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2-Arylureidophenyl-4-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)triazines as highly potent and selective ATP competitive mTOR inhibitors: Optimization of human microsomal stability

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ABSTRACT

Isosteric replacement of one of the 3,5-ethylene-bridged morpholines in 2-arylureidophenyl-4,6-di(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)triazines led to significant improvements in human microsomal stability. 3-*R*-Me-morpholine and tetrahydropyran were identified as preferred isosteres for the bridged morpholine. Combination of tetrahydropyran substitution with an *N*-Me-piperazinophenylureido group led to **27**, that selectively suppressed mTOR biomarkers in vivo and possessed excellent efficacy in a murine xenograft model.

Rapamycin and its analogs ('rapalogs') have shown clinical efficacy in the treatment of cancer. These compounds are allosteric inhibitors of a complex between the mammalian Target Of Rapamycin (mTOR) and raptor (mTOR complex 1, mTORC1).[1] Inhibition of mTORC1 signaling leads to increased Akt activity through a feedback mechanism, resulting in potential anti-apoptotic effects.[2] In addition, although the rapalogs strongly inhibit mTORC1 signaling to S6K, they only have marginal effects on mTORC1 signaling to 4EBP.[3,4] It is expected that increased anti-tumor efficacy may be obtained with ATP competitive active site inhibitors of mTOR. Such inhibitors would disrupt mTORC1 signaling to both S6K as well as 4EBP. Moreover, a second functional complex of mTOR, mTORC2, which is not affected by rapalogs,[5] contributes to Akt activation by phosphorylation of Akt at serine 473.[6] ATP competitive mTOR inhibitors would be expected to disrupt mTORC2-mediated phosphorylation of serine 473 and thus prevent the feedback activation of Akt observed for the rapalogs. Therefore, several groups,[7–11] including our own,[12–18] have recently embarked on an intense search for ATP competitive mTOR inhibitors.

In the preceding Letter[19] we presented the design and synthesis of di-(3-oxa-8-azabicyclo[3.2.1]octan-8-yl)-arylureidophenyl-triazines as potent and selective mTOR inhibitors (e.g., **1**, Table 1). Compound **1** is a highly potent and selective inhibitor with excellent activity in murine models of human tumor xenografts. We generally observed excellent PK parameters (high AUC, low clearance) for compounds with good microsomal stability. Although **1** displayed excellent stability in nude mouse microsomes ($t_{1/2}$ >30 min), its clinical development was precluded by its rapid degradation by human microsomes ($t_{1/2}$ <5 min). The discrepancy between microsomal stability in the pre-clinical efficacy species (nude mouse) and the target species (human) suggests that the CYP isoforms responsible for the major metabolic pathways differ between these two species. In this Letter, we describe our efforts aimed at increasing the human microsomal stability of derivatives of **1**, while maintaining the excellent potency and selectivity characteristics.

In the case of pyrazolopyrimidine mTOR inhibitors, we had shown that the human microsomal stability could be enhanced by variation of the polar substituent at the 4-position of the phenylureidophenyl group.[12,15,17] Initial efforts on the triazine series therefore centered on this group as well. The compounds listed in Table 1 were prepared according to the schemes in our preceding Letter.[19] A variety of different polar groups were examined, resulting in all cases in highly potent inhibitors of both mTOR.[20] All compounds also potently inhibited proliferation of a prostate tumor cell line (LNCaP) that is characterized by hyperactive PI3K-Akt-mTOR signaling.[21] We have previously demonstrated that cellular proliferation inhibition in this model correlates with inhibition of mTOR signaling.[15] Replacement of the *N*-methyl piperazine with a

