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Discovery of Ethyl Ketone-Based Highly Selective HDACs 1, 2, 3 Inhibitors for HIV Latency Reactivation with Minimum Cellular Potency Serum Shift and Reduced hERG Activity

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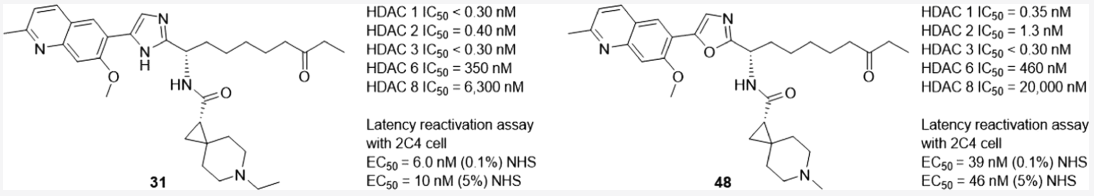

ABSTRACT: We describe the discovery of histone deacetylase (HDACs) 1, 2, and 3 inhibitors with ethyl ketone as the zinc-binding group. These HDACs 1, 2, and 3 inhibitors have good enzymatic and cellular activity. Their serum shift in cellular potency has been minimized, and selectivity against hERG has been improved. They are also highly selective over HDACs 6 and 8. These inhibitors contain a variety of substituted heterocycles on the imidazole or oxazole scaffold. Compounds **31** and **48** stand out due to their good potency, high selectivity over HDACs 6 and 8, reduced hERG activity, optimized serum shift in cellular potency, and good rat and dog PK profiles.

INTRODUCTION

World Health Organization estimated that globally 37.9 million people are living with Human Immunodeficiency Virus (HIV), which causes Acquired Immune Deficiency Syndrome (AIDS).[1] Even though the current treatment of combined antiretroviral therapy (cART) can reduce the virus to undetectable levels, due to the existence of HIV latency, life-long treatment is required for patients as any disruption will lead to the rebound of HIV. HIV latency is HIV-infected memory CD4+ T-cells that are resting and transcriptionally silent.[2] The half-life of these latently infected resting memory CD4+ T-cells has been calculated to be 44 months,[3,4] leading to estimates that it would take in excess of 70 years of cART therapy to fully eradicate this reservoir. Life-long cART treatment causes multiple issues for patients, including financial burden, side effects, and long-term toxicities. Complete cure of HIV infection remains an unmet medical need and is the focus of much research effort. One approach toward a complete HIV cure is a strategy known as "shock and kill".[5] In this strategy, the latently infected CD4+ T-cells are first "shocked" to induce the production of viral proteins. Once decorated with viral proteins, the HIV-infected memory T-cells can be distinguished from uninfected CD4+ T-cells and become susceptible to any selective "kill" strategies. The "shock" part of the strategy can be achieved by latency-reversing agents (LRAs). Known LRAs are histone deacetylase (HDAC) inhibitors, protein kinase C (PKC) agonists, histone methylation (HMT) inhibitors, DNA methyltransferase (DNMT) inhibitors, bromodomain and extra terminal (BET) domain proteins (BET) inhibitors, and disulfiram.[6]

HDACs are enzymes that are essential for histone deacetylation.[7,8] Deacetylation of the lysine residues in histones results in a condensed chromatin structure and therefore reduces the gene expression. HDAC inhibitors prevent the deacetylation of lysine residues in histones, and therefore prevent the chromatin from transitioning into the condensed status. Through this mechanism, HDAC inhibitors can be used to regulate gene expression. Early studies on HDAC inhibitors were focused on oncology programs and several inhibitors have been approved to treat cancers.[9,10] Recently HDAC inhibitors have been used in the "shock" therapy for the eradication of HIV latency. Proof of concept for shocking the HIV latency in clinical studies has been achieved

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by HDAC inhibitors vorinostat (suberoylanilide hydroxamic acid/SAHA),[11] panobinostat,[12] and romidepsin.[13] After administration of HDAC inhibitors, statistically significant increases of HIV mRNA level in resting CD4+ T-cells as well as plasma viraemia were observed.[14] Clinical studies with "shock and kill" strategies combined so far resulted in mixed results. A clinical trial with a combination of romidepsin, Vacc-4x, and recombinant human granulocyte macrophage colony-stimulating factor (rhuGM-CSF) vaccination reduced the total HIV-1 DNA level and infectious units per million (IUPM), but not the integrated HIV-1 DNA level.[15] Another clinical trial evaluating antiretroviral therapy alone versus antiretroviral therapy with a "kick and kill" approach on patients with recent HIV infection identified no statistically difference in the mean total HIV DNA between the two groups. However, the efficacy of the "kick and kill" strategy has not been disproved, instead, more powerful kick or kill agents would be desired for future clinic studies.[16]

HDACs are zinc-dependent enzymes with four classes. Early HDAC inhibitors using the hydroxamic acid group as the zinc-binding group. They are not selective and potent on both class I (HDACs 1, 2, 3, and 8) and II (HDACs 4, 5, 6, 7, 9, and 10) isozymes. Due to the known on- and off-target undesired effects, their use as chronic treatments remains a concern.[17] Recent research has focused on the development of isozyme selective HDAC inhibitors. Our initial effort in this field established ethyl ketone-based HDAC inhibitor **1** (Figure 1, Table 1) for oncology purpose.[18] Our continued effort has shifted to HIV latency reactivation. The Jurkat model of HIV latency using 2C4 cells was adopted as the latency reactivation assay to evaluate the cellular potency against HIV latency. This assay measures the reactivation of a quiescent HIV provirus by quantification of the luciferase reporter gene. The cell line was generated according to the same method as Jurkat HIV T-cell line, which used an eGFP reporter gene.[19−21] The assay was performed with 0.1 or 5% normal human sera (NHS). Addition of 0.1% NHS is necessary for Jurkat 2C4 cells to live longer. Addition of 5% NHS was to measure the serum shift of the compounds' cellular potency. Minimum serum shift in cellular potency is desirable. Recently, we have reported that inhibitions of HDACs 1, 2, and 3 are sufficient to show cellular latency reactivation activity in 2C4 cells. In that effort, compound **2** (Figure 1, Table 1) was identified as an early lead.[22] Compound **2** also utilizes an ethyl ketone group (the hydrate form) as the zinc-binding group. Compared to **1**, compound **2** had improved HDACs 1, 2, and 3 enzymatic activity as well as cellular potency. However, compound **2** is an inhibitor of cardiac hERG.[23] The ratio of its hERG IC_{50} to its cell EC_{50} with 5% NHS is only 100×. It also shows a serum shift (3.2×) in cellular potency between 0.1% NHS and 5% NHS. The rat PK profile of **2** needs further improvement. The unbound C_{max} in the rat PK study at 10 mg/kg (mpk) dose was 7.5 nM (C_{max} = 627 nM, plasma protein binding (PPB) = 98.8%), which is only 0.09x of the cell EC_{50} with 5% NHS. Its unbound C_{24h} in the same rat PK study is 0.11 nM (C_{24h} = 9 nM), which cannot cover its IC_{50} against HDAC 2. Among the potency against HDACs 1, 2, and 3, the IC_{50} value against HDAC 2 is normally the highest among the HDAC inhibitors in the series explored in this study. On the other side, these inhibitors are normally more active against HDAC 6 than HDAC 8. Therefore, the IC_{50} ratio between the HDAC 6 over HDAC 2 is used to assess the selectivity of HDACs 1, 2, and 3 over HDACs 6 and 8. The IC_{50} ratio of HDAC 6 over HDAC 2 for **2** is 114×, which is only marginally acceptable.

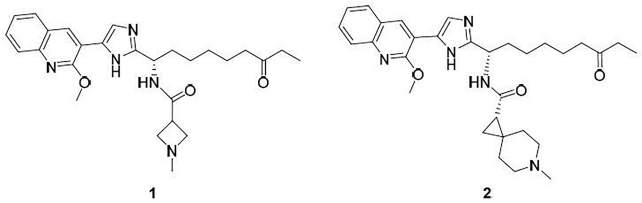

Figure 1. HDAC inhibitors **1** and **2**.

To move an HDAC inhibitor to further development, we were targeting an inhibitor with reduced serum shift (<1.5×) between the cellular potency data in 0.1% NHS and 5% NHS, improved selectivity over hERG IC_{50} (>500× over cell EC_{50}), and improved selectivity of HDAC 2 over HDAC 6 (>100×). Ideally, we are also targeting to achieve the unbound C_{max} in rat PK study at 10 mpk dose covers the cell EC_{50}, and the unbound C_{24h} covers the IC_{50}'s against HDACs 1, 2, and 3. These requirements had been established to ensure a good efficacy and safety profiles of the lead compounds moving to the next stage of development.

RESULTS AND DISCUSSION

Our initial effort was to explore different heterocycles as the substituents on the imidazole moiety, and their potency profiles are summarized in Table 2. Compound **3** was a side product obtained during the preparation of **2**, and it was potent against HDACs 1, 2, and 3 but 3× less potent than **2** in the latency reactivation assay. The *N*-methyl analogue **4** was as potent as **3** but was 2× less selective against hERG. Compound **5** contained a 1-methylquinolin-4(1*H*)-one moiety and showed no serum shift in cellular potency and no hERG activity. However, its enzymatic and cellular potency was reduced as well. Removing the methoxy group of the 2-methoxyquinoline moiety in **2** led to 3-quinoline derivative **6**, which was 3× to 5× weaker in both enzymatic activity against HDACs 1, 2, and 3, and cellular activity than **2**. Other quinoline analogues (**7**−**9**) were all potent against HDACs 1, 2, and 3, and **8** showed the best cellular activity among them. Interestingly, 6-quiloline analogue **7** was not active against hERG, but both 7-isoquinoline analogue (**6**) and 5-isoquinoline analogue (**8**) had their EC_{50}'s against hERG in the low double-digit μM range. Analogues with fused-bicyclic hetero-

Table 1. Reported Potency Data of HDAC Inhibitors 1 and 2

	HDAC IC_{50} (nM)					2C4 cell EC_{50} (nM)		
ID	1	2	3	6	8	0.1% NHS	5% NHS	hERG IC_{50} (μM)
1	13	18	12	680	>10 000	110	480	ND[a]
2	<0.30	1.4	<0.30	160	3 400	26	83	8.3

[a]ND = not determined.

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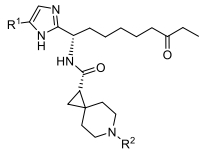
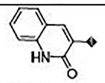
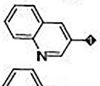
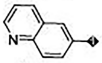
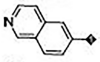
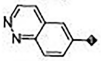
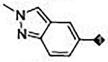
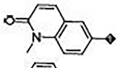
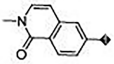
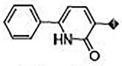
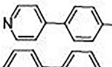
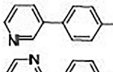
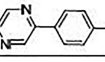
Table 2. HDAC and hERG Activities of Compounds 3−21[a]



ID	R^1	R^2	HDAC IC_{50} (nM) 1	2	3	6	8	2C4 cell EC_{50} (nM) 0.1% NHS	5% NHS	hERG IC_{50} (μM)
3		Me	<1.5	<1.5	<1.5	550	>4,500	140	240	28
4		Me	<1.5	2.5	<1.5	660	>4,500	ND[b]	ND	11
5		Me	8.4	41	10	450	>4,500	9,300	11,000	>60
6		Me	0.98	5.8	1.1	1,500	8,600	250	280	11
7		Me	0.53	3.1	0.63	690	4,400	120	97	>60
8		Et	<1.5	<1.5	<1.5	990	2,800	24	32	12
9		Et	4.1	23	5.2	260	2,900	310	840	16
10		Me	7.9	35	6.2	84	3,600	760	640	>60
11		Me	12	34	4.6	10,000	13,000	3,200	2,700	ND
12		Me	3.6	9.9	3.8	1,800	3,400	11,000	13,000	ND
13		Me	3.3	11	2.2	1,200	3,100	220	180	40
14		Me	3.6	19	2.3	7,600	9,700	220	240	58
15		Me	0.38	3.3	0.54	5,600	8,900	140	170	>60
16		Me	1.3	5.4	1.2	>1,500	>15,000	260	310	>60
17		Me	<0.30	1.6	<0.30	2,000	5,600	100	130	>60
18		Et	0.48	5.0	0.67	45,000	>45,000	210	160	3
19		Me	0.28	3.1	0.46	7,700	10,000	51	46	6
20		Me	0.31	2.5	0.45	4,400	5,500	19	29	4
21		Me	0.55	4.1	0.75	7,800	9,200	50	58	21

[a]All reported potency values are the average of at least two independent measurements with standard deviation less than 3-fold of the reported mean. [b]ND = not determined.

cycles with two nitrogen atoms were also explored. Quinoxaline derivative **10**, cinnoline derivative **11**, and 1,8-naphthyridine derivative **12** were all 10× less potent than **7** against HDACs 1, 2, and 3. Analogues substituted with 3a,7a-dihydrobenzo[*d*]thiazole (**13**), 3a,7a-dihydrobenzo[*d*]oxazole (**14**), and 2-methyl-2*H*-indazole (**15**) were also prepared and they were all similar in enzymatic and cellular potency to **7**. 1-Methylquinolin-2(1*H*)-one group (**16**) and 2-methylisoquinolin-1(2*H*)-one group (**17**) were also incorporated. Both **16** and **17** showed single-digit nM IC_{50}'s against HDACs 1, 2, and 3, and double-digit nM EC_{50}'s in the cellular potency assay. Their serum shift in cellular potency was reduced to less than 1.5×. Both **16** and **17** showed hERG IC_{50}'s greater than 60 μM. Nonfused bicyclic heterocycles (**18**−**21**) were also explored and their potency profile are summarized in Table 2 as well. Although their IC_{50}'s against HDACs 1, 2, and 3 were in the single-digit nM or sub-nM range, and their cellular potency EC_{50}'s were in the double-digit EC_{50}'s range, they showed

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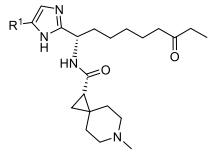
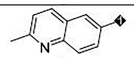
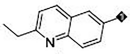
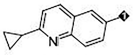
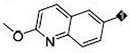
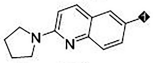
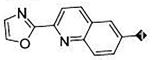
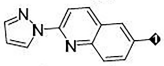
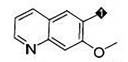
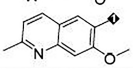
Table 3. HDAC and hERG Activities of Compounds 22−30[a]



ID	R^1	HDAC IC_{50} (nM)					2C4 cell EC_{50} (nM)		hERG
		1	2	3	6	8	0.1% NHS	5% NHS	IC_{50} (μM)
22		<0.30	< 1.5	0.33	1,500	7,200	27	29	43
23		0.32	2.3	0.34	2,900	1,600	60	51	19
24		<0.30	1.1	<0.30	7,800	5,400	61	55	4.8
25		0.40	2.2	0.41	4,200	8,300	42	59	3.3
26		0.30	2.0	0.40	1,500	>45,000	65	32	28
27		0.50	2.1	0.51	>150	>1,500	98	100	15
28		0.90	4.2	1.0	>150	>1,500	130	200	1.2
29		<0.30	1.4	0.36	200	3,700	37	81	51
30		<1.5	<1.5	<1.5	320	3,900	17	21	16

[a]All reported potency values are the average of at least two independent measurements with standard deviation less than 3-fold of the reported mean.

single-digit μM EC_{50}'s against hERG except **21** with hERG IC_{50} as 21 μM.

Compound **7** in Table 2 stood out as it showed good enzymatic and cellular activity, no serum shift in cellular potency, and no hERG activity. Analogues of **7** were synthesized and their HDAC and hERG activities are provided in Table 3. Introduction of a methyl group next to the nitrogen of the quinoline led to **22**, which was 3× more potent in the latency reactivation assay than **7**. Encouraged by this data, ethyl (**23**), cyclopropyl (**24**), methoxy (**25**), and pyrrolidyl (**26**) analogues were also prepared. They all showed sub-nM IC_{50}'s against HDACs 1, 2, 3, and double-digit nM EC_{50}'s in cellular potency assay. However, their EC_{50}'s against hERG were in the double-digit μM range. Oxazole (**27**) and pyrazole (**28**) moieties were also introduced to the same position, but both inhibitors had poor selectivity against hERG. Compound **22** was soaked into the crystal of HDAC 2 enzyme and the overlay of its X-ray structure with **2** was shown in Figure 2. Both compounds overlaid with each other well. There should be space available for **22** to incorporate a methoxy group at the C-7 position of the quinoline. This was confirmed by the potency data of **29**, which contained a 7-methoxyquinoline moiety. Then, 7-methoxy-2-methylquinoline derivative **30** was prepared and indeed it showed good cellular potency with the serum shift as 1.2×. However, it still had moderate activity against hERG.

Due to the good cellular potency of **30**, we followed up with its analogues that had different substitutions on nitrogen atom of the 6-azaspiro[2.5]octane moiety. Their potency profiles are summarized in Table 4. The ethyl analogue **31** and isopropyl analogue **32** showed sub-nM IC_{50}'s against HDACs 1, 2, and 3 as well as single-digit or double-digit nM EC_{50}'s in cellular potency assay. They showed moderate to weak potency against

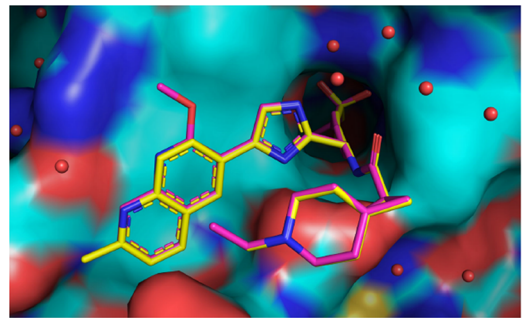


Figure 2. Overlay of the X-ray structures of **2** (purple) and **22** (yellow) in the crystal of HDAC 2. Access codes for X-ray coordinates in RCSB Protein Data Bank (PDB) database are 6WBZ (**2**) and 7JS8 (**22**). The authors will release the atomic coordinates upon article publication.

HDACs 6 and 8 with IC_{50}'s over 350 nM. Moreover, their EC_{50}'s against hERG are greater than 40 μM. Compound **31** was the first compound showed a single-digit nM EC_{50} value in the latency reactivation assay in this series. The cyclopropylmethyl analogue **33** and cyclobutyl analogue **34** were both similar in potency to **31** and **32**, but their EC_{50}'s against hERG were in the single-digit μM range. Comparing the cellular data of **29** and **30** with the data of their corresponding analogues without the 7-methoxy substitution in the quinoline moiety (**7** and **22**), the 7-methoxy substitution in the quinoline seemed to improve the potency in the HIV latency reactivation assay. Therefore, a methoxy group was introduced to a similar position of the heterocycles we explored in Tables 2 and 3. The enzymatic and cellular EC_{50}'s as well as hERG activity are summarized in Table 4. The 2-ethyl-7-methoxyquinoline (**35**)

https://doi.org/10.1021/acs.jmedchem.0c02150
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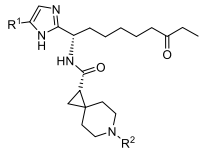
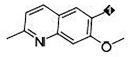
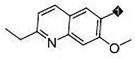
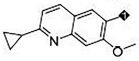
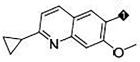
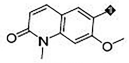
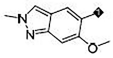
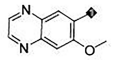
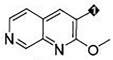
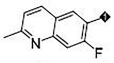
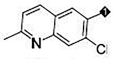
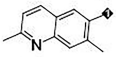
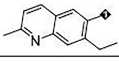
Table 4. HDAC and hERG Activities of Compounds 31−45[a]



ID	R^1	R^2	HDAC IC_{50} (nM)					2C4 Cell EC_{50} (nM)		hERG
			1	2	3	6	8	0.1% NHS	5% NHS	IC_{50} (μM)
31		Et	<0.30	0.40	<0.30	350	6,300	6.0	10	42
32		*i*-Pr	<0.30	0.59	<0.30	460	4,500	24	13	>60
33		*c*-$PrCH_2$-	<0.30	<0.30	<0.30	270	>900	8.0	10	8.4
34		*c*-Bu	<0.30	<0.30	<0.30	310	>900	7.0	14	8.9
35		*c*-$PrCH_2$-	<0.30	<0.30	<0.30	300	>900	10	5.0	ND[b]
36		*c*-$PrCH_2$-	<0.30	<0.30	<0.30	620	>900	11	7.0	ND
37		Me	<0.30	0.60	<0.30	4,300	3,800	26	19	11
38		Et	<0.30	0.70	<0.30	>1,500	>4,500	120	140	>60
39		Et	12	44	5.0	560	3,100	970	1,400	>60
40		Et	4.5	16	2.6	310	>900	480	650	25
41		Me	<0.30	2.3	0.37	750	3,600	93	110	26
42		Me	<0.30	1.4	<0.30	>900	>900	32	40	24
43		Me	1.5	14	1.5	>900	>900	35	52	10
44		Me	<0.30	0.70	<0.30	>900	>900	34	34	ND
45		Me	<0.30	2.2	0.54	>900	>900	30	39	21

[a]All reported potency values are the average of at least two independent measurements with standard deviation less than 3-fold of the reported mean. ND = not determined.

and 2-cyclopropyl-7-methoxyquinoline (**36**−**37**) analogues showed single-digit or double-digit nM EC_{50}'s in the cellular potency assay. The other methoxy substituted heterocycles (**38**−**41**) were less potent in the latency reactivation assay than **31**. We also explored other substitution at the 7-position of the quinoline. The 7-methoxy group of **31** was replaced with fluoro, chloro, methyl, and ethyl groups (**42**−**45**). All of these were less potent than **31** in the cellular assay and more active to hERG. However, they all showed good selectivity against HDACs 6 and 8.

