



Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Incorporation of water-solubilizing groups in pyrazolopyrimidine mTOR inhibitors: Discovery of highly potent and selective analogs with improved human microsomal stability

David J. Richard^{a,*}, Jeroen C. Verheijen^a, Kevin Curran^a, Joshua Kaplan^a, Lourdes Toral-Barza^b, Irwin Hollander^b, Judy Lucas^b, Ker Yu^b, Arie Zask^a

^a Chemical Sciences, Wyeth Research, 401 N. Middletown Rd, Pearl River, NY 10965, United States

^b Oncology Research, Wyeth Research, 401 N. Middletown Rd, Pearl River, NY 10965, United States

ARTICLE INFO

Article history:

Received 30 September 2009

Revised 20 October 2009

Accepted 21 October 2009

Available online 25 October 2009

Keywords:

mTOR

PI3K

Kinase

ABSTRACT

A series of highly potent and selective pyrazolopyrimidine mTOR inhibitors which contain water-solubilizing groups attached to the 6-arylethidophenyl moiety have been prepared. Such derivatives displayed superior potency to those in which these appendages were attached to alternative sites. In comparison to unfunctionalized arylethidophenyl compounds, these analogs demonstrated enhanced cellular potency and significantly improved stability towards human microsomes, resulting in an mTOR inhibitor with impressive efficacy in a xenograft model with an intermittent dosing regimen.

© 2009 Elsevier Ltd. All rights reserved.

The serine–threonine kinase mTOR (mammalian Target of Rapamycin) is a component of the phosphoinositide 3-kinase (PI3K) signaling pathway which plays a key role in regulation of cellular growth and has been extensively investigated as an oncology target.¹ In response to increased levels of insulin, nutrients, and energy supply, mTOR mediates proliferation by activation of a number of downstream proteins.^{2,3} The clinical efficacy of derivatives of the natural product rapamycin, such as ToriselTM, has validated mTOR inhibition as an anti-cancer treatment. mTOR signaling involves two distinct complexes, mTOR/raptor (mTORC1) and mTOR/riCTOR (mTORC2). Signaling through mTORC1 results in phosphorylation of proteins such as S6K and 4E-BP1, whereas mTORC2 activates AKT by phosphorylation of serine 473. Rapamycin and its analogs are allosteric inhibitors of mTORC1 but have no effect on mTORC2. In contrast, a small molecule which binds at the ATP-binding site of mTOR would inhibit both complexes. Selective inhibition of mTORC1 by rapamycin results in upregulation of AKT by a negative feedback mechanism,⁴ whereas inhibition of mTORC2 is expected to result in reduced AKT activity. As AKT activation has been correlated with anti-apoptotic effects,^{5,6} inhibition of both mTORC1 and mTORC2 may result in increased anti-proliferative efficacy compared with mTORC1 inhibition alone.

Phosphatidylinositol 3-kinase (PI3K) is a membrane-bound lipid kinase which is found upstream of mTOR. As compounds

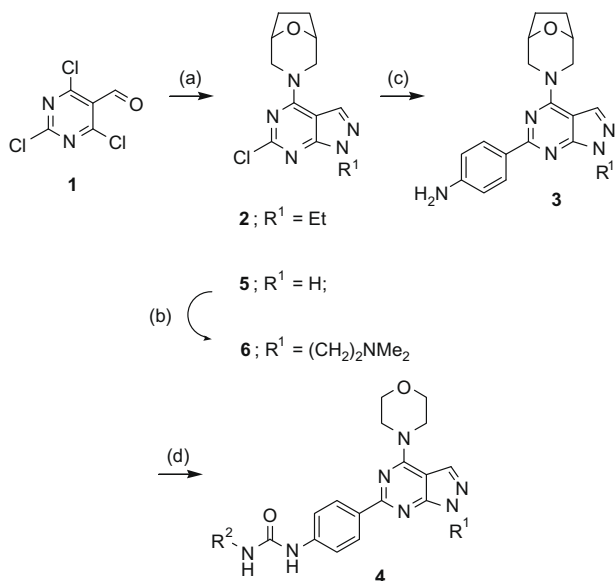
which decrease mTOR activity without affecting PI3K activity may demonstrate improved tolerability relative to dual mTOR/PI3K inhibitors, selectivity over PI3K was deemed desirable. Recent efforts,^{7–11} including our own,^{12–14} have resulted in the first examples of ATP-competitive mTOR inhibitors which show selectivity over PI3K.

