

# Optical isomers of statins enantiospecifically activate pregnane X receptor PXR and induce CYP2A6, CYP2B6 and CYP3A4 in human hepatocytes



Martina Stepankova, Aneta Dorcakova and Zdenek Dvorak

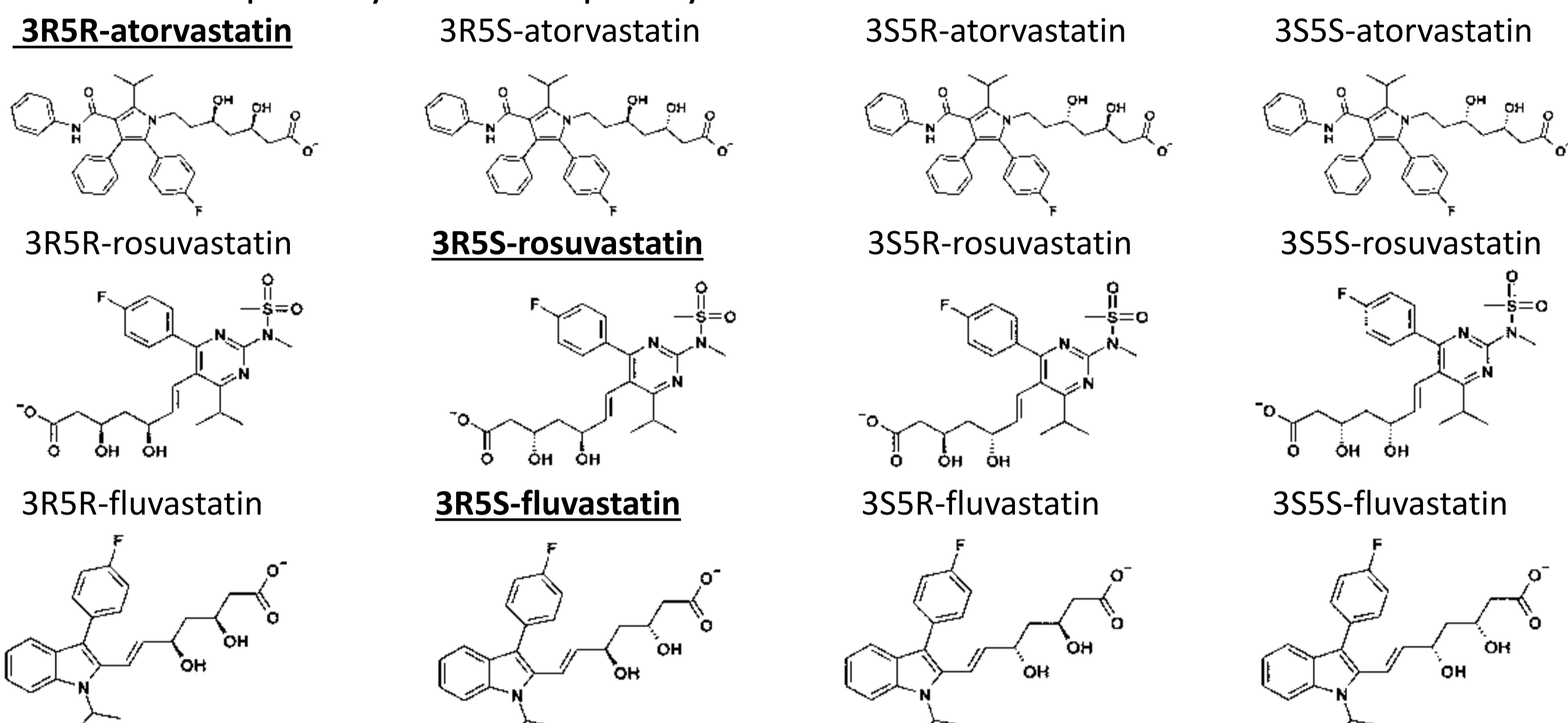
Email: m.korhonova@gmail.com

Department of Cell Biology and Genetics, Faculty of Science, Palacky University, Slechtitelu 27, 783 71 Olomouc, Czech Republic

## 1. Introduction:

Statins are drugs used for the treatment of hypercholesterolemia. Statins have two chiral centers in their molecules, thus they form four enantiomers: 3R5R-, 3R5S-, 3S5R- and 3S5S-. Regarding the most frequently prescribed statins, following enantiopure formulations are used in the clinics: 3R5R-atorvastatin, 3R5S-rosuvastatin and 3R5S-fluvastatin (Figure 1). Individual enantiomers of one drug can qualitatively and quantitatively differ in their biological activities. In some cases, only one enantiomer is responsible for the therapeutic effect, while the opposite one may be inactive or can cause undesired or even toxic effects. Therefore, it is of value to study *in vitro* effects of individual enantiomers.

