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Discovery of Novel, Induced-Pocket Binding Oxazolidinones as Potent, Selective, and Orally Bioavailable Tankyrase Inhibitors

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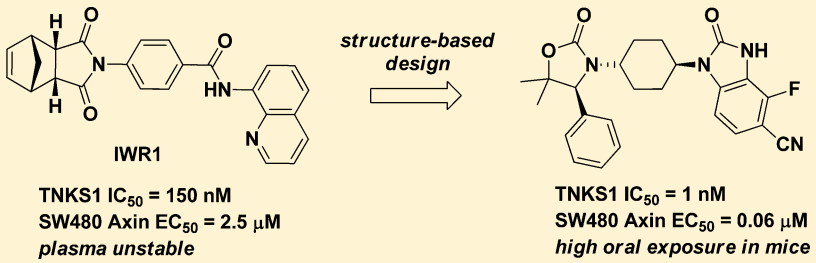
Supporting Information



ABSTRACT: Tankyrase (TNKS) is a poly-ADP-ribosylating protein (PARP) whose activity suppresses cellular axin protein levels and elevates β-catenin concentrations, resulting in increased oncogene expression. The inhibition of tankyrase (TNKS1 and 2) may reduce the levels of β-catenin-mediated transcription and inhibit tumorigenesis. Compound **1** is a previously described moderately potent tankyrase inhibitor that suffers from poor pharmacokinetic properties. Herein, we describe the utilization of structure-based design and molecular modeling toward novel, potent, and selective tankyrase inhibitors with improved pharmacokinetic properties (**39**, **40**).

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the third leading cause of cancer death in both men and women in the United States and the fourth leading cause of cancer death worldwide.[1] The dysregulation of the wnt/β-catenin signaling pathway is believed to be the initiating event for the vast majority of CRCs where truncating mutations of the Adenomatous polyposis coli (APC) gene are the most common alteration, occurring in approximately 80% of cases, while loss-of-function AXIN2 (axis inhibition protein 2) mutations and activating mutations in CTNNB1 (the gene which encodes β-catenin) account for the aberrant Wnt pathway activity in most of the remaining 20%.[2−5] Many wnt/β-catenin pathway alterations lead to sustained β-catenin stabilization in the absence of exogenous wnt signals, thereby blocking the normal differentiation of colonic epithelial stem cells and promoting uncontrolled proliferation and tumor initiation.[6] The concentration of β-catenin is regulated by a cytoplasmic multicomponent complex known as the β-catenin destruction complex. The tumor suppressor proteins axin and APC bind β-catenin and enable phosphorylation events catalyzed by glycogen synthase kinase 3β (GSK3β) and casein kinase 1α (CK1α). Phosphorylated β-catenin is subsequently degraded by the proteosome. Truncated APC is unable to effectively facilitate the phosphorylation of β-catenin, resulting in its nuclear translocation and the overexpression of β-catenin/TCF-regulated genes. Mutations in axin similarly lead to a nonfunctional β-catenin destruction complex while activating mutations in β-catenin prevent its phosphorylation and subsequent degradation.

TNKS1 and 2 (PARP-5a, PARP-5b) are members of the poly-ADP-ribose polymerase (PARP) family of enzymes which catalyze the transfer of NAD^+ units to their substrate proteins.[7−10] The resulting poly-ADP-ribose tagged (PARsylated) proteins are altered in size, charge, and function. Tankyrase was first described as a positive regulator of telomere length, acting by PARsylating telomere repeat binding factor 1 (TRF1), which subsequently dissociates from telomeric DNA to allow access to telomerase.[11] More recently, TNKS was identified as a positive regulator of the wnt signaling pathway via its interaction with axin protein. Tankyrase catalyzes the PARsylation of axin, ultimately resulting in axin protein degradation and thus elevated levels of β-catenin and increased expression of β-catenin/TCF-regulated oncogenes. The inhibition of TNKS produces elevated axin protein levels and

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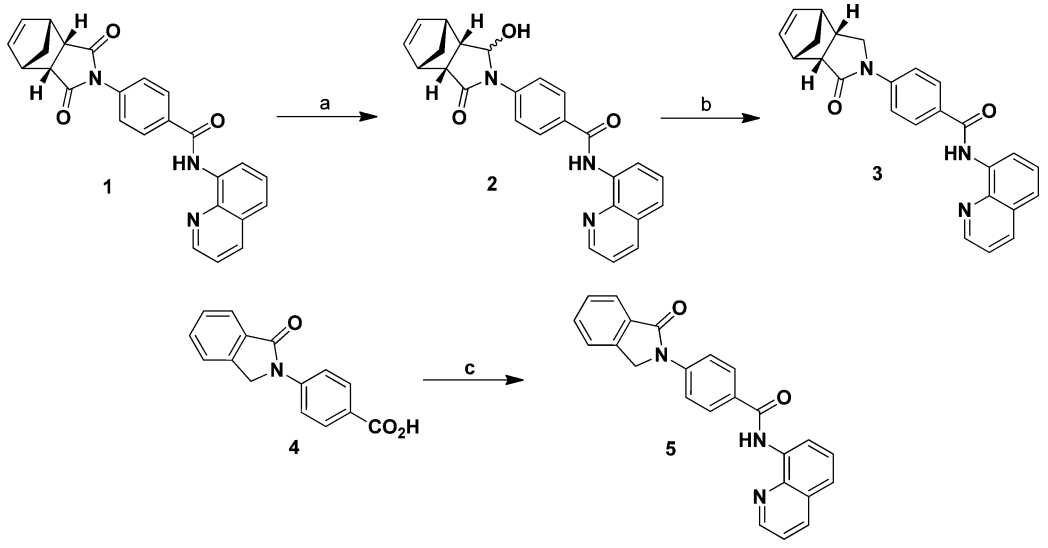
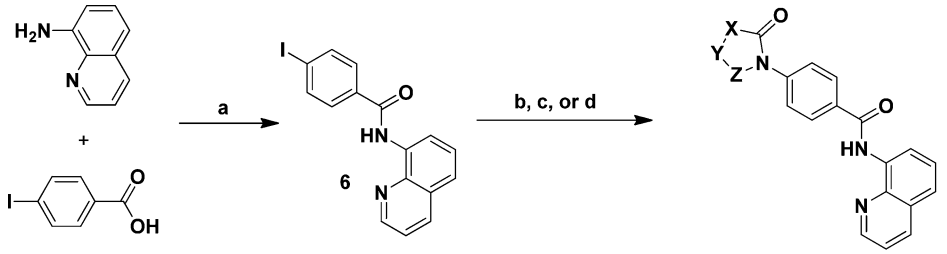
Scheme 1. Synthesis of Lactam Molecules 3 and 5[a]



[a]Reagents and conditions: (a) MP-BH_4, DCM/MeOH, 60 °C, 37%; (b) PhSeH, TFA, DCM, reflux, 7%; (c) quinolin-8-amine, HATU, DIEA, DMF, 24%.

Scheme 2. Synthesis of Modified Lactam Analogues 7−18[a]



e

7: X = N-Ph; Y, Z = CH_2; 9%
8: X, Z = CH_2; Y = CH-Ph (rac); 59%
9: X, Y = CH_2; Z = CH-Ph (rac); 77%
10: X, Y = CH_2; Z =CH-Ph (*R*)
11: X, Y = CH_2; Z = CH-Ph (*S*)
12: X, Y = CH_2; Z = $C(Me)_2$; 3%
13: X, Y = CH_2; Z = CHMe (rac); 43%
14: X, Y = CH_2; Z = CH-*o*-F Phenyl (rac); 53%
15: X, Y = CH_2; Z = CH-*m*-F Phenyl (rac); 61%
16: X, Y = CH_2; Z = CH-*p*-F Phenyl (rac); 42%
17: X = O; Y = CH_2; Z = CH-Ph (*S*); 20%
18: X = O; Y = $C(Me)_2$; Z = CH-Ph (*S*); 35%

[a]Reagents and conditions: (a) HATU, DIEA, DMF, 64%; (b) substituted lactam or oxazolidinone, CuI, DMEDA, K_3PO_4, 1,4-dioxane, microwave, 140 °C (conditions used for preparations of **7**, **12**, **13**, **17**, **18**, yields in scheme); (c) substituted lactam or oxazolidinone, CuI, DMEDA, Cs_2CO_3, 1,4-dioxane, microwave, 140 °C (conditions used for preparations of **8**−**11**, **15**, **16**, yields in scheme); (d) substituted lactam or oxazolidinone, CuI, (1*R*,2*R*)-N^1,N^2-dimethylcyclohexane-1,2-diamine, Cs_2CO_3, toluene, 100 °C (conditions used for preparation of **14**, yield in scheme); (e) chiral separation.

reduced levels of cellular β-catenin, ultimately inhibiting the growth of APC mutant cell colonies.[12,13] Therefore, it is hypothesized that the inhibition of TNKS may offer a novel approach toward the treatment of CRC. Herein we describe the identification of potent, selective, and orally bioavailable TNKS inhibitors utilizing structure-based design principles.

CHEMISTRY

The synthesis of lactam variants of a previously described inhibitor IWR1 (**1**) are described in Scheme 1.[14,15] The synthesis of racemic lactam (**3**) was accomplished by a stepwise reduction protocol wherein borohydride reduction with solid-supported borohydride (MP-BH_4) afforded alcohol **2** and subsequent reduction via trifluoroacetic acid (TFA) activation and reaction with phenyl selenol afforded lactam **3**. Lactam **5**, devoid of a bridged bicycle, was prepared under standard 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU)-mediated amide formation conditions with commercially available carboxylic acid **4** and quinolin-8-amine.

The modified and substituted lactam analogues **7**−**18** were synthesized by a general protocol (Scheme 2). The common aryl iodide intermediate (**6**) was prepared by standard HATU coupling conditions with 4-iodobenzoic acid and quinolin-8-amine. Copper-mediated carbon−nitrogen bond formation

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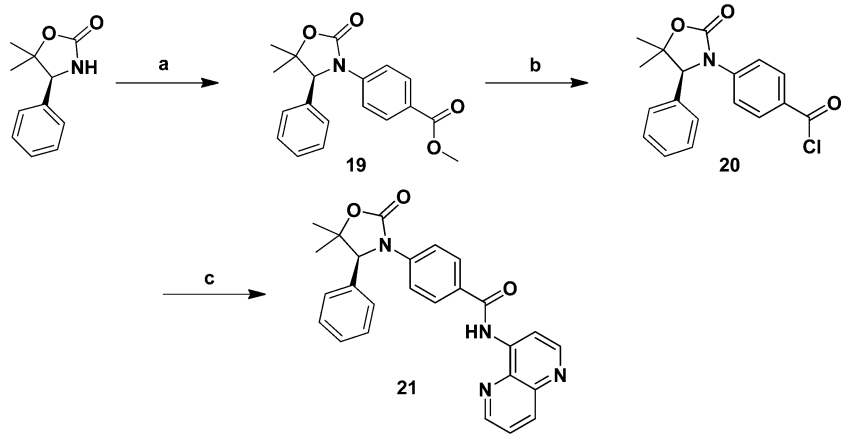
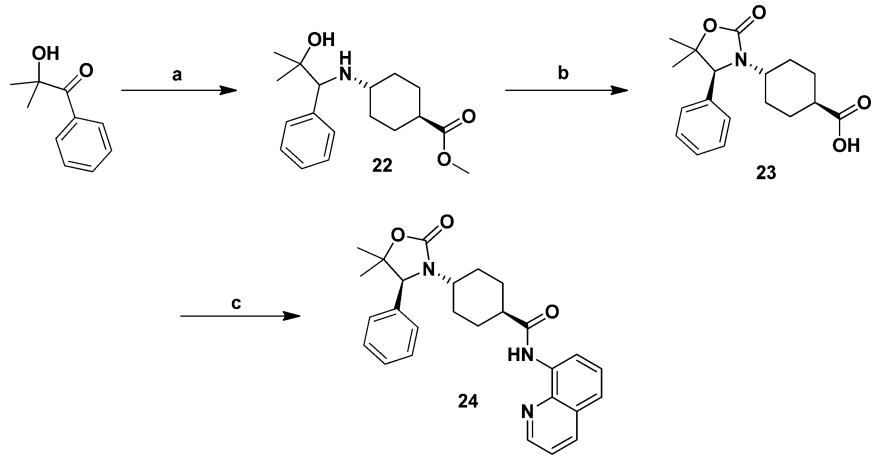
Scheme 3. Synthesis of Naphthyridine Amide 21[a]



[a]Reagents and conditions: (a) methyl 4-iodobenzoate, CuI, DMEDA, Cs_2CO_3, 1,4-dioxane, microwave, 140 °C, 93%; (b) (1) LiOH, THF/MeOH/H_2O, 50 °C, 48%; (2) $SOCl_2$, DCM; (c) 1,5-naphthyridin-4-amine, DIEA, DCM, 40 °C, 96%.

Scheme 4. Synthesis of *trans*-Cyclohexyl Amide 24[a]



[a]Reagents and conditions: (a) (1*R*,4*R*)-methyl 4-aminocyclohexanecarboxylate hydrochloride, $NaBH(OAc)_3$, DCE, 30%; (b) (1) triphosgene, DIEA, THF, 0 °C; (2) enantiomer separation; (3) LiOH, THF/MeOH/H_2O, 50 °C, 100%; (c) quinolin-8-amine, HATU, DIEA, DMF, 38%.

conditions were broadly utilized to deliver **7**−**18** in varying yields.

The synthesis of naphthyridine amide **21** is described in Scheme 3. Copper-mediated coupling of commercial (*S*)-5,5-dimethyl-4-phenyloxazolidin-2-one with methyl 4-iodobenzoate afforded ester **19** in good yield. Subsequent ester hydrolysis and treatment with thionyl chloride provided penultimate acid chloride **20**, which was transformed smoothly to naphthyridine amide **21** in high yield.

The synthesis of saturated amide **24** is described in Scheme 4. Reductive amination of commercial 2-hydroxy-2-methyl-1-phenylpropan-1-one with (1*R*,4*R*)-methyl 4-aminocyclohexanecarboxylate hydrochloride provided amino alcohol **22**. Triphosgene-mediated cyclization, separation of the enantiomers, and ester hydrolysis yielded oxazolidinone **23**. Chiral separations were accomplished using preparative SFC (supercritical fluid) chromatography with the stationary phase and percent cosolvent used for each separation selected through method development screening. Standard peptide coupling conditions furnished saturated amide analogue **24**.

Analogues in which the amide is "reversed" relative to **24** were prepared in a convergent manner, as described in Scheme 5. Reductive amination of (*S*)-methyl 2-amino-2-phenylacetate hydrochloride with *tert*-butyl (4-oxocyclohexyl)carbamate produced amino ester **25** in moderate yield as a mixture of geometric isomers. The addition of methyl Grignard yielded tertiary alcohol **26**, which was cyclized to oxazolidinone **27** with triphosgene. It was at this stage that the cis and trans cyclohexyl isomers (~1:1 cis/trans) were separated by silica gel chromatography. Removal of the *tert*-butoxycarbonyl (Boc) group under standard acidic conditions afforded penultimate amine **28**. Partial racemization of the oxazolidinone phenyl group occurred during this sequence. (Studies with a related analogue indicated that partial racemization occurred at the reductive amination step. Reversal of the reductive amination and Grignard reaction sequence prevented racemization entirely.) Standard peptide coupling conditions utilizing either HATU or propylphosphonic anhydride (T3P) furnished amides **29**−**31**.

The benzimidazolone containing analogues **34**−**41** were prepared by a robust, three-step protocol depicted in Scheme 6. Nucleophilic aromatic substitution reaction (S_NAr) of primary amine **28** with the appropriately substituted ortho-halo nitroarene substrate provided nitro amine **32**. The nitro

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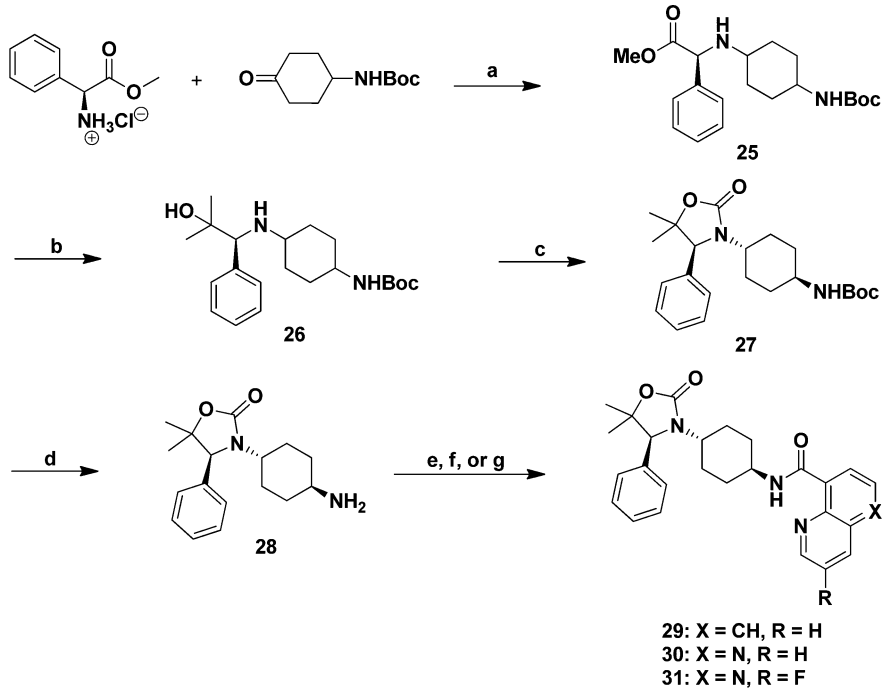
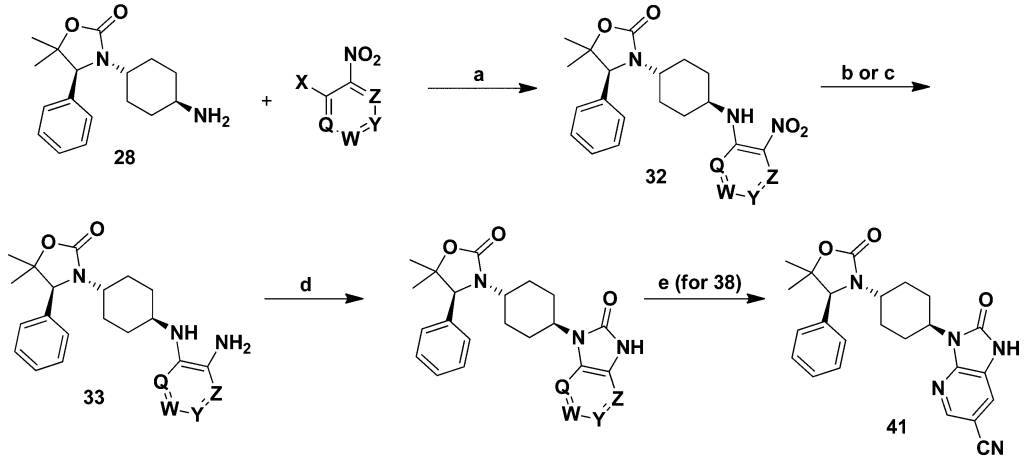
Scheme 5. Synthesis of Cyclohexyl "Reverse" Amides 29 − 31[a]



[a]Reagents and conditions: (a) $NaBH(OAc)_3$, DME, RT, 67%; (b) MeMgBr, THF, 0 °C to RT, 43%; (c) triphosgene, DIEA, THF, 0 °C to RT, 50%; (d) (1) TFA, DCM, RT, 100%; (2) enantiomer separation; (e) (for **29**) quinoline-8-carboxylic acid, HATU, DIEA, DCM, RT, 73%; (f) (for **30**) 1,5-naphthyridine-4-carboxylic acid, HATU, DIEA, DMF, RT, 17%; (g) (for **31**) 7-fluoro-1,5-naphthyridine-4-carboxylic acid,[16] T3P, Et_3N, DMF, 35%.

Scheme 6. General Synthetic Route toward Benzimidazolones 34−41[a]



[a]Reagents and conditions: (a) Et_3N, CH_3CN, 80 °C; (b) Raney Ni, $NH_2NH_2-H_2O$, EtOH, 40 °C (toward **34**, **35**, **38**); (c) $SnCl_2$, EtOH, 80 °C (toward **36**, **37**, **39**, **40**); (d) triphosgene, DIEA, THF, 0 °C to RT or RT (3-step yields in scheme); (e) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 120 °C, 94%.

group was reduced to aniline **33** in the presence of Raney nickel and hydrazine hydrate or stannous chloride in ethanol.[17] Cyclization to benzimidazoles **34**−**40** was effected with carbonyldiimidazole (CDI) and diisopropylethyl amine (DIEA) in tetrahydrofuran. Cyanation of bromo-substituted benzimidazolone **38** with zinc cyanide under palladium catalyzed conditions afforded **41** in high yield.

