Design, Synthesis and Cytotoxic Evaluation of 2-Imino-2H-Chromene-3-Carboxamide Derivatives Containing Benzyl-1,2,3-Triazole Ring as Novel Cytotoxic Agents

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Introduction: Cancer is an important leading causes of death worldwide. Although some prevention programs have been organized in many regions in the world, new cancer incidences are increasing. Chemotherapy is the most well-known method for treatment of cancer. Resistance to chemotherapy drugs, their low efficiency, highly adverse effects, and economic issues have raised efforts to discover new anticancer targets. Aldoketoreductase is a NADPH-dependent reductase that is up-regulated in hepatocellular carcinomas. Iminochromenes have been reported as promising compounds in the inhibition of human isoform of aldoketoreductase (AKR1B10). 1,2,3-triazoles containing compounds show cytotoxic effects in previous studies. In this study, click synthesis method is applied to raise up selectivity and yield of the reaction while producing 1,2,3-triazole ring. The cytotoxic assays were then performed to evaluate the potential anticancer effects of the compounds.

Material and Methods: Nine different derivatives of 2-imino-2H-chromene-3-carboxamide have been synthesized via four chemical steps. In first three steps, iminochromene carboxamide backbone is produced. In the last step, using click synthesis method, the iminochromene carboxamide backbone is bounded to different benzylic derivatives via a methylene-1,2,3-triazole bridge. The final compounds are purified using various purification methods; such as plate chromatography and recrystallization. The compounds are then verified throughout IR, Mass and ^{1}H-NMR spectrometry. MTT assay have been used on MCF-7 cell lines in order to evaluate cytotoxic effects of the compounds. Docking studies have been performed to deeply investigate the type of ligand's interactions with AKR1B10 active site, using AutoDock 4.2 as a molecular docking software.

Results: Click synthesis yields to a highly pure products. In cytotoxic assay, the synthesized compounds show noticeable effects in comparison to cisplatin and doxorubicin. 2-imino-2H-chromene-3-carboxylic acid-[1-(4-nitobenzyl)-1H-[1,2,3]-triazole-4-yl methyl] amide with an IC_{50} of 14.7±2.51 is the most potent derivative. Electron-withdrawing substitutions is preferable in cytotoxic effects compared to electron-donating ones. Binding inhibitory constants of the compounds are calculated to be about 0.5-5.23 nM in *in-silico* studies.

Discussion and conclusion: π – π stacking interaction of Trp112 to iminochromene ring, cation-π stacking interactions of His111 to benzylic group, and dipole-dipole interactions of Val301 to carbonyl group are the most important interactions due to docking studies. There are some differences between docking data and MTT-assay data which are probably because of electronic moiety of the cells, solubility and polarizability of the compounds, the effects of the compounds on other signaling pathways in cells,

etc. Nevertheless, favorable binding energies of the compounds to the enzyme's active site are calculated to be about ((-11.6kcal/mol)-(-12.69kcal/mol)) compared to main ligand, zopolrestate which possesses -11.35kcal/mol free binding energy. AKR1B10 has an important role in cell differentiation processes. Inhibition of the enzyme, increases the amounts of available retinoic acid for the cells and therefor prohibits cell proliferation.
Compounds that are containing electron-withdrawing substitutions have shown the most cytotoxic effects. C_3 and C_4 positions on benzyl group does not show any meaningful difference on IC_{50} of corresponding compounds. Electron-donating and bulk groups on benzyl moiety reduces the cytotoxic effects of the compounds.

Keywords: 2-imino-2H-chromene-3-carboxamide derivatives, Aldoketoreductase, MTT assay, Molecular docking, Click synthesis