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# Second-Generation Antibacterial Benzimidazole Ureas: Discovery of a Preclinical Candidate with Reduced Metabolic Liability

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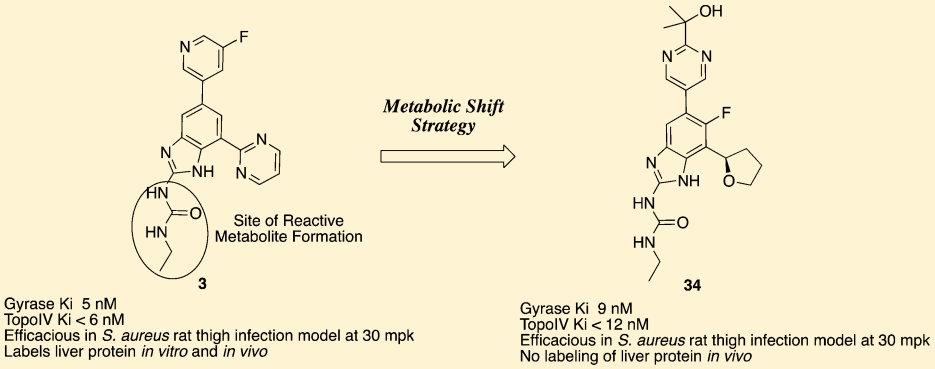
Supporting Information




**ABSTRACT:** Compound **3** is a potent aminobenzimidazole urea with broad-spectrum Gram-positive antibacterial activity resulting from dual inhibition of bacterial gyrase (GyrB) and topoisomerase IV (ParE), and it demonstrates efficacy in rodent models of bacterial infection. Preclinical in vitro and in vivo studies showed that compound **3** covalently labels liver proteins, presumably via formation of a reactive metabolite, and hence presented a potential safety liability. The urea moiety in compound **3** was identified as being potentially responsible for reactive metabolite formation, but its replacement resulted in loss of antibacterial activity and/or oral exposure due to poor physicochemical parameters. To identify second-generation aminobenzimidazole ureas devoid of reactive metabolite formation potential, we implemented a metabolic shift strategy, which focused on shifting metabolism away from the urea moiety by introducing metabolic soft spots elsewhere in the molecule. Aminobenzimidazole urea **34**, identified through this strategy, exhibits similar antibacterial activity as that of **3** and did not label liver proteins in vivo, indicating reduced/no potential for reactive metabolite formation.

## INTRODUCTION

Over the past decade, the introduction of a handful of new antibacterial agents as well as the implementation of programs aimed at minimizing the spread of antibiotic-resistant bacterial strains and limiting the overuse of novel antibiotics has produced some short-term successes in the battle against drug resistance.[1] However, resistance to antibiotics remains a global human threat with the potential for catastrophic consequences in the future. Infections caused by drug resistant Gram-positive organisms, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), remain a critical issue not only because of the morbidity and mortality caused by these infections but also because of the associated economic cost.[1−3] Thus, an unmet medical need remains for a safe antibiotic suitable for extended use to treat serious bacterial infections.[4,5]

We previously reported the discovery of a novel class of dual-targeting benzimidazole ureas that inhibit DNA gyrase and topoisomerase IV and have potent antibacterial activity.[6] This work and subsequent studies led to the identification of several potential preclinical candidates (Figure 1 and Table 1). Compound **1** was extensively characterized and demonstrated excellent activity in rodent models of infection,[6] but it was shown to be a potent CYP3A4 inhibitor and suffered from a short half-life and poor physicochemical properties.[7] Compound **2** had similar antibacterial properties as those of compound **1**, with a lower serum shift and lack of CYP inhibition. Its solubility profile was improved, but its oral bioavailability was suboptimal.[7] Compound **3** resolved the above issues, exhibited potent activity against a broad range of Gram-positive organisms, and showed efficacy in rodent models of infection as well as an ADME profile compatible with oral and intravenous administration. The ability

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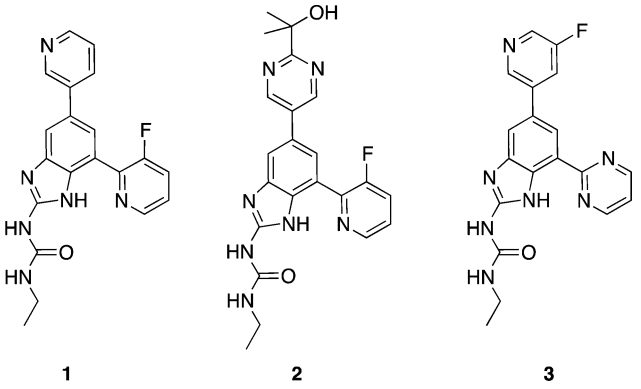

1 2 3

**Figure 1.** Structures of lead aminobenzimidazole ureas **1**–**3**.

**Table 1. Enzyme Inhibition and Antibacterial Profile of Aminobenzimidazole Ureas 1–3**[a]

| | compound | | |
|---|---|---|---|
| | **1** | **2** | **3** |
| *E. coli* Gyrase $K_i$ (nM) | 6 | <4 | 5 |
| *S. aureus* TopoIV $K_i$ (nM) | 6 | <6 | <6 |
| *S. aureus* MIC ($\mu$g/mL) | 0.031 | 0.031 | 0.016 |
| *S. aureus* MIC + HS ($\mu$g/mL) | 1 | 0.25 | 0.25 |
| *S. pneumoniae* MIC ($\mu$g/mL) | ≤0.008 | ≤0.008 | ≤0.008 |
| *E. faecalis* MIC ($\mu$g/mL) | 0.016 | 0.032 | <0.008 |
| *H. influenzae* MIC ($\mu$g/mL) | 1 | 1 | 1 |

[a]HS, 50% human serum.

to dose a Gram-positive agent intravenously would allow use in the hospital for the treatment of infections caused by methicillin-resistant *S. aureus* as well as vancomycin-resistant enterococci. We deemed the option for oral dosing essential for a novel Gram-positive agent because, in addition to providing the possibility of step-down use outside of the hospital setting, there is a need for new drugs active against methicillin-resistant *S. aureus* and fluoroquinolone-resistant *Streptococcus pneumoniae* in the community setting, where intravenous drugs are not easily administered. Compound **3**, when dosed BID orally in a 72 h thigh infection model (*S. aureus*), produced substantial reductions in bacterial CFUs (colony forming units; ≥4 log), demonstrating clear in vivo bactericidality at doses ≥25 mg/kg.[8] However, in vitro and in vivo studies showed that compound **3** presented a potential safety liability, as it was shown to covalently label liver proteins, presumably via the formation of a reactive metabolite. We report herein a second-generation aminobenzimidazole urea with an improved metabolic profile, identified via the implementation of a metabolic shift strategy.

## RESULTS AND DISCUSSION

As part of our preclinical evaluation of compound **3**, $^{14}$C-labeled **3** was synthesized and shown to covalently label liver proteins in vitro in mouse, rat, dog, monkey, and human liver microsomes (Figure 2) following methods published in the literature.[9] Labeling occurred in the presence of NADPH but not in its absence, suggesting that the formation of the reactive species was mediated by CYP oxidation of compound **3**.

To evaluate the potential of compound **3** to form a reactive metabolite in vivo, covalent binding of **3** to rat liver proteins was studied.[8] Male rats were dosed with 90 mg/kg of unlabeled **3** for 6 or 13 days. On day 7 or 14, the animals received 100 $\mu$Ci of $^{14}$C-labeled **3** (specific activity of 1.43 mCi/mmol). A separate

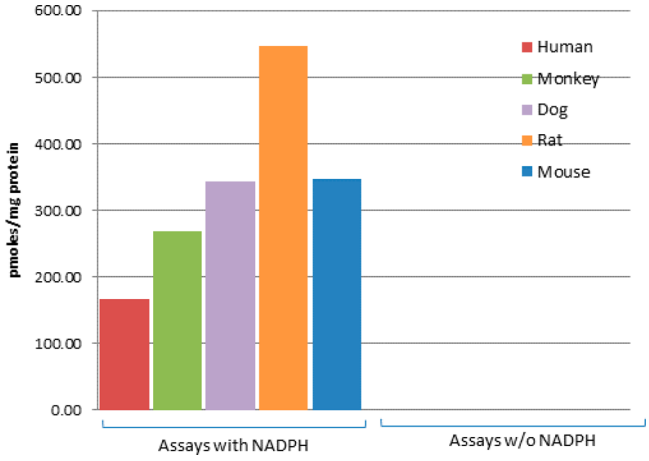


**Figure 2.** In vitro labeling of human, monkey, dog, rat, and mouse microsomal liver proteins by $^{14}$C-labeled **3** incubated at 30 $\mu$M for 1 h in the presence or absence of NADPH. Covalent binding was observed only in the presence of NADPH.

cohort of rats received a single dose of the radiolabeled compound. Four hours after the radiolabeled compound was dosed, all animals were euthanized, and the amount of radiolabel associated with liver proteins was determined (Figure 3). A

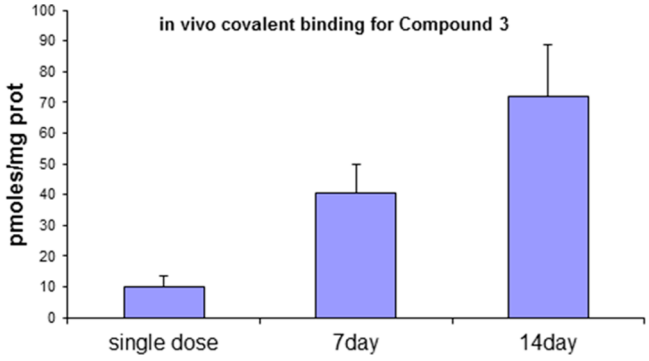


**Figure 3.** Covalent labeling of liver proteins in vivo following 0, 6, or 13 days of dosing with compounds **3**, followed by one dose of $^{14}$C-labeled **3**, resulting in a time-dependent increase in amount of radiolabel incorporation.

time-dependent increase in the amount of radiolabel bound to liver proteins was observed, with levels above 50 pmol/mg at 14 days. The observed time-dependent increase in amount of radiolabel incorporated suggests potential induction of the CYP enzyme(s) responsible for the oxidation of **3** that led to reactive metabolite formation.

**Compound 3 Metabolites.** To identify the putative reactive metabolite of **3**, metabolite identification studies were conducted in vitro in rat, dog, monkey, and human liver microsomes and S9 liver fractions. In vivo plasma samples from rat, dog, and monkey were also examined, leading to a proposed route of metabolism of **3** via dehydrogenation of the ethyl urea moiety (M − 2, **4**) and subsequent epoxidation (M + 14, **5**) (Figure 4). A M + 14 metabolite, shown to be present in vitro in all species in liver microsomes and S9 fractions, was thought to be epoxide **5**, the reactive metabolite responsible for protein covalent labeling. Interestingly, this metabolite, while present in monkey and dog plasma, was not present in rat plasma samples. A metabolite of mass M − 2 was also present in plasma at low levels in all species.


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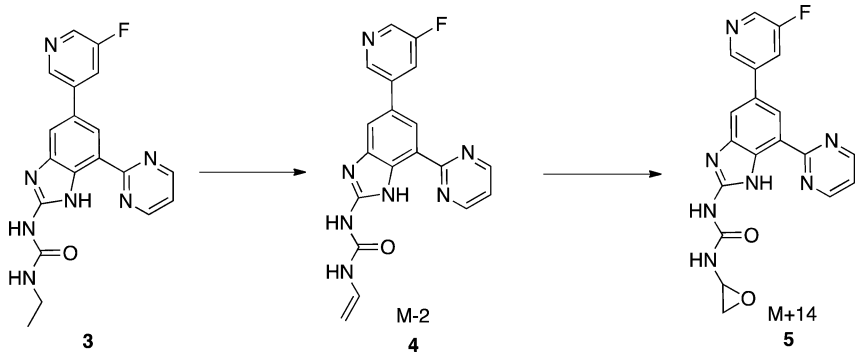


**Figure 4.** Initial proposed route of metabolism of **3**.

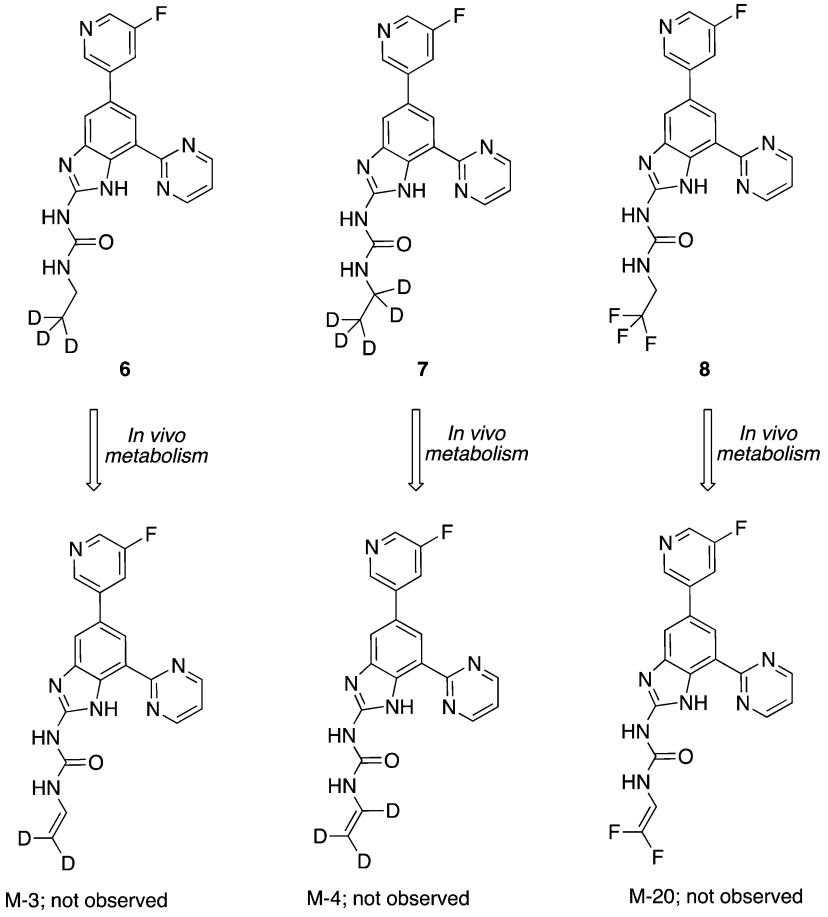


**Figure 5.** Structures of tool compounds **6**–**8**. In vivo metabolism according to Figure 3 was envisioned to yield depicted metabolites M − 3 for **6**, M − 4 for **7**, and M − 20 for **8**. Instead, M − 2, M − 3, and M − 2 metabolites were observed, respectively.

Three follow-up compounds were prepared to investigate the metabolism further: trideuterated analogue **6**, penta-deuterated analogue **7**, and trifluoroethyl analogue **8** (Figure 5).

These compounds were each dosed orally in rat at 30 mg/kg, and plasma, bile, urine, and feces samples were analyzed to identify their metabolites. In the case of deuterated analogues **6** and **7**, the expected metabolites (of mass M − 3 and M − 4, respectively) resulting from dehydrogenation of the urea's ethyl group were not observed. Instead, species of mass M − 2 (for compound **6**) and M − 3 (for compound **7**) were found as major metabolites in plasma, bile, urine, and feces. Trifluoroethyl urea **8** also yielded a metabolite of mass M − 2. These findings led us to hypothesize that metabolism of **3** resulted in the formation of imine **9** (Figure 6), another potential metabolite of mass M − 2, formed via $\alpha$-hydroxylation of the ethyl substituent in **3**, followed by water elimination. This route of metabolism also explained the findings for compounds **6**–**8** shown in Figure 5. Attempted de novo synthesis of imine **9** did not result in isolation of the desired compound; rather, compound **10**, also of mass M − 2, resulting from cyclization of imine **9** was isolated. The structure of compound **10** was confirmed by 2D NMR experiments (see Supporting Information). Synthesized compound **10** was shown to be identical to the metabolite obtained from compound **3** in vivo by coelution experiments, and it showed the same fragmentation pattern in mass spectrometry (see Supporting Information). Additional MS/MS studies of the observed M + 14 metabolites suggested that these metabolites arise from oxidation of compound **10** (Figure 6, see Supporting Information).


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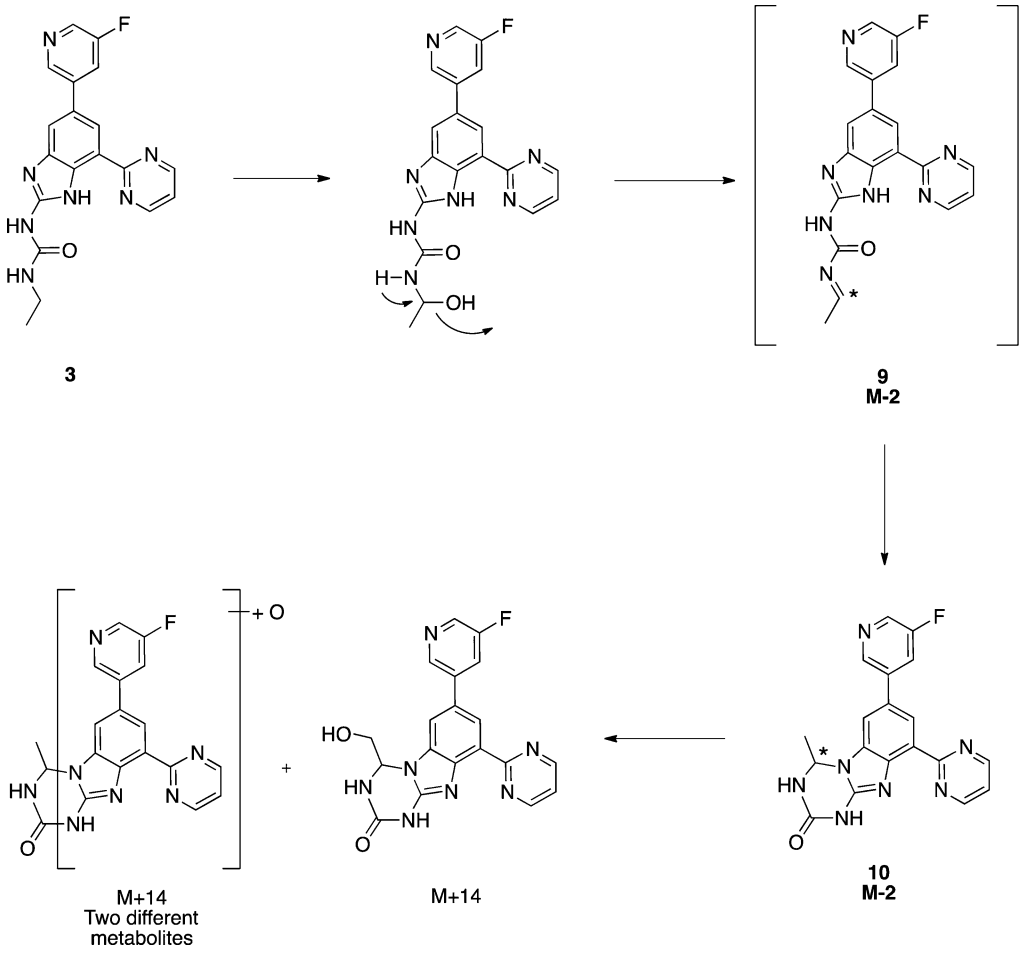


**Figure 6.** M − 2 metabolite formation. Initial oxidation takes place on the aliphatic carbon attached to the urea moiety in **3**. Subsequent loss of water and cyclization leads to **10**. The asterisk-labeled carbon atoms in structures **9** and **10** denote activated centers susceptible to nucleophilic attack. Three M + 14 metabolites are formed by oxidation of compound **10**.

Compound **6** was also shown to produce corresponding M + 13 metabolites with structures similar to those obtained from compound **3**. Our conclusion from these studies is that compound **10** is the M − 2 metabolite obtained from compound **3**. We also hypothesize that the initial observed M + 14 metabolite is not the proposed structure **5** (Figure 4) but instead results from further oxidation of compound **10** (Figure 6), although this was not unequivocally determined through chemical synthesis.

Incubation of compound **3** in individual recombinant CYP supersomes at 1 and 10 $\mu$M was shown to produce compound **10** (Figure 7). Incubations containing CYP3A4, CYP1A2, and CYP2D6 showed formation of compound **10** at 10 $\mu$M, confirming that compound **10** is formed via oxidative metabolism mediated by P450 enzymes.

Our conclusion from these studies is that cyclized intermediate **10**, formed via CYP-mediated oxidation of **3** followed by loss of water and intramolecular cyclization, is likely to be the putative reactive metabolite formed upon dosing of compound **3**. It is, however, also possible that transient imine **9** itself acts as the reactive metabolite.

**Addressing Reactive Metabolite Formation: Attempts To Replace the Urea Moiety.** Our first approach toward identifying an aminobenzimidazole analogue with antibacterial activity comparable to that of **3** and reduced potential for reactive metabolite formation focused on identifying a replacement of the presumed site for reactive metabolite formation in compound **3**, namely, the ethyl urea moiety. As observed for previously reported aminobenzimidazole analogues,[6] the crystal structure of **3** bound to bacterial *S. aureus* GyrB highlights several key

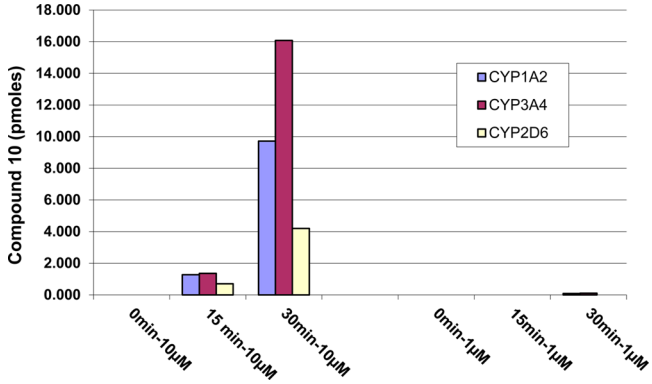


**Figure 7.** Incubations of compound **3** in supersomes. Compound **10** was not formed in the experiments without NADPH. Incubations containing recombinant CYP2B6, CYP2C9, CYP2C8, and CYP2C19 did not show the formation of compound **10**. Incubations with CYP3A4, CYP1A2, and CYP2D6 showed formation of compound **10**. Incubations containing 1 $\mu$M compound **3** did not show formation of the imine metabolite. The amount of metabolite formed increased significantly at 10 $\mu$M at 30 min, and the highest amount was seen in incubations containing CYP3A4 supersomes.


