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Discovery of Potent, Selective, and Orally Bioavailable Inhibitors of USP7 with In Vivo Anti-Tumor Activity

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ABSTRACT. USP7 is a promising target for cancer therapy as its inhibition is expected to decrease function of oncogenes, increase tumor suppressor function, and enhance

immune function. Using a structure-based drug design strategy, a new class of reversible USP7 inhibitors has been identified that is highly potent in biochemical and cellular assays and extremely selective for USP7 over other deubiquitinases (DUBs). The succinimide was identified as a key potency-driving motif, forming two strong hydrogen-bonds to the allosteric pocket of USP7. Re-design of an initial benzofuran-amide scaffold yielded a simplified ether series of inhibitors, utilizing acyclic conformational control to achieve proper amine placement. Further improvements were realized upon replacing the ether-linked amines with carbon-linked morpholines, a modification motivated by Free Energy Perturbation (FEP+) calculations. This led to the discovery of compound **41**, a highly potent, selective, and orally bioavailable USP7 inhibitor. In xenograft studies, compound **41** demonstrated tumor growth inhibition in both p53 wildtype and p53 mutant cancer cell lines, demonstrating that USP7 inhibitors can suppress tumor growth through multiple different pathways.

INTRODUCTION

Ubiquitin-specific protease 7 (USP7), also known as HAUSP, is an enzyme which cleaves ubiquitin groups from specific ubiquitinated client proteins.[1] This de-ubiquitination activity can rescue the client proteins from proteasome-mediated degradation and result in a net increase in concentration of these proteins. Through this mechanism, USP7 is proposed to stabilize the oncogenic E3 ubiquitin ligase MDM2, a negative regulator of the tumor suppressor p53.[2] USP7 has also been reported to play a role in stabilizing FOXP3, an essential transcription factor for the development and function of regulatory T cells, and hence may play a role in the suppression of immune responses to tumors.[3] Additionally, USP7 may stabilize several proteins involved in DNA damage repair.[4] Pharmacological inhibition of USP7 may therefore be beneficial for cancer therapy through multiple mechanisms.

A number of USP7-selective reversible inhibitors have recently been reported.[5] These include 4-hydroxy piperidine inhibitors **1-4** (Figure 1) disclosed by Hybrigenics,[6] FORMA Therapeutics,[7] Almac Discovery,[8] Servier,[9] and others,[10] as well as tri-aryl inhibitors such as **5** disclosed by Genentech.[11,12] 4-hydroxy piperidine inhibitors such as **2**-**4** have been reported to bind to an allosteric pocket in the “palm” domain of USP7

(Figure 2). Small molecule binding to this allosteric site is believed to stabilize an inactive conformation of USP7, preventing USP7 catalytic activity.[7] The tri-aryl inhibitors such as **5** are reported to bind in a different allosteric pocket in the "palm" domain of USP7.

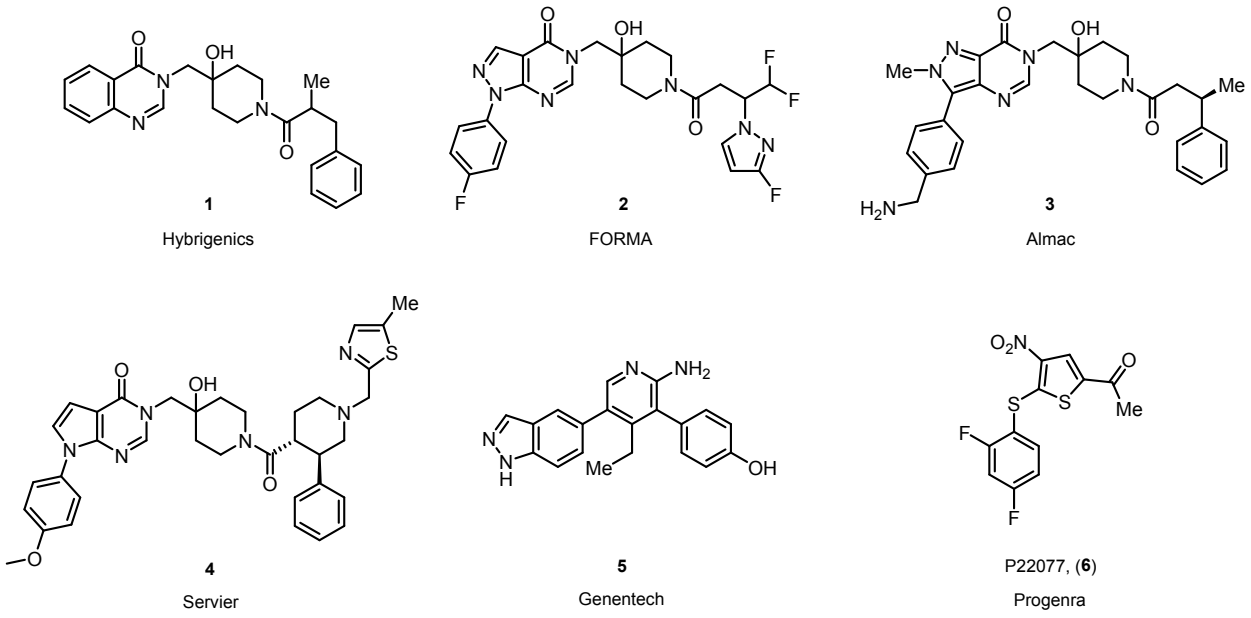


Figure 1. Examples of previously described USP7 inhibitors.

Many studies interrogating the biological functions of USP7 have employed irreversible active-site dual inhibitors of USP7 and USP47, such as P22077 (**6**, Figure 1).[13,14] Potent, DUB-selective, and reversible inhibitors of USP7 of diverse chemotypes would be useful tools in clarifying and assessing the many potential biological roles of

USP7 and its relevance as a target for human cancer therapy. Here we report an optimization campaign resulting in a new class of highly potent, selective, reversible, and bioavailable inhibitors of USP7. These inhibitors have recently aided the clarification of the biological activities of USP7.[15]

RESULTS AND DISCUSSION

Pyridylbenzofurans as Starting Chemical Matter

Our drug discovery campaign for USP7 inhibitors began with an evaluation of compounds derived from a focused library screen of known DUB inhibitors as well as other structurally related compounds. Anticipating DUB-selectivity to be a major challenge for a USP7 program based on the conserved nature of DUB active sites, early chemical matter was tested for biochemical activity against USP7 as well as against USP1, UCH-L3, and USP47. Among the USP7-active compounds evaluated, the 7-pyridylbenzofuran compound **7** (Figure 2) stood out as an attractive starting point for optimization due to its relatively high selectivity for USP7 over related de-ubiquitinating enzymes. Interestingly, co-crystallizations of 4-hydroxy piperidine compound **1** and

benzofuran **7** with USP7 revealed that both inhibitors occupy the same allosteric pocket of the palm region, even though they belong to different chemotypes (Figure 2).

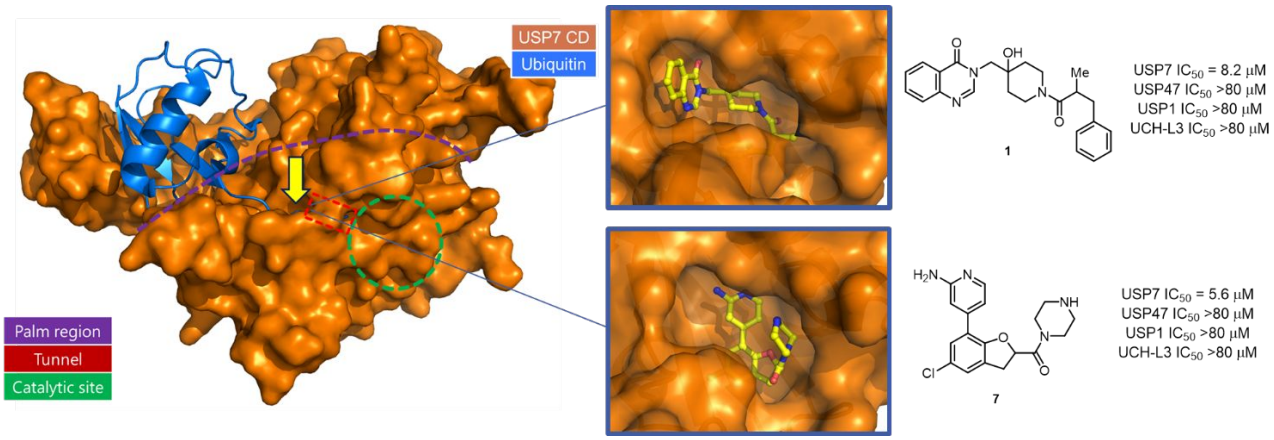


Figure 2. (Left) Schematic of the catalytic domain of USP7 (orange) bound to ubiquitin (blue), with the palm region (below purple dashed line), tunnel area (red box), and catalytic site (green circle) highlighted. The yellow arrow indicates the compound binding region highlighted in the structures on the right. (Right) Comparison of co-crystal structures of 4-hydroxy piperidine inhibitor **1** and pyridylbenzofuran compound **7**. Both inhibitors bind to the same allosteric site of USP7 and are selective inhibitors of USP7. The PDB code for **1** bound to USP7 is 6VN4. The PDB code for **7** bound to USP7 is 6VN5.

Benzofuran compound **7** and 4-hydroxy piperidine compound **1** form many similar interactions with the USP7 protein despite their distinct structures. Both compounds form hydrogen-bonds with the backbone nitrogen of Val-296, the phenol moiety of Tyr-465, and the carboxylate of Asp-295. Importantly, despite the two compounds similar potencies, there appeared to be interactions made by compound **1** with USP7 that were not made by compound **7** (Figure 3), suggesting that it should be possible to enhance compound **7**'s affinity towards USP7 by introducing novel elements that would take advantage of additional interactions in the binding pocket. It was hypothesized that the potency of a compound such as benzofuran **7** could be improved by introducing functionality which could target the backbone hydrogen bond donor of Phe-409 with an appropriately linked hydrogen-bond acceptor, or which could better engage the glutamine side chain of Gln-297.

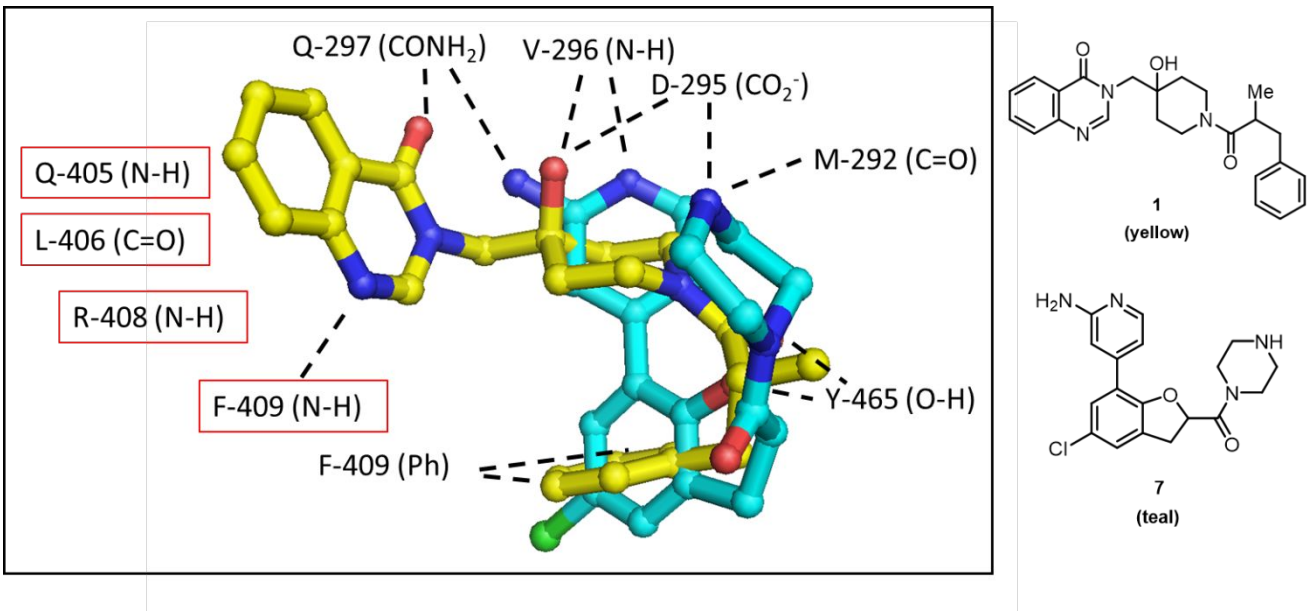


Figure 3. Comparison of USP7 binding interactions of 4-hydroxy piperidine inhibitor **1** and pyridylbenzofuran inhibitor **7**. Boxed residues indicate potential hydrogen-bonding interactions that are not utilized by compound **7**. The PDB code for **1** bound to USP7 is 6VN4. The PDB code for **7** bound to USP7 is 6VN5.

WaterMap[16] analysis of the holo structure (ligand removed) revealed several unfavorable hydration sites in the binding site of **7** (Figure 4). Unfavorable hydration sites can be characterized as sites that have a predicted free energy ΔG of > 0 kcal/mol upon adding water while favorable hydration sites have a predicted free energy ΔG of < 0 kcal/mol. Binding of compound **7** likely displaces several of the water molecules as

shown by the magnified hydration sites of Figure 4A, including a high-energy site filled by the aryl chloride moiety of **7**. However, sites adjacent to the aminopyridine are not displaced by the ligand, corroborating the proposed hypothesis that adding appropriate functionality in this region could lead to improved potency. This was further confirmed by a WaterMap analysis with compound **7** present in the complex, showing that the ligand indeed does not displace water sites adjacent to the aminopyridine (Figure 4B). In fact, the free energy ΔG of these sites is even more unfavorable than predicted from the structure with ligand removed, likely due to a loss of entropy as the waters are somewhat trapped between the ligand and protein. The combined structural and computational analysis strongly suggests that efforts to improve the potency of compound **7** should be focused on changes to the upper pyridine section of the molecule.

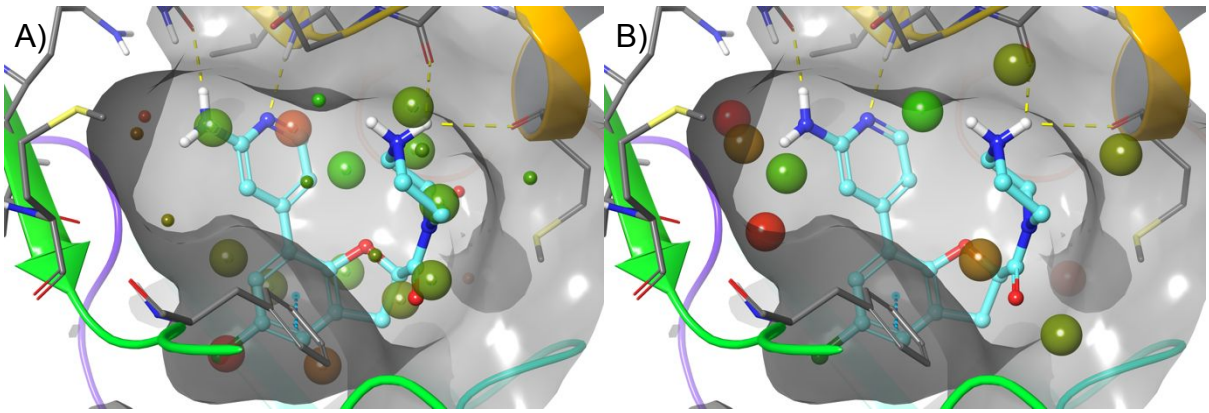
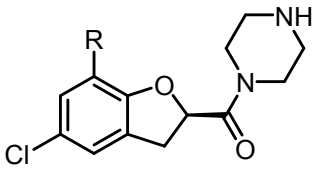


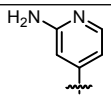
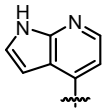
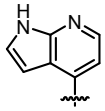
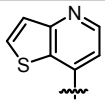
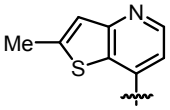
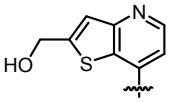
Figure 4. WaterMap analysis of USP7 binding pocket. Hydration sites are shown as spheres colored by their predicted free energies (ΔG). Green spheres indicate a favorable free energy ΔG while red spheres indicate an unfavorable ΔG. (A) Hydration sites calculated with the ligand (compound **7**) removed. Overlay of compound **7** indicates that the magnified (enlarged) spheres are predicted to be displaced by compound **7** while the other (smaller) spheres are not. (B) Hydration sites with compound **7** present. Three unfavorable water sites (red/orange) near the 2-aminopyridyl headgroup of **7** suggest opportunities to further improve the potency of this inhibitor. WaterMap analysis based on co-crystal structure of compound **7** and USP7, PDB code 6VN5.

Addition to the Pyridyl Moiety Improves Potency

Several bicyclic analogs of **7** were first prepared as scaffolds for a potential hydrogen-bond acceptor (Table 1). Azaindole **8** was isoefficient to 2-aminopyridine **7**, having increased potency of comparable magnitude to its increased lipophilicity. In agreement with the selective co-crystallization of the *R*-isomer from racemic 2-aminopyridine **7** with USP7, asymmetric synthesis of the enantiomers indicated that the *R*-isomer **9** was significantly more active against USP7, while the *S*-isomer ***ent*-9** was found to be inactive. Alternative bicyclic scaffolds to the azaindole motif were surveyed. Thienopyridine **10** was found to be more potent and isoefficient to the corresponding aza-indole, but with one less hydrogen-bond donor. Substitution of the thienopyridine with a methyl group (compound **11**) did not lead to a gain in potency, but substitution with a hydroxymethylene group (compound **12**) did lead to an improvement in potency while simultaneously reducing lipophilicity. Consistent with the binding mode of these inhibitors to an allosteric pocket uniquely accessible in USP7, these inhibitors generally did not display significant activity against USP47 or USP1.[8]

Table 1. Evaluation of heterocyclic scaffolds[a]



Compound	R Group	USP7 IC_{50} (nM)[b]	USP47 IC_{50} (μM)[c]	USP1 IC_{50} (μM)[d]	cLogP[e]	LipE[f]
7[g]		5600	>80	>80	2.2	2.9
8[g]		930	>80	>80	3.1	2.9
9		450	>80	>80	3.1	3.2
10		90	>40	>40	3.9	3.1
11		130	>80	>80	4.4	2.5
12		43	>40	>40	2.9	4.5

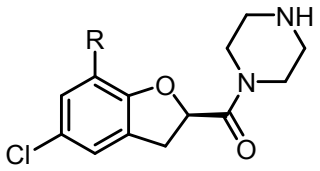
[a]Data represent the mean of $n = 2$ or more determinations. [b]USP7 biochemical assay.

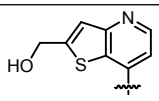
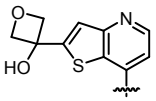
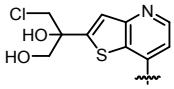
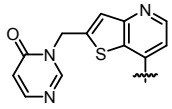
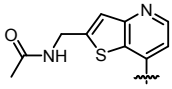
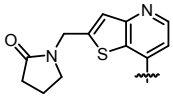
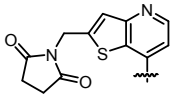
[c]USP47 biochemical assay. [d]USP1 biochemical assay. [e]Calculated using ChemDraw.

[f]LipE = $p(IC_{50})$ – cLogP. [g]Racemic compound.

To further improve potency toward USP7, compounds with an additional hydrogen bond donor or acceptor near the thienopyridine were explored. It was expected that additional binding affinity could be achieved in this region by simultaneously forming interactions with the backbone N–H motifs of either Arg-408 or Phe-409 and the amide side chain of Gln-297. Interactions with these two residues are evident in co-crystal structures of USP7 with 4-hydroxy piperidine inhibitor **1** (see Figure 3) but are not fully utilized by thienopyridine alcohol **12**. Introduction of an oxetane with the intention of engaging the side chain of Gln-297 resulted in compound **13** which was not more potent than compound **12** (Table 2). Serendipitously, the chlorohydrin **14** resulting from HCl-mediated chlorinolysis of **13** was found to be significantly more active against USP7 while decreasing overall lipophilicity. Co-crystallization of **14** with USP7 confirmed that this compound was forming the originally desired contacts with Arg-408 and Gln-297 while binding in a reversible manner (Figure 5).

Table 2. Evaluation of heteroatom-containing headgroups[a]



Compound	R Group	USP7 IC_{50} (nM)[b]	USP47 IC_{50} (μM)[c]	cLogP[d]	LipE[e]
12		43	>40	2.9	4.5
13		73	>40	2.8	4.3
14		9	>40	2.6	5.4
15		27	>5	2.3	5.3
16		25	>80	2.7	4.9
17		26	>20	2.8	4.8
18		0.18	>2	2.7	7.0

[a]Data represent the mean of *n* = 2 or more determinations. [b]USP7 biochemical assay.

