Abstract

Modern oncology, particularly in the fields of radiotherapy and phototherapy, faces a challenge: how to effectively and selectively eliminate cancer cells while minimizing damage to healthy tissues. In response to these needs, photodynamic therapy (PDT) has emerged as a rapidly advancing treatment modality. PDT combines light, a photosensitizer, and molecular oxygen within tissues to initiate reactions leading to cancer cell death. Despite its potential, the effectiveness of PDT is limited by hypoxia – a characteristic oxygen deficiency in the microenvironment of solid tumors. Therefore, the development of novel, more efficient photosensitizers working under hypoxia is timely and well justified.

This PhD thesis investigates two synthetic uridine analogs: 5-bromo-4-thio-2′-deoxyuridine (BrSdU) and 5-iodo-4-thio-2′-deoxyuridine (ISdU). Due to the presence of sulfur and halogen atoms, these compounds exhibit distinctive photochemical properties that enable their selective activation by UVA light – radiation known for its deeper tissue penetration and reduced cytotoxicity toward healthy cells. The aim of the study was to elucidate the photosensitizing mechanism of BrSdU/ISdU using the models of increasing complexity: isolated modified nucleosides, site-specifically modified DNA, and, finally, living cells.

In the first stage of the study, stationary photolysis of BrSdU and ISdU was performed in buffered solutions, leading to the identification of multiple photoreaction products, including disulfide dimers. Comprehensive structural characterization was achieved through HPLC, LC-MS, and 1H NMR analyses, supported by TD-DFT calculations, which together confirmed the high photoreactivity of the studied compounds. Notably, the stability of certain photoproducts varied with increasing UVA irradiation dose, indicating possible secondary reactions.

The next part focused on investigating the photochemical properties of BrSdU when incorporated into DNA. Synthetic double-stranded oligonucleotide models containing site-specific BrSdU modifications were employed. Unlike classical halogenated nucleosides such as BrdU and IdU, BrSdU did not induce DNA strand breaks via long-range photoinduced electron transfer. Instead, stable photoadducts featuring covalent bonds between nucleobases were observed, indicating an alternative reaction pathway likely involving the sulfur atom. These results provide new insights into the unique photochemistry of halogenated 4-thiouridine derivatives and highlight their potential for application in cancer therapies.

As the final phase of the study, the photosensitizing effects of BrSdU and ISdU were investigated at a cellular level using human prostate cancer cells. Both compounds were non-cytotoxic on their own. However, when combined with UVA irradiation, they significantly decreased cell viability, especially ISdU. Flow cytometry provided detailed insights into the photosensitizers and UVA light synergistic mechanism of action, revealing: (1) cell cycle arrest in the G2/M phase, a typical response to severe DNA damage that serves as a protective mechanism by preventing the replication of compromised genetic material; (2) induction of early apoptosis, confirming the effectiveness of PS combined with UVA; (3) increased γH2A.X levels, a marker of DNA double-strand breaks and (4) elevated reactive oxygen species (ROS), suggesting oxidative mechanisms in the cytotoxic effects of BrSdU/ISdU and UVA action.

In conclusion, this research clearly demonstrates that BrSdU and ISdU exhibit photochemical and photobiological properties, making them promising candidates as next-generation photosensitizers in photodynamic cancer therapy. Their selective activation by UVA light and effectiveness even under hypoxic conditions make them well-suited for the treatment of solid tumors. These findings provide a strong foundation for further studies, including detailed analyses of photoreaction mechanisms in biological environments and comparative analyses with classical photosensitizers. An interesting direction involves quantum-chemical calculations comparing the excited states of BrSdU and BrdU, which may help elucidate the role of the sulfur atom in determining photoreactivity and selectivity. Ultimately, the development of such compounds may contribute to more precise, effective, and safer light-based cancer treatment strategies.