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interactions contributing to its affinity: a hydrogen bond between the C5-fluoropyridine nitrogen and Arg136, a cation−$\pi$ stacking interaction between the C5-fluoropyridine and Arg76, and a bidentate hydrogen bond between the urea moiety and Asp73 (Figure 8). Additionally, an intramolecular hydrogen bond

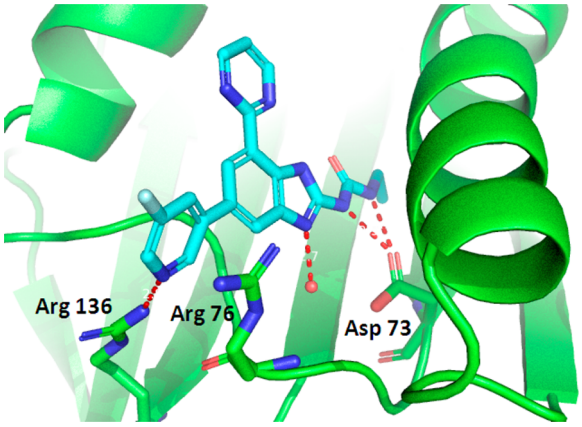


**Figure 8.** Crystal structure of compound **3** in complex with *S. aureus* gyrase B (PDB ID: 4P8O). The depicted interactions are (a) a bidentate hydrogen bond between the urea NH's of **3** and Asp73, (b) a hydrogen bond between the pyridine nitrogen of **3** and Arg136, (c) cation−$\pi$ stacking interaction between the pyridine ring of **3** and Arg76. Also depicted is a hydrogen bond between one of the benzimidazole nitrogens and a conserved water molecule.

helps to maintain coplanarity between the pyrimidine and the benzimidazole rings, a feature previously shown to be optimal for dual activity against gyrase B and topoisomerase IV for the aminobenzimidazoles.[6]

With these elements in mind, we designed over 40 analogues of compound **3** incorporating small urea mimics, due to the steric constraints in this portion of the ATP binding site, with the potential to maintain at least one hydrogen bond with Asp73. Also essential to the design was the lack of potential for these compounds to afford M − 2 imines upon metabolism. These analogues were synthesized, and their MIC's against *S. aureus* were measured (Figure 9).

In most instances, the MIC's of these analogues against *S. aureus* were greater than 8 $\mu$g/mL. Very small changes to the ethyl urea moiety in compound **3** resulted in significant loss of antibacterial activity, as exemplified by amides **12** and **13** (Table 2, *S. aureus* MICs > 8 $\mu$g/mL). Only two analogues, carbamate **14** and amide **15**, maintained MICs against *S. aureus* below 0.1 $\mu$g/mL (Table 2). However, both compounds exhibited poor properties, including poor solubility, large shifts in MICs when tested in the presence of 50% human serum, and/or lack of oral exposure; hence, these compounds were not pursued further.

**Addressing Compound 3 Reactive Metabolite Formation: Metabolic Shift Strategy.** Having been unsuccessful in identifying a suitable replacement for the urea moiety in compound **3**, we devised what we termed a metabolic shift strategy, in which we attempted to shift metabolism away from the urea by introducing a diverse set of metabolically labile groups in our lead compounds.[10] Utilizing the SAR knowledge gained in the work leading to the identification of our preclinical candidates,[6] we identified two C7 substituents as new starting points toward aminobenzimidazole ureas with different metabolic profiles: 1-pyrazole and 2-tetrahydrofuran. Both of these C7 moieties were attractive for several reasons: (a) they maintain planarity between the benzimidazole core and the C7 substituent, and an intramolecular hydrogen bond between the C7 substituent and the aminobenzimidazole NH helps achieve ligand preorganization (Figure 10); (b) they are structurally diverse from previously investigated C7 substituents and

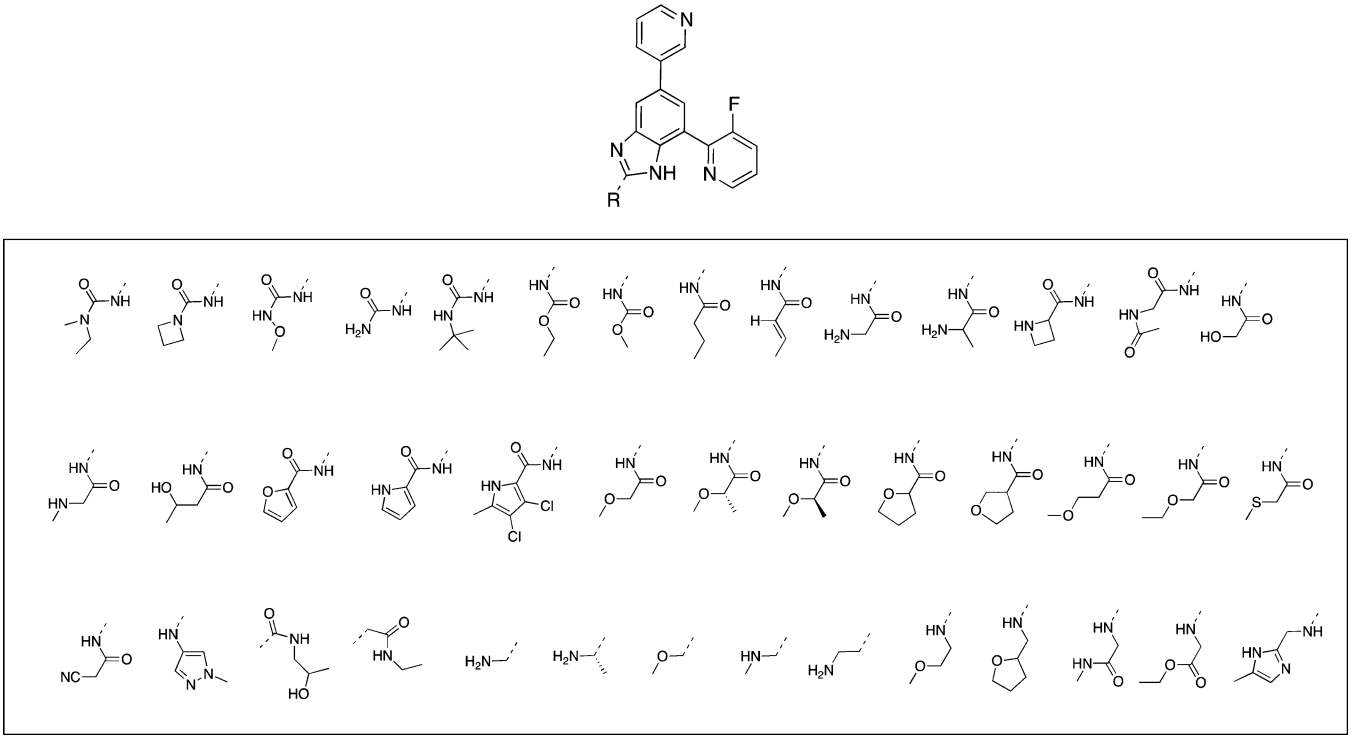

**Figure 9.** Urea mimics of compound **3**. All compounds were designed to eliminate the potential for formation of M − 2 imines (see **4** in Figure 3).


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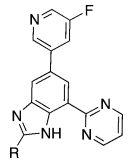
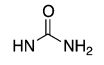
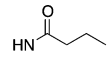
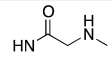
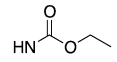
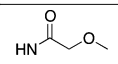
**Table 2. Antibacterial Profiles of Selected Urea Replacements**[a]



| | | | Minimum inhibitory concentration (MIC) (μg/mL) | | | | |
|---|---|---|---|---|---|---|---|
| Cpd | R | cLogP/PSA | *S. aureus* | *S. aureus* + HS | *E. faecalis* | *E. faecium* | *S. pneumoniae* |
| **11** |  | 2.0/122 | > 8 | > 8 | ND | ND | ND |
| **12** |  | 2.2/92 | > 8 | > 8 | ND | ND | ND |
| **13** |  | 1.5/105 | > 8 | > 8 | > 8 | > 8 | > 8 |
| **14** |  | 1.6/101 | 0.016 | >8 | 0.016 | 0..032 | 0.008 |
| **15** |  | 2.3/114 | 0.064 | 8 | 0.032 | 0.064 | 0.032 |

[a]HS, 50% human serum.

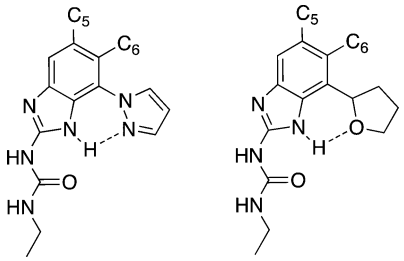


**Figure 10.** Intramolecular hydrogen bond in the C7-pyrazoles and C7-tetrahydrofurans resulting in ligand preorganization.

therefore are likely to have a different metabolic profile; and (c) both of these substituents present lower cLogP's, which is advantageous, as higher LogP values have been correlated with increased toxicity.[11] We have demonstrated previously that for the aminobenzimidazoles, maintaining planarity between the aminobenzimidazole core and the substituent at C7 is key for dual inhibition of gyrase and topoisomerase IV.[6] Other dual inhibitors of bacterial gyrase and topoisomerase IV that bind to the ATP-binding site have been reported in the literature.[12−17] Series that are isosteric to the aminobenzimidazoles exist,[13−15,17] and conformational analysis of representative compounds from these series suggest that their lowest-energy conformation is planar as well, independently of whether the analysis is performed on the bound or unbound state. To drive our optimization effort, we focused primarily on MICs against *S. aureus* because, historically, this Gram-positive organism had been the most difficult to inhibit effectively with the aminobenzimidazole series. MIC assays were run under Clinical and Laboratory Standards Institute (CLSI) conditions as well as in the presence of 50% human serum to assess the effect of protein binding on compound potency (serum-shifted MIC). A serum-shifted MIC against *S. aureus* of 0.5 μg/mL or lower was deemed to be acceptable to justify compound progression. Additionally, we evaluated potential for acceptable pharmacokinetics by assessing oral exposure in rat after administration of a 30 mg/kg dose of compound in an appropriate vehicle. Finally, our optimization approach focused on exploring solubilizing substituents in order to maintain potential for IV dosing.

**1-Pyrazole at C7.** In the C7 pyrazole series, the substituent at C5 was investigated (Table 3). Compound **16** with a 3-pyridine moiety at C5, which maintains a hydrogen bond with Arg136, showed a reasonable MIC against *S. aureus*, but it showed a 16-fold serum shift. Introduction of a fluorine atom at C6 (pyrazole **17**), which improved antibacterial potency as well as affinity for gyrase B and topoisomerase IV in earlier series,[6] did not result in a significant improvement in *S. aureus* MIC. However, oral exposure was improved 2-fold and therefore we focused exclusively on C6-fluorobenzimidazole pyrazoles. Next, introduction of polarity at C5 was investigated in an effort to improve MICs and solubility. Solubilizing groups on the pyridine moiety were tolerated, as shown by the activity of compound **18**. Introduction of a methyl substituent at the 4-position of the pyrazole in **18** (compound **19**) resulted in a 4-fold improvement in the *S. aureus* MIC. However, this modification resulted in reduced oral exposure. As in our earlier report,[6] pyridones continued to show excellent antimicrobial activity, as exemplified by compound **20** (*S. aureus* MIC = 0.016 μg/mL), but failed to show acceptable oral exposure. Solubilizing substituents other than amines, particularly alcohols, were also investigated. Compound **21** showed a MIC against *S. aureus* of 0.125 μg/mL and could be improved slightly by the addition of a methyl group on the C5 substituent, as in **22**. Resolution of **22** yielded compounds **23** and **24**. The (*S*)-isomer **23** was 4-fold more potent than the (*R*)-isomer **24** against *S. aureus* and showed acceptable oral exposure. However, compound **23** exhibited a high serum shift (16-fold) and was not investigated further.

**2-Tetrahydrofuranyl Substituent at C7.** The potency of the 2-tetrahydrofuranyl C7 series was first evaluated with compounds **25** (3-pyridyl at C5) and **26** (5-pyrimidinyl substituent at C5) (Table 4). Both showed moderate to good activity against


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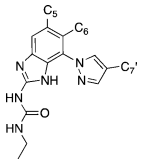
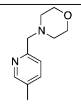
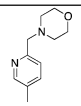
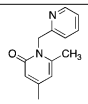
**Table 3. C7 Pyrazole Series SAR**[a]



| | | | | | Minimum inhibitory concentration μg/mL | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Cmpd | C5 | C6 | C7' | cLogP/ PSA | *S. aureus* | *S. aureus* + HS | *E. faecalis* | *E. faecium* | *S. pneumoniae* | Rat PO AUC μg.h/mL (normalized to 30 mpk) |
| **16** |  | H | H | 1.9/101 | 0.25 | 4 | 0.063 | - | 0.016 | 5.7 |
| **17** |  | F | H | 2.1/101 | 0.25 | 2 | 0.063 | - | 0.032 | 10.8 |
| **18** |  | F | H | 1.9/113 | 0.25 | 1 | 0.125 | 0.5 | 0.016 | 13.8 |
| **19** |  | F | Me | 2.4/113 | 0.063 | 0.5 | 0.063 | 0.25 | 0.016 | 1.9 |
| **20** |  | F | Me | 2.7/123 | 0.016 | 0.125 | 0.016 | 0.032 | < 0.008 | 0.2 |
| **21** |  | F | H | 2.0/121 | 0.125 | 3 | 0.125 | 0.5 | 0.032 | ND |
| **22** |  | F | H | 2.5/121 | 0.063 | 2 | 0.125 | 0.25 | 0.032 | 7.2 |
| **23** |  | F | H | 2.5/121 | 0.063 | 1 | 0.063 | 0.5 | 0.016 | 8.4 |
| **24** |  | F | H | 2.5/121 | 0.25 | 4 | 0.063 | 0.25 | 0.063 | ND |

[a]HS, 50% human serum; ND, not determined.

*S. aureus* and produced low serum shifts when tested in the presence of 50% human serum. As in the case of the C7 pyrazoles, introduction of a pyridone substituent at C5 resulted in an improvement in antibacterial activity and a minimal serum shift. However, neither of the two pyridones (**27** and **28**) showed good exposure when dosed orally in rat. On the basis of previous SAR, C5-azine alcohols were also investigated. Introduction of a *gem*-dimethyl hydroxymethyl substituent at the 6-position of the pyridine ring (compound **29**) resulted in a *S. aureus* MIC of 0.5 and a 4-fold serum shift. The corresponding pyrimidine (compound **30**) showed a slightly improved *S. aureus* MIC and a serum shift of only 2, probably due to this compound's higher polar surface area. Addition of a C6 fluorine atom (compound **33**) produced the expected further improvement in antibacterial activity, along with a higher but still acceptable serum shift of 8. Resolution of compounds **30** and **33** yielded **31** and **32** and **34** and **35**, respectively. In both cases, the (*R*)-isomers were more potent than the (*S*)-isomers (the *R* stereochemistry for both of these compounds was confirmed through small molecule X-ray crystallography; see Supporting Information for the small molecule X-ray structure of compound **34**). When dosed at 1 mg/kg in rat (in 25% dimethylacetamide/30% propylene glycol/5% polysorbate-80/40% water as the vehicle), the IV clearance for **34** was 49 mL/min/kg, and the half-life was 1.9 h. Oral bioavailability in rat was 48% following a 3 mg/kg PO dose (vehicle: 0.5% methylcellulose in water). The rat IV PK parameters of compound **34** were similar to those of compound **3** (clearance = 31 mL/min/kg; half-life = 1.1 h when dosed at 1 mg/kg in 5% dextrose in water at pH 3). Compound **34** was selected for further evaluation because of its overall antibacterial profile and its adequate oral exposure in rat.

**In Vitro and in Vivo Antibacterial Profile of Compound 34.** Compound **34** was shown to be a potent dual inhibitor of


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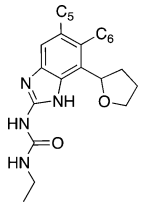
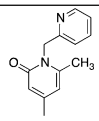
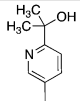
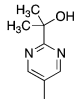
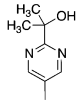
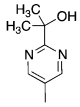
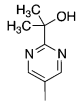
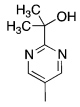
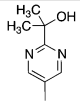
**Table 4. C7 THF Series SAR**[a]



| | | | | | Minimum inhibitory concentration (MIC) μg/mL | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Cpd** | **C5** | **C6** | THF stereochemistry | cLogP/ PSA | *S. aureus* | *S. aureus* + *HS* | *E. faecalis* | *E. faecium* | *S. pneumoniae* | Rat PO AUC @30 mpk μg.h/mL |
| **25** |  | H | racemate | 2.2/92 | 1 | 4 | 0.25 | - | 0.125 | ND |
| **26** |  | H | racemate | 1.5/105 | 1 | 2 | 0.5 | 1 | 0.125 | 10.5 |
| **27** |  | H | racemate | 1.6/101 | 0.75 | 0.75 | 0.25 | 0.25 | 0.016 | BQL |
| **28** |  | H | racemate | 2.3/114 | 0.125 | 0.25 | 0.125 | 0.25 | < 0.008 | BQL |
| **29** |  | H | racemate | 2.5/112 | 0.5 | 2 | 0.25 | 1 | 0.063 | 2.6 |
| **30** |  | H | racemate | 2.1/125 | 0.25 | 0.5 | 0.25 | 0.5 | 0.032 | 5.5 |
| **31** |  | H | (*R*) | 2.1/125 | 0.125 | 0.25 | 0.063 | 0.5 | 0.032 | 4.3 |
| **32** |  | H | (*S*) | 2.1/125 | 1 | 2 | 1 | 2 | 0.5 | ND |
| **33** |  | F | racemate | 2.3/125 | 0.063 | 0.5 | 0.125 | 0.5 | 0.032 | ND |
| **34** |  | F | (*R*) | 2.3/125 | 0.032 | 0.25 | 0.032 | 0.125 | < 0.008 | 11.7 |
| **35** |  | F | (*S*) | 2.3/125 | 0.5 | 4 | 0.5 | 2 | 0.25 | ND |

[a]HS: 50% human serum; BQL: below quantitation levels; ND, not determined.

*S. aureus* gyrase and *S. aureus* topoisomerase IV with $K_i$'s of <12 and 9 nM, respectively. In a panel of Gram-positive organisms and selected Gram-negative organisms (Table 5), **34** exhibited antibacterial potencies generally below 0.25 μg/mL; this profile


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**Table 5. Antibacterial Activity of Compound 34 against Selected Gram-Positive and -Negative Microorganisms**

| species | strain/assay conditions | MIC ($\mu$g/mL) |
|---|---|---|
| Gram-Positive | | |
| *Staphylococcus aureus* | ATCC 29213 | 0.016 |
| +50% human serum | | 0.125 |
| *S. epidermidis* | ATCC 12228 | ≤0.008 |
| *Enterococcus faecalis* | ATCC 29212 | 0.016 |
| *E. faecium* | ATCC 49624 | 0.063 |
| *Streptococcus pneumoniae* | ATCC 10015 | ≤0.008 |
| Gram-Negative | | |
| *Haemophilus influenzae* | ATCC 49247 | 0.5 |
| *Moraxella catarrhalis* | ATCC 25238 | ≤0.016 |

was well-aligned with our target indications, which included bacteremia, endocarditis, and osteomyelitis. In addition, compound **34** showed bactericidal activity against key organisms, exhibited low resistance frequencies, and maintained activity against multidrug resistant organisms.[7]

The potential of **34** to treat skin and skin structure infections was evaluated in a neutropenic rat thigh infection model (*S. aureus* ATCC 29213).[6,18] In untreated animals, the bacterial loads in thigh tissue increased by 1.41 and 1.62 $\log_{10}$ colony forming units (CFUs) at 8 and 24 h (LC, late controls), respectively, as compared to the bacterial load at the start of dosing (EC, early controls). Administration of **34** to infected animals resulted in a dose-related and time-dependent antibacterial effect (Figure 11). All doses of compound **34** provided statistically significant decreases versus the 24 h late controls, indicating bacteriostatic activity for all doses. Compared with initial burden prior to treatment (EC), median bacterial density in the thigh after 24 h of treatment with the two higher doses (30 and 60 mg/kg) of compound **34** was significantly reduced (at least −1 $\log_{10}$ difference versus EC), indicating modest bactericidal compound activity. Bacterial killing was time-dependent; it was less pronounced after the shorter 8 h treatment with 30 and 60 mg/kg of compound **34** (approximately −0.6 $\log_{10}$ in median bacterial density versus EC). The q12h doses of 30 and 60 mg/kg of compound **34** provided similar decreases in *S. aureus* thigh burdens as that of 30 mg/kg q12h of the comparator moxifloxacin.

**Metabolism of Compound 34.** The metabolism of compound **34** was investigated in vitro through incubations with mouse, rat, dog, monkey, and human liver microsomes and hepatocytes and in vivo through studies in bile duct-cannulated rats that received a single oral dose of the compound. Metabolites were analyzed on multiple MS platforms, including high-resolution LC-MS/MS. As summarized in Figure 12, compound **34** was metabolized primarily via oxidation followed by glucuronidation (see Supporting Information). Only a very low amount of the M − 2 cyclized metabolite (<2%) was observed.

To study the potential of compound **34** to form reactive metabolites, covalent binding of **34** to rat liver protein was evaluated in vivo.[9] Male rats were dosed with 90 mg/kg of unlabeled **34** for 6 or 13 days. On day 7 or 14, the animals received 100 $\mu$Ci of $^{14}$C-labeled **34** (specific activity of 55 mCi/mmol). A separate cohort of rats received a single dose of the radiolabeled compound. Four hours after the radiolabeled compound was dosed, all animals were euthanized, and the amount of radiolabel associated with liver proteins was determined. The amount of radiolabel bound to liver protein in all samples was less than 5 pmol/mg of protein, suggesting that **34** has very low potential to covalently bind to rat liver proteins in vivo (Figure 13). Taken in isolation, the lack of a time-dependent increase in radiolabel incorporation with compound **34** could be due to a reduction of potential CYP induction with compound **3**. However, the lack of liver labeling after a single dose of compound **34** supports a metabolic shift from the ethyl group of compound **3** to another position in compound **34**.

Taken together, these studies suggest that metabolism of **34** occurs away from the urea moiety. Studies in bile duct-cannulated rats have shown metabolism of compounds **3** and **34** to be mainly hepatic. Because of their similar rat IV clearance numbers, **3** and **34** are cleared at the same rate but through different pathways, supporting a metabolic shift from compound **3** to compound **34**.

