Inhibition of lysyl oxidase like 2 reduces collagen accumulation and collagen cross-links in CCl_4-induced liver fibrosis

W Jarolimek; A Buson; L Cao; M Deodhar; A Findlay; J Foot; A Greco; J Moses; H Schilter; C Turner; T Yow; W Zhou
Pharmaxis Ltd. 20 Rodborough Road, French's Forest, NSW, Australia

Introduction
Lysyl oxidases are predominantly involved in the cross-linking of collagen and elastin in physiological and pathophysiological conditions. The lysyl oxidase family contains 5 members and lysyl oxidase like 2 (LOXL2) is a validated drug target as it is upregulated in various fibrotic diseases. Therefore, inhibition of LOXL2 is a promising mechanism to resolve fibrosis.
CCl_4-induced liver fibrosis is a useful model to study the role of extracellular matrix formation in the maintenance or progression of the disease.

Pharmaxis has developed small molecule mechanism-based inhibitors that are selective for LOXL2 over ubiquitous LOX and have drug-like properties.

Cmpd A is a selective inhibitor

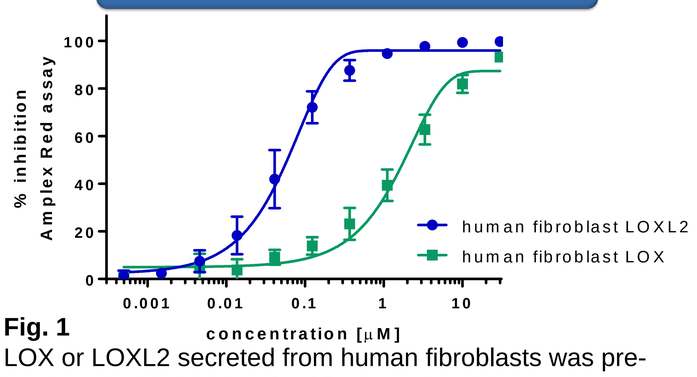


Fig. 1
LOX or LOXL2 secreted from human fibroblasts was pre-incubated with inhibitor for 30 min and activity was measured in physiological buffer solution.

Inhibition [nM]		
	rec human LOXL2	24
	rec mouse LOXL2	20
	human LOXL2	40
	human LOX	1800
	bovine LOX	1600
	rec human LOXL1	1700
	rec human LOXL3	38
	rec human LOXL4	100

Table 1
Pharmacology of Cmpd A against other lysyl oxidases
No inhibition (IC50 >30 µM) of other amine oxidases (SSAO, MAO-A and MAO-B).

PK profile in CCl_4 model

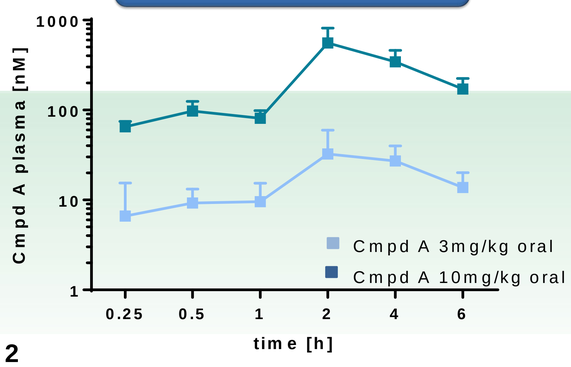
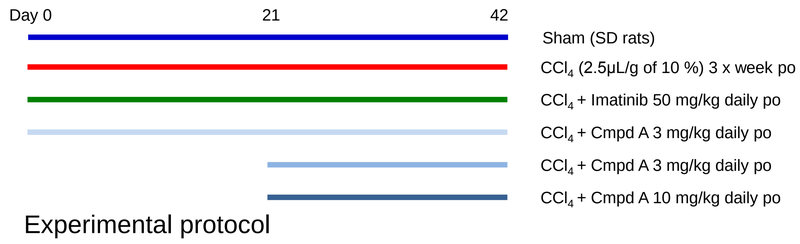


Fig. 2
PK profile after oral application of Cmpd A in animals treated with CCl_4 for 3 weeks



Experimental protocol

Cmpd A improves liver function

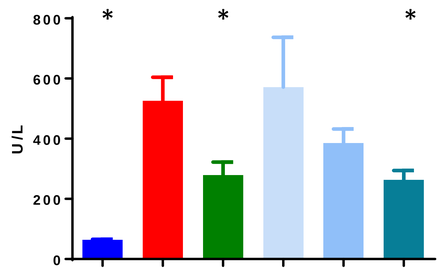


Fig. 3
Plasma ALT levels after 6 weeks of CCl_4 treatment

Cmpd A is anti-fibrotic

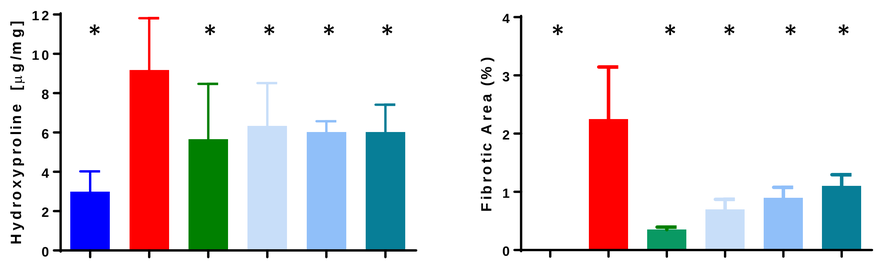


Fig. 4
Fibrosis was measured by total hydroxyproline content (left) and area of fibrosis as quantified through Picrosirius Red stain (right).