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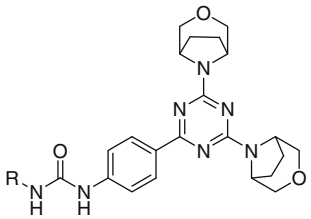
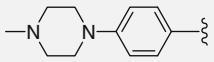
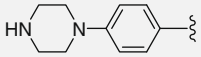
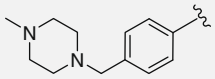
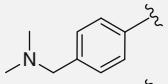
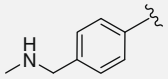
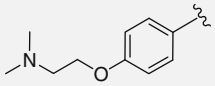
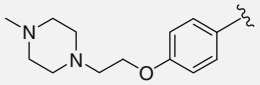
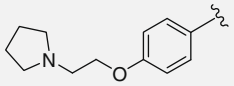
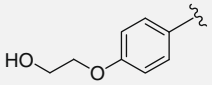
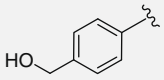
Table 1
The effect of different substituents at the 4-position of the phenylureidophenyl group on activity, selectivity and microsomal stability

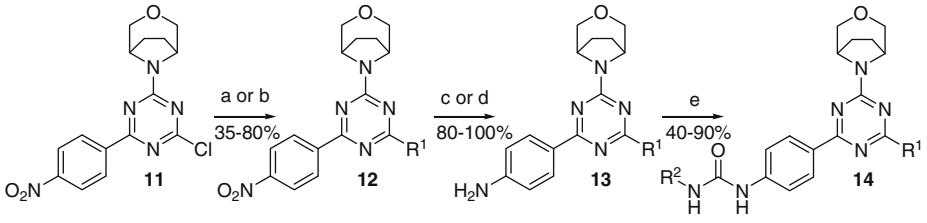


Compd	R	mTOR IC_{50}[a] (nM)	PI3K-α IC_{50}[a] (nM)	Sel.[b]	LNCaP cell IC_{50} (nM)	Mouse microsome $T_{1/2}$[c] (min)	Human microsome $T_{1/2}$[c] (min)
1		1.0 ± 0.1	899 ± 107	892	1.3	>30	2.5
2		0.57 ± 0.01	947	1661	<0.8	>30	4
3		1.0 ± 0.2	1127	1127	4.0	>30	3
4		1.1 ± 0.3	499	460	5.0	>30	6
5		0.89 ± 0.09	226	255	3.0	>30	6
6		1.6 ± 0.2	955	579	3.0	>30	2.5
7		0.9 ± 0.3	483	537	0.8	13	3
8		0.8 ± 0.2	389	458	0.8	>30	4
9		0.26 ± 0.06	961	3768	0.8	>30	<1
10		0.18 ± 0.02	253	1406	<0.8	>30	<1

[a] Mean ± SEM.
[b] Selectivity (IC_{50} PI3K-α/IC_{50} mTOR).
[c] Nude mouse or human microsomal stability.

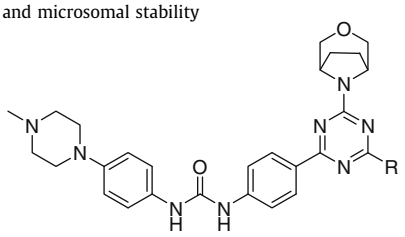
secondary amine (**2**) resulted in an equipotent compound with excellent selectivity over PI3K-α.[22] Introduction of a second basic amine, as in benzylic piperidine **3**, was well tolerated. Smaller benzylic amines (**4** and **5**) were slightly less selective. Aminoethoxy substituents (**6**–**8**) also showed slightly less selectivity than the piperazinophenyl compounds. Primary alcohols led to extremely potent compounds with excellent selectivity over PI3K-α (**9** and **10**). Unfortunately, all compounds in Table 1 still suffered from poor stability in human microsomes.[23]

