Bioorganic & Medicinal Chemistry Letters 22 (2012) 5995–5998

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Modulation of cofilin phosphorylation by inhibition of the Lim family kinases

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ARTICLE INFO

Article history:
Received 1 May 2012
Revised 27 June 2012
Accepted 2 July 2012
Available online 21 July 2012

Keywords:
LIMK1
LIMK2
Cofilin phosphorylation
Actin cytoskeleton

ABSTRACT

A series of aminothiazoles that are potent inhibitors of LIM kinases 1 and 2 is described. Appropriate choice of substituents led to molecules with good selectivity for either enzyme. An advanced member of the series was shown to effectively interfere with the phosphorylation of the LIM kinases substrate cofilin. Consistent with the important role of the LIM kinases in regulating cytoskeletal structure, treated cells displayed dramatically reduced F-actin content.

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The actin cytoskeleton plays a key role in a number of cellular processes including migration, cytokinesis, and endocytosis. Given the important functions of the cytoskeleton, the dynamics of actin polymerization are highly regulated. One important class of actin-binding proteins is the ADF/cofilins. These proteins bind to F-actin and facilitate depolymerization. The key biological function of these proteins is illustrated by the fact that knockout of the n-cofilin gene is embryonically lethal in mouse.[1] The actin severing activity of cofilin is regulated by an inactivating phosphorylation on serine-3. Four kinases are known to phosphorylate cofilin on serine-3, the two LIM domain-containing protein kinases, LIMK1[2] and LIMK2, and the related testis-specific protein kinases, TESK1 and TESK2.[3] Recently, small molecule LIMK2 inhibitors have been reported as potential therapeutics for treatment of intraocular pressure and associated glaucoma.[4]

A number of studies indicate that inhibition of the LIMKs or TESKs might be of value as new targets for cancer chemotherapy. LIMK1 has been suggested to be an important regulator of invasion and metastatic growth.[5] A recent definitive study using siRNA and a small molecule inhibitor delineated the critical role played by LIMK1/2 in invasive path generation by tumor and tumor-associated stromal cells.[6] Overexpression of LIMK1 has also been suggested to make PC12 cells resistant to apoptosis.[7] Additionally, the tumor suppressor LATS1 has been reported to be a negative modulator of LIMK1.[8] LIMK2 has recently been shown to play an important role in normal mitotic spindle formation and decreased LIMK2 expression may augment tumor cell sensitivity to microtubule destabilizing agents.[9] For these reasons, we initiated a program to identify selective inhibitors of LIMK1/2.

A limited screening effort of our in house chemical library led to the identification of series of 2-aminothiazol-5-yl-pyridimidines (**1**) with moderate activity for LIMK1. Given the earlier success in converting aminothiazoles to advanced drug candidates,[10] we decided to attempt the optimization of these leads for LIMK inhibition. Based on the crystal structure of these inhibitors bound to the p38 kinase,[11] we proposed a similar binding mode for LIMK and

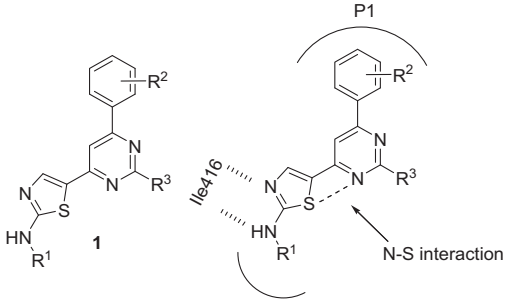

Figure 1. Proposed interactions of 1 with LIMK1.

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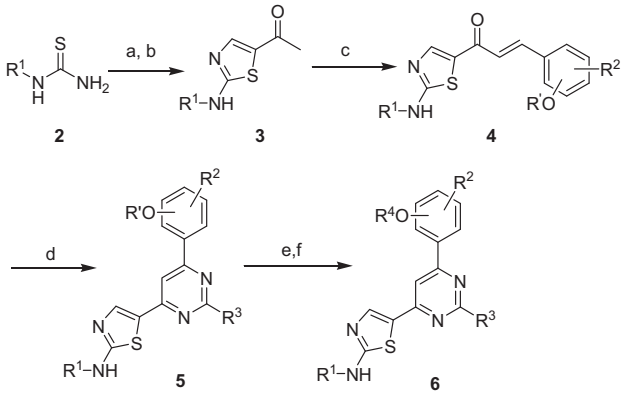
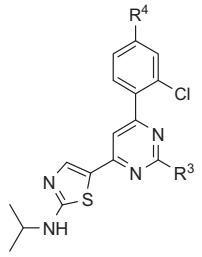
0960-894X/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.bmcl.2012.07.002



Scheme 1. Reagents and conditions: (a) DMF-DMA, EtOH, 95%; (b) chloroacetone, CH_3CN, 94%; (c) substituted benzaldehyde, KOH, EtOH, 40 °C, 70–85%; (d) $R^3C(=NH)NH_2$ hydrochloride, DBU, DMF, 90 °C, 15–30%; (e) For R̂ = Bn: TFA, 60 °C, 80–95%; for R̂ = Me: BBr_3, DCE, 85–96%; (f) 1° alkyl iodide, K_2CO_3, DMF, 30–60% or 2° alkyl bromide, NaI, NaH, DMF, 12-40%.

Table 1
Isopropylaminothiazol-5-yl pyrimidines: Effect of substituents on the 6-phenyl ring



Compds	R^3	R^4	LIMK1 IC_{50}, nM[a]	p38α IC_{50}, nM[a]
9	2-Pyridyl	OEt	485	3940
10	2-Pyridyl	H	252	4
11	2-Fluorophenyl	OEt	>50000	590
12	2,5-Pyrazinyl	OEt	368	1999

[a] IC_{50} values were derived from dose response curves generated from triplicate data points.

