Novel Dual-Targeting Benzimidazole Urea Inhibitors of DNA Gyrase and Topoisomerase IV Possessing Potent Antibacterial Activity: Intelligent Design and Evolution through the Judicious Use of Structure-Guided Design and Stucture−Activity Relationships

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The discovery of new antibacterial agents with novel mechanisms of action is necessary to overcome the problem of bacterial resistance that affects all currently used classes of antibiotics. Bacterial DNA gyrase and topoisomerase IV are well-characterized clinically validated targets of the fluoroquinolone antibiotics which exert their antibacterial activity through inhibition of the catalytic subunits. Inhibition of these targets through interaction with their ATP sites has been less clinically successful. The discovery and characterization of a new class of low molecular weight, synthetic inhibitors of gyrase and topoisomerase IV that bind to the ATP sites are presented. The benzimidazole ureas are dual targeting inhibitors of both enzymes and possess potent antibacterial activity against a wide spectrum of relevant pathogens responsible for hospital- and community-acquired infections. The discovery and optimization of this novel class of antibacterials by the use of structure-guided design, modeling, and structure−activity relationships are described. Data are presented for enzyme inhibition, antibacterial activity, and in vivo efficacy by oral and intravenous administration in two rodent infection models.

Introduction

Resistant Gram-positive organisms have emerged over the past 2 decades and pose significant public health concerns because of the serious types of infections they cause, the vulnerable patient populations they infect, and their ability to spread within the hospital environment and from the hospital to the community. Clinically relevant organisms include methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, penicillin-resistant *Streptococcus*, macrolide-resistant *Streptococcus*, and more recently, fluoroquinolone-resistant *Staphylococcus* and *Streptococcus*.[1−5] Data from the National Nosocomial Infections Surveillance system[6] suggest that the prevalence of methicillin-resistant *S. aureus* infections in hospital ICUs has increased steadily over the past decade to a value exceeding 60% in 2004. Similarly, vancomycin-resistant *Enterococcus* infections have reached a level exceeding 30%. A recent troubling trend is the increase of clinical isolates that are multidrug resistant.[7,8]

Bacterial resistance has been demonstrated against all of the commonly prescribed classes of antibiotics.[1] Antibiotic sales are comprised primarily of multiple generations of β-lactams (cephalosporins, penicillins, penems, and monobactams), fluoroquinolones, and macrolides.[9] A major driver for continual modification of these scaffolds has been to stay one step ahead of resistance mechanisms, a strategy that is rapidly approaching the point of diminishing returns largely because resistance mechanisms to earlier members of a given class of antibiotics often impact, to some degree, the effectiveness of subsequent generations of molecules from the same class. Identifying new scaffold classes that hit new molecular targets is the obvious approach to maintain a pipeline of effective antibiotics.[10] In the past 35 years, only two antibiotics representing new scaffold classes linezolid and daptomycin have been approved in the U.S. This creates a compelling need for new classes of antibacterial agents with novel mechanisms of action. While genomics has offered the potential to identify novel druggable antibacterial targets, this has not yet proven successful.[1]

DNA gyrase and topoisomerase IV (topoIV[a]) have long been recognized as attractive targets for antibiotics.[11−13] Each enzyme is a heterotetrameric type II topoisomerase characterized by its ability to alter chromosome structure through breaking and rejoining double stranded DNA; in addition, each enzyme is independently essential for bacterial DNA replication. Gyrase is primarily responsible for introducing negative supercoils into conformationally constrained DNA, while topoIV primarily resolves linked chromosome dimers at the conclusion of DNA replication.[12,14−16] The fluoroquinolones inhibit the catalytic subunits of gyrase (GyrA) and/or topoIV (ParC).[12] The associated subunits responsible for supplying the energy necessary for catalytic turnover/resetting of the enzymes via ATP hydrolysis are GyrB (gyrase) and ParE (topoIV), respectively.[17] The ATP binding sites in these subunits have been less successfully exploited as antibacterial targets with the exception of the natural product coumarins, e.g., novobiocin (Scheme 1) and cyclothialadines.[18] The benzimidazole ureas described in

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[a] Abbreviations: topoIV, topoisomerase IV; MIC, minimum inhibitory conventration; CFU, colony-forming unit; ADPNP, 5′-adenylyl-β,γ-imidodiphosphate.

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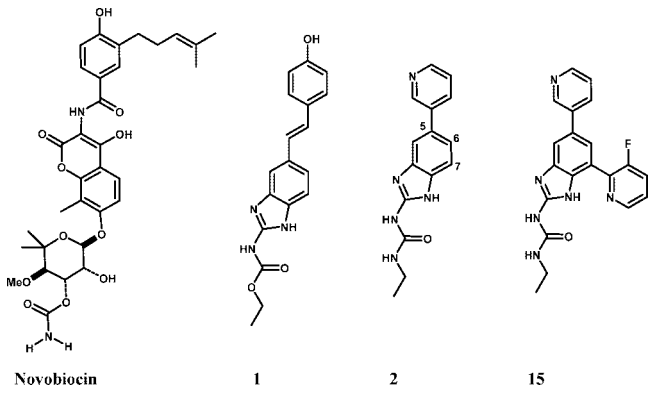
Scheme 1



this study were designed and optimized to target these same ATP binding sites in the GyrB and ParE subunits.

To maximize effectiveness and longevity of an antibiotic, as well as to gain acceptance for use as an empiric monotherapy, it is important that a new antibacterial agent suppress the appearance of resistant strains. One way to accomplish this is for a compound to work by interacting with more than one essential target such that multiple mutations or other significant genetic alterations are needed for clinically relevant resistance.[19−21] The statistical likelihood of significant simultaneous mutations in two independently essential targets is low (∼1 in 10^{14} bacteria).[22] Dual inhibition of gyrase and topoIV is, therefore, expected to minimize the frequency of resistant mutations. Biochemical, genetic, and structural data show that the benzimidazole ureas are potent dual targeting agents of both enzymes.[23−25]

Compound **1** (Scheme 1) was identified in a high-throughput ATPase assay targeting the GyrB subunit.[26] Approximately 30 000 compounds prefiltered for druglike properties[27] were used as the screening deck. Both publically available[28] and in-house crystal structures of novobiocin bound to the *E. coli* GyrB subunit served as the initial guide for ligand optimization. While the use of structure-guided design has proven itself with respect to optimizing enzyme inhibitors,[29] there are certainly additional factors that contribute to antibacterial potency. Foremost among these factors are permeability into the bacteria and avoidance of bacterial efflux.

Results and Discussion

All compounds were evaluated for enzymatic inhibition and antibacterial potency. Relative serum binding potential was evaluated by *S. aureus* MIC assays containing 50% human serum (Table 1).

The benzimidazole carbamate, **1**, was identified as a micromolar GyrB inhibitor with MIC values greater than 16 μg/mL against all organisms tested (Scheme 1, Table 1). Docking of **1** into the *S. aureus* X-ray structure from which novobiocin was removed immediately suggested two types of structural modifications (Figure 1). First, it appeared that modification of the carbamate oxygen of **1** to a nitrogen (urea) might change a seemingly repulsive interaction into an attractive one that would further contribute to an extensive hydrogen bond network involving Asp-73, Thr-165 (*E. coli* gyrase numbering), and a highly conserved structural water (Figure 1B). Since the benzimidazole portion of the scaffold also contributes to this hydrogen bond network, it was thought that this would further provide an optimization path to potent, low MW inhibitors. The benzimidazole urea scaffold is positioned deep in the ATP binding site and maximizes overlap with the adenine ring of the nonhydrolyzable ATP analogue, ADPNP (Figure 1C). It was expected that this would reduce the selection of resistant mutants in this region of the binding site, since they would affect the viability of the organism if the binding of ATP were perturbed.

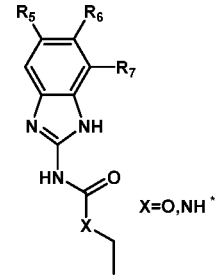
The second design principle to emerge from this initial analysis was that the hydrogen bond interaction between Arg-136 and the exocyclic carbonyl oxygen of the novobiocin coumarin ring (*Figure 1*A) could potentially be mimicked by a heterocyclic hydrogen bond acceptor directly attached to the benzimidazole urea core (*Figure 1*D).

Compound **2** (Table 1) was the end result of this initial design and provided an approximately 250-fold improvement in K_i against *E. coli* gyrase (i.e., from 20 μM to 81 nM) and approximately 15-fold in K_i against *S. aureus* gyrase (i.e., from 2 μM to 130 nM). This compound showed modest antibacterial potency with an MIC of 16 μg/mL against *S. aureus* and 2 μg/mL against *S. pneumoniae* (Table 1). Interestingly, **2** also showed some weak inhibitory potency against *E. coli* topoIV with a K_i of 2.3 μM. While compound **2** failed to show antibacterial activity against the Gram-negative organism, *E. coli* (MIC > 64 μg/mL), it did show an MIC of 16 μg/mL against the respiratory Gram-negative pathogen, *H. influenzae*. (For most compounds presented in this paper, there was a general lack of antibacterial potency against *E. coli*. This has been previously attributed to the effects of compound-mediated efflux.)[23]

Selected SAR at the 5-, 6-, and 7-Positions of the Benzimidazole Urea Scaffold. Examination of the model of **2** bound in the ATP binding site suggested that a variety of substitutions might be tolerated at both the 5- and 6- positions of the benzimidazole core. A limited number of examples of substituents at each of these positions are presented to illustrate specific points; however, it should be noted that many additional analogues were synthesized and have contributed to the overall SAR understanding.

5-Position SAR. As mentioned above, formation of a hydrogen bond between the 3-pyridyl nitrogen of **2** and the side chain of Arg-136 appeared to mimic an interaction observed with novobiocin. Compound **3** lacks the ability to form such a hydrogen bond and consequently loses 5-fold in gyrase K_i, compared with **2**, accompanied by a decrease in antibacterial potency. However, compound **4** contains a 5-pyrimidinyl substituent and shows modest improvements relative to **2** in terms of K_i against both gyrase and topoIV that translates to an improvement in overall MIC. Compound **5** represents several imidazole amide analogues wherein the design intended the amide carbonyl oxygen to act as a hydrogen bond acceptor with Arg-136. This substitution resulted in approximately 10- to 20-fold improvement in K_i against gyrase and a 30-fold improvement against topoIV. These improvements in K_i relative to **2** resulted in a 4-fold improvement in antibacterial potency against *S. aureus* and *H. influenzae* and a 30-fold improvement in MIC against *S. pneumoniae*. This hydrogen bond was confirmed by crystallography with later structures (Figure 2A, compound **19**).[30] It appeared that the exocyclic nature of the imidazole amide carbonyl allowed for a more optimal hydrogen-bonding pattern with the Arg-136 guanidino group. It is also interesting that compounds **4** and **5** began to show improved antibacterial activity against *H. influenzae* that was reflected by their improvement in enzyme inhibitory potency against gyrase. In addition to the modifications at the 5-position, the 6-fluoro substituent of compounds **5** and **6** may have also contributed to the improvement in both enzyme inhibition and antibacterial potency relative to **2**.

Table 1. Substituent Effects on Gyrase and Topoisomerase IV Inhibition and Antibacterial Activities[a]



				enzyme inhibiton data K_i (μM)			minimum inhibitory concentration (μg/mL)			
							S. aureus			
compd	5-position	6-position	7-position	*S. aureus* gyrase	*E. coli* gyrase	*E. coli* topoIV	− HS	+ HS	*S. pneumoniae*	*H. influenzae*
novobiocin	NA	NA	NA	0.01	0.014	0.11	0.125	>16	2	0.063
1	4-hydroxystyrylphenyl	H	H	2	20	>60	>16	>16	>16	>16
2	3-pyridyl	H	H	0.13	0.081	2.3	16	>16	2	16
3	phenyl	H	H	0.47	0.4	9.5	>16	>16	>16	>16
4	5-pyrimidinyl	H	H	0.049	0.02	1.1	4	4	0.5	2
5	1-imidazole-4-cyclopropylamide	F	H	0.010	<0.004	0.078	4	8	0.063	4
6	3-pyridyl	F	H	0.038	0.033	0.68	1	16	0.25	16
7	3-pyridyl	methyl	H	0.085	0.061	1.4	16	>16	0.5	16
8	3-pyridyl	methoxy	H	0.19	0.13	3.5	16	>16	2	>16
9	3-pyridyl	piperidinyl	H	0.081	0.12	3.3	16	>16	2	>16
10	3-pyridyl	H	methyl ester	0.008	<0.004	0.035	0.063	0.5	0.016	0.5
11	3-pyridyl	H	methyl amide	0.026	0.005	0.15	16	>16	0.063	16
12	3-pyridyl	H	1-pyrazole	0.015	<0.004	0.046	0.063	4	0.008	1
13	3-pyridyl	H	2-pyridyl	0.007	<0.004	0.014	0.031	2	0.004	2
14	3-pyridyl	H	3-pyridyl	0.017	0.006	1.3	4	16	0.5	>16
15	3-pyridyl	H	3-fluoropyridin-2-yl	0.014	<0.004	0.023	0.031	0.5	0.004	1
16	3-pyridyl	H	benzyloxy	0.035	0.018	0.39	2	>16	1	>16
17	2-methoxy-5-pyrimidinyl	methoxy	H	0.044	0.015	0.68	2	8	0.125	4
18	5-pyrimidinyl	H	1-pyrazole	0.016	<0.004	0.058	0.031	0.25	<0.008	0.25
19	1-imidazole-4-cyclopropylamide	H	1-pyrazole	0.01	<0.004	0.012	0.5	4	0.003	0.25
20	1-imidazole-4-cyclopropylamide	H	2-pyridyl	0.012	<0.004	0.007	0.063	0.25	0.002	0.125
21	4-(1-methyl-2-pyridone)	H	3-fluoropyridin-2-yl	0.023	<0.004	0.011	0.016	0.032	<0.008	0.125

[a] *S. aureus* strain ATCC 29213; *S. pnemoniae* strain ATCC 10015; HS = 50% human serum; *H. influenzae* strain ATCC 51907; compound **1** is a carbamate (X = O); compounds **2**−**21** are ureas (X = NH).

6-Position SAR. Exploration at the 6-position showed that a variety of substituents were well tolerated with respect to gyrase and topoIV with minimal effect on either K_i or antibacterial potency (Table 1). As mentioned above, the 6-fluoro analogue, **6**, showed a 3-fold improvement in K_i relative to **2**, for both gyrase and topoIV, but showed a 16-fold improvement in MIC against *S. aureus* and an 8-fold improvement against *S. pneumoniae*. Presumably, the fluorine has either increased bacterial permeability or decreased efflux potential for this compound with respect to these two organisms.

A docking model of the 6-methyl analogue, **7**, further suggested that a variety of substituents might be tolerated at both the 5- and 6-positions of the benzimidazole core (Figure 2B). This model also suggested that the preferred orientation of the 5-aryl substituent (i.e., with the 5-aryl ∼40−50° relative to benzimidazole core) can be maintained by any substituent at the 6-position that does not perturb this "preorganized" conformation.[31] The 6-piperidinyl (compound **9**, as well as other larger substituents, data not shown) did not cause any additional loss in potency. Another important insight provided by this structure is that it should be possible to fill the space adjacent to the 7-position of the benzimidazole.

7-Position SAR. As suggested in the above section, it was desirable to explore the region of the ATP site adjacent to the 7-position because of the apparent unfilled space. The 7-carbomethoxy compound **10** showed a 15- to 20-fold improvement in K_i against gyrase and an even greater than expected 65-fold improvement against topoIV. This compound was the first example of a benzimidazole urea with highly potent enzyme inhibition against both gyrase and topoIV (Table 1). Dual targeting further resulted in a dramatic improvement in overall antibacterial potency with MICs of 0.063, 0.016, and 0.5 μg/mL against *S. aureus*, *S. pneumoniae,* and *H. influenzae*, respectively. Surprisingly, compound **11**, the *N*-methylcarboxamide analogue of **10**, also showed potent gyrase inhibition; however, it was less active against topoIV (K_i = 150 nM) relative to compound **10** (K_i = 35 nM). One possible explanation for this reduced topoIV inhibitory potency might be repulsion between the amide proton with the 6-position proton causing the methylamide moiety to rotate slightly out of plane (∼15°) with respect to the benzimidazole core.

On the basis of the observed differences between the amide and ester, the hypothesis began to emerge that coplanarity at the 7-position provided optimal enzyme inhibitory potency against topoIV. The 7-aryl analogues **12**−**14** were synthesized to test this hypothesis and to further explore the steric limits of this region. All of these compounds were equivalent to **10** with respect to gyrase inhibitory potency; compounds **12** and **13** were

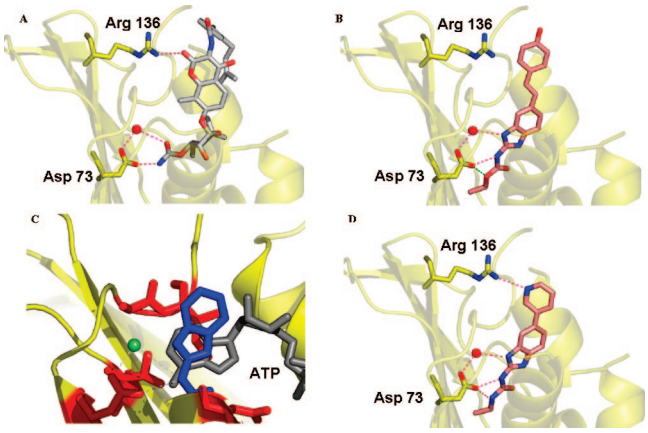


Figure 1. (A) Crystallographic data showing key binding interactions of novobiocin with selected *E. coli* gyrase ATP binding site residues. (B) Model of compound **1** docked into the ATP site highlighting the interactions of the benzimidazole carbamate with a highly conserved water and possible improvements suggested by the model. Potential repulsion between the carbamate oxygen and Asp-73 is shown in green. (C) Superposition of the modeled benzimidazole core with the adenine moiety of ADPNP (from X-ray structure 1EI1[33]). (D) Model of compound **2** docked into the ATP site highlighting the potential interactions that might simultaneously mimic those of novobiocin while improving the strength of the hydrogen bond network involving Asp-73 and a highly conserved water molecule (Thr-165 is not shown but also stabilizes the conserved water via a hydrogen bond).

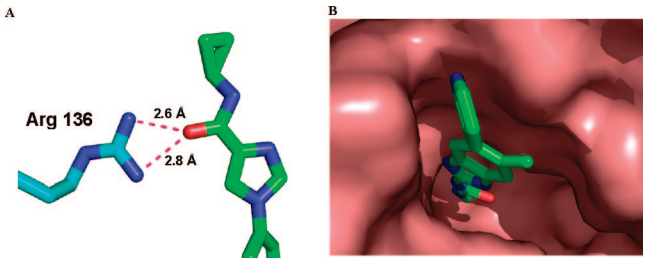


Figure 2. (A) Amide carbonyl oxygen of compound **19** making a bifurcated hydrogen bond with Arg-136. (B) Model of compound **7** bound to *S. aureus* gyrase; solvent exposure of 6-position and conformational effect of 6-methyl substituent on orientation of 5-pyridyl.

also equivalent to **10** with respect to topoIV inhibitory potency. However, the regioisomeric compound **14** showed a 35-fold loss in topoIV K_i relative to **10**. Quantum mechanical rotational barrier calculations[32] performed at 6-31G* (Figure 3A) lend support for this topoIV coplanarity requirement. Compound **10** shows a clear energetic preference for the methyl ester to be perfectly coplanar. Similarly, compound **12** also showed a global energy minimum corresponding to a roughly coplanar pyrazole ring. However, compound **14** with its 3-pyridyl substituent at the 7-position shows preferred minima that are 60° and 120° out of plane. Interestingly, compounds **10** and **12** also appear to have this coplanar minimum stabilized by an intramolecular hydrogen bond between the imidazole NH and either the carbonyl oxygen of **10** or 2-pyrazole nitrogen of **12** (Figure 3B). The 3-pyridyl nitrogen of **14** would not be able to make such an intramolecular hydrogen bond, and coplanarity would be further destabilized by van der Waals repulsion between the imidazole NH and the 2H proton of the 3-pyridyl ring. If the pyridine nitrogen of **14** is moved over one position (i.e., a 2-pyridyl) as with **13**, the intramolecular hydrogen bond would occur, thereby resulting in restored coplanarity and potent topoIV inhibitory potency. These observations allowed the use of rotational barrier calculations in a prospective manner to rank synthetic candidates possessing direct linked 7-aryl substituents. One compound that met the criteria for coplanarity was the 3-fluoropyridin-2-yl compound, **15** (Scheme 1). While compound **15** was almost identical to the des-fluoro analogue, **13**, in terms of both dual enzyme inhibition and general antibacterial potency, the MIC against *S. aureus* was less affected by the addition of human serum (Table 1). Finally, compound **16** with a benzyloxy substituent at the 7-position showed an approximately 5-fold increase in K_i against gyrase and approximately 10-fold increase against topoIV relative to **10**. Possible explanations for this observation include that the benzyloxy group has exceeded the optimum size for this region of the ATP binding site and/or was unable to maintain the required planarity in this region of the binding site because of the additional torsional degrees of freedom and lack of ability to achieve a constraining intramolecular hydrogen bond as described above. Some of the structural similarities and differences between GyrB and ParE in this region of the binding site will be discussed in further detail in the next section.

