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4-Methylpteridinones as orally active and selective PI3K/mTOR dual inhibitors

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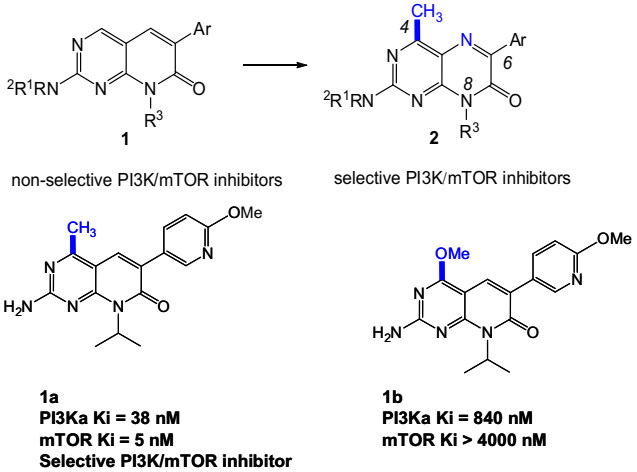
ABSTRACT

Pteridinones were designed based on a non-selective kinase template. Because of the uniqueness of the PI3K and mTOR binding pockets, a methyl group was introduced to C-4 position of the peteridinone core to give compounds with excellent selectivity for PI3K and mTOR. This series of compounds were further optimized to improve their potency against PI3Kα and mTOR. Finally, orally active compounds with improved solubility and robust in vivo efficacy in tumor growth inhibition were identified as well.

The phosphatidylinositol-3-kinase (PI3K)/mammalian target of rapamycin (mTOR) signaling pathway plays a central role in driving tumor cell proliferation, survival, angiogenesis and metastasis by activating mutation, deletion or amplification on one or several of its components.[1] PI3Ks are lipid kinases that regulate cellular growth and metabolism by phosphorylation of the 3-hydroxy of phosphatidylinositol to generate phosphatidylinositol triphosphate (PIP_3). One of the pivotal downstream effectors, Akt, is activated by PIP_3 via membrane recruitment and phosphorylation at Thr-308 by the 3-phosphoinositide-dependent protein kinase-1 (PDK-1). The PIP_3 formation is also responsible for facilitating the second phosphorylation at Ser473 of Akt by mTOR–rictor complex (mTORC2).[2] Among different subtype of PI3Ks, PI3Kα is the primary target for the treatment of cancers since functional loss of PTEN (phosphatase and tensin homolog protein is encoded by PTEN gene, the most commonly mutated tumor-suppressor gene in cancer after p53), oncogenic mutations in the PIK3CA gene encoding PI3Kα, amplification of the PIK3CA gene and over-expression of Akt have been established in many human cancers.[3]

mTOR is a mammalian serine/threonine kinase and a member of PI3K like kinase (PIKK) family of proteins. Rapamycin and its analogs (e.g., RAD001 (everolimus), CCI-779 (temsirolimus), and AP23573 (deforolimus)) have demonstrated anti-tumor activity in clinical trials.[4] Temsirolimus was approved in May 2007 for the treatment of advanced kidney cancer.[5] mTOR exists in two different complexes,[6] mTORC1, a rapamycin sensitive complex signaling to S6K1 and 4E-BP1 and mTORC2, an rapamycin insensitive complex signals to Akt as mentioned earlier. The existence of this rapamycin insensitive component of the mTOR signaling pathway thus provides new opportunities to inhibit mTOR.

A small molecule inhibitor that targets mTOR kinase should prevent signaling through both mTORC1 and mTORC2 to have a



Scheme 1. From non-selective to selective PI3K/mTOR inhibitors.

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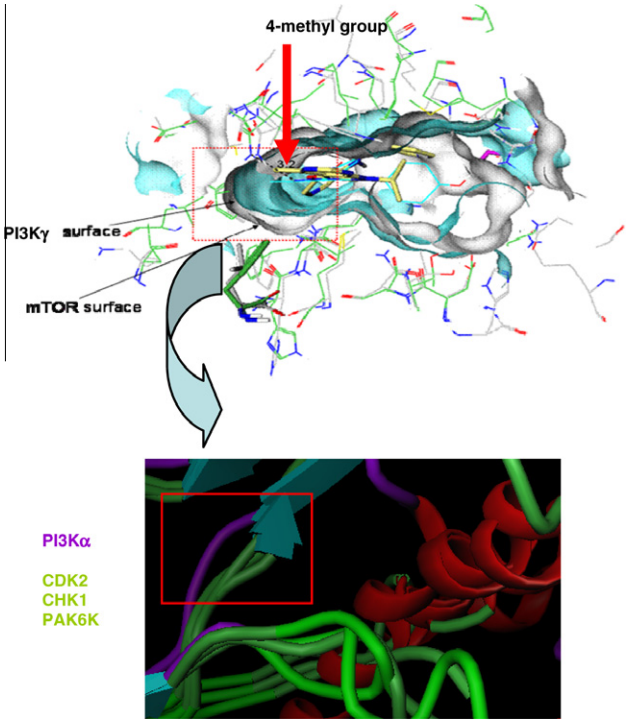
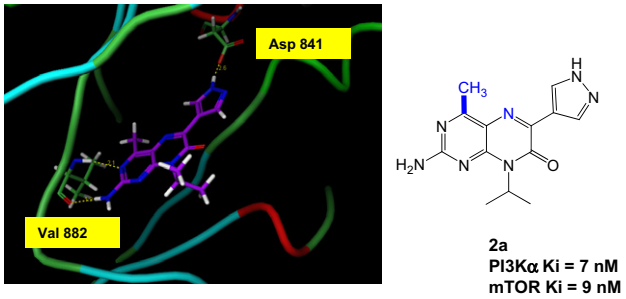


