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Design, synthesis, and testing of an 6-O-linked series of benzimidazole based inhibitors of CDK5/p25

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disease resulting in cognitive and behavioral impairment. The two classic pathological hallmarks of AD include extraneuronal deposition of amyloid β (Aβ) and intraneuronal formation of neurofibrillary tangles (NFTs). NFTs contain hyperphosphorylated tau. Tau is the major microtubule-associated protein in neurons and stabilizes microtubules (MTs). Cyclin dependent kinase 5 (CDK5), when activated by the regulatory binding protein p25, phosphorylates tau at a number of proline-directed serine/threonine residues, resulting in formation of phosphorylated tau as paired helical filaments (PHFs) then in subsequent deposition of PHFs as NFTs. Beginning with the structure of Roscovitine, a moderately selective CDK5 inhibitor, we sought to conduct structural modifications to increase inhibitory potency of CDK5 and increase selectivity over a similar enzyme, cyclin dependent kinase 2 (CDK2). The design, synthesis, and testing of a series of 1-isopropyl-4-aminobenzyl-6-ether-linked benzimidazoles is presented.

1. Background

Alzheimer's disease is a progressive neurodegenerative disease without a cure. It is estimated that 4.5 million Americans have Alzheimer's disease.[1] This is twice as many as in 1980, but less than the estimated 11 million persons whom are estimated to be afflicted by 2050. This increase in number represents a shift in age demographics where increased life-expectancy increases the number of persons afflicted with neurodegenerative diseases. A parallel increase in the cost of caring for persons with Alzheimer's disease is also anticipated. The average lifetime cost for an individual with Alzheimer's disease is currently $175,000[2] and these costs will only increase.

The pathological hallmarks for Alzheimer's disease have been known for some time.[3–8] Post-mortem examination of persons with Alzheimer's disease exhibit two types of aberrant proteins: amyloid β 42 (Aβ 42 and neurofibrillary tangles (NFT). The extra neuronal precipitation of Aβ was initially recognized by the birefringence induced by the amyloid-detecting histological stain congo red. Currently inhibiting the generation and deposition of Aβ represents a very hopeful target for therapy of Alzheimer's disease.[9–12] Specifically, inhibition of the processing enzymes β-secretase (BACE)[8,9,13–16] and γ-secretase[14,15,17–22] represent major current targets for potential therapeutic intervention. These hold the hope of reducing the biosynthesis of Aβ 42, preventing the deposition of Aβ plaques, and reducing associated toxicities. NFTs are intraneuronal depositions of insoluble proteins. The major component of these NFTs is the structural protein tau. Tau can be phosphorylated at multiple residues, but phosphorylation of Ser202 and/or Thr205 is the phospho-tau form that exists in NFT's. This hyperphosphorylated tau form is the major component of neurofibrillary tangles and is present in all confirmed cases of Alzheimer's disease. The origin this hyperphosphorylated tau is still a complex issue but a coherent theory is emerging.[23–27]

The current understanding of tau hyperphosphorylation is that the following residues are phosphorylated in PHF tau: Thr181, Ser199, Thr205, Thr121, Thr217, and Thr231. These phosphorylations can be accomplished by sequential action of PKA, CaMKII and then GSK-3β, or by CDK5 and then GSK-3β, or by CDK5 alone. Suggestions have been made to target multiple kinases, and several groups are currently pursuing these strategies. Additionally, there have been reports that initial priming of tau by CDK5 facilitates additional phosphorylation by GSK-3β.[28,29] CDK5 has also been implicated in phosphorylation of p53, activation of the ERK signaling pathway, MEF-2 activation, and peroxiredoxin[30–33] up-regulation. These later two results are particularly interesting as they could provide a link to other neurodegenerative diseases with aberrant tau phosphorylation. Additionally it appears that CDK5 mediated

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tau hyperphosphoryaltion may induce an increase of sAPP production and perhaps Aβ production.

There are increasing indications that tau can be hyperphosphorylated by CDK5/p25 selectively[5] in response to excitotoxic events[34] including Aβ,[35] and that increased soluble amyloid precursor protein (APP) synthesis (the peptide inappropriately directed toward Aβ 42 in pathologic states) may be a response to these events. Thus the development of selective CDK5/p25 inhibitors would contribute significantly to an understanding of the role of tau hyperphosphorylation in Alzheimer's disease and could provide early proof of concept for the therapeutic strategy of inhibiting CDK5/p25 to effect a disease-modifying treatment for neurodegenerative disease including Altzheimer's disease.

Cyclin-dependent kinases (CDKs) have been known for some time and are the focus of several drug-development programs.[36–43] The most actively researched CDK is CDK2.[40,44,45] CDK2 has been examined in the context of anti-cancer agents. All CDK's have several layers of intrinsic inhibition and require multiple interactions for activation. A PSTAIRE helix is found in the primary sequence of all CDKs and is inserted between two beta-sheets domains of the CDK. Displacement of this helix exposes the ATP binding site.[46–48] Displacement of this helix is effected by the obligate cyclin; cyclin binding repositions the PSTAIRE helix to an interface between the cyclin and the CDK permitting both ATP binding and then substrate binding. In many cases there may be several cyclins capable of activating a given CDK. Different cyclins may confer different substrate specificities upon the CDKs. This implies a different induced active site unique to each dimeric complex. Additional phosphorylation of a Thr residue of an extended 'T loop' may also be required.

CDK5 has a PSSALRE sequence for the 'PSTAIRE' motif,[48] this provided the basis for its initial identification as a CDK. CDK5 is distinct in several ways from other members of the CDK family. First the tissue distribution is mostly localized to the brain and the testis (in rats).[48–51] Second, CDK5 does not require phosphorylation of the 'T loop' (specifically Ser159) to generate an active enzymatic complex.[48] In fact it has been shown that phosphorylation of this residue prevents effector cyclins from binding. In the case of CDK5, hydrophobic and ionic interactions assemble the cyclin/CDK complex. CDK5 binds to and is activated by cyclin D, a membrane bound protein p35, or by a 208 residue C-terminal proteolytic product of p35:p25. The complex CDK5/p25 has consistently been shown to hyperphosphorylate tau protein and produce cell death.[52–55] Administration of CIP, a 125-residue portion of p35, was shown to selectively inhibit CDK5, reduce tau phosphorylation, and protect against Aβ induced apoptosis. Tau replacement strategies with the tubulin stabilizer paclitaxel also reduced Aβ induced apoptotic cell death mediated by tau hyperphosphorylation.[7,56,57] This raises the possibility that CDK5 mediated tau hyperphosphorylation causes both NFT formation and the loss of cytoskeletal integrity that facilitates Aβ mediated apoptotic cell death. With the capability to clone and express CDK5 it was found that CDK5/p25 but not the CDK5/p35 complex[35,53–55,58–60] is capable of hyperphosphorylating tau, specifically at the Ser202 and Thr205 residues.[5]

There are several X-ray crystal structures of CDK5 published and deposited into the PDB. The first X-ray crystal structure was obtained and deposited by Tarricone.[48] This confirmed the phosphorylation state of Ser159 and established the nature of the CDK/p25 interaction. The remaining structures by Mappelli[47] are highly similar with respect to the protein domains, but have different inhibitors diffused into the ATP binding pocket. These compounds, (R) roscovitine **1**, aloisine A **2**, and indirubin 3′-oxime **3**, were obtained from other CDK SAR studies and inhibit other CDKs and other kinases as well. R-roscovitine (Figs. 1 and 2) was

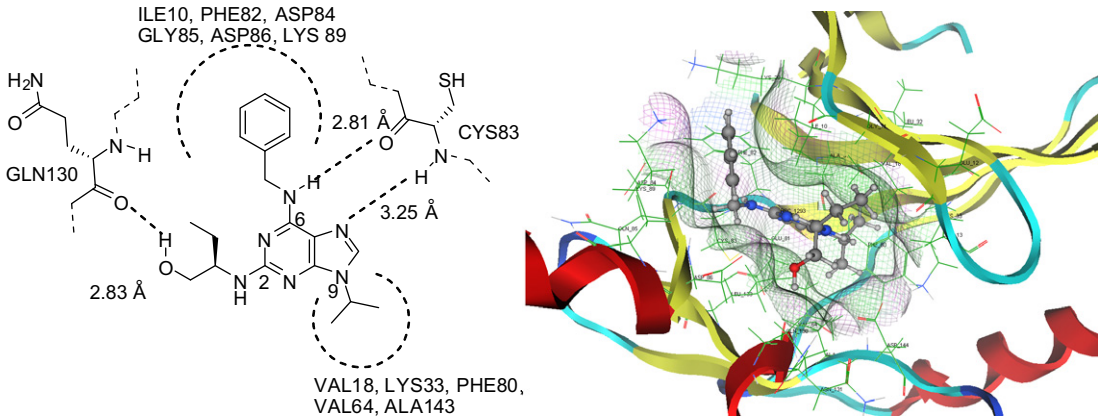


Figure 1. Interactions between roscovitine **1** and CDK5[47] (from PDB: 1UNL).

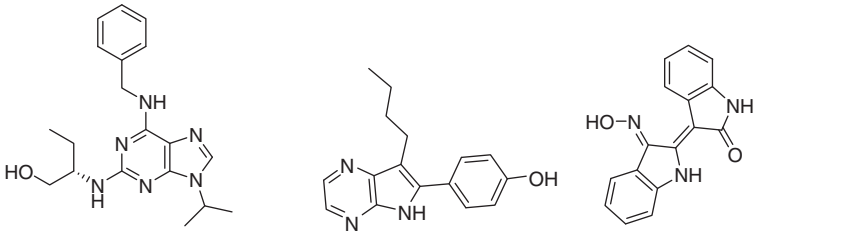


Figure 2. Known inhibitors that have been co-crystallized in CDK5/p25.[47]

originally developed by analog synthesis on the adenine core for CDK2 inhibition[39,47] and was found to bind deeply into the ATP binding site of CDK5 and inhibited CDK5/p25 with an IC_{50} of 160 nM. Further kinetic experiments with several CDK5/p25 inhibitors identified different modes of enzyme inhibition[46] for compounds **2** and **3**.

Several research groups are currently working on CDK5 inhibitors.[61,62] The sources for many of these compounds are prior projects exploring ATP-binding kinases. The following centroids have been used for the development of CDK5 inhibitors: pyrrolopyrimidines, benzthiazoles, indazoles, aminoimidazoles, aminothiazoles, and paullinones.[42] Many of these compounds were prepared in the context of CDK2 inhibitors and were likely identified via high-throughput screens detecting CDK5 inhibition. We sought to systematically examine the benzimidazole scaffold, a known adenine mimic, but one that is underutilized both in medicinal chemistry[63–66] and in CDK inhibition[67,68] specifically.

2. Molecular modeling

(R)-Roscovitine is a moderately active inhibitor of CDK5 (IC_{50} 160 nM). Although it was first designed and developed as a CDK2A inhibitor (IC_{50} 700 nM[40]), roscovitine has displays preferential inhibition for CDK5/p25 over other CDKs.[38,40] Additionally compounds **2** and **3**, although they have been co-crystalized with CDK5,[47] yet exhibit preferential inhibition of CDK2. Our goal was to explore the utility of the benzimidazole core as a purine mimic using molecular modeling and analysis of the published crystal structure of (R)-roscovitine-CDK5/p25 (PDB ID: 1UNL) (Fig. 1)[47] as a starting point. Based on 1UNL, we proposed that the most significant bonding pattern between CDK5 and roscovitine is from the purine N7 (H-bond acceptor) and the exocylic NH at C6 of roscovitine (H-bond donor) to the amide NH and the backbone amide carbonyl of Cys83, respectively. Purine nitrogens N1, N3 and the exocyclic NH at C2 are not within hydrogen bonding distance of any polar functionality on CDK5. In addition, the floor of the purine-binding region is derived from Leu133 and the top of the purine-binding region is derived from Ile10. This suggests that replacement of the purine of roscovitine with a benzimidazole and the exocylic 2-NH at C2 on the purine with an ether linkage (Fig. 3) would not only retain the key H-bonding patterns but also add hydrophobic interaction with Ile10 and Leu133. This strategy could increase selectivity as the exocylic NH at C2 of roscovitine forms an important H-bond with Glu131 of CDK2. This sort of ether substitution has been extensively employed in the exploration of receptor tyrosine kinases.[69,70] The polar and non-polar ribose binding pockets can be further explored with various substitutions from the C6 position of the benzimidazole core.

The molecular modeling was conducted in a manner parallel to prior studies.[71,72] The PDB file for 1UNL was uploaded, protons and valencies were examined. The structure of roscovitine was both directly modified to the benzimidazole core or was computer docked with the automated docking protocol MOE-dock.[73] Amber 98 was used as the forcefield for protein calculations, MMFF94 was used as the forcefield for small molecule calculations. Complementary results were obtained for both docking strategies. Two strategies were utilized for analysis of the inhibitor–protein complex. Scoring using a principle-components method,[71,72] or using the standard MOE evaluation that generated a calculated pK_i, with the MMFF94 forcefield using triangulation for the final fit. Selected compounds are presented in Table 1, and the values for this second scoring function strategy are presented in Table 2.

