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Discovery and Biological Profiling of Potent and Selective mTOR Inhibitor GDC-0349

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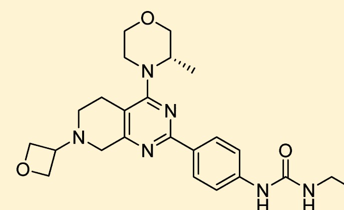
Supporting Information

ABSTRACT: Aberrant activation of the PI3K-Akt-mTOR signaling pathway has been observed in human tumors and tumor cell lines, indicating that these protein kinases may be attractive therapeutic targets for treating cancer. Optimization of advanced lead **1** culminated in the discovery of clinical development candidate **8h**, GDC-0349, a potent and selective ATP-competitive inhibitor of mTOR. GDC-0349 demonstrates pathway modulation and dose-dependent efficacy in mouse xenograft cancer models.



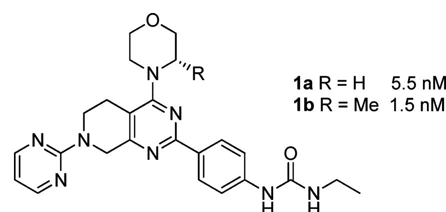
8h GDC-0349
mTOR Ki = 3.8 nM
selective against 266 kinases
efficacious in vivo

KEYWORDS: *Mammalian target of rapamycin, mTOR, TDI, urea bioisostere*

The mammalian target of rapamycin (mTOR) is an important component of the PI3K-Akt signaling pathway that regulates cell growth and proliferation.[1] Since aberrant activation of this pathway is found in many types of cancer, inhibition of PI3K, Akt, and mTOR has become an attractive strategy for treating cancer.[2,3] mTOR is a serine/threonine kinase belonging to the phosphoinositide kinase-related kinase (PIKK) family. A mTORC1 complex is formed when mTOR associates with RAPTOR and mLST8. A mTORC2 complex is formed by mTOR associating with RICTOR, mLST8, PPR5, and SIN1. The natural product rapamycin and its analogs (rapalogs) only inhibit the mTORC1 complex and are efficacious in reducing tumor cell growth, leading to two rapalogs being approved for oncology indications.[4] However, the mTORC1 complex negatively affects upstream insulin signaling through IRS1/2 via activation of the phosphorylated substrate S6K.[5] This negative feedback loop can activate a growth factor signaling cascade that includes mTORC2 and may limit the efficacy of rapalogs. Small molecule mTOR kinase inhibitors that inhibit both mTORC1 and mTORC2 may be more efficacious than the rapalogs.[6−10]

We recently reported the discovery of potent, selective, ATP-competitive mTOR inhibitors exemplified by compound **1** (Scheme 1).[11,12] Examination of the structure−activity relationship (SAR) and docking into a homology model of mTOR revealed key structural features of this tetrahydroquinazoline

Scheme 1



(THQ) class of inhibitors required for potency: (a) the morpholine oxygen atom binds to the hinge of the mTOR kinase domain; (b) the two N−H and carbonyl groups of the urea moiety hydrogen-bond with both Asp2195 and Lys2187, respectively; (c) the ethyl group of the urea imparts selectivity over PI3K; and (d) the (*S*)-methyl on the morpholine enhances the selectivity of **1b** by occupying a hydrophobic cavity formed by Trp2239 that is absent in PI3K (Supporting Information Figure S1). However, compound **1** exhibited potent time-dependent inhibition (TDI) of cytochrome P450 (CYPs), especially CYP3A4, and it possessed relatively high free plasma clearance in animals. As TDI can lead to drug−drug

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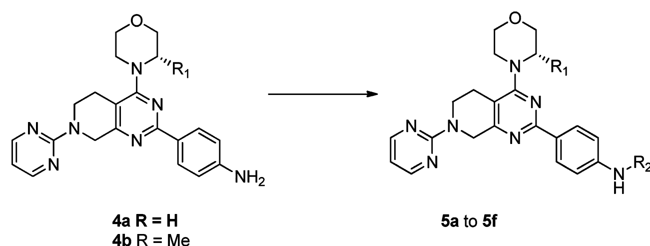
interactions and potential toxicity,[13,14] we decided to identify compounds that are devoid of TDI. Here we report our efforts to improve the above properties while maintaining/improving the potency, leading to the discovery of a drug development candidate.

