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Design, synthesis and biological evaluation of renin inhibitors guided by simulated annealing of chemical potential simulations

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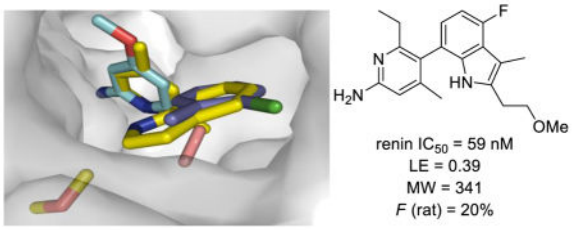
Abstract

We have applied Simulated Annealing of Chemical Potential (SACP) to a diverse set of ~150 very small molecules to provide insights into new interactions in the binding pocket of human renin, a historically difficult target for which to find low MW inhibitors with good bioavailability. In one of its many uses in drug discovery, SACP provides an efficient, thermodynamically principled method of ranking chemotype replacements for scaffold hopping and manipulating physicochemical characteristics for drug development. We introduce the use of Constrained Fragment Analysis (CFA) to construct and analyze ligands composed of linking those fragments with predicted high affinity. This technique addresses the issue of effectively linking fragments together and provides a predictive mechanism to rank order prospective inhibitors for synthesis. The application of these techniques to the identification of novel inhibitors of human renin is described. Synthesis of a limited set of designed compounds provided potent, low MW analogs (IC_{50}s < 100 nM) with good oral bioavailability (F >20–58%).

Graphical abstract



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Supplementary Material

List of fragments used in SACP; correlation plot of the validation set in 2G1Y; torsion angle analysis of representative heterocycles.

Keywords

Renin; Fragment-based simulations; Simulated Annealing of Chemical Potential; Hypertension; Physicochemical properties

1. Introduction

Detailed investigations of the enormous costs and the extensive amount of time involved in drug discovery have called into question the benefits of early optimization efforts which were aimed at creating potent, single-digit nanomolar compounds.[1–3] There is substantial evidence that early efforts to drive hyper-potency typically lead to large and hydrophobic compounds.[4] Gleeson[5] argues that such compounds inherently possess a dichotomy between physicochemical parameters associated with potency and those associated with desirable ADME characteristics. Analysis by Wenlock[6] indicated that in the development pipeline, failure of oral drugs was associated with the higher MW and LogP candidates. Another analysis by Oprea[7] added solubility to Wenlock's analysis and confirmed that drug-like molecules are more likely to be low MW (<425) compounds.

A prominent and salient example of the tension between the early drive for very high potency and the difficulty of developing a real drug is the decades-long search for a renin inhibitor to treat hypertension. The Renin–Angiotensin–Aldosterone System (RAAS) plays a significant role in hypertension by regulating blood pressure and extracellular fluid volume.[8, 9] It has been shown that blockade of the RAAS, either with an angiotensin converting enzyme inhibitor (ACEi) or with an angiotensin II (AngII) AT1 receptor blocker (ARB) reduces blood pressure (BP). Another method for therapeutic intervention of the RAAS pathway would be directly target renin, the first and rate-limiting step in the system. After more than 20 years of research in the pharmaceutical industry, aliskiren, a sub-nanomolar compound with less than 3% oral bioavailability[10] in humans, was finally approved as the only marketed renin inhibitor to date. Aliskiren has a molecular weight of 551, which is a contributor to its poor *in vivo* properties and one of the reasons for the continuing research on better alternatives. Yokokawa[11] has comprehensively summarized the progress between 2009–2012 on investigations into creating renin inhibitors with a better balance of potency to physicochemical properties. While these efforts have produced promising new molecules, there is still no marketed alternative to aliskiren, which is a testament to how difficult it has been to achieve both potency and desirable physicochemical properties simultaneously.

The analyses of Gleeson,[5] Wenlock[6] and Oprea[7] suggest that a compound with MW<425 and a potency of around 50 nM promises to have the right characteristics for drug development. One approach adopted by the fragment-based drug design (FBDD) community[12, 13] has been to start with low affinity, small molecular weight compounds. Multiple distinct fragments predicted to bind in several adjacent pockets can be linked together to form higher molecular weight, higher affinity compounds. Ligand efficiency (LE),[14] a metric used to describe affinity per molecular unit, is very useful to compare different scaffolds during this process. Renin, however, presents a unique challenge for

fragment-based drug design, because many very potent high molecular weight renin inhibitors have been reported. Designing compounds that conform to the Gleeson-Wenlock-Oprea constraints requires doing the opposite of standard FBDD, i.e. instead of growing fragments to make larger molecules, existing molecules must be shrunk by finding smaller fragments that can replace the larger fragments without materially degrading binding affinity. This task is quite difficult, because if the molecular weight is to be reduced by more than 100 Daltons, then multiple fragment replacements must be performed and thus the design process must be exceptionally robust, since even one incorrect fragment substitution could abolish all affinity. *In silico* Simulated Annealing of Chemical Potential[15] (SACP) is well suited to this problem, because it can efficiently screen chemical interactions in a specific protein environment and rank desirable opportunities for scaffold hopping, plus it is not limited to the solubility requirements of experimentally applied FBDD approaches.

Significant efforts over more than two decades by several companies were invested in developing non-peptidomimetic renin inhibitors such as the piperidines (**2**),[16] piperazinones (**3** and **4**)[17] and ureas (**5**) (Figure 1).[18] We chose the 2IKO[19] X-ray protein structure as the input for SACP, because the diaminopyrimidine scaffolds (**6–8**) reported by Pfizer[19] had lower pKa's (~8.0) compared to the more basic amines inherent in many renin inhibitors like **1** (pKa = 9.8) (Table 1). Maintaining basicity closer to physiological pH was expected to provide enhanced oral absorption.[20] Our goals were three-fold: find replacements for the diaminopyrimidine head group with similar pKa values; find neutral fragments with equal or higher affinity than the tetrahydroquinoline and dihydrobenzoxazinone moieties; and, keep the MW and cLogP of the inhibitors low to enhance the physicochemical characteristics for drug development.[5–7] The SACP technology was applied to the renin structure 2IKO using 150 fragments, resulting in the design of a small set of molecules with MW<425 and cLogP<4 predicted to have 100 nM affinity or better for renin. We were able to accomplish all the goals with a minimum of effort. Oral bioavailability from 20–58% was obtained with 341<MW<412 and 2.2<cLogP<3.8.

2. Materials and Methods

2.1. Computational methods

SACP is a way to determine the binding sites and the 3-dimensional orientations of small molecular fragments on the target protein, with binding affinity distinguished by excess chemical potential (average free energy per molecule). A major strength of the SACP technique is its ability to identify and reliably rank non-obvious fragment substitution patterns and chemotype replacements, including so-called scaffold hopping. This enables optimizing the properties of a particular class of molecules without appreciably degrading affinity or ligand efficiency – a central goal of lead optimization. Because SACP simulates how individual fragments separately interact with the protein, it suffers the same limitation as experimental NMR and X-ray fragment methods – it, by itself, provides no information about whether or not two different fragments predicted to bind with high affinity in adjacent sites on the protein can be chemically linked. Previously we demonstrated that binding sites can be identified using SACP to determine localized regions on a protein that have high affinity for a diversity of fragments and a low affinity for water ("hot-spots").[21] Once such a

site is determined, SACP can then be applied with a binding site biasing option, including a detailed balance correction, to oversample a critical locality on a protein in order to get more information, not just on affinity, but also orientations of the fragment relative to the protein and other fragments. Such biased sampling is essential to discover poses in narrow clefts of the protein ("letter-in-envelope"). Chemical potential (μ) is a free energy metric, and because the method is thermodynamically principled, one can develop an understanding of the requirements of the ligand-protein interaction. By comparing the individual fragments' chemical potentials in binding sites, one can predict their relative affinities and reliably prioritize which compounds to make. Resources can be conserved by focusing on the higher affinity fragments, thereby reducing the number of compounds to make and test compared to conventional empirical SAR efforts.

To address the fundamental linkage problem, we have generalized SACP to a process called constrained fragment annealing (CFA).[21] CFA grand canonical sampling goes one step further than SACP by biasing the sampling of two (or more) fragments simultaneously to oversample binding modes to the protein that also place the fragments in correct binding geometries[22] for linkage. This distorts each fragment's binding mode to the protein from its optimal position and thus the chemical potential of the individual fragment-protein interaction is raised. The best candidates for linkage from CFA occur when two adjacent fragments align with good bond lengths and angles with the minimal distortion of the optimal binding chemical potential of the individual fragments. This methodology of using SACP followed by CFA is a theoretically grounded method to first assess which individual fragments bind with the highest affinity at a particular site on a protein and then to assess if two distinct high affinity fragments at adjacent sites on the protein should be linked. CFA allows one to answer the question of where a full ligand, composed of two or more assembled fragments identified by SACP, wants to interact and in what orientation relative to the protein. The relative predicted affinity (FE Pred) of a ligand is calculated by summation of the fragments' individual chemical potential values less the solvation penalty ($\Delta\Delta G_s$)[23] for moving the ligand from bulk solvent to the interaction site.

2.2. Chemistry

The general strategy for construction of the aminopyridyl-substituted bicylic heterocycles was to prepare the appropriately substituted aminopyridines and bicyclic heterocycles separately and to couple at or near the final step via a traditional Suzuki reaction. Which coupling partner in this penultimate reaction contained the boronic ester acid depended on experimentation or availability of starting material. The substituted 2-aminopyridines utilized in this paper were commercially available or prepared as shown in Scheme 1 through bromination of the requisite pyridines. Synthesis of the tetrahydroquinoline derivatives **12–15** was accomplished by coupling of the known boronic acid ester **42**[24] with aminopyridines **41a–d**.

Coupling of the simple unsubstituted or 3-methylindoles was carried out using the commercially available indoleboronic acid esters **43a–b** to provide **16–20** as shown in Scheme 2. Preparation of fluoro-substituted indoles was initiated using the Bartoli indole synthesis[25] to provide the 3-methylindoles **46a–c** in one step (Scheme 3). For the simple

mono- and difluoroindole analogs, Suzuki coupling was consistently unsuccessful or resulted in a very poor yield of product when the boronic acid ester was contained on the indole coupling partner. However, synthesis of **21–23** was accomplished when the 5-bromo-2-aminopyridines were converted to their corresponding boronates **47a–b** and reacted with the requisite bromofluoroindoles **46a–c**.

Elaboration of the 2-position of the indoles with substituents extending into the S3sp region was performed by first formylating or iodinating the indole ring, followed by further extension via Wittig or Shonogashira chemistries as shown in Scheme 4.

For the synthesis of **24**, it was found that protection of the indole NH was necessary to complete elaboration of the 2-position, as shown in Scheme 5. Following deprotection, **53** was smoothly transformed into the final compound. Similarly, coupling of the aminopyridine boronates **50a–e** and **51** with the appropriate substituted bromoindoles was readily accomplished, followed by hydrogenation of the side chains to provide **25–31** (Scheme 6).

Preparation of the benzoxazinone derivatives **32–34** was accomplished by coupling of the known boronates **54a–b**[18b] with the requisite bromoaminopyridines (Scheme 7). Synthesis of **35** required the assembly of the necessary boronate from **55**.

3. Results and Discussion

To validate the technological fit of SACP to the design of renin inhibitors, compounds **6a–d** and **7a–d** were deconstructed into their corresponding fragments, and CFA was applied using the 2IKO structure. By definition, a plot of the absolute free energy of the binding interaction versus the log of the inhibition constant (pK_i) should be a straight line. Instead of pK_i we use pIC_{50}, which is easier to obtain and is proportional to the pK_i if the assay conditions are kept the same. For the literature compounds, a plot of FE Pred versus the pIC_{50} data shows an excellent correlation ($R^2 = 0.82$) (Figure 2). Although 2IKO contains a weak ligand (**8**; $IC_{50} = 4$ μM)[19] that lacks a sidechain in the S3 selectivity pocket ($S3^{sp}$), the validation demonstrates that SACP can predict relative affinity, even in regions not occupied by an X-ray ligand. The crystal structure of **7a** bound to renin (2G1Y), with an alkyl ether occupying the $S3^{sp}$ site was also available. Validation of the same set of data in the 2G1Y structure gave a comparable correlation ($R^2 = 0.84$)[26] (Supporting Information). Experience has shown that it is not uncommon to observe three-fold variability in measured IC_{50} values for the same compound based on day-to-day experimental variability in assays. To this extent, we add error bars of $pIC_{50} = 0.5$ to the graphs to represent that three-fold variance. If we can reliably predict the affinity of the compounds within the variance of the biological assay, the model can be used to predict the affinity of the next compound to be made and, just as importantly, which compounds would likely be less potent and need not be made. Using a reliable predictive model also allows the chemist to select compounds for synthesis that would maintain or enhance those key physicochemical characteristics considered pertinent to drug development.

Our first goal was to replace the diaminopyrimidine group present in literature compounds **6–8**. This heterocycle is protonated in its interaction with the aspartates at the catalytic site

of renin. The usual set of fragments used in SACP consists of about 150 neutral fragments ranging from extremely small fragments such as methane, water, methanol and acetamide to moderately large fused heterocycles with a maximum MW of 170. The fragments are chosen to represent readily prepared functional groups commonly found in drugs. Experience has shown that the overlap of this small group of fragments readily suggests alternative or more complicated moieties that are readily analyzed by CFA. This set was supplemented by 15 protonated fragments predicted to have pKa's <8.5. A full list of the fragments simulated can be found in the Supporting Information. Fragments **9–11** were used for simulations. Two protonated pyridine fragments, **9a** and **10a**, were compared to the pyrimidine head group **11a** (Figure 3). **9a** and **10a** showed predicted binding affinity comparable to **11a** in the simulations. Of interest was the resulting replacement of the polar 4-amino substituent in **11** with the neutral Me and OMe substituents. An ethane fragment with good predicted affinity was found adjacent to the substituted aminopyridines, and could be linked to the pyridine 6-position such that the orientation of the Et group in **9b** and **10b** corresponded exactly to the orientation of that in the pyrimidine of **8** in the 2IKO X-ray structure. Subsequent CFA simulations predicted improved affinity for the ethyl analogs **9b** and **10b** as expected from filling of the lipophilic pocket between Val31 and Val122 (Table 2).

In the 2IKO structure the 4-amino group of **8** interacts via hydrogen bonds to the O-atom on the side chain of Ser79 and O-atom of the backbone carbonyl of Thr80. Medicinal chemists involved in structure-based design emphasize the introduction of polar functionalities in ligands for H-bond interactions with a protein to increase the enthalpy of the interactions. In fact, in a discussion of the X-ray structures of **7c–d** and **8**, the authors refer to this H-bonded network as contributing significantly to the binding enthalpy of the interactions.[19] Here the SACP suggests two fragment structures with considerably less polarity and less or no ability to H-bond to these amino acids, yet with similar predicted binding affinity. Although the pyrimidines **11a–b** have better enthalpic interactions than **9a–b** or **10a–b** with Ser79 and Thr80, the difference in the enthalpic interactions is not as pronounced when the whole protein is considered (Table 2, column 4). More importantly, the solvation penalty (Table 2, column 3) plays a larger role in determining the overall relative affinity. Designed compounds **12–15** were synthesized and tested and compared to the literature compound **6c** (Table 3). Gratifyingly, the potencies for replacement of the pyrimidine head group by the pyridine analogs **13** and **15** were maintained. The importance of the 6-Et group was clearly shown by the much lower activity seen for compounds **12** and **14**. We note that the 6-ethylpyrimidine-2,4-diamine replacement by the 4-methyl or 4-methoxy 6-ethylpyridin-2-amine was relatively straight forward, and could have been discovered in a standard medicinal chemistry program, but the computation allowed us to explore many possibilities and only make and test the highest ranked compounds.

