Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

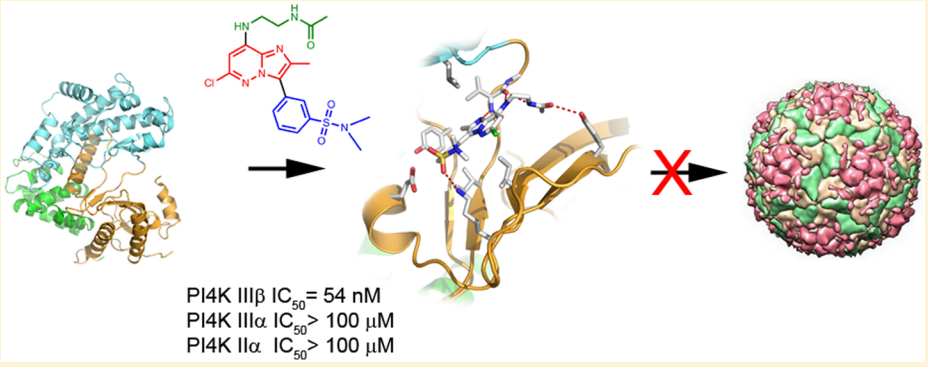
Highly Selective Phosphatidylinositol 4-Kinase IIIβ Inhibitors and Structural Insight into Their Mode of Action

Ivana Mejdrová,[†,§] Dominika Chalupská,[†,§] Martin Kögler,[†,§] Michal Šála,[†] Pavla Plačková,[†] Adriana Baumlová,[†] Hubert Hřebabecký,[†] Eliška Procházková,[†] Milan Dejmek,[†] Rémi Guillon,[†] Dmytro Strunin,[†] Jan Weber,[†] Gary Lee,[‡] Gabriel Birkus,[‡] Helena Mertlíková-Kaiserová,[†] Evzen Boura,*[,†] and Radim Nencka*[,†]

[†]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Gilead Sciences & IOCB Research Centre, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

[‡]Gilead Sciences, Inc., 333 Lakeside Drive, Foster City, California 94404, United States

Supporting Information



ABSTRACT: Phosphatidylinositol 4-kinase IIIβ is a cellular lipid kinase pivotal to pathogenesis of various RNA viruses. These viruses hijack the enzyme in order to modify the structure of intracellular membranes and use them for the construction of functional replication machinery. Selective inhibitors of this enzyme are potential broad-spectrum antiviral agents, as inhibition of this enzyme results in the arrest of replication of PI4K IIIβ-dependent viruses. Herein, we report a detailed study of novel selective inhibitors of PI4K IIIβ, which exert antiviral activity against a panel of single-stranded positive-sense RNA viruses. Our crystallographic data show that the inhibitors occupy the binding site for the adenine ring of the ATP molecule and therefore prevent the phosphorylation reaction.

INTRODUCTION

Phosphatidylinositol 4-phosphate (PI4P) is the most abundant monophosphoinositide in eukaryotic cell. It decorates the Golgi complex and trans-Golgi networks (TGN) and also has a role at the plasma membrane and endosomes.[1] In addition, PI4P is the precursor for the synthesis of other phosphoinositides, most notably phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), the hallmark of the plasma membrane, and phosphatidylinositol 3,4,5-trisphosphate ($PI(3,4,5)P_3$), a key secondary messenger molecule. The synthesis of PI4P is carried out by phosphatidylinositol 4-kinases (PI4Ks).[2,3] Mammalian genomes contain two types of PI4Ks, type II (PI4K IIα and PI4K IIβ) and type III (PI4K IIIα and PI4K IIIβ) kinases that share homology with phosphatidylinositol 3-kinases (PI3Ks). Recently, crystal structures were solved for PI4K IIα[4,5] and for PI4K IIIβ.[6]

Type II PI4Ks are palmitoylated enzymes stably associated with the membrane. They are present at various endomembranes such as the TGN, early and late endosomes, as well as at the plasma membrane.[7,8] These enzymes are critical to intracellular trafficking[9,10] and interact with clathrin adaptor complexes (PI4K IIα with AP-3 and PI4K IIβ with AP1).[11,12] In contrast, type III kinases are soluble cytoplasmic proteins that are recruited to the membrane via protein−protein interactions. Efr, YPP1/TTC7, and TMEM150 direct the PI4K IIIα recruitment to the plasma membrane, where it is responsible for PI4P synthesis.[13,14] The Golgi resident proteins ACBD3 and GBF1/Arf recruit PI4K IIIβ to the Golgi,[15,16] where this enzyme is mostly responsible for the biogenesis of the Golgi pool of PI4P although type II PI4Ks also contribute to this pool.[17,18] The type III PI4Ks are essential host factors for a plethora of single-stranded positive-sense (ss(+)RNA) viruses[19] and are potential drug targets.

ss(+)RNA viruses hijack PI4Ks to produce membranous organelles (referred to as "membranous webs" or "replication

Received: December 15, 2014
Published: April 21, 2015

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

factories") highly enriched in PI4P, which facilitate the assembly of functional viral replication machinery. For example, the hepatitis C virus (HCV) hijacks both PI4K IIIα[20] and PI4K IIIβ,[21,22] whereas various members of the *Picornaviridae* family, including coxsackieviruses and rhinoviruses,[23−26] and those of the *Coronaviridae* family,[27] were shown to depend on PI4K IIIβ. In spite of the broad dependence on PI4Ks, several different molecular mechanisms of hijacking have recently been described. The NS5A protein of HCV directly binds to PI4K IIIα and stimulates its activity,[28] whereas the 3A protein of the Aichi virus hijacks PI4K IIIβ through interaction with its binding partner, the ACBD3 protein.[15] The recruitment of PI4K IIIβ to Coxsackievirus B3 replication factories occurs through an unknown mechanism that is independent of ACBD3.[29] PI4Ks are therefore potential drug targets as essential host factors for the replication of ss(+)RNA viruses. A possible impediment to these strategies, however, is that genetic inactivation of type III PI4Ks is detrimental for animals.[30,31] A window of possibility relies on the fact that RNA viruses require every bit of the cell's PI4K activity (and even up regulate PI4Ks) for replication, whereas partially compromised PI4K activity may be well tolerated in animals, especially if only for a short time period.[19] To clarify whether a therapeutic window exists for PI4K enzyme inhibition, potent and selective inhibitors of the PI4K enzymes are needed.

Although several potent inhibitors of PI4K type III enzymes have been discovered recently, most of them targeted PI4K IIIα.[32,33] The studies focused on PI4K IIIβ suffer from a lack of detailed structure−activity relationship (SAR) and structural data, which would allow for a better understanding of the mode of action of these compounds.[34−36]

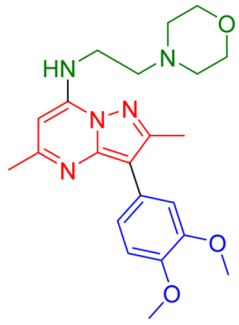
We present an extensive study on a broad series of novel PI4K IIIβ inhibitors. We show that these compounds are highly selective based on biochemical characterization. We also assess the effects of these inhibitors against selected viruses in cell-based assays. To gain detailed knowledge of the molecular details of their inhibitory mechanisms, we solved the crystal structures of PI4K IIIβ enzyme bound to an archetypical inhibitor and to ATP. These structures revealed the mechanism of PI4K IIIβ enzyme inhibition by these compounds at the atomic level.

RESULTS

Chemistry. We prepared a series of 65 novel compounds based on the screening hit **2a** (T-00127-HEV1) discovered by Arita et al.[24] We sequentially modified all three parts of the molecule: the amine side chain 1 (Figure 1, in green), bicyclic central core (Figure 1, in red), and aromatic side chain 2 (Figure 1, in blue).

First, we focused on the effect of diverse modifications of the side chain 1 using the original pyrazolo[1,5-*a*]pyrimidine central core. Most of the derivatives were prepared starting from the compound **1**[37] by simple nucleophilic substitution (DIPEA, EtOH, 75 °C) of the chlorine atom at position 7 of the bicyclic central core (Scheme 1), which proceeds smoothly with high to excellent yields. An additional series of the inhibitors was prepared by derivatization of the ethyl-enediamino chain with various substituents (Scheme 2, compounds **3**−**9**). Analogous derivatives **10** and **11** were obtained by acetylation from appropriate starting materials **2f** and **2k**, respectively.

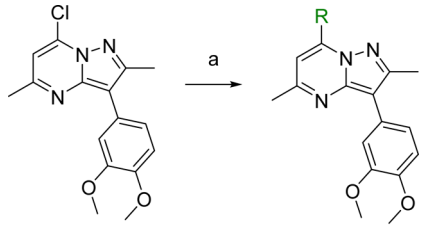
We also prepared compounds with sulfur and oxygen atoms instead of the NH-group at position 7 in order to investigate



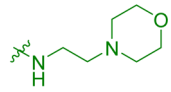
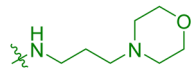
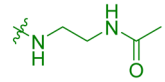
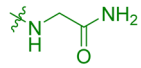
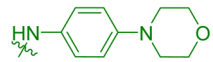
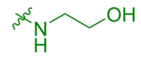
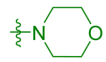
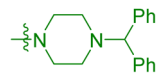
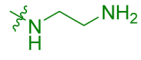
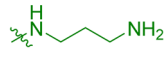
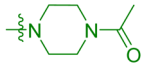
2a (T-00127-HEV1)

Figure 1. Screening hit as a model compound for SAR study.

Scheme 1. Synthesis of C7-Modified Pyrazolopyrimidines[a]



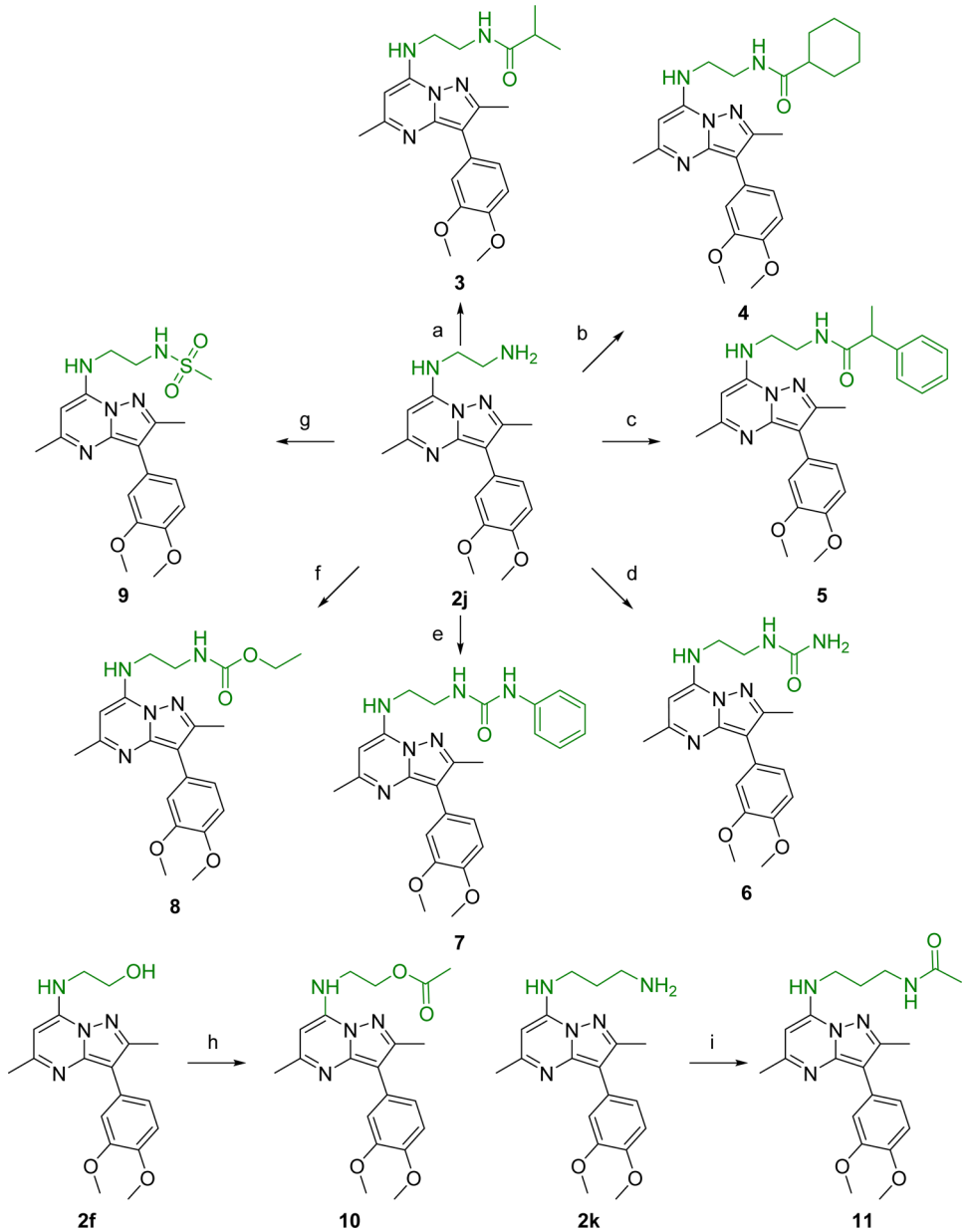
1 **2a-l**

Compnd	R	Yield
2a		89%
2b		80%
2c		87%
2d		82%
2e		65%
2f		88%
2g		75%
2h		77%
2i	NH_2	74%
2j		91%
2k		92%
2l		90%

[a]Reagents and conditions: (a) amine, EtOH, heating.

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

Scheme 2. Preparation of Various *N*-Modified Ethylenediamino Derivatives 3−11[a]



[a]Reagents and conditions: (a) isobutyric acid, DCC, CH_2Cl_2, 0 °C to rt, 20 h, 74%; (b) cyclohexanecarbonyl chloride, Et_3N, CH_2Cl_2, 0 °C, 2 h, 72%; (c) 2-phenylpropionic acid, DCC, CH_2Cl_2, 0 °C to rt, 20 h, 81%; (d) KCNO, aq HCl·H_2O, MeOH, 50 °C, 40 h, 75%; (e) phenyl isocyanate, CH_2Cl_2, 24 h, 89%; (f) ClCOOEt, Et_3N, CH_2Cl_2, 0 °C, 1.5 h, 61%; (g) methanesulfonyl chloride, Et_3N, CH_2Cl_2, 0 °C, 5 h and rt 4 h, 83%; (h) Ac_2O, Et_3N, CH_2Cl_2, 0 °C, 20 h, 71%; (i) Ac_2O, aq $NaHCO_3$, $CHCl_3$, 0 °C, 80%.

the influence of putative hydrogen bond with the protein. The thio derivatives were prepared either by reaction with thiourea and subsequent alkylation with chloroethyl morpholine to obtain analogue **13** or by direct nucleophilic substitution using appropriate thiophenol yielding derivative **14**. The oxygen analogue **15** of the parent compound **2a** was prepared also by a nucleophilic substitution under basic conditions (NaH/DMF) (Scheme 3).

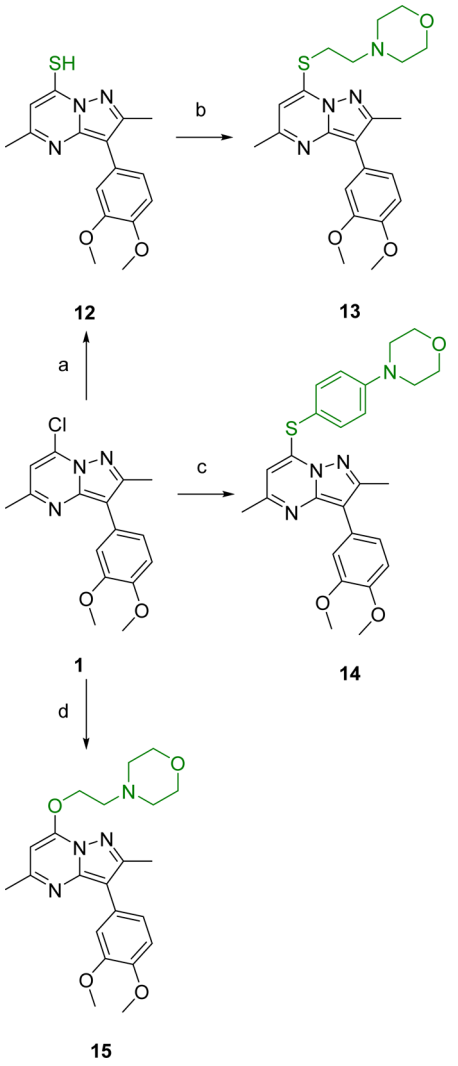
Second, we turned our attention to the modification of the central bicyclic core. We prepared analogues of the parent compound with imidazo[1,2-*a*]pyridine (**16**) (Scheme 4), imidazo[1,2-*b*]pyridazine (**17**) (Scheme 5), and pyrazolo[1,5-*a*][1,3,5]triazine (**18**) central core (Scheme 7).

The synthesis of imidazo[1,2-*a*]pyridine derivative started from compound **19**,[38] which was easily iodinated using NIS (Scheme 4). Suzuki cross-coupling reaction followed by a microwave-assisted Hartwig−Buchwaldg reaction with 4-(2-aminoethyl)morpholine yielded the desired analogue **16**. However, this compound is surprisingly unstable and decomposes quite rapidly.

The preparation of the compound **17** started from dichloro derivative **22**.[39] Because the reactivity of the chlorine atoms differs significantly, treatment with 4-(2-aminoethyl)-morpholine afforded the desired monosubstituted product **23** in high yield. This compound was utilized for subsequent preparation of the methylated derivative **24** (Scheme 5). A number of diverse methylation reactions failed to give this

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

Scheme 3. Synthesis of C7-Modified Pyrazolopyrimidines[a]



[a]Reagents and conditions: (a) thiourea, EtOH, reflux, 15 h, 85%; (b) 4-(2-chloroethyl)-morpholine hydrochloride, K_2CO_3, DMF, 77%; (c) 4-(morpholin-4-yl)benzenethiol, K_2CO_3, DMF, 43%; (d) 4-(2-hydroxyethyl)morpholine, NaH, DMF, 58%.

derivative. Finally, the transfer of a methyl group was accomplished by reacting compound **23** with DABAL-Me_3 (a reactive complex between one molecule of DABCO and two molecules of $AlMe_3$)[40] under Pd-catalyzed cross-coupling conditions. This way, key intermediate **24** was prepared in moderate 42% yield. Iodination and Suzuki coupling with 3,4-dimethoxyphenyl boronic acid afforded title compound **17** (Scheme 5).

Because compound **17** exerted interesting inhibitory activity in the PI4K IIIβ enzymatic assay, the preparation of analogues with modified substituents at position 6 was the logical further step. The derivative **23** served as a suitable precursor for the preparation of analogues bearing either a hydrogen or chlorine atom instead of the methyl group at this position (Scheme 5). The synthesis of the former derivative (**28**) was accomplished by simple hydrogenolysis followed by iodination and Suzuki reaction. The latter compound (**30a**) was obtained in the similar fashion without the hydrogenolysis step. In this case, we prepared also several other related derivatives with this structural pattern summarized in Scheme 6 using intermediate **29** subdued to Suzuki cross-coupling reactions.

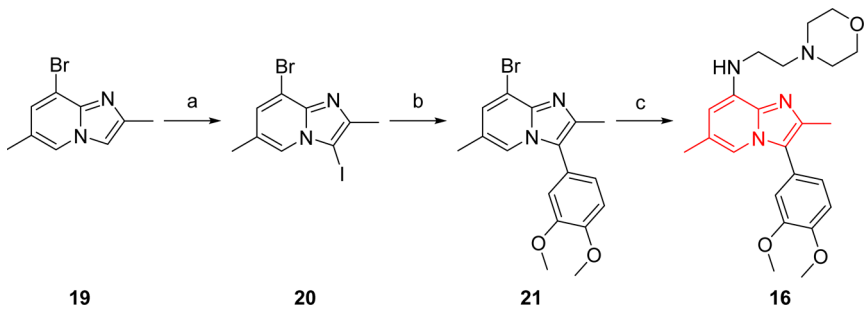
Derivative **32** with the last bicyclic core, pyrazolo[1,5-*a*][1,3,5]triazine, was obtained starting from bromide **31**[41] by cross-coupling reaction with 3,4-dimethoxyphenylboronic acid. Subsequent chlorination followed by immediate substitution of the chlorine atom with the appropriate amine afforded the desired analogue of **2a**, compound **18**, and derivative **33** (Scheme 7).

On the basis of our preliminary results from PI4K IIIβ assay, with respect to synthetic feasibility and potential metabolic decomposition, we selected 6-chloro-2-methylimidazo[1,2-*b*]-pyridazine as the most suitable central core and [2-(acetylamino)ethyl]amino group as side chain 1 for further optimization of the aromatic side chain 2. The key intermediate **35** for this study was prepared by iodination with NIS, giving compound **34** (Scheme 5), followed by selective nucleophilic substitution of the chlorine at position 8 with 2-amino-ethylacetamide.

Majority of compounds form the last series was prepared by Suzuki cross-coupling reaction starting from the compound **35** (Scheme 8).

Another subseries of derivatives modified in the aromatic side chain was prepared by derivatization of suitable precursors obtained by Suzuki couplings. First, we modified the amino group of the compound **36u**. In particular, we obtained a few acylated derivatives (**37**–**39**) either by reaction with acyl anhydrides or chlorides (Scheme 9). Subsequently, reaction with isopropylisocyanate afforded the substituted urea derivative **40** and the compounds **41** and **42** were prepared by reductive amination and alkylation of compound **36u**, respectively (Scheme 10).

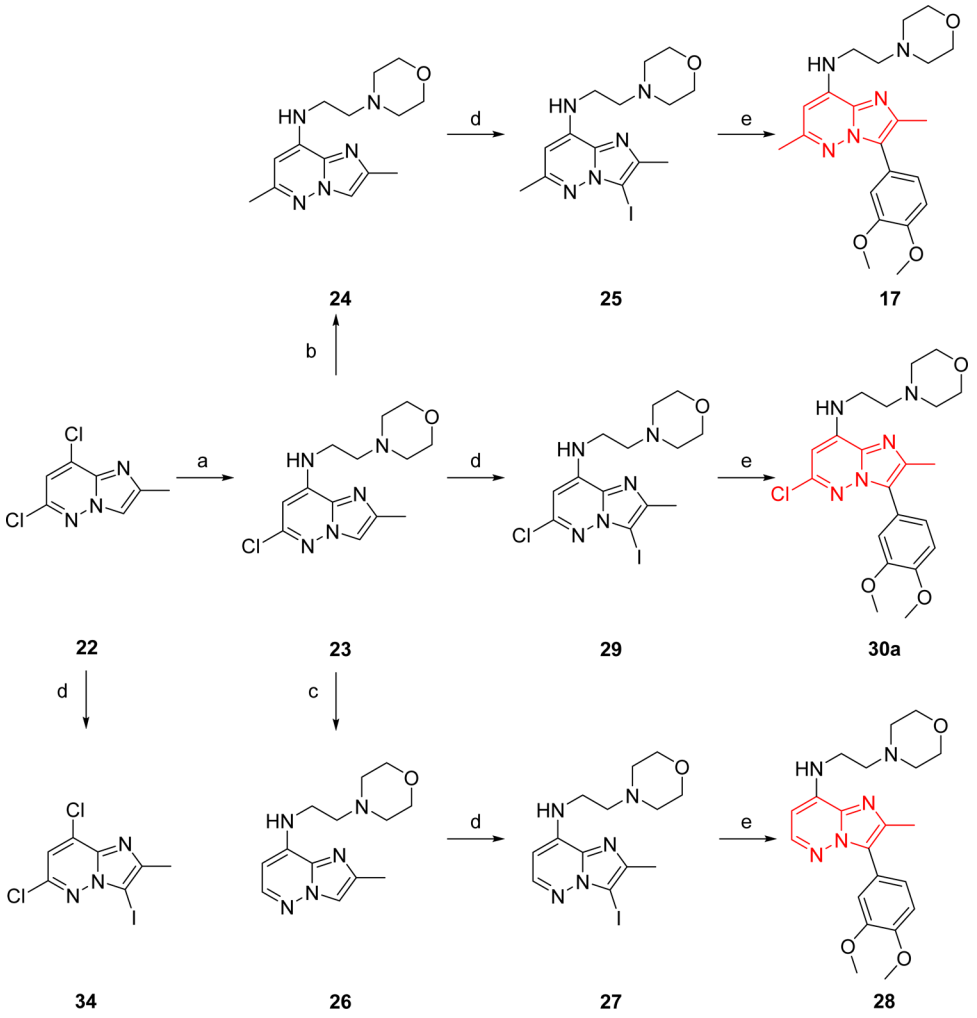
Scheme 4. Preparation of Bicyclic Analogue Imidazo[1,2-*a*]pyridine 16[a]



[a]Reagents and conditions: (a) NIS, DMF, rt, 30 min, yield 89%; (b) 3,4-dimethoxy-phenylboronic acid, Pd(dppf)Cl_2, Na_2CO_3, dioxane/H_2O (4:1), 16 h, 60%; (c) 4-(2-aminoethyl)morpholine, $Pd_2(dba)_3$, MeDalPhos, *t*-ButONa, dioxane, MW, 30 min, 150 °C, 72%.

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767–3793

Scheme 5[a]



[a]Reagents and conditions: (a) 4-(2-aminoethyl)morpholine, DIPEA, CH_3CN, 80 °C, sealed vessel, 16 h, 87%; (b) DABAL-Me_3, $Pd_2(dba)_3$, X-Phos, THF, rt to 80 °C, overnight, 42%; (c) H_2, Pd/C, THF:MeOH, rt, 21 h; (d) NIS, AcOH/CH_2Cl_2 or DMF, (49% for **25**, 41% for **27**, 41% for **29**, 95% for **34**); (e) 3,4-dimethoxyphenylboronic acid, $Pd(PPh_3)_4$, 1 M K_2CO_3, dioxane, 95 °C, overnight (60% for **17**, 83% for **28**, 77% for **30a**).

We also prepared 3-(3,4-dihydroxyphenyl) derivative **43** by the demethylation of **30a**, albeit in very low yield (Scheme 11). Reduction of the formyl functions in compounds **36e** and **36i** with $NaBH_4$ gave the corresponding hydroxymethyl derivatives **44** and **45**, respectively, in moderate yields.

To introduce highly polar group, the cyano moiety in compound **36h** was successfully reduced to the primary amine **46**, which was isolated as its hydrochloride salt.

The analogue of compounds **36a** with hydrogen instead of the chlorine atom at the position 6 of the imidazo[1,2-*b*]pyridazine core (**47**) was prepared by simple hydrogenolysis of **36a** (Scheme 12).

Finally, the derivative **49** was obtained by ammonolysis of compound **34** and subsequent Suzuki coupling as described in Scheme 12.

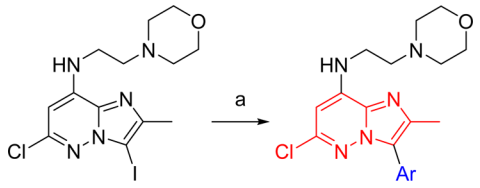
Novel PI4K III*β* Inhibitors and Their Antiviral Activity. To characterize the potency of this series of inhibitors in vitro, we used the luminescent ADP-Glo kinase assay.[42] For the presentation of the biological activities of the whole series, we have selected 10 compounds which exerted the highest inhibitory activities against PI4K III*β* in vitro (Figure 2).

We found that the IC_{50} values for the top 10 compounds were in the range of 50−130 nM against PI4K III*β*. Further, we determined whether the compounds were isoform specific for PI4K III*β*, a necessity for evaluating therapeutic potential. We have thus used the ADP-Glo kinase assay to measure the IC_{50} values for two structurally and functionally related enzymes, PI4K III*α* and PI4K II*α*. We found that none of the top 10 compounds inhibited PI4K III*α* and PI4K II*α* with an IC_{50} less than 10 *μ*M (These data are summarized in Table 1 for the top 10 compounds, and in Supplementary Results, Supporting Information, Table 2, for the remaining 55).

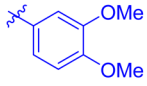
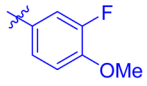
Next, we focused on determining the antiviral activity of the compounds. For this, we used an assay based on the virus-induced cytopathic effect in HeLa cells (in detail in Experimental Methods). We tested the activity of the compounds against two important members of the *Picornaviridae* family, Coxsackievirus B3 (CVB3), and human rhinovirus (HRV) and two distinct genotypes of HCV (*Flaviviridae*), genotype 1b and 2a. For each virus tested except for HCV 2a several of the compounds exhibited IC_{50} values below 1 *μ*M as summarized in Table 2 and Supporting Information, Table 3.

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

Scheme 6. Suzuki Cross-Coupling Reaction of Morpholino Ethyl Derivative 29[a]

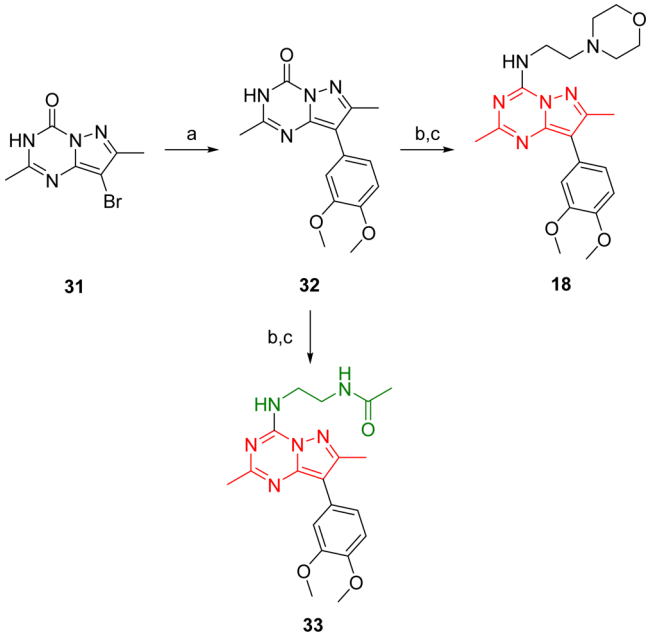


29 30a-c

Compd	Ar	Yield
30a		77%
30b		76%
30c		56%

[a]Reagents and conditions: (a) Ar-B(OH)$_2$, Pd(PPh$_3$)$_4$, 1 M K$_2$CO$_3$, dioxane, heating.

Scheme 7. Synthesis of Pyrazolo[1,5-*a*][1,3,5]triazine Derivatives[a]



[a]Reagents and conditions: (a) 3,4-dimethoxyphenylboronic acid, Pd(dppf)Cl$_2$, Na$_2$CO$_3$, dioxane/H$_2$O (4:1), 16 h, 60%; (b) POCl$_3$, dimethylaniline, 120 °C, 20 h; (c) amine, DCM, 0 °C−rt, 16 h (yields over two steps: 68% for **18**, 65% for **33**).

