Bioorganic & Medicinal Chemistry Letters 20 (2010) 2654–2657

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Triazines incorporating (*R*)-3-methylmorpholine are potent inhibitors of the mammalian target of rapamycin (mTOR) with selectivity over PI3Kα

David J. Richard [a,*], Jeroen C. Verheijen [a], 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 20 January 2010
Revised 4 February 2010
Accepted 8 February 2010
Available online 11 February 2010

Keywords:
mTOR
PI3K
Kinase inhibitors
Cancer
Oncology

ABSTRACT

Potent inhibitors of the mammalian target of rapamycin (mTOR) which contain the triazine scaffold and the (*R*)-3-methyl morpholine moiety have been identified. Such compounds also demonstrated good selectivity over the related lipid kinase PI3Kα. Incorporation of additional functionality at the 4-position of the arylureidophenyl ring resulted in compounds with enhanced cellular activity.

© 2010 Elsevier Ltd. All rights reserved.

The mammalian target of rapamycin (mTOR) is a serine–threonine kinase which is a member of the phosphatidylinositide kinase related kinase (PIKK) subfamily.[1,2] One of the two functional complexes of mTOR, mTORC1, phosphorylates a number of downstream proteins which result in translation initiation, most notably the proteins S6K and 4E-BP1. Rapamycin and its analogs, such as Torisel™, exert their anti-proliferative effects through inhibition of mTORC1. The second complex, mTORC2, however, is unaffected by this class of inhibitors.[1] As mTORC2 activates AKT and increased levels of phosphorylated AKT have been correlated with anti-apoptotic effects,[3,4] inhibition of both mTORC1 and mTORC2 may result in increased anti-proliferative efficacy compared with mTORC1 inhibition alone. A number of research groups,[5–9] including our own,[10–16] have therefore embarked upon the search for ATP-competitive inhibitors of mTOR.

We have recently demonstrated that triazines bearing two 3,5-bridged morpholines may function as potent inhibitors of mTOR.[17] The metabolic instability of the 3,5-bridged morpholine group towards human microsomes prompted a search for alternative morpholine derivatives. Analogs which contained one 3,5-bridged morpholine and either the (*R*)-3-methyl morpholine or tetrahydropyran group successfully maintained the potency of bis-3,5-bridged morpholine derivatives but displayed improved human microsomal stability.[18]

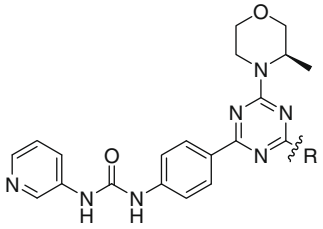
Metabolite identification studies demonstrated that the ethylene bridge of the 3,5-bridged morpholine was the primary site of metabolism.[18] Therefore, the complete elimination of this functional group could be an alternative strategy to achieve increased microsomal stability. We have recently presented several morpholine derivatives, including the (*R*)-3-methyl morpholine group, that can lead to selective mTOR inhibitors on a pyrazolopyrimidine scaffold.[16] In this Letter we demonstrate that triazines in which the 3,5-bridged morpholine has been substituted with a (*R*)-3-methyl morpholine are effective inhibitors of mTOR. The synthesis of these compounds was accomplished using methods which have been previously described.[17,18]

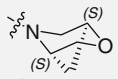
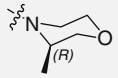
Our initial efforts focused upon the preparation of substituted triazines which possessed one (*R*)-3-methyl morpholine moiety and one additional morpholine derivative (Table 1). Molecular modeling[19] has confirmed that the triazine series of mTOR inhibitors formed the same enzyme binding interactions as did the previously described pyrazolopyrimidine series.[12] Importantly, to maintain selectivity over PI3Kα, the second appendage must not effectively allow for hydrogen bond formation with the PI3Kα hinge region valine (Val2240).[10] The 2,5-bridged morpholine derivative (**1**) met this criteria and showed good potency against mTOR. 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 MDA468 breast cancer cells.[20] Analog **1** displayed IC_{50}s of 250 and 300 nM, respectively, in these cellular assays. Use of (*R*)-3-methylmorpholine (**2**) or tetrahydropyran (**3**)

* Corresponding author. Tel.: +1 845 602 2143; fax: +1 845 602 5561.
E-mail address: david.j.richard@pfizer.com (D.J. Richard).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.02.029

Table 1
Triazine-based mTOR inhibitors containing one 3*R*-methylmorpholine and a morpholine analog



Compd	R	mTOR IC_{50}[a] (nM)	PI3Kα IC_{50}[a] (nM)	Selectivity (over PI3Kα)	LNCaP cell IC_{50} (nM)	MDA468 cell IC_{50} (nM)
1		3.6	1896	525	250	300
2		0.2	644	3009	12	100
3		0.6	593	941	2	80

[a] Average IC_{50}. The average error for IC_{50} determinations was <25%.