For compound **15** we considered that the Ser79 hydroxyl could either rotate or interact with the methoxy substituent of **15** via a water-mediated H-bond, accounting for some of the affinity observed. Molecular dynamics simulations of compounds **13** and **15** were performed to investigate the stability of the cavity created by the flexible loop containing Ser79 and Thr80. Over a 10 ns simulation time course the cavity was maintained, no water molecules penetrated this site, and the Ser79 hydroxyl did not rotate. When comparing the affinity of

6c to **13** and **15**, these results suggest that the improved enthalpic interaction due to the amino group is offset by the solvation penalty required by the interaction. Here we show that free energy ranking coupled with an efficient solvation penalty algorithm can discriminate the contributions from various chemotypes and suggest non-obvious, sometimes counterintuitive alternatives for synthesis.

Attention then focused on using the SACP of neutral fragments to find replacements for the tetrahydroquinoline and benzoxazinone moieties in compounds **6–7**. Based on their ranking and orientation relative to the 5-position of the ligand heterocycle in the 2IKO model, two fragments were of greatest interest - indole and benzimidazole. Indole was favored due to its smaller solvation penalty in this site. The orientation of the indole fragment when linked to the pyrimidine of the X-ray structure was close to the global minimum of the assembled compound **16**. Interestingly, the indole NH interacts with the protein forming an H-bond to the carbonyl of Gly223. In the 2G1R structure it is the amide NH of **7b** that interacts with Gly223, albeit in a different H-bonded configuration. A 2-chlorotoluene fragment, although ranked considerably weaker than indole, overlapped the benzo region of the indole fragment. This prompted us to analyze the interactions of over 30 halogenated benzimidazoles, indoles, and 3-alkyllindoles. This represents a common theme in design using such fragment data - larger fragments are suggested by the relative superposition of smaller overlapping fragments. This was seen here for multiple fragment orientations and was previously described for a methane fragment, highlighting the importance of the ethyl side chain of the aminoheterocycles interacting at the catalytic site. Using CFA, it is possible to analyze and rank such composite fragments not present in the original dataset within a few hours. The results of the analysis indicated that the fluoro-3-methylindole fragment patterns were preferred. Designed compounds **17–23** were synthesized and tested. The improvement in LE from **16** to **18,** where the protonated heterocycle was the only change, was encouraging. Also of note was the 36-fold improvement in activity seen by judicious use of a single extra heavy atom going from **17** to **19** and then to **20** as supported by the simulation data. The differences observed in the fluorosubstitution patterns **21–23** were within the error of the bioassay.

The 2-position of the indole was ideally positioned to explore $S3^{sp}$. The fragments we found in our simulation set occupying this pocket, alkanes (C1-C3), dimethyl ether, acetamides and some simple 5-membered ring heterocyles, replicate those previously reported in the literature. Analysis of alkyl substitution off the 2-position of the indole indicated that this 6,5-bicyclic ring system was more tolerant of a wide range of torsion angles compared to the 6,6-benzoxazinone and tetrahydroquinoline ring systems (see Supporting Information). The orientation of the indole allowed a linkage to an alkyl ether fragment that resulted in ligand design **26**, with a substitution pattern containing one less rotatable bond for the extension into $S3^{sp}$ than that found in the tetrahydroquinolines **6a–c** and the benzoxazinones **7a** and **7c**. The reduction in the overall entropy of this side chain by removal of a rotatable bond was expected to improve the affinity of the interaction as well. Further extension of the side chain to a different alkyl ether fragment in an alternative orientation of higher predicted affinity provided **30**. Upon testing **30** was found to be comparable in activity to **26,** illustrating the trade-off between affinity and entropy associated with the longer side chain.

The correlation plot for the indoles containing the 2-amino-6-ethyl-4-methylpyridyl moiety is shown in Figure 4. The predicted binding pose of the most potent indole derivative **27** illustrates the interactions with Gly223 and the $S3^{sp}$ pocket compared to the 2IKO and 2G1R X-ray ligands (Figures 5 and 6).

Unlike the alkyl ether substitutions, linkage from the indole to an acetamide or a heterocyclic fragment could not be achieved without imposing a large conformational penalty upon binding. This was predicted by CFA and exemplified by the much poorer affinity observed for **31**. Using predictive ranking of the substituents, it was possible to drive the potency to 59 nM by synthesizing only 13 compounds (Table 4) while maintaining LE.

Having established the substituted pyridine as a replacement for the pyrimidine in the tetrahydroquinoline scaffold, and considering its activity in the indole series, we examined this head group replacement in the potent benzoxazinone Pfizer scaffold. The 6-ethyl-2-aminopyridine groups improved affinity 2- to 3-fold,[28] with a modest improvement in LE (Table 5, 7b *vs* **32** and **34**). The SACP data suggested that an appropriately orientated amide would have a higher affinity in the $S3^{sp}$ pocket than an ether. The amides **32** and **33** and the carbamate **35** do indeed show higher affinity than the ether analog **34** because, in contrast to the indole analog **31,** they can adopt a low energy conformation when linked to the benzoxazinone scaffold.

The three most potent compounds, **27**, **32** and **33,** were chosen for pharmacokinetic studies in rats. Data is shown in Table 6. All three compounds achieved maximum concentrations upon oral dosing that were many times greater than their corresponding in vitro renin IC_{50} values, and they demonstrated reasonable half-lives and good oral bioavailability. In Table 7, we compare key measurements of ligand optimization (potency, MW, and cLogP) for the synthesized compounds with those presented in the literature for compound **7b**. Since the values obtained for the standards used were three to seven times higher than reported elsewhere, the LE values were normalized to **7b** (Table 7, column 7). Aliskiren **1, 7c** and **7d** were not as efficient as **7b** at interacting with the protein, and their bioavailability was poor, ranging from 2–12%. Manipulation of the side chain of **7b** to give **7e** improved bioavailability at the expense of LE. In addition cLogP increased significantly to >5. In contrast, compounds **27**, **32** and **33** maintained or improved LE relative to **7b**, exhibited a lower cLogP range (2.2–3.8) and resulted in oral bioavailabilities from 20–58%.

4. Conclusion

These results demonstrate that the SACP and CFA techniques can be utilized as effective medicinal chemistry design tools to rapidly search for, and rank novel chemotype and substituent replacements. The data generated frequently suggests non-obvious ligands and substitutions. With a predictive tool in hand, only a limited number of compounds need to be assessed for affinity. The combination of SACP+CFA has broad applicability to all aspects of discovery and optimization provided structural information is available. It is complementary to experimental FBDD approaches, allowing one to assess alternative chemotype replacements that might be too insoluble to be addressed early in the experimental phase or *a priori* to rank the effectiveness of adding functionality. Applying

these techniques, we were able to achieve our initial goals of lower MW and cLogP with new fragments, and to efficiently identify novel inhibitors of human renin with increased LE and comparable or improved bioavailability relative to literature comparators.

5. Experimental

5.1. General methods

All solvents and reagents were obtained from commercially available sources and used without further purification. Reactions were carried out under nitrogen atmosphere unless otherwise stated. NMR was performed on a Varian Oxford system using either tetramethylsilane (TMS) or the appropriate solvent reference as an internal standard. Column chromatography was carried out using 230–400 mesh silica gel. Preparative HPLC was performed on a Gilson GX-281 system using conditions specified in the experimental descriptions. Analytical LC/MS was performed on an Agilent 1200 purification system coupled to an Agilent 6110 quadrupole mass spectrometer (column: Halo 2.7 μm C18 50 × 4.6 mm; injection volume: 0.8 μL; flow rate: 1.8 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 5:95 CH_3CN:water (0.05% v/v formic acid) to 95:5 CH3CN:water (0.05% v/v formic acid) over 1 min, with a hold for 1 min). All compounds were isolated as amorphous solids unless noted otherwise. The purity of all compounds was determined to be >95% by HPLC analysis. Experiments were performed at BioDuro, LLC.

5.2. 5-(1-(3-Methoxypropyl)-1,2,3,4-tetrahydroquinolin-7-yl)- 4,6-dimethylpyridin-2-amine (12)—A mixture of **42** (180 mg, 0.54 mmol), 2-amino-5-bromo-4,6-dimethylpyridine (109 mg, 0.54 mmol), tetrakis(triphenylphosphine)palladium(0) (31 mg, 0.03 mmol), and aqueous Na_2CO_3 solution (2 N, 0.7 mL) in dioxane (5 mL) was stirred at 80 °C overnight under argon. The mixture was cooled to room temperature, filtered, and the filtrate was concentrated under vacuum. The crude residue was purified by silica gel column chromatography, eluting with 20:1 petroleum ether: EtOAc, to give 88 mg of impure product. The resulting material was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 30:70 CH_3CN:water (0.1% v/v TFA) to 50:50 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **12** (53 mg, 30%) as the TFA salt as a yellow oil. ^1H NMR (300 MHz, $CDCl_3$) δ 14.72 (br s, 2H), 7.17 (d, 1H, J = 6.0 Hz), 6.84 (s, 1H), 6.69 (d, 1H, J = 6.0 Hz), 6.52 (s, 1H), 5.32 (br s, 5H), 3.47–3.44 (m, 6H), 3.31 (s, 3H), 2.92 (m, 2H), 2.28 (s, 3H), 2.13–2.08 (m, 5H), 1.94 (m, 2H). LCMS 326 $[M + H]^+$.

5.3. 6-Ethyl-5-(1-(3-methoxypropyl)-1,2,3,4-tetrahydroquinolin-7-yl)-4-methylpyridin-2-amine (13)—A mixture of **42** (200 mg, 0.6 mmol), 2-amino-5-bromo-6-ethyl-4-methylpyridine (130 mg, 0.6 mmol), tetrakis(triphenylphosphine)palladium(0) (35 mg, 0.03 mmol), and aqueous Na_2CO_3 solution (2 *N*, 0.7 mL) in dioxane (5 mL) was stirred at 80 °C overnight under argon. The mixture was cooled to room temperature, filtered, and the filtrate was concentrated under vacuum. The crude residue was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 25:75

CH_3CN:water (0.1% v/v TFA) to 50:50 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **13** (12 mg, 4%) as the TFA salt as a yellow oil. ^{1}H NMR (300 MHz, CD_3OD) δ 8.45 (s, 1H), 6.92 (d, 1H, J = 0.6 Hz), 6.56 (s, 1H), 6.32 (s, 1H), 6.23 (d, 1H, J = 0.9 Hz), 3.28–3.27 (m, 2H), 3.26–3.24 (m, 3H), 2.81–2.76 (m, 2H), 2.45–2.41 (m, 2H), 2.01 (s, 3H), 1.95–1.93 (m, 2H), 1.78–1.76 (m, 2H), 1.09 (t, 3H, J = 7.5 Hz). LCMS 340 $[M + H]^+$.

5.4. 4-Methoxy-5-(1-(3-methoxypropyl)-1,2,3,4-tetrahydroquinolin-7-yl)pyridin-2-amine (14)

(41c): A mixture of 2-amino-4-methoxypyridine (2.4 g, 19 mol) and N-bromosuccinimide (3.1 g, 17 mmol) was dissolved in acetic acid (6 mL) and stirred at room temperature for 1 h. The reaction mixture was concentrated under vacuum, basified with saturated aqueous K_2CO_3 and extracted with EtOAc. The combined organics were dried, concentrated under vacuum, and purified by silica gel column chromatography, eluting with 50:1 CH_2Cl_2:MeOH, to give **41c** (1.85 g, 47%). LCMS 203 [M+H]+.

A mixture of **42** (100 mg, 0.3 mmol), **41c** (61 mg, 0.3 mmol), tetrakis(triphenylphosphine)palladium(0) (18 mg, 0.015 mmol), and aqueous Na_2CO_3 solution (2 *N*, 0.3 mL) in dioxane (5 mL) was stirred at 80 °C overnight under argon. The mixture was cooled to room temperature, filtered, and the filtrate was concentrated under vacuum. The crude residue was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 25:75 CH_3CN:water (0.1% v/v TFA) to 50:50 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **14** (25 mg, 19%) as the TFA salt as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.65 (s, 1H), 6.98 (d, 1H, J = 9.0 Hz), 6.77 (s, 1H), 6.64 (d, 1H, J = 9.0 Hz), 3.97 (s, 3H), 3.48–3.34 (m, 6H), 3.32 (s, 3H), 2.81–2.77 (m, 2H), 2.03–1.95 (m, 2H), 1.92–1.83 (m, 2H). LCMS 328 $[M+H]^+$.

5.5. 6-Ethyl-4-methoxy-5-(1-(3-methoxypropyl)-1,2,3,4-tetrahydroquinolin-7-yl)pyridin-2-amine (15)

(37): A solution of sodium methoxide in MeOH (33 mL, 1*M*, 33 mmol) was added to a suspension of 2,4,6-trichloropyridine (**36**) (6.0 g, 33 mmol) in MeOH (82 mL) and stirred for 5 h at room temperature. The reaction mixture was poured into water (200 mL). The solid was removed by filtration, and the filtrate was concentrated under vacuum to give **37** (4.8 g, 80%) as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.19 (s, 2H), 3.89 (s, 3H). LCMS 178.0, 180.1 $[M + 1]^+$.

(38): A mixture of **37** (2.2 g, 12.5 mmol), benzophenone imine (2.7 g, 15.0 mmol), palladium (II) acetate (280 mg, 1.2 mmol), 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (777 mg, 1.2 mmol) and Cs_2CO_3 (8.1 g, 25.0 mmol) in toluene (30 mL) was stirred at 100 °C overnight under argon. The mixture was cooled to room temperature and filtered. The filtrate was concentrated under vacuum and the resulting residue was purified by silica gel column chromatography, eluting with a gradient of 50:1 to 10:1 petroleum ether:EtOAc, to give **38** (900 mg, 20%) as a brown oil. LCMS 323.1 $[M + 1]^+$.

(39): A mixture of **38** (900 mg, 2.8 mmol), 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (650 mg, 4.2 mmol), tetrakis(triphenylphosphine)palladium(0) (650 mg, 0.56 mmol) and Cs_2CO_3 (1.8 g, 5.6 mmol) in toluene/water (4:1, 20 mL) was stirred at 100 °C overnight under argon. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under vacuum and the resulting residue was purified by silica gel column chromatography, eluting with a gradient of 200:1 to 100:1 CH_2Cl_2:MeOH, to give the desired vinylpyridine (460 mg, 52%) as a brown oil. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.68 - 7.66 (m, 2H), 7.57 - 7.44 (m, 3H), 7.31 - 7.29 (m, 3H), 7.17 - 7.149 (m, 2H), 6.61 - 6.52 (m, 2H), 6.16 (d, 1H, J = 2.1 Hz), 6.02 (dd, 1H, J_1 = 17.4 Hz, J_2 = 2.1 Hz), 5.31 (dd, 1H, J_1 = 10.6 Hz, J_2 = 1.5 Hz), 3.68 (s, 3H). LCMS 315.1 $[M + 1]^+$. A solution of the vinylpyridine (460 mg, 1.46 mmol) in 1*M* HCl/THF (10 mL) was stirred overnight at room temperature. The solvent was evaporated under reduced pressure to give the hydrochloride salt of **39** (160 mg, 73%) as a white solid. LCMS 151.2 $[M + 1]^+$.