Analogues containing benzimidazolone moieties were alternately prepared by synthesizing the amino-cyclohexyl benzimidazolone portion prior to constructing the oxazolidinone, as outlined in Scheme 7. Amines **43** and **44** were prepared beginning with an S_NAr reaction of *tert*-butyl (1*R*,4*R*)-4-aminocyclohexyl)carbamate with the appropriately substituted ortho-fluoro nitroarene under basic conditions. Sub-

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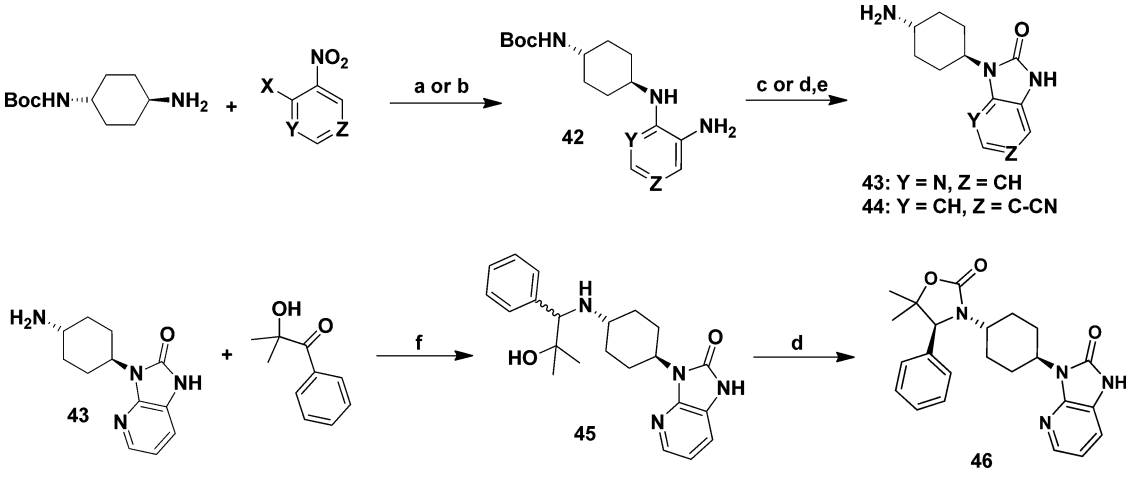
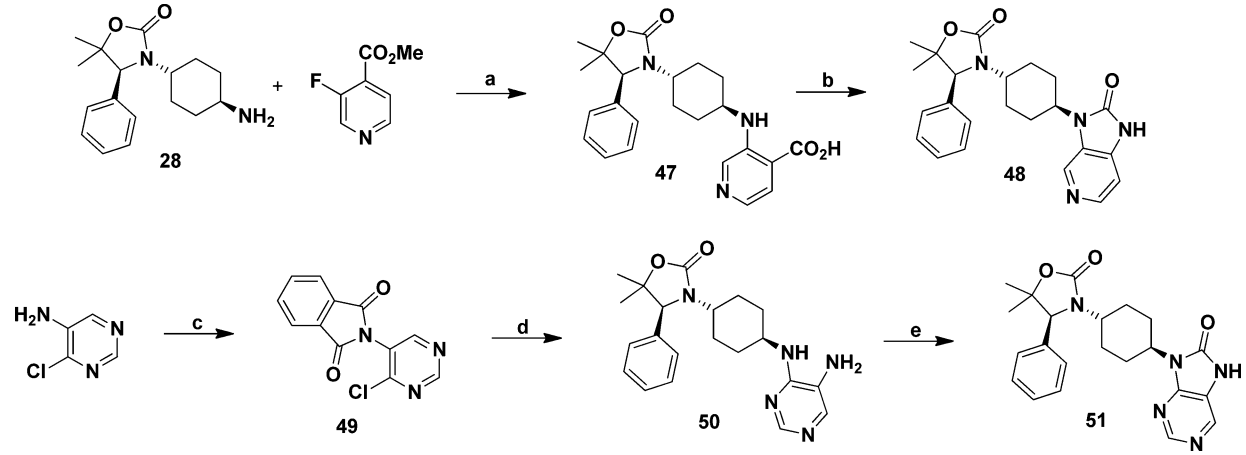
Scheme 7. Synthesis of aza-Benzimidazolone 46[a]



[a]Reagents and conditions: (a) (toward **43**) (1) 2-chloro-3-nitropyridine, Et_3N, CH_3CN, 80 °C, 60%; (2) $SnCl_2$, EtOH, 80 °C, 62%; (b) (toward **44**) (1) 4-chloro-3-nitrobenzonitrile, K_2CO_3, DMF, 60 °C, 90%; (2) Zn powder, NH_4Cl, THF/MeOH/H_2O, RT, 89%; (c) (toward **43**) (1) CDI, DIEA, THF, 0 °C, 67%; (2) TFA, DCM, RT, 82%; (d) triphosgene, DIEA, THF, RT, 22%; (e) HCl in 1,4-dioxane, 10% MeOH in $CHCl_3$, 0 °C, 100%; (f) $NaBH(OAc)_3$, CH_3COOH, DMF, 60 °C, 47%; (g) (1) triphosgene, DIEA, THF, RT, 15%; (2) enantiomer separation.

Scheme 8. Synthesis of aza-Benzimidazolones 48 and 51[a]



[a]Reagents and conditions: (a) (1) methyl 3-fluoroisonicotinate, Et_3N, CH_3CN, 80 °C, 69%; (2) LiOH, THF/MeOH/H_2O, 50 °C, 75%; (b) DPPA, Et_3N, THF, 80 °C, 12%; (c) phthalic anhydride, Et_3N, THF/toluene (1:1), 100 °C, 77%; (d) (1) **28**, Et_3N, CH_3CN, 80 °C, 68%, (2) NH_2NH_2/H_2O, EtOH, 100 °C, 73%; (e) CDI, DIEA, THF, RT, 20%.

sequent nitro reduction afforded diamine **42**, which was cyclized to the benzimidazolone using CDI. Cleavage of the Boc protecting group afforded amines **43** and **44** in good overall yields. Reductive amination of amine **43** with 2-hydroxy-2-methyl-1-phenylpropan-1-one provided racemate **45** in modest yield. Cyclization with triphosgene and chiral separation provided oxazolidinone **46**.[18]

The syntheses of aza-benzimidazolone analogues **48** and **51** are described in Scheme 8. S_NAr of amine **28** with methyl 3-fluoroisonicotinate and subsequent lithium hydroxide-mediated ester hydrolysis provided amino acid **47**. Exposure of **47** to diphenylphosphoryl azide (DPPA) in the presence of triethylamine effected a Curtius rearrangement, with the resulting isocyanate undergoing intramolecular cyclization with the proximal amine to produce benzimidazolone **48**. Protection of 4-chloropyrimidin-5-amine as phthalimide **49** followed by S_NAr with amine **28** and phthalimide cleavage afforded diamine **50**. Cyclization with CDI provided purinone **51**.

RESULTS AND DISCUSSION

To validate the hypothesis that tankyrase inhibition is a suitable approach for the treatment of CRC, potent and selective pharmacological tools must be identified for proof of concept studies in tumor-bearing mice. Toward this end, we focused on driving cellular potency in an APC mutant CRC cell line while optimizing rodent pharmacokinetics—first in vitro with routine monitoring of stability in liver microsomes and plasma, and later in vivo toward achieving suitable unbound exposure levels following oral dosing in mice.

Known TNKS inhibitor IWR1 (**1**) was the starting point for our structure activity relationship (SAR) evaluations.[14,15] In our hands, benchmark **1** was found to inhibit TNKS1,2 at submicromolar levels while demonstrating high levels of selectivity over PARP 1,2 (>100-fold). In cellular assays, treatment with **1** effected the accumulation of axin modestly (EC_{50} = 2.5 μM) and more strongly inhibited β-catenin (IC_{50} = 0.25 μM) (Table 1). Inhibition of β-catenin-dependent gene

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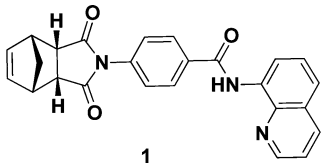
Table 1. Profile of 1



1

assay	potency (μM)
TNKS1, TNKS2 IC_{50}	0.15, 0.039
PARP1, PARP2 IC_{50}	>85, >170
SW480 Axin EC_{50}	2.5
SW480 β-catenin IC_{50}	0.25
HLM, RLM CLint (μL/min/mg)	41, 69
mouse plasma ($t_{1/2}$, min)	15

transcription was observed in non-APC mutant HEK-293-STF cells, but only weakly in APC mutant SW480 cells.

The cocrystal structure of **1** with TNKS1 was obtained, revealing a nontraditional PARP inhibitory binding motif.[19,20] The critical hydrogen bonding contacts identified were one hydrogen bond between TNKS residue Tyr1213 and one of the imide carbonyl groups as well as one hydrogen bonding contact between Asp1198 and the amide carbonyl. The bicyclic moiety engages in significant hydrophobic contacts, as suggested by the observed stereopreference (*exo* diastereomer TNKS1 IC_{50} = 1 μM vs 0.15 μM for *endo*). The quinoline moiety engages in hydrophobic stacking interactions with His1201 (see Figure 1).

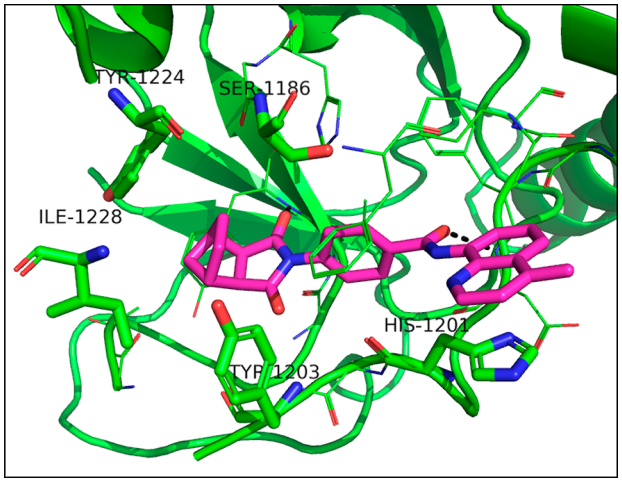
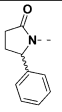
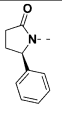
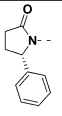


Figure 1. Representation of the cocrystal structure of **1** with TNKS1. Highlighted are the hydrogen bonding contacts between an imide carbonyl and the amide carbonyl of the ligand and protein as well as the hydrophobic interactions of the bicyclic moiety with the protein.

Compound **1** functioned as a useful in vitro tool compound; however, it suffered from poor pharmacokinetic properties along with moderate cellular potency and was therefore unsuitable for use as an in vivo tool. Consistent with previously reported plasma stability results, studies indicated that both the imide and amide moieties were labile sites ($t_{1/2}$ = 15 min, mouse plasma).[15,21]

With the information gleaned from the plasma stability study and the cocrystal structure, we proceeded with the goals of stabilizing the molecule and improving potency. Because only one imide carbonyl group engaged the protein and the imide functionality was deemed unstable, one imide carbonyl group

Table 2. IWR to Phenyl Lactam SAR[a]

Entry		TNKS1 - IC_{50} (μM)*	SW480 β-catenin IC_{50} (μM)*	SW480-Axin EC_{50} (μM)*	HLM , RLM CLint (μL/min/mg)
1		0.15	0.25	2.5	41 , 69
3		1.25	NA	NA	160 , 345
5		>20	NA	NA	124 , NA
7		>20	NA	NA	NA , >399
8		>20	NA	NA	224 , 297
9		1.6	NA	>25	147 , >399
10		0.54	>10	>25	114 , >399
11		>20	NA	NA	260 , >399
12		1.3	7.3	>25	<14 , 31
13		>20	NA	NA	16 , 163

[a]NA = not measured. *See the Supporting Information for individual data variance and *n* values.

was replaced with a methylene (**3**, racemic mixture) which reduced potency (Table 2). Further modification of the bicyclic moiety to an aromatic group (**5**) abrogated activity. Investigation of 5-membered succinimide replacements containing one H-bond acceptor and a hydrophobic substituent were explored using focused libraries. While replacing the fused aromatic ring with a pendant phenyl group α (**7**) or β to the carbonyl (**8**) did not provide measurable potency, attachment at the γ position (racemate **9**) afforded weak TNKS inhibition (1.6 μM). This represented the first nonbicyclic analogue prepared which displayed inhibitory activity. Resolution and analysis of the separated enantiomers revealed a strong stereopreference for a single enantiomer (**10**: IC_{50} = 0.54 μM; **11**: IC_{50} > 20 μM). The importance of the phenyl group was confirmed by reduced activity observed with small substituents *gem*-dimethyl (**12**) and methyl substitution (**13**).

Phenyl lactam **10** suffered from poor microsomal stability, particularly in rat liver microsomes (CL_{int} > 399 μL/min/mg). On the basis of the possibility that the phenyl ring may provide sites for oxidative metabolism, a fluoro-walk was performed

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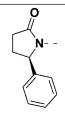
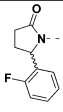
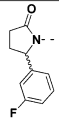
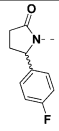
Table 3. Phenyl Lactam SAR

Entry		TNKS1 - IC_{50} (μM)*	SW480 β-catenin IC_{50} (μM)*	SW480-Axin EC_{50} (μM)*	HLM , RLM CLint (μL/min/mg)
10		0.54	>10	>25	114 , >399
14		4.3	NA	>25	104 , >399
15		1.5	NA	>25	137 , >399
16		0.4	1.1	2.0	79 , >399

*See the Supporting Information for individual data variance and *n* values.

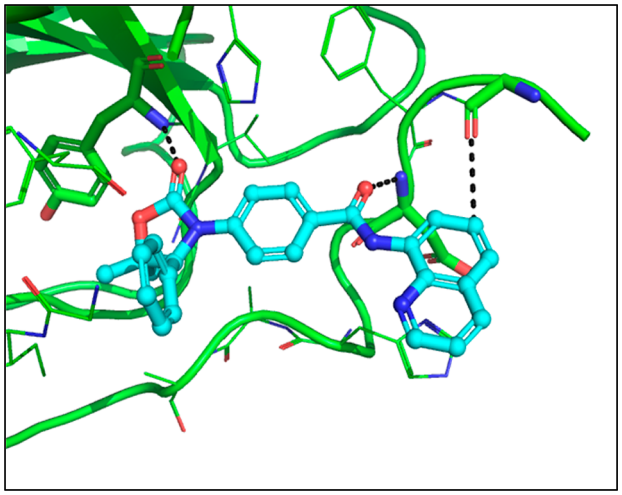


Figure 2. Representation of the cocrystal structure of **18** with TNKS1 highlighting hydrogen bonding contacts between the ligand and protein.

(**14**–**16**) which presented the para position (**16**) as optimal for potency. Whereas **16** was not stable in microsomes, markedly improved mouse plasma stability was observed ($t_{1/2}$ **16** = 262 min vs **1** = 15 min). Subsequent efforts were directed toward attenuating potential oxidation by systematically replacing or substituting the methylene units of the lactam moiety (Table 3). Replacement of the α methylene with an oxygen (**17**) did not yield improvements in intrinsic stability or potency; however, further substituting the β methylene with geminal dimethyl groups (**18**) improved intrinsic stability (HLM, RLM CLint = 41, 83 μL/min/mg), resulting in modest clearance in rat (1.3 L/hr/kg) (see Table 4).

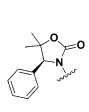
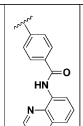
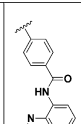
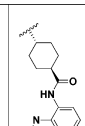
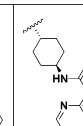
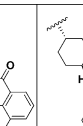
Although **18** represented an improvement in PK relative to lead **10**, the cellular potency was weak (SW480 TBC = 2.1 μM). Additionally, **18** was very selective over PARPs 1, 2, 3,

Table 4. Phenyl Lactam to *gem*-Dimethyl Phenyl Oxazolidinone[a]

Entry		TNKS1 - IC_{50} (μM)*	SW480 β-catenin IC_{50} (μM)*	SW480-Axin EC_{50} (μM)*	HLM , RLM CLint (μL/min/mg)
10		0.54	1.57	>25	114 , >399
17		1.22	4	>25	104 , >399
18		0.20	2.1	10.5	41 , 83

[a]NA = not measured. *See the Supporting Information for individual data variance and *n* values.

Table 5. Modification of the Biaryl Amide Motif

						
Entry	**18**	**21**	**24**	**29**	**30**	**31**
TNKS1 - IC_{50} (μM)*	0.20	0.018	0.016	0.01	0.004	0.004
SW480 β-catenin IC_{50} (μM)*	2.1	0.189	0.39	0.20	0.073	0.271
Axin EC_{50} (μM)*	10.5	1.01	0.64	0.56	0.23	0.19
HLM , RLM CLint (μL/min/mg)	41 , 83	55 , 61	25 , 112	<14 , 52	36 , 67	51 , 87
Mouse Plasma Stability (t ½, min)	312	238	>500	NA	NA	NA
Solubility (μg/ml) (0.01N HCl , SIF (pH 6.8) , PBS (pH 7.4))	5 , 10 , 1	171 , 9 , 1	94 , 24 , 5	37 , 58 , 19	NA	94 , 156 , 81
Papp (ave AB/BA) (μcm/s) / ER[a]	8.1 , 1.4	17.1 , 1	22.1 , 1.3	13.4 , 0.8	NA	NA
Plasma Protein Binding: rat , mouse (% bound)	99.9 , NA	NA	NA	97.3 , 97.7	91.6 , 90.8	NA , 91.8
Mouse PO PK (30 mg/kg)[b]: AUCt (μM*hr) , Cmax (μM)	NA	0.17 , 0.37	0.13 , 0.25	NA	0.258 , 0.22	2.24 , 1.3

[a]MDR1-LLC-PK1/LLC-PK1 or porcine renal epithelial cells expressing human Pgp. [b]CD-1 mice were dosed orally at 30 mpk in 10 mL/kg of vehicle (10% pluronic F68, 30% HPBCD, 60% water, pH 2.5 adjusted with methanesulfonic acid for **21**, **24**, **30**; pH adjusted to 10.0 with sodium hydroxide for **31**). NA = not measured. *See the Supporting Information for individual data variance and *n* values.

and 6 (>10 μM).[22] We obtained a cocrystal structure of **18** with TNKS which indicated an aryl C−H−carbonyl interaction between the C−H of the 6-position of the quinoline and the carbonyl group of Gly1196 (Figure 2). Toward enhancing that interaction by providing a more polarized C−H bond relative to quinoline **18**, naphthyridine **21** was prepared. Gratifyingly, this point mutation afforded an order of magnitude boost in enzyme and cellular potencies. Improvements in solubility in

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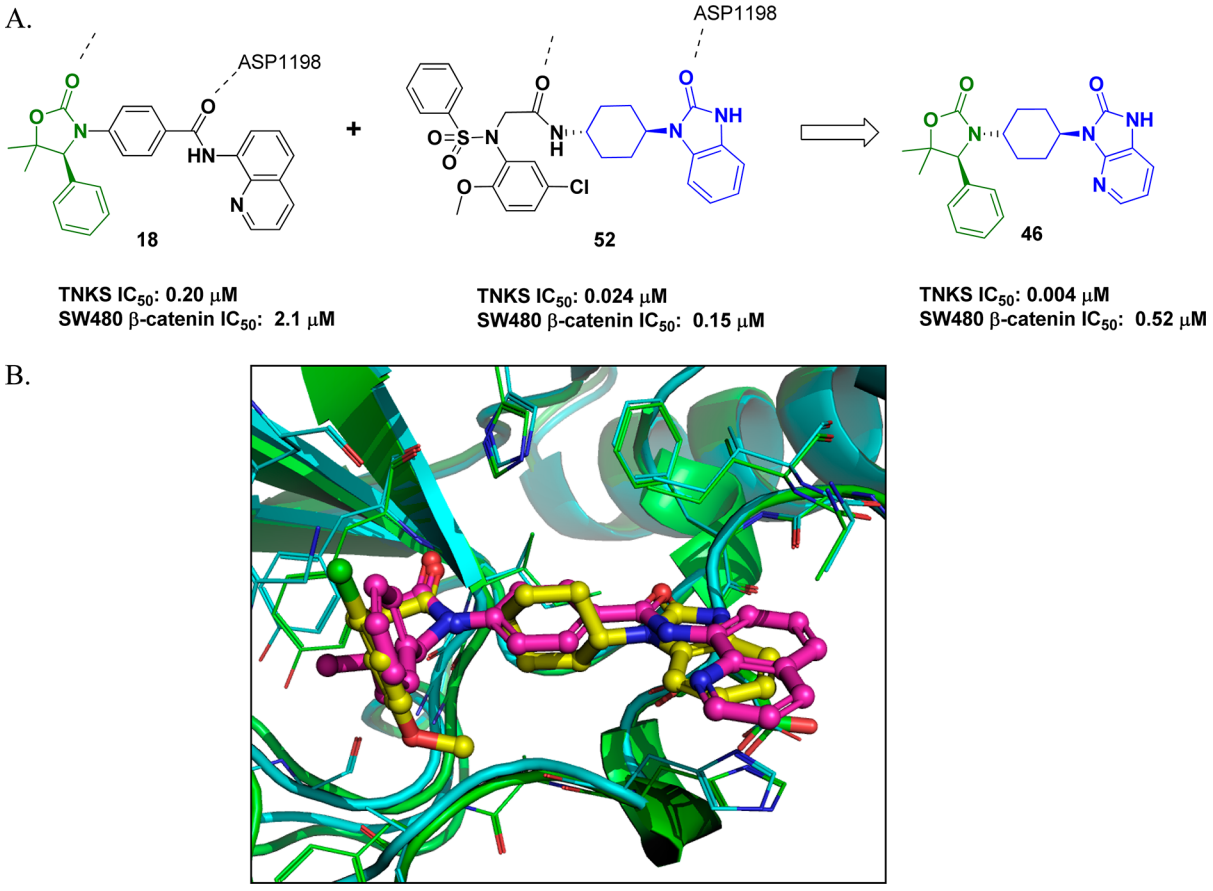

Figure 3. (A) Chemdraw representation of the hybridization of **18** with HTS hit **52** to produce **46**. (B) Overlay of the TNKS1 cocrystal structure of **18** (pink) and **52** (yellow).

acidic media, and permeability were also realized along with moderate plasma stability and clearance (Rat IV CL = 1.3 L/hr/kg) (see Table 5).