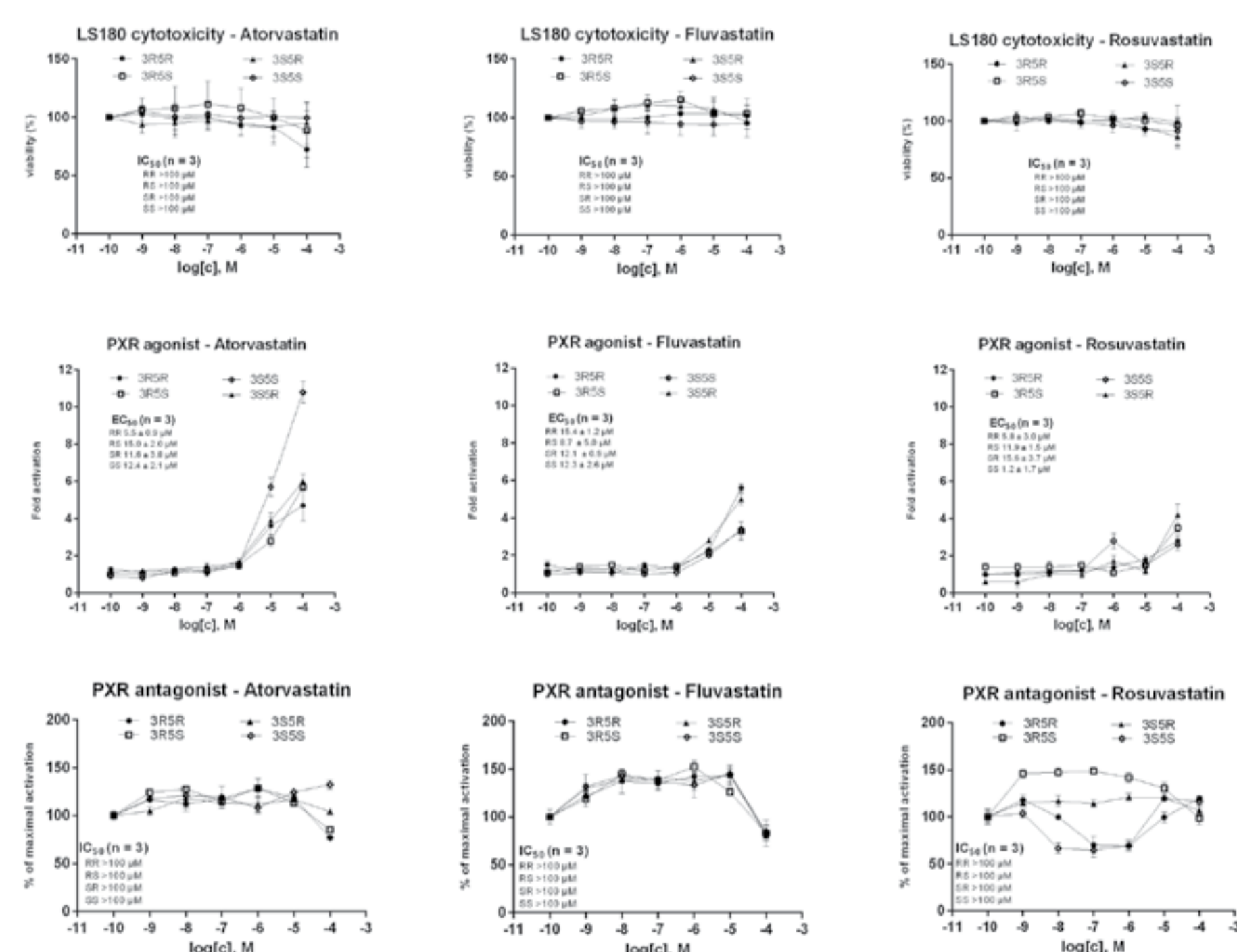
We investigated enantiospecific interactions of four enantiopure forms of atorvastatin, rosuvastatin and fluvastatin with main transcriptional regulators of drug-metabolizing enzymes - aryl hydrocarbon receptor (AhR) (data not shown), glucocorticoid receptor (GR) (data not shown) and pregnane X receptor (PXR). Agonist and antagonist activities of tested compounds towards AhR, PXR and GR were determined using human reporter cell lines. Moreover, we have measured expression of drug-metabolizing enzymes CYPs on mRNA and protein level in primary human hepatocytes.



