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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 of human renin is described. Synthesis of a limited set of designed compounds provided potent, low MW analogs (IC<sub>50</sub>s < 100 nM) with good oral bioavailability (F >20-58%).

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### 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.<sup>1-3</sup> There is substantial evidence that early efforts to drive hyper-potency typically lead to large and hydrophobic compounds.<sup>4</sup> Gleeson<sup>5</sup> argues that such compounds inherently possess a dichotomy between physicochemical parameters associated with potency and those associated with desirable ADME characteristics. Analysis by Wenlock<sup>6</sup> indicated that in the development pipeline, failure of oral drugs was associated with the higher MW and LogP candidates. Another analysis by Oprea<sup>7</sup> 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.<sup>8, 9</sup> 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<sup>10</sup> 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<sup>11</sup> 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,<sup>5</sup> Wenlock<sup>6</sup> and Oprea<sup>7</sup> 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<sup>12, 13</sup> 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),<sup>14</sup> a metric used to describe affinity per molecular unit, is very useful to compare different

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Figure 1. Examples of non-peptidomimetic renin inhibitors.

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<sup>15</sup> (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.

Table 1. 2,4-Diaminopyrimidine Inhibitors

H <sub>2</sub> N N	NH <sub>2</sub> R <sup>1</sup> H <sub>2</sub> N N	NH <sub>2</sub>			
Cmpd	$\mathbf{R}^{1}$	Х	Y	$R^2$	R <sup>3</sup>
6a	(CH <sub>2</sub> ) <sub>3</sub> OMe	0	0	-	-
6b	(CH <sub>2</sub> ) <sub>3</sub> OMe	0	H,H	-	-
6c	(CH <sub>2</sub> ) <sub>3</sub> OMe	$\operatorname{CH}_2$	H,H	-	-
6d	(CH <sub>2</sub> ) <sub>2</sub> NHCOMe	$\operatorname{CH}_2$	H,H	-	-
7a	(CH <sub>2</sub> ) <sub>3</sub> OMe	-	-	Me	Me
7b	(CH <sub>2</sub> ) <sub>2</sub> NHCOMe	-	-	Me	Me
7c	(CH <sub>2</sub> ) <sub>3</sub> OMe	-	-	Me	F
					F
7d	(CH <sub>2</sub> ) <sub>2</sub> NHCOMe	-	-	Me	F
7e	(CH <sub>2</sub> ) <sub>3</sub> CF <sub>3</sub>	-	-	Me	F
					F

Significant efforts over more than two decades by several companies were invested in developing non-peptidomimetic renin inhibitors such as the piperidines (2),<sup>16</sup> piperazinones (3 and 4)<sup>17</sup> and ureas (5) (Figure 1).<sup>18</sup> We chose the  $2IKO^{19}$  X-ray protein structure as the input for SACP, because the diaminopyrimidine scaffolds (6-8) reported by Pfizer<sup>19</sup> 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.<sup>20</sup> 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.<sup>5-7</sup> 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 3dimensional 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").<sup>21</sup> 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 ("letterin-envelope"). Chemical potential  $(\mu)$  is a free energy metric, and because the method is thermodynamically principled, one can develop an understanding of the requirements of the ligandprotein 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).<sup>21</sup> 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<sup>22</sup> 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

Scheme 1. Synthesis of 7-(2-Aminopyridyl)tetrahydroquinoline Analogs<sup>a</sup>



<sup>a</sup>Reagents and conditions. (a) NaOMe, MeOH; (b) Ph<sub>2</sub>CNH, Pd(OAc)<sub>2</sub>, BINAP, Cs<sub>2</sub>CO<sub>3</sub>, toluene, 100 °C; (c) (i) Pd(PPh<sub>3</sub>)<sub>4</sub>, Cs<sub>2</sub>CO<sub>3</sub>, toluene/H<sub>2</sub>O, 100 °C; (ii) HCl, THF; (d) H<sub>2</sub>, Pd, MeOH; (e) Br<sub>2</sub>, AcOH (**40b**) or NBS, CH<sub>3</sub>CN (**40c-d**); (f) Pd(PPh<sub>3</sub>)<sub>4</sub>, aq. Na<sub>2</sub>CO<sub>3</sub> or Cs<sub>2</sub>CO<sub>3</sub>, dioxane, 80-100 °C.

