mg, 816.55 umol, 1 *eq*). The reaction was degassed and purged with nitrogen for 3 times, and then the mixture was stirred at 25°C for 10 h. The solution was concentrated under reduce pressure to give a residue. The residue was purified by prep-TLC (Dichloromethane: Methanol: TEA=20: 1: 0.2) and then was purified by SFC (column: AD(250mm*30mm,10um);mobile phase: [0.1%NH₃H₂O ETOH];B%: 55%-55%,min , FlowRate(ml/min) : 80mL/min,) to yield the title compound (0.2 g, 374.88 umol, 45.91% yield). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.36 (br s, 1 H), 8.26 (s, 1 H), 8.18 (s, 1 H), 8.10 (br d, *J*=8.5 Hz, 1 H), 7.89 (br d, *J*=4.5 Hz, 2 H), 7.76 (d, *J*=8.5 Hz, 1 H), 4.04 - 4.12 (m, 1 H), 3.96 (dd, *J*=13.1, 9.0 Hz, 1 H), 3.74 (s, 3 H), 2.81 - 2.92 (m, 1 H), 2.35 (s, 2 H), 2.26 (s, 3 H),

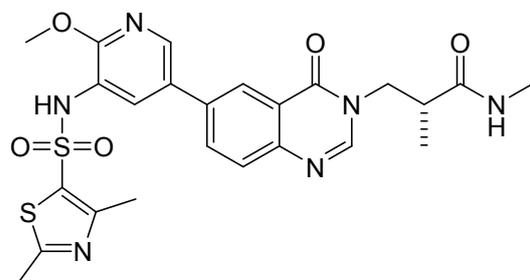
1.08 (d, $J=7.0$ Hz, 3 H), MS: 527.1(M+1)⁺.

(*R*)-3-(6-(6-methoxy-5-(3-methylthiophene-2-sulfonamido)pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**39**)



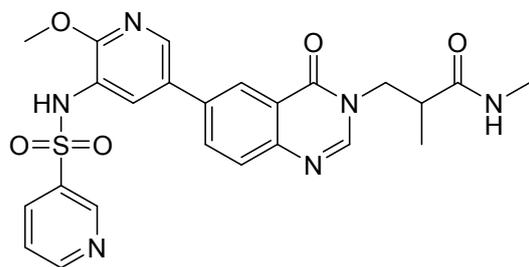
The title compound refers to the synthesis method of compound **38**, and was purified by SFC (column: AD(250mm*50mm,10um);mobile phase: [0.1%NH₃H₂O ETOH];B%: 55%-55%,min). ¹H NMR (400MHz, CDCl₃): δ 8.31 (d, $J=2.0$ Hz, 1H), 8.15 - 8.05 (m, 3H), 7.98 (d, $J=2.3$ Hz, 1H), 7.90 - 7.69 (m, 2H), 7.28 (br s, 1H), 7.19 - 7.00 (m, 1H), 5.58 (br s, 1H), 5.40 - 5.23 (m, 1H), 4.28 - 4.12 (m, 1H), 4.12 - 4.01 (m, 1H), 3.98 (s, 3H), 3.10 (br dd, $J=7.4, 14.4$ Hz, 1H), 1.33 (d, $J=7.0$ Hz, 3H), MS: 528.1(M+1)⁺.

(*R*)-3-(6-(5-(2,4-dimethylthiazole-5-sulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**40**)



