Left hand side exploration of novel bacterial topoisomerase inhibitors to improve selectivity against hERG binding

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Supporting information

EXPERIMENTAL SECTION

Figures

Figure 1a Known novel bacterial topoisomerase inhibitors **1 a-h**

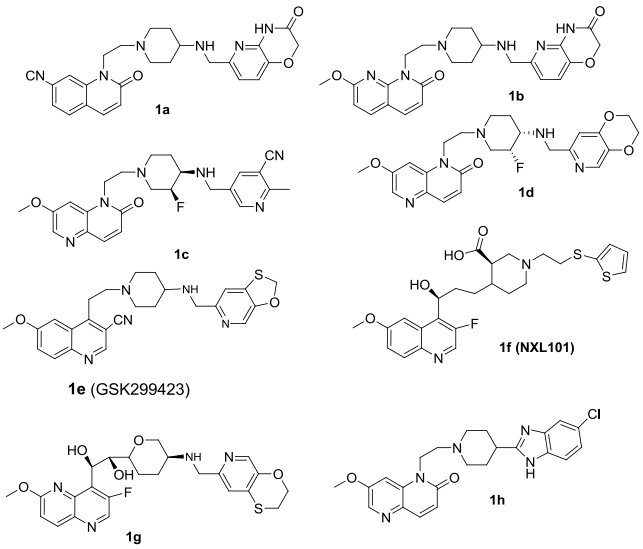


Figure 2a. Compound **19** is bactericidal against intracellular *Mtb* growing in THP-1 macrophages.

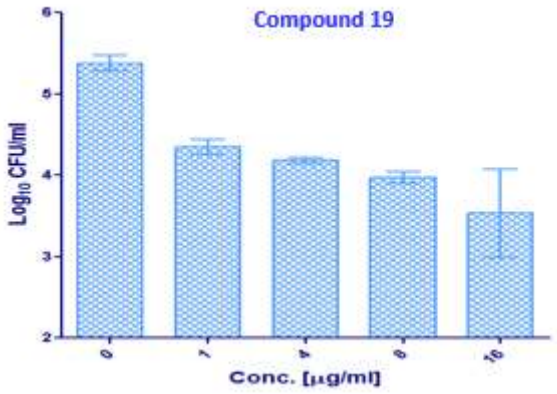
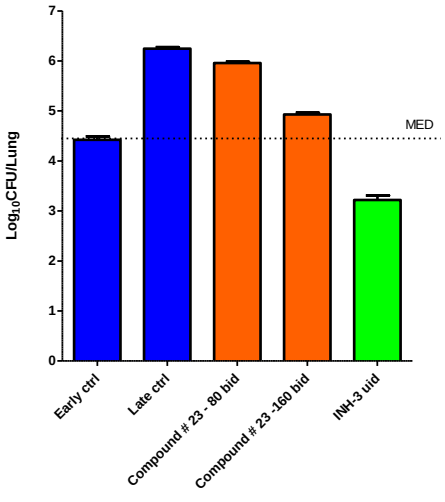


Figure 3a. Compound **19** is active against replicating *Mtb* following oral administration in an acute mouse model of TB



Tables

Table 1a. Naphthyridones: DMPK Properties

Entry	**10**	**11**	**18**	**19**
Solubility (μM)	>1000	>1000	>1000	>1000
Hu PPB (% free)	58	9	32	41
Caco-2: Papp A−B/B−A (1×10^{-6} cm/s)	1.1/6.3	1.2/11	4.5/11	4.3/8.9
	Mouse oral PK (80mg/kg) with ABT			
AUC (μg.h/mL)	ND	1.6	ND	32.6
Cmax (μM)\	ND	0.47	ND	21

Computational methods

Ligand preparation

Throughout the studies, CORINA (http://www.molecular-networks.com/products/corina)

was used for generating 3D structures from smiles and Omega 2.2.0 (OpenEye Scientific Software, Santa Fe, New Mexico 87507) was used for generating conformers. Default settings were retained for both the tools.

Homology modeling

Mycobacterium tuberculosis Gyrase subunit A (*Mtb* GyrA) was built by homology modeling using MOE 2013.12 version.[1] The published *Staphylococcus aureus* DNA gyrase Subunit A (*Sau* GyrA) crystal structure was retrieved from Protein Data Bank (PDB ID: 2XCS 2.1Å) and examined for 1) ligand interaction and followed by 2) similarity with *Mtb* GyrA.

Determining the antimycobacterial properties. *M. tuberculosis* H37Rv ATCC 27294 was used for all the studies and was grown and tested as described earlier.[2] The minimum inhibitory concentration (MIC) was determined as described earlier.[3]

Determination of IC_{50} for the supercoiling activity of *M. tuberculosis* H37Rv gyrase holoenzyme. DNA supercoiling assay was performed in 96-well PCR plates (Bio-rad) in 30 µL volume. Assay mix contained 12.5 nM *M. tuberculosis* H37Rv holoenzyme, 50 ng of relaxed pBR322 DNA, 40 mM HEPES-KOH pH 7.5, 100 mM potassium glutamate, 15 mM KCl, 1 mM spermidine, 10 mM $MgCl_2$, 4 mM DTT, 8% glycerol, 0.36 mg/mL BSA and 75 µM ATP. The assay was started with the addition of DNA and ATP mix and continued for 90 min at 37°C. The reaction was stopped by addition of 0.75 µL Proteinase K (20 mg/mL, 40 U/mg) and 3 µL of 2% SDS followed by incubation at 37 ºC for 1 hour. This was followed by the addition of 4 µL of 10 X DNA-loading dye to samples followed by loading onto the gel. Supercoiled and relaxed forms of DNA were separated by gel electrophoresis for 16 h at 1 V/cm, on a 0.8 % agarose in buffer containing 45 mM Tris-borate and 1 mM EDTA. The gel was stained for 10 min with a solution containing 0.7 µg/mL ethidium bromide in water, destained in water for 30 min followed by imaging and quantitation of the gel bands.

Determination of Resistance Frequency. Spontaneous resistant mutants were raised against compound **1a, 1c, ciprofloxacin** and **moxifloxacin** using a single step selection method as described.[4] Briefly, a mid-logarithmic phase culture of Mtb H37Rv was centrifuged and concentrated 100-fold to achieve a bacterial number of ~10^{10} CFU/mL. Varying dilutions of the bacterial culture were plated on to compound-containing plates. Appropriate dilutions of the bacterial culture were also plated on drug-free Middlebrook 7H11 agar to enumerate the bacterial numbers in the culture. Plates were incubated for 4 weeks at 37°C and the CFUs in drug-free plates were enumerated. The drug-containing plates were incubated for up to 6-8 weeks at 37°C to enumerate the number of resistant colonies.

