SYNTHESES OF METHOTREXATE-HYBRID COMPOUNDS FOR TARGET PROFILING OF SMALL MOLECULES WITH MASPIT

D. De Clercq[1*], M. Risseeuw[1], S. Lievens[2],
J. Tavernier[2], S. Van Calenbergh[1]

[1]Laboratory for Medicinal Chemistry, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium
[2]Cytokine Receptor Lab, Ghent University, Albert Baertsoenkaai 3, B-9000 Ghent, Belgium

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, that may contribute to their therapeutic efficacy. Finally, such methods also allow to profile small molecules against a series of related intracellular targets (e.g., kinases).

A recently developed assay, Mammalian Small molecule-Protein Interaction Trap (MASPIT) (1) provides a new tool for swift proteome-wide screening for intracellular targets of known small molecules. The principle of MASPIT is based on the JAK/STAT signaling pathway of the cytokine receptor (CR). A small molecule, or bait, is tethered to methotrexate (MTX) via a PEG linker. The MTX moiety allows immobilization of the bait onto the DHFR unit which is fused to the CR, thus allowing screening the bait against a collection of chimeric-prey proteins. Upon binding of the bait to a prey protein, the JAK/STAT signal cascade is activated, resulting in the expression of a luciferase reporter gene allowing a facile photometric read out.

We set out to synthesize alkyne-functionalized analogues of blockbuster drugs (simvastatin, propranolol and tamoxifen) and the small molecule reversine, paying close attention to SAR. These analogues were conjugated with an azide containing MTX-reagent via the Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition (CLICK chemistry). The resulting MTX-conjugates are currently being evaluated in the MASPIT assay.

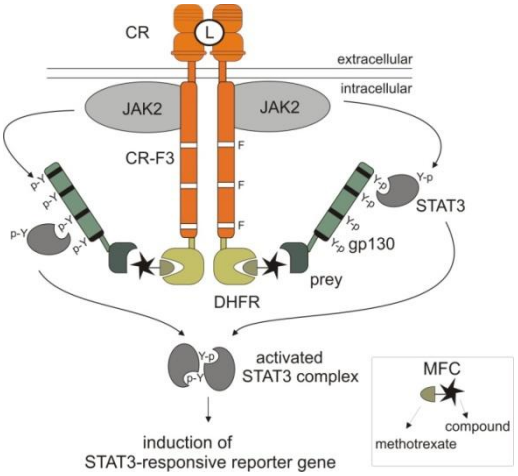

Fig. 1: Outline of the MASPIT system.

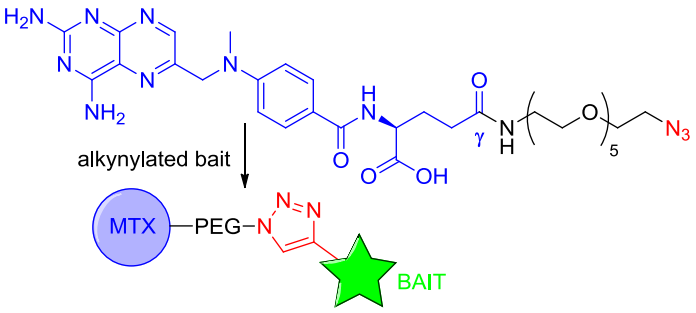

Fig. 2: General structure of MTX fusion compound prepared via CuAAC.

REFERENCES

(1) Caligiuri, M.; Molz, L.; Liu, Q.; Kaplan, F.; Xu, J. P.; Majeti, J. Z.; Ramos-Kelsey, R.; Murthi, K.; Lievens, S.; Tavernier, J.; Kley, N. *MASPIT: Three-Hybrid Trap for Quantitative Proteome Fingerprinting of Small Molecule-Protein Interactions in Mammalian Cells. Chem. Biol.* **2006**, *13*, 711–722.