**Inhibition of lysyl oxidase like 2 reduces collagen accumulation and collagen cross-links in CCl₄-induced liver fibrosis**

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**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. CCl₄-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.

**Table 1**

<table>
<thead>
<tr>
<th>Inhibition [nM]</th>
<th>rec human LOXL2</th>
<th>rec mouse LOXL2</th>
<th>human LOXL2</th>
<th>human LOX</th>
<th>rec human LOXL1</th>
<th>rec human LOXL3</th>
<th>rec human LOXL4</th>
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<td>24</td>
<td>20</td>
<td>40</td>
<td>1800</td>
<td>1600</td>
<td>1700</td>
<td>38</td>
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</table>

**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.

**Fig. 2**

PK profile after oral application of Cmpd A in animals treated with CCl₄ for 3 weeks

**Fig. 3**

Plasma ALT levels after 6 weeks of CCl₄ treatment

**Fig. 4**

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

**Fig. 5**

Col1A1 expression was significantly upregulated in CCl₄-treated animals and reduced by drug treatment. Except for CTGF all tested mRNAs were increased in CCl₄ animals and imatinib or 3mg/kg Cmpd A for 6 weeks similarly reduced the overexpression.

**Fig. 6**

Biosynthesis of collagen 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 CCl₄-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.