Dissecting the Role of the S2 Non-Canonical Binding Pocket on Farnesoid X Receptor Activity

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Introduction

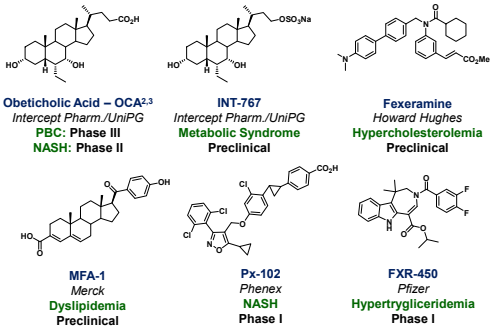
Physiological Role and Target Genes of FXR[1]

Physiological Function	⬆ Upregulated Genes		⬇ Downregulated Genes	
Bile acid homeostasis	*BAAT* *BACS* *BSEP* *CYP3A4* *FGF15/9* *IBABP* *MDR2*	*MDR3* *MRP2* *OATP8* *OST-α,β* *SHP* *SULT2A1* *UGT2B4*	*ASBT* *CYP7A1* *CYP8B1* *LRH-1*	*NTCP* *OAT2* *UGT2B7*
Cholesterol and lipid metabolism	*ApoC-I* *ApoC-II* *ApoC-IV* *ApoE* *C3* *FAS* *Insig-2* *PLTP*	*PDK4* *PPARα* *SRB-1* *Syndecan-1* *VLDLR*	*ANGPTL3* *ApoA-I* *ApoC-III* *HL* *HNF4A* *MTP* *Paraoxonase 1* *SREPB-1c*	
Glucose metabolism	*AKR1B7* *GLUT4*	*GSK3* *PEPCK*	*FBP-1* *G6Pase*	*PEPCK*
Inflammatory response			*NF-kB*	
Regulation of coagulation	*Fibrinogen*	*Kininogen*		
Vascular remodeling	*DDAH-1* *eNOS*	*ICAM-1* *VCAM-1*	*Endothelin-1*	
Antibacterial activity	*CAMP* *CAR12*	*IL-18* *iNOS*		

Therapeutic Applications Associated with FXR Modulation

- Liver diseases
- Metabolic syndrome
- Atherosclerosis
- Diabesity
- Inflammatory bowel diseases
- Hepatic cancer

Compounds in Preclinical and Clinical Phase



Obeticholic Acid – OCA[2,3]
Intercept Pharm./UniPG
PBC: Phase III
NASH: Phase II

INT-767
Intercept Pharm./UniPG
Metabolic Syndrome
Preclinical

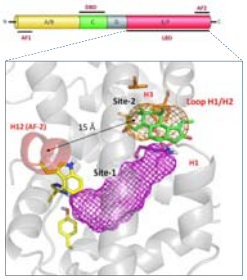
Fexeramine
Howard Hughes
Hypercholesterolemia
Preclinical

MFA-1
Merck
Dyslipidemia
Preclinical

Px-102
Phenex
NASH
Phase I

FXR-450
Pfizer
Hypertriglyceridemia
Phase I

The FXR Ligand Binding Domain: S1 and S2 Pocket[4]



- Canonical binding pocket (S1) hosts both endogenous and synthetic agonists.
- Non-canonical binding pocket (S2) may be occupied by guggulsterones (GSs) and extended side chain of bile acids resulting in partial agonism and gene-selective modulation

Approaches to Nuclear Receptor Modulation

- Affecting Relative Efficacy (Partial Agonists, Inverse Agonists)
- **Affecting the ability to activate or to repress individual sets of genes through selective co-regulator recruitment (eventually in specific tissue)** ⟹ **SBARMs[5]**
- Targeting Non-Genomic Effects
- Modulating Pharmacokinetics and Tissue Distribution
- Others: poly-pharmacology, receptor kinetics, subtypes, post-translational modification

Aims of the Work

In this work, we report our ongoing endeavours in confirming the hypothesis of the non-canonical S2 binding pocket by determining both the biochemical and transactivation response upon co-administration of GSs and FXR agonists. Furthermore, complex computational study composed by docking and molecular dynamic simulations will performed in view of the amino acidic residues involved in this "alternative" FXR modulation.

Results

Investigation of FXR S2 Binding Pocket: *E*-GS in combination with OCA

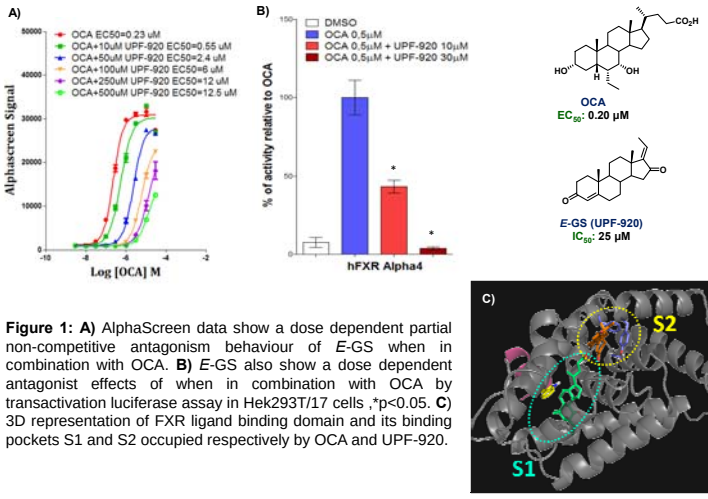


Figure 1: A) AlphaScreen data show a dose dependent partial non-competitive antagonism behaviour of *E*-GS when in combination with OCA. **B)** *E*-GS also show a dose dependent antagonist effects of when in combination with OCA by transactivation luciferase assay in Hek293T/17 cells ,*p<0.05. **C**) 3D representation of FXR ligand binding domain and its binding pockets S1 and S2 occupied respectively by OCA and UPF-920.