**Figure 1:** Four individual enantiomers of atorvastatin, rosuvastatin and fluvastatin are shown in the figure. Clinically used enantiopure forms are underlined.

## 2. Effects of statin enantiomers on transcriptional activity of PXR in LS180 cells.

Prior to gene reporter assays, we examined the cytotoxicity of tested compounds in human colon adenocarcinoma LS180 cell line. For this purpose, the cells were incubated for 24 h with individual enantiomers of atorvastatin, fluvastatin and rosuvastatin at concentrations ranging from 100 pM to 100 μM. The vehicle was DMSO (0.1% v/v). The data are the mean ± SD from experiments performed in three consecutive passages of cells and are expressed as percentage of viability of control cells. The values of IC<sub>50</sub> were calculated where appropriate and they are indicated in plots (Figure 2, upper panels). Transcriptional activity of PXR was tested in LS180 cells transiently transfected with p3A4-luc reporter construct, incubated for 24 h with individual statin enantiomers in concentration ranging from 100 pM to 100 μM in the absence (agonist mode – middle panels) or presence (antagonist mode – lower panels) of rifampicin (RIF; 10 μM). The vehicle was DMSO (0.1% v/v). After the treatments, cells were lysed and luciferase activity was measured. Treatments were performed in triplicates. Data are expressed as a fold induction of luciferase activity over control cells (agonist mode) or as a percentage of maximal activation attained by RIF (antagonist mode). The values of EC<sub>50</sub> and IC<sub>50</sub> from *n* independent cell passages were calculated where appropriate and the average values are indicated in plots. Representative gene reporter assays are shown.



**Figure 2:** Effects of statin enantiomers on transcriptional activity of human pregnane X receptor.

Transcriptional activity of PXR was dose-dependently induced by all tested statins, and the potency and efficacy between individual optical isomers varied substantially. Ligand-inducible transcriptional activity of PXR was influenced by all tested statins, again differentially, depending on statin and optical isomer.