(41d): A suspension of **39** (160 mg, 1.06 mmol) and 12% palladium on carbon (20 mg) in MeOH (10 mL) was hydrogenated under 1 atm of hydrogen (balloon) with stirring overnight at room temperature. The reaction mixture was filtered, and the filtrate was concentrated under vacuum. The resulting residue was diluted with CH_2Cl_2 (30 mL) and washed with saturated aqueous $NaHCO_3$ (30 mL). The organic phase was washed with brine (20 mL), dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography, eluting with a gradient of 200:1 to 50:1 CH_2Cl_2:MeOH, to give **40d** (120 mg, 74%) as a yellow oil. LCMS $[M+1]^+$ 153.1. To a solution of **40d** (120 mg, 1.06 mmol) in CH_3CN (10 mL) was added N-bromosuccinimide (126 mg, 0.71 mmol), and the reaction mixture was stirred in the dark at room temperature for 2 h, then diluted with water (30 mL) and extracted with ethyl acetate (3 × 30 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4 and concentrated under vacuum to give **41d** (145 mg, 80%) as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d_6)δ 5.97 (br s, 2H), 5.94 (s, 1H), 3.76 (s, 3H), 2.63 (q, 2H, J = 8.0 Hz), 1.13 (t, 3H, J = 8.0 Hz). LCMS 231.0, 233.0 $[M + 1]^+$.

A mixture of **41d** (120 mg, 0.52 mmol), **42** (172 mg, 0.52 mmol), tetrakis(triphenylphosphine)palladium(0) (120 mg, 0.11 mmol) and Cs_2CO_3 (338 mg, 10.4 mmol) in dioxane (10 mL) and water (2.5 mL) was stirred at 100 °C overnight under argon. The mixture was cooled to room temperature, filtered, and the filtrate was concentrated under vacuum. The crude residue was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 20:80 CH_3CN:water (0.1% v/v TFA) to 55:45 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **15** (50 mg, 27%) as the TFA salt as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.58 (br s, 1H), 11.50 (br s, 1H), 7.50 (br s, 1H), 7.06 (d, 1H, J = 7.8 Hz), 6.79 (s, 1H), 6.65 (d, 1H, J = 7.7 Hz), 6.10 (s, 1H), 3.72 (s, 3H), 3.45 - 3.31 (m, 6H), 3.22 (s, 3H), 2.83 (t, 2H, J = 6.5 Hz), 2.45 (q, 2H, J = 7.5 Hz), 2.07 - 1.99 (m, 2H), 1.89 - 1.82 (m, 2H), 1.08 (t, 3H, J = 7.5 Hz). LCMS 356.3 $[M + 1]^+$.

5.6. 6-Ethyl-5-(1H-indol-7-yl)pyrimidine-2,4-diamine (16)

(44): A mixture of 2,4-diamino-6-ethylpyrimidine (543 mg, 3.93 mmol), N-iodosuccinimide (884 mg, 3.93 mmol) in methanol (10 mL) was stirred at room temperature for 30 min, at which time analysis by TLC showed the starting material was completely consumed. The resulting mixture was concentrated and purified by silica gel chromatography, eluting with 50:50 petroleum ether:EtOAc, to give **44** (400 mg, 40 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.29 (br s, 2H), 6.03 (br s, 2H), 2.57 (q, 2H, J = 7.5 Hz), 1.09 (t, 3H, J = 7.5 Hz). LCMS 265.1 $[M + H]^+$.

A mixture of **44** (200 mg, 0.758 mmol), indole-7-boronic acid pinacol ester (184.1 mg, 0.758 mmol), tetrakis(triphenylphosphine)palladium(0) (87.5 mg, 0.076 mmol), and Na_2CO_3 (201 mg, 1.89 mmol) in dioxane/water (4:1, 10 mL) was stirred at 100 °C overnight under argon. The reaction mixture was cooled to room temperature and concentrated under vacuum. The resulting residue was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 3 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 25:75 CH_3CN:water (0.1% v/v TFA) to 45:55 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **16** (17 mg, 6%) as the TFA salt. ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.06 (br s, 1H), 8.05 (br s, 1H), 7.63 (d, 1H, J = 8.7 Hz), 7.34 (t, 1H, J = 2.7 Hz), 7.10 (t, 1H, J = 7.5 Hz), 6.94 (d, 1H, J = 7.5 Hz), 6.56 (br s, 1H), 6.52–6.50 (m, 1H), 2.20 - 2.02 (m, 2H), 0.94 (t, 3H, J = 7.5 Hz). LCMS 254.1 $[M + H]^+$.

5.7. 5-(1H-Indol-7-yl)-4,6-dimethylpyridin-2-amine (17)—A mixture of indole-7-boronic acid pinacol ester (**43a**) (243 mg, 1 mmol), **41a** (200 mg, 1 mmol), tetrakis(triphenylphosphine)palladium(0) (58 mg, 0.05 mmol), and aqueous Na_2CO_3 solution (2*M*, 1.25 mL) in dioxane (5 mL) was stirred at 100 °C for 14 h under argon. The mixture was cooled to room temperature, poured into ice water (20 mL) and extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with brine, dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by preparative TLC (20:1 CH_2Cl_2:MeOH), then recrystallized (5:1 hexane:EtOAc, 6 mL) to give **17** (60 mg, 25%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.45 (br s, 1H), 7.65 (d, 1H, J = 9.0 Hz), 7.20 - 7.14 (m, 2H), 6.93 (d, 1H, J = 9.0 Hz), 6.61 5.97 (m, 1H), 6.35 (s, 1H), 4.47 (br s, 2H), 2.08 (s, 3H), 1.92 (s, 3H). LCMS 238.1 $[M + H]^+$.

5.8. 6-Ethyl-5-(1H-indol-7-yl)-4-methylpyridin-2-amine (18)—A mixture of indole-7-boronic acid pinacol ester (**43a**) (203 mg, 0.8 mmol), **41b** (150 mg, 0.7 mmol), tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.03 mmol), and aqueous Na_2CO_3 solution (2*M*, 0.9 mL) in dioxane (10 mL) was stirred at 100 °C under argon overnight. The mixture was cooled to room temperature, filtered, and concentrated under vacuum. The crude residue was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 10:90 CH_3CN:water (0.1% v/v TFA) to 50:50 CH_3CN:water (0.1% v/v TFA) over 10min) to give **18** (65 mg, 26%) as the TFA salt as a pale yellow solid. ^{1}H NMR (300 MHz, CD_3OD) δ 8.49 (*s*, 1H), 7.62 (*d*, 1H, J = 7.5 Hz), 7.20 (*d*, 1H, J = 3.6 Hz), 7.11 (*t*, 1H, J = 7.5 Hz), 6.89 (*d*, 1H, J = 7.2 Hz), 6.72 (*s*, 1H), 6.52

(*d*, 1H, *J* = 3.0 Hz), 2.51–2.34 (*m*, 2H), 1.95 (*s*, 3H), 1.03 (*t*, 3H, *J* = 1.5 Hz). LCMS 252 [M + H] $^{+}$.

5.9. 4,6-Dimethyl-5-(3-methyl-1H-indol-7-yl)pyridin-2-amine (19)—To a solution of **43b** (prepared from 7-bromo-3-methylindole according to Koshino, N. et al. (Sumitomo Chemical Co.). Nitrogen-Containing Aromatic Compounds and Metal Complexes. PCT Application WO 2011/052805 A1, 5 May 2011) (70 mg, 0.27 mmol), **41a** (65 mg, 0.327 mmol), and Cs_2CO_3 (176 mg, 0.54 mmol) in 3:1 dioxane:water (4 mL) was addded tetrakis(triphenylphosphine)palladium(0) (31 mg, 0.027 mmol) quickly under argon. The mixture was heated at 100 °C under argon overnight. Water (10 mL) was added and the resulting mixture was extracted with EtOAc (3 × 5 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under vacuum. The product residue was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 25:75 CH_3CN:water (0.1% v/v TFA) to 60:40 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **19** (20 mg, 30%) as the TFA salt as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 10.04 (br s, 1H), 7.58 (d, 1H, *J* = 9.0 Hz), 7.13 (t, 1H, *J* = 7.2 Hz), 6.99 (s, 1H), 6.89 (d, 1H, *J* = 9.0 Hz), 6.81 (s, 1H), 2.33 (s, 3H), 2.14 (s, 3H), 2.01 (s, 3H). LC-MS 252.2 [M + H] $^{+}$.

5.10. 6-Ethyl-4-methyl-5-(3-methyl-1H-indol-7-yl)pyridin-2-amine (20)—To a mixture of 3-methylindole-7-boronic acid pinacol ester (**43b**) (prepared from 7-bromo-3-methylindole according to Koshino, N. et al. Nitrogen-Containing Aromatic Compounds and Metal Complexes. PCT Application WO 2011/052805 A1, 5 May 2011) (70 mg, 0.27 mmol), **41b** (70 mg, 0.327 mmol), and Cs_2CO_3 (176 mg, 0.54 mmol) in dioxane/water (3:1, 4 mL) was added tetrakis(triphenylphosphine)palladium(0) (31 mg, 0.027 mmol) quickly under argon. The reaction mixture was stirred at 100 °C under argon overnight and cooled to room temperature. Water (10 mL) was added and the resulting mixture was extracted with EtOAc (3 × 5 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under vacuum. The crude residue was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 20:80 CH_3CN:water (0.1% v/v TFA) to 70:30 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **20** (15 mg, 21%) as the TFA salt as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 10.08 (br s, 1H), 7.58 (d, 1H, *J* = 9.0 Hz), 7.13 - 7.10 (m, 1H), 6.98 (d, 1H, *J* = 1.2 Hz), 6.89 (dd, 1H, J_1 = 7.2 Hz, J_2 = 1.2 Hz), 6.81 (d, 1H, *J* = 0.9 Hz), 2.53–2.35 (m, 2H), 2.33 (d, 3H, *J* = 0.6 Hz), 1.97 (d, 3H, *J* = 1.2 Hz), 1.05 (t, 3H, *J* = 7.5 Hz). LCMS 266.2 [M + H] $^{+}$.

5.11. 6-Ethyl-5-(4-fluoro-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine (21)

(46a): To a suspension of 1-bromo-4-fluoro-2-nitrobenzene (**45a**) (2.0 g, 9.12 mmol) in THF (40 mL) at −78 °C was added 1-propenylmagnesium bromide (60 mL, 1*M* in THF, 60 mmol). The reaction mixture was warmed to room temperature, stirred for 2 h, and quenched with saturated aqueous NH_4Cl. The mixture was extracted with EtOAc (3 × 50 mL), and the combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, and concentrated under vacuum to afford **46a** (1.1 g, 55%) as a yellow solid. ^{1}H NMR (300

MHz, DMSO-d_6) δ 11.21 (br s, 1H), 7.32 - 6.96 (m, 2H), 6.68 (dd, 1H, J_1 = 12.0 Hz, J_2 = 9.0 Hz), 2.35 (s, 3H). GCMS 226, 228 $[M]^+$.

(47b): A mixture of **41b** (1.4 g, 6.5 mmol), 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi(1,3,2-dioxaborolane) (2.5 g, 9.8 mmol), tetrakis(triphenylphosphine)palladium(0) (2.3 g, 1.9 mmol) and potassium acetate (1.28 g, 13 mmol) in DMSO (15 mL) was stirred at 120 °C overnight under argon. The mixture was cooled to room temperature and poured into water (30 mL). The aqueous phase was extracted with EtOAc (3 × 100 mL), and the combined organic phases were washed with water (50 mL) and brine (50 mL), dried over Na_2SO_4, concentrated under vacuum and purified by silica gel column chromatography, eluting with 100:1 CH_2Cl_2: methanol, to give **47b** (0.4 g, 24%) as a brown oil. LC-MS 263 $[M + H]^+$.

A mixture of **46a** (150 mg, 0.66 mmol), **47b** (150 mg, 0.79 mmol), tris(dibenylideneacetone)dipalladium(0) (60 mg, 0.066 mmol), 2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (X-Phos) (40 mg, 0.066 mmol) and Cs_2CO_3 (430 mg, 1.32 mmol) in dioxane/water (4:1, 15 mL) was stirred at 100 °C overnight under argon. The mixture was cooled to room temperature, filtered, and concentrated under vacuum. The crude product was purified by silica gel column chromatography, eluting with a gradient of 200:1 to 50:1 CH_2Cl_2:MeOH, to give **21** (50 mg, 20%) as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (brs, 1H), 6.79 - 6.76 (m, 3H), 6.32 (s, 1H), 4.46 (s, 2H), 2.47 (s, 3H), 2.39 - 2.23 (m, 2H), 1.86 (s, 3H), 1.00 (t, 3H, J = 7.5 Hz). LCMS 284.1 $[M + H]^+$.

5.12. 6-Ethyl-5-(5-fluoro-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine (22)

(46b): To a solution of 2-bromo-4-fluoro-1-nitrobenzene (**45b**) (13.7 g, 62.5 mmol) in THF (20 mL) at −60 °C was added 1-propenylmagnesium bromide (500 mL, 0.5*M* in THF, 250 mmol) dropwise. The reaction mixture was stirred at −60 °C for 1 h, quenched with saturated aqueous NH_4Cl (100 mL) and extracted with EtOAc (2 × 500 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under vacuum. The crude residue was purified by silica gel column chromatography, eluting with 50:1 petroleum ether: EtOAc, to give **46b** (5.6 g, 39%) as a light brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (*s*, 1H), 7.17–7.12 (*m*, 2H), 7.06 (*s*, 1H), 2.27 (*d*, 3H, J = 1.6 Hz). GC-MS 227 $[M]^+$.

A mixture of **46b** (80 mg, 0.35 mmol), **47b** (113 mg, 0.42 mmol), tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.035 mmol), and Cs_2CO_3 (228 mg, 0.7 mmol) in dioxane (10 mL) and water (2 mL) was stirred at 100 °C overnight under argon, then cooled to room temperature. Water (10 mL) was added and the mixture was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 60:40 CH_3CN:water (0.05% v/v NH_4OH) to 90:10 CH_3CN:water (0.05% v/v NH_4OH) over 10 min) to give **22** (33 mg, 33%) as a light brown solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.17 (*dd*, 1H, J = 10.0 Hz, J = 4.0 Hz), 6.99 (*s*, 1H), 6.65 (*dd*, 1H, J = 8.0 Hz, J = 4.0 Hz), 6.45 (*s*, 1H), 2.42–2.21 (*m*, 5H), 1.86 (*s*, 3H), 0.99 (*t*, 3H, J = 8.0 Hz). LCMS 284.1 $[M + H]^+$.

5.13. 5-(4,5-Difluoro-3-methyl-1H-indol-7-yl)-6-ethyl-4-methylpyridin-2-amine (23)

(46c): To a mixture of 2-bromo-4,5-difluoronitrobenzene (**45c**) (238 mg, 1.0 mmol) in THF (10 mL) at 0 °C under nitrogen was added 1-propenylmagnesium bromide (20 mL, 0.5*M* in THF, 10 mmol) dropwise. The reaction mixture was stirred at room temperature overnight, poured into ice water (20 mL), and extracted with EtOAc (3 x10 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4, and concentrated under vacuum. The crude product was purified by silica gel column chromatography to afford **46c** (85 mg, 35%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.00 (*br s*, 1H), 7.12–7.17 (*m*, 1H), 2.41 (*s*, 3H).