**Scheme 2.** Synthesis of 7-(2-Aminopyridyl)- and 7-(2,4 Diaminopyrimidyl)indoles<sup>a</sup>



<sup>a</sup>Reagents and conditions. (a) **41a** or **41b**, Pd(PPh<sub>3</sub>)<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub> or Cs<sub>2</sub>CO<sub>3</sub>, dioxane/H<sub>2</sub>O, 100 °C. (b) Pd(PPh<sub>3</sub>)<sub>4</sub>, Na<sub>2</sub>CO<sub>3</sub>, dioxane/H<sub>2</sub>O, 100 °C.

Scheme 3. Synthesis of Fluoroindole Analogs<sup>a</sup>



<sup>a</sup>Reagents and conditions. (a) 1-propenylMgBr, THF, -78 <sup>°</sup>C or 0 <sup>°</sup>C to rt; (b) **47b**, Pd<sub>2</sub>(dba)<sub>3</sub>/X-Phos or Pd(PPh<sub>3</sub>)<sub>4</sub>, Cs<sub>2</sub>CO<sub>3</sub>, dioxane/H<sub>2</sub>O, 100 <sup>°</sup>C; (c) bis(pinacolato)diboron, Pd(dppf)Cl<sub>2</sub> or Ph(PPh<sub>3</sub>)<sub>4</sub>, KOAc, DMSO, 120 <sup>°</sup>C. 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 aminopyridylsubstituted 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

Scheme 4. Synthesis of 2-Substituted Indole Intermediates<sup>a</sup>



<sup>*a*</sup>Reagents and conditions. (a) POCl<sub>3</sub>, DMF, DCE, 0 °C to rt; (b)  $Ph_{3}P^{+}CH_{2}YX'$ , BuLi, THF, -78 °C or 0 °C to rt; (c)  $I_{2}$ , AgOTf, THF, -78 °C; (d) 3-methoxyprop-1-yne, Pd( $Ph_{3}P$ ), Cl<sub>3</sub>, CuI, Et<sub>3</sub>N, DMF.



<sup>*a*</sup>Reagents and conditions. (a) NaH, SEMCl,  $CH_2Cl_2$ , 0 <sup>*o*</sup>C; (b) (i) NaBH<sub>4</sub>, MeOH, THF, 0 <sup>*o*</sup>C; (ii) NaH, MeI, THF, 0 <sup>*o*</sup>C to RT; (iii) TBAF, ethylenediamine, THF; (c) **47b**, Pd(PPh<sub>3</sub>)<sub>4</sub>, Cs<sub>2</sub>CO<sub>3</sub>, dioxane/H<sub>2</sub>O, 100 <sup>*o*</sup>C.

Scheme 6. Synthesis of Indole Analogs 25-31<sup>a</sup>



<sup>*a*</sup>Reagents and conditions. (a) **47a** or **47b**,  $Pd(PPh_3)_4$ ,  $Na_2CO_3$  or  $Cs_2CO_3$ , dioxane/H,O, 100 °C; (b) H<sub>2</sub>, Pd/C, MeOH.

Scheme 7. Synthesis of 6-(2-Aminopyridyl)benzoxazinone Analogs<sup>a</sup>



<sup>a</sup>Reagents and conditions. (a)  $Pd(PPh_{3})_{4}$ , LiCl, CsOHH<sub>2</sub>O, aq. dioxane, 100 °C; (b)  $Br(CH_{2})_{2}NHCO_{2}Me$ , NaH, 15-cr-5, DMF; (c) (i) bis(pinacolato)diboron,  $Pd(dppf)Cl_{2}$ , KOAc, dioxane, 85 °C; (ii) **41b**,  $Pd(dppf)Cl_{3}$ , K<sub>3</sub>PO<sub>4</sub>7H<sub>3</sub>O, toluene, 100 °C.