The cocrystal structure suggested that the central phenyl moiety did not undergo any direct interaction with the protein. We speculated that replacing the phenyl group with a trans cyclohexyl might result in retention of potency and gains in plasma stability and solubility.[15,23,24] As hypothesized, saturated derivative **24** was equipotent to **18** and offered improved plasma stability ($t_{1/2}$ > 500) and solubility (Table 5). Toward eliminating potential aniline metabolites while reducing the oxidative potential of the quinoline moiety, the reversed amide analogue was prepared (**29**).[25] Compound **29** had retained potency with a significantly increased free fraction (2.7% vs 0.1% unbound for **18**).

Toward further improving potency via the H-bond donor properties of the hydrogen at the 2-position, as described earlier, naphthyridine **30** was prepared, affording improved enzyme and cellular potency (~2-fold) but poor oral exposure in mouse PO PK studies (C_{max} @ 30 mg/kg = 0.22 μM, AUC = 0.258 μM h). Further blocking sites of potential oxidative metabolism yielded fluorinated analogue **31**, which featured retained potency with improved solubility relative to **29**. Furthermore, improvements in oral exposure were observed in PO PK studies (C_{max} @ 30 mg/kg =2.24 μM, AUC = 1.3 μM h).

Despite the vast improvements in cellular potency and PK achieved, the amide containing molecules generally suffer from moderate intrinsic clearance. In addition to optimizing the oxazolidinone amide series, we sought isosteric amide replacements in hopes of improving microsomal stability and oral exposure. An interesting observation presented the possibility of using a benzimidazolone in this regard based on the identification of **52** from a TNKS1 auto-PARsylation high throughput screen. We speculated that **52** may bind in an induced pocket[20] with the amide and benzimidazolone carbonyl groups engaging the protein in a similar manner as **18**, which was subsequently confirmed by the TNKS1/**52** cocrystal structure. Overlay of the TNKS cocrystal structure of HTS hit molecule **52** with the cocrystal structure of **18** revealed that the carbonyl groups of the benzimidazolone and amide moieties both engage ASP1198 in a hydrogen bonding contact. Furthermore, the electron poor quinoline ring of **18** engaged in a favorable stacking interaction with His1201 and overlaid well with the benzimidazolone phenyl ring of **52**. Hybridization of the oxazolidinone portion of **18** with the cyclohexyl benzimidazolone of **52** afforded **46** (Figure 3).

Lead benzimidazolone hybrid **46** possessed good enzyme and β-catenin potency but weak axin activity (IC_{50} = 3.6 μM). Toward improving stacking interactions with His1201, we performed an aza-walk, preparing all four possible aza-benzimidazolone analogues (Table 6).[26] In general, good enzyme potency (≤12 nM) was observed, with notable improvements in cellular potency with the 3-aza analogue **34** (β-catenin, Axin IC_{50} = 0.14, 0.23 μM, respectively) as well as improved oral exposure (~3-fold greater C_{max} vs **31**). The cellular potency was on par with our top amide lead (**31**, β-catenin, Axin IC_{50} = 0.27, 0.19 μM, respectively), with

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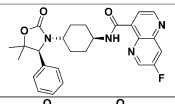
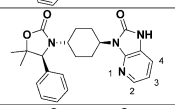
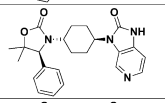
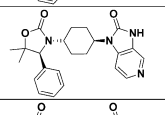
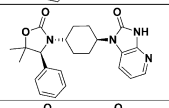
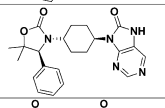
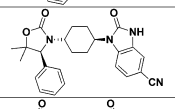
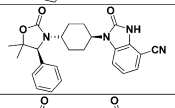
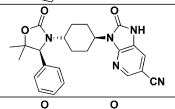
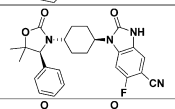
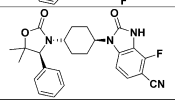
Table 6. Benzimidazolone SAR

Entry	Structure	TNKS1 - IC_{50} (μM)*	SW480 β-catenin IC_{50} (μM)*	SW480-Axin EC_{50} (μM)*	HLM , RLM CLint (μL/min/mg)	Mouse PO PK (30 mg/kg)[a]: AUCt (μM*hr) , Cmax (μM)
31		0.004	0.27	0.19	51 , 87	2.24 , 1.31
46		0.012	0.52	3.6	14 , 280	NA
48		0.004	1.71	NA	<14 , 35	NA
34		0.003	0.14	0.23	<14 , 50	9.96 , 3.26
35		0.011	1.1	2.0	<14 , 44	NA
51		0.005	0.29	0.39	<14 , 43	NA
36		0.001	0.054	0.073	<14 , 22	33.5 , 6.0
37		0.004	0.27	0.45	<14 , 47	NA
41		0.143	0.17	0.80	NA , 186	NA
39		0.003	0.067	0.12	<14 , 19	89.8 , 6.9
40		0.001	0.15	0.061	<14 , 45	101 , 27.4

[a]CD-1 mice were dosed orally at 30 mpk in 10 mL/kg of vehicle (10% pluronic F68, 30% HPBCD, 60% water, pH 2.0 adjusted with methanesulfonic acid for **34**; pH 2.5 adjusted with methanesulfonic acid for **39**; pH adjusted to 10.0 with sodium hydroxide for **31**, **36**, **40**). NA = not measured. *See the Supporting Information for individual data variance and *n* values.

improved microsomal stability. Toward realizing an additive effect, pyrimidine **51** was prepared, but no improvements in potency were observed relative to **34**.

The introduction of a cyano group at the 3-position of the benzimidazole (**36**) provided improved enzyme and cellular potencies (3-fold) and increased exposure in mouse PK studies (C_{max} = 6.0 μM with a 30 mg/kg PO dose). Cyano substitution at the 4-position (**37**) reduced potency (~4-fold) relative to **36**. Lead **36** was incubated with fresh rat hepatocytes, and metabolite analysis suggested that the primary oxidative liability was the benzimidazolone aryl moiety. While replacement of the C−H at the 1 position with nitrogen (**41**) afforded reduced potency (TBC potency: 172 nM vs 54 nM), substituting the positions ortho to the cyano group with a fluorine (**39**, **40**) yielded retained potency with significantly improved oral exposure for **40** (C = 16.3 and 5.9 μM at 1 and 6 h postdose, respectively). However, the mean blood concentration of **40** was only 2.2 and 0.1 μM at 1 and 6 h postdose, respectively, after 3 days of BID administration at 50 mg/kg to female mice. The low exposure after multiple dosing may potentially be due to the induction of metabolic enzymes, as the compound was a strong activator of rat pregnane X receptor (PXR).

In conclusion, efforts to modify IWR-like TNKS inhibitors toward improved cellular potency and pharmacokinetics in rodents for potential in vivo proof of concept studies have led to the identification of lead molecules within amide and benzimidazoline series (Table 7) that have good selectivities

Table 7. Expanded Data Set and Comparison of Top Lead Molecules

			
Entry	**31**	**39**	**40**
TNKS1 IC_{50} (μM)*	0.004	0.003	0.001
SW480 β-catenin IC_{50} (μM)*	0.27	0.067	0.15
SW480-Axin EC_{50} (μM)[a,*]	0.19	0.116	0.061
PARP1 , PARP2 IC_{50} (μM)	>85 , >170	>85 , 2.2	>85 , >170
HLM , RLM CLint (μL/min/mg)	51 , 87	<14 , 19	<14 , 45
Solubility (μg/mL) (0.01N HCl , SIF (pH 6.8) , PBS (pH 7.4))	94 , 156 , 81	5 , 11 , 3	13 , 24 , 12
Plasma Protein Binding (mouse) (% bound)	91.8	95.9	94.9
CYP 3A4 inh. IC_{50} (μM)	>27	7.6	5.4
PXR (rat) (POC @ 10 μM, %)	3	79	74
Mouse PO PK[b], C_{max} (μM)	1.31	6.9	27.4
Mouse PO $C_{max(unbound)}$/Axin EC_{50}[c]	0.57	2.4	23

[a]Assay run in the presence of 10% FBA. [b]CD-1 mice were dosed orally at 30 mpk in 10 mL/kg of vehicle (10% pluronic F68, 30% HPBCD, 60% water, pH 2.5 adjusted with methanesulfonic acid for **39**; pH adjusted to 10.0 with sodium hydroxide for **31**, **40**). [c]fu in 10% FBS >0.5. NA = not measured. *See the Supporting Information for individual data variance and *n* values.

over PARP1/2. Amide containing lead (**31**) had moderate cellular potencies but poor oral exposure in mice. Disubstituted benzimidazoles (**39**, **40**) had significantly improved cellular potencies relative to **1** (~40-fold more potent Axin EC_{50} values) and produced unbound exposure levels exceeding cellular IC_{50} values when dosed orally in mice (mouse PO C_{max}(unbound)/Axin EC_{50} = 23 for **40**). Subsequent efforts toward producing molecules in this series with extended target coverage suitable for proof-of-concept pharmacodynamic studies in mice will be reported in due course.

EXPERIMENTAL SECTION

Axin2 Accumulation Assay. TNKS inhibition results in Axin2 accumulation into distinct cytoplasmic foci which were visualized and quantified using a high content imaging system (Cellomics ArrayScan). SW480 cells were grown under normal culture conditions (RPMI 1640, 10% HI FBS, and 1× Sodium Pyruvate). On the day of the assay, cells were plated at 2500 cells per well in 60 μL of assay media in Perkin-Elmer Black 384 ViewPlates (Fisher, 509052489). TNKS compounds were diluted to generate a 22-point dose titration in media and incubated with the cells for 24 h at 37 °C, 5% CO_2. The next day the cells were fixed for 15 min in 4% paraformaldehyde and 0.1% Triton, washed in PBS, and blocked in PBS with 0.1% Tween-20 and 1% Normal Goat Serum. The cells were stained with an Axin2 primary antibody (Sigma, SAB1100677-200UL) at 1:10000 overnight at 4 °C and an Alexa 488-labeled secondary antibody (Invitrogen, A11008). The nuclei were visualized with Hoechst dye. The Axin2 foci were quantified on the Cellomics ArrayScan (a variation of the compartmental analysis protocol was optimized, and data was analyzed

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using MEAN_RingSpotAvgIntenCh2), and EC_{50} values were calculated. Assay standards: 18 runs with a standard which yielded a mean of 0.311 μM with a standard deviation of 0.173. Eighteen runs with another standard which yielded a mean of 0.114 μM with a standard deviation of 0.065.

Quantitative Total β-Catenin Assay. TNKS inhibition results in degradation of the total pool of β-catenin in SW-480 colorectal cells. SW480 cells do not express E-cadherin and thus do not have membrane associated ("non-signaling") β-catenin which interferes with Wnt pathway activity analysis. Cells were seeded at 10,000/well in CellBIND 96-well in 60 μL of normal growth medium (MEM alpha supplemented with 10% heat inactivated FBS, GlutaMAX, pyruvate, and 10 mM HEPES). A 10-point, 3-fold dilution series for each TNKS inhibitor was constructed, and 20 μL of each diluted compound was transferred to the plated SW-480 cells (resulting in a final vehicle (DMSO) concentration of 0.1%). The plates were incubated at 37 °C for 40−48 h, after which the media was removed and the cells were lysed with 75 μL/well MSD lysis buffer. A goat anti-rabbit MSD plate (catalog no. L41RA-1) was coated with 25 μL of 5 μg/mL of Cell Signaling antitotal β-catenin polyclonal (catalog no. 9562, lyophilized, carrier-free special order) and incubated overnight in a cold room with gentle shaking. The plate was then blocked with 150 μL of Blocker "A" per well and washed 4 times with 150 μL/well TBS-T wash buffer (150 mM NaCl, 50 mM Tris, pH 7.5, 0.02% Tween-20). Cell lysates (75 μL) were transferred to prepared MSD plates and incubated at 4 °C overnight with gentle shaking. The next day MSD plates were washed with TBS-T wash buffer and the antitotal β-catenin mAb (catalog no. 610153) detection antibody conjugated to SULFO-TAG was added. The detection antibody was incubated for 1 h at room temperature with vigorous shaking, after which plates were washed and processed for analysis by the addition of 150 μL/well MSD Read 4X Buffer T with surfactant. The plates were read, and the data was captured on the SECTOR Imager 6000. Assay standards: 54 runs with a standard which yielded a mean of 0.157 μM with a standard deviation of 0.116; 38 runs with another standard which yielded a mean of 0.063 μM with a standard deviation of 0.041.

Tankyrase 1 and 2 Assays. The tankyrase 1 biochemical activity of the compounds was assayed in the following assay buffer (50 mM MOPS pH 7.5, 100 mM NaCl, 2.5 mM $MgCl_2$, 0.01% Tween-20, 0.05% BSA, and 1 mM DTT) as follows: 0.25 nM of 6XHIS-tankyrase1 (1091-1325) was incubated in the presence of compound (DMSO 1.85% final) in a Perkin-Elmer 384 well Proxiplate PlusTM (cat. no. 6008289) with 400 nM of NAD for 60 min at RT. The assay was then stopped with the above assay buffer containing a 0.6 μM inhibitor and the following detection components: 0.05 μg/mL monoclonal anti-PAR antibody (Trevigen cat. no. 4335-MC-01K-AC) prebound for 60 min with 0.63 μg/mL protein G AlphaLisa acceptor bead (Perkin-Elmer cat. no. AL102M) and 5 μg/mL AlphaLisa nickel chelate donor bead (Perkin-Elmer cat. no. AS101M). The assay was incubated for 16 h at RT in the dark and read on a Perkin-Elmer Envision multi label reader using the default program set with laser excitation at 680 nM and emission at 615 nM. Assay standards: 66 runs with a standard which yielded a mean of 0.0086 μM with a standard deviation of 0.00038; 42 runs with a different standard which yielded a mean of 0.0013 μM with a standard deviation of 0.00064.

PARP 1 and 2 Assays. PARP1 biochemical assay was purchased as a kit from Trevigen (cat. no. 4676-096-K) and used per the manufacturer's recommendations. PARP2 biochemical assay was purchased as a kit from BPS (cat. no. 80552) and used per the manufacturer's recommendations.

CHEMISTRY

General. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Anhydrous solvents were obtained from Aldrich and used directly. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. Purity (3 min methods only) and reaction analyses were measured using Agilent 1100 Series high performance liquid chromatography (HPLC) systems with UV detection at 254 and 215 nm (System A: Agilent Zorbax SB-C18 3.0 mm × 50 mm, 3.5 μm, 5−95% CH_3CN in H_2O with 0.1% TFA for 3.6 min at 1.5 mL/min or Halo Phenyl-Hexyl, 3 mm × 50 mm, 2.7 μm, 5−95% CH_3CN in H_2O with 0.1% TFA for 1.01 min at 2.0 mL/min; System B: Waters Xbridge C18, 3 mm × 50 mm, 3.5 μm, 5−95% CH_3CN in H_2O with 0.1% formic acid for 3.6 min at 1.5 mL/min). Purities for final compounds were >95%. Exact mass confirmation was performed on an Agilent 1200 series high performance liquid chromatography (HPLC) system (Santa Clara, CA, US) by flow injection analysis, eluting with a binary solvent system A and B (A, water with 0.1% FA; B, ACN with 0.1% FA) under isocratic conditions (50% A/50% B) at 0.2 mL/min with MS detection by an Agilent 6510-Q time-of-flight (TOF) mass spectrometer (Santa Clara, CA, US). Silica gel chromatography was generally performed with prepacked silica gel cartridges (Biotage, Teledyne-Isco, or Interchim). Chiral method development was performed on an analytical Thar SFC/MS. Preparative chiral separations were performed on Thar SFC Prep 80 or SFC Prep 350 instruments. Library purification methods: Preparative LC/MS: Waters autopurification system; liquid transfer system, Tecan; drying system, Genevac; preparative column, Xbridge (19 mm × 100 mm, C18, 10 μm); flow rate, 40 mL/min; general gradient, 5−95% B, 0.1% additive in both A and B, 10 min gradient; mobile phase A, water; mobile phase B, acetonitrile; additive, TFA or NH_4OH. ^1H NMR spectra were recorded on a Bruker AV-400 (400 MHz) spectrometer or a Varian 400 MHz spectrometer at ambient temperature, or the NMR spectra were collected with a Bruker Avance III spectrometer operating at a proton frequency of 500.13 MHz using a 10 mL Protasis CapNMR flow probe. NMR samples were delivered to the flow probe using a Protasis One-Minute NMR Automation system comprised of a Discovery Tower Sample Manager and a Waters Liquid Handler made by CTC, Switzerland (model 2777). All observed protons are reported as parts per million (ppm) downfield from tetramethylsilane (TMS) or other internal reference in the appropriate solvent indicated. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants, and number of protons. Low-resolution mass spectral (MS) data were determined on an Agilent 1100 Series LCMS with UV detection at 254 and 215 nm and a low resonance electrospray mode (ESI).

Synthetic Details. 4-((3a*R*,4*S*,7*R*,7a*S*)-1-Oxo-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindol-2(3H)-yl)-*N*-(quinolin-8-yl)benzamide (3). To a vial charged with **1** (122 mg, 0.298 mmol) was added MeOH (1192 μL) and DCM (1192 μL) and MP-BH_4 (macroporous borohydride) (114 mg, 0.358 mmol). The mixture was shaken overnight at 60 °C, providing about 60% conversion to desired product. An additional aliquot of DCM, MeOH, and MP-BH_4 (same quantities as above) was added, and the mixture was heated and shaken at 80 °C for 4 h, providing complete conversion. The mixture was filtered through cotton and washed with DCM and MeOH. The filtrate was dried under reduced pressure and the crude material purified by MPLC, 25g column ramping DCM/MeOH/NH_4OH (90:10:1) in DCM from 0% to 100%, leading to coelution of product with impurities. The material was repurified using a 15 μm spherical silica column (Interchim) with the same gradient, leading to complete isolation of desired product cleanly, obtained as a yellow oil (±)-4-((3a*S*,4*R*,7-*S*,7a*R*)-1-hydroxy-3-oxo-3a,4,7,7a-tetrahydro-1H-4,7-methanoi-

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soindol-2(3H)-yl)-*N*-(quinolin-8-yl)benzamide (45 mg, 0.109 mmol, 36.7% yield). *m/z* (ESI) 412.2 $(M + H)^+$.