Although the correlation between the results from the enzymatic assay and the cell-based assay was not very tight, there was a clear rise in antiviral potency as PI4K IIIβ inhibitory activity increased. The sensitivity of various viruses to the inhibition of the PI4K IIIβ showed significant differences. The HCV genotype 1b displayed some sensitivity, whereas the effects of PI4K IIIβ inhibition on replication of HCV 2a were rather limited. The most potent compound in the series was derivative **36t**, which inhibited PI4K IIIβ with IC$_{50}$ = 54 nM and exerted significant effect in all cell-based assays. Derivative **36t** was the most active compound in HVC 2a assay (EC$_{50}$ = 10.6 μM) and the second most active against HCV1b (EC$_{50}$ = 0.087 μM). Furthermore, this compound showed strong protection against CVB3-induced cytopathic effects in HeLa cells with half-maximal effective concentration (EC$_{50}$) value of 145 nM. This compound was also evaluated in a panel of phosphatidylinositol 3-kinases (PI3Kα, PI3Kβ, PI3Kδ, and PI3Kγ). None of these kinases was inhibited with IC$_{50}$ up to 10 μM.

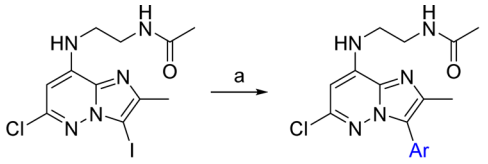
Structural Analysis of the Inhibition Mode of PI4K IIIβ. To determine how the inhibitors function at the atomic level, we solved the crystal structure of PI4K IIIβ either with an inhibitor or bound to ATP. We selected several inhibitors of archetypical chemical structure and reasonable solubility for crystallographic trials. The best crystals were obtained with inhibitor **49** but still diffracted only to 3.5 Å resolution. The structure was solved by molecular replacement using chain A of the crystal structure of PI4K IIIβ with Rab11 (PDB code: ID0L) without the PIK93 inhibitor to reduce bias. Because of the rather low resolution the unbiased $F_o - F_c$ map contoured at 3σ did not provide enough information to unambiguously place the **49** inhibitor (Supporting Information, Figure 3). However, the density for the **49** inhibitor was clearly visible in the $F_o - F_c$ map contoured at 2σ and allowed for unambiguous placement of the **49** inhibitor (Figure 3a). We have refined the structure to R = 20.89% and R_{free} = 25.16%. To confirm that the result of manual docking and refinement in Coot[43] is correct, we calculated the R factors for a structure where the **49** inhibitor is rotated by 180° and refined into the $F_o - F_c$ density. The obtained values (R = 21.19% and R_{free} = 26.07%) are significantly worse and argue for correct placement of the ligand.

Crystals with ATP bound diffracted to 3.3 Å resolution, and the structure was solved in the same way as the structure with **49** inhibitor and refined to R = 18.25% and R_{free} = 24.41%. However, we did not observe density for the whole ATP molecule, only for the adenine ring (Figure 3b), despite the better resolution. We, therefore, concluded that the ribose ring and the three phosphate groups are disordered in our structure. The density for adenine at 3.3 Å, in principle, allows positioning the adenine ring in multiple orientations. To confirm that the best fit obtained in Coot[43] was the right solution, we compared the structure of PI4K IIIβ with the structure of PI4K IIα, where the ATP molecule is clearly resolved.[4] We have used the C-lobes of PI4K IIα and PI4K IIIβ for superposition, taking advantage of the fact that they are structurally very similar. The superposition revealed that the adenine rings in both structures are in a similar orientation (Supplementary Figure 2), supporting the placement of the adenine ring in the presented structure.

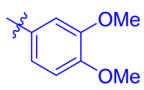
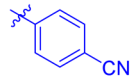
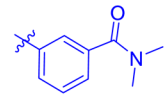
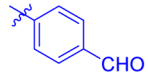
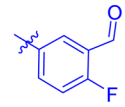
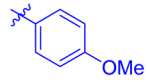
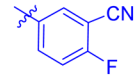
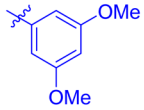
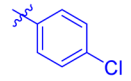
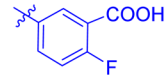
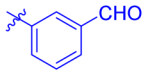
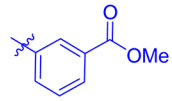
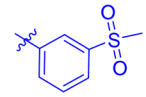
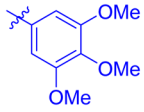
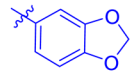
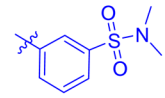
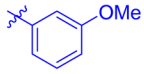
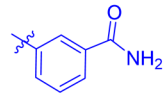
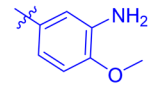
Both **49** and the adenine ring lie in a binding canyon of PI4K IIIβ held in place by a combination of hydrophobic interactions and hydrogen bonds, water bridges surely also contribute (for instance, the hydroxyl group of Tyr^{598} and the N6 of the adenine ring are in perfect distance and orientation for a water bridge), but water molecules were not modeled due to limited resolution of our crystal structures (Figure 4a,b). Superposition of the two structures revealed that compound **49** and the adenine ring occupy the same pocket (Figure 4c), suggesting that this family of inhibitors function by sterically blocking the

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

Scheme 8. Aryl Derivatives Prepared by Suzuki Coupling Reaction[a]



35 36a-u

Compd	Ar	Yield	Compd	Ar	Yield	Compd	Ar	Yield
36a		97%	**36h**		92%	**36o**		78%
36b		85%	**36i**		91%	**36p**		55%
36c		60%	**36j**		77%	**36q**		73%
36d		32%	**36k**		40%	**36r**		29%
36e		82%	**36l**		40%	**36s**		57%
36f		96%	**36m**		40%	**36t**		65%
36g		96%	**36n**		78%	**36u**[b]		80%

[a]Reagents and conditions: (a) Ar−B(OH)$_2$, Pd(PPh$_3$)$_4$, K$_2$CO$_3$, dioxane/H$_2$O (4:1), 95 °C, 16 h. [b]Pinacol ester of boronic acid was used as reagent.

binding of the ATP's adenine ring rather than the phosphate moiety and therefore interfering with the course of the reaction.

The *N*-lobe is colored in orange, the C-lobe in cyan, and the helical domain and the $F_o - F_c$ map in green. In the detailed view, carbon atoms are colored silver, nitrogen in blue, chlorine in green, and oxygen in red.

Docking Study of the Inhibitors. We performed docking studies with selected inhibitors to determine putative parts of the inhibitor that could be further modified to enhance the affinity to the enzyme and to explain the results of our structure−activity relationship (SAR) study at the atomic level. The results of our docking study largely explain the outcomes of the SAR study.The conformation of **36t** with the lowest predicted energy fits into the enzyme's ATP binding site and forms three hydrogen bonds with Val^{613} in the hinge region (Figure 4d). The amide oxygen of this residue forms two hydrogen bonds with both nitrogen moieties of side chain 1. The hydrogen atom bound to amide nitrogen of Val^{613} interacts with nitrogen atom of the inhibitor's central bicyclic core, which facilitates effective interaction with the enzyme (Figure 4d). Two additional hydrogen bonds with residues Tyr^{400} and Lys^{564} are responsible for the effect of the sulfonamide moiety (the aromatic side chain) and importance of the amide oxygen atom (the amine side chain), respectively.

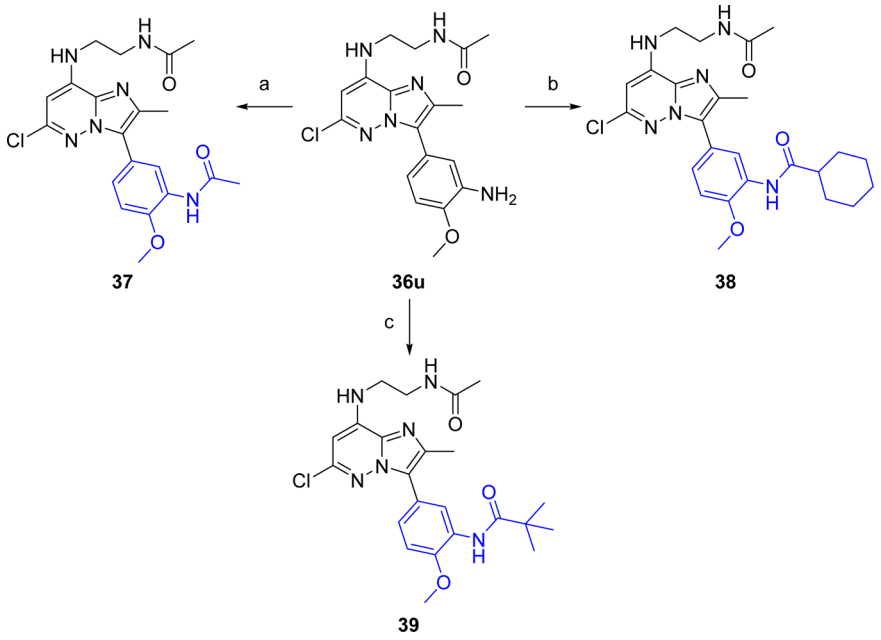
DISCUSSION

Selective inhibitors of PI4K IIIβ are critically needed as PI4K IIIβ is a promising target for development of broad-spectrum antiviral agents for treatment of diseases caused by viral pathogens such as SARS, MERS, HCV, and HRV. Furthermore, effective inhibitors of this class of enzymes can serve as much-needed tools to understand various processes connected with membrane signaling and mechanisms of membrane trafficking in numerous cellular structures. Therefore, such compounds can revolutionize the treatment of viral diseases and sharpen our knowledge of cell biology of organelles.

We show that substitution of all three moieties (amine side chains, bicyclic central core, and aromatic side chain) on the parent molecule has significant impact on the activity of this family of inhibitors (Figure 5). Our study suggests that for substitutions the most suitable side chains at position 1 (the amino side chain) are the 2-aminoethyl acetate and the 2-aminoethylacetamide groups. Because the ester substituent is most likely metabolically unstable, we focused our efforts on

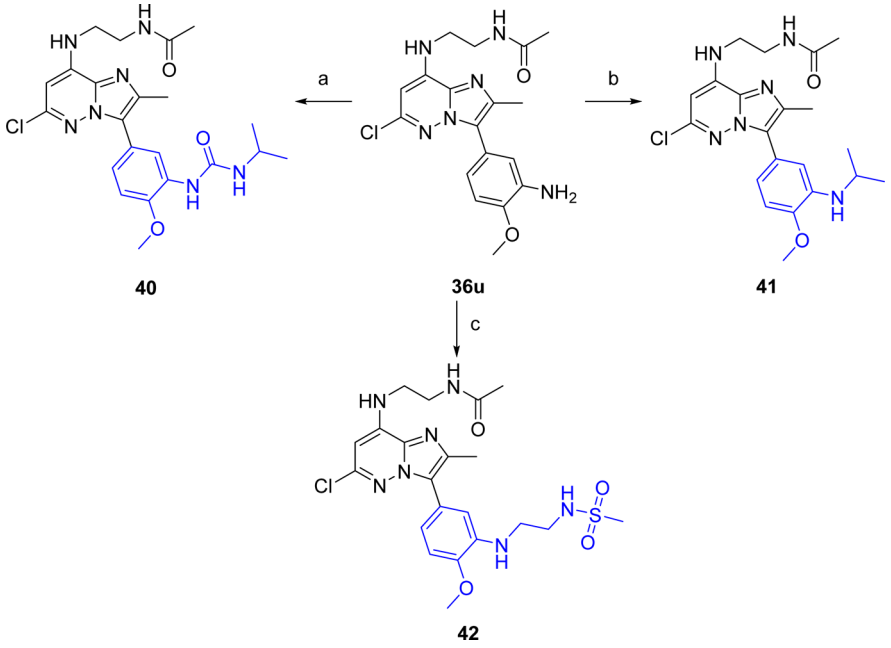
DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

Scheme 9. Acylated Derivatives Preparation[a]



[a]Reagents and conditions: (a) Ac_2O, DIPEA, CH_2Cl_2, 0 °C, 92%; (b) cyclohexanoyl chloride, DIPEA, CH_2Cl_2, 0 °C, 63%; (c) pivaloyl chloride, DIPEA, CH_2Cl_2, 0 °C, 48%.

Scheme 10. Preparation of Derivatives with Substituted Aromatic Part of the Molecule[a]

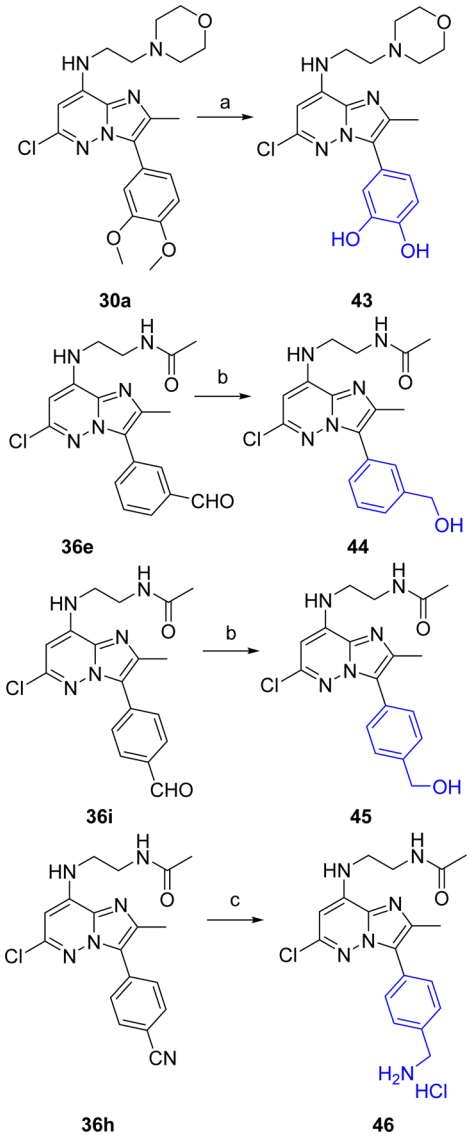


[a]Reagents and conditions: (a) acetone, $NaBH_3CN$, MeOH, 76%; (b) isopropylisocyanate, THF, 6 h, reflux, 86%; (c) *N*-(2-chloroethyl)-methanesulfonamide, K_2CO_3, toluene, 90 °C, 45%.

the amide and the central core of the molecule. These optimizations revealed two important factors: First, simple scaffold hopping significantly influences the activity, thus the derivatives with imidazo[1,2-*b*]pyridazine exert higher potency than the members of pyrazolo[1,5-*a*]pyrimidine series, whereas the compounds with other central cores possess lower affinity toward PI4K III*β*. Second, small variations of the substituent at position 6 of both imidazo[1,2-*b*]pyridazine and pyrazolo[1,5-*a*]pyrimidine core do not significantly affect the activity. Generally, hydrogen, methyl, and chlorine atoms were all tolerated, with only marginal differences in their inhibitory activities. We subsequently screened a large series of compounds, with aromatic side chain 2 modified to investigate the most suitably decorated arene moiety. Although derivatives with the dimethoxybenzene group were among the most potent inhibitors, the phenyl groups bearing a strong hydrogen bond acceptor, such as sulfonamide or aldehyde, at the meta position relative to the central core significantly contributed to the

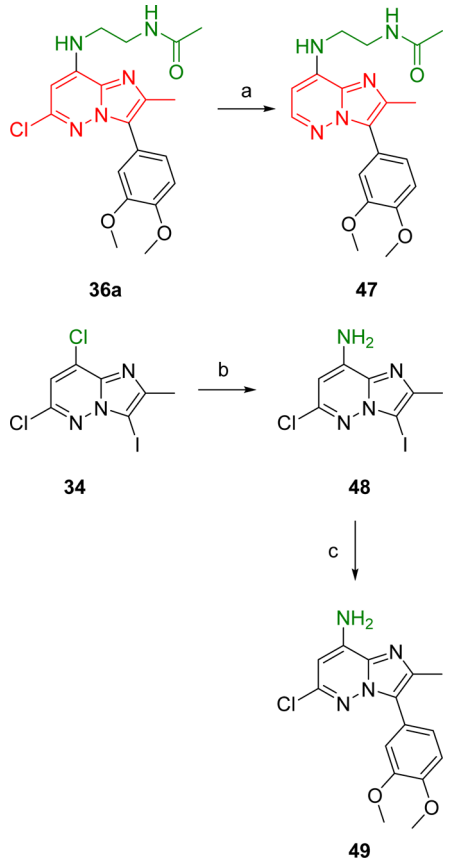
DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

Scheme 11. Modification of the Aromatic Side Chain[a]



[a]Reagents and conditions: (a) BBr_3, CH_2Cl_2, −78 °C to rt, overnight, 13%; (b) $NaBH_4$, MeOH/CH_2Cl_2, rt, 1 h, 30% for **33** and 40% for **34**; (c) (i) H_2 (3.5 bar), Raney-Ni, NH_3/EtOH, rt, overnight, (ii) HCl/Et_2O, CH_2Cl_2, 0 °C, 71%.

Scheme 12. Preparation of Derivatives 47 and 49[a]



[a]Reagents and conditions: (a) H_2 (5 bar), $Pd(OH)_2/C$, Et_3N, THF:MeOH (1:1), 63%; (b) NH_3/EtOH, MW, 140 °C, 1.5 h, 77%; (c) (3,4-dimethoxyphenyl)boronic acid, $Pd(PPh_3)_4$, 1 M K_2CO_3, dioxane, 90 °C, overnight, 80%.

enhanced affinity toward the enzyme. Using this approach, we identified a number of novel inhibitors of PI4K IIIβ with the most interesting compound being **36t** with IC_{50} = 54 ± 15 nM. This compound displayed antiviral activity against members of both the *Picornaviridae* and the *Filoviridae* families, with activities being in the nanomolar level for CVB3 and HCV 1b and in micromolar level for HRVM and HCV 2a. The results in both enzymatic and antiviral cell-based assays showed the clear superiority over the starting hit T-00127-HEV1 (**2a**).

Very recently, the first crystal structure of PI4K IIIβ in complex with a nonselective lipid kinase inhibitor, PIK93 was solved by Burke et al.[6,44] Our extensive synthetic studies yielding a set of molecular tools now allowed us to solve the structure of PI4K IIIβ bound to a potent and selective molecule, namely compound **49**, which possesses a significantly different structure than PIK93. In addition, we were able to obtain a crystal structure with a bound ATP even though a significant part of the ATP molecule is disordered in the structure, probably because it would be stabilized by the catalytically important C-terminus of the enzyme, which impedes crystallization and thus is missing in the crystallized construct.[6] This crystal structure suggests the correct orientation and electrostatic interactions of the adenine moiety in the hinge region. The superposition of the structure with **49** and ATP clarifies the precise mode of action of this type of inhibitors. They compete with the natural binding site for ATP adenine ring, preventing the binding of ATP and hence inhibiting the lipid phosphorylation reaction. The crystal structures also suggest that the binding of the inhibitor causes conformational changes in the ATP binding site. In particular, residues Glu^{572} and Lys^{564} seem in closer proximity when binding ATP due to a weak electrostatic interaction. However, when the inhibitor **49** is bound, the Lys^{564} residue adopts a conformation that allows for an effective hydrogen bond with one of the methoxy groups of the inhibitor's aromatic side chain. Our docking studies suggest that this interaction can be exploited for the design of inhibitors with significantly enhanced affinity toward the enzyme as demonstrated in the example of the most active compound, **36t**. In the case of compound **36t**, the lysine residue Lys^{564} is significantly attracted by the strongly electronegative oxygen atom of the sulfonamide moiety. The docking studies support the importance of the amide side chain, which can significantly elevate the enthalpic contribution to the interaction of the

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

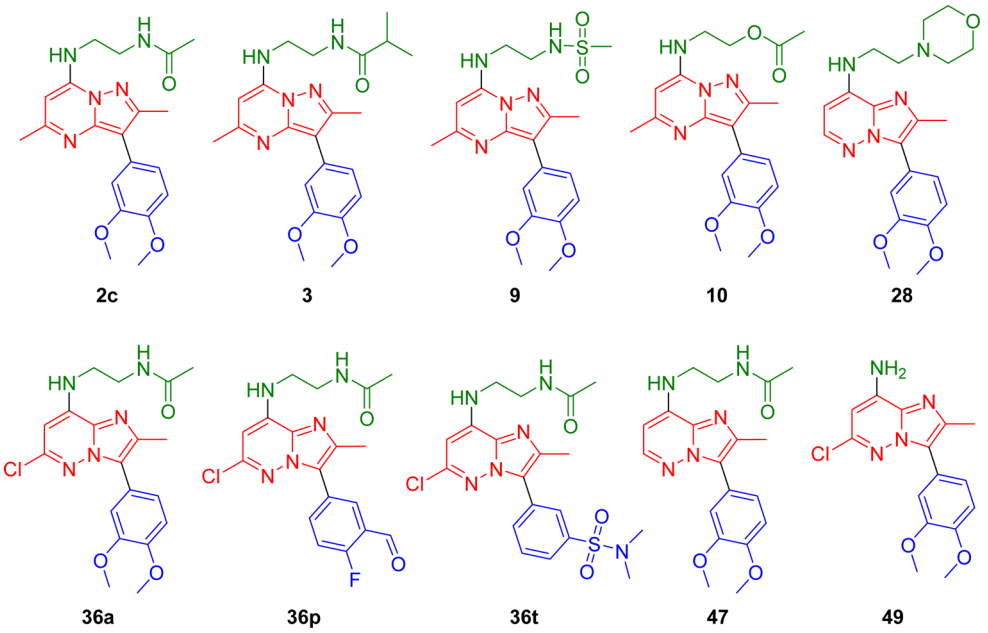


Figure 2. Structures of the selected subseries of inhibitors. The amine side chains are in green, bicyclic central core in red, and aromatic side chain in blue.

Table 1. Inhibitory Activity of Selected Compounds against Members of PI4K Family

compd	PI4K IIIβ IC_{50} (μM)	PI4K IIIα IC_{50} (μM)	PI4K IIα IC_{50} (μM)
2c	0.070 ± 0.020	59.45 ± 0.06	>100
3	0.120 ± 0.010	62.14 ± 0.84	>100
9	0.120 ± 0.010	29.31 ± 0.96	>100
10	0.067 ± 0.009	29.52 ± 3.40	>100
28	0.093 ± 0.012	27.46 ± 2.47	>100
36a	0.090 ± 0.011	82.67 ± 18.64	>100
36p	0.089 ± 0.003	>100	>100
36t	0.054 ± 0.015	>100	>100
47	0.092 ± 0.006	33.67 ± 0.49	>100
49	0.083 ± 0.025	>100	>100
T-00127-HEV1[a]	0.150 ± 0.010	74.55 ± 3.08	≥100

[a]References 24, 26.

enzyme and inhibitor. While we cannot explain the specificity of our compounds against PI4K IIIα on the structural level as the PI4K IIIα crystal structure is not available, we can explain why the prepared compounds do not inhibit PI4K IIα. The structural superposition of these two enzymes (Supporting Information, Figure 3) reveals that from the two most critical residues for inhibitor binding (K564 and E572) only the glutamate is conserved.

CONCLUSION

In conclusion, we present highly potent inhibitors of PI4K IIIβ with excellent selectivity. The most active compound, **36t** (IC_{50} = 54 nM), possesses outstanding selectivity against both the most homologous enzyme, PI4K IIIβ, and the nonhomologues enzyme, the PI4K IIα that catalyzes the same reaction. This compound has high potential as exquisite chemical biology tool for efficient studies of PI4Ks in cells and in animal models as well as an excellent starting point in the search for broad-spectrum antiviral agents against ss(+)RNA viruses and potential therapeutics of other human diseases. Through our crystallographic and computational studies, we revealed the molecular mechanism of the inhibitors and their disruption of

Table 2. Antiviral Activity against Selected ss(+)RNA Viruses

compd	CVB3 EC_{50} (μM)	HRVM EC_{50} (μM)	HCV 1b EC_{50} (μM)	HCV 2a EC_{50} (μM)	HeLa CC_{50} (μM)
2c	1.23 ± 0.03	2.9 ± 0.1	1.03 ± 1.17	>44	>50
3	11.0 ± 0.1	1.62 ± 0.04	1.21 ± 0.22	>44	>50
9	1.43 ± 0.01	3.0 ± 0.1	0.91 ± 0.44	42.5 ± 0.5	>50
10	0.86 ± 0.08	1.38 ± 0.05	0.96 ± 0.67	>44	>50
28	0.50 ± 0.07	0.88 ± 0.10	0.37 ± 0.06	17.9 ± 0.1	>50
36a	0.75 ± 0.03	1.09 ± 0.14	0.29 ± 0.07	31.8 ± 0.2	>50
36p	>50	24.3 ± 0.1	5.3 ± 0.1	13.6 ± 0.1	>50
36t	0.145 ± 0.034	1.03 ± 0.35	0.087 ± 0.067	10.6 ± 0.1	>50
47	0.28 ± 0.04	2.9 ± 0.1	0.36 ± 0.09	27.9 ± 0.2	>50
49	0.023 ± 0.004	0.89 ± 0.10	0.21 ± 0.09	26.5 ± 0.21	1.75 ± 0.17
T-00127-HEV1[a]	3.38 ± 0.05	2.5 ± 0.1	1.03 ± 0.12	>44	>50
MK-0608[b]	ND	5.1 ± 1.5	0.21 ± 0.07	0.21 ± 0.10	>50

[a]References 24, 26. [b]7-Deaza-2′-C-methyladenosine (MK-0608), ref 36.

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

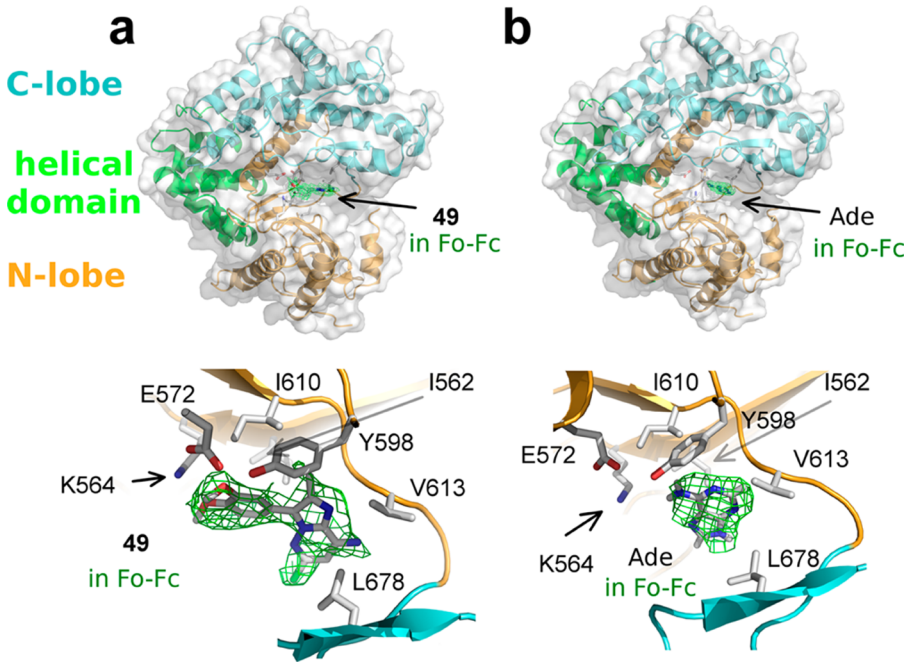


Figure 3. Crystal structures of **49** and ATP bound to PI4K IIIβ. (a) Compound **49** in the unbiased $F_o - F_c$ map contoured at 2σ. Top: view on the whole enzyme. Bottom: detailed view of the binding pocket. (b) The adenine ring of the ATP in the unbiased $F_o - F_c$ map contoured at 2σ. Top: view on the whole enzyme. Bottom: detailed view of the binding pocket.

ATP binding. Finally, we explain the role of their structural features for interaction with PI4K IIIβ.

EXPERIMENTAL METHODS

Synthesis of Novel Inhibitors. *General Chemical Procedures.* Melting points were determined on a Büchi melting point B-540 apparatus. Microwave syntheses were carried out in a CEM Discover instrument with a single0-mode cavity and focused microwave heating (microwave power supply 0−300 W, 1 W increments, sealed vessel mode, pressure range 0−20 bar). NMR spectra were measured on a Bruker Avance II-600 and/or Bruker Avance II-500 instruments (600 or 500.0 MHz for ^{1}H and 150 or 125 MHz for ^{13}C) in hexadeuterodimethyl sulfoxide and referenced to the solvent signal (δ 2.50 and 39.70, respectively). Mass spectra were measured on a LTQ Orbitrap XL (Thermo Fischer Scientific) using electrospray ionization (ESI) and a GCT Premier (Waters) using EI. HPLC-MS spectra were obtained on a Shimadzu LCMS-2020 LC-MS. The elemental analyses (summarized in Supporting Information, Table 1) were obtained on a PerkinElmer CHN Analyzer 2400, series II Sys (PerkinElmer) and X-ray fluorescence spectrometer SPECTRO iQ II (SPECTRO Analytical Instruments, Germany). All of the compounds in the series had purity higher than 95% (determined either by elemental analysis or HPLC-MS). Column chromatography and thin-layer chromatography (TLC) were performed using Silica Gel 60 (Fluka) and Silufol Silica Gel 60 F_{254} foils (Merck), respectively. Solvents were evaporated at 2 kPa and bath temperature 30−60 °C. The compounds were dried at 13 Pa and 50 °C.

General Procedure for Introduction of the N-Substituent to Position 7. Chloro derivative **1**[35] (150 mg, 0.47 mmol) was dissolved in ethanol (5 mL) and DIPEA (0.25 mL, 1.41 mmol) together with an appropriate amine (2 equiv). Reaction mixture was then stirred at 75 °C for 12 h (or until consumption of the starting material). Reaction mixture was then evaporated, and the residue was chromatographed (silica gel, 50 g) with an appropriate mobile phase and the solid was recrystallized.

*3-(3,4-Dimethoxyphenyl)-2,5-dimethyl-N-(2-morpholinoethyl)-pyrazolo[1,5-a]pyrimidin-7-amine (**2a**).* Mobile phase: ethyl acetate → ethyl acetate:toluene:acetone:ethanol (17:4:3:1). Crystallized from ethanol−water. Yield: 172 mg (89%); mp 170−170.5 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.42 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.23 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 6.96 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.63 (t, $J_{NH-1'}$ = 5.0 Hz, 1H, NH), 5.78 (s, 1H, H-6), 3.95 (s, 3H, 3″-OCH_3), 3.91 (s, 3H, 4″-OCH_3), 3.78 (m, 4H, morph-$O(CH_2)_2$), 3.44 (q, $J_{1'-2'}$ = $J_{1'-NH}$ = 5.7 Hz, 2H, H-1′), 2.75 (t, $J_{2'-1'}$ = 6.1 Hz, 2H, H-2′), 2.66 (s, 3H, 2-CH_3), 2.55 (m, 4H, morph-$N(CH_2)_2$), 2.50 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.3 (C-5), 150.9 (C-2), 148.8 (C-3″), 147.2 (C-4″), 146.2 (C-3a), 145.7 (C-7), 126.1 (C-1″), 120.9 (C-6″), 112.4 (C-2″), 111.4 (C-5″), 107.0 (C-3), 85.3 (C-6), 66.9 (morph-O(**C**$H_2)_2$), 56.3 (C-2′), 55.9 (4″-OCH_3), 55.9 (3″-OCH_3), 53.3 (morph-N(**C**$H_2)_2$), 38.2 (C-1′), 25.3 (5-CH_3), 14.5 (2-CH_3). Anal. ($C_{22}H_{29}N_5O_3$) C, H, N. ESI MS, *m*/*z* (rel%): 412 (100) [M + H]. HRMS: calcd for [M + H], 412.23432; found, 412.23434.