## CONCLUSIONS

Aminobenzimidazole ureas are a novel class of antibacterial agents that exert their potent activity via dual inhibition of both bacterial gyrase B and topoisomerase IV. The development of an early preclinical candidate (**3**) from this series was halted due to evidence for the formation of a reactive metabolite in vivo. By implementing a metabolic shift strategy to circumvent the metabolic path responsible for reactive metabolite formation, we have identified a second-generation aminobenzimidazole urea

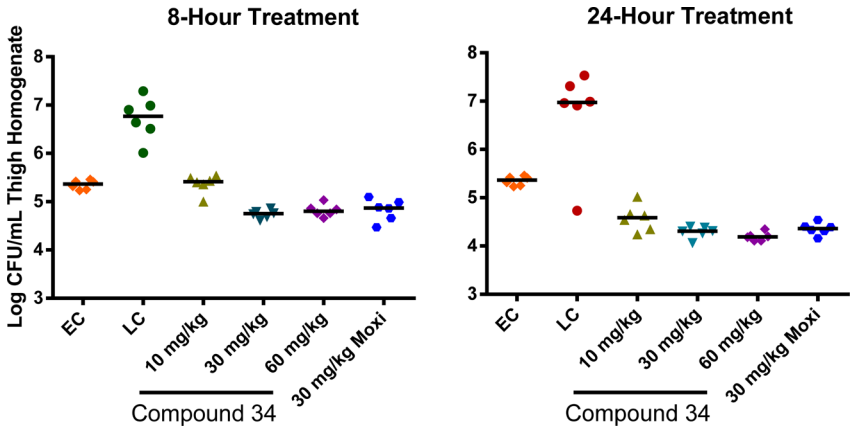


**Figure 11.** Efficacy of compound **34** in a neutropenic *S. aureus* thigh infection model. Compound **34** was dosed q12h at 10, 30, and 60 mg/kg, and moxifloxacin was dosed q12h at 30 mg/kg. Compound **34** at 30 and 60 mg/kg provided similar reduction in bacterial burden as that of moxifloxacin at 30 mg/kg at both 8 and 24 h post-treatment.


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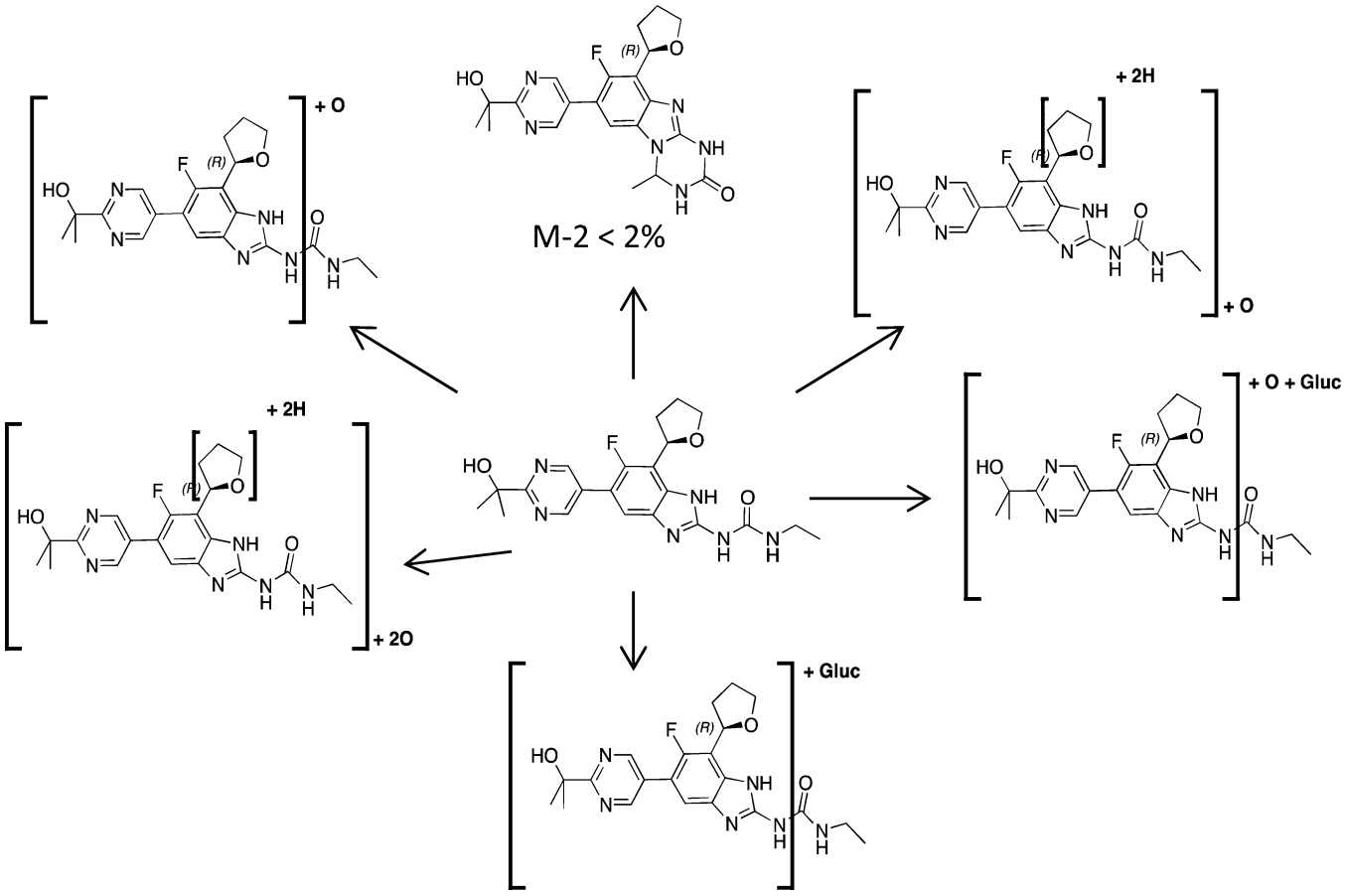


**Figure 12.** Putative metabolites of compound **34** from in vitro and in vivo experiments.

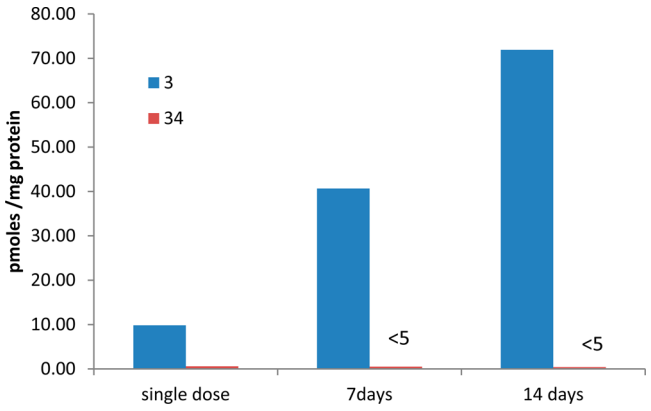


**Figure 13.** Covalent labeling of liver proteins in vivo following 0, 6, or 13 days of dosing with compounds **3** or **34**, followed by one dose of $^{14}$C-labeled **3** or **34**. Experiments with compound **34** did not result in incorporation of radiolabel, whereas studies with compound **3** resulted in a time-dependent increase in the amount of radiolabel incorporation.

(**34**) devoid of the metabolic liability of compound **3**. Compound **34** has been selected as a preclinical candidate, and studies are ongoing toward first-in-human clinical trials. Results will be reported in due course.

## EXPERIMENTAL SECTION

**Syntheses of Benzimidazole Ureas.** Benzimidazole ureas were generally synthesized according to the methods published in ref 6. All syntheses proceeded to an appropriately substituted *ortho*-phenylenediamine intermediate that was then converted to the ethyl urea according to the methods reported in refs 6, 19, and 20. *Ortho*-phenylenediamines were synthesized by reduction of the corresponding *ortho*-nitroanilines. C7 substituents were generally introduced via a Suzuki coupling (pyridine and tetrahydrofuran) or via $S_NAr$ of an appropriately substituted phenyl halide (pyrazole). C5 substituents were installed via Suzuki couplings.

The preparation of compound **2** started with conversion of bromoiodopyrimidine **36** to alcohol **37** (Scheme 1). Coupling of **37** to **38**[6] under Miyaura−Suzuki conditions afforded nitroaniline **39**. Reductive hydrogenation followed by condensation with reagent **41**[6,19] afforded compound **2**.

The synthesis of **6** and **7** required the preparation of deuterated versions of the urea forming reagents **43a** and **43b**.[20] Condensation with *ortho*-phenylenediamine **44**[20] at pH 3.5 afforded **6** and **7**, respectively (Scheme 2).

The synthesis of compounds **8**, **10**, and **11** proceeded through intermediate **45** obtained via condensation of amine **44** with commercially available methyl *N*-(*N*-methoxycarbonyl-C-methylsulfanylcarbonimidoyl)carbamate. Compounds **8** and **11** were synthesized via aminolysis of **45** with trifluoroethylamine or ammonia, respectively. Treatment of **11** with acetaldehyde under dehydrating conditions yielded tricycle **10** (Scheme 3).

The synthesis of compounds **12**−**15** required the introduction of amide or carbamate moieties in the last step. For compound **12**, this was achieved by preparing the amide transfer reagent **46** and condensing it with diamine **44**. Carbamate **14** was prepared in the same manner as that for compound **45**, using the commercially available 1,3-bis(ethoxycarbonyl)-*S*-methylisothiourea (Scheme 4). Another convenient way to prepare amides was via aminobenzimidazole intermediate **47**, prepared efficiently by hydrolysis of compound **3** (Scheme 5). Coupling of **47** with the desired carboxylic acid under standard amide coupling conditions yielded compounds **13** and **15**.


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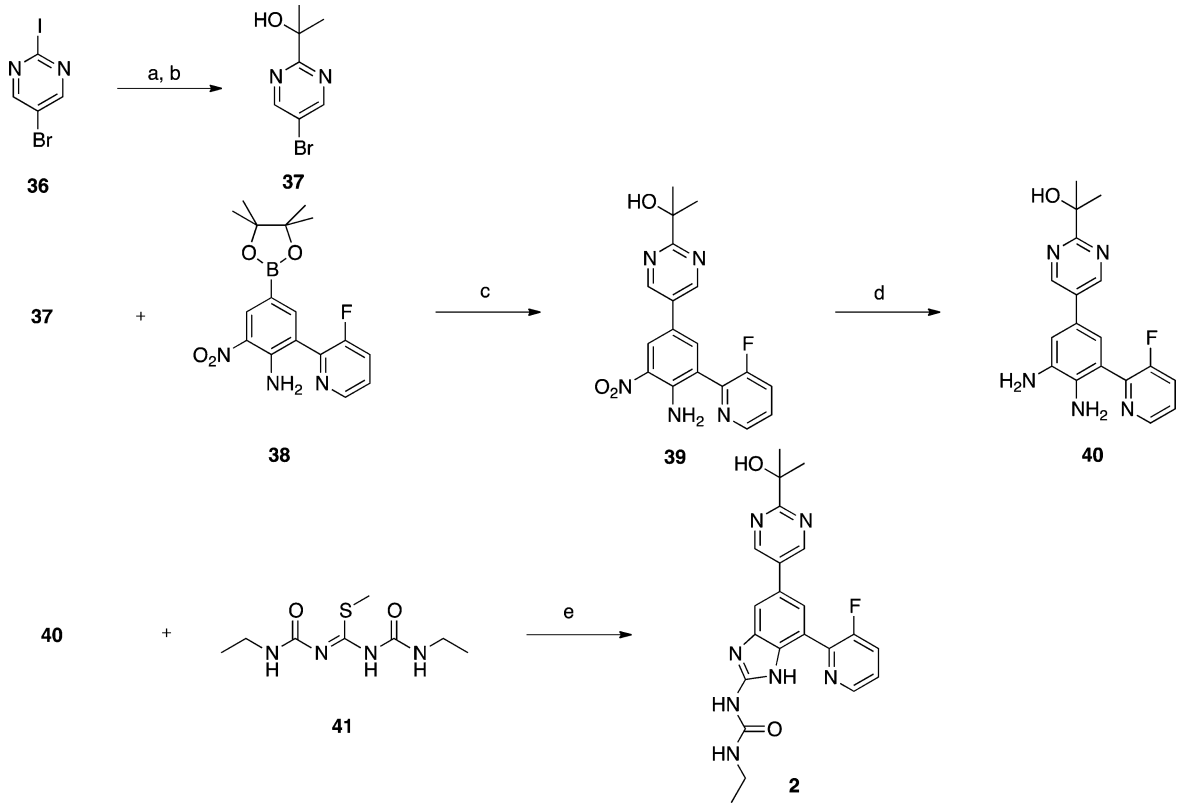
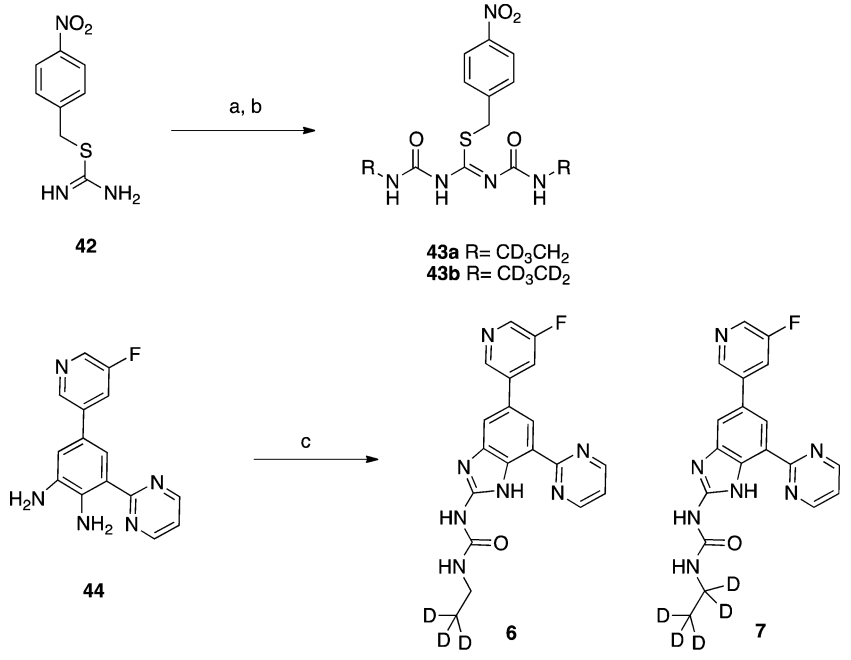
**Scheme 1.** [a]




[a](a) *n*-BuLi, $CH_2Cl_2$, −65 °C; (b) acetone, $CH_2Cl_2$, −65 °C to rt; (c) $Pd(PPh_3)_4$, $Na_2CO_3$, DMF, reflux; (d) 45 psi $H_2$, Raney Ni, methanol; (e) pH 3.5 buffer, 1,4-dioxane, reflux.

**Scheme 2.** [a]




[a](a) DIPEA, CDI, DMF; (b) $d_3$-diethylamine hydrochloride or $d_5$-diethylamine hydrochloride; (c) **43a** or **43b**, pH 3.5 buffer, DME, 80 °C.

The synthesis of compound **17** (Scheme 6) started from 2,3,6-trifluoroaniline, which was brominated and then oxidized to afford **50**. Treatment of **50** with the anion of pyrazole resulted in the exclusive displacement of the fluorine at the 2-position in **50**, to afford **51**. Treatment of **51** with ammonia yielded bromonitroaniline **52a**, which was converted to nitroaniline **53** by Suzuki coupling with the desired boronic acid. Subsequent reduction of **53** and condensation of the intermediate *ortho*-phenylenediamine completed the synthesis of **17**.

Ureas **18**−**22** were prepared in a similar fashion (Schemes 7−9). Coupling of boronate **55** with **52a** or **52b** afforded the desired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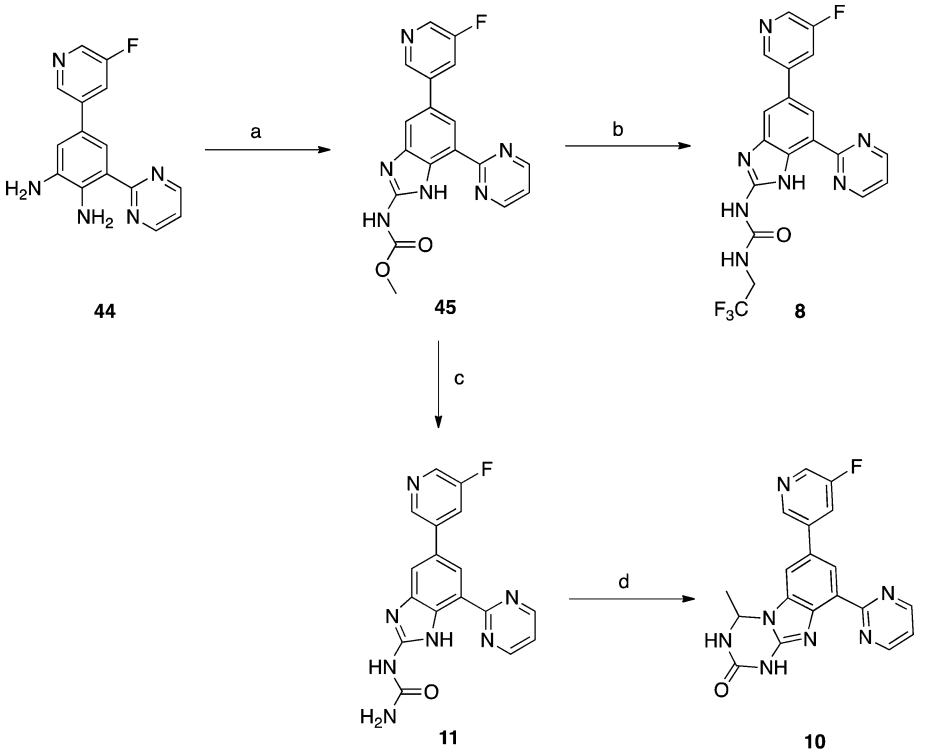
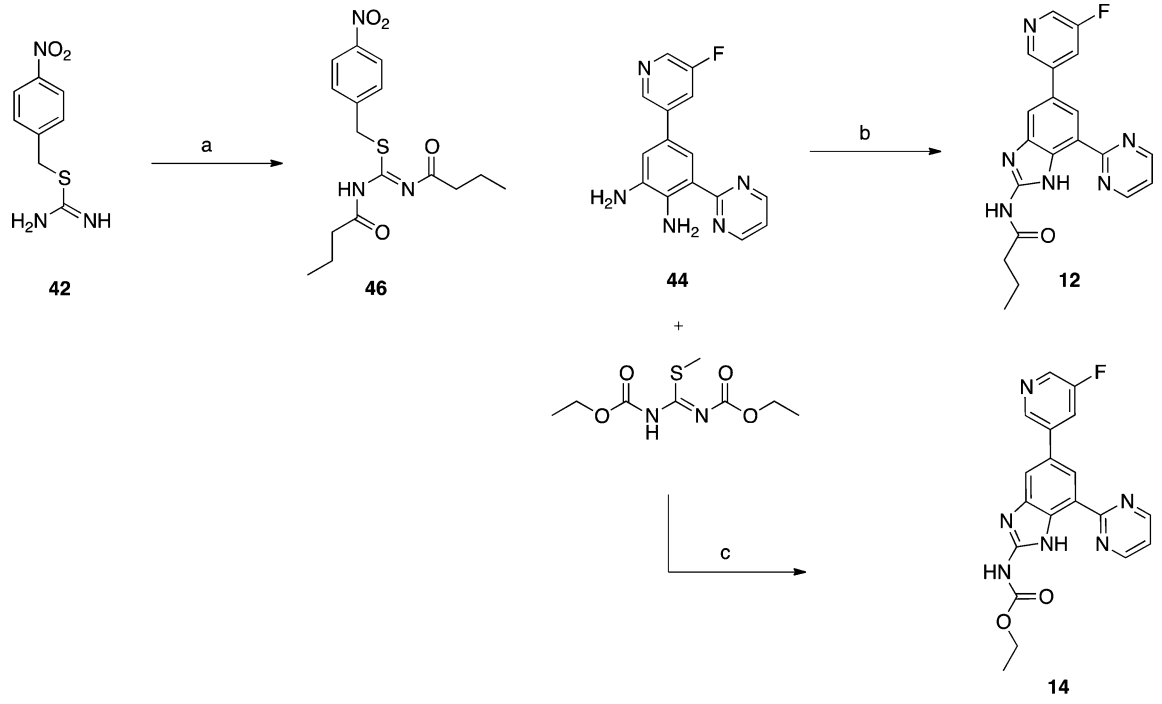
**Scheme 3.** [a]




[a](a) Methyl *N*-(*N*-methoxycarbonyl-C-methylsulfanyl-carbonimidoyl)carbamate, pH 3.5 buffer, 1,4-dioxane, reflux; (b) 2,2,2-trifluoroethanamine, NMP; (c) ammonia, NMP, 120 °C; (d) acetaldehyde, TFA, 4 Å sieves, rt.

**Scheme 4.** [a]




[a](a) Butyric anhydride, 2 N NaOH(aq), DME, rt; (b) **46**, pH 3.5 buffer, DME, rt; (c) pH 3.5 buffer, 1,4-dioxane, reflux.

nitroanilines, which were converted to **18** and **19**, respectively, using the same two-step process described for **17**. Coupling of triflate **57** with bromonitroaniline **52b** afforded **58**, which was converted to **20**. Compound **21** was prepared via coupling of 1-(5-bromo-2-pyridyl)ethanol to the boronate of **52a**. For the synthesis of **22**, boronate **60** was coupled to bromide **52a**. Ureas **23** and **24** were obtained via SFC separation of **22**.