Varieties of heterocycles were explored in the imidazole series. Selected heterocycles were incorporated with the oxazole core as well and their HDAC and hERG activity data are provided in Table 5. The oxazole analogues were not as potent as the imidazole derivatives in both enzymatic and cellular assays. However, some compounds, such as **48** and **49**, still showed good enzymatic and cellular potency as well as reduced hERG activity.

Compounds with good cellular potency and reduced hERG activity were screened in rat cassette PK studies. Compounds showed good clearance were tested in full rat and dog PK studies. The PK parameters are provided in Table 6. Imidazole analogues (**22**, **29**−**32**) all showed bioavailability lower than 10% with 7 have the highest value of 7.9%. Oxazole analogues (**48**, **49**, **51**, **52**, **54**, **56**) showed improved bioavailability than the imidazole analogues with values greater than 20% except **52**, which had a value of 6.9%. The improved PK exposure of oxazole analogues could be explained by their greater membrane permeability compared to the imidazole analogues. For example, the measured Papp value of **48** and **49** are 19×10^{-6} cm/s and 16×10^{-6} cm/s, respectively. However, the measured Papp value of **31** is only 2.8×10^{-6} cm/s. Among the imidazole analogues, the PK profile of compound **31** is reasonable considering its single-digit nM EC_{50} in the latency reactivation assay and reasonable C_{max} in both rat and dog PK studies. Among the oxazole analogues, compound **48** showed the best PK profile with bioavailability of 78% in rat and 42% in dog.

CONCLUSIONS

Extensive structure−activity relationship (SAR) studies have been done on the different heterocyclic substitutions on the imidazole or oxazole moieties as well as different alkyl substitution on the 6-azaspiro[2.5]octane moiety. Novel HDAC inhibitors have been identified with improved enzymatic activity against HDACs 1, 2, and 3, cellular potency in the latency reactivation assay, and minimized serum shift in cellular potency between 0.1 or 5% NHS. Many of these

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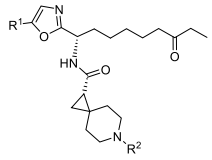
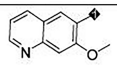
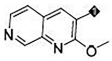
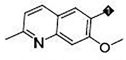
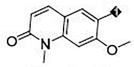
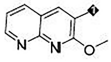
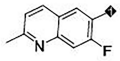
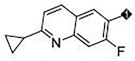
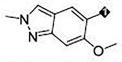
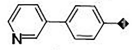
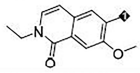
Table 5. HDAC and hERG Activities of Compounds 46−56[a]



ID	R^1	R^2	HDAC IC_{50} (nM) 1	2	3	6	8	2C4 cell EC_{50} (nM) 0.1% NHS	5% NHS	hERG IC_{50} (μM)
46		Me	<1.5	3.2	<1.5	74	17,000	120	170	>60
47		Et	0.45	3.2	0.49	300	24,000	62	80	40
48		Me	0.35	1.3	<0.30	460	20,000	39	46	34
49		Et	<0.30	1.1	<0.30	220	39,000	26	24	51
50		Et	0.49	2.4	0.40	310	>15,000	130	140	>60
51		Et	<1.5	2.2	<1.5	83	7,600	91	78	12
52		Me	1.3	5.1	1.3	>900	>900	160	200	30
53		Me	1.8	5.9	1.9	4,200	24,000	210	240	3.3
54		Et	7.3	30	2.3	160	27,000	280	300	>60
55		Me	3.3	13	<1.5	3,100	9,700	190	220	4.1
56		Et	<1.5	2.0	<1.5	970	>15,000	85	51	>60

[a]All reported potency values are the average of at least two independent measurements with standard deviation less than 3-fold of the reported mean.

Table 6. Pharmacokinetic Profiles of Compounds 22, 29−32, 48, 49, 51, 52, 54, and 56[a]

		IV			PO					
ID	species	dose (mpk)	$T_{1/2}$ (h)	CL (mL/min/Kg)	dose (mpk)	AUC (μM·h)	T_{max} (h)	C_{max} (μM)	C_{24h} (μM)	*F*%
22	rat	2	2.9	45	10	0.010	0.30	0.0040	0.00	0.10
29	rat	2	9.5	61	10	0.060	2.1	0.020	0.0010	2.0
30	rat	2	33	12	10	0.98	5.0	0.17	0.0089	2.5
31	rat	2	13	51	10	0.47	2.1	0.097	0.0032	7.9
32	rat	2	24	60	10	0.020	2.1	0.0090	0.0010	0.60
48	rat	2	4.8	25	10	10	4.0	2.5	0.028	78
49	rat	2	5.4	65	10	1.1	5.0	0.15	0.0072	25
51	rat	2	3.9	6	10	9.7	3.0	2.1	0.0092	20
52	rat	2	1.9	71	10	0.30	2.0	0.063	0.0010	6.9
54	rat	2	5.1	49	10	2.3	4.0	0.51	0.0036	37
56	rat	2	6.5	100	10	0.60	5.0	0.12	0.0022	22
31	dog	1	19	24	2	0.10	1.3	0.026	0.0012	4.1
48	dog	1	43	6.0	2	0.57	3.0	0.072	0.0033	42
49	dog	1	91	2.9	2	0.070	3.0	0.015	0.0020	11

[a]Male Wistar Han rats or Beagle dogs were used. Compound was dosed IV in 60% PEG200 and PO in 10% Tween.

HDAC inhibitors have reduced hERG activity as well. Compound **48** stands out as it meets all of the set requirements for moving into further development. Its serum shift between cell EC_{50}'s at 0.1% or 5% NHS was reduced to 1.2×. The ratio of its hERG IC_{50} over cell EC_{50} at 5% NHS is 740×. The ratio of its HDAC 6 IC_{50} over HDAC 2 IC_{50} is 350×. The PK profile of **48** also meets our requirement. Its unbound C_{max} in rat PK study at 10 mpk is 120 nM, which is 2.6× over its cell EC_{50} at 5% NHS. Its unbound C_{24h} is 1.3 nM, which covers its IC_{50}'s against HDACs 1, 2, and 3. Compound **31** is another HDAC inhibitor of high interest due to its excellent selectivity over hERG and the fact that it meets majority of the requirement. Its serum shift was reduced to 1.7×, which is only slightly above 1.5×. Its selectively over hERG IC_{50} is 4200×, which far exceeds the 500× set requirement. Its HDAC 2 selectivity over HDAC 6 is 880×.

https://doi.org/10.1021/acs.jmedchem.0c02150
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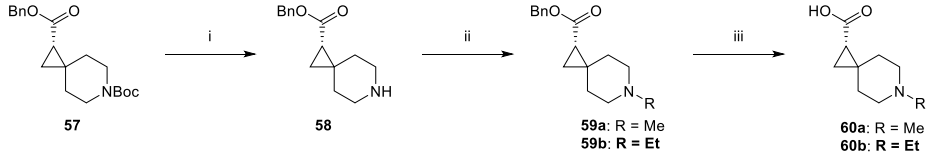
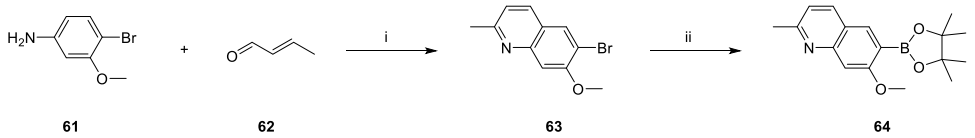
Scheme 1. Preparation of Intermediates 60a and 60b[a]



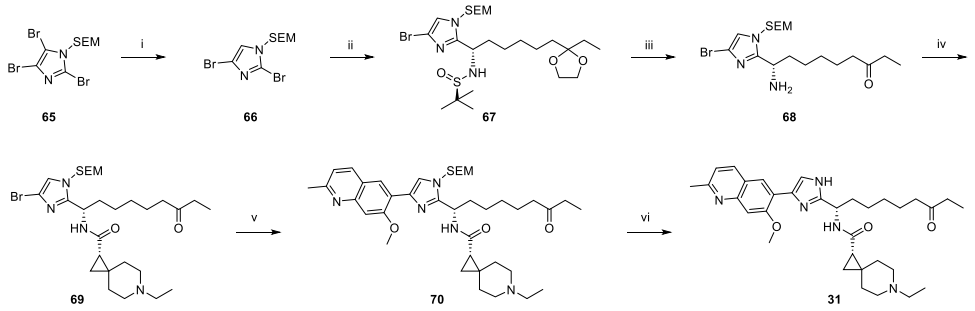
[a]Reagents and conditions: (i) trifluoroacetic acid (TFA), dichloromethane (DCM), 25 °C, 4 h; (ii) **59a**: formaldehyde, MeOH, 25 °C, 2 h, then $NaBH(AcO)_3$, 1 h, 99% for two steps; **59b**: acetaldehyde, MeOH, 30 °C, 32 h, then $NaBH(AcO)_3$, 30 °C, 16 h, 91% for two steps; (iii) **60a**: H_2 (15 psi), Pd/C (10%), MeOH, 24 °C, 1.5 h, 96%; **60b**: H_2 (15 psi), Pd/C (10%), MeOH, 18 °C, 2 h, 99%.

Scheme 2. Preparation of Intermediate 64[a]



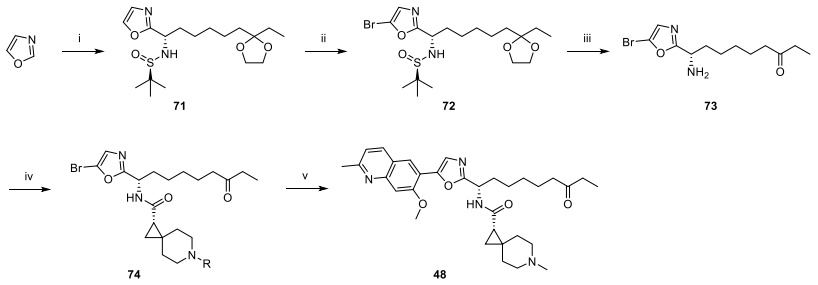
[a]Reagents and conditions: (i) HCl, water, 110 °C, 2 h, 54%; (ii) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, 1,4-dioxane, 80 °C, 12 h, 71%.

Scheme 3. Preparation of Compound 31[a]



[a]Reagents and conditions: (i) *n*-BuLi (2 equiv), THF, −78 °C, 10 min; H_2O (1 equiv), −78 to −50 °C, 1 h; −78 °C, Br_2 (1.1 equiv), 30 min, 29%; (ii) *n*-BuLi, THF, −78 °C, then (*R*)-*N*-[(1*E*)-6-(2-ethyl-1,3-dioxolan-2-yl)hexylidene]-2-methylpropane-2-sulfinamide, 54%; (iii) HCl, 25 °C, 8 h, 72%; (iv) **60b**, HATU, Et_3N, DMF, 25 °C, 2 h; (v) **69**, **64**, $PdCl_2$(DTBPF), K_3PO_4, THF, 70 °C, 2 h, 86%; (vi) TFA, rt, 2 h, 96%.

Scheme 4. Preparation of Compound 48[a]



[a]Reagents and conditions: (i) *n*-BuLi, BH_3·THF, (*R*)-*N*-[(1*E*)-6-(2-ethyl-1,3-dioxolan-2-yl)hexylidene]-2-methylpropane-2-sulfinamide, THF, −78 °C, 53%; (ii) *t*-BuLi, CBr_4, THF, −78 °C, 51%, (iii) HCl, MeOH, 98%; (iv) **60a**, HATU, Et_3N, DMF, 25 °C, 2 h; (v) **74**, **64**, $PdCl_2$(DTBPF), K_3PO_4, THF, 70 °C, 2 h, 46%.

The PK profile of **31** marginally meets the requirements. Its unbound C_{max} in rat PK study at 10 mpk is 3 nM, which is 0.3× over its cell EC_{50} at 5% NHS. Its unbound C_{24h} is 0.09 nM, which can cover its IC_{50}'s against HDACs 1 and 3, but not HDAC 2. Compounds **31** and **48** have been identified as advanced leads and are being evaluated in safety studies and biological models for HIV latency reactivation. The *in vitro* target engagement and histone acetylation studies have been published recently.[24] The *in vivo* histone acetylation studies in rats will be published in due course.

CHEMISTRY

Compounds listed in Tables 2−4 were prepared in similar chemistry, which was exemplified by the preparation of **31** outlined in Schemes 1−3. Scheme 1 describes the preparation of (*S*)-6-methyl-6-azaspiro[2.5]octane-1-carboxylic acid (**60a**) and (*S*)-6-ethyl-6-azaspiro[2.5]octane-1-carboxylic acid (**60b**). Compound **57** was prepared based on the procedures published.[22] The Boc group was removed, and the methyl group (**59a**) or ethyl group (**59b**) was introduced by reductive amination. The benzyl group was removed to give acid intermediates **60a** and **60b** respectively. 7-Methoxy-2-methyl-

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6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (**64**) was prepared by the chemistry outlined in Scheme 2. Ring closure of 4-bromo-3-methoxyaniline and (*E*)-but-2-enal gave 6-bromo-7-methoxy-2-methylquinoline (**63**) in moderate yield. Boronic ester **64** was obtained from **63** via the Suzuki coupling reaction with bis(pinacolato)diboron. With intermediates **60b** and **64** in hand, compound **31** was prepared based on the chemistry outlined in Scheme 3. Treatment of **65** with 2 equiv of *n*-BuLi generated a dianion intermediate, which was converted to a monoanion intermediate by the addition of one equivalent of water. Addition of bromine to the monoanion intermediate gave dibromide **66**. Lithium-halogen exchange with *n*-BuLi to generate the imidazole anion, which added to (*R*)-*N*-[(1*E*)-6-(2-ethyl-1,3-dioxolan-2-yl)-hexylidene]-2-methylpropane-2-sulfinamide[25] to give **67**. Deprotection of the amine and ketone under acidic condition afforded intermediate **68**, which coupled with **60b** to give the common intermediates **69**. Coupling reaction between **69** and **64** gave **70**, which afforded **31** after the SEM group was removed. Compounds listed in Tables 2−4 were prepared by the same chemistry outlined in Schemes 1−3 with or without adjustment of the synthetic sequence. The boronic ester intermediates used for preparation of compounds listed in Tables 2−4 were either purchased or prepared from the corresponding bromides using the same chemistry of step 2 in Scheme 2. For any bromides that were not commercially available, their preparations are described in the Experimental Section.

The preparation of oxazole analogues in Table 5 was exemplified by the preparation of **48** as outlined in Scheme 4. The oxazole anion generated by the treatment of oxazole with *n*-BuLi added to (*R*)-*N*-[(1*E*)-6-(2-ethyl-1,3-dioxolan-2-yl)-hexylidene]-2-methylpropane-2-sulfinamide to give **71**. Deprotonation of **71** with *t*-BuLi followed by the addition of CBr_4 afforded **72**. Treatment of **72** under acidic condition gave amine **73**, which coupled with **60a** to give common intermediate **74**. Compound **48** was obtained by the Suzuki coupling reaction of **74** with **64**.

EXPERIMENTAL SECTION

Chemicals purchased from commercial suppliers were used as received unless specified. Silica gel column chromatography was carried out on ISCO CombiFlash Companion. Prepacked silica gel cartridges were used. Thin-layer chromatography (TLC) visualization was performed under 254 nm ultraviolet light. Reversed-phase preparative high-performance liquid chromatography (HPLC) purifications were done on a Gilson 215 Liquid Handler with Unpoint software, typically with a Sun Fire Prep C18 OBD 5 am 19 × 50 mm column. The following method was typically used, run time: 15 min. flow rate: 14 mL/min. Mobile phase: 10 to 90% CH_3CN/H_2O, with 0.1% TFA. UV detection at 254 or 210 nm was typically used. SFC separations were normally done on a TharSFC instrumentation. Columns and separation conditions were specified in each separation. Nuclear magnetic resonance spectra were obtained on a Varian 400 MHz or Bruker 400 MHz spectrometers. Spectra were taken at ambient temperature. Chemical shifts were assigned using residual solvent signal as internal standard and are reported in parts per million (ppm). Resonance patterns are reported using the following notations: br (broad), s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). The purity of all final compounds was determined to be >95% according to the liquid chromatography−mass spectrometry (LCMS) data obtained on Agilent 1200 Series HPLC equipped with DAD and 6110 single quadrupole MSD and ELSD with Agilent TC-C18, 50 × 2.1 mm, 5 μm for acid methods, Waters X-Bridge ShieldRP18, 50 × 2.1 mm 5 μm for basic methods.

Human HDAC Enzymatic and HIV Latency Assay Procedure. The procedures for human HDAC enzyme inhibitor Fluor-de-Lys assay and HIV latency reactivation model with 2C4 cell assay have been published in separated papers.[26,27] All reported potency values are the average of at least two independent measurements with standard deviation less than 3-fold of the reported mean. hERG data were obtained based on the published method.[28]

Procedure for Crystal Soaking of 22 with HDAC 2 Protein. Crystallization of Human HDAC 2 protein (Proteros, Cat. No. PR-0105) was performed by hanging drop vapor diffusion with seeding at 293 K. Drops contained equal volumes of protein and crystallization buffer containing 25% PEG 3350, 0.2 M ammonium sulfate, 0.1 M Hepes pH 7.5. Crystal soaking solutions contained compound **22** at a concentration of 4 mM with crystallization buffer. HDAC 2 crystals were harvested and transferred to the soaking solution and incubated for 24 h prior to flash freezing in liquid nitrogen. Diffraction data were processed with AutoProc (Global Phasing), and structures were refined using AutoBuster with ligand geometry restraints derived from Grade. Compound **2** was soaked with HDAC 2 protein in the same method. The X-ray structures of **2** and **22** in the crystal of HDAC 2 have been deposited in RCSB Protein Data Bank (PDB) database. Their access codes are 6WBZ (**2**) and 7JS8 (**22**), respectively. The authors will release the atomic coordinates upon article publication.

Rat Cassette PK Screening Generic Procedure. Plasma pharmacokinetic parameters for clearance, volume of distribution, half-life, and mean residence time (MRT) were determined in rats from IV cassette administration studies. Two male rats typically weighing 225−260 grams, were fasted overnight prior to dosing. Compounds were prepared for IV dosing by addition to a vehicle, depending on the dose used. For a typical preparation, 1 mg per mL (IV) of up to five test compounds were added to vehicle composed of 20% dimethyl sulfoxide (DMSO), 60% poly(ethylene glycol) 400 (PEG400), and 20% water. IV formulation was administered to two rats via precannulated jugular vein. Blood was collected by precannulated artery, typically at predose, 2, 8, 15, 30 min, 1, 2, 4, 6, and 8 h postdose. Samples were collected in K2EDTA tubes, stored on ice, and centrifuged. Plasma was transferred to a micro titer plate and stored at −70 °C until analysis. Plasma samples were extracted using protein precipitation and analyzed by liquid chromatography separation followed by mass spec detection (LCMS/MS), using a standard curve for each compound. Plasma pharmacokinetic parameters were calculated by noncompartmental methods.

Rat PK Screening Generic Procedure. Plasma pharmacokinetic parameters for clearance, volume of distribution, half-life, and oral bioavailability were determined in rats from oral administration and IV administration studies. Four male rats, typically weighing 225−260 g, were fasted overnight prior to dosing. Compounds were prepared for oral and IV dosing by addition to a vehicle, depending on the dose used. For a typical preparation, 1 mg per mL (IV) or 1.5 mg per mL (oral) of test compound was added to vehicle composed of 20% dimethyl sulfoxide (DMSO), 60% poly(ethylene glycol) 400 (PEG400), and 20% water. IV formulation was administered to two rats via precannulated jugular vein, and oral dosing was administered to two rats via oral gavage. Blood was collected by precannulated artery, typically at predose, 2, 8, 15, 30 min, 1, 2, 4, 6, 8, and 24 h postdose for IV, and at predose, 15, 30 min, 1, 2, 4, 6, 8, and 24 h for oral dosing. Samples were collected in K2EDTA tubes, stored on ice, and centrifuged. Plasma was transferred to a micro titer plate and stored at −70 °C until analysis. Plasma samples were extracted using protein precipitation and analyzed by liquid chromatography separation followed by mass spec detection (LCMS/MS), using a standard curve for each compound. Plasma pharmacokinetic parameters were calculated for IV and oral dosing data by noncompartmental methods. Oral bioavailability was determined as the ratio of the dose-normalized plasma area under the curve (AUC) following oral dosing vs IV dosing.