*3-(3,4-Dimethoxyphenyl)-2,5-dimethyl-N-(3-morpholinopropyl)-pyrazolo[1,5-a]pyrimidin-7-amine (**2b**).* Mobile phase: ethyl acetate → ethyl acetate:toluene:acetone:ethanol (17:4:4:1). Crystallized from ethyl acetate. Yield 160 mg (80%); mp 105.5−116 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 8.06 (t, $J_{NH-1'}$ = 4.6 Hz), 7.43 (d, $J_{2''-6''}$ = 1.9 Hz, 1H, H-2″), 7.23 (dd, $J_{6''-2''}$ = 1.9, $J_{6''-5''}$ = 8.2 Hz, 1H, H-6″), 6.96 (d, $J_{5''-6''}$ = 8.2 Hz, 1H, H-5″), 5.75 (s, 1H, H-6), 3.97−3.94 (m, 7H, 3″-OCH_3, morph-$O(CH_2)_2$), 3.91 (s, 3H, 4″-OCH_3), 3.49 (m, 2H, H-1′), 2.63−2.53 (m, 9H, H-3′, 2-CH_3, morph-$N(CH_2)_2$), 2.49 (s, 3H, 5-CH_3), 1.93 (pent, $J_{2'-1'}$ = $J_{2'-3'}$ = 5.8 Hz, 2H, H-2′). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 159.3 (C-5), 150.7 (C-2), 148.8 (C-3′), 147.1 (C-4″), 146.2 (C-3a), 146.1 (C-7), 126.3 (C-1″), 120.9 (C-6″), 112.4 (C-2″), 111.4 (C-5″), 106.7 (C-3), 84.8 (C-6), 66.6 (morph-O(**C**$H_2)_2$), 58.0 (C-3′), 56.0 and 55.8 (4″-OCH_3 and 3″-OCH_3), 53.9 (morph-N(**C**$H_2)_2$), 42.3 (C-1′), 25.4 (5-CH_3), 24.0 (C-2′), 14.4 (2-CH_3). Anal. ($C_{23}H_{31}N_5O_3$) C, H, N. ESI MS, *m*/*z* (rel%): 426 (100) [M + H]. HRMS: calcd for [M + H], 426.24997; found, 426.24991.

*N-(2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)amino)ethyl)acetamide (**2c**).* Mobile phase: ethyl acetate → ethyl acetate:acetone:ethanol:H_2O (20:3:1.2:0.8). Crystallized from ethyl acetate. Yield 156 mg (87%); mp 163−164 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.06 (t, J_{NH-2} = 5.6 Hz, 1H, NHCO), 7.76 (t, J_{NH-1} = 6.2 Hz, 1H, 7′-NH), 7.41 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.21 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 7.01 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.08 (s, 1H, H-6′), 3.79 (s, 3H, 3″-OCH_3), 3.78 (s, 3H, 4″-OCH_3), 3.42 (m, 2H, H-1), 3.29 (m, 2H, H-2), 2.52 (s,

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

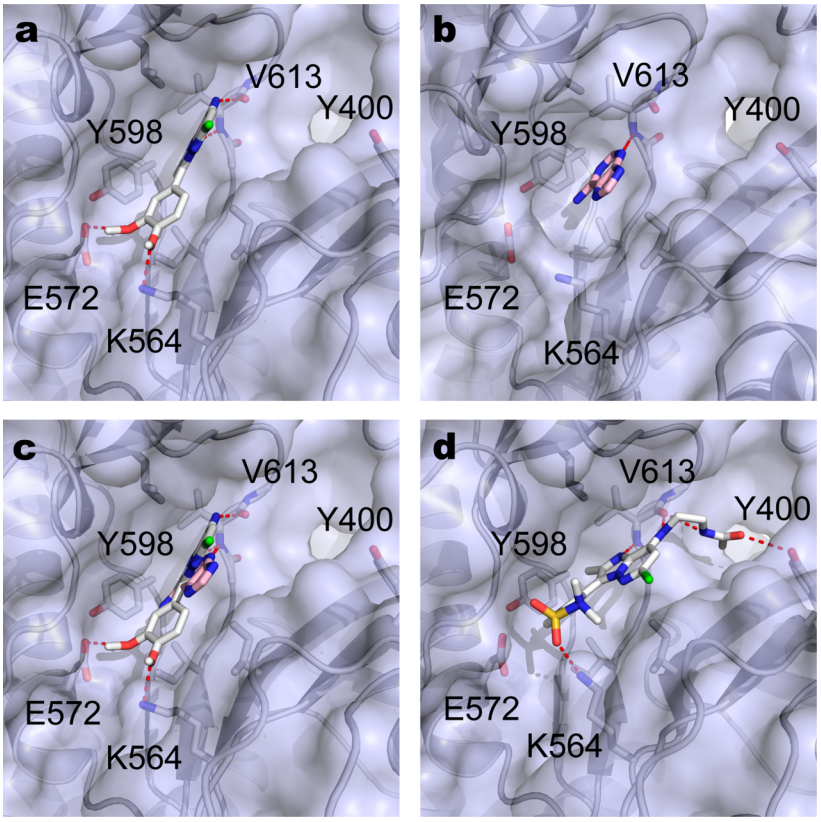


Figure 4. Structural insight into the inhibition of ATP approach to ATP binding site. (a) Compound **49** in the ATP binding site: the inhibitor appears to be strongly bound in the ATP binding site based on strong hydrogen bonds to amino acid residues Val^{613}, Lys^{564}, and Glu^{572} and significant structural match with the binding canyon. (b) ATP bound to PI4K IIIβ (a detailed view into ATP binding site) residues Glu^{572} and Lys^{564} seem to be in close proximity, suggesting existence of electrostatic interaction between these two residues with opposite polarity; this conformation of the protein allows effective binding of the nucleobase due to extensive shape complementarity. (c) Superposition of ATP and **49** in the biding site, which clearly shows these two ligands occupy the same area of the enzyme and compete with each other at the ATP binding site. (d) Docking study of the most potent inhibitor in the study derivative **36t** fits nicely into the binding canyon of the enzyme, formed by numerous lipophilic residues. In particular, the aromatic side chain of the inhibitor stays in T-shape conformation to the Tyr^{598}, suggesting significant $\pi-\pi$ interaction. In comparison with the binding mode of **49**, compound **36t** seems to form a stronger hydrogen bond with Lys^{564} and two additional hydrogen bonds with Val^{613} and Tyr^{400}.

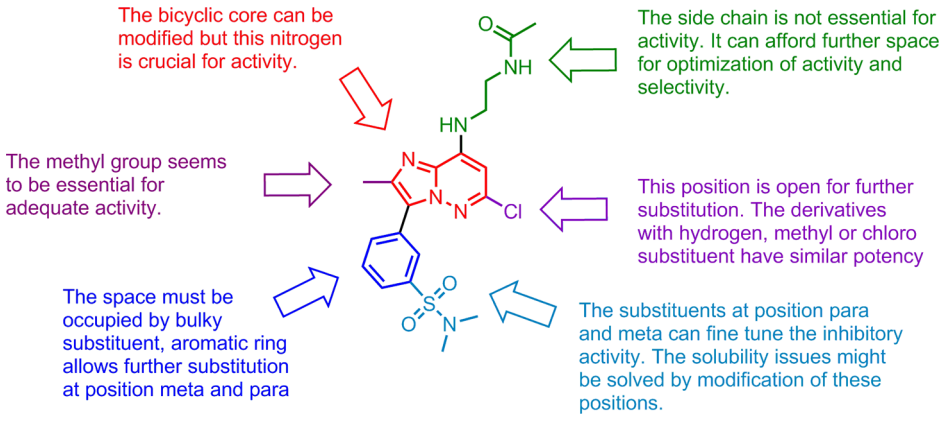


Figure 5. Schematic representation of the results of our SAR study.

3H, 2′-CH_3), 2.38 (s, 3H, 5′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (CO), 158.8 (C-5′), 149.9 (C-2′), 148.7 (C-3″), 147.0 (C-4″), 146.1 and 146.2 (C-3a′ and C-7′), 126.3 (C-1″), 120.6 (C-6″), 112.7 (C-2″), 112.2 (C-5″), 105.6 (C-3′), 85.2 (C-6′), 55.8 and 55.7 (3″-OCH_3 and 4″-OCH_3), 41.1 (C-1), 38.2 (C-2), 25.1 (5′-CH_3), 22.8 ($COCH_3$), 14.7 (2′-CH_3). Anal. ($C_{20}H_{25}N_5O_3 \cdot 0.25H_2O$) C, H, N. ESI MS, *m*/*z* (rel%): 384 (100) [M + H]. HRMS: calcd for [M + H], 384.20302; found, 384.20302.

*2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)amino)acetamide (**2d**).* Mobile phase: ethyl acetate → ethyl acetate:toluene:acetone:ethanol (17:4:4:1). Crystallized from ethyl acetate. Yield 137 mg (82%); mp 229.5−231 °C. ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.67 (t, J_{NH-CH_2} = 6.0 Hz), 7.57 (bs, 1H, $CONH_2$b), 7.41 (d, $J_{2'-6'}$ = 2.0 Hz, 1H, H-2′), 7.25 (bs, 1H, $CONH_2$a), 7.23 (dd, $J_{6'-2'}$ = 2.0, $J_{6'-5'}$ = 8.3 Hz, 1H, H-6′), 7.01 (d, $J_{5'-6'}$ = 8.3 Hz, 1H, H-5′), 5.88 (s, 1H, C-6), 3.96 (d, J_{CH_2-NH} = 6.0 Hz,

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

2H, CH_2CO), 3.79 (s, 3H, 3′-OCH_3), 3.78 (s, 3H, 4′-OCH_3), 2.53 (s, 3H, 2-CH_3), 2.38 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 ($CONH_2$), 158.8 (C-5), 150.0 (C-2), 148.7 (C-3′), 147.0 (C-4′), 146.2 (C-7), 146.0 (C-3a), 126.2 (C-1′), 120.6 (C-6′), 112.7 (C-2′), 112.2 (C-5′), 105.6 (C-3), 85.6 (C-6), 55.7 and 55.8 (3′-OCH_3 and 4′-OCH_3), 44.1 ($NHCH_2$), 25.1 (5-CH_3), 14.7 (2-CH_3). Anal. ($C_{18}H_{21}N_5O_3 \cdot 0.33H_2O$) C, H, N. ESI MS, m/z (rel%): 356 (100) [M + H]. HRMS: calcd for [M + H], 356.17172; found, 356.17176.

*3-(3,4-Dimethoxyphenyl)-2,5-dimethyl-N-(4-morpholinophenyl)-pyrazolo[1,5-a]pyrimidin-7-amine (**2e**).* After 12 h, the same amount of the reagents was added and heating continued for another 12 h. Reaction mixture was cooled down, and product started to crystallize. Yield 140 mg (65%); mp 175.5−176 °C. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.79 (bs, 1H, NH), 7.42 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.28 (d, $J_{2'-3'}$ = 8.9 Hz, 2H, H-2′), 7.24 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 6.98 (d, $J_{3'-2'}$ = 8.9 Hz, 2H, H-3′), 6.97 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.01 (s, 1H, H-6), 3.95 (s, 3H, 3″-OCH_3), 3.92 (s, 3H, 4″-OCH_3), 3.89 (m, 4H, morph-O(CH_2)$_2$), 3.21 (m, 4H, morph-N(CH_2)$_2$), 2.62 (s, 3H, 2-CH_3), 2.45 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.6 (C-5), 151.0 (C-2), 149.8 (C-4′), 148.9 (C-3″), 147.3 (C-4″), 146.3 (C-3a), 144.7 (C-7), 128.4 (C-1′), 125.9 (C-1″), 125.8 (C-2′), 121.0 (C-6″), 116.4 (C-3′), 112.5 (C-2″), 111.5 (C-5″), 107.4 (C-3), 86.4 (C-6), 66.8 (morph-O(CH_2)$_2$), 56.0 and 55.9 (3″-OCH_3 and 4″-OCH_3), 49.2 (morph-N(CH_2)$_2$), 25.4 (5-CH_3), 14.4 (2-CH_3). Anal. ($C_{26}H_{29}N_5O_3$) C, H, N. ESI MS, m/z (rel %): 460 (100) [M + H]. HRMS: calcd for [M + H], 460.23432; found, 460.23434.

*2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)amino)ethanol (**2f**).* Mobile phase: ethyl acetate. Crystallized from ethyl acetate. Yield 142 mg (88%); mp 183.5−184 °C. ^1H NMR (600 MHz, DMSO-d_6) δ (ppm): 7.48 (t, J_{NH-2} = 6.1 Hz, 1H, NH), 7.42 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.22 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 7.00 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.09 (s, 1H, H-6′), 4.90 (t, J_{OH-1} = 5.5 Hz, 1H, 1-OH), 3.79 (s, 3H, 3′-OCH_3), 3.78 (s, 3H, 4″-OCH_3), 3.64 (m, 2H, H-1), 3.42 (m, 2H, H-2), 2.52 (s, 3H, 2′-CH_3), 2.39 (s, 3H, 5′-CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 158.8 (C-5′), 149.9 (C-2′), 148.6 (C-3″), 146.9 (C-4″), 146.2 (C-7′), 146.0 (C-3′a), 126.3 (C-1″), 120.6 (C-6″), 112.6 (C-2″), 112.2 (C-5″), 105.5 (C-3′), 85.4 (C-6′), 59.5 (C-1), 55.7 and 55.8 (3″-OCH_3 and 4″-OCH_3), 44.0 (C-2), 25.1 (5′-CH_3), 14.7 (2′-CH_3). Anal. ($C_{18}H_{22}N_4O_3$) C, H, N. ESI MS, m/z (rel%): 343 (100) [M + H]. HRMS: calcd for [M + H], 343.17647; found, 343.17654.

*4-(3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)morpholine (**2g**).* Mobile phase: toluene: ethyl acetate 11:9. Crystallized from ethyl acetate. Yield 130 mg (75%); mp 170.5−171 °C. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.38 (d, $J_{2'-6'}$ = 2.0 Hz, 1H, H-2′), 7.22 (dd, $J_{6'-2'}$ = 2.0, $J_{6'-5'}$ = 8.3 Hz, 1H, H-6′), 6.97 (d, $J_{5'-6'}$ = 8.3 Hz, 1H, H-5′), 5.97 (s, 1H, H-6), 3.99 (m, 4H, morph-O(CH_2)$_2$), 3.94 (s, 3H, 3′-OCH_3), 3.92 (s, 3H, 4′-OCH_3), 3.71 (m, 4H, morph-N(CH_2)$_2$), 2.59 (s, 3H, 2-CH_3), 2.53 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.1 (C-5), 151.1 (C-2), 149.7 (C-7), 148.8 (C-3′), 147.4 (C-4′), 125.7 (C-1′), 121.2 (C-6′), 112.6 (C-2′), 111.4 (C-5′), 107.2 (C-3), 93.0 (C-6), 66.3 (morph-O(CH_2)$_2$), 55.9 and 56.0 (3′-OCH_3 and 4′-OCH_3), 48.4 (morph-N(CH_2)$_2$), 25.1 (5-CH_3), 14.5 (2-CH_3), C-3a was not detected. Anal. ($C_{20}H_{24}N_4O_3$) C, H, N. ESI MS, m/z (rel%): 369 (100) [M + H]. HRMS: calcd for [M + H], 369.19212; found, 369.19215.

*7-(4-Benzhydrylpiperazin-1-yl)-3-(3,4-dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]pyrimidine (**2h**).* Mobile phase: toluene:ethyl acetate 4:1. Crystallized from ethyl acetate. Yield 193 mg (77%). ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.48−7.45 (m, 4H, Ph-*o*), 7.38 (d, $J_{2'-6'}$ = 2.1 Hz, 1H, H-2′), 7.32−7.28 (m, 4H, Ph-*m*), 7.22−7.19 (m, 2H, Ph-*p*), 7.20 (dd, $J_{6'-2'}$ = 2.1, $J_{6'-5'}$ = 8.3 Hz, 1H, H-6′), 6.95 (d, $J_{5'-6'}$ = 8.3 Hz, 1H, H-5′), 5.94 (s, 1H, H-6), 4.34 (s, 1H, C**H**Ph_2), 3.93 (s, 3H, 3′-OCH_3), 3.91 (s, 3H, 4′-OCH_3), 3.73 (m, 4H, H-1″), 2.68 (t, $J_{2''-1''}$ = 4.7 Hz, 4H, H-2″), 2.59 (s, 3H, 2-CH_3), 2.53 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 158.9 (C-5), 150.8 (C-2), 149.8 (C-7), 148.8 (C-3′), 147.9 (C-3a), 147.3 (C-4′), 142.3 (Ph-*i*), 128.6 (Ph-*m*), 127.9 (Ph-*o*), 127.1 (Ph-*p*), 125.9 (C-1′), 121.1 (C-6′), 112.5 (C-2′), 111.4 (C-5′), 106.9 (C-3), 93.1 (C-6), 76.2 (**C**HPh_2), 55.8 and 56.0 (3′-OCH_3 and 4′-OCH_3), 51.2 (C-2″), 48.3 (C-1″), 25.2 (5-CH_3), 14.5 (2-CH_3). Anal. ($C_{20}H_{24}N_4O_3 \cdot 0.33EtOAc$) C, H, N. ESI MS, m/z (rel%): 534 (100) [M + H]. HRMS: calcd for [M + H], 534.28635; found, 534.28633.

*3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]pyrimidin-7-amine (**2i**).* Chloro derivative **1** (260 mg, 0.82 mmol) was dissolved in ethanolic ammonia (3.5 M, 5.5 mL) and heated in microwave reactor at 120 °C for 1 h. Reaction mixture was evaporated and chromatographed on silica gel column (75 g) in ethyl acetate:toluene (7:3). Solid was crystallized from ethyl acetate, affording the product **2i**. Yield 181 mg (74%); mp 192−194 °C. ^1H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.39 (d, $J_{2'-6'}$ = 2.0 Hz, 1H, H-2′), 7.22 (dd, $J_{6'-2'}$ = 2.0 Hz, $J_{6'-5'}$ = 8.3 Hz, 1H, H-6′), 6.96 (d, $J_{5'-6'}$ = 8.3 Hz, 1H, H-5′), 5.88 (s, 1H, H-6), 5.63 (s, 2H, NH_2), 3.94 (s, 3H, 3′-OCH_3), 3.90 (s, 3H, 4′-OCH_3), 2.59 (s, 3H, 2-CH_3), 2.47 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 159.4 (C-5), 151.4 (C-2), 148.8 (C-3′), 147.2 (C-4′), 146.4 (C-3a), 145.6 (C-7), 125.9 (C-1′), 121.0 (C-6′), 112.4 (C-2′), 111.4 (C-5′), 106.9 (C-3), 88.2 (C-6), 55.9 (4′-OCH_3), 55.8 (3′-OCH_3), 25.0 (5-CH_3), 14.4 (2-CH_3). Anal. ($C_{16}H_{18}N_4O_2$) C, H, N. ESI MS, m/z (rel%): 299 (100) [M + H]. HRMS: calcd for [M + H], 299.15025; found, 299.15045.

*N1-(3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)ethane-1,2-diamine (**2j**).* Chloro derivative **1** (1.81 g, 5.7 mmol) was dissolved in ethanol (100 mL), and ethane-1,2-diamine (7.6 mL, 114 mmol) was added and reaction mixture was heated to 75 °C for 1.5 h and evaporated. Residue was chromatographed (silica gel, 250 g) in ethyl acetate:acetone:ethanol:water (17:3:3:2) + 1% Et_3N to give off the oily product **2j**. Solid powder was obtained after a sonication with ethyl acetate.Yield 1.77 g (91%); mp 183.5−184 °C. ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.86 (m, 1H, 7-NH), 7.41 (d, $J_{2''-6''}$ = 1.9 Hz, 1H, H-2″), 7.22 (dd, $J_{6''-2''}$ = 1.9, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 7.01 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.27 (s, 1H, C-6′), 3.79 (s, 3H, 3″-OCH_3), 3.78 (s, 3H, 4″-OCH_3), 3.66 (m, 2H, H-1), 3.04 (t, 2H, J_{2-1} = 6.3 Hz, H-2), 2.53 (s, 3H, 2-CH_3), 2.40 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 159.0 (C-5′), 149.9 (C-2′), 148.7 (C-3″), 147.0 (C-4″), 146.1 and 146.1 (C-3a′and C-7′), 126.2 (C-1″), 120.6 (C-1″), 112.7 (C-2″), 112.2 (C-5″), 105.6 (C-3′), 85.6 (C-6′), 55.7 and 55.8 (3′-OCH_3 and 4′-OCH_3), 39.3 (C-1), 38.1 (C-2), 25.1 (5-CH_3), 14.7 (2-CH_3). Anal. ($C_{18}H_{23}N_5O_2$) C, H, N. ESI MS, m/z (rel%): 342 (100) [M + H]. HRMS: calcd for [M + H], 342.19245; found, 342.19271.

*N1-(3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)propane-1,3-diamine (**2k**).* Chloro derivative **1** (267 mg, 0.84 mmol) was dissolved in ethanol (20 mL) and propane-1,3-diamine (1.4 mL, 16.8 mmol) was added, and reaction mixture was heated to 75 °C for 1.5 h and evaporated. Residue was chromatographed (silica gel, 75 g) in ethyl acetate:acetone:ethanol:water (17:3:3:2) + 1% Et_3N to give **2k** as an oily product. Solid powder was obtained after sonication with ethyl acetate. Yield: 275 mg (92%); mp 200−204 °C. ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.89 (bs, 1H, 7-NH), 7.41 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.21 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 7.01 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.15 (s, 1H, C-6′), 3.79 (s, 3H, 3″-OCH_3), 3.78 (s, 3H, 4″-OCH_3), 3.47 (m, 2H, H-1), 2.85 (m, 2H, H-3), 2.52 (s, 3H, 2-CH_3), 2.39 (s, 3H, 5-CH_3), 1.92 (m, 2H, H-2). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 158.9 (C-5′), 149.9 (C-2′), 148.6 (C-3″), 146.9 (C-4″), 146.1 (C-3a′), 145.9 (C-7′), 126.6 (C-1″), 120.6 (C-6″), 112.7 (C-2″), 112.2 (C-5″), 105.6 (C-3′), 85.4 (C-6′), 55.7 and 55.8 (3″-OCH_3 and 4″-OCH_3), 38.4 (C-1), 36.8 (C-3), 26.8 (C-2), 25.1 (5-CH_3), 14.7 (2-CH_3). Anal. ($C_{19}H_{25}N_5O_2$) C, H, N. ESI MS, m/z (rel%): 356 (100) [M + H]. HRMS: calcd for [M + H], 356.20810; found, 356.20812.

*1-(4-(3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)piperazin-1-yl)ethanone (**2l**).* Chloro derivative **1** (303 mg, 0.95 mmol) was dissolved in DMF (10 mL), and piperazine (822 mg, 9.5 mmol) was added. Reaction mixture was stirred at rt for 3 h and evaporated. Residue was coevaporated with DMF (3 × 20 mL) and then dissolved in chloroform (70 mL). Organic phase was washed with water (30 mL), and the water phase was re-extracted with chloroform (70 mL). Combined organic phases were dried (Na_2SO_4)

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

and evaporated. Crude intermediate was immediately used in the following step. The intermediate was dissolved in CH_2Cl_2 (13 mL) and Et_3N (0.4 mL, 2.9 mmol) at 0 °C. Then acetic anhydride (180 μL, 1.9 mmol) and reaction mixture was stirred at 0 °C for 3 h. Reaction was poured into satd aq solution of $NaHCO_3$ (35 mL), and the mixture was extracted with chloroform (2 × 70 mL). The organic phase was dried (Na_2SO_4) and evaporated. Chromatography of the residue on silica gel column (75 g) with ethyl acetate → ethyl acetate:toluene:acetone:ethanol (17:4:3:1) afforded the product **2l**. The solid was recrystallized from ethyl acetate. Yield: 348 mg (90%); mp 186.5−187 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.37 (d, $J_{2''-6''}$ = 1.8 Hz, 1H, H-2″), 7.20 (dd, $J_{6''-2''}$ = 1.8, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 7.02 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.29 (s, 1H, H-6′), 3.79 (s, 3H, 3″-OCH_3), 3.78 (s, 3H, 4″-OCH_3), 3.77 (m, 2H, H-1a), 3.68−3.63 (m, 6H, H-1b, H-2), 2.52 (s, 3H, 2′-CH_3), 2.44 (s, 3H, 5′-CH_3), 2.07 (s, 3H, $COCH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 168.7 (CO), 158.9 (C-5′), 149.9 (C-2′), 148.9 (C-7′), 148.6 (C-3″), 147.5 (C-3a), 147.2 (C-4″), 125.7 (C-1″), 120.9 (C-6″), 112.9 (C-2″), 111.1 (C-5″), 106.0 (C-3), 93.6 (C-6′), 55.7 and 55.8 (3′-OCH_3 and 4′-OCH_3), 47.6 and 47.8 (2 × C-1), 40.4 and 45.2 (2 × C-2), 24.8 (5′-CH_3), 21.4 ($CO\mathbf{C}H_3$), 14.7 (2′-CH_3). Anal. ($C_{22}H_{27}N_5O_3$) C, H, N. ESI MS, *m*/*z* (rel%): 432 (100) [M + Na]. HRMS: calcd for [M + H], 410.21867; found, 410.21875.

*N-(2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)amino)ethyl)isobutyramide (**3**).* Compound **2j** (288 mg, 0.84 mmol) was dissolved in CH_2Cl_2 (25 mL), and isobutyric acid (86 μL, 0.92 mmol) was added. The reaction mixture was cooled down to 0 °C, and DCC (209 mg, 1 mmol) was added in one portion. Reaction mixture was slowly allowed to warm to rt and then stirred for 20 h. Precipitated solid was filtered off, and the filtrate was evaporated. Residue was chromatographed on a silica gel column (100 g) with ethyl acetate → ethyl acetate:toluene:acetone:ethanol (17:4:4:1) to obtain product **3**, which was recrystallized from MeOH−Et_2O in a freezer. Yield: 266 mg (74%); mp 135−136 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.40 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.22 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 6.96 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.39 (m, 1H, 7′-NH), 5.95 (m, 1H, NHCO), 5.87 (s, 1H, H-6′), 3.94 (s, 3H, 3″-OCH_3), 3.91 (s, 3H, 4″-OCH_3), 3.57−3.55 (m, 4H, H-1, H-2), 2.57 (s, 3H, 2′-CH_3), 2.49 (s, 3H, 5′-CH_3), 2.35 (sept, $J_{CH(CH_3)_2-CH_3}$ = 6.9 Hz, 1H, C**H**$(CH_3)_2$), 1.15 (d, $J_{CH_3-CH(CH_3)_2}$ = 6.9 Hz, 6H, CH(C**H**$_3)_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 177.8 (CO), 159.5 (C-5′), 150.9 (C-2′), 148.8 (C-3″), 147.2 (C-4″), 146.1 (C-3′a), 145.8 (C-7′), 125.9 (C-1″), 120.9 (C-6″), 112.4 (C-2″), 111.4 (C-5″), 107.1 (C-3′), 85.2 (C-6′), 55.8 and 55.9 (3″-OCH_3 and 4″-OCH_3), 41.5 (C-1), 39.0 (C-2), 35.5 (**C**H$(CH_3)_2$), 25.4 (5′-CH_3), 19.5 (CH(**C**H$_3)_2$), 14.4 (2′-CH_3). Anal. ($C_{22}H_{29}N_5O_3$) C, H, N. ESI MS, *m*/*z* (rel%): 412 (100) [M + H]. HRMS: calcd for [M + H], 412.23432; found, 412.23448.

*N-(2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)amino)ethyl)cyclohexanecarboxamide (**4**).* To a solution of compound **2j** (257 mg, 0.75 mmol) in CH_2Cl_2 (10 mL) and Et_3N (137 μL, 0.98 mmol) at 0 °C was added cyclohexanecarboxylic acid chloride (131 μL, 0.98 mmol), and the reaction mixture was stirred at 0 °C for 2 h. The reaction mixture was poured into a satd aq solution of $NaHCO_3$ (35 mL) and extracted with chloroform (70 mL). The organic phase was dried (Na_2SO_4) and evaporated. Chromatography of the residue on a silica gel column (75 g) with ethyl acetate afforded the product **4**. Solid was recrystallized from ethyl acetate. Yield 244 mg (72%); mp 135.5−136 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.89 (t, $J_{NH-1'}$ = 5.3 Hz, 1H, NHCO), 7.75 (t, $J_{NH-2'}$ = 6.0 Hz, 1H, 7″-NH), 7.41 (d, $J_{2'''-6'''}$ = 1.4 Hz, 1H, H-2‴), 7.22 (dd, $J_{6'''-2'''}$ = 1.4, $J_{6'''-5'''}$ = 8.2 Hz, 1H, H-6‴), 7.01 (d, $J_{5'''-6'''}$ = 8.2 Hz, 1H, H-5‴), 6.07 (s, 1H, H-6″), 3.79 (s, 3H, 3‴-OCH_3), 3.78 (s, 3H, 4‴-OCH_3), 3.41 (m, 2H, H-2′), 3.29 (m, 2H, H-1′), 2.52 (s, 3H, 2″-CH_3), 2.38 (s, 3H, 5″-CH_3), 2.04 (m, 1H, H-1), 1.71−1.63 (m, 4H, H-2a, H-3a), 1.59 (m, 1H, H-4a), 1.30 (m, 2H, H-2b), 1.24−1.09 (m, 3H, H-3b, H-4b). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 175.8 (CO), 158.7 (C-5″), 149.8 (C-2″), 148.6 (C-3‴), 146.9 (C-4‴), 146.3 (C-7″), 146.1 (C-3″a), 126.3 (C-1‴), 120.6 (C-6‴), 112.7 (C-2‴), 112.2 (C-5‴), 105.5 (C-3″), 85.3 (C-6″), 55.7 and 55.7 (3‴-OCH_3 and 4‴-OCH_3), 41.1 (C-2′), 38.2 (C-1′), 29.3 (C-2), 25.7 (C-4), 25.5 (C-3), 25.1 (5″-CH_3), 14.6 (2″-CH_3). Anal. ($C_{25}H_{33}N_5O_3$) C, H, N. ESI MS, *m*/*z* (rel%): 452 (100) [M + H]. HRMS: calcd for [M + H], 452.26562; found, 452.26566.

