

CRT Licensing Opportunity



Inhibitors of LIMK1 and LIMK2

- Emerging target for therapeutic intervention in cancer and ocular disease
- Inhibition of LIMK leads to reduction in growth of invasive tumours *in vivo*
- Potent and selective late hit-to-lead inhibitors of LIMK1/2 available
- Demonstrated activity in cellular assays for tumour cell invasion and growth

SMALL MOLECULES | Hit-to-Lead

June 2011

Commercial Opportunity

CRT are seeking a commercial partner to undertake further development of an exciting collection of potent pre-clinical LIMK1/2 inhibitors with potential application in cancer and ocular disease. Two chemical series are subject to recent priority patent filings.

Therapeutic Rationale

By virtue of their role as effectors of Rho and cdc42 pathways involving ROCK, PAK1, PAK4 and MRCK, the LIM kinases are implicated as key regulators of the cytoskeleton (Figure 1). Several reports have also suggested cross-talk between LIMK and the TGF- β superfamily, key mediators of fibrotic responses. LIMK is also implicated in chemoresistance (1,2), including as a direct downstream target of p53 in response to DNA damage (3)

LIMK1/2 are ser/thr kinases which are upregulated in some tumours including metastatic breast and prostate (3, 4). Over-expression of LIMK has been demonstrated to increase tumour cell migration and invasion and to increase tumour growth, angiogenesis and metastasis *in vivo* (3). Conversely, abrogation of LIMK function results in decreased breast cancer cell motility and formation of osteolytic bone lesions in an animal model of invasion (5). In a zebrafish pancreatic xenograft model, LIMK 1/2 knockdown blocked invasion and metastasis, and significantly reduced angiogenesis (6).

Downregulation of LIMK1 has been demonstrated to reduce inflammation in a mouse model of ocular surgery. Furthermore, small molecule inhibition or genetic deletion of LIMK2 is effective in reducing intraocular pressure in mouse models, a key

risk factor in glaucoma disease progression.

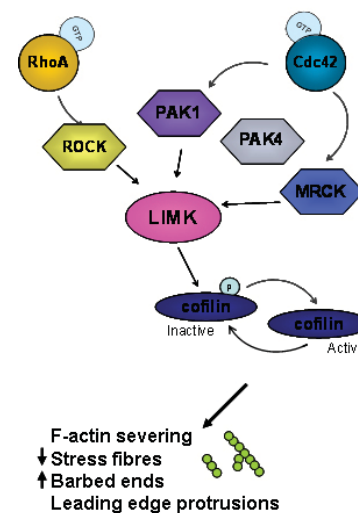


Figure 1: LIMK signalling pathways

Potent and Selective LIMK1/2 Inhibitors

CRT's Discovery Laboratories and the Australian consortium Cancer Therapeutics Pty Ltd. have collaborated to develop two late hit-to-lead compound series with low nM activity against LIMK1/2. The compounds were identified through an HTRF-based screen of CRT's fully synthetic compound library. An extensive medicinal chemistry effort has been carried out resulting in impressive potency and selectivity over a wide panel of other kinases. Compounds from the lead series are amongst the most

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potent LIMK inhibitors reported in the literature to date, some having sub-nM IC_{50} s *in vitro*, and are in novel chemical space.

Compounds from the lead series demonstrate drug-like physicochemical properties including low molecular weight, with high membrane permeability and metabolic stability.

Sensitisation to chemotherapies

The compounds exhibit low uM potency in breast cancer cells showing dose dependent inhibition of phosphorylation of the LIMK substrate cofilin (Figure 2).

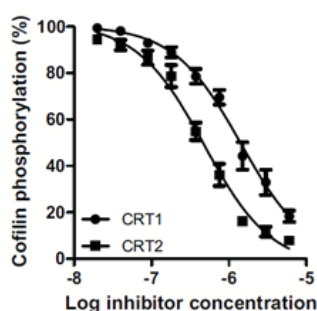


Figure 2: Reduction of cofilin phosphorylation in MCF7 breast cancer cells by LIMK inhibitors.

Analysis of chemosensitivity to 55 anticancer drugs across 39 human cancer cell lines revealed that LIMK2 expression was significantly correlated to resistance to 18 widely-used drugs (8). CRT LIMK inhibitors have been demonstrated to synergise with a number of chemotherapies to increase SubG1 populations and apoptosis (Figure 3).

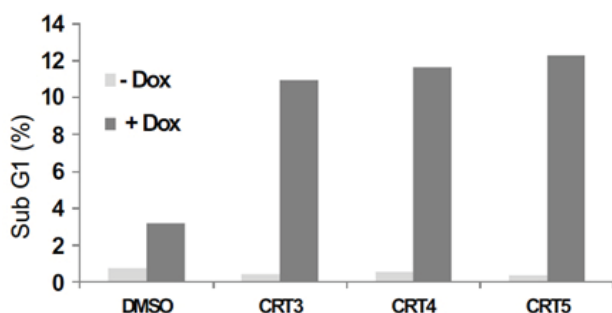


Figure 3: LIMK inhibitors increase apoptosis (measured by SubG1 population) in MCF7 cells treated with doxorubicin.

Inhibition of invasion

Treatment of MDA-MB-231 breast cancer cells with the compounds significantly reduces their ability to invade a matrigel plug in an inverse *in vitro* invasion assay (Figure 4). Furthermore, the inhibitors effectively reduce fibroblast-led collective invasion in a co-culture organotypic model (data not shown).

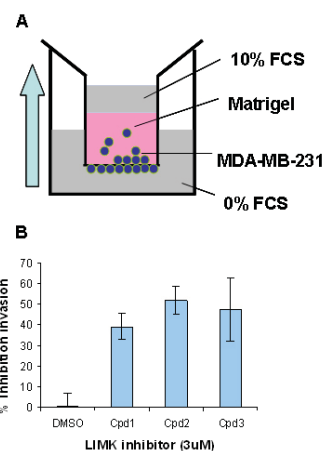


Figure 4: LIMK inhibitors block breast cancer cell invasion

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