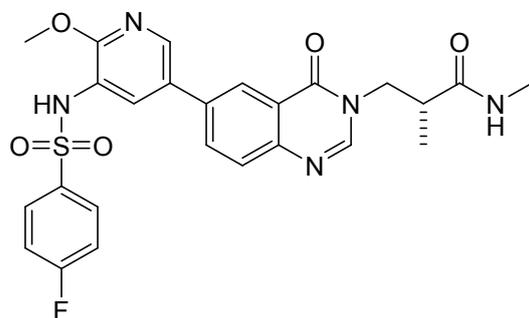
The title compound refers to the synthesis method of compound **38**, and was purified by SFC(Column: AD(250mm*30mm,10um), Condition: 0.1%NH₃H₂O ETOH, B%: 55%-55%, Flow Rate:80mL/min). ¹H NMR (400MHz, CDCl₃): 8.40(d, $J=2$ Hz, 1H), 8.22 (d, $J=2$ Hz, 1H), 8.13 (s, 1H), 8.09 (d, $J=2.4$ Hz, 1H), 7.89(d, $J=2.4$ Hz, 1H), 7.80-7.82 (m, 1H), 7.14 (m, 1H), 5.56 (s, 1H), 4.17-4.21 (m, 1H), 4.03-4.08 (m, 1H), 3.97 (s, 3H), 2.93-2.99 (m, 1H), 2.76 (s, 3H), 2.64 (s, 3H), 1.29 (d, $J=6.8$ Hz, 1H), MS: 543.1(M+1)⁺.

3-(6-(6-methoxy-5-(pyridine-3-sulfonamido)pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**41**)



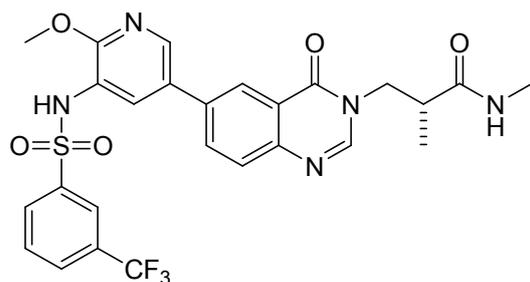
The title compound refers to the synthesis method of compound **38**. ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.87 (d, *J*=1.8 Hz, 1 H), 8.75 (br d, *J*=3.8 Hz, 1 H), 8.22 - 8.28 (m, 2 H), 8.18 (s, 1 H), 8.03 - 8.13 (m, 2 H), 7.85 - 7.94 (m, 2 H), 7.75 (d, *J*=8.5 Hz, 1 H), 7.58 (dd, *J*=7.9, 4.9 Hz, 1 H), 4.03 - 4.12 (m, 1 H), 3.92 - 4.00 (m, 1 H), 3.65 (s, 3 H), 2.79 - 2.93 (m, 1 H), 2.48 (br s, 3 H), 1.08 (d, *J*=7.0 Hz, 3 H), MS: 509.1(M+1)⁺.

(*R*)-3-(6-(5-(4-fluorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**42**)



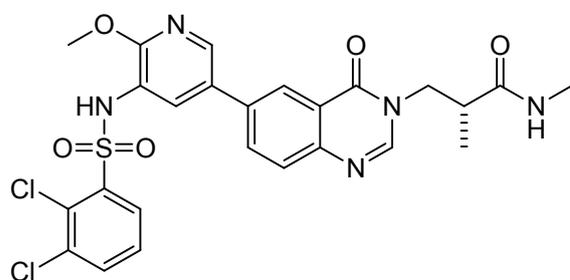
The title compound refers to the synthesis method of compound **38**, and was purified by SFC (Column: AD(250mm*30mm,10um), Condition: 0.1%NH₃H₂O ETOH, B%: 55%-55%, Flow Rate:80mL/min). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.31 - 8.36 (m, 1 H), 8.25 (d, *J*=2.0 Hz, 1 H), 8.19 (s, 1 H), 8.07 (dd, *J*=8.5, 2.0 Hz, 1 H), 7.87 - 7.93 (m, 2 H), 7.82 (dd, *J*=8.8, 5.3 Hz, 1 H), 7.79 - 7.84 (m, 1 H), 7.41 (t, *J*=8.8 Hz, 2 H), 4.02 - 4.13 (m, 1 H), 3.97 (dd, *J*=13.2, 9.2 Hz, 1 H), 3.69 (s, 3 H), 2.81 - 2.94 (m, 1 H), 2.48 - 2.49 (m, 3 H), 1.08 (d, *J*=7.0 Hz, 3 H), MS: 526.1(M+1)⁺.

