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Discovery of new small molecule inhibitors targeting isocitrate dehydrogenase 1 (IDH1) with blood-brain barrier (BBB) penetration

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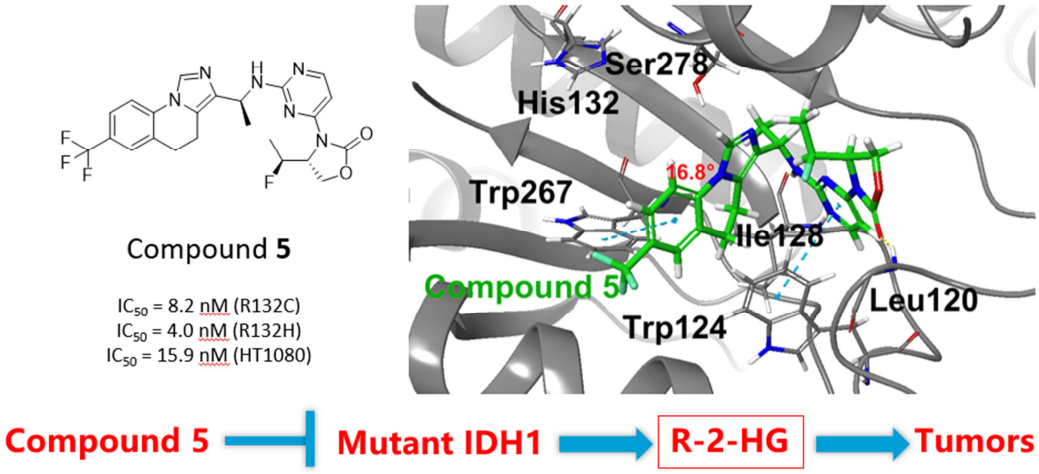
KEYWORDS: Isocitrate dehydrogenase 1, small molecule inhibitors, glioblastoma, acute myeloid leukemia, blood-brain barrier, cancer therapy

GRAPHIC ABSTRACT



ABSTRACT

Isocitrate dehydrogenase (IDH), which catalyzes the conversion of isocitrate to α-ketoglutarate, is one of key enzymes in the tricarboxylic acid cycle (TCA). Abundant evidence has shown that IDH mutations occur in various tumors. Substitution of specific amino acids, such as R132 in IDH1 or R172 in IDH2, in the active site alters the function of IDH, converting the α-ketoglutarate(α-KG) to 2-hydroxyglutarate (2-HG) and leading to abnormal cell metabolism. Because the IDH mutations involve multiple brain tumors, IDH inhibitors have to breach the blood-brain barrier (BBB) and reach the site of action at the minimal effective concentration in order to treat brain tumors. The requirement may limit the clinical application in glioblastoma of the current IDH1 inhibitor, for example, AG-120. Consequently, we have designed and synthesized a novel serial of mutant IDH1 inhibitors with improved activity and BBB penetration. Compound **5** exhibits excellent inhibitory activity both in the enzyme (R132C IC_{50} = 8.2 nM; R132H IC_{50} = 4.0 nM) and in the HT1080 cell line (IC_{50} = 15.9 nM). It also shows good BBB penetration with satisfactory PK and PD

profiling in a CDX model, which makes it a potential preclinical candidate to treat glioblastoma.

INTRODUCTION

Isocitrate dehydrogenase (IDH) is a key enzyme in the tricarboxylic acid (TCA) cycle and plays an important role in energy metabolism.[1] In humans, there are three isoforms of IDH enzyme (IDH1, IDH2, IDH3).[2,3] IDH1, present mainly in the cytosol, is NADP-dependent and performs the conversion of isocitrate to α-ketoglutarate (α-KG) through an oxidative decarboxylation process.[4] With hotspot mutations in the active site, such as R132H and R132C, IDH1 performs an erroneous catalytic conversion and accumulates the oncometabolite (R)-2-hydroxyglutatrate (R-2-HG).[5–9]

Increased R-2-HG levels not only act as a biomarker for accurate diagnosis of the IDH mutation,[10,11] but also promote tumor formation through a variety of mechanisms. Numerous studies have confirmed that R-2-HG can affect many cellular enzymes that regulate histone and DNA methylation levels, and this in turn is highly associated with tumorigenesis.[12–14] Most recently, the crucial role of R-2-HG in immunity has been reported.[15,16] R-2-HG may also acts as immune suppressor in the tumor microenvironment,[17] which suggests a significant antitumor potential stemming from inhibition of mutant IDH.

In the last decade, with the development of large-scale tumor genetic sequencing and the development of precision medicine, IDH mutations with the neomorphic activity have been identified as the important driving mutations in a variety of cancers, including malignancies

such as acute myeloid leukemia (AML),[6,18–20] gliomas,[8,10,21,22] and intrahepatic cholangiocarcinoma.[23] Secondary glioblastoma multiforme (secondary GBM) is an extremely malignant cancer that begins in the brain. At present, the treatment of GBM is limited to surgery and radiotherapy.[24] Because most antitumor drugs have difficulty passing the blood-brain barrier, there are few effective drugs in this context and the application of some tyrosine kinase inhibitors as second-line treatment is also apparently subject to certain obstacles.[25] An oral alkylating agent, temozolomide, widely used in GBM chemotherapy, has been shown to be more effective in patients with MGMT methylation.[26–28] Despite intense treatment with temozolomide, the cancer usually recurs and the median survival time is only 15 months.[29] There is therefore an urgent need for new drugs that can effectively control this serious disease. Since IDH1 mutations are present in more than 70% of low-grade gliomas and secondary glioblastoma,[30] IDH inhibitors could bring new breakthroughs in the treatment of this type of cancer. Acute myeloid leukemia (AML) is another fatal leukemia caused by arrest of differentiation of the immature myeloblast and can progress very rapidly. With the help of tumor genomic analysis, some specific mutations that lead to AML have been identified and mutant IDH has been shown to block cell differentiation.[31,32] Thus inhibitors against IDH mutations are designed for use in AML patients with IDH mutations, a group which accounts for 20% of all AML patients.[32] At present, with the AG-221 (enasidenib), a mutant IDH2 inhibitor, approved by the US FDA, this treatment concept targeting mutant IDH has been affirmed.[33]

Currently, some IDH1 inhibitors are undergoing clinical trials, among which AG-120 (ivosidenib) and IDH-305 have progressed considerably. Both of these compounds can

significantly reduce D-2-HG levels in mice with xenograft tumors.[34,35] Compared to AG-120, IDH-305 appears to be superior in respect of BBB penetration. Therefore, based on IDH-305, we carried out further chemical modification by means of computer-assisted drug design (CADD). In order to enhance the affinity of the compounds to the mutant IDH1, we attempted to link the bipyridyl moiety of IDH-305 and change the dihedral angles in the structure to produce a new π-π stacking with the W267 residue in IDH-305. Compound **5** exhibits great biochemical and cellular activities. The pharmacokinetic (PK) and pharmacodynamics (PD) profiles were also evaluated in mouse models. Compound **5** effectively lower the 2-HG level *in vivo*. Notably, **5** is brain permeable and holds great potential as a preclinical candidate to treat brain tumors with IDH1 mutations.

RESULTS AND DISCUSSION

Design strategies

In the initial stage of structural modification, we used the Maestro program in Schrödinger software to explore the appropriate transformation method. According to the reported X-ray co-crystal structure (PDB: 6b0z), consisting of compound IDH-305 and protein mutant IDH1, there are 4 hydrogen bonds within the pocket and π-π stacking between the W124 and the pyrimidine ring (Figure 1A).[35] The red area shows key atoms that participate in hydrogen bonds and these core areas were retained before beginning further modification. Docking to the protein template of some hypothetical small molecular structures was then examined. The first alternative began with a connection to the bipyridyl moiety. A six-membered alkyl ring was thought to be the most suitable ring with which to create a new π-π stacking between the

terminal pyridyl ring and W267. However, the calculated results show that this may conflict with W124 and cause a conformational change (Figure 1B and 1C). We then resized the internal pyridyl ring to a five-membered imidazole and found that the calculated results support a new π-π stacking without such a clash (Figure 1E). We also tried to change the trifluoromethyl group to chlorine and a carbon to oxygen. The product docked in the pockets with a similar pose to that of IDH-305 (Figure 1D). These changes created a smart dihedral angle (12.7° in compound **1** and 16.8° in compound **5**)**,** which are both much smaller than the angle in IDH-305 (55.3°). The saturated heterocyclic ring can effectively reduce the dihedral angle between the two aromatic rings while retaining a certain flexibility. This new π-π stacking appears to lead to improvement in the activity of compounds.

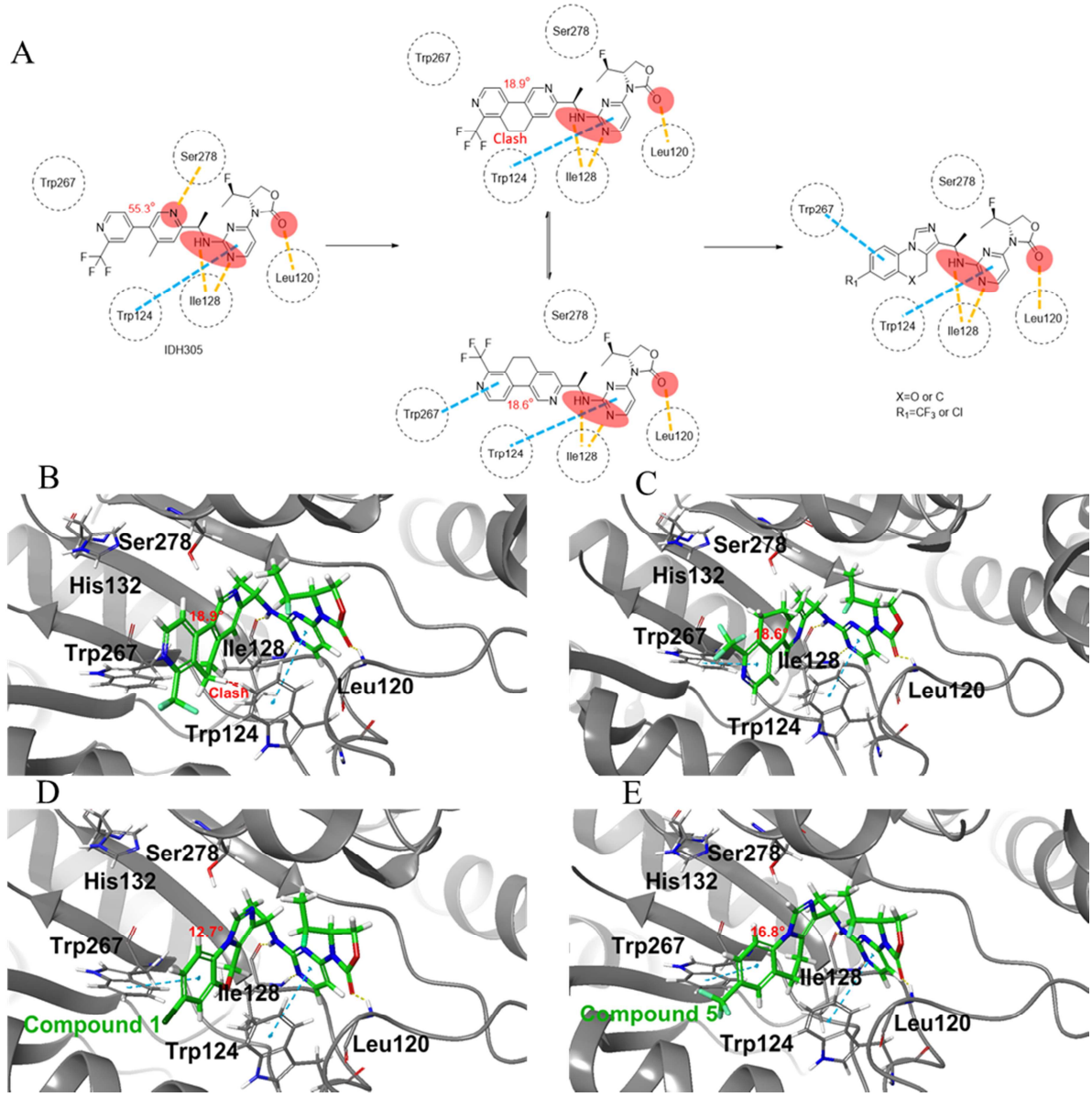
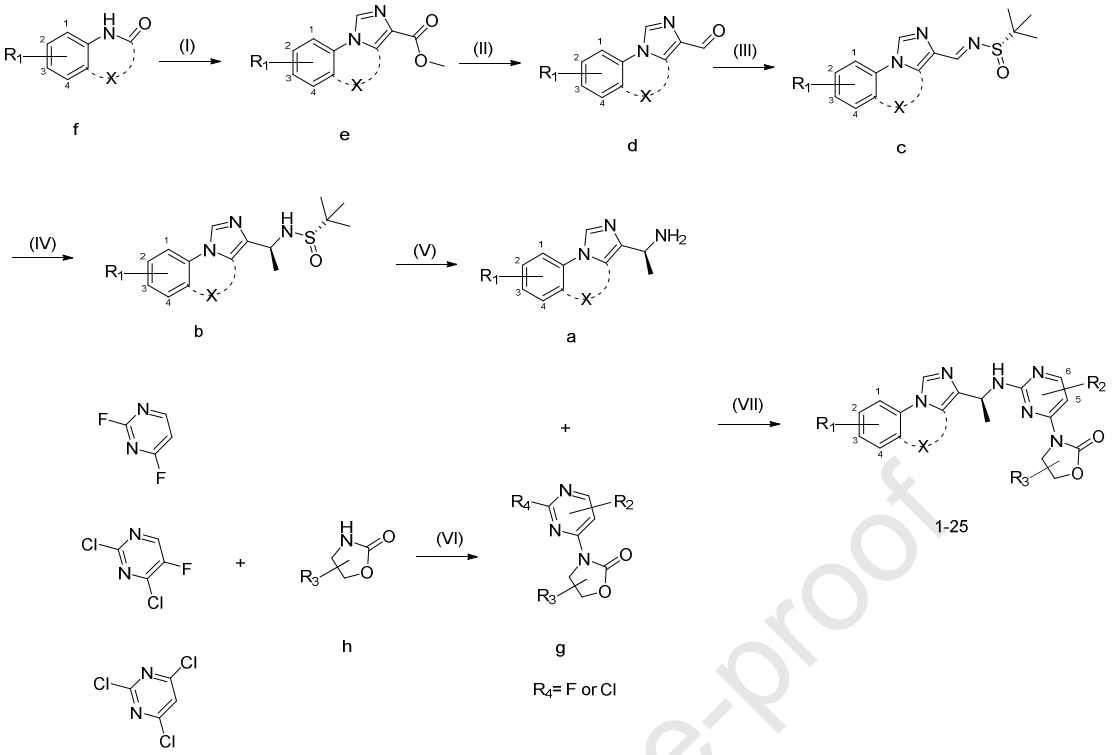


Figure 1. (A) Conceptual design of new compounds. **(B, C)** Docking and possible binding modes shown with the aid of Maestro software. A six-member alkyl ring between the bipyridyl moiety may conflict with W124 and cause conformational change. All molecule backbones are shown in green. The R132H IDH1 backbone comes from PDB bank (PDB: 6b0z) and is grey. The π-π stacking is shown with blue dashed lines. Steric clashes are shown as red dashed lines. Hydrogen bonds are shown as yellow dashed lines. **(D)** Compound **1** docks with mutant IDH1. **(E)** Compound **5** docks with mutant IDH1.

Chemistry

Compounds **1-24** were synthesized and subjected to structure-activity relationship (SAR) studies. All these compounds consist of different amino side chains with a heterocyclic-fused structure and, in addition, a 4-oxazolidinone pyrimidine moiety. Both fragments were synthesized separately following the general route described in Scheme 1. For modification of the amino side chains, we used different commercially available materials to synthesize lactams as the starting substrates. Imidazole rings were then formed with diethyl chlorophosphite and methyl isocyanoacetate. After DIBALH reduction to aldehydes (Dess-Martin periodinane was used to counter overreduction), a methyl group was introduced, creating a chiral carbon, by a Grignard reagent with the help of chiral induction of (S)-*tert*-butylsulfinamide. Amino side chains were successfully formed after alcoholysis of the inductive group. The synthesis of different substituted oxazolidinones uses the same published route as was used for IDH-305 and its derivatives [35]. Finally, the oxazolidinone was linked to the halogenated pyrimidine and the two fragments were combined to obtain the desired compounds **1-24** (Table 1-4).



Scheme 1. General route for synthesis of compounds **1-25**. Reagents and reaction conditions: (I) NaHMDS, diethyl chlorophosphite, methyl isocyanoacetate, THF, -30□, 2 h (II) DIBALH (DMP for overreduction), DCM, -78□, 40 min (III) (S)-(-)-2-Methyl-2-propanesulfinamide, $Ti(OEt)_4$, THF, RT, overnight (IV) MeMgBr, THF, -78□, 1 h (V) HCl, MeOH, rt, 2 h (VI) NaH, DMF, RT, 2h (VII)DIPEA, DMSO, 100□, 1.5 h.