Cmpd A reduces fibrotic markers

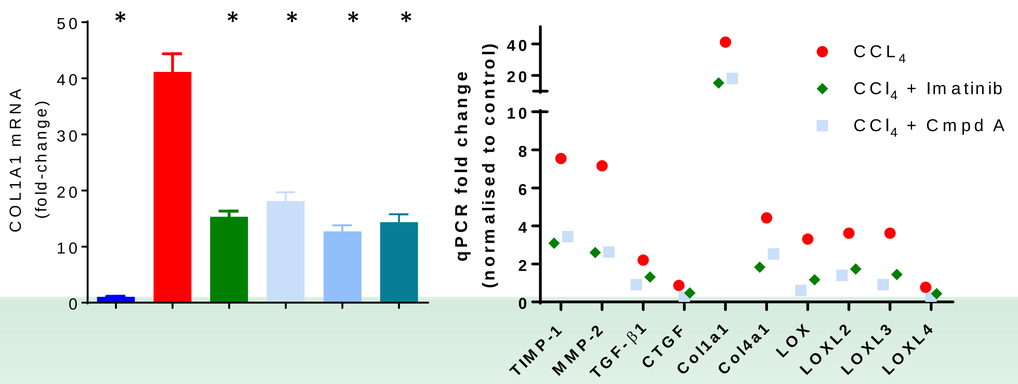
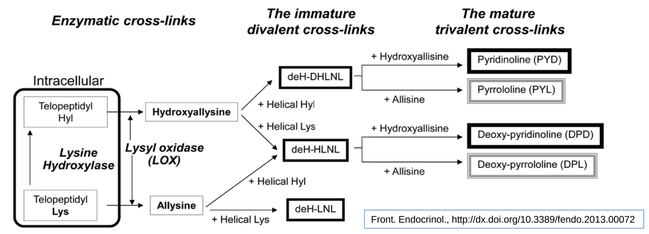


Fig. 5
Col1A1 expression was significantly upregulated in CCl_4-treated animals and reduced by drug treatment. Except for CTGF all tested mRNAs were increased in CCl_4 animals and imatinib or 3mg/kg Cmpd A for 6 weeks similarly reduced the overexpression.

Collagen telopeptides are cross-linked by lysyl oxidases to generate di- and trivalent products.



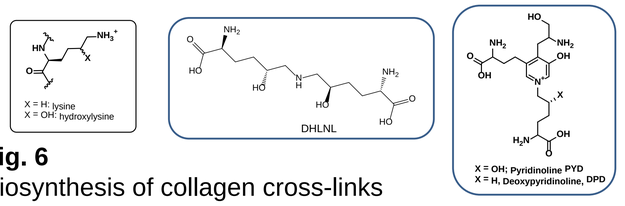


Fig. 6
Biosynthesis of collagen cross-links

Cmpd A reduces number of cross-links

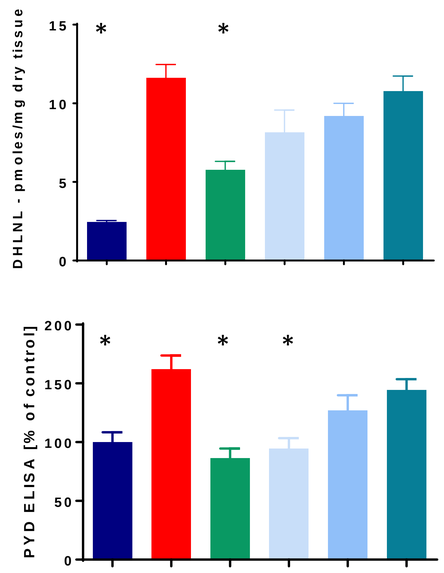


Fig. 7
Changes in the number of cross-links as measured by LC/MS/MS (DHNLN) or ELISA (pyd).

Summary

Pharmaxis has developed small molecules that selectively inhibit LOXL2 with nanomolar potency and:

- *Improve liver function and reduce fibrosis*
- *Dampen CCl_4-induced gene expression of major drivers in fibrosis*
- *Immature and mature cross-links are reduced by LOXL2 inhibitors but 6 weeks of treatment are required to have a significant effect on mature cross-links*

LOXL2 inhibitors reduce the oxidation of lysine residues and, therefore, enable degradation of non cross-linked collagen. This reduces all hallmarks of fibrosis. Already cross-linked collagen is slowly degraded.

pharmaxis