Investigation of FXR S2 Binding Pocket: *E*-GS in combination with UPF-930

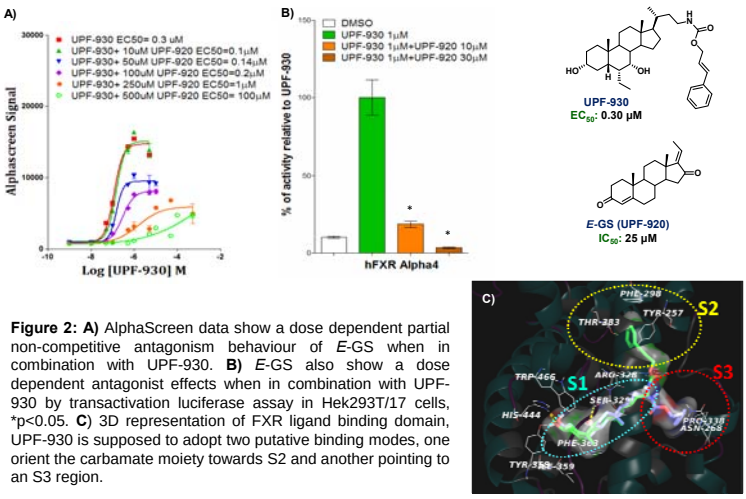


Figure 2: A) AlphaScreen data show a dose dependent partial non-competitive antagonism behaviour of *E*-GS when in combination with UPF-930. **B)** *E*-GS also show a dose dependent antagonist effects when in combination with UPF-930 by transactivation luciferase assay in Hek293T/17 cells, *p<0.05. **C**) 3D representation of FXR ligand binding domain, UPF-930 is supposed to adopt two putative binding modes, one orient the carbamate moiety towards S2 and another pointing to an S3 region.

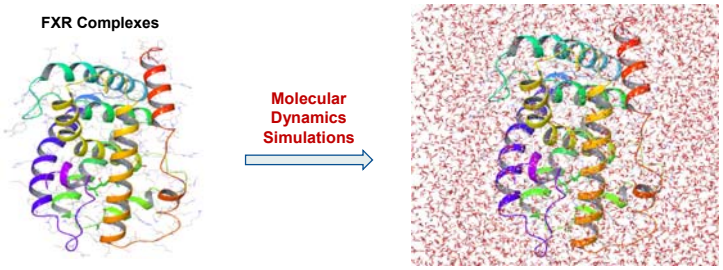


Figure 3: Molecular dynamic simulation: The systems will be solvated with SPC water and a production run of 100ns will be analyzed in terms of energies of interactions, ligands and protein stability.

Discussion

Our data confirm the hypothesis determined by computational study of the FXR non-canonical S2 binding pocket. Here we show how *E*-GS is able to antagonize the effects of different FXR agonists (i.e. OCA and UPF-930) through the occupancy of the S2 non-canonical binding pocket. Moreover, as hypothesized by *in silico* studies, an additional S3 binding pocket might exists since the side chain of UPF-930 can switch from S2 to S3 when *E*-GS is in S2. The validation of the molecular dynamic simulations performed in view of the amino acidic residues involved in this "alternative" FXR modulation, combined with the use of steroidal chemical tools, will be very helpful to design a new class of FXR modulators for the treatment of metabolic or inflammatory disorders.

References

[1] a) Matsubara, T. *et al. Mol. Cell. Endocrinol.* **2013**, *368*, 17-29. b) Shaik, F. B. *Inflamm Res.* **2015**, *64*, 9-20. [2] a) Pellicciari, R. *et al. J. Med. Chem.* **2002**, *45*, 3569-3572. b) Mi, L. Z. *et al. Mol Cell.* **2003**, 11, 1093-100. [3] a) Hirschfield, G. M. *et al. Gastroenterology* **2015**, 148, 751-61.e8. b) Mudaliar, S. *et al. Gastroenterology* **2013**, 145, 574-82.e1. c) Adorini, L. *et al. Drug Discov Today.* **2012** 17(17-18):988-97. [4] a) Meyer, U. *et al. J. Med. Chem.* **2005**, 48, 6948-6955. b) Pellicciari, R. *et al. J. Med. Chem.* **2006**, 49, 4208-4215. c) Gioiello, A. *et al. Bioorg. Med. Chem.* **2011**, 19, 2650-2658. d) *Yang L. et al. Biochim. Biophys. Acta* **2014** 1844(9):1684-93. [5]Cui, J. *et al. J. Biol. Chem.* **2003**, 278, 10214-10220.

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