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Short communication

Docking studies of neurokinin-1 receptor antagonists as an anticancer target

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Introduction

Over the last two decades, research regarding Neurokinin-1 (NK1) receptor has been pursued aggressively to develop drugs that might be useful for a branch of pharmacologic purposes including; anticancer, antiviral and antiemetic and dozens of molecules have been entered into various phases of clinical trials. The endogenous ligand neuropeptide Substance P (SP) selectively binds to NK1 receptor at the plasma membrane (Munoz et al. 2011). SP is an undecapeptide that belongs to the tachykinin peptide family and widely distributed in both the central and the peripheral nervous system of mammals. Activation of NK1 receptor by SP stimulates G-protein mediated signaling pathways that are crucial for regulating cellular excitability and function such as cAMP accumulation, arachidonic acid mobilization and phosphatidylinositol turnover. It has been shown that activation of Akt suppresses apoptosis and stimulation of NK1 receptor by SP induces phosphorylation on Akt or Protein Kinase B (PKB) activity in human glioblastoma cells. After binding to the NK1 receptor in tumor cells, SP induces mitogenesis and inhibits apoptosis. Hence NK1 receptor antagonists can lead to apoptosis and inhibit tumor cell proliferation. Antagonists of these receptors inhibit the development of metastasis by blocking the activation of NK1 receptor by SP. It is shown that NK1 receptors are overexpressed in tumor cells and their antagonists such as aprepitant, L-733,060, and L-732,138 have antitumor activity against several human cancer cell lines such as melanoma, neuroblastoma, glioma, retinoblastoma, pancreatic, larynx, gastric and colon carcinomas (Munoz et al. 2011).

It has been demonstrated that binding sites of peptide antagonists and non-peptide antagonists of NK1receptor are different than each other. SP and peptide NK1 receptor antagonists bind to the extracellular terminal region of the receptor, but non-peptide NK1 receptor antagonists bind to intracellular part of the enzyme between transmembrane helices (Munoz et al. 2011). Ligand binding pocket of an NK1 receptor is a hydrophobic core between the loops of transmembrane TM III-VII. Several residues, such as Gln165 (TM IV), His197 (TM V), His265 (TM VI) and Tyr287 (TM VII) are involved in the binding of many non-peptide antagonists of the NK1 receptor. The other residues that are contributed in non-peptide antagonist binding are Ser169, Glu193, Lys194, Phe264, Phe267, Pro271 and Tyr272 (Almeida et al. 2004).

Over the last decade our group have been designed, synthesized, and working on the new anticancer active compounds. Some of our previously synthesized benzoxazole and benzamide compounds showed significant inhibitory activity for human DNA Topoisomerases and Glutathione S-transferases and also anticancer effects observed on various cell cultures (Pinar et al. 2004).

In this research, we aimed to search the activity of our previously synthesized compound, 2-[4-(4-ethylbenzamido)phenyl]benzothiazole (BSN009), to the new anticancer target NK1 receptor and to identify the binding site features and modes of NK1 receptor and the non-peptide antagonists including our synthesized compound using molecular docking study.

Materials and methods

The cytotoxic activity of tested compounds (BSN009, CP-96345, L-733,060, L-732,138, and aprepitant) were assayed using the MTT colorimetric protocol. MTT is cleaved to formazan by the "succinate-tetrazolium reductase" system (EC 1.3.99.1) which belongs to the mitochondrial respiratory chain and is active only in viable cells. Human

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colon carcinoma cell line HT-29 (ATCC, HTB-38), breast cancer cell line MCF-7 (ATCC, HTB-38), human cervical carcinoma cell line HeLa (ATCC, CCL-2) and mouse embryonic fibroblast cell line NIH3T3 (ATCC, CRL-1658) were used in this study for cytotoxicity experiments.

To analyze the binding site features of the tested compounds the molecular docking studies were performed by using CDocker working with Accelrys Discovery Studio (DS) 3.5 software.

The homology model of the NK1 receptor with CP-96345 was developed by Evers and Klebe. For preparation of protein and ligands the target protein was taken, hydrogens were added and their positions were optimized using all atom CHARMm forcefield and the Powell method available in DS 3.5 protocol. The minimized protein was defined as the receptor using the binding site module. The binding site was defined from the cavity finding method which was modified to accommodate all the important interacting residues in non-peptide antagonist binding site of the NK1 receptor. The protein was held rigid while the ligands were allowed to be flexible during refinement. The docking and scoring methodology was first validated by docking of ligand CP-96345. The docked position of CP-96345 overlaps well with the homology model position,

Results and discussion

Our previously synthesized compound, 2-[4-(4-ethylbenzamido)phenyl]benzothiazole (BSN009) was found as an active compound at a concentration of 50 μM as a result of MTT assay and inhibited colon cancer cell lines growth about by 57.53%. On the other hand, it has also been found that BSN-009 had no toxic effect on the normal cell line.

As a result of the molecular docking studies; BSN-009 was shown similar binding modes with NK1 receptor as known antagonists L-733,060, aprepitant, and L-732,138. The tested 2-substituted benzothiazole, BSN009, has hydrogen bonds with Gln165 (2,39 Angstrom) and His197 (1,83 Angstrom) like other non-peptide antagonists of NK1 receptor Oxygen atom of carbonyl group of BSN009 makes an H bond with His197. The phenyl ring of the benzamide group of BSN009 has a pi-cation interactions with His187. Binding energy values (kcal/mol-1) of BSN009 and aprepitant, which is a well-known NK1 receptor antagonist, is close to each other.

Conclusion

In conclusion, the performed molecular docking study elucidated that Gln165, His197, His265 and Tyr287 are crucial amino acids in the non-peptide binding site of the NK1 receptor. As a result of the molecular docking study and cytotoxic experiments, it can be concluded that BSN009 may be a good anticancer drug candidate as an NK1 receptor antagonist and is worthy to carry on the anticancer in vivo studies. This study also provide a model to design novel and more potent antitumor agents as NK1 receptor antagonists.

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