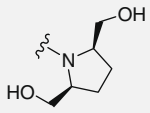
Metabolite identification studies revealed that the 3,5-ethylene bridge on the morpholine group was the primary site of metabolism. This observation prompted us to explore compounds where one of the 3,5-bridged morpholines had been replaced with another substituent. The synthesis of these compounds is depicted in Scheme 1.[24] Chloride **11** (prepared as described in the preceding Letter[19]) was treated with an amine in the presence of base (for compounds **15**–**19**) or subjected to Stille coupling conditions with tributyl(3,6-dihydro-2*H*-pyran-4-yl)stannane (for compounds **20**



Scheme 1. R1 and R2 are as defined in the tables. Reagents and conditions: (a) amine, NEt_3; (b) tributyl(3,6-dihydro-2*H*-pyran-4-yl)stannane, $Pd(PPh_3)_4$, toluene; (c) H_2, Pd/C; (d) Fe, AcOH; (e) triphosgene, NEt_3, then R^2NH_2.

Table 2
The effect of morpholine replacements on activity, selectivity and microsomal stability



Compd	R	mTOR[a] IC_{50} (nM)	PI3K-α IC_{50}[a] (nM)	Sel.[b]	LNCaP cell IC_{50} (nM)	Mouse microsome $T_{1/2}$[c] (min)	Human microsome $T_{1/2}$[c] (min)
15		0.7 ± 0.1	1028	1546	<0.8	>30	5.5
16		5.4 ± 0.9	2506	460	50	>30	>30
17		6.6 ± 0.7	4766	728	240	–	–
18		8.8 ± 0.9	592	67	800	–	–
19		1.2 ± 0.2	665 ± 106	578	6	>30	18
20		0.7 ± 0.2	51 ± 8	78	25	–	–
21		0.52 ± 0.05	777 ± 129	1494	0.8	>30	27

[a] Mean ± SEM.
[b] Selectivity (IC_{50} PI3K-α/IC_{50} mTOR).
[c] Nude mouse or human microsomal stability.

and **21**). The resulting nitro compound **12** was reduced to the aniline (**13**) by hydrogenation (for **15**–**19**, **21**) or by treatment with iron and acetic acid (**20**). Aniline **13** was converted into urea **14** by treatment with triphosgene and an amine.

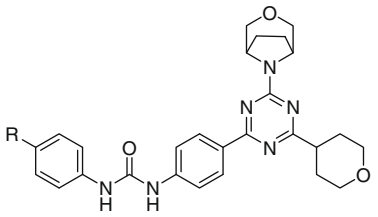
Replacement of the 3,5-bridged morpholine with a ring-opened analog (**15**, Table 2) resulted in a potent inhibitor that was selective over PI3K-α but still suffered from poor stability in human microsomes. In contrast, replacement of the bridged morpholine with isopropylamine (**16**) led to excellent stability in this assay. Unfortunately, the latter substitution also resulted in decreased potency. Attempts to enhance potency with other small alkylamine substituents (e.g., **17**) were not met with success. It should be noted that compound **16** still contains one bridged morpholine, a potential source for microsomal degradation. The data in Table 2 suggests that replacement of a single bridged morpholine may be sufficient to provide increased microsomal stability. A possible explanation for this observation may be that one of the bridged morpholines plays a key role in presenting the second bridged morpholine to CYP-450 in a spatial orientation that leads to rapid oxidation. A related series of compounds in which both bridged morpholines have been replaced with isosteres is discusses in our accompanying Letter.[25]

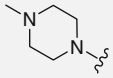
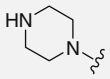
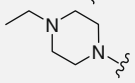
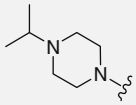
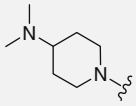
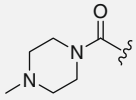
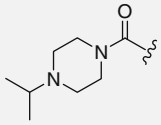
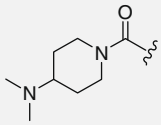
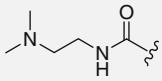
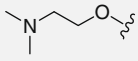
Since compounds **1** and **15** both displayed excellent potency, and both were equipped with a morpholine-like substituent, we focused our efforts on substituents that resembled morpholine. In our accompanying Letter,[19] we have shown that the selectivity of di-morpholino-triazines depends on the nature of both morpholine groups. Thus, because of the *C*2 symmetrical nature of the triazine inhibitors, the presence of a single morpholine derivative capable of forming the hinge region hydrogen bond to PI3K-α will result in decreased selectivity. Therefore, we selected morpholine derivatives that were not expected to potently bind to PI3K-α.[26,27]

