Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis and biological characterization of selective LIMK inhibitors

Sandro Boland [a,*], Arnaud Bourin [a], Jo Alen [a], Jacques Geraets [a], Pieter Schroeders [a], Karolien Castermans [a], Nele Kindt [a], Nicki Boumans [a], Laura Panitti [a], Jessica Vanormelingen [a], Silke Fransen [a], Sarah Van de Velde [b], Olivier Defert [a]

[a] Amakem Therapeutics N.V., Agoralaan Abis, 3590 Diepenbeek, Belgium
[b] Laboratory of Ophthalmology, KU Leuven, 3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 22 May 2015
Revised 30 June 2015
Accepted 2 July 2015
Available online xxxx

Keywords:
Kinase inhibitor
LIMK
ROCK
SAR
LX-7101

ABSTRACT

Inhibitors of LIM kinases are considered of interest for several indications, including elevated intraocular pressure (IOP), cancer, or infection by HIV-1. LX-7101 (Lexicon Pharmaceuticals) was advanced to Phase-I clinical trials as an IOP-lowering agent for treatment of glaucoma. We here discuss the design, synthesis and evaluation of LIMK inhibitors based on a pyrrolopyrimidine scaffold, which represent close analogs of LX-7101. Exploration of structure–activity relationships revealed that many of such compounds, including LX-7101, cause potent inhibition of LIMK1 and LIMK2, and also ROCK2 and PKA. Molecular variations around the various structural elements of LX-7101 were attempted. Substitution on position 6 of the pyrrolopyrimidine scaffold led to the identification of LX-7101 analogs displaying good selectivity versus ROCK, PKA and Akt.

The LIM kinase (Lin-11/Isl-1/Mec-3 domain-containing protein kinase) family consists of two members: LIM kinase 1 (LIMK1)[1] and LIM kinase 2 (LIMK2).[2] LIMK1 and LIMK2 are closely related proteins containing two N-terminal LIM domains, a PDZ domain with two nuclear export signals and one C-terminal kinase domain. The LIM domains are protein-binding domains that are frequently found among cytosolic proteins interacting with the actin cytoskeleton.[3] Consistent with the presence of these LIM domains, both LIM kinases have been shown to influence the architecture of the actin cytoskeleton by regulating the activity of the cofilin family proteins cofilin1, cofilin2 and destrin.[4] Regulation of actin reorganization and contractility allows cells to control their shape, movement, division and secretion. An important group of effectors regulating this process is composed of the small GTPases from the Rho Family. Active Rho GTPases will allow signal transmission towards Rho-associated, coiled-coil containing protein kinases (ROCK1 and ROCK2) and/or p21-activated protein kinases (PAK, PAK2 and PAK4).[5] Both PAKs and ROCKs can phosphorylate and activate LIMKs, which will in turn influence the architecture of the actin cytoskeleton via their action on cofilin1, cofilin2 and destrin. Given the position of LIMK in biological cascades, LIMK inhibitors have been considered of interest for several indications, including cancer,[5] elevated intraocular pressure (IOP) and glaucoma,[6,7] pulmonary hypertension.[8] Recent research has also highlighted a potential role of LIMK in viral diseases.[9] Indeed, inhibition of actin polymerization affects human immunodeficiency virus type 1 (HIV-1) binding and entry into host cells. It has also been shown that LIMK1 is activated by HIV-1 in order to initiate viral infection.[10] Depletion of ROCK1 or inhibition of LIMK1 by a synthetic peptide reduced the release or retroviral particles, as well as cell–cell transmission events.[11]

In spite of those multiple potential indications, the number of reported LIMK inhibitor series remains modest, in comparison to more common kinase targets. The most potent examples of LIMK inhibitors reported to date are based on pyrrolopyrimidines[6,7,12,13] or 2-aminothiazole scaffolds.[14–17] A series of non-competitive (Type III) LIMK inhibitors was also recently reported.[18] From a clinical perspective, LX-7101[7] (Lexicon Pharmaceuticals, Fig. 1) is to date the only LIMK inhibitor that reached clinical trials. LX-7101 was evaluated in a Phase-I trial as an IOP-lowering agent for treatment of glaucoma. This compound however retains non-negligible activity against ROCKs.[6] As ROCK inhibitors themselves represent IOP-lowering agents,[19] it is therefore unclear whether the observed effects of LX-7101 result from activity against LIMKs, ROCKs, or both targets. Selective LIMK inhibitors of sufficient stability and

* Corresponding author.
E-mail address: sandro.boland@amakem.com (S. Boland).