A mixture of **46c** (110 mg, 0.45 mmol), **47b** (128 mg, 0.49 mmol), tetrakis(triphenylphosphine)palladium(0) (140 mg, 0.12 mmol), and Cs_2CO_3 (398 mg, 1.22 mmol) in water (2 mL) and dioxane (10 mL) was stirred at 100 °C overnight under argon. The reaction mixture was poured into ice water (15 mL) and extracted with EtOAc (3 × 5 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 30:70 CH_3CN:water (0.1% v/v TFA) to 50:50 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **23** (11 mg, 8%) as the TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 7.04 (*s*, 1H), 6.87–6.92 (*m*, 1H), 6.84 (*s*, 1H), 2.38–2.51 (*m*, 5H), 2.01 (*s*, 3H), 1.09 (*t*, 3H, *J*= 8.0 Hz). LCMS 302 [M + H] $^+$.

5.14. 6-Ethyl-5-(2-(methoxymethyl)-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine (24)

(52): To a suspension of sodium hydride (160 mg, 54 wt. % in oil, 3.6 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C under nitrogen was added **48d** (237 mg, 1 mmol). After warming to room temperature over 1 h, a solution of 2-(trimethylsilyl)ethoxymethyl chloride (200 mg, 1.2 mmol) in dry CH_2Cl_2 (2mL) was added. The mixture was stirred for 1 h at room temperature. Water (15mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried, concentrated under vacuum, and purified by silica gel column chromatography, eluting with 20:1 petroleum ether: EtOAc, to give **52** (368 mg, 100%) as a brown oil. ^{1}H NMR (300 MHz, DMSO) δ 10.19 (*s*, 1H), 7.82 (*d*, 1H, *J* = 6.0 Hz), 7.67 (*d*, 1H, *J* = 6.0 Hz), 7.14–7.09 (*m*, 1H), 6.26 (*s*, 2H), 3.43–3.32 (*m*, 2H), 2.61 (*s*, 3H), 0.76–0.71 (*m*, 2H), 0.14 (*s*, 9H). LCMS 392 [M + Na]$^+$. The reaction was repeated on a similar scale (1.14 mmol of **48d**) to provide an additional 410 mg of **52**.

(53): To a solution of **52** (740 mg, 2.0 mmol) dissolved in THF/MeOH (1:1, 10 mL) was added sodium borohydride (90 mg, 2.4 mmol) in portions at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and purified by silica gel column chromatography, eluting with 8:1 petroleum ether:EtOAc, to give the desired alcohol (560 mg, 70%) as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (*dd*, 1H, *J* = 7.8 Hz, 0.9 Hz), 7.41 (*dd*, 1H, *J* = 7.6 Hz, 0.8 Hz), 7.01–6.96 (*m*, 1H), 7.14–7.09 (*m*, 1H), 6.12 (*s*, 2H), 4.80 (*s*, 2H), 3.66–3.60 (*m*, 2H), 2.34 (*s*, 3H), 0.92–0.86 (*m*, 2H), −0.05 (*s*, 9H). LCMS 332 [M + H] $^+$.

To a suspension of sodium hydride (78 mg, 54 wt. % in oil, 1.76 mmol) in dry THF (10 mL) cooled to 0 °C under nitrogen was added the alcohol prepared above (500 mg, 1.35 mmol). After stirring for 1 h at 0 °C, methyl iodide (0.7 mL, 10.8 mmol) was added. The reaction mixture was stirred at room temperature overnight. Water was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were dried and concentrated under vacuum. The crude product was purified by silica gel column chromatography, eluting with 10:1 petroleum ether:EtOAc to give the desired SEM-protected methyl ether (510 mg, 98%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (*dd*, 1H, J = 7.8 Hz, 1.1 Hz), 7.39 (*dd*, 1H, J = 7.7 Hz, 1.1 Hz), 6.97–6.92 (*m*, 1H), 5.95 (*s*, 2H), 4.66 (*s*, 2H), 3.58–3.53 (*m*, 2H), 3.33 (*s*, 3H), 2.31 (*s*, 3H), 0.91–0.85 (*m*, 2H), −0.06 (*s*, 9H).

A mixture of the SEM-protected methyl ether prepared above (250 mg, 0.65 mmol), ethylenediamine (118 mg, 1.95 mmol) and tetrabutylammonium fluoride (3.9 mL, 1*M* in THF, 3.9 mmol) in THF (1 mL) was stirred at 70 °C under nitrogen for 4 h. The reaction mixture was cooled to room temperature, concentrated under vacuum, and purified by preparative TLC to give **53** (143 mg, 87%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.23 (*s*, 1H), 7.48 (*d*, 1H, J = 9.0 Hz), 7.34–7.31 (*m*, 1H, J = 9 Hz), 7.01–6.96 (*m*, 1H), 4.62 (*s*, 2H), 3.40 (*s*, 3H), 2.29 (*s*, 3H).

A mixture of **53** (188 mg, 0.74 mmol), **47b** (195 mg, 0.74 mmol), tetrakis(triphenylphosphine)palladium(0) (43 mg, 0.04 mmol) and Cs_2CO_3 (604 mg, 1.86 mmol) in dioxane (5 mL) and water (1 mL) was stirred at 100 °C for overnight under argon. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under vacuum and purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 35:65 CH_3CN:water (0.05% v/v NH_4OH) to 55:45 CH_3CN:water (0.05% v/v NH_4OH) over 10 min) to give **24** (33 mg, 14%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 10.44 (*s*, 1H), 7.42 (*d*, 1H, J = 6.0 Hz), 7.04–7.00 (*m*, 1H), 6.78 (*d*, 1H, J = 6.0 Hz), 6.26 (*s*, 1H), 5.73 (*s*, 2H), 4.43 (*s*, 2H), 3.19 (*s*, 3H), 2.26 (*s*, 3H), 2.23–2.07 (*m*, 2H), 1.73 (*s*, 3H), 0.93–0.89 (*m*, 3H). LCMS 310 [M +H] $^+$.

5.15. 5-(2-(2-Methoxyethyl)-3-methyl-1H-indol-7-yl)-4,6-dimethylpyridin-2-amine (25)

(48d): Phosphorus oxychloride (523 mg, 2.50 mmol) was added dropwise into DMF (456 mg, 6.25 mmol) at 0 °C under nitrogen, stirred at 0 °C for 1 h, and treated with a solution of **46d** (523 mg, 2.5 mmol) in 1,2-dichloroethane (10 mL). The resulting mixture was heated at reflux for 4 h, and cooled to room temperature. Saturated aqueous sodium acetate (20 mL) was added and the mixture was heated at reflux for an additional 4 h. The reaction mixture was cooled to room temperature, diluted with water (20 mL), and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography, eluting with a gradient of 30:1 to 20:1 petroleum ether:EtOAc, to give **48d** (279 mg, 47%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.06 (s, 1H), 8.83

(br s, 1H), 7.65 (d, 1H, J = 9.0 Hz), 7.54 (dd, 1H, J_1 = 9.0 Hz, J_2 = 3.0 Hz), 7.05 (t, 1H, J = 9.0 Hz), 2.64 (s, 3H). LCMS 237.9, 239.9 $[M + H]^+$.

(50d): To a suspension of (methoxymethyl)triphenylphosphonium chloride (515 mg, 1.5 mmol) in dry THF (5 mL) was added *n*-butyllithium (0.75 mL, 2.2*M* in hexane, 1.65 mmol) at 0 °C under nitrogen. The mixture was stirred at 0 °C for 1 h and a solution of **48d** (237 mg, 1 mmol) in THF (5 mL) was added quickly. The reaction mixture was stirred at room temperature overnight and saturated aqueous NH_4Cl (20 mL) was added. The mixture was extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by silica gel column chromatography, eluting with a gradient of 100:0 to 5:95 petroleum ether:EtOAc, to give **50d** (125 mg, 47%) as a white solid. LCMS 266.0, 268.1 $[M + H]^+$. A small sample of **50d** (10 mg, mixture of isomers) was purified by preparative TLC, eluting with 20:1 petroleum ether:EtOAc, to afford the *Z* isomer exclusively: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.26 (br s, 1H), 7.46 (d, 1H, J = 7.8 Hz), 7.38–7.31 (m, 1H), 6.98 (t, 1H, J = 7.8 Hz), 6.22 (d, 1H, J = 9.0 Hz), 5.52 (d, 1H, J = 9.0 Hz), 3.88 (s, 3H), 2.31 (s, 3H).

(47a): To a mixture of **41a** (200 mg, 1 mmol), 4,4,4′,4′,5,5,5′,5′-octamethyl-2,2′-bi(1,3,2-dioxaborolane) (508 mg, 2 mmol) and potassium acetate (196 mg, 2 mmol) in DMSO (3 mL) was added [1,1′-bis-(diphenylphosphino)ferrocene]dichloropalladium(II) (220 mg, 0.3 mmol) quickly under argon. The mixture was stirred at 120 °C under overnight. The reaction mixture was cooled to room temperature, poured into water (20 mL), and extracted with EtOAc (3 × 10 mL). The combined organic phases were washed with brine (2 × 10 mL), dried over Na_2SO_4 and concentrated under vaccum. The product residue was purified by silica gel column chromatography, eluting with 15:1 petroleum ether: EtOAc, to give **47a** (41 mg, 16%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.13 (s, 1H), 4.60 (br s, 2H), 2.50 (s, 3H), 2.31 (s, 3H), 1.36 (s, 12H). LC-MS 249.3 $[M + H]^+$. A second batch using 180 mg of **41a** gave an additional 102 mg (45%) of **47a**.

A mixture of **50d** (90 mg, 0.34 mmol), **47a** (127 mg, 0.40 mmol), tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.034 mmol), and aqueous Na_2CO_3 solution (0.51 mL, 2*M*) in dioxane (5 mL) was stirred at 100 °C overnight under argon. The reaction mixture was cooled to room temperature, poured into ice water (15 mL), and extracted with EtOAc (3 × 5 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by preparative TLC, eluting with 5:1 petroleum ether:EtOH, to give the desired (*E*)- and (*Z*)-5-(2-(2-methoxyvinyl)-3-methyl-1H-indol-7-yl)-4,6-dimethylpyridin-2-amine (80 mg, 77%). LCMS 308.0 $[M + H]^+$.

A mixture of the olefins prepared above (80 mg, 0.26 mmol) and a catalytic amount of 10% palladium on carbon in MeOH (5 mL) was stirred at room temperature under an atmosphere of hydrogen (balloon) for 2 h. The reaction mixture was filtered and the filtrate was concentrated under vacuum. The crude product was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 30:70 CH_3CN:water (0.1% v/v TFA) to 38:62 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **25** (4 mg, 3%) as the

TFA salt. ^{1}H NMR (400 MHz, CD_3OD) δ 9.91 (br s, 1H), 7.53 (d, 1H, J = 8.0 Hz), 7.12 (t, 1H, J = 8.0 Hz), 6.86 (d, 2H, J = 8.0 Hz), 3.61 (t, 2H, J = 8.0 Hz), 3.32 (s, 3H), 2.98 (t, 2H, J = 8.0 Hz), 2.29 (s, 3H), 2.17 (s, 3H), 2.07 (s, 3H). LCMS 310.2 $[M + H]^+$.

5.16. 6-Ethyl-5-(2-(2-methoxyethyl)-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine (26)—A mixture of **50d** (90 mg, 0.34 mmol), **47b** (134 mg, 0.40 mmol), tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.034 mmol), and aqueous Na_2CO_3 solution (0.51 mL, 2*M*) in dioxane (5 mL)) was stirred at 100 °C overnight under argon. The reaction mixture was cooled to room temperature, poured into ice water (15 mL), and extracted with EtOAc (3 × 5 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by preparative TLC, eluting with 5:1 petroleum ether:EtOH, to give the desired (*E*)- and (*Z*)-6-ethyl-5-(2-(2-methoxyvinyl)-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine (90 mg, 82%). LCMS 322.1 $[M + H]^+$.

A mixture of the olefins prepared above (90 mg, 0.28 mmol) and a catalytic amount of 10% palladium on carbon in MeOH (15 mL) was stirred at room temperature under an atmosphere of hydrogen (balloon) for 2 h. The reaction mixture was filtered and the filtrate was concentrated under vacuum. The crude product was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 30:70 CH_3CN:water (0.1% v/v TFA) to 50:50 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **26** (5 mg, 4%) as the TFA salt as a yellow oil. ^{1}H NMR (400 MHz, CD_3OD) δ 9.93 (br s, 1H), 7.53 (d, 1H, J = 8.0 Hz), 7.12 (t, 1H, J = 8.0 Hz), 6.86 (d, 2H, J = 8.0 Hz), 3.60 (t, 2H, J = 8.0 Hz), 3.35 - 3.30 (m, 3H), 2.97 (t, 2H, J = 6.0 Hz), 2.54 - 2.40 (m, 2H), 2.29 (s, 3H), 2.02 (s, 3H), 1.09 (t, 3H, J = 8.0 Hz). LCMS 324.3 $[M + H]^+$.

5.17. 6-Ethyl-5-(4-fluoro-2-(2-methoxyethyl)-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine (27)

(48a): Phosphorus oxychloride (1.01 g, 6.6 mmol) was added to DMF (482 mg, 6.6 mmol) at 0 °C, followed by a solution of **46a** (500 mg, 2.2 mmol) in 1,2-dichloroethane (8 mL). The reaction mixture was heated at reflux for 5 h, followed by the addition of saturated aqueous sodium acetate solution (10 mL), and the reaction was continued at reflux for an additional 1 h. The reaction mixture was cooled to room temperature and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography, eluting with 10:1 petroleum ether: EtOAc, to give **48a** (477 mg, 65%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.03 (*s* 1H), 8.83 (*br s*, 1H), 7.42–7.38 (*m*, 1H), 6.72–6.66 (*m*, 1H), 2.75 (*d*, 3H, J = 4.0 Hz).

(50a): To a solution of (methoxymethyl)triphenylphosphonium chloride (349 mg, 1.02 mmol) in THF (5 mL) was added *n*-BuLi (0.36 mL, 0.892 mmol, 2.5 M in *n*-hexane) at 0 °C. The mixture was stirred at 0 °C for 1 h. The crude (methoxymethylene)triphenylphosphorane product solution was used in the next step without any purification.

A solution of **48a** (65 mg, 0.255 mmol) in THF (3 mL) was added to the crude (methoxymethylene)triphenylphosphorane solution above, and the reaction mixture was stirred at room temperature overnight. Water (5 mL) was added and the mixture was extracted with EtOAc (3 × 3 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The resulting residue was purified by preparative TLC (10:1 petroleum ether: EtOAc) to give **50a** (26 mg, 36%) as a light brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.26 (*br s*, 1H), 7.13–7.09 (*m*, 1H), 6.62–6.56 (*m*, 1H), 6.24 (*d*, 1H, J = 8.8 Hz), 5.46 (*d*, 1H, J = 8.8 Hz), 3.90 (*s*, 3H), 2.39 (*s*, 3H).

To a solution of **50a** (150 mg, 0.53 mmol), **47b** (171 mg, 0.64 mmol) and Cs_2CO_3 (346 mg, 1.06 mmol) in dioxane (10 mL) and water (2 mL) was added tetrakis(triphenylphosphine)palladium(0) (61 mg, 0.053 mmol) under argon. The mixture was stirred at 100 °C under argon overnight, then cooled to room temperature. Water (10 mL) was added and the mixture was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by preparative TLC, eluting with 10:1 CH_2Cl_2:MeOH, to give the desired (*E*)- and (*Z*)-6-ethyl-5-(4-fluoro-2-(2-methoxyvinyl)-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine (58 mg, 32%) as a light brown oil. LCMS 340.1 $[M + H]^+$.