The spontaneous rate of resistance was calculated by dividing the number of colonies on drug-containing plates (at a given concentration) divided by the total number of viable bacteria estimated on drug-free plates. Resistant colonies were randomly picked from the drug containing plates and grown in complete 7H9 broth to determine their level of resistance against the parent compound, close analogs and TB drugs with different mechanisms of action.

Genetic mapping of mutations conferring resistance to N-linked aminopeperidinyl alkyl quinolones and naphthyridones. In order to map the genetic mutations arising following compound exposure and mutant selection, genomic DNA from microbiologically well characterized colonies were isolated. The entire *gyrA* gene of resistant mutants and wild-type Mtb H37Rv were PCR amplified using standard conditions from boiled supernatants using specific Mtb primers to amplify the entire *gyrA* gene. PCR was performed with cycling parameters of 94 °C for 30 s, 60°C for 30 s, and 72°C for 2 min for 30 cycles in a DNA Engine Dyad cycler (Bio-Rad). PCR products were purified from the gel (PCR purification kit, Qiagen), quantitated, and sequenced (Microsynth). The sequences from the resistant clones were aligned against the wild-type H37Rv *gyrA* gene using Vector NTI software to detect mutations in the target gene.

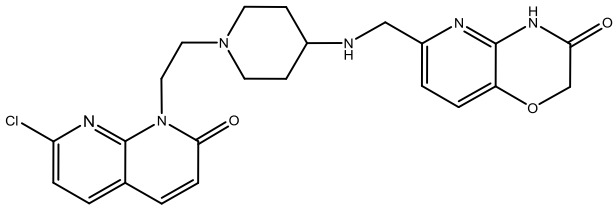
hERG assay

Compounds were tested on voltage-gated ion channels using the medium-throughput electrophysiology IonWorks™ device as described previously. [5]

General chemical methods. All commercial reagents and solvents were used without further purification. Analytical thin-layer chromatography (TLC) was performed on SiO_2 plates on alumina. Visualization was accomplished by UV irradiation at 254 and 220 nm. Flash column chromatography was performed using the Biotage Isolera flash purification system with SiO_2 60 (particle size 0.040−0.055 mm, 230−400 mesh). Purity of all final derivatives for biological testing was confirmed to be >95% as determined using the following conditions: a Shimadzu HPLC instrument with a Hamilton reverse phase column (HxSil, C18, 3μm, 2.1 mm × 50 mm (H2)). Eluent A: 5% CH_3CN in H_2O, eluent B: 90% CH_3CN in H_2O. A flow rate of 0.2 mL/min was used with UV detection at 254 and 214 nm. The structure of the intermediates and end products was confirmed by ^1H NMR and mass spectroscopy. Proton magnetic resonance spectra were determined in DMSO- d_6 unless otherwise stated, using Bruker DRX-300 or Bruker DRX-400 spectrometers, operating at 300 MHz, or 400 MHz, respectively. Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad peak. LCMS data was acquired using Agilent LCMS VL series. Source: ES ionization, coupled with an Agilent 1100 series HPLC system and an Agilent 1100 series PDA as the

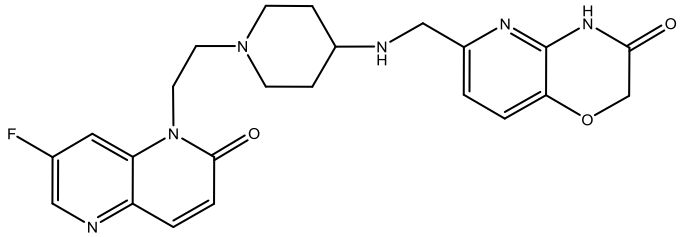
front end. HRMS data was acquired using an Agilent 6520, Quadrupole-Time of flight tandem mass spectrometer (Q-Tof MS/MS) coupled with an Agilent 1200 series HPLC system.

6-((1-(2-(7-chloro-2-oxo-1,8-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (Ia)



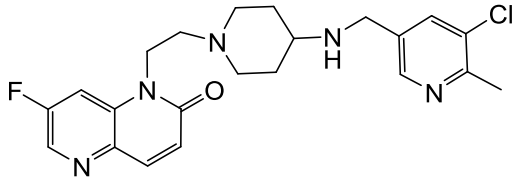
The compound **Ia** (CAS: 1033589-08-0) was synthesized as reported earlier (WO 2008071964)

6-((1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (Ib)



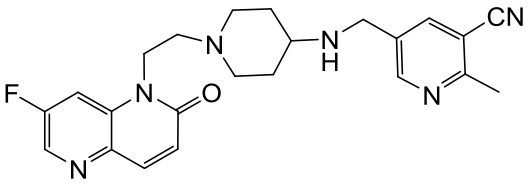
The compound **Ib** (CAS: 1003943-19-8) was synthesized as reported earlier (WO 2008009700)

1-(2-(4-((5-chloro-6-methylpyridin-3-yl)methylamino)piperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (Ic)



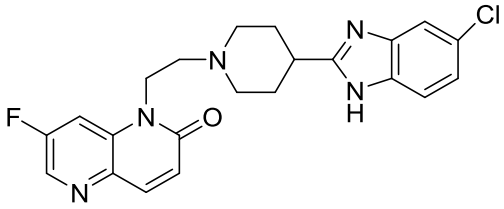
The compound **Ic** (CAS: 1173896-62-2) was synthesized as reported earlier (WO 2009090222)

5-((1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2-methylnicotinonitrile (Id)



Compound **Id** (CAS: 1610628-32-4) was synthezised as mono acetate salt as reported earlier.[1]

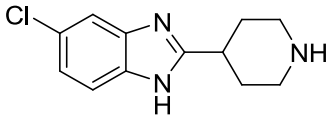
1-(2-(4-(5-chloro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one(Ie)



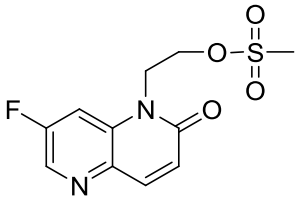
Intermediate **Ie** was synthesized using analogues procedures reported earlier.[6]

Step 1 : Synthesis of 5-chloro-2-(piperidin-4-yl)-1H-benzo[d]imidazole

Step1 intermediate, 5-chloro-2-(piperidin-4-yl)-1H-benzo[d]imidazole was synthesized (CAS: 578709-06-5) as reported earlier.[6]