[c]USP47 biochemical assay. [d]Calculated using ChemDraw. [e]LipE = *p*(IC_{50}) – cLogP.

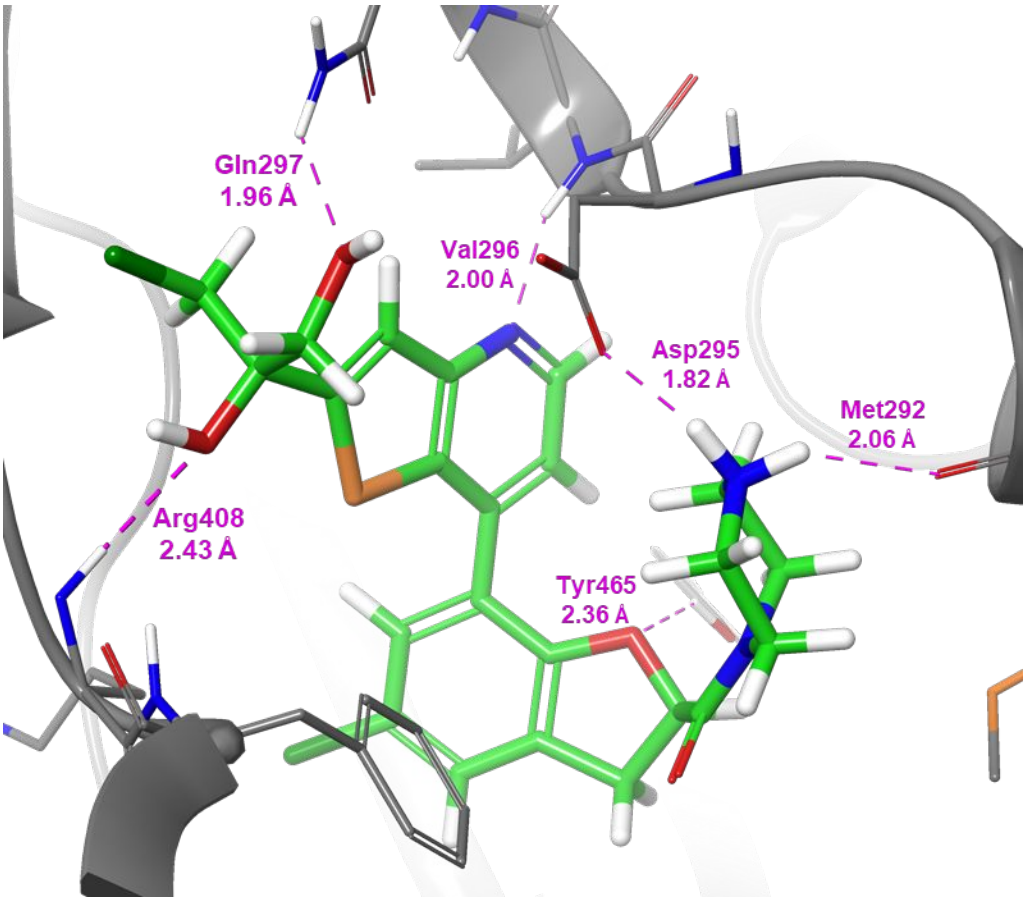


Figure 5. Compound **14** co-crystal structure with USP7, highlighting key interactions with the binding pocket. The PDB code for **14** bound to USP7 is 6VN6.

Alternative functional groups to the chloro-diol present in compound **14** were evaluated (Table 2). The pyrimidinone side chain, whose derivatives are present in the hydroxy-piperidine compounds **1-4**, resulted in compound **15** which was found to have slightly better potency than alcohol **12.** Amide or lactam-containing molecules **16** and **17** were not found to have improved potency. However, the addition of a second hydrogen-

bond acceptor resulted in succinimide **18**, a compound with sub-nanomolar biochemical activity against USP7 and excellent lipophilic efficiency. X-ray co-crystallization confirmed that this succinimidyl functional group was making both interactions with Phe-409 and Gln-297 (Figure 6). No effect was observed on the potency of USP7 inhibition with pre-incubation of the succinimide-based inhibitor, indicating that the compound behaves as a reversible inhibitor as suggested by the crystal structure.[17]

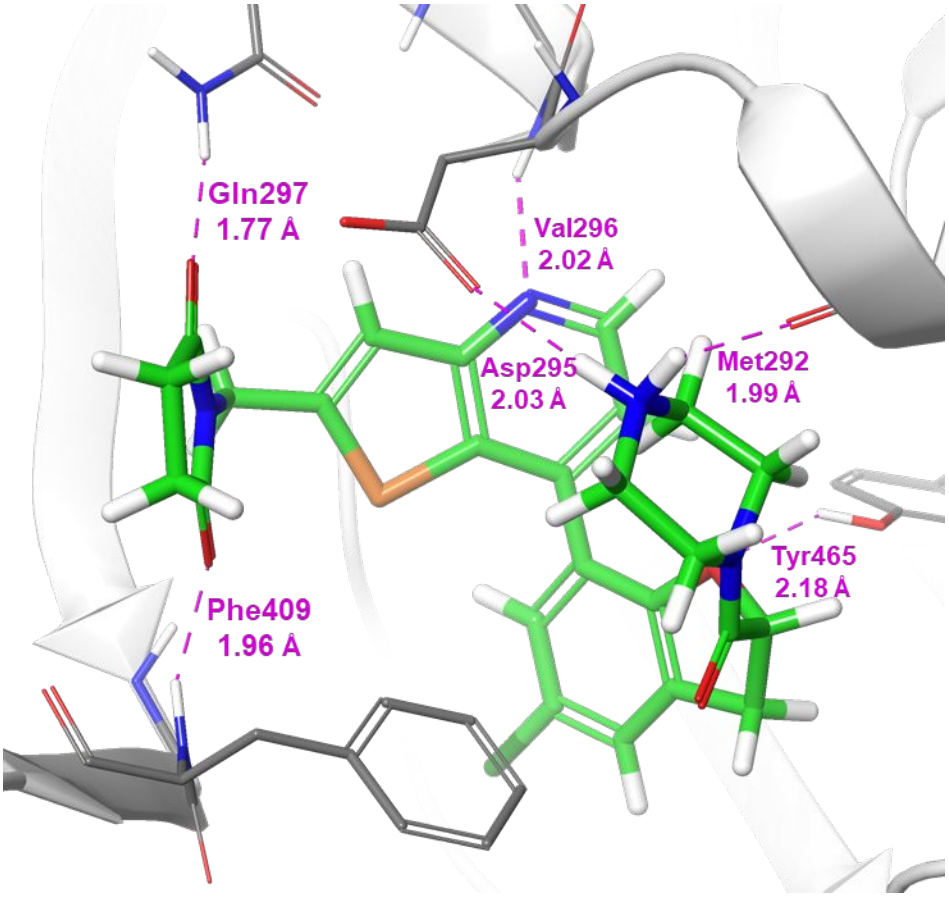


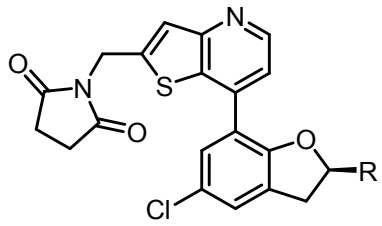
Figure 6. X-ray co-crystal structure of compound **18** with USP7, highlighting key interactions with the binding pocket. The PDB code for **18** bound to USP7 is 6VN2.

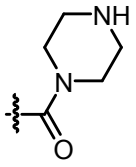
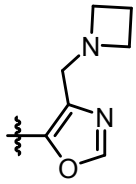
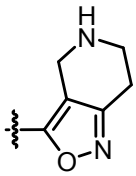
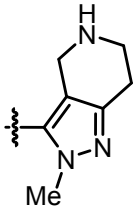
A Simplified Ether Series

Compound **18** was evaluated in rodent PK and found to have very high clearance and poor oral exposure, despite reasonable stability in an in vitro hepatocyte stability assay (Table 3). The low oral exposure was suspected to be in part due to transporter-mediated efflux resulting from the basic amine moiety of the molecule; however, compounds without the basic amine motif, which makes multiple key interactions with Asp-101 and a backbone carbonyl, were orders of magnitude less active against USP7. Additionally, stability to incubation with hepatocytes was still found to be low for these compounds. Several isosteric analogs of **18** were prepared where the amide linker was replaced with a heterocyclic linker in an attempt to improve permeability and stability, a subset of which are shown in Table 3. Oxazole **19** was found to be less potent than amide **18** and be less stable to hepatocytes. Isoxazole **20** was found to have good potency but poor metabolic stability, clearance, and oral exposure. Pyrazole **21** was

found to be more stable to hepatocytes, but unfortunately, this did not extend to a significantly reduced clearance in vivo.

Table 3. Evaluation of piperazine-amide replacements



Compound	R Group	USP7 IC_{50} (nM)[a,b]	Hepatocyte stability: % remaining (R / H)[c]	IV Clearance (L/hr/kg)[d]	PO AUC (hr*ng/mL)[d]
18		0.18	14% / 37%	11.5	0
19		13	2% / 14%	N.D.	N.D.
20		0.38	4% / 26%	5.8	0
21		1.1	42% / 63%	4.7	0

[a]Data represent the mean of *n* = 2 or more determinations. [b]USP7 biochemical assay. [c]% remaining after 60-minute incubation with rat (R) or human (H) hepatocytes. [d]Sprague-Dawley rats, dose of 0.5 mg/kg IV, 2 mg/kg PO. N.D. = not determined.

Having been unsuccessful at improving PK properties by simple amide replacement, we were motivated to make more significant modifications to the dihydrofuran-amide scaffold that had been omnipresent in our inhibitors. Additionally, these dihydrofuran-containing compounds required relatively lengthy syntheses, resulting in a slower discovery effort (see Scheme 2). A simplified and metabolically stable replacement for this bottom portion was desired, but one that maintained the key contacts with the binding pocket (as highlighted in Figure 6).

It was believed that a simplified structure such as ether-linked pyrrolidine **22** might maintain the desired binding interactions while providing better metabolic stability, a simpler synthesis, and reduced molecular weight (Figure 7). However, this modification resulted in a 1000-fold potency loss. It became quite clear that while many aspects of the dihydrofuran-amide did not interact with the binding pocket, it served an

important scaffolding role to help place the basic nitrogen in a productive spatial orientation. Computational modeling indicated that the lowest energy conformation of dihydrofuran-amide **18** is one in which the piperazine ring is oriented upward, *syn*- to the pyridine ring system – the same conformation that is adopted upon binding to USP7 (see Figure 6). Conversely, the ground state of simplified system **22** has the basic nitrogen directed *anti*- to the pyridine ring system. This is a non-productive conformation and a significant energy penalty is required to achieve proper amine placement in the binding pocket. To help overcome this unproductive ground state conformation, A-(1,3) strain was leveraged by installing a methyl group ortho- to the ether linkage (as in pyrrolidine **23**). This raises the energy of the global minimum conformation of **23** relative to its bound conformation, increasing the overall free energy of binding with USP7. We were pleased to see that this simple modification results in a greater than 100-fold potency gain. A USP7 co-crystal structure of **23** was obtained and showed the pyrrolidine nitrogen making the same contacts with Asp-295 and Met-292 as the previous piperazine-amide (Figure 8). Gratifyingly, compound **23** also demonstrated moderate clearance and non-zero oral bioavailability.

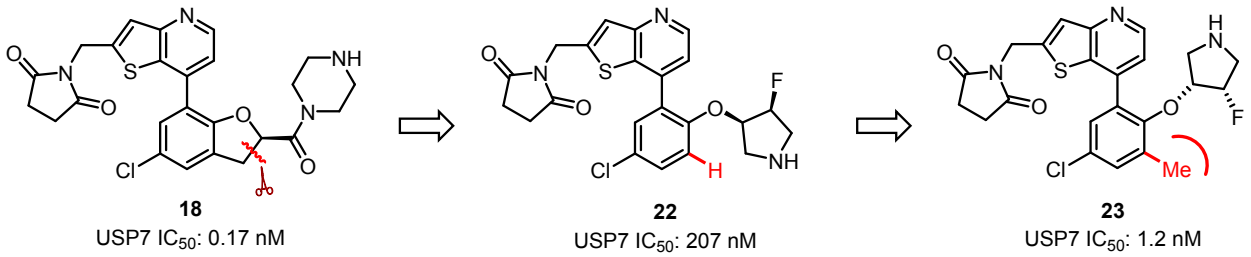


Figure 7. A scaffold switch from the benzofuran-amide to a simplified ether is productive with the inclusion of an ortho-methyl group.

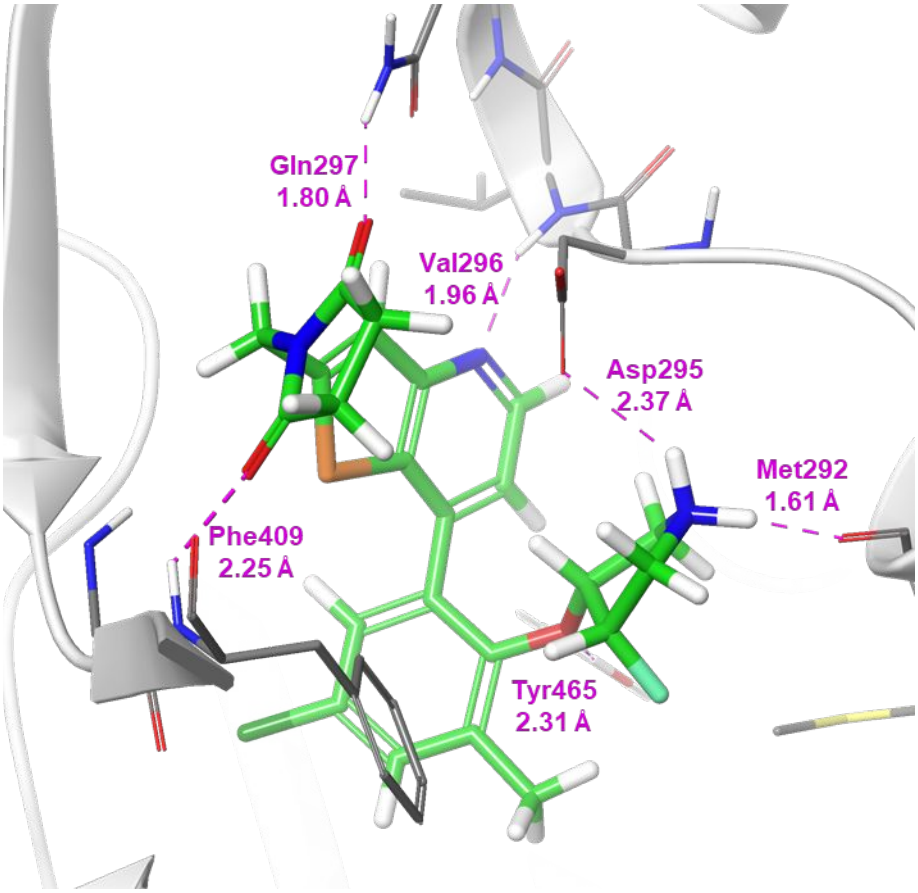
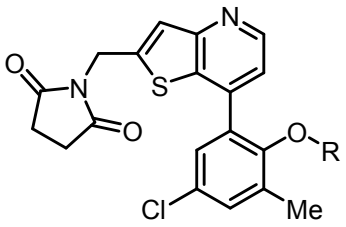
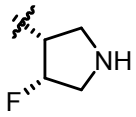
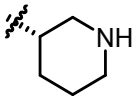
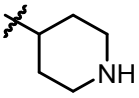
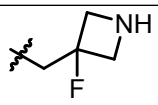
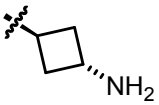
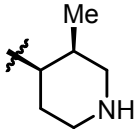


Figure 8. X-ray co-crystal structure of ether **23** with USP7, highlighting key interactions with the binding pocket. The PDB code for **23** bound to USP7 is 6VN3.

This structural simplification enabled extensive SAR exploration of the basic amine portion of these molecules. Analogues were assessed based on their biochemical USP7 inhibition as well as in a cellular assay to measure elevated p53 levels.[18] This was accomplished by utilizing a p53 response element-driven luciferase reporter assay in engineered RKO cells. In addition to pyrrolidines such as **23**, many other ring systems were well tolerated with respect to USP7 potency (Table 4). 3- and 4-piperidinyl compounds such as **24** and **25** were both tolerated, as were azetidines such as **26**. Even exocyclic amines such as cyclobutane **27** showed potent USP7 inhibition. However, most of these compounds still demonstrated only moderate clearance and bioavailability. After screening many substituents on these ring systems, it was found that a single methyl group afforded a drastic change in the PK profile of the 4-substituted-piperidine set of compounds. Relative to piperidine **25**, methyl-piperidine **28** had 5-fold lower rat IV clearance and 20-fold greater PO AUC. The methylated compound **28** was also more potent in line with its greater lipophilicity relative to the parent compound **25**.

Table 4. Highlights of compounds in the simplified ether series



Compound	R Group	USP7 IC_{50} (nM)[a,b]	p53 EC_{50} (nM)[a,c]	cLogP[d]	LipE[e]	IV clearance (L/hr/kg)[f]	PO AUC (hr*ng/mL)[f]
23		1.2	560	3.5	5.4	2.1	130
24		4.6	180	3.7	4.6	4.9	170
25		8.0	400	3.1	5.0	0.33	44
26		1.5	320	3.4	5.4	N.D.	N.D.
27		3.4	150	3.2	5.3	N.D.	N.D.
28		2.1	150	3.6	5.1	0.07	860

[a]Data represent the mean of n = 2 or more determinations. [b]USP7 biochemical assay.

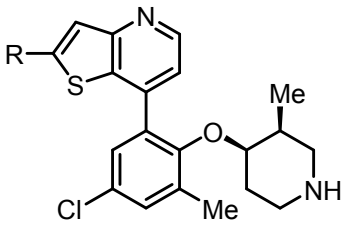
[c]p53-reporter gene assay in RKO cells. [d]Calculated using ChemDraw. [e]LipE = $p(IC_{50})$ –

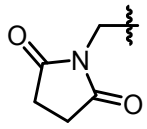
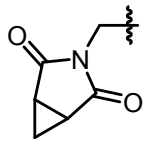
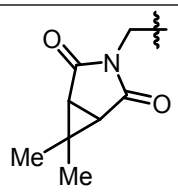
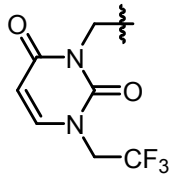
cLogP. [f]Sprague-Dawley rats, dose of 0.5 mg/kg IV, 2 mg/kg PO. N.D. = not determined.

Re-examination of the Succinimide Side Chain

Having established this new piperidine ether as providing an improved PK profile relative to the benzofuran series, the succinimide side chain was re-evaluated. Although the succinimide was a key potency-driver, this moiety was also chemically labile and slowly hydrolyzed under aqueous conditions. Fortunately, substitution on the succinimide backbone with a cyclopropyl ring mitigated hydrolysis while maintaining potency (**29**, Table 5). Further succinimide substitution with a gem-dimethylcyclopropyl ring resulted in a compound with greater biochemical potency in line with an increase in lipophilicity (**30**), as well as with greatly enhanced chemical stability. Replacement of the succinimide for a substituted uracil as in **31** also resulted in a potent compound; however, the PK properties for uracil-containing compounds were sub-optimal, in part due to greater efflux.

Table 5. Cyclic imide SAR



Compound	R Group	USP7 IC_{50} (nM)[a,b]	p53 EC_{50} (nM)[a,c]	cLogP[d]	LipE[e]	IV clearance (L/hr/kg)[f]	PO AUC (hr*ng/mL)[f]
28		2.1	150	3.6	5.1	0.07	860
29		1.1	170	3.8	5.1	N.D.	N.D.
30		0.18	65	5.1	4.6	0.11	4700
31		0.26	42	4.2	5.4	1.0	52

[a]Data represent the mean of *n* = 2 or more determinations. [b]USP7 biochemical assay. [c]p53-reporter gene assay in RKO cells. [d]Calculated using ChemDraw. [e]LipE = $p(IC_{50})$ – cLogP. [f]Sprague-Dawley rats, dose of 0.5 mg/kg IV, 2 mg/kg PO. N.D. = not determined.