To a solution of the olefin mixture above (58 mg, 0.17 mmol) in MeOH (3 mL) was added 10% palladium on carbon (58 mg), and the mixture was stirred under an atmosphere of hydrogen (balloon) at room temperature overnight, filtered and concentrated under vacuum. The crude product was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 70:30 CH_3CN:water (0.05% v/v NH_4OH) to 75:25 CH_3CN:water (0.05% v/v NH_4OH) over 10 min) to give **27** (32 mg, 55%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 6.68–6.66 (*m*, 2H), 6.44 (*s*, 1H), 3.56 (*t*, 2H, J = 8.0 Hz), 3.31 (*s*, 3H), 2.91 (*t*, 2H, J = 8.0 Hz), 2.39–2.24 (*m*, 5H), 1.85 (*s*, 3H), 0.98 (*t*, 2H, J = 8.0 Hz). LCMS 342.1 $[M + H]^+$.

Alternative synthesis of **27**. To a solution of **50a** (500 mg, 1.767 mmol), bis(pinacolato)boron (897 mg, 3.534 mmol) and potassium acetate (346 mg, 3.543 mmol) in DMSO (10 mL) was added [1,1′-bis-(diphenylphosphino)ferrocene]dichloropalladium(II) (130 mg, 0.177 mmol) under argon. The reaction mixture was heated to 120 °C overnight under argon, and cooled to room temperature. Water (100 mL) was added and the mixture was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (2 × 20 mL), dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography, eluting with 50:1 petroleum ether: EtOAc, to give the desired indole pinacol ester (212 mg, 36%) as a brown oil. LCMS: 332.2 $[M + H]^+$.

To a solution of the indole boronic ester prepared above (212 mg, 0.64 mmol), **41b** (164 mg, 0.77 mmol) and Cs_2CO_3 (417 mg, 1.28 mmol) in dioxane (10 mL) and water (2 mL) was added tetrakis(triphenylphosphine)palladium(0) (74 mg, 0.064 mmol) under argon. The mixture was stirred at 100 °C under argon overnight, and cooled to room temperature. Water (50 mL) was added and the mixture was extracted with EtOAc (3 × 20 mL). The combined

organic layers were dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography, eluting with 50:1 petroleum ether: EtOAc, to give (*E*)- and (*Z*)-6-ethyl-5-(4-fluoro-2-(2-methoxyvinyl)-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine (200 mg, 92%) as a brown oil. LCMS 340.0 $[M + H]^+$.

To a solution of the olefin mixture prepared above (200 mg, 0.59 mmol) in MeOH (5 mL) was added 10% palladium on carbon (400 mg) and the mixture was stirred under an atmosphere of hydrogen (balloon) at room temperature overnight. The reaction mixture was filtered and the filtrate was concentrated under vacuum. The crude product was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 70:30 CH_3CN:water (0.05% v/v NH_4OH) to 90:10 CH_3CN:water (0.05% v/v NH_4OH) over 10 min) to give the desired product as the free amine. The free amine was dissolved in MeOH, treated with concentrated HCl, and lyophilized to give **27** (37 mg, 19%) as the HCl salt as a white solid. ^1H NMR (400 MHz, CD_3OD) δ 6.83 (*s*, 1H), 6.78–6.75 (*m*, 2H), 3.58 (*t*, 2H, *J* = 8.0 Hz), 3.33 (*s*, 3H), 2.94 (*t*, 2H, *J* = 8.0 Hz), 2.51–2.40 (*m*, 5H), 2.01 (*s*, 3H), 1.09 (*t*, 3H, *J* = 8.0 Hz). LCMS 342.1 $[M + H]^+$.

5.18. 6-Ethyl-5-(5-fluoro-2-(2-methoxyethyl)-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine (28)

(48b): Phosphorus oxychloride (0.6 mL, 6.6 mmol) was added to DMF (0.5 mL, 6.6 mmol) at 0 °C, followed by a solution of **46b** (500 mg, 2.2 mmol) in 1,2-dichloroethane (8 mL). The cooling bath was removed, and the reaction mixture was heated at reflux overnight, followed by cooling to room temperature. Sodium hydroxide solution was added until a pH 8–9 was reached. The reaction mixture was extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic layers were dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography, eluting with 10:1 petroleum ether: EtOAc, to give **48b** (70 mg, 13%) as a yellow solid. 1H NMR (300 MHz, $CDCl_3$) δ 10.05 (*s*, 1H), 8.78 (*s*, 1H), 7.38–7.29 (*m*, 2H), 2.59 (*s*, 3H).

(50b): To a solution of **48b** (400 mg, 1.57 mmol) in THF (5 mL) was added a solution of (methoxymethylene)triphenylphosphorane in THF (5.49 mmol) (prepared as for **50a**), and the reaction mixture was stirred at room temperature overnight. Water (10 mL) was added and the mixture was extracted with EtOAc (3 × 5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by preparative TLC, eluting with 10:1 petroleum ether: EtOAc, to give **50b** (220 mg, 50%) as a light brown oil. ^1H NMR (300 MHz, $CDCl_3$) δ 9.16 (*s*, 1H), 7.07–7.04 (*m*, 2H), 6.24 (*d*, 1H, *J* = 6.9 Hz), 5.48 (*d*, 1H, *J* = 6.6 Hz), 3.90 (*s*, 3H), 2.21 (*d*, 3H, *J* = 1.8 Hz).

To a solution of **50b** (200 mg, 0.71 mmol), **47b** (227 mg, 0.85 mmol) and Cs_2CO_3 (461 mg, 1.41 mmol) in dioxane (10 mL) and water (2 mL) was added tetrakis(triphenylphosphine)palladium(0) (82 mg, 0.071 mmol) under argon. The reaction mixture was stirred at 100 °C under argon overnight and cooled to room temperature. Water (10 mL) was added and the mixture was extracted with EtOAc (2 × 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under vacuum. The crude product

was purified by preparative TLC, eluting with 16:1 CH_2Cl_2:MeOH, to give the desired (*E*)- and (*Z*)-6-ethyl-5-(5-fluoro-2-(2-methoxyvinyl)-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine (70 mg, 29%) as a brown oil. LCMS: 340.1 $[M + H]^+$.

To a solution of the olefin mixture above (70 mg, 0.21 mmol) in MeOH (3 mL) was added 10% palladium on carbon (140 mg) and the mixture was stirred under an atmosphere of hydrogen (balloon) at room temperature overnight. The catalyst was filtered and the filtrate was concentrated under vacuum. The crude product was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 70:30 CH_3CN:water (0.05% v/v NH_4OH) to 99:1 CH_3CN:water (0.05% v/v NH_4OH) over 10 min) to give **28** (15 mg, 21%) as a white solid. ^1H NMR (400 MHz, CD_3OD) δ 7.08 (*dd*, 1H, J = 10.0 Hz, J = 4.0 Hz), 6.57 (*dd*, 1H, J = 10.0 Hz, J = 4.0 Hz), 6.45 (*s*, 1H), 3.57 (*t*, 2H, J = 8.0 Hz), 3.30 (*s*, 3H), 2.93 (*t*, 2H, J = 8.0 Hz), 2.42–2.23 (*m*, 5H), 1.87 (*s*, 3H), 1.00 (*t*, 3H, J = 8.0 Hz). LCMS 342.1 $[M + H]^+$.

5.19. 5-(4,5-Difluoro-2-(2-methoxyethyl)-3-methyl-1H-indol-7-yl)-6-ethyl-4-methylpyridin-2-amine (29)

(48c): Phosphorus oxychloride (1.84 g, 12 mmol) was added dropwise into DMF (876 mg, 12 mmol) at 0 °C under an atmosphere of nitrogen. The reaction mixture was stirred at 0 °C for 1 h, followed by the addition of a solution of **46c** (984 mg, 4 mmol) in 1,2-dichloroethane (20 mL). The reaction mixture was heated at reflux for 4 h, cooled to room temperature, and treated with saturated aqueous sodium acetate (20 mL). The reaction mixture was heated at reflux for an additional 4 h, cooled to room temperature, and water (20 mL) was added. The mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by silica gel column chromatography, eluting with a gradient of 30:1 to 20:1 petroleum ether:EtOAc, to give **48c** (234 mg, 21%). ^1H NMR (300 MHz, $CDCl_3$) δ 10.03 (*br s*, 1H), 8.80 (*br s*, 1H), 7.36–7.42 (m, 1H), 2.75 (*s*, 3H).

(50c): To a suspension of (methoxymethyl)triphenylphosphonium chloride (879 mg, 2.56 mmol) in THF (5 mL) was added *n*-butyllithium (1.16 mL, 2.2*M* in hexane, 2.56 mmol) at 0 °C under nitrogen. The reaction mixture was stirred at 0 °C for 1 h, and a solution of **48c** (234 mg, 1 mmol) in THF (5 mL) was added quickly. The reaction mixture was allowed to stir at room temperature overnight, followed by the addition of saturated aqueous NH_4Cl (20 mL). The mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by silica gel column chromatography, eluting with a gradient of 100:0 to 5:95 petroleum ether:EtOAc, to give **50c** (64 mg, 21%) as a white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 9.18 (*brs*, 1H), 7.05–7.10 (*m*, 1H), 6.26 (*d*, 1H, J = 9.0 Hz), 5.45 (*d*, 1H, J = 9.0 Hz), 3.90 (*s*, 3H), 2.37 (*s*, 3H). The reaction was repeated with 95 mg of **48c** to provide an additional 27 mg (25%) of **50c**.

A mixture of **50c** (91 mg, 0.37 mmol), **47b** (117 mg, 0.44 mmol), tetrakis(triphenylphosphine)palladium(0) (118 mg, 0.11 mmol), and Cs_2CO_3 (362 mg, 1.11

mmol) in water (1 mL) and dioxane (5 mL) was stirred at 100 °C overnight under argon. The reaction mixture was cooled to room temperature, poured into ice water (15 mL), and extracted with EtOAc (3 × 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by preparative TLC, eluting with 5:1 petroleum ether: EtOH, to give the desired (*E*)- and (*Z*)-5-(4,5-difluoro-2-(2-methoxyvinyl)-3-methyl-1H-indol-7-yl)-6-ethyl-4-methylpyridin-2-amine (78 mg, 59%). LCMS 358 $[M + H]^+$.

A mixture of the olefin prepared above (78 mg, 0.22 mmol) and 10% palladium on carbon (30 mg) in MeOH (15 mL) was stirred at room temperature under an atmosphere of hydrogen (balloon) for 2 h. The reaction mixture was filtered and the filtrate was concentrated under vacuum. The crude product was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 30:70 CH_3CN:water (0.1% v/v Et_3N) to 50:50 CH_3CN:water (0.1% v/v Et_3N) over 10 min) to give **29** (12 mg, 15%). ^1H NMR (400 MHz, CD_3OD) δ 6.63–6.67 (*m*, 1H), 6.45 (*s*, 1H), 3.56 (*t*, 2H, J = 8.0 Hz), 3.31 (*s*, 3H), 2.91 (*t*, 2H, J = 8.0 Hz), 2.22–2.29 (*m*, 5H), 1.87 (*s*, 3H), 1.00 (*t*, 3H, J = 8.0 Hz). LCMS 324.3 $[M + H]^+$.

5.20. 6-Ethyl-5-(2-(3-methoxypropyl)-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine (30)

(49): Iodine (279 mg, 1.1 mmol) was added in one portion to a solution of **46d** (209 mg, 1 mmol) in dry THF (10 mL) at −78 °C. After 3 min, silver trifluoromethanesulfonate (283 mg, 1.1 mmol) was added in one portion. The reaction mixture was stirred at −78 °C for 3 h and $NaHCO_3$ (185 mg, 2.2 mmol) was added. The reaction mixture was allowed to warm to room temperature. After 30 min, the mixture was filtered through Celite and rinsed with EtOAc. To the filtrate was added a mixture of saturated aqueous sodium thiosulfate solution and saturated aqueous $NaHCO_3$ solution (1:1, 30 mL). The organic layer was separated and dried, and then concentrated and purified to give **49** (200 mg, 60%) as a brown oil. ^1H NMR (300 MHz, $CDCl_3$) δ 8.08 (*s*, 1H), 7.43 (*d*, 1H, J = 9.0 Hz), 7.28 (*s*, 1H), 7.00 - 6.97 (*m*, 1H), 7.27 (*s*, 3H). LCMS 335 $[M + H]^+$.

(51): A mixture of **49** (200 mg, 0.6 mmol), copper (I) iodide (23 mg, 0.12 mmol) and bis(triphenylphosphine)palladium(II) dichloride (42 mg, 0.06 mmol) in DMF/triethylamine (1:1, 2 mL) under argon was heated to 50 °C, and then 3-methoxyprop-1-yne (0.05 mL, 0.5 mmol) was added. The mixture was stirred overnight at 50 °C under argon and cooled to room temperature. The mixture was treated with 1% aqueous HCl and extracted with EtOAc. The combined organic layers were dried and purified to give **51** (71 mg, 43%) as a pale yellow solid. ^1H NMR (300 MHz, DMSO) δ 11.55 (*s*, 1H), 7.50 (*d*, 1H, J = 6.0 Hz,), 7.36–7.34 (*m*, 1H), 6.98–6.93 (*m*, 1H), 4.42 (*s*, 2H), 3.37 (*s*, 3H), 2.29 (*s*, 3H). LCMS 278 $[M + H]^+$.

A mixture of **51** (111 mg, 0.4 mmol), **47b** (126 mg, 0.48 mmol), tetrakis(triphenylphosphine)palladium(0) (23 mg, 0.02 mmol) and Cs_2CO_3 (326 mg, 1.0 mmol) in dioxane (5 mL) and water (1 mL) was stirred at 100 °C overnight under argon. The

reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under vacuum and purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 25:75 CH_3CN:water (0.1% v/v TFA) to 50:50 CH_3CN:water (0.5% v/v NH_4OH) over 10 min) to give the desired 6-ethyl-5-(2-(3-methoxyprop-1-yn-1-yl)-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine (26 mg, 20%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 7.59 (*d*, 1H, *J* = 9.0 Hz), 7.12-7.07 (*m*, 1H), 6.88 (*d*, 1H, *J* = 9.0 Hz), 6.43 (*s*, 1H), 4.37 (*s*, 2H), 3.43 (*s*, 3H), 2.38 (*s*, 3H), 2.35–2.19 (*m*, 2H), 1.84 (*s*, 3H), 0.99 - 0.94 (*m*, 3H). LCMS 334 [M +H] $^+$. The reaction was repeated on the same scale to provide an additional 25 mg of 6-ethyl-5-(2-(3-methoxyprop-1-yn-1-yl)-3-methyl-1H-indol-7-yl)-4-methylpyridin-2-amine.

A mixture of the alkyne prepared above (35 mg, 0.1 mmol) and 10% palladium on carbon (10 mg) in MeOH (2 mL) was stirred for 4 h at room temperature under an atmosphere of hydrogen (balloon). The reaction mixture was filtered and the filtrate was concentrated under vacuum. The crude product was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 25:75 CH_3CN:water (0.1% v/v TFA) to 50:50 CH_3CN:water (0.5% v/v NH_4OH) over 10 min) to give **30** (7 mg, 17%) as a white solid. ^{1}H NMR (400 MHz, DMSO) δ 10.16 (*s*, 1H), 7.32 (*d*, 1H, *J* = 4.0 Hz), 6.99–6.95 (*m*, 1H), 6.68 (*d*, 1H, *J* = 8.0 Hz), 6.25 (*s*, 1H), 5.71 (*s*, 2H), 3.27–3.24 (*m*, 2H), 3.20 (*s*, 3H), 2.67–2.63 (*m*, 2H), 2.23–2.10 (*m*, 5H), 1.79–1.73 (*m*, 5H), 0.93–0.89 (*m*, 3H). LCMS 338 [M +H] $^+$.