Dog PK Screening Generic Procedure. Plasma pharmacokinetic parameters for clearance, volume of distribution, half-life, mean residence time (MRT), and oral bioavailability were determined in dogs from oral administration and IV administration studies. Four

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male dogs, typically weighing 8−12 kg, were fasted overnight prior to dosing. Compounds were prepared for oral and IV dosing by addition to a vehicle, depending on the dose used. For a typical preparation, 1 mg per mL (IV) or 1.5 mg per mL (oral) of test compound was added to vehicle composed of 20% dimethyl sulfoxide (DMSO), 60% poly(ethylene glycol) 400 (PEG400), and 20% water. IV formulation was administered to two dogs via the saphenous or cephalic vein, and oral dosing was administered to two dogs via oral gavage. Blood was collected by the cephalic or jugular vein, typically at predose, 2, 8, 15, 30 min, 1, 2, 4, 6, 8, and 24 h postdose for IV, and at predose, 15, 30 min, 1, 2, 4, 6, 8, and 24 h for oral dosing. Samples were collected in K2EDTA tubes, stored on ice, and centrifuged. Plasma was transferred to a micro titer plate and stored at −70 °C until analysis. Plasma samples were extracted using protein precipitation and analyzed by liquid chromatography separation followed by mass spec detection (LCMS/MS), using a standard curve for each compound. Plasma pharmacokinetic parameters were calculated for IV and oral dosing data by noncompartmental methods. Oral bioavailability was determined as the ratio of the dose-normalized plasma area under the curve (AUC) following oral dosing vs IV dosing.

***N*-((*S*)-1-(5-(2-Hydroxyquinolin-3-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (3).** To a 4 mL vial with pressure release cap was added *N*-((*S*)-1-(5-(2-methoxyquinolin-3-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (150 mg, 0.282 mmol), HCl (23.17 μL, 0.282 mmol), water (1411 μL), and THF (1411 μL). The reaction mixture was stirred at 25 °C for 16 h. The product was purified by C18 chromatography (30 g, CH_3CN in water with 0.1% TFA: 0−90%) to give (*S*)-*N*-((*S*)-1-(5-(2-hydroxyquinolin-3-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide, 2TFA (**3**, the second peak, 13.1 mg, 6.23%), LCMS (ESI) calcd for $C_{30}H_{39}N_5O_3$ $[M + H]^+$: 518.3, found: 518.4, R_t = 0.76 min. ^1H NMR (500 MHz, methanol-d_4) δ 8.73, 8.57 (s and s, 1H), 8.14−8.11 (m, 1H), 7.80−7.76 (m, 1H), 7.69−7.53 (m, 1H), 7.50−7.37 (m, 1H), 7.33−7.32 (m, 1H), 5.15−5.13 (m, 1H), 3.62−3.38 (m, 2H), 3.38−3.19 (m, 2H), 3.80 (m, 1H), 2.57−2.31 (m, 4H), 2.31−2.20 (m, 1H), 2.20−1.74 (m, 6H), 1.70−1.47 (m, 3H), 1.39−1.18 (m, 6H), 1.09−0.99 (m, 4H).

(*S*)-6-Methyl-*N*-((*S*)-1-(5-(1-methyl-4-oxo-1,4-dihydroquinolin-3-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (4). 1-Methyl-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinolin-2(1*H*)-one was prepared from 3-bromo-1-methylquinolin-2(1*H*)-one based on the same method as **64**. Compound **4** was prepared based on the same method of **31**. LCMS (ESI) calcd for $C_{31}H_{41}N_5O_3$ $[M + H]^+$: 532.3, found: 532.44, R_t = 0.88 min. ^1H NMR (500 MHz, methanol-d_4) δ 8.49 (brs, 1H), 7.82 (brs, 1H), 7.72−7.71 (m, 1H), 7.60−7.57 (m, 3H), 7.33−7.31 (m, 1H), 6.97 (s, 1H), 5.19−5.03 (m, 1H), 3.81 (s, 3H), 2.46−2.25 (m, 6H), 2.15−1.84 (m, 3H), 1.66−1.31 (m, 11H), 1.28−0.96 (m, 6H), 0.83−0.78 (m, 3H).

(*S*)-6-Methyl-*N*-((*S*)-1-(5-(1-methyl-4-oxo-1,4-dihydroquinolin-3-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (5). Br_2 (1.5 mL, 30.3 mmol) was added to a mixture of quinolin-4(1*H*)-one (4 g, 27.6 mmol) in AcOH (50 mL) at 25 °C, and the mixture was stirred at room temperature (rt) for 2 h. The mixture was filtered off. The filter cake was washed with NH_4OH and dried under vacuum to get 3-bromoquinolin-4(1*H*)-one as a white solid (5.4 g, 21.69 mmol, 79%). ^1H NMR (400 MHz, DMSO-d_6) δ 8.47 (s, 1H), 8.14 (d, *J* = 7.9 Hz, 1H), 7.68 (t, 1H), 7.61 (d, 1H), 7.38 (t, 1H). NaH (1.446 g, 36.2 mmol) was added to a mixture of 3-bromoquinolin-4(1*H*)-one (5.4 g, 24.10 mmol) in THF (60 mL) at 0 °C. The mixture was stirred at 0 °C for 10 min, and MeI (2.69 mL, 43.0 mmol) was added. After the mixture was stirred at rt for 1.5 h the mixture was quenched with saturated aqueous NH_4Cl (50 mL). The solid was filtered, and the filter cake was dried under vacuum to get 3-bromo-1-methylquinolin-4(1*H*)-one as a white solid (4.1 g, 15.50 mmol, 64%). ^1H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.26−8.20 (m, 1H), 7.84−7.77 (m, 1H), 7.75−7.69 (m, 1H), 7.52−7.45 (m, 1H), 3.88 (s, 1H). (1-Methyl-4-oxo-1,4-dihydroquinolin-3-yl)boronic acid was prepared based on the same method as **64**. Compound **5** was prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{31}H_{41}N_5O_3$ $[M + H]^+$: 532.3, found: 532.3, R_t = 2.077 min. ^1H NMR (400 MHz, methanol-d_4) δ 8.77−8.67 (m, 1H), 8.49−8.39 (m, 1H), 7.93−7.76 (m, 3H), 7.62−7.53 (m, 1H), 5.22−5.07 (m, 1H), 4.05 (s, 3H), 3.58−3.39 (m, 2H), 3.25−2.99 (m, 2H), 2.86 (s, 3H), 2.68−2.56 (m, 1H), 2.47 (s, 4H), 2.37−2.15 (m, 1H), 2.13−1.75 (m, 5H), 1.67−1.45 (m, 3H), 1.44−1.14 (m, 5H), 1.13−0.92 (m, 3H).

(*S*)-6-Methyl-*N*-((*S*)-7-oxo-1-(5-(quinolin-3-yl)-1*H*-imidazol-2-yl)nonyl)-6-azaspiro[2.5]octane-1-carboxamide (6). Prepared by the same method of **31**. LCMS (ESI) calcd for $C_{30}H_{39}N_5O_2$ $[M + H]^+$: 502.3, found: 502.4, R_t = 0.77 min. ^1H NMR (500 MHz, methanol-d_4) δ 9.27−9.24 (m, 1H), 8.75 (s, 1H), 8.07−7.97 (m, 2H), 7.91 (s, 1H), 7.81 (s, 1H), 7,68−7.51 (m, 1H), 5.09−5.05 (m, 1H), 3.50−3.42 (m, 2H), 3.34−3.02 (m, 2H), 2.65−2.47 (m, 4H), 2.25−2.19 (m, 1H), 2.02−1.81 (m, 5H), 1.54−1.39 (m, 11H), 0.98−0.71 (m, 4H).

(*S*)-6-Methyl-*N*-((*S*)-7-oxo-1-(5-(quinolin-6-yl)-1*H*-imidazol-2-yl)nonyl)-6-azaspiro[2.5]octane-1-carboxamide (7). Prepared by the same method of **31**. LCMS (ESI) calcd for $C_{30}H_{39}N_5O_2$ $[M + H]^+$: 502.3, found: 502.5, R_t = 0.67 min. ^1H NMR (500 MHz, methanol-d_4) δ 8.79−8.78 (m, 1H), 8.37−8.35 (m, 1H), 8.26 (brs, 1H), 8.18 (brs, 1H), 8.03−8.01 (m, 1H), 7.55−7.52 (m, 3H), 6.99 (s, 1H), 5.03 (m, 1H), 2,68−2.59 (m, 3H), 2.48−2.36 (m, 4H), 2.22 (s, 3H), 1.98−1.87 (m, 3H), 1.70−1.29 (m, 10H), 1.13−0.82 (m, 6H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(isoquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (8). Prepared by the same method of **31**. LCMS (ESI) calcd for $C_{31}H_{41}N_5O_2$ $[M + H]^+$: 516.3, found: 516.3, R_t = 0.68 min. ^1H NMR 1H (500 MHz, methanol-d_4) δ 9.73 (brs, 1H), 8.70−8.67 (m, 1H), 8.61 (brs, 1H), 8.57−8.55 (m, 1H), 8.42−8.38 (m, 3H), 8.24−8.23 (m, 2H), 5.15−5.11 (m, 1H), 3.57−3.54 (m, 2H), 3.19−2.98 (m, 4H), 2.48−2.44 (m, 4H), 2.44−2.42 (m, 1H), 2.08−2.07 (m, 2H), 1.95−1.86 (m, 2H), 1.59−1.52 (m, 4H), 1.38−1.20 (m, 9H), 1.03−0.97 (m, 3H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(isoquinolin-7-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (9). Prepared by the same method of **31**. LCMS (ESI) calcd for $C_{31}H_{41}N_5O_2$ $[M + H]^+$: 516.3, found: 516.4, R_t = 0.75 min. ^1H NMR (500 MHz, methanol-d_4) δ 9.12 (brs, 1H), 8.87−8.81 (m, 1H), 8.54−8.51 (m, 1H), 8.31−8.28 (m, 1H), 8.20−8.12 (m, 2H), 7.89−7.87 (m, 1H), 5.12−5.10 (m, 1H), 3.58−3.55 (m, 2H), 3.19−2.90 (m, 3H), 2.48−2.42 (m, 4H), 2.30−2.10 (m, 1H), 1.96−1.82 (m, 4H), 1.60−1.53 (m, 4H), 1.38−1.33 (m, 7H), 1.23−1.18 (m, 3H), 1.08−0.97 (m, 3H).

(*S*)-6-Methyl-*N*-((*S*)-7-oxo-1-(5-(quinoxalin-6-yl)-1*H*-imidazol-2-yl)nonyl)-6-azaspiro[2.5]octane-1-carboxamide (10). 6-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)quinoxaline was prepared from 6-bromoquinoxaline based on the same method as **64**. **10** was prepared based on the same method as **31**. White solid. LCMS (ESI) calcd for $C_{29}H_{38}N_6O_2$ $[M + H]^+$: 503.3, found: 503.3, R_t = 0.865 min. ^1H NMR (400 MHz, $CDCl_3$) δ 8.78 (d, *J* = 17.2 Hz, 2H), 7.98−8.19 (m, 2H), 7.40 (s, 1H), 6.75 (d, *J* = 7.5 Hz, 1H), 4.98 (d, *J* = 7.1 Hz, 1H), 2.24−2.48 (m, 8H), 2.17 (brs, 3H), 1.93−2.11 (m, 2H), 1.52−1.80 (m, 4H), 1.37 (dd, *J* = 7.1, 18.3 Hz, 7H), 1.19 (brs, 1H), 1.03 (t, *J* = 7.3 Hz, 3H), 0.83 (dd, *J* = 4.3, 7.2 Hz, 1H).

(*S*)-*N*-((*S*)-1-(5-(Cinnolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (11). See Scheme S1 in the Supporting Information for the synthetic scheme.

Step 1. *t*-BuOK (0.510 g, 4.55 mmol) was added to a solution of (bromomethyl)triphenylphosphonium bromide (1.98 g, 4.55 mmol) in THF (30 mL) at −78 °C. The mixture was stirred at −78 °C for 1 h and 2,5-dibromobenzaldehyde (1 g, 3.79 mmol) was added. The reaction was gradually warmed up to rt and stirred at rt for another 12 h. Aqueous NH_4Cl (saturated, 10 mL) was added, and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column flash chromatography eluting with EtOAc in

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petroleum ether (0−50%) to give (*Z*)-1,4-dibromo-2-(2-bromovinyl)-benzene (0.9 g, 49%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 1.8 Hz, 1H), 7.45 (d, J = 8.6 Hz, 1H), 7.34−7.28 (m, 1H), 7.12 (s, 1H), 6.64 (d, J = 8.2 Hz, 1H).

Step 2. Diethyl hydrazine-1,2-dicarboxylate (0.517 g, 2.93 mmol) was added to a mixture of copper(I) iodide (0.0280 g, 0.147 mmol), K_2CO_3 (0.507 g, 3.67 mmol), *N*1,*N*2-dimethylethane-1,2-diamine (0.0260 g, 0.293 mmol), and (*Z*)-1,4-dibromo-2-(2-bromovinyl)-benzene (0.500 g, 1.47 mmol) in 1,4-dioxane (5 mL) and water (1 mL). The mixture was degassed under vacuum and refilled with N_2 three times. After the mixture was stirred at 90 °C for 10 h, it was cooled down and filtered. The filter cake was washed with EtOAc (50 mL). The filtrate was concentrated to dryness. The crude product was purified by silica gel column chromatography eluting with EtOAc in petroleum ether (0−20%) to give diethyl 6-bromocinnoline-1,2-dicarboxylate (150 mg, 23%) as a yellow solid. LCMS (ESI) calcd for $C_{14}H_{15}BrN_2O_4$ $[M + H]^+$: 355.0, 357.0, found: 354.9, 356.9, R_t = 1.192 min.

Step 3. Diethyl 6-bromocinnoline-1,2-dicarboxylate (150 mg, 0.422 mmol) was added to a mixture of Pd(dppf)Cl_2 (30.9 mg, 0.0420 mmol), potassium acetate (104 mg, 1.06 mmol), and 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi(1,3,2-dioxaborolane) (139 mg, 0.549 mmol) in 1,4-dioxane (5 mL) and water (1 mL). The mixture was degassed under vacuum and refilled with N_2 three times. The mixture was stirred to 90 °C for 10 h and was cooled down. It was filtered, and the filter cake was washed with EtOAc (50 mL). The filtrate was concentrated to dryness. The crude product was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (0−50%) to give diethyl 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)cinnoline-1,2-dicarboxylate (130 mg, 61%) as a yellow solid. LCMS (ESI) calcd for $C_{20}H_{27}BN_2O_6$ $[M + H]^+$: 403.2, found: 403.2, R_t = 1.255 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74−7.66 (m, 1H), 7.59−7.52 (m, 1H), 7.47−7.37 (m, 1H), 7.29 7.20 (m, 1H), 6.22−6.01 (m, 1H), 4.39−4.06 (m, 4H), 1.32 (s, 12H), 1.26−1.21 (m, 9H).

Step 4. A mixture of diethyl 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)cinnoline-1,2-dicarboxylate (130 mg, 0.323 mmol), (*R*)-*N*-((*S*)-1-(5-bromo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-6-(2-ethyl-1,3-dioxolan-2-yl)hexyl)-2-methylpropane-2-sulfinamide (225 mg, 0.388 mmol), K_3PO_4 (206 mg, 0.970 mmol), and Pd(DTBPF)Cl_2 (21.0 mg, 0.0320 mmol) in THF (10 mL) and water (1 mL) was degassed under vacuum and refilled with N_2 three times. The mixture was stirred at 80 °C for 12 h and was concentrated to dryness. The crude product was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (0−100%) to give diethyl 6-(2-((*S*)-1-((*R*)-1,1-dimethylethylsulfinamido)-6-(2-ethyl-1,3-dioxolan-2-yl)hexyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-5-yl)cinnoline-1,2-dicarboxylate (120 mg, 46%) as a yellow solid. LCMS (ESI) calcd for $C_{38}H_{61}N_5O_8SSi$ $[M + H]^+$: 776.4, found: 776.3, R_t = 1.267 min.

Step 5. A mixture of diethyl 6-(2-((*S*)-1-((*R*)-1,1-dimethylethylsulfinamido)-6-(2-ethyl-1,3-dioxolan-2-yl)hexyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-5-yl)cinnoline-1,2-dicarboxylate (120 mg, 0.155 mmol) and NaOH (0.2 mL, 1.000 mmol) in EtOH (2 mL) was heated to 70 °C for 12 h in air. The mixture was cooled down, diluted with DCM (20 mL), washed with water (10 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure to give the (*R*)-*N*-((*S*)-1-(5-(cinnolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-6-(2-ethyl-1,3-dioxolan-2-yl)hexyl)-2-methylpropane-2-sulfinamide (100 mg) as a brown oil. It was used in the next step without further purification. LCMS (ESI) calcd for $C_{32}H_{51}N_5O_4SSi$ $[M + H]^+$: 630.3, found: 630.3, R_t = 1.209 min.

Step 6. TFA (2 mL, 26.0 mmol) was added to a stirred mixture of (*R*)-*N*-((*S*)-1-(5-(cinnolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)-methyl)-1*H*-imidazol-2-yl)-6-(2-ethyl-1,3-dioxolan-2-yl)hexyl)-2-methylpropane-2-sulfinamide (100 mg, 0.159 mmol) in DCM (2 mL) at 16 °C, and the mixture was stirred at 16 °C for 1 h. The solvent was evaporated under reduced pressure to give (*S*)-9-amino-9-(5-(cinnolin-6-yl)-1*H*-imidazol-2-yl)nonan-3-one (100 mg) as a brown oil. It was used in the next step without further purification. LCMS (ESI) calcd for $C_{20}H_{25}N_5O$ $[M + H]^+$: 352.2, found: 352.1, R_t = 0.778 min.

Step 7. (*S*)-9-amino-9-(5-(cinnolin-6-yl)-1*H*-imidazol-2-yl)nonan-3-one (100 mg, 0.285 mmol) was added to a mixture of HATU (130 mg, 0.341 mmol), (*S*)-6-methyl-6-azaspiro[2.5]octane-1-carboxylic acid (53 mg, 0.313 mmol), and DIEA (0.1 mL, 0.573 mmol) in DCM (3 mL), and the mixture was stirred at 16 °C for 2 h. The residue was purified by preparative C18 HPLC eluting with acetonitrile in water (10−90% with 0.1% TFA), then by chiral SFC eluting with 35% EtOH with 0.05% DEA in CO_2 on Chiralcel AS column to give (*S*)-*N*-((*S*)-1-(5-(cinnolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (**11**, 10 mg, 6.7%) as a yellow solid. LCMS (ESI) calcd for $C_{29}H_{38}N_6O_2$ $[M + H]^+$: 503.3, found: 503.3, R_t = 0.788 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 9.28−9.14 (m, 1H), 8.44−8.23 (m, 3H), 8.20−8.08 (m, 1H), 7.77−7.66 (m, 1H), 5.06−4.95 (m, 1H), 3.13−2.98 (m, 2H), 2.71−2.64 (m, 1H), 2.61−2.49 (m, 2H), 2.49−2.38 (m, 4H), 2.01−1.92 (m, 2H), 1.81−1.71 (m, 3H), 1.62−1.49 (m, 3H), 1.38−1.24 (m, 8H), 1.20−1.12 (m, 1H), 1.01−0.93 (m, 3H).

(*S*)-*N*-((*S*)-1-(5-(1,8-Naphthyridin-3-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (12). 3-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-1,8-naphthyridine was prepared from 3-bromo-1,8-naphthyridine based on the same method as **64**. **12** was prepared based on the same method as **31**. Yellow solid. LCMS (ESI) calcd for $C_{29}H_{38}N_6O_2$ $[M + H]^+$: 503.3, found: 503.3, R_t = 1.958 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 9.67−9.89 (m, 1H), 9.36−9.49 (m, 2H), 9.23−9.35 (m, 1H), 8.19−8.39 (m, 2H), 5.03−5.17 (m, 1H), 3.38−3.71 (m, 2H), 3.02−3.17 (m, 1H), 2.89−3.00 (m, 2H), 2.82−2.88 (m, 1H), 2.39−2.53 (m, 4H), 2.02−2.32 (m, 3H), 1.74−2.00 (m, 3H), 1.51−1.64 (m, 3H), 1.24−1.48 (m, 5H), 1.12−1.21 (m, 1H), 1.04−1.10 (m, 1H), 0.95−1.03 (m, 3H).

(*S*)-*N*-((*S*)-1-(5-(Benzo[*d*]thiazol-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (13). 6-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]thiazole was prepared from 6-bromobenzo[*d*]thiazole based on the same method as **64**. **13** was prepared based on the same method as **31**. LC/MS: calcd for $C_{28}H_{37}N_5O_2S$ $[M + H]^+$: 508.3, found: 508.2 $[M + H^+]$, R_t = 0.802 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 9.45−9.54 (m, 1H), 8.51−8.73 (m, 1H), 8.16−8.25 (m, 1H), 7.91−8.09 (m, 2H), 5.02−5.11 (m, 1H), 3.58−3.72 (m, 1H), 3.39−3.55 (m, 1H), 2.98−3.25 (m, 1H), 2.74−2.96 (m, 4H), 2.46−2.47 (m, 4H), 1.76−2.30 (m, 5H), 1.47−1.68 (m, 3H), 1.24−1.45 (m, 5H), 1.11−1.23 (m, 1H), 1.10−0.98 (m, 4H).