Out of the above compounds, gem-dimethylcyclopropylsuccinimide **30** had the best combination of potency and PK properties. Additionally, **30** was found to be potent against the MM.1S and H526 cell lines. It is well-established that the MM.1S cell line, a p53-wildtype multiple myeloma cell line, is sensitive to USP7 inhibition and other mechanisms of p53 activation.[7,9] Additionally, we have recently discovered that certain p53-mutant cancer cell lines are also sensitive to USP7 inhibitors, including H526, a small-cell lung cancer cell line.[15] This activity is presumably mediated through a different mechanism other than p53 elevation since the p53 pathway is expected to be non-functional in H526 cells.[19] Furthermore, target engagement was demonstrated for compound **30** in MM.1S cells, highlighting that our inhibitors are capable of preventing USP7 from binding and conjugating to an active-site ubiquitin probe.[20]

Compound **30** possessed significant cytotoxic activity against both the MM.1S and H526 cell lines, demonstrating a CC_{50} of 140 nM and 440 nM, respectively. Additionally, **30** was tested against a wide variety of DUBs and was found to have exquisite selectivity for USP7. Compound **30** showed an IC_{50} >10 μM for all the >40

DUBs that were assayed, corresponding to a >50,000-fold selectivity for USP7 over the other DUBs.[21] Unfortunately, **30** also exhibited some general cytotoxicity, observed by sharply reduced ATP levels at 3.4 μM in RKO cells, suggestive of physicochemical toxicity. Additionally, substantial hERG inhibition was observed with this compound in a simple hERG binding assay (0.4 μM) as well as mild CYP3A4 inhibition (6.2 μM). These sub-optimal properties along with the above-mentioned cytotoxicity encouraged further SAR exploration into a somewhat different chemical space from ether-linked compound **30**.

Carbon-Linked Compounds Replace the Ether Series

The ether oxygen atom had been omnipresent in our USP7 inhibitors up to this point (first in the benzofurans and later in the simplified ethers) because it was assumed that the buried hydrogen bond to Tyr-465 (as observed in co-crystal structures with USP7) was critical for binding activity. The importance of this interaction, however, had never been experimentally established. Free Energy Perturbation (FEP+) calculations[22] had been successfully used throughout this program to assess different modifications in

order to help prioritize compounds, especially in occasions involving challenging or lengthy syntheses. Using FEP+, the relative binding free energy was calculated for compounds where the ether oxygen had been replaced with a nitrogen or carbon atom. Much to our surprise, carbon-linked analogues were predicted to be equipotent to the standard ether-linked compounds; nitrogen-linked analogues were predicted to be even more potent. These calculations were validated when aniline **33** and methylene-analogue **34** were synthesized and assayed (Figure 9). Compared to ether-linked compound **32**, carbon-linked compound **34** was slightly more potent. Nitrogen-linked compound **33** displayed marked improvements in potency, especially in the cellular p53 assay. This phenomenon was rationalized by re-analyzing the USP7 crystal structure, which revealed a small hole on the side of the protein opposite to the ligand binding site which enables Tyr-465 to be solvent exposed.[23] This could help make up for loss of hydrogen bond when moving to the carbon-linker and enable additional hydrogen bonding when Tyr-465 switches from an H-bond donor to an H-bond acceptor when bound to aniline **33**.

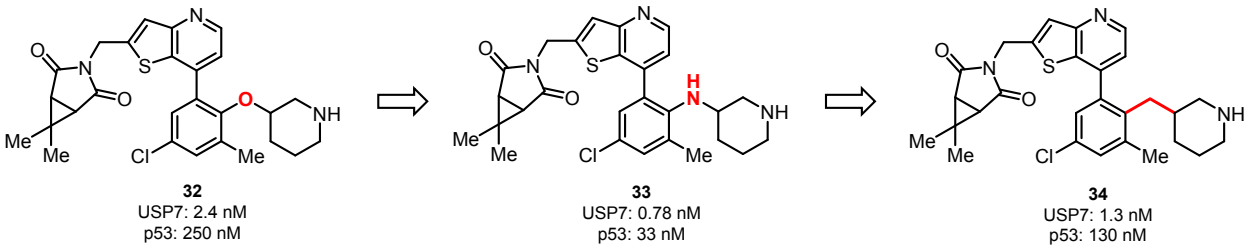


Figure 9. Biochemical and cellular potencies with oxygen-, nitrogen-, and carbon-linked compounds.

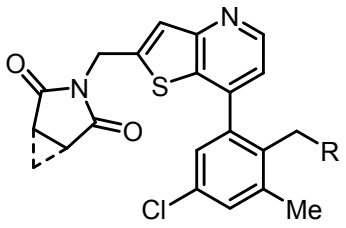
SAR of the new nitrogen-linked series showed that while these compounds were quite potent, they had poor PK properties, likely in part due to an additional hydrogen-bond donor. Additionally, this series was undesirable because it introduced an aniline toxicophore. The carbon-linked series was found to be more promising – by removing a heteroatom from the linker position, it enabled the introduction of a heteroatom into the piperidine ring to forge either piperazines or morpholines. These saturated heterocycles would result in less basic compounds and help mitigate the hERG inhibition seen in ether-linked compound **30**.

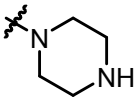
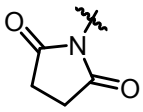
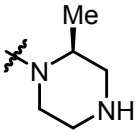
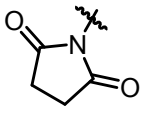
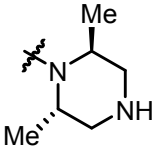
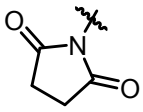
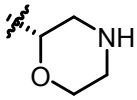
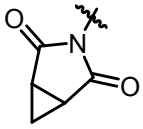
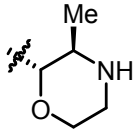
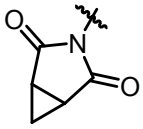
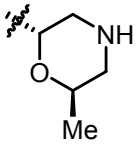
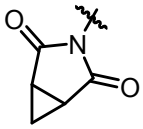
Initially, simple piperazine **35** was not a very potent compound (Table 6). However, the addition of methyl groups to the 2- and 6-positions of the piperazine ring

significantly influenced potency, as evidenced from the progression of piperazine **35** to methyl piperazine **36** to di-methyl piperazine **37**. It is likely that these groups aid in reinforcing the U-shape required to properly position the basic amine in the binding pocket. Dimethyl piperazine **37** displayed excellent PK with low clearance and a large PO exposure and showed a hERG IC_{50} of 2.7 μM, almost a 10-fold improvement over ether **30**. However, cellular potencies against the MM.1S and H526 cell lines were sub-optimal for compound **37**.

The morpholine series yielded extremely potent USP7 inhibitors (Table 6). Unsubstituted morpholine **38** demonstrated remarkable potency, displaying an enzymatic potency of 160 pM. As with the piperazine series, the reduced basicity and lipophilicity of morpholine **38** resulted in a further reduction in hERG inhibition, displaying an IC_{50} of 8 μM. Unlike the piperazine series, the addition of methyl groups at either the 3- or 6-positions did not result in a dramatic increase in potency (see compounds **39** and **40**).

Table 6. Effects of methyl substitutions in the piperazine and morpholine series[a]



Compound	R Group	Imide	USP7 IC_{50} (nM)[b]	p53 EC_{50} (nM)[c]	MM.1S CC_{50} (nM)[d]	H526 CC_{50} (nM)[e]	cLogP[f]	LipE[g]
35			32	1200	N.D.	N.D.	3.4	4.1
36			6.6	280	N.D.	N.D.	3.9	4.3
37			0.32	60	135	2200	4.4	5.1
38			0.16	35	210	3600	3.4	6.4
39[h]			1.5	210	310	N.D.	3.9	4.9
40[h]			0.39	60	120	N.D.	3.9	5.5

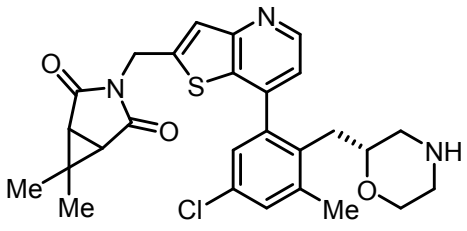
[a]Data represent the mean of n = 2 or more determinations. [b]USP7 biochemical assay. [c]p53-reporter gene assay in RKO cells. [d]Cytotoxicity assay in MM.1S cells. [e]Cytotoxicity

assay in H526 cells. [f]Calculated using ChemDraw. [g]LipE = $p(IC_{50})$ – cLogP. [h]Racemic compound. N.D. = not determined.

In Vivo Xenograft Studies

With these new promising piperazine and morpholine series, the most potent cyclic imide side chains were re-evaluated. Among the compounds surveyed, morpholine **41** demonstrated the optimal balance of cellular potencies and PK properties and was selected for in vivo xenograft studies (Table 7). Several other compounds (such as piperazine **37**) had excellent PK properties but lacked sufficient potency in the MM.1S and H526 cellular assays. Compound **41** showed excellent activity in cellular assays and maintained a good therapeutic index as demonstrated by a high RKO CC_{50}. While compound **41** displayed excellent PK properties in rat, murine PK studies indicated that **41** had slightly worse oral exposure in NOD-SCID or nude mice – animals required for the subsequent xenograft studies.[24] Consequently, an elevated oral dose of 50 mg/kg BID was chosen for these in vivo efficacy studies.[25]

Table 7. Properties of compound **41**.



41

						PO AUC @ 30 mg/kg (hr*ng/mL)		
USP7 IC_{50}	p53 EC_{50}	MM.1S CC_{50}	H526 CC_{50}	RKO CC_{50}	hERG IC_{50}	Balb/C	NOD-SCID	Nude
0.44 nM	25 nM	89 nM	450 nM	13 μM	4.2 μM	9,400	5,500	4,600

The ability of compound **41** to suppress tumor growth was assessed in an MM.1S xenograft model using NOD-SCID mice. When given a 50 mg/kg oral dose of compound **41** twice daily, nearly complete tumor growth inhibition was observed (Figure 10a). This dose was generally well tolerated among the animals, but in the few cases where body weight dropped to less than 90% of their original weight, a dosing holiday was given. This resulted in a rebound of the animal's body weight, whereupon regular dosing was resumed.

Tumor growth inhibition in the MM.1S xenograft model is suspected to be primarily mediated through increases in p53, but a subset of p53-mutant cell lines are also sensitive to USP7 inhibition.[15] Compound **41** inhibited the growth of p53-mutant small cell lung cancer cell line H526 at a CC_{50} of 450 nM, implying that tumor growth suppression might be observed in an in vivo setting. For the H526 xenograft model, athymic nude mice were administered twice daily oral doses of compound **41** for 24 days (Figure 10b). At 50 mg/kg, significant tumor growth inhibition was observed which resulted in strikingly improved survival as compared to the vehicle control group. Since compound **41** is less potent towards H526 cells than MM.1S cells in vitro, it is not surprising that there is slightly inferior tumor growth inhibition in the H526 xenograft model as compared to the MM.1S study. Nevertheless, this in vivo activity demonstrates that USP7 inhibitors can suppress tumor growth through multiple different pathways, potentially widening the utility of USP7 inhibitors beyond p53-sensitive tumors.

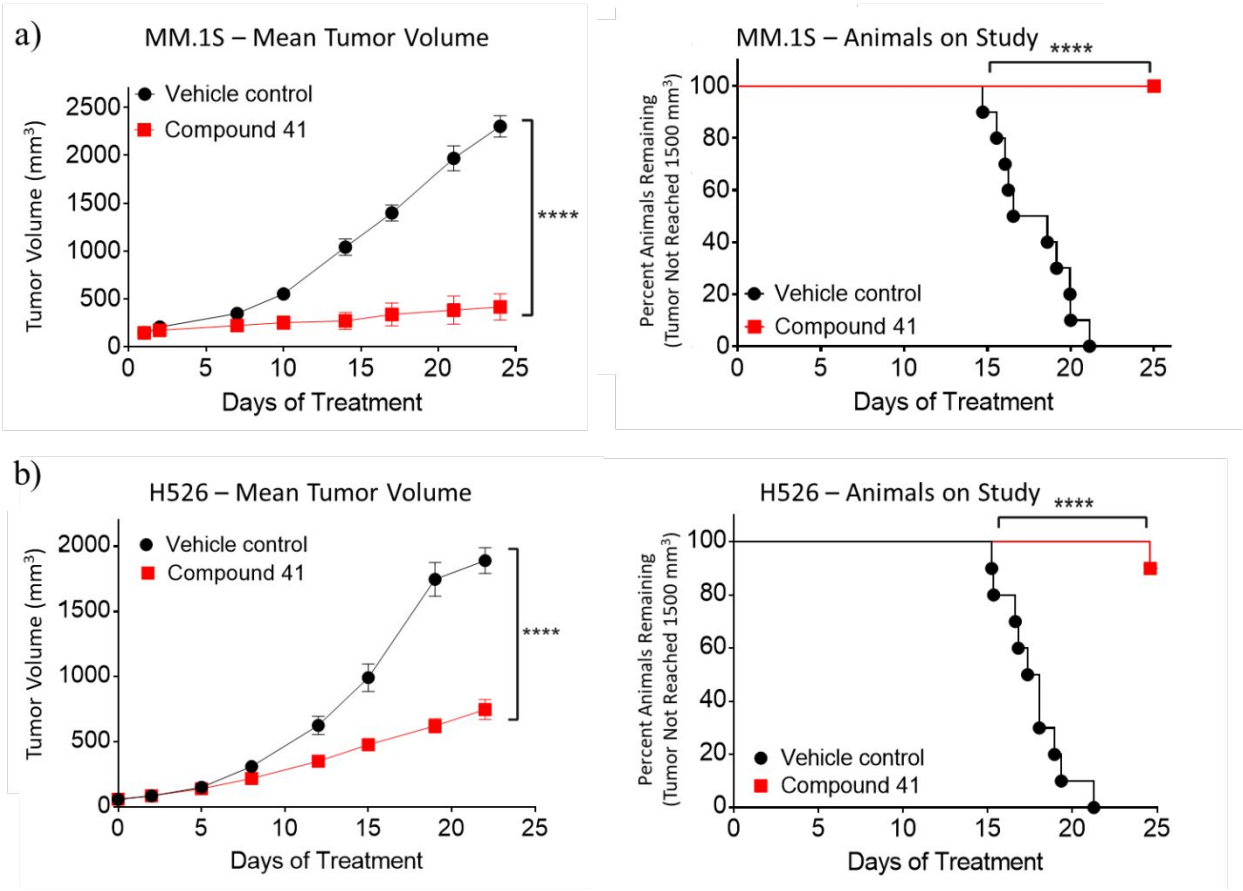
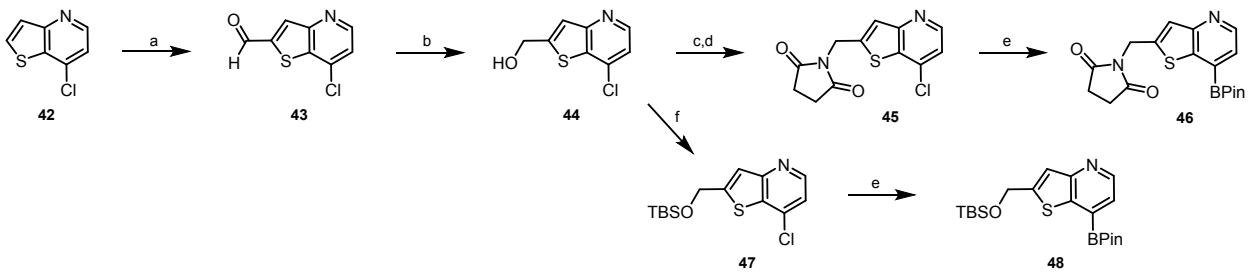


Figure 10. Xenograft studies with (a) NOD-SCID mice engrafted with MM.1S tumors, or (b) athymic nude mice engrafted with H526 tumors. For each study, compound **41** was dosed PO at 50 mg/kg BID for the indicated time and tumor volume was measured. If the tumor volume exceeded 1500 mm^3, the animals were taken down from the study (**** $p < 0.0001$).

SYNTHETIC CHEMISTRY

USP7 inhibitors were synthesized in a convergent manner by palladium-catalyzed coupling between the upper thienopyridine piece and the lower aryl portion. The thienopyridine pieces were synthesized according to Scheme 1. Commercial thienopyridine chloride **42** was elaborated to aldehyde **43** by trapping the corresponding lithium anion with DMF at low temperature.[26] Simple reduction of aldehyde **43** produced alcohol **44**, which could be converted to succinimidyl side chain **45** via chlorination and subsequent S_N2 displacement. This piece could be borylated to produce boronic ester **46** for subsequent Suzuki coupling. Alternatively, alcohol **44** can be protected as the silyl ether **47** and borylated to produce **48**. The silyl ether moiety was useful for late-stage diversification of the imide side chain.

Scheme 1. Synthesis of thienopyridine pieces.

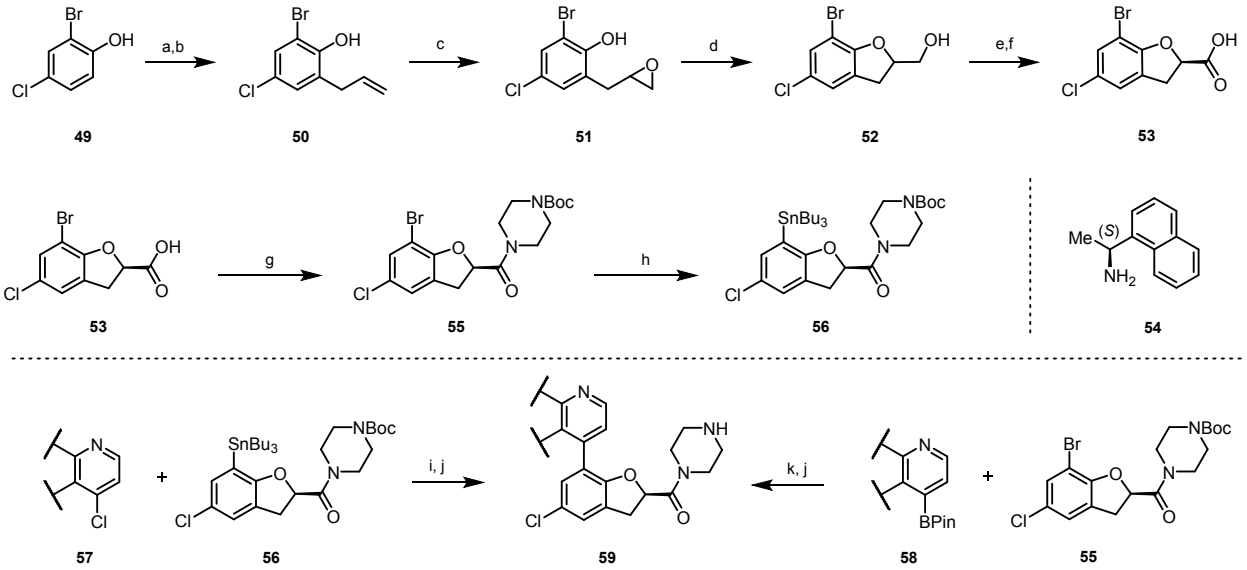


Reagents: (a) *n*-BuLi, -78 ºC, DMF, 90%. (b) $NaBH_4$, MeOH, 96%. (c) $SOCl_2$, DCM,

99%. (d) pyrrolidine-2,5-dione, K_2CO_3, acetone, 63%. (e) B_2Pin_2, $Pd(dppf)Cl_2$, dppf, KOAc, dioxane, 110 °C, 83-99%. (f) TBS-Cl, imidazole, DMF, 85%.

The benzofuran-amide portion was prepared from 2-bromo-4-chlorophenol (**49**, Scheme 2). Phenol **49** was allylated with allyl bromide and was converted to 2-allylphenol **50** by a Claisen rearrangement.[27] Epoxidation with *m*-CPBA afforded **51**, which underwent facile cyclization under basic conditions to afford alcohol **52**. TEMPO-catalyzed oxidation of **52** afforded the racemic carboxylic acid, which was enantioenriched to the (*R*)-acid **53** by classical resolution with napthylamine **54**, affording **53** in 99% ee on multi-gram scale. This enantioenriched acid was converted to piperazine-amide **55** by T3P coupling. This compound could be converted to the aryl stannane **56** for subsequent Stille coupling. The final products were prepared via one of two protocols – via Stille coupling of chloropyridine **57** and stannane **56** followed by acidic deprotection, or via Suzuki coupling of pyridine boronic ester **58** and aryl bromide **55** followed by deprotection under acidic conditions.

Scheme 2. Synthesis of benzofuran-amide USP7 inhibitors.

