UHPLC-MS/MS screening of the hTEAD surface for the identification of novel allosteric inhibitors with anticancer activity

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The Hippo signalling metabolic control pathway represents an emerging topic in tumour suppression regulation and regenerative medicine. This pathway, activated by extracellular anti-proliferative signals, is regulated by a cytosolic phosphorylation cascade of four main proteins with serin-threonine kinase activity [1]. At the endpoint of this cascade, phosphorylation of YAP (yes associated protein)/TAZ (Tafazzin) paralogues proteins, act as TEAD1-4 (transcriptional enhancer factor TEF) coactivators. TEAD Ser127 phosphorylation activates YAP/TAZ proteasomal degradation, and this prevents its migration to the nucleus for YAP-TEAD interaction, thus preventing the transcription of genes activating cell proliferation. The hTEAD-4 isoform, the most represented in solid tumor masses, shows three distinct binding surfaces and a lipid pocket, usually occupied by a palmitic/myristic fatty acid forming a thioester with side residue of Cys365 [2]. Despite being a promising pharmaceutical target, the disruption of the YAP-TEAD complex is still under preliminary investigation, and further protein reactivity studies are necessary to better understand how the pathway can be inhibited. Our studies focus on characterizing existing cysteine-based pocket of the hTEAD4-YAP binding domain (YBD) surface by analysing the reactivity of its four cysteine residues (Cys310, Cys335, Cys367, Cys410). These residues are nearby the area where YAP binds but are not fully hindered and therefore show some thiol-like reactivity. As Cys335 is closest to *interface-3,* the region that naturally recognises the YAP Ω-loop sequence, compounds able to interact near this area may suggest novel allosteric binding sites for a new inhibitor class. For this reason, we used UHPLC-HRMS (Orbitrap Q-Ex) and HPLC-UV-ELSD to characterize recombinant hTEAD4, its naturally myristoylated fraction, and its ability to bind a small library of disulphides and thiols with different chemical properties through exposed cysteines residues. Among these ligands, we demonstrated that only a few compounds reacted by forming a disulphide bond. Mass spectrometry ($ddMS^2$) experiment on hTEAD tryptic peptides proved that the ligands display high selectivity towards Cys335. No disulphide is formed with Cys367, not even in the non-myristoylated fraction, probably because of the high lipophilic nature of its pocket. We confirmed the results with an intact protein MS^1 experiment and measured the same mass shift. Ongoing X-ray crystallography experiments will provide the structural bases for an HIT optimization medicinal chemistry program.

Additional ongoing work consists in the exploration of a larger thiophenol-based library by medium throughput MS screening methods, to study the influence of those ligands on the non-covalent interaction of YAP with TEAD. The short-term perspectives of this work are the identification of an allosteric inhibitor useful for YAP-TEAD complex disruption, the confirmation and quantitative assessment of inhibitor binding with an orthogonal assay and the achievement of structural information for starting a hit-optimization medicinal chemistry program.

[1] Santucci M, Vignudelli T, et al. The Hippo Pathway and YAP/TAZ-TEAD Protein-Protein Interaction as Targets for Regenerative Medicine and Cancer Treatment. J Med Chem. 2015 Jun 25;58(12):4857-73. In the frame of AIRC IG16944.

[2] Yueh C, Rettenmaier J, et al. Atlas: Druggability Analysis of Potential Allosteric Sites in Kinases. J Med Chem. 2019 Jul 25;62(14):6512-6524.
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