http://dx.doi.org/10.1016/j.bmcl.2015.07.009
0960-894X/

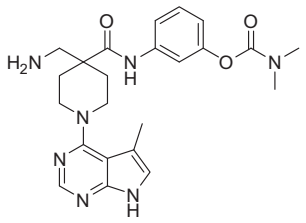


Figure 1. Structure of the LIMK inhibitor LX-7101.

solubility for formulation and administration as eye drops could reportedly not be identified by Lexicon.[7] Selectivity between LIMK and ROCK can however be achieved, as demonstrated by a recent series of bis-aryl urea derivatives incorporating the pyrrolopyrimidine scaffold but displaying strong selectivity versus ROCK.[13] Herein, we report the synthesis and evaluation of new analogs of LX-7101, alongside with the identification and biological characterization of a selective LIMK inhibitor. Our synthetic efforts were focused on modulation of the amine, of the pyrrolopyrimidine scaffold, and on replacement of the carbamate moiety of LX-7101. The latter was attempted with the purpose of optimizing interactions with residues located around the Glycine-rich loop or beyond, which are not conserved between LIMKs and ROCKs. Ester chains were favored in our design, as they left open the possibility of a soft drug approach for avoiding ROCK-associated side effects,[20] in case the ROCK activity observed with LX-7101 could not be eliminated.

Compounds were prepared in 2–5 steps starting from commercially available piperidine derivatives (Fig. 2). Kinase hinge-binding motifs were introduced to the piperidine derivatives through S_NAr reaction of aryl chloride which occurred selectively on the piperidine nitrogen atom.[21] When the aryl groups were not introduced at the last step of the synthesis, isopropanol was replaced by DMSO/water as solvent, providing better solubility of the starting materials, allowing higher temperature and thereby accelerating the reaction. Moreover, hydrolysis of the methyl ester occurred in situ under these conditions, reducing the number of steps for some of the compounds. Otherwise, the carboxylic acid could be generated by a saponification reaction using aqueous NaOH. The amine function was methylated via a reductive amination with formaldehyde. Amide formation with selected anilines could be achieved preferably at low temperature in DCM using T3P as activating agent. For the compounds LX-7101, **1** and **27**, a Boc-protection of the 4-aminomethyl piperidine was required prior to the final coupling reaction.

Variations around the amine and carbamate moieties are summarized in Table 1. Compounds were evaluated against LIMK1 and 2, ROCK2 and PKA. Compounds generally appeared more potent against LIMK2 than against LIMK1. This finding is in line with previous reports regarding LX-7101 and structurally related compounds.[6,7] Replacement of the carbamate moiety of LX-7101 by a methyl ester did not result in significant loss of on-target potency against LIMK1 or LIMK2, as illustrated by **1**. However, both compounds displayed strong activity against ROCK2, and also PKA.

Methylation of the amine function was well tolerated with respect to LIMK activity (LX-7101 vs **2**, or **1** vs **3**). However, both **2** and **3** retained significant activity against ROCK2 or PKA Removal of the methylene between the amine and the piperidine ring resulted in compounds **4** and **5**. Those modifications were again tolerated by LIMK1, but resulted in a significant loss of potency versus LIMK2. As a result, **4** or **5** did not display improved selectivity, in spite of their reduced potency against PKA and ROCK2. Introduction of short spacers between the phenyl and ester moieties (**6**–**7**) did not eliminate activity against PKA and ROCK2. Displacement of the methyl ester on position 4 (**8**) was rapidly put as second priority, as it reduced potency against LIMK2 while maintaining (ROCK2) or even increasing (PKA) off-target activities. Modulation around the alkyl ester (**9**–**12**) had some minor impact on the IC_{50} values for LIMK, ROCK and PKA, but did not result in compounds that could be considered selective.

In kinases, modifications of the hinge-binding motif often have a profound impact on potency and selectivity. In 2004, Aronov and Murko published a 5-point pharmacophore for kinase frequent hitters, wherein four points involved interaction with the kinase hinge region.[22] Pyrrolopyrimidines represent a very common kinase scaffold, with over 1100 references in literature. This includes over 110 references wherein the pyrrolopyrimidine displays a six-membered, nitrogen-containing heterocycle on position 4 (see Supporting information). We believed this situation illustrated, besides a potentially crowded IP space, the overall promiscuity of this scaffold. Consequently, we investigated replacement of the pyrrolopyrimidine as a potential strategy to improve selectivity (Table 2).