(*S*)-*N*-((*S*)-1-(5-(Benzo[*d*]oxazol-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (14). 6-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzo[*d*]oxazole was prepared from 6-bromobenzo[*d*]oxazole based on the same method as **64**. White solid. LC/MS: MS (ESI) m/z: 246.1 $[M + H^+]$, R_t = 1.122 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 1H), 8.04 (s, 1H), 7.74−7.86 (m, 2H), 1.37 (s, 12H). **14** was prepared based on the same method as **31**. Yellow oil. LC/MS: calcd for $C_{28}H_{37}N_5O_3$ $[M + H]^+$: 492.3, found: 492.3, R_t = 0.792 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.45 (s, 1H), 7.97−8.07 (m, 1H), 7.66−7.83 (m, 2H), 7.39−7.47 (m, 1H), 4.97−5.03 (m, 1H), 2.28−2.72 (m, 9H), 2.22 (brs, 4H), 1.87−2.02 (m, 3H), 1.23−1.73 (m, 7H), 1.08−1.13 (m, 1H), 0.98−0.96 (m, 4H), 0.79−0.87 (m, 1H).

(*S*)-6-Methyl-*N*-((*S*)-1-(5-(2-methyl-2*H*-indazol-5-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (15). Prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{29}H_{40}N_6O_2$ $[M + H]^+$: 505.3, found: 505.1, R_t = 2.25 min. ^{1}H NMR (500 MHz, methanol-d_4) δ 8.18 (s, 1H), 7.98 (s, 1H), 7.66−7.60 (m, 2H), 7.30 (s, 1H), 5.03−5.00 (m, 1H), 4.20 (s, 3H), 2.65 (brs, 2H), 2.46−2.40 (m, 6H), 2.43 (s, 3H), 1.98−1.91 (m, 3H), 1.72−1.62 (m, 6H), 1.43−1.29 (m, 4H), 1.14−1.12 (m, 1H), 0.99−0.97 (m, 3H), 0.87−0.84 (m, 1H).

(*S*)-6-Methyl-*N*-((*S*)-1-(5-(1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (16). Prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{31}H_{41}N_5O_3$ $[M + H]^+$: 532.3, found: 532.3, R_t

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= 2.724 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (brs, 1H), 7.95 (brs, 1H), 7.75 (brs, 1H), 7.47 (brs, 2H), 6.78−6.75 (m, 1H), 5.18 (brs, 1H), 3.73 (s, 3H), 3.66−3.33 (m, 2H), 3.07−2.63 (m, 4H), 2.44−2.40 (m, 6H), 2.10−2.00 (m, 4H), 1.35−1.22 (m, 7H), 1.05−0.93 (m, 6H).

(*S*)-6-Methyl-*N*-((*S*)-1-(5-(2-methyl-1-oxo-1,2-dihydroisoquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]-octane-1-carboxamide (17). Prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{31}H_{41}N_5O_3$ [M + H]$^+$: 532.3, found: 532.4, R_t = 0.922 min. ^{1}H NMR (500 MHz, methanol-d_4) δ 8.41−8.39 (m, 1H), 8.11−8.01 (m, 2H), 7.90−7.84 (m, 1H),7.47−7.45 (m, 1H), 6.74−6.69 (m, 1H), 5.11−5.10 (m, 1H), 3.64 (s, 3H), 3.51−3.49 (m, 2H), 3.32-3-31 (m, 1H), 3.20−3.00 (m, 1H), 2.48−2.44 (m, 4H), 2.30−2.04 (m, 4H), 1.94−1.82 (m, 3H), 1.60−1.56 (m, 3H), 1.38−1.22 (m, 6H), 1.01−0.98 (m, 4H).

(*S*)-6-Ethyl-*N*-((*S*)-7-oxo-1-(5-(2-oxo-6-phenyl-1,2-dihydropyridin-3-yl)-1*H*-imidazol-2-yl)nonyl)-6-azaspiro[2.5]octane-1-carboxamide (18). Prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{33}H_{43}N_5O_3$ [M + H]$^+$: 558.3, found: 558.5, R_t = 0.85 min. ^{1}H NMR (500 MHz, methanol-d_4) δ 8.22−8.15 (m, 1H), 8.04−8.02 (m, 1H), 7.73 (brs, 2H), 7.55−7.54 (m, 3H), 6.80−6.78 (m, 1H), 5.13−5.11 (m, 1H), 3.56−3.38 (m, 3H), 3.30−3.03 (m, 4H), 2.48−2.42 (m, 4H), 2.36−1.81 (m, 5H), 1.59−1.21 (m, 11H), 1.07−0.96 (m, 4H).

(*S*)-6-Methyl-*N*-((*S*)-7-oxo-1-(5-(4-(pyridin-4-yl)phenyl)-1*H*-imidazol-2-yl)nonyl)-6-azaspiro[2.5]octane-1-carboxamide (19). Prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{32}H_{41}N_5O_2$ [M + H]$^+$: 528.3, found: 528.5, R_t = 0.66 min. ^{1}H NMR (500 MHz, methanol-d_4) δ 8.57−8.56 (m, 2H), 7.85 (brs, 2H), 7.79−7.74 (m, 2H),7.73−7.72 (m, 2H), 7.43 (s, 1H), 5.04−5.01 (m, 1H), 2.47−2.42 (m, 6H), 2.30 (brs, 1H), 2.25 (s, 3H), 1.98−1.93 (m, 3H), 1.67−1.60 (m, 6H), 1.42−1.33 (m, 5H), 1.12−1.10 (m, 1H), 1.00-098 (m, 3H), 0.83−0.81 (m, 1H).

(*S*)-6-Methyl-*N*-((*S*)-7-oxo-1-(5-(4-(pyridin-3-yl)phenyl)-1*H*-imidazol-2-yl)nonyl)-6-azaspiro[2.5]octane-1-carboxamide (20). Prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{32}H_{41}N_5O_2$ [M + H]$^+$: 528.3, found: 528.5, R_t = 0.66 min. ^{1}H NMR (500 MHz, methanol-d_4) δ 8.83 (s, 1H), 8.51−8.50 (m, 1H), 8.13−8.10 (m, 1H), 7.85−7.83 (m, 2H), 7.70−7.68 (m, 2H), 7.53−7.50 (m, 1H), 7.41 (s, 1H), 5.04−5.01 (m, 1H), 2.46−2.42 (m, 7H), 2.22 (s, 3H), 1.98−1.92 (m, 3H), 1.68−1.58 (m, 6H), 1.36−1.34 (m, 5H), 1.13−1.11 (m, 1H), 1.01−0.98 (m, 3H), 0.84−0.83 (m, 1H).

(*S*)-6-Methyl-*N*-((*S*)-7-oxo-1-(5-(4-(pyrazin-2-yl)phenyl)-1*H*-imidazol-2-yl)nonyl)-6-azaspiro[2.5]octane-1-carboxamide (21). Prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{31}H_{40}N_6O_2$ [M + H]$^+$: 529.3, found: 529.5, R_t = 0.83 min. ^{1}H NMR (500 MHz, methanol-d_4) δ 8.84−8.81 (m, 2H), 8.41−8.39 (m, 2H), 7.83−7.78 (m, 2H), 7.45 (s, 1H), 7.33−7.31 (m, 1H), 5.02−4.99 (m, 1H), 2.85 (brs, 2H), 2.65−2.64 (m, 1H), 2.45−2.41 (m, 8H), 1.98−1.81 (m, 3H), 1.75−1.71 (m, 4H), 1.69−1.67 (m, 2H), 1.44−1.35 (m, 4H), 1.15−1.13 (m, 1H), 0.99−0.97 (m, 3H), 0.89−0.87 (m, 1H).

(*S*)-6-Methyl-*N*-((*S*)-1-(5-(2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (22). Prepared based on the same method as **31**. HRMS (ESI) calcd for $C_{31}H_{41}N_5O_2$ [M + H]$^+$ 516.3260, found 516.3338. ^{1}H NMR (500 MHz, methanol-d_4) δ 8.22−8.18 (m, 2H), 8.10−8.08 (m, 1H), 7.94−7.92 (m, 1H), 7.51 (s, 1H), 7.42−7.40 (m, 1H), 5.04−5.02 (m, 1H), 2.71 (s, 3H), 2.61 (brs, 2H), 2.46−2.41 (m, 5H), 2.23 (s, 3H), 2.02−1.92 (m, 2H), 1.70−1.55 (m, 6H), 1.38−1.33 (m, 6H), 1.14−1.12 (m, 1H), 1.00−0.97 (m, 3H), 0.86−0.84 (m, 1H).

(*S*)-*N*-((*S*)-1-(5-(2-Ethylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (23). See Scheme S2 in the Supporting Information for the synthetic scheme.

Step 1. *n*-BuLi (6.6 mL, 16.50 mmol) was added to a mixture of 6-bromo-2-chloroquinoline (2.0 g, 8.25 mmol) and triisopropyl borate (4 mL, 17.23 mmol) in THF (20 mL) at −78 °C, and the mixture was stirred at rt for 12 h. Hydrochloric acid (1 M, 8.0 mL, 8.0 mmol) was added, and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic fractions were washed with brine (20 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was recrystallized from petroleum ether (15 mL). The solid was collected and dried *in vacuo* to give (2-chloroquinolin-6-yl)boronic acid (1.7 g, 94%) as a light-gray solid. LCMS (ESI) calcd for $C_9H_7BClNO_2$ [M + H]$^+$: 208.0, found: 208.0, R_t = 0.998 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.42−8.50 (m, 1H), 8.37 (s, 2H), 8.16 (d, J = 8.2 Hz, 1H), 7.90 (d, J = 8.6 Hz, 1H), 7.58 (d, J = 8.6 Hz, 1H).

Step 2. $PdCl_2$(DTBPF) (5.0 mg, 7.7 μmol) and potassium phosphate (197 mg, 0.928 mmol) were added to a mixture of (*S*)-*tert*-butyl (1-(5-(2-chloroquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)carbamate (150 mg, 0.309 mmol) and potassium trifluoro-(vinyl)borate (83 mg, 0.619 mmol) in THF (2 mL) and water (0.2 mL). The mixture was stirred at 85 °C for 8 h, cooled down, diluted with EtOAc (8 mL), washed with brine (3 × 10 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (0−100%) to give (*S*)-*tert*-butyl (7-oxo-1-(5-(2-vinylquinolin-6-yl)-1*H*-imidazol-2-yl)nonyl)carbamate (100 mg, 68%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (brs, 1H), 8.22 (brs, 1H), 8.12 (brs, 1H), 8.04 (brs, 2H), 7.59 (d, J = 8.6 Hz, 1H), 7.33−7.38 (m, 1H), 7.03 (dd, J = 11.0, 17.6 Hz, 1H), 6.27 (d, J = 17.6 Hz, 1H), 5.65 (d, J = 11.0 Hz, 1H), 5.16 (brs, 1H), 4.68 (brs, 1H), 2.37−2.47 (m, 4H), 2.21 (brs, 1H), 1.98 (brs, 1H), 1.60 (brs, 4H), 1.47 (s, 9H), 1.35−1.42 (m, 2H), 1.05 (t, J = 7.2 Hz, 3H).

Step 3. [1,1-Bis(di-*tert*-butylphosphino)ferrocene]palladium(II) dichloride (5.0 mg, 7.7 μmol) and potassium phosphate (197 mg, 0.928 mmol) was added to a stirred mixture of (*S*)-*tert*-butyl (1-(5-(2-chloroquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)carbamate (150 mg, 0.309 mmol), and potassium trifluoro(vinyl)borate (83.0 mg, 0.619 mmol) in THF (2 mL) and water (0.2 mL) at rt (20 °C), and the mixture was stirred at 85 °C for 8 h. TLC showed starting material was consumed and a new product was formed. The mixture was cooled down, diluted with EtOAc (8 mL), washed with brine (3 × 10 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (0−100%) to give (*S*)-*tert*-butyl (7-oxo-1-(5-(2-vinylquinolin-6-yl)-1*H*-imidazol-2-yl)nonyl)carbamate (100 mg, 0.210 mmol, 67.8%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.05 (br. s., 1H), 8.22 (br. s., 1H), 8.12 (br. s., 1H), 8.04 (br. s., 2H), 7.59 (d, J = 8.61 Hz, 1H), 7.33−7.38 (m, 1H), 7.03 (dd, J = 10.96, 17.61 Hz, 1H), 6.27 (d, J = 17.61 Hz, 1H), 5.65 (d, J = 10.96 Hz, 1H), 5.16 (br. s., 1H), 4.68 (br. s., 1H), 2.37−2.47 (m, 4H), 2.21 (br. s., 1H), 1.98 (br. s., 1H), 1.60 (br. s., 4H), 1.47 (s, 9H), 1.35−1.42 (m, 2H), 1.05 (t, J = 7.24 Hz, 3H).

Step 4. 10% Pd−C (10 mg, 0.094 mmol) was added to a stirred mixture of (*S*)-*tert*-butyl (7-oxo-1-(5-(2-vinylquinolin-6-yl)-1*H*-imidazol-2-yl)nonyl)carbamate (150 mg, 0.315 mmol) in EtOH (1 mL) at rt, and the mixture was stirred at rt for 2 h under H_2 balloon. The mixture was filtered, and the filter cake was washed with MeOH (3 × 10 mL). The filtrate was concentrated to dryness. The residue was purified by silica gel column flash chromatography, eluting with EtOAc in petroleum ether (0−100%) to give (*S*)-*tert*-butyl (1-(5-(2-ethylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)carbamate (100 mg, 66%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.03 (brs, 1H), 8.21 (brs, 1H), 8.09 (d, J = 7.7 Hz, 1H), 8.02 (brs, 2H), 7.34 (s, 1H), 7.30 (d, J = 8.4 Hz, 1H), 5.15 (brs, 1H), 4.68 (brs, 1H), 3.00 (q, J = 7.8 Hz, 2H), 2.39−2.44 (m, 4H), 2.21 (brs, 1H), 1.98 (brs, 1H), 1.57−1.61 (m, 3H), 1.47 (s, 9H), 1.41 (t, J = 7.6 Hz, 5H), 1.05 (t, J = 7.3 Hz, 3H).

Step 5. TFA (0.5 mL, 6.49 mmol) was added to a stirred mixture of (*S*)-*tert*-butyl (1-(5-(2-ethylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)carbamate (40 mg, 0.084 mmol) in DCM (2.5 mL) at 20 °C, and the mixture was stirred at rt for 1 h. The mixture was concentrated to give (*S*)-9-amino-9-(5-(2-ethylquinolin-6-yl)-1*H*-imidazol-2-yl)nonan-3-one (32 mg of crude) as a yellow oil. LCMS

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(ESI) calcd for $C_{23}H_{30}N_4O$ $[M + H]^+$: 379.2, found: 0.759, R_t = 379.2 min.

Step 6. (*S*)-9-amino-9-(5-(2-ethylquinolin-6-yl)-1*H*-imidazol-2-yl)-nonan-3-one (79 mg, 0.209 mmol) was added to a stirred mixture of Et_3N (0.090 mL, 0.646 mmol), HATU (79 mg, 0.209 mmol), (*S*)-6-methyl-6-azaspiro[2.5]octane-1-carboxylic acid (53 mg, 0.313 mmol) in DMF (1 mL) at rt, and the mixture was stirred at rt for 1 h. The residue was purified by preparative HPLC (reversed-phase C-18 column), eluting with acetonitrile in water (10−90% with 0.05% $NH_3{\cdot}H_2O$) to give (*S*)-*N*-((*S*)-1-(5-(2-ethylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (**23**, 60 mg, 52%) as a white solid. LCMS (ESI) calcd for $C_{32}H_{43}N_5O_2$ $[M + H]^+$: 530.3, found: 530.3, R_t = 1.443 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86−8.24 (m, 4H), 7.27−7.36 (m, 2H), 6.60 (brs, 1H), 4.89−5.01 (m, 1H), 3.00 (q, *J* = 7.8 Hz, 2H), 2.25−2.59 (m, 7H), 2.15−2.22 (m, 3H), 2.03 (d, *J* = 7.8 Hz, 4H), 1.63−1.85 (m, 3H), 1.53−1.62 (m, 2H), 1.28−1.52 (m, 8H), 1.21 (d, *J* = 4.7 Hz, 1H), 1.03 (t, *J* = 7.2 Hz, 3H), 0.84 (dd, *J* = 4.3, 7.4 Hz, 1H).

(*S*)-*N*-((*S*)-1-(5-(2-Cyclopropylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (24). Prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{33}H_{43}N_5O_2$ $[M + H]^+$: 542.3, found: 542.2, R_t = 1.651 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.86−8.80 (m, 1H), 8.69−8.58 (m, 1H), 8.45−8.42 (m, 1H), 8.39−8.37 (m, 1H), 8.25−8.23 (m, 1H), 8.10−8.08 (m, 1H), 5.10−5.08 (m, 1H), 3.50−3.23 (m, 3H), 3.23−3.06 (m, 2H), 2.88−2.85 (m, 1H), 2.48−2.43 (m, 5H), 2.25−2.11 (m, 3H), 1.92−1.87 (m, 3H), 1.59−1.57 (m, 5H), 1.39−1.20 (m, 4H), 1.20−1.10 (m, 4H), 0.99−0.97 (m, 4H).

(*S*)-*N*-((*S*)-1-(5-(2-Methoxyquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (25). Prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{31}H_{41}N_5O_3$ $[M + H]^+$: 532.3, found: 532.5, R_t = 0.89 min. ^{1}H NMR (500 MHz, methanol-d_4) δ 8.11−8.09 (m, 2H), 7.99−7.97 (m, 1H), 7.82−7.80 (m, 1H), 7.42 (s, 1H), 6.95−6.93 (m, 1H), 5.04−5.01 (m, 1H), 4.05 (s, 3H),2.61 (brs, 2H), 2.46−2.42 (m, 5H), 2.24 (s, 3H), 1.98−1.91 (m, 2H), 1.71−1.54 (m, 6H), 1.44−1.29 (m, 6H), 1.14−1.12 (m, 1H), 1.00−0.97 (m, 3H), 0.86−0.84 (m, 1H).

(*S*)-6-Methyl-*N*-((*S*)-7-oxo-1-(5-(2-(pyrrolidin-1-yl)quinolin-6-yl)-1*H*-imidazol-2-yl)nonyl)-6-azaspiro[2.5]octane-1-carboxamide (26). See Scheme S3 in the Supporting Information for the synthetic scheme.

Step 1. A solution of 6-bromo-2-chloroquinoline (1.00 g, 4.12 mmol) and pyrrolidine (0.88 g, 12.4 mmol) in DMF (10 mL) was stirred at 100 °C for 3 h. The reaction mixture was evaporated *in vacuo*, diluted with water (100 mL), and extracted with EtOAc (3 × 50 mL). The organic layers were combined, washed with brine (80 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica column chromatography eluting with EtOAc in petroleum ether (6.6%) to afford 6-bromo-2-(pyrrolidin-1-yl)quinoline (1.09 g, 96%) as a light-yellow solid. ESI-MS *m*/*z* $[M + H]^+$: 279.0, R_t = 0.877 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (d, *J* = 9.0 Hz, 1H), 7.71 (s, 1H), 7.56 (s, 2H), 6.73 (d, *J* = 9.4 Hz, 1H), 3.61 (brs, 4H), 2.05 (t, *J* = 6.5 Hz, 4H).

Step 2. A mixture of 6-bromo-2-(pyrrolidin-1-yl)quinoline (1.06 g, 3.82 mmol), bis(pinacolato)diboron (1.94 g, 7.63 mmol), potassium acetate (0.75 g, 7.63 mmol), and Pd(dppf)Cl_2 (0.28 g, 0.38 mmol) in 1,4-dioxane (30 mL) was stirred at 70 °C under N_2 for 8 h. The reaction mixture was cooled down and filtered through celite. The filtrate was diluted with water (150 mL) and extracted with EtOAc (3 × 80 mL). The organic layers were combined, washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica column chromatography eluting with EtOAc in petroleum ether (20%) to afford 2-(pyrrolidin-1-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (0.56 g, 46%) as a light-yellow solid. ESI-MS *m*/*z* $[M + H]^+$: 325.2, R_t = 1.020 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.85 (dd, *J* = 16.0, 8.6 Hz, 2H), 7.64 (d, *J* = 7.0 Hz, 1H), 6.68 (d, *J* = 9.0 Hz, 1H), 3.61 (brs, 4H), 2.02 (t, *J* = 6.3 Hz, 4H), 1.35 (s, 12H).

Step 3. A mixture of 2-(pyrrolidin-1-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) quinoline (0.18 g, 0.56 mmol), (*S*)-*N*-((*S*)-1-(5-bromo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (0.27 g, 0.46 mmol), potassium phosphate monohydrate (0.27 g, 1.16 mmol), and Pd(dppf)Cl_2 (0.03 g, 0.05 mmol) in THF/H_2O (6.4 mL/1.6 mL) was stirred at 70 °C under N_2 atmosphere for 12 h. The reaction mixture was cooled and filtered through celite. The resulting filtrate was diluted with water (120 mL) and extracted with EtOAc (60 mL × 2). The organic layers were combined, washed with brine (100 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by prep-TLC (SiO_2, DCM/MeOH = 6:1) to afford (*S*)-6-methyl-*N*-((*S*)-7-oxo-1-(5-(2-(pyrrolidin-1-yl)quinolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)nonyl)-6-azaspiro[2.5]octane-1-carboxamide (0.32 g, 40%) as a light-yellow solid. ESI-MS *m*/*z* $[M + H]^+$: 701.5, R_t = 1.060 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.86 (m, 2H), 7.66−7.76 (m, 1H), 7.18 (s, 1H), 6.91 (brs, 1H), 6.73 (d, *J* = 9.3 Hz, 1H), 5.70 (d, *J* = 11.0 Hz, 1H), 5.00−5.21 (m, 2H), 3.64 (d, *J* = 4.8 Hz, 4H), 3.49−3.60 (m, 2H), 2.73 (brs, 1H), 2.53 (brs, 1H), 2.33−2.45 (m, 4H), 1.91−2.25 (m, 11H), 1.52−1.67 (m, 3H), 1.39−1.49 (m, 2H), 1.33 (brs, 3H), 1.18−1.28 (m, 3H), 1.03 (t, *J* = 7.3 Hz, 3H), 0.80−0.99 (m, 4H), 0.01 (s, 9H).

