

Design, synthesis and biological evaluation of tamoxifen-fusion compounds for the optimization of MASPIT, a three-hybrid target deconvolution assay

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Methods that allow high throughput identification of cellular targets of biologically active small molecules are valuable assets in pharmaceutical research. They are useful in mechanism of action studies of hits identified via phenotypic screening. Alternatively, they may uncover 'off-target' proteins of established drugs, that may contribute to their therapeutic efficacy or unwanted side effects. Finally, such methods also allow to identify potential novel therapeutic applications of existing drugs.

Mammalian Small molecule-Protein Interaction Trap (MASPIT) is a three-hybrid approach that enables swift proteome-wide screening for intracellular targets of small molecules based on the JAK/STAT signaling pathway of the cytokine receptor (CR).¹ MASPIT employs DHFR, fused to the CR to present a methotrexate (MTX) fusion compound (MFC) to the intracellular environment. This allows the screening of a small molecule bait against a collection of chimeric prey proteins. As a result of the interaction between the bait and a prey protein, the JAK/STAT pathway is activated, resulting in the expression of the luciferase reporter gene.

A *conditio sine qua non* for successful MASPIT analysis is the availability of appropriate synthetic probes.² In this presentation we will discuss our efforts in optimizing the MASPIT system's selectivity and sensitivity via the synthesis and biological evaluation of tamoxifen-fusion compounds, comprising immobilizing moieties different from the known MTX anchor.

¹ Caligiuri, M. *et al.*, *Chem. Biol.* **2006**, *13*, 711-722.

² Risseeuw, M.D.P. *et al.*, *ChemMedChem.* **2013**, *8*, 521-526.