Hinge Binder Scaffold Hopping Identifies Potent Calcium/Calmodulin-Dependent Protein Kinase Kinase 2 (CAMKK2) Inhibitor Chemotypes

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CAMKK2 is a serine/threonine kinase and an activator of AMPK whose dysregulation is linked with multiple diseases. Unfortunately, STO-609, the tool inhibitor commonly used to probe CAMKK2 signaling, has limitations. To identify promising scaffolds as starting points for the development of high-quality CAMKK2 chemical probes, we utilized a hinge-binding scaffold hopping strategy to design new CAMKK2 inhibitors. Starting from the potent but promiscuous disubstituted 7-azaindole GSK650934 (CAMKK2 IC50 = 3 nM), a total of 32 compounds, composed of single ring, 5,6-, and 6,6-fused heteroaromatic cores were synthesized. The compound set was specifically designed to probe interactions with the kinase hinge-binding residues. These compounds were evaluated in vitro in biochemical and cellular assays for CAMKK2 inhibition. Compared to GSK650394 and STO-609, thirteen of our compounds displayed similar or better CAMKK2 inhibitory potency in vitro, while compounds 13g and 45 had greatly improved selectivity for CAMKK2 across the kinome. Our systematic survey of hinge binding chemotypes identified several potent and selective inhibitors of CAMKK2 to serve as starting points for medicinal chemistry programs aimed at the identification of CAMKK2 chemical probes and clinical candidates

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CAMKK2 hinge binder scaffold hopping ChemRxiv.docx (19.71 MiB)
Hinge Binder Scaffold Hopping Identifies Potent Calcium/Calmodulin-Dependent Protein Kinase Kinase 2 (CAMKK2) Inhibitor Chemotypes

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ABSTRACT:
CAMKK2 is a serine/threonine kinase and an activator of AMPK whose dysregulation is linked with multiple diseases. Unfortunately, STO-609, the tool inhibitor commonly used to probe CAMKK2 signaling, has limitations. To identify promising scaffolds as starting points for the development of high-quality CAMKK2 chemical probes, we utilized a hinge-binding scaffold hopping strategy to design new CAMKK2 inhibitors. Starting from the potent but promiscuous disubstituted 7-azaindole GSK650934 (CAMKK2 IC\textsubscript{50} = 3 nM), a total of 32 compounds, composed of single ring, 5,6-, and 6,6-fused heteroaromatic cores were synthesized. The compound set was specifically designed to probe interactions with the kinase hinge-binding residues. These compounds were evaluated \textit{in vitro} in biochemical and cellular assays for CAMKK2 inhibition. Compared to GSK650394 and STO-609, thirteen of our compounds
displayed similar or better CAMKK2 inhibitory potency *in vitro*, while compounds 13g and 45 had greatly improved selectivity for CAMKK2 across the kinome. Our systematic survey of hinge binding chemotypes identified several potent and selective inhibitors of CAMKK2 to serve as starting points for medicinal chemistry programs aimed at the identification of CAMKK2 chemical probes and clinical candidates.

**INTRODUCTION:** Calcium (Ca\(^{2+}\))/calmodulin-dependent protein kinase kinase 2 (CAMKK2) is a serine/threonine kinase that is one of the calmodulin (CaM)-binding proteins of the CaMK family.\(^1\)\(^-\)\(^3\) Ca\(^{2+}\) is an important second messenger that aids in the regulation of a wide range of signaling events in part through binding to its intracellular receptor CaM.\(^4\) Ca\(^{2+}\)-bound CaM modulates various cellular responses *via* activation of an array of downstream proteins including CAMKK2.\(^5\) Upon activation, CAMKK2 phosphorylates and activates its substrates including CAMK1, CAMK4, AMP-activated protein kinase (AMPK) and in some cases AKT. This signal transduction results in the regulation of many important physiological and pathological processes.\(^6\)\(^-\)\(^{16}\)

Due to CAMKK2’s important role in cell signaling, dysregulation of CAMKK2 has been implicated in several diseases. Aberrant activation and overexpression of CAMKK2 has been linked to multiple cancer types including prostate, breast, ovarian, gastric and hepatic cancers.\(^8\)\(^-\)\(^{14}\).\(^{17,\ 18}\) Knockdown of CAMKK2 *via* siRNA or pharmacological inhibition of CAMKK2 reduced cell proliferation, migration and invasion as well as induced apoptosis and cell cycle arrest in
numerous cancer cell lines and tumor models. Mechanistically, CAMKK2 is an important regulator of metabolic and inflammatory processes, which are contributory factors to its impact on cancer growth.

In addition, therapeutic interventions targeting CAMKK2 may be beneficial beyond oncology. For example, hepatocellular carcinoma (HCC) is often preceded by non-alcoholic fatty liver disease (NAFLD), which is driven by several risk factors including obesity and type 2 diabetes. Inhibition of CAMKK2 reduced food intake in mice, and Camkk2-null mice are leaner than wild-type mice when fed a high-fat diet. Consistent with these findings, CaMKK2 was recently shown to be inhibited by Liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist that decreases food intake and promotes weight loss. In relation to skeletal disease, CAMKK2 is expressed in osteoblasts and osteoclasts, which play an essential role in bone tissue maintenance. Inhibition of CAMKK2 stimulated bone formation and reversed age-associated decline in bone health by promoting osteoblast differentiation and inhibiting osteoclast differentiation. Taken together, these findings suggest that inhibition of CAMKK2 may be effective for the treatment of a variety of diseases including various types of cancers, metabolic disorders, and bone diseases like osteoporosis. While CAMKK2 has emerged as an attractive therapeutic target, there remains a shortage of high-quality CAMKK2 inhibitors, which has impaired progress in the field.
At present, almost all CAMKK2 related pharmacological studies rely on the use of the ATP-competitive inhibitor STO-609 to study CAMKK2 signaling events (Figure 1). However, STO-609 is not an ideal chemical tool. It binds to multiple kinases beyond CAMKK2. When screened against a panel of over 400 wild-type human kinases (KINOMEscan®, Eurofins DiscoverX) at 1 μM, STO-609 bound with strong affinity to 14 kinases (<20% activity remaining). Amongst these kinases were CDKL2, GRK3 and CK2 which are all overexpressed in several cancer types. In addition to these collateral kinase targets, STO-609 potently activates the aryl hydrocarbon receptor. STO-609 is a planar molecule with poor aqueous solubility, yet it is routinely used at high doses in cell culture (>10 μM) and in vivo. Due to the liabilities of STO-609, the field would benefit from the discovery and development of potent and highly selective small molecule inhibitors of CAMKK2 as high-quality probes.

Figure 1: Structures of known CAMKK2 inhibitors: STO-609 and GSK650394.

We recently conducted an extensive literature search for CAMKK2 inhibitors to identify starting points for medicinal chemistry optimization. A promising series of potent CAMKK2 inhibitors was recently disclosed by GlaxoSmithKline (GSK). The 7-azaindole GSK650394 (also called a pyrrolopyridine) (Figure 1) was a potent CAMKK2 inhibitor (IC$_{50}$ = 0.63 nM). The
co-crystal structure of GSK650394 with CAMKK2 (PDB 6BKU) revealed the critical role of the carboxylic acid and cyclopentyl moiety (Figure 2-A), which was consistent with the reported SAR (structure-activity relationship) studies. The co-crystal structure reveals that the nitrogen atoms of the pyridine and pyrrole rings act as hydrogen bond acceptor and donor pairs respectively, and form interactions with the protein backbone of the ATP binding site. The acid functionality contributes to critical hydrogen bonding interaction with both the protonated amine of Lys194 as well as the carboxylate group of Glu236 in a water-mediated manner. Additionally, CH-π interactions (aromatic edge-face interactions) between the aromatic ring of gatekeeper Phe267 with both the CH in the 2-position of the 7-azaindole and the methine-group in the ortho-position of the 3-aryl moiety of the inhibitor stabilize GSK650394 in this orientation. The pendant phenyl group in the 5-position of the 7-azaindole scaffold occupies a hydrophobic pocket which can potentially be exploited to gain selectivity, potency and modulate physical properties to optimize pharmacokinetic parameters. Although a crystal structure of CAMKK2 co-crystallized with ATP is not available, the cyclopentyl group seems to mimic at least the position of the ribosyl moiety of ATP based on the proximity of the cyclopentyl group to the P-loop of CAMKK2. The 7-azaindole pharmacophore in GSK650394 is commonly found in kinase inhibitors and is considered a powerful but sometimes promiscuous hinge binder. When screened against a panel of 334 kinases (Reaction Biology Corporation), GSK650394 inhibited 29 kinases by more than 90%, limiting its utility as a tool compound for studying CAMKK2 or any other kinase.
**Figure 2:** A) Binding mode of the co-crystallized ligand GSK650394 in the ATP-binding site of CAMKK2 (PDB-ID: 6BKU). Image was generated with PyMOL®. The protein is colored in grey. Blue-dashed lines indicate H-bond interactions; green-dashed lines display CH-π interactions. GSK650394 is shown as purple sticks and the water molecule as red sphere. Oxygen and nitrogen atoms are colored in red and blue, respectively. B) Structure of GSK650394 showing important sites for development of new CAMKK2 inhibitors.

To improve the kinase selectivity of GSK650394, we targeted replacement of the 7-azaindole with other heterocycles that would modulate interactions with the hinge-binding residues and in some cases reduce interaction with the hinge-binding residues. Our compound design strategy is depicted in Figure 2-B. Our central hypothesis was based on the belief that the 3-position aryl ring, decorated with the carboxylic acid and cyclopentyl groups, plays a dominant role in CAMKK2 recognition and that inhibitors with enhanced selectivity would arise from compounds with the binding contribution from the hinge binder diminished. This led to the synthesis and evaluation of structurally diverse novel chemotypes as potential inhibitors of CAMKK2 (Figure 3).
Thus, we performed a scaffold-hopping exercise wherein the hinge-binding 7-azaindole core of GSK650394 was substituted with structurally diverse alternate hinge-binding moieties. We retained the ortho-cyclopentyl benzoic acid moiety hypothesizing that the interactions observed in the crystal structure would bias the new set towards CAMKK2 affinity.

Figure 3: New molecules based on scaffold hopping from GSK650394 designed to interrogate several aspects of hinge binding interactions between CAMKK2 and the proposed inhibitors. Top row: Inhibitors retaining the topology of GSK650394 (5,6-fused ring systems with the cyclopentyl substituted benzoic acid moiety attached to the 5-membered ring). Middle row: 5,6-fused systems with the cyclopentyl substituted benzoic acid moiety appended to the 6-membered ring. Bottom row: Inhibitors with ring expansion (6,6-fused ring system) or ring deletion (single 6 membered ring as the hinge binding core).

RESULTS:

Chemistry: To develop analogs of GSK650394 that could be potent and selective CAMKK2 inhibitor scaffolds, all our synthesized compounds incorporated the critical pharmacophore,
ortho-cyclopentyl benzoic acid. In parallel, to investigate the importance of the pendant phenyl ring, we synthesized matched pairs with and without this group. We theorized that removal of the phenyl ring could significantly lower the cLogP and increase ligand efficiency if it was dispensable without being detrimental to potency. To this end, we first synthesized the pinacol borate esters 3 and 4 to enable attachment to the various hinge binding pharmacophores via Suzuki coupling reactions (Scheme 1). 4-Bromo-2-fluorobenzoic acid 1 underwent a Grignard reaction with cyclopentylmagnesium bromide to afford the ortho-cyclopentyl substituted benzoic acid 2, which was then converted into the corresponding pinacol boronate ester using Miyaura borylation conditions yielding 3. The analogous methyl ester 4 was made in a similar fashion following esterification of 2 in methanol in the presence of thionyl chloride.

**Scheme 1**: Synthesis of pinacol boronate esters 3 and 4.

\[ \text{i Reagents and conditions: a) Cyclopentylmagnesium bromide, THF, -10–25 °C, 5 h then aq. HCl; b) PdCl}_2(dppf)-\text{CH}_2\text{Cl}_2, \text{KOAc, bis(pinacolato)diboron, 100 °C, 2 h; c) SOCl}_2, \text{MeOH, 16 h, 75 °C.} \]

3- and 3,5-substituted fused 6,5-ring systems:

Initial analogs were fused 5,6-ring systems with 3- and 3,5- substitution patterns that retained the topology of GSK650394 but modify the nature and/or location of heteroatoms in the ring system and thus can alter the compound’s ability to form H-bond interactions with the hinge
region. GSK650394 is commercially available, but its matched pair 7, truncated at the 5-position, required synthesis (Scheme 2). 3-Bromo-7-azaindole 5 was converted to the silylethoxymethyl (SEM)-protected azaindole, and then subjected to Suzuki-Miyaura conditions to afford cyclopentyl analog 6. SEM deprotection followed by base-mediated saponification afforded the target azaindole 7.

The N-methyl analogs of GSK650394 and 7 were synthesized as depicted in Scheme 3. To access 10, 5-chloro-3-iodo azaindole 8b was N-alkylated. Consecutive Suzuki reactions with the ortho-cyclopentyl methyl benzoate 4 and then phenyl boronic acid placed the key pharmacophore in the 3 position, and a phenyl ring in the 5-position. Saponification of the methyl ester proceeded smoothly affording azaindole 10. Coupling of boronate ester 4 with 3-bromo-N-methyl azaindole followed by saponification of the methyl ester afforded target molecule 11.

Synthesis of the structurally similar furopyridine and thienopyridine cores is described in Scheme 4. Nicotinic acid derivatives 12a and 13a were converted into the ethyl esters 12-14b and then treated with ethyl glycolate or ethyl thioglycolate in the presence of sodium hydride (NaH) to give the respective furopyridines or thienopyridines 12-14c. A one-pot saponification-decarboxylation of the β-keto esters afforded the 2-unsubstituted heterocycles 12-14d. These aryl alcohols were converted to the corresponding triflates 12-14d which were able to undergo Suzuki-Miyaura reactions to yield analogs 12f, 13g and 14g. 12g was obtained directly from the commercially available 3-bromothieno[2,3-b]pyridine 15e using Suzuki-Miyaura reaction.
Scheme 2: Synthesis of azaindole 7, the matched pair for GSK650394.\textsuperscript{1}

\textsuperscript{1}Reagents and conditions: a) SEM-Cl, NaH, DMF, 0–21 °C, 2 h; b) 4, Pd(OAc)$_2$, K$_3$PO$_4$, P(Cy)$_3$, PhMe/H$_2$O, 80 °C, 16 h; c) CF$_3$COOH, CH$_2$Cl$_2$, then NaOAc, EtOH, 21 °C, 24 h; d) Aq. NaOH, MeOH, 75 °C, 1 h then aq. HCl.

Scheme 3: Synthesis of N-methyl azaindole hinge binders 10 and 11\textsuperscript{1}

\textsuperscript{1}Reagents and conditions: a) 3, Pd$_2$(dba)$_3$, XPhos, Cs$_2$CO$_3$, dioxane, H$_2$O, 120 °C, 16 h; b) Aq. LiOH, dioxane, 100 °C, 16 h, thenaq. HCl; c) 4, PdCl$_2$(dppe)-CH$_2$Cl$_2$, Cs$_2$CO$_3$, dioxane, H$_2$O, rt, 16 h; d) PhB(OH)$_2$, Pd$_2$(dba)$_3$, XPhos, Cs$_2$CO$_3$, dioxane, H$_2$O, 120 °C, 16 h; e) Aq. LiOH, dioxane, 100 °C, 16 h, thenaq. HCl.

Scheme 4: General route to the furo- and thienopyridines.\textsuperscript{1}
Reagents and conditions: a) \((\text{EtO})_3\text{CH}\), PhMe, 100 °C, 2 h or \(\text{H}_2\text{SO}_4\), EtOH, reflux, 24 h; b) Ethyl glycolate or ethylthioglycolate, NaH, DME, 0-75 °C, 2.5 h; c) Aq. NaOH, EtOH then aq. HCl, 100 °C, 2 h; d) Tf\(_2\), DIPEA, CH\(_2\)Cl\(_2\), -10-25 °C, 3 h; e) \(\text{4, Pd(PPh}_3\text{)}_4\), Na\(_2\)CO\(_3\), MeOH/CH\(_2\)Cl\(_2\), 90 °C, 1 h; f) PhB(OH)\(_2\), Pd(PPh\(_3\))\(_4\), Na\(_2\)CO\(_3\), MeOH/CH\(_2\)Cl\(_2\), 90 °C, 1 h or PhB(OH)\(_2\), Pd\(_2\)(dba)\(_3\), XPhos, Cs\(_2\)CO\(_3\), dioxane/H\(_2\)O 90 °C, 16 h; g) Aq. NaOH, MeOH then Aq. HCl, 1 h; h) \(\text{3, Pd(PPh}_3\text{)}_4\), Na\(_2\)CO\(_3\), MeOH/CH\(_2\)Cl\(_2\), 90 °C, 1 h.

The subsequent set of fused 5,6-ring derivatives synthesized in Scheme 5 (pyrazolopyridines and pyrazolopyrimidines) dramatically altered the placement of the heteroatoms based on the parent compound. Analogs \(\text{19 and 20}\) were obtained by stepwise Suzuki couplings, first with \(\text{4}\) and then phenylboronic acid, followed by methyl ester hydrolysis. The monosubstituted complementary matched pairs were synthesized from commercially available 3-bromopyrazolo[1,5-a]pyridine or –pyrimidine via Suzuki couplings with \(\text{4}\) to afford \(\text{21 and 22}\).
The same set of reaction conditions were used to afford the triazolopyridazine analogs 25 and 26 (Scheme 6).

Scheme 5: Synthesis of pyrazolopyridine and pyrazolopyrimidine analogs.¹

¹Reagents and conditions: a) 3, Pd₂(dba)₃, XPhos, Cs₂CO₃, dioxane, H₂O, 120 °C, 16 h; 17 via b) 4, Pd₂(dba)₃, XPhos, Cs₂CO₃, dioxane, H₂O, 120 °C, 16 h; 18 via c) PdCl₂(dppf)-CH₂Cl₂, Cs₂CO₃, dioxane, H₂O, 80 °C, 16 h; d) PhB(OH)₂, Pd(PPh₃)₄, Cs₂CO₃, dioxane, H₂O, 120 °C, 30 min, µW; e) Aq. LiOH, dioxane, 100 °C, 16 h then Aq. HCl.

Scheme 6: Synthesis of the triazolopyridazine analogs 25 and 26.¹

¹Reagents and conditions: a) 3, Pd₂(dba)₃, XPhos, Cs₂CO₃, dioxane/H₂O, 90 °C, 16 h; b) 4, Pd(PPh₃)₄, K₂CO₃, dioxane/H₂O, 90 °C, 16 h; c) Pd₂(dba)₃, XPhos, Cs₂CO₃, dioxane/H₂O, 90 °C, 16 h; d) Aq. NaOH, MeOH 75 °C, 1 h then Aq. HCl.

2- and 2,4-substituted fused 5,6-ring systems:

The first scaffolds in this category were N-methyl azaindoles and are described in Scheme 7.

The disubstituted azaindole 29 was obtained from coupling phenylboronic acid at the more
reactive 2-position of 2-iodo-4-chloro-N-methyl azaindole followed by coupling with intermediate 4 at the 4-position. Saponification of the methyl ester afforded acid 29. The monosubstituted compound 30 was synthesized from commercially available 4-chloro-N-methyl-7-azaindole with a Suzuki coupling reaction followed by saponification of the methyl ester.

**Scheme 7: Synthesis of 2,4-substituted N-methyl azaindoles**

| Reagents and conditions: a) 4, Pd(PPh₃)₄, Cs₂CO₃, dioxane/H₂O, 120 °C, 30 min; b) Aq. LiOH, dioxane, 100 °C, 16 h then aq. HCl; c) PhB(OH)₂, Pd(PPh₃)₄, Cs₂CO₃, dioxane/H₂O, 120 °C, 30 min; d) 4, Pd(PPh₃)₄, Cs₂CO₃, dioxane/H₂O, 120 °C, 30 min; e) Aq. LiOH, dioxane, 100 °C, 16 h then aq. HCl. |

Imidazopyridine analogs were obtained from initial coupling of 2,3-diamino-4-chloropyridine with 4 to afford the aryl-substituted pyridine 32 (Scheme 8). Imidazopyridine 33 was synthesized by condensation of diamine 32 with trimethyl orthoformate in methanol, followed by saponification of the ester to afford the desired carboxylic acid. The 2-aryl analog 34 was obtained by converting benzoic acid into an activated ester with 1,1'-carbonyldiimidazole (CDI) and addition of diamine 32 to form a putative amide intermediate that underwent a ring closure.
under thermal conditions resulting in the imidazopyridine intermediate. Ester hydrolysis yielded the desired imidazopyridine 34.

**Scheme 8**: Synthesis of the imidazopyridine hinge binders.¹

¹Reagents and conditions: a) 4, Pd$_2$(dba)$_3$, XPhos, Cs$_2$CO$_3$, dioxane, H$_2$O, 100 °C, 16 h; b) CH(OMe)$_3$, H$_2$NSO$_3$, MeOH, rt, 1 h; c) Aq. LiOH, dioxane, 100 °C, 16 h then aq. HCl; d) Benzoic acid, CDI, DMF, 0 °C, 30 min then 32, 200 °C, 10 min; e) Aq. LiOH, dioxane, 100 °C, 16 h then aq. HCl.

