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Discovery of 3-(3-(4-(1-Aminocyclobutyl)phenyl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine (ARQ 092): An Orally Bioavailable, Selective, and Potent Allosteric AKT Inhibitor

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Abstract

The work in this paper describes the optimization of the 3-(3-phenyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine chemical series as potent, selective allosteric inhibitors of AKT kinases, leading to the discovery of ARQ 092 (**21a**). The co-crystal structure of compound **21a** bound to full-length AKT1 confirmed the allosteric mode of inhibition of this chemical class and the role of the cyclobutylamine moiety. Compound **21a** demonstrated high enzymatic potency against AKT1, AKT2 and AKT3, as well as potent cellular inhibition of AKT activation and the phosphorylation of the downstream target PRAS40. Compound **21a** also served as a potent inhibitor of the AKT1-E17K mutant protein and inhibited tumor growth in a human xenograft mouse model of endometrial adenocarcinoma.

Introduction

The AGC kinases AKT1, AKT2 and AKT3 are key mediators of the PI3K/AKT/mTOR signaling pathway, which promotes diverse physiological processes such as proliferation, migration, anti-apoptotic survival and protein synthesis.[1] The activation of the PI3K/AKT/mTOR signaling network is commonly observed in several human cancers and can be induced through a variety of mechanisms, including the overexpression of upstream receptors, PI3KCA-activating mutations, the loss of PTEN function, and the overexpression or mutational activation of AKT.[2] The serine/threonine AKT kinases represent a pivotal node connecting the PI3K and mTOR pathways, suggesting that AKT kinases are compelling targets for the treatment of human cancers and non-oncology pathologies. Multiple efforts have been made to discover small molecule inhibitors of AKT, including both allosteric inhibitors that target full-length AKT and ATP-competitive inhibitors that are designed to bind the ATP binding pocket of the catalytic domain.[3] Allosteric inhibitors offer a unique advantage over ATP-competitive inhibitors, in that very high kinase selectivity can be achieved by targeting a distinct allosteric pocket in AKT formed by the pleckstrin homology (PH) and kinase domains. In contrast to allosteric inhibitors, which inhibit the phosphorylation of Thr308 and Ser473 in AKT, ATP-competitive inhibitors paradoxically induce the hyper-phosphorylation of these two regulatory sites in cells and *in vivo*; however, the clinical significance of this observation is not yet understood.[4]

We recently reported the pre-clinical characterization of ARQ 092 (**21a**)[5], a highly selective, allosteric inhibitor of AKT.[6] Compound **21a** showed strong affinity for un-phosphorylated full-length AKT1 and potently inhibited the phosphorylated form of full-length AKT isoforms. In a large panel of cell lines derived from various tumor types, **21a** showed potent anti-proliferative activity in cell lines containing PIK3CA/PIK3R1 mutations compared to those with wild-type

(wt) PIK3CA/PIK3R1 or PTEN loss. Moreover, **21a** also exhibited strong anti-tumor activity in mouse xenograft models implanted with cell lines carrying a PIK3CA mutation and in a patient-derived tumor mouse model harboring an AKT1-E17K activating mutation. Both animal studies demonstrated the desirable pharmacokinetic properties of **21a** which is under investigation in clinical trials targeting advanced solid tumors and recurrent malignant lymphoma. Compound **21a** is also being investigated for treatment of Proteus syndrome, a disease driven by AKT-E17K mutation.[7]

In our previous study, we demonstrated that optimizing activity against AKT1, AKT2 and AKT3 was critical to achieving cellular potency; however, the most potent benzamide analogs of the imidazo[4,5-*b*]pyridine series had very low exposure in the plasma and tumors in mouse xenograft models. Therefore, the final phase of the optimization was then focused on optimizing the pharmacokinetics and demonstrating *in vivo* activity in tumor xenografts. One critical optimization step in the early stage of discovery of this chemical series was the reduction to a methyl group (**4a)** from a 4-*t*-butyl group observed in the initial hit (**4b**), followed by the addition of a primary anime to the methyl group (**6**). These two small side-chain modifications significantly improved the biochemical potency of the compounds and incorporated the substituents necessary for further optimization. Compound **6** became the cornerstone from which an extensive optimization campaign was undertaken, part of which has been previously disclosed with a focus on substitutions at the 5- and 6-position of the imidazo[4,5-*b*]pyridine ring system, as well as the substitution of the benzylic amine amide.[8]

In this communication, we describe the additional structure-activity relationships (SARs) that led to the discovery of **21a**, its characterization *in vitro* and *in vivo* and its binding mode based on the co-crystallization of **21a** with AKT1. To probe the structural features associated with this

highly biologically active scaffold, we previously reported the screening and early optimization of a series of 3-(3-phenyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amines as potent AKT inhibitors.[8] The SAR-guided optimization of the imidazo[4,5-b]pyridine series using *in vitro* and *in vivo* assessments yielded highly selective AKT inhibitors. *In vivo* characterization employed both pharmacokinetic and pharmacodynamic analyses, and the lead inhibitors demonstrated oral bioavailability in mice and were well tolerated, with robust inhibition of AKT activation, as well as downstream AKT signaling. The screening paradigm in our previous study used a biophysical thermal shift assay to identify binders of AKT1[9], followed by a biochemical assay using the un-phosphorylated AKT1 protein to recapitulate the binding event measured by the thermal shift assay. This was achieved by pre-incubating the test compounds with the un-phosphorylated protein, followed by *in situ* activation. AKT activation entails the translocation of the protein to the cell membrane and the binding of the PH domain of AKT to phosphoinositides, upon which a conformational change permits the phosphorylation of Thr308 (for AKT1) in the activation loop by phosphoinositide-dependent kinase 1 (PDK1).[10] The full activation of AKT is achieved when Ser473 (for AKT1) in the hydrophobic motif (HM) domain is phosphorylated by the mTORC2 complex.[11] For the biochemical assay, un-phosphorylated full-length proteins (AKT1 (1-480), AKT2 (1-481) and AKT3 (1-479)) were pre-incubated with the test compounds, followed by the successive addition of the activating proteins PDK1 and MAPKAPK2 (a Ser473-phosphorylating kinase that replaces the mTORC2 complex), lipid vesicles, and ATP before addition of the substrate, biotinylated crosstide. The biochemical activities of the test compounds described in this work against the two activating kinases (PDK1 and MAPKAPK2) were determined to ascertain whether the observed AKT inhibition was due specifically to AKT binding.[12]

Additionally, the biochemical inhibition of full-length, pre-activated AKT1, AKT2 and AKT3 was probed for all compounds to further validate the inhibitory potency.[13]

Synthesis

From commercially available 2-chloro-3-nitropyridine (**1a**) and substituted 2-chloro-3-nitropyridines (**1b-f**), the displacement of the chlorine atom with substituted anilines in DMSO or 1,4-dioxane in the presence of a mild base (diisopropylethylamine or trimethylamine), under heating, provided compounds **2a-c** and **3a-e** with good yields (Schemes 1 and 2). A one-pot condensation/cyclization of the substituted 2-anilino-3-nitropyridines (**2a-c**, **3a-e**) with 2-amino-nicotinaldehyde under reducing conditions using sodium hydrosulfite in a DMSO/methanol mixture (2:1) at 100 °C afforded compounds **4a-c** and **5a-e** with yields varying from 25-75%. Intermediates **4c** and **5a-e** were Boc-deprotected using 4 M HCl in 1,4-dioxane at room temperature, producing **6** and **7a-e** in quantitative yields as HCl salts.

As shown in Scheme 3, intermediate **5d** was reacted with phenyl boronic acid, 3-acetamidophenyl boronic acid or 4-acetamidophenyl boronic acid in a Suzuki coupling reaction, affording compounds **8a-d** with good yields.[14] Standard Boc-deprotection with HCl produced compounds **9a-d** in quantitative yields as HCl salts. Alternatively, intermediate **5e** was reacted with (pyridin-3-yl)boronic acid under the same Suzuki conditions, affording the Boc-protected compound **8e** with a substituent at the 6-position. Standard deprotection with HCl yielded **9e**.

Synthesis of the gem-dimethyl compound **17** is depicted in Scheme 4. Racemic 2-methyl-2-propanesulfinamide (**10**) was reacted with acetone in the presence of $Ti(OEt)_4$, affording the N-propan-2-ylidene **11** with a 47% yield.[15] Halogen-metal exchange using 4-bromo-N,N-bis(trimethylsilyl)aniline and BuLi at a low temperature generated the 4-lithio species, which was trapped with **11**. *In situ* cleavage of the Si-N bonds afforded the aniline **12** with a modest

yield.[5] Following a displacement reaction using **1e**, the resulting compound **13** was subjected to a de-protection/re-protection sequence, yielding the Boc-protected intermediate **14** with excellent yields. Cyclization of **14** with 2-aminonicotinaldehyde under reducing conditions produced **15** with moderate yields. As described above, a Suzuki coupling reaction yielded **16**, and a standard Boc-deprotection using HCl gave the final product **17**.

The synthesis of the amino-cyclobutyl compounds **21a-c**, **22** and **23** is shown in Scheme 5. The preparation of *tert*-butyl (1-(4-amino phenyl)cyclobutyl)carbamate is described[5] and is now commercially available. Using the starting nitropyridine **1e** and *tert*-butyl (1-(4-amino phenyl)cyclobutyl)carbamate in a displacement reaction produced **18** with moderate yields. Cyclization under reducing conditions with 2-aminonicotinaldehyde afforded the intermediate **19**. A Suzuki coupling reaction using phenyl boronic acid, 3-acetamidophenyl boronic acid or 4-acetamidophenyl boronic acid produced **20a-c** with good yields. Standard Boc-deprotection with HCl afforded the final compounds **21a-c** in quantitative yields. Compound **22** was the result of a conventional amine acylation using acetic anhydride in pyridine. Subjecting the deprotected compound **21b** to HCl in dioxane for an extended period (3 days at 50 ^{o}C) produced the de-acylated compound **23**.

The replacement of the amino group appending the four-membered ring with a carboxylic acid methyl ester (**26**) or a carboxamide (**27**) is shown in Scheme 6. Suzuki coupling (**24**→**25**) was executed prior to the reductive cyclization with the 2-amino-nicotinaldehyde, and compound **26** was obtained with a good overall yield (46% over three steps). Hydrolysis followed by EDC coupling produced **27** with a modest yield.

Results and Discussion

The structure-activity-relationship described here was focused on two regions of the 3-(3-phenyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine series: the substitution around the pyridine ring and substitution at the 4-position of the phenyl ring. The SAR of substitutions of the pyridine ring is shown in Table 2. The introduction of a methyl group at the 7- (**7a**) or 6-position (**7b**) led to improved potency; however, methyl groups at the 5- or 6-position (**7c**) were not tolerated. A similar effect was noticed in previous SAR studies using the benzylamine amides[8], which showed little difference between the two positions on the imidazo[4,5-*b*]pyridine core. Compounds **7a** and **7b** displayed very modest inhibitory activity against AKT2 and AKT3. The presence of a chlorine atom at the 5-position (**7d**) or a bromine atom at the 6-position (**7e**) did not significantly affect the potency against AKT1. Interestingly, the introduction of a phenyl ring at the 5-position dramatically improved the activity against all three isoforms, while a pyridyl substitution at the 6-position also increased potency against AKT1 but spared AKT2 and AKT3 by 100 fold and >300 fold, respectively.

After identifying the key position for improving activity against the AKT isoforms, substitutions at the 5-position were further explored. A limited set of substitutions is exemplified with compounds **9b**, **9c** and **9d**. The introduction of more polar groups at the *para* or *meta* position was well tolerated and offered similar levels of AKT inhibition and isozyme selectivity.

Combining the SAR points from compounds **4b**, **6** and **9a**, we were determined to make the hybrid compound **17**, which proved to be more arduous than anticipated. A small amount was finally isolated, and the compound showed potent inhibitory activity against AKT1 (Table 3, IC_{50} = 0.0054 μM). Further investigation into substituting the benzylic position led to compound **21a**, in which a cyclobutylamine replaces the gem-dimethyl substitution, and the potency against

all three AKT isoforms remarkably improved. The role of the free amino group was confirmed when we tested the acetylated compound **22,** which showed a 10-fold loss of potency against AKT1. Replacing the acetamide group in **22** with a reverse acetamide (**27**) was tolerated and showed similar potency; however, the introduction of an ester moiety (**26**) at this position was deleterious. A similar SAR trend was observed in our previous study: the acetamide analogue of **6** was active compared to non-polar or polar substituents that lacked hydrogen donors (compound **8d** of reference 11, IC_{50} = 0.81 μM).[8] Thus, the introduction of substituents at the benzylic position appeared to change the previously defined SAR when a basic nitrogen is present.

The extension of the cyclobutylamine to compound **9b** to produce **21b** showed comparable inhibitory activity to that of **21a** against all AKT isozymes (Table 4). The removal of the acetamide (**23**) did not change the IC_{50} values against inactive AKT. Finally, introducing a pyridine nitrogen in the aromatic ring of the 5-position substituent (**21c**) modestly affected the activities against AKT2 and AKT3.

The biochemical potencies of the compounds against inactive, un-phosphorylated AKT were compared with those against active, phosphorylated AKT isoforms in the final stage of optimization. As shown in Table 4, with the exception of **21c**, the compounds showed similar inhibitory potency for the enzymatically distinct forms of AKT. The presence of a nitrogen atom in the aromatic substituent at position 5 appeared to be detrimental to the activity against AKT2 and AKT3. In contrast, as reported in our previous study, the initial hits of this chemical series were preferentially highly active in the assay utilizing only the un-phosphorylated form of AKT. Therefore, further lead optimization was continued with the same assay format. Finally, the fully optimized, highly potent inhibitors were capable of inhibiting both the active and inactive forms

of AKT with equivalent potency, and we believe that this functionality in the inhibitors is necessary to target constitutive AKT kinase activity in cancer cells.

To define the binding mode of these potent AKT inhibitors and to understand the interactions of the phenyl cyclobutylamine and phenyl groups at the 5-position, the crystal structure of un-phosphorylated AKT1 was determined in complex with compound **21a** (Figure 1).[16] The overall conformation of AKT1 was very similar to the previously determined structure[8], adopting an identical auto-inhibitory conformation[17], and the inhibitor (**21a**) occupied an allosteric pocket at the interface between the kinase and PH domains. The aminopyridine moiety of the (3-phenyl-3*H*-imidazo[4,5-*b*]-pyridin-2-yl)pyridin-2-amine core forms hydrogen bonds with the disordered αC-helix and the β4-strand, and the core forms a key hydrophobic interaction with Trp80 in the PH domain. The phenyl ring of phenylcyclobutylamine makes a direct hydrophobic contact with Tyr272 in the highly-conserved YRD motif of the kinase domain. The primary amine of cyclobutylamine forms bidentate hydrogen bonds with the main chain carbonyl of Tyr 72 and the carboxylate group of Asp274, and this interaction facilitates the steric orientation of the cyclobutyl group towards the hydrophobic side chain of Ile 84 and the methyl group of Thr82 in the PH domain. Therefore, a strong proton donor on the benzylic group is critical for the interaction; thus, replacing this side chain with either with a single proton donor group, such as an amide in **27,** or a strong proton acceptor, such as the methyl ester group in **26**, would be detrimental. The phenyl moiety at the 5-position contacts the acyl side chains of Gln79 and Asn53, and thus, polar substituents (**9b**, **9c**, **9d**, **21b** and **23**) on this hydrophobic phenyl ring are well tolerated, as these groups project towards the solvent. The crystal structure further explains the advantage of these interactions that led to activity improvement at the 5- position but not at

the 6- or 7-positions, which is exemplified by **7a**, **7b** and **7c**, where the methyl substituents are directly exposed to the highly polar solvent region.