(*R*)-3-(6-(6-methoxy-5-(3-(trifluoromethyl)phenylsulfonamido)pyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**43**)



The title compound refers to the synthesis method of compound **38**, and was purified by SFC (column: AD(250mm*30mm,10um); mobile phase: [0.1%NH₃H₂O ETOH];B%: 55%-55%,min, FlowRate (mL/min): 80mL/min). ¹H NMR (400 MHz, DMSO-d₆): δ 8.41 (d, *J*=2.3 Hz, 1 H), 8.28 (d, *J*=2.0 Hz, 1 H), 8.20 (s, 1 H), 8.08 (s, 3 H), 7.99 (br d, *J*=8.0 Hz, 1 H), 7.95 - 7.97 (m, 1 H), 7.87 - 7.95 (m, 1 H), 7.78 - 7.84 (m, 1 H), 7.76 (d, *J*=8.1 Hz, 1 H), 4.08 (dd, *J*=13.2, 5.6 Hz, 1 H), 3.93 - 4.02 (m, 1 H), 3.59 (s, 3 H), 2.82 - 2.91 (m, 1 H), 2.48 (br s, 3 H), 1.08 (d, *J*=6.8 Hz, 3 H), MS: 576.1(M+1)⁺.

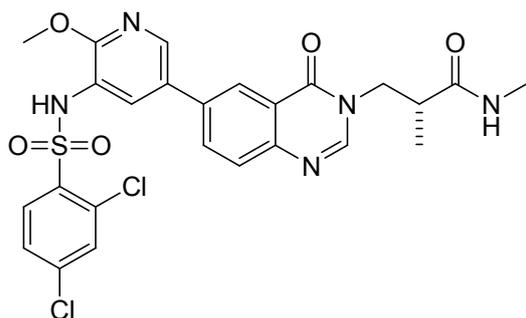
(*R*)-3-(6-(5-(2,3-dichlorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**44**)



The title compound refers to the synthesis method of compound **38**, and was purified by SFC (column: DAICEL CHIRALPAK AS-H(250mm*30mm,5um); mobile phase: [Neu-MeOH];B%: 42%-42%,5min). ¹H NMR (400MHz, METHANOL-d₄): δ 8.32 (d, *J*=1.5 Hz, 1H), 8.22 (d, *J*=2.2 Hz, 1H), 8.17 (s, 1H), 8.08 - 7.94 (m, 3H), 7.85 - 7.69 (m, 2H), 7.42 (t, *J*=8.0 Hz, 1H), 4.23 (dd, *J*=5.0, 13.3 Hz, 1H), 4.02 (dd, *J*=9.9, 13.5 Hz, 1H), 3.82 (s, 3H), 3.08 - 2.92 (m, 1H), 2.61 (s, 3H), 1.23 (d, *J*=7.1 Hz, 3H), MS: 576.0 (M+1)⁺.

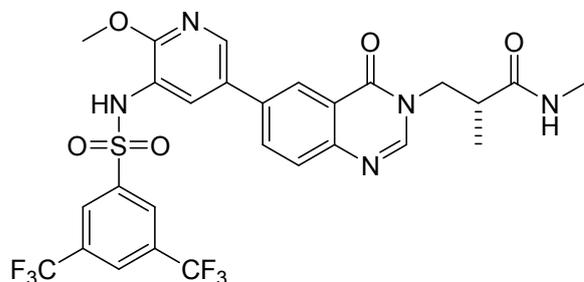
(*R*)-3-(6-(5-(2,4-dichlorophenylsulfonamido)-6-methoxypyridin-3-yl)-4-

oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**45**)