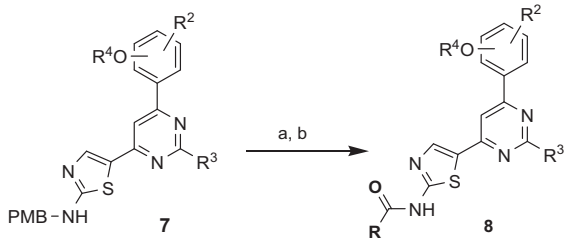
hypothesized that LIMK activity and selectivity could be garnered through modulation of the substituents at R^1, R^2 and R^3 (Fig. 1).

The typical synthesis for the inhibitors is outlined in Scheme 1. The appropriately substituted thiourea **2** was condensed with DMF–DMA and then cyclized with chloroacetone to generate aminothiazoloketone **3**. The ketone then reacted under Claisen-Schmidt conditions to give an intermediate enone **4** which was directly converted to the pyrimidine **5** by cyclization with a substituted carboxamidine. The products **5** could be further derivatized by deprotection of the phenol moiety followed by realkylation using classical techniques. An alternate synthesis of this class of kinase inhibitors has also been recently reported.[12]

The *N*-acylated analogs were prepared by a minor modification of the chemical sequence (Scheme 2). The core pyrimidine was constructed as described above using commercially available *p*-methoxybenzylthiourea. The resulting pyrimidine **7** could then be deprotected with TFA and acylated to give the desired amides, carbamates and ureas.

Initial SAR analysis of the hits indicated that selectivity over p38α could be achieved through modulation of the substituents on the 6-phenyl ring (R^4) in conjunction with a 2-pyridyl ring at R^3 (Table 1). Specifically, introduction of ethoxy at R^4 position retained LIMK1 activity while significantly improving the selectivity over p38α (**9**, **10**). Bioisosteric replacement of the 2-pyridyl ring with 2-fluorophenyl (**11**) negated the LIMK1 activity while replacement of the 2-pyridyl with a 2-pyrazinyl ring (**12**) slightly improved biochemical activity while maintaining selectivity. The 2-pyrazinyl ring was then fixed at R^3 for the duration of this work.

With an angle on selectivity in hand, we next sought to more thoroughly examine the SAR around 6-phenyl ring (Table 2). Modulation of the size of the 4-substituent had an effect on the overall



Scheme 2. Reagents and conditions: (a) TFA, 60 °C, 80–90%; (b) RCOCl, TEA, THF, 40–55%; or ROCOCl, TEA, THF, 40–50%; or RNCO, TEA, THF, 40–60% or PhOCOCl,TEA,THF, 45–60%, then RNH_2 40 °C, 30–50%.

LIMK1/2 activity. Smaller groups (**13**, **14**) diminished overall activity while larger straight chain alkyl groups (**15**) maintained activity albeit with no improvement in potency. Interestingly, introduction of branched alkoxy groups in this position afforded compounds (**16**, **17**) that were selective for LIMK1 over the three other closely related LIM family members (LIMK2, TESK1/2). Incorporation of an additional *ortho* chlorine atom on the phenyl ring (**18**, R^2 = OMe) significantly increased LIMK1/2 activity by 40- to 70-fold respectively. For compounds containing a 2, 6-dichlorophenyl ring, increasing the alkoxyether chain length or utilizing branched ethers (**19–21**) resulted in reduced LIMK1/2 potencies. Unlike the analogs in monochlorophenyl system, the branched ethers (**20**, **21**) retained moderate LIMK2 activity. Replacement of the 2-chlorine substituent with methyl moiety (**16** vs **22**) modestly improved LIMK1 activity but resulted in diminished LIMK1/2 selectivity. Importantly, moving the alkoxy group from 4-position of the phenyl ring to 5-position reversed the LIMK1/2 selectivity (**23**–**25**) affording compounds that were modestly more active (>5-fold) against LIMK2 than LIMK1.

We next shifted our efforts to investigate the SAR of the aminothiazole side chain. As shown in Table 3, introduction of a methyl carbamate on the aminothiazole produced analogs (**26**, **33**) that showed similar potencies against LIMK1/2 without (**26**) or weakly potent (**33**) TESK1/2 activity. The methyl amide analogs (**27**, **32**, **35**) were more potent LIMK1/2 inhibitors than the corresponding carbamates. Introduction of a urea side chain resulted in compounds (**29**, **30**, **31**) which were not only very potent LIMK1/2 inhibitors, but also showed moderate TESK1/2 activities. In particular, compound **31** showed well balanced activities against both the LIMK family and the TESK family members. Interestingly, when R^1 was a 2, 6-dichloro-4-methoxyphenyl group, both amide (**34**) and urea (**35**) analogs strongly inhibited LIMK1/2 enzymes without displaying any binding activities against TESK1/2. These two compounds were the most selective LIMK1/2 inhibitors prepared.

Excellent selectivity for other kinase families could also be achieved with members of this inhibitor class. When compound **31** was evaluated against an Ambit panel of kinases[14] at 10 μM, excellent selectivity was observed. Of the 307 kinases evaluated, compound **31** only displayed significant binding to 8 members of the panel resulting in control affinity of <5%: Alk4 (4.6%), DDR1 (0.2%), Fused (3.0%), Kit (0.4%), Kit-V559D (0.4%), LIMK2 (0.0%), TGFBR1 (1.0%) and Tie2 (1.1%). No affinity for p38α (100%) was observed.

Subsequent to our SAR investigations, an X-ray structure of the kinase domain of LIMK1 was published.[15] This new information was used to generate a model of **31** bound to its target. As shown