To a flask charged with 4-((3a*S*,4*R*,7*S*,7a*R*)-1-hydroxy-3-oxo-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindol-2(3H)-yl)-*N*-(quinolin-8-yl)benzamide (36 mg, 0.087 mmol) was added DCM (700 μL), TFA (10.11 μL, 0.131 mmol) and benzeneselenol (37.2 μL, 0.350 mmol). The orange solution was refluxed overnight. The resulting orange solution was dried under reduced pressure and the crude residue purified by a 25g column ramping DCM/MeOH/NH_4OH (90:10:1) in DCM from 0% to 100%, leading to coelution of product with a selenium containing impurity. The mixture was repurified using the Gilson RP-HPLC, ramping ACN in H_2O (10−90%, 0.1% TFA), providing product as a white film upon drying. The material was converted to its free-base using a 2g SCX-2 (strong cation exchange) column, washing first with MeOH and then with 2 M NH_3 in MeOH, providing a white film upon drying. The film obtained was lyophilized from MeOH/H_2O, providing product as a fluffy light yellow solid 4-((3a*R*,4*S*,7-*R*,7a*S*)-1-oxo-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindol-2(3H)-yl)-*N*-(quinolin-8-yl)benzamide (2.5 mg, 6.32 μmol, 7.23% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.61 (s, 1H), 8.95−9.02 (m, 1H), 8.72 (d, J = 7.5 Hz, 1H), 8.47 (d, J = 8.2 Hz, 1H), 8.01 (d, J = 8.8 Hz, 2H), 7.86 (d, J = 8.8 Hz, 2H), 7.72−7.77 (m, 1H), 7.62−7.72 (m, 2H), 6.22−6.28 (m, 1H), 6.17 (dd, J = 2.8, 5.7 Hz, 1H), 3.94 (t, J = 10.1 Hz, 1H), peak under water, 2.90−3.00 (m, 1H), 1.42−1.53 (m, 2H). *m/z* (ESI) 396.2 $(M + H)^+$, HRMS calcd for $C_{25}H_{21}N_3O_2$ $(M + H)^+$ 396.1712, found 396.1708.

4-(1-Oxoisoindolin-2-yl)-*N*-(quinolin-8-yl)benzamide (5). A resealable tube was charged with phthalaldehyde (0.474 g, 3.54 mmol), 4-aminobenzoic acid (0.485 g, 3.54 mmol), 4-aminobenzoic acid (0.485 g, 3.54 mmol), and EtOH (10 mL). The mixture was heated at 90 °C for 6 h. The pale yellow precipitate was isolated via vacuum filtration and then washed with EtOH and diethyl ether and dried under reduced pressure to afford crude 4-(1-oxoisoindolin-2-yl)benzoic acid (0.222 g, 0.877 mmol, 25%), which was used without further purification.

To a flask charged with crude 4-(1-oxoisoindolin-2-yl)-benzoic acid (0.217 g, 0.857 mmol) was added DMF (5.0 mL) followed by DIPEA (0.449 mL, 2.57 mmol), 8-aminoquinoline (0.130 g, 0.900 mmol), and HATU (0.326 g, 0.857 mmol). The mixture was stirred overnight at RT. The resulting yellow suspension was then filtered through a membrane, washing with MeOH to afford a solid. Purification by SFC afforded 4-(1-oxoisoindolin-2-yl)-*N*-(quinolin-8-yl)benzamide (0.079 g, 0.208 mmol, 24%) as a pale yellow solid in 95% HPLC purity. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.69 (s, 1 H), 9.02 (d, J = 2.7 Hz, 1 H), 8.76 (d, J = 7.6 Hz, 1 H), 8.49 (d, J = 8.2 Hz, 1 H), 8.21−8.14 (m, 4 H), 7.85 (dd, J = 7.6 Hz, 1 H), 7.77−7.66 (m, 5 H), 7.60 (m, 1 H), 5.15 (s, 2 H). *m/z* (ESI) 380.2 $(M + H)^+$, HRMS calcd for $C_{24}H_{17}N_3O_2$ $(M + H)^+$ 380.1399, found 380.1392.

4-(2-Oxo-3-phenyl-1-imidazolidinyl)-*N*-8-quinolinyl-benzamide (7). Compound **7** was prepared as described for compound **9** using 1-phenylimidazolidin-2-one (1 equiv) to afford 4-(2-oxo-3-phenyl-1-imidazolidinyl)-*N*-8-quinolinylbenzamide (0.04 g, 98 mmol, 12% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.64 (s, 1H), 9.00 (dd, J = 4.2, 1.7 Hz, 1H), 8.74 (dd, J = 7.6, 1.3 Hz, 1H), 8.47 (dd, J = 8.3, 1.6 Hz, 1H), 8.05−8.10 (m, 2H), 7.87−7.92 (m, 2H), 7.63−7.77 (m, 5H), 7.37−7.44 (m, 2H), 7.07−7.14 (m, 1H), 4.02−4.10 (m, 4H). LC-MS (ESI) *m/z*: 409.2. HRMS calcd for $C_{25}H_{20}N_4O_2$ $(M + H)^+$ 409.1664, found 409.1657.

4-((4*R*)-2-Oxo-4-phenyl-1-pyrrolidinyl)-*N*-8-quinolinyl-benzamide (8). Compound **8** was prepared as described for compound **9** using (4*R*)-phenylpyrrolidin-2-one (1 equiv) to afford 4-((4*R*)-2-oxo-4-phenyl-1-pyrrolidinyl)-*N*-8-quinolinyl-benzamide (0.19 g, 0.466 mmol, 58% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1H), 8.99 (dd, J = 4.2, 1.7 Hz, 1 H), 8.73 (dd, J = 7.6, 1.3 Hz, 1H), 8.47 (dd, J = 8.4, 1.6 Hz, 1H), 8.04−8.10 (m, 2H), 7.92−7.99 (m, 2H), 7.72−7.77 (m, 1H), 7.63−7.72 (m, 2H), 7.41−7.47 (m, 2H), 7.35−7.41 (m, 2H), 7.25−7.32 (m, 1H). LC-MS (ESI) *m/z*: 408.2 (M + 1). HRMS calcd for $C_{26}H_{21}N_3O_2$ $(M + H)^+$ 408.1712, found 408.1706.

4-(2-Oxo-5-phenylpyrrolidin-1-yl)-*N*-(quinolin-8-yl)-benzamide (9). A mixture of 4-iodobenzoic acid (9.26 g, 37.3 mmol), DIEA (13.05 mL, 74.7 mmol), HATU (7.10 g, 18.67 mmol), and quinolin-8-amine (7 g, 48.6 mmol) in DMF (124 mL) was stirred under nitrogen overnight at room temperature. The reaction mixture was diluted with saturated aqueous sodium bicarbonate (100 mL) and water (300 mL), yielding a yellow/brown precipitate which was filtered, washed with water (100 mL), and dried under high vacuum to obtain 4-iodo-*N*-(quinolin-8-yl)benzamide (9 g, 24.05 mmol, 64.4% yield) as a yellow brown solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.65 (s, 1 H), 8.98 (dd, J = 1.6, 4.3 Hz, 1 H), 8.70 (dd, J = 1.4, 7.6 Hz, 1 H), 8.47 (dd, J = 1.7, 8.3 Hz, 1 H), 8.03−7.98 (m, 2 H), 7.83−7.79 (m, 2 H), 7.76 (dd, J = 1.3, 8.2 Hz, 1 H), 7.71−7.62 (m, 2 H).

To a flask charged with 4-iodo-*N*-(quinolin-8-yl)benzamide (0.30 g, 0.802 mmol), 5-phenylpyrrolidin-2-one (0.13 g, 0.802 mmol), and cesium carbonate (0.784 g, 2.405 mmol) was added dioxane (9 mL), and the mixture was purged with nitrogen prior to the addition of copper(I) iodide (0.153 g, 0.802 mmol) and N^1,N^2-dimethylethane-1,2-diamine (0.141 g, 1.604 mmol) and repurging with nitrogen and then heating in the microwave at 140 °C for 2 h. The resulting reaction mixture was filtered through a frit and purified by MPLC by eluting with EtOAc/hexanes and DCM (5%) as an additive to obtain compound **9** (0.25 g, 0.614 mmol, 77% yield) as a white solid. Compound **9** was separated using ChiralPak AD-H (2 × 15 cm), 35% isopropanol (0.1%, DEA), 100 bar, 60 mL/min, 220 nm, inj vol: 1−3 mL, 15 mg/mL methanol, to obtain compounds **10** and **11** as white solids. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.54 (s, 1H), 8.95 (dd, J = 4.3, 1.7 Hz, 1H), 8.67 (dd, J = 7.6, 1.4 Hz, 1H), 8.44 (dd, J = 8.4, 1.6 Hz, 1H), 7.90−7.95 (m, 2H), 7.60−7.75 (m, 5H), 7.29−7.37 (m, 4H), 7.20−7.27 (m, 1H), 5.58 (dd, J = 7.8, 4.8 Hz, 1H), 2.56−2.74 (m, 3H), 1.85−1.93 (m, 1H). LC-MS (ESI) *m/z*: 408.2 (M + 1). HRMS calcd for $C_{26}H_{21}N_3O_2$ $(M + H)^+$ 408.1712, found 408.1711.

4-(2,2-Dimethyl-5-oxo-1-pyrrolidinyl)-*N*-8-quinolinyl-benzamide Trifluoroacetic Acid (12). Compound **12** was prepared as described for compound **9** using 5,5-dimethylpyrrolidin-2-one (1 equiv) to afford 4-(2,2-dimethyl-5-oxo-1-pyrrolidinyl)-*N*-8-quinolinylbenzamide trifluoroacetic acid (1.2 mg, 0.003 mmol, 0.5% yield) as a tan solid following purification with TFA modified RP-HPLC. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.68 (s, 1 H), 9.02−8.95 (m, 1 H), 8.73 (dd, J = 1.3, 7.6 Hz, 1 H), 8.49 (dd, J = 1.7, 8.3 Hz, 1 H), 8.13−8.04 (m, 2 H), 7.77 (dd, J = 1.3, 8.3 Hz, 1 H), 7.73−7.62 (m, 2 H), 7.45−7.37 (m, 2 H), 2.58−2.51 (m, 2 H), 2.04 (m, 2 H), 1.27 (s, 6 H). *m/z* (ESI) 360.0 $(M + H)^+$.

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4-(2-Methyl-5-oxo-1-pyrrolidinyl)-*N*-8-quinolinylbenzamide (13). Compound **13** was prepared as described for compound **9** using 5-methylpyrrolidin-2-one (1 equiv) to afford 4-(2-methyl-5-oxo-1-pyrrolidinyl)-*N*-8-quinolinylbenzamide (0.008 g, 24 mmol, 4% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.64 (s, 1H), 8.96−9.00 (m, 1H), 8.73 (d, J = 7.6 Hz, 1H), 8.46 (d, J = 8.2 Hz, 1H), 8.06 (d, J = 8.7 Hz, 2H), 7.72−7.80 (m, 3H), 7.63−7.71 (m, 2H), 4.50−4.58 (m, 1H), 2.65 (ddd, J = 16.9, 9.4, 7.4 Hz, 1H), 2.42−2.49 (m, 1H), 2.28−2.38 (m, 1H), 1.73 (td, J = 8.3, 4.8 Hz, 1H), 1.21 (d, J = 6.2 Hz, 3H). LC-MS (ESI) m/z: 346.2 (M + 1). HRMS calcd for $C_{21}H_{19}N_3O_2$ (M + H)$^+$ 346.1555, found 346.1561.

4-(2-(4-Fluorophenyl)-5-oxopyrrolidin-1-yl)-*N*-(quinolin-8-yl)benzamide (14). Compound **14** was prepared as described for compound **9** using 5-(4-fluorophenyl)pyrrolidin-2-one (1 equiv), affording 4-(2-(4-fluorophenyl)-5-oxopyrrolidin-1-yl)-*N*-(quinolin-8-yl)benzamide (29 mg, 0.068 mmol, 42.1% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.54 (s, 1 H), 8.95 (dd, J = 1.7, 4.3 Hz, 1 H), 8.67 (d, J = 6.5 Hz, 1 H), 8.44 (dd, J = 1.5, 8.3 Hz, 1 H), 7.94 (s, 2 H), 7.74−7.61 (m, 5 H), 7.37 (dd, J = 5.4, 8.7 Hz, 2 H), 7.15 (t, J = 8.8 Hz, 2 H), 5.61−5.57 (m, 1 H), 2.74−2.55 (m, 3 H), 1.92−1.86 (m, 1 H). m/z (ESI) 426.2 (M + H)$^+$. HRMS calcd for $C_{26}H_{20}FN_3O_2$ (M + H)$^+$ 426.1618, found 426.1617.

4-(2-(3-Fluorophenyl)-5-oxopyrrolidin-1-yl)-*N*-(quinolin-8-yl)benzamide (15). Compound **15** was prepared as described for compound **9** using 5-(3-fluorophenyl)pyrrolidin-2-one (1 equiv), affording 4-(2-(3-fluorophenyl)-5-oxopyrrolidin-1-yl)-*N*-(quinolin-8-yl)benzamide (460 mg, 1.081 mmol, 60.5% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1 H), 8.95 (dd, J = 1.7, 4.2 Hz, 1 H), 8.67 (dd, J = 1.2, 7.6 Hz, 1 H), 8.45 (dd, J = 1.6, 8.3 Hz, 1 H), 7.95 (d, J = 8.8 Hz, 2 H), 7.75−7.60 (m, 5 H), 7.41−7.33 (m, 1 H), 7.21−7.14 (m, 2 H), 7.06 (dt, J = 2.2, 8.4 Hz, 1 H), 5.61 (dd, J = 5.0, 7.5 Hz, 1 H), 2.76−2.56 (m, 3 H), 1.95−1.87 (m, 1 H). m/z (ESI) 426.2 (M + H)$^+$. HRMS calcd for $C_{26}H_{20}FN_3O_2$ (M + H)$^+$ 426.1618, found 426.1619.

4-(2-(2-Fluorophenyl)-5-oxopyrrolidin-1-yl)-*N*-(quinolin-8-yl)benzamide (16). To a solution of ethyl 4-(2-fluorophenyl)-4-oxobutanoate (500 mg, 2.2 mmol) in methanol (5 mL) were added ammonium acetate (4.62 g, 60 mmol) and sodium borohydride (250 mg, 4.4 mmol) at ambient temperature, and the reaction mixture was stirred for 2 h. The resulting reaction mixture was stirred at 80 °C for 12 h. Reaction progress was monitored by the TLC system: 50% ethyl acetate in hexane. After reaction completion the reaction mixture was concentrated under reduced pressure. The resulting reaction mixture was adjusted to pH ∼12 by treatment with 6 N NaOH solution and then ethyl acetate. The ethyl acetate layer was concentrated and the crude material purified by column chromatography with 30% ethyl acetate in hexane as the eluent to afford 5-(2-fluorophenyl)pyrrolidin-2-one (300 mg, 75% yield) as a colorless liquid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.08 (s, 1H), 7.47−7.10 (m, 4H), 5.00−4.83 (m, 1H), 2.24 (t, J = 8.0 Hz, 2H), 2.02−1.67 (m, 1H). m/z (ESI) 179 (M + H)$^+$.

To a mixture of 4-iodo-*N*-(quinolin-8-yl)benzamide (see protocol for compound **9**) (50 mg, 0.13 mmol), 5-(2-fluorophenyl)pyrrolidin-2-one (23 mg, 0.13 mmol), and (1*R*,2*R*)-N^1,N^2-dimethylcyclohexane-1,2-diamine (36 mg, 0.26 mmol) in toluene (2 mL) was added CuI (24 mg, 0.13 mmol) and cesium carbonate (210 mg, 0.65 mmol) at ambient temperature, and the resulting reaction mixture was stirred for 12 h at 100 °C. Reaction progress was monitored by the TLC system: 40% ethyl acetate in hexane. Upon completion, the reaction was concentrated under reduced pressure, and then the crude material was purified by column chromatography using 20% ethyl acetate in hexane as the eluent to afford 4-(2-(2-fluorophenyl)-5-oxopyrrolidin-1-yl)-*N*-(quinolin-8-yl)-benzamide (30 mg, 52.8% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1H), 8.95 (d, J = 2.9 Hz, 1H), 8.67 (d, J = 7.4 Hz, 1H), 8.45 (d, J = 8.1 Hz, 1H), 7.96 (d, J = 8.7 Hz, 2H), 7.81−7.55 (m, 5H), 7.52−7.09 (m, 3H), 7.09−7.03 (m, 1H), 5.80 (d, J = 7.5 Hz, 1H), 3.05−2.60 (m, 4H), 2.01−1.63 (m, 1H). m/z (ESI) 426.0 (M + H)$^+$, HRMS calcd for $C_{26}H_{20}FN_3O_2$ (M + H)$^+$ 426.1618, found 426.1611.

4-((4*S*)-2-Oxo-4-phenyl-1,3-oxazolidin-3-yl)-*N*-8-quinolinylbenzamide Trifluoroacetic Acid (17). Compound **17** was prepared as described for compound **9** using (*R*)-5-phenylpyrrolidin-2-one (1 equiv) to afford 4-((4*S*)-2-oxo-4-phenyl-1,3-oxazolidin-3-yl)-*N*-8-quinolinylbenzamide trifluoroacetic acid as a white solid following purification with TFA modified RP-HPLC (57 mg, 0.109 mmol, 20% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.55 (s, 1 H), 8.95 (dd, J = 1.5, 4.1 Hz, 1 H), 8.67 (d, J = 6.8 Hz, 1 H), 8.44 (dd, J = 1.5, 8.4 Hz, 1 H), 7.97 (d, J = 8.8 Hz, 2 H), 7.76−7.58 (m, 5 H), 7.45−7.35 (m, 4 H), 7.32 (d, J = 6.9 Hz, 1 H), 5.83 (dd, J = 5.4, 8.6 Hz, 1 H), 4.90 (t, J = 8.6 Hz, 1 H), 4.20 (dd, J = 5.3, 8.5 Hz, 1 H). m/z (ESI) 410.4 (M + H)$^+$. HRMS calcd for $C_{25}H_{19}N_3O_3$ (M + H)$^+$ 410.1504, found 410.15.

4-((4*S*)-5,5-Dimethyl-2-oxo-4-phenyl-1,3-oxazolidin-3-yl)-*N*-8-quinolinylbenzamide (18). Compound **18** was prepared as described for compound **9** using (*S*)-5,5-dimethyl-4-phenyloxazolidin-2-one from Aldrich. Purification was done by preparative LC-MS with 0.1% NH_4OH in ACN and water as mobile phase. The pure fractions were dried under reduced pressure to afford ((4*S*)-5,5-dimethyl-2-oxo-4-phenyl-1,3-oxazolidin-3-yl)-*N*-8-quinolinylbenzamide as a brown solid (82 mg, 0.187 mmol, 35% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.55 (s, 1 H), 8.95 (dd, J = 1.6, 4.2 Hz, 1 H), 8.67 (dd, J = 1.2, 7.6 Hz, 1 H), 8.45 (dd, J = 1.6, 8.3 Hz, 1 H), 7.96 (d, J = 8.8 Hz, 2 H), 7.81−7.56 (m, 5 H), 7.48−7.17 (m, 5 H), 5.57 (s, 1 H), 3.17 (d, J = 5.1 Hz, 1 H), 1.66 (s, 3 H), 0.93 (s, 3 H). m/z (ESI) 438.3 (M + H)$^+$. HRMS calcd for $C_{27}H_{23}N_3O_2$ (M + H)$^+$ 438.1817, found 438.1813.

(*S*)-4-(5,5-Dimethyl-2-oxo-4-phenyloxazolidin-3-yl)-*N*-(1,5-naphthyridin-4-yl)benzamide (21). To a round-bottom flask was added (*S*)-5,5-dimethyl-4-phenyloxazolidin-2-one (1.717 g, 8.98 mmol, Sigma Aldrich), methyl 4-iodobenzoate (2.353 g, 8.98 mmol), and dioxane (90 mL) followed by potassium phosphate, tribasic (9.53 g, 44.9 mmol), and copper(I) iodide (1.710 g, 8.98 mmol). The vessel was purged with nitrogen, followed by the addition of *N*,*N*′-dimethylethylenediamine (1.933 mL, 17.96 mmol). The suspension was heated to reflux overnight, yielding a tan suspension. The mixture was filtered through Celite, and the filtrate was dried under reduced pressure, providing a light green oil (*S*)-methyl 4-(5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)benzoate (**19**) (2.72 g, 8.36 mmol, 93% yield), which will be used as is in the next step. ^{1}H NMR (400 MHz, chloroform-d) δ 7.94−7.90 (m, 2 H), 7.55−7.51 (m, 2 H), 7.39−7.32 (m, 3 H), 7.21−7.16 (m, 2 H), 5.04 (s, 1 H), 3.86 (s, 3 H), 1.70 (s, 3 H), 1.02 (s, 3 H).

