Bioorganic & Medicinal Chemistry Letters 20 (2010) 2644–2647

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of 2-ureidophenyltriazines bearing bridged morpholines as potent and selective ATP-competitive mTOR inhibitors

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ARTICLE INFO

Article history:
Received 21 January 2010
Revised 5 February 2010
Accepted 8 February 2010
Available online 13 February 2010

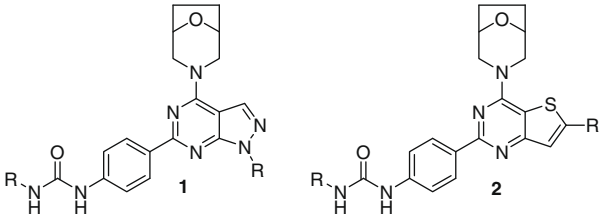
Keywords:
mTOR
PI3K
Kinase
Inhibitor
Cancer
Oncology
Antiproliferative

ABSTRACT

Incorporation of bridged morpholines in monocyclic triazine PI3K/mTOR inhibitors gave compounds with increased mTOR selectivity relative to the corresponding morpholine analogs. Compounds with ureidophenyl groups gave highly potent and selective mTOR inhibitors. Potency and selectivity was demonstrated both in vitro and in vivo through biomarker suppression studies. Select compounds exhibited potent inhibition of tumor growth in nude mouse xenograft assays upon PO and IV administration.

The mammalian target of rapamycin (mTOR) is a member of the phosphoinositide-3-kinase (PI3K)-related kinases (PIKKs), high molecular mass serine/threonine protein kinases. mTOR is frequently upregulated in cancer and is a clinically validated cancer treatment target.[1–4] Development of dual-pan PI3K/mTOR inhibitors has also been an area of intense research activity.[4–6] However, as PI3K is upstream of mTOR in the signaling pathway and its isoforms are involved in a number of biological processes, highly potent and specific ATP-competitive mTOR inhibitors may be better tolerated, and exhibit a higher therapeutic index for enhanced clinical efficacy. The extensive conservation of the ATP-binding pockets of mTOR and PI3K makes the development of specific mTOR inhibitors particularly challenging. Recently, we[7–13] and others[14–18] reported on selective mTOR inhibitors.

We previously reported that bridged morpholines on pyrazolopyrimidine (**1**)[10,11] and thienopyrimidine (**2**)[13] scaffolds with a *para*-ureidophenyl substituent led to potent mTOR inhibitors with greater selectivity for mTOR versus PI3K than the corresponding morpholine containing analogs. Molecular modeling suggests that a single amino acid difference (Phe961Leu)[19] in the hinge regions of PI3K and mTOR leads to a deeper pocket in mTOR relative to PI3K that can accommodate the additional steric bulk of the morpholine bridge.[10] We have extended these discoveries to other scaffolds and report here and in the accompanying papers[20,21] our findings with mTOR inhibitors based on a triazine scaffold.



Analogs were synthesized as shown in Schemes 1 and 2. Treatment of cyanuric chloride with one equivalent of morpholine or a bridged morpholine (**3**–**5**) gave mono-substituted dichlorotriazines (Scheme 1). Addition of another equivalent of a different morpholine gave the di-substituted triazine. Alternatively, two equivalents of a morpholine could be added to give the di-substituted chlorotriazine. Coupling with 4-aminophenylboronic acid under Suzuki coupling conditions followed by reaction with triphosgene and an amine gave the corresponding ureas. Analogs **6**–**8** were prepared by reaction of the di-morpholine chlorotriazine intermediate with 2-(difluoromethyl)-1*H*-benzo[*d*]imidazole.

Analogs could also be synthesized from 4-nitrophenyltriazine dichloride (Scheme 2). Reaction of the dichloride with a morpholine derivative gave displacement of chloride to provide the