The synthesis of the C7 tetrahydrofuranyl derivatives proceeded through intermediates **61** (for C6 = H) or **62** (where C6 = F) (Scheme 10). The syntheses of **61** and **62** are reported in ref 14. Similar to the C7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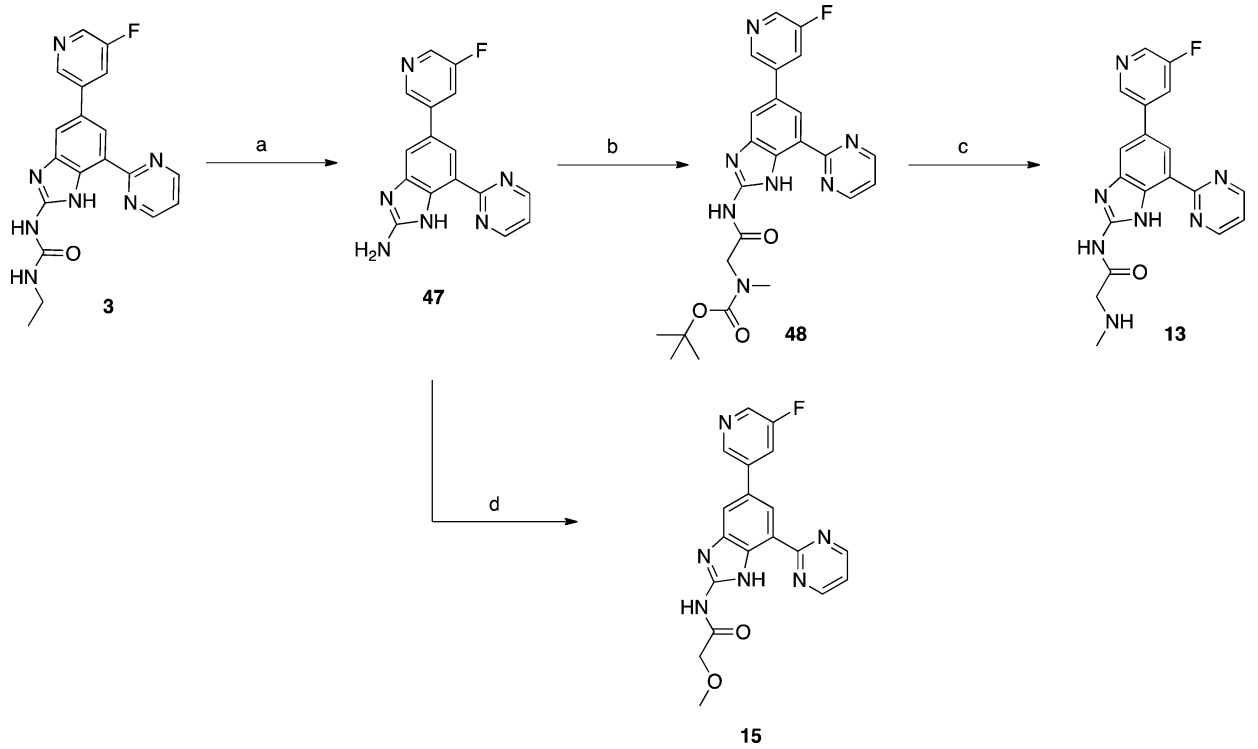
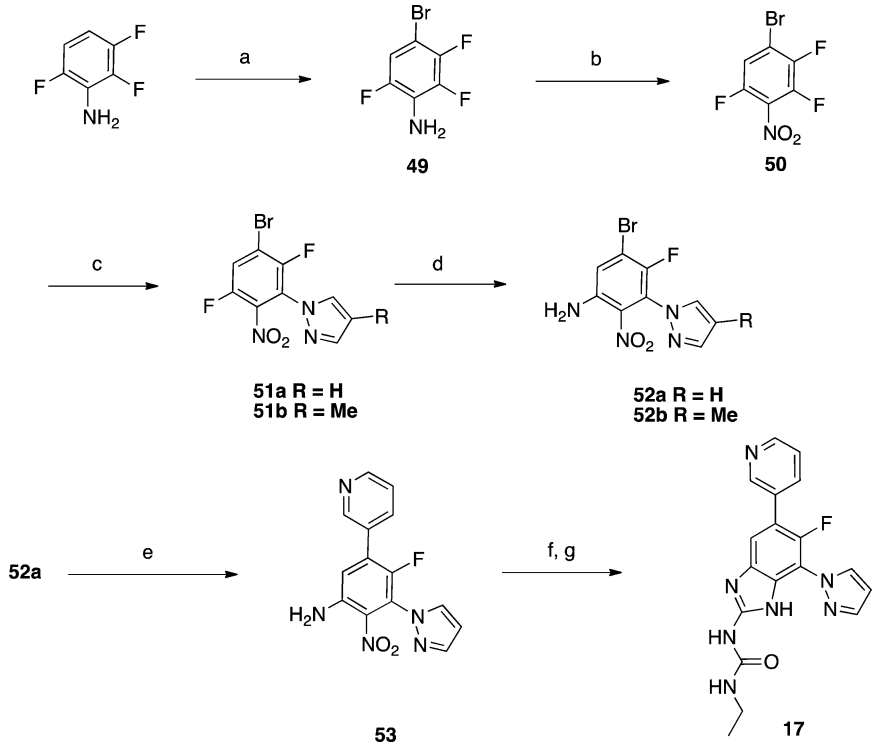
**Scheme 5.** [a]




[a](a) 12 M HCl, 110 °C; (b) N-BOC-sarcosine, HBTU, HOBt, DIPEA, DMF, rt; (c) HCl, $Et_2O$, $CH_2Cl_2$, MeOH, rt; (d) 2-methoxyacetic acid, HBTU, HOBt, DIPEA, DMF, rt.

**Scheme 6.** [a]




[a](a) NBS, DMF, rt; (b) $NaBO_3$, AcOH, 55 °C; (c) pyrazole or 4-methyl-1*H*-pyrazole, NaH, THF, 0 °C to rt; (d) $NH_3$, EtOH, reflux; (e) 3-pyridineboronic acid 1,3-propanediol ester, $Pd(PPh_3)_4$, $NaHCO_3$, DME, reflux; (f) $H_2$, 10% Pd/C, EtOAc, 55 psi; (g) **41**, pH 3.5 buffer, 1,4-dioxane, 90 °C.

pyrazole analogues, coupling of **61** or **62** to the desired coupling partner afforded compounds **25**–**30** and **33**. SFC separation of **30** and **33** yielded enantiomers **31** and **32** and enantiomers **34** and **35**, respectively (Scheme 11). The stereochemistry of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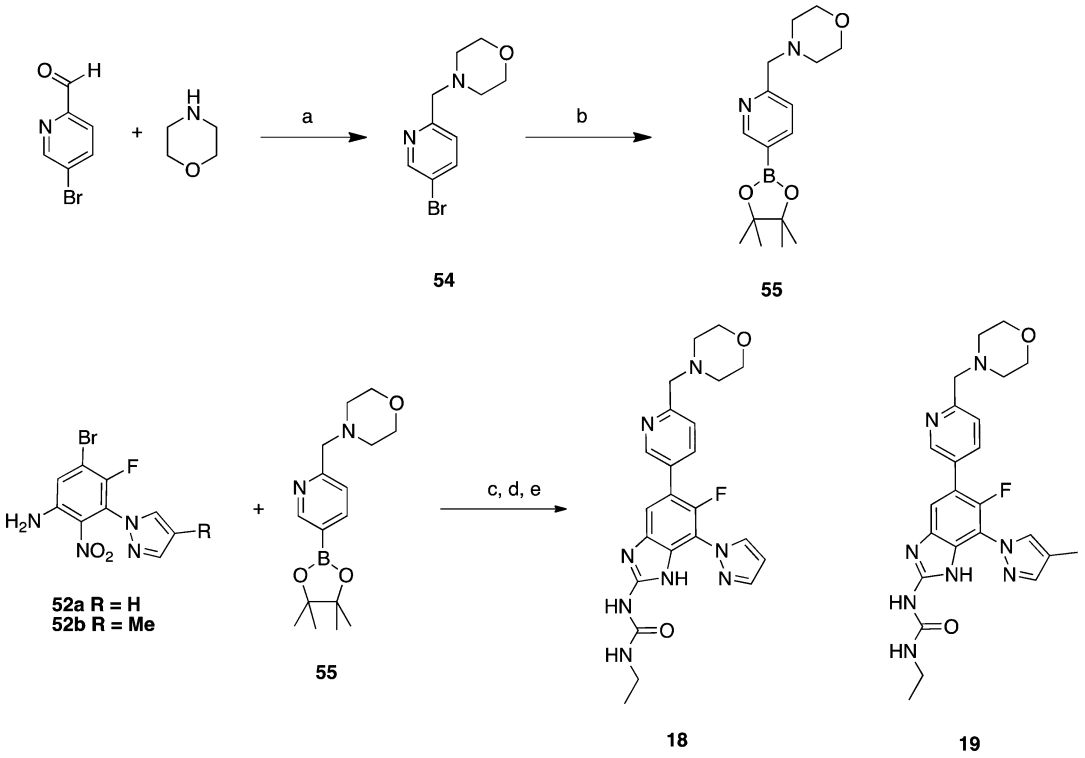
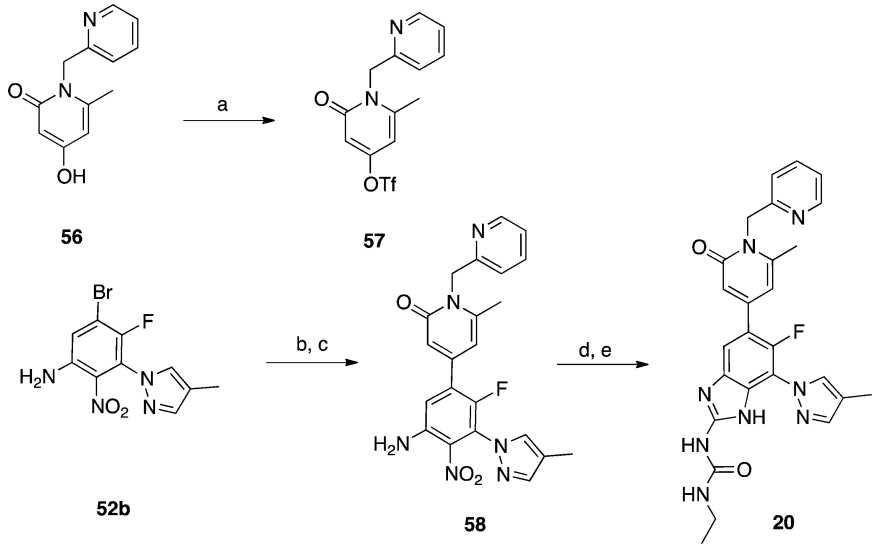
**Scheme 7.** [a]




[a](a) $NaB(OAc)_3H$, DCE; (b) bis(pinacolato)diboron, $PdCl_2(Cy_3P)_2$, KOAc, 1,4-dioxane, 130 °C; (c) $Na_2CO_3$, $Pd(PPh_3)_4$, DME, 90 °C; (d) Ra Ni, 48 psi $H_2$, MeOH; (e) **41**, pH 3.5 buffer, 1,4-dioxane, 90 °C.

**Scheme 8.** [a]




[a](a) 1,1,1-Trifluoro-*N*-phenyl-*N*-(trifluoromethylsulfonyl)methanesulfonamide, $Et_3N$, DMF, rt; (b) bis(pinacolato)diboron, KOAc, $PdCl_2(Cy_3P)_2$, 2-Me THF, 130 °C; (c) **57**, $Pd(PPh_3)_4$, $Na_2CO_3$, LiCl, DME, 100 °C; (d) Ra Ni, 45 psi $H_2$, MeOH; (e) **41**, pH 3.5 buffer, dioxane, 90 °C.

**31** and **34** was determined by obtaining small molecule X-ray structures.

**Chemistry.** All commercial reagents and anhydrous solvents were obtained from commercial sources and were used without further purification, unless otherwise specified. Proton and carbon NMR spectra ($^1H$ NMR and $^{13}C$ NMR) were recorded on a Bruker Avance instrument with a QNP probe using TMS as the internal standard in the indicated deuterated solvent. Preparative HPLC isolations were performed using Agilent 1100 mass-directed purification chromatography system with 0.1% TFA in acetonitrile/water mobile phase. LC-MS analyses were performed on a Waters Acquity UPLC system with a binary pump, a photodiode array detector, and an SQD Mass Spectrometer using the following methods: Waters CSH $C_{18}$ column with water/acetonitrile/0.1% TFA; Waters Xbridge $C_8$ column with water/acetonitrile/0.1% TFA; Waters Xbridge $C_8$ column with water/acetonitrile/50 mM NH4OAc/pH 9; Waters Xselect perfluorophenyl column with water/acetonitrile/0.1% TFA. The purity of the final compounds was determined by HPLC and/or LC-MS and was 95% or greater unless specified otherwise. High-resolution mass measurements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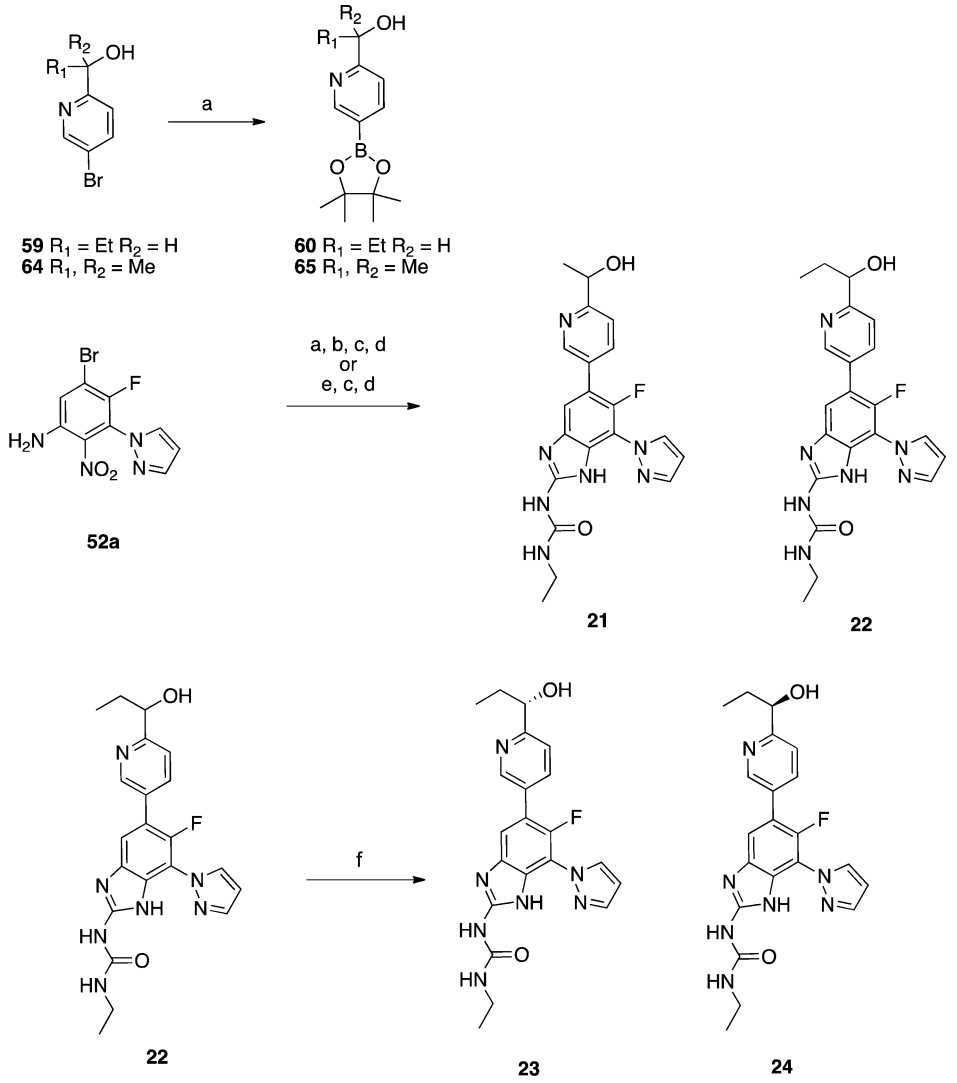
**Scheme 9.** [a]




[a](a) Bis(pinacolato)diboron, KOAc, $PdCl_2(Cy_3P)_2$, 2-Me-THF, microwave, 130 °C; (b) 1-(5-bromo-2-pyridyl)ethanol, $Pd(PPh_3)_4$, $Na_2CO_3$, DME, 100 °C; (c) Ra Ni, MeOH, 40 psi $H_2$; (d) **41**, pH 3.5 buffer, dioxane, 90 °C; (e) **60**, $PdCl_2$(dppf), $NaHCO_3$, 2-Me-THF, microwave, 130 °C; (f) SFC separation.

were performed on a Thermo QExactive mass spectrometer with a heated electrospray source operated in positive ion mode.

**2-(5-Bromopyrimidin-2-yl)propan-2-ol (37).** A 5 L flask containing 2.0 L of $CH_2Cl_2$ was cooled to −65 °C under nitrogen, and *n*-butyllithium (294.8 mL of 2.5 M, 0.737 mol) was added dropwise. 5-Bromo-2-iodo-pyrimidine (**36**, 200 g, 0.702 mol) in 500 mL of $CH_2Cl_2$ was added dropwise over 20 min. The solution was stirred for 15 min, and a precipitate formed. Acetone (257.8 mL, 3.510 mol) was added at once, and the precipitate dissolved. The reaction mixture was stirred for 30 min at −65 °C, warmed to room temperature, and then quenched with 700 mL of aqueous 2 M $NaHPO_4$. The aqueous phase was extracted with $CH_2Cl_2$, and the combined organic phases were washed with brine and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel eluted with 30% ethyl acetate/hexanes to yield 38 g (25%) of **37** as a yellow solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.76 (s, 2H), 4.33 (s, 1H), 1.58 (s, 6H) ppm.

**2-(5-(4-Amino-3-(3-fluoropyridin-2-yl)-5-nitrophenyl)-pyrimidin-2-yl)propan-2-ol (39).** A mixture of 2-(5-bromopyrimidin-2-yl)propan-2-ol (**37**, 17.84 g, 0.0822 mol), 2-(3-fluoropyridin-2-yl)-6-nitro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**38**,[6] 29.52 g, 0.822 mol), tetrakis(triphenylphosphine) palladium(0) (3.79 g, 0.0033 mol), and sodium carbonate (2 M, 0.822 L, 0.1644 mol) in DMF (0.55 L) was stirred at 100 °C for 1.5 h. The reaction was partitioned between ethyl acetate and water and filtered through Celite. The aqueous layer was extracted with ethyl acetate. The combined organic phases were washed with water and brine, dried over sodium sulfate, and concentrated to a slurry under reduced pressure. The slurry was triturated with water and then with isopropyl alcohol. The resulting solid was filtered and dried under vacuum to afford 27.8 g (91%) of **39** as an orange solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.10 (s, 2H), 8.57 (d, 1H), 8.52 (s, 1H), 8.00 (s, 2H), 7.96 (m, 1H), 7.68 (t, 1H), 7.43 (m, 1H), 4.61 (s,1H), 1.64 (s, 6H) ppm. MS, *m*/*z*: 370 $(M + H)^+$.

**2-(5-(3,4-Diamino-5-(3-fluoropyridin-2-yl)phenyl)pyrimidin-2-yl)propan-2-ol (40).** To a solution of 2-(5-(3-amino-5-(3-fluoropyridin-2-yl)-4-nitrophenyl)pyrimidin-2-yl)propan-2-ol (**39**, 5.05 g, 13.67 mmol) in methanol (750 mL) was added Raney nickel (4.0 mL of suspension in water). The mixture was placed under 45 psi of hydrogen on a Parr apparatus and shaken for 4 h. The catalyst was removed, and the reaction was filtered and concentrated under vacuum to afford 4.64 g (quant.) of **40** as a tan solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.05 (s, 2H), 8.53 (d, 1H), 7.58 (t, 1H), 7.31 (m, 2H), 6.96 (s, 1H), 4.79 (s, 2H), 4.71 (s, 1H), 3.44 (s, 2H), 1.64 (s, 6H) ppm. MS, *m*/*z*: 340 $(M + H)^+$.