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<sup>25</sup> 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 2position, 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<sub>i</sub>) should be a straight line. Instead of pK<sub>i</sub> 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



Figure 2. Correlation plot of predicted relative Free Energies versus pIC<sub>50</sub> for compounds 6a-d and 7a-d.

compounds, a plot of FE Pred versus the pIC<sub>50</sub> data shows an excellent correlation ( $R^2 = 0.82$ ) (Figure 2). Although 2IKO contains a weak ligand (8;  $IC_{50} = 4 \mu M)^{19}$  that lacks a sidechain in the S3 selectivity pocket (S3<sup>sp</sup>), 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<sup>sp</sup> 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<sub>50</sub> 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).



Figure 3. Protonated amines interacting at the catalytic site.

 Table 2. Relative Interaction Values for Protonated Amines Compared to 11b.

				Rel. ΔH
	Rel. FE		Rel. ∆H	Ser79
Compound	Pred	Rel. $\Delta\Delta G_s$	protein <sup>a</sup>	$+Thr80^{b}$
	(kcal/mol)	(kcal/mol)	(kcal/mol)	(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

<sup>a</sup>Enthalpic interaction energy between fragment and entire protein. <sup>b</sup>Enthalpic interaction energy between fragment and Ser79+Thr80 only.

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 Oatom 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.<sup>19</sup> 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 6ethylpyrimidine-2,4-diamine replacement by the 4-methyl or 4methoxy 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.

**Table 3.** Comparison of Heterocyclic Groups Interacting with the Catalytic Aspartates.

P	N H <sub>2</sub> N		R <sup>2</sup>	0	
Cmpd	Х	$\mathbf{R}^1$	$R^2$	IC <sub>50</sub> (µM)	Ligand Efficiency
6с	Ν	Et	NH <sub>2</sub>	$0.512^{a,17}$	0.34
12	СН	Me	Me	16.8	0.27
13	СН	Et	Me	0.491	0.34
14	СН	Н	OMe	>100	-
15	СН	Et	OMe	0.500	0.33
1 (Aliskiren)	-	-	-	$0.003^{b,27}$	0.30

 ${}^{a}$ Lit. IC<sub>50</sub> = 0.691  $\mu$ M, K<sub>d</sub> = 0.535  $\mu$ M.  ${}^{b}$ Lit. IC<sub>50</sub> = 0.0006  $\mu$ M.

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 2chlorotoluene 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-3methylindole 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<sup>sp</sup>. 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<sup>sp</sup> pocket compared to the 2IKO and 2G1R X-ray ligands (Figures 5 and 6).



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 21KO showing the interaction of the indole NH with Gly223, and the ether penetrating S3<sup>sp</sup>.



**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.

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.

 $G \qquad R^4 \qquad R^3$ 

Table 4. SAR of the indoles that were designed, synthesized, and tested.

$H_{2N} X Y HN R^{1}$									
Cmpd	X	Y	G	R	R <sup>2</sup>	R <sup>3</sup>	$R^4$	IC <sub>50</sub> (μΜ)	LE
16	Ν	$\mathrm{NH}_2$	Et	Н	Н	Н	Н	29.2	0.32
17	СН	Me	Me	Н	Н	Н	Н	124	0.30
18	СН	Me	Et	Н	Н	Н	Н	10.2	0.36
19	CH	Me	Me	Н	Me	Н	Н	8.53	0.36
20	СН	Me	Et	Н	Me	Н	Н	3.37	0.37
21	СН	Me	Et	Н	Me	F	Н	4.52	0.35
22	СН	Me	Et	Н	Me	Н	F	8.26	0.33
23	СН	Me	Et	Н	Me	F	F	7.76	0.32
24	CH	Me	Et	CH <sub>2</sub> OMe	Me	Н	Н	3.9	0.32
25	СН	Me	Me	(CH <sub>2</sub> ) <sub>2</sub> OMe	Me	Н	Н	1.33	0.35
26	СН	Me	Et	(CH <sub>2</sub> ) <sub>2</sub> OMe	Me	Н	Н	0.23	0.38
27	СН	Me	Et	(CH <sub>2</sub> ) <sub>2</sub> OMe	Me	F	Н	0.06	0.39
28	СН	Me	Et	(CH <sub>2</sub> ) <sub>2</sub> OMe	Me	Н	F	0.36	0.35
29	СН	Me	Et	(CH <sub>2</sub> ) <sub>2</sub> OMe	Me	F	F	0.11	0.36
30	СН	Me	Et	(CH <sub>2</sub> ) <sub>3</sub> OMe	Me	Н	Н	0.22	0.36
31	СН	Me	Et	(CH <sub>2</sub> ) <sub>2</sub> CONHMe	Me	Н	Н	1.93	0.29