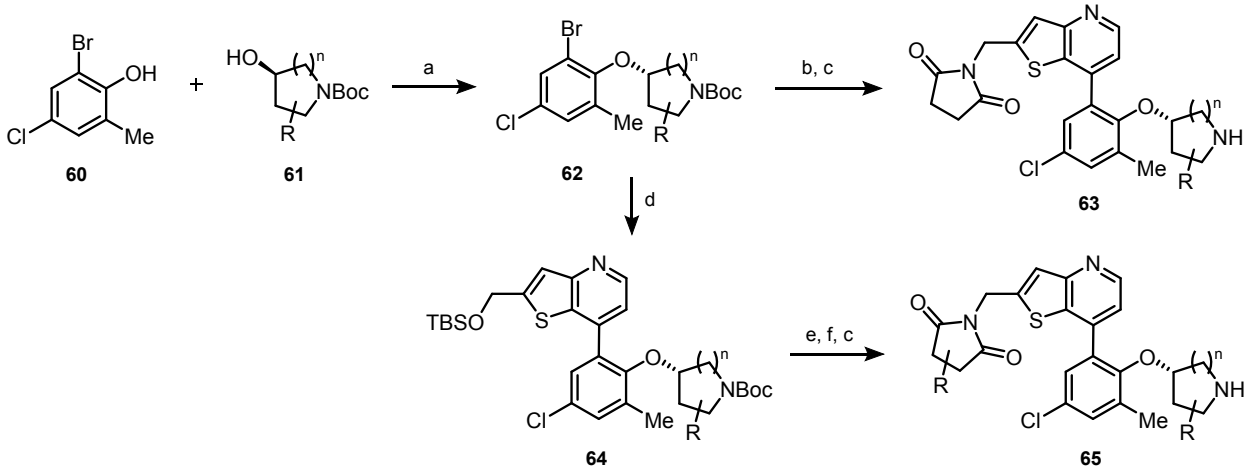


(a) allyl bromide, K_2CO_3, acetone, 55 ºC. (b) mesitylene, 180 ºC, 73% over 2 steps. (c) *m*-CPBA, DCM. (d) KOH, MeOH/H_2O. (e) TEMPO (cat.), KBr (cat.), NaOCl, $NaHCO_3$, THF, H_2O, 0ºC (52% over 3 steps). (f) **54**, MeOH, recrystallization (x2), 42%. (g) *N*-Boc-piperazine, 1-methylimidazole, propanephosphonic anhydride, EtOAc, 81%. (h) $PdCl_2(PPh_3)_2$ (cat.), $(Sn(n\text{-}Bu)_3)_2$, 1,4-dioxane, 110 ºC, 72%. (i) $Pd(PPh_3)_2Cl_2$, Cu_2O, 1,4-dioxane, 100 ºC. (j) HCl or TFA, DCM, 90-99%. (k) $Pd(PPh_3)_2Cl_2$, aq. Na_2CO_3, EtOH, PhMe, 85 °C.

To prepare the simplified ether analogues, phenol **60** and substituted alcohol **61** were brought together in a Mitsunobu reaction to form ether **62** (Scheme 3). Suzuki

coupling with boronic ester **46** followed by protecting group removal afforded the final compound **63**. Analogs with diverse imide side chains could also be generated by late-stage substitution via intermediates such as TBS-ether **64**. Accordingly, Suzuki coupling of aryl bromide **62** with boronic ester **48** yielded coupled product **64**. Silyl cleavage followed by chlorination with methanesulfonyl chloride enabled facile displacement by a range of cyclic imides or other nucleophiles. Deprotection under acidic conditions produced the final compounds.

Scheme 3. Synthesis of the simplified ether series of compounds.



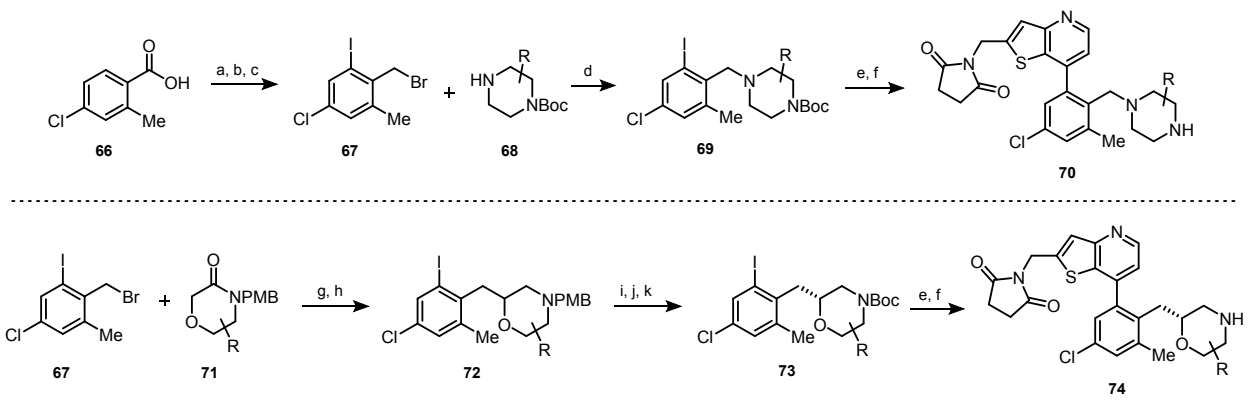
Reagents: (a) PPh_3, DIAD, THF, 51-80%. (b) **46**, $Pd(PPh_3)_2Cl_2$, aq. Na_2CO_3, EtOH, PhMe, 85 °C, 20-69%. (c) TFA or HCl, DCM, 90-99%. (d) **48**, $Pd(PPh_3)_2Cl_2$, aq.

Na_2CO_3, EtOH, PhMe, 85 °C, 21-61%. (e) TBAF, THF, 67-94%. (f) MsCl, DIPEA, DCM, then cyclic imide, Cs_2CO_3, MeCN, 60 °C, 45-65%.

Synthesis of the piperazine series of inhibitors is outlined in Scheme 4. Starting from 4-chloro-2-methylbenzoic acid (**66**), large amounts of benzyl bromide **67** could be prepared via a 3-step sequence of carboxylate-directed C–H iodination, reduction, and bromination. Treatment of benzyl bromide **67** with mono-Boc-protected piperazine **68** and sodium hydride afforded benzylic piperazine **69**. Suzuki coupling with boronic ester **46** followed by deprotection afforded the final compounds.

The morpholine-containing compounds were synthesized through alkylation of PMB-protected morpholinone **71** with benzyl bromide **67**, followed by lactam reduction to give racemic morpholine **72** (Scheme 4). Protecting group exchange from PMB to Boc and then chiral separation afforded chiral morpholine **73**. Suzuki coupling with boronic ester **46** followed by deprotection gave the final compounds.

Scheme 4. Synthesis of carbon-linked compounds: piperazines and morpholines.

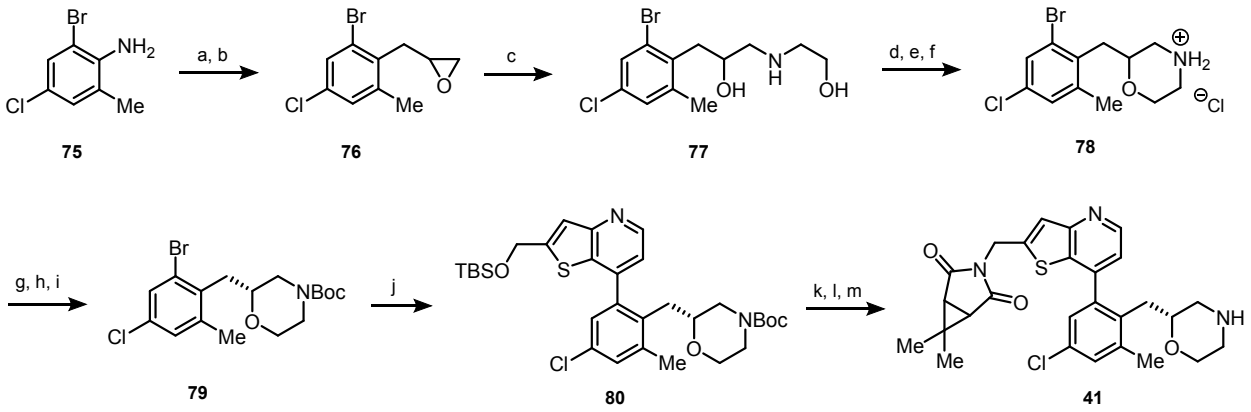


Reagents: (a) NIS, $Pd(OAc)_2$, DMF, 120 °C, 30%. (b) borane-DMS, THF, 50 °C, 73%. (c) PBr_3, DCM, 88%. (d) NaH, DMF, 42-83%. (e) **46**, $Pd(PPh_3)_2Cl_2$, aq. Na_2CO_3, EtOH, PhMe, 85 °C, 20-69%. (f) TFA, or HCl, DCM 90-99%. (g) LDA, THF, -78 °C, 43-62%. (h) borane-THF, THF, 70 °C, 62-88%. (i) chloroethyl chloroformate, DCE, then MeOH, 70 °C, 84-98%. (j) Boc_2O, imidazole, THF, 63-99%. (k) chiral SFC separation.

In order to synthesize multi-gram quantities of compound **41** for in vivo studies, an improved synthetic route to enantiopure morpholine **79** was designed (Scheme 5). Readily available 2-bromo-4-chloro-6-methylaniline (**75**) was first converted to epoxide **76** through a two-step procedure of allylation[28] followed by epoxidation. Epoxide **76** could be opened regioselectively with ethanolamine to give amino-diol **77**. Boc protection followed by Mitsunobu ring closure and treatment with HCl afforded racemic morpholine **78**. From epoxide **76**, morpholine **78** can be accessed in 59% yield over 4

steps without column chromatographic purification. Neutralization of the HCl salt generated the morpholine free base which was utilized to develop a large-batch resolution/recrystallization procedure. The addition of 0.5 equivalents of *N*-Ac-D-Leu allowed for enrichment of the desired morpholine enantiomer, and repeated recrystallization followed by Boc protection enabled production of chiral morpholine **79** in 99% ee on multi-gram scale. Standard Suzuki coupling with boronic ester **48** afforded compound **80** that was converted into compound **41** by silyl cleavage, chlorination, displacement with 6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione, and deprotection under acidic conditions.

Scheme 5. Synthetic route to access multi-gram quantities of enantiopure compound **41**.



Reagents: (a) allyl bromide, tBuONO, MeCN, 73%. (b) ^{m}CPBA, DCM, 63%. (c) ethanol amine, THF, 60 °C. (d) Boc_2O, imidazole, THF. (e) DIAD, PPh_3, MTBE. (f) HCl, DCM, 59% over 4 steps. (g) NaOH, DCM. (h) *N*-Ac-D-Leu (0.5 equiv), DCE, recrystallization (x3). (i) Boc_2O, Et_3N, DCM, 31% over 3 steps. (j) **48**, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, EtOH, PhMe, 85 °C. (k) TBAF, THF, 78% over 2 steps. (l) MsCl, DIPEA, DCM, then 6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione, Cs_2CO_3, MeCN, 60 °C. (m) HCl, DCM, 65% over 2 steps.

CONCLUSION

Co-crystal structures of USP7 with two micromolar inhibitors of different chemotypes revealed both compounds bound to the same allosteric site adjacent to the catalytic site of the enzyme. Careful analysis of the binding of a novel pyridylbenzofuran series suggested possibilities for establishing additional interactions and displacing unfavorable water molecules within this site. Introduction of a succinimide side chain to a thienopyridine functionality resulted in significantly increased potency. In order to improve the pharmacokinetic properties of these inhibitors, non-benzofuran chemotypes were developed. Key to this success was the re-design of the benzofuran-amide template to a simplified ortho-methyl ether series of compounds employing acyclic conformational control. Drug-like properties and cellular potencies were further improved by switching from ether-linked compounds to carbon-linked piperazines and morpholines, leading to the discovery of compound **41**. This USP7 inhibitor demonstrates marked tumor growth inhibition in both p53-wild type and p53-mutant tumors, suggesting that USP7 inhibition can suppress tumor growth in vivo through both p53 dependent and independent mechanisms. Further elucidation of these intricate

pathways could delineate a future clinical pathway for these potent, selective, and orally bioavailable USP7 inhibitors.

EXPERIMENTAL SECTION

General Chemistry.

All chemicals were purchased from commercial suppliers and used as received. An inert atmosphere of nitrogen or argon was used for reactions involving air or moisture sensitive reagents. Analytical thin layer chromatography (TLC) was performed using 2.5 x 7.5 cm Merck silica gel 60 F_{254} thin layer plates (EMD Millipore 1.15341.0001) visualized using combinations of UV visualization, p-anisaldehyde, potassium permanganate, and/or iodine staining. Silica gel column chromatography was performed using Teledyne ISCO RediSep Rf normal phase (35−70 μm) silica gel columns on a Teledyne ISCO CombiFlash Rf or CombiFlash Rf+ purification system (detection at 254 nm). Reversed-phase preparative HPLC was carried out using a Gemini-NX-C18 column (10 μm, 250 x 30 mm, Phenomenex, Torrance, CA) eluting with a linear gradient from 5 to 100% acetonitrile in water containing 0.1% trifluoroacetic acid over 30 minutes on a Teledyne ISCO EZ Prep, Teledyne ISCO ACCQPrep HP125, or Agilent 1200 Series purification system. Analytical reverse phase HPLC was performed using a Gemini-NX-C18 column (5 μm, 250 x 4.6 mm, Phenomenex, Torrance, CA) eluting with MeCN in water with 0.1% TFA on an Agilent 1200 Series purification system (detection at 254 nm). Proton NMR spectra were recorded on a Varian Oxford 400 MHz spectrometer and carbon NMR spectra were recorded at 101 MHz. Chemical shifts are expressed in δ ppm referenced to tetramethylsilane (δ = 0 ppm). Abbreviations used in describing peak signal multiplicity are as follows: s = singlet, d = doublet, dd = double doublets, t = triplet, q = quartet, m = multiplet, br = broad peak. Analytical LC-MS was

performed using a ZORBAX SB-C18 column (1.8 μm, 2.1 x 50 mm, 600 bar, Agilent, Santa Clara, CA) eluting with a linear gradient from 0% to 100% B over 2 min and then 100% B for 3 min (A = 5% MeCN in H2O with 0.1% formic acid, B = MeCN + 0.1% formic acid, flow rate 0.4 mL/min) using an Agilent 1260 Infinity II LC System (detection at 254 nm) equipped with an Agilent 6120 Quadrupole LC/MS in electrospray ionization mode (ESI+). The purity of all compounds used in bioassays was determined by this method to be >95% pure.

7-chlorothieno[3,2-b]pyridine-2-carbaldehyde (43). To a solution of 7-chlorothieno[3,2-b]pyridine (65.2 g, 0.384 mol) in anhydrous THF (0.64 L, 0.6 M) at –78 ºC was added a solution of *n*-BuLi (0.2 L, 0.5 mol, 1.3 equiv.) over 0.5 hr using an addition funnel. The mixture was stirred at –78 ºC for 2 hr before the addition of DMF (98 mL, 1.27 mol, 3.3 equiv.). The mixture was stirred for an additional 2 hr before warming to 0 ºC. 2 N aq. HCl (0.8 L) was added to acidify the mixture. The resulting precipitate was collected by filtration and dried in vacuo to yield the desired product as a yellow solid (68.5 g, 90% yield).

(7-chlorothieno[3,2-b]pyridin-2-yl)methanol (44). To a stirred suspension of aldehyde **43** (73.7 g, 0.373 mol) in methanol (1.1 L, 0.33 M) at 0 ºC was carefully added $NaBH_4$ (28.2 g, 0.746 mol, 2 equiv.) in portions over 30 min. The mixture was allowed to stir for an additional 30 min at 0 ºC. The reaction was monitored by LCMS to completion, and the mixture was concentrated in vacuo. The residue was triturated with H_2O (1 L) and the resulting solid was collected by filtration and dried in vacuo to yield the desired product (71.6 g, 96% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.54 (d, *J* = 5.1 Hz, 1H), 7.44 (t, *J* = 1.1 Hz, 1H), 7.26 (d, *J* = 5.2 Hz, 1H), 5.01 (d, *J* = 1.1 Hz, 2H). [M+H] 200.0.

1-((7-chlorothieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (45). To a stirred suspension of alcohol **44** (21.6 g, 108.2 mmol) in dichloromethane (200 mL) was added $SOCl_2$ (23.5 mL, 324.6 mmol, 3.0 equiv.) at room temperature. The mixture was stirred at room temperature for 20 h. Upon completion, the mixture was concentrated in vacuo to afford the desired chloride (27.5 g, 99% yield). To a stirred suspension of this alkyl chloride (7.0 g, 32.1 mmol) and K_2CO_3 (17.74 g, 128.4 mmol, 4.0 equiv.) in acetone (215 mL) was added pyrrolidine-2,5-dione (7.0 g, 70.6 mmol, 2.2 equiv.). The mixture

was heated to 80 ºC and stirred for 24 h and cooled to room temperature. The mixture was then filtered over celite, rinsing with 10% MeOH in DCM. The filtrate was concentrated in vacuo and purified by flash column chromatography (30-80% ethyl acetate/DCM) to afford the desired product as a white solid (5.7 g, 63% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.56 (d, *J* = 5.1 Hz, 1H), 7.55 (t, *J* = 0.8 Hz, 1H), 7.26 (d, *J* = 5.0 Hz, 1H), 4.96 (s, 2H), 2.78 (s, 4H). [M+H] 281.0.

1-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (46). To thienopyridine chloride **45** (185 mg, 0.66 mmol), bis(pinacolato)diboron (160 mg, 0.6 mmol), Pd(dppf)Cl_2 (44 mg, 0.06 mmol), and potassium acetate (176 mg, 1.8 mmol) was added 1,4-dioxane (3 mL). The resulting mixture was heated to 100 ºC for 3 h. The reaction mixture was diluted with water (5 mL), extracted with EtOAc (2x), dried over sodium sulfate, filtered, and concentrated under reduced pressure to afford the title compound (161 mg, 99% yield) which was used directly for the next Suzuki coupling (general procedure B).

2-(((tert-butyldimethylsilyl)oxy)methyl)-7-chlorothieno[3,2-b]pyridine (47). Alcohol **44** (30 g, 150.3 mmol) was suspended in DCM (500 mL) followed by the addition of DIEA (105 mL, 601.2 mmol), TBSCl (49.8 g, 330.6 mmol), and DMAP (3.67 g, 30 mmol). The mixture was allowed to stir at room temperature for 16 hrs. Upon completion, MeOH (100 mL) was added to the mixture and stirred for 2 hrs at room temperature. The reaction mixture was concentrated in vacuo and dissolved in DCM (500 mL). The organic layer was washed with 2N HCl (2 x 200 mL), H_2O (1 x 200 mL), and brine (1 x 200 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give the product (40.2 g, 85%).

2-(((tert-butyldimethylsilyl)oxy)methyl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridine (48). Thienopyridine chloride **47** (40.2 g, 128.1 mmol), bis(pinacolato)diboron (65 g, 256.1 mmol), potassium acetate (37.7 g, 384.3 mmol), and Pd(dppf)$_2$$Cl_2$ (4.7 g, 6.4 mmol) were suspended in dioxane (173 mL). The mixture was degassed by bubbling with N_2 for 30 mins then heated to 100 °C for 4.5 hrs. Upon completion, the mixture was cooled to room temperature then MeOH (200 mL) was added. The mixture was stirred at room temperature for 1 hr before slowly poured into

water (1 L) with vigorous stirring. The solid was filtered and triturated with hexanes yielding the product (20.8 g). The hexane layer was concentrated in vacuo and the resulting solid was triturated with hexanes yielding the product (22.2 g, combined yield 83%).

2-allyl-6-bromo-4-chlorophenol (50). To a solution of 2-bromo-4-chlorophenol (300 g, 1.45 mol) in acetone (0.6 L) was added allyl bromide (184 g, 1.05 equiv.) and K_2CO_3 (220 g, 1.1 equiv.). The mixture was stirred at 55 ºC for 2 hr, then cooled to room temperature. The mixture was filtered and washed with acetone (0.6 L). The filtrate was dried and concentrated to afford the desired allylated product as an oil (358 g), which was used directly in the following step. This oil (358 g) was dissolved in mesitylene (80 mL) and stirred at 180 ºC for 24 hr. The mixture was cooled to room temperature and the mesitylene was removed by concentration in vacuo. The desired product was isolated by distillation (90-95 ºC @ 1 torr) to afford the product as a colorless oil (262 g, 73% over 2 steps).

2-bromo-4-chloro-6-(oxiran-2-ylmethyl)phenol (51). To a solution of alkene **50** (180 g, 0.73 mol) in DCM (800 mL) at 0 ºC was added *m*-CPBA (263 g, 1.05 equiv.) in portions. The mixture was allowed to stir at room temperature overnight. The reaction was quenched with 2M aq. NaS_2O_3 solution and half sat. aq. $NaHCO_3$ with stirring. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo to afford the product (190 g), which was used directly in the following step.

(7-bromo-5-chloro-2,3-dihydrobenzofuran-2-yl)methanol (52). To a stirred solution of oxirane **51** (180 g) in methanol (800 mL) and water (50 mL) was added solid KOH (42 g, 1.1 equiv.) in portions. The mixture was stirred at room temperature overnight. The mixture was concentrated under reduced pressure, diluted with water (800 mL) and ethyl acetate (800 mL), and acidified to ~pH 2 with 6N aq. HCl. The organic layer was washed with water, sat. aq. $NaHCO_3$, and brine. The organic layer was dried over sodium sulfate and concentrated in vacuo to afford the product (190 g) which was used directly in the following step.