The simple, non-substituted pyrrolopyrimidine **13** was first made for comparison. While this compound was essentially similar to **3** with respect to LIMK1, it displayed reduced activity against

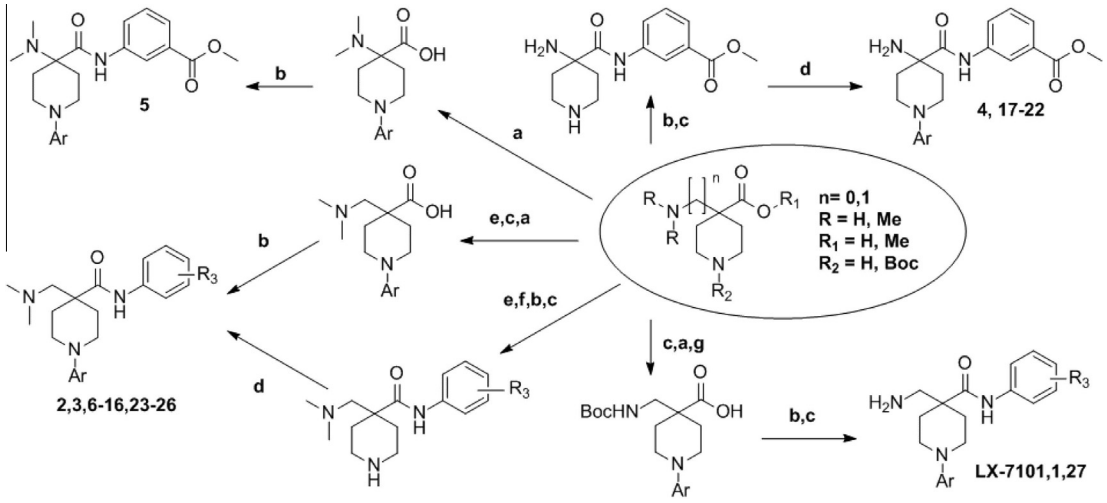
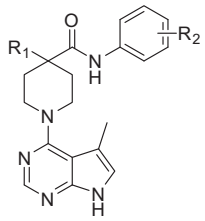


Figure 2. Overview of compound synthesis. (a) ArCl, DIPEA, DMSO/water (1:1), 140 °C, 16 h; (b) $ArNH_2$, T3P, DMAP, DCM, −15 °C, 16 h; (c) DCM/TFA (10:1), rt, 48 h; (d) ArCl, DIPEA, *i*PrOH, 120 °C, 48 h; (e) CH_2O, $NaBH_3CN$, MeOH, rt, 2 h; (f) NaOH, EtOH/water (1:1), reflux, 16 h; (g) Boc_2O, NaOH (1 M)/dioxane (1:2), 0 °C–rt, 2 h.

Table 1
Structure–activity relationships for variation around the amine and carbamate moieties of LX-7101



Compound	R_1	R_2	IC_{50} LIMK1 (nM)	IC_{50} LIMK2 (nM)	IC_{50} ROCK2 (nM)	IC_{50} PKA (nM)
LX-7101	CH_2NH_2	3-$OCONMe_2$	24 ± 3	1.6 ± 0.8	10 ± 4	<1
1	CH_2NH_2	3-CO_2Me	16 ± 5	<1	<1	<1
2	CH_2NMe_2	3-$OCONMe_2$	19 ± 3	<1	40 ± 7	2.6 ± 0.5
3	CH_2NMe_2	3-CO_2Me	27 ± 5	1.5 ± 0.7	8.9 ± 1.6	24 ± 6
4	NH_2	3-CO_2Me	18 ± 4	7.4 ± 2.6	100 ± 16	81 ± 18
5	NMe_2	3-CO_2Me	37 ± 5	12 ± 4	63 ± 6.8	150 ± 28
6	CH_2NMe_2	3-OCH_2CO_2Me	38 ± 6	<1	7.9 ± 2.0	5.4 ± 1.3
7	CH_2NMe_2	3-CH_2CO_2Me	57 ± 12	<1	56 ± 11	7.0 ± 1.9
8	CH_2NMe_2	4-CO_2Me	41 ± 8	7.7 ± 2.9	7.2 ± 0.7	2.7 ± 0.5
9	CH_2NMe_2	3-CO_2*n*-Pr	50 ± 13	2.1 ± 0.4	41 ± 6.2	5.8 ± 0.8
10	CH_2NMe_2	3-CO_2*i*-Pr	41 ± 11	2.2 ± 0.9	53 ± 36	22 ± 3.2
11	CH_2NMe_2	3-CO_2*sec*-Bu	50 ± 13	4.6 ± 1.7	137 ± 23	24 ± 2.6
12	CH_2NMe_2	3-$CO_2CH_2C{\equiv}CH$	7.6 ± 1.4	<1	16 ± 3	2.2 ± 0.5