Selected Mix and Match Examples. Once it was established that direct-linked 7-aryl substituents allowed for potent dual targeting of gyrase and topoIV that translated into significant improvements in antibacterial potency, many analogues were prepared with key examples highlighted below. However, one compound that does not possess a 7-position substituent but is still noteworthy is compound **17** that contains a 2-methoxy-5-pyrimidinyl substituent at the 5-position and a methoxy group at the 6-position. This compound showed a modest 3- to 5- fold improvement in K_i vs **2**. Most importantly, its overall antibacterial potency (Table 1) combined with favorable pharmacokinetic profile served to clearly link in vitro potency with exposure and pharmacologic response (data not shown) and validated the potential of the class.

Compound **18** is a hybrid of compounds **4** and **12** showing almost identical enzyme inhibitory potency and antibacterial activity as **12** with an improvement in the *H. influenzae* MIC and a lower serum effect. Another interesting compound containing a pyrazole at the 7-position is **19**. It was rationalized that since the imidazole amide imparted a greater degree of enzyme inhibitory potency relative to a 3-pyridyl or 5-pyrimidinyl substituent (compare **5** with compounds **2** and **4**, Table 1) this compound when combined with a 7-aryl moiety might be very potent. Compound **19** proved to be quite potent and showed a 5-fold improvement in K_i against topoIV relative to **18**; however, this did not result in an improvement in antibacterial activity. In an attempt to improve the antibacterial activity against *S. aureus*, the 2-pyridyl, compound **20**, was tried in place of the pyrazole at the 7-position since compound **13** retained excellent antibacterial potency against *S. aureus*. Compound **20** turned out to be one of the best compounds in this study in terms of potent dual targeting of gyrase and topoIV as well as excellent antibacterial potency. As mentioned earlier, it is believed that the increase in enzyme inhibitory potency of the imidazole amide analogues is largely due to the ability of the amide carbonyl oxygen to make stronger hydrogen bonds with Arg-136. In this context, the 2-pyridone analogue, **21** also attempted to mimic this optimized interaction. Compound **21** showed almost identical dual targeting inhibition of gyrase and topoIV as compared with **20** and also showed a slight improvement in antibacterial potency against *S. aureus*.

Compounds **18** and **19** contributed to a better understanding of the SAR surrounding dual targeting of gyrase and topoIV and the translation into antibacterial potency. More specifically, these two compounds helped elucidate the structural understand-

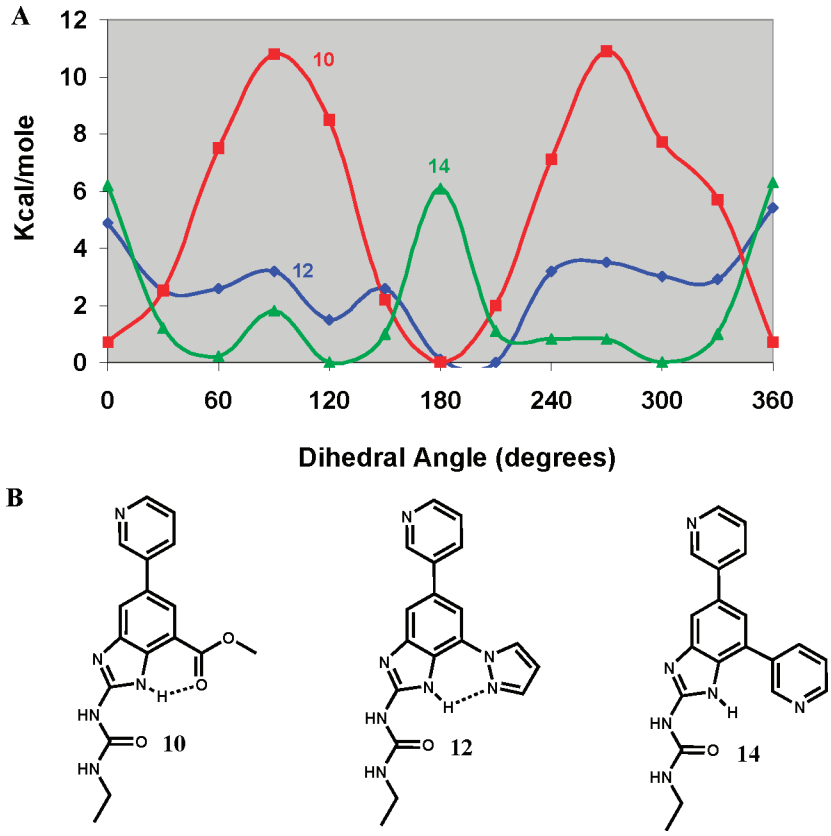


Figure 3. (A) Rotational barrier for compounds **10**, **12**, and **14**. The torsion described is defined by the atoms comprising C6 and C7 on the benzimidazole, the attachment atom and adjacent atoms for the 7-substituent. All rotational barrier calculations were performed at the HF/6-31G* level of theory with Gaussian 98. (B) Chemical structures of compounds **10** and **12** illustrating intramolecular hydrogen bond that contributes to ligand preorganization required for optimal topoIV enzyme inhibitory potency. Compound **14** is not capable of making this intramolecular hydrogen bond.

ing of 7-aryl substitution as it applies to dual targeting. Both compounds contain a pyrazole at the 7-position of the benzimidazole core although they differ with respect to their 5-position substituents. The X-ray crystal structures were solved for compound **18** complexed with GyrB and compound **19** complexed with ParE.[30] It has been previously shown that in GyrB, the Ile-78 and Ile-94 side chains form hydrophobic packing interactions; in ParE, Ile-78 is replaced by a methionine (Met-74).[25] It is plausible that this difference combined with the residue packing adjacent to these residues causes the ParE "wall" to extend further into the binding site (Figure 4), thereby narrowing the region that a direct-linked 7-aryl substituent can occupy. The net result of this relatively narrowed portion of the binding site in ParE is the requirement for a greater degree of coplanarity of any 7-aryl substituent for optimal inhibitory potency against topoIV. The ability of several of these compounds (e.g., **10**, **12**, **13**, **15**, **18**−**21**) to make intramolecular hydrogen bonds further imparts ligand preorganization in terms of this required coplanarity.

In Vivo Efficacy for Compound 15. In addition to the in vivo data presented in this study, an extensive microbiological characterization of compound **15** (Scheme 1) has been recently reported.[23,24] In these studies, **15** demonstrated dual targeting activity in a variety of bacteria, exhibited in vitro bactericidality, and showed low in vitro resistance frequencies. Most importantly, compound **15** maintained potent antibacterial activity against key multidrug resistant Gram-positive organisms.

The potential for compound **15** to be effective against skin and skin structure infections was demonstrated in a thigh infection model (Figure 5A). A single intravenous bolus dose

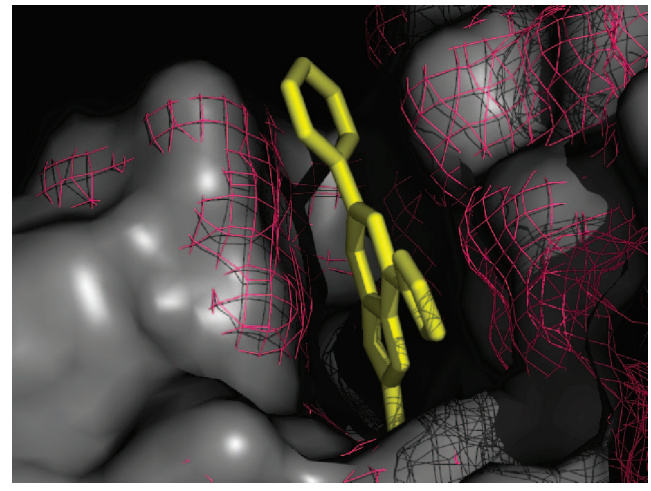


Figure 4. Molecular surface of GyrB ATP binding site (gray) from crystallographic complex with compound **18** (yellow). The corresponding portion of the ParE ATP site from the crystallographic complex with compound **19** is shown as a purple mesh.

of 50 mg/kg compound **15** reduced bacterial density in thigh tissue by 1.5 mean $\log_{10}$ CFUs respectively against *S. aureus* ATCC 29213 at 6 h after dosing compared to the same hour vehicle-treated control value (both $P < 0.01$); a reduction of 0.5 mean $\log_{10}$ CFU was achieved when compared to the 0 h vehicle-treated control value (both $P < 0.01$). At 6 h after dosing, the effect of linezolid at 50 mg/kg was equivalent to that observed with compound **15** relative to both 0 h or same hour vehicle-treated controls (both $P < 0.01$). The data from

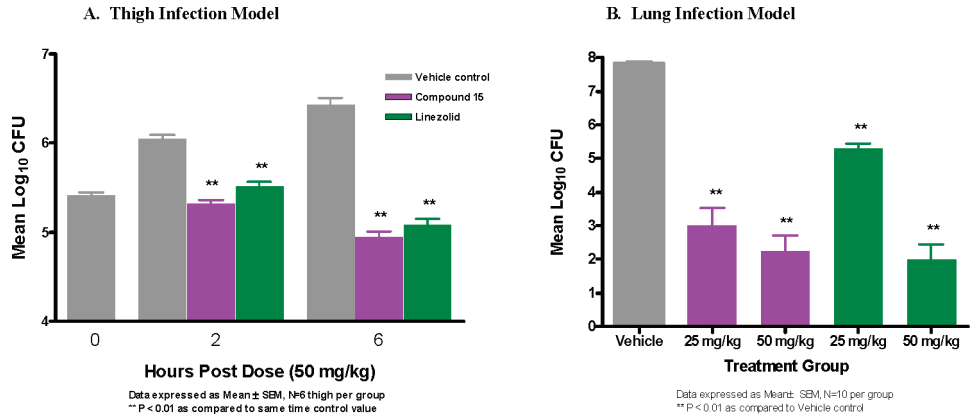


Figure 5. Results for vehicle controls are in gray, compound **15** (purple bars), and linezolid (green bars). (A) Thigh infection: bar graph showing reduction in mean $\log_{10}$ CFU from thigh tissue at 2 and 6 h postdose. Compound **15** and linezolid were administered as a single intravenous bolus at a dose of 50 mg/kg. (B) Lung (*pneumococcal* pneumonia) infection: bar graph showing reduction in mean $\log_{10}$ CFU from lung tissue at 72 h postinfection. Compound **15** and linezolid were administered as a two oral doses at 18 and 26 h postinfection. Relevant pharmacokinetic parameters in rat are as follows: Cl = 14.4 mL/min/kg, $T_{1/2}$ = 0.7 h, V_{ss} = 1.6 L/kg, F = 88.4%.

this single dose, short time course study indicate that compound **15** distributes to the thigh tissue and provides a reduction in bacterial density comparable to a relevant clinical standard.

In a lung infection model of pneumonia, orally administered compound **15** at doses of 25 and 50 mg/kg given at 18 and 26 h postinfection resulted in a significant reduction of mean $\log_{10}$ CFU in lung tissue at 72 h postinfection by 4.8 and 5.6 $\log_{10}$ units, respectively, compared with vehicle-treated controls ($P < 0.01$ for all treatment groups). The mean $\log_{10}$ values for total CFU, measured in lung extracts at 72 h postinfection, are summarized in Figure 5B. Overall, the trend analysis for the mean $\log_{10}$ CFU reduction exhibited by compound **15** over the two oral doses appeared to be dose related. At these same doses, linezolid reduced mean $\log_{10}$ CFU in lung tissue at 72 h postinfection by 2.5 and 5.9 $\log_{10}$ units, respectively, compared with vehicle-treated controls ($P < 0.01$ for all treatment groups). At the 25 mg/kg dose, the superior antibacterial potency of compound **15** against *S. pneumoniae* has clearly translated to a greater than 2 $\log_{10}$ reduction in lung bacterial density relative to linezolid, while at the 50 mg/kg dose, the effects were comparable.

Conclusion

History has shown many times over that familiarity with a well-accepted antibiotic class has helped usher successive generations of that class into widespread use by the medical community. This phenomenon spans all of the major classes of antibiotics: β-lactams, fluoroquinolones, cephalosporins, and macrolides. This study represents the prospective design of dual targeting inhibitors of both gyrase and topoisomerase IV using tightly coupled insights from structural information, modeling, and SAR.

There is no de facto expectation that an improvement in enzyme inhibitory potency will result in greater antibacterial potency, since the properties governing permeability and efflux may be different from those imparting target affinity. A clear understanding of SAR can be further complicated by the possibility that different compounds can exhibit differing degrees of enzyme inhibition across different bacterial species, and in the present work, K_i values have only been determined for two species (i.e., *S. aureus* and *E. coli*). Nonetheless, in the case of the benzimidazole ureas, improvements in enzyme inhibitory potency (especially when dual targeting was introduced) generally led to enhancements in antibacterial potency against Gram-positive organisms.

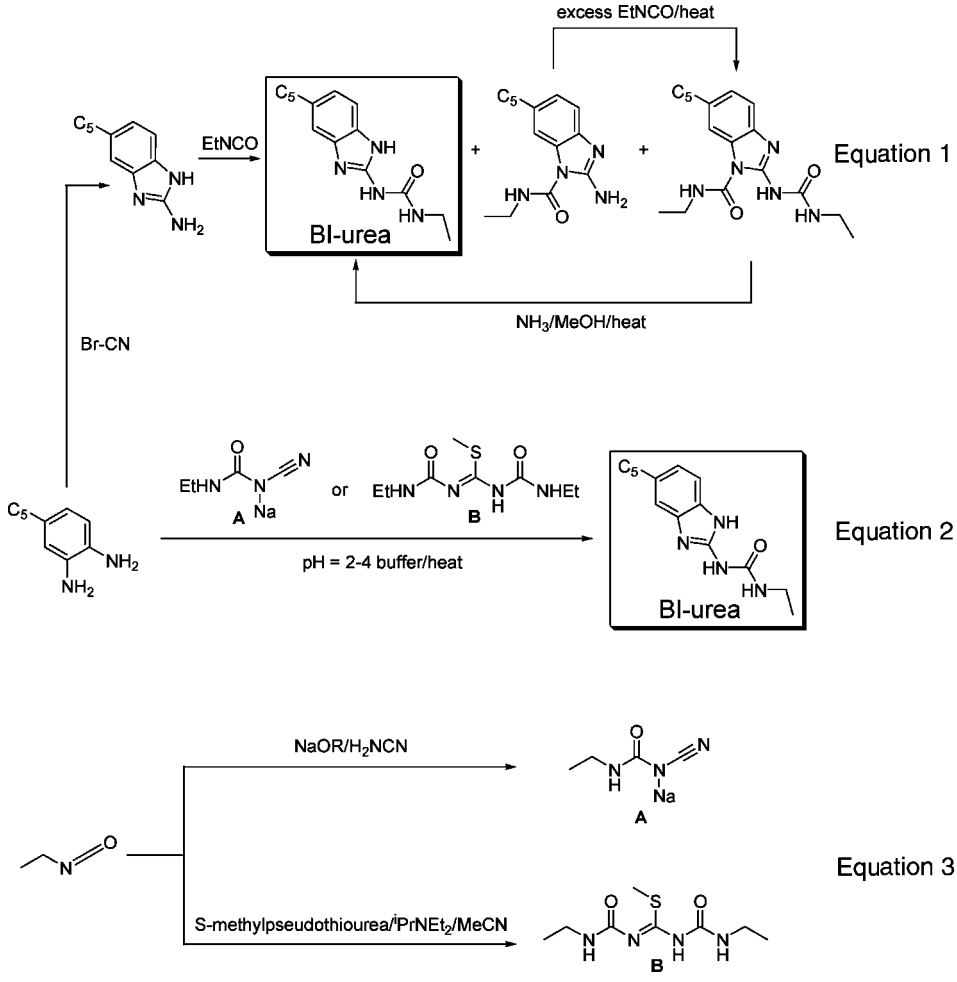
Substituent changes at the 5- and 6-positions of the benzimidazole core dramatically improved enzyme inhibitory and antibacterial potencies relative to initial compounds in the series. However, an appropriate 7-substituent capable of forming a preorganized intramolecular hydrogen bond, while at the same time making complementary hydrophobic interactions with the enzyme, appeared to be the key structural requirement for potent dual targeting. In the case of most Gram-negative organisms, however, potent enzyme inhibition did not translate to very potent antibacterial activity with the exception of the respiratory pathogens, *H. influenzae* and *M. catarrhalis* (data not shown). Increased susceptibilities of *E. coli* mutant strains with either improved permeability (*impA* mutant) or decreased efflux (*tolC* mutant), suggested that efflux and, to a lesser degree, poor permeability are responsible for the relative lack of antibacterial potency in *E. coli* and other Gram-negative organisms.[23]

The benzimidazole ureas are a novel class of dual targeting agents exerting their antibacterial effect via simultaneous inhibition of the ATPase function of gyrase and topoisomerase IV. Several of the compounds presented in this study possess potent antibacterial activity against clinically relevant, multidrug resistant organisms.[23] Compound **15** was shown in previous studies to possess low *in vitro* spontaneous resistance frequencies and demonstrated bactericidality against all organisms tested. In the current study, this compound was shown to be effective in rodent models of skin and skin structure infection and pneumonia when dosed by both intravenous and oral routes. In summary, the benzimidazole ureas represent one of the most exciting recent chemical advances in the antibacterial field. As a novel chemical class, the benzimidazole ureas have the potential to become clinically viable antibiotics, directly addressing the resistance problem by their novel dual-targeting mechanism of action.

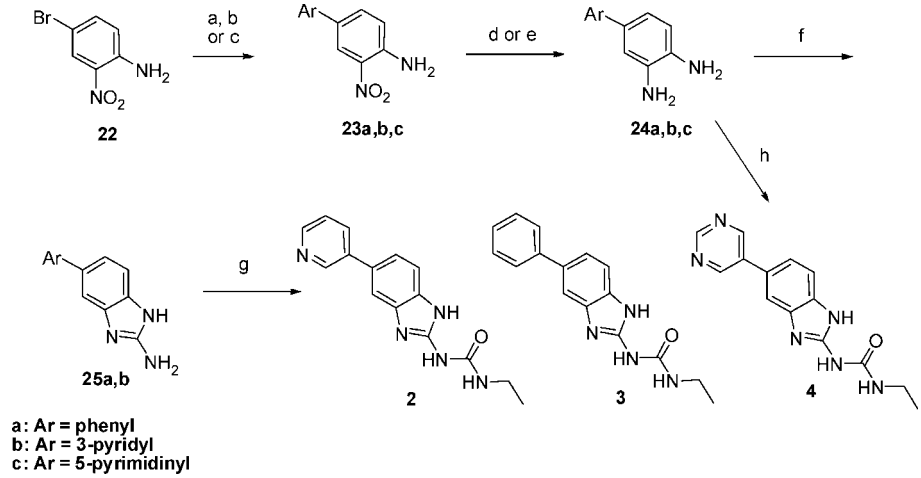
Experimental Section

Synthesis of Benzimidazole Ureas. The general synthetic plan to support SAR exploration within the benzimidazole urea (BI-urea) series is outlined in Scheme 2. Each approach involved initial placement of the various C5, C6, and C7 functional groups followed by manipulation of the masked *o*-phenylenediamine to yield the desired BI-urea. While our initial stepwise approach to converting functionalized *o*-phenylenediamines to the BI-urea core did yield final compounds, reaction efficiencies occasionally suffered. In some cases complex mixtures of mono- and bis-urea adducts were formed (Scheme 2, eq 1). If this was the dominant outcome, reactions were driven to the bis-urea intermediates and then selectively endo-

Scheme 2



Scheme 3[a]

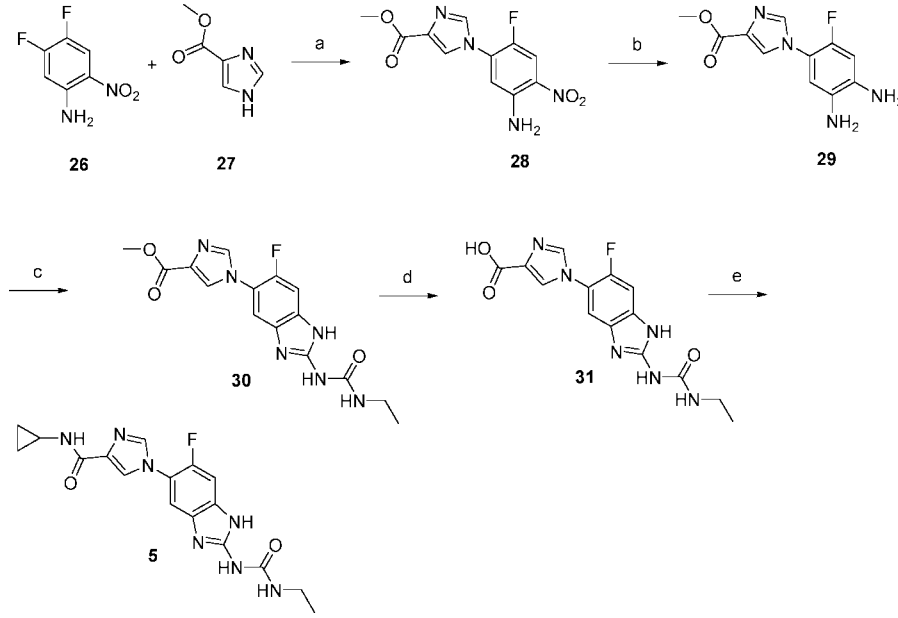


[a] (a) Bis(pinacolotto)diboron, KOAc, Pd(dppf)Cl_2, DMSO, 70 °C; (b): Ar−Br, Pd(PPh_3)$_4$, K_3PO_4, DMSO, 100 °C; (c) pyridin-3-yl-3-boronic acid, Pd(dppf)Cl_2, K_3PO_4, DMF, 120 °C; (d) 5% Pd−C, H_2, EtOH, EtOAc; (e) $NaHSO_3$, aq EtOH, reflux; (f) BrCN, MeOH, room temp; (g) **25a**,**b**, EtNCO, THF, reflux; (h) **24c**, reagent **A**, aq HCl, *p*-dioxane, reflux.

deacylated to yield desired product using ammonia. Further pursuit of milder and more expeditious methods identified reagents **A** and **B** (Scheme 2, eqs 2 and 3) that allowed for facile one-step conversions of *o*-phenylenediamines directly to BI-ureas in high yields.[34]

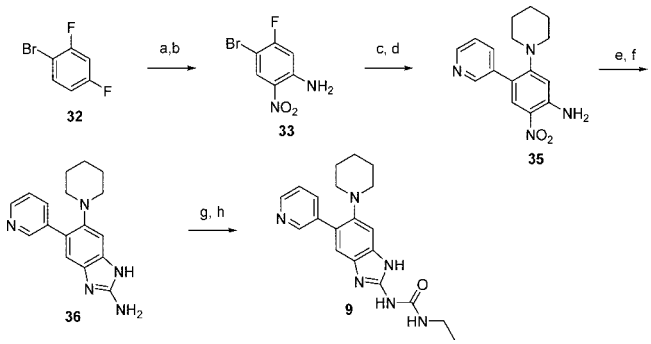
Functionalization of the C5-position was most often accomplished either by direct Suzuki couplings for C5-carbon linked aromatic groups or by nucleophilic aromatic substitution of activated C5-fluorobenzenes for C5-nitrogen linked aryls. The preparation of compounds **2** and **3** started with conversion of 2-nitro-4-bromoaniline **22** to the corresponding biaryls **23a** and **23b** by direct cross-couplings with 3-pyridyl- and phenylboronates under standard conditions (Scheme 3). Alternatively, **22** could be converted to its C4-boronate under conditions described by Miyaura[35] et al. and used either directly in a one pot, two-step sequence or as an isolated intermediate to prepare other biaryls, including **23c**. Reduction of

Scheme 4[a]



[a] (a) Na_2CO_3, DMF, 125 °C; (b) 50 psi of H_2, Raney Ni, MeOH; (c) reagent **B**, pH 3.5 buffer, dioxane, reflux; (d) 6 N HCl, reflux; (e) DIEA, HBTU, cyclopropylamine, DMF, room temp.

