Structure based approach for search of protein-protein interaction disruptors as inhibitors of GITRL-GITR signaling

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The tumor necrosis factor (TNF) superfamily (TNFSF) contains about thirty structurally related receptors (TNFSFRs) and about twenty protein ligands, able to bind to one or more of these receptors. Because these receptors play important roles in inflammatory and immune modulation, they represent potential pharmacological targets for treatment of autoimmune diseases, transplant rejection, or cancer. Nowadays, the development of biological drugs (i.e. antibodies, decoy receptors) is the most successful strategy for inhibition of TNFSFR signaling; e.g. infliximab, adalimumab, certolizumab, golimumab, etanercept. However, development of new small molecules or repurposing of already approved drugs able to interfere with protein-protein interaction (PPI) between a TNFSFR and a corresponding proteinaceous ligand represent an alternative strategy that may improve patient compliance and reduce costs. The GITR receptor signaling is involved in several inflammatory and autoimmune diseases, including rheumatoid arthritis and Crohn's diseases (1). GITR activation implies the formation of a homotrimer able to interact with a homotrimer GITRL. Our study was aimed at finding small molecules able to interfere with GITRL-GITR interaction by searching potential disruptors of GITRL monomer-monomer interaction, thereby inhibiting the formation of the active trimer. A similar strategy has been previously used to identify small molecules able to inhibit CD40 signaling by disruption of CD40L monomer-monomer interactions (2, 3). The crystal structure of GITRL (PDB:3B93) was edited in order to fill missing loops and residues side chain by means of Maestro suite of Schrödinger package©. The putative binding pocket of PPI disruptors, was recognized by two approaches: i. identification of monomer-monomer interfaces with highest displacement during simplified molecular dynamics simulation (elastic network model); ii. protein contact network analysis, in order to find hot-spot residues important for PPI stability (4). Virtual screening of the library Asinex® "PPI designed inhibitors" (8639 structures) was carried out on the most promising pocket of GITRL trimer. The virtual screening protocol was characterized by three sequential docking calculation with increasing precision: Glide HTVS, Glide SP, Glide XP (Schrodinger©). Compounds were then rescored (predicted ΔGbind) with MM-GBSA applying to protein, ligands and protein-ligands complexes an implicit salvation model. Virtual screening identified 8 molecules from the Asinex® library as hit-compounds, these compounds have cycloheptane-; pyridinone-; pyrimidine- carboxyamide moieties.

References

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