5.21. 3-(7-(6-Amino-2-ethyl-4-methylpyridin-3-yl)-3-methyl-1H-indol-2-yl)-N-methylpropanamide (31)

(50e): To a suspension of (2-(methylamino)-2-oxoethyl)triphenylphosphonium bromide (828 mg, 2.0 mmol) (prepared from heating 2-bromo-N-methylacetamide (1 eq) and triphenylphosphine (1 eq) in toluene at reflux overnight, followed by cooling and filtration to give the desired solid salt) in dry THF (5 mL) at −78 °C under nitrogen was added *n*-butyllithium (0.91 mL, 2.2*M* in hexane, 2.0 mmol). The reaction mixture was allowed to warm to room temperature and stir for 1 h, followed by the rapid addition of a solution of **48d** (237 mg, 1 mmol) in THF (5 mL). The reaction mixture was stirred at room temperature overnight, quenched with saturated aqueous NH_4Cl (10 mL), and extracted with EtOAc (3 × 5 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by silica gel column chromatography, eluting with a gradient of 15:1 to 5:1 petroleum ether:EtOAc, to give **50e** (93 mg, 32%) as a mixture of isomers. A small sample of **50e** (10 mg, mixture of isomers) was purified by preparative TLC, eluting with 3:1 petroleum ether:EtOAc, to afford the *E* isomer exclusively: ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.96 (br s, 1H), 7.49 (d, 1H, *J* = 9.0 Hz), 7.38 (d, 1H, *J* = 9.0 Hz), 6.93 (t, 1H, *J* = 9.0 Hz), 6.85 (d, 1H, *J* = 15.0 Hz), 5.75 (br s, 1H), 6.64 (d, 1H, *J* = 15.0 Hz), 2.98 (d, 1H, *J* = 6.0 Hz), 2.40 (s, 3H).

A mixture of **50e** (93 mg, 0.32 mmol), **47b** (125 mg, 0.38 mmol), tetrakis(triphenylphosphine)palladium(0) (37 mg, 0.03 mmol), and Cs_2CO_3 (311 mg, 0.95

mmol) in dioxane (5 mL) and water (1 mL) was stirred at 100 °C overnight under argon. The reaction mixture was cooled to room temperature, poured into ice water (10 mL), and extracted with EtOAc (3 × 5 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by preparative TLC, eluting with 3:1 petroleum ether:EtOH, to give the desired (*E*)- and (*Z*)-3-(7-(6-amino-2-ethyl-4-methylpyridin-3-yl)-3-methyl-1H-indol-2-yl)-N-methylacrylamide (73 mg, 66%). LCMS 349.2 $[M + H]^+$.

A mixture of the olefins prepared above (73 mg, 0.21 mmol) and a catalytic amount of 10% palladium on carbon in MeOH (5 mL) was stirred at room temperature under an atmosphere of hydrogen (balloon) for 2 h. The reaction mixture was filtered and concentrated under vacuum. The crude product was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 30:70 CH_3CN:water (0.1% v/v TFA) to 33:67 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **31** (6 mg, 6%) as the TFA salt. ^1H NMR (400 MHz, CD_3OD) δ 7.52 (d, 1H, J = 8.0 Hz), 7.12 (t, 1H, J = 8.0 Hz), 6.86 (d, 2H, J = 8.0 Hz), 3.00 - 2.98 (m, 2H), 2.68 (s, 3H), 2.49 - 2.42 (m, 2H), 2.28 (s, 3H), 2.01 (s, 3H), 1.08 (t, 3H, J = 8.0 Hz). LCMS 351.2 $[M + H]^+$.

5.22. N-(2-(6-(6-Amino-2-ethyl-4-methylpyridin-3-yl)-2,2-dimethyl-3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)ethyl)acetamide (32)—A mixture of **54b** (448 mg, 1.15 mmol), **41b** (248 mg, 1.15 mmol), tetrakis(triphenylphosphine)palladium(0) (133 mg, 0.115 mmol), cesium hydroxide monohydrate (579 mg, 3.45 mmol) and lithium chloride (146 mg, 3.45 mmol) in a mixture of dioxane (18 mL) and water (3 mL) was stirred at 100 °C for 12 h under argon. The reaction mixture was cooled to room temperature and concentrated under vacuum to give 1.1 g of a crude product mixture. The crude residue was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 5 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 30:70 CH_3CN:water (0.1% v/v TFA) to 70:30 CH_3CN:water (0.1% v/v TFA) over 10 min) to give the desired product as the free base. The free base was dissolved in concentrated HCl (10 mL) and lyophilized to give **32** (120 mg, 26%) as the HCl salt as a white solid. ^1H NMR (400 MHz, CD_3OD) δ 7.28 (*s*, 1 H), 7.13 (*d*, 1H, J = 8.1 Hz), 6.90 (*dd*, 1H, J = 1.8, 8.1 Hz), 6.77 (*s*, 1H), 4.04 (*t*, 2H, J = 7.2 Hz), 3.34 (*t*, 2H, J = 7.2 Hz), 2.58 (*q*, 2H, J = 7.5 Hz), 2.15 (*s*, 3H), 1.90 (*s*, 3H), 1.52 (*s*, 6H), 1.18 (*t*, 3H, J = 7.5 Hz). LCMS 397 $[M + H]^+$.

5.23. N-(2-(6-(6-Amino-2-ethyl-4-methoxypyridin-3-yl)-2,2-dimethyl-3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)ethyl)acetamide (33)—A mixture of **54b** (224 mg, 0.58 mmol), **41d** (134 mg, 0.58 mmol), tetrakis(triphenylphosphine)palladium(0) (67 mg, 0.058 mmol), cesium hydroxide monohydrate (292 mg, 1.74 mmol) and lithium chloride (74 mg, 1.74 mmol) in dioxane (12 mL) and water (1.5 mL) was stirred at 100 °C for 12 h under argon. The reaction mixture was cooled to room temperature and concentrated under vacuum to give 80 mg of a crude product mixture. The crude residue was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 5 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear

gradient of 30:70 CH_3CN:water (0.1% v/v TFA) to 40:60 CH_3CN:water (0.1% v/v TFA) over 10 min) to give the desired product as the free base. The free base was dissolved in 2N aqueous HCl (2 mL) and lyophilized to give **33** (40 mg, 17%) as the HCl salt as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.24 (*s*, 1 H), 7.04 (*d*, 1H, *J* = 8.1 Hz), 6.94 (*dd*, 1H, *J* = 1.8, 8.1 Hz), 6.37 (*s,* 1H), 4.02 (*t*, 2H, *J* = 7.2 Hz), 3.89 (*s*, 3H), 3.38 (*m*, 2H), 2.59 (*q*, 2H, *J* = 7.5 Hz), 1.88 (*s*, 3H), 1.52 (*s*, 6H), 1.20 (*t*, 3H, *J* = 7.5 Hz). LCMS 413 $[M + H]^+$.

5.24. 6-(6-Amino-2-ethyl-4-methylpyridin-3-yl)-4-(3-methoxypropyl)-2,2-dimethyl-2H-benzo[b][1,4]oxazin-3(4H)-one (34)—A mixture of **54a** (140 mg, 0.37 mmol), **41b** (66.7 mg, 0.31 mmol), tetrakis(triphenylphosphine)palladium(0) (36 mg, 0.03 mmol), cesium hydroxide monohydrate (156 mg, 0.93 mmol) and lithium chloride (39.4 mg, 0.93 mmol) in dioxane (10 mL) and water (2 mL) was stirred at 100 °C for 12 h under argon. The reaction mixture was cooled to room temperature and concentrated under vacuum to give 100 mg of a crude product mixture. The crude residue was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 5 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 25:75 CH_3CN:water (0.1% v/v TFA) to 45:55 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **34** (30 mg, 25%) as the TFA salt as a yellow oil. ^{1}H NMR (400 MHz, CD_3OD) δ 7.12 (*s*, 1 H), 7.10 (*d*, 1H, *J* = 8.4 Hz), 6.89 (*dd*, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 6.80 (*s,* 1H), 4.04 (*t*, 2H, *J* = 5.1 Hz), 3.33 (*t*, 2H, *J* = 1.2 Hz), 3.32 (*s*, 3H), 2.57 (*q*, 2H, *J* = 8.4 Hz), 2.13 (*s*, 3H), 1.88 (*t*, 2H, *J* = 2.8 Hz), 1.51 (*s*, 6H), 1.19 (*t*, 3H, *J* = 7.2 Hz). LCMS 384 $[M + H]^+$.

5.25. Methyl (2-(6-(6-amino-2-ethyl-4-methylpyridin-3-yl)-2,2-dimethyl-3-oxo-2H-benzo[b][1,4]oxazin-4(3H)-yl)ethyl)carbamate (35)

(56): A mixture of **55** (prepared according to Powell, *et al.*[18b]) (200 mg, 0.78 mmol) and sodium hydride (37.5 mg, 60 wt %, 0.94 mmol) in DMF (10 mL) was stirred at 0 °C for 15 min, followed by the addition of 15-crown-5 (0.1 mL) and methyl (2-bromoethyl)carbamate (171 mg, 0.94 mmol). The reaction mixture was warmed to room temperature, stirred for 12 h, poured into water (50 mL), and extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under vacuum. The resulting residue was purified by silica gel column chromatography, eluting with 2:1 petroleum ether: EtOAc, to give **56** (0.22 g, 79%) as a white solid. LCMS 357, 359 $[M + H]^+$.

A mixture of **56** (0.2 g, 0.56 mmol), bis(pinacolato)boron (0.17 g, 0.67 mmol), [1,1′-bis-(diphenylphosphino)ferrocene]dichloropalladium(II) (46 mg, 0.056 mmol) and potassium acetate (165 mg, 1.68 mmol) in dioxane (10 mL) was stirred under argon at 85 °C for 12 h. The resulting mixture was cooled to room temperature and concentrated under vacuum. The resulting residue was purified by silica gel column chromatography, eluting with 2:1 petroleum ether: EtOAc to give the desired boronic ester (0.18 g, 80%) as a white solid. LCMS 405 $[M + H]^+$. A mixture of the boronic ester (100 mg, 0.25 mmol), **41b** (54 mg, 0.25 mmol), [1,1′-bis-(diphenylphosphino)ferrocene]dichloropalladium(II) (20 mg, 0.025 mmol), and potassium phosphate tribasic heptahydrate (253 mg, 0.75 mmol) in toluene (10 mL) was stirred at 100 °C for 12 h under argon. The reaction mixture was cooled to room

temperature and concentrated under vacuum to give 280 mg of a crude product mixture. The crude residue was purified by preparative HPLC (column: Phenomenex Gemini 5 μm C18 150 × 21.2 mm; injection volume: 5 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 30:70 CH_3CN:water (0.1% v/v TFA) to 80:20 CH_3CN:water (0.1% v/v TFA) over 10 min) to give **35** (35 mg, 34%) as a TFA salt as a white solid. ^1H NMR (400 MHz, CD_3OD) δ 7.15 (*s*, 1 H), 7.08 (*d*, 1H, *J* = 8.4 Hz), 6.89 (*dd*, 1H, *J* = 2.0, 8.4 Hz), 6.79 (*s*, 1H), 4.02 (*t*, 2H, *J* = 2.8 Hz), 3.54 (*s*, 3H), 3.31 (*t*, 2H, *J* = 1.6 Hz), 2.54 (*q*, 2H, *J* = 7.6 Hz), 2.12 (*s*, 3H), 1.50 (*s*, 6H), 1.18 (*t*, 3H, *J* = 7.6 Hz). LCMS 413 $[M + H]^+$.

5.26. Renin Inhibition Assay

Synthesized compounds were tested for inhibition against human renin using the commercially available SensoLyte® 520 Renin Assay Kit (Fluorimetric), catalog #72040, from AnaSpec, Inc. (Fremont, CA). All assays were performed with at least duplicate IC_{50} determinations according to the manufacturer's instructions by BPS Bioscience (San Diego, CA). Final concentration of DMSO was 1% in all assays. Fluorescence intensity was measured using a Tecan Infinite M1000 microplate reader. Data analysis and curve fitting was performed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). All IC_{50} assays included a concentration response for commercially obtained aliskiren hydrochloride (standard) included on the same assay plate.

5.27. Rat In Vivo Pharmacokinetic Studies

All studies were performed by BioDuro, LLC under IACUC-approved protocols using 8 week-old jugular-cannulated Sprague Dawley rats weighing between 250 and 350 g. Single intravenous bolus doses were administered via jugular cannula, and single oral doses were administered via gavage. Blood samples were collected for analysis at 2 min (iv only), 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, and 24 h after dosing. Plasma concentrations were determined by LC/MS/MS after precipitation with acetonitrile. Test compounds (as their HCl salts) were formulated in an aqueous 5% glucose solution (D5W) at a concentration of 1 mg/mL, and dosed at 1 mg/kg body weight for the intravenous study arms and 10 mg/kg body weight for the oral study arms. Data are reported as the mean (n = 3).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

SACP	simulated annealing of chemical potential
CFA	constrained fragment analysis

FE Pred	predicted relative free energy
$\Delta\Delta G_S$	relative solvation change
D5W	5% dextrose (glucose) in water
Vd_{ss}	steady-state volume of distribution
Cl	clearance
C_{max}	maximum concentration

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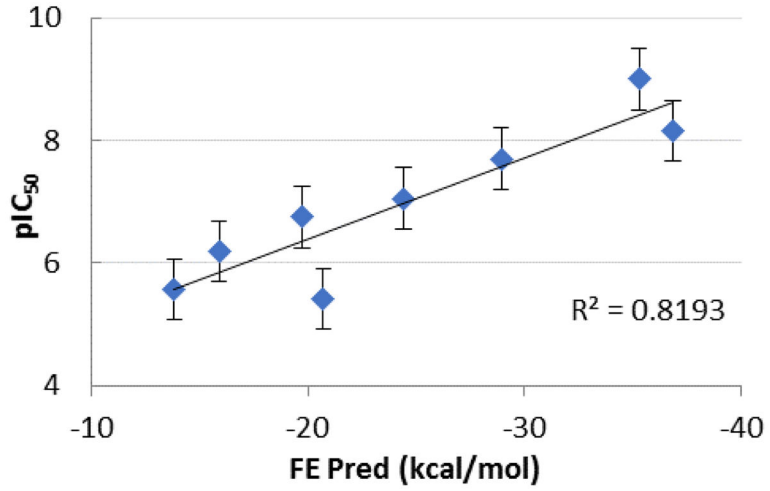
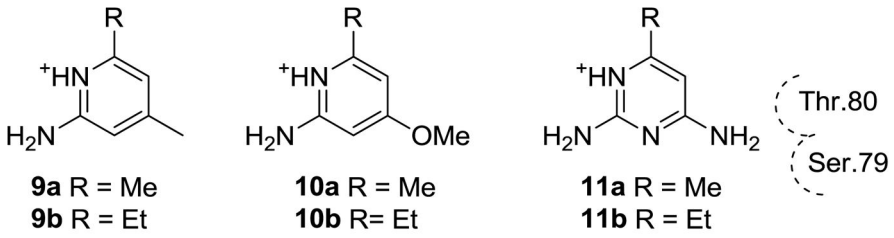


Figure 1.
Examples of non-peptidomimetic renin inhibitors.



9a R = Me **9b** R = Et **10a** R = Me **10b** R= Et **11a** R = Me **11b** R = Et

Figure 2.

Correlation plot of predicted relative Free Energies versus pIC_{50} for compounds **6a–d** and **7a–d**.