Mono- and disubstituted thienopyrimidine analogs of the [3,2-\textit{d}] and [2,3-\textit{d}] core scaffolds were synthesized from commercially available starting materials (Scheme 9). The monosubstituted analogs 38 and 42 were easily accessed in one-step via Suzuki coupling using commercially available 35a and 39a, respectively. 37 was synthesized by first coupling 35b with phenylboronic acid to afford 2-aryl-substituted intermediate 36 which was then coupled with 3 to yield the disubstituted thieno[3,2-\textit{d}]pyrimidine. The reaction sequence was reversed in the synthesis of the disubstituted thieno[2,3-\textit{d}]pyrimidine 41, since the chlorine substituent in 39b was found to be more reactive than the thiophene bromine. Intermediate 4 was coupled to the 4-
position of 39b affording 40. Subsequent Suzuki coupling with phenylboronic acid, followed by saponification of the ester yielded disubstituted analog 41.

Scheme 9: Synthesis of thieno[3,2-d]pyrimidine and thieno[2,3-d]pyrimidine hinge binders."

\textsuperscript{1}Reagents and conditions: a) 3, Pd(PPh\textsubscript{3})\textsubscript{4}, Cs\textsubscript{2}CO\textsubscript{3}, dioxane/water, 125 °C, µW, 15 min; b) PhB(OH)\textsubscript{2}, NaHCO\textsubscript{3}, CsF, Pd(PPh\textsubscript{3})\textsubscript{4}, dioxane/H\textsubscript{2}O, 95 °C, 3 h; c) 3, Pd(PPh\textsubscript{3})\textsubscript{4}, Cs\textsubscript{2}CO\textsubscript{3}, dioxane/water, 125 °C, 15 min; d) 3, Pd(PPh\textsubscript{3})\textsubscript{4}, Cs\textsubscript{2}CO\textsubscript{3}, dioxane/water, 125 °C, µW, 15 min; e) 4, Pd(PPh\textsubscript{3})\textsubscript{4}, Cs\textsubscript{2}CO\textsubscript{3}, dioxane/water, 145 °C, µW, 15 min; f) PhB(OH)\textsubscript{2}, NaHCO\textsubscript{3}, CsF, Pd(PPh\textsubscript{3})\textsubscript{4}, dioxane/H\textsubscript{2}O, 80 °C, 4 h; g) Aq. LiOH, dioxane, 100 °C, 16 h then aq. HCl.

_Synthesis of substituted fused 6,6-ring hinge binders:_

This set of hinge binders moves from 5,6-fused ring systems to 6,6-fused systems. The disubstituted quinoline analog 45 was synthesized in a straightforward manner from commercially available 4,6-dichloroquinoline 43b via sequential Suzuki couplings with 4 and phenylboronic acid followed by saponification of the methyl ester. The monosubstituted quinoline
46 was synthesized in one-step via Suzuki coupling between the commercially available 43a and 3.

The quinazoline hinge binder derivatives were obtained via similar routes utilized in the synthesis of the quinolines (Scheme 10) with the exception of compound 49. The disubstituted quinazoline 49 was prepared from 4-chloroquinazolin-6-ol 47b with initial installation of boronate 4 and subsequent conversion of the hydroxy group of 48 into a triflate which readily underwent Suzuki coupling with phenylboronic acid. Saponification then afforded the target compound. The 4-aryl-2-methylquinoline 52 and 1,6-naphthyridine 54 analogs were synthesized in one step via Suzuki coupling with 3 (Scheme 10-c).

Scheme 10: Synthesis of quinoline and quinazoline hinge binders.¹
Reagents and conditions: a) 3, Pd\(_2\)(dba)\(_3\), XPhos, Cs\(_2\)CO\(_3\), dioxane/H\(_2\)O, 90 °C, 16 h; b) 4, Pd\(_2\)(dba)\(_3\), XPhos, Cs\(_2\)CO\(_3\), rt, 16 h; c) PhB(OH)\(_2\), Pd\(_2\)(dba)\(_3\), XPhos, Cs\(_2\)CO\(_3\), 120 °C, 16 h; d) Aq. LiOH, dioxane, 100 °C, 16 h then aq. HCl; e) 4, Pd(PPh\(_3\))\(_4\), K\(_2\)CO\(_3\), dioxane/H\(_2\)O, 120 °C, 16 h; f) Aq. LiOH, dioxane, 100 °C, 16 h then aq. HCl; g) 4, Pd(PPh\(_3\))\(_4\), K\(_2\)CO\(_3\), dioxane/H\(_2\)O, 90 °C, 16 h; h) i. DIPEA, Tf\(_2\)O, CH\(_2\)Cl\(_2\), 0–21 °C, 2 h, ii. PhB(OH)\(_2\), Pd(PPh\(_3\))\(_4\), K\(_2\)CO\(_3\), CH\(_2\)Cl\(_2\)/MeOH, 90 °C, 1 h; i) Aq. NaOH, MeOH, 75 °C, 1 h then aq. HCl; j) 4, Pd\(_2\)(dba)\(_3\), XPhos, Cs\(_2\)CO\(_3\), dioxane/H\(_2\)O, 90 °C, 16 h.

Synthesis of pyrimidine hinge binders:

The final set of hinge binders we explored consisted of substituted pyrimidines. The monosubstituted pyrimidine 56 was obtained via Suzuki coupling of 55 with 3 (Scheme 11-a). 2-phenyl-4-aryl pyrimidine 58 was synthesized from 2-phenyl-4-chloropyrimidine 57 by Suzuki coupling with 3. The synthesis of the aminopyrimidines 61 and 62 is described in Scheme 11-b. Starting from commercially available 4-chloropyrimidin-2-amine 59, Suzuki coupling with 4
afforded 60. Saponification of the resulting methyl ester afforded 61. 60 was utilized in a Buchwald-Hartwig coupling with bromobenzene and subsequent ester hydrolysis yielded 2-phenylaminopyrimidine 62.

Scheme 11: Synthesis of pyrimidine and aminopyrimidine hinge binders.

Reagents and conditions: a) 3, Pd(PPh$_3$)$_4$, Cs$_2$CO$_3$, 1,2-DME, H$_2$O, 120 °C, 0.5 h. b) 4, Pd(PPh$_3$)$_4$, Cs$_2$CO$_3$, 1,2-DME, H$_2$O, 120 °C, 0.5 h; c) Aq. LiOH, dioxane, 100 °C, 16 h then aq. HCl; d) PhBr, Pd$_2$(dba)$_3$, XantPhos, NaO'Bu, PhMe, 80 °C, 19 h.

Assays used for in vitro compound affinity evaluation:

DSF Assay: We used a thermal-shift assay (Differential Scanning Fluorimetry, DSF) to detect protein-ligand interaction. For many proteins, including kinases, this is a useful high-throughput method to identify compounds that bind to a protein of interest, and in many cases the melting temperature correlates with binding affinity. CAMKK1 and CAMKK2 are ~60% identical at the amino acid sequence level. We measured DSF $\Delta T_m$ for all analogs against both CAMKK2 and CAMKK1 using the purified kinase domains of these two proteins.
In vitro CAMKK2 enzyme inhibition assay: We used purified recombinant CAMKK2 in an assay that measured the transfer of radiolabeled phosphate from [γ-32P]-ATP to a synthetic CAMKK2 substrate (CAMKKtide). Initially, we evaluated CAMKK2 inhibitory activity of all synthesized compounds at three different concentrations (10 nM, 100 nM, 1000 nM) to rank compounds and provide preliminary dose response information. In the PoC experiments, the assay with no inhibitor present serves as the control. Half-maximal inhibitory concentrations (IC₅₀) were then determined for analogs with percent of control (PoC) values <10 at the 1 µM screening concentration. STO-609 and GSK650394 were routinely used as reference compounds in the assay to ensure the assay was performing correctly and CAMKK2 inhibition was observed.

In vitro compound screening results:

The initial set of compounds evaluated was focused on those with similar topology to GSK650394. Data in Table 1 depicts compounds, the DSF data for CAMKK1 and CAMKK2, the enzyme inhibition data (PoC) at three concentrations, and CAMKK2 enzyme inhibition IC₅₀ data for selected compounds. These data allowed us to evaluate compound effect on CAMKK2 protein stabilization (DSF ΔTₘ) as a surrogate for binding affinity as well as compound effect on enzymatic activity.
Removal of the phenyl group from the 5-position of GSK650394 led to a decrease in protein melting temperature (7: DSF $\Delta T_m = 15.5$ °C vs. GSK650394: DSF $\Delta T_m = 20.7$ °C) as well as enzyme inhibitory activity ($IC_{50} = 26$ nM vs. 3 nM), implying that the phenyl group or an appropriately adorned phenyl group may be useful to enhance CAMKK2 enzyme affinity and/or activity. Similar pairs of analogs for the different hinge-binders allowed for direct comparison (Table 1).

**Table 1:** DSF and enzyme inhibition data of CAMKK2 inhibitors of 3- and 3,5-substituted fused 5,6-ring systems.

| ID | Hinge Binder | Hinge Binder | X | DSF $\Delta T_m$ [°C] | CAMKK2 PoC | IC$_{50}$ [nM] |
|----|--------------|--------------|---|----------------------|-------------|---------------|
|    |              |              |   | CAMKK 1 | CAMKK 2 | 1 µM | 0.1 µM | 0.01 µM |     |
| 1  | Azaindole    | Ph           | 15.1 | 20.7 | 3          | 4          | 14       | 3      |
| 2  | Azaindole    | H            | 10.1 | 15.5 | 0          | 6          | 83       | 26     |
| 3  | N-methyl azaindole | Ph | 5.0 | 3.1 | 101 | 102 | 103 | NG |
| 4  | N-methyl azaindole | H | -0.9 | -0.4 | 95 | 100 | 103 | NG |
| 5  | Furopyridine  | Ph           | 8.3 | 15.5 | 7          | 46         | 71       | 65     |
| 6  | Furopyridine  | H            | 3.2 | 7.4  | 28         | 71         | 97       | NG     |
| 7  | Thienopyridine| Ph           | 10.1 | 13.9 | 3          | 14         | 30       | 5      |
| 8  | Thienopyridine| H            | 5.1  | 8.0  | 19         | 75         | 101      | NG     |
| 9  | Pyrazolopyridine| Ph | 6.8 | 12.6 | 9          | 60         | 85       | 145    |
| 10 | Pyrazolopyridine| H | 6.3 | 12.0 | 5          | 37         | 81       | 44     |
| 11 | Pyrazolopyridin e| Ph | 10.6 | 14.8 | 4          | 18         | 71       | 21     |
| 12 | Pyrazolopyridin e| H | 5.0  | 9.8  | 16         | 65         | 100      | NG     |
| 13 | Triazolopyridazine| Ph | 4.7 | 5.1  | 58         | 72         | 91       | NG     |
R = *ortho*-cyclopentyl benzoic acid moiety; DSF (Differential Scanning Fluorimetry); PoC (Percent of Control = percent of enzyme activity remaining, when compared with control); IC\textsubscript{50} (half-maximal Inhibitory Concentrations); NG (Not generated); DSF results are a mean of n = 3 runs. IC\textsubscript{50} values were generated in an 8 point full dose response assay.

The goal of this project was to discover alternate scaffolds for CAMKK2 with improved kinome selectivity that can be used as starting points for medicinal chemistry optimization programs. We hypothesized that carefully modulating the hydrogen bonding interactions at the hinge-binding region *via* a variety of heterocyclic scaffolds could identify new series that maintained CAMKK2 affinity and improved selectivity profiles. Kinase inhibitors with multiple hinge binding interactions can suffer from off-target effects, owing to their potential for poor selectivity across the kinome.\textsuperscript{39, 41, 50} However, careful modification of even promiscuous starting points can in some cases lead to desired levels of selectivity. There are examples demonstrating that modifications to the hydrogen bonding interactions between a kinase inhibitor and the hinge region of the kinase can improve selectivity without severely impacting potency.\textsuperscript{41} *N*-Methyl azaindoles \textbf{10} and \textbf{11} (Table 1), offer a particularly drastic change in hinge binding by blocking the H-bonding donor property of the pyrrole ring. This resulted in a significant drop in CAMKK2 melting temperature and a complete loss in enzyme inhibition potency *in vitro*. This suggested that the steric bulk of the *N*-methyl group is not accommodated within the binding pocket of CAMKK2 when the cyclopentyl benzoic acid moiety is attached at the 3-position of the azaindole scaffold.
Further structural modifications to the hinge-binding moiety that maintained the fused 5,6-ring system are outlined here. Compounds 13g and 12f replaced the pyrrole ring with a furan ring. Compounds 14g and 12g replaced the pyrrole ring of GSK650394 with a thiophene ring. The pyridine nitrogen that makes a key hydrogen bond with the hinge is maintained, but the NH hydrogen bonding opportunity has been removed. Compounds 19, 20, 21, 22, 25, and 26 all maintain the 5,6-fused core but have key differences from GSK650394. They do not have a nitrogen in a position analogous to the nitrogen in the 7-position of GSK650394 thus necessitating different hinge binding interactions. They also incorporate nitrogen atoms in other locations within the 5,6-ring system.

The DSF data demonstrated that many of the compounds interacted with CAMKK1 in addition to CAMKK2. All pairs of analogs exhibited appreciably greater $\Delta T_m$ for CAMKK2 than CAMKK1, with the exception of 10 which recorded a DSF $\Delta T_m$ of 5.0 °C and 3.1 °C for CAMKK1 and CAMKK2 respectively. One can usefully compare thermal stability data for different compounds on the same protein. However, comparisons across different proteins are confounded by intrinsic differences in the thermal stabilization. It appears that CAMKK1 has a smaller window of stabilization, but we do not presently know whether the lower $\Delta T_m$ values for CAMKK1 relative to CAMKK2 will be reflected in a binding potency preference for the latter.

To further understand the propensity for these compounds to bind CAMKK1, we are currently pursuing a CAMKK1 enzyme assay to accurately measure *in vitro* inhibition.
Generally, compounds that induced large shifts in melting temperatures (DSF $\Delta T_m \geq 12 \, ^\circ C$) were potent inhibitors of CAMKK2 and had IC$_{50}$ values $\leq 145$ nM in the enzyme assay. Data from the enzymatic assay generally corroborated with results from the DSF screen. Nearly all of the phenyl-substituted analogs of active CAMKK2 inhibitor scaffolds showed greater enzyme potency than their corresponding truncated analogs. An exception was compound 19 (phenyl version) which had an IC$_{50} = 145$ nM compared to an IC$_{50} = 44$ nM for compound 21 (truncated version). Replacement of the pyrrole ring in the azaindole with either a furan or thiophene ring retained potency in the phenyl-substituted analogs (13g IC$_{50} = 65$ nM, 14g IC$_{50} = 5$ nM), but their corresponding truncated analogs (12f, 12g) were relatively inactive. Hence, the choice of a hinge-binder plays a role in the type of substitutions that are tolerated. Both versions of the triazolopyridazine hinge binder (25, 26) showed no activity. We hypothesize that this is due to repulsion between the lone pair on the nitrogen in the 2-position and a backbone protein carbonyl (carbonyl from E268 in CAMKK2). In many kinase/inhibitor co-crystal structures, this carbonyl is engaged in hydrogen bonding with an NH from the inhibitor or at least pointing towards a polarized CH on the inhibitor. In summary, four scaffolds (furopyridine, thienopyridine, pyrazolopyridine and pyrazolopyrimidine) in this set exhibited excellent CAMKK2 enzyme inhibition, with 14g (IC$_{50} = 5$ nM) exhibiting comparable enzymatic inhibitory activity to GSK650394 (IC$_{50} = 3$ nM).
To further evaluate fused 5,6-ring structures as CAMKK2 inhibitors we switched from 3,5- to 2,4-ring substitutions as depicted in Table 2. N-Methyl azaindoles 29 and 30 were the first pair of analogs synthesized. Interestingly, these two N-methyl substituted azaindole analogs were well tolerated, displaying a DSF $\Delta T_m >11$ °C with CAMKK2 and good CAMKK2 enzyme inhibitory activity (29 $IC_{50} = 120$ nM, 30 $IC_{50} = 56$ nM). This result is in stark contrast to the 3,5-N-methyl azaindoles 10 and 11 in Table 1. The truncated analog 30 was significantly more potent than its corresponding phenyl analog 29.

Table 2: DSF and enzyme inhibition of CAMKK2 inhibitors of 2- and 2,4-substituted fused 6,5-ring systems.

| ID | Hinge Binder | Hinge Binder | X | DSF $\Delta T_m$ [°C] | CAMKK2 PoC | IC$_{50}$ [nM] |
|----|--------------|--------------|---|----------------------|------------|----------------|
|    |              |              |   | CAMKK1 | CAMKK2 | 1 µM | 0.1 µM | 0.01 µM |
| 29 | N-methyl azaindole | Ph | 5.2 | 11.7 | 6 | 64 | 84 | 120 |
| 30 |              | H | 6.5 | 12.1 | 5 | 30 | 97 | 56 |
| 34 | Imidazopyridine | Ph | 11.7 | 20.5 | 0 | 8 | 78 | 23 |
| 33 |              | H | 5.2 | 9.0 | 11 | 66 | 97 | 193 |
| 37 | Thienopyrimidine | Ph | 13.6 | 20.6 | 0 | 68 | 101 | 169 |
| 38 |              | H | 8.5 | 13.7 | 0 | 17 | 73 | 24 |
| 41 | Thienopyrimidine | Ph | 6.2 | 10.7 | 12 | 75 | 91 | 232 |
| 42 |              | H | 5.5 | 10.0 | 2 | 24 | 77 | 31 |

R = ortho-cyclopentyl benzoic acid moiety; DSF (Differential Scanning Fluorimetry); PoC (Percent of Control = percent of enzyme activity remaining, when compared with control); IC$_{50}$
(half-maximal inhibitory concentrations); DSF results are a mean of n = 3 runs. IC_{50} values were generated in an 8-point full dose response assay.

The trends observed in the thermal shifts for the 3,5-substituted analogs in Table 1 held with the 2,4-substituted analogs of Table 2 in that they regularly showed greater stabilization of CAMKK2. Incorporation of an extra nitrogen into the pyrrole ring to create imidazopyridine analogs 33 and 34 was well tolerated, with the phenyl-substituted analog demonstrating higher potency (34 IC_{50} = 23 nM) than the corresponding non-phenyl version (33 IC_{50} = 193 nM). Similar to the N-methyl azaindole 30, non-phenyl substituted thienopyrimidines (38 IC_{50} = 24 nM, 42 IC_{50} = 31 nM) were more potent than their corresponding phenyl-substituted analogs (37 IC_{50} = 169 nM, 41 IC_{50} = 232 nM). For both [3,2-d] analogs, with the sulfur oriented away from the hinge binding region, a significant increase in change of melting temperatures was observed (37 at 20.6 °C, 38 at 13.7 °C), whereas the [2,3-d] analogs 41 and 42 with the sulfur oriented towards the hinge seem to bind with lower affinity (41 DSF ΔT_m 10.7 °C, 42 DSF ΔT_m 10.0 °C).

Next, we explored replacement of the 5,6-bicyclic cores with 6,6-bicyclic structures. Quinazolines and quinolines are frequently used as kinase hinge binding heterocycles and were identified as active scaffolds for CAMKK2 inhibition (Table 3). Both quinoline 45, with a phenyl in the 6-position, and 46, unsubstituted in the 6-position, inhibited CAMKK2 (45 IC_{50} = 137 nM, IC_{50} 46 = 13 nM). The potency of the low molecular weight compound 46 suggests further
exploration may be fruitful. Likewise, the two quinazolines 49 (IC\textsubscript{50} = 12 nM) and 50 (IC\textsubscript{50} = 96 nM) were also potent CAMKK2 inhibitors.

As expected, introduction of a methyl group at the 2-position of 52 resulted in a complete loss of CAMKK2 enzyme activity. The steric bulk of the 2’ methyl group is not tolerated and hinders the ability of the quinolines nitrogen to effectively participate in hydrogen bonding with the hinge region. Naphthyridine 54 showed poor CAMKK2 enzymatic activity.

**Table 3:** Tested CAMKK2 inhibitors of fused 6,6-ring systems with their scaffold types.

| Entry | Hinge Binder | Hinge Binder | X | DSF ΔT\textsubscript{o} [°C] | CAMKK2 PoC |
|-------|--------------|--------------|---|-----------------------------|------------|
|       |              |              |   | CAMKK1 | CAMKK2 | 1 µM | 0.1 µM | 0.01 µM | IC\textsubscript{50} [nM] |
| 45    | Quinoline    | Ph           |   | 8.4    | 13.1   | 8    | 62     | 92      | 137     |
| 46    |              | H            |   | 9.5    | 14.5   | 3    | 5      | 51      | 13      |
| 49    | Quinazoline  | Ph           |   | 5.8    | 10.3   | 5    | 28     | 48      | 12      |
| 50    |              | H            |   | 4.8    | 9.9    | 10   | 51     | 96      | 96      |
| 52    | 2-Methylquinoline | H         | -0.9 | 0.2 | 97 | 93 | 98 | NG |
| 54    | 1,6-Naphthyridine | - | 4.1 | 8.4 | 86 | 91 | 102 | NG |

R = ortho-cyclopentyl benzoic acid moiety; DSF (Differential Scanning Fluorimetry); PoC (Percent of Control = percent of enzyme activity remaining, when compared with control); IC\textsubscript{50} (half-maximal inhibitory concentrations); NG (Not generated); DSF results are a mean of n = 3 runs. IC\textsubscript{50} values were generated in an 8 – point full dose response assay.
Finally, we evaluated a small set of pyrimidines, well-known kinase ATP-competitive inhibitor scaffolds, for their CAMKK2 inhibitory activity (Table 4). Pyrimidine 56, with a phenyl in the 2-position, had a CAMKK2 IC<sub>50</sub> = 21 nM. The 2-anilino-pyrimidine 62 was also potent with CAMKK2 IC<sub>50</sub> = 51 nM and recorded the highest shift in melting temperature at 22.4 °C. Pyrimidine 58, unsubstituted in the 2-position, lost considerable activity relative to 56. 2-amino-pyrimidine 61 retained CAMKK2 potency (IC<sub>50</sub> = 108 nM).