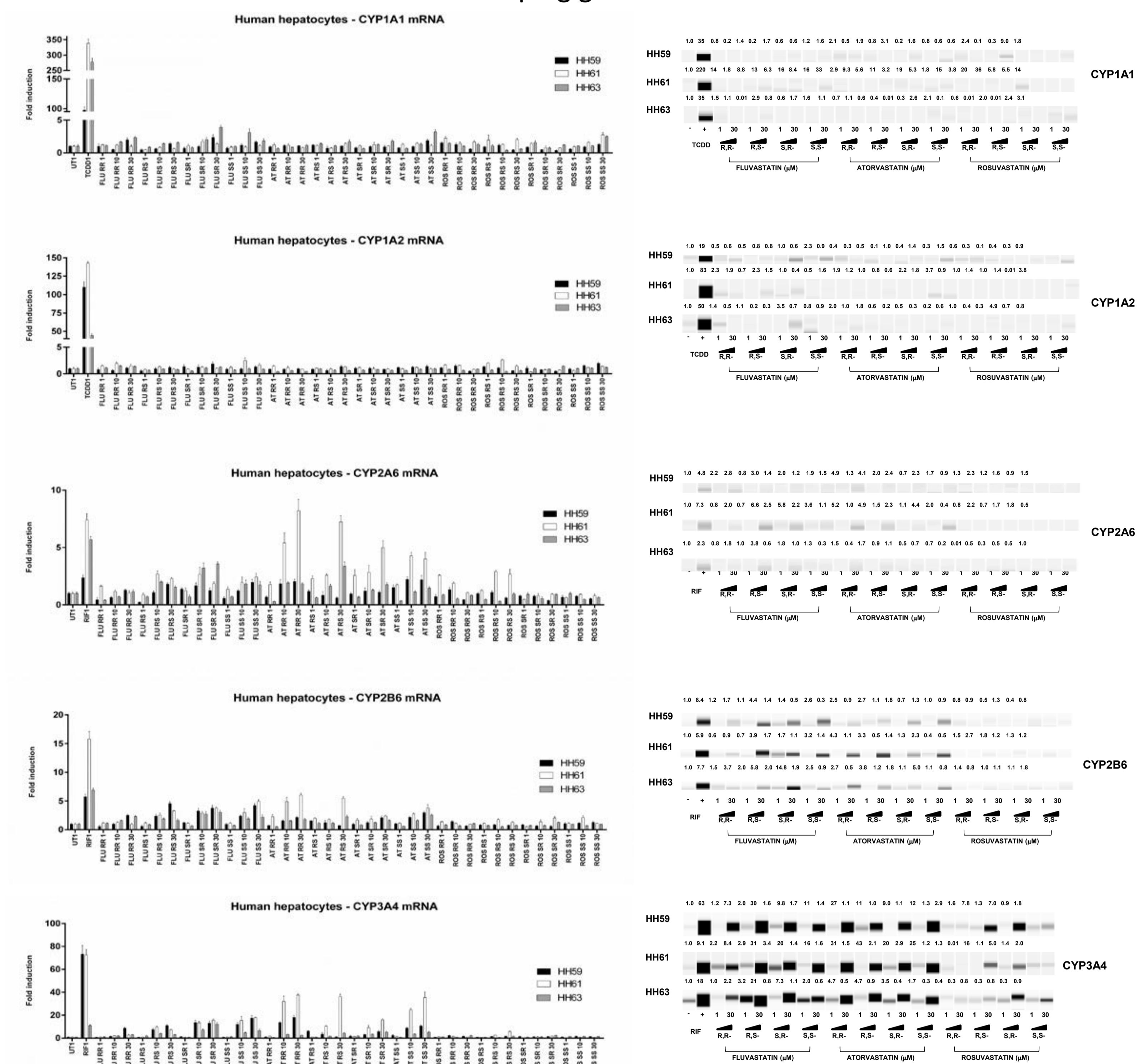
## References:

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## 3. Effects of statin enantiomers on the expression of drug-metabolizing cytochromes P450 in primary human hepatocytes

We examined a capability of statin enantiomers to induce transcriptionally regulated drug-metabolizing cytochromes P450 in three human hepatocytes cultures (HH59, HH61, HH63). Hepatocytes were treated for 24 h (for determination of mRNA) or 48 h (for determination of proteins) with optical isomers of tested statins (1 μM, 10 μM, 30 μM), rifampicin (RIF; 10 μM) and vehicle (DMSO; 0.1% v/v). Measurements were performed in triplicates. Gene expression was normalized to GAPDH as a housekeeping gene.