Scheme 5[a]



[a] (a) KNO_3, concd H_2SO_4, 0 °C to room temp; (b) 0.5 N NH_3, dioxane, sealed tube, 70 °C; (c) piperidine, MeCN, 80 °C; (d) pyridine-3- boronic acid pinacol ester, aq $NaHCO_3$, $Pd(PPh_3)_4$, DME, 90 °C; (e) 10% Pd−C, 40 psi of H_2, MeOH, HOAc; (f) NCBr, MeCN, MeOH, room temp; (g) excess EtNCO, MeCN, 70 °C; (h) 7 N NH_3, MeOH.

the resulting *o*-nitroanilines to their corresponding *o*-phenylenediamines (**24a**, **24b**, **24c**) was accomplished by several means including catalytic hydrogenation and stoichiometric methods using inorganic reducing agents. Following a two-step sequence, **24a** and **24b** were first converted to their corresponding aminobenzimidazoles using cyanogen bromide then acylated with ethyl isocyanate to provide **2** and **3**. Alternatively, **24c** was directly converted to **4** in a single step using reagent **A**.

Preparation of **5** began with an SnAr reaction between **26** and **27** under alkaline conditions (Scheme 4) to provide adduct **28**. Subsequent reduction to the *o*-phenylenediamine **29** and direct conversion to the BI-urea using reagent **B** provided **30**. Ester hydrolysis to **31** and conversion to the corresponding carboxamide provided compound **5**.

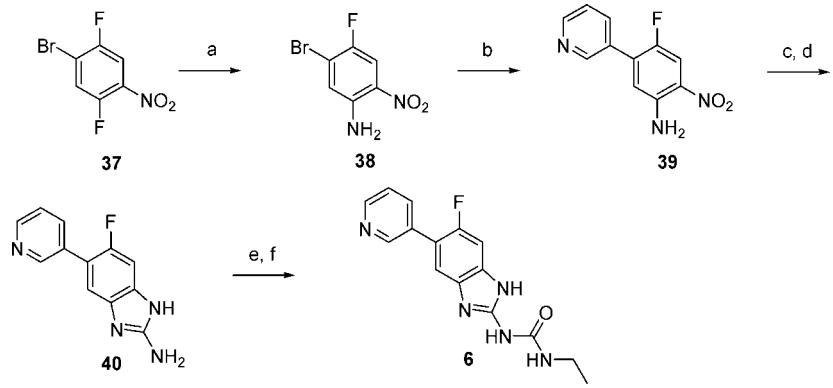
C6 exploration normally began with placement of the C6-substituent from commercial starting materials followed by incorporation of the C5-aryl and subsequent conversion to the BI-urea final products. Preparation of the 1-piperidinyl analogue **9** (Scheme 5) began with nitration of **32** followed by a regioselective *o*-fluoro SnAr reaction with ammonia to give nitroaniline **33**. Exchange of the second fluorine with the more nucleophilic piperidine followed by Suzuki coupling to introduce the C5 aryl provided *o*-nitroaniline **35**. Following reduction and cyanogen bromide treatment the aminobenzimidazole **36** was obtained. Preparation of the C6 fluoro analogue **6** began with the commercially available bromodifluoronitrobenzene **37**. Initial *o*-fluoro SnAr reaction followed by installation of the C5 aryl via Suzuki coupling provided *o*-nitroaniline **39**, which was similarly reduced and treated with cyanogen bromide to yield the aminobenzimidazole **40** (Scheme 6). The aminobenzimidazoles **44a** and **44b** were similarly prepared from the appropriately functionalized nitrobromoanilines **41a** and **41b** through a Suzuki/reduction/cyanogen bromide sequence (Scheme 7). The conversion of **44a** and **44b** to the desired BI-ureas **7** and **8** was effected with simple acylation using ethyl isocyanate in refluxing THF. However, under nearly identical conditions the conversions of **36** and **40** to the desired BI-ureas provided complex mixtures of mono- and diacylated products. To circumvent this issue, these reactions were driven to their fully diacylated intermediates and then regioselectively endodeacylated with ammonia to provide **9** and **6**, respectively. The BI-urea analogue **17** was prepared from **41c** using the most expeditious Suzuki/reduction/reagent **A** sequence.

Preparation of the C7-carbonyl substituted analogues began with nitration of bromoanthranilate **45** to provide **46** (Scheme 8). Introduction of the C5-aryl via Suzuki coupling provided **47**, which was then reduced and directly converted to the BI-urea **10** using the cyanamide derived reagent **A**. Subsequent methyl ester hydrolysis of **10** and conversion to the primary carboxamide under standard amide forming conditions provided **11**.

Preparation of the C7-ether analogue **16** began with bromination of the hydroxynitroaniline **49** followed by chemoselective ether formation providing **50** (Scheme 9). From this point BI-urea **16** was obtained through the usual sequence of Suzuki coupling to introduce the C5-aryl, reduction, and then cyanogen bromide treatment. The resulting aminobenzimidazole was then exhaustively acylated and then treated with ammonia to effect the regioselective endourea cleavage.

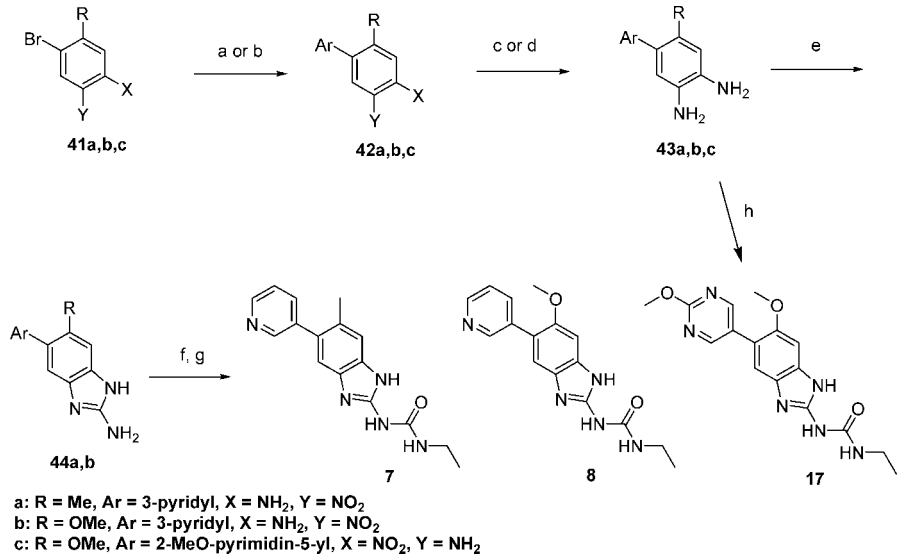
The preparation of the C5 and C7 bis-N-linked heteroaromatic analogue **19** began with the two-step sequence involving initial regeoselective para-sulfonation[36] followed by ortho-nitration using potassium nitrate in trifluoroacetic anhydride as solvent[37] of aniline **54** through an in situ generated trifluoroacetanilide to provide **56**. To our surprise, cleavage of the sulfate blocking group occurred under the nitration reaction conditions (Scheme 10) rather than in a subsequent step involving heat and strong aqueous acid. As the expected nitrated sulfonic acid intermediate was never observed,

Scheme 6[a]



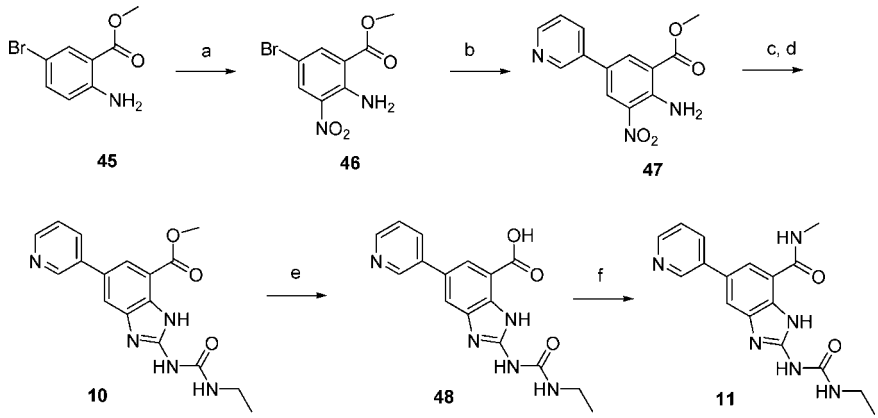
[a] (a) NH_3, MeOH, reflux; (b) pyridine-3-boronic acid pinacol ester, aq $NaHCO_3$, $Pd(PPh_3)_4$, DME, 90 °C; (c) 10% Pd−C, 40 psi of H_2, MeOH, room temp; (d) NCBr, MeCN, MeOH, room temp; (e) excess EtNCO, MeCN, 70 °C; (f) 7 N NH_3, MeOH.

Scheme 7[a]



[a] (a) 3-Pyridineboronic acid pinacol ester, aq $NaHCO_3$, $Pd(PPh_3)_4$, DME, 90 °C; (b): 2-methoxy-pyrimidine-5-pinacol boronate, $Pd(PPh_3)_2Cl_2$, aq $NaHCO_3$, DME, reflux; (c) 10% Pd−C, 40 psi of H_2, MeOH, HOAc; (d) Et_3N, HCO_2H, Pd−C, MeOH, reflux; (e) NCBr, MeCN, room temp; (f) excess EtNCO, MeCN, 70 °C; (h) 7 N NH_3, MeOH; (h) **43c**, reagent **A**, aq H_2SO_4, reflux.

Scheme 8[a]

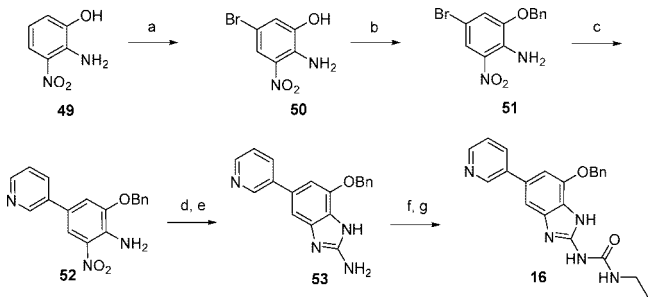


[a] (a) TFAA, KNO_3, 0 °C; (b) 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine, aq $NaHCO_3$, $Pd(PPh_3)_4$, DME, 90 °C; (c) 10% Pd−C, 40 psi of H_2, MeOH, HOAc; (d) reagent **A**, aq H2SO4, reflux; (e) 6 N HCl, reflux; (f) PyBrOP, $MeNH_2$, DMAP, NMP.

it is presumed that loss of SO_3 may have been facilitated by using the strongly electrophilic trifluoroacetic anhydride as solvent. Acid catalyzed cleavage for the trifluoroacetyl group followed by introduction of the 1-pyrazolyl substituent at C7 via a regioselective anionic SnAr reaction yielded **58**. Incorporation of the C5 imidazole amide group was similarly effected under more forcing conditions to provide nitroaniline **59**.

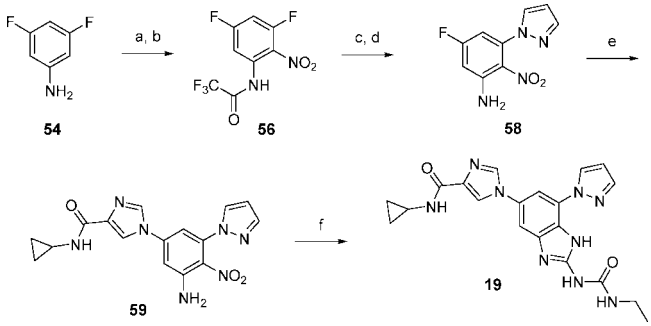
The preparation of analogues **12** and **18** began with oxidation of aniline **59** to the corresponding nitro analogue **60** (Scheme 11). Sequential ortho SnAr displacements with ammonia then anionic

Scheme 9[a]



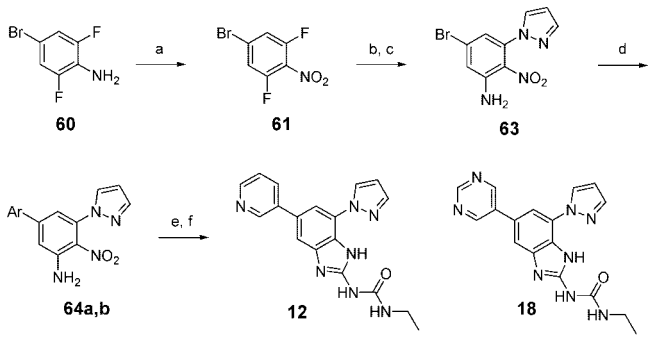
[a] (a) Br_2, HOAc, room temp; (b) K_2CO_3, benzyl bromide, DMF, room temp; (c) pyridine-3-boronic acid 1,3-propanediol cyclic ester, $Pd(PPh_3)_4$, $NaHCO_3$, DME, 90 °C; (d) sodium hydrosulfite, EtOH, water, 70 °C; (e) NCBr, CH_3CN, MeOH, room temp; (f) EtNCO, CH_3CN, room temp; (g) NH_3, MeOH, 60 °C.

Scheme 10[a]



[a] (a) H_2SO_4, 190 °C; (b) trifluoroacetic anhydride, KNO_3, room temp; (c) 6 N HCl, 100 °C; (d) NaH, pyrazole, DMF, 50 °C; (e) **87**, Na_2CO_3, DMF, 95 °C; (f) Pd−C, 45 psi of H_2, 2 N HCl; (g) **A**, aq H_2SO_4, 100 °C.

Scheme 11[a]



a: Ar = pyridin-3-yl
b: Ar = pyrimidin-5-yl

[a] (a) $NaBO_3$, HOAc, 100 °C; (b) NaH, 1*H*-pyrazole, DMF; (c) NH_3, ethanol, sealed tube, 80 °C; (d) $ArB(OR)_2$ or $ArBEt_2$, $Pd(PPh_3)_4$, $NaHCO_3$, aq DME, 90 °C; (e) 40 psi of H_2, $Pd(OH)_2$−C, MeOH; (f) **A**, pH 3.5 buffer, 100 °C.

pyrazole provided bromide **63**. Introduction of the C5 aryl substituent via Suzuki coupling yielded the nitroanline intermediates **64a,b**. Reduction of nitroanilines **59**, **64a**, and **64b** followed by treatment with reagent **A** gave the final BI-urea analogues **19**, **12**, and **18** respectively.

The preparation of C7-aryl substituted analogues **15** and **21** was effected by initial Suzuki couplings between the requisite halo-heteroaromatics and boronate **65** to provide the biaryl **66** (Schemes 12 and 13). Following a three-step sequence involving para-bromination, then ortho-nitration, and finally amide cleavage, intermediate **69** was obtained. While direct Suzuki coupling to this bromide was possible, it was also advantageous to prepare the C5-boronate[35] analogue **70** to allow for more diversification at the C5 position of the BI core. Suzuki couplings with either **69** or **70** provided **71a**,**b** in high yields. Reduction of the resulting nitroanilines and conversion to the final BI-ureas using reagent **A** gave analogues **15** and **21**, respectively.

An alternative approach to incorporating the C7 aryl functionality was also developed starting with introduction of the C5 aryl substituent via a Suzuki coupling to **76** (Scheme 14). Regioselective bromination of **77** introduced the C7-bromide that could participate in a subsequent organometallic cross-coupling with either 2-pyridylzinc chloride or 3-pyridylboronic acid to provide **79a** and **79b**, respectively. The resulting nitroanilines were then reduced and directly converted to the desired BI-ureas **13** and **14** using reagent **A**.

Finally, the synthesis of analogue **20** with C5-nitrogen linked azaheteroaromatics and C7-carbon linked heteroaryls began with the regioselective nitration of bromodifuorobenzene **80** (Scheme 15). Ortho-selective SnAr displacement of **81** using ammonia provided **82**, which was then cross-coupled with 2-pyridylzinc chloride under Negishi conditions to give **83**. Sequential introduction of the C5 imidazolylamide under anionic SnAr conditions using **87**[38] (Scheme 16) followed by acid catalyzed cleavage of the *tert*-butyl group yielded **85**. Final conversion to the BI-urea **20** was then achieved via the two-step sequence of reduction and treatment of the resulting phenylenediamine with reagent **B**.

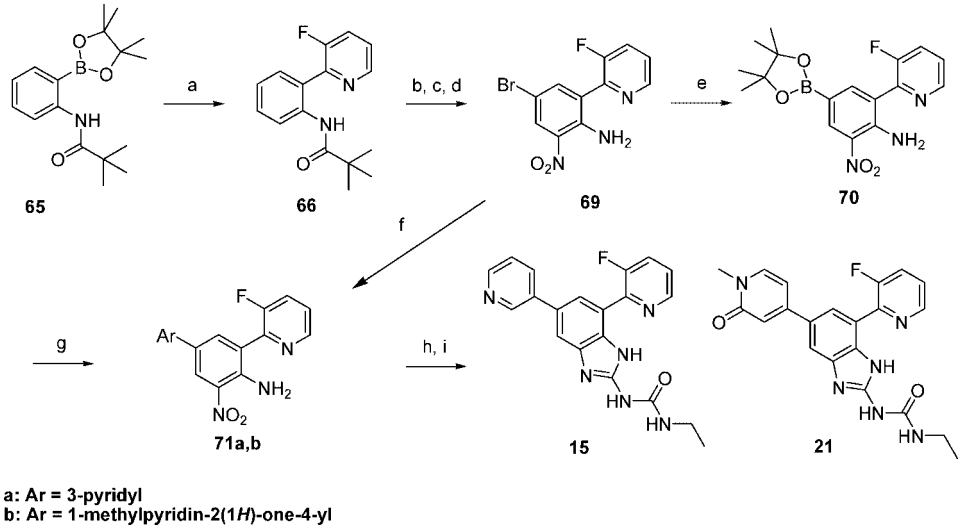
Chemistry. Proton NMR spectra were recorded on a Bruker Advance instrument with a QNP probe using TMS as the internal standard in the indicated deuterated solvent. LC−MS analyses were performed on a Waters ZQ or ZMD or QuatroII mass spectrometer using the electrospray (ESI) ionization technique. Preparative HPLC isolations performed using Agilent 1100 mass-directed purification chromatography system with 0.1% TFA in acetonitrile/water mobile phase and Waters Sunfire C_{18} stationary phases. All commercially available reagents were used without further purification. Purity assessment for final compounds based on two orthogonal HPLC methods. Method A consisted of the following: column, 4.6 mm × 50 mm Waters YMC Pro-C18 column, 5 μm, 120A. Mobile phases are as follows: A, H_2O with 0.2% formic acid; B, acetonitrile with 0.2% formic acid; gradient, 10% B to 90% B in 3 min with 5 min run time. Flow rate is 1.5 mL/min. Method B consisted of the following: column: 4.6 mm × 50 mm Waters Symmetry C18 column, 5 μm. Mobile phases are as follows: A, H_2O with 10 mM ammonium formate; B, ACN with 10 mM ammonium formate; gradient, 10% B to 90% B in 3 min with 5 min run time. Flow rate is 1.5 mL/min.