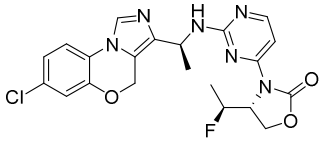
SAR studies

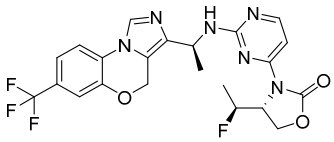
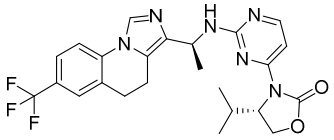
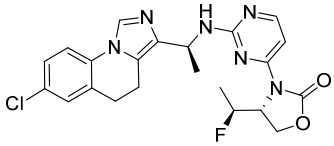
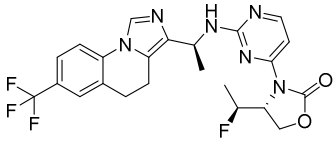
A recombinant IDH1 enzyme assay was used to assess the ability of all compounds to inhibit the enzymatic activity of mutant IDH1. Diaphorase and resazurin can induce a secondary reaction for accurate quantification of NADPH residuals. Cellular activity was determined in HT1080 cells by a commercially available kit. The amount of 2-HG present in the samples can be measured directly. A selection of compounds with good enzymatic inhibitory activity

(IC_{50}<20 nM) were tested for cellular activity.

Compounds **1-5** were synthesized and tested in the enzyme assay and all were found to have good potency (Table 1). The results also met the expectations of the computer-aided design. Some of them have even lower IC_{50} values than AG-120 and they are all better than the prototype compound IDH-305. Compound **3**, with IC_{50}= 1.0 nM has the highest enzyme inhibitory activity. The structural changes among compounds **1, 2, 4, 5** do not cause significant changes in enzyme inhibitory activity. However, substitution of a trifluoromethyl group leads to better cellular activity than chlorine substitution. The oxygen substitution in heterocyclic ring has less impact. Further structural modification and screening based on compound **1-5** continued. The structural modification area is mainly divided into the four groups, X, R_1, R_2 and R_3.

Table 1. Recombinant IDH1 Enzyme Assay Activity and 2-HG Inhibition Assay Activity of compound **1-5** [a]

Compound	Structure	Recombinant IDH1 Enzyme Assay (resazurin/diaphorase/NADPH system)[b]		2-HG inhibiton in HT1080 [b]
		R132C IC_{50} (nM)	R132H IC_{50} (nM)	IC_{50} (nM)
1		14.4	2.2	60.3

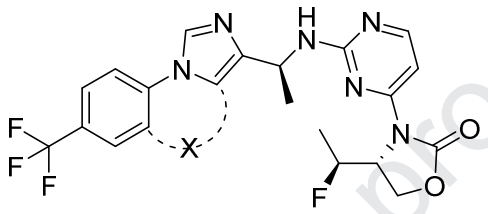
2		9.4	2.7	12.0
3		9.3	1.0	12.5
4		15.3	2.3	53.7
5		8.2	4.0	15.9
IDH-305		50.2	49.6	52.7
AG-120		23.0	3.5	25.4

[a]See the experimental section for assay details. [b]The given IC_{50} values are mean values of at least three experiments. The deviations were within 10%.

In group X (Table 2), the ethylene group was replaced by a methyl group (**10, 14**) or by a suitable bioisostere group (**8, 13, 21**). Due to the steric limitations imposed by I128 and W124 (Figure 1B), any expansion results in loss of potency, which is consistent with the results of the CADD. Compound **8** shows a slightly increased cellular activity with a smaller sulfur atom linker. This may result from the enhanced planarity of its amino side chain (**8:** ClogP=3.69 vs. **5:** ClogP=3.22). However, the structural rigidity may be too strong to create a new π-π stacking with W267 and this will finally limit its enzyme activity (Figure 2A). Compound **13** has a more flexible seven-membered ring, which may result in triple π-π

stacking with W124 (Figure 2B). Therefore, a saturated six-membered ring was determined to be the most suitable linking group for this spatial region. It is flexible, producing a suitable dihedral angle (**1**: 12.7°, **5**: 16.8° π-π stacking with W267).

Table 2. Recombinant IDH1 Enzyme Assay Activity and 2-HG Inhibition Assay Activity and calculated dihedral angles of compound **8, 10, 13, 14, 21** [a]



Compound	X Group Structure	Calculated Dihedral Angle	Calculated π-π stacking	Recombinant IDH1 Enzyme Assay (resazurin/diaphorase/ NADPH system) [b]		2-HG Inhibiton in HT1080 [b]
				R132C IC_{50}(nM)	R132H IC_{50}(nM)	IC_{50}(nM)
8	-S-	-0.3°	None	17.3	19.2	9.7
10	$-CH(CH_3)CH_2-$	-17.7° or -18.5°	None	93.5	128.5	
13	$-CH_2CH_2CH_2-$	-49.5°	W124	43.5	44.3	35.5
14	$-CH_2CH(CH_3)-$	-15.2° or -19.5°	None	119.1	119.8	
21	$-SCH_2-$	23.0°	W267	24.1	23.8	17.0

[a]See the experimental section for assay details. [b]The given IC_{50} values are mean values of at least three experiments. The deviations were within 10%.

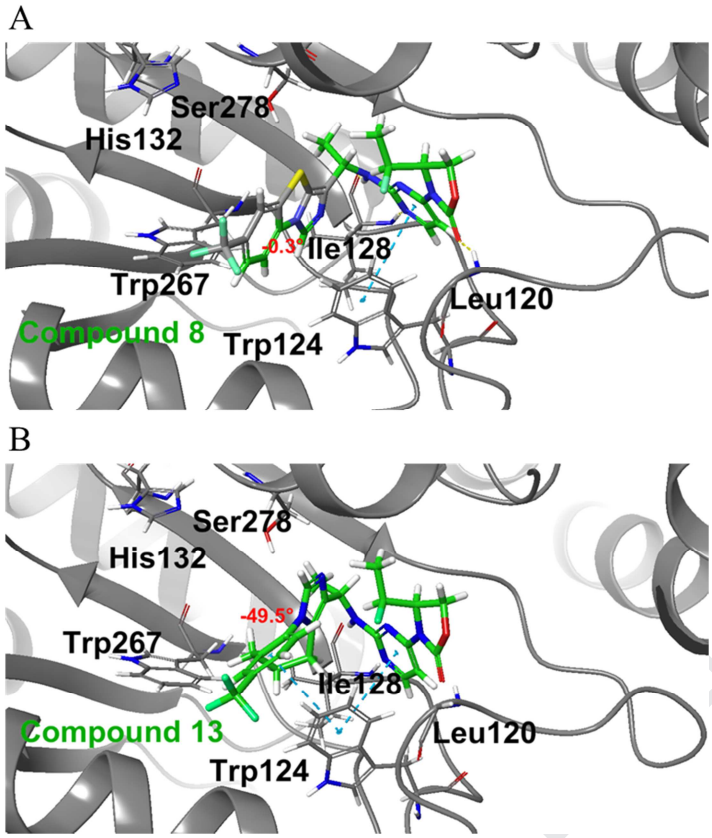
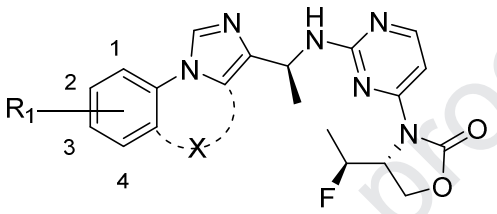


Figure 2. (A) Compound **8** docks with mutant IDH1. **(B)** Compound **13** docks with mutant IDH1.

Then we also tested different substituents on the terminal benzene ring. According to the SAR of IDH-305, the group R_1 is critical to the physicochemical properties of the compound. Low polarity substituents, such as *tert*-butyl, will increase the penetration to brain but such compounds will be easily cleared [35]. Therefore, some other hydrophobic groups were tried (Table 3). When compounds **1, 5, 23, 24** were compared, it was found that, in addition to hydrophobic interactions, strong electron-withdrawing effects are also important contributors to the activity of the compound and it was suspected that the trifluoromethyl group reduces the electron density of the benzene ring and favors the formation of the π-π stacking with W267. Compound **11, 12, 22** were synthesized as compounds with rich or medium electronic

substitution but they failed to show the same level of activity as compound **2**. The change in the position of the trifluoromethyl group (**15**) leads to maintenance of the activity level but substitution by the 4-chlorine (**9**) decreased the activity.

Table 3. Recombinant IDH1 Enzyme Assay Activity and 2-HG Inhibition Assay Activity of compounds **9, 11, 12, 15, 23-25** [a]

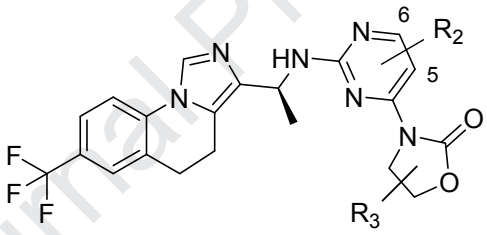


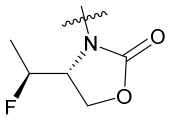
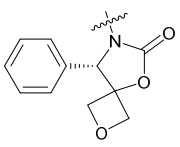
Compound	Structure		Recombinant IDH1 Enzyme Assay (resazurin/diaphorase/NADPH system) [b]		2-HG inhibiton in HT1080 [b]
	R_1	X	R132C IC_{50}(nM)	R132H IC_{50}(nM)	IC_{50}(nM)
9	4-Cl	$-CH_2CH_2-$	81.9	24.1	
11	3-cyclopropyl	$-OCH_2-$	24.8	33.4	166.6
12	3-cyano	$-OCH_2-$	393.9	478.5	
15	2-CF_3	$-CH_2CH_2-$	8.0	18.4	13.2
22	3-OCH_3	$-OCH_2-$	153.8	61.9	
23	3-CH_3	$-CH_2CH_2-$	16.3	42.7	21.9
24	3-F	$-OCH_2-$	44.5	47.8	

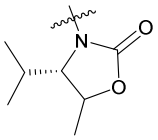
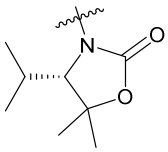
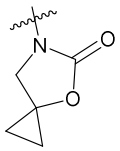
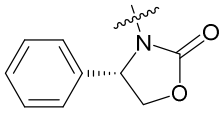
[a]See the experimental section for assay details. [b]The given IC_{50} values are mean values of at least three experiments. The deviations were within 10%.

Compounds **6** and **7** with smaller halogen substitution of R_2 on the pyrimidine ring (Table 4), such as fluorine or chlorine, are both tolerated. At group R_3, most other substitution on oxazolidinones failed to improve the potency. Surprisingly, compound **20** with a phenyl substituent has the highest potency in cells (IC_{50}= 6.6 nM). This may be due to the further improvement of ClogP (3.86). Although current research has difficulty providing an accurate of its conformation in the pocket, this may provide new ideas for further structural modification.

Table 4. Recombinant IDH1 Enzyme Assay Activity and 2-HG Inhibition Assay Activity of compound **6, 7, 16-20** [a]



Compound	Structure		Recombinant IDH1 Enzyme Assay (resazurin/diaphorase/NADPH system) [b]		2-HG inhibiton in HT1080 [b]
	R_2	R_3	R132C IC_{50}(nM)	R132H IC_{50}(nM)	IC_{50}(nM)
6	5-F		17.0	2.1	20.3
7	6-Cl		23.6	2.12	34.6
16			9.7	33.8	140.5

17		15.0	42.0	86.8
18		18.8	50.8	26.3
19		5858	>10000	
20		14.2	15.5	6.6

[a]See the experimental section for assay details. [b]The given IC_{50} values are mean values of at least three experiments. The deviations were within 10%.

DMPK results

Because of the excellent activity in the recombinant IDH1 enzyme assay of both R132C and R132H, compounds **1-6** were selected for further *in vivo* pharmacokinetic testing and comparison with AG-120 (Table 5). Trifluoromethyl-substituted compounds (**2**, **5**) not only have better R132C inhibitory activity and 2-HG inhibitory activity than chlorine-substituted compounds (**1**, **4**) (Table 1), but also have lower clearance, higher exposure and bioavailability. Despite its isopropyl substitution in the R_3 region (**3**) has a more enhanced ability to inhibit the R132H IDH1 enzyme than the fluoroethyl group, but its clearance is increased significantly and its AUC is extremely low. This is consistent with the study of IDH-305.[35] Compound **6**, with a fluorine substituent on the pyrimidine, has a similar

pharmacokinetic profile to that of compound **5**. After 1 mg/kg IV administration in mice, compound **5** showed a moderate plasma clearance (23±1 mL min^{-1} kg^{-1}), $T_{1/2}$ (1.5 ± 0.1 hr) and MRT (1.6 ± 0.1 hr). Its mean Vss was 2.3 ± 0.2 L kg^{-1}. About 30 min after PO administration of 5 mg/kg (Tmax 0.4 ± 0.1 hr), the plasma concentration of compound **5** reached Cmax (1690 ± 241 nM). The PO exposure (AUClast 11041 ± 2593 nM*hr) was close to AG-120 in our study. Compound **5** was also considered to be the most promising compound for higher dosage PK profiling. A xenograft model derived from HT1080 cells was established for PD testing. We measured the concentration of compound **5** in plasma, brain and tumor tissue and compared them with those of AG-120 (Figure 3A-3C). Although the plasma exposures of compound **5** and AG-120 in plasma were relatively close, the exposure of compound **5** was significantly higher than that of AG-120 in the brain and the tumor. This indicates that compound **5** has better targeting ability and has greater potential for use in the treatment of brain tumors. The same CDX model was used for PD testing. It was found that compound **5** could achieve a more effective R-2-HG reduction than AG-120 within 3-20 h after administration (Figure 3D).

Table 5. *In vivo* mouse PK profiles of compound **1-6** and AG-120.[a]

Compound		**1**	**2**	**3**	**4**	**5**	**6**	AG-120
Mouse IV	Cl(mL min^{-1} kg^{-1})	39	30	109	50	23	21	9
	Vss(L kg^{-1})	2.3	5.2	3.0	4.3	2.3	2.1	1.5
	$T_{1/2}$(hr)	1.0	2.1	0.5	1.7	1.5	1.4	2.5
	MRTinf(hr)	1.0	2.9	0.4	1.4	1.6	1.6	2.8

	AUClast(hr*nM)	917	1022	312	717	1439	1520	2972
	AUCinf(hr*nM)	930	1113	323	735	1472	1543	3258
Mouse PO	Tmax(hr)	0.4	1.7	0.3	0.3	0.4	0.9	0.4
	Cmax(nM)	1206	1122	730	932	1690	1703	2264
	$T_{1/2}$(hr)	2.4	2.5	2.0	3.4	2.5	2.5	3.5
	AUClast(hr*nM)	5008	8464	1650	4019	11041	12700	11162
	AUCinf(hr*nM)	5048	9237	1761	4673	11057	12719	12952
	F(%)	109	152	109	117	150	165	80

[a]Data represents the mean of three replicates.

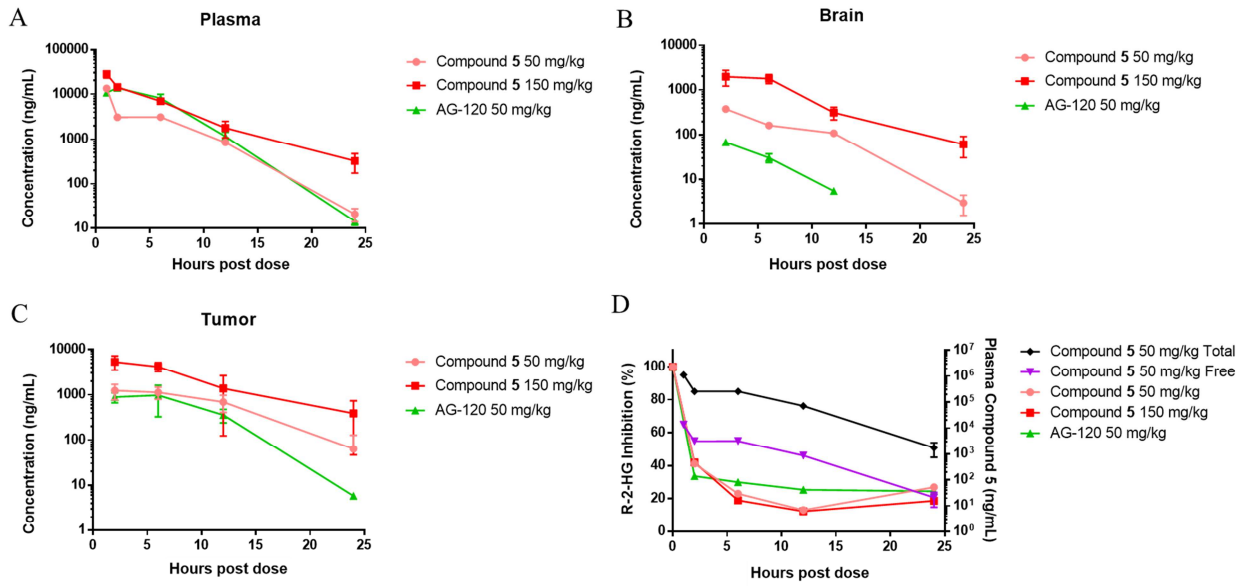


Figure 3. **(A-C)** Drug concentration in plasma, brain and tumor tissues tested in HT1080 xenograft mouse model after single dose. **(D)** Free and estimated total plasma compound **5** concentration (mean±SD) was presented following a single 50mg/kg dose of compound **5**. Percent inhibition of 2-HG (mean±SEM) in HT1080 xenograft tumor samples in the case of

the single dose of 50 mg/kg and 150 mg/kg respectively. AG-120 was compared at a single 50 mg/kg dose.

