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In vitro validation of novel drug candidate EGFR inhibitor in treatment of colorectal cancer employing 2D and 3D cell culture techniques

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Colorectal cancer (CRC) is deemed to be the third leading cause of cancer-related deaths worldwide with an overall mortality rate of 10%. One of the major causes of CRC is overexpression of EGFR, and currently, there are very few effective medications available to target it. This study intends to validate the potency of 2-((1,6-dimethyl-4-oxo-1,4-dihydropyridin-3-yl)oxy)-N-(1H-indol-4-yl)acetamide (E1) which has previously been reported as an effective EGFR inhibitor via *in silico* approaches. Anticancer investigation, toxicity analysis, indirect ELISA studies, clonogenic assay, live-dead assay, Reactive Oxygen Species (ROS) assay, and flow cytometry analysis were conducted. Compound E1 inhibited colorectal cancer cells by 38% at an LC_{50} of 156.0365 µg/mL, while inhibiting normal cells by only 15% at an LC_{50} of 418.3150 µg/mL. Activity of BRAF (downstream antibody of EGFR in the MAPK pathway) was reduced three-fold compared to control samples, demonstrating the compound's efficacy. Colony formation ability of the cancer cells was reduced by four-fold while the ROS contents were declined by 23 times in the presence of E1. Cell cycle arrest was confirmed at G0/G1 phase through flow cytometry analysis. RT-PCR studies also confirmed the EGFR inhibition ability of E1. 3D cell culture validation of E1 established strong anticancer inhibition ability for the compound E1. The nominated compound E1 thus can be considered as a novel and potential EGFR inhibitor.

Keywords: Anticancer evaluation, Toxicity analysis, Indirect ELISA, Apoptosis, Cell cycle arrest, RT-PCR