Investigation of the mechanisms of cytotoxic activity of thiourea derivatives

Cancer cells are a paradoxical, distorted copy of normal cells, exploiting mechanisms that are fundamental to human life and survival. Therefore, cancer treatment remains one of the greatest challenges of modern medicine. Among the numerous therapeutic strategies, chemotherapy still plays a key role, despite the limitations of low selectivity, drug resistance and variable bioavailability. The present study focuses on evaluating the antitumour potential of structurally diverse groups of 1,3-disubstituted thiourea derivatives containing electro-negative substituents in the terminal phenyl rings. Such modifications may affect the cytotoxicity, bioavailability and selectivity of the compounds. The resulting thiourea derivatives were subjected to in vitro cytotoxic screening tests against primary and metastatic colon (SW 480 and SW 620), prostate (PC3) and leukaemia (K-562) cancer cells. Structure-activity relationship analysis allowed the selection of the most promising compounds, which were then subjected to detailed studies assessing the mechanisms of action. From the group of 3- (trifluoromethyl)phenylthioureas, particularly derivatives 1, 2, 3, 8 and 9 showed strong cytotoxic activity and high selectivity against colorectal cancer cells, both primary and metastatic. At the same time, these analogues showed strong pro-apoptotic effects and inhibition of IL-6 secretion associated with tumor progression. On the basis of biological activity, two compounds - dihalogen derivative 2 and 4-trifluoromethylthiourea 8 - were selected, which had the lowest IC_{50} and the highest selectivity index (SI). Further studies of metabolic pathways showed that both derivatives activated caspase 3/7 and also affected the inhibition of the NF-κB pathway, which was associated with a reduction in VEGF levels and a potential anti-angiogenic effect. In addition, significant pro-oxidant effects of both compounds were confirmed. Metabolomic analysis, on the other hand, confirmed particularly beneficial changes in the metabolic profile in SW620 metastatic colorectal cancer cells and, in the case of derivative 2, also in prostate cancer cells. Interestingly, these compounds also showed strong bioactivity in spheroids under 3D culture conditions, which may suggest their favourable bioavailability. Meanwhile, compounds 1a, 2b, 3a, 3b, 4a, 5d and 5j from the second pool of halogenated bisphenylthiourea derivatives tested showed strong cytotoxic effects ($IC_{50} < 10.7$ μM), comparable to cisplatin. Of these, derivatives 3b and 5j showed selective activity against cancer cells of the SW480 lineage, and compound 5d against PC3 cells, while remaining non-toxic to normal cells. All compounds tested inhibited IL-6 secretion in colorectal cancer (SW480, SW620) and prostate cancer (PC3) cells, and increased free radical levels, what together with impairing antioxidant mechanisms, may have potentiated their cytotoxic effect. Changes in the cell cycle were also observed, with compounds

1a, 3a and 5d increasing the percentage of cells in the sub-G1 phase, compound 3a in G0/G1 and 2b in G2/M, which may suggest successively induction of apoptosis or necrosis, prevention of division or DNA damage. The dichlorophenyl derivative 3a and the monosubstituted thioureas 1a and 5j showed the greatest anticancer potential. Promising effects of selected thiourea derivatives were observed from in vitro cytotoxicity tests, indicating their potential as candidates for further preclinical studies in the context of both solid and hematological cancer therapy.