Step 4. A solution of (*S*)-6-methyl-*N*-((*S*)-7-oxo-1-(5-(2-(pyrrolidin-1-yl)quinolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)nonyl)-6-azaspiro[2.5]octane-1-carboxamide (129 mg, 0.184 mmol) in TFA (5 mL) was stirred at 20 °C for 2 h. The reaction mixture was concentrated and purified by C18 prep-HPLC eluting with acetonitrile in water (7−37%, with 0.1% TFA). The collected product was concentrated, and 1 M HCl (0.8 mL) was added into the residue, which was lyophilized to afford (*S*)-6-methyl-*N*-((*S*)-7-oxo-1-(5-(2-(pyrrolidin-1-yl)quinolin-6-yl)-1*H*-imidazol-2-yl)nonyl)-6-azaspiro[2.5]octane-1-carboxamide hydrochloride (**26**, 72 mg, 64%) as light-yellow powder. LCMS (ESI) calcd for $C_{34}H_{46}N_6O_2$ $[M + H]^+$: 571.4, found: 571.3, R_t = 0.847 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.45−8.43 (m, 1 H), 8.40−8.37 (m, 1 H), 8.30−8.20 (m, 1H), 8.08−8.04 (m, 1 H), 7.96 (d, *J* = 7.6 Hz, 1 H), 7.32 (d, *J* = 10.0 Hz, 1H), 5.07−5.02 (m, 1 H), 3.85−3.82 (m, 4H), 3.54−3.52 (m, 1 H), 3.51−3.48 (m, 2H), 3.34−2.95 (m, 2H), 2.85 (s, 3 H), 2.55−2.48 (m, 4H), 2.60−2.00 (m, 7H), 1.95−1.55 (m, 3H), 1.53−1.49 (m, 3H), 1.48−1.44 (m, 4H), 1.40−1.35 (m, 1H), 1.15−0.88 (m, 3H).

(*S*)-6-Methyl-*N*-((*S*)-1-(5-(2-(oxazol-2-yl)quinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (27). Prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{33}H_{40}N_6O_3$ $[M + H]^+$: 569.3, found: 569.3, R_t = 2.157 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.53 (dd, *J* = 8.9, 13.3 Hz, 1H), 8.37−8.47 (m, 1H), 8.26−8.37 (m, 2H), 8.13−8.26 (m, 2H), 8.03 (s, 1H), 7.51 (s, 1H), 5.09 (m, 1H), 3.41−3.59 (m, 2H), 3.24 (d, *J* = 10.1 Hz, 1H), 3.02−3.13 (m, 1H), 2.83 (d, *J* = 19.6 Hz, 3H), 2.61 (brs, 1H), 2.43−2.51 (m, 3H), 2.28 (brs, 1H), 2.09 (brs, 2H), 1.79−1.97 (m, 3H), 1.49−1.66 (m, 3H), 1.16−1.43 (m, 5H), 0.78−1.10 (m, 4H).

(*S*)-*N*-((*S*)-1-(5-(2-(1*H*-Pyrazol-1-yl)quinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (28). See Scheme S4 in the Supporting Information for the synthetic scheme.

Step 1. A mixture of 6-bromo-2-chloroquinoline (1.00 g, 4.12 mmol), 1*H*-pyrazole (0.84 g, 12.36 mmol), and Cs_2CO_3 (2.69 g, 8.24 mmol) in DMF (30 mL) was stirred at 100 °C for 17 h. The reaction mixture was evaporated *in vacuo*. To the residue was added water (150 mL) and the aqueous layer was extracted with EtOAc (3 × 60 mL). The organic layers were combined, washed with brine (80 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by silica column chromatography eluting with EtOAc in petroleum ether (6.6%) to afford 6-bromo-2-(1*H*-pyrazol-1-yl)-quinoline (0.95 g, 84%) as a light-pink solid. LCMS (ESI) calcd for $C_{12}H_8BrN_3$ $[M + H]^+$: 274.0, found: 273.9. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.53 (s, 1H), 7.74−7.82 (m, 2H), 7.83−7.91 (m, 1H), 7.98 (d, *J* = 1.96 Hz, 1H), 8.14−8.28 (m, 2H), 8.77 (d, *J* = 1.96 Hz, 1H).

Step 2. Potassium acetate (1164 mg, 11.86 mmol) was added to the mixture of $PdCl_2$(dppf) (400 mg, 0.547 mmol), 6-bromo-2-(1*H*-

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pyrazol-1-yl)quinoline (1300 mg, 4.74 mmol) and BPD (2200 mg, 8.66 mmol) in 1,4-dioxane (30 mL). The reaction mixture was stirred at 70 °C under N_2 for 8 h, filtered, and concentrated *in vacuo*. The residue was purified by silica column eluting with EtOAc in petroleum ether (20%) to afford 2-(1*H*-pyrazol-1-yl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (1.6 g, 89%) as a light-yellow solid. LCMS (ESI) calcd for $C_{18}H_{20}BN_3O_2$ $[M + H]^+$: 322.2, found: 322.0.

Step 3. Compound **28** was prepared based on the same method as **31**. Calcd for $C_{33}H_{41}N_7O_2$ $[M + H]^+$: 568.3, found: 568.3, R_t = 0.879 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.83 (s, 1H), 8.50−8.52 (m, 2H), 8.17−8.36 (m, 2H), 8.11 (s, 1H), 8.01 (d, J = 10 Hz, 1H), 7.85 (s, 1H), 6.63 (s, 1H), 5.04−5.08 (m, 1H), 3.64−3.66 (m, 1H), 3.45−3.48 (m, 2H), 3.10−3.31 (m, 2H), 2.43−2.50 (m, 4H), 1.92−2.47 (m, 6H), 1.57−1.60 (m, 3H), 1.20−1.40 (m, 5H), 0.99−1.20 (m, 6H).

(*S*)-*N*-((*S*)-1-(5-(7-Methoxyquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (29). See Scheme S5 in the Supporting Information for the synthetic scheme.

Step 1. A solution of H_2SO_4 (5.3 mL, 99 mmol) in water (6 mL) was added to 3-nitrobenzenesulfonic acid (5.1 g, 25.1 mmol) and propane-1,2,3-triol (6.3 mL, 86 mmol) to give a thick gray suspension. The suspension was heated to 110 °C and 4-bromo-3-methoxyaniline (5.0 g, 24.75 mmol) was added. Additional amount of water (6 mL), propane-1,2,3-triol (6 mL), and H_2SO_4 (6 mL) were added, and the reaction temperature was further increased to 140 °C. After 5 h, the mixture became a homogeneous dark-brown solution. The mixture was cooled to rt, poured into ice, and the pH was adjusted to between 8 and 9 by addition of aq. NaOH (20%). The aqueous layer was extracted with EtOAc (3 × 20 mL), and the combined organic layers were washed with brine (2 × 30 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (0−70%) to give 6-bromo-7-methoxyquinoline (2.95 g, 12.4 mmol, 50%) as a yellow solid. LCMS (ESI) calcd for $C_{10}H_8BrNO$ $[M + H]^+$: 238.0, found: 237.9, R_t = 1.031 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.86 (d, J = 2.7 Hz, 1H), 7.98−8.09 (m, 2H), 7.47 (s, 1H), 7.31 (dd, J = 4.3, 8.22 Hz, 1H), 4.06 (s, 3H).

Step 2. $PdCl_2$(dppf) (123 mg, 0.168 mmol) was added to a stirred mixture of BPD (450 mg, 1.77 mmol), potassium acetate (495 mg, 5.04 mmol), and 6-bromo-7-methoxyquinoline (400 mg, 1.68 mmol) in 1,4-dioxane (5 mL), stirred at 75 °C for 4 h, and cooled down to rt. It was diluted with EtOAc (10 mL), washed with brine (3 × 10 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (10−50%) to give (7-methoxyquinolin-6-yl)boronic acid (230 mg, 1.13 mmol, 67%) as a brown oil. LCMS (ESI) calcd for $C_{10}H_{10}BNO_3$ $[M + H]^+$: 204.1, found: 204.0, R_t = 0.810 min.

Step 3. $PdCl_2$(DTBPF) (35 mg, 0.054 mmol) was added to a stirred mixture of K_3PO_4 (314 mg, 1.47 mmol), (7-methoxyquinolin-6-yl)boronic acid (100 mg, 0.493 mmol), (*S*)-*N*-((*S*)-1-(5-bromo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (288 mg, 0.493 mmol) in THF (1 mL) and water (0.5 mL), stirred at 70 °C for 4 h under N_2, and cooled down to rt. It was diluted with EtOAc (10 mL), washed with water (3 × 10 mL), dried with Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column flash chromatography eluting with MeOH in DCM (0−30%) to give (*S*)-*N*-((*S*)-1-(5-(7-methoxyquinolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (120 mg, 0.181 mmol, 37%) as a yellow oil. LCMS (ESI) calcd for $C_{37}H_{55}N_5O_4Si$ $[M + H]^+$: 662.4, found: 662.5, R_t = 1.087 min.

Step 4. (*S*)-*N*-((*S*)-1-(5-(7-methoxyquinolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (110 mg, 0.166 mmol) was added to TFA (2 mL) and stirred at rt for 2 h. The TFA was removed, and the residue was purified by preparative C18 HPLC eluting with acetonitrile in water (10−90% with 0.05% $NH_3{\cdot}H_2O$) to give (*S*)-*N*-((*S*)-1-(5-(7-methoxyquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (60 mg) as a white solid. 2,3-Dihydroxysuccinic acid (17 mg, 0.113 mmol) in MeCN (2 mL) was added to a solution of (*S*)-*N*-((*S*)-1-(5-(7-methoxyquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (60 mg) in MeCN (1 mL). MeCN was removed to give (*S*)-*N*-((*S*)-1-(5-(7-methoxyquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide 2,3-dihydroxysuccinate (**29**, 76 mg, 0.110 mmol, 66%) as a yellow solid. LCMS (ESI) calcd for $C_{31}H_{41}N_5O_3{\cdot}C_4H_6O_6$ $[M + H]^+$: 532.3, found: 532.3, R_t = 1.911 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.73 (d, J = 3.3 Hz, 1H), 8.40 (s, 1H), 8.31 (d, J = 7.9 Hz, 1H), 7.66 (s, 1H), 7.38−7.50 (m, 2H), 5.05 (t, J = 7.5 Hz, 1H), 4.44 (s, 2H), 4.11 (s, 3H), 3.31−3.34 (m, 4H), 3.02−3.03 (m, 1H), 2.70 (brs, 3H), 2.37−2.50 (m, 4H), 1.73−2.06 (m, 6H), 1.31−1.65 (m, 7H), 1.20 (brs, 1H), 0.98 (t, J = 7.4 Hz, 3H).

(*S*)-*N*-((*S*)-1-(5-(7-Methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (30). Prepared based on the same method as **31**. HRMS (ESI) calcd for $C_{32}H_{43}N_5O_3$ $[M + H]^+$: 546.3366, found: 546.3439, R_t = 1.900 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.94−9.08 (m, 1H), 8.89 and 8.67 (s, s, 1H), 8.11 and 8.06 (s, s, 1H), 7.83 (dd, J = 2.20, 8.38 Hz, 1H), 7.68 (s, 1H), 5.07−5.14 (m, 1H), 4.25 (d, J = 4.19 Hz, 3H), 3.40−3.55 (m, 2H), 3.39−3.66 (m, 1H), 3.02−3.26 (m, 1H), 3.00 (s, 3H), 2.81−2.93 (m, 3H), 2.41−2.53 (m, 4H), 1.75−2.31 (m, 6H), 1.21−1.64 (m, 8H), 1.12−1.64 (m, 1H), 0.95−1.10 (m, 3H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(4-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (31). TFA (4 mL, 51.9 mmol) was added to a solution of (*S*)-6-ethyl-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (250 mg, 0.362 mmol) at rt, and the mixture was stirred at rt for 2 h. All of the volatiles were removed by an evaporator to give (*S*)-6-ethyl-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]-octane-1-carboxamide as a brown oil (240 mg, 96%) HRMS (ESI) calcd for $C_{33}H_{45}N_5O_3$ $[M + H]^+$: 560.3522, found: 560.3597. ^{1}H NMR (400 MHz, methanol-d_4) δ 9.09−8.93 (m, 1H), 8.91−8.54 (m, 1H), 8.22−7.98 (m, 1H), 7.95−7.77 (m, 1H), 7.75−7.46 (m, 1H), 5.22−5.03 (m, 1H), 4.25 (s, 3H), 3.70−3.42 (m, 2H), 3.20−3.11 (m, 1H), 3.09−2.89 (m, 4H), 2.82−2.61 (m, 1H), 2.46 (m, 4H), 2.29−1.74 (m, 6H), 1.57 (m, 3H), 1.43−1.23 (m, 6H), 1.20−1.08 (m, 3H), 1.06−0.90 (m, 4H). ^{13}C NMR (101 MHz, methanol-d_4) δ 213.0, 175.7, 171.2, 158.8, 158.1, 149.5, 147.0, 137.0, 131.7, 124.9, 122.4, 121.7, 120.3, 119.9, 105.0, 72.9, 55.0, 51.5, 51.4, 51.2, 41.4, 35.1, 33.4, 32.3, 28.3, 26.2, 25.5, 25.0, 24.3, 23.2, 23.0, 16.7, 8.1, 6.7.

(*S*)-6-Isopropyl-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (32). Acetone (1 mL, 0.188 mmol) was added to (*S*)-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (100 mg, 0.188 mmol) at rt (15 °C), and the mixture was stirred at 15 °C for 4 h. $NaBH(OAc)_3$ (355 mg, 1.881 mmol) was added, and the mixture was stirred at 15 °C for 18 h. The residue was purified by preparative reversed-phase HPLC eluting with acetonitrile/water with 0.05% $NH_3{\cdot}H_2O$ to give (*S*)-6-isopropyl-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide as a colorless solid (60 mg, 0.079 mmol, 42.3%). The solid was dissolved in MeOH (2 mL) and L-(+)-tartaric acid (16 mg, 0.107 mmol) was added. The mixture was concentrated to give (*S*)-6-isopropyl-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide 2,3-dihydroxysuccinate (40 mg, 0.047 mmol, 44.9%) as a colorless solid. HRMS (ESI) calcd for $C_{34}H_{47}N_5O_3{\cdot}C_4H_6O_6$ $[M + H]^+$: 574.3679, found 574.3744. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.35 (brs, 1H), 8.22 (d, J = 8.22 Hz, 1H), 7.61−7.66 (m, 1H), 7.42 (s, 1H), 7.32 (d, J = 8.41 Hz, 1H), 5.09 (brs, 1H), 4.45 (s, 3H), 4.08 (s, 3H), 3.28−3.30 (m, 10H), 3.28−3.28 (m, 2H), 3.02 (brs, 1H), 2.70 (s, 3H), 2.37−

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2.48 (m, 4H), 2.13−2.29 (m, 1H), 1.99 (brs, 2H), 1.72−1.92 (m, 2H), 1.55 (d, J = 7.04 Hz, 2H), 1.25−1.40 (m, 5H), 0.94−0.98 (m, 1H), 0.96 (t, J = 7.24 Hz, 5H).

(1*S*)-6-(Cyclopropylmethyl)-*N*-{(1*S*)-1-[5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl]-7-oxononyl}-6-azaspiro[2.5]octane-1-carboxamide (33). Cyclopropanecarbaldehyde (25 mg, 0.357 mmol) was added to a solution of (*S*)-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide 2,2,2-trifluoroacetate (125 mg, 0.194 mmol) in MeOH (2 mL), and the mixture was stirred at rt for 1 h. $NaBH(OAc)_3$ (123 mg, 0.581 mmol) was added, and the mixture was stirred at rt for 24 h. Water (5 mL) was added, and the mixture was extracted with ethyl acetate (3 × 5 mL). The organic layers were combined and washed with saturated aqueous $NaHCO_3$ (5 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by preparative reversed-phase HPLC eluting with acetonitrile/water with 0.05% $NH_3{\cdot}H_2O$ to give (*S*)-6-(cyclopropylmethyl)-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide as a white solid (50 mg, 43%). The solid was dissolved in acetonitrile (2 mL) and L-(+)-tartaric acid (10 mg, 0.067 mmol) was added. The mixture was dried by lyophilization to give (*S*)-6-(cyclopropylmethyl)-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (2*R*,3*R*)-2,3-dihydroxysuccinate as a white solid. LCMS (ESI) calcd for $C_{35}H_{47}N_5O_3$ [M + H]$^+$: 586.4, found: 586.1. 1H NMR (400 MHz, methanol-d_4) δ 8.40 (brs, 1H), 8.23 (d, J = 8.2 Hz, 1H), 7.65 (s, 1H), 7.43 (s, 1H), 7.34 (d, J =8.2 Hz, 1H), 5.07 (t, J = 7.6 Hz, 1H), 4.45 (s, 2H), 4.10 (s, 3H), 3.50 (brs, 3H), 3.13 (brs, 2H), 2.71 (s, 4H), 2.38−2.50 (m, 4H), 1.73−2.35 (m, 6H), 1.08−1.68 (m, 9H), 0.98 (t, J = 7.2 Hz, 4H), 0.72 (brs, 1H), 0.43 (brs, 2H), −0.03 (brs, 2H).

(1*S*)-6-Cyclobutyl-*N*-{(1*S*)-1-[5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl]-7-oxononyl}-6-azaspiro[2.5]octane-1-carboxamide (34). A mixture of cyclobutanone (25 mg, 0.357 mmol) and (*S*)-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide 2,2,2-trifluoroacetate (125 mg, 0.194 mmol) in MeOH (2 mL) was stirred at rt for 1 h. $NaBH(OAc)_3$ (123 mg, 0.581 mmol) was added, and the mixture was stirred at rt for 24 h. Water (5 mL) was added, and the aqueous layer was extracted with ethyl acetate (3 × 5 mL). The combined organic layers were washed with aqueous $NaHCO_3$ (saturated, 1 × 5 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by preparative reversed-phase HPLC eluting with acetonitrile/water with 0.05% $NH_3{\cdot}H_2O$ to give (*S*)-6-cyclobutyl-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]-octane-1-carboxamide (40 mg, 33%) as a white solid. The solid was dissolved in acetonitrile (2 mL) and L-(+)-tartaric acid (11 mg, 0.073 mmol) was added. The mixture was dried by lyophilization to give (*S*)-6-cyclobutyl-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (2*R*,3*R*)-2,3-dihydroxysuccinate as a white solid. LCMS (ESI) calcd for $C_{35}H_{47}N_5O_3$ [M + H]$^+$: 586.4, found: 586.1, R_t = 1.666 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.45−8.59 (m, 1H), 8.25−8.36 (m, 1H), 7.63−7.77 (m, 1H), 7.45 (s, 1H), 7.32−7.41 (m, 1H), 5.01−5.16 (m, 1H), 4.48 (s, 2H), 4.11 (s, 3H), 2.88−3.10 (m, 2H), 2.72 (s, 3H), 2.44 (d, J = 6.7 Hz, 5H), 1.67−2.35 (m, 11H), 1.16−1.64 (m, 10H), 0.96−0.99 (m, 5H).

(*S*)-6-(Cyclopropylmethyl)-*N*-((*S*)-1-(4-(2-ethyl-7-methoxyquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]-octane-1-carboxamide (35). 6-Bromo-2-ethyl-7-methoxyquinoline was prepared from 4-bromo-3-methoxyaniline and (*E*)-pent-2-enal based on the same method as **63**. Compound **35** was prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{36}H_{49}N_5O_3$ [M + H]$^+$: 600.3, found: 600.2, R_t = 1.654 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.37 (s, 1H), 8.22 (d, J = 8.4 Hz, 1H), 7.61 (s, 1H), 7.44 (s, 1H), 7.35 (d, J = 8.4 Hz, 1H), 5.06−5.01 (m, 1H), 4.43 (s, 2H), 4.08 (s, 3H), 3.53−3.46 (m, 1H), 3.12−3.10 (m, 2H), 2.99−2.97 (m, 3H), 2.45−2.43 (m, 2H), 2.42−2.39 (m, 6H), 2.25−2.10 (m, 2H), 2.00−1.79 (m, 5H), 1.57−1.54 (m, 3H), 1.39−1.35 (m, 9H), 0.98−0.94 (m, 4H), 0.68−0.67 (m, 1H), 0.40−0.30 (m, 2H), −0.07 (m, 1H).