Table 4: Substituted pyrimidines and 4-aminopyrimidines evaluated for binding affinity and CAMKK2 inhibition.

| ID | Hinge Binder | Hinge Binder | X | DSF ΔT<sub>m</sub> [°C] | CAMKK2 PoC | IC<sub>50</sub> [nM] |
|----|--------------|--------------|---|-----------------------|-------------|-------------------|
|    |              |              |   |                       | CAMKK 1     | CAMKK 2 | 1 µM | 0.1 µM | 0.01 µM |
| 56 | Pyrimidine   | Ph           |   | 6.9                   | 13.7        | 0      | 10   | 73   | 21     |
| 58 |              | H            |   | 2.0                   | 7.1         | 26     | 84   | 98   | NG     |
| 62 | Aminopyrimidine | Ph       |   | 14.9                  | 22.4        | 0      | 21   | 97   | 51     |
| 61 |              | H            |   | 5.3                   | 10.1        | 8      | 53   | 95   | 108    |

R = ortho-cyclopentyl benzoic acid moiety; DSF (Differential Scanning Fluorimetry); PoC (Percent of Control = percent of enzyme activity remaining, when compared with control); IC<sub>50</sub> (half-maximal inhibitory concentrations); NG (Not generated); DSF results are a mean of n = 3 runs. IC<sub>50</sub> values were generated in an 8 – point full dose response assay.

X-ray crystallography and in silico docking studies

In order to more fully understand the binding modes and to plan optimization studies on these scaffolds, we turned to X-ray crystallography and in silico docking analysis of key molecules. We
previously reported the crystal structure of 7-azaindole GSK650394 bound to CAMKK2 (PDB ID 6BKU).\textsuperscript{51} A crystal structure of the closely related 7-azaindole GSK650393 is also available (PDB ID 6CMJ).\textsuperscript{25} These two structures clearly demonstrate a hydrogen bond interaction between the NH of the pyrrole in the 7-azaindole to the carbonyl group of Glu268 at the CAMKK2 hinge region. The nitrogen atom at the 7-position forms an interaction with the hinge via hydrogen bond with the NH of Val270. We were able to obtain a crystal structure of furopyridine 13g (PDB ID 5UY6). Furopyridine 13g displays a similar binding mode to GSK650394. The 5,6-fused ring systems are oriented in the same way in the CAMKK2 active site, and the pyridine moieties of the two heterocycles form comparable hydrogen bond interactions with the NH group of Val270, and the furan oxygen atom and pyrrole NH group are in the same region of space. Although we have no crystal structure of the thienopyridine 14g, we hypothesize that it would likely bind in a similar fashion.

To assess the binding modes of 14g and other compounds, we performed \textit{in silico} docking studies with the docking software Glide\textsuperscript{®} version 2014 (Schrödinger\textsuperscript{®}).\textsuperscript{52} For our docking studies, we utilized three of the X-ray crystal structures of CAMKK2 (PDB-ID: 6BKU, 5UY6 and 5UYJ). We based our choice of the CAMKK2 PDB protein structure to use for our modeling on the structural similarity between the co-crystallized ligand and the compound we planned to use in the \textit{in silico} docking. In order to ensure reliability of the docking protocol and the ensuing results for hypothesis generation, the co-crystallized ligands GSK650394, 13g, and
UNC10244803 (2-cyclopentyl-4-(7-methoxyquinolin-4-yl)benzoic acid, CAMKK2 enzyme IC\textsubscript{50} = 33 nM) were removed from the binding site and the ligands were re-docked into the binding pocket. The docking poses were then compared with the original pose from the crystal structures. The Root Mean Square Deviation (RMSD) value was lower than 1 Å indicating a reliable docking procedure. The predicted binding modes of active compounds (Figure 3) show conserved H-bonds or close proximity between the compound’s carboxylate group and both the protonated amine of Lys194 as well as the carboxylate group of Glu236 in a water-mediated manner, analogous to what was observed in the crystal structures. The overall orientation of the tested compounds and their interactions with the hinge region is also of interest.

**Figure 3:** X-ray structures and *in silico* docking. *In silico* docking was performed with Glide\textsuperscript{®} and images were generated with PyMOL\textsuperscript{®}. The protein is colored in grey. Blue-dashed lines indicate H-bond interactions while green-dashed lines display CH-π interactions and orange-
dashed lines refer to sulfur-σ hole bonding. Docked ligands are shown as yellow sticks or orange sticks, co-crystallized ligands as purple sticks, and the water molecule as a red sphere. Oxygen and nitrogen atoms are colored in red and blue, respectively.

3A: Predicted binding mode of 14g (yellow) using protein structure from PDB-ID 5UY6 compared to the co-crystallized ligand 13g (purple). 3B: Predicted binding modes of 46 (yellow) using protein structure from PDB-ID 5UYJ and co-crystallized ligand UNC10244803 (purple). 3C: Predicted binding mode of 56 using protein structure from PDB-ID 5UYJ. 3D: Predicted binding mode of 22 using protein structure from PDB-ID 6BKU. 3E: Predicted binding modes of 10 (orange) and 29 (yellow) using protein structure from PDB-ID 6BKU (light gray) and 5UYJ (dark gray) respectively. 3F: Predicted binding mode of 38 using protein structure from PDB-ID 5UYJ.

As expected, the predicted binding mode of thienopyridine 14g overlaid almost perfectly with the pose of furopyridine 13g in the X-ray structure (Figure 3A). The backbone NH group of Val270 and the thienopyridine N-atom are well positioned for a hydrogen bond interaction. Interestingly, there appears to be an additional favorable interaction between the sulfur atom of 14g and the carbonyl group of Glu268. Inter- and intra-molecular interactions between sulfur and oxygen atoms are relatively common and can be used to the medicinal chemists’ advantage.53, 54
We were also able to obtain a crystal structure of quinoline UNC10244803 (PDB ID: 5UYJ). The quinoline nitrogen atom is positioned to form a hydrogen bond with the NH group of Val270. The predicted binding pose of 46 (Figure 3B) is highly comparable to the binding mode of the co-crystallized ligand UNC10244803. Similar to the quinoline 46, the 2-phenyl-pyrimidine 56 has only one heteroatom that is capable of forming hydrogen bonds with the hinge (Figure 3C). The N-atom of the pyridine in 1-position of 56 shows an H-bond with the NH-group of Val270. Together with the presumed H-bond formed between the protonated N-atom of Lys194 and the carboxylate group of 56, these two interactions anchor 56 in the active site. In this orientation the phenyl ring in the 2-position is tolerated by the binding pocket. 2-aryl-pyrimidines are much less common as kinase inhibitors than 2-anilinopyrimidines and so warrant further exploration. The 2-position phenyl ring of 56 is adjacent to Leu269 of CAMKK2. We speculate that kinases incorporating amino acids with larger side chain such as Phe or Tyr in this location at the hinge region will be less tolerant of this scaffold, perhaps offering a path to enhance selectivity.

Pyrazolopyrimidine 22 (Figure 3D) forms one hinge binding interaction between the N-atom in position 1 and the backbone NH-group of Val270. The distance between the NH$_3^+$-group of Lys194 and the carboxylate group of 22 is greater compared to the predicted binding modes of the other compounds (Figures 3B and 3E). The N-methyl group of compound 10 (Figure 3E) seems to preclude binding of this compound in the same orientation as GSK650394. This
compound is inactive in the enzyme assay, and the docking predicts a flipped orientation of the compound. N-methyl compound 29, however, retains activity and in silico docking result suggests a conformation that allows the pyridyl nitrogen atom to interact with the NH group of Val270. The methyl group in this orientation is tolerated, perhaps analogously to the 2-phenyl of compound 56. This flipped binding mode is possibly due to the shift of the attachment position of the cyclopentyl benzoic acid moiety from the 5-position of 10 to the 4-position in 29. The hinge binding interactions shown in the predicted binding mode of thienopyrimidine 38 are comparable (Figure 3F) to those of 56. Additionally, the orientation of 38 is further stabilized by an interaction that is formed between the O-atom of the carbonyl moiety of Ile171 and the S-atom of the thienopyrimidine scaffold.

**Compound properties:**

A number of key physicochemical screens and calculations are considered in early-stage drug discovery, including lipophilicity, pKa, solubility, permeability and stability. Solubility is one of the most critical physicochemical properties as poor solubility can lead to an underestimation of activity, inaccurate SAR, inaccurate in vitro ADMET test results, and downstream compound development issues. Highlighting the critical importance of solubility, it has been shown that 87% of commercial drugs have solubility ≥65 μg/mL, but only 7% had solubility ≤20 μg/mL. Therefore, solubility data can identify a series liability that needs to be fixed and highlight promising series with enhanced solubility. This information can help guide optimization strategies. Ligand efficiency metrics are also a commonly used tool that can guide hit-to-lead
optimization for drug candidates.\textsuperscript{58, 59} To this end, we calculated ligand efficiency (LE) and lipophilic ligand efficiency (LLE) and collected kinetic solubility data for compounds with IC\textsubscript{50} values < 150 nM in the CAMKK2 enzyme assay (Table 5).\textsuperscript{60}

Ligand efficiency values are influenced by molecular size; hence, it was unsurprising to see two clear ranges depending on the presence or absence of the phenyl ring. The hinge binders without the phenyl ring had ligand efficiency values ranging from 0.41 to 0.46. These are smaller compounds with molecular weights between 300 and 325, and generally LE values above 0.3 are acceptable for this MW range.\textsuperscript{58} The LLE values for these compounds were between 3.02 to 4.43. The aminopyridine 61 had the highest LE and LLE values, of 0.46 and 4.43 respectively. 7 and 38 both had comparable LE values of 0.45, but significantly lower LLE values of 3.82 and 3.61.

Both the LE and LLE metrics the phenyl-substituted analogs have lower values. This is reflective of the increased atom count and higher cLogP values due to the additional lipophilicity of the phenyl ring. The LE values are between 0.32 and 0.40 for these compounds. GSK650394 has the highest LE and LLE values of 0.40 and 2.83 respectively. The analogous thienopyridine 14g has the next highest LE value of 0.39, but a lower LLE value of 1.98. The quinoline 45 had the lowest LE and LLE values of 0.32 and 0.31 respectively. Solely relying on ligand efficiency metrics to select compounds is unadvisable as many other factors, such as solubility, underpin successful drug development. A future direction could be to focus on polar substituents on the
Pendant phenyl to lower logP, enhance solubility, and mitigate metabolic liability. Likewise, non-aromatic substituents could be explored to access this same region of the active site.

**Table 5**: Ligand efficiency metrics, cLogP and solubility data for potent CAMKK2 inhibitors.

| ID   | CAMKK 2 enzyme IC50 [nM] | CAMKK2 NB IC50 [nM] | cLogP | Lipophilic ligand efficiency | Solubility [μg/mL] |
|------|--------------------------|----------------------|-------|-----------------------------|-------------------|
| STO-609 | 58                        | NG                   | 3.59  | 0.41                        | 3.61              | 60                |
| GSK650394 | 3                         | <3                  | 5.67  | 0.40                        | 2.83              | 2                 |
| 7     | 26                        | NG                   | 3.78  | 0.45                        | 3.82              | -                 |
| 13g   | 65                        | 700 ± 9.3            | 5.68  | 0.34                        | 1.52              | 77                |
| 14g   | 5                         | 210 ± 24             | 6.32  | 0.39                        | 1.98              | 1.5               |
| 19    | 145                       | 530 ± 95             | 6.01  | 0.32                        | 0.79              | 42                |
| 20    | 21                        | 190 ± 19             | 5.17  | 0.36                        | 2.53              | 50.5              |
| 21    | 44                        | 200 ± 11             | 4.13  | 0.44                        | 3.27              | 48.3              |
| 29    | 120                       | 240 ± 7              | 6.06  | 0.32                        | 0.84              | -                 |
| 30    | 56                        | 470 ± 44             | 3.97  | 0.42                        | 3.33              | 40                |
| 34    | 23                        | 8.1 ± 0.8            | 5.42  | 0.36                        | 2.18              | 55.1              |
| 38    | 24                        | 170 ± 18             | 3.99  | 0.45                        | 3.61              | 55.4              |
| 42    | 31                        | 1400 ± 250           | 3.78  | 0.45                        | 3.72              | 62.6              |
| 45    | 137                       | 540 ± 62             | 6.59  | 0.32                        | 0.31              | 38                |
| 46    | 13                        | 140 ± 12             | 4.7   | 0.45                        | 3.2               | 64.4              |
| 49    | 12                        | 890 ± 71             | 5.86  | 0.36                        | 2.04              | 70.4              |
| 50    | 96                        | 1900 ± 200           | 3.97  | 0.40                        | 3.03              | -                 |
| 56    | 21                        | 290 ± 23             | 4.68  | 0.41                        | 3.02              | 19.4              |
| 61    | 108                       | 950 ± 110            | 2.57  | 0.46                        | 4.43              | -                 |
| 62    | 51                        | <3                  | 5.38  | 0.37                        | 1.92              | 10.6              |

NB = data from the NanoBRET in cell target engagement assay
Of the phenyl substituted analogs, GSK650394 and 14g were the most potent, with IC$_{50}$ values of 3 and 5 nM respectively, and had the best LE and LLE values. Although promising in this regard, these compounds have extremely poor solubility values of 2 and 1.5 µg/mL respectively. Optimization of these compounds will thus require extensive structure-property-relationship (SPR) studies in parallel to SAR studies to develop them into useful tools with adequate solubility for further study. Interestingly, the furopyridine 13g had the highest measured solubility value of 77 µg/mL. Substitution of the azaindole’s NH with an oxygen greatly improved solubility. The effect was reversed when a more lipophilic sulfur atom is incorporated into the same position, 14g. The pyrimidine analogs 56 and 62 are also only modestly soluble (10.6 and 19.4 µg/mL). Optimization to tool compounds will likely require the introduction of solubilizing functional groups. A number of the compounds tested had reasonable solubility ranging from 38–55 µg/mL. Four compounds had promising solubility above that recorded for STO-609, 60 µg/mL. These were the thienopyrimidine 42 (62.6 µg/mL) and quinoline 46 (64.4 µg/mL), both without the pendant phenyl ring. The phenyl substituted quinazoline 49 has a solubility of 70.4 µg/mL, second only to the furopyridine 13g. Overall, the solubility of several potent compounds was encouraging but will need to be monitored as optimization proceeds.

**Compound selectivity:**

To further understand their kinase selectivity, compounds 13g and 45 were profiled in a panel of over 400 wild-type human kinases using Eurofins DiscoverX’s KINOMEscan® technology.$^{50}$
Freemont, CA USA. \textbf{13g}, chosen as representative of the 6,5-bicyclic chemotypes, retains the topology of GSK650394, but has modified hydrogen bonding properties. GSK650394 was previously profiled in a panel of 334 kinases (Reaction Biology Corporation) and at 1 μM inhibited 47 kinases by over 80% (29 kinases by 90%). In the DiscoverX binding assay at a screening concentration of 1 μM, \textbf{13g} showed a substantial increase in selectivity by only inhibiting two kinases by >80%, CAMKK2 (88%) and CAMKK1 (81%) (Figure 4). The selectivity score ($S_{20}$) value for \textbf{13g} is 0.004, whereas GSK650394 (albeit in a different assay format) has an $S_{20}$ value of 0.16. In a dose response experiment in the DiscoverX binding assay \textbf{13g} had a CAMKK2 disassociation constant ($K_d$) of 34 nM. At 1 μM, only four other kinases are moderately inhibited (>50%) by \textbf{13g}; ACVR2B (70%), FLT3 (66%), LZK (66%) and PIP5K2C (66%). Although the thienopyridine \textbf{14g} was more potent than the furopyridine, its poor solubility led us to deprioritize selectivity screening at this point.

We chose to profile quinoline \textbf{45} as it offered a very different scaffold with varied hydrogen bonding within the hinge region and the pendant ortho-cyclopentyl benzoic acid moiety moved relative to the hinge binding nitrogen of the furopyridine scaffold \textbf{13g} (or parent azaindole GSK650394). \textbf{45} screened at 1 μM bound to only two kinases, WNK2 and CAMKK2, by >80% providing an $S_{20} = 0.004$. The WNK2 result needs to be followed up with a $K_d$ determination using this platform and checked in an orthogonal assay to determine if it is a true target of this compound or a false positive. Both \textbf{13g} and \textbf{45} showed high selectivity as indicated by their
respective selectivity scores (S score). These results illustrate excellent selectivity for the furopyridine and quinoline chemotypes.

**Figure 4:** *In vitro* kinase selectivity profile of 13g and 45 at 1 µM (Eurofins DiscoverX KINOMEScan®). TREEspot interaction maps for 13g and 45 profiled against > 400 human kinase targets. Results for primary screen binding interactions are reported as percent (%) control, where lower values are indication of stronger hits in the matrix. S-score is a quantitative measure of a compound’s selectivity at a particular screening concentration, calculated by dividing the number of kinases that a particular compound binds to at a chosen threshold by the total number of distinct kinases tested. This $S_{20} \ (1 \ \mu M) = 0.004$ means 0.4% of the kinases tested had a PoC<20 at an inhibitor screening concentration of 1 µM.

**CAMKK2 NanoBRET Cellular Target Engagement:**

Having demonstrated potent CAMKK2 inhibition and favorable selectivity profiles in *in vitro* assays, we next profiled these compounds for quantitative engagement of CAMKK2 in cells
(Table 5). To this end, we developed a CAMKK2 NanoBRET target engagement assay.\textsuperscript{61} This assay uses bioluminescence resonance energy transfer (BRET) between nanoluciferase (NL) fused to the kinase domain of CAMKK2 (BRET donor) and a tracer molecule that binds to the ATP-binding site of the kinase (BRET acceptor). The addition of cell penetrant CAMKK2 inhibitors leads to displacement of the tracer molecule and a quantifiable reduction in BRET signal. This assay is conducted in living cells, and the observation of binding not only depends on affinity between compound and CAMKK2 but also on compound cell penetrance. The parent molecule, GSK650394, was very potent in this assay (NB IC\textsubscript{50} <3 nM). Several additional key compounds were evaluated in this assay (Table 5, SI Section 3.4). A key takeaway here is that all of the compounds that advanced to this assay (those with CAMKK2 enzyme IC\textsubscript{50} < 150 nM) demonstrated measurable CAMKK2 target engagement in cells. Potency was below 500 nM for thienopyridine 14g (210 nM), pyrazolopyrimidines 20 and 21 (190 nM and 200 nM), N-methyl azaindoles 29 and 30 (240 nM and 470 nM), imidazopyridine 34 (8 nM), thienopyrimidine 38 (170 nM), quinoline 46 (140 nM), 2-phenylpyrimidine 56 (290 nM), and 2-amino-pyrimidine 62 (<3 nM). Results for exemplar compounds from five different scaffolds (13g, 45, 46, 56, 62) are depicted in Figure 5.
Figure 5: CAMKK2 NanoBRET dose-response curves for compounds 13g, 45, 46, 56, and 62.

On-target cellular effect (Phosphorylation inhibition assay): We also assessed the impact of our inhibitors on phosphorylation downstream from CAMKK2 using western blot analysis with C4-2 prostate cancer cells to provide evidence of a phenotypic and on-target effect of our CAMKK2 inhibitors in intact cells. AMPK (Thr172) was chosen because in intact cells, CAMKK2, but not the related CAMKK1, can readily phosphorylate this substrate. We performed preliminary Western blot analysis at a single inhibitor concentration of 1 µM for the 18 compounds with CAMKK2 enzyme IC$_{50}$ < 150 nM along with STO-609. These results are shown in Figure 6.
**Figure 6**: Western blots of the 18 compounds with IC\(_{50}\) < 150 nM in the CAMKK2 enzyme assay, together with STO-609, screened at a single inhibitor concentration = 1 µM.

Based on these results compounds 7, 20, 29, 34, 38, 46, 56, and 62 showed the most robust reduction of the p-AMPK band and were advanced into full dose response experiments in this same assay to provide IC\(_{50}\) estimates for inhibition of phosphorylation of AMPK at Thr172. The IC\(_{50}\) values were determined from dose response experiments using 0 – 10 µM of compound. Western blots for the most potent compound, 62, are depicted in **Figure 7a**. Additional dose response data can be found in the supplemental material. The calculated IC\(_{50}\) values for all eight compounds are shown in **Figure 7b**. Compounds 62 (IC\(_{50}\) = 10 nM), 46 (IC\(_{50}\) = 390 nM), 56 (IC\(_{50}\) = 790 nM), 20 (IC\(_{50}\) = 900 nM), and 29 (IC\(_{50}\) 980 nM) all have IC\(_{50}\) values for inhibition of phosphorylation of AMPK at Thr172 below 1 µM suggesting that optimization into compounds with potent cellular activity will be achievable for these series.
**Figure 7.** a. Dose response for inhibition of phosphorylation of AMPK at Thr172 in C4-2 prostate cancer cells by compound 62. b. Calculated IC\textsubscript{50} values for inhibition of phosphorylation of AMPK at Thr172 in C4-2 prostate cancer cells for the top compounds from the single concentration experiment depicted in Figure 6.