We have recently prepared a series of pyrazolopyrimidines which contain substituted morpholines at the 4-position of the pyrazolopyrimidine core.¹⁵ This modification resulted in dramatically improved selectivity over PI3K in comparison with analogs which contained an unsubstituted morpholine ring. The previously disclosed compounds were efficacious in various xenograft tumor models following daily oral dosing.^{16,17} In certain cases, intermittent iv administration of anti-tumor drugs may be preferred over daily oral administration, requiring adequate solubility for formulation. In this Letter we report a thorough examination of the effect of introduction of water-solubilizing groups (WSGs) at various positions of pyrazolopyrimidines that contain substituted morpholines, with the aim of producing potent and selective inhibitors with improved physicochemical and pharmaceutical properties, suitable for intermittent iv administration.

Pyrazolopyrimidines containing the 1-ethyl substituent were prepared by modification of our previously described route (Scheme 1).^{13,14} Trichloropyrimidine aldehyde **1** was converted to pyrazolopyrimidine **2** by treatment with ethyl hydrazine followed by bridged morpholine in a two-step, one-pot process. Suzuki coupling provided an aniline (**3**) which was subsequently

* Corresponding author. Tel.: +1 845 602 2143; fax: +1 845 602 5561.

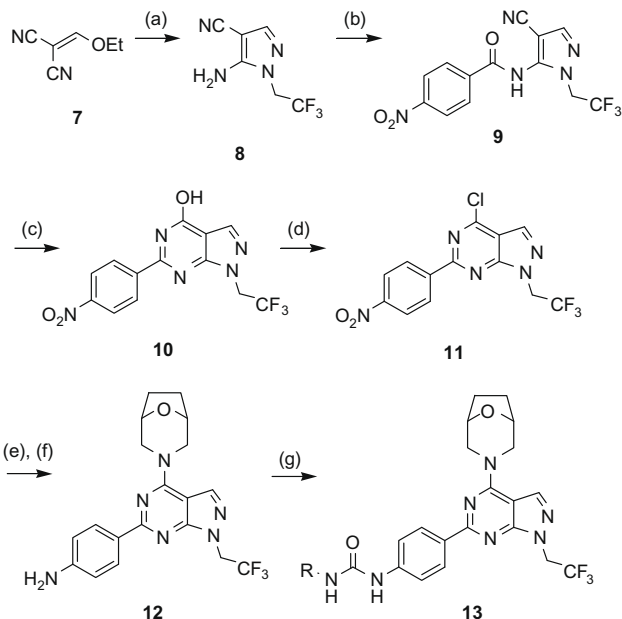
E-mail address: richard4@wyeth.com (D.J. Richard).



Scheme 1. Reagents and conditions: (a) ethyl hydrazine, triethylamine, ethanol, CH_2Cl_2 , then 8-oxa-3-azabicyclo[3.2.1]octane hydrochloride ($\text{R}^1 = \text{Et}$); hydrazine, triethylamine, ethanol, CH_2Cl_2 , then 8-oxa-3-azabicyclo[3.2.1]octane hydrochloride ($\text{R}^1 = \text{H}$); (b) 2-(dimethylamino)ethanol, PPh_3 , DIAD, CH_2Cl_2 ; (c) 4-aminophenylboronic acid pinacol ester, $\text{Pd}(\text{PPh}_3)_4$, Na_2CO_3 , toluene, ethanol; (d) triphosgene, CH_2Cl_2 , then R^2NH_2 .

transformed to the desired urea (**4**) by reaction with triphosgene and the appropriate amine. The synthesis of an *N,N*-dimethylethyl analog was accomplished in a similar fashion. However, in this case a pyrazolopyrimidine lacking substitution at the 1-position (**5**), prepared by reaction of **1** with hydrazine, was functionalized under Mitsunobu conditions to install the alkylamine, yielding intermediate **6**. Suzuki coupling and urea formation then provided the final compound (**4**).