Step2 : Synthesis of 2-(7-fluoro-2-oxo-1, 5-naphthyridin-1(2H)-yl) ethyl methanesulfonate



Step2 intermediate, 2-(7-fluoro-2-oxo-1, 5-naphthyridin-1(2H)-yl) ethyl methanesulfonate (CAS: 1610628-07-3) was synthesized as reported earlier.[1]

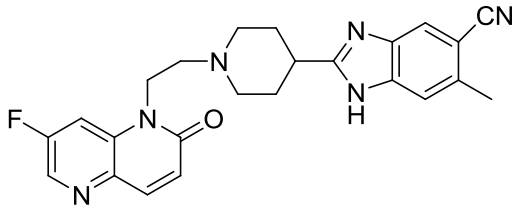
Step 3: Synthesis of 1-(2-(4-(5-chloro-1H-benzo[d]imidazol-2-yl) piperidin-1-yl) ethyl)-7-fluoro-1, 5-naphthyridin-2(1H)-one (**1e**)

In a 25ml round-bottomed flask 2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl methanesulfonate (0.33 mmol), 5-chloro-2-(piperidin-4-yl)-1H-benzo[d]imidazole hydrochloride (0.25 mmol) and Sodium carbonate (155 mg, 1.25 mmol) were taken in DMF (3 mL). The resulting suspension was stirred at 90 0C for 2hrs. Progress of reaction was monitored by LCMS and LCMS profile showed the formation of product.

The reaction mixture was diluted with ethyl acetate and washed with water, brine and dried over sodium sulphate. The organic fraction was evaporated under vacuum and the crude product was purified by reverse phase column chromatography

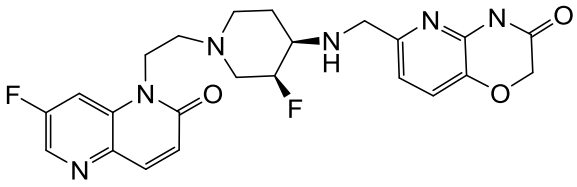
^{1}H NMR (300 MHz, DMSO- d_6) δ 1.52-1.87 (m, 2H), 1.95-2.0 (m, 2H), 2.10-2.33 (m, 2 H), 2.60 (m. 2H), 2.73-2.99(m, 1H), 2.96-3.19(m, 2H), 4.40-4.50 (m, 2H), 6.85 (d, *J* =9.80 Hz, 1 H), 7.38-7.73 (m, 2H), 7.90-8.2 (m, 3H), 8.60 (s, 1H), 12.71 (br s, 1H). HRMS (ESI-TOF): calcd for $C_{22}H_{21}Cl_FN_5O$ $(M+H)^{+,}$ 426.89240; obsd, 426. 83551. HPLC purity: 95.6%.

2-(1-(2-(7-fluoro-2-oxo-1, 5-naphthyridin-1(2H)-yl) ethyl) piperidin-4-yl)-6-methyl-1H-benzo[d]imidazole-5-carbonitrile (If)



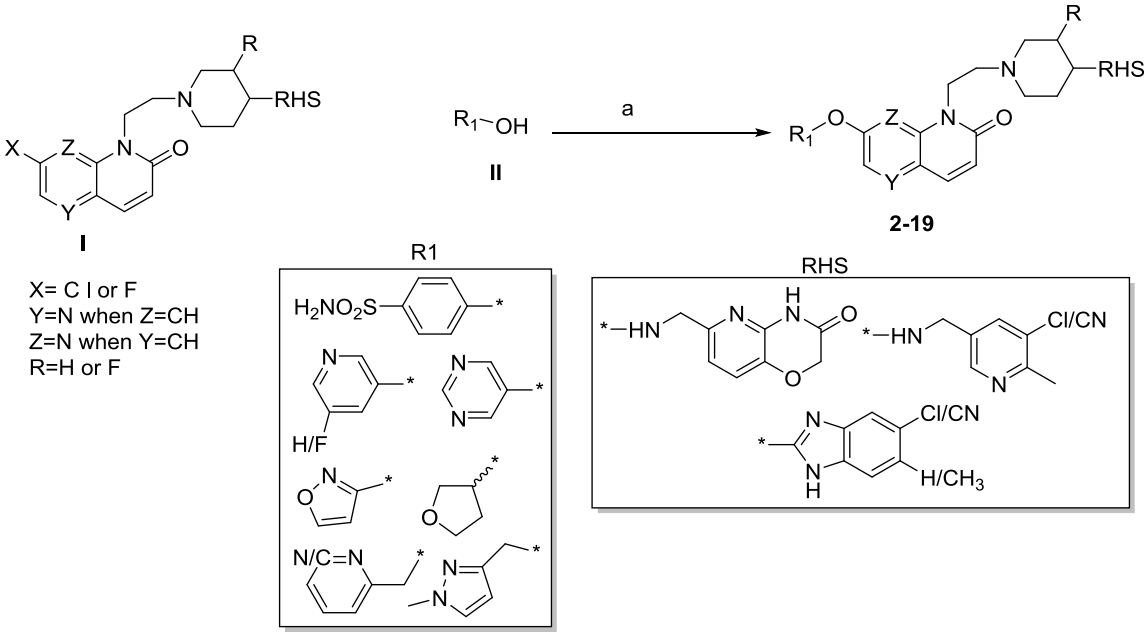
Compound **If** (CAS: 1613053-90-9) was synthezised as reported earlier.[6]

6-(((3S,4R)-3-fluoro-1-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one(Ig)



Compound **Ig** (CAS: 1610628-67-4) was synthezised as reported earlier.[1]

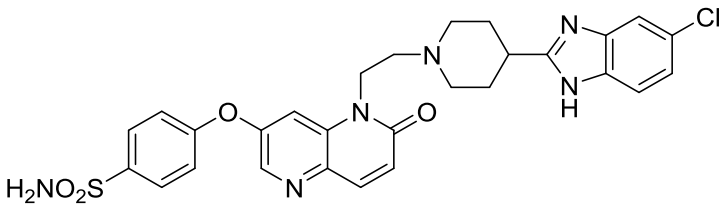
Compound **2-19** were synthesized using the following generic scheme.



