Published in final edited form as:

J Med Chem. 2018 August 23; 61(16): 7065–7086. doi:10.1021/acs.jmedchem.8b00084.

Identification of the 4-position of 3-alkynyl and 3-heteroaromatic substituted pyridine methanamines as a key modification site eliciting increased potency and enhanced selectivity for cytochrome P-450 2A6 inhibition.

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Abstract

Cigarette smoking causes nearly one in every five deaths in the United States. The development of a specific inhibitor of cytochrome P450 2A6 (CYP2A6), the major nicotine-metabolizing enzyme in humans, which could be prescribed for the cessation of cigarette smoking, has been undertaken. To further refine the structure activity relationship of CYP2A6, previously synthesized 3-alkynyl and 3-heteroaromatic substituted pyridine methanamines were used as lead compounds. Isosteric pyridine replacement and appendage of all available positions around the pyridine ring with a methyl group was performed to identify a modification that would increase CYP2A6 inhibition potency which led to **4g** ($IC_{50} = 0.055$ mM) as a primary analogue. Potent compounds were evaluated for CYP selectivity, human liver microsomal half-life and compliance with the rules of five. Top compounds (i.e., **6i**, $IC_{50} = 0.017$ mM, >120 min half-life) are poised for further development as treatments for smoking and tobacco use cessation.

Graphical Abstract

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Supporting Information

The supporting information is available free of charge on the ACS publications website at <http://pubs.acs.org>. ^1H and ^{13}C NMR spectra, the UPLC/UV and UPLC/MS traces for the synthesized nicotine analogues (PDF). SMILES codes of final compounds including CYP IC_{50} s and half-life for calculations (CSV)

Notes:

The authors declare no conflict of financial interests.

Keywords

Nicotine; Cytochrome P450 2A6; Cotinine; Smoking Cessation; Tobacco Use

Introduction:

According to the World Health Organization, worldwide tobacco use causes nearly 6 million deaths per year and current trends show that tobacco use will cause more than 8 million deaths annually by 2030.¹ Cigarette smoking is responsible for more than 480,000 deaths per year in the United States, including nearly 42,000 deaths resulting from secondhand smoke exposure. This is about one in five deaths annually, or 1,300 deaths every day.² More deaths are caused each year by tobacco use than by human immunodeficiency virus (HIV), illegal drug use, alcohol use, motor vehicle injuries, suicides and murders combined.³ Smoking causes an estimated 90% of all lung cancer deaths in men and 80% of all lung cancer deaths in women. Hence, if smoking ceased altogether, one of every three cancer deaths in the United States would not occur.

More people in the United States are addicted to nicotine than to any other drug and, in 2010, 69% of all current U.S. adult cigarette smokers reported that they wanted to quit completely.⁴ Unfortunately, according to the National Institutes of Drug Abuse, only about 6 percent of smokers are able to quit in a given year.⁵ It is, therefore, of utmost importance to identify novel smoking cessation strategies.

Nicotine is the molecule responsible for the psychopharmacological effects and the addictive properties of tobacco and cigarette smoking. Currently, the most often used smoking cessation strategies are nicotine replacement therapies including: patches, gum, inhalers, nasal spray and lozenges alone or in combination with the $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist, varenicline. A recent study determined that 12 weeks of treatment with a nicotine patch, the drug varenicline, or combination nicotine replacement therapy produced no significant differences in confirmed rates of smoking abstinence at 26 or 52 weeks.⁶ Moreover, varenicline is associated with a high incidence of side effects that reduce medication compliance (nausea, constipation, sleep disorders)⁷, although recent studies have found controversial results,⁸ past studies associated this treatment with less-frequent, but severe, psychiatric contraindications such as violent behavior and suicidal tendencies.^{9, 10}

Smokers adjust their tobacco use to maintain a certain blood and brain level of nicotine.^{11–13} Because of this, modulation of the enzymatic degradation of nicotine may have potential as a cessation strategy. After entering the circulation, nicotine is eliminated mainly by

metabolism to its major metabolite, cotinine. Nicotine to cotinine metabolism occurs in a two-step reaction, where nicotine is first oxidized to nicotine- 5'(1')-iminium ion, mainly catalyzed by CYP2A6.¹⁴ The intermediate iminium ion is then further oxidized to cotinine, in a second step, by either CYP2A6 or the cytosolic enzyme, aldehyde oxidase.¹⁵ It has been shown that individuals with decreased or loss-of-function CYP2A6 genotypes smoke less.^{16, 17} It has been hypothesized that specific inhibition of CYP2A6 will be well tolerated in humans.¹⁸ By mimicking the effects of these naturally-occurring variants in the human population through the use of a pharmacological agent (a CYP2A6 inhibitor), it is anticipated that individual smokers would experience increased levels of nicotine in blood, and a corresponding decrease in levels of smoking/tobacco use. An individual's exposure to the detrimental and carcinogenic effects of tobacco and cigarette smoke would decrease accordingly.^{19–22} The increase in nicotine plasma half-life should delay the onset of withdrawal symptoms typically felt by the tobacco user, affording this individual the opportunity to delay their subsequent tobacco use, potentially resulting in a corresponding decrease in the behavioral addiction component, i.e., the act of smoking itself. Therefore, the smoker may also have a larger chance for success in quitting altogether. The goal of the present study was to develop agents that can specifically inhibit CYP2A6 and potentially be used for the cessation of cigarette smoking and tobacco use. Previous drug discovery studies have identified a pharmacophore consisting of a pyridine ring connected to a primary methanamine via various linker motifs including an alkyne (Figure 1), furan, thiophene, isoxazole or pyrazole on the 3-position. What has not been performed is the search for an acceptable position on the pyridine ring to be utilized as a site for modification to increase the potency and selectivity of the new compounds for CYP2A6. To address this, a traditional structure activity relationship (SAR) was undertaken. Each available ring position was modified with a common group (methyl in this instance, Figure 1) to identify the position to be elaborated. The 4-position of the pyridine ring was identified and the further modification of this position led to a set of lead compounds with all of the characteristics of an orally available drug that can be more carefully characterized in pre-clinical studies as potential new smoking cessation agents.

Chemistry:

The synthesis of all of the compounds tested is shown in Scheme 1. At the time of the experiments, all of the aryl halides were commercially available. Compounds **4a–4q** were synthesized by the Sonogashira reaction between the select aryl halide and NH-Boc propargylamine, utilizing conditions described for the previous round of inhibitors.^{21, 22} The intermediates, **1a–1q**, were deprotected with trifluoroacetic acid in dichloromethane, as previously described.^{21, 22} Once the protecting groups were removed, the compounds were treated with anhydrous hydrogen chloride in ethyl ether to afford the hydrochloride salts. The compounds, in the salt form, were all isolated as solids that were highly soluble in aqueous based buffers for use in the subsequent biological assays.

Compounds **5g**, **5i–5q** and **6g**, **6i–6q** were analogously prepared by the Suzuki coupling of the appropriate aryl halide and, for compounds **2g**, **2i–2q**, (5-(((tert-butoxycarbonyl)amino)methyl)furan-2-yl)boronic acid or, for compounds **3g**, **3i–3q**, (5-(((tert-butoxycarbonyl)amino)methyl)thiophen-2-yl)boronic acid. These compounds were

subsequently deprotected as described above for **1a-1q**. The hydrochloride salts were formed by treating the free base amines with anhydrous hydrogen chloride in ethyl ether to afford the furan-linked (**5g**, **5i-5q**) and thiophene linked (**6g**, **6i-6q**) target compounds. Two compounds containing two- (compounds **4k** and **4l**), three- (compounds **4p** and **6o**) and four-nitrogen atoms (compounds **4q** and **5q**) were analyzed for chlorine content by combustion analysis. It was determined that all of the protonatable nitrogen atoms formed the hydrochloride salts under these conditions. Compounds that contain 4-nitrogens were determined to be intermediate between tri- and tetra-hydrochlorides.

Results and Discussion:

1.CYP2A6 inhibitor screening

Previously, Denton, et al. prepared a library of compounds in an effort to delineate the initial pharmacophore of CYP2A6 using 5-substituted, 6-substituted and unsubstituted 3-heteroaromatic pyridine analogues of nicotine.^{21, 22} This study revealed the repetitive pharmacophore for the most active and selective inhibitors to consist of a 3-substituted pyridine ring connected to a primary methanamine via a linker consisting of an alkyne, a 1,5-substituted furan or a 1,5-substituted thiophene. Using these previous structure, activity and selectivity data, as well as the availability of N-Boc propargylamine, the compound consisting of the 3-pyridyl-alkyne-mathanamine (**4a**) was chosen as the reference agent for the current study.

In an effort to identify an allowable site for modification of the pyridine ring portion of the reference compound **4a**, a series of compounds consisting of the fused ring heterocyclic 4-substituted isoquinoline (**4b**), the 3-substituted quinoline (**4c**), the 5-substituted pyrimidine (**4d**) and the methyl substituted pyridines **4e-4h** were prepared as described in Scheme 1. Since the active site of CYP2A6 is much smaller than most other CYPs (nearly four times smaller than CYPs 2C8, 2C9 and 3A4)²³, it is important to determine the structural modifications allowable for retention of CYP2A6 binding and, therefore, enzyme inhibition, which should allow for selectivity over other CYP isoforms. In addition, the existing co-crystal structures²² were utilized to help visualize potential steric contacts of new lead compounds.

As a pre-screening, all compounds were assayed at 1 μ M and 10 μ M for inhibition of CYP2A6 mediated coumarin 7-hydroxylation in pooled, human liver microsomes by ultraperformance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS). For those agents that exhibited >50% inhibition of activity at 10 μ M, IC₅₀ determinations were performed using multiple concentrations (0.005, 0.01, 0.1, 0.5, 1, 5, 10, 25 and 100 μ M) of inhibitor.

As shown in Table 1, isoquinoline (**4b**) or quinoline (**4c**) replacement of the pyridine ring decreased or eliminated inhibition of CYP2A6 activity (IC₅₀ values greater than 10 μ M) as compared to the reference compound (**4a**), presumably by steric repulsion of these compounds within the CYP2A6 active site. A similar lack of inhibition was observed when replacing the pyridine ring with the sterically similar 5-substituted pyrimidine ring (**4d**). As determined by previous X-ray crystallographic analysis of related nicotine analogues co-

crystallized within the active site of CYP2A6, the nitrogen of the pyridine moiety consistently forms a hydrogen bond pair with residue Asn 297, which lines the small cavity of the CYP2A6 active site.²² The pyrimidine nitrogen is much less basic than that of the pyridine, thus decreasing its ability to accept hydrogen bonds, which may be the factor detracting from the inhibitory activity of compound **4d**. Although, in related compounds prepared previously,²² when the pyridine ring of the active compound was replaced by a phenyl ring and, analogous to compound **4d**, the alkyne moiety was replaced by a furan (compound **22**, reference 1) or a thiophene (compound **23**, reference 1) the activity decreased substantially but not completely, as was the case for compound **4d** (IC₅₀ of compound **1a** ref 1, pyridine, furan analogue = 0.08 μ M, IC₅₀ of compound **22** ref 1, phenyl, furan analogue = 0.6 μ M, IC₅₀ of compound **2a** ref 1, pyridine, thiophene analogue = 0.1 μ M, IC₅₀ of compound **23** ref 1, phenyl, thiophene analogue = 0.6 μ M). Because of the comparable size of the pyrimidine and pyridine rings and the substantial, but not complete, decrease in potency when replacing the pyridine ring with a phenyl ring, the lack of productive binding, and thus, enzyme inhibition by compound **4d**, is likely caused by an electronic repulsion of the nitrogen of the 3-position of the pyrimidine ring with an important residue of the active site of CYP2A6. Due to the lack of potency of the pyrimidine containing compounds, they were not considered leads. In future rounds of SAR, to gain a better understanding of how this class of compounds interact with the CYP2A6 active site, they may be investigated further.

To assess the relative effect of substitutions on the 2-, 4-, 5- and 6-position of the pyridine ring, methyl groups were incorporated at positions 2 (compound **4h**), 4 (compound **4g**), 5 (compound **4f**) and 6 (compound **4e**). Substitution of a methyl group at the 4-position caused the most potent inhibition of CYP2A6 with an IC₅₀ value of 0.055 μ M. Substitution of methyl groups for the hydrogens at the 2- and 5-position afforded compounds with no inhibitory activity (no inhibition at 10 μ M) while moderate inhibition (IC₅₀ value = 5.5 μ M) was observed by addition of a methyl group at the 6- position of the pyridine ring (compound **4e**; Table 1).

The methyl group of compound **4g** was exchanged for a methoxy group (compound **4j**) to explore both the increased size and change in electronic nature of the group (from van Der Waals interactions only to van Der Waals plus hydrogen bond accepting). This change resulted in a nearly two orders of magnitude decrease in inhibition potency (from IC₅₀ = 0.055 μ M to IC₅₀ = 3.4 μ M). To determine if the size or electronics were responsible for the decreased inhibition potency of compound **4j**, the 4-position was substituted with an ethyl group (**4i**). While good inhibition of CYP2A6 activity was still observed, the IC₅₀ value of this new compound decreased substantially from that of the methyl substitution - from 0.055 μ M to 0.83 μ M. This data indicates that the decreased inhibition of analogue **4j** may be due to electronic (oxygen lone pairs) rather than steric factors (the ethyl and methoxyl groups are roughly the same steric volume). The placement of a propyl group (compound **4k**) and a phenyl group (compound **4l**) at the 4-position of the pyridine ring resulted in similar decreases in inhibitory activity against CYP2A6 with IC₅₀ values of 0.44 μ M and 0.13 μ M, respectively. Although there is potential for off-target, CYP-mediated metabolism of the phenyl group (epoxidation/hydroxylation) and the methyl, ethyl and propyl groups (benzylic

hydroxylation), the high inhibitory activity of these compounds supported a strategy of keeping the molecular weight of the compounds low. This feature will allow for future refinement of lead compounds, while staying within the molecular weight parameter of Lipinski's rule of five (MW less than 500 amu).

To further glean information about the availability of space in the active site of CYP2A6 around substituents appended to the 4-position of the ligands, some molecular modeling was performed. Although the alkyne linker was chosen as the reference compound in this study, an examination of the X-ray co-crystal structure of CYP2A6 complexed with compound **5a** (PDB ID 2FDW) was used as the basis of the computational analysis. It has been reported that phenylalanine (F) residues 107, 209 and 480 are important for binding of inhibitors of this class (3-pyridyl-linker-mathanamine) with CYP2A6.²² Using crystal structure 2FDW as a template, a molecular model was built with a phenyl ring appended to the 4-position of compound **5a**, using the PyMOL Molecular Graphics System (Version 2.0, Schrödinger, LLC). Additionally, a local minimization was performed using Biovia Discovery Studio (Dassault Systèmes BIOVIA, San Diego, CA). The resulting, minimized structure is shown in Figure 2. After minimization, the phenyl ring of the new Compound (**5l**) is accommodated nicely within an "aromatic nest" comprised of residues F07, F209 and F480. The model suggests positive pi-pi interactions of the ligand's phenyl group with end-to-face interactions with F107 and F480 as well as potential face-to-face interactions with F209. Although the model, and the *in vitro* data obtained, suggest a positive interaction between the phenyl group and the aromatic nest (compound **4l**, $IC_{50} = 0.13 \mu M$, as a reminder, at this point in the study, the alkyne linker was being employed in lieu of not having a crystal structure available containing the alkyne linker; therefore, the furan linker was used as a surrogate), compound **4g**, with a methyl group at the 4-position ($IC_{50} = 0.055 \mu M$) was indeed, a better inhibitor. The use of molecular modeling is a good tool in predicting "potential" compounds as ligands in any system, although the need for synthesis and testing is never without need to confirm, or deny, any computational outcomes that may be predicted. With the data obtained by the combination of 1) wet chemistry with *in vitro* binding assays to prove inhibition by the synthesized compounds, 2) analysis of previously obtained X-ray crystal structures to provide physical evidence of ligand binding and 3) the use of molecular modeling to append the previously bound ligands with functional groups to assess ability of the lipophilic pocket, or "aromatic nest" to accommodate functional groups in this region of the active site, a series of compounds, substituted at the 4-position with various aromatic moieties, was prepared and evaluated for *in vitro* inhibition of CYP2A6.

The phenyl group of compound **4l** ($IC_{50} = 0.13 \mu M$) was replaced with the smaller, but still aromatic, hydrogen bond accepting furan-2-yl (compound **4m**) and furan-3-yl (compound **4n**) groups, which afforded IC_{50} values of $0.56 \mu M$ and $0.67 \mu M$, respectively. The placement of the oxygen atom in the aromatic ring, in these two compounds, does not make a significant difference in inhibition potency, indicating that there could be ample space to account for the lone pair of electrons in the active site for both of these positions. As shown in Figure 2, a phenyl group located within the "aromatic nest" of CYP2A6 indicates the availability of space in this region of the active site to accommodate the lone pair of electrons from the furan rings.

An additional set of agents was synthesized where the pyridine ring was substituted at the 4-position with pyridin-4-yl (compound **4o**), pyridin-3-yl (compound **4p**) and pyrimid-5-yl (**4q**) groups, which afforded IC₅₀ values of 3.6 μ M, 0.59 μ M and 2.7 μ M, respectively. The high variability in inhibition potency, with small changes in the steric and electronic properties of the moieties extending from the pyridine 4-position, makes prediction of potency with change in moiety at this position difficult. Even with the use of a molecular model, it is unlikely that these compounds would have been excluded from the synthesis and testing portion of the project; they were, therefore, synthesized and tested. When the phenyl group (**4l**, IC₅₀ = 0.13 μ M) was replaced by a ring of approximately the same size (pyridine), where the electronegative and hydrogen bond-accepting nitrogen atom was in the 4-position, the inhibition potency dropped approximately 28-fold (compound **4o**, IC₅₀ = 3.6 μ M). Placement of the nitrogen atom at the 3-position, which more closely resembles the placement of the oxygen atom of compounds **4m** and **4n** (IC₅₀ = 0.56 and 0.67 μ M, respectively) afforded an inhibitor with nearly the same inhibition potency (compound **4p**, IC₅₀ = 0.59 μ M). However, when the pyrimid-5-yl moiety was added (compound **4q**), which, again, retains the same size as the phenyl group, a nearly 21-fold decrease in inhibitor potency was observed (compound **4q**, IC₅₀ = 2.7 μ M). This may be due to the change in electronegativity versus the phenyl moiety, but when comparing this compound to compound **4p** (IC₅₀ = 0.59 μ M) which, within the pyridin-3-yl substituent, incorporates a nitrogen atom in the same region of space, the inhibition potencies are dramatically different. Although the nitrogen atom of the pyrimidin-5-yl group is in the same location of the 6-membered ring as the nitrogen atom of the pyridin-3-yl group and close to that of the 2- (compound **4m**) and 3-furanyl (compound **4n**) analogues, the electronic attributes of the pyrimidyl group are much different than a pyridine (as discussed above), which may account for the variability in inhibitor potency. In future rounds of synthesis and testing, these attributes will be more fully addressed. The development of a new computational docking model, based on the crystal structures obtained previously, will also be employed.

Based on the SAR data obtained with the above series of alkyne linker-containing compounds, where substituents at the 4-position (compounds **4g**, **4i-4q**) exhibited relatively good inhibition activity against CYP2A6, we further explored the SAR by replacing the alkyne linking group with either a furan (compounds **5g**, **5i-5q**) or thiophene (compounds **6g**, **6i-6q**) linker group. Other than compounds **5j**, **5q** and **6q**, whose IC₅₀ values were determined to be 1.0 μ M, 1.3 μ M and 1.2 μ M, respectively, all of the thiophene and furan derivatives tested were effective inhibitors of CYP2A6 with IC₅₀ values below 1 μ M (Table 1). Compounds **6i**, **6k**, **5i**, and **6n** displayed the most potent IC₅₀ values of 0.017 μ M, 0.042 μ M, 0.051 μ M and 0.076 μ M, respectively. These data, as well as the molecule model constructed (see Figure 2), suggest that there is ample space above the pyridine ring for secondary lipophilic and/or aromatic interactions between the ligand and the active site of the enzyme. To reaffirm that the compounds are interacting with the heme iron atom utilizing lone pair electrons from a nitrogen atom, compounds **4e**, **4f**, **4g** and **4h**, each containing a methyl group at available carbons at positions 2, 4, 5 and 6 within the pyridine ring and exhibiting highly variable IC₅₀ values against CYP2A6, were tested to determine spectral binding characteristics.²² All compounds were determined to have a Type II binding mode to the truncated version of CYP2A6 (termed 'CYP2A6dh'), indicating the same

binding modality as all of the compounds previously tested in this structural series (Supporting Information, Figure 1). In addition, these data suggest that differences in heme coordination do not account for the observed differences in CYP2A6 inhibitory activities observed with these different compounds. However, besides identification of the pyridine 4-position as a site for succinct SAR development, at this time it is difficult to make a linear correlation between pyridine 4-position functional group and inhibition potency. Experiments to further refine this SAR are currently underway.

