Supporting Information

Structure-Activity Relationship Study of QL47 – a Broad-spectrum Anti-viral Agent

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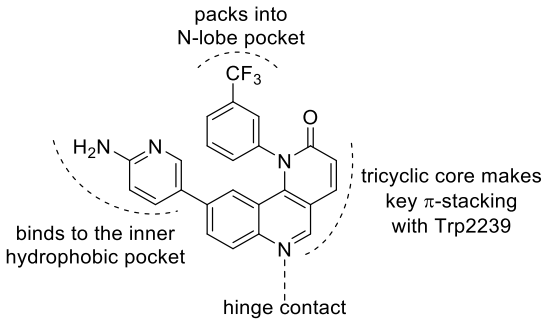
Table S1. Mouse microsomal stability and *in vivo* pharmacokinetic properties of QL47.

Route	**Dose (mg/kg)**	**$T_{1/2}$ (hrs)**	**T_{max} (hrs)**	**C_{max} (ng/mL)**	**AUC_{last} (ng x hr/mL)**	**AUC_{inf} (ng x hr/mL)**	**CL (mL/min/kg)**	**V_{ss} (L/kg)**	**F (%)**	**Microsome stability: $T_{1/2}$ (min)**
i.v.	0.5	0.07		310.24	22.74	22.83	365	1.03		1.2
i.p.	1		0.08	14.86	5.22	5.95				
p.o.	10								0	

Figure S1. Binding mode and co-crystal structure of Torin2 with mTOR kinase domain. (**A**) Chemical structure of Torin2. Key interactions of Torin2 with the ATP-binding pocket of mTOR kinase domain are shown. (**B**) Co-crystal structure of Torin2-mTOR. Torin2 is shown as a stick representation with C in black, O in red, N in blue, and F in cyan. mTOR backbone is shown in red with N in blue. Hinge contact is shown in green.

A



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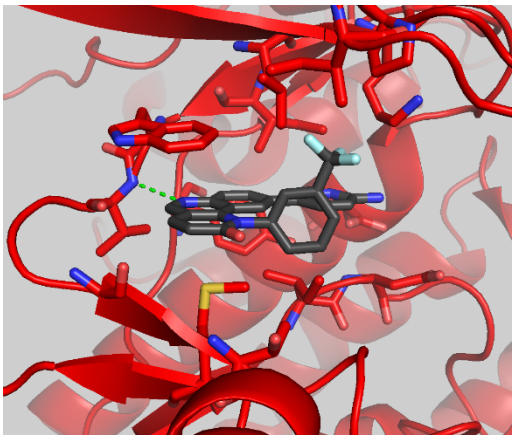
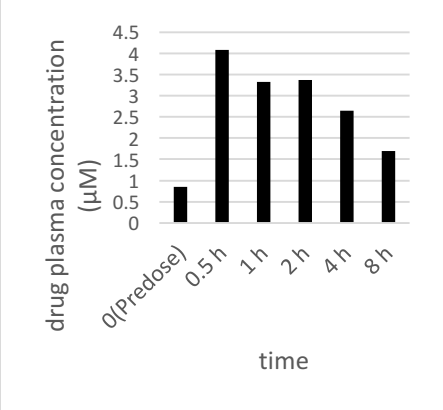


Figure S2. In vivo tolerability study of YKL-04-085. 14 Days study in 4 mice with 40 mg/kg IP dosing once daily. Drug formulation: 2 mg/mL solution in 4.3 mM HCl acidified saline. (**A**) Plasma drug concentration was measured on day 14, and the average data of 4 mice are shown. (**B**) Body weight of the 4 mice over 14 day's treatment.

A



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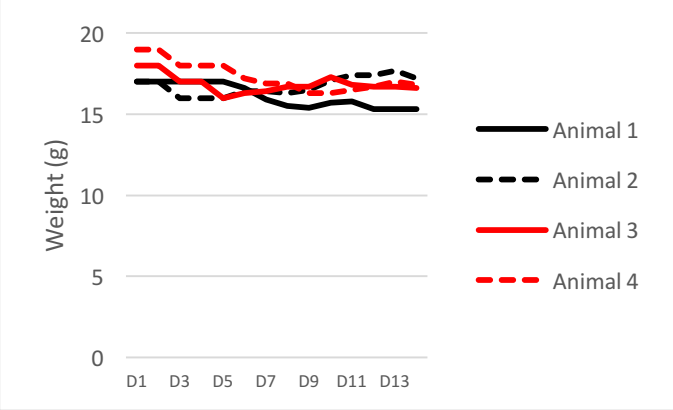


Materials and Methods

Cell lines

Human liver-derived Huh7 cells and African green monkey kidney-derived Vero cells were cultured in Dulbecco modified Eagle medium (DMEM) with 10% fetal bovine serum (FBS) at 37 °C with 5% CO_2. Baby hamster kidney-derived BHK-21 cells were grown in minimum essential Eagle medium (MEM-α) supplemented with 5% FBS. C6/36 cells, a continuous mosquito cell line derived from *Aedes albopictus* embryonic tissue, were grown in Leibovitz medium (L-15) containing 10% FBS at 28 °C.

Viruses

All work with infectious virus was performed in a biosafety level 2 (BSL2) laboratory using additional safety practices as approved by the Harvard Committee on Microbiological Safety.
Dengue virus serotype 2 strain New Guinea C (DENV2) was a kind gift from Lee Gerhke (Massachusetts Institute of Technology). West Nile virus strain Kunjin (WNV) was obtained from Michael Diamond (Washington University in Saint Louis). Poliovirus strain Chat (PV) was obtained from ATCC (VR-1562). Vesicular stomatitis virus strain Indiana (VSV) was gifted by Sean Whelan (Harvard Medical School).

Cytotoxicity assay

Huh7 cells were seeded at a density of 10,000 cells/well in a 96-well format. Small molecules were serially diluted in DMEM supplemented with 2% FBS, in presence of DENV2 diluted to a multiplicity of infection (MOI) of 1. The mixture was added to the cells, and the plates were incubated at 37 °C for 24 hours. The cytotoxicity was measured by quantitation of the ATP present, using a CellTiter-Glo luminescent cell viability assay (Promega) according to the manufacturer's instructions. The values for the concentrations that lead to 50% cytotoxicity (CC_{50}) were calculated using the nonlinear fit variable slope model (GraphPad Software).

Virus infections

All DENV2 and WNV infectivity assays were performed on Huh7 cells, while PV and VSV infectivity assays were performed on Vero cells. Cells were infected by incubation of a confluent monolayer with viral inoculum diluted in EBSS at MOI of 1 for 1 hour at 37 °C. The inoculum was then removed and the cells were washed twice with PBS. DMEM containing 2% FBS and

the indicated concentrations of small molecules was then overlaid on cells. The viral supernatants were harvested at 24 hours post-infection (DENV2 and WNV), 12 hours post-infection (PV) or 6 hours post-infection (VSV), and stored at -80 °C.

For DENV2 IC_{90} determination, cells were treated with a range of small molecules concentrations. The yield of infectious particles produced at 24 hours post-infection was quantified by focus-forming assay, and the results were plotted relative to DMSO-treated samples. The concentrations that lead to 90% DENV2 inhibition (IC_{90}) were calculated using the nonlinear fit variable slope model (GraphPad Software).

The antiviral testing for respiratory syncytial virus (RSV) was performed by IBT Bioservices. Vero cells were seeded in a 96-well plate and then treated with the indicated concentration of small molecule, prior to infection with RSV strain A2 at a MOI of 0.01. At 7 days post-infection, the cells were fixed and stained with a solution of crystal violet in glutaric dialdehyde. The optical density was determined after solubilizing the crystal violet in 100 µL of 50% ethanol. The percentage of plaque formation was calculated using the uninfected cells control as 0%, and the DMSO control as 100%.

Focus-forming assay (FFA).

BHK-21 cells were seeded in 24-well plates. Aliquots from DENV2 infections were thawed, tenfold dilutions in EBSS were prepared, and 100 µL of each dilution was added to the cells. The plates were incubated for 1 hour at 37 °C and rocked every 15 min. Unadsorbed virus was removed by a PBS wash, after which 1 mL of MEM-α supplemented with 1.05% carboxymethyl cellulose (CMC), 10 mM HEPES buffer, 44 mM sodium bicarbonate, and 2% FBS was added to each well, followed by incubation at 37 °C for 3 days. The CMC overlay was aspirated, and the cells were washed with PBS and fixed with methanol for 15 min at −20 °C. After fixation, the cells were washed with PBS and incubated for 1 hour at room temperature with monoclonal antibody 6F3.1 against DV2 core protein (generously provided by John Aaskov (Queensland University of Technology).[1] The cells were then washed and incubated with HRP-conjugated anti-mouse IgG antibody (Bio-Rad laboratories). The plates were developed with the Vector VIP peroxidase substrate kit (Vector Laboratories) according to the manufacturer's instructions.