**1-Ethyl-3-(7-(3-fluoropyridin-2-yl)-5-(2-(2-hydroxypropan-2-yl)-pyrimidin-5-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (2).** 2-(5-(3,4-Diamino-5-(3-fluoropyridin-2-yl)phenyl)pyrimidin-2-yl)propan-2-ol (**40**, 4.6 g,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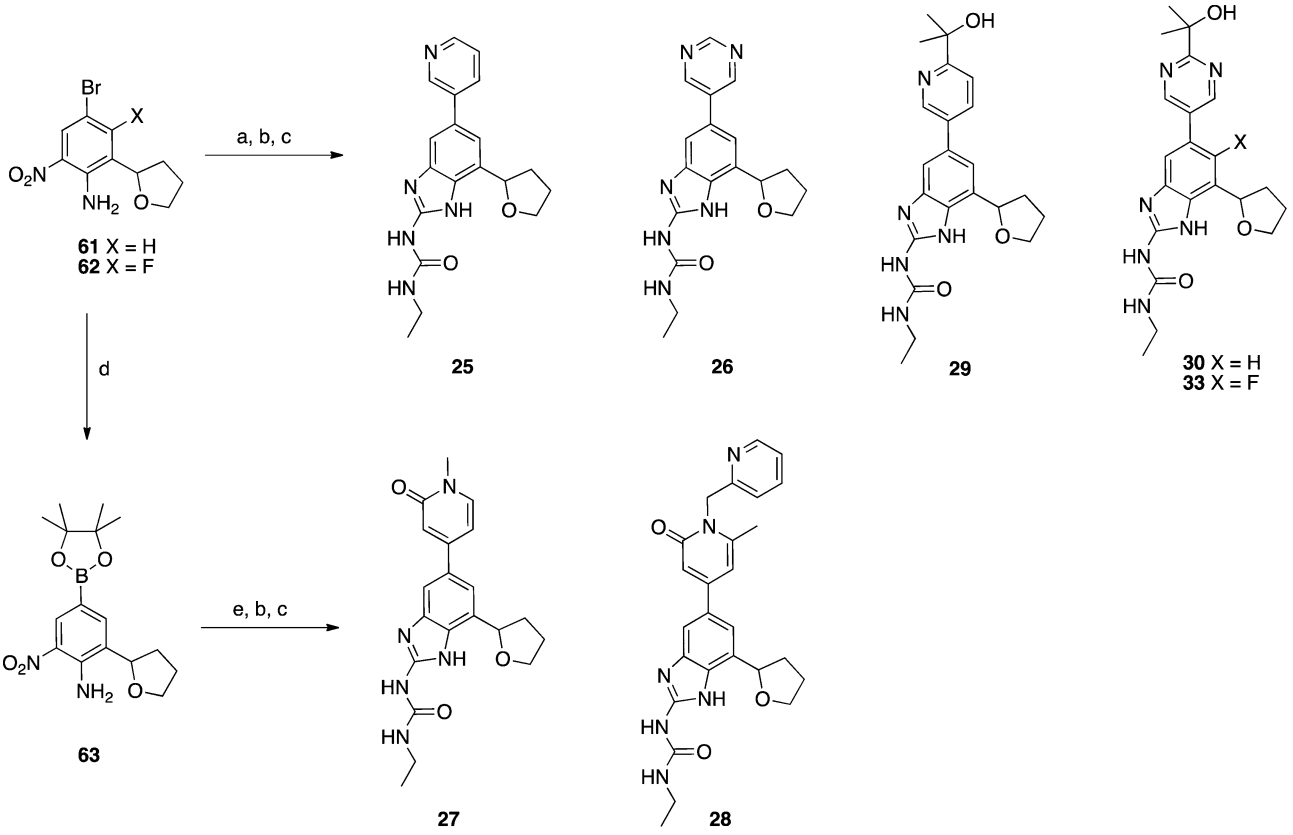
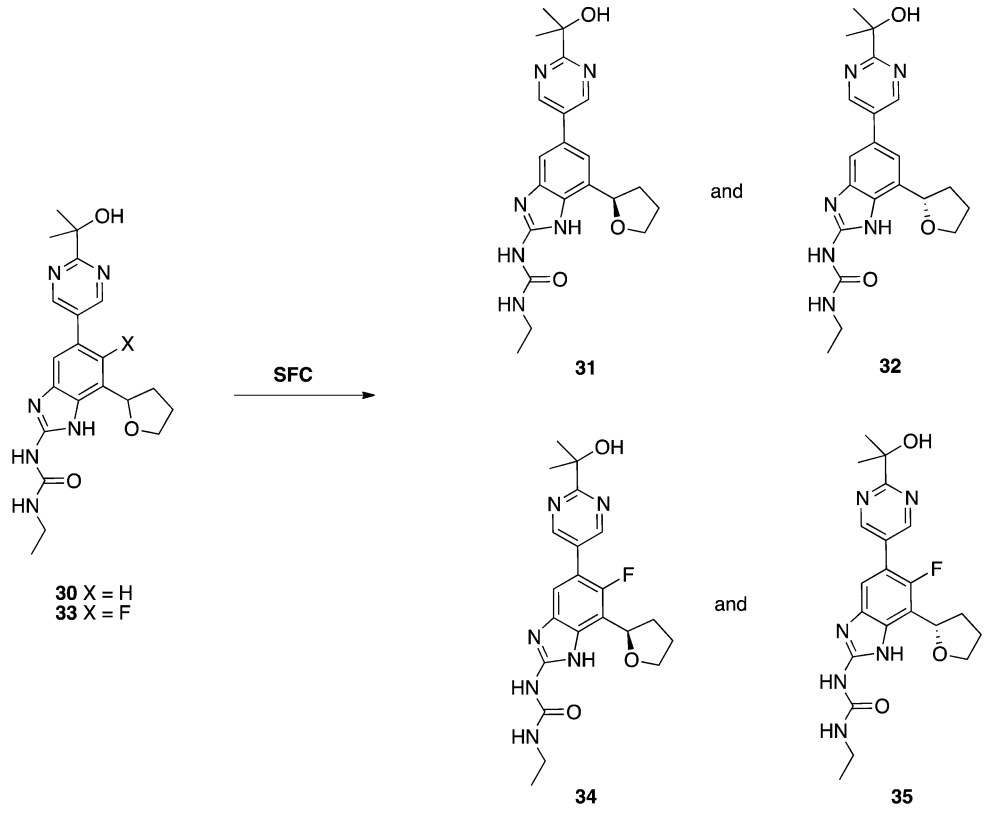
**Scheme 10.** [a]




[a](a) $ArBR_2$ or $ArB(OR)_2$, Pd catalyst, base; (b) reduction catalyst, $H_2$; (c) **41**, pH 3.5 buffer, dioxane, 90 °C; (d) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, DME, reflux; (e) ArOTf, Pd catalyst, base.

**Scheme 11**





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13.55 mmol) was combined with a 1 M solution of 1-ethyl-3-(*N*-(ethylcarbamoyl)-C-methylsulfanyl-carbonimidoyl)urea (**41**)[6,19] in dioxane (14.9 mL, 14.9 mmol) and 300 mL of pH 3.5 buffer (prepared with 57.5 g $NaOAc{\cdot}3H_2O$ and 400 mL of 1 N $H_2SO_4$), and the mixture was stirred at 105 °C for 8 h. The reaction was cooled to room temperature, diluted with water, and brought to pH 7.5 by adding solid $NaHCO_3$. The mixture was allowed to stir for 20 min, and the resulting solid was collected by filtration, washed successively with water and ethyl acetate, and dried in vacuo to afford 2.67 g of **2** as an off-white solid. This solid was suspended in EtOH (25 mL), and 2 equiv of methanesulfonyl chloride (1.41 g, 12.3 mmol) was added. The mixture was warmed to 40 °C, stirred until homogeneous, filtered, and concentrated in vacuo. The resulting solid was dissolved in dichloromethane and added dropwise to a stirred solution of diethyl ether (200 mL) to afford an off-white suspension. The solid was collected by filtration and dried in vacuo to afford 3.93 g (46%) of **2** as the bis-mesylate salt. $^1$H NMR (DMSO-$d_6$, 500 MHz) $\delta$ 11.3 (br, 1H), 9.18 (s, 2H), 8.70 (br d, $J$ = 4.5 Hz, 1H), 8.24 (s, 1H), 8.04 (d, $J$ = 1.5 Hz, 1H), 8.03 (m, 1H), 7.72 (br t, $J$ = 5 Hz, 1H), 7.67 (quint, $J$ = 4.0 Hz, 1H), 3.27 (m, 2H), 2.48 (s, 6H, 2 MsOH), 1.58 (s, 6H), 1.16 (t, $J$ = 7.0 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 436.1897, found $m/z$: 436.1888.

**1-Ethyl-3-(5-(5-fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (3).** See refs 21 and 22.

**1-Ethyl-3-(*N*-(ethylcarbamoyl)-C-(*para*-nitrobenzylsulfanyl-carbonimidoyl)urea-$d_6$ (43a).** To a solution of CDI (959 mg, 5.9 mmol) and DIEA (1.82 g, 2.453 mL, 14.08 mmol) in DMF was added at 0 °C commercially available **42** (823 mg, 2.8 mmol). The reaction mixture was stirred at 0 °C for 1 h. To the dark purple solution was added 2,2,2-$d_3$-diethylamine hydrochloride (500 mg, 5.913 mmol), and the mixture was warmed to rt and stirred for 16 h. The reaction mixture was diluted with water (50 mL), EtOAc (50 mL), and hexanes (15 mL). The phases were separated, and the aqueous layer was extracted twice with EtOAc. The combined organic extracts were washed with saturated aqueous $KHSO_4$, water, and brine. The organic phase was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was diluted in EtOAc/$Et_2O$, and hexanes was added until cloudiness was observed. The mixture was then cooled to ca. −15 °C over 90 min, and the resulting precipitate was filtered. The filtrate was concentrated in vacuo to afford **43b** (0.34 g, 34%). MS, $m/z$: 360.22 $(M + H)^+$.

**1-Ethyl-3-(*N*-(ethylcarbamoyl)-C-(*para*-nitrobenzylsulfanyl-carbonimidoyl)urea-$d_{10}$ (43b).** A solution of CDI (7.81 g, 48.2 mmol) in DMF (75 mL) was cooled to −5 °C, and **41**[6] (6.72 g, 22.9 mmol) was added. The reaction mixture was stirred for 1 h. To the solution was added $d_5$-diethylamine hydrochloride (4.17 g, 48.2 mmol). The reaction mixture was warmed to room temperature and stirred for 16 h. Water (75 mL) and isopropyl acetate (75 mL) were added. Heptane (10 mL) was added, and the phases were separated. The lower aqueous phase was extracted with isopropyl acetate (2 × 10 mL), and the combined organic phases were washed with saturated aqueous $NaHSO_4$ solution, then water, and then saturated aqueous NaCl solution. The organic phase was dried ($Na_2SO_4$) and filtered, and the solvent was removed in vacuo to give 3.68 g (44%) of the desired product **43b**, which was carried to the next step without further purification.

**1-$d_3$-Ethyl-3-(5-(5-fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (6).** To a suspension of **44**[20] (132 mg, 0.47 mmol) in DME (3 mL) was added a solution of **43a** (337 mg, 0.9376 mmol) in DME (3 mL). To the mixture was added buffer (pH 3.5, NaOAc/1 N $H_2SO_4$). The mixture was stirred at 90 °C for 6 h. After 6 h, the beige reaction mixture was cooled to room temperature, diluted with water, and filtered. The solid was triturated in hot EtOAc, filtered, and washed with MeOH, water, and $Et_2O$ in succession to afford a 90% pure solid, which was resolubilized in hot DMSO/2 N aq HCl and was purified by reverse-phase HPLC Gilson to yield **6** (33 mg, 14%). The final product was converted to its esylate salt by dilution in MeOH and addition of 1 equiv of ethanesulfonic acid. $^1$H NMR (300.0 MHz, $CDCl_3$) $\delta$ 8.94 (d, $J$ = 4.9 Hz, 2H), 8.77 (s, 1H), 8.71 (d, $J$ = 1.6 Hz, 1H), 8.52 (d, $J$ = 2.6 Hz, 1H), 7.83 (d, $J$ = 1.6 Hz, 1H), 7.75−7.71 (m, 1H), 7.32 (t, $J$ = 4.9 Hz, 1H), 6.15 (s, 1H), 3.35 (br d, $J$ = 5.4 Hz, 2H), 3.03 (q, $J$ = 7.4 Hz, 2H) and 1.42 (t, $J$ = 7.4 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 381.1667, found $m/z$: 381.1665.

**1-$d_5$-Ethyl-3-(5-(5-fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (7).** To a round-bottomed flask was added **44** (1.91 g, 6.7 mmol) and pH 3.5 aqueous phosphate buffer (23 mL). A solution of **43b** (3.68 g, 10.1 mmol) in DME (17 mL) was added, and the solution was warmed to 80 °C for 3 h. The reaction mixture was cooled to ambient temperature, and the mixture was filtered. The solids were rinsed with water and dried to afford 2.31 g (90% yield) of **7**. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 12.09 (s, 1H), 10.03 (s, 1H), 9.04 (dd, $J$ = 4.9, 1.0 Hz, 2H), 8.86 (t, $J$ = 1.8 Hz, 1H), 8.57 (d, $J$ = 2.6 Hz, 1H), 8.49 (d, $J$ = 1.7 Hz, 1H), 8.12 (dt, $J$ = 10.4, 2.3 Hz, 1H), 7.98 (d, $J$ = 1.7 Hz, 1H), 7.59−7.48 (m, 1H), 7.34 (s, 1H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 383.1792, found $m/z$: 383.1791.

**Methyl (5-(5-Fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1*H*-benzo-[*d*]imidazol-2-yl)carbamate (45).** A suspension of **44** (4 g, 14.2 mmol) and methyl *N*-(*N*-methoxycarbonyl-C-methylsulfanyl-carbonimidoyl)-carbamate (3.5 g, 17.1 mmol) in 1,4-dioxane (70 mL) and buffer pH 3.5 (20 mL, stock solution made from 1 N $H_2SO_4$ and NaOAc) was stirred at reflux for 4 h. The crude reaction mixture was filtered while warm, and the beige solid was washed successively with water (100 mL), acetone (15 mL), EtOAc (15 mL), and $Et_2O$ (100 mL) and dried in vacuo to afford **45** (5.33 g, quant.) as a white solid. $^1$H NMR (300 MHz, TFA) $\delta$ 9.19 (dd, $J$ = 1.2, 5.5 Hz, 2H), 8.95 (s, 1H), 8.62 (s, 1H), 8.52 (m, 2H), 8.27 (s, 1H), 7.79 (t, $J$ = 5.5 Hz, 1H), 3.74 (s, 3H) ppm. An aliquot of 227 mg was then salted as the monoesylate salt. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.00 (d, $J$ = 4.9 Hz, 2H), 8.91 (d, $J$ = 0.8 Hz, 1H), 8.83 (d, $J$ = 1.6 Hz, 1H), 8.64 (d, $J$ = 2.5 Hz, 1H), 8.01−7.97 (m, 2H), 7.40 (t, $J$ = 4.9 Hz, 1H), 4.01 (s, 3H), 3.07 (q, $J$ = 7.4 Hz, 2H), 1.45 (t, $J$ = 7.4 Hz, 3H) ppm. MS, $m/z$: 365.25 $(M + H)^+$.

**1-(5-(5-Fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1*H*-benzo[*d*]-imidazol-2-yl)-3-(2,2,2-trifluoroethyl)urea (8).** To a suspension of **45** (330 mg, 0.89 mmol) in NMP (20 mL) was added 2,2,2-trifluoroethylamine (2.64 g, 26.6 mmol), and the mixture was heated at 120 °C in a sealed Parr flask for 5 h. The resulting clear yellow solution was cooled to room temperature and poured into water (70 mL). The resulting suspension was stirred for 1 h and filtered, and the off-white solid was washed with water (2 × 50 mL) and then MeOH (10 mL), which partially redissolved the product, explaining the low yield. The resulting white solid (89.7 mg, 23%) was then converted to the monoesylate salt. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.98 (d, $J$ = 4.9 Hz, 2H), 8.80 (m, 1H), 8.79 (dd, $J$ = 1.6, 13.6 Hz, 1H), 8.55 (d, $J$ = 2.6 Hz, 1H), 7.94 (d, $J$ = 1.6 Hz, 1H), 7.79−7.74 (m, 1H), 7.37 (t, $J$ = 4.9 Hz, 1H), 7.09 (t, $J$ = 6.2 Hz, 1H), 4.05−3.94 (m, 2H), 3.08 (q, $J$ = 7.4 Hz, 2H),1.45 (t, $J$ = 7.4 Hz, 2H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 432.1196, found $m/z$: 432.1190.

**1-(5-(5-Fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1*H*-benzo[*d*]-imidazol-2-yl)urea (11).** To a suspension of **45** (280 mg, 0.75 mmol) in NMP (20 mL) was bubbled ammonia (384.9 mg, 22.60 mmol) for 10 min. The mixture was heated at 120 °C in a sealed Parr flask for 5 h. The resulting clear yellow solution was then cooled to rt and poured into water (70 mL). The resulting suspension was stirred for 1 h and filtered, and the off-white solid was washed with water (2 × 50 mL), triturated in hot MeOH for 2 h, and then triturated with hot DMF (70 °C). The insoluble solid was collected to yield 19.7 mg of **11** (5.7%) as the free base, which was converted to its monoesylate salt. $^1$H NMR (300 MHz, MeOD) $\delta$ 9.03 (d, $J$ = 4.8 Hz, 2H), 8.83 (s, 1H), 8.74 (s, 1H), 8.58 (d, $J$ = 2.2 Hz, 1H), 8.06 (m, 2H), 7.53 (t, $J$ = 4.8 Hz, 1H), 2.73 (q, $J$ = 7.4 Hz, 2H), 1.25 (t, $J$ = 7.4 Hz, 3H) ppm. MS, $m/z$: 350.14 $(M + H)^+$.

**7-(5-Fluoropyridin-3-yl)-4-methyl-9-(pyrimidin-2-yl)-3,4-dihydrobenzo[4,5]imidazo[1,2-*a*][1,3,5]triazin-2(1*H*)-one (10).** To a solution of **11** (9.8 mg, 0.028 mmol) in TFA (1.5 mL) were added in succession excess acetaldehyde (6.176 mg, 7.868 μL, 0.140 mmol) and 4Å molecular sieves. The reaction mixture was stirred at rt for 3 days, concentrated in vacuo, and purified by reverse-phase HPLC to afford **10** (2.4 mg, 23%) as a white solid. $^1$H NMR (800 MHz, DMSO-$d_6$) $\delta$ 9.00 (d, 2H), 8.93 (s, 1H), 8.61 (Hb, 1H), 8.40 (Hd, 1H), 8.22 (m, 1H), 8.20 (m, 1H), 7.54 (m, 1H), 6.08 (m, 1H), 1.65 (d, 3H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 162.50, 159.44 (d, $J$ = 254.98 Hz), 158.75, 157.58, 148.96, 147.56, 143.96, 137.06, 136.30 (d, $J$ = 23.75 Hz), 131.05, 130.00, 125.26, 122.34, 121.10 (d, $J$ = 18.75 Hz), 120.05, 111.16, 61.79, 22.84 ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 376.1322, found $m/z$: 376.1317.


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***N*-[*N*-Butanoyl-C-[4-nitrophenyl)methylsulfanyl]carbonimidoyl]-butanamide (46).** The hydrobromide salt of **42** (2.82 g, 9.65 mmol), butanoyl butanoate (6.11 g, 4.33 mL, 38.6 mmol), water (15 mL), and DME (24 mL) were charged in a 50 mL flask. The pH of the mixture was adjusted to 8 with a 2 N aqueous NaOH solution, and the resulting biphasic reaction mixture was stirred at room temperature for 16 h. The residue was diluted with EtOAc (50 mL), and the biphasic mixture was stirred for 10 min and separated, and the organics were washed with a saturated aqueous solution of $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated in vacuo to afford an off-white solid. The solid was triturated with $Et_2O$/hexanes, filtered, and dried in vacuo to yield **46** (2.61 g, 83%) as an off-white solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.18−8.14 (m, 2H), 7.57−7.53 (m, 2H), 4.32 (s, 2H), 2.48 (t, $J$ = 6.9 Hz, 2H), 2.37 (t, $J$ = 7.0 Hz, 2H), 1.71−1.67 (m, 5H), 0.96 (t, $J$ = 7.3 Hz, 6H) ppm. $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$ 186.8, 171.9, 167.8, 146.9, 144.9, 129.8, 123.2, 43.2, 39.9, 34.4, 18.3, 17.8, 13.6, 13.3 ppm. MS, $m/z$: 352.28 $(M + H)^+$.

***N*-(5-(5-Fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1*H*-benzo[*d*]-imidazol-2-l)butyramide (12).** A suspension of **44** (400 mg, 1.42 mmol) and **46** (600 mg, 1.71 mmol), 1,4-dioxane (10 mL), and pH 3.5 buffer (10 mL, stock solution made from 1 N $H_2SO_4$ and NaOAc) was stirred for 8 h at reflux. The reaction mixture was cooled to room temperature and filtered, and the beige solid was washed successively with water (50 mL) and MeOH (70 mL) and then dried in vacuo to yield **12** (500 mg, 94%) as a beige solid, which was converted to its monoesylate salt. $^1$H NMR (300.0 MHz, $CDCl_3$) $\delta$ 9.00 (d, $J$ = 4.9 Hz, 2H), 8.83−8.82 (m, 2H), 8.57 (d, $J$ = 2.7 Hz, 1H), 7.94 (d, $J$ = 1.6 Hz, 1H), 7.39 (t, $J$ = 4.9 Hz, 1H), 3.07 (q, $J$ = 7.4 Hz, 2H), 2.70 (t, $J$ = 7.4 Hz, 2H), 1.85 (q, $J$ = 7.4 Hz, 2H), 1.46 (t, $J$ = 7.4 Hz, 3H), 1.07 (t, $J$ = 7.4 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 377.1526, found $m/z$: 377.1522.

**5-(5-Fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1*H*-benzo[*d*]-imidazol-2-amine (47).** A mixture of **3** (15 g, 30.8 mmol) in aqueous HCl (12 M, 150 mL) was heated in a sealed tube at 110 °C for 48 h. The reaction mixture was cooled to 0 °C and brought to pH 8 by the addition of 6 N aqueous NaOH. The white solid was collected by filtration, washed with water, and dried in vacuo to afford 9 g (95%) of **47**. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 11.12 (br s, 1H), 8.99 (d, $J$ = 4.8 Hz, 2H), 8.81 (s, 1H), 8.53 (d, $J$ = 2.7 Hz, 1H), 8.29 (d, $J$ = 1.7 Hz, 1H), 8.07−8.02 (m, 1H), 7.69 (d, $J$ = 1.7 Hz, 1H), 7.47 (t, $J$ = 4.9 Hz, 1H), 6.56 (s, 2H) ppm. MS, $m/z$ 307.04 $(M + H)^+$.

***tert*-Butyl (2-((5-(5-Fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)amino)-2-oxoethyl)(methyl)carbamate (48).** To a solution of 5-(5-fluoro-3-pyridyl)-7-pyrimidin-2-yl-1*H*-benzimidazol-2-amine (**47**, 60 mg, 0.20 mmol) in DMF were added (benzotriazol-1-yloxy-dimethylamino-methylene)-dimethylammonium hexafluorophosphate (96.6 mg, 0.25 mmol), 1-hydroxybenzotriazole (34.4 mg, 0.25 mmol), *N*-ethyl-*N*-isopropyl-propan-2-amine (58.2 mg, 78.5 $\mu$L, 0.45 mmol), and 2-(*tert*-butoxycarbonyl(methyl)amino)acetic acid (48.2 mg, 0.25 mmol). The reaction mixture was stirred for 18 h at room temperature. The solids were then filtered, washed with DMF (1 mL × 2), methanol (1 mL × 2), and dichloromethane (1 mL × 2), and dried in vacuo to provide **48** (60 mg, 64%) as a white solid. MS, $m/z$: 478.40 $(M + H)^+$.

***N*-(5-(5-Fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1*H*-benzo[*d*]-imidazol-2-yl)-2-(methylamino)acetamide (13).** To a solution of **48** (60 mg) in methanol (1 mL) and dichloromethane (1 mL) was added 2 mL of 2.0 M hydrogen chloride in diethyl ether. The solution was stirred at room temperature for 2 h. The resulting solid was collected by filtration, washed with DMF (1 mL × 2), methanol (1 mL × 2), and dichloromethane (1 mL × 2), and dried in vacuo to afford **13** (40 mg, 71%) as a white solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.19−8.14 (m, 3H), 8.03 (d, $J$ = 1.7 Hz, 2H), 7.94 (dd, $J$ = 1.4, 2.5 Hz, 1H), 7.28 (d, $J$ = 1.7 Hz, 1H), 6.64 (t, $J$ = 4.9 Hz, 1H), 3.92 (d, $J$ = 1.4 Hz, 2H), 3.40 (s, H), 2.45−2.40 (m, H), 1.99 (d, $J$ = 1.6 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 378.1479, found $m/z$: 378.1473.