We next examined the *in vitro* anti-proliferative activity of the compounds in multiple cell lines where the PI3K/AKT pathway is upregulated due to mutational activation; A2780 (ovarian carcinoma), AN3CA (endometrial adenocarcinoma), IGROV-1 (ovarian adenocarcinoma) and LNCaP (prostate adenocarcinoma) were all monitored using a standard MTS assay. Compound **9a** exhibited micromolar anti-proliferative activity (Table 5), while its cyclobutylamine analog showed a 10-35 fold increase in activity, being most potent against the IGROV-1 cell line. The acetamide analog **21b** was less active against the A2780, AN3CA and LNCaP cell line yet was best at inhibiting the proliferation of IGROV-1 ovarian cells (IC_{50} = 0.16 μM). The corresponding amine **23** was also very potent in the MTS assay. Consistent with its biochemical inhibition of AKT, the pyridine analog **21c** was markedly less active in the cell lines, with the exception of IGROV-1 cells.

We assessed the ability of compounds **21a-c** to inhibit AKT1 phosphorylation at residues S473 and T308, as well as the downstream PRAS40 residue T246 in cells using Western blot analysis. Compounds **21a** and **21b** showed excellent inhibition of p-AKT (S473) and p-AKT (T308) in both AN3CA and A2780 cells (Table 6). The inhibition of the downstream protein p-PRAS40 (T246) was observed with **21a** (IC_{50} = 0.31 μM), whereas **21b** did not significantly inhibit the phosphorylation of PRAS40 (IC_{50} = >3 μM).

Table 7 shows CYP450 isoform inhibition and microsomal stability using human, mouse and dog liver microsome preparations. Compounds **9a-b**, **21a-c** and **23** did not significantly inhibit CYP450 1A2, 2C8, 2D6 and 3A4. Compounds **9a** and **9b** inhibited CYP450 2C9 with a sub-micromolar IC_{50} value, while **9a** also inhibited CYP450 2C19 (IC_{50} = <1 μM). All compounds

were adequately stable in liver microsomes (human, mouse and dog). In general, we found that compounds from this chemical series possess adequate *in vitro* ADME properties, and compound **21a** in particular showed good biochemical inhibition, cellular knockdown of AKT phosphorylation and ADME properties. In a mouse pharmacokinetic study, (po at 100 mg/kg, iv at 5 mg/kg), compound **21a** showed an oral bioavailability of 23%. An *in vivo* pharmacodynamic assessment of **21a** using NCr-M nude mice implanted with AN3CA tumor xenografts was reported in our recent publication.[6] Compound **21a** resulted in 99%, 95% and 58% reductions in p-AKT (S473), p-AKT (T306) and p-PRAS40 (T246), respectively, after tumor-bearing mice were treated with 100 mg/kg po. The inhibition of phosphorylation was sustained at eight hours. The plasma concentration of compound **21a** at one hour was 2.1 μM and decreased to 0.26 μM at 8 hours, while in the tumor, the concentration was 21.0 μM at one hour and 9.6 μM at 8 hours. The concentrations in the tumor tissues were significantly higher than in the plasma, indicating a marked preference for tissue accumulation compared with the vasculature compartment.

The efficacy of compound **21a** has been probed in a previous communication, in which it was shown to have strong tumor growth inhibition in mouse xenografts of endometrial, breast cancer cell lines and patient-derived tumor models.[6]

Definitive single dose pharmacokinetic studies were conducted in rats and monkeys (Table 9). Compound **21a** showed good absolute oral bioavailability in rats and monkeys with F values of 62% and 49%, respectively. The compound was more slowly absorbed in rats compared to monkeys with T_{max} values of 8.0 hr for rats versus 4.3 hr for monkeys. The half-life was also longer in rats compared to monkeys with $t_{1/2}$ values of 17 hr in rats versus 7 hr in monkeys. The C_{max} was 198 and 258 ng/mL and the AUC_{inf} was 5496 and and 2960 hr*ng/mL in rats and monkeys, respectively.

In summary, compound **21a** is a highly optimized drug candidate with desirable biochemical potency and cellular inhibitory activity across multiple tumor types, as well as sustained *in vivo* target inhibition and good oral bioavailability. Consistent with targeting a distinct allosteric site, as revealed by its co-crystal structure with AKT1, **21a** demonstrated a very high selectivity when profiled against a large panel of kinases.[6]

Experimental Section

Chemistry: All reagents and solvents were purchased from commercially available sources and used without further purification. All reactions were carried out according to the indicated procedures and conditions. Reactions were monitored by LC/MS analysis and/or thin-layer chromatography (TLC) on silica-coated glass plates (EMD silica gel 60 F_{254}) with the indicated eluent. The compounds were visualized using UV light (254 nm). LC/MS analysis was performed on a Shimadzu HPLC/UV (214 nm and/or 254 nm wavelength) system coupled to ELSD (Sedex 75, Sedere) and MS (ZQ, Micromass) detectors. Compounds were dissolved in 100% DMSO and separated on a Zorbax SB-C8 rapid resolution cartridge (30 × 4.6 mm, 3.5 µm, 3 mL/min flow rate) using acetonitrile/water as the mobile phase with 0.1% TFA as a modifier. The gradient started at 5% acetonitrile and increased linearly to 95% acetonitrile over 1.9 min, with a 0.3 min hold at 95%, and the cartridge was re-equilibrated to the original conditions in a total of 2.5 min. The purity of the compounds selected for the *in vivo* studies was determined using an Agilent HP1100 system with a YMC C18 column (4.6 mm x 100 mm, 5 µm, 1 mL/min flow rate) and acetonitrile/water as the mobile phase with 0.1% TFA as a modifier. The gradient started at 2% acetonitrile and increased linearly to 30% acetonitrile over 7 min, followed by a linear increase to 80% acetonitrile by 12 min and a hold at 80% for 16 min. Purity was determined at 254 nm and was found to be > 95%. Library compounds were purified by reverse

phase chromatography on a preparative LC/UV/MS system (High Throughput Purification) using a mass-triggered fractionation method. Compounds were eluted from the HPLC column (MACCEL 120-10-C18 SH, 10 μm, 20 mm ID x 50 mm) at 88 mL/min with an acetonitrile/water gradient using 0.1% TFA as a modifier. Nuclear magnetic resonance (^{1}H NMR**)** spectra were recorded on a Varian Mercury Plus NMR spectrometer operating at 400.13 MHz for ^{1}H using a 5 mm ASW PFG probe capable of detecting ^{1}H, ^{13}C, ^{31}P, and ^{15}N nuclei. The proton chemical shifts (ppm) were in reference to the tetramethylsilane standard (0 ppm). The NMR data are reported with these descriptions: *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet; *m*, multiplet; and *br*, broad peak.

3-Nitro-*N*-(*p*-tolyl)pyridin-2-amine (2a). 2-Chloro-3-nitropyridine **1** (100 mg, 0.63 mmol) was dissolved in dioxane (4 mL) in a round-bottom flask. *p*-Tolylaniline (68 mg, 0.63 mmol) was then added, followed by DIEA (0.22 mL) addition. The reaction mixture was heated to 80°C for 24 h, and the reaction was stopped when complete as observed by LCMS. After cooling to room temperature, the solvent was removed under reduced pressure to afford an oily residue. The residue was dissolved in ethyl acetate (20 mL) and washed with water and brine (5 mL each). The organic phase was separated and dried over Na_2SO_4. After filtration, the solvent was removed under reduced pressure. The crude product was carried on to the next step without further purification. MS *m/z* 230 $[M+H]^+$.

***N*-(4-(*tert*-Butyl)phenyl)-3-nitropyridin-2-amine (2b). 2b** was prepared from **1** as described for **2a** using 4-(*tert*-butyl)aniline. The crude product was purified by column chromatography (1% MeOH/DCM, silica gel) to give **2b** (1.55 g, 89 %) as a red solid. Mp 156-158 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.92 (s, 1H), 8.51 (dd, J =14.5, 5.6 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 7.38 (d, J = 8.5 Hz, 1H), 6.96 (dd, J = 8.1, 4.6 Hz, 1H), 1.29 (s, 9H); MS *m/z* 272 $[M+H]^+$.

***tert*-Butyl (4-((3-nitropyridin-2-yl)amino)benzyl)carbamate (2c). 2c** was prepared from **1** as described for **2a** using *tert*-butyl (4-aminobenzyl)carbamate. The crude product was purified by column chromatography (1% MeOH/DCM, silica gel). MS *m/z* 345 $[M+H]^+$.

***tert*-Butyl (4-((4-methyl-3-nitropyridin-2-yl)amino)benzyl)carbamate (3a). 3a** was prepared from 2-chloro-4-methyl-3-nitropyridine (**1b)** as described for **2a** using *tert*-butyl (4-aminobenzyl)carbamate. The crude product was used directly in the next step without purification. MS *m/z* 359 $[M+H]^+$.

***tert*-Butyl (4-((5-methyl-3-nitropyridin-2-yl)amino)benzyl)carbamate (3b). 3b** was prepared from 2-chloro-5-methyl-3-nitropyridine (**1c)** as described for **2a** using *tert*-butyl (4-aminobenzyl)carbamate. The crude product was used directly in the next step without purification. MS *m/z* 359 $[M+H]^+$.

***tert*-Butyl (4-((5,6-dimethyl-3-nitropyridin-2-yl)amino)benzyl)carbamate (3c). 3c** was prepared from 2-chloro-5,6-dimethyl-3-nitropyridine (**1d)** as described for **2a** using *tert*-butyl (4-aminobenzyl)carbamate. The crude product was used directly in the next step without purification. MS *m/z* 373 $[M+H]^+$.

***tert*-Butyl (4-((6-chloro-3-nitropyridin-2-yl)amino)benzyl)carbamate (3d). 3d** was prepared from 2,6-dichloro-3-nitropyridine (**1e)** as described for **2a** using *tert*-butyl (4-aminobenzyl) carbamate. The crude product was used directly in the next step without purification. MS *m/z* 378 $[M+H]^+$.

***tert*-Butyl (4-((5-bromo-3-nitropyridin-2-yl)amino)benzyl)carbamate (3e). 3e** was prepared from 5-bromo-2-chloro-3-nitropyridine (**1f)** as described for **2a** using *tert*-butyl (4-aminobenzyl) carbamate. The crude product was used directly in the next step without purification. Mp 178-180 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.95 (s, 1H), 8.65 (m, 1H), 8.54 (s, 1H), 7.51 (d, J =

6.4 Hz, 2H), 7.23 (d, *J* = 6.4 Hz, 2H), 4.21-4.12 (m, 2H), 1.41 (s, 9H); MS *m/z* 367, 369 [M(-*t*-Bu)+H, M(-*t*-Bu)+2+H]$^+$.

3-(3-(*p*-Tolyl)-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine (4a). Crude 3-nitro-N-(*p*-tolyl)pyridin-2-amine **2a** was dissolved in DMSO (4 mL) and MeOH (2 mL) in a round bottom flask. 2-aminonicotinaldehyde 4 (77 mg, 1.1 equivalent) and $Na_2S_2O_4$ (274 mg) were added to the flask. The reaction mixture was heated to 100°C for 18 h. After cooling to room temperature, the reaction mixture was diluted with dichloromethane (50 mL) and washed with water and brine. The organic phase was separated and dried over Na_2SO_4. After filtration, the solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography (EtOAc/hexane) to afford 20 mg of 3-(3-(*p*-tolyl)-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine (**4a**) as an off-white solid. Mp 186-188 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.29-8.28 (dd, *J* = 5.0, 1.6 Hz, 1H), 8.18-8.15 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.96-7.95 (dd, *J* = 4.7, 1.6 Hz, 1H), 7.37-7.33 (dd, *J* = 8.21, 5.09 Hz, 1H), 7.31-7.26 (m, 4H), 7.20-7.17 (dd, *J* = 7.8, 1.9 Hz, 1H), 6.96 (s, 2H), 6.39-6.36 (dd, *J* = 7.8, 4.6 Hz, 1H), 2.36 (s, 3H); MS *m/z* 302 [M+H]$^+$.

3-(3-(4-(*tert*-Butyl)phenyl)-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine (4b). 4b was prepared as described for **4a** using **2b** and 2-aminonicotinaldehyde. Mp 175-180 ºC; ^{1}H NMR (400M Hz, Acetone-d_6) δ 8.44–8.42 (dd, *J* = 4.7, 1.2 Hz, 1H), 8.26-8.23 (dd, *J* = 8.2,1.6 Hz, 1H), 8.05-8.02 (dd, *J* = 6.3, 1.6 Hz, 1H), 7.75-7.73 (dd, *J* = 7.8, 1.6 Hz, 1H), 7.70-7.68 (dd, *J* = 6.6, 2.3 Hz, 2H), 7.57-7.55 (dd, *J* = 8.6, 1.9 Hz, 2H), 7.48-7.44 (dd, J = 8.2, 4.7 Hz, 1H), 6.82-6.78 (dd, *J* = 7.8, 6.3 Hz, 1H), 1.41 (s, 9H). MS *m/z* 344 [M+H]$^+$; Anal. ($C_{21}H_{21}N_5$·1.5 TFA·0.1 acetone) calcd: C, 56.10; H, 4.48; N 13.46. Found: C, 56.32; H, 4.25; N, 13.70.

***tert*-Butyl (4-(2-(2-aminopyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-3-yl)benzyl)carbamate (4c). 4c** was prepared as described for **4a** using **2c** and 2-aminonicotinaldehyde. Mp 172-173 ºC;

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (dd, J = 4.8, 1.5 Hz, 1H), 8.20 (dd, J = 8.0, 1.5 Hz, 1H), 7.99 (dd, J = 4.8, 1.8 Hz, 1H), 7.49 (t, J = 6.3 Hz, 1H), 7.43 – 7.32 (m, 5H), 7.20 (dd, J = 7.7, 1.9 Hz, 1H), 6.99 (s, 2H), 6.39 (dd, J = 7.7, 4.8 Hz, 1H), 4.21 (d, J = 6.2 Hz, 2H), 1.41 (s, 9H); MS *m/z* 417 $[M+H]^+$.