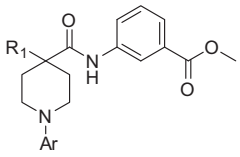
LIMK2 and stronger activity against both ROCK2 and PKA. However, this effect was dependent on the amine displayed on the piperidine moiety, as no real difference could be observed between **4** and **17** in terms of PKA inhibition. The less common pyrazolopyrimidine and pyrazolopyridine isosters **14** and **15** had decreased potency against the four tested kinases, and therefore did not display selectivity towards LIMK. Replacement of the original scaffold by a 6-oxo-pyrrolidinopyrimidine resulted in **16**, which was essentially inactive or weakly active against all four kinases. A series of compounds representing more drastic changes on the hinge-binding moiety was also investigated. Simplification of the pyrrolopyrimidine into a pyrimidine resulted in a complete loss of activity (**18**), which was not rescued by presence of a 6-amino (**19**) or 2-amino (**20**) group. Addition of a carboxamide was also attempted, by analogy to reported aminothiazoles.[14] Compound **21** displayed some degree of selectivity for LIMK, but displayed too little on-target potency to be considered useful.

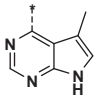
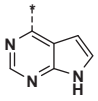
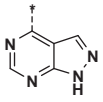
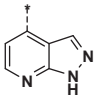
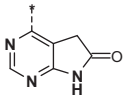
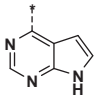
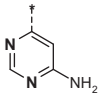
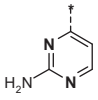
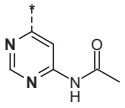
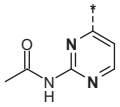
As we could identify no suitable replacement for the pyrrolopyrimidine structure, we investigated the effect of substitutions on this scaffold (Table 3). Small substitutions on position 5 such as cyano or fluoro did not result in more selective compounds (**23** or **24** vs **13**). However, the mere addition of a methyl group on position 6 resulted in **25**, which displayed encouraging selectivity against both ROCK2 and PKA. This newfound selectivity was further increased by combining methyl groups in positions 5 and 6 (**26**) and could be fully transferred to LX-7101 (**27** vs LX-7101). This effect of methyl groups on selectivity appeared of special interest to us. Indeed, introduction of a 6-Me was previously reported as being tolerated in structurally related LIMK2 inhibitors, but the corresponding compound was not evaluated against ROCK.[6] The 5,6-dimethylpyrrolopyrimidine fragment only appears in a small fraction of the references describing pyrrolopyrimidine derivatives as kinase inhibitors and was not reported among analogs of LX-7101.[6,7,12] However, the 6-methyl and 5,6-dimethyl substitution patterns were previously reported to favor selectivity for Akt (PKB) over PKA for structurally related pyrrolopyrimidines.[23] This suggested that such substitution patterns might still allow, or even favor activity of the compounds against Akt and prompted us to evaluate the activity of LX-7101, **26** and **27** against Akt1. Interestingly, LX-7101 displayed potent inhibition of Akt1 under the tested conditions (IC_{50} < 1 nM), which to our knowledge was previously unreported. Meanwhile, neither **26** nor **27** displayed significant inhibition of Akt1 under the same conditions (IC_{50} > 1000 nM for both compounds).

Docking simulations provide a likely explanation for the observed selectivity of **27**. As expected, the preferred binding mode (Fig. 3) positions **27** in the ATP-binding site of LIMK2, with the pyrrolopyrimidine moiety acting as kinase hinge binder, while the phenyl group occupies a vacant space under the Glycine-rich loop. However, its orientation is opposite to the one seen for structurally related compounds in PKA or PKB,[21] with position 6 facing the solvent-exposed front pocket, instead of the back pocket and gatekeeper residue. This leaves position 6 freely available for substitution in LIMK, while little or no space is available in the PKA/PKB orientation. The orientation proposed in LIMK is unlikely to be allowed in PKA, PKB or ROCK, as access to the front pocket is prevented by a C-terminal loop that is typical of AGC kinases. With respect to other interactions, the pyrrolopyrimidine moiety forms two classical hydrogen bonds with the hinge region (I408, backbone). Other polar contacts involve the carbamate moiety (F341, F342, backbone NH), the amine moiety (N456, side chain, D469, side chain) and the amide moiety (K360, side chain). Refinement of the complex structure proposed through docking also suggests the possibility of a water-mediated hydrogen bond with the gatekeeper residue T405. Interestingly, the piperidine moiety is markedly rotated with respect to the plane defined by the pyrrolopyrimidine structure, a conformation that is likely favored by the 5-Me substituent. Several of our findings are in line with those recently reported for bis-aryl urea LIMK inhibitors.[13] However, some diverging observations can be made with respect to the effect of methyl groups. We here found that a 5-Me substitution on the pyrrolopyrimidine had little or no effect on LIMK1 (**3** vs **13**, **4** vs **17**), but could affect selectivity versus ROCK2 and PKA, depending on the amine present on the piperidinyl moiety. On the other hand the effect of a 6-Me substitution on the pyrrolopyrimidine appeared less clear-cut than in bis-aryl urea series, as full selectivity against ROCK2 could only be achieved through a 5,6-dimethyl substitution pattern. Those differences in SAR might result from diverging conformational constraints between the two series. In particular, the presence of a 5-Me group should