CONCLUSION

AML and secondary GBM are two very difficult tumors and current clinical drug treatment options are very limited. IDH1 and IDH2 are both considered to be promising drug targets for the treatment of these two malignancies and the IDH2 targeted therapeutic drug, enasidenib, has been successfully marketed. Through computer aided drug design, a promising π-π stacking with W267 was revealed that may enhance the activity in the co-crystal structure of IDH-305. To verify this hypothesis, we designed and synthesized compounds **1-24**, analogs with saturated heterocyclic linking groups. All compounds were screened by the recombinant IDH1 enzyme assay and the best were selected for cellular activity and PK profiling comparison. The modelling studies assisted the design of a first group of compounds with linkers in six-membered rings (compounds **1-5**). All five compounds exhibit excellent inhibitory potency against mutant IDH1. Compound **3** has the best activity but is readily metabolized *in vivo*. Another 20 compounds were designed for in-depth exploration of their SARs. A trifluoromethyl group on the benzene ring may produce a more favorable π-π stacking and a moderately flexible linking group also contributes to the proper dihedral angle. Further structural modifications can be made with compound **6** and compound **20** because they maintain relatively good activity and have more substitutable positions.

Compound **5** demonstrated good pharmacokinetic properties in the single-dose PK assay. Subsequently the pharmacokinetic profile with higher dosages and pharmacodynamics profile

were realized in the CDX model. Compound **5** exhibits higher exposure than AG-120 in the brain and tumor and significantly reduces R-2-HG in tumors, suggesting good blood-brain barrier permeability and therapeutic value in brain tumors with IDH1 mutation. More experiments for toxicity and selectivity will be conducted and verified in the future.

EXPERIMENTAL SECTION

General methods for Chemistry All reagents and starting materials were either commercially available or prepared according to literature reported procedures. All reactions were monitored by thin layer chromatography (TLC) on silica gel 60 F254 plates and visualized under UV light or used $KMnO_4$ staining. NMR spectra were obtained on a Bruker AV-400 spectrometer (^{1}H NMR at 400 MHz, ^{13}C NMR at 101 MHz, and ^{19}F NMR at 376 MHz). Chemicals shifts (δ) were reported in parts per million and referenced to the residual solvent peaks ($CDCl_3$ 7.26 ppm in ^{1}H NMR spectra and 77.16 ppm in ^{13}C NMR). Coupling constants (J) values were in hertz, and the splitting patterns were abbreviated as follows: s for singlet; b for broad; d for doublet; t for triplet; q for quartet; and m for multiplet. Yields were of purified product and were not optimized. Purity of all tested compounds was higher than 95%. Purity and low resolution electrospray ionization (ESI) mass spectra were recorded on an Agilent 1260/6120 ES-LCMS system. The column was a Phenomenex Luna C18, 100A, 2.0 50 mm, 5 μm. Mobile phase: A (H_2O + 0.1% HCOOH); B (ACN). Gradient: 30% B increase to 95% B within 1 min, 95% B to 100% B within 0.1 min; 100% B for 2.4

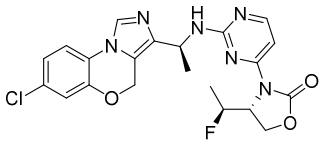
min. Flow rate 0.50 mL/min. Column Temperature: 40 □. Sig=254nm. High resolution ESI-MS were performed on an Thermo Fisher Scientific LTQ FTICR-MS instrument.

General method for combination of amino side chains and oxazolidinone fragments.

a (less than 100mg, 1.0 equiv) and **g** (1.2 equiv) were dissolved in anhydrous DMSO (1.5mL). DIPEA (3 equiv) was added and the mixture was heated to 100 □ for 1.5 h. The mixture was then extracted with EtOAc and H_2O. The organic layer was washed with H_2O and saturated NaCl solution and dried over Na_2SO_4. The mixture was then concentrated and purified via flash chromatography on silica gel to get the desired compounds (**1-24**). All final products were lyophilized to give out white powder.

*(R)-3-(2-(((S)-1-(7-chloro-4H-benzo[b]imidazo[1,5-d][1,4]oxazin-3-yl)ethyl)amino)pyrimidin-4-yl)-4-((S)-1-fluoroethyl)oxazolidin-2-one (**1**)*

Compound **1** was synthesized from **1a** and **1g**. **1a** was synthesized from 7-chloro-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one in a similar progress to that described for the preparation of **2a.**

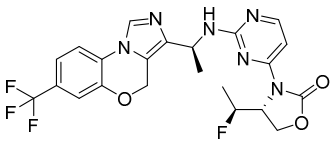


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.19 (d, *J* = 5.8 Hz, 1H), 7.89 (s, 1H), 7.46 (d, *J* = 5.8 Hz, 1H), 7.33 (d, *J* = 8.5 Hz, 1H), 7.07 (d, *J* = 2.1 Hz, 1H), 7.01 (dd, *J* = 8.5, 2.2 Hz, 1H), 5.68 (br, 1H), 5.37 – 4.78 (m, 4H), 4.74 – 4.58 (m, 1H), 4.51 (d, *J* = 3.3 Hz, 1H), 4.36 (t, *J* = 9.0 Hz, 1H), 1.60 (d, *J* = 6.9 Hz, 3H), 1.24 (br, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ

-197.63 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.78 , 159.04 , 157.20 , 154.21 , 146.60 , 138.91 , 132.05 , 130.52 , 122.80 , 119.01 , 117.57 , 116.26 , 99.53 , 87.25 (d, *J* = 174.4 Hz), 62.45 , 61.86 (d, *J* = 6.3 Hz), 57.70 (d, *J* = 19.6 Hz), 44.62 , 21.39 , 16.83 (d, *J* = 21.6 Hz). ES-LCMS m/z: 459.0 $(M+H)^+$. HRMS m/z: calcd for C21H21ClFN6O3 459.1342; found, 459.1341. 99.63% purity.

*(R)-4-((S)-1-fluoroethyl)-3-(2-(((S)-1-(7-(trifluoromethyl)-4H-benzo[b]imidazo[1,5-d][1,4]oxazin-3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one（**2**）*

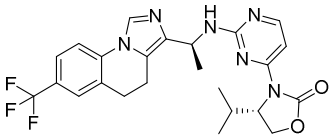
Compound **2** was synthesized from **2a** and **1g**.



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.11 (d, *J* = 5.8 Hz, 1H), 7.90 (s, 1H), 7.44 (d, *J* = 8.1 Hz, 1H), 7.38 (d, *J* = 5.8 Hz, 1H), 7.27 – 7.19 (m, 2H), 6.70 – 5.40 (br, 1H), 5.34 – 4.78 (m, 4H), 4.59 (dd, *J* = 26.7, 8.5 Hz, 1H), 4.42 (dd, *J* = 8.9, 3.3 Hz, 1H), 4.29 (t, *J* = 9.0 Hz, 1H), 1.55 (d, *J* = 6.9 Hz, 3H), 1.17 (d, *J* = 17.8 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.57 , -196.92 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.80 , 158.92 , 157.17 , 154.15 , 145.89 , 139.37 , 130.85 , 128.98 (q, *J* = 33.3 Hz), 126.55 , 123.41 (q, *J* = 272.2 Hz), 119.59 (q, *J* = 3.9 Hz), 117.61 , 115.98 (q, *J* = 3.8 Hz), 115.73 , 99.37 , 87.24 (d, *J* = 174.2 Hz), 62.41 , 61.80 (d, *J* = 6.2 Hz), 57.66 (d, *J* = 19.5 Hz) , 44.10 , 21.25 , 16.74 (d, *J* = 21.6 Hz). ES-LCMS m/z: 493.0 $(M+H)^+$. HRMS m/z: calcd for C22H21F4N6O3 493.1606; found, 493.1606. 98.41% purity.

*(S)-4-isopropyl-3-(2-(((S)-1-(7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one (**3**)*

Compound **3** was synthesized from **3a** and **3g**. **3g** was synthesized from 2,4-dichloropyrimidine and (S)-4-isopropyloxazolidin-2-one in a similar progress to that described for the preparation of **1g.**

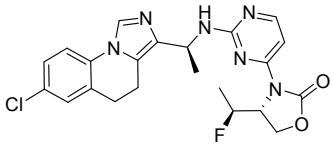


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.18 (d, *J* = 5.8 Hz, 1H), 7.99 (s, 1H), 7.59 – 7.46 (m, 3H), 7.43 (d, *J* = 5.8 Hz, 1H), 5.70 (br, 1H), 5.19 (br, 1H), 4.68 (br, 1H), 4.35 – 4.20 (m, 2H), 3.18 – 2.82 (m, 4H), 2.51 (br, 1H), 1.59 (d, *J* = 6.8 Hz, 3H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.81 (d, *J* = 6.5 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.31 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 161.03 , 158.83 , 157.42 , 154.91 , 139.09 , 136.64 , 131.09 , 128.71 , 127.82 (q, *J* = 32.9 Hz), 126.72 (q, *J* = 3.6 Hz), 125.10 (q, *J* = 3.5 Hz), 123.89 (q, *J* = 271.9 Hz), 122.37 , 115.94 , 99.30 , 63.13 , 58.65 , 43.93 , 27.75 , 26.65 , 21.67 , 19.01 , 18.24 , 14.34 . ES-LCMS m/z: 487.0 $(M+H)^{+}$. HRMS m/z: calcd for C24H26F3N6O2 487.2064; found, 487.2063. 100% purity.

*(R)-3-(2-(((S)-1-(7-chloro-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)-4-((S)-1-fluoroethyl)oxazolidin-2-one (**4**)*

Compound **4** was synthesized from **4a** and **1g**. **4a** was synthesized from (2,5-dichlorophenyl)boronic acid in a similar progress to that described for the preparation of

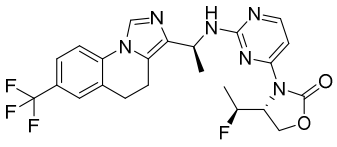
3a.



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.21 (d, *J* = 5.7 Hz, 1H), 7.93 (s, 1H), 7.46 (d, *J* = 5.7 Hz, 1H), 7.35 – 7.24 (m, 3H), 5.75 (br, 1H), 5.17 (br, 2H), 4.82 – 4.61 (m, 1H), 4.54 (dd, *J* = 8.8, 3.4 Hz, 1H), 4.39 (t, *J* = 8.9 Hz, 1H), 3.20 – 2.75 (m, 4H), 1.58 (d, *J* = 6.8 Hz, 3H), 1.33 (d, *J* = 19.1 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -197.81 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.82 , 159.07 , 157.12 , 154.28 , 138.62 , 132.54 , 131.09 , 130.66 , 129.85 , 129.49 , 127.82 , 122.29 , 116.93 , 99.12 , 87.28 (d, *J* = 174.0 Hz), 61.85 (d, *J* = 6.3 Hz), 57.74 (d, *J* = 19.6 Hz) , 43.64 , 26.52 , 21.70 , 19.07 , 16.90 (d, *J* = 21.7 Hz). ES-LCMS m/z: 457.0 $(M+H)^{+}$. HRMS m/z: calcd for C22H23ClFN6O2 457.1550; found, 457.1549. 98.80% purity.

*(R)-4-((S)-1-fluoroethyl)-3-(2-(((S)-1-(7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin -3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one (**5**)*

Compound **5** was synthesized from **3a** and **1g**.

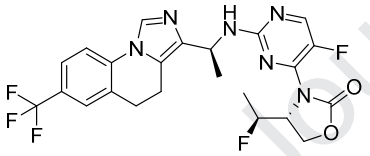


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.19 (s, 1H), 7.98 (s, 1H), 7.60 – 7.39 (m, 4H), 5.72 (br, 1H), 5.16 (br, 2H), 4.76 – 4.61 (m, 1H), 4.52 (d, *J* = 8.4 Hz, 1H), 4.37 (t, *J* = 8.6 Hz, 1H), 3.15 – 2.80 (d, *J* = 63.1 Hz, 4H), 1.57 (d, *J* = 6.6 Hz, 3H), 1.32 (d, *J* = 21.3 Hz, 3H). ^{19}F

NMR (376 MHz, Chloroform-*d*) δ -62.35 , -197.86 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.84 , 159.08 , 157.16 , 154.25 , 139.03 , 136.58 , 131.01 , 128.70 , 127.83 (q, *J* = 32.8, 32.2 Hz), 126.81 – 126.59 (m), 125.08 (q, *J* = 3.4 Hz), 123.87 (q, *J* = 271.9 Hz), 122.39 , 115.92 , 99.18 , 87.27 (d, *J* = 174.7 Hz), 61.84 (d, *J* = 6.3 Hz), 57.74 (d, *J* = 19.6 Hz) , 43.57 26.60 , 21.63 , 19.02 , 16.89 (d, *J* = 21.7 Hz). ES-LCMS m/z: 491.0 $(M+H)^+$. HRMS m/z: calcd for C23H23F4N6O2 491.1813; found, 491.1811. 100% purity.

*(R)-3-(5-fluoro-2-(((S)-1-(7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)-4-((S)-1-fluoroethyl)oxazolidin-2-one (**6**)*

Compound **6** was synthesized from **3a** and **6g**. **6g** was synthesized from **1h** and 2,4-dichloro-5-fluoropyrimidine in a similar progress to that described for the preparation of **1g.**

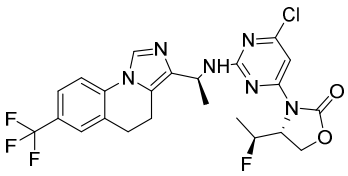


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.20 (d, *J* = 2.8 Hz, 1H), 7.98 (s, 1H), 7.58 – 7.46 (m, 3H), 5.86 (br, 1H), 5.15 – 4.87 (m, 2H), 4.75 – 4.61 (m, 1H), 4.50 (p, *J* = 8.6 Hz, 2H), 3.13 – 2.84 (m, 4H), 1.56 (d, *J* = 6.8 Hz, 3H), 1.27 (dd, *J* = 23.2, 6.3 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.31 , -152.16 , -194.12 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 157.54 (d, *J* = 2.4 Hz), 153.69 , 147.88 (d, *J* = 11.8 Hz), 145.39 (d, *J* = 12.3 Hz), 143.94 (d, *J* = 254.2 Hz), 138.77 , 136.56 , 131.08 , 128.68 , 127.82 (q, *J* = 32.9 Hz), 126.71 (q, *J* = 3.7 Hz), 123.87 (q, *J* = 271.9 Hz), 125.08 (q, *J* = 3.8 Hz), 122.55 , 115.96 , 86.18 (d, *J* = 176.8 Hz), 62.90 (d, *J* = 5.9 Hz), 58.40 (d, *J* = 20.3 Hz), 44.57 , 26.58 , 21.42 , 19.00 , 16.58 (d, *J* = 21.9 Hz). ES-LCMS m/z: 509.0 $(M+H)^+$. HRMS m/z:

calcd for C23H22F5N6O2 509.1719; found, 507.1718. 97.20% purity.

*(R)-3-(6-chloro-2-(((S)-1-(7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)-4-((S)-1-fluoroethyl)oxazolidin-2-one (**7**)*

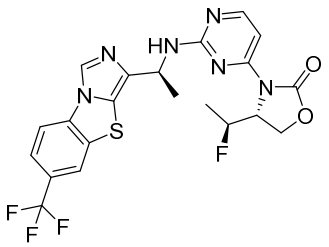
Compound **7** was synthesized from **3a** and **7g**. **7g** was synthesized from **1h** and 2,4,6-trichloropyrimidine in a similar progress to that described for the preparation of **1g.**



^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.97 (s, 1H), 7.60 – 7.45 (m, 4H), 5.92 (d, *J* = 7.3 Hz, 1H), 5.40 – 4.87 (m, 2H), 4.81 – 4.28 (m, 3H), 3.39 – 2.77 (m, 4H), 1.59 (d, *J* = 6.5 Hz, 3H), 1.42 – 1.23 (m, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.30 , -197.78 (dp, *J* = 47.9, 24.0, 23.5 Hz). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 162.11 , 160.24 , 157.93 , 153.96 , 138.25 , 136.55 , 131.16 , 129.04 , 127.83 (q, *J* = 33.4 Hz), 126.74 (q, *J* = 3.7 Hz), 125.01 , 123.86 (q, *J* = 271.9 Hz), 123.57 , 115.91 , 98.09 , 87.15 (d, *J* = 174.1 Hz), 61.89 (d, *J* = 6.0 Hz), 57.99 (d, *J* = 19.4 Hz), 43.25 , 26.69 , 20.95 , 18.93 , 16.77 (d, *J* = 21.4 Hz). ES-LCMS m/z: 525.0 $(M+H)^{+}$. HRMS m/z: calcd for C23H22ClF4N6O2 525.1423; found, 525.1435. 100% purity.