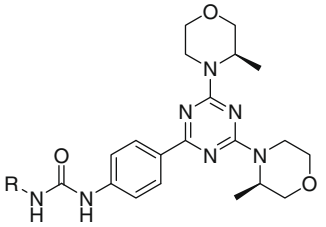
resulted in enhanced activity against mTOR and a corresponding increase in cellular activity. Both compounds also exhibited enhanced selectivity over PI3Kα.

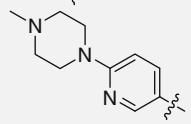
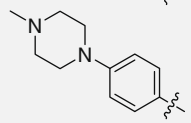
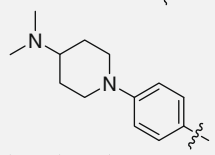
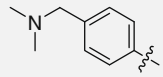
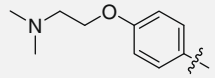
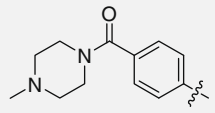
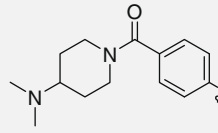
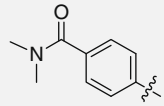
The activity of the 3-pyridyl urea, bis-(*R*)-3-methylmorpholine **2** prompted a thorough investigation of alternative urea derivatives. It has been demonstrated with related pyrazolopyrimidine analogs that the ureidophenyl moiety forms three crucial hydrogen bonds within the enzyme binding pocket.[10] The presence of alkyl ureas on the triazine scaffold resulted in compounds with significantly reduced potency relative to aryl ureas (data not shown); therefore, our efforts centered upon aryl ureas. The 4-pyridyl urea **4** (Table 2) displayed comparable potency against mTOR to the 3-pyridyl analog but reduced selectivity over PI3Kα. In an effort to produce analogs with improved physicochemical properties, the inclusion of a water-solubilizing group at the pyridyl urea was investigated. The addition of such groups has been previously shown to result in compounds with enhanced cellular activity, particularly in the case of piperazine derivatives.[17,18] The *N*-methylpiperazine derivative **5** displayed slightly decreased mTOR activity but maintained good cellular potency. Modification of the pyridyl urea to a phenyl urea (**6**) proved to be well-tolerated, and derivatives of phenyl ureas were therefore further explored.

A number of other bis-(*R*)-3-methylmorpholine triazines with functionalized phenyl ureas were prepared. The inclusion of a basic amine appendage, attached either through a nitrogen, carbon, or oxygen linker, resulted in analogs with good enzyme and cellular potency and selectivity over PI3Kα in the range of 150–400-fold (**7, 8, 9**). Addition of a substituted amide at the 4-position of the phenylureidophenyl moiety resulted in compounds which were subnanomolar against mTOR and displayed enhanced cellular potency, an effect which has previously been observed in related dual mTOR and PI3K inhibitors.[21] This trend was observed with urea derivatives with a variety of structural features, as demonstrated by **10**, **11**, and **12**.

As shown in Table 1, the tetrahydropyran group was also identified as a substituent which resulted in potent mTOR inhibitors (as in **3**). Inclusion of an *N*-methyl piperazine substituent at the pyridyl urea resulted in reduced mTOR potency and cellular activity

Table 2
Bis-3*R*-methylmorpholine triazine analogs

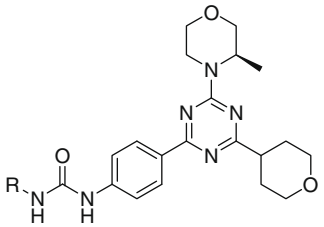


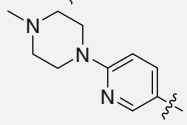
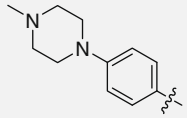
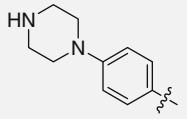
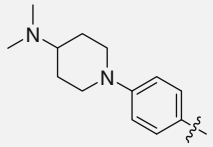
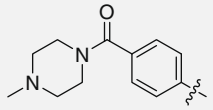
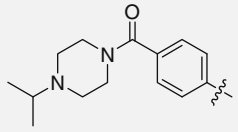
Compd	R	mTOR IC_{50}[a] (nM)	PI3Kα IC_{50}[a] (nM)	Selectivity (over PI3Kα)	LNCaP cell IC_{50} (nM)	MDA468 cell IC_{50} (nM)
2		0.2	644	3009	12	100
4		0.2	173	1116	7	40
5		1.7	846	489	12	40
6		1.1	330	300	3	10
7		1.9	287	153	40	80
8		2.0	413	204	21	50
9		0.7	314	417	8	45
10		1.7	76	45	<0.8	<0.8
11		0.5	55	120	1	5
12		0.5	41	74	<0.8	<0.8

[a] Average IC_{50}. The average error for IC_{50} determinations was <25%.

(**13**, Table 3). However, the piperazine phenyl urea **14** displayed activity comparable to **3** and significantly improved activity against the MDA468 cell line. This result led us to explore piperazine analogs in greater detail. Removal of the methyl group was well-tolerated (**15**), as was relocation of the basic piperazine nitrogen outside of the six-membered ring (**16**). The enhanced cellular potency of 4-amidophenyl urea analogs which was observed in the bis-(*R*)-3-methylmorpholine system was evident on the tetrahydropyran series as well. As such, two piperazine amides (**17** and **18**) showed subnanomolar cellular activity against both LNCaP and MDA468.

