Bioorganic & Medicinal Chemistry Letters 19 (2009) 5898–5901

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Identification and optimisation of novel and selective small molecular weight kinase inhibitors of mTOR

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ARTICLE INFO

Article history:
Received 14 July 2009
Revised 18 August 2009
Accepted 19 August 2009
Available online 21 August 2009

Keywords:
Mammalian target of rapamycin
mTOR
Kinase inhibitors
Phosphatidylinositol 3-kinase
PI3K

ABSTRACT

A pharmacophore mapping approach, derived from previous experience of PIKK family enzymes, was used to identify a hit series of selective inhibitors of the mammalian target of rapamycin (mTOR). Subsequent refinement of the SAR around this hit series based on a tri-substituted triazine scaffold has led to the discovery of potent and selective inhibitors of mTOR.

The mammalian target of rapamycin (mTOR) is a key target in the development of antitumour therapies.[1] Activated by growth factor/mitogenic stimulation activation of the phosphatidylinositol 3-kinase (PI3K)/Akt signalling pathway mTOR is a central regulator of cell growth and proliferation. This PI3K-Akt-mTOR pathway is one of the most frequently dysregulated pathways in cancer.[2] mTOR, a serine/threonine kinase of approximately 289 kDa in size, is a member of the evolutionary conserved eukaryotic PI3K like kinase (PIKK) family of proteins, for example, DNA-PK (DNA dependent protein kinase) and ATM (Ataxia-telangiectasia mutated).[3–5]

The known mTOR inhibitor Rapamycin and its analogues (RAD001, CCI-779, AP23573) bind to the FKBP12/rapamycin complex binding domain (FRB), resulting in suppression of signalling to the downstream targets p70S6K and 4E-BP1.[6,7] The potent but non-specific inhibitors of PI3K, LY294002 and wortmannin, have also been shown to inhibit the kinase function of mTOR; however, in this case the catalytic domain of the protein is targeted.[8]

Recently it has been shown that mTOR can exist in an alternative, rapamycin insensitive, complex that signals to Akt.[9] The existence of both a rapamycin sensitive complex (mTORC1) and a rapamycin insensitive complex (mTORC2) may provide an explanation for the differences observed in the earlier work of Brunn et al.[8] and Edinger et al.[10] Therefore, it is proposed that a compound directly targeting the kinase domain of mTOR would inhibit signalling through both mTORC1 and mTORC2 and as such the compound would exhibit a different spectrum of pharmacology compared with rapamycin. As such a number of groups, including our own, have sought to identify selective inhibitors of mTORC1 and mTORC2.[11,12]

Herein, we report the discovery and optimisation of a series of ATP-competitive morpholino-pyrimidines and triazines as potent and selective inhibitors of mTOR kinase.

Our search for inhibitors of mTOR began with a pharmacophore analysis derived from previous experience of another member of the PIKK family; DNA-PK.[13] Additional filtering constraints based on physicochemical properties known to increase the chance of drug-like properties were also considered[14] and the resulting model applied to a database search of over 1 million commercially available compounds. 108 candidates were subsequently nominated for purchase and screened for inhibitory activity against mTOR kinase.[15] 13 compounds exhibited an IC_{50} <15 μM and 2 compounds exhibited an IC_{50} <0.5 μM. In particular bis-morpholine-triazine (**1**) was considered an attractive start point for further chemical optimisation with an mTOR IC_{50} of 0.27 μM[16] and complete selectivity against the other PIKK family members tested (PI3Kα and ATM IC_{50} >10 μM), Figure 1.

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doi:10.1016/j.bmcl.2009.08.069

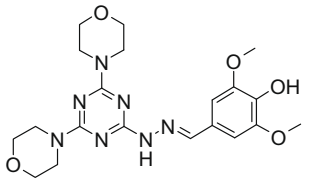


Figure 1. Structure of initial hit compound (**1**).