Selectivity for CYP2A6 versus other drug metabolizing CYPs.

To refine the potential lead candidate pool and further explore the druglikeness of the new compounds, the pre-clinical parameter of specificity of compounds with substitutions at the pyridine 4-position for CYP2A6 was explored. For these studies, the inhibitory activity of the compounds against eight major human xenobiotic metabolizing CYPs was determined (CYPs 3A4, 2D6, 2C9, 2C19, 1A2, 2B6, 2C8 and 2E1). The IC₅₀ value of each compound was determined for each CYP and compared to the IC₅₀ obtained for that compound against CYP2A6. Using the values obtained, the selectivity ratio was calculated (IC₅₀ for any given CYP_{hepatic}/IC₅₀ for CYP2A6). As shown in Table 2, the selectivity ratio was greater than 5.0 against all enzymes tested for several alkyne-linked compounds (**4g**, **4i**, **4k**, **4l**, **4p** and **4q**). Compound **4o** demonstrated a selectivity ratio of <5.0 against one enzyme (CYP2D6) while **4m**, **4n** and **4j** demonstrated a selectivity ratio of <5.0 for at least two CYPs (CYPs 3A4, 1A2 and 2E1; 2C19 and 1A2 and 2C9 and 2B6, respectively). Compounds **4g**, **4l**, **4p** and **4q** exhibited very high selectivity for CYP2A6, with IC₅₀ ratios ranging from >650 for CYP2C9 to >44 for CYP2D4 for **4g**, >300 for CYP3A4 to >22 for 2C9 for **4l**, from >616 for CYP2B6 to >23 for CYP2D6 for **4p** and >19 for CYP2C8 to >9 for CYP3A4 for **4q**. Also analyzed was the pyridine-6-methyl-containing compound **4e**, which exhibited a selectivity ratio of <5.0 for CYPs 2C19, 1A2 and 2B6.

In addition to the alkyne-linked compounds, thiophene- and furan-linked analogues were also very selective. Compounds **5g**, **6g**, **5i**, **6i**, **5n**, **5o**, **6o**, **5p**, **5q** and **6q** exhibited CYP_{hepatic}/CYP2A6 IC₅₀ ratios of <5.0 for no more than one tested hepatic CYP (Table 2). Compounds **5p**, **5o** and **6o** exhibited very high selectivity for CYP2A6, with IC₅₀ ratios of >236 for CYP3A4 to >12 for CYP1A2 for **5p**, >167 for CYP2E1 to >22 for CYP2D6 for compound **5o** and >647 for CYP3A4 to >10 for CYP2B6 for compound **6o**. Together, these data suggest that several of these agents are highly inhibitory and selective for CYP2A6. Of the 37 novel compounds tested in this study, 18 of them exhibit highly potent inhibition of CYP2A6 and selectivity over at least 7 of 8 major drug metabolizing enzymes, functional attributes that propel them into the lead candidate pool.

Stability of compounds in human liver microsomes.

The most potent and selective nicotine analogues were defined as those agents that exhibited an IC₅₀ <5.0 μ M against CYP2A6 while exhibiting a CYP_{hepatic}/CYP2A6 selectivity ratio of <5.0 for no more than one other major hepatic CYP enzyme. Although a compound with an IC₅₀ value of 1 μ M will not likely be developed as a drug, the relatively high (5 μ M) IC₅₀ value was chosen as to give a wider breadth of compounds to determine these initial metabolism parameters. To examine the stability of these 18 nicotine analogues, compounds

(10 μ M) were incubated with human liver microsomes for up to 2 h. Of the analogues tested, only compounds **4g**, **4i**, **5g** and **6g**, exhibited half-lives ($T_{1/2}$) of less than 25 min and were removed from further testing and development (Table 3). Agents **4q**, **5i**, **5p**, **5o**, **5q** and **6q** were the most stable, all exhibiting a $T_{1/2}$ of >120 min. It is important to note the similarity of the pyridine 4-position functional groups on the most metabolically unstable compounds. Compounds **4g**, **5g** and **6g**, all of which possess a methyl group at the 4-position of the pyridine ring, exhibited $T_{1/2}$ s of 8.4, 21 and 11 min, respectively. It is likely that the methyl group at the 4-position reduces metabolic half-life of the compounds due to ease of hydroxylation by one or more CYPs present in the human liver microsomes. In addition, while compound **4i**, which possesses an ethyl group at the 4-position, exhibits a low $T_{1/2}$ (9.8 min), its isosteric counterparts (compounds **5i** and **6i**) exhibit much longer half-lives of >120 min and 40 min, respectively. Due to the rigidity of the linker portion of the compounds, the smaller the functional group at the 4-position (methyl versus ethyl) may allow for more movement within the active site, allowing for oxidation at alternative sites of the ethyl versus methyl analogues. Although these compounds were removed from the lead candidate pool, they will serve as good tools for the future delineation of the metabolic fate of these compounds upon treatment with human liver microsomes [i.e., NMR analysis can be used to determine specific sites of oxidation of the inhibitors (substrates, in this case)] leading to future rounds of SAR development. Additional evidence for this potential metabolic route is provided by compounds containing a furan moiety at the 4-position of the pyridine ring. Furan rings have high potential to be substrates of a number of hepatic cytochrome P-450s, yet, all of the pyridine-4-furan-3-yl-containing compounds examined in the present study exhibited a high metabolic half-life, indicating these as lead compounds for future development. These findings, collectively, will serve as the basis for subsequent rounds of SAR development.

Assessment of Druglikeness.

The 18 most potent and selective agents were also characterized for their druglikeness (Table 3). In terms of Lipinski's rule of five, each of the 18 compounds fall within the acceptable parameters of having a molecular weight less than 500, a LogP value <5, a total polar surface area of less than 140 Å, less than 5 hydrogen bond donors and less than 10 hydrogen bond acceptors, as well as the more recent parameters of fewer than 10 rotatable bonds. In fact, the compounds have room for further development. The molecular weights are low enough that more fragments can be added to the lead compounds in an effort to optimize the biological and physiochemical properties of the lead compounds. In terms of druglikeness, all of the new compounds have the potential to be orally bioavailable drug candidates. Although some of the lead-compounds do not follow the more stringent "lead-like" parameters being used in the field of fragment-based screening, (i.e., no more than three hydrogen bond donors and no more than three hydrogen bond acceptors), these compounds will still remain in the lead pool as they will be final compounds, not fragments.

Conclusion:

The results obtained in this study suggest that 13 new analogues of nicotine (compounds **4k**, **4l**, **4o**, **4p**, **4q**, **5i**, **5n**, **5o**, **5p**, **5q**, **6i**, **6o**, and **6q**; see Table 4), developed as selective CYP2A6 inhibitors for the modulation of nicotine metabolism, exhibit all of the necessary

attributes of lead drug candidates. The compounds are potent and selective inhibitors of CYP2A6, exhibit metabolic stability, and contain all of the physiochemical parameters necessary to propel them into the next stage of drug development. It is predicted that, when a candidate drug is administered in cigarette smokers, for example, this agent will inhibit CYP2A6, resulting in a longer *in vivo* half-life for nicotine as compared to smokers with unaltered nicotine metabolism. This increase in nicotine plasma half-life should delay the onset of withdrawal symptoms typically felt by a smoker, affording this individual the opportunity to delay their subsequent tobacco use, potentially resulting in a corresponding decrease in the behavioral addiction component - the act of smoking itself. Therefore, the smoker may have a larger chance for success in quitting altogether. An added benefit of this course of treatment is that, since a smoker will potentially reduce the number of cigarettes smoked, their exposure to the disease-causing and carcinogenic effects of tobacco and cigarette smoke will also be decreased. The lead compounds described in this study will be further tested in pre-clinical drug development models and serve as lead structures for further refinement of future smoking cessation agents.

Experimental Section:

General Procedures:

Chemistry—Unless otherwise noted, all materials for the synthetic chemistry portion were obtained from commercial suppliers (Combi-Blocks, CombiPhos, Fisher Scientific, Sigma-Aldrich or VWR) and used without further purification. The ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE 300 or 500 MHz spectrometer using CDCl_3 or D_2O as the solvent. Chemical shifts (δ) for ^1H NMR are reported in ppm relative to the residual solvent signal (7.26 ppm, CDCl_3 , 4.63 ppm, D_2O), coupling constants (J) are reported in hertz and peak multiplicities are reported as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br s (broad singlet). For ^{13}C NMR of the final products, compound **4g** was used to assign the chemical shift to be used, subsequently, as the internal standard, as follows: to a solution of compound **4g** in D_2O was added *t*-butanol to a final concentration of ca. 0.5% and the spectrum was recorded. The peak corresponding to the methyl singlet of *t*-butanol was set to 30.695 ppm and the methylene proton of the compound was determined to be 30.76 ppm. This chemical shift was assigned to the methylene peak of each final product as the reference point. Thin layer chromatography (TLC) was performed on glass backed, 0.20 mm silica gel MF254 plates (Agela Technologies, USA) and column chromatography was performed using KP-SIL silica gel (Biotage, USA) and flash column chromatography was performed on Biotage prepacked columns or Yamazen Hi-Flash columns using either the automated flash chromatography system Biotage Isolera One (Biotage AB, Uppsala, Sweden) or Yamazen Smart Flash EPCLC AI-580S (Yamazen Corporation, Osaka, Japan). The purities of all the final compounds were of >95% as determined by UPLC analysis unless otherwise indicated. Analytical UPLC and HRMS measurements were performed on a Waters Acquity UPLC coupled to a Xevo G2-S QToF mass spectrometer. Separations were achieved with an ACQUITY UPLC_{MS} HSS T3 column (2.1 mm $\times$ 100 mm, 1.8 μm). Standard conditions utilized a binary solvent system consisting of solvents A (5 mM ammonium formate and 2 mM formic acid) and B (methanol) set at a flow rate of 0.4 mL/min for a total run of 9 min. The gradient elution was as follows: Initial:

95.0:5.0 (A:B, v/v), 0.5 min; 95.0:5.0 (A:B, v/v), 5.0 min; 10.0:90.0 (A:B, v/v), 7.00 min; 95.0:5.0 (A:B, v/v), 9.00 min; 95.0:5.0 (A:B, v/v). All tested compounds had purities of >95% as judged by the UPLC analysis described above (UV detection at 254 nm) except as noted. For HRMS, the mass spectrometer was employed. ¹H NMR, ¹³C NMR and mass spectra were consistent with the assigned structures. To confirm the number of hydrochlorides associated with the compounds, chlorine analysis was performed by Midwest Microlab (Indianapolis, IN).

The synthesis of each agent is described below.

tert-Butyl (3-(isoquinolin-4-yl)prop-2-yn-1-yl)carbamate (4b-Boc).—To a 100 mL round bottom flask containing N-Boc-propargylamine (902. mg, 5.80 mmol) under a blanket of argon(g) was added 40 mL of degassed 1-propanol followed by tetrakis(triphenylphosphine)palladium(0) (268. mg, 0.230 mmol) and cuprous iodide (110. mg, 0.580 mmol). To the vigorously stirred suspension was added sodium carbonate (801. mg, 7.55 mmol) dissolved in degassed water (ca. 2.0 mL). The flask was purged with argon(g), stirred at ambient temperature for 10 min followed by the addition of a solution of 3-bromo-isoquinoline (1.13 g, 5.40 mmol) in 4 mL of degassed 1-propanol. The mixture was refluxed under argon(g) for 24 h. The contents of the flask were transferred to a sintered glass funnel containing anhydrous Na₂SO₄. The crude material was eluted with methanol. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v to EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 50:50, R_f = 0.44) to afford **4b-Boc** (936. mg, 71.1% yield) as a red oil: ¹H NMR (CDCl₃) δ 9.19 (s, 1 H), 8.66 (s, 1 H), 8.26–8.19 (m, 2 H), 8.02–7.96 (m, 1 H), 7.82–7.74 (m, 1 H), 7.70–7.61 (m, 1 H), 4.32 (s, 2 H), 1.50 (s, 9 H).

3-(Isoquinolin-4-yl)prop-2-yn-1-amine dihydrochloride (4b).—To a solution of **4b-Boc** (56.1 mg, 0.200 mmol) in anhydrous dichloromethane (ca. 1.5 mL) was added trifluoroacetic acid (4 mL) and the resultant solution was stirred at ambient temperature for 1.5 h. The mixture was diluted with 20 mL of water, transferred to a separatory funnel and the organics were discarded. The aqueous portion was washed with dichloromethane (2 X 40 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (2 X 50 mL). The combined organics were dried over anhydrous MgSO₄, filtered and the solvent was removed *in vacuo*. This material was dissolved in 40 mL of diethyl ether and treated with 5 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford **4b** (58.6 mg, 68.1% yield) as a white solid: ¹H NMR (300 MHz, D₂O) δ 9.32 (s, 1 H), 8.45 (s, 1 H), 8.15 – 8.22 (m, 2 H), 7.99 – 8.06 (m, 1 H), 7.77 – 7.84 (m, 1 H), 4.13 (s, 2 H); ¹³C NMR (75 MHz, D₂O) δ 147.4, 137.6, 137.2, 135.6, 131.1, 130.6, 126.6, 125.0, 118.2, 90.7, 78.7, 29.6; HRMS (ESI) *m/z* calcd for C₁₂H₁₁N₂ [M + H]⁺ 183.0922, found 183.0952.

tert-Butyl (3-(quinolin-3-yl)prop-2-yn-1-yl)carbamate (4c-Boc).—To a Biotage 2.0–5.0 mL microwave tube containing N-Boc-propargylamine (845. mg, 5.40 mmol) under a blanket of argon(g) was added cuprous iodide (103. mg, 0.540 mmol) followed by tetrakis(triphenylphosphine)palladium(0) (320. mg, 0.277 mmol) followed by degassed 1-propanol (2.0 mL). To the vigorously stirred suspension was added sodium carbonate (750.

mg, 7.08 mmol) dissolved in degassed water (ca. 2.0 mL). The tube was purged with argon_(g), stirred at ambient temperature for 10 min followed by the addition of a solution of 3-bromo-quinoline (1.13 g, 5.43 mmol). The tube was purged with argon_(g), capped, placed in a Biotage Initiator⁺ microwave and heated to 100 °C for 15 min on normal absorption level. The contents of the flask were transferred to a sintered glass funnel containing anhydrous Na₂SO₄. The crude material was eluted with dichloromethane followed by ethyl acetate. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v to EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 25:75, R_f = 0.34) to afford **4c-Boc** (325. mg, 21.0% yield) as a red oil: ¹H NMR (CDCl₃) δ 8.90–8.87 (m, 1 H), 8.20–8.17 (m, 1 H), 8.11–8.05 (m, 1 H), 7.78–7.67 (m, 2 H), 7.58–7.51 (m, 1 H), 4.22 (br s, 2 H), 1.47 (s, 9 H).

3-(Quinolin-3-yl)prop-2-yn-1-amine dihydrochloride (4c).—To a solution of **4c-Boc** (112. mg, 0.390 mmol) in anhydrous dichloromethane (ca. 1.5 mL) was added trifluoroacetic acid (4 mL) and the resultant solution was stirred at ambient temperature for 1.5 h. The mixture was diluted with 20 mL of water, transferred to a separatory funnel and the organics were discarded. The aqueous portion was washed with dichloromethane (2 X 40 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (3 X 50 mL). The combined organics were dried over anhydrous MgSO₄, filtered and the solvent was removed *in vacuo*. This material was dissolved in 40 mL of ethyl ether and treated with 5 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford **4c** (58.6 mg, 68.1% yield) as a white solid: ¹H NMR (D₂O) δ 8.77–8.68 (m, 1 H), 7.63 (m, 1 H), 8.60–8.47 (m, 1 H), 7.80–7.50 (m, 4 H), 4.07 (s, 2 H); ¹³C NMR (75 MHz, D₂O) δ 148.4, 147.9, 138.6, 136.3, 131.3, 129.8, 128.4, 116.8, 87.1, 81.6, 30.8; HRMS (ESI) *m/z* calcd for C₁₂H₁₁N₂ [M + H]⁺ 183.0922, found 183.0921.

tert-Butyl (3-(pyrimidin-5-yl)prop-2-yn-1-yl)carbamate (4d-Boc).—To a Biotage 2.0–5.0 mL microwave tube containing N-Boc-propargylamine (633. mg, 4.08 mmol) under a blanket of argon_(g) was added cuprous iodide (77.0 mg, 0.408 mmol) followed by tetrakis(triphenylphosphine)palladium(0) (260. mg, 0.225 mmol) followed by degassed 1-propanol (1.5 mL). To the vigorously stirred suspension was added sodium carbonate (562. mg, 5.3 mmol) dissolved in degassed water (ca. 2.0 mL). The tube was purged with argon_(g), stirred at ambient temperature for 10 min followed by the addition of a solution of 5-bromopyrimidine (645. mg, 4.08 mmol) in hot degassed 1-propanol (1.5 mL). The tube was purged with argon_(g), capped, placed in a Biotage Initiator⁺ microwave and heated to 100 °C for 15 min on normal absorption level. The contents of the flask were transferred to a sintered glass funnel containing anhydrous Na₂SO₄. The crude material was eluted with dichloromethane followed by methanol. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v R_f = 0.33) to afford **4d-Boc** (560. mg, 59.0% yield) as a red oil: ¹H NMR (500 MHz, CDCl₃) δ 9.06 (s, 1 H), 8.69 (s, 2 H), 7.21 (m, 2 H), 5.29 (br s, 1 H), 4.13 (s, 2 H), 1.40 (s, 9 H).

3-(Pyrimidin-5-yl)prop-2-yn-1-amine di/trihydrochloride (4d).—To a solution of **4d-Boc** (118. mg, 0.513 mmol) in anhydrous dichloromethane (ca. 1.5 mL) was added trifluoroacetic acid (3 mL) and the resultant solution was stirred at ambient temperature for

2 h. To the mixture was added 3 mL of 1 N HCl and the mixture was vigorously stirred for 10 min. The mixture was diluted with 20 mL of water, transferred to a separatory funnel and the organics were discarded. The aqueous portion was washed with dichloromethane (2 X 30 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (3 X 35 mL). The combined organics were dried over anhydrous MgSO₄, filtered and the solvent was removed *in vacuo*. The residue was passed through a short pad of silica gel and this material was dissolved in 40 mL of ethyl ether and treated with 5 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford **4d** (109. mg, 77% yield) as a white solid: as a 2:1 mixture of the monohydrochloride to dihydrochloride: ¹H NMR (300 MHz, D₂O) δ 8.94 (mono-HCl, s, 1 H), 8.75 (mono-HCl, s, 2 H), 8.15 (di-HCl, s, 1 H), 6.87 (di-HCl, s, 1 H), 7.21 (m, 2 H), 5.59 (di-HCl, s, 1 H), 3.94 (mono-HCl, s, 2 H), 3.82 (di-HCl, s, 2 H). Free Base NMR: ¹H NMR (300 MHz, CDCl₃) δ 8.99 (s, 1 H), 8.62 (s, 2 H), 1.41 (br s, 2 H); **Note:** We will not be characterizing this by ¹³C NMR or UPLC for percent purity due to lack of activity and mixture of salt forms; HRMS (ESI) *m/z* calcd for C₇H₈N₃ [M + H]⁺ 134.0718, found 134.0717.

tert-Butyl (3-(6-methylpyridin-3-yl)prop-2-yn-1-yl)carbamate (4e-Boc).—To a Biotage 2.0–5.0 mL microwave tube containing N-Boc-propargylamine (474. mg, 2.98 mmol) under a blanket of argon(g) was added cuprous iodide (56.6 mg, 0.297 mmol) followed by tetrakis(triphenylphosphine)palladium(0) (172. mg, 0.149 mmol) followed by degassed 1-propanol (2.0 mL). To the vigorously stirred suspension was added sodium carbonate (410. mg, 3.87 mmol) dissolved in a minimum amount of water (ca. 1.3 mL). The tube was purged with argon(g), stirred at ambient temperature for 10 min followed by the addition of a solution of 3-bromo-6-methylpyridine (512. mg, 2.97 mmol) in degassed 1-propanol. The tube was purged with argon(g), capped, placed in a Biotage Initiator⁺ microwave and heated to 100 °C for 15 min on normal absorption level. The contents of the flask were transferred to a sintered glass funnel containing anhydrous Na₂SO₄. The crude material was eluted with dichloromethane followed by methanol. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v to EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 50:50, R_f = 0.48) to afford **4e-Boc** (349. mg, 47.6% yield) as a dark red oil: ¹H NMR (500 MHz, CDCl₃) δ 8.52 (d, *J* = 1.6 Hz, 1 H), 7.55 (dd, *J* = 2.2, 7.88 Hz, 1 H), 7.07 (d, *J* = 8.2 Hz, 1 H), 2.52 (s, 2 H), 1.50 (s, 9 H).