*(R)-4-((S)-1-fluoroethyl)-3-(2-(((S)-1-(6-(trifluoromethyl)benzo[d]imidazo[5,1-b]thiazol-3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one **(8)***

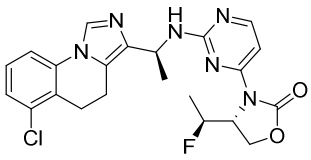
Compound **8** was synthesized from **8a** and **1g**.



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.29 (s, 1H), 8.24 (d, *J* = 5.8 Hz, 1H), 7.78 (s, 1H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.51 (d, *J* = 5.8 Hz, 1H), 6.21 (br, 1H), 5.17 (br, 1H), 4.65 (dd, *J* = 26.7, 7.4 Hz, 1H), 4.58 – 4.40 (m, 2H), 4.36 (t, *J* = 8.9 Hz, 1H), 1.67 (d, *J* = 6.9 Hz, 3H), 1.06 (br, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -61.85 , -197.40 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 161.11 , 159.32 , 157.18 , 154.15 , 134.87 , 134.58 , 133.17 , 128.33 (q, *J* = 33.3 Hz), 126.34 , 123.82 , 123.62 (q, *J* = 272.5 Hz), 123.52 (q, *J* = 3.7 Hz), 121.94 (q, *J* = 3.9 Hz), 112.80 , 99.59 , 87.26 (d, *J* = 174.3 Hz), 61.80 (d, *J* = 6.5 Hz), 57.75 (d, *J* = 19.5 Hz), 46.29 , 21.08 , 16.80 (d, *J* = 21.5 Hz). ES-LCMS m/z: 495.1 $(M+H)^{+}$. HRMS m/z: calcd for C21H19F4N6O2S 495.1221; found, 495.1220. 100% purity.

(R)-3-(2-(((S)-1-(6-chloro-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)-4-((S)-1-fluoroethyl)oxazolidin-2-one ***(9)***

Compound **9** was synthesized from **9a** and **1g**.

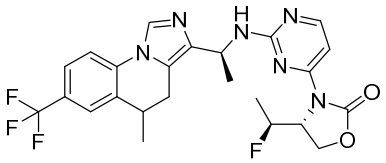


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.21 (d, *J* = 5.7 Hz, 1H), 7.95 (s, 1H), 7.46 (d, *J* = 5.7 Hz, 1H), 7.34 (dd, *J* = 7.0, 2.2 Hz, 1H), 7.31 – 7.19 (m, 2H), 5.81 (br, 1H), 5.18 (br, 2H), 4.85 – 4.60 (m, 1H), 4.54 (dd, *J* = 8.9, 3.4 Hz, 1H), 4.40 (t, *J* = 8.9 Hz, 1H), 3.25 – 2.73 (m, 4H),

1.59 (d, *J* = 6.8 Hz, 3H), 1.47 – 1.18 (m, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -197.64 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.80 , 159.03 , 157.08 , 154.24 , 138.43 , 135.13 , 134.70 , 130.92 , 128.17 , 126.65 , 126.32 , 122.23 , 114.21 , 99.05 , 87.27 (d, *J* = 174.1 Hz), 61.82 (d, *J* = 6.3 Hz), 57.71 (d, *J* = 19.6 Hz), 43.60 , 23.57 , 21.65 , 18.59 , 16.86 (d, *J* = 21.6 Hz). ES-LCMS m/z: 457.1 $(M+H)^+$. HRMS m/z: calcd for C22H23ClFN6O2 457.1550; found, 457.1548. 100% purity.

(4R)-4-((S)-1-fluoroethyl)-3-(2-(((1S)-1-(5-methyl-7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one ***(10)***

Compound **10** was synthesized from **10a** and **1g**.

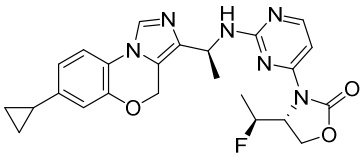


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.20 (dd, *J* = 5.7, 2.0 Hz, 1H), 7.99 (s, 1H), 7.55 (d, *J* = 5.6 Hz, 2H), 7.49 (d, *J* = 8.8 Hz, 1H), 7.45 (dd, *J* = 5.7, 4.0 Hz, 1H), 5.72 (br, 1H), 5.17 (br, 2H), 4.69 (dd, *J* = 26.7, 7.1 Hz, 1H), 4.59 – 4.44 (m, 1H), 4.37 (t, *J* = 8.9 Hz, 1H), 3.19 – 2.68 (m, 3H), 1.57 (dd, *J* = 6.8, 2.4 Hz, 3H), 1.45 – 1.10 (m, 6H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.25 , -197.47 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.81 (d, *J* = 3.7 Hz), 159.06 , 157.15 (d, *J* = 3.7 Hz), 154.28 , 140.07 , 135.73 , 133.58 (d, *J* = 8.6 Hz), 130.84 , 128.10 (q, *J* = 32.8 Hz), 125.62 – 125.30 (m), 125.03 (q, *J* = 3.8 Hz), 123.91 (q, *J* = 272.0 Hz), 121.42 , 116.06 , 99.20 , 89.02 – 85.30 (m), 61.83 (d, *J* = 5.7 Hz), 57.74 (d, *J* = 19.4 Hz), 43.65 , 31.49 (d, *J* = 17.1 Hz), 26.68 , 21.81 (d, *J* = 23.0 Hz), 19.86 (d, *J* = 51.6 Hz), 16.94 (dd, *J* = 21.6, 5.5 Hz). ES-LCMS m/z: 505.1 $(M+H)^+$. HRMS m/z: calcd for

C24H25F4N6O2 505.1970; found, 505.1966. 98.66% purity.

(R)-3-(2-(((S)-1-(7-cyclopropyl-4H-benzo[b]imidazo[1,5-d][1,4]oxazin-3-yl)ethyl)amino)pyrimidin-4-yl)-4-((S)-1-fluoroethyl)oxazolidin-2-one ***(11)***

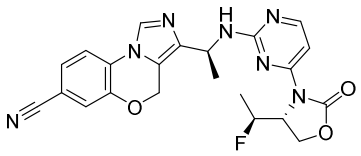
Compound **11** was synthesized from **11a** and **1g**.



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.19 (d, *J* = 5.7 Hz, 1H), 7.87 (s, 1H), 7.46 (d, *J* = 5.7 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 6.79 – 6.70 (m, 2H), 5.71 (br, 1H), 5.31 – 4.89 (m, 4H), 4.65 (dd, *J* = 25.8, 7.7 Hz, 1H), 4.50 (dd, *J* = 8.7, 2.9 Hz, 1H), 4.36 (t, *J* = 9.0 Hz, 1H), 1.84 (tt, *J* = 8.5, 5.1 Hz, 1H), 1.60 (d, *J* = 6.8 Hz, 3H), 1.24 (br, 3H), 0.96 (q, *J* = 6.3 Hz, 2H), 0.66 (q, *J* = 4.9 Hz, 2H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -197.72 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.84 , 159.08 , 157.17 , 154.22 , 146.06 , 143.82 , 138.40 , 130.28 , 121.64 , 120.14 , 117.91 , 115.29 , 115.19 , 99.43 , 87.27 (d, *J* = 174.2 Hz), 62.19 , 61.84 (d, *J* = 6.4 Hz), 57.70 (d, *J* = 19.6 Hz), 44.46 , 21.56 , 16.82 (d, *J* = 21.6 Hz), 15.31 , 9.56 . ES-LCMS m/z: 465.1 $(M+H)^{+}$. HRMS m/z: calcd for C24H26FN6O3 465.2045; found, 465.2043. 98.96% purity.

3-((S)-1-((4-((R)-4-((S)-1-fluoroethyl)-2-oxooxazolidin-3-yl)pyrimidin-2-yl)amino)ethyl)-4H-benzo[b]imidazo[1,5-d][1,4]oxazine-7-carbonitrile ***(12)***

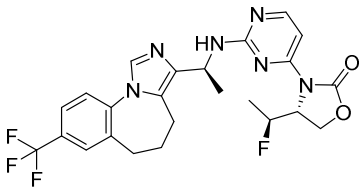
Compound **12** was synthesized from **12a** and **1g**.



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.17 (d, *J* = 5.8 Hz, 1H), 7.96 (s, 1H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.45 (d, *J* = 5.7 Hz, 1H), 7.38 – 7.31 (m, 2H), 5.64 (br, 1H), 5.40 – 4.95 (m, 4H), 4.72 – 4.56 (m, 1H), 4.49 (dd, *J* = 8.8, 3.2 Hz, 1H), 4.36 (t, *J* = 9.0 Hz, 1H), 1.59 (d, *J* = 6.9 Hz, 3H), 1.25 (d, *J* = 16.6 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -197.61 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.69 , 158.98 , 157.18 , 154.16 , 145.96 , 139.57 , 131.09 , 127.54 , 126.77 , 122.32 , 117.86 , 117.48 , 116.19 , 110.21 , 99.55 , 87.22 (d, *J* = 174.3 Hz), 62.52 , 61.84 (d, *J* = 6.3 Hz), 57.67 (d, *J* = 19.5 Hz), 43.98 , 21.15 , 16.80 (d, *J* = 21.6 Hz). ES-LCMS m/z: 450.0 $(M+H)^{+}$. HRMS m/z: calcd for C22H21FN7O3 450.1684; found, 450.1683. 100% purity.

(R)-4-((S)-1-fluoroethyl)-3-(2-(((S)-1-(8-(trifluoromethyl)-5,6-dihydro-4H-benzo[f]imidazo[1, 5-a]azepin-3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one ***(13)***

Compound **13** was synthesized from **13a** and **1g**.

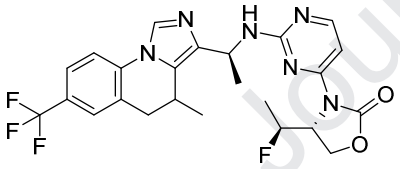


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.19 (d, *J* = 5.7 Hz, 1H), 7.67 (s, 1H), 7.61 (d, *J* = 8.2 Hz, 1H), 7.57 (s, 1H), 7.45 (d, *J* = 5.7 Hz, 1H), 7.39 (d, *J* = 8.1 Hz, 1H), 5.77 (br, 1H), 5.19 (br, 2H), 4.80 – 4.60 (m, 1H), 4.52 (dd, *J* = 8.8, 3.4 Hz, 1H), 4.38 (t, *J* = 8.7 Hz, 1H), 2.78 – 2.49 (m, 4H), 2.25 – 1.80 (m, 2H), 1.58 (d, *J* = 6.7 Hz, 3H), 1.33 (dd, *J* = 22.9, 5.4 Hz, 3H).

^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.38 , -197.95 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.76 , 159.07 , 157.12 , 154.29 , 139.43 , 139.32 , 135.61 , 134.35 , 130.00 (q, *J* = 32.8 Hz), 127.60 (q, *J* = 3.6 Hz), 126.24 , 125.12 (q, *J* = 3.8 Hz), 123.80 (q), 123.02 , 99.09 , 87.24 (d, *J* = 173.9 Hz), 61.83 (d, *J* = 6.2 Hz), 57.75 (d, *J* = 19.4 Hz), 43.50 , 30.62 , 29.15 , 22.21 , 19.85 , 16.94 (d, *J* = 21.7 Hz). ES-LCMS m/z: 505.1 $(M+H)^+$. HRMS m/z: calcd for C24H25F4N6O2 505.1970; found, 505.1970. 97.78% purity.

(4R)-4-((S)-1-fluoroethyl)-3-(2-(((1S)-1-(4-methyl-7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one ***(14)***

Compound **14** was synthesized from **14a** and **1g**. **14a** was synthesized from 2-chloro-5-(trifluoromethyl)phenylboronic acid and methacrylamide in a similar progress to that described for the preparation of **3a**.

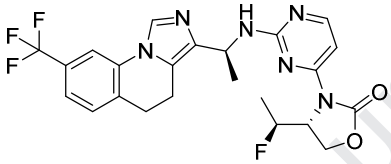


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.20 (d, *J* = 5.7 Hz, 1H), 7.98 (s, 1H), 7.55 (s, 2H), 7.49 (d, *J* = 8.6 Hz, 1H), 7.44 (d, *J* = 5.7 Hz, 1H), 5.76 (br, 1H), 5.45 – 5.00 (m, 2H), 4.69 (d, *J* = 21.5 Hz, 1H), 4.52 (dt, *J* = 8.5, 3.7 Hz, 1H), 4.37 (t, *J* = 8.6 Hz, 1H), 3.70 – 3.35 (d, *J* = 63.6 Hz, 1H), 3.07 (dd, *J* = 15.5, 5.4 Hz, 1H), 2.85 2.70 (m, 1H), 1.58 (d, *J* = 6.7 Hz, 3H), 1.34 (dd, *J* = 23.1, 6.6 Hz, 3H), 1.11 (d, *J* = 7.1 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.28 , -197.88 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.74 (d, *J* = 3.4 Hz), 159.09 , 157.15 (d, *J* = 1.7 Hz), 154.27 , 138.43 (d, *J* = 12.8 Hz), 135.99 (d, *J* = 6.3 Hz), 131.01 (d, *J*

= 19.8 Hz), 127.97 (q, *J* = 32.9 Hz), 127.50 (q, *J* = 3.6 Hz), 127.23 (d, *J* = 9.5 Hz), 126.69 , 125.09 (q, *J* = 3.7 Hz), 123.87 (q, *J* = 272.0 Hz), 115.65 (d, *J* = 5.4 Hz), 99.16 , 87.26 (d, *J* = 174.1 Hz), 61.83 (d, *J* = 6.2 Hz), 57.75 (d, *J* = 19.4 Hz), 43.39 , 34.10 (d, *J* = 2.6 Hz), 24.83 (d, *J* = 21.3 Hz), 21.99 (d, *J* = 13.6 Hz), 19.96 , 16.87 (d, *J* = 21.7 Hz). ES-LCMS m/z: 505.0 $(M+H)^+$. HRMS m/z: calcd for C24H25F4N6O2 505.1970; found, 505.1968. 100% purity.

(R)-4-((S)-1-fluoroethyl)-3-(2-(((S)-1-(8-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin -3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one ***(15)***

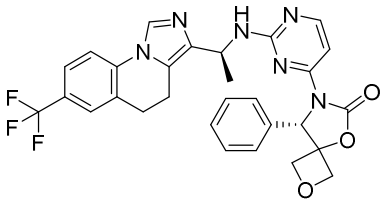
Compound **15** was synthesized from **15a** and **1g**. **15a** was synthesized from 2-chloro-4-trifluoromethylphenylboronic acid in a similar progress to that described for the preparation of **3a**.



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.19 (d, *J* = 5.7 Hz, 1H), 7.99 (s, 1H), 7.61 (s, 1H), 7.44 (d, *J* = 5.7 Hz, 1H), 7.41 (s, 2H), 5.74 (br, 1H), 5.17 (br, 2H), 4.80 – 4.58 (m, 1H), 4.52 (dd, *J* = 8.9, 3.4 Hz, 1H), 4.37 (t, *J* = 8.9 Hz, 1H), 3.15 – 2.80 (m, 4H), 1.57 (d, *J* = 6.8 Hz, 3H), 1.41 – 1.23 (m, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.58 , -198.01 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.82 , 159.04 , 157.14 , 154.28 , 138.90 , 134.31 , 132.04 , 130.83 , 130.36 (q, *J* = 33.0 Hz), 130.13 , 123.68 (q, *J* = 272.3 Hz), 122.48 (q, *J* = 3.8 Hz), 112.70 (q, *J* = 3.8 Hz), 99.17 , 87.28 (d, *J* = 174.2 Hz), 61.84 (d, *J* = 6.3 Hz), 57.74 (d, *J* = 19.5 Hz), 43.52 , 26.69 , 21.66 , 18.97 , 16.89 (d, *J* = 21.6 Hz). ES-LCMS m/z: 491.1 $(M+H)^+$. HRMS m/z: calcd for C23H23F4N6O2 491.1813; found, 491.1811. 100% purity.