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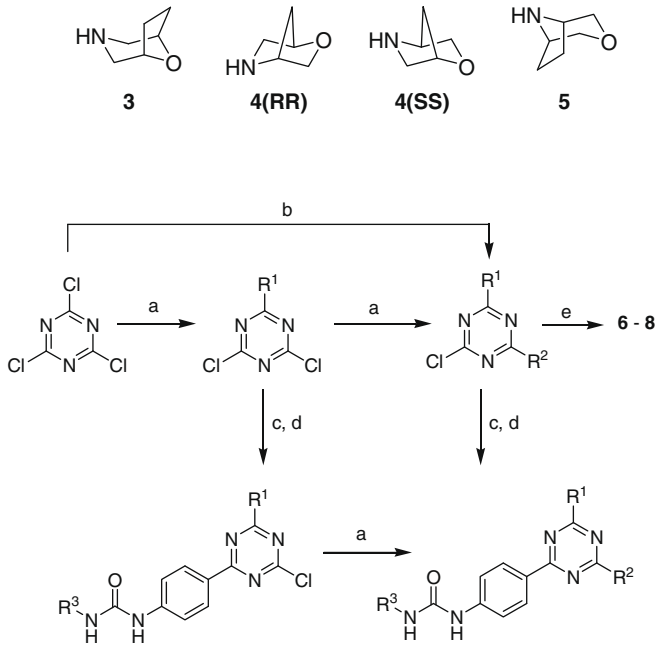
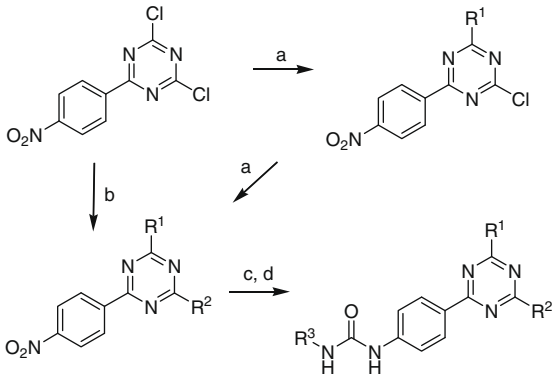
doi:10.1016/j.bmcl.2010.02.045



Scheme 1. R^1, R^2 and R^3 are as defined in the tables. (a) One equiv morpholine or **3**–**5**, Et_3N; (b) two equiv morpholine or **3**–**5**, Et_3N; (c) 4-aminophenylboronic acid, Pd(0), sodium carbonate; (d) triphosgene, triethylamine, then R^3NH_2; (e) 2-(difluoromethyl)-1*H*benzo[*d*]imidazole.

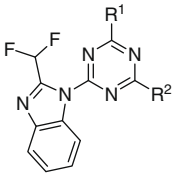


Scheme 2. R^1, R^2 and R^3 are as defined in the tables: (a) 1 equiv morpholine or **3**–**5**, Et_3N; (b) 2 equiv morpholine or **3**–**5**, Et_3N; (c) H_2, Pd/C; (d) triphosgene, Et_3N, then R^3NH_2.

mono-substituted triazine. The remaining chloride could be displaced by a different morpholine derivative. Alternatively, both chlorines could be displaced simultaneously by treatment with two equivalents of a morpholine derivative. Hydrogenation of the nitro group followed by conversion of the resulting aniline to the urea as described above gave the desired analogs.

ZSTK474 (**6**), a dual PI3K/mTOR inhibitor, is a triazine with two morpholines and a difluoromethylbenzimidazole group (Table 1).[22] Molecular modeling studies have shown that a single amino acid difference between PI3K and mTOR (Phe961Leu)[19] leads to a difference in the depth of the binding pocket that can accommodate a bridged morpholine in mTOR but not in PI3K.[10] Based on this observation, we predicted that replacement of the morpholines in **6** with bridged morpholines would lead to increased mTOR selectivity due to the deeper binding pocket in mTOR relative to PI3K. Replacement of both morpholines with a 2,6-bridged morpholine (**3**) gave **7** with a 19-fold increase in selectivity for mTOR over PI3K (Table 1). Consistent with the molecular modeling, mTOR was able to accommodate the bridged morpholine in its deeper binding pocket. In contrast, the PI3K potency decreased significantly. Replacement of only one of the morpholines with a bridged morpholine (**3**) gave **8** with a substantially lesser degree

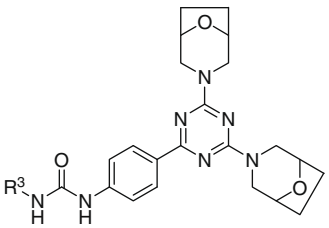
Table 1

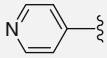
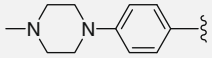


Compd	R^1	R^2	mTOR[a] (nM)	PI3K[a] (nM)	Selectivity[b]	LNCap[a] (nM)
6	Morph.	Morph.	45 ± 19	6.5 ± 0.5	0.15	210
7	3	3	44 ± 5.5	124 ± 40	2.8	680
8	Morph.	3	60 ± 15	24 ± 2	0.41	180

[a] Average IC_{50} ± SEM.
[b] PI3K IC_{50}/mTOR IC_{50}.

