IDENTIFICATION OF A 2-PROPANOL ANALOGUE MODULATING THE NON-ENZYMATIC FUNCTION OF INDOLEAMINE 2,3-DIOXYGENASE 1

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INTRODUCTION: Indoleamine 2,3dioxygenase 1(IDO1) is a metabolic enzyme that catalyzes the conversion of the essential amino acid tryptophan (Trp) into a series of immunoactive catabolites, collectively known as kynurenines. Through the depletion of Trp and the generation of kynurenines, IDO1represents a key regulator of the immune responses involved in physiologic homeostasis as well as in neoplastic and autoimmune pathologies. The IDO1enzyme has been described as an important immune checkpoint to be targeted by catalytic inhibitors in the treatment of cancer. In contrast, a defective expression/activity of the enzyme has been demonstrated in autoimmune diseases. Beside its catalytic activity, the IDO1protein is endowed with an additional function associated with the presence of two immunoreceptor tyrosine-based inhibitory motifs (ITIMs), which, once phosphorylated, bind SHP phosphatases and mediate a long-term immunoregulatory activity of IDO1.

Methods: Herein,we report the screening of a focused library of molecules bearing a propanol core by a protocol combining microscale thermophoresis (MST) analysis and a cellular assay.

Results: As a result, the combined screening identified a 2-propanolol analogue, VIS351, as the first potent activator of the ITIM-mediated function of the IDO1enzyme. VIS351displayed a good dissociation constant (Kd=1.90 μM) for IDO1and a moderate cellular inhibitor activity (IC_{50}=11.463μM), although it did not show any catalytic inhibition of the recombinant IDO1enzyme. Because we previously demonstrated that the enzymatic and non-enzymatic (i.e., ITIM-mediated) functions of IDO1reside in different conformations of the protein, we hypothesized that in the cellular system VIS351may shift the dynamic conformational balance towards the ITIM-favoring folding of IDO1, resulting in the activation of the signaling rather than catalytic activity of IDO1. We demonstrated that VIS351activated the ITIM-mediated signaling of IDO1also in mouse plasmacytoid dendritic cells, conferring those cells an immunosuppressive
phenotype detectable *in vivo*.

Conclusions: The manuscript describes for the first time a small molecule as a positive modulator of IDO1signaling function, paving the basis for an innovative approach to develop first-in-class drugs acting on the IDO1target.