**CONCLUSION:** Through a hinge-binder scaffold hopping strategy, we have designed, synthesized and biologically evaluated a series of 32 inhibitors consisting of 5,6-bicyclic, 6,6-bicyclic, and single ring inhibitors of CAMKK2 that are based on the 7-azaindole GSK650394. These inhibitors were designed with the aim of retaining CAMKK2 potency and improving the kinase selectivity of the promiscuous azaindole by modulating the strong H-bond interactions with the hinge-binding residues. Additionally, we also sought to identify inhibitors with improved physicochemical properties, drug likeness, and selectivity all while retaining CAMKK2 inhibitory potency. The DSF data revealed a general trend wherein CAMKK2 showed larger ΔT\textsubscript{m} values than CAMKK1, with CAMKK1 exhibiting a smaller range of stabilization with this set of compounds. None of our compounds are completely selective for CAMKK2 over CAMKK1 or vice versa. This result is not surprising given the high degree of sequence similarity between the two enzymes. Further evaluation of our compounds in a CAMKK1 enzyme assay would be beneficial to help understand any potential for CAMKK2 versus CAMKK1 selectivity. Several
compounds with similar or better potency than GSK650394 and STO-609 have been identified, some of which also showed improved physicochemical properties. Kinome-wide profiling revealed that the furopyridine 13g and quinoline 45 were highly selective for CAMKK2 across the human kinome. Our work has shown that kinase inhibitors with weakened hinge-binding interactions can show highly selective kinase inhibition. As a result, we have identified several CAMKK2 scaffolds with alternate hinge binding moieties that hold promise for the development of high quality CAMKK2 chemical probes.

EXPERIMENTAL:

BIOLOGY:

*Protein expression and purification/DSF assay:* Small molecule screening by DSF were performed as described previously. Briefly, the DSF assay was performed in 96-well format. Purified CAMKK1 or CAMKK2 was diluted to 2 μM kinase in 100 mM potassium phosphate pH 7.5, 150 mM NaCl, and 10% glycerol supplemented with 5 × SYPRO Orange (Invitrogen, Carlsbad, CA, USA). All assay experiments used 19.5 μL of 2 μM kinase and SYPRO Orange mixture. Compounds solubilized in DMSO were used at 12.5 μM final concentration, with a 2.5% concentration of DMSO per well. PCR plates were sealed using optically clear films and transferred to a C1000 thermal cycler with CFX-96 RT-PCR head (BioRad, Hercules, CA, USA). The fluorescence intensity was measured over a temperature gradient from 25 to 95 °C at a constant rate of 0.05 °C/s. Curve fitting and protein melting temperatures were calculated based
on a Boltzmann function fitting to experimental data (GraphPad Prism 8). Protein with the addition of 2.5% DMSO was used as a reference. All experiments were carried out in triplicate and the mean of the $\Delta T_m$ is reported. Compounds that provided negative values are presented as having a $\Delta T_m$ of 0 °C.

**CAMKK2 enzyme assay**: CAMKK2 activity was determined by measuring the transfer of radiolabeled phosphate from $[\gamma^{32}\text{P}]-\text{ATP}$ to a synthetic peptide substrate (CaMKKtide) as previously described. Briefly, purified recombinant CAMKK2 (100 pM) was incubated in assay buffer (50 mM HEPES [pH 7.4], 1 mM DTT, 0.02% [v/v] Brij-35) containing 200 μM CaMKKtide (Genscript), 100 μM CaCl₂, 1 μM CaM (Sigma-Aldrich, Castle Hill, NSW, Australia) 200 μM $[\gamma^{32}\text{P}]-\text{ATP}$ (Perkin Elmer, Boston, MA, USA), 5 mM MgCl₂ (Sigma-Aldrich, Castle Hill, NSW, Australia) and various concentrations of inhibitors (0–1 μM) in a standard 30 μL assay for 10 min at 30 °C. Reactions were terminated by spotting 15 μl onto P81 phosphocellulose paper (GE Lifesciences, Paramatta, NSW, Australia) and washing extensively in 1% phosphoric acid (Sigma-Aldrich, Castle Hill, NSW, Australia). Radioactivity was quantified by liquid scintillation counting.

**CAMKK2 NanoBRET assay**: To quantify the cellular activity of these inhibitors we developed a CAMKK2 NanoBRET target engagement assay. Briefly this assay utilizes a nanoluciferase (NL) fused to the kinase domain of CAMKK2. This NL kinase fusion is then transiently transfected into HEK293 cells and after 24 hours tracer is added to the cells. When the tracer and
the NL-CAMKK2 fusion come into proximity they create a BRET signal that can be competed in
dose-dependent manner by the addition of cell-penetrant CAMKK2 inhibitors.

*Western blot analysis*: C4-2 cells were plated in 6-well plates in IMEM medium containing
0.5% FBS. After 72 hours, the cells were then treated with the compounds for 24 hours before
the media was aspirated and wells were washed twice in ice cold PBS. Cells were lysed using
RIPA buffer containing phosphatase and protease inhibitor cocktail while rotating for 30 minutes
at 4 °C. In each lane, 30 µg/well of protein lysate was loaded into a 10% SDS-PAGE gel and run
for 1 hour and 30 minutes. Gels were then transferred overnight in a TRIS-glycine/methanol
transfer buffer onto a PVDF membrane at 4 °C. Membranes were blocked, incubated with
primary overnight at 4 °C, washed, incubated with secondary at room temperature for 1 hour,
washed, and then developed on an Azure Biosystems C-600 imager.

[Cell Signaling: Phospho-AMPKα (Thr172) (40H9) Rabbit mAb: Cat#: 2535; AMPKα
(D5A2) Rabbit mAb Cat#: 5831; BD Bioscience: CAMKK mouse mAb Cat# 610544;
Sigma: GAPDH rabbit pAb: Cat# G9545; Secondary antibody: Goat Anti-Rabbit IgG (H + L)-
HRP Conjugate was from Bio-Rad (Cat#:1706515).]

**CHEMISTRY**

*General chemistry information*: All reagents and solvents, unless specifically stated, were used
as obtained from their commercial sources without further purification. Solvents were degassed
with nitrogen for cross-coupling reactions. Air and moisture sensitive reactions were performed
under an inert atmosphere using nitrogen in a previously oven-dried or flame-dried reaction flask, and addition of reagents were done using a syringe. All microwave (µW) reactions were carried out in a Biotage Initiator EXP US 400W microwave synthesizer. Thin layer chromatography (TLC) analyses were performed using 200 µm pre-coated sorbtech fluorescent TLC plates and spots were visualized using UV light. High resolution mass spectrometry samples were analyzed with a ThermoFisher Q Exactive HF-X (ThermoFisher, Bremen, Germany) mass spectrometer coupled with a Waters Acquity H-class liquid chromatograph system. All HRMS were obtained via electrospray ionization (ESI). Column chromatography was undertaken with a Biotage Isolera One or Prime instrument. Nuclear magnetic resonance (NMR) spectrometry was run on a varian Inova 400 MHz or Bruker Avance III 700 MHz spectrometer equipped with a TCI H-C/N-D 5 mm cryoprobe and data was processed using the MestReNova processor. Chemical shifts are reported in ppm with residual solvent peaks referenced as internal standard.

**4-Bromo-2-cyclopentylbenzoic acid (2)**

4-Bromo-2-fluorobenzoic acid (2.00 g, 9.00 mmol) was dissolved THF (20.0 mL) and cooled to 0 °C. Cyclopentylmagnesium bromide solution (16.0 mL of a 2M solution, 32.0 mmol) was added dropwise. The reaction was stirred at 0 °C for 4 hours. 2 M HCl (25.0 mL) was then slowly added to the solution followed by EtOAc (40.0 mL). The organic phase was separated, concentrated and purified using column chromatography (10% EtOAc/hexane) to afford 4-bromo-2-cyclopentylbenzoic acid 2 (1.70g, 71%), as a pure white solid. The NMR data for this compound matches that previously reported.\(^{38}\)

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 13.07 (s, 1H), 7.60–7.56 (m, 2H), 7.45 (dd, \(J = 8.3, 2.1\) Hz, 1H), 3.68 (tt, \(J = 9.5, 7.5\) Hz, 1H), 2.03–1.94 (m, 2H), 1.82–1.72 (m, 2H), 1.67–1.46 (m, 4H). \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 168.9, 148.6, 131.2, 131.1, 129.5, 128.6, 125.1, 41.2, 34.2, 25.2; LCMS [M+H]^+: \(m/\zeta\) 269.0

**2-Cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid (3)**
To a stirred solution of 4-bromo-2-cyclopentylbenzoic acid 2 (2.50 g, 9.30 mmol) and bis(pinacolato)diboron (2.80 g, 11.0 mmol) in dioxane (50.0 mL) were added PdCl$_2$(dppf)∙CH$_2$Cl$_2$ (0.76 g, 0.96 mmol) and KOAc (3.60 g, 37.0 mmol). The solution was heated to 95 °C for 2 hours. Once cooled to rt, the solution was diluted with EtOAc and filtered through a celite pad. The solution was concentrated, and the crude product purified by column chromatography (10% EtOAc/hexane) to afford 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid 3 (2.30 g, 78 %), as an off white solid.

1H NMR (400 MHz, DMSO-d$_6$) δ 13.00 (s, 1H), 7.69 (d, $J = 1.1$ Hz, 1H), 7.60 (d, $J = 7.6$ Hz, 1H), 7.53 (dd, $J = 7.6, 1.1$ Hz, 1H), 3.62 (tt, $J = 9.8, 7.4$ Hz, 1H), 2.05–1.95 (m, 2H), 1.83–1.73 (m, 2H), 1.69–1.45 (m, 4H), 1.29 (s, 12H); 13C NMR (100 MHz, DMSO-d$_6$) δ 169.7, 144.4, 134.9, 132.1, 131.5, 128.2, 83.9, 41.2, 34.4, 25.2, 24.7; LCMS [M+H]$^+$: m/z 317.2.

Methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (4)

To a stirred solution of methyl 4-bromo-2-cyclopentylbenzoate SI1 (1.50 g, 5.29 mmol) and bis(pinacolato)diboron, (1.88 g, 7.42 mmol) in dioxane (35.0 mL) were added Pd(dppf)Cl$_2$ (194 mg, 0.26 mmol) and KOAc (1.56 g, 15.9 mmol). The solution was heated to 100 ºC for 2 h. Once cooled to rt the solution was filtered through a pad of celite and washed with 100 mL of EtOAc. The volatiles were removed in vacuo and the crude residue was purified via column chromatography (0–5% EtOAc/hexane) to afford Methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 4 (1.37 g, 78%) as oil which slowly solidified into a white solid under vacuum.

1H NMR (400 MHz, DMSO-d$_6$) δ 7.70 (d, $J = 1.1$ Hz, 1H), 7.60 (d, $J = 7.7$ Hz, 1H), 7.56 (d, $J = 1.1$ Hz, 1H), 3.83 (s, 3H), 3.50 (tt, $J = 9.9, 7.5$ Hz, 1H), 2.04–1.93 (m, 2H), 1.85–1.70 (m, 2H), 1.70–1.44 (m, 4H), 1.30 (s, 12H); 13C NMR (100 MHz, DMSO-d$_6$) δ 168.3, 144.6, 133.6, 132.2, 131.6, 128.3, 84.0, 52.2, 41.3, 34.3, 25.2, 24.7; LCMS [M+H]$^+$: m/z 330.2.

Methyl 2-cyclopentyl-4-(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)benzoate (6)

To a stirred solution of 3-bromo-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,3-b]pyridine SI2 (450 mg, 1.37 mmol) and methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-
yl)benzoate 4 (681 mg, 2.06 mmol) in PhMe (8.0 mL) and H₂O (1.00 mL) was added Pd(OAc)₂ (15.4 mg, 68.7 µmol), K₃PO₄ (934 mg, 4.40 mmol) and P(Cy)₃ (38.6 mg, 137 µmol). The reaction mixture heated to 100 °C for 18 h and allowed to cool to rt, filtered through a pad of celite and washed with EtOAc (10 mL). The filtrate was concentrated in vacuo, and the crude was purified by column chromatography eluting with 0–30% EtOAc/hexane to afford methyl 2-cyclopentyl-4-(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,3-b]pyridin-3-yl)benzoate 6 (545 mg, 88%), as a light yellow solid.

LCMS [M+H]+: m/z 327.3.

2-Cyclopentyl-4-(1H-pyrrolo[2,3-b]pyridin-3-yl)benzoic acid (7):

Methyl 2-cyclopentyl-4-(1H-pyrrolo[2,3-b]pyridin-3-yl)benzoate SI3 (100 mg, 0.312 mmol) was dissolved in methanol (8.00 mL) followed by the addition of 50% NaOH solution (170 µL, 3.12 mmol). The mixture was heated to 75 °C for 1 h, allowed to cool, and extracted with diethyl ether (20.0 mL). The aqueous layer was then acidified to pH 5 with 1M HCl and extracted with CH₂Cl₂ (15 mL). The organic layer was then dried (Na₂SO₄), filtered and concentrated in vacuo to afford 2-cyclopentyl-4-(1H-pyrrolo[2,3-b]pyridin-3-yl)benzoic acid 7 (85.0 mg, 89 %) as a pale yellow solid.

¹H NMR (400 MHz, DMSO-d₆) δ 12.64 (s, 1H), 12.12–12.08 (m, 1H), 8.32–8.27 (m, 2H), 8.03 (d, J = 2.6 Hz, 1H), 7.78–7.71 (m, 2H), 7.60 (dd, J = 8.1, 1.8 Hz, 1H), 7.22 (dd, J = 8.0, 4.7 Hz, 1H), 3.92–3.81 (m, 1H), 2.07 (d, J = 14.0 Hz, 2H), 1.82 (t, J = 5.8 Hz, 2H), 1.73–1.61 (m, 4H).

¹³C NMR (101 MHz, DMSO-d₆) δ 169.3, 148.3, 147.1, 142.3, 138.1, 130.4, 128.6, 128.2, 125.2, 124.2, 123.2, 117.7, 116.4, 113.8, 41.2, 34.4, 25.3; HRMS calcd for C₁₉H₁₈N₂O₂ [M+H]+: m/z 307.1447; found 307.1426. MP Range 238–242 °C.

Methyl 4-(5-chloro-1-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-2-cyclopentylbenzoate (9)

5-Chloro-3-iodo-1-methyl-1H-pyrrolo[2,3-b]pyridine (100 mg, 0.34 mmol), methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (113 mg, 0.34 mmol), Pd(dppf)Cl₂·CH₂Cl₂ (28.0 mg, 34.0 µmol) and Cs₂CO₃ (334 mg, 1.00 mmol) were loaded into a microwave vial. A 3:1 mixture of dioxane:water (2 ml) was added before the vial flushed with nitrogen and capped. The solution was stirred at rt for 16 h. Once cooled the solution was diluted with EtOAc (2.00 mL) and water (2.00 mL). The layers were separated, and the aqueous phase
extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. The crude was purified via column chromatography (5–20% EtOAc/hexane) to afford methyl 4-(5-chloro-1-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-2-cyclopentylbenzoate 9 (217 mg, 69%) as a white solid.

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.34 (d, $J = 2.2$ Hz, 1H), 8.15 (d, $J = 2.0$ Hz, 1H), 7.86 (d, $J = 8.1$ Hz, 1H), 7.60 (d, $J = 1.6$ Hz, 1H), 7.49 (s, 1H), 7.42 (dd, $J = 8.1$, 1.7 Hz, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 3.92–3.84 (m, 1H), 2.31–2.06 (m, 2H), 1.92–1.80 (m, 2H), 1.80–1.71 (m, 2H), 1.71–1.59 (m, 4H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 168.5, 148.6, 146.3, 141.7, 137.6, 130.8, 128.5, 128.3, 127.6, 125.0, 124.4, 123.6, 119.3, 114.2, 52.0, 41.7, 34.9, 31.8, 25.7; HRMS calcd for C$_{21}$H$_{22}$N$_2$O$_2$Cl [M+H]$^+$: m/z 369.1369 found m/z 369.1348; MP Range: 144–148 ºC.

2-Cyclopentyl-4-(1-methyl-5-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)benzoic acid (10) Methyl 4-(5-chloro-1-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl)-2-cyclopentylbenzoate 9 (100 mg, 0.27 mmol), phenylboronic acid (40.0 mg, 0.32 mmol), Pd$_2$(dba)$_3$ (12.0 mg, 13.0 µmol), XPhos (13.0 mg, 27.0 µmol) and Cs$_2$CO$_3$ (265 mg, 0.81 mmol) were loaded into a microwave vial. A 3:1 mixture of dioxane:water (2 ml) was added, the vial was flushed with nitrogen and capped. The solution was heated to 120 ºC for 16 h. Once cooled the solution was diluted with EtOAc (2 mL) and water (2.00 mL). The layers were separated, and the aqueous phase extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. The crude was purified via column chromatography (5–10% EtOAc/hexane) to afford the methyl ester intermediate. The methyl ester was saponified in a solution of aq. LiOH (1.00 mL, 1M) and dioxane (1.00 mL) at 100 ºC for 16 h. The solution was cooled to rt, diluted with water (2 mL) then acidified with aq. HCl (1M) until pH 4. The solution was extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. The solid was washed with cold water (5.00 mL) followed by hexane (10.0 mL) and dried to afford the 2-cyclopentyl-4-(1-methyl-5-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)benzoic acid 10 (77.0 mg, 72%) as an off-white solid.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.75 (br s, 1H), 8.63 (d, $J = 2.1$ Hz, 1H), 8.43 (d, $J = 2.1$ Hz, 1H), 8.15 (s, 1H), 7.83–7.67 (m, 5H), 7.50 (dd, $J = 8.4$, 7.0 Hz, 2H), 7.43–7.34 (m, 1H), 3.94–3.85 (m, 1H), 3.92 (s, 3H), 2.12–1.99 (m, 2H), 1.88–1.76 (m, 2H), 1.75–1.58 (m, 4H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.7, 148.0, 147.7, 142.4, 139.2, 138.2, 131.0, 130.0, 129.6, 129.5,
128.9, 127.6, 126.1, 124.5, 123.6, 117.9, 113.3, 41.4, 34.8, 31.6, 25.8; HRMS calcd for 
C_{26}H_{25}N_{2}O_{2} [M+H]^+: m/z 397.1916, found m/z 397.1911; MP Range: 199–201 ºC.

2-Cyclopentyl-4-(1-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl)benzoic acid (11)
3-Bromo-1-methyl-1H-pyrrolo[2,3-b]pyridine (100 mg, 0.47 mmol), methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate (180 mg, 0.54 mmol), Pd_{2}(dba)_{3} (22.0 mg, 24 µmol), XPhos (23.0 mg, 48.0 µmol) and Cs_{2}CO_{3} (540 mg, 1.66 mmol) were loaded into a 
microwave vial. A 3:1 mixture of dioxane/water (2.00 ml) was added and the vial was flushed 
with nitrogen and capped. The solution was heated to 120 ºC for 16 h. Once cooled the solution 
was diluted with EtOAc (2.0 mL) and water (2.00 mL). The layers were separated, and the 
aqueous phase extracted with EtOAc (2 × 3.00 mL). The combined organics were dried 
(Na_{2}SO_{4}), filtered and concentrated in vacuo. The crude was purified via column 
chromatography (0–20% EtOAc/hexane) to afford the methyl ester intermediate. The methyl 
ester was saponified in a solution of dioxane/water (2.00 ml) was added and the vial was flushed 
with nitrogen and capped. The solution was heated to 120 ºC for 16 h. Once cooled the solution 
was diluted with EtOAc (2.0 mL) and water (2.00 mL). The layers were separated, and the 
aqueous phase extracted with EtOAc (2 × 3.00 mL). The combined organics were dried 
(Na_{2}SO_{4}), filtered and concentrated in vacuo. The crude was purified via column 
chromatography (0–20% EtOAc/hexane) to afford the methyl ester intermediate. The methyl 
ester was saponified in a solution of aq. LiOH (2.00 mL, 1.00 M) and dioxane (2.00 mL) at 100 ºC for 16 h. The solution was cooled to rt, diluted with water (2.00 mL) then acidified with aq. 
HCl (1.00 M) until pH 4. The solution was extracted with EtOAc (2 × 3.00 mL). The combined 
organics were dried (Na_{2}SO_{4}), filtered and concentrated in vacuo. The solid was washed with 
cold water (5.00 mL) followed by hexane (10.0 mL) and dried to afford 11 (113 mg, 75%) as an 
off-white solid.

\[^1^H\text{NMR (400 MHz, DMSO-}d_6\text{)} \delta 8.33 (d, J = 2.2 Hz, 1H), 8.29 (d, J = 2.2 Hz, 1H), 8.20 (s, 1H), 
7.75 (d, J = 8.1 Hz, 1H), 7.65 (d, J = 1.8 Hz, 1H), 7.59 (dd, J = 8.1, 1.8 Hz, 1H), 3.91–3.80 (m, 
1H) 3.87 (s, 3H), 2.12–1.96 (m, 2H), 1.82 (m, 2H), 1.71–1.59 (m, 4H); \[^{13}\text{C NMR (100 MHz,} 
\text{DMSO-}d_6\text{)} \delta 169.3, 147.0, 146.3, 141.0, 136.9, 130.7, 130.5, 129.1, 126.8, 124.0, 123.4, 123.1, 
118.2, 112.3, 41.1, 34.3, 31.3, 25.3; \text{HRMS calcd for C}_{20}H_{21}N_{2}O_{2} [M+H]^+: m/z 321.1603, found 
m/z 321.1596; MP Range: 181–184 ºC.