Plaque-formation assay (PFA)

WNV titers were quantified by plaque-formation assay (PFA) on BHK-21 cells. PV and VSV titers were quantified by PFA on Vero cells. Cells were seeded in 24-well plates to form a

confluent monolayer. Aliquots from viral stocks or from infections were thawed at room temperature. Tenfold dilutions in EBSS were prepared, and 100 μL of each dilution was added to the cells. The plates were incubated for 1 hour at 37 °C and rocked every 15 min. Unadsorbed virus was removed, after which 1 mL of DMEM (Vero cells) or MEMα (BHK-21 cells) supplemented with 1.05% carboxymethyl cellulose (CMC), 44 mM sodium bicarbonate, and 2% FBS was added to each well, followed by incubation at 37 °C for 3 days (WNV) or 2 days (PV and VSV). The CMC overlay was aspirated, and the cells were washed with PBS and stained with crystal violet. The number of plaque-forming units (PFU) per milliliter was evaluated by counting the number of plaques.

Reporter replicon assay.

The mutagenized plasmid pDENrep-FH that expressed the DENV2(GVD) reporter replicon RNA was described previously.[2] *In vitro* transcripts were synthesized from PstI-linearized plasmids using the T7-Scribe Standard RNA IVT kit (CellScript) and m7G(5')ppp(5')A RNA cap structure analog (New England BioLabs) according to the manufacturers' instructions. Huh7 cells seeded in a 24-well plate were treated with the indicated concentrations of small molecules, and immediately transfected with *in vitro* transcripts using the Lipofectamine MessengerMAX transfection reagent (ThermoFisher Scientific). At 6 hours post-transfection, the cells were collected, and the samples were processed according to the instructions in the luciferase assay system (Promega). The firefly luciferase signal was read using a Perkin-Elmer EnVision plate reader and reported as a function of the DMSO treated samples.

Hepatic microsomal stability.

In vitro metabolic study was conducted at DMPK core of the Scripps Research Institute, Florida. Microsome stability was evaluated by incubating 1 μM compound with 1 mg/mL hepatic microsomes in 100 mM KP_i, pH 7.4. The reaction was initiated by adding NADPH (1 mM final concentration). Aliquots are removed at 0, 5, 10, 20, 40, and 60 minutes and added to acetonitrile (5X v:v) to stop the reaction and precipitate the protein. NADPH dependence of the reaction was evaluated with the addition of no-NADPH control samples. At the end of the assay, the samples were centrifuged through a Millipore Multiscreen Solvinter 0.45 micron low binding PTFE hydrophilic filter plate and analyzed by LC-MS/MS. Data was log transformed and represented as half-life.

***In vivo* pharmacokinetic studies.**

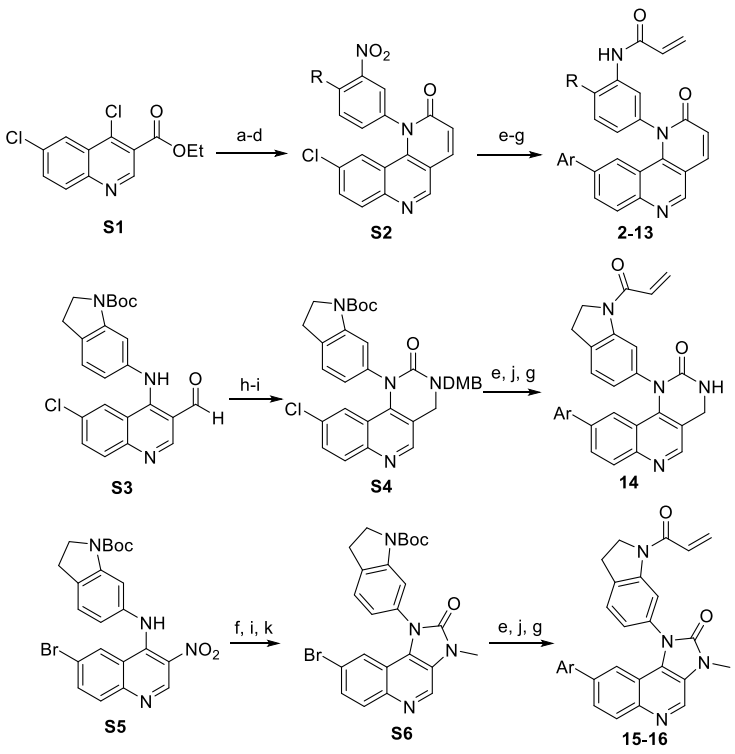
Male Swiss albino mice were administered with compound solution formulation in normal saline intravenously (i.v.) *via* tail vein at a dose of 2 mg/kg, or orally (p.o.) at a dose of 10 mg/kg, or *via* intraperitoneal route (i.p.) at doses of 10 mg/kg and 50 mg/kg. Blood samples (approximately 60 µL) were collected under light isoflurane anesthesia from retro orbital plexus at Predose, 0.08, 0.25, 0.5, 1, 2, 4, 8 and 24 hr (i.v. and i.p.) and Predose, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hr (p.o.). Plasma samples were separated by centrifugation of whole blood and stored below -70 ºC until bioanalysis. All samples were processed for analysis by protein precipitation using acetonitrile and analyzed with fit-for-purpose LC/MS/MS method (LLOQ – 2.46 ng/mL). Pharmacokinetic parameters were calculated using the noncompartmental analysis tool of Phoenix WinNonlin® (Version 6.3).

Chemistry Experimental Procedures

General experimental

All reactions were monitored by thin layer chromatography (TLC) with 0.25 mm E. Merck pre-coated silica gel plates (60 F_{254}) and Waters LCMS system (Waters 2998 UV/Visible Detector, Waters SQ Detector 2, Waters 515 HPLC pump, Waters 2545 Binary Gradient Module, Waters System Fluidics Organizer, Waters 2767 Sample Manager) using SunFire™ C18 column (4.6 x 50 mm, 5 µm particle size): solvent gradient = 95% A at 0 min, 1% A at 5 min; solvent A = 0.035% TFA in Water; solvent B = 0.035% TFA in MeOH; flow rate : 1.5 mL/min. Purification of reaction products was carried out by flash chromatography using CombiFlash®Rf with Teledyne Isco Redi*Sep*®Rf High Performance Gold or Silicycle Silia*Sep*™ High Performance columns (4 g, 12 g, 24 g, 40 g or 80 g). The purity of all compounds was over 95% and was analyzed with Waters LCMS system. ^{1}H NMR spectra were obtained using a 600 MHz Varian Inova-600 spectrometer or 500 MHz Bruker Advance III spectrometer or 400 MHz Varian Inova-400 spectrometer. Chemical shifts are reported relative to dimethyl sulfoxide (δ = 2.50 ppm) for ^{1}H NMR. Data are reported as (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets).

Scheme 1A.



Reagent and conditions: (a) 4-methyl-3-nitroaniline, dioxane, 100 ^{o}C; (b) $NaBH_4$, EtOH, 0 ^{o}C - rt; (c) MnO_2, DCM, rt; (d) triethyl phosphonoacetate, K_2CO_3, EtOH, 100 ^{o}C; (e) R-B(OH)$_2$, $Pd(PPh_3)_2Cl_2$, tBuXPhos, Na_2CO_3, dioxane, H_2O, 80 ^{o}C; (f) $SnCl_2$, EtOAc, 70 ^{o}C; (g) acryloyl chloride, THF/$NaHCO_3$ 1:1, 0 ^{o}C. (h) 2,4-dimethoxybenzylamine, $Na(OAc)_3BH$, AcOH, THF, rt; (i) triphosgene, DIEA, DCM, 0 ^{o}C; (j) TFA, DCM, rt; (k) NaH, MeI, THF, 0 ^{o}C.

Typical procedure from intermediate **S1** to **S2**

Ethyl 4,6-dichloroquinoline-3-carboxylate (**S1**) (8.10 g, 30 mmol) and 4-methyl-3-nitroaniline (4.56 g, 30 mmol) was mixed with 1,4-dioxane (100 mL) and stirred at 100 ^{o}C overnight. The mixture was cooled and concentrated. The residue was extracted with EtOAc and sat. $NaHCO_3$. The organic layer was washed with H_2O, and brine, dried (Na_2SO_4), and concentrated to give crude ethyl 6-chloro-4-((4-methyl-3-nitrophenyl)amino)quinoline-3-carboxylate as a yellow solid which was used directly for the next step. MS (*m*/*z*): 386.2 [M+1].