2-Nitro-4-(pyridin-3-yl)benzenamine (23b). To a solution of **22** (217 mg, 1 mmol) in 6 mL of DMF was added successively 3-pyridineboronic acid (148 mg, 1.2 mmol), potassium phosphate (276 mg, 1.3 mmol), and palladium dichloro-dppf (83 mg, 0.1 mmol). The mixture was stirred at 95 °C for 24 h and allowed to cool to ambient temperature. It was diluted with 50 mL of ethyl acetate and filtered through a pad of Celite. The filtrate was washed successively with saturated aqueous sodium bicarbonate, water, brine, was dried over magnesium sulfate, and was concentrated in vacuo. The residue was purified by column chromatography over silica gel eluted with ethyl acetate−hexanes (gradient from 1:4 to 5:1) to afford **23b** (117 mg, 54% yield) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.80 (d, 1H), 8.55 (m, 1H), 8.35 (d, 1H), 7.85 (dd, 2H), 7.65 (dd, 2H), 7.35 (m, 1H), 6.95 (d, 1H), 6.25 (broad s, 2H).

4-(Pyridin-3-yl)benzene-1,2-diamine (24b). To a solution of **23b** (117 mg, 0.544 mmol) in EtOAc (13 mL) was added 10% palladium on carbon (51 mg). The resulting mixture was shaken in a Parr bottle under a 40 psi of hydrogen and then filtered through Celite. The solids were washed with EtOAc and the combined filtrates concentrated in vacuo to afford compound **24b** which was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.80 (d, 1H), 8.45 (m, 1H), 7.75 (m, 1H), 7.25 (m, 1H), 6.95 (m, 2H), 6.80 (m, 1H), 3.25 (br s, 2H).

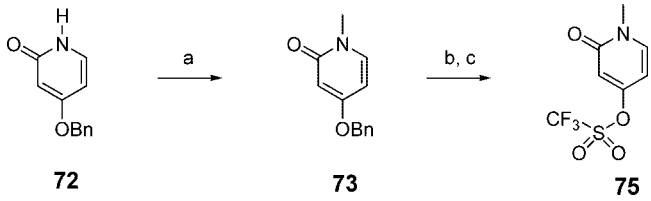
5-(Pyridin-3-yl)-1*H*-benzo[*d*]imidazol-2-amine (25b). To a solution of compound **24b** (0.54 mmol) in THF (2.5 mL) was added 5 mL of methanol and 5 mL of water, followed by an acetonitrile solution of cynaogen bromide (0.116 mL, 0.580 mmol) at ambient

Scheme 12[a]



[a] (a) Ar−Br, $Pd(PPh_3)_4$, $NaHCO_3$, aq DME, 100 °C; (b) Br_2, HOAc, room temp; (c) HNO_3, TFAA, TFA, 0 °C; (d) 6 N HCl, reflux; (e) bis(pinacoloto)diboron, KOAc, $Cl_2Pd(dppf)_2$, dioxane, 100 °C; (f) 3-pyridyl-1,3-propanediol boronate ester, $Pd(PPh_3)_4$, Na_2CO_3, aq DME, reflux; (g) **75**, LiCl, $Pd(PPh_3)_4$, Na_2CO_3, DMF, 95 °C; (h) Pd−C, 45 psi of H_2, MeOH, EtOAc; (i) **A**, aq H_2SO_4, 100 °C.

Scheme 13[a]



[a] (a) Na_2CO_3, MeI, acetone, room temp; (b) H_2, $Pd(OH)_2$−C, MeOH, room temp; (c) Tf_2NPh, TEA, MeCN, room temp.

temperature. The mixture was stirred overnight and then diluted with 50 mL of water and 5 mL of 2 N NaOH and extracted with three 50 mL portions of ethyl acetate. The combined organic extracts were washed sequentially with 1 N NaOH, brine, dried over $MgSO_4$, and concentrated in vacuo to provide **25b** (50 mg, 41% crude yield) as a white solid which was used without further purification. ^{1}H NMR (500 MHz, MeOD): δ 8.8 (m, 1H), 8.45 (m, 1H), 8.05 (m, 1H), 7.45 (m, 2H), 7.25 (m, 2H), 4.9 (broad s, 3H).

1-Ethyl-3-(5-(pyridin-3-yl)-1*H*-benzo[d]imidazol-2-yl)urea (2). To a solution of **25b** (50 mg, 0.24 mmol) in THF (2 mL) was added ethyl isocyanate (0.022 mL, 0.29 mmol). The mixture was heated at 80 °C overnight and then returned to ambient temperature. The crude reaction was diluted with 20 mL of ethyl acetate, washed sequentially with 1 N sodium hydroxide and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by preparatory HPLC to afford **2** (25 mg, 16% yield) as a white solid. Purity, method A 98%; method B 98%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.0 (s, 1H), 8.65 (d, J = 4.0 Hz, 1H), 8.35 (d, J = 8.7 Hz, 1H), 7.85 (s, 1H), 7.7 (dd, J = 8.7, 4.0 Hz, 1H), 7.65 (s, 1H), 7.6 (m, 1H), 7.55 (m, 1H), 3.25 (m, 2H), 1.15 (7, J = 7.5 Hz, 3H). MS, m/z: 282 $(M + H)^+$.

4-Phenyl-2-nitroaniline (23a). To a solution of **22** (6 mmol, 1.30 g) in 36 mL of DMF was added successively phenylboronic acid (7.2 mmol, 878 mg), potassium phosphate (7.8 mmol, 1.66 g), and palladium dichloro-dppf (0.6 mmol, 489 mg). The mixture was stirred at 95 °C for 24 h and allowed to cool to ambient temperature. It was diluted with 200 mL of ethyl acetate and filtered through a pad of Celite. The filtrate was washed successively with saturated aqueous sodium bicarbonate, water, brine, was dried over magnesium sulfate, and was concentrated in vacuo. The residue was purified by column chromatography over silica gel eluted with ethyl acetate−hexanes (gradient from 1:4 to 5:1) to afford **23a** (635 mg, 50%) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.4 (d, 1H), 7.7 (dd, 1H), 7.55 (m, 2H), 7.45 (m, 2H), 7.35 (m, 1H), 6.9 (d, 1H), 6.1 (br s, 2H).

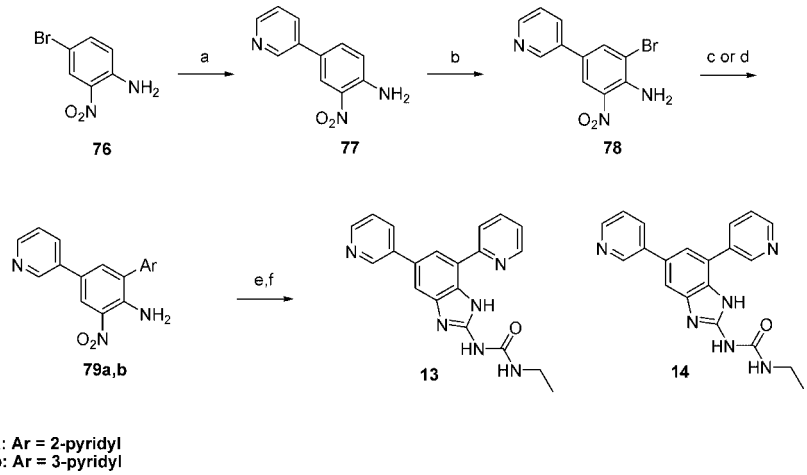
5-Phenyl-1*H*-benzo[*d*]imidazol-2-amine (25a). To a solution of **23a** (200 mg, 0.93 mmol) in 9 mL of EtOH and 6 mL of water was added 1.42 g of $NaHSO_3$. The mixture was stirred at 80 °C for 90 min, cooled to ambient temperature, and partitioned between 100 mL of ethyl acetate and 50 mL of water. The aqueous layer was extracted with three 50 mL portions of ethyl acetate. The combined organic extracts were dried over magnesium sulfate and concentrated in vacuo to afford **24a** (152 mg, 89%) as an oil, which was used without further purification.

A solution of **24a** (0.83 mmol) in 5 mL of THF was treated with 8 mL of methanol and 8 mL of water, followed by 178 μL of a 5 M solution of cynaogen bromide in acetonitrile. The mixture was stirred at ambient temperature overnight, diluted with 80 mL of water and 8 mL of 2 N NaOH, extracted with three 50 mL portions of ethyl acetate. The combined organic extracts were washed with aqueous 1 N NaOH, brine, dried ($MgSO_4$), and concentrated in vacuo to afford **25a** (118 mg, 68%) as an oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.6 (broad s, 1H), 7.45 (m, 2H), 7.25 (m, 4H), 7.15 (m, 1H), 7.0 (m, 2H), 6.1 (m, 1H).

1-Ethyl-3-(5-phenyl-1*H*-benzo[*d*]imidazol-2-yl)urea (3). To a solution of **25a** (40 mg, 0.19 mmol) in 2 mL of THF was added ethyl isocyanate (0.34 mmol, 27 μL). The mixture was heated at 80 °C overnight, allowed to cool at ambient temperature, diluted with 20 mL of ethyl acetate, washed with aqueous 1 N sodium hydroxide, brine, was dried ($MgSO_4$), and was concentrated in vacuo. The residue was purified by preparatory HPLC to afford **3** (15 mg, 28% yield) as a white solid. Purity, method A 90%; method B 90%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.0 (s, 1H), 8.65 (d, J = 4.0 Hz, 1H), 8.35 (d, J = 8.7 Hz, 1H), 7.85 (s, 1H), 7.7 (dd, J = 8.7, 4.0 Hz, 1H), 7.65 (s, 1H), 7.6 (m, 1H), 7.55 (m, 1H), 3.25 (m, 2H), 1.15 (q, J = 7.5 Hz, 3H). MS, m/z: 281 $(M + H)^+$

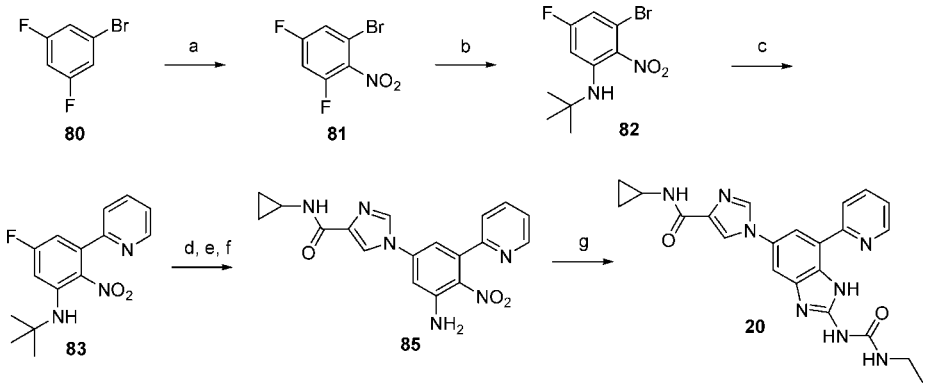
2-Nitro-4-pyrimidin-5-ylphenylamine (23c). In a sealed tube, **22** (0.32 g, 1.5mmol) and bis(pinacolotto)diboron (0.46 g, 1.8 mmol) were dissolved in DMSO (3 mL) and treated with potassium acetate (0.44 g, 4.5 mmol) and (0.1 g, 0.12 mmol) before heating overnight at 70 °C in an oil bath. 5-Bromopyrimidine (0.24 g, 1.5 mmol), finely ground potassium phosphate (0.96 g, 4.5 mmol), $Pd(dppf)Cl_2$ (0.1 g), and DMSO (3 mL) were added to the cooled mixture, sealed, heated at 100 °C for 18 h, then returned to ambient temperature. The cooled mixture was poured into water (100 mL) and extracted thrice with EtOAc. The combined organic extracts were washed thrice with water, dried over $MgSO_4$, filtered, then concentrated to a yellow-orange solid which was purified by silica

Scheme 14[a]



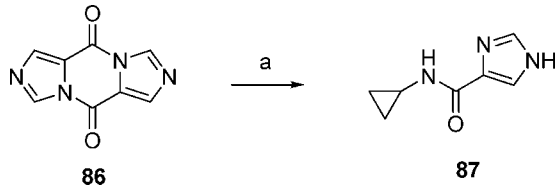
[a] (a) 3-Pyridine-1,3-propanediol boronic ester, aq $NaHCO_3$, $Pd(PPh_3)_4$, DME, 90 °C; (b) Br_2, HOAc, room temp; (c) 3-(1,3,2-dioxaborinan-2-yl)pyridine, $Pd(PPh_3)_4$, $NaHCO_3$, aq DME, 90 °C; (d) 2-pyridylzinc chloride, $Pd(PPh_3)_4$, THF, DMF, reflux; (e) H2, Pd−C, EtOAc; (f) reagent **A**, aq H_2SO_4, 100 °C.

Scheme 15[a]



[a] (a) HNO_3, H_2SO_4, room temp; (b) $^tBuNH_2$, dioxane, sealed tube, 80 °C; (c) 2-pyridylzinc chloride, $Pd(PPh_3)_4$, THF, DMF, 90 °C; (d) **87**, Na_2CO_3, DMF, 120 °C; (e) HCl, MeOH, reflux; (f) 40 psi of H_2, $Pd(OH)_2$−C, MeOH; (g) reagent **B**, pH 3.5 buffer, 100 °C.

Scheme 16[a]



[a] (a) Cyclopropylamine, dioxane, sealed tube, 80 °C.

gel chromatography (1:0 to 49:1 CH_2Cl_2/MeOH gradient) to afford **23c** (0.21 g, 66% yield) as a yellow solid. ^{1}H NMR: (500 MHz, $CDCl_3$): δ 9.2 (s, 1H), 8.9 (s, 2H), 8.4 (s, 1H), 7,6 (d, 1H), 6.9 (d, 1H), 6.2 (broad s, 2H). MS, *m/z*: 217 $(M + H)^+$; 215 $(M - H)^-$.

4-Pyrimidin-5-ylbenzene-1,2-diamine (24c). A solution of **23c** in EtOAc (30 mL) and EtOH (30 mL) in a Parr bottle was treated with 5% Pd−C (0.1 g) before pressurizing to 45 psi with hydrogen. After 3 h, the mixture was filtered through a syringe filter disk and concentrated to give **24c** (0.18 g, 100% yield) as yellow solid. MS, *m/z*: 187 $(M + H)^+$.

1-Ethyl-3-(5-pyrimidin-5-yl-1*H*-benzoimidazol-2-yl)urea (4). To a solution of **24c** (0.66 g, 0.35 mmol) in a mixture of water (5 mL) and *p*-dioxane (5 mL) was added a solution of reagent **A** (0.5 mmol) which had been preacidified to pH 3 with 3 M H_2SO_4. The mixture was refluxed for 16 h, returned to ambient temperature, and diluted with water producint a precipitate. The solids were filtered and further washed with water, then concentrated to dryness to give **4** (0.05 g, 50% yield) as an off-white. Purity, method A 98%; method B 98%. ^{1}H NMR (500 MHz, DMSO-d_6): 10.0 (broad s, 1H), 9.1 (m, 3H), 7.8 (s, 1H), 7.4−7.5 (m, 2H), 7.2 (broad s, 1H), 3.2 (q, 2H), 1.1 (t, 3H). MS, *m/z*: 283 $(M + H)^+$; 281 $(M - H)^-$.

Methyl 1-(5-Amino-2-fluoro-4-nitrophenyl)-1*H*-imidazole-4-carboxylate (28). To a solution of **27** (54.31 g, 0.312 moles) and **26** (43.27 g, 0.343 moles) in DMF (1.3 L) was added solid sodium carbonate (36.4 g, 0.343 moles), and the mixture was heated to 100 °C for 7 h under N_2 atmosphere and then stirred overnight at ambient temperature. The mixture was then poured into water/ice (5 L), resulting in a precipitate. The solids were stirred vigorously at 0 °C for 20 min, then filtered. Solids were washed with water and dried in vacuo at 50 °C to give **28** (82.5 g, 97.4% yield) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.28 (s, 1H), 8.20 (s, 1H), 8.05 (d, 1H), 7.51 (s, 2H), 7.29 (d, 1H), 3.81 (s, 3H). MS, *m/z*: 281$(M + H)^+$.

1-(2-(3-Ethylureido)-6-fluoro-1*H*-benzo[*d*]imidazol-5-yl)-1*H*-imidazole-4-carboxylic Acid (31). A suspension of **28** (4.2 g, 0.015 moles) and Raney nickel (0.42 g) in methanol (150 mL) was placed under 45 psi of hydrogen gas on a Parr shaker for 4 h. The mixture was then filtered through Celite to give a clear solution. The solids were washed with MeOH. The combined filtrates were concentrated in vacuo to the intermediate phenylenediamine **29** (3.3 g, 88% yield crude) which was then diluted with pH ∼3.5 buffer (1 N H_2SO_4 buffered with NaOAc) and treated with solid reagent **B** (4.60 g, 0.0198 mol). The resulting mixture was heated to 100 °C for 5 h, then returned to ambient temperature. The reaction pH was adjusted to ∼7 with addition of aqueous $NaHCO_3$ resulting in the precipitation of the methyl ester of crude **30**. The solids were taken up in 6 N HCl (50 mL) and heated to reflux. After 2.5 h the ester hydrolysis was complete. The mixture was cooled to ambient

temperature and concentrated in vacuo. The resulting product was azeotroped thrice with ethanol and pumped dry to yield **31** (3.0 g, 60% yield) as an off-white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.9 (s, 1H), 10.08 (s, 1H), 8.20 (s, 1H), 8.05 (s, 1H), 7.55 (d, 1H), 7.42 (d, 1H), 7.03 (m, 1H), 3.8 (s, 3H), 3.21 (dt, 2H), 1.13 (t, 3H). MS, *m*/*z*: 333 $(M + H)^+$.

1-(5-(4-(Cyclopropylcarbamoyl)-1*H*-imidazol-1-yl)-6-fluoro-1*H*-benzo[*d*]imidazol-2-yl)-3-ethylurea (5). To a solution of **31** (400 mg, 1.2 mmol) and diisopropylethylamine (0.5 mL, 2.88 mmol) in DMF (1.6 mL) were added cyclopropylamine (0.12 mL, 1.8 mmol) and HBTU (680 mg, 1.8 mmol), and the resulting mixture was stirred at ambient temperature for 15 h. The mixture was then poured onto water (30 mL), resulting in a precipitate that was vigorously stirred for 1 h. The heterogeneous mixture was then filtered, and the solids were washed with copious amounts of water. The solids were then dried in vacuo, then washed sequentially with toluene, then hexanes providing **5** (200 mg, 45% yield) as a white solid. Purity, method A 97%; method B 97%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.9 (m, 1H), 10.06 (s, 1H), 8.20 (s, 1H), 8.05 (s, 1H), 7.55 (m, 1H), 7.41 (d, J = 10.8 Hz, 1H), 6.98 (m, 1H), 3.23 (dq, J = 11.7, 9.0 Hz, 2H), 2.84 (m, 1H), 1.12 (dd, J = 9.0 Hz, 3H), 0.65 (m, 2H), 0.60 (m, 2H). MS, *m*/*z*: 372 $(M + H)^+$

5-Bromo-4-fluoro-2-nitroaniline (38). To a solution of **37** (1 g, 4.2 mmol) in DMF (10 mL) was added $(NH_4)_2CO_3$ (2 g, 21 mmol). The resulting mixture was heated at 90 °C for 18 h, then cooled to ambient temperature and quenched with water (10 mL). The product was extracted thrice with EtOAc. The combined organic extracts were dried over Na_2SO_4, filtered, concentrated in vacuo and the residue was purified by silica gel chromatography (7:3, hexanes/EtOAc) to afford **38** (600 mg) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.90 (d, 1H), 7.10 (d, 1H), 6.0 (s, 2H). MS, *m*/*z*: 245$(M + H)^+$.

5-Methoxyl-4-(pyridin-3-yl)-2-nitroaniline (39). To a solution of **38** (286 mg, 1.2 mmol) in DMSO (10 mL) was added pyridine-3-boronic acid (177 mg, 1.44 mmol), potassium phosphate (1.3 g, 6 mmol), and tetrakis(triphenylphosphine)palladium (0.1 equiv). The resulting mixture was heated at 95 °C for 24 h, returned to ambient temperature, then quenched with water (10 mL). The product was extracted thrice with EtOAc, the combined organic extracts dried over Na_2SO_4 and then concentrated in vacuo, and the residue was purified by silica gel chromatography (7:3 to 1:1 hexanes/EtOAc gradient) to afford **39** (120 mg, 41% yield) as a yellow solid. MS, *m*/*z*: 234 $(M + H)^+$.