Trifluoroethyl pyrazolopyrimidines were synthesized by the route depicted in Scheme 2. Trifluoroethylpyrazole **8** was gener-



Scheme 2. Reagents and conditions: (a) trifluoroethyl hydrazine, ethanol, water; (b) *p*-NO₂PhCOCl, CH_2Cl_2 , CH_3CN ; (c) 30% H_2O_2 , 2.5 N aq NaOH, ethanol; (d) POCl_3 , cat. DMF; (e) 8-oxa-3-azabicyclo[3.2.1]octane hydrochloride, triethylamine, ethanol; (f) Pd/C, ethyl acetate, THF; (g) triphosgene, CH_2Cl_2 , then selected amine.

ated by the reaction of a malonitrile derivative (**7**) with trifluoroethyl hydrazine. Acylation of the resulting amine, followed by cyclization, provided the pyrazolopyrimidine core (**10**). A sequence involving chlorination, amine displacement, reduction, and urea formation was then utilized to provide the final products (**13**).

To determine the optimal site for the addition of water-solubilizing groups, pyrazolopyrimidine analogs were prepared in which a basic amine was incorporated at two different positions. The choice of sites was influenced by molecular modeling in an mTOR homology model, derived from X-ray crystallographic data of pyrazolopyrimidines bound to PI3K γ , that suggested that solvent could be accessed by groups extending from either the 1-position or the 6-ureidophenyl position.¹³ The effects of introduction of basic amines at these positions were explored for alkyl- and arylureido compounds. When a basic amine was introduced at the 1-position of alkyl ureido **14**, the resulting analog **15** displayed significantly decreased mTOR activity (see Table 1). The mTOR inhibitors in this study were also examined for their growth inhibitory properties in two PTEN deficient cell lines (resulting in overactive PI3K-AKT-mTOR signaling): LNCAP prostate cancer cells and MDA-468 breast cancer cells. We have previously shown that mTOR inhibitors of this class lead to potent inhibition of proliferation of cell lines driven by hyperactive PI3K-AKT-mTOR signaling, whereas cell lines that do not rely on hyperactive PI3K-AKT-mTOR signaling are less sensitive.¹⁴ In comparison with analog **14**, dimethylamino-substituted analog **15** showed reduced cellular activity. Similar results were obtained for introduction of basic amines at the 6-ureidophenyl position; dimethylaminoethyl urea **16** possessed poor mTOR and cellular activity compared to ethyl urea **14**.

In the case of arylureido compounds, introduction of a basic amine at the 1-position also resulted in significantly reduced activity (compare **17** and **18**). In sharp contrast, incorporation of a basic amine at the *para* position of the arylureidophenyl group (**19**) led to an inhibitor with sub-nanomolar mTOR and cellular potency.

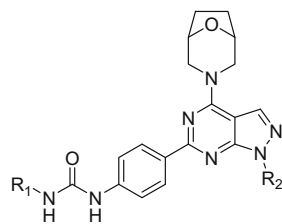
Interestingly, PI3K α activity was significantly less affected by the addition of basic amines than was mTOR activity, particularly in the case of dimethylaminoethyl urea **16** (compare to **14**). This suggests utility for aminoalkyl substituents in the design of PI3K α inhibitors.

The potency of functionalized 3-pyridyl urea **19** prompted a more thorough investigation of analogs containing various groups at this position. Substitution by small amines (as in **20** and **21**, Table 1) resulted in retention of potency and selectivity but a decrease in cellular activity. Cell activity was restored by the inclusion of a larger group, as shown by morpholine derivative **22**. This analog was also notable for its extraordinary selectivity over PI3K α . Incorporation of a basic amine in the form of an ether-linked morpholine resulted in **23** which was equipotent to piperazine derivative **19**.

Analogues with basic, aliphatic amines (**19** and **23**) displayed excellent solubility at pH 3 (>100 $\mu\text{g}/\text{ml}$), thereby facilitating formulation of these compounds for *in vivo* administration. In addition, these compounds displayed marked improvement in human microsomal stability. Whereas 3-pyridyl urea **17** exhibited a half-life of only 4 min in this assay, piperazine-substituted pyridyl urea **19** showed a half-life of greater than 30 min. Previous studies of this class of mTOR inhibitors have demonstrated a good correlation between increased microsomal stability and reduced *in vivo* clearance.^{13–15} This finding suggested that incorporation of appropriate substituents at this site could have significant effects on the pharmacokinetic properties of this class of compounds.