Please cite this article in press as: Boland, S.; et al. *Bioorg. Med. Chem. Lett.* (2015), http://dx.doi.org/10.1016/j.bmcl.2015.07.009

Table 2
Structure–activity relationships for variation around the kinase scaffold of LX-7101



Compound	R_1	Ar	IC_{50} LIMK1 (nM)	IC_{50} LIMK2 (nM)	IC_{50} ROCK2 (nM)	IC_{50} PKA (nM)
3	CH_2NMe_2		27 ± 5	1.5 ± 0.7	8.9 ± 1.6	24 ± 6
13	CH_2NMe_2		23 ± 16	13 ± 1.8	1.8 ± 0.5	1.1 ± 0.2
14	CH_2NMe_2		282 ± 38	193 ± 16	62 ± 25	5.1 ± 0.9
15	CH_2NMe_2		65 ± 35	274 ± 66	11 ± 2	3.8 ± 0.6
16	CH_2NMe_2		1000 ± 350	1860 ± 330	>10,000[a]	1130 ± 360
17	NH_2		31 ± 7	9.6 ± 3.2	67 ± 7.5	69 ± 11
18	NH_2		>1000[a]	>10,000[a]	>1000[a]	>1000[a]
19	NH_2		945 ± 275	4560 ± 670	2060 ± 530	>1000
20	NH_2		>1000[a]	>10,000[a]	416 ± 70	>1000[a]
21	NH_2		239 ± 44	494 ± 114	>1000[a]	>1000[a]
22	NH_2		>10,000[a]	>10,000[a]	>1000[a]	>1000[a]

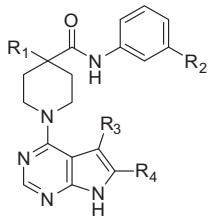
[a] Highest tested concentration.

influence preferential orientation of the piperidine moiety and, in turn, of the amine and carbamoylphenyl side chains. Meanwhile, the bis-aryl urea series displays a *para*-substituted phenyl structure instead of the piperidine, which favors a more linear shape that would be less influenced by presence of a 5-Me group. A comparison between the binding modes of the two chemical series is presented as Supporting information.

At this stage, we had identified a clear possibility to obtain selective LIMK inhibitors. Subsequent work on 5,6-dimethyl pyrrolopyrimidines however had to take into consideration several patent applications, which focus on other kinase targets. Those applications do not exemplify compounds such as **26**, **27**, or the corresponding substitution pattern, but contain broad disclosures.[25] Ironically, one of such documents contained granted claims to compounds described as Akt inhibitors, even though we could demonstrate selectivity against Akt for **26** and **27**. The results of our medicinal chemistry efforts towards further selective LIMK inhibitors will be reported in an upcoming publication.

As we had identified compounds with improved selectivity against ROCK2 and PKA, we chose to further investigate whether or not this newfound selectivity was reflected in functional assays. Cofilin is one of the protein substrates of LIMKs, and cofilin

Table 3
Structure–activity relationships for substitutions on pyrrolopyrimidine



Compound	R_1	R_2	R_3	R_4	IC_{50} LIMK1 (nM)	IC_{50} LIMK2 (nM)	IC_{50} ROCK2 (nM)	IC_{50} PKA (nM)
LX-7101	CH_2NH_2	$OCONMe_2$	Me	H	24 ± 3	1.6 ± 0.8	10 ± 4	<1
3	CH_2NMe_2	CO_2Me	Me	H	27 ± 5	1.5 ± 0.7	8.9 ± 1.6	24 ± 6
13	CH_2NMe_2	CO_2Me	H	H	23 ± 16	13 ± 2	1.8 ± 0.5	1.1 ± 0.2
23	CH_2NMe_2	CO_2Me	C≡N	H	25 ± 10	4.0 ± 0.6	2.5 ± 0.4	<1
24	CH_2NMe_2	CO_2Me	F	H	20 ± 7	2.8 ± 0.7	5.7 ± 1.7	<1
25	CH_2NMe_2	CO_2Me	H	Me	23 ± 8	8.0 ± 1.9	652 ± 227	332 ± 106
26	CH_2NMe_2	CO_2Me	Me	Me	26 ± 6	1.6 ± 0.5	>1000[a]	>1000[a]
27	CH_2NH_2	$OCONMe_2$	Me	Me	22 ± 4	1.4 ± 0.6	>1000[a]	>1000[a]

[a] Highest tested concentration.

