Discovery of novel and selective inhibitor leads by targeting an allosteric site in Insulin-Regulated Aminopeptidase

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Insulin-Regulated aminopeptidase is a zinc-dependent aminopeptidase with several important biological functions and an emerging pharmaceutical target for cognitive enhancement and immune system regulation. Aiming to discover lead-like IRAP inhibitors with enhanced selectivity versus homologous enzymes, we chose to target an allosteric site at the C-terminal domain pocket of IRAP. We compiled a library of 2.6 million commercially available compounds from the ZINC database, with MW of 200–350, cLogP <3.0 and lacking reactive groups or PAINS. This library was employed in molecular docking at the target pocket of IRAP and the corresponding pocket of the homologous enzyme, ERAP1. From this search we identified compounds with the highest predicted affinity for IRAP and filtered them for the highest possible selectivity versus ERAP1. After visual inspection of the top-ranked complexes, 305 compounds were selected for further investigation by molecular dynamics simulations and the MM–GB/PBSA methodology. Based on these results, we selected 33 compounds for *in vitro* evaluation. These compounds were evaluated using two orthogonal functional assays: one using a small fluorigenic substrate and one by following the degradation of Oxytocin, a natural peptidic substrate of IRAP. Our *in vitro* evaluation suggested that several of the compounds tested are capable of inhibiting IRAP, but the inhibition profile was highly dependent on substrate size, consistent with the allosteric nature of the targeted site. Overall, our results describe several novel leads as IRAP inhibitors and suggest that the C-terminal domain pocket of IRAP is a valid target for developing highly selective IRAP inhibitors.