7-bromo-5-chloro-2,3-dihydrobenzofuran-2-carboxylic acid (*rac*-53). To a solution of alcohol **52** (180 g, 0.68 mol) in THF (600 mL) and sat. aq. $NaHCO_3$ (600 mL) at 0 ºC was added TEMPO (21.6 g, 0.2 equiv.) and potassium bromide (16.2 g, 0.2 equiv.). 13% aq. NaOCl (900 mL, 3.0 equiv.) was then added dropwise via addition funnel to the stirred mixture. The mixture was warmed to room temperature, and the reaction was monitored by TLC. After 8 hr, the mixture was diluted with MTBE (500 mL), water (800 mL), and 2M aq. potassium carbonate (100 mL), and the organic layer was removed. The aqueous layer was acidified to pH 2 with 6M aq. HCl, and the mixture was extracted with MTBE (2 x 500 mL). The organic extracts were dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was triturated with a mixture of MTBE/hexanes (1:5) and collected by filtration to afford the desired racemic acid as an off-white solid (105 g, 52% over 3 steps).

(*R*)-7-bromo-5-chloro-2,3-dihydrobenzofuran-2-carboxylic acid (53). To a solution of racemic acid ***rac*-53** (155 g, 0.56 mol) in methanol (500 mL) was slowly added a solution of (*S*)-1-(naphthalen-1-yl)ethan-1-amine (101 g, 0.59 mol, 1.05 equiv.) in methanol (500 mL). The resulting salt (255 g) was recrystallized from hot methanol (3 L) to afford an isomerically enriched salt (100 g) after collecting the solid by filtration. The filtrate was concentrated and recrystallized from methanol to afford a second crop of isomerically enriched salt (20 g). The combined salts (120 g) were treated with 1 N aq. NaOH (200 mL) and washed with MTBE (2 x 200 mL). The aqueous layer was acidified to pH 2 and extracted with EtOAc (2 x 200 mL). The combined organics were washed with water, brine, dried over sodium sulfate and concentrated to afford the title enantioenriched acid as a white solid (65 g, 42% yield, 99% ee).

tert-butyl (*R*)-4-(7-bromo-5-chloro-2,3-dihydrobenzofuran-2-carbonyl)piperazine-1-carboxylate (55). To a suspension of (*R*)-acid **53** (4.16 g, 15 mmol), N-Boc-piperazine (3.07 g, 16.5 mmol) and methylimidazole (3.59 mL, 45 mmol) in EtOAc (30 mL) was added a solution of T3P (50% by wt. in EtOAc, 17.9 mL, 30 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was diluted with aq. NaOH solution (1M, 150 mL), extracted with EtOAc (2 x 100 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. The crude residue was purified by flash

column chromatography (20-100% EtOAc in hexanes) to afford the title compound (5.4 g, 81% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.27 (s, 1H), 7.12 (s, 1H), 5.47 (dd, *J* = 9.9, 6.6 Hz, 1H), 4.05 (dd, *J* = 16.2, 6.6 Hz, 1H), 3.99 – 3.89 (m, 2H), 3.81 – 3.63 (m, 2H), 3.53 – 3.18 (m, 5H), 1.48 (s, 9H). [M+Na] 467.0.

tert-butyl (R)-4-(5-chloro-7-(tributylstannyl)-2,3-dihydrobenzofuran-2-carbonyl)piperazine-1-carboxylate (56). A solution of aryl bromide **55** (3.0 g, 6.73 mmol) and bis(triphenylphosphine)palladium (II) chloride (236 mg, 0.337 mmol) in dioxane (67 mL) was evacuated/backfilled with argon (3x). To this mixture was added tributyl(tributylstannyl)stannane (5.86 g, 10.1 mmol). The resulting mixture was stirred at 110 ºC for 18 h. The reaction mixture was allowed to cool to room temperature and solvent was removed under reduced pressure. The crude residue was purified by column chromatography (0-50% EtOAc in hexanes) to afford the title compound (3.2 g, 72% yield) as a clear oil. [M+H] 657.0.

General Procedure A (Stille Coupling). To a solution of stannane **56** (0.23 mmol) and appropriate chloropyridine (for example, **45**, **47**, or **57**, 0.23 mmol) in 1,4-dioxane (1.5 mL) was added $PdCl_2(PPh_3)_2$ (16 mg, 0.02 mmol) and copper oxide (33 mg, 0.23 mmol). The resulting mixture was sparged with argon for 5 min and heated to 100 ºC with stirring for 6 h. The solvent was removed under reduced pressure and the crude residue was purified by flash column chromatography (0-10% MeOH in DCM) to afford the coupled product.

General Procedure B (Suzuki Coupling). A mixture of pyridine boronic ester (for example, **46**, **48**, or **58**, 0.86 mmol), appropriate aryl bromide or iodide (for example, **55**, **62**, **69**, **73**, or **79**, 0.86 mmol), dichlorobis(triphenylphosphine)palladium(II) (60 mg, 0.09 mmol), and 1M aqueous sodium carbonate (2.5 mL) in toluene:ethanol (5 mL, 1:1 v/v) was heated to 85 ºC and stirred vigorously for 1-15 h. The reaction mixture was diluted in EtOAc (40 mL), then water (20 mL) and brine (20 mL) were added and the organic layer was separated, dried over $MgSO_4$, and concentrated. The crude residue was purified by column chromatography (0-10% MeOH in DCM) to afford the coupled product.

General Procedure C (Boc-Deprotection with HCl). Desired Boc-amine (0.7 mmol) was dissolved in DCM (4 mL), then HCl (4M in dioxane, 2 mL) was added. The resulting mixture was stirred at room temperature until complete conversion was achieved (1-3 h). The solvents were then removed in vacuo and residue was dried on high vacuum for 12 h to afford the final compound as the HCl salt.

General Procedure D (Boc-Deprotection with TFA). Desired Boc-amine (0.63 mmol) was dissolved in DCM (6 mL) at which time trifluoroacetic acid (1 mL) was added dropwise. The resulting mixture was stirred at room temperature until complete conversion was reached (1-3 h). The solvents were removed in vacuo and residue was dried on high vacuum for 12 h to afford the final compound as the TFA salt. If necessary, purification by reverse phase chromatography (0-100% MeCN in water) was performed.

General Procedure E (Preparation of Free Bases). The desired hydrochloride or TFA salt was dissolved in MeOH/DCM (20/80, 2mL) and filtered through a PL-HCO3 MP SPE column, eluting with MeOH/DCM (20/80). The solvents were removed in vacuo and residue was dried on high vacuum for 12 h to afford the final compound as the free base.

(7-(2-Aminopyridin-4-yl)-5-chloro-2,3-dihydrobenzofuran-2-yl)(piperazin-1-yl)methanone (7). The title compound was synthesized following general procedure B with tert-butyl 4-(7-bromo-5-chloro-2,3-dihydrobenzofuran-2-carbonyl)piperazine-1-carboxylate (***rac*-55**) and 2-aminopyridine-4-boronic acid pinacol ester followed by general procedure C to give the desired compound as the HCl salt (20 mg, 50% yield). ^{1}H NMR (400 MHz, Methanol-d_4): δ ppm 7.87 (d, J = 6.9 Hz, 1H), 7.50 (d, J = 2.1 Hz, 1H), 7.45 – 7.39 (m, 2H), 7.33 (dd, J = 6.9, 1.7 Hz, 1H), 5.88 (dd, J = 9.9, 6.6 Hz, 1H), 4.04 – 3.75 (m, 4H), 3.65 – 3.54 (m, 2H), 3.39 – 3.26 (m, 4H). [M+H] 359.1.

(5-chloro-7-(1H-pyrrolo[2,3-b]pyridin-4-yl)-2,3-dihydrobenzofuran-2-yl)(piperazin-1-yl)methanone (8). The title compound was synthesized following general procedure B with tert-butyl 4-(7-bromo-5-chloro-2,3-dihydrobenzofuran-2-carbonyl)piperazine-1-carboxylate (***rac*-55**) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrrolo[2,3-

b]pyridine followed by general procedures C and E to give the desired compound as the free base (25 mg, 70% yield). ^{1}H NMR (400 MHz, Methanol-d_4): δ ppm 8.23 (bs, 1H), 7.43 (d, *J*=3.6Hz, 1H), 7.40 (d, *J*=2Hz, 1H), 7.32 (s, 1H), 7.28 (s, 1H), 6.55 (d, *J*=3.6Hz, 1H), 5.69 (dd, *J*=9.6, 6.8Hz, 1H), 3.76-2.56 (m, 10H). [M+H] 383.0.

(*R*)-(5-chloro-7-(1H-pyrrolo[2,3-b]pyridin-4-yl)-2,3-dihydrobenzofuran-2-yl)(piperazin-1-yl)methanone (9). The title compound was synthesized following general procedure B with tert-butyl (*R*)-4-(7-bromo-5-chloro-2,3-dihydrobenzofuran-2-carbonyl)piperazine-1-carboxylate (**55**) and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrrolo[2,3-b]pyridine followed by general procedure C to give the desired compound as the HCl salt (165 mg, 0.431 mmol, 63% yield). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.44 (d, *J*=6.2 Hz, 1H), 7.81 (d, *J*=6.1 Hz, 1H), 7.74 (d, *J*=3.6 Hz, 1H), 7.56 (d, *J*=2.2 Hz, 1H), 7.47 (s, 1H), 7.03 (d, *J*=3.6 Hz, 1H), 5.91 – 5.81 (m, 1H), 4.02 – 3.76 (m, 4H), 3.77 – 3.69 (m, 1H), 3.61 – 3.54 (m, 1H), 3.43 – 3.04 (m, 4H). [M+H] 383.0.

(*R*)-(5-chloro-7-(thieno[3,2-b]pyridin-7-yl)-2,3-dihydrobenzofuran-2-yl)(piperazin-1-yl)methanone (10). The title compound was synthesized following general procedure A with tert-butyl 4-[5-chloro-7-tributylstannyl-2,3-dihydrobenzofuran-2-carbonyl]piperazine-1-carboxylate (**56**) and 7-chlorothieno[3,2-b]pyridine and purified by reverse phase HPLC using water (0.1% TFA) and acetonitrile (0.1% TFA) as the eluent followed by general procedure D to give the desired compound as the TFA salt (2.5 mg, 4% yield). ^{1}H NMR (400 MHz, Methanol-d_4, hydrochloric acid salt): δ ppm 8.64 (s, 1H), 8.21 (bs, 1H), 7.86 (bs, 1H), 7.22 (bs, 1H), 7.67 (bs, 1H), 7.53 (s, 1H), 5.89 (m, 1H), 3.86-3.09 (m, 10H). [M+H] 400.0.

[(2*R*)-5-chloro-7-(2-methylthieno[3,2-b]pyridin-7-yl)-2,3-dihydrobenzofuran-2-yl]-piperazin-1-yl-methanone (11). The title compound was synthesized following general procedure A with tert-butyl 4-[5-chloro-7-tributylstannyl-2,3-dihydrobenzofuran-2-carbonyl]piperazine-1-carboxylate (**56**) and 7-chloro-2-methyl-thieno[3,2-b]pyridine followed by general procedure C. The title compound was isolated by flash column chromatography (silica gel, 0-10% MeOH in DCM + 0.1M NH_3) (14 mg, 29% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.61 (d, *J* = 4.9 Hz, 1H), 7.44 (d, *J* = 2.1 Hz, 1H), 7.36 – 7.19 (m, 3H), 5.46 (dd, J = 9.9, 7.1 Hz, 1H), 4.06 – 3.89 (m, 1H), 3.89 – 3.70 (m, 1H),

3.70 – 3.53 (m, 1H), 3.48 – 3.29 (m, 2H), 2.93 – 2.82 (m, 1H), 2.82 – 2.67 (m, 1H), 2.63 (s, 3H), 2.60 – 2.46 (m, 1H), 2.46 – 2.00 (m, 2H). [M+H] 414.1.

(*R*)-(5-chloro-7-(2-(hydroxymethyl)thieno[3,2-b]pyridin-7-yl)-2,3-dihydrobenzofuran-2-yl)(piperazin-1-yl)methanone (12). The title compound was synthesized following general procedure A with tert-butyl 4-[5-chloro-7-tributylstannyl-2,3-dihydrobenzofuran-2-carbonyl]piperazine-1-carboxylate (**56**) and (7-chlorothieno[3,2-b]pyridin-2-yl)methanol and purified by reverse phase HPLC using water (0.1% TFA) and acetonitrile (0.1% TFA) as the eluent followed by general procedures D and E successively to yield the desired compound as the free base. ^{1}H NMR (400 MHz, Methanol-d_4): δ ppm 8.75 (d, J=4.4Hz, 1H), 7.63 (d, J=4.8Hz, 1H), 7.61 (d, J=2.0Hz, 1H), 7.57 (s, 1H), 7.54 (d, J=2.0Hz, 1H), 5.88 (dd, J=9.6, 6.8Hz, 1H), 5.06 (s, 2H), 3.92-3.40 (m, 6H), 2.92-2.70 (m, 4H). [M+H] 430.0.

(*R*)-(5-chloro-7-(2-(3-hydroxyoxetan-3-yl)thieno[3,2-b]pyridin-7-yl)-2,3-dihydrobenzofuran-2-yl)(piperazin-1-yl)methanone (13). Step 1: To a solution of 7-chlorothieno[3,2-b]pyridine (1.0 g, 5.9 mmol) in THF (20 mL) at -78 ºC was added a solution of *n*-BuLi (2.5M in hexanes, 1.3 equiv, 3.1 mL). The solution was stirred for 20 min before the addition of 3-oxetanone (850 mg, 11.8 mmol, 2.0 equiv). The solution was allowed to stir for 1 hr prior to quenching with sat. aq. NH_4Cl (50 mL). The mixture was extracted with ethyl acetate (2 x 50 mL) and the combined organic layers were dried, filtered, and concentrated to afford a crude compound, which was recrystallized from 80% ethyl acetate in hexanes to afford 3-(7-chlorothieno[3,2-b]pyridin-2-yl)oxetan-3-ol as white crystals (1.12 g, 79% yield). [M+H] 242.0. Step 2: The title compound was synthesized following general procedure A with 3-(7-chlorothieno[3,2-b]pyridin-2-yl)oxetan-3-ol and stannane **56** followed by general procedure D to give the desired compound as the TFA salt (31 mg, 43% yield). [M+H] 472.0.

((2*R*)-5-chloro-7-(2-(1-chloro-2,3-dihydroxypropan-2-yl)thieno[3,2-b]pyridin-7-yl)-2,3-dihydrobenzofuran-2-yl)(piperazin-1-yl)methanone (14). The title compound was synthesized following general procedures A with stannane **56** and 3-(7-chlorothieno[3,2-b]pyridin-2-yl)oxetan-3-ol followed by general procedure C to give the desired compound as the HCl salt (27 mg, 30% yield). [M+H] 507.9.

(*R*)-3-((7-(5-chloro-2-(piperazine-1-carbonyl)-2,3-dihydrobenzofuran-7-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrimidin-4(3H)-one (15). Step 1: 7-chloro-2-(chloromethyl)thieno[3,2-b]pyridine (300 mg, 1.38 mmol) was dissolved in DMF (9.2 mL), then 1H-pyrimidin-6-one (264 mg, 2.75 mmol) and potassium carbonate (570.29mg, 4.13 mmol) were added. The reaction was stirred for 15 h. Water was added and the mixture was extracted with ethyl acetate. Then, the organics were washed with water (x2) and brine, dried, concentrated, and purified by column chromatography (1 to 5% MeOH in DCM) to yield 3-((7-chlorothieno[3,2-b]pyridin-2-yl)methyl)pyrimidin-4(3H)-one (267 mg, 70%). Step 2: The title compound was synthesized following general procedure A with 3-((7-chlorothieno[3,2-b]pyridin-2-yl)methyl)pyrimidin-4(3H)-one and stannane **56** followed by general procedure C to give the desired compound as the HCl salt. ^{1}H NMR (400 MHz, Methanol-d_4) δ 9.14 (s, 1H), 8.97 (d, J = 6.0 Hz, 1H), 8.18 (d, J = 6.1 Hz, 1H), 8.06 (d, J = 7.0 Hz, 1H), 7.95 (s, 1H), 7.65 (d, J = 2.1 Hz, 1H), 7.56 (dd, J = 2.2, 1.1 Hz, 1H), 6.67 (d, J = 6.9 Hz, 1H), 5.91 (dd, J = 9.7, 7.0 Hz, 1H), 5.70 (d, J = 0.7 Hz, 2H), 3.95 – 3.10 (m, 10H). [M+H] 508.0.

(*R*)-N-((7-(5-chloro-2-(piperazine-1-carbonyl)-2,3-dihydrobenzofuran-7-yl)thieno[3,2-b]pyridin-2-yl)methyl)acetamide (16). Step 1: To a mixture of (7-chlorothieno[3,2-b]pyridin-2-yl)methanol (0.5 g, 2.5 mmol), triphenylphosphine (0.78 g, 1.2 equiv.), phthalimide (0.44 g, 1.2 equiv) in THF was added diisopropyl azodicarboxylate (0.66 mL, 1.3 equiv.). The reaction was stirred for 2 hr before being concentrated, charged with DCM (10 mL), and filtered to afford 2-((7-chlorothieno[3,2-b]pyridin-2-yl)methyl)isoindoline-1,3-dione as an off-white solid (0.47 g, 57% yield) which was used directly in the following step. Step 2: The above solid (0.47 g, 1.43 mmol) was dissolved in ethanol (10 mL) and hydrazine (0.4 mL, 5 equiv.) was added. The reaction mixture was heated to 70 ºC for 1 h. The reaction mixture was concentrated in vacuo, diluted with water (10 mL) and ethyl acetate (25 mL), and the aqueous layer was back-extracted with ethyl acetate (25 mL). The combined organic layers were washed with brine, dried over sodium sulfate, filtered, and concentrated to afford (7-chlorothieno[3,2-b]pyridin-2-yl)methanamine (0.28 g, 99% yield) which was used directly in the following step. Step 3: The above material (0.28 g, 1.41 mmol) was dissolved in THF (5 mL), then

triethylamine (0.3 mL, 1.5 equiv.), acetic anhydride (0.036 mL, 2.2 equiv.), and pyridine (110 mg, 1.0 equiv.) were added. The solution was allowed to stir overnight. The solution was concentrated in vacuo, diluted with water (10 mL), and extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with brine, dried, filtered, and concentrated to afford a residue which was purified by flash column chromatography (silica gel, 100% ethyl acetate) to afford N-((7-chlorothieno[3,2-b]pyridin-2-yl)methyl)acetamide (0.19 g, 56% yield). Step 4: The title compound was synthesized following general procedure A with N-((7-chlorothieno[3,2-b]pyridin-2-yl)methyl)acetamide and stannane **56** followed by general procedure C to give the desired compound as the HCl salt. ^{1}H NMR (400 MHz, Methanol-d_4): δ ppm 8.93 (bs, 1H), 8.12 (s, 1H), 7.72 (s, 1H), 7.63 (s, 1H), 7.54 (s, 1H), 5.90 (bs, 1H), 4.80 (s, 2H), 3.95-3.12 (m, 10H), 2.04 (s, 3H). [M+H] 471.0.

(*R*)-1-((7-(5-chloro-2-(piperazine-1-carbonyl)-2,3-dihydrobenzofuran-7-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidin-2-one (17). Step 1: To 2-pyrrolidone (117 mg, 1.38 mmol, 2.0 equiv.) in DMF (2.3 mL) was added sodium hydride (41 mg, 1.03 mmol, 60% dispersion in mineral oil). After 20 min, 7-chloro-2-(chloromethyl)thieno[3,2-b]pyridine was added in one portion. After 30 min, the reaction mixture was quenched with sat. aq. ammonium chloride (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layers were concentrated and the residue purified by flash column chromatography (100% EA, then 0-10% MeOH in DCM) to afford 1-[(7-chlorothieno[3,2-b]pyridine-2-yl)methyl]pyrrolidine-2-one as a thick oil (35 mg, 19% yield). Step 2: The title compound was synthesized following general procedure A with 1-[(7-chlorothieno[3,2-b]pyridine-2-yl)methyl]pyrrolidine-2-one and stannane **56** followed by general procedures D and E to give the desired compound as the free base. ^{1}H NMR (400 MHz, Methanol-d_4): δ ppm 8.63 (bs, 1H), 7.54 (s, 1H), 7.45 (s, 1H), 7.44 (s, 1H), 7.39 (s, 1H), 5.74 (dd, J=9.6, 6.8Hz, 1H), 4.59 (s, 2H), 3.74-3.39 (m, 10H), 2.43 (t, J=8.0Hz, 2H), 2.09 (quin, J=7.2Hz, 2H). [M+H] 497.0.