The proposed compounds retained the isopropyl group found at the 9-N atom of roscovitine but are designated 1-N on the benzimidazole core based CDK5 inhibitors. The ribose/triphosphate binding pocket for CDK5 is the first area where we would like to explore structural variations. As such, compounds **4–8** examined point variations on the alkoxy chain resulting from movement of the ethyl chain, deletion of the ethyl chain, or capping the hydroxyl group with a methoxy group. Compounds **9–11** examine addition of an ammonium group in the proximity of Asp86. This is precedented by the work of Toogood.[74–76] He was able to optimize inhibitor activity at CDK4 by reaching across from the purine-binding region to the magnesium ion binding site on the cusp of the ribose binding pocket. Additionally this strategy anchored the hydrogen-bonding motif that mimics the purine core with a bulky aliphatic group (cyclopentyl); this is analogous to our incorporation of the 1-N isopropyl group. Compounds **12–15** explore the possibility of including an alkyl or alkoxy side chain to access a hydrophobic binding region proximal to the roscovitine binding region and uniquely utilized by the butyl chain of aloisine A as identified in its CDK5 cocrystal structure.[47]

3. Chemical discussion

To prepare the desired functionalization at the 1, 4, and 6 positions on the benzimidazole core (Table 1), synthesis began with the previously described 1-isopropyl-4-nitro-6-methoxybenzimidazole

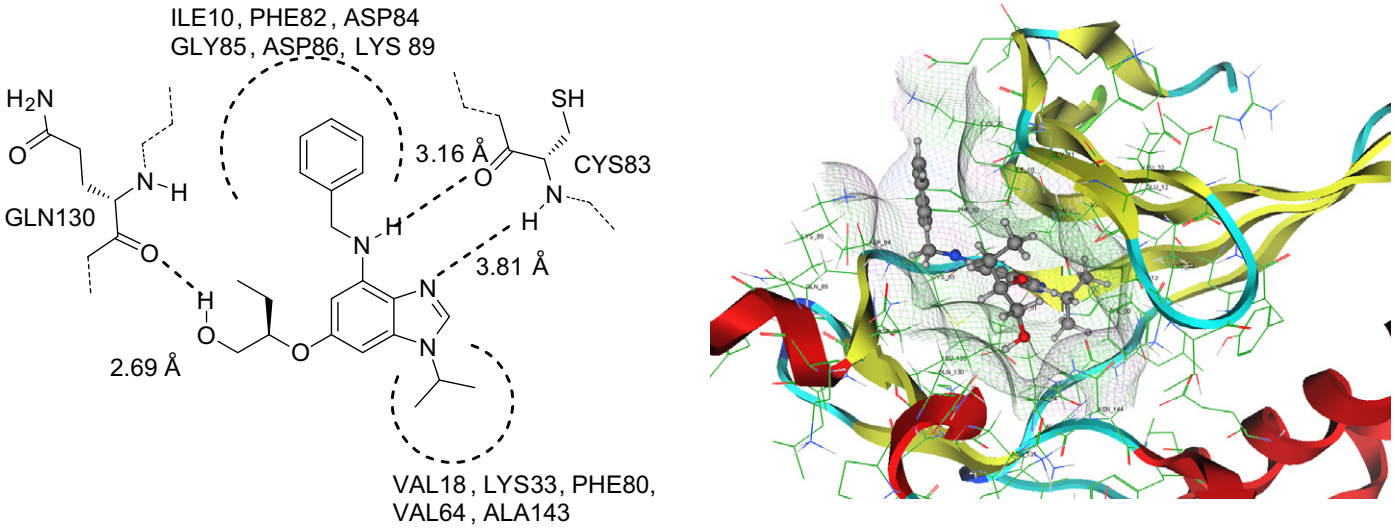
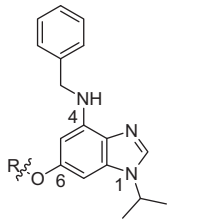


Figure 3. Interactions between benzimidazole analog **4** and CDK5 (derived from PDB: 1UNL[47]).

Table 1
Proposed compounds



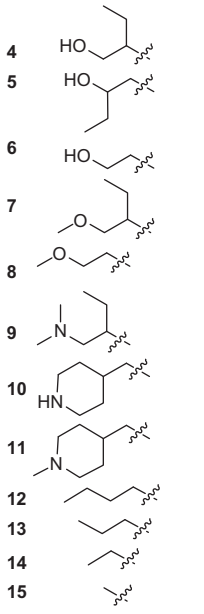


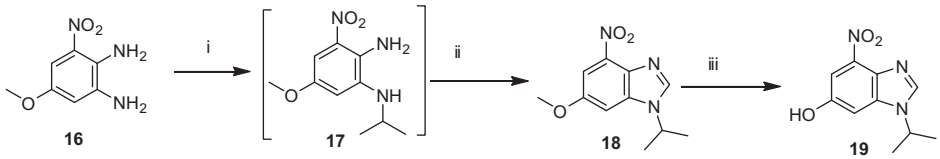
Table 2
Scoring of proposed compounds

Compound	Calcd pK_i	Proposed side chain interactions
4	7.783	H-bonding with Gln130 and Asp144
5	7.690	Gln130
6	7.624	H-bonding with Asp144
7	7.892	Hydrophobic interactions with Gln130 backbone
8	7.385	Hydrophobic interactions with glycine-rich loop
9	9.436	Ionic interaction with Asp144
10	9.422	Ionic interaction with Asp144
11	9.753	Ionic interaction with Asp144
12	7.839	Hydrophobic interactions with glycine-rich loop
13	7.310	Hydrophobic interactions with glycine-rich loop
14	7.166	Hydrophobic interactions with Gln130 backbone
15	7.065	Hydrophobic interactions with Gln130 backbone
1 roscovitine	7.971	H-bonding with Gln130 amide

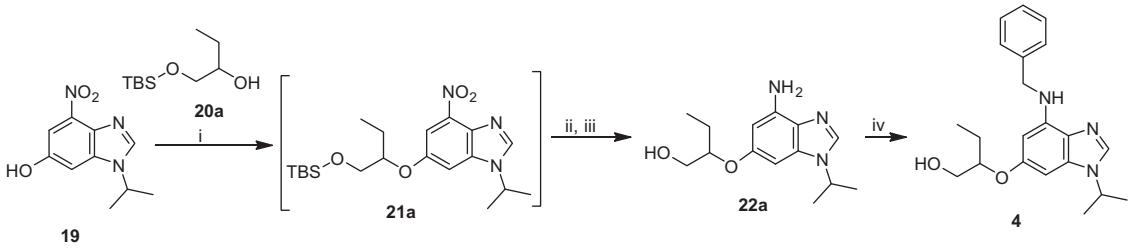
18.[77] The key feature in the preparation of this compound was regioselective alkylation on the 1-amine of **16** prior to cyclization. This was affected by reductive alkylation with acetone on the more nucleophilic amine which appears *meta* to the electron-withdrawing nitro group. The initial synthesis[77] utilized sodium triformylborohydride generated in situ from sodium borohydride and formic acid to effect the reductive alkylation with acetone then crude **17** was cyclized to the corresponding benzimidazole **18**. Subsequently it was found that the more traditional reductive alkylation reagent sodium triacetoxyborohydride could be employed. This improved procedure shown in Scheme 1 is presented in the Section 6. The advantages of this second method are the commercial availability and improved stability of sodium triacetoxyborohydride. Sodium triformylborohydride was stable as a 1 M solution in THF at 0 °C, but not at 23 °C. The possible competition of acetate versus formate in the subsequent Philips cyclization was examined in parallel experiments: (1) reductive alkylation with sodium triformylborohydride then cyclization with a formic acid HCl mixture, or (2) reductive alkylation with sodium triacetoxyborohydride then cyclization with an acetic acid and HCl mixture, or finally (3) reductive alkylation with sodium triacetoxyborohydride then cyclization with a formic acid HCl mixture. From these studies it was found that the relative rate of cyclization with acetic acid was approximately 100 times slower versus formic acid. No 2-methylbenzamidazole product was found in the collected product when the sequence of sodium triacetoxyborohydride mediated reductive alkylation followed by formic acid HCl Philips cyclization was employed. A second improvement was the utilization of BHT. Initial Philips cyclization on dimine **17** required careful exclusion of oxygen to reduce oxidative polymerization, but use of catalytic BHT dramatically increased yield, increased purity, and stopped the presumably radical mediated oxidative polymerization.

Hydrobromic acid catalyzed deprotection of the methyl phenyl ether **18** afforded the corresponding phenol **19** in 93% yield under microwave conditions. Other deprotective systems were examined including conventional heating with HBr, BBr_3, or BBr_3 with anisole. These conditions were all lower yielding.

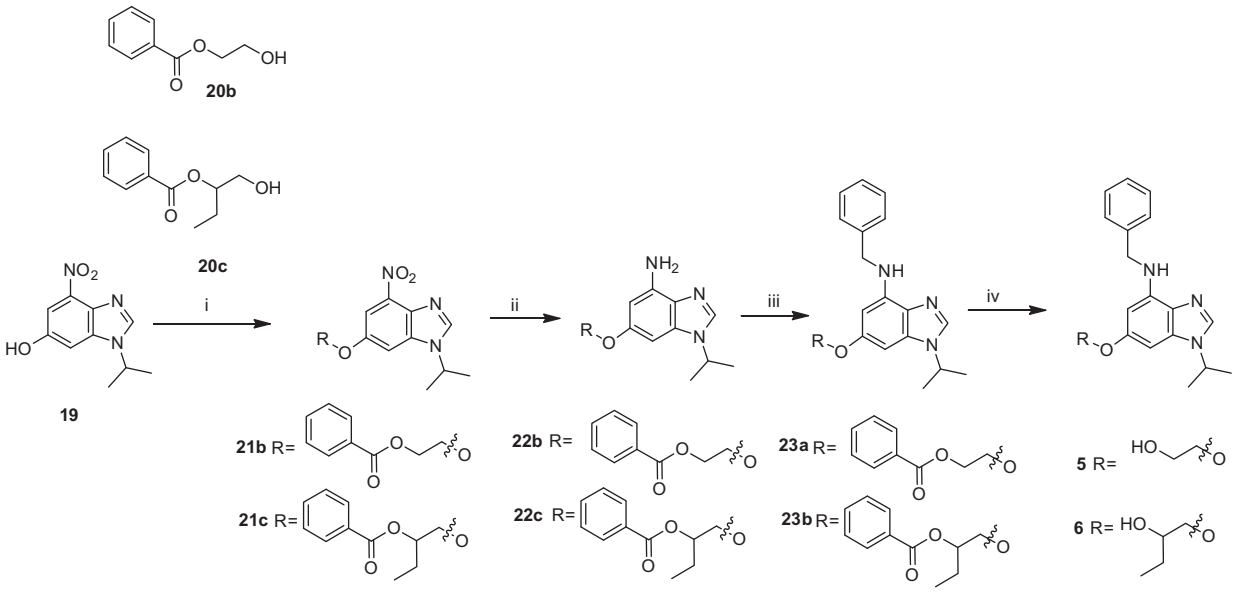
Alkylation of the 6-phenol of **19** was achieved via standard Mitsunobu coupling conditions as shown in Schemes 2–5 or by abstraction of the phenolic proton of **19** using sodium hydride in DMF followed by addition of the required halide as shown in Scheme 6. Selection of the alkylation method employed was determined by the commercial availability of the required electrophile. Compound **18** was used directly to generate the methoxy compound **15** as previously described.[78] When Mitsunobu reaction conditions were employed, it was found to be necessary to add the DIAD in two portions to prolong the length of time that the electrophile was available for reaction. In general the compounds prepared via simple alkylation could be characterized as the 4-nitro ether analogs, but the compounds prepared via the Mitsunobu route typically co-eluted with the side product triphenylphosphine oxide and it was found expedient to fully characterize the subsequent product after reduction. This conversion was efficiently achieved with catalytic palladium on carbon under 50 psi of H_2 with shaking to give amino derivatives **22a**–**k**. Mono-alkylation on the 4-amine was achieved using a single equivalent of benzaldehyde with sodium triacetoxyborohydride in dichloroethane. Use of an acid catalyst or use of a more aggressive hydride source resulted in dialkylation. This sequence of 6-O alkylation, 4-nitro reduction, and finally 4-alkylation represents the optimal sequence of reaction events. All possible variations were examined including temporary protection of the 4-benzylamine. A requirement for the 4-nitro group for a successful Mitsunbou alkylation on the 6-hydroxy of **19** can be explained as arising from the electron-withdrawing nature of the nitro group on the normally electron rich benzimidazole system and the consequent lowering of pK_a of the 6-hydroxyl group of **19**. More acidic phenols are known to be better substrates for the Mitsunobu reaction as this facilitates attack on the transient electrophile. Although the TBS group was found expedient to generate **4** as shown in Scheme 2, this strategy was not successful in preparing compounds **5** and **6**. Use of **20b** to prepare compound **5** was successful and permitted partial characterization of the intermediate ethers. Transient protection of the more accessible 1-hydroxyl group of 1,2-butane diol, benzoylation of its secondary 2-hydroxyl group, and finally deprotection gave **20c**[79] and permitted synthesis of **6**,[79] a known compound. Hydrolysis of the benzoate esters **23a** and **23b** under standard conditions gave **5** and **6** as shown in Scheme 3. Scheme 4 presents the synthesis of compounds **7**–**9**. These compounds do not have



Scheme 1. Synthesis of 1-isopropyl-4-nitro-6-hydroxybenzimidazole **19**. Reagents and conditions: (i) $Na(AcO)_3BH$, acetone, formic acid, THF, 23 °C, 12 h; (ii) formic acid, concd HCl, BHT, 100 °C, 15 min; (iii) 48% HBr, 120 °C, 2.5 h, microwave.