To a mixture of crude ethyl 6-chloro-4-((4-methyl-3-nitrophenyl)amino)quinoline-3-carboxylate in EtOH (100 mL) was added $NaBH_4$ (11.3 g, 300 mmol) slowly at 0 ^{o}C. The resulting solution was stirred at rt overnight. Solvent was removed and the residue was extracted with EtOAc and H_2O. The organic layer was washed with H_2O, brine, dried and concentrated to give crude (6-chloro-4-((4-methyl-3-nitrophenyl)amino)quinolin-3-yl)methanol as a yellow solid which was used directly for the next step. MS (*m*/*z*): 344.2 [M+1].

To a mixture of crude (6-chloro-4-((4-methyl-3-nitrophenyl)amino)quinolin-3-yl)methanol in DCM (80 mL) was added MnO_2 (40 g) and stirred at rt overnight. The resulting mixture was filtered through Celite and washed with DCM. The filtrate was concentrated to give crude 6-chloro-4-((4-methyl-3-nitrophenyl)amino)quinoline-3-carbaldehyde as a yellow solid which was used directly for the next step. MS (*m*/*z*): 342.2 [M+1].

The crude 6-chloro-4-((4-methyl-3-nitrophenyl)amino)quinoline-3-carbaldehyde was dissolved in EtOH (100 mL) and added triethyl phosphonoacetate (13.44 g, 60 mmol), followed by K_2CO_3 (12.4 g, 90 mmol). The mixture was stirred at 100 ^{o}C overnight. Solvent was removed and the residue was extracted with EtOAc and H_2O. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), and concentrated. The crude was purified by column chromatography on silica gel (0-50% EtOAc in hexane) to give intermediate **S2** as a yellow solid (5.1 g, 46% over four steps). MS (*m*/*z*): 366.8 [M+1].

General procedure from intermediate **S2** to compound **2-13**

To a solution of 9-chloro-1-(4-methyl-3-nitrophenyl)benzo[*h*][1,6]naphthyridin-2(1*H*)-one (**S2**) (50 mg, 0.137 mmol) in 1,4-dioxane (2 mL) was added R-$B(OH)_2$ (0.21 mmol), tBuXPhos (5.8 mg, 0.014 mmol), and sat. Na_2CO_3 (0.5 mL). The resulting mixture was degassed, followed by the addition of $Pd(PPh_3)_2Cl_2$ (9.8 mg, 0.014 mmol). The reaction was sealed and heated at 80 ^oC overnight. The mixture was cooled, filtered, and concentrated. The residue was purified by column chromatography on silica gel (0-10% MeOH in DCM) to give Suzuki product as a yellow solid (50-85% yield).

The Suzuki product isolated from the previous step was dissolved in EtOAc (2 mL) and $SnCl_2{\cdot}2H_2O$ (5 eq.) was added. The mixture was stirred at 70 ^oC overnight. The mixture was poured into sat. $NaHCO_3$ and extracted with $CHCl_3$/iPrOH (4:1) twice. The combined organic layers were washed with H_2O and brine, dried (Na_2SO_4), and concentrated to give crude aniline, which was used for the next step without further purification.

The resulting aniline was dissolved in THF (1 mL) and sat. $NaHCO_3$ (1 mL) was added. The mixture was cooled to 0 ^oC, and acryloyl chloride (3-5 eq.) was added dropwise while monitoring with LCMS. After completion of the reaction, the mixture was extracted with $CHCl_3$/iPrOH (4:1), washed with H_2O and brine, dried (Na_2SO_4), and concentrated. The residue was purified by reverse phase HPLC (0-100% MeOH in H_2O) to give compound **2-13** as an off-white or yellow solid (35-56% yield over two steps).

Typical procedure from intermediate **S3** to **14**

To a solution of *tert*-Butyl 6-((6-chloro-3-formylquinolin-4-yl)amino)indoline-1-carboxylate (**S3**) (137 mg, 0.323 mmol) in THF (3 mL) was added 2,4-dimethoxybenzylamine (108 mg, 0.097 mL, 0.646 mmol), followed by AcOH (5 drops). $Na(OAc)_3BH$ (342 mg, 1.62 mmol) was added and the reaction was stirred at rt overnight. The resulting mixture was diluted with $CHCl_3$/iPrOH (4:1) and washed with sat. $NaHCO_3$ twice, and brine. The organic layer was dried (Na_2SO_4), concentrated, and purified by reverse phase HPLC (0-100% MeOH in H_2O) to give reductive amination product as a TFA salt (194 mg, 40%). MS (*m*/*z*): 574.3 [M+1].

The resulting product obtained from the previous step (194 mg, 0.24 mmol) and DIEA (309 mg, 0.42 mL, 2.4 mmol) was dissolved in DCM (3 mL) and cooled to 0 ^oC. Triphosgene (72 mg, 0.24 mmol) was added and stirred at 0 ^oC for 1 h. The mixture was diluted with DCM and washed with sat. $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), concentrated, and purified by

flash column chromatography on silica gel (0-5% MeOH in DCM) to give intermediate **S4** as a yellow solid (120 mg, 83%). MS (*m*/*z*): 601.2 [M+1].

To a solution of *tert*-butyl 6-(9-chloro-2-oxo-3,4-dihydropyrimido[5,4-*c*]quinolin-1(2*H*)-yl)indoline-1-carboxylate (**S4**) (30 mg, 0.05 mmol) in 1,4-dioxane (1 mL) was added (1-methyl-1*H*-pyrazol-4-yl)boronic acid (12.6 mg, 0.10 mmol), tBuXPhos (3.2 mg, 0.0075 mmol), and sat. Na_2CO_3 (0.25 mL). The resulting mixture was degassed, followed by the addition of $Pd(PPh_3)_2Cl_2$ (3.5 mg, 0.005 mmol). The reaction was sealed and heated at 80 ^oC overnight. The mixture was cooled, filtered, and concentrated. The residue was purified by column chromatography on silica gel (0-10% MeOH in DCM) to give Suzuki product as a yellow solid (20 mg, 62%). MS (*m*/*z*): 647.4 [M+1].

To a solution of the Suzuki product obtained from the previous step (20 mg, 0.031 mmol) in DCM (1 mL) was added TFA (1mL) and stirred at rt overnight. The reaction was concentrated and dissolved in THF (1 mL), followed by the addition of sat. $NaHCO_3$ (1 mL). The mixture was cooled to 0 ^oC, and acryloyl chloride was added dropwise while monitoring with LCMS. After completion of the reaction, the mixture was extracted with $CHCl_3$/iPrOH (4:1), washed with H_2O and brine, dried (Na_2SO_4), and concentrated. The residue was purified by reverse phase HPLC (0-100% MeOH in H_2O) to give compound **14** as a yellow solid (5.6 mg, 41% over two steps).

Typical procedure from intermediate **S5** to **15-16**

tert-Butyl 6-((6-bromo-3-nitroquinolin-4-yl)amino)indoline-1-carboxylate (**S5**) (500 mg, 1.03 mmol) was dissolved in EtOAc (10 mL) and $SnCl_2{\cdot}2H_2O$ (1.16 g, 5.15 mmol) was added. The mixture was stirred at 70 ^oC for 5 h. The mixture was poured into sat. $NaHCO_3$ and extracted with $CHCl_3$/iPrOH (4:1) twice. The combined organic layers were washed with H_2O and brine, dried (Na_2SO_4), and concentrated to give crude aniline, which was used for the next step without further purification. MS (*m*/*z*): 455.2 [M+1].

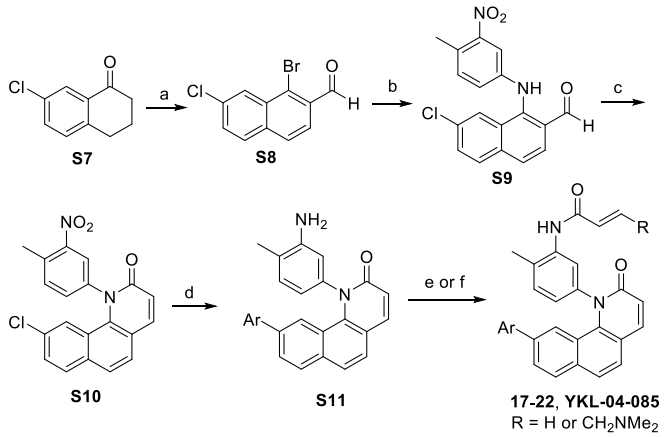
The resulting product obtained from the previous step and TEA (1.04 g, 1.43 mL, 10.3 mmol) was dissolved in DCM (20 mL) and cooled to 0 ^oC. Triphosgene (306 mg, 1.03 mmol) was added and stirred at 0 ^oC for 1 h. The mixture was diluted with DCM and washed with sat. $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), concentrated, and purified by flash column chromatography on silica gel (0-5% MeOH in DCM) to give cyclized product as a yellow solid (372 mg, 75% over two steps). MS (*m*/*z*): 481.1 [M+1].