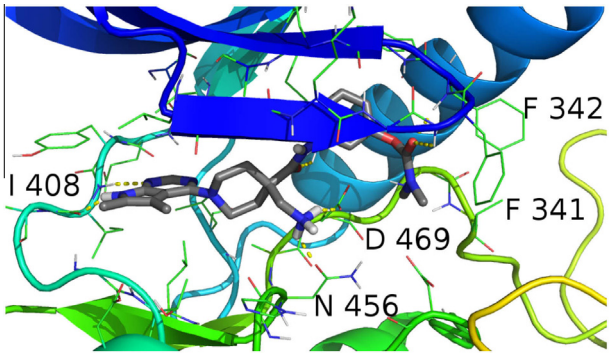


Figure 3. Proposed binding mode of **27** in LIMK2, as suggested by docking.

phosphorylation therefore represents a cellular marker of LIMK activity.[4] The effects of LX-7101 and **27** on cofilin phosphorylation were evaluated in Hela cells, using a *Surefire*® assay developed by Perkin Elmer. Under the tested conditions, both compounds displayed comparable efficacy, in the nanomolar range, which is in line with the comparable on-target activity of the compounds (Table 4). Monitoring of MLC phosphorylation[24] represented a logical counterscreen for ROCK inhibition, since MLC is phosphorylated by ROCKs, but not LIMKs. In this assay, LX-7101 had a clear concentration-dependent effect (EC_{50} = 2300 nM). Several non-selective inhibitors such as **2**, **3** or **6** also affected MLC phosphorylation in the low micromolar or sub-micromolar range. In contrast, **26** and **27** did not affect MLC phosphorylation at the highest tested concentrations, confirming the selectivity observed in on-target assays. Based on those results, selectivity of LX-7101 versus ROCK appears higher in terms of functional activity than in terms of on-target potency. This observation appears in line with the higher K_m of LIMKs for ATP, which would result in a weaker competition at physiological ATP concentrations.[7]

LX-7101 and **27** were further evaluated in vivo for their IOP-lowering effects (Fig. 4). Ocular normotensive New Zealand White (NZW) rabbits were used. Both compounds were formulated as 0.5% w/v solutions in a PEG400/Water (1:1) vehicle, adjusted to pH 7.0 and applied topically as 40 µl eye drop (200 µg/eye drop). All animals (n = 5 for each experiment) received the test compound in one eye and placebo (vehicle) in the contralateral eye. Both studies were done blinded. Under such conditions, animals receiving

Table 4
Cell-based activity of LX-7101, **26** and **27**

Compound	EC_{50} MLC-PP[a] (nM)	EC_{50} cofilin-PP[a]
LX-7101	2300	8.7
26	>10,000[b]	NT[b]
27	>10,000[b]	9.0

[a] Data represents the average of two experiments, each run in triplicate.
[b] Highest concentration tested. NT: not tested.

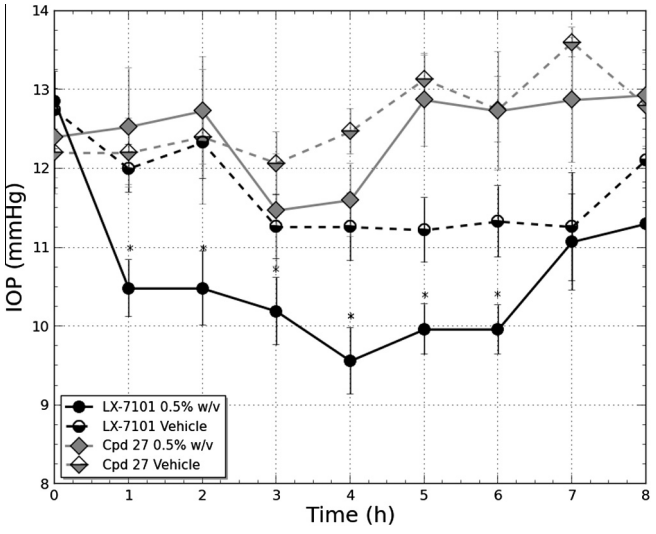


Figure 4. IOP-lowering efficacy of LX-7101 and **27** in ocular normotensive NZW rabbits.

the non-selective LX-7101 displayed a significant IOP reduction at time points ranging from 1 h to 6 h post administration. However, some animals showed clear conjunctival hyperemia (redness). Meanwhile, animals receiving the more selective **27** at an equivalent concentration did not display a significant IOP reduction (vs vehicle) at any time point.