3-(6-Methylpyridin-3-yl)prop-2-yn-1-amine dihydrochloride (4e).—To a solution of **4e-Boc** (282. mg, 1.14 mmol) in anhydrous dichloromethane (ca. 10 mL) was added trifluoroacetic acid (1 mL) and the resultant solution was stirred at ambient temperature for 15 min. To the mixture was added 50 mL of 1 N HCl and the mixture was vigorously stirred for 10 min. The mixture was transferred to a separatory funnel, the organics were discarded and the aqueous phase was washed with dichloromethane (2 X 25 mL). The aqueous portion was transferred to an Erlenmeyer flask, the pH was adjusted to ca. 10 using 10 N NaOH, ca. 50 mL of brine was added followed by ca. 75 mL of EtOAc and the mixture was vigorously stirred for 5–10 min. The organics were collected and the aqueous portion was extracted with EtOAc (100 mL). The combined organics were dried over anhydrous MgSO₄, filtered and the solvent was removed *in vacuo*. The residue was chromatographed on silica gel (MeOH/CH₂Cl₂, 10/90, v/v; TLC: MeOH/CH₂Cl₂, 10/90, R_f = 0.23) to afford **4e** (107. mg,

64.1% yield) as a red oil: ^1H NMR (300 MHz, D_2O) δ 8.59 (d, J = 1.9 Hz, 1 H), 8.29 (dd, J = 2.0, 8.4 Hz, 1 H), 7.69 (dd, J = 0.6, 8.5 Hz, 1 H), 3.96 (s, 2 H), 2.60 (s, 3 H); ^{13}C NMR (D_2O) δ 156.7, 150.8, 145.6, 130.4, 121.7, 89.1, 82.0, 32.0, 21.7; HRMS (ESI) m/z calcd for $\text{C}_9\text{H}_{11}\text{N}_2$ $[\text{M} + \text{H}]^+$ 147.0922, found 147.0927.

***tert*-Butyl (3-(5-methylpyridin-3-yl)prop-2-yn-1-yl)carbamate (4f-Boc).**—To a Biotage 2.0–5.0 mL microwave tube containing N-Boc-propargylamine (372. mg, 2.34 mmol) under a blanket of argon(g) was added cuprous iodide (44.6 mg, 0.234 mmol) followed by tetrakis(triphenylphosphine)palladium(0) (135. mg, 0.117 mmol) followed by degassed 1-propanol (2.0 mL). To the vigorously stirred suspension was added sodium carbonate (410. mg, 3.87 mmol) dissolved in a minimum amount of water (ca. 1.3 mL). The tube was purged with argon(g), stirred at ambient temperature for 10 min followed by the addition of a solution of 3-bromo-5-methylpyridine (402. mg, 2.34 mmol) in degassed 1-propanol. The tube was purged with argon(g), capped, placed in a Biotage Initiator⁺ microwave and heated to 100 °C for 15 min on normal absorption level. The contents of the flask were transferred to a sintered glass funnel containing anhydrous Na_2SO_4 . The crude material was eluted with dichloromethane followed by methanol. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v; TLC: EtOAc/Hex, 25:75, R_f = 0.12) to afford **4f-Boc** (342. mg, 59.0% yield) as a yellow solid: ^1H NMR (500 MHz, CDCl_3) δ 8.45 (s, 1 H), 8.35 (s, 1 H), 7.49 (s, 1 H), 5.05 (br s, 1 H), 4.14 (br s, 2 H), 2.28 (s, 3 H), 1.45 (s, 9 H).

3-(5-Methylpyridin-3-yl)prop-2-yn-1-amine dihydrochloride (4f).—To a solution of **4f-Boc** (76.4 mg, 0.310 mmol) in anhydrous dichloromethane (ca. 1.5 mL) was added trifluoroacetic acid (3 mL) and the resultant solution was stirred at ambient temperature for 3 h. To the mixture was added 2 mL of 1 N HCl and the mixture was vigorously stirred for 10 min. The mixture was diluted with 20 mL of water, transferred to a separatory funnel and the organics were discarded. The aqueous portion was washed with dichloromethane (2 X 30 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (3 X 35 mL). The combined organics were dried over anhydrous MgSO_4 , filtered and the solvent was removed *in vacuo*. The residue was chromatographed on silica gel (MeOH/ CH_2Cl_2 , 10/90, v/v; TLC: MeOH/ CH_2Cl_2 , 10/90, v/v, streak, R_f = 0.114 – 0.409) to afford the compound as the free base. This material was dissolved in 40 mL of ethyl ether and treated with 5 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford **4f** (41.6 mg, 73.4% yield) as a white solid: ^1H NMR (300 MHz, D_2O) δ 8.57 (s, 1 H), 8.45 (s, 1 H), 8.31 (s, 1 H), 3.97 (s, 2 H), 2.35 (s, 3 H); ^{13}C NMR (75 MHz, D_2O) δ 150.6, 142.3, 142.2, 140.3, 122.8, 86.3, 80.9, 30.8, 19.8; HRMS (ESI) m/z calcd for $\text{C}_9\text{H}_{11}\text{N}_2$ $[\text{M} + \text{H}]^+$ 147.0922, found 147.0912.

***tert*-Butyl (3-(4-methylpyridin-3-yl)prop-2-yn-1-yl)carbamate (4g-Boc).**—To a Biotage 2.0–5.0 mL microwave tube containing N-Boc-propargylamine (541. mg, 3.49 mmol) under a blanket of argon(g) was added cuprous iodide (66.0 mg, 0.353 mmol) followed by tetrakis(triphenylphosphine)palladium(0) (200. mg, 0.174 mmol) followed by degassed 1-propanol (2.0 mL). To the vigorously stirred suspension was added sodium carbonate (481. mg, 4.53 mmol) dissolved in a minimum amount of water (ca. 1.5 mL). The

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tube was purged with argon(g), stirred at ambient temperature for 10 min followed by the addition of a solution of 3-bromo-4-methylpyridine (600. mg, 3.49 mmol) in degassed 1-propanol. The tube was purged with argon(g), capped, placed in a Biotage Initiator⁺ microwave and heated to 100 °C for 15 min on normal absorption level. The contents of the flask were transferred to a sintered glass funnel containing anhydrous Na₂SO₄. The crude material was eluted with dichloromethane followed by methanol. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v to EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 50:50, R_f = 0.42) to afford **4g-Boc** (455. mg, 53.0% yield) as a red oil: ¹H NMR (500 MHz, CDCl₃) δ 8.55 (s, 1 H), 8.36 (s, 1 H), 7.11 (s, 1 H), 4.97 (br s, 1 H), 4.18 (br s, 2 H), 2.39 (s, 3 H), 1.46 (s, 9 H).

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3-(4-Methylpyridin-3-yl)prop-2-yn-1-amine dihydrochloride (4g).—To a solution **4g-Boc** (94. mg, 0.38 mmol) in anhydrous dichloromethane (ca. 1.5 mL) was added trifluoroacetic acid (3 mL) and the resultant solution was stirred at ambient temperature for 3 h. To the mixture was added 3 mL of 1 N HCl and the mixture was vigorously stirred for 10 min. The mixture was diluted with 20 mL of water, transferred to a separatory funnel and the organics were discarded. The aqueous portion was washed with dichloromethane (2 X 30 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (3 X 35 mL). The combined organics were dried over anhydrous MgSO₄, filtered and the solvent was removed *in vacuo*. This material was dissolved in 40 mL of ethyl ether and treated with 5 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford **4g** (51. mg, 74% yield) as a white solid: ¹H NMR (300 MHz, D₂O) δ 8.66 (s, 1 H), 8.45 (s, 1 H), 7.77 (s, 1 H), 4.07 (s, 2 H), 2.56 (s, 3 H); ¹³C NMR (75 MHz, D₂O) δ 163.1, 144.8, 141.4, 128.8, 123.1, 91.8, 79.9, 30.8, 22.1; HRMS (ESI) *m/z* calcd for C₉H₁₁N₂ [M + H]⁺ 147.0922, found 147.0926.

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tert-Butyl (3-(2-methylpyridin-3-yl)prop-2-yn-1-yl)carbamate (4h-Boc).—To a Biotage 2.0–5.0 mL microwave tube containing N-Boc-propargylamine (413. mg, 2.66 mmol) under a blanket of argon(g) was added degassed DME/EtOH 50:50 (2.5 mL) followed by cuprous iodide (46.0 mg, 0.242 mmol) followed by tetrakis(triphenylphosphine)palladium(0) (290. mg, 0.252 mmol). To the vigorously stirred suspension was added triethylamine (734. mg, 7.26 mmol). The tube was purged with argon(g), stirred at ambient temperature for 10 min followed by the addition of a solution of 3-bromo-2-methylpyridine (416. mg, 2.42 mmol). The tube was purged with argon(g), capped, placed in a Biotage Initiator⁺ microwave and heated to 150 °C for 15 min on normal absorption level. The contents of the flask were filtered through a compressed bed of celite and the crude material was eluted with dichloromethane. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v; TLC: EtOAc/Hex, 25:75, v/v R_f = 0.17) to afford **4h-Boc** (192. mg, 32.0% yield) as a red oil: ¹H NMR (500 MHz, CDCl₃) δ 8.34 (d, *J* = 3.6 Hz, 1 H), 7.55 (dd, *J* = 1.5, 7.7 Hz, 1 H), 6.98 (dd, *J* = 4.9, 7.5 Hz, 1 H), 5.31 (br s, 1 H), 4.13 (d, *J* = 4.9 Hz, 2 H), 2.56 (s, 3 H), 1.40 (s, 9 H).

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3-(2-Methylpyridin-3-yl)prop-2-yn-1-amine dihydrochloride (4h).—To a solution of **4h-Boc** (192. mg, 0.780 mmol) in anhydrous dichloromethane (ca. 1.5 mL) was added trifluoroacetic acid (3 mL) and the resultant solution was stirred at ambient temperature for

2 h. To the mixture was added 4 mL of 1 N HCl and the mixture was vigorously stirred for 10 min. The mixture was diluted with 20 mL of water, transferred to a separatory funnel and the organics were discarded. The aqueous portion was washed with dichloromethane (2 X 30 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (3 X 35 mL). The combined organics were dried over anhydrous MgSO₄, filtered and the solvent was removed *in vacuo*. The residue was chromatographed on silica gel (MeOH/CH₂Cl₂, 10/90, v/v; TLC: MeOH/CH₂Cl₂, 10/90, v/v, *R_f* = 0.23) This material was dissolved in 40 mL of ethyl ether and treated with 5 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford **4h** (109. mg, 77.0% yield) as a white solid: ¹H NMR (300 MHz, D₂O) δ 8.43–8.35 (m, 2 H), 7.71–7.66 (m, 1 H), 4.02 (s, 2 H), 2.68 (s, 3 H); ¹³C NMR (75 MHz, D₂O) δ 157.5, 149.8, 141.2, 125.5, 123.4, 91.6, 80.4, 30.8, 19.8; HRMS (ESI) *m/z* calcd for C₉H₁₁N₂ [M + H]⁺ 147.0922, found 147.0873.

tert-Butyl (3-(4-ethylpyridin-3-yl)prop-2-yn-1-yl)carbamate (4i-Boc).—To a Biotage 2.0–5.0 mL microwave tube containing N-Boc-propargylamine (224. mg, 1.44 mmol) under a blanket of argon(g) was added degassed DME/EtOH 50:50 (0.5 mL) followed by a slurry of tetrakis(triphenylphosphine)palladium(0) (280. mg, 0.242 mmol) in degassed DME/EtOH 50:50 (1.5 mL), followed by cuprous iodide (27.0 mg, 0.144 mmol). To the vigorously stirred suspension was added sodium carbonate (300. mg, 1.57 mmol) dissolved in a minimum amount of degassed water (ca. 1.5 mL), the tube was purged with argon(g), stirred at ambient temperature for 10 min, followed by the addition of a solution of 3-bromo-4-ethylpyridine (269. mg, 1.44 mmol). The tube was purged with argon(g), capped, placed in a Biotage Initiator⁺ microwave and heated to 150 °C for 15 min on normal absorption level. The contents of the flask were transferred to a sintered glass funnel containing anhydrous Na₂SO₄ and the crude material was eluted with dichloromethane. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v; TLC: EtOAc/Hex, 25:75, v/v, *R_f* = 0.20) to afford **4i-Boc** (141. mg, 50.6% yield) as a yellow oil: ¹H NMR (500 MHz, CDCl₃) δ 8.54 (s, 1 H), 8.39 (d, *J* = 5.4 Hz, 1 H), 7.10 (d, *J* = 5.0 Hz, 1 H), 5.10 (br s, 1 H), 4.17 (s, 2 H), 2.74 (q, *J* = 7.6 Hz, 2 H), 1.45 (s, 9 H), 1.21 (t, *J* = 7.6 Hz, 3 H).

3-(4-Ethylpyridin-3-yl)prop-2-yn-1-amine dihydrochloride (4i).—To solution **4i-Boc** (69.5 mg, 0.260 mmol) in anhydrous dichloromethane (ca. 3.0 mL) was added trifluoroacetic acid (3 mL) and the resultant solution was stirred at ambient temperature for 2 h. To the mixture was added 3 mL of 1 N HCl and the mixture was vigorously stirred for 10 min. The mixture was diluted with 20 mL of water and transferred to a separatory funnel, the organics were discarded and the aqueous phase was washed with dichloromethane (2 X 40 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (2 X 40 mL). The combined organics were dried over anhydrous MgSO₄, filtered and the solvent was removed *in vacuo*. This material was dissolved in 40 mL of ethyl ether and treated with 5 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford **4i** (49.0 mg, 93% yield) as an off white solid: ¹H NMR (500 MHz, D₂O) δ 8.69 (s, 1 H), 8.51 (d, *J* = 5.7 Hz, 1 H), 7.84 (d, *J* = 6.0 Hz, 1 H), 4.07 (s, 2 H), 2.93 (q, *J* = 7.6 Hz, 2 H), 1.18 (t, *J* = 7.6 Hz, 3 H); ¹³C NMR (D₂O) δ 168.7,

144.8, 141.4, 127.4, 122.8, 91.8, 79.5, 30.8, 29.0, 13.2; HRMS (ESI) m/z calcd for $C_{10}H_{13}N_2$ $[M + H]^+$ 161.1079, found 161.1082.

tert-Butyl (3-(4-methoxypyridin-3-yl)prop-2-yn-1-yl)carbamate (4j-Boc).—To a Biotage 2.0–5.0 mL microwave tube containing N-Boc-propargylamine (212. mg, 1.37 mmol) under a blanket of argon(g) was added degassed DME/EtOH 50:50 (0.3 mL) followed by a slurry of tetrakis(triphenylphosphine)palladium(0) (109. mg, 0.094 mmol) in degassed DME/EtOH 50:50 (2.0 mL), followed by cuprous iodide (29.0 mg, 0.152 mmol). To the vigorously stirred suspension was added sodium carbonate (290. mg, 2.73 mmol) dissolved in a minimum amount of degassed water (ca. 1.5 mL), the tube was purged with argon(g), stirred at ambient temperature for 10 min, followed by the addition of a solution of 3-bromo-4-methoxypyridine (257. mg, 1.37 mmol). The tube was purged with argon(g), capped, placed in a Biotage Initiator⁺ microwave and heated to 150 °C for 15 min on normal absorption level. The contents of the flask were transferred to a sintered glass funnel containing anhydrous Na_2SO_4 and the crude material was eluted with dichloromethane. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v; TLC: EtOAc/Hex, 25:75, v/v, R_f = 0.23) to afford **4j-Boc** (272. mg, 76.3% yield) as a yellow oil: ^1H NMR (500 MHz, $CDCl_3$) δ 8.48 (s, 1 H), 8.40 (d, J = 5.7 Hz, 1 H), 6.77 (d, J = 6.0 Hz, 1 H), 4.93 (br s, 1 H), 4.20 (br s, 2 H), 3.93 – 3.88 (m, 3 H), 1.46 (s, 9 H).

3-(4-Methoxypyridin-3-yl)prop-2-yn-1-amine dihydrochloride (4j).—To a solution of **4j-Boc** (272. mg, 1.03 mmol) in anhydrous dichloromethane (ca. 3.0 mL) was added trifluoroacetic acid (4 mL) and the resultant solution was stirred at ambient temperature for 3 h. To the mixture was added 3 mL of 1 N HCl and the mixture was vigorously stirred for 10 min. The mixture was diluted with 20 mL of water, transferred to a separatory funnel and the organics were discarded. The aqueous portion was washed with dichloromethane (2 X 30 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (2 X 40 mL). The combined organics were dried over anhydrous $MgSO_4$, filtered and the solvent was removed *in vacuo*. This material was dissolved in 40 mL of ethyl ether and treated with 5 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford the product **4j** (140. mg, 68.3% yield) as a white solid: ^1H NMR (500 MHz, D_2O) δ 8.44 (s, 1 H), 8.35 (d, J = 5.7 Hz, 1 H), 6.74 (d, J = 6.0 Hz, 1 H), 3.87 (s, 3 H), 3.65 (s, 2 H); ^{13}C NMR (D_2O) δ 173.4, 145.6, 144.2, 112.2, 110.8, 91.1, 77.1, 59.4, 30.8; HRMS (ESI) m/z calcd for $C_9H_{10}N_2O$ $[M + H]^+$ 163.0871, found 163.0881.

tert-Butyl (3-(4-propylpyridin-3-yl)prop-2-yn-1-yl)carbamate (4k-Boc).—To a Biotage 2.0–5.0 mL microwave tube containing N-Boc-propargylamine (147. mg, 0.950 mmol) under a blanket of argon(g) was added degassed DME/EtOH 50:50 (3.0 mL) followed by a slurry of tetrakis(triphenylphosphine)palladium(0) (145. mg, 0.125 mmol) in degassed DME/EtOH 50:50 (1.0 mL) followed by cuprous iodide (30.0 mg, 0.157 mmol). To the vigorously stirred suspension was added sodium carbonate (215. mg, 2.02 mmol) dissolved in degassed water (ca. 1.5 mL). The tube was purged with argon(g), stirred at ambient temperature for 10 min followed by the addition of a solution of 3-bromo-4-propylpyridine (190. mg, 0.950 mmol). The tube was purged with argon(g), capped, placed in a Biotage

Initiator⁺ microwave and heated to 150 °C for 15 min on normal absorption level. The contents of the flask were transferred to a sintered glass funnel containing anhydrous Na₂SO₄ and the crude material was eluted with dichloromethane. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v; TLC: EtOAc/Hex, 25:75, v/v, *R_f* = 0.10) to afford **4k-Boc** (69.0 mg, 29.4% yield) as a red oil: ¹H NMR (500 MHz, CDCl₃) δ 8.55 (s, 1 H), 8.38 (d, *J* = 5.0 Hz, 1 H), 7.08 (d, *J* = 4.7 Hz, 1 H), 4.94 (br s, 1 H), 4.27 – 4.07 (m, 2 H), 2.79 – 2.59 (m, 2 H), 1.75 – 1.56 (m, 2 H), 1.52 – 1.39 (m, 9 H), 1.03 – 0.84 (m, 3 H).