The resulting product isolated from the previous step (100 mg, 0.21 mmol) was dissolved in DMF (2 mL) and cooled to 0 ^{o}C. NaH (60% on mineral oil, 16.6 mg, 0.42 mmol) was added slowly and stirred at 0 ^{o}C for 30 min. Methyl iodide (45 mg, 0.02 mL, 0.315 mmol) was added dropwise and stirred at 0 ^{o}C for 1 h. The mixture was quenched with MeOH, and extracted with EtOAc, washed with sat. $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), concentrated, and purified by flash column chromatography on silica gel (0-5% MeOH in DCM) to give intermediate **S6** as a yellow solid (82 mg, 79%). MS (*m*/*z*): 495.2 [M+1].

To a solution of *tert*-butyl 6-(8-bromo-2-oxo-2,3-dihydro-1*H*-imidazo[4,5-*c*]quinolin-1-yl)indoline-1-carboxylate (**S6**) (16 mg, 0.036 mmol) in 1,4-dioxane (1 mL) was added (1-methyl-1*H*-pyrazol-4-yl)boronic acid (8.9 mg, 0.071 mmol), tBuXPhos (1.5 mg, 0.0036 mmol), and sat. Na_2CO_3 (0.25 mL). The resulting mixture was degassed, followed by the addition of $Pd(PPh_3)_2Cl_2$ (2.5 mg, 0.0036 mmol). The reaction was sealed and heated at 80 ^{o}C overnight. The mixture was cooled, filtered, and concentrated. The residue was purified by column chromatography on silica gel (0-10% MeOH in DCM) to give Suzuki product as a yellow solid (14.3 mg, 80%). MS (*m*/*z*): 497.3 [M+1].

To a solution of the Suzuki product obtained from the previous step (14.3 mg, 0.029 mmol) in DCM (1 mL) was added TFA (1mL) and stirred at rt overnight. The reaction was concentrated and dissolved in THF (1 mL), followed by the addition of sat. $NaHCO_3$ (1 mL). The mixture was cooled to 0 ^{o}C, and acryloyl chloride was added dropwise while monitoring with LCMS. After completion of the reaction, the mixture was extracted with $CHCl_3$/iPrOH (4:1), washed with H_2O and brine, dried (Na_2SO_4), and concentrated. The residue was purified by reverse phase HPLC (0-100% MeOH in H_2O) to give compound **15** as a yellow solid (10.9 mg, 83% over two steps).

Scheme 1B.



Reagent and conditions: (a) i. PBr_3, DMF, $CHCl_3$, 70 °C; ii. DDQ, toluene, 110 °C; (b) 4-methyl-3-nitroaniline, $Pd_2(dba)_3$, BINAP, Cs_2CO_3, toluene, 80 °C; (c) triethyl phosphonoacetate, K_2CO_3, EtOH, 100 °C; (d) i. Ar-$B(OH)_2$, $Pd(PPh_3)_2Cl_2$, tBuXPhos, Na_2CO_3, dioxane, H_2O, 80 °C; ii. $SnCl_2$, EtOAc, 70 °C; (e) acryloyl chloride, THF/$NaHCO_3$ 1:1, 0 °C; (f) i. 4-bromocrotonyl chloride, DIEA, acetonitrile, 0 °C; ii. $NHMe_2$, NMP, 50 °C.

Typical procedure from **S7** to **17-22**, and **YKL-04-085**

To a solution of PBr_3 (5.59 g, 1.95 mL, 20.7 mmol) in $CHCl_3$ (40 mL) was added DMF (1.77 g, 1.87 mL, 24.2 mmol) dropwise at 0 °C and stirred at this temperature for 2 h. A solution of 7-chlorotetralone (**S7**) (1.46 g, 8.07 mmol) in $CHCl_3$ (15 mL) was added at 0 °C and heated at 70 °C for 2 h. The solution was cooled and diluted with DCM, washed with sat. $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4), and concentrated to give aldehyde as a yellow oil (1.74 g, 80%), which was dissolved directly in toluene (45 mL). DDQ (4.03 g, 17.8 mmol) was added and the mixture was refluxed for 3 d. The mixture was cooled and filtered through Celite and washed with DCM. The filtrate was concentrated and purified by flash column chromatography on silica gel (0-30% EtOAc in hexane) to provide intermediate **S8** as a yellow solid (680 mg, 31% over two steps). MS (*m*/*z*): 269.0 [M+1].

To a solution of 1-bromo-7-chloro-2-naphthaldehyde (**S8**) (300 mg, 1.1 mmol) in toluene (15 mL) was added 4-methyl-3-nitroaniline (168 mg, 1.1 mmol), BINAP (126 mg, 0.20 mmol), and Cs_2CO_3 (360 mg, 1.1 mmol). The resulting mixture was degassed, followed by the addition of $Pd_2(dba)_3$ (60 mg, 0.066 mmol). The reaction was sealed and heated at 80 °C for 3 h. The mixture was cooled, filtered, and concentrated. The residue was purified by column chromatography on silica gel (0-50% EtOAc in hexane) to give intermediate **S9** as a yellow solid (201 mg, 51%). MS (*m*/*z*): 341.1 [M+1].

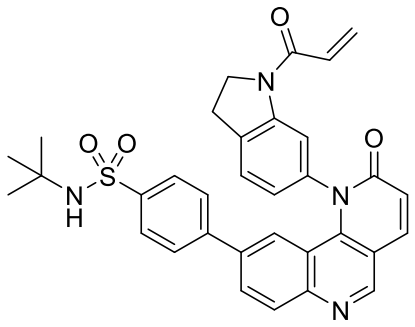
7-Chloro-1-((4-methyl-3-nitrophenyl)amino)-2-naphthaldehyde (190 mg, 0.56 mmol) was dissolved in EtOH (15 mL) and added triethyl phosphonoacetate (250 mg, 1.12 mmol), followed by K_2CO_3 (232 mg, 1.68 mmol). The mixture was stirred at 100 ^oC overnight. Solvent was removed and the residue was extracted with EtOAc and H_2O. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), and concentrated. The crude was purified by column chromatography on silica gel (0-50% EtOAc in hexane) to give intermediate **S10** as a brownish solid (107 mg, 50%). MS (*m*/*z*): 365.1 [M+1].

To a solution of 9-chloro-1-(4-methyl-3-nitrophenyl)benzo[*h*]quinolin-2(1*H*)-one (**S10**) (30 mg, 0.082 mmol) in 1,4-dioxane (1.2 mL) was added (1-methyl-1*H*-pyrazol-4-yl)boronic acid (31 mg, 0.247 mmol), tBuXPhos (7.0 mg, 0.016 mmol), and sat. Na_2CO_3 (0.3 mL). The resulting mixture was degassed, followed by the addition of $Pd(PPh_3)_2Cl_2$ (8.6 mg, 0.0123 mmol). The reaction was sealed and heated at 80 ^oC overnight. The mixture was cooled, filtered, and concentrated to give crude Suzuki product as a yellow solid. MS (*m*/*z*): 411.2 [M+1].

To a solution of the crude Suzuki product obtained from the previous step in EtOAc (2 mL) was added $SnCl_2{\cdot}2H_2O$ (93 mg, 0.41 mmol). The mixture was stirred at 70 ^oC for overnight. The mixture was poured into sat. $NaHCO_3$ and extracted with $CHCl_3$/iPrOH (4:1) twice. The combined organic layers were washed with H_2O and brine, dried (Na_2SO_4), and concentrated to give crude aniline, which was used for the next step without further purification.

The resulting aniline was dissolved in CH_3CN (1 mL) and DIEA (21 mg, 0.03 mL, 0.164 mmol) was added. The mixture was cooled to 0 ^oC, and 4-bromocrotonyl chloride (3-5 eq.) was added dropwise while monitoring with LCMS. After completion of the reaction, the mixture was extracted with $CHCl_3$/iPrOH (4:1), washed with H_2O and brine, dried (Na_2SO_4), and concentrated. The residue was dissolved in NMP (1 mL) and dimethylamine solution (2 M in THF, 0.2 mL, 0.41 mmol) was added. The solution was heated at 50 ^oC for 1 h. The reaction was directly purified by reverse phase HPLC (0-100% MeOH in H_2O) to give compound **YKL-04-085** as a yellow solid (13.2 mg, 27% over four steps).