Reagents and conditions: a) Cs_2CO_3, DMSO, 130^oC

Scheme 1: **Synthesis of compound 2-19**

4-((5-(2-(4-(5-chloro-1H-benzo[d]imidazol-2-yl) piperidin-1-yl) ethyl)-6-oxo-5, 6-dihydro-1, 5-naphthyridin-3-yl) oxy) benzenesulfonamide (2)



In a 10mL round-bottomed flask, 4-hydroxybenzenesulfonamide (0.18 mmol) and cesium carbonate (117 mg, 0.36 mmol) were taken in DMSO (25 mL) to give a colorless suspension and stirred at room temperature for 30 mins. Then, 1-(2-(4-(5-chloro-1H-benzo[d]imidazol-2-

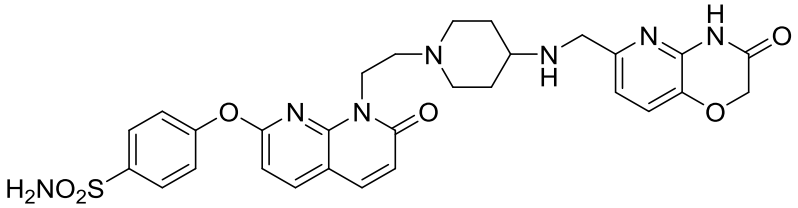
yl)piperidin-1-yl)ethyl)-7-fluoro-1,5-naphthyridin-2(1H)-one (0.18 mmol) was added and the resulting reaction miture was heated to 120 °C for overnight. Progress of reaction was monitored by LCMS, which indicated required product formation. Water was added to the reaction mixture and extracted using 10% Methanol in DCM (4X50ml). The combined organic layers were concentrated to dryness and purified by reverse phase chromatography (Gilson) to obtain 4-((5-(2-(4-(5-chloro-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)ethyl)-6-oxo-5,6-dihydro-1,5-naphthyridin-3-yl)oxy)benzenesulfonamide.

Yield: 30mg, 34 %

^{1}H NMR (300 MHz, DMSO- d_6) δ 1.66-1.77 (m, 2H), 1.93-1.97 (m, 2H), 2.10-2.19 (m, 4H), 2.81-2.93 (m, 3H), 4.28(t, *J*=6.4 Hz, 2H), 6.81(d, *J*=9.6 Hz, 1H), 7.43-7.71 (m, 5H), 7.95(d, *J*=9.8 Hz, 1H), 8.03 (s, 1H), 8.41-8.46 (m, 2H), 8.59 (s, 1H), 11.80 (br s, 1H). HRMS (ESI-TOF): calcd for $C_{28}H_{27}ClN_6O_4S$ $(M+H)^+$, 579.15030; obsd, 579.15792. HPLC purity: 97.3%.

.

4-(7-oxo-8-(2-(4-((3-oxo-3,4-dihydro-2H-pyrido[3,2-b][1,4]oxazin-6-yl)methylamino)piperidin-1-yl)ethyl)-7,8-dihydro-1,8-naphthyridin-2-yloxy)benzenesulfonamide (3)

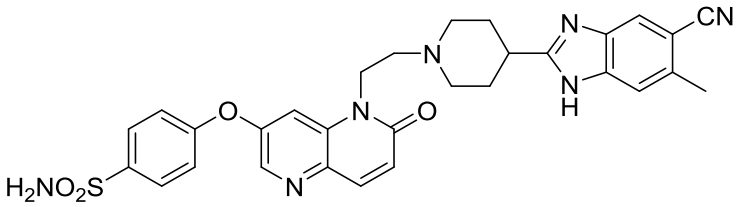


In a 50 mL round-bottomed flask, 4-hydroxybenzenesulfonamide (0.194 g, 1.12 mmol) was dissolved in DMF (1 mL) and K_2CO_3 (0.206 g, 1.49 mmol) was added and stirred for 2 minutes. Then, Intermediate **Ia** (350mg, 0.75 mmol), was added and heated to 90°C for overnight. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product. The reaction mixture was concentrated under vacuum and the residue was diluted with 15% MeOH/DCM and washed with saturated sodium bicarbonate solution. Organic layer was separated and concentrated under vacuum. The purification was done using reverse phase column chromatography (GILSON Prep system) using acetonitrile ammonium acetate buffer as eluent (gradient elution) afforded the title compound (25 mg, 5.53 %).

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 1.10 (d, *J*=10.09 Hz, 2 H) 1.68 (br s, 2 H) 1.76 (br s, 3 H) 1.91 (s, 2 H) 2.24 - 2.33 (m, 2 H) 3.18 (s, 3 H) 3.67 (s, 2 H) 4.04 - 4.18 (m, 3 H) 4.61 (s, 2 H) 6.57 (d,

J=9.77 Hz, 2 H) 7.04 (dd, *J*=19.86, 8.20 Hz, 2 H) 7.30 (d, *J*=8.20 Hz, 1 H) 7.47 (d, *J*=8.20 Hz, 2 H) 7.92 (dd, *J*=15.13, 8.83 Hz, 2 H) 8.26 (d, *J*=8.51 Hz, 1 H). HRMS (ESI-TOF): calcd for $C_{29}H_{31}N_7O_6S$ $(M+H)^+$, 606.20565; obsd, 606.20583. HPLC purity: 95.7 %.

4-(5-(2-(4-(5-cyano-6-methyl-1H-benzo[d]imidazol-2-yl)piperidin-1-yl)ethyl)-6-oxo-5,6-dihydro-1,5-naphthyridin-3-yloxy)benzenesulfonamide (4)

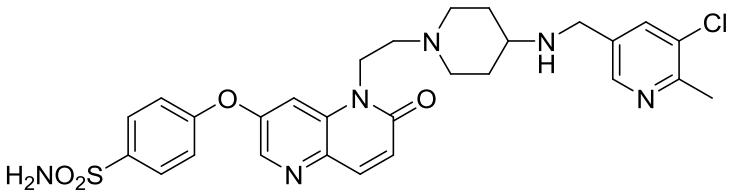


Compound **4** was synthesized from intermediate **If** and 4-hydroxybenzene sulfonamide using procedure analogues to compound **2**

Yield: 30mg, 29%

^{1}H NMR (300 MHz, DMSO- d_6) δ 1.57-1.81 (m, 2H), 1.87-2.02 (m, 2H), 2.10-2.19 (m, 2H), 2.45 (s, 3H), 2.55-2.764 (m, 3H), 2.74-3.03 (m, 3H), 4.30(t, *J*=6.4 Hz, 2H), 6.84(d, *J*=9.6 Hz, 1H), 7.53-7.66 (m, 2H), 7.40(d, *J*=9.7 Hz, 2H), 7.80(s, 1H), 7.82-8.10 (m, 4H), 8.45 (s, 1H), 12.1 (br s, 1H). HRMS (ESI-TOF): calcd for $C_{30}H_{29}N_7O_4S$ $(M+H)^+$, 584.20353; obsd, 584.20715. HPLC purity: 95.4%.

