Journal of Medicinal Chemistry

Article

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Rational Design of Novel Highly Potent and Selective Phosphatidylinositol 4-Kinase IIIβ (PI4KB) Inhibitors as Broad-Spectrum Antiviral Agents and Tools for Chemical Biology

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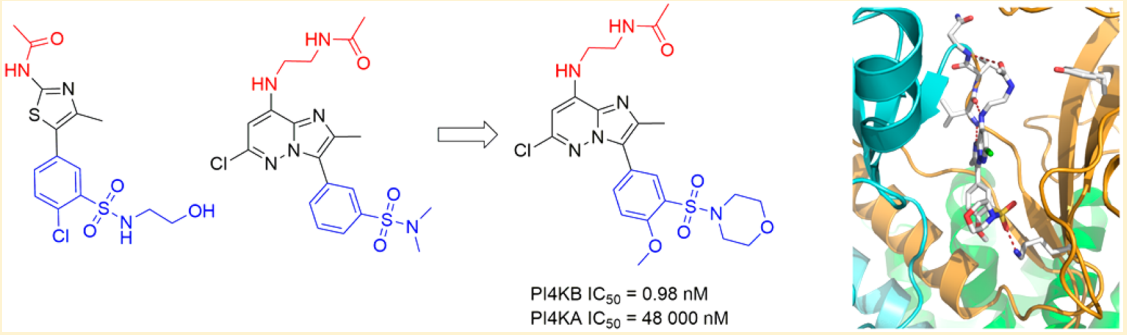
Supporting Information



ABSTRACT: Phosphatidylinositol 4-kinase IIIβ (PI4KB) is indispensable for the replication of various positive-sense single stranded RNA viruses, which hijack this cellular enzyme to remodel intracellular membranes of infected cells to set up the functional replication machinery. Therefore, the inhibition of this PI4K isoform leads to the arrest of viral replication. Here, we report on the synthesis of novel PI4KB inhibitors, which were rationally designed based on two distinct structural types of inhibitors that bind in the ATP binding side of PI4KB. These "hybrids" not only excel in outstanding inhibitory activity but also show high selectivity to PI4KB compared to other kinases. Thus, these compounds exert selective nanomolar or even subnanomolar activity against PI4KB as well as profound antiviral effect against hepatitis C virus, human rhinovirus, and coxsackievirus B3. Our crystallographic analysis unveiled the exact position of the side chains and explains their extensive contribution to the inhibitory activity.

INTRODUCTION

Positive-sense single stranded RNA (+RNA) viruses replicate at specialized compartments termed replication factories or membranous webs.[1] These membrane-derived compartments are specifically enriched in the phosphatidylinositol 4-phosphate (PI4P) lipid.[2] Humans have four kinases that synthesize PI4P, two typical lipid kinases, PI4KA and PI4KB (also known as PI4K IIIα and PI4K IIIβ), and two type II (or atypical) kinases, PI4K2A and PI4K2B (also known as PI4K IIα and PI4K IIβ).[3,4] In 2009, Berger and colleagues used siRNA screen to identify essential host factors for the replication of hepatitis C virus (HCV) and found PI4KA to be an essential host factor for HCV.[5] Since then PI4KB has also been reported to be an essential host factor for a plethora of +RNA viruses including SARS coronavirus, kobuvirus, poliovirus, coxsackievirus B3, and human rhinovirus.[6−8] Surprisingly, so far PI4K2A or PI4K2B have never been reported as a viral cofactor. These enzymes are palmitoylated and can behave as integral transmembrane proteins; thus, it might be too difficult for a virus to manipulate them.

Now it is generally accepted that most +RNA viruses hijack PI4K kinase to produce PI4P-enriched membranes.[9] Viruses have evolved different strategies of PI4K hijacking. The aforementioned

Received: October 5, 2016
Published: December 8, 2016

DOI: 10.1021/acs.jmedchem.6b01465
J. Med. Chem. 2017, 60, 100−118

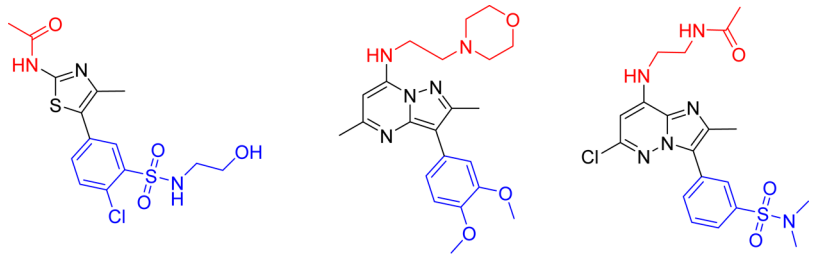


Figure 1. Structures of PI4KB inhibitors.

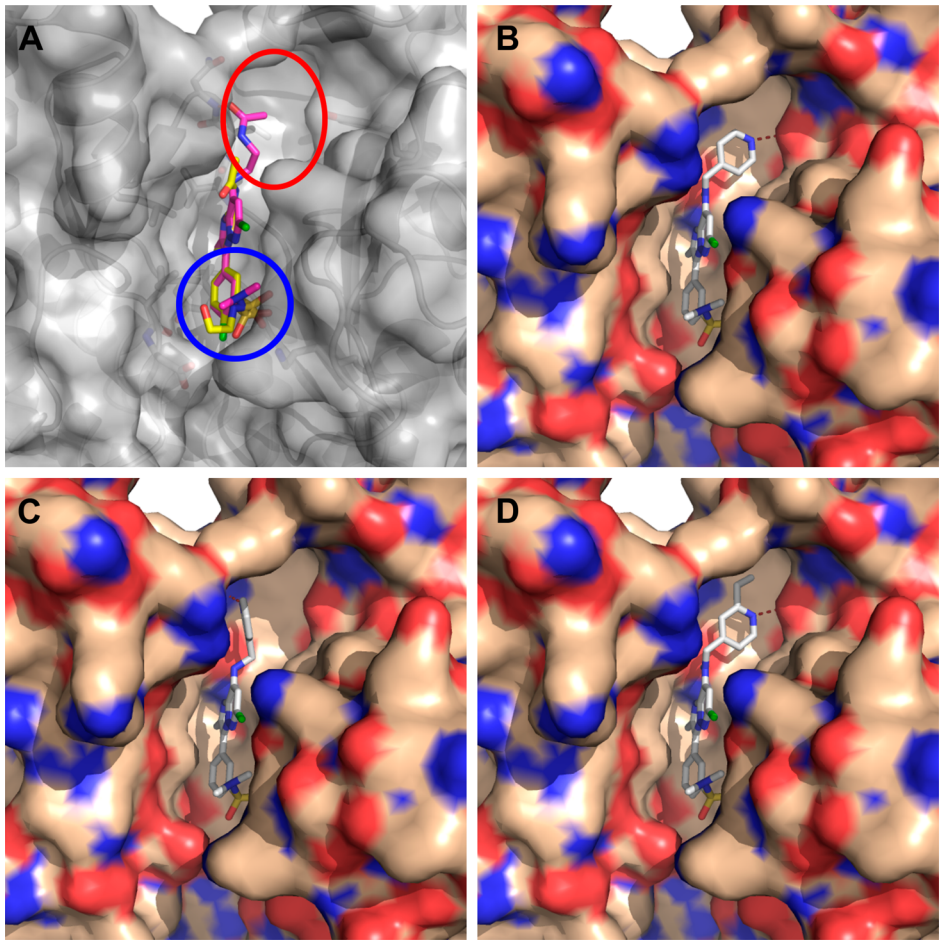


Figure 2. Design of new series of PI4KB inhibitors. (A) Overlay of the crystal structure of PI4KB with **1** (yellow) and the docking model of the preferred conformation of **3** (magenta) unveils two sites of possible modifications. The red region can easily accommodate a large aromatic substituent. The blue region appears open for further optimization of the aromatic part of the molecule. (B–D) Docking poses of derivatives **4**, **5**, and **8**, respectively. The figure was prepared using PyMOL.

HCV uses its NS5A protein to directly hijack PI4KA.[10] Many picornaviruses use their nonstructural 3A protein to hijack PI4KB. For instance, the 3A protein from the poliovirus, coxsackievirus, rhinovirus, or Aichi virus binds to the Golgi residing ACBD3 (acyl-CoA binding domain containing 3) protein, a known binding partner of the PI4KB.[8,11,12] However, picornaviruses have also evolved additional means of PI4KB hijacking that are ACBD3 independent.[13−15]

PI4Ks are considered a potential pharmacological target because of their importance in the life cycle of various +RNA viruses. The discovery of their importance for +RNA viruses aroused big interest in PI4Ks in the scientific community. During the last two years crystal structures of P4KB, PI4K2A, and PI4K2B have become available,[16−19] providing critical information for structure aided inhibitor design. The last missing piece is the structure of PI4KA perhaps due to difficulties in the expression of the recombinant protein.[20] So far only a study that used experiment guided homology modeling is available.[21]

In the past few years, both academia and pharma invested substantial amounts of time in developing small molecule inhibitors targeting PI4KA and PI4KB. One of the first compounds, **1** (PIK93), has attracted attention because of its selectivity toward

DOI: 10.1021/acs.jmedchem.6b01465
J. Med. Chem. 2017, 60, 100−118

PI4KB;[22,23] however, **1** has also notable activity toward PI3 kinases.[24] Later it was used as a starting point for developing isoform specific inhibitors. A compound resembling **1** was reported to inhibit PI4KA with IC_{50} = 450 nM but the selectivity was rather poor (IC_{50} = 8000 nM for PI4KB).[25] Recently, very potent inhibitors of PI4KA were reported, IC_{50} = ~1 nM for the best compound; however, the selectivity was not optimal (IC_{50} = 250 nM for PI4KB).[26] Perhaps the best inhibitor of PI4KA was reported by GlaxoSmithKline, which had an IC_{50} of 5 nM against PI4KA and negligible activity against PI4KB.[27] We and others have also reported PI4KB inhibitors with varying degrees of potency and specificity[28−32] with best compounds having an IC_{50} between 10 and 20 nM. Important questions regarding the drugability of these enzymes remain as knockout of these enzymes is detrimental to the animals.[25,33] However, upregulated PI4K activity is needed for a successful viral infection. Total long time inhibition of either PI4KA or PI4KB might be toxic, but a short time dose (few days or a week) that would inhibit the enzymes only partially might be well tolerated and still fatal for the virus. This is especially the case for PI4KB because PI4K2A can take over many of its roles at the Golgi.[34,35] Further studies are needed to decide whether PI4KB is a valid pharmacological target, and these studies will need very potent and extremely specific inhibitors.

In this study, we present the design and synthesis of novel PI4KB inhibitors that exert high inhibitory potency and outstanding selectivity in comparison to related lipid kinases. Furthermore, our prototypical compounds show absolutely no significant inhibition of any other kinases at 1 μM concentration in kinome-wide screen (Figure 3).

RESULTS AND DISCUSSION

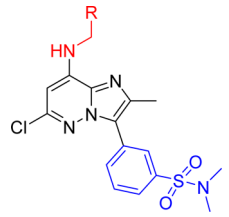
Design of Inhibitors. Generally, specific PI4KB inhibitors targeting the ATP binding site can be divided into two structural groups. First, several compounds derived from **1** exert both significant affinity and high selectivity toward this enzyme. Second, derivatives with a bicyclic central core related to **2** (T-00127-HEV1)[36] were also identified as potent and selective inhibitors of PI4KB.

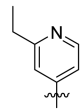
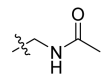
Structural studies reported by us and Burke et al.[16,28] proved that both these types of compounds bind in a similar manner in the ATP binding site and their structures can be easily overlaid and, in principle, merged. This observation, together with subsequent docking studies, led to the design of this second generation of PI4KB inhibitor. These newly designed inhibitors combine features of both these aforementioned compounds into a single molecule. Originally, these compounds were designed based on simple fusion of **1** and our compound **3** (MI14) (Figures 1 and 2), which exerted a unique selectivity profile in our previous studies.[28] Subsequently, we tried to optimize the side chains on both combinatorial and rational bases.

Our previous crystal structures revealed a lateral pocket, which is occupied by the amino side-chain of the molecule (Figure 2A, in red). Evidently, this pocket can be accommodated by significantly more bulky substituent than the (2-acetamidoethyl)amino side-chain employed in our original study.

Therefore, we investigated the effect of several related aromatic moieties that gave the best results in our docking experiments (Table 1 and Figure 2). Interestingly, our docking results suggest that compounds with various amino side-chains

Table 1. Correlation of the Inhibitory Potencies of the Derivatives with Modified "Amino Side Chain" with the Results Obtained from the Docking Experiments Using AutoDock Vina Software



Compd	R	PI4KB IC_{50} (nM)[a]	AutoDock Vina Score
4		26.4	9.9
5		74.6	9.8
6		145.4	9.7
7		33.7	10.2
8		12.5	10.4
3		54.0	9.3

[a]Determined by three independent experiments.

can exploit different sites of the binding canyon. In particular, the derivatives bearing the (2-acetamidoethyl)amino side-chain, for example, **3**, and compounds with pyridine attached at position 3 tend to form an interaction with Asn600 whereas the analogs attached at position 4 bind to Tyr385 on the opposite side of the cleft.

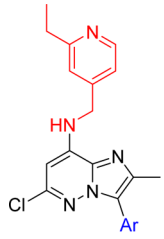
Due to the significant effect on selectivity, the aromatic side chain (Figure 2A, in blue) was more difficult to target by means of rational design, and therefore this part of the molecule was elaborated by a combinatorial approach.

Inhibition of Phosphatidylinositol 4-Kinases. Initially we have focused our attention on the PI4KB potency and selectivity toward specific PI4K isoforms. The enzymatic activity was determined using a luminescent ADP-Glo kinase assay.[37]

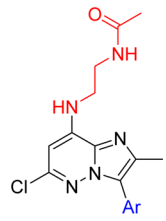
First, we determined the inhibitory activity of derivatives with the optimized "amine side-chain" of the molecule, and it was encouraging to see similar trends in both the activity predicted by our docking experiments performed in AutoDock Vina software[38] and the observed inhibition constants in enzymatic assay. The data obtained for derivatives with 3-(*N*,*N*-dimethylsulfamoyl)phenyl "aromatic side-chain" are summarized in the Table 1. This part of the study shows that

DOI: 10.1021/acs.jmedchem.6b01465
J. Med. Chem. 2017, 60, 100−118

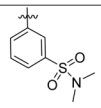
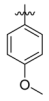
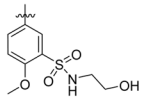
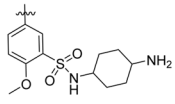
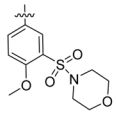
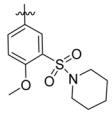
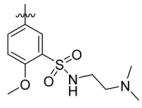
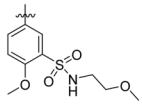
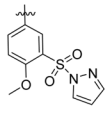
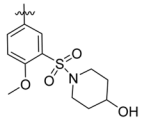
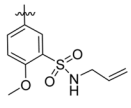
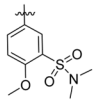
Table 2

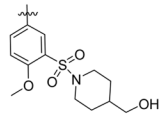
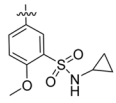
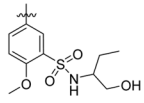
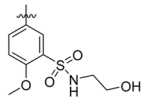
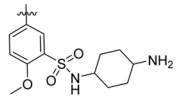
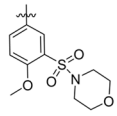
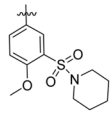
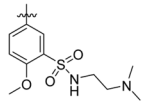
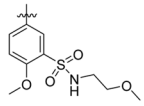
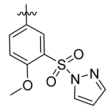
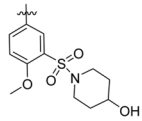
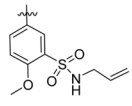


Series A



Series B

Compd	Ar	Series	PI4KB IC_{50} (nM)[a]	PI4KA % of control (IC_{50} (nM))	PI4K2A % of control (IC_{50} (nM))
8		A	12.5	ND	ND
9		A	444.1	102	101
10		A	6.1	42 (13 400)	106
11		A	15.7	9 (553)	101
12		A	17.6	70	97
13		A	59.2	80	99
14		A	9.0	14 (1 128)	100
15		A	17.2	50	104
16		A	19.1	77	112
17		A	6.2	58	109
18		A	8.6	58	105
19		A	27.3	74	106

Compd	Ar	Series	PI4KB IC_{50} (nM)[a]	PI4KA % of control (IC_{50} (nM))	PI4K2A % of control (IC_{50} (nM))
20		A	41.5	61	115
21		A	56.0	84	114
22		A	5.3	59	104
23		B	22.0	63	112
24		B	26.9	13 (660)	102
25		B	0.98	93 (48 000)	88
26		B	189.4	62	60
27		B	93.5	32 (4 860)	105
28		B	134.8	80	107
29		B	163.3	98	83
30		B	43.8	72	88
31		B	19.9	77	93

DOI: 10.1021/acs.jmedchem.6b01465
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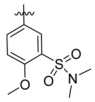
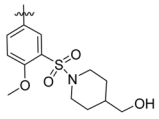
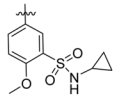
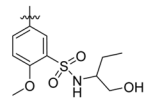
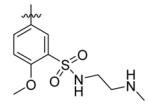
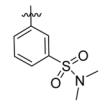
Table 2. continued

Compd	Ar	Series	PI4KB IC_{50} (nM)[a]	PI4KA % of control (IC_{50} (nM))	PI4K2A % of control (IC_{50} (nM))
32		B	2.4	72	104
33		B	14.9	43 (8 190)	85
34		B	105.8	80	108
35		B	7.23	34 (12 280)	77
36		B	146.9	44 (17 070)	73
3		B	54	(>100 000)	(>100 000)

[a]Determined by three independent experiments.

the position of the nitrogen atom on the phenyl ring can significantly contribute to the efficiency of the inhibition, which significantly correlated with results obtained by the docking experiments. Thus, the derivative connected to the central core at position 4 of the pyridine ring (compound **4**) exerted significantly higher potency in comparison with its counterparts attached at position 2 or 3, compounds **6** and **5**, respectively. Subsequently, we also tried to optimize the substituent on the pyrimidine ring. Since there is significant space next to the pyrimidine core, we gradually introduced methyl and ethyl group at the position 2 of the pyrimidine of the side chain, which seemed to fit the best into this cavity of the enzyme based on the docking experiments. Derivative **8** exerted significantly elevated inhibitory activity in comparison to **3** with retention of excellent selectivity toward PI4KB with respect to the most similar isoenzyme, PI4KA.