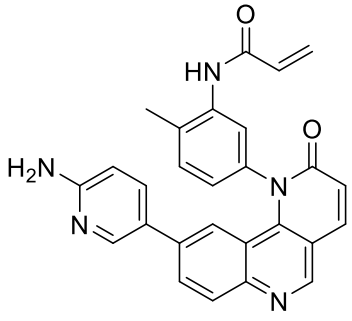
Compound characterization



1

4-(1-(1-acryloylindolin-6-yl)-2-oxo-1,2-dihydrobenzo[*h*][1,6]naphthyridin-9-yl)-*N*-(*tert*-butyl)benzenesulfonamide (1)

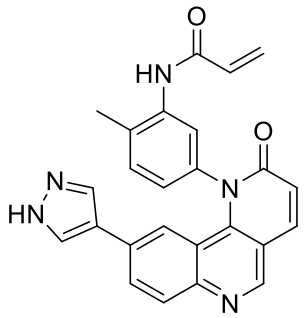
Compound **1** was prepared according to the reported procedure.[3] ^{1}H NMR (DMSO-*d6*, 500 MHz): δ 9.16 (s, 1H), 8.32 (d, *J* = 9.5 Hz, 1H), 8.17 (s, 1H), 8.13 (d, *J* = 8.5 Hz, 1H), 8.05 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.66 (s, 1H), 7.57 (d, *J* = 7.0 Hz, 1H), 7.34 (d, *J* = 8.5 Hz, 2H), 7.31 (d, *J* = 1.5 Hz, 1H), 7.14 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.93 (d, *J* = 9.5 Hz, 1H), 6.75 (dd, *J* = 16.5, 10.5 Hz, 1H), 6.23 (dd, *J* = 16.5, 1.5 Hz, 1H), 5.82 (d, *J* = 11.5 Hz, 1H), 4.47 – 4.40 (m, 1H), 4.32 – 4.26 (m, 1H), 3.39 – 3.34 (m, 1H), 3.33 – 3.27 (m, 1H), 1.10 (s, 9H). MS (*m*/*z*): 579.2 [M+1].



2

***N*-(5-(9-(6-aminopyridin-3-yl)-2-oxobenzo[*h*][1,6]naphthyridin-1(2*H*)-yl)-2-methylphenyl)acrylamide (2)**

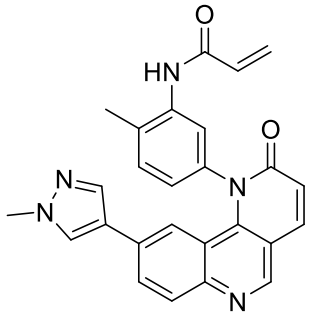
^{1}H NMR (DMSO-*d6*, 500 MHz): δ 9.85 (s, 1H), 9.10 (s, 1H), 8.25 (d, *J* = 9.5 Hz, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.98 (d, *J* = 2.0 Hz, 1H), 7.95 (d, *J* = 2.0 Hz, 1H), 7.93 (d, *J* = 2.0 Hz, 1H), 7.53 – 7.51 (m, 2H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.17 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.04 (d, *J* = 2.0 Hz, 1H), 6.91 (d, *J* = 9.0 Hz, 1H), 6.87 (d, *J* = 9.0 Hz, 1H), 6.46 (dd, *J* = 17.0, 10.5 Hz, 1H), 6.16 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.70 (d, *J* = 10.5 Hz, 1H), 2.33 (s, 3H). MS (*m*/*z*): 448.3 [M+1].



3

***N*-(2-methyl-5-(2-oxo-9-(1*H*-pyrazol-4-yl)benzo[*h*][1,6]naphthyridin-1(2*H*)-yl)phenyl)acrylamide (3)**

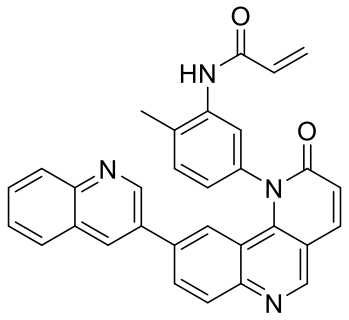
^{1}H NMR (DMSO-*d6*, 400 MHz): δ 12.92 (br, 1H), 9.73 (s, 1H), 8.98 (s, 1H), 8.21 (d, *J* = 9.8 Hz, 1H), 7.94 – 7.87 (m, 2H), 7.69 (m, 1H), 7.62 (s, 1H), 7.48 (d, *J* = 8.2 Hz, 1H), 7.32 (m, 1H), 7.13 (m, 1H), 7.01 (s, 1H), 6.83 (d, *J* = 9.4 Hz, 1H), 6.51 (m, 1H), 6.13 (d, *J* = 17.2 Hz, 1H), 5.68 (d, *J* = 10.2 Hz, 1H), 2.38 (s, 3H). MS (*m*/*z*): 422.3 [M+1].



4

***N*-(2-methyl-5-(9-(1-methyl-1*H*-pyrazol-4-yl)-2-oxobenzo[*h*][1,6]naphthyridin-1(2*H*)-yl)phenyl)acrylamide (4)**

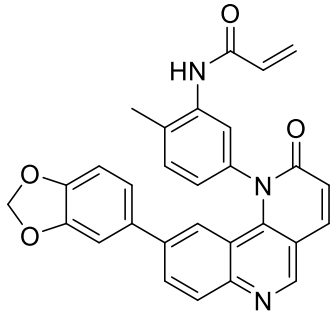
^{1}H NMR (DMSO-*d6*, 400 MHz): δ 9.85 (s, 1H), 9.01 (s, 1H), 8.25 (d, *J* = 9.4 Hz, 1H), 7.97 (d, *J* = 8.6 Hz, 1H), 7.87 (dd, *J* = 8.6, 1.6 Hz, 1H), 7.67 (s, 1H), 7.65 (s, 1H), 7.51 (d, *J* = 8.2 Hz, 1H), 7.34 (s, 1H), 7.13 (dd, *J* = 8.2, 2.4 Hz, 1H), 7.04 (d, *J* = 2.0 Hz, 1H), 6.87 (d, *J* = 9.8 Hz, 1H), 6.54 (dd, *J* = 16.8, 10.2 Hz, 1H), 6.20 (dd, *J* = 17.2, 2.0 Hz, 1H), 5.74 (d, *J* = 10.6 Hz, 1H), 3.81 (s, 3H), 2.41 (s, 3H). MS (*m*/*z*): 435.2 [M+1].



5

***N*-(2-methyl-5-(2-oxo-9-(quinolin-3-yl)benzo[*h*][1,6]naphthyridin-1(2*H*)-yl)phenyl)acrylamide (5)**

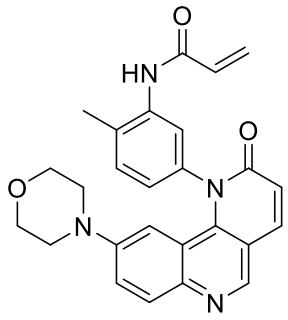
^{1}H NMR (DMSO-*d6*, 500 MHz): δ 9.71 (s, 1H), 9.16 (s, 1H), 8.78 (d, *J* = 2.0 Hz, 1H), 8.28 (d, *J* = 9.5 Hz, 1H), 8.19 – 8.13 (m, 3H), 7.99 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 7.5 Hz, 1H), 7.73 (m, 1H), 7.67 (s, 1H), 7.61 (m, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.33 (d, *J* = 1.5 Hz, 1H), 7.21 (dd, *J* = 8.0, 2.0 Hz, 1H), 6.89 (d, *J* = 9.5 Hz, 1H), 6.52 (dd, *J* = 16.8, 10.4 Hz, 1H), 6.12 (dd, *J* = 16.8, 1.5 Hz, 1H), 5.68 (d, *J* = 10.4 Hz, 1H), 2.28 (s, 3H). MS (*m*/*z*): 483.2 [M+1].



6

***N*-(5-(9-(benzo[*d*][1,3]dioxol-5-yl)-2-oxobenzo[*h*][1,6]naphthyridin-1(2*H*)-yl)-2-methylphenyl)acrylamide (6)**

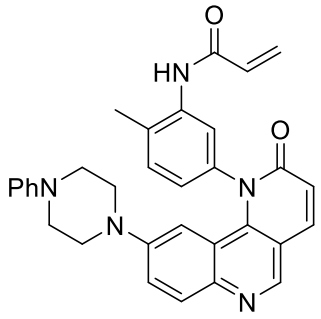
^{1}H NMR (DMSO-*d6*, 500 MHz): δ 9.69 (s, 1H), 9.11 (s, 1H), 8.30 (d, *J* = 9.5 Hz, 1H), 8.05 (d, *J* = 9.0Hz, 1H), 7.96 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.71 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 7.23 – 7.20 (m, 2H), 6.92 – 6.90 (m, 2H), 6.76 – 6.73 (m, 2H), 6.58 (dd, *J* = 17.0, 10.0 Hz, 1H), 6.19 (dd, *J* = 17.0, 1.5 Hz, 1H), 5.76 (s, 2H), 5.74 (d, *J* = 10.0 Hz, 1H), 2.44 (s, 3H). MS (*m*/*z*): 476.2 [M+1].