4-(5-(2-(4-((5-chloro-6-methylpyridin-3-yl) methylamino)piperidin-1-yl)ethyl)-6-oxo-5,6-dihydro-1,5-naphthyridin-3-yloxy)benzenesulfonamide(5)



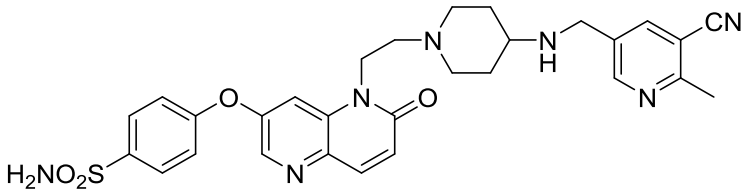
Compound **5** was synthesized from intermediate **Ic** and 4-hydroxybenzene sulfonamide using procedure analoues to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.04 - 1.21 (m, 2 H) 1.61 - 1.76 (m, 4H) 1.94 (t, *J*=10.36 Hz, 2 H) 2.24 - 2.35 (m, 3H) 2.42 - 2.48 (m, 2H) 2.77 (dd, *J*=5.93, 5.37 Hz, 2H) 3.69 (s, 2H) 4.25 (t, *J*=6.59 Hz, 2H) 6.81 (d, *J*=9.80 Hz, 1H) 7.26 - 7.50 (m, 4H) 7.70 (d, *J*=1.88 Hz, 1H) 7.77 - 7.83 (m, 1H) 7.89

(d, *J*=8.85 Hz, 2H) 7.95 (d, *J*=9.80 Hz, 1H) 8.40 (d, *J*=2.07 Hz, 1H) 8.34 (d, *J*=1.51 Hz, 1H). HRMS (ESI-TOF): calcd for $C_{28}H_{31}ClN_6O_4S$ $(M+H)^+$, 583.18160; obsd, 583.18991. HPLC purity: 97.2 %.

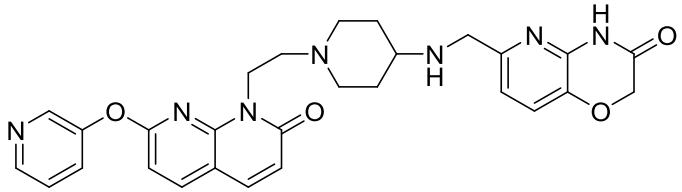
4-(5-(2-(4-((5-cyano-6-methylpyridin-3-yl)methylamino)piperidin-1-yl)ethyl)-6-oxo-5,6-dihydro-1,5-naphthyridin-3-yloxy)benzenesulfonamide (6)

Compound **6** was synthesized **Id** and 4-hydroxybenzene sulfonamide using procedure similar to compound **2**



^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.10 - 1.30 (m, 2H) 1.65 - 1.85 (m, 4H) 1.88 - 2.03 (m, 2H) 2.36 - 2.47 (m, 2H) 2.66 (s, 3 H) 2.76 - 2.88 (m, 2H) 3.81 (br s, 2H) 4.26 (t, *J*=6.50 Hz, 2H) 6.82 (d, *J*=9.61 Hz, 1H) 7.28 - 7.37 (m, 2H) 7.40 (br s, 2H) 7.67 - 7.75 (m, 1H) 7.84 - 7.93 (m, 2H) 7.96 (d, *J*=9.80 Hz, 1H) 8.19 (br s, 1H) 8.41 (d, *J*=2.26 Hz, 1H) 8.69 (s, 1H). HRMS (ESI-TOF): calcd for $C_{29}H_{31}ClN_7O_4S$ $(M+H)^+$, 574.21918; obsd, 574.22337. HPLC purity: 98.1 %.

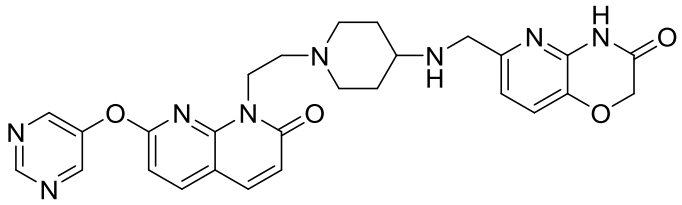
6-((1-(2-(2-oxo-7-(pyridin-3-yloxy)-1,8-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (7)



Compound **7** was synthesized from intermediate **Ia** and 3-hydroxy pyridine using procedure similar to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.11 - 1.25 (m, 2H) 1.67 - 1.78 (m, 4H) 2.25 – 2.35 (m, 4H) 2.45 - 2.5 (m, 2H) 3.69 (s, 2H) 4.31 (t, *J*=7.06 Hz, 2H) 4.60 (s, 2H) 6.66 (d, *J*=9.61 Hz, 1H) 7.01 (dd, *J*=4.80, 1.70, 0.85 Hz, 2H) 7.29 (d, *J*=8.10 Hz, 1H) 7.51 (dd, *J*=7.54, 4.90, 1.13 Hz, 1H) 7.81 (d, *J*=2.07 Hz, 1H) 7.89(d, *J*=7.91 Hz, 1H) 8.32 (d, *J*=2.45 Hz, 1H) 8.51 (d, J=7.91Hz, 1H) 8.67 (d, *J*=2.07 Hz, 1H) 11.14 (br s, 1H). HRMS (ESI-TOF): calcd for $C_{28}H_{29}N_7O_4$ $(M+H)^+$, 528.22810; obsd, 528.22710. HPLC purity: 95.1%.

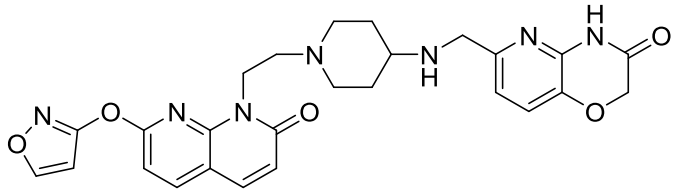
6-((1-(2-(2-oxo-7-(pyrimidin-5-yloxy)-1, 8-naphthyridin-1(2H)-yl) ethyl) piperidin-4-ylamino) methyl)-2H-pyrido [3, 2-b][1, 4] oxazin-3(4H)-one (8)



Compound **8** was synthesized from intermediate **Ia** and 5-hydroxy pyrimidine using procedure similar to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.15 (m, 3H) 1.70 -1.83 (m, 4H) 1.89 (s, 3H) 2.35-2.40 (m, 3 H) 3.68 (s, 2H) 4.19 (t, *J*=6.97 Hz, 2H) 4.61 (s, 2 H) 6.79 (d, *J*=9.80 Hz, 1H) 7.02 (d, *J*=8.10 Hz, 1H) 7.25 (d, 1H) 7.30 (d, *J*=8.10 Hz, 1H) 7.89 (d, *J*=2.07 Hz, 1H) 8.31 (d, *J*=9.80 Hz, 1H) 8.92 (s, 2H) 9.12 (s, 1H). HRMS (ESI-TOF): calcd for $C_{27}H_{28}N_8O_4$ $(M+H)^+$, 529.22335; obsd, 529.23177. HPLC purity: 97.4%.