3-(4-propylpyridin-3-yl)prop-2-yn-1-amine dihydrochloride (4k).—To a solution of **4k-Boc** (69. mg, 0.25 mmol) in anhydrous dichloromethane (ca. 1.5 mL) was added trifluoroacetic acid (3 mL) and the resultant solution was stirred at ambient temperature for 3 h. To the mixture was added 3 mL of 1 N HCl and the mixture was vigorously stirred for 10 min. The mixture was diluted with 20 mL of water, transferred to a separatory funnel and the organics were discarded. The aqueous portion was washed with dichloromethane (2 X 30 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (2 X 40 mL). The combined organics were dried over anhydrous MgSO₄, filtered and the solvent was removed *in vacuo*. This material was dissolved in 40 mL of ethyl ether and treated with 5 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford **4k** (37. mg, 70% yield) as an off white solid: ¹H NMR (500 MHz, D₂O) δ 8.68 (br s, 1 H), 8.48 (d, *J* = 5.0 Hz, 1 H), 7.81 (d, *J* = 6.0 Hz, 1 H), 4.09 – 4.01 (m, 2 H), 2.89 (t, *J* = 7.6 Hz, 2 H), 1.73 – 1.51 (m, 2 H), 0.92 – 0.70 (m, 3 H); HRMS (ESI) *m/z* calcd for C₁₁H₁₄N₂ [M + H]⁺ 175.1235, found 175.1233.

tert-Butyl (3-(4-phenylpyridin-3-yl)prop-2-yn-1-yl)carbamate (4l-Boc).—To a Biotage 2.0–5.0 mL microwave tube containing N-Boc-propargylamine (167. mg, 1.07 mmol) under a blanket of argon(g) was added degassed DME/EtOH 50:50 (1.0 mL) followed by a slurry of tetrakis(triphenylphosphine)palladium(0) (102. mg, 0.0883 mmol) in degassed DME/EtOH 50:50 (1.0 mL) followed by cuprous iodide (25.0 mg, 0.131 mmol). To the vigorously stirred suspension was added sodium carbonate (253. mg, 2.38 mmol) dissolved in degassed water (ca. 1.5 mL). The tube was purged with argon(g), stirred at ambient temperature for 10 min followed by the addition of a solution of 3-bromo-4-phenylpyridine (252. mg, 1.07 mmol). The tube was purged with argon(g), capped, placed in a Biotage Initiator⁺ microwave and heated to 120 °C for 15 min on normal absorption level. The contents of the flask were transferred to a sintered glass funnel containing anhydrous Na₂SO₄. The crude material was eluted with dichloromethane. The solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v; TLC: EtOAc/Hex, 25:75, v/v, *R_f* = 0.16) to afford **4l-Boc** (220. mg, 66.3% yield) as a red oil: ¹H NMR (500 MHz, CDCl₃) δ 8.73 (br s, 1 H), 8.55 (br s, 1 H), 7.68 – 7.57 (m, 2 H), 7.53 – 7.38 (m, 3 H), 7.36 – 7.27 (m, 1 H), 4.73 (br s, 1 H), 4.11 – 3.99 (m, 2 H), 1.48 – 1.42 (m, 9 H).

3-(4-Phenylpyridin-3-yl)prop-2-yn-1-amine dihydrochloride (4l).—To a solution of **4l-Boc** (168. mg, 0.540 mmol) in anhydrous dichloromethane (ca. 1.5 mL) was added trifluoroacetic acid (3 mL) and the resultant solution was stirred at ambient temperature for

3 h. To the mixture was added 2 mL of 1 N HCl and the mixture was vigorously stirred for 10 min. The mixture was diluted with 20 mL of water, transferred to a separatory funnel and the organics were discarded. The aqueous portion was washed with dichloromethane (2 X 30 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (2 X 35 mL). The combined organics were dried over anhydrous MgSO_4 , filtered and the solvent was removed *in vacuo*. This material was dissolved in 40 mL of ethyl ether and treated with 5 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford **4l** (95.7 mg, 71.1% yield) as a white solid: ^1H NMR (300 MHz, D_2O) δ 8.80 (s, 1 H), 8.66 – 8.45 (m, 1 H), 7.81 (d, J = 6.2 Hz, 1 H), 7.71 – 7.52 (m, 2 H), 7.52 – 7.27 (m, 3 H), 3.87 (s, 2 H); ^{13}C NMR (D_2O) δ 161.3, 146.5, 141.4, 135.8, 132.7, 130.3, 130.2, 128.5, 121.2, 90.7, 80.8, 30.8; HRMS (ESI) m/z calcd for $\text{C}_{14}\text{H}_{12}\text{N}_2$ [$\text{M} + \text{H}$] $^+$ 209.1079, found 209.1081.

tert-Butyl [3-(4-furan-2-yl-pyridin-3-yl)-prop-2-ynyl]-carbamate (4m-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3-bromo-4-(furan-2-yl)pyridine (87.0 mg, 0.388 mmol), N-Boc-propargylamine (61.0 mg, 0.393 mmol), CuI (20.0 mg, 0.105 mmol) and bis(triphenylphosphine)palladium(II) dichloride (27.0 mg, 0.0385 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/EtOH 50:50 (2.0 mL) and degassed Et_3N (165. μL , 1.18 mmol). The vial was capped, placed in a Biotage Initiator $^+$ microwave and heated to 140 $^\circ\text{C}$ for 15 min on normal absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with CH_2Cl_2 (25 mL), washed with water (20 mL), followed by saturated NaCl (20 mL), dried over Na_2SO_4 , gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 10:90, v/v to EtOAc/Hex, 50:50, v/v; TLC: 50% EtOAc/hexane, R_f = 0.56) to afford **4m-Boc** (39.0 mg, 34.2% yield) as a semisolid: ^1H NMR (500 MHz, CDCl_3) δ 8.20 – 9.20 (m, 2 H), 7.66 (s, 1 H), 7.54 (s, 1 H), 7.50 (m, 1 H), 6.49 (dd, J = 3.6 Hz, J = 1.8 Hz, 1 H), 5.07 (br s, 1 H), 4.19 (d, J = 5.3 Hz, 2 H), 1.41 (s, 9 H).

3-(4-Furan-2-yl-pyridin-3-yl)-prop-2-ynylamine dihydrochloride (4m).—To a solution of **4m-Boc** (39.0 mg, 0.131 mmol) in dichloromethane (0.75 mL), cooled to 0 $^\circ\text{C}$ in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 $\times$ 25 mL). The organic solution was dried over Na_2SO_4 , gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **4m** (32.0 mg, 90.3% yield) as a yellow solid: ^1H NMR (500 MHz, D_2O) δ 8.69 (s, 1 H), 8.44 (dd, J = 0.79, 6.5 Hz, 1 H), 8.13 (d, J = 6.6 Hz, 1 H), 7.85 (d, J = 3.8 Hz, 1 H), 7.74 – 7.81 (m, 1 H), 6.66 (dd, J = 1.6, 3.8 Hz, 1 H), 4.11 (s, 2 H); ^{13}C NMR (125 MHz, D_2O) δ 149.7, 148.2, 147.2, 145.9, 140.9, 122.0, 121.2, 115.0 (2 C), 91.9, 81.3, 30.8; HRMS calcd for $\text{C}_{12}\text{H}_{11}\text{N}_2\text{O}$ [$\text{M} + \text{H}$] $^+$ 199.0871, found 199.0870.

tert-Butyl [3-(4-furan-3-ylpyridin-3-yl)-prop-2-ynyl]-carbamate (4n-Boc).—To a 5 mL microwave vial (Biotage) was added 3-bromo-4-(furan-3-yl)pyridine (83.0 mg, 0.370 mmol), N-Boc-propargylamine (59.0 mg, 0.380 mmol), CuI (9.0 mg, 0.0472 mmol) and bis(triphenylphosphine)palladium(II) dichloride (25.0 mg, 0.0356 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/EtOH 50:50 (2.0 mL) and degassed Et₃N (155. μ L, 1.11 mmol). The vial was capped and placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 15 min on normal absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with EtOAc (25 mL), washed with water (25 mL), followed by saturated NaCl (20 mL), dried over Na₂SO₄, gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 10:90, v/v to EtOAc/Hex, 50:50, v/v; TLC: 50% EtOAc/hexane, R_f = 0.44) to afford **4n-Boc** (40.0 mg, 36.3% yield) as a yellow solid: ¹H NMR (500 MHz, CDCl₃) δ 8.65 (s, 1 H), 8.47 (d, *J* = 5.0 Hz, 1 H), 8.29 (s, 1 H), 7.51 (t, *J* = 1.7 Hz, 1 H), 7.31 (d, *J* = 5.4 Hz, 1 H), 6.86 (dd, *J* = 0.95, 1.9 Hz, 1 H), 4.20 (d, *J* = 5.4 Hz, 2 H), 1.47 (s, 9 H).

3-(4-Furan-3-yl-pyridin-3-yl)-prop-2-ynylamine dihydrochloride (4n).—To a solution of **4n-Boc** (40.0 mg, 0.134 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 $\times$ 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **4n** (14.0 mg, 39.1% yield) as a yellow solid: ¹H NMR (500 MHz, D₂O) δ 8.72 (s, 1 H), 8.51 (s, 1 H), 8.47 (d, *J* = 6.3 Hz, 1 H), 7.95 (d, *J* = 6.3 Hz, 1 H), 7.58 (t, *J* = 1.7 Hz, 1 H), 6.98 (d, *J* = 1.9 Hz, 1 H), 4.06 (s, 2 H); ¹³C NMR (125 MHz, D₂O) δ 151.8, 147.7, 147.0, 146.2, 141.2, 125.7, 122.3, 118.6, 110.2, 91.6, 81.5, 30.8; HRMS calcd for C₁₂H₁₁N₂O [M+H]⁺ 199.0871, found 199.0870.

tert-Butyl (3-([4,4'-bipyridin]-3'-yl)prop-2-yn-1-yl)carbamate (4o-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3'-bromo-4, 4'-bipyridine (99.0 mg, 0.421 mmol), N-Boc-propargylamine (68.0 mg, 0.438 mmol), CuI (10.0 mg, 0.0913 mmol) and bis(triphenylphosphine)palladium(II) dichloride (29.0 mg, 0.0413 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/EtOH 50:50 (2.0 mL) and degassed Et₃N (175. μ L, 1.26 mmol). The vial was capped and placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 15 min on normal absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with EtOAc (30 mL), washed with water (15 mL), followed by saturated NaCl (15 mL), dried over Na₂SO₄, gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 50:50, v/v to 100 % EtOAc, TLC: 100% EtOAc, R_f = 0.52) to afford **4o-Boc** (39.0 mg, 30% yield) as a yellow semisolid: ¹H NMR (500 MHz, CDCl₃) δ 8.78 (s,

1 H), 8.74 (d, $J = 5.4$ Hz, 2 H), 8.62 (d, $J = 5.1$ Hz, 1 H), 7.54 (d, $J = 5.4$ Hz, 2 H), 7.31 (d, $J = 5.1$ Hz, 1 H), 4.97 (br s, 1 H), 4.09 (d, $J = 5.2$ Hz, 2 H), 1.46 (s, 9 H).

3-([4,4'-Bipyridin]-3'-yl)prop-2-yn-1-amine trihydrochloride (4o).—To a solution of **4o-Boc** (39.0 mg, 0.126 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **4o** (19.0 mg, 47% yield) as a blue solid: ¹H NMR (500 MHz, D₂O) δ 8.88 (s, 1H), 8.83 (d, $J = 6.94$ Hz, 2H), 8.70 (d, $J = 5.67$ Hz, 1H), 8.25 (d, $J = 6.94$ Hz, 2H), 7.80 – 7.86 (m, 1H), 3.88 (s, 2H); ¹³C NMR (125 MHz, D₂O) δ 154.8, 151.2, 151.0, 146.9, 143.2 (2 C), 128.7 (2 C), 126.9, 120.1, 91.3, 80.7, 30.8; HRMS calcd for C₁₃H₁₂N₃ [M+H]⁺ 210.1031, found 210.1052, UPLC (254 nm) > 95%.

tert-Butyl (3-([3,4'-bipyridin]-3'-yl)prop-2-yn-1-yl)carbamate (4p-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3'-bromo-3, 4'-bipyridine (116. mg, 0.493 mmol), N-Boc-propargylamine (76.5 mg, 0.493 mmol), CuI (12.0 mg, 0.0630 mmol) and bis(triphenylphosphine)palladium(II) dichloride (36.0 mg, 0.0513 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/EtOH 50:50 (2.0 mL) and degassed Et₃N (205. μL, 1.47 mmol). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 15 min on normal absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with EtOAc (30 mL), washed with water (15 mL), followed by saturated NaCl (15 mL), dried over Na₂SO₄, gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 50:50, v/v to 100% EtOAc; TLC: 100% EtOAc, R_f = 0.36) to afford **4p-Boc** (49.0 mg, 32.2% yield) as a brown semisolid: ¹H NMR (500 Hz, CDCl₃) δ 8.83 (d, $J = 1.9$ Hz, 1 H), 8.77 (s, 1 H), 8.68 (dd, $J = 4.9, 1.5$ Hz, 1 H), 8.61 (d, $J = 5.1$ Hz, 1 H), 7.99 (d, $J = 7.9$ Hz, 1 H), 7.42 (ddd, $J = 7.9, 4.9, 0.4$ Hz, 1 H), 7.32 (d, $J = 5.1$ Hz, 1 H), 4.87 (br s, 1 H), 4.08 (d, $J = 4.7$ Hz, 2 H), 1.45 (s, 9 H).

3-([3,4'-Bipyridin]-3'-yl)prop-2-yn-1-amine trihydrochloride (4p).—To a solution of **4p-Boc** (49.0 mg, 0.158 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual

solvent was removed *in vacuo* to afford **4p** (21.0 mg, 42.1% yield) as a white solid: ^1H NMR (500 MHz, D_2O) δ 8.97 – 8.99 (m, 1 H), 8.75 – 8.77 (m, 1 H), 8.72 – 8.75 (m, 1 H), 8.68 – 8.71 (m, 1 H), 8.56 – 8.61 (m, 1 H), 7.96 – 8.05 (m, 1 H), 7.60 – 7.66 (m, 1 H), 3.86 (s, 2 H); ^{13}C NMR (125 MHz, D_2O) δ 152.7, 148.7, 148.0, 147.2, 144.0, 143.2, 137.1, 128.4, 126.1, 119.3, 89.7, 81.8, 30.8; HRMS calcd for $\text{C}_{13}\text{H}_{12}\text{N}_3$ $[\text{M}+\text{H}]^+$ 210.1031, found 210.1036.

***tert*-Butyl (3-(4-(pyrimidin-5-yl)pyridin-3-yl)prop-2-yn-1-yl)carbamate (4q-Boc).**

—To a 2.0–5.0 mL microwave tube (Biotage) was added 5-(3-bromopyridin-4-yl)pyrimidine (140. mg, 0.593 mmol), *N*-Boc-propargylamine (93.1 mg, 0.600 mmol), CuI (17.0 mg, 0.0892 mmol) and bis(triphenylphosphine)palladium(II) dichloride (40.0 mg, 0.0570 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/EtOH 50:50 (2.0 mL) and degassed Et_3N (250. μL , 1.79 mmol). The vial was capped and placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 15 min on normal absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with EtOAc (30 mL), washed with water (15 mL), followed by saturated NaCl (15 mL), dried over Na_2SO_4 , gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 30:70, v/v to 100 % EtOAc; TLC: 100% EtOAc, R_f = 0.28) to afford **4q-Boc** (69.0 mg, 37.4% yield) as a brown semisolid: ^1H NMR (500 MHz, CDCl_3) δ 9.29 (s, 1 H), 9.01 (s, 2 H), 8.81 (s, 1 H), 8.66 (d, J = 5.1 Hz, 1 H), 7.33 (d, J = 5.1 Hz, 1 H), 5.05 (br s, 1 H), 4.11 (d, J = 4.9 Hz, 2 H), 1.46 (s, 9 H).

3-(4-(Pyrimidin-5-yl)pyridin-3-yl)prop-2-yn-1-amine tri/tetrahydrochloride (4q).

—To a solution of **4q-Boc** (69.0 mg, 0.222 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 $\times$ 25 mL). The organic solution was dried over Na_2SO_4 , gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **4q** (35.0 mg, 49.2% yield) as a light yellow solid: ^1H NMR (500 MHz, D_2O) δ 9.15 (s, 1 H), 9.05 (s, 2 H), 8.91 (s, 1 H), 8.71 (d, J = 6.0 Hz, 1 H), 7.98 (d, J = 6. Hz, 1 H), 3.91 (s, 2 H); ^{13}C NMR (125 MHz, D_2O) δ 159.8, 158.1 (2 C), 152.9, 148.3, 144.1, 131.4, 128.0, 121.5, 91.5, 80.3, 30.8; HRMS calcd for $\text{C}_{12}\text{H}_{11}\text{N}_4$ $[\text{M}+\text{H}]^+$ 211.0984, found 211.0995.

***tert*-Butyl ((5-(4-methylpyridin-3-yl)furan-2-yl)methyl)carbamate (5g-Boc).**—To a Biotage 2.0–5.0 mL microwave tube containing 5-((*tert*-butoxycarbonyl)aminomethyl)furan-2-boronic acid (134. mg, 0.609 mmol) under a blanket of argon(g) was added degassed DME/ H_2O /EtOH 7:3:2 (1.0 mL) followed by *trans*-dichlorobis(triphenylphosphine)palladium(II) (17.1 mg, 0.0113 mmol) followed by a solution of sodium carbonate (89.1 mg, 0.832 mmol) in degassed H_2O (0.4 mL). The resultant mixture was stirred under argon(g) for 5 min followed by the addition of 3-

bromo-4-methylpyridine (0.0683 mL, 0.611 mmol). The tube was purged with argon(g), capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 2 min on normal absorption level. The contents of the flask were transferred to an Erlenmeyer flask containing 5 g of anhydrous Na₂SO₄, with the aid of CH₂Cl₂, and subsequently diluted to 50 mL with additional CH₂Cl₂. The Na₂SO₄ was removed by gravity filtration, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 50:50, v/v, *R*_f = 0.29) to afford **5g-Boc** (143. mg, 89.4% yield) as a red oil: ¹H NMR (300 MHz, CDCl₃) δ 8.81 (s, 1 H), 8.35 (d, *J* = 5.1 Hz, 1 H), 7.22 – 7.02 (m, 1 H), 6.52 (d, *J* = 3.2 Hz, 1 H), 6.33 (d, *J* = 3.2 Hz, 1 H), 5.07 (br s, 1 H), 4.37 (d, *J* = 5.8 Hz, 2 H), 2.46 (s, 3 H), 1.55 – 1.22 (m, 9 H).

(5-(4-Methylpyridin-3-yl)furan-2-yl)methanamine dihydrochloride (5g).—To a solution of **5g-Boc** (143. mg, 0.502 mmol) in anhydrous dichloromethane (ca. 1.5 mL) was added trifluoroacetic acid (3 mL) and the resultant solution was stirred at ambient temperature for 3 h. To the mixture was added 2 mL of 1 N HCl and the mixture was vigorously stirred for 10 min. The mixture was diluted with 20 mL of water, transferred to a separatory funnel and the organics were discarded. The aqueous portion was washed with dichloromethane (2 X 30 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (2 X 35 mL). The combined organics were dried over anhydrous MgSO₄, filtered and the solvent was removed *in vacuo*. This material was dissolved in 40 mL of ethyl ether and treated with 5 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford **5g** (12.9 mg, 11.1% yield) as a white solid: ¹H NMR (300 MHz, D₂O) δ (s, 1 H), 8.33 (dd, *J* = 1.9, 6.0 Hz, 1 H), 7.77 (dd, *J* = 6.0, 2.6 Hz, 1 H), 6.99 – 6.77 (m, 1 H), 6.74 – 6.48 (m, 1 H), 4.18 (s, 2 H), 2.60 (d, *J* = 4.9 Hz, 3 H); ¹³C NMR (75 MHz, D₂O) δ 170.1, 151.1, 143.3, 142.6, 133.0, 132.8, 124.4, 110.1, 108.5, 30.8, 17.2; HRMS calcd for C₁₁H₁₃N₂O [M+H]⁺ 189.1028, found 189.1057.

tert-Butyl ((5-(4-ethylpyridin-3-yl)furan-2-yl)methyl)carbamate (5i-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3-bromo-4-ethylpyridine (219. mg, 1.18 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)furan-2-yl)boronic acid (284. mg, 1.18 mmol) and bis(triphenylphosphine)palladium(II) dichloride (9.0 mg, 0.013 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/EtOH 50:50 (2.0 mL) and degassed Et₃N (175. μL, 1.26 mmol). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with EtOAc (30 mL), washed with water (15 mL), followed by saturated NaCl (15 mL), dried over Na₂SO₄, gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 5:95, v/v to EtOAc/Hex, 50:50, v/v; TLC: 50% EtOAc in hexane, *R*_f = 0.36) to afford **5i-Boc** (211. mg, 59.3% yield) as a brown semisolid: ¹H NMR (500 MHz, CDCl₃) δ 8.72 (s, 1 H), 8.37 (d, *J* = 5.1 Hz, 1 H), 7.19 (d, *J* = 5.1 Hz, 1 H), 6.52 (d, *J* = 3.3 Hz, 1 H), 6.35 (m, 1 H), 5.74 (br s, 1 H), 2.83 (m, 2 H), 1.46 (s, 9H), 1.24 (t, *J* = 7.5 Hz, 3 H).

