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Identification of 5,6-substituted 4-aminothieno[2,3-*d*]pyrimidines as LIMK1 inhibitors

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ABSTRACT

4-Aminobenzothieno[3,2-*d*]pyrimidines were previously identified in a high throughput screening campaign as LIMK1 inhibitors. Scaffold reversal led to the identification of a series of simple 5,6-substituted 4-aminothieno[2,3-*d*]pyrimidines with low micromolar inhibition of LIMK1.

The LIMK family of proteins is comprised of two family members LIMK1 and LIMK2. These kinases are recognised by two common zinc finger LIM motifs, a PDZ and a catalytic domain. The cysteine rich LIM domains are highly conserved and the catalytic domains share 70% homology.[1]

LIMK proteins are mediators of actin polymerisation and microtubule disassembly. LIMK 1 and 2 are actin binding kinases that phosphorylate cofilin and control the balance of polymerization of globular actin into filamentous actin. Deregulation of this pathway is governed by protein phosphatase Slingshot homolog 1 (SSH1) that de-phosphorylates cofilin. The finite balance of LIMK and SSH1 within microenvironments in the cell determines the reorganisation of the filamentous actin cytoskeleton, and regulation of cell morphology and motility.[2]

Actin cytoskeleton dynamics has been associated with highly metastatic and invasive tumor cells. Studies have found that LIMK1 is highly expressed in a number of malignant tumor cell lines, and has been shown to play a critical role in metastasis. These studies have led to the belief that disruption of the dynamic equilibrium of phospho and non-phospho cofilin will result in reduced metastasis and invasiveness. Hence the development of a small molecule inhibitor of LIMK1, will not only provide insights into the precise role of LIMK1, but may provide a novel approach to cancer therapy.

There is good evidence to suggest the LIMK1 is a viable therapeutic cancer target. However, only a few small molecule inhibitors of LIMK 1 and 2 have been described in literature. Bristol Myers Squibb (BMS) disclosed two classes of inhibitor, pyrazolo series and a 5-thiazolopyrimidine series.[3,4] Some of the pyrazolo series of inhibitors were shown to be potently cytotoxic but it was determined that this was due to off-target (anti-microtubule) activity and that LIMK inhibition did not result in inhibition of cellular proliferation.[3] As BMS were not interested in an anti-metastatic program, development of this series was halted. In an independent study, Scott et al.[5] utilised the BMS pyrazolo inhibitor in a number of invasive functional assays. In this study it was observed that although motility was not affected by inhibition of LIMK in cancer associated fibroblasts, the inhibitor did disrupt invadopodia formation, impairing the ability of path generation by leading cells in collective invasion.

Lexicon pharmaceuticals are the only other organization to disclose LIMK1 and 2 inhibitors; however, these compounds are described for treatment of ocular hypertension and glaucoma.[6–8] Results from this study are ongoing.

More recently we described aminobenzothieno[3,2-*d*]pyrimidines as potent inhibitors of LIMK1.[9,10] Our primary focus in this campaign was to develop a LIMK1 inhibitor as a potential metastatic therapeutic. The series originated from a high throughput screen of a diverse 40000 compound library, whereby a 3-aminothieno[2,3*b*]pyridine-2-carboxamide **1** scaffold was identified and transformed into a 4-aminopyridine **3** with concomitant increase in potency (Fig. 1 and Table 1). Through other iterations it was found that the pyridyl nitrogen atom could be replaced with carbon (**2** and **4**) and further that the thiophene sulfur atom could be replace with a nitrogen atom (**5**). Further substitutions around the aminobenzothieno[3,2-*d*]pyrimidine core revealed that the

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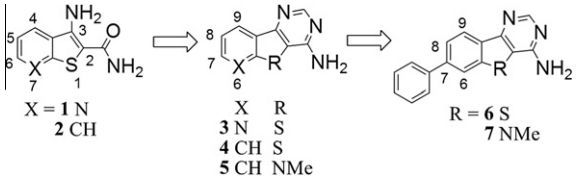


Figure 1. Previous progression summary of LIMK1 inhibitors.

Table 1
LIMK1 inhibition of compounds **1–7**

Compound	IC_{50}, μM	Compound	IC_{50}[a], μM
1	37	**5**	4
2	33	**6**	0.30
3	9	**7**	0.26
4	7		

[a] KinaseGlo assay format[9,10]

7–phenyl compounds **6** and **7** gave a marked increase in potency and could accommodate solubilizing groups to improve physicochemical properties.[9]

Herein we report our efforts on scaffold hopping to give 4-aminothieno[2,3-*d*] pyrimidines **12–15** (Fig. 2). Investigation of the retro synthetic route of such a core revealed the synthon was a 2-aminothiophene. 2-Aminothiophenes are classically constructed utilizing the multi-component Gewald reaction. The Gewald reaction consists of a base mediated reaction of typically using 2-cyanoacetate, sulfur and a ketone (Scheme 1). Given that there are a vast number of ketones readily available, this would allow a simple point of variation to the initial scaffold to probe SAR.