(*R*)-1-((7-(5-chloro-2-(piperazine-1-carbonyl)-2,3-dihydrobenzofuran-7-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (18). The title compound was synthesized following general procedure A using stannane **56** (100 mg, 0.156 mmol) and 1-((7-

chlorothieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**45**) followed by general procedures D and E to afford the desired compound as the free base (15 mg, 0.026 mmol, 17% yield). 1H NMR (400 MHz, Methanol-d_4): δ ppm 8.93 (bs, 1H), 8.08 (bs, 1H), 7.78 (s, 1H), 7.64 (s, 1H), 7.57 (s, 1H), 5.93 (m, 1H), 5.14 (s, 2H), 3.99-3.10 (m, 10H), 2.84 (s, 4H). [M+H] 511.0.

(*R*)-1-((7-(2-(4-(azetidin-1-ylmethyl)oxazol-5-yl)-5-chloro-2,3-dihydrobenzofuran-7-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (19). Step 1: To a solution of (2*R*)-7-bromo-5-chloro-2,3-dihydrobenzofuran-2-carboxylic acid (**53**, 1.0 g, 3.6 mmol) in DMF (5.5 mL) was added potassium carbonate (2.0 g, 14.4 mmol), diphenylphosphoryl azide (1.01 mL, 4.7 mmol), and ethyl isocyanoacetate (0.79 mL, 7.2 mmol). After stirring for 15 hours, the reaction was diluted with water (50 mL) and brine (50 mL) and extracted with EtOAc (2 x 50 mL). The organics were dried, concentrated, and purified by column chromatography (20-100% EtOAc/hexane) to yield ethyl 5-[(2*R*)-7-bromo-5-chloro-2,3-dihydrobenzofuran-2-yl]oxazole-4-carboxylate (389 mg, 29%). Step 2: To a solution of ethyl 5-[(2*R*)-7-bromo-5-chloro-2,3-dihydrobenzofuran-2-yl]oxazole-4-carboxylate (190 mg, 0.53 mmol) in THF (4.5 m) was added water (0.5 mL) and sodium borohydride (100 mg, 2.65 mmol). After stirring vigorously for 3 days, the reaction was diluted with water and extracted with EtOAc (2 x 5 mL). The organics were dried, concentrated, and purified by column chromatography (0-100% EtOAc/hexane) to yield [5-[(2*R*)-7-bromo-5-chloro-2,3-dihydrobenzofuran-2-yl]oxazol-4-yl]methanol (77 mg, 44%). Step 3: [5-[(2*R*)-7-bromo-5-chloro-2,3-dihydrobenzofuran-2-yl]oxazol-4-yl]methanol (60 mg, 0.18 mmol) and 1-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**46**, 81 mg, 0.22 mmol) were utilized with general procedure B to afford 1-[[7-[(2*R*)-5-chloro-2-[4-(hydroxymethyl)oxazol-5-yl]-2,3-dihydrobenzofuran-7-yl]thieno[3,2-b]pyridin-2-yl]methyl]pyrrolidine-2,5-dione (7.5 mg, 8%). Step 4: To a solution of 1-[[7-[(2*R*)-5-chloro-2-[4-(hydroxymethyl)oxazol-5-yl]-2,3-dihydrobenzofuran-7-yl]thieno[3,2-b]pyridin-2-yl]methyl]pyrrolidine-2,5-dione (7.5 mg, 0.015 mmol) in DCM (0.3 mL) was added sodium bicarbonate (2.5 mg, 0.03 mmol) and Dess-Martin periodinane (7.4 mg, 0.017 mmol). This solution was stirred for 1 hour, then filtered through Celite and

concentrated, then purified by column chromatography (0-100% EtOAc/hexane) to yield (*R*)-5-(5-chloro-7-(2-((2,5-dioxopyrrolidin-1-yl)methyl)thieno[3,2-b]pyridin-7-yl)-2,3-dihydrobenzofuran-2-yl)oxazole-4-carbaldehyde (3 mg, 40%). Step 5: To a solution of (*R*)-5-(5-chloro-7-(2-((2,5-dioxopyrrolidin-1-yl)methyl)thieno[3,2-b]pyridin-7-yl)-2,3-dihydrobenzofuran-2-yl)oxazole-4-carbaldehyde (3 mg, 0.006 mmol) in DCE (0.3 mL) was added azetidine HCl (2.9 mg, 0.03 mmol) and sodium triacetoxyborohydride (2.6 mg, 0.01 mmol). After stirring for 2 hours, the reaction was quenched with 1 M NaOH (1 mL) and extracted with DCM (3 x 1 mL). The organics were dried and concentrated and purified by column chromatography (1-10% MeOH/DCM) to give the desired product as the free base. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.78 (d, *J* = 4.9 Hz, 1H), 8.32 (s, 1H), 7.65 (s, 1H), 7.62 (d, *J* = 2.4 Hz, 1H), 7.60 (d, *J* = 2.2 Hz, 1H), 7.58 (d, *J* = 4.9 Hz, 1H), 6.33 (dd, *J* = 9.8, 7.4 Hz, 1H), 5.10 (s, 2H), 3.91 (dd, *J* = 16.3, 9.9 Hz, 1H), 3.76 (dd, *J* = 16.3, 7.4 Hz, 1H), 3.68 (d, *J* = 8.1 Hz, 2H), 3.40 – 3.30 (m, 4H), 2.93 (s, 4H), 2.15 (p, *J* = 7.1 Hz, 2H). [M+H] 535.0.

(*R*)-1-((7-(5-chloro-2-(4,5,6,7-tetrahydroisoxazolo[4,3-c]pyridin-3-yl)-2,3-dihydrobenzofuran-7-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (20). Step 1: To a stirred solution of (2*R*)-7-bromo-5-chloro-2,3-dihydrobenzofuran-2-carboxylic acid (**53**, 9.37 g, 33.8 mmol) in DCM (68 mL) at 0 ºC was added DMF (2 drops), followed by oxalyl chloride (5.8 mL, 2.0 equiv.) dropwise over 3 min. The solution was allowed to warm to room temperature over 20 min and concentrated in vacuo to give the acid chloride (10 g). In a separate flask, a solution of tert-butyl 4-oxopiperidine-1-carboxylate (10.8 g, 54.2 mmol, 1.6 equiv.) in anhydrous THF (125 mL) at –78 ºC was added a solution of LiHMDS (54.2 mL, 1 M in THF, 54.2 mmol, 1.6 equiv.). The solution was stirred at –78 ºC for 50 min before the addition of a solution of the above acid chloride (10.0 g, 33.9 mmol) in THF (5 mL). The resulting mixture was stirred at –78 ºC for 1 hr, then warmed to 0 ºC. The reaction was quenched with half sat. aq. NH_4Cl at 0 ºC and the aqueous layer was extracted with ethyl acetate (2 x 200 mL). The combined organic layers were concentrated and the crude product was purified by column chromatography (5-25% EtOAc/hexanes) to afford tert-butyl 3-((*R*)-7-bromo-5-chloro-2,3-dihydrobenzofuran-2-carbonyl)-4-oxopiperidine-1-carboxylate (6.8 g, 44%). Step 2:

To a solution of tert-butyl 3-((*R*)-7-bromo-5-chloro-2,3-dihydrobenzofuran-2-carbonyl)-4-oxopiperidine-1-carboxylate (3.3 g, 7.2 mmol) in EtOH (30 mL) was added $NH_2OH{\cdot}HCl$ (746 mg, 10.8 mmol, 1.5 equiv.), followed by $NaOAc{\cdot}3H_2O$ (2.94 g, 21.6 mmol, 3.0 equiv.). The mixture was heated to 82 ºC for 2 hr, then cooled to room temperature and concentrated in vacuo. The residue was diluted with water (100 mL) and extracted with ethyl acetate (2 x 100 mL). The combined organic layers were washed with sat. aq. $NaHCO_3$, dried over sodium sulfate, filtered, and concentrated to give the oxime (3.4 g). To this oxime (3.4 g, 7.2 mmol) in DCM (40 mL) was added MsCl (1.81 g, 15.9 mmol) and triethylamine (4.38 mL, 31.0 mmol). The mixture was stirred at room temperature for 2 hr and concentrated in vacuo. The residue was extracted with ethyl acetate (200 mL) and the organic layer was washed with 1M aq. HCl (100 mL), water (100 mL), and brine (100 mL). The organic layer was concentrated and the residue was purified by column chromatography (5-30% EtOAc/hexanes) to afford tert-butyl (*R*)-3-(7-bromo-5-chloro-2,3-dihydrobenzofuran-2-yl)-6,7-dihydroisoxazolo[4,3-c]pyridine-5(4H)-carboxylate (1.8 g, 55%). Step 3: To tert-butyl (*R*)-3-(7-bromo-5-chloro-2,3-dihydrobenzofuran-2-yl)-6,7-dihydroisoxazolo[4,3-c]pyridine-5(4H)-carboxylate (6.0 g, 13.2 mmol), bis(pinacolato)diboron (4.18 g, 16.5 mmol, 1.25 equiv.), potassium acetate (1.62 g, 16.5 mmol, 1.25 equiv.), and 1,1'-bis(diphenylphosphino)ferrocene-palladium(II) dichloride (530 mg, 0.72 mmol, 0.06 equiv.) was added 1,4-dioxane (60 mL) in a 150 mL sealed tube. The vessel was heated to 100 ºC for 6 hr, then cooled to room temperature, diluted with ethyl acetate (100 mL), and filtered through a pad of celite, washing with ethyl acetate. The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography (0-100% EtOAc/hexanes) to afford tert-butyl (*R*)-3-(5-chloro-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3-dihydrobenzofuran-2-yl)-6,7-dihydroisoxazolo[4,3-c]pyridine-5(4H)-carboxylate as a brown foam (5.35 g, 81% yield). Step 4: To a mixture of 1-((7-chlorothieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**45**, 109 mg, 0.39 mmol), tert-butyl (*R*)-3-(5-chloro-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2,3-dihydrobenzofuran-2-yl)-6,7-dihydroisoxazolo[4,3-c]pyridine-5(4H)-carboxylate (390 mg, 0.78 mmol, 2.0 equiv.), Xphos (18.5 mg, 0.04 mmol, 0.1 equiv.), $Pd(OAc)_2$ (8.7 mg, 0.04 mmol, 0.1 equiv.), and K_3PO_4 (247 mg, 1.16 mmol, 3.0 equiv.) was added THF (1.1 mL) and water (0.2 mL). The mixture was

sparged with Ar for 5 min and heated to 60 ºC with stirring for 5 h in a sealed vial. The mixture was cooled to room temperature and diluted with water (10 mL) and ethyl acetate (10 mL). The organic layer was concentrated and purified by flash column chromatography (50-100% ethyl acetate/hexanes) to afford tert-butyl (*R*)-3-(5-chloro-7-(2-((2,5-dioxopyrrolidin-1-yl)methyl)thieno[3,2-b]pyridin-7-yl)-2,3-dihydrobenzofuran-2-yl)-6,7-dihydroisoxazolo[4,3-c]pyridine-5(4H)-carboxylate (49 mg, 20% yield). Step 5: The title compound was synthesized following general procedure C with tert-butyl (*R*)-3-(5-chloro-7-(2-((2,5-dioxopyrrolidin-1-yl)methyl)thieno[3,2-b]pyridin-7-yl)-2,3-dihydrobenzofuran-2-yl)-6,7-dihydroisoxazolo[4,3-c]pyridine-5(4H)-carboxylate (49 mg, 0.08 mmol) to give the desired compound as the HCl salt (44 mg, 99% yield). 1H NMR (400 MHz, Methanol-d_4) δ 8.89 (s, 1H), 7.93 (s, 1H), 7.73 (s, 1H), 7.61 (s, 2H), 6.22 (s, 1H), 5.04 (s, 2H), 4.28 – 4.12 (m, 1H), 3.89 – 3.43 (m, 5H), 3.18 – 3.08 (m, 2H), 2.77 (s, 4H). [M+H] 521.0.

(R)-1-((7-(5-chloro-2-(2-methyl-4,5,6,7-tetrahydro-2H-pyrazolo[4,3-c]pyridin-3-yl)-2,3-dihydrobenzofuran-7-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (21). Step 1: To a solution of tert-butyl 3-((*R*)-7-bromo-5-chloro-2,3-dihydrobenzofuran-2-carbonyl)-4-oxopiperidine-1-carboxylate (5 g, 10.9 mmol) in ethanol (50 mL) was added anhydrous hydrazine (1.4 g, 43.7 mmol, 4 equiv). The mixture was heated to 62 ºC for 1.5 h with stirring. The mixture was cooled, concentrated and partitioned between EtOAc and H_2O. The organic layer is washed with sat. aq. $NaHCO_3$, concentrated and dried over sodium sulfate to afford tert-butyl (*R*)-3-(7-bromo-5-chloro-2,3-dihydrobenzofuran-2-yl)-2,4,6,7-tetrahydro-5H-pyrazolo[4,3-c]pyridine-5-carboxylate as a white solid (4.9 g, 98%). [M+H] = 454. Step 2: To a stirred suspension of (*R*)-3-(7-bromo-5-chloro-2,3-dihydrobenzofuran-2-yl)-2,4,6,7-tetrahydro-5H-pyrazolo[4,3-c]pyridine-5-carboxylate (1.6 g, 3.5 mmol) and K_2CO_3 (1.46 g, 10.6 mmol, 3.0 equiv) in DMF (10 mL) was added methyl iodide (548 uL, 8.8 mmol, 2.5 equiv). The resulting suspension was stirred overnight. The mixture was quenched with water and extracted w/ EtOAc (3 x 50 mL). The combined organic layers were washed with water (100 mL) and concentrated in vacuo. The crude mixture was purified by flash column chromatography (0-40% EtOAc in hexanes) to afford tert-butyl (*R*)-3-(7-bromo-5-chloro-

2,3-dihydrobenzofuran-2-yl)-2-methyl-2,4,6,7-tetrahydro-5H-pyrazolo[4,3-c]pyridine-5-carboxylate (958 mg, 58% yield). [M+H] 468. Step 3: The title compound was synthesized following general procedure B with tert-butyl (*R*)-3-(7-bromo-5-chloro-2,3-dihydrobenzofuran-2-yl)-2-methyl-2,4,6,7-tetrahydro-5H-pyrazolo[4,3-c]pyridine-5-carboxylate and pyridine boronic ester **46** followed by general procedure C to give the desired product as the HCl salt. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.60 (d, J = 4.9 Hz, 1H), 7.46 (t, J = 0.8 Hz, 1H), 7.43 – 7.39 (m, 3H), 5.90 (dd, J = 9.4, 8.0 Hz, 1H), 4.93 (d, J = 0.9 Hz, 2H), 3.91 (q, J = 15.8, 14.7, 14.7 Hz, 2H), 3.73 (s, 3H), 3.70 – 3.55 (m, 2H), 3.43 – 3.34 (m, 2H), 2.90 (t, J = 6.1 Hz, 2H), 2.74 (s, 4H). [M+H] 534.0.

General Procedure F (Mitsunobu Reaction). Diisopropyl azodicarboxylate (0.3 mL, 1.5 mmol) was added to a stirring mixture of 2-bromo-4-chloro-6-methylphenol (**60**, 0.22 g, 1.0 mmol), triphenylphosphine (0.39 g, 1.5 mmol), and an appropriate substituted alcohol (**61,** 1.2 mmol) in THF (3 mL) and stirred overnight. The reaction mixture was concentrated and purified by column chromatography (0-30% EtOAc in hexane) to give the desired product (**62**).

1-((7-(5-Chloro-2-(((3R,4S)-4-fluoropyrrolidin-3-yl)oxy)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (22). The title compound was synthesized following general procedure F using 2-bromo-4-chlorophenol and tert-butyl (3S,4S)-3-fluoro-4-hydroxypyrrolidine-1-carboxylate, followed by general procedure B with 1-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**46**), then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.66 (m, 1H), 7.53 (m, 3H), 7.45 (m, 1H), 7.32 (m, 1H), 5.46 (m, 1H), 5.19 (m, 1H), 4.96 (s, 2H), 3.69 (m, 1H), 3.59 (m, 2H), 3.16 (m, 2H), 2.71 (s, 4H). [M+H] 462.0.

1-((4-(5-Chloro-2-(((3R,4S)-4-fluoropyrrolidin-3-yl)oxy)-3-methylphenyl)thieno[3,2-d]pyrimidin-6-yl)methyl)pyrrolidine-2,5-dione (23). The title compound was synthesized following general procedure F using 2-bromo-4-chloro-6-methylphenol (**60**) and tert-butyl (3S,4S)-3-fluoro-4-hydroxypyrrolidine-1-carboxylate, followed by general procedure B with 1-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**46**), then general procedure D to give the desired

product. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.68 (d, J = 4.9 Hz, 1H), 7.88 – 7.64 (m, 2H), 7.51 – 7.26 (m, 2H), 5.02 – 4.93 (m, 1H), 4.73 – 4.38 (m, 1H), 4.06 – 3.79 (m, 1H), 3.30 (s, 4H), 2.99 – 2.74 (m, 2H), 2.64 – 2.52 (m, 1H), 2.47 (s, 3H), 2.44 – 2.26 (m, 1H), 2.01 – 1.81 (m, 2H). [M+H] 474.1.

(S)-1-((7-(5-Chloro-3-methyl-2-(piperidin-3-yloxy)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (24). The title compound was synthesized following general procedure F using 2-bromo-4-chloro-6-methylphenol (**60**) and tert-butyl (R)-3-hydroxypiperidine-1-carboxylate, followed by general procedure B with 1-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**46**) then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.80 (d, J = 5.3 Hz, 1H), 7.68 – 7.58 (m, 2H), 7.50 (dd, J = 2.7, 0.8 Hz, 1H), 7.41 (dd, J = 2.6, 0.6 Hz, 1H), 5.01 (d, J = 0.9 Hz, 2H), 3.81 – 3.70 (m, 1H), 3.06 – 2.94 (m, 2H), 2.94 – 2.85 (m, 1H), 2.83 – 2.71 (m, 5H), 2.43 (d, J = 0.7 Hz, 3H), 1.61 (tt, J = 17.2, 9.2 Hz, 2H), 1.44 – 1.24 (m, 2H). [M+H] 470.0.

1-((7-(5-Chloro-3-methyl-2-(piperidin-4-yloxy)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (25). The title compound was synthesized following general procedure F using 2-bromo-4-chloro-6-methyl-phenol (**60**) and tert-butyl 4-hydroxypiperidine-1-carboxylate, followed by general procedure B with 1-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**46**), then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.77 (d, J = 5.2 Hz, 1H), 7.66 – 7.56 (m, 2H), 7.49 (dq, J = 2.8, 0.7 Hz, 1H), 7.38 (dd, J = 2.7, 0.7 Hz, 1H), 5.00 (d, J = 1.0 Hz, 2H), 3.93 – 3.83 (m, 1H), 2.93 – 2.78 (m, 3H), 2.76 (s, 4H), 2.42 (d, J = 0.7 Hz, 3H), 1.83 – 1.68 (m, 2H), 1.58 – 1.44 (m, 2H). [M+H] 470.0.

1-((7-(5-Chloro-2-((3-fluoroazetidin-3-yl)methoxy)-3-methylphenyl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (26). The title compound was synthesized following general procedure F using 2-bromo-4-chloro-6-methylphenol (**60**) and tert-butyl 3-fluoro-3-(hydroxymethyl)azetidine-1-carboxylate, followed by general procedure B with 1-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**46**), then general procedure D to give the desired product. ^{1}H NMR (400

MHz, MeOH-d4) δ 8.66 (m, 1H), 7.51 (m, 1H), 7.42 (m, 2H), 7.34 (m, 1H), 4.96 (m, 2H), 3.70 (s, 2H), 3.47 (m, 2H), 3.21 (m, 2H), 2.75 (s, 4H), 2.39 (s, 3H). [M+H] 475.0.

1-((7-(2-((1r,3r)-3-aminocyclobutoxy)-5-chloro-3-methylphenyl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (27). The title compound was synthesized following general procedure F using 2-bromo-4-chloro-6-methylphenol (**60**) and cis-tert-butyl (3-hydroxycyclobutyl)carbamate, followed by general procedure B with 1-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**46**), then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.77 (d, J = 5.0 Hz, 1H), 7.60 (s, 1H), 7.57 (dd, J = 5.2, 1.0 Hz, 1H), 7.46 (dd, J = 2.6, 0.8 Hz, 1H), 7.37 (dd, J = 2.7, 0.8 Hz, 1H), 5.00 (s, 2H), 4.32 – 4.23 (m, 1H), 3.52 (dq, J = 8.6, 4.3 Hz, 1H), 2.77 (s, 4H), 2.37 (s, 3H), 2.23 – 2.14 (m, 2H), 2.06 – 1.96 (m, 2H). [M+H] 456.0.