Scheme 2. Synthesis of **4** via Mitsunobu coupling with a TBS-protective group strategy. Reagents and conditions: (i) DIAD, PPh_3, DMF, 23 °C, 12 h; (ii) Pd/C, 50 psi H_2, EtOH, 23 °C, 5 h; (iii) HCl, EtOH; (iv) $Na(AcO)_3BH$, DCE, benzaldehyde, 23 °C, 12 h.



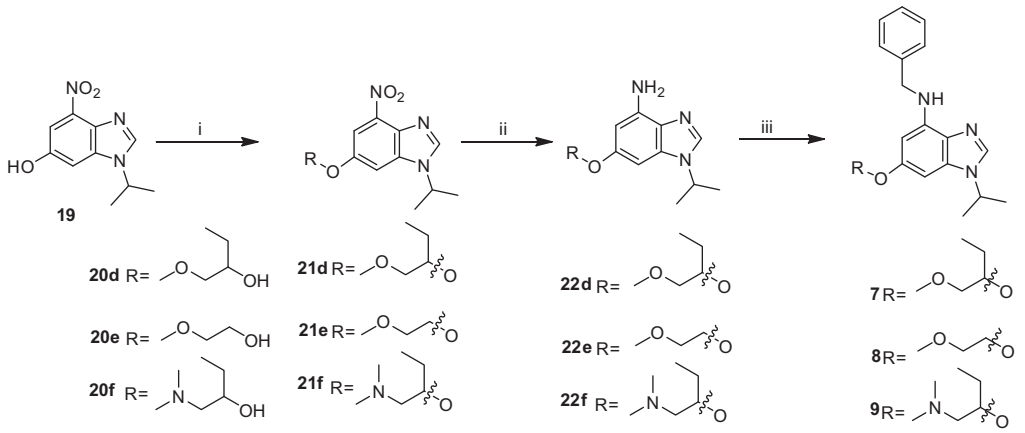
Scheme 3. Synthesis of 6-ether derivatives via Mitsunobu coupling with benzoate-protective group strategy. Reagents and conditions: (i) DIAD, PPh_3, DMF, 23 °C, 12 h; (ii) Pd/C, 50 psi H_2, EtOH, 23 °C, 5 h; (iii) $Na(AcO)_3BH$, DCE, benzaldehyde, 23 °C, 12 h; (iv) 5% KOH/MeOH, 23 °C, 12 h.

exchangeable protons and were prepared without protective group manipulation.

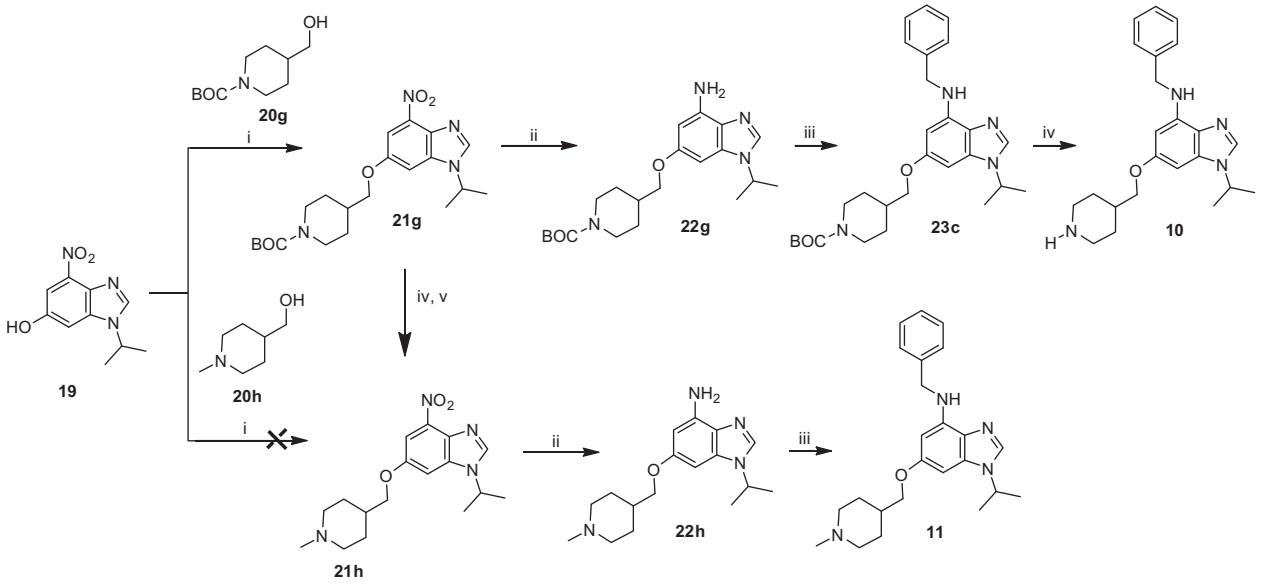
Piperidine compounds **10** and **11** were prepared as shown in Scheme 5. Compound **10** was prepared from BOC-protected precursor **20g** with acid-catalyzed deprotection in the final step. Attempts to synthesize compound **11** via the intended Mitsunobu coupling partner **20f** were not synthetically tractable. However, compound **21h** could be prepared conveniently from the protected precursor **21g** by acid-catalyzed BOC deprotection followed by Eschweiler–Clarke methylation. Reduction of **21h** then reductive alkylation gave **11**.

4. Biological assay

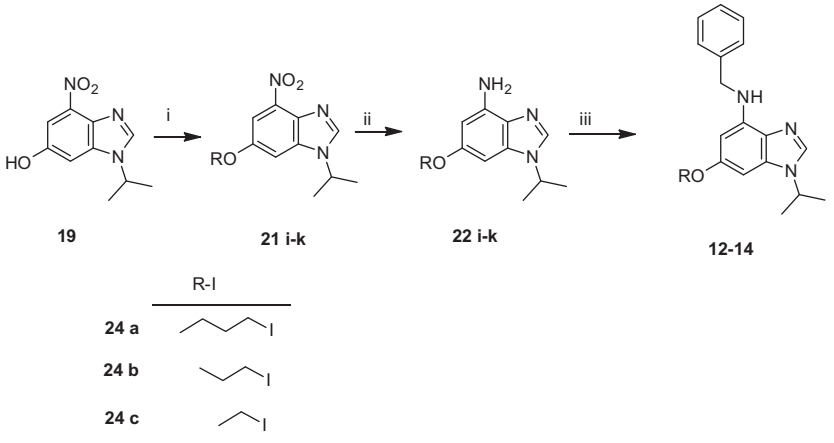
The compounds were assayed using an established protocol[39] with mammalian CDK5 and p25 cloned and stably co-expressed and isolated as glutathione-S-transferase (GST) fusion proteins. Isolation of the expressed protein utilized a glutathione-agarose affinity column chromatography and kinase activity was assayed in 25 mM Mops Buffer (pH 7.2) with 60 mM β-glycerophosphate, 5 mM EDTA, 15 mM $MgCl_2$, and 1 mM DTT. The protein histone H1 (Sigma; 1 mg/mL) was used as the substrate with 15 μm [γ-33P] ATP (3000 μCi/mmol; 1 mCi/mL) to a final volume of 30 μL. After a 10 min incubation period at 30 °C, 25 μL aliquots of supernatant were spotted onto 2.5 × 3 cm pieces of Whatman P81 phosphocellulose paper. After 20 s the filter paper was washed five times (for at least 5 min each time) with a solution of 10 mL phosphoric acid/liter of H_2O. The activity of the wet filter papers was counted in the presence of 1 mL ACS (Amersham) scintillation fluid to determine the amount of substrate phosphorylation. IC_{50} values were determined with probit analysis and experimentally determined values which were inconsistent with symmetrical binding curves were assigned semi-quantitative values. Of the compounds assayed (Table 3), only one compound, **4**, was deemed



Scheme 4. Direct synthesis of 6-ether derivatives via Mitsunobu coupling. Reagents and conditions: (i) DIAD, PPh_3, DMF, 23 °C, 12 h; (ii) Pd/C, 50 psi H_2, EtOH, 23 °C, 5 h; (iii) $Na(AcO)_3BH$, DCE, benzaldehyde, 23 °C, 12 h.



Scheme 5. Synthesis of 6-ether derivatives via Mitsunobu coupling requiring BOC-protective group strategy. Reagents and conditions: (i) DIAD, PPh_3, DMF, 23 °C, 12 h; (ii) Pd/C, 50 psi H_2, EtOH, 23 °C, 5 h; (iii) $Na(AcO)_3BH$, DCE, benzaldehyde, 23 °C, 12 h; (iv) 2 N HCl/EtOH, 23 °C, 2 h; (v) formic acid, formaldehyde, 120 °C, 6 min, microwave.



Scheme 6. Synthesis of 6-ether derivatives via direct alkylation on the 6-pheonolic group. Reagents and conditions: (i) NaH, DMF, 20 °C, 2 h; (ii) Pd/C, 50 psi H_2, EtOH, 23 °C, 5 h; (iii) $Na(AcO)_3BH$, DCE, benzaldehyde, 23 °C, 12 h.

Table 3a
Biological data: (a) Inhibition of CDK5/p25

Compound	CDK5/p25 IC_{50} (μM)
4	13
5	>100
6	>100
7	>10
8	>100
9	>100
10	>100
11	>100
12	>100
13	>100
14	>100
15	>10
1 (R) roscovitine	0.16

Compounds were first tested at 10 and 100 μM. Dose–response curves were run and IC_{50} values determined for those compounds displaying more than 50% inhibition at 100 μM.

of adequate activity to carry on to analysis with other kinases. Compound **4** was assayed against CDK1/cyclin B, glycogen synthase kinase 3α/β (GSK3), casein kinase 1 (CK1), DYRK1A, and CDK2/cyclin A. Analysis of these data indicates an affinity for DYRK1A and CDK2/cyclinA within an order of magnitude of the affinity for CDK5.

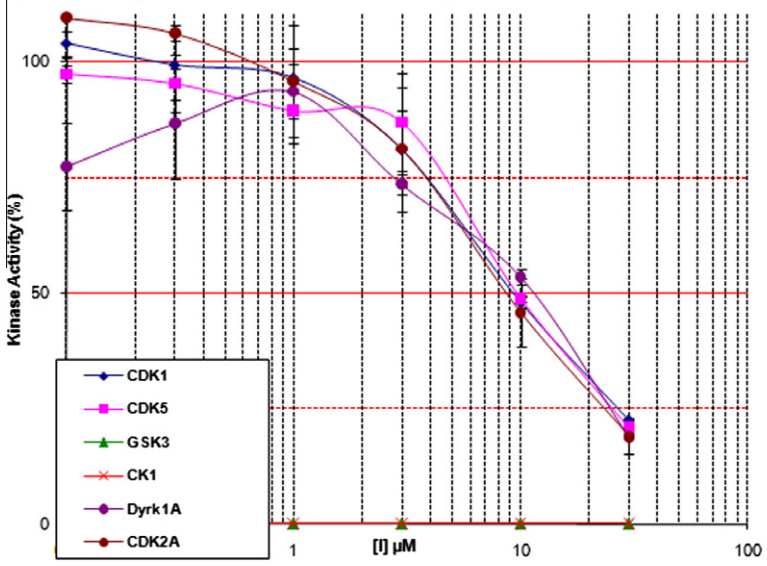
5. Discussion

These compounds were significantly less active than predicted with the Madura scoring protocol or the standard MOE evaluation. The principal components protocol and derived from the interaction of HIV protease inhibitors with their target aspartyl protease. Additional contributions to binding affinity derived primarily from hydrophobic interactions. This strategy of a retained interaction and point variations had suggested favorable analogy for this project. The most likely failing of this strategy were assumptions that the hydrogen bond donor–acceptor pair represented by the 4 NH proton and the 3-N lone pair on the benzimidazole core may have been overstated in their magnitude with this scoring system. The parallel difficulties with the MOE scoring protocol suggests that this system may have features not well accounted using this model. A desolvation parameter, though implicit in the scoring functions, may need to be modified as these compounds were quite hydrophobic. Finally, there may be the possibility of internal hydrogen bonding from the N4 amine to the lone pair of the N3 nitrogen.

Of the compounds prepared only compound **4** had appreciable affinity for CDK5. The activity of compound **4** is consistent with our proposed binding orientation. The absolute requirement for the terminal hydroxyl group is consistent with the prior SAR of purine-based ATP-site inhibitors of CDK5. Capping the alcohol with a methoxy group clearly removed any activity. Curiously removing or moving the ethyl group also decreased activity. There are multiple examples of X-ray crystal structures confirming the benzimidazole core acting as either a hydrogen bond donor or as a hydrogen bond acceptor at the ATP binding site of a kinase.[80–85] Additional studies have examined the interaction of a benzimidazole core with pendant substituents utilizing molecular modeling techniques.[86–89] In many of these cases an additional hydrogen-bonding interaction from the benzimidazole was provided from the 5 or 2 position. Additional interactions were then subsequently obtained by optimizing interactions from these positions. Possible paths forward in the presented series include modification of the 4-benzyl group to optimize interactions with the enzyme surface, modification on the N4 substituent to reduce possible internal hydrogen bonding and increase the effectiveness of the H-bond donor–acceptor pair, increasing the pK_a of the 4-amino group, or incorporation of new additional interactions with the enzyme surface. Interestingly a series of benzimidazoles including compounds **4** and **15** were tested against another serine/threonine kinase, MEK5.[78] The early SAR from those studies or presented here suggest a very divergent SAR for MEK5 versus CDK5: **4** is moderately active against CDK5 but inactive against MEK5, **15** is inactive against CDK5 but active against MEK5. Clearly additional work is required. The observed activity is consistent with the proposed binding orientation and the core postulate that a benzimidazole core can mimic an adenine core.

