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Discovery of 2-arylthieno[3,2-*d*]pyrimidines containing 8-oxa-3-azabicyclo[3.2.1]octane in the 4-position as potent inhibitors of mTOR with selectivity over PI3K

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ABSTRACT

2-Aryl-4-morpholinothieno[3,2-*d*]pyrimidines are known PI3K inhibitors. This class of compounds also potently inhibited the homologous enzyme mTOR. Replacement of the morpholine group in these compounds with an 8-oxa-3-azabicyclo[3.2.1]octane group led to mTOR inhibitors with selectivity over PI3K. Optimization of the 2-aryl substituent led to the discovery of 2-(4-ureidophenyl)-thienopyrimidines as highly potent (IC_{50} <1 nM) mTOR inhibitors with excellent selectivity (up to >1000-fold) over PI3K and good potency in a cellular proliferation assay (IC_{50} <50 nM).

PI3K and mTOR are key components of the PI3K-Akt-mTOR signaling cascade, one of the most frequently upregulated pathways in tumor biology. Consequently, inhibitors of mTOR and/or PI3K have recently been the focus of an intense effort towards the development of novel anticancer medicines.[1,2] 2-Aryl-4-morpholinothieno[3,2-*d*]pyrimidines (e.g., **1**, **2** and GDC-0941, Fig. 1) have been described as potent inhibitors of the various PI3K-isoforms.[3–6] We recently reported that pyrazolopyrimidines with a similar substitution pattern as **1** and **2** potently inhibited the homologous enzyme, mTOR. On the pyrazolopyrimidines, introduction of isosteres for the morpholine and phenol/aminopyrimidine substituents could lead to increased mTOR potency and selectivity.[7–11] Here, we report the application of those findings to the thienopyrimidine scaffold, to give novel, highly potent mTOR inhibitors with selectivity over PI3K.

Compounds **1** and **2** were prepared following published procedures[3,4] and assayed against PI3K-α and mTOR,[12] revealing that these leads are dual inhibitors of PI3K and mTOR, with moderate potency (Table 1).

Scheme 1 describes the synthesis of thienopyrimidines carrying bridged morpholine analog **6**.[13] 3-Aminothiophene-2-carboxamide **3** was condensed with triphosgene to give thienopyrimidine **4**. Chlorination of **4** with phosphorus oxychloride proceeded smoothly to give **5** in near-quantitative yield. Regioselective nucleophilic displacement of the 4-chloride with **6**, followed by Suzuki coupling gave access to target compounds **8a–h**.

Introduction of the ethylene-bridged morpholine **6** on the thienopyrimidine core led to increased selectivity for mTOR over PI3K-α (Table 2). Compounds **8a** and **8b** inhibited mTOR with similar potency compared to the corresponding 4-morpholinothienopyrimidines **1** and **2**. In contrast, compounds **8a** and **8b** were significantly less potent against PI3K-α than **1** and **2**, resulting in sevenfold (**8a** compared to **1**) or 31-fold (**8b** compared to **2**) increased selectivity over PI3K-α. We have previously described similar decreases in PI3K potency upon introduction of ethylene-bridged morpholine **6** on pyrazolopyrimidines, which was ascribed to a steric clash with Phe961. This residue is smaller (Leu) in mTOR, thus avoiding the steric clash.[11] When the *meta*-phenol (**8a**) was replaced with a *para*-phenol (**8c**), a modest increase in potency against mTOR was observed. In addition, the potency against PI3K-α was decreased, resulting in significantly increased selectivity for mTOR. Next, we addressed whether the pyrimidine ring nitrogens in **8b** contributed to mTOR activity. Removal of one of the nitrogens, resulting in aminopyridine **8d**, led to a small increase in mTOR potency and a pronounced increase in selectivity over PI3K-α. In contrast, removal of both nitrogens (to give aniline **8e**) resulted in decreased mTOR inhibitory activity, while maintaining