***tert*-Butyl (4-(2-(2-aminopyridin-3-yl)-7-methyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)benzyl) carbamate (5a). 5a** was prepared from **3a** as described for **4a**. Mp 160-162 ºC; ^{1}H NMR (400 MHz, CD_3OD) δ: 8.17 (d, J = 5.1 Hz, 1H), 7.96 (dt, J = 5.1, 1.4 Hz, 1H), 7.45 (d, J = 7.8 Hz, 2H), 7.37-7.31 (m, 3H), 7.24 (d, J = 5.1 Hz, 1H), 6.49-6.45 (m, 1H), 4.31 (br.s, 2H), 2.74 (s, 3H), 1.46 (s, 9H); MS *m/z* 431 $[M+H]^+$.

***tert*-Butyl (4-(2-(2-aminopyridin-3-yl)-6-methyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)benzyl) carbamate (5b). 5b** was prepared from **3b** as described for **4a**. Mp 211-212 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.16 (dd, J = 2.0, 0.8 Hz, 1H), 8.04–7.95 (m, 2H), 7.49 (t, J = 6.2 Hz, 1H), 7.36 (s, 4H), 7.17 (dd, J = 7.7, 1.9 Hz, 1H), 7.02 (s, 2H), 6.38 (dd, J = 7.7, 4.8 Hz, 1H), 4.21 (d, J = 6.2 Hz, 2H), 2.45 (d, J = 0.8 Hz, 3H), 1.41 (s, 9H), 1.34; MS *m/z* 431 $[M+H]^+$.

***tert*-Butyl (4-(2-(2-aminopyridin-3-yl)-5,6-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)benzyl) carbamate (5c). 5c** was prepared from **3c** as described for **4a**. The crude product was used without purification. MS *m/z* 445 $[M+H]^+$.

***tert*-Butyl (4-(2-(2-aminopyridin-3-yl)-5-chloro-3*H*-imidazo[4,5-*b*]pyridin-3-yl)benzyl) carbamate (5d). 5d** was prepared from **3d** as described for **4a**. Mp 235-237 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.26 (d, J = 8.6 Hz, 1H), 7.99 (dd, J = 4.7, 2.0, 1H), 7.49 (t, J = 6.1 Hz, 1H), 7.45 (d, J = 8.2 Hz, 1H), 7.42-7.35 (m, 4H), 7.21 (dd, J = 7.8, 2.0 Hz, 1H), 6.92 (s, 2H), 6.41 (dd, J = 7.4, 4.7 Hz, 1H), 4.22 (d, J = 6.1 Hz, 2H), 1.41 (s, 9H); MS *m/z* 451 $[M+H]^+$.

***tert*-Butyl 4-(2-(2-aminopyridin-3-yl)-6-bromo-3*H*-imidazo[4,5-*b*]pyridine-3-yl)benzyl carbamate (5e)**. **5e** was prepared from **3e** as described for **4a**. The product was purified by column chromatography (1-5% methanol in dichloromethane) followed by re-crystallization in 1:1 ethyl acetate/hexanes. Mp 176-178 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (d, J = 2.0 Hz, 1H), 8.40 (d, J = 2.4 Hz, 1H), 7.99 (dd, J = 4.7, 2.0 Hz, 1H), 7.48 (t, J = 5.6 Hz, 1H), 7.40-7.31 (m, 3H), 7.23 (dd, J = 7.8, 2.0 Hz, 1H), 6.97 (s, 2H), 6.40 (dd, J = 7.7, 4.4 Hz, 1H), 4.121 (d, J = 6.2 Hz, 2H), 1.41 (s, 9H). MS *m/z* 495, 497 $[M+H, M+2+H]^+$.

3-(3-(4-(Aminomethyl)phenyl)-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine (6). HCl (4 M in dioxane, 7.2 mL, 28.8 mmol) was added to a solution of **4c** (0.7 g, 1.7 mmol) in methanol (5 mL), and the mixture was stirred at room temperature for 4 h. The solution was concentrated under reduced pressure to yield **6** (0.82 g, quantitative yield) as a hydrochloric acid salt. Mp 255-257 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (m, 3H), 8.30 (dd, J = 8.0, 1.4 Hz, 1H), 8.12 (dd, J = 6.2, 1.6 Hz, 1H), 7.87 (d, J = 6.6 Hz, 1H), 7.65 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.4 Hz, 2H), 7.48 (dd, J = 8.0, 4.7 Hz, 1H), 6.85 (dd, J = 7.1, 6.6 Hz, 1H), 4.12 (d, J = 5.6 Hz, 2H), 3.81 (s, 2H). MS *m/z* 317 $[M+H]^+$; Anal. ($C_{18}H_{16}N_6 \cdot 4.6$ HCl$\cdot 4.2$ $H_2O \cdot 0.8$ Et_2O) calcd: C, 41.13; H, 6.02; N, 13.58. Found: C, 41.12; H, 6.02; N, 13.58.

3-(3-(4-(Aminomethyl)phenyl)-7-methyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (7a). 7a was prepared from **5a** as described for **6**. Mp 210-212 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.52 (s, 3H), 8.45 (s, 2H), 8.27 (d, J = 4.7 Hz, 1H), 8.14 (dd, J = 6.3, 1.6 Hz, 1H), 7.91 (dd, J = 7.4, 1.6 Hz, 1H), 7.67–7.64 (m, 2H), 7.57–7.53 (m, 2H), 7.31 (d, J = 5.4 Hz, 1H), 6.88 (dd, J = 7.4, 6.2 Hz, 1H), 4.10 (m, 2H), 2.70 (s, 3H); MS *m/z* 331 $[M+H]^+$.

3-(3-(4-(Aminomethyl)phenyl)-6-methyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (7b). 7b was prepared from **5b** as described for **6**. Mp 220-230 ºC; ^{1}H NMR (400

MHz, DMSO-d_6) δ: 8.65 (s, 3H), 8.53 (s, 2H), 8.29 (dd, *J* = 2.0, 0.7 Hz, 1H), 8.16 (dd, *J* = 6.2, 1.7 Hz, 1H), 8.12 (dd, *J* = 2.0, 0.9 Hz, 1H), 7.91 (dd, *J* = 7.5, 1.6 Hz, 1H), 7.75–7.64 (m, 2H), 7.62–7.48 (m, 2H), 6.90 (dd, *J* = 7.5, 6.2 Hz, 1H), 4.10 (q, *J* = 5.8 Hz, 2H), 2.49 (s, 3H); MS *m/z* 331 $[M+H]^+$; Anal. ($C_{19}H_{18}N_6 \cdot 3.43$ HCl·0.43 EtOAc) calcd: C, 50.45; H, 5.08; N, 17.04. Found: C, 50.42; H, 5.21; N, 17.07.

3-(3-(4-(Aminomethyl)phenyl)-5,6-dimethyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (7c). 7c was prepared from **5c** as described for **6**. Mp 103-112 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.91 (s, 2H), 7.45-7.40 (m, 2H), 7.32-7.26 (m, 2H), 7.11 (d, *J* = 7.2 Hz, 1H), 6.99 (m, 2H), 6.38 (dd, *J* = 7.2, 6.3 Hz, 1H), 3.80 (s, 2H), 3.40-3.15 (br.s, 3H), 2.41 (s, 3H), 2.35 (s, 3H); MS *m/z* 345 $[M+H]^+$.

3-(3-(4-(Aminomethyl)phenyl)-5-chloro-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (7d). 7d was prepared from **5d** as described for **6**. Mp 208-211 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.55 (s, 3H), 8.37 (dd, *J* = 8.4, 1.0 Hz, 1H), 8.18 – 8.12 (m, 1H), 7.97 – 7.91 (m, 1H), 7.69 (d, *J* = 8.0 Hz, 2H), 7.61 – 7.51 (m, 3H), 6.90 (t, *J* = 6.9 Hz, 1H), 4.11 (d, *J* = 5.9 Hz, 2H), 3.57 (s, 2H); MS *m/z* 351 $[M+H]^+$; Anal. ($C_{18}H_{15}N_6Cl \cdot 2.58$ HCl·0.16 EtOAc) calcd: C, 48.78; H, 4.14; N, 18.31. Found: C, 48.80; H, 4.32; N, 18.31.

3-(3-(4-(Aminomethyl)phenyl)-6-bromo-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (7e). 7e was prepared from **5e** as described for **6**. Mp. 221-223ºC; ^{1}H NMR (DMSO-d_6) 400 M Hz δ: 8.60 (d, *J* = 2.0 Hz, 1H), 8.56 (bs, 2H), 8.51 (d, *J* = 2.4 Hz, 1H), 8.38 (bs, 2H), 8.13 (dd, *J* = 2.0 Hz and 4.7 Hz, 1H), 7.95 (dd, *J* = 1.6 Hz and 7.4 Hz, 1H), 7.63 (d, *J* = 8.6 Hz, 2H), 7.53 (d, *J* = 8.6 Hz, 2H), 6.89 (dd, *J* = 6.2 Hz and 7.4 Hz, 1H), 4.17 (app q, *J* = 5.5 Hz, 1H); MS *m/z* 394, 396 $[M+H, M+2+H]^+$.

***tert*-Butyl (4-(2-(2-aminopyridin-3-yl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)benzyl) carbamate (8a).** *tert*-Butyl (4-(2-(2-aminopyridin-3-yl)-5-chloro-3*H*-imidazo[4,5-*b*]pyridin-3-yl)benzyl)carbamate (**5d**) (5.1 g, 11.33 mmol) and phenyl boronic acid (2.76 g, 22.6 mmol) in suspension (200 mL toluene/200 mL EtOH) were treated with saturated aqueous $NaHCO_3$ (33 mL), and the mixture was degassed for 5 min. The catalyst $Pd(PPh_3)_4$ (0.51 g, 0.44 mmol) was added, and the reaction mixture was stirred overnight at 100 °C. Upon cooling to room temperature, the mixture was extracted with DCM (3 x 100 mL). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and then with water. The solution was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (hexanes/ethyl acetate, 5:95). The *tert*-butyl (4-(2-(2-aminopyridin-3-yl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)benzyl)carbamate (**8a**) was obtained as dark red crystals (4.7 g, 84%). Mp 209-212 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.25 (dd, J = 8.4, 2.3 Hz, 1H), 8.04 – 7.94 (m, 4H), 7.50 – 7.33 (m, 8H), 7.20 (dd, J = 7.8, 1.9 Hz, 1H), 7.00 (s, 2H), 6.40 (dd, J = 7.6, 4.9 Hz, 1H), 4.22 (d, J = 6.2 Hz, 2H), 1.39 (s, 9H); MS *m/z* 493 $[M+H]^+$.

***tert*-Butyl (4-(5-(3-acetamidophenyl)-2-(2-aminopyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-3-yl)benzyl)carbamate (8b). 8b** was prepared from **5d** as described for **8a** using (3-acetamidophenyl)boronic acid. The crude product was purified by column chromatography (95:5 ethyl acetate/methanol). MS *m/z* 550 $[M+H]^+$.

***tert*-Butyl (4-(5-(4-acetamidophenyl)-2-(2-aminopyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-3-yl)benzyl)carbamate (8c). 8c** was prepared from **5d** as described for **8a**, using (4-acetamidophenyl)boronic acid. The crude product was purified by column chromatography (5:95 hexanes/ethyl acetate). MS *m/z* 550 $[M+H]^+$.

***tert*-Butyl (4-(2-(2-aminopyridin-3-yl)-5-(1*H*-pyrazol-4-yl)-3*H*-imidazo[4,5-*b*]pyridin-3-yl) benzyl)carbamate (8d). 8d** was prepared from **5d** as described for **8a** using (1*H*-pyrazol-4-yl)boronic acid. Mp. 243-244 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12,99 (s, 1H), 8.19 (s, 1H), 8.12 (d, J = 8.8 Hz, 1H), 7.95 (d, J = 5.2 Hz, 1H), 7.92 (s, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.47 (t, J = 5.6 Hz, 1H), 7.40-7.33 (m, 4H), 7.13 (d, J = 7.2 Hz, 1H), 6.94 (s, 2H), 6.36 (t, J = 6.0 Hz, 1H), 4.20 (d, J = 6.4Hz, 2H), 1.39 (s, 9H); MS *m/z* 483 $[M+H]^+$.

***tert*- Butyl (4-(2-(2-aminopyridin-3-yl)-6-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-3-yl) benzyl)carbamate (8e). 8e** was prepared from **5e** as described for **8a** using (pyridin-3-yl)boronic acid. The crude residue was purified by silica gel chromatography (2-20% methanol in ethyl acetate), yielding 3.9 g of **8e** (7.9 mmol, 99% yield). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.98 (d, J = 1.9 Hz, 1H), 8.64 (d, J = 2.0 Hz, 1H), 8.59 (dd, J = 1.6 Hz and 4.7 Hz, 1H), 8.54 (d, J = 2.0 Hz, 1H), 8.20 (m, 1H), 7.98 (m, 1H), 7.52 (m, 2H), 7.48-7.34 (m, 4H), 7.23 (m, 1H), 7.01 (s, br, 2H), 6.38 (m, 1H), 4.19 (m, 2H), 1.38 (s, 9H); MS *m/z* 438, 494, $[M-(t\text{-Bu})+H, M+H]^+$.

3-(3-(4-(Aminomethyl)phenyl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (9a). 9a was prepared from **8a** as described for **6**. Mp 215-218 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.52 (s, 3H), 8.35 (d, J = 8.2 Hz, 1H), 8.16 – 8.11 (m, 1H), 8.05 (t, J = 8.5 Hz, 3H), 7.90 (d, J = 7.0 Hz, 1H), 7.68 (d, J = 7.8 Hz, 2H), 7.65 – 7.59 (m, 2H), 7.50 – 7.37 (m, 3H), 6.88 (q, J = 5.7, 4.9 Hz, 1H), 4.68 (s, 2H), 4.11 (d, J = 5.9 Hz, 2H); MS *m/z* 393 $[M+H]^+$; Anal. ($C_{24}H_{20}N_6 \cdot 3.14$ HCl $\cdot 0.45$ EtOAc) calcd: C, 56.69; H, 4.93; N, 15.38. Found: C, 56.68; H, 5.18; N, 15.37.

N-(3-(3-(4-(Aminomethyl)phenyl)-2-(2-aminopyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)phenyl)acetamide hydrochloride (9b). 9b was prepared from **8b** as described for **6**. Mp 245-248 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.16 (s, 1H), 8.47 (s, 3H), 8.39 (s, 2H), 8.35 (d, J =

8.1 Hz, 1H), 8.18 (d, *J* = 2.6 Hz, 1H), 8.15 – 8.08 (m, 1H), 7.89 (d, *J* = 8.1 Hz, 2H), 7.63 (dt, *J* = 11.8, 7.5 Hz, 5H), 7.36 (t, *J* = 7.9 Hz, 1H), 6.87 (t, *J* = 6.6 Hz, 1H), 4.10 (d, *J* = 5.9 Hz, 2H), 2.04 (s, 3H); MS *m/z* 450 $[M+H]^+$; Anal. ($C_{26}H_{23}N_7O \cdot 3.35\ HCl$) calcd: C, 54.63; H, 4.65; N, 17.15. Found: C, 54.62; H, 4.67; N, 16.81.