(S)-8-phenyl-7-(2-(((S)-1-(7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)-2,5-dioxa-7-azaspiro[3.4]octan-6-one ***(16)***

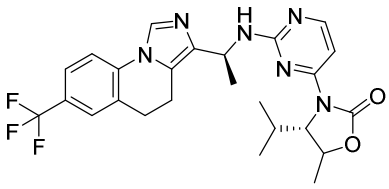
Compound **16** was synthesized from **3a** and **16g**.



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 (d, *J* = 5.7 Hz, 1H), 7.92 (s, 1H), 7.46 (dt, *J* = 18.7, 8.4 Hz, 3H), 7.35 (d, *J* = 5.6 Hz, 1H), 7.30 – 7.05 (m, 5H), 5.80 – 5.40 (m, 2H), 5.20 – 4.59 (m, 3H), 4.52 (d, *J* = 8.4 Hz, 1H), 4.03 (d, *J* = 8.2 Hz, 1H), 2.94 – 2.05 (m, 4H), 1.48 (br, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.29 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.83 , 159.18 , 156.47 , 152.77 , 138.61 , 136.58 , 136.26 , 130.96 , 129.26 , 128.78 , 128.65 , 127.77 (q, *J* = 33.0 Hz), 126.64 (q, *J* = 3.4 Hz), 126.24 , 125.06 (q, *J* = 3.9 Hz), 123.87 (q, *J* = 272.0 Hz), 122.42 , 115.92 , 98.95 , 82.84 (d, *J* = 100.2 Hz), 76.84 , 65.18 , 43.93 , 26.49 , 21.30 , 18.68 . ES-LCMS m/z: 563.2 $(M+H)^{+}$. HRMS m/z: calcd for C29H26F3N6O3 563.2013; found, 563.2013. 100% purity.

(4S)-4-isopropyl-5-methyl-3-(2-(((S)-1-(7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one ***(17)***

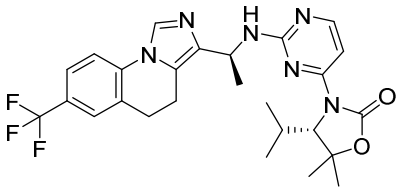
Compound **17** was synthesized from **3a** and **17g**.



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.16 (d, *J* = 5.7 Hz, 1H), 7.98 (s, 1H), 7.57 – 7.45 (m, 3H), 7.41 (d, *J* = 5.7 Hz, 1H), 5.71 (br, 1H), 5.19 (br, 1H), 4.72 (br, 2H), 3.18 – 2.82 (m, 4H), 2.22 (br, 1H), 1.57 (d, *J* = 6.8 Hz, 3H), 1.51 (d, *J* = 6.3 Hz, 3H), 0.93 (d, *J* = 6.3 Hz, 6H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.30 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.92 , 158.91 , 157.98 , 154.88 , 139.10 , 136.60 , 131.01 , 128.66 , 127.72 (q, *J* = 32.9 Hz), 126.65 (q, *J* = 3.7 Hz), 125.04 (q, *J* = 3.9 Hz), 123.86 (q, *J* = 272.0 Hz), 122.37 , 115.91 , 99.11 , 76.17 , 61.34 , 43.98 , 28.58 , 26.58 , 21.59 , 20.36 , 19.00 , 17.96 , 14.76 . ES-LCMS m/z: 501.2 $(M+H)^{+}$. HRMS m/z: calcd for C25H28F3N6O2 501.2220; found, 501.2228. 100% purity.

(S)-4-isopropyl-5,5-dimethyl-3-(2-(((S)-1-(7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one ***(18)***

Compound **18** was synthesized from **3a** and **18g**.

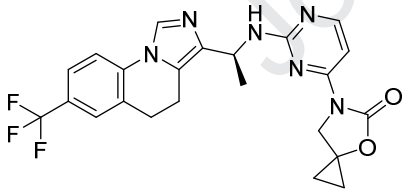


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.15 (d, *J* = 5.7 Hz, 1H), 7.98 (s, 1H), 7.57 – 7.45 (m, 3H), 7.40 (d, *J* = 5.7 Hz, 1H), 5.70 (br, 1H), 5.18 (br, 1H), 4.47 (s, 1H), 3.15 – 2.80 (m, 4H),

2.18 (br, 1H), 1.56 (d, *J* = 6.8 Hz, 3H), 1.50 (s, 3H), 1.35 (s, 3H), 0.90 (d, *J* = 6.4 Hz, 6H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.29 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.86 , 158.78 , 158.55 , 154.33 , 139.07 , 136.59 , 131.01 , 128.66 , 127.68 (q, *J* = 32.9 Hz), 126.63 (q, *J* = 3.7 Hz), 125.01 (q, *J* = 3.8 Hz), 123.84 (q, *J* = 271.9 Hz), 122.37 , 115.89 , 99.28 , 82.51 , 66.00 , 43.73 , 29.62 , 29.09 , 26.56 , 21.62 , 21.53 , 20.89 , 18.98 , 17.60 . ES-LCMS m/z: 515.2 $(M+H)^+$. HRMS m/z: calcd for C26H30F3N6O2 515.2377; found, 515.2374. 100% purity.

(S)-6-(2-((1-(7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)-4-oxa-6-azaspiro[2.4]heptan-5-one ***(19)***

Compound **19** was synthesized from **3a** and **19g**.

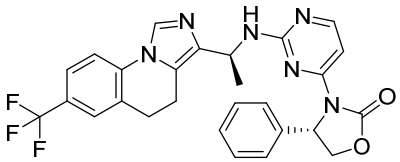


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.16 (d, *J* = 5.7 Hz, 1H), 7.98 (s, 1H), 7.58 – 7.46 (m, 3H), 7.40 (d, *J* = 5.7 Hz, 1H), 5.80 (br, 1H), 5.20 (br, 1H), 4.16 (s, 2H), 3.15 – 2.84 (m, 4H), 1.57 (d, *J* = 6.8 Hz, 3H), 1.26 (br, 2H), 0.80 (br, 2H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.28 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 161.06 , 158.56 , 157.42 , 153.97 , 139.04 , 136.64 , 130.98 , 128.67 , 127.72 (q, *J* = 32.9 Hz), 126.66 (q, *J* = 3.7 Hz), 125.04 (q, *J* = 3.9 Hz), 123.87 (q, *J* = 272.0 Hz), 122.45 , 115.94 , 98.70 , 60.16 , 49.45 , 43.81 , 26.61 , 21.42 ,

19.03 , 10.90 , 10.84 . ES-LCMS m/z: 471.1 $(M+H)^+$. HRMS m/z: calcd for C23H22F3N6O2 471.1751; found, 471.1747. 100% purity.

(S)-4-phenyl-3-(2-(((S)-1-(7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one ***(20)***

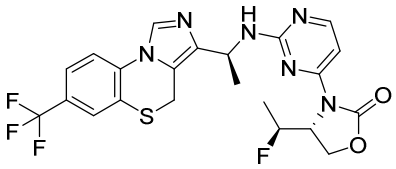
Compound **20** was synthesized from **3a** and **20g**. **20g** was synthesized from (S)-(+)-2-phenylglycinol in a similar progress to that described for the preparation of **19g.**



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.15 (d, *J* = 5.7 Hz, 1H), 7.96 (s, 1H), 7.59 – 7.44 (m, 4H), 7.35 – 7.14 (m, 5H), 6.03 – 5.48 (m, 2H), 5.25 – 4.50 (m, 2H), 4.24 (dd, *J* = 8.6, 3.6 Hz, 1H), 3.00 – 2.20 (m, 4H), 1.53 (d, *J* = 6.4 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.27. ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.82 , 158.96 , 156.96 , 154.80 , 140.41 , 138.63 , 136.58 , 130.89 , 129.02 , 128.66 , 128.04 , 127.70 (q, *J* = 32.8 Hz), 126.61 (q, *J* = 3.7 Hz), 125.61 , 125.00 (q, *J* = 3.8 Hz), 123.88 (q, *J* = 272.0 Hz), 122.37 , 115.89 , 98.96 , 70.36 , 58.11 , 43.82 , 26.47 , 21.21 , 18.58 . ES-LCMS m/z: 521.1 $(M+H)^+$. HRMS m/z: calcd for C27H24F3N6O2 521.1907; found, 521.1905. 98.39% purity.

(R)-4-((S)-1-fluoroethyl)-3-(2-(((S)-1-(7-(trifluoromethyl)-4H-benzo[b]imidazo[1,5-d][1,4]thiazin-3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one ***(21)***

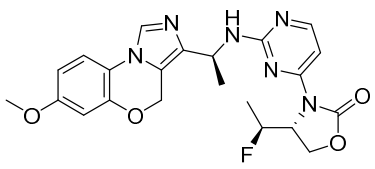
Compound **21** was synthesized from **21a** and **1g**.



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.20 (d, *J* = 5.8 Hz, 1H), 7.95 (s, 1H), 7.71 (s, 1H), 7.57 – 7.43 (m, 3H), 5.59 (br, 1H), 5.20 (br, 2H), 4.67 (dd, *J* = 26.6, 7.0 Hz, 1H), 4.52 (dd, *J* = 8.9, 3.4 Hz, 1H), 4.37 (t, *J* = 9.0 Hz, 1H), 4.29 – 3.96 (m, 2H), 1.62 (d, *J* = 6.8 Hz, 3H), 1.31 (d, *J* = 22.4 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.64 , -197.75 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.75 , 158.92 , 157.25 , 154.23 , 138.55 , 135.71 , 133.08 , 128.56 (q, *J* = 33.4 Hz), 126.78 (q, *J* = 3.3 Hz), 126.75 , 124.28 (q, *J* = 3.7 Hz), 123.45 (q, *J* = 272.4 Hz), 120.35 , 118.09 , 99.58 , 87.25 (d, *J* = 174.4 Hz), 61.88 (d, *J* = 6.4 Hz), 57.73 (d, *J* = 19.7 Hz), 43.31 , 22.53 , 21.20 , 16.90 (d, *J* = 21.8 Hz). ES-LCMS m/z: 509.1 $(M+H)^{+}$. HRMS m/z: calcd for C22H21F4N6O2S 509.1377; found, 509.1375. 99.72% purity.

(R)-4-((S)-1-fluoroethyl)-3-(2-(((S)-1-(7-methoxy-4H-benzo[b]imidazo[1,5-d][1,4]oxazin-3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one ***(22)***

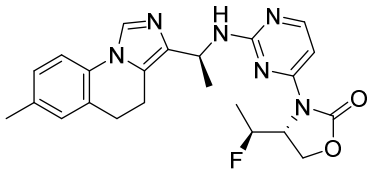
Compound **22** was synthesized from **22a** and **1g**. **22a** was synthesized from 2-amino-5-methoxyphenol in a similar progress to that described for the preparation of **2a.**



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.18 (d, *J* = 5.8 Hz, 1H), 7.84 (s, 1H), 7.44 (d, *J* = 5.8 Hz, 1H), 7.30 (d, *J* = 8.7 Hz, 1H), 6.62 – 6.54 (m, 2H), 5.82 (br, 1H), 5.34 – 4.92 (m, 4H), 4.75 – 4.56 (m, 1H), 4.48 (dd, *J* = 8.8, 3.3 Hz, 1H), 4.34 (t, *J* = 9.0 Hz, 1H), 3.76 (s, 3H), 1.59 (d, *J* = 6.9 Hz, 3H), 1.21 (br, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -197.64 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.85 , 159.02 , 158.59 , 157.14 , 154.18 , 147.10 , 138.38 , 130.02 , 117.83 , 117.52 , 116.08 , 108.56 , 103.83 , 99.33 , 87.26 (d, *J* = 174.4 Hz), 62.34 , 61.80 (d, *J* = 6.3 Hz), 57.67 (d, *J* = 19.5 Hz), 55.69 , 44.00 , 21.53 , 16.77 (d, *J* = 21.6 Hz). ES-LCMS m/z: 455.1 $(M+H)^{+}$. HRMS m/z: calcd for C22H24FN6O4 455.1838; found, 455.1836. 99.15% purity.

(R)-4-((S)-1-fluoroethyl)-3-(2-(((S)-1-(7-methyl-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)oxazolidin-2-one ***(23)***

Compound **23** was synthesized from **23a** and **1g**. **23a** was synthesized from 2-chloro-5-methylphenylboronic acid in a similar progress to that described for the preparation of **3a.**

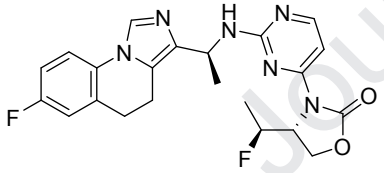


^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.18 (d, *J* = 5.7 Hz, 1H), 7.91 (s, 1H), 7.42 (d, *J* = 5.7 Hz, 1H), 7.26 (d, *J* = 8.6 Hz, 1H), 7.10 – 7.03 (m, 2H), 5.83 (br, 1H), 5.14 (br, 2H), 4.79 – 4.60 (m, 1H), 4.51 (dd, *J* = 8.9, 3.4 Hz, 1H), 4.36 (t, *J* = 8.9 Hz, 1H), 3.10 – 2.75 (m, 4H), 2.31 (s, 3H), 1.56 (d, *J* = 6.8 Hz, 3H), 1.30 (d, *J* = 18.9 Hz, 3H). ^{19}F NMR (376 MHz,

Chloroform-*d*) δ -197.88 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 160.82 , 159.03 , 157.06 , 154.27 , 138.07 , 135.61 , 131.55 , 130.44 , 130.01 , 128.22 , 127.85 , 122.46 , 115.47 , 98.93 , 87.29 (d, *J* = 174.1 Hz), 61.81 (d, *J* = 6.3 Hz), 57.71 (d, *J* = 19.5 Hz), 43.63 , 26.54 , 21.75 , 21.00 , 19.33 , 16.84 (d, *J* = 21.6 Hz). ES-LCMS m/z: 437.2 $(M+H)^+$. HRMS m/z: calcd for C23H26FN6O2 437.2096; found, 437.2094. 95.56% purity.

(R)-3-(2-(((S)-1-(7-fluoro-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethyl)amino)pyrimidin-4-yl)-4-((S)-1-fluoroethyl)oxazolidin-2-one ***(24)***

Compound **24** was synthesized from **24a** and **1g**. **24a** were synthesized from 2-Chloro-5-fluorobenzeneboronic acid in a similar progress to that described for the preparation of **2a.**



^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.21 (d, *J* = 5.7 Hz, 1H), 7.91 (s, 1H), 7.46 (d, *J* = 5.7 Hz, 1H), 7.36 (dd, *J* = 8.5, 4.7 Hz, 1H), 7.08 – 6.96 (m, 2H), 5.66 (d, *J* = 7.7 Hz, 1H), 5.17 (br, 2H), 4.70 (dd, *J* = 26.2, 7.4 Hz, 1H), 4.53 (dd, *J* = 8.8, 3.4 Hz, 1H), 4.38 (t, *J* = 8.9 Hz, 1H), 2.95 (d, *J* = 70.2 Hz, 4H), 1.58 (d, *J* = 6.8 Hz, 3H), 1.33 (dd, *J* = 22.9, 5.6 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -116.15 , -197.71 . ^{13}C NMR (101 MHz, Chloroform-*d*) δ 161.48 , 160.83 , 159.04 , 157.11 , 154.27 , 138.47 , 130.53 , 130.35 , 130.29 (d, *J* = 2.8 Hz), 122.25 , 117.09 (d, *J* = 8.5 Hz), 116.36 (d, *J* = 22.8 Hz), 114.51 (d, *J* = 23.2 Hz), 99.08 ,

87.28 (d, *J* = 174.3 Hz), 61.83 (d, *J* = 6.3 Hz), 57.73 (d, *J* = 19.6 Hz), 43.67 , 26.78 , 21.73 , 19.09 , 16.89 (d, *J* = 21.7 Hz). ES-LCMS m/z: 441.2 $(M+H)^+$. HRMS m/z: calcd for C22H23F2N6O2 441.1845; found, 441.1848. 100% purity.

Synthetic methods for amino side chains

(S)-1-(7-(trifluoromethyl)-4H-benzo[b]imidazo[1,5-d][1,4]oxazin-3-yl)ethan-1-amine ***(2a)***

2-amino-5-(trifluoromethyl)phenol (2.0 g, 11.29 mmol) and potassium carbonate (4.7 g, 34.00 mmol) were dissolved in anhydrous acetonitrile (20 mL) under N_2. Chloroacetyl chloride (1.4 g, 12.40 mmol) was added dropwise. The mixture was refluxed at 85 □ and stirred for 2 h. Then the mixture was concentrated by rotary evaporation. H_2O and EtOAc were added to the residue for extraction. The organic layer was dried over anhydrous Na_2SO_4 and purified via flash chromatography on silica gel to get 1.7 g (Yield 69.3%) of 7-(trifluoromethyl)-2*H*-benzo[*b*][1,4]oxazin-3(4*H*)-one **(2f)** as a powder. ES-LCMS m/z: 218.0 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.98 (s, 1H), 7.23 (d, *J* = 7.1 Hz, 2H), 6.95 (d, *J* = 8.3 Hz, 1H), 4.68 (s, 2H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.20.