Ethyl 2-chloronicotinate (12b)
To a solution of 2-chloronicotinic acid (5.00 g, 31.7 mmol) in toluene (35.0 mL) was added 
dropwise with stirring triethyl orthoacetate (17.5 mL, 95.2 mmol). The mixture was heated to 
reflux for 16 h and allowed to cool to room temperature and the resultant solution washed with 
sat. NaHCO_{3} (50.0 mL). The organic phase was dried with MgSO_{4} and the solvent removed in 
vacuo to afford the title compound, ethyl 2-chloronicotinate 12b (5.40 g, 92 %), as a clear oil.
1H NMR (400 MHz, DMSO-d$_6$) δ 8.58 (dd, $J$ = 4.8, 2.0 Hz, 1H), 8.24 (dd, $J$ = 7.7, 2.0 Hz, 1H), 7.57 (dd, $J$ = 7.7, 4.8 Hz, 1H), 4.35 (q, $J$ = 7.1 Hz, 2H), 1.32 (t, $J$ = 7.1 Hz, 3H); 13C NMR (101 MHz, DMSO-d$_6$) δ 164.1, 152.2, 147.8, 140.2, 127.1, 123.2, 61.8, 13.9; LCMS [M+H]$^+$: m/z 186.0.

**Ethyl 5-bromo-2-chloronicotinate (13b)**

To a solution of 5-bromo-2-chloronicotinic acid (10.0 g, 42.0 mmol) in ethanol (60.0 mL) was added slowly H$_2$SO$_4$ (9.10 g, 5.0 mL, 93.0 mmol). The reaction mixture was heated to reflux for 16 h. The mixture was concentrated in vacuo, re-dissolved in aq. NaHCO$_3$ (150 mL) and extracted with EtOAc (2 x 300 mL). The combined organic layers were dried over Na$_2$SO$_4$ and concentrated to give the desired product, 13b (10.5 g, 92%) as a light yellowish oil.

1H NMR (400 MHz, DMSO-d$_6$) δ 8.76 (d, $J$ = 2.5 Hz, 1H), 8.47 (d, $J$ = 2.5 Hz, 1H), 4.35 (q, $J$ = 7.1 Hz, 2H), 1.33 (t, $J$ = 7.1 Hz, 3H); 13C NMR (100 MHz, DMSO-d$_6$) δ 162.88, 152.79, 146.65, 142.12, 128.34, 118.85, 62.24, 13.83; LCMS [M+H]$^+$: m/z 186.0.

**Ethyl 2,5-dichloronicotinate (14b)**

Prepared following the procedure for 12b using 2,5-dichloronicotinic acid (10.0 g, 42.0 mmol). After purification ethyl 2,5-dichloronicotinate 14b (11.0 g, 99%) was afforded as a clear oil.

1H NMR (400 MHz, DMSO-d$_6$) δ 8.69 (d, $J$ = 2.6 Hz, 1H), 8.38 (d, $J$ = 2.6 Hz, 1H), 4.35 (q, $J$ = 7.1 Hz, 2H), 1.33 (t, $J$ = 7.1 Hz, 3H); 13C NMR (100 MHz, DMSO-d$_6$) δ 162.88, 152.79, 146.65, 142.12, 128.0, 62.24, 13.83; LCMS [M+H]$^+$: m/z 220.1.

**Ethyl 3-hydroxyfuro[2,3-b]pyridine-2-carboxylate (12c)**

To a suspension of sodium hydride, 60% dispersed in mineral oil, (5.60 g, 0.14 mol) in 1,2-dimethoxyethane (60.0 mL) was added ethyl 2-hydroxyacetate (13.0 mL, 0.13 mol) under ice cooling and stirring. The ice bath was removed, and the solution was allowed warm to rt. After 30 minutes, a solution of ethyl 2-chloronicotinate (10.0 g, 54.0 mmol) in 100 mL 1,2-dimethoxyethane was slowly added and the mixture was heated to 75 °C for 2 hr. The solvent was removed in vacuo and the residual solid re-dissolved in aq. NaHCO$_3$ solution (150 mL) and EtOAc (250 mL). The aq. layer was acidified with AcOH (pH 4) and extracted with CH$_2$Cl$_2$ (3 x 200 mL). The combined organic phases were dried over Na$_2$SO$_4$ and concentrated in vacuo. Purification by flash column chromatography (10% EtOAc/hexane) afforded ethyl 3-
hydroxyfuro[2,3-b]pyridine-2-carboxylate 12c (8.5 g, 76%) as a pale white solid, which was used in subsequent step without further characterization. LCMS [M+H]^+: m/z 208.1.

**Ethyl 5-bromo-3-hydroxyfuro[2,3-b]pyridine-2-carboxylate (13c)**
Prepared following the procedure for 12c using ethyl 5-bromo-2-chloronicotinate 13b (18.0 g, 68.0 mmol). After purification ethyl 5-bromo-3-hydroxyfuro[2,3-b]pyridine-2-carboxylate 13c (19.0 g, 76%) was afforded as a light brown solid.

^1H NMR (400 MHz, DMSO-d_6) δ 11.34–11.30 (m, 1H), 8.58 (d, J = 2.3 Hz, 1H), 8.53 (d, J = 2.3 Hz, 1H), 4.32 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H); ^13C NMR (100 MHz, DMSO-d_6) δ 158.7, 156.7, 148.8, 144.7, 133.5, 127.2, 115.9, 114.3, 60.4, 14.3; LCMS [M+H]^+: m/z 286.0.

**Ethyl 5-chloro-3-hydroxythieno[2,3-b]pyridine-2-carboxylate (14c)**
Prepared following the procedure for 12c using ethyl 2,5-dichloronicotinate 14b (15.0 g, 68.0 mmol) and ethyl 2-mercaptoacetate (15.0 mL, 0.14 mmol). After purification ethyl 5-chloro-3-hydroxythieno[2,3-b]pyridine-2-carboxylate 14c (12.4 g, 71%) was afforded as a light orange solid.

^1H NMR (400 MHz, DMSO-d_6) δ 11.04 (s, 1H), 8.73 (d, J = 2.4 Hz, 1H), 8.41 (d, J = 2.4 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H); ^13C NMR (100 MHz, DMSO-d_6) δ 162.6, 155.7, 152.2, 149.4, 130.3, 127.7, 126.7, 105.5, 61.1, 14.2; LCMS [M+H]^+: m/z 258.0.

**Furo[2,3-b]pyridin-3(2H)-one (12d)**
A mixture of ethyl 3-hydroxyfuro[2,3-b]pyridine-2-carboxylate 12c (6.00 g, 30.0 mmol) in ethanol (100 mL) and sodium hydroxide (5.00 g, 100 mmol) dissolved in 15.0 mL of water was refluxed for 1 hour. After evaporation of the solvent, the yellow-orange crystalline mass was dissolved in water (100 mL), acidified to pH 2-3 with conc. HCl and heated to reflux for 45 minutes. Once cooled, the solution was neutralized with sodium bicarbonate and extracted with chloroform. The organic was dried (Na_2SO_4), filtered and the solvent removed in vacuo to afford furo[2,3-b]pyridin-3(2H)-one 12d (2.70 g, 67%) as a light brown solid. The title compound was isolated as a mixture of keto-enol tautomers (9:1).

^1H NMR (400 MHz, DMSO-d_6) δ 9.71 (s, 0H), 8.60 (dd, J = 4.9, 2.0 Hz, 1H), 8.15 (dd, J = 7.5, 1.9 Hz, 1H), 7.27–7.22 (m, 1H), 4.89 (s, 2H); ^13C NMR (101 MHz, DMSO-d_6) δ 197.7, 177.1, 156.9, 134.3, 118.8, 113.6, 74.6; LCMS [M+H]^+: m/z 136.1.

**5-Bromofuro[2,3-b]pyridin-3(2H)-one (13d)**
Prepared following the procedure for 12d using ethyl 5-bromo-3-hydroxyfuro[2,3-b]pyridine-2-carboxylate 13c (10.0 g, 35.0 mmol). After purification 5-bromofuro[2,3-b]pyridin-3(2H)-one 13d (6.30 g, 84%) was afforded as a brown solid.

\[^1H\text{NMR (400 MHz, DMSO-}d_6\text{)} \delta 8.71 (d, J = 2.5 Hz, 1H), 8.42 (d, J = 2.5 Hz, 1H), 4.96 (s, 2H); \]^13C NMR (100 MHz, DMSO) \delta 196.4, 175.6, 156.9, 136.3, 115.5, 113.2, 75.8; LCMS [M+H]^+: m/z 213.4.

5-Chlorothieno[2,3-b]pyridin-3(2H)-one (14d)
Prepared following the procedure for 12d using ethyl 5-chloro-3-hydroxythieno[2,3-b]pyridine-2-carboxylate 14c (3.0 g, 12 mmol). After purification 5-chlorothieno[2,3-b]pyridin-3(2H)-one 14d (1.0 g, 50%) was isolated as an orange solid composed of keto- and enol-isomers.

\[^1H\text{NMR (400 MHz, DMSO-}d_6\text{)} \delta 10.46 (s, 1H), 8.77 (d, J = 2.5 Hz, 1H), 8.56 (dd, J = 2.3, 0.5 Hz, 1H), 8.16 (d, J = 2.4 Hz, 1H), 8.11 (d, J = 2.5 Hz, 1H), 6.70 (d, J = 0.5 Hz, 1H), 4.18 (s, 2H); \]^13C NMR (101 MHz, DMSO-\text{d}_6\text{)} \delta 196.8, 172.1, 156.5, 154.7, 145.4, 145.3, 133.2, 127.9, 127.8, 126.9, 126.0, 100.9, 40.8; LCMS [M+H]^+: m/z 186.4.

Furo[2,3-b]pyridin-3-yl trifluoromethanesulfonate (12e)
A solution of 12d (450 mg, 3.33 mmol) in CH\text{Cl}_2 (8.00 mL) was cooled to 0 °C. DIPEA (0.70 mL, 4.00 mmol) was added followed by dropwise addition of trifluoromethanesulfonic anhydride (788 µL, 4.66 mmol). The reaction was slowly warmed to room temperature and stirred for 2 h, then quenched with water. The aqueous phase was extracted with CH\text{Cl}_2 and the combined organic extracts were dried, filtered and concentrated in vacuo. The residue was purified by column chromatography (10% EtOAc/hexane) to afford furo[2,3-b]pyridin-3-yl trifluoromethanesulfonate 12e (640 mg, 72%) as a brown oil. This material was used in subsequent step without further characterization.

LCMS [M+H]^+: m/z 268.2.

5-Bromofuro[2,3-b]pyridin-3-yl trifluoromethanesulfonate (13e)
Prepared following the procedure for 12e using 5-bromofuro[2,3-b]pyridin-3(2H)-one 13d (10.0 g, 47.0 mmol). After purification 5-bromofuro[2,3-b]pyridin-3-yl trifluoromethanesulfonate 13e (12.8 g, 79%) was isolated as a light brown oil which solidified upon standing.
\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \( \delta \) 8.50 (dd, \( J = 2.2, 0.4 \ Hz, 1H \)), 8.14 (d, \( J = 2.2 \ Hz, 1H \)), 7.92 (s, 1H); LCMS [M+H]\textsuperscript{+}: m/z 347.1.

5-Chlorothieno[2,3-b]pyridin-3-yl trifluoromethanesulfonate (14e)
Prepared following the procedure for 12e using 5-chlorothieno[2,3-b]pyridin-3(2H)-one 14d (800 mg, 4.31 mmol). After purification 5-Chlorothieno[2,3-b]pyridin-3-yl trifluoromethanesulfonate 14e (1.00 g, 74%) was isolated as a brown oil. This material was used in subsequent step without further characterization.
LCMS [M+H]\textsuperscript{+}: m/z 318.4.

2-Cyclopentyl-4-(furo[2,3-b]pyridin-3-yl)benzoic acid (12f)
A microwave vial was loaded with furo[2,3-b]pyridin-3-yl trifluoromethanesulfonate 12e (250 mg, 936 \( \mu \)mol), 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid 3 (444 mg, 1.40 mmol), \( \text{Pd(PPh}_3\text{)}_4 \) (11.0 mg, 9.50 \( \mu \)mol), sodium carbonate (397 mg, 3.74 mmol), MeOH (2.00 mL) and \( \text{CH}_2\text{Cl}_2 \) (0.50 mL). The vial was sealed and heated to 90 \( \circ \)C for 2 hr. The solution was neutralized with aq. HCl (10 mL) and then extracted with ethyl acetate (2 x 20.0 mL). The organic phases were combined and dried (\( \text{Na}_2\text{SO}_4 \)), filtered and concentrated \textit{in vacuo}. The crude was purified via column chromatography (30% EtOAc/hexane) to afford 12f (217 mg, 76 \%) as an off-white solid.

\textsuperscript{1}H NMR (400 MHz, DMSO-\textit{d}_6) \( \delta \) 12.94 (s, 1H), 8.67 (s, 1H), 8.41 (d, \( J = 6.3 \ Hz, 2H \)), 7.81 – 7.75 (m, 2H), 7.67 (dd, \( J = 8.0, 1.8 \ Hz, 1H \)), 7.52–7.46 (m, 1H), 3.84–3.73 (m, 1H), 2.07–2.03 (m, 2H), 1.86–1.81 (m, 2H), 1.73–1.62 (m, 4H); \textsuperscript{13}C NMR (101 MHz, DMSO-\textit{d}_6) \( \delta \) 169.2, 161.9, 147.0, 144.5, 143.0, 133.7, 131.0, 130.2, 124.8, 123.9, 120.2, 119.9, 117.4, 41.3, 34.3, 25.2; HRMS calcd for C\textsubscript{19}H\textsubscript{17}NO\textsubscript{3}[M+H]\textsuperscript{+}: m/z 308.1287; found 308.1266. MP Range 164–168 \( \circ \)C.

Methyl 4-(5-bromofuro[2,3-b]pyridin-3-yl)-2-cyclopentylbenzoate (13f)
Prepared following the procedure for 12f using 5-bromofuro[2,3-b]pyridin-3-yl trifluoromethanesulfonate 13e (100 mg, 0.29 mmol) and 4 (95.4 mg, 0.29 mmol). After
purification methyl 4-(5-bromofuro[2,3-b]pyridin-3-yl)-2-cyclopentylbenzoate **13f** (68 mg, 59%) was isolated as an off-white solid.

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 8.74 (s, 1H), 8.56 (d, \(J = 1.8\) Hz, 1H), 8.48 (d, \(J = 1.6\) Hz, 1H), 7.75 (d, \(J = 8.2\) Hz, 2H), 7.69 (dd, \(J = 7.9, 1.7\) Hz, 1H), 3.85 (s, 3H), 3.71–3.58 (m, 1H), 2.08–1.96 (m, 2H), 1.87–1.76 (m, 2H), 1.73–1.61 (m, 4H); \(^{13}\)C NMR (101 MHz, DMSO-\(d_6\)) \(\delta\) 167.9, 160.5, 147.0, 144.9, 144.8, 133.4, 132.3, 130.2, 130.0, 125.0, 124.1, 119.6, 119.5, 115.3, 52.2, 41.4, 34.2, 25.2.

**Methyl 4-(5-chlorothieno[2,3-b]pyridin-3-yl)-2-cyclopentylbenzoate, (14f)**

Prepared following the procedure for **12f** using 5-chlorothieno[2,3-b]pyridin-3-yl trifluoromethanesulfonate **14e** (950 mg, 2.99 mmol) and 4 (988 mg, 2.99 mmol). After purification methyl 4-(5-chlorothieno[2,3-b]pyridin-3-yl)-2-cyclopentylbenzoate **14f** (870 mg, 78%) was isolated as a near white solid.

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 8.69 (d, \(J = 2.3\) Hz, 1H), 8.27–8.24 (m, 2H), 7.79 (d, \(J = 8.0\) Hz, 1H), 7.66 (d, \(J = 1.8\) Hz, 1H), 7.57 (dd, \(J = 8.0, 1.7\) Hz, 1H), 3.87 (s, 3H), 3.71–3.61 (m, 1H), 2.08–2.01 (m, 2H), 1.85–1.75 (m, 2H), 1.69–1.62 (m, 4H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 168.0, 159.5, 147.1, 145.4, 137.1, 133.6, 131.3, 130.2, 130.0, 129.7, 128.1, 128.0, 126.6, 125.3, 52.2, 41.4, 34.3, 25.2.

**2-Cyclopentyl-4-(5-phenylfuro[2,3-b]pyridin-3-yl)benzoic acid (13g)**

4-(5-Bromofuro[2,3-b]pyridin-3-yl)-2-cyclopentylbenzoic acid, **13f** (48.0 mg, 0.12 mmol), phenylboronic acid (30.0 mg, 0.25 mmol), Na\(_2\)CO\(_3\) (40.0 mg, 0.37 mmol) and Pd(PPh\(_3\))\(_4\) (14.0 mg, 12 µmol) were dissolved in a 4:1 mixture of dioxane (2.5 mL) and heated to 90 °C under nitrogen for 16 h. The mixture was neutralized to pH 6-7 with aq. HCl and extracted with ethyl acetate (5.00 mL). The combined organics were dried (Na\(_2\)SO\(_4\)), filtered and concentrated in vacuo. The crude residue was purified using flash column chromatography (30% EtOAc/hexane) to obtain the methyl ester intermediate which was immediately dissolved in MeOH (3.0 mL), added aq. NaOH (small excess) and heated to 70 °C for 1 hr. After cooling H\(_2\)O (4.0 mL) was added and the mixture extracted with diethyl ether (2 × 4.0 mL). The aqueous phase was acidified to pH 6 with aq. HCl, extracted with CH\(_2\)Cl\(_2\) (6.00 mL) and concentrated to
afford 2-cyclopentyl-4-(5-phenylfuro[2,3-b]pyridin-3-yl)benzoic acid 13g (39 mg, 82 %) a white solid.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.95 (s, 1H), 8.72 (s, 1H), 8.67 (d, $J = 2.2$ Hz, 1H), 8.53 (d, $J = 2.2$ Hz, 1H), 7.82 (d, $J = 1.5$ Hz, 1H), 7.81–7.79 (m, 4H), 7.56–7.50 (m, 2H), 7.47–7.41 (m, 1H), 3.86–3.77 (m, 1H), 2.11–2.01 (m, 2H), 1.87–1.79 (m, 2H), 1.73–1.62 (m, 4H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 169.3, 161.6, 147.0, 143.8, 143.2, 137.5, 133.6, 132.9, 131.0, 130.3, 129.1, 128.2, 127.8, 127.5, 125.0, 124.1, 120.2, 117.6, 41.2, 34.4, 25.3; HRMS calcd for C$_{25}$H$_{21}$NO$_3$[M+H]$^+$: m/z 384.1600; found 384.1593; MP Range 259–262 °C.

2-Cyclopentyl-4-(5-phenylthieno[2,3-b]pyridin-3-yl)benzoic acid (14g)

Methyl 4-(5-chlorothieno[2,3-b]pyridin-3-yl)-2-cyclopentylbenzoate 14f (150 mg, 403 µmol), phenylboronic acid (73.8 mg, 605 µmol), Pd$_2$(dba)$_3$ (18.5 mg, 20.2 µmol), dicyclohexyl(2',4',6'-triisopropyl-[1,1'-biphenyl]-2-yl)phosphane (28.8 mg, 60.5 µmol), Cs$_2$CO$_3$ (394 mg, 1.21 mmol) were dissolved in dioxane (3.00 mL) and H$_2$O (0.8 mL). The solution was heated to 90 °C for 16 h. The reaction was allowed to cool to rt, neutralized to pH 7 with aq. HCl and extracted with ethyl acetate (15 mL). The combined organic phases were dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. The crude was purified by column chromatography (25% EtOAc/hexane) to afford the methyl ester intermediate. This material was dissolved in MeOH (6 mL), added aq. NaOH (small excess) and heated to 70 °C for 1 h. After cooling H$_2$O (8 mL) was added and the mixture extracted with diethyl ether (2 × 10.0 mL). The aqueous phase was acidified to pH 6, extracted with CH$_2$Cl$_2$ (10 mL) and concentrated to afford 2-cyclopentyl-4-(5-phenylthieno[2,3-b]pyridin-3-yl)benzoic acid 14g (116 mg, 72%) as a yellow solid.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.94 (s, 1H), 8.96 (d, $J = 2.1$ Hz, 1H), 8.38 (d, $J = 2.2$ Hz, 1H), 8.17 (s, 1H), 7.83–7.77 (m, 3H), 7.72 (d, $J = 1.7$ Hz, 1H), 7.62 (dd, $J = 8.0$, 1.7 Hz, 1H), 7.55–7.50 (m, 2H), 7.47–7.41 (m, 1H), 3.87–3.78 (m, 1H), 2.09–2.04 (m, 2H), 1.83–1.78 (m, 2H), 1.71–1.63 (m, 4H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) δ 169.4, 160.6, 147.0, 145.8, 137.3, 137.3, 134.4, 132.5, 131.2, 130.4, 130.1, 129.2, 128.1, 128.1, 127.3, 126.5, 125.2, 125.3, 41.2, 34.5, 25.3; HRMS calcd for C$_{25}$H$_{21}$NO$_2$S [M+H]$^+$: m/z 400.1371, found 400.1365; MP Range: 269–273 °C.
2-Cyclopentyl-4-(thieno[2,3-b]pyridin-3-yl)benzoic acid (12g)

Prepared following the procedure for 12f using 3-bromothieno[2,3-b]pyridine 15e (50.0 mg, 0.23 mmol). After purification 2-cyclopentyl-4-(thieno[2,3-b]pyridin-3-yl)benzoic acid 12g (66.0 mg, 88%) was afforded as a light brown solid.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.99 (s, 1H), 8.65 (dd, $J = 4.6, 1.6$ Hz, 1H), 8.27 (dd, $J = 8.2, 1.6$ Hz, 1H), 8.10 (s, 1H), 7.80 (d, $J = 8.0$ Hz, 1H), 7.64 (d, $J = 1.8$ Hz, 1H), 7.56–7.50 (m, 2H), 3.85–3.76 (m, 1H), 2.08–2.03 (m, 2H), 1.82–1.78 (m, 2H), 1.72–1.58 (m, 4H); $^{13}$C NMR (176 MHz, DMSO-$d_6$) δ 172.6, 164.7, 150.1, 149.9, 140.4, 137.4, 134.5, 133.7, 133.4, 133.1, 129.5, 128.4, 128.4, 123.4, 44.4, 37.5, 28.4; HRMS calcd for C$_{19}$H$_{17}$NO$_2$S [M+H]$^+$: m/z 324.1058; Found 324.1054. MP Range 194–197 ºC.

