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Quinazolines with intra-molecular hydrogen bonding scaffold (iMHBS) as PI3K/mTOR dual inhibitors

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ABSTRACT

Intra-molecular hydrogen bonding was introduced to the quinazoline motif to form a pseudo ring (intra-molecular H-bond scaffold, iMHBS) to mimic our previous published core structures, pyrido[2.3-*D*]pyrimidin-7-one and pteridinone, as PI3K/mTOR dual inhibitors. This design results in potent PI3K/mTOR dual inhibitors and the purposed intra-molecular hydrogen bonding structure is well supported by co-crystal structure in PI3Kγ enzyme. In addition, a novel synthetic route was developed for these analogs.

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] mTOR is a mammalian serine/threonine kinase and a member of PI3K like kinase (PIKK) family of proteins. Rapamycin analogs (rapalogues) such as RAD001 (everolimus), CCI-779 (temsirolimus), and AP23573 (deforolimus)) have demonstrated anti-tumor activity in clinical trials.[2] mTOR exists in two different complexes,[3] mTORC1, a rapamycin sensitive complex signaling to S6K1 and 4E-BP1, and mTORC2, an rapamycin insensitive complex signals to Akt. Studies have shown that rapalogues are protein–protein inhibitors of mTOR through mTORC1 but not mTORC2.[4] 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 broad and advantageous spectrum of pharmacology over rapamycin. There are also reports that rapalogues led to PI3K/AKT activation and attenuated the agents' antitumor activities.[5] This complexity results from the negative regulation of the insuline/IGF-1 receptor signaling by mTORC1.[6] Recently, several PI3K inhibitors and rapalogues combination studies indicate the rapalogue-induced AKT activation was prevented and resulted in a synergistic inhibition of tumor growth. Furthermore, AKT activation induced by rapalogues has been ascribed to drug resistance.[7] All these data suggest a PI3K and mTOR dual inhibitor will be a useful tool to understand the resistance in the clinic and as a promising agent for the treatment of cancers.

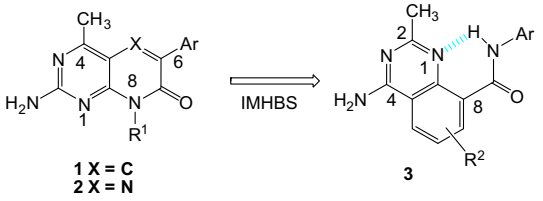

Figure 1. New quinazoline scaffold, **3** as IMHBS for templates **1** and **2**.

In the process to find other novel leads for our PI3K/mTOR dual inhibitor program and to strengthen chemical leads' IP position, we decided to rationally re-design our templates from the previously reported series, pyrido[2.3-*D*]pyrimidin-7-one (**1**) and pteridinone (**2**) cores.[8,9] We employed a de-construction approach to break the bi-cyclic ring in template **1** and **2** to a new quinazoline ring template **3** in Figure 1. A secondary amide side chain was introduced to the C-8 position to form a pseudo-ring through the intra-molecular

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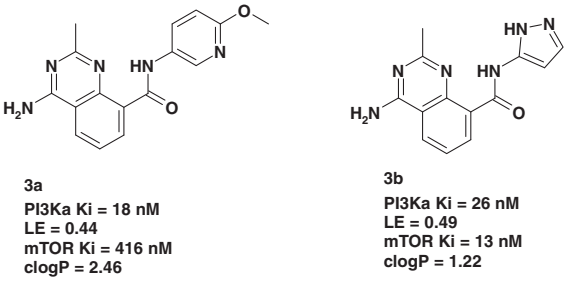


Figure 2. The first round of quinazoline IMHBS, compound **3a** and **3b**.

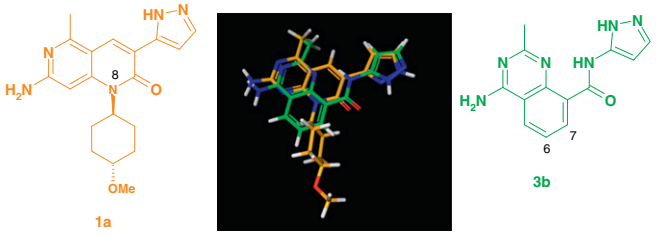


Figure 4. PI3Kg co-crystal structure overlay between compound **3b** (code: 3PRZ) and **1a** (code: 3PRE).

