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Phosphatidylinositol 4-kinases (PI4Ks) catalyze the transfer of phosphate from ATP to position 4 of phosphatidylinositol, resulting in the formation of phosphatidylinositol 4-phosphate (PI4P). This important phosphoinositide is involved in numerous cellular processes such as vesicular budding and membrane dynamics. Apart from these signaling roles, PI4P also serves as a crucial intermediate in the synthesis of other phosphoinositides, including phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) and phosphatidylinositol 4,5,6-trisphosphate (PI(4,5,6)P3). Mammalian cells contain four different PI4K isoforms catalogued into class II (PI4K II) and class III (PI4K III). Furthermore, the crystal structures of PI4K IIα, IIβ, and PI4K IIIβ,22–24 were solved during the last year, thus allowing further optimization of inhibitor efficiency and selectivity.25

Generally, two distinct types of selective PI4K IIIβ inhibitors have been introduced (Fig. 1). Firstly, some compounds can be regarded as derivatives of PIK93 possessing a five-membered central core connected to an aromatic side chain. In contrast, the second type of PI4K IIIβ inhibitors with the archetypal example being T-00127-HEV1 is characterized by a bicyclic central core and a similar aromatic sidechain.3 Within this study, we have used purine derivatives and several other structurally related bicycles as potential analogs of the latter group. Our major goals were to complete the structure–activity relationship study and to understand the role of methyl substituents on the central purine core. Although our initial results indicated that the purine analog is approximately six times less active than the parent compound T-00127-HEV1,6 we decided to use this structural pattern to explore mainly the potential substitution at positions 8 and 2 because of easy access to a vast variety of these derivatives.

Our exploration of the SAR started with the preparation of 7-deaza derivative 6. The 6-chloro-7-deazapurine 2 served as the starting material to be treated with 3,4-dimethoxyphenylboronic acid...
acid 3 under Chan–Lam cross-coupling conditions, which afforded compound 4 (Scheme 1).

Nucleophilic substitution followed by hydrochloride formation gave the desired analog 6, as depicted in Scheme 1. The synthesis of 6-aminoalkyl-substituted 9-aryl-8-azapurine derivatives 14 and 15 started from commercially available 4,6-dichloro-5-aminopyrimidine 7 and 4,6-dichloro-2-methyl-5-aminopyrimidine 8, respectively. Nucleophilic displacement with 3,4-dimethoxyaniline followed by the formation of the 8-azapurine moiety furnished the key intermediates 12 and 13. The treatment of these compounds with 4-(2-aminoethyl)morpholine and conversion into hydrochloride salts (ethereal HCl in CH₂Cl₂ at 0 °C) afforded the desired compounds 14 and 15 (Scheme 1).

Subsequently, the SAR of purine derivatives began with the variation of the substituents at position 8 (H, CH₃, isopropyl, cyclohexenyl, cyclohexanyl, etc.). We used a built-up strategy similar to procedures previously reported by us and others, starting from 3,4-dimethoxaniline. Compound 1 was prepared in three steps, including the reaction of 3,4-dimethoxaniline with 2-methyl-4,6-dichloro-5-aminopyrimidine 8, giving derivative 11, an imidazole ring-closure reaction (both under microwave irradiation), yielding compound 17, and the nucleophilic replacement of the chlorine atom with 4-(2-aminoethyl)morpholine 5. A similar reaction sequence was used for the preparation of compound 19—analog bearing hydrogen at position 8. In this case, we directly prepared purine derivative 18 in one step by the reaction of the 3,4-dimethoxaniline 9 with 2-methyl-4,6-dichloro-5-formylaminopyrimidine 16 under microwave irradiation. The chlorine atom was then again replaced by amine 5 to afford analog 19.

Compound 11 was also utilized in the subsequent preparation of derivatives 22 and 23, which were obtained in two steps—iron (III) chloride/silica gel-mediated imidazole ring formation and the subsequent nucleophilic displacement of the chlorine at position 6 of the purine skeleton (Scheme 2). Compound 23 was also easily converted to analog 24 by palladium-catalyzed hydrogenation.