(*S*)-6-(Cyclopropylmethyl)-*N*-((*S*)-1-(4-(2-ethyl-7-methoxyquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]-octane-1-carboxamide (36). See Scheme S6 in the Supporting Information for the synthetic scheme.

Step 1. $AgNO_3$ (43 mg, 0.253 mmol), cyclopropanecarboxylic acid (37 mg, 0.430 mmol) and 6-bromo-7-methoxyquinoline (100 mg, 0.420 mmol) were added to a solution of H_2SO_4 (0.05 mL, 0.938 mmol) in water (3 mL) at 20 °C, and the mixture was warmed up to 70 °C and stirred at 70 °C for 6 h. $(NH_4)_2S_2O_8$ (288 mg, 1.260 mmol) in water (2 mL) was added dropwise, and the mixture was stirred at 70 °C for 12 h, cooled down to rt, and diluted with EtOAc (10 mL). It was washed with water (3 × 10 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column flash chromatography eluting with EtOAc /petroleum ether (0−60%) to give 6-bromo-2-cyclopropyl-7-methoxyquinoline as a colorless oil (10 mg, 8.6%). LCMS (ESI) calcd for $C_{13}H_{12}BrNO$ [M + H]$^+$: 278.0, found: 278.0. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (s, 1H), 7.85 (d, J = 8.4 Hz, 1H), 7.34 (s, 1H), 7.07 (d, J = 8.4 Hz, 1H), 4.03 (s, 3H), 2.16−2.26 (m, 1H), 1.09−1.15 (m, 3H), 0.85 (dd, J = 5.7, 19.85 Hz, 1H).

Step 2. $PdCl_2$(dppf) (30 mg, 0.041 mmol) was added to a mixture of BPD (110 mg, 0.433 mmol) and 6-bromo-2-cyclopropyl-7-methoxyquinoline (100 mg, 0.360 mmol) in 1,4-dioxane (1 mL) at rt and was stirred at 80 °C for 4 h under N_2. The mixture was cooled down to rt, diluted with EtOAc (10 mL), washed with brine (3 × 10 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (0−70%) to give (2-cyclopropyl-7-methoxyquinolin-6-yl)boronic acid (40 mg, 46%) as a colorless oil. LCMS (ESI) calcd for $C_{13}H_{14}BNO_3$ [M + H]$^+$: 244.1, found: 244.1.

Step 3. Compound **36** was prepared based on the same procedure as **31**. LCMS (ESI) calcd for C37H49N5O3 [M + H]$^+$: 612.3, found: 612.1, R_t = 1.694 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.33 (s, 1H), 8.14 (d, J = 8.4 Hz, 1H), 7.62 (s, 1H), 7.42 (s, 1H), 7.13 (d, J = 8.8 Hz, 1H), 5.05 (t, J = 7.3 Hz, 1H), 4.45 (s, 2H), 4.08 (s, 3H), 3.48−3.46 (m, 2H), 3.14−3.12 (m, 2H), 2.69−2.68 (m, 2H), 2.46−2.44 (m, 4H), 2.27−2.26 (m, 2H), 2.02−2.01 (m, 2H), 1.59−1.57 (m, 3H), 1.52−1.51 (m, 3H), 1.36−1.35 (m, 4H), 1.16−1.14 (m, 6H), 0.99−0.97 (m, 4H), 0.68−0.67 (m, 1H), 0.43−0.05 (m, 3H).

(*S*)-*N*-((*S*)-1-(5-(2-Cyclopropyl-7-methoxyquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (37). Prepared based on the same method as **36** as yellow solid. LCMS (ESI) calcd for $C_{34}H_{45}N_5O_3{\cdot}ClH$ [M + H]$^+$: 572.4, found: 572.4, R_t = 2.002 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.59−8.93 (m, 2H), 8.06 (d, J = 18.1 Hz, 1H), 7.70 (s, 1H), 7.46 (dd, J = 2.2, 8.6 Hz, 1H), 5.09 (brs, 1H), 4.24 (d, J = 4.2 Hz, 3H), 3.40−3.68 (m, 3H), 2.99−3.25 (m, 2H), 2.82−2.94 (m, 4H), 2.52−2.63 (m, 1H), 2.41−2.52 (m, 4H), 2.05−2.20 (m, 2H), 1.88−1.96 (m, 2H), 1.27−1.68 (m, 11H), 1.13−1.22 (m, 1H), 0.94−1.09 (m, 3H), 0.80 (t, J = 7.5 Hz, 1H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(7-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro-[2.5]octane-1-carboxamide (38). 6-Bromo-7-methoxy-1-methylquinolin-2(1*H*)-one was prepared based on published procedure.[21] Compound **38** was prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{33}H_{45}N_5O_4$ [M + H]$^+$: 576.3, found: 576.2, R_t = 2.110 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.38 (brs, 0.5H), 8.09 (brs, 0.5H), 8.00−7.90 (m, 1H), 7.88−7.75 (m, 1H), 7.18 (brs, 1H), 6.63 (d, J = 9.4 Hz, 1H), 5.07 (brs, 1H), 4.15 (d, J = 4.1 Hz, 3H), 3.80 (brs, 3H), 3.62−3.40 (m, 2H), 3.23−3.11 (m, 3H), 3.07−2.89 (m, 1H), 2.72−2.56 (m, 2H), 2.26−1.97 (m, 2H), 1.96−1.70 (m, 4H), 1.56 (m, 3H), 1.42−1.21 (m, 7H), 1.18−1.09 (m, 3H), 1.06−0.85 (m, 4H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(5-methoxy-2-methyl-2*H*-indazol-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (39). See Scheme S7 in the Supporting Information for the synthetic scheme.

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Step 1. Trimethyloxonium tertafluoroborate (0.782 g, 5.28 mmol) was added to a solution of 6-bromo-5-methoxy-1*H*-indazole (1.00 g, 4.40 mmol) in EtOAc (20 mL) and stirred at rt for 2 h. Water (50 mL) was added, and the aqueous layer was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with brine (2 × 50 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (20%) to give 6-bromo-5-methoxy-2-methyl-2*H*-indazole as an orange solid (800 mg, 72%). LCMS (ESI) calcd for $C_9H_9BrN_2O$ $[M + H]^+$: 241.0, found: 242.9. ^1H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.90 (s, 1H), 7.19 (s, 1H), 4.11 (s, 3H), 3.83 (s, 3H).

Step 2. Potassium acetate (855 mg, 8.71 mmol) and BPD (1475 mg, 5.81 mmol) were added to a stirred mixture of 6-bromo-5-methoxy-2-methyl-2*H*-indazole (700 mg, 2.90 mmol) in 1,4-dioxane (10 mL) at rt under N_2, followed by addition of $PdCl_2$(dppf) (212 mg, 0.290 mmol). The mixture was stirred at 80 °C for 18 h and filtered. The filter cake was washed with EtOAc (40 mL), and the filtrate was concentrated under vacuum. It was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (25%) to give 5-methoxy-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2*H*-indazole (920 mg, 56%) as a red solid. LCMS (ESI) calcd for $C_{15}H_{21}BN_2O_3$ $[M + H]^+$: 289.1, found: 289.1.

Step 3. Compound **39** was prepared based on the same procedure as **31**. Red solid. LCMS (ESI) calcd for $C_{31}H_{44}N_6O_3$ $[M + H]^+$: 549.3, found: 549.2. ^1H NMR (400 MHz, methanol-d_4) δ 8.34 (d, J = 4.2 Hz, 1H), 8.18 (s, 0.5H), 8.00 (s, 0.5H), 7.88 (d, J = 12 Hz, 1H), 7.36 (s, 1H), 5.12−5.05 (m, 1H), 4.27 (d, J = 3.2 Hz, 3H), 4.00 (d, J = 2.9 Hz, 3H), 3.60−3.33 (m, 2H), 3.28−3.14 (m, 2H), 3.08−2.91 (m, 1H), 2.73−2.58 (m, 1H), 2.51−2.38 (m, 5H), 2.25−1.86 (m, 7H), 1.63−1.47 (m, 3H), 1.43−1.28 (m, 6H), 1.23−1.14 (m, 3H), 1.07−0.96 (m, 4H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(7-methoxyquinoxalin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (40). *n*-BuLi (1 mL, 2.500 mmol) was added to a solution of 6-bromo-7-methoxyquinoxaline (500 mg, 2.091 mmol) in THF (10 mL) at −78 °C. The mixture was stirred at −78 °C for 1 h. Triisopropyl borate (787 mg, 4.18 mmol) in THF (5 mL) was added at −78 °C, and the mixture was stirred at −78 °C for 2 h. The mixture was quenched with saturated aqueous NH_4Cl (5 mL) and extracted with EtOAc (10 mL). The organic layer was washed with 10% EtOAc in petroleum ether and filtered to give (7-methoxyquinoxalin-6-yl)boronic acid as a white solid (40 mg, 0.098 mmol, 4.7%). LCMS (ESI) calcd for $C_9H_9BN_2O_3$ $[M + H]^+$: 205.0, found: 205.1, R_t = 0.288 min. Compound **40** was prepared based on the same method as **31**. Yellow solid. LCMS (ESI) calcd for $C_{31}H_{42}N_6O_3$ $[M + H]^+$: 547.3, found: 547.1, R_t = 2.151 min. ^1H NMR (400 MHz, methanol-d_4) δ 8.74−8.88 (m, 2H), 8.39−8.63 (m, 1H), 7.91−8.02 (m, 1H), 7.56−7.66 (m, 1H), 5.03−5.15 (m, 1H), 4.12−4.20 (m, 3H), 3.51−3.61 (m, 1H), 2.91−3.25 (m, 4H), 2.39−2.50 (m, 4H), 2.16−2.36 (m, 1H), 1.97−2.15 (m, 2H), 1.80−1.94 (m, 3H), 1.46−1.64 (m, 3H), 1.08−1.43 (m, 10H), 0.91−1.07 (m, 4H).

(*S*)-*N*-((*S*)-1-(5-(2-Methoxy-1,7-naphthyridin-3-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (41). LDA (1.873 mL, 3.75 mmol) was added to a solution of 2-methoxy-1,7-naphthyridine (400 mg, 2.497 mmol) and triisopropyl borate (939 mg, 4.99 mmol) in THF (3 mL) at −78 °C, and the mixture was stirred at −78 °C for 3 h and 18 °C for 10 h. The reaction was quenched with saturated aqueous NH_4Cl (10 mL) and extracted with EtOAc (10 mL × 3). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated. The product was purified by flash silica gel chromatography (20 g, MeOH/DCM: 0−10%) to give (2-methoxy-1,7-naphthyridin-3-yl)boronic acid as a white solid (100 mg, 20%). ^1H NMR (400 MHz, methanol-d_4) δ 9.17−9.10 (m, 1H), 8.45−8.39 (m, 1H), 8.30−8.23 (m, 1H), 7.85−7.79 (m, 1H), 4.12 (s, 1H). LCMS (ESI) calcd for $C_9H_9BN_2O_3$ $[M + H]^+$: 205.1, found: 205.1. Compound **41** was prepared based on the same method as **31**. LCMS (ESI) calcd for $C_{30}H_{40}N_6O_3$ $[M + H]^+$: 533.3, found: 533.3, R_t = 1.980 min. ^1H NMR (400 MHz, methanol-d_4) δ 9.09 (s, 1H), 8.80−8.72 (m, 1H), 8.42−8.37 (m, 1H), 7.83−7.78 (m, 1H), 7.77 (s, 1H), 5.08−4.99 (m, 1H), 4.25 (s, 3H), 2.57−2.40 (m, 7H), 2.35−2.25 (m, 1H), 2.19−2.11 (m, 3H), 2.06−1.94 (m, 2H), 1.70−1.52 (m, 7H), 1.50−1.29 (m, 6H), 1.02−0.94 (m, 3H).

(1*S*)-*N*-{(1*S*)-1-[5-(7-Fluoro-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl]-7-oxononyl}-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (42). 4-Bromo-3-fluoroaniline (500 mg, 2.63 mmol) was added to a solution of *p*-chloranil (0.776 g, 3.16 mmol) in butanol (5.0 mL) and HCl (37%, 5 mL, 60.9 mmol) at rt, and the temperature was increased to 120 °C. (*E*)-but-2-enal (9.31 mL, 113 mmol) in BuOH (0.3 mL) was added dropwise. The mixture was stirred at 120 °C for 40 min. After cooling to rt, it was diluted with H_2O (10 mL) and extracted with EtOAc (3 × 10 mL). The aqueous layer was neutralized with NaOH (37%) to pH> 9 and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by preparative reversed-phase HPLC eluting with acetonitrile/water with 0.225% HCOOH to give 6-bromo-7-fluoro-2-methylquinoline as a white solid (200 mg, 32%). ^1H NMR (400 MHz, methanol-d_4) δ 8.67 (d, J = 8.6 Hz, 1H), 8.54 (d, J = 7.0 Hz, 1H), 7.85 (d, J = 9.0 Hz, 1H), 7.76 (d, J = 8.6 Hz, 1H), 2.89 (s, 3H). LCMS (ESI) calcd for $C_{10}H_7BrFN$ $[M + H]^+$: 240.0, found: 240.0. Compound **42** was prepared based on the same method as **31**. White solid. LCMS (ESI) calcd for $C_{31}H_{40}FN_5O_2$ $[M + H]^+$: 534.3, found: 534.3. ^1H NMR (400 MHz, $CDCl_3$) δ 8.46 (brs, 1H), 8.07 (d, J = 8.2 Hz, 1H), 7.67 (d, J = 12.9 Hz, 1H), 7.47 (d, J = 3.9 Hz, 1H), 7.24 (d, J = 8.6 Hz, 1H), 6.54 (d, J = 7.4 Hz, 1H), 4.97 (q, J = 7.4 Hz, 1H), 2.72 (s, 3H), 2.35−2.49 (m, 5H), 2.20−2.34 (m, 3H), 2.17 (s, 3H), 2.04 (d, J = 7.8 Hz, 3H), 1.59 (td, J = 7.2, 14.48 Hz, 4H), 1.30−1.51 (m, 6H), 1.21 (d, J = 4.3 Hz, 1H), 1.03 (t, J = 7.4 Hz, 3H), 0.83 (dd, J = 4.3, 7.43 Hz, 1H).

(*S*)-*N*-((*S*)-1-(5-(7-Chloro-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (43). See Scheme S8 in the Supporting Information for the synthetic scheme.

Step 1. (*E*)-but-2-enal (2.03 g, 29.1 mmol) was added dropwise to a stirred mixture of 4-bromo-3-chloroaniline (4.00 g, 19.4 mmol) and conc. HCl (12.7 mL, 155 mmol) in water (4.0 mL) at 110 °C, and the mixture was stirred at 110 °C for 2 h. Then, the mixture was neutralized with NH_4OH till pH = 7. The organic phase was extracted with DCM (3 × 50 mL). The combined organic layers were dried with $MgSO_4$, filtered, and concentrated. The residue was purified by preparative C-18 HPLC eluting with acetonitrile in water (10−90% with 0.1% TFA) to give 6-bromo-7-chloro-2-methylquinoline (1492 mg, 5.82 mmol, 30.0%) as a white solid and 6-bromo-5-chloro-2-methylquinoline (1577 mg, 6.15 mmol, 31.7%) as a white solid.

Step 2. $PdCl_2$(dppf) (61 mg, 0.084 mmol) was added to a stirred mixture of KOAc (494 mg, 5.03 mmol), BPD (724 mg, 2.85 mmol), and 6-bromo-7-chloro-2-methylquinoline (430 mg, 1.68 mmol) in dioxane (10 mL), and the mixture was stirred at 70 °C for 1 h under N_2. The solvent was evaporated under reduced pressure. The residue was purified by silica gel, eluting with EtOAc in petroleum ether (50%) to give 7-chloro-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (500 mg, 1.65 mmol, 98%) as a black solid. LCMS (ESI) calcd for $C_{16}H_{19}BClNO_2$ $[M + H]^+$: 304.1, found: 304.1, R_t = 1.041 min.

Step 3. $PdCl_2$(DTBPF) (52 mg, 0.081 mmol) was added to a mixture of (*S*)-*tert*-butyl 1-(((*S*)-1-(5-bromo-1-((2-(trimethylsilyl)-ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)carbamoyl)-6-azaspiro[2.5]octane-6-carboxylate (600 mg, 0.896 mmol), 7-chloro-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (408 mg, 1.344 mmol), and K_3PO_4 (570 mg, 2.69 mmol) in 1,4-dioxane (12.0 mL) and water (0.5 mL). Then, the mixture was stirred at 70 °C under N_2 for 4 h. The reaction mixture was cooled to 26 °C and filtered through celite. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (20−50%) to give (*S*)-*tert*-butyl 1-(((*S*)-1-(5-(7-chloro-2-methylquinolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)-carbamoyl)-6-azaspiro[2.5]octane-6-carboxylate (550 mg, 0.718

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mmol, 80%) as a yellow oil. LCMS (ESI) calcd for $C_{41}H_{60}ClN_5O5Si$ $[M + H]^+$: 766.4, found: 766.5, R_t = 1.318 min.

Step 4. (*S*)-*tert*-butyl-1-(((*S*)-1-(5-(7-chloro-2-methylquinolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)carbamoyl)-6-azaspiro[2.5]octane-6-carboxylate (200 mg, 0.261 mmol) was added to TFA (1 mL, 0.261 mmol), and the mixture was stirred at 20 °C for 2 h. The mixture was concentrated to dryness to give crude (*S*)-*N*-((*S*)-1-(5-(7-chloro-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (140 mg, 0.261 mmol), which was used without further purification. LCMS (ESI) calcd for $C_{30}H_{38}ClN_5O_2$ $[M + H]^+$: 536.3, found: 536.3, R_t = 0.832 min.

Step 5. Formaldehyde (1 mL, 12.32 mmol) was added to a mixture of (*S*)-*N*-((*S*)-1-(5-(7-chloro-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (140 mg, 0.261 mmol) in MeOH (3 mL), and the mixture was stirred at 20 °C for 2 h. $NaBH(OAc)_3$ (247 mg, 1.2 mmol) was added, and the mixture was stirred at 20 °C for 3 h. The reaction mixture was concentrated, and the residue was purified by preparative C18 HPLC eluting with acetonitrile in water (10−90%, with 0.05% $NH_3{\cdot}H_2O$) to give (*S*)-*N*-((*S*)-1-(5-(7-chloro-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (75 mg, 0.136 mmol, 52.2%) as a white solid. LCMS (ESI) calcd for $C_{31}H_{40}ClN_5O_2$ $[M + H]^+$: 550.3, found: 550.4, R_t = 0.863 min. L-Tartaric acid (17 mg, 0.12 mmol) was added to (*S*)-*N*-((*S*)-1-(5-(7-chloro-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (65 mg, 0.12 mmol) in water (2 mL) at 16 °C, and the mixture was stirred at the same temperature for 15 min. The mixture was lyophilized to give (*S*)-*N*-((*S*)-1-(5-(7-chloro-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide 2*R*,3*R*-dihydroxysuccinate (**43**, 80 mg, 0.11 mmol) as a yellow oil. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.25−8.31 (m, 2H), 8.05 (s, 1H), 7.58−7.62 (m, 1H), 7.43−7.49 (m, 1H), 5.03−5.054 (m, 1H), 4.42 (s, 2H), 3.02−3.12 (m, 1H), 2.72 (s, 6H), 2.39−2.50 (m, 4H), 1.75−2.08 (m, 6H), 1.31−1.63 (m, 8H), 1.27−1.32 (m, 2H), 1.17−1.24 (m, 1H), 0.99 (t, J = 7.3 Hz, 4H).

(*S*)-*N*-((*S*)-1-(5-(2,7-Dimethylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (44). Prepared based on the same method of **31**. LCMS (ESI) calcd for $C_{32}H_{43}N_5O_2$ $[M + H]^+$: 530.3, found: 530.2, R_t = 1.945 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.23 (d, J = 8.6 Hz, 1H), 8.04 (s, 1H), 7.83 (s, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.29 (s, 1H), 5.03 (t, J = 7.0 Hz, 1H), 4.42 (s, 2H), 3.42−4.13 (m, 1H), 2.98−3.22 (m, 2H), 2.63−2.95 (m, 6H), 2.60 (s, 3H), 2.41−2.49 (m, 4H), 2.08−2.34 (m, 1H), 1.74−2.07 (m, 6H), 1.53−1.62 (m, 2H), 1.28−1.50 (m, 5H), 1.19 (brs, 1H), 0.95−1.02 (m, 4H).

(1*S*)-*N*-{(1*S*)-1-[5-(7-Ethyl-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl]-7-oxononyl}-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (45). See Scheme S9 in the Supporting Information for the synthetic scheme.

Step 1. $PdCl_2$(DTBPF) (52 mg, 0.081 mmol) was added to a mixture of (*S*)-*tert*-butyl 1-(((*S*)-1-(5-bromo-1-((2-(trimethylsilyl)-ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)carbamoyl)-6-azaspiro[2.5]octane-6-carboxylate (600 mg, 0.896 mmol), 7-chloro-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (408 mg, 1.34 mmol), and K_3PO_4 (570 mg, 2.69 mmol) in dioxane (12.0 mL) and water (0.5 mL). After the mixture was stirred at 70 °C under N_2 for 4 h, it was cooled to rt and filtered through Celite. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (10−50%) to give (*S*)-*tert*-butyl 1-(((*S*)-1-(5-(7-chloro-2-methylquinolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)carbamoyl)-6-azaspiro[2.5]octane-6-carboxylate (550 mg, 80%) as a yellow oil. LCMS (ESI) calcd for $C_{41}H_{60}ClN_5O_5Si$ $[M + H]^+$: 766.4, found: 766.5.