Therefore, this motive was used in our subsequent studies focused on the "aromatic side-chain" of the molecule. Here, we focused primarily on the additional effect of introducing the substituent vicinal to the sulfonamide moiety and character of the sulfonamide moiety itself (both based on the **1**/**3** hybrid design) (Table 2, compounds **8** and **9**).

The installation of the sulfonamide moiety equivalent to **1** presented immediate success in enhancing the inhibitory activity, and compound **10** exerted significant activity while still retaining the excellent selectivity. This series (Table 2, series A) gave a number of interesting compounds with single digit nanomolar inhibitory activity and showed that the substitution of the sulfonamide moiety has a significant impact on the selectivity toward PI4KB as well. The most active compounds of the series were derivatives **10**, **17**, and **22**, which all exerted low nanomolar activity against PI4KB with outstanding selectivity. All these compounds shared the same sulfonamide substituent, a free hydroxy group.

Series B of this study was mostly driven by our curiosity. Besides the derivatives with the optimized "amino side-chain", we prepared derivatives with the original 2-acetamidoethylamino substituent as well in order to see how substantial is the impact of the more efficient but less flexible pyridine side-chain. To our surprise, some of the analogs within the series provided extremely high inhibitory activity against PI4KB with an outstanding PI4KB/PI4KA selectivity ratio with the most active compound being derivative **25**, which exerts subnanomolar activity against PI4KB (IC_{50} = 0.98 nM) with PI4KA IC_{50} = 48 000 nM.

Antiviral Screening. The obtained compounds were also screened against selected +RNA viruses: human rhinovirus 1 (HRV1), coxsackie virus B3 (CVB3), hepatitis C virus (HCV, 1b and 2a genotypes), and Middle East respiratory syndrome coronavirus (MERS-CoV).

For clarity, we present only the results for the compounds that had an IC_{50} lower than 20 nM in the enzymatic assays (Table 2, additional data can be found in Supporting Information, Tables 2 and 3).

Antiviral screening unveiled a significant difference in activity of the A and B series (Table 3). Generally, the members of the

Table 3

compd	series	HRV1 EC_{50} (nM)	CVB3 EC_{50} (nM)	HCV 1b EC_{50} (nM)	HCV 2a EC_{50} (nM)	HeLa CC_{50} (nM)
8	A	62	1100	393	8689	15354
10	A	75	58	29	14402	18333
11	A	69	56	9196	26540	5757
12	A	<10	83	1025	25988	25729
14	A	<10	151	350	8506	5558
15	A	<10	<20	416	27940	9144
16	A	<10	138	954	>44444	61931
17	A	<10	17	20	20400	8399
18	A	<10	12	667	20147	6853
22	A	<10	476	<20	9439	17850
25	B	655	38	1033	>44444	89223
31	B	990	5310	2681	35923	445413
32	B	716	1320	594	>44444	84176
33	B	5421	5000	1826	>44444	131179
35	B	6756	1661	3415	>44444	>50000

A series exerted extremely high activity against HRV1, which in most cases reached the detection limit of the assay (EC_{50} lower than 100 nM for all presented compounds). Furthermore, most of the members of this series exerted significant inhibitory effect toward CVB3 and HCV 1b. It is noteworthy, that derivatives **10**, **17**, and **22**, which were the most active members of this series in the enzymatic assay, also exerted steady nanomolar activities in these three antiviral assays. Notably, the effect on HCV 2a is rather negligible, which is consistent with genotype

DOI: 10.1021/acs.jmedchem.6b01465
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dependent insensitivity of HCV to knockdown of PI4KB.[39] In this respect, the inactivity can be regarded as proof of selectivity toward PI4KB, as HCV 2a is significantly more susceptible to inhibition of PI4KA.

In contrast, the compounds from series B exerted significantly lower nominal activity in cell-based assays. However, their toxicities were shifted in a similar fashion suggesting that the compounds suffered from limited permeation through the cell membranes and therefore cannot reach the side of the action. However, compound **25**, the derivative exerting the highest activity in the enzymatic assay, was potent enough to inhibit the sensitive viruses at the submicromolar or low micromolar level.

None of the compounds exerted significant antiviral effect against MERS-CoV, which seems to be in contrast with the reported implication of PI4KB in the replication of pathogenic coronaviruses such as severe acute respiratory syndrome coronavirus (SARS-CoV).[6]

Inhibition of Other Kinases by Compound 10. Kinome-wide profiling of compound **10** was performed by Carna Biosciences, Inc. This screen unveiled a striking selectivity toward PI4KB since activity of 318 tested protein kinases was not inhibited at 1 μM concentration of compound **10** to more than to 50% of the control (Figure 3A). Additionally, this compound was also screened against a panel of lipid kinases (PI4KA, PI4K2A, PI3Kα, PI3Kβ, PI3Kδ, PI3Kγ), and none of these lipid kinases was inhibited with an IC_{50} lower than 10 μM (Figure 3B).

Chemistry. Synthesis of the target compounds started by the preparation of iodinated imidazo[1,2-*b*]pyridazine derivatives with the appropriate amine side chain (Scheme 1).

First, we substituted the original aminoethylacetamide side chain with positional isomers of pyridinylmethanamine by means of nucleophilic aromatic substitution (EtOH, DIPEA, 70 °C) of halogenated imidazo[1,2-*b*]pyridazine derivatives (Scheme 1) in moderate to quantitative yields. In consideration of the enzymatic binding site, the simple pyridine moiety was replaced by either 2-methyl or 2-ethylpyridine, which achieved better scores in our docking studies and filled up the cavity of PI4KB with higher efficiency than unsubstituted the pyridines (see above). Both 2-methyl and 2-ethylpyridinylmethanamines were prepared from 2-chloroisonicotinonitrile in two steps following the procedure of Ceccarelli et al.[40]

This series of iodo derivatives served as a modular toolbox for further exploration of SAR focused on the aromatic side chain. Initially, we selected the aryl moiety that displayed the best biological activity among our first series of PI4KB inhibitors, the 3-(*N*,*N*-dimethylsulfamoyl)phenyl. Within this

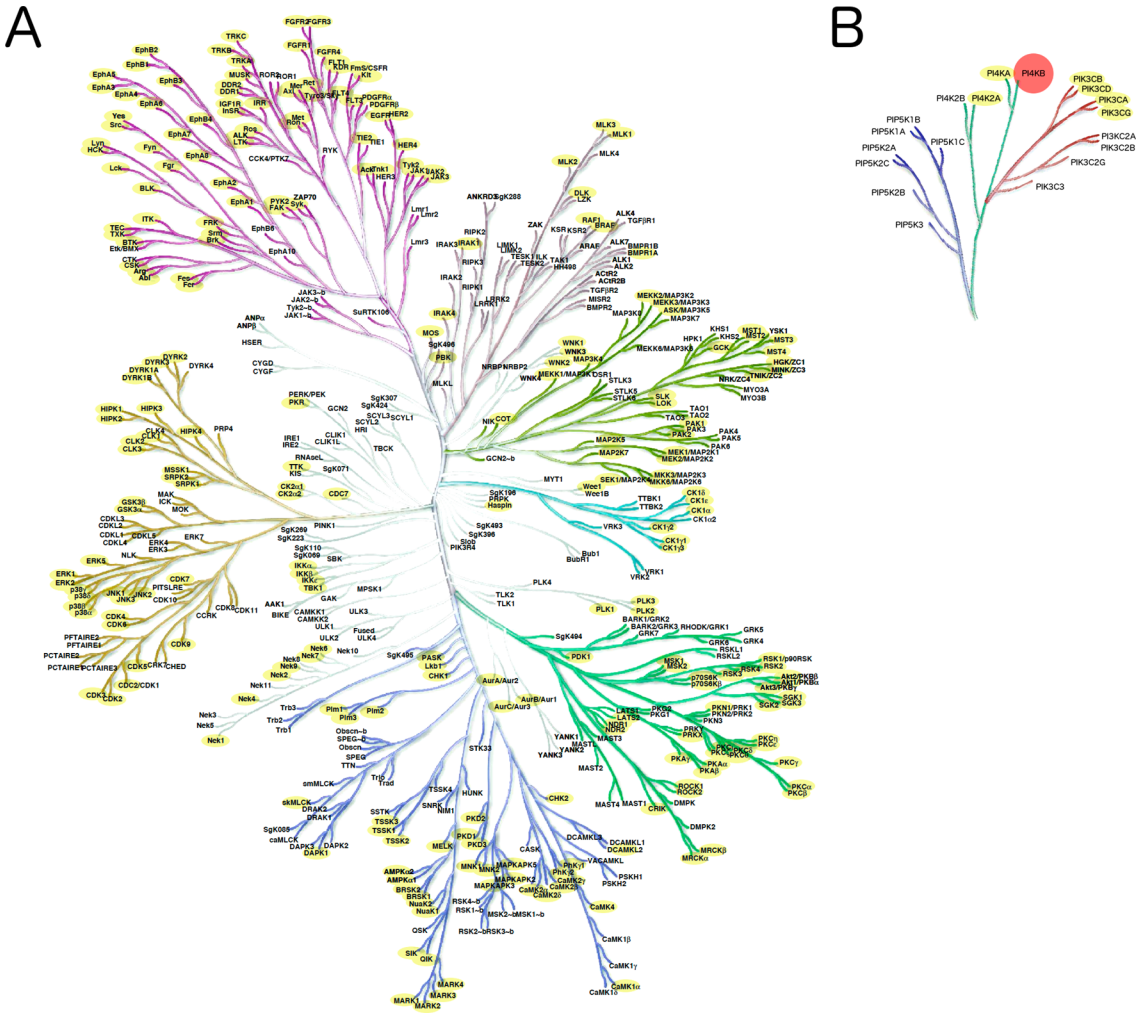

Figure 3. Graphical representation of the kinome-wide profiling of compound **10**. The tested kinases are depicted in yellow. Only PI4KB was significantly inhibited at 1 μM concentration.

DOI: 10.1021/acs.jmedchem.6b01465
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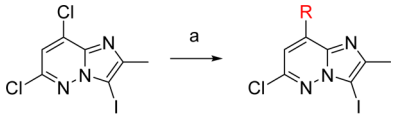
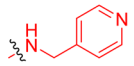
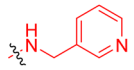
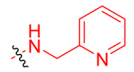
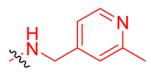
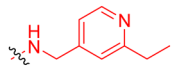
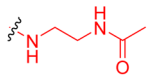
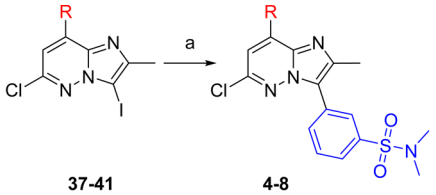
Scheme 1. Preparation of Iodinated Precursors 37−42 by Selective Nucleophilic Displacement of One Chlorine atom[a]



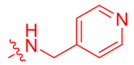
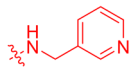
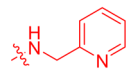
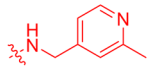
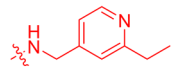
Compd	R	Yield
37		86 %
38		95 %
39		97 %
40		65 %
41		85 %
42		90%

[a]Reagents and conditions: (a) R−H, DIPEA, EtOH, heating.

Scheme 2. Preparation of Derivatives 4−8 Utilizing Cross-Coupling[a]



37-41 **4-8**

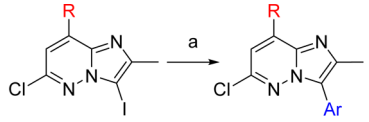
Compnd	Starting compnd	R	Yield
4	**37**		86%
5	**38**		80%
6	**39**		80%
7	**40**		80%
8	**41**		83%

[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.

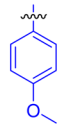
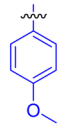
part of the study, we also prepared derivatives with various other aromatic substituents identified as potentially interesting in our previous work. The majority of these derivatives were prepared by simple Suzuki cross-coupling of six iododerivatives with the appropriate boronic acid or its pinacol ester (Scheme 2); the results are summarized in the Supporting Information.

Derivatives **9** and **43** (Scheme 3) prepared by the Suzuki coupling procedure were chlorosulfonylated (0 °C, CH_2Cl_2) to provide derivatives **44** and **45** in quantitative yield. Crude derivatives **44** and **45** were treated with various primary and secondary, cyclic, or acyclic amines (DIPEA, CH_2Cl_2, rt) providing *p*-methoxysulfoamides in moderate to good yields. Results are summarized in Scheme 4.

Scheme 3. Synthesis of Derivatives 9 and 43 by Cross-Coupling Reactions with Corresponding Boronic Acids[a]



41 or 42 **9 and 43**

Compnd	Starting compnd	Ar	Yield
9	**41**		90%
43	**42**		99%

[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.

Binding Mode of Novel Inhibitors−Structural Analysis. We used protein crystallography to gain insight into the binding mode of our new inhibitors. PI4KB contains unstructured regions that impede crystallization.[41] Thus, we adopted the strategy introduced by Burke et al.:[16] we removed the disordered loops and crystallized PI4KB together with Rab11 (wt or Q70L mutant) (Figure 4, Supporting Information, Figure S1). Using this strategy, we were able to obtain five crystal structures with members of series B (compounds **23**, **24**, **25**, **33**, and **35**) (Table 4). Generally, our crystallographic analysis was in very good agreement with our docking studies. It revealed that the binding mode of the ligand can be severely influenced by the sulfonamide substituent. Although water molecules probably play an important role in the interaction of both "amino side-chain" and "aromatic side-chain", we did not model the water molecules due to limited resolution of our crystal structures. In particular, the position of the "amino side-chain", although quite remote from the "aromatic side chain", differs significantly depending on the sulfonamide moiety attached. Thus, the (2-acetamidoethyl)amino side-chain can directly form hydrogen bonds with Asn615 (ligand **25**, Figure 4D), Tyr385 (ligand **33**, Figure 4E), or even Ala616 (ligand **35**, Figure 4F). However, it seems to be clear that the interaction with these residues can be mediated also through a water bridge, which seems to be the case for compounds **23** and **24** (Figure 4B,C, respectively). In addition, the sulfonamide moiety can form hydrogen bonds with Gly675 or Asn676 as is obvious from the crystal structures containing

DOI: 10.1021/acs.jmedchem.6b01465
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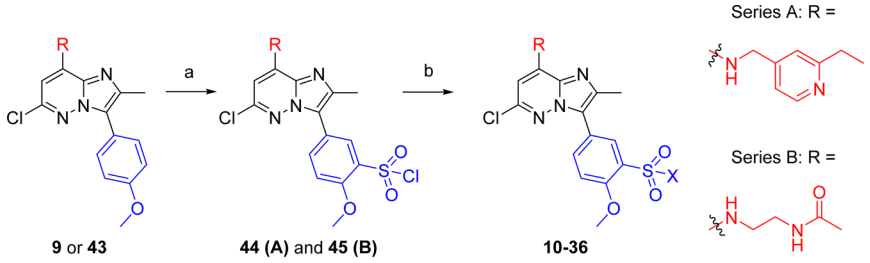
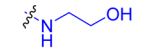
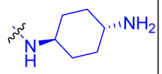
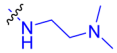
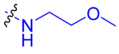
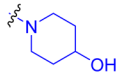
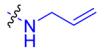
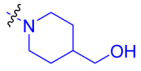
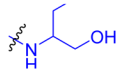
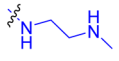
Scheme 4. Preparation of Sulfonamide Derivatives 10−36[a]



X	Compd	Series	Yield	Compd	Series	Yield
	**10**	A	66%	**23**	B	75%
	**11**	A	50%	**24**	B	64%
	**12**	A	87%	**25**	B	77%
	**13**	A	94%	**26**	B	60%
	**14**	A	90%	**27**	B	75%
	**15**	A	93%	**28**	B	78%
	**16**	A	75%	**29**	B	73%
	**17**	A	85%	**30**	B	68%
	**18**	A	92%	**31**	B	77%
	**19**	A	84%	**32**	B	70%
	**20**	A	71%	**33**	B	62%
	**21**	A	76%	**34**	B	79%
	**22**	A	63%	**35**	B	66%
				**36**	B	61%

[a]Reagents and conditions: (a) chlorosulfonic acid, rt, 16 h, quant.; (b) amine (X−H), DIPEA, CH_2CL_2, rt, 16 h.

derivatives **24** and **33** (Figure 4C,E, respectively). In contrast, the interaction with these two residues can be also provided through water molecules as might be the case for compound **25** (Figure 4D). An interesting difference was observed for structurally related compounds **23** (Figure 4B) and **35** (Figure 4F). Compound **23** forms the interaction with Lys584 through hydrogen bonding with the oxygen bonded on the sulfur atom whereas the hydroxyl group on the rear of the sulfonamide side-chain provides the interaction with the same residue in the case of compound **35**. Therefore, it seems that the exact ligand's position and the efficiency of its interaction with the kinase is highly dependent on the sulfonamide substituent and can be fine-tuned by careful selection of this moiety. We also hypothesize that this effect is responsible for the fluctuation in the results observed for these diverse sulfonamide moieties in both series we prepared.