Building upon the success observed with 4-piperazine substituted pyridyl urea **19**, an investigation of the effect of substitution on the 4-position of phenyl ureas was conducted (Table 2). Piperazinophenyl urea **25** was comparable to unsubstituted phenyl urea derivative **24** with respect to mTOR activity. However, the piperazine group provided an advantage over the unsubstituted

Table 1
Introduction of water-solubilizing groups at the 1- and 6-arylidophenyl positions



Compd	R ¹	R ²	mTOR IC ₅₀ ^a (nM)	PI3Kα IC ₅₀ ^a (nM)	Selectivity (over PI3Kα)	LNCaP cell IC ₅₀ (nM)	MDA468 cell IC ₅₀ (nM)
14			0.6	1249	2226	16	80
15			13	3125	240	280	1800
16			51	836	16	350	1250
17			0.1	148	1115	<0.8	1.2
18			2.7	235	89	50	220
19			0.7	196	275	<0.7	0.8
20			0.9	270	302	17	200
21			1.5	282	282	6	80
22			0.3	2154	7302	0.8	8
23			1.0	373	383	<0.7	0.8

^a Average IC₅₀. The average error for IC₅₀ determinations was <25%.

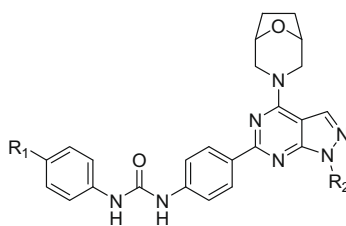
phenyl analog in terms of both cellular activity and human microsomal stability. The presence of a basic, aliphatic amine appeared to be required for enhanced stability, as shown by the decreased stability of pyrrolidine **26** relative to **25**. An analog containing the trifluoroethyl substituent at the 1-position as well as a piperazinophenyl urea (**27**) also displayed excellent activity and good stability. The secondary amine **28** showed activity equivalent to that of the *N*-methyl analog **27**.

The solvent-exposed nature of the 4-aryl position was used to incorporate other WSGs such as acyclic basic amines. Both di- and tri-methylethylenediamine derivatives (**29** and **30**, Table 3)

were somewhat less potent against mTOR than the unsubstituted analog (**24**, Table 2), although the cellular activity of **30** was superior. Amines **29** and **30** also exhibited greater human microsomal stability (14 min in each case) than **24** (human microsomal stability of 5 min). Dimethylbenzylamine **31** showed reduced cellular activity but increased stability. Modification to a primary amine (**32**) led to an analog which was highly potent and stable. The incorporation of an additional basic nitrogen in the form of a piperazine was well-tolerated (**33**). *N,N*-Dimethylethyl ether **34** was equipotent to the unsubstituted phenyl urea **24** (Table 2) but showed significantly improved human microsomal stability.

Table 2

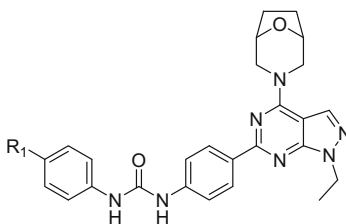
Piperazine and pyrrolidine substitution at the 6-phenylureido- position



Compd	R ¹	R ²	mTOR IC ₅₀ ^a (nM)	PI3Kα IC ₅₀ ^a (nM)	Selectivity (over PI3Kα)	LNCaP cell IC ₅₀ (nM)	MDA468 cell IC ₅₀ (nM)	Micros. Stab. (t _{1/2} , human, min)
24	H	CH ₃	0.3	66	212	0.8	12	5
25	N-methylpiperazine	CH ₃	0.3	51	188	<0.7	<0.7	>30
26	pyrrolidine	CH ₃	6.0	1436	243	55	120	5
27	N-methylpiperazine	CF ₃	0.6	150	237	<0.8	1.5	24
28	4-methylpiperazine	CF ₃	0.2	76	318	<0.8	1.0	—

^a Average IC₅₀. The average error for IC₅₀ determinations was <25%.**Table 3**

Incorporation of other basic or polar substituents



Compd	R ¹	mTOR IC ₅₀ ^a (nM)	PI3Kα IC ₅₀ ^a (nM)	Selectivity (over PI3Kα)	LNCaP cell IC ₅₀ (nM)	MDA468 cell IC ₅₀ (nM)	Micros. Stab. (t _{1/2} , human, min)
29	N-methyl-N-(2-methyl-2-oxoethyl)pyrrolidine	1.7	207	122	2.8	7	14
30	N-methyl-N-(2-methyl-2-oxoethyl)piperazine	0.9	256	295	0.8	0.8	14
31	N-methyl-N-(2-methyl-2-oxoethyl)pyrrolidine	1.1	263	239	7	30	29
32	2-aminoethyl	0.2	21	95	<0.8	<0.8	>30
33	4-methylpiperazine	0.7	320	451	<0.8	0.8	>30
34	N-methyl-N-(2-methyl-2-oxoethyl)pyrrolidine	0.8	136	172	<0.7	8	>30
35	2-hydroxyethyl	0.1	40	335	<0.7	<0.7	11