2f (1.6 g, 7.37 mmol) was dissolved in anhydrous THF (24 mL) under N_2, in a Dewar flask at -30 □. At that temperature, 2.0 M NaHMDS (4.42 mL, 8.84 mmol, 2.0 M in THF) was added dropwise and the mixture was stirred for 30 min. Then diethyl chlorophosphite (2.54 g, 14.74 mmol) was added dropwise and the mixture was stirred at -30 □ for 30 min. After removal from the Dewar flask, the reaction was stirred at rt for another 30 min. It was then cooled to -30 □ and methyl isocyanoacetate (1.46 g, 14.74 mmol) and 2.0M NaHMDS (7.37 mL, 14.74 mmol) were added The temperature was slowly increased to rt and the mixture was stirred for

2 h. Saturated ammonium chloride solution was added to quench the reaction. The mixture was diluted with H_2O and extracted with EtOAc. The EtOAc solution was washed with saturated aqueous NaCl and dried over Na_2SO_4, filtered and concentrated in vacuo and purified via flash chromatography on silica gel to get 1.25 g (Yield 57.1%) of methyl 7-(trifluoromethyl)-4*H*-benzo[*b*]imidazo-[1,5-*d*][1,4]- oxazine-3- carboxylate **(2e)** as a yellow solid. ES-LCMS m/z: 299.0 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.08 (s, 1H), 7.59 (d, *J* = 8.3 Hz, 1H), 7.44 – 7.32 (m, 2H), 5.59 (s, 2H), 3.95 (s, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.78.

2e (1.15g, 3.87 mmol) was dissolved in anhydrous DCM (35 mL) under N_2, at -78 □. DIBALH (10.32 mL, 15.48 mmol, 1.5 M in toluene) was added to the solution dropwise. After 40 min, cold MeOH was added at -78□ to quench the reaction. Then saturated aqueous potassium sodium tartrate was added and the mixture was stirred overnight. The mixture was extracted with DCM. The organic layer was concentrated and purified via flash chromatography on silica gel to get 503 mg (Yield 48.6%) of 7-(trifluoromethyl)-4*H*-benzo[*b*]imidazo[1,5-*d*][1,4]oxazine-3-carbaldehyde **(2d)**. ES-LCMS m/z: 269.0 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.01 (s, 1H), 8.23 (s, 1H), 7.64 (d, *J* = 8.2 Hz, 1H), 7.45 – 7.37 (m, 2H), 5.60 (s, 2H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.85.

$Ti(OEt)_4$ (1.47 g, 6.44 mmol) was added to a stirred solution of **2d** (480 mg, 1.79 mmol) and (S)-(-)-2-Methyl-2-propanesulfinamide (390 mg, 3.22 mmol) in THF (3 mL) and the reaction

was stirred at rt overnight. Brine was added to the mixture and which was then extracted with EtOAc. The organic layer was separated and dried over Na_2SO_4. The solution was then concentrated and purified via flash chromatography on silica gel to get 550 mg (Yield 82.7%) of (S,E)-2-methyl-N-((7-(trifluoromethyl)-4*H*-benzo[*b*]imidazo-[1,5-*d*][1,4]oxazin-3-yl)methylene)propane-2-sulfinamide **(2c)**. ES-LCMS m/z: 372.0 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.66 (s, 1H), 8.11 (s, 1H), 7.59 (d, *J* = 8.1 Hz, 1H), 7.42 – 7.35 (m, 2H), 5.64 – 5.44 (m, 2H), 1.26 (s, 9H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.74.

MeMgBr (8.88 mL, 8.88 mmol) was added dropwise at -78 □, under N_2, to a stirred solution of **2c** (550 mg, 1.48 mmol) in anhydrous THF (30 mL). After 1 h, saturated aqueous ammonium chloride was added to quench the reaction and the mixture was extracted with EtOAc and H2O. The organic layer was concentrated and purified via flash chromatography on silica gel to get 453 mg (Yield 78.9%) of (S)-2-methyl-N-((S)-1-(7-(trifluoromethyl)-4*H*-benzo[*b*]imidazo[1,5-*d*][1,4]oxazin-3-yl)ethyl)propane-2-sulfi namide **(2b)**. ES-LCMS m/z: 388.0 $(M+H)^+$.

2b (453 mg, 1.17 mmol) was dissolved in MeOH (4.5 mL) under N_2. HCl (0.45 mL) was added and the mixture was stirred for 2 h at rt. H_2O was added and the mixture was concentrated in vacuum to remove the MeOH. 1N aqueous sodium hydroxide was added to pH11. The mixture was extracted with EtOAc and dried over anhydrous Na_2SO_4. The organic layer was filtered and concentrated in vacuum to get 287 mg (Yield 86.7%) of a viscous liquid. The residue **(2a)** was placed into small bottles for the next step. ES-LCMS m/z: 267.0

$(M\text{-}NH)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.96 (s, 1H), 7.52 (d, *J* = 8.3 Hz, 1H), 7.40 – 7.28 (m, 2H), 5.35 (s, 2H), 4.17 (q, *J* = 6.7 Hz, 1H), 1.90 (br, 2H), 1.45 (d, *J* = 6.6 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.54.

(S)-1-(7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethan-1-amine ***(3a)***

(2-chloro-5-(trifluoromethyl)phenyl)boronic acid (1.65 g, 7.35 mmol), acrylamide (0.52 g, 7.32 mmol), allylpalladium chloride dimer (134 mg, 0.37 mmol), chloro(1,5-cyclooctadiene)rhodium(I) dimer (145 mg, 0.29 mmol), X-PHOS(350 mg, 0.73 mmol) and potassium phosphate (3.40 g, 0.73 mmol) were dissolved in 2-methyl-2-butanol mixed with MeOH (10:1) (20 mL) The mixture was heated to 110 □ and stirred for 18 h. The cooled mixture was then filtered through a thin pad of silica gel and washed with EtOAc. The filtrate was concentrated and purified via flash chromatography on silica gel to get 1.2 g (Yield 73.8%) of 6-(trifluoromethyl)-3,4-dihydroquinolin-2(1*H*)-one **(3f)** as a pale yellow solid. ES-LCMS m/z: 216.0 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.34 (s, 1H), 7.44 (d, *J* = 7.8 Hz, 2H), 6.92 (d, *J* = 8.1 Hz, 1H), 3.03 (t, *J* = 7.6 Hz, 2H), 2.69 (dd, *J* = 8.4, 6.8 Hz, 2H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -61.93.

Fragment **3a** was obtained from **3f** in a process similar to that described for the preparation of **2a**. If the ester group is reduced to an alcohol, one or more equivalents Dess-Martin periodinane could be added to the solvent of alcohol product in DCM for 1 h. ES-LCMS m/z: 265.1 $(M\text{-}NH)^+$. ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.35.

(S)-1-(6-(trifluoromethyl)benzo[d]imidazo[5,1-b]thiazol-3-yl)ethan-1-amine ***(8a)***

2-bromo-4-(trifluoromethyl)aniline (8 g, 33.33 mmol.), CuI (318 mg, 1.67 mmol) and sodium trifluoromethanesulfinate (10.4 g, 66.66 mmol) was dissolved in toluene (100 mL) in a sealed bottle. The solution was bubbled with nitrogen and diethyl phosphite (9.206 g, 66.66 mmol) was added. The mixture was stirred at 110 □. After 16 h, the mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed by saturated aqueous NaCl and dried over anhydrous Na_2SO_4. It was filtered and concentrated in vacuo and then purified via flash chromatography on silica gel to get 5.04 g (Yield 53.6%) of 2-bromo-1-isothiocyanato-4-(trifluoromethyl)benzene **(8i)** as a light green solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.85 (s, 1H), 7.55 (d, *J* = 8.3 Hz, 1H), 7.35 (d, *J* = 8.3 Hz, 1H).

8i (5.04 g, 17.87 mmol), CuCl (177 mg, 1.79 mmol) and $CsCO_3$ (11.64 g, 35.74 mmol) was dissolved in toluene (50 mL) under N_2 in a round bottom flask. Then methyl isocyanoacetate was added and the mixture was warmed to 110 □ for 3 h. The mixture was concentrated and extracted with EtOAc and H_2O. The organic layer was washed by saturated aqueous NaCl and dried over anhydrous Na_2SO_4. It was filtered and concentrated in vacuo and then it was purified via flash chromatography on silica gel to get 2.33 g (Yield 43.5%) of methyl 6-(trifluoromethyl)benzo[*d*]imidazo[5,1-*b*]thiazole-3- carboxylate (**8e**) as a yellow solid. ES-LCMS m/z: 301.0 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.40 (s, 1H), 8.04 (s, 1H), 7.92 (d, *J* = 8.5 Hz, 1H), 7.79 (d, *J* = 8.4 Hz, 1H), 4.00 (s, 3H).

Fragment **8a** was obtained from **8e** in a process similar to that described for the preparation of **2a**. If the ester group is reduced to the alcohol, one or more equivalents Dess-Martin periodinane could be added to the solvent of the alcohol product in DCM for 1 h. ES-LCMS

m/z: 269.0 $(M\text{-}NH)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.27 (s, 1H), 7.84 (s, 1H), 7.72 (d, *J* = 8.4 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 1H), 4.29 (d, *J* = 6.2 Hz, 1H), 1.74 (br, 2H), 1.50 (d, *J* = 6.4 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -61.73.

*(S)-1-(6-chloro-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethan-1-amine (**9a**)*

3-chloro-2-iodoaniline (5 g, 19.73 mmol), AIBN (1.29 g, 7.89 mmol) and tributyltin (9.28 g, 31.88 mmol) were dissolved under N_2 in anhydrous DMSO (30 mL). The mixture was cooled to 0□ and methyl acrylate (8.2 g, 95.25 mmol) was added dropwise. The reaction mixture was heated to 120 □ and stirred for 12 h. After cooling to rt, cold H_2O was added to quench the reaction. EtOAc was added for extraction. The organic layer was washed with saturate aqueous NaCl and dried over Na_2SO_4. The mixture was then concentrated and purified via flash chromatography on silica gel to get 1.78 g (Yield 49.7%) of 5-chloro-3,4-dihydroquinolin-2(1H)-one (**9f**) as a brown-white solid ES-LCMS m/z: 182.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.61 (s, 1H), 7.09 (t, *J* = 7.9 Hz, 1H), 7.03 (dd, *J* = 8.1, 1.2 Hz, 1H), 6.78 (dd, *J* = 7.7, 1.1 Hz, 1H), 3.13 – 3.03 (m, 2H), 2.65 (dd, *J* = 8.4, 7.0 Hz, 2H).

Fragment **9a** was obtained from **9f** in a process similar to that described for the preparation of **2a**. If the ester group is reduced to alcohol, one or more equivalents Dess-Martin periodinane could be added to the solution of the alcohol product in DCM for 1 h. ES-LCMS m/z: 231.0 $(M\text{-}NH)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.96 (s, 1H), 7.41 – 7.31 (m, 1H), 7.30 – 7.21 (m, 2H), 4.15 (p, *J* = 6.8 Hz, 1H), 3.01 (dtdd, *J* = 29.6, 14.9, 11.9, 6.8 Hz, 4H), 2.30 (br, 2H), 1.50 (d, *J* = 6.7 Hz, 3H).

(1S)-1-(5-methyl-7-(trifluoromethyl)-4,5-dihydroimidazo[1,5-a]quinolin-3-yl)ethan-1-amine

(10a)

[2-amino-5-(trifluoromethyl)phenyl]boronic acid (5 g, 24.40 mmol), trans-methyl crotonate (1.22 g, 12.20 mmol), chloro(1,5-cyclooctadiene)rhodium(I) dimer (183 mg, 0.37 mmol) and potassium hydroxide (5.18 g, 24.40 mmol) were dissolved under N_2 in anhydrous 1,4-dioxane (34 mL). The mixture was refluxed for 6 h. After cooling to rt, the reaction mixture was filtered through a pad of silica gel and washed with EtOAc. The filtrate was washed with saturated aqueous ammonium chloride, The organic layer was dried over Na_2SO_4. The mixture was then concentrated and purified via flash chromatography on silica gel to get 713 mg (Yield 12.7%) 4-methyl-6-(trifluoromethyl)-3,4-dihydroquinolin-2(1H)-one (**10f**) as yellow-brown oily liquid. ES-LCMS m/z: 230.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.04 (s, 1H), 7.40 (s, 1H), 6.95 (d, *J* = 8.2 Hz, 1H), 6.49 (d, *J* = 8.4 Hz, 1H), 3.12 (p, *J* = 6.6 Hz, 1H), 2.70 (dd, *J* = 16.3, 5.9 Hz, 1H), 2.41 (dd, *J* = 16.3, 7.4 Hz, 1H), 1.30 (d, *J* = 7.0 Hz, 3H).

Fragment **10a** was obtained from **10f** in a progress similar to that described for the preparation of **2a**. If the ester group is reduced to alcohol, one or more equivalents Dess-Martin periodinane could be added to the solution of the alcohol product in DCM for 1 h. ES-LCMS m/z: 279.1 $(M-NH)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.97 (s, 1H), 7.55 (d, *J* = 8.0 Hz, 2H), 7.49 (d, *J* = 8.2 Hz, 1H), 4.15 – 4.05(m, 1H), 3.17 – 3.07 (m, 1H), 3.06 – 2.96 (m, 1H), 2.82 – 2.69 (m, 1H), 1.99 (br, 2H), 1.44 (d, *J* = 6.7 Hz, 3H), 1.27 (dd, *J* = 6.9, 3.8 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.27.

(S)-1-(8-(trifluoromethyl)-5,6-dihydro-4H-benzo[f]imidazo[1,5-a]azepin-3-yl)ethan-1- amine

(13a)

A round-bottom flask was fitted with a stirbar, and 4-amino-3-bromobenzotrifluoride (15 g, 62.49 mmol) and $Pd(PPh_3)_4$ (2.16 g, 1.87 mmol) . DMF (150 mL) and allyltributyltin (21.46 ml, 69.23 mmol) were added under N_2 in that order, giving a mixture that was then warmed to 80 □. After 19 h, the reaction mixture was cooled to rt, and extracted with EtOAc and H_2O. The organic extracts were then combined and washed with aqueous saturated NaCl, and dried over Na_2SO_4, filtered and concentrated in vacuo that was purified via flash chromatography on silica gel to get 8.147g (Yield 65%) of 2-allyl-4-(trifluoromethyl)aniline (**13l**) as a brown oil. ES-LCMS m/z: 202.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.35 – 7.29 (m, 2H), 6.70 (d, *J* = 8.0 Hz, 1H), 5.95 (ddt, *J* = 16.4, 10.1, 6.1 Hz, 1H), 5.26 – 5.08 (m, 2H), 3.98 (br, 2H), 3.33 (d, *J* = 6.1 Hz, 2H).

13l (8 g, 39.76 mmol) was dissolved under N_2 in THF (80 mL). Triethylamine (6.84 g, 67.59 mmol) and acrylyl chloride (4.32 g, 47.71 mmol) were added dropwise at 0 □. The mixture was stirred and the temperature was increased to rt slowly over 1 h. Another 1 h later, the mixture was concentrated and H_2O and EtOAc were added for extraction. The organic layer was washed with saturated aqueous NaCl then dried over Na_2SO_4. It was then concentrated and purified via flash chromatography on silica gel to get 5.9 g (Yield 58.1%) N-(2-allyl-4-(trifluoromethyl)phenyl)acrylamide (**13k**) as a white solid. ES-LCMS m/z: 256.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.35 – 8.19 (m, 1H), 7.57 – 7.48 (m, 2H), 7.45 (s, 1H), 6.42 (dd, *J* = 16.9, 0.8 Hz, 1H), 6.19 (dd, *J* = 16.9, 10.3 Hz, 1H), 5.99 (ddt, *J* =

16.2, 10.1, 6.1 Hz, 1H), 5.81 (dd, *J* = 10.3, 0.9 Hz, 1H), 5.29 (dd, *J* = 10.1, 1.4 Hz, 1H), 5.23 – 5.10 (m, 1H), 3.47 (d, *J* = 6.0 Hz, 2H).

A round-bottom flask containing the product **13k** (5.9 g, 23.11 mmol) was fitted with a stirbar and dichloromethane (150 mL) and Grubbs II catalyst (1.18 g, 1.39 mmol) were added, and the resulting solution was stirred for 18 h at rt. The mixture was then concentrated in vacuo and purified via flash chromatography on silica gel to get 3.51 g (Yield 66.8%) of 7-(trifluoromethyl)-1,5-dihydro-2*H*-benzo[*b*]azepin-2-one (**13j**) as a purple solid. ES-LCMS m/z: 228.0 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.76 (br, 1H), 7.50 (d, *J* = 8.2 Hz, 1H), 7.42 (s, 1H), 7.26 (d, *J* = 8.5 Hz, 1H), 6.67 (dt, *J* = 11.0, 6.8 Hz, 1H), 6.03 (d, *J* = 10.9 Hz, 1H), 3.45 (d, *J* = 6.8 Hz, 2H).