*N-(2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)amino)ethyl)-2-phenylpropanamide (**5**).* Compound **2j** (248 mg, 0.73 mmol) was dissolved in CH_2Cl_2 (15 mL), and 2-phenylpropanoic acid (100 μL, 0.80 mmol) was added. The reaction mixture was cooled down to 0 °C, and DCC (181 mg, 0.88 mmol) was added in one portion. The reaction mixture was slowly allowed to warm to rt and then stirred for 20 h. Precipitated solid was filtered, and filtrate was evaporated. Residue was chromatographed on silica gel column (100 g) with ethyl acetate:toluene (10:1) to obtain the product **5**, which was recrystallized from ethyl acetate. Yield: 280 mg (81%); mp 154−155 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.42 (d, $J_{2'''-6'''}$ = 2.0 Hz, 1H, H-2‴), 7.29−7.21 (m, 6H, H-6‴, Ph-*o*, Ph-*m*, Ph-*p*), 6.96 (d, $J_{5'''-6'''}$ = 8.4 Hz, 1H, H-5‴), 6.28 (t, $J_{NH-1'}$ = 5.8 Hz, 7′-NH), 5.83 (s, 1H, H-6″), 5.75 (m, 1H, N**H**CO), 3.95 (s, 3H, 3‴-OCH_3), 3.92 (s, 3H, 4‴-OCH_3), 3.55 (q, $J_{COCH-CH_3}$ = 7.2 Hz, 1H, COC**H**), 3.52−3.49 (m, 4H, H-1′, H-2′), 2.58 (s, 3H, 2″-CH_3), 2.49 (s, 3H, 5″-CH_3), 1.53 (d, J_{CH_3-COCH} = 7.2 Hz, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 175.1 (CO), 159.5 (C-5″), 150.8 (C-2″), 148.8 (C-3‴), 147.3 (C-4‴), 146.1 (C-3″a), 145.7 (C-7″), 140.9 (Ph-*i*), 128.9 (Ph-*m*), 127.5 (ph-*o*), 127.4 (Ph-*p*), 126.0 (C-1‴), 120.9 (C-6‴), 112.4 (C-2‴), 111.5 (C-5‴), 107.1 (C-3″), 85.1 (C-6″), 56 and 55.8 (3‴-OCH_3 and 4‴-OCH_3), 47.0 (CO**C**HCH$_3$), 41.3 (C-1′), 39.1 (C-2′), 25.4 (5″-CH_3), 18.4 (CH_3), 14.4 (2″-CH_3). Anal. ($C_{27}H_{31}N_5O_3$) C, H, N. ESI MS, *m*/*z* (rel%): 474 (100) [M + H]. HRMS: calcd for [M + H], 474.24997; found, 474.25006.

*1-(2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)amino)ethyl)urea (**6**).* Starting material **2j** (255 mg, 0.75 mmol) was dissolved in a mixture of methanol (1.5 mL) and aq HCl (3.6 mL, 1 M). The reaction mixture was stirred at rt, and a solution of the KCNO (91 mg, 1.13 mmol) in water (2 mL) was added dropwise. After 2 h at rt, the reaction mixture was heated to 50 °C for 20 h. A second portion of KCNO (200 mg, solid) was added, and heating (50 °C) continued for another 20 h. Reaction mixture was neutralized and diluted with satd aq $NaHCO_3$ (30 mL). This solution was extracted with chloroform (2 × 60 mL). Organic phases were dried (Na_2SO_4) and evaporated. Residue was crystallized from acetone to afford of the product **6**. Yield: 216 mg (75%); mp 212−213 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.77 (t, J_{NH-2} = 5.8 Hz, 1H, 7′-NH), 7.42 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.22 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 7.01 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.16 (t, J_{NH-1} = 5.8 Hz, CON**H**), 6.09 (s, 1H, H-6′), 5.57 (s, 2H, CON**H**$_2$), 3.79 (s, 3H, 3′-OCH_3), 3.78 (s, 3H, 4″-OCH_3), 3.37 (m, 2H, H-2), 3.26 (m, 2H, H-1), 2.52 (s, 3H, 2′-CH_3), 2.39 (s, 3H, 5′-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 14.7 (2′-CH_3), 25.1 (5′-CH_3), 38.6 (C-1), 42.3 (C-2), 55.7 and 55.8 (3″-OCH_3 and 4″-OCH_3), 85.3 (C-6′), 105.5 (C-3′), 112.2 (C-5″), 112.6 (C-2″), 120.6 (C-6″), 126.3 (C-1″), 146.1 (C-3′a), 146.3 (C-7′), 146.9 (C-4″), 148.6 (C-3″), 149.8 (C-2′), 158.8 (C-5′), 159.2 (CO). Anal. ($C_{19}H_{24}N_6O_3 \cdot 0.5CH_3COCH_3$) C, H, N. ESI MS, *m*/*z* (rel%): 385 (100) [M + H]. HRMS: calcd for [M + H], 385.19827; found, 385.19850.

*1-(2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)amino)ethyl)-3-phenylurea (**7**).* Compound **2j** (253 mg, 0.74 mmol) was dissolved in dry CH_2Cl_2 (7 mL) under argon atmosphere. Phenyl isocyanate (121 μL, 1.1 mmol) was added, and the reaction mixture was stirred at rt for 24 h. The reaction mixture was evaporated, and residue was chromatographed on silica gel column (75 g) in ethyl acetate:toluene (1:1) → ethyl acetate to obtain the product **7**. Solid was recrystallized from ethyl acetate. Yield: 302 mg (89%); mp 125−127 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.55 (s, 1H, N**H**Ph), 7.82 (t, J_{NH-2} = 5.8 Hz, 1H, 7′-NH), 7.42 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.39 (m, 2H, Ph-*o*), 7.20 (m, 3H, H-6″, Ph-*m*), 7.01 (d, $J_{5''-6''}$ = 8.5 Hz, 1H, H-5″), 6.89 (m, 1H, Ph-*p*), 6.16 (t, J_{NH-1} = 5.8 Hz, CON**H**), 6.10 (s, 1H, H-6′), 3.79 (s, 3H, 3′-OCH_3), 3.78 (s, 3H, 4″-OCH_3), 3.47 (m, 2H, H-2), 3.39 (m, 2H, H-1), 2.53 (s,

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

3H, 2′-CH_3), 2.33 (s, 3H, 5′-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 158.8 (C-5′), 155.7 (NHCONH), 149.8 (C-2′), 148.6 (C-3″), 146.9 (C-4″), 146.3 (C-7′), 146.1 (C-3′a), 140.6 (Ph-*i*), 128.8 (Ph-*m*), 126.3 (C-1″), 121.3 (Ph-*p*), 120.6 (C-6″), 118.0 (Ph-*o*), 112.6 (C-2″), 112.2 (C-5″), 105.5 (C-3′), 85.3 (C-6′), 55.8 and 55.7 (3″-OCH_3 and 4″-OCH_3), 41.8 (C-2), 38.6 (C-1), 25.1 (5′-CH_3), 14.7 (2′-CH_3). Anal. ($C_{25}H_{48}N_6O_3$·0.66EtOAc) C, H, N. ESI MS, *m*/*z* (rel %): 461 (100) [M + H]. HRMS: calcd for [M + H], 461.22957; found, 461.22972.

*Ethyl (2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)amino)ethyl)-carbamate (**8**).* Starting material **2j** (252 mg, 0.79 mmol) was dissolved in CH_2Cl_2 (10 mL) and cooled down to 0 °C. To the reaction mixture was then sequentially added Et_3N (143 μL, 1 mmol) followed by ClCOOEt (99 μL, 1 mmol), and the reaction mixture was stirred at 0 °C for 1.5 h and then diluted with chloroform (60 mL). The organic phase was washed with water (35 mL), dried (Na_2SO_4), and evaporated. Chromatography of the residue on silica gel column (100 g) with ethyl acetate:toluene (5:1) afforded the product **8**. Crystalline powder was obtained after sonication of the compound from the mixture of ethyl acetate−pentane. Yield: 199 mg (61%); mp 170−171 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.76 (t, $J_{NH\text{-}2}$ = 5.9 Hz, 1H, 7′-NH), 7.42 (d, $J_{2''-6''}$ = 1.7 Hz, 1H, H-2″), 7.27 (t, $J_{NH\text{-}1}$ = 5.2 Hz, CON**H**), 7.22 (dd, $J_{6''-2''}$ = 1.7, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 7.01 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.08 (s, 1H, H-6′), 3.99 (q, $J_{OCH_2\text{-}CH_3}$ = 7.0 Hz, 2H, OC**H**$_2$CH$_3$), 3.79 (s, 3H, 3′-OCH_3), 3.78 (s, 3H, 4″-OCH_3), 3.43 (m, 2H, H-2), 3.23 (m, 2H, H-1), 2.52 (s, 3H, 2′-CH_3), 2.38 (s, 3H, 5′-CH_3), 1.14 (t, $J_{CH_3\text{-}OCH_2}$ = 7.0 Hz, 3H, OCH$_2$C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 158.7 (C-5′), 156.7 (CONH), 149.9 (C-2′), 148.6 (C-3″), 146.9 (C-4′), 146.2 and 146.1 (C-7′a and C-3′), 126.3 (C-1″), 120.6 (C-6″), 112.6 (C-2″), 112.2 (C-5″), 105.5 (C-3′), 85.1 (C-6′), 59.9 (O**C**H$_2$CH$_3$), 55.8 and 55.7 (4″-OCH_3 and 3″-OCH_3), 41.1 (C-2), 39.6 (C-1), 25.1 (5′-CH_3), 14.8 and 14.7 (OCH$_2$**C**H$_3$, 2′-CH_3). Anal. ($C_{21}H_{27}N_5O_3$) C, H, N. ESI MS, *m*/*z* (rel%): 414 (100) [M + H]. HRMS: calcd for [M + H], 414.21358; found, 414.21351.

*N-(2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)amino)ethyl)methanesulfonamide (**9**).* To a solution of the compound **2j** (253 mg, 0.74 mmol) in CH_2Cl_2 (10 mL) and Et_3N (134 μL, 0.96 mmol) at 0 °C was added mesyl chloride (75 μL, 0.96 mmol), and reaction mixture was stirred at 0 °C for 5 h and then for additional 4 h at rt. Reaction was poured into a satd aq solution of $NaHCO_3$ (35 mL) and extracted with chloroform (70 mL). The organic phase was dried (Na_2SO_4) and evaporated. Chromatography of the residue on a silica gel column (75 g) with ethyl acetate afforded the product **9**. Crystalline powder was obtained after sonication of the compound from ether. Yield: 258 mg (83%); mp 208.5−209.5 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.75 (t, $J_{NH\text{-}1}$ = 6.3 Hz, 1H, 7′-NH), 7.42 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.22 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.4 Hz, 1H, H-6″), 7.21 (t, $J_{NH\text{-}2}$ = 6.0 Hz, 1H, NHSO$_2$), 7.01 (d, $J_{5''-6''}$ = 8.4 Hz, 1H, H-5″), 6.11 (s, 1H, H-6′), 3.79 (s, 3H, 3″-OCH_3), 3.78 (s, 3H, 4″-OCH_3), 3.49 (m, 2H, H-1), 3.23 (m, 2H, H-2), 2.91 (s, 3H, SO_2CH_3), 2.53 (s, 3H, 2′-CH_3), 2.40 (s, 3H, 5′-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 158.8 (C-5′), 149.9 (C-2′), 148.6 (C-3″), 146.9 (C-4″), 146.0 (C-3a′, C-7′), 126.2 (C-1″), 120.6 (C-6″), 112.7 (C-2″), 112.2 (C-5″), 105.6 (C-3′), 85.3 (C-6′), 55.8 and 55.7 (4″-OCH_3 and 3″-OCH_3), 41.6 (C-1), 41.5 (C-2), 39.6 (SO_2CH_3), 25.2 (5′-CH_3), 14.7 (2′-CH_3). Anal. ($C_{19}H_{25}N_5SO_3$) C, H, N. ESI MS, *m*/*z* (rel%): 420 (100) [M + H]. HRMS: calcd for [M + H], 420.17000; found, 420.16994.

*2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)amino)ethyl Acetate Hydrochloride (**10**).* To a solution of the compound **2f** (163 mg, 0.48 mmol) in CH_2Cl_2 (12.5 mL) and Et_3N (134 μL, 0.96 mmol) at 0 °C was added acetic anhydride (68 μL, 0.72 mmol), and the reaction mixture was stirred for 20 h at rt. Then same amount of the reagents was added and stirring continued for another 12 h and reaction was evaporated. Chromatography of the residue on silica gel column (75 g) with toluene:ethyl acetate (1:1) afforded the product **10** as viscous oil. Crystalline powder was obtained after conversion of the compound to hydrochloride salt (HCl/ether). Yield: 130 mg (71%); mp 186.5−188.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 13.50 (bs, 1H, HCl), 9.81 (bs, 1H, 7′-NH), 7.09 (d, $J_{5''-6''}$ = 8.4 Hz, 1H, H-5″), 7.06 (d, $J_{2''-6''}$ = 1.9 Hz, 1H, H-2″), 7.00 (dd, $J_{6''-2''}$ = 1.9, $J_{6''-5''}$ = 8.4 Hz, 1H, H-6″), 6.68 (s, 1 H, H-6′), 4.29 (t, J_{1-2} = 5.3 Hz, 2H, H-1), 3.84 (q, J_{2-1} = $J_{2\text{-}NH}$ = 5.3 Hz, 2H, H-2), 3.82 (2s, 2 × 3H, 3″-OCH_3, 4″-OCH_3), 2.56 (s, 3H, 5′-CH_3), 2.43 (s, 3H, 2′-CH_3), 2.00 (s, 3H, $COCH_3$). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 170.5 (CO), 155.3 (C-5′), 153.8 (C-2′), 148.9 (C-3″), 148.8 (C-7′), 148.6 (C-4″), 137.1 (C-3a′), 122.2 (C-6″), 121.6 (C-1″), 113.9 (C-2″), 112.2 (C-5″), 105.5 (C-3′), 85.3 (C-6′), 62.1 (C-1), 55.8 and 55.7 (4″-OCH_3 and 3″-OCH_3), 41.7 (C-2), 20.9 (CO**C**H$_3$), 20.0 (5′-CH_3), 13.4 (2′-CH_3). Anal. ($C_{20}H_{25}ClN_4O_4$·H_2O) C, H, N. ESI MS, *m*/*z* (rel%): 385 (100) [M + H]. HRMS: calcd for [M + H], 385.18703; found, 385.18714.

*N-(3-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)amino)propyl)acetamide Hydrochloride (**11**).* Starting material **2k** (281 mg, 0.79 mmol) was dissolved in chloroform (20 mL) and mixed with a satd aq solution of $NaHCO_3$ (8 mL). To this mixture was added dropwise at 0 °C a solution of Ac_2O (83 μL, 0.87 mmol) in chloroform (3 mL), and reaction mixture was stirred at 0 °C for 2 h. Then the reaction mixture was diluted with water (20 mL) and extracted with chloroform (2 × 70 mL). Combined organic phases were dried (Na_2SO_4) and evaporated. Residue was chromatographed on silica gel column (75 g) in ethyl acetate → ethyl acetate:acetone:ethanol:H_2O (20:3:1.2:0.8), and it was obtained an oily product. The oily residue was treated with etheral HCl, and crystalline product **11** was filtered off. Yield 252 mg (80%); mp 174−180 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 13.63 (bs, 1H, HCl), 9.84 (t, $J_{NH\text{-}3}$ = 5.9 Hz, 1H, 7′-NH), 7.76 (t, $J_{NH\text{-}1}$ = 5.8 Hz, 1H, NHCO), 7.10 (d, $J_{5''-6''}$ = 8.2 Hz, 1H, H-5″), 7.03 (d, $J_{2''-6''}$ = 1.7 Hz, 1H, H-2″), 6.98 (dd, $J_{6''-2''}$ = 1.7, $J_{6''-5''}$ = 8.2 Hz, 1H, H-6″), 6.71 (s, 1H, H-6′), 3.82 (s, 3H, 3″-OCH_3), 3.81 (s, 3H, 4″-OCH_3), 3.16 (m, 2H, H-3), 3.12 (m, 2H, H-3), 2.54 (s, 3H, 5′-CH_3), 2.42 (s, 3H, 2′-CH_3), 1.83 (s, 3H, $COCH_3$), 1.79 (m, 2H, H-2). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 169.6 (CO), 154.8 (C-5′), 153.9 (C-2′), 148.9 (C-3″), 148.7 (C-4″), 148.5 (C-7′), 136.6 (C-3a′), 122.3 (C-6″), 121.4 (C-1″), 113.9 (C-2″), 112.3 (C-5″), 105.4 (C-3′), 87.2 (C-6′), 55.9 and 55.7 (4″-OCH_3 and 3″-OCH_3), 40.4 (C-3), 36.0 (C-1), 28.7 (C-2), 22.8 (CO**C**H$_3$), 19.7 (5′-CH_3), 13.3 (2′-CH_3). Anal. ($C_{21}H_{28}ClN_5O_3$) C, H, N. ESI MS, *m*/*z* (rel%): 398 (100) [M + H]. HRMS: calcd for [M + H], 398.21867; found, 398.21894.

*3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidine-7-thiol (**12**).* A solution of chloro derivative **1** (160 mg, 0.51 mmol) and thiourea (66 mg, 0.87 mmol) in ethanol (10 mL) was heated to reflux for 15 h, and then the reaction mixture was cooled down to 0 °C. Precipitated solid was filtered and thoroughly washed with ethanol and ether to afford the product **12**. Yield: 136 mg (85%); mp 264.5−266 °C (decomp). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 12.67 (bs, 1H, SH), 7.08 (d, $J_{5'-6'}$ = 8.3 Hz, 1H, H-5′), 7.06 (bs, 1H, H-2′), 6.95 (dd, $J_{6'-2'}$ = 1.7, $J_{6'-5'}$ = 8.2 Hz, 1H, H-6′), 6.63 (s, 1H, H-6), 3.81 (s, 3H, 4′-OCH_3), 3.80 (s, 3H, 3′-OCH_3), 2.34 (s, 3H, 2-CH_3), 2.29 (s, 3H, 5-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 152.1 (C-2), 148.9 (C-3′), 148.3 (C-4′), 144.3 (C-5), 122.8 (C-1′), 122.3 (C-6′), 113.7 (C-2′), 112.3 (C-5′),111.4 (C-6), 103.5 (C-3), 55.8 and 55.7 (4′-OCH_3 and 3′-OCH_3), 18.3 (5-CH_3), 13.3 (2-CH_3), carbons 3a and 7 were not detected. Anal. ($C_{16}H_{17}N_3SO_2$) C, H, N. ESI MS, *m*/*z* (rel%): 338 (100) [M + Na]. HRMS: calcd for [M + H], 316.11142; found, 316.11138.

*4-(2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)thio)ethyl)morpholine (**13**).* Thioderivative **12** (100 mg, 0.32 mmol) was combined with 4-(2-chloroethyl)morpholine hydrochloride (89 mg, 0.48 mmol) in DMF (5 mL), and K_2CO_3 (132 mg, 0.96 mmol) was added. Resulted mixture was heated for 24 h at 75 °C and evaporated. Residue was partitionated between chloroform (70 mL) and water (50 mL). The organic phase was dried (Na_2SO_4) and evaporated. Product was isolated by column chromatography (silica gel, 50 g) with ethyl acetate → ethyl acetate:toluene:acetone:ethanol (17:4:3:1) to afford the product **13**. Solid was recrystallized from ether. Yield: 106 mg (77%); mp 150−152 °C. ^{1}H NMR (500 MHz,

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

$CDCl_3$) δ (ppm): 7.35 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.22 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 6.98 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.49 (s, 1H, H-6′), 3.94 (s, 3H, 3″-OCH_3), 3.92 (s, 3H, 4″-OCH_3), 3.75 (m, 4H, morph-O(CH_2)$_2$), 3.28 (m, 2H, H-1), 2.84 (m, 2H, H-2), 2.64 (s, 3H, 2′-CH_3), 2.57 (s, 3H, 5′-CH_3), 2.56 (m, 4H, morph-N(CH_2)$_2$). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 157.0 (C-5′), 150.1 (C-2′), 148.9 (C-3″), 147.6 (C-4″), 145.4 (C-7′), 125.2 (C-1″), 121.2 (C-6″), 112.5 (C-2″), 111.4 (C-5″), 108.1 (C-3′), 103.3 (C-6′), 66.8 (morph-O($\mathbf{C}H_2$)$_2$), 56.1 (C-2), 55.9 and 56.0 (3″-OCH3, 4″-OCH_3), 53.3 (morph-N($\mathbf{C}H_2$)$_2$), 28.2 (C-1), 25.1 (5′-CH_3), 14.4 (2′-CH_3), C-3′a was not detected. Anal. ($C_{22}H_{28}N_4SO_3$) C, H, N. ESI MS, m/z (rel%): 451 (100) [M + Na]. HRMS: calcd for [M + H], 429.19549; found, 429.19549.

*4-(4-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)thio)phenyl)morpholine (**14**).* 4-(Morpholin-4-yl)-benzenethiol (110 mg, 0.56 mmol) and K_2CO_3 (130 mg, 0.94 mmol) were heated at 65 °C in DMF (5 mL) for 0.5 h. Chloro derivative **1** (150 mg, 0.47 mmol) was then added in one portion, and the reaction mixture was heated at 90 °C for 18 h. The reaction mixture was evaporated and partitionated between ethyl acetate (75 mL) and water (50 mL). The water phase was then extracted with ethyl acetate (2 × 70 mL). The combined organic phases were dried (Na_2SO_4) and evaporated. Residue was chromatographed (silica gel, 80 g) with toluene−ethyl acetate (2:1). Product **14** was obtained as a solid after a crystallization from ethyl acetate. Yield: 97 mg (43%); mp 214−215 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.56 (d, J_{2-3} = 9.0 Hz, 2H, H-2), 7.36 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.23 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 7.02 (d, J_{3-2} = 9.0 Hz, 2H, H-3), 6.98 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 5.87 (s, 1H, H-6′), 3.94 (s, 3H, 3″-OCH_3), 3.92 (s, 3H, 4″-OCH_3), 3.90 (m, 4H, morph-O(CH_2)$_2$), 3.30 (m, 4H, morph-N(CH_2)$_2$), 2.67 (s, 3H, 2′-CH_3), 2.41 (s, 3H, 5′-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 157.3 (C-5′), 152.7 (C-4), 152.1 (C-2′), 150.6 (C-1), 148.9 (C-3″), 147.6 (C-4″), 145.5 (C-3′a), 137.5 (C-2), 125.4 (C-1″), 121.2 (C-6″), 116.1 (C-3), 113.9 (C-7′), 112.5 (C-2″), 111.5 (C-5″), 107.8 (C-3′), 103.5 (C-6′), 66.6 (morph-O($\mathbf{C}H_2$)$_2$), 56.0 and 55.9 (4″-OCH3, 3″-OCH_3), 48.0 (morph-N($\mathbf{C}H_2$)$_2$), 25.1 (5′-CH_3), 14.4 (2′-CH_3). Anal. ($C_{26}H_{28}N_4SO_3$) C, H, N. ESI MS, m/z (rel%): 499 (100) [M + Na]. HRMS: calcd for [M + H], 477.19549; found, 477.19544.

*4-(2-((3-(3,4-Dimethoxyphenyl)-2,5-dimethylpyrazolo[1,5-a]-pyrimidin-7-yl)oxy)ethyl)morpholine (**15**).* 4-(2-Hydroxyethyl)-morpholine (0.16 mL, 1.3 mmol) was dissolved in DMF (2 mL), and the solution was cooled to 0 °C. Sodium hydride (46 mg, 1.14 mmol, 60% dispersion in mineral oil) was added, and the reaction mixture was stirred at rt for 0.5 h. A solution of chloroderivative **1** (164 mg, 0.52 mmol) in DMF (2 mL + 1 mL for rinsing of the flask) was slowly added during 30 min, and then the reaction mixture was stirred for 2 h. Reaction was quenched with water (1 mL) and evaporated. Residue was taken into ethyl acetate (80 mL), and the organic phase was washed with water (2 × 30 mL). The organic phase was dried (Na_2SO_4) and evaporated. Product **15** was isolated by column chromatography (silica gel, 50 g) with ethyl acetate → ethyl acetate:acetone:ethanol:H_2O (20:3:1.2:0.8) and crystallized from ethyl acetate. Yield: 125 mg (58.3%); mp 139−140 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.36 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.22 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 6.97 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.05 (s, 1H, H-6′), 4.50 (t, J_{1-2} = 6.1 Hz, H-1), 3.94 (s, 3H, 3″-OCH_3), 3.92 (s, 3H, 4″-OCH_3), 3.75 (m, 4H, morph-O(CH_2)$_2$), 3.03 (t, J_{2-1} = 6.1 Hz, 2H, H-2), 2.65 (m, 4H, morph-N(CH_2)$_2$), 2.62 (s, 3H, 2′-CH_3), 2.56 (s, 3H, 5′-CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ (ppm): 160.3 (C-5′), 153.9 (C-7′), 152.5 (C-2′), 148.8 (C-3″), 147.6 (C-4′), 125.3 (C-1″), 121.2 (C-6″), 112.4 (C-2″), 111.4 (C-5″), 108.1 (C-3′), 87.4 (C-6′), 67.4 (C-1), 66.7 (morph-O($\mathbf{C}H_2$)$_2$), 56.5 (C-2), 55.9 and 55.8 (4″-OCH_3 and 3″-OCH_3), 54.0 (morph-N($\mathbf{C}H_2$)$_2$), 25.5 (5′-CH_3), 14.5 (2′-CH_3), C-3a was not detected. Anal. ($C_{22}H_{28}N_4O_4{\cdot}H_2O$) C, H, N. ESI MS, m/z (rel%): 435 (100) [M + Na]. HRMS: calcd for [M + H], 413.21833; found, 413.21868.

*8-Bromo-3-(3,4-dimethoxyphenyl)-2,6-dimethylimidazo[1,2-a]-pyridine (**21**).* *N*-Iodosuccinimide (2.03 g, 9 mmol) was added to the solution of 8-bromo-3-iodo-2,6-dimethylimidazo[1,2-*a*]pyridine[38] (1.35 g, 6 mmol) in DMF (120 mL), and the reaction mixture was stirred overnight at rt for 1 h. The reaction mixture was diluted with water (400 mL) and extracted with ethyl acetate (3 × 200 mL). Combined organic phases were dried over sodium sulfate and evaporated. Crude product was then directly used in the next step. The intermediate was dissolved in dioxane (88 mL) and water (22 mL), and sodium carbonate (1.77 g, 16.7 mmol) and boronic acid (1.1 g, 6 mmol) were added. Reaction mixture was three times degassed and purged with argon. To the reaction mixture Pd(dppf)Cl_2 (220 mg, 0.27 mmol) was added in one portion, and the mixture was stirred at 95 °C overnight under argon atmosphere. After cooling to ambient temperature, the mixture was partitioned between ethyl acetate (400 mL) and brine (200 mL). Organic phase was dried over anhydrous sodium sulfate and evaporated. The crude product was chromatographed on a silica gel column (200 g) in hexanes:ethyl acetate (3:2). It obtained 975 mg (45%, two steps) of an off-white solid. An analytical sample was obtained after recrystallization from hot ethyl acetate. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.03 (m, 1H, H-5), 7.45 (m, 1H, H-7), 7.14 (d, $J_{5'-6'}$ = 8.4, 1H, H-5′), 7.06 (d, $J_{2'-6'}$ = 1.9, 1H, H-2′), 7.02 (dd, $J_{6'-5'}$ = 8.4, $J_{6'-2'}$ = 1.9, 1H, H-6′), 3.83 (s, 3H, 4′-OCH_3), 3.80 (s, 3H, 3′-OCH_3), 2.33 (s, 3H, 2-CH_3), 2.24 (s, 3H, 6-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6DMSO-d_6) δ (ppm): 149.31 (C-3′), 149.1 (C-4′), 140.5 (C-2), 140.2 (C-8a), 129.2 (C-7), 122.9 (C-3), 122.3 (C-6′), 121.9 (C-6), 121.1 (C-5), 121.1 (C-1′), 113.1 (C-2′), 112.5 (C-5′), 109.5 (C-8), 55.8 (3′-OCH_3), 55.8 (4′-OCH_3), 17.5 (6-CH_3), 14.0 (2-CH_3). HRMS: calcd for [M + H], 361.05462; found, 361.05470.

*3-(3,4-Dimethoxyphenyl)-2,6-dimethyl-N-(2-morpholinoethyl)-imidazo[1,2-a]pyridin-8-amine (**16**).* Compound **21** (250 mg, 0.69 mmol), Me-DalPhos (42 mg, 0.1 mmol), $Pd_2(dba)_3$ (21 mg, 3% mol), and *t*-BuONa (94 mg, 0.98 mmol) were combined in a microwave vial and purged with argon. To this mixture, a solution of morpholinoethylamine (110 μL, 0.84 mmol) in degassed dioxane (6.5 mL) was added and reaction vessel was heated in microwave reactor for 2 h at 150 °C. Reaction mixture was evaporated and chromatographed on a silica gel column (100 g) in ethyl acetate → ethyl acetate:acetone:ethanol:water (19:3:1.2:0.8). It obtained 113 mg (40%) of the yellowish solid. This compound is rather unstable and difficult to store. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.30 (m, 1H, H-5), 7.12 (d, $J_{5'-6'}$ = 8.0, 1H, H-5′), 6.97−6.99 (m, 2H, H-2′, H-6′), 5.99 (m, 1H, H-7), 5.70 (t, $J_{NH-1''}$ = 5.5, 1H, NH), 3.82 (s, 3H, 4′-OCH_3), 3.79 (s, 3H, 3′-OCH_3), 3.60 (m, 4H, morph-O(CH_2)$_2$), 3.29 (m, 2H, H-1″), 2.61 (t, $J_{2''-1''}$ = 6.3, 2H, H-2″), 2.44 (bs, 4H, morph-N(CH_2)$_2$), 2.30 (s, 3H, 2-CH_3), 2.16 (s, 3H, 6-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 149.2 (C-3′), 148.6 (C-4′), 137.3 (C-2), 136.4 (C-8a), 136.2 (C-8), 122.5 (C-6), 122.1 (C-6′), 122.0 (C-1′), 121.7 (C-3), 113.1 (C-2′), 109.5 (C-5), 99.6 (C-7), 66.4 (morph-O(CH_2)$_2$), 56.7 (C-2″), 55.8 (3′-OCH_3), 55.7 (4′-OCH_3), 53.4 (morph-N(CH_2)$_2$), 39.3 (C-1″), 18.7 (6-CH_3), 13.9 (2-CH_3). HRMS: calcd for [M + H], 411.23907; found, 411.23901.

*6-Chloro-2-methyl-N-(2-morpholinoethyl)imidazo[1,2-b]-pyridazin-8-amine (**23**).* To a solution of **22** (1.5 g, 7.368 mmol) in CH_3CN (25 mL) were added DIPEA (1.97 mL, 11.31 mmol) and 4-(2-aminoethyl)morpholine (1.25 mL, 9.52 mmol). The reaction mixture was stirred at 80 °C for 16 h and cooled, and the solvent was evaporated. The residue was partitioned between $CHCl_3$ (70 mL) and satd NH_4Cl (70 mL), the layers were separated, and the aqueous layer was extracted with $CHCl_3$ (1 × 70 mL). The combined organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$:MeOH = 97:3), affording compound **23**, which was recrystallized from hot EtOAc to obtain an analytical sample. Yield: 2.014 g, (92%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.73 (q, J_{3-CH3} = 0.9 Hz, 1H, H-3), 7.48 (t, $J_{NH-1'}$ = 5.7 Hz, 1H, 8-NH), 6.18 (s, 1H, H-7), 3.56 (m, 4H, O-CH_2), 3.39 (m, 2H, H-1′), 2.55 (t, $J_{2'-1'}$ = 6.4 Hz, 2H, H-2′), 2.42 (m, 4H, N-CH_2), 2.30 (s, 3H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 147.0 (C-6), 142.9 (C-8), 139.8 (C-2), 131.6 (C-9), 114.8 (C-3), 90.8 (C-7), 66.4 (O-CH_2), 56.5 (C-2′), 53.4 (N-CH_2), 38.3 (C-1′), 14.4 (2-CH_3). Anal. ($C_{13}H_{18}ClN_5O$) C, H, N. HRMS: calcd for [M + H], 388.06288; found, 388.06284.