hydrogen bonding between the quinazoline core N-1 nitrogen and the C-8 amide hydrogen atom to lock the quinazoline ring and to mimic the original bi-cyclic ring space in templates **1** and **2**. In addition, the C-8 amide side chain nicely overlaps with the C-6 aryl groups in **1** or **2**. For kinase selectivity reason, we engineered the important methyl group to the C-2 of the quinazoline template which is corresponding to the C-4 methyl group in **1** or **2**. This methyl group plays a crucial role on dual PI3K/mTOR inhibitors selectivity over regular protein kinases as was reported recently.[8c,9] We named this type designed scaffold as iMHBS (*i*ntra-*m*olecular *h*ydrogen *b*onding *s*caffold). This type of design has been used in small molecule drug discovery[10] or in mimicking biologically active protein conformation.[11] This approach should allow us a quick entry to this newly designed iMHBS by leveraging the SAR with our knowledge on series **1** and **2**, since these templates share common chemistry vectors/handles and a similar conformation.

To implement this iMHBS strategy, we decided to make compounds **3a** and **3b** which represent an amalgamation of these ideas. These two compounds have R^2 = H for the ease of synthesis to allow rapid validation of our hypothesis. Indeed, both compounds **3a** and **3b** have good to moderate binding potency for either PI3Kα or mTOR and with excellent ligand efficacy[12] around 0.45 based on PI3Kα Ki as shown in Figure 2.

Encouraged by this result, we then focused our efforts on potency improvement by expanding the SAR efforts on the C-6/C-7 and C-8 of iMHBS **3** which correspond to the important pharmacophores of C-6 aryl and N-8 R_1 groups in series **1** and **2**, respectively. Compounds **3a** and **3b** were submitted for co-crystallization with the PI3Kγ enzyme under a soaking condition to confirm the iMHBS design. Much to out delight, the co-crystal structures of compounds **3a** and **3b** confirmed our design hypothesis as shown in Figure 3. These X-ray structures showed compound conformations which are favorable in making the intra-molecular H-bonding; the N–N distances between N-1 nitrogen and C-8 amide nitrogen are 2.4(3a)/2.5(3b) Å, with N-1 and amide free hydrogen distances 1.5(3a)/1.7(3b) Å and bond angels of 134°(3a)/132°(3b) (Fig. 3). These observed geometers are within the range of well-established H-bond geometries.[13] As was observed in series **1** and **2** earlier,[8c,9] the N-3 nitrogen and C-4 free NH_2 are making critical interaction with the hinge Val-882 in PI3Kγ. In addition, the C-8 carbonyl group picks up an extra interaction with Lys-833 in PI3Kγ. Both Val-882 and Lys-833 are conserved in PI3Kα as the corresponding Val-851 and Lys-802, respectively.

When we superimposed the co-crystal structures of iMHBS, **3b** and compound **1a** from series **1** together as shown in Figure 4, it became evident and once again supported our modeling that modifications on C-6 and C-7 of iMHBS **3** should provide us with compounds that mimicking the N-8 R^1 groups in series **1** or **2**.

In general and as shown in Table 1, the PI3Kα binding potency improves with C-6 methyl substitution and deteriorates badly with C-7 methyl substitution when compared with their corresponding parent compounds, R_1 = H such as **3d, 3c** versus **3a**; **3f, 3e** versus **3b**; **3n, 3m** versus **3l** and **3q, 3p** versus **3o**. When we tried to further elaborate the C-6 methyl group by bringing in solvating

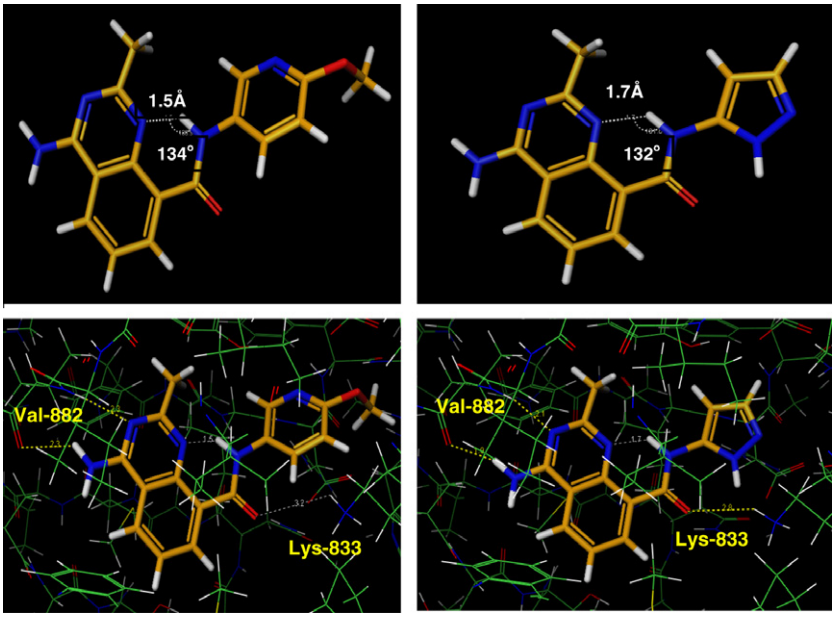
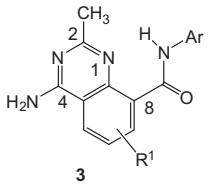
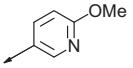
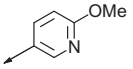
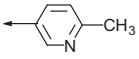
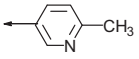
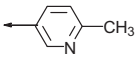
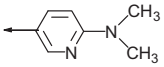
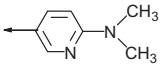
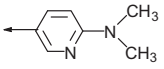
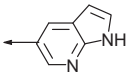
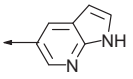
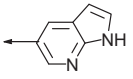