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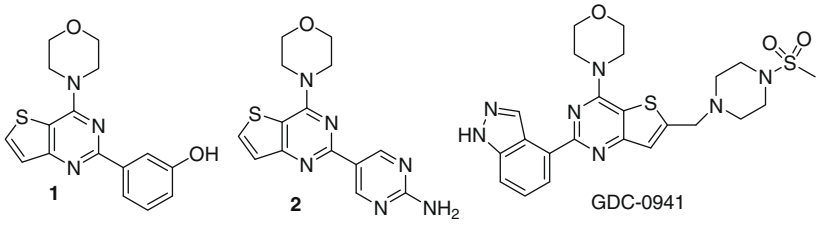
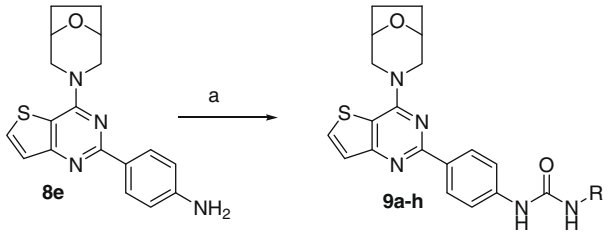


Figure 1. Representative examples of 2-arylthieno[3,2-*d*]pyrimidine PI3K inhibitors.

selectivity over PI3K-α. A further increase in selectivity was observed for indole **8f** and acetamide **8g** (392-fold and 165-fold selective over PI3K-α, respectively). Replacement of the acetamide in **8g** with a methyl carbamate (**8h**) resulted in further enhanced potency and selectivity over PI3K-α.

In case of the pyrazolopyrimidine scaffold, we have shown that the mTOR activity could be increased by replacement of the carbamoylphenyl group with a ureidophenyl group, which was able to form three hydrogen bonds to the enzyme (two bonds between urea NHs and an aspartate, one bond between the urea carbonyl and a lysine).[7,10] Based on these observations, the ureidophenyl group was explored as an isostere for the carbamoylphenyl moiety in **8h**. As illustrated in Scheme 2, treatment of aniline **8e** with triphosgene followed by addition of excess amine gave target ureidophenyl compounds **9a–h**. A dramatic increase in potency was observed for methyl ureidophenyl **9a** (Table 3) compared to methyl carbamoylphenyl **8h** (Table 2). The ureidophenyl compound inhibited the mTOR enzyme at sub-nanomolar concentrations while maintaining excellent selectivity over PI3K-α. Cellular proliferation was also inhibited at much lower concentrations than with carbamoylphenyl **8h**. The dramatic increase in potency observed when the ureidophenyl group was introduced on the thienopyrimidine core suggests that these compounds interact with their target enzymes in a manner similar to the pyrazolopyrimidines.



Scheme 2. Reagents: (a) (1) triphosgene, CH_2Cl_2, NEt_3; (2) RNH_2, 22–89%.

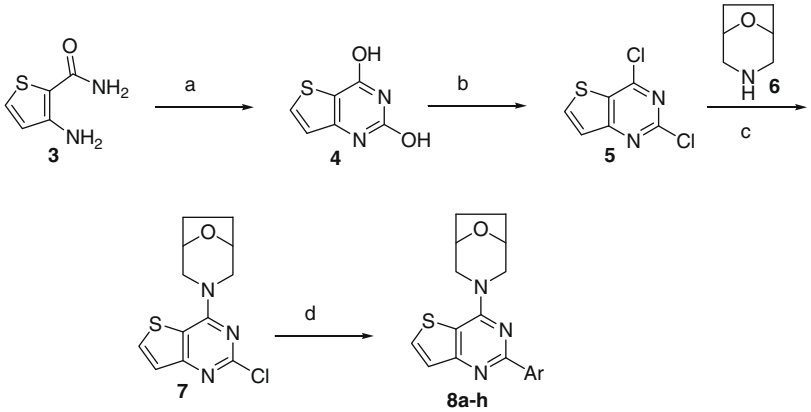
Based on the promising activity of methyl ureido compound **9a**, additional ureido compounds were investigated (Table 3). Alkylureido compounds (e.g., **9b**–**9e**) were very potent (<1 nM) and highly selective (>1000-fold over PI3K-α) mTOR inhibitors with good to excellent potency in a cellular proliferation assay (IC_{50}: 12–180 nM). Aryl ureido compounds (**9f–9h**) were also potent mTOR inhibitors with somewhat decreased selectivity over PI3K-α, although pyridinyl ureido compounds **9g** and **9h** both retained high selectivity (>100-fold) over PI3K-α and displayed excellent potency against cellular proliferation.