To a flask charged with (*S*)-methyl 4-(5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)benzoate (2.70 g, 8.30 mmol) was added THF (48.5 mL), MeOH (48.5 mL), and water (48.5 mL),

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respectively. To the resulting turbid solution was added LiOH (0.994 g, 41.5 mmol), and the resulting mixture was heated at 50 °C overnight. The resulting mixture was cooled in an ice water bath and brought to pH ∼2 by the addition of 2 N HCl (∼15 mL), leading to a light yellow solution to which water was added (∼100 mL), leading to the formation of a white precipitate which was collected via vacuum filtration and washed with excess water. After drying under high vacuum overnight, product was obtained as a white solid (*S*)-4-(5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)benzoic acid (1.25 g, 4.02 mmol, 48.4% yield). NMR indicates that the product formed as a THF adduct with a 3:2 product to THF ratio. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.76 (br s, 1 H), 7.87−7.81 (m, 2 H), 7.63−7.57 (m, 2 H), 7.41−7.34 (m, 2 H), 7.34−7.30 (m, 1 H), 7.30−7.19 (m, 2 H), 5.51 (s, 1 H), 1.63 (s, 3 H), 0.91 (s, 3 H).

To a vial charged with (*S*)-4-(5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)benzoic acid (0.200 g, 0.642 mmol) was added DCM (2.57 mL) followed by thionyl chloride (0.469 mL, 6.42 mmol). The resulting solution was sealed and shaken at 40 °C for 2 h. LC-MS indicated near complete conversion to desired product (primarily methyl ester peak identified). The mixture was dried under reduced pressure, providing product (**20**) as a light yellow solid. *m*/*z* (ESI) 326.2 (M + H)$^+$ (methyl ester ionization observed).

To a vessel charged with (*S*)-4-(5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)benzoyl chloride (**20**) (50 mg, 0.152 mmol) was added DCM (606 μL), 1,5-naphthyridin-4-amine (Astatech) (24 mg, 0.167 mmol), and DIEA (55.6 μL, 0.318 mmol), respectively. The vessel was sealed and shaken overnight at 40 °C. The mixture was dried under reduced pressure and purified using a 25 g, 15 μm spherical silica column (Interchim) ramping 0−100% EtOAc in heptane to afford product, which was lyophilized from MeOH/H_2O to yield (*S*)-4-(5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)-*N*-(1,5-naphthyridin-4-yl)benzamide (64 mg, 96%) as an off-white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1 H), 9.01 (dd, *J* = 1.6, 4.2 Hz, 1 H), 8.93 (d, *J* = 5.0 Hz, 1 H), 8.54 (d, *J* = 5.1 Hz, 1 H), 8.46 (dd, *J* = 1.6, 8.5 Hz, 1 H), 8.01−7.96 (m, 2 H), 7.89 (dd, *J* = 4.3, 8.6 Hz, 1 H), 7.76−7.70 (m, 2 H), 7.43−7.37 (m, 2 H), 7.36−7.22 (m, 3 H), 5.57 (s, 1 H), 1.67 (s, 3 H), 0.94 (s, 3 H). *m*/*z* (ESI) 439.2 (M + H)$^+$. HRMS calcd for $C_{26}H_{22}N_4O_3$ (M + H)$^+$ 439.1770, found 438.1775.

(1*S*,4*R*)-4-((*S*)-5,5-Dimethyl-2-oxo-4-phenyloxazolidin-3-yl)-*N*-(quinolin-8-yl)cyclohexanecarboxamide (24). To a flask charged with 2-hydroxy-2-methyl-1-phenylpropan-1-one (1.570 mL, 10.33 mmol) was added DCE (20.65 mL) and methyl *trans*-4-aminocyclohexanecarboxylate hydrochloride (1.00 g, 5.16 mmol), and the reaction mixture was cooled in an ice water bath prior to the addition of sodium triacetoxyhydroborate (1.094 g, 5.16 mmol). The mixture was stirred for 1 h. The mixture was diluted with water, transferred to a separatory funnel, and extracted with DCM (2×). The combined organics were dried with Na_2SO_4, filtered, and dried under reduced pressure, yielding a colorless oil. The oil was purified with a 10g SCX-2 column, washing with MeOH and then with 2 M NH_3 in MeOH. LC-MC of the basic wash shows *m*/*z* of 304/306, suggestive of an imine product, which is strange, since the initial crude LC-MS indicated the amine mass cleanly. NMR suggests amine product, obtained as a sticky white solid (±)-(1*R*,4*R*)-methyl 4-((2-hydroxy-2-methyl-1-phenylpropyl)amino)cyclohexanecarboxylate (**22**; 0.570 g, 1.866 mmol, 36.1% yield). There were about 20% impurity present as well, possibly imine. The material was used without further purification. *m*/*z* (ESI) 306.3 (M + H)$^+$.

To a flask charged with (1*R*,4*R*)-methyl 4-((2-hydroxy-2-methyl-1-phenylpropyl)amino)cyclohexanecarboxylate (2.785 g, 9.12 mmol) was added tetrahydrofuran (36.5 mL) and DIEA (4.78 mL, 27.4 mmol). The mixture was cooled in an ice water bath prior to the addition of triphosgene (2.71 g, 9.12 mmol), yielding a white suspension which was allowed to stir and slowly warm to room temperature (2 h, ice melt). LC-MS indicated about 75% conversion, which was the same as after 1 h. The mixture was cooled back to 0 °C, and an additional 0.5 equiv of triphosgene was added. After 1 h additional conversion was not observed. To the mixture was added additional DIEA (1.5 equiv). After 30 min LC-MS indicated complete conversion to desired product. The suspension was added carefully to a stirred flask containing aqueous NH_4Cl and ice. The mixture was transferred to a separatory funnel and extracted with EtOAc (2×). The combined organics were dried with Na_2SO_4, filtered, and dried under reduced pressure, providing a light yellow crude solid (quantitative yield). Enantiomer separation: system, Prep SFC-2, Chiralpak AD-H, 2 cm × 25 cm, 20% methanol w/0.2% DEA; 80 mL/min flow rate (pressure drop = 55 bar); 220 nm detection; 100 bar BPR; sample dissolved in 50 mL of methanol. Test Injections: 500 μL, 1 mL, 1.5 mL, sample processed with 1.5 mL injection volume and 3.5 min cycle time. Absolute stereochemical determination: Compound **24** had previously been prepared in a more stepwise asymmetrical manner (not described). One of the separated enantiomers from this separation was converted to **24**, and the retention time compared with that of **24** prepared from the asymmetric route to determine absolute stereochemistry. *m*/*z* (ESI) 332.3 (M + H)$^+$.

To a vial charged with (1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexanecarboxylic acid (**23**, 0.028 g, 0.088 mmol) was added HATU (0.034 g, 0.088 mmol), quinolin-8-amine (0.013 g, 0.088 mmol), DMF (0.353 mL), and DIEA (0.015 mL, 0.088 mmol), respectively. The vessel was sealed and shaken at room temperature overnight. The resulting mixture was dried under reduced pressure and purified with a 25 HP spherical silica column, ramping DCM/MeOH (90:10) in DCM (0−40%, isocratic at 40%, detection at 215 nm), providing product as a white film, (1*R*,4*R*)-4-((*R*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)-*N*-(quinolin-8-yl)-cyclohexanecarboxamide (0.015 g, 0.034 mmol, 38.3% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1 H), 8.90 (dd, *J* = 1.7, 4.3 Hz, 1 H), 8.57 (dd, *J* = 1.3, 7.7 Hz, 1 H), 8.39 (dd, *J* = 1.7, 8.4 Hz, 1 H), 7.66−7.59 (m, 2 H), 7.57−7.50 (m, 1 H), 7.46−7.40 (m, 2 H), 7.40−7.15 (m, 3 H), 4.62 (s, 1 H), 3.51−3.41 (m, 1 H), 2.58−2.52 (m, 1 H), 2.03−1.95 (m, 1 H), 1.92−1.75 (m, 3 H), 1.64 (d, *J* = 10.9 Hz, 1 H), 1.59−1.50 (m, 1 H), 1.50−1.47 (m, 3 H), 1.47−1.36 (m, 1 H), 1.27−1.13 (m, 1 H), 0.81 (s, 3 H). *m*/*z* (ESI) 444.3 (M + H)$^+$, HRMS calcd for $C_{27}H_{29}N_3O_3$ (M + H)$^+$ 444.2287, found 444.2288.

(*S*)-3-((1*R*,4*S*)-4-Aminocyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (28). To a flask charged with 4-*N*-boc-aminocyclohexanone (Combi-Blocks) (11.00 g, 51.6 mmol) was added DCE (206 mL) followed by (*S*)-(+)-2-phenylglycine methyl ester hydrochloride (Aldrich) (10.40 g, 51.6 mmol). The resulting mixture was stirred at room temperature for 15 min prior to the addition of sodium triacetoxyborohydride (21.86 g, 103 mmol). The resulting suspension was stirred overnight at room temperature, leading to conversion to desired product (∼1:1 cis/trans). To the

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mixture was added water, and the resulting mixture was transferred to a separatory funnel. The mixture was extracted with EtOAc (2×). The combined organics were dried with Na_2SO_4, filtered, and dried under reduced pressure. The crude material was purified with a 100g SNAP column, ramping DCM/MeOH (90:10) in DCM (0−25%), then isocratic at 25% (monitoring at 215 nm) to yield product as a mixture of isomers as a yellow solid (±)-(*S*)-methyl 2-(((1*R*,4*S*)-4-((*tert*-butoxycarbonyl)amino)cyclohexyl)amino)-2-phenylacetate (12.43 g, 34.3 mmol, 66.5% yield).

A flask charged with (±)-(*S*)-methyl 2-(((1*R*,4*S*)-4-((*tert*-butoxycarbonyl)amino)cyclohexyl)amino)-2-phenylacetate (12.4 g, 34.2 mmol) was dried under high vacuum and placed under nitrogen. Then, THF (98 mL) was added and the resulting solution cooled in an ice water bath prior to the addition of methylmagnesium bromide (1 M in THF) (16.32 g, 137 mmol) over 15 min. The mixture was allowed to slowly warm to room temperature overnight, providing a yellow suspension. The mixture was carefully added to a mixture of ice, saturated aqueous NH_4Cl, and EtOAc with stirring. The mixture was transferred to a separatory funnel with the aid of MeOH and extracted with EtOAc (2×). The combined organics were dried with $MgSO_4$, filtered, and dried under reduced pressure. The residue was purified with a 100g SNAP column, ramping DCM/MeOH (90:10) in DCM from 0 to 30%, then isocratic at 30% (monitoring at 215 nm), providing product as a yellow solid as a 3:1 mixture of isomers (±)-*tert*-butyl ((1*S*,4*R*)-4-(((*S*)-2-hydroxy-2-methyl-1-phenylpropyl)-amino)cyclohexyl)carbamate (5.28 g, 14.57 mmol, 42.6% yield) (note: partial racemization of the phenyl bonded center occurs during this two-step protocol).

To a flask charged with (±)-*tert*-butyl ((1*S*,4*R*)-4-(((*S*)-2-hydroxy-2-methyl-1-phenylpropyl)amino)cyclohexyl)carbamate (5.2 g, 14.34 mmol) was added tetrahydrofuran (57.4 mL) and DIEA (12.53 mL, 71.7 mmol). The mixture was cooled in an ice water bath prior to the addition of triphosgene (4.26 g, 14.34 mmol). The resulting yellow suspension was allowed to stir and warm slowly to room temperature overnight. LC-MS of the yellow suspension indicated product as the main peak (observed as carbamic acid) with consumption of starting material.

To the mixture was added water, and the resulting orange solution was transferred to a separatory funnel, diluted with brine, and extracted with EtOAc (2×). The combined organics were dried under reduced pressure and purified with a 200g (15 μm spherical silica, Interchim) column, flow rate 65 mL/min, ramping DCM/MeOH (90:10) in DCM, from 0 to 30%, then isocratic at 30% (monitoring at 215 nm), providing some separation of regioisomers with peak slicing yielding the trans/cis product in 50% yield (~4:1).

To a flask charged with (±)-*tert*-butyl ((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)carbamate (2.81 g, 7.23 mmol) was added DCM (28.9 mL) followed by TFA (5.57 mL, 72.3 mmol). The resulting mixture was stirred for 3 h at room temperature.

The resulting mixture was dried under reduced pressure and purified with a 40g HP spherical silica column (Interchim), ramping DCM/MeOH (90:10) in DCM (0−100%) (monitoring at 215 nm) to provide product as a white foam (quantitative conversion). Chiral purification: column, Chiralpak AD-H, 5 cm × 30 cm; mobile phase, 20% methanol w/0.2% diethylamine/80% CO_2; flow rate, 350 mL/min; sample dissolution, 72 mg/mL in 1:1 dichloromethane/methanol, processed with 1.25 mL injections. Absolute stereochemistry establishment: only partial racemization had occurred in this sequence. We began with enantiopure (*S*) material, and therefore, the major enantiomer was the (*S*) enantiomer. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.79−7.64 (m, 2 H), 7.45−7.38 (m, 2 H), 7.38−7.32 (m, 1 H), 7.32−7.10 (m, 2 H), 4.59 (s, 1 H), 3.41−3.32 (m, 1 H), 2.84 (tt, *J* = 3.8, 11.6 Hz, 1 H), 1.94 (td, *J* = 3.2, 12.5 Hz, 1 H), 1.86−1.74 (m, 3 H), 1.59−1.52 (m, 1 H), 1.45 (s, 3 H), 1.37−1.07 (m, 3 H), 0.78 (s, 3 H). *m*/*z* (ESI) 289.2 $(M + H)^+$.

***N*-((1*S*,4*R*)-4-((*S*)-5,5-Dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)quinoline-8-carboxamide (29).** To a vial charged with **28** (0.750 g, 2.60 mmol) was added DCM (10.40 mL), DIEA (1.363 mL, 7.80 mmol), 8-quinoline carboxylic acid (Aldrich) (0.450 g, 2.60 mmol), and HATU (0.989 g, 2.60 mmol), respectively. The resulting suspension was shaken overnight at room temperature, leading to conversion to desired product according to LC-MS with more minor and polar impurities visible.

The resulting brown solution was dried under reduced pressure and purified with a 40g HP spherical silica column (15 μm spherical, Interchim), ramping DCM/MeOH (90:10) in DCM (0−30%, then isocratic at 30%, monitoring at 215 nm), providing product along with impurity coelution. The material was repurified (same column type) with a ramp of EtOAc in heptane (10% DCM throughout) (0−100%), leading to separation of some minor impurities, but NMR revealed ~10% starting quinoline carboxylic acid. The white solid was dissolved in DCM and extracted with 1 M Na_2CO_3 (3×). The organic phase was dried with Na_2SO_4, filtered, and dried under reduced pressure, providing a *N*-((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)quinoline-8-carboxamide (0.846 g, 1.907 mmol, 73.3% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.80 (d, *J* = 7.6 Hz, 1 H), 9.00 (dd, *J* = 1.9, 4.3 Hz, 1 H), 8.53 (ddd, *J* = 1.7, 7.7, 8.9 Hz, 2 H), 8.17 (dd, *J* = 1.6, 8.2 Hz, 1 H), 7.72 (t, *J* = 7.7 Hz, 1 H), 7.66 (dd, *J* = 4.3, 8.3 Hz, 1 H), 7.48−7.40 (m, 2 H), 7.40−7.12 (m, 3 H), 4.67 (s, 1 H), 3.71 (s, 1 H), 3.58−3.46 (m, 1 H), 2.08 (td, *J* = 3.0, 12.4 Hz, 1 H), 1.96−1.82 (m, 3 H), 1.62−1.53 (m, 1 H), 1.51−1.37 (m, 4 H), 1.37−1.27 (m, 1 H), 1.26−1.14 (m, 1 H), 0.81 (s, 3 H). *m*/*z* (ESI) 444.2 $(M + H)^+$, HRMS calcd for $C_{27}H_{29}N_3O_3$ $(M + H)^+$ 444.2287, found 444.2297.

***N*-((1*S*,4*R*)-4-((*S*)-5,5-Dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-1,5-naphthyridine-4-carboxamide (30).** To a vial charged with **28** (0.052 g, 0.180 mmol) was added DCM (0.721 mL), DIEA (0.094 mL, 0.541 mmol), 1,5-naphthyridine-4-carboxylic acid (Life Chemicals Building Blocks) (0.031 g, 0.180 mmol), and HATU (0.069 g, 0.180 mmol), respectively. The resulting suspension was shaken overnight at room temperature. The resulting mixture was dried under reduced pressure and purified using a 25g HP-spherical silica column (15 um), ramping DCM in MeOH (90:10) in DCM from 0 to 30%, then isocratic at 30%, affording 32 mg of product with minor impurities present. The material was repurified using a 2g SCX-2 column, washing with MeOH and then 2 M NH_3 in MeOH. The basic wash was dried under reduced pressure and lyophilized from MeOH/H_2O to yield product as a white powder *N*-((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-1,5-naphthyridine-4-carboxamide (0.009 g, 0.020 mmol, 11.23% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.25 (d, *J* = 7.7 Hz, 1 H), 9.13 (d, *J* = 4.4 Hz, 1 H), 9.07 (dd, *J* = 1.7, 4.2 Hz, 1 H), 8.57 (dd, *J* = 1.7, 8.5 Hz, 1 H), 8.20 (d, *J* = 4.4 Hz, 1 H), 7.89 (dd, *J* = 4.2, 8.5

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Hz, 1 H), 7.46−7.40 (m, 2 H), 7.39−7.16 (m, 3 H), 4.66 (s, 1 H), 3.77−3.67 (m, 1 H), 3.56−3.45 (m, 1 H), 2.10−2.03 (m, 1 H), 1.94−1.83 (m, 3 H), 1.62−1.55 (m, 1 H), 1.48 (s, 3 H), 1.47−1.42 (m, 1 H), 1.34 (dq, J = 3.2, 12.5 Hz, 1 H), 1.26−1.16 (m, 1 H), 0.81 (s, 3 H). m/z (ESI) 445.3 $(M + H)^+$. m/z (ESI) 444.3 $(M + H)^+$. HRMS calcd for $C_{26}H_{28}N_4O_3$ $(M + H)^+$ 445.2239, found 445.2235.

***N*-((1*S*,4*R*)-4-((*S*)-5,5-Dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-7-fluoro-1,5-naphthyridine-4-carboxamide (31).** To a solution of 5-fluoropyridin-3-amine (2 g, 0.01 mol) in ethanol (20 mL), 2, 2′-dimethyl-1, 3-dioxane-4,6-dione (Meldrum's acid) (2.44 g, 0.02 mol) and triethyl orthoformate (2.64 g, 0.01 mol) were added, and the reaction mixture was heated to reflux for 3 h with constant stirring. The reaction was monitored by TLC (TLC eluent: 40% EtOAc in petroleum ether, UV active). After the completion of the reaction, the mixture was allowed to cool to ambient temperature, yielding a white precipitate. The precipitate was filtered, washed with ethanol (20 mL), and dried under vacuum to afford 5-(((5-fluoropyridin-3-yl)amino)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione as an off white solid (1.2 g, 25%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.33 (d, J = 14.4 Hz, 1H), 8.71 (s, 1H), 8.65 (d, J = 14.4 Hz, 1H), 8.47 (d, J = 2.4 Hz, 1H), 8.17−8.13 (m, 1H), 1.68 (s, 6H).

To a stirred solution of dowtherm A at 250 °C was added 5-(((5-fluoropyridin-3-yl)amino)methylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (1 g, 0.003 mol) portionwise over a period of 10 min. The reaction was monitored by TLC (TLC eluent: 50% EtOAc in petroleum ether, UV active). After completion of the reaction, the mixture was cooled to ambient temperature and treated with diethyl ether to obtain a gray precipitate. The precipitate was stirred for 5−10 min and filtered, washed with diethyl ether, and dried under vacuum to obtain 7-fluoro-1,5-naphthyridin-4-ol as a tan colored solid (0.4 g, 65%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.68 (br s, 1H), 9.43 (s, 1H), 8.75 (t, J = 6.4 Hz, 1H), 8.59 (d, J = 8, 1H), 6.99 (d, J = 17.2 Hz, 1H). m/z (ESI) 165.1 $(M + H)^+$.