Table 2



Compd	R^3	mTOR[a] (nM)	PI3K[a] (nM)	Sel.[b]	LNCap[a] (nM)
9	Me—	2.5 ± 0.5	680	272	190
10	Et—	2.8 ± 0.6	2692	961	180
11		2.6 ± 0.5	2125	802	90
12		0.50 ± 0.01	142	282	45
13		1.7 ± 0.4	87 ± 9	51	2.3

[a] Average IC_{50} ± SEM.
[b] PI3K IC_{50}/mTOR IC_{50}.

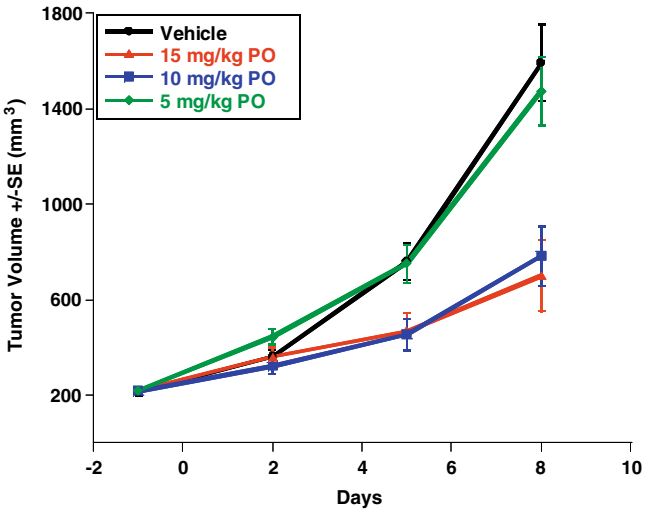


Figure 1. Efficacy of **13** in the U87MG xenograft model.

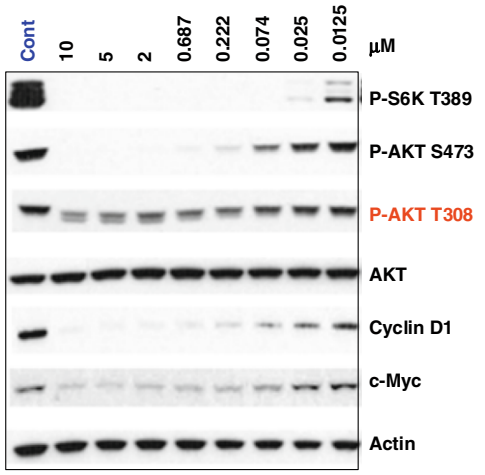


Figure 2. In vitro signaling inhibition of **16** in MDA361 cells.

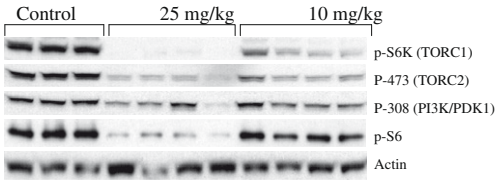


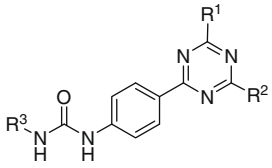
Figure 3. In vivo signaling inhibition of **16** in MDA361 xenografts, 8 h after IV dose. At 10 mpk a strong inhibition of TORC1 and TORC2 and negligible inhibition of PI3K/PDK1 was seen. At 25 mpk near complete inhibition of TORC1 and TORC2 and little inhibition of PI3K signaling was seen.

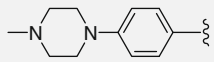
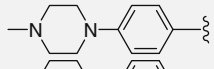
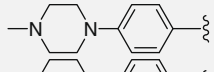
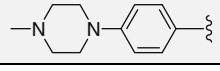
of increase in mTOR selectivity due to the ability of either morpholine or **3** to access the hinge region.

We were encouraged by these results and reasoned that replacement of the benzimidazole in **7** with a 4-ureidophenyl group, which molecular modeling based on the X-ray structure of PI3Kγ has shown to form 3 hydrogen bonds to mTOR on a pyrazolopyrimidine scaffold,[7] would lead to an increase in mTOR potency. Attachment of a 4-methylureidophenyl group to the triazine, in addition to the 2,6-ethylene bridged morpholines, led to a potent and selective inhibitor **9** with good cell activity (Table 2). As seen previously,[7] further increases in selectivity were obtained with analogs containing the ethyl and cyclopropyl ureas (**10** and **11**, respectively). A 4-pyridyl urea gave a subnanomolar mTOR inhibitor **12** while a 4-methylpiperazine arylurea gave **13** with single digit nanomolar inhibition of proliferation of a prostate tumor cell line (LNCap) that is characterized by hyperactive PI3K-Akt-mTOR signaling.[23] We have previously demonstrated that cellular proliferation inhibition in this model correlates with inhibition of mTOR signaling.[8] The data in Table 2 reveal that compounds with similar enzyme IC_{50}s can possess drastically different cellular proliferation IC_{50}s (cf. **9–11**, **13**), possibly reflecting differences in cellular permeability and intracellular distribution. On the basis of its in vitro potency, **13** was assayed in a nude mouse U87MG xenograft model and showed potent inhibition of tumor growth upon oral dosing daily X 5 (Fig. 1). On day 8, tumor/control was 44% for the 10 mpk group.