Scheme 2. Top: the 4-methyl analog of compounds **1**, **1a**, was docking to PI3Kγ and mTOR (PI3K surface: light blue, mTOR surface: light gray); bottom: PI3Kα ATP binding site (purple color) vs protein kinases (green color: CDK2, CHK1 and PAK6K).

broad and advantageous spectrum of pharmacology over mTORC1 specific rapamycin. Given the pivotal role of the PI3Kα/Akt/mTOR pathway in the biology of human cancers, we believe a dual PI3Kα/mTOR inhibitor can provide a great understanding in cancer treatment.

Finding leads for this PI3K/mTOR dual inhibitor project, we started with promiscuous kinase hits with a general structure as compound **1** in Scheme 1. To improve compound selectivity against other kinases, PI3K ATP binding site was analyzed and compared with other protein kinase ATP binding sites. It is interesting to find out that there is an additional small binding pocket in the PI3Ks and it is not available in regular protein kinases as shown in Scheme 2. This additional binding pocket should be able to accommodate a larger molecule and give us the selectivity that



Scheme 3. Co-crystal structure of compound **2a** and PI3Kγ.

we were looking for. From molecular modeling and docking studies, a small methyl group at C-4 position of compound **1** such as compound **1a** will orient the C-4 methyl group to fill this unique PI3K/mTOR binding pocket as shown in the top graph of Scheme 2. Compound **1a**[7] was made to test this model and indeed this compound shows excellent selectivity against other kinases in our kinase screening panels which covers more than sixty different kinases. The compound also has good and moderate binding affinity on PI3Kα and mTOR, respectively. This unique binding pocket for PI3K/mTOR turns out to be very small as we had predicted earlier since compound **1b** which has 4-methoxy instead of 4-mehtyl at C-4 position does not bind to mTOR at >4 μM and has much weaker binding affinity for PI3Kα as well.

To further increase structural diversity from **1**, we decided to replace the pyridopyrimidinone with pteridinone as the core structure (compound **2** in Scheme 1) while keeping the critical C-4 methyl group for selectivity. Based on earlier SAR learning from compound **1**,[7] we jump started to make compound **2a** (Scheme 3) with the pteridinone core and hoped the SAR transfer well between these two series. Indeed, compound **2a** shows good potency on PI3Kα and mTOR with great selectivity against other kinases as compound **1a**. We then focused on this 4-methyl-pteridinone series to improve both PI3K and mTOR potency with acceptable ADME properties.

To improve PI3Kα and mTOR potency, small alkyl groups at R^1 and R^2 are preferred. From the co-crystal structure of compound **2a** and PI3Kγ[8] (Scheme 3, PDB: 3OAW), it is consistent with our docking model and indicated that the C-2 amino and N-3 interact with Val 882 in the hinge region. Additionally, the pyrazole group at C-6 position picks up an addition interaction with Asp 841 (Scheme 3). Both Val 882 and Asp 841 are conserved in PI3Kα as

