Bioorganic & Medicinal Chemistry Letters 27 (2017) 3395–3398

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis and biological evaluation of novel 3*H*-imidazole [4,5-*b*] pyridine derivatives as selective mTOR inhibitors

Lingzhi Zhang [a,c], Tantan Bu [a,c], Xiaobo Bao [a,b], Tingting Liang [a,b], Yiran Ge [b], Yungen Xu [a,b,*], Qihua Zhu [a,b,*]

[a] Jiangsu Key Laboratory of Drug Design and Optimization, China Pharmaceutical University, Nanjing 21009, China
[b] Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 21009, China

ARTICLE INFO

Article history:
Received 20 February 2017
Revised 22 May 2017
Accepted 2 June 2017
Available online 3 June 2017

Keywords:
Mammalian target of rapamycin
mTOR inhibitors
3*H*-Imidazo [4,5-*b*] pyridine
Antitumor
Synthesis

ABSTRACT

A series of 3*H*-imidazo [4,5-*b*] pyridines derivatives were designed and synthesized as selective mTOR inhibitors. The systematic optimization of the molecules resulted in the identification of two compounds **10d** and **10n** with nanomolar mTOR inhibitory activity and selectivity over PI3Kα. Besides, compounds **10d** and **10n** demonstrated attractive potency against human breast cancer cells (MCF-7) and human ovarian cancer cell (A2780).

© 2017 Elsevier Ltd. All rights reserved.

Introduction

The mammalian target of rapamycin (mTOR) is a typical serine/threonine protein kinase including two structural and functional distinct complexes: mTOR complex 1 (TORC1) and mTOR complex 2 (TORC2). It plays an important role in regulating multiple signaling pathways, including the mitotic signal, the nutrient sensing signal and the insulin signal. The activity of mTOR is strongly regulated in response to physiological conditions, and aberrant mTOR signaling occurs in multiple pathologies, including diabetes, cancer and neurodegenerative diseases.[1] Therefore, mTOR has been considered as a target for the treatment of human cancer. In the past decades, a number of mTOR inhibitors have already been approved or are under clinical development,[2] including rapamycin,[3] temsirolimus,[4] everolimus,[5] AZD8055,[6] GDC-0349,[7] INK128[8] and OSI-027[9] (Fig. 1).

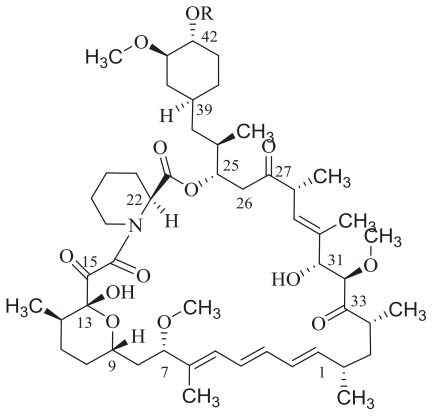
Wendy Lee and coworkers at *Genentech Inc.* identified imidazolopyrimidine **1** as a lead with moderate mTOR potency, low selectivity versus PI3Kα and PI3Kδ (Fig. 2).[10] They reported the first hit to lead optimization of this compound and demonstrated that the replacement of morpholine and aminopyrimidine by a substituted morpholine and an aryl urea on related imidazolopyrimidine scaffold (compound **2**) significantly improved mTOR potency and selectivity over PI3K kinases.[11] In order to identify novel mTOR inhibitor with excellent anti-tumor activity, further studies on analogous of **2** were carried out in this research. Based on the SAR of this series that both morpline and urea moiety play important roles in the mTOR potency, imidazolopyrimidine scaffold was replaced by 3*H*-imidazo [4,5-*b*] pyridine and a novel series of 3*H*-imidazo [4,5-*b*] pyridine derivatives were synthesized as mTOR inhibitors in our study.

The preparation of target compounds **10a**–**p** was described in Scheme 1. The target compounds were synthesized from commercially available 4,6-dichloropyridin-2-amine through six steps. Nitration of **3** with mixed acids of fuming nitric acid and sulfuric acid afforded nitro compound **4**,[12] and then reactions with morpholine or (*S*)-3-methylmorpholine at room temperature gave intermediates **5a** or **5b**. The diamino compounds **6a** or **6b**, which obtained by reduction of the nitro group by $SnCl_2$, were heated with formic acid or acetic acid to give the compounds **7a**–**d**. The key intermediates **8a**–**q**, which obtained by substitution of amino group by RI or RBr, underwent standard Suzuki coupling with pinacol ester **9** to give target compounds **10a**–**p**.