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

*2,6-Dimethyl-N-(2-morpholinoethyl)imidazo[1,2-b]pyridazin-8-amine (**24**).* To a solution of DABCO (90 mg, 0.8 mmol) in 2 mL of freshly distilled THF, $AlMe_3$ (2 M in hexanes, 0.8 mL, 1.6 mmol) was added dropwise and the mixture was stirred at rt for 30 min. A solution of **23** (296 mg, 1 mmol), $Pd_2(dba)_3$ (46 mg, 0.05 mmol), and X-Phos (48 mg, 0.10 mmol) in 7 mL of freshly distilled THF was subsequently added to the solution, and the reaction mixture was stirred in a sealed tube at 80 °C overnight. The mixture was cooled to 0 °C and quenched with satd NH_4Cl diluted with acetone and MeOH and filtered over Celite. The filtrate was evaporated to near dryness and partitioned between EtOAc and H_2O. The aqueous layer was extracted twice with EtOAc, and the combined organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate:acetone:ethanol:water (20:3:1:1)), and compound **24** was obtained as an off-white solid. Yield: 115 mg (42%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.61 (s, 1H, H-3), 6.83 (s, 1H, 8-NH), 5.94 (s, 1H, H-7), 3.58 (m, 4H, O−CH_2), 3.35 (m, 2H, H-1′), 2.56 (t, $J_{2'-1'}$ = 5.6 Hz, 2H, H-2′), 2.43 (m, 4H, N-CH_2), 2.30 (s, 3H, 2-CH_3), 2.29 (s, 3H, 6-CH_3). Anal. ($C_{14}H_{21}N_5O$) C, H, N.

*3-Iodo-2,6-dimethyl-N-(2-morpholinoethyl)imidazo[1,2-b]pyridazin-8-amine (**25**).* Compound **24** (210 mg, 0.763 mmol) was dissolved in dry CH_2Cl_2 (8 mL) and AcOH (320 μL), and the solution was cooled to 0 °C. *N*-Iodosuccinimide (180 mg, 0.8 mmol) was added in one portion, and the reaction mixture was stirred at 0 °C for 30 min and at rt for 2 h. The reaction mixture was diluted with $CHCl_3$ and treated with satd Na_2CO_3:10% aq $Na_2S_2O_3$ = 1:1. The aqueous layer was extracted 1× with $CHCl_3$, and the combined organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5)), affording compound **25** as an off-white solid. Yield: 151 mg (49%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.13 (bs, 1H, 8-NH), 6.90 (s, 1H, H-7), 3.77 (t, $J_{CH_2\text{-}CH_2}$ = 4.4 Hz, 4H, O-CH_2), 3.40 (m, 2H, H-1′), 2.74 (t, $J_{2'-1'}$ = 6.0 Hz, 2H, H-2′), 2.55 (bs, 4H, N-CH_2), 2.49 (s, 3H, 2-CH_3), 2.46 (s, 3H, 6-CH_3). ^{13}C NMR (125 MHz, CDCl3) δ (ppm): 153.6 (C-6), 143.3 (C-2), 141.1 (C-8), 134.9 (C-9), 93.4 (C-7), 68.5 (C-3), 66.7 (O-CH_2), 56.6 (C-2′), 53.5 (N-CH_2), 39.0 (C-1′), 22.4 (6-CH_3), 14.9 (2-CH_3). Anal. ($C_{14}H_{20}IN_5O$) C, H, N.

General Procedure for Suzuki Reaction: Preparation of Compounds 17, 28 and 30a−c. A suspension of iodine derivative **25**, **27**, or **29** (1 equiv) and the corresponding boronic acid (1.1 equiv) in dioxane:1 M K_2CO_3 = 4:1 was purged repeatedly with argon, and $Pd(PPh_3)_4$ (5 mol %) was added to the mixture. The reaction mixture was degassed again and subsequently stirred at 95−105 °C between 14 and 22 h. The mixture was allowed to cool and diluted with $CHCl_3$ and H_2O. The layers were separated, and the aqueous layer was extracted with $CHCl_3$; the combined organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography, and the product was recrystallized to afford an analytically pure sample.

*3-(3,4-Dimethoxyphenyl)-2,6-dimethyl-N-(2-morpholinoethyl)-imidazo[1,2-b]pyridazin-8-amine (**17**).* This compound has been prepared according to the general procedure described for Suzuki reaction. The following reagents, amounts, and conditions have been employed: compound **25** (149 mg, 0.371 mmol), 3,4-dimethoxyphenylboronic acid (75 mg, 0.412 mmol), and $Pd(PPh_3)_4$ (21 mg, 0.0182 mmol) in 1 M K_2CO_3 (1.2 mL) and dioxane (4 mL). The reaction mixture was stirred at 95−100 °C overnight and worked up as described above, including silica gel column chromatography (ethyl acetate:acetone:ethanol:water (20:3:1:1)), yielding compound **17** as a white solid. Recrystallization from hot EtOAc/MeOH afforded an analytically pure sample. Yield: 109 mg (71%); mp 161.3−162.0 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.29 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.17 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 7.07 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.88 (t, $J_{NH\text{-}1'}$ = 5.6 Hz, 1H, 8-NH), 6.01 (s, 1H, H-7), 3.81, 3.78 (2s, 2 × 3H, 3″-O-CH_3, 4″-O-CH_3), 3.59 (m, 4H, O-CH_2), 3.38 (m, 2H, H-1′), 2.58 (t, $J_{2'-1'}$ = 6.4 Hz, 2H, H-2′), 2.44 (bs, 4H, N-CH_2), 2.41 (s, 3H, 2-CH_3), 2.31 (s, 3H, 6-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 152.05 (C-6), 148.48 (C-3″), 148.28 (C-4″), 141.63 (C-8), 136.55 (C-2), 131.44 (C-9), 124.22 (C-3), 122.12 (C-1″), 121.91 (C-6″), 113.14 (C-2″), 111.86 (C-5″), 91.46 (C-7), 66.43 (O-CH_2), 56.53 (C-2′), 55.75 (3″-O-CH_3, 4″-O-CH_3), 53.44 (N-CH_2), 38.94 (C-1′), 22.03 (6-CH_3), 14.81 (2-CH_3). Anal. ($C_{22}H_{29}N_5O_3$) C, H, N. HRMS: calcd for [M + H], 412.23432; found, 412.23428.

*2-Methyl-N-(2-morpholinoethyl)imidazo[1,2-b]pyridazin-8-amine (**26**).* To a solution of compound **23** (590 mg, 2 mmol) in THF (12 mL) and MeOH (12 mL) was added 10% Pd/C (60 mg) and NEt_3 (560 μL, 4 mmol), and the reaction mixture was stirred under a H_2 atmosphere (3 atm) in an autoclave at rt for 21 h. The autoclave was flushed with Ar, and then the mixture was filtered over Celite to remove the catalyst and washed with MeOH and acetone. The solvent was evaporated and the residue was partitioned between $CHCl_3$ and satd $NaHCO_3$ (40 mL each), and the layers were separated. The aqueous layer was extracted with $CHCl_3$ (1 × $CHCl_3$), and the combined organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5) → ethyl acetate:acetone:ethanol:water (20:3:1:1)), yielding compound **26** as an off-white solid. Recrystallization from hot EtOAc afforded an analytically pure sample. Yield: 337 mg (64%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.93 (d, J_{6-7} = 5.4 Hz, 1H, H-6), 7.71 (s, 1H, H-3), 6.98 (bs, 1H, 8-NH), 6.02 (d, J_{7-6} = 5.4 Hz, 1H, H-7), 3.57 (bs, 4H, O-CH_2), 3.38 (bs, 2H, H-1′), 2.55 (t, $J_{2'-1'}$ = 6.1 Hz, 2H, H-2′), 2.43 (m, 4H, N-CH_2), 2.31 (s, 3H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 144.1 (C-6), 141.9 (C-8), 139.3 (C-2), 133.1 (C-9), 114.2 (C-3), 90.0 (C-7), 66.4 (O-CH_2), 56.5 (C-2′), 53.4 (N-CH_2), 38.9 (C-1′), 14.4 (2-CH_3). Anal. ($C_{13}H_{19}N_5O$) C, H, N. HRMS: calcd for [M + H], 388.06288 $(M + H)^+$; found, 388.06284.

*3-Iodo-2-methyl-N-(2-morpholinoethyl)imidazo[1,2-b]pyridazin-8-amine (**27**).* To a solution of compound **26** (409 mg, 1.565 mmol) in dry DMF (16 mL), *N*-iodosuccinimide (423 mg, 1.88 mmol) was added in one portion and the reaction mixture was stirred at rt overnight. The solvent was evaporated to near dryness, coevaporated with toluene, and the residue was partitioned between $CHCl_3$ and 10% aq $Na_2S_2O_3$ (25 mL each). The layers were separated, the aqueous layer was extracted with $CHCl_3$ (1 × 25 mL), and the combined organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5)), which furnished compound **27** as an off-white solid. Trituration with ether and filtration/wash yielded an analytically pure sample. Yield: 248 mg (41%); mp 130 °C (decomposed). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.06 (d, J_{6-7} = 5.6 Hz, 1H, H-6), 7.12 (t, $J_{NH\text{-}1'}$ = 5.7 Hz, 1H, 8-NH), 6.13 (d, J_{7-6} = 5.6 Hz, 1H, H-7), 3.57 (m, 4H, O-CH_2), 3.38 (m, 2H, H-1′), 2.55 (t, $J_{2'-1'}$ = 6.7 Hz, 2H, H-2′), 2.42 (m, 4H, N-CH_2), 2.35 (s, 3H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 144.6 (C-6), 143.1 (C-2), 141.9 (C-8), 135.6 (C-9), 91.0 (C-7), 70.8 (C-3), 66.4 (O-CH_2), 56.4 (C-2′), 53.4 (N-CH_2), 39.1 (C-1′), 14.8 (2-CH_3). Anal. ($C_{13}H_{18}IN_5O$) C, H, N. HRMS calcd for $C_{13}H_{19}IN_5O$ *m*/*z*: HRMS calcd for [M + H], 388.06288; found, 388.06284.

*3-(3,4-Dimethoxyphenyl)-2-methyl-N-(2-morpholinoethyl)-imidazo[1,2-b]pyridazin-8-amine (**28**).* This compound has been prepared according to the general procedure for Suzuki reaction. The following reagents, amounts, and conditions have been employed: compound **27** (174 mg, 0.449 mmol), 3,4-dimethoxyphenylboronic acid (90 mg, 0.494 mmol), and $Pd(PPh_3)_4$ (26 mg, 0.0225 mmol) in 1 M K_2CO_3 (1.5 mL) and dioxane (5 mL). The reaction mixture was stirred at 95−100 °C overnight and worked up as described above, including silica gel column chromatography (ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5)), yielding compound **28** as a white solid. Recrystallization from hot MeOH afforded an analytically pure sample. Yield: 149 mg (83%); mp 169.1−169.9 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.99 (d, J_{6-7} = 5.5 Hz, 1H, H-6), 7.24 (d, $J_{2''-6''}$ = 2.0 Hz, H-2″), 7.18 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.2 Hz, 1H, H-6″), 7.07 (d, $J_{5''-6''}$ = 8.2 Hz, 1H, H-5″), 7.04 (t, $J_{NH\text{-}1'}$ = 5.7 Hz, 1H, 8-NH), 6.09 (d, J_{7-6} = 5.5 Hz, 1H, H-7), 3.81 (s, 3H, 4″-O-CH_3), 3.78 (s, 3H, 3″-O-CH_3), 3.59 (m, 4H, O-CH_2), 3.41 (m, 2H, H-1′), 2.58 (t, $J_{2'-1'}$ = 6.5 Hz, 2H, H-2′), 2.44 (m, 4H, *N*-CH_2), 2.43 (s, 3H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 148.5 (C-3″), 148.4 (C-4″),

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

144.0 (C-6), 142.1 (C-8), 136.9 (C-2), 132.3 (C-9), 124.5 (C-3), 122.1 (C-6″), 121.9 (C-1″), 113.2 (C-2″), 111.9 (C-5″), 90.2 (C-7), 66.4 (O-CH_2), 56.5 (C-2′), 55.8, 55.8 (3″-O-CH_3), 4″-O-CH_3), 53.4 (*N*-CH_2), 39.0 (C-1′), 14.7 (2-CH_3). Anal. ($C_{21}H_{27}N_5O_3$) C, H, N. HRMS: calcd for [M + H], 398.21867; found, 398.21860.

*6-Chloro-3-iodo-2-methyl-N-(2-morpholinoethyl)imidazo[1,2-b]-pyridazin-8-amine (**29**).*[45] To a solution of compound **23** (705 mg, 2.39 mmol) in CH_2Cl_2 (25 mL) and AcOH (1 mL) at 0 °C was added *N*-iodosuccinimide (699 mg, 3.10 mmol), and the reaction mixture was stirred at 0 °C for 5 min and at rt for 2.5 h. The mixture was diluted with CH_2Cl_2 (25 mL) and washed with 10% aq $Na_2S_2O_3$ (40 mL). The layers were separated, and the aqueous layer was extracted with $CHCl_3$ (2 × 40 mL). The combined layer was subsequently dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography and eluted with Michal 17, which gave compound **29** as an off-white solid. Recrystallization from hot MeOH/$CHCl_3$ afforded an analytical sample. Yield: 412 mg (41%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.59 (t, $J_{NH\text{-}1'}$ = 5.8 Hz, 1H, 8-NH), 6.29 (s, 1H, H-7), 3.56 (m, 4H, O-CH_2), 3.40 (m, 2H, H-1′), 2.54 (t, $J_{2'-1'}$ = 6.4 Hz, 2H, H-2′), 2.42 (m, 4H, *N*-CH_2), 2.34 (s, 3H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 147.7 (C-6), 143.7 (C-2), 142.9 (C-8), 134.3 (C-9), 91.8 (C-7), 71.8 (C-3), 66.4 (O-CH_2), 56.4 (C-2′), 53.4 (N-CH_2), 38.6 (C-1′), 14.9 (2-CH_3). Anal. ($C_{13}H_{17}ClIN_5O$) C, H, N. HRMS: calcd for [M + H], 388.06288; found, 388.06284.

*6-Chloro-3-(3,4-dimethoxyphenyl)-2-methyl-N-(2-morpholinoethyl)imidazo[1,2-b]pyridazin-8-amine (**30a**).* This compound has been prepared according to the general procedure described above. The following reagents, amounts, and conditions have been employed: compound **29** (200 mg, 0.474 mmol), 3,4-dimethoxyphenylboronic acid (95 mg, 0.523 mmol), and $Pd(PPh_3)_4$ (27 mg, 0.0234 mmol) in 1 M K_2CO_3 (1.2 mL) and dioxane (4 mL). The reaction mixture was stirred at 100 °C for 16 h and worked up as described above, including silica gel column chromatography ($CHCl_3$:MeOH = 97:3), yielding compound **30a** as a white solid. Recrystallization from hot MeOH/$CHCl_3$ afforded an analytically pure sample. Yield: 157 mg (77%); mp 195.8−196.9 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.25 (d, $J_{2''-6''}$ = 1.9 Hz, H-2″), 7.18 (dd, $J_{6''-5''}$ = 8.3, $J_{6''-2''}$ = 1.9 Hz, 1H, H-6″), 7.16 (bs, 1H, NH), 7.11 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.21 (s, 1H, H-7), 3.85, 3.82 (2*s*, 6H, 3″-O-CH_3, 4″-O-CH_3), 3.61 (m, 4H, O-CH_2), 3.48 (m, 2H, H-1′), 2.56 (t, $J_{2'-1'}$ = 6.2 Hz, 2H, H-2′), 2.49 (m, 4H, *N*-CH_2), 2.43 (s, 3H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 148.8 and 148.7 (C-3″and C-4″), 146.6 (C-6), 142.8 (C-8), 137.1 (C-2), 130.6 (C-9), 124.8 (C-3), 122.0 (C-6″), 121.1 (C-1″), 113.8 (C-2″), 112.5 (C-5″), 90.8 (C-7), 66.0 (O-CH_2), 56.2 (C-2′), 55.8 and 55.73 (3″-O-CH_3 and 4″-O-CH_3), 53.0 (*N*-CH_2), 39.2 (C-1′), 14.2 (2-CH_3). Anal. ($C_{21}H_{17}ClIN_5O$) C, H, N. HRMS: calcd for [M + H], 432.17969; found, 432.17957.

*6-Chloro-3-(3-fluoro-4-methoxyphenyl)-2-methyl-N-(2-morpholinoethyl)imidazo[1,2-b]pyridazin-8-amine (**30b**).* This compound was prepared according to the general procedure described above. The following reagents, amounts, and conditions were employed: compound **29** (200 mg, 0.474 mmol), 3-fluoro-4-methoxyphenylboronic acid (89 mg, 0.523 mmol), and $Pd(PPh_3)_4$ (27 mg, 0.0234 mmol) in 1 M K_2CO_3 (1.2 mL) and dioxane (4 mL). The reaction mixture was stirred at 95 °C for 16 h and at 100 °C for 4 h and worked up as described above, including silica gel column chromatography ($CHCl_3$:MeOH = 97:3), yielding compound **30b** as a white solid. Recrystallization from hot MeOH/$CHCl_3$ afforded an analytically pure sample. Yield: 152 mg (76%); mp 195.0−195.8 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.49 (dd, $J_{2''-6''}$ = 2.1, $J_{2''\text{-}F}$ = 12.8 Hz, H-2″), 7.42 (ddd, $J_{6''-5''}$ = 8.5, $J_{6''-2''}$ = 2.1, $J_{6''\text{-}F}$ = 1.2 Hz, 1H, H-6″), 7.30 (d, $J_{5''-6''}$ = 8.5, $J_{5''\text{-}F}$ = 9.1 Hz, 1H, H-5″), 7.23 (m, 1H, NH), 6.21 (s, 1H, H-7), 3.93 (s, 3H, 4″-O-CH_3), 3.60 (m, 4H, O-CH_2), 3.48 (m, 2H, H-1′), 2.63 (t, $J_{2'-1'}$ = 6.4 Hz, 2H, H-2′), 2.48 (m, 4H, *N*-CH_2), 2.43 (s, 3H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 151.2 (d, $J_{3''\text{-}F}$ = 243.9 Hz, C-3″), 146.8 (C-6), 146.64 (d, $J_{4''\text{-}F}$ = 10.8 Hz, C-4″), 142.8 (C-8), 137.5 (C-2), 130.8 (C-9), 125.4 (d, $J_{6''\text{-}F}$ = 3.4 Hz, C-6″), 123.5 (d, $J_{3\text{-}F}$ = 1.7 Hz, C-3), 121.3 (d, $J_{1''\text{-}F}$ = 7.6 Hz, C-1″), 116.3 (d, $J_{2''\text{-}F}$ = 19.4 Hz, C-2″), 114.2 (d, $J_{5''\text{-}F}$ = 2.2 Hz, C-5″), 91.1 (C-7), 66.1 (O−CH_2), 56.2 (C-2′), 56.2 (4″-O-CH_3), 53.1 (*N*-CH_2), 39.2 (C-1′), 14.2 (2-CH_3). Anal. ($C_{20}H_{23}ClFN_5O_3$) C, H, N. HRMS: calcd for [M + H], 420.15971; found, 420.15963.

*6-Chloro-3-(pyridin-3-yl)-2-methyl-N-(2-morpholinoethyl)-imidazo[1,2-b]pyridazin-8-amine (**30c**).* This compound was prepared according to the general procedure described above. The following reagents, amounts, and conditions were employed: compound **29** (229 mg, 0.543 mmol), 3-pyridineboronic acid (73 mg, 0.594 mmol), and $Pd(PPh_3)_4$ (31 mg, 0.027 mmol) in 1 M K_2CO_3 (1.5 mL) and dioxane (6 mL). The reaction mixture was stirred at 95 °C overnight and at 105 °C for 8 h and worked up as described above, including silica gel column chromatography (ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5) → ethyl acetate:acetone:ethanol:water (20:3:1:1)), yielding compound **30c** as a white solid. Recrystallization from hot EtOAc/MeOH afforded an analytically pure sample. Yield: 107 mg (53%); mp 179.1−180.0 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.84 (dd, $J_{2''-5''}$ = 0.8, $J_{2''-4''}$ = 2.3 Hz, H-2″), 8.59 (dd, $J_{6''-4''}$ = 1.7, $J_{6''-5''}$ = 4.8 Hz, 1H, H-6″), 8.07 (ddd, $J_{4''-6''}$ = 1.7, $J_{4''-2''}$ = 2.3, $J_{4''-5''}$ = 7.9 Hz, 1H, H-4″), 7.66 (t, $J_{NH\text{-}1'}$ = 5.8 Hz, 1H, 8-NH), 7.56 (ddd, $J_{5''\text{-}2''}$ = 0.8, $J_{5''-6''}$ = 4.8, $J_{5''-4''}$ = 7.9 Hz, 1H, H-5″), 6.31 (s, 1H, H-7), 3.57 (m, 4H, O-CH_2), 3.44 (m, 2H, H-1′), 2.57 (t, $J_{2'-1'}$ = 6.3 Hz, 2H, H-2′), 2.46 (m, 4H, *N*-CH_2), 2.42 (s, 3H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 149.6 (C-2″), 148.7 (C-6″), 147.4 (C-6), 143.2 (C-8), 138.7 (C-2), 136.5 (C-4″), 131.7 (C-9), 125.0 (C-3″), 123.8 (C-5″), 122.1 (C-3), 91.6 (C-7), 66.4 (O-CH_2), 56.5 (C-2′), 53.4 (*N*-CH_2), 38.9 (C-1′), 14.5 (2-CH_3). Anal. ($C_{18}H_{21}ClN_6O$) C, H, N. HRMS: calcd for [M + H], 373.15381; found, 373.15371.

8-(3,4-Dimethoxyphenyl)-2,7-dimethylpyrazolo[1,5-*a*]-[1,3,5]triazin-4(3*H*)-one (32). A mixture 8-bromo-2,7-dimethylpyrazolo[1,5-*a*][1,3,5]triazin-4(3*H*)-one (**23**) (700 mg, 2.9 mmol), Na_2CO_3 (436 mg, 4.4 mmol), boronic acid (757 mg, 4.4 mmol), and Pd(dppf)Cl_2 (232 mg, 10%) in deoxygenated dioxane (30 mL) and water (7.5 mL) was heated under argon atmosphere for 16 h at 95 °C. After cooling to ambient temperature, the mixture was partitioned between ethyl acetate (250 mL) and brine (100 mL). Water phase was extracted with ethyl acetate (2 × 200 mL). Organic phases were combined, dried over anhydrous sodium sulfate, and evaporated. The crude product was chromatographed on a silica gel column (200 g) in ethyl acetate → ethyl acetate:toluene:acetone:ethanol (17:4:3:1). Product **24** was obtained after recrystallization from ethyl acetate as an off-white solid. Yield: 653 mg (75%); mp 251.5−252 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.36 (bs, 1H, NH), 7.22 (d, $J_{2'-6'}$ = 1.9 Hz, 1H, H-2′), 7.12 (dd, $J_{6'-2'}$ = 1.9, $J_{6'-5'}$ = 8.3 Hz, 1H, H-6′), 7.02 (d, $J_{5'-6'}$ = 8.3 Hz, 1H, H-5′), 3.78 (s, 6H, 4′-OCH_3, 3′-OCH_3), 2.42 (s, 3H, 7-CH_3), 2.32 (s, 3H, 2-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 154.3 (C-2), 152.8 (C-7), 148.7 (C-3′), 147.9 (C-4′), 145.4 (C-8a), 144.1 (C-4), 124.0 (C-1′), 121.2 (C-6′), 112.7 (C-2′), 112.1 (C-5′), 110.3 (C-8), 55.7 (3′-OCH_3, 4′-OCH_3), 21.2 (2-CH_3), 14.5 (7-CH_3). Anal. ($C_{15}H_{15}N_4O_3$) C, H, N. ESI MS, *m*/*z* (rel%): 23 (100) [M + Na]. HRMS: calcd for [M + Na], 323.11146; found, 323.11154.

8-(3,4-Dimethoxyphenyl)-2,7-dimethyl-*N*-(2-morpholinoethyl)pyrazolo[1,5-*a*][1,3,5]triazin-4-amine (18). Compound **32** (300 mg, 1 mmol) was suspended in $POCl_3$ (7 mL) and dimethylaniline (0.4 mL), and the reaction mixture was heated at 120 °C for 20 h, cooled down, and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (20 mL) at 0 °C and 2-morpholinoethanamine (0.92 mL, 7 mmol), and the reaction mixture was stirred for 16 h. Then reaction mixture was diluted with solution of satd aq sodium bicarbonate (50 mL) and extracted with ethyl acetate (2 × 150 mL). Combined organic phases were dried with sodium sulfate and evaporated. Residue was chromatographed on silica gel column (100 g) in ethyl acetate → ethyl acetate:toluene:acetone:ethanol (17:4:3:2) to afford product **18** which was recrystallized from ethyl acetate. Yield: 280 mg (68%); mp 126−126.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.43 (t, $J_{NH\text{-}2'}$ = 5.8 Hz, 1H, NH), 7.31 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.17 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 7.02 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 3.79 (s, 3H, 3″-OCH_3), 3.78 (s, 3H, 4″-OCH_3), 3.63 (m, 2H, H-2′), 3.55 (m, 4H, morph-O(CH_2)$_2$), 2.56 (t, $J_{1'-2'}$ =

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

6.5 Hz, 2H, H-1′), 2.51 (s, 3H, 7-CH_3), 2.44 (bs, 4H, morph-N(CH_2)$_2$), 2.38 (s, 3H, 2-CH_3). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 163.0 (C-2), 152.1 (C-7), 148.7 (C-3″), 148.2 (C-4), 147.4 (C-4″),146.0 (C-8a), 124.9 (C-1″), 120.9 (C-6″), 112.7 (C-2″), 112.2 (C-5″), 106.9 (C-8), 66.4 (morph-O(CH_2)$_2$), 57.2 (C-1′), 55.9 and 55.8 (3″-OCH3, 4″-OCH_3), 53.4 (morph-N(CH_2)$_2$), 37.1 (C-2′), 26.0 (2-CH_3), 14.5 (7-CH_3). Anal. ($C_{21}H_{28}N_6O_3$) C, H, N. ESI MS, m/z (rel%): 413 (100) [M + H]. HRMS: calcd for [M + H], 413.22957; found, 413.22980.

***N*-(2-(8-(3,4-Dimethoxyphenyl)-2,7-dimethylpyrazolo[1,5-*a*]-[1,3,5]triazin-4-ylamino)ethyl)acetamide (33).** Compound **32** (300 mg, 1 mmol) was suspended in $POCl_3$ (7 mL) and dimethylaniline (0.4 mL), and the reaction mixture was heated at 120 °C for 20 h, cooled down, and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (20 mL) at 0 °C and *N*-(2-aminoethyl)-acetamide (0.67 mL, 7 mmol), and the reaction mixture was stirred for 16 h. Then reaction mixture was diluted with solution of satd aq sodium bicarbonate (50 mL) and extracted with ethyl acetate (2 × 150 mL). Combined organic phases were dried with sodium sulfate and evaporated. Residue was chromatographed on silica gel column (100 g) in ethyl acetate → ethyl acetate:toluene:acetone:ethanol (17:4:3:2) to afford product **33** as a solid which was recrystallized from ethyl acetate. Yield: 250 mg (65%); mp 203.5−204 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ (ppm): 8.59 (t, $J_{NH\text{-}2}$ = 5.8 Hz, 1H, NH), 7.98 (t, $J_{NH\text{-}1}$ = 5.7 Hz, 1H, NHCO), 7.31 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.18 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″),7.02 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 3.79 (s, 3H, 3″-OCH_3), 3.78 (s, 3H, 4″-OCH_3), 3.56 (m, 2H, H-2), 3.32 (m, 2H, H-1), 2.52 (s, 3H, 7′-CH_3), 2.39 (s, 3H, 2′-CH_3), 1.79 (s, 3H, $COCH_3$). ^{13}C NMR (150 MHz, DMSO-d_6) δ (ppm): 169.7 (CO), 162.9 (C-2′), 152.0 (C-7′), 148.7 (C-3″), 148.4 (C-4′), 147.4 (C-4″), 146.1 (C-8′a), 124.9 (C-1″), 120.9 (C-6″), 112.7 (C-2″), 112.2 (C-5″), 106.8 (C-8′), 55.7 and 55.8 (3″-OCH3, 4″-OCH_3), 40.1 (C-2), 38.3 (C-1), 26.0 (2′-CH_3), 22.8 ($COCH_3$), 14.5 (7′-CH_3). Anal. ($C_{20}H_{25}N_5O_3$) C, H, N. ESI MS, m/z (rel%): 407 (100) [M + Na]. HRMS: calcd for [M + H], 385.19827; found, 385.19833.

6,8-Dichloro-3-iodo-2-methylimidazo[1,2-*b*]pyridazine (34). To the suspension of 6,8-dichloro-2-methylimidazo[1,2-*b*]pyridazine (1.155 g, 5.78 mmol) in DMF (8 mL), NIS (1.43 g, 6.36 mmol) was added in one portion, followed by acetic acid (0.5 mL, 8.67 mmol). The reaction mixture was stirred overnight at 65 °C, cooled to rt, and partitioned between DCM/$Na_2S_2O_3$ (100 mL each). The inorganic phase was washed with DCM (2 × 50 mL), and the combined organic phases were dried over sodium sulfate and evaporated. Oily residue was dissolved in minimum amount of DCM, adsorbed onto silica, and purified by flash column chromatography (hexane/EtOAc 15−25%), affording **34** as yellow solid. An analytical sample was obtained after recrystallization from hot acetone. Yield: 1.745 g (93%); mp 127.1−127.7 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.81 (s, 1 H, H-7), 2.43 (s, 3 H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 148.5 (C-2), 145.4 (C-6), 138.0 (C-8), 132.6 (C-9), 118.2 (C-7), 75.1 (C-3), 15.3 (2-CH_3). Anal. ($C_7H_4Cl_2N_3I$) C, H, N. HRMS: calcd for [M + H], 327.88997; found, 327.89011.

***N*-(2-((6-Chloro-3-iodo-2-methylimidazo[1,2-*b*]pyridazin-8-yl)amino)ethyl)acetamide (35).** To a solution of **34** (0.43 g, 1.3112 mmol) in EtOH (5 mL), DIPEA (0.365 mL, 1.6 equiv) and *N*-(2-aminoethyl)acetamide (0.2 mL, 1.6 equiv) were subsequently added and the mixture was heated in a microwave reactor at 100 °C for 2 h. Silica gel column chromatography (EtOAc:acetone:EtOH:water, 20:3:1.6:0.4) afforded product **26** as a yellowish solid. Recrystallization from hot MeOH/$CHCl_3$ yielded an analytically pure sample. Yield: 0.465 g (90%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.03 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1H, NH-CO), 7.87 (t, $J_{NH\text{-}2}$ = 6.0 Hz, 1H, 8′-NH), 6.30 (s, 1H, H-7′), 3.33 (bs, 2H, H-2), 3.23 (m, 2H, H-1), 2.34 (s, 3H, 2′-CH_3), 1.79 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 147.7 (C-6′), 143.7 (C-2′), 143.1 (C-8′), 134.3 (C-9′), 91.7 (C-7′), 71.7 (C-3′), 41.9 (C-2), 37.8 (C-1), 22.8 ($COCH_3$), 14.9 (2′-CH_3). HRMS: calcd for [M + H], 393.99261; found, 393.99255.