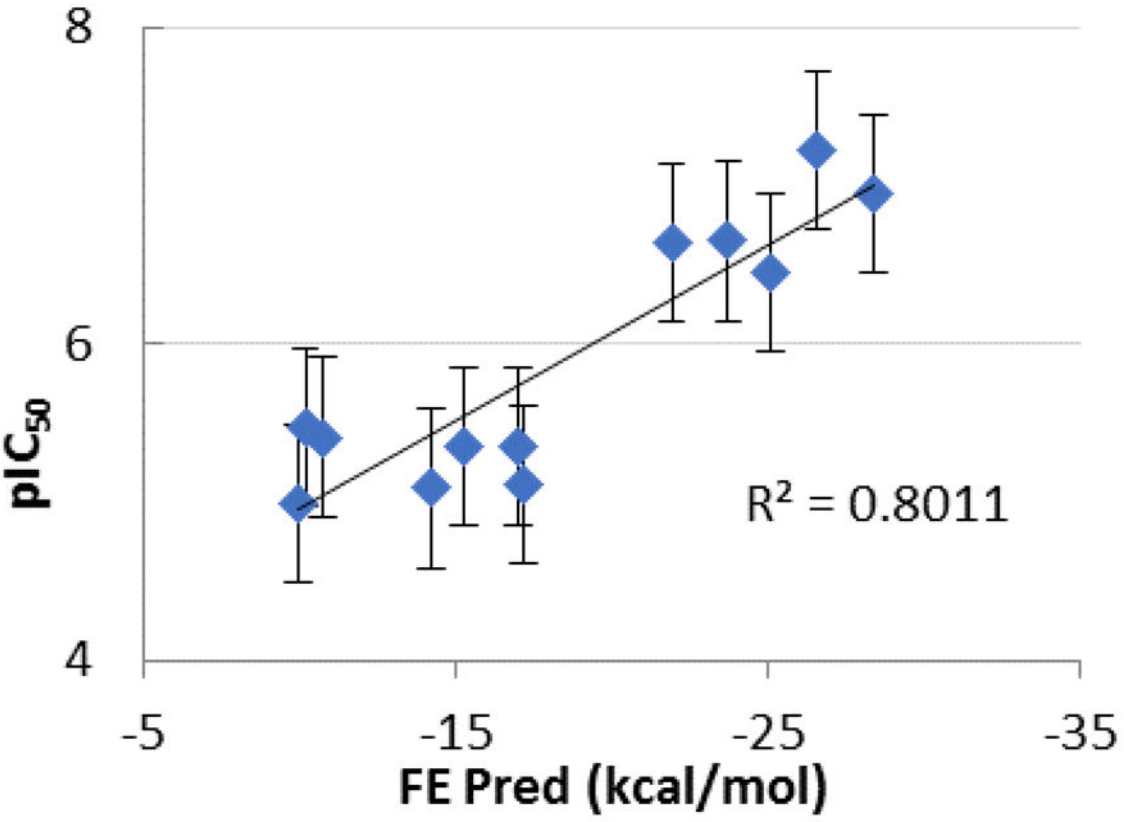


Figure 3.
Protonated amines interacting at the catalytic site.

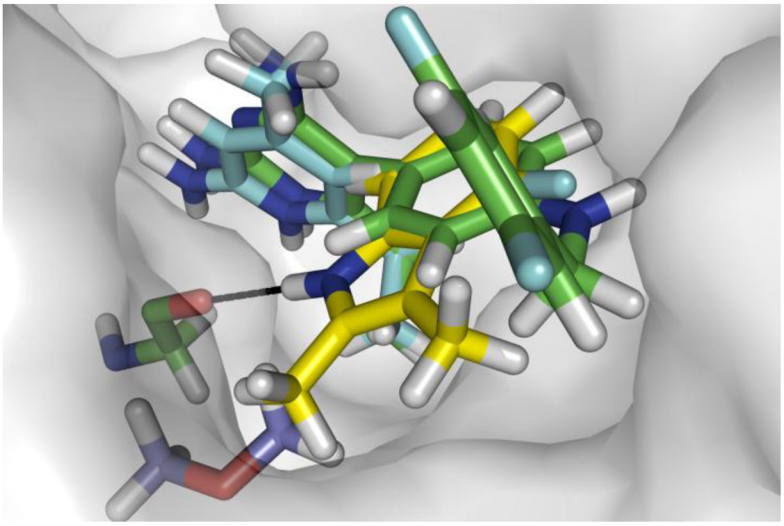


Figure 4.

Correlation plot of versus predicted relative Free Energies versus pIC_{50} for compounds **17–30** where G=Et.

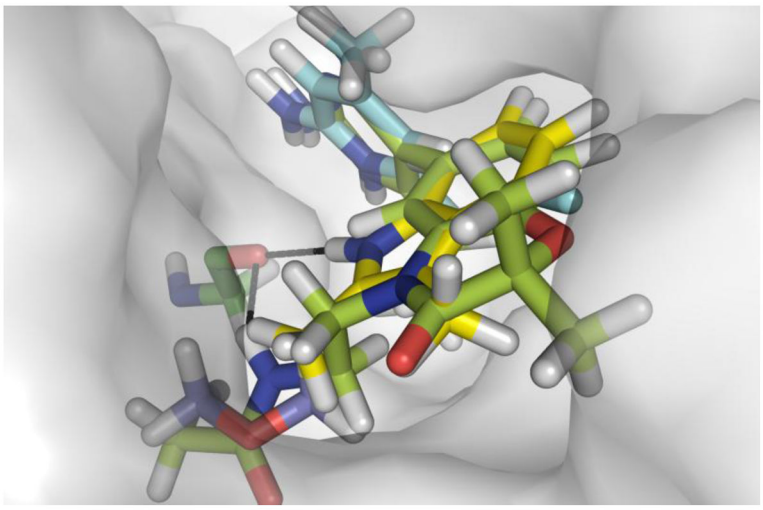


Figure 5.

Predicted binding pose of the fragments comprising 27 (cyan, yellow, blue) compared to 8 (green) in 2IKO showing the interaction of the indole NH with Gly223, and the ether penetrating $S3^{sp}$.

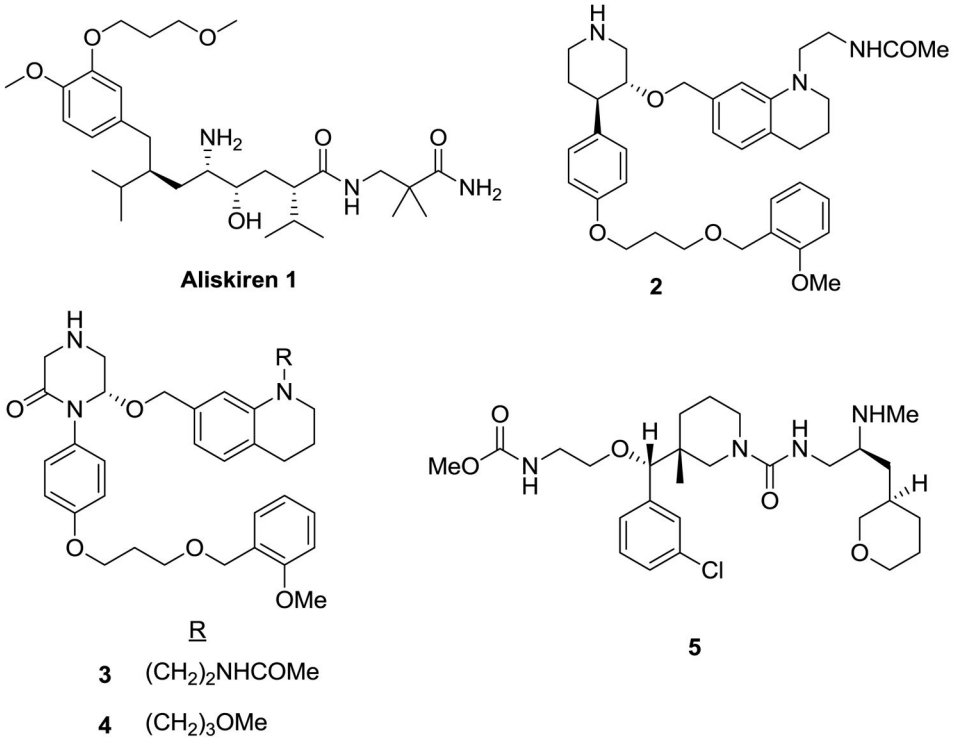
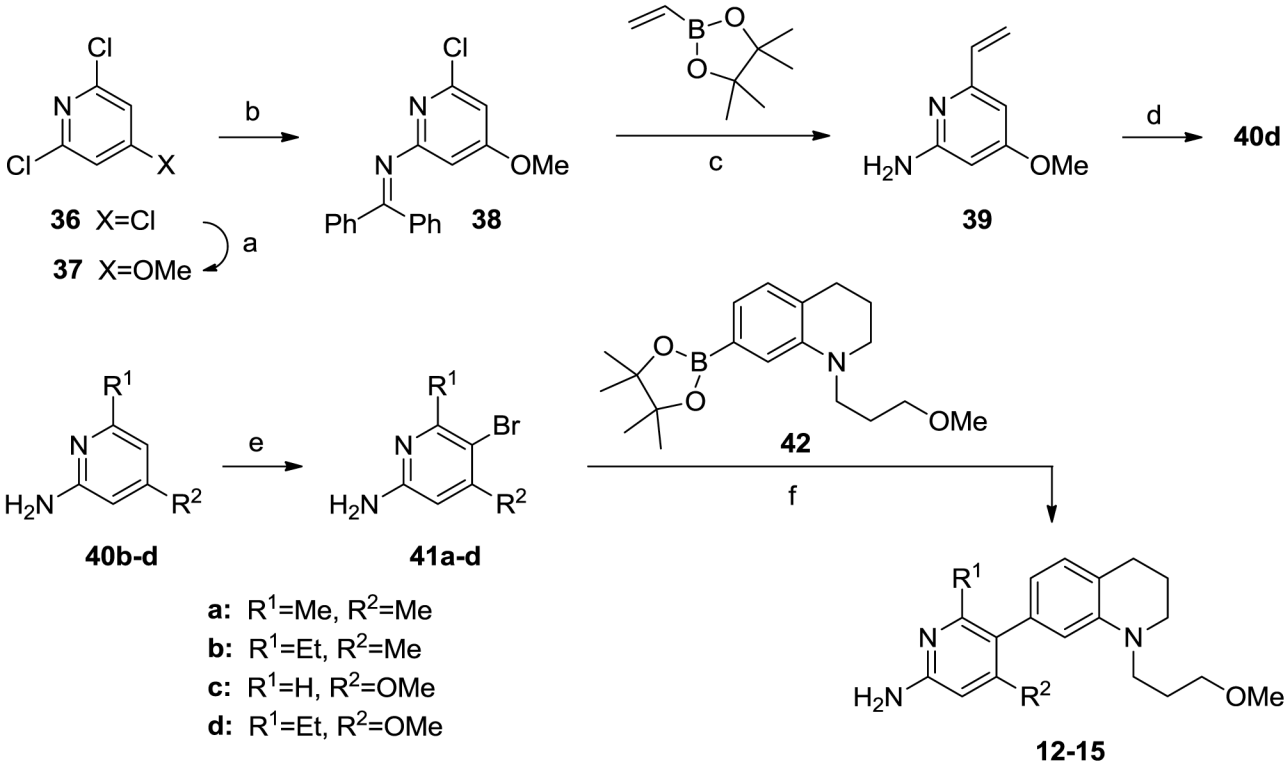


Figure 6.

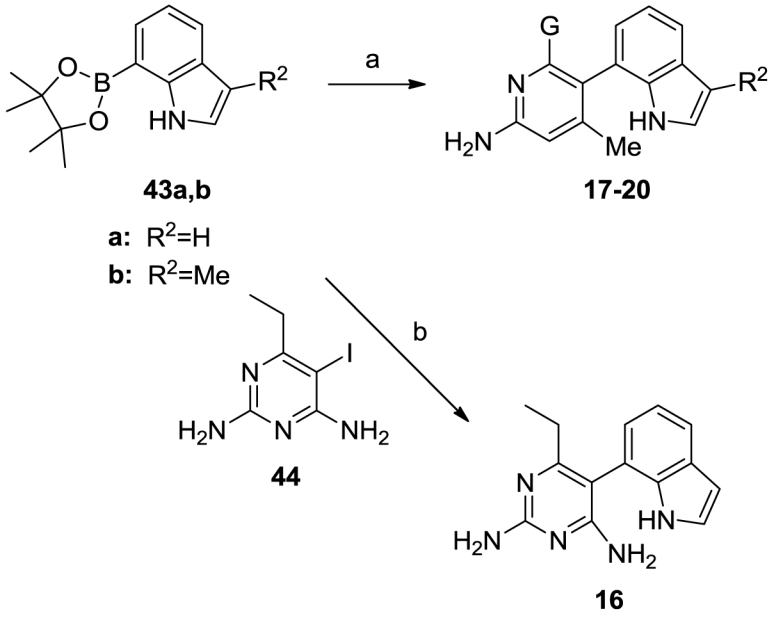
Predicted binding pose of **27** (cyan, yellow, blue) compared to **7d** (light green) in 2G1R showing the two different NH interactions with Gly223.



Scheme 1.

Synthesis of 7-(2-Aminopyridyl)tetrahydroquinoline Analogs[a]

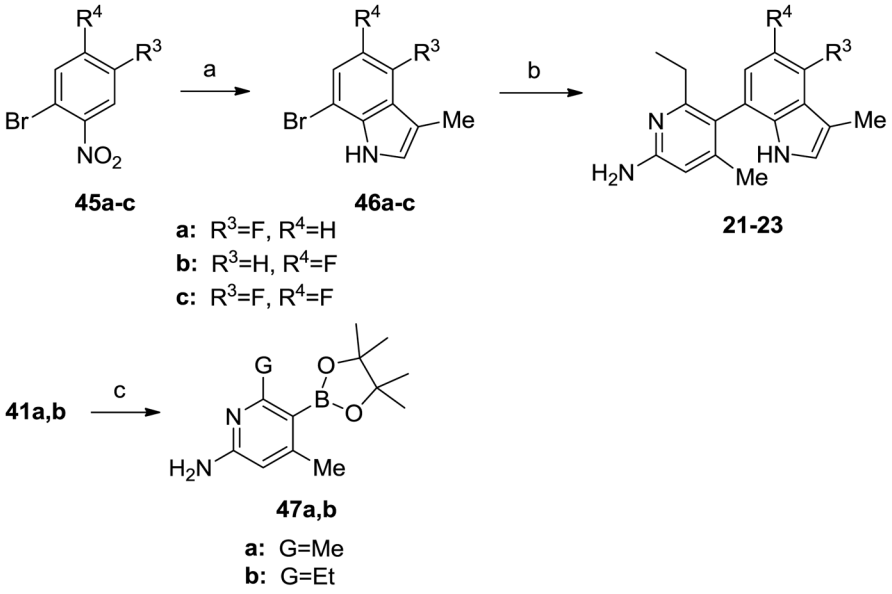
[a]Reagents and conditions. (a) NaOMe, MeOH; (b) Ph_2CNH, $Pd(OAc)_2$, BINAP, Cs_2CO_3, toluene, 100 °C; (c) (i) $Pd(PPh_3)_4$, Cs_2CO_3, toluene/H_2O, 100 °C; (ii) HCl, THF; (d) H_2, Pd, MeOH; (e) Br_2, AcOH (**40b**) or NBS, CH_3CN (**40c–d** °C.); (f) $Pd(PPh_3)_4$, aq. Na_2CO_3 or Cs_2CO_3, dioxane, 80–100 °C.



