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Evaluation of the molecular mechanism of action of novel tetrahydroacridine and cyclopentaquinoline derivatives as potential anticancer compounds.

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ABSTRACT

Lung cancer is not only the most commonly diagnosed cancers worldwide but is still the leading cause of cancer-related death. It is an aggressive tumor with a 5 year survival rate of less than 15% demonstrating that current therapy is still ineffective. Therefore, the search for better chemotherapeutic agents with advanced activity against lung cancer is needed. Acridine derivatives interact with DNA through intercalation, which causes the inhibition of replication, transcription and DNA repair and subsequently may lead to cell cycle arrest or apoptosis.

The aim of the dissertation was to evaluate the anticancer properties of 32 novel tetrahydroacridine and cyclopentaquinoline derivatives on the growth inhibition of human lung adenocarcinoma cells and identify the molecular mechanisms of action of the most effective compounds. These synthesized compounds contained 6-hydrazinonicotinic acid or 4-fluorobenzoic acid moiety and differed from each other in length of the aliphatic chain containing from 2 to 9 carbon atoms.

We found that new tetrahydroacridine and cyclopentaquinoline derivatives containing 4-fluorobenzoic acid were much more effective in inhibition of lung cancer cells growth in comparison with compounds with 6-hydrazinonicotinic acid moiety. Interestingly, their efficacy was correlated with increasing number of carbon atoms in the aliphatic chain. Based on the IC_{50} values of tested acridine derivatives we selected 6 the most effective compounds from each group for further evaluation. To characterize mode of actions of the most effective compounds we investigated their effects on cell cycle progression and apoptosis. The results indicated that inhibition of A549 cell growth by tetrahydroacridine and cyclopentaquinoline derivatives with 4-fluorobenzoic acid moiety was associated with a cell cycle arrest at G0/1 phase and with induction of caspase 3-dependent apoptosis. Similar acridine derivatives with 6-hydrazinonicotinic acid also caused lung cancer cells death due to apoptosis, however, they had no significant effect on a cell cycle progression. For this reason, 4 novel acridine derivatives with 4-fluorobenzoic acid moiety were selected to identify the molecular mechanisms of action. The results showed that 4 selected tetrahydroacridine and cyclopentaquinoline derivatives with 4-fluorobenzoic acid induced rapid activation of ATM kinase in response to DNA damage resulting in phosphorylation of histone H2A.X and p53. Studies on the

molecular mechanisms of apoptosis showed that the tested compounds significantly decreased the mitochondrial membrane potential values, as well as increased expression of pro-apoptotic protein Bax and down-regulation of anti-apoptotic protein Bcl-2, suggesting that the induction of apoptosis by these compounds is associated with activation of the mitochondrial pathway. The study on the molecular mechanisms of inhibition of cancer cell proliferation showed that cyclopentaquinoline derivatives with 4-fluorobenzoic acid moiety induced G0/1 cell cycle arrest by a p21-dependent pathway. In contrast, treatment of cancer cells with tetrahydroacridine derivatives had no significant effects on p21 expression. Moreover, exposure of cancer cells to the compounds with 4-fluorobenzoic acid resulted in inhibition of the CDK2 kinase activity leading to inhibition of cell cycle progression from G1 to S phase. The tested compounds also significantly attenuated the migration rates of lung cancer cells.

In conclusion, the results from this project indicate that tetrahydroacridine and cyclopentaquinoline derivatives with 4-fluorobenzoic acid containing 9 carbon atoms can be great candidates for further evaluation as a lung cancer therapy in preclinical study because they efficiently suppressed growth of lung cancer cells by induction of cell cycle arrest as well as induction of apoptosis. Moreover, these studies gained new knowledge that fluorobenzoic acid moiety and length of the aliphatic linker enhance anticancer activity and have impact on the mode of actions of acridine-based compounds.