Table 3b
Biological data: (b) Selectivity of inhibition for **4**: dose–response curves run on selected kinases

b) Selectivity of inhibition for **4**: Dose-response curves run on selected kinases.



6. Experimental

All solvents and reagents were used as received unless noted otherwise. Tetrahydrofuran (THF) was distilled from Na-benzophenone ketyl radical under a blanket of argon prior to use. All reactions were conducted in dry glassware and under an atmosphere of argon unless otherwise noted. Microwave reactions were conducted in a sealed tube and utilized a multimode Milestone Start apparatus for irradiation with power and control parameters as noted. Melting points were determined on a MelTemp apparatus and are uncorrected. All proton NMR spectra were obtained with a 500 or 400 MHz Oxford spectrospin cryostat, controlled by a Bruker Avance system, and were acquired using Bruker Topsin 2.0 acquisition software. Acquired FIDs were analyzed using MestReC 3.2. Elemental analyses were conducted by Atlantic Microlabs and are ±0.4 of theoretical. All HRMS mass spectral analyses were conducted at Duquesne University with a nano ESI chip cube TOF HRMS and are ±0.004 of theoretical. All ^{1}H NMR spectra were taken $CDCl_3$ unless otherwise noted and are reported as ppm relative to TMS as an internal standard. Coupling values are reported in Hertz.

6.1. 1-Isopropyl-6-methoxy-4-nitro-1*H*-benzo[*d*]imidazole (18)

A 500 mL one-neck flask was charged with 5-methoxy-3-nitrobenzene-1,2-diamine **16** (5.0 g, 27.3 mmol), $NaHB(OAc)_3$ (17.36 g, 81.9 mmol), THF (100 mL), acetone (7.93 g, 137 mmol), and formic acid (3.77 g, 81.9 mmol). The mixture was stirred overnight. The solvent was removed in vacuo, and the dark red residue was dissolved in formic acid (31 mL). BHT (20.0 mg, 0.10 mmol) was added, and then the mixture was cooled to 0 °C. Concentrated HCl (87 mL) was added, and the mixture was quickly heated to reflux with a heating mantle. After maintaining reflux temperature for 15 min, the solvent was removed in vacuo at 80 °C. This solution was cooled to ambient temperature, neutralized with 50% NaOH (aq) to pH 8 then extracted with EA (4 × 50 mL). The combined extracts were washed with NaCl (aq, satd) (3 × 10 mL), dried over Na_2SO_4, decanted, and then the solvent was removed under reduced pressure to give a brown solid, which was subjected to SiO_2 column chromatography (Hex/EtOAc 1:1) to afford 4.50 g (74%) of **18** as a yellow solid. R_f 0.32 (CH_2Cl_2/MeOH/NH_4OH 100:10:0.1). Mp 127.2–128.1 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12 (s, 1H), 7.21 (d, J = 2.3 Hz, 1H), 7.79 (d, J = 2.3 Hz, 1H), 4.58–4.65 (m, 1H), 3.95 (s, 3H), 1.65 (d, J = 6.8 Hz, 6H), consistent with the literature.[77]

6.2. 1-Isopropyl-4-nitro-1*H*-benzo[*d*]imidazol-6-ol (19)

A 50 mL microwave reactor tube was charged with 1-isopropyl-6-methoxy-4-nitro-1*H*-benzo[*d*]imidazole **18** (1.00 g, 4.25 mmol) and 20 mL of 48% HBr sealed and then subjected to 250 W of microwave irradiation for 2.5 h, maintaining an internal temperature of 120 °C. The solvent was removed under reduced pressure, and the resulting yellow solid was dissolved in minimum amount (2 mL) of H_2O. Solid $NaHCO_3$ was added in small portions until pH 6. The resultant yellow solid was collected on #1 Whatmann filter paper, washed twice with 2.0 mL of ice-cold H_2O, and then dried in vacuo (150 torr, 12 h) to afford 0.92 g (97%) of **19** as a yellow solid. R_f 0.36 (CH_2Cl_2/MeOH/NH_4OH 100:10:0.1). Mp 206–207 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.16 (s, 1H), 8.45 (s, 1H), 7.51 (d, J = 2.0 Hz, 1H), 7.40 (d, J = 2.0 Hz, 1H), 4.68–4.75 (m, 1H), 1.50 (d, J = 6.8 Hz, 6H). Anal. Calcd for $C_{10}H_{11}N_3O_3$: C, 54.29; H, 5.01; N, 19.00; O, 21.70. Found: C, 51.84; H, 4.73; N, 18.07 plus 0.131 HBr.

6.3. 2-(4-Amino-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)-butan-1-ol (22a)

A dry one-necked flask was charged with 1-isopropyl-4-nitro-1*H*-benzo[*d*]imidazol-6-ol **19** (334.0 mg, 1.5 mmol), 1-(*tert*-butyldimethylsilyloxy)butan-2-ol **20a** lit[90] (817.5 mg, 4.0 mmol), Ph_3P (985.0 mg, 3.8 mmol), and 4 mL of anhydrous DMF. This solution was cooled to 0 °C. Neat DIAD (0.4 mL, 2.8 mmol) was added with slow dropwise addition. The solution was stirred for 30 min at 0 °C then an additional 2 h at 23 °C. The reaction was then cooled to 0 °C and an additional quantity of DIAD (0.4 mL, 2.8 mmol) was added in one portion at 0 °C. The reaction was stirred for 30 min at 0 °C, then at 23 °C for 12 h. The reaction mixture taken up into 10 mL of EA and 10 mL K_2CO_3 (aq, satd). The mixture was extracted three times each with 10 mL portions of EA and the combined organic extracts were washed with NaCl (aq, satd) and dried over Na_2SO_4. The extract was decanted and the solvent was removed under reduced pressure to afford crude **21a**. This crude material was dissolved into 75 mL of EtOH and added to a Parr hydrogenation vessel previously charged with 150.1 mg Pd/C (10% w/w). After three vacuum/purge cycles with H_2, the vessel was charged to 50 psi with H_2 and shaken for 5 h. The mixture was filtered through a pad of Celite and then the pad was washed with an additional 25 mL of EtOH. A 10 mL portion of concd HCl was added to the 100 mL of resultant filtrate at 0 °C and was stirred at 23 °C for 12 h. The solvent was removed under reduced pressure and the resultant material was taken up into 50 mL of 1 N HCl (aq) (pH 1) and washed three times each with 10 mL portions of EA, neutralized then adjusted with 6 N NaOH (aq) to a pH of 10. The mixture was then extracted (3 × 15 mL) with Et_2O, washed with (2 × 5 mL) NaCl (aq, satd), and dried over Na_2SO_4. The organic phase was decanted and the solvent was removed under reduced pressure to give 264.6 mg (67%) of **22a** as a pale brown, hygroscopic solid. Mp 35.5–36.9 °C. SiO_2 TLC R_f 0.44 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^{1}H NMR ($CDCl_3$): δ 7.75 (s, 1H), 6.36 (d, J = 2.0 Hz, 1H), 6.23 (d, J = 2.0 Hz, 1H), 4.45 (m, 1H), 4.36 (s, 2H, NH_2), 4.26 (m, 2H), 3.47 (m, 1H), 2.08 (s, 1H, OH) 1.66 (m, 2H), 1.57 (d, J = 6.8 Hz, 6H), 0.98 (t, J = 7.6 Hz, 3H). Anal. Calcd for $C_{14}H_{21}N_3O_2{\cdot}1H_2O$: C, 59.77; H, 8.24; N, 14.94. Found: C, 59.91; H, 7.93; N, 14.64.

6.4. 2-(4-(Benzylamino)-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)butan-1-ol hydrochloride (4)

Compound **22a** (80.0 mg, 0.3 mmol) and $NaBH(OAc)_3$ (650.0 mg, 3.0 mmol) were added to 2.5 mL of DCE. Benzaldehyde (36 μL, 1.2 mmol) was added in one portion and the mixture was stirred for 12 h at 23 °C. The reaction mixture was quenched with the addition of 1 mL of $NaHCO_3$ (aq, satd) and then stirred for an additional 10 min. The mixture was treated with 5 mL 5% K_2CO_3 (aq) and extracted three times with 15 mL portions of Et_2O. The combined organic extracts were washed with 10 mL of NaCl (aq, satd) and dried (Na_2SO_4). The extract was decanted and the solvent was removed under reduced pressure. The crude material was isolated on SiO_2 and eluted with hexane/EA (1:4). The HCl salt was prepared by the addition of 2 mL of ethereal HCl, then recrystallized from EA to give 46.1 mg (40%) of a white solid. Mp: 148.7–149 °C. SiO_2 TLC R_f 0.5 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^{1}H NMR ($CDCl_3$): δ 8.79 (s, 1H), 7.69 (s, 1H, NH), 7.41 (d, J = 7.6 Hz, 2H), 7.31 (d, J = 7.2 Hz, 2H), 7.28 (m, 1H), 6.26 (d, J = 2.0 Hz, 1H), 6.07 (d, J = 2.0 Hz, 1H), 4.63 (m, 1H), 4.54 (s, 1H), 4.14 (m, 2H), 3.70 (m, 1H), 2.69 (s, 1H, NH) 1.66 (d, J = 6.8 Hz, 6H), 1.57 (m, 2H), 0.86 (t, J = 7.6 Hz, 3H). Anal. Calcd for $C_{21}H_{28}ClN_3O_2{\cdot}1/8H_2O$: C, 64.32; H, 7.26; N, 10.71. Found: C, 64.27; H, 7.30; N, 10.61.

6.5. 2-(4-(Benzylamino)-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)ethanol (21b)

An oven dried one-neck flask was charged with 1-isopropyl-4-nitro-1*H*-benzo[*d*]imidazol-6-ol **19** (166.0 mg, 0.75 mmol), 1-hydroxybutan-2-yl benzoate **20b** (388 mg, 2.0 mmol), Ph_3P (493 mg, 1.9 mmol), and 4.0 mL of anhydrous DMF. The resulting solution was cooled to 0 °C. Neat DIAD (0.2 mL, 1.4 mmol) was added with dropwise at 0 °C. The solution was stirred for 30 min at 0 °C for additional 2 h at rt. The reaction was then cooled to 0 °C and a second amount of DIAD (0.2 mL, 1.4 mmol) was added at 0 °C. The reaction was stirred for 30 min at 0 °C then at rt for 12 h. After completion of reaction (TLC), 10 mL of EA and 10 mL satd K_2CO_3 (aq) (pH >9) were added to the reaction mixture and extracted (3 × 10 mL) with EA. The combined organic extracts were washed with NaCl (aq, satd) (3 × 5 mL) and dried over Na_2SO_4. The organic extract was decanted, and the solvent was removed under reduced pressure to afford 3.30 g of **21b** as an off-white solid which was carried on directly to the next step. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.99–8.03 (m, 5H), 7.79 (d, J = 2.27 Hz, H), 7.28 (d, J = 2.30 Hz, 1H), 5.38–5.44 (m, 1H), 4.55 (m, 1H), 4.20–4.40 (m, 2H), 1.71–1.79 (m, 2H), 1.58 (dd, J = 4.72, 6.73 Hz, 6H), 1.05 (t, J = 7.46 Hz, 3H).

6.6. 2-(4-(Benzylamino)-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)ethanol (22b)

A Parr hydrogenation vessel was charged with 100 mg of Pd/C (10% w/w) and then solution of crude **21g** in 100 mL of EtOH was added to the reaction vessel. After three vacuum/purge cycles with H_2, the reaction vessel was shaken at 50 psi on a Parr hydrogenation apparatus for 12 h. The reaction mixture was filtered through a pad of Celite and the pad was washed with an additional 10 mL of EtOH. The solvent was removed under reduced pressure to afford a resultant brown oil that was treated with 50 mL of 1 N HCl (aq) (pH <1) and washed with EA (3 × 10 mL). The aqueous layer was then basified (pH >9) with 5% K_2CO_3 (aq) and extracted (3 × 15 mL) with EA. The combined EA layers were washed with NaCl (aq, satd) (3 × 10 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure to give 280 mg of **22b** as a clear film which was carried on to the next step directly. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (dd, J = 1.31, 8.45 MHz, 2H), 7.74 (s, 1H), 7.55–7.57 (m, 1H), 7.48–7.51 (m, 2H), 6.58 (br s, 2H), 6.30 (d, J = 2.06 Hz, 1H), 6.21 (d, J = 2.09 Hz, 1H), 5.34–5.41 (m, 1H), 4.43 (m, 1H), 4.14–4.23 (ddd, J = 4.46 Hz, J = 10.26, 20.23 MHz, 2H), 1.89–1.95 (m, 2H), 1.54 (dd, J = 3.67, 6.74 Hz, 6H), 1.05 (t, J = 7.43 Hz, 3H).