7

***N*-(2-methyl-5-(9-morpholino-2-oxobenzo[*h*][1,6]naphthyridin-1(2*H*)-yl)phenyl)acrylamide (7)**

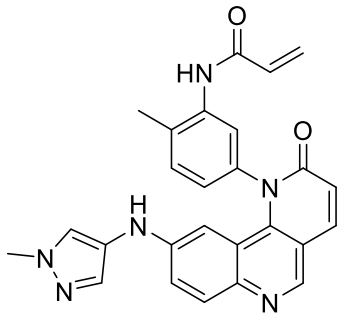
^{1}H NMR (500 MHz, DMSO-d_6): δ 9.64 (s, 1H), 8.82 (s, 1H), 8.15 (d, *J* = 9.4 Hz, 1H), 7.79 (d, *J* = 9.2 Hz, 1H), 7.57 (s, 1H), 7.43 (dd, *J* = 9.2, 2.6 Hz, 1H), 7.41 (d, *J* = 8.1 Hz, 1H), 7.06 (dd, *J* = 8.1, 2.3 Hz, 1H), 6.77 (d, *J* = 9.4 Hz, 1H), 6.49 (dd, *J* = 17.0, 10.3 Hz, 1H), 6.43 (d, *J* = 2.6 Hz, 1H), 6.16 (dd, *J* = 17.0, 2.0 Hz, 1H), 5.69 (dd, *J* = 10.2, 2.0 Hz, 1H), 3.53 (ddd, *J* = 5.6, 4.1, 1.4 Hz, 4H), 2.74 – 2.65 (m, 2H), 2.65 – 2.58 (m, 2H), 2.26 (s, 3H). MS (*m*/*z*): 441.2 [M+1].



8

***N*-(2-methyl-5-(2-oxo-9-(4-phenylpiperazin-1-yl)benzo[*h*][1,6]naphthyridin-1(2*H*)-yl)phenyl)acrylamide (8)**

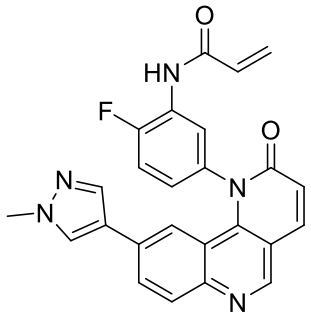
^{1}H NMR (500 MHz, DMSO-d_6): δ 9.77 (s, 1H), 8.87 (s, 1H), 8.22 (d, J = 9.4 Hz, 1H), 7.88 (d, J = 9.2 Hz, 1H), 7.68 (s, 1H), 7.55 (dd, J = 9.2, 2.6 Hz, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.24 (dd, J = 8.7, 7.3 Hz, 2H), 7.12 (dd, J = 8.1, 2.3 Hz, 1H), 6.97 – 6.90 (m, 2H), 6.86 – 6.79 (m, 2H), 6.66 – 6.55 (m, 1H), 6.54 (d, J = 2.7 Hz, 1H), 6.23 (dd, J = 17.0, 1.9 Hz, 1H), 5.81 – 5.73 (m, 1H), 3.17 – 3.02 (m, 4H), 2.92 (qd, J = 13.3, 12.6, 5.6 Hz, 4H), 2.38 (s, 3H). MS (*m*/*z*): 516.3 [M+1].



9

***N*-(2-methyl-5-(9-((1-methyl-1H-pyrazol-4-yl)amino)-2-oxobenzo[*h*][1,6]naphthyridin-1(2*H*)-yl)phenyl)acrylamide (9)**

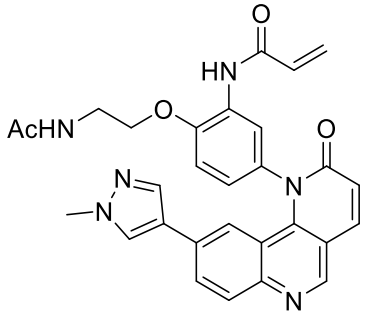
^{1}H NMR (500 MHz, DMSO-d_6): δ 9.39 (s, 1H), 8.98 (s, 1H), 8.22 (d, J = 9.5 Hz, 1H), 7.85 (d, J = 9.0 Hz, 1H), 7.78 (s, 1H), 7.42 (s, 1H), 7.32 (s, 1H), 7.30 (d, J = 2.4 Hz, 1H), 7.20 (d, J = 8.1 Hz, 1H), 7.12 (d, J = 8.0 Hz, 1H), 7.01 (s, 1H), 6.88 (d, J = 9.5 Hz, 1H), 6.63 – 6.52 (m, 1H), 6.34 (d, J = 2.3 Hz, 1H), 6.17 (dd, J = 16.8, 2.0 Hz, 1H), 5.72 (d, J = 10.4 Hz, 1H), 3.85 (s, 3H), 2.55 (s, 3H). MS (*m*/*z*): 451.2 [M+1].



10

***N*-(2-fluoro-5-(9-(1-methyl-1*H*-pyrazol-4-yl)-2-oxobenzo[*h*][1,6]naphthyridin-1(2*H*)-yl)phenyl)acrylamide (10)**

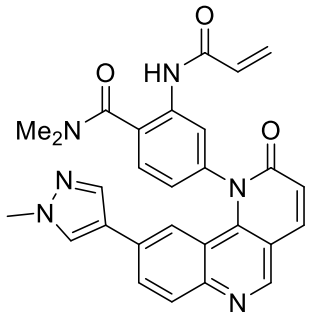
^{1}H NMR (500 MHz, DMSO-d_6): δ 10.37 (s, 1H), 9.12 (s, 1H), 8.30 (d, J = 9.5 Hz, 1H), 8.15 (dd, J = 6.9, 2.7 Hz, 1H), 8.03 (d, J = 8.6 Hz, 1H), 7.94 (dd, J = 8.7, 1.9 Hz, 1H), 7.85 (s, 1H), 7.64 (dd, J = 10.6, 8.7 Hz, 1H), 7.32 (s, 1H), 7.32 – 7.29 (m, 1H), 7.12 (d, J = 1.9 Hz, 1H), 6.94 (d, J = 9.4 Hz, 1H), 6.64 (dd, J = 17.0, 10.3 Hz, 1H), 6.25 (dd, J = 17.0, 1.9 Hz, 1H), 5.80 (dd, J = 10.2, 1.9 Hz, 1H), 3.85 (s, 3H). MS (*m*/*z*): 440.2 [M+1].



11

***N*-(2-(2-acetamidoethoxy)-5-(9-(1-methyl-1*H*-pyrazol-4-yl)-2-oxobenzo[*h*][1,6]naphthyridin-1(2*H*)-yl)phenyl)acrylamide (11)**

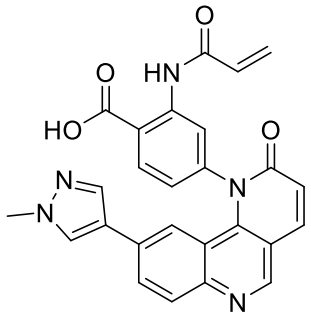
^{1}H NMR (500 MHz, DMSO-d_6): δ 9.36 (s, 1H), 9.00 (s, 1H), 8.25 – 8.15 (m, 3H), 7.93 (d, J = 8.5 Hz, 1H), 7.83 (dd, J = 8.6, 1.8 Hz, 1H), 7.66 (s, 1H), 7.29 (d, J = 8.7 Hz, 1H), 7.21 (d, J = 0.8 Hz, 1H), 7.11 (dd, J = 8.6, 2.6 Hz, 1H), 7.06 (d, J = 1.9 Hz, 1H), 6.84 (d, J = 9.4 Hz, 1H), 6.71 (dd, J = 17.0, 10.2 Hz, 1H), 6.14 (dd, J = 17.0, 1.9 Hz, 1H), 5.71 (dd, J = 10.2, 1.8 Hz, 1H), 4.13 (t, J = 5.4 Hz, 2H), 3.76 (s, 3H), 3.56 – 3.49 (m, 2H), 1.85 (s, 3H). MS (*m*/*z*): 523.2 [M+1].