Table 1
Representative compounds **2**. **2b–g** on the C-6 aryl modifications

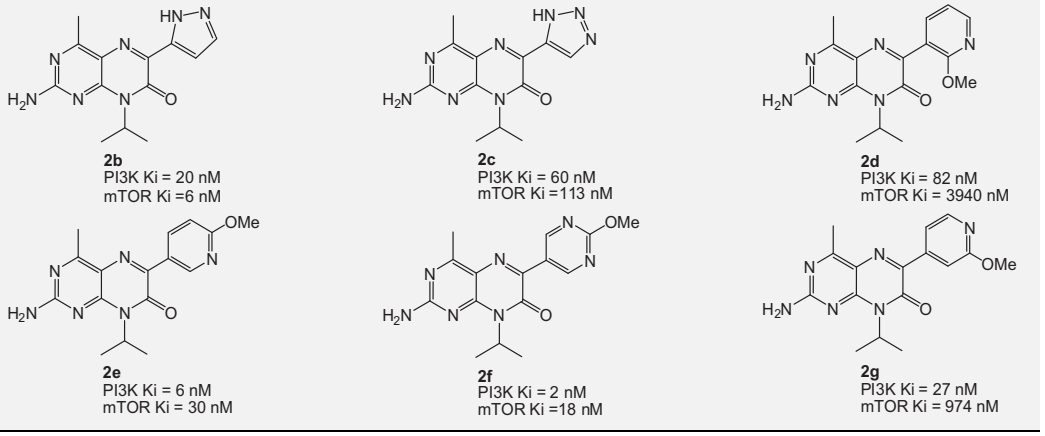
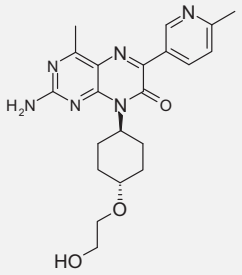
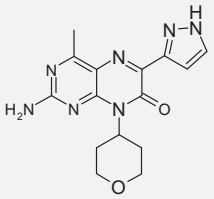


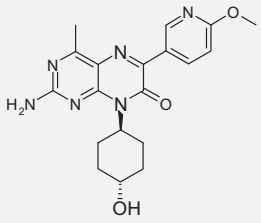
Table 2
Representative compounds **2**. **2h–l**



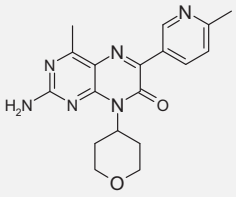
2h
PI3K Ki: 2.75 nM
mTOR Ki: 5.11 nM
S473 pAKT IC_{50}: 5.74 nM
HLM ER <0.3
RLM ER <0.3
DLM ER <0.3
RRCK = 21



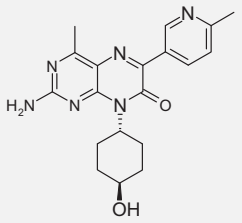
2i
PI3K Ki: 46 nM
mTOR Ki: 0.8 nM
S473 pAKT IC_{50}: 68 nM
HLM ER<0.2
RLM ER = 0.5
DLM ER = 0.2



2j
PI3K Ki: 2.8 nM
mTOR Ki: 6.8 nM
S473 pAKT IC_{50}: 14.3 nM
HLM ER < 0.2
DLM ER = 0.2
RRCK = 32



2k
PI3K Ki: 13 nM
mTOR Ki: 5 nM
S473 pAKT IC_{50}: 25 nM
HLM ER <0.2
RLM ER = 0.3
DLM ER = 0.1



2l
PI3K Ki: 2.8 nM
mTOR Ki: 0.85 nM
S473 pAKT IC_{50}: 5.7 nM
HLM ER < 0.2
RRCK = 32

Val 851 and Asp 810. However, there is some subtle SAR on mTOR and PI3K potency in the C-6 binding pocket. Small changes in the C-6 aryl group will have profound effects on either PI3K or mTOR potency such as examples shown in Table 1.

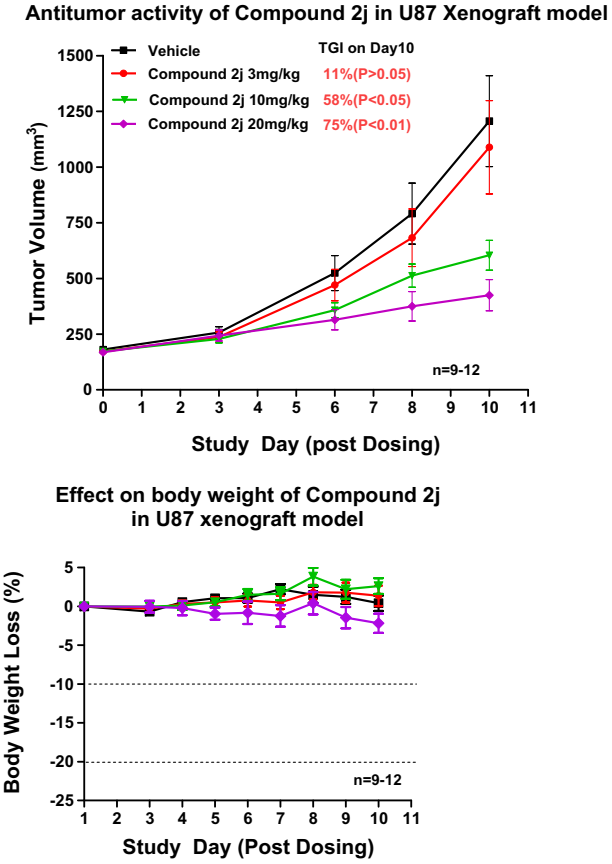
Nevertheless, all the compounds are still selective against most of other protein kinases.