CONCLUSION

We rationally designed and prepared a series of novel PI4KB inhibitors. These compounds were designed by merging two structural patterns of known PI4KB inhibitors, **3**, which excels in high selectivity toward this target, and **1**, which is a highly active inhibitor of PI4KB with limited selectivity. This design approach proved to be highly effective, and we have obtained numerous compounds with excellent inhibitory activity and maintained selectivity toward the title enzyme. The most active compound, derivative **25**, exerts subnanomolar activity in the enzymatic assay and excellent selectivity in comparison to other PI4Ks. To the best of our knowledge, this compound possesses the highest inhibitory activity ever reported for PI4KB with outstanding selectivity vs PI4KA (SI = 50 000), Unfortunately, this compound did not meet our expectations in cell-based antiviral assays, probably due to lower cell permeability. Therefore, we selected compound **10**, which has single digit nanomolar

DOI: 10.1021/acs.jmedchem.6b01465
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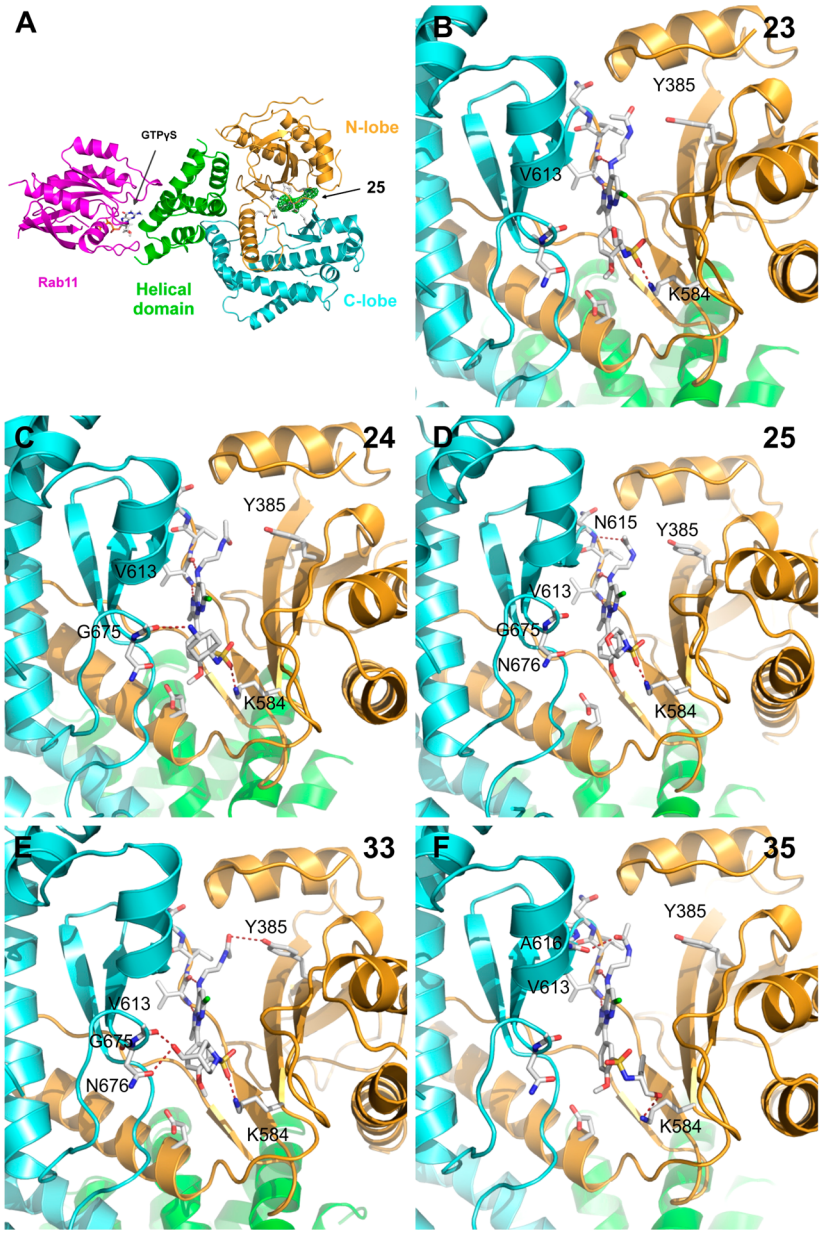

Figure 4. Structural analysis of newly the prepared inhibitors. (A) Structure of the whole crystallized complex of PI4KB with Rab11. (B–F) Binding modes of inhibitors **23**, **24**, **25**, **33**, and **35**. The figure was prepared using PyMOL.

activity in the enzymatic assay, significant selectivity in comparison to the other PI4Ks, and consistent antiviral response in cell-based assays for kinome-wide profiling. This compound did not inhibit any other protein or lipid kinases at the 1 μM level and proved to be an extremely selective inhibitor of PI4KB (Figure 3).

In addition, our crystallographic analysis showed the proper positioning of the (2-acetamidoethyl)amino side chain for the first time (Figure 4), which may explain the differences in activities of the two discussed series. This side chain interacts with residue Asn600 in the hinge region rather than with residue Tyr385 in the opposite side of the binding canyon, which is probably exploited by the different types of amino side chain, for example, pyridine based ones.

In conclusion, we have clearly demonstrated that this rational approach can be utilized to enhance activity and selectivity of novel PI4KB inhibitors, which exert antiviral activities against various +RNA viruses such as human rhinoviruses, enteroviruses, and flaviviruses (namely, HCV). Surprisingly, the compounds exert no inhibition of Middle East respiratory syndrome coronavirus (MERS-CoV), despite the possible implication of PI4KB in coronavirus replication reported previously.[6] However, the reported compounds can serve as potential broad spectrum antiviral agents and also as highly specific tools for chemical biology.

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 Dicover instrument with a single-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 or Bruker Avance II-500 instruments (600.1 or 500.0 MHz for ^{1}H and 150.9 or 125.7 MHz for ^{13}C) in

DOI: 10.1021/acs.jmedchem.6b01465
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Table 4. Statistics of Crystallographic Data Collection and Refinement

crystal	PI4KIIIβ/wtRab11 + **25**	PI4KIIIβ/wtRab11 + **33**	PI4KIIIβ/Q70L-Rab11 + **24**	PI4KIIIβ/Q70L-Rab11 + **23**	PI4KIIIβ/Q70L-Rab11 + **35**
PDB code	5FBL	5FBQ	5FBR	5FBV	5FBW
space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
cell dimensions	*a* = 49.5 Å, *b* = 104.9 Å, *c* = 188.0 Å	*a* = 48.8 Å, *b* = 101.2 Å, *c* = 188.5 Å	*a* = 49.3 Å, *b* = 103.9 Å, *c* = 187.6 Å	*a* = 48.5 Å, *b* = 102.8 Å, *c* = 186.1 Å	*a* = 49.1 Å, *b* = 104.1 Å, *c* = 187.6 Å
X-ray source	BESSY ID 14-1	BESSY ID 14-1	BESSY ID 14-1	BESSY ID 14-1	BESSY ID 14-1
wavelength, Å	0.9796	0.9796	0.9796	0.9796	0.9796
resolution, Å	47.01−3.37 (3.49−3.37)	48.85−3.79 (3.92−3.78)	47.65−3.28 (3.40−3.28)	46.97−3.28 (3.40−3.28)	47.47−3.48 (3.61−3.48)
no. of unique reflns	14419 (1382)	9508 (865)	15037 (1135)	14745 (1381)	12696 (1166)
$I/\sigma(I)$	14.11 (1.71)	8.33 (1.45)	12.75 (1.35)	7.63 (0.95)	6.77 (0.87)
$I/\sigma(I)$ = 2 at resolution, Å	3.43	3.85	3.32	3.45	3.67
R_{means}, %	11.3	21.1	12.8	23.0	19.1
data completeness, %	99.54 (97.19)	96.34 (91.53)	97.42 (76.48)	99.01 (95.11)	98.32 (93.65)
multiplicity	3.85	5.40	6.33	3.44	4.27
R_{work}, %	24.28 (35.68)	25.18 (35.94)	22.61 (35.26)	23.71 (39.66)	24.62 (40.30)
R_{free}, %	26.88 (34.96)	30.44 (38.12)	26.38 (38.44)	28.14 (43.98)	28.31 (42.35)
rms bond angle deviation, deg	0.004	0.003	0.004	0.012	0.005
rms bond angle deviation, Å	1.01	0.89	0.91	1.54	1.15
Ramachandran (outliers/favored)	0%/96%	0%/97%	0%/95%	0%/96%	0%/96%

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. The elemental analyses were obtained on a PerkinElmer CHN Analyzer 2400, Series II Sys (PerkinElmer) and X-ray fluorescence spectrometer SPECTRO iQ II (SPECTRO Analytical Instruments, Germany). 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. All prepared compounds had ≥95% purity.

General Procedure A. Introduction of the N-Substituent to Position 7. 6,8-Dichloro-3-iodo-2-methylimidazo[1,2-*b*]pyridazine (prepared as described previously[30]) (150 mg, 0.47 mmol) was dissolved in EtOH (5 mL) and DIPEA (0.25 mL, 1.41 mmol) together with an appropriate amine (2 equiv). The reaction mixture was then stirred at 75 °C for 12 h (or until consumption of the starting material). The reaction mixture was subsequently evaporated, the residue was chromatographed (silica gel, 50 g) using an appropriate mobile phase, and the solid was recrystallized providing derivatives **37**−**42**.

*General Procedure B. Preparation of Derivatives **4**−**9** and **43** via Suzuki Coupling Reaction.* Iodo derivatives **37**−**42** were suspended in 1,4-dioxane (8 mL, ∼0.4 mmol) followed by the addition of the appropriate boronic acid/pinacol ester (1.05 equiv), and a solution of 1 M Na_2CO_3(aq) (2 mL) was added. The reaction mixture was degassed three times, Pd(dppf)Cl_2 (5 mol %) was added quickly, and the mixture was degassed once more, heated to 90 °C, and stirred until the completion of the reaction (3 h to overnight). After cooling to rt, the mixture was diluted with water/brine (1:1), and the aqueous phase was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic phases were dried over Na_2SO_4, concentrated *in vacuo*, and purified by flash column chromatography providing compounds **4**−**9** and **43**. Derivatives **4**−**8** were prepared as their HCl salts in order to enhance solubility in aqueous medium for the evaluation of biological activity.

*General Procedure C. Preparation of Sulfonamide Derivatives **10**−**36**.* Sulfonyl chloride derivative **44** or **45** was dissolved in anhydrous CH_2Cl_2, and DIPEA (1.5 equiv) was added followed by the addition of the appropriate amine derivative (1.5 equiv) (except for compounds when the sulfonyl chloride was added to the solution of amine (excess) and DIPEA in CH_2Cl_2). The reaction mixture was stirred at rt until completion of the reaction (monitored by TLC). The reaction mixture was purified by flash column chromatography, and analytically pure samples were obtained after recrystallization.

*4-(((6-Chloro-3-(3-(N,N-dimethylsulfamoyl)phenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)methyl)pyridin-1-ium Chloride Hydrochloride (**4**).* Prepared according to general procedure B. Mobile phase EtOAc/EtOH (10−20%). Recrystallized from EtOH/$CHCl_3$. Yield: 154 mg (86%) as an off-white solid; mp 211.6−212.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.66 (t, J_{NH-CH2} = 6.4 Hz, 1 H, NH), 8.52 (m, 2 H, H-2″), 8.07 (td, J_{2-4} = J_{2-6} = 1.6 Hz, J_{2-5} = 0.5 Hz, 1 H, H-2), 7.99 (dt, J_{4-5} = 7.6 Hz, J_{4-2} = J_{4-6} = 1.6 Hz, 1 H, H-4), 7.81 (td, J_{5-4} = J_{5-6} = 7.6 Hz, J_{5-2} = 0.5 Hz, 1 H, H-5), 7.76 (dt, J_{6-5} = 7.6 Hz, J_{6-4} = J_{6-2} = 1.6 Hz, 1 H, H-6), 7.36 (m, 2 H, H-3″), 6.20 (s, 1 H, H-7′), 4.64 (bs, 2 H, C**H**$_2$-NH), 2.69 (s, 6 H, N(CH_3)$_2$), 2.51 (s, 3 H, 2′-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 149.89 (C-2″), 147.33 (C-4″), 147.13 (C-6′), 143.15 (C-8′), 141.32 (C-3′), 138.92 (C-2′), 134.93 (C-1), 133.22 (C-4), 131.57 (C-9′), 129.83 (C-5), 129.63 (C-3), 127.74 (C-2), 126.62 (C-6), 123.41 (C-3′), 122.25 (C-3″), 92.29 (C-7′), 44.17 (NH-**C**H$_2$), 37.87 (N(CH_3)$_2$), 14.66 (2′-CH_3). HRMS: calcd for [M + H], 457.12080; found, 457.12086. HCl salt: mp 230.2 (decomp). Anal. ($C_{21}H_{23}Cl_3N_6O_2S$·0.66H_2O) C, H, N.

*3-(((6-Chloro-3-(3-(N,N-dimethylsulfamoyl)phenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)methyl)pyridin-1-ium Chloride Hydrochloride (**5**).* Prepared according to general procedure B. Mobile phase: EtOAc/EtOH (10−20%). Recrystallized from EtOH. Yield 148 mg (80%) as an off-white solid; mp 193.0−194.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.66 (t, J_{NH-CH2} = 6.3 Hz, 1 H, NH), 8.65 (d, $J_{2''-4''}$ = 2.2 Hz, 1 H, H-2″), 8.47 (dd, $J_{6''-5''}$ = 4.8 Hz, $J_{6''-4''}$ = 1.7 Hz, 1 H, H-6″), 8.06 (t, J_{2-4} = J_{2-6} = 1.8 Hz, 1 H, H-2), 7.98 (dt, J_{4-5} = 7.5 Hz, J_{4-2} = J_{4-6} = 1.6 Hz, 1 H, H-4), 7.74−7.82 (m, 3 H, H-4″, H-5, H-6), 7.37 (dd, $J_{5''-6'}$ = 4.8 Hz, $J_{5''-4''}$ = 7.8 Hz, 1 H, H-5″), 6.32 (s, 1 H, H-7′), 4.63 (bs, 2 H, C**H**$_2$-NH), 2.68 (s, 6 H, N(CH_3)$_2$), 2.50 (s, 3 H, 2′-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 149.00 (C-2″), 148.60 (C-6″), 147.15 (C-6′), 142.97 (C-8′), 138.84 (C-2′), 135.15 (C-4″), 134.92 (C-1), 133.21 (C-4), 131.60 (C-9′), 129.81 (C-5), 129.64 (C-3), 127.73 (C-2), 126.60 (C-6), 123.78 (C-5″), 123.78 (C-5″), 123.37 (C-3′), 92.15 (C-7′), 42.79 (NH-**C**H$_2$), 37.87 (N(CH_3)$_2$), 14.65 (2′-CH_3). HRMS: calcd for

DOI: 10.1021/acs.jmedchem.6b01465
J. Med. Chem. 2017, 60, 100−118

[M + H], 457.12080; found, 457.12084. HCl salt: mp 170 °C (decomp). Anal. ($C_{21}H_{23}Cl_3N_6O_2S·0.5H_2O$) C, H, N.

*2-(((6-Chloro-3-(3-(N,N-dimethylsulfamoyl)phenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)methyl)pyridin-1-ium Chloride Hydrochloride (**6**).* Prepared according to general procedure B. Mobile phase: EtOAc/EtOH (10−15%). Recrystallized from MeOH/$CHCl_3$. Yield: 142 mg (80%) as an off-white solid; mp 203.6−204.6 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.56 (ddd, $J_{6''\text{-}5''}$ = 4.9 Hz, $J_{6''\text{-}4''}$ = 1.8 Hz, $J_{6''\text{-}3''}$ = 0.9 Hz, 1 H, H-6″), 8.48 (t, J_{NH-CH2} = 6.2 Hz, 1 H, NH), 8.07 (td, J_{2-4} = J_{2-6} = 1.6 Hz, J_{2-5} = 0.5 Hz, 1 H, H-2), 7.99 (dt, J_{4-5} = 7.6 Hz, J_{4-2} = J_{4-6} = 1.6 Hz, 1 H, H-4), 7.75−7.83 (m, 3 H, H-4″, H-5, H-6), 7.38 (dm, $J_{3''\text{-}4''}$ = 7.8 Hz, 1 H, H-3″), 7.31 (ddd, $J_{5''\text{-}6''}$ = 4.9 Hz, $J_{5''\text{-}4''}$ = 7.5 Hz, $J_{5''\text{-}3''}$ = 1.1 Hz, 1 H, H-5″), 6.21 (s, 1 H, H-7′), 4.68 (bs, 2 H, C**H**$_2$-NH), 2.69 (s, 6 H, N(CH_3)$_2$), 2.50 (s, 3 H, 2′-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 157.23 (C-2″), 149.23 (C-6″), 147.11 (C-6′), 143.21 (C-8′), 138.90 (C-2′), 137.23 (C-4″), 134.93 (C-1), 133.20 (C-4), 131.62 (C-9′), 129.83 (C-5), 129.65 (C-3), 127.71 (C-2), 126.60 (C-6), 123.36 (C-3′), 122.71 (C-5″), 121.50 (C-3″), 92.35 (C-7′), 47.23 (NH-**C**H$_2$), 37.88 (N(CH_3)$_2$), 14.69 (2′-CH_3). HRMS: calcd for [M + H], 457.12080; found, 457.12092. HCl salt: foam. Anal. ($C_{21}H_{23}Cl_3N_6O_2S·0.5H_2O$) C, H, N.

*4-(((6-Chloro-3-(3-(N,N-dimethylsulfamoyl)phenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)methyl)-2-methylpyridin-1-ium Chloride Hydrochloride (**7**).* Prepared according to general procedure B. Mobile phase: EtOAc/EtOH (10−20%). Yield: 143 mg (80%) as an off-white foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.62 (t, J_{NH-CH2} = 6.0 Hz, 1 H, NH), 8.37 (d, $J_{6''\text{-}5''}$ = 5.0 Hz, 1 H, H-6″), 8.07 (m, 1 H, H-2), 7.98 (dm, J_{4-5} = 7.5 Hz, 1 H, H-4), 7.81 (m, 1 H, H-5), 7.76 (dm, J_{6-5} = 7.9 Hz, 1 H, H-6), 7.22 (bs, 1 H, H-3″), 7.15 (dd, $J_{5''\text{-}6''}$ = 5.0 Hz, $J_{5''\text{-}3''}$ = 1.5 Hz, 1 H, H-5″), 6.17 (s, 1 H, H-7′), 4.59 (bs, 2 H, C**H**$_2$-NH), 2.69 (s, 6 H, N(CH_3)$_2$), 2.51 (s, 3 H, 2′-CH_3), 2.43 (s, 3 H, 2″-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 158.13 (C-2″), 149.24 (C-6″), 147.55 (C-4″), 147.13 (C-6′), 143.17 (C-8′), 138.90 (C-2′), 134.93 (C-1), 133.21 (C-4), 131.58 (C-9′), 129.84 (C-5), 129.64 (C-3), 127.74 (C-2), 126.62 (C-6), 123.40 (C-3′), 121.30 (C-3″), 92.19 (C-7′), 44.23 (NH-**C**H$_2$), 37.88 (N(CH_3)$_2$), 24.25 (2″-CH_3), 14.68 (2′-CH_3). HRMS: calcd for [M + H], 471.13645; found, 471.13651. HCl salt: mp 216.6 °C (decomp). Anal. ($C_{22}H_{25}Cl_3N_6O_2S·0.5H_2O$). C, H, N.