To a suspension of 7-fluoro-1,5-naphthyridin-4-ol (400 mg, 2.43 mmol) in DMF (8 mL) was added PBr_3 (660 mg, 2.43 mmol) dropwise at 50 °C for 10 min. After complete addition of PBr_3, the suspension became homogeneous, and a brown colored precipitate was obtained over a period of 30 min. The reaction was monitored by TLC (TLC eluent: 50% EtOAc in petroleum ether). After completion of reaction, the mixture was cooled to ambient temperature to generate more precipitate. The precipitate was filtered and washed with Et_2O (15 mL) and dried under vacuum to afford a brown colored hydrobromide salt of 8-bromo-2-methoxy[1, 5]naphthyridine which was converted to free base by treatment with saturated aqueous bicarbonate solution (10 mL) and product extraction into EtOAc (2 × 25 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to afford 8-bromo-3-fluoro-1,5-naphthyridine as a brown solid (200 mg, 36%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.19 (d, J = 2.4 Hz, 1H), 8.84 (d, J = 4.8 Hz, 1H), 8.44 (dd, J = 2.8, 9.2 Hz, 1H), 8.23 (d, J = 4.8 Hz, 1H). m/z (ESI) 227.1 $(M + H)^+$.

In a sealed tube, to a solution of 8-bromo-3-fluoro-1,5-naphthyridine (1.7 g, 0.0074 mol) in DMF (17 mL) were added dppf (207 mg, 0.0003 mol) and palladium acetate (84 mg, 0.0003 mol). The mixture was purged with argon for 5−10 min. Then, DIPEA (3.4 mL, 2 times) and acetic formic anhydride (3.4 mL, 2 times) were added slowly at RT. The reaction mixture was stirred for 16 h at 90 °C. After completion of the reaction, monitored by TLC (TLC eluent: 50% EtOAc in petroleum ether, UV active), the reaction mixture was cooled to ambient temperature and concentrated. The crude material was taken in 20% NaOH solution and extracted with ethyl acetate. Then the aqueous layer was acidified with 1 N HCl at 0−5 °C and then extracted with ethyl acetate. The extract was washed with water and saturated NaCl solution and dried over sodium sulfate. The organic solvent was evaporated to afford 7-fluoro-1,5-naphthyridine-4-carboxylic acid (950 mg, 66%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.2 (br s, 1H), 9.20 (d, J = 2.8 Hz, 1H), 9.16 (d, J = 4.4 Hz, 1H), 8.49 (dd, J = 2.8 Hz, 9.6 Hz, 1H), 7.98 (d, J = 4 Hz, 1H). m/z (ESI) 193.2 $(M + H)^+$.

To a vial charged with (*S*)-3-((1*R*,4*S*)-4-aminocyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one **28** (0.073 g, 0.253 mmol) was added DMF (1.013 mL), Et_3N (0.039 mL, 0.278 mmol), 7-fluoro-1,5-naphthyridine-4-carboxylic acid (0.049 g, 0.253 mmol), and 1-propanephosphonic acid cyclic anhydride (0.161 mL, 0.253 mmol), respectively. The resulting light brown solution was shaken at room temperature. After 4 h additional 1-propanephosphonic acid cyclic anhydride (0.161 mL, 0.253 mmol) was added, and the mixture was shaken overnight at room temperature. The mixture was dried under reduced pressure and purified using a 25g HP spherical silica column (15 μm, Interchim), ramping DCM/MeOH (90:10) in DCM from 0 to 100% with product elution occurring toward the 100% polar eluent along with a minor impurity (10−20%), which was more polar and visible by TLC and LC-MS. The material was repurified by dissolving in MeOH and using RP-HPLC (Gilson), ramping ACN in H_2O (10−90%, 0.1% TFA), affording separation of impurities. The product containing eluents were transferred to a separatory funnel, diluted with EtOAc, and extracted with saturated aqueous $NaHCO_3$. The organic layer was dried with Na_2SO_4, filtered, and dried under reduced pressure. The film obtained was lyophilized from MeOH/H_2O, providing product as an off-white solid *N*-((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)-cyclohexyl)-7-fluoro-1,5-naphthyridine-4-carboxamide (0.041 g, 0.089 mmol, 35.0% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.57 (d, J = 7.7 Hz, 1 H), 9.15 (d, J = 2.8 Hz, 1 H), 9.13 (d, J = 4.4 Hz, 1 H), 8.44 (dd, J = 2.8, 9.5 Hz, 1 H), 8.05 (d, J = 4.4 Hz, 1 H), 7.46−7.15 (m, 5 H), 4.66 (s, 1 H), 3.70 (tdt, J = 3.9, 7.6, 11.5 Hz, 1 H), 3.53−3.41 (m, 1 H), 2.08−1.99 (m, 1 H), 1.92−1.81 (m, 2 H), 1.61−1.53 (m, 1 H), 1.47 (s, 3 H), 1.45−1.13 (m, 4 H), 0.80 (s, 3 H). m/z (ESI) 463.3 $(M + H)^+$. HRMS calcd for $C_{26}H_{27}FN_4O_3$ $(M + H)^+$ 463.2145, found 463.215.

(*S*)-5,5-Dimethyl-3-((1*R*,4*S*)-4-(2-oxo-2,3-dihydro-1H-imidazo[4,5-*c*]pyridin-1-yl)cyclohexyl)-4-phenyloxazolidin-2-one (34). To a flask charged with **28** (3.00 g, 7.46 mmol) was added acetonitrile (24.85 mL), triethylamine (2.078 mL, 14.91 mmol), and 4-chloro-3-nitropyridine (Aldrich) (1.206 g, 7.60 mmol), respectively. The resulting orange solution was shaken at 80 °C overnight, leading to 80−90% conversion according to LC-MS. Additional 4-chloro-3-nitropyridine was added (0.4 equiv, 480 mg), and stirring and heating were continued. After 30 min no additional conversion had occurred. Additional triethylamine (2.078 mL, 14.91 mmol) was added, and heating and stirring were continued at 80 °C. After 1 h LC-MS of the red/orange solution indicated consumption of starting material. The mixture was cooled in an ice water bath and stirred at 0 °C, and saturated aqueous NH_4Cl was added. The mixture was transferred to a separatory

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funnel and extracted with EtOAc (2×). The combined organics were dried with Na_2SO_4, filtered, and dried under reduced pressure. The crude residue obtained was purified with an 80g HP spherical silica column (15 μm spherical), ramping EtOAc in heptane (0−100%, 5% DCM throughout), leading to the isolation of product as a yellow solid 5,5-dimethyl-3-((1*R*,4*R*)-4-((3-nitropyridin-4-yl)amino)cyclohexyl)-4-phenyloxazolidin-2-one (1.83 g, 4.46 mmol, 59.8% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (s, 1 H), 8.25 (d, J = 6.3 Hz, 1 H), 8.00 (d, J = 7.2 Hz, 1 H), 7.49−7.29 (m, 4 H), 7.23−7.06 (m, 1 H), 6.64 (d, J = 6.3 Hz, 1 H), 4.39 (s, 1 H), 3.50−3.34 (m, 2 H), 2.24−2.05 (m, 3 H), 2.04−1.95 (m, 1 H), 1.91−1.80 (m, 1 H), 1.63−1.56 (m, 1 H), 1.46−1.26 (m, 2 H), 0.94 (s, 3 H). m/z (ESI) 411.2 $(M + H)^+$.

To a flask charged with (*S*)-5,5-dimethyl-3-((1*R*,4*S*)-4-((3-nitropyridin-4-yl)amino)cyclohexyl)-4-phenyloxazolidin-2-one (1.82 g, 4.43 mmol) was added EtOH (89 mL), Raney nickel, slurry, in water (4.88 mL, 740 mmol) (added roughly using pipet). The flask was sealed and placed under nitrogen, and the reaction mixture was heated to 40 °C. To the resulting yellow solution was added hydrazine hydrate solution (2.071 mL, 66.5 mmol) dropwise. The resulting mixture was stirred for 3 h at 40 °C. The mixture was filtered through Celite, which was washed with MeOH. The filtrate was dried under reduced pressure, providing an off-white foam after drying under high vacuum overnight, which was used directly in the next step (quantitative yield). m/z (ESI) 381.4 $(M + H)^+$.

To a flask charged with 3-((1*R*,4*R*)-4-((3-aminopyridin-4-yl)amino)cyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (1.60 g, 4.21 mmol) was added THF (16.82 mL) and DIEA (2.57 mL, 14.72 mmol). The mixture was cooled in an ice water bath prior to the addition of CDI (2.387 g, 14.72 mmol). The resulting mixture was allowed to stir and warm slowly to room temperature (ice melt) over 2 h. To the yellow solution was added saturated aqueous NH_4Cl, and the mixture was transferred to a separatory funnel and extracted with EtOAc (2×). The combined organics were washed with brine, dried with Na_2SO_4, filtered, and dried under reduced pressure. The crude residue obtained was purified with a 40g HP spherical silica column (Interchim), ramping DCM/MeOH/NH_4OH (90:10:1) in DCM from 0 to 45%, then isocratic at 45%, affording the title compound as a white solid (1.3 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.15 (d, J = 0.6 Hz, 1 H), 8.08 (d, J = 5.4 Hz, 1 H), 7.46−7.39 (m, 3 H), 7.39−7.35 (m, 1 H), 7.28 (br s, 2 H), 4.66 (s, 1 H), 4.04 (tt, J = 3.8, 12.4 Hz, 1 H), 3.68 (tt, J = 3.9, 11.6 Hz, 1 H), 2.25−2.03 (m, 2 H), 2.02−1.83 (m, 2 H), 1.77−1.68 (m, 1 H), 1.61 (s, 3 H), 1.48 (s, 3 H), 1.29 (dq, J = 3.1, 12.7 Hz, 1 H), 0.80 (s, 3 H). m/z (ESI) 407.2 $(M + H)^+$. HRMS calcd for $C_{23}H_{26}N_4O_3$ $(M + H)^+$ 407.2083, found 407.2089.

(*S*)-5,5-Dimethyl-3-((1*R*,4*S*)-4-(2-oxo-2,3-dihydro-1H-imidazo[4,5-*b*]pyridin-1-yl)cyclohexyl)-4-phenyloxazolidin-2-one (35). To a vial charged with (*S*)-3-((1*R*,4*S*)-4-aminocyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (**28**; 0.560 g, 1.942 mmol) (this intermediate **28** lot existed as a ∼3:1 *S*/*R* mixture of phenyl chirality) was added acetonitrile (6.47 mL), triethylamine (0.541 mL, 3.88 mmol), and 3-fluoro-2-nitropyridine (Matrix Scientific) (276 mg, 1.94 mmol), respectively. The resulting orange solution was shaken at 80 °C for 4 h, leading to conversion to the desired product as the major species according to LC-MS with about 50% conversion. The mixture was shaken for 48 h at 80 °C, providing complete conversion to desired product according to LC-MS. The mixture was dried under reduced pressure and purified using a 25g SNAP column (Biotage), ramping EtOAc in heptane (0−100%, with 10% DCM throughout), providing product as a yellow foam (*S*)-5,5-dimethyl-3-((1*R*,4*S*)-4-((2-nitropyridin-3-yl)amino)cyclohexyl)-4-phenyloxazolidin-2-one (0.714 g, 1.739 mmol, 90% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (dd, J = 1.3, 3.9 Hz, 1 H), 7.66 (dd, J = 1.2, 8.9 Hz, 1 H), 7.53 (dd, J = 4.0, 8.7 Hz, 1 H), 7.46−7.39 (m, 2 H), 7.38−7.15 (m, 3 H), 4.60 (s, 1 H), 3.53−3.41 (m, 2 H), 2.02 (td, J = 3.1, 12.8 Hz, 1 H), 1.98−1.79 (m, 3 H), 1.58 (d, J = 10.5 Hz, 1 H), 1.47 (s, 3 H), 1.46−1.37 (m, 1 H), 1.37−1.25 (m, 2 H), 0.78 (s, 3 H). m/z (ESI) 411.2 $(M + H)^+$.

To a flask charged with (*S*)-5,5-dimethyl-3-((1*R*,4*S*)-4-((2-nitropyridin-3-yl)amino)cyclohexyl)-4-phenyloxazolidin-2-one (0.702 g, 1.710 mmol) was added EtOH (34.2 mL), Raney nickel, slurry, in water (1.884 mL, 286 mmol) (added using pipet). The flask was sealed and placed under nitrogen, and heated to 40 °C. To the resulting yellow solution was added hydrazine hydrate solution (0.799 mL, 25.7 mmol) dropwise. The resulting mixture was stirred overnight at 40 °C.

The resulting mixture was filtered through Celite which was washed with MeOH. The filtrate was dried under reduced pressure and the material purified with SCX-2 (5g), washing with MeOH, then 2 M NH_3 in MeOH, but much material came through the column with the initial MeOH wash. The washes were combined and dried under reduced pressure. The crude residue was purified with a 50g SNAP column (Biotage), ramping MeOH in DCM from 0 to 25%, leading to isolation of product as a purple film (*S*)-3-((1*R*,4*S*)-4-((2-aminopyridin-3-yl)amino)cyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (0.580 g, 1.524 mmol, 89% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45−7.38 (m, 2 H), 7.38−7.23 (m, 3 H), 7.21 (dd, J = 1.5, 4.9 Hz, 1 H), 6.52 (d, J = 6.8 Hz, 1 H), 6.38 (dd, J = 4.9, 7.6 Hz, 1 H), 5.36 (s, 2 H), 4.61 (s, 1 H), 4.38 (d, J = 7.4 Hz, 1 H), 3.53−3.42 (m, 1 H), 3.06−2.95 (m, 1 H), 2.03 (td, J = 3.2, 12.9 Hz, 1 H), 1.92−1.76 (m, 3 H), 1.58−1.49 (m, 1 H), 1.46 (s, 3 H), 1.26−1.03 (m, 3 H), 0.78 (s, 3 H). m/z (ESI) 381.4 $(M + H)^+$.

To a flask charged with (*S*)-3-((1*R*,4*S*)-4-((2-aminopyridin-3-yl)amino)cyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (0.560 g, 1.472 mmol) was added THF (5.89 mL), DIEA (0.566 mL, 3.24 mmol), and CDI (0.525 g, 3.24 mmol), respectively. The dark mixture was stirred at 45 °C overnight, providing ∼70% conversion according to LC-MS, with clean conversion and starting material remaining. An additional 1 equiv of DIEA and CDI were added, and heating continued at 45 °C for 2 h. The dark purple solution was dried under reduced pressure and purified with a 50g SNAP column (Biotage), ramping DCM/MeOH (90:10) in DCM from 0 to 30%, then isocratic at 30%, providing elution of product. Using the following conditions the enantiomers were successfully separated (AD-H (2 × 20 cm) column, 25% MeOH (0.1% DEA)/CO_2; 100 bar; 70 mL/min; 220 nm detection; injection volume, 1 mL; 18 mg/mL, 4:1 MeOH/DCM). The NMR showed the presence of substantial diethyl amine. Each of the enantiomers were dissolved in DCM/trace MeOH and extracted with H_2O (2×). The organics were dried with Na_2SO_4, filtered, and dried under reduced pressure, then lyophilized from MeOH/H_2O, affording the title compound **35** as a light pink solid (*S* enantiomer, peak 2, major enantiomer −235 mg, 39%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.47 (br s, 1 H), 7.86 (d, J = 3.9 Hz, 1 H), 7.67 (d, J = 7.9 Hz, 1 H), 7.42 (d, J = 6.5 Hz, 2 H), 7.39−7.13 (m, 3 H), 6.97−6.88 (m, 1 H),

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4.66 (s, 1 H), 4.11−3.98 (m, 1 H), 3.74−3.60 (m, 1 H), 2.24−1.86 (m, 4 H), 1.74 (d, J = 11.2 Hz, 1 H), 1.60 (t, J = 12.6 Hz, 2 H), 1.48 (s, 3 H), 1.28 (q, J = 11.3 Hz, 1 H), 0.80 (s, 3 H). m/z (ESI) 407.1 $(M + H)^+$. HRMS calcd for $C_{23}H_{26}N_4O_3$ $(M + H)^+$ 407.2083, found 407.2084.

1-((1*S*,4*R*)-4-((*S*)-5,5-Dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-2-oxo-2,3-dihydro-1H-benzo[*d*]-imidazole-5-carbonitrile (36). To a vial charged with (*S*)-3-((1*R*,4*S*)-4-aminocyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (**28**; 0.150 g, 0.520 mmol) (this lot existed as a ∼3:1 *S*/*R* mixture of phenyl chirality) was added acetonitrile (1.734 mL), triethylamine (0.145 mL, 1.040 mmol), and 4-fluoro-3-nitrobenzonitrile (Alfa Aesar) (0.086 g, 0.520 mmol), respectively. The resulting orange solution was shaken at 80 °C overnight. The resulting mixture was dried under reduced pressure and the crude material purified with a 25g SNAP column, ramping EtOAc in heptane from 0 to 100% with 10% DCM throughout, providing product as a yellow solid 4-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)-cyclohexyl)amino)-3-nitrobenzonitrile (0.172 g, 0.396 mmol, 76% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.47 (d, J = 2.0 Hz, 1 H), 8.25 (d, J = 7.4 Hz, 1 H), 7.58−7.51 (m, 1 H), 7.47−7.28 (m, 4 H), 7.14 (br s, 1 H), 6.85 (d, J = 9.2 Hz, 1 H), 4.39 (s, 1 H), 3.50−3.33 (m, 2 H), 2.24−2.06 (m, 3 H), 2.02−1.93 (m, 1 H), 1.90−1.79 (m, 1 H), 1.62−1.49 (m, 4 H), 1.47−1.26 (m, 2 H), 0.92 (s, 3 H). m/z (ESI) 435.2 $(M + H)^+$.

To a flask charged with 4-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-3-nitrobenzonitrile (0.172 g, 0.396 mmol) was added ethanol (2.64 mL) followed by tin(II) chloride (0.225 g, 1.188 mmol). The resulting yellow suspension was heated overnight at 80 °C. LC-MS of the resulting yellow solution indicates product as a major species along with minor impurities which are close to product in retention time. The solution was cooled to room temperature and filtered through a Si-thiol column. The filtrate was dried under reduced pressure and purified with a 25g SNAP column, ramping DCM/MeOH (90:10) in DCM from 0 to 30%, then isocratic at 30% to yield product along with minor impurities which had coeluted. The material obtained was used without further purification. Product was obtained as a yellow oil 3-amino-4-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)benzonitrile (0.117 g, 0.289 mmol, 73.1% yield). m/z (ESI) 405.2 $(M + H)^+$.

To a flask charged with 3-amino-4-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-benzonitrile (0.878 g, 2.171 mmol) was added THF (8.68 mL) and DIEA (1.327 mL, 7.60 mmol). The resulting solution was cooled in an ice water bath prior to the addition of CDI (1.23 g, 7.60 mmol). The resulting mixture was stirred for 3 h and allowed to slowly warm to ambient temperature (ice melt), providing a greenish suspension. LC-MS indicated complete conversion to product. The mixture was dried under reduced pressure and purified with a 40g HP spherical silica column (15 μm spherical, Interchim), ramping DCM/MeOH (90:10) in DCM from 0 to 30%, then isocratic at 30%, yielding partial separation of clean product obtained as a white film as well as a darker eluting mixture primarily containing product. The dark mixture was purified with an SCX-2 column (5g), washing with MeOH, leading to elution of a yellow solution containing product. After drying, the pale yellow solid was triturated with diethyl ether, and the off-white solid obtained was combined with the clean material from the initial column. NMR revealed still some very minor impurities in the aliphatic region. The solid was ground with a spatula and triturated again with ether, providing a white solid 1-((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-2-oxo-2,3-dihydro-1H-benzo[*d*]imidazole-5-carbonitrile (790 mg, 85%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.53 (d, J = 8.3 Hz, 1 H), 7.49−7.34 (m, 4 H), 7.29 (d, J = 8.3 Hz, 3 H), 4.65 (s, 1 H), 4.12−4.04 (m, 1 H), 3.70−3.61 (m, 1 H), 2.27−2.07 (m, 2 H), 2.02−1.85 (m, 2 H), 1.73 (d, J = 11.6 Hz, 1 H), 1.68−1.54 (m, 2 H), 1.48 (s, 3 H), 1.36−1.25 (m, 1 H), 0.82−0.77 (m, 3 H). m/z (ESI) 431.1 $(M + H)^+$. HRMS calcd for $C_{25}H_{26}N_4O_3$ $(M + H)^+$ 431.2083, found 431.2083.