Synthesis of the 7-aza tetrahydroquinazolines (7-aza-THQ) with diversified R_2 groups is outlined in Scheme 2. Treatment

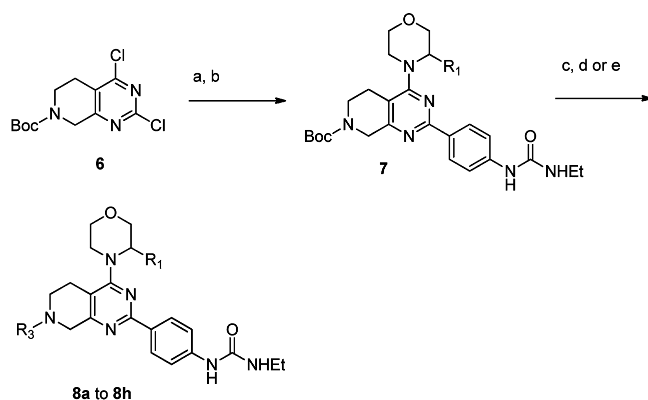
Scheme 2[a]



[a]Reagents and conditions: For **5a**, phosgene, TEA, then 2-fluoroethylamine; For **5b**: cat. Pd(OAc)2, 2-chloropyrimidine, Cs_2CO_3, dioxane, 160 °C, microwave. For synthesis of **5c**- **5f**, see supporting material.

of aniline **4** with phosgene followed by 2-fluoroethylamine gave **5a**. Palladium-mediated cross-coupling with 2-chloropyrimidine gave **5b**. Synthesis of the 7-aza-THQ with diversified R_3 groups is outlined in Scheme 3. After the 4-chlorine atom in dichloride

Scheme 3[a]



[a]Reagents and conditions: (a) morpholine or (*S*)-3-morpholine, DIPEA, DMF; (b) 4-(EtNHCONH)Ph boronic ester, $Pd(PPh_3)_4$, base, 120 °C; (c) TFA, DCM; (d) when R_3 = heteroaromatic: R_3Cl, DMF, 80-115 °C; (e) when R_3 = alkyl: alkyl ketone, then $NaBH(OAc)_3$, 1,2-dichloroethane.

6 was selectively displaced with a morpholine or (*S*)-3-methylmorpholine, the 2-chlorine atom participated in Suzuki coupling with a boronic ester to give biphenyl **7**. The Boc protecting group was removed and the R_3 group was introduced via either a nucleophilic replacement or a reductive amination to afford final compounds **8**. Final compounds were tested in both mTOR and PI3K-α enzymatic assays, and inhibitory potency is reported as K_i values.[11,12] Antiproliferative activities (reported as EC_{50} values) were measured in a PC3 prostate cancer cell line, where the PI3K-AKt-mTOR pathway is activated via the loss of the tumor suppressor PTEN.

Our first effort was to modify or replace the ethyl urea group with the hope to improve the metabolic stability, as deethylated product of **1a** was observed as one of the metabolites in the in vitro liver microsome metID study (data not shown). Introduction of an electron-withdrawing fluorine atom at the end of the ethyl group led to compound **5a**, which retained potency and selectivity over PI3K-α (Table 1). A pyrimidine

Table 1. SAR of the Right-Hand Moiety of 7-aza-THQ[a]

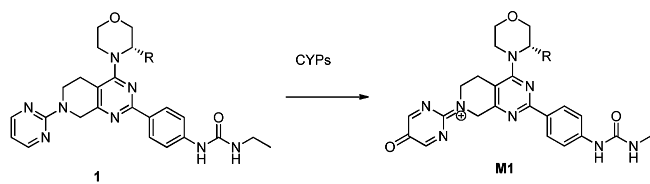
Cpd	R_1	R_2	mTOR K_i	PI3Kα selectivity[b]	PC3 prolif EC_{50}
1a	H	-CONHEt	5.5	60x	310
1b	Me	-CONHEt	1.5	500x	147
5a	H	-CONHCH2-CH2F	2.7	75x	58
5b	H		220	4x	ND[c]
5c	Me		2.9	510x	139
5d	Me		13.4	140x	667
5e	H		6.6	93x	1,300
5f	Me		0.4	630x	25

[a]Potencies in nanomolar. [b]Ratio of K_i values of PI3Kα over mTOR. [c]ND: not determined.