One ADME drawback in this series is solubility, and fortunately the R^3 (N-8 position) points to the solvent exposed surface area according to co-crystal structures. Therefore, we can modify R^3 to modulate ADME properties of pteridinones to improve compounds' solubility.

After mixing and matching, compounds **2h–l** were made with solvating groups at R^3 for better solubility, and with promising heteroaryl groups at C-6 to interact with Asp 810 residue in PI3Kα for better potency. All the compounds **2h–l** still keep their excellent selective against other kinases as **1a**. Compounds **2h–l** are potent binders for both PI3Kα and mTOR with K_i <15 nM, and all the compounds are potent in our cellular functional assay, phosphorylation of S473 of AKT in BT20 cell line. In addition, these compounds have improved solubility and excellent microsomal stability across different species as shown in Table 2.

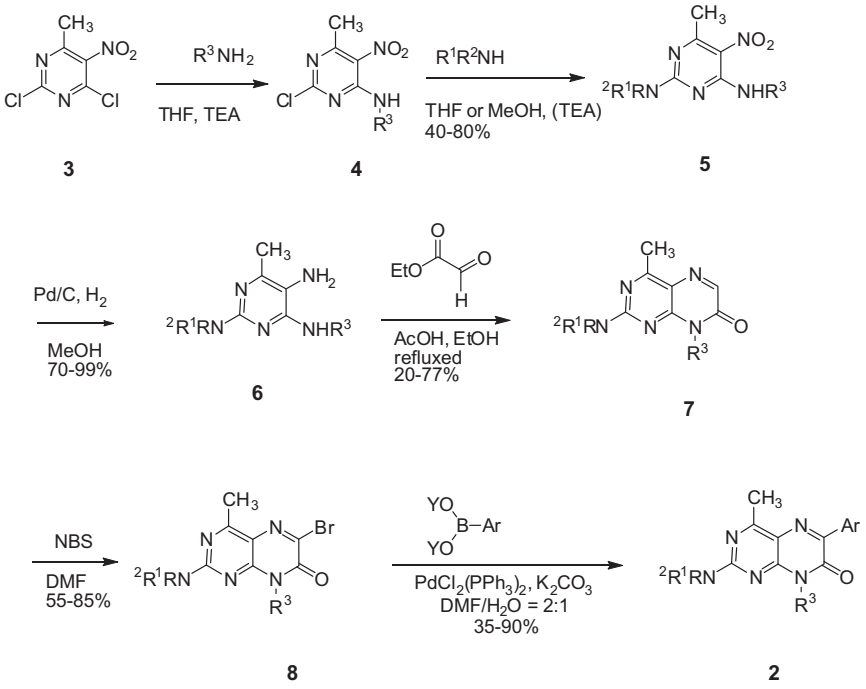
Compound **2j** was picked for our in vivo tumor growth inhibition (TGI) studies because of favorable human PK predictions. A shown in Scheme 4, a nice dose–response curve was observed in the TGI studies when **2j** was orally dosed in U87 (glioma cell line) mouse xenograft model. Additionally, there was no effect on body weight in the same studies.

General synthesis of compound **2** is described below and as shown on Scheme 5. Compounds of general structure **4** are prepared from 2,4-dichloro-6-methyl-5-nitropyrimidine (**3**) with ammonia or optionally substituted amines in THF and triethylamine as the base to yield compound **4**. Compound **4** without purification are then treated with ammonia or optionally substituted amines under the same condition as the first N-alkylation with or without triethylamine base to generate compound **5** in 40–



Scheme 4. TGI and body weight studies of compound **2j**.

80% yield in two steps. The nitro group in compound **5** is reduced with Pd/C under hydrogenation in MeOH to give compound **6** with



Scheme 5. Synthesis of compounds **2**.

free amino group in 70–99% yield. Compound **6** are cyclized with ethyl glyoxalate and acetic acid in refluxing ethanol to give compound **7** in 20–77% yield which are subsequently treated with NBS in DMF to yield brominated compound **8** in 55–85% yield. Finally, compound **6** are reacted with an appropriate aryl boronic acid or ester under the Suzuki–Miyaura coupling[9] condition with catalytic amount of $PdCl_2$ $(PPh_3)_2$ and 2 equiv of K_2CO_3 in the DMF/H_2O = 2:1 mixed solvent system at 100 °C to generate compound **2** in 35–90% yield.

In summary, from a non-selective kinase template, 2-aminopyridopyrimidinone (**1**), 4-methylpteridinones were designed based on a small special pocket within PI3K and mTOR binding site to give us a series of selective PI3K/mTOR dual inhibitors. After optimization in compound potency and solubility, Compounds with good cellular potency were found. In addition, several orally active compounds were identified with robust efficacy in our tumor growth inhibition models as well.

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