Figure 3. Co-crystal structures of compound **3a** (left, code:3PS6) and **3b** (right, code: 3PRZ) in PI3Kγ.

Table 1
SAR of IMBHS **3** and highlighted compounds with in vitro PI3Kα and mTOR binding potency



3

Compound	R^1	Ar	PI3Kα K_i (nM)	mTOR K_i (nM)
3c	7-CH_3		8%@ 100 nM	15%@ 1 μM
3d	6-CH_3		0.5	265
3e	7-CH_3		4550	984
3f	6-CH_3		7	3
3g	6-CH_2—N⟨O⟩		15	13
3h	6-CH_2NMe_2		188	1330
3i	H		0.5	31
3j	7-CH_3		4550	3250
3k	6-CH_3		0.5	66
3l	H		54	57%@ 1 μM
3m	7-CH_3		0%@ 100 nM	ND
3n	6-CH_3		12	162
3o	H		1	5
3p	7-CH_3		631	216
3q	6-CH_3		0.3	17
3r	H		6	21
3s	7-CH_3		6%@ 100 nM	ND
3t	6-CH_3		5	84

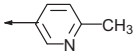
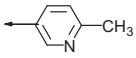
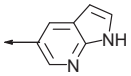
ND = not determined; please see Ref. 8a for assay condition.

groups, it resulted in compounds with lower binding affinity such as **3g, 3h** versus **3f**. From this exercise, we did find potent PI3Kα/mTOR dual inhibitors such as **3f**, **3g** and **3o**. Interestingly, potent PI3Kα selective compounds such as **3d**, **3k** and **3i** were identified as well. These compounds have binding selectivity against mTOR from 60- to 440-fold. One thing to note is that in our PI3Kα

enzymatic assay, 0.5 nM was our lowest detection limit. Therefore, these compounds could be more potent in PI3Kα binding and more selective against mTOR. In addition, iMHBS **3** has great selectivity against regular protein kinases because of the C-2 methyl group for the reason that we mentioned earlier.

In addition to good binding, compounds with good cellular potency were also observed as shown in Table 2. The table lists compounds with cellular potency less than 120 nM in BT20 cell line for the phosphorylation of S473 residue of AKT.

Table 2
Selective IMBHS **3** with cellular potency in BT20 cell line of phosphorylation of AKT s473