**Table 5.** Comparison of benzoxazinones that were designed, synthesized, and tested.

$R^1$		~°~	$\vdash$
N		<sup>№</sup> R <sup>3</sup>	0
$H_2N^{\prime} X^{\prime}$	$R^2$		

Cmpd	Х	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	IC <sub>50</sub> (µM)	LE
7b	Ν	Et	$\mathrm{NH}_2$	(CH <sub>2</sub> ) <sub>2</sub> NHCOMe	0.147	0.33
32	СН	Et	Me	(CH <sub>2</sub> ) <sub>2</sub> NHCOMe	0.069	0.35
33	СН	Et	OMe	(CH <sub>2</sub> ) <sub>2</sub> NHCOMe	0.045	0.33
34	СН	Et	Me	(CH <sub>2</sub> ) <sub>3</sub> OMe	0.267	0.31
35	СН	Et	Me	(CH <sub>2</sub> ) <sub>2</sub> NHCO <sub>2</sub> Me	0.121	0.30

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,<sup>28</sup> 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<sup>sp</sup> 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<sub>50</sub> values, and they demonstrated reasonable halflives 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 nonobvious 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 x 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<sub>3</sub>CN: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-4,6dimethylpyridine (109 mg, 0.54 mmol), tetrakis(triphenylphosphine)palladium(0) (31 mg, 0.03 mmol), and aqueous Na<sub>2</sub>CO<sub>3</sub> 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  $\mu$ m C18 150  $\times$  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<sub>3</sub>CN:water (0.1% v/v TFA) to 50:50 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give 12 (53 mg, 30%) as the TFA salt as a yellow oil. <sup>1</sup>H NMR  $(300 \text{ MHz}, \text{CDCl}_3) \delta 14.72 \text{ (br s, 2H)}, 7.17 \text{ (d, 1H, J} = 6.0 \text{ Hz}),$ 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]<sup>+</sup>.

## 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-6ethyl-4-methylpyridine (130 mg, 0.6 mmol). tetrakis(triphenylphosphine)palladium(0) (35 mg, 0.03 mmol), and aqueous Na<sub>2</sub>CO<sub>3</sub> 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  $\times$  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<sub>3</sub>CN:water (0.1% v/v TFA) to 50:50 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give 13 (12 mg, 4%) as the TFA

Table 6.	Rat Pharmacokinetic Data.	

Cmpd	$t_{1/2}^{a}$ (h)	$Vd_{ss}^{a}$ (L/kg)	Cl <sup>a</sup> (mL/kg min)	$\%F^b$	AUC <sup>b</sup> (ngh/mL)	$C_{\rm max}^{\ \ b}$ (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
	4 h4 0 1	1 2 4	1 1 1 1 1 1 1 1 1			

<sup>a</sup>1 mg/kg iv dose. <sup>b</sup>10 mg/kg po dose. Compounds dosed as their HCl salts; vehicle = D5W.

Table 7. Comparison of Some Key Metrics								
Cmpd	Measured IC <sub>50</sub>	Literature IC <sub>50</sub>	MW	cLogP	LE	Relative LE cf. 7b	%F (rat)	
	(µM)	(µM)						
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 <sup>c</sup>	
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	

<sup>*a*</sup>Based on measured IC<sub>50</sub>. <sup>*b*</sup>Based on literature IC<sub>50</sub>. <sup>*c*</sup>Not determined.

salt as a yellow oil. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD)  $\delta$  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]<sup>+</sup>.