The morpholine motif is a common pharmacophore present in many inhibitors of the PIKK family[17,18] and, at least for the case of the prototypical PI3K inhibitor LY294002, the morpholine oxygen has been shown to participate directly in a key hydrogen bonding interaction in the ATP-binding site of PI3Kγ.[19] By analogy we reasoned that one of the morpholine oxygens present in (**1**) may also form a key interaction with residue Val 133 in the hinge region of the mTOR kinase domain. Initial modifications of (**1**), outlined in Table 1, were therefore designed to explore this hypothesis. Removal of the oxygen from a single morpholine to give (**2**) resulted in a moderate loss of activity; however, the removal of oxygen from both morpholine rings (**3**) showed a significant loss of potency. These findings are consistent with our view of one of the morpholines being involved in a key hydrogen bonding interaction and possibly suggests that the other morpholine motif could be completely removed to maximise ligand efficiency; a tactic that was employed later in our template optimisation. Interestingly the trizaine ring could be replaced by pyrimidine with only a modest effect on potency (**4**).

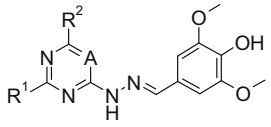
Next our attention turned to replacement of the hydrazone functionality in (**1**). Initial studies aimed at finding a more chemically stable hydazone replacement identified the cyclised analogue (**5**), Figure 2. This compound displayed significantly improved potency against mTOR with an IC_{50} of 0.006 μM in the enzyme assay and high levels of selectivity against PI3Kα (IC_{50} >10 μM).

Further exploration of heterocyclic replacements for the hydrazone motif was carried out with the monosubstituted pyrimidine scaffold, Table 2.

Contrary to our initial expectations the switch to the monosubstituted pyrimidine scaffold resulted in a considerable loss in potency (**5** vs **6**). The nature of the heterocycle appears to have a significant impact on the mTOR potency, presumably due to the subtle differences in the vectors of the two pendant rings as well as the differing electronics. Switching to the pyrazole (**7**) or the thiophene (**8**) resulted in a significant loss of potency whereas the pyridine (**9**) and thiazole (**10**) examples showed similar levels of potency. Interestingly the alternative thiazole isomer (**11**) and the furan (**12**) showed significantly improved potency.

Clearly the pyrimidine scaffold exemplified in (**6**) can provide alternative regioisomers. To determine the influence of this arrangement the reference compound (**13**) was prepared, Figure 3. A loss in potency with respect to compound (**6**) was noted which encouraged the continued use of the 2-morpholine substitution pattern for subsequent SAR investigation.

Table 1
Modification of the trazine portion of the molecule



Compds	R^1	R^2	A	mTOR IC_{50} (μM)
2			N	0.75
3			N	5.9
4			CH	0.66

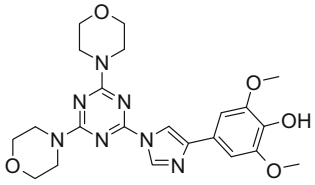
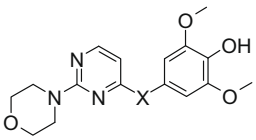


Figure 2. Structure of compound (**5**).

Table 2
Modifications to the central ring heterocycle



Compds	X	mTOR IC_{50} (μM)
6		0.46
7		<50% at 10 μM
8		<50% at 10 μM
9		0.55
10		0.56
11		0.18
12		0.052

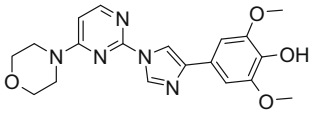


Figure 3. Structure of compound (**13**), mTOR IC_{50} = 0.57 μM.

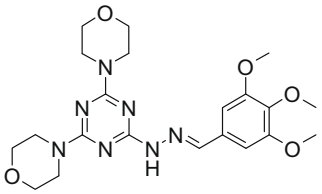


Figure 4. Structure of compound (**14**), mTOR IC_{50} = 30 μM.

