ML290, a small allosteric biased agonist at RXFP1

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Introduction: Relaxin, an insulin-like peptide hormone that is the cognate ligand at relaxin family peptide receptor 1 (RXFP1) has vasodilator and anti-fibrotic properties and is in Phase III clinical trials for the treatment of acute heart failure. ML290 (2-Isopropoxy-N-(2-(3-(trifluoromethylsulfonyl)phenylcarbamoyl)phenyl)benzamide) is a small molecule allosteric agonist acting at RXFP1[1] but relatively little is known of its signaling profile.

***Method*:** This study examined ML290-mediated MAPK, cAMP and cGMP signalling using Surefire or Alphascreen kits in human cells endogenously and recombinantly expressing RXFP1[2] and longer-term actions on markers of fibrosis including MMP2 expression and Smad2/3 phosphorylation in human cardiac fibroblasts (HCFs)[2]. Data points represent mean±SEM of *n* experiments.

***Results*:** ML290 (1μM) did not affect the rate of association or dissociation of ^{125}I-H2 relaxin, but did increase total binding to RXFP1 from 102.7±7.3% to 135.2±6.5% ($P<0.05$, n=6). In HEK-RXFP1 cells, ML290 stimulated cAMP accumulation and phosphorylation of p38MAPK but did not promote cGMP accumulation or phosphorylation of ERK1/2 and JNK1/2/3. In human primary vascular cells, ML290 increased cAMP and cGMP accumulation but not p-ERK1/2 in coronary artery (HCAEC) and umbilical vein endothelial cells (HUVEC), in umbilical artery (HUASMC) and umbilical vein smooth muscle cells (HUVSMC) but not in umbilical artery endothelial cells that do not display cell surface expression of RXFP1. In human cardiac fibroblasts (HCF), ML290 increased cGMP accumulation but had no effect on p-ERK1/2 and given chronically also activated MMP-2 expression and inhibited TGF-β1-induced Smad2 and Smad3 phosphorylation. ML290 increased p-p38MAPK only in smooth muscle but not endothelial cells. In vascular cells, ML290 was ten times more potent for cGMP accumulation and p-p38MAPK than for cAMP accumulation. ML290-mediated cAMP and cGMP accumulation was inhibited by NF449 ($G\alpha_s$ inhibitor) but not NF023 ($G\alpha_i$ inhibitor) in all vascular cells. In BRET studies ML290 caused strong coupling of RXFP1 to $G\alpha_s$ and $G\alpha_{oB}$ but weak coupling to $G\alpha_{i3}$.

Table 1 pEC_{50} values for signalling pathways activated by H2 relaxin and ML290 (n=3-7)

	pERK1/2	pERK1/2	p-p38MAPK	cGMP	cAMP
	H2-relaxin	ML290	ML290	ML290	ML290
HEK-RXFP1	9.5 ± 0.3	NE	9.3 ± 0.6	NE	6.4 ± 0.1
HCAEC	ND	NE	NE	7.2 ± 0.4	6.1 ± 0.3
HUVEC	9.2 ± 0.4$^{\#}$	NE	NE	7.2 ± 0.5	6.1 ± 0.5
HUASMC	9.1 ± 0.4$^{\#}$	NE	8.6 ± 0.6	7.2 ± 0.5	6.1 ± 0.7

HUVSMC	9.2 ± 0.4[#]	NE	8.5 ± 1.0	7.2 ± 0.6	6.2 ± 0.5

***Conclusion**:* ML290 exhibited signalling and system bias at RXFP1 displaying a signalling profile indicative of vasodilator and anti-fibrotic properties. Understanding the signalling profile of drugs acting at RXFP1 is vital for drug development targeting this receptor.

References:

1. Xiao *et al.,* (2013) *Nature Comm* **4**: 1-7

2. Sarwar M *et al.* (2015) *Br J Pharmacol* **172**: 1005-19