The title compound refers to the synthesis method of compound **38**, and was purified by SFC (column: OJ(250mm*30mm,5um); mobile phase: [0.1%NH₃H₂O ETOH];B%: 30%-30%,min). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 10.33 (br s, 1 H), 8.37 (s, 1 H), 8.26 (d, *J*=2.0 Hz, 1 H), 8.19 (s, 1 H), 8.07 (dd, *J*=8.5, 2.5 Hz, 1 H), 7.86 - 7.95 (m, 4 H), 7.76 (d, *J*=8.5 Hz, 1 H), 7.58 (dd, *J*=8.5, 2.0 Hz, 1 H), 4.04 - 4.13 (m, 1 H), 3.90 - 4.02 (m, 1 H), 3.69 (s, 3 H), 2.87 (br dd, *J*=15.1, 6.5 Hz, 1 H), 2.49 (br s, 3 H), 1.09 (d, *J*=7.0 Hz, 3 H) MS: 576.0(M+1)⁺.

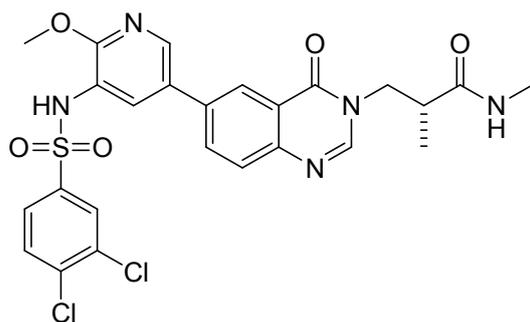
(*R*)-3-(6-(5-(3,5-bis(trifluoromethyl)phenyl)sulfonamido)-6-methoxypyridin-3-yl)-4-oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**46**)



The title compound refers to the synthesis method of compound **38**, and was purified by SFC (column: C2 250mm*30mm,10um;mobile phase: [0.1%NH₃H₂O MEOH];B%: 40%-40%,min, FlowRate(ml/min): 80mL/min). ¹H NMR (400 MHz, DMSO-*d*₆): δ 8.39 (br s, 1 H), 8.29 (s, 2 H), 8.22 - 8.28 (m, 2 H), 8.18 (s, 1 H), 8.04 (dd, *J*=8.5, 2.0 Hz, 1 H), 7.87 - 7.94 (m, 2 H), 7.74 (d, *J*=8.5 Hz, 1 H), 4.02 - 4.12 (m, 1 H), 3.96 (dd, *J*=13.2, 9.2 Hz, 1 H), 3.62 (s, 3 H), 2.82 - 2.91 (m, 1 H), 2.48 (br s, 3 H), 1.08 (d, *J*=6.8 Hz, 3 H). MS: 644.1(M+1)⁺.

(*R*)-3-(6-(5-(3,4-dichlorophenyl)sulfonamido)-6-methoxypyridin-3-yl)-4-

oxoquinazolin-3(4*H*)-yl)-*N*,2-dimethylpropanamide (**47**)



The title compound refers to the synthesis method of compound **38**, and was purified by SFC (Column: Chiralpak AS-H 250*30mm i.d. 5u; Mobile phase: A for CO₂ and B for MeOH(0.1%NH₃H₂O). ¹H NMR (400MHz, METHANOL-d₄): δ 8.41 (d, *J*=1.8 Hz, 1H), 8.29 (d, *J*=2.2 Hz, 1H), 8.19 (s, 1H), 8.11 - 8.04 (m, 2H), 7.97 (s, 1H), 7.78 (d, *J*=8.3 Hz, 1H), 7.71 - 7.64 (m, 2H), 4.24 (dd, *J*=4.8, 13.6 Hz, 1H), 4.03 (dd, *J*=9.9, 13.4 Hz, 1H), 3.77 (s, 3H), 2.99 (br d, *J*=9.6 Hz, 1H), 2.62 (s, 3H), 1.24 (d, *J*=6.6 Hz, 3H)