### 5.4. 4-Methoxy-5-(1-(3-methoxypropyl)-1,2,3,4tetrahydroquinolin-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<sub>2</sub>Cl<sub>2</sub>: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<sub>2</sub>CO<sub>3</sub> 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  $\times$  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<sub>3</sub>CN:water (0.1% v/v TFA) to 50:50 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give 14 (25 mg, 19%) as the TFA salt as a white solid. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD)  $\delta$  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. <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ )  $\delta$  7.19 (s, 2H), 3.89 (s, 3H). LCMS 178.0, 180.1 [M + 1]<sup>+</sup>.

(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]<sup>+</sup>.

(39). A mixture of 38 (900 mg, 2.8 mmol), 4,4,5,5tetramethyl-2-vinyl-1,3,2-dioxaborolane (650 mg, 4.2 mmol), tetrakis(triphenylphosphine)palladium(0) (650 mg, 0.56 mmol) and Cs<sub>2</sub>CO<sub>3</sub> (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<sub>2</sub>Cl<sub>2</sub>:MeOH, to give the desired vinylpyridine (460 mg, 52%) as a brown oil. <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ )  $\delta$ 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]<sup>+</sup>. 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]<sup>+</sup>.

(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 CH2Cl2 (30 mL) and washed with saturated aqueous NaHCO<sub>3</sub> (30 mL). The organic phase was washed with brine (20 mL), dried over Na2SO4 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<sub>2</sub>Cl<sub>2</sub>: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<sub>3</sub>CN (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 x 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. <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ )  $\delta$  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]<sup>+</sup>.

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<sub>2</sub>CO<sub>3</sub> (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  $150 \times 21.2$  mm; injection volume: 4 mL; flow rate: 20 mL/min; wavelength: 214 nm and 254 nm; elution: linear gradient of 20:80 CH<sub>3</sub>CN:water (0.1% v/v TFA) to 55:45 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give 15 (50 mg, 27%) as the TFA salt as a brown solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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]<sup>+</sup>.

#### 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 %). <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  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]<sup>+</sup>.

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<sub>2</sub>CO<sub>3</sub> (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<sub>3</sub>CN:water (0.1% v/v TFA) to 45:55 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give **16** (17 mg, 6%) as the TFA salt. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  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]<sup>+</sup>.

#### 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<sub>2</sub>CO<sub>3</sub> solution (2M, 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 x 10 mL). The combined organic phases were washed with brine, dried over Na2SO4 and concentrated under vacuum. The crude product was purified by preparative TLC (20:1 CH<sub>2</sub>Cl<sub>2</sub>:MeOH), then recrystallized (5:1 hexane:EtOAc, 6 mL) to give 17 (60 mg, 25%) as a white solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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]<sup>+</sup>.

### 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 mmol), mg, 0.8 mmol), 41b (150 mg, 0.7 tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.03 mmol), and aqueous Na<sub>2</sub>CO<sub>3</sub> solution (2M, 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  $\mu$ m C18 150  $\times$  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<sub>3</sub>CN:water (0.1% v/v TFA) to 50:50 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give 18 (65 mg, 26%) as the TFA salt as a pale yellow solid. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD) 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<sub>2</sub>CO<sub>3</sub> (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 x 5 mL). The combined organic phases were dried over Na2SO4 and concentrated under vacuum. The product residue was purified by preparative HPLC (column: Phenomenex Gemini 5 µm C18 150  $\times$  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<sub>3</sub>CN:water (0.1% v/v TFA) to 60:40 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give **19** (20 mg, 30%) as the TFA salt as a white solid. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD)  $\delta$  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]<sup>+</sup>.