Methyl 4-(5-bromopyrazolo[1,5-a]pyridin-3-yl)-2-cyclopentylbenzoate (17)

5-Bromo-3-iodopyrazolo[1,5-a]pyridine (200 mg, 0.62 mmol) and methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 4 (194 mg, 0.60 mmol) were dissolved in a 3:1 solution of dioxane:water (4.00 mL). Pd$_2$(dba)$_3$ (28 mg, 31 µmol), XPhos (30.0 mg, 62.0 µmol) and Cs$_2$CO$_3$ (605 mg, 1.90 mmol) were added. The mixture was stirred at rt for 16 h. The aqueous phase was removed and the solution concentrated in vacuo. The crude was purified via column chromatography (0–20% EtOAc/hexane) to afford ethyl 4-(5-bromopyrazolo[1,5-a]pyridin-3-yl)-2-cyclopentylbenzoate 17 (189 mg, 76%) as a mustard yellow solid.

$^1$H NMR (400 MHz, CDCl$_3$) δ 8.36 (dd, $J = 7.3, 0.8$ Hz, 1H), 8.16 (s, 1H), 7.94 (dd, $J = 2.1, 0.8$ Hz, 1H), 6.91 (ddd, $J = 7.3, 2.1, 0.4$ Hz, 1H), 3.93 (s, 3H), 3.91–3.83 (m, 1H), 2.24–2.11 (m, 2H), 1.93–1.81 (m, 2H), 1.81–1.72 (m, 2H), 1.71–1.61 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 168.5, 148.7, 141.6, 137.6, 135.8, 130.9, 129.7, 128.4, 125.3, 123.7, 119.7, 118.7, 116.0, 112.4, 52.0, 41.7, 34.8, 25.7; HRMS calcd for C$_{20}$H$_{20}$N$_2$O$_2$Br [M+H]$^+$: m/z 399.0708, found m/z 399.0705; MP Range: 182–184 ºC.

Methyl 4-(5-chloropyrazolo[1,5-a]pyrimidin-3-yl)-2-cyclopentylbenzoate (18)

5-Chloro-3-iodopyrazolo[1,5-a]pyrimidine (500 mg, 1.79 mmol) and methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 4 (591 mg) were dissolved in a 10:1 mixture of dioxane:water (10.0 mL). Pd(dppf)Cl$_2$:CH$_2$Cl$_2$ (73.0 mg, 0.9 mmol) and Cs$_2$CO$_3$ (1.75 g, 5.37 mmol) were added and the solution heated to 80 ºC for 16 h. The solution was allowed to cool to rt, the aqueous phase was removed, and the organic was concentrated in vacuo. The crude
was purified via column chromatography (10–20% EtOAc/hexane) to afford the methyl 4-(5-chloropyrazolo[1,5-a]pyrimidin-3-yl)-2-cyclopentylbenzoate 18 (270 mg, 44%) as a bright yellow solid.

1H NMR (400 MHz, CDCl₃) δ: 8.59 (d, J = 7.2 Hz, 1H), 8.48 (s, 1H), 8.12 (d, J = 1.5 Hz, 1H), 7.92–7.78 (m, 2H), 6.86 (d, J = 7.2 Hz, 1H), 3.99–3.86 (m, 4H), 2.28–2.04 (m, 2H), 1.97–1.84 (m, 2H), 1.84–1.61 (m, 2H); 13C NMR (100 MHz, CDCl₃) δ 168.6, 150.8, 148.6, 143.9, 136.5, 134.4, 130.6, 128.3, 124.5, 122.8, 110.1, 109.4, 51.9, 41.7, 34.8, 25.8; HRMS calcd for C₁₉H₁₉N₃O₂Cl [M+H]⁺: m/z 356.1165, found m/z 356.1161; MP Range: 163–166 ºC.

2-Cyclopentyl-4-(5-phenylpyrazolo[1,5-a]pyrimidin-3-yl)benzoic acid (19)

Methyl 4-(5-bromopyrazolo[1,5-a]pyridin-3-yl)-2-cyclopentylbenzoate 17 (100 mg, 0.25 mmol), phenylboronic acid (37.0 mg, 0.30 mmol), Pd(PPh₃)₄ (29.0 mg, 25.0 µmol) and Cs₂CO₃ (245 mg, 0.75 mmol) were loaded into a microwave vial. A 3:1 mixture of dioxane:water (1.50 mL) was added and the vial flushed with nitrogen and sealed. The mixture was irradiated at 120 ºC for 30 min. Once cooled the solution was diluted with EtOAc (2.00 mL) and water (2.00 mL). The layers were separated, and the aqueous phase extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na₂SO₄), filtered and concentrated in vacuo. The crude was purified via column chromatography (5–10% EtOAc/hexane) to afford the methyl ester intermediate. The methyl ester was saponified in a solution of aq. LiOH (1.00 mL, 1.00 M) and dioxane (1.00 mL) at 100 ºC for 16 h. The solution was cooled to rt, diluted with water (2.00 mL) then acidified with aq. HCl (1M) until pH 4. The solution was extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na₂SO₄), filtered and concentrated in vacuo. The solid was washed with cold water (5.00 mL) followed by hexane (10.0 mL) and dried to afford the acid (68.0 mg, 71%) as a yellow solid.

1H NMR (700 MHz, DMSO-d₆) δ 8.83 (d, J = 6.9 Hz, 1H), 8.50 (s, 1H), 8.10 (s, 1H), 8.02 (s, 1H), 7.79 (d, J = 7.8 Hz, 1H), 7.72 (s, 1H), 7.67 (d, J = 7.6 Hz, 1H), 7.53 (t, J = 6.9 Hz, 2H), 7.45 (t, J = 6.8 Hz, 1H), 7.33 (d, J = 6.8 Hz, 1H), 3.91–3.85 (m, 1H), 2.10-2.01 (m, 2H), 1.88-1.78 (m, 2H), 1.73-1.60 (m, 4H); 13C NMR (176 MHz, DMSO-d₆) δ 169.2, 147.2, 141.4, 137.6, 136.9, 136.5, 135.7, 131.5, 131.4, 130.5, 129.7, 129.3, 129.1, 128.9, 128.7, 128.5, 126.7, 124.5, 123.4, 113.5, 111.9, 111.7, 41.1, 34.3, 25.3; HRMS calcd for C₂₅H₂₃N₂O₂ [M+H⁺]: m/z 383.1759, found m/z 383.1754; MP Range: 153–155 ºC.

2-Cyclopentyl-4-(5-phenylpyrazolo[1,5-a]pyrimidin-3-yl)benzoic acid (20)
Methyl 4-(5-chloropyrazolo[1,5-a]pyrimidin-3-yl)-2-cyclopentylbenzoate 18 (100 mg, 0.28 mmol), phenylboronic acid (41.0 mg, 0.33 mmol), Pd(PPh₃)₄ (32.0 mg, 28.0 µmol) and Cs₂CO₃ (275 mg, 0.84 mmol) were loaded into a microwave vial. A 3:1 mixture of dioxane:water (2.00 ml) was added and the vial was flushed with nitrogen and capped. The solution was irradiated at 120 °C for 30 min. Once cooled the solution was diluted with EtOAc (2.00 mL) and water (2.00 mL). The layers were separated, and the aqueous phase extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na₂SO₄), filtered and concentrated in vacuo. The crude was purified via column chromatography (5–10% EtOAc/hexane) to afford the methyl ester intermediate. The methyl ester was saponified in a solution of aq. LiOH (1.00 mL, 1.00 M) and dioxane (1.00 mL) at 100 °C for 16 h. The solution was cooled to rt, diluted with water (2.00 mL) then acidified with aq. HCl (1.00 M) until pH 4. The solution was extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na₂SO₄), filtered and concentrated in vacuo. The solid was washed with cold water (5.00 mL) followed by hexane (10.0 mL) and dried to afford 2-cyclopentyl-4-(5-phenylpyrazolo[1,5-a]pyrimidin-3-yl)benzoic acid 20 (87.0 mg, 81%) as a bright yellow solid.

1H NMR (700 MHz, DMSO-d₆) δ 12.94 (s, 1H), 9.24 (dd, J = 7.6, 2.8 Hz, 1H), 8.87 (s, 1H), 8.65 (s, 1H), 8.39–8.28 (m, 2H), 7.95 (d, J = 8.1 Hz, 1H), 7.84–7.72 (m, 2H), 7.61–7.55 (m, 3H), 3.98–3.91 (m, 1H), 2.15–2.09 (m, 2H), 1.93–1.86 (m, 2H), 1.77–1.68 (m, 4H); 13C NMR (176 MHz, DMSO-d₆) 169.2, 155.9, 147.3, 144.3, 143.6, 137.0, 136.6, 135.3, 131.0, 130.1, 129.0 (2 × ArCH), 128.4, 127.2 (2 × ArCH), 123.8, 122.1, 108.2, 105.9, 41.0 34.7, 25.6; HRMS calcd for C₂₄H₂₂N₃O₂ [M+H]+: m/z 384.1712, found m/z 384.1707; MP Range: 210–214 °C.

2-Cyclopentyl-4-(pyrazolo[1,5-a]pyridin-3-yl)benzoic acid (21)
3-Bromopyrazolo[1,5-a]pyridine (100 mg, 0.50 mmol), 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid 3 (193 mg, 0.61 mmol), Pd₂(dba)₃ (23.0 mg, 25.0 µmol), XPhos (24.0 mg, 51.0 µmol) and Cs₂CO₃ (579 mg, 1.80 mmol) were loaded into a microwave vial. A 3:1 mixture of dioxane:water (3.00 mL) was added and the vial flushed with nitrogen and sealed. The mixture was heated to 120 °C for 16 h. Once cooled the solution was diluted with EtOAc (2.00 mL) and water (2.00 mL). The mixture was acidified to pH 4 with 1.00 M aq. HCl solution. The layers were separated, and the aqueous phase extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na₂SO₄), filtered and concentrated in vacuo. The crude was
purified via column chromatography (50–100% EtOAc/hexane) to afford 2-cyclopentyl-4-(pyrazolo[1,5-a]pyridin-3-yl)benzoic acid 21 (64.0 mg, 41%) as a yellow solid.

$^{1}$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 12.78 (br s, 1H), 8.76 (d, $J = 7.0$ Hz, 1H), 8.47 (s, 1H), 7.94 (d, $J = 9.0$ Hz, 1H), 7.77 (d, $J = 8.1$ Hz, 1H), 7.67 (d, $J = 1.6$ Hz, 1H), 7.57 (dd, $J = 8.1$, 1.7 Hz, 1H), 7.39 (ddd, $J = 8.9$, 6.7, 1.0 Hz, 1H), 6.99 (td, $J = 6.9$, 1.1 Hz, 1H), 6.99 (td, $J = 6.9$, 1.1 Hz, 1H), 3.90–3.79 (m, 1H), 2.09–1.97 (m, 2H), 1.88-1.76 (m, 2H), 1.73-1.58 (m, 4H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 169.2, 147.1, 140.8, 136.3, 135.8, 130.5, 129.6, 128.7, 125.4, 124.2, 123.2, 117.1, 112.7, 110.9, 41.2, 34.3, 25.2; HRMS (ESI) calcd for C$_{19}$H$_{19}$N$_2$O$_2$ [M+H]$^+$: m/z 307.1446, found m/z 307.1439; MP Range: 167–169 °C.

2-Cyclopentyl-4-(pyrazolo[1,5-a]pyrimidin-3-yl)benzoic acid 22

2-Bromopyrazolo[1,5-a]pyrimidine (150 mg, 0.75 mmol), 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid 3 (287 mg, 0.90 mmol), Pd(PPh$_3$)$_4$ (87.0 mg, 75.0 µmol) and Cs$_2$CO$_3$ (864 mg, 2.65 mmol) were loaded into a microwave vial. A 3:1 mixture of dioxane:water (2.00 ml) was added and the vial was flushed with nitrogen and capped. The solution was irradiated at 120 ºC for 30 min. Once cooled the solution was diluted with EtOAc (2.00 mL) and water (2.00 mL). The mixture was acidified to pH 4 with 1M aq. HCl solution. The layers were separated, and the aqueous phase extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na$_2$SO$_4$), filtered and concentrated in vacuo. The crude was purified via column chromatography (50–100% EtOAc/hexane) to afford 2-cyclopentyl-4-(pyrazolo[1,5-a]pyrimidin-3-yl)benzoic acid 22 (137 mg, 59%) as a bright yellow solid.

$^{1}$H NMR (700 MHz, DMSO-$d_6$): $\delta$ 12.74 (br s, 1H), 9.24–9.12 (d, $J = 6.0$ Hz, 1H), 8.87 (d, $J = 2.9$ Hz, 1H), 8.72 (s, 1H), 8.25 (s, 1H), 8.03 (d, $J = 8.2$ Hz, 1H), 7.75 (dd, $J = 8.1$, 2.8 Hz, 1H), 7.14 (dt, $J = 7.4$, 3.5 Hz, 1H), 3.89–3.83 (m, 1H), 2.08–2.01 (m, 2H), 1.88–1.82 (m, 2H), 1.71–1.63 (m, 4H); $^{13}$C NMR (176 MHz, DMSO-$d_6$) $\delta$ 169.3, 150.9, 146.9, 144.5, 143.2, 136.6, 135.0, 130.1, 128.6, 123.6, 122.5, 109.0, 108.2, 41.2, 34.3, 25.2; HRMS calcd for C$_{18}$H$_{18}$N$_3$O$_2$ [M+H]$^+$: m/z 308.1399, found m/z 308.1393; MP Range: 196–199 ºC.

Methyl 4-(6-chloro-[1,2,4]triazolo[4,3-b]pyridazin-3-yl)-2-cyclopentylbenzoate (24)

3-bromo-6-chloro-[1,2,4]triazolo[4,3-b]pyridazine (924 mg, 3.96 mmol), methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 4 (1.31 g, 3.96 mmol), K$_2$CO$_3$ (1.64 g, 11.9 mmol) and Pd(PPh$_3$)$_4$ (320 mg, 0.28 mmol) in a mixture of dioxane (12.0 mL) and water (3.00 mL) was degassed and put under N$_2$ atmosphere. The reaction mixture was heated to 90 ºC and stirred for 16 h. The reaction mixture was diluted with aq. HCl (50.0 mL) to pH 6 and
extracted with EtOAc (2 × 75.0 mL) and the combined organic phases were concentrated **in vacuo**. The crude residue was purified **via** column chromatography (35% EtOAc/hexane) to afford methyl 4-(6-chloro-[1,2,4]triazolo[4,3-b]pyridazin-3-yl)-2-cyclopentylbenzoate 24 (1.20 g, 85%) as a grayish solid. 24 was used in the next step without further characterization.

LCMS [M+H]+: m/z 356.2.

2-cyclopentyl-4-(6-phenyl-[1,2,4]triazolo[4,3-b]pyridazin-3-yl)benzoic acid (25)

Following the procedure for 14g using 4-(6-chloro-[1,2,4]triazolo[4,3-b]pyridazin-3-yl)-2-cyclopentylbenzoate 24 (220 mg, 0.62 mmol). After purification cyclopentyl-4-(6-phenyl-[1,2,4]triazolo[4,3-b]pyridazin-3-yl)benzoic acid 25 (200 mg, 84%) was afforded as a near yellow solid.

^1H NMR (400 MHz, DMSO-d6) δ 13.14 (s, 1H), 8.74 (d, J = 1.7 Hz, 1H), 8.58 (d, J = 9.8 Hz, 1H), 8.32 (dd, J = 8.2, 1.7 Hz, 1H), 8.21–8.18 (m, 2H), 8.07 (d, J = 9.8 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.65–7.59 (m, 3H), 3.91–3.82 (m, 1H), 2.15–2.11 (m, 2H), 1.88–1.83 (m, 2H), 1.72–1.66 (m, 4H); ^13C NMR (100 MHz, DMSO-d6) δ 169.2, 153.5, 146.6, 146.4, 144.5, 134.1, 133.0, 131.1, 129.7, 129.1, 128.7, 127.4, 125.6, 125.2, 124.2, 119.9, 41.1, 34.7, 25.6; HRMS calcd for C_{23}H_{20}N_{4}O_{2}[M+H]+: m/z 385.1665; found 385.1659; MP Range 228–231 °C.

4-((1,2,4)Triazolo[4,3-b]pyridazin-3-yl)-2-cyclopentylbenzoic acid (26)

3-bromo-[1,2,4]triazolo[4,3-b]pyridazine (150 mg, 754 µmol), methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 4 (373 mg, 1.13 mmol), XPhos (53.9 mg, 113 µmol), Cs_2CO_3 (516 mg, 1.58 mmol) and Pd_2(dba)_3 (34.5 mg, 0.04 mmol) in a 3:1 mixture of dioxane:water (4.00 mL) was heated to 90 °C under N_2 atmosphere for 16 h. The reaction mixture was diluted with aq. HCl (25.0 mL, pH 6) and extracted with EtOAc (2 × 50.0 mL), the organic phases were combined, concentrated and purified using flash column chromatography (40% EtOAc/hexane) to afford 4-((1,2,4)triazolo[4,3-b]pyridazin-3-yl)-2-cyclopentylbenzoic acid 26 (178.0 mg, 77%) as an off-white solid.

^1H NMR (400 MHz, DMSO-d6) δ 13.14 (s, 1H), 8.79 (dd, J = 4.3, 1.5 Hz, 1H), 8.50–8.46 (m, 2H), 8.29 (dd, J = 8.2, 1.7 Hz, 1H), 7.86 (d, J = 8.2 Hz, 1H), 7.44 (dd, J = 9.4, 4.3 Hz, 1H), 3.86–3.75 (m, 1H), 2.14–2.04 (m, 2H), 1.89–1.79 (m, 2H), 1.74–1.58 (m, 4H); ^13C NMR (100 MHz, DMSO-d6) δ 169.2, 146.8, 146.4, 146.3, 145.1, 133.1, 129.6, 128.6, 125.4, 125.3, 124.1, 120.9,
HRMS calcd for C_{17}H_{16}N_{4}O_{2} [M+H]^+; m/z 309.1352; found 309.1346; MP Range 208–211°C.

4-Chloro-1-methyl-2-phenyl-1H-pyrrolo[2,3-b]pyridine (28)

4-Chloro-2-iodo-1-methyl-1H-pyrrolo[2,3-b]pyridine (75.0 mg, 0.25 mmol), phenylboronic acid (31.0 mg, 0.25 mmol), Cs_{2}CO_{3} (251 mg, 0.77 mmol) and Pd(PPh_{3})_{4} (30.0 mg, 0.02 mmol) were loaded into a microwave vial. A 3:1 mixture of dioxane:water (2.00 ml) was added and the vial was flushed with nitrogen and capped. The solution was irradiated at 120 °C for 30 min. Once cooled the solution was diluted with EtOAc (2.00 mL) and water (2.00 mL). The layers were separated and the aqueous phase extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na_{2}SO_{4}), filtered and concentrated in vacuo. The crude was purified via column chromatography (10% EtOAc/hexane) to afford 4-chloro-1-methyl-2-phenyl-1H-pyrrolo[2,3-b]pyridine 28 (58.0 mg, 93%) as a white-grey solid.

1H NMR (400 MHz, CDCl_{3}) δ 8.23 (d, J = 5.2 Hz, 1H), 7.63–7.40 (m, 5H), 7.12 (d, J = 5.2 Hz, 1H), 6.63 (s, 1H), 3.89 (s, 3H); 13C NMR (101 MHz, CDCl_{3}) δ 149.6, 142.6, 142.5, 135.3, 131.7, 129.1(2 × ArCH), 128.7 (2 × ArCH), 128.6, 119.9, 116.2, 97.9, 30.3; MP Range: 113–116 °C.