General Procedure for Suzuki Coupling Reactions: Preparation of Compounds 36a−u. A round-bottom flask was charged with **34** and appropriate boronic acid derivative (1.1 equiv), 1,4-dioxane:water (4:1), and potassium carbonate (3 equiv). A flask was equipped with stir bar and rubber septum, stirred at RT, and degassed 3 times and purged with argon. To the reaction mixture, $Pd(PPh_3)_4$ (5 mol %) was added in one portion and the suspension was refluxed for 2 h and then stirred at 95 °C overnight under argon atmosphere. Upon completion of the reaction (monitored by TLC), the mixture was cooled to rt, diluted with water, and extracted with DCM (3 × 50 mL). Combined organic phases were dried over sodium sulfate, evaporated, and purified by column chromatography.

*N-(2-((6-Chloro-3-(3,4-dimethoxyphenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**36a**).* Mobile phase: ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5). Recrystallization from hot MeOH. Yield 237 mg (97%) as an off-white solid; mp 192.4−194.2 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.05 (t, $J_{NH\text{-}1}$ = 5.7 Hz, 1H, NH-CO), 7.81 (t, $J_{NH\text{-}2}$ = 6.0 Hz, 1H, 8′-NH), 7.20 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 7.15 (dd, $J_{6''-2''}$ = 2.0, $J_{6''-5''}$ = 8.3 Hz, 1H, H-6″), 7.10 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.26 (s, 1H, H-7′), 3.82 (s, 3H, 4″-O-CH_3), 3.78 (s, 3H, 3″-O-CH_3), 3.36 (m, 2H, H-2), 3.26 (m, 2H, H-1), 2.41 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 148.8 (C-4″), 148.6 (C-3″), 147.1 (C-6′), 143.3 (C-8′), 137.4 (C-2′), 130.9 (C-9′), 125.1 (C-3′), 122.2 (C-6″), 121.0 (C-1″), 113.2 (C-2″), 112.0 (C-5″), 90.9 (C-7′), 55.8 (3″-O-CH_3, 4″-O-CH_3), 41.8 (C-2), 37.9 (C-1), 22.8 ($COCH_3$), 14.6 (2′-CH_3). Anal. ($C_{19}H_{22}ClN_5O_3$) C, H, N. HRMS: calcd for [M + H], 404.14839; found, 404.14827.

*N-(2-((6-Chloro-2-methyl-3-(pyridin-3-yl)imidazo[1,2-b]-pyridazin-8-yl)amino)ethyl)acetamide (**36b**).* Mobile phase: ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5) → ethyl acetate:acetone:ethanol:water (20:3:1:1). Recrystallization from hot MeOH. Yield: 178 mg (85%) as a white solid; mp 211.1−212.4 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.84 (dd, $J_{2''-5''}$ = 0.8, $J_{2''-4''}$ = 2.2 Hz, 1H, H-2″), 8.60 (dd, $J_{6''-5''}$ = 4.8, $J_{6''-4''}$ = 1.6 Hz, 1H, H-6″), 8.07 (ddd, $J_{4''-6''}$ = 1.6, $J_{4''-2''}$ = 2.2, $J_{4''-5''}$ = 7.9 Hz, 1H, H-4″), 8.05 (t, $J_{NH\text{-}1}$ = 5.7 Hz, 1H, NH−CO), 7.94 (t, $J_{NH\text{-}2}$ = 6.1 Hz, 1H, 8′-NH), 7.56 (ddd, $J_{5''\text{-}2''}$ = 0.8, $J_{5''-6''}$ = 4.8, $J_{5''-4''}$ = 7.9 Hz, 1H, H-5″), 6.32 (s, 1H, H-7′), 3.37 (m, 2H, H-2), 3.26 (m, 2H, H-1), 2.44 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 149.6 (C-2″), 148.7 (C-6″), 147.4 (C-6′), 143.4 (C-8′), 138.7 (C-2′), 136.5 (C-4″), 131.7 (C-9′), 125.0 (C-3″), 123.8 (C-5″), 122.1 (C-3′), 91.5 (C-7′), 41.8 (C-2), 37.8 (C-1), 22.8 ($COCH_3$), 14.5 (2′-CH_3). Anal. ($C_{16}H_{17}ClN_6O$) C, H, N. HRMS: calcd for [M + H]: 345.12251, found 345.12254

*N-(2-((6-Chloro-2-methyl-3-(thiophen-3-yl)imidazo[1,2-b]-pyridazin-8-yl)amino)ethyl)acetamide (**36c**).* Mobile phase: ethyl acetate:toluene:acetone:ethanol (17:4:4:1) → ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5). Recrystallization from hot MeOH. Yield: 143 mg (67%) as a white solid; mp 197.4−199.0 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.05 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1H, NH-CO), 7.95 (dd, $J_{2''-4''}$ = 1.2, $J_{2''\text{-}5''}$ = 3.0 Hz, 1H, H-2″), 7.85 (t, $J_{NH\text{-}2}$ = 6.1 Hz, 1H, 8′-NH), 7.71 (dd, $J_{5''\text{-}2''}$ = 3.0, $J_{5''-4''}$ = 5.0 Hz, 1H, H-5″), 7.60 (dd, $J_{4''-2''}$ = 1.2, $J_{4''-5''}$ = 5.0 Hz, 1H, H-4″), 6.29 (s, 1H, H-7′), 3.37 (bs, 2H, H-2), 3.26 (m, 2H, H-1), 2.49 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 147.2 (C-6′), 143.3 (C-8′), 137.7 (C-2′), 130.9 (C-9′), 128.7 (C-3″), 127.4 (C-4″), 126.2 (C-5″), 123.7 (C-2″), 121.4 (C-3′), 91.0 (C-7′), 41.8 (C-2), 37.9 (C-1), 22.8 ($COCH_3$), 15.3 (2′-CH_3). Anal. ($C_{15}H_{16}ClN_5OS$) C, H, N. HRMS: calcd for [M + H], 350.08369; found, 350.08372.

*N-(2-((6-Chloro-3-(3,5-dimethoxyphenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**36d**).* Mobile phase: ethyl acetate:toluene:acetone:ethanol (17:4:4:1) → ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5). Recrystallization from hot MeOH. Yield: 112 mg (46%) as a white solid; mp 160.5−161.0 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1H, NH−CO), 7.86 (t, $J_{NH\text{-}2}$ = 6.0 Hz, 1H, 8′-NH), 6.78 (d, $J_{2''-4''}$ = 2.3 Hz, 2H, H-2″), 6.57 (t, $J_{4''-2''}$ = 2.3 Hz, 1H, H-4″), 6.28 (s, 1H, H-7′), 3.79 (s, 6H, 3″-O-CH_3), 3.37 (bs, 2H, H-2), 3.26 (m, 2H, H-1), 2.43 (s, 3H, 2′-

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 160.5 (C-3″), 147.1 (C-6′), 143.3 (C-8′), 138.0 (C-2′), 131.2 (C-9′), 130.3 (C-1″), 124.8 (C-3′), 107.5 (C-2″), 99.6 (C-4″), 91.1 (C-7′), 55.5 (3″-O-CH_3), 41.8 (C-2), 37.8 (C-1), 22.8 ($COCH_3$), 14.8 (2′-CH_3). Anal. ($C_{19}H_{22}ClN_5O_3$) C, H, N. HRMS: calcd for [M + H], 404.14839; found, 404.14815.

*N-(2-((6-Chloro-3-(3-formylphenyl)-2-methylimidazo[1,2-b]-pyridazin-8-yl)amino)ethyl)acetamide (**36e**).* Mobile phase: ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5). Recrystallization from hot EtOAc. Yield: 234 mg (quant) as a white solid; mp 185.6−186.6 °C. ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.09 (s, 1H, CHO), 8.17 (t, $J_{2''-6''} = J_{2''-4''} = 1.7$ Hz, 1H, H-2″), 8.05 (t, $J_{NH-1} = 5.7$ Hz, 1H, NH-CO), 7.99 (ddd, $J_{6''-4''} = 1.2$, $J_{6''-2''} = 1.7$, $J_{6''-5''} = 7.7$ Hz, 1H, H-6″), 7.94 (dm, $J_{4''-5''} = 7.7$ Hz, 1H, H-4″), 7.92 (t, $J_{NH-2} = 6.1$ Hz, 1H, 8′-NH), 7.76 (t, $J_{5''-4''} = J_{5''-6''} = 7.7$ Hz, 1H, H-5″), 6.32 (s, 1H, H-7′), 3.38 (m, 2H, H-2), 3.27 (m, 2H, H-1), 2.45 (s, 3H, 2′-CH_3), 1.81 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 193.3 (−CHO), 170.0 (HN-C═O), 147.3 (C-6′), 143.4 (C-8′), 138.5 (C-2′), 136.6 (C-3″), 135.0 (C-6″), 131.5 (C-9′), 130.0 (C-2″), 129.6 (C-1″), 129.5 (C-5″), 128.8 (C-4″), 123.8 (C-3′), 91.4 (C-7′), 41.8 (C-2), 37.8 (C-1), 22.8 ($COCH_3$), 14.6 (2′-CH_3). Anal. ($C_{18}H_{18}ClN_5O_2$) C, H, N. HRMS: calcd for [M + H], 372.12218; found, 372.12163.

*N-(2-((6-Chloro-2-methyl-3-(3,4,5-trimethoxyphenyl)imidazo-[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**36f**).* Mobile phase: ethyl acetate:toluene:acetone:ethanol (17:4:4:1) → ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5). Recrystallization from hot MeOH. Yield: 285 mg (96%) as a white solid; mp 154.5−155.4 °C. ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, $J_{NH-1} = 5.5$ Hz, 1H, NH-CO), 7.85 (t, $J_{NH-2} = 6.1$ Hz, 1H, 8′-NH), 6.92 (s, 2H, H-2″), 6.28 (s, 1H, H-7′), 3.81 (s, 6H, 3″-O-CH_3), 3.73 (s, 3H, 4″-O-CH_3), 3.37 (bs, 2H, H-2), 3.26 (m, 2H, H-1), 2.45 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 153.0 (C-3″), 147.1 (C-6′), 143.3 (C-8′), 137.8 (C-2′), 137.5 (C-4″), 131.0 (C-9′), 125.0 (C-3′), 124.0 (C-1″), 107.1 (C-2″), 91.0 (C-7′), 60.3 (4″-O-CH_3), 56.2 (3″-O-CH_3), 41.8 (C-2), 37.9 (C-1), 22.8 ($COCH_3$), 14.7 (2′-CH_3). Anal. ($C_{20}H_{24}ClN_5O_4$) C, H, N. HRMS: calcd for [M + H], 456.14090; found, 456.14077.

*N-(2-((6-Chloro-3-(3-methoxyphenyl)-2-methylimidazo[1,2-b]-pyridazin-8-yl)amino)ethyl)acetamide (**36g**).* Mobile phase: ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5). Recrystallization from hot MeOH. Yield: 219 mg (96%) as a white solid; mp 186.3−187.0 °C. ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, $J_{NH-1} = 5.7$ Hz, 1H, NH-CO), 7.86 (t, $J_{NH-2} = 6.1$ Hz, 1H, 8′-NH), 7.43 (t, $J_{5''-4''} = J_{5''-6''} = 8.1$ Hz, 1H, H-5″), 7.21−7.19 (m, 2H, H-2″, H-6″), 6.99 (dm, $J_{4''-5''} = 8.1$ Hz, H-4″), 6.29 (s, 1H, H-7′), 3.80 (s, 3H, 3″-O-CH_3), 3.37 (m, 2H, H-2), 3.26 (m, 2H, H-1), 2.43 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 159.3 (C-3″), 147.2 (C-6′), 143.3 (C-8′), 138.0 (C-2′), 131.2 (C-9′), 129.9 (C-1″), 129.7 (C-5″), 124.8 (C-3′), 121.6 (C-6″), 115.1 (C-2″), 113.3 (C-4″), 91.1 (C-7′), 55.4 (3″-O-CH_3), 41.8 (C-2), 37.9 (C-1), 22.8 ($COCH_3$), 14.7 (2′-CH_3). Anal. ($C_{18}H_{20}ClN_5O_2$) C, H, N. HRMS: calcd for [M + H], 396.11977; found, 396.11976.

*N-(2-((6-Chloro-3-(4-cyanophenyl)-2-methylimidazo[1,2-b]-pyridazin-8-yl)amino)ethyl)acetamide (**36h**).* Mobile phase: ethyl acetate:toluene:acetone:ethanol (17:4:4:1) → ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5). Recrystallization from hot MeOH/$CHCl_3$. Yield: 207 mg (92%) as a white solid; mp 218.0−219.5 °C. ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, $J_{NH-1} = 5.6$ Hz, 1H, NH-CO), 7.97 (m, 2H, H-2″), 7.96 (t, $J_{NH-2} = 6.2$ Hz, 1H, 8′-NH), 7.88 (m, 2H, H-3″), 6.34 (s, 1H, H-7′), 3.36 (m, 2H, H-2), 3.26 (m, 2H, H-1), 2.46 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 147.5 (C-6′), 143.4 (C-8′), 139.5 (C-2′), 133.4 (C-1″), 132.5 (C-2″), 132.0 (C-9′), 129.4 (C-3″), 123.3 (C-3′), 119.1 (C-4″), 109.9 (CN), 91.8 (C-7′), 41.8 (C-2), 37.8 (C-1), 22.8 ($COCH_3$), 14.9 (2′-CH_3). Anal. ($C_{18}H_{17}ClN_6O$) C, H, N. HRMS: calcd for [M + H], 369.12251; found, 369.12252.

*N-(2-((6-Chloro-3-(4-formylphenyl)-2-methylimidazo[1,2-b]-pyridazin-8-yl)amino)ethyl)acetamide (**36i**).* Mobile phase: ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5). Recrystallization from hot MeOH. Yield: 206 mg (91%) as a white solid; mp 193.9−195.2 °C. ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.05 (s, 1H, CHO), 8.06−8.02 (m, 3H, H-3″, NH−CO), 7.94 (t, $J_{NH-2} = 6.0$ Hz, 1H, 8′-NH), 7.91 (m, 2H, H-2″), 6.34 (s, 1H, H-7′), 3.37 (m, 2H, H-2), 3.27 (m, 2H, H-1), 2.48 (s, 3H, 2′-CH_3), 1.81 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 192.8 (CHO), 170.0 (HN-C═O), 147.4 (C-6′), 143.4 (C-8′), 139.4 (C-2′), 135.0 (C-4″), 134.6 (C-1″), 131.9 (C-9′), 129.7 (C-3″), 129.2 (C-2″), 123.9 (C-3′), 91.7 (C-7′), 41.8 (C-2), 37.8 (C-1), 22.8 ($COCH_3$), 15.0 (2′-CH_3). Anal. ($C_{18}H_{18}ClN_5O_2$) C, H, N. HRMS: calcd for [M + H], 372.12218; found, 372.12207.

*N-(2-((6-Chloro-3-(4-methoxyphenyl)-2-methylimidazo[1,2-b]-pyridazin-8-yl)amino)ethyl)acetamide (**36j**).* Mobile phase: ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5). Recrystallization from hot MeOH. Yield: 88 mg (39%) as a white solid; mp 210.1−212.0 °C. ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, $J_{NH-1} = 5.6$ Hz, 1H, NH-CO), 7.82 (t, $J_{NH-2} = 6.0$ Hz, 1H, 8′-NH), 7.54 (m, 2H, H-2″), 7.08 (m, 2H, H-3″), 6.26 (s, 1H, H-7′), 3.82 (s, 3H, 4″-O-CH_3), 3.36 (bs, 2H, H-2), 3.26 (m, 2H, H-1), 2.39 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 159.0 (C-4″), 147.1 (C-6′), 143.3 (C-8′), 137.2 (C-2′), 130.8 (C-9′), 130.7 (C-2″), 125.0 (C-3′), 120.8 (C-1″), 114.1 (C-3″), 90.8 (C-7′), 55.4 (4″-O-CH_3), 41.7 (C-2), 37.9 (C-1), 22.8 ($COCH_3$), 14.5 (2′-CH_3). Anal. ($C_{18}H_{20}ClN_5O_2$) C, H, N. HRMS: calcd for [M + H], 374.13783; found, 374.13761.

*N-(2-((6-Chloro-3-(4-chlorophenyl)-2-methylimidazo[1,2-b]-pyridazin-8-yl)amino)ethyl)acetamide (**36k**).* Mobile phase: ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5). Recrystallization from hot EtOAc/MeOH. Yield: 171 mg (77%) as a white solid; mp 211.5−212.7 °C. ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, $J_{NH-1} = 5.7$ Hz, 1H, NH-CO), 7.82 (t, $J_{NH-2} = 6.0$ Hz, 1H, 8′-NH), 7.67 (m, 2H, H-2″), 7.58 (m, 2H, H-3″), 6.30 (s, 1H, H-7′), 3.37 (m, 2H, H-2), 3.26 (m, 2H, H-1), 2.42 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 147.3 (C-6′), 143.3 (C-8′), 138.2 (C-2′), 132.5 (C-4″), 131.4 (C-9′), 130.9 (C-2″), 128.7 (C-3″), 127.5 (C-1″), 123.8 (C-3′), 91.3 (C-7′), 41.8 (C-2), 37.8 (C-1), 22.8 ($COCH_3$), 14.6 (2′-CH_3). Anal. ($C_{17}H_{17}Cl_2N_5O$) C, H, N. HRMS: calcd for [M + H], 378.08829; found, 378.08806.

*Methyl 3-(8-((2-Acetamidoethyl)amino)-6-chloro-2-methylimidazo[1,2-b]pyridazin-3-yl)benzoate (**36l**).* Mobile phase: EtOAc:acetone:EtOH:water (20:3:1.2:0.8). Recrystallized from hot MeOH. Yield: 64 mg (40%); mp 181−181.9 °C. ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.22 (td, $J_{2-4,2-6} = 1.8$, $J_{2-5} = 0.4$ Hz, 1H, H-2), 8.06 (t, $J_{NH-2} = 5.6$ Hz, 1H, NH-CO), 7.99 (ddd, $J_{6-5} = 7.8$, $J_{6-2} = 1.8$, $J_{6-4} = 1.2$ Hz, 1H, H-6), 7.92 (ddd, $J_{4-2} = 1.8$, $J_{4-6} = 1.2$, $J_{4-5} = 7.8$ Hz, 1H, H-4), 7.91 (t, $J_{NH-1} = 6.1$ Hz, 1H, 8′-NH), 7.69 (td, $J_{5-4,\ 5-6} = 7.8$, $J_{5-2} = 0.4$ Hz, 1H, H-5), 6.32 (s, 1H, H-7′), 3.89 (s, 3H, 1-$COOCH_3$), 3.38 (m, 2H, H-1″), 3.27 (m, 2H, H-2″), 2.43 (s, 3H, 2′-CH_3), 1.81 (s, 3H, $NHCOCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 166.3 (1-CO), 147.4 (C-6′), 143.4 (C-8′), 138.4 (C-2′), 133.9 (C-4), 131.5 (C-9′), 130.1 (C-1), 129.7 (C-2), 129.3 (C-5), 129.3 (C-3), 128.5 (C-6), 123.9 (C-3′), 91.4 (C-7′), 52.6 (CO-O-CH_3), 41.8 (C-1″), 37.9 (C-2″), 22.8 (NH-CO-CH_3), 14.6 (2′-CH_3). Anal. ($C_{19}H_{21}O_3N_5Cl$) C, H, N. HRMS: calcd for [M + H], 402.13274; found, 402.13316.

*N-(2-((3-(Benzo[d][1,3]dioxol-5-yl)-6-chloro-2-methylimidazo-[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**36m**).* Mobile phase: EtOAc:acetone:EtOH:water (20:3:1.2:0.8). Recrystallized from hot MeOH. Yield: 80 mg (40%); mp 183.2−183.9 °C. ^1H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, $J_{NH-1} = 5.7$ Hz, 1H, NH-CO), 7.83 (t, $J_{NH-2} = 6.1$ Hz, 1H, 8′-NH), 7.17 (m, 1H, H-4″), 7.06−7.07 (M, 2H, H-6″, H-7″), 6.26 (s, 1H, H-7′), 6.09 (s, 2H, 2″), 3.35 (m, 2H, H-2), 3.25 (m, 2H, H-1), 2.39 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $NHCOCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 147.5 (C-6′), 147.1 (C-1″, C-3″), 143.3 (C-8′), 137.5 (C-2′), 130.9 (C-9′), 124.9 (C-3′), 123.5 (C-6″), 122.2 (C-5″), 109.7 (C-4″), 108.6 (C-7″), 101.5 (C-2″), 91.0 (C-7′), 41.8 (C-2), 37.9 (C-1), 22.8 (NH-CO-CH_3), 14.6 (2′-CH_3). Anal. ($C_{18}H_{18}ClN_5O_3$·0.66MeOH) C, H, N. HRMS: calcd for [M + H], 388.11709; found, 388.11769.

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

*3-(8-((2-Acetamidoethyl)amino)-6-chloro-2-methylimidazo[1,2-b]pyridazin-3-yl)benzamide (**36n**).* Mobile phase: EtOAc:acetone:EtOH:water (20:3:1.2:0.8). Recrystallized from hot MeOH. Yield: 80 mg (40%) as a white solid; mp 263.5−264.6 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.09 (td, $J_{2-4,2-6}$ = 1.8, J_{2-5} = 0.4 Hz, 1H, H-2), 8.07 (bs, 2H, CO-NH_2), 8.06 (t, J_{NH-2} = 5.8 Hz, 1H, NH-CO), 7.91 (ddd, J_{6-5} = 7.8, J_{6-2} = 1.8, J_{6-4} = 1.2 Hz, 1H, H-6), 7.89 (t, J_{NH-1} = 6.1 Hz, 1H, 8′-NH), 7.78 (ddd, J_{4-2} = 1.8, J_{4-6} = 1.2, J_{4-5} = 7.8 Hz, 1H, H-4), 7.61 (td, $J_{5-4,5-6}$ = 7.8, J_{5-2} = 0.4 Hz, 1H, H-5), 7.47 (bs, 2H, CO-NH_2^B), 6.31 (s, 1H, H-7′), 3.38 (m, 2H, H-1″), 3.27 (m, 2H, H-2″), 2.43 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $NHCOCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 167.8 (CO-NH), 147.3 (C-6′), 143.4 (C-8′), 138.1 (C-2′), 134.7 (C-1), 132.1 (C-4), 131.4 (C-9′), 128.8 (C-3), 128.7 (C-5), 127.0 (C-6), 124.6 (C-3′), 91.3 (C-7′), 41.8 (C-1″), 37.9 (C-2″), 22.8 (NH-CO-CH_3), 14.5 (2′-CH_3). Anal. ($C_{18}H_{19}O_2N_6Cl$) C, H, N. HRMS: calcd for [M + H], 387.13308; found, 387.13363.

*3-(8-((2-Acetamidoethyl)amino)-6-chloro-2-methylimidazo[1,2-b]pyridazin-3-yl)-N,N-dimethylbenzamide (**36o**).* Mobile phase: EtOAc:acetone:EtOH:water (20:3:1.2:0.8). Oily residue triturated with diethyl ether. Yield: 0.206 g (78%) as a pinkish solid; mp 159.5−160.9 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.06 (t, J_{NH-1} = 5.3 Hz, 1H, NH-CO), 7.89 (t, J_{NH-2} = 5.8 Hz, 1H, 8′-NH), 7.70 (dm, J_{4-5} = 7.9 Hz, H-4), 7.66 (m, 1H, H-2), 7.59 (t, J_{5-6} = J_{5-4} = 7.9 Hz, 1H, H-5), 7.44 (dm, J_{6-5} = 7.9 Hz, 1H, H-6), 6.30 (s, 1H, H-7′), 3.36 (m, 2 H, H-1″), 3.26 (m, 2H, H-2″), 3.01 (s, 6H, *N*-$(CH_3)_2$, 2.44 (s, 3H, 2′-CH_3), 1.80 (s, 3H, CO-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.1 (C═OCH_3), 169.9 (C═ON$(CH_3)_2$), 147.3 (C-6′), 143.4 (C-8′), 138.2 (C-2′), 136.7 (C-1), 131.4 (C-9′), 130.1 (C-4), 128.8 (C-5), 128.5 (C-3), 127.6 (C-2), 126.7 (C-6), 124.4 (C-3′), 91.3 (C-7′), 41.8 (C-1″), 39.3 (N-CH_3^A), 37.9 (C-2″), 35.1 (N-CH_3^B), 22.8 (NH-CO-CH_3), 14.6 (2′-CH_3). Anal. ($C_{20}H_{23}O_2N_6Cl$) C, H, N. HRMS: calcd for [M + H], 415.16438; found, 415.16425.

*N-(2-((6-Chloro-3-(4-fluoro-3-formylphenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**36p**).* Mobile phase: EtOAc:acetone:EtOH:water (20:3:1.2:0.8).Yield: 0.140 g (55%) as an off-white solid; mp 177.6−178.9 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.27 (CHO), 8.07 (dd, $J_{2''-6''}$ = 2.4, $J_{2''-F}$ = 6.7 Hz, 1H, H-2″), 8.05 (t, J_{NH-1} = 5.7 Hz, 1H, NH-CO), 8.01 (ddd, $J_{6''-2''}$ = 2.4,$J_{6''-5''}$ = 8.7,$J_{6''-F}$ = 5.1 Hz, 1H, H-6″), 7.92 (t, J_{NH-2} = 6.0 Hz, 1H, 8′-NH), 7.58 (dd, $J_{5''-6''}$ = 8.7,$J_{5''-F}$ = 10.5 Hz, 1H, H-5″), 6.32 (s, 1H, H-7′), 3.37 (m, 2H, H-2″), 3.26 (m, 2H, H-1″), 2.42 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 188.0 (d, J_{C-F} = 3.9, CHO), 170.1 (C═O), 162.7 (d, $J_{4'-F}$ = 259.8, C-4″), 147.4 (C-6′), 143.4 (C-8′), 138.4 (C-2′), 137.4 (d, $J_{4'-F}$ = 9.3, C-4″), 131.5 (C-9′), 129.8 (d, $J_{2''-F}$ = 2.1, C-2″), 125.8 ($J_{1''-F}$ = 3.1, C-1″), 124.1 (d, $J_{3''-F}$ = 9.0, C-3″), 123.1 (C-3′), 117.4 (d, $J_{5''-F}$ = 21.1, C-5″), 91.5 (C-7′), 41.8 (C-2″), 37.9 (C-1″), 22.8 (CO-CH_3), 14.5 (2′-CH_3). Anal. ($C_{18}H_{18}O_2N_5ClF$) C, H, N. HRMS: calcd for [M + H], 390.11276; found, 390.11310.

*N-(2-((6-Chloro-3-(3-cyano-4-fluorophenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**36q**).* Mobile phase: EtOAc:acetone:EtOH:water (20:3:1.2:0.8). Recrystallized from hot MeOH:$CHCl_3$ (10:1). Yield 0.181 g (73%); mp 224.5−225.6 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.18 (dd, $J_{2''-6''}$ = 2.2, $J_{2''-F}$ = 6.2 Hz, 1H, H-2″), 8.03−8.07 (m, 2H, NH-CO), 7.94 (t, J_{NH-2} = 6.0 Hz, 1H, 8′-NH), 7.69 (t, $J_{5''-6'}$ = $J_{5''-F}$ = 9.1 Hz, 1H, H-5″), 6.33 (s, 1H, H-7′), 3.37 (m, 2H, H-2″), 3.26 (m, 2H, H-1″), 2.42 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.1 (C═O), 161.8 (d, $J_{4''-F}$ = 257.5, C-4″), 147.5 (C-6′), 143.4 (C-8′), 138.8 (C-2′), 136.8 (d, $J_{6''-F}$ = 8.8, C-6″), 134.2 (C-2″), 131.6 (C-9′), 126.4 (d, $J_{1''-F}$ = 3.7, C-1″), 122.3 (C-3′), 117.2 (d, $J_{5''-F}$ = 19.9, C-5″), 114.1 (CN), 100.8 (d, $J_{3''-F}$ = 15.6, C-3″), 91.6 (C-7′), 41.8 (C-2″), 37.8 (C-1″), 22.8 (CO-CH_3), 14.5 (2′-CH_3). Anal. ($C_{18}H_{17}ON_6ClF$) C, H, N. HRMS: calcd for [M + H], 387.11309; found, 387.11368.

*4-(8-((2-Acetamidoethyl)amino)-6-chloro-2-methylimidazo[1,2-b]pyridazin-3-yl)-2-fluorobenzoic Acid (**36r**).* Mobile phase: $CHCl_3$:MeOH:CH_3COOH (9:1:0.1). Triturated with MeOH. Yield: 0.082 g (29%); mp 268.8−270.2 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.05 (t, J_{NH-2} = 5.6 Hz, 1H, NH-CO), 7.99 (t, J_{6-5} = J_{6-F} = 8.1 Hz, 1H, H-6), 7.95 (t, J_{NH-1} = 5.9 Hz, 1H, 8′-NH), 7.65 (dd, J_{3-F} = 12.3, J_{3-5} = 1.4 Hz, 1H, H-3), 7.62 (dd, J_{5-3} = 1.4, J_{5-6} = 8.1 Hz, 1H, H-5), 6.35 (s, 1H, H-7′), 3.37 (m, 2H, H-1″), 3.26 (m, 2H, H-2″), 2.48 (s, 3H, 2′-CH_3), 1.80 (s, 3H, CO-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.1 (NH-CO), 165.0 (d, J_{C-F} = 3.1 Hz, COOH), 161.1 (d, J_{2-F} =256.1 Hz, C-2), 147.5 (C-6′), 143.4 (C-8′), 139.6 (C-2′), 134.8 (d, J_{4-F} = 9.7 Hz, C-4), 132.2 (d, J_{6-F} = 1.8 Hz, C-6), 132.0 (C-9′), 124.6 (d, J_{5-F} = 3.4 Hz, C-5), 122.9 (C-3′), 118.2 (d, J_{1-F} = 10.1 Hz, C-1), 116.6 (d, J_{3-F} = 24.1 Hz, C-3), 91.8 (C-7′), 41.8 (C-1″), 37.9 (C-2″), 22.8 (NH-CO-CH_3), 15.1 (2′-CH_3). Anal. ($C_{18}H_{17}O_3N_5ClF{\cdot}H_2O$) C, H, N. HRMS: calcd for [M + H], 406.10767; found, 406.10802.