6-Fluoro-5-pyridin-3-yl-1*H*-benzoimidazol-2-ylamine (40). To a solution of compound **39** (120 mg, 0.52 mmol) in ethyl acetate (20 mL) was added 10% palladium on carbon (catalytic amount). The resulting suspension was placed in a Parr hydrogenation apparatus under 40 psi of hydrogen gas while shaking at ambient temperature for 2 h. The catalyst was removed by filtration and the filtrate concentrated in vacuo to afford the reduced product (75 mg, 0.37 mmol) as an off-white solid. MS, *m*/*z*: 204 $(M + H)^+$. To the above reduced product (0.37 mmol) in MeCN/MeOH/water solution (5/4/1, 10 mL) was added BrCN (0.44 mmol, 1.2 equiv), and the resulting mixture was stirred at ambient temperature for 18 h. The mixture was then partitioned between EtOAc and 1 N NaOH, and the organic phase was washed sequentially with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography (1:0 to 49:1 EtOAc/MeOH gradient) to afford compound **40** (25 mg, 90% yield) as a white solid. MS, *m*/*z*: 229 $(M + H)^+$.

1-Ethyl-3-(6-fluoro-5-pyridin-3-yl-1*H*-benzoimidazol-2-yl)urea (6). To a solution compound **40** (25 mg, 0.1 mmol) in THF (5 mL) was added ethyl isocyanate (0.13 mmol). The resulting mixture was heated to reflux overnight, returned to ambient temperature, then concentrated in vacuo. The crude product was purified by preparative HPLC to afford compound **6** (6 mg) as a white solid. Purity, method A 97%; method B 97%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.79 (s, 1H), 8.61 (d, J = 5.1 Hz, 1H), 8.17 (d, J = 7 Hz, 1H), 7.63 (m, 2H), 7.43 (d, J = 10.1 Hz, 1H), 6.30 (s, 1H, NH), 3.20 (m, 2H), 1.07 (t, J = 7.2 Hz, 3H). MS, *m*/*z*: 300 $(M + H)^+$.

5-Methyl-4-(pyridin-3-yl)-2-nitroaniline (42a). To a solution of **41a** (347 mg, 1.5 mmol) in DMSO (10 mL) was added pyridine-3-boronic acid pinacol ester (203 mg, 1.65 mmol), potassium phosphate (1.59 g, 5 mmol), and tetrakis(triphenylphosphine)-palladium (0.1 equiv). The resulting mixture was heated at 95 °C for 72 h, then returned to ambient temperature and quenched with water (10 mL). The resulting crude mixture was extracted thrice with EtOAc, the combined organic extracts were then dried over Na_2SO_4, filtered and concentrated in vacuo, and the residue was purified by silica gel chromatography (1:1 hexanes/EtOAc) to afford **42a** (140 mg, 41% yield) as a yellow solid. MS, *m*/*z*: 230 $(M + H)^+$.

5-Methyl-4-pyridin-3-yl-benzene-1,2-diamine (43a). To a solution of compound **42a** (140 mg, 0.61 mmol) in ethyl acetate (20 mL) was added 10% palladium on carbon (100 mg). The resulting suspension was placed in a Parr hydrogenation apparatus under 40 psi of hydrogen gas while shaking at ambient temperature for 2 h. The catalyst was removed by filtration and the filtrate concentrated in vacuo to afford compound **43a** (83 mg, 62% yield) as a white solid. MS, *m*/*z*: 200 $(M + H)^+$.

6-Methyl-5-(pyridin-3-yl)-1*H*-benzo[*d*]imidazol-2-amine (44a). To a solution compound **43a** (83 mg, 0.42 mmol) in MeCN/MeOH/water (5/4/1, 100 mL) was added BrCN (0.5 mmol from 5 M solution in MeCN), and the resulting mixture was stirred at ambient temperature for 18 h. The mixture was partitioned between EtOAc and 1 N NaOH, and the organic phase was sequentially washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography (1:0 to 9:1 EtOAc/MeOH gradient) to afford compound **44a** (40 mg, 43% yield) as a light-brown solid.

1-Ethyl-3-(6-methyl-5-pyridin-3-yl-1*H*-benzoimidazol-2-yl)urea (7). To a solution compound **44a** (40 mg, 0.4 mmol) in THF (5 mL) was added ethyl isocyanate (28 μL, 0.3 mmol). The resulting mixture was heated to reflux overnight then returned to ambient temperature and concentrated in vacuo. The crude product was directly purified by silica gel chromatography (1:0 to 19:1 EtOAc/MeOH gradient) to afford compound **7** (5 mg, 9% yield) as a white solid. Purity, method A 92%; method B 92%. ^{1}H NMR (500 MHz, CD_3CN): δ 8.65 (m, 2H), 8.08 (dd, J = 8.0 Hz, 1H), 7.69 (dd, J = 7.8, 5.3 Hz, 1H), 7.46 (s, 1H), 7.38 (s, 1H), 3.62 (m, 2H), 2.89 (m, 2H), 2.25 (s, 3H), 1.14 (t, J = 7.4 Hz, 3H), 1.07 (t, J = 7.2 Hz, 3H). MS, *m*/*z*: 296 $(M + H)^+$.

5-Methoxyl-4-(pyridin-3-yl)-2-nitroaniline (42b). To a solution of **41b** (495 mg, 2 mmol) in DMSO (10 mL) was added pyridine-3-boronic acid 1,3-propanediol cyclic ester (342 mg, 2.1 mmol), potassium phosphate (2.1 g, 10 mmol), and tetrakis(triphenylphosphine)palladium (0.1 equiv). The resulting mixture was heated at 95 °C for 48 h, then returned to ambient temperature and quenched with water (10 mL). The crude mixture was extracted thrice with EtOAc, the combined organic extracts were then dried over Na_2SO_4, filtered, concentrated in vacuo, and the residue was purified by silica gel chromatography (EtOAc) to afford **42b** (450 mg, 46% yield) as a yellow solid. MS, *m*/*z*: 246 $(M + H)^+$.

5-Methoxyl-4-pyridin-3-ylbenzene-1,2-diamine (43b). To a solution of compound **42b** (450 mg, 1.84 mmol) in ethyl acetate (20 mL) was added 10% palladium on carbon (100 mg). The resulting suspension was placed in a Parr hydrogenation apparatus under 40 psi of hydrogen gas while shaking at ambient temperature for 2 h. The catalyst was removed by filtration, and the filtrate concentrated in vacuo to afford compound **43b** (290 mg, 73% yield) as a white solid. MS, *m*/*z*: 216 $(M + H)^+$.

7-Methoxy-5-pyridin-3-yl-1*H*-benzoimidazol-2-ylamine (44b). To a solution compound **43b** (140 mg, 0.65 mmol) in MeCN/MeOH/water (5/4/1, 100 mL) was added BrCN (0.78 mmol from 5 M solution in MeCN), and the resulting mixture was stirred at ambient temperature for 18 h. The mixture was partitioned between EtOAc and NaOH (1 N) and the aqueous phase extracted thrice with EtOAc. The combined organic extracts were sequentially washed with water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product were purified by silica gel chromatography (1:0 to 9:1 EtOAc/MeOH gradient) to afford **44b** (95 mg,

61%) as a pale-yellow solid. ^{1}H NMR (500 MHz, CD_3CN): δ 8.77 (d, 1H), 8,54 (m, 1H), 7.95 (m, 1H), 7.43 (m, 1H), 7.23 (s, 1H), 7.07 (s, 1 H), 5.12 (m, 2H), 3.86 (t, 3H). MS, *m/z*: 241 $(M + H)^+$.

1-Ethyl-3-(7-methoxyl-5-pyridin-3-yl-1*H*-benzoimidazol-2-yl)urea (8). To a solution compound **44b** (95 mg, 0.4 mmol) in THF (1 mL) was added ethyl isocyanate (63 μL, 0.8 mmol). The resulting mixture was heated to reflux overnight, returned to ambient temperature, concentrated in vacuo, then treated with 7 N NH_3 in MeOH (3 mL) at 55 °C for 1 h. The ambient temperature reaction mixture was concentrated in vacuo and purified by silica gel chromatography (1:0 to 19:1 EtOAc/MeOH gradient) to afford compound **8** (42 mg, 34% yield) as a white solid. Purity, method A 90%; method B 90%. ^{1}H NMR (CD_3CN): δ 8.70 (d, J = 1.6 Hz, 1H), 8.48 (dd, J = 4.8, 1.6 Hz, 1H), 7.87 (m, 1H), 7.36 (m, 1H), 7.13 (s, 1H), 3.80 (s, 3H), 3.28 (m, 2H), 1.15 (t, J = 7.2 Hz, 3H). MS, *m/z*: 312 $(M + H)^+$.

4-Methoxy-5-(2-methoxypyrimidin-5-yl)-2-nitroaniline (42c). To a mechanically stirring suspension of 2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidine (119 g, 0.506 mol) and **41c** (125 g, 0.506 mol) in 1,2-dimethoxyethane (506 mL) was added *trans*-dichlorobis(triphenylphosphine)palladium(II) (36 g, 0.0506 mol) followed by saturated aqueous $NaHCO_3$ (~1 M, 759 mL, 0.759 mol). The reaction mixture was stirred at reflux for 6 h (analyzed by HPLC), diluted with water (1.3 L), cooled using an ice−water bath while stirring for 30 min and then allowed to stand at 5 °C for 2 h, and collected the precipitate by filtration. The solid was washed with water until neutral pH, washed with hexane, and dried under high vacuum. The resulting solids were then slurried in $CHCl_3$, stirred at reflux for 1.5 h, allowed to cool to ambient temperature, then collected by filtration, washed with $CHCl_3$, and dried under high vacuum to give **42c** (101 g, 72% yield) as an orange powdery solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.69 (s, 2H), 7.67 (s, 1H), 6.78 (s, 1H), 5.98 (broad s, 2H), 4.08 (s, 3H), 3.83 (s, 3H).

4-Methoxy-5-(2-methoxypyrimidin-5-yl)benzene-1,2-diamine (43c). To a mechanically stirring orange suspension of **42c** (6.15 g, 22.28 mmol) and methanol (22 mL) was added triethylamine (13.9 mL, 100.26 mmol) followed by 10% palladium on activated carbon (50% wet, 0.22 g). Formic acid (96%, 3.8 mL, 95.80 mmol) was added slowly via dropping funnel over 5−10 min while the mixture was simultaneously slowly heated. After complete addition, stirring was continued at reflux for 2 h (all of the orange color had disappeared, leaving a greenish black solution, and TLC indicated that the reaction was complete). It was allowed to cool, diluted with methanol, filtered, and the filtrate was concentrated under reduced pressure. The residue was dissolved in a minimal amount of aqueous 2 N HCl and washed thrice with CH_2Cl_2. The stirring aqueous phase was then neutralized (pH 5−8) with slow addition of solid $NaHCO_3$, giving rise to a light-brown precipitate which was extracted five times with CH_2Cl_2. The combined organic extracts were washed with brine, dried over Na_2SO_4/$MgSO_4$, filtered, and concentrated in vacuo to give **43c** (5.15 g, 94% yield) as a light greenish brown solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.57 (s, 2H), 6.55 (s, 1H), 6.37 (s, 1H), 4.82 (broad s, 2H), 4.18 (broad s, 2H), 3.92 (s, 3H), 3.62 (s, 3H).

1-Ethyl-3-(6-methoxy-5-(2-methoxypyrimidin-5-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (17). To **43c** (13.6 g, 55.3 mmol) in a three-neck round-bottom flask (leaving plenty of head space for frothing) was added a solution of concentrated H_2SO_4 (4.6 mL, 165.9 mmol) in water (130 mL), giving rise to a slight exotherm. To this mechanically stirring suspension was added a mixture of the monosodium salt of 1-cyano-3-ethylurea reagent **A** (14.9 g, 110.6 mmol) in water (60 mL + 27 mL rinse). An immediate mild frothing was observed which lasted only momentarily. The reaction mixture was stirred vigorously at a gentle reflux overnight (it was very important to adjust the pH to between 3 and 6). When the mixture was heated, the solids went into solution and then an off-white tan precipitate formed giving rise to substantial frothing. When the reaction was complete (as analyzed by HPLC and LCMS), it was allowed to cool to ambient temperature and the precipitated solid collected by filtration, washed with water, followed by acetone, and then dried under high vacuum giving **17** (17.0 g, 90% yield) as a tan solid. Purity, method A 92%; method B 92%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.40−11.75 (very broad s, 1H), 9.76−10.04 (very broad s, 1H), 8.68 (s, 2H), 7.33 (s, 1H), 7.23−7.31 (broad s, 1H), 7.14 (s, 1H), 3.95 (s, 3H), 3.77 (s, 3H), 3.21 (quintet, J = 7 Hz, 2H), 1.11 (t, J = 7 Hz, 3H). MS, *m/z*: 343 $(M + H)^+$; 341 $(M - H)^-$.

1-Bromo-2,4-difluoro-5-nitrobenzene (33). A 0 °C solution of **32** (3.0 g, 15.5 mmol) in concentrated sulfuric acid (16 mL) was treated with potassium nitrate (1.60 g, 15.7 mmol) portionwise and the reddish mixture stirred at 0 °C for 5 min and then at ambient temperature for 3 h. The mixture was carefully poured directly onto ice, then extracted with ethyl acetate. The organic phase was washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated in vacuo to give (3.66 g, 99%) of crude product as a brown oil which was used directly in the next step. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (t, 1 H), 7.14 (dd, 1 H). The intermediate bromo-2,4-difluoro-5-nitrobenzene (3.35 g, 14.0 mmol) was dissolved in 0.5 N ammonia in dioxane (30 mL, 15 mmol) and the mixture heated in a sealed tube at 70 °C for 48 h. The mixture was cooled to ambient temperature, then evaporated in vacuo to give crude compound **33** as a yellow solid which was not purified further. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.40 (d, 1H), 6.6 (d, 1H), 6.2 (broad s, 2H).

4-Bromo-2-nitro-5-(piperidin-1-yl)benzenamine (34). A solution of **33** (500 mg, 2.13 mmol) in CH_3CN (5 mL) was treated with excess piperidine (1.05 mL, 10.7 mmol) and the yellow mixture heated at 80 °C for 2 h and then returned to ambient temperature. The mixture was partitioned between EtOAc and saturated $NaHCO_3$ and the organic layer washed with brine, dried over Na_2SO_4, filtered, and evaporated in vacuo to give 583 mg of an orange oil. The crude product was purified by silica gel chromatography (4:1 hexanes/ethyl acetate) to give **34** (450 mg, 70%) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.10 (s, 1H), 7.5 (broad s, 2H), 6.55 (s, 1H), 3.0 (m, 4H), 1.7 (m, 4H), 1.55 (m, 2H) ppm.

2-Nitro-5-(piperidin-1-yl)-4-(pyridin-3-yl)benzenamine (35). A solution of **34** (450 mg, 1.5 mmol) and pyridine-3-boronic acid pinacol ester (318 mg, 1.95 mmol) in DME (6 mL) and saturated $NaHCO_3$ (3 mL, 3.0 mmol) was treated with $Pd(PPh_3)_4$ (87 mg, 0.075 mmol) and the biphasic suspension heated at 80 °C for 12 h. The mixture was partitioned between CH_2Cl_2 and saturated $NaHCO_3$, the organic phase was washed with brine, dried over anhydrous Na_2SO_4, and evaporated in vacuo to give 572 mg of a brown oil. Crude product was chromatographed on silica gel (9:1 to 4:1 CH_2Cl_2/EtOAc gradient) to give **35** (373 mg, 83%) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.80 (s, 1H), 8.55 (m, 1H), 7.95 (m, 1H), 7.85 (s, 1H), 7.6 (s, 2H), 7.4 (m, 1H), 6.5 (s, 1H), 2.75 (m, 4H), 1.4 (m, 6H).

6-(Piperidin-1-yl)-5-(pyridin-3-yl)-1*H*-benzo[*d*]imidazol-2-amine (36). A suspension of **35** (273 mg, 0.92 mmol) in methanol (4 mL) and acetic acid (2 drops) was treated with 10% Pd/C (70 mg) and the black suspension shaken on a Parr apparatus under 45 psi of hydrogen for 12 h. Catalyst was removed by filtration, and the filtrate was reduced in volume to give a solution of crude diamine which was used immediately in the next step. A solution of the crude diamine (~0.9 mmol) in methanol (4 mL) was treated with cyanogen bromide (3.7 mmol from 5 M solution in MeCN) and the red-brown mixture stirred at ambient temperature under an N_2 atmosphere for 25 min. The reaction mixture was added directly, purified by silica gel and chromatography (95:5 CH_2Cl_2/MeOH, to 93:7 CH_2Cl_2:7N NH_3 in MeOH gradient) to give **36** (66 mg, 26% yield) as an oily brown solid. MS, *m/z*: 294 $(M + H)^+$

1-Ethyl-3-(6-(piperidin-1-yl)-5-(pyridin-3-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (9). A solution **36** (66 mg, 0.24 mmol) in dry CH_3CN (0.5 mL) was treated with excess ethyl isocyanate (200 μL) and the brown mixture heated at 70 °C for 3 h. The mixture was evaporated in vacuo to give crude bis-urea as an oil which was then immediately treated with 7 N NH_3 in MeOH (2 mL) and heated at 70 °C for 1 h. The mixture was returned to ambient temperature, evaporated in vacuo and then purified by column chromatography on silica gel (98:2 to 96:4 to 95:5 CH_2Cl_2/MeOH gradient) to give

28 mg of product as an off-white solid. Crude product was suspended in water, stirred for 20 min, then collected by filtration, rinsed twice with water, and dried in vacuo to give **9** (22 mg, 26%) as a white solid. Purity, method A 94%; method B 94%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 11.40 (broad s, 1H), 9.8 (broad s, 1H), 8.90 (1H, s), 8.50 (dd, J = 1.8, 2.6 Hz, 1H), 8.0 (dt, J = 1.6, 8.4 Hz, 1H), 7.4 (dd, J = 2.2, 7.4 Hz, 1H), 7.3 (broad s, 1H), 7.2 (s, 1H), 7.1 (s, 1H), 3.2 (q, J = 8.4 Hz, 2H), 2.7 (m, 4H), 1.3 (m, 6H), 1.1 (t, J = 8.4 Hz, 3H). MS, m/z: 365 $(M + H)^+$.

Methyl-2-amino-5-bromo-3-nitrobenzoate (46). To a 0−5 °C solution of trifluoroacetic anhydride (60 mL) was slowly added **45** (5 g, 21.73 mmol), resulting in a beige precipitate formed during the addition. After the mixture was stirred for 15 min, solid potassium nitrate was added in (2.64 g, 26.08 mmol) in one portion. The ice bath was then removed, and the resulting mixture was allowed to stir overnight at ambient temperature. The resulting crude reaction was then concentrated to dryness, dissolved in ethyl acetate, and carefully neutralized with excess sodium bicarbonate. The organic layer was washed with brine, dried over Na_2SO_4, filtered, then concentrated to dryness. The resulting crude solid (6.39 g) was heated to 65 °C in a mixture of methanol (150 mL) and 6 N hydrochloric acid (50 mL) for 3 h. The mixture was cooled to ambient temperature and concentrated to dryness to provide **46** (3.5 g, 59% yield) as a light-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.52 (s, 1H), 8.43 (s, 1H), 3.94 (s, 3H). MS, m/z: 275 $(M + H)^+$.

Methyl-2-amino-3-nitro-5-(pyridine-3-yl)benzoate (47). To a solution of **46** (0.4 g, 1.095mmol) in dimethoxyethane (8 mL) were added pyridin-3-ylboronic acid (0.2 g, 1.64 mmol), tetrakis(triphenylphosphine)palladium (0.125 g, 0.109mmol), and 1 M aqueous sodium bicarbonate (2.18 mL, 2.18mmol). The resulting mixture was refluxed overnight, then diluted with saturated sodium bicarbonate and extracted thrice with ethyl acetate. The combined organic extracts were subsequently washed with brine, dried over Na_2SO_4, filtered, concentrated in vacuo, and purified by column chromatography on silica gel (1:0 to 0: EtOAc/CH_2Cl_2 gradient) to provide **47** (0.169 g, 57% yield) as a pale-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.81 (s, 1H), 8.67 (s, 1H), 8.63 (d, 1H), 8.52 (s, 1H), 7.88 (d, 1H), 7.41 (dd, 1H), 3.96 (s, 3H). MS, m/z: 274 $(M + H)^+$.

Methyl-2-(3-ethylureido)-5-(pyridine-3-yl)-1*H*-benzo[*d*]imidazole-7-carboxylate (10). A slurry of 10% palladium on carbon (0.035 g) and **47** (0.167 g, 0.611mmol) in ethanol was subjected to 40 psi of H_2 in a Parr shaker for 4 h. The depressurized mixture was then filtered through Celite and concentrated to dryness to provide the corresponding phenylenediamine (0.110 g) which was then suspended in water buffered to pH 3 with 1 N sulfuric acid and treated with reagent **A**. The mixture was refluxed overnight, cooled to ambient temperature, and the resulting solids were collected via filtration. Further purification by column chromatography on silica gel (100% ethyl acetate, then switching to 9:1 CH_2Cl_2/7 N ammonia/methanol) provided 0.055 g of material that was dissolved in methanol and reprecipitated with diethyl ether to provide **10** (0.01 g, 5% yield) as a white solid. Purity, method A 98%; method B 98%. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.5 (broad s, 1H), 9.89 (broad. s, 1H), 8.91 (broad s, 1H), 8.56 (dd, J = 4.7 and 1.5 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H), 8.01 (s, 1H), 7.9 (d, J = 1.7 Hz, 1H), 7.40−7.51 (m, 2H), 3.96 (s, 3H), 3.25 (q, J = 7.0, 5.6 Hz, 2H), 1.13 (t, J = 7.2 Hz, 3H). MS, m/z: 340 $(M + H)^+$; 338 $(M - H)^-$.