## 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<sub>2</sub>CO<sub>3</sub> (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 x 5 mL). The combined organic phases were dried over Na2SO4 and concentrated under vacuum. The crude residue was purified by preparative HPLC (column: Phenomenex Gemini 5  $\mu$ m C18 150  $\times$  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<sub>3</sub>CN:water (0.1% v/v TFA) to 70:30 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give **20** (15 mg, 21%) as the TFA salt as a white solid. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD)  $\delta$  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]<sup>+</sup>.

#### 5.11. 6-Ethyl-5-(4-fluoro-3-methyl-1H-indol-7-yl)-4methylpyridin-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 1propenylmagnesium 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<sub>4</sub>Cl. The mixture was extracted with EtOAc (3 x 50 mL), and the combined organic layers were washed with brine (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated under vacuum to afford 46a (1.1 g, 55%) as a yellow solid. <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  11.21 (br s, 1H), 7.32 - 6.96 (m, 2H), 6.68 (dd, 1H, *J*<sub>1</sub> = 12.0 Hz, *J*<sub>2</sub> = 9.0 Hz), 2.35 (s, 3H). GCMS 226, 228 [M]<sup>+</sup>.

(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 x 100 mL), and the combined organic phases were washed with water (50 mL) and brine (50 mL), dried over Na<sub>2</sub>SO<sub>4</sub>, concentrated under vacuum and purified by silica gel column chromatography, eluting with 100:1 CH<sub>2</sub>Cl<sub>2</sub>: 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<sub>2</sub>CO<sub>3</sub> (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<sub>2</sub>Cl<sub>2</sub>:MeOH, to give **21** (50 mg, 20%) as a brown solid. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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]<sup>+</sup>.

## 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<sub>4</sub>Cl (100 mL) and extracted with EtOAc (2 x 500 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> 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. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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]<sup>+</sup>.

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<sub>2</sub>CO<sub>3</sub> (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 x 5 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The crude product was purified by preparative HPLC (column: Phenomenex Gemini 5  $\mu$ m C18 150  $\times$  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<sub>3</sub>CN:water (0.05% v/v NH<sub>4</sub>OH) to 90:10 CH<sub>3</sub>CN:water (0.05% v/v NH<sub>4</sub>OH) over 10 min) to give 22 (33 mg, 33%) as a light brown solid. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  7.17 (*dd*, 1H, J = 10.0 Hz, J = 4.0Hz), 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-4methylpyridin-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<sub>2</sub>SO<sub>4</sub>, and concentrated under vacuum. The crude product was purified by silica gel column chromatography to afford **46c** (85 mg, 35%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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<sub>2</sub>CO<sub>3</sub> (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 x 5 mL). The combined organic phases were washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The crude product was purified by preparative HPLC (column: Phenomenex Gemini 5  $\mu$ m 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<sub>3</sub>CN:water (0.1% v/v TFA) to 50:50 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give **23** (11 mg, 8%) as the TFA salt. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  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]<sup>+</sup>.

## 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<sub>2</sub>Cl<sub>2</sub> (30 mL) at 0 °C under nitrogen was added 48d (237 mg, 1 mmol). After warming to room h, a solution temperature over of 2-1 (trimethylsilyl)ethoxymethyl chloride (200 mg, 1.2 mmol) in dry CH<sub>2</sub>Cl<sub>2</sub> (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 x 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. <sup>1</sup>H NMR (300 MHz, DMSO)  $\delta$  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. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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]<sup>+</sup>.

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<sub>2</sub>Cl<sub>2</sub>. 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. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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<sub>2</sub>CO<sub>3</sub> (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  $\mu$ m 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<sub>3</sub>CN:water (0.05% v/v NH<sub>4</sub>OH) to 55:45 CH<sub>3</sub>CN:water

(0.05% v/v NH<sub>4</sub>OH) over 10 min) to give **24** (33 mg, 14%) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  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]<sup>+</sup>.

### 5.15. 5-(2-(2-Methoxyethyl)-3-methyl-1H-indol-7-yl)-4,6dimethylpyridin-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<sub>2</sub>Cl<sub>2</sub> (3 x 50 mL). The combined organic phases were washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> 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. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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]<sup>+</sup>.