To investigate the importance of the phenolic functionality present in these molecules, the reference compound (**14**) was prepared, Figure 4. This compound showed a dramatic loss of potency compared to the initial hit (**1**) suggesting that the presence of this hydrogen bond donor is critical for activity.

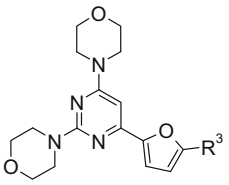
More detailed studies of the SAR in this portion of the molecule were conducted on the optimised pyrimidinylfuranyl template, Table 3.

In line with previous SAR the 4-hydroxy-3,5-dimethoxyphenyl compound (**15**) displays excellent potency. Paring down the trisubstituted system to give either compound (**16**) or (**17**) proved detrimental to potency. Investigation into alternative functional groups with the capacity to act as a hydrogen bond donor appeared to show some promise. In the case of the benzyl alcohol (**19**) the additional methylene spacer presumably allows the positioning of the hydroxyl group to make the putative hydrogen bond with the enzyme necessary for activity. Attempts to find phenolic bioisosteres, such as (**20**) and (**21**) proved unsuccessful.

A selection of the most potent mTOR inhibitors were further profiled in a cellular screen using the growth inhibition of U87MG cells as the readout, Table 4. However, despite excellent levels of enzyme potency and selectivity these compounds showed disappointing levels of activity in cells. Although the reasons for the disappointing cell activity are as yet unknown, it is tempting to speculate that the presence of phenolic functionality in the molecules results in compromised cell permeability. Indeed a difference in apparent permeability (Papp) was observed between compounds (**17**) and (**19**) in an MDCKII assay,[20,21] which may be regarded as supporting this hypothesis (Papp in the A-B direction was 2.2×10^{-6} cm/s and 33×10^{-6} cm/s, respectively). Improving the cellular potency remains an important goal for the further optimisation of this series as does more fully exploring the selectivity profile of this series.

For the analogues described above synthetic access was variously through modification of mono, di or trichloropyrimidine or triazine (**27**, **29**, **32** or **35**) as shown in Scheme 1.

Table 3
Modification of the phenolic functionality



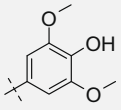
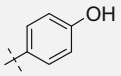
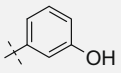
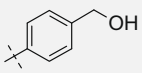
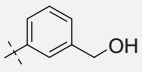
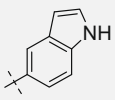
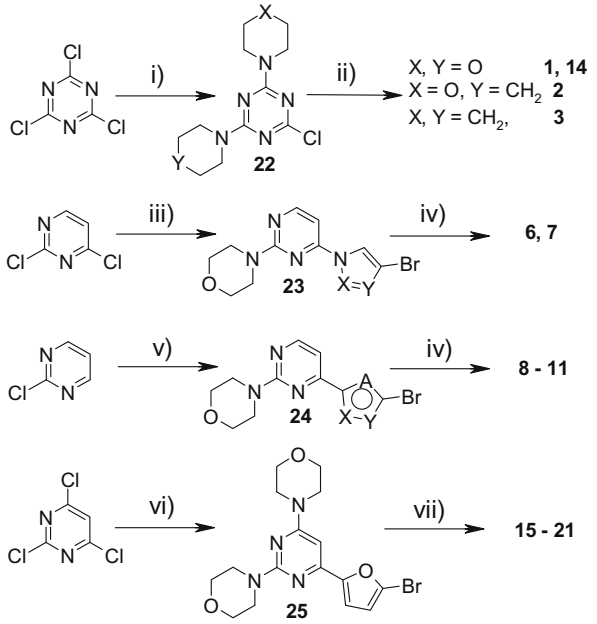
Compds	R^3	mTOR IC_{50} (μM)
15		0.023
16		0.55
17		1.5
18		5.5
19		0.51
20		0.52
21		>10