(5-(4-Ethylpyridin-3-yl)furan-2-yl)methanamine dihydrochloride (5i).—To a solution of **5i-Boc** (61.1 mg, 0.202 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **5i** (39.0 mg, 70.1% yield) as a yellow solid. ¹H NMR (500 MHz, D₂O) δ 8.77 (s, 1 H), 8.38 (d, *J* = 6.3 Hz, 1 H), 7.8 (d, *J* = 6.0 Hz, 1 H), 6.85 (d, *J* = 3.5 Hz, 1 H), 6.62 (d, *J* = 3.5 Hz, 1 H), 4.17 (s, 2 H), 2.93 (q, *J* = 7.5 Hz, 2 H), 1.16 (t, *J* = 7.4 Hz, 3 H); ¹³C NMR (125 MHz, D₂O) δ 155.5, 143.3, 142.9, 134.6, 134.5, 123.7, 121.9, 109.5, 108.4, 30.8, 22.5, 7.1; HRMS calcd for C₁₂H₁₅N₂O [M+H]⁺ 203.1184, found 203.1183.

tert-Butyl ((5-(4-methoxypyridin-3-yl)furan-2-yl)methyl)carbamate (5j-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3-bromo-4-methoxypyridine (131. mg, 0.697 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)furan-2-yl)boronic acid (168. mg, 0.698 mmol) and bis(triphenylphosphine)palladium(II) dichloride (28.0 mg, 0.0399 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/H₂O/EtOH (7:3:2, v/v:v, 2.0 mL) and degassed 2 M Na₂CO₃ (0.7 mL). The vial was capped and placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with EtOAc (30 mL), washed with water (15 mL), followed by saturated NaCl (15 mL), dried over Na₂SO₄, gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 5:95, v/v to EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 50:50, v/v, R_f = 0.30) to afford **5j-Boc** (162. mg, 76.2% yield) as a reddish semisolid: ¹H NMR (500 MHz, CDCl₃) δ 8.92 (s, 1 H), 8.38 (d, *J* = 5.8 Hz, 1 H), 6.84 – 6.86 (m, 2 H), 6.32 (m, 1 H), 5.07 (br s, 1 H), 4.38 (d, *J* = 5.4 Hz, 2 H), 3.98 (s, 3 H), 1.47 (s, 9 H).

(5-(4-Methoxypyridin-3-yl)furan-2-yl)methanamine dihydrochloride (5j).—To a solution of **5j-Boc** (162. mg, 0.532 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.75 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (7.5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **5j** (72.0 mg, 49.1% yield) as a brown solid: ¹H NMR (500 MHz, D₂O) δ 8.60 (s, 1 H), 8.21 (d, *J* = 6.3 Hz, 1 H), 7.11

(s, 1 H), 6.89 (d, $J = 3.5$ Hz, 1 H), 6.52 (d, $J = 3.5$ Hz, 1 H), 4.13 (s, 2 H), 3.91 (s, 3 H); ^{13}C NMR (125 MHz, D_2O) δ 158.7, 142.2, 141.5, 141.2, 137.6, 111.8, 108.6, 108.2, 103.4, 51.6, 30.8; HRMS calcd for $\text{C}_{11}\text{H}_{13}\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$ 205.0977, found 205.0974.

tert-Butyl ((5-(4-propylpyridin-3-yl)furan-2-yl)methyl)carbamate (5k-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3-bromo-4-propylpyridine (167. mg, 0.835 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)furan-2-yl)boronic acid (202. mg, 0.838 mmol) and bis(triphenylphosphine)palladium(II) dichloride (29.0 mg, 0.0413 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/ H_2O /EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na_2CO_3 (0.7 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with EtOAc (30 mL), washed with water (15 mL), followed by saturated NaCl (15 mL), dried over Na_2SO_4 , gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 5:95, v/v to EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 50:50, v/v, $R_f = 0.30$) to afford **5k-Boc** (97.0 mg, 37.4% yield) as a yellow semisolid: ^1H NMR (500 MHz, CDCl_3) δ 8.84 (s, 1 H), 8.39 (d, $J = 5.1$ Hz, 1 H), 7.13 (d, $J = 5.1$ Hz, 1 H), 6.48 (d, $J = 2.9$ Hz, 1 H), 6.34 (m, 1 H), 5.29 (br s, 1 H), 4.38 (d, $J = 5.4$ Hz, 2 H), 2.76 (t, $J = 7.8$ Hz, 2 H), 1.62 (m, 2 H), 1.46 (s, 9 H), 0.98 (t, $J = 7.4$ Hz, 3 H).

(5-(4-Propylpyridin-3-yl)furan-2-yl)methanamine dihydrochloride (5k).—To a solution of **5k-Boc** (97.0 mg, 0.311 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.75 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 hours. The reaction mixture was subsequently chilled in an external ice-water bath followed by dropwise adding saturated sodium carbonate (7.5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 $\times$ 25 mL). The organic solution was dried over Na_2SO_4 , gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **5k** (54.0 mg, 81.3% yield) as a brown solid: ^1H NMR (500 MHz, D_2O) δ 8.80 (s, 1 H), 8.38 (d, $J = 6.3$ Hz, 1 H), 7.81 (d, $J = 6.3$ Hz, 1 H), 6.85 (d, $J = 3.8$ Hz, 1 H), 6.62 (s, 1 H), 4.18 (s, 2 H), 2.90 (t, $J = 7.6$ Hz, 1 H), 1.57 (m, $J = 7.5$ Hz, 2 H), 0.82 (t, $J = 7.4$ Hz, 3 H); ^{13}C NMR (125 MHz, D_2O) δ 155.1, 143.1, 142.1, 133.6, 132.9, 123.9, 122.9, 109.4, 108.1, 30.8, 30.5, 16.3, 7.8; HRMS calcd for $\text{C}_{13}\text{H}_{17}\text{N}_2\text{O}$ $[\text{M}+\text{H}]^+$ 217.1341, found 213.1346.

tert-Butyl ((5-(4-phenylpyridin-3-yl)furan-2-yl)methyl)carbamate (5l-Boc).—To a Biotage 2.0–5.0 mL microwave tube containing 5-(((tert-butoxycarbonyl)aminomethyl)furan-2-yl)boronic acid (205. mg, 0.849 mmol) under a blanket of argon(g) was added degassed DME/ H_2O /EtOH 7:3:2 (1.50 mL) followed by *trans*-dichlorobis(triphenylphosphine)palladium(II) (16.0 mg, 0.0231 mmol) followed by a solution of sodium carbonate (132. mg, 1.25 mmol) in degassed H_2O (0.65 mL). The resultant mixture was stirred under argon(g) for 5 minutes followed by the addition of a solution of 3-bromo-4-phenylpyridine (221. mg, 0.946 mmol) in degassed DME/ H_2O /EtOH

7:3:2 (1.0 mL). The tube was purged with argon(g), capped and placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 4 min on normal absorption level. The contents of the flask were transferred to an Erlenmeyer flask containing 5 g of anhydrous Na₂SO₄, with the aid of CH₂Cl₂, and subsequently diluted to 50 mL with additional CH₂Cl₂. The Na₂SO₄ was removed by gravity filtration, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v; TLC: EtOAc/Hex, 25:75, v/v, *R*_f = 0.13–0.21) to afford **5l-Boc** (118. mg, 40.3% yield) as a colorless oil: ¹H NMR (300 MHz, CDCl₃) δ 8.80 (br s, 1 H), 8.37 (br s, 1 H), 7.30 – 7.21 (m, 5 H), 7.18 – 7.02 (m, 5 H), 5.96 (d, *J* = 3.0 Hz, 1 H), 5.65 (d, *J* = 3.0 Hz, 1 H), 4.64 (br s, 1 H), 4.05 (d, *J* = 5.3 Hz, 2 H), 1.41 – 1.16 (m, 9 H).

(5-(4-Phenylpyridin-3-yl)furan-2-yl)methanamine dihydrochloride (5l).—To a solution of **5l-Boc** (118. mg, 0.336 mmol) in anhydrous dichloromethane (ca. 4 mL) was added trifluoroacetic acid (4 mL) and the resultant solution was stirred at ambient temperature for 2.5 h. The mixture was transferred to a separatory funnel followed by 5 mL of 1 N HCl, 10 mL of H₂O and 45 mL of dichloromethane. The organics were discarded and the aqueous portion was washed with dichloromethane (45 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (2 X 50 mL). The combined organics were dried over anhydrous MgSO₄, filtered and the solvent was removed *in vacuo*. This material was dissolved in 40 mL of diethyl ether and treated with 5 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford **5l** (28.2 mg, 29.1% yield) as a white solid: ¹H NMR (300 MHz, D₂O) δ 8.90 (br s, 1 H), 8.49 (br s, 1 H), 7.74 (br s, 1 H), 7.53 – 7.29 (m, 3 H), 7.26 (br s, 2 H), 6.43 – 6.16 (m, 1 H), 5.92 (br s, 1 H), 4.03 (s, 2 H); ¹³C NMR (75 MHz, D₂O) δ 168.0, 150.6, 145.8, 143.3, 142.8, 135.0, 134.5, 131.4, 125.5, 124.4, 123.2, 117.3, 115.1, 109.7, 108.4, 30.8; HRMS calcd for C₁₆H₁₅N₂O [M+H]⁺ 251.1184, found 251.1211.

tert-Butyl ((5-(4-(furan-2-yl)pyridin-3-yl)furan-2-yl)methyl)carbamate (5m-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3-bromo-4-(furan-2-yl)pyridine (83.1 mg, 0.371 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)furan-2-yl)boronic acid (89.0 mg, 0.369 mmol) and bis(triphenylphosphine)palladium(II) dichloride (27.0 mg, 0.0385 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/H₂O/EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na₂CO₃ (0.7 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with CH₂Cl₂ (25 mL), washed with water (20 mL), followed by saturated NaCl (20 mL), dried over Na₂SO₄, gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel using a gradient elution (EtOAc/Hex, 10:90, v/v to EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 50:50, v/v, *R*_f = 0.36) to afford **5m-Boc** (53.0 mg, 43.3% yield) as a yellow semisolid: ¹H NMR (500 MHz, CDCl₃) δ 8.59 (s, 1 H), 8.50 (d, *J* = 5.4 Hz, 1 H), 7.62 (d, *J* = 6.1 Hz, 1 H), 7.46 (d, *J* = 1.3 Hz, 1 H), 6.39 (m, 1 H), 6.31 (m, 1 H), 6.09 (d, *J* = 3.1 Hz, 1 H), 4.89 (br s, 1 H), 4.25 (d, *J* = 4.4 Hz, 2 H), 1.38 (s, 9 H).

(5-(4-(Furan-2-yl)pyridin-3-yl)furan-2-yl)methanamine dihydrochloride (5m).—

To a solution of **5m-Boc** (53.0 mg, 0.156 mmol) in dichloromethane (1 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **5m** (31.0 mg, 63.3% yield) as a yellow solid: ¹H NMR (500 MHz, D₂O) δ 8.45 – 8.53 (m, 1 H), 8.37 – 8.44 (m, 1 H), 7.76 – 7.86 (m, 1 H), 7.48 – 7.61 (m, 1 H), 6.58 (d, *J* = 3.2 Hz, 1 H), 6.48 – 6.53 (m, 1 H), 6.42 – 6.48 (m, 1 H), 6.05 – 6.28 (m, 1 H), 4.00 – 4.19 (m, 2 H); ¹³C NMR (125 MHz, D₂O) δ 144.4, 143.5, 142.6, 141.4, 141.2, 140.2, 139.2, 135.4, 118.1, 116.9, 110.2, 108.1, 107.2, 30.8; HRMS calcd for C₁₄H₁₃N₂O₂ [M+H]⁺ 241.0977, found 241.0979.

tert-Butyl ((5-(4-(furan-3-yl)pyridin-3-yl)furan-2-yl)methyl)carbamate (5n-Boc).

—To a 2.0–5.0 mL microwave tube (Biotage) was added 3-bromo-4-(furan-3-yl)pyridine (75.0 mg, 0.335 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)furan-2-yl)boronic acid (81.0 mg, 0.336 mmol) and bis(triphenylphosphine)palladium(II) dichloride (25.0 mg, 0.0356 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/H₂O/EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na₂CO₃ (0.7 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with EtOAc (25 mL), washed with water (20 mL), followed by saturated NaCl (20 mL), dried over Na₂SO₄, gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel using a gradient elution (EtOAc/Hex, 10:90, v/v to EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 50:50, v/v, R_f = 0.36) to afford **5n-Boc** (43.0 mg, 38.4% yield) as a semisolid: ¹H NMR (500 MHz, CDCl₃) δ 8.78 (s, 1 H), 8.51 (d, *J* = 5.1 Hz, 1 H), 7.45–7.46 (m, 2 H), 7.28 (d, *J* = 5.1 Hz, 1 H), 6.26–6.31 (m, 3 H), 4.98 (br s, 1 H), 4.30 (d, *J* = 5.2 Hz, 2 H), 1.46 (s, 9 H).

(5-(4-(Furan-3-yl)pyridin-3-yl)furan-2-yl)methanamine dihydrochloride (5n).—

To a solution of **5n-Boc** (43.0 mg, 0.131 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **5n** (26.0 mg, 63.1% yield) as a yellow solid mixture of the 2-furan/3-furan substituted products at a ratio of

13/87 as determined by the ratio of the methylene peaks at 4.16 ppm (2-furanyl) and 4.09 ppm (3-furanyl), respectively: ^1H NMR (500 MHz, D_2O) δ 8.86 (s, 1 H), 8.62 (d, J = 6.2 Hz, 1 H), 8.01 (d, J = 6.2 Hz, 1 H), 7.84 (m, 1 H), 7.63 (dd, J = 1.7 Hz, 1 H), 6.74 (d, J = 3.5 Hz, 1 H), 6.71 (d, J = 3.5 Hz, 1 H), 6.47 (m, 1 H), 4.25 (s, 2 H); ^{13}C NMR (125 MHz, D_2O) δ 143.3 – 104.7: Aromatic carbons, only a select number of peaks are labeled for clarity – 30.8; HRMS calcd for $\text{C}_{14}\text{H}_{13}\text{N}_2\text{O}_2$ $[\text{M}+\text{H}]^+$ 241.0977, found 241.0977.

***tert*-Butyl ((5-([4,4'-bipyridin]-3'-yl)furan-2-yl)methyl)carbamate (5o-Boc).**—To a 2.0–5.0 mL microwave tube (Biotage) was added 3'-bromo-4,4'-bipyridine (63.0 mg, 0.268 mmol), (5-(((*tert*-butoxycarbonyl)amino)methyl)furan-2-yl)boronic acid (65.0 mg, 0.270 mmol) and bis(triphenylphosphine)palladium(II) dichloride (24.0 mg, 0.0342 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/ H_2O /EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na_2CO_3 (0.75 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, diluted with EtOAc (30 mL), washed with water (15 mL) followed by saturated NaCl (15 mL), dried over Na_2SO_4 , gravity filtered and the solvent was removed *in vacuo* and the residue was chromatographed on silica gel using a gradient elution (EtOAc/Hex, 10:90, v/v to 100% EtOAc; TLC: 25% EtOAc/Hex, v/v, R_f = 0.19) to afford the product **5o-Boc** (28.0 mg, 30.3% yield) as a yellow semisolid: ^1H NMR (500 MHz, CDCl_3) δ 8.97 (s, 1 H), 8.68 (d, J = 6.0 Hz, 2 H), 8.59 (d, J = 5.0 Hz, 1 H), 7.23 (d, J = 6.0 Hz, 2 H), 7.19 (d, J = 5.0 Hz, 1 H), 6.16 (m, 1 H), 5.95 (d, J = 2.8 Hz, 1 H), 4.81 (br s, 1 H), 4.21 (d, J = 5.2 Hz, 2 H), 1.46 (s, 9 H).

(5-([4,4'-Bipyridin]-3'-yl)furan-2-yl)methanamine trihydrochloride (5o).—To a solution of **5o-Boc** (28.0 mg, 0.0797 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for one hour, diluted with water (20 mL) and extracted with dichloromethane (2 $\times$ 25 mL). The organic solution was dried over Na_2SO_4 , gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **5o** (18.0 mg, 63.3% yield) as a yellow solid: ^1H NMR (500 MHz, D_2O) δ 9.07 (s, 1 H), 8.80 (d, J = 6.31 Hz, 2 H), 8.69 (d, J = 5.99 Hz, 1 H), 8.04 (d, J = 6.62 Hz, 2 H), 7.89 (d, J = 5.7 Hz, 1 H), 6.45 (d, J = 3.2 Hz, 1 H), 6.25 (d, J = 3.5 Hz, 1 H), 4.02 (s, 2 H); ^{13}C NMR (125 MHz, D_2O) δ 149.8, 144.7, 142.9, 141.8, 137.8 (2 C), 137.2, 136.6, 123.3, 123.0, 122.5 (2 C), 110.8, 108.8, 30.8; HRMS calcd for $\text{C}_{15}\text{H}_{14}\text{N}_3\text{O}$ $[\text{M}+\text{H}]^+$ 252.1137, found 252.1139.

***tert*-Butyl ((5-([3,4'-bipyridin]-3'-yl)furan-2-yl)methyl)carbamate (5p-Boc).**—To a 2.0–5.0 mL microwave tube (Biotage) was added 3'-bromo-3,4'-bipyridine (104. mg, 0.442 mmol), (5-(((*tert*-butoxycarbonyl)amino)methyl)furan-2-yl)boronic acid (106. mg, 0.440 mmol) and bis(triphenylphosphine)palladium(II) dichloride (31.0 mg, 0.0442 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/ H_2O /EtOH

(7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na₂CO₃ (0.75 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, diluted with EtOAc (30 mL), washed with water (15 mL) followed by saturated NaCl (15 mL), dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* and the residue was chromatographed on silica gel using a gradient elution (EtOAc/Hex, 50:50, v/v to 100% EtOAc; TLC: 100% EtOAc, R_f = 0.22) to afford the product **5p-Boc** (57.0 mg, 37.4% yield) as a yellow solid: ¹H NMR (500 MHz, CDCl₃) δ 8.87 (s, 1 H), 8.59 (d, *J* = 4.1 Hz, 1 H), 8.50 (d, *J* = 5.0 Hz, 1 H), 8.47 (m, 1 H), 7.53 (ddd, *J* = 7.8, 1.9, 1.9 Hz, 1 H), 7.29 (dd, *J* = 7.8 Hz, *J* = 5.0 Hz, 1 H), 7.15 (d, *J* = 5.0 Hz, 1 H), 6.08 (m, 1 H), 5.87 (d, *J* = 3.1 Hz, 1 H), 4.81 (br s, 1 H), 4.12 (d, *J* = 5.3 Hz, 2 H), 1.38 (s, 9 H).