*3-(6-Chloro-8-(((2-ethylpyridin-4-yl)methyl)amino)-2-methylimidazo[1,2-b]pyridazin-3-yl)-N,N-dimethylbenzenesulfonamide Hydrochloride (**8**).* Prepared according to general procedure B. Mobile phase: EtOAc/EtOH (10−20%). Yield: 143 mg (83%) as an off-white foam (CH_2Cl_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.62 (t, J_{NH-CH2} = 6.5 Hz, 1 H, NH), 8.41 (dd, $J_{6''\text{-}5''}$ = 5.1 Hz, $J_{6''\text{-}3''}$ = 0.6 Hz, 1 H, H-6″), 8.07 (td, J_{2-4} = J_{2-6} = 1.8 Hz, J_{2-5} = 0.4 Hz, 1 H, H-2), 7.99 (dm, J_{4-5} = 7.6 Hz, 1 H, H-4), 7.81 (m, 1 H, H-5), 7.76 (dm, J_{6-5} = 7.8 Hz, 1 H, H-6), 7.25 (bs, 1 H, H-3″), 7.16 (dd, $J_{5''\text{-}6''}$ = 5.1 Hz, $J_{5''\text{-}3''}$ = 1.5 Hz, 1 H, H-5″), 6.19 (s, 1 H, H-7′), 4.60 (bs, 2 H, C**H**$_2$-NH), 2.72 (q, $J_{CH2-CH3}$ = 7.6 Hz, 2 H, **CH**$_2$CH$_3$), 2.69 (s, 6 H, N(CH_3)$_2$), 2.51 (s, 3 H, 2′-CH_3), 1.20 (t, $J_{CH2-CH3}$ = 7.6 Hz, 3 H, CH_2**CH**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 163.01 (C-2″), 149.25 (C-6″), 147.61 (C-4″), 147.12 (C-6′), 143.18 (C-8′), 138.99 (C-2′), 134.94 (C-1), 133.21 (C-4), 131.57 (C-9′), 129.83 (C-5), 129.64 (C-3), 127.73 (C-2), 126.61 (C-6), 123.39 (C-3′), 120.40 (C-3″), 119.55 (C-5″), 92.20 (C-7′), 44.31 (NH-**C**H$_2$), 37.87 (N(CH_3)$_2$), 30.73 (**C**H$_2$CH$_3$), 14.67 (2′-CH_3), 13.91(CH_2**C**H$_3$). HRMS: calcd for [M + H], 485.15210; found, 485.15208. HCl salt: mp 193.4−196.1 °C, Anal. ($C_{23}H_{26}Cl_2N_6O_2S·0.33H_2O$) C, H, N.

*6-Chloro-N-((2-ethylpyridin-4-yl)methyl)-3-(4-methoxyphenyl)-2-methylimidazo[1,2-b]pyridazin-8-amine (**9**).* Prepared according to general procedure B. Mobile phase (EtOAc/EtOH 0−10%). Yield: 0.987 g (90%) as an off-white solid; mp 168.8−169.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.51 (t, J_{NH-CH2} = 6.2 Hz, 1 H, NH), 8.40 (d, $J_{6''\text{-}5''}$ = 5.1 Hz, 1 H, H-6″), 7.55 (m, 2 H, H-2′), 7.24 (bs, 1 H, H-3″), 7.16 (dd, $J_{5''\text{-}6''}$ = 5.1 Hz, $J_{5''\text{-}3''}$ = 1.4 Hz,1H, H-5″), 7.08 (m, 2 H, H-3′), 6.10 (s, 1H, H-7), 4.58 (bs, 2 H, C**H**$_2$-NH), 3.82 (s, 3 H, 4′-OCH_3); 2.71 (q, $J_{CH2-CH3}$ = 7.7 Hz, 2 H, C**H**$_2$CH$_3$), 2.42 (s, 3 H, 2-CH_3), 1.20 (t, 3 H, $J_{CH3-CH2}$ = 7.7 Hz, CH_2C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 162.99 (C-2″), 159.04 (C-4′), 149.24 (C-6″), 147.84 (C-4″), 146.84 (C-6), 143.03 (C-8), 1387.48 (C-2), 130.75 (C-9), 130.71 (C-2′), 125.10 (C-3), 120.74 (C-1′), 120.41 (C-3″), 119.56 (C-5″), 114.14 (C-3′), 91.48 (C-7), 55.38 (4′-O−CH_3), 44.29 (NH-**C**H$_2$), 30.73 (**C**H$_2$−CH_3), 14.53 (2-CH_3), 13.93 (CH_2−**C**H$_3$). Anal. ($C_{22}H_{22}ClN_5O·0.5EtOH$) C, H, N. HRMS: calcd for [M + H], 408.15856; found, 408.15868.

*5-(6-Chloro-8-(((2-ethylpyridin-4-yl)methyl)amino)-2-methylimidazo[1,2-b]pyridazin-3-yl)-N-(2-hydroxyethyl)-2-methoxybenzenesulfonamide (**10**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH 0−20%. Yield 264 mg (66%); yellowish foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.54 (t, J_{NH-CH2} = 6.4 Hz, 1 H, NH−CH2), 8.40 (d, $J_{6''\text{-}5''}$ = 5.1 Hz, 1 H, H-6″), 7.97 (d, J_{6-4} = 2.3 Hz, 1 H H-6), 7.88 (dd, J_{4-3} = 8.6 Hz,J_{4-6} = 2.3 Hz, 1 H, H-4), 7.40 (d, J_{3-4} = 8.6 Hz, 1 H, H-3), 7.24 (bs, 1 H, H-3″), 7.18 (t, $J_{NH-CH2CH3}$ = 5.9 Hz, 1 H, NH−CH_2CH_2), 7.16 (dm, $J_{5''\text{-}6''}$ = 5.1 Hz, 1 H, H-5″), 6.15 (s, 1 H, H-7′), 4.67 (t, J_{OH-CH2} = 5.7 Hz, 1 H, CH_2OH), 4.59 (bs, 2 H, C**H**$_2$-NH), 3.97 (s, 3 H, OCH_3), 2.88 (m, 2 H, CH_2CH_2NH), 2.72 (q, $J_{CH2-CH3}$ = 7.4 Hz, 2 H, CH_2CH_3), 2.44 (s, 3 H, 2′-CH_3), 1.20 (t, 3 H, $J_{CH3-CH2}$ = 7.4 Hz, CH_2CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 163.02 (C-2″), 155.90 (C-2), 149.26 (C-6″), 147.71 (C-4″), 147.04 (C-6′), 143.10 (C-8′), 137.97 (C-2′), 135.02 (C-4), 131.10 (C-9′), 129.84 (C-6), 128.21 (C-5), 123.72 (C-3′), 120.43 (C-1), (C-6), 120.40 (C-3″), 119.56 (C-5″), 113.56 (C-3), 91.87 (C-7′), 60.05 (CH_2−OH), 56.58 (O−CH_3), 45.40 (CH_2−CH_2−NH), 44.30 (CH_2−NH-8′), 30.74 (CH_3−CH_2), 14.51 (2′-CH_3), 13.93 (CH_2CH_2). Anal. ($C_{24}H_{27}ClN_6O_4S.H_2O$) C, H, N. HRMS: calcd for [M + H], 531.15758; found, 531.15762.

*N-(4-Aminocyclohexyl)-5-(6-chloro-8-(((2-ethylpyridin-4-yl)methyl)amino)-2-methylimidazo[1,2-b]pyridazin-3-yl)-2-methoxybenzenesulfonamide (**11**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH 10−30%. Yield: 87 mg (50%); mp 136.6−138.8 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.57 (bs, 1 H, NH-8′), 8.40 (dd, $J_{6''\text{-}5''}$ = 5.1 Hz, $J_{6''\text{-}3''}$ = 0.6 Hz, 1 H, H-6″), 7.97 (d, J_{6-4} = 2.3 Hz, 1 H, H-6), 7.85 (dd, J_{4-6} = 2.3 Hz, J_{4-3} = 8.5 Hz, 1 H, H-4), 7.24 (bs,1 H, H-3″), 7.15 (dd, $J_{5''\text{-}6''}$ = 5.1 Hz, $J_{5''\text{-}3''}$ = 1.6 Hz, 1 H, H-5″), 6.15 (s, 1 H, H-7′), 4.59 (s, 2 H, NH−CH_2), 4.59 (s, 2 H, C**H**$_2$-NH), 3.96 (s, 3 H, OCH_3), 2.95 (m, 2 H, CH-NH), 2.71 (q, $J_{CH2-CH3}$ = 7.6 Hz, 2 H, CH_2CH_3), 2.43 (s, 3 H, 2′-CH_3), 2.38 (m, 1 H, CH-NH_2), 1.56−1.67 (m, 4 H, CH_2^a-CH-NH, CH_2^a-CH-NH_2), 1.18−1.27 (m, 5 H, CH_2^b-CH-NH, CH^b-CH_2), 0.91 (m, 2 H, CH_2^b-CH-NH_2). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 163.02 (C-2″), 155.92 (C-2), 149.28 (C-6″), 147.72 (C-4″), 147.05 (C-6′), 143.11 (C-8′), 137.92 (C-2′), 134.88 (C-4), 131.10 (C-9′), 129.76 (C-6), 129.67 (C-5), 123.82 (C-3′), 120.40 (C-1), 119.57 (C-5″), 119.57 (C-5″), 113.27 (C-3), 91.84 (C-7′), 56.39 (O−CH_3), 52.61 (CH-NH), 49.53 (**C**H-NH_2), 44.30 (NH-**C**H$_2$), 35.24 (CH_2−CH-NH_2), 32.14 (CH−CH-NH), 30.76 (CH_2−CH_3), 14.48 (2′-CH_3), 13.96 (CH_2−CH_3). Anal. ($C_{28}H_{34}ClN_7O_3S·0.5H_2O$) C, H, N. HRMS: calcd for [M + H], 584.22051; found 584.22052.

*6-Chloro-N-((2-ethylpyridin-4-yl)methyl)-3-(4-methoxy-3-(morpholinosulfonyl)phenyl)-2-methylimidazo[1,2-b]pyridazin-8-amine (**12**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (10−20%). Yield: 103 mg (87%); mp 173.9−175.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.59 (t, J_{NH-CH2} = 6.2 Hz, 1 H, NH-8′), 8.40 (dd, $J_{6''\text{-}5''}$ = 5.1 Hz, $J_{6''\text{-}3''}$ = 0.6 Hz, 1 H, H-6″), 8.00 (d, $J_{2'\text{-}6'}$ = 2.3 Hz, 1 H, H-2′), 7.91 (dd, $J_{6'\text{-}5'}$ = 8.7 Hz, $J_{6'\text{-}2'}$ = 2.3 Hz, 1 H, H-6′), 7.43 (d, $J_{5'\text{-}6'}$ = 8.7 Hz, 1 H, H-5′), 7.24 (bs, 1 H, H-3″), 7.16 (dd, $J_{5''\text{-}6''}$ = 5.1 Hz, $J_{5''\text{-}3''}$ = 1.4 Hz, 1 H, H-5″), 6.15 (s, 1 H, H-7), 4.59 (bs, 2 H, 8-NH-C**H**$_2$), 3.96 (s, 3 H, 4′-OCH_3), 3.61 (m, 4 H, CH_2−O), 3.11 (m, 4 H, CH_2−N), 2.71 (q, $J_{CH2-CH3}$ = 8.1 Hz, 2 H, CH_2−CH_3), 2.45 (s, 3 H, 2-CH_3), 1.19 (t, $J_{CH2-CH3}$ = 8.1 Hz, 3 H, CH_2C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 162.99 (C-2″), 156.40 (C-4′), 149.21 (C-6″), 147.87 (C-4″), 147.09 (C-6), 143.15 (C-8), 138.02 (C-2), 135.67 (C-6′), 131.55 (C-2′), 131.18 (C-9), 124.86 (C-1), 123.52 (C-3), 120.65 (C-5), 119.62 (C-5″), 113.82 (C-5′), 91.90 (C-7), 66.08 (CH_2−O), 56.55 (4′-O−CH_3), 46.10 (CH_2−N), 44.33 (8-NH−CH_2), 30.74 (CH_2−CH_3), 14.48 (2-CH_3), 13.97 (CH_2−**C**H$_3$). Anal. ($C_{26}H_{29}ClN_6O_4S·0.5EtOH$) C, H, N. HRMS: calcd for [M + H], 557.17327; found 557.17323.

DOI: 10.1021/acs.jmedchem.6b01465
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6-Chloro-N-((2-ethylpyridin-4-yl)methyl)-3-(4-methoxy-3-(piperidin-1-ylsulfonyl)phenyl)-2-methylimidazo[1,2-b]pyridazin-8-amine (**13**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (5−10%). Yield: 108 mg (94%) as an off-white foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.59 (t, J_{NH-CH2} = 6.3 Hz, 1 H, NH-8), 8.40 (dd, $J_{6‴-5‴}$ = 5.1 Hz, $J_{6‴-3‴}$ = 0.6 Hz, 1 H, H-6‴), 7.98 (d, $J_{2′-6′}$ = 2.3 Hz, 1 H, H-2′), 7.88 (dd, $J_{6′-5′}$ = 8.6 Hz, $J_{6′-2′}$ = 2.3 Hz, 1 H, H-6′), 7.40 (d, $J_{5′-6′}$ = 8.6 Hz, 1 H, H-5′), 7.25 (bs, 1 H, H-3‴), 7.16 (dd, $J_{5‴-6‴}$ = 5.1 Hz, $J_{5‴-3‴}$ = 1.5 Hz, 1 H, H-5‴), 6.15 (bs, 2 H, H-7), 3.94 (s, 3 H, 4′-OCH$_3$), 3.12 (m, 4 H, NH-1″), 2.71 (q, $J_{CH2-CH3}$ = 7.6 Hz, 2 H, CH$_2$−CH$_3$), 2.45 (s, 3 H, 2-CH$_3$), 1.51 (m, 4 H, H-2″), 1.44 (m, 2 H, H-3″), 1.19 (t, $J_{CH2-CH3}$ = 7.6 Hz, 3 H, CH$_2$C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 162.94 (C-2‴), 156.30 (C-4′), 149.14 (C-6‴), 147.99 (C-4‴), 143.15 (C-8), 137.98 (C-2), 135.27 (C-6′), 131.33 (C-2′), 131.15 (C-9), 125.87 (C-1), 123.61 (C-3), 120.53 (C-5), 120.51 (C-3‴), 119.65 (C-5‴), 113.69 (C-5′), 91.88 (C-7), 56.49 (4′-O−CH$_3$), 46.68 (C-1″), 44.32 (8-NH−CH$_2$), 30.70 (CH$_2$−CH$_3$), 25.48 (C-2″), 23.39 (C-3″), 14.55 (2-CH$_3$), 13.97 (CH$_2$−**C**H$_3$). Anal. ($C_{27}H_{31}ClN_6O_3S$) C, H, N. HRMS: calcd for [M + H], 555.19396, found 555.19399.

5-(6-Chloro-8-(((2-ethylpyridin-4-yl)methyl)amino)-2-methylimidazo[1,2-b]pyridazin-3-yl)-N-(2-(dimethylamino)ethyl)-2-methoxybenzenesulfonamide (**14**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (20−50%). Yield: 109 mg (90%); mp 193.6−195.7 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.59 (t, J_{NH-CH2} = 6.5 Hz, 1 H, NH-8″), 8.40 (dd, $J_{6‴-5‴}$ = 5.0 Hz, 1 H, H-6‴), 7.98 (d, J_{6-4} = 2.3 Hz, 1 H, H-6), 7.88 (dd, J_{4-3} = 8.7 Hz, J_{4-6} = 2.3 Hz, 1 H, H-4), 7.41 (d, J_{3-4}= 8.7 Hz, 1 H, H-3), 7.35 (bs, 1 H, SO$_2$−NH), 7.24 (bs, 1 H, H-3‴), 7.15 (dd, $J_{5‴-6‴}$ = 5.0 Hz, $J_{5‴-3‴}$ = 1.2 Hz, 1 H, H-5‴), 6.15 (s, 1 H, H-7″), 4.59 (bs, 2 H, 8″-NH-C**H**$_2$) 3.98 (s, 3 H, 2-OCH$_3$), 3.02 (m, 2 H, H-1′), 2.71 (q, $J_{CH2-CH3}$ = 7.6 Hz, 2 H, C**H**$_2$-CH$_3$), 2.58 (bs, 2 H, H-2′), 2.44 (s, 3 H, 2″-CH$_3$), 2.29 (s, 6 H, N-(CH$_3$)$_2$), 1.19 (t, $J_{CH2-CH3}$ = 7.6 Hz, 3 H, CH$_2$C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 163.03 (C-2‴), 155.95 (C-2), 149.30 (C-6‴), 147.76 (C-4‴), 147.07 (C-6″), 143.13 (C-8″), 138.01 (C-2″), 135.24 (C-4), 131.13 (C-9″), 129.95 (C-6), 127.86 (C-1), 123.73 (C-3″), 120.48 (C-5), 120.43 (C-3‴), 119.59 (C-5‴), 113.38 (C-3), 91.86 (C-7″), 57.33 (C-2′), 56.65 (2-O−CH$_3$), 44.30 (8″-NH−CH$_2$), 44.13 (N-(CH$_3$)$_2$), 30.79 (CH$_2$−CH$_3$), 14.54 (2″-CH$_3$), 13.99 (CH$_2$−**C**H$_3$). Anal. ($C_{26}H_{32}ClN_7O_3S$) C, H, N. HRMS: calcd for [M + H], 558.20486, found 558.20493.