(continued on next page)

Table 3 (continued)

Compd	R ¹	mTOR IC ₅₀ ^a (nM)	PI3K α IC ₅₀ ^a (nM)	Selectivity (over PI3K α)	LNCaP cell IC ₅₀ (nM)	MDA468 cell IC ₅₀ (nM)	Micros. Stab. (t _{1/2} , human, min)
36		0.1	68	570	<0.7	<0.7	10
37		0.3	109	350	<0.7	<0.7	23
38		0.2	102	586	<0.7	4	>30
39		0.2	175	972	<0.7	<0.7	6

^a Average IC₅₀. The average error for IC₅₀ determinations was <25%.

In addition to basic amines, the incorporation of other polar functional groups was investigated. The presence of hydroxymethyl and hydroxyethyl substituents (as in **35** and **36**, Table 3) provided analogs which were exceptionally potent in both enzyme and cell assays but displayed modest human microsomal stability. Hydrazides **37** and **38** were highly potent and stable. The selectivity enhancement observed by morpholine substitution with 3-pyridyl urea analogs was also exhibited in the phenyl urea series. Thus, analog **39** displayed comparable mTOR binding but reduced PI3K affinity (compare to **24**, Table 2).

Piperazine-substituted phenyl urea **27** was notable for its promising activity and selectivity over PI3K α . This compound was also highly selective against the other PI3K isoforms, as shown in Table 4. This derivative also displayed excellent selectivity over a variety of other kinases; of the 242 kinases examined, none showed greater than 25% inhibition at 2 μ M concentration of **27**.¹⁸

The selectivity observed at the enzymatic level was also evident when **27** was examined at the cellular level. As seen in Figure 1, this compound resulted in complete inhibition of signaling by mTORC1 (P-S6K T389) and significant inhibition of mTORC2 (P-AKT-S473) at a concentration of 25 nM.¹⁹ Phosphorylation of the PI3K/PDK1 biomarker AKT-T308 was not affected, illustrating the highly selective nature of **27**. These data correlated well with inhibition of cellular proliferation, as was previously also observed.^{13–15}

Compound **27** showed good microsomal stability in the desired efficacy species ($T_{1/2}$ >30 min in nude mouse). The pharmacokinetic profile of **27** was characterized by low clearance (7 ml/min/

kg), low Vss (1.2 L/kg), moderate half-life (2.9 h) and high exposure (12,466 h ng/ml) following an iv dose of 5 mg/kg in nude mice. Inhibition of mTOR signaling in vivo was studied in an MDA-361 breast adenocarcinoma nude mouse xenograft model.¹⁹ Eight hours after a 25 mg/kg IV dose, **27** showed complete suppression of TORC1 signaling (p70-S6K), resulting in significant down-regulation of its substrate pS6. In addition, near complete inhibition of TORC2 was observed (p-AKT S473). The strong inhibition of mTOR signaling correlated with good plasma exposure at the 8 h time point (2499 ng/ml).

Daily iv dosing of compound **27** in an MDA-361 nude mouse xenograft model was well-tolerated and resulted in excellent inhibition of tumor growth at low doses (10–25 mg/kg). We also examined use of this inhibitor on an intermittent basis, as such a schedule would be more clinically relevant. We were gratified to find that intermittent (every 5th day) intravenous administration of a 20 mg/kg dose resulted in complete tumor stasis ($p < 0.005$) (Fig. 2). To the best of our knowledge, this finding represents the first example of in vivo anti-tumor efficacy following intermittent dosing of an ATP-competitive mTOR inhibitor.

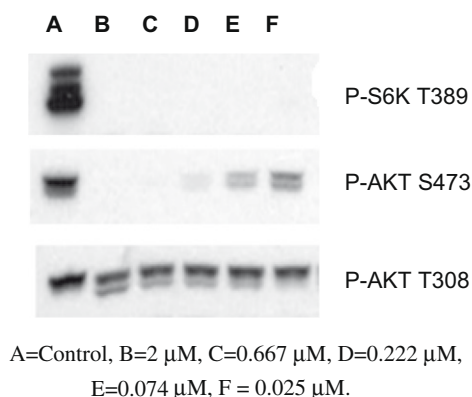


Figure 1. Inhibition of mTOR signaling following treatment of MDA-361 cells with **27**.