(5-([3,4'-Bipyridin]-3'-yl)furan-2-yl)methanamine trihydrochloride (5p).—To a solution of **5p-Boc** (57.0 mg, 0.162 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **5p** (36.0 mg, 62.1% yield) as a yellow solid: ¹H NMR (500 MHz, D₂O) δ 8.88 (s, 1 H), 8.64 (dd, *J* = 1.4, 5.52 Hz, 1 H), 8.63 (d, *J* = 2.2 Hz, 1 H), 8.53 (d, *J* = 5.4 Hz, 1 H), 8.13 – 8.25 (m, 1 H), 7.76 (ddd, *J* = 8.0, 5.5, 1.0 Hz, 1 H), 7.57 – 7.62 (m, 1 H), 6.40 (d, *J* = 3.5 Hz, 1 H), 6.08 (d, *J* = 3.5 Hz, 1 H), 4.00 (s, 2 H); ¹³C NMR (125 MHz, D₂O) δ 143.8, 143.3, 140.1 (2 C), 140.0 (2 C), 138.9, 138.4, 131.2, 122.2, 121.9, 121.5, 108.9, 108.3, 30.8; HRMS calcd for C₁₅H₁₄N₃O [M+H]⁺ 252.1137, found 252.1143.

tert-Butyl ((5-(4-(pyrimidin-5-yl)pyridin-3-yl)furan-2-yl)methyl)carbamate (5q-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 5-(3-bromopyridin-4-yl)pyrimidine (102. mg, 0.430 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)furan-2-yl)boronic acid (104. mg, 0.431 mmol) and bis(triphenylphosphine)palladium(II) dichloride (29.0 mg, 0.0413 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/H₂O/EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na₂CO₃ (0.75 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, diluted with EtOAc (30 mL), washed with water (15 mL) followed by saturated NaCl (15 mL), dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* and the residue was chromatographed on silica gel using a gradient elution (EtOAc/Hex, 30:70, v/v to 100% EtOAc; TLC: 100% EtOAc, R_f = 0.28) to afford the product **5q-Boc** (79.0 mg, 52.4% yield) as a yellow semisolid: ¹H NMR (500 MHz, CDCl₃) δ 9.18 (s, 1 H), 8.87 (s, 1 H), 8.60 (s, 2 H), 8.55 (d, *J* = 5.0 Hz, 1 H), 7.17 (d, *J* = 5.0 Hz, 1 H), 6.15 (d, *J* = 2.3 Hz, 1 H), 6.06 (d, *J* = 2.3 Hz, 1 H), 4.91 (br s, 1 H), 4.11 (d, *J* = 5.4 Hz, 2 H), 1.37 (s, 9 H).

(5-(4-(Pyrimidin-5-yl)pyridin-3-yl)furan-2-yl)methanamine tri/tetrahydrochloride (5q).—To a solution of **5q-Boc** (79.0 mg, 0.224 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **5q** (37.0 mg, 46.2% yield) as a light yellow solid: ¹H NMR (500 MHz, D₂O) δ 9.13 (s, 1 H), 8.98 (s, 1 H), 8.76 (s, 2 H), 8.62 (d, *J* = 5.7 Hz, 1 H), 7.84 (d, *J* = 5.7 Hz, 1 H), 6.46 (d, *J* = 3.2 Hz, 1 H), 6.24 (d, *J* = 3.2 Hz, 1 H), 4.02 (s, 2 H); ¹³C NMR (125 MHz, D₂O) δ 153.4, 151.7 (2 C), 144.0, 142.8, 141.5, 137.4, 136.9, 126.8, 123.5, 123.4, 109.9, 108.6, 30.8; HRMS calcd for C₁₄H₁₃N₄O [M+H]⁺ 253.1089, found 253.1092.

tert-Butyl ((5-(4-methylpyridin-3-yl)thiophen-2-yl)methyl)carbamate (6g-Boc).—To a Biotage 2.0–5.0 mL microwave tube containing 5-((tert-butoxycarbonyl)aminomethyl)thiophene-2-boronic acid (131. mg, 0.509 mmol) under a blanket of argon(g) was added degassed DME/H₂O/EtOH 7:3:2 (1.0 mL) followed by *trans*-dichlorobis(triphenylphosphine)palladium(II) (19.1 mg, 0.0273 mmol) followed by a solution of sodium carbonate (82.2 mg, 0.775 mmol) in degassed H₂O (0.38 mL). The resultant mixture was stirred under argon(g) for 5 min followed by the addition of 3-bromo-4-methylpyridine (0.051 mL, 0.462 mmol). The tube was purged with argon(g), capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 2 min on normal absorption level. The contents of the flask were transferred to an Erlenmeyer flask containing 5 g of anhydrous Na₂SO₄, with the aid of CH₂Cl₂, and subsequently diluted to 50 mL with additional CH₂Cl₂. The Na₂SO₄ was removed by gravity filtration, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 50:50, v/v, *R*_f = 0.36) to afford **6g-Boc** (119. mg, 84.4% yield) as a red oil: ¹H NMR (300 MHz, CDCl₃) δ 8.54 (s, 1 H), 8.39 (d, *J* = 5.1 Hz, 1 H), 7.16 (d, *J* = 4.9 Hz, 1 H), 6.96 – 6.92 (m, 2 H), 5.09 (br s, 1 H), 4.49 (d, *J* = 5.8 Hz, 2 H), 2.42 (s, 3 H), 1.46 (s, 9 H).

(5-(4-Methylpyridin-3-yl)thiophene-2-yl)methanamine dihydrochloride (6g).—To a solution of **6g-Boc** (119. mg, 0.393 mmol) in anhydrous dichloromethane (ca. 1.5 mL) was added trifluoroacetic acid (3 mL) and the resultant solution was stirred at ambient temperature for 3 h. To the mixture was added 2 mL of 1 N HCl and the mixture was vigorously stirred for 10 min. The mixture was diluted with 20 mL of water, transferred to a separatory funnel and the organics were discarded. The aqueous portion was washed with dichloromethane (2 X 30 mL), the pH was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (2 X 35 mL). The combined organics were dried over anhydrous MgSO₄, filtered and the solvent was removed *in vacuo*. This material was dissolved in 40 mL of ethyl ether and treated with 5 mL of ethereal HCl. The resulting

precipitate was collected by centrifugation and dried *in vacuo* to afford **6g** (26.7 mg, 28.4% yield) as a white solid: ^1H NMR (300 MHz, D_2O) δ 8.56 (d, J = 6.8 Hz, 1 H), 8.39 (br. s., 1 H), 7.88 – 7.65 (m, 1 H), 7.25 – 7.03 (m, 2 H), 4.29 (d, J = 7.9 Hz, 2 H), 2.50 (d, J = 10.9 Hz, 3 H); ^{13}C NMR (75 MHz, D_2O) δ 152.5, 133.7, 132.3, 130.4, 129.2, 127.0, 123.8, 123.7, 122.2, 30.8, 14.8; HRMS calcd for $\text{C}_{11}\text{H}_{13}\text{N}_2\text{S}$ $[\text{M}+\text{H}]^+$ 205.0799, found 205.0846.

tert-Butyl ((5-(4-ethylpyridin-3-yl)thiophen-2-yl)methyl)carbamate (6i-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3-bromo-4-ethylpyridine (107. mg, 0.575 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)thiophen-2-yl)boronic acid (147. mg, 0.572 mmol) and bis(triphenylphosphine)palladium(II) dichloride (8.0 mg, 0.011 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/ H_2O /EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na_2CO_3 (0.75 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, diluted with water (20 mL), extracted with dichloromethane (2×20 mL), dried over Na_2SO_4 , gravity filtered and the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 5:95, v/v to EtOAc/Hex, 50:50, v/v; TLC: 50% EtOAc in hexane, R_f = 0.33) to afford the product **6i-Boc** (69.0 mg, 38.3% yield) as a yellow oil: ^1H NMR (500 MHz, CDCl_3) δ 8.47 (s, 1 H), 8.43 (m, 1 H), 7.24 (d, J = 5.1 Hz, 1 H), 6.96 (m, 1 H), 6.90 (d, J = 3.5 Hz, 1 H), 5.45 (br s, 1 H), 4.50 (m, 2 H), 2.77 (q, J = 7.6 Hz, 2 H), 1.48 (s, 9 H), 1.20 (t, J = 7.6 Hz, 3 H).

(5-(4-Ethylpyridin-3-yl)thiophen-2-yl)methanamine dihydrochloride (6i).—To a solution of **6i-Boc** (69.0 mg, 0.217 mmol) in dichloromethane (0.5 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2×20 mL). The organic solution was dried over Na_2SO_4 , gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in dichloromethane (0.5 mL) and treated with ethereal HCl (1 mL, dropwise). The solid material was collected via filtration and the residual solvent was removed *in vacuo* to afford **6i** (40.5 mg, 64.4% yield) as a yellow solid: ^1H NMR (500 MHz, D_2O) δ 8.45 (s, 1 H), 8.40 (d, J = 5.4 Hz, 1 H), 7.47 (d, J = 5.0 Hz, 1 H), 7.26 (d, J = 3.5 Hz, 1 H), 7.13 (d, J = 3.8 Hz, 1 H), 4.42 (s, 2 H), 2.78 (q, J = 7.6 Hz, 2 H), 1.14 (t, J = 7.6 Hz, 3 H); ^{13}C NMR (125 MHz, D_2O) δ 147.7, 141.5, 140.6, 132.8, 128.4, 123.4, 123.3, 121.9, 117.8, 30.8, 19.1, 6.9. HRMS calcd for $\text{C}_{12}\text{H}_{15}\text{N}_2\text{S}$ $[\text{M}+\text{H}]^+$ 219.0956, found 219.0963.

tert-Butyl ((5-(4-methoxypyridin-3-yl)thiophen-2-yl)methyl)carbamate (6j-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3-bromo-4-methoxypyridine (173. mg, 0.918 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)thiophen-2-yl)boronic acid (235. mg, 0.916 mmol) and bis(triphenylphosphine)palladium(II) dichloride (35.0 mg, 0.0499 mmol). The vial was purged with argon for 5 min followed the addition of degassed DME/ H_2O /EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na_2CO_3 (0.7 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal

absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with EtOAc (30 mL), washed with water (15 mL), followed by saturated NaCl (15 mL), dried over Na₂SO₄, gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 5:95, v/v to EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 50:50, v/v, R_f = 0.32) to afford **6j-Boc** (244. mg, 83.4% yield) as a light yellow oil: ¹H NMR (500 MHz, CDCl₃) δ 8.67 (s, 1 H), 8.37 (d, *J* = 5.7 Hz, 1 H), 7.32 (d, *J* = 3.7 Hz, 1 H), 6.93 (m, 1 H), 6.86 (d, *J* = 5.7 Hz, 1 H), 5.32 (br s, 1 H), 4.49 (m, 2 H), 3.94 (s, 3 H), 1.47 (s, 9 H).

(5-(4-Methoxypyridin-3-yl)thiophen-2-yl)methanamine dihydrochloride (6j).—To a solution of **6j-Boc** (244. mg, 0.762 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.75 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (7.5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **6j** (139. mg, 62.3% yield) as an off-white solid: ¹H NMR (500 MHz, D₂O) δ 8.27 (s, 1 H), 8.04 (d, *J* = 5.7 Hz, 1 H), 7.17 (d, *J* = 3.8 Hz, 1 H), 6.93 (d, *J* = 3.8 Hz, 1 H), 6.82 (d, *J* = 6.0 Hz, 1 H), 4.09 (s, 2 H), 3.72 (s, 3 H); ¹³C NMR (125 MHz, D₂O) δ 154.5, 141.9, 140.0, 130.3, 128.9, 121.2, 119.4, 111.9, 100.3, 48.4, 30.8; HRMS calcd for C₁₁H₁₃N₂OS [M+H]⁺ 221.0749, found 221.0748.

tert-Butyl ((5-(4-propylpyridin-3-yl)thiophen-2-yl)methyl)carbamate (6k-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3-bromo-4-propylpyridine (153. mg, 0.765 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)thiophen-2-yl)boronic acid (196. mg, 0.762 mmol) and bis(triphenylphosphine)palladium(II) dichloride (27.0 mg, 0.0385 mmol). The vial was purged with argon for 5 min followed the addition of degassed DME/H₂O/EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na₂CO₃ (0.7 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with EtOAc (30 mL), washed with water (15 mL), followed by saturated NaCl (15 mL), dried over Na₂SO₄, gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 5:95, v/v to EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 50:50, v/v, R_f = 0.30) to afford **6k-Boc** (95.0 mg, 38.4% yield) as a yellow semisolid: ¹H NMR (500 MHz, CDCl₃) δ 8.50 (s, 1 H), 8.44 (d, *J* = 5.1 Hz, 1 H), 7.18 (d, *J* = 5.1 Hz, 1 H), 6.96 (m, 1 H), 6.88 (d, *J* = 3.5 Hz, 1 H), 5.25 (bs, 1 H), 4.51 (m, 2 H), 2.70 (t, *J* = 7.8 Hz, 2 H), 1.59 (m, 2 H), 1.47 (s, 9 H), 0.93 (t, *J* = 7.3 Hz, 3 H).

(5-(4-Propylpyridin-3-yl)thiophen-2-yl)methanamine dihydrochloride (6k).—To a solution of **6k-Boc** (95.0 mg, 0.296 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.75 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction

mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (7.5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **6k** (51.0 mg, 77.2% yield) as an off-white solid: ¹H NMR (500 MHz, D₂O) δ 8.65 (s, 1 H), 8.56 (d, *J* = 6.0 Hz, 1 H), 7.86 (d, *J* = 6.0 Hz, 1 H), 7.31 (d, *J* = 3.8 Hz, 1 H), 7.23 (d, *J* = 3.8 Hz, 1 H), 4.45 (s, 2 H), 2.81 – 3.04 (m, 2 H), 1.48 – 1.75 (m, 2 H), 0.88 (t, *J* = 7.4 Hz, 3 H); ¹³C NMR (125 MHz, D₂O) δ 154.4, 136.1, 134.6, 130.0, 129.9, 126.2, 123.5, 123.3, 120.5, 30.8, 28.6, 16.1, 6.4; HRMS calcd for C₁₃H₁₇N₂S [M+H]⁺ 233.1112, found 233.1120.

***tert*-Butyl ((5-(4-phenylpyridin-3-yl)thiophene-2-yl)methyl)carbamate (6l-Boc).**

—To a Biotage 2.0–5.0 mL microwave tube containing 5-((*tert*-butoxycarbonyl)aminomethyl)thiophene-2-boronic acid (245. mg, 0.971 mmol) under a blanket of argon(g) was added degassed DME/H₂O/EtOH 7:3:2 (0.75 mL) followed by *trans*-dichlorobis(triphenylphosphine)palladium(II) (22.0 mg, 0.0310 mmol) followed by a solution of sodium carbonate (160. mg, 1.51 mmol) in degassed H₂O (0.75 mL). The resultant mixture was stirred under argon(g) for 5 minutes followed by the addition of a solution of 3-bromo-4-phenylpyridine (250. mg, 1.07 mmol) in degassed DME/H₂O/EtOH 7:3:2 (1.50 mL). The tube was purged with argon(g), capped and placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 2 min on normal absorption level. The contents of the flask were transferred to an Erlenmeyer flask containing 5 g of anhydrous Na₂SO₄, with the aid of CH₂Cl₂, and subsequently diluted to 50 mL with additional CH₂Cl₂. The Na₂SO₄ was removed by gravity filtration, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel (EtOAc/Hex, 25:75, v/v; TLC: EtOAc/Hex, 25:75, v/v, *R_f* = 0.13) to afford **6l-Boc** (193. mg, 52.4% yield) as a yellow oil: ¹H NMR (300 MHz, CDCl₃) δ 8.70 (br s, 1 H), 8.55 (d, *J* = 4.3 Hz, 1 H), 7.45 – 7.18 (m, 6 H), 6.76 (d, *J* = 3.4 Hz, 1 H), 6.62 (d, *J* = 3.6 Hz, 1 H), 5.23 (br s, 1 H), 4.52 – 4.20 (m, 2 H), 1.45 (s, 9 H).

(5-(4-Phenylpyridin-3-yl)thiophen-2-yl)methanamine dihydrochloride (6l).—To a

solution of **6l-Boc** (193. mg, 0.526 mmol) in anhydrous dichloromethane (ca. 5 mL) was added trifluoroacetic acid (3 mL) and the resultant solution was warmed to reflux and stirred until complete conversion was determined by TLC analysis (1 h). The mixture was cooled to rt, transferred to a separatory funnel followed by 10 mL of H₂O, 10 mL of 1 N HCl and dichloromethane (40 mL). The organics were discarded and the aqueous layer was washed with 40 mL of dichloromethane. The pH of the aqueous layer was adjusted to ca. 10 using 10 N NaOH and extracted with dichloromethane (2 X 50 mL). The combined organics were dried over anhydrous MgSO₄, filtered and the solvent was removed *in vacuo*. The residue was chromatographed on 10 g of silica gel (CH₃OH/CH₂Cl₂, 10:90, v/v; TLC: CH₃OH/CH₂Cl₂, 10:90, v/v, *R_f* = 0.12). This material was dissolved in 40 mL of ethyl ether and treated with 3 mL of ethereal HCl. The resulting precipitate was collected by centrifugation and dried *in vacuo* to afford **6l** (87.7 mg, 55.4% yield) as a white solid: ¹H NMR (300 MHz, D₂O) δ 8.71 (s, 1 H), 8.53 (d, *J* = 6.0 Hz, 1 H), 7.75 (d, *J* = 6.0 Hz, 1 H), 7.42 – 7.09 (m, 5

H), 6.97 (d, $J = 3.8$ Hz, 1 H), 6.89 (d, $J = 3.8$ Hz, 1 H), 4.17 – 4.05 (m, 2 H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 148.2, 137.4, 135.9, 135.8, 133.2, 130.6, 130.5, 124.1, 123.9, 123.8, 123.5, 123.2 (2), 12.4, 118.3, 30.8; HRMS calcd for $\text{C}_{16}\text{H}_{15}\text{N}_2\text{S}$ $[\text{M}+\text{H}]^+$ 267.0956, found 267.0956.

tert-Butyl ((5-(4-(furan-2-yl)pyridin-3-yl)thiophen-2-yl)methyl)carbamate (6m-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3-bromo-4-(furan-2-yl)pyridine (76.0 mg, 0.339 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)thiophen-2-yl)boronic acid (87.0 mg, 0.338 mmol) and bis(triphenylphosphine)palladium(II) dichloride (25.0 mg, 0.0356 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/H₂O/EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na₂CO₃ (0.75 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, transferred to a separatory funnel, diluted with CH₂Cl₂ (25 mL), washed with water (20 mL), followed by saturated NaCl (20 mL), dried over Na₂SO₄, gravity filtered, the solvent was removed *in vacuo* and the residue was chromatographed on silica gel using a gradient elution (EtOAc/Hex, 15:85, v/v to EtOAc/Hex, 50:50, v/v; TLC: EtOAc/Hex, 50:50, v/v, $R_f = 0.44$) to afford **6m-Boc** (59.0 mg, 50.4% yield) as a yellow semisolid: ^1H NMR (500 MHz, CDCl₃) δ 8.51 (d, $J = 5.5$ Hz, 1 H), 8.46 (s, 1 H), 7.71 (d, $J = 5.5$ Hz, 1 H), 7.44 (m, 1 H), 6.91 (d, $J = 2.8$ Hz, 1 H), 6.82 (d, $J = 3.5$ Hz, 1 H), 6.31 (dd, $J = 3.4, 1.8$ Hz, 1 H), 6.03 (d, $J = 3.4$ Hz, 1 H), 5.01 (br s, 1 H), 4.42 (m, 2 H), 1.40 (s, 9 H).

(5-(4-(Furan-2-yl)pyridin-3-yl)thiophen-2-yl)methanamine dihydrochloride (6m).