5-(6-Chloro-8-(((2-ethylpyridin-4-yl)methyl)amino)-2-methylimidazo[1,2-b]pyridazin-3-yl)-2-methoxy-N-(2-methoxyethyl)benzenesulfonamide (**15**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (5−15%). Yield: 110 mg (93%), yellowish foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.58 (t, J_{NH-CH2} = 6.4 Hz, 1 H, NH-8″), 8.40 (dd, $J_{6‴-5‴}$ = 5.1 Hz,$J_{6‴-3‴}$ = 0.6 Hz, 1 H, H-6‴), 7.97 (d, J_{6-4} = 2.3 Hz, 1 H, H-6), 7.86 (dd, J_{4-3} = 8.7 Hz, J_{4-6} = 2.3 Hz, 1 H, H-4), 7.39 (t, $J_{NH-1′}$= 6.0 Hz, 1 H, NH-1′), 7.38 (d, J_{3-4} = 8.7 Hz, 1 H, H-3), 7.24 (bs, 1 H, H-3‴), 7.16 (dd, $J_{5‴-6‴}$ = 5.1 Hz, $J_{5‴-3‴}$ = 1.6 Hz, 1 H, H-5‴), 6.14 (s, 1 H, H-7″), 4.59 (bs, 2 H, 8″-NH-C**H**$_2$) 3.97 (s, 3 H, 2-OCH$_3$), 3.29 (t, $J_{2′-1′}$ = 5.9 Hz, 2 H, H-2′), 3.12 (s, 3 H, 2′-OCH$_3$), 3.02 (q, $J_{1′-2′}$ = $J_{1′-NH}$ = 6.0 Hz, 2 H, H-1′), 2.71 (q, $J_{CH2-CH3}$ = 7.7 Hz, 2 H, C**H**$_2$-CH$_3$), 2.44 (s, 3 H, 2″-CH$_3$), 1.19 (t, $J_{CH2-CH3}$ = 7.7 Hz, 3 H, CH$_2$C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 163.00 (C-2‴), 155.99 (C-2), 149.23 (C-6‴), 147.86 (C-4‴), 147.07 (C-6″), 143.12 (C-8″), 137.98 (C-2″), 135.00 (C-4), 131.12 (C-9″), 129.78 (C-6), 128.59 (C-1), 123.80 (C-3″), 120.45 (C-3‴), 120.39 (C-5), 119.61 (C-5‴), 113.30 (C-3), 91.85 (C-7″), 70.75 (C-2′), 58.01 (2′-O−CH$_3$), 56.57 (2-O−CH$_3$), 44.32 (8″-NH−CH$_2$), 42.52 (C-1′), 30.75 (CH$_2$−CH$_3$), 14.52 (2″-CH$_3$), 13.97 (CH$_2$−**C**H$_3$). Anal. ($C_{25}H_{29}ClN_6O_4S$·0.33EtOAc) C, H, N. HRMS: calcd for [M + H], 545.17323, found 545.17324.

3-(3-((1H-Pyrazol-1-yl)sulfonyl)-4-methoxyphenyl)-6-chloro-N-((2-ethylpyridin-4-yl)methyl)-2-methylimidazo[1,2-b]pyridazin-8-amine (**16**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (5−15%). Yield: 86 mg (75%); mp 214.4−216.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.58 (t, J_{NH-CH2} = 6.3 Hz, 1 H, NH-8), 8.49 (dd, $J_{5‴,3‴-4‴}$ = 2.8 Hz, $J_{5‴-3‴}$ = 0.6 Hz, 1 H, H-5‴ or H-3‴), 8.42 (dd, $J_{6′-5′}$ = 5.1 Hz, $J_{6′-3′}$ = 0.5 Hz, 1 H, H-6′), 8.13 (d, $J_{2″-6″}$ = 2.3 Hz, 1 H, H-2″), 8.02 (dd, $J_{6″-5″}$ = 8.7 Hz, $J_{6″-2″}$ = 2.3 Hz, 1 H, H-6″),7.86 (dd, $J_{5‴,3‴-4‴}$ = 1.6 Hz, $J_{5‴-3‴}$ = 0.6 Hz, 1 H, H-5‴ or H-3‴), 7.41 (d, $J_{5″-6″}$= 8.7 Hz, 1 H, NH-5″),7.28 (bs, 1 H, H-3′), 7.19 (dd, $J_{5′-6′}$ = 5.1 Hz, $J_{5′-3′}$ = 1.2 Hz, 1 H, H-5′), 6.60 (dd, $J_{4‴-3‴,5‴}$ = 1.6 Hz, $J_{4‴-3‴,5‴}$ = 2.8 Hz, 1 H, H-4″),6.18 (s, 1 H, H-7), 4.61 (bs, 2 H, 8-NH-C**H**$_2$),3.83 (s, 3 H, 4″-OCH$_3$), 2.73 (q, $J_{CH2-CH3}$ = 7.6 Hz, 2H, C**H**$_2$-CH$_3$), 2.45 (s, 3 H, 2-CH$_3$), 1.21 (t, $J_{CH2-CH3}$ = 7.6 Hz, 3 H, CH$_2$C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 162.80 (C-2′), 156.57 (C-4″), 148.92 (C-6′), 147.13 (C-6), 145.47 (C-5‴ or C-3‴), 143.11 (C-8), 138.34 (C-2), 137.79 (C-6″), 133.93 (C-3‴ or C-5‴), 131.28 (C-9), 130.41 (C-2″), 124.26 (C-3″), 122.91 (C-3), 121.13 (C-1″), 120.56 (C-3′), 119.68 (C-5′), 114.28 (C-5″), 108.39 (C-4‴), 92.04 (C-7), 56.89 (4″-O−CH$_3$), 44.33 (8-NH−CH$_2$), 30.55 (CH$_2$−CH$_3$), 14.50 (2-CH$_3$), 13.87 (CH$_2$−**C**H$_3$). Anal. ($C_{25}H_{24}ClN_7O_3S$·0.33EtOAc) C, H, N. HRMS: calcd for [M + H], 538.14226, found 538.14227.

1-((5-(6-Chloro-8-(((2-ethylpyridin-4-yl)methyl)amino)-2-methylimidazo[1,2-b]pyridazin-3-yl)-2-methoxyphenyl)sulfonyl)-piperidin-4-ol (**17**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (20−50%). Yield: 103 mg (85%); mp 196.8−197.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.55 (t, J_{NH-CH2} = 6.3 Hz, 1 H, NH-8″), 8.40 (dd, $J_{6‴-5‴}$ = 5.1 Hz, $J_{6‴-3‴}$ = 0.7 Hz, 1 H, H-6‴), 7.99 (d, $J_{6′-4′}$ = 2.3 Hz, 1 H, H-6′), 7.89 (dd, $J_{4′-5′}$ = 8.7 Hz, $J_{4′-6′}$ = 2.3 Hz, 1 H, H-4′), 7.41 (d, $J_{3′-4′}$ = 8.7 Hz, 1 H, H-3′), 7.24 (bs, 1 H, H-3‴), 7.15 (dd, $J_{5‴-6‴}$ = 5.1 Hz, $J_{5‴-3‴}$ = 1.4 Hz, 1 H, H-5‴), 6.15 (s, 1 H, H-7″), 4.59 (bs, 2 H, 8-NH-C**H**$_2$), 3.94 (s, 3 H, 2′-OCH$_3$), 3.58 (m, 1 H, H-4), 3.39−3.44 (m, 2 H, H-2a), 2.91−2.97 (m, 2 H, H-2b), 2.72 (q, $J_{CH2-CH3}$ = 7.6 Hz, 2H, C**H**$_2$-CH$_3$), 2.45 (s, 3 H, 2″-CH$_3$), 1.71−1.76 (m, 2 H, H-3a), 1.35−1.42 (m, 2 H, H-3b), 1.20 (t, $J_{CH2-CH3}$ = 7.6 Hz, 3 H, CH$_2$C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 163.00 (C-2‴), 156.24 (C-2′), 149.25 (C-6‴), 147.67 (C-4‴), 147.00 (C-6″), 143.11 (C-8″), 137.93 (C-2″), 135.24 (C-4′), 131.26 (C-6′), 131.11 (C-9″), 125.99 (C-1′), 123.56 (C-3″), 120.52 (C-5′), 120.37 (C-3‴), 119.54 (C-5‴), 113.69(C-3′), 91.84 (C-7″), 64.73 (C-4), 56.47 (2′-O−CH$_3$), 44.31 (NH−CH$_2$), 43.38 (C-2), 33.84 (C-3), 30.73 (CH$_2$−CH$_3$), 14.49 (2″-CH$_3$), 13.90 (CH$_2$−**C**H$_3$). Anal. ($C_{27}H_{31}ClN_6O_4S$·0.5EtOH) C, H, N. HRMS: calcd for [M + H], 571.18888; found 571.18891.

N-Allyl-5-(6-chloro-8-(((2-ethylpyridin-4-yl)methyl)amino)-2-methylimidazo[1,2-b]pyridazin-3-yl)-2-methoxybenzenesulfonamide (**18**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (5−10%). Yield: 108 mg (92%); mp 170.0−171.0 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.54 (t, J_{NH-CH2} = 6.4 Hz, 1 H, NH-8′), 8.40 (dd, $J_{6″-5″}$ = 5.2 Hz, 1 H, H-6″), 7.97 (d, J_{6-4} = 2.1 Hz, 1 H, H-6), 7.85 (dd, J_{4-3} = 8.7 Hz, J_{4-6} = 2.1 Hz, 1 H, SO$_2$−NH), 7.37 (d, J_{3-4}= 8.7 Hz, 1 H, H-3), 7.24 (bs, 1 H, H-3″), 7.16 (dd, $J_{5″-6″}$ = 5.2 Hz, $J_{5″-3″}$ = 1.4 Hz, 1 H, H-5″), 6.14 (s, 1 H, H-7′), 5.69 (m, 1 H, CH═CH$_2$), 5.12 (dq, $J_{CH}^{T}{}_{-CH}$ = 17.1 Hz, J_G = ^4J = 1.7 Hz, 1 H, CH═CH^{trans}), 4.98 (dq, $J_{CH}^{C}{}_{-CH}$ = 10.3 Hz, J_G = ^4J = 1.7 Hz, 1 H, CH═CH^{cis}), 4.60 (bs, 2 H, 8′-NH-C**H**$_2$), 3.97 (s, 3 H, 2-OCH$_3$), 3.53 (m, 2 H, SO$_2$−NH-C**H**$_2$), 2.71 (q, $J_{CH2-CH3}$ = 7.6 Hz, 2 H, C**H**$_2$-CH$_3$), 2.44 (s, 3 H, 2′-CH$_3$), 1.19 (t, $J_{CH2-CH3}$ = 7.6 Hz, 3 H, CH$_2$C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 163.00 (C-2″), 155.97 (C-2), 149.23 (C-6″), 147.72 (C-4″), 147.03 (C-6′), 143.09 (C-8′), 137.94 (C-2′), 134.97 (C-4), 134.71 (**C**H═CH$_2$), 131.09 (C-9′), 129.90 (C-6), 128.72 (C-1), 123.77 (C-3′), 120.39 (C-3″), 120.35 (C-5), 119.55 (C-5″), 116.33 (CH═**C**H$_2$), 113.27 (C-3), 91.83 (C-7′), 56.50 (2-O−CH$_3$), 45.37 (SO$_2$NH-C**H**$_2$), 44.34 (8′-NH-C**H**$_2$), 30.72 (2″-C**H**$_2$−CH$_3$), 14.46 (2′-CH$_3$), 13.90 (CH$_2$−**C**H$_3$). Anal. ($C_{25}H_{27}ClN_6O_3S$·0.33EtOAc) C, H, N. HRMS: calcd for [M + H], 527.16266; found 527.16272.

5-(6-Chloro-8-(((2-ethylpyridin-4-yl)methyl)amino)-2-methylimidazo[1,2-b]pyridazin-3-yl)-2-methoxy-N,N-dimethylbenzenesulfonamide (**19**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (5−10%). Yield: 103 mg (84%); mp 164.3−166.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.59 (t, J_{NH-CH2} = 6.4 Hz, 1 H, NH-8′), 8.40 (dd, $J_{6″-5″}$ = 5.0 Hz, 1 H, H-6″), 7.99 (d, J_{6-4} = 2.3 Hz, 1 H, H-6), 7.90 (dd, J_{4-6} = 2.3 Hz, J_{4-3} = 8.7 Hz, 1 H, H-4), 7.43 (d, J_{3-4}= 8.7 Hz, 1 H, H-3), 7.24 (bs, 1 H,

DOI: 10.1021/acs.jmedchem.6b01465
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H-3″), 7.16 (dd, $J_{5''\text{-}6''}$ = 5.0 Hz, $J_{5''\text{-}3''}$ = 1.4 Hz, 1 H, H-5″), 6.15 (s, 1 H, H-7′), 4.59 (bs, 2 H, 8′-NH-C**H**$_2$), 3.96 (s, 3 H, 2-OC**H**$_3$), 2.77 (s, 6 H, N-(C**H**$_3$)$_2$), 2.71 (q, $J_{CH2\text{-}CH3}$ = 7.9 Hz, 2 H, C**H**$_2$-CH$_3$), 2.45 (s, 3 H, 2-CH$_3$), 1.20 (t, $J_{CH2\text{-}CH3}$ = 7.9 Hz, 3 H, CH$_2$C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 163.01 (C-2″), 156.34 (C-2), 149.27 (C-6″), 147.81 (C-4″), 147.07 (C-6′), 143.15 (C-8′), 137.99 (C-2′), 135.37 (C-4), 131.48 (C-6), 131.15 (C-9′), 125.37 (C-1), 123.60 (C-3′), 120.57 (C-5), 120.45 (C-3″), 119.61 (C-5″), 113.71(C-3), 91.90 (C-7′), 56.53 (2-O−CH$_3$), 44.29 (NH−CH$_2$), 37.57 (N-(**C**H$_3$)$_2$, 30.77 (**C**H$_2$−CH$_3$), 14.54 (2-CH$_3$), 13.99 (CH$_2$−**C**H$_3$). Anal. (C$_{24}$H$_{27}$ClN$_6$O$_3$S0.6EtOAc) C, H, N. HRMS: calcd for [M + H], 515.16266; found, 515.16271.

(1-((5-(6-Chloro-8-(((2-ethylpyridin-4-yl)methyl)amino)-2-methylimidazo[1,2-b]pyridazin-3-yl)-2-methoxyphenyl)sulfonyl)-piperidin-4-yl)methanol (**20**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (10−15%). Yield: 95 mg (71%); mp 203.3−204.5 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.57 (t, J_{NH-CH2} = 6.3 Hz, 1 H, NH), 8.41 (dd, $J_{6'''\text{-}5'''}$ = 5.0 Hz, $J_{6'''\text{-}3'''}$ = 0.6 Hz, 1 H, H-6‴), 7.99 (d, $J_{6'\text{-}4'}$ = 2.3 Hz, 1 H, H-6′), 7.88 (dd, $J_{4'\text{-}3'}$ = 8.6 Hz, $J_{4'\text{-}6'}$ = 2.3 Hz, 1 H, H-4′), 7.41 (d, $J_{3'\text{-}4'}$ = 8.6 Hz, 1 H, H-3′), 7.25 (bs, 1 H, H-3‴), 7.16 (dd, $J_{5'''\text{-}6'''}$ = 5.0 Hz, $J_{5'''\text{-}3'''}$ = 1.4 Hz, 1 H, H-5‴), 6.15 (s, 1 H, H-7″), 4.59 (bs, 2 H, NH−CH$_2$), 4.49 (t, J_{OH-CH2} = 5.1 Hz, 1 H, CH$_2$−OH), 3.94 (s, 3 H, O−CH$_3$), 3.72 (dm, J_{gem}= 12.3 Hz, 2 H, H-2a), 3.22 (m, 2 H, C**H**$_2$OH), 2.72 (q, $J_{CH2\text{-}CH3}$ = 7.5 Hz, 2 H, CH$_2$−CH$_3$), 2.59 (m, 2 H, H-2b), 2.45 (s, 3 H, 2″-CH$_3$), 1.69 (dm, J_{gem} = 13.5 Hz, 2 H, H-3a), 1.42 (m, 2 H, H-4), 1.20 (t, $J_{CH2\text{-}CH3}$ = 7.5 Hz, 3 H, CH$_2$−C**H**$_3$), 1.10 (m, 2 H, H-3b). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 162.97 (C-2‴), 156.27 (C-2′), 149.19 (C-6‴), 147.85 (C-4‴), 147.03 (C-6″), 143.13 (C-8″), 137.96 (C-2″), 135.24 (C-6′), 131.24 (C-6′), 131.112 (C-9″), 126.04 (C-1′), 123.60 (C-3″), 120.50 (C-5′), 119.60 (C-5‴), 113.69 (C-3′), 91.86 (C-7″), 65.56 (C−CH$_2$OH), 56.48 (O−CH$_3$), 45.90 (C-2), 44.31 (CH$_2$NH), 37.81 (C-4), 30.71 (**C**H$_2$−CH$_3$), 28.62 (C-3), 14.53 (2′-CH$_3$), 13.94 (CH$_2$−**C**H$_3$). Anal. (C$_{21}$H$_{23}$ClN$_6$O$_4$S0.5H$_2$O) C, H, N. HRMS: calcd for [M + H], 585.20453; found, 585.20456.

5-(6-Chloro-8-(((2-ethylpyridin-4-yl)methyl)amino)-2-methylimidazo[1,2-b]pyridazin-3-yl)-N-cyclopropyl-2-methoxybenzenesulfonamide (**21**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (40−65% + 1% TEA). Yield: 117 mg (76%); mp 189.8−191.4 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.56 (t, J_{NH-CH2} = 6.3 Hz, 1 H, NH), 8.40 (dd, $J_{6''\text{-}5''}$ = 5.1 Hz, $J_{6''\text{-}3''}$ = 0.7 Hz, 1 H, H-6″), 8.01 (d, J_{6-4} = 2.3 Hz, 1 H, H-6), 7.89 (dd, J_{4-6} = 2.3 Hz, J_{4-3} = 8.6 Hz, 1 H, H-4), 7.73 (d, $J_{CH\text{-}NH}$ = 2.6 Hz, 1 H, CH-NH), 7.40 (d, J_{3-4} = 8.6 Hz, 1 H, H-3), 7.24 (bs, 1 H, H-3″), 7.16 (dd, $J_{5''\text{-}6''}$ = 5.1 Hz, $J_{5''\text{-}3''}$ = 1.6 Hz, 1 H, H-5″), 6.15 (s, 1 H, H-7′), 4.59 (bs, 2 H, NH−CH$_2$), 3.97 (s, 3 H, O−CH$_3$), 2.72 (q, $J_{CH2\text{-}CH3}$ = 7.6 Hz, 2 H, CH$_2$−CH$_3$), 2.45 (s, 3 H, 2′-CH$_3$), 2.16 (m, 1 H, CH^{cyclpr}), 1.20 (t, $J_{CH2\text{-}CH3}$ = 7.6 Hz, 3 H, CH$_2$−C**H**$_3$), 0.44−0.46 (m, 4 H, CH$_2^{cyclpr}$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 163.02 (C-2″), 156.04 (C-2), 149.28 (C-6″), 147.71 (C-4″), 147.05 (C-6′), 143.11 (C-8′), 137.95 (C-2′), 135.21 (C-4), 131.11 (C-9′), 130.57 (C-6), 127.77 (C-1), 123.76 (C-3′), 120.43 (C-5), 120.40 (C-3″), 119.57 (C-5″), 113.33 (C-3), 91.83 (C-7′), 56.53 (O−CH$_3$), 44.30 (CH$_2$NH), 30.75 (**C**H$_2$−CH$_3$), 24.24 (CH^{cyclpr}), 14.49 (2′-CH$_3$), 13.95 (CH$_2$−**C**H$_3$), 5.29 (CH$_2^{cyclpr}$). Anal. (C$_{25}$H$_{27}$ClN$_6$O$_3$S·0.5H$_2$O) C, H, N. HRMS: calcd for [M + H], 527.16266; found, 527.16268.