N-(4-(3-(4-(Aminomethyl)phenyl)-2-(2-aminopyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)phenyl)acetamide hydrochloride (9c). 9c was prepared from **8c** as described for **6**. Mp 227-230 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.25 (s, 1H), 8.58 (s, 3H), 8.33 (d, *J* = 8.4 Hz, 1H), 8.16 (dd, *J* = 6.4, 1.7 Hz, 1H), 8.01 (t, *J* = 7.9 Hz, 3H), 7.90 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.75 – 7.59 (m, 6H), 6.90 (t, *J* = 6.9 Hz, 1H), 5.80 (s, 2H), 4.13 (d, *J* = 5.8 Hz, 2H), 2.07 (s, 3H); MS *m/z* 450 $[M+H]^+$; Anal. ($C_{26}H_{23}N_7O \cdot 3.35\ HCl \cdot 0.28\ EtOAc \cdot 0.3\ H_2O$) calcd: C, 54.33; H, 4.90; N, 16.35. Found: C, 54.35; H, 4.96; N, 16.11.

3-(3-(4-(Aminomethyl)phenyl)-5-(1*H*-pyrazol-4-yl)-3H-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (9d). 9d was prepared from **8d** as described for **6**. Mp. 264-269 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.50 (br, 4H), 8.43 (br, 1H), 8.22 (d, *J* = 8.4 Hz, 1H), 8.13-8.09 (m, 3H), 7.85 (d, *J* = 7.6 Hz, 1H), 7.77 (d, *J* = 8.8 Hz, 1H), 7.66 (d, *J* = 8.0 Hz, 2H), 7.58 (d, *J* = 8.8 Hz, 2H), 6.86 (t, *J* = 7.2 Hz, 1H), 4.10 (q, *J* = 5.2 Hz, 2H); MS *m/z* 383 $[M+H]^+$.

3-(3-(4-(Aminomethyl)phenyl)-6-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (9e). 9e was prepared from **8e** as described for **6**. ^{1}H NMR (400 MHz, (CD_3OD) δ: 9.38 (d, *J* = 2.0 Hz, 1H), 9.11 (d, *J* = 2.0 Hz, 1H), 8.95-8.93 (m, 1H), 8.89 (d, *J* = 2.0 Hz, 1H), 8.78 (m, 1H), 8.83-8.25 (m, 1H), 8.06-8.04 (m, 1H), 7.88-7.86 (m, 1H), 7.77-7.66 (m, 4H), 6.86-6.83 (m, 1H), 4.23 (m, 2H), MS *m/z* 394 $[M+H]^+$.

2-Methyl-N-(propan-2-ylidene)propane-2-sulfinamide (11). $Ti(OEt)_4$ (5.36 mL) was added dropwise over the course of 15 min to a solution of 2-methyl-2-propanesulfinamide (**10**) (1.47 g,

12.1 mmol) and acetone (0.98 mL) in THF (24 mL) chilled to 0 ºC. After being stirred at room temperature for 3 days, the reaction mixture was quenched by the rapid addition of an ice-cooled saturated sodium bicarbonate solution. The suspension was filtered through glass-fiber filter paper and was washed with ethyl acetate. The filtrate was washed with sat. aq. $NaHCO_3$ solution and brine. The organics were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (10-60% ethyl acetate in hexanes) yielded 2-methyl-N-(propan-2-ylidene)propane-2-sulfinamide (**11**) (0.92 g, 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.34 (s, 3H), 2.18 (s, 3H), 1.23 (s, 9H).

N-(2-(4-Aminophenyl)propan-2-yl)-2-methylpropane-2-sulfinamide (12). A solution of n-butyllithium in hexanes (1.65 M, 5.2 mL) was added dropwise to a solution of 4-bromo-N,N-bis(trimethylsilyl)aniline (2.55 mL) in THF (14 mL) at -78 ºC. The resulting mixture was stirred for 1.5 h at -78 ºC, after which a solution of N-isopropylidene-2-methylpropane-2-sulfinamide (**11**) in THF (2.8 mL) was added dropwise at -78 ºC. The reaction mixture was stirred for an additional 1 h at -78 ºC and then allowed to warm to room temperature. After being stirred for 18 h at room temperature, the reaction mixture was poured into an ice-cooled sat. aq. $NaHCO_3$ solution. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with a sat. aq. $NaHCO_3$ solution and brine and then dried over Na_2SO_4. The dried solution was filtered, and the filtrate was concentrated to dryness. The residue was diluted with diisopropylether and extracted with a 1 M citric acid solution. The aqueous extracts were combined and alkalinized with solid sodium bicarbonate to pH>7. The basic aqueous layer was extracted with ethyl acetate, and the organic layer was washed with brine. The organics were dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (50-100% ethyl acetate in hexanes) produced N-(2-(4-aminophenyl)propan-2-

yl)-2-methylpropane-2-sulfinamide (**12**) (173 mg, 12%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.26 (d, *J* = 8.7 Hz, 2H), 6.65 (d, *J* = 8.7 Hz, 2H), 3.66 (brs, 1H), 3.31 (s, 1H), 1.67 (s, 3H), 1.64 (s, 3H), 1.20 (s, 9H).

N-(2-(4-((6-Chloro-3-nitropyridin-2-yl)amino)phenyl)propan-2-yl)-2-methylpropane-2-sulfinamide (13). 13 was prepared from 2,6-dichloro-3-nitropyridine (**1e)** as described for **2a** using N-(2-(4-aminophenyl)propan-2-yl)-2-methylpropane-2-sulfinamide (**12**). The crude product was purified by column chromatography (30-100% ethyl acetate in hexanes). MS *m/z* 411 $[M+H]^+$.

***tert*-Butyl (2-(4-((6-chloro-3-nitropyridin-2-yl)amino)phenyl)propan-2-yl)carbamate (14).** A solution of HCl in dioxane (4 M, 130 μL) was added dropwise to a solution of N-(1-(4-((6-chloro-3-nitropyridin-2-yl)amino)phenyl)-1-methylethyl)-2-methylpropane-2-sulfinamide (**13**) (107 mg, 0.26 mmol) in DCM (5 mL) at room temperature. The reaction mixture was stirred for 30 min. Hexane was added to the suspension, and the solid was filtered to yield an N-(4-(1-amino-1-methylethyl)phenyl)-6-chloro-3-nitropyridin-2-amine hydrochloride intermediate. Sat. aq. $NaHCO_3$ solution (6 mL) was added to a suspension of N-(4-(1-amino-1-methylethyl)phenyl)-6-chloro-3-nitropyridin-2-amine hydrochloride in THF (3 mL) chilled to 0 ºC, followed by dropwise addition of a solution of di-*tert*-butyl dicarbonate (167 mg) in THF (3 mL). After being stirred at room temperature overnight, the reaction mixture was extracted with ethyl acetate. The organic layer was washed with sat. aq. $NaHCO_3$ solution and brine. The organics were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (3-30% ethyl acetate in hexanes) produced *tert*-butyl (2-(4-((6-chloro-3-nitropyridin-2-yl)amino)phenyl) propan-2-yl)carbamate (**14**) (99 mg, 2 steps 93%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.25 (br.s, 1H), 8.46 (d, *J* = 8.7Hz, 1H), 7.61 (d, *J* = 8.7 Hz, 2H), 7.43 (d,

J = 8.7 Hz, 2H), 6.79 (d, *J* = 8.7 Hz, 1H), 4.94 (brs, 1H), 1.64 (s, 6H), 1.50-1.00 (m, 9H); MS *m/z* 351 [M-(t-Bu)+H].

***tert*-Butyl (2-(4-(2-(2-aminopyridin-3-yl)-5-chloro-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl) propan-2-yl)carbamate (15). 15** was prepared as described for **4a** using **14** and 2-aminonicotinaldehyde. Purification by column chromatography (10-100% ethyl acetate in hexanes) produced *tert*-butyl (2-(4-(2-(2-aminopyridin-3-yl)-5-chloro-3*H*-imidazo[4,5-b]pyridin-3-yl)phenyl)propan-2-yl)carbamate (**15**) (30 mg, 26%). MS *m/z* 479 $[M+H]^+$.

***tert*-Butyl (2-(4-(2-(2-aminopyridin-3-yl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl) propan-2-yl)carbamate (16). 16** was prepared as described for **8a** using **15** and phenyl boronic acid. Purification by column chromatography (30-100% ethyl acetate in hexanes) yielded *tert*-butyl (2-(4-(2-(2-aminopyridin-3-yl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl) propan-2-yl)carbamate (**16**) (27 mg, 81%). MS *m/z* 521 $[M+H]^+$.

3-(3-(4-(2-Aminopropan-2-yl)phenyl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine (17). 17 was prepared from **16** as described for **6**. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.90-8.70 (m, 3H), 8.37 (d, *J* = 8.3 Hz, 2H), 8.15 (dd, *J* = 6.0 Hz and 1.8 Hz, 1H,), 8.08-8.03 (m, 3H), 7.86 (dd, *J* = 7.3 Hz and 1.4 Hz, 1H), 7.79 (d, *J* = 8.7 Hz, 2H), 7.67 (d, *J* = 8.7 Hz, 2H), 7.52-7.47 (m, 2H), 7.46-7.41 (m, 1H), 6.89 (dd, *J* = 7.3 Hz and 6.4 Hz, 1H), 1.70 (s, 6H); MS *m/z* 421 $[M+H]^+$.

***tert*-Butyl (1-(4-((6-chloro-3-nitropyridin-2-yl)amino)phenyl)cyclobutyl)carbamate (18).** A solution of 2,6-dichloro-3-nitropyridine (**1e**) (5.11 g, 26.4 mmol) in DMA (50 mL) and triethylamine (5 mL) cooled to 0 ºC was added dropwise to a solution of *tert*-butyl (1-(4-amino phenyl)cyclobutyl)carbamate (6.3 g, 24.0 mmol)) in DMA (25 mL). The reaction mixture was stirred at 0 ºC for one hour and then at room temperature overnight. The reaction mixture was

then diluted with water (250 mL) and extracted with ethyl acetate (2 x 200 mL). The organics were combined and washed with a sat. aq. $NaHCO_3$ solution, water and brine. The organics were dried over Na_2SO_4 and concentrated *in vacuo*. Purification by column chromatography (15% ethyl acetate in hexanes) produced *tert*-butyl (1-(4-((6-chloro-3-nitropyridin-2-yl)amino)phenyl)cyclobutyl)carbamate (**18**) as an orange solid (5.05 g, 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.05 (s, 1H), 8.52 (d, J = 8.8Hz, 1H), 7.56 – 7.52 (m, 2H), 7.42 – 7.37 (m, 3H), 6.98 (d, J = 8.8 Hz, 1H), 2.47 – 2.34 (m, 4H), 2.04 – 1.96 (m, 1H), 1.84 – 1.74 (m, 1H), 1.30 (bs, 9H); MS *m/z* 419 $[M+H]^+$.

***tert*-Butyl (1-(4-(2-(2-aminopyridin-3-yl)-5-chloro-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl) cyclobutyl)carbamate (19). 19** was prepared as described for **6** using **18** and 2-aminonicotinaldehyde. ^{1}H NMR (400M Hz, DMSO-d_6) δ 8.26 (d, J = 8.0 Hz, 1H), 8.00 – 7.96 (m, 1H), 7.69 (bs, 1H), 7.54 – 7.35 (m, 5H), 7.24 – 7.08 (m, 1H), 7.04 – 6.96 (m, 2H), 6.32 – 6.28 (m, 1H), 2.48 – 2.35 (m, 4H), 2.06 – 1.96 (m, 1H), 1.86 – 1.76 (m, 1H), 1.40 – 1.06 (m, 9H); MS *m/z* 491 $[M+H]^+$.

***tert*-Butyl (1-(4-(2-(2-aminopyridin-3-yl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)cyclobutyl)carbamate (20a). 20a** was prepared from **19** as described for **8a** using phenylboronic acid. The crude product was purified by column chromatography (10-100% ethyl acetate in hexanes), yielding **20a** with some impurities. The solid was re-crystallized from ethyl acetate, affording an off-white solid (7.2 g). Mp 111-116 °C; ^{1}H NMR (400M Hz, DMSO-d_6) δ: 8.23 (d, J = 8.0 Hz, 1H), 8.04 – 7.98 (m, 3H), 7.94 (d, J = 8.0 Hz, 1H), 7.55 (d, J = 8.8 Hz, 2H), 7.46 – 7.35 (m, 6H), 7.18 – 7.14 (m, 1H), 6.90 (bs, 1H), 6.33 (dd, J = 7.6Hz and 4.4 Hz, 1H), 2.48 – 2.40 (m, 4H), 2.09 – 2.00 (m, 1H), 1.89 – 1.79 (m, 1H), 1.30 (m, 9H); MS *m/z* 533 $[M+H]^+$.

***tert*-Butyl (1-(4-(5-(3-acetamidophenyl)-2-(2-aminopyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)cyclobutyl)carbamate (20b). 20b** was prepared from **19** as described for **8a** using (3-acetamidophenyl)boronic acid. Purification by column chromatography (0-10% methanol in dichloromethane) yielded **20b** as a brown solid with some co-eluting impurities. The fractions with the product were combined and concentrated to produce a dark brown solid. The solid was triturated with ethyl acetate to afford a tan solid (2.6 g, 70%). Mp 160-163 °C; ^{1}H NMR (400M Hz, DMSO-d_6) δ: 8.23 (d, J = 8.0 Hz, 1H), 8.04 – 7.98 (m, 3H), 7.94 (d, J = 8.0 Hz, 1H), 7.55 (d, J = 8.8 Hz, 2H), 7.46 – 7.35 (m, 6H), 7.18 – 7.14 (m, 1H), 6.90 (bs, 1H), 6.33 (dd, J = 7.6Hz and 4.4 Hz, 1H), 2.48 – 2.40 (m, 4H), 2.09 – 2.00 (m, 1H), 1.89 – 1.79 (m, 1H), 1.30 (m, 9H); MS *m/z* 590 $[M+H]^+$; Anal. ($C_{34}H_{35}N_7O_3 \cdot 0.18$ EtOAc) calcd: C, 68.87; H, 6.07; N, 16.19. Found: C, 69.12; H, 6.34; N, 15.94.