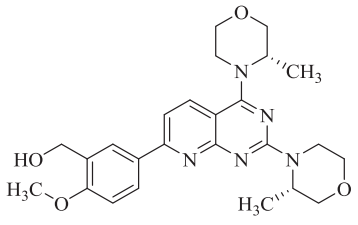
All target compounds were evaluated in vitro for their mTOR enzyme inhibition activity. The results were summarized in Table 1. As shown in Table 1, all target compounds except compound **10l**

* Corresponding authors at: Jiang Su Key Laboratory of Drug Design and Optimization, Department of Medicinal Chemistry, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China.
E-mail addresses: xu_yungen@hotmail.com (Y. Xu), zhuqihua@vip.126.com (Q. Zhu).
[c] Both authors contributed equally to this work.

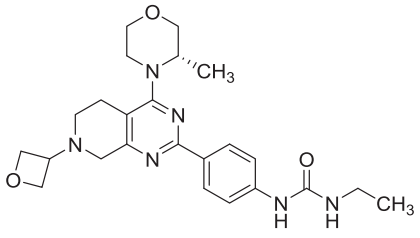
http://dx.doi.org/10.1016/j.bmcl.2017.06.010
0960-894X/© 2017 Elsevier Ltd. All rights reserved.



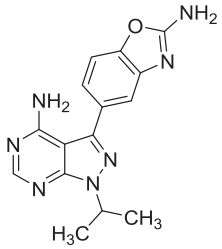
Rapamycin: R=H;
Temsirolimus: R=COCH(CH$_2$OH)$_2$CH$_3$
Everolimus: R=CH$_2$CH$_2$OCH$_3$



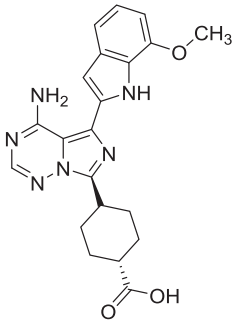
AZD8055, Phase 1



GDC-0349, Phase 1

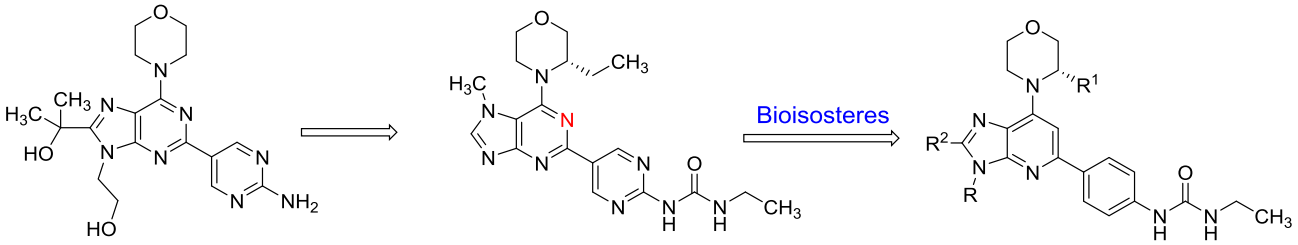


INK 128, Phase 1



OSI-027, Phase 1

Fig. 1. Structures of representative mTOR inhibitors.



1
mTOR *Ki*=72 nM
PI3Kα *Ki*=2 nM, PI3Kα/mTOR=0.03
PI3Kδ *Ki*=4 nM, PI3Kδ/mTOR=0.06

2
mTOR *Ki*=4 nM
PI3Kα *Ki*>10,000 nM, PI3Kα/mTOR>2600
PI3Kδ *Ki*>10,000 nM, PI3Kδ/mTOR>2600