**Ethyl (5-(5-Fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1*H*-benzo-[*d*]imidazol-2-yl)carbamate (14).** A suspension of **44** (1.18 g, 4.20 mmol) and ethyl *N*-(*N*-ethoxycarbonyl-C-methylsulfanyl-carbonimidoyl)carbamate (1.18 g, 5.04 mmol) in dioxane (25 mL) and buffer pH 3.5 (25 mL, prepared from 1 N $H_2SO_4$ and NaOAc) was stirred at reflux overnight. The crude reaction mixture was filtered while warm, and the beige solid was successively washed with water (100 mL) and methanol (75 mL) to afford 1.19 g of **14** (52%) as free base, which was converted to the monoesylate salt. $^1$H NMR (300.0 MHz, $CDCl_3$) $\delta$ 9.00 (d, $J$ = 4.9 Hz, 2H), 8.88 (s, 1H), 8.81 (d, $J$ = 1.6 Hz, 1H), 8.61 (d, $J$ = 2.6 Hz, 1H), 7.96−7.90 (m, 2H), 7.40 (t, $J$ = 4.9 Hz, 1H), 4.45 (q, $J$ = 7.1 Hz, 2H), 3.07 (q, $J$ = 7.4 Hz, 2H), 1.48−1.42 (m, 6H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 379.1319, found $m/z$: 379.1312.

***N*-(5-(5-Fluoropyridin-3-yl)-7-(pyrimidin-2-yl)-1*H*-benzo[*d*]-imidazol-2-yl)-2-methoxyacetamide (15).** To compound **47** (68.9 mg, 0.23 mmol) in DMF (3 mL) were added 2-methoxyacetic acid (26.4 mg, 23 $\mu$L, 0.29 mmol), DIEA (66.9 mg, 90 $\mu$L, 0.52 mmol), 1-hydroxybenzotriazole (39.6 mg, 0.29 mmol), and HBTU (111.0 mg, 0.29 mmol). The reaction mixture was stirred at rt for 18 h. The resulting solid was collected, washed with DMF (2 mL), MeOH (2 mL), and $CH_2Cl_2$ (3 mL), dried in vacuo, and salted as the monoesylate salt to afford **15** (70 mg, 64%). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 9.04 (d, $J$ = 5.1 Hz, 2H), 8.92 (s, 1H), 8.86 (s, 1H), 8.59 (d, $J$ = 2.1 Hz, 1H), 8.14 (m, 2H), 7.55 (t, $J$ = 4.8 Hz, 1H), 4.34 (s, 2H), 3.62 (s, 3H), 2.80 (q, $J$ = 7.5 Hz, 2 H), 1.30 (t, $J$ = 7.5 Hz, 3 H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 379.1319, found $m/z$: 379.1318.

**4-Bromo-2,3,6-trifluoro-aniline (49).** To a solution of 2,3,6-trifluoroaniline (20 g, 136.0 mmol) in DMF (300.0 mL) was added *N*-bromosuccinimide (29.05 g, 163.2 mmol). The mixture was allowed to stir at room temperature for 2 h, diluted with iPrOAc, washed with water (3×) and brine, dried ($Na_2SO_4$), and concentrated in vacuo. The residue was purified by column chromatography over silica gel (120 g) and eluted with a 0 to 25% $CH_2Cl_2$/hexanes gradient to afford **49** (22.36 g, 72%) as a brown crystalline solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.02 (ddd, $J$ = 9.8, 5.5, 2.5 Hz, 1H), 3.90 (s, 2H) ppm.

**1-Bromo-2,3,5-trifluoro-4-nitrobenzene (50).** To a suspension of sodium perborate tetrahydrate (26.02 g, 443 mmol) in glacial acetic acid (450 mL) at 55 °C was added a solution of 4-bromo-2,3,6-trifluoro-aniline (20 g, 88.5 mmol) in acetic acid (75 mL) dropwise. The mixture was stirred at 55 °C for 2 h, and an additional 5 equiv of sodium perborate tetrahydrate (25.5 g, 0.435 mol) was added. The reaction mixture was stirred for 1.5 h and concentrated in vacuo. The residue was diluted with water and extracted with diethyl ether (2×). The combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography over silica gel (120 g) and eluted with a 0 to 5% DCM/hexanes gradient to afford 8.53 g (38%) of **50** as an orange solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.46−7.31 (m, 1H) ppm.

**1-(3-Bromo-2,5-difluoro-6-nitrophenyl)-1*H*-pyrazole (51a).** To an ice cold suspension of sodium hydride (1.68 g, 42.2 mmol) in THF (90 mL) was added 1*H*-pyrazole (2.39 g, 35.2 mmol). The reaction mixture was stirred for 20 min and then added to an ice cold solution of 1-bromo-2,3,5-trifluoro-4-nitro-benzene (**50**, 9 g, 35.2 mmol) in THF (75 mL). The resulting mixture was stirred for 1.5 h, quenched with water, and concentrated in vacuo. The residue was extracted with diethyl ether, and the organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over 120 g of silica gel and eluted with a 0 to 25% ethyl acetate/petroleum ether gradient to afford **51** (8.25 g, 77%) as a light yellow solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.93−7.84 (m, 1H), 7.79 (d, $J$ = 1.5 Hz, 1H), 7.58 (dd, $J$ = 8.2, 5.3 Hz, 1H), 6.62−6.54 (m, 1H) ppm.

**1-(3-Bromo-2,5-difluoro-6-nitrophenyl)-4-methyl-1*H*-pyrazole (51b).** To an ice cold suspension of sodium hydride (1.58 g, 39.6 mmol) in THF (90 mL) was added 4-methyl-1*H*-pyrazole (2.71 g, 33.0 mmol). The mixture was stirred at 0 °C for 20 min and then added to an ice cold solution of 1-bromo-2,3,5-trifluoro-4-nitrobenzene (8.45 g, 33.0 mmol) in THF (75 mL). The reaction mixture was stirred for 2 h, quenched with water, and concentrated under reduced pressure. The residue was extracted with diethyl ether, and the organics were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography over 120 g of silica gel and eluted with a 0 to 8% ethyl acetate/petroleum ether gradient to afford 9.0 g (86%) of **51b** as a light orange solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ = 7.63−7.57 (m, 1H), 7.54−7.44 (m, $J$ = 8.2, 5.3, 1H), 2.16 (s, 2H) ppm.


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**5-Bromo-4-fluoro-2-nitro-3-(1*H*-pyrazol-1-yl)aniline (52a).** 1-(3-Bromo-2,5-difluoro-6-nitro-phenyl)pyrazole (**51**, 7.5 g, 24.67 mmol) was dissolved in 15 mL of ethanol and 7 M ammonia in methanol (35.24 mL, 246.7 mmol). The reaction was stirred at 60 °C overnight. An additional portion of 7 M ammonia in methanol (35.24 mL, 246.7 mmol) was added, and stirring was continued for 24 h. Another portion of ammonia in methanol was added, and stirring was continued overnight. The reaction mixture was evaporated in vacuo to afford a bright yellow solid, which was recrystallized from dichloromethane/hexanes to afford 5.25g of **52** as a bright yellow solid. A 1.31 g second crop was collected to yield a total of 6.56 g (84%) of **52**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.74−7.62 (m, 2H), 7.03 (d, $J$ = 5.7 Hz, 1H), 6.48−6.42 (m, 1H), 4.99 (s, 2H) ppm.

**5-Bromo-4-fluoro-3-(4-methyl-1*H*-pyrazol-1-yl)-2-nitroaniline (52b).** A suspension of 1-(3-bromo-2,5-difluoro-6-nitrophenyl)-4-methyl-1*H*-pyrazole (9.0 g, 28.30 mmol) and ammonia in methanol (40.4 mL of 7 M, 283.0 mmol) in ethanol (15 mL) was heated to 60 °C and allowed to stir overnight. An additional 5 equiv of 7 M $NH_3$ in MeOH was added, and the mixture was heated to 80 °C for 48 h. The reaction was cooled to rt and concentrated in vacuo. The residue was diluted with water and extracted with diethyl ether (2×). The combined organics were dried ($MgSO_4$) and concentrated in vacuo. The residue was purified by column chromatography over 120 g of silica gel and eluted with a 0 to 30% ethyl acetate−hexanes gradient to afford 5.1 g (56%) of **52b** as a yellow solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ = 7.61−7.46 (m, 2H), 7.06 (d, $J$ = 5.7, 1H), 4.97 (s, 2H), 2.17 (s, 3H) ppm.

**4-Fluoro-2-nitro-3-(1*H*-pyrazol-1-yl)-5-(pyridin-3-yl)aniline (53).** A mixture of **52** (175 mg, 0.58 mmol), 3-pyridineboronic acid 1,3-propanediol ester (114 mg, 0.70 mmol), and palladium tetrakis triphenylphosphine (69 mg, 0.06 mmol) in 1.16 mL of 1 M $NaHCO_3$ and 4 mL of DME was stirred at 70 °C overnight. The mixture was diluted with EtOAc, washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography over silica gel (30 g) and eluted with ethyl acetate−hexanes, 3:1, then 4:1, then 5:1, to afford 100 mg (57%) of **53** as an orange solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.82 (m, 1H), 8.70 (m, 1H), 8.20 (m, 1H), 8.05 (m, 1H), 7.80 (m, 1H), 7.65 (m, 1H), 7.20 (m, 1H), 6.55, (s, 1H), 6.50 (br s, 2H) ppm.

**1-Ethyl-3-(6-fluoro-7-(1*H*-pyrazol-1-yl)-5-(pyridin-3-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (17).** To a solution of 200 mg (0.67 mmol) of **53** in 20 mL of EtOAc was added 10% palladium on carbon (50 mg). The mixture was shaken on a Parr apparatus under 45 psi of hydrogen for 3h, filtered over Celite, and concentrated in vacuo. To a solution of the residue in 1.8 mL of 1,4-dioxane were added 1-ethyl-3-(*N*-(ethylcarbamoyl)-C-methylsulfanyl-carbonimidoyl)urea (155 mg, 0.67 mmol) and 20 mL of pH 3.5 buffer. The mixture was heated at 95 °C overnight, cooled to room temperature, and neutralized by addition of saturated aqueous $NaHCO_3$. The resulting solid was collected by filtration, washed with hot water (100 mL), and purified by column chromatography over silica gel (5 to 100% of $CH_2Cl_2$/20% MeOH/$CH_2Cl_2$) to afford **17** (50 mg, 20%) as an off-white solid. $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.79 (s, 1H), 8.57 (d, $J$ = 4.7 Hz, 1H), 8.27 (br, 1H), 8.10 (dd, $J$ = 7.7, 2.1 Hz, 1H), 7.89 (s, 1H), 7.65−7.45 (m, 2H), 6.62 (s, 1H), 4.84 (brs, 2H), 1.21 (t, $J$ = 7.2 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 366.1479, found $m/z$: 366.1476.

**4-[(5-Bromo-2-pyridyl)methyl]morpholine (54).** To a solution of 5-bromopyridine-2-carbaldehyde (25 g, 134.4 mmol) and morpholine (23.42 g, 23.4 mL, 268.8 mmol) in 1,2-dichloroethane (224 mL) was added sodium triacetoxyborohydride (56.97 g, 268.8 mmol). The resulting mixture was stirred at room temperature for 16 h and then partitioned between EtOAc and saturated $NaHCO_3$. The organic phase was washed with brine, dried ($Na_2SO_4$), and concentrated in vacuo. The crude product was purified by silica gel chromatography (0−25% EtOAc/hexanes gradient) to afford **54** (21.7 g, 62%) as an off-white crystalline solid. $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.58 (d, $J$ = 2.3 Hz, 1H), 7.97 (dd, $J$ = 8.4, 2.3 Hz, 1H), 7.48 (d, $J$ = 8.4 Hz, 1H), 3.76−3.65 (m, 4H), 3.61 (s, 2H), 2.54−2.41 (m, 4H) ppm.

**4-((5-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)methyl)morpholine (55).** To a suspension of **54** (16.5 g, 63.5 mmol), bis(pinacolato)diboron (19.36 g, 76.2 mmol), and KOAc (9.35 g, 95.3 mmol) was added dichlorobis(tricyclohexylphosphine)-palladium(II) (5.0 mol %, 710 mg) in 1,4-dioxane (254 mL) . The mixture was degassed for 15 min before and after addition of the catalyst, heated at 130 °C for 18 h, cooled to ambient temperature, filtered through a pad of Celite, and washed three times with EtOAc. The combined filtrates were concentrated in vacuo to afford **55** (19.3 g, 80%) as green/brown solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.69 (s, 1H), 7.98 (dd, $J$ = 7.7, 1.7 Hz, 1H), 7.47 (d, $J$ = 7.7 Hz, 1H), 3.58 (d, $J$ = 4.7 Hz, 4H), 2.46−2.35 (m, 4H), 1.31 (s, 12H) ppm.

**1-Ethyl-3-(6-fluoro-5-(6-(morpholinomethyl)pyridin-3-yl)-7-(1*H*-pyrazol-1-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (18).** A mixture of **52a** (300 mg, 1.0 mmol), 4-[[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-pyridyl]methyl]morpholine (568 mg, 1.5 mmol), $Na_2CO_3$ (1.5 mL of 2.0 M, 3.0 mmol), and $Pd(PPh_3)_4$ (115 mg, 0.1 mmol) in DME (5 mL) was degassed with $N_2$ and heated to 80 °C for 14 h. The reaction mixture was diluted with EtOAc, washed with water, filtered though a layer of Celite, and concentrated in vacuo. The crude product was loaded onto a 12 g silica gel cartridge eluted with 0−10% MeOH/DCM to afford 4-fluoro-5-[6-(morpholinomethyl)-3-pyridyl]-2-nitro-3-pyrazol-1-yl-aniline (380 mg, 0.95 mmol, 96%). MS, $m/z$: 399.27 $(M + H)^+$. To a solution of 4-fluoro-5-[6-(morpholinomethyl)-3-pyridyl]-2-nitro-3-pyrazol-1-yl-aniline (380 mg, 0.95 mmol) in MeOH (30 mL) was added Raney nickel (82 mg, 0.95 mmol). The reaction mixture was shaken on a Parr apparatus for 2 h under 40 psi of hydrogen, filtered through a layer of Celite, and concentrated in vacuo to afford 4-fluoro-5-[6-(morpholinomethyl)-3-pyridyl]-3-pyrazol-1-yl-benzene-1,2-diamine (350 mg, 99%). MS, $m/z$: 369.3 $(M + H)^+$. To a mixture of 4-fluoro-5-[6-(morpholinomethyl)-3-pyridyl]-3-pyrazol-1-yl-benzene-1,2-diamine (350 mg, 0.97 mmol) and 1-ethyl-3-(*N*-(ethylcarbamoyl)-C-methyl-sulfanyl-carbonimidoyl)urea (272 mg, 1.17 mmol) in dioxane (5 mL) was added pH 3.5 buffer (12 mL). The mixture was heated at 90 °C for 15 h. The reaction mixture was cooled to room temperature and neutralized with saturated aqueous $NaHCO_3$. The resulting precipitate was collected by filtration, washed with $H_2O$, EtOAc, and $Et_2O$, and dried in vacuo to afford **18** as the free base, which was converted to the bis-HCl salt (220 mg, 38%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.89 (s, 1H), 8.34 (s, 1H), 8.18 (d, $J$ = 8.0 Hz, 1H), 7.93 (s, 1H), 7.82 (d, $J$ = 8.0 Hz, 1H), 7.58 (t, $J$ = 10.4 Hz, 2H), 6.65 (s, 1H), 4.58 (s, 2H), 3.90 (s, 3H), 3.26 (dt, $J$ = 15.8, 10.9 Hz, 5H), 1.25−1.05 (m, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 465.2163, found $m/z$: 465.2157.

**1-Ethyl-3-(6-fluoro-7-(4-methyl-1*H*-pyrazol-1-yl)-5-(6-(morpholinomethyl)pyridin-3-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (19).** To a suspension of **55** (2.41 g, 6.35 mmol), **52b** (1.0 g, 3.17 mmol), and $Na_2CO_3$ (3.17 mL of 2.0 M, 6.35 mmol) was added $Pd(PPh_3)_4$ (183 mg, 0.159 mmol) in DME (10.6 mL). The mixture was degassed for 10 min and then heated at 90 °C for 14 h. The mixture was cooled to ambient temperature and partitioned between EtOAc and water. The organic phase was dried ($Na_2SO_4$) and concentrated in vacuo. The crude residue was purified by silica gel chromatography (0−10% 7 N $NH_3$/MeOH/$CH_2Cl_2$ gradient) to afford the desired nitroaniline adduct (1.09 g, 83%) as a dark brown oil. MS, $m/z$: 413.32 $(M + H)^+$. A suspension of the above nitroaniline (1.09 g, 2.643 mmol) and Raney nickel (3.0 mL, suspension in water) in MeOH (18.8 mL) was placed under 48 psi of hydrogen on a Parr shaker for 2 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo to afford the diamine (0.83g, 82%) as a dark gray solid. MS, $m/z$: 383.38 $(M + H)^+$. To a solution of the above diamine (0.83g, 2.168 mmol) and 1-ethyl-3-(*N*-(ethylcarbamoyl)-C-methylsulfanyl-carbonimidoyl)urea (655 mg, 2.82 mmol) in 1,4-dioxane (12.0 mL) was added pH 3.5 buffer (36 mL, stock solution prepared from 1 M $H_2SO_4$ and NaOAc). The resulting mixture was stirred at 90 °C for 16 h. Saturated sodium bicarbonate was added to the reaction, and the mixture was stirred for 10 min. The resulting solid was collected by filtration, washed twice with water, and dried in vacuo to afford **19** (0.64 g, 58%) as a beige solid. $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.68 (s, 1H), 8.11−7.89 (m, 2H), 7.65 (s, 1H), 7.56 (d, $J$ = 8.1 Hz, 1H), 7.47 (d, $J$ = 6.2 Hz, 1H), 3.65 (s, 2H), 3.64−3.58 (m, 4H), 3.21 (dt, $J$ = 13.9, 7.1 Hz, 2H), 2.49−2.43 (m, 4H), 2.14 (s, 3H), 1.11 (t, $J$ = 7.2 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 479.2319, found $m/z$: 479.2316.


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**6-Methyl-2-oxo-1-(pyridin-2-ylmethyl)-1,2-dihydropyridin-4-yl Trifluoromethanesulfonate (57).** To a mixture of commercially available 4-hydroxy-6-methyl-1-(2-pyridylmethyl)pyridin-2-one (**56**, 6.9 g, 31.91 mmol) and triethylamine (3.88 g, 5.34 mL, 38.3 mmol) in DMF (50 mL) was added 1,1,1-trifluoro-*N*-phenyl-*N*-(trifluoromethylsulfonyl)methanesulfonamide (12.54 g, 35.1 mmol). The reaction mixture was stirred at rt for 8 h and concentrated in vacuo. The residue was purified by column chromatography over silica gel (80 g) eluted with a 0 to 60% ethyl acetate/hexanes gradient to afford 10.1 g (90%) of compound **57**. MS, *m*/*z*: 348.84 $(M + H)^+$.

**4-(5-Amino-2-fluoro-3-(4-methyl-1*H*-pyrazol-1-yl)-4-nitrophenyl)-1-(pyridin-2-ylmethyl)pyridin-2(1*H*)-one (58).** A mixture of 5-bromo-4-fluoro-3-(4-methyl-1*H*-pyrazol-1-yl)-2-nitroaniline (**52b**, 250 mg, 0.7934 mmol), 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (302 mg, 1.190 mmol), potassium acetate (233 mg, 2.38 mmol), and dichlorobis(tricyclohexylphosphine)palladium(II) (30 mg, 0.04 mmol) in 2-Me THF (3.2 mL) was degassed with a nitrogen stream in a 5 mL high-pressure vial for 20 min. The tube was sealed and heated to 130 °C for 7 h. The reaction mixture was then cooled to rt, filtered through a pad of Florisil, eluted with ethyl acetate and 20%MeOH/DCM, and concentrated in vacuo. The resulting oil was dissolved in ethyl acetate, washed with saturated $NaHCO_3$ ($Na_2SO_4$), and concentrated in vacuo. The residue was combined with [2-methyl-6-oxo-1-(2-pyridylmethyl)-4-pyridyl] trifluoromethanesulfonate (276 mg, 0.793 mmol), LiCl (134 mg, 3.170 mmol), tetrakis(triphenylphosphine)palladium(0) (137 mg, 0.119 mmol), and sodium carbonate (595 μL of 2 M, 1.189 mmol) in dimethoxyethane (4.7 mL) and degassed with a nitrogen stream for 20 min, and was then heated to 90 °C overnight. The mixture was cooled to rt and partitioned between ethyl acetate and water. The organic phase was washed with brine, dried ($Na_2SO_4$), and concentrated in vacuo. The residue was purified by column chromatography over 12 g of silica gel eluted with ethyl acetate to afford 128 mg (37%) of **58**. $^1H$ NMR (300 MHz, $CDCl_3$) δ 8.54 (d, *J* = 4.8 Hz, 1H), 7.66 (td, *J* = 7.7, 1.7 Hz, 1H), 7.54 (d, *J* = 4.2 Hz, 2H), 7.31 (d, *J* = 7.7 Hz, 1H), 7.24−7.10 (m, 1H), 6.86 (d, *J* = 6.1 Hz, 1H), 6.64 (s, 1H), 6.21 (s, 1H), 5.44 (s, 2H), 5.00 (s, 1H), 2.47 (s, 1H), 2.17 (s, 1H) ppm.

**1-Ethyl-3-(5-fluoro-4-(4-methyl-1*H*-pyrazol-1-yl)-6-(2-oxo-1-(pyridin-2-ylmethyl)-1,2-dihydropyridin-4-yl)-1*H*-benzo[*d*]-imidazol-2-yl)urea (20).** To a solution of **58** (128 mg, 0.295 mmol) in methanol (20 mL) was added Raney nickel (1.0 mL of suspension in water). The mixture was placed under 45 psi of hydrogen in a Parr apparatus and allowed to shake for 3 h. The catalyst was removed, and the reaction mixture was filtered and concentrated under vacuum. The residue was combined with 1-ethyl-3-(*N*-(ethylcarbamoyl)-C-methylsulfanyl-carbonimidoyl)urea (75.2 mg, 0.324 mmol), pH 3.5 buffer (prepared with 57.5 g $NaOAc{\cdot}3H_2O$ and 400 mL of 1 N $H_2SO_4$), and 1,4-dioxane (1 mL) and was stirred at 105 °C for 8 h. The reaction mixture was cooled to rt, diluted with water,and neutralized by adding solid $NaHCO_3$. The mixture was allowed to stir for 20 min, and the resulting precipitate was collected by filtration and washed with water (2×) and ethyl acetate to yield 91 mg (61%) of **20** as free base, which was converted to the bis-HCl salt by dilution in $CH_2Cl_2$/MeOH, addition of 4 N HCl/dioxane (0.5 mL), and lyophilization. $^1H$ NMR (300 MHz, DMSO-$d_6$) δ 10.49 (s,1H), 8.57 (d, *J* = 4.2, 1H), 8.10 (t, *J* = 6.7, 1H) 7.90 (s, 1H) 7.53 (d, *J* = 6.0, 1H), 7.43−7.25 (m, 4H), 6.48 (d, *J* = 9.6, 2H), 5.40 (s, 2H), 3.30−3.12 (m, 2H), 2.43 (s, 3H), 2.15 (s, 3H), 1.11 (t, *J* = 7.2, 3H) ppm. HRMS calcd. for $(M + H)^+$, *m*/*z*: 501.2163, found *m*/*z*: 501.2159.

