Syntheses of MTX-fusion compounds for target profiling of small molecules with MASPIT

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Methods that allow high throughput identification of cellular targets of 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, which may contribute to their therapeutic efficacy. Finally, such methods also allow profiling small molecules against a series of related intracellular targets (e.g. kinases).

Mammalian Small molecule-Protein Interaction Trap (MASPIT)[1] provides a new tool for swift proteome-wide screening for intracellular targets of known small molecules. This three-hybrid system is based on the JAK/STAT signaling pathway of the cytokine receptor and enables the identification of mammalian cytosolic proteins that interact with a small molecule of interest (see the poster of Lievens *et al.* for more details on the biology aspects and applications of the MASPIT technology).

In this poster we present a scalable synthesis of a versatile methotrexate (MTX) reagent equipped with an azide ligation handle that allows rapid γ-selective conjugation to yield MTX fusion compounds (MFCs) appropriate for MASPIT.[2] We selected three structurally diverse pharmacologically active compounds (tamoxifen, reversine and FK506) as model baits. After acetylene functionalization of these baits (paying close attention to SAR), MFCs were synthesized via a CuAAC reaction, demonstrating the general applicability of the MTX reagent.

In analytical mode, MASPIT was able to give concentration-dependent reporter signals for the established target proteins. Furthermore, we demonstrate that the sensitivity obtained with the new MTX reagent was significantly stronger than that of a previously used non-regiomeric conjugate mixture. Finally, the FK506 MFC was explored in a cellular array screen for targets of FK506. Out of a pilot collection of nearly 2000 full-length human ORF preys, FKBP12, the established target of FK506, emerged as the prey protein that gave the highest increase in reporter signal. This indicates that our newly developed synthetic strategy for the straightforward generation of MFCs is a promising asset to uncover new intracellular targets using MASPIT cellular array screening[3].

References

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