A round-bottom flask containing the product of **13j** (3.51 g, 15.45 mmol) was fitted with a stirbar. Tetrahydrofuran (30 mL), EtOH (30 mL) and 10% Pd/C (512 mg) were added under N_2. The reaction vessel was flushed with H_2, then placed under a balloon of H_2 and stirred at room temperature for 18 h. The reaction mixture was then filtered through celite and concentrated in vacuo to give a solid that was purified via flash chromatography on silica gel to get 3.153 g (Yield 89.0%) of 7-(trifluoromethyl)-1,3,4,5-tetrahydro-2*H*-benzo[*b*]azepin-2-one (**13f**) as a white solid. ES-LCMS m/z: 230.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.15 – 7.90 (m, 1H), 7.55 – 7.45 (m, 2H), 7.08 (d, *J* = 8.5 Hz, 1H), 2.87 (t, *J* = 7.2 Hz, 2H), 2.40 (t, *J* = 7.2 Hz, 2H), 2.28 (p, *J* = 7.9, 7.3 Hz, 2H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.20.

Fragment **13a** was obtained from **13f** in a process similar to that described for the preparation

of **2a**. If the ester group is reduced to alcohol, one or more equivalents of Dess-Martin periodinane could be added to the solvent of the alcohol product in DCM for 1 h. ES-LCMS m/z: 279.1 $(M\text{-}NH)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.66 (s, 1H), 7.61 (d, *J* = 8.2 Hz, 1H), 7.57 (s, 1H), 7.40 (d, *J* = 8.1 Hz, 1H), 4.17 – 4.02 (m, 1H), 2.73 – 2.57 (m, 4H), 2.13 (tt, *J* = 13.4, 6.5 Hz, 2H), 2.05 – 1.90 (br, 2H), 1.46 (d, *J* = 6.6 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.37.

*(S)-3-(1-aminoethyl)-4H-benzo[b]imidazo[1,5-d][1,4]oxazine-7-carbonitrile (**12a**)*

(S)-N-((S)-1-(7-bromo-4*H*-benzo[*b*]imidazo[1,5-*d*][1,4]oxazin-3-yl)ethyl)-2-methylpropane-2-sulfinamide (**25b**) was obtained from 2-amino-5-bromophenol in a similar progress to that described for the preparation of **2a**. If the ester group is reduced to the alcohol, one or more equivalents of Dess-Martin periodinane could be added to the solution of the alcohol product in DCM for 1 h. ES-LCMS m/z: 398.0 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.90 (s, 1H), 7.30 – 7.24 (m, 2H), 7.17 (dd, *J* = 8.5, 2.1 Hz, 1H), 5.29 – 5.21 (m, 2H), 4.52 (p, *J* = 6.6 Hz, 1H), 3.85 (d, *J* = 6.0 Hz, 1H), 1.57 (d, *J* = 6.7 Hz, 3H), 1.22 (s, 9H).

25b (200 mg, 0.50 mmol), tetrakis(triphenylphosphine)palladium (35 mg, 0.03 mmol) and zinc cyanide (35 mg, 0.30 mmol) were dissolved under N_2 in anhydrous DMF (1 mL). The reaction mixture was heated to 80 □ for 6 h. After cooling, 2 N ammonium hydroxide and EtOAc were added for extraction. The organic layer was washed by saturated aqueous NaCl and dried over Na_2SO_4. Then it was concentrated and purified via flash chromatography on silica gel to get 166.7 mg (Yield 97.1%) of (S)-N-((S)-1-(7-cyano-4*H*-benzo[*b*]imidazo[1,5-*d*][1,4]oxazin-3-yl)ethyl)-2-methylpropane-2-sulfinamide (**12b**) as a

light yellow liquid. ES-LCMS m/z: 345.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.02 (s, 1H), 7.54 (d, *J* = 8.7 Hz, 1H), 7.35 (dq, *J* = 4.0, 1.7 Hz, 2H), 5.41 – 5.27 (m, 2H), 4.51 (p, *J* = 6.7 Hz, 1H), 4.00 (d, *J* = 6.7 Hz, 1H), 1.58 (d, *J* = 6.8 Hz, 3H), 1.24 (s, 9H).

Fragment **12a** was obtained from **12b** in a process similar to that described for the preparation of **2a** but the HCl was changed to a solution in dioxane of hydrochloride acid, with the same volume. ES-LCMS m/z: 224.1 $(M-NH)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.99 (s, 1H), 7.56 – 7.51 (m, 1H), 7.39 – 7.34 (m, 2H), 5.40 – 5.36 (m, 2H), 4.17 (q, *J* = 6.7 Hz, 1H), 1.73 (br, 2H), 1.45 (d, *J* = 6.6 Hz, 3H).

*(S)-1-(7-cyclopropyl-4H-benzo[b]imidazo[1,5-d][1,4]oxazin-3-yl)ethan-1-amine (**11a**)*

25b (140 mg, 0.35 mmol), palladium (II) acetate (11 mg, 0.018 mmol), butyldi-1-adamantylphosphine (10 mg, 0.028 mmol), potassium cyclopropyltrifluoroborate (78 mg, 0.53 mmol) and cesium carbonate (342 mg, 1.05 mmol) were dissolved under N_2 in mixed toluene and H_2O solvent (10:1) (5mL). The reaction mixture was heated to 100 □ for 7 h. After cooling, H_2O and EtOAc were added for extraction. The organic layer was washed with saturated aqueous NaCl and dried over Na_2SO_4. Then it was concentrated and purified via flash chromatography on silica gel to get 86.9 mg (Yield 69.0%) of (S)-N-((S)-1-(7-cyclopropyl-4*H*-benzo[*b*]imidazo[1,5-*d*][1,4]oxazin-3-yl)ethyl)-2-methylpropane-2-sulfinamide (**11b**) as a yellow solid. ES-LCMS m/z: 360.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.88 (s, 1H), 7.34 – 7.23 (m, 1H), 6.81 – 6.71 (m, 2H), 5.29 – 5.15 (m, 2H), 4.53 (p, *J* = 6.6 Hz, 1H), 3.91 (d, *J* = 5.4 Hz, 1H), 1.86 (ddd, *J* = 13.4, 8.4, 5.1 Hz, 1H), 1.57 (d, *J* = 6.7 Hz, 3H), 1.22 (s, 9H), 1.03 – 0.91 (m, 2H), 0.74 – 0.61 (m,

2H).

Fragment **11a** was obtained from **11b** in a process similar to that described for the preparation of **2a** except the HCl was changed to dioxane solution of hydrochloride acid with the same volume. ES-LCMS m/z: 239.2 $(M\text{-}NH)^+$. ^1H NMR (400 MHz, Chloroform-*d*) δ 7.88 (s, 1H), 7.32 – 7.27 (m, 1H), 6.80 – 6.75 (m, 2H), 5.24 (s, 2H), 4.17 – 4.10 (m, 1H), 1.91 – 1.78 (m, 3H), 1.45 (d, *J* = 6.6 Hz, 3H), 1.01 – 0.94 (m, 2H), 0.72 – 0.65 (m, 2H).

*(S)-1-(7-(trifluoromethyl)-4H-benzo[b]imidazo[1,5-d][1,4]thiazin-3-yl)ethan-1-amine (**21a**)*

6-(Trifluoromethyl)benzo[*d*]thiazol-2-amine (6.8 g, 31.16 mmol) was dissolved in THF (60 mL) under N_2. Isoamyl nitrite (8.03 g, 68.55 mmol) was added dropwise and the mixture was refluxed for 1.5 h. Then the mixture was poured into ice-H_2O (60 mL) and extracted with EtOAc. The organic layer was washed by saturated aqueous NaCl and dried over Na_2SO_4. It was then concentrated and purified via flash chromatography on silica gel to get 6 g (Yield 43.2%) of 6-(trifluoromethyl)benzo[*d*]thiazole (**21n**) as a yellow oil. ES-LCMS m/z: 204.0 $(M+H)^+$.

21n was dissolved under N_2 in EtOH (72 mL). 85% hydrazine hydrate (13.2 g, 223.25 mmol) was added and the mixture was refluxed for 3 h. It was then cooled to 0 □ and H_2O (60 mL) was added. 50% acetic acid aqueous solution was added to pH 6-7 and the mixture was extracted with DCM. The organic layer was washed with saturated aqueous NaCl and dried over Na_2SO_4. Then it was concentrated to get 5.6 g (Yield 98.2%) of 2-amino-5-(trifluoromethyl)benzenethiol (**21m**) as a yellow oil without further purification. ES-LCMS m/z: 194.0 $(M+H)^+$.

21m (5 g, 25.88 mmol) and sodium acetate trihydrate (3.2 g, 23.52 mmol) were dissolved under N_2 in EtOH (50 mL). Chloroacetic acid (2.9 g, 30.69 mmol) was added dropwise and the mixture was refluxed for 2.5 h. After reaction was complete, the mixture was poured into ice-H2O (100 mL) and stirred for 30 min. It was then filtered and the precipitate was purified via flash chromatography on silica gel to get 3.7 g (Yield 61.4%) of 7-(trifluoromethyl)-2H-benzo[*b*][1,4]thiazin-3(4*H*)-one (**21f**) as a yellow solid. ES-LCMS m/z: 234.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.36 (s, 1H), 7.59 (s, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.00 (d, *J* = 8.3 Hz, 1H), 3.48 (s, 2H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.27.

Fragment **21a** was obtained from **21f** in a process similar to that described for the preparation of **2a.** If the ester group is reduced to alcohol, one or more equivalents Dess-Martin periodinane could be added to the solution of the alcohol product in DCM for 1 h. ES-LCMS m/z: 283.0 $(M-NH)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.97 (s, 1H), 7.71 (s, 1H), 7.57 – 7.48 (m, 2H), 4.21 (q, *J* = 6.1 Hz, 1H), 4.09 (s, 2H), 2.24 (br, 2H), 1.49 (d, *J* = 6.6 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -62.62.

Compounds with other amino side chains including **1a** and **22a** were synthesized in a process similar to that described for the preparation of **2a**. Compounds **4a, 14a, 15a, 23a** and **24a** were synthesized in a process to similar that described for the preparation of **3a**.

Synthetic methods for oxazolidinone fragments.

*(R)-4-((S)-1-fluoroethyl)-3-(2-fluoropyrimidin-4-yl)oxazolidin-2-one (**1g**)*

N-benzyloxycarbonyl-O-*tert*-butyl-L-threonine dicyclohexylamine salt (10.0 g, 20.38 mmol) was dissolved in THF (200 mL) under N_2 and cooled to -25 □. Isobutyl chloroformate (3.3 g, 24.16 mmol) and N-methylmorpholine (2.5 g, 24.71 mmol) were added and the mixture was stirred at -25 °C for 15 min. It was then filtered and the filtrate was cooled to -20 °C, sodium borohydride (1.5 g, 39.65 mmol) was added, and H_2O (40 mL) was slowly added dropwise over about 0.5-1 hour. After the dropwise addition was completed, the reaction mixture was allowed to warm to rt then stirred for 30 min. The reaction solution was poured into of ice-H2O (200 mL), and extracted twice with EtOAc (200 mL). The organic layer was combined, washed by H_2O and saturated aqueous NaCl. It was then dried over Na_2SO_4 and concentrated to get benzyl ((2R,3R)-3-(*tert*-butoxy)-1-hydroxybutan-2-yl)- carbamate (**1r**) which was used directly in the next step without further purification. ES-LCMS m/z: 318.2 $(M+Na)^+$.

1r (6.0 g, 20.31 mmol) was dissolved in DMF (100 mL) under N_2 and cooled to 0°C. Sodium hydride (60%, 1.6 g, 40.00 mmol) was added portionwise and stirred for 30 min. p-Methoxybenzyl chloride (4.8 g, 30.65 mmol) and tetrabutylammonium iodide (0.8 g, 2.16 mmol) were added and the reaction mixture was warmed to rt and stirred overnight. After the reaction was completed, the reaction solution was quenched in ice-H_2O (200 mL) then extracted with EtOAc. The organic layer was washed by H_2O and saturated aqueous NaCl and dried over Na_2SO_4. Then it was concentrated and purified via flash chromatography on silica gel to get 5.9 g (Yield 94.5%) of (R)-4-((R)-1-(*tert*-butoxy)ethyl)-3-(4-methoxy-benzyl)oxazolidin-2-one (**1q**) as a yellow oil. ES-LCMS m/z: 308.1 $(M+H)^+$.

1q (5.9 g, 19.19 mmol) was dissolved in DCM (40 mL), trifluoroacetic acid (40 mL) was added, and the mixture was stirred under N_2 for 30 min. The reaction solution was concentrated, and the concentrate was mixed with DCM (50 mL). This was repeated three times then the trifluoroacetic acid was removed. The product was purified via flash chromatography on silica gel to get 3.4g (Yield 70.7%) of (R)-4-((R)-1-hydroxy-ethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (**1p**) as a white solid. ES-LCMS m/z: 252.1 $(M+H)^+$.

1p (3.4 g, 13.53 mmol) was dissolved in MeCN (45 mL) and cooled to 0 ° C under N_2. Nonafluorobutanesulfonyl fluoride (7.4 mL, 40.59 mmol), triethylamine (17.0 mL, 121.77 mmol) and triethylamine trihydrofluoride (6.8 mL, 40.59 mmol), were added and stirring was continued at 0 ° C for 1 h. The reaction mixture was poured into ice-H_2O (90 mL) and extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl and dried over Na_2SO_4. Then it was concentrated and purified via flash chromatography on silica gel to get 2.8 g (Yield 81.4%) of (R)-4-((S)-1-fluoroethyl)-3-(4-methoxybenzyl)oxazolidin-2-one (**1o**) as an oil. ES-LCMS m/z: 254.1 $(M+H)^+$.

1o (2.8 g, 11.05 mmol) was added to trifluoroacetic acid (56 mL) and the mixture was heated to 65 □ overnight. After the reaction was completed, the reaction mixture was concentrated, and the residue was purified via flash chromatography on silica gel to get 1.4 g (Yield 95.1%) of (R)-4-((S)-1-fluoroethyl)oxazolidin-2-one (**1h**) as a light blue oil. ES-LCMS m/z: 134.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.24 (s, 1H), 4.69 – 4.40 (m, 2H), 4.31 (dd, *J* = 9.1, 4.9 Hz, 1H), 3.87 (ddt, *J* = 13.5, 9.4, 5.0 Hz, 1H), 1.31 (dd, *J* = 24.2, 6.3 Hz, 3H).

1h (850 mg, 6.38 mmol) and 2,4-difluoropyrimidine (740 mg, 6.37 mmol) were added to DMF (10 mL) under N_2 and the mixture was cooled to 0□. After adding sodium hydride (310 mg, 7.75 mmol) and stirring for 30 min, the reaction mixture was warmed to rt and stirred for 2 h. After the reaction was completed, the reaction mixture was poured into ice-H2O (30 mL) then extracted with EtOAc. The organic phase was combined and washed once with H_2O (60 mL) and once with brine (60 mL). The organic layer was washed with H_2O and saturated aqueous NaCl and dried over Na_2SO_4. it was then concentrated and purified via flash chromatography on silica gel to get 1.1 g (Yield 75.2%) of white crystalline solid (**1g**). ES-LCMS m/z: 230.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.50 (dd, *J* = 5.8, 2.1 Hz, 1H), 8.18 (dd, *J* = 5.8, 3.7 Hz, 1H), 5.32 (dqd, *J* = 49.5, 6.6, 1.2 Hz, 1H), 4.77 (dddd, *J* = 26.6, 9.1, 3.4, 1.2 Hz, 1H), 4.64 (dd, *J* = 9.0, 3.4 Hz, 1H), 4.50 (td, *J* = 9.0, 1.1 Hz, 1H), 1.42 (dd, *J* = 23.2, 6.6 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -43.67 , -197.57 (dq, *J* = 49.9, 26.6, 25.0 Hz).

*(S)-3-(2-fluoropyrimidin-4-yl)-4-isopropyl-5,5-dimethyloxazolidin-2-one (**18g**)*

Methyl N-(tert-butoxycarbonyl)-L-valinate (5 g, 21.62 mmol) was dissolved in anhydrous THF (50 mL) under N_2. A solution of methyl magnesium bromide (25.2 mL, 75.67 mmol, 3.0 M in Et_2O) was added dropwise at 0 °C. After 10 min, the ice bath was removed and the mixture was stirred at rt for 48 h. The mixture was then quenched by dropwise addition of a saturated ammonium chloride solution at 0 □ and extracted with EtOAc, dried over Na_2SO_4 and concentrated. Potassium *tert*-butoxide (222 mg, 1.99 mmol) was added to the residue and dissolved in anhydrous THF (13 mL). The mixture was stirred at rt for 3 h. Saturated

ammonium chloride solution was added and the mixture was extracted with EtOAc. The organic layer was washed with H_2O and saturated aqueous NaCl and dried over Na_2SO_4. It was then concentrated and purified via flash chromatography on silica gel to get 148.7 mg of (S)-4-isopropyl-5,5-dimethyloxazolidin-2-one (**18h**) as a white solid. ES-LCMS m/z: 158.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.34 (s, 1H), 3.20 (d, *J* = 8.5 Hz, 1H), 1.89 – 1.74 (m, 1H), 1.48 (s, 3H), 1.38 (s, 3H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 3H).