12

2-acrylamido-*N*,*N*-dimethyl-4-(9-(1-methyl-1*H*-pyrazol-4-yl)-2-oxobenzo[*h*][1,6]naphthyridin-1(2*H*)-yl)benzamide (12)

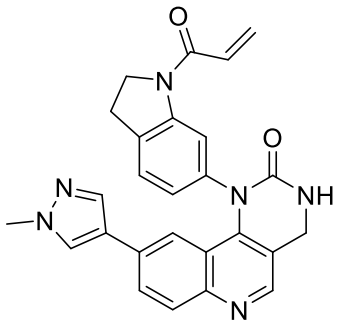
^{1}H NMR (500 MHz, DMSO-d_6): δ 10.05 (s, 1H), 8.99 (s, 1H), 8.22 (d, J = 9.5 Hz, 1H), 7.94 (d, J = 8.6 Hz, 1H), 7.85 (dd, J = 8.6, 1.8 Hz, 1H), 7.68 (d, J = 2.1 Hz, 1H), 7.60 (d, J = 0.9 Hz, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.48 (s, 1H), 7.21 (dd, J = 8.1, 2.0 Hz, 1H), 7.13 (d, J = 1.8 Hz, 1H), 6.84 (d, J = 9.4 Hz, 1H), 6.43 (dd, J = 17.0, 10.2 Hz, 1H), 6.16 (dd, J = 17.0, 1.9 Hz, 1H), 5.69 (dd, J = 10.3, 1.9 Hz, 1H), 3.80 (s, 3H), 2.94 (s, 3H), 2.81 (s, 3H). MS (*m*/*z*): 493.2 [M+1].



13

2-acrylamido-4-(9-(1-methyl-1*H*-pyrazol-4-yl)-2-oxobenzo[*h*][1,6]naphthyridin-1(2*H*)-yl)benzoic acid (13)

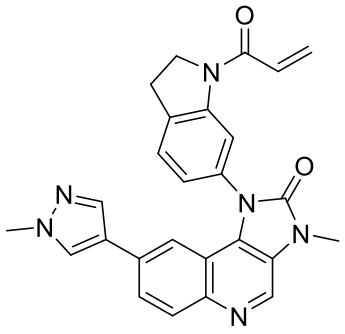
^{1}H NMR (600 MHz, DMSO-d_6): δ 9.03 (s, 1H), 8.56 (s, 1H), 8.27 (d, J = 9.4 Hz, 1H), 7.97 (d, J = 8.5 Hz, 1H), 7.88 (d, J = 8.5 Hz, 1H), 7.47 – 7.32 (m, 2H), 7.24 – 7.06 (m, 4H), 7.01 (s, 1H), 6.90 (d, J = 9.4 Hz, 1H), 6.34 – 6.19 (m, 1H), 6.20 – 6.08 (m, 1H), 5.79 – 5.68 (m, 1H), 3.80 (s, 3H). MS (*m*/*z*): 466.2 [M+1].



14

1-(1-acryloylindolin-6-yl)-9-(1-methyl-1*H*-pyrazol-4-yl)-3,4-dihydropyrimido[5,4-c]quinolin-2(1*H*)-one (14)

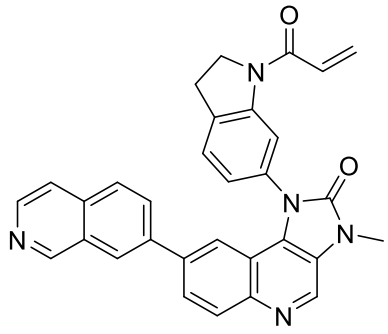
^{1}H NMR (500 MHz, DMSO-d_6): δ 8.87 (s, 1H), 8.25 (s, 1H), 8.08 (d, J = 8.8 Hz, 1H), 8.04 (s, 1H), 7.97 (d, J = 8.6 Hz, 1H), 7.80 (s, 1H), 7.45 (d, J = 7.9 Hz, 1H), 7.24 (s, 2H), 7.21 (d, J = 8.1 Hz, 1H), 6.76 (dd, J = 16.6, 10.3 Hz, 1H), 6.26 (dd, J = 16.7, 2.0 Hz, 1H), 5.83 (dd, J = 10.3, 2.1 Hz, 1H), 4.66 (s, 2H), 4.33 (t, J = 8.5 Hz, 2H), 3.83 (s, 3H), 3.28 (t, J = 8.6 Hz, 2H). MS (*m*/*z*): 451.2 [M+1].



15

1-(1-acryloylindolin-6-yl)-3-methyl-8-(1-methyl-1*H*-pyrazol-4-yl)-1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-one (15)

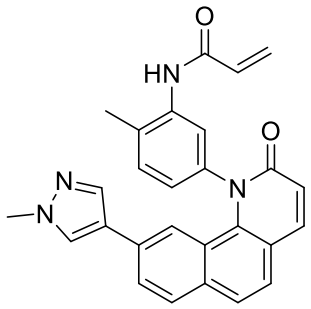
^{1}H NMR (500 MHz, DMSO-d_6): δ 9.24 (s, 1H), 8.37 (s, 1H), 8.13 (d, J = 8.9 Hz, 1H), 8.03 (dd, J = 9.0, 1.9 Hz, 1H), 7.90 (s, 1H), 7.66 (d, J = 7.9 Hz, 1H), 7.37 (dd, J = 7.8, 2.0 Hz, 1H), 7.31 (d, J = 0.8 Hz, 1H), 7.21 (d, J = 1.8 Hz, 1H), 6.79 (dd, J = 16.7, 10.3 Hz, 1H), 6.27 (dd, J = 16.6, 2.0 Hz, 1H), 5.85 (dd, J = 10.4, 2.0 Hz, 1H), 4.42 (t, J = 8.6 Hz, 2H), 3.85 (s, 3H), 3.64 (s, 3H), 3.44 – 3.37 (m, 2H). MS (*m*/*z*): 451.2 [M+1].



16

1-(1-acryloylindolin-6-yl)-8-(isoquinolin-7-yl)-3-methyl-1,3-dihydro-2*H*-imidazo[4,5-*c*]quinolin-2-one (16)

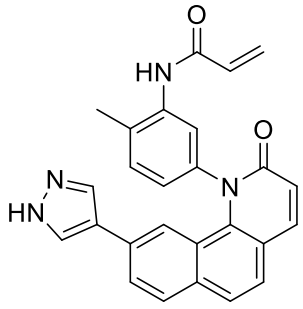
^{1}H NMR (500 MHz, DMSO-d_6): δ 9.45 (s, 1H), 9.28 (s, 1H), 8.61 (d, J = 6.0 Hz, 1H), 8.43 (s, 1H), 8.32 (s, 1H), 8.29 (s, 2H), 8.16 (d, J = 8.6 Hz, 1H), 8.08 (d, J = 5.9 Hz, 1H), 7.79 (d, J = 8.4 Hz, 1H), 7.69 (d, J = 7.8 Hz, 1H), 7.58 (s, 1H), 7.42 (dd, J = 7.9, 2.0 Hz, 1H), 6.80 (dd, J = 16.7, 10.4 Hz, 1H), 6.32 – 6.21 (m, 1H), 5.89 – 5.79 (m, 1H), 4.50 – 4.32 (m, 2H), 3.66 (s, 3H), 3.43 – 3.33 (m, 2H). MS (*m*/*z*): 498.2 [M+1].



17

***N*-(2-methyl-5-(9-(1-methyl-1*H*-pyrazol-4-yl)-2-oxobenzo[*h*]quinolin-1(2*H*)-yl)phenyl)acrylamide (17)**

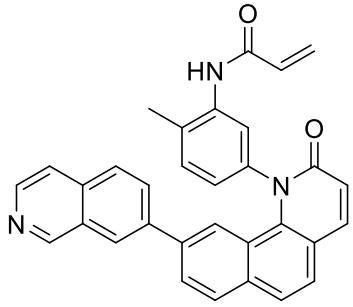
^{1}H NMR (500 MHz, DMSO-d_6): δ 9.81 (s, 1H), 8.13 (d, J = 9.4 Hz, 1H), 7.92 (d, J = 8.4 Hz, 1H), 7.78 – 7.68 (m, 3H), 7.66 (s, 2H), 7.46 (d, J = 8.1 Hz, 1H), 7.39 (d, J = 0.8 Hz, 1H), 7.34 (d, J = 1.5 Hz, 1H), 7.03 (dd, J = 8.2, 2.2 Hz, 1H), 6.80 (d, J = 9.3 Hz, 1H), 6.64 – 6.51 (m, 1H), 6.24 (dd, J = 17.0, 1.9 Hz, 1H), 5.77 (d, J = 10.3 Hz, 1H), 3.82 (s, 3H), 2.39 (s, 3H). MS (*m*/*z*): 435.2 [M+1].



18

***N*-(2-methyl-5-(2-oxo-9-(1*H*-pyrazol-4-yl)benzo[*h*]quinolin-1(2*H*)-yl)phenyl)acrylamide (18)**

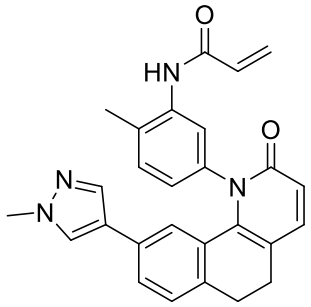
^{1}H NMR (500 MHz, DMSO-d_6): δ 12.93 (s, 1H), 9.71 (s, 1H), 8.14 (d, J = 9.4 Hz, 1H), 7.93 (d, J = 8.4 Hz, 1H), 7.79 – 7.67 (m, 3H), 7.64 (s, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.40 (s, 1H), 7.37 – 7.30 (m, 1H), 7.15 – 7.06 (m, 1H), 6.80 (d, J = 9.4 Hz, 1H), 6.58 (dd, J = 17.1, 10.1 Hz, 1H), 6.20 (dd, J = 17.1, 2.0 Hz, 1H), 5.74 (d, J = 10.2 Hz, 1H), 2.41 (s, 3H). MS (*m*/*z*): 421.2 [M+1].