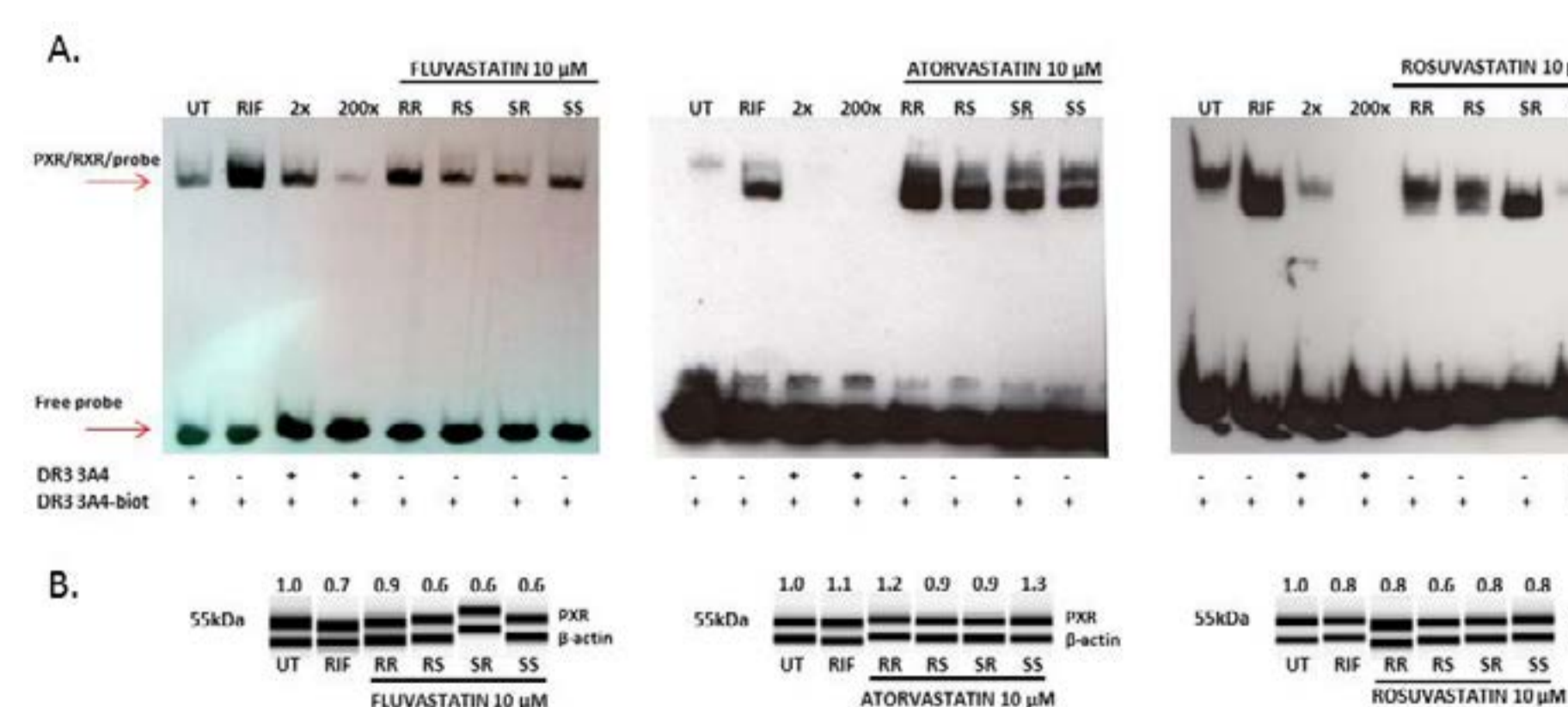


**Figure 3:** Effects of statin enantiomers on the expression of drug-metabolizing cytochromes P450 in primary human hepatocytes

The expression of CYP1A1 and CYP1A2 in human hepatocytes was not influenced by tested statins. All statins induced CYP2A6, CYP2B6 and CYP3A4, and the effects on CYP2C9 were rather modulatory. The effects varied between statins and enantiomers and induction potency decreased in order: atorvastatin (RR>RS=SR>SS) > fluvastatin (SR>RS=SS>RR) >> rosuvastatin (only RS active).

## 4. Binding of PXR to DNA - electrophoretic mobility shift assay

We tested whether the effects of statins on PXR-CYP3A4 signaling pathway involves also changes in the formation of PXR/RXRα-DNA complex. PXR-mediated gene activation requires direct binding of the PXR-RXRα heterodimeric complex to the response elements in the gene promoter. Maximal induction of CYP3A4 gene expression apparently requires an additional DR3 nuclear receptor-binding element 1(dNR1; -7733/-7719) in a distal xenobiotic responsive enhancer module<sup>2,3</sup>. Nuclear extracts from LS174T cells treated by DMSO (0.1% v/v), RIF (10 μM) and individual enantiomers of atorvastatin, rosuvastatin and fluvastatin at concentration 10 μM were incubated with biotin-labeled double-stranded oligonucleotide corresponding to the DR3 PXR response element in the CYP3A4 promoter and resolved on non-denaturing gel. The specificity of PXR-RXRα binding was confirmed by competition with non-labeled double-stranded DR3 oligonucleotide.



**Figure 4:** Effect of statin enantiomers on the binding of PXR/RXRα complex to the DR3 motif of human CYP3A4 gene promoter.

Positive control RIF strongly stimulated a formation of PXR/RXRα-DNA-binding complex. All tested compounds increased binding of PXR/RXRα to the DR3 module in comparison with vehicle (Figure 4A). Immunoblot analysis confirmed that equal amounts of PXR proteins were used in the gel shift assay (Figure 4B).

## 5. Conclusion:

We have investigated enantioselective effects of statins on the expression of xenobiotic-metabolizing human enzymes. The data showed that the potential for drug-drug interactions involving induction of P450s is higher for clinically used optical isomers of rosuvastatin, atorvastatin and fluvastatin, as compared to their respective enantiomers, which are not in therapeutic use.