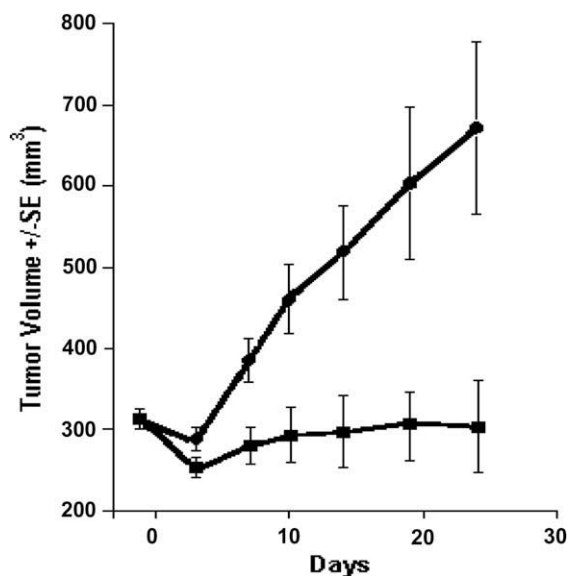


Figure 2. Tumor growth in a nude mouse xenograft model of MDA-361 cells for vehicle (circles) and compound **27** (squares). **27** was dosed at 20 mg/kg IV on days 1, 5, 9, 13, 17 and 21.

Table 4
Inhibition of mTOR and PI3K isoforms by **27**

Compd	mTOR IC ₅₀ (nM)	PI3K α IC ₅₀ ^a (nM)	PI3K β IC ₅₀ ^a (nM)	PI3K γ IC ₅₀ ^a (nM)	PI3K δ IC ₅₀ ^a (nM)
27	0.6	150	>10,000	>10,000	945

^a Average IC₅₀. The average error for IC₅₀ determinations was <25%.

In summary, a variety of potent and selective pyrazolopyrimidine mTOR inhibitors have been prepared. These analogs contained water-solubilizing groups attached to the aryl ring of arylureidophenyl pyrazolopyrimidines. The presence of such moieties resulted in compounds which were generally of equivalent or greater potency against the mTOR enzyme than the unsubstituted derivatives and possessed excellent activity in a cellular proliferation assay. The inclusion of a basic amine significantly enhanced human microsomal stability, a trend which was observed for a variety of functional groups regardless of structure (nitrogen, carbon, and oxygen containing linkers). The combination of excellent potency and stability resulted in an inhibitor (**27**) that demonstrated impressive efficacy in a xenograft model with an intermittent dosing regimen. The highly selective nature of this analog was demonstrated by complete abrogation of mTOR signaling in MDA-361 tumor cells without affecting PI3K signaling. These results demonstrate that modifications of the arylureido moiety may be employed to optimize the physicochemical, pharmaceutical and pharmacokinetic properties of this class of mTOR inhibitors.

Acknowledgements

The authors thank Dr. Li Di and Susan Li for human microsome assays, Angela Bretz for mouse microsome assays, Dr. Inder Chaudhary for pharmacokinetic and plasma exposure studies, Wei-Guo Zhang for mTOR assay development, and Robert Mahoney and Kenneth Kim for biomarker and xenograft data.