1-((7-(5-Chloro-3-methyl-2-(((3R,4S)-3-methylpiperidin-4-yl)oxy)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (28). The title compound was synthesized following general procedure F using 2-bromo-4-chloro-6-methylphenol (**60**) and tert-butyl (3R,4R)-4-hydroxy-3-methylpiperidine-1-carboxylate, followed by general procedure B with 1-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**46**), then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.79 (s, 1H), 7.62 – 7.51 (m, 2H), 7.44 (d, J = 2.6 Hz, 1H), 7.32 (d, J = 2.6 Hz, 1H), 4.97 (s, 2H), 4.12 – 3.98 (m, 1H), 2.88 (dd, J = 12.6, 4.0 Hz, 1H), 2.74 (s, 4H), 2.67 – 2.46 (m, 3H), 2.44 (s, 3H), 2.12 – 1.80 (m, 2H), 1.58 – 1.38 (m, 2H), 0.92 (d, J = 6.9 Hz, 3H). [M+H] 484.0.

General Procedure G (Late-Stage Chlorination/Displacement). Step 1: To a solution of TBS-ether (such as **64** or **80**, 0.2 mmol) in THF (2 mL) was added tetra-n-butylammonium fluoride (0.3 mL, 0.3 mmol). This solution was allowed to stir at room temperature for 1 hour. The solution was concentrated and purified by column chromatography (1-4% MeOH/DCM) to give the primary alcohol. Step 2: This primary alcohol (~0.2 mmol) was dissolved in DCM (2 mL) after which N,N-diisopropylethylamine (0.11 mL, 0.6 mmol) and methanesulfonyl chloride (0.02 mL, 0.3 mmol) were sequentially added. After stirring at room temperature for 12 hours, the

mixture was diluted with DCM (5 mL) and washed with 1 N HCl (1 x 5 mL), sat aq. $NaHCO_3$ (1 x 5 mL), and brine (1 x 5 mL). The organics were dried over Na_2SO_4 and concentrated to give the benzylic chloride. To this crude material in acetonitrile (2 mL) was added cesium carbonate (195 mg, 0.6 mmol) and the desired cyclic imide (0.6 mmol) and heated to 60 °C for 3 hours. After cooling, the reaction was quenched with water along with a small amount of trifluoroacetic acid and purified directly by reverse phase chromatography to give the desired product.

3-((7-(5-chloro-3-methyl-2-(((3S,4R)-3-methylpiperidin-4-yl)oxy)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)-3-azabicyclo[3.1.0]hexane-2,4-dione (29). The title compound was synthesized following general procedure F using 2-bromo-4-chloro-6-methylphenol (**60**) and tert-butyl (3R,4R)-4-hydroxy-3-methylpiperidine-1-carboxylate, then general procedure B with 2-(((tert-butyldimethylsilyl)oxy)methyl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridine (**48**), followed by general procedure G with 3-azabicyclo[3.1.0]hexane-2,4-dione, then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.76 (d, J = 5.1 Hz, 1H), 7.58 – 7.51 (m, 2H), 7.46 (d, J = 2.7 Hz, 1H), 7.34 (d, J = 2.7 Hz, 1H), 4.83 (s, 2H), 4.09 (s, 1H), 2.89 (dd, J = 12.6, 3.9 Hz, 1H), 2.64 (dd, J = 11.1, 6.5 Hz, 1H), 2.60 (d, J = 3.6 Hz, 1H), 2.58 (d, J = 3.6 Hz, 1H), 2.46 (s, 3H), 2.03 – 1.87 (m, 2H), 1.66 – 1.57 (m, 1H), 1.59 – 1.49 (m, 2H), 1.47 – 1.38 (m, 1H), 0.95 (d, J = 6.9 Hz, 3H). [M+H] 496.0.

3-((7-(5-chloro-3-methyl-2-(((3S,4R)-3-methylpiperidin-4-yl)oxy)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione (30). The title compound was synthesized following general procedure F using 2-bromo-4-chloro-6-methylphenol (**60**) and tert-butyl (3R,4R)-4-hydroxy-3-methylpiperidine-1-carboxylate, then general procedure B with 2-(((tert-butyldimethylsilyl)oxy)methyl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridine (**48**), followed by general procedure G with 6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione, then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.76 (d, J = 5.1 Hz, 1H), 7.57 (s, 1H), 7.53 (d, J = 5.0 Hz, 1H), 7.46 (d, J = 2.7 Hz, 1H), 7.34 (d, J = 2.7 Hz, 1H), 4.88 (s, 2H), 4.08 (s, 1H), 2.90 (dd, J = 12.7, 4.0 Hz, 1H), 2.68 – 2.52 (m,

1H), 2.50 (s, 2H), 2.53 – 2.41 (m, 1H), 2.46 (s, 3H), 2.08 – 1.85 (m, 2H), 1.62 – 1.43 (m, 2H), 1.24 (s, 3H), 1.11 (s, 3H), 0.94 (d, *J* = 7.0 Hz, 3H). [M+H] 524.0.

3-((7-(5-Chloro-3-methyl-2-(((3S,4R)-3-methylpiperidin-4-yl)oxy)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)-1-(2,2,2-trifluoroethyl)pyrimidine-2,4(1H,3H)-dione (31). The title compound was synthesized following general procedure F using 2-bromo-4-chloro-6-methylphenol (**60**) and tert-butyl (3R,4R)-4-hydroxy-3-methylpiperidine-1-carboxylate, then general procedure B with 2-(((tert-butyldimethylsilyl)oxy)methyl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridine (**48**), followed by general procedure G with 1-(2,2,2-trifluoroethyl)pyrimidine-2,4(1H,3H)-dione, then general procedure D to give the desired product as the TFA salt. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.77 (d, *J* = 5.1 Hz, 1H), 7.70 – 7.60 (m, 2H), 7.55 (d, *J* = 5.1 Hz, 1H), 7.46 (d, *J* = 2.6 Hz, 1H), 7.34 (d, *J* = 2.7 Hz, 1H), 5.88 (d, *J* = 8.0 Hz, 1H), 5.43 (s, 2H), 4.71 – 4.47 (m, 2H), 4.02 (s, 1H), 2.91 – 2.75 (m, 1H), 2.73 – 2.58 (m, 1H), 2.45 (s, 3H), 2.47 – 2.36 (m, 1H), 2.10 – 1.81 (m, 2H), 1.63 – 1.36 (m, 2H), 0.89 (d, *J* = 6.7 Hz, 3H). [M+H] 579.0.

3-((7-(5-chloro-3-methyl-2-(piperidin-3-yloxy)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione (32). The title compound was synthesized following general procedure F using 2-bromo-4-chloro-6-methylphenol (**60**) and tert-butyl 3-hydroxypiperidine-1-carboxylate, then general procedure B with 2-(((tert-butyldimethylsilyl)oxy)methyl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridine (**48**), followed by general procedure G with 6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione, then general procedure D to give the desired product. [M+H] 510.0.

3-((7-(5-chloro-3-methyl-2-(piperidin-3-ylamino)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione (33). Step 1: Sodium triacetoxyborohydride (12.96 g, 61.1 mmol) was added to a solution of 2-bromo-4-chloro-6-methylaniline (3.37 g, 15.3 mmol) and 1-Boc-3-piperidone (9.14 g, 34.0 mmol) and acetic acid (7 mL, 122 mmol) in DCM (20 mL) and the reaction mixture was stirred at 60 °C overnight. The reaction was quenched with 1N NaOH (20 mL) and extracted with EtOAc (3 x 60 mL). The combined organic layers were washed with brine (60 mL),

concentrated, and purified by column chromatography (0-100% EtOAc in hexanes) to give tert-butyl 3-((2-bromo-4-chloro-6-methylphenyl)amino)piperidine-1-carboxylate as a light brown viscuous oil (2.1 g). Step 2: The title compound was synthesized following general procedure B with tert-butyl 3-((2-bromo-4-chloro-6-methylphenyl)amino)piperidine-1-carboxylate and 2-(((tert-butyldimethylsilyl)oxy)methyl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridine (**48**), followed by general procedure G with 6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione, then general procedure D to give the desired product as the TFA salt. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.64 (d, J = 4.9 Hz, 1H), 7.47 (s, 1H), 7.41 (d, J = 4.9 Hz, 1H), 7.26 (d, J = 2.6 Hz, 1H), 7.08 (d, J = 2.6 Hz, 1H), 5.48 (s, 2H), 2.67 – 2.55 (m, 2H), 2.47 (s, 2H), 2.43 – 2.30 (m, 2H), 2.33 (s, 3H), 2.13 (dd, J = 12.0, 9.3 Hz, 1H), 1.48 – 1.40 (m, 2H), 1.21 (s, 3H), 1.06 (s, 3H), 1.04 – 0.97 (m, 2H). [M+H] 509.0.

3-((7-(5-chloro-3-methyl-2-(piperidin-3-ylmethyl)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione (34). Step 1: A solution of Boc-4-piperidone (3.0 g, 15 mmol) and 1-phenylethylamine (1.82 g, 15 mmol) in toluene (75 mL) was heated to reflux under a Dean Stark apparatus for 12 hours. After cooling, the reaction mixture was concentrated to yield tert-butyl 4-((1-phenylethyl)imino)piperidine-1-carboxylate (4.5 g, 99%). Step 2: A solution of tert-butyl 4-(1-phenylethylimino)piperidine-1-carboxylate (2.0 g, 6.6 mmol) in THF (33 mL) was cooled to -78 °C, then lithium diisopropylamide (2 M, 3.0 mL, 6.0 mmol) was added dropwise. After stirring for 30 minutes at -78 °C, a solution of 1-bromo-2-(bromomethyl)-5-chloro-3-methylbenzene (2.0 g, 6.6 mmol) in THF (10 mL) was added dropwise. After stirring for 20 minutes at -78 °C, the reaction mixture was allowed to slowly warm to room temperature over the course of 1 hour. The reaction was quenched with NH_4Cl (20 mL), extracted with EtOAc, and concentrated. This crude material was dissolved in methanol (10 mL), after which 1 M HCl (10 mL) was added. This mixture was stirred at room temperature overnight. Partial Boc cleavage was also observed, so re-subjection to standard Boc protection conditions can be employed. The product was purified by column chromatography (0-30% EtOAc in hexane) to yield tert-butyl 3-(2-bromo-4-

chloro-6-methylbenzyl)-4-oxopiperidine-1-carboxylate (0.9 g, 62%). Step 3: A solution of tert-butyl 3-(2-bromo-4-chloro-6-methylbenzyl)-4-oxopiperidine-1-carboxylate (200 mg, 0.48 mmol) and 4-methylbenzenesulfonhydrazide (107 mg, 0.58 mmol) in ethanol (2.4 mL) was heated to reflux for 6 hours. After cooling, the reaction mixture was concentrated. This crude material was dissolved in THF (2.4 mL) and cooled to 0 °C. Sodium borohydride (280 mg, 7.5 mmol) was added slowly, then the reaction was heated to reflux for 15 hours. After cooling, water (5 mL) was added and extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over sodium sulfate, filtered, concentrated, and purified by column chromatography (0-50% EtOAc/hexane) to yield tert-butyl 3-(2-bromo-4-chloro-6-methylbenzyl)piperidine-1-carboxylate (130 mg, 64%). Step 4: The title compound was synthesized following general procedure B with tert-butyl 3-(2-bromo-4-chloro-6-methylbenzyl)piperidine-1-carboxylate and 2-(((tert-butyldimethylsilyl)oxy)methyl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridine (**48**), followed by general procedure G with 6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione, then general procedure D to give the desired product as the TFA salt. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.75 (d, J = 5.0 Hz, 1H), 7.58 (s, 1H), 7.48 – 7.38 (m, 2H), 7.18 (d, J = 6.6 Hz, 1H), 4.85 (s, 2H), 3.18 (d, J = 12.6 Hz, 1H), 3.00 (dd, J = 22.6, 12.5 Hz, 1H), 2.81 – 2.59 (m, 2H), 2.50 (d, J = 2.7 Hz, 2H), 2.47 (s, 3H), 2.43 – 2.28 (m, 2H), 1.81 – 1.54 (m, 2H), 1.54 – 1.27 (m, 2H), 1.24 (s, 3H), 1.09 (d, J = 6.7 Hz, 3H), 0.98 – 0.79 (m, 1H). [M+H] 508.0.

2-(bromomethyl)-5-chloro-1-iodo-3-methylbenzene (67): Step 1: 4-Chloro-2-methylbenzoic acid (100 g, 586 mmol) and NIS (158 g, 703 mmol) were dissolved in DMF (1 L, 0.6M) at which time $Pd(OAc)_2$ (13.2 g, 58.6 mmol) was added. The mixture was heated to 120 °C for 16 h, cooled, concentrated. The mixture was partitioned between EtOAc and water, and the organic phase washed with brine, dried over Na_2SO_4, and concentrated. The crude mixture was purified by silica gel chromatography to yield 4-chloro-2-iodo-6-methylbenzoic acid (51 g, 172 mmol, 30%). Step 2: The above material was dissolved in THF (325 mL), cooled to 0 °C, and added borane DMS (34.4 mL, 344 mmol). The mixture was heated to 50 °C and stirred for 20 h. Upon completion, the mixture was cooled to 0 °C then quenched carefully with

MeOH. The mixture was concentrated, and water was added to precipitate the product. The solid was purified by silica gel chromatography to furnish (4-chloro-2-iodo-6-methylphenyl)methanol (35.3 g, 125 mmol, 73%). Step 3: The above material was dissolved in DCM (410 mL), cooled to 0 °C, and added PBr_3 (23.6 mL, 250 mmol). The mixture was allowed to warm to room temperature and stirred 3 h. The mixture was cooled to 0 °C then quenched with sat. $NaHCO_3$. The layers were separated, and the aqueous layer extracted with DCM (3 x 200 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated to afford the title compound (38 g, 88%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.71 (d, *J* = 2.1 Hz, 1H), 7.17 (d, *J* = 2.1 Hz, 1H), 4.65 (s, 2H), 2.47 (s, 3H).

General Procedure H (Piperazine Substitution). To desired mono-Boc-protected piperazine (**68**, 2.0 mmol) in DMF (5 mL) was added sodium hydride (0.16 g, 4.0 mmol). The mixture was stirred for 5 min at which time 2-(bromomethyl)-5-chloro-1-iodo-3-methylbenzene (**67**, 0.83 g, 2.4 mmol) was added. The mixture was stirred for 35 min and partitioned between sat. NH_4Cl (2 mL), H2O (3 mL), and 50% EtOAc/Hexane (15 mL). The organic layer was separated, concentrated and purified by silica gel chromatography (0-40% EtOAc in hexane) to afford the desired product (**69**).

1-((7-(5-chloro-3-methyl-2-(piperazin-1-ylmethyl)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (35). The title compound was synthesized following general procedure H with 2-(bromomethyl)-5-chloro-1-iodo-3-methylbenzene (**67**) and tert-butyl piperazine-1-carboxylate, followed by general procedure B with 1-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**46**), then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.99 (bs, 1H), 7.97 (s, 1H), 7.85 (s, 1H), 7.59 (s, 1H), 7.39 (s, 1H), 5.08 (s, 2H), 4.15 (bs, 1H), 3.71 (bs, 1H), 3.02 (bs, 4H), 2.78 (s, 4H), 2.68 (bs, 4H), 2.60 (s, 3H). [M+H] 469.0.

(S)-1-((7-(5-chloro-3-methyl-2-((2-methylpiperazin-1-yl)methyl)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (36). The title compound was synthesized following general procedure H with 2-(bromomethyl)-5-chloro-1-iodo-3-methylbenzene (**67**) and tert-butyl (S)-3-methylpiperazine-1-carboxylate, followed by general procedure

B with 1-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**46**), then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.87 (bs, 1H), 7.70 (s, 1H), 7.66 (s, 1H), 7.47 (s, 1H), 7.27 (s, 1H), 5.03 (s, 2H), 4.29-4.08 (m, 4H), 3.11-2.91 (m, 2H), 2.77 (s, 4H), 2.64-2.56 (m, 1H), 2.52 (s, 3H), 2.45-1.99 (m, 3H), 1.27 (d, J = 7.2 Hz, 3H). [M+H] 483.0.

1-((7-(5-chloro-2-(((2S,6S)-2,6-dimethylpiperazin-1-yl)methyl)-3-methylphenyl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (37). The title compound was synthesized following general procedure H with 2-(bromomethyl)-5-chloro-1-iodo-3-methylbenzene (**67**) and tert-butyl (3S,5S)-3,5-dimethylpiperazine-1-carboxylate, followed by general procedure B with 1-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)pyrrolidine-2,5-dione (**46**), then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d4) δ 9.00 (s, 1H), 8.02 (s, 1H), 7.93 (s, 1H), 7.55 (s, 1H), 7.38 (s, 1H), 5.07 (s, 2H), 4.33-3.95 (m, 2H), 3.15-2.54 (m, 4H), 2.79 (s, 3H), 2.68 (d, J = 11.0 Hz, 4H), 0.93 (bs, 3H), 0.84 (bs, 3H). [M+H] 497.1.

General Procedure I (Morpholine Synthesis). Step 1: To diisopropylamine (0.31 mL, 2.2 mmol) in THF (10 mL) at 0 °C was added n-butyllithium (2.5 M in hexane, 0.88 mL, 2.2 mmol) dropwise and the solution was stirred at 0 °C for 45 min. The mixture was cooled to -78 °C at which time substituted PMB-morpholinone (**71**, 2.0 mmol) in THF (5 mL) was added dropwise. After stirring at -78 °C for 1 h, 2-(bromomethyl)-5-chloro-1-iodo-3-methylbenzene (**67**, 1.04 g, 3.0 mmol) in THF (5 mL) was added dropwise. The mixture was allowed to warm to rt and stirred 16 h. To this was added sat. aq. NH_4Cl (20 mL), extracted with EtOAc (2 x 20 mL), dried over Na_2SO_4 and concentrated. Silica gel chromatography afforded the alkylated morpholinone. Step 2: To the above alkylated morpholinone (1.58 mmol) in THF (3 mL) was added borane tetrahydrofuran (2.0 M in THF, 2.37 mL, 4.73 mmol) dropwise. The mixture was stirred at 70 °C overnight, then cooled to 0 °C and quenched carefully with 1:1 THF/H_2O (10 mL). Sat. aq. Rochelle's salt (10 mL) was added and the mixture vigorously stirred at rt. The mixture was extracted with EtOAc (2 x 20 mL) and purified by silica gel chromatography to furnish

the desired morpholine (**72**). Step 3: To the above morpholine (**72**, 1.39 mmol) in DCE (13 mL) was added chloroethyl chloroformate (0.45 mL, 4.15 mmol). The mixture was stirred overnight and concentrated. Methanol (13 mL) was added and the mixture was heated to 70 °C for 3 h. The mixture was cooled, concentrated, and purified by silica gel chromatography (2-10% MeOH/DCM) to afford the N-H morpholine (cleavage of PMB group). This material was then dissolved in THF (6 mL) after which di-*tert*-butyl dicarbonate (455 mg, 2.08 mmol) and imidazole (283 mg, 4.15 mmol) were added. After stirring at room temperature overnight, the mixture was concentrated and directly purified by column chromatography (5-30% EtOAc/hexane) to give the desired Boc-morpholine (**73**). For enantioenriched material, the racemic morpholine was subjected to chiral SFC separation.