Aryl members of the 8-substituted series were prepared from 8-bromo derivative 25, which had been obtained by lithiation with LDA at −78 °C, followed by quenching the resulting lithiated species with 1,2-dibromotetrachloroethane. With this important precursor in hand, a small set of 8-aryl and 8-heteroaryl-substituted derivatives has been prepared (Scheme 3 and Table 1). Moreover,
compound 25 was treated with phenylacetylene under classic Sonogashira conditions, affording compound 31. Furthermore, the compounds 29 and 30 were subjected to catalytic hydrogenation and reduction, respectively, which provided derivatives 32 and 33 in good yields (depicted in Scheme 4).

The lithiation strategy was also used for the preparation of 8-iodo derivative 34. In this case, an excess of LDA enables direct preparation of this derivative, which can be subsequently easily transformed into 8-methoxy analog 35 by nucleophilic substitution using sodium methoxide in methanol (Scheme 5). In contrast, a one-pot lithiation of compound 18 followed by the addition of DMF and reduction of the resulting aldehyde led to hydroxymethyl derivative 36, which was converted to the desired final compound 37 by the nucleophilic displacement of the chlorine atom at position 6. The 8-substituted series was finalized by the preparation of 8-trifluoroderivative, which was performed by the treatment of diamine 11 with trifluoroacetic anhydride, followed by heating with the pyridine dioxane mixture, which led to the 6-oxo derivative 38. This compound was converted into the target compound 39 by (benzotriazol-1-yl)oxiridine (BOP)-catalyzed amination reaction.

The second part of our work focused on the synthesis of a series of compounds modified at position 2. Firstly, a simplified derivative with hydrogen atom at position 2 was prepared. The synthesis of this compound was accomplished starting from compound 10 with the procedure being similar to that used for compound 1, consisting of imidazole-ring construction followed by amino sidechain installation. The desired product 41 was obtained in very good overall yield. The whole series of 2-substituted derivatives was prepared via the crucial intermediate 45, which was prepared starting from diaminopyrimidine derivative 42 and 3,4-dimethoxyaniline.

Table 1
The preparation of the derivatives 26–31 via Scheme 1

| Compd | R   | Method | Yield (%) |
|-------|-----|--------|-----------|
| 26    | S   | A      | 46        |
| 27    | F   | A      | 94        |
| 28    | O   | A      | 89        |
| 29    | OBn | A      | 96        |
| 30    | O   | A      | 75        |
| 31    | Ph  | B      | 57        |

Scheme 2. The reagents and conditions: (i) R-CHO, FeCl₃/SiO₂, dioxane, rt (1 h)–100 °C (16 h); (ii) 5, DIPEA, i-PrOH, 120 °C, MW; (iii) H₂, Pd(OH)₂, MeOH, rt, 12 h.

Scheme 3. The reagents and conditions: (i) (a) LDA, THF, –78 °C, 30 min; (b) CCl₂BrCCl₂Br, THF, 2 h; (ii) method (A) R-B(OH)₂, PdCl₂(dppf), Na₂CO₃, dioxane/H₂O, 95 °C, 16 h; method (B) phenylacetylene, PdCl₂(PPh₃)₂, Cul, NEt₃, THF, 60 °C, 28 h (see Table 1).

Table 1
The preparation of the derivatives 26–31 via Scheme 1

| Compd | R   | Method | Yield (%) |
|-------|-----|--------|-----------|
| 26    | S   | A      | 46        |
| 27    | F   | A      | 94        |
| 28    | O   | A      | 89        |
| 29    | OBn | A      | 96        |
| 30    | O   | A      | 75        |
| 31    | Ph  | B      | 57        |