*N-(2-((6-Chloro-2-methyl-3-(3-(methylsulfonyl)phenyl)imidazo[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**36s**).* Mobile phase: EtOAc:MeOH (10−15%). Recrystallization from hot MeOH:$CHCl_3$ (7:1). Yield: 0.150 g (57%); mp 218.9−219.3 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.20 (t, $J_{2''-4''}$ = $J_{2''-6''}$ = 1.7 Hz, 1H, H-2″), 8.06 (t, J_{NH-1} = 1.7 Hz, 1H, NH−CO), 8.01 (dt, $J_{6''-5''}$ = 7.8, $J_{6''-2''}$ = $J_{6''-4''}$ = 1.7 Hz, 1H, H-6″), 7.95 (dt, $J_{4''-5''}$ = 7.8, $J_{4''-2''}$ = $J_{4''-6''}$ = 1.7 Hz, 1H, H-4″), 7.94 (t, J_{NH-2} = 5.8 Hz, 1H, 8′-NH), 7.81 (t, $J_{5''-4''}$ = $J_{5''-6''}$ = 7.8 Hz, 1H, H-5″), 6.34 (s, 1H, H-7′), 3.37 (m, 2H, H-2″), 3.27 (m, 2H, H-1″), 2.46 (s, 3H, 2′-CH_3), 1.81 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.1 (C═O), 147.5 (C-6′), 143.4 (C-8′), 141.3 (C-3″), 138.8 (C-2′), 134.0 (C-6″), 131.7 (C-9′), 129.9 (C-1″), 127.1 (C-2″), 126.2 (C-4″), 123.4 (C-3′), 91.6 (C-7′), 43.7 (SO_2CH_3), 41.8 (C-2″), 37.9 (C-1″), 22.8 (CO-CH_3), 14.7 (2′-CH_3). Anal. ($C_{18}H_{20}O_3N_5ClS$) C, H, N. HRMS: calcd for [M + H], 422.10481; found, 422.10486.

*N-(2-((6-Chloro-3-(3-(N,N-dimethylsulfamoyl)phenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)-acetamide (**36t**).* Mobile phase: EtOAc:acetone:EtOH:water (20:3:1.2:0.8). Recrystallized from MeOH:$CHCl_3$ (10:1). Yield 0.791 g (65%) as an off-white solid; mp 240−240.7 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.06 (m, 1H, H-2″), 8.05 (t, J_{NH-1} = 5.6 Hz, 1H, NH-CO), 7.98 (dt, (t, $J_{6''-5''}$ = 7.7, $J_{6''-2''}$ =$J_{6''-4''}$ = 1.4 Hz, 1H, H-6″, 7.95 (t, J_{NH-2} = 6.0 Hz, 1H, 8′-NH), 7.81 (t, $J_{5''-4''}$ = $J_{5''-6''}$ = 7.8 Hz, 1H, H-5″), 6.34 (s, 1H, H-7′), 3.38 (m, 2H, H-2″), 3.26 (m, 2H, H-1″), 2.69 (s, 6H, $N(CH_3)_2$), 2.48 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.1 (C═O), 147.4 (C-6′), 143.5 (C-8′), 138.7 (C-2′), 134.9 (C-3″), 133.3 (C-6″), 131.7 (C-9′), 129.9 (C-5″), 129.8 (C-1″), 127.7 (C-2″), 126.6 (C-4″), 123.3 (C-3′), 91.6 (C-7′), 41.8 (C-2″), 37.9 (C-1″), 22.8 ($CO-CH_3$), 14.7 (2′-CH_3). Anal. ($C_{19}H_{23}ClN_6O_3S$) C, H, N. HRMS: calcd for [M + H], 451.13136; found, 451.13144.

2-Methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-aniline. 5-Bromo-2-methoxyaniline (0.518 mg, 2.56 mmol) was dissolved in DMSO (8 mL). $B_2(pin)_2$ (1.3 g, 5.12 mmol) and AcOK (1.43 g, 14.59 mmol) were added in one portion, and the suspension was degassed 3 times and refilled with argon. Pd(dppf)Cl_2·DCM (0.1 g, 5 mol %) was added quickly, and the mixture was degassed once more. The reaction was then heated up to 80 °C and stirred under argon atmosphere overnight. After cooling to rt, the reaction mixture was partitioned between EtOAc/brine and the water phase was extracted twice more with EtOAc. Combined organic phases were dried over sodium sulfate and evaporated, coevaporated with toluene, and then the residue was adsorbed onto silica gel and purified by column chromatography (hexane:EtOAc 4:1) to provide brownish oil. Yield: 370.4 mg (58%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.00 (d, J_{6-4} = 1.6, 1H, H-6), 6.90 (dd, J_{4-6} = 1.6, J_{4-3} = 7.9 Hz, 1H, H-4), 6.77 (d, J_{3-4} = 7.9 Hz, 1H, H-3), 4.66 (bs, 2H, 1-NH_2), 3.77 (s, 3H, O−CH_3), 1.25 (s, 12H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 149.2 (C-2), 137.1 (C-1), 128.8 (C-4), 120.2 (C-5), 120.0 (C-6), 109.9 (C-3), 83.2 (C-$(CH_3)_2$), 55.3 (O−CH_3), 24.9 (CH_3). HRMS: calcd for [M + H], 250.16090; found, 250.16095.

*N-(2-((3-(3-Amino-4-methoxyphenyl)-6-chloro-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**36u**).*[46] To the suspension of **35** (0.228 g, 0.58 mmol) in 1,4-dioxane (8 mL) was added solution of 2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

aniline (0.217 g, 0.87 mmol) followed by solution of K_2CO_3 (2 mL, 1M). Reaction mixture was degassed 3 times, then $Pd(PPh_3)_4$ (5 mol %) was added quickly and the mixture was degassed once more and stirred under argon atmosphere at 90 °C overnight. After completion of the reaction, it was cooled to rt, diluted with water, and extracted with EtOAc (50 mL). The water phase was extracted twice more with EtOAc (2 × 25 mL), and the combined organic phases were dried over sodium sulfate and evaporated. Oily residue was dissolved in MeOH/$CHCl_3$ mixture and adsorbed onto silica gel and purified by column chromatography (EtOAc:acetone:EtOH:water 20:3:1.6:0.4). Product **36u** was obtained as a brownish powder. Yield: 182 mg (80%); mp 214.7−215.5 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, $J_{NH\text{-}2}$ = 5.8 Hz, 1H, NH-CO), 7.78 (t, $J_{NH\text{-}1}$ = 6.0 Hz, 1H, 8′-NH), 6.92 (d, J_{6-5} = 8.4 Hz, H-6), 6.87 (d, J_{3-5} = 2.1 Hz, 1H, H-3), 6.75 (dd, J_{5-3} = 2.1, J_{5-6} = 8.4 Hz, 1H, H-5), 6.23 (s, 1H, H-7′), 4.85 (bs, 2H, 2-NH_2), 3.82 (s, 3H, 1-O-CH_3), 3.33 (m, 2H, H-2″), 3.25 (m, 2H, H-2″), 2.36 (s, 3H, 2′-CH_3), 1.80 (s, 3H, CO-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.9 (C═O), 146.9 (C-6′), 146.4 (C-1), 143.2 (C-8′), 137.7 (C-2), 137.0 (C-2′), 130.6 (C-9′), 125.9 (C-3′), 121.0 (C-4), 117.8 (C-5), 114.8 (C-3), 110.6 (C-6), 90.6 (C-7′), 55.6 (O-CH_3), 41.7 (C-1″), 37.9 (C-2″), 22.8 (CO-CH_3), 14.6 (2′-CH_3). HRMS: calcd for [M + H], 389.14873; found, 389.14905.

***N*-(2-((3-(3-Acetamido-4-methoxyphenyl)-6-chloro-2-methylimidazo[1,2-*b*]pyridazin-8-yl)amino)ethyl)acetamide (37).** Compound **36u** (201 mg, 0.52 mmol) was dissolved in DCM (5 mL), followed by addition of DIPEA (0.1 mL, 0.62 mmol). Acetanhydride (0.06 mL, 0.62 mmol) was then addend dropwise at 0 °C. After the addition, the mixture was allowed to warm to rt and stirred overnight. Then it was diluted with DCM, washed with H_2O, dried over sodium sulfate, evaporated to minimal volume, and adsorbed onto silica gel. Column chromatography (EtOAc:MeOH 6:1) afforded the product **37**. An analytical sample was obtained after recrystallization from MeOH:$CHCl_3$ (10:1) as violet crystals. Yield: 204 mg (92%); mp 145−146.2 °C (decomposed). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 9.27 (s, 1H, 3″-NH), 8.16 (d, 1H, H-2″), 8.03 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1H, NH-CO), 7.81 (t, $J_{NH\text{-}2}$ = 6.0 Hz, 1H, 8′-NH), 7.36 (dd, $J_{6''-5''}$ = 8.5, $J_{6''-2''}$ = 2.0 Hz, 1H, H-6″), 7.18 (d, $J_{5''-6''}$ = 8.5 Hz, 1H, H-5″), 6.25 (s, 1H, H-7′), 3.90 (s, 3H, 4″-OCH_3), 3.36 (m, 2H, H-2), 3.26 (m, 2H, H-1), 2.39 (s, 3H, 2′-CH_3), 2.10 (s, 3 H, 3″-NH-CO-CH_3), 1.80 (s, 3 H, 1-NH-CO-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.9 (1-NH-CO), 168.8 (3″-NH-CO), 149.4 (C-4″), 147.0 (C-6′), 143.3 (C-8″), 137.3 (C-2′), 130.8 (C-9′), 127.4 (C-3″), 125.5 (C-6″), 125.0 (C-3′), 123.2 (C-2″), 120.4 (C-1″), 111.3 (C-5″), 90.8 (C-7′), 57.1 (4″-O-CH_3), 41.7 (C-2), 37.9 (C-1), 24.0 (3″-NH-CO-CH_3), 22.8 (1-NHCO-CH_3), 14.4 (2′-CH_3). Anal. ($C_{20}H_{23}ClN_6O_3$) C, H, N. HRMS: calcd for [M + H], 431.15929; found, 431.15935.

***N*-(5-(8-((2-Acetamidoethyl)amino)-6-chloro-2-methylimidazo[1,2-*b*]pyridazin-3-yl)-2-methoxyphenyl)-cyclohexanecarboxamide (38).** Compound **36u** (201 mg, 0.52 mmol) was dissolved in DCM (5 mL), followed by addition of DIPEA (0.1 mL, 0.62 mmol). Cyclohexanoyl chloride (0.07 mL, 0.62 mmol) wad then added dropwise at 0 °C. After the addition, the mixture was allowed to warm to rt and stirred for 1 h. Then it was diluted with DCM, washed with H_2O, dried over sodium sulfate, evaporated to minimal volume, and adsorbed onto silica gel. Column chromatography (EtOAc:acetone:EtOH:water 20:3:1.6:0.4) afforded off-white foam, which was triturated with ether, sonicated for a few minutes, and the formed suspension was filtered, washed with ether, and dried, affording the product **38** as an off white solid. Yield: 162 mg (63%); mp 164.6−165.2 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 9.04 (s, 1H, 1′-NH), 8.17 (d, $J_{6'-4'}$ = 2.1 Hz, 1H, H-6′), 8.03 (t, $J_{NH\text{-}2'''}$ = 5.6 Hz, 1H, 2‴-NH−CO), 7.81 (t, $J_{NH\text{-}1'''}$ = 6.0 Hz, 1H, 8″-NH), 7.34 (dd, $J_{4'-3'}$ = 8.5, $J_{4'-6'}$ = 2.1 Hz, H-4′), 7.18 (d, $J_{3'-4'}$ = 8.5 Hz, 1H, H-3′), 6.25 (s, 1H, H-7″), 3.90 (s, 3H, 2′-OCH_3), 3.36 (m, 2H, H-1‴), 3.26 (m, 2H, H-2‴), 2.54 (m, 2H, H-1), 2.39 (s, 3H, 2″-CH_3), 1.81 (s, 3H, NH-CO-CH_3), 1.79 (m, 2H, 2a), 1.73 (dm, J_{GEM} = 12.9 Hz, 2H, 3a), 1.64 (dm, J_{GEM} = 12.2 Hz, 4a), 1.38 (m, 2H, 2b), 1.27 (m, 2H, 3b), 1.18 (m, 4b). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 174.7 (1′-NH-CO), 169.9 (2″-NH-CO), 149.4 (C-2′), 147.0 (C-6″), 143.3 (C-8″), 137.8 (C-2″), 130.8 (C-9″), 127.6 (C-5′), 125.4 (C-4′), 125.1 (C-3″), 123.0 (C-6′), 120.4 (C-1′), 111.2 (C-3′), 90.8 (C-7″), 56.1 (2′-O-CH_3), 44.5 (C-1), 41.7 (C-1‴), 37.9 (C-2‴), 29.5 (C-2), 25.6 (C-4), 25.4 (C-3), 22.8 (NH-CO-CH_3), 14.4 (2″-CH_3). Anal. ($C_{25}H_{31}ClN_6O_3$·0.5H_2O) C, H, N. HRMS: calcd for [M + H], 499.22189; found, 499.22195.

***N*-(5-(8-((2-Acetamidoethyl)amino)-6-chloro-2-methylimidazo[1,2-*b*]pyridazin-3-yl)-2-methoxyphenyl)-pivalamide (39).** Compound **36u** (164 mg, 0.42 mmol) was dissolved in DCM (5 mL) followed by addition of DIPEA (0.1 mL, 0.5 mmol). Pivaloyl chloride (0.078 mL, 0.5 mmol) was then added dropwise at 0 °C. After the addition, the mixture was allowed to warm to rt and stirred overnight. Then the mixture was evaporated to minimal volume, diluted with EtOAc, and washed with water. The water phase was extracted twice more with EtOAc, dried over sodium sulfate, evaporated, and adsorbed onto silica gel from acetone. Column chromatography (EtOAc:acetone:EtOH:water 20:3:1.6:0.4) afforded off-white foam. Yield: 95 mg (48%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.51 (s, 1H, 1-NH), 8.09 (d, J_{6-4} = 2.2 Hz, 1H, H-6), 8.03 (t, $J_{NH\text{-}2''}$ = 5.8 Hz, 1H, 2″-NH-CO), 7.81 (t, $J_{NH\text{-}1}$ = 6.0 Hz, 1H, 8-NH), 7.38 (dd, J_{4-3} = 8.5, J_{4-6} = 2.2 Hz, H-4), 7.21 (d, J_{3-4} = 8.5 Hz, 1H, H-3), 6.26 (s, 1H, H-7′), 3.91 (s, 3H, 2-OCH_3), 3.36 (bs, 2H, H-1″), 3.26 (m, 2H, H-2″), 2.40 (s, 3H, 2″-CH_3), 1.81 (s, 3H, CO-CH_3), 1.24 (s, 9H, $CH(CH_3)_3$. ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 176.3 (1-NH-CO), 169.9 (2″-NH-CO), 149.9 (C-2), 147.1 (C-6′), 143.3 (C-8′), 137.3 (C-2′), 130.9 (C-9′), 127.4 (C-1), 125.9 (C-4), 125.0 (C-3′), 123.2 (C-6), 120.6 (C-5), 111.3 (C-3), 90.8 (C-7′), 56.3 (2-O-CH_3), 41.8 (C-1″), 39.5 ($CH(CH_3)_3$, 37.9 (C-2″), 27.4 (CH_3)$_3$, 22.8 (NH-CO-CH_3), 14.5 (2′-CH_3). Anal. ($C_{23}H_{29}ClN_6O_3$·0.75EtOAc) C, H, N. HRMS: calcd for [M + H]: 473.20624, found 473.20629.

***N*-(2-((6-Chloro-3-(3-(3-isopropylureido)-4-methoxyphenyl)-2-methylimidazo[1,2-*b*]pyridazin-8-yl)amino)ethyl)acetamide (40).** Compound **36u** (178 mg, 0.46 mmol) was dissolved in THF (10 mL), followed by addition of TEA (0.19 mL, 1.38 mmol) and isopropylisocyanate (0.144 mL, 0.92 mmol)). The reaction mixture was refluxed for 6 h. Then the mixture was cooled to rt, evaporated to minimal volume, diluted with EtOAc, and washed with water. The water phase was extracted twice more with EtOAc, dried over sodium sulfate, evaporated, and adsorbed onto silica gel. Column chromatography (EtOAc:acetone:EtOH:water 20:3:1.2:0.8) afforded off-white foam. Yield: 187 mg (86%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.30 (d, $J_{2''-6''}$ = 2.1 Hz, 1H, H-2″), 8.04 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1H, 1-NH), 7.90 (s, 1H, 3″-NH), 7.80 (t, $J_{NH\text{-}2}$ = 6.1 Hz, 1H, 2-NH−CO), 7.15 (dd, $J_{6''-5''}$ = 8.4, $J_{6''-2''}$ = 2.1 Hz, 1H, H-6″), 7.10 (d, $J_{5''-6''}$ = 8.4 Hz, 1H, H-5″), 6.24 (s, 1H, H-7′), 3.90 (s, 3H, 4″-OCH_3), 3.73 (m, 1H, NH-$CH(CH_3)_2$), 3.36 (m, 2H, H-2), 3.26 (m, 2H, H-1), 2.38 (s, 3H, 2′-CH_3), 1.80 (s, 3H, NH-CO-CH_3), 1.08 (d, J_{CH-CH_3} = 6.5 Hz, 6H, NH-CH-$(CH_3)_2$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.9 (NH-CO), 154.6 (CO-NH-Pr), 147.1 (C-4″), 147.0 (C-6′), 143.2 (C-8′), 137.1 (C-2′), 130.7 (C-9′), 129.6 (C-1″), 125.6 (C-3′), 122.2 (C-6″), 120.7 (C-3″), 119.1 (C-2″), 110.6 (C-5″), 90.7 (C-7′), 56.0 (4″-O-CH_3), 41.7 (C-2), 41.0 (NH-CH-$(CH_3)_2$), 37.9 (C-1), 23.2 (NH-CH-$(CH_3)_2$), 22.8 (NH-CO-CH_3), 14.5 (2′-CH_3). Anal. ($C_{22}H_{28}ClN_7O_3$) C, H, N. HRMS: calcd for [M + H], 474.20149; found, 474.20150.

***N*-(2-((6-Chloro-3-(3-(isopropylamino)-4-methoxyphenyl)-2-methylimidazo[1,2-*b*]pyridazin-8-yl)amino)ethyl)acetamide (41).** A round-bottom flask was charged with **36u** (178 mg, 0.46 mmol) and diluted with MeOH (2 mL) and acetone (0.08 mL), followed by an addition of $NaBH_3CN$ (43 mg, 0.69 mmol). Acetic acid (0.065 mL, 1.15 mmol) was added, and the reaction mixture was stirred at rt overnight. Then pH was adjusted to 2 by diluted HCl and stirred for 7 h, after which pH was adjusted to 7 with diluted KOH and the mixture was extracted with chloroform (35 mL). The water phase was extracted with chloroform (2 × 30 mL), and the combined organic phases were dried over sodium sulfate and evaporated. Residue was dissolved in a small amount of DCM, adsorbed on silica gel, and purified by column chromatography (EtOAc:acetone:EtOH:water 20:3:1.2:0.8). Residue was triturated with ether, and the formed suspension was filtered and washed with ether, affording **41** as an off-

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

white solid. Yield: 150 mg (76%); mp 165.2−166.3 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.03 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1H, 1-NH), 7.76 (t, $J_{NH\text{-}2}$ = 6.0 Hz, 1H, 8-NH), 6.94 (d, $J_{5''-6''}$ = 8.2 Hz, 1H, H-5″), 6.79 (d, $J_{2''-6''}$ = 1.9 Hz, 1H, H-2″), 6.77 (dd, $J_{6''-5''}$ = 8.2, $J_{6''-2''}$ = 1.9 Hz, 1H, H-6″), 6.24 (s, 1H, H-7′), 4.46 (d, J_{NH-CH} = 8.2 Hz, 1H, 3″-NH), 3.84 (s, 3H, 4″-OCH_3), 3.57 (m, 1H, CH-$(CH_3)_2$), 3.36 (bs, 2H, H-2), 3.26 (m, 2H, H-1), 2.41 (s, 3H, 2′-CH_3), 1.80 (s, 3H, NH−CO−CH_3), 1.19 (d, J_{CH-CH_3} = 6.3 Hz, 6H, CH-$(CH_3)_2$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.9 (NH-CO), 146.9 (C-6′), 146.2 (C-4′), 143.2 (C-8′), 137.0 and 136.9 (C-2′ and C3″), 130.6 (C-9′), 125.9 (C-3′), 121.2 (C-1″), 116.8 (C-6″), 110.9 (C-2″), 109.8 (C-5″), 90.6 (C-7′), 55.6 (4″-O-CH_3), 43.2 (CH-$(CH_3)_2$), 41.7 (C-2), 37.9 (C-1), 22.8 (CO-CH_3), 22.5 (NH-CH-$(CH_3)_2$), 14.6 (2′-CH_3). Anal. ($C_{21}H_{27}ClN_6O_2$) C, H, N. HRMS: calcd for [M + H], 431.19568; found, 431.19573.

***N*-(2-((6-Chloro-3-(4-methoxy-3-((2-(methylsulfonamido)ethyl)amino)phenyl)-2-methylimidazo[1,2-*b*]pyridazin-8-yl)amino)ethyl)acetamide (42).** Compound **36u** (0.17 g, 0.437 mmol) was diluted in dry toluene (3 mL), followed by addition of *N*-(2-chloroethyl)methanesulfonamide (0.112 g, 0.554 mmol) in toluene and K_2CO_3 (0.06 g, 0.437 mmol). The reaction mixture was stirred at 90 °C overnight. After cooling to rt, the solvent was evaporated and the residue diluted with DCM and washed with water. The organic phase was dried over sodium sulfate, evaporated to minimal volume, adsorbed onto silica gel, and purified by column chromatography (EtOAc:acetone:EtOH:water 20:3:1.2:0.8), affording **42** as an of off-white foam. Yield: 100 mg (45%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.05 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1H, 1-NH), 7.79 (t, $J_{NH\text{-}2}$ = 6.1 Hz, 1H, 8-NH), 6.98 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.83 (dd, $J_{6''-5''}$ = 8.3 Hz, 1H, H-5″), 6.81 (d, $J_{2''-6''}$ = 2.0 Hz, 1H, H-2″), 6.26 (s, 1H, H-7′), 5.37 (t, $J_{NH\text{-}1''}$ = 6.0 Hz, 1H, 3″-NH), 3.86 (s, 3H, 4″-OCH_3), 3.32−3.38 (m, 4H, H-1, H-1‴), 3.26 (m, 2H, H-2), 3.02 (m, 2H, H-2‴), 2.40 (s, 3H, 2′-CH_3), 1.80 (s, 3H, NH-CO-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.9 (NH-CO), 146.9 (C-6′), 146.6 (C-4″), 143.2 (C-8′), 137.2 (C-3″), 137.0 (C-2′), 130.6 (C-9′), 125.9 (C-3′), 121.2 (C-1″), 117.8 (C-6″), 110.3 (C-2″), 110.1 (C-5″), 90.6 (C-7′), 55.6 (4″-O-CH_3), 41.8 (C-1), 40.5 (C-1‴), 38.0 (C-2‴), 22.8 (CO-CH_3), 14.6 (2′-CH_3). Anal. ($C_{21}H_{28}ClN_7O_4S$·0.5EtOAc) C, H, N. HRMS: calcd for [M + H], 431.19568; found, 431.19573.

***N*-(2-((6-Chloro-3-(3-(hydroxymethyl)phenyl)-2-methylimidazo[1,2-*b*]pyridazin-8-yl)amino)ethyl)acetamide (44).** Compound **36e** (134 mg, 0.36 mmol) was dissolved in CH_2Cl_2 (4 mL) and MeOH (1 mL), and $NaBH_4$ (74 mg, 1.95 mmol) was added in one portion. The reaction mixture stirred at rt for 1 h and was subsequently quenched through addition of satd NH_4Cl (1 mL) and H_2O (1 mL), and the resulting suspension was stirred at rt for 10 min before being diluted with $CHCl_3$ (30 mL) and H_2O (15 mL). The layers were separated, and the aqueous layer was extracted twice with $CHCl_3$ (2 × 20 mL) and the organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5)), and the product was recrystallized from hot MeOH to afford compound **33** as a white solid. Yield: 40 mg (30%); mp 185.2−186.4 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.05 (t, $J_{NH\text{-}1}$ = 5.7 Hz, 1H, NH-CO), 7.85 (t, $J_{NH\text{-}2}$ = 6.0 Hz, 1H, 8′-NH), 7.55 (m, 1H, H-2″), 7.51−7.45 (m, 2H, H-5″, H-6″), 7.36 (dm, $J_{4''-5''}$ = 6.9 Hz, 1H, H-4″), 6.28 (s, 1H, H-7′), 5.28 (t, $J_{OH\text{-}CH_2}$ = 5.8 Hz, 1H, CH_2OH), 4.57 (d, $J_{CH_2\text{-}OH}$ = 5.8 Hz, 2H, CH_2-OH), 3.37 (m, 2H, H-2), 3.26 (m, 2H, H-1), 2.41 (s, 3H, 2′-CH_3), 1.81 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 147.2 (C-6′), 143.3 (C-8′), 143.0 (C-3″), 137.8 (C-2′), 131.1 (C-9′), 128.4 (C-1″), 128.4 and 127.8 (C-5″and C-6″), 127.3 (C-2″), 126.2 (C-4″), 125.2 (C-3′), 91.1 (C-7′), 63.0 (CH_2-OH), 41.8 (C-2), 37.9 (C-1), 22.8 ($COCH_3$), 14.6 (2′-CH_3). Anal. ($C_{17}H_{17}Cl_2N_5O$) C, H, N. HRMS: calcd for [M + H], 374.13783; found, 374.13713.

***N*-(2-((6-Chloro-3-(4-(hydroxymethyl)phenyl)-2-methylimidazo[1,2-*b*]pyridazin-8-yl)amino)ethyl)acetamide (45).** Compound **36i** (131 mg, 0.35 mmol) was dissolved in CH_2Cl_2 (4 mL) and MeOH (1 mL), and $NaBH_4$ (72 mg, 1.9 mmol) was added in one portion. The reaction mixture stirred at rt for 1 h and was subsequently quenched through addition of satd NH_4Cl (1 mL) and H_2O (1 mL), and the resulting suspension was stirred at rt for 10 min before being diluted with $CHCl_3$ (30 mL) and H_2O (15 mL). The layers were separated, and the aqueous layer was extracted twice with $CHCl_3$ (2 × 20 mL) and the organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography (ethyl acetate:acetone:ethanol:water (21:3:0.5:0.5)), and the product was recrystallized from hot MeOH to afford compound **45** as a white solid. Yield: 52 mg (40%); mp 203.8−204.5 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.05 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1H, NH-CO), 7.85 (t, $J_{NH\text{-}2}$ = 6.0 Hz, 1H, 8′-NH), 7.58 (m, 2H, H-2″), 7.46 (m, 2H, H-3″), 6.27 (s, 1H, H-7′), 5.26 (t, $J_{OH\text{-}CH_2}$ = 5.8 Hz, 1H, CH_2OH), 4.56 (d, $J_{CH_2\text{-}OH}$ = 5.8 Hz, 2H, CH_2-OH), 3.37 (m, 2H, H-2), 3.26 (m, 2H, H-1), 2.41 (s, 3H, 2′-CH_3), 1.80 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.0 (C═O), 147.1 (C-6′), 143.3 (C-8′), 142.4 (C-4″), 137.7 (C-2′), 131.1 (C-9′), 129.1 (C-2″), 127.0 (C-1″), 126.7 (C-3″), 125.1 (C-3′), 91.0 (C-7′), 62.9 (CH_2-OH), 41.8 (C-2), 37.9 (C-1), 22.8 ($COCH_3$), 14.6 (2′-CH_3). Anal. ($C_{17}H_{17}Cl_2N_5O$) C, H, N. HRMS: calcd for [M + H], 374.13783; found, 374.13785.

(4-(8-((2-Acetamidoethyl)amino)-6-chloro-2-methylimidazo-[1,2-*b*]pyridazin-3-yl)phenyl)methanaminium Chloride (46). To a solution of compound **36h** (130 mg, 0.352 mmol) in 4 M ethanolic ammonia (10 mL) was added a catalytic amount of Raney-Ni, and the reaction mixture was stirred under H_2 (50 psi) at rt for 16 h. The catalyst was filtered off over Celite and washed with MeOH, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate:acetone:ethanol:water (18:3:2.5:1.5) → ethyl acetate:acetone:ethanol:water (17:3:3:2) + 1% NEt_3) to give the title compound ,which was converted into its hydrochloride through treatment with ethereal HCl in CH_2Cl_2 and a small amount of MeOH. The sticky product thus obtained was triturated with acetone and minute amounts of MeOH, affording title compound **46** as an off-white solid. Yield: 102 mg (71%); mp 254.1−255.3 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.70 (m, 1H, 8′-NH), 8.65 (m, 3H, NH_3^+), 8.12 (t, $J_{NH\text{-}2''}$ = 5.8 Hz, 1H, NH-CO), 7.71 (s, 2H, H-3), 7.71 (s, 2H, H-2), 6.79 (s, 1H, H-7′), 4.10 (m, 2H, 1-CH_2), 3.45 (m, 2H, H-1″), 3.30 (m, 2H, H-2″), 2.49 (s, 3H, 2′-CH_3), 1.82 (s, 3H, $COCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.1 (C═O), 149.7 (C-6′), 141.4 (C-8′), 135.3 (C-1), 131.9 (C-2′), 130.1 (C-3), 129.5 (C-2), 129.2 (C-9′), 126.0 (C-4), 125.3 (C-3′), 95.9 (C-7′), 42.1 (C-1″, 1-CH_2), 37.4 (C-2″), 22.9 ($COCH_3$), 11.9 (2′-CH_3). Anal. ($C_{18}H_{22}Cl_2N_6O$) C, H, N. HRMS: calcd for [M + H], 373.15381; found, 373.15349.

***N*-(2-((3-(3,4-Dimethoxyphenyl)-2-methylimidazo[1,2-*b*]-pyridazin-8-yl)amino)ethyl)acetamide (47).** Compound **36a** (272 mg, 0.67 mmol) was dissolved in THF:MeOH (8 mL, 1:1), and $Pd(OH)_2$ 20%/C (47 mg, 10 mol %) was added followed by addition of TEA (0.187 mL, 1.35 mmol). The mixture was stirred under the atmosphere of H_2 in an autoclave (5 bar) overnight. The reaction mixture was then filtrated over pad of Celite, washed with MeOH, and evaporated. Recrystallization from hot MeOH provided the product **47** as an off-white solid. Yield: 156 mg (63%); mp 153.5−154 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.05 (t, $J_{NH\text{-}2}$ = 5.5 Hz, 1H, NH-CO), 7.99 (d, $J_{6'-7'}$ = 5.5 Hz, 1H, H-6′), 7.32 (t, $J_{NH\text{-}1}$ = 5.9 Hz, 1H, 8′-NH), 7.24 (d, $J_{2''-6''}$ = 2.0 Hz, H-2″), 7.18 (dd, $J_{6''-5''}$ = 8.3, $J_{6''-2''}$ = 2.0 Hz, 1H, H-6″), 7.07 (d, $J_{5''-6''}$ = 8.3 Hz, 1H, H-5″), 6.12 (d, $J_{7'-6'}$ = 5.5 Hz, 1H, H-7′), 3.81 (s, 3H, 4″-OCH_3), 3.78 (s, 3H, 3″-OCH_3), 3.35 (m, 2H, H-2), 3.28 (m, 2H, H-1), 2.43 (s, 3H, 2′-CH_3), 1.81 (s, 3H, $NHCOCH_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.9 (C═O), 148.6 (C-3″), 148.4 (C-4″), 144.0 (C-6′), 142.3 (C-8′), 136.9 (C-2′), 132.4 (C-9′), 124.5 (C-3′), 122.1 (C-6″), 121.9 (C-1″), 113.2 (C-2″), 111.9 (C-5″), 90.1 (C-7′), 55.8 (4′-OCH_3), 55.8 (3′-OCH_3), 41.7 (C-2″), 37.9 (C-1″), 22.9 (CO-CH_3), 14.8 (2′-CH_3). Anal. ($C_{19}H_{23}ClN_5O_3$) C, H, N. HRMS: calcd for [M + H], 370.18737; found, 370.18749.