***tert*-Butyl (1-(4-(2-(2-aminopyridin-3-yl)-5-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-3-yl) phenyl)cyclobutyl)carbamate (20c). 20c** was prepared from **19** as described for **8a** using pyridin-3-ylboronic acid. Purification by column chromatography (0-10% methanol in dichloromethane) produced **20c** with some impurities. The solid was triturated with ethyl acetate to yield a yellow solid (0.084 g, 79%). Mp 163-164 °C; ^{1}H NMR (400M Hz, DMSO-d_6) δ: 9.21 (dd, J = 2.4, 0.8 Hz, 1H), 8.56 (dd, J = 4.8, 1.6 Hz, 1H), 8.34 (ddd, J = 8.1, 2.3, 1.6 Hz, 1H), 8.29 (d, J = 8.3 Hz, 1H), 8.05 (d, J = 8.4 Hz, 1H), 7.97 (dd, J = 4.8, 1.9 Hz, 1H), 7.55 – 7.48 (m, 2H), 7.45 (dt, J = 11.8, 4.2 Hz, 3H), 7.04 (s, 2H), 6.31 (dd, J = 7.7, 4.8 Hz, 1H),4.45-2.40 (m, 4H), 2.10-1.90 (m, 1H), 1.85-1.75 (s, 1H), 1.33 (s, 9H); MS *m/z* 534 $[M+H]^+$.

3-(3-(4-(1-Aminocyclobutyl)phenyl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (21a). 21a was prepared from **20a** as described for **6**. The product was suspended in Et_2O and then filtered off, producing 3-(3-(4-(1-aminocyclobutyl)phenyl)-5-

phenyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (**21a**) as a white solid. Mp 280-285 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.94 (s, 3H), 8.47 (bs, 1H) 8.38 (d, J = 8.8Hz, 1H), 8.19 – 8.15 (m, 1H), 8.10 – 8.03 (m, 3H), 7.93 – 7.88 (m, 1H), 7.76 (d, J = 8.4 Hz, 1H), 7.69 (d, J = 8.4 Hz, 2H), 7.52 – 7.40 (m, 3H), 6.92 (t, J = 7.6 Hz, 1H), 2.70 – 2.57 (m, 4H), 2.29 – 2.20 (m, 1H), 1.90 – 1.80 (m, 1H); MS *m/z* 433 $[M+H]^+$; Anal. ($C_{27}H_{24}N_6 \cdot 3.06$ HCl·0.01 dioxane · 0.03 diethylether) calcd: C, 59.61; H, 5.28; N, 15.36. Found: C, 59.62; H, 5.05; N, 15.36.

N-(3-(3-(4-(1-Aminocyclobutyl)phenyl)-2-(2-aminopyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)phenyl)acetamide hydrochloride (21b). 21b was prepared from **20b** as described for **6**. The product was suspended in Et_2O and then filtered off, producing N-(3-(3-(4-(1-aminocyclobutyl)phenyl)-2-(2-aminopyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)phenyl)acetamide hydrochloride (**21b**) as a yellow solid. Mp 233-237 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 10.25 (s, 1H), 8.98 (bs, 3H), 8.56–8.43 (m, 2H), 8.38 (d, J = 8.4 Hz, 1H), 8.24–8.22 (m, 1H), 8.18 (dd, J = 4.8 Hz and 1.6 Hz, 1H), 7.96–7.92 (m, 1H), 7.79–7.66 (m, 6H), 7.33 (t, J = 8.0 Hz, 1H), 6.93 (dd, J = 7.6 Hz and 6.0 Hz 1H), 2.70–2.58 (m, 4H), 2.32–2.20 (m, 1H), 2.08 (s, 3H), 1.92–1.82 (m, 1H); MS *m/z* 433 $[M+H]^+$; Anal. ($C_{29}H_{27}N_7O \cdot 3.36$ HCl·0.23 dioxane · 0.06 diethylether) calcd: C, 56.89; H, 5.19; N, 15.40. Found: C, 56.89; H, 5.18; N, 15.40.

3-(3-(4-(1-Aminocyclobutyl)phenyl)-5-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (21c). 21c was prepared from **20c** as described for **6**. The product was suspended in Et_2O and then filtered off, yielding 3-(3-(4-(1-aminocyclobutyl)phenyl)-5-(pyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (**21c**) as a yellow solid. Mp 283-287 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.45 (s, 1H), 9.10–9.00 (m, 4H), 8.90 (d, J = 4.8 Hz, 1H), 8.59–8.49 (m, 3H), 8.33 (d, J = 8.8 Hz, 1H),

8.20 (d, *J* = 6.0 Hz, 1H), 8.10–8.04 (m, 1H), 7.94 (d, *J* = 7.6 Hz, 1H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.71 (d, *J* = 8.8 Hz, 2H), 6.95 (t, *J* = 6.4 Hz, 1H), 2.64 (t, *J* = 8.0 Hz, 4H), 2.32 – 2.20 (m, 1H), 1.92 – 1.82 (m, 1H); MS *m/z* 434 $[M+H]^+$; Anal. ($C_{26}H_{23}N_7O \cdot 3.94$ HCl·0.26 dioxane) calcd: C, 54.12; H, 4.87; N, 16.34. Found: C, 54.12; H, 5.11; N, 16.33.

N-(1-(4-(2-(2-Aminopyridin-3-yl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)cyclobutyl)acetamide (22). 3-(3-(4-(1-aminocyclobutyl)phenyl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (**21a**) (22 mg, 0.05 mmol) was dissolved in pyridine (1.0 mL), followed by the addition of acetic anhydride (4.7 μL, 0.05 mmol). The mixture was stirred overnight at room temperature, diluted with DCM/DMF (1:1, 2 mL) and then stirred for an additional 12 h. The mixture was diluted with DMSO, filtered and purified by HPLC to afford N-(1-(4-(2-(2-aminopyridin-3-yl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)cyclobutyl)acetamide (**22**) (14 mg, 57%) as a white powder after freeze drying. NMR (400 MHz, DMSO-d_6) δ: 8.53 (1H, s), 8.27 (1H, d, *J* = 8.6 Hz), 8.03 (2H, d, *J* = 7.5 Hz), 8.00 (1H, dd, *J* = 5.2, 1.7 Hz), 7.98 (1H, d, *J* = 8.0 Hz), 7.54 (2H, d, *J* = 8.6 Hz), 7.48 (2H, t, *J* = 7.5 Hz), 7.43 (2H, d, *J* = 8.6 Hz), 7.40 (1H, t, *J* = 8.6 Hz), 7.13 (1H, dd, *J* = 8.0, 1.7 Hz), 6.99 (2H, s), 6.38 (1H, dd, *J* = 7. 5, 4.6 Hz), 2.51-2.45 (4H, m), 2.09-2.00 (1H, m), 1.92-1.83 (1H, m); MS *m/z* 475 $[M+H]^+$.

3-(3-(4-(1-Aminocyclobutyl)phenyl)-5-(3-aminophenyl)-3*H*-imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (23). HCl in dioxane (4.0 M, 1 mL) was added to a solution of N-(3-(3-(4-(1-aminocyclobutyl) phenyl)-2-(2-aminopyridin-3-yl)-3*H*-imidazo[4,5-*b*]pyridin-5-yl)phenyl) acetamide (**21b**) (0.180 g, 0.42 mmol) in dichloromethane (5 mL) and methanol (1 mL). The reaction was capped and heated at 50ºC for 3 days. Upon completion, the reaction was diluted with ether (20 mL), and 3-(3-(4-(1-aminocyclobutyl)phenyl)-5-(3-aminophenyl)-3*H*-

imidazo[4,5-*b*]pyridin-2-yl)pyridin-2-amine hydrochloride (**23**) was filtered from the mixture as a yellow solid (0.162 g, 87%). Mp decomposed at 240 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.96 (bs, 3H), 8.44–8.30 (m, 3H), 8.16 (bs, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 8.01–8.79 (m, 2H), 7.89 (d, *J* = 5.2 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 2H), 7.68 (d, *J* = 8.0Hz, 2H), 7.57–7.50 (m, 1H), 7.32–7.28 (m, 1H), 6.94–6.86 (m, 1H), 2.72–2.58 (m, 4H), 2.32–2.20 (m, 1H), 1.92–1.82 (m, 1H); MS *m/z* 448 $[M+H]^+$; Anal. ($C_{27}H_{25}N_7 \cdot 3.73\ HCl \cdot 0.17$ dioxane $\cdot$ $0.73\ CH_2Cl_2$) calcd: C, 51.66; H, 4.81; N, 14.84. Found: C, 51.66; H, 4.81; N, 14.85.

Methyl 1-(4-((6-chloro-3-nitropyridin-2-yl)amino)phenyl)cyclobutane-1-carboxylate (24). 24 was prepared as described for **2a** using **1e** and methyl 1-(4-aminophenyl)cyclobutane-1-carboxylate. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.27 (s, 1H), 8.47 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.63 (dd, *J* = 8.6, 1.1 Hz, 2H), 7.35 (dd, *J* = 8.6, 1.1 Hz, 2H), 6.81 (dd, *J* = 8.6, 1.1 Hz, 1H), 3.67 (d, *J* = 1.7 Hz, 3H), 2.88-2.83 (m, 2H), 2.56-2.49 (m, 2H), 2.10-2.00 (m, 1H), 1.94-1.85 (m, 1H); MS *m/z* 362 $[M+H]^+$.

Methyl 1-(4-((3-nitro-6-phenylpyridin-2-yl)amino)phenyl)cyclobutane-1-carboxylate (25). 25 was prepared as described for **8a** using **24** and phenylboronic acid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.33 (s, 1H), 8.59 (d, *J* = 8.7 Hz, 1H), 8.08-8.03 (m, 2H), 7.76 (dt, *J* = 9.2, 2.4 Hz, 2H), 7.53-7.49 (m, 3H), 7.36 (dt, *J* = 8.9, 2.3 Hz, 2H), 7.31 (d, *J* = 8.7 Hz, 1H), 3.68 (s, 3H), 2.90-2.83 (m, 2H), 2.60-2.51 (m, 2H), 2.12-2.00 (m, 1H), 1.97-1.86 (m, 1H); MS *m/z* 404 $[M+H]^+$.

Methyl 1-(4-(2-(2-aminopyridin-3-yl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl) cyclobutane-1-carboxylate (26). 26 was prepared as described for **4a** using **25** and 2-aminonicotinaldehyde. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.27 (d, *J* = 8.6 Hz, 1H), 8.04–8.02 (m, 2H), 8.01 (dd, *J* = 4.9, 2.0 Hz, 1H), 7.98 (d, *J* = 8.6 Hz, 1H), 7.57–7.50 (m, 1H), 7.50–7.44

(m, 6H), 7.40 (tt, J = 7.2, 1.5 Hz, 1H), 7.14 (dd, J = 7.4, 1.7 Hz, 1H), 6.98 (s, 2H), 6.40 (dd, J = 7.7, 4.9 Hz, 1H), 3.64 (s, 3H), 2.81–2.75 (m, 2H), 2.55-2.51 (m, 2H), 2.04–1.94 (m, 1H), 1.92–1.83 (m, 1H); MS *m/z* 476 $[M+H]^+$.

1-(4-(2-(2-Aminopyridin-3-yl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl)cyclobutane-1-carboxamide (27). Methyl 1-(4-(2-(2-aminopyridin-3-yl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl) cyclobutane-1-carboxylate (**26**) (209 mg, 0.44 mmol) was dissolved in THF/MeOH/aq. NaOH 1 N (2:2:1 by volume, 25 mL), and the mixture was stirred for one hour at 65 °C. The reaction mixture was cooled to 0 °C and acidified with 1 N HCl (5 mL). The mixture was diluted with water and extracted with $CHCl_3$. The organics were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was treated with CH_2Cl_2 and hexanes, producing 1-(4-(2-(2-aminopyridin-3-yl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl)phenyl) cyclobutane-1-carboxylic acid as a white powder (170 mg, 84%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.51 (br. s, 1H), 8.30 (d, *J* = 8.0 Hz, 1H), 8.06–8.03 (m, 3H), 8.00 (d, *J* = 8.0 Hz, 1H), 7.51–7.44 (m, 7H), 7.41 (t, J = 8.0 Hz, 1H), 7.36 (d, J = 7.4 Hz, 2H), 6.53 (dd, J = 7.7, 5.4 Hz, 1H), 2.79–2.72 (m, 2H), 2.49-2.44 (m, 2H), 2.04–1.94 (m, 1H), 1.89–1.81 (m, 1H); MS *m/z* 462 $[M+H]^+$.

The carboxylic acid (35 mg, 0.076 mmol) was dissolved in DMF (1 mL) and then treated with HOBt (10.3 mg, 0.076 mmol), triethylamine (32 μL, 0.23 mmol) and EDC (29 mg, 0.15 mmol). The reaction mixture was stirred at room temperature for 8 h. An ammonia solution in MeOH (7 M, 10 eq.) was added, and the mixture was stirred at room temperature for one hour. Water was then added, and the mixture was extracted with $CHCl_3$. The organics were dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by HPLC (acetonitrile/HCOOH buffer). After evaporation, 1-(4-(2-(2-aminopyridin-3-yl)-5-phenyl-3*H*-imidazo[4,5-*b*]pyridin-3-yl) phenyl)cyclobutane-1-carboxamide (**27**) was obtained (15 mg, 43%) as a white solid. ^{1}H NMR

(400 MHz, DMSO-d_6) δ: 8.27 (d, J = 8.0 Hz, 1H), 8.03 (d, J = 8.0 Hz, 2H), 8.01 (dd, J = 4.6, 1.7 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.51–7.44 (m, 6H), 7.40 (tt, J = 7.2, 1.5 Hz, 1H), 7.29 (s, 1H), 7.17 (dd, J = 7.4, 1.7 Hz, 1H), 6.96 (s, 3H), 6.40 (dd, J = 7.7, 4.9 Hz, 1H), 2.78–2.71 (m, 2H), 2.42-2.36 (m, 2H), 1.91–1.73 (m, 2H); MS *m/z* 461 $[M+H]^+$.

General Experimental Details

Interference compounds: All test compounds passed the PAINS filter. The screening was performed by Molecular Forecaster as part of a computational analysis of the entire ArQule Compound Library.

Protein expression and purification: Full-length AKT isoform constructs (AKT1 (2-480), AKT2 (2-481) and AKT3 (2-479)) and ΔPH-AKT1 (144-480) were expressed in Sf9 cells using a baculovirus vector. The frozen cell pellets were thawed and re-suspended in lysis buffer containing 25 mM Tris, pH 8.0, 100 mM NaCl, 25 mM imidazole, 10% glycerol, and protease inhibitors (Sigma #S8820). Cells were lysed by sonication, the lysate was centrifuged, and the clarified supernatant was loaded onto a Ni-NTA affinity column. Following washing with buffer containing 25 mM Tris, pH 8.0, 500 mM NaCl, and 25 mM imidazole, the protein was eluted in buffer containing 25 mM Tris, 100 mM NaCl, and 400 mM imidazole, pH 8.0. The protein was concentrated and purified on a Superdex 200 gel-filtration column. The concentrated protein (10 mg/ml) was stored in buffer containing 25 mM Tris, pH 8.0, 100 mM NaCl, and 10% glycerol at -80 °C.