Table 4
Cellular activity with selected potent inhibitors

Compds	mTOR IC_{50} (μM)	PI3Kα IC_{50} (μM)	U87MG GI_{50} (μM)
5	0.021	>10	24
11	0.18	>10	21
12	0.052	>10	3.7
15	0.023	>10	16



Scheme 1. Reagents and conditions: (i) morpholine or piperidine, acetone, 0 °C or (a) morpholine (50% aqueous solution), $MeOCH_2CH_2OMe$, −15 °C; (b) piperidine, K_2CO_3, DMF, −5 °C; (ii) (a) hydrazine hydrate, ethanol, reflux; (b) 4-hydroxy-3,5-dimethoxybenzaldehyde, TsOH, ethanol, reflux; (iii) (a) 4-bromoimidazole or 4-bromopyrazole, K_2CO_3, DMF, 0 °C; (b) morpholine, NaH, DMF, 0 °C then microwave 120 °C; (iv) 4-hydroxy-3,5-dimethoxyphenyl boronic acid, K_2CO_3, $Pd(Ph_3)_4$, dioxane, microwave 150 °C; (v) (a) dibromoheterocyle, *n*BuLi, DDQ, Et_2O, −78 °C to rt; (b) morpholine, NaH, DMF, 0 °C then microwave 120 °C; (vi) morpholine, ethanol, Et_3N, 0 °C to rt; (vii) (a) 2-furanbornic acid, K_2CO_3, $Pd(Ph_3)_4$, dioxane, microwave 150 °C; (b) NBS, DMF, 0 °C; (c) aryl boronic acid, K_2CO_3, $Pd(Ph_3)_4$, dioxane, microwave 150 °C.

Cyanuric chloride can be sequentially reacted with the appropriate amines to furnish intermediate (**22**). The remaining halogen can be displaced by hydrazine and condensed with the appropriate aldehyde to furnish the desired hydrazone containing compounds. An analogous route could be used to obtain (**4**). Bromoheterocycles could be introduced by either the nucleophilic attack of nitrogen heterocycles on dichloropyrimidine or by deprotonation of 2-chloropyrimidine and subsequent reaction with dibromoheterocycles in the presence of DDQ. Subsequent reaction with morpholine furnished intermediates (**23**) and (**24**). Installation of the 4-hydroxy-3,5-dimethoxyphenyl motif was achieved using Suzuki coupling reactions. An analogous route was used to furnish (**5**) and (**13**) Stirring 2,4,6-trichloropyrimidine with morpholine resulted in reaction in both the 2- and 4-positions. The remaining halogen could be reacted with 2-furanboronic acid in a Suzuki coupling reaction and the subsequent material brominated to furnish intermediate (**25**). A variety of aryl groups were subsequently introduced using Suzuki couplings. An analogous route was used to furnish (**12**).

In conclusion, we have identified a novel series of PIKK family selective inhibitors of mTOR based on a hit found through a pharmacophore analysis. The systematic optimisation of the molecules has resulted in the identification of a number of extremely potent and selective inhibitors of the enzyme mTOR kinase.

Acknowledgements

The authors would like to thank Dr Lisa Smith and Mrs Sharon Maguire for their help in producing the biological data presented.