Scheme 4. The reagents and conditions: (i) H₂, Pd(OH)₂/C, THF/EtOH, rt, 16 h; (ii) NaBH₄, CH₂Cl₂/MeOH, 0 °C to rt, 16 h.
In this case, conventional heating provided better yields of coupling product 43 than the microwave-assisted built-up procedure. The subsequent closure of the imidazole ring under microwave conditions afforded a complex mixture of compounds with a partially or fully acetylated amino group in position 2 (44). Therefore, the crude mixture was heated under acidic conditions, which led to the desired intermediate 45 in 60% yield. The final 2-aminoderivative 46 was then obtained by the nucleophilic substitution in similar fashion as in the previous cases in 65% yield. Compound 45 served as a suitable starting material also for the preparation of iodo derivative 48, which was acquired in two successive steps—iodine introduction via the Sandmeyer reaction and the subsequent nucleophilic displacement of the chlorine at position 6. Compound 48 served as a starting material for the preparation of a small library of compounds with variously modified position 2 (Scheme 6 and Table 2).

The aromatic and heteroaromatic substituents were installed by Suzuki coupling reactions, the alkynes were introduced by Sonogashira cross-coupling and the cyano derivative was obtained by a palladium-catalyzed reaction with Bu₃SnCN. The OMe derivative 60 was prepared by the nucleophilic displacement of the iodine with sodium methoxide. Finally, a homo-derivative of the parent

Scheme 5. The reagents and conditions: (i) (a) LDA, THF, −78 °C, 30 min; (b) I₂, THF, −78 °C; (ii) MeONa, MeOH, reflux, 72 h; (iii) (a) LDA, THF, −78 °C, 30 min; (b) DMF, THF, −78 °C; (c) NaH₂, THF–H₂O; (iv) 5, DIPEA, MW, CH₃CN, 150 °C, 45 min; (v) (a) (CF₃CO)₂O, DCM, pyridine; (b) pyridine, dioxane, 100 °C; (vi) 5, BOP reagent, DBU, CH₃CN; (vii) CH₃CH(OEt)₃, Aoc₂O, MW, 120 °C, 75 min; (viii) 5, DIPEA, EtOH, 75 °C.

Scheme 6. The reagents and conditions: (i) 9, DIPEA, n-BuOH, 3 d; (ii) CH₃CH(OEt)₃, Ac₂O, MW, 120 °C, 75 min; (iii) HCl, THF–H₂O, reflux, 1 h; (iv) 5, DIPEA, EOH, 75 °C; (v) CuI, isomyl nitrite, CH₂J₂, THF. (vi) method (A) R-B(OH)₂, PdCl₂(dpdpf), Na₂CO₃, dioxane/H₂O, 95 °C, 16 h; method (B) (1) TMS–acetylene, PdCl₂(PPh₃)₂, Cu, Et₃N, DMF, 60 °C, o/n, (2) K₂CO₃, THF–MeOH (1:4), rt, 1.5 h; method (C) phenylacetylene, PdCl₂(PPh₃)₂, Cu, Et₃N, DMF, 65 °C; (D) Bu₃SnCN, Pd[PPh₃]₄, DMF, 17 h, 120 °C; (E) MeONa, MeOH, reflux, 72 h; (see Table 2).
The preparation of the derivatives 49–60 via Scheme 6

| Compd | R      | Method | Yield (%) |
|-------|--------|--------|-----------|
| 49    |        |        | 64        |
| 50    |        |        | 60        |
| 51    |        |        | 68        |
| 52    |        |        | 62        |
| 53    | A      | 54°    | 54b       |
| 54    |        |        | 60        |
| 55    |        |        | 58        |
| 56    |        |        | 66        |
| 57    | B      | 55°    | 55b       |
| 58    | CN     | C      | 79        |
| 59    | CN     | D      | 92        |
| 60    | OCH₃   | E      | 60        |

* Derivative 53 was prepared from its benzylated precursor similarly to derivative 32. (Step 2: H₂, Pd(OH)₂/C, THF/EtOH, rt, 16 h, yield over two steps.)

The effect of the compounds on PI4K IIIβ and PI4K IIIα was measured using the PI4K ADP-Glo assay. Most of the compounds exerted no or minimal effect on PI4K IIIα, whereas the inhibitory activity against PI4K IIIβ was strongly dependent on the substitution at both investigated positions. We initially measured inhibition at 10 μM and subsequently IC₅₀ values if the residual activity was lower than 25% of control.