The finding that a selective LIMK inhibitor such as **27** did not display IOP-lowering efficacy obviously came as a surprise. Indeed, LIMK inhibitors have in multiple occasions been reported as IOP-lowering agents in several patent applications[12,16] and

journal articles.[7,8,14] However, those results were generated in animal models featuring a higher IOP baseline than for NZW rabbits. For instance, a baseline IOP of 28 mmHg was reported for rats used in the evaluation of bis-aryl urea LIMK inhibitors;[13] and LX-7101 was evaluated in a dexamethasone-induced ocular hypertensive mouse model.[7] This point might be of importance, because ROCK inhibitors (and by extension LIMK inhibitors) normally target the trabecular outflow of aqueous humor;[26] which is a pressure-dependent contribution.[27] Consequently, LIMK inhibitors might not be able to display their full efficacy in NZW ocular normotensive rabbits which have a baseline IOP around 12 mmHg.

In spite of such considerations, the observation remains that LX-7101 displays efficacy in normotensive NZW rabbits while its very close and LIMK-selective analog **27** does not. In terms of in vitro activity, the compounds have comparable effects against LIMK1, LIMK2 and cofilin phosphorylation. However, **27** and LX-7101 differed significantly in their off-target activity profile. While **27** displays clear selectivity vs ROCK2, LX-7101 inhibits ROCK in enzymatic and cell-based assays. With ROCK inhibitors representing known IOP-lowering agents,[18] it is tempting to conclude that at least part of the observed IOP-lowering effects of LX-7101 result from ROCK inhibition. Indeed, the EC_{50} we observe for LX-7101 in the MLC-phosphorylation assay is comparable to its reported C_{max} in aqueous humor during ocular PK experiments (1500 nM).[7] Further, the observation that some of the animals receiving LX-7101 displayed conjunctival hyperemia, which is a typical side effect of most ROCK inhibitors, also supports that hypothesis.

In conclusion, we have here presented the synthesis and biological evaluation of a series of LIMK inhibitors based on the pyrrolopyrimidine scaffold. Those compounds represent close analogs of LX-7101, Lexicon's candidate for the treatment of glaucoma. We here showed that many of such derivatives, as well as LX-7101 display nanomolar potency against LIMK1 and LIMK2, and also ROCK2 and PKA. Such non-selective derivatives, including LX-7101 could inhibit ROCK at low-micromolar concentrations in a cell-based assay. Exploration of structure–activity relationships resulted in the identification of 5,6-dimethyl-pyrrolopyrimidines derivatives with improved selectivity. However, elimination of side-activities also abolished IOP-lowering efficacy in ocular normotensive NZW rabbits. Our findings suggest that at least part of the IOP-lowering effects of LX-7101 could be attributed to ROCK inhibition rather than LIMK inhibition. Additionally, it is likely that LIMK inhibitors should preferentially be tested in animal models with high baseline IOP. Further evaluation of selective LIMK-inhibitors should allow a better separation between LIMK-associated and ROCK-associated effects in in vitro or in vivo assays than with LX-7101.

Acknowledgment

The authors wish to thank Dr. Jean-Marie Stassen for his contribution and advice during the review of this manuscript.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.07.009.