Step 2. $PdCl_2$(DTBPF) (33 mg, 0.051 mmol) was added to a mixture of (*S*)-*tert*-butyl 1-(((*S*)-1-(5-(7-chloro-2-methylquinolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)carbamoyl)-6-azaspiro[2.5]octane-6-carboxylate (203 mg, 0.265 mmol), 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (210 mg, 1.364 mmol), and K_3PO_4 (189 mg, 0.890 mmol) in THF (3 mL) and water (0.3 mL), and the mixture was stirred at 80 °C for 6 h. To the mixture was added another batch of 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (200 mg), K_3PO_4 (190 mg), $PdCl_2$(DTBPF) (30 mg), bubbled with N_2, and stirred at 80 °C for 9 h. To the mixture was added one more batch of 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (180 mg), K_3PO_4 (190 mg), $PdCl_2$(DTBPF) (33 mg), and bubbled with N_2, and stirred at 80 °C for another 20 h. The mixture was diluted with water (30 mL), extracted with DCM (3 × 15 mL). The combined organic layers were washed with brine (15 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column flash chromatography eluting with MeOH in DCM (0−10%). The product obtained was purified one more time by preparative C18 HPLC eluting with acetonitrile in water (10−90% with 0.1% TFA) to give (*S*)-*tert*-butyl 1-(((*S*)-1-(5-(2-methyl-7-vinylquinolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)carbamoyl)-6-azaspiro[2.5]octane-6-carboxylate (70 mg, 32%) as a yellow oil. LCMS (ESI) calcd for $C_{43}H_{63}N_5O_5Si$ $[M + H]^+$: 758.5, found: 758.4.

Step 3. 10% Pd−C (80 mg, 0.075 mmol) was added to a mixture of (*S*)-*tert*-butyl 1-(((*S*)-1-(5-(2-methyl-7-vinylquinolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)-carbamoyl)-6-azaspiro[2.5]octane-6-carboxylate (70 mg, 0.092 mmol) in MeOH (15 mL), and the mixture was stirred at rt for 2 h under H_2 (15 psi). The mixture was filtered, and the filter cake was washed with MeOH (30 mL). The filtrate was concentrated to give (*S*)-*tert*-butyl 1-(((*S*)-1-(5-(7-ethyl-2-methylquinolin-6-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)-carbamoyl)-6-azaspiro[2.5]octane-6-carboxylate (65 mg, 93%) as a yellow oil, which was used in the next step without further purification. LCMS (ESI) calcd for $C_{43}H_{65}N_5O_5Si$ $[M + H]^+$: 760.5, found: 760.5.

Step 4. TFA (4.0 mL, 52 mmol) was added to (*S*)-*tert*-butyl 1-(((*S*)-1-(5-(7-ethyl-2-methylquinolin-6-yl)-1-((2-(trimethylsilyl)-ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)carbamoyl)-6-azaspiro[2.5]octane-6-carboxylate (65 mg, 0.086 mmol), and the mixture was stirred at rt for 3 h. TFA was removed under reduced pressure to give (*S*)-*N*-((*S*)-1-(5-(7-ethyl-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (45 mg) as a yellow oil, which was used in the next step without further purification. LCMS (ESI) calcd for $C_{32}H_{43}N_5O_2$ $[M + H]^+$: 530.3, found: 530.4.

Step 5. A mixture of (*S*)-*N*-((*S*)-1-(5-(7-ethyl-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (45 mg, 0.085 mmol) and formaldehyde (0.5 mL, 6.72 mmol) in MeOH (2 mL) was stirred at rt for 8 h. Then, $NaBH(OAc)_3$ (103 mg, 0.486 mmol) was added portion-wise. After the mixture was stirred at rt for another 1 h, it was diluted with DMF (3 mL) and purified by preparative C18 HPLC eluting with acetonitrile in water (10−90% with 0.1% TFA). The obtained product (20 mg) was purified one more time by preparative C18 HPLC eluting with acetonitrile in water (10−90% with 0.05% $NH_3{\cdot}H_2O$) to give (*S*)-*N*-((*S*)-1-(5-(7-ethyl-2-methylquinolin-6-yl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (**45**, 11 mg, 24%) as a yellow oil. LCMS (ESI) calcd for $C_{33}H_{45}N_5O_2$ $[M + H]^+$: 544.4, found: 544.4. ^{1}H NMR (400 MHz, MeOD) δ 8.19 (d, J = 8.22 Hz, 1H), 7.92 (brs, 1H), 7.85 (s, 1H), 7.37 (d, J = 8.61 Hz, 1H), 7.15 (brs, 1H), 5.02 (t, J = 7.63 Hz, 1H), 2.97−2.99 (m, 2H), 2.71 (s, 3H), 2.40−2.56 (m, 7H), 2.34 (brs, 1H), 2.17 (s, 3H), 1.88−2.05 (m, 2H), 1.67 (brs, 2H), 1.51−1.62 (m, 4H), 1.29−1.41 (m, 5H), 1.24 (t, J = 7.63 Hz, 3H), 1.11 (t, J = 4.89 Hz, 1H), 0.99 (t, J = 7.24 Hz, 3H), 0.82 (dd, J = 4.50, 8.02 Hz, 1H).

(*S*)-*N*-((*S*)-1-(5-(7-Methoxyquinolin-6-yl)oxazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (46). Prepared based on the same method as **48**. Yellow solid. LCMS (ESI) calcd for $C_{31}H_{40}N_4O_4{\cdot}ClH$ $[M + H]^+$: 533.3, found: 533.1, R_t = 2.118 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 9.20−9.27 (m, 1H), 9.19−9.36 (m, 1H), 9.09 (d, J = 5.3 Hz, 1H), 7.97 (dd, J = 5.7, 8.16

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Hz, 1H), 7.80 (d, J = 8.8 Hz, 1H), 7.72 (d, J = 2.2 Hz, 1H), 5.17 (d, J = 6.6 Hz, 1H), 4.29 (s, 3H), 3.44−3.60 (m, 2H), 3.00−3.17 (m, 2H), 2.89 (d, J = 17.4 Hz, 3H), 2.42−2.52 (m, 4H), 2.32 (m, 2H), 1.86−2.09 (m, 5H), 1.46−1.65 (m, 3H), 1.31−1.46 (m, 4H), 1.18−1.31 (m, 1H), 0.96−1.03 (m, 3H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(2-methoxy-1,7-naphthyridin-3-yl)-oxazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (2*R*,3*S*)-2,3-dihydroxysuccinate (47). Prepared based on the same method as **48**. Yellow solid. LCMS (ESI) calcd for $C_{31}H_{41}N_5O_4$ [M + H]$^+$: 548.3, found: 548.1, R_t = 2.201 min. 1H NMR (400 MHz, methanol-d_4) δ 9.14 (s, 1H), 8.61 (s, 1H), 8.46 (d, J = 5.29 Hz, 1H), 7.90 (d, J = 5.51 Hz, 1H), 7.76 (s, 1H), 5.10−5.24 (m, 1H), 4.45 (s, 2H), 4.28 (s, 3H), 3.11−3.23 (m, 2H), 2.38−2.53 (m, 5H), 2.03−2.15 (m, 1H), 1.88−2.00 (m, 3H), 1.77−1.87 (m, 1H), 1.11−1.68 (m, 14H), 0.99 (t, J = 7.39 Hz, 4H), 0.88−1.08 (m, 1H).

(*S*)-*N*-((*S*)-1-(5-(7-Methoxy-2-methylquinolin-6-yl)oxazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (48). To a 10 mL vial was added a solution of (*S*)-*N*-((*S*)-1-(5-bromooxazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (150 mg, 0.330 mmol), 7-methoxy-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (119 mg, 0.396 mmol), potassium phosphate (210 mg, 0.990 mmol), and $PdCl_2$(DTBPF) (22 mg, 0.034 mmol). The vial was degassed and refilled with nitrogen three times. Dioxane (2 mL) and water (0.05 mL) were sealed in a 10 mL vial with nitrogen, and the reaction mixture was stirred at 70 °C for 2 h. The reaction mixture was concentrated to dryness and the product. After it was cooled down, the solvents was removed under reduced pressure and the product was purified by preparative reversed-phase HPLC eluting with acetonitrile/water with 0.1% TFA to give (*S*)-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-oxazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide as a yellow solid (80 mg, 0.128 mmol, 38.8%). HRMS (ESI) calcd for $C_{32}H_{42}N_4O_4$ [M + H]$^+$: 547.3206, found 547.3288. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.96−9.07 (m, 1H), 8.58−8.69 (m, 1H), 7.78 (dd, J = 7.7, 12.57 Hz, 2H), 7.65 (d, J = 2.9 Hz, 1H), 5.15 (t, J = 6.2 Hz, 1H), 4.26 (s, 3H), 3.42−3.60 (m, 2H), 2.99 (s, 3H), 2.88 (d, J = 13.45 Hz, 3H), 2.40−2.52 (m, 4H), 2.25−2.38 (m, 1H), 1.83−2.13 (m, 5H), 1.17−1.65 (m, 10H), 0.95−1.08 (m, 4H). ^{13}C NMR (101 MHz, methanol-d_4) δ 212.9, 175.4, 171.4, 164.1, 159.9, 157.1, 147.7, 147.3, 137.1, 126.2, 124.3, 121.5, 120.6, 118.1, 105.5, 72.7, 55.1, 53.9, 53.6, 42.4, 41.4, 35.1, 32.7, 32.5, 28.3, 26.1, 25.3, 25.1, 24.1, 23.2, 17.0, 6.7.

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-oxazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (49). Prepared based on the same method as **48**. HRMS (ESI) calcd for $C_{33}H_{44}N_4O_4$ [M + H]$^+$: 561.3363, found 561.3435. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.26−8.24 (m, 2H), 7.58 (s, 1H), 7.43 (s,1H), 7.35−7.32 (m, 1H), 5.18−5.14 (m, 1H), 4.40 (s, 2H), 4.11 (s, 3H), 3.11−3.09 (m, 2H), 2.70 (s, 3H), 2.48−2.41 (m, 4H), 1.97−1.93 (m, 5H), 1.85−1.80 (m, 1H), 1.60−1.38 (m, 11H), 1.23 (brs, 4H), 1.00−0.96 (m, 4H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(7-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)oxazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]-octane-1-carboxamide (50). Prepared based on the same method as **48**. Yellow solid. LCMS (ESI) calcd for $C_{33}H_{44}N_4O_5$ [M + H]$^+$: 577.7, found: 577.7, R_t = 1.008 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.08−8.16 (m, 1H), 8.02 (dd, J = 2.31, 9.37 Hz, 1H), 7.58 and 7.65 (s, s, 1H), 7.14 (s, 1H), 6.62 (d, J = 9.48 Hz, 1H), 5.11−5.21 (m, 1H), 4.15 (s, 3H), 3.79 (s, 3H), 3.47−3.57 (m, 1H), 2.95−3.26 (m, 4H), 2.41−2.52 (m, 4H), 1.79−2.13 (m, 6H), 1.22−1.62 (m, 12H), 0.94−1.08 (m, 4H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(7-methoxyquinolin-6-yl)oxazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (51). Prepared based on the same method as **48**. Yellow oil. HRMS (ESI) calcd for $C_{32}H_{42}N_4O_4$ [M + H]$^+$: 547.3206, found 547.3285. ^{1}H NMR (400 MHz, methanol-d_4) δ 9.17−9.28 (m, 1H), 9.08 (d, J=4.19 Hz, 1H), 8.65−8.78 (m, 1H), 7.95 (m, 1H), 7.79 (s, 1H), 7.71 (s, 1H), 5.10−5.17 (m, 1H), 4.29 (s, 3H), 3.38−3.69 (m, 2H), 2.93−3.31 (m, 5H), 2.46 (s, 4H), 2.23−2.37 (m, 1H), 1.81−2.17 (m, 5H), 1.17−1.66 (m, 11H), 0.95−1.15 (m, 3H),

(*S*)-*N*-((*S*)-1-(5-(7-Fluoro-2-methylquinolin-6-yl)oxazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (2*R*,3*R*)-2,3-dihydroxysuccinate (52). Prepared based on the same method as **48**. White solid. HRMS (ESI) calcd for $C_{31}H_{39}FN_4O_3$ [M + H]$^+$: 535.3006, found 535.3090. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.35 (dd, J = 4.5, 7.8 Hz, 2H), 7.70 (d, J = 12.5 Hz, 1H), 7.55 (d, J = 4.1 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 5.14−5.21 (m, 1H), 4.48 (s, 2H), 3.49 (brs, 1H), 2.96−3.15 (m, 1H), 2.85 (s, 3H), 2.73 (s, 3H), 2.41−2.51 (m, 4H), 1.90−2.35 (m, 5H), 1.83 (t, J = 6.7 Hz, 1H), 1.34−1.66 (m, 7H), 1.15−1.32 (m, 2H), 0.99 (t, J = 7.3 Hz, 4H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(7-methoxy-1-methyl-2-oxo-1,2-dihydroquinolin-6-yl)oxazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]-octane-1-carboxamide (53). See Scheme S10 in the Supporting Information for the synthetic scheme.

Step 1. Tricyclohexylphosphine (121 mg, 0.432 mmol) and diacetoxypalladium (48 mg, 0.214 mmol) were added to a mixture of potassium phosphate tribasic (917 mg, 4.32 mmol), cyclopropylboronic acid (185 mg, 2.159 mmol), and 6-bromo-7-fluoro-2-iodoquinoline (760 mg, 2.159 mmol) in toluene (5 mL), and the mixture was stirred at 100 °C for 8 h. The mixture was cooled down, diluted with EtOAc (10 mL), washed with brine (3 × 15 mL), dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (0−10%) to give 6-bromo-2-cyclopropyl-7-fluoroquinoline (300 mg, 1.13 mmol, 52%) as a yellow solid. LCMS (ESI) calcd for $C_{12}H_9BrFN$ [M + H]$^+$: 266.0, found: 267.7, R_t = 1.082 min.

Step 2. *n*-BuLi (1.5 mL, 3.75 mmol) was added to a stirred mixture of triisopropyl borate (424 mg, 2.255 mmol) and 6-bromo-2-cyclopropyl-7-fluoroquinoline (300 mg, 1.127 mmol) in THF (2 mL) at −78 °C, and the mixture was gradually warmed up to rt and stirred at rt for 4 h. Aqueous ammonium chloride (20%, 10 mL) was added, and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine (15 mL), dried over Na_2SO_4, filtered, and the filtrate was evaporated under reduced pressure. The residue was purified by silica gel column flash chromatography eluting with MeOH in DCM (0−10%) to give (2-cyclopropyl-7-fluoroquinolin-6-yl)boronic acid (160 mg, 0.693 mmol, 61%) as a yellow oil. LCMS (ESI) calcd for $C_{12}H_{11}BFNO_2$ [M + H]$^+$: 232.1, found: 231.7, R_t = 0.902 min.

Step 3. $PdCl_2$(DTBPF) (40 mg, 0.061 mmol) was added to a mixture of K_3PO_4 (358 mg, 1.69 mmol), (*S*)-*N*-((*S*)-1-(5-bromooxazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (250 mg, 0.550 mmol), and (2-cyclopropyl-7-fluoroquinolin-6-yl)boronic acid (130 mg, 0.563 mmol) in THF (3 mL) and water (0.5 mL) at rt, and the mixture was stirred at 70 °C for 6 h. The mixture was concentrated to 3 mL and purified by preparative C18 HPLC eluting with acetonitrile in water (10−90% with 0.1% TFA) to give (*S*)-*N*-((*S*)-1-(5-(2-cyclopropyl-7-fluoroquinolin-6-yl)oxazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (65 mg, 0.112 mmol, 20%) as a yellow solid. HCl (1 M in water, 2.3 mL, 0.230 mmol) was added to the above product in MeCN (2 mL) at rt. The product was lyophilized to give (*S*)-*N*-((*S*)-1-(5-(2-cyclopropyl-7-fluoroquinolin-6-yl)oxazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide hydrochloride (**53**, 60 mg, 0.096 mmol, 83%) as a yellow solid. LCMS (ESI) calcd for $C_{33}H_{41}FN_4O_3$·ClH [M + H]$^+$: 561.3, found: 561.3, R_t = 2.042 min. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.97−9.04 (m, 1H), 8.63−8.75 (m, 1H), 7.99 (d, J = 11.0 Hz, 1H), 7.67 (dd, J = 4.1, 13.5 Hz, 1H), 7.57 (d, J = 8.6 Hz, 1H), 5.08−5.20 (m, 1H), 3.39−3.60 (m, 2H), 3.02−3.11 (m, 1H), 2.81−2.94 (m, 3H), 2.52−2.63 (m, 1H), 2.38−2.50 (m, 3H), 2.22−2.32 (m, 1H), 1.79−2.09 (m, 5H), 1.68 (d, J = 5.5 Hz, 2H), 1.15−1.62 (m, 11H), 0.88−1.12 (m, 4H), 0.78 (t, J = 7.2 Hz, 1H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(5-methoxy-2-methyl-2*H*-indazol-6-yl)oxazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (54). Prepared based on the same method as **39** and **48**. White solid. HRMS (ESI) calcd for $C_{31}H_{43}N_5O_4$·ClH [M + H]$^+$: 550.3115, found 550.3886. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.37 (brs, 1H), 8.00 (d, J = 10.1 Hz, 1H), 7.62 (d, J = 4.9 Hz, 1H), 7.33 (s, 1H),

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5.17−5.11 (m, 1H), 4.28 (d, J = 3.1 Hz, 3H), 4.04−3.99 (m, 3H), 3.62−3.51 (m, 2H), 3.37−3.33 (m, 1H), 3.22−2.98 (m, 4H), 2.76 (d, J = 3.5 Hz, 1H), 2.50−2.40 (m, 3H), 2.37−2.19 (m, 1H), 2.09−1.91 (m, 6H), 1.89−1.79 (m, 1H), 1.61−1.53 (m, 4H), 1.51−1.25 (m, 6H), 1.23−1.17 (m, 2H), 1.07−0.96 (m, 4H).

(*S*)-6-Methyl-*N*-((*S*)-7-oxo-1-(5-(4-(pyridin-3-yl)phenyl)-oxazol-2-yl)nonyl)-6-azaspiro[2.5]octane-1-carboxamide (55). Prepared based on the same method as **48**. LCMS (ESI) calcd for $C_{32}H_{40}N_4O_3$ $[M + H]^+$: 529.3, found: 529.3, R_t = 2.161 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (d, J = 1.6 Hz, 1H), 8.61 (d, J = 3.9 Hz, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.61−7.75 (m, 4H), 7.39 (dd, J = 5.1, 7.8 Hz, 1H), 7.28 (s, 1H), 6.91 (d, J = 7.8 Hz, 1H), 5.23−5.29 (m, 1H), 2.85−3.36 (m, 3H), 2.72 (s, 3H), 2.40 (tt, J = 3.6, 6.9 Hz, 3H), 1.80−2.10 (m, 6H), 1.53−1.69 (m, 4H), 1.23−1.48 (m, 6H), 1.03 (t, J = 7.2 Hz, 3H), 0.95 (brs, 1H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(2-ethyl-7-methoxy-1-oxo-1,2-dihydroisoquinolin-6-yl)oxazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]-octane-1-carboxamide (56). See Scheme S11 in the Supporting Information for the synthetic scheme.

Step 1. HATU (3.61 g, 9.5 mmol) and TEA (1.042 g, 10.32 mmol) were added to a stirred mixture of 4-bromo-3-methoxybenzoic acid (2 g, 8.6 mmol) in DCM (20 mL) at rt, and the mixture was stirred at rt for 15 min. Then, 2,2-dimethoxyethan-1-amine (997 mg, 9.5 mmol) was added. The solution was stirred for 1 h. Then, the solvent was removed to give 4-bromo-*N*-(2,2-dimethoxyethyl)-3-methoxybenzamide (2.72 g crude) as a brown oil, which was used in the next step without further purification.

Step 2. H_2SO_4 (20 mL) was added slowly to stirring 4-bromo-*N*-(2,2-dimethoxyethyl)-3-methoxybenzamide (2.72 g crude, 8.6 mmol), and the mixture was stirred at rt for 2 h. The reaction was poured into ice water. The resulting precipitate was collected by filtration and the filter cake was washed with water (50 mL) and dried to afford 6-bromo-7-methoxyisoquinolin-1(2*H*)-one (1.52 g crude) as a yellow solid, which was used without further purification in the next step.

Step 3. Iodoethane (1.4 g, 9.0 mmol) was added to a stirred mixture of 6-bromo-7-methoxyisoquinolin-1(2*H*)-one (1.52 g, 5.98 mmol) and Cs_2CO_3 (2.9 g, 8.97 mmol) in DMF (20 mL), and the mixture was stirred at rt for 2 h. The mixture was filtered, and the filter cake was washed with DMF (10 mL). The filtrate was poured into water (50 mL) and the precipitate was collected with filtration and the filter cake was washed with water (50 mL) and dried to give 6-bromo-2-ethyl-7-methoxyisoquinolin-1(2*H*)-one (1.6 g, crude), which was used in the next step without further purification. LCMS (ESI) calcd for $C_{12}H_{12}BrNO_2$ $[M + H]^+$: 282.0, found: 284.0.