6-Chloro-3-iodo-2-methylimidazo[1,2-*b*]pyridazin-8-amine (48). Compound **34** (141 mg, 4.3 mmol) was diluted with ethanolic

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

Table 3. Statistics of Crystallographic Data Collection and Refinement[a]

crystal	PI4K IIIβ + ATP	PI4K IIIβ + MI103
space group	P 4_3	P 4_3
cell dimensions (Å)	a = 108.3, b = 108.3, c = 55.1	a = 108.4, b = 108.4, c = 55.1
X-ray source	BESSY ID 14-1	BESSY ID 14-1
wavelength (Å)	0.918409	0.918417
resolution (Å)	48.45−3.318 (3.436−3.318)	49.14−3.407 (3.529−3.407)[b]
no. of unique reflections	9684 (953)	8935 (850)
I/σ (I)	6.59 (2.35)	5.81 (1.73)
R_{merge}	24.2	39.8
data completeness (%)	99.79 (99.06)	99.81 (98.15)
mltiplicity	4.5	7.5
R_{work} (%)	18.25 (23.83)	20.89 (27.77)
R_{free} (%)	24.41 (33.34)	25.16 (34.40)
rms bond angle deviation (deg)	0.012	0.013
rms bond angle deviation (Å)	1.59	1.11

[a]Numbers in parentheses refer to the highest resolution shell of the respective data set.

[b]I/σ (I) = 2 at resolution 3.56 Å.

ammonia (4.5 mL, 4M) and heated in a microwave reactor at 140 °C for 90 min. After completion of the reaction (monitored by TLC, EtOAc:acetone:EtOH:H_2O 20:3:1:1), the reaction mixture was directly adsorbed on silica gel and purified by column chromatography (hexane/EtOAc 3:2 to 1:1). Recrystallization from hot MeOH afforded greenish crystals of **48**. Yield 108 mg (77%); mp 244.4−245.7 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.40 (bs, 2H, 8-NH_2), 6.18 (s, 1H, H-7), 2.34 (s, 3H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 147.1 (C-6), 144.3 (C-8), 144.0 (C-2), 134.4 (C-9), 94.5 (C-7), 71.9 (3), 14.9 (2-CH_3). Anal. ($C_7H_7ClN_4I$) C, H, N. HRMS: calcd for [M + H], 308.93984; found, 308.93992.

6-Chloro-3-(3,4-dimethoxyphenyl)-2-methylimidazo[1,2-*b*]-pyridazin-8-amine (49). Compound **48** (176 mg, 0.57 mmol) was dissolved in 1,4-dioxane (8 mL) and (3,4-dimethoxyphenyl)boronic acid (124 mg, 0.68 mmol) followed by 1 M potassium carbonate solution (2 mL) was added. The reaction mixture was stirred and degassed 3 times, after which $Pd(PPh_3)_4$ (5 mol %) was added, and the mixture was degassed once more, heated up to 90 °C, and stirred overnight. After cooling to rt, the mixture was diluted with water and extracted with EtOAc (2 × 50 mL), and the combined organic phases were dried over sodium sulfate and evaporated. Residue was dissolved in a minimal volume of DCM/MeOH, adsorbed on silica gel, and purified by column chromatography (EtOAc). Recrystallization from hot MeOH/$CHCl_3$ 6:1 afforded analytical sample **49** as an off-white solid. Yield: 145 mg (80%); mp 230.9−231.7 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 7.31 (bs, 2H, 8-NH_2), 7.21 (d, $J_{2'-6'}$ = 2.0, 1H, H-2′), 7.16 (dd, $J_{6'-2'}$ = 2.0, $J_{6'-5'}$ = 8.3, 1H, H-6′), 7.10 (d, $J_{5'-6'}$ = 8.3, 1H, H-5′), 6.15 (s, 1H, H-7), 3.82 (s, 3H, 3′-O-CH_3), 3.78 (s, 3H, 4′-O-CH_3), 2.42 (s, 3H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 148.7 and148.6 (C3′ and C4′), 146.5 (C-6), 144.4 (C-8), 137.7 (C-2), 131.0 (C-9), 125.1 (C-3), 122.1 (C-6′), 121.1 (C-1′), 113.2 (C-2′), 112.0 (C-5′), 93.7 (C-7), 55.8 and 55.8 (3′-O-CH_3 and 4′-O-CH_3), 14.7 (2-CH_3). Anal. ($C_{15}H_{15}ClN_4O_2$·0.17$CHCl_3$) C, H, N.

Protein Expression and Purification. The PI4K IIIβ was expressed and purified using protocols developed in our laboratory.[4,47,48] Briefly, the protein was affinity purified by using Ni-NTA resin (QIAGEN). Upon cleavage of the 6× His affinity tag, the PI4K IIIβ was further purified at Superdex 200 column (GE Healthcare) in 20 mM citrate, pH = 5.5, 200 mMNaCl, 3 mM β-mercaptoethanol. PI4K IIIβ was concentrated to ∼5 mg/mL, flash frozen in liquid nitrogen, and stored at −80 °C until use.

Crystallization and Structure Determination. Before setting up crystal drops, the protein was supplemented with 5 mM ATP and 2 mM $MgCl_2$, or in the case of the structure with MI103 inhibitor bound, the PI4K IIIβ was incubated overnight at 4 °C with 0.5 mM MI103. The crystals grew 5 days at 293 K in sitting drops consisting of a 1:1 mixture of the protein and a well solution (100 mM MOPS/HEPES-Na pH 7.5, 10% w/v PEG 4000, 30 mM diethylene glycol, 30 mM triethylene glycol, 20% v/v glycerol). The crystals were directly frozen in liquid nitrogen, and complete data sets were collected at the MX-14.1 beamLine at BESSY II, Berlin. The structures were solved by molecular replacement using the PI4K IIIβ in complex with PIK93 (PDB code 4D0L) and refined using Phenix[49] to R_{work} = 18.25% and R_{free} = 24.41% for the structure with ATP bound and to R_{work} = 20.89% and R_{free} = 25.16% for the structure with **49** bound as detailed in Table 3.

Inhibition of the Activity of PI4Ks. The lipid kinase activity was determined by ADP-Glo kinase assay (Promega) measuring the amount of ADP produced during the kinase reaction. Reactions were carried out in a total volume of 5 μL and contained PI4K enzyme (final concentration for PI4K IIIβ was 4 ng/μL, for PI4K IIIα was 2 ng/μL, and for PI4K IIα was 2 ng/μL) in kinase buffer (20 mM TRIS pH 7.5, 5 mM $MgCl_2$, 0.2% Triton-X, 0.1 mg/mLBSA, 2 mM DTT), PI/PS (lipid kinase substrate) in kinase buffer (final concentration = 50 μM), and inhibitors (10 mM stock solutions in DMSO were diluted with kinase buffer to final concentration dependent on inhibitor's activity, e.g., 400−0.01, 300−0.0001, 150−0.00001 μM for PI4K IIIβ), and the reaction was started by adding ATP in kinase buffer (final concentration 100 μM). This reaction was carried out for 60 min/25 °C, and the amount of hydrolyzed ATP was measured according to the manufacturer's protocol (ADP/Glo reagent was added to terminate the kinase reaction and deplete the remaining ATP, then Kinase Detection Reagent was added to simultaneously convert ADP to ATP and allow the newly synthesized ATP to be measured using a luciferase/luciferin reaction). Luminescence was measured using spectrophotometer TECAN infinite M 1000.

Screening of Antiviral Activity. The anticoxsackie activity was measured by determining the extent to which the test compounds inhibited virus-induced cytopathic effect in HeLa cells. Briefly, 3-fold serial dilutions of compounds were added in triplicate in a 96-well plate with 30000 HeLa cells plated a day before in DMEM with L-glutamine supplemented with 2% fetal bovine serum (both GE Healthcare, Little Chalfont, UK), 100 U/mL of penicillin, and 100 μg/mL of streptomycin (Sigma-Aldrich, St. Louis, USA). After 1 h incubation, Coxsackie B3 virus (strain Nancy, ATCC, Manassas, USA) was added at multiplicity of infection at 0.005 IU/cell. Following 3 days incubation at 37 °C in a 5% CO_2 incubator, the cell viability was determined by addition of XTT solution (Sigma-Aldrich) for 4 h and the absorbance of newly formed orange formazan solution was measured using Victor X3 plate reader (PerkinElmer). Drug concentrations required to reduce viral cytopathic effect by 50% (EC_{50}) were calculated using nonlinear regression analysis from plots of percentage cell viability versus $\log_{10}$ drug concentration using GraphPad Prism v.6.02 (GraphPad Software, La Jolla, USA).

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

The antirhinovirus activity of test compounds was measured in H1-HeLa cells. Compounds were prepared in replicate 3-fold serial dilutions in DMSO (384-well format), and 0.1 μL of these dilutions were transferred acoustically to assay plates with an Echo instrument. H1-HeLa cells in RPMI medium supplemented with 10% heat inactivated FBS and antibiotics were premixed in batch with mixture of HRV1A, HRV14, and HRV16 at a $TCID_{90}$ of 4× for each strain. After three day incubation at 33 °C, virus-induced cytopathic effects were determined by a Cell-Titer Glo viability assay (Promega, Madison, WI).

Anti-HCV activity of compounds was determined in multiplex assay using HUH 7-lunet, stably replicating I389luc-ubi-neo/NS3-3′/ET genotype 1b replicon and 2aLucNeo-25 cell line encoding a genotype 2a JFH-1 replicon. Cells were maintained in Dulbecco DMEM medium supplemented with GlutaMAX (Invitrogen), 10% FBS (not heat-inactivated), 1 mg/mL G-418, Pen-Strep, and nonessential amino acids. Cells were plated into 384-well assay plates with 1600 cells per well and treated with serial dilutions of compounds. Following three day incubation, the activity of Renilla luciferase was quantified using the Dual-Glo luciferase assay system from Promega (Promega, Madison, WI).

Docking. The 3D structures of the docked molecules were built using ACD/ChemSketch 12.01,[50] and the geometry was optimized with MOPAC2012[51] using the PM7 method. The necessary format conversions were performed using OpenBabel.[52] The preparation of the pdbqt files was done by standard procedure using AutoDock Tools 1.5.6. The docking runs were performed in AutoDock Vina using the default scoring function. Docking of the ligands into the binding pocket was performed in 24 × 34 × 30 $Å^3$ search space centered at 25, 33, and 0.5 Å and exhaustiveness 100.[53] Because the position of several important residues varies in the two crystal structures, we decided to use flexible docking into the structure with MI103 in order to simulate flexibility of the enzyme (4WAG strucute was used for this purpose). We set Lys^{564} and Tyr^{400} as flexible residues that resulted in obtaining the most reliable results of the docking. The docking results of the top five compounds are summarized in Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

Detailed SAR discussion, results of biochemical assays for remaining compounds, and supplementary figures. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b00499.

Accession Codes

The atomic coordinates and structure factors have been deposited in the RCSB Protein Data Bank, www.pdb.org (accession codes 4WAE and 4WAG).

■ AUTHOR INFORMATION

Corresponding Authors

*For E.B.: phone, +420-220-183-465; E-mail, boura@uochb.cas.cz.

*For R.N.: phone, +420-220-183-265; E-mail, nencka@uochb.cas.cz.

Author Contributions

§I.M., D.C. and M.K. contributed equally to this work.

Notes

The authors declare the following competing financial interest(s): The project was partially founded by a private company Gilead Sciences, Inc.

■ ACKNOWLEDGMENTS

We are grateful to Dr. Tamas Balla, Dr. David Murray, Dr. Eric Lansdon, and Michael Downey for critical reading of our manuscript. We are grateful to Eva Zborníková for measurement of HPLC-MS spectra. The project was supported by the Czech Science Foundation (registration no. 15-09310S to I.M., D.C., M.Š., P.P. and R.N. and 15-21030Y to A.B. and E.B) and by the Academy of Sciences Czech Republic (RVO: 61388963). MarieCurie FP7-PEOPLE-2012-CIG project number 333916 and Project InterBioMed LO1302 from Ministry of Education of the Czech Republic are also acknowledged. The work was supported by Gilead Sciences, Inc. The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under BioStruct-X (grant agreement no. 283570). We thank H.Z.B. for the allocation of synchrotron radiation beamtime.

■ ABBREVIATIONS USED

ACBD3, Golgi resident protein GCP60; Ade, adenine; AP, clathrin-associated adaptor protein complex; Arf, ADP-ribosylation factor; CVB3, Coxsackievirus B3; Erf3, eukaryotic release factor 3; GBF1, Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1; HRV, human rhinovirus; MERS, Middle East respiratory syndrome coronavirus; PI4K, phosphatidylinositol 4-kinase; PI4P, phosphatidylinositol 4-phosphate; SARS, severe acute respiratory syndrome coronavirus; TNG, trans-Golgi network

■ REFERENCES

(1) Balla, T. Phosphoinositides: Tiny Lipids with Giant Impact on Cell Regulation. *Phys. Rev.* **2013**, *93*, 1019–1137.

(2) Balla, A.; Balla, T. Phosphatidylinositol 4-kinases: old enzymes with emerging functions. *Trends Cell. Biol.* **2006**, *16*, 351–361.

(3) Minogue, S.; Waugh, M. The Phosphatidylinositol 4-Kinases: Don't Call it a Comeback. In *Phosphoinositides I: Enzymes of Synthesis and Degradation*; Balla, T., Wymann, M., York, J. D., Eds.; Springer Verlag: New York, 2012; Volume *58*, pp 1–24.

(4) Baumlova, A.; Chalupska, D.; Różycki, B.; Jovic, M.; Wisniewski, E.; Klima, M.; Dubankova, A.; Kloer, D. P.; Nencka, R.; Balla, T.; Boura, E. The crystal structure of the phosphatidylinositol 4-kinase IIα. *EMBO Rep.* **2014**, *15*, 1085–1092.

(5) Zhou, Q.; Li, J.; Yu, H.; Zhai, Y.; Gao, Z.; Liu, Y.; Pang, X.; Zhang, L.; Schulten, K.; Sun, F.; Chen, C. Molecular insights into the membrane-associated phosphatidylinositol 4-kinase II alpha. *Nature Commun.* **2014**, *5*, 3552 DOI: 10.1038/ncomms4552.

(6) Burke, J. E.; Inglis, A. J.; Perisic, O.; Masson, G. R.; McLaughlin, S. H.; Rutaganira, F.; Shokat, K. M.; Williams, R. L. Structures of PI4KIIIβ complexes show simultaneous recruitment of Rab11 and its effectors. *Science* **2014**, *344*, 1035–1038.

(7) Wang, Y. J.; Wang, J.; Sun, H. Q.; Martinez, M.; Sun, Y. X.; Macia, E.; Kirchhausen, T.; Albanesi, J. P.; Roth, M. G.; Yin, H. L. Phosphatidylinositol 4 phosphate regulates targeting of clathrin adaptor AP-1 complexes to the golgi. *Cell* **2003**, *114*, 299–310.

(8) Minogue, S.; Waugh, M. G.; De Matteis, M. A.; Stephens, D. J.; Berditchevski, F.; Hsuan, J. J. Phosphatidylinositol 4-kinase is required for endosomal trafficking and degradation of the EGF receptor. *J. Cell Sci.* **2006**, *119*, 571–580.

(9) Jović, M.; Kean, M. J.; Dubankova, A.; Boura, E.; Gingras, A.-C.; Brill, J. A.; Balla, T. Endosomal sorting of VAMP3 is regulated by PI4K2A. *J. Cell Sci.* **2014**, *127*, 3745–3756.

(10) Jović, M.; Kean, M. J.; Szentpetery, Z.; Polevoy, G.; Gingras, A.-C.; Brill, J. A.; Balla, T. Two phosphatidylinositol 4-kinases control lysosomal delivery of the Gaucher disease enzyme, β-glucocerebrosidase. *Mol. Biol. Cell* **2012**, *23*, 1533–1545.

(11) Craige, B.; Salazar, G.; Faundez, V. Phosphatidylinositol-4-kinase type II alpha contains an AP-3-sorting motif and a kinase domain that are both required for endosome traffic. *Mol. Biol. Cell* **2008**, *19*, 1415–1426.

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767–3793

(12) Wieffer, M.; Uhalte, E. C.; Posor, Y.; Otten, C.; Branz, K.; Schutz, I.; Mossinger, J.; Schu, P.; Abdelilah-Seyfried, S.; Krauss, M.; Haucke, V. PI4K2 beta/AP-1-Based TGN-Endosomal Sorting Regulates Wnt Signaling. *Curr. Biol.* **2013**, *23*, 2185−2190.
(13) Wu, X.; Chi, R. J.; Baskin, J. M.; Lucast, L.; Burd, C. G.; De Camilli, P.; Reinisch, K. M. Structural Insights into Assembly and Regulation of the Plasma Membrane Phosphatidylinositol 4-Kinase Complex. *Dev. Cell* **2014**, *28*, 19−29.
(14) Chung, J.; Nakatsu, F.; Baskin, J. M.; De Camilli, P. Plasticity of PI4KIIIalpha interactions at the plasma membrane. *EMBO Rep.* **2015**, *16*, 312−20.
(15) Sasaki, J.; Ishikawa, K.; Arita, M.; Taniguchi, K. ACBD3-mediated recruitment of PI4KB to picornavirus RNA replication sites. *EMBO J.* **2012**, *31*, 754−766.
(16) Godi, A.; Pertile, P.; Meyers, R.; Marra, P.; Di Tullio, G.; Iurisci, C.; Luini, A.; Corda, D.; De Matteis, M. A. ARF mediates recruitment of PtdIns-4-OH kinase-[bgr] and stimulates synthesis of PtdIns(4,5)-P2 on the Golgi complex. *Nature Cell Biol.* **1999**, *1*, 280−287.
(17) Audhya, A.; Emr, S. D. Stt4 PI 4-kinase localizes to the plasma membrane and functions in the Pkc1-mediated MAP kinase cascade. *Dev. Cell* **2002**, *2*, 593−605.
(18) Strahl, T.; Hama, H.; DeWald, D. B.; Thorner, J. Yeast phosphatidylinositol 4-kinase, Pik1, has essential roles at the Golgi and in the nucleus. *J. Cell Sci.* **2005**, *171*, 967−979.
(19) Altan-Bonnet, N.; Balla, T. Phosphatidylinositol 4-kinases: hostages harnessed to build panviral replication platforms. *Trends Cell Biol.* **2012**, *37*, 293−302.
(20) Berger, K. L.; Cooper, J. D.; Heaton, N. S.; Yoon, R.; Oakland, T. E.; Jordan, T. X.; Mateu, G.; Grakoui, A.; Randall, G. Roles for endocytic trafficking and phosphatidylinositol 4-kinase III alpha in hepatitis C virus replication. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106*, 7577−7582.
(21) LaMarche, M. J.; Borawski, J.; Bose, A.; Capacci-Daniel, C.; Colvin, R.; Dennehy, M.; Ding, J.; Dobler, M.; Drumm, J.; Gaither, L. A.; Gao, J.; Jiang, X.; Lin, K.; McKeever, U.; Puyang, X.; Raman, P.; Thohan, S.; Tommasi, R.; Wagner, K.; Xiong, X.; Zabawa, T.; Zhu, S.; Wiedmann, B. Anti-Hepatitis C Virus Activity and Toxicity of Type III Phosphatidylinositol-4-Kinase Beta Inhibitors. *Antimicrob. Agents Chemother.* **2012**, *56*, 5149−5156.
(22) Borawski, J.; Troke, P.; Puyang, X. L.; Gibaja, V.; Zhao, S. C.; Mickanin, C.; Leighton-Davies, J.; Wilson, C. J.; Myer, V.; CornellaTaracido, I.; Baryza, J.; Tallarico, J.; Joberty, G.; Bantscheff, M.; Schirle, M.; Bouwmeester, T.; Mathy, J. E.; Lin, K.; Compton, T.; Labow, M.; Wiedmann, B.; Gaither, L. A. Class III Phosphatidylinositol 4-Kinase Alpha and Beta Are Novel Host Factor Regulators of Hepatitis C Virus Replication. *J. Virol.* **2009**, *83*, 10058−10074.
(23) Greninger, A. L.; Knudsen, G. M.; Betegon, M.; Burlingame, A. L.; DeRisi, J. L. The 3A Protein from Multiple Picornaviruses Utilizes the Golgi Adaptor Protein ACBD3 To Recruit PI4KIII beta. *J. Virol.* **2012**, *86*, 3605−3616.
(24) Arita, M.; Kojima, H.; Nagano, T.; Okabe, T.; Wakita, T.; Shimizu, H. Phosphatidylinositol 4-Kinase III Beta Is a Target of Enviroxime-Like Compounds for Antipoliovirus Activity. *J. Virol.* **2011**, *85*, 2364−2372.
(25) Alastruey-Izquierdo, A.; Mellado, E.; Pelaez, T.; Peman, J.; Zapico, S.; Alvarez, M.; Rodriguez-Tudela, J. L.; Cuenca-Estrella, M.; Grp, F. S. Phosphatidylinositol 4-Kinase III Beta Is Essential for Replication of Human Rhinovirus and Its Inhibition Causes a Lethal Phenotype in Vivo. *Antimicrob. Agents Chemother.* **2013**, *57*, 3358−3368.
(26) Mello, C.; Aguayo, E.; Rodriguez, M.; Lee, G.; Jordan, R.; Cihlar, T.; Birkus, G. Multiple Classes of Antiviral Agents Exhibit in Vitro Activity against Human Rhinovirus Type C. *Antimicrob. Agents Chemother.* **2014**, *58*, 1546−1555.
(27) Yang, N.; Ma, P.; Lang, J.; Zhang, Y.; Deng, J.; Ju, X.; Zhang, G.; Jiang, C. Phosphatidylinositol 4-Kinase III*β* Is Required for Severe Acute Respiratory Syndrome Coronavirus Spike-Mediated Cell Entry. *J. Biol. Chem.* **2012**, *287*, 8457−8467.
(28) Berger, K. L.; Kelly, S. M.; Jordan, T. X.; Tartell, M. A.; Randall, G. Hepatitis C Virus Stimulates the Phosphatidylinositol 4-Kinase III Alpha-Dependent Phosphatidylinositol 4-Phosphate Production That Is Essential for Its Replication. *J. Virol.* **2011**, *85*, 8870−8883.
(29) Dorobantu, C. M.; van der Schaar, H. M.; Ford, L. A.; Strating, J. R. P. M.; Ulferts, R.; Fang, Y.; Belov, G.; van Kuppeveld, F. J. M. Recruitment of PI4KIII*β* to Coxsackievirus B3 Replication Organelles Is Independent of ACBD3, GBF1, and Arf1. *J. Virol.* **2014**, *88*, 2725−2736.
(30) Bojjireddy, N.; Botyanszki, J.; Hammond, G.; Creech, D.; Peterson, R.; Kemp, D. C.; Snead, M.; Brown, R.; Morrison, A.; Wilson, S.; Harrison, S.; Moore, C.; Balla, T. Pharmacological and genetic targeting of pPI4KA reveals its important role in maintaining plasma membrane PtdIns4p and PtdIns(4,5)p2 levels. *J. Biol. Chem.* **2014**, *289*, 6120−6132 DOI: 10.1074/jbc.M113.531426.
(31) Vaillancourt, F. H.; Brault, M.; Pilote, L.; Uyttersprot, N.; Gaillard, E. T.; Stoltz, J. H.; Knight, B. L.; Pantages, L.; McFarland, M.; Breitfelder, S.; Chiu, T. T.; Mahrouche, L.; Faucher, A.-M.; Cartier, M.; Cordingley, M. G.; Bethell, R. C.; Jiang, H.; White, P. W.; Kukolj, G. Evaluation of Phosphatidylinositol-4-kinase III alpha as a Hepatitis C Virus Drug Target. *J. Virol.* **2012**, *86*, 11595−11607.
(32) Waring, M. J.; Andrews, D. M.; Faulder, P. F.; Flemington, V.; McKelvie, J. C.; Maman, S.; Preston, M.; Raubo, P.; Robb, G. R.; Roberts, K.; Rowlinson, R.; Smith, J. M.; Swarbrick, M. E.; Treinies, I.; Winter, J. J. G.; Wood, R. J. Potent, selective small molecule inhibitors of type III phosphatidylinositol-4-kinase alpha-but not beta-inhibit the phosphatidylinositol signaling cascade and cancer cell proliferation. *Chem. Commun.* **2014**, *50*, 5388−5390.
(33) Leivers, A. L.; Tallant, M.; Shotwell, J. B.; Dickerson, S.; Leivers, M. R.; McDonald, O. B.; Gobel, J.; Creech, K. L.; Strum, S. L.; Mathis, A.; Rogers, S.; Moore, C. B.; Botyanszki, J. Discovery of Selective Small Molecule Type III Phosphatidylinositol 4-Kinase Alpha (P14KIII alpha) Inhibitors as Anti Hepatitis C (HCV) Agents. *J. Med. Chem.* **2014**, *57*, 2091−2106.
(34) Keaney, E. P.; Connolly, M.; Dobler, M.; Karki, R.; Honda, A.; Sokup, S.; Karur, S.; Britt, S.; Patnaik, A.; Raman, P.; Hamann, L. G.; Wiedmann, B.; LaMarche, M. J. 2-Alkyloxazoles as potent and selective PI4KIII*β* inhibitors demonstrating inhibition of HCV replication. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3714−3718.
(35) MacLeod, A. M.; Mitchell, D. R.; Palmer, N. J.; de Poel, H. V.; Conrath, K.; Andrews, M.; Leyssen, P.; Neyts, J. Identification of a Series of Compounds with Potent Antiviral Activity for the Treatment of Enterovirus Infections. *ACS Med. Chem. Lett.* **2013**, *4*, 20−24.
(36) Spickler, C.; Lippens, J.; Laberge, M.-K.; Desmeules, S.; Bellavance, É.; Garneau, M.; Guo, T.; Hucke, O.; Leyssen, P.; Neyts, J.; Vaillancourt, F. H.; Décor, A.; O'Meara, J.; Franti, M.; Gauthier, A. Phosphatidylinositol 4-Kinase III Beta Is Essential For Replication of Human Rhinovirus and Its Inhibition Causes a Lethal Phenotype in Vivo. *Antimicrob. Agents Chemother.* **2013**, *57*, 3358−3368.
(37) Chen, C.; Wilcoxen, K. M.; Huang, C. Q.; McCarthy, J. R.; Chen, T.; Grigoriadis, D. E. Optimization of 3-phenylpyrazolo-1,5-*a* pyrimidines as potent corticotropin-releasing factor-1 antagonists with adequate lipophilicity and water solubility. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3669−3673.
(38) Kazock, J. Y.; Enguehard-Gueiffier, U.; Thery, I.; Gueiffier, A. Evaluation of the 2-substituent effect on the reactivity of the 8-haloimidazo 1,2-*a* pyridine series towards Suzuki-type cross-coupling reaction. *Bull. Chem. Soc. Jpn.* **2005**, *78*, 154−159.
(39) LeClercq, L. C. L., ; Bartolomé-Nebreda, J. M.; Conde-Ceide, S.; Van Gool, M. L. M. Inhibitors of phosphodiesterase 10 enzyme. WO2014009305 A1, 2014.
(40) Cooper, T.; Novak, A.; Humphreys, L. D.; Walker, M. D.; Woodward, S. User-friendly methylation of aryl and vinyl halides and pseudohalides with DABAL-Me-3. *Adv. Synth. Catal.* **2006**, *348*, 686−690.
(41) Zuev, D.; Mattson, R. J.; Huang, H.; Mattson, G. K.; Zueva, L.; Nielsen, J. M.; Kozlowski, E. S.; Huang, X. S.; Wu, D.; Gao, Q.; Lodge, N. J.; Bronson, J. J.; Macor, J. E. Potential CRF1R PET Imaging Agents: *N*-Fluoroalkyl-8-(6-methoxy-2-methylpyridin-3-yl)-2,7-di-

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793

methyl-*N*-alkylpyrazolo-1,5-*a*-1,3,5 triazin-4-amines. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2484−2488.
(42) Tai, A. W.; Bojjireddy, N.; Balla, T. A homogeneous and nonisotopic assay for phosphatidylinositol 4-kinases. *Anal. Biochem.* **2011**, *417*, 97−102.
(43) Debreczeni, J. E.; Emsley, P. Handling ligands with Coot. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2012**, *68*, 425−430.
(44) Rozycki, B.; Boura, E. Large, dynamic, multi-protein complexes: a challenge for structural biology. *J. Phys.: Condens. Matter* **2014**, *26*, 463103 DOI: 10.1088/0953-8984/26/46/463103.
(45) Pastor-Fernández, J.; Bartolomé-Nebreda, J. M.; MacDonald, G. J.; Conde-Ceide, S.; Delgado-González, Ó.; Vanhoof, G. C. P.; Van Gool, M. L. M.; Martín-Martín, M. L.; Alonso-De Diego, S. A. Imidazo[l,2-*b*]pyridazine derivatives and their use as PDE10 inhibitors. WO 2011/051342 A1, 2011.
(46) Walsh, J. J.; Shah, R.; McCormack, E. M.; Hudson, G. J.; White, M.; Stack, G. D.; Moran, B. W.; Coogan, A.; Breen, E. C. Tubulin binding agents. WO 2013/026942, 2013.
(47) Nemecek, D.; Boura, E.; Wu, W.; Cheng, N.; Plevka, P.; Qiao, J.; Mindich, L.; Heymann, J. B.; Hurley, J. H.; Steven, A. C. Subunit Folds and Maturation Pathway of a dsRNA Virus Capsid. *Structure* **2013**, *21*, 1374−1383.
(48) Boura, E.; Rezabkova, L.; Brynda, J.; Obsilova, V.; Obsil, T. Structure of the human FOXO4−DBD−DNA complex at 1.9 A resolution reveals new details of FOXO binding to the DNA. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2010**, *66*, 1351−1357.
(49) Adams, P. D.; Afonine, P. V.; Bunkoczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L. W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr., Sect. D: Biol. Crystallogr.* **2010**, *66*, 213−221.
(50) *ACD/ChemSketch*, 12.01; Advanced Chemistry Development, Inc.: Toronto, Ontario, Canada, 2014; www.acdlabs.com.
(51) Stewart J. P. *MOPAC2012*, version 7.263W; Stewart Computational Chemistry: Colorado Springs, CO, 2012; HTTP://OpenMOPAC.net, .
(52) O'Boyle, N.; Banck, M.; James, C.; Morley, C.; Vandermeersch, T.; Hutchison, G. Open Babel: An open chemical toolbox. *J. Cheminform.* **2011**, *3*, 33 DOI: 10.1186/1758-2946-3-33.
(53) Trott, O.; Olson, A. J. Software News and Update AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. *J. Comput. Chem.* **2010**, *31*, 455−461.

DOI: 10.1021/acs.jmedchem.5b00499
J. Med. Chem. 2015, 58, 3767−3793