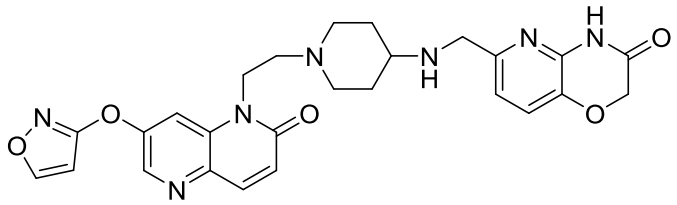
6-((1-(2-(7-(isoxazol-3-yloxy)-2-oxo-1,8-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one(9)



Compound **9** was synthesized from intermediate **Ia** and 3-hydroxy isoxazole using procedure similar to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.16 (d, *J*=9.80 Hz, 2H) 1.25 (br s, 1H) 1.72 (d, *J*=10.74 Hz, 2 H) 1.80 - 1.97 (m, 2H) 2.24 - 2.44 (m, 3H) 2.68 (d, *J*=11.11 Hz, 2H) 3.67 (s, 2H) 4.11 - 4.31 (m, 2H) 4.61 (s, 2H) 6.62 (d, *J*=9.42 Hz, 1H) 6.86 (d, *J*=1.88 Hz, 1H) 7.01 (d, *J*=8.10 Hz, 1H) 7.14 (d, *J*=8.29 Hz, 1H) 7.30 (d, *J*=8.10 Hz, 1H) 7.96 (d, *J*=9.61 Hz, 1H) 8.31 (d, *J*=8.48 Hz, 1H) 8.99 (d, *J*=1.88 Hz, 1H) 11.15 (br s, 1H). HRMS (ESI-TOF): calcd for $C_{26}H_{27}N_7O_5$ $(M+H)^+$, 518.21072; obsd, 518.21487. HPLC purity: 97.2%.

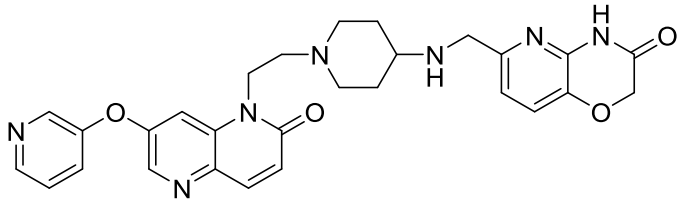
6-((1-(2-(7-(isoxazol-3-yloxy)-2-oxo-1, 5-naphthyridin-1(2H)-yl) ethyl) piperidin-4-ylamino) methyl)-2H-pyrido [3, 2-b][1,4]oxazin-3(4H)-one (10)



Compound **10** was synthesized from intermediate **Ib** and 3-hydroxy isoxazole using procedure similar to compound **2**

^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.0-1.18 (m, 2H) 1.71 (br s, 1H) 1.9-2.05 (m, 3H) 2.33 (br s, 2H) 2.85 (d, *J*=11.54 Hz, 3H) 3.66 (br s, 2H) 4.30 (t, *J*=6.78 Hz, 2H) 4.61 (s, 3H) 6.71 (d, *J*=1.51 Hz, 1H) 6.85 (d, *J*=9.54 Hz, 1H) 7.01 (d, *J*=8.03 Hz, 1H) 7.30 (d, *J*=8.03 Hz, 1H) 7.91 - 8.09 (m, 2H) 8.57 (d, *J*=2.01 Hz, 1H) 8.95 (d, *J*=1.51 Hz, 1 H) 11.18 (s, 1H). HRMS (ESI-TOF): calcd for $C_{26}H_{27}N_7O_5$ $(M+H)^+$, 518.21072; obsd, 518.21482. HPLC purity: 98.1%.

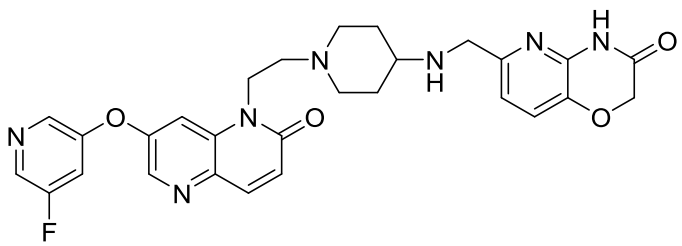
6-((1-(2-(2-oxo-7-(pyridin-3-yloxy)-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (11)



Compound **11** was synthesized from intermediate **Ib** and 3-hyrdoxy pyridine using procedure similar to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.31 - 1.43 (m, 4H) 1.80 - 1.92 (m, 2H) 1.99 - 2.07 (m, 2 H) 2.33-2.36 (m, 2H) 2.82 - 2.93 (m, 2 H) 3.87 - 3.98 (m, 2 H) 4.24 - 4.34 (m, 2H) 4.70 (s, 2H) 6.84 (d, *J*=9.80 Hz, 1H) 7.13 (d, *J*=8.10 Hz, 2H) 7.41 (d, *J*=8.29 Hz, 1H) 7.52 - 7.64 (m, 2H) 7.74 (dd, *J*=7.63, 2.17 Hz, 1H) 8.00 (d, *J*=9.61 Hz, 1H) 8.46 (d, *J*=2.26 Hz, 1H) 8.54 (dd, *J*=4.62, 1.22 Hz, 1H) 8.62 (d, *J*=2.64 Hz, 1H). HRMS (ESI-TOF): calcd for $C_{28}H_{29}N_7O_4$ $(M+H)^+$, 528.22810; obsd, 528.23641. HPLC purity: 96.7%.