6.7. 2-(4-(Benzylamino)-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)ethyl benzoate (23a)

A dry round bottom flask was charged with 2-(4-amino-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)ethyl benzoate **22g** (280 mg, 0.75 mmol), $NaHB(OAc)_3$ (317.9 mg, 1.5 mmol), benzaldehyde (85 μL, 0.85 mmol, 1.15 equiv) and 5.0 mL of DCE. This mixture was stirred at rt for 12 h, quenched with 10 mL $NaHCO_3$ (satd, aq), then extracted (3 × 10 mL) with EA. The combined organic extracts were washed (2 × 5 mL) with NaCl (aq, satd) and dried over Na_2SO_4. Evaporation of the solvent gave a brown solid, which was subjected to SiO_2 flash column chromatography (Hexanes/EtOAc 1:1) to afford 40 mg of **23a** (11%) as a brown solid. Mp 116.1–116.9 °C R_f 0.71 (CH_2Cl_2/MeOH/NH_4OH 100:10:0.1). ^{1}H NMR ($CDCl_3$) for $C_{28}H_{31}N_3O_3$: δ 8.07 (dd, J = 1.35, 8.39 Hz, 1H), δ 7.71 (s, 1H), 7.54–7.59 (m, 1H), 7.39–7.46 (m, 4H), 7.29–7.35 (m, 2H), 7.26 (m, 1H), 6.26 (d, J = 2.07 Hz, 1H), 6.08 (d, J = 2.05 Hz, 1H), 5.39 (m, 1H), 5.25 (t, J = 5.51 Hz, 1H), 4.45–4.52 (m, 4H), 4.13–4.22 (ddd, 2H, J = 5.0, 10.3, 22.9 Hz, 2H), 1.89–1.94 (m, 2H), 1.59 (dd, J = 3.5, 6.8 Hz, 6H), 1.07 (t, J = 7.39 Hz, 3H). HRMS (EI) calcd for $C_{28}H_{31}N_3O_3$: 457.2438 [M+H$^+$], found 457.2438 [M+H$^+$].

6.8. 2-(4-(Benzylamino)-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)ethanol hydrochloride (5)

2-(4-(Benzylamino)-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)ethyl benzoate **23a** was dissolved into 2.0 mL of 5% KOH in MeOH and stirred for 12 h. The reaction progress was assayed by TLC. The reaction was quenched with 5 mL of NH_4OH (satd, aq) and extracted (3 × 10 mL) with EA. The combined EA extracts were washed (1 × 5 mL) with NaCl (aq, satd) and dried over Na_2SO_4. Removal of the solvent under reduced pressure gave 30 mg of **5** as an off white solid, which was dissolved in 2.0 mL Et_2O. A slight excess of freshly prepared ethereal HCl was slowly added to form the corresponding hydrochloride salt. Recrystallization from EtOH gave 15 mg (65% yield) of **5** as a white solid. Mp 140–142 °C R_f 0.62 (CH_2Cl_2/MeOH/NH_4OH 100:10:0.1). ^{1}H NMR (MeOD) for $C_{21}H_{27}N_3O_2$: δ 9.23 (s, 1H), 7.42–7.46 (d, J = 7.24 Hz, 2H), 7.38–7.35 (t, J = 7.4 Hz, 2H), 7.30 (t, J = 7.24 Hz, 1H), 6.67 (d, J = 1.71 Hz, 1H), 6.37 (d, J = 1.85 Hz, 1H), 4.92 (m, 1H), 4.49 (s, 2H), 3.96–4.1 (ddd, J = 5.2, 9.6, 16.0 Hz, 2H), 3.80–3.86 (m, 1H), 1.68 (d, J = 6.70 Hz, 6H), 1.50–1.65 (m, 2H), 1.00–1.05 (t, J = 7.44 Hz, 3H). Anal. Calcd for $C_{21}H_{28}ClN_3O_2$: C, 64.69; H, 7.24; N, 10.78. Found: C, 60.60; H, 7.10; N, 9.90 plus 1.46% H_2O.

6.9. 2-(1-Isopropyl-4-nitro-1*H*-benzo[*d*]imidazol-6-yloxy)ethyl benzoate (21c)

An oven dried one-neck flask was charged with 1-isopropyl-4-nitro-1*H*-benzo[*d*]imidazol-6-ol **19** (334.0 mg, 1.5 mmol), 2-hydroxyethyl benzoate **20c** (665 mg, 4.0 mmol), Ph_3P (985.0 mg, 3.8 mmol), and 4.0 mL of anhydrous DMF. The resulting solution was cooled to 0 °C. Neat DIAD (0.4 mL, 2.8 mmol) was added with dropwise at 0 °C. Then this solution was stirred for 30 min at 0 °C then for an additional 2 h at 23 °C. The reaction was cooled to 0 °C and a second amount of DIAD (0.4 mL, 2.8 mmol) was added at 0 °C. The reaction was stirred for 30 min at 0 °C then at rt for 12 h. After completion of reaction (TLC), 10 mL of EA and 10 mL satd K_2CO_3 (aq) (pH >9) were added to the reaction mixture and extracted with EA (3 × 10 mL). The organic layers were combined and washed with NaCl (aq, satd) (3 × 5 mL) then dried (Na_2SO_4). The organic layer was decanted and the solvent was removed under reduced pressure to give 2.0 g of **21c** as a light brown oil which was carried on directly in the next step. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.13 (s, 1H), 8.01–8.02 (m, 5H), 7.85 (d, J = 2.29 Hz, 1H), 7.29 (d, J = 2.29 Hz, 1H), 5.23 (m, 1H), 4.73–4.76 (dd, J = 4.13, 5.27 Hz, 2H), 3.81–3.84 (dd, J = 5.25, 6.19 Hz, 2H), 1.62 (d, J = 6.74 Hz, 6H).

6.10. 2-(4-Amino-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)-ethyl benzoate (22c)

A Parr hydrogenation vessel was charged with 115 mg of Pd/C (10% w/w) then a solution of crude **21c** (Th 1.5 mmol) in 75 mL of EtOH. After three vacuum/purge cycles with H_2, the reaction vessel was subjected to hydrogenation at 50 psi for 12 h on a Parr hydrogenation apparatus. The reaction mixture was filtered through a pad of Celite, and then the pad was washed with an additional 10 mL of EtOH. The solvent was removed under reduced pressure. The resultant brown oil was treated with 50 mL of 1 N HCl (aq) (pH <1) and washed with EA (3 × 10 mL), this aqueous layer was then basified with 5% K_2CO_3 (aq) (pH ~9–10) and then extracted with EA (3 × 15 mL). The combined EA layers were washed with NaCl (aq, satd) (3 × 10 mL) and dried over Na_2SO_4. The solvent was removed under reduced vacuum to give 0.110 g of **22c** as a colorless film which was carried on directly. R_f 0.45

(CH_2Cl_2/MeOH/NH_4OH 100:10:0.1). ^1H NMR ($CDCl_3$) for $C_{19}H_{21}N_3O_3$: δ 8.075 (dd, J = 1.31, 8.37 Hz, 2H), δ 7.76 (s, 1H), 7.65–7.71 (m, 2H), 7.54–7.59 (m, 2H), 7.42–7.51 (m, 4H), 6.33 (d, J = 2.12 Hz, 1H), 6.25 (d, J = 2.14 Hz, 1H), 5.00 (td, J = 6.3, 12.6 Hz, 1H), 4.62–4.71 (dd, J = 4.3, 5.4 Hz, 2H), 4.37 (br peak, 2H) 4.33–4.36 (dd, J = 4.3, 5.3 Hz, 2H), 1.58 (d, J = 6.76 Hz, 6H).

6.11. 2-(4-(Benzylamino)-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)ethyl benzoate (23b)

A dry round bottom flask was charged with 2-(4-amino-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)ethyl benzoate **22c** (110 mg, 0.3 mmol), $NaBH(OAc)_3$ (127.16 mg, 0.6 mmol), benzaldehyde (33 μL, 0.33 mmol) and 5.0 mL of DCE. The mixture was stirred at room temperature for 12 h. A second quantity of $NaBH(OAc)_3$ (41.0 mg, 0.2 mmol, 0.66 equiv) and benzaldehyde (11 μL, 0.11 mmol, 0.33 equiv) were added to the reaction mixture to drive the reaction to completion over the next 3 h (TLC). The reaction was quenched with 10 ml $NaHCO_3$ (satd, aq) and the mixture was extracted (3 × 15 mL) with EA. The combined organic extracts were washed (2 × 5 mL) with NaCl (aq, satd) and dried (Na_2SO_4). Evaporation of the solvent gave a brown solid, which was subjected to SiO_2 flash column chromatography (Hexanes/EtOAc 1:1) to afford 40 mg of **23b** (31%) as a white solid. Mp 141.5–142.8 °C. R_f 0.7 (CH_2Cl_2/MeOH/NH_4OH 100:10:0.1). ^1H NMR ($CDCl_3$) for $C_{26}H_{27}N_3O_3$: δ 8.07 (dd, J = 1.3, 8.4 Hz, 1H), δ 7.73 (s, 1H), 7.55–7.60 (m, 1H), 7.40–7.46 (m, 4H), 7.31–7.35 (m, 2H), 7.27 (m, 1H), 6.26 (d, J = 2.07 Hz, 1H), 6.10 (d, J = 2.04 Hz, 1H), 5.29 (t, J = 5.76 Hz, 1H), 4.67 (dd, J = 4.33, 5.31 Hz, 2H) 4.45–4.52 (m, 3H), 4.23–4.28 (dd, J = 3.70, 5.95 Hz, 2H), 1.58 (d, J = 6.76 Hz, 6H). Anal. Calcd for $C_{26}H_{27}N_3O_3$: C, 65.41; H, 7.49; N, 10.40. Found: C, 65.43; H, 7.35; N, 10.33.

6.12. 2-(4-(Benzylamino)-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)ethanol (6)

2-(4-(Benzylamino)-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)-ethyl benzoate **23b** was dissolved in 2.0 ml of 5% KOH in MeOH and reaction mixture was stirred for 12 h. The reaction was quenched with 5 mL of NH_4Cl (satd, aq) and extracted (3 × 10 mL) with EA. The combined EA extracts were washed with NaCl (aq, satd) and dried (Na_2SO_4). Removal of the solvent under reduced pressure afforded an off white solid that was subjected to SiO_2 flash column chromatography (Hexanes/EtOAc; 1:1) to afford 18 mg of **6** as a white solid. Recrystallization from DCM/Hexanes provided **6** as white needles in 56% yield. Mp 140–142 °C. R_f 0.65 (CH_2Cl_2/MeOH/NH_4OH 100:10:0.1). ^1H NMR ($CDCl_3$) for $C_{19}H_{23}N_3O_2$: δ 7.74 (s, 1H), 7.41–7.43 (m, 2H), 7.31–7.36 (m, 2H), 7.25–7.29 (m, 1H), 6.23 (d, J = 2.11 Hz, 1H), 6.06 (d, J = 2.07 Hz, 1H), 5.33 (br peak, 1H), 4.45–4.55 (m, 3H), 4.08–4.14 (dd, 2H, J = 3.9, 5.2 Hz), 3.94–3.97 (dd, J = 3.4, 5.51 Hz, 2H), 1.59 (d, J = 6.75 Hz, 6H). Anal. Calcd for $C_{19}H_{23}N_3O_2$: C, 70.13; H, 7.12; N, 12.91. Found: C, 67.88; H, 6.93; N, 12.34 plus 0.6% H_2O.

6.13. 1-Isopropyl-6-(1-methoxybutan-2-yloxy)-4-nitro-1*H*-benzo-[*d*]imidazole (21d)

An oven dried one-neck flask was charged with 1-isopropyl-4-nitro-1*H*-benzo[*d*]imidazol-6-ol **19** (334.0 mg, 1.5 mmol), 1-methoxybutan-2-ol **20d** (0.5 ml, 4.0 mmol), Ph_3P (985.0 mg, 3.8 mmol), and 4.0 mL of anhydrous DMF. The solution was cooled to 0 °C. Neat DIAD (0.4 mL, 2.8 mmol) was added with dropwise addition. The solution was stirred for 30 min at 0 °C then the reaction was stirred for an additional 2 h at rt. The reaction was then cooled to 0 °C and a second quantity of DIAD (0.4 mL, 2.8 mmol) was added at 0 °C. The reaction was stirred for 30 min at 0 °C then at rt until the reaction was complete (TLC). The reaction mixture was poured into 10 mL of EA and 10 mL satd K_2CO_3 (aq) (pH >9). This was extracted with EA (3 × 10 mL) and the combined organic extracts were washed with NaCl (aq, satd) (3 × 5 mL) and dried (Na_2SO_4). The extract was decanted and the solvent was removed under reduced pressure to give crude **21d** which was carried on directly. ^1H NMR (400 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.83 (d, J = 2.24 Hz, 1H), 7.36 (d, J = 2.23 Hz, 1H), 4.55–4.62 (sept, J = 6.7 Hz, 1H), 4.37–4.43 (m, 1H), 3.42–3.49 (m, 2H), 3.37 (s, 3H), 1.72–1.78 (m, 2H), 1.61 (d, J = 6.78 Hz, 6H), 1.00 (t, J = 7.47 Hz, 3H).

6.14. 1-Isopropyl-6-(1-methoxybutan-2-yloxy)-1*H*-benzo[*d*]-imidazol-4-amine (22d)

Crude **21d** was dissolved into 25 mL of EtOH and added to a Parr hydrogenation vessel previously charged with 90 mg of Pd/C (10% w/w). After three vacuum/purge cycles with H_2, the vessel was charged to 50 psi with H_2 and shaken for 12 h. The mixture was then filtered through a pad of Celite, the pad was washed with an additional 10 mL of EtOH, and then the solvent was removed under reduced pressure. The resultant oil was treated with 50 mL of 1 N HCl (aq) (pH <1) and then washed with EA (3 × 10 mL). The aqueous phase was neutralized, made basic with 5% K_2CO_3 (aq) (pH ~9–10), and then extracted with (3 × 15 mL) of EA. The combined EA layers were washed with 1 N HCl (3 × 10 mL), basified with 50% NaOH (aq) (pH ~12), and then extracted with Et_2O (3 × 15 mL). These combined Et_2O layers were washed with NaCl (aq, satd) (3 × 10 mL) and dried (Na_2SO_4). The organic phase was decanted and the solvent was removed under reduced pressure to afford 220.0 mg of crude **22d** as a light brown film carried on directly. ^1H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 6.38 (d, J = 2.07 Hz, 1H), 6.25 (d, J = 2.07 Hz, 1H), 4.42–4.52 (m, 1H), 4.33 (br s, 1H), 4.26–4.33 (td, J = 5.5, 10.9 Hz, 1H), 3.52–3.62 (ddd, J = 4.91, 10.17, 24.31 Hz, 2H), 3.41 (s, 3H), 1.73–1.82 (m, 2H), 1.58 (d, J = 6.76 Hz, 6H), 0.96–1.01 (t, J = 7.47 Hz, 3H).

