Dipartimento di Farmacia

Università degli Studi di Napoli Federico II

PROGETTO PER IL XXXIX° Ciclo di Dottorato in Scienza del Farmaco

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Title: Development of Ligands targeting Adenosine receptor A2A

Background - G protein-coupled receptors (GPCRs) are cell membrane proteins responsible for the transduction of a broad range of signals from outside to inside the cell. Operatively, they bind signaling molecules (i.e., endogenous and exogenous ligands) at extracellular orthosteric sites and transmit the ligand-induced allosteric signal to the intracellular binding site for effector proteins such as G proteins. A pharmacologically relevant member of GPCRs is the adenosine A2A receptor which regulates many vital functions in different districts of our body. The A2A pathway plays an important role in heart activity[1] as well as in glutamate and dopamine release in the brain, particularly in regions that are involved in motor control and cognitive function.[2] In addition, A2A can suppress immune cells, thereby protecting tissue from inflammation.[3] However, many solid tumors hijack the A2A pathway, promoting immune escape of tumor cells in the tumor microenvironment. Therefore, blocking A2A can inhibit the progression of a variety of solid tumors.[4] In this background, understanding how ligands bind to A2A is of paramount relevance for rational design of new therapeutic agents for a large number of diseases, including cancer, inflammatory, cardiovascular, Parkinson's and Alzheimer's diseases.[5-7] So far, drug discovery has focused on the extracellular orthosteric binding and more recently on allosteric binding sites, with the ambition to design ligands capable of selectively promoting the GPCR interaction with a specific G protein subtype, thus activating a certain cellular pathway over the others (biased signaling) and eventually improving their activity and toxicity profile.

Project - At variance with previous studies, we target the GPCR intracellular binding site for the G protein with the aim to develop a small library of ligands endowed with a selective binding affinity for a specific GPCR-G protein couple. First, compounds, from different databases, will be docked to the intracellular binding site of Adenosine A2A GPCR for Gs protein. The best ligands, considering their binding energy and the interactions established with the protein, will be purchased and experimentally evaluated using NMR ligand/protein binding experiments, such as Saturation Transfer Difference (STD) and WaterLOGSY (WL) experiments. To this aim, A2A, Gs protein and some rationally designed mutants of them will be expressed in *P. pastoris*, purified, and resuspended in suitable detergent media. Subsequently, the compounds that exhibit binding will be pharmacologically characterized, for their intrinsic activity (agonist, partial agonist, inverse agonist, antagonist), using the cAMP assay in A2A-transfected hek293 cells.

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References

1. Paganelli F, Gaudry M, Ruf J, Guieu R. Recent advances in the role of the adenosinergic system in coronary artery disease. *Cardiovasc Res.* 2021;117(5):1284-1294. doi:10.1093/cvr/cvaa275
2. Morelli M, Di Paolo T, Wardas J, Calon F, Xiao D, Schwarzschild MA. Role of adenosine A2A receptors in parkinsonian motor impairment and l-DOPA-induced motor complications. *Prog Neurobiol.* 2007;83(5):293-309. doi: 10.1016/j.pneurobio.2007.07.001
3. Ohta A, Sitkovsky M. Role of G-protein-coupled adenosine receptors in downregulation of inflammation and protection from tissue damage. *Nature*. 2001;414(6866):916-920. doi:10.1038/414916a
4. Sun C, Wang B, Hao S. Adenosine-A2A Receptor Pathway in Cancer Immunotherapy. *Front Immunol*. 2022;13:837230. doi:10.3389/fimmu.2022.837230
5. Dall'Igna OP, Porciúncula LO, Souza DO, Cunha RA, Lara DR. Neuroprotection by caffeine and adenosine A2A receptor blockade of beta-amyloid neurotoxicity. *Br J Pharmacol*. 2003;138(7):1207-1209. doi:10.1038/sj.bjp. 0705185
6. Gołembiowska K, Dziubina A. Striatal adenosine A(2A) receptor blockade increases extracellular dopamine release following l-DOPA administration in intact and dopamine-denervated rats. *Neuropharmacology.* 2004;47(3): 414-426. doi:10.1016/j.neuropharm.2004.04.018
7. Headrick JP, Ashton KJ, Rose'meyer RB, Peart JN. Cardiovascular adenosine receptors: expression, actions and interactions. *Pharmacol Ther.* 2013;140(1):92-111. doi:10.1016/j.pharmthera.2013.06.002