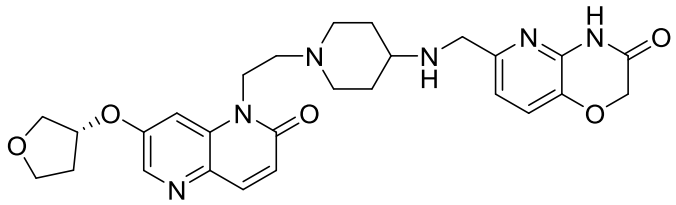
6-((1-(2-(7-(5-fluoropyridin-3-yloxy)-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (12)



Compound **12** was synthesized intermediate **Ib** and 5-fluro-3-hydroxy pyridine using procedure similar to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.88 - 2.14 (m, 4H) 2.38 (d, *J*=12.62 Hz, 2H) 3.08 (d, *J*=10.55 Hz, 2H) 3.32 (br s, 1H) 3.77 (d, *J*=11.30 Hz, 2H) 4.19 (br s, 2H) 4.63 (t, *J*=6.22 Hz, 2 H) 4.71 (s, 2H) 6.89 (d, *J*=9.80 Hz, 1H) 7.22 (d, *J*=8.10 Hz, 1H) 7.46 (d, *J*=8.10 Hz, 1H) 7.74 (d, *J*=10.17 Hz, 1H) 8.03 (d, *J*=9.80 Hz, 1 H) 8.16 (br s, 1 H) 8.39 - 8.53 (m, 2 H) 9.55 (br s, 1 H) 10.75 (br s, 1H) 11.37 (s, 1H). HRMS (ESI-TOF): calcd for $C_{28}H_{28}FN_7O_4$ $(M+H)^+$, 546.22204; obsd, 546.22663. HPLC purity: 97.8%.

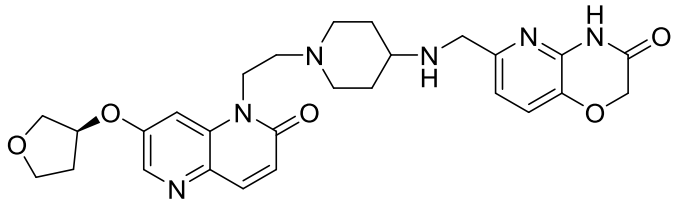
(R)-6-((1-(2-(2-oxo-7-(tetrahydrofuran-3-yloxy)-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (13)



Compound **13** was synthesized from intermediate **Ib** and (R)-tetrahydrofuran-3-ol using procedure similar to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.1 - 1.3 (m, 3H) 1.7 - 1.8 (m, 2H) 1.9 - 2.1 (m, 4H) 2.3 - 2.5 (m, 3H) 2.8-3.0(m, 2H) 3.6(s, 2H) 3.7-3.8(m, 1H) 3.9-4.0 (m, 3H) 4.2-4.4 (m, 2H) 4.6 (s, 2H) 5.4(m, 1H) 5.8 (s, 1H) 6.6 (d, *J*=9.20 Hz, 1H) 7.1 (d, *J*=8.0 Hz, 1H) 7.25(d, *J*=8.15Hz, 1H) 7.9(d, *J*=9.90 Hz, 1H) 8.3(s, 1H) 11.25(br s, 1H). HRMS (ESI-TOF): calcd for $C_{27}H_{32}N_6O_5(M+H)^+$, 521.24342; obsd, 521.24313. HPLC purity: 95.8%.

(S)-6-((1-(2-(2-oxo-7-(tetrahydrofuran-3-yloxy)-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (14)

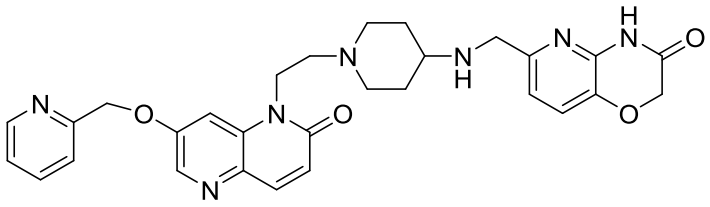


Compound **14** was synthesized from intermediate **Ib** and (S)-tetrahydrofuran-3-ol using procedure similar to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.1 - 1.3 (m, 3H) 1.7 - 1.8 (m, 2H) 1.9 - 2.1 (m, 4H) 2.26 - 2.47 (m, 3H) 2.8-2.99(m, 2H) 3.69(s, 2H) 3.75-3.8(m,1H) 3.9-4.0 (m, 3H) 4.2-4.4 (m, 2 H) 4.6 (s, 2H)

5.39(m, 1H), 5.79(s, 1H) 6.61 (d, *J*=9.20 Hz, 1H) 7.01 (d, *J*=8.0 Hz, 1H) 7.27 (d, *J*=8.16Hz, 1H) 7.85(d, *J*=9.91 Hz, 1H) 8.3(s, 1H) 11.23(br s, 1H). HRMS (ESI-TOF): calcd for $C_{27}H_{32}N_6O_5(M+H)^+$, 521.24342; obsd, 521.24313. HPLC purity: 97.6%.

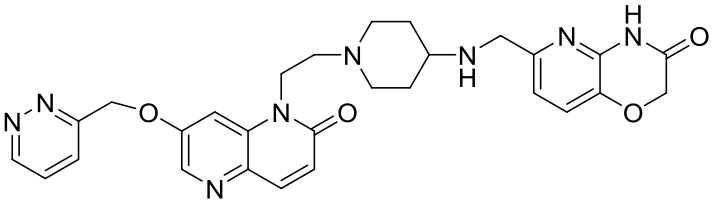
6-((1-(2-(2-oxo-7-(pyridin-2-ylmethoxy)-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (15)



Compound **15** was synthesized from intermediate **Ib** and pyridin-2-ylmethanol using procedure similar to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.20 - 1.32 (m, 2H) 1.70 - 1.81 (m, 2H) 1.93 - 2.05 (m, 2H) 2.31 - 2.39 (m, 2H) 2.40 - 2.48 (m, 2H) 2.80 - 2.92 (m, 2H) 3.69 (s, 2H) 4.31 (t, *J*=7.06 Hz, 2H) 4.60 (s, 2H) 5.46 (s, 2H) 6.66 (d, *J*=9.61 Hz, 1H) 7.01 (d, *J*=8.10 Hz, 1H) 7.29 (d, *J*=8.10 Hz, 1H) 7.37 (dd, *J*=7.54, 4.90, 1.13 Hz, 1H) 7.51 (d, *J*=2.07 Hz, 1H) 7.58 (d, *J*=7.91 Hz, 1H) 7.82 - 7.92 (m, 2H) 8.38 (d, *J*=2.45 Hz, 1H) 8.60 (dd, *J*=4.80, 1.70, 0.85 Hz, 1H) 11.14 (br s, 1 H). HRMS (ESI-TOF): calcd for $C_{29}H_{31}N_7O$ $(M+H)^+$, 542.24375; obsd, 542.24271. HPLC purity: 95.4%.

