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Synthesis, spectral, photophysical and photochemical properties of tricyclic thiopurine analogues with potential biological applications

ABSTRACT

Chemical compounds of natural origin are the basis for the design of active substances used in molecular biology and for therapeutic and diagnostic purposes. The continuously growing number of cancer cases and the observation of side effects resulting from the use of certain drugs create the need to search for new active substances with improved properties.

The aim of this dissertation was to synthesize and characterize the biological activity and spectral, photophysical, and photochemical properties of a new, previously undescribed group of tricyclic purine thioanalogues.

The synthesis of five 1,N^2-etheno guanine thioanalogues: TEGuo (9-thio-1,N^2-ethenoguanosine), TEGua (9-thio-1,N^2-ethenoguanine), 6MeTEGuo (6-methyl-9-thio-1,N^2-ethenoguanosine), 6MeTEGua (6-methyl-9-thio-1,N^2-ethenoguanine), 6MeTEG (2',3',5'-tri-O-acethyl-6-methyl-9-thio-1,N^2-ethenoguanosine) and two guanosine thioderivatives of the 1,N^2-ethano type: DTEGuo (6,9-dithio-1,N^2-ethanoguanosine), DTEG (2',3',5'-tri-O-acethyl-6,9-dithio-1,N^2-ethanoguanosine) was carried out. The structures of the obtained compounds were confirmed by mass spectrometry (ESI-MS) and nuclear magnetic resonance spectroscopy (^{1}H and ^{13}C NMR).

The ADME profile of the obtained compounds and their pharmacokinetic properties were characterized, which allowed the entire group of compounds to be classified as drug-like substances. In the next step of the research, the compounds were tested for anticancer activity against the HeLa cancer cell line. Their activity against mouse fibroblast cells NIH/3T3 was also examined. On the basis of the obtained results, the selectivity indexes were calculated.

The characterization of spectral and photophysical properties of tricyclic thiopurines was carried out based on UV-vis absorption and emission spectroscopy and time-resolved spectroscopic methods (TCSPC and LFP). The combination of thiocarbonyl chromophore and tricyclic structure shifts their absorption region above 360 nm, allowing selective excitation of the obtained

compounds when present in biological systems. The greatest shift of absorption maximum (λ_{max} ~ 410 nm) was observed for DTEGuo and DTEG due to the presence of two thiocarbonyl groups in their structure. Tricyclic thiopurines exhibit fluorescence from the second singlet excited state S_2 ($\pi\pi^*$) like other aromatic thiocarbonyl compounds. The exceptions are DTEG and DTEGuo, for which direct, simultaneous fluorescence from two different singlet excited states: the S_2 ($\pi\pi^*$) state and S_1 ($n\pi^*$) state was observed. This is an unusually infrequent phenomenon for aromatic thioketones due to their small radiative rate constant ($k_{S1 \rightarrow S0} \sim 10^5\ s^{-1}$) and their large quantum yields of intersystem crossing from the S_1 state to the T_1 state ($\Phi_{ISC} \sim 1$), which disable the fluorescence from the S_1 state, allowing only the observation of relatively intense fluorescence occurring from the S_2 state. The low fluorescence quantum yields for all of the tricyclic thiopurines do not allow for monitoring their distribution in organisms based on their emitted radiation. Their low fluorescence quantum yields result from their very efficient intersystem crossing leading to the formation of an excited triplet state with near unity quantum yield ($\Phi_{ISC} \sim 0.9$). Efficient formation of a long-lived T_1 triplet state ($\tau_T \sim 2.0\ \mu s$) allowed for an efficient generation of singlet oxygen with a quantum yield around $\Phi_\Delta \sim 0.6$ in ACN. Singlet oxygen sensitization was not observed for either DTEG or DTEGuo as a result of their relatively short triplet lifetimes in the range of hundreds of nanoseconds ($\tau_T \sim 100$ ns).

Tricyclic thiopurines undergo photochemical reactions under irradiation with wavelengths $\lambda > 350$ nm (water and phosphate buffer in the presence of air) with a quantum yield $\Phi_{PR} \sim 0.01$. Six main photoproducts were identified and different formation mechanisms were proposed. Unlike the other aromatic thioketones, DTEG and DTEGuo are photostable under irradiation at $\lambda = 410$ nm in the presence of air and in argon-saturated solution.

One of the most important achievements of this work is the observation of very efficient formation of tricyclic thiopurine derivatives in a direct one-step reaction of 6-thioguanine/6-thioguanosine with chloroacetaldehyde in water (pH 6.0 – 7.0). We proved that these products could be formed in the organisms of patients undergoing thiopurine-based therapy, even at low concentrations of thiopurine and high concentrations of aldehydes. It should be mentioned that thiopurines are among the one hundred most commonly used drugs in the world. This suggests the possibility that these newly formed tricyclic analogues can affect the therapeutic effect of thiopurines.

The results described within this thesis indicate that most of the synthesized compounds constitute a group of potential candidates for use in cancer chemotherapy as well as in chemotherapy combined with photodynamic therapy. The detailed spectral, photophysical, photochemical characterization and preliminary biological evaluation provide information about the properties of this group of compounds. These results constitute a basis for initiating systematic studies of the processes that the tricyclic thiopurine analogues can undergo in organisms and are crucial in the context of their use as potential therapeutics.