**1-(5-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-propan-1-ol (60).** A mixture of commercially available 1-(5-bromopyridin-2-yl)propan-1-ol (**59**, 400 mg, 1.85 mmol), bis(pinacolato)diboron (541 mg, 2.13 mmol), potassium acetate (545 mg, 5.55 mmol), and dichloro-bis (tricyclohexylphosphoranyl)palladium (69 mg, 0.09 mmol) in 2-methyltetrahydrofuran (7.4 mL) was degassed under a nitrogen stream for 10 min. The mixture was microwaved for 45 min at 135 °C, cooled to rt, and filtered over Celite. The filtrate was concentrated to dryness, dissolved in minimal ethyl acetate, and dropped into cold hexanes while stirring vigorously. The resulting precipitate was removed by filtration, and the filtrate was concentrated to afford 620 mg of **60** as a crude yellow oil that was used without further purification. MS, *m*/*z*: 182.16 $(M + H)^+$ (boronic acid).

**1-Ethyl-3-(6-fluoro-5-(6-(1-hydroxyethyl)pyridin-3-yl)-7-(1*H*-pyrazol-1-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (21).** 5-Bromo-4-fluoro-2-nitro-3-(1*H*-pyrazol-1-yl)aniline (**52a**, 3 g, 9.47 mmol), 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (2.77 g, 10.9 mmol), dichloro-bis(tricyclohexylphosphoranyl)palladium (350 mg, 0.47 mmol), and KOAc (2.787 g, 28.40 mmol) were each split evenly among 3 microwave tubes, suspended in 13 mL of solvent, and degassed for 10 min. The reactions were heated for 45 min at 135 °C and cooled to room temperature, filtered, and concentrated in vacuo to afford a dark brown solid. This material was dissolved in minimal EtOAc and dropped into an Erlenmeyer flask containing hexanes stirring vigorously. The resulting tan solid was filtered and dried to afford 550 mg of boronate. MS, *m*/*z*: 267.20 $(M + H)^+$ (boronic acid). A mixture of 4-fluoro-2-nitro-3-pyrazol-1-yl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-aniline (200 mg, 0.57 mmol), 1-(5-bromo-2-pyridyl)ethanol (116 mg, 0.57 mmol), sodium hydrogen carbonate (960 μL of 1.2 M, 1.15 mmol), and $PdCl_2(Cy_3P)_2$ (50 mg, 0.06 mmol) in DMF (2 mL) was degassed with a stream of nitrogen for 10 min. The reaction mixture was refluxed for 10 h, diluted with EtOAc, washed with brine, dried ($Na_2SO_4$), and concentrated in vacuo. The residue was purified by column chromatography over a 12 g silica gel cartridge and eluted with a 0 to 100% EtOAc/hexanes gradient to afford the desired nitroaniline (70 mg, 36%). MS, *m*/*z*: 344.20 $(M + H)^+$. A mixture of nitroaniline (70 mg, 0.204 mmol) and Raney nickel (20 mg, 0.20 mmol) in MeOH (20 mL) was shaken for 1 h under 40 psi of hydrogen. The catalyst was removed by filtration though a layer of Celite. The filtrate was concentrated in vacuo the give the desired diamine (60 mg, 94%). MS, *m*/*z*: 314.20 $(M + H)^+$. To a mixture of the above diamine (60 mg, 0.19 mmol) and 1-ethyl-3-(*N*-(ethylcarbamoyl)-C-methylsulfanyl-carbonimidoyl)urea (53 mg, 0.23 mmol) in dioxane (1 mL) was added pH 3.5 buffer (10 mL). The mixture was stirred for 15 h at 90 °C and neutralized with saturated aqueous $NaHCO_3$. The resulting precipitate was collected by filtration, washed with $H_2O$ and EtOAc, and dried in vacuo. This free base of **21** was converted to the bis-HCl salt (56 mg, 0.11 mmol, 58%). $^1H$ NMR (300 MHz, $CD_3OD$) δ 9.04 (s, 1H), 8.86 (d, *J* = 8.3 Hz, 1H), 8.48 (s, 1H), 8.22 (d, *J* = 8.4 Hz, 1H), 8.03 (s, 1H), 7.79 (d, *J* = 5.7 Hz, 1H), 6.74 (s, 1H), 5.32 (dd, *J* = 13.4, 6.7 Hz, 1H), 3.3−3.4 (q, 2H), 1.25 (t, 3H) ppm. HRMS calcd. for $(M+H)^+$, *m*/*z*: 410.1741, found *m*/*z*: 410.1739.

**1-Ethyl-3-(6-fluoro-5-(6-(1-hydroxypropyl)pyridin-3-yl)-7-(1*H*-pyrazol-1-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (22).** A mixture of crude 1-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-propan-1-ol (**60**, 270 mg, 1.03 mmol), 5-bromo-4-fluoro-2-nitro-3-(1*H*-pyrazol-1-yl)aniline (**52a**, 325 mg, 1.03 mmol), sodium bicarbonate (170 mg, 2.05 mmol), and $PdCl_2$(dppf) (84 mg, 0.10 mmol) was microwaved for 20 min at 120 °C. The crude mixture was filtered over Celite, and the Celite pad was washed with ethyl acetate. The filtrate was concentrated to dryness and purified by column chromatography over silica gel eluted with a 25 to 100% gradient of ethyl acetate in hexanes to afford 220 mg (60%) of the desired nitroaniline as a brown oil. MS, *m*/*z*: 358.36 $(M + H)^+$. To a solution of the above nitroaniline (220 mg, 0.62 mmol) in methanol (15 mL) was added Raney nickel. The mixture was shaken on a Parr apparatus under 40 psi of hydrogen for 90 min. The reaction mixture was filtered, concentrated to dryness, purified by column chromatography over silica gel, and eluted with 10% methanol in dichloromethane to afford 81 mg (40%) of the diamine. MS, *m*/*z*: 328.28 $(M + H)^+$. To the above diamine (81 mg, 0.247 mmol) in 4 mL of a 2:1 mixture of pH 3.5 buffer ($NaOAc/H_2SO_4$/water) and dioxane was added 3-(*N*-(ethylcarbamoyl)-C-methylsulfanyl-carbonimidoyl)-urea (75 mg, 0.322 mmol) . The reaction was stirred at 90 °C for 2 h. The mixture was cooled to room temperature and diluted with water. The resulting brown precipitate was filtered and dried to give 33 mg (32%) of **22** as the free base. This free base was dissolved in a minimal amount of methanol and treated with aqueous hydrochloric acid (1.2 equiv of 6 N, 15 uL, 0.09 mmol). The resulting mixture was diluted with water and lyophilized to afford 36 mg (32%) of desired product as the mono hydrochloride salt. $^1H$ NMR (300 MHz, DMSO-$d_6$) δ 8.93


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(s, 1H), 8.62 (d, $J$ = 8.2 Hz, 1H), 8.35 (s, 1H), 8.05 (d, $J$ = 8.4 Hz, 1H), 7.94 (d, $J$ = 1.5 Hz, 1H), 7.73−7.54 (m, 2H), 6.60 (s, 1H), 4.96 (dd, $J$ = 7.2, 4.8 Hz, 1H), 3.31−3.11 (m, 2H), 1.85 (dtd, $J$ = 28.4, 13.7, 7.2 Hz, 2H), 1.22−1.04 (m, 3H), 0.94 (t, $J$ = 7.3 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 424.1897, found $m/z$: 424.1896.

**(+)-(*S*)-1-Ethyl-3-(6-fluoro-5-(6-(1-hydroxypropyl)pyridin-3-yl)-7-(1*H*-pyrazol-1-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (23) and (−)-(*R*)-1-Ethyl-3-(6-fluoro-5-(6-(1-hydroxypropyl)pyridin-3-yl)-7-(1*H*-pyrazol-1-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (24).** 150 mg of **22** was separated by chiral SFC using 40% MeOH + 0.2% TEA @ 170 mL/min on a Chiralpak IC (30 × 150); detection: 220 nm. 180 mg of **23** and 84 mg of **24** were obtained as off-white solids. The purity of **23** was assessed at 99.4% using a Chiralpak IC (4.6 × 100), eluted with 40% MeOH + 0.2% TEA, detection: 220 nm, and ee calculated to be 99.9%, and the purity of **24** was 98.8%, with ee of 99.7%, using the same analytical method. Both compounds were converted to their monomesylate salt. Analytical data for **23**: $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.85 (s, 1H), 8.50 (d, $J$ = 8.4, 1H), 8.29 (t, $J$ = 2.3, 1H), 7.95 (d, $J$ = 8.4, 1H), 7.87 (d, $J$ = 1.7, 1H), 7.54 (d, $J$ = 5.9, 1H), 6.66−6.56 (m, 1H), 4.95 (dd, $J$ = 7.5, 4.6, 2H), 3.32−3.28 (m, 2H), 2.74 (s, 3H), 2.11−1.92 (m, 1H), 1.92−1.76 (m, 1H), 1.20 (t, $J$ = 7.2, 3H), 1.04 (t, $J$ = 7.4, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 424.1897, found $m/z$: 424.1895. $[\alpha]_D^{26}$ = 16.0 ($c$ 0.1, MeOH). Analytical data for **24**: $^1$H NMR (300 MHz, TFA) $\delta$ 8.74 (s, 1H), 8.50 (d, $J$ = 8.6, 1H), 8.13 (d, $J$ = 2.5, 1H), 8.08 (d, $J$ = 2.5, 1H), 7.97 (d, $J$ = 5.6, 1H), 7.80 (d, $J$ = 8.5, 1H), 6.75 (d, $J$ = 2.5, 1H), 5.02 (dd, $J$ = 8.1, 4.4, 1H), 3.09 (q, $J$ = 7.2, 2H), 1.90−1.57 (m, 2H), 0.94 (t, $J$ = 7.3, 3H), 0.80 (t, $J$ = 7.3, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 424.1897, found $m/z$: 424.1890. $[\alpha]_D^{26}$ = −15.0 ($c$ 0.1, MeOH).

**1-Ethyl-3-(5-(pyridin-3-yl)-7-(tetrahydrofuran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (25).** A mixture of 4-bromo-2-nitro-6-tetrahydrofuran-2-yl-aniline (**61**,[21] 138 mg, 0.48 mmol), $PdCl_2(PPh_3)_2$ (10 mg, 0.024 mmol), 2 M sodium carbonate (0.43 mL), and 3-pyridyldiethylborane (92 mg, 0.62 mmol) in DME (4 mL) was heated at 140 °C in the microwave for 15 min. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate, and filtered through Celite. The filtrate was sequentially washed with saturated sodium bicarbonate, water, and brine, dried ($Na_2SO_4$), and concentrated in vacuo. The residue was purified by column chromatography over silica gel and eluted with a gradient of 40 to 80% ethyl acetate in hexanes to afford the intermediate nitroaniline (100 mg, 73%). To a solution of the nitroaniline (99 mg, 0.35 mmol) in MeOH (20 mL) was added a catalytic amount of $PtO_2$. The reaction mixture was stirred under 1 atm of $H_2$ for 1 h, filtered through Celite, and concentrated to dryness to provide the intermediate diamine (70 mg, 78%), which was used as is. The above diamine (70 mg, 0.27 mmol) and 3-(*N*-(ethylcarbamoyl)-C-methylsulfanyl-carbonimidoyl)urea (135 mg, 0.54 mmol) were dissolved in dioxane (3 mL) and pH 3.5 buffer (6 mL, stock solution made from 1 N $H_2SO_4$ and NaOAc). The mixture was refluxed for 4 h, cooled to ambient temperature, neutralized by addition of 1 M aqueous $NaHCO_3$, and extracted with ethyl acetate 3 times. The combined organic extracts were washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by column chromatography over silica gel and eluted with a 10 to 20% gradient of methanol in methylene chloride to afford a brown oil. Crystallization of this oil using acetonitrile/ethyl acetate afforded **25** (33 mg, 35%) as a yellow solid. $^1$H NMR ($CD_3OD$, 500 MHz) $\delta$ 8.80 (s, 1H), 8.48 (dd, 1H), 8.10 (m, 1H), 7.58 (d, 1H), 7.50 (dd, 1H), 7.34 (br s, 1H), 5.30 (m, 1H), 4.23 (m, 1H), 4.01 (m, 1H), 3.34 (q, 2H), 2.52 (m, 1H), 2.13 (m, 2H), 1.98 (m, 1H), 1.22 (t, 3H) ppm. MS, $m/z$ 352.2 $(M + H)^+$.

**1-Ethyl-3-(5-(pyrimidin-5-yl)-7-(tetrahydrofuran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (26).** A mixture of 4-bromo-2-nitro-6-tetrahydrofuran-2-yl-aniline (**61**,[21] 500 mg, 1.74 mmol), $Pd(PPh_3)_4$ (201.1 mg, 0.17 mmol), 2 M sodium carbonate (2.6 mL), and 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine (538.2 mg, 2.61 mmol) in DME (10.00 mL) was heated at 100 °C for 18 h. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate, and filtered through Celite. The filtrate was sequentially washed with saturated sodium bicarbonate, water, and brine, dried ($Na_2SO_4$), and concentrated in vacuo. The residue was purified by column chromatography over silica gel eluted with a gradient of 20 to 100% ethyl acetate in hexanes to afford the intermediate nitroaniline (250 mg, 50.2%). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 9.20 (s, 1H), 8.39 (d, $J$ = 2.2 Hz, 1H), 7.58 (d, $J$ = 2.0 Hz, 1H), 7.28 (s, 1H), 7.13 (s, 2H), 4.93 (t, $J$ = 7.2 Hz, 1H), 4.17 (dt, $J$ = 8.2, 7.0 Hz, 1H), 4.00 (dt, $J$ = 8.5, 6.9 Hz, 1H), 2.43−2.28 (m, 1H), 2.25−2.07 (m, 3H) ppm. MS, $m/z$: 287.23 $(M + H)^+$. To a solution of the above Suzuki adduct in MeOH and EtOAc (~100 mL; 1:2) was added 10% Pd/C (0.087 mmol). The reaction mixture was stirred under $H_2$ (45 psi) for 2 h. The depressurized mixture was filtered through Celite and concentrated to dryness to provide the intermediate diamine (170 mg, 76%), which was used as is. MS, $m/z$: 257.18 $(M + H)^+$. The above diamine and 3-(*N*-(ethylcarbamoyl)-C-methylsulfanyl-carbonimidoyl)urea (154.1 mg, 0.66 mmol) were dissolved in dioxane (3 mL) and pH 3.5 buffer (6 mL, stock solution made from 1 N $H_2SO_4$ and NaOAc). The mixture was refluxed for 3 h, cooled to ambient temperature, and neutralized by addition of 1 M aqueous $NaHCO_3$. The resulting solids were collected by filtration, washed with hot water (200 mL), dried in vacuo, and salted as the monomesylate salt to afford **26** (144 mg, 45%). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 9.19 (s, 1H), 9.13 (s, 2H), 7.81 (d, $J$ = 1.5 Hz, 1H), 7.69−7.59 (m, 1H), 5.28 (t, $J$ = 7.3 Hz, 1H), 4.25 (dd, $J$ = 14.4, 7.5 Hz, 1H), 4.03 (dd, $J$ = 14.9, 7.5 Hz, 1H), 3.36 (dd, $J$ = 9.6, 4.9 Hz, 2H), 2.75 (s, 3H), 2.58 (tt, $J$ = 12.4, 6.3 Hz, 1H), 2.13 (dt, $J$ = 14.9, 5.6 Hz, 2H), 1.95 (ddd, $J$ = 16.5, 12.0, 8.1 Hz, 1H), 1.23 (t, $J$ = 7.2 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 353.1726, found $m/z$: 353.1724.

**2-Nitro-6-(tetrahydrofuran-2-yl)-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (63).** A solution of **61**[21] (3.91 g, 13.6 mmol), bis(pinacolato)diboron (3.80 g, 14.98 mmol), and potassium acetate (4.01 g, 14.86 mmol) in 39 mL of DME was degassed with a stream of nitrogen for 1 h. 1,1′-Bis(diphenylphosphino)ferrocenepalladium(II) dichloride (556 mg, 0.68 mmol) was added, and the reaction mixture was stirred at reflux for 1.5 h. The mixture was then allowed to cool to rt, diluted with hexanes, filtered through a plug of Florisil, eluted with EtOAc, and concentrated to dryness in vacuo to afford 6.47 g of a dark amber oil. This oil was purified by ISCO silica gel chromatography eluting with 0 to 35% EtOAc/hexanes to afford 4.24 g (93%) of **63** as an orange glass. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.6 (m, 1H), 7.7 (m, 1H), 7.1 (br s, 2H), 4.8 (m, 1H), 4.1 (m, 1H), 3.9 (m, 1H), 2.3−2.0 (m, 4H), 1.3 (s, 12H) ppm. MS, $m/z$: 335.45 $(M + H)^+$.

**1-Ethyl-3-(5-(1-methyl-2-oxo-1,2-dihydropyridin-4-yl)-7-(tetrahydrofuran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (27).** A mixture of (1-methyl-2-oxo-4-pyridyl) trifluoromethanesulfonate (923 mg, 3.59 mmol), 2-nitro-6-tetrahydrofuran-2-yl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**63**, 1000 mg, 2.99 mmol), LiCl (508 mg, 11.97 mmol), $Pd(PPh_3)_4$ (519 mg, 0.45 mmol), and 2 M aqueous $Na_2CO_3$ (3 mL) in DME (25 mL) was purged with $N_2$ for 2 min and then heated at 100 °C for 48 h. The reaction mixture was then cooled to ambient temperature, diluted with ethyl acetate, and filtered through Celite. The filtrate was sequentially washed with saturated sodium bicarbonate, water, and brine, dried ($Na_2SO_4$), and concentrated in vacuo. The residue was purified by column chromatography over silica gel eluted with a 1 to 10% gradient of MeOH in $CH_2Cl_2$ to afford the intermediate nitroaniline (650 mg, 69%). MS, $m/z$: 315.32 $(M + H)^+$. To the above Suzuki adduct in MeOH and EtOAc (100 mL; 1:2) was added 10% Pd/C (219 mg, 0.21 mmol). The reaction mixture was stirred under 45 psi of $H_2$ for 4 h. The depressurized mixture was filtered through Celite and concentrated to dryness to provide the intermediate diamine (400 mg, 68% yield). MS, $m/z$: 286.26 $(M + H)^+$. The above diamine and 3-(*N*-(ethylcarbamoyl)-C-methylsulfanyl-carbonimidoyl)urea (326 mg, 1.4 mmol) were dissolved in dioxane (6.5 mL) and pH 3.5 buffer (13 mL, stock solution made from 1 N $H_2SO_4$ and NaOAc). The mixture was refluxed for 4 h, cooled to ambient temperature, and neutralized with 1 M aqueous $NaHCO_3$. The resulting solids were collected by filtration and washed with hot water (200 mL). The solids were dried and salted as mono-HCl salt to afford **27** (450 mg, 76%). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.08 (d, $J$ = 7.0 Hz, 1H), 7.87 (s, 1H), 7.68 (s, 1H), 7.22 (dd, $J$ = 6.9, 1.8 Hz, 1H), 7.15 (s, 1H), 5.29 (t, $J$ = 7.4 Hz, 1H), 4.26 (dd, $J$ = 14.6, 7.3 Hz, 1H), 4.03 (dd, $J$ = 14.7, 7.7 Hz, 1H), 3.82 (s, 2H), 3.42−3.33 (m, 2H), 2.60 (td, $J$ = 12.1, 6.8 Hz, 1H), 2.24−2.04


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(m, 2H), 1.94 (ddd, $J$ = 16.5, 12.0, 8.0 Hz, 1H), 1.23 (t, $J$ = 7.2 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 382.1879, found $m/z$: 382.1879.

**1-Ethyl-3-(5-(6-methyl-2-oxo-1-(pyridin-2-ylmethyl)-1,2-dihydropyridin-4-yl)-7-(tetrahydrofuran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (28).** A mixture of [2-methyl-6-oxo-1-(2-pyridylmethyl)-4-pyridyl] trifluoromethanesulfonate (**57**, 1.60 g, 4.55 mmol), 2-nitro-6-tetrahydrofuran-2-yl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (**63**, 1.22 g, 3.64 mmol), LiCl (617 mg, 14.6 mmol), $Pd(PPh_3)_4$ (630 mg, 0.55 mmol), and 2 M aqueous $Na_2CO_3$ (3.6 mL) in DME (30 mL) was purged with $N_2$ for 2 min and then heated at 100 °C for 48 h. The reaction mixture was then cooled to ambient temperature, diluted with ethyl acetate, and filtered through Celite. The filtrate was sequentially washed with saturated sodium bicarbonate, water, and brine, dried ($Na_2SO_4$), and concentrated in vacuo. The residue was purified by column chromatography over silica gel eluted with a 1 to 10% gradient of MeOH in $CH_2Cl_2$ to afford the intermediate nitroaniline (1.2 g, 81%). MS, $m/z$: 407.25 $(M + H)^+$. To the above Suzuki adduct in MeOH and EtOAc (100 mL; 1:2) was added 10% Pd/C (314 mg, 0.30 mmol). The reaction mixture was stirred under $H_2$ (45 psi) for 4 h. The depressurized mixture was filtered through Celite and concentrated to dryness to provide the intermediate diamine (910 mg, 82%). MS, $m/z$: 377.27 $(M + H)^+$. The above diamine 3-(*N*-(ethylcarbamoyl)-C-methylsulfanyl-carbonimidoyl)urea (3.20 g, 1.38 mmol) were dissolved in 1,4-dioxane (6.5 mL) and pH 3.5 buffer (13 mL, stock solution made from 1 N $H_2SO_4$ and NaOAc). The mixture was refluxed for 4 h, cooled to ambient temperature, and neutralized with 1 M aqueous $NaHCO_3$. The resulting solids were collected by filtration, washed with hot water (200 mL), and dried to afford **28** (450 mg, 76%). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.59 (d, $J$ = 5.1 Hz, 1H), 7.67 (td, $J$ = 7.9, 1.3 Hz, 2H), 7.21(dd, $J$ = 14.1, 5.8 Hz, 4H), 6.63 (d, $J$ = 1.3 Hz, 1H), 5.51 (s, 2H), 5.20 (d, $J$ = 7.5 Hz, 1H), 4.31−4.13 (m, 1H), 4.06−3.87 (m, 1H), 3.42−2.99 (m, 2H), 2.59−2.31 (m, 4H), 2.04 (dd, $J$ = 19.4, 13.0 Hz, 3H), 1.04 (t, $J$ = 7.2 Hz, 3H) ppm. MS, $m/z$: 473.32 $(M + H)^+$.