Step 4. Potassium acetate (1.660 g, 17 mmol) and BPD (2.16 g, 8.51 mmol) were added to a mixture of 6-bromo-2-ethyl-7-methoxyisoquinolin-1(2*H*)-one (1.60 g, 5.67 mmol) in 1,4-dioxane (20 mL) at rt. The mixture was replaced with N_2 and $PdCl_2$(dppf) (0.414 g, 0.567 mmol) was added. The mixture was stirred at 80 °C for 18 h. After it was cooled down, the mixture was filtered, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether (30%) to give 2-ethyl-7-methoxy-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinolin-1(2*H*)-one (0.92 g, 49%) as a yellow solid. LCMS (ESI) calcd for $C_{18}H_{24}BNO_4$ [M + H]+: 330.2, found: 330.2.

Step 5. Compound **56** was prepared based on the method as **48**. HRMS (ESI) calcd for $C_{33}H_{44}N_4O_5$ $[M + H]^+$: 591.3468, found 591.3537. ^{1}H NMR (400 MHz, methanol-d_4) δ 8.04 (s, 1H), 7.89 (s, 1H), 7.65 (s, 1H), 7.33 (d, J = 7.28 Hz, 1H), 6.77 (d, J = 7.72 Hz, 1H), 5.13−5.18 (m, 1H), 4.43 (s, 3H), 4.07−4.14 (m, 5H), 3.37−3.64 (m, 2H), 3.14 (m, 4H), 2.41−2.51 (m, 4H), 2.06 (brs, 1H), 1.88−1.89 (m, 2H), 1.80 (brs, 1H), 1.22−1.62 (m, 16H), 0.99 (t, J = 7.28 Hz, 4H).

(*S*)-Benzyl 6-Azaspiro[2.5]octane-1-carboxylate (58). TFA (4.5 mL, 60.6 mmol) was added to a solution of (*S*)-1-benzyl 6-*tert*-butyl 6-azaspiro[2.5]octane-1,6-dicarboxylate (3.0 g, 8.68 mmol) in DCM (30 mL), and the mixture was stirred at rt for 4 h. The solvents were removed by an evaporator to give crude (*S*)-benzyl 6-azaspiro[2.5]octane-1-carboxylate (3.1 g), which was used directly for the next step without further purification. LCMS (ESI) calcd for $C_{15}H_{19}NO_2$ $[M + H]^+$: 246.1, found: 246.1.

(*S*)-Benzyl 6-Methyl-6-azaspiro[2.5]octane-1-carboxylate (59a). Formaldehyde (18.77 g, 231 mmol) was added to a solution of (*S*)-benzyl 6-azaspiro[2.5]octane-1-carboxylate (9.9 g, 28.9 mmol) in MeOH (100 mL), and the mixture was stirred at rt for 2 h. Sodium triacetoxyhydroborate (18.39 g, 87 mmol) was added, and the mixture was stirred at rt for 1 h. The solvent was removed by an evaporator. Water (100 mL) was added, and the mixture was extracted with ethyl acetate (50 mL × 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (50 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column flash chromatography eluting with MeOH in DCM from 0 to 10% to give (*S*)-benzyl 6-methyl-6-azaspiro[2.5]octane-1-carboxylate as a yellow oil (7.5 g, 99% for two steps). LCMS (ESI) calcd for $C_{16}H_{21}NO_2$ [M + H]$^+$: 260.3, found: 260.1.

(*S*)-Benzyl 6-ethyl-6-azaspiro[2.5]octane-1-carboxylate (59b). Acetaldehyde (21 mL, 149 mmol) was added to a solution of (*S*)-benzyl 6-azaspiro[2.5]octane-1-carboxylate (6.2 g, 18.11 mmol) in MeOH (60 mL), and the mixture was stirred at 30 °C for 16 h. More acetaldehyde (4.0 mL) was added, and the mixture was stirred at 30 °C for another 16 h. Sodium triacetoxyhydroborate (11.52 g, 54.3 mmol) was added, and the mixture was stirred at 30 °C for 16 h. The solvent was removed by an evaporator. Water (50 mL) was added, and the mixture was extracted with ethyl acetate (35 mL × 3). The combined organic layers were washed with saturated aqueous $NaHCO_3$ (35 mL × 2), brine (35 mL × 2), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash chromatography eluting with MeOH in DCM from 0 to 10% to give (*S*)-benzyl 6-ethyl-6-azaspiro[2.5]octane-1-carboxylate as a yellow oil (4.5 g, 91% for two steps). LCMS (ESI) calcd for $C_{17}H_{23}NO_2$ [M + H]$^+$: 274.2, found: 274.1.

(*S*)-6-Methyl-6-azaspiro[2.5]octane-1-carboxylic acid (60a). To a 100 mL three-neck round-bottom flask was added (*S*)-benzyl 6-methyl-6-azaspiro[2.5]octane-1-carboxylate (7.5 g, 28.9 mmol) in MeOH (75 mL) and Pd/C (10%, wet, 520 mg, 0.489 mmol) under Ar. The suspension was degassed under vacuum and refilled with nitrogen three times. The mixture was stirred under H_2 (15 psi) at 24 °C for 90 min and filtered. The filter cake was washed with MeOH (20 mL × 2) and the filtrate was concentrated to give (*S*)-6-methyl-6-azaspiro[2.5]octane-1-carboxylic acid as a colorless oil (4.7 g, 96%). LCMS (ESI) calcd for $C_9H_{15}NO_2$ $[M + H]^+$: 169.1, found: 246.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.88 (brs, 3H), 2.73 (brs, 1H), 2.50 (s, 3H), 1.77 (brs, 2H), 1.51 (m, 3H), 0.95 (m, 2H).

(*S*)-6-Ethyl-6-azaspiro[2.5]octane-1-carboxylic acid (60b). To a solution of (*S*)-benzyl 6-ethyl-6-azaspiro[2.5]octane-1-carboxylate (4.5 g, 16.46 mmol) in MeOH (45 mL) was added Pd/C (10%, wet, 300 mg, 0.282 mmol) under Ar. The suspension was degassed under vacuum and refilled with N_2 three times. The mixture was then stirred under H_2 (15 psi) at 18 °C for 2 h and filtered. The filter cake was washed with MeOH (15 mL × 3). The filtrate was concentrated to give (*S*)-6-ethyl-6-azaspiro[2.5]octane-1-carboxylic acid as a colorless oil (3.0 g, 99%), which was used without further purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 2.60−2.71 (m, 2H), 2.54−2.60 (m, 2H), 2.50 (brs, 2H), 1.65−1.78 (m, 2H), 1.48 (dd, J = 5.6, 7.4 Hz, 3H), 1.06 (t, J =7.2 Hz, 3H), 0.87−0.92 (m, 1H), 0.81−0.87 (m, 1H).

6-Bromo-7-methoxy-2-methylquinoline (63). A suspension of 4-bromo-3-methoxyaniline (15.00 g, 74.2 mmol) and concentrated HCl (52.5 mL, 639 mmol) in water (60 mL) was heated to 110 °C. (*E*)-but-2-enal (8.23 g, 117 mmol) was added dropwise into the above mixture over 30 min. The reaction mixture was stirred at 110 °C for 2 h and was cooled to rt. Aqueous ammonia (28%, 300 mL) was added, and the mixture was extracted with ethyl acetate (100 mL × 3). The combined organic layers were washed with brine (200 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column flash chromatography eluting with EtOAc in petroleum ether from 10 to 40% to give **63** as a pale brown solid (10.12 g, 40 mmol, 54%). LCMS (ESI) calcd for $C_{11}H_{10}BrNO$ [M + H]$^+$: 252.0, found: 251.9.

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7-Methoxy-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (64). $Pd(dppf)Cl_2$ (85 mg, 0.116 mmol) was added to a solution of potassium acetate (405 mg, 4.13 mmol), 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi(1,3,2-dioxaborolane) (786 mg, 3.09 mmol), and 6-bromo-7-methoxy-2-methylquinoline (520 mg, 2.063 mmol) in 1,4-dioxane (8 mL). The mixture was stirred at 80 °C for 12 h under nitrogen and concentrated. The residue was purified by silica gel column flash chromatography, eluting with 50% EtOAc in petroleum ether to give 7-methoxy-2-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline (440 mg, 71%) as a brown gum. LCMS (ESI) calcd for $C_{17}H_{22}BNO_3$ $[M + H]^+$: 300.2, found: 300.2. ^1H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.98 (d, J = 8.22 Hz, 1H), 7.34 (s, 1H), 7.14 (d, J = 8.22 Hz, 1H), 3.97 (s, 3H), 2.72 (s, 3H), 1.41 (s, 12H).

2,4-Dibromo-1-((2-(Trimethylsilyl)ethoxy)methyl)-1*H*-imidazole (66). To a 10 L three-neck round-bottom flask was added a solution of 2,4,5-tribromo-1-[[2-(trimethylsilyl)ethoxy]methyl]-1*H*-imidazole (420 g, 966 mmol) in tetrahydrofuran (4000 mL). It was cooled to −78 °C, and *n*-BuLi (2.49 M in hexane, 783 mL, 1950 mmol) was added dropwise with stirring. The mixture was stirred at −78 °C for 10 min. Water (17.4 g, 966.67 mmol) was added. The mixture was slowly warmed to −50 °C over 1 h and cooled back to −78 °C again. Br_2 (170 g, 1.06 mol) was added, and the reaction was stirred at −78 °C for another 30 min. The reaction was then quenched by the addition of 2 L of water. The resulting solution was extracted with ethyl acetate (2 L × 3), and the organic layers were combined. The resulting mixture was washed with brine (1 L). The mixture was dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was applied onto a silica gel column with ethyl acetate in petroleum ether (1:30) to afford 2,4-dibromo-1-[[2-(trimethylsilyl)ethoxy]methyl]-1*H*-imidazole (100 g, 29%) as a yellow solid. LCMS (ESI) calcd for $C_9H_{16}Br_2N_2OSi$ $[M + H]^+$:356.9, found: 256.9.

(*R*)-*N*-((*S*)-1-(4-Bromo-1-((2-(Trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-6-(2-ethyl-1,3-dioxolan-2-yl)hexyl)-2-methylpropane-2-sulfinamide (67). To a 5 L three-neck round-bottom flask was added a solution of 2,4-dibromo-1-[[2-(trimethylsilyl)-ethoxy]methyl]-1*H*-imidazole (120 g, 336.96 mmol) in tetrahydrofuran (1200 mL). It was cooled to −78 °C and *n*-BuLi (138 g, 2.15 mol) was added dropwise, followed by (*R*)-*N*-[(1*E*)-6-(2-ethyl-1,3-dioxolan-2-yl)hexylidene]-2-methylpropane-2-sulfinamide (102 g, 336.12 mmol). The solution was gradually warm up to -30 °C and stirred for 30 min. The reaction was quenched by the addition of 1 L of water. The resulting solution was extracted with ethyl acetate (1 L × 3), and the organic layers were combined, washed with brine (500 mL), dried over anhydrous sodium sulfate, and concentrated under vacuum. The product was purified by silica gel Prep-HPLC eluting with 50−70% CH_3CN in water with 0.5% NH_4HCO_3 to give 130 g product. The product was purified again by Chiral-Prep-HPLC (Prep SFC 350, column: CHIRALPAK 250 cm × 25 cm; mobile phase: 20% MeOH in CO_2) to give (*R*)-*N*-[(1*S*)-1-(4-bromo-1-[[2-(trimethylsilyl)ethoxy]methyl]-1*H*-imidazol-2-yl)-6-(2-ethyl-1,3-dioxolan-2-yl)hexyl]-2-methylpropane-2-sulfinamide as a yellow oil (106 g, 54%). LCMS (ESI) calcd for $C_{24}H_{46}BrN_3O_4SSi$ $[M + H]^+$: 582.2, found: 582.1.

(*S*)-9-Amino-9-(4-bromo-1-((2-(Trimethylsilyl)ethoxy)-methyl)-1*H*-imidazol-2-yl)nonan-3-one (68). To a 3 L three-neck round-bottom flask was added a solution of (*R*)-*N*-[(1*S*)-1-(4-bromo-1-[[2-(trimethylsilyl)ethoxy]methyl]-1*H*-imidazol-2-yl)-6-(2-ethyl-1,3-dioxolan-2-yl)hexyl]-2-methylpropane-2-sulfinamide (130 g, 223.87 mmol) in tetrahydrofuran (1300 mL). It was cooled to 0−5 °C, and hydrochloric acid (32%, 28 g, 246.3 mmol) was added dropwise. The solution was warmed up to rt and stirred for 8 h. It was diluted with 1000 mL of ice water and extracted with ethyl acetate (500 mL × 3). The combined organic layer was washed with brine (500 mL), dried over anhydrous sodium sulfate, and concentrated under vacuum. The product was purified by silica gel column eluting with EtOH/DCM (1/30). The product was dissolved in DCM (500 mL), and hydrochloric acid (16 g) in EtOAc (100 mL) was added. The mixture was washed with brine, dried over Na_2SO_4, and concentrated to afford (9*S*)-9-amino-9-(4-bromo-1-[[2-(trimethylsilyl)ethoxy]methyl]-1*H*-imidazol-2-yl)nonan-3-one hydrochloride as a yellow oil (75.6 g, 72%). LCMS (ESI) calcd for $C_{18}H_{34}BrN_3O_2Si$ $[M + H]^+$: 432.2, found: 434.0. ^1H NMR (300 MHz, DMSO-d_6) δ 8.29 (brs, 3H), 7.51 (s, 1H), 5.54 (d, J = 11.1 Hz, 1H), 5.23 (d, J = 11.2 Hz, 1H), 4.40 (t, J = 6.9 Hz, 1H), 3.44 (m, 2H), 2.41- 2.26 (m, 4H), 1.84 (brs, 2H), 1.36 (m, 2H), 1.11 (m, 5H), 0.94- 0.68 (m, 5H), −0.06 (s, 9H).

(*S*)-*N*-((*S*)-1-(5-Bromo-1-((2-(Trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-ethyl-6-azaspiro[2.5]octane-1-carboxamide (69). HATU (0.967 g, 2.54 mmol) and Et_3N (1.6 mL, 11.48 mmol) were added to a solution of (*S*)-6-ethyl-6-azaspiro[2.5]octane-1-carboxylic acid (0.466 g, 2.54 mmol) in DMF (15 mL) at rt and stirred for 15 min. (*S*)-9-amino-9-(5-bromo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)nonan-3-one (1.0 g, 2.3 mmol) was added and stirred at rt for 1 h. Water (10 mL) was added, and the aqueous layer was extracted with ethyl acetate (10 mL × 3). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel flash chromatography eluting with MeOH in DCM from 1 to 10% to give **69** as a yellow oil (970 mg, 66%). LCMS (ESI) calcd for $C_{28}H_{49}BrN_4O_3Si$ $[M + H]^+$: 597.3 and 599.3, found: 597.3 and 599.3. ^1H NMR (400 MHz, $CDCl_3$) δ 6.87 (s, 1H), 6.15−6.27 (m, 1H), 5.54−5.66 (m, 1H), 5.08−5.17 (m, 1H), 5.01−5.07 (m, 1H), 3.44−3.56 (m, 2H), 2.47−2.56 (m, 1H), 2.27−2.45 (m, 8H), 1.74−2.01 (m, 4H), 1.62−1.73 (m, 1H), 1.44−1.61 (m, 3H), 1.18−1.42 (m, 6H), 1.11−1.17 (m, 1H), 1.06 (s, 6H), 0.83−0.97 (m, 2H), 0.74−0.82 (m, 1H), 0.00 (s, 9H).

(*S*)-6-Ethyl-*N*-((*S*)-1-(5-(7-methoxy-2-methylquinolin-6-yl)-1-((2-(Trimethylsilyl)ethoxy)methyl)-1*H*-imidazol-2-yl)-7-oxononyl)-6-azaspiro[2.5]octane-1-carboxamide (70). $PdCl_2$(DTBPF) (27 mg, 0.041 mmol) was added to a mixture of **64a** (128 mg, 0.427 mmol), **69** (250 mg, 0.418 mmol), and K_3PO_4 (266 mg, 1.255 mmol) in THF (3 mL) and water (0.3 mL) at rt under a nitrogen atmosphere. The mixture was stirred at 70 °C for 2 h and cooled down to rt. Water (15 mL) was added, and the aqueous layer was extracted with ethyl acetate (10 mL × 3). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography eluting with MeOH in DCM from 0 to 10% to give **70** as a brown oil (250 mg, 86%). LCMS (ESI) calcd for $C_{39}H_{59}N_5O_4Si$ $[M + H]^+$: 690.4, found: 690.4.

(*R*)-*N*-((*S*)-6-(2-Ethyl-1,3-dioxolan-2-yl)-1-(oxazol-2-yl) hexyl)-2-methylpropane-2-sulfinamide (71). BH_3·THF (1.0 *M*, 1.8 mL, 1.800 mmol) was added to a solution of oxazole (148 mg, 2.142 mmol) in THF (5 mL), and the mixture was stirred at rt for 1 h under N_2 before it was cooled to −78 °C. *n*-BuLi (2.5 M in hexane, 0.7 mL, 1.750 mmol) was added. The mixture was stirred at −78 °C for 1 h. (*R*,*E*)-*N*-(6-(2-ethyl-1,3-dioxolan-2-yl)hexylidene)-2-methylpropane-2-sulfinamide (500 mg, 1.648 mmol) in THF (1 mL) was added dropwise, and the mixture was stirred at −78 °C for 2 h. Saturated aqueous NH_4Cl (0.2 mL) was added, and the aqueous layer was extracted with ethyl acetate (10 mL × 3). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel chromatography eluting with MeOH in DCM from 0 to 10% to give **71** as a yellow oil (326 mg, 53%). LCMS (ESI) calcd for $C_{18}H_{32}N_2O_4S$ $[M + H]^+$: 373.2, found: 373.1.

Preparation of (*R*)-*N*-((*S*)-1-(5-Bromooxazol-2-yl)-6-(2-ethyl-1,3-dioxolan-2-yl)hexyl)-2-methylpropane-2-sulfinamide (72). *t*-BuLi (1.3 M in hexane, 3.3 mL, 4.29 mmol) was added to a solution of **71** (400 mg, 1.074 mmol) in THF (4 mL) at −78 °C, and the mixture was stirred at −78 °C for 1 h under N_2. CBr_4 (1068 mg, 3.22 mmol) in THF (0.5 mL) was added slowly, and the mixture was stirred at −78 °C for 1 h. Saturated aqueous NH_4Cl (2 mL) was added, and the mixture was extracted with DCM (6 mL × 3). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel chromatography eluting with EtOAc in petroleum ether from 50

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to 100% to give **72** as a brown oil (246 mg, 51%). LCMS (ESI) calcd for $C_{18}H_{31}BrN_2O_4S$ $[M + H]^+$: 451.1, found: 453.1.

(*S*)-9-Amino-9-(5-bromooxazol-2-yl)nonan-3-one (73). HCl (4 M in MeOH, 0.1 mL, 0.4 mmol) was added to a solution of **72** (91 mg, 0.202 mmol) in MeOH (1 mL), and the mixture was stirred at rt for 5 min. Saturated aqueous $NaHCO_3$ (1 mL) was added, and the mixture was extracted with ethyl acetate (3 mL × 3). The combined organic layers were washed with brine (2 mL), dried over Na_2SO_4, filtered, and concentrated to give **73** as a yellow oil (60 mg, 98%), which was used without further purification. LCMS (ESI) calcd for $C_{12}H_{19}BrN_2O_2$ $[M + H]^+$: 303.1, found: 303.0.

(*S*)-*N*-((*S*)-1-(5-Bromooxazol-2-yl)-7-oxononyl)-6-methyl-6-azaspiro[2.5]octane-1-carboxamide (74). Et_3N (1.026 mL, 7.36 mmol) was added to a solution of **73** (500 mg, 1.472 mmol) in DMF (1 mL); then, this solution was added to **60a** (299 mg, 1.766 mmol) in DMF (1 mL). Then, T_3P (1405 mg, 2.208 mmol) was added. The mixture was stirred at rt for 16 h and diluted with DCM (15 mL). It was washed with brine (15 mL × 3), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography eluting with MeOH in DCM from 0 to 10% to give **74** as a light-yellow oil (500 mg, 1.076 mmol, 73.1%). LCMS (ESI) calcd for $C_{21}H_{32}BrN_3O_3$ $[M + H]^+$: 454.2, found: 456.2, 470.2, R_t = 0.977 min.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c02150.

Schemes S1−S11 for the synthesis of compounds **11**, **23**, **26**, **28**, **29**, **36**, **39**, **43**, **45**, **53**, and **56**; ^1H NMR spectra of compounds **3**−**56**; and ^{13}C NMR and LCMS spectra of compounds **31** and **48** (PDF)

Molecular formula strings (CSV)

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■ ABBREVIATIONS USED

BPD, bis(pinacolato)diboron; *c*-Pr, cyclopropyl; *c*-Bu, cyclobutyl; DCM, dichloromethane; HDAC, histone deacetylase (HDAC)

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