19

***N*-(5-(9-(isoquinolin-7-yl)-2-oxobenzo[*h*]quinolin-1(2*H*)-yl)-2-methylphenyl)acrylamide (19)**

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.76 (s, 1H), 9.26 (s, 1H), 8.51 (d, J = 5.7 Hz, 1H), 8.19 (d, J = 9.4 Hz, 1H), 8.12 (d, J = 8.5 Hz, 1H), 8.04 – 7.97 (m, 2H), 7.96 (d, J = 8.6 Hz, 1H), 7.90 – 7.80 (m, 3H), 7.72 (d, J = 1.6 Hz, 1H), 7.69 (s, 1H), 7.61 (dd, J = 8.6, 1.9 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.16 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 9.3 Hz, 1H), 6.59 (dd, J = 17.0, 10.4 Hz, 1H), 6.20 (d, J = 17.2 Hz, 1H), 5.75 (d, J = 10.3 Hz, 1H), 2.34 (s, 3H). MS (*m*/*z*): 482.2 [M+1].

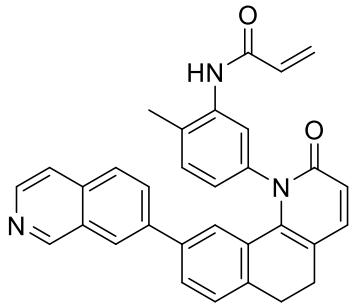


20

***N*-(2-methyl-5-(9-(1-methyl-1*H*-pyrazol-4-yl)-2-oxo-5,6-dihydrobenzo[*h*]quinolin-1(2*H*)-yl)phenyl)acrylamide (20)**

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.75 (s, 1H), 7.64 (d, J = 2.3 Hz, 1H), 7.59 (s, 1H), 7.53 (d, J = 9.2 Hz, 1H), 7.40 (s, 1H), 7.30 (dd, J = 7.7, 1.7 Hz, 1H), 7.26 – 7.19 (m, 2H), 6.90 (d, J = 1.7 Hz, 1H), 6.84 (dd, J = 8.1, 2.2 Hz, 1H), 6.60 (dd, J = 17.0, 10.2 Hz, 1H), 6.48 (d, J = 9.1 Hz, 1H),

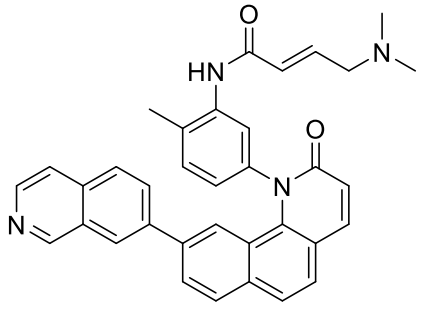
6.30 (dd, *J* = 17.1, 2.0 Hz, 1H), 5.80 (d, *J* = 10.6 Hz, 1H), 3.76 (s, 3H), 2.85 – 2.72 (m, 2H), 2.68 – 2.59 (m, 1H), 2.58 – 2.52 (m, 1H), 2.24 (s, 3H). MS (*m*/*z*): 437.2 [M+1].



21

***N*-(5-(9-(isoquinolin-7-yl)-2-oxo-5,6-dihydrobenzo[*h*]quinolin-1(2*H*)-yl)-2-methylphenyl)acrylamide (21)**

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.73 (s, 1H), 9.42 (s, 1H), 8.57 (d, *J* = 6.1 Hz, 1H), 8.13 (d, *J* = 6.1 Hz, 1H), 8.08 (d, *J* = 8.7 Hz, 1H), 7.99 (s, 1H), 7.71 – 7.64 (m, 2H), 7.63 (dd, *J* = 7.8, 1.9 Hz, 1H), 7.58 (d, *J* = 9.2 Hz, 1H), 7.47 (d, *J* = 7.8 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.24 (d, *J* = 1.9 Hz, 1H), 7.01 (d, *J* = 7.6 Hz, 1H), 6.58 (dd, *J* = 17.1, 10.3 Hz, 1H), 6.53 (d, *J* = 9.2 Hz, 1H), 6.24 (dd, *J* = 16.9, 2.0 Hz, 1H), 5.77 (d, *J* = 10.3 Hz, 1H), 2.90 (t, *J* = 7.2 Hz, 2H), 2.72 – 2.57 (m, 2H), 2.31 (s, 3H). MS (*m*/*z*): 484.2 [M+1].

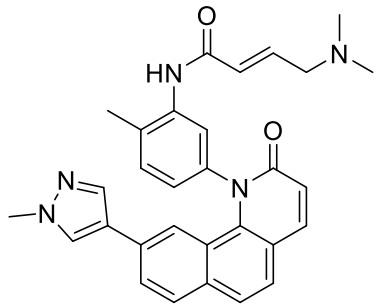


22

(*E*)-4-(dimethylamino)-*N*-(5-(9-(isoquinolin-7-yl)-2-oxobenzo[*h*]quinolin-1(2*H*)-yl)-2-methylphenyl)but-2-enamide (22)

^{1}H NMR (500 MHz, DMSO-d_6): δ 9.94 (s, 1H), 9.37 (s, 1H), 8.56 (d, *J* = 5.8 Hz, 1H), 8.20 (d, *J* = 9.4 Hz, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 8.10 (s, 1H), 8.06 – 7.95 (m, 3H), 7.91 – 7.81 (m, 2H),

7.73 (s, 1H), 7.70 – 7.62 (m, 2H), 7.51 (d, *J* = 8.1 Hz, 1H), 7.20 (dd, *J* = 8.0, 2.2 Hz, 1H), 6.85 (d, *J* = 9.3 Hz, 1H), 6.75 – 6.64 (m, 1H), 6.61 (d, *J* = 15.4 Hz, 1H), 3.92 (d, *J* = 7.0 Hz, 2H), 2.78 (s, 6H), 2.36 (s, 3H). MS (*m*/*z*): 539.3 [M+1].



YKL-04-085

(*E*)-4-(dimethylamino)-*N*-(2-methyl-5-(9-(1-methyl-1*H*-pyrazol-4-yl)-2-oxobenzo[*h*]quinolin-1(2*H*)-yl)phenyl)but-2-enamide (YKL-04-085)

^{1}H NMR (600 MHz, DMSO-d_6): δ 9.95 (s, 1H), 8.11 (d, *J* = 9.3 Hz, 1H), 7.90 (d, *J* = 8.4 Hz, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.70 – 7.66 (m, 2H), 7.65 (s, 1H), 7.62 (s, 1H), 7.46 (d, *J* = 8.0 Hz, 1H), 7.35 (s, 1H), 7.30 (s, 1H), 7.05 (d, *J* = 8.3 Hz, 1H), 6.77 (d, *J* = 9.3 Hz, 1H), 6.73 – 6.65 (m, 1H), 6.58 (d, *J* = 15.3 Hz, 1H), 3.90 (d, *J* = 7.0 Hz, 2H), 3.80 (s, 3H), 2.76 (s, 6H), 2.38 (s, 3H). MS (*m*/*z*): 492.3 [M+1].

1. Bulich, R.; Aaskov, J. G., Nuclear localization of dengue 2 virus core protein detected with monoclonal antibodies. *The Journal of general virology* **1992,** *73 (Pt 11)*, 2999-3003.
2. de Wispelaere, M.; LaCroix, A. J.; Yang, P. L., The Small Molecules AZD0530 and Dasatinib Inhibit Dengue Virus RNA Replication via Fyn Kinase. *Journal of Virology* **2013,** *87* (13), 7367-7381.
3. Wu, H.; Wang, W.; Liu, F.; Weisberg, E. L.; Tian, B.; Chen, Y.; Li, B.; Wang, A.; Wang, B.; Zhao, Z.; McMillin, D. W.; Hu, C.; Li, H.; Wang, J.; Liang, Y.; Buhrlage, S. J.; Liang, J.; Liu, J.; Yang, G.; Brown, J. R.; Treon, S. P.; Mitsiades, C. S.; Griffin, J. D.; Liu, Q.; Gray, N. S., Discovery of a Potent, Covalent BTK Inhibitor for B-Cell Lymphoma. *ACS Chemical Biology* **2014,** *9* (5), 1086-1091.