**2-(5-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)propan-2-ol (65).** A microwave tube was charged with commercially available 2-(5-bromo-2-pyridyl)propan-2-ol (796 mg, 3.684 mmol), bis(dipinacolato)diboron (982 mg, 3.87 mmol), dichloro-bis (tricyclohexylphosphoranyl) palladium (136 mg, 0.18 mmol), KOAc (1.08 g, 11.1 mmol), and 2-methyltetrahydrofuran (14.9 mL). The mixture was degassed for 10 min and then heated in a microwave for 45 min at 135 °C. The crude mixture was allowed to cool and was filtered on Florisil (eluted with $CH_2Cl_2$/MeOH). The filtrate was washed with saturated aqueous $NaHCO_3$, dried ($MgSO_4$), and concentrated in vacuo. The residue was then used without further purification. MS, $m/z$: 181.93 $(M + 1)^+$ (boronic acid).

**1-Ethyl-3-(5-(6-(2-hydroxypropan-2-yl)pyridin-3-yl)-7-(tetrahydrofuran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (29).** A mixture of crude 2-[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-pyridyl]-propan-2-ol (**65**, 500 mg, 1.9 mmol), 4-bromo-2-nitro-6-tetrahydrofuran-2-yl-aniline (**61**, 469 mg, 1.58 mmol), $Pd(PPh_3)_4$ (183 mg, 0.158 mmol), and sodium carbonate (2.37 mL of 2 M, 4.75 mmol) in 1,2-dimethoxyethane (10 mL) was heated at reflux for 8 h. The dark yellow reaction mixture was cooled to rt and diluted with water, saturated aqueous $NaHCO_3$, and $CH_2Cl_2$. The phases were separated and dried ($MgSO_4$), and the organics were concentrated in vacuo. The residue was purified by ISCO (40 g $SiO_2$, $CH_2Cl_2$ to 60% EtOAc in $CH_2Cl_2$) to yield 259 mg of the desired product (257 mg, 48%) as a yellow oil that solidified upon standing. MS, $m/z$: 344.23 $(M + 1)^+$. A solution of the above nitroaniline (259 mg, 0.75 mmol) in MeOH (20 mL)/EtOAc (30 mL) was placed ion a Parr flask, and 10% Pd/C (wet, Degussa, 200 mg, 1.88 mmol) was added. The mixture was shaken in a Parr apparatus under 45 psi of hydrogen for 4 h. The reaction mixture was filtered and concentrated in vacuo to afford the desired diamine (230 mg, 97%), which was used as is. MS, $m/z$: 314.25 $(M + 1)^+$. To a solution of crude diamine (230 mg, 0.734 mmol) in 1,4-dioxane (4.4 mL) was added 1-ethyl-3-(*N*-(ethylcarbamoyl)-C-methylsulfanylcarbonimidoyl)urea (222 mg, 0.954 mmol) and pH 3.5 buffer (10 mL). The reaction solution was stirred at 90 °C for 10 h, cooled to rt, diluted with water (∼20 mL), and neutralized with saturated aqueous $NaHCO_3$ (∼15 mL). The resulting turbid reaction mixture was extracted twice with $CH_2Cl_2$/MeOH (9:1). The organics were dried ($MgSO_4$) and concentrated in vacuo. The residue was dissolved in $CH_2Cl_2$ and purified by ISCO (24 g $SiO_2$, DCM to 4% 7 N $NH_3$/MeOH in DCM) to afford a beige solid, which was triturated in hot $Et_2O$ to yield the free base of **29** (140 mg, 47%) as an off-white solid. This was converted to the bis mesylate salt. $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.95 (d, $J$ = 1.6 Hz, 1H), 8.90 (dd, $J$ = 8.6, 2.2 Hz, 1H), 8.23 (d, $J$ = 8.6 Hz, 1H), 7.92 (d, $J$ = 1.5 Hz, 1H), 7.72 (d, $J$ = 0.8 Hz, 1H), 5.31 (t, $J$ = 7.3 Hz, 1H), 4.26 (dd, $J$ = 14.4, 7.5 Hz, 1H), 4.03 (dd, $J$ = 14.9, 7.6 Hz, 1H), 3.36 (dd, $J$ = 12.0, 4.8 Hz, 2H), 2.73 (s, 6H), 2.61 (dd, $J$ = 12.0, 5.1 Hz, 1H), 2.12 (ddd, $J$ = 9.7, 7.3, 4.3 Hz, 2H), 2.04−1.88 (m, 1H), 1.73 (s, 6H), 1.22 (dd, $J$ = 11.4, 4.1 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 410.2192, found $m/z$: 410.2188.

**1-Ethyl-3-(5-(2-(2-hydroxypropan-2-yl)pyrimidin-5-yl)-7-(tetrahydrofuran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (30).** A mixture of 4-bromo-2-nitro-6-tetrahydrofuran-2-yl-aniline (**61**, 500 mg, 1.74 mmol), $Pd(PPh_3)_4$ (201 mg, 0.17 mmol), 2 M aqueous sodium carbonate (2.6 mL), and commercially available 2-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-yl)propan-2-ol (608 mg, 2.30 mmol) in DME (12.50 mL) was heated at 100 °C for 18 h. The reaction mixture was then cooled to ambient temperature, diluted with ethyl acetate, and filtered through Celite. The filtrate was sequentially washed with saturated sodium bicarbonate, water, and brine, dried ($Na_2SO_4$), and concentrated in vacuo. The residue was purified by column chromatography over silica gel eluted with a 20 to 100% gradient of EtOAc in hexanes to afford the intermediate nitroaniline (900 mg, 75%). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.89 (s, 2H), 8.36 (d, $J$ = 2.2 Hz, 1H), 7.57 (d, $J$ = 2.1 Hz, 1H), 7.11 (s, 2H), 4.93 (t, $J$ = 7.2 Hz, 1H), 4.68 (s, 1H), 4.15 (ddd, $J$ = 10.2, 7.7, 5.4 Hz, 1H), 4.04−3.94 (m, 1H), 2.43−2.26 (m, 1H), 2.16 (ddd, $J$ = 9.4, 6.5, 1.9 Hz, 3H), 1.64 (s, 6H) ppm. MS, $m/z$: 345.18 $(M + H)^+$. To the above Suzuki adduct in MeOH and EtOAc (∼100 mL; 1:2) was added 10% Pd/C (278 mg, 0.26 mmol). The reaction mixture was stirred under $H_2$ (45 psi) for 2 h. The depressurized mixture was then filtered through Celite and concentrated to dryness to provide the intermediate diamine (565 mg, 69%). MS, $m/z$: 315.26 $(M + 1)^+$. The above diamine and 1-ethyl-3-(*N*-(ethylcarbamoyl)-C-methylsulfanylcarbonimidoyl)urea (417 mg, 1.80 mmol) were dissolved in dioxane (6 mL) and buffer pH 3.5 (18 mL, stock solution made from 1 N $H_2SO_4$ and NaOAc). The resulting mixture was refluxed for 3 h, cooled to ambient temperature, and neutralized with 1 M aqueous $NaHCO_3$. The resulting beige solids were collected by filtration, washed with hot water (200 mL), dried in vacuo, and salted as the monomesylate salt to afford **30** (576 mg, 62% yield). $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 9.07 (s, 2H), 7.79 (d, $J$ = 1.5 Hz, 1H), 7.62 (s, 1H), 5.41−5.21 (m, 1H), 4.32−4.17 (m, 1H), 4.04 (dd, $J$ = 14.7, 8.0 Hz, 1H), 3.38 (d, $J$ = 7.2 Hz, 2H), 2.72 (s, 3H), 2.66−2.52 (m, 1H), 2.12 (d, $J$ = 7.6 Hz, 2H), 2.03−1.89 (m, 1H), 1.64 (s, 6H), 1.23 (t, $J$ = 7.2 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 411.2145, found $m/z$: 411.2140.

Urea **30** (576 mg) was dissolved in 23 mL of methanol (solution concentration of 25 mg/mL) and separated into its two enantiomers using chiral SFC (preparative conditions: 50% MeOH + 0.2% DEA @ 170 mL/min on an IC (30*150), 100 bar, 35C, 220 nm, 3.0 mL injections). The enantiomers were salted to afford compound **32** (223 mg, 48.5%, ee 100%) and **31** (228 mg, 48.6%, ee 100%) as the monomesylate salts (analytical conditions: 40% MeOH + 0.2% DEA @ 5 mL/min on an IC (4.6*100), 100 bar, 35C, 220 nm; ee's calculated using analytical chromatogram peak areas).

**(−)-(*S*)-1-Ethyl-3-(5-(2-(2-hydroxypropan-2-yl)pyrimidin-5-yl)-7-(tetrahydrofuran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (32).** $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 9.08 (s, 2H), 7.80 (d, $J$ = 1.2 Hz, 1H), 7.62 (s, 0H), 5.30 (t, $J$ = 7.4 Hz, 1H), 4.24 (dd, $J$ = 14.5, 7.5 Hz, 1H), 4.04 (dd, $J$ = 14.7, 7.7 Hz, 1H), 3.36 (dd, $J$ = 10.3, 4.1 Hz, 2H), 2.72 (s, 3H), 2.67−2.51 (m, 1H), 2.28−2.04 (m, 2H), 2.03−1.88 (m, 1H), 1.64 (s, 6H), 1.23 (t, $J$ = 7.2 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 411.2145, found $m/z$: 411.2143. $[\alpha]_D^{25} = -17.0$ ($c$ 0.1, MeOH).

**(+)-(*R*)-1-Ethyl-3-(5-(2-(2-hydroxypropan-2-yl)pyrimidin-5-yl)-7-(tetrahydrofuran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (31).** $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 9.08 (s, 2H), 7.80 (d, $J$ = 1.3 Hz, 1H), 7.70−7.43 (m, 1H), 5.42−5.21 (m, 1H), 4.25 (d, $J$ = 7.5 Hz, 1H), 4.03 (d, $J$ = 6.8 Hz, 1H), 3.36 (dd, $J$ = 10.2, 4.3 Hz, 2H), 2.73 (s, 3H), 2.59 (s, 1H), 2.29−2.05 (m, 2H), 1.96 (m, 1H), 1.64 (s, 3H), 1.23


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(t, $J$ = 7.2 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 411.2145, found $m/z$: 411.2142. $[\alpha]_D^{26}$ = 17.0 ($c$ 0.1, MeOH).

**1-Ethyl-3-(6-fluoro-5-(2-(2-hydroxypropan-2-yl)pyrimidin-5-yl)-7-(tetrahydrofuran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (33).** A suspension of 4-bromo-3-fluoro-6-nitro-2-tetrahydrofuran-2-yl-aniline (**62**,[21] 9.41 g, 30.8 mmol), 2-[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-yl]propan-2-ol (8.96 g, 33.9 mmol), saturated aqueous $NaHCO_3$ (77 mL, 93 mmol), and tetrakis-(triphenylphosphine) palladium(0) (1.78 g, 1.54 mmol) in 1,4-dioxane (94 mL) was stirred at 108 °C overnight, cooled to rt, and diluted with ethyl acetate. The organics were washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. To a stirred solution of the residue in 20 mL of ethanol was added 100 mL of hexanes. After 1 h of stirring, the precipitate was collected, washed with hexanes, and dried in vacuo to afford the intermediate nitroaniline (8.88 g, 79%). MS, $m/z$: 365.25 $(M + 1)^+$. To a solution of nitroaniline (8.88 g, 24.5 mmol) in methanol (44 mL) was added triethylamine (6.8 mL, 49 mmol) and 5% Pd/C (50% wet, 1.78 g). The reaction mixture was placed on a Parr shaker under 45 psi of hydrogen overnight. Filtration through Celite and purification by column chromatography ($SiO_2$, ethyl acetate) afforded the intermediate diamine (7.22 g, 89%). MS, $m/z$: 333.34 $(M + 1)^+$. To the diamine (7.22 g, 21.7 mmol) in 1,4-dioxane (36 mL) was added pH 3.5 buffer (72 mL). After addition of 1-ethyl-3-(*N*-(ethylcarbamoyl)-*C*-methylsulfanyl-carbonimidoyl)urea (**41**, 6.05 g, 26.1 mmol), the reaction was heated at 108 °C for 1.5 h. Following cooling to rt and addition of aqueous sodium bicarbonate solution (144 mL), the mixture was filtered, and the collected solid was washed twice with water (2 × 10 mL) and dried in vacuo to afford **33** (7.90 g, 85%). MS, $m/z$: 429.45 $(M + 1)^+$.

Urea **33** (7.50 g) was dissolved in 2330 mL of methanol/methylene chloride 1:1 (solution concentration of 3 mg/mL) and, after filtration through a 0.2 $\mu$m nylon membrane, was separated into its two enantiomers using chiral SFC (preparative conditions: 70% MeOH + 0.2% DEA at 175 mL/min on an IC (30*150), 100 bar, 35C, 220 nm, 12 mL injections) to afford 3.72 g (40%) of (+)-(*R*)-1-ethyl-3-(6-fluoro-5-(2-(2-hydroxypropan-2-yl)pyrimidin-5-yl)-7-(tetrahydrofuran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (**34**), with an ee of 100%, and 3.70 g (40%) of (−)-(*S*)-1-ethyl-3-(6-fluoro-5-(2-(2-hydroxypropan-2-yl)-pyrimidin-5-yl)-7-(tetrahydrofuran-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)-urea (**35**), with an ee of 100% (analytical conditions: 70% MeOH + 0.2% DEA at 5 mL/min on an IC (4.6*100), 100 bar, 35C, 220 nm; ee's calculated using analytical chromatogram peak areas). Analytical data for **34**: $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.95 (d, $J$ = 1.6 Hz, 2H), 7.45 (d, $J$ = 6.4 Hz, 1H), 5.44−5.32 (m, 1H), 4.27 (q, $J$ = 7.3 Hz, 1H), 4.01 (q, $J$ = 7.3 Hz, 1H), 3.36−3.30 (m, 2H), 2.65−2.49 (m, 1H), 2.20−2.06 (m, 2H), 2.00−1.83 (s, 1H), 1.63 (s, 6H), 1.21 (t, $J$ = 7.2 Hz, 3H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ 173.18, 156.28, 156.26, 152.61 (d, $J$ = 240.87 Hz), 152.18, 146.99, 128.03, 126.69, 117.96 (d, $J$ = 16.25 Hz), 115.72 (d, $J$ = 20 Hz), 112.80, 74.42, 72.64, 68.20, 34.46, 32.74, 29.59, 25.43, 14.74 ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 429.2050, found $m/z$: 429.2039. $[\alpha]_D^{26}$ = 16 ($c$ 0.1, MeOH). Analytical data for **35**: $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 8.94 (d, $J$ = 1.6 Hz, 2H), 7.44 (d, $J$ = 6.4 Hz, 1H), 5.44−5.33 (m, 1H), 4.27 (q, $J$ = 7.3 Hz, 1H), 4.01 (q, $J$ = 7.3 Hz, 1H), 3.36−3.30 (m, 2H), 2.65−2.48 (m, 1H), 2.20−2.06 (m, 2H), 2.00−1.4 (s, 1H), 1.62 (s, 6H), 1.20 (t, $J$ = 7.2 Hz, 3H) ppm. HRMS calcd. for $(M + H)^+$, $m/z$: 429.2050, found $m/z$: 429.2039. $[\alpha]_D^{26}$ = −14.0 ($c$ 0.1, MeOH).

**$K_i$ Determination.** The details of the enzyme inhibition assays can be found in ref 23.

**Small Molecule X-ray Structure Determination.** Colorless crystals of **34** were obtained by crystallization from ethanol. A crystal with dimensions of 0.15 × 0.15 × 0.10 mm$^3$ was mounted on a Bruker APEX II CCD diffractometer with Cu Ka radiation. Diffraction data were collected to a resolution of 0.85 Å using 0.5° steps and 30 s exposure for each frame. Data were collected at 100 (2) K using a nitrogen open-flow system. Integration of intensities and refinement of cell parameters were accomplished using APEX software. The structure was solved by direct methods and refined by the SHELXTL package.

**Susceptibility Testing.** All bacteria were obtained from the American Type Culture Collection (ATCC; Manassas, VA). Minimal inhibitory concentration (MIC) determinations were performed in liquid medium in 96-well microtiter plates according to the methods described by the Clinical Laboratory and Standards Institute (CLSI[23]) with modifications as described previously.[23] To measure the relative effects of serum on compound susceptibility, human serum (U.S. Biological, Swampscott, MA) was added to liquid medium to a final concentration of 50% in assays performed with *S. aureus* ATCC 29213. All test methods met acceptable standards based on recommended quality control ranges for all comparator antibiotics and the appropriate ATCC strain.

**In Vitro Covalent Binding Experiments.** Thirty micromolar $^{14}$C-radiolabeled **3** (specific activity: 0.43 mCi/mmole) was incubated with microsomes from rat, dog, monkey, and mouse liver in the presence of NADPH for 1 h. Incubation was quenched with acetonitrile, and the precipitated protein was washed exhaustively until the supernatant from the washes were devoid of unbound radioactivity. The remaining protein pellet was dissolved in 1 N NaOH in a water bath at 60 °C for at least 1 h or until completely dissolved. Ten microliters of the dissolved sample was then analyzed on a liquid scintillation counter to determine the amount of covalently bound radioactivity. An aliquot of the dissolved protein was reserved for determination of protein concentration using the Bradford assay. The amount of radioactivity was normalized to the protein content in every sample and expressed as picomoles per milligram of protein.

**In Vivo Covalent Binding Experiments.** Unlabeled **3** was dosed to male rats at 90 mg/kg for 6 or 13 days. On day 7 or 14, the animals received 100 $\mu$Ci of $^{14}$C-labeled **3** (specific activity: 55mCi/mmol). A separate cohort of rats received a single dose of the radiolabeled compound. Four hours after the radiolabeled compound was dosed, all animals were euthanized, and their livers were removed and weighed. An aliquot of the livers was homogenized in 0.1 M phosphate buffer using a Potter-Elvehjem tissue grinder. The liver homogenate was then centrifuged at 9000*g* to separate the S9 fraction. An aliquot of the S9 fraction was then washed exhaustively with acetonitrile until the supernatant was devoid of unbound radioactivity, as determined by liquid scintillation counting. The remaining protein pellet was dissolved in 1 N NaOH in a water bath at 60 °C for at least 1 h or until completely dissolved. Ten microliters of the dissolved sample was then analyzed on a liquid scintillation counter to determine the amount of covalently bound radioactivity. An aliquot of the dissolved protein was reserved for determination of protein concentration using the Bradford assay. The amount of radioactivity was normalized to the protein content in every sample and expressed as picomoles per milligram of protein. A similar experiment was conducted with compound **34**.

**Incubations in Supersomes.** Compound **3** (1 and 10 $\mu$M) was incubated with individual recombinant CYP supersomes in the presence and absence of NADPH. The incubations were carried out for either 0, 15, and 30 min. Samples were analyzed by LC-MS/MS, and the formation of compound **10** was quantified.

**Description of the Neutropenic Rat Thigh Infection Model.** The methicillin-susceptible *S. aureus* (MSSA) strain ATCC 29213 was obtained from the American Type Culture Collection (ATCC). The frozen bacterial test isolate was subcultured twice onto standard microbiological agar media (trypticase soy agar with 5% sheep blood) prior to inoculation. The second plating occurred less than 24 h before use in the thigh infection model. Immediately prior to injection into thighs, several bacterial colonies were suspended in 0.9% saline to approximate a 0.5 McFarland turbidity standard and then diluted to the target concentration of $10^7$ colony forming units (CFU)/mL for inoculation. Several dilutions of this inoculum were plated again to verify the infection dose. Neutropenia was induced via an intraperitoneal injection of 150 mg/kg of the immunosuppressant cyclophosphamide (Baxter Healthcare Corp.) at a volume of 1 mL per animal 3 days prior to bacterial infection. On day 0, the rats (121−159 g) were infected by an intramuscular injection (0.2 mL) into both rear thighs using a suspension of $10^7$ CFU/mL *S. aureus* ATCC 29213 in normal saline. Compound dosing commenced at the designated time 0 at 2 h post-inoculation, when colonization was expected to be stable. Three rats ($n$ = 3) per thigh collection time point (8 and 24 h post treatment initiation) were administered one of the following five test formulations: vehicle (20% cavitron/1% HPMCAS-MG), **34** at 10, 30, or 60 mg/kg in vehicle, or moxifloxacin (Moxi) at 30 mg/kg in saline. Each treatment


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was administered via oral gavage in a 10 mL/kg dosing volume at 0 and 12 h (q12h). Thigh tissues from untreated controls were harvested 2 h after infection at time 0 (early control, EC). Thighs from vehicle- (late controls, LC) and compound-treated animals were harvested after 8 or 24 h of treatment. Both rear thighs ($n$ = 2) of each animal were harvested, rinsed with sterile saline, weighed, placed in 50 mL sterile normal saline, and homogenized. Approximately half of the unfiltered homogenate was frozen for subsequent drug concentration analysis. The other half of the homogenized sample volume was passed through a large pore filter to remove cartilage and large clumped pieces of tissue. The filtrate was then 10-fold serially diluted in saline, cultured onto agar media plates (trypticase soy agar with 5% sheep blood), and incubated at 37 °C for 18−24 h. Colony forming units (CFU) were enumerated as CFU/mL homogenate, and the median for each treatment group ($n$ = 6, i.e., two thighs from three animals each) was calculated. As a measure of thigh burden, the median CFU/mL thigh homogenate for each compound-treated group was compared to both the initial (time 0) bacterial density (EC) and that of the harvest time-matched vehicle controls (LC). To obtain statistics, the data were analyzed using a Kruskal−Wallis nonparametric test followed by a Dunn's post-test to compare all data sets in an experiment (Prism Software). Significant differences were noted with their $p$ values represented as follows: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

**Ethics Regulation of Laboratory Animals.** All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and with approval of the Vertex Pharmaceuticals Institutional Animal Care and Use Committee (IACUC).

## ■ ASSOCIATED CONTENT

### Supporting Information

Two-dimensional NMR characterization of compound **10**; coelution data of synthesized compound **10** and M − 2 metabolite; small molecule X-ray structure of compound **34**; MS/MS data supporting the structure of the M + 14 metabolites of compound **3**, and of the metabolites of compound **34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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