group replacement led to significantly reduced mTOR potency (**5b**), presumably due to the loss of the hydrogen-bond donating group of the urea NH. Triazolone **5c**, designed to mimic the urea group, retained substantial mTOR potency and selectivity over PI3K-α compared to **1b**. Introduction of a squaramide led to compound **5d** with ~9-fold potency reduction. Pyridone **5e** has a K_i of 6.6 nM, but the antiproliferative activity is reduced, likely caused by higher plasma protein binding. Pyrimidone **5f** exhibited a K_i of 0.4 nM and 630-fold selectivity over PI3K-α. It also demonstrated high antiproliferative potency (25 nM) in PC3 cells. However, none of the compounds in Table 1 is superior to **1b** in terms of overall profile (e.g., **5f** displayed high in vivo clearance of 78 mL/(min·kg) in mouse and no oral bioavailability) and, therefore, was not profiled further.

Although we were unable to experimentally identify the reactive metabolites, we hypothesized that one of the possibilities is the formation of diaza quinone iminium[15] **M1** via CYP-mediated oxidation of the aminopyrimidine moiety, which would lead to TDI (Scheme 4).

Scheme 4



This led our attention to modifying the left-hand side of the molecule. Analogs with a fluorine atom at the C5 position of pyrimidine (**8a**), regioisomers of pyrimidine (**8b** and **8c**), or an imidazole ring (**8d**) were designed to block the formation of the diaza quinone iminium species (Table 2). These four analogs maintained potency and selectivity. Replacing the pyrimidine ring by an isopropyl group (**8e**) or a cyclopentyl group (**8f**) decreased potency relative to **1b**. The nitrogen atom with alkyl groups attached is quite basic, which could bring potential liabilities, such as blockade of the hERG channel.[16] Indeed, compound **8e**, with a pK_a of 7.6, has an hERG IC_{50} of 8.5 μM (patch clamp assay). Compounds **8g** and **8h**, with reduced basicity of the nitrogen atom, maintained most of the potency and selectivity of **1b**. Introduction of an oxetane group reduced the basicity of **8h** (pK_a = 5.0), which translated into diminished hERG liability (IC_{50} > 100 μM).

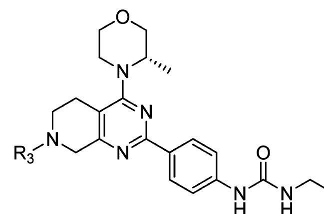
Compounds **8a–h** were tested in a TDI shift assay in human liver microsomes in the presence of cofactor NADPH with midazolam as a CYP3A4 substrate.[17] When a reactive metabolite is formed and reacts with CYP during a 30-min preincubation with the compound, a left shift of the CYP activity curve is observed compared with no pre-incubation and the compound is designated as TDI positive. TDI IC_{50} is calculated from this shifted curve. When there is no shift with 30-min preincubation, the compound is designated as TDI negative. Of the three compounds with modified pyrimidines in Table 2, two (**8a** and **8b**) had a similar level of TDI compared to **1b** and one compound (**8c**) had significantly less TDI (Table 2). Compounds with alkyl groups (**8e** and **8h**) or a sulfonamide (**8g**) had decreased TDI potency compared to **1b**, consistent with the hypothesis that minimizing the formation of diaza quinone iminium could decrease TDI.

Compound **8h** is a remarkably selective mTOR inhibitor, with less than 25% inhibition of 266 kinases, including all isoforms of PI3K when tested at Invitrogen at 1 μM (Supporting Information Table S1). Compound **8h** has ~10-fold reduced free plasma clearance in both mice (100 mL/min/kg) and rats (171 mL/min/kg in rat) compared to **1b** (1818 mL/min/kg in mice, 1538 mL/min/kg in rats).

Compound **8h** inhibited downstream markers of mTOR, including phospho-4EBP1 and phospho-Akt(s473) in an in vivo PK/PD study in mouse (Supporting Information Figure S2), consistent with an inhibition of both mTORC1 and mTORC2 complexes.