(50d). То а 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<sub>4</sub>Cl (20 mL) was added. The mixture was extracted with EtOAc (3 x 10 mL). The combined organic phases were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, 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: <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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 x 10 mL). The combined organic phases were washed with brine (2 x 10 mL), dried over Na<sub>2</sub>SO<sub>4</sub> 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. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) 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]<sup>+</sup>. 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<sub>2</sub>CO<sub>3</sub> 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 x 5 mL). The combined organic phases were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, 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]<sup>+</sup>.

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  $\mu$ m C18 150  $\times$  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<sub>3</sub>CN:water (0.1% v/v TFA) to 38:62 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give **25** (4 mg, 3%) as the TFA salt. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  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]<sup>+</sup>.

## 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<sub>2</sub>CO<sub>3</sub> 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 x 5 mL). The combined organic phases were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, 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]<sup>+</sup>.

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  $\mu$ m C18 150  $\times$  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<sub>3</sub>CN:water (0.1% v/v TFA) to 50:50 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give 26 (5 mg, 4%) as the TFA salt as a yellow oil. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  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]<sup>+</sup>.

## 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  $^{\circ}$ 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<sub>2</sub>Cl<sub>2</sub> (3 x 10 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> 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. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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 x 3 mL). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> 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. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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<sub>2</sub>CO<sub>3</sub> (346 mg, 1.06 mmol) in dioxane (10 mL) and (2added water mL) was 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 x 5 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The crude product was purified by preparative TLC, eluting with 10:1 CH<sub>2</sub>Cl<sub>2</sub>:MeOH, to give the desired (E)- and (Z)-6-ethyl-5-(4-fluoro-2-(2-methoxyvinyl)-3methyl-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<sub>3</sub>CN:water (0.05% v/v NH<sub>4</sub>OH) to 75:25 CH<sub>3</sub>CN:water (0.05% v/v NH<sub>4</sub>OH) over 10 min) to give **27** (32 mg, 55%) as a white solid. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  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]<sup>+</sup>.

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 x 20 mL). The combined organic layers were washed with brine (2 x 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<sub>2</sub>CO<sub>3</sub> (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 x 20 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> 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]<sup>+</sup>.

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  $\times$  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<sub>3</sub>CN:water (0.05% v/v NH<sub>4</sub>OH) to 90:10 CH<sub>3</sub>CN:water  $(0.05\% \text{ v/v NH}_4\text{OH})$  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. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$ 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]<sup>+</sup>.

## 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<sub>2</sub>Cl<sub>2</sub> (3 x 20 mL), and the combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> 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. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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 solution added mL) was а 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 x 5 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> 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. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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 x 10 mL). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated under vacuum. The crude product was purified by preparative TLC, eluting with 16:1 CH<sub>2</sub>Cl<sub>2</sub>: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]<sup>+</sup>.

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  $\mu$ m 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<sub>3</sub>CN:water (0.05% v/v NH<sub>4</sub>OH) to 99:1 CH<sub>3</sub>CN:water (0.05% v/v NH<sub>4</sub>OH) over 10 min) to give **28** (15 mg, 21%) as a white solid. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  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]<sup>+</sup>.

### 5.19. 5-(4,5-Difluoro-2-(2-methoxyethyl)-3-methyl-1H-indol-7yl)-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<sub>2</sub>Cl<sub>2</sub> (3 x 50 mL). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, 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%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 10.03 (br s, 1H), 8.80 (br s, 1H), 7.36-7.42 (m, 1H), 2.75 (s, 3H).

То (50c). suspension of а (methoxymethyl)triphenylphosphonium chloride (879 mg, 2.56 mmol) in THF (5 mL) was added n-butyllithium (1.16 mL, 2.2M 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<sub>4</sub>Cl (20 mL). The mixture was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> 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. <sup>1</sup>H NMR (300 MHz,  $CDCl_3$ )  $\delta$  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<sub>2</sub>CO<sub>3</sub> (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 x 5 mL). The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, 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]<sup>+</sup>.