Table 3
Triazine analogs bearing the 3*R*-methylmorpholine and tetrahydropyran groups



Compd	R	mTOR IC_{50}[a] (nM)	PI3Kα IC_{50}[a] (nM)	Selectivity (over PI3Kα)	LNCaP cell IC_{50} (nM)	MDA468 cell IC_{50} (nM)
3		0.6	593	941	2	80
13		2.3	220	96	40	52
14		0.7	414	572	1	6
15		0.8	269	340	2	9
16		1.7	402	237	16	38
17		1.0	43	41	<0.8	<0.8
18		0.7	90	131	<0.8	<0.8

[a] Average IC_{50}. The average error for IC_{50} determinations was <25%.

In summary, a variety of potent triazine mTOR inhibitors which showed excellent cellular potency have been prepared. Analogs containing the (*R*)-3-methylmorpholine substituent and a pyridylureidophenyl group displayed greater than 500-fold selectivity for mTOR over the related lipid kinase PI3Kα. The addition of basic amines at the 4-position of the ureidophenyl ring was well-tolerated and offers the opportunity to develop mTOR inhibitors with improved physicochemical properties. Amide derivatives at this site resulted in reduced selectivity over PI3Kα but enhanced cellular activity.

Acknowledgments

The authors thank Wei-Guo Zhang and Lourdes Toral-Barza for mTOR assays and Irwin Hollander for PI3K assays. We also thank Drs. Tarek Mansour, Semiramis Ayral-Kaloustian, John Ellingboe, Robert Abraham, and James Gibbons for program support and contributions.

References and notes

1. Petroulakis, E.; Mamane, Y.; Le Bacquer, O. L.; Shahbazian, D.; Sonenberg, N. *Br. J. Cancer* **2006**, *94*, 195.
2. Baldo, P.; Cecco, S.; Giacomin, E.; Lazzarini, R.; Ros, B.; Marastoni, S. *Curr. Cancer Drug Targets* **2008**, *8*, 647.
3. Songyang, Z.; Baltimore, D.; Cantley, L. C.; Kaplan, D. R.; Franke, T. F. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 11345.
4. Carracedo, A.; Pandolfi, P. P. *Oncogene* **2008**, *27*, 5527.
5. Feldman, M. E.; Apsel, B.; Uotila, A.; Loewith, R.; Knight, Z. A.; Ruggero, D.; Shokat, K. M. *PLoS Biol.* **2009**, *7*, 371.
6. 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.
7. 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.
8. Menear, K. A.; Gomez, S.; Malagu, K.; Bailey, C.; Blackburn, K.; Cockroft, 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.
9. 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.; Molyneaux, 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.

10. Zask, A.; Verheijen, J.; Kaplan, J.; Curran, K.; Richard, D.; Brooijmans, N.; Lucas, J.; Toral-Barza, L.; Zhang, W.-G.; Hollander, I.; Gibbons, J.; Abraham, R.; Ayral-Kaloustian, S.; Mansour, T.; Yu, K. *J. Med Chem.* **2009**, *52*, 5013.
11. 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.
12. Verheijen, J. C.; 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. S.; Yu, K.; Zask, A. *J. Med. Chem.* **2009**, *52*, 8010.
13. Verheijen, J. C.; Yu, K.; Toral-Barza, L.; Hollander, I.; Zask, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 375.
14. Richard, D. J.; Verheijen, J. C.; Curran, K.; Kaplan, J.; Brooijmans, N.; Toral-Barza, L.; Hollander, I.; Yu, K.; Zask, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6830.
15. 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. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1440.
16. Zask, A.; Kaplan, J.; Verheijen, J. C.; Richard, D. J.; Curran, K.; Brooijmans, N.; Bennett, E. M.; Toral-Barza, L.; Hollander, I.; Ayral-Kaloustian, S.; Yu, K. *J. Med. Chem.* **2009**, *52*, 7942.
17. Zask, A.; Verheijen, J. C.; Richard, D. J.; Kaplan, J.; Curran, K.; Toral-Barza, L.; Lucas, J.; Hollander, I.; Yu, K. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2644.
18. Verheijen, J. C.; Richard, D. J.; Curran, K.; Kaplan, J.; Yu, K.; Zask, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2648.
19. Zask, A.; Brooijmans, N., unpublished results.
20. Biological methods for determination of cellular mTOR signaling inhibition and efficacy in nude mouse xenograft models have been described in Ref. 11 and supporting information therein.
21. Dehnhardt, C. M.; Venkatesan, A. M.; Delos Santos, E.; Chen, Z.; Santos, O.; Ayral-Kaloustian, S.; Brooijmans, N.; Mallon, R.; Hollander, I.; Feldberg, L.; Lucas, J.; Chaudhary, I.; Yu, K.; Gibbons, J.; Abraham, R.; Mansour, T. S. *J. Med. Chem.* **2010**, *53*, 798.