Protein Characterization (AKT1, AKT2 and AKT3). Phosphorylation at Thr308 and Ser473 in AKT1 (1-480) was analyzed as described below. The proteins were subjected to solution-phase digestion, and peptide phosphorylation was determined by LC/MS and LC/MS/MS analysis. Proteins (50 μg) were diluted in 50 mM ammonium bicarbonate buffer, pH 8,

containing 5 mM DTT, 3 M urea, 5% acetonitrile and endoprotease (trypsin, 1:20 w/w or GluC, 1:10 w/w ratio) and digested overnight. The digests were separated on a BEH C18 column (1.0 x 50 mm, 1.7 μm) equilibrated at 40 °C and mass analyzed on a QTOF mass spectrometer. Elution was performed using a water/acetonitrile gradient with a 0.1% formic acid modifier, starting from 2% and reaching 40% acetonitrile over 14 min, followed by 40 to 50% acetonitrile from 14 to 16 min, 50 to 80% acetonitrile from 16 to 18 min, held at 80% for 2 min and re-equilibrated with 2% acetonitrile for another 3 min. The MS spectra of the peptides were de-convoluted using the MaxEnt3 software package (Masslynx, Waters). For the inactive AKT proteins, no phosphorylation was detected at Thr308/Thr309/Thr305, and less than 10% of Ser473/Ser474/Ser472 was detected as phosphorylated (in AKT1, AKT2 and AKT3, respectively). Following activation with PDK1 and MAPKAP2 in the presence of lipids, MS analysis identified 60% of Thr308 sites and 80% of Ser473 sites as being phosphorylated.

Assay Descriptions:

Lipid Vesicle Preparation. Lipid vesicles were prepared from PtdIns(3,4,5)P_3, dipalmitoyl and a synthetic phospholipid blend of DOPS/DOPC. A 1.0 mg/mL solution of PtdIns(3,4,5)P_3 and a 16.7 mg/mL suspension of DOPS/DOPC in 10 mM Hepes, pH 7.4, were prepared immediately before use. The DOPS/DOPC suspension (800 μL) and the PtdIns(3,4,5)P_3 solution (964 μL) were mixed and then diluted in 18.2 mL of 10 mM Hepes, pH 7.4, and mixed well. This mixture (400 μM DOPS/400 μM DOPC/40 μM PtdIns(3,4,5)P_3) was sonicated on ice for 30 sec at 75% power until the mixture clarified. Aliquots were prepared and frozen at -80 °C until use.

AKT 1(1-480), AKT2(1-481) and AKT3(1-479) Alpha Screen Assays. AKT activity was assayed using a GSK3-derived biotinylated peptide substrate, crosstide (biotin-GRPRTSSFAEG), and AlphaScreen™ (Amplified Luminescent Proximity Homogeneous

Assay) technology. Test compounds and controls were prepared in 10% DMSO at 10-fold the desired final concentration, and 2.5 μL of each compound was added to each well of a reaction plate (Corning 96-well half-area solid white nonbinding surface plate). Full-length un-phosphorylated AKT was diluted in assay buffer (50 mM Tris, pH 8.0, 0.02 mg/mL BSA, 10 mM $MgCl_2$, 1 mM EGTA, 10% glycerol, 0.2 mM Na_3VO_4, 1 mM DTT, 0.1 mM β-glycerophosphate, and 0.2 mM NaF) and added to each well at a volume of 17.5 μL for a final concentration of 8 nM (AKT1), 63 nM (AKT2), or 13 nM (AKT3) in the 25 μL reaction. After a 20-min pre-incubation at room temperature, the kinase reaction was initiated by the addition of 5 μL of the activation mixture diluted in assay buffer, containing biotinylated crosstide, PDK1, MAPKAPK2, DOPS/DOPC, PtdIns(3,4,5)P3, and ATP for a final concentration of 60 nM biotinylated crosstide, 0.1 nM (AKT1, AKT3) or 0.3 nM (AKT2) PDK1, 0.7 nM (AKT1), 1.3 nM (AKT2), or 0.4 nM (AKT3) MAPKAPK2, 5.5 μM DOPS, 5.5 μM DOPC, 0.5 μM PtdIns(3,4,5)P3, and 50 μM (AKT1, AKT2) or 18 μM (AKT3) ATP. The plates were incubated for 30 min at room temperature, and the reactions were stopped in the dark by the addition of 10 μL of stop/detection mixture prepared in assay buffer, containing EDTA, AlphaScreen™ Streptavidin Donor and Protein A acceptor beads, and phospho-AKT substrate antibody at final concentrations of 10 mM EDTA, 500 ng/well of both AlphaScreen™ Streptavidin donor beads and Protein A acceptor beads, and phospho-AKT substrate antibody at a final dilution of 1:350. Assay plates were incubated for 90 min at room temperature in the dark, and the plates were read on a Perkin Elmer Envision Multilabel plate reader (excitation wavelength: 640 nm, emission wavelength: 570 nm). All IC_{50} values reported are the geometric mean of at least $n = 2$ determinations.

***In vitro* Western Blot Pharmacodynamic Assay (p-Ser473, p-Thr308, p-PRAS40).** Cellular activity was determined by measuring the phosphorylation status of Ser473, Thr308 and the AKT substrate p-PRAS40 using A2780 and AN3CA cell lines. A2780 and AN3CA cells were plated in 6-well plates at 60-75% confluency and incubated at 37 °C overnight in 5% CO_2. Upon reaching 75-90% confluency, the cells were treated with the test compound, following a three-fold dilution scheme, for one hour with a final DMSO concentration of less than 0.2% v/v. Cells were stimulated with 100 ng/mL EGF and 100 nM insulin for fifteen minutes. The medium was removed, and 150 µL of 1X E-Page Loading Buffer (Cat#EPBUF-01, Invitrogen) was added. Cells were scraped and transferred to a deep-well 96-well plate and lysed by sonication at medium power four to six times in 30-sec bursts. Samples were run on EPage™ gels and transferred to PVDF membranes using the iBlot™ system (Invitrogen). Membranes were washed for 3 min in dH_2O, blocked for 30 min in Odyssey Blocking Buffer (Cat#927-40000, LiCor), and then incubated overnight at 4 °C in Odyssey Blocking/TBST Buffer (50 mM Tris, pH 7.4, 150 mM NaCl, and 0.05% Tween 20) (1:3) with antibodies against p-AKT (Cat#9271 and Cat#9275, Cell Signaling Technology), phospho-PRAS40 (Cat#2640, Cell Signaling Technology), and β-actin (Cat#A2228, Sigma). After three washes for 5 min each in TBST buffer, the membranes were incubated with the appropriate secondary antibodies labeled with near-infrared or Alexa Fluor dyes (Cat#611-132-122 goat anti-rabbit IgG H+L, Cat#610-132-121 goat anti-mouse IgG H+L, Rockland (Cat#A21076 goat anti-rabbit IgG H+L, Cat#A21057 goat anti-mouse IgG H+L, Molecular Probes). After a one-hour incubation, the membranes were washed three times for five minutes each and then scanned using an Odyssey infrared scanner (LiCor). The intensities of the bands were quantified using the accompanying software. The data were normalized to the

untreated controls, the dose-response curves were fitted to a four-parameter logistic equation, and the IC_{50} values were determined.

Cellular Anti-proliferation Assay (MTS). Anti-proliferative cellular assays were conducted using the CellTiter Non-Radioactive Cell Proliferation Assay developed by Promega, which utilizes the production of formazan from a tetrazolium compound by live cells. AN3CA and A2780 cells were obtained from the ATCC. AN3CA cells were cultured in DMEM, and A2780 cells were cultured in RPMI supplemented with 10% FBS, 100 units/mL penicillin, 100 µg/mL streptomycin, and 2 mM glutamine at 37 °C under 5% CO_2. Cells were plated in 96-well plates at 2,000-10,000 cells/well, cultured for 24 h, and treated with the test compound for 72 h at a final DMSO concentration no greater than 0.5% v/v. PMS stock reagent (0.92 mg/mL in DPBS) was diluted 20-fold in MTS stock reagent (2 mg/mL in DPBS), and this MTS/PMS mixture was diluted 5-fold into each well of the 96-well plate. The plates were incubated for 3-4 h, and the absorbance of formazan was measured at 490 nm. The data were normalized to the untreated controls, the dose-response curves were fit to a four-parameter logistic equation, and the IC_{50} values were determined. All IC_{50} values reported are the geometric mean of at least two independent determinations.

CYP450 Inhibition Assay. Human liver microsomes (0.25 mg/mL), probe substrates [3 µM midazolam (3A4), 5 µM bufuralol (2D6), 100 µM tolbutamide (2C9), 10 µM paclitaxel (2C8), 80 µM S-mephenytoin (2C19), and 50 µM phenacetin (1A2)], 3.3 mM $MgCl_2$, 1 mM NADPH, and the compound (0.1 - 10 µM) dissolved in DMSO (0.1% final) were incubated for 10 min, after which acetonitrile containing reserpine (internal standard) was used to stop the reaction. The solvents were evaporated under a constant stream of nitrogen gas at 35 °C, and the resulting compound was reconstituted in 100 µL of water for LC/MS/MS analysis. An inhibitors cocktail

consisting of 0.5 μM ketoconazole (3A4), 1 μM sulfaphenazole (2C9), 1 μM quinidine (2D6), 2 μM quercetin (2C8), 1 μM nootkatone (2C19), and 0.5 μM α-naphthoflavone (1A2) was used as a positive control. Incubations containing only DMSO (no compound) served as the 100% activity control. The percent inhibition was calculated based on the signal for the 100% activity control, and the IC_{50} values were either estimated or determined using fit-model 205 (one-site dose response) in XLfit4 (Guildford, Surrey, UK).

Cross-species NADPH-dependent Microsomal Stability Assay. Human, CD-1 mouse and Beagle dog liver microsomes (0.25 mg/mL), 3.3 mM $MgCl_2$ and a NADPH-regenerating system (0.4 units/mL G6PDH, 1.3 mM $NADP^+$, and 3.3 mM G6P) were incubated with 1 μM of compound for 0, 3, 6, 10, 15, or 30 min. The incubations were stopped with acetonitrile containing warfarin (internal standard), and the samples were analyzed by LC/MS/MS. The peak area ratios were used to determine the percent remaining at each time point compared with time zero. The half-life values were estimated using the equation for mono-exponential decay. Midazolam and propranolol (female mouse only) were used as positive controls.

Crystallization, Data Collection, and Refinement. A 6xHis-AKT1 (2-446) with a TEV protease site construct was cloned in a baculovirus vector and expressed in Sf9 cells. To aid the crystallization, three mutations were introduced on the linker region (E114A, E115A, and E116A) in the AKT1 (2-446) construct. The protein was purified using a similar protocol as described above for the AKT isoforms and the 6xHis tag was removed using TEV protease. Co-crystals of **21a** were obtained with the following conditions: 16% butanol, 10mM ammonium sulfate, 0.1% 2-mercaptoethanol, 15% ethylene glycol, 50 mM Tris, pH 7.5. Diffraction data were collected at NSLS on the X29 beamline, and the data were processed using Mosflm and scaled using Scala. The structure was solved by molecular replacement using the Phaser program

with search models consisting of AKT2 (1MRV) and the AKT1 PH domain (2UVM). The initial model was built using ARP/wARP program, and the model was subsequently improved through iterative cycles of manual model building with Coot and refinement with ARP/wARP or Refmac5.

PDB ID code: **21a** bound to AKT1, 5KCV

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Notes

The authors declare no competing financial interest.

Abbreviations Used

mTOR, mammalian target of rapamycin; PTEN, phosphatase and tensin homolog; PH, pleckstrin homology; PRAS40, proline-rich AKT substrate, 40 kDa; PDK1, phosphoinositide-dependent kinase 1; mTORC2, mammalian target of rapamycin complex 2; HM, hydrophobic motif; MAPKAPK2, mitogen-activated protein kinase activated protein kinase 2; ELSD, evaporative light scattering detector; DIEA, diisopropylethylamine; HOBt, hydroxybenzotriazole; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HCl, hydrochloric acid; QTOF, quadrupole-time-of-flight; DOPS, 1,2-dioleoyl-sn-glycero-3-phospho-L-serine; DOPC, 1,2-dioleoyl-sn-glycero-3-phosphocholine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; GSK-3, glycogen synthase kinase 3; EGTA, ethylene glycol tetraacetic acid; FBS, fetal bovine serum; MTS, (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium); PMS, phenazine methosulfate; HLM, human liver mcrosome; MLM, mouse liver microsome; DLM, dog liver microsome; M, male; F, female; DPBS, Dulbecco's phosphate-buffered saline; TEV, tobacco etch virus.

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12. All 3-(3-phenyl-3*H*-imidazo[4,5-*b*]pyridin-2yl)pyridin-2-amine compounds tested for biochemical inhibition of PDK1 and MAPKAPK2 showed IC_{50} between 10-100 μM.

13. The biochemical IC_{50} determination against full-length AKT1, AKT2 and AKT3 for the test compounds described in this paper were performed at Reaction Biology (Malvern, PA). The compounds were tested in duplicate, in a 10-dose mode with 3-fold serial dilution starting at 10 or 100 μM (based on the IC_{50} data generated internally with the un-phosphorylated proteins), in the presence of 10 μM ATP. Curve fits were performed where the enzyme activities at the highest concentration of test compounds were less than 65% of enzyme activity relative to DMSO controls.

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16. The crystallographic data described here has been deposited with the Protein Data Bank (PDB code for **21a** bound to AKT1: 5KCV).