—To a solution of **6m-Boc** (59.0 mg, 0.166 mmol) in dichloromethane (1 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 $\times$ 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **6m** (40.0 mg, 73.3% yield) as a yellow solid as a mixture of the 2-furan/3-furan substituted products at a ratio of 76/23 as determined by the ratio of the methylene peaks at 4.30 ppm (2-furanyl) and 4.23 ppm (3-furanyl), respectfully: ^1H NMR (500 MHz, D₂O) δ 5.96 – 8.76 (m, 8 H), 4.10 – 4.44 (m, 2 H); ^{13}C NMR (125 MHz, D₂O) δ 140.9 – 105.5: Aromatic carbons, only a select number of peaks are labeled for clarity – 30.8; HRMS calcd for $\text{C}_{14}\text{H}_{13}\text{N}_2\text{OS}$ $[\text{M}+\text{H}]^+$ 257.0749, found 257.0746.

tert-Butyl [5-(4-furan-3-yl-pyridin-3-yl)thiophen-2-ylmethyl]carbamate (6n-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3-bromo-4-(furan-3-yl)pyridine (80.0 mg, 0.357 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)thiophen-2-yl)boronic acid (91.0 mg, 0.354 mmol) and bis(triphenylphosphine)palladium(II) dichloride (26.0 mg, 0.037 mmol). The vial was purged with argon for 5 min followed by the addition

of degassed DME/H₂O/EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na₂CO₃ (0.75 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, diluted with dichloromethane (25 mL), washed with water (20 mL) followed by saturated NaCl (20 mL), dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was purified by flash chromatography using a gradient elution (EtOAc/Hex, 10:90, v/v to EtOAc/Hex, 50:50, v/v; TLC: 50% EtOAc/hexane, R_f = 0.34) to afford the product **6n-Boc** (70.0 mg, 55.4% yield) as an off-white solid: ¹H NMR (500 MHz, CDCl₃) δ 8.58 (s, 1 H), 8.53 (d, *J* = 5.1 Hz, 1 H), 7.38 (dd, *J* = 1.7 Hz, 1 H), 7.35 (m, 1 H), 7.32 (d, *J* = 5.1 Hz, 1 H), 6.90 (d, *J* = 3.4 Hz, 1 H), 6.82 (d, *J* = 3.4 Hz, 1 H), 6.32 (m, 1 H), 5.19 (br s, 1 H), 4.48 (d, *J* = 4.9 Hz, 2 H), 1.46 (s, 9 H).

(5-(4-(Furan-3-yl)pyridin-3-yl)thiophen-2-yl)methanamine dihydrochloride (6n).

—To a solution of **6n-Boc** (70.0 mg, 0.196 mmol) in dichloromethane (1 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **6n** (41.0 mg, 64.4% yield) as a yellow solid: ¹H NMR (500 MHz, D₂O) δ 8.46 (s, 1 H), 8.40 (d, *J* = 5.7 Hz, 1 H), 7.66 (d, *J* = 5.7 Hz, 1 H), 7.33 – 7.43 (m, 2 H), 7.09 (d, *J* = 3.8 Hz, 1 H), 6.96 (d, *J* = 3.5 Hz, 1 H), 6.29 (dd, *J* = 2.1, 1.8 Hz, 1 H), 4.25 (s, 2 H); ¹³C NMR (125 MHz, D₂O) δ 139.6, 139.2, 137.6, 137.3 (2 C), 131.5, 129.9, 123.5, 123.0 (2 C), 118.2, 115.2, 103.2, 30.8; HRMS calcd for C₁₄H₁₃N₂OS [M+H]⁺ 257.0749, found 257.0763.

tert-Butyl ((5-([4,4'-bipyridin]-3'-yl)thiophen-2-yl)methyl)carbamate (6o-Boc).—

To a 2.0–5.0 mL microwave tube (Biotage) was added 3'-bromo-4, 4'-bipyridine (72.0 mg, 0.306 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)thiophen-2-yl)boronic acid (78.0 mg, 0.303 mmol) and bis(triphenylphosphine)palladium(II) dichloride (22.0 mg, 0.0313 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/H₂O/EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na₂CO₃ (0.75 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, diluted with EtOAc (30 mL), washed with water (15 mL) followed by saturated NaCl (15 mL), dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was purified by flash chromatography using a gradient elution (EtOAc/Hex, 50:50, v/v to 100% EtOAc; TLC: 100% EtOAc, R_f = 0.20) to afford the product **6o-Boc** (43.0 mg, 39.3% yield) as a yellow semisolid: ¹H NMR (500 MHz, CDCl₃) δ 8.76 (s, 1 H), 8.60 – 8.64 (m, 3 H), 7.25 (d, *J* = 5.0 Hz, 1 H), 7.20 (d, *J* = 5.8 Hz, 2 H), 6.79 (d, *J* = 3.4 Hz, 1 H), 6.60 (d, *J* = 3.4 Hz, 1 H), 5.12 (br s, 1 H), 4.41 (d, *J* = 5.1 Hz, 2 H), 1.45 (s, 9 H).

(5-([4,4'-Bipyridin]-3'-yl)thiophen-2-yl)methanamine trihydrochloride (6o).—To a solution of **6o-Boc** (43.0 mg, 0.117 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by dropwise adding saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **6o** (31.0 mg, 70.1% yield) as a yellow solid: ¹H NMR (500 MHz, D₂O) δ 8.85 (s, 1 H), 8.71 (d, *J* = 5.7 Hz, 1 H), 8.62 – 8.68 (m, 2 H), 7.89 – 7.95 (m, 2 H), 7.84 (d, *J* = 5.7 Hz, 1 H), 7.01 (d, *J* = 3.8 Hz, 1 H), 6.84 (d, *J* = 3.8 Hz, 1 H), 4.19 (s, 2 H); ¹³C NMR (125 MHz, D₂O) δ 147.9, 142.3, 139.2, 137.5, 135.7 (2 C), 131.6, 129.2, 124.8, 124.7, 124.0, 121.1 (2 C), 120.2, 30.8; HRMS calcd for C₁₅H₁₄N₃S [M + H]⁺ 268.0908, found 269.0905.

tert-Butyl ((5-([3,4'-bipyridin]-3'-yl)thiophen-2-yl)methyl)carbamate (6p-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 3'-bromo-3, 4'-bipyridine (77.0 mg, 0.328 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)thiophen-2-yl)boronic acid (84.0 mg, 0.327 mmol) and bis(triphenylphosphine)palladium(II) dichloride (22.0 mg, 0.0313 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/H₂O/EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na₂CO₃ (0.75 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, diluted with EtOAc (30 mL), washed with water (15 mL) followed by saturated NaCl (15 mL), dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was purified by flash chromatography using a gradient elution (EtOAc/Hex, 50:50, v/v to 100% EtOAc; TLC: 100% EtOAc, R_f = 0.22) to afford the product **6p-Boc** (49.0 mg, 41.1% yield) as a yellow semisolid: ¹H NMR (500 Hz, CDCl₃) δ 8.56 – 8.80 (m, 4 H), 7.63 (d, *J* = 7.8 Hz, 1 H), 7.32 – 7.35 (m, 2 H), 6.80 (d, *J* = 3.3 Hz, 1 H), 6.65 (d, *J* = 3.3 Hz, 1 H), 4.94 (br s, 1 H), 4.40 (d, *J* = 5.0 Hz, 2 H), 1.45 (s, 9 H).

(5-([3,4'-Bipyridin]-3'-yl)thiophen-2-yl)methanamine trihydrochloride (6p).—To a solution of **6p-Boc** (49.0 mg, 0.133 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by dropwise adding saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2 × 25 mL). The organic solution was dried over Na₂SO₄, gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **6p** (68.0 mg, 79.4% yield) as a yellow solid. ¹H NMR (500 MHz, D₂O) δ 8.84 (s, 1 H), 8.63 – 8.73 (m, 3 H), 8.32 (d, *J* = 8.5 Hz, 1 H), 7.78

– 7.93 (m, 2 H), 7.02 (d, $J = 3.8$ Hz, 1 H), 6.90 (d, $J = 3.8$ Hz, 1 H), 4.18 (s, 2 H); ^{13}C NMR (125 MHz, D_2O) δ 141.9, 139.1, 138.5, 137.1, 136.9, 136.2, 131.6, 129.4, 129.0, 125.5, 124.7, 124.0, 121.0, 120.5, 30.8; HRMS calcd for $\text{C}_{15}\text{H}_{14}\text{N}_3\text{S}$ $[\text{M}+\text{H}]^+$ 268.0908, found 268.0910.

tert-Butyl ((5-(4-(pyrimidin-5-yl)pyridin-3-yl)thiophen-2-yl)methyl)carbamate (6q-Boc).—To a 2.0–5.0 mL microwave tube (Biotage) was added 5-(3-bromopyridin-4-yl)pyrimidine (108. mg, 0.458 mmol), (5-(((tert-butoxycarbonyl)amino)methyl)thiophen-2-yl)boronic acid (121. mg, 0.471 mmol) and bis(triphenylphosphine)palladium(II) dichloride (29.0 mg, 0.0413 mmol). The vial was purged with argon for 5 min followed by the addition of degassed DME/ H_2O / EtOH (7:3:2, v:v:v, 2.0 mL) and degassed 2 M Na_2CO_3 (0.75 mL). The vial was capped, placed in a Biotage Initiator⁺ microwave and heated to 140 °C for 5 min on normal absorption. The contents of the flask were cooled to rt, diluted with EtOAc (30 mL), washed with water (15 mL) followed by saturated NaCl (15 mL), dried over Na_2SO_4 , gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was purified by flash chromatography using a gradient elution (EtOAc/Hex , 30:70, v/v to 100% EtOAc ; TLC: 100% EtOAc , $R_f = 0.29$) to afford the product **6q-Boc** (84.0 mg, 50.1% yield) as a light yellow semisolid: ^1H NMR (500 MHz, CDCl_3) δ 9.21 (s, 1 H), 8.79 (s, 1 H), 8.68 (d, $J = 5.0$ Hz, 2 H), 8.66 (s, 2 H), 7.30 (d, $J = 5.0$ Hz, 1 H), 6.83 (m, 1 H), 6.68 (d, $J = 3.4$ Hz, 1 H), 5.02 (br s, 1 H), 4.41 (d, $J = 5.1$ Hz, 2 H), 1.45 (s, 9 H).

(5-(4-(Pyrimidin-5-yl)pyridin-3-yl)thiophen-2-yl)methanamine tri/tetrahydrochloride (6q).—To a solution of **6q-BOC** (84.0 mg, 0.228 mmol) in dichloromethane (0.75 mL), cooled to 0 °C in an external ice-water bath, was added trifluoroacetic acid (0.5 mL). The ice bath was removed and the resultant solution was stirred at ambient temperature for 2 h. The reaction mixture was subsequently chilled in an external ice-water bath followed by the dropwise addition of saturated sodium carbonate (5 mL). The resultant mixture was stirred at room temperature for 1 h, diluted with water (20 mL) and extracted with dichloromethane (2×25 mL). The organic solution was dried over Na_2SO_4 , gravity filtered and the solvent was removed *in vacuo* to afford the crude material which was dissolved in 0.5 mL of dichloromethane and treated with ethereal HCl (1 mL, dropwise). The supernatant was discarded and the residual solvent was removed *in vacuo* to afford **6q** (68.0 mg, 79.4% yield) as a yellow solid: ^1H NMR (500 MHz, D_2O) δ 9.06 (s, 1 H), 8.86 (s, 1 H), 8.71 (d, $J = 5.7$ Hz, 1 H), 8.68 (s, 2 H), 7.92 – 8.02 (m, 1 H), 7.04 (d, $J = 3.8$ Hz, 1 H), 6.96 (d, $J = 3.8$ Hz, 1 H), 4.19 (s, 2 H); ^{13}C NMR (125 MHz, D_2O) δ 151.5, 150.4 (2 C), 143.0, 136.9, 135.3, 131.8, 129.1, 126.2, 124.8, 124.4, 124.0, 121.4, 30.8; HRMS calcd for $\text{C}_{14}\text{H}_{13}\text{N}_4\text{S}$ $[\text{M}+\text{H}]^+$ 269.0861, found 269.0858.

CYP Inhibition Assays:

Pooled human liver microsomes were obtained from Xenotech, LLC (Lenexa, Kansas). NADPH-regenerating system (1.3 mM NADP, 3.3 mM glucose 6-phosphate and 0.4 U/mL glucose 6-phosphate dehydrogenase) was from Corning; Bedford, MA. Coumarin, phenacetin, bupropion, amodiaquine, diclofenac, omeprazole, dextromethorphan, chlorzoxazone, midazolam, furafylline, tranlycypromine, clopidogrel, montelukast, sulfaphenazole, tranlycypromine, quinidine, chlomethiazole, ketoconazole and methoxsalen

were all purchased from Sigma-Aldrich; St. Louis, MO. LCMS-grade methanol, acetonitrile and formic acid were obtained from Fisher Scientific (Waltham, MA).

The inhibition activities of the nicotine analogues against CYP2A6 was determined using either 1 μ M or 10 μ M of each analogue as an initial screen. Incubations were performed in pooled human liver microsomes (mixed gender, pool of 50 donors) in incubations using coumarin as the CYP2A6 probe substrate. Coumarin was prepared as 20 mM stock solution in DMSO and stored in aliquots at -80°C . Inhibition assays for each new compound against CYP2A6 were performed after pre-incubation of a reaction mixture containing pooled human liver microsomes (0.1 mg/mL), the test compound (1 or 10 μ M), coumarin (2.5 μ M; approximating the known K_M for CYP2A6 against coumarin), 100 mM potassium phosphate buffer (pH 7.4) and magnesium chloride (3 mM) in a final reaction volume of 50 μ L for 5 min in a 37°C water bath. The DMSO in the final incubation mixture was 0.1%. The reaction was initiated by the addition of an NADPH-regenerating system (1.3 mM NADP, 3.3 mM glucose 6-phosphate and 0.4 U/mL glucose 6-phosphate dehydrogenase) and incubated for 15 min at 37°C . Reactions were terminated by the addition of 50 μ L of stop solution (acetonitrile/methanol; 1:1, v/v). Samples were mixed on a vortex mixer and centrifuged at $13,200 \times g$ for 15 min at room temperature. The supernatant ($\sim 75 \mu\text{L}$) was then transferred to a UPLC sample vial, and the metabolite (7-hydroxycoumarin) was detected using an ultra-performance liquid chromatograph (UPLC; Waters Acquity; Waters Corp, Milford, MA) coupled to a triple-quadrupole mass spectrometer (Waters Xevo TDQ; Waters Corp, Milford, MA) by multiple reaction monitoring (MRM) analysis as described below.

As a positive control for every CYP2A6 inhibition experiment, 1 μ M methoxsalen or tranilcypromine was added instead of the test agent. As a negative control experiment, only vehicle (0.1% DMSO) was added (no inhibitor or test compound was added).

Inhibition assays for CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4 were performed as described above for CYP2A6 using phenacetin, bupropion, amodiaquine, diclofenac, omeprazole, dextromethorphan, chlorzoxazone and midazolam, respectively, as the corresponding probe substrates; with concentrations indicated in Table 5. Positive controls for the inhibition of each enzyme included: furafylline (1 μ M), clopidogrel (1 μ M), montelukast (5 μ M), sulfaphenazole (1 μ M), tranilcypromine (10 μ M), quinidine (1 μ M), chlomethiazole (10 μ M) and ketoconazole (1 μ M) for CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4, respectively (see Table 5).

The activity of CYPs 3A4 (midazolam), 2D6 (dextromethorphan), 2C9 (diclofenac), 2C19 (omeprazole) and 1A2 (phenacetin) was determined using a cocktail method containing five probe substrates in a single reaction in incubation mixtures performed essentially as described above for CYP2A6, with test compounds, reference inhibitor or vehicle control and the cocktail containing the five probe substrates (indicated above) at a concentration similar to their known, respective, K_M values, as indicated in Table 5. The metabolites of CYPs 3A4, 2D6, 2C9, 2C19 and 1A2, [1-hydroxymidazolam, dextrophan, 4'-hydroxydiclofenac, 5-hydroxyomeprazole and acetaminophen, respectively] were detected simultaneously on five different channels using ultra-performance liquid chromatograph

(UPLC; Waters Acquity; Waters Corp, Milford, MA) coupled to an electron triple-quadrupole mass spectrometer (Waters Xevo TDQ; Waters Corp, Milford, MA) by MRM analysis as described in Table 6.

The measurement of CYP 2B6, 2C8 and 2E1 activity was determined individually in human liver microsomes. The test compound, positive control inhibitor or vehicle control were pre-incubated in a reaction mixture as described above for other CYP enzymes using bupropion (100 μ M), amodiaquine (2 μ M) or chlorzoxazone (100 μ M) for CYPs 2B6, 2C8 and 2E1, respectively.

Determination of IC₅₀ values:

For those agents that exhibited >50% inhibition of activity for any given CYP at <10 μ M, IC₅₀ determinations were performed using multiple concentrations (0.005, 0.01, 0.1, 0.5, 1, 5, 10, 25 and 100 μ M) of test compound in 15 min, 37 °C incubations as described above. The final concentration of DMSO in the reaction mixture was always 0.1%. Peak areas corresponding to the probe metabolite were determined and the percentage of control activity was calculated by comparing the peak area in incubations containing the agents to incubations containing only the vehicle control. IC₅₀ values were calculated using Graph Pad Prism software version 6.

Analytical Conditions:

As described above, probe substrate metabolites for all enzymatic reactions were detected using an UPLC-MS/MS system (Waters Xevo TDQ Acquity UPLC-MS/MS system) by MRM analysis. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (100% methanol). Samples (2–5 μ L) were injected onto an Acquity UPLC column (C₁₈, 1.7 μ m, 2.1 X 100 mm) from Waters Corp. The 6 min program was as follows: 2 min at 95% A:5% B followed by a linear gradient for 2 min to 5% A:95% B, 1 min at 5% A:95% B and re-equilibration for 1 min at 95% A:5% B. The flow rate was 0.4 mL/min and the column temperature was 40 °C.

Human Liver Microsomal Stability Determinations:

Each nicotine analogue (10 μ M) was incubated at 37 °C with 12.5 μ g of human liver microsomal protein containing 7 μ M MgCl₂, 3 μ L NADPH-regenerating system (1.3 mM NADP, 3.3 mM glucose 6-phosphate and 0.4 U/mL glucose 6-phosphate dehydrogenase), and 50 mM potassium phosphate buffer (pH 7.4) in a total volume of 137.5 μ L. Aliquots of 25 μ L were taken at 0, 15, 30, 60 and 120 min, and added to 25 μ L of 1:1 methanol:acetonitrile. After vortexing, samples were centrifuged at 17,000 x g for 15 min at 4 °C. The supernatant (~40 μ L) was then transferred to a clean UPLC sample vial, with the remaining concentration of each nicotine analogue determined by monitoring of the molecular ion, [M + H]⁺ of each agent on the UPLC-MS using the same system and chromatography conditions as described above. Half-lives were calculated using the one-phase exponential decay equation (GraphPad 6.0).

CYP2A6dh Protein Preparation

Approximately 50 ng of CYP2A6dh plasmid DNA (generously provided by Eric F. Johnson, The Scripps Research Institute) was transformed into DH5 α -competent *E. Coli* (a kind gift from Weihang Chai, Washington State University) using standard protocols. The transformed bacteria were diluted with 1 mL LB broth (with no antibiotics, VWR, Radnor, PA) and incubated in a 37 °C water bath for 1 hour with no shaking. Cells were pelleted at 13000 g for 1 minute. All but 100 μ L of the media was aspirated and the cells were re-suspended in the remaining 100 μ L, spread onto an LB/amp isolation plate and incubated at 37 °C overnight.

A single colony from this plate was inoculated into 5 mL of Super Broth (VWR, Radnor, PA) containing 50 μ g/mL ampicillin (VWR, Radnor, PA) the resultant mixture was shaken at 225 rpm overnight at 37 °C. The next day, 3.5 mL of the overnight culture was seeded into 500 mL of Terrific Broth (VWR, Radnor, PA) containing 50 μ g/mL ampicillin and this mixture was shaken at 225 rpm and 37 °C until the OD₆₀₀ nm of the culture reached 0.35. The heme precursor aminolevulinic acid (ALA, Ark Pharm, Arlington Heights, IL) was added to a final concentration of 0.5 mM, and when the OD₆₀₀ of the culture reached 0.45 nm, expression was induced by the addition of 1 mM isopropyl- β -D-thiogalactopyranoside (Biosynth, Itasca, IL). The resultant bacterial suspension was shaken at 190 rpm at 30 °C for 48 hours.