6.15. *N*-Benzyl-1-isopropyl-6-(1-methoxybutan-2-yloxy)-1*H*-benzo[*d*]imidazol-4-amine hydrochloride (7)

A dry round bottom flask was charged with 1-isopropyl-6-(1-methoxybutan-2-yloxy)-1*H*-benzo[*d*]imidazol-4-amine **22d** (200.0 mg, 0.72 mmol), $NaHB(OAc)_3$ (307.3 mg, 1.45 mmol), benzaldehyde (80 μL, 0.80 mmol) and 5.0 mL of DCE. The mixture was stirred at room temperature for 24 h, then a second amount of $NaBH(OAc)_3$ (40.0 mg, 0.19 mmol) and benzaldehyde (25 μL, 0.25 mmol) were added to drive the reaction to completion over the next 2 h. The reaction mixture was quenched with the addition of 10 mL 5% K_2CO_3 (aq) (pH ~9–10) and the mixture was extracted with (3 × 10 mL) of EA. The combined extracts were washed with 5 mL of NaCl (aq, satd) and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave crude **7** as a brown solid, which was subjected to SiO_2 flash column chromatography (Hexanes/EtOAc/Et_3N 1:1:0.005) to afford 200 mg of **7** (76%) as a colorless oil. Conversion of **7** to its corresponding hydrochloride salt was achieved in 1.0 mL EA with gaseous HCl to give 220 mg of **7** as a white solid mp 129–130 °C. R_f 0.7 (CH_2Cl_2/MeOH/NH_4OH 100:10:0.1). ^1H NMR ($CDCl_3$) for $C_{22}H_{29}N_3O_2$: δ 7.27 (s, 1H), 7.41–7.43 (m, 2H), 7.31–7.34 (m, 2H), 7.26 (m, 1H), 6.32 (d, J = 2.05 Hz, 1H), 6.10 (d, J = 2.04 Hz, 1H), 5.30 (br peak, 1H), 4.45–4.52 (m, 3H), 4.23–4.28 (m, 1H), 3.49–3.59 (dd, J = 4.85, 10.16, 23.14 Hz, 2H), 3.37 (s, 3H), 1.70–1.74 (m, 2H), 1.59 (d, J = 6.76 Hz, 6H) 0.96–1.00 (t, J = 7.47 Hz, 3H). Anal. Calcd for $C_{22}H_{30}ClN_3O_2$: C, 65.41; H, 7.49; N, 10.40. Found: C, 65.43; H, 7.35; N, 10.33.

6.16. 1-Isopropyl-6-(2-methoxyethoxy)-1*H*-benzo[*d*]imidazol-4-amine (22e)

A dry one-necked flask was charged with 1-isopropyl-4-nitro-1*H*-benzo[*d*]imidazol-6-ol, **19** (334.0 mg, 1.5 mmol), 2-methoxyethanol **20e** (0.5 mL, 6.9 mmol), Ph_3P (985.0 g, 3.8 mmol), and 4 mL of anhydrous DMF. This solution was cooled to 0 °C. Neat DIAD (0.4 mL, 2.8 mmol) was added with drop-wise addition over 15 min. The solution was stirred for 30 min at 0 °C, and then the ice-bath was removed. The reaction mixture was stirred for an additional 2 h at 23 °C. The reaction was then cooled to 0 °C and an additional quantity of DIAD (0.4 mL, 2.8 mmol) was added at 0 °C in one portion. The reaction was stirred for 30 min at 0 °C and then at 23 °C for 12 h. The reaction mixture was poured into 10 mL EA and 10 mL K_2CO_3 (aq, satd), extracted three times with 10 mL portions of EA, and then the combined organic extracts were washed with NaCl (aq, satd) and dried over Na_2SO_4. The extract was decanted and the solvent was removed under reduced pressure. Crude **21e** was dissolved into 75 mL of EtOH and added to a Parr hydrogenation vessel previously charged with Pd/C (10% w/w, 119.8 mg). After three vacuum/purge cycles with H_2, the vessel was charged to 50 psi with H_2 and shaken for 5 h on a Parr hydrogenation apparatus. The mixture was filtered through a pad of Celite and then the pad was washed with an additional 25 mL of EtOH. The solvent was removed under reduced pressure to give a brown oil. This was poured into 50 mL of 1 N HCl (aq) (pH 1) and washed three times with 10 mL portions of EA. The aqueous phase was neutralized and then basified with 6 N NaOH (aq) to a pH of 10 and then extracted three times with 15 mL portions of Et_2O. The combined Et_2O extracts were washed with NaCl (aq, satd) and dried over Na_2SO_4. The solvent was decanted and then removed under reduced pressure to give 150.5 mg (40%) of **22e** as a pale brown, hygroscopic solid. Mp 32.9–34.1 °C. SiO_2 TLC R_f 0.31 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 7.75 (s, 1H), 6.31 (d, J = 2.0 Hz, 1H), 6.23 (d, J = 2.0 Hz, 1H), 4.48 (m, 1H), 4.14 (t, J = 6.4 Hz, 2H), 3.76 (t, J = 3.6 Hz, 2H), 3.46 (s, 3H), 1.57 (d, J = 6.8 Hz, 6H). Anal. Calcd for $C_{13}H_{19}N_3O_2 \cdot 1/5H_2O$: C, 61.74; H, 7.73; N, 16.61. Found: C, 61.99; H, 7.81; N, 16.26.

6.17. *N*-Benzyl-1-isopropyl-6-(2-methoxyethoxy)-1*H*-benzo[*d*]-imidazol-4-amine oxalate (8)

1-Isopropyl-6-(2-methoxyethoxy)-1*H*-benzo[*d*]imidazol-4-amine **22e** (120.0 mg, 0.48 mmol) and solid $NaBH(OAc)_3$ (279.7 mg, 1.4 mmol) were added to 2.5 mL of DCE. Benzaldehyde (53.6 μL, 0.5 mmol) was added in one portion and the mixture was stirred for 12 h. The reaction mixture was quenched with the addition of 1 mL of $NaHCO_3$ (aq, satd) and then stirred for an additional 10 min. The mixture was taken up into 50 mL of 5% of K_2CO_3 (aq, satd) and then extracted three times with 15 mL portions of Et_2O. The combined extracts were washed with NaCl (aq, satd) and then dried over Na_2SO_4. The extract was decanted and the solvent was removed under reduced pressure. The crude material was isolated on SiO_2 and eluted with hexanes/EA (1:3) to give 69.3 mg of a clear film. This was dissolved into 2.5 mL of EA, followed by addition of a solution of anhydrous oxalic acid (20.1 mg, 0.22 mmol) in 2.5 mL of EA to afford 82.6 mg (40%) of **8** as a white crystalline solid. Mp 164.6–165.1 °C. SiO_2 TLC R_f 0.55 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR (MeOD-d_4): δ 8.90 (s, 1H), 7.41 (d, J = 7.6 Hz, 2H), 7.33 (t, J = 7.2 Hz, 2H), 7.25 (t, J = 7.6 Hz, 1H), 6.56 (d, J = 2.0 Hz, 1H), 6.19 (d, J = 2.0 Hz, 1H), 4.88 (m, 1H), 4.47 (s, 2H), 4.11 (t, J = 2.0 Hz, 2H), 3.72 (t, J = 2.8 Hz, 2H), 3.40 (s, 3H), 1.64 (d, J = 6.8 Hz, 6H). Anal. Calcd for $C_{22}H_{27}N_3O_6 \cdot 1/6H_2O$: C, 61.10; H, 6.37; N, 9.72. Found: C, 61.08; H, 6.31; N, 9.78.

6.18. 1-(Dimethylamino)butan-2-ol (20f)

An oven dried 250 mL round bottom flask was cooled under argon and charged with a 2 M solution of dimethylamine in THF (30 mL, 60 mmol). The reaction mixture was cooled to −78 °C and then 2 M *n*-BuLi in THF (25 mL, 50 mmol) was added slowly over 15 min maintaining the internal temperature of the reaction below −50 °C. The reaction was then permitted to warm to 23 °C and then stirred at 23 °C for 1 h. The reaction mixture was cooled to −78 °C and (±)2-ethyloxirane (4.0 mL, 46 mmol) was added over 5 min. Then the reaction mixture was permitted to warm to 23 °C and then stirred for additional 12 h. A mixture of 3:1 NH_4OH/NH_4Cl was added and the mixture was extracted (3 × 15 mL) with Et_2O, washed (3 × 10 mL) with NaCl (aq, satd), and then dried (Na_2SO_4). The extract was decanted and then removed under reduced pressure to give 1.23 g (88%) of **20f** as a brownish-yellow oil. bp: 149.8–151.1 °C (lit[91] 152–153 °C). ^1H NMR ($CDCl_3$): δ 3.46 (m, 1H), 2.51 (m, 2H), 2.31 (s, 6H), 1.51 (m, 2H), 0.95 (t, 3H).

6.19. *N*-Benzyl-6-(1-(dimethylamino)butan-2-yloxy)-1-isopropyl-1*H*-benzo[*d*]imidazol-4-amine hydrochloride (9)

A dry one-necked flask was charged with 1-isopropyl-4-nitro1*H*-benzo[*d*]imidazol-6-ol **19** (334.0 mg, 1.5 mmol), 1-(dimethylamino)butan-2-ol **20f** (468.6 mg, 4.0 mmol), Ph_3P (985.0 mg, 3.8 mmol), and 4 mL of anhydrous DMF. This solution was cooled to 0 °C. Neat DIAD (0.4 mL, 2.8 mmol) was added with slow drop-wise addition. The solution was stirred for 30 min at 0 °C, the ice-bath was removed, and the reaction was stirred for an additional 2 h at 23 °C. The reaction was then cooled to 0 °C and an additional quantity of DIAD (0.4 mL, 2.8 mmol) was added in one portion at 0 °C. The reaction was stirred for 30 min at 0 °C, then at 23 °C for 12 h. The reaction mixture taken up into 10 mL of EA and 10 mL of K_2CO_3 (aq, satd). This mixture was extracted three times with 10 mL portions of EA and the combined organic extracts were washed with 10 mL of NaCl (aq, satd) and then dried over Na_2SO_4. The extract was decanted and the solvent was removed under reduced pressure. The HCl salt was prepared with the addition of 2 mL of ethereal HCl. Crude **21f** was dissolved into 75 mL of EtOH and was added to a Parr hydrogenation vessel previously charged with Pd/C (10% w/w, 91.3 mg). After three vacuum/purge cycles with H_2, the vessel was charged to 50 psi with H_2 and shaken for 5 h. The mixture was filtered through a pad of Celite and then the pad was washed with an additional 25 mL of EtOH. A 10 mL portion of concd HCl was added to the filtrate at 0 °C. The reaction was stirred at 23 °C for 12 h. The solvent was removed under reduced pressure. The crude oil was taken up into 50 mL of 1 N HCl (aq) (pH 1) and washed three times with 10 mL portions of EA, neutralized and adjusted with 6 N NaOH (aq) to a pH of 10 and then extracted three times with 15 mL portions of Et_2O. The combined Et_2O layers were washed with NaCl (aq, satd) and dried (Na_2SO_4). The organic phase was decanted and the solvent was removed under reduced pressure to give crude **22f** which was used directly to the next step. ^1H NMR ($CDCl_3$): δ 8.31 (s, 1H), 6.83 (d, J = 2.0 Hz, 1H), 6.44 (d, J = 2.0 Hz, 1H), 6.42 (s, 1H, NH), 4.44 (m, 1H), 4.52 (s, 1H), 2.92 (s, 6H), 3.62 (m, 1H), 1.70 (m, 2H), 1.68 (d, J = 6.8 Hz, 6H), 1.20 (m, 2H), 1.11 (t, J = 7.6 Hz, 1H).

6-(1-(Dimethylamino)butan-2-yloxy)-1-isopropyl-1*H*-benzo[*d*]imidazol-4-amine, **22c**, (84.0 mg, 0.39 mmol) and solid $NaBH(OAc)_3$ (650.0 mg, 3.0 mmol) were added to 2.5 mL of DCE. Benzaldehyde

(31 μL, 1.2 mmol) was added in one portion and the mixture was stirred for 12 h. The reaction mixture was quenched with the addition of 1 mL of $NaHCO_3$ (aq, satd) and stirred for 10 min. The mixture was taken up into 5% K_2CO_3 (aq) and then extracted three times with 15 mL of Et_2O. The combined organic extracts were washed with NaCl (aq, satd) and then dried over Na_2SO_4. The extract was decanted and the solvent was removed under reduced pressure. The crude material was isolated on SiO_2 and eluted with hexane/EA (1:4). The HCl salt of **9** was prepared with dropwise addition of 2 mL of ethereal HCl to give a white, hygroscopic solid. Mp 88.2–89.5 °C. SiO_2 TLC R_f 0.47 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR (MeOD-d_4): δ 9.31 (s, 1H), 7.42 (q, 2H), 7.35 (d, J = 7.2 Hz, 2H), 7.29 (q, 1H), 6.83 (d, J = 2.0 Hz, 1H), 6.44 (d, J = 2.0 Hz, 1H), 6.42 (s, 1H, NH), 4.44 (m, 1H), 4.52 (s, 1H), 2.92 (s, 6H), 3.62 (m, 1H), 1.70 (m, 2H), 1.68 (d, J = 6.8 Hz, 6H), 1.20 (m, 2H), 1.11 (t, J = 7.6 Hz, 1H). Anal. Calcd for $C_{23}H_{34}Cl_2N_4O{\cdot}2.5H_2O$: C, 55.42; H, 7.89; N, 11.24. Found: C, 55.38; H, 7.62; N, 11.15.