1-((1*S*,4*R*)-4-((*S*)-5,5-Dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-2-oxo-2,3-dihydro-1H-benzo[*d*]-imidazole-4-carbonitrile (37). To a vial charged with 3-((1*R*,4*R*)-4-aminocyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one 2,2,2-trifluoroacetate (**28**; 0.300 g, 0.746 mmol) (this lot existed as a ∼3:1 *S*/*R* mixture of phenyl chirality) was added acetonitrile (2.485 mL), DIEA (0.260 mL, 1.491 mmol), and 3-chloro-2-nitrobenzonitrile (Biogene Organics) (0.136 g, 0.746 mmol), respectively. The vial was sealed and heated to 80 °C overnight, providing an orange/brown solution and about 50% conversion according to LC-MS. Additional DIEA (0.260 mL, 1.491 mmol) was added, and heating and shaking continued. After 1 additional hourr at 80 °C, no additional conversion was observed by LC-MS. The mixture was heated to 110 °C overnight, leading to conversion to product along with two major impurities which have similar retention times to product. The mixture was dried under reduced pressure and purified with a 25g column, ramping DCM/MeOH/NH_4OH (90:10:1) in DCM from 0 to 15%, then 15% isocratic, which yielded some separation of impurities but not complete separation. The material (∼50% purity) was used without further purification, isolated as an orange/brown solid 3-(((1*R*,4*R*)-4-(5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-2-nitrobenzonitrile (0.180 g, 0.414 mmol, 55.6% yield). m/z (ESI) 435.0 $(M + H)^+$.

To a flask charged with 3-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-2-nitrobenzonitrile (0.180 g, 0.414 mmol) was added ethanol (2.76 mL) followed by tin(II) chloride (0.236 g, 1.243 mmol). The resulting yellow suspension was heated at 80 °C for 2 h, providing an orange brown solution. LC-MS indicated product as a major species along with impurities (present in starting material) which are close to product in retention time. The solution was cooled to room temperature and filtered through a Si-thiol column. The filtrate was dried under reduced pressure and purified with a 25g SNAP column, ramping DCM/MeOH (90:10) in DCM from 0 to 50%, then isocratic at 50% to yield product along with the impurities which coeluted (most impurities present from crude). The material was further purified with a 2g SCX-2 column, washing with MeOH and then 2 M NH_3 in MeOH to yield a brown oil 2-amino-3-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)-cyclohexyl)amino)benzonitrile (0.124 g, 0.307 mmol, 74.0% yield) which had been enriched in purity relative to after MPLC (∼75% pure). The material was used as is without further purification. m/z (ESI) 405.0 $(M + H)^+$.

To a vial charged with 2-amino-3-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-benzonitrile (0.124 g, 0.307 mmol) was added THF (1.226 mL), DIEA (0.187 mL, 1.073 mmol), and CDI (0.174 g, 1.073 mmol), respectively. The dark mixture was stirred for 1 h at room temperature, yielding no conversion according to LC-MS.

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The mixture was heated to 60 °C. After 2 h LC-MS indicated about 30% conversion. Heating and shaking at 60 °C was continued overnight. LC-MS indicated product as the main species.

The dark solution was dried under reduced pressure and purified with RP-HPLC, ramping ACN in H_2O (10−90%, 0.1% TFA), affording incomplete purification. The product containing eluents were dried under reduced pressure and repurified. Separation #1: Chiralpak OJ-H, 2 cm × 25 cm, 80% CO_2/20% methanol w/0.2% DEA, 80 mL/min, 254 nm; dissolution, 20 mL of 3:1 MeOH/DCM; sample processed with 0.25 mL injections and 2.75 min cycle time. Separation #2: Chiralpak IA, 2 cm × 25 cm, 75% CO_2/25% methanol w/0.2% DEA, 80 mL/min, 215 nm; dissolution, 10 mL of 1:1 MeOH/DCM; sample processed with 0.15 mL injections and 5.25 min cycle time. Desired major enantiomer eluted first. Product contained a minor amount of DEA. The material was passed through a 2g SCX-2 column and the initial wash dried under reduced pressure and lyophilized from MeOH/H_2O, providing product as a white solid 1-((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-2-oxo-2,3-dihydro-1H-benzo[*d*]imidazole-4-carbonitrile (17 mg, 0.039 mmol, 12.88% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.90 (s, 1 H), 7.66 (d, J = 7.8 Hz, 1 H), 7.46−7.39 (m, 2 H), 7.39−7.15 (m, 4 H), 7.07 (t, J = 8.0 Hz, 1 H), 4.65 (s, 1 H), 4.11−4.02 (m, 1 H), 3.66 (d, *J* = 11.8 Hz, 1 H), 2.26−2.06 (m, 2 H), 2.03−1.84 (m, 2 H), 1.80−1.69 (m, 1 H), 1.67−1.55 (m, 2 H), 1.48 (s, 3 H), 1.30 (dq, J = 3.7, 12.8 Hz, 1 H), 0.80 (s, 3 H). *m*/*z* (ESI) 431.1 (M + H)$^+$. HRMS calcd for $C_{25}H_{26}N_4O_3$ (M + H)$^+$ 431.2083, found 431.2082.

1-((1*S*,4*R*)-4-((*S*)-5,5-Dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-6-fluoro-2-oxo-2,3-dihydro-1H-benzo[*d*]imidazole-5-carbonitrile (39). A vial charged with (*S*)-3-((1*R*,4*S*)-4-aminocyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (**28**; 0.184 g, 0.638 mmol), acetonitrile (1.734 mL), and DIEA (0.181 mL, 1.04 mmol) was cooled to 0 °C, and 2,4-difluoro-5-nitrobenzonitrile (Aldrich) (0.078 g, 0.342 mmol) was added. The vial was sealed, and the temperature was slowly raised to room temperature over 16 h, providing a brown solution with product as the main species according to LC-MS. The solution was dried under reduced pressure and purified with a 25g SNAP column (Biotage), ramping DCM/MeOH (90:10) in DCM (0−25%, then isocratic at 30%, 215 nm detection), providing 4-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-2-fluoro-5-nitrobenzonitrile (0.282 g, 0.623 mmol, 98% yield) as a yellow solid. *m*/*z* (ESI) 453.3 (M + H)$^+$.

To a flask charged with 4-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-2-fluoro-5-nitrobenzonitrile (0.282 g, 0.623 mmol) was added ethanol (6.23 mL) and tin(II) chloride (0.019 mL, 0.405 mmol), and the resulting yellow suspension was shaken for 3 h at 80 °C. LC-MS indicated complete conversion. The solution was dried under reduced pressure and purified with a 25g SNAP column (Biotage), ramping DCM/MeOH (90:10) in DCM from 0 to 30%, then isocratic at 30% to provide 5-amino-4-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-amino)-2-fluorobenzonitrile (0.258 g, 0.611 mmol, 98% yield) as yellow brown solid. *m*/*z* (ESI) 423.3 (M + H)$^+$.

To a flask charged with 5-amino-4-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-2-fluorobenzonitrile (0.258 g, 0.611 mmol) was added THF (2.043 mL), DIEA (0.372 mL, 2.137 mmol), and CDI (0.347 g, 2.137 mmol). The mixture was stirred at room temperature for 16 h. LC-MS indicated complete reaction. The solution was dried under reduced pressure and purified with a 25g SNAP column (Biotage), ramping DCM/MeOH (90:10) in DCM from 0 to 40%, then isocratic at 40% to provide product 1-((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)-cyclohexyl)-6-fluoro-2-oxo-2,3-dihydro-1H-benzo[*d*]imidazole-5-carbonitrile as a light yellow solid (0.151 g, 0.337 mmol, 55% yield).^{1}H NMR (400 MHz, DMSO-d_6) δ 11.34 (br s, 1 H), 7.73 (d, *J* = 10.5 Hz, 1H), 7.45−7.2 (m, 6 H), 4.65 (s, 1 H), 4.13−4.03 (m, 1 H), 3.77−3.68 (m, 1 H), 2.28−2.07 (m, 2 H), 2.02−1.83 (m, 2 H), 1.75−1.55 (m, 2 H), 1.48 (s, 3 H), 1.38−1.25 (m, 1 H), 0.80 (s, 3 H). *m*/*z* (ESI) 449.1 (M + H)$^+$. HRMS calcd for $C_{25}H_{25}FN_4O_3$ (M + H)$^+$ 449.1989, found 449.1985.

1-((1*S*,4*R*)-4-((*S*)-5,5-Dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-4-fluoro-2-oxo-2,3-dihydro-1H-benzo[*d*]imidazole-5-carbonitrile (40). To a vial charged with (*S*)-3-((1*R*,4*S*)-4-aminocyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (**28**; 0.114 g, 0.395 mmol) was added acetonitrile (1.318 mL) and triethylamine (0.110 mL, 0.791 mmol). The resulting solution was cooled in an ice water bath. In a separate vial, 2,4-difluoro-3-nitrobenzonitrile (0.076 g, 0.415 mmol) was dissolved in ACN (200 μL) and added slowly to the cooled solution of amine. The mixture was allowed to slowly warm to room temperature overnight (ice melt). The resulting orange solution was dried under reduced pressure and purified with a 25g SNAP column, ramping DCM/MeOH (90:10) in DCM from 0 to 30%, then isocratic at 30% (detection at 215 nm) to provide product, obtained as a yellow solid 4-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-2-fluoro-3-nitrobenzonitrile (0.161 g, 0.356 mmol, 90% yield) with about 20% impurity present. The material was used as is for a subsequent step. *m*/*z* (ESI) 453.4 (M + H)$^+$.

To a vial charged with 4-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-2-fluoro-3-nitrobenzonitrile (0.161 g, 0.356 mmol) was added EtOH (1.423 mL) and tin(II) chloride (0.202 g, 1.067 mmol), respectively. The suspension was heated at 80 °C for 3 h, yielding an orange solution. The resulting mixture was dried under reduced pressure, and the residue obtained was purified with a 25g SNAP column, ramping DCM/MeOH (90:10) in DCM (0−30%, then isocratic at 30%, detection at 215 nm), leading to isolation of product, obtained as an orange oil (145 mg, 96%). *m*/*z* (ESI) 423.4 (M + H)$^+$.

To a flask charged with 3-amino-4-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-2-fluorobenzonitrile (0.145 g, 0.343 mmol) was added THF (1.373 mL) and DIEA (0.210 mL, 1.201 mmol) and CDI (0.195 g, 1.201 mmol), respectively. The mixture was shaken at room temperature for 1.5 h. The mixture was dried under reduced pressure and the residue purified with a 25g HP spherical silica column (15 μm spherical silica, Interchim), ramping DCM/MeOH (90:10) in DCM from 0 to 25%, then isocratic at 25%, with detection at 215 nm, providing product as a white solid (118 mg, 77%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.94 (br s, 1 H), 7.52−7.13 (m, 7 H), 4.67−4.62 (m, 1 H), 4.09 (tt, *J* = 3.4, 12.4 Hz, 1 H), 3.66 (tt, *J* = 3.8, 11.7 Hz, 1 H), 2.40−2.05 (m, 2 H), 2.03−1.84 (m, 2 H), 1.81−1.55 (m, 3 H), 1.48 (s, 3 H), 1.36−1.21 (m, 1 H), 0.80 (s, 3 H). *m*/*z* (ESI) 449.1 (M + H)$^+$, HRMS calcd for $C_{25}H_{25}FN_4O_3$ (M + H)$^+$ 449.1989, found 449.1987. See Supporting Information Figures

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S1 and S2 for IR spectrum and NMR proton, carbon peak assignments, respectively.

3-((1*S*,4*R*)-4-((*S*)-5,5-Dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-2-oxo-2,3-dihydro-1H-imidazo[4,5-*b*]pyridine-6-carbonitrile (41). To a vial charged with (*S*)-3-((1*R*,4*S*)-4-aminocyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (**28**; 0.194 g, 0.673 mmol) was added acetonitrile (2.242 mL), DIEA (0.235 mL, 1.345 mmol), and 5-bromo-2-chloro-3-nitropyridine (Matrix Scientific) (0.168 g, 0.706 mmol,) respectively. The vial was sealed and heated to 90 °C for 2 h, providing a brown solution with product as the main species according to LC-MS. The solution was dried under reduced pressure and purified with a 25g SNAP column, ramping DCM/MeOH (90:10) in DCM (0−30%, then isocratic at 30%, detection at 215 nm), providing (*S*)-3-((1*R*,4*S*)-4-((5-bromo-3-nitropyridin-2-yl)amino)cyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (0.315 g, 0.644 mmol, 96% yield) as a yellow/orange solid. *m*/*z* (ESI) 490.1 $(M + H)^+$.

To a flask charged with (*S*)-3-((1*R*,4*S*)-4-((5-bromo-3-nitropyridin-2-yl)amino)cyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (0.310 g, 0.633 mmol) was added EtOH (12.67 mL), Raney 3202 nickel, slurry, in water (0.698 mL, 106 mmol) (added roughly using pipet). The flask was sealed and placed under nitrogen, and heated to 40 °C. To the resulting yellow solution was added hydrazine hydrate solution (0.296 mL, 9.50 mmol) dropwise. The resulting mixture was stirred at 40 °C overnight. LC-MS of the cloudy white suspension indicated complete conversion to desired product. The mixture was cooled to room temperature and filtered through Celite, which was washed with MeOH. The filtrate was dried under reduced pressure and then purified using a 5g SCX-2 column, washing with MeOH and then 2 M NH_3 in MeOH. The basic was dried under reduced pressure, providing (*S*)-3-((1*R*,4*S*)-4-((3-amino-5-bromopyridin-2-yl)amino)cyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (0.270 g, 0.588 mmol, 93% yield) as a purple film. *m*/*z* (ESI) 459.1 $(M + H)^+$.

To a flask charged with (*S*)-3-(4-((3-amino-5-bromopyridin-2-yl)amino)cyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (0.270 g, 0.588 mmol) was added THF (2.351 mL), DIEA (0.359 mL, 2.057 mmol), and CDI (0.334 g, 2.057 mmol), respectively. The mixture was shaken at room temperature overnight. LC-MS of the dark solution indicated complete reaction. The mixture was dried under reduced pressure and purified with a 25g HP 15 μm spherical silica column (Interchim), ramping DCM/MeOH (90:10) in DCM (0−30%, then isocratic at 30%, detection at 215 nm), yielding product as an off-white solid, (*S*)-3-((1*S*,4*R*)-4-(6-bromo-2-oxo-1H-imidazo[4,5-*b*]pyridin-3(2H)-yl)cyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (0.230 g, 0.474 mmol, 81% yield) upon drying. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.27 (br s, 1 H), 8.00 (d, J = 2.0 Hz, 1 H), 7.52−7.14 (m, 6 H), 4.67 (s, 1 H), 4.12−4.01 (m, 1 H), 3.52−3.40 (m, 1 H), 2.42−2.17 (m, 2 H), 2.01−1.83 (m, 2 H), 1.75 (d, J = 12.0 Hz, 1 H), 1.61 (t, J = 13.2 Hz, 2 H), 1.47 (s, 3 H), 1.33−1.16 (m, 1 H), 0.81 (s, 3 H). *m*/*z* (ESI) 485.1 $(M + H)^+$.

To a 0.5−2.0 mL microwave vial charged with (*S*)-3-((1*S*,4*R*)-4-(6-bromo-2-oxo-1H-imidazo[4,5-*b*]pyridin-3(2H)-yl)cyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (0.200 g, 0.412 mmol) was added DMF (1.648 mL) followed by zinc cyanide (0.026 mL, 0.412 mmol) and [1,1-bis-(diphenylphosphino)ferrocene]palladium(II) chloride, complex with dichloromethane (1:1) (0.034 g, 0.041 mmol). The resulting mixture was purged with argon, ,sealed and irradiated for 30 min at 160 °C. LC-MS indicated that no conversion had occurred. To the mixture was added $Pd(PPh_3)_4$ (0.048 g, 0.041 mmol) and 2 equiv of $Zn(CN)_2$. The vessel was purged with argon and heated to 120 °C for 90 min, yielding complete conversion according to LC-MS.

The suspension was cooled to room temperature and filtered through a 5g SCX-2 column, washing with MeOH, then 2 M NH_3 in MeOH. The product eluted during the initial MeOH wash. This wash was dried under reduced pressure and the crude brown oil purified with a 25g HP spherical silica column (15 μm, Interchim), ramping DCM/MeOH (90:10) in DCM (0−30%, then isocratic at 30%, detection at 215 nm), yielding an off-white solid with a minor yellow discoloration (168 mg). The material was triturated with MeOH and washed with a small volume of MeOH to yield 3-((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-2-oxo-2,3-dihydro-1H-imidazo[4,5-*b*]pyridine-6-carbonitrile as a white solid (56 mg, 31%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.56 (br s, 1 H), 8.38 (d, J = 1.9 Hz, 1 H), 7.67 (d, J = 1.9 Hz, 1 H), 7.47−7.17 (m, 5 H), 4.67 (s, 1 H), 4.13 (tt, J = 3.8, 12.3 Hz, 1 H), 3.53−3.41 (m, 1 H), 2.41−2.17 (m, 2 H), 2.01−1.84 (m, 2 H), 1.83−1.73 (m, 1 H), 1.71−1.58 (m, 2 H), 1.47 (s, 3 H), 1.35−1.20 (m, 1 H), 0.81 (s, 3 H). *m*/*z* (ESI) 432.0 $(M + H)^+$, HRMS calcd for $C_{23}H_{25}BrN_4O_3$ $(M + H)^+$ 485.1188, found 485.1185.

(*S*)-5,5-Dimethyl-3-((1*R*,4*S*)-4-(2-oxo-1H-imidazo[4,5-*b*]pyridin-3(2H)-yl)cyclohexyl)-4-phenyloxazolidin-2-one (46). To a flask charged with toluene (37.3 mL) was added *trans*-*N*-boc-1,4-cyclohexanediamine (2.00 g, 9.33 mmol), potassium carbonate (1.290 g, 9.33 mmol), and 2-fluoro-3-nitropyridine (Matrix Scientific) (1.326 mL, 9.33 mmol). The resulting bright yellow solution was heated overnight at 120 °C, affording a bright yellow suspension. The mixture was diluted with water, transferred to a separatory funnel, and extracted with EtOAc (2×). The combined organics were dried with Na_2SO_4, filtered, and dried under reduced pressure, providing a bright yellow solid which was triturated with DCM, providing product as a bright yellow solid *tert*-butyl ((1*R*,4*R*)-4-((3-nitropyridin-2-yl)amino)cyclohexyl)carbamate (1.51 g, 4.49 mmol, 48.1% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.49 (dd, J = 1.8, 4.5 Hz, 1 H), 8.41 (dd, J = 1.8, 8.4 Hz, 1 H), 8.06 (d, J = 7.7 Hz, 1 H), 6.79−6.69 (m, 2 H), 4.13−4.00 (m, 1 H), 3.29−3.20 (m, 1 H), 2.01−1.92 (m, 2 H), 1.82 (d, J = 10.6 Hz, 2 H), 1.55−1.41 (m, 2 H), 1.38 (s, 9 H), 1.35−1.21 (m, 2 H). *m*/*z* (ESI) 281.2 $(M + H)^+$.

To a flask charged with *tert*-butyl ((1*R*,4*R*)-4-((3-nitropyridin-2-yl)amino)cyclohexyl)carbamate (1.500 g, 4.46 mmol) was added EtOH (12.74 mL) and tin(II) chloride (2.54 g, 13.38 mmol). The resulting yellow suspension was heated to 80 °C under nitrogen overnight, providing a light brown solution containing primarily product according to LC-MS. The mixture was dried under reduced pressure and purified with a 50g SNAP column (Biotage), ramping DCM/MeOH (90:10) in DCM from 0 to 100% (215 nm detection), yielding product as an orange foam. The material was purified with a 10g SCX-2 column washing with MeOH, then with NH_3 in MeOH, providing product as a brown flaky solid *tert*-butyl ((1*R*,4*R*)-4-((3-aminopyridin-2-yl)amino)cyclohexyl)carbamate (0.840 g, 2.74 mmol, 61.5% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.31 (dd, J = 1.6, 5.2 Hz, 1 H), 6.71 (dd, J = 7.8, 12.8 Hz, 2 H), 6.36 (t, J = 6.2 Hz, 1 H), 4.87 (br s, 2 H), 3.73 (s, 1 H), 3.23 (br s, 1 H), 1.97 (d, J = 8.2 Hz, 2 H), 1.80 (d, J = 7.7 Hz, 2 H), 1.38 (s, 9 H), 1.35−1.17 (m, 4 H). *m*/*z* (ESI) 307.2 $(M + H)^+$.