Scheme 2.

Synthesis of 7-(2-Aminopyridyl)- and 7-(2,4 Diaminopyrimidyl)indoles[a]

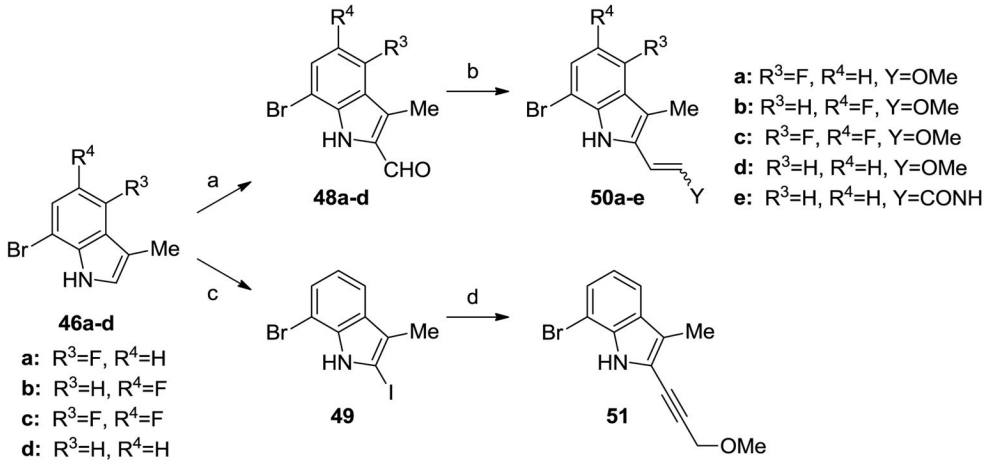
[a]Reagents and conditions. (a) **41a** or **41b**, $Pd(PPh_3)_4$, Na_2CO_3 or Cs_2CO_3, dioxane/H_2O, 100 °C. (b) $Pd(PPh_3)_4$, Na_2CO_3, dioxane/H_2O, 100 °C.



Scheme 3.

Synthesis of Fluoroindole Analogs[a]

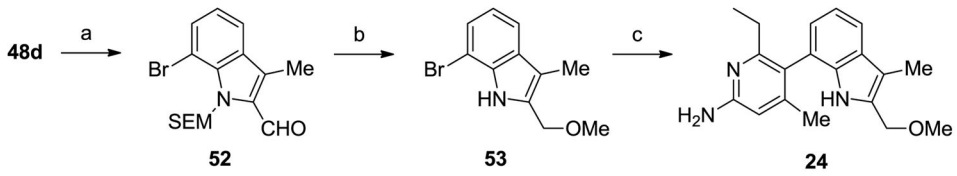
[a]Reagents and conditions. (a) 1-propenylMgBr, THF, −78 °C or 0 °C to rt; (b) **47b**, $Pd_2(dba)_3$/X-Phos or $Pd(PPh_3)_4$, Cs_2CO_3, dioxane/H_2O, 100 °C. (c) bis(pinacolato)diboron, $Pd(dppf)Cl_2$ or $Ph(PPh_3)_4$, KOAc, DMSO, 120 °C.



Scheme 4.

Synthesis of 2-Substituted Indole Intermediates[a]

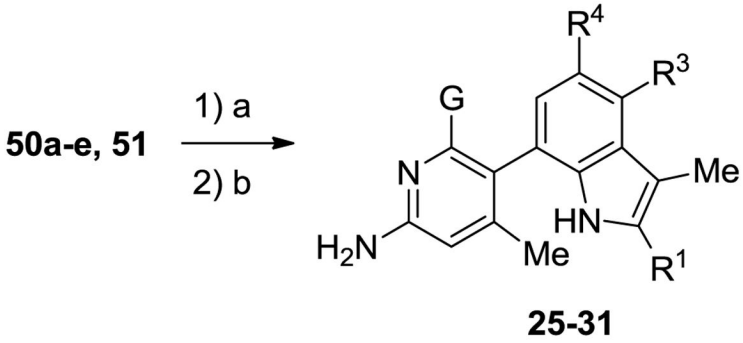
[a]Reagents and conditions. (a) $POCl_3$, DMF, DCE, 0 °C to rt; (b) $Ph_3P^+CH_2YX^-$, BuLi, THF, −78 °C or 0 °C to rt; (c) I_2, AgOTf, THF, −78 °C; (d) 3-methoxyprop-1-yne, $Pd(Ph_3P)_2Cl_2$, CuI, Et_3N, DMF.



Scheme 5.

Synthesis of Indole **24**[a]

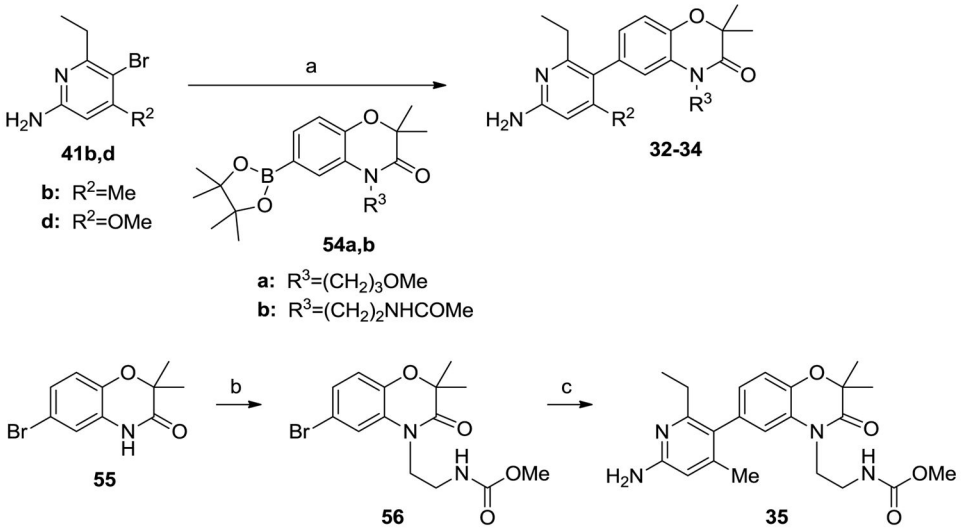
[a]Reagents and conditions. (a) NaH, SEMCl, CH_2Cl_2, 0 °C; (b) (i) $NaBH_4$, MeOH, THF, 0 °C; (ii) NaH, MeI, THF, 0 °C to RT; (iii) TBAF, ethylenediamine, THF; (c) **47b**, $Pd(PPh_3)_4$, Cs_2CO_3, dioxane/H_2O, 100 °C.



Scheme 6.

Synthesis of Indole Analogs **25–31**[a]

[a]Reagents and conditions. (a) **47a** or **47b**, $Pd(PPh_3)_4$, Na_2CO_3 or Cs_2CO_3, dioxane/H_2O, 100 °C; (b) H_2, Pd/C, MeOH.



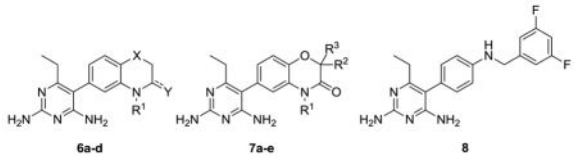
Scheme 7.

Synthesis of 6-(2-Aminopyridyl)benzoxazinone Analogs[a]

[a]Reagents and conditions. (a) $Pd(PPh_3)_4$, LiCl, CsOH H_2O, aq. dioxane, 100 °C; (b) Br $(CH_2)_2NHCO_2Me$, NaH, NaH, 15-cr-5, DMF; (c) (i) bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, dioxane, 85 °C; (ii) **41b**, $Pd(dppf)Cl_2$, K_3PO_4 $7H_2O$, toluene, 100 °C.

Table 1

2,4-Diaminopyrimidine Inhibitors



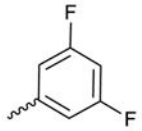
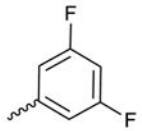
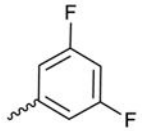
Cmpd	R^1	X	Y	R^2	R^3
6a	$(CH_2)_3OMe$	O	O	-	-
6b	$(CH_2)_3OMe$	O	H,H	-	-
6c	$(CH_2)_3OMe$	CH_2	H,H	-	-
6d	$(CH_2)_2NHCOMe$	CH_2	H,H	-	-
7a	$(CH_2)_3OMe$	-	-	Me	Me
7b	$(CH_2)_2NHCOMe$	-	-	Me	Me
7c	$(CH_2)_3OMe$	-	-	Me	
7d	$(CH_2)_2NHCOMe$	-	-	Me	
7e	$(CH_2)_3CF_3$	-	-	Me	

Table 2

Relative Interaction Values for Protonated Amines Compared to **11b**.

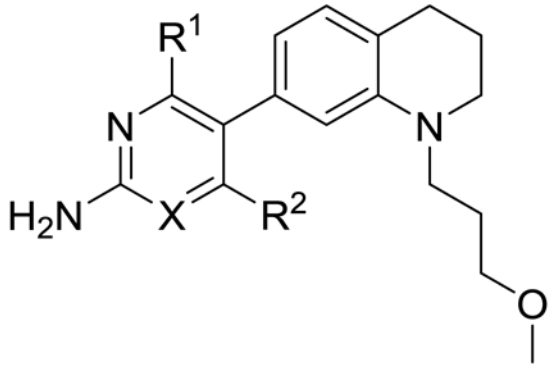
Compound	Rel. FE Pred (kcal/mol)	Rel. $\Delta\Delta G_s$ (kcal/mol)	Rel. ΔH protein[a] (kcal/mol)	Rel. ΔH Ser79 +Thr80[b] (kcal/mol)
10b	−2.0	−5.0	1.7	8.4
9b	−1.6	−4.5	−0.3	7.5
11b	0.0	0.0	0.0	0.0
10a	10.7	7.7	4.5	11.5
11a	13.2	13.3	2.5	0.0
9a	13.9	8.2	3.9	7.6

[a] Enthalpic interaction energy between fragment and entire protein.

[b] Enthalpic interaction energy between fragment and Ser79+Thr80 only.

Table 3

Comparison of Heterocyclic Groups Interacting with the Catalytic Aspartates.



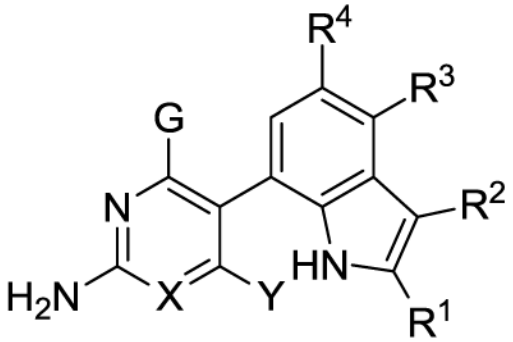
Cmpd	X	R^1	R^2	IC_{50} (μM)	Ligand Efficiency
6c	N	Et	NH_2	0.512[a,17]	0.34
12	CH	Me	Me	16.8	0.27
13	CH	Et	Me	0.491	0.34
14	CH	H	OMe	>100	-
15	CH	Et	OMe	0.500	0.33
1 (Aliskiren)	-	-	-	0.003[b,27]	0.30

[a] Lit. IC_{50} = 0.691 μM, K_d = 0.535 μM.

[b] Lit. IC_{50} = 0.0006 μM.

Table 4

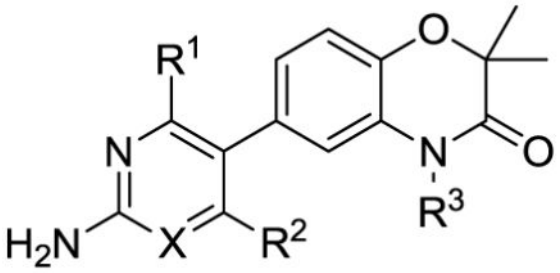
SAR of the indoles that were designed, synthesized, and tested.



Cmpd	X	Y	G	R^1	R^2	R^3	R^4	IC_{50} (μM)	LE
16	N	NH_2	Et	H	H	H	H	29.2	0.32
17	CH	Me	Me	H	H	H	H	124	0.30
18	CH	Me	Et	H	H	H	H	10.2	0.36
19	CH	Me	Me	H	Me	H	H	8.53	0.36
20	CH	Me	Et	H	Me	H	H	3.37	0.37
21	CH	Me	Et	H	Me	F	H	4.52	0.35
22	CH	Me	Et	H	Me	H	F	8.26	0.33
23	CH	Me	Et	H	Me	F	F	7.76	0.32
24	CH	Me	Et	CH_2OMe	Me	H	H	3.9	0.32
25	CH	Me	Me	$(CH_2)_2OMe$	Me	H	H	1.33	0.35
26	CH	Me	Et	$(CH_2)_2OMe$	Me	H	H	0.23	0.38
27	CH	Me	Et	$(CH_2)_2OMe$	Me	F	H	0.06	0.39
28	CH	Me	Et	$(CH_2)_2OMe$	Me	H	F	0.36	0.35
29	CH	Me	Et	$(CH_2)_2OMe$	Me	F	F	0.11	0.36
30	CH	Me	Et	$(CH_2)_3OMe$	Me	H	H	0.22	0.36
31	CH	Me	Et	$(CH_2)_2CONHMe$	Me	H	H	1.93	0.29

Table 5

Comparison of benzoxazinones that were designed, synthesized, and tested.



Cmpd	X	R^1	R^2	R^3	IC_{50} (μM)	LE
7b	N	Et	NH_2	$(CH_2)_2NHCOMe$	0.147	0.33
32	CH	Et	Me	$(CH_2)_2NHCOMe$	0.069	0.35
33	CH	Et	OMe	$(CH_2)_2NHCOMe$	0.045	0.33
34	CH	Et	Me	$(CH_2)_3OMe$	0.267	0.31
35	CH	Et	Me	$(CH_2)_2NHCO_2Me$	0.121	0.30

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Table 6

Rat Pharmacokinetic Data.

Cmpd	$t_{1/2}$[a] (h)	VD_{ss}[a] (L/kg)	Cl[a] (mL/kg min)	%F[b]	AUC[b] (ngh/mL)	C_{max}[b] (ng/mL)
27	2.2	10	68	20	473	87
32	1.0	3.4	66	58	1810	529
33	2.0	5.4	56	39	1493	331

[a] 1 mg/kg iv dose.

[b] 10 mg/kg po dose. Compounds dosed as their HCl salts; vehicle = D5W.

Table 7

Comparison of Some Key Metrics

Cmpd	Measured IC_{50} (μM)	Literature IC_{50} (μM)	MW	cLogP	LE	Relative LE *cf.* 7b	%*F* (rat)
1	0.003	0.0006	551	3.5	0.30[a]/0.32[b]	0.9	2.4
7b	0.147	0.020	385	2.8	0.33[a]/0.37[b]	1	nd[c]
7c	-	0.007	483	4.3	0.32	0.86	0.4
7d	-	0.001	496	3.3	0.34	0.91	8
rac-**7e**	-	0.141	521	5.0	0.25	0.68	12
(S)-**7e**	-	0.048	521	5.0	0.27	0.73	74
27	0.059	-	341	3.8	0.39	1.18	20
32	0.069	-	396	3.6	0.35	1.06	58
33	0.045	-	412	2.2	0.33	1.05	39

[a] Based on measured IC_{50}.

[b] Based on literature IC_{50}.

[c] Not determined.