It had previously been shown[3] that substitution at the 2-position of the thiophene ring (cf. GDC-0941, Fig. 1) could be used to optimize aqueous solubility and other physicochemical properties. Based on this report, we explored the effect of these substitutions on our series. As shown in Scheme 3, the target compounds were accessible from 2-chlorothienopyrimidine **7**. Formylation of **7** gave aldehyde **10**. Reductive amination of **10**

Table 1
Inhibitory concentrations (IC_{50}'s) of thienopyrimidines **1** and **2** against mTOR, PI3K-α and LNCaP cellular proliferation

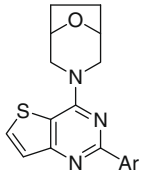
Compds	mTOR IC_{50}[a] (nM)	PI3K-α IC_{50}[b] (nM)	Sel.[c]	LNCaP cell IC_{50} (nM)
1	49 ± 13	41	0.9	1,300
2	61 ± 3	8.5	0.14	680

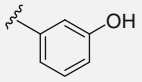
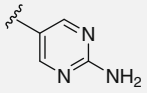
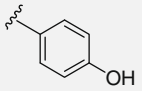
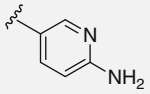
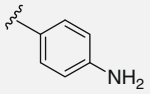
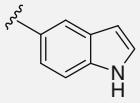
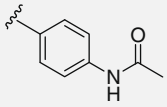
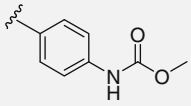
[a] Mean ± SEM.
[b] IC_{50} determinations were done in triplicate at eight (half-log) concentrations. The average error was <50%.
[c] Selectivity (IC_{50} PI3K-α/IC_{50} mTOR).



Scheme 1. Reagents and conditions: (a) triphosgene, dioxane, 80 °C; (b) $POCl_3$, 120 °C, 99% from **3**; (c) CH_2Cl_2, EtOH, NEt_3, 93%; (d) $ArB(OR)_2$, $Pd(PPh_3)_4$, toluene, EtOH, aq Na_2CO_3, microwave, 120 °C, 26–87%.

Table 2
Inhibitory concentrations (IC_{50}'s) of thienopyrimidines against mTOR, PI3K-α and LNCaP cellular proliferation



Compds	Ar	mTOR IC_{50}[a] (nM)	PI3K-α IC_{50}[b] (nM)	Sel.[c]	LNCaP cell IC_{50} (nM)
8a		58 ± 4	399	6.9	2800
8b		57 ± 8	246	4.4	1200
8c		32 ± 4	6270	199	2800
8d		35 ± 4	1767	51	1250
8e		100	>10,000	>100	4300
8f		22	8619	392	1350
8g		26.5 ± 0.7	4372	165	1700
8h		11 ± 4	6199	549	1850

[a] Mean ± SEM.
[b] IC_{50} determinations were done in triplicate at eight (half-log) concentrations. The average error was <50%.
[c] Selectivity (IC_{50} PI3K-α/IC_{50} mTOR).

gave access to **11**, which was converted into 2-(4-nitrophenyl)-thienopyrimidine **12** under Suzuki coupling conditions. Reduction of the nitro group, followed by urea formation, gave access to target compounds **13a–d**. Alternatively, 2-chlorothienopyrimidine **10** could be reacted first under Suzuki conditions. Reductive amination of the resulting intermediate **14** gave **15**. Conversion of the nitrophenyl group in **15** into ureidophenyl compounds **16a–d** was effected as described for the conversion of **12** into **13a–d**.