References and notes

1. Mizuno, K.; Okano, I.; Ohashi, K.; Nunoue, K.; Kuma, K.; Miyata, T.; Nakamura, T. *Oncogene* **1994**, *9*, 1605.
2. Okano, I.; Hiraoka, J.; Otera, H.; Nunoue, K.; Ohashi, K.; Iwashita, S.; Hirai, M.; Mizuno, K. *J. Biol. Chem.* **1995**, *270*, 31321.
3. Bach, I. *Mech. Dev.* **2000**, *91*, 5.
4. Bernard, O. *Int. J. Biochem. Cell Biol.* **2007**, *39*, 1071.
5. Manetti, F. *Med. Res. Rev.* **2012**, *32*, 968.
6. Harrison, B. A.; Whitlock, N. A.; Voronkov, M. V.; Almstead, Z. Y.; Gu, K. J.; Mabon, R.; Gardyan, M.; Hamman, B. D.; Allen, J.; Gopinathan, S.; McKnight, B.; Crist, M.; Zhang, Y.; Liu, Y.; Courtney, L. F.; Key, B.; Zhou, J.; Patel, N.; Yates, P. W.; Liu, Q.; Wilson, A. G.; Kimball, S. D.; Crosson, C. E.; Rice, D. S.; Rawlins, D. B. *J. Med. Chem.* **2009**, *52*, 6515.
7. Harrison, B. A.; Almstead, Z. Y.; Burgoon, H.; Gardyan, M.; Goodwin, N. C.; Healy, J.; Liu, Y.; Mabon, R.; Marinelli, B.; Samala, L.; Zhang, Y.; Stouch, T. R.; Whitlock, N. A.; Gopinathan, S.; McKnight, B.; Wang, S.; Patel, N.; Wilson, A. G.; Hamman, B. D.; Rice, D. S.; Rawlins, D. B. *ACS Med. Chem. Lett.* **2015**, *6*, 84.
8. Dai, Y. P.; Bongalon, S.; Tian, H.; Parks, S. D.; Mutafova-Yambolieva, V. N.; Yamboliev, I. A. *Vasc. Pharmacol.* **2006**, *44*, 275.
9. Manetti, F. *Drug Discovery Today* **2012**, 81.
10. Vorster, P. J.; Guo, J.; Yoder, A.; Wang, W.; Zheng, Y.; Xu, X.; Yu, D.; Spear, M.; Wu, Y. *J. Biol. Chem.* **2011**, *286*, 12554.
11. Wen, X.; Ding, L.; Wang, J. J.; Qi, M.; Hammonds, J.; Chu, H.; Chen, X.; Hunter, E.; Spearman, P. *J. Virol.* **2014**, *88*, 6906.
12. Burgoon, H. A.; Goodwin, N. C.; Harrison, B. A.; Healy, J. P.; Liu, Y.; Mabon, R.; Marinelli, B.; Rawlins, D. B.; Rice, D. S.; Whitlock, N. A. WO/2009/131940, 2009.
13. Yin, Y.; Zheng, K.; Eid, N.; Howard, S.; Jeong, J.-H.; Yi, F.; Guo, J.; Park, C. M.; Bibian, M.; Wu, W.; Hernandez, P.; Park, H.; Wu, Y.; Luo, J.-L.; LoGrasso, P. V.; Feng, Y. *J. Med. Chem.* **2015**, *58*, 1846.
14. Wrobelski, S. T.; Lin, S.; Leftheris, K.; He, L.; Seitz, S. P.; Lin, T. A.; Vaccaro, W. WO/2006/084017, 2006.
15. Ross-Macdonald, P.; de Silva, H.; Guo, Q.; Xiao, H.; Hung, C. Y.; Penhallow, B.; Markwalder, J.; He, L.; Attar, R. M.; Lin, T. A.; Seitz, S.; Tilford, C.; Wardwell-Swanson, J.; Jackson, D. *Mol. Cancer. Ther.* **2008**, *7*, 3490.
16. Goodwin, N. C.; Rawlins, D. B. WO/2011/091204, 2011.
17. He, L.; Seitz, S. P.; Trainor, G. L.; Tortolani, D.; Vaccaro, W.; Poss, M.; Tarby, C. M.; Tokarski, J. S.; Penhallow, B.; Hung, C. Y.; Attar, R.; Lin, T. A. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5995.
18. Goodwin, N. C.; Cianchetta, G.; Burgoon, H. A.; Healy, J.; Mabon, R.; Strobel, E. D.; Allen, J.; Wang, S.; Hamman, B. D.; Rawlins, D. B. *ACS Med. Chem. Lett.* **2015**, *6*, 53.
19. Mandell, K. J.; Kudelka, M. R.; Wirostko, B. *Rev. Ophthalmol.* **2011**, *6*, 611.
20. Boland, S.; Defert, O.; Alen, J.; Bourin, A.; Castermans, K.; Kindt, N.; Boumans, N.; Panitti, L.; Van de Velde, S.; Stalmans, I.; Leysen, D. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6442.
21. Caldwell, J. J.; Davies, T. G.; Donald, A. *J. Med. Chem.* **2008**, *51*, 2147.
22. Aronov, A. M.; Murcko, M. A. *J. Med. Chem.* **2004**, *47*, 5616.
23. Blake, J. F.; Kallan, N. C.; Xiao, D.; Xu, R.; Bencsik, J. R.; Skelton, N. J.; Spencer, K. L.; Mitchell, I. S.; Woessner, R. D.; Gloor, S. L.; Risom, T.; Gross, S. D.; Martinson, M.; Morales, T. H.; Vigers, G. P.; Brandhuber, B. J. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5607.
24. Schröter, T.; Griffin, E.; Weiser, A.; Feng, Y.; LoGrasso, P. *Biochem. Biophys. Res. Commun.* **2008**, *374*, 356.
25. (a) Davies, T. G.; Garrett, M. D.; Boyle, R. G.; Collins, I. WO/2007/125321; (b) Berdini, V.; Boyle, R. G.; Saxty, G.; Walker, D. W.; Woodhead, S. J.; Wyatt, P. G.; Caldwell, J.; Collins, I.; Da Fonseca, T. F.; Donald, A. WO/2006/046024.
26. Kopczynski, C. C.; Epstein, D. L. *J. Ocul. Pharmacol. Ther.* **2014**, *30*, 85.
27. Goel, M.; Picciani, R. G.; Lee, R. K.; Bhattacharya, S. K. *Open Ophthalmol. J.* **2010**, *4*, 52.