6.20. *tert*-Butyl-4-((4-(benzylamino)-1-isopropyl-1*H*-benzo[*d*]-imidazol-6-yloxy)methyl)piperidine-1-carboxylate (21g)

A dry one-necked flask was charged with 1-isopropyl-4-nitro1*H*-benzo[*d*]imidazol-6-ol **19** (334.0 mg, 1.5 mmol), *tert*-butyl 4-(hydroxymethyl)piperidine-1-carboxylate **20g** (861.1 mg, 4.0 mmol), Ph_3P (985.0 mg, 3.8 mmol), and 4 mL of anhydrous DMF. This solution was cooled to 0 °C. Neat DIAD (0.4 mL, 2.8 mmol) was added with slow dropwise addition. The solution was stirred for 30 min at 0 °C then the reaction was stirred for an additional 2 h at 23 °C. The reaction was cooled to 0 °C and an second quantity of DIAD (0.4 mL, 2.8 mmol) was added in one portion at 0 °C. The reaction was stirred for 30 min at 0 °C, then at 23 °C for 12 h. The reaction mixture taken up into a mixture of 10 mL of EA and 10 mL K_2CO_3 (aq, satd) and extracted (3 × 10 mL) with EA. The combined organic extracts were washed with 10 mL of NaCl (aq, satd) and dried over Na_2SO_4. The extract was decanted and the solvent was removed under reduced pressure. Crude **21a** was dissolved into 75 mL of EtOH and added to a Parr hydrogenation vessel previously charged with Pd/C (10% w/w, 145.7 mg). After three vacuum/purge cycles with H_2, the vessel was charged to 50 psi with H_2 and shaken for 5 h. The mixture was filtered through a pad of Celite and then the pad was washed with an additional 25 mL of EtOH. Crude **21g** was carried directly to next step. ^1H NMR ($CDCl_3$): δ 7.61 (s, 1H), 6.18 (d, J = 2.0 Hz, 1H), 6.02 (d, J = 2.0 Hz, 1H), 5.27 (s, 1H, NH), 4.47 (s, 1H), 4.14 (m, 1H), 3.78 (d, 2H), 2.71 (m, 1H), 1.70 (m, 2H), 1.80 (m, 2H), 1.57 (d, J = 6.8 Hz, 6H), 1.46 (s, 9H), 1.30 (m, 2H), 1.25 (m, 2H).

Crude **21g** (83.3 mg) and solid $NaBH(OAc)_3$ (650.0 mg, 3.0 mmol) were added to 5 mL of DCE. Benzaldehyde (24 μL, 1.2 mmol) was added in one portion and the mixture was stirred for 12 h. The reaction mixture was quenched with the addition of 1 mL of $NaHCO_3$ (aq, satd) and then stirred for an additional 10 min. The mixture was taken up into 5% K_2CO_3 (aq) and extracted three times with 15 mL portions of Et_2O. The combined organic extracts were washed with NaCl (aq, satd) and then dried (Na_2SO_4). The extract was decanted off and the solvent was removed under reduced pressure. The crude material was isolated on SiO_2 and eluted with hexane/EA (1:4) to give 72.1 mg (10%) of **23c** as a white hygroscopic solid. Mp 81.1–81.5 °C. SiO_2 TLC R_f 0.57 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 7.61 (s, 1H), 7.41 (d, J = 7.6 Hz, 2H), 7.31 (d, J = 7.2 Hz, 2H), 7.28 (q, 1H), 6.18 (d, J = 2.0 Hz, 1H), 6.02 (d, J = 2.0 Hz, 1H), 5.27 (s, 1H, NH), 4.47 (s, 1H), 4.14 (m, 1H), 3.78 (d, 2H), 2.71 (m, 1H), 1.70 (m, 2H), 1.80 (m, 2H), 1.57 (d, J = 6.8 Hz, 6H), 1.46 (s, 9H), 1.30 (m, 2H), 1.25 (m, 2H). Anal. Calcd for $C_{28}H_{38}Cl_4N_3O{\cdot}1H_2O$: C, 67.71; H, 8.12; N, 11.28. Found: C, 68.09; H, 7.77; N, 11.26.

6.21. *N*-Benzyl-1-isopropyl-6-(piperidin-4-ylmethoxy)-1*H*-benzo[*d*]imidazol-4-amine dihydrochloride (10)

tert-Butyl 4-((4-(benzylamino)-1-isopropyl-1*H*-benzo[*d*]imidazol-6-yloxy)methyl)piperidine-1-carboxylate **23c** (71.1 mg, 0.15 mmol) was dissolved into 50 mL EtOH and 5 mL of concd HCl at 0 °C. The reaction was stirred at 23 °C for 12 h. The solvent was then removed under reduced pressure. Recrystallization from MeOH afforded 60 mg (83%) of a white solid. Mp 218–220 °C. ^1H NMR (MeOD-d_4): δ 9.26 (s, 1H), 7.51 (s, 1H, NH) 7.42 (q, 2H), 7.35 (d, J = 7.2 Hz, 2H), 7.28 (q, 1H), 6.66 (d, J = 2.0 Hz, 1H), 6.42 (s, 1H, NH), 6.25 (d, J = 2.0 Hz, 1H), 4.48 (m, 1H), 3.95 (s, 1H), 3.43 (s, 2H), 3.30 (m, 1H), 3.01 (m, 2H), 2.14 (m, 2H), 2.13 (m, 2H), 1.58 (d, J = 6.8 Hz, 6H), 1.28 (m, 2H). Anal. Calcd for $C_{23}H_{32}Cl_2N_4O{\cdot}2.5H_2O$: C, 55.64; H, 7.51; N, 11.21. Found: C, 55.40; H, 7.38; N, 11.26.

6.22. 1-Isopropyl-6-((1-methylpiperidin-4-yl)methoxy)-4-nitro-1*H*-benzo[*d*]imidazole (21h)

Carbamate **21a** (650.0 mg, 1.6 mmol) was dissolved into 30 mL of EtOH and 3 mL of concd HCl was added dropwise at 0 °C. The reaction was stirred at 23 °C until the reaction was complete as determined by TLC. The solvent was removed under reduced pressure and resulting solid was then dissolved into 50 mL of 1 N HCl (aq) and washed three times with 10 mL portions of EA, neutralized, then adjusted to a pH 10 with 6 N NaOH (aq). This mixture was extracted three times with 15 mL portions of $CHCl_3$. The combined organic extracts were washed with a 5 mL aliquot of NaCl (aq, satd) and then dried (Na_2SO_4). The organic phase was decanted and the solvent was removed under reduced pressure. The crude yellow solid (100 mg, 0.38 mmol) obtained was dissolved in 0.7 mL of formic acid, 1 mL of 38% aqueous formaldehyde, and then subjected to 250 Watt microwave irradiation to a temperature of 120 °C for 5.5 min. After cooling the solvent was removed and the resultant yellow oil was taken up into 10 mL K_2CO_3 (aq, satd) and 10 mL EA. This was extracted three times with 10 mL portions of EA, washed with 5 mL of NaCl (aq, satd), and then dried (Na_2SO_4). The extract was decanted and the solvent was removed under reduced pressure to give 150.2 mg (30%) of **21h** as a brown solid. Mp 205–211 °C. SiO_2 TLC R_f 0.31 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 8.42 (s, 1H), 7.77 (d, J = 2.0 Hz, 1H), 7.23 (d, J = 2.0 Hz, 1H), 4.64 (m, 1H), 3.98 (d, J = 5.6 Hz, 2H), 3.61 (d, J = 10.0 Hz, 1H), 2.74 (s, 3H), 2.68 (m, 2H), 2.06 (m, 2H), 1.95 (m, 2H), 1.65 (d, J = 6.8 Hz, 6H), 1.26 (m, 2H). Anal. Calcd for $C_{17}H_{24}N_4O_3$: C, 61.43; H, 7.28; N, 16.86. Found: C, 61.45; H, 7.21; N, 16.58.

6.23. 1-Isopropyl-6-((1-methylpiperidin-4-yl)methoxy)-1*H*-benzo[*d*]imidazol-4-amine (22h)

Compound **21h** (148.3 mg, 0.4 mmol) was dissolved into 75 mL of EtOH and added to a Parr hydrogenation vessel previously charged with Pd/C (10% w/w, 8.1 mg). After three vacuum/purge cycles with H_2, the vessel was charged to 50 psi with H_2 and shaken for 5 h. The mixture was filtered through a pad of Celite and then the pad was washed with an additional 25 mL of EtOH. A 10 mL portion of concd HCl was added to resultant filtrate at 0 °C. The solvent was removed under reduced pressure. The resultant solid was dissolved into 10 mL K_2CO_3 (aq, satd) and extracted three times with 10 mL portions of Et_2O. The combined organic extracts were washed three times with NaCl (aq, satd) and then dried over Na_2SO_4. The organic phase was decanted and the solvent was removed under reduced pressure to give 76.7 mg (51.1%) of **22h** as a yellow, hygroscopic solid. Mp 37.0–38.5 °C. SiO_2 TLC R_f 0.22

(CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 7.73 (s, 1H), 6.23 (d, J = 2.0 Hz, 1H), 6.17 (d, J = 2.0 Hz, 1H), 4.64 (m, 1H), 3.98 (d, J = 5.6 Hz, 2H), 3.61 (d, J = 10.0 Hz, 1H), 2.74 (s, 3H), 2.68 (m, 2H), 2.06 (m, 2H), 1.95 (m, 2H), 1.65 (d, J = 6.8 Hz, 6H), 1.26 (m, 2H). Anal. Calcd for $C_{17}H_{26}N_4O$: C, 67.52; H, 8.67; N, 18.53. Found: C, 67.45; H, 8.41; N, 18.58.

6.24. *N*-Benzyl-1-isopropyl-6-((1-methylpiperidin-4-yl)methoxy)-1*H*-benzo[*d*]imidazol-4-amine hydrochloride (11)

Compound **22h** (63.7 mg, 0.16 mmol) and solid $NaBH(OAc)_3$ (650.0 mg, 3.0 mmol) were added to 5 mL of DCE. Benzaldehyde (18 μL, 1.2 mmol) was added in one portion and the mixture was stirred for 12 h. The reaction mixture was quenched with the addition of 1 mL of $NaHCO_3$ (aq, satd) and stirred for 10 min. The mixture was taken up into 5% K_2CO_3 (aq) and then extracted three times with 15 mL portions of Et_2O. The combined organic extracts were washed with 10 mL of NaCl (aq, satd) and then dried over Na_2SO_4. The extract was decanted and the solvent was removed under reduced pressure. The crude material was isolated on SiO_2 and eluted with EA. The HCl salt was prepared from 2 mL of ethereal HCl to give 38.1 mg (46%) of **9** as a white hygroscopic solid. Mp 92.1–92.7 °C. SiO_2 TLC R_f 0.39 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR (MeOD-d_4): δ 9.26 (s, 1H), 7.51 (s, 1H, NH) 7.42 (q, 2H), 7.35 (d, J = 7.2 Hz, 2H), 7.28 (q, 1H), 6.66 (d, J = 2.0 Hz, 1H), 6.25 (d, J = 2.0 Hz, 1H), 4.48 (m, 1H), 3.95 (s, 1H), 3.43 (d, 2H), 3.30 (m, 1H), 3.01 (m, 2H), 2.88 (s, 3H), 2.14 (m, 2H), 2.13 (m, 2H), 1.58 (d, 6H), 1.28 (m, 2H). Anal. Calcd for $C_{24}H_{34}Cl_2N_4O{\cdot}4H_2O$: C, 53.63; H, 7.88; N, 10.42. Found: C, 53.31; H, 7.59; N, 10.31.

6.25. 6-Butoxy-1-isopropyl-4-nitro-1*H*-benzo[*d*]imidazole (21i)

A dry 100 mL one-necked flask was charged with 1-isopropyl-4-nitro-1*H*-benzo[*d*]imidazol-6-ol, **19** (400.0 mg, 1.8 mmol), 1-iodobutane, **23i**, (0.3 mL, 2.72 mmol), and 2.5 mL of DMF. NaH (130.2 mg, 5.4 mmol) was added at 0 °C in two portions. This solution was stirred at 0 °C for 30 min and then at 23 °C for an additional 2 h. The reaction mixture was poured into a mixture of 10 mL EA and 10 mL K_2CO_3 (aq, satd). The mixture was extracted (3 × 10 mL) with EA and the combined extracts were washed with (2 × 5 mL) of NaCl (aq, satd) then dried (Na_2SO_4). The organic phase was decanted and the solvent was removed under reduced pressure. The residue was isolated on a SiO_2 column with hexanes/EA (1:2). Recrystallization from EA and hexane gave 200.6 mg (40%) of **21i** as a yellow solid. Mp 73.9–74.2 °C. SiO_2 TLC R_f 0.55 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 8.11 (s, 1H), 7.79 (d, J = 2.0 Hz, 1H), 7.21 (d, J = 2.0 Hz, 1H), 4.61 (m, 1H), 4.08 (t, J = 6.4 Hz, 2H), 1.84 (m, 2H), 1.64 (d, J = 6.8 Hz, 2H), 1.53 (m, 6H), 1.01 (t, J = 7.2 Hz, 3H). Anal. Calcd for $C_{14}H_{19}N_3O_3$: C, 60.63; H, 6.91; N, 15.15. Found: C, 60.67; H, 6.92; N, 15.14.