2-(3-Ethylureido)-5-(pyridine-3-yl)-1*H*-benzo[*d*]imidazole-7-carboxylate (48). A solution of **10** (0.143 g, 0.421mmol) in 6 N hydrochloric acid (5 mL) was refluxed for 16 h, then cooled to ambient temperature and concentrated in vacuo to provide **48** (0.128 g, 94% yield) as a white solid.

2-(3-Ethylureido)-*N*-methyl-5-(pyridine-3-yl)-1*H*-benzo[*d*]imidazole-7-carboxamide (11). To an ambient temperature mixture of **48** (0.0125 g, 0.031mmol) in dimethylformamide (1 mL) was added bromo tris-pyrolidino-hexafluorophosphate (0.029 g, 0.063mmol), methyamine hydrochloride (0.263 g, 0.094 mmol), diisopropylethylamine (0.32 mL, 0.188mmol), and a catalytic amount of dimethylaminopyridine. After being stirred overnight, the mixture was directly submitted for reverse phase HPLC to provide **11** (0.0022 g, 21% yield) as a white solid. Purity, method A 98%; method B 98%. ^{1}H NMR (500 MHz, CD_3OD): δ 9.16 (d, J = 1.6 Hz, 1H), 8.79−8.85 (m, 2H), 8.19 (d, J = 1.2 Hz, 1H,), 8.09-.10 (m, 1H), 8.02 (d, J = 1.2 Hz, 1H), 3.33 (q, J = 14.5 and 7.3 Hz, 2H), 3.31 (s, 3H), 1.22 (t, J = 7.3 Hz, 3H). MS, m/z: 339 $(M + H)^+$; 337 $(M - H)^-$.

2-Amino-5-bromo-3-nitrophenol (50). To a stirred solution of **49** (3.0 g, 19.5mmol) in acetic acid (45 mL) was added bromine (3.1 g, 19.5mmol) in acetic acid (25 mL) over 1 h. The resulting mixture was allowed to stir for 18 h under nitrogen at ambient temperature. The mixture was made homogeneous with the addition of ethanol (100 mL) followed by the addition of ethyl acetate (300 mL). The solution was then neutralized with the addition of 2 N NaOH and then reacidified to pH 3 with the addition of 6 N HCl. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and the resulting filtrate was concentrated to dryness under vacuum to provide **50** (4.35 g, 82% yield) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.6 (broad s, 1H), 6.35 (broad s, 2H), 7.00 (s, 1H), 7.91 (s, 1H).

2-(Benzyloxy)-4-bromo-6-nitrobenzenamine (51). To a stirred mixture of **50** (0.33 g, 1.2mmol) and potassium carbonate (0.46 g, 3.1mmol) was added benzyl bromide (0.21 g, 1.2mmol). The mixture was allowed to stir under nitrogen at ambient temperature for 18 h. The mixture was partitioned between ethyl acetate and water. The organic phase was washed 5 times with water, brine, dried over Na_2SO_4, filtered, then concentrated to dryness under vacuum. The resulting residue was purified by column chromatography on silica gel using (19:1 hexanes/EtOAc) to give **51** (0.21 g, 53% yield) as a dark-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.07 (s, 2H), 6.44 (broad s, 2H), 7.02 (s, 1H), 7.42 (m, 5H), 7.89 (s, 1H).

2-(Benzyloxy)-6-nitro-4-(pyridin-3-yl)benzenamine (52). To a suspension of **51** (0.21 g, 0.64mmol), pyridine-3-boronic acid-1, 3-propanediolcyclic ester (0.14 g, 0.84mmol), and 1 M sodium bicarbonate (1.3 mL) was added tetrakistriphenylphosphine palladium (0.07 g, 0.06 mmol) in ethylene glycol dimethyl ether. The mixture was heated to reflux and allowed to stir for 5 h, then cooled to ambient temperature. The mixture was diluted with ethyl acetate and filtered through Celite. The filtrate was sequentially washed with saturated sodium bicarbonate, water, and brine, then dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel 95.5:0.5 CH_2Cl_2/MeOH) to give **52** (0.10 g, 50% yield) as a dark-yellow solid. ^{1}H NMR (500 MHz, MeOH-d_4): δ 5.24 (s, 2H), 7.21 (s, 1H), 7.34−7.5 (m, 6H), 7.91 (d, 1H), 8.5 (d, 1H), 8.71 (s, 1H).

7-(Benzyloxy)-5-(pyridin-3-yl)-1*H*-benzo[*d*]imidazol-2-amine (53). To a solution of **52** (0.10 g, 0.31mmol) in ethanol (15 mL) and water (2 mL) at 70 °C was added sodium sulfite (0.43 g, 2.49mmol) in water (2 mL). The mixture was allowed to stir for 30 min, then cooled to ambient temperature. The solvent was evaporated under reduced pressure, and the resulting residue was purified by column chromatography on silica gel (0.5:5.0:94.5 NH_4OH/MeOH/CH_2Cl_2). The resulting diamine (0.07 g, 0.23mmol) was dissolved in acetonitrile (4 mL) and methanol (1 mL), and to it was added cyanogen bromide (0.46 mmol from 5 M solution in MeCN). The resulting mixture was allowed to stir for 2 h. The solvent was evaporated under reduced pressure and the resulting residue was purified by column chromatography on silica gel (95:5 CH_2Cl_2/7 N ammonia in methanol) to provide **53** (0.05 g, 46% yield) as an off-white solid. ^{1}H NMR (500 MHz, MeOD): δ 5.28 (s, 2H), 6.84 (s, 1H), 7.13 (s, 1H), 7.32−7.45 (m, 4H), 7.50 (d, 2H), 7.91 (d, 1H), 8.48 (d, 1H), 8.76 (s, 1H).

1-(7-(Benzyloxy)-5-(pyridin-3-yl)-1*H*-benzo[*d*]imidazol-2-yl)-3-ethylurea (16). To a mixture of **53** (0.05 g, 1.5mmol) in acetonitrile (5 mL) was added an excess of ethyl isocyanate (1 mL). The mixture was heated to 70 °C and allowed to stir for 3 h. The mixture was returned to ambient temperature, concentrated in vacuo, and the resulting residue was suspended in 7 N ammonia in methanol (5 mL). The mixture was heated to 60 °C and allowed to stir for 3 h, then cooled to ambient temperature and concentrated in vacuo. The resulting residue was purified by column chromatography on

silica gel (97:3 CH_2Cl_2/7 N ammonia−methanol). The chromatographed material was the triturated with water, filtered, and dried under high vacuum to give **16** (0.05 g, 86% yield) as an off-white solid. Purity, method A 98%; method B 98%. ^{1}H NMR (MeOH-d_4): δ 1.20 (t, J = 7.2 Hz, 3H), 3.30 (q under H_2O peak, 2H), 5.39 (s, 2H), 7.03 (s, 1H), 7.31 (m, 2H), 7.39 (t, J = 2.3 Hz, 2H), 7.49 (m, 1H), 8.06 (d, J = 4.7 Hz, 1H), 8.47 (m, 1H), 8.77 (s, 1H). MS, m/z: 388 $(M + H)^+$.

2,2,2-Trifluoro-*N*-(3,5-difluoro-2-nitrophenyl)acetamide (56). A mixture of **54** (19.4 g, 150 mmol) in concentrate sulfuric acid (22 mL) was heated with stirring at 190 °C for 5 h and then returned to ambient temperature. The mixture was poured onto ice and the resulting precipitate was filtered and washed with cold water, affording 24.7 g of the sulfated intermediate as a gray solid. A small fraction of the resulting product (2.0 g, 13.6mmol) was dissolved in trifluoroacetic anhydride (18 g, 85.0mmol), allowed to stir for 1 h at ambient temperature, then treated with solid potassium nitrate (1.8 g, 17.8mmol) and allowed to stir for an additional 18 h. The resulting crude reaction was poured slowly onto ice and extracted 4 times with CH_2Cl_2. The combined organics were dried over Na_2SO_4, and the solvent was evaporated in vaccuo. The crude sample was purified by column chromatography on silica gel (19:1 hexanes/EtOAc) to provide **56** (2.94 g, 75% yield) as a dark-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.91 (m, 1H), 8.23 (m, 1H), 10.55 (broad s, 1H).

3,5-Difluoro-2-nitrobenzenamine (57). A mixture of **56** (0.41 g, 1.5mmol) and 6 N HCl (15 mL) was heated to reflux and allowed to stir for 5 h. The mixture was cooled to ambient temperature and poured into ice-cold aqueous sodium carbonate and extracted 4 times with CH_2Cl_2 and then a mixture of ethyl acetate and ethanol (90:10). The combined organics were dried over Na_2SO_4, filtered, then concentrated in vacuo to give **57** (0.17 g, 64% yield) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.89 (broad s, 2H), 6.29 (m, 2H).

5-Fluoro-2-nitro-3-(1*H*-pyrazol-1-yl)benzenamine (58). To a solution of **57** (0.20 g, 1.2mmol) and pyrazole (0.094 g, 1.38 mmol) in dimethylformamide (5 mL) was added sodium hydride, 60% dispersion in mineral oil (0.055 g, 1.38 mmol). The mixture was heated to 50 °C and allowed to stir for 18 h, returned to ambient temperature, then quenched by the addition of aqueous ammonium chloride. The crude mixture was extracted with twice with EtOAc, the combined organics were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The resulting residue was purified by column chromatography on silica gel (4:1 hexanes/EtOAc) to provide **58** (0.064 g, 25% yield) as a dark-yellow solid. ^{1}H NMR ($CDCl_3$): δ 5.3 (broad s, 2H) 6.45−6.68 (m, 3H) 7.65 (d, 1H) 7.71 (d, 1H).

1-(3-Amino-4-nitro-5-(1*H*-pyrazol-1-yl)phenyl)-*N*-cyclopropyl-1*H*-imidazole-4-carboxamide (59). To a mixture of **58** (0.063 g, 0.28 mmol) and **87** (0.064 g, 0.43 mmol) in dimethyformamide (4 mL) was added sodium carbonate (0.045 g, 0.43 mmol). The mixture was heated to 95 °C and allowed to stir for 18 h, then returned to ambient temperature. Water was added to the mixture and the heterogeneous reaction allowed to stir for 30 min. The mixture was allowed to settle, and the aqueous solution was decanted. The resulting solids were triturated with water (2 × 30 mL) and filtered with a methanol wash. The mother liquor was concentrated in vacuo and the resulting solid was triturated with a 10 mL solution of ethyl acetate/hexanes (1:1), filtered, and combined with the original filtered solid to give **59** (0.06 g, 96% yield) as a dark-yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.64 (m, 4H, 2.84 (m, 1H), 6.52 (broad s, 2H), 6.55 (t, 1H), 7.21 (m, 2H), 7.70 (s, 1H), 8.10 (d, 1H), 8.23 (s, 1H), 8.33 (d, 1H), 8.38 (s, 1H).

1-(5-(4-(Cyclopropylcarbamoyl)-1*H*-imidazol-1-yl)-7-(1*H*-pyrazol-1-yl)-1*H*-benzo[*d*]imidazol-2-yl)-3-ethylurea (19). A mixture of **59** (0.06 g, 0.17 mmol) and 10% Pd−C (10 mg) in methanol (20 mL) was placed under an atmosphere of hydrogen (45 psi) using a Parr apparatus. The mixture was shaken for 2 h, the catalyst was filtered off, and the solvent was evaporated under vacuum. The resulting solid mostly dissolved in 1 N sulfuric acid (1.3 mL), and to it was added reagent **A** (0.5 mmol). Then 1 N sulfuric acid was added dropwise until the pH was approximately 3, then heated to reflux and allowed to stir for 18 h. The mixture was cooled to ambient temperature, resulting in a heterogeneous mixture. The solvent was decanted, and the remaining solids were triturated twice with water. The resulting solid was further purified by preparative HPLC (10−90% gradient of acetonitrile−water with 0.1% TFA), then converted to the bis-HCl salt by dissolving purified product in 2 N hydrochloric acid (1.5 mL) and methanol (5 mL) and stirring the mixture for 5 min. The solvent was removed in vacuo and residual water was removed azeotropically with methanol to give **19** (24 mg, 32% yield as bis-HCl salt) as an off-white solid. Purity, method A 96%; method B 96%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 0.62 (m, 2H), 0.71 (m, 2H), 1.11 (t, J = 7.2 Hz, 3H), 2.89 (m, 1H), 3.23 (q, J = 6.7 Hz, 2H), 6.69 (t, J = 2.3 Hz, 1H), 7.4 (broad s, 1H), 7.67 (s, 1H), 7.95 (s, 1H), 8.07 (m, 1H), 8.52 (m, 2H), 8.89 (broad s, 1H), 9.07 (s, 1H), 10.66 (broad s, 1H).

5-Bromo-1,3-difluoro-2-nitrobenzene (61). To a suspension of sodium perborate tetrahydrate (1.04 g, 5 mmol) in acetic acid (20 mL), stirred at 55 °C, was added a solution of **60** in acetic acid (10 mL) over 1 h in a dropwise fashion. After being stirred at 55 °C for an additional 3 h, the solution was allowed to cool to ambient temperature and filtered. The filtrate was poured into ice and extracted twice with ethyl acetate. The combined organic extracts were washed successively with water (5 times), brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (20:1 hexanes/EtOAc) to provide **61** (780 mg, 66% yield) as a tan solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.32 (dt, 2H).

1-(5-Bromo-3-fluoro-2-nitrophenyl)-1*H*-pyrazole (62). To a stirring 0 °C suspension of sodium hydride (44 mg, 1.1 mmol, 60% oil dispersion) in THF (4 mL) was added a solution of pyrazole (72 mg, 1.05 mmol) in THF (1 mL). The resulting mixture was stirred at 0 °C for 5 min, then treated with a THF solution of **61** (238 mg, 1 mmol) dropwise over 10 min. The resulting mixture was warmed to ambient temperature and allowed to stir for 1 h. The reaction was then quenched by addition of water (1 mL), partitioned between water and ethyl acetate, and the phases were separated. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (6:1 hexanes/EtOAc) to provide **62** (240 mg, 86% yield) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.55 (t, 1H), 7.45 (d, 1H), 7.60 (s, 1H), 7.80 (m, 2H). MS, m/z: 289 $(M + H)^+$; 287 $(M - H)^-$.

5-Bromo-2-nitro-3-pyrazol-1-ylphenylamine (63). To a solution of **62** (240 mg, 0.84 mmol) in ethanol (3 mL) was added ammonia (3 mL, 2 N in methanol). The resulting mixture was heated in a sealed tube at 80 °C for 16 h, returned to ambient temperature and pressure, then concentrated in vacuo. The residue was purified by column chromatography on silica gel (3:1 hexanes/EtOAc) to provide **63** (205 mg, 86%yield) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.20 (broad s, 2H), 6.50 (t, 1H), 6.9 (d, 1H), 7.1 (d, 1H), 7.7 (d, 1H), 7.8 (d, 1H). MS, m/z: 285 $(M + H)^+$; 283 $(M - H)^-$.

2-Nitro-3-pyrazol-1-yl-5-pyridin-3-ylphenylamine (64a). To a solution of **63** (200 mg, 0.71 mmol) in THF (8 mL) was added 3-pyridyldiethyl borane (157 mg), (tetrakistriphenylphosphine) palladium(0) (84 mg), and sodium carbonate (2.2 mmol from 2 M aqueous). The resulting mixture was stirred at 70 °C overnight, then cooled to ambient temperature. The reaction mixture was diluted with ethyl acetate (100 mL) and sequentially washed with water then brine, dried over $MgSO_4$, filtered, then concentrated in vacuo. The resulting residue was purified by column chromatography on silica gel (3:1 to 1:8 hexanes/EtOAc slow gradient) to afford **64a** (120 mg, 60% yield) as a yellow solid. ^{1}H NMR (DMSO-d_6): δ 6.45 (broad s, 2H), 6.55 (t, 1H), 7.1 (s, 1H), 7.25 (s, 1H), 7.55 (m, 1H), 7.7 (s, 1H), 8.1 (dt, 1H), 8.3 (d, 1H), 8.7 (d, 1H), 8.9 (s, 1H).

1-Ethyl-3-(7-pyrazol-1-yl-5-pyridin-3-yl-1*H*-benzoimidazol-2-yl)urea (12). A suspension of **64a** (120 mg, 0.40 mmol) and 10% Pd (12 mg) in ethyl acetate (10 mL) was placed in a Parr hydrogenator under a hydrogen pressure of 45 psi. The mixture

was shaken for 16 h, filtered, and the filtrate was concentrated in vacuo. The resulting residue was diluted with H_2SO_4 (1.6 mL of 1 N), combined with reagent **A** (0.8 mL from 1 M solution), and heated at 95 °C for 4 h. The mixture was then cooled to ambient temperature, concentrated in vacuo, and purified by preparative HPLC to afford **12** as a bis-TFA salt. This resulting salt was suspended in aqueous $NaHCO_3$, stirred for 10 min, and filtered. The resulting solids were washed with water and dried under high vacuum to afford freebase **12** (69 mg, 50% yield) as a white solid. Purity, method A 98%; method B 98%. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.76 (broad s, 1H), 9.28 (d, J = 1.5 Hz, 1 H), 9.06 (d, J = 2.5 Hz, 1H), 8.83 (dd, J = 5.5 and 1.3 Hz, 1H), 8.78 (d, J = 7.9 Hz, 1H), 8.18 (d, J = 1.3 Hz, 1H), 8.02 (dd, J = 8.1 and 5.5 Hz, 1H), 7.94 (d, J = 1.5 Hz, 1H), 7.82 (d, J = 1.5 Hz, 1H), 7.59 (broad s, 1H), 6.69 (dd, J = 2.6 and 1.9 Hz, 1H), 3.25 (m, 2H), 1.14 (t, J = 7.2 Hz, 3H). MS, *m/z*: 348 $(M + H)^+$; 346 $(M - H)^-$.

2-Nitro-3-(1*H*-pyrazol-1-yl)-5-(pyrimidin-5-yl)benzenamine (64b). To a solution of **63** (100 mg, 0.43 mmol) in DME (5 mL) was added successively (1.3 mL of 1 M aqueous $NaHCO_3$), 5-pyrimidine boronic acid (65 mg, 0.52 mmol), and palladium tetrakistriphenylphosphine (50 mg, 0.04 mmol). The resulting mixture was stirred at 80 °C overnight, cooled to ambient temperature, and partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (3:1 hexanes/EtOAc) to afford **64b** (90 mg, 85%) as an orange solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.25 (s, 1H), 9.1 (s, 2H), 7.2 (broad s, 2H), 7.1 (s, 1H), 7.05 (d, 1H).

1-(7-(1*H*-Pyrazol-1-yl)-5-(pyrimidin-5-yl)-1*H*-benzo[*d*]imidazol-2-yl)-3-ethylurea (18). To a solution of **64b** (100 mg, 0.35 mmol) in ethyl acetate (15 mL) was added of 10% $Pd(OH)_2$−C (15 mg, catalytic). The mixture was shaken under a 45 psi hydrogen atmosphere for 16 h, filtered over Celite, and concentrated in vacuo. To a solution of the residual oil in 1 N sulfuric acid (1.4 mL) and water (3 mL) was added reagent **A** (0.7 mL of a 1 M solution). The mixture was stirred at reflux for 3.5 h, cooled to ambient temperature, and concentrated in vacuo. The residue was purified by preparative HPLC to afford **18** (42 mg, 20%) as an off white solid. Purity, method A 98%; method B 98%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.25 (broad s, 1H), 9.2 (broad s, 1H), 9.15 (s, 2H), 9.05 (broad s, 1H), 8.05 (s, 1H), 7.85 (broad s, 1H), 7.75 (s, 1H), 7.25 (broad s, 1H), 6.65 (s, 1H), 3.25 (m, 2H), 1.15 (t, J = 7.2 Hz, 3H). MS, *m/z*: 349 $(M + H)^+$; 347 $(M - H)^-$.

