EFFECTS OF STEPWISE TERMINAL NH_2-METHYLATION OF ESTRONE-SALICYLALDEHYDE–THIOSEMICARBAZONE AND COPPER COORDINATION, SOLUTION SPECIATION, ANTICANCER ACTIVITY AND REDOX ACTIVITY.

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Thiosemicarbazones (TSCs) as excellent metal chelators are a class of organic compounds with structural diversity and broad spectrum of pharmacological activities, such as antiproliferative, antiviral, antibacterial, antimalarial and antifungal effect [1]. Also, their metal complex can be more active than the free ligand, and some side effects may decrease upon complexation. In addition, the complex can exhibit bioactivities, which are not shown by the free ligand. Previously, a tridentate estrone-salicylaldehyde TSC hybrid molecule (estrone-TSC) was developed in addition to an analogous bicyclic derivative (thn-TSC), which were cytotoxic against the hormone-responsive MCF-7 breast cancer cell lines (IC_{50}: thn-TSC: 3.7 μM, estrone-TSC: 6.4 μM). Their Cu(II) complexes showed more significant cytotoxicity than the ligands as 1-2 orders of magnitude lower IC_{50} values were obtained for the complexes against a series of human cancer cell lines [2]. Disubstitution of the terminal NH_2 groups in case of several α-N-pyridine thiosemicarbazones could result in highly increased anticancer activity (e.g. dimethylated Triapine, DpC, Dp44mT) [3]. Based on this finding in this work the *N*-terminally mono- and dimethylated derivatives of estrone-TSC and thn-TSC (Chart 1) and their Cu(II) complexes were aimed to prepare to obtain more effective compounds.

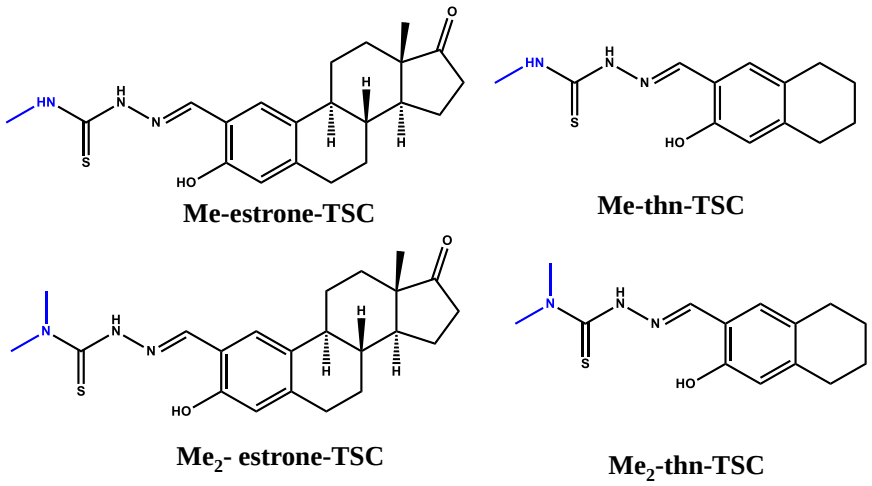

Chart 1. Chemical structures of the investigated ligands.

The solution stability and structure of the complexes were determined using UV-visible spectrophotometry and electron paramagnetic resonance spectroscopy. Due to the limited water solubility of the compounds UV-titrations were performed in a 30% (v/v) DMSO/H_2O solvent mixture in order to determine the pK_a values of the ligands and the stability constants of the complexes. Additionally, their anticancer activity was studied via *in vitro* cytotoxicity and ROS generation assays, and the cell proliferation, apoptosis and its caspase-dependence were also screened on 3D cancer spheroids, which are considered to mimic better the main features of human solid tumours compared with traditional 2D cultures.

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