E. Coli were harvested by centrifugation at 6285g for 10 min at 4 °C and resuspended in lysis buffer [20 mM potassium phosphate (pH 7.4, VWR, Radnor, PA), 4.8 mM CHAPS (3-((3-cholamidopropyl) dimethylammonio)-1-propanesulfonate, BioVision, Milpitas, CA), 20% glycerol (VWR, Radnor, PA), 1 mM phenylmethylsulfonyl fluoride (PMSF, VWR, Radnor, PA) and 10 mM 2-mercaptoethanol (2-ME, VWR, Radnor, PA)] in a volume equal to 10% of the original bacterial culture volume. Spheroplasts were produced by treating the suspension for 30 min with 0.2 mg/mL of lysozyme (VWR, Radnor, PA) with stirring at 4 °C. Subsequently, an equal volume of cold water was added slowly to the stirring mixture. After stirring an additional 10 min at 4 °C, the resulting spheroplasts were pelleted by centrifugation at 10,000g for 15 min at 4 °C, the supernatant was discarded, and the spheroplasts were frozen using a dry ice / acetone bath. The frozen pellets were thawed on ice and suspended in cold 500 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol, 0.5 mM PMSF and 10 mM 2-ME in a volume equal to 8% of the original bacterial culture volume. The suspension was sonicated (Branson sonifier 250, constant power mode) using three 45 second pulses at 20% power, with 60 seconds of cooling on ice in between. The resultant material was centrifuged at 38,465g for 60 minutes at 4 °C. The protein was coordinated to nickel-nitrilotriacetic acid resin (1 mL, G-Biosciences, St Louis, MO) that had been thoroughly washed with loading buffer (100 mM potassium phosphate, pH 7.4, 20% glycerol, 200 mM NaCl and 4.8 mM CHAPS) by inverting the supernatant in the presence of the resin in a 50 mL centrifuge tube at 4 °C for 30 minutes. The resin was collected via centrifugation and transferred to the 1 mL tube in which it was received. To isolate the protein, the resin was washed with 2 column volumes (CV) of loading buffer followed by 2 CV wash buffer (100 mM potassium phosphate, pH 7.4, 20% glycerol, 200 mM NaCl, 4.8 mM CHAPS, 8 mM histidine) two times, and subsequently eluted in three

fractions: Fraction 1, 2 CV of elution buffer (10 mM potassium phosphate, pH 7.4, 20% glycerol, 100 mM NaCl, 4.8 mM CHAPS, 80 mM histidine and 2 mM EDTA); Fraction 2, 2 CV of elution buffer and Fraction 3, 4 CV of elution buffer. Presence of the protein in the eluent fractions was confirmed by UV/Vis spectrophotometry. A spectrum (200–750 nm) was obtained showing a λ_{max} at 418 nm.

The protein from elution fraction 3 was diluted 1:3 with assay buffer (50 mM potassium phosphate, pH 7.4), and 750 μL was transferred to a blank cuvette and 750 μL was transferred to a sample cuvette. A spectrum was recorded (UV-1700 PharmaSpec UV-VIS Spectrophotometer, Shimadzu) from 350–450 nm to confirm a baseline. 7.5 μL of DI water was added to the blank cuvette while 7.5 μL of a 10 mM solution (100 μM , final concentration) of the test compound (dissolved in DI water) was added to the sample cuvette. Both were mixed using a pipette set to 650 μL and the spectrum was recorded to afford the difference spectrum for each test compound.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

The authors gratefully acknowledge Todd T. Talley (T3 BioSciences, Moses Lake, WA) for his technical expertise in the analysis of X-ray crystal structures and molecular modeling performed in this work. The authors would like to thank Eric F. Johnson and Mei-Hui Hsu (The Scripps Research Institute, San Diego, CA) for the plasmid encoding CYP2A6dh. We also thank Megan Chastain and Weihang Chai (Washington State University, Spokane, WA) for the kind donation of competent DH5a cells used in this study and for Megan's technical expertise in the protein transformation studies. The authors wish to acknowledge Devika Gates, Laken Kruger, Matt Ryplewski and Dunxin Shen (College of Pharmacy, Washington State University, Spokane, WA) for their technical assistance in the preparation of CYP2A6dh protein. We would also like to thank the Mass Spectrometry and Nuclear Magnetic Resonance Core Facilities at Washington State University-Spokane for their support and help with analysis. This work was supported in part by the Health Sciences and Services Authority of Spokane, WA.

Abbreviations Used:

CYP	Cytochrome P-450
HLM	human liver microsomes
MRM	multiple reaction monitoring

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Figure 1.

Starting with a previous lead²², the 4-position of the pyridine ring was identified as a point of further

Figure 2. Local area minimized structure of CYP2A6 with compound **5I** in the active site. The “aromatic nest” consisting of residues F107, F209, and F480 accommodate the phenyl ring of the ligand.

Scheme 1.

Nicotine analogues prepared and tested for CYP inhibitory activity and selectivity.

Table 1.

Inhibitory values of nicotine analogues for select cytochrome P-450s.

Compd	CYP [IC ₅₀ ± SD (μM)]									
	2A6	3A4	2D6	2C9	2C19	1A2	2B6	2C8	2E1	
4a	0.16 ± 0.03	4.0 ± 0.20	6.1 ± 1.4	30 ± 7.0	7.6 ± 1.6	5.4 ± 0.67	22 ± 4.0	6.4 ± 1.5	19 ± 2.5	
4b	>10 ^a	ND ^b	ND	ND	ND	ND	ND	ND	ND	
4c	>10 ^a	ND	ND	ND	ND	ND	ND	ND	ND	
4d	>10 ^a	ND	ND	ND	ND	ND	ND	ND	ND	
4e	5.5 ± 0.50	>50 ^c	>50 ^c	>50 ^c	13 ± 2.1	6.1 ± 0.70	13 ± 2.5	>50 ^c	29 ± 1.2	
4f	>10 ^a	ND	ND	ND	ND	ND	ND	ND	ND	
4g	0.055 ± 0.016	5.6 ± 0.36	2.4 ± 0.37	36 ± 4.8	8.0 ± 2.0	11 ± 2.5	20 ± 2.5	8.6 ± 2.0	19 ± 3.3	
4h	>10 ^a	ND	ND	ND	ND	ND	ND	ND	ND	
4i	0.83 ± 0.12	>50 ^c	6.6 ± 1.8	>50 ^c	22 ± 3.6	>50 ^c	19 ± 2.5	25 ± 3.6	15 ± 3.0	
4j	3.4 ± 0.33	>50 ^c	>50 ^c	28 ± 6.2	9.3 ± 2.8	25 ± 5.0	11 ± 1.6	>50 ^d	18 ± 4.1	
4k	0.44 ± 0.01	>50 ^c	2.8 ± 0.09	32 ± 4.1	11 ± 2.5	>50 ^c	4.1 ± 0.70	20 ± 3.3	32 ± 3.0	
4l	0.13 ± 0.02	39 ± 5.5	5.0 ± 2.0	3.0 ± 0.15	12 ± 2.0	8 ± 2.1	3.0 ± 0.01	14 ± 3.0	15 ± 3.1	
4m	0.56 ± 0.06	11 ± 0.45	3.9 ± 0.64	2.8 ± 0.27	3.7 ± 0.47	1.4 ± 0.36	5.0 ± 0.23	7.5 ± 1.2	1.9 ± 0.25	
4n	0.67 ± 0.07	6.4 ± 0.18	4.6 ± 1.3	6.3 ± 0.44	3.1 ± 0.88	2.0 ± 0.30	3.5 ± 0.43	21 ± 12	4.2 ± 1.1	
4o	3.6 ± 0.69	>25 ^d	12 ± 3.9	>25 ^d	>25 ^d	21 ± 5.4	>25 ^d	>50 ^c	>25 ^d	
4p	0.59 ± 0.07	92 ± 29	14 ± 3.4	48 ± 14	31 ± 3.7	31 ± 9.4	364 ± 97	>25 ^d	45 ± 11	
4q	2.7 ± 0.64	>25 ^d	>25 ^d	>25 ^d	>25 ^d	>25 ^d	>25 ^d	>50 ^c	>25 ^d	
5g	0.39 ± 0.03	29 ± 4.1	21 ± 2.3	36 ± 5.1	34 ± 3.2	2.3 ± 0.66	5.8 ± 0.34	79 ± 12	12 ± 0.66	
5i	0.051 ± 0.013	181 ± 32	3.0 ± 0.61	59 ± 12	0.26 ± 0.03	2.0 ± 0.85	1.30 ± 0.40	73 ± 7.9	0.92 ± 0.05	
5j	1.0 ± 0.18	>25 ^d	12 ± 3.9	>50 ^d	4.1 ± 0.33	1.3 ± 0.15	3.9 ± 0.46	16 ± 0.69	5.9 ± 0.63	
5k	0.14 ± 0.02	4.9 ± 0.33	1.7 ± 0.27	19 ± 2.9	2.0 ± 0.14	0.37 ± 0.06	0.37 ± 0.06	0.26 ± 0.024	1.9 ± 0.61	
5l	0.40 ± 0.01	5.2 ± 0.35	0.85 ± 0.06	2.6 ± 0.21	4.3 ± 0.07	0.29 ± 0.03	0.18 ± 0.07	5.2 ± 0.80	7.0 ± 0.62	
5m	0.29 ± 0.03	29 ± 3.6	3.0 ± 0.98	2.0 ± 0.25	2.2 ± 0.27	0.26 ± 0.07	0.12 ± 0.01	5.4 ± 0.17	9.4 ± 0.62	

Compd	CYP [IC ₅₀ ± SD (μM)]								
	2A6	3A4	2D6	2C9	2C19	1A2	2B6	2C8	2E1
5n	0.11 ± 0.02	24 ± 6.5	3.6 ± 0.83	4.8 ± 0.99	3.4 ± 0.73	1.4 ± 0.50	0.23 ± 0.07	>25 ^d	1.3 ± 0.05
5o	0.30 ± 0.03	39 ± 3.8	6.5 ± 1.8	>50 ^c	12 ± 0.25	7.9 ± 3.2	13 ± 3.9	>25 ^d	>50 ^c
5p	0.38 ± 0.08	90 ± 22	12 ± 2.4	28 ± 4.3	13 ± 0.72	4.4 ± 0.46	7.8 ± 1.6	>25 ^d	31 ± 7.6
5q	1.3 ± 0.19	>25 ^d	>25 ^d	>25 ^d	>25 ^d	7.6 ± 2.4	>25 ^d	>50 ^c	>50 ^c
6g	0.22 ± 0.01	37 ± 4.8	20 ± 4.0	28 ± 1.9	27 ± 3.3	3.8 ± 1.1	0.85 ± 0.15	80 ± 5.4	2.0 ± 0.19
6i	0.017 ± 0.005	13 ± 2.6	4.5 ± 1.0	16 ± 3.8	0.84 ± 0.21	4.0 ± 0.90	0.093 ± 0.041	1.5 ± 0.37	0.23 ± 0.04
6j	0.55 ± 0.06	>25 ^d	10 ± 1.8	>50 ^c	0.92 ± 0.12	1.4 ± 0.28	4.6 ± 0.61	>25 ^d	4.5 ± 0.64
6k	0.042 ± 0.012	4.3 ± 0.64	2.7 ± 0.43	8.5 ± 1.5	1.1 ± 0.09	0.19 ± 0.04	0.087 ± 0.014	2.2 ± 0.91	1.5 ± 0.27
6l	0.41 ± 0.02	5.3 ± 0.79	0.76 ± 0.14	2.9 ± 0.34	4.4 ± 0.61	0.91 ± 0.1	0.060 ± 0.026	1.1 ± 0.10	6.8 ± 0.95
6m	0.16 ± 0.03	7.0 ± 0.60	>50 ^c	4.4 ± 0.62	1.4 ± 0.23	0.20 ± 0.004	0.073 ± 0.015	3.8 ± 1.3	3.1 ± 0.67
6n	0.076 ± 0.001	5.2 ± 0.35	>50 ^c	6.1 ± 1.4	0.81 ± 0.29	0.27 ± 0.003	0.062 ± 0.006	4.8 ± 2.0	5.5 ± 1.0
6o	0.33 ± 0.03	214 ± 21	12 ± 1.6	43 ± 5.6	11 ± 3.3	4.8 ± 0.63	3.5 ± 0.40	>25 ^d	31 ± 9.6
6p	0.46 ± 0.01	42 ± 0.87	16 ± 4.7	23 ± 5.1	9.1 ± 2.1	1.6 ± 0.59	1.2 ± 0.12	3.0 ± 1.0	37 ± 2.4
6q	1.2 ± 0.24	39 ± 11	8.2 ± 2.1	>50 ^c	27 ± 0.90	25 ± 3.3	40 ± 5.0	>25 ^d	>50 ^c

^aNo inhibition was observed at 10 μM.

^bND, not determined due to lack of inhibition of CYP2A6 at 10 μM.

^cNo inhibition was observed at 50 μM.

^dNo inhibition was observed at 25 μM.

Table 2.IC₅₀ ratios for major hepatic CYP enzymes versus CYP2A6^a.

Compd	CYP isoform							
	3A4	2D6	2C9	2C19	1A2	2B6	2C8	2E1
4a	25	38	197	47	34	138	42	120
4b	ND ^b	ND	ND	ND	ND	ND	ND	ND
4c	ND	ND	ND	ND	ND	ND	ND	ND
4d	ND	ND	ND	ND	ND	ND	ND	ND
4e	>9.1 ^c	>9.1 ^c	>9.1 ^c	2.4	1.1	2.2	>9.1 ^c	5.3
4f	ND	ND	ND	ND	ND	ND	ND	ND
4g	108	44	655	145	200	364	156	345
4h	ND	ND	ND	ND	ND	ND	ND	ND
4i	>60 ^c	8.0	>60 ^c	27	>60 ^c	23	30	18
4j	>15 ^c	>15 ^c	8.2	2.7	7.4	3.2	>15 ^c	5.3
4k	>114 ^c	6.4	73	25	>114 ^c	9.3	45	73
4l	300	39	23	92	62	23	108	115
4m	2.0	7.0	5.0	6.6	2.5	8.9	13	3.4
4n	9.6	6.9	9.4	4.6	3.0	5.2	31	6.3
4o	>9.7 ^c	3.3	>9.7 ^c	>9.7 ^c	5.8	>9.7 ^c	>19 ^c	>9.7 ^c
4p	156	24	81	53	53	617	>42 ^c	76
4q	>9.3 ^c	>9.3 ^c	>9.3 ^c	>9.3 ^c	>9.3 ^c	>9.3 ^c	>19 ^c	>9.3 ^c
5g	74	54	92	87	5.9	15	203	31
5i	3549	59	1157	5.1	39	25	1431	18
5j	>25 ^c	12	>50 ^c	4.1	1.3	3.9	16	5.9
5k	35	12	136	14	2.6	1.9	30	14
5l	13	2.1	6.5	11	0.73	0.45	13	18
5m	100	10	6.9	7.6	1.0	0.41	19	32
5n	218	33	44	31	13	2.1	>227 ^c	12
5o	130	22	>167 ^c	40	26	43	>83 ^c	>167 ^c
5p	237	32	74	34	12	21	>66 ^c	82
5q	>19 ^c	>19 ^c	>19 ^c	>19 ^c	5.8	>19 ^c	>38 ^c	>38 ^c
6g	168	91	127	123	17	3.9	364	9.1
6i	765	265	941	49	235	5.5	88	14
6j	>45 ^c	18	>90 ^c	1.7	2.5	8.4	45	8.2
6k	102	64	202	26	4.5	2.1	52	36
6l	13	1.9	7.1	11	.046	0.15	2.7	17
6m	44	>312 ^c	28	8.8	1.3	0.46	24	19

Compd	CYP isoform							
	3A4	2D6	2C9	2C19	1A2	2B6	2C8	2E1
6n	68	>658 ^c	80	11	3.6	0.82	63	72
6o	648	36	130	33	15	11	>76 ^c	94
6p	91	35	50	20	3.5	2.6	6.5	80
6q	33	6.8	42	23	21	33	>21 ^c	>42 ^c

^aValues = (IC₅₀ CYP_{hepatic} CYP)/(IC₅₀ CYP2A6).

^bND, not determined due to lack of potency against CYP2A6 at 10 μM.

^cThe ratio is greater than the number indicated because the inhibitor exhibited an IC₅₀ value higher than the maximum concentration tested for the selected CYP.

Table 3.

Metabolic half-lives in human live microsomes and physiochemical properties of druglikeness (Rule of Five parameters) of nicotine analogues.

Compd	T _{1/2} (min) ^a	cLogP ^b	MW	tPSA ^b	# of H-bond Donors	# of H-Bond Acceptors
4a	>120	-0.443	132.17	38.38	1	2
4g	8.4	0.056	146.19	38.38	1	2
4i	9.8	0.585	160.22	38.38	1	2
4k	39	1.114	174.25	38.38	1	2
4l	48	1.445	208.26	38.38	1	2
4o	36	0.056	209.25	50.74	1	3
4p	46	0.056	209.25	50.74	1	3
4q	>120	-0.898	210.24	63.10	1	4
5g	21	1.070	188.23	47.61	1	3
5i	>120	1.599	202.26	47.61	1	3
5n	39	1.335	240.26	56.84	1	4
5o	>120	0.770	251.29	59.97	1	4
5p	>120	0.770	251.29	59.97	1	4
5q	>120	-0.184	252.28	72.33	1	5
6g	11	1.579	204.29	38.38	1	2
6i	40	2.108	218.32	38.38	1	2
6o	32	1.271	267.35	50.74	1	3
6q	>120	0.314	268.34	63.10	1	4

^aCompounds (10 µM) were incubated with human liver microsomes (12.5 µg of protein) for up to 2 h, and the half-life was calculated as the time when half the peak area under the UPLC/MS curve remained. The experimental details are described in the Experimental Section.

^bCalculated with Chemdraw Professional version 15.1.0.144

Table 4.

Lead compounds for future development as smoking cessation agents.

Compd	Structure	CYP2A6 IC ₅₀ (μM)	Selectivity Criteria ^a	Half-life (min)
4k		0.44	Pass	39
4l		0.13	Pass	48
4o		3.6	2D6 ^b	36
4p		0.59	Pass	46
4q		2.7	Pass	>120
5i		0.051	Pass	>120
5n		0.11	2B6 ^c	39

Compd	Structure	CYP2A6 IC ₅₀ (μM)	Selectivity Criteria ^a	Half-life (min)
5o		0.30	Pass	>120
5p		0.38	Pass	>120
5q		1.3	Pass	>120
6i		0.017	Pass	40
6o		0.33	Pass	32
6q		1.2	Pass	>120

^aPass, all compounds have a selectivity ratio of >5 for inhibition of CYP2A6 versus the other CYPs tested.

^b2D6 = The selectivity ratio was <5 for inhibition of CYP2A6 versus CYP2D6.

^c2B6 = The selectivity ratio was <5 for inhibition of CYP2A6 versus CYP2B6.

Table 5.

Probe substrates, concentrations and reference inhibitors used in CYP450 assays.

Enzyme	Probe Substrate	Substrate Concentration (μM)	Metabolite	Positive Control Inhibitor
CYP1A2	Phenacetin	10	Acetaminophen	Furafylline
CYP2A6	Coumarin	2.5	7-hydroxycoumarin	Tranilcypromine
CYP2B6	Bupropion	100	Hydroxybupropion	Clopidogrel
CYP2C8	Amodiaquine	2	Desethylamodiaquine	Montelukast
CYP2C9	Diclofenac	10	4'-hydroxydiclofenac	Sulfaphenazole
CYP2C19	Omeprazole	1.0	5-hydroxyomeprazole	Tranilcypromine
CYP2D6	Dextromethorphan	5	Dextrophan	Quinidine
CYP2E1	Chlorzoxazone	100	6-hydroxychlorzoxazone	Chlomethiazole
CYP3A4	Midazolam	5	1-hydroxymidazolam	Ketoconazole

Table 6.

Mass spectrometry parameters for CYP substrates.

Enzyme	Probe Substrate Metabolite	MRM Transition	Cone voltage	Collision voltage	Mode
CYP1A2	Acetaminophen	152>110	30	20	positive
CYP2A6	Hydroxycoumarin	161>133	30	15	positive
CYP2B6	Hydroxybupropion	256>139	25	30	positive
CYP2C8	Desethylamodiaquine	328>283	30	35	positive
CYP2C9	Hydroxydiclofenac	312>266	30	20	positive
CYP2C19	Hydroxyomeprazole	362>214	30	20	positive
CYP2D6	Dextrophan	258>199	30	20	positive
CYP2E1	Hydroxychlorzoxazone	184>120	30	20	negative
CYP3A4	Hydroxymidazolam	342>324	30	20	positive