References and notes

1. Huang, S.; Houghton, P. J. *Curr. Opin. Pharmacol.* **2003**, *3*, 371.
2. Yap, T. A.; Garrett, M. D.; Walton, M. I.; Raynaud, F.; de Bono, J. S.; Workman, P. *Curr. Opin. Pharmacol.* **2008**, *8*, 393.
3. Brown, E. J.; Albers, M. W.; Shin, T. B.; Ichikawa, K.; Keith, C. T.; Lane, W. S.; Schreiber, S. L. *Nature* **1994**, *369*, 756.
4. Sabatini, D. M.; Erdjument-Bromage, H.; Lui, M.; Tempst, P.; Snyder, S. H. *Cell* **1994**, *78*, 35.
5. Abraham, R. T. *Curr. Opin. Immunol.* **1996**, *8*, 412.
6. Fingar, D. C.; Salama, S.; Tsou, C.; Harlow, E.; Blenis, J. *Gene Dev.* **2002**, *16*, 1472.
7. Fingar, D. C.; Richardson, C. J.; Tee, A. R.; Cheatham, L.; Tsou, C.; Blenis, J. *Mol. Cell. Biol.* **2004**, *24*, 200.
8. Brunn, G. J.; Williams, J.; Sabers, C.; Wiederrecht, G.; Lawrence, J. C., Jr.; Abraham, R. T. *EMBO J.* **1996**, *15*, 5256.
9. Sarbassov, D. D.; Ali, S. M.; Sabatini, D. M. *Curr. Opin. Cell Biol.* **2005**, *17*, 596.
10. Edinger, A. L.; Linardic, C. M.; Chiang, G. G.; Thompson, C. B.; Abraham, R. T. *Cancer Res.* **2003**, *63*, 8451.
11. 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.
12. Feldman, M. E.; Apsel, B.; Uotila, A.; Loewith, R.; Knight, Z.; Ruggero, D.; Shokat, K. M. *PLoS Biol.* **2009**, *7*, 371.
13. Hollick, J. J.; Rigoreau, L. J. M.; Cano-Soumillac, C.; Cockcroft, X.; Curtin, N. J.; Frigerio, M.; Golding, B. T.; Guiard, S.; Hardcastle, I. R.; Hickson, I.; Hummersone, M. G.; Menear, K. A.; Martin, N. M. B.; Matthews, I.; Newell, D. R.; Ord, R.; Richardson, C. J.; Smith, G. C. M.; Griffin, R. J. *J. Med. Chem.* **2007**, *50*, 1958.
14. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feency, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
15. Inhibition of mTOR kinase was assayed using full-length mTOR (extracted from HeLa cytoplasmic extract by immunoprecipitation) with a recombinant 4E-BP1 protein substrate and an ATP concentration of 50 μM. Detection of phosphorylated product through an ELISA format was essentially as described in Ref. 8 Figures quoted are the mean of at least 2 determinations and variability around the mean was <50%.
16. mTOR Kinase inhibition experiments were conducted at increasing concentrations of inhibitor (**1**) (50, 100, 200, 350, 500 nM) in varying concentrations of ATP (10–100 μM). The data was plotted using a Lineweaver-Burke double reciprocal plot and revealed characteristic behaviour of competitive inhibition.
17. Verheijen, J. C.; Zask, A. *Drugs Future* **2007**, *32*, 537.
18. Hickson, I.; Zhao, Y.; Richardson, C. J.; Green, S. J.; Martin, N. M.; Orr, A. I.; Reaper, P. M.; Jackson, S. P.; Curtin, N. J.; Smith, G. C. *Cancer Res.* **2004**, *64*, 9152.
19. Walker, E. H.; Pacold, M. E.; Perisic, O.; Stephens, L.; Hawkins, P. T.; Wymann, M. P.; Williams, R. L. *Mol. Cell.* **2000**, *6*, 909.
20. Monolayers of MDCKII (Madin Darby Canine Kidney Cells) cells transfected with the human MDR1 (Multi-Drug Resistance 1) transporter protein were cultured in transwells and used to study the permeability and efflux potential of compounds (**17**) and (**19**) at a concentration of 10 μM. Since neither compound showed significant levels of efflux in this assay, the Papp in the A to B direction is considered to provide a measure of the passive permeability of the compounds in these cells.
21. Keogh, J. P.; Kunta, J. R. *Eur. J. Pharm. Sci.* **2006**, *27*, 543.