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  $\mu$ m 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<sub>3</sub>CN:water (0.1% v/v Et<sub>3</sub>N) to 50:50 CH<sub>3</sub>CN:water (0.1% v/v Et<sub>3</sub>N) over 10 min) to give **29** (12 mg, 15%). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  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]<sup>+</sup>.

## 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<sub>3</sub> (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<sub>3</sub> 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. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  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]<sup>+</sup>.

(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. <sup>1</sup>H NMR (300 MHz, DMSO)  $\delta$  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]<sup>+</sup>.

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<sub>3</sub>CN:water (0.1% v/v TFA) to 50:50 CH<sub>3</sub>CN:water (0.5% v/v NH<sub>4</sub>OH) 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. <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD)  $\delta$  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]<sup>+</sup>. 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  $\mu$ m 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<sub>3</sub>CN:water (0.1% v/v TFA) to 50:50 CH<sub>3</sub>CN:water (0.5% v/v NH<sub>4</sub>OH) over 10 min) to give **30** (7 mg, 17%) as a white solid. <sup>1</sup>H NMR (400 MHz, DMSO)  $\delta$  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]<sup>+</sup>.

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

(50e). То a suspension of (2-(methylamino)-2oxoethyl)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, 2.2M 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 The reaction mixture was stirred at room THF (5 mL). temperature overnight, quenched with saturated aqueous NH4Cl (10 mL), and extracted with EtOAc (3 x 5 mL). The combined organic phases were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, 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<sub>3</sub>)  $\delta$  12.96 (br s, 1H), 7.49 (d, 1H, J = 9.0Hz), 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 x 5 mL). The combined organic phases were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, 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]<sup>+</sup>.

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<sub>3</sub>CN:water (0.1% v/v TFA) to 33:67 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give **31** (6 mg, 6%) as the TFA salt. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  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]<sup>+</sup>.

#### 5.22. N-(2-(6-(6-Amino-2-ethyl-4-methylpyridin-3-yl)-2,2dimethyl-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  $\mu m$  C18 150  $\times$  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<sub>3</sub>CN:water (0.1% v/v TFA) to 70:30 CH<sub>3</sub>CN: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. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  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)-2,2dimethyl-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  $\mu$ m C18 150  $\times$  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<sub>3</sub>CN:water (0.1% v/v TFA) to 40:60 CH<sub>3</sub>CN: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. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  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-(3methoxypropyl)-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<sub>3</sub>CN:water (0.1% v/v TFA) to 45:55 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give **34** (30 mg, 25%) as the TFA salt as a yellow oil. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  7.12 (*s*, 1 H), 7.10 (*d*, 1H, *J* = 8.4 Hz), 6.89 (*dd*, 1H, *J*<sub>1</sub> = 8.4 Hz, *J*<sub>2</sub> = 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]<sup>+</sup>.

#### 5.25. Methyl (2-(6-(6-amino-2-ethyl-4-methylpyridin-3-yl)-2,2dimethyl-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.*<sup>18b</sup>) (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 x 100 mL). The combined organic layers were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> 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]<sup>+</sup>.

A mixture of 56 (0.2 g, 0.56 mmol), bis(pinacolato)boron [1,1'-bis-(0.17)0.67 mmol), g, (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  $\times$  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<sub>3</sub>CN:water (0.1% v/v TFA) to 80:20 CH<sub>3</sub>CN:water (0.1% v/v TFA) over 10 min) to give 35 (35 mg, 34%) as a TFA salt as a white solid. <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD)  $\delta$  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]<sup>+</sup>.

#### 5.26. Renin Inhibition Assay.

Synthesized compounds were tested for inhibition against human renin using the commercially available SensoLyte<sup>®</sup> 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<sub>50</sub> 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 IACUCapproved 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 HCI 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).

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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.

#### 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<sub>ss</sub>, steady-state volume of distribution; Cl, clearance;  $C_{\text{max}}$ , maximum concentration.

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### **Graphical Abstract**