***N*-[2-(3-Fluoropyridin-2-yl)phenyl]-2,2-dimethylpropionamide (66).** A 3 L flask was charged with boronic acid **65** as a tetrahydrate (92.1 g, 314 mmol), chlorofluoropyridine (37.6 g, 286 mmol), $NaHCO_3$ (48.0 g, 572 mmol), and $Pd(PPh_3)_4$ (3.3 g, 2.86 mmol). Water (300 mL) and dimethoxyethane (300 mL) were added, and the mixture was heated slowly to 83 °C (internal temperature) over a 1 h period with overhead stirring. After ∼2 h all solids had dissolved. The mixture was allowed to stir for an additional 10 h. The mixture was cooled to ambient temperature and stirred overnight, after which time a thick gum had formed. The crude mixture was diluted with water (2 L) and stirred for an additional 2 h. The mixture was then allowed to rest without stirring until the gum had settled to the bottom of the flask. The liquid phase was removed via vacuum, then replaced with 0.1 N NaOH and stirred for 15 min. The gum was allowed to settle and the liquid removed via vacuum. The gum was then similarly washed three times with water, then transferred to a 1 neck flask as an acetone solution. The mixture was concentrated in vacuo and azeotroped five times with ethyl acetate. No further purification was performed on intermediate **66**. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.21 (s, 1H), 7.99 (dd, J = 0.9, 8.1 Hz, 1H), 7.89 (m, 1H), 7.60 (ddd, J = 1.5, 3.3, 7.8 Hz, 1H), 7.54 (quintet, J = 3.9 Hz, 1H), 7.46 (dt, J = 1.8, 7.8 Hz, 1H), 7.25 (dt, J = 1.2, 7.8 Hz, 1H), 1.10 (s, 9H). MS, *m/z*: 273.14 $(M + H)^+$.

***N*-[4-Bromo-2-(3-fluoropyridin-2-yl)phenyl]-2,2-dimethylpropionamide (67).** To an ambient temperature suspension of **66** (∼77 mmol) in acetic acid (300 mL) was added bromine (12 mL, 228 mmol) as a solution in 50 mL of acetic acid over a 1 h period. The heterogeneous mixture was stirred at ambient temperature for 5 h, over which time a thick precipitate formed. The mixture was then poured over ice, diluted with 1 N $Na_2S_2O_3$ (500 mL), and stirred for 1 h. The solids were filtered, resuspended in water (2 L), stirred for 1 h, then filtered and washed with water again. The resulting solids were pumped to dryness at 50 °C, resuspended in HOAc (400 mL), and treated with bromine (4 mL, 76 mmol) in acetic acid solution (20 mL) over a 20 min period. The resulting heterogeneous mixture was stirred for 5 h, then quenched and treated as described above. The resulting solids were vaccuum-dried at 50 °C to afford **67** (19.1 g, 72% yield) as a tan powder. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 10.13 (s, 1H), 8.58 (d, J = 1.5 Hz, 1H), 7.90 (m, 2H), 7.56 (t, J = 2.4 Hz, 1H), 7.65 (dd, J = 2.4, 8.7 Hz, 1H), 7.57 (quintet, J = 4.2 Hz, 1H), 1.10 (s, 9H). MS, *m/z*: 351.03 and 353.08 $(M + H)^+$

***N*-[4-Bromo-2-(3-fluoropyridin-2-yl)-6-nitrophenyl]-2,2-dimethylpropionamide (68).** To a suspension of **67** (6.45 g, 18.4 mmol) in TFA (100 mL) and TFAA (25.5 mL, 183.6 mmol) at 0 °C was added a TFA solution (30 mL) of 90% fuming nitric acid (2.46 mL, 55.1 mmol) over a 45 min period. The mixture was then stirred at 0 °C for a total of 4 h. The crude mixture (now homogeneous) was poured into ice, producing a pasty mass. The mixture was diluted to 500 mL total volume with water, treated with 50 mL of methanol, and vigorously stirred for 12 h. The resulting solids were filtered, washed with copious amounts of water, then dried in vacuo at 50 °C to afford **68** (6.1 g, 82% yield) as a tan powder. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 9.59 (s, 1H), 8.57 (d, J = 1.5 Hz, 1H), 8.28 (d, J = 2.4 Hz, 1H), 8.05 (dd, J = 0.9, 2.4 Hz, 1H), 7.85 (dt, J (q, J = 1.2, 8.4 Hz, 1H), 7.58 (quintet, J = 4.2 Hz) 1.10 (s, 9H). MS, *m/z*: 396.09 $(M + H)^+$

4-Bromo-2-(3-fluoropyridin-2-yl)-6-nitrophenylamine (69). A suspension of **68** (522 g, 1.32 mol) was suspended in 6 M hydrochloric acid (5 L), and the reaction mixture was heated at reflux for 5 h. Pivalic acid and water were collected using a Dean−Stark trap. After 5 h the LCMS results still indicated the presence of starting material. Concentrated hydrochloric acid and water (1 L each) were added, and the mixture was heated at reflux until the starting material was consumed (additional 3 h). The mixture was cooled overnight, and the solid was collected by vacuum filtration and rinsed with water (5 L). The filter cake was suspended in 10% sodium carbonate (5 L) and stirred for 30 min. The solid was collected by vacuum filtration and rinsed with water (5 L). The solid was dried in a convection oven at 50 °C for 48 h to provide the product **8** (378.2, 92% yield) as an orange-yellow solid. ^{1}H NMR (CDCl3, 500 MHz): δ 8.53 (m, 1H), 8.40 (d, J = 3.0 Hz, 1H), 7.81 (t, J = 3.0 Hz, 1H), 7.79 (br s, 2H), 7.63 (m, 1H), 7.41 (quintet, J = 4.0 Hz, 1H). MS, *m/z*: 311.76 and 313.76 $(M + H)^+$

2-(3-Fluoropyridin-2-yl)-6-nitro-4-(pyridin-3-yl)benzenamine (71a). To a heterogeneous mixture of **69** (4.0 g, 12.82 mmol) and 3-(1,3,2-dioxaborinan-2-yl)pyridine (2.7 g, 16.66 mmol) in DME/water (50:25 mL) was added solid Na_2CO_3 (2.7 g, 25.63 mmol) and $Pd(PPh_3)_4$ (0.75 g, 0.64 mmol). The resulting mixture was stirred at reflux for 6 h and then returned to ambient temperature, diluted with EtOAc, then slowly acidified with 6 N HCl with stirring. The phases were separated, and the aqueous phase was washed thrice with EtOAc. The resulting aqueous extract was filtered to separate out insoluble matter and then slowly basified with ammonium hydroxide to pH ∼10. The resulting heterogeneous mixture was stirred at room temperature for 5 h and solids were isolated via filtration, washed with copious amounts of water, and dried under high vacuum to afford **71a** (3.65 g, 92% yield) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz): major rotomer δ 8.91 (d, J = 2.5 Hz, 1H), 8.61 (dt, J = 1.5, 4.5 Hz, 1H), 8.54 (dd, J = 2.5 Hz, 1H), 8.11 (m, 1H), 8.03 (t, J = 2.5 Hz, 1H), 7.95 (m, 1H), 7.62 (quintet, J = 4.0 Hz, 1H), 7.50 (m, 2H), 7.46 (m, 1H). MS, *m/z*: 310.9 $(M + H)^+$; 309.37 $(M - H)^-$.

1-Ethyl-3-(7-(3-fluoropyridin-2-yl)-5-(pyridin-3-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (15). To a suspension of **71a** (350 mg, 1.13 mmol) in EtOAc (10 mL) was added Pd−C (∼50 mg). The suspension shaken in a Parr bottle under 45 psi of hydrogen for 1 h. The

reaction mixture was diluted with EtOAc (25 mL) and filtered on 2.7 μm glass microfiber filter. The filtrate was concentrated in vacuo to give the crude intermediate diamine (245 mg), which was used without further purification for the next step. MS, *m/z*: 281 $(M + H)^+$. A 50 mL round-bottom flask equipped with a reflux condenser and a magnetic stirrer was charged with 1 N aqueous H_2SO_4 (3.5 mL), crude diamine (245.0 mg), and reagent **A** (4.52 mmol from 1 M solution). The pH of the reaction mixture was then adjusted to 3 with additional 1 N aqueous H_2SO_4 (3.5 mL). The mixture was heated to 100 °C and stirred at that temperature for 10 h. The resulting mixture was cooled to ambient temperature and filtered to give an off-white solid, which was washed with water, 1 N ammonium hydroxide, water, EtOAc, and MeOH in succession to afford **15** (125 mg, 29% yield) as an off-white solid. Purity, method A 98%; method B 98%. ^{1}H NMR (500 MHz, CD_3OD): δ 8.86 (d, $J = 2.0$ Hz, 2H), 8.69 (m, 1H), 8.52 (d, $J = 5.0$ Hz, 1H), 8.16 (m, 2H), 7.79 (m, 2H), 7.56 (m, 1H), 7.50 (m, 1H), 3.55 (m, 2H), 1.23, (t, $J = 7.2$ Hz, 3H). MS, *m/z*: 377 $(M + H)^+$.

2-(3-Fluoropyridin-2-yl)-6-nitro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (70). To a solution of compound **69** (430 mg, 1.4 mmol) in dioxane (8 mL) was added bis(pinacoloto)diboron (462 mg, 1.82 mmol), KOAc (274 mg, 2.8 mmol), and $PdCl_2(dppf)_4$ (0.1 equiv). The resulting mixture was heated at 160 °C (microwave) for 12 min. The crude reaction was concentrated in vacuo and the resulting residue was purified by column chromatography on silica gel (1:1 to 0:1 hexanes/EtOAc gradient) to afford **70** (360 mg, 71% yield) as a yellow solid, which was directly used for the next step. MS, *m/z*: 360 $(M + H)^+$.

4-(Benzyloxy)-1-methylpyridin-2(1*H*)-one (73). To a 500 mL Parr bottle with Teflon cap was added **72** (8.04 g, 0.04 mol) which was partially dissolved in 50 mL of acetone. Then 11.6 g of potassium carbonate (0.082 mol) was added followed by 3.0 mL of iodomethane (0.048 mol) and the mixture was sealed and heated to 50 °C for 16 h. After the mixture was cooled to ambient temperature, the solids were filtered, washed twice with acetone, and the filtrate was treated with 1 g of activated charcoal. After filtration through a 1 in. plug of Celite and after being washed with acetone, the resulting residue was purified by column chromatography on silica gel (1:1 to 0:1 hexanes/EtOAc gradient) to provide **73** (7.06 g, 82%) as an off-white solid. IR (cm^{-1}): C═O (1652), C(O)═N (1592), CH_3 (1378), C−O (1226, 1210). ^{1}H NMR (500 MHz, acetone-d_6): δ 7.4−7.5 (m, 3H), 7.35 (t, 2H), 7.3 (m, 1H), 5.9 (d, 1H), 5.85 (s, 1H), 3.3 (s, 3H).

4-Hydroxy-1-methylpyridin-2(1*H*)-one (74). In a 500 mL Parr bottle, 6.52 g of **73** (0.03 mol) was dissolved in 100 mL of methanol. After charging with 0.5 g of 5% Pd/C, the mixture was pressurized to 48 psi with hydrogen and shaken for 1.25 h, at which time no further hydrogen uptake occurred. Filtration through Celite and concentration gave **74** (2.75 g, 73% yield) as a white solid. MS, *m/z*: 126 $(M + H)^+$; 124 $(M - H)^-$.

1-Methyl-2-oxo-1,2-dihydropyridin-4-yl Trifluoromethanesulfonate (75). A partial solution of **74** (4.46 g, 0.036 mol) in a mixture of DMF (30 mL) and dichloromethane (30 mL) was stirred for 30 min before 6.0 mL of triethylamine was added all at once. Complete solution occurred rapidly. The resulting solution was treated with solid *N*-phenyl-bis(trifluoromethanesulfonimide) (13.99 g, 0.0392 mol) added in 3 portions over 5 min. Some warming of the flask was noted. The solution was stirred at ambient temperature for 3.5 h before dilution with EtOAc. The solution was washed thrice with water, brine and dried over $MgSO_4$. Filtration and concentration in vacuo gave an oil (~19 g) which was purified by column chromatography on silica gel (3:1 to 1:1 hexanes/EtOAc gradient) to provide **75** (9.0 g, 98% yield) as an oil. ^{1}H NMR (500 MHz, acetone-d_6): δ 7.9 (d, 1H), 6.4 (s, 1H), 6.3 (d, 1H), 3.5 (s, 3H). MS, *m/z*: 258 $(M + H)^+$; 255 $(M - H)^-$.

4-(4-Amino-3-(3-fluoropyridin-2-yl)-5-nitrophenyl)-1-methylpyridin-2(1*H*)-one (71b). To a solution of compound **75** (386 mg, 1.5 mmol) in DME (20 mL) was added boronate ester **70** (359 mg, 1.0 mmol), sodium carbonate (6 mmol from 2 M solution), LiCl (126 mg, 3 mmol), and tetrakis(triphenylphosphine)palladium (174 mg, 0.15mmol). The resulting mixture was heated at 90 °C for 4 h, then cooled to ambient temperature. The solids were collected, washed sequentially with hot water and then 1:1 hexanes/EtOAc, and dried to afford the biaryl product (200 mg, 59% yield) as a yellow solid. To a solution of this adduct (200 mg, 0.59 mmol) in ethyl acetate (20 mL) was added 10% palladium on carbon (catalytic amount). The resulting suspension was placed in a Parr hydrogenation apparatus under 40 psi of hydrogen gas while shaking at ambient temperature for 1 h. The catalyst was removed by filtration and the filtrate concentrated in vacuo to afford **71b** (130 mg, 0.42 mmol) as an off-white solid. MS, *m/z*: 311 $(M + H)^+$.

1-Ethyl-3-(7-(3-fluoropyridin-2-yl)-5-(1-methyl-2-oxo-1,2-dihydropyridin-4-yl)-1*H*-benzo[*d*]imidazol-2-yl)urea (21). To an aqueous solution of **71b** (130 mg, 0.42 mmol) in sulfuric acid (0.84 mL of 1 N solution) was added solid reagent **A** (113 mg, 0.84 mmol) and enough sulfuric acid to adjust the mixture to pH 3. The resulting mixture was heated at 100 °C for 8 h and then cooled to ambient temperature, resulting in a precipitate. The solids were collected via filtration and washed with water, dried in vacuo, further washed with EtOAc followed by 9:1 EtOAc/MeOH to afford compound **21** (100 mg, 59% yield) as a white solid. Purity, method A 98%; method B 98%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.72 (m, 1H), 8.43 (d, $J = 1.4$ Hz, 1H), 8.00 (d, $J = 1.4$ Hz, 1H), 7.91 (m, 1H), 7.88 (m, 1H), 7.60 (m, 1H), 6.99 (d, $J = 2$ Hz, 1H), 6.96 (dd, $J = 7.0$, 2.1 Hz, 1H), 3.36 (m, 2H), 3.72 (s, 3H), 1.24 (t, $J = 7.3$ Hz, 3H). MS, *m/z*: 407 $(M + H)^+$.

4-(Pyridin-3-yl)-2-nitroaniline (77). To a solution of **76** (4.8 g, 22 mmol) in DME (100 mL) was added pyridine-3-boronic acid 1,3-propanediol cyclic ester (4.0 g, 24 mmol), sodium bicarbonate (45 mmol from 1 M solution), and tetrakis(triphenylphosphine)-palladium (1.27 g, 1.1 mmol). The resulting mixture was heated at 90 °C for 8 h and then cooled to ambient temperature. The solids were collected, washed sequentially with water and then 19:1 hexanes/EtOAc, and dried to afford **77** (4.7 g, 100% yield) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$):δ 8.8 (d, 1H), 8.55 (m, 1H), 8.35 (d, 1H), 7.85 (dd, 1H), 7.65 (dd, 1H), 7.35 (m, 1H), 6.95 (d, 1H), 6.25 (broad s, 2H).

2-Bromo-6-nitro-4-pyridin-3-ylphenylamine (78). To a solution of **77** (1.3 g, 9 mmol) in acetic acid (25 mL) was added bromine (1.58 g, 9.9 mmol) as a solution in acetic acid (5 mL). The resulting mixture was stirred at ambient temperature for 1 h and then poured into ice. The resulting solids were collected via filtration and washed with water, then dissolved in EtOAc and sequentially washed with NaOH (2 N, 20 mL), water, and brine. The organic phase was then dried over $MgSO_4$, filtered, and concentrated in vacuo. The concentrate was purified by column chromatography on silica gel (1:1 hexanes/EtOAc)] to afford **78** (0.8 g, 30% yield) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.83 (d, 1H), 8.55 (m, 1H), 8.41 (d, 1H), 8.15 (d, 1H), 7.96 (m, 1H), 7.41 (m, 1H), 6.80 (broad s, 2H). MS, *m/z*: 294 $(M + H)^+$.

2-Nitro-6-pyridin-2-yl-4-pyridin-3-ylphenylamine (79a). A mixture of **78** (100 mg, 0.34 mmol), 2-pyridylznic bromide (2.05 mmol, from 0.5 M solution in THF) and tetrakis(triphenylphosphine)-palladium (40 mg, 0.034 mmol) in THF (10 mL) was heated at 100 °C for 18 h. The mixture was returned to ambient temperature and quenched with water. The product was extracted thrice with EtOAc, the combined organic extracts were then dried over Na_2SO_4, filtered, and concentrated in vacuo, and the residue was purified by column chromatography on silica gel (EtOAc) to provide **79a** (75 mg, 75% yield) as a yellow solid. MS, *m/z*: 293 $(M + H)^+$.

1-Ethyl-3-(7-pyridin-2-yl-5-pyridin-3-yl-1*H*-benzoimidazol-2-yl)urea (13). To a solution of **79a** (75 mg, 0.26 mmol) in ethyl acetate (20 mL) was added 10% Pd−C (50 mg). The resulting suspension was placed in a Parr hydrogenation apparatus under 40 psi of hydrogen gas while shaking at ambient temperature for 1 h. The catalyst was removed by filtration through Celite and the filtrate concentrated in vacuo to give the corresponding phenylenediamine (50 mg, 0.19 mmol) which was directly combined with solid reagent **A** (100 mg, 0.76 mmol) and sulfuric acid (0.76 mL, 1 N) in water (1 mL). The resulting mixture was treated with enough sulfuric acid to adjust the mixture to pH 3 and was heated at 100 °C for 8 h. The reaction mixture was then cooled to ambient temperature,

resulting in a precipitate. The solids were collected, washed with water, and dried in vacuo. The solids were then purified by column chromatography on silica gel (1:0 to 9:1 EtOAc/MeOH gradient) to afford **13** (27 mg, 29% yield) as a white solid. Purity, method A 95%; method B 95%. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.92 (d, J = 1.8 Hz, 1H), 8,80 (m, 1H), 8.52 (m, 1H), 8,30 (m, 1H), 8.21 (d, J = 8 Hz, 1H), 8.04 (s, 1 H), 7.94 (m, 1H), 7.75 (s, 1H), 7.56 (d, J = 7.9 Hz, 1H), 7.37 (m, 2H), 3.36 (q, 7.3 Hz, 2H), 1.24 (t, J = 7.3 Hz, 3H). (M + 1). MS, m/z: 359 $(M + H)^+$.

2-Nitro-6-pyridin-3-yl-4-pyridin-3-ylphenylamine (79b). To a solution of **78** (50 mg, 0.17 mmol) in DME (10 mL) was added pyridine-3-boronic acid 1,3-propanediol cyclic ester (42 mg, 0.25 mmol), potassium phosphate (2.1 g, 10 mmol), and tetrakis(triphenylphosphine)palladium (20 mg, 0.017 mmol). The resulting mixture was heated at 90 °C for 18 h, then cooled to ambient temperature. The reaction was quenched with water and extracted thrice with EtOAc. The combined organic extracts were then dried over Na_2SO_4, filtered, concentrated in vacuo and the residue was purified by silica gel chromatography eluting with ethyl acetate to afford **79b** (50 mg, 100% yield) as a yellow solid. MS, m/z: 293 $(M + H)^+$.

1-Ethyl-3-(7-pyridin-3-yl-5-pyridin-3-yl-1*H*-benzoimidazol-2-yl)urea (14). To a solution of **79b** (50 mg, 0.17 mmol) in ethyl acetate (20 mL) was added 10% Pd−C (catalytic amount). The resulting suspension was placed in a Parr hydrogenation apparatus under 40 psi of hydrogen gas while shaking at ambient temperature for one hour. The catalyst was removed by filtration and the filtrate concentrated in vacuo to afford the intermediate phenylelediamine (42 mg, 0.16 mmol). MS, m/z: $(M + H)^+$ 263. To a solution of above product (42 mg, 0.16 mmol) and sulfuric acid (0.76 mL, 1 N) in water (1 mL) was added solid reagent **A** (102 mg, 0.76 mmol). Enough sulfuric acid was added dropwise to achieve pH 3. The resulting mixture was heated at 100 °C for 8 h and then cooled to ambient temperature, resulting in a precipitate. The solids were collected, washed with water, and dried in vacuo. The solids were purified by column chromatography on silica gel (1:0 to 9:1 EtOAc/MeOH gradient) to afford compound **14** (10 mg, 16% yield) as a white solid. Purity, method A 90%; method B 90%. ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.32 (s, 1H), 8.95 (d, J = 2.1 Hz, 1H), 8.53 (m, 3H), 8.12 (m, 1H), 7.74 (d, J = 2.1 Hz, 1H), 7.68 (s, 1H), 7.49 (m, 2H), 3.22 (m, 2H), 1.13 (t, J = 7.2 Hz, 3H). MS, m/z: 359 $(M + H)^+$.