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To a flask charged with *tert*-butyl ((1*R*,4*R*)-4-((3-aminopyridin-2-yl)amino)cyclohexyl)carbamate (0.830 g, 2.71 mmol) was added THF (10.84 mL) and triethylamine (0.793 mL, 5.69 mmol). The resulting suspension was cooled in an ice water bath prior to the addition of triphosgene (0.804 g, 2.71 mmol), leading to the immediate formation of a precipitate, product according to LC-MS. To the glob of solid was added additional THF (~15 mL) and a small amount of water. The resulting solid was collected via vacuum filtration and washed with water, followed by diethyl ether, and dried under high vacuum, providing product as a white solid (420 mg). The filtrate was analyzed by LC-MS, which indicated the presence of additional product. The mixture was transferred to a separatory funnel and extracted with EtOAc (2×). The combined organics were dried with Na_2SO_4, filtered, and dried under reduced pressure, providing additional product (180 mg) as a light tan solid with slightly lower purity than that of the precipitated material (total −600 mg, 67%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.04 (s, 1 H), 7.93 (dd, J = 1.4, 5.2 Hz, 1 H), 7.26 (dd, J = 1.3, 7.7 Hz, 1 H), 6.97 (dd, J = 5.2, 7.7 Hz, 1 H), 6.77 (d, J = 7.3 Hz, 1 H), 4.18 (tt, J = 4.0, 12.4 Hz, 1 H), peak under H_2O in DMSO (1H), 2.47−2.27 (m, 2 H), 1.90 (d, J = 11.6 Hz, 2 H), 1.68 (d, J = 10.8 Hz, 2 H), 1.39 (s, 9 H), 1.36−1.25 (m, 2 H). m/z (ESI) 355.2 $(M + Na)^+$.

To a vial charged with *tert*-butyl ((1*R*,4*R*)-4-(2-oxo-1H-imidazo[4,5-*b*]pyridin-3(2H)-yl)cyclohexyl)carbamate (0.600 g, 1.805 mmol) was added DCM (7.22 mL) and TFA (1.391 mL, 18.05 mmol), providing a light peach solution, which was stirred at room temperature. After 1 h, LC-MS indicated product as the primary species. The solution was dried under reduced pressure, and the residue obtained was purified with a 5g SCX-2 column, washing with MeOH followed by 2 M NH_3 in MeOH. The basic wash was dried under reduced pressure to provide product 3-((1*R*,4*R*)-4-aminocyclohexyl)-1H-imidazo-[4,5-*b*]pyridin-2(3H)-one (0.343 g, 1.477 mmol, 82% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.92 (dd, J = 1.5, 5.2 Hz, 1 H), 7.26 (dd, J = 1.5, 7.6 Hz, 1 H), 6.96 (dd, J = 5.2, 7.7 Hz, 1 H), 4.19 (tt, J = 3.9, 12.3 Hz, 1 H), 2.70−2.60 (m, 1 H), 2.41 (dq, J = 3.3, 12.8 Hz, 2 H), 1.88 (d, J = 11.5 Hz, 2 H), 1.64 (d, J = 11.2 Hz, 2 H), 1.25−1.11 (m, 2 H). m/z (ESI) 233.2 $(M + H)^+$.

To a flask charged with 3-((1*R*,4*R*)-4-aminocyclohexyl)-1H-imidazo[4,5-*b*]pyridin-2(3H)-one (0.335 g, 1.442 mmol) was added DMF (5.77 mL), 1-phenyl-2-hydroxy-2-methyl-1-propanone (Aldrich) (0.219 mL, 1.442 mmol), and acetic acid (0.091 mL, 1.586 mmol), respectively, and the resulting suspension was stirred for 15 min prior to the addition of sodium triacetoxyborohydride (0.611 g, 2.88 mmol). The resulting suspension was stirred overnight at 60 °C, leading to the formation of product along with significant reduced alcohol and starting amine still remaining. To the mixture was added additional ketone (2 equiv) and sodium triacetoxyborohydride (0.611 g, 2.88 mmol), and stirring at 60 °C continued. After 2 h of stirring, LC-MS indicated starting material consumption and additional product present. To the mixture was added water, and the material was transferred to a separatory funnel and extracted with EtOAc (2×). The combined organics were dried with Na_2SO_4, filtered, and dried under reduced pressure, providing the crude residue as an oil. The crude residue was purified with a 25g SNAP column (Biotage), ramping DCM/MeOH (90:10) in DCM from 0 to 30%, then isocratic at 30%, providing impurity elution, then ramping to 100% polar eluent to provide product, obtained as a sticky white goo upon drying, (±)-3-((1*R*,4*R*)-4-((2-hydroxy-2-methyl-1-phenylpropyl)-amino)cyclohexyl)-1H-imidazo[4,5-*b*]pyridin-2(3H)-one (0.255 g, 0.670 mmol, 46.5% yield). m/z (ESI) 381.2 $(M + H)^+$.

A vial charged with (±)-3-((1*R*,4*R*)-4-((2-hydroxy-2-methyl-1-phenylpropyl)amino)cyclohexyl)-1H-imidazo[4,5-*b*]pyridin-2(3H)-one (0.245 g, 0.644 mmol) and a stir bar was dried under reduced pressure and placed under nitrogen prior to the addition of THF (2.58 mL) and DIEA (0.236 mL, 1.352 mmol). The resulting suspension was cooled in an ice water bath prior to the addition of triphosgene (0.065 g, 0.219 mmol). The resulting suspension was stirred at 0 °C and allowed to slowly warm to room temperature. The mixture was cooled back in an ice water bath, and additional triphosgene was added (130 mg) and stirring continued. After 15 min, LC-MS indicated cyclization had occurred along with overacylation, as indicated by the masses of the overacylated acid and ester. After 20 min of stirring at room temperature, no additional conversion was observed. Therefore, an additional 0.5 equiv of triphosgene was added (98 mg), and stirring at room temperature continued. After another hour, only minor additional conversion was observed. To the reaction was added MeOH, and the mixture was dried under reduced pressure. The crude material was purified with a 25g HP spherical silica column (15 μm), ramping DCM/MeOH (90:10) in DCM from 0 to 30%, then isocratic at 30%, then to 100%, providing product (38 mg, 15%) along with overacylated material. Enantiomer resolution: column, Chiralpak AS, 5 μm, 2 cm i.d. × 40 cm length; mobile phase, 35% methanol w/0.2% diethylamine/65% CO_2; flow rate, 65 mL/min; detection, 293 nm; injection size, 3 mg in 400 μL of 1:1 DCM/methanol. Absolute configurations not experimentally elucidated but assigned on the basis of potency discrepancies (*S* more potent than *R*) and elution order comparisons to related analogues. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.02 (b. s, 1 H), 7.91−7.87 (m, 1 H), 7.46−7.40 (m, 2 H), 7.40−7.34 (m, 1 H), 7.34−7.19 (m, 3 H), 6.95 (dd, J = 5.2, 7.7 Hz, 1 H), 4.68 (s, 1 H), 4.14−4.06 (m, 1 H), 3.48 (t, J = 11.6 Hz, 1 H), 2.46−2.36 (m, 1 H), 2.35−2.24 (m, 1 H), 1.99−1.85 (m, 2 H), 1.74 (d, J = 12.6 Hz, 1 H), 1.68−1.54 (m, 2 H), 1.48 (s, 3 H), 1.31−1.21 (m, 1 H), 0.82 (s, 3 H). m/z (ESI) 407.1 $(M + H)^+$, HRMS calcd for $C_{23}H_{26}N_4O_3$ $(M + H)^+$ 407.2083, found 407.2087.

(*S*)-5,5-Dimethyl-3-((1*R*,4*S*)-4-(2-oxo-1H-imidazo[4,5-*c*]pyridin-3(2H)-yl)cyclohexyl)-4-phenyloxazolidin-2-one (48). To a vial charged with (*S*)-3-((1*R*,4*S*)-4-aminocyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (**28**; 0.181 g, 0.628 mmol) was added acetonitrile (2.51 mL), triethylamine (0.262 mL, 1.883 mmol), and methyl 3-fluoroisonicotinate (Biogene Organics) (0.107 g, 0.690 mmol). The vessel was sealed and shaken overnight at 80 °C over 72 h. The mixture was dried under reduced pressure and purified with a 25g SNAP column, ramping DCM/MeOH (90:10) in DCM (0−25%, then isocratic at 25%, detection at 215 nm), providing product with minor impurities as a light brown oil methyl 3-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)-amino)isonicotinate (0.184 g, 0.434 mmol, 69.2% yield). m/z (ESI) 424.4 $(M + H)^+$

To a vial charged with methyl 3-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-isonicotinate (0.182 g, 0.430 mmol) was added THF (2.51 mL), MeOH (2.51 mL), water (2.51 mL), and LiOH (0.051 g, 2.149 mmol), respectively. The mixture was heated to 50 °C for 2 h. The light brown solution was diluted with water, acidified

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with 2 N HCl, and extracted with EtOAc (2×) and DCM (2×). The combined organics were dried with Na_2SO_4, filtered, and dried under reduced pressure, providing product as an off-white solid 3-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)isonicotinic acid (0.132 g, 0.322 mmol, 75% yield). *m*/*z* (ESI) 410.3 $(M + H)^+$.

To a flask charged with 3-(((1*S*,4*R*)-4-((*S*)-5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)isonicotinic acid (0.128 g, 0.313 mmol) was added THF (1.563 mL), triethylamine (0.061 mL, 0.438 mmol), and diphenyl phosphoryl azide (0.094 mL, 0.438 mmol), respectively. The mixture was heated and shaken at 80 °C overnight. The resulting mixture was dried under reduced pressure and purified with a 25g SNAP column, ramping DCM/MeOH (90:10) in DCM (0−100%), providing product eluting at 100% polar eluent which had coeluted with phosphitic acid side product from DPPA (∼20%). Repurification with a HP spherical silica column (25g, 15 μm spherical, Interchim) was only able to partially separate the impurity (∼70 mg with ∼5% impurity by LC-MS). The material was dissolved in DMSO/MeOH and purified with Gilson RP-HPLC, ramping ACN in H_2O (25−80%, 0.1% TFA throughout).

Product eluents were directly free-based with a 5g SCX-2 column washing with MeOH and then 2 M NH_3 in MeOH. The basic wash was dried under reduced pressure, then lyophilized from MeOH/H_2O, yielding product as a white solid (*S*)-5,5-dimethyl-3-((1*R*,4*S*)-4-(2-oxo-1H-imidazo[4,5-*c*]-pyridin-3(2H)-yl)cyclohexyl)-4-phenyloxazolidin-2-one (0.015 g, 0.037 mmol, 11.81% yield). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.43−11.22 (m, 1 H), 8.56 (s, 1 H), 8.13 (d, *J* = 5.3 Hz, 1 H), 7.51−7.06 (m, 5 H), 7.01 (d, *J* = 5.1 Hz, 1 H), 4.66 (s, 1 H), 4.06 (t, *J* = 12.1 Hz, 1 H), 3.76−3.65 (m, 1 H), 2.26−2.04 (m, 2 H), 2.03−1.85 (m, 2 H), 1.76 (d, *J* = 11.9 Hz, 1 H), 1.71−1.53 (m, 3 H), 1.48 (s, 2 H), 1.39−1.20 (m, 1 H), 0.80 (s, 3 H). *m*/*z* (ESI) 407.4 $(M + H)^+$, HRMS calcd for $C_{23}H_{26}N_4O_3$ $(M + H)^+$ 407.2083, found 407.2089.

(*S*)-5,5-Dimethyl-3-((1*R*,4*S*)-4-(8-oxo-7H-purin-9(8H)-yl)cyclohexyl)-4-phenyloxazolidin-2-one (51). To a vial charged with 4-chloropyrimidin-5-amine (Frontier Scientific) (0.500 g, 3.86 mmol) was added THF (6.43 mL), toluene (6.43 mL), triethylamine (1.345 mL, 9.65 mmol), and phthalic anhydride (1.310 mL, 13.51 mmol). The mixture was heated to 100 °C for 72 h. The crude mixture was dried under reduced pressure and purified with a 50g SNAP column (Biotage), ramping EtOAc in heptane from 0 to 45%, then isocratic at 45% to elute product, obtained as a yellow solid 2-(4-chloropyrimidin-5-yl)isoindoline-1,3-dione (0.773 g, 2.98 mmol, 77% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (d, *J* = 0.4 Hz, 1 H), 9.09 (d, *J* = 0.4 Hz, 1 H), 8.12−8.05 (m, 2 H), 8.04−7.96 (m, 2 H). *m*/*z* (ESI) 260.1 $(M + H)^+$.

To a vial charged with 2-(4-chloropyrimidin-5-yl)isoindoline-1,3-dione (0.327 g, 1.259 mmol) was added acetonitrile (4.20 mL), triethylamine (0.369 mL, 2.64 mmol), and 3-((1*R*,4*R*)-4-aminocyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one 2,2,2-trifluoroacetate (0.507 g, 1.259 mmol) (**28** as a mixture of S:R enantiomers ∼3:2), respectively. The vessel was sealed and shaken overnight at 80 °C, providing a dark solution containing product as the primary species according to LC-MS. The solution was dried under reduced pressure and purified with a 40g HP spherical silica column (Interchim), ramping EtOAc in heptane (0−100%, 5% DCM throughout), leading to isolation of product as a mixture of trans/cis isomers with minor impurities as a light yellow foam 2-(4-(((1*R*,4*R*)-4-(5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)-pyrimidin-5-yl)isoindoline-1,3-dione (0.435 g, 0.850 mmol, 67.5% yield). *m*/*z* (ESI) 512.3 $(M + H)^+$.

To a vial charged with 2-(4-(((1*R*,4*R*)-4-(5,5-dimethyl-2-oxo-4-phenyloxazolidin-3-yl)cyclohexyl)amino)pyrimidin-5-yl)-isoindoline-1,3-dione (0.435 g, 0.850 mmol) was added ethanol (3.40 mL) and hydrazine hydrate (0.132 mL, 4.25 mmol). The vial was sealed and heated to 100 °C for 2 h, yielding a white suspension. LC-MS indicated complete conversion to product cleanly. The solid was collected via vacuum filtration and washed with DCM/MeOH. The precipitate was primarily 2,3-dihydrophthalazine-1,4-dione. The filtrate contained product along with phthalazinedione. The filtrate was purified with a 5g SCX-2 column, washing with MeOH, then with 2 M NH_3 in MeOH. The basic wash was dried under reduced pressure, providing product as a white foam 3-((1*R*,4*R*)-4-((5-amino-pyrimidin-4-yl)amino)cyclohexyl)-5,5-dimethyl-4-phenyloxazo-lidin-2-one (0.236, 73%). *m*/*z* (ESI) 382.3 $(M + H)^+$.

To a vial charged with 3-((1*R*,4*R*)-4-((5-aminopyrimidin-4-yl)amino)cyclohexyl)-5,5-dimethyl-4-phenyloxazolidin-2-one (0.330 g, 0.865 mmol) was added THF (3.46 mL), DIEA (0.529 mL, 3.03 mmol), and CDI (0.491 g, 3.03 mmol), respectively. The mixture was shaken at room temperature for 2 h, providing a yellow solution which contained primarily product according to LC-MS. The material was loaded directly onto a load column and purified with a 25g HP spherical silica gel column (Interchim) ramping DCM/MeOH/NH_4OH (90:10:1) in DCM from 0 to 35%, then isocratic at 35%, yielding product as a white foam upon drying. NMR is consistent with a product as a mixture of isomers. The material was repurified twice more as follows: column, Chiralpak OD-H (2 cm × 15 cm); gradient, 20% MeOH (0.2% DEA)/CO_2; 100 bar; flow, 65 mL/min; detection, 220 nm; injection volume, 0.75 mL, 12 mg/mL; dissolution, 1:1 DCM/MeOH. The desired enantiomer (major product) was the first eluting. Second purification: column, Chiralpak AD-H, 2 cm × 15 cm; mobile phase, 75% CO_2/25% MeOH w/0.2% DEA; flow rate, 80 mL/min; detection, 279 nm; injection volume, 0.5 mL; dissolution, 5 mL of 1:1 DCM/MeOH. The product was lyophilized from MeOH/H_2O yielding a white powder (S)-5,5-dimethyl-3-((1*R*,4*S*)-4-(8-oxo-7H-purin-9(8H)-yl)cyclohexyl)-4-phenyloxazolidin-2-one (0.069 g, 0.169 mmol, 19.58% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.20 (br s, 1 H), 8.56−8.48 (m, 1 H), 8.21 (s, 1 H), 7.51−7.41 (m, 2 H), 7.40−7.10 (m, 3 H), 4.68 (s, 1 H), 4.07 (tt, *J* = 3.6, 12.3 Hz, 1 H), 3.54−3.43 (m, 1 H), 2.41−2.17 (m, 2 H), 2.02−1.86 (m, 2 H), 1.79 (d, *J* = 12.4 Hz, 1 H), 1.64 (d, *J* = 10.6 Hz, 2 H), 1.48 (s, 3 H), 1.35−1.17 (m, 1 H), 0.81 (s, 3 H). *m*/*z* (ESI) 408.0 $(M + H)^+$, HRMS calcd for $C_{22}H_{25}N_5O_3$ $(M+H)^+$ 408.2035, found 408.2044.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Table S1 provides enzyme and cellular data including individual standard deviation and *n* values. Figure S1 provides the IR spectrum of **40**, and Figure S2 and Table S2 provide the proton/carbon NMR assigments for **40**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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ABBREVIATIONS USED

TNKS, tankyrase; PARP, poly(ADP-ribose) polymerase; CRC, colorectal cancer; APC, adenomatous polyposis coli; AXIN2, axis inhibition protein 2; CTNNB1, gene which encodes β-catenin; GSK3β, glycogen synthase kinase 3β; CK1α, casein kinase 1α; TCF, T-cell specific transcription factor; TRF1, telomere repeat binding factor 1; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIEA, *N*-ethyl-*N*-isopropylpropan-2-amine; DMEDA, N^1,N^2-dimethylethane-1,2-diamine; DEA, diethylamine; MP-BH_4, macroporous borohydride; ACN, acetonitrile; SFC, supercritical fluid; SCX-2, strong cation exchange prepacked resin (propylsulfonic acid functionalized); DPPA, diphenyl phosphoryl azide; T3P, 1-propanephosphonic anhydride; S_NAR, nucleophilic aromatic substitution; CDI, 1,1′-carbonyldiimidazole

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(16) See the Experimental Section for the preparation of **31** for the synthetic details of 7-fluoro-1,5-naphthyridine-4-carboxylic acid.

(17) For cyano-substituted substrates, Raney nickel was avoided in favor of tin chloride to mitigate over-reduction.

(18) The absolute stereochemistry was established on the basis of the knowledge that only partial racemization occurred in the synthetic sequence toward common intermediate **28**. We began with the (*S*), and therefore, the major enantiomer must be the (*S*) enantiomer. Some analogues were prepared using enantiopure **28**, and some were separated at the end of the synthetic sequence.

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(21) Compound **1** was incubated in fresh rat plasma at a concentration of 10 μM at 37 °C for 2 h. Following protein precipitation, LC/MS/MS analysis was conducted. The primary mass identified was consistent with imide ring hydrolysis, and the secondary mass was consistent with amide hydrolysis.

(22) Measured at BPS Bioscience Inc. as follows: In general, all assays were done by following the BPS PARP assay kit protocols. The enzymatic reactions for PARP 1, 2, 3, and 6 were conducted in duplicate at room temperature for 1 h in a 96 well plate coated with histone substrate. 50 μL of reaction buffer (Tris·HCl, pH 8.0) contains NAD+, biotinylated NAD+, activated DNA, a PARP enzyme, and the test compound. After the enzymatic reactions, a plate was washed three times with 200 μL of PBST. 50 μL of Streptavidin-horseradish peroxidase was added to each well, and the plate was incubated at room temperature for an additional 30 min. 100 μL of developer reagents were added to wells, and luminescence was measured using a BioTek SynergyTM 2 microplate reader.

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(25) The HOMO−LUMO gap for **24** is 4.20 eV, and the HOMO−LUMO gap for **29** is 4.45 eV. Band gaps were calculated at the time-dependent B3LYP/6-311+G(d,p) level of theory by using Gaussian 03 software. The calculated band gaps for *N*-(quinolin-8-yl)acetamide and *N*-methylquinoline-8-carboxamide groups are 3.60 and 3.78 eV, respectively. The increased band gap for *N*-methylquinoline-8-carboxamide groups suggests that the carboxamide analogue is less susceptible to oxidative metabolism than the acetamide analogue.

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