To evaluate the proposal a 5,6-six membered carbocycle 4-aminothieno[2,3-*d*] pyrimidine **13** was synthesized. The Gewald reaction utilising ethyl 2-cyanoacetate allowed high yielding access to the intermediate 2-aminothiophene ester **9**. The aminoacetate moiety was then converted to the hydroxypyrimidine **10** using formamidine acetate at high temperature. Conversion of the hydroxypyrimidine **10** to the 4-chloropyrimidine **11** was performed using phosphorous oxychloride, and this was subsequently converted to the 4-aminopyrimdines **12–15** using ammonia in 1, 4-dioxane at 100 °C (Scheme 1).

Compound **13** was evaluated in an 11 point titration to assess its inhibition of LIMK1 and was shown to possess an IC_{50} of 4 μM. This activity was comparable to the activity of the aminobenzothieno[3,2-*d*]pyrimidine **4**. To further evaluate the thieno[2,3-*d*] pyrimidine scaffold an acyclic, five and seven membered 5,6-carbocycle were also synthesised and evaluated for LIMK1 inhibition. The five membered analogue **14** demonstrated a slight decrease in activity, the acyclic derivative **12** was similar in activity, while the seven membered analogue **15** displayed a slight increase in potency compared with **13** (Table 2).

We then elaborated SAR at the 2 and 4 positions. 2-Substituted compounds **16–24** were purchased commercially (Table 3), while the 4-substituted compounds **25–32** (Table 4) were synthesized utilising the 4-chloro aminothieno[2,3-*d*] pyrimidine intermediate with the appropriate amine, analogous to that represented by ammonium hydroxide in Scheme 1. The results are shown in Tables 3 and 4, and demonstrate the substitution at both the 2 and 4 positions have a detrimental effect on activity. This led us to believe that the binding mode of the thieno[2,3-*d*] pyrimidine compounds may be similar to the original aminobenzothieno[3,2-*d*] pyrimidine series as substituents in equivalent positions also abrogated activity.[9,10]

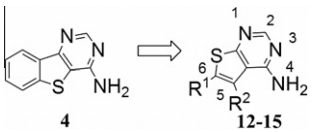
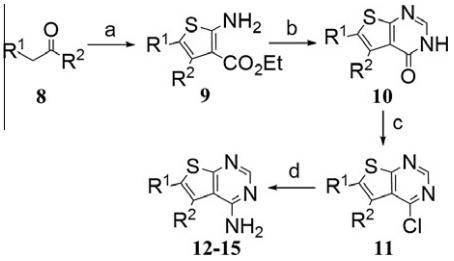
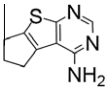
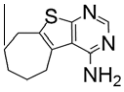


Figure 2. LIMK1 inhibitor scaffold hopping.



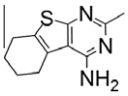
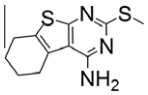
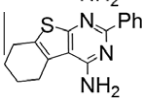
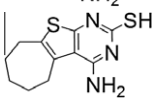
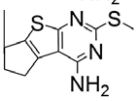
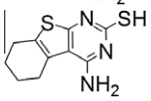
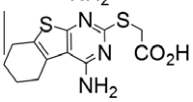
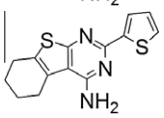
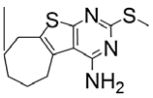
Scheme 1. Synthesis of compounds **12–15**. Reagents and conditions: (a) ethyl 2-cyanoacetate, Et_2NH, S_8, EtOH, 25 °C; (b) formamide, formamidine acetate, 160 °C; (c) $POCl_3$, 90 °C; (d) aq NH_4OH, dioxane, 100 °C, μW.

Table 2
LIMK1 inhibition [a,b] of early compounds **12–15**

#	Structure	IC_{50}	#	Structure	IC_{50}
12		4.4	**13**		4.0
14		5.3	**15**		2.5

[a]KinaseGlo and TR-FRET assay formats[9,10].
[b]IC_{50} values are μM.

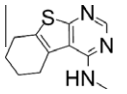
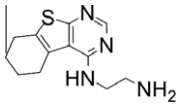
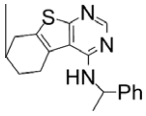
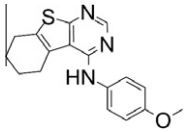
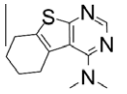
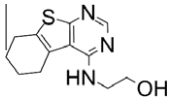
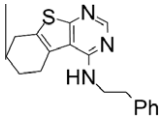
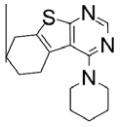
Table 3
LIMK1 inhibition [a,b] of 2-substituted compounds **16–24**

#	Structure	IC_{50}	#	Structure	IC_{50}
16		>100	**17**		>100
18		>100	**19**		>100
20		>100	**21**		>100
22		>100	**23**		>100
24		>100			

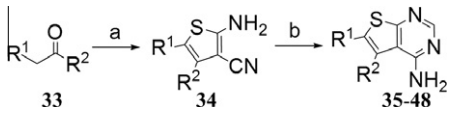
[a]KinaseGlo and TR-FRET assay formats.[9,10]
[b]IC_{50} values are μM.