1-Bromo-3,5-difluoro-2-nitrobenzene (81). To an ambient temperature, heterogeneous mixture of **80** (6.0 mL, 52.1 mmol) in neat sulfuric acid (10 mL) was added fuming nitric acid (2.5 mL, 62.5 mmol) dropwise over a 1 h period. The resulting mixture was stirred for an additional hour, resulting in a precipitate that progressively inhibited stirring. The resulting solids were broken up with vigorous stirring and the mixture was then poured onto ice, producing a sticky solid. The mixture was allowed to warm to ambient temperature, diluted with hexanes/ethyl acetate (1:2 v/v), and stirred until all solids dissolved. The organic phase was washed with brine, dried over Na_2SO_4, and filtered through a short pad of silica gel with 1:1 hexanes/ethyl acetate. The filtrate was then concentrated in vacuo at 0 °C until solids formed providing **81** (11.2 Grams, 90% yield) as a light-yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.92 (ddd, 1H), 7.88 (ddd, 1H).

***N*-*tert*-Butyl-3-bromo-5-fluoro-2-nitrobenzenamine (82).** To a solution of **81** (3.0 g, 12.61 mmol) in dioxane (10 mL) was added *tert*-butylamine (3.3 mL, 31.56 mmol), and the mixture was sealed in a high-pressure tube. The mixture was then heated to 65 °C for 5 h and then returned to ambient temperature and pressure. The mixture was then diluted with water and 1:1 hexanes/EtOAc and stirred until all solids had dissolved. The resulting phases were then separated, the organic phase was then washed with brine, dried over Na_2SO_4, filtered through a short pad of silica gel with 1:1 hexanes/EtOAc, and concentrated in vacuo. The crude product was then purified by column chromatography on silica gel (3:1 to 2:1 hexanes/CH_2Cl_2 gradient) to provide **82** (2.95 g, 81% yield) as an orange oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ 6.98 (dd, 1H), 6.93 (dd, 1H), 5.81 (s, 1H), 1.35 (s, 9H).

***N*-*tert*-Butyl-5-fluoro-2-nitro-3-(pyridin-2-yl)benzenamine (83).** To an ambient temperature solution of **82** (1.0 g, 3.44 mmol) in DMF (5 mL) was added $Pd(PPh_3)_4$ (200 mg, 0.172 mmol) and then 2-pyridylzinc bromide (5.15 mmol as 0.5 M solution in THF). The resulting mixture was heated to reflux for 8 h and then returned to ambient temperature. The crude reaction was then diluted with ethyl acetate and saturated $NaHCO_3$, and the resulting slurry was vigorously stirred for 30 min. Celite was added, and stirring was continued for an additional 30 min. The heterogeneous mixture was filtered through a pad of fresh Celite, and the solids were washed sequentially with ethyl acetate, water, then ethyl acetate again. The combined filtrates were separated, and the organic phase was dried over Na_2SO_4, filtered through a short pad of silica gel with ethyl acetate, and concentrated in vacuo. The resulting crude product was then purified by column chromatography on silica gel (30:1 to 9:1 CH_2Cl_2/ethyl acetate slow gradient) to provide **83** (600 mg, 60% yield) as an orange oil. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.52 (d, 1H), 7.91 (ddd, 1H), 7.61 (dd, 1H), 7.40 (ddd, 1H), 7.01 (dd, 1H), 6.72 (dd, 1H), 6.33 (s, 1H), 1.40 (s, 9H).

1-(3-(*tert*-Butylamino)-4-nitro-5-(pyridin-2-yl)phenyl)-*N*-cyclopropyl-1*H*-imidazole-4-carboxamide (84). To a solution of **83** (300 mg, 1.03 mmol) and **87** (190 mg, 1.24 mmol) in DMF (3 mL) was added solid Na_2CO_3 (130 mg. 1.24 mmol) at ambient temperature. The resulting mixture was heated to 120 °C for 8 h and then returned to ambient temperature. The crude mixture was diluted with water (20 mL), the resulting heterogeneous mixture was stirred for 20 min, and the solids were then filtered. The solids were washed with copious amounts of water and concentrated in vacuo to provide **84** (400 mg, 92% yield) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.57 (d. 1H), 8.48 (s, 1H), 8.40 (s, 1H), 8.12 (d, 1H), 7.96 (dd, 1H), 7.82 (d, 1H), 7.41 (dd, 1H), 7.22 (d, 1H), 7.21 (d, 1H), 6.20 (s, 1H), 2.85 (m, 1H), 1.48 (s, 9H), 0.65 (m, 2H), 0.61 (m, 2H).

1-(3-Amino-4-nitro-5-(pyridin-2-yl)phenyl)-*N*-cyclopropyl-1*H*-imidazole-4-carboxamide (85). A solution of **84** (400 mg, 0.95 mmol) in MeOH (20 mL) and 2 N HCl (5 mL, 10 mmol) was heated to reflux for 2 h and then returned to ambient temperature. The resulting mixture was then concentrated in vacuo and diluted with water (50 mL) and treated with enough solid $NaHCO_3$ to raise the pH to ~7.0. The resulting heterogeneous mixture was then stirred for 30 min and filtered. The solids were washed with copious amounts of water and concentrated in vacuo to provide **85** (312 mg, 90% yield) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.54 (d, 1H), 8.40 (s, 1H), 8.23 (s, 1H), 8.12 (d, 1H), 7.95 (dd, 1H), 7.80 (d, 1H), 7.41 (dd, 1H), 7.28 (d, 1H), 7.15 (d, 1H), 6.51 (s, 2H), 2.82 (m, 1H), 0.66 (m, 2H), 0.61 (m, 2H).

***N*-Cyclopropyl-1*H*-imidazole-4-carboxamide (87).** A mixture of **86** (5.0 g, 26.6 mmol) and cyclopropylamine (3.9 mL, 55.8 mmol) in dioxane was heated to 80 °C in a sealed tube for 4 h. The mixture was cooled to ambient temperature and concentrated in vacuo, providing a sticky solid. The crude product was azeotroped thrice with ethanol and the resulting solids were triturated with toluene, filtered, and dried under high vacuum to afford **87** (7.2 Grams, 90% yield) as an off-white solid. ^{1}H NMR (500 MHz, MeOH-d_4): δ 7.7 (s, 1H), 7.6 (s, 1H), 2.8 (m, 1H) 0.8 (m, 2H) 0.6 (m, 2H).

1-(6-(4-(Cyclopropylcarbamoyl)-1*H*-imidazol-1-yl)-4-(pyridin-2-yl)-1*H*-benzo[*d*]imidazol-2-yl)-3-ethylurea (20). To a partial suspension of **85** (100 mg, 0.275 mmol) in MeOH (20 mL) was added Raney Ni (~0.2 mL of slurry) in a Parr bottle. The mixture was exposed to 45 psi of H_2 for 2 h with shaking and then purged of excess H_2. The resulting black solution was filtered through a 0.5 μm polycarbonate membrane, and the solids were washed with MeOH. The filtrate was concentrated in vacuo, and the resulting waxy solid was diluted with dioxane (2 mL) and pH 3.5 buffer (5 mL), treated with reagent **B** (83 mg, 0.358 mmol), and heated to reflux for 6 h. The crude mixture was then cooled to ambient temperature, diluted with 1 M $NaHCO_3$ (20 mL), and the heterogeneous mixture was stirred for 20 min. The solids were then collected via filtration, washed sequentially with water and 9:1 EtOH/water, then dried in vacuo to provide **20** (62 mg, 52% yield) as an off-white solid. Purity, method A 91%; method B 91%. ^{1}H

NMR (500 MHz, MeOH-d_4): δ 8.96 (s, 1H), 8.89 (d, $J = 5.1$ Hz, 1H), 8.47 (d, $J = 8.2$, 1H), 8.37 (m, 2H), 8.20 (ddd, $J = 7.8$, 7.8, 2.3 Hz, 1H), 7.93 (s, 1H), 7.63 (dd, $J = 7.0$, 6.6 Hz, 1H), 3.37 (q, $J = 7.1$ Hz, 1H), 2.88 (m, 1H), 1.24 (t, $J = 6.3$ Hz, 3H), 0.87 (m, 2H), 0.68 (m, 2H). MS, *m/z*: 431 $(M + H)^+$; 429 $(M - H)^-$.

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

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.

Computational Methods. Docking calculations were performed with the ICM program (Molsoft LLC).[39] Compounds **1** and **2** were docked using unconstrained docking with the docking region defined by the crystallographic position of novobiocin. Subsequent docking calculations employed harmonic constraints applied to the benzimidazole urea portion of the molecules to the crystallographic position of related molecules.[30] The docking and postprocessing were performed according to previous methods.[40] Final pose selection was determined on the basis of both nonbond interaction energies and strain energies relative to the closest local minimum, as well as visual inspection. All quantum mechanical calculations described were performed with Gaussian 98 at the HF/6-31G* level of theory.[32]

In Vivo Efficacy. Description of the Pneumococcal Pneumonia Model. Briefly, *S. pneumoniae* strain ATCC 6303, serotype 3, was cultured in enriched broth overnight and the concentration of the bacterial suspension adjusted to 4.51×10^7 CFU/mL. The MICs of compound **15** and linezolid against this strain of *S. pneumoniae* were 0.008 and 0.4 μg/mL, respectively. On day 0, male Sprague−Dawley rats (185−224 g) were anesthetized and inoculated with 0.2 mL of bacterial suspension (0.9×10^7 CFU) by intratracheal administration. At 18 h postinfection, the rats were randomly assigned into treatment groups (10 per group). Compound **15** was formulated in 5% dextrose in water (D5W) for oral administration. All the compounds were administered twice, at 18 and 26 h postinfection (q8h×2). At 72 h after infection, all animals were sacrificed and the lungs were removed and homogenized for CFU determination. The CFU data are expressed as mean $\log_{10}$ CFU ± SEM; lung tissue CFU $\log_{10}$ reductions were analyzed by a one-way analysis of variance (ANOVA) to detect the overall compound effect, followed by Dunnett's pairwise comparison between groups. Undetectable CFU levels in the raw data were assigned a $\log_{10}$ value of 0.90 by convention.

Description of the Thigh Infection Model. Male Sprague−Dawley rats (175−200 g) were used in the study. Neutropenia was induced by intraperitoneal injections of cyclophosphamide given twice prior to infection (150 mg/kg, at 4 days prior to infection, and 50 mg/kg, at 1 day prior to infection). The thigh infections were generated by an intramuscular injection of a 0.2 mL suspension of 1×10^7 CFU/mL of *S. aureus* ATCC 29213 in normal saline. The MICs of compound **15** and linezolid against this strain of *S. aureus* were 0.031 and 4.0 μg/mL, respectively. A single intravenous bolus of compound **15** or linezolid (50 mg/kg) or D5W (vehicle control) was administered 2 h postinfection via the tail vein. Thigh tissues were harvested and homogenized 6 h after dosing (8 h postinfection) for CFU determination. The 0 h control samples were collected 2 h after infection, prior to dosing. Data are presented as the mean $\log_{10}$ CFU ± SEM. A one-way ANOVA test was used to detect the overall compound effect, followed by Dunnett's pairwise comparison between vehicle and treatment groups.

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Supporting Information Available: LC/MS data for compounds **2**−**21**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Barrett, C. T.; Barrett, J. F. Antibacterials: are the new entries enough to deal with the emerging resistance problems? *Curr. Opin. Biotechnol.* **2003**, *14*, 621–626.
(2) Jacobs, M. R. Worldwide trends in antimicrobial resistance among common respiratory tract pathogens. *Pediatr. Infect. Dis. J.* **2003**, *22*, S109−19.
(3) Livermore, D. M. The need for new antibiotics. *Clin. Microbiol. Infect.* **2004**, *10* (Suppl. 4), 1–9.
(4) Aspa, J.; Rajas, O.; Rodriguez de Castro, F.; Blanquer, J.; Zalacain, R.; Fenoll, A.; de Celis, R.; Vargas, A.; Rodriguez Salvanes, F.; Espana, P. P.; Rello, J.; Torres, A. Drug-resistant *pneumococcal* pneumonia: clinical relevance and related factors. *Clin. Infect. Dis.* **2004**, *38*, 787–798.
(5) Pottumarthy, S.; Fritsche, T. R.; Jones, R. N. Comparitive activity of oral and parenteral cephalosporins tested against multidrug-resistant *Streptococcus pneumoniae*: report from the SENTRY Antimicrobial Surveillance Program (1997−2003). *Diagn. Microbiol. Infect. Dis.* **2005**, *51*, 147–150.
(6) National Nosocomial Infections Surveillance (NNIS) System Report (data summary from January 1992 through June 2004, issued October 2004). http://www.cdc.gov/ncidod/dhqp/pdf/nnis/2004NNISreport.pdf.
(7) Meyer, A. L. Prospects and challenges of developing new agents for tough Gram-negatives. *Curr. Opin. Pharmacol.* **2005**, *5* (5), 490–494.
(8) Alanis, A. J. Resistance to antibiotics: are we in the post-antibiotic era? *Arch. Med. Res.* **2005**, *36* (6), 697–705.
(9) Goossens, H.; Ferech, M.; Coenen, S.; Stephens, P. Comparison of outpatient systemic antibacterial use in 2004 in the United States and 27 European countries. *Clin. Infect. Dis.* **2007**, *44*, 1091–1095.
(10) Thomson, C. J.; Power, E.; Ruebsamen-Waigmann, H.; Labischinski, H. Antibacterial research and development in the 21st century—an industry perspective of the challenges. *Curr. Opin. Microbiol.* **2004**, *7*, 445–450.
(11) Maxwell, A. DNA gyrase as a drug target. *Trends Microbiol.* **1997**, *5*, 102–109.
(12) Drlica, K.; Zhao, X. DNA gyrase, topoisomerse IV and the 4-quinolones. *Microbiol. Mol. Biol. Rev.* **1997**, *61*, 377–392.
(13) Heisig, P. Inhibitors of bacterial topoisomerases: mechanisms of action and resistance and clinical aspects. *Planta Med.* **2001**, *67*, 3–12.
(14) Kato, J.-I.; Nishimura, Y.; Imamura, R.; Niki, H.; Hiraga, S.; Suzuki, H. New topoisomerases essential for chromosome segregation in *E. coli*. *Cell* **1990**, *63*, 393–404.
(15) Deibler, R. W.; Rahmati, S.; Zechiedrich, E. L. Topoisomerase IV, alone, unknots DNA in *E. coli*. *Genes Dev.* **2001**, *15*, 748–761.
(16) Zechiedrich, E. L.; Khodrusky, A. B.; Bachellier, S.; Schneider, R.; Chen, D.; Lilley, D. M. J.; Cozzarelli, N. R. Roles of topoisomerases in maintaining steady-state DNA supercoiling in *Escherichia coli*. *J. Biol. Chem.* **2000**, *11*, 8103–8113.
(17) Champoux, J. J. DNA topoisomerases: structure, function and mechanism. *Annu. Rev. Biochem.* **2001**, *70*, 369–413.
(18) Maxwell, A.; Lawson, D. M. The ATP-binding site of type II topoisomerases as a target for antibacterial drugs. *Curr. Top. Med. Chem.* **2003**, *3*, 283–303.
(19) Ince, D.; Zhang, X.; Silver, L. C.; Hooper, D. C. Topoisomerase targeting with and resistance to gemifloxacin in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **2003**, *47*, 274–282.
(20) Strahilevitz, J.; Hooper, D. C. Dual-targeting of topoisomerase IV and gyrase to reduce mutant selection: direct testing of the paradigm by using WCK-1734, a new fluoroquinolone, and ciprofloxacin. *Antimicrob. Agents Chemother.* **2005**, *49*, 1949–1956.
(21) Canu, A.; Leclercq, R. Overcoming bacterial resistance by dual target inhibition: the case of streptoGramins. *Current Drug Targets: Infect. Disord.* **2001**, *1* (2), 215–225.
(22) Estimated from the average mutation rate (where one can detect a phenotype, such as resistance) of 10^{-6} to 10^{-7} for any gene in a given organism. Thus, it would require a bacterial population of approximately 10^{14} for simultaneous mutations to occur in two different gene products.
(23) Mani, N.; Gross, C. H.; Parsons, J. D.; Hanzelka, B.; Müh, U.; Mullin, S.; Liao, Y.; Grillot, A.-L.; Stamos, D.; Charifson, P. S.; Grossman,

T. H. In vitro characterization of the antibacterial spectrum of novel bacterial type II topoisomerase inhibitors of the aminobenzimidazole class. *Antimicrob. Agents Chemother.* **2006**, *50* (4), 1228–1237.
(24) Grossman, T. H.; Bartels, D.; Mullin, S.; Gross, C. H.; Parsons, J. D.; Liao, Y.; Grillot, A.-L.; Stamos, D.; Olson, E.; Charifson, P. S.; Mani, N. Dual-targeting of GyrB and ParE by VRT-125856 and VRT-752586, representatives of a novel class of antibacterial compounds. *Antimicrob. Agents Chemother.* **2007**, *51* (2), 657–666.
(25) Bellon, S.; Parsons, J. D.; Wei, Y.; Hayakawa, K.; Swenson, L. L.; Charifson, P. S.; Lippke, J. A.; Aldape, R.; Gross, C. H. Crystal structures of *Escherichia coli* topoisomerase IV ParE subunit (24 and 43 kilodaltons): a single residue dictates differences in novobiocin potency against topoisomerase IV and DNA gyrase. *Antimicrob. Agents Chemother.* **2004**, *48* (5), 1856–1864.
(26) Gross, C. H.; Parsons, J. D.; Grossman, T. H.; Charifson, P. S.; Bellon, S.; Jernee, J.; Dwyer, M.; Chambers, S. P.; Markland, W.; Botfield, M.; Raybuck, S. A. Active-site residues of *Escherichia coli* DNA gyrase required in coupling ATP hydrolysis to DNA supercoiling and amino acid substitutions leading to novobiocin resistance. *Antimicrob. Agents Chemother.* **2003**, *47* (3), 1037–1046.
(27) Walters, W. P.; Murcko, M. A. Library Filtering Systems and Predicition of Drug-like Properties. In *Virtual Screeing for Bioactive Molecules*; Böhm, H.-J., Schneider, G., Eds.; Wiley-VCH: Weinheim, Germany, 2000; pp 15−32.
(28) Holdgate, G. A.; Tunnicliffe, A.; Ward, W. H.; Weston, S. A.; Rosenbrock, G.; Barth, P. T.; Taylor, I. W.; Pauptit, R. A.; Timms, D. The entropic penalty of ordered water accounts for weaker binding of the antibiotic novobiocin to a resistant mutant of DNA gyrase: a thermodynamic and crystallographic study. *Biochemistry* **1997**, *36*, 9663–9673.
(29) Murcko, M. A.; Caron, P. R.; Charifson, P. S. Structure based drug design. *Annu. Rep. Med. Chem.* **1999**, *34*, 297–306.
(30) Wei, Y.; Bellon, S.; Hayakawa, K.; Swenson, L. L.; Lippke, J. A.; Grillot, A.-L.; Ronkin, S.; Stamos, D.; Charifson, P. S. Unpublished results.
(31) The torsion angles between the 3-pyridyl at the 5-position and the benzimidazole core were−38°,−37°, and−51° for 6-H, F, and Me, respectively.
(32) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Baboul, A. G.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.11.4; Gaussian, Inc.: Pittsburgh, PA, 1998.
(33) Brino, L.; Urzhumtsev, A.; Mousli, M.; Bronner, C.; Mitschler, A.; Oudet, P.; Moras, D. Dimerization of *Escherichia coli* DNA-gyrase B provides a structural mechanism for activating the ATPase catalytic center. *J. Biol. Chem.* **2000**, *275*, 9468–9475.
(34) Drumm, J. E.; Deininger, D.; LeTiran, A.; Wang, T.; Grillot, A-L.; Liao, Y.; Ronkin, S. M.; Stamos, D.; Tang, Q.; Tian, S.-K.; Oliver-Shaffer, P. Facile preparation of fused ring azolylureas. *Tetrahedron Lett.* **2007**, *48* (31), 5535–5538.
(35) Ishiyama, J.; Murata, M.; Miyaura, N. Palladium(0)-catalyzed cross-coupling reaction of alkoxydiboron with haloarenes: a direct procedure for arylboronic esters. *J. Org. Chem.* **1995**, *60* (23), 7508–7510.
(36) Smith, W. T.; Steinle, E. T. Some substituted benzimidazoles. *J. Am. Chem. Soc.* **1953**, *75*, 1292–1294.
(37) Kher, S. M.; Cai, S. X.; Weber, E.; Keana, J. F. W. Regiospecific oxidative nitration of 3,4-dihydro-6,7-disubstituted quinoxalin-2(1*H*)-ones gives 1,4-dihydro-5-nitro-6,7-disubstituted quinoxaline-2,3-diones, potent antagonists at the NMDA/glycine site. *J. Org. Chem.* **1995**, *60*, 5838–5842.
(38) Kasina, S.; Nematollahi, J. New and simple synthesis of some 4-substituted imidazoles. *Synthesis* **1975**, *3*, 162–163.
(39) Totrov, M.; Abagyan, R. Flexible protein−ligand docking by global energy optimization in internal coordinates. *Proteins* **1997**, (Suppl. 1), 215–220. ICM is distributed by Moloft LLC.
(40) Perola, E.; Walters, W. P.; Charifson, P. S. A detailed comparison of current docking and scoring methods on systems of pharmaceutical relevance. *Proteins: Struct., Funct., Bioinf.* **2004**, *56* (2), 235–249.

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