2-Cyclopentyl-4-(1-methyl-2-phenyl-1H-pyrrolo[2,3-b]pyridin-4-yl)benzoic acid (29)

4-Chloro-1-methyl-2-phenyl-1H-pyrrolo[2,3-b]pyridine 28 (60.0 mg, 0.25 mmol), methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 4 (98.0 mg, 0.30 mmol), Pd(PPh_{3})_{4} (29.0 mg, 25.0 µmol) and Cs_{2}CO_{3} (242 mg, 0.74 mmol) were loaded into a microwave vial. A 3:1 mixture of dioxane:water (2.00 ml) was added and the vial was flushed with nitrogen and capped. The solution was irradiated at 120 °C for 30 min. Once cooled the solution was diluted with EtOAc (2.00 mL) and water (2.00 mL). The layers were separated, and the aqueous phase extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na_{2}SO_{4}), filtered and concentrated in vacuo. The crude was purified via column chromatography (5–10% EtOAc/hexane) to afford the methyl substituted ester intermediate. The methyl ester was saponified in a solution of aq. LiOH (1.00 mL, 1.00 M) and dioxane (1.00 mL) at 100 °C for 16 h. The solution was cooled to rt, diluted with water (2.00 mL) then acidified with aq. HCl (1.00 M) until pH 4. The solution was extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na_{2}SO_{4}), filtered and concentrated in vacuo. The solid was washed with cold water.
(5.00 mL) followed by hexane (10.0 mL) and dried to afford 2-cyclopentyl-4-(1-methyl-2-phenyl-1H-pyrrolo[2,3-b]pyridin-4-yl)benzoic acid \(29\) (79.0 mg, 80%) as a brown-yellow solid. \(^1\)H NMR (700 MHz, DMSO-\(d_6\)) \(\delta\) 8.40 (d, \(J = 4.9\) Hz, 1H), 7.82 (d, \(J = 8.0\) Hz, 1H), 7.80 (d, \(J = 1.7\) Hz, 1H), 7.71 (t, \(J = 7.7\) Hz, 3H), 7.56 (t, \(J = 7.5\) Hz, 2H), 7.50 (t, \(J = 7.4\) Hz, 1H), 7.33 (d, \(J = 4.9\) Hz, 1H), 6.73 (s, 1H), 3.89 (s, 3H), 3.86–3.76 (m, 1H), 2.12–2.04 (m, 2H), 1.84–1.77 (m, 2H), 1.70–1.59 (m, 4H); \(^{13}\)C NMR (176 MHz, DMSO-\(d_6\)) \(\delta\) 169.3, 149.5, 146.7, 143.0, 142.2, 140.8, 139.4, 131.8, 131.6, 130.0, 128.9 (2 × ArCH), 128.8 (2 × ArCH), 128.6, 126.5, 125.5, 117.5, 115.1, 98.1, 41.2, 34.4, 29.9, 25.3; HRMS calcd for C\(_{26}\)H\(_{25}\)N\(_2\)O\(_2\) [M+H]\(^+\): m/z 397.1916, found m/z 397.1908; MP Range: 187–189 °C.

2-Cyclopentyl-4-(1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)benzoic acid (30)

4-Chloro-1-methyl-1H-pyrrolo[2,3-b]pyridine (75.0 mg, 0.45 mmol), methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate \(4\) (180 mg, 0.54 mmol), Pd(PPh\(_3\))\(_4\) (52.0 mg, 45.0 µmol) and Cs\(_2\)CO\(_3\) (440 mg, 1.40 mmol) were loaded into a microwave vial. A 3:1 mixture of dioxane:water (3.00 ml) was added and the vial was flushed with nitrogen and capped. The solution was irradiated at 120 °C for 30 min. Once cooled the solution was diluted with EtOAc (2.00 mL) and water (2.00 mL). The layers were separated, and the aqueous phase extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na\(_2\)SO\(_4\)), filtered and concentrated in vacuo. The crude was purified via column chromatography (5–10% EtOAc/hexane) to afford the methyl ester intermediate. The methyl ester was saponified in a solution of aq. LiOH (1.00 mL, 1.00 M) and dioxane (1.00 mL) at 100 °C for 16 h. The solution was cooled to rt, diluted with water (2.00 mL) then acidified with aq. HCl (1.00 M) until pH 4. The solution was extracted with EtOAc (2 × 3.00 mL). The combined organics were dried (Na\(_2\)SO\(_4\)), filtered and concentrated in vacuo. The solid was washed with cold water (5.00 mL) followed by hexane (10.0 mL) and dried to afford 2-cyclopentyl-4-(1-methyl-1H-pyrrolo[2,3-b]pyridin-4-yl)benzoic acid \(30\) (97.0 mg, 67%) as a pale yellow solid. \(^1\)H NMR (700 MHz, DMSO-\(d_6\)) \(\delta\) 13.01 (s, 1H), 8.36 (s, 1H), 7.81 (d, \(J = 7.6\) Hz, 1H), 7.76 (s, 1H), 7.67–7.61 (m, 2H), 7.27 (s, 1H), 6.57 (s, 1H), 3.87 (s, 3H), 3.80 (dd, \(J = 19.7, 12.5\) Hz, 1H), 2.13–2.00 (m, 2H), 1.87–1.74 (m, 2H), 1.71–1.56 (m, 4H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)) \(\delta\) 169.3, 147.9, 146.6, 142.6, 140.8, 139.9, 131.7, 131.0, 129.9, 126.5, 125.4, 117.5, 114.3, 97.8, 41.1, 34.4, 31.2, 25.3; HRMS calcd for C\(_{26}\)H\(_{25}\)N\(_2\)O\(_2\) [M+H]\(^+\): m/z 321.1603, found m/z 321.1595; MP Range: 201–203 °C.
Methyl 2-cyclopentyl-4-(2,3-diaminopyridin-4-yl)benzoate (32)
4-Chloropyridine-2,3-diamine (103 mg, 0.71 mmol) and methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 4 (250 mg, 0.75 mmol) were dissolved in a 10:1 mixture of dioxane:H₂O (10.0 mL). Pd₂(db₃)₃ (69.0 mg, 75 µmol), XPhos (72.0 mg, 150 µmol) and Cs₂CO₃ (740 mg, 2.30 mmol) were added. The solution was heated to 100 ºC for 16 h. Once cooled to rt the aqueous phase was removed and the organic concentrated in vacuo. The crude was purified via column chromatography (25–75% EtOAc/hexane) to afford methyl 2-cyclopentyl-4-(2,3-diaminopyridin-4-yl)benzoate 32 (169 mg, 71%) as a dark brown solid.

1H NMR (400 MHz, CDCl₃) δ 7.82 (d, J = 8.0 Hz, 1H), 7.66 (d, J = 5.3 Hz, 1H), 7.47 (d, J = 1.8 Hz, 1H), 7.31–7.23 (dd, 1H), 6.61 (d, J = 5.3 Hz, 1H), 4.63 (br s, 2H), 3.93 (s, 3H), 3.86–3.71 (m, 1H), 3.61 (br s, 2H), 2.19–2.07 (m, 2H), 1.90–1.73 (m, 2H), 1.75–1.65 (m, 2H), 1.65–1.50 (m, 2H); 13C NMR (100 MHz, CDCl₃) δ 168.5, 149.1, 148.4, 140.6, 136.8, 133.8, 130.4, 127.2, 126.6, 125.5, 116.3, 52.2, 41.7, 35.0, 25.7; HRMS calcd for C₁₈H₂₂N₃O₂ [M+H]⁺: m/z 312.1712, found m/z 312.1704; MP Range: 125–129 ºC.

2-Cyclopentyl-4-(3H-imidazo[4,5-b]pyridin-7-yl)benzoic acid (33)
Methyl 2-cyclopentyl-4-(2,3-diaminopyridin-4-yl)benzoate 32 (100 mg, 0.32 mmol) was dissolved in MeOH (1 mL). Sulfamic acid (3.00 mg, 32.0 µmol) and trimethyl orthoformate (85.0 mg, 0.80 mmol) were added and the mixture stirred at rt for 1 h. The solvent was removed in vacuo and the residue was dissolved in EtOAc (10.0 mL) and water (10.0 mL). The phases were separated and the aqueous phase was extracted with EtOAc (2 × 10.0 mL). The combined organics were dried (Na₂SO₄), filtered and concentrated in vacuo. The crude residue was purified by column chromatography (50–100% EtOAc/hexane). The isolated methyl ester was dissolved in dioxane (1.00 mL) and aq LiOH (1.00 mL of a 1.00 M solution). The solution was heated to 100 ºC for 16 h, cooled and diluted with EtOAc (3.00 mL) and water (3.00 mL). The mixture was acidified to pH 4 with 1.00 M aq. HCl solution. The layers were separated, and the aqueous phase extracted with EtOAc (3 × 3.00 mL). The combined organics were dried (Na₂SO₄), filtered and concentrated in vacuo to afford 2-cyclopentyl-4-(3H-imidazo[4,5-b]pyridin-7-yl)benzoic acid 33 (45 mg, 46%) as a white solid.

1H NMR (700 MHz, DMSO-d₆) δ 13.23 (s, 1H), 8.51 (s, 1H), 8.45 (s, 1H), 8.41 (d, J = 4.7 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.59 (d, J = 4.7 Hz, 1H), 3.80 (t, J = 8.7 Hz, 1H), 2.10–2.03 (br m, 2H), 1.87–1.73 (br m, 2H), 1.67 (m, 4H); 13C NMR (176 MHz, DMSO-d₆) δ 171.9, 169.4, 148.8, 146.1, 144.2, 143.8, 138.2, 136.5, 132.1, 131.8, 129.3, 127.6,
2-Cyclopentyl-4-(2-phenyl-3H-imidazo[4,5-b]pyridin-7-yl)benzoic acid (34)
Benzoic acid (47.0 mg, 0.38 mmol) and CDI (63.0 mg, 0.38 mmol) were loaded into a microwave vial and dissolved in DMF (1.0 mL). The solution was cooled to 0 °C and stirred for 30 min. Methyl 2-cyclopentyl-4-(2,3-diaminopyridin-4-yl)benzoate 4 (100 mg, 0.32 mmol) was added and the vial sealed. The mixture was heated to 200 °C for 10 min, allowed to cool to rt and water (3.00 mL) was added causing a precipitate to form. The precipitate was filtered, washed with cold water (10.0 mL) and hexane (10.0 mL). The precipitate was dissolved in dioxane (1.00 mL) and aq LiOH (1.00 mL of a 1.00 M solution). The solution was heated to 100 °C for 16 h then diluted with EtOAc (3.00 mL) and water (3.00 mL). The mixture was acidified to pH 4 with 1M aq. HCl solution. The layers were separated, and the aqueous phase extracted with EtOAc (3 × 3.00 mL). The combined organics were dried (Na₂SO₄), filtered and concentrated in vacuo. The crude was purified via column chromatography (50–100% EtOAc/hexane) to afford 2-cyclopentyl-4-(2-phenyl-3H-imidazo[4,5-b]pyridin-7-yl)benzoic acid 34 (93.0 mg, 76%) as a white solid.

1H NMR (700 MHz, DMSO-$_d_6$) δ 8.55–8.44 (m, 2H), 8.33 (d, $J = 6.3$ Hz, 2H), 8.05 (d, $J = 6.7$ Hz, 1H), 7.84 (d, $J = 7.8$ Hz, 1H), 7.67 (s, 1H), 7.61 (d, $J = 6.6$ Hz, 3H), 3.93–3.79 (m, 1H), 2.18–2.05 (m, 2H), 1.93–1.83 (m, 2H), 1.82–1.66 (m, 4H); 13C NMR (176 MHz, DMSO-$_d_6$) δ 169.4, 150.2, 146.6, 137.7, 132.4, 131.1, 129.5, 129.1, 128.0, 127.1, 125.6, 116.2, 41.1, 39.5, 34.5, 25.4; HRMS calcd for C$_{24}$H$_{22}$N$_3$O$_2$ [M+H]$^+$: m/z 384.1712, found m/z 384.1704; MP Range: 196–199 °C.

4-Chloro-6-iodothieno[3,2-d]pyrimidine (35b)
An oven dried 250 mL three-neck round-bottom flask provided with a pressure-equalizing addition funnel was cooled under N$_2$. 4-Chlorothieno[3,2-d]pyrimidine 35a (3.40 g, 20.0 mmol) was added under positive N$_2$ pressure, followed by the addition of anhydrous THF (65.0 mL). The solution was cooled down to -78 °C, LDA (12.0 mL, 24.0 mmol,) was added dropwise over 5 minutes, and the mixture was stirred at -78 °C for 20 min. A solution of I$_2$ (6.08 g, 24.0 mmol) in anhydrous THF (15.0 mL) was added dropwise over 10 min using the equalizing addition funnel. The resulting mixture was stirred at -78 °C for 30 min, allowed to warm up to rt over 1 h then stirred for 16 h. The reaction was quenched with saturated NH$_4$Cl/ice and diluted with
The layers were separated, and the aqueous layer was extracted with CHCl₃ (2 × 50.0 mL). The combined organics were rinsed with aq. Na₂S₂O₃ (2 × 25.0 mL) and sat. NaCl solution (2 × 25.0 mL), dried (Na₂SO₄), filtered and concentrated in vacuo. The crude was purified via column chromatography (0–100% hexane/EtOAc) affording 4-chloro-6-iodothieno[3,2-d]pyrimidine 35b (4.60 g, 68%) as a clear brown solid.

**4-Chloro-6-phenylthieno[3,2-d]pyrimidine (36)**

4-Chloro-6-iodothieno[3,2-d]pyrimidine 35b (1.01 mmol, 300 mg), phenylboronic acid (1.01 mmol, 125 mg), NaHCO₃ (1.01 mmol, 85.0 mg), CsF (1.01 mmol, 155 mg) and Pd(Ph₃)₄ (35.0 mg, 0.03 mmol) were dissolved in a 3:1 mixture of dioxane:water (7.00 mL). The reaction mixture was stirred at reflux for 3 h. The volatiles were removed under reduced pressure and the crude was purified via column chromatography (hexane/EtOAc 0–30%) to afford 4-chloro-6-phenylthieno[3,2-d]pyrimidine 36 (175 mg, 70%) as a white solid.

**2-Cyclopentyl-4-(6-phenylthieno[3,2-d]pyrimidin-4-yl)benzoic acid (37)**

A 5 mL microwave vial was loaded with pyrimidine 36 (55.0 mg, 0.22 mmol) and 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid 3 (70.0 mg, 0.22 mmol), Cs₂CO₃ (0.45 mmol, 150 mg), Pd(Ph₃)₄ (5 mol%, 13.0 mg). The solids were dissolved in a solution of 1,4-dioxane/water (3:1, 1.50 mL). The vial was sealed and the reaction mixture was irradiated at 125 °C for 15 min. The volatiles were removed in vacuo, remaining solids were suspended in ice/water before the pH adjusted to 2–3 with 2M HCl. After an initial purification over silica gel, the compound was further purified by recrystallization from diethyl ether, affording 2-cyclopentyl-4-(6-phenylthieno[3,2-d]pyrimidin-4-yl)benzoic acid 37 (12.0 mg, 15%) as white solid.

**1H NMR (400 MHz, DMSO-d₆) δ 13.25 (s, 1H), 9.30 (s, 1H), 8.23 (s, 2H), 8.07 (dd, J = 8.1, 1.8 Hz, 1H), 8.00 (dd, J = 7.1, 2.5 Hz, 2H), 7.89 (d, J = 8.1 Hz, 1H), 7.62–7.52 (m, 3H), 3.80 (p, J = 8.3 Hz, 1H), 2.18–2.06 (m, 2H), 1.92–1.78 (m, 2H), 1.75–1.60 (m, 4H); 13C NMR (176 MHz, DMSO-d₆) δ 169.2, 162.98, 157.7, 155.2, 153.6, 146.6, 138.9, 134.4, 131.9, 130.6, 129.8, 129.5,
2-Cyclopentyl-4-(thieno[3,2-d]pyrimidin-4-yl)benzoic acid (38)

A 5 mL microwave vial was loaded with 4-chlorothieno[3,2-d]pyrimidine 35a (0.41 mmol, 70.0 mg), 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid 3 (0.41 mmol, 130 mg), Cs₂CO₃ (0.82 mmol, 270 mg), Pd(Ph₃)₄ (5 mol%, 25.0 mg). The solids were dissolved in a solution of 1,4-dioxane/water (3:1, 1.50 mL). The vial was sealed and the reaction mixture was irradiated at 125 °C for 15 min. The volatiles were removed in vacuo, and the remaining solids were suspended with ice/water before the pH adjusted to 2–3 with 2M HCl(aq). The crude was purified via column chromatography (CH₂Cl₂/MeOH/HOAc, 95:4:1–90:9:1) affording 2-cyclopentyl-4-(thieno[3,2-d]pyrimidin-4-yl)benzoic acid 38 (70.0 mg, 48%) as a white solid.

1H NMR (400 MHz, DMSO-d₆) δ 13.24 (s, 1H), 9.31 (s, 1H), 8.60 (d, J = 5.5 Hz, 1H), 8.22 (d, J = 1.7 Hz, 1H), 8.04 (dd, J = 8.1, 1.7 Hz, 1H), 7.88 (d, J = 8.1 Hz, 1H), 7.76 (d, J = 5.5 Hz, 1H), 3.80 (p, J = 8.4, 7.9 Hz, 1H), 2.16–2.06 (m, 2H), 1.89–1.79 (m, 2H), 1.74–1.60 (m, 4H); 13C NMR (176 MHz, DMSO-d₆) δ 169.2, 162.1, 158.3, 154.7, 146.6, 139.0, 138.9, 134.4, 129.8, 127.6, 126.6, 125.5, 124.5, 41.2, 34.5, 25.4; HRMS calcd for C₁₈H₁₆N₂O₂S [M+H]+ 325.1011, found 325.1011; MP Range: 205–209 °C.

Methyl 4-(6-bromothieno[2,3-d]pyrimidin-4-yl)-2-cyclopentylbenzoate (40).

A 5 mL microwave vial was loaded with 6-bromo-4-chlorothieno[2,3-d]pyrimidine 39b (0.40 mmol, 100 mg), methyl 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 4 (0.36 mmol, 120 mg), Cs₂CO₃ (0.60 mmol, 195 mg) and Pd(Ph₃)₄ (5 mol%, 23.0 mg). The solids were dissolved in solution of 1,4-dioxane/water (3:1, 1.5 mL). The vial was sealed and the reaction mixture irradiated at 145 °C for 15 min. The volatiles were removed in vacuo, and the crude was purified via column chromatography (0-5% hexane/EtOAc) to afford methyl 4-(6-bromothieno[2,3-d]pyrimidin-4-yl)-2-cyclopentylbenzoate 40 (50.0 mg, 30%) as a white solid.

1H NMR (400 MHz, DMSO-d₆) δ 8.95 (s, 1H), 8.22 (s, 1H), 7.98 (d, J = 1.9 Hz, 1H), 7.83–7.76 (m, 2H), 3.86 (s, 3H), 3.63–3.57 (m, 1H), 2.08–2.00 (m, 2H), 1.90–1.81 (m, 2H), 1.75–1.63 (m, 4H).

2-Cyclopentyl-4-(6-phenylthieno[2,3-d]pyrimidin-4-yl)benzoic acid (41)

Methyl 4-(6-bromothieno[2,3-d]pyrimidin-4-yl)-2-cyclopentylbenzoate 40 (0.11 mmol, 45.0 mg), phenylboronic acid (0.12 mmol, 15.0 mg), Cs₂CO₃ (0.38 mmol, 52.0 mg), Pd(Ph₃)₄ (5 mol%, 6.00 mg), were dissolved in a solution of 1,4-dioxane/water (3:1, 2.00 mL). The reaction
mixture was stirred at reflux for 3 h. The volatiles were removed in vacuo and the crude was purified via column chromatography (0–10% EtOAc/hexane). The material was further purified using preparative TLC silica plates affording methyl 2-cyclopentyl-4-(6-phenylthieno[2,3-d]pyrimidin-4-yl)benzoate (25 mg, 55%) as a white solid. The methyl ester was saponified as follows: Methyl 2-cyclopentyl-4-(6-phenylthieno[2,3-d]pyrimidin-4-yl)benzoate (0.06 mmol, 25.0 mg) was dissolved in 1,4-dioxane (2.50 mL). LiOH (0.30 mmol, 7.00 mg) was dissolved in H2O (0.50 mL) and added to the solution. This mixture was stirred at 80 °C for 4 h. Solvents were removed in vacuo, and the material was suspended in ice/water, and the pH adjusted to 2-3 with 2M HCl. The resulting solid was filtered, thoroughly rinsed with water, air and vacuum dried affording 2-cyclopentyl-4-(6-phenylthieno[2,3-d]pyrimidin-4-yl)benzoic acid 41 (17.0 mg, 70%) as a white solid.

1H NMR (400 MHz, DMSO-d6) δ 13.11 (s, 1H), 9.16 (s, 1H), 8.16 (s, 1H), 8.13–8.06 (m, 2H), 7.83 (dd, J = 4.3, 2.4 Hz, 2H), 7.78–7.74 (m,1H), 7.68–7.63 (m, 3H), 3.68–3.62 (m, 1H), 2.10–1.98 (m, 2H), 1.87–1.79 (m, 2H), 1.70–1.62 (m, 4H); 13C NMR (176 MHz, DMSO-d6) δ 169.1, 168.8, 159.9, 153.4, 146.8, 142.8, 136.9, 134.9, 130.7, 130.2, 129.4, 129.0, 128.9, 126.7, 124.9, 124.4, 117.7, 41.4, 34.2, 25.1; HRMS (HESI) calcd for C24H20N2O2S [M+H]+ 401.1324, found 401.1330; MP Range: >250 °C.

2-Cyclopentyl-4-(thieno[2,3-d]pyrimidin-4-yl)benzoic acid (42).
Prepared following the procedure for 38 using 4-chlorothieno[2,3-d]pyrimidine 35a (70.0 mg, 0.41 mmol) and 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid 3 (130 mg, 0.41 mmol). After purification 2-cyclopentyl-4-(thieno[2,3-d]pyrimidin-4-yl)benzoic acid 42 (72.0 mg, 55%) was afforded as a white solid.

1H NMR (400 MHz, DMSO-d6) δ 9.19 (s, 1H), 8.09 (d, J = 6.1 Hz, 1H), 7.98 (d, J = 1.6 Hz, 1H), 7.87–7.79 (m, 2H), 7.68 (d, J = 6.1 Hz, 1H), 3.83–3.70 (m, 1H), 2.12–2.02 (m, 2H), 1.86–1.74 (m, 4H); 13C NMR (176 MHz, DMSO-d6) δ 169.3, 169.3, 159.2, 153.1, 146.2, 133.9, 129.5, 129.3, 127.4, 127.4, 126.4, 120.7, 41.3, 34.4, 25.3; HRMS (HESI) calcd for C18H16N2O2S [M+H]+ 325.1011, found 325.1013; MP Range: 146–149.5 °C.