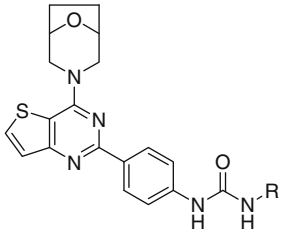
The data in Table 4 reveals the effects of substitution on the thiophene ring. Introduction of a methylpiperazinomethyl group led to compounds with comparable IC_{50}'s against PI3K-α (compare Tables 3 and 4). Unfortunately, this particular substitution was not well tolerated by mTOR. Thus, compounds **13a–d** were 10–20-fold less potent inhibitors of mTOR than the corresponding unsubstituted thienopyrimidines. Consequently, the selectivity of compounds **13a–d** was significantly decreased. Replacement of the methylpiperazine with a methylsulfonylpiperazine led to an approximately 10-fold increase in activity, both against mTOR and PI3K-α, resulting in potent mTOR inhibitors with moderate to good selectivity over PI3K-α (**16a–d**). Similar increases in potency where previously described following introduction of methylsulfonylpiperazine, and were explained by the formation of hydrogen bonds between the sulfonyl oxygens and K802 and A805.[3] 3-Pyridinyl ureidophenyl **16c** was a highly potent dual inhibitor of mTOR and PI3K with excellent cellular activity, whereas cyclopropyl ureido compound **16b** retained high selectivity for mTOR over PI3K-α and possessed excellent enzyme and cellular potency.

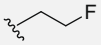
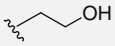
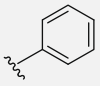
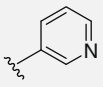
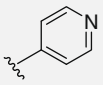
In conclusion, we have shown that introduction of an 8-oxa-3-azabicyclo[3.2.1]octane group onto a thienopyrimidine scaffold greatly increased selectivity for mTOR over PI3K-α. In addition, replacement of the aminopyrimidine or phenol substituent in the 6-position with 4-ureidophenyl groups gave access to highly potent (<1 nM) and selective (>1000-fold over PI3K-α) mTOR inhibitors.

Acknowledgment

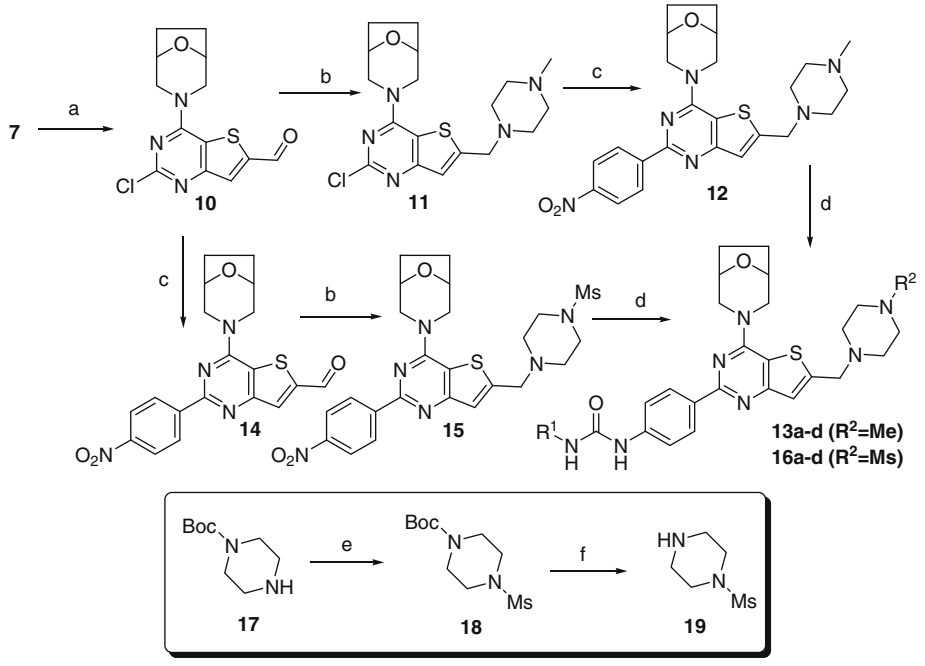
The authors are grateful to Wei-Guo Zhang for mTOR assay development.