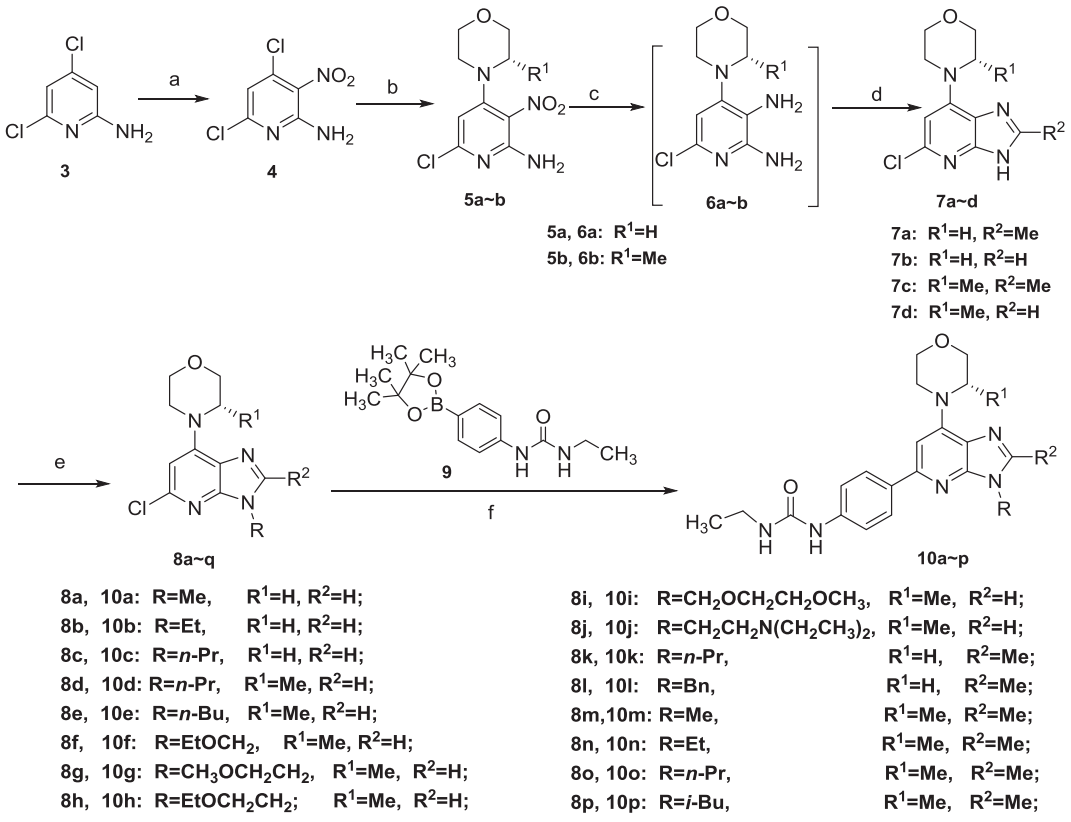
Target compounds 10a~p

Fig. 2. Structures and design strategy for target compounds **10a**–**p**.

demonstrated varied inhibitory activities against mTOR at the concentration of 0.1 μM. Among these compounds, compound **10c** (IC_{50} = 399 nM), which contained an *n*-propyl group at R position, showed higher inhibitory activity than methyl and ethyl substituted compounds **10a** and **10b**. When the hydrogen atom at R^1 position was substituted by methyl, compound **10d** (IC_{50} = 290 nM) exhibited good inhibitory activity. But with the increasing size of R (**10e**–**10j**), the inhibitory activity decreased, which indicated that smaller R group was optimal for mTOR enzyme inhibitory activity. Similar phenomenon was observed when R^1 was hydrogen atom and R^2 was methyl, *n*-propyl substituted analogs **10k** showed higher mTOR inhibitory activity than **10l**. When R^1 and R^2 were both substituted by methyl, compound **10n** (IC_{50} = 332 nM) with ethyl substituted at R position showed the highest mTOR enzyme inhibition activity compared with **10m**, **10o** and **10p**.

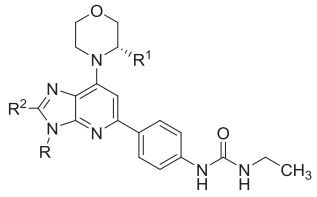
Based on the above results, compounds **10d** and **10n** were chosen to evaluate their PI3Kα enzyme inhibition activity. As shown in Table 2, none of them displayed significant inhibition against PI3Kα at the concentration of 10.0 μM. Therefore, **10d** and **10n**, as potent mTOR inhibitors, simultaneously exhibited acceptable selectivity over PI3Kα.

Encouraged by the favorable enzymatic activity of **10d** and **10n**, compounds **10d** and **10n** were selected to evaluate their anti-proliferative efficacies against several human tumor cell lines, including human breast cancer cells (MCF-7) and human ovarian cancer cell (A2780). GDC-0349, a selective mTOR inhibitor under phase I clinical trial, was employed as the positive control. As illustrated in Table 3, compounds **10d** and **10n** exhibit different degrees of inhibitory activity against MCF-7 and A2780 cell lines, though less efficient than the positive drug GDC-0349. Compounds **10n**



Scheme 1. Synthesis route for target compound **10a**–**p**. Regents and conditions: (a) fuming HNO_3, *conc.* H_2SO_4, 0–5 °C, 12 h; (b) DCM, DIEA, morpholine or (*S*)-3-methylmorpholine, r.t., 2 h; (c) $SnCl_2{\cdot}2H_2O$/HCl, THF, 65 °C, 4 h; (d) formic acid or HOAc/PPA, reflux, 24 h; (e) RI or RBr, K_2CO_3, DMF, r.t., 12 h; (f) **9**, K_2CO_3, $Pd(PPh_3)_4$, DMF, H_2O, 90 °C, 20 h.