5-(6-Chloro-8-(((2-ethylpyridin-4-yl)methyl)amino)-2-methylimidazo[1,2-b]pyridazin-3-yl)-N-(1-hydroxybutan-2-yl)-2-methoxybenzenesulfonamide (**22**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (10−20%). Yield: 102 mg (63%); mp 181.8−183.7 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.55 (t, J_{NH-CH2} = 6.7 Hz, 1 H, NH), 8.40 (dd, $J_{6'''\text{-}5'''}$ = 5.1 Hz, $J_{6'''\text{-}3'''}$ = 0.7 Hz, 1 H, H-6‴), 7.98 (d, J_{6-4} = 2.3 Hz, 1 H, H-6), 7.87 (dd, J_{4-6} = 2.3 Hz, J_{4-3} = 8.8 Hz, 1 H, H-4), 7.37 (d, J_{3-4} = 8.8 Hz, 1 H, H-3), 7.24 (bs, 1 H, H-3‴), 7.15 (dd, $J_{5'''\text{-}6'''}$ = 5.1 Hz, $J_{5'''\text{-}3'''}$ = 1.5 Hz, 1 H, H-5″), 7.12 (d, $J_{NH\text{-}2'}$ = 7.7 Hz, 1 H, SO$_2$NH), 6.14 (s, 1 H, H-7″), 4.56−4.61 (m, 3 H, 1′−OH, 8″-NH$_2$), 3.96 (s, 2 H, O−CH$_3$), 3.31 (m, 1 H, H-1′a), 2.99 (m, 1 H, H-2′), 2.71 (q, $J_{CH2\text{-}CH3}$ = 7.5 Hz, 2 H, C**H**$_2$-CH$_3$), 1.52 + 1.29 (m, 2 H, 2′-C**H**$_2$-CH$_3$), 1.20 (t, $J_{CH2\text{-}CH3}$ = 7.5 Hz, 3 H, CH$_2$−C**H**$_3$), 0.71 (t, $J_{CH3\text{-}CH2}$ = 7.4 Hz, 3 H, CH$_2$−C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 163.03 (C-2‴), 155.90 (C-2), 149.28 (C-6‴), 147.73 (C-4‴), 147.04 (C-6″), 143.10 (C-8″), 137.93 (C-2″), 134.86 (C-4), 131.10 (C-9″), 129.74 (C-6), 129.25 (C-1), 123.81 (C-3″), 120.40 (C-5), 120.23 (C-5), 119.57 (C-5‴), 113.17 (C-3), 91.83 (C-7″), 63.18 (C-1′), 57.04 (C-2′), 56.39 (2-O−CH$_3$), 44.31 (CH$_2$−NH), 30.76 (CH$_2$CH$_3$), 14.54 (2″-CH$_3$), 13.95 (CH$_2$−**C**H$_3$), 9.89 (2′- CH$_2$−**C**H$_3$). Anal. (C$_{26}$H$_{31}$ClN$_6$O$_4$S·0.5EtOAc) C, H, N. HRMS: calcd for [M + H], 559.18888; found, 559.18889.

N-(2-((6-Chloro-3-(3-(N-(2-hydroxyethyl)sulfamoyl)-4-methoxyphenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)-acetamide (**23**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (10−20%). Recrystallized from EtOH. Yield: 111 mg (75%) as an off white solid; mp 208.9−210.6 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.05 (t, 1 H, NH−CO), 7.97 (d, $J_{2''\text{-}6''}$ = 2.2 Hz, 1 H, H-2″), 7.86−7.90 (m, 2 H, H-6″, 8′-NH), 7.40 (d, $J_{5''\text{-}6''}$ = 8.9 Hz, 1 H, H-5″), 7.17 (bs, 1 H, SO$_2$−NH), 6.30 (s, 1 H, H-7′), 4.67 (t, $J_{OH\text{-}1'''}$ = 5.6 Hz 1 H, OH), 3.97 (s, 3 H, 4″-O−CH$_3$), 3.35−3.41 (m, 4 H, H-2, 1‴), 3.26 (m, 2 H, H-1), 2.88 (m, 2 H, 2‴), 2.42 (s, 3 H, 2′-CH$_3$), 1.81 (s, 3 H, CO-C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.95 (C═O), 155.86 (C-4″), 147.25 (C-6′), 143.34 (C-8′), 137.72 (C-2′), 135.00 (C-6″), 131.18 (C-9′), 129.81 (C-2″), 128.19 (C-3″), 123.56 (C-3′), 120.52 (C-1″), 113.34 (C-5″), 91.19 (C-7′), 60.06 (C-1‴), 56.58 (4″-O−CH$_3$), 41.79 (C-2), 37.85 (C-1), 22.80 (CO-**C**H$_3$), 14.50 (2′-CH$_3$). Anal. (C$_{20}$H$_{25}$ClN$_6$O$_5$S·0.33H$_2$O) C, H, N. HRMS: calcd for [M + Na], 519.11879; found, 519.11905.

N-(2-((3-(3-(N-(4-Aminocyclohexyl)sulfamoyl)-4-methoxyphenyl)-6-chloro-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)-acetamide (**24**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH/H$_2$O (3:2:0.5 + TEA 1%). Yield: 115 mg (64%) as an off white foam. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.10 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1 H, NH−CO), 7.98 (d, $J_{2''\text{-}6''}$ = 2.3 Hz, 1 H, H-2″), 7.88 (m, 1 H, 8′-NH), 7.86 (dd, $J_{6''\text{-}2''}$ = 2.3 Hz, $J_{6''\text{-}5''}$ = 8.6 Hz, 1 H, H-6″), 7.48 (m, 1 H, SO$_2$NH), 7.38 (d, $J_{5''\text{-}6''}$ = 8.6 Hz, 1 H, H-5″), 3.97 (s, 3 H, 4″-O−CH$_3$), 3.37 (bs, 2 H, H-2), 3.26 (m, 2 H, H-1), 3.00 (m, 1 H, H-1‴), 2.81 (m, 1 H, H-4‴), 2.41 (s, 3 H, 2′-CH$_3$), 1.86 (m, 2 H, H-3‴a), 1.81 (s, 3 H, CO-C**H**$_3$), 1.70 (m, 2 H, H-2a‴), 1.20−1.33 (m, 4 H, H-3b‴, H-2b‴). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.98 (C═O), 155.84 (C-4″), 147.26 (C-6′), 143.34 (C-8′), 137.69 (C-2′), 134.94 (C-6″), 131.17 (C-9′), 129.67 (C-2″), 129.59 (C-3″), 123.62 (C-3′), 120.44 (C-1″), 113.33 (C-5″), 91.19 (C-7′), 56.43 (4″-O-**C**H$_3$), 51.70 (C-1‴), 48.49 (C-4‴), 41.79 (C-2), 37.86 (C-1), 31.13 (C-3‴), 29.82 (C-2‴), 22.80 (CO-**C**H$_3$), 14.48 (2′-CH$_3$). Anal. (C$_{24}$H$_{33}$ClN$_7$O$_4$S.H$_2$O) C, H, N. HRMS: calcd for [M + H], 550.19978; found, 550.19980.

N-(2-((6-Chloro-3-(4-methoxy-3-(morpholinosulfonyl)phenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**25**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (10−20%). Recrystallized from EtOH. Yield: 106 mg (77%) as an off white solid; mp 200.0−202.7 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, J_{NH-CO} = 5.6 Hz, 1 H, NH−CO), 7.99 (d, $J_{2''\text{-}6''}$ = 2.3 Hz, 1 H, H-2″), 7.92 (dd, $J_{6''\text{-}2''}$ = 2.3 Hz, $J_{6''\text{-}5''}$ = 8.7 Hz, 1 H, H-6″), 7.90 (t, $J_{NH\text{-}2}$ = 6.0 Hz, 1 H, 8′-NH), 7.44 (d, $J_{5''\text{-}6''}$ = 8.7 Hz, 1 H, H-5″), 6.30 (s, 1 H, H-7′), 3.97 (s, 3 H, 4″-O−CH$_3$), 3.62 (m, 4 H, CH$_2$O), 3.37 (bs, 2 H, H-2), 3.26 (m, 2 H, H-1), 3.13 (m, 4 H, CH$_2$−N), 2.43 (s, 3 H, 2′-CH$_3$), 1.80 (s, 3 H, CO-C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.93 (C−O), 156.32 (C-4″), 147.25 (C-6′), 143.36 (C-8′), 137.73 (C-2′), 135.61 (C-6″), 131.46 (C-2″), 124.89 (C-3″), 123.33 (C-3′), 120.70 (C-1″), 113.81 (C-5″), 91.21 (C-7′), 66.05 (CH$_2$O), 56.54 (4″-O-**C**H$_3$), 46.06 (CH$_2$N), 41.79 (C-2), 37.84 (C-1), 22.79 (CO-**C**H$_3$), 14.49 (2′-CH$_3$). Anal. (C$_{22}$H$_{27}$ClN$_6$O$_5$S·0.5H$_2$O) C, H, N. HRMS: calcd for [M + Na], 545.13444; found, 545.13446.

N-(2-((6-Chloro-3-(4-methoxy-3-(piperidin-1-ylsulfonyl)phenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**26**). Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (5−20%). Yield: 99 mg (60%) as an off white foam. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.04 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1 H, NH−CO), 7.98 (d, $J_{2''\text{-}6''}$ = 2.3 Hz, 1 H, H-2″), 7.87−7.90 (m, 2 H, 8′-NH, H-6″), 7.41 (d, $J_{5''\text{-}6''}$ = 8.8 Hz, 1 H, H-5″), 6.30 (s, 1 H, H-7′), 3.95 (s, 3 H,

DOI: 10.1021/acs.jmedchem.6b01465
J. Med. Chem. 2017, 60, 100−118

4″-O−CH_3), 3.36 (m, 2 H, H-2), 3.26 (m, 2 H, H-1), 3.11−3.14 (m, 4 H, H-2‴), 2.42 (s, 3 H, 2′-CH_3), 1.80 (s, 3 H, CO-C**H**$_3$), 1.50−1.55 (m, 4 H, H-3‴), 1.43−1.48 (m, 2 H, H-4‴). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.95 (C═O), 156.23 (C-4″), 147.22 (C-6′), 143.36 (C-8′), 137.69 (C-2′), 135.21 (C-6″), 131.24 (C-2″), 131.19 (C-9′), 125.89 (C-3″), 123.42 (C-3′), 120.59 (C-1″), 113.67 (C-5″), 91.19 (C-7′), 56.48 (4″-O-**C**H$_3$), 46.64 (C-2‴), 41.78 (C-2), 37.84 (C-1), 25.45 (C-3‴), 23.37 (C-4‴), 22.79 (CO-**C**H$_3$), 14.48 (2′-CH_3). Anal. ($C_{23}H_{29}ClN_6O_4S$·0.5EtOH) C, H, N. HRMS: calcd for [M + H], 521.17323; found, 521.17316.

*N-(2-((6-Chloro-3-(3-(N-(2-(dimethylamino)ethyl)sulfamoyl)-4-methoxyphenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)-ethyl)acetamide (**27**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH/H_2O (3:2:0.5 + TEA 1%). Triturated with diethyl ether. Yield: 125 mg (75%) as an off white solid; mp 222.0−223.7 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.10 (t, $J_{NH\text{-}1}$ = 5.3 Hz, 1 H, NH−CO), 8.01 (d, $J_{2''\text{-}6''}$ = 2.3 Hz, 1 H, H-2″), 7.88−7.92 (m, 2 H, H-6″, 8′-NH), 7.79 (t, $J_{NH\text{-}1}$ = 6.0 Hz, 1 H, SO_2NH), 7.73 (d, $J_{5''\text{-}6''}$ = 8.9 Hz, 1 H, H-5″), 6.31 (s, 1 H, H-7′), 4.01 (s, 3 H, 4″−O−CH_3), 3.37 (bs, 2 H, H-2), 3.26 (m, 2 H, H-1), 3.22 (m, 2 H, H-1‴), 3.15 (m, 2 H, H-2‴), 2.75 (s, 6 H, N(CH_3)$_2$), 2.42 (s, 3 H, 2′-CH_3), 1.81 (s, 3 H, CO-C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.97 (C═O), 155.89 (C-4″), 147.26 (C-6′), 143.34 (C-8′), 137.79 (C-2′), 135.42 (C-9′), 130.02 (C-2″), 127.30 (C-3″), 123.45 (C-3′), 120.64 (C-1″), 113.52 (C-5″), 91.22 (C-7′), 56.73 (4″-O-**C**H$_3$), 56.16 (C-2‴), 42.55 (N(CH_3)$_2$), 41.79 (C-2), 37.96 (C-1‴), 37.83 (C-1), 22.80 (CO-**C**H$_3$), 14.53 (2′-CH_3). Anal. ($C_{22}H_{30}ClN_7O_4S$) C, H, N. HRMS: calcd for [M + H], 524.18413; found, 524.18416.

*N-(2-((6-Chloro-3-(4-methoxy-3-(N-(2-methoxyethyl)sulfamoyl)-phenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)-acetamide (**28**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (10−20%). Recrystallized from EtOH. Yield: 130 mg (78%) as an off white solid; mp 181.4−183.7 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.04 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1 H, NH−CO), 7.96 (t, $J_{2''\text{-}6''}$ = 2.3 Hz, 1 H, H-2″), 7.88 (t, $J_{NH\text{-}2}$ = 6.0 Hz, 1 H, 8′-NH), 7.86 (dd, $J_{6''\text{-}2''}$ = 2.3 Hz, $J_{6''\text{-}5''}$ = 8.7 Hz, 1 H, H-6″), 7.39 (d, $J_{5''\text{-}6''}$ = 8.7 Hz, 1 H, H-5″), 6.30 (s, 1 H, H-7′), 3.97 (s, 3 H, 4″-O−CH_3), 3.36 (bs, 2 H, H-2), 3.30 (t, $J_{2'''\text{-}1'''}$ = 5.9 Hz, 2 H, H-2‴), 3.26 (m, 2 H, H-1), 3.12 (s, 3 H, 2‴-O−CH_3), 3.02 (q, 2 H, H-1‴), 2.41 (s, 3 H, 2′-CH_3), 1.80 (s, 3 H, CO-C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.95 (C═O), 155.93 (C-4″), 147.24 (C-6′), 143.34 (C-8′), 137.70 (C-2′), 134.95 (C-6″), 131.17 (C-9′), 129.71 (C-2″), 128.58 (C-3″), 123.62 (C-3′), 120.45 (C-1″), 113.29 (C-5″), 91.18 (C-7′), 70.72 (C-2‴), 57.99 (2‴-O-**C**H$_3$), 56.55 (4″−O−CH_3), 42.49 (C-1‴), 41.78 (C-2), 37.85 (C-1), 22.80 (CO-**C**H$_3$), 14.46 (2′-CH_3). Anal. ($C_{21}H_{27}ClN_6O_5S$·0.25H_2O) C, H, N.HRMS: calcd for [M + Na], 533.13444; found, 533.13446.

*N-(2-((3-(3-((1H-Pyrazol-1-yl)sulfonyl)-4-methoxyphenyl)-6-chloro-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)-acetamide (**29**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (10−20%). Recrystallized from EtOH. Yield: 115 mg (73%) as an off white solid; mp 211.0−212.9 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.50 (dd, $J_{3'',5''\text{-}4''}$ = 2.8 Hz, $J_{3''\text{-}5''}$ = 0.6 Hz, 1 H, H-3″ or 5″), 8.12 (d, $J_{2''\text{-}6''}$ = 2.3 Hz, 1 H, H-2″), 8.02 (dd, $J_{6''\text{-}2''}$ = 2.3 Hz, $J_{6''\text{-}5''}$ = 8.7 Hz, 1 H, H-6″), 7.93 (t,J_{NH2} = 5.9 Hz, 1 H, 8′-NH), 7.87 (dd, $J_{3''',5'''\text{-}4'''}$ = 1.5 Hz, $J_{3'''\text{-}5'''}$ = 0.6 Hz, 1 H, H-5‴ or 3‴), 7.41 (d, $J_{5''\text{-}6''}$ = 8.7 Hz, 1 H, H-5″), 6.60 (dd, $J_{4'''\text{-}5''',3'''}$ = 2.8 Hz, $J_{4'''\text{-}3''',5'''}$ = 1.5 Hz, 1 H, H-4‴), 6.33 (s, 1 H, H-7′), 3.83 (s, 3 H, 4″-O−CH_3), 3.36 (bs, 2 H, H-2), 3.27 (m, 2 H, H-1), 2.43 (s, 3 H, 2′-CH_3), 1.81 (s, 3 H, CO-C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.95 (C═O), 156.54 (C-4″), 147.35 (C-6′), 145.50 (C-5‴ or 3‴), 143.35 (C-8′), 138.07 (C-2′), 137.78 (C-6″), 133.98 (C-3‴ or 5‴), 131.35 (C-9′), 130.37 (C-2″), 124.23 (C-3″), 122.75 (C-3′), 121.20 (C-1″), 114.27 (C-5″), 108.41 (C-4‴), 91.40 (C-7′), 56.90 (4″-O-**C**H$_3$), 41.80 (C-2), 37.84 (C-1), 22.80 (CO-**C**H$_3$), 14.49 (2′-CH_3). Anal. ($C_{21}H_{22}ClN_7O_4S$·0.33EtOH) C, H, N. HRMS: calcd for [M + H], 504.12153; found, 504.12155.

*N-(2-((6-Chloro-3-(3-((4-(hydroxymethyl)piperidin-1-yl)sulfonyl)-4-methoxyphenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)-ethyl)acetamide (**30**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (10−20%). Recrystallized from EtOH. Yield: 110 mg (68%) as an off-white solid; mp 215.8−217.5 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, J_{NH-CO} = 5.6 Hz, 1 H, NH−CO), 7.98 (d, $J_{2''\text{-}6''}$ = 2.3 Hz, 1 H, H-2″), 7.87−7.90 (m, 2 H, 8′-NH, H-6″), 7.42 (d, $J_{5''\text{-}6''}$ = 8.8 Hz, 1 H, H-5″), 6.30 (s, 1 H, H-7′), 4.72 (d, $J_{OH\text{-}4'''}$ = 4.1 Hz, 1 H, OH), 3.95 (s, 3 H, 4″-O−CH_3), 3.58 (m, 1 H, H-4‴), 3.35−3.44 (m, 4 H, H-2‴a, 2), 3.26 (m, 2 H, H-1), 2.94 (m, 2 H, H-2‴b), 2.42 (s, 3 H, 2′-CH_3), 1.80 (s, 3 H, CO-C**H**$_3$), 1.71−1.77 (m, 2 H, H-3‴a), 1.35−1.43 (m, 2 H, H-3‴b). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.94 (C−O), 156.21(C-4″), 147.22 (C-6′), 143.36 (C-8′), 137.69 (C-2′), 135.24 (C-6″), 131.12 (C-2″), 125.94 (C-3″), 123.41 (C-3′), 120.60 (C-1″), 113.69 (C-5″), 91.19 (C-7′), 64.75 (H-4‴), 56.48 (4″-O-**C**H$_3$), 43.41 (C-2‴), 41.78 (C-2), 37.84 (C-1), 33.86 (C-3‴), 22.79 (CO-**C**H$_3$), 14.48 (2′-CH_3). Anal. ($C_{24}H_{31}ClN_6O_5S.H_2O$) C, H, N. HRMS: calcd for [M + Na], 559.15009; found, 559.15002.

*N-(2-((3-(3-(N-Allylsulfamoyl)-4-methoxyphenyl)-6-chloro-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**31**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (0−20%). Yield: 112 mg (77%) as an off white solid; mp 193.8−194.2 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, J_{NH-CO} = 5.6 Hz, 1 H, NH−CO), 7.95 (d, $J_{2''\text{-}6''}$ = 2.3 Hz, 1 H, H-2″), 7.88 (t, 1 H, 8′-NH), 7.86 (dd, $J_{6''\text{-}5''}$ = 8.7 Hz, $J_{6''\text{-}2''}$ = 2.3 Hz, 1 H, H-6″), 7.60 (t, J_{NH-CH2} = 5.6 Hz, 1 H, SO_2−NH), 7.38 (d, $J_{5''\text{-}6''}$ = 8.7 Hz, 1 H, H-5″), 6.30 (s, 1 H, H-7′), 5.69 (m, 1 H, C**H**═CH_2), 5.13 (dd, J_{GEM} = 1.7 Hz, $J_{HT\text{-}H}$ = 17.1 Hz, 1 H, CH^{trans}), 4.98 (dd, J_{GEM} = 1.7 Hz, $J_{HC\text{-}H}$ = 10.3 Hz, 1 H, CH^{cis}), 3.95 (s, 3 H, O−CH_3), 3.53 (m, 1 H, SO_2−NH−CH_2), 3.26 (m, 2 H, H-1), 2.41 (s, 3 H, 2′-CH_3), 1.80 (s, 3 H, CO-C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.93 (C−O), 155.91 (C-4″), 147.23 (C-6′), 143.34 (C-8′), 137.68 (C-2′), 134.97 (C-6″), 134.76 (CH═CH_2), 131.16 (C-2″), 128.68 (C-3″), 123.61 (C-3′), 120.42 (C-1″), 116.35 (CH═**C**H$_2$), 113.28 (C-5″), 91.17 (C-7′), 56.51 (O-**C**H$_3$), 45.37 (SO_2NH-**C**H$_2$), 41.78 (C-2), 37.84 (C-1), 22.79 (CO-**C**H$_3$), 14.45 (2′-CH_3). Anal. ($C_{21}H_{25}ClN_6O_4S$·0.33EtOAc) C, H, N. HRMS: calcd for [M + H], 493.14193; found, 493.14188.

*N-(2-((6-Chloro-3-(3-(N,N-dimethylsulfamoyl)-4-methoxyphenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**32**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (5−10%). Recrystallized from EtOH. Yield: 110 mg (70%) as an off white solid; mp 226.9−229.0 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, $J_{NH\text{-}1}$ = 6.0 Hz, 1 H, NH−CO), 7.99 (d, $J_{2''\text{-}6''}$ = 2.3 Hz, 1 H, H-2″), 7.87−7.91 (m, 2 H, H-6″, 8′-NH), 7.43 (d, 1 H, H-5″), 6.30 (s, 1 H, H-7′), 3.96 (s, 3 H, 4″-O−CH_3), 3.36 (bs, 2 H, H-2), 3.26 (m, 2 H, H-1), 2.78 (s, 6 H, N-(CH_3)$_2$), 2.43 (s, 3 H, 2′-CH_3), 1.80 (s, 3 H, CO-C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.97 (C−O), 156.28 (C-4″), 147.24 (C-6′), 143.37 (C-8′), 137.73 (C-2′), 135.31 (C-6″), 131.42 (C-2″), 131.21 (C-9′), 125.39 (C-3″), 123.42 (C-3′), 120.65 (C-1″), 113.68 (C-5″), 91.22 (C-7′), 56.51 (4″-O-**C**H$_3$), 41.79 (C-2), 37.85 (C-1), 37.85 (N(CH_3)$_2$), 22.80 (CO-**C**H$_3$), 14.48 (2′-CH_3). Anal. ($C_{20}H_{25}ClN_6O_4S$) C, H, N. HRMS: calcd for [M + H], 481.14193; found, 481.14194.

*N-(2-((6-Chloro-3-(3-((4-(hydroxymethyl)piperidin-1-yl)sulfonyl)-4-methoxyphenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)-ethyl)acetamide (**33**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (10−20%). Yield: 89 mg (62%) as an off white solid; mp 202.5−207.3 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1 H, NH−CO), 7.99 (d, $J_{2''\text{-}6''}$= 2.3 Hz, 1 H, H-2″), 7.87−7.90 (m, 2 H, H-6″, 8′-NH), 7.41 (d, 1 H, H-5″), 6.30 (s, 1 H, H-7′), 3.95 (s, 3 H, 4″-O−CH_3), 3.73 (dm, J_{GEM} = 12.5 Hz, 2 H, H-2a‴), 3.37 (m, 2 H, H-2), 3.27 (m, 2 H, H-1), 3.23 (m, 2 H, CH_2OH), 2.60 (m, 2 H, H-2b‴), 2.43 (s, 3 H, 2′-CH_3), 1.80 (s, 3 H, CO-C**H**$_3$), 1.70 (dm, J_{GEM} = 13.0 Hz, 2 H, H-3a‴), 1.43 (m, 1 H, H-4‴), 1.11 (m, 2 H, H-3b‴). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 170.22 (C−O), 156.49 (C-4″), 147.49 (C-6′), 143.36 (C-8′), 137.97 (C-2′), 135.47 (C-9′), 131.45 (C-2″), 126.30 (C-3″), 123.70 (C-3′), 120.85 (C-1″), 113.94 (C-5″), 91.47 (C-7′), 65.82 (CH_2OH), 56.74 (4″-O-**C**H$_3$), 46.17 (C-2‴), 42.06 (C-2), 38.13 (C-1), 38.09 (C-4‴), 28.89 (C-3‴), 23.07 (CO-**C**H$_3$), 14.77 (2′-CH_3).

DOI: 10.1021/acs.jmedchem.6b01465
J. Med. Chem. 2017, 60, 100−118

Anal. ($C_{24}H_{31}ClN_6O_5S·0.5H_2O$) C, H, N. HRMS: calcd for [M + Na], 573.16574; found, 573.16524.

*N-(2-((6-Chloro-3-(3-(N-cyclopropylsulfamoyl)-4-methoxyphenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)acetamide (**34**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (10−20%). Recrystallized from EtOH. Yield: 126 mg (79%) as an off white solid; mp 213.0−215.9 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.04 (t, $J_{NH\text{-}1}$ = 5.7 Hz, 1 H, NH−CO), 8.00 (d, $J_{2''\text{-}6''}$ = 2.3 Hz, 1 H, H-2″), 7.87−7.90 (m, 2 H, H-6″, 8′-NH), 7.72 (d, J_{NH-CH} = 2.5 Hz, 1 H, SO_2−NH), 7.40 (d, $J_{5''\text{-}6''}$ = 8.8 Hz, 1 H, H-5″), 6.30 (s, 1 H, H-7′), 3.97 (s, 3 H, 4″-O−CH_3), 3.37 (bs, 2 H, H-2), 3.26 (m, 2 H, H-1), 2.42 (s, 3 H, 2′-CH_3), 2.16 (m, 1 H, CH^{cypr}), 1.81 (s, 3 H, CO-C**H**$_3$), 0,44−0.47 (m, 4 H, CH_2^{cypr}). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.94 (C═O), 155.99 (C-4″), 147.24 (C-6′), 143.34 (C-8′), 137.69 (C-2′), 135.17 (C-6″), 131.18 (C-9′), 130.53 (C-2″), 127.76 (C-3″), 123.60 (C-3′), 120.51 (C-1″), 113.31 (C-5″), 91.19 (C-7′), 56.53 (4″-O−CH_3), 41.79 (C-2), 37.85 (C-1), 24.24 (CH^{cypr}), 22.79 (CO-**C**H$_3$), 14.46 (2′-CH_3), 5.29 (CH_2^{cypr}). Anal. ($C_{21}H_{25}ClN_6O_4S·0.5H_2O$) C, H, N. HRMS: calcd for [M + H], 493.14193; found, 493.14185.

*N-(2-((6-Chloro-3-(3-(N-(1-hydroxybutan-2-yl)sulfamoyl)-4-methoxyphenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)amino)ethyl)-acetamide (**35**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH (5−20%). Recrystallized from EtOH. Yield: 110 mg (66%) as an off white solid; mp 200.1−201.6 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.05 (t, $J_{NH\text{-}1}$ = 5.7 Hz, 1 H, NH−CO), 7.98 (d, $J_{2''\text{-}6''}$ = 2.4 Hz, 1 H, H-2″), 7.85−7.89 (m, 2 H, H-6″, 8′-NH), 7.37 (d, $J_{5''\text{-}6''}$ = 8.8 Hz, 1 H, H-5″), 7.12 (d, J_{NH-CH} = 7.7 Hz, 1 H, NH−CH), 6.30 (s, 1 H, H-7′), 4.59 (t, J_{OH-CH2} = 5.5 Hz, 1 H, CH_2O**H**), 3.96 (s, 3 H, 4″-O−CH_3), 3.36 (m, 2 H, H-2), 3.30 (m, 2 H, C**H**$_2^a$_OH), 3.26 (m, 2 H, H-1), 3.18 (m, 2 H, C**H**$_2^b$_OH), 2.99 (m, 1 H, NH−CH), 2.41 (s, 3 H, 2′-CH_3), 1.80 (s, 3 H, CO-C**H**$_3$), 1.53 + 1.29(m, 2 H, CH_2CH_3), 0.72 (t, $J_{CH3\text{-}CH2}$ = 7.4 Hz, 3 H, C**H**$_3$$CH_2$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.95 (C═O), 155.84 (C-4″), 147.23 (C-6′), 143.33 (C-8′), 137.67 (C-2′), 134.82 (C-6″), 131.16 (C-9′), 129.69 (C-2″), 129.24 (C-3″), 123.64 (C-3′), 120.30 (C-1″), 113.15 (C-5″), 91.16 (C-7′), 63.17 (CH_2OH), 57.03 (NH−CH), 56.38 (4″-O-**C**H$_3$), 41.79 (C-2), 37.86 (C-1), 24.02 (CH$_3$-**C**H$_2$), 22.80 (CO−CH_3), 14.50 (2′-CH_3), 9.88 (CH_2**C**H$_3$). Anal. ($C_{22}H_{29}ClN_6O_5S·0.5H_2O$) C, H, N. HRMS: calcd for [M + Na], 547.15009; found, 547.14934.

*N-(2-((6-Chloro-3-(4-methoxy-3-(N-methyl-N-(2-(methylamino)-ethyl)sulfamoyl)phenyl)-2-methylimidazo[1,2-b]pyridazin-8-yl)-amino)ethyl)acetamide (**36**).* Prepared according to general procedure C. Mobile phase: EtOAc/EtOH/H_2O (3:2:0.25 + TEA 1%). Triturated with DEE. Yield: 102 mg (61%) as an off-white solid; mp 179.3−181.1 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.73 (bs, 2 H, NH_2), 8.08 (t, $J_{NH\text{-}1}$ = 5.6 Hz, 1 H, NH−CO), 8.03 (d, $J_{2''\text{-}6''}$ = 2.3 Hz, 1 H, H-2″), 7.93 (dd, $J_{6''\text{-}2''}$ = 2.3 Hz, $J_{6''\text{-}5''}$ = 8.7 Hz, 1 H, H-6″), 7.90 (t, $J_{NH\text{-}2}$ = 6.2 Hz, 1 H, 8′-NH), 7.46 (d, $J_{5''\text{-}6''}$ = 8.7 Hz, 1 H, H-5″), 6.32 (s, 1 H, H-7′), 3.99 (s, 3 H, 4″-O−CH_3), 3.45 (m, 2 H, CH_3N−C**H**$_2$), 3.36 (m, 2 H, H-2), 3.26 (m, 2 H, H-1), 3.12 (m, 2 H, N−CH_2), 2.82 (s, 3 H, N−CH_3), 2.59 (s, 3 H, NH-C**H**$_3$), 22.43 (s, 3 H, 2′-CH_3), 1.80 (s, 3 H, CO-C**H**$_3$). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.97 (C═O), 156.08 (C-4″), 147.27 (C-6′), 143.36 (C-8′), 137.78 (C-2′), 135.69 (C-6″), 131.39 (C-2″), 131.23 (C-9′), 125.54 (C-3″), 123.32 (C-3′), 120.78 (C-1″), 113.82 (C-5″), 91.24 (C-7′), 56.65 (4″-O-**C**H$_3$), 46.39 (CH$_3$N-**C**H$_2$), 46.16 (N−CH_2), 41.80 (C-2), 37.82 (C-1), 35.37 (CH_2NH-**C**H$_3$), 32.80 (NH-C**H**$_3$), 22.80 (CO-**C**H$_3$), 14.51 (2′-CH_3). Anal. ($C_{22}H_{30}ClN_7O_4S.H_2O$) C, H, N. HRMS: calcd for [M + H], 524.18413; found, 524.18420.

*6-Chloro-3-iodo-2-methyl-N-(pyridin-4-ylmethyl)imidazo[1,2-b]-pyridazin-8-amine (**37**).* Prepared according to general procedure A. Mobile phase: EtOAc/MeOH (10−20%). Recrystallization from EtOH. Yield: 1.21 g (86%); mp 136.2−137.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.58 (t, J_{NH-CH2} = 6.3 Hz, 1 H, NH), 8.50 (m, 2 H, H-2′), 7.33 (m, 2 H, H-3′), 6.17 (s, 1 H, H-7), 4.60 (d, J_{NH-CH2} = 6.3 Hz, 2 H, C**H**$_2$-NH), 2.37 (s, 3 H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 149.88 (C-2′), 147.46 (C-6), 147.25 (C-4′), 143.99 (C-2), 142.86 (C-8), 134.18 (C-9), 122.21 (C-3′), 92.36 (C-7), 72.00 (C-3), 44.19 (NH-**C**H$_2$), 14.87 (2-CH_3). Anal. ($C_{15}H_{15}ClIN_5$.H_2O) C, H, N. HRMS: calcd for [M + H], 506.08149; found, 506.08148.

*6-Chloro-3-iodo-2-methyl-N-(pyridin-3-ylmethyl)imidazo[1,2-b]-pyridazin-8-amine (**38**).* Prepared according to general procedure A. Mobile phase: EtOAc/MeOH (10−20%). Recrystallization from EtOAc. Yield: 1.15 g (95%); mp 154.1−155.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.61 (dd, $J_{2'\text{-}4'}$ = 2.3 Hz, $J_{2'\text{-}5'}$ = 0.8 Hz, 1 H, H-2′), 8.56 (t, J_{NH-CH2} = 6.2 Hz, 1 H, NH), 8.46 (dd, $J_{6'\text{-}5'}$ = 4.8 Hz, $J_{6'\text{-}4'}$ = 1.7 Hz, 1 H, H-6′), 7.76 (ddd, $J_{4'\text{-}5'}$ = 7.9 Hz, $J_{4'\text{-}6'}$ = 1.7 Hz, $J_{4'\text{-}2'}$ = 2.3 Hz, 1 H, H-4′), 7.35 (ddd, $J_{5'\text{-}4'}$ = 7.9 Hz, $J_{5'\text{-}6'}$ = 4.8 Hz, $J_{5'\text{-}2'}$ = 0.8 Hz, 1 H, H-5′), 6.29 (s, 1 H, H-7), 4.60 (d, J_{NH-CH2} = 6.2 Hz, 2 H, C**H**$_2$-NH), 2.36 (s, 3 H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 149.27 (C-2′), 148.89 (C-6′), 147.79 (C-6), 144.21 (C-2), 142.98 (C-8), 135.41 (C-4′), 134.50 (C-9), 134.05 (C-3′), 124.07 (C-5′), 92.52 (C-7), 72.22 (C-3), 43.12 (NH-**C**H$_2$), 15.14 (2-CH_3). Anal. ($C_{13}H_{11}ClIN_5$.0.5EtOAc) C, H, N. HRMS: calcd for [M + H], 399.98204; found, 399.98217.

*6-Chloro-3-iodo-2-methyl-N-(pyridin-2-ylmethyl)imidazo[1,2-b]-pyridazin-8-amine (**39**).* Prepared according to general procedure A. Mobile phase: EtOAc/MeOH (10−15%). Recrystallization from MeOH. Yield: 1.13 g (97%); mp 159.6−161.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.54 (ddd, $J_{6'\text{-}5'}$ = 4.8 Hz, $J_{6'\text{-}4'}$ = 1.8 Hz, $J_{6'\text{-}3'}$ = 0.9 Hz, 1 H, H-6′), 8.40 (t, J_{NH-CH2} = 6.2 Hz, 1 H, NH), 7.76 (td, $J_{4'\text{-}5'}$ = $J_{4'\text{-}3'}$ = 7.7 Hz, $J_{4'\text{-}6'}$ = 1.8 Hz, 1 H, H-4′), 7.34 (dm, $J_{3'\text{-}4'}$ = 7.7 Hz, 1 H, H-3′), 7.29 (ddd, $J_{5'\text{-}6'}$ = 4.8 Hz, $J_{5'\text{-}4'}$ = 7.7 Hz, $J_{5'\text{-}3'}$ = 1.0 Hz, 1 H, H-5′), 6.19 (s, 1 H, H-7), 4.64 (bs, 2 H, C**H**$_2$-NH), 2.37 (s, 3 H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 157.14 (C-2′), 149.21 (C-6′), 147.43 (C-6), 143.96 (C-2), 142.92 (C-8), 137.21 (C-4′), 134.23 (C-9), 122.70 (C-5′), 121.47 (C-3′), 92.44 (C-7), 71.91 (C-3), 47.21 (NH-**C**H$_2$), 14.88 (2-CH_3). Anal. ($C_{13}H_{11}ClIN_5$.0.66MeOH) C, H, N. HRMS: calcd for [M + H], 399.98204; found, 399.98223.

*6-Chloro-3-iodo-2-methyl-N-((2-methylpyridin-4-yl)methyl)-imidazo[1,2-b]pyridazin-8-amine (**40**).* Prepared according to general procedure A. Mobile phase: EtOAc/MeOH, yellowish solid. Yield: 1.221 g (65%) as an off-white solid; mp 155.8−158.2 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.54 (t, J_{NH-CH2} = 6.4 Hz, 1 H, NH), 8.36 (dd, $J_{6'\text{-}5'}$ = 5.1 Hz, $J_{6'\text{-}3'}$ = 0.6 Hz, 1 H, H-6′), 7.18 (dd, $J_{3'\text{-}5'}$ = 1.8 Hz, $J_{3'\text{-}6'}$ = 0.6 Hz, 1 H, H-3′), 7.11 (dd, $J_{5'\text{-}3'}$ = 1.8 Hz, $J_{5'\text{-}6'}$ = 5.1 Hz, 1 H, H-5′), 6.14 (s, 1 H, H-7), 4.55 (d, J_{NH-CH2} = 6.4 Hz, 2 H, C**H**$_2$-NH), 2.42 (s, 3 H, 2′-CH_3), 2.37 (s, 3 H, 2-CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 158.11 (C-2′), 149.22 (C-6′), 147.46 (C-6, 4′), 143.96 (C-2), 142.88 (C-8), 134.19 (C-9), 121.25 (C-3′), 119.33 (C-5′), 92.28 (C-7), 71.97 (C-3), 44.24 (NH-**C**H$_2$), 24.23 (2′-CH_3), 14.87 (2-CH_3). Anal. ($C_{14}H_{13}ClIN_5$.0.17EtOH) C, H, N. HRMS: calcd for [M + H], 413.99769; found, 413.99776.

*6-Chloro-N-((2-ethylpyridin-4-yl)methyl)-3-iodo-2-methylimidazo[1,2-b]pyridazin-8-amine (**41**).* Prepared according to general procedure A. Mobile phase petrolether/EtOAc (60−90%). Yield: 1.205 g (85%) as yellowish solid; mp 144.2−145.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.54 (t, J_{NH-CH2} = 6.1 Hz, 1 H, NH), 8.39 (d, $J_{6'\text{-}5'}$ = 5.0 Hz, 1 H, H-6′), 7.21 (s, 1 H, H-3′), 7.13 (d, $J_{5'\text{-}6'}$ = 5.0 Hz, 1 H, H-5′), 6.16 (s, 1 H, H-7), 4.57 (bs, 2 H, C**H**$_2$-NH), 2.70 (q, $J_{CH2\text{-}CH3}$ = 7.4 Hz, 2 H, CH_2CH_3), 2.37 (s, 3 H, 2-CH_3), 1.18 (t, $J_{CH3\text{-}CH2}$ = 7.4 Hz, 3 H, CH_2CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 162.99 (C-2′), 149.19 (C-6′), 147.71 (C-6), 144.01 (C-2), 142.92 (C-8), 134.22 (C-9), 120.42 (C-3′), 119.60 (C-5′), 92.32 (C-7), 72.02 (C-3), 44.35 (NH-**C**H$_2$), 30.70 (**C**H$_2$−CH_3), 14.90 (2-CH_3), 13.95 (CH_2−**C**H$_3$). Anal. ($C_{15}H_{15}ClIN_5$) C, H, N. HRMS: calcd for [M + H], 506.08149; found, 506.08148.

*N-(2-((6-Chloro-3-iodo-2-methylimidazo[1,2-b]pyridazin-8-yl)-amino)ethyl)acetamide (**42**).* Prepared according to general procedure A. Mobile phase: EtOAc/acetone/EtOH/water (20:3:1.6:0.4). Yellowish solid. Recrystallization from hot MeOH/$CHCl_3$. 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

DOI: 10.1021/acs.jmedchem.6b01465
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($COCH_3$), 14.9 (2′-CH_3). HRMS: calcd. for [M + H]: 393.99261, found 393.99255. Anal. ($C_{11}H_{13}ClIN_5O$) C, H, N.

*N-(2-((6-Chloro-3-(4-methoxyphenyl)-2-methylimidazo[1,2-b]-pyridazin-8-yl)amino)ethyl)acetamide (**43**).* Prepared according to general procedure B. Mobile phase: EtOAc/EtOH (10−20%). Yield: 484 mg (99%) as an off-white solid; mp 210.9−212.0 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.04 (t, $J_{NH\text{-}1}$ = 5.6, 1 H, 1-NH), 7.82 (t, $J_{NH\text{-}2}$ = 6.0, 1 H, 2-NH), 7.53−7.56 (m, 2 H, H-2″), 7.07−7.10 (m, 2 H, H-3″), 6.26 (s, 1 H, H-7′), 3.82 (s, 3 H, O−CH_3), 3.34 (m, 2 H, H-2), 3.26 (m, 2 H, H-1), 2.39 (s, 3 H, 2′- CH_3), 1.80 (s, 3 H, CO−CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 169.96 (C═O), 159.02 (C-4″), 147.06 (C-6′), 143.29 (C-8′), 137.23 (C-2′), 130.84 (C-9′), 130.72 (C-2″), 124.96 (C-3′), 120.84 (C-1″), 114.15 (C-3″), 90.85 (C-7′), 55.41 (O−CH_3), 41.74 (C-2), 37.88 (C-1), 22.80 (CO−CH_3), 14.52 (2′-CH_3). Anal. ($C_{18}H_{20}ClN_5O_2.0.25H_2O$) C, H, N. HRMS: calcd for [M + H], 374.13783; found, 374.13784.

*5-(6-Chloro-8-(((2-ethylpyridin-4-yl)methyl)amino)-2-methylimidazo[1,2-b]pyridazin-3-yl)-2-methoxybenzenesulfonyl Chloride (**44**).* Compound **9** (270 mg, 0.662 mmol) was dissolved in dry CH_2Cl_2 (5 mL); cooled ice/brine mixture and chlorosulfonic acid (4 mL, in excess) was added slowly. The reaction mixture was stirred in an ice bath for another 30 min and then allowed to warm to RT after which it was stirred another 4 h. The reaction mixture was poured carefully onto ice, pH was adjusted to 7 with saturated $NaHCO_3$ solution, and the mixture was extracted with CH_2Cl_2, 3×. Combined organic phases were dried over sodium sulfate and evaporated, and the crude product was used without further purification since no starting material was detected (TLC, satisfied purity of ^{1}H NMR). Analytical sample was obtained after the flash column chromatography.

Mobile phase: EtOAc/EtOH. Yield 10−15% as a off-white solid; mp 258 °C (decomp). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.47 (dm, $J_{6''\text{-}5''}$ = 5.2 Hz, 1 H, H-6″), 8.14 (d, $J_{6\text{-}4}$ = 2.3 Hz, 1 H, H-6), 8.01 (dd, $J_{4\text{-}6}$ = 2.3 Hz,$J_{4\text{-}3}$ = 8.7 Hz, 1 H, H-4), 7.21 (d, $J_{3\text{-}4}$ = 8.7 Hz, H-3), 7.09 (bs, 1 H, H-3″), 7.04 (dm, $J_{5''\text{-}6''}$ = 5.2 Hz, 1 H, H-5″), 6.33 (m, 1 H, CH_2−N**H**), 5.89 (s, 1 H, H-7′), 4.49 (d, 2 H, $J_{CH2\text{-}NH}$ = 6.1 Hz, 2 H, C**H$_2$**-NH), 4.06 (s, 3 H, OCH_3); 2.78 (q, $J_{CH2\text{-}CH3}$ = 7.6 Hz, 2 H, CH_2CH_3), 2.46 (s, 3 H, 2′-CH_3), 1.25 (t, $J_{CH3\text{-}CH2}$ = 7.6 Hz, 3 H, CH_2CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 164.42 (C-2″), 156.69 (C-2), 149.75 (C-6″), 147.95 (C-6′), 145.69 (C-4″), 142.09 (C-8′), 138.95 (C-2′), 137.78 (C-4), 131.91 (C-5), 131.27 (C-9′), 130.33 (C-6), 123.63 (C-3′), 121.08 (C-1), 120.19 (C-3″), 119.17 (C-5″), 113.54 (C-3), 93.16 (C-7′), 56.85 (O−CH_3), 45.79 (NH-**C**H_3), 31.30 (**C**H_2−CH_3), 14.51 (2′-CH_3), 13.88 (CH_2−**C**H_3). Anal. ($C_{22}H_{21}Cl_2N_5O_3S$) C, H, N. HRMS: calcd for [M + H], 506.08149; found, 506.08148.

*5-(8-((2-Acetamidoethyl)amino)-6-chloro-2-methylimidazo[1,2-b]pyridazin-3-yl)-2-methoxybenzenesulfonyl Chloride (**45**).* Chlorosulfonic acid (5 mL, in excess) was cooled in the ice bath and vigorously stirred while derivative **43** (333 mg, 0.8907 mmol) was added in small portions. After the completion of the addition, the reaction mixture was kept under a drying tube and stirred at rt overnight. Then the reaction mixture was carefully poured onto ice and extracted with CH_2Cl_2 (100 mL, 2 × 50 mL). Organic layers were dried over sodium sulfate and evaporated to provide brownish foam. Crude MI310 was used in the next step without further purification. Analytical sample was obtained by flash column chromatography.

Mobile phase EtOAc/EtOH (10−20%). ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 8.12 (d, $J_{6\text{-}4}$ = 2.4, 1 H, H-6), 8.00 (dd, $J_{4\text{-}6}$ = 2.4, $J_{4\text{-}3}$ = 8.9, 1 H, H-4), 7.21 (d, $J_{3\text{-}4}$ = 8.9, 1 H, H-3), 6.37 (bs, 1 H, 8′-NH), 6.07 (s, 1 H, H-7′), 5.99 (bs, 1 H, NH−CO), 4.06 (s, 3 H, CO−CH_3), 3.52 (m, 2 H, H-2″), 3.45 (m, 2 H, H-1″), 2.44 (s, 3 H, 2′- CH_3), 1.95 (s, 3 H, CO−CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm): 171.06 (C═O), 156.70 (C-2), 148.37 (C-6′), 142.30 (C-8′), 138.22 (C2′), 137.84 (C-4), 131.86 (C-1), 131.24 (C-9′), 130.31 (C-6), 123.45 (C-3′), 120.92 (C-5), 113.54 (C-3′), 92.89 (C-7′), 56.86 (2-O−CH_3), 42.66 (C-1″), 38.67 (C-2″), 23.22 (CO−CH_3), 14.31 (2′-CH_3). HRMS: calcd for [M + H], 472.06076; found, 472.06079.

Protein Expression and Purification. PI4KB was expressed and purified as before.[28] Rab11 wt and Q70L mutant were expressed using earlier established protocols.[42,43] Briefly, the proteins were expressed in *Escherichia coli* BL21 Star. The bacteria were grown at 37 °C in autoinduction media until OD reached one. Then the temperature was lowered to 18 °C, and the bacteria were cultured for 16 additional hours. The cells were harvested and lysed in lysis buffer (50 mM Tris, pH 8, 300 mM NaCl, 3 mM βME, 20 mM imidazole, 10% glycerol) using EmulsiFlex-C3 (Avestin) followed by nickel affinity chromatography. The 6xHisTag or the 6xHis-GB1 solubility tags were removed by TEV protease. Finally, the proteins were purified using size exclusion chromatography (SEC) on Superdex200 (PI4KB) or Superdex75 (Rab11) columns in SEC buffer (10 mM Tris, pH 8, 200 mM NaCl, 0.5 mM TCEP). Purified proteins were concentrated to 10 mg/mL and stored in −80 °C until needed.

Crystallographic Analysis. Diffraction quality crystals grew in 3−7 days in a vapor diffusion sitting drop. Data sets were collected at the BESSY 14.1 MX beamline using Pilatus 6 M detector.[44] The crystals diffracted to 3.3−3.8 Å and belonged to the orthorhombic spacegroup $P2_12_12_1$. The diffraction data were indexed, integrated, and scaled using XDSAPP.[45] The structures were solved by molecular replacement using **1** bound PI4KB as a search model.[16] Subsequently, the structures were refined in Phenix[46] and Coot[47] to excellent R_{work}, and R_{free} (given the resolution) and to good stereochemistry as summarized in Table 4.

Enzymatic Assays. For the measurement enzymatic activity of the lipid kinases (PI4Ks), we used ADP-Glo kinase assay (Promega) with determination of ADP generated during the reaction as reported previously.[28] The activity of other protein and lipid kinases was determined by Carna Biosciences, Inc. by standardized protocols.

Antiviral Activity Screening. The screening of anti-HCV and anti-coxsackie activity was performed as reported previously.[28]

For the analysis of anti-rhinovirus activity of the selected compounds, HeLa cells were seeded in 96-well plates and incubated with different concentrations (50, 10, 5, 1, 0.5, 0.1, 0.05, 0.01 μM) of the indicated compound and infected (or mock infected) with rhinovirus 1A at a multiplicity of infection (MOI) of 0.1 pfu/cell. All experiments were done in quadruplicate. After 3 days of incubation at 33 °C, the virus-induced cytopathic effect (CPE) was determined by cell viability assay (MTT assay, Sigma-Aldrich). To determine CC_{50} values, the MTT value(s) measured for cells treated with the respective compounds were normalized using the MTT values obtained for untreated cells. The cytotoxic concentration 50% (CC_{50}) (that is, the concentration of a given compound that reduces the cell viability to 50%) was determined with the "Sigma Plot 8.0" software package (Systat Software GmbH, Germany) by plotting the percentages of cell viability (after 3 days of incubation at 33 °C) as a function of concentration of the compound in the cell culture medium. To calculate effective concentrations (EC_{50}) for specific compounds, the MTT values of virus-infected and treated cells were compared to those determined for treated cells (without virus infection). In other words, the calculation of EC_{50} values was based on the (additional) virus-specific cytopathology in infected cells in the presence of the respective compound (at the indicated concentration) in the cell culture medium.

Docking. The 3D structures of the docked molecules were built using ACD/ChemSketch 12.01,[48] and the geometry was optimized with MOPAC2016[49] using PM7 method. The necessary format conversions were performed using OpenBabel.[50] 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 1.1 using the default scoring function. Since the position of several important residues varies in the reported crystal structures, we decided to use flexible docking into the structure with **1** in order to simulate flexibility of the enzyme (pdb code 4D0L). Docking of the ligands into the binding pocket was performed in 30 × 28 × 28 Å^2 search space centered at 7.7, 334.1, and 10.1 Å and exhaustiveness 50.[38] We set Tyr385 and Lys549 as flexible residues that resulted in obtaining the most reliable results of the docking.

DOI: 10.1021/acs.jmedchem.6b01465
J. Med. Chem. 2017, 60, 100−118

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b01465.

Detailed SAR discussion, results of biochemical assays for remaining compounds and supplementary figures (PDF)
Molecular formula strings and some data (CSV)

Accession Codes

The atomic coordinates and structure factors have been deposited in the RCSB Protein Data Bank, www.pdb.org (accession codes 5FBL, 5FBQ, 5FBR, 5FBV, and 5FBW). Authors will release the atomic coordinates and experimental data upon article publication.

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Notes

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

■ ACKNOWLEDGMENTS

The work of I.M., D.Ch., P.P., M.D., and R.N. was supported by Czech Science Foundation (Registration No. 15-09310S). The work of D.C., A.B., and E.B. was supported by MarieCurie FP7-PEOPLE-2012-CIG project number 333916. The work was supported by Gilead Sciences, Inc. The project was also supported by the Academy of Sciences of the Czech Republic (RVO 61388963). The work of C.M. and J.Z. was supported by the German Center for Infection Research (DZIF, TTU Emerging Infections). We thank HZB for the allocation of synchrotron radiation beamtime. We are grateful to the MX 14.1 and MX 14.2 scientists for their help and support during data acquisition. We thank Lenka Klouckova for technical assistance during protein purification. We are also grateful to Michael Downey for critical reading of the manuscript.

■ ABBREVIATIONS

CCR2, CC chemokine receptor 2; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5; TLC, thin layer chromatography; PI4KB, phosphatidylinositol 4-kinase IIIβ; PI4KA, phosphatidylinositol 4-kinase IIIα; PI4K2A, phosphatidylinositol 4-kinase IIα; HCV, hepatitis C virus; HRV, human rhinovirus; CVB3, coxsackie 3B virus; ATP, adenosine triphosphate; ADP, adenosine diphosphate; EtOH, ethanol; DIPEA, diisopropylethylamine; MERS-CoV, Middle East respiratory syndrome coronavirus; SARS-CoV, severe acute respiratory syndrome coronavirus; ND, not determined

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