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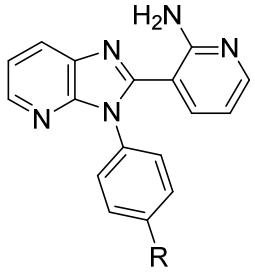
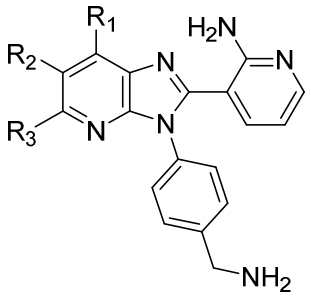
Table 1. Biochemical Results of Early Compounds[a]



		IC_{50} (μM)[b]		
Compd	R	AKT1	AKT2	AKT3
4a	Me	0.72	20	>100
4b	*t*-Bu	5.9	16	71
6	CH_2NH_2	1.7	9.3	>100

[a] See Experimental Section for assay details
[b] Assay conducted with un-phosphorylated enzymes

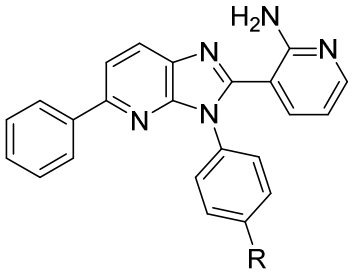
Table 2. Structure-Activity Relationship for Substitution on the Pyridine Ring[a]

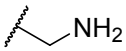
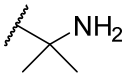
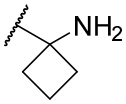
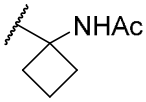
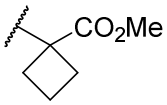
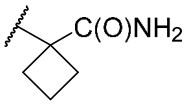


Compd	R_1	R_2	R_3	IC_{50} (μM)[b] AKT1	AKT2	AKT3
6	H	H	H	1.7	9.3	>100
7a	Me	H	H	0.5	NT	NT
7b	H	Me	H	0.27	5.3	>10
7c	H	Me	Me	>1	NT	NT
7d	H	H	Cl	0.27	1.9	2.6
7e	H	Br	H	0.32	NT	NT
9a	H	H	Ph	0.0037	0.033	0.43
9b	H	H	3-NHAc-Ph	0.013	0.024	0.086
9c	H	H	4-NHAc-Ph	0.072	0.079	0.70
9d	H	H	1H-pyrazol-4-yl	0.017	0.014	0.065
9e	H	pyridin-3-yl	H	0.036	3.5	>10

[a] See Experimental Section for assay details.
[b] Assay conducted with un-phosphorylated enzymes
NT means Not Tested

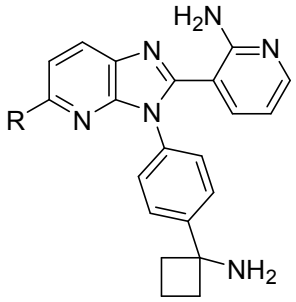
Table 3. Structure-Activity Relationship for Substitution at the Benzylic Position[a]



Compd	R	IC_{50} (μM) [b] AKT1	AKT2	AKT3
9a		0.0037	0.033	0.43
17		0.0054	NT	NT
21a		0.0027	0.014	0.0081
22		0.035	NT	NT
26		0.54	NT	NT
27		0.035	NT	NT

[a] See Experimental Section for assay details.
[b] Assay conducted with un-phosphorylated enzymes
NT means Not Tested

Table 4. Structure-Activity Relationship 5-Substituted Analogs[a]



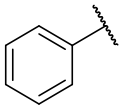
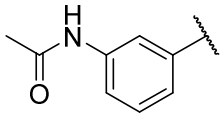
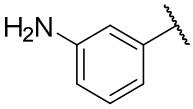
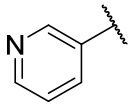
Compd	R	IC_{50} (μM) for un-phosphorylated and phosphorylated forms of AKT isoforms					
		AKT1		AKT2		AKT3	
		Inactive	Active	Inactive	Active	Inactive	Active
21a		0.0027	0.0050	0.014	0.0045	0.0081	0.016
21b		0.0028	0.0023	0.0071	0.0032	0.0054	0.022
23		0.0019	0.0026	0.0054	0.0028	0.0040	0.0090
21c		0.006	0.034	0.0025	0.036	0.026	0.34

Table 5. *In Vitro* Anti-Proliferative Activity of Selected Compounds[a]

	MTS cell proliferation, IC_{50} (μM)			
Compd	A2780	AN3CA	IGROV-1	LNCAP
9a	7.5	13	7.5	NT
21a	0.73	0.71	0.21	0.90
21b	1.4	1.6	0.16	1.5
23	0.18	0.59	NT	0.13
21c	14	6.1	0.40	13

[a] See Experimental Section for assay details.
NT means Not Tested

Table 6. *In Vitro* Cellular PD of Selected Compounds in AN3CA Cells[a]

Compd	p-AKT (S473) IC_{50} (μM)	p-AKT (T308) IC_{50} (μM)	p-PRAS40 (T246) IC_{50} (μM)
21a	0.040	0.061	0.31
21b	0.041[b]	0.037[b]	> 3.0[b]
21c	0.056	0.063	0.64

[a] See Experimental Section for assay details.
[b] In A2780 cell line.

Table 7. *In Vitro* ADME Properties of Selected Compounds[a]

Compd	CYP 450, IC_{50} (μM)						Liver microsomal stability at 1.0 μM $T_{1/2}$ (min)			Solubility (μM)
	1A2	2C8	2C9	2C19	2D6	3A4	HLM	MLM (F/M)	DLM (F/M)	
9a	>10	>10	<1	<1	>10	>10	39	NT	NT	188
9b	>10	>20	0.1	>5	>20	>20	NT	45 / >60	>60 / >60	≥200
21a	20	20	7.9	8.0	15	20	55	18 / 17	28 / 31	170
21b	>10	1-10	1-10	>10	1-10	>10	>60	>60 / >60	50 / >60	160
23	>20	>20	>20	>20	>20	>20	>60	>60 / >60	>60 / >60	138
21c	>20	>20	10	NT	11	18	>30	37 / 31	21 / >30	178

[a] See Experimental Section for assay details.
NT means Not Tested

Table 8. *In vivo* Pharmacokinetic and Pharmacodynamic Results for **21a** in Tumor Bearing Mice (AN3CA Cell Line)[a]

		% inhibition				
po dose (mg/kg)	time (h)	p-AKT (S473)	p-AKT (T308)	p-PRAS40 (T246)	plasma concn (μM)	tumor concn (μM)
100	1	99.2	95.0	57.7	2.1	21.0
100	8	98.6	96.3	35.9	0.26	9.6
200	1	99.2	93.3	50.5	2.7	35.8
200	8	99.7	90.8	42.5	1.0	27.4

[a] See Experimental Section for assay details.

Table 9. Single Dose Pharmacokinetic Results for **21a** in Rats and Monkeys

PK Parameters	Rats (5 mg/kg)*	Monkeys (10 mg/kg)*
T_{max} (hr)	8.0	4.3
$t_{1/2}$ (hr)	17	7
C_{max} (ng/mL)	198	258
AUC_{inf}(hr*ng/mL)	5496	2960
F (%)	62	49

*Dosed in a solution formulation (compound **21a** in 0.01 M phosphoric acid)

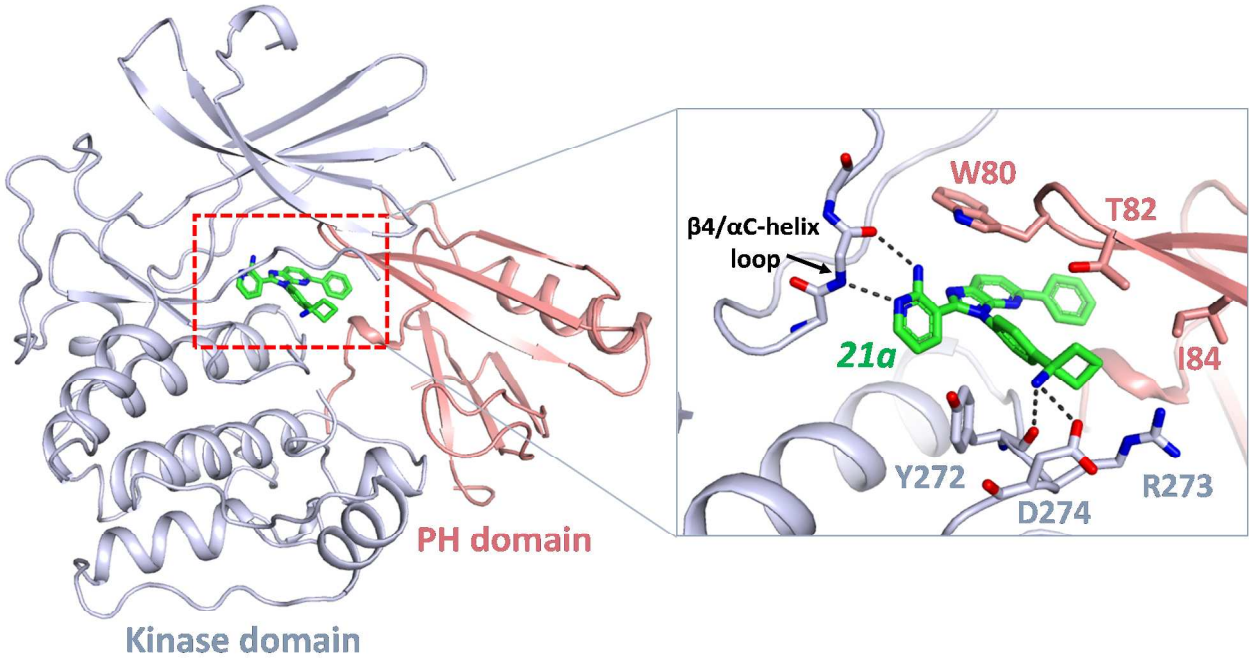
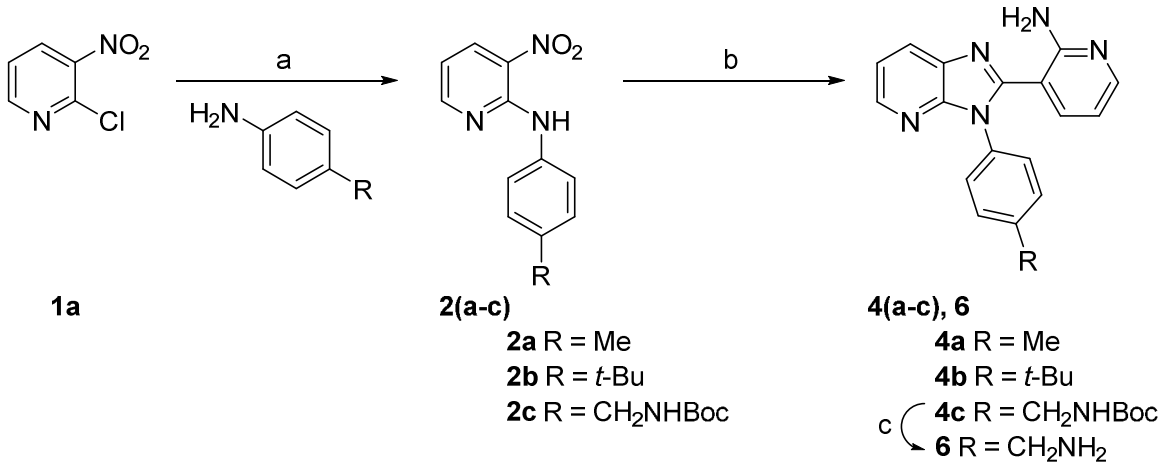


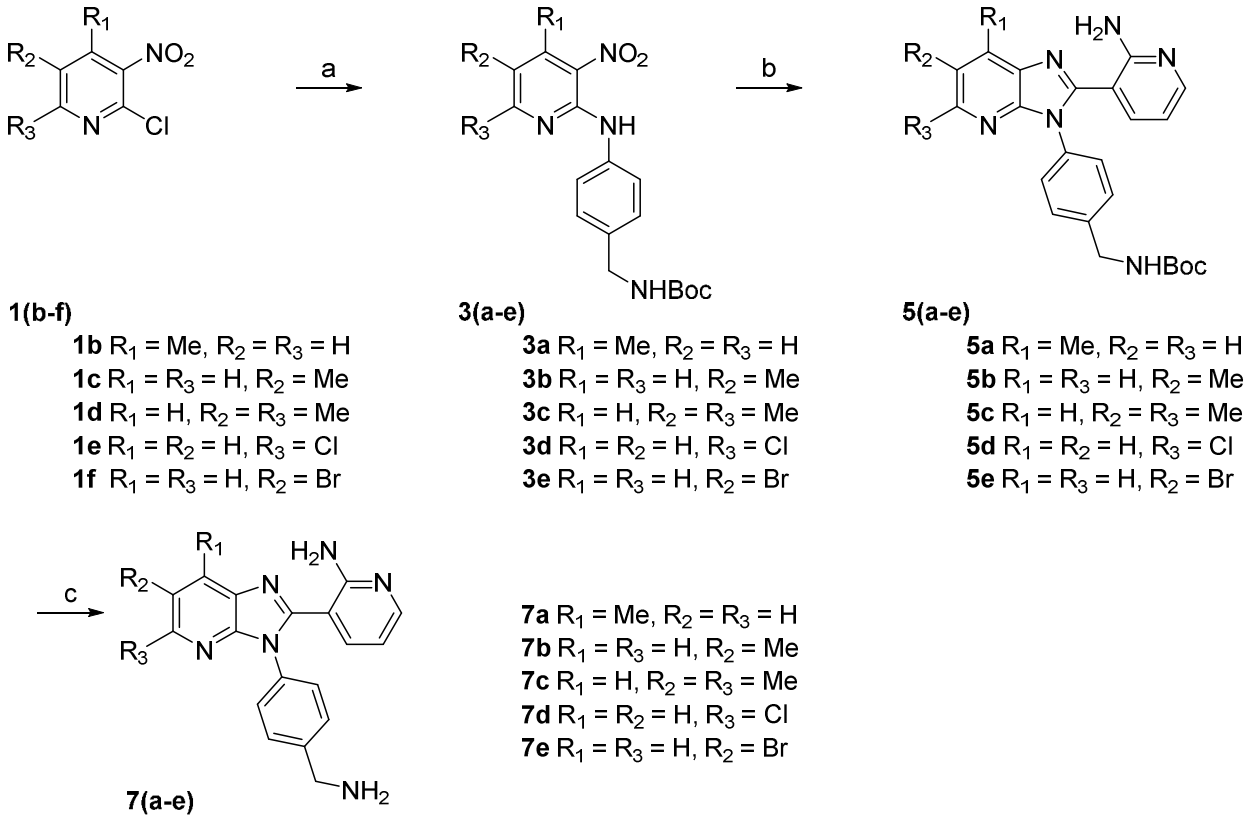
Figure 1. Co-crystal structure of **21a** with AKT1 (PDB accession code 5KCV). Compound **21a** binds to the allosteric pocket formed by the kinase and PH domains. The amino-pyrimidine group of the core moiety forms a bidentate hydrogen bonding interaction with the main chain atoms of loop of β4-strand and αC-helix. The phenylcyclobutylamine side chain associates with the conserved YRD motif of kinase domain by polar and non-polar interactions.