Table 1
The structures and mTOR enzyme inhibition activity of compounds **10a**–**p**.



Compound	R	R^1	R^2	Inhibition (%)[a]	IC_{50} (nM)
10a	Me	H	H	14	ND[b]
10b	Et	H	H	20	422
10c	*n*-Pr	H	H	23	399
10d	*n*-Pr	Me	H	33	290
10e	*n*-Bu	Me	H	19.5	425
10f	$EtOCH_2$	Me	H	22.5	403
10g	$MeOCH_2CH_2$	Me	H	16	ND
10h	$EtOCH_2CH_2$	Me	H	12.5	ND
10i	$CH_3OCH_2CH_2OCH_2$	Me	H	20	410
10j	$Et_2NCH_2CH_2$	Me	H	14	ND
10k	*n*-Pr	H	Me	15	ND
10l	Bn	H	Me	2	ND
10m	Me	Me	Me	13	ND
10n	Et	Me	Me	30	332
10o	*n*-Pr	Me	Me	19	423
10p	*i*-Bu	Me	Me	19	430
GDC-0349				59	

[a] The mTOR inhibition of compounds at 100 nM.
[b] ND: Not determined.

Table 2
The inhibitory activities of compounds **10d** and **10n** against mTOR and PI3Kα.

Compound	IC_{50} (μM)	
	mTOR	PI3Kα
10d	0.29	>10.0
10n	0.33	>10.0
GDC-0349	0.039	4.2

Table 3
Cellular activity with selected potent inhibitors **10d** and **10n**.

Compound	IC_{50} (μM)	
	MCF-7	A2780
10d	0.064 ± 0.020	3.11 ± 1.72
10n	0.018 ± 0.0087	2.71 ± 1.15
GDC-0349	0.0058 ± 0.0028	0.33 ± 0.14

(IC_{50} = 0.018 ± 0.0087 μM) exhibits the best inhibitory activity of human breast cancer cells MCF-7 and human ovarian cancer cell A2780, revealing its potential for further improvement.

Conclusion

In summary, a novel series of 3*H*-imidazole [4,5-*b*] pyridine derivatives as selective mTOR inhibitors have been designed and synthesized. Two target compounds were evaluated for their mTOR inhibitory activity. In general, compounds **10d** and **10n** with nanomolar mTOR inhibitory activity also demonstrated attractive potency against the tested cell lines, including MCF-7 and A2780. Besides, compounds **10d** and **10n** were identified to be selective over PI3Kα. Further studies will be carried out in the near future.

Acknowledgments

We are thankful for the financial support of Outstanding Scientific and Technological Innovation Team of Jiangsu Province of China (2015).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.06.010.

References

1. Dazert E, Hall MN. *Curr Opin Cell Biol*. 2011;23:744.
2. Mark EW, Kulik G. *Bioorg Med Chem*. 2013;21:4063.
3. Vézina C, Kudelsk A, Sehgal SN. *J Antibiot*. 1975;10:721.
4. Hudes G, Carducci M, Tomczak P, et al. *N Engl J Med*. 2007;356:2271.
5. Papadimitrakopoulou V, Soria JC, Douillard JY, et al. *ASCO Annu Meet Proc*. 2007;25:7589.
6. Pike KG, Malaqu K, Hummersone MG, et al. *Bioorg Med Chem Lett*. 2013;23:1212.
7. Raynaud FI, Eccles SA, Patel S, et al. *Mol Cancer Then.*. 2009;8:1725.
8. Zhang H, Dou J, Yu Y, et al. *Apoptosis*. 2015;20:50.
9. Wagle N, Grabiner BC, Van AEM, et al. *Cancer Discovery*. 2014;4:554.
10. Lee W, Ortwine DF, Bergeron P, et al. *Bioorg Med Chem Lett*. 2013;23:5097.
11. (a) Verheijen JC, Yu K, Toral-Barza L, Hollander I, Zask A. *Bioorg Med Chem Lett*. 2010;20:375;
(b) Curran KJ, Verheijen JC, Kaplan J, et al. *Bioorg Med Chem Lett*. 2010;20:1440.
12. Stupple PA, Walker SR, Pinson JA, et al. WO 2014128465; 2014.