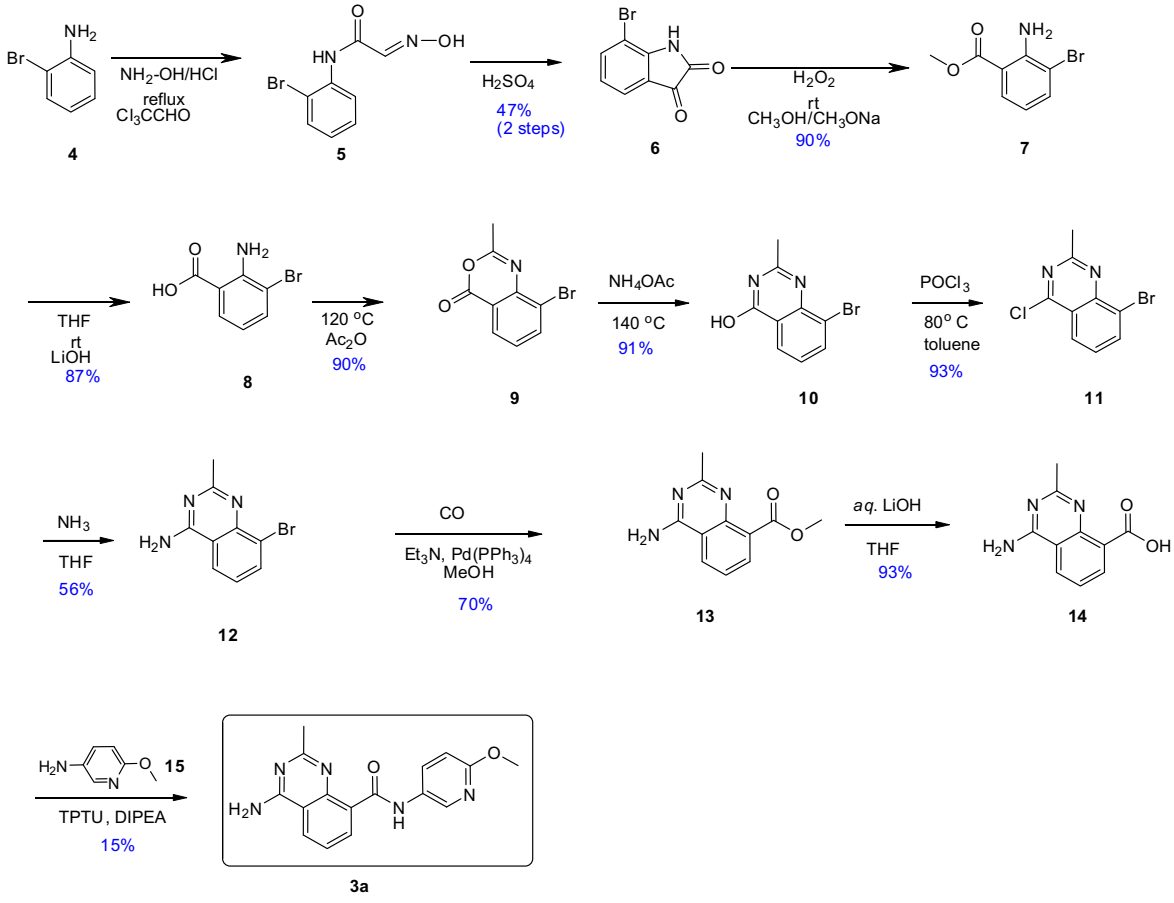
Compound	R^1	Ar	IC_{50} (nM) pAKT-s473 (BT20)
3f	6-CH_3		35
3i	H		112
3k	6-CH_3		88
3q	6-CH_3		43
3t	6-CH_3		24

Please see Ref. 8a for assay condition.

The synthesis of IMHBS, **3**, turned out to be more challenging than we originally expected, and a novel synthetic route was developed to realize our designs. The synthesis is depicted in Scheme 1 by using compound **3a** as an example.

2-Bromoaniline (**4**) in a hot HCl solution was treated with trichloroacetaldehyde monohydrate in water in the presence hydroxylamine hydrochloride and sodium sulfate to give isonitrosoacetanilide **5**. The intermediate **5** was treated with concentrated H_2SO_4 to give the known cyclized compound **6** in 44% yield in two steps according to the literature procedure.[14] Compound **6** was then dissolved in methanol, and treated with hydrogen peroxide and sodium methoxide to give methyl ester compound **7** in 90% yield. Compound **7** was hydrolyzed with lithium hydroxide to give free acid **8** in 87% yield. The amino acid **8** was treated with acetic anhydride at 120 °C to give cyclized product **9** in 90% yield. The oxazin-4-one **9** was then treated with ammonium acetate at 140 °C to give quinazolin-4-ol **10** in 91% yield. Compound **10** in toluene was chlorinated with $POCl_3$ and *N*,*N*-dimethylaniline to give chloroquinazoline **11** in 93% yield which was subsequently treated with ammonium hydroxide in refluxed THF to give compound **12** in 56% yield. Compound **12** was subjected to palladium catalyzed carbonylation in methanol to give methyl ester **13** which was hydrolyzed to free carboxylic acid **14** with lithium hydroxide in excellent yield. Compound **14** was then coupled with amine **15** to give our final target, compound **3a**.

In summary, an iMHBS was designed based on quinazoline template to inhibit both PI3K and mTOR. Co-crystal structures of **3a** and **3b** confirmed our iMHBS design. In addition to developing a novel synthetic route to these molecules, a series of potent and selective dual PI3K/mTOR inhibitors were discovered.



Scheme 1. Synthesis of compound **3a**.

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