When dosed orally once daily in athymic mice in a MCF7-neo/Her2 tumor xenograft model (PI3K mutation), compound **8h** inhibited tumor growth in a dose-dependent manner, achieving stasis (99% TGI) at the maximum tolerated dose

Table 2. SAR of the N-Substitution of 7-aza-THQ[a]



Cpd	R_3	mTOR K_i	PI3Kα selectivity[b]	PC3 Prolif EC_{50}	TDI IC_{50}[c]
1b		1.5	500x	147	0.9
8a		3.2	510x	118	1.4
8b		2.4	810x	118	3.3
8c		2.7	1,850x	129	8.4
8d		1.4	970x	72	>10
8e	iPr	9.6	840x	890	>10
8f		4.8	540x	353	ND[d]
8g	MeSO2	4.2	88x	245	negative
8h		3.8	790x	270	>10

[a]Potency and EC_{50} in nM. [b]Ratio of K_i values of PI3Kα over mTOR. [c]IC_{50} value (in μM) is with imidazolam as the probe. [d]ND: not determined.

(Figure 1). Body weight change was less than 10% up to the highest dose. Compound **8h** was also efficacious in other xenograft models, including PC3 (PTEN null) and 786-0 (VHL mutant) (Supporting Information Figure S3). Similar levels of tumor growth inhibition were achieved when **8h** was administered once every three days at higher doses compared to once every day (Supporting Information Figure S4). While the single agent mTOR inhibitor (GDC-0349), PI3K inhibitor (GDC-0941),[18] or MEK (GDC-0973)[19] inhibitor showed only modest tumor growth delay in the A549 mouse xenograft model (KRas mutant, LKB1 deficient), combination of mTOR and MEK inhibitors achieved the same level of tumor stasis as the combination of PI3K and MEK inhibitors (Figure 2). All the combinations were well tolerated and caused no significant change in body weight.

In summary, we report here further optimization of our advanced lead compound **1**. Urea bioisosteric replacements have been identified that possess equal or better mTOR potency, as exemplified by **5e** and **5f**. SAR efforts culminated in the discovery of selective and potent mTOR kinase inhibitors,

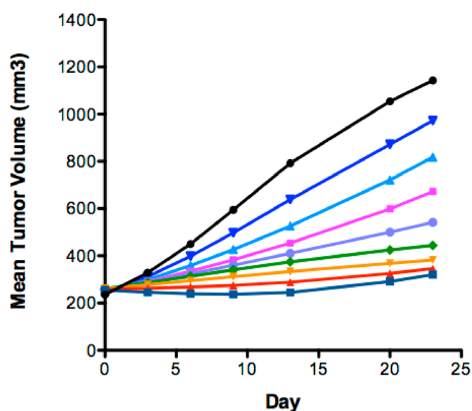


Figure 1. Dose-dependent tumor growth inhibition by compound **8h** dosed orally in the MCF7-neo/Her-2 mouse xenograft breast cancer model. The dosages from top to bottom are 0 (vehicle), 10, 20, 30, 40, 50, 60, 70, and 80 mg/kg QD, respectively.

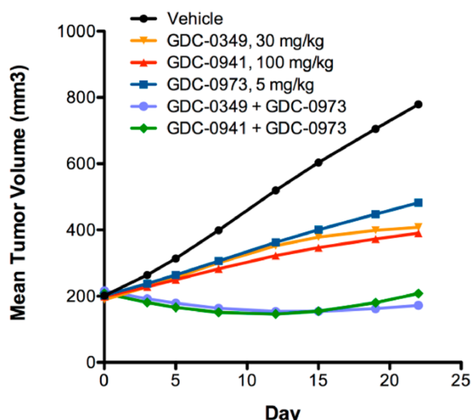


Figure 2. Combination of mTOR (GDC-0349) or PI3K (GDC-0941) inhibitor with MEK inhibitor GDC-0973 dosed orally QD increases tumor growth inhibition in the A549 mouse xenograft lung cancer model.

as exemplified by **8h**, which exhibits favorable free plasma clearance and is devoid of time-dependent inhibition of CYPs and hERG liabilities. Compound **8h** demonstrates modulation of pathway PD markers and is efficacious in several mouse xenograft models of neoplastic growth as either a single agent or combination therapy. On the basis of its desirable overall profile, compound **8h** was chosen as the drug development candidate GDC-0349.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedures and characterization data, kinase selectivity of **8h**, TDI assay conditions, PK-PD figures, and additional in vivo efficacy studies. 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

mTOR, mammalian target of rapamycin; PI3K, phosphatidylinositol-3-kinase; TDI, time-dependent inhibition; THQ, tetrahydroquinazoline

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