Indeed, thiomorpholine **18** did not result in potent inhibition of PI3K. Unfortunately, the potency of **18** against mTOR was not increased compared to **16** and **17**. In contrast, 3-*R*-Me-morpholine, another poor binder of PI3K,[26] led to good mTOR and cellular potency while remaining selective over PI3K-α (**19**). Dihydropyran **20** was even more potent against mTOR. As expected,[27] **20** also possessed significantly increased potency against PI3K-α, resulting in decreased selectivity. We have previously demonstrated that reduction of the olefin in the dihydropyran group leads to poor PI3K-α binding.[27] When the same strategy was applied to **20**, resulting tetrahydropyran **21** maintained mTOR potency but lost the ability to potently inhibit PI3K-α. The data in Table 2 reveal that compounds with similar enzyme IC_{50}s can possess drastically different cellular proliferation IC_{50}s (cf. **15** and **20**), presumably reflecting differences in cellular permeability and intracellular distribution. Both 3-*R*-Me-morpholine derivative **19** and tetrahydropyran **21** combined excellent mTOR and LNCaP potency and selectivity over PI3K-α with significantly increased human microsomal stability. Therefore, additional analogs of **19** and **21** were explored.

Table 3 lists analogs containing the tetrahydropyran group. Replacement of the tertiary amine in **21** with a secondary amine (**22**) resulted in decreased selectivity and cellular potency. Ethylpiperazine **23** was equipotent to the methyl analog (**21**) and displayed excellent stability in human microsomes. The cellular potency of isopropyl analog **24** was somewhat decreased, which could be restored by moving the amine to an exocyclic position (**25**). The latter substitution resulted in a moderate loss of microsomal stability. As observed previously,[28] inhibitors containing

Table 3
Inhibitors containing the tetrahydropyran substituent



Compd	R	mTOR IC_{50}[a] (nM)	PI3K-α IC_{50}[a] (nM)	Sel.[b]	LNCaP cell IC_{50} (nM)	Mouse microsome $T_{1/2}$[c] (min)	Human microsome $T_{1/2}$[c] (min)
21		0.52 ± 0.05	777 ± 129	1494	0.8	>30	27
22		0.535 ± 0.005	294	550	20	>30	27
23		0.56 ± 0.02	574 ± 26	1025	<0.8	>30	>30
24		0.88 ± 0.04	660 ± 16	751	18	>30	>30
25		0.56 ± 0.04	382 ± 14	682	1.0	>30	20
26		0.81 ± 0.07	79 ± 18	97	<0.8	>30	>30
27		0.46 ± 0.02	148 ± 17	325	0.9	>30	>30
28		0.34 ± 0.06	77 ± 7	226	<0.8	>30	>30
29		0.14 ± 0.02	54 ± 6.0	377	<0.8	>30	>30
30		0.7 ± 0.1	876	1208	9	>30	20

[a] Mean ± SEM.
[b] Selectivity (IC_{50} PI3K-α/IC_{50} mTOR).
[c] Nude mouse or human microsomal stability.

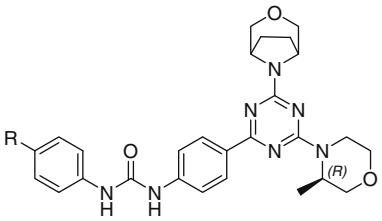
benzamido water solubilizing groups were extremely potent against cellular proliferation and possessed excellent microsomal stability (**26**–**29**). Although methylpiperazino benzamide **26** suffered from a 15-fold loss in selectivity compared to **21**, other amides maintained high selectivity over PI3K-α (**27**–**29**). Dimethylaminoethoxy analog **30** was less potent in the cellular assay and was only moderately stable in the microsomal assay.