Methyl 4-(6-chloroquinolin-4-yl)-2-cyclopentylbenzoate (44)
4,6-Dichloroquinoline (1.00 g, 5.05 mmol) and 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 4 (1.58 g, 4.80 mmol) were dissolved in a 10:1 solution of dioxane:water (27.5 mL). Pd2(dba)3 (231 mg, 0.25 mmol), XPhos (240 mg, 0.51 mmol) and Cs2CO3 (4.94 g, 15.2 mmol) were added and the mixture stirred for 16 h at 25 °C. The solution
was diluted with EtOAc (20.0 mL) and water (25.0 mL). The layers were separated and the aqueous was extracted with EtOAc (3 × 20.0 mL). The combined organics were dried (Na₂SO₄), filtered and concentrated in vacuo. The crude was purified via column chromatography (10–30% EtOAc/hexane) to afford methyl 4-(6-chloroquinolin-4-yl)-2-cyclopentylbenzoate 44 (1.54 g, 83%) as a white solid.  

**1H NMR** (400 MHz, CDCl₃) δ 8.96 (d, J = 4.4 Hz, 1H), 8.15 (d, J = 9.0 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 2.3 Hz, 1H), 7.69 (dd, J = 9.0, 2.3 Hz, 1H), 7.51 (d, J = 1.6 Hz, 1H), 7.42–7.33 (m, 2H), 3.97 (s, 3H), 3.89–3.78 (m, 1H), 2.23–2.12 (m, 2H), 1.86–1.69 (m, 4H), 1.63 (ddd, J = 16.2, 12.9, 8.8 Hz, 2H); **13C NMR** (100 MHz, CDCl₃) δ 168.5, 145.0, 148.1, 147.2, 146.9, 140.4, 133.0, 131.5, 131.1, 130.5, 130.0, 128.1, 127.2, 126.4, 124.5, 121.8, 77.0, 52.3, 41.8, 35.9, 25.7; HRMS calcd for C₂₂H₂₁ClNO₂ [M+H]+: m/z 366.1260 found m/z 366.1246; MP Range: 137–139 °C.

**2-Cyclopentyl-4-(6-phenylquinolin-4-yl)benzoic acid (45)**  
Methyl 4-(6-chloroquinolin-4-yl)-2-cyclopentylbenzoate 44 (100 mg, 0.27 mmol) and phenylboronic acid (66.0 mg, 0.32 mmol) were dissolved in a 10:1 solution of dioxane:water (12 mL). Pd₂(dba)₃ (25 mg, 0.03 mmol), XPhos (29.0 mg, 0.06 mmol) and Cs₂CO₃ (264 mg, 0.81 mmol) were added and the solution heated to 80 °C for 16 h. Once cooled the solution was diluted with EtOAc (10 mL) and water (10 mL). The layers were separated and the aqueous was extracted with EtOAc (3 × 10 mL). The combined organics were dried (Na₂SO₄), filtered and concentrated in vacuo. The crude was purified via column chromatography (10-30% EtOAc/hexane) to afford methyl 2-cyclopentyl-4-(6-phenylquinolin-4-yl)benzoate as an intermediate. The intermediate methyl ester was dissolved in dioxane (1 mL) and aq. LiOH (1 mL of a 1M solution). The solution was heated to 100 ºC for 16 h then acidified to pH 4 with 1M aq. HCl causing a precipitate to form. The solid was collected by filtration, washed with cold water (4 mL) and Et₂O (10 mL), dried under vacuum to afford 2-cyclopentyl-4-(6-phenylquinolin-4-yl)benzoic acid 45 (81 mg, 76%) as an off-white solid.  

**1H NMR** (400 MHz, DMSO-d₆) δ 9.15 (d, J = 4.8 Hz, 1H), 8.39 (d, J = 8.6 Hz, 1H), 8.32 (d, J = 8.7 Hz, 1H), 8.13 (d, J = 1.7 Hz, 1H), 7.87 (d, J = 7.9 Hz, 1H), 7.84–7.79 (m, 1H), 7.74–7.69 (m, 3H), 7.59 (dd, J = 8.0, 1.6 Hz, 1H), 7.51 (t, J = 7.4 Hz, 2H), 7.47–7.41 (m, 1H), 3.88–3.72 (m, 1H), 2.14–2.01 (m, 2H), 1.84–1.71 (m, 2H), 1.71–1.50 (m, 4H); **13C NMR** (176 MHz, DMSO) δ 169.3, 146.4, 145.2, 139.8, 138.9, 138.9, 138.8, 132.8, 130.7, 129.6, 129.3, 129.2, 128.3, 128.1, 127.1,
2-Cyclopentyl-4-(quinolin-4-yl)benzoic acid (46)
Prepared following the procedure for 21 using 4-chloroquinoline (100 mg, 611 µmol). After purification 2-cyclopentyl-4-(quinolin-4-yl)benzoic acid 46 (160 mg, 83%) was afforded as a white solid.

H NMR (400 MHz, DMSO-\(d_6\)) δ 13.09 (s, 1H), 8.97 (d, \(J = 4.4\) Hz, 1H), 8.14–8.11 (m, 1H), 7.8–7.78 (m, 3H), 7.62 (ddd, \(J = 8.3, 6.9, 1.3\) Hz, 1H), 7.56 (d, \(J = 1.7\) Hz, 1H), 7.50 (d, \(J = 4.4\) Hz, 1H), 7.44 (dd, \(J = 7.9, 1.7\) Hz, 1H), 3.85–3.73 (m, 1H), 2.11–2.00 (m, 2H), 1.81–1.71 (m, 2H), 1.70–1.54 (m, 4H); C NMR (100 MHz, DMSO-\(d_6\)) δ 169.4, 150.2, 148.1, 146.8, 146.6, 140.0, 132.1, 129.6, 129.6, 129.5, 127.8, 127.2, 126.7, 125.7, 125.2, 121.5, 41.2, 34.5, 25.3; HRMS calcd for C\(_{21}\)H\(_{19}\)NO\(_2\) [M-H]: m/z 316.1338; found 316.1339; MP Range 259–263 °C.

Methyl 2-cyclopentyl-4-(6-hydroxyquinazolin-4-yl)benzoate (48)
Prepared following the procedure for 32 using 4-chloroquinazolin-6-ol 47b (50.0 mg, 0.28 mmol). After purification methyl 2-cyclopentyl-4-(6-hydroxyquinazolin-4-yl)benzoate 48 (63.0 mg, 65%) was afforded as a thick oil.

H NMR (400 MHz, DMSO-\(d_6\)) δ 9.42 (s, 1H), 8.16 (d, \(J = 9.0\) Hz, 1H), 8.10 (dd, \(J = 9.0, 2.3\) Hz, 1H), 7.99–7.98 (m, 1H), 7.88–7.84 (m, 3H), 7.71 (dd, \(J = 7.9, 1.7\) Hz, 1H), 3.70–3.62 (m, 1H), 2.13–2.01 (m, 2H), 1.81–1.75 (m, 2H), 1.72–1.57 (m, 4H); C NMR (101 MHz, DMSO-\(d_6\)) δ 167.9, 166.2, 154.7, 149.0, 146.4, 138.9, 134.8, 132.6, 132.3, 130.8, 129.4, 128.4, 127.1, 125.2, 122.9, 52.4, 41.3, 34.4, 25.2.

2-Cyclopentyl-4-(6-phenylquinazolin-4-yl)benzoic acid (49)
Methyl 2-cyclopentyl-4-(6-phenylquinazolin-4-yl)benzoate SI4 (170 mg, 0.41 mmol) was dissolved in MeOH (3.5 mL) and aq. NaOH (1 mL). The resulting solution was heated to 70 °C for 1 hr and allowed to cool to rt, added water (4 mL) and extracted with ether (2 x 10 mL). The aqueous phase was acidified with 1N HCl (pH 6) and then extracted with EtOAc. The organic
phase was dried (Na$_2$CO$_3$) and concentrated to afford 2-cyclopentyl-4-(6-phenylquinazolin-4-yl)benzoic acid 49 (150 mg, 91%) as a light yellow solid.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.19 (s, 1H), 9.39 (s, 1H), 8.39 (dd, $J = 8.8, 2.1$ Hz, 1H), 8.24–8.19 (m, 2H), 7.92 (d, $J = 1.7$ Hz, 1H), 7.87 (d, $J = 7.9$ Hz, 1H), 7.78–7.73 (m, 3H), 7.51 (t, $J = 7.4$ Hz, 2H), 7.45 (d, $J = 7.3$ Hz, 1H), 3.86–3.75 (m, 1H), 2.12–2.04 (m, 2H), 1.80–1.74 (m, 2H), 1.69–1.60 (m, 4H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 169.4, 167.0, 154.4, 149.9, 146.1, 139.8, 139.0, 138.8, 133.5, 133.4, 129.3, 129.2, 128.5, 128.3, 127.2, 127.1, 123.7, 122.5, 41.2, 34.5, 25.3; HRMS calcd for C$_{26}$H$_{22}$N$_2$O$_2$ [M-H]: m/z 393.1607; Found 393.1603; MP Range: 265–269 °C.

2-Cyclopentyl-4-(quinazolin-4-yl)benzoic acid (50)

4-Chloroquinazoline (100 mg, 0.60 mmol), 2-cyclopentyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzoate 4 (240 mg, 0.72 mmol), Pd(PPh$_3$)$_4$ (70.0 mg, 60.0 μmol) and Cs$_2$CO$_3$ (0.60 g, 1.80 mmol) were loaded into a microwave vial. A solution of dioxane/water (6.0 mL of a 10:1 mix) was added and the vial flushed with nitrogen. The mixture was irradiated at 100 °C for 30 minutes. Once cooled, the aqueous layer was removed and the remaining volatiles were removed in vacuo. The crude was purified via column chromatography and the intermediate ester isolated. The ester was dissolved in dioxane (1 mL) and aq. LiOH (1 mL of a 1M solution). The solution was heated to 100 °C for 16 h then acidified to pH 4 with 1M aq. HCl causing a precipitate to form. The solid was collected by filtration, washed with cold H$_2$O then dried under vacuum to afford the quinazoline (128 mg, 67%) as a pale white solid.

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.38 (s, 1H), 8.15–8.03 (m, 3H), 7.85 (d, $J = 7.9$ Hz, 1H), 7.78 (ddd, $J = 9.7, 6.8, 1.4$ Hz, 3H), 7.66 (ddd, $J = 7.9, 1.7$ Hz, 1H), 3.83–3.73 (m, 1H), 2.11–2.02 (m, 2H), 1.81–1.71 (m, 2H), 1.70–1.57 (m, 4H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 169.3, 167.0, 154.3, 150.3, 146.0, 139.0, 134.4, 133.5, 129.1, 128.6, 128.4, 128.2, 127.08, 126.6, 122.3, 41.3, 34.4, 25.2; HRMS calcd for C$_{20}$H$_{19}$N$_2$O$_2$ [M+H]: m/z 319.1446 found m/z 319.1427; MP Range: 120–122 °C.

2-Cyclopentyl-4-(2-methylquinolin-4-yl)benzoic acid (52)
Prepared following the procedure for 21 using 4-chloro-2-methylquinoline 51 (200 mg, 1.13 mmol). After purification 2-cyclopentyl-4-(2-methylquinolin-4-yl)benzoic acid 52 (290 mg, 77%) was afforded as an off white solid.

1H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 13.08 (s, 1H), 8.01 (dd, \(J = 8.5, 1.5\) Hz, 1H), 7.81 (d, \(J = 7.9\) Hz, 1H), 7.78–7.72 (m, 2H), 7.56–7.52 (m, 2H), 7.43–7.39 (m, 2H), 3.86–3.74 (m, 1H), 2.70 (s, 3H), 2.12–2.00 (m, 2H), 1.78–1.74 (m, 2H), 1.67–1.56 (m, 4H);

13C NMR (176 MHz, DMSO-\(d_6\)) \(\delta\) 172.6, 161.6, 151.0, 150.0, 149.3, 143.2, 135.4, 132.6, 132.5, 132.0, 130.8, 129.7, 129.3, 128.1, 127.2, 125.3, 44.4, 37.6, 28.4, 27.9; HRMS calcd for \(\text{C}_{22}\text{H}_{21}\text{NO}_{2}\) [M-H]: \(m/z\) 332.1651; found 332.1642. MP Range: 271–275 °C.

2-Cyclopentyl-4-(1,6-naphthyridin-4-yl)benzoic acid (54)

Prepared following the procedure for 21 using 4-chloro-1,6-naphthyridine 53 (80.0 mg, 0.49 mmol). After purification 2-cyclopentyl-4-(1,6-naphthyridin-4-yl)benzoic acid 54 (110 mg, 54%) was afforded as a yellow solid.

1H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 13.14 (s, 1H), 9.24–9.15 (m, 2H), 8.79 (d, \(J = 5.8\) Hz, 1H), 8.01 (dd, \(J = 5.9, 0.9\) Hz, 1H), 7.84 (d, \(J = 7.9\) Hz, 1H), 7.71–7.65 (m, 2H), 7.54 (dd, \(J = 7.9, 1.8\) Hz, 1H), 3.84–3.73 (m, 1H), 2.09–2.04 (m, 2H), 1.80–1.71 (m, 2H), 1.71–1.56 (m, 4H);

13C NMR (176 MHz, DMSO-\(d_6\)) \(\delta\) 169.88, 155.32, 150.85, 150.45, 147.84, 146.88, 146.7, 138.4, 133.1, 129.9, 128.4, 127.3, 123.5, 122.3, 121.5, 41.6, 34.8, 25.6; HRMS calcd for \(\text{C}_{20}\text{H}_{18}\text{N}_{2}\text{O}_{2}\) [M-H]: \(m/z\) 319.1447; found 319.1427. MP Range 195–200 °C.

2-Cyclopentyl-4-(pyrimidin-4-yl)benzoic acid (56)

4-Chloropyrimidine (162 mg, 1.40 mmol) and \(\text{Cs}_2\text{CO}_3\) (1.32 g, 4.00 mmol) were dissolved in 1,2-DME (4.00 mL) and \(\text{H}_2\text{O}\) (0.70 mL). \(\text{Pd(PPh}_3\text{)}_4\) (78 mg, 68 \(\mu\)mol) and 3 (503 mg, 1.60 mmol) were added and the solution stirred at 120 °C for 30 min. The resulting suspension was filtered through Celite® under vacuum. The filter cake was washed with ethyl acetate and the filtrate was dried (\(\text{Na}_2\text{SO}_4\)), filtered and concentrated in vacuo. The crude product was purified by column chromatography (0–50% EtOAc(0.01% acetic acid)/hexane) to afford 2-cyclopentyl-4-(pyrimidin-4-yl)benzoic acid 56 (29 mg, 8%) as a white solid.
1H NMR (700 MHz, DMSO-d6): δ 13.14 (br s, 1H), 9.28 (s, 1H), 8.92–8.87 (m, 1H), 8.25 (s, 1H), 8.20–8.16 (m, 1H), 8.06 (d, J = 7.7 Hz, 1H), 7.77 (d, J = 7.7 Hz, 1H), 3.72 (p, J = 8.0 Hz, 1H), 2.09–2.01 (m, 2H), 1.87–1.79 (m, 2H), 1.73–1.59 (m, 4H); 13C NMR (176 MHz, DMSO-d6): δ 169.3, 158.8, 158.8, 158.3, 146.4, 134.5, 129.7, 125.1, 124.2, 117.7, 41.4, 34.4, 25.3; HRMS calcd for C16H15N2O2 [M-H]−: m/z 267.1138, found 267.1139; MP Range: 245 °C.

2-Cyclopentyl-4-(2-phenylpyrimidin-4-yl)benzoic acid (58)

4-Chloro-2-phenylpyrimidine (155 mg, 0.81 mmol) and Cs2CO3 (775 mg, 2.40 mmol) were dissolved in 1,2-DME (2.40 mL) and H2O (0.40 mL). Pd(PPh3)4 (48.0 mg, 42.0 μmol) and 3 (307 mg, 0.97 mmol) were added and the solution stirred at 120 °C for 30 min. The resulting suspension was filtered through Celite® under vacuum. The filter cake was washed with ethyl acetate and the filtrate was dried (Na2SO4), filtered and concentrated in vacuo. The crude product was purified by column chromatography (0–10% EtOAc(0.01% acetic acid)/hexane) to afford 2-cyclopentyl-4-(2-phenylpyrimidin-4-yl)benzoic acid 58 (21 mg, 8%) as a white solid.

1H NMR (700 MHz, DMSO-d6): δ 13.16 (br s, 1H), 9.04–8.95 (m, 1H), 8.52 (d, J = 6.0 Hz, 2H), 8.33 (s, 1H), 8.21 (d, J = 7.6 Hz, 1H), 8.15–8.07 (m, 1H), 7.82 (d, J = 7.6 Hz, 1H), 7.63–7.53 (m, 3H), 3.76 (p, J = 8.8 Hz, 1H), 2.12–2.03 (m, 2H), 1.90–1.82 (m, 2H), 1.75–1.65 (m, 4H); 13C NMR (176 MHz, DMSO-d6): δ 169.4, 163.4, 162.3, 158.9, 146.4, 138.5, 137.2, 134.5, 131.0, 129.7, 128.8, 127.8, 125.3, 124.3, 115.7, 41.4, 34.4, 25.3; HRMS calcd for C22H21N2O2 [M+H]+: m/z 345.1598, found 345.1600; MP Range: >250 °C.

Methyl 4-(2-aminopyrimidin-4-yl)-2-cyclopentylbenzoate (60)

4-Chloropyrimidin-2-amine (501 mg, 3.90 mmol) and Cs2CO3 (3.81 mg, 12.0 mmol) were dissolved in 1,2-DME (8.00 mL) and H2O (2.00 mL). Pd(PPh3)4 (238 mg, 0.21 mmol) and 4 (1.50 g, 4.50 mmol) were added and the solution was irradiated at 120 °C for 30 min. The resulting suspension was filtered through Celite® under vacuum. The filter cake was washed with ethyl acetate and the filtrate was dried (Na2SO4), filtered and concentrated in vacuo. The crude product was purified by column chromatography (20% EtOAc/hexane) to afford methyl 4-(2-aminopyrimidin-4-yl)-2-cyclopentylbenzoate 60 (523 mg, 45%) as a white solid.
4-(2-Aminopyrimidin-4-yl)-2-cyclopentylbenzoic acid hydrochloride (61·HCl)

Methyl 4-(2-aminopyrimidin-4-yl)-2-cyclopentylbenzoate 60 (40.0 mg, 0.13 mmol) was dissolved in 1,4-dioxane (1.00 mL). 1M LiOH (1.00 mL) was added and the solution stirred for at 100 °C for 17 h. HCl (6.00 M, 6.00 mL) was added resulting in precipitate formation. The precipitate was filtered and washed with HCl (6.00 M). The precipitate was dried reduced pressure to afford 4-(2-aminopyrimidin-4-yl)-2-cyclopentylbenzoic acid hydrochloride 61·HCl (35.0 mg, 85%) as a white solid.

2-Cyclopentyl-4-(2-(phenylamino)pyrimidin-4-yl)benzoic acid hydrochloride (62·HCl)

Methyl 2-cyclopentyl-4-(2-(phenylamino)pyrimidin-4-yl)benzoate SI5 (20 mg, 54 μmol) was dissolved in 1,4-dioxane (1.0 mL). 1M LiOH (1.0 mL) was added and the solution stirred for at 100 °C for 16 h. HCl (6.0 M, 6.0 mL) was added resulting in precipitate formation. The precipitate was filtered and washed with HCl (6.0 M). The precipitate was dried reduced pressure to afford 2-cyclopentyl-4-(2-(phenylamino)pyrimidin-4-yl)benzoic acid hydrochloride (62·HCl) (12 mg, 63%) as a yellow solid.
1H), 7.33–7.27 (m, 2H), 7.01–6.96 (m, 1H), 3.77 (p, \(J = 9.6\) Hz, 1H), 2.13–2.03 (m, 2H), 1.89–1.80 (m, 2H), 1.73–1.61 (m, 4H); \(^{13}\)C NMR (176 MHz, DMSO-\(d_6\)): \(\delta 169.3, 162.6, 160.1, 159.3, 146.4, 140.5, 139.0, 134.0, 129.6, 128.4, 125.1, 124.0, 121.5, 119.0, 108.3, 41.2, 34.5, 25.4\); HRMS calcd for C\(_{22}\)H\(_{22}\)N\(_3\)O\(_2\) [M+H]+: \(m/z 360.4365\), found 360.1707; MP Range: >250 °C.

ASSOCIATED CONTENT

The supporting information contains mass spectrometry methods, compound characterization spectra, NanoBRET binding curves and Western blots. The experimental contains detailed synthetic procedures for compound synthesis. This material if available free of charge from https://pubs.acs.org.

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ABBREVIATIONS

CAMKK2, calcium-calmodulin protein kinase kinase 2; AMPK, AMP-activated protein kinase; AKT, protein kinase B (PKB); siRNA, small interfering RNA; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; GRK3, G protein-coupled receptor kinase 3; CK2, casein kinase 2; CDKL2, cyclin-dependent kinase-like 3; AhR, aryl hydrocarbon receptor; ATP, adenosine triphosphate; SEM, trimethylsilylhexoxymethyl; CDI, 1,1-carboadiimazole; DME, dimethoxyethane; DSF, differential scanning fluorimetry; PoC, percentage of control; ADMET, absorption, distribution, metabolism, excretion and toxicology; LE, ligand efficiency; LLE, lipophilic ligand efficiency; DMSO, dimethylsulfoxide; BRET, bioluminescence resonance energy transfer.
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