We next focused our attention to the making alterations to the 5 and 6 positions. In order to make the synthesis amenable to producing analogues more efficiently, we endeavored to shorten the initial synthetic route used from the four steps used in Scheme 1 to two steps (Scheme 2). This was made possible by simply using malonitrile in place of ethyl 2-cyanoacetate. This modification gave the 2-amino-3-nitrile thiophene **34**, which was then transformed

Table 4
LIMK1 inhibition [a,b] of 4-substituted compounds **25–32**

#	Structure	IC_{50}	#	Structure	IC_{50}
25		>100	**26**		>100
27		>100	**28**		>100
29		>100	**30**		>100
31		>100	**32**		>100

[a] KinaseGlo and TR-FRET assay formats.[9,10]
[b] IC_{50} values are μM.



Scheme 2. Synthesis of compounds **35–48**. Reagents and conditions: (a) malonitrile, Et_2NH, S_8, EtOH, 70 °C; (b) formamidine acetate, formamide, 160 °C.

directly into the 4-aminopyrimidine (Scheme 2). However it was noted that the yields of Gewald reaction using malonitrile, compared with using ethyl 2-cyanoacetate (Scheme 1), were dramatically reduced and more byproducts observed. Using this synthetic approach analogues **35–52** were produced (Table 5). Compounds **44** and **45** are mixture of regioisomers as a result of the lack of regio control from the Gewald reaction. The isomers were unable to be separated, so were submitted for LIMK1 evaluation as a mixture. Compounds **49–52** were produced from compound **47** either via hydrolysis or aminolysis.

Of the analogues presented in Table 5, it appeared that a number changes to the 5,6-substitution pattern were tolerated. However, it appeared any heteroatom substituted into the 5,6-carbocycle system was slightly detrimental to activity and polar extensions such as those in **47**, **49–52** led to either a slight (**52**) or complete (**51**) loss of activity. Hydrophobicity was clearly preferable for activity and several simple hydrophobic compounds (**35**, **37–39**, **41**, **45**, **46** and **48**) returned single digit micromolar activity. Indeed, the regioisomeric mixture of compound **44** was the most potent and returned an IC_{50} of 660 nM. The regioisomer responsible for biological activity remains to be determined.

In summary, we have identified 5,6-substituted 4-aminothieno[2,3-*d*] pyrimidines as a promising lead series, which possesses moderate LIMK inhibition. In particular, compound **44**, with a molecular weight of only 253, exhibits submicromolar LIMK1 inhibitory activity with an IC_{50} of 0.66 μM and has potential for significant optimization of drug-like properties.

Acknowledgments

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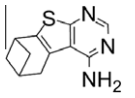
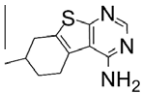
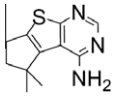
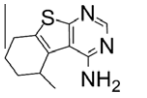
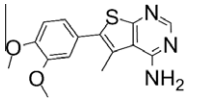
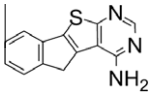
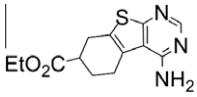
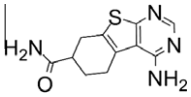
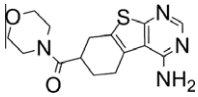
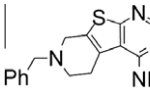
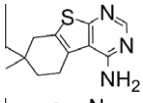
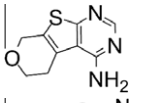
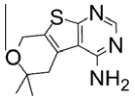
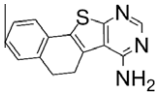
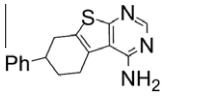
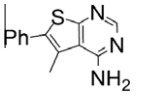
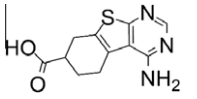
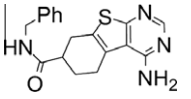
Table 5
LIMK1 inhibition [a,b] of 5,6-substituted compounds **35–48**

#	Structure	IC_{50}	#	Structure	IC_{50}
35		1.8	**36**		18
37		2.1	**38**		2.7
39		3.2	**40**		11
41		2.3	**42**		9.1
43		8.6	**44**[c]		0.66
45		2.8	**46**		3.5
47		12.7	**48**		4.3
49		>20	**50**		17
51		>20	**52**		9.5

[a] KinaseGlo and TR-FRET assay formats.[9,10]
[b] IC_{50} values are μM.
[c] A mixture of regioisomers.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.050.

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