6-((1-(2-(2-oxo-7-(pyridazin-3-ylmethoxy)-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (16)

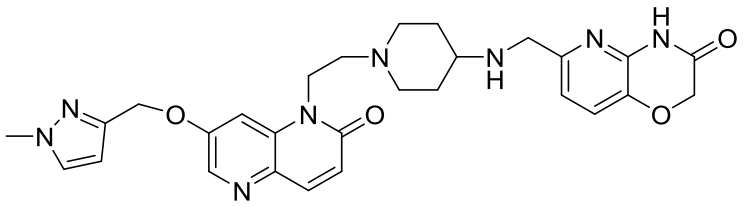


Compound **16** was synthesized from intermediate **Ib** and pyridazin-3-ylmethano using procedure similar to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.21 - 1.3 (m, 2H) 1.70 - 1.80 (m, 2H) 1.93 - 2.1 (m, 2 H) 2.31 - 2.39 (m, 2H) 2.40 - 2.48 (m, 2H) 2.80 - 2.92 (m, 2H) 3.68 (s, 2H) 4.31 (t, *J*=7.1 Hz, 2H) 4.59 (s, 2H) 5.80 (s, 2H) 6.50 (d, J=9.42 Hz, 1H) 6.87 (d, J=8.48 Hz, 1H) 7.00 (d, J=7.91 Hz, 1H) 7.27 (d,

J=8.10 Hz, 1H) 7.70 - 7.76 (m, 3H) 7.86 (d, J=9.61 Hz, 1H) 8.11 (d, J=8.48 Hz, 1H) 8.28 (s, 1H). HRMS (ESI-TOF): calcd for $C_{28}H_{30}N_8O_4$ $(M+H)^+$, 543.24236; obsd, 543.24705.

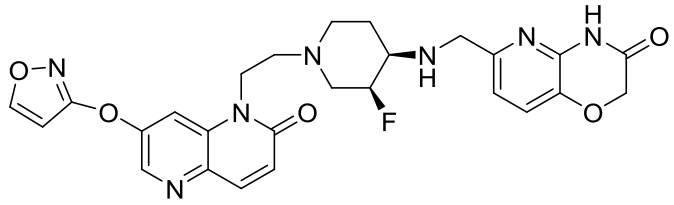
6-((1-(2-(7-((1-methyl-1H-pyrazol-3-yl)methoxy)-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (17)



Compound **17** was synthesized from intermediate **Ib** and (1-methyl-1H-pyrazol-3-yl) methanol using procedure similar to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.16 - 1.34 (m, 2H) 1.71 - 1.84 (m, 2H) 2.07 (t, *J*=10.46 Hz, 2H) 2.34 - 2.46 (m, 1H) 2.53 - 2.61 (m, 2H) 2.86 - 2.98 (m, 2H) 3.70 (s, 2H) 3.84 (s, 3H) 4.34 (t, *J*=6.59 Hz, 2H) 4.60 (s, 2H) 5.28 (s, 2H) 5.75 (s, 1H) 6.37 (d, *J*=2.07 Hz, 1H) 6.65 (d, *J*=9.61 Hz, 1H) 7.01 (d, *J*=8.10 Hz, 1H) 7.29 (d, *J*=8.10 Hz, 1H) 7.54 - 7.62 (m, 1H) 7.69 (d, *J*=1.88 Hz, 1H) 7.85 (d, *J*=9.61 Hz, 1H) 8.30 (d, *J*=1.88 Hz, 1H) 11.13 (br s, 1H). HRMS (ESI-TOF): calcd for $C_{28}H_{32}N_8O$ $(M+H)^+$, 545.25801; obsd, 545.25461. HPLC purity: 97.2%.

6-(((3S,4R)-3-fluoro-1-(2-(7-(isoxazol-3-yloxy)-2-oxo-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (18)

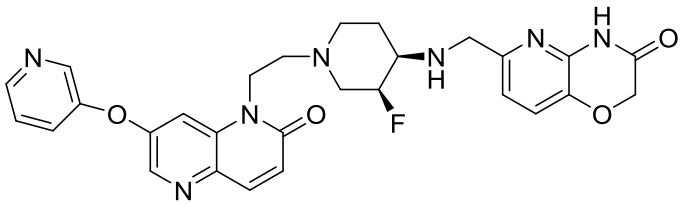


Compound **18** was synthesized from intermediate **Ig** and 3-hydroxy isoxazole using procedure similar to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) d ppm 1.51-1.60 (m, 2H) 2.12 -2.35 (m, 4H) 2.41-2.45 (m, 1H) 2.82 - 2.85 (m, 1H) 3.11 -3.18 (m, 1H), 3.75 -3.78 (m, 2H) 4.25 -4.30 (m , 2H) 4.7 (s, 3H) 6.65 (d, *J*=1.6 Hz, 1H) 6.85 (d, *J*=9.32 Hz 1H) 7.01 (d, *J*=8.1 Hz, 1H) 7.34 (d, *J*=8.11 Hz, 1H) 7.79 (d, *J*=9.65 Hz, 1H)

8.10 (d, *J*=2.05 Hz, 1H) 8.61(d, *J*=1.65 Hz 1H) 9.01 (s, 1H) 11.25 (s, 1H). HRMS (ESI-TOF): calcd for $C_{26}H_{26}FN_7O_5$ $(M+H)^+$, 536.19795; obsd, 536.19783. HPLC purity: 96.7%.

6-(((3S,4R)-3-fluoro-1-(2-(2-oxo-7-(pyridin-3-yloxy)-1,5-naphthyridin-1(2H)-yl)ethyl)piperidin-4-ylamino)methyl)-2H-pyrido[3,2-b][1,4]oxazin-3(4H)-one (19)



Compound **19** was synthesized from intermediate **Ig** and 3-hydroxy pyridine using procedure similar to compound **2**

^{1}H NMR (300 MHz, DMSO-d_6) δ1.70 - 1.85 (m, 2H), 1.95– 2.0 (m, 2H), 2.20 (t, *J*=11.4 Hz, 2H), 2.61 (t, *J*=11.1 Hz, 2H), 2-84-2.87 (m, 1H), 3.06 (d, *J*=10.7 Hz, 2H), 3.35 (s, 2H), 4.0 (s, 2H), 4.43(t, J=6.8 Hz, 2H), 6.70(d, J=9.80 Hz, 1H), 7.35-7.57(m, 5H), 7.90-7.95 (m, 3H), 8.30 (s, 1H), 12.20 (br s, 1H). HRMS (ESI-TOF): calcd for $C_{28}H_{28}FN_7O_4$ $(M+H)^+$, 546.22204; obsd, 546.22705. HPLC purity: 97.1%.

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