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Optimization of chemical-biological tools for characterizing the activity of dissociative inhibitor of human thymidylate synthase

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Human thymidylate synthase (hTS) catalyzes the reductive methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) which requires methylene tetrahydrofolate (mTHF) as a cofactor [1]. This enzyme has been targeted in cancer treatment because of its crucial role. Drugs such as 5-fluorouracil (5-FU) and folate analogues like raltitrexed can bind covalently to the active site of hTS. However, this mechanism often leads to an increase in the expression of hTS within the cell, resulting in drug resistance. Over the years, it was shown that the hTS is a homodimer of 76 kDa consisting of two identical subunits, each weighing 37 kDa. Each subunit contributes to the formation of two active

sites and can exist in different states. The dimer can switch between an active state and an inactive state, the dimer is in equilibrium with its monomeric form with a Kd around 6-8 nM. All these states can contribute to compose the broad interactome with different biomolecular targets within the cell and trigger eventually different specific mechanism. To overcome drug resistance, our research group has focused on finding new ways to switch the dimer to monomer equilibrium and reduce the enzyme catalytic activity causing its overexpression [2]. We aim to target the interface of the dimer to shift the equilibrium towards the inactive monomeric form of hTS. In order to characterize the dissociative character of these newly synthesized

compounds to shift the equilibrium toward the monomeric form of the protein and inactivate it, different chemical-biological tools were set up: fluorescence-resonance energy tarnsfer (FRET) experiments, kinetic assays with dedicate algorithms, site specific mutagenesis and more recently dialysis experiments. The present work present the results from the different assays for some representative inhibitors and focus specifically on the recent results on the dialysis experiments. By conjugating the protein with a fluorescent probe we were able to monitor its movement in the dialysis device and detect some interesting events such as the dissociative effect of the dimer destabilizer.In conclusion , we succeed in developing a new chemical biological tools to characterize the dissociative capacity of these newly shythesized inhibitor , which rapresent a promisimg Medicinal Chemistry strategy .

References:

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