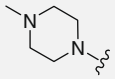
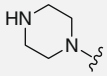
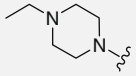
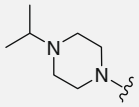
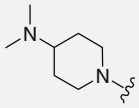
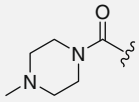
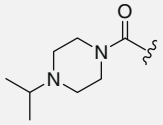
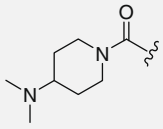
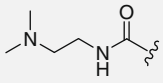
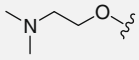
Similar trends were observed for analogs incorporating 3-*R*-Memorpholine (Table 4). Thus, N-linked water solubilizing groups led to potent compounds with good selectivity over PI3K-α (**19**, **31**–**34**). Amide-linked substituents resulted in increased potency but decreased selectivity (**35**–**38**). Compounds **36** and **37** combined excellent potency and stability with good selectivity (>200-fold) over PI3K-α. The dimethylaminoethoxy group again resulted in the least potent analog (**39**).

Based on its excellent potency, selectivity over PI3K-α and microsomal stability across different species, compound **27** was selected for further evaluation. First, we explored whether **27** was selective over other kinases in addition to PI3K-α. The IC_{50} against another PI3K isoform, PI3K-γ, was 1256 nM (2730-fold selective). In addition, when **27** was screened against an in-house panel of 21 kinases, none of them were inhibited at IC_{50}s below 50 μM (>100,000-fold selectivity).[29] In addition to its excellent stability in human microsomes, **27** was highly stable in human hepatocytes as well, as evidenced by low clearance (0.0083 mL/min/million cells) and a high half-life (>60 min). Finally, the potential for cardiac effects and drug–drug interactions was explored. Inhibitory concentrations of **27** against hERG and various cytochrome P450 isoforms (1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4) were greater than 30 μM (>65,000-fold higher than the mTOR IC_{50}). The only CYP isozyme that was inhibited below 30 μM was CYP 2C8, with an IC_{50} of 3 μM. The latter value is still more than 6,500-fold higher than the mTOR IC_{50}.

Next, we evaluated the effects of **27** in vivo. The pharmacokinetics of **27** following intravenous administration (5 mg/kg) were

Table 4
Inhibitors containing the 3-*R*-Me-morpholine substituent



Compd	R	mTOR IC_{50}[a] (nM)	PI3K-α IC_{50}[a] (nM)	Sel.[b]	LNCaP cell IC_{50} (nM)	Mouse microsome $T_{1/2}$[c] (min)	Human microsome $T_{1/2}$[c] (min)
19		1.2 ± 0.2	665 ± 106	578	6	>30	18
31		0.40 ± 0.04	286 ± <1	706	<0.8	>30	17
32		1.2 ± 0.1	1006	838	1.0	>30	23
33		1.25 ± 0.05	970	776	6.0	>30	>30
34		0.90 ± 0.06	443 ± 68	494	8.0	>30	20
35		0.57 ± 0.05	60 ± 7	105	<0.8	>30	>30
36		0.42 ± 0.07	102 ± 18	245	<0.8	21	>30
37		0.25 ± 0.03	52 ± 1	212	<0.8	>30	>30
38		0.10 ± 0.02	43 ± 3	407	<0.8	>30	20
39		1.7 ± 0.1	610	359	12	>30	12

[a] Mean ± SEM.
[b] Selectivity (IC_{50} PI3K-α/IC_{50} mTOR).
[c] Nude mouse or human microsomal stability.