3-((7-(5-chloro-3-methyl-2-(((R)-morpholin-2-yl)methyl)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)-3-azabicyclo[3.1.0]hexane-2,4-dione (38). The title compound was synthesized following general procedure I with 2-(bromomethyl)-5-chloro-1-iodo-3-methylbenzene (**67**) and 4-(4-methoxybenzyl)morpholin-3-one, followed by general procedure B with 3-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)-3-azabicyclo[3.1.0]hexane-2,4-dione, then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.87 (dd, J = 5.8, 2.6 Hz, 1H), 7.81 – 7.63 (m, 2H), 7.49 (d, J = 2.2 Hz, 1H), 7.24 (dd, J = 13.2, 2.3 Hz, 1H), 4.88 (d, J = 6.5 Hz, 2H), 3.89 (dd, J = 13.0, 3.7 Hz, 1H), 3.65 – 3.38 (m, 2H), 3.21 – 3.02 (m, 2H), 2.95 (ddd, J = 17.8, 13.6, 3.6 Hz, 1H), 2.87 – 2.62 (m, 3H), 2.59 (dt, J = 8.1, 3.3 Hz, 2H), 2.48 (s, 3H), 1.63 (tdd, J = 7.9, 4.6, 3.0 Hz, 1H), 1.47 (p, J = 3.6 Hz, 1H). [M+H] 482.0.

rac-3-((7-(5-chloro-3-methyl-2-(((2R,3R)-3-methylmorpholin-2-yl)methyl)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)-3-azabicyclo[3.1.0]hexane-2,4-dione (39). Step 1: To diisopropylamine (2.3 mL, 16.4 mmol) in THF (50 mL) at 0 °C was added n-butyllithium (2.5 M in hexane, 6.6 mL, 16.4 mmol) dropwise and the solution was stirred at 0 °C for 45 min. The mixture was cooled to -78 °C at which time tert-butyl 3-oxomorpholine-4-carboxylate (3.0 g, 14.9 mmol) in THF (10 mL) was added dropwise. After stirring at -78 °C for 1 h, 2-(bromomethyl)-5-chloro-1-iodo-3-methylbenzene (**67**, 10.3 g, 30 mmol) in THF (20 mL) was added dropwise. The mixture was allowed to

warm to rt and stirred for 2 h. To this was added sat. aq. NH_4Cl (100 mL), extracted with EtOAc (2 x 100 mL), dried over Na_2SO_4 and concentrated. Silica gel chromatography afforded tert-butyl 2-(4-chloro-2-iodo-6-methylbenzyl)-3-oxomorpholine-4-carboxylate (2.64 g, 30%). Step 2: The above compound (800 mg, 1.7 mmol) was dissolved in diethyl ether (15 mL) and cooled to -78 °C. Then, methylmagnesium bromide (3 M, 1.14 mL, 3.4 mmol) was added dropwise and the solution was stirred for 3 h. The reaction was quenched with sat. aq. NH_4Cl (20 mL), extracted with EtOAc (2 x 20 mL), and purified by column chromatography to yield tert-butyl (2-((1-(4-chloro-2-iodo-6-methylphenyl)-3-oxobutan-2-yl)oxy)ethyl)carbamate (510 mg, ~90% purity). Step 3: The above material (510 mg) was dissolved in methanol (10 mL) and then conc. HCl (0.72 mL, 8.6 mmol) was added. After stirring for 15 h, the mixture was concentrated, then dissolved in DCE (10 mL). To this was added sodium triacetoxyborohydride (725 mg, 3.4 mmol) and a drop of acetic acid. After stirring for 15 h, the mixture was concentrated and purified by column chromatography to yield trans-2-(4-chloro-2-iodo-6-methylbenzyl)-3-methylmorpholine (200 mg, 32% over 2 steps). Step 4: The above compound (200 mg, 0.54 mmol) along with di-tert-butyl dicarbonate (143 mg, 0.65 mmol) and 4-dimethylaminopyridine (73 mg, 0.60 mmol) were dissolved in acetonitrile (5 mL) and stirred for 15 h. After diluting with DCM (50 mL), the solution was washed with 1N HCl (1 x 30 mL), aq. $NaHCO_3$ (1 x 30 mL) and brine (1 x 30 mL), then purified by column chromatography to yield tert-butyl (2R,3R)-2-(4-chloro-2-iodo-6-methylbenzyl)-3-methylmorpholine-4-carboxylate (186 mg, 73%). Step 5: The title compound was synthesized following general procedure B with the above compound and 3-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)-3-azabicyclo[3.1.0]hexane-2,4-dione, then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.77 (d, J = 5.2 Hz, 1H), 7.59 – 7.46 (m, 2H), 7.43 (dd, J = 4.0, 2.2 Hz, 1H), 7.20 (dd, J = 12.3, 2.2 Hz, 1H), 4.84 (s, 2H), 4.37 – 4.28 (m, 1H), 4.16 (ddd, J = 12.0, 6.1, 2.5 Hz, 1H), 3.60 (dd, J = 13.1, 3.6 Hz, 1H), 3.45 – 3.31 (m, 1H), 3.16 – 3.03 (m, 2H), 2.98 – 2.82 (m, 2H), 2.62 – 2.55 (m, 2H), 2.47 (s, 3H), 1.65 – 1.43 (m, 2H), 0.96 (dd, J = 12.8, 6.5 Hz, 3H). [M+H] 496.0.

rac-3-((7-(5-chloro-3-methyl-2-(((2R,6R)-6-methylmorpholin-2-yl)methyl)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)-3-azabicyclo[3.1.0]hexane-2,4-dione (40). The title compound was synthesized following general procedure I with 2-(bromomethyl)-5-chloro-1-iodo-3-methylbenzene (**67**) and 4-(4-methoxybenzyl)-6-methylmorpholin-3-one, followed by general procedure B with 3-((7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridin-2-yl)methyl)-3-azabicyclo[3.1.0]hexane-2,4-dione, then general procedure D to give the desired product. ^{1}H NMR (400 MHz, Methanol-d4) δ 8.73 (d, J = 5.1 Hz, 1H), 7.53 (d, J = 1.4 Hz, 1H), 7.51 – 7.38 (m, 2H), 7.20 (d, J = 25.8 Hz, 1H), 4.81 (d, J = 3.0 Hz, 2H), 3.72 – 3.41 (m, 2H), 3.19 – 2.85 (m, 3H), 2.66 – 2.51 (m, 5H), 2.47 (d, J = 2.8 Hz, 3H), 1.61 (td, J = 8.1, 4.6 Hz, 1H), 1.43 (p, J = 4.8 Hz, 1H), 1.14 – 0.79 (m, 3H). [M+H] 496.0.

2-(2-bromo-4-chloro-6-methylbenzyl)oxirane (76). Step 1: 2-bromo-4-chloro-6-methylaniline (60 g, 272 mmol) was added over 30 min to a cooled, stirring solution of tBuONO (48.5 mL, 408 mmol) and allyl bromide (353 mL, 4081 mmol) in acetonitrile (272 mL) with maintaining internal temperature < 5 °C. The mixture was then stirred at room temperature for 20 hours. Upon completion, the mixture was concentrated then partitioned between H_2O/EtOAc. The layers were separated and the aqueous extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, then concentrated. The crude was purified by silica gel plug (100% hexanes) to yield the allyl arene (49 g, 73%). Step 2: This material (49 g, 201 mmol) was dissolved in DCM (400 mL) then cooled to 0 °C. mCPBA (69.4 g, 301.8 mmol) was added to the stirring mixture in portions over 35 mins with maintaining internal temperature < 5 °C. The mixture was then stirred at room temperature for 20 hours. Upon completion, the mixture was cooled to 0 °C and quenched with sat. $Na_2S_2O_3$ with internal temperature < 20 °C. The excess m-chlorobenzoic acid was filtered off. The layers were separated and the aqueous was extracted with DCM (2 x 100 mL). The combined organic layers were washed with sat. $NaHCO_3$, dried over Na_2SO_4, then concentrated. The crude was purified by column chromatography to yield the title compound (33.1 g, 63%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.43 (d, *J* = 2.2 Hz, 1H),

7.12 (d, *J* = 2.2 Hz, 1H), 3.23 – 3.13 (m, 2H), 3.09 (dd, *J* = 15.4, 6.0 Hz, 1H), 2.75 (dd, *J* = 5.0, 3.7 Hz, 1H), 2.50 (dd, *J* = 4.9, 2.4 Hz, 1H), 2.38 (s, 3H).

Large-scale synthesis of tert-butyl (R)-2-(2-bromo-4-chloro-6-methylbenzyl)morpholine-4-carboxylate (79). Step 1: Ethanol amine (30 mL) was added to 2-(2-bromo-4-chloro-6-methylbenzyl)oxirane (**76**, 13g, 49.7 mmol) in THF (30 mL) at room temperature. The reaction mixture was stirred at 60 °C for 16 h, then poured into water (1 L) and stirred for 1 h. The resultant white solid was filtrated and rinsed with water (400 mL) to give the crude diol (**77**). Solid can be wet for next step. Step 2: In a 1L flask, diol **77** was suspended in THF (200 mL) and di-*tert*-butyl dicarbonate (16.3 g, 74 mmol) was added portion wise. This was stirred at room temperature for 1 h. Imidazole (3.4 g, 49.7 mmol) was added and the solvent was partially removed on the rotavap for 15 min at 40 °C. The crude mixture was diluted in 50% ethyl acetate/hexanes (400 mL) and rinsed 2 times with 1M HCl (300 mL) and once with brine (100 mL). The organics were dried over sodium sulfate, filtered, and concentrated to afford a white solid (~19 g, 90%). Step 3: In a 1L flask, this solid (19 g, 44.8 mmol) was suspended in MTBE (250 mL). This was heated at 50 °C for 20 min to obtain a light cloudy solution. At 0 °C, triphenylphosphine (14.1 g, 53.8 mmol) was added in one portion. When the triphenylphosphine was all dissolved, diisopropyl azodicarboxylate (10.6 mL, 53.8 mmol) was added dropwise over 15 min at 0 °C. The reaction was stirred overnight at room temperature and a solid precipitated over time. The solution was concentrated and a thick gum was obtained. This was triturated with hexanes (500 mL) to obtain a white solid. The solid was collected by filtration and dried to obtain a white/light yellow solid (19g, 105%) – about 80% pure by NMR as the Boc-morpholine. Step 4: The above Boc-morpholine (~19g, 46 mmol) was dissolved in DCM (200 mL). 4N HCl in dioxane (30 mL) was added and the reaction was stirred at room temperature for 16 h. White solid slowly formed over time. Diethyl ether (200 mL) was added and the reaction was stirred for 15 min. The solid was filtered and rinsed with ether (50 mL) to give the racemic morpholine as the HCl salt (**78**, 9.1 g, 29.3 mmol, 59% from epoxide **76**). Step 5: The morpholine HCl salt was dissolved in DCM and rinsed with 1M NaOH. The organic phase was collected, dried with Na_2SO_4, and concentrated to afford the morpholine free

base as a colorless gum. Step 6: The morpholine free base was dissolved in DCE (100 mg morpholine per 1 mL DCE). *N*-Ac-D-Leu (unnatural enantiomer, 0.5 equivalents rel. to free base) was added and the DCE solution stirred and warmed until the solution became homogeneous. Stirring was stopped and the solution was removed from heat. Heptane (equal volume to DCE) was then added and the solution was swirled until homogeneous. The solution was allowed to stand, cooling to room temperature. After 1-2 h, large amounts of crystalline white solid appeared. The mixture was allowed to stand overnight. The solid was then collected by filtration (this solid is typically 80% ee in the first batch) and the filtrate (containing mostly the undesired enantiomer) was set aside. The solid was returned to the original flask and dissolved in hot DCE (85-90 °C, 100 mg morpholine per 1 mL DCE). Once homogeneous, the solution was removed from the heat and heptane (equal volume to DCE) was then added to the warm mixture and the solution was swirled until homogeneous. The solution was allowed to stand, cooling to room temperature. Once most of the solid had emerged from solution, the solid was collected by filtration (typically 95% ee in the second crystallization) and the filtrate was added to the previous filtrate and set aside. The recrystallization procedure was repeated a third time to afford solid of >99% ee. Step 7: To the above enantioenriched morpholine (5.55g, 11.62 mmol) in DCM (50 mL) was added triethylamine (4.84mL, 34.85 mmol) and di-tert-butyl dicarbonate (3.8g, 17.42 mmol). This was stirred at room temperature for 1 hour, then imidazole (1.19 g, 17.4 mmol) was added. After stirring for an additional 30 min, the mixture was diluted with 1M aq. HCl and stirred vigorously for 1 min. The organic layer was washed with water, then 1M NaOH, then the aqueous layer was back extracted 2x with DCM. The combined organics were dried, filtered, and concentrated to give the final product, enantioenriched morpholine **79** (31% over 3 steps). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.42 (d, *J* = 2.2 Hz, 1H), 7.10 (d, *J* = 2.4 Hz, 1H), 3.94 (bs, 1H), 3.80 (dd, *J* = 11.7, 3.4 Hz, 2H), 3.62 (bs, 1H), 3.40 (td, *J* = 11.8, 2.8 Hz, 1H), 2.98 – 2.83 (m, 3H), 2.72 (bs, 1H), 2.38 (s, 3H), 1.45 (s, 9H). [M+Na] 426.0.

3-((7-(5-chloro-3-methyl-2-(((R)-morpholin-2-yl)methyl)phenyl)thieno[3,2-b]pyridin-2-yl)methyl)-6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione (41). The title compound

was synthesized following general procedure B with enantioenriched morpholine **79** and 2-(((tert-butyldimethylsilyl)oxy)methyl)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thieno[3,2-b]pyridine (**48**), followed by general procedure G with 6,6-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione, then general procedure C to give the desired product. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.88 (t, J = 4.8, 3.8 Hz, 1H), 7.81 – 7.65 (m, 2H), 7.49 (d, J = 2.3 Hz, 1H), 7.22 (dd, J = 11.2, 2.3 Hz, 1H), 4.93 (d, J = 5.0 Hz, 2H), 3.89 (dd, J = 13.0, 3.7 Hz, 1H), 3.67 – 3.39 (m, 2H), 3.20 – 3.02 (m, 2H), 3.02 – 2.89 (m, 1H), 2.88 – 2.54 (m, 3H), 2.52 (d, J = 3.6 Hz, 2H), 2.48 (s, 3H), 1.25 (d, J = 2.0 Hz, 3H), 1.13 (d, J = 8.5 Hz, 3H). [M+H] 510.0.

X-ray Co-crystallography. Amino acids 208-560 of human USP7 were expressed in E.coli and purified using a GST-Tag. The GST-Tag was cleaved off via PreScission protease after affinity purification and the GST-tag was removed by an additional affinity purification step on GSTrap. Finally, a size exclusion chromatography step was performed using a S-200 26/60 column in 20mM Tris 8.0, 100mM NaCl, 10% Glycerol, 5mM DTT buffer and the sample was concentrated to 27 mg/ml. Crystallization was performed at 4 °C by vapor diffusion method at a protein concentration of 17 mg/ml and a ligand concentration of 10 mM (10% DMSO). Incubation time for the soak was 2 days. The crystallization reservoir condition contained 22.00 %w/v PEG 3350 and 0.50 M NH4 Acetate. Crystals were cryo-protected by 25% Glycerol in reservoir solution. Diffraction data were collected at the Swiss Light Source (SLS).

Computational Details. The co-crystal structure of compound **7** in complex with USP7 was prepared for modeling at pH 7.3 using the Protein Preparation Wizard in Maestro 11.2 (Schrödinger, LLC, New York, NY, 2017). This method first adds hydrogens, assigns bond orders to the ligand and then optimizes the hydrogen-bonding network of the receptor. An all-atom minimization is then performed, with a 0.3Å heavy-atom RMSD cutoff applied using the OPLS3 forcefield.[29] WaterMap, described in detail elsewhere,[30,31] uses a molecular dynamics approach to predict the thermodynamic properties of water molecules in a protein environment. This includes each water site's entropy, enthalpy, and free energy. WaterMap calculations were run with default

settings (2ns simulation time with waters analyzed with 10 Å of the ligand), using the prepared co-crystal structure of compound **7** in complex with USP7 (PDB code 6VN5), both with and without the ligand present. FEP+ calculations were carried out using the 2017-2 release of the Schrödinger Suite with the OPLS3 forcefield. Default lambda schedules and REST[32] regions were used in FEP+ while simulation time ranged from 10ns to 15ns, to allow for convergence. Ligands were prepared using LigPrep[33] and docked using Glide[34] with default settings. In cases where ligand alignment was insufficient, flexible ligand alignment in Maestro was used starting from the native ligand conformation as reference.

USP7 Biochemical Assay: A 25 µl reaction volume containing recombinant full-length USP7 (62 pM) in 20 mM HEPES pH 7.3, 150 mM NaCl, 1 mM TCEP, and 125 µg/ml BSA was assembled in wells of 384 well plates. Compounds were dispensed with a Hewlett Packard D300 digital dispenser (1% final DMSO). Following a 30-minute incubation at room temperature, ubiquitin-rhodamine (BostonBiochem) was added with the D300 to a final concentration of 100 nM and the reaction was allowed to proceed for 1 hour at room temperature protected from light. The reaction was stopped by the addition of 5 µl 1M acetic acid. Rhodamine fluorescence was measured using an Envision plate reader (Perkin Elmer) and IC_{50} values were determined by non-linear regression using a 4-parameter fit in the Dotmatics software package. Values presented are the average of n = 2 or more determinations, where the value of each determination is within a 3-fold difference of each other.

DUB Selectivity Profiling: Deubiquitinase selectivity profiling was conducted by Ubiquigent (Dundee, UK) using a similar assay protocol with the following changes: assay buffer was 40 mM Tris/HCl pH 7.4, 5% glycerol, 0.005%Tween-20, 1 mM DTT, 0.05 mg/ml ovalbumin, and the reaction was stopped by the addition of 5 µl 100 mM N-Ethylmaleimide.

p53-Luciferase Reporter Gene Assay: RKO cells stably transfected with a p53 luciferase reporter vector (Signosis) were seeded at 2500 cells per well in 25µl of recommended media in 384-well black-walled tissue plates (Greiner). Compounds were added with a D300 digital dispenser (0.5% final DMSO). Following an 18-hour

incubation, p53-dependent luciferase levels were measured via Bright-Glo Luciferase (Promega), following the manufacturer's instructions, using a CLARIOstar plate reader (BMG LABTECH). IC_{50} values were determined by non-linear regression using a 4-parameter fit in the Dotmatics software package.

Cell Culture, Cell Treatments, and Viability Assays (MM.1S, H526, RKO). All cell lines were purchased from American Type Culture Collection. For all cell lines, identity was confirmed by short tandem repeat profiling and culturing was performed in recommended growth medium. For studies evaluating cellular effects of compound treatment, 250 to 2,000 cells in 40 µl of recommended medium were seeded per well in 384-well plates (Corning 3764). DMSO-solubilized compounds were added in duplicate in a two-fold dilution series using a D300e Digital Dispenser (Hewlett-Packard). Following a 5-day incubation, cell viability was measured using CellTiter-Glo (Promega) following the manufacturer's instructions. Luminescence was measured using a CLARIOstar plate reader (BMG LABTECH) and normalized to that of DMSO-treated cells. IC_{50} values were determined by non-linear regression using a 4-parameter fit in Prism (GraphPad Software).

Animal experiments: MM.1S and H526 xenograft studies were conducted at Crown Bio (Beijing, China) and RAPT Therapeutics, respectively, according to the guidelines approved by the respective Institutional Animal Care and Use Committees (IACUC). MM.1S cells were inoculated in PBS into irradiated NOD/SCID mice, while H526 cells were inoculated in PBS/Matrigel (Corning) into Nu/Nu mice (Jackson Laboratories). At the start of the study, NOD/SCID mice were 9-10 weeks old and 17.2-22.3 g in weight, while Nu/Nu mice were 7 weeks old and 18.1-25.4 g in weight. Mice were randomized into groups when mean tumor size reached 150 mm^3 (MM.1S) or 50-100 mm^3 (H526), respectively, with 10 mice per group. Drug administration by oral gavage was started on day of randomization. Tumor volumes and body weights were subsequently measured twice per week. Tumor volumes were calculated using the formula: $V = 0.5(A \times B^2)$, where A and B are the long and short diameters of the tumor, respectively. If the animal's body weight dropped to less than 90% of the original weight, a dosing holiday

was given until their body weight surpassed the 90% mark. All animals were included in the tumor growth plots, regardless of whether they received a dosing holiday.

ANCILLARY INFORMATION

Supporting Information.

The following files are available free of charge: molecular formula strings; biochemical and cellular potency correlation; target engagement for compound **30**; DUB selectivity profile for compound **30**; view of USP7 showing solvent-exposed Tyr-465; PK curves for compound **41**.

Accession Codes

The coordinates and structure factors for compounds **1**, **7**, **14**, **18**, and **23** in complex with USP7 have been deposited (PDB codes 6VN4, 6VN5, 6VN6, 6VN2, and 6VN3, respectively). Authors will release the atomic coordinates upon article publication.

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Notes

The authors declare the following competing financial interests: The majority of authors of this manuscript are or were employees of RAPT Therapeutics, Inc. (formerly known as FLX Bio, Inc.).

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Abbreviations

USP7, ubiquitin-specific protease 7; DUB, deubiquitinase; MDM2, mouse double minute 2 homolog; p53, tumor protein p53; FOXP3, forkhead box P3; USP1, ubiquitin-specific

protease 1; UCH-L3, ubiquitin carboxy-terminal hydrolase isozyme L3; USP47, ubiquitin-specific protease 47; LipE, lipophilic efficiency; IV, intravenous; PO, per os (administered orally); AUC, area under the curve; SAR, structure-activity relationship; PK, pharmacokinetics; hERG, human ether-a-go-go-related gene potassium ion channel; CYP, cytochrome P450; FEP+, free energy perturbation; NOD-SCID, non-obese diabetic-severe combined immunodeficient, DMF, dimethylformamide, *m*-CPBA, meta-chloroperoxybenzoic acid; TEMPO, 2,2,6,6-Tetramethylpiperidin-1-yl)oxyl; T3P, propylphosphonic acid anhydride; TBS, tert-butyldimethylsilyl; PMB, para-methoxybenzyl; TLC, thin layer chromatography.

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[23] See Supporting Figure 4 for a visualization of solvent-exposed Tyr-465.

[24] See Supporting Figure 5 for PK curves of compound **41** in different mouse species.

[25] Compound **41** is measured to be 95.3% bound to mouse plasma proteins as measured by ultracentrifugation.

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Table of Contents graphic: