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Development of First Lead Structures for Phosphoinositide 3-Kinase-C2γ Inhibitors

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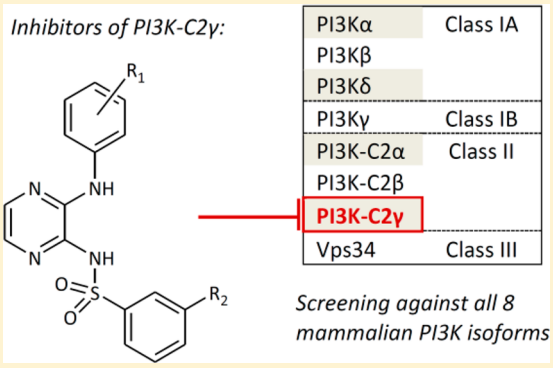
Supporting Information

ABSTRACT: The importance of complete elucidation of the biological functions of phosphoinositide 3-kinases (PI3K) was realized years ago. They generate 3-phosphoinositides, which are known to function as important second messengers in many inter- and intracellular signaling pathways. However, the functional role of class II PI3Ks is still unclear. Herein, we describe the synthesis of a panel of compounds that were tested against all eight mammalian PI3K-isoforms. We found inhibitors with some selectivity for class II PI3K-C2γ and also compounds with preferred inhibition of class II PI3K-C2β, providing structural leads to develop selective tool compounds.



INTRODUCTION

Phosphoinositide 3-kinases (PI3K) are lipid kinases phosphorylating phosphoinositides at the 3′-OH position of the inositol ring, giving rise to PtdIns(3)P, PtdIns(3,4)P_2, and PtdIns(3,4,5)P_3.[1] These lipids are known to function as second messengers and contribute to signaling pathways involved in cell growth, proliferation, survival, metabolism, cytoskeletal rearrangement, and vesicle trafficking.[2] There are eight different isoforms of these lipid kinases in mammals, which are divided into three classes depending on their sequence homology and substrate specifity.[3] Of these, class I has been the most commonly investigated.

Members of class I exist as heterodimers containing a catalytic and a regulatory subunit. Class I is further subdivided into class I_A (α-, β-, and δ-isoform) based on their catalytic subunits (p110α, -β and -δ) and class I_B (γ-isoform, p110γ). The catalytic subunits of class I_A PI3Ks form heterodimers with noncatalytic regulatory p85-subunits, and the sole class I_B isoform PI3Kγ associates with one of the two noncatalytic subunits p87 (also known as p84) or p101.[4] Their preferred substrate in vivo is PtdIns(4,5)P_2, generating the second messenger PtdIns(3,4,5)P_3 (also known as PIP_3).[3] Distribution of PI3Kα and -β is almost ubiquitous,[5] whereas PI3Kγ and -δ are mainly expressed in leukocytes.[5,6] Because PI3Ks are involved in tumorigenesis and inflammatory processes, their inhibition has been established as a target of study and small molecule inhibitors are already being investigated in clinical trials.[7]

The only class III member, Vps34 (vacuolar protein sorting), was originally discovered in *Saccharomyces cerevisiae*, but it is conserved from yeast and plants to mammals.[8] It exists as a constitutive heterodimer with Vps15, and the expression of this enzyme seems to be ubiquitous.[9] Its substrate in vitro and in vivo is PtdIns. The main functions of the kinase known to date all concern endosomal trafficking.

Class II is the least understood class of PI3Ks. This class consists of three isoforms named PI3K-C2α, -C2β, and -C2γ, which were discovered only by PCR and not by cellular function.[10−12] In contrast to the other PI3Ks, these kinases exist as monomers, with PI3K-C2α and -C2β being almost ubiquitously expressed[13] and PI3K-C2γ having a much more restrictive expression pattern. High levels have been found in liver, breast, prostate, and salivary glands.[9] They are able to phosphorylate PtdIns and PtdIns(4)P in vitro, but in vivo there is a strong preference for PtdIns.[2] In the past few years, researchers have gained some insight into the physiological roles of PI3K-C2α and -C2β, revealing their involvement in some disease-related processes. The two isoforms have responded to cytokines like EGF and PDGF[14] as well as

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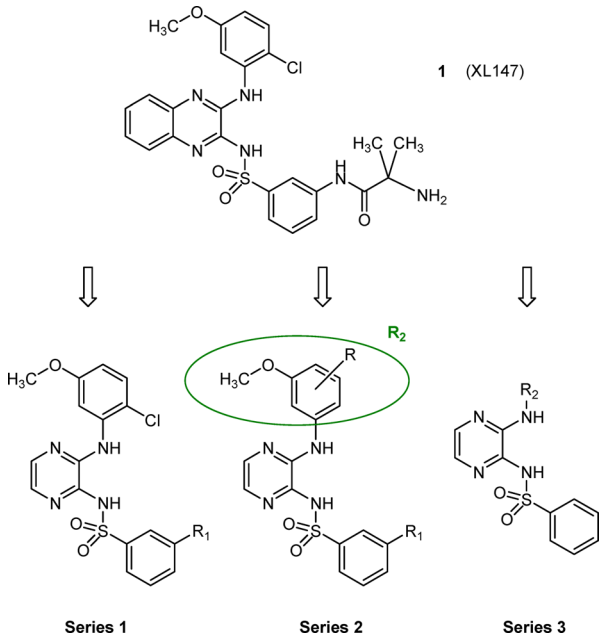
insulin,[15] integrin,[16] and chemokine MCP-1.[17] PI3K-C2β overexpression was recently shown to participate to increased PKB activity in cancer malignancies like acute myeloid leukemia (AML), glioblastoma multiforme, and small-cell lung cancer (SCLC).[18] Furthermore, a role for PI3K-C2γ in hepatitis C virus replication has been discovered,[19] showing that these kinases are potential drug targets. Even though the expression of PI3K-C2γ is restricted to only a few tissues, its biological role remains unclear. Besides genetic knockout-experiments, selective inhibitors have proved to be useful pharmacological tools for the investigation of the biological function of an enzyme. In contrast to class I PI3Ks, no isoform-specific inhibitors for class II PI3Ks have been reported.[7] The lack of crystal structures of these kinases represents a handicap in designing new potential inhibitors. Herein, we describe the synthesis of a panel of compounds and the results of an initial testing against all eight mammalian PI3Ks, revealing promising new approaches for PI3K research.

CHEMISTRY

Starting from a core structure of a known pan-class I-inhibitor (**1**, XL147, displayed IC_{50} values in the range of 23−383 nM for class I PI3Ks[20]), we pursued different strategies (Chart 1).

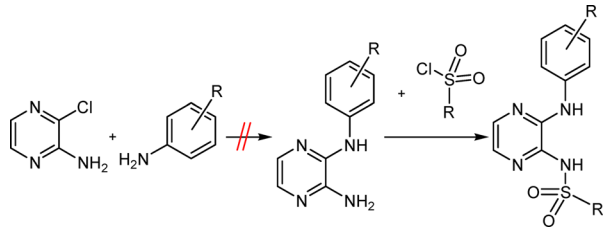
Chart 1. Three Series of Compounds Were Synthesized Derived from a Reduced Core-Structure of 1 (XL147)[21,22]



Docking studies with known crystal structures of class I PI3Ks showed no interaction of chinoxaline with the kinase, so the core was reduced to the pyrazine. We synthesized a series of compounds with high similarity to the lead compound by varying the substituent at the sulfonamide (series 1). Furthermore, we varied the substituents at the 3-methoxyaniline in different combinations with substituents at the benzenesulfonamide (series 2), and for series 3, we varied substituents at the pyrazin-2-ylamine.

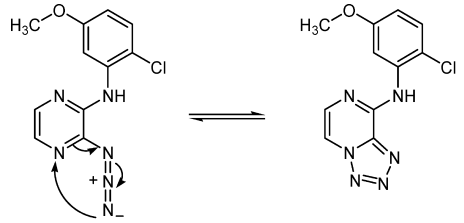
Because a direct two-step synthesis starting from 2-amino-3-chloro-pyrazine was not successful (Scheme 1), we developed a new synthetic strategy.

Scheme 1. Direct Two-Step Approach to Build the Desired New Core-Structure Was Not Successful



A general synthetic approach has been established beginning with commercially available 2,3-dichloropyrazine and the corresponding aniline derivatives. In Scheme 3, the established synthetic route is represented by the example of the synthesis of compound **2**. Because pyrazines are quite electron deficient, nucleophilic substitution of chlorine is possible under basic conditions. Here, 2-chloro-5-methoxyaniline was activated with the strong non-nucleophilic base Na-HMDS. Fortunately, no second substitution of **2a** was observed, probably because the resulting product was not sufficiently electron deficient for a second attack of the nucleophile. This result is in accordance with the failure of direct reaction of the aniline derivatives with 2-amino-3-chloro-pyrazine (Scheme 1). In the next step, the remaining chlorine has to be converted into an amine for the subsequent reaction with sulfonyl chloride derivatives. Any kind of direct substitution with an N-nucleophile failed, so we made a detour via the corresponding azides, which are known to be susceptible for reductions and form the corresponding amines. Compound **2a** was therefore heated with sodium azide in DMF to replace the chlorine by an azide function.[23] Interestingly, infrared spectroscopy showed no typical azide absorption and NMR spectroscopy confirmed the formation of a tetrazolopyrazine **2b** (Scheme 2). Azidopyrazines isomerize to ring-closed tetrazolopyrazines depending on substituents, solvent, and temperature.[24]

Scheme 2. Azidopyrazines Exist at Equilibrium with the Isomeric Ring-Closed Tetrazolopyrazines



Tetrazolopyrazines are very stable, so subsequent reduction needed more rigorous conditions than expected. We finally succeeded in formation of **2c** by refluxing the reaction mixture with $Sn(II)Cl_2$ in concentrated HCl. These conditions were used for all chloro-substituted tetrazolopyrazines because catalytic reduction with Pd/C and H_2 caused the cleavage of the chlorine as side reaction. For all tetrazolopyrazines not comprising an aromatic chloro-substituent, the catalytical reduction with palladium on activated carbon with H_2 at elevated temperature was the method of choice.

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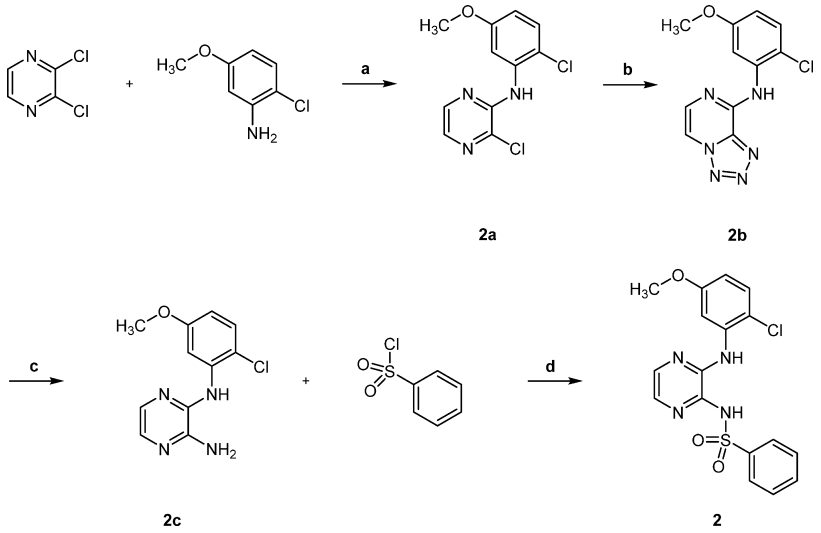
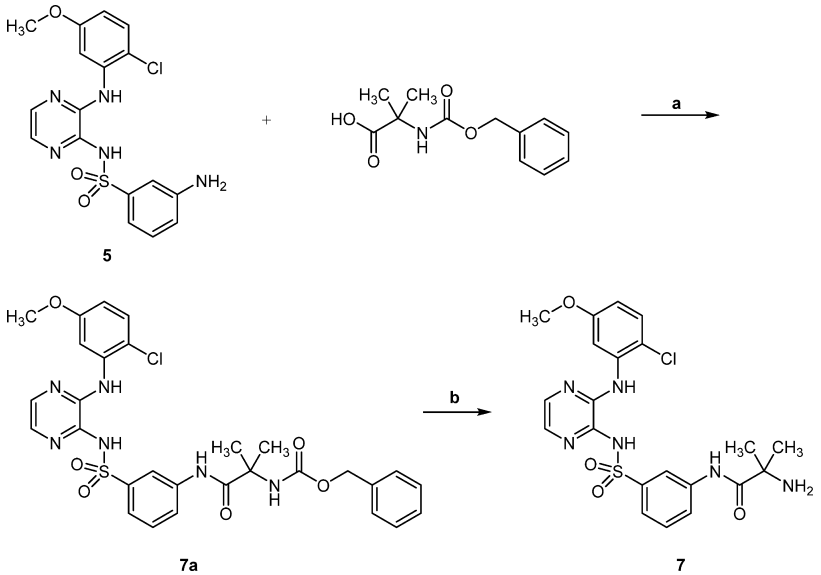
Scheme 3. Synthesis of Compound 2 as an Example of the Established Synthetic Route[a]



[a]Reactands and conditions: (a) Na-HMDS, THF, 0 °C → room temperature; (b) NaN_3, DMF, 100 °C; (c) Sn(II)Cl_2 in concd HCl, reflux; (d) pyridine, room temperature.

Scheme 4. Amidation of Compound 5 with *N*-Carbobenzyloxy-2-methylalanine with Subsequent Deprotection Led to Compound 7[a]



[a]Reactands and conditions: (a) DCC in dry DCM, room temperature; (b) Pd/C, H_2 in ethyl acetate, room temperature, 3 h.

Subsequently, construction of the sulfonamide functionality could be performed by stirring **2c** with the desired sulfonyl chloride in pyridine at room temperature.

The benzenesulfonamides with an amino- or an acetamide substituent in *m*-position (**5**, **11**, **16**, **21**, and **26** or **6**, **12**, **17**, **22**, and **27**, respectively) were prepared by reducing the corresponding nitro-substituted compounds (**4**, **10**, **15**, **20**, and **25**) and acetylating them with acetic anhydride.

Compound **7** was prepared by amidation of compound **5**. Therefore, the amino-function of 2-methylalanine was protected and subsequently the carboxylic acid had to be activated. Amidation with *N*-Boc-protected 2-methylalanine could not be achieved, neither with DCC nor with CDI or as acid chloride. So we used *N*-carbobenzyloxy-2-methylalanine instead, and amidation was successful after activation with DCC. Deprotection could be achieved by hydrogenation with Pd/C and H_2 (Scheme 4).

Methyl 3-(chlorosulfonyl)benzoate was needed as precursor for methyl carboxylate compounds **8**, **13**, **18**, **23**, and **28** and carboxylic acid compounds **9**, **14**, **19**, **24**, and **29**. Therefore, 3-(chlorosulfonyl)benzoic acid was activated with oxalyl chloride to increase electrophilicity of the carbonyl compared to that of sulfonyl chloride. After a short reaction time with methanol of 15 min the reaction was quenched, so selective esterification of the carboxylic function was achieved without formation of methyl sulfonate. The corresponding carboxylic acids were subsequently prepared by alkaline hydrolysis.

Preparation of compound **30** was achieved by demethylation of compound **2** with BBr_3 in a nearly quantitative manner.

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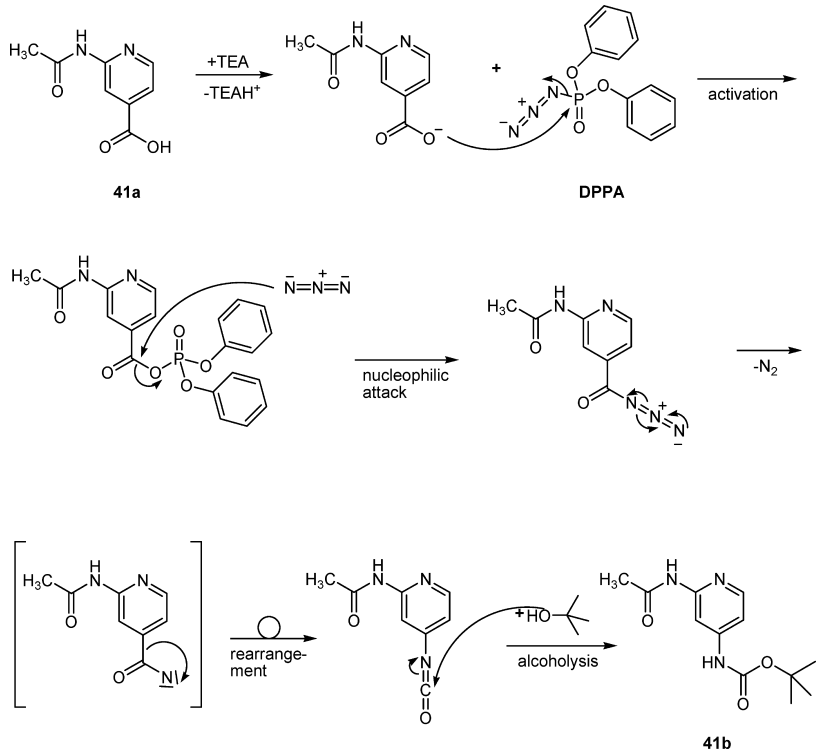
Scheme 5. Curtius Rearrangement and Alcoholysis of 41a Leads to *N*-Boc Protected Compound 41b[a25–27]



[a]Reactands and conditions: triethylamine (TEA) and diphenylphosphoryl azide (DPPA) in toluene, 75 °C, 1 h, *tert*-butanol, reflux, 2 h.

Some aniline derivatives were not commercially available. The precursor of compounds **36** and **37** was prepared by protecting 3-nitroaniline with di-*tert*-butyldicarbonate and subsequent reduction of the nitro functionality. With this, intermediate synthesis was carried out as described in Scheme 3. Cleavage of the *N*-Boc protection group succeeded quantitatively using trifluoroacetic acid.

The precursor of compounds **41** and **42** was synthesized starting with 2-amino-isonicotinic acid. First, the amino function was protected by acetylation with acetic anhydride. Then the carboxylic function was converted to a Boc-protected amino function by Curtius rearrangement (Scheme 5): The acid was deprotonated with triethylamine and activated with diphenylphosphoryl azide. By nucleophilic attack of the azide anion, a carbonyl azide was formed, which separates N_2 at elevated temperatures and rearranges to an isocyanate.

Alcoholysis of isocyanates with *tert*-butanol leads to *N*-Boc-protected amines which can be easily purificated by silica flash chromatography. The following cleavage of the protection group was achieved quantitatively by using trifluoroacetic acid. Compounds **41** and **42** were subsequently prepared according to Scheme 3.

RESULTS AND DISCUSSION

Selectivity Screening. The compounds were screened in a nonradiometric ADP Glo Assay against eight PI3K isoforms by singlicate measurement at the concentration of 10 μM to obtain preliminary information about potency and selectivity. Assay conditions and performance of the screening are specified in the Supporting Information.

Results of the three series of compounds are shown in Tables 1, 2, and 3.

None of the shown compounds inhibited class III PI3K Vps34 or class II PI3K-C2α. Only a few compounds showed slight effects on the activity of class I_A PI3K associated with the noncatalytic p85 subunits and monomeric class I_B PI3Kγ.

As mentioned before, class I_B PI3Kγ exists as one of the two heterodimers p101/p110γ or p87/p110γ in vivo. Recent results indicated that these dimers showed unequal conformational alterations of several regions within the catalytic p110γ subunit and/or lipids.[28] On the basis of this background, we also tested the compounds against the activity of the physiologically relevant heterodimeric PI3Kγ enzymes. With this approach, we identified several other compounds with inhibitory effects against the kinase as compared to the data obtained from the monomeric enzyme. Nevertheless, the inhibitory effects were not impressive as compared to inhibition of other isoforms. The results of these tests are available in the Supporting Information.

Interestingly, the compounds with the new scaffold preferentially inhibit the nearly unexplored isoforms PI3K-C2γ and to some extent PI3K-C2β.

With series 1 candidates, we examined the influence of substitution at the benzenesulfonamide (Table 1). Surprisingly, the closest derivative to **1** (XL147), compound **7**, showed no inhibition of class I PI3Ks. Rather, **7** slightly inhibited PI3K-C2γ. Because there is no data available for selectivity of XL147 beyond class I PI3Ks, we do not know whether this reduced scaffold also improves inhibition of C2γ. Compounds **2** (R_1 = −H) and **6** (R_1 = −acetamide) showed an inhibition similar to **7**, so the presence of a free amine in the side chain appears not to favor further interactions with the kinase. Notably, the best inhibition was achieved by the compounds with amine- (**5**) and carboxylic acid residue (**9**). These compounds are quite different in nature concerning their interaction; perhaps the

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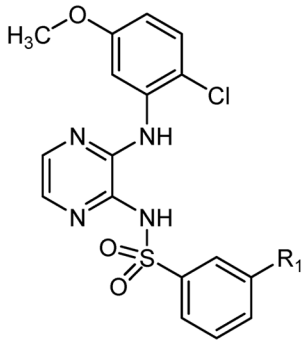
Table 1. Series 1, Varying Substituents at the *m*-Position of the Benzensulfonamide[a]



Series 1

	Class	I_A			I_B	II			III
cpd	R_1	p110α/ p85α	p110β/ p85α	p110δ/ p85α	p110γ	PI3K-C2α	PI3K-C2β	PI3K-C2γ	Vps34
2	-H	96	86	82	100	93	68	57	93
3	-Cl	83	80	83	93	88	81	83	85
4	$-NO_2$	93	92	88	114	92	97	70	97
5	$-NH_2$	76	97	77	115	94	82	34	99
6	-acetamide	87	84	75	234	91	84	46	92
7	-2-methylalanine-amide	85	87	86	80	90	84	56	91
8	-methyl carboxylate	103	88	92	97	90	84	64	94
9	-carboxylic acid	65	154	68	343	90	97	26	88

[a]Residual activity of class II PI3K-C2γ and -C2β is given in %; 25−45% is indicated in yellow, 45−70% in red. Testing was performed at a concentration of 10 μM by singlicate measurement.

residue is exposed to the solvent and hence improves inhibition potency to some extent. These two compounds also showed some slight effects on class I_A PI3Ks α and δ.

In series 2, several substituents at the benzenesulfonamide were combined with different 3-methoxyphenyl-substituents at the pyrazine-2-ylamine. The results of inhibition studies at 10 μM (Table 2) showed a significant difference in inhibition potency against PI3K-C2γ between the distinct 3-methoxyphenyl-derivatives. The mean inhibition of the 3-methoxyphenyl-compounds (**10**−**14**) of series 2 and the 2-chloro-5-methoxyphenyl-derivatives of series 1 were comparable, while 5-methoxy-2-methylphenyl-substituted compounds (**20**−**24**) showed no inhibition in any of the tested kinases. This indicates that the substituents in the *o*-position strongly affected binding to the kinase, probably due to the influence on rotation of the residue. Interestingly, 3,5-dimethoxyphenyl-substitutents (**25**−**29**) were the most potent inhibitors of PI3K-C2γ in this series; probably a further interaction can be formed. Compounds **27** and **28** also inhibit class I_A PI3Ks to some extent, but compounds **25** and **26** seem to inhibit PI3K-C2γ in a selective way.

Methyl carboxylate substitution at the benzenesulfonamide was not tolerated, suggesting that binding of these compounds is sterically hindered. Here also no concrete correlation of the properties of substituents and inhibition effect can be seen; residues at pyrazine-2-ylamine seemed to have a greater effect on potency of the inhibitors.

We therefore prepared a panel of compounds with broader variation at pyrazine-2-ylamine (series 3), and the residual kinase activity after treatment with 10 μM of the inhibitors is shown in Table 3.

Here, the importance of an H-bond-acceptor in the appropriate position is evident. All compounds lacking an H-bond-acceptor showed no inhibition of any tested kinase. To our surprise, demethylation of **2** greatly improved inhibition of C2β, while inhibition of C2γ remained unaffected. The compound had no effects on any other isoform. Compounds **38** and **40** also showed a preference for C2β over C2γ. Compound **38** does not affect any other kinase, while compound **40** slightly inhibits class I_A PI3Kβ. Compound **41** contained a typical hinge-binder motif, and indeed it was the most nonselective compound in our screening, as it displayed equal potency against C2β and C2γ and furthermore inhibited class I_B PI3Kγ (displaying remaining kinase activity of approximately 40%).

Eleven compounds were further tested against a panel of 60 protein kinases. Only **30**, **38**, and **40** showed slight inhibition of p38α, but no additional protein kinases were inhibited. Complete testing results are available in the Supporting Information.

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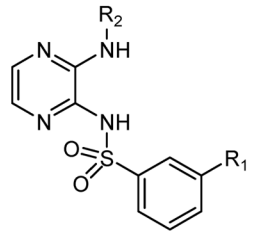
Table 2. Testing Results of Series 2, Combination of Residues at Position 2 of Pyrazine (R_2) and Benzenesulfonamide (R_1)[a]



Series 2

R_2		
3-methoxyphenyl-	cpd 10-14	
2,5-dimethoxyphenyl-	cpd 15-19	
5-methoxy-2-methylphenyl-	cpd 20-24	
3,5-dimethoxyphenyl-	cpd 25-29	

	Class	I_A			I_B	II			III
cpd	R_1	p110α/p85α	p110β/p85α	p110δ/p85α	p110γ	PI3K-C2α	PI3K-C2β	PI3K-C2γ	Vps34
10	-NO_2	89	125	79	86	100	76	42	94
11	-NH_2	86	140	81	107	92	86	47	91
12	-acetamide	92	90	91	158	93	95	60	108
13	-methyl carboxylate	94	88	81	99	93	79	67	96
14	-COOH	86	82	84	90	91	89	68	94
15	-NO_2	92	73	90	81	94	91	90	92
16	-NH_2	74	82	60	157	89	66	17	89
17	-acetamide	84	102	74	121	86	79	29	98
18	-methyl carboxylate	83	77	89	88	93	74	67	82
19	-COOH	82	104	83	78	98	85	29	98
20	-NO_2	94	95	99	121	104	87	94	100
21	-NH_2	96	89	84	164	93	90	99	93
22	-acetamide	100	93	84	101	91	93	89	93
23	-methyl carboxylate	92	78	89	108	94	87	96	92
24	-COOH	101	89	95	135	92	99	89	91
25	-NO_2	83	506	78	594	92	86	23	92
26	-NH_2	89	88	87	97	90	83	41	96
27	-acetamide	69	74	56	78	88	71	11	93
28	-methyl carboxylate	72	70	64	111	86	76	14	78
29	-COOH	93	85	89	104	96	95	86	94

[a]Residual activity of the kinases up to 25% is marked in green, 25−45% in yellow, 45−70% in red. Testing was performed at an inhibitor concentration of 10 μM by singlicate measurement.

Dose-Dependent Inhibition of PI3K-C2β and -C2γ. Four compounds of this first set were additionally tested as dose-dependent on the inhibition of the two isoforms PI3K-C2β and -C2γ. The IC_{50} values were determined in a nonradiometric ADP Glo Assay at 10 concentrations from 100 μM to 3 nM by singlicate measurements (Table 4). Assay conditions and performance of the screening as well as the raw data are specified in the Supporting Information.

Compounds **17** and **19** showed more than 10-fold selectivity for PI3K-C2γ over -C2β (Table 4). The IC_{50} values against PI3K-C2γ are in the range of 3.5 μM, whereas PI3K-C2β was inhibited with an IC_{50} of 44.8 or 61.2 μM, respectively. The inhibition profile of compound **26** revealed an IC_{50} of 0.34 μM against PI3K-C2γ with about 80-fold selectivity over PI3K-C2β (IC_{50} 28.8 μM, Scheme 6).

Compound **30** showed preferred inhibition the PI3K-C2β isoform in the selectivity screening. Indeed determination of the IC_{50} confirmed 10-fold selectivity over PI3K-C2γ (IC_{50} of 2.71 or 23.2 μM, respectively).

CONCLUSION

We established a general synthetic route to obtain a panel of compounds. Three series of compounds were synthesized and screened against all eight mammalian isoforms of PI3Ks. We found several compounds which show 60−80% inhibition of PI3K-C2γ activity at 10 μM, whereas they did not affect any of the other tested protein kinases. The most promising compound **26** showed an IC_{50} of 0.34 μM with over 80-fold selectivity over PI3K-C2β.

Furthermore, we found three compounds with preferred inhibition for PI3K-C2β (**30**, **38**, and **40**). Here, 10-fold

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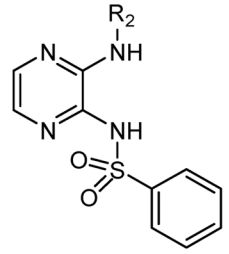
Table 3. Testing Results of Compounds of Series 3, Varying Substituents at Position 2 of the Pyrazine[a]



Series 3

	Class	I_A			I_B	II			III
cpd	R_2	p110α/ p85α	p110β/ p85α	p110δ/ p85α	p110γ	PI3K-C2α	PI3K-C2β	PI3K-C2γ	Vps34
30	2-chloro-5-hydroxyphenyl-	84	80	81	90	90	19	63	96
31	phenyl-	94	74	84	97	91	93	94	96
32	2,5-dichlorophenyl-	92	93	91	98	94	78	90	93
33	3-chlorophenyl-	94	98	87	108	98	94	96	101
34	2-chlorophenyl-	97	85	89	100	91	97	93	99
35	4-chlorophenyl-	87	118	95	288	96	95	124	109
36	3-aminophenyl-	83	82	84	115	85	79	81	82
37	-phenyl-3-acetamide	91	75	84	97	88	86	89	93
38	pyridin-3-yl-	97	106	90	111	98	49	97	95
39	pyridin-4-yl-	93	193	89	107	94	84	89	93
40	2-chloro-pyridin-4-yl-	87	65	83	90	87	27	56	98
41	-pyridin-4-yl-2-acetamide	71	79	62	42	80	19	18	114
42	2-amino-pyridin-4-yl-	93	147	93	102	87	82	80	93

[a]Residual activity of the kinases up to 25% is marked in green, 25−45% in yellow, and 45−70% in red. Testing was performed at an inhibitor concentration of 10 μM by singlicate measurement.

selectivity of PI3K-C2β over -C2γ could be confirmed with IC_{50} values of 2.71 and 23.2 μM, respectively. Because these three compounds also showed side effects on a protein kinase (p38α), we can assume that the binding pockets of these two different types of kinases show similarities.

No crystal structure of class II PI3Ks is available to date, so interactions of the compounds with the kinase can only be assumed. Reducing the scaffold from quinoxaline to pyrazine reduced potency of class I inhibitors. Polar substituents at the *m*-position of the benzenesulfonamide moiety slightly improved inhibition potency for PI3K-C2γ, but no direct interaction seems to be formed. An appropriate H-bond acceptor at the phenyl residue at pyrazine-2-ylamine is essential for inhibition. For PI3K-C2γ selectivity, the 3-methoxyphenyl substitution is crucial, and substituents in position 2 of the phenylamino-pyrazin-2-yl-residue have an influence on inhibitor potency, probably due to rotation effects.

Even with limited test data available, the results may provide a lead to the development of the first selective compounds for further investigation of the biological roles of class II PI3K-C2γ.

EXPERIMENTAL SECTION

All reagents and (anhydrous) solvents are commercially available and were used without further purification. Purification was performed on normal phase silica using Davisil LC60A 20−45 μm silica from Grace for the column, and separation was conducted by an automated flash chromatography system (LaFlash). ^1H spectra were obtained with Bruker Avance 200 at 200 MHz or with Bruker 400 Avance at 400 MHz, respectively. The spectra were obtained in the indicated solvent and calibrated against the residual proton peak of the deuterated solvent. Chemical shifts (δ) are reported in parts per million relative to

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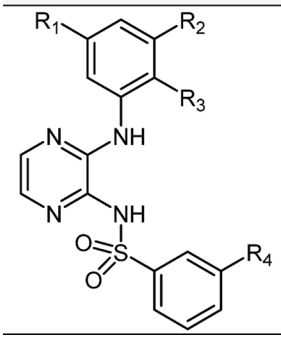
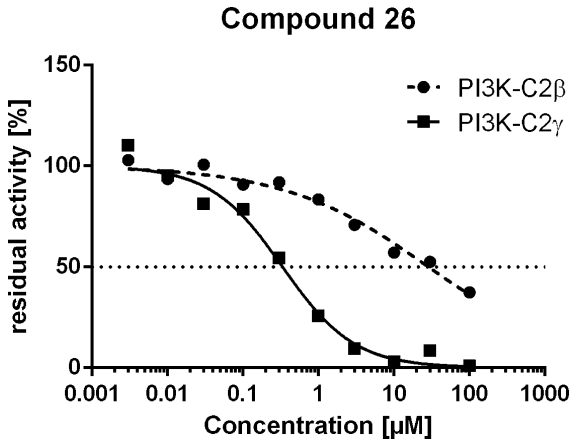
Table 4. IC_{50} Values of Four Compounds against PI3K-C2β and -C2γ[a]

	cpd	R_1	R_2	R_3	R_4	PI3K-C2β	PI3K-C2γ
						IC_{50} [μM]	
	17	$-OCH_3$	-H	$-OCH_3$	-acetamide	44.8	3.41
	19	$-OCH_3$	-H	$-OCH_3$	-COOH	61.2	3.61
	26	$-OCH_3$	$-OCH_3$	-H	$-NH_2$	28.8	0.34
	30	-OH	-H	-Cl	-H	2.71	23.2

[a]Inhibitions curves were determined at 10 concentrations in semilog steps from 100 μM to 3 nM by singlicate measurement. IC_{50} values up to 5 μM are marked in green, from 5 to 25 μM in yellow, and from 25 to 50 μM in red.

Scheme 6. Dose-Dependent Inhibition Curves of Compound 26



tetramethylsilane. GC-MS analyses were carried out on a Hewlett-Packard HP 6890 series GC-system equipped with a HP-5MS capillary column (0.25 μm film thickness, 30 m × 250 μm) and a HP 5973 mass selective detector (electron ionization). Helium was used as carrier gas. EI-MS data were obtained using a Finnigan Sector Field mass spectrometer. FAB-MS, Finnigan MAT TSQ 70; matrix, 3-nitrobenzyl alcohol.

Purity of the tested compounds was confirmed by reverse phase HPLC on Merck Hitachi. Separation was conducted with a Lichrospher RP18 column. Detection: Merck Hitachi L-4250 UV–vis detector at 254 nm. The method used had a flow of 1 mL/min and a gradient of 0.01 M KH_2PO_4, pH 2.3, and methanol from 60/40 to 15/85. All shown compounds have a purity >95% determined by HPLC.

For detailed syntheses and characterization of all shown compounds and their precursors see Supporting Information.

3-Chloro-*N*-(2-chloro-5-methoxyphenyl)pyrazin-2-amine (intermediate 2a). 2-Chloro-5-methoxyaniline (1.2 equiv) was dissolved in dry THF under argon and cooled to 0 °C. Then 1.2 equiv Na-HMDS was added dropwise and the reaction mixture was stirred for 1 h. After addition of 2,3-dichloropyrazine (1 equiv) in dry THF, the solution was allowed to warm to room temperature and stirred for 2 h. The mixture was quenched with a saturated solution of ammonium chloride, and the product was extracted with ethyl acetate. The combined organic layers were dried over Na_2SO_4, and solvent was evaporated. Purification of the desired compound was achieved by flash chromatography using ethyl acetate/petroleum ether 5/95 as eluent, affording 64% of the desired compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.77 (s, 3H), 6.81 (m, 1H), 7.43 (m, 1H), 7.44 (s, 1H), 7.50 (s, 1H), 7.84 (s, 1H), 8.47 (s, 1H). GC-MS (m/z): 269 [M^+].

***N*-(2-Chloro-5-methoxyphenyl-)tetrazolo[1,5-*a*]pyrazin-8-amine (intermediate 2b).** Intermediate **2a** (1 equiv) and sodium azide (2.5 equiv) were dissolved in DMF and heated to 100 °C overnight. After cooling to room temperature, the reaction mixture was poured into ice–water, resulting in precipitation of the product, which could be filtered and dried. Without further purification, 93% of compound **2b** were obtained. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.78 (s, 3H), 6.91 (d, 1H, J = 8.8 Hz), 7.44 (s, 1H), 7.48 (d, 1H, J = 8.8 Hz), 7.76 (d, 1H), 8.69 (d, 1H), 10.05 (s, 1H). EI-MS (m/z): 276 [M^+].

***N*-(2-Chloro-5-methoxyphenyl-)pyrazin-2,3-diamine (intermediate 2c).** Intermediate **2b** (1 equiv) was dissolved in concentrated HCl and refluxed after addition of 10 equiv $Sn(II)Cl_2$ for 3 h. The mixture had to be cooled to room temperature, diluted with water, and then neutralized with K_2CO_3. Extraction with DCM (until there was no further product extracted) and evaporation of the solvent yielded 87% of compound **2c** with sufficient purity. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.72 (s, 3H), 4.51 (s, 2H), 6.43 (d, 1H, J = 8.4 Hz), 6.65 (s, 1H), 7.19 (d, 1H, J = 8.4 Hz), 7.58 (s, 1H), 7.62 (s, 1H), 7.69 (s, 1H). GC-MS (m/z): 250 [M^+].

***N*-{3-[(2-Chloro-5-methoxyphenyl)amino]pyrazin-2-yl}-benzenesulfonamide (2).** Intermediate **2c** (1 equiv) and benzenesulfonyl chloride (3 equiv) were dissolved in dry pyridine, and the reaction mixture was stirred overnight at room temperature. The reaction was quenched by adding water and acidifying the solution with HCl. The product was extracted with ethyl acetate and dried over Na_2SO_4, and the solvent was evaporated. Purification of the desired compound was achieved by flash chromatography using DCM/methanol 95/5 as eluent, affording 65% of the desired compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.75 (s, 3H), 6.67 (d, 1H, J = 8.8 Hz), 7.20 (s, 1H), 7.40 (d, 1H, J = 8.8 Hz), 7.47 (s, 1H), 7.61 (m, 3H), 7.98 (d, 2H), 8.22 (s, 1H), 8.87 (s, 1H), 12.63 (bs, 1H). FT-ICR-MSFT-ICR-MS (m/z): 413 [M + Na]$^+$.

***N*-{3-[(2-Chloro-5-methoxyphenyl)amino]pyrazin-2-yl}-3-nitrobenzenesulfonamide (4).** Intermediate **2c** (1 equiv) and 3-nitrobenzenesulfonyl chloride (3 equiv) were dissolved in dry pyridine, and the reaction mixture was stirred overnight at room temperature. The reaction was quenched by adding water and acidifying the solution with HCl. The product was extracted with ethyl acetate and dried over Na_2SO_4, and the solvent was evaporated. Purification of the desired compound was achieved by flash chromatography using DCM as eluent, affording 57% of the desired compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 3.76 (s, 3H), 6.69 (dd, 1H, J = 8.8 Hz), 7.22 (s, 1H), 7.42 (d, 1H, J = 8.8 Hz), 7.52 (s, 1H), 7.89 (t, 1H, J = 8.0 Hz), 8.20 (s, 1H), 8.39 (d, 1H, J = 8.0 Hz), 8.46 (d, 1H, J = 8.0 Hz), 8.70 (s, 1H), 8.86 (s, 1H), 12.84 (bs, 1H). FAB-MS (m/z): 434 [M − H]$^-$.

3-Amino-*N*-{3-[(2-chloro-5-methoxyphenyl)amino]pyrazin-2-yl}benzenesulfonamide (5). At room temperature, compound **4**

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was dissolved in ethyl acetate, then Pd/C (10% (m/m)) was added and H_2 was led through the reaction mixture. After 3 h of stirring, Pd/C was filtered and the solvent was removed under reduced pressure. The crude product was purified by silica flash chromatography using ethyl acetate/*n*-hexane as eluent, affording 47% of the desired compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.82 (s, 3H), 6.56 (dd, 1H), 6.84 (m, 2H), 7.30 (m, 5H), 8.38 (d, 1H), 8.96 (s, 1H); $-NHSO_2-$ was not detected. FAB-MS (*m*/*z*): 404 $[M - H]^-$.

***N*-{3-[({3-[(2-Chloro-5-methoxyphenyl)amino]pyrazin-2-yl}amino)sulfonyl]phenyl}acetamide (6).** Compound **5** (1 equiv) was dissolved in water with an excess of acetic anhydride (5000 equiv) and refluxed for 1 h. Subsequently, the reaction mixture was brought to pH 5 with K_2CO_3 and extracted with ethyl acetate. The organic layers were combined and dried over Na_2SO_4, and the solvent was evaporated under reduced pressure. Remaining acetic acid was codestilled with ethyl acetate to yield 100% of the desired compound with sufficient purity. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 2.04 (s, 3H), 3.74 (s, 3H), 6.66 (dd, 1H), 7.20 (m, 1H), 7.43 (m, 3H), 7.62 (d, 1H, *J* = 7.9 Hz), 7.73 (d, 1H, *J* = 7.9 Hz), 8.24 (m, 2H), 8.85 (s, 1H), 10.21 (s, 1H), 12.55 (bs, 1H). FAB-MS (*m*/*z*): 446 $[M - H]^-$.

Cbz-*N*^{1}-{3-[({3-[(2-chloro-5-methoxyphenyl)amino]pyrazin-2-yl}amino)sulfonyl]phenyl}-2-methylalaninamide (7a). Compound **5** (1 equiv) and *N*-carbobenzyloxy-2-methylalanine (1.5 equiv) were dissolved in dry DCM, and dicyclohexylcarbodiimide (DCC) in DCM (1.5 equiv) was added dropwise. The solution was stirred at room temperature, and further *N*-Carbobenzyloxy-2-methylalanine and DCC were added until all of compound **5** was converted (HPLC). The resulting precipitate was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by silica flash chromatography using ethyl acetate/petroleum ether with a gradient of 35/65 for 1 h, then 65/35 as eluent, affording 37% of the desired compound. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 1.41 (s, 6H), 3.75 (s, 3H), 5.00 (s, 2H), 6.65 (d, 1H), 7.34 (m, 10H), 7.64 (d, 1H, *J* = 7.4 Hz), 7.87 (d, 1H, *J* = 7.4 Hz), 8.29 (m, 2H), 8.91 (s, 1H), 9.83 (s, 1H), 12.67 (bs, 1H). FAB-MS (*m*/*z*): 255 $[M - H]^-$.

N^1-{3-[({3-[(2-Chloro-5-methoxyphenyl)amino]pyrazin-2-yl}amino)sulfonyl]phenyl}-2-methylalaninamide (7). At room temperature, compound **7a** was dissolved in ethyl acetate, then Pd/C (10% (m/m)) was added and H_2 was led through the reaction mixture. After 3 h of stirring, Pd/C was filtered and the solvent was removed under reduced pressure. The crude product was purified by silica flash chromatography using first ethyl acetate/*n*-hexane 70/30 as eluent then ethyl acetate/methanol 90/10 to afford 60% of the desired compound. ^{1}H NMR (200 MHz, MeOD-d_3): δ = 1.70 (s, 6H), 3.63 (s, 3H), 6.61 (d, 1H, *J* = 8.4 Hz), 7.08 (s, 1H), 7.28 (d, 1H, *J* = 8.4 Hz), 7.46 (s, 1H), 7.72 (m, 4H), 8.02 (d, 1H, *J* = 7.2 Hz), 8.10 (s, 1H), 8.37 (s, 1H); $-NHSO_2-$ and $-NH_2$ were not detected. EI-MS (*m*/*z*): 490 $[M^+]$.

Methyl 3-Chlorosulfonylbenzoate. Under argon, 3-chlorosulfonylbenzoic acid (1 equiv) was dissolved in dry DCM, 3 drops of DMF were added, and then oxalyl chloride (1.5 equiv) was added dropwise under vigorous stirring. The reaction was stirred at room temperature for 1.5 h, then dry methanol (5 equiv) was added. After 15 min, the solvent was removed under reduced pressure and the residue was purified immediately by silica flash chromatography using ethyl acetate/petroleum ether 20/80 as eluent. We obtained 58% of the desired compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.99 (s, 3H), 7.74 (t, 1H, *J* = 7.8 Hz), 8.21 (d, 1H, *J* = 7.8 Hz), 8.40 (d, 1H, *J* = 7.8 Hz), 8.68 (s, 1H).

Methyl 3-[({3-[(2-Chloro-5-methoxyphenyl)amino]pyrazin-2-yl}amino)sulfonyl]benzoate (8). Compound **2c** (1 equiv) and methyl 3-chlorosulfonylbenzoate (3 equiv) were dissolved in dry pyridine, and the reaction mixture was stirred overnight at room temperature. The reaction was quenched by adding water and acidifying the solution with HCl. The product was extracted with ethyl acetate and dried over Na_2SO_4, and the solvent was evaporated. The crude product was purified by silica flash chromatography using ethyl acetate/*n*-hexane 35/65 as eluent, affording 38% of the desired compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.82 (s, 3H), 3.95 (s, 3H), 6.56 (dd, 1H, *J* = 8.8 Hz), 6.93 (d, 1H, *J* = 4.2 Hz), 7.27 (d, 1H, *J* = 8.8 Hz), 7.40 (d, 1H, *J* = 4.2 Hz), 7.61 (t, 1H), 8.22 (m, 2H), 8.38 (m, 1H), 8.69 (m, 1H), 8.93 (s, 1H); $-NHSO_2-$ was not detected. FAB-MS (*m*/*z*): 447 $[M - H]^-$.

3-[({3-[(2-Chloro-5-methoxyphenyl)amino]pyrazin-2-yl}amino)sulfonyl]benzoic Acid (9). Compound **8** was dissolved in 1,4-dioxane, then a 2 M solution of sodiumhydroxide in water (10 equiv of NaOH) was added and the solution was stirred at room temperature for 2.5 h. The reaction was quenched by adding water and acidified with 2 M HCl. The product was extracted with ethyl acetate and the organic layers were dried over Na_2SO_4, and the solvent was removed under reduced pressure. The desired product was obtained to 100% in sufficient purity. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 3.74 (s, 3H), 6.66 (dd, 1H, *J* = 8.9 Hz), 7.20 (d, 1H, *J* = 3.6 Hz), 7.39 (d, 1H, *J* = 8.9 Hz), 7.48 (d, 1H, *J* = 3.6 Hz), 7.71 (t, 1H, *J* = 7.8 Hz), 8.17 (m, 3H), 8.48 (m, 1H), 8.84 (s, 1H), 12.74 (bs, 1H), 13.43 (bs, 1H). FAB-MS (*m*/*z*): 433 $[M - H]^-$.

2-(Acetylamino)isonicotinic Acid (Intermediate 41a). 4-Aminoisonicotinic acid was suspended in acetic anhydride, a few drops of sulfuric acid were added, and the reaction mixture was heated overnight at 90 °C. Acetic acid and excess acetic anhydride were evaporated under reduced pressure as completely as possible, water was added to the residue, and the mixture was stirred and cooled for 15 min. The desired product could be filtered and dried to afford 60% of sufficient purity. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 2.11 (s, 3H), 7.49 (dd, 1H, *J* = 5.0 Hz), 8.45 (d, 1H, *J* = 5.0 Hz), 8.55 (s, 1H), 10.71 (s, 1H). FAB-MS (*m*/*z*): 179 $[M + H]^+$.

***tert*-Butyl-2-(acetylamino)pyridin-4-ylcarbamate (Intermediate 41b).** At room temperature under argon, intermediate **41a** (1 equiv) was suspended in dry toluene. Diphenylphosphorylazide (1 equiv) and triethylamine (1.2 equiv) were added dropwise, and then the reaction mixture was warmed to 75 °C for 1 h. Afterward, an excess of dried *tert*-butanol (ca. 7 equiv) was added and the mixture was refluxed for 3 h. Then water was added and brought to pH 8−9 with a 10% solution of sodium hydroxide in water. The layers were separated, and the water was extracted 3 times with ethyl acetate. The organic layers were combined and dried over Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by silica flash chromatography using ethyl acetate/petroleum ether 67/33 as eluent to yield 62% of the desired compound. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.51 (s, 9H), 2.23 (s, 3H), 7.71 (d, 1H), 8.13 (m, 2H), 8.66 (s, 1H), 10.15 (s, 1H). GC-MS (*m*/*z*): 251 $[M^+]$.

***N*-(4-Aminopyridin-2-yl)acetamide (Intermediate 41c).** At room temperature, intermediate **41b** (1 equiv) was dissolved in 1,2-dichloroethane, trifluoroacetic acid (3 equiv) was added dropwise, and the mixture was refluxed for 48 h. After reducing the volume of the reaction mixture, the precipitate could be filtered. The precipitate was solved in water and basified with K_2CO_3. The water was extracted 3 times with ethyl acetate, the organic layers were combined and dried over Na_2SO_4, and the solvent was removed under reduced pressure to yield 82% of the desired compound with sufficient purity. ^{1}H NMR (200 MHz, DMSO-d_6): δ = 2.00 (s, 3H), 6.01 (s, 2H), 6.18 (dd, 1H, *J* = 5.6 Hz), 7.29 (m, 1H), 7.69 (d, 1H, *J* = 5.6 Hz), 9.93 (s, 1H). FAB-MS (*m*/*z*): 152 $[M + H]^+$.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis and characterization of compounds **2**−**42** and their intermediates. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

CDI, 1,1′-carbonyldiimidazole; cpd, compound; MCP-1, monocyte chemotactic protein 1; Na-HMDS, sodium hexamethyldisilazane/sodium-bis(trimethylsilyl)amide; Pd/C, palladium on activated carbon; PDGF, platelet derived growth factor; PtdIns, phosphatidyl inositide; Vps, vacuolar protein sorting

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