In an effort to synthesize analogs that maintained the potent activity of **13** but were more selective for mTOR we investigated other bridged morpholine systems. Incorporation of chiral 2,5-methylene bridged morpholines **4(RR)** and **4(SS)** gave analogs **14** and **15**, respectively. As seen previously with the pyrazolopyrimidine scaffold,[10] the analog incorporating the SS enantiomer was

Table 3



Compd	R^1	R^2	R^3	mTOR[a] (nM)	PI3K[a] (nM)	Sel.[b]	LNCap[a] (nM)
14	**4(RR)**	**4(RR)**		130	1819	14	550
15	**4(SS)**	**4(SS)**		35	3438	98	80
16	**5**	**5**		1.0 ± 0.1	899 ± 107	899	1.3
17	**3**	**5**		2.0	150	75	<0.8

[a] Average IC_{50} ± SEM.
[b] PI3K IC_{50}/mTOR IC_{50}.

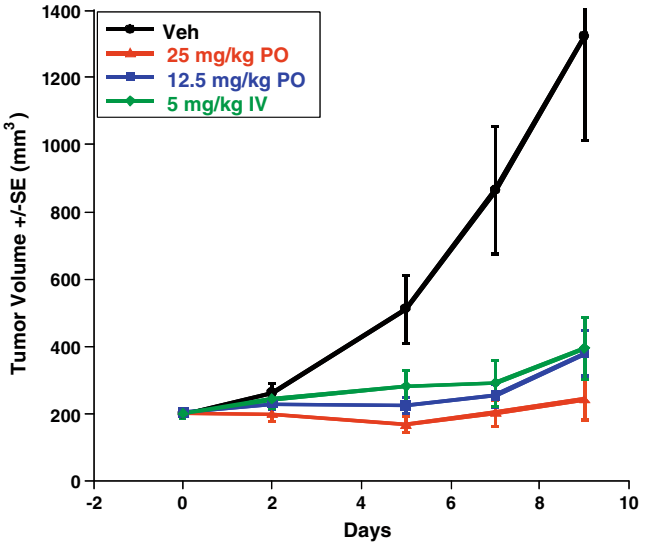


Figure 4. Efficacy of **16** in theU87MG xenograft model.

more potent for mTOR than the analog with the RR enantiomer and displayed higher selectivity versus PI3K (Table 3). However, **15** did not meet our potency or selectivity requirements and was not evaluated further. We had seen previously[10] with the pyrazolopyrimidine scaffold that the 3,5-ethylene bridged morpholine **5** gave analogs that had comparable potency and selectivity to those with the 2,6-bridged morpholine **3**. In contrast, in the case of the triazine ring system, when the 3,5-ethylene bridged morpholine **5** was incorporated, an analog **16** with a 10-fold increase in selectivity was obtained (Table 3). As expected, the analog **17** with one of each bridged morpholine **3** and **5** did not see an increase in selectivity due to the accessibility of both **3** and **5** to the hinge region (Table 3). The mTOR selectivity of **16** translated into in vitro cellular selectivity with the mTOR biomarker S473 being preferentially suppressed with respect to the PI3K T308 biomarker (Fig. 2). In vivo suppression of the S473 biomarker in preference to the T308 biomarker was also seen after 8 h upon dosing nude mice bearing MDA361 tumors with 10 or 25 mpk of **16** (Fig. 3). In the U87MG nude mouse xenograft assay, **16** showed potent inhibition of tumor growth with daily X5 oral dosing (Fig. 4). At 5 mpk, on day 9 a tumor/control ratio of 31% was achieved.

In summary, incorporation of bridged morpholines onto the monocyclic triazine scaffold bearing a ueridophenyl group gave potent and selective mTOR inhibitors. The 3,5-ethylene bridged morpholine gave more selective inhibitors than the 2,6-ethylene bridged morpholines. Potent and selective analog **16** was shown to selectively suppress mTOR biomarkers both in vitro in MDA361 cells and in vivo in nude mice bearing U87MG tumors. Testing of **16** in nude mice bearing U87MG xenografts gave potent activity upon IV or PO dosing.

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