Our data clearly show two trends (Table 3). Firstly, any substitution at position 8 led to a decrease of inhibitory activity in comparison with the parent methyl derivative. The position is only opened for a small substituent, and, besides the methyl derivative only the 8-bromo derivative 25 exerted mediocre inhibitory activity. All the other bigger substituents resulted in a complete loss of inhibitory activity.

Secondly, we observed an interesting structure–activity relationship regarding position 2. Once again, small substituents, e.g., the amino, nitrile or acetylene group, are tolerated while with the growth of the substituent the activity generally drops. Interestingly, derivatives with hydrogen-bond acceptor groups as a meta-or para-substituent on phenyl rings, such as compounds 51 and 52, regain the inhibitory activity. This suggests the formation of a novel interaction between the ligand and the enzyme.

Moreover, we were able to crystallize PI4K IIIβ in complex with inhibitors and ATP during the preparation of this Letter, which allows deeper understanding of the observed structure–activity relationship. We performed a series of docking studies based on these recently published structural data in order to elucidate these phenomena. The removal of the nitrogen atom at position 7 is accompanied by significant drop of activity (compound 6), which can be easily explained by missing hydrogen bond between the core of the inhibitor and amide moiety of Val613 and steric hindrance of additional hydrogen atom, which causes additional distortion of the amino sidechain and loss of another hydrogen contact with carboxyl moiety of Val613 (Supporting Fig. 1B). The docking results nicely explain also the observed lack of activity of 8-substituted derivatives caused by a limited space of the binding cavity, which is appropriate for the accommodation of a methyl group (Supporting Figs. 1 and 2). The partial loss of activity of compounds 14 and 15 with the additional nitrogen at position 8 could be explained by repulsion of electron pair of this nitrogen atom, which occupy this small cavity, and carbonyl group of Glu611 (Supporting Fig. 1C and D).
Our data also indicate that the carbonyl oxygen of the amide group attached to the phenyl substituent at position 2 of derivative 51 can easily form a hydrogen bond with Ser618 and enhance the inhibitory effect of the inhibitor (Fig. 2). Similarly, compound 52 can easily accommodate the position with the carbonyl moiety in close proximity to the Ser618, although an alternative binding mode with an amide hydrogen interacting with the carbonyl group of Gly675 might also be possible according to our docking results (Supporting Fig. 3).

Apart from the enzymatic studies, we also evaluated the antiviral activity of the compounds against a panel of (+)ssRNA viruses containing Coxsackievirus B3 (CVB3), human rhinovirus (HRV), hepatitis C virus (HCV). In the 8-substituted series, the antiviral activity largely correlated with the observed inhibitory activity and hepatitis C virus (HCV). In the 8-substituted series, the antiviral activity of the compounds against a panel of (+)ssRNA viruses (Supporting Fig. 3).

In conclusion, we have prepared a series of novel purine derivatives in order to study the effects of substituents at positions 8 and 2 on inhibitory activity against PI4K IIIβ. Our study clearly proves that position 8 is not suitable for any extensive modification with the methyl group being the optimum. On the other hand, the opposite side of the purine scaffold (position 2) can be decorated by various substituents. Although we have observed a significant drop of inhibitory activity for derivatives with simple aromatic rings, the analogs bearing appropriately substituted phenyls have exerted restored inhibitory activities against the title enzyme and have also proved to be the most potent in antiviral screening against various (+)ssRNA viruses.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.04.002.

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Figure 2. The docking study of (A) derivative 51 and (B) derivative 52.
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30. None of the compounds significantly inhibited (less than 50% of control) PI4K
IIIα at 10 μM.

31. The docking runs were performed in Autodock Vina, using the default scoring
function in similar fashion as reported previously (coordinates from 4WAG,
search space 26 x 26 x 26 Å centered at 31.5, 28, –19 Å and exhaustiveness
100). The figure was prepared in PyMol.

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