References and notes

- Liu, P.; Cheng, H.; Roberts, T. M.; Zhao, J. J. *Nat. Rev. Drug Disc.* **2009**, *8*, 627.
- Guertin, D. A.; Sabatini, D. M. *Cancer Cell* **2007**, *12*, 9.
- Chiang, G. G.; Abraham, R. T. *Trends Mol. Med.* **2007**, *13*, 433.
- Carracedo, A.; Pandolfi, P. P. *Oncogene* **2008**, *27*, 5527.
- Songyang, Z.; Baltimore, D.; Cantley, L. C.; Kaplan, D. R.; Franke, T. F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11345.
- Kennedy, S. G.; Wagner, A. J.; Conzen, S. D.; Jordán, J.; Bellacosa, A.; Tsichlis, P. N.; Hay, N. *Gene Dev.* **1997**, *11*, 701.
- Feldman, M. E.; Apsel, B.; Uotila, A.; Loewith, R.; Knight, Z. A.; Ruggero, D.; Shokat, K. M. *PLoS Biol.* **2009**, *7*, 371.
- Thoreen, C. C.; Kang, S. A.; Chang, J. W.; Liu, Q.; Zhang, J.; Gao, Y.; Reichling, L. J.; Sim, T.; Sabatini, D. M.; Gray, N. S. *J. Biol. Chem.* **2009**, *284*, 8023.
- García-Martínez, J. M.; Moran, J.; Clarke, R. G.; Gray, A.; Cosulich, S. C.; Chresta, C. M.; Alessi, D. R. *Biochem. J.* **2009**, *421*, 29.
- Menear, K. A.; Gomez, S.; Malagu, K.; Bailey, C.; Blackburn, K.; Cockcroft, X.-L.; Ewen, S.; Fundo, A.; Le Gall, A.; Hermann, G.; Sebastian, L.; Sunose, M.; Presnot, T.; Torode, E.; Hickson, I.; Martin, N. M. B.; Smith, G. C. M.; Pike, K. G. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5898.
- Malagu, K.; Duggan, H.; Menear, K.; Hummersone, M.; Gomez, S.; Bailey, C.; Edwards, P.; Drzewiecki, J.; Leroux, F.; Quesada, M. J.; Hermann, G.; Maine, S.; Molyneux, C.-A.; Le Gall, A.; Pullen, J.; Hickson, I.; Smith, L.; Maguire, S.; Martin, N.; Smith, G.; Pass, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5950.
- Yu, K.; Toral-Barza, L.; Shi, C.; Zhang, W.-G.; Lucas, J.; Shor, B.; Kim, J.; Verheijen, J.; Curran, K.; Malwitz, D. J.; Cole, D. C.; Ellingboe, J.; Ayral-Kaloustian, S.; Mansour, T. S.; Gibbons, J. J.; Abraham, R. T.; Nowak, P.; Zask, A. *Cancer Res.* **2009**, *69*, 6232.
- Zask, A.; Verheijen, J. C.; Curran, K.; Kaplan, J.; Richard, D. J.; Nowak, P.; Malwitz, D. J.; Brooijmans, N.; Bard, J.; Svenson, K.; Lucas, J.; Toral-Barza, L.; Zhang, W.-G.; Hollander, I.; Gibbons, J. J.; Abraham, R. T.; Ayral-Kaloustian, S.; Mansour, T. S.; Yu, K. *J. Med. Chem.* **2009**, *52*, 5013.
- Verheijen, J.; Richard, D. J.; Curran, K.; Kaplan, J.; Lefever, M.; Nowak, P.; Malwitz, D. J.; Brooijmans, N.; Toral-Barza, L.; Zhang, W.-G.; Hollander, I.; Ayral-Kaloustian, S.; Mansour, T.; Yu, K.; Zask, A. *J. Med. Chem.* **2009**, in press, doi:10.1021/jm9013828.
- Zask, A.; Kaplan, J.; Verheijen, J. C.; Richard, D. J.; Curran, K.; Brooijmans, N.; Bennett, E.; Toral-Barza, L.; Hollander, I.; Ayral-Kaloustian, S.; Yu, K. *J. Med. Chem.* **2009**, in press, doi:10.1021/jm901415X.
- Curran, K. J.; Verheijen, J. C.; Kaplan, J.; Richard, D. J.; Toral-Barza, L.; Hollander, I.; Lucas, J.; Ayral-Kaloustian, S.; Yu, K.; Zask, A. Unpublished results.
- Yu, K.; Shi, C.; Toral-Barza, L.; Lucas, J.; Shor, B.; Kim, J. E.; Zhang, W.-G.; Mahoney, R.; Gaydos, C.; Tardio, L.; Kim, S. Y.; Conant, R.; Curran, K.; Kaplan, J.; Verheijen, J.; Ayral-Kaloustian, S.; Mansour, T. S.; Abraham, R. T.; Zask, A.; Gibbons, J. J. Unpublished results.
- An assay of the activity of **27** against a panel of 242 protein kinases was performed by SelectScreen™ profiling (Invitrogen).
- Biological methods for determination of cellular mTOR signaling inhibition and efficacy in nude mouse xenograft models have been described in Ref. ¹³ and Supplementary data therein.