Fragment **18g** was obtained from **18h** in a process similar to that described for the preparation of **1g.** ES-LCMS m/z: 254.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.44 (dd, *J* = 5.8, 2.3 Hz, 1H), 8.13 (dd, *J* = 5.8, 3.8 Hz, 1H), 4.54 (d, *J* = 3.5 Hz, 1H), 2.27 (ddp, *J* = 10.4, 7.0, 3.5 Hz, 1H), 1.57 (s, 3H), 1.42 (s, 3H), 1.01 (d, *J* = 7.1 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -44.13.

*6-(2-fluoropyrimidin-4-yl)-4-oxa-6-azaspiro[2.4]heptan-5-one (**19g**)*

1-Aminomethyl-1-cyclopropanol (5 g, 57.39 mmol) and carbonyldiimidazole (9.31 g, 57.39 mmol) were dissolved in anhydrous THF (170 mL) and stirred at rt overnight. The mixture was concentrated to get 7.24 g of 4-oxa-6-azaspiro[2.4]heptan-5-one (**19h**), as a yellow oil which was used directly in the next step without further purification. ES-LCMS m/z: 114.2 $(M+H)^+$.

Fragment **19g** was obtained from **19h** in a process similar to that described for the preparation of **1g.** ES-LCMS m/z: 210.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.47

(dd, *J* = 5.7, 2.1 Hz, 1H), 8.14 (dd, *J* = 5.7, 3.8 Hz, 1H), 4.27 (s, 2H), 1.38 – 1.30 (m, 2H), 0.92 – 0.85 (m, 2H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -43.97.

*(4S)-3-(2-fluoropyrimidin-4-yl)-4-isopropyl-5-methyloxazolidin-2-one (**17g**)*

N-Boc-L-valine (5 g, 23.01 mmol) was dissolved in anhydrous DCM (100 mL). DCC (6.17 g, 29.91 mmol) and HOBT (3.42 g, 25.31 mmol) were added and 10 min later, morpholine (3 g, 34.52 mmol) was added and the mixture was stirred at rt for 17 h. It was then filtered, and the filtrate was washed with 1 M aqueous HCl and saturated aqueous $NaHCO_3$. It was then dried over Na_2SO_4 and purified via flash chromatography on silica gel to get 5.43 g (Yield 82.4%) of *tert*-butyl (S)-(3-methyl-1-morpholino-1-oxobutan- 2-yl)carbamate (**17u**) as a transparent viscous liquid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.31 (d, *J* = 9.1 Hz, 1H), 4.37 (dd, *J* = 9.2, 5.9 Hz, 1H), 3.74 – 3.41 (m, 8H), 1.88 (dq, *J* = 13.2, 6.7 Hz, 1H), 1.38 (s, 9H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.85 (d, *J* = 6.8 Hz, 3H).

17u (5.43 g, 18.96 mmol) was dissolved in anhydrous THF (90 mL) under N_2. Methyl magnesium bromide solution (47.4 mL, 47.40 mmol, 1.0 M) in THF was added dropwise at 0□. The mixture was stirred at 0 ° C for 1 h and at rt for 1 h. It was quenched with 1 M diluted aqueous HCl at 0 □ and extracted with Et_2O. The extract was concentrated and purified via flash chromatography on silica gel to get 728 mg (Yield 17.8%) of *tert*-butyl (S)-(2-methyl-4-oxopentan-3-yl)carbamate (**17t**) as a light yellow liquid. ES-LCMS m/z: 116.2 $(M+H-100)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.12 (d, *J* = 7.2 Hz, 1H), 4.24 (dd, *J* = 8.5, 3.8 Hz, 1H), 2.15 (s, 4H), 1.39 (s, 9H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.74 (d, *J* = 6.9 Hz, 3H).

17t (728 mg, 3.38 mmol) was dissolved in anhydrous MeOH (7 mL) at 0□. Sodium borohydride (256 mg, 6.76 mmol) was added portionwise. The mixture was stirred at rt for 1 h. The reaction was then quenched with 1 M aqueous HCl and extracted with EtOAc and H_2O. The organic layer was dried over Na_2SO_4 and concentrated to get 765 mg of *tert*-butyl ((3S)-2-hydroxy-4-methylpentan-3-yl)carbamate (**17s**) as a white solid. The residue was used directly in the next step without further purification. ES-LCMS m/z: 118.2 $(M+H-100)^+$.

Fragment **17g** was obtained from **17s** in a process to similar that described for the preparation of **18g.** ES-LCMS m/z: 240.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.45 (dd, *J* = 5.7, 2.1 Hz, 1H), 8.13 (dd, *J* = 5.6, 3.9 Hz, 1H), 4.88 – 4.75 (m, 2H), 2.30 (heptd, *J* = 6.9, 2.8 Hz, 1H), 1.62 – 1.55 (m, 3H), 1.04 (d, *J* = 7.1 Hz, 3H), 1.00 (d, *J* = 7.0 Hz, 3H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -44.09.

*1.15. (S)-7-(2-fluoropyrimidin-4-yl)-8-phenyl-2,5-dioxa-7-azaspiro[3.4]octan-6-one (**16g**)*

3-oxetanone (1 g, 13.88 mmol), triethylamine (281 mg, 2.78 mmol) were placed in a round-bottom flask TMS-CN (1.65 g, 16.66 mmol) was added slowly (exothermic). After stirring for 1 h, the mixture was concentrated and the residue was dissolved in Et_2O (10 mL) then a solution of phenylmagnesium bromide (6.01 mL, 18.04 mmol, 3.0 M) in Et_2O was added. Et_2O (5 mL) was added and the mixture was stirred for 4 h. Slowly added 3mL MeOH (3 mL) and sodium borohydride (630 mg, 16.66 mmol) were added slowly. MeOH (12 mL) was then added slowly, with gassing and then the mixture was stirred overnight. H_2O (6 mL) and 10% aqueous HCl (20 mL) were added carefully and, after 4 h of vigorous stirring, Et_2O was added. The organic layer was extracted with 10% aqueous HCl (20 mL), and the aqueous

layer was washed twice with Et_2O. 6N sodium hydroxide solution was added to the aqueous, and the milky white solution was extracted once with DCM, once with EtOAc/THF (1:1) and twice with EtOAc. Then it was washed separately with saturated $NaHCO_3$ aqueous and dried over Na_2SO_4. It was then concentrated to get 1.99 g of (S)-7-(2-fluoropyrimidin-4-yl)-8-phenyl-2,5-dioxa-7- azaspiro[3.4]octan-6-one (**16v**) as a yellow liquid. ES-LCMS m/z: 180.1 $(M+H)^+$. The residue was used directly in the next step without further purification.

Fragment **16g** was obtained from **16v** in a process similar to that described for the preparation of **19g.** ES-LCMS m/z: 302.1 $(M+H)^+$. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.46 (dd, *J* = 5.7, 2.1 Hz, 1H), 8.13 (dd, *J* = 5.7, 3.7 Hz, 1H), 7.43 – 7.28 (m, 5H), 5.87 (s, 1H), 5.00 (dd, *J* = 7.8, 0.8 Hz, 1H), 4.88 (dd, *J* = 7.8, 0.9 Hz, 1H), 4.68 (dd, *J* = 8.6, 0.9 Hz, 1H), 4.20 (dd, *J* = 8.6, 1.0 Hz, 1H). ^{19}F NMR (376 MHz, Chloroform-*d*) δ -43.59.

Other fragments include **3g, 6g** and **7g** which were synthesized in a process similar to that described for the preparation of **1g.** Compound **20g** was synthesized in a process similar to that described for the preparation of **19g**.

Molecular docking

The moleclue docking was performed with Schrodinger Maestro. IDH1 protein structure was extracted from published IDH1 structure (PDB: 6b0z). The IDH1 protein and ligand were prepared by following all steps in Maestro Protein Preparation Wizard. Protein and ligand protonation states were set at pH 7.0 and at pH 7.0+/-2.0 using PROPKA and Epik,

respectively. The H-bond assignment was optimized by choosing sample water orientations. The structure was split into protein and ligand, and then the receptor grid was created by Glide centered on IDH-305. Compound molecules were docked to the protein and scored the five conformations with the lowest energy. The interaction between ligands and protein was reported as default setting. The dihedral angels was measured in the conformation with the lowest energy.

Biological Evaluation

Expression and purification of IDH1 wt and mutants. IDH1 wt and mutants were purified as described before.[36] Recombinant proteins were overexpressed in *E. coli* BL21 by addition of 1 mM IPTG overnight at 18 °C. Cells were resuspended in 20 mM Tris (pH 7.4), containing 500 mM NaCl, 1 mM phenylmethylsulphonyl fluoride (PMSF), 0.1% Triton X-100, 5 mM β-mercaptoethanol and 10% glycerol and lysed using a microfluidizer. The lysate was loaded onto a $Ni2^{+}$ column (GE Healthcare). After washing with 10 column volumes of 20 mM Tris (pH 7.4), 500 mM NaCl, 5 mM β-mercaptoethanol, 50 mM imidazole and 10% glycerol, the protein was eluted with elution buffer (20 mM HEPES, pH 7.4, 250 mM NaCl, 250 mM imidazole, 10% glycerol) and loaded on a Superdex200(GE Healthcare) gel filtration column (GE Healthcare) equilibrated with 50 mM Tris (pH 7.5), 200 mM NaCl , 5 mM β-mercaptoethanol and 10% glycerol. Protein purity was assessed by SDS-PAGE.

Cell culture Cells were grown in DMEM supplemented with 10% heat-inactivated fetal calf

serum and 1% penicillin/streptomycin (P/S) in humidified atmosphere at 37□ with 5% CO_2.

Recombinant IDH1 Enzyme Assays. Briefly, compounds were added to the plate followed by 200 ng IDH1-R132C in 50 μL 1X Buffer (50 mM K_2HPO_4, 40 mM $NaHCO_3$, 5 mM $MgCl_2$, 10% glycerol, 0.03% BSA). After 60 min of incubation at room temperature, 50 μL of substrate mix (1x Buffer, 20 μM NADPH, 2 mM α-KG) was added to initiate the reaction. The plate was rapidly transferred to a Spectramax plate reader and NADPH consumption was measured for 60 min (excitation =340 nm, emission = 450 nm) in kinetic mode. Then 50 μL of diaphorase/resazurin-coupled detection buffer (1x Buffer, 36 μg/mL diaphorase, 30 μM resazurin) was added. The plate was transferred to a Spectramax plate reader after 10 min and the fluorescence product resorufin was measured (excitation= 540 nm, emission = 590 nm) in endpoint mode. 1X Buffer for IDH1-R132H enzyme assay was 150 mM NaCl, 20 mM Tris-HCl, 10 mM $MaCl_2$, 0.05% BSA. Compounds were allowed to equilibrate with 100 ng IDH1-R132H for 60 min before addition of the substrates (1x Buffer, 20 μM NADPH, 1 mM α-KG) and detection reagents (1x Buffer, 36 μg/mL diaphorase, 30 μM resazurin).

2-HG production in HT1080 cell assay. HT1080 cells were plated in 24 well plates and incubated overnight. Compounds were prepared in 100% dimethyl sulfoxide (DMSO) and added to each well. After 48 hours of incubation with compounds, the supernatants were removed and filtered using a 10 kD spin column (Millipore) to remove enzymatic activity. The flow was used for measurement. Determination of 2-HG produced by cells with inhibitors was performed using a PicoProbeTM D-2-Hydroxyglutarate (D2HG) Assay Kit from BioVison. The enzymatic reactions were incubated for 60 min at 37°C and measured at

the fluorescence at Ex/Em = 535/587 nm in a Spectramax plate reader.

Pharmacokinetics Studies in rodents. Male ICR mice (6-8 weeks, 20-25 g) (Shanghai BK Laboratory Animal Co., LTD.) were used in the experiments. All animal experiments were performed in accordance with IACUC protocol (IACAC NO. IRCBC-2017-003) or the regulations effective in the the Interdisciplinary research center on biology and chemistry. Nine ICR mice received 1 mg/kg by slow intravenous injection or 5 mg/kg orally. Blood was collected at multiple time points (5 min (iv),15 min, 30 min, 1 h, 2 h, 4 h, 8 h and 24 h) postdose and transferred to a EDTA tube. The blood was centrifuged at 5000 rpm for 15 min, and the plasma was transferred to a polypropylene tube, capped, and stored frozen (−20 °C) for parent compound analysis.

In the PK studies, plasma samples were collected at multiple time points to assess brain penetration. Protein precipitation was employed for sample preparation. A 20 μL aliquot of sample was subjected to protein precipitation using 100 μL of acetonitrile containing 100 ng/mL of internal standard (Dexamethasone). After vortex and centrifugation for 10 min at 3000 rpm, the supernatant (60 μL) was transferred to a 1 mL 96-well plate, followed by the addition of 60 μL of water. The analysis was conducted by using LCMS(Agilent Technologies 1290 Infinity II，Palo Alto, CA, USA)coupled with mass spectrometric(SCIEX TRIPLE QUAD 3500, Applied Biosystems ,Grand Island, NY, USA)detection. All pharmacokinetic (PK) parameters were carried out by PKSlover using noncompartmental analyses basing on the concentration− time data.

HT1080 Xenograft tumor models. The female athymic balb/c nude mice were purchased

from shanghai BK animal facility. HT1080 cells were cultured in Dulbecco's Modified Eagle Medium (Thermo Fisher Scientific, C11995500BT), supplemented with 10% FBS (gibco, 10091-148) and 1% Pen Strep Glutamine (gibco,10378-016). Cells were confirmed to be devoid of mycoplasma before use. To establish HT1080 xenografts ,cells were resuspended in PBS and mixed with Matrigel (BD Bioscience) (1:1 v/v) before injecting 100 μl containing 5 × 106 cells subcutaneously into right flanks of Balb/c nude mice.

***In vivo* pharmacokinetic/pharmacodynamics.** Female nude mice bearing HT1080 tumors were treated with a compound, followed by blood and tumor tissue collections at various time points post treatment. The plasma concentration of the compound and concentration of 2-HG in the brain and tumor tissue were determined using sensitive LC/MS/MS methods. The percent inhibition in tumor tissue of treated mice relative to vehicle treat mice was determined.

Compound **5** was used in pharmacokinetic/pharmacodynamics relationship in animals bearing HT1080 xenograft tumors. Mice were assigned into groups using a randomized block design (12 mice per group, considering the nature of model, animal welfare and preliminary statistical analysis). Tumor volume was determined by measuring the length (L) and perpendicular width (W), with calipers and calculated using the formula: 0.5 × L × W2. No blinding is included. The compound was formulated as a suspension formulation (0.5% MC+ 0.5% TW80 in water) and administered orally by gavage at a dose volume of 10 ml/kg twice per day with 8 h in-between for PK/PD study for a week. At the last day, the animals were given the first dose administration (i.e., the second dose was not given). For serum PK

analysis 100 μl of blood samples were collected from each animal by orbital sinus bleeding. For analysis of compound levels and PD in tissues, tumors and brains were collected as the time points (2 h, 6 h, 12 h and 24 h post last dosing) and frozen immediately in liquid nitrogen. When applicable, results are presented as mean ± s.e.m. Graphing and statistical analysis was performed using GraphPad Prism 5.00 (GraphPad Software).

In the PK/PD studies, brain and tumor samples were collected at multiple time points to assess brain penetration. Tissues were homogenized in 400 μL PBS (Beyotime, C0221A). Protein precipitation was employed for sample preparation. A 20 μL aliquot of sample (plasma or brain homogenate) was subjected to protein precipitation using 100 μL of acetonitrile containing 100 ng/mL of internal standard (Dexamethasone). After vortex and centrifugation for 10 min at 3000 rpm, the supernatant (60 μL) was transferred to a 1 mL 96-well plate, followed by the addition of 60 μL of water. The analysis was conducted by using LCMS (Agilent Technologies 1290 Infinity II , Palo Alto, CA, USA) coupled with mass spectrometric (AB SCIEX TRIPLE QUAD 3500, Applied Biosystems ,Grand Island, NY, USA) detection. All pharmacokinetic (PK) parameters were carried out by PKSlover using noncompartmental analyses basing on the concentration− time data.

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Notes

The authors declare no competing financial interest.

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Highlights

1. A series of 24 new mutant IDH1 inhibitors have been synthesized and tested.
2. Compound **5** exhibits excellent inhibitory activity both in the enzyme and in cells.
3. Compound **5** is demonstrated good pharmacodynamics and pharmacokinetics properties.
4. Compound **5** exhibits higher exposure than AG-120 in the brain and tumor.
5. Compound **5** is effective in inhibiting 2-HG in HT1080 xenograft tumor samples.