Table 3
Inhibitory concentrations (IC_{50}'s) of thienopyrimidines **9a–h** against mTOR, PI3K-α and LNCaP cellular proliferation



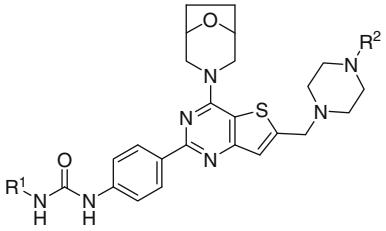
Compds	R	mTOR IC_{50}[a] (nM)	PI3K-α IC_{50}[b] (nM)	Sel.[c]	LNCaP cell IC_{50} (nM)
9a		0.9 ± 0.1	423	492	85
9b		0.7 ± 0.1	1262	1856	12
9c		0.7 ± 0.2	825	1107	87
9d		0.34 ± 0.03	324	982	180
9e		0.68 ± 0.05	1359	2013	40
9f		1.25 ± 0.07	82	66	140
9g		0.29 ± 0.01	119	410	27
9h		0.44 ± 0.07	80	182	10

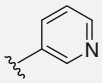
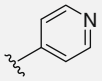
[a] Mean ± SEM.
[b] IC_{50} determinations were done in triplicate at eight (half-log) concentrations. The average error was <50%.
[c] Selectivity (IC_{50} PI3K-α/IC_{50} mTOR).



Scheme 3. Reagents and conditions: (a) BuLi (1.2 equiv), THF, −78 °C; then DMF (1.5 equiv), 91%; (b) $NaHB(OAc)_3$, HOAc, 1,2-dichloroethane, *N*-Me-piperazine or *N*-Ms-piperazine (**19**), 76% (**11**), 54% (**15**); (c) 4,4,5,5-tetramethyl-2-(4-nitrophenyl)-1,3,2-dioxaborolane, $Pd(PPh_3)_4$, DME, aq Na_2CO_3, 90 °C, 83% (**12**), 51% (**14**); (d) (1) H_2, Pd/C, CH_2Cl_2, *i*PrOH; (2) triphosgene, CH_2Cl_2, NEt_3; (3) R^1NH_2, 20–55%; (e) MsCl, NEt_3, CH_2Cl_2, 94%; (f) TFA, CH_2Cl_2, 99%.

Table 4
Inhibitory concentrations (IC_{50}'s) of thienopyrimidines **13a–d** and **16a–d** against mTOR, PI3K-α and LNCaP cellular proliferation



Compds	R^1	R^2	mTOR IC_{50}[a] (nM)	PI3K-α IC_{50}[b] (nM)	Sel.[c]	LNCaP cell IC_{50} (nM)
13a		Me	20 ± 2	322	17	500
16a		Ms	1.4 ± 0.7	46	33	120
13b		Me	16 ± 4	874	56	400
16b		Ms	1.0 ± 0.1	179	176	32
13c		Me	4.7 ± 0.3	148	31	148
16c		Ms	0.6 ± 0.1	12	21	22
13d		Me	3.7 ± 0.9	86	23	200
16d		Ms	0.45 ± 0.04	8.3	19	120

[a] Mean ± SEM.
[b] IC_{50} determinations were done in triplicate at eight (half-log) concentrations. The average error was <50%.
[c] Selectivity (IC_{50} PI3K-α/IC_{50} mTOR).

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