Scheme 1[a]



[a]Reagents and conditions: (a) substituted anilines, DIEA, DMSO or 1,4-dioxane, 80°C, overnight; (b) 2-amino-nicotinaldehyde, $Na_2S_2O_4$, DMSO, MeOH, 100°C, overnight; (c) 4.0 M HCl in 1,4-dioxane, 1,4-dioxane, room temperature, overnight

Scheme 2[a]



1(b-f)

1b R_1 = Me, R_2 = R_3 = H
1c R_1 = R_3 = H, R_2 = Me
1d R_1 = H, R_2 = R_3 = Me
1e R_1 = R_2 = H, R_3 = Cl
1f R_1 = R_3 = H, R_2 = Br

3(a-e)

3a R_1 = Me, R_2 = R_3 = H
3b R_1 = R_3 = H, R_2 = Me
3c R_1 = H, R_2 = R_3 = Me
3d R_1 = R_2 = H, R_3 = Cl
3e R_1 = R_3 = H, R_2 = Br

5(a-e)

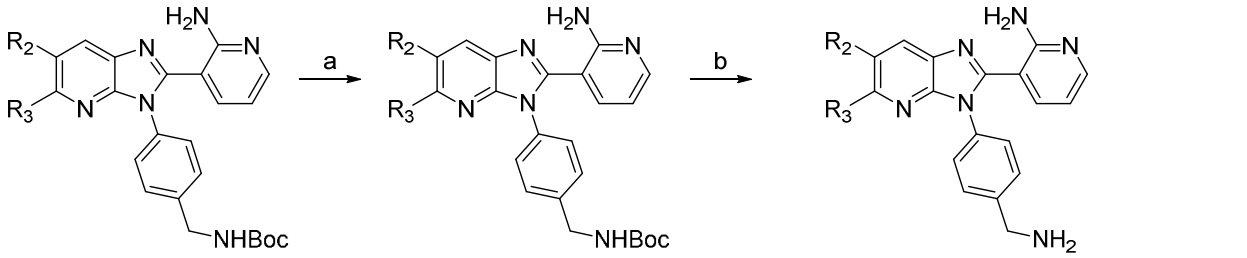
5a R_1 = Me, R_2 = R_3 = H
5b R_1 = R_3 = H, R_2 = Me
5c R_1 = H, R_2 = R_3 = Me
5d R_1 = R_2 = H, R_3 = Cl
5e R_1 = R_3 = H, R_2 = Br

7(a-e)

7a R_1 = Me, R_2 = R_3 = H
7b R_1 = R_3 = H, R_2 = Me
7c R_1 = H, R_2 = R_3 = Me
7d R_1 = R_2 = H, R_3 = Cl
7e R_1 = R_3 = H, R_2 = Br

[a]Reagents and conditions: (a) *tert*-butyl (4-aminobenzyl)carbamate, DIEA, DMSO or 1,4-dioxane, 80°C, overnight; (b) 2-amino-nicotinaldehyde, $Na_2S_2O_4$, DMSO, MeOH, 100°C, overnight; (c) 4.0 M HCl in 1,4-dioxane, 1,4-dioxane, room temperature, overnight

Scheme 3[a]



5(d,e)

5d R_2 = H, R_3 = Cl

5e R_2 = Br, R_3 = H

8(a-e)

8a R_2 = H, R_3 = phenyl

8b R_2 = H, R_3 = 3-phenylacetamide

8c R_2 = H, R_3 = 4-phenylacetamide

8d R_2 = H, R_3 = 1*H*-pyrazol-4-yl

8e R_2 = pyridin-3-yl, R_3 = H

9(a-e)

9a R_2 = H, R_3 = phenyl

9b R_2 = H, R_3 = 3-phenylacetamide

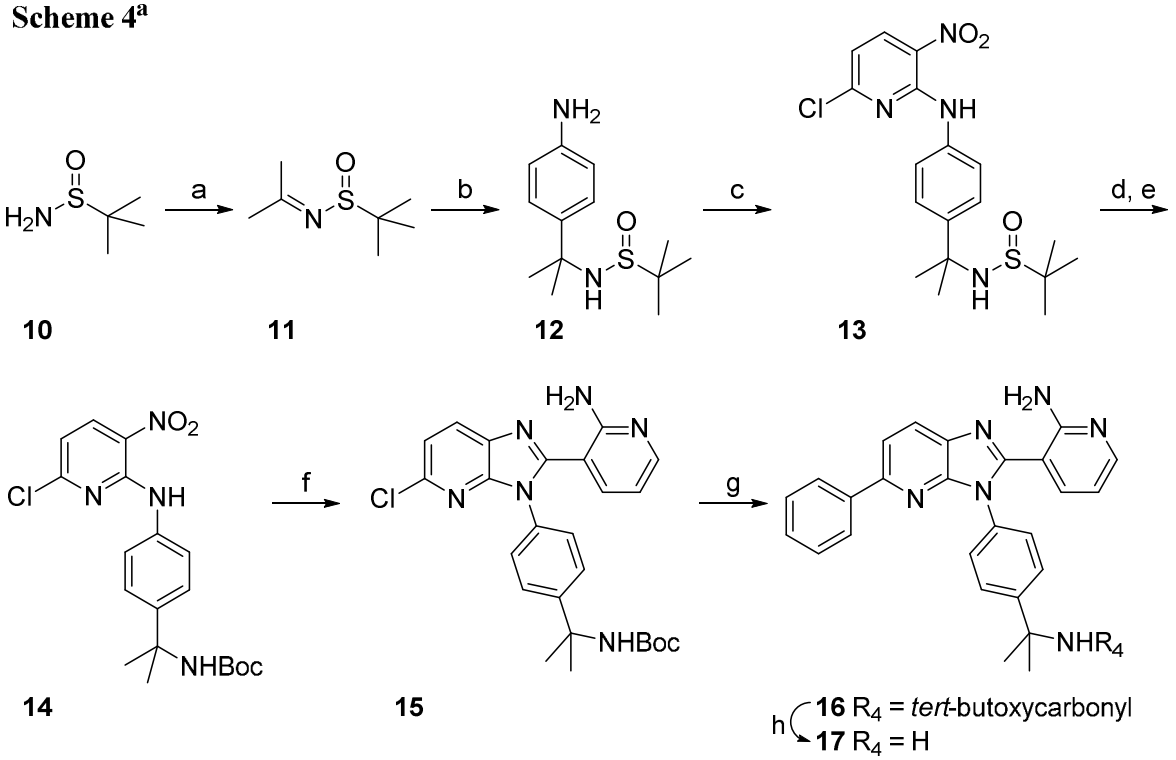
9c R_2 = H, R_3 = 4-phenylacetamide

9d R_2 = H, R_3 = 1*H*-pyrazol-4-yl

9e R_2 = pyridin-3-yl, R_3 = H

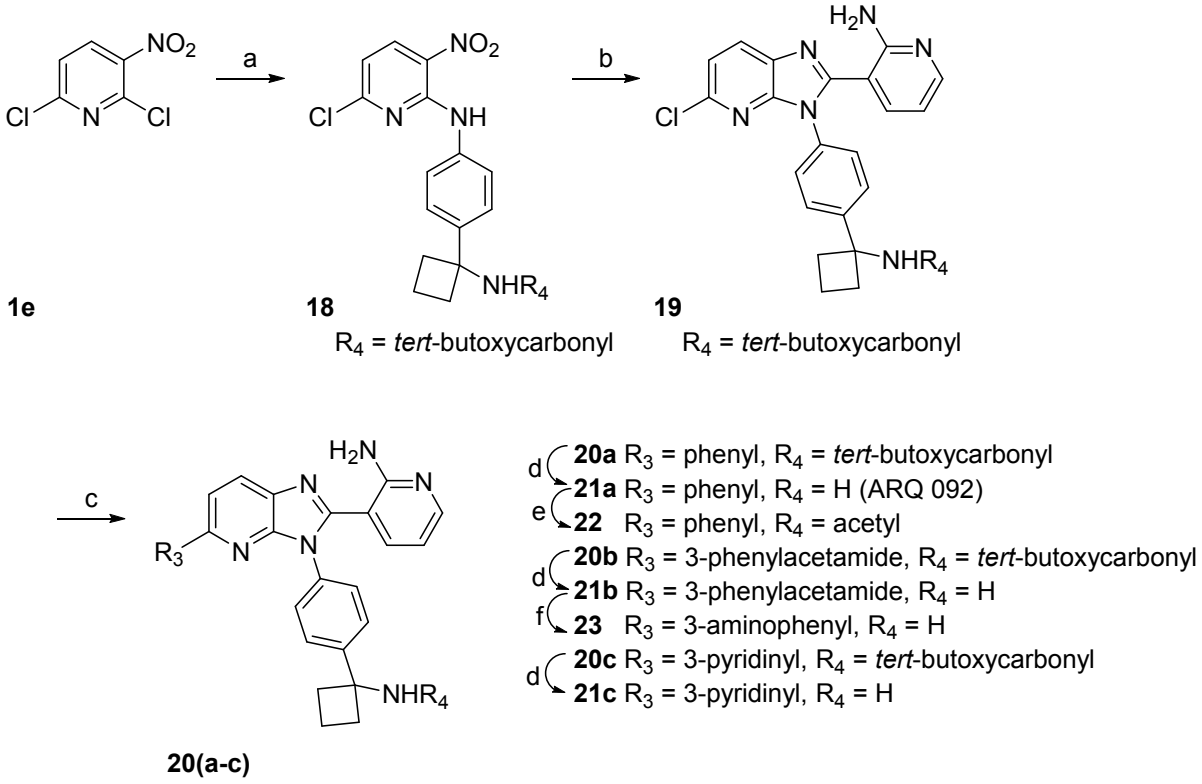
[a]Reagents and conditions: (a) aryl/heteroaryl boronic acid, toluene, EtOH, aq. $NaHCO_3$, $Pd(PPh_3)_4$, 100°C, overnight; (b) 4.0 M HCl in 1,4-dioxane, 1,4-dioxane, room temperature, overnight

Scheme 4[a]



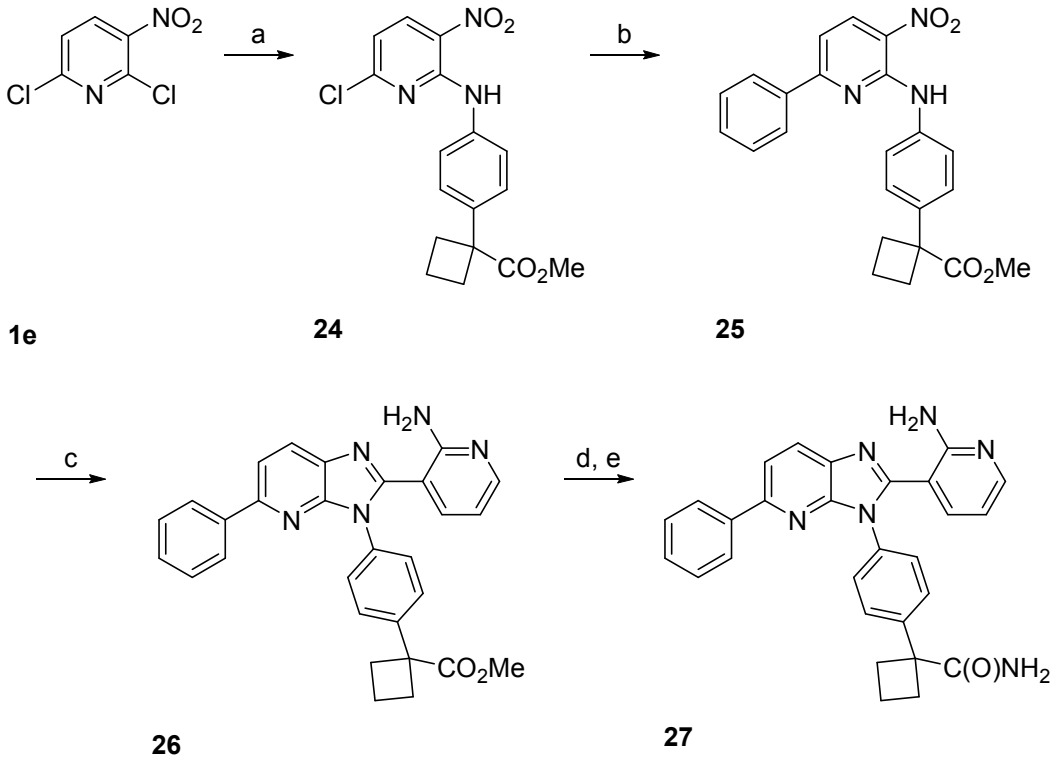
[a]Reagents and conditions: (a)$Ti(OEt)_4$, acetone, THF; (b) 4-bromo-N,N-bis(trimethylsilyl)aniline, BuLi, THF, -78 °C; (c) **1e**, Et_3N, DMA, 0-20 °C, overnight; (d) 4.0 M HCl in 1,4-dioxane, DCM, 30 min.; (e) di-*t*-Butyl dicarbonate, $NaHCO_3$, THF; (f) 2-amino-nicotinaldehyde, $Na_2S_2O_4$, DMSO, MeOH, 100°C, overnight; (g) phenyl boronic acid, $Pd(PPh_3)_4$, $NaHCO_{3\ aq.}$, EtOH, toluene, 100°C, overnight; (h) 4.0 M HCl in 1,4-dioxane, 1,4-dioxane, room temperature, 1 hr

Scheme 5[a]



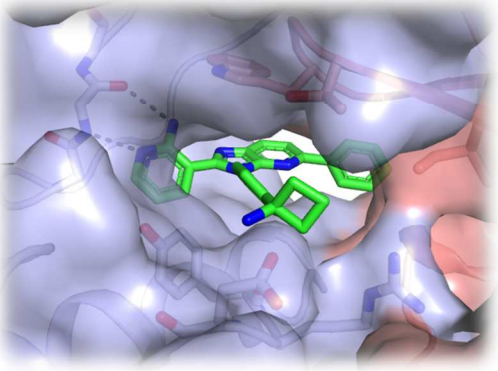
[a]Reagents and conditions: (a) *tert*-butyl (1-(4-aminophenyl)cyclobutyl)carbamate, DIEA, DMSO or 1,4-dioxane, 80^{o}C, overnight; (b) 2-amino-nicotinaldehyde, $Na_2S_2O_4$, DMSO, MeOH, 100^{o}C, overnight; (c) substituted phenyl boronic acid, $Pd(PPh_3)_4$, $NaHCO_{3\ aq.}$, EtOH, toluene, 100^{o}C, overnight; (d) 4.0 M HCl in 1,4-dioxane, 1,4-dioxane, room temperature, overnight; (e) ; (f) 4.0 M HCl in 1,4-dioxane, 1,4-dioxane, MeOH, 50 ^{o}C, 3 days

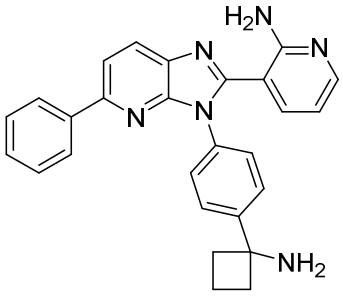
Scheme 6[a]



[a]Reagents and conditions: (a)methyl 1-(4-aminophenyl)cyclobutane-1-carboxylate , DIEA, EtOH, 45°C, overnight; (b) phenyl boronic acid, $Pd(PPh_3)_4$, $NaHCO_{3\ aq.}$, EtOH, toluene, 100°C, overnight; (c) 2-amino-nicotinaldehyde, $Na_2S_2O_4$, DMSO, MeOH, 100°C, overnight; (d) NaOH 1N, THF, MeOH, 65°C; (e) EDC, HOBt, TEA, ammonia, DMF

Table of Content Graphic





21a

Potency in biochemical assay

AKT1 IC_{50}	5.0 nM
AKT2 IC_{50}	4.5 nM
AKT3 IC_{50}	16 nM