6.26. 6-Butoxy-1-isopropyl-1*H*-benzo[*d*]imidazol-4-amine (22i)

Compound **21i** (170.3 mg, 0.61 mmol) was dissolved into 75 mL of EtOH and added to a Parr hydrogenation vessel previously charged with Pd/C (10% w/w, 8.5 mg). After three vacuum/purge cycles with H_2, the vessel was charged to 50 psi with H_2 and shaken for 5 h. The mixture was filtered through a pad of Celite and then washed with an additional 25 mL of EtOH. The solvent was removed under reduced pressure to give 133.4 mg (88%) of **22i** as a pale brown, hygroscopic solid. Mp 33.9–35.1 °C. SiO_2 TLC R_f 0.34 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 7.74 (s, 1H), 6.25 (d, J = 2.0 Hz, 1H), 6.19 (d, J = 2.0 Hz, 1H), 4.47 (m, 1H), 3.96 (t, J = 6.4 Hz, 2H), 1.78 (m, 2H), 1.57 (d, J = 6.8 Hz, 6H), 1.56 (m, 2H), 0.97 (t, J = 7.2 Hz, 3H). Anal. Calcd for $C_{14}H_{21}N_3O$: C, 67.98; H, 8.56; N, 16.99. Found: C, 68.23; H, 8.54; N, 16.82.

6.27. *N*-Benzyl-6-butoxy-1-isopropyl-1*H*-benzo[*d*]imidazol-4-amine (12)

Compound **22i** (110.0 mg, 0.44 mmol) and solid $NaBH(OAc)_3$ (282.8 mg, 1.3 mmol) were suspended in 2.5 mL of DCE. Benzaldehyde (54.0 μL, 0.5 mmol) was added in one portion and the mixture was stirred for 12 h. The reaction mixture was quenched with the addition of 1 mL of $NaHCO_3$ (aq, satd) and stirred for an additional 10 min. The mixture was taken up into 25 mL of 5% K_2CO_3 (aq, satd) and then extracted three times with 15 mL portions of Et_2O. The combined extracts were washed with three 5 mL portions of NaCl (aq, satd) and then dried (Na_2SO_4). The extract was decanted and the solvent was removed under reduced pressure. The crude material was isolated on SiO_2 with hexanes/EA (1:3). This material was recrystallized from EA and hexanes to give 75.0 mg (50%) of **12** as a white crystalline solid. Mp 64.5–65.9 °C. SiO_2 TLC R_f 0.61 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 7.70 (s, 1H), 7.41 (d, J = 7.6 Hz, 2H), 7.31 (t, J = 7.2 Hz, 2H), 6.20 (d, J = 2.0 Hz, 1H), 6.05 (d, J = 2.0 Hz, 1H), 5.23 (m, 1H), 4.48 (d, J = 6.8 Hz, 2H), 3.95 (t, J = 6.8 Hz, 2H), 1.77 (m, 2H), 1.58 (d, J = 6.8 Hz, 6H), 1.51 (m, 2H), 0.97 (t, J = 7.6 Hz, 3H). Anal. Calcd for $C_{21}H_{27}N_3O{\cdot}1/7H_2O$: C, 74.18; H, 8.09; N, 12.36. Found: C, 74.17; H, 7.98; N, 12.37.

6.28. 1-Isopropyl-4-nitro-6-propoxy-1*H*-benzo[*d*]imidazole (21j)

A dry 100 mL one-necked flask was charged with 1-isopropyl-4-nitro-1*H*-benzo[*d*]imidazol-6-ol, **19**, (250.0 mg, 1.1 mmol), 1-iodopropane **23j** (0.22 mL, 2.30 mmol), and 2 mL of DMF. NaH (81.4 mg, 3.4 mmol) was added at 0 °C in two portions. This solution was stirred at 0 °C for 30 min and at 23 °C for an additional 2 h. The reaction mixture was poured into a mixture of 10 mL EA and 10 mL of K_2CO_3 (aq, satd). The mixture was extracted three times with 10 mL portions of EA and the combined extracts were washed with 10 mL of NaCl (aq, satd) and dried (Na_2SO_4). The extract was decanted and the solvent was removed under reduced pressure. The crude material (180.9 mg) was isolated on SiO_2 and eluted with hexanes/EA (1:3). Recrystallization from EA gave 163.6 mg (55%) of **23j** as a yellow solid. Mp 121.1–121.9 °C. SiO_2 TLC R_f 0.51 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 8.11 (s, 1H), 7.79 (d, J = 2.0 Hz, 1H), 7.21 (d, J = 2.0 Hz, 1H), 4.61 (m, 1H), 4.05 (q, J = 6.8 Hz, 2H), 1.89 (m, 2H), 1.64 (d, J = 6.8 Hz, 6H), 1.09 (t, J = 7.2 Hz, 3H). Anal. Calcd for $C_{13}H_{17}N_3O_3$: C, 59.30; H, 6.51; N, 15.96. Found: C, 59.21; H, 6.54; N, 15.58.

6.29. 1-Isopropyl-6-propoxy-1*H*-benzo[*d*]imidazol-4-amine (22j)

Compound **21j** (150.4 mg, 0.57 mmol) was dissolved into 75 mL of EtOH and added to a Parr hydrogenation vessel previously charged with Pd/C (10% w/w, 7.7 mg). After three vacuum/purge cycles with H_2, the vessel was charged to 50 psi with H_2 and shaken for 5 h. The mixture was filtered through a pad of Celite and then the pad was washed with an additional 25 mL of EtOH. The solvent was removed under reduced pressure to give 119.6 mg (90%) of **22j** as a pale brown, hygroscopic solid. Mp 30.2–30.9 °C. SiO_2 TLC R_f 0.32 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 7.74 (s, 1H), 6.27 (d, J = 2.0 Hz, 1H), 6.20 (d, J = 2.0 Hz, 1H), 4.47 (m, 1H), 3.93 (t, J = 6.4 Hz, 2H), 1.83 (m, 2H), 1.59 (d, J = 6.8 Hz, 6H), 1.05 (t, J = 7.2 Hz, 3H). Anal. Calcd for $C_{13}H_{19}N_3O{\cdot}1/5H_2O$: C, 65.91; H, 8.25; N, 17.74. Found: C, 66.11; H, 8.21; N, 17.39.

6.30. *N*-Benzyl-1-isopropyl-6-propoxy-1*H*-benzo[*d*]imidazol-4-amine (13)

Compound **22j** (100.0 mg, 0.43 mmol) and solid $NaBH(OAc)_3$ (272.6 mg, 1.3 mmol) were suspended in 2.5 mL of DCE. Benzaldehyde (53.0 μL, 0.5 mmol) was added in one portion and the mixture was stirred for 12 h. The reaction mixture was quenched with the addition of 1 mL of $NaHCO_3$ (aq, satd) and stirred for an additional 10 min. The mixture was taken up into 25 mL of 5% K_2CO_3 (aq, satd) and then extracted three times with 15 mL portions of Et_2O. The combined extracts were washed three times with 5 mL portions of NaCl (aq, satd) and then dried (Na_2SO_4). The extract was decanted and the solvent was removed under reduced pressure. Crude **13** was isolated on SiO_2 and eluted with hexanes/EA. This material was recrystallized from EA and hexanes gave 77.0 mg (55%) of **13** as a white crystalline solid. Mp 102.4–104 °C. SiO_2 TLC R_f 0.58 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 7.70 (s, 1H), 7.40 (d, J = 7.6 Hz, 2H), 7.31 (t, J = 7.2 Hz, 2H), 6.19 (d, J = 2.0 Hz, 1H), 6.05 (d, J = 2.0 Hz, 1H), 5.23 (m, 1H), 4.46 (d, J = 6.8 Hz, 2H), 3.90 (t, J = 6.4 Hz, 2H), 1.80 (m, 2H), 1.57 (d, J = 6.8 Hz, 6H), 1.02 (t, J = 7.6 Hz, 3H). Anal. Calcd for $C_{20}H_{25}N_3O$: C, 74.35; H, 7.90; N, 12.97. Found: C, 74.27; H, 7.79; N, 12.99.

6.31. 6-Ethoxy-1-isopropyl-4-nitro-1*H*-benzo[*d*]imidazole (21k)

A dry 100 mL one-necked flask was compound **19** (250.0 mg, 1.1 mmol), ethyl iodide, **23k**, (0.2 mL, 2.05 mmol) and 2 mL of DMF. NaH (81.4 mg, 3.4 mmol) was added at 0 °C in two portions. This suspension was stirred at 0 °C for 30 min and at 23 °C for an additional 2 h. The reaction mixture was poured into 10 mL EA and 10 mL K_2CO_3 (aq, satd). The mixture was extracted three times each with 10 mL portions of EA and the combined extracts were washed with 5 mL of NaCl (aq, satd) and then dried (Na_2SO_4). The extract was decanted and the solvent was removed under reduced pressure. The residue was loaded onto a SiO_2 column and eluted with hexanes/EA (1:3). Recrystallization from EA and hexane gave 169.0 mg (60%) of **21k** as a yellow solid. Mp 82.7–82.9 °C. SiO_2 TLC R_f 0.52 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 8.11 (s, 1H), 7.79 (d, J = 2.0 Hz, 1H), 7.21 (d, J = 2.0 Hz, 1H), 4.61 (m, 1H), 4.16 (q, J = 6.8 Hz, 2H), 1.64 (d, J = 6.8 Hz, 6H), 1.48 (t, J = 7.2 Hz, 3H). Anal. Calcd for $C_{12}H_{15}N_3O_3 \cdot 1/5H_2O$: C, 57.00; H, 6.14; N, 16.62. Found: C, 56.93; H, 6.00; N, 16.49.

6.32. 6-Ethoxy-1-isopropyl-1*H*-benzo[*d*]imidazol-4-amine (22k)

Compound **21k** (150.0 mg, 0.60 mmol) was dissolved into 75 mL of EtOH and added to a Parr hydrogenation vessel previously charged with Pd/C (10% w/w, 8.1 mg). After three vacuum/purge cycles with H_2, the vessel was charged to 50 psi with H_2 and shaken for 5 h. The mixture was filtered through a pad of Celite and the pad was washed with an additional 25 mL of EtOH. The solvent was removed under reduced pressure to give 128.0 mg (97%) of **22k** as a pale brown, hygroscopic solid. Mp 37.2–38.0 °C. SiO_2 TLC R_f 0.30 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 7.75 (s, 1H), 6.27 (d, J = 2.0 Hz, 1H), 6.20 (d, J = 2.0 Hz, 1H), 4.47 (m, 1H), 4.05 (q, J = 6.8 Hz, 2H), 1.57 (d, J = 6.8 Hz, 1H), 1.42 (t, J = 7.2 Hz, 3H). Anal. Calcd for $C_{12}H_{17}N_3O \cdot 1/3H_2O$: C, 63.98; H, 7.90; N, 18.05. Found: C, 63.83; H, 7.77; N, 17.78.

6.33. *N*-Benzyl-6-ethoxy-1-isopropyl-1*H*-benzo[*d*]imidazol-4-amine (14)

Compound **22k** (110.0 mg, 0.50 mmol) and $NaBH(OAc)_3$ (318.9 mg, 1.5 mmol) were suspended in 2.5 mL of DCE. Benzaldehyde (61.0 μL, 0.6 mmol) was added in one portion and the mixture was stirred for 12 h. The reaction mixture was quenched with the addition of 1 mL of $NaHCO_3$ (aq, satd) then stirred for an additional 10 min. The mixture was taken up into 25 mL of 5% K_2CO_3 (aq, satd) and extracted three times with 15 mL portions of Et_2O. The combined extracts were washed (3 × 5 mL) with NaCl (aq, satd) and then dried (Na_2SO_4). The extract was decanted and the solvent was removed under reduced pressure. The crude material was isolated on SiO_2 eluted with hexane/EA (1:3). This material was recrystallized from EA and hexanes to give 77.6 mg (50%) of **14** as a white crystalline solid. Mp: 124.1–124.7 °C. SiO_2 TLC R_f 0.57 (CH_2Cl_2/5% CH_3OH/0.1% NH_4OH). ^1H NMR ($CDCl_3$): δ 7.70 (s, 1H), 7.40 (d, J = 7.6 Hz, 2H), 7.32 (t, J = 7.2 Hz, 2H), 6.19 (d, J = 2.0 Hz, 1H), 6.03 (d, J = 2.0 Hz, 1H), 5.25 (m, 1H), 4.46 (m, 2H), 4.02 (q, J = 6.8 Hz, 2H), 1.57 (d, J = 6.8 Hz, 1H), 1.39 (t, J = 7.2 Hz, 3H). Anal. Calcd for $C_{19}H_{23}N_3O$: C, 73.76; H, 7.49; N, 13.58. Found: C, 73.49; H, 7.12; N, 13.64.

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