characterized by low clearance (5 mL/min/kg), a moderate volume of distribution (1.3 L/kg) and a moderate to long half-life (3.4 h), resulting in high exposure (AUC_{0-inf}: 16,654 h*ng/mL). Intravenous administration of **27** (25 mg/kg) to nude mice bearing MDA361 tumor xenografts[30] resulted in complete inhibition of mTORC1 signaling, lasting at least 8 h, as evidenced by the lack of phosphorylation of S6K and its downstream substrate S6 (Fig. 1). Phosphorylation of the mTORC2 substrate, Akt (S473) was also significantly inhibited. The selective nature of inhibitor **27** was exemplified by the lack of suppression of phosphorylation of the PI3K/PDK1 biomarker Akt (T308). Mean plasma and tumor concentrations of **27** at the 8 h time point were 2161 ng/mL and 2474 ng/mL, respectively.

Administration of low intravenous doses of **27** (3 mg/kg, qd) for five days to nude mice bearing human glioblastoma U87MG xenografts resulted in pronounced tumor growth arrest (Fig. 2). To the

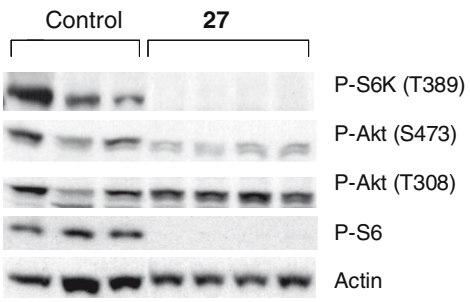


Figure 1. Inhibition of mTOR signaling in vivo following a single dose of mTOR kinase inhibitor **27** (25 mg/kg, iv). Nude mice bearing sc MDA361 tumors were injected iv with vehicle or 25 mg/kg of **27**. Tumor lysates were prepared 8 h after injection and were immunoblotted with antibodies against P-S6K (T389), P-Akt (S473), P-Akt (T308), P-S6 and β-actin.

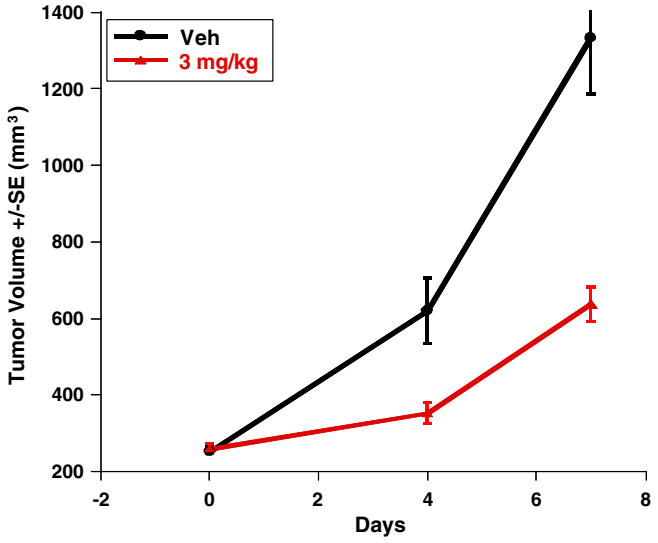


Figure 2. Inhibition of U87MG tumor growth following administration of **27** (3 mg/kg, iv, qd, days 0–4).

best of our knowledge, efficacy at these low doses has not been demonstrated previously for ATP competitive mTOR inhibitors.

In conclusion, isosteric replacement of the 3-oxa-8-azabicyclo[3.2.1]octane group in triazine mTOR inhibitors led to compounds that combined potent and selective inhibition of mTOR with excellent microsomal stability. A representative inhibitor, compound **27**, displayed potent and selective in vivo inhibition of mTOR leading to efficacy in murine tumor xenograft models at unprecedentedly low doses.

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30. In vivo methods for determination of biomarker inhibition and efficacy in nude mouse xenograft models have been described before; see Ref. 12.