

# Determination of the molecular structure of compounds with pharmacological potential using the methodology based on X-ray absorption spectroscopy

The aim of the research was the synthesis, structural characterization, as well as microbiological and cytotoxic activity evaluation of copper and silver complexes with bioactive tetrazole derivative ligands in solutions. The binding site between copper and silver ions and two bioactive tautomeric forms of 1,5-substituted tetrazole-derivative ligand has been investigated (Fig. 1.). After coordination to metal ions an increase in the activity has been observed. Since the potential of pharmacological action depends on the chemical structure, there was an interest in detailed investigation of complexes' molecular structure. The novelty of the research was structural characterization of new 1,5-disubstituted tetrazole-derivative complexes with Cu and Ag ions in cell culture medium, used to simulate the living cell environment. In order to mimic *in vivo* environment of a cell complexes in form of solvates have been tested. Methodology based on the X-ray absorption spectroscopy (XAS) has been applied (Fig. 2.)

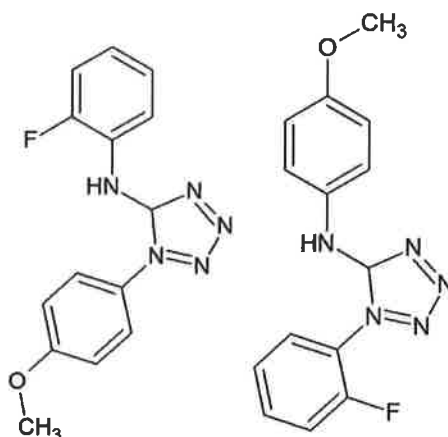


Figure 1. Schematic structure of two tautomeric forms of 1,5-substituted tetrazole-derivative ligand used in coordination reaction.

The motivation of the presented research is the dependence of the biological activity of the compound on its chemical structure. In general, the microbiological/cytotoxic activity of a metal complex, and thus its pharmacological potential, is increased in comparison with parent ligand. Although the quality of healthcare has increased together with the invention of antibiotics, nowadays the antimicrobial resistance remains a global and critical healthcare issue [1]. Biofilm, multidimensional structure impenetrable for immune cells and chemotherapeutics and associated mainly with artificial devices, complicates hospital care. Therefore, the development of new therapeutic agents, active not only on the surface, but also inhibiting cell's growth in the core of the biofilm is needed. Without effective antimicrobial drugs medical procedures become risky. There is a real threat that healthcare will return to the situation from times when antibiotics were not discovered yet. Greater effort is required in research and development of the novel class of compounds effective against pathogens [2, 3].

Tetrazole derivatives represent a group of synthetic, heterocycle compounds, whose molecule is a five-membered ring of one carbon and four nitrogen atoms. Their wide range of biological

activity and polynitrogen planar structure allows for binding with various biomolecules, thus making them perfect template aimed at the development of new bioactive compounds [4–10]. They are also hardly affected in the metabolic processes, what makes them possible to create products capable of reaching the target without undergoing undesirable transformations [11]. Moreover, various structural modifications may be applied to increase the biological activity of a compound, i.e. halogen or aromatic substituent incorporation into organic framework [2,12] and coordination to metal ions. For example Ag(I) complexes, already known from antimicrobial activities, perform better cytotoxicity in comparison with cisplatin with less toxicity and greater selectivity toward cancer cells. While Cu(II) complexes exhibit antitumor properties as well, and are promising for amoebiasis and animalarial therapy [13–19].

In coordination process ligands are donors of electron pairs, i.e. through oxygen, nitrogen or sulfur atoms. Different types of chelate compounds, with different coordination numbers can be thereby obtained [19]. However, not only the presence of metal ion determines the biological activity of compound. The chemical state of metal ion, type and number of coordinating ligands next to complexes' coordination geometry also define its properties. As already mentioned, solvent molecules coordinating to metal ions as ligands, may change the properties of chemical compound. Since the potential of pharmacological action depends on the chemical structure, there is an interest in detailed investigation of the complex's molecular structure, what enables structure–activity relationship studies. Mechanisms of drug action offered by metal complexes and proper identification of so called pharmacophores (active parts of metallo-drug crucial for its activity) may be an effective strategy against microorganisms which become increasingly resistant to organic drugs [13,22–24]. Advances of spectroscopic methods together with coordination chemistry and modern molecular biology development allow for multidisciplinary research and fruitful application of its achievements for modern pharmacology, pointing the way to rational drug design [13].

Objectives of my research involved synthesis, structural studies and microbiological as well as cytotoxic activity evaluation of new bioactive 1,5-disubstituted tetrazole–derivative complexes with copper and silver ions in a form of solvates.

Synthesis of complexes has been performed in a dedicated laboratory in Institute of Physics Polish Academy of Sciences. As ligands in the electrochemical coordination reaction 1- (2-Fluorophenyl) -N- (4-methoxyphenyl) -1H-tetrazole-5-amine (MD) and N- (2-fluorophenyl) -1- (4-methoxyphenyl) -1H-tetrazol-5-amine (MG) have been used. Electrochemical synthesis has been performed in order to obtain uncontaminated compounds. It was carried out in ethanol environment using copper, silver and platinum electrodes. The MD and MG ligand were differed in the substituents position in the tetrazole ring. With powder products solvate samples have been prepared (with 20mM and 10mM concentrations, with analogy to biological tests requirements).

Microbiological tests, for solvates at milimolar range, have been carried out by determining minimal inhibitory concentration. MTT assays, for solvates at micromolar range, have been performed for cytotoxic activity determination. Microbiological and cytotoxic tests have been performed in dedicated laboratory of Medical University of Warsaw. Compared to the low biological activity of tetrazole ligands, the copper complexes showed promising cytotoxic activity as well as selective activity against the metastatic prostate cancer cell line. The microbial activity of the silver complexes turned out to be comparable with the commercially used antibiotic. From the structure-activity relationship research point of view, there is an interest to investigate in the local atomic environment around metal ions in the presented complexes.

The aim of the study was to resolve molecular structure of coordination site in presented non-crystalline copper and silver complexes. FTIR spectra of ligands and complexes have been collected for powder samples in the 400–4000  $\text{cm}^{-1}$  range. Elemental analysis has been performed in order to establish metal to ligand ratio. Since the XAS method requires the use of synchrotron radiation the measurements of studied solvates have been performed in collaboration with XAFS group from Elettra synchrotron in Italy and XAFS group from The Synchrotron Light Research Institute (SLRI)

in Thailand. Samples in the form of solution and powder have been scanned in fluorescence and transmission modes respectively. For data processing the dedicated IFEFFIT software and the FEFF 9.6 code have been applied [26–28]. As a theoretical support DFT calculations have been provided as well [29].

Determining the chemical structure of a compound in a form of a solution is not a trivial issue. For this purpose multi-technique approach methodology (Fig.2.) has been used. It combines spectroscopic, analytical and computational methods of quantum chemistry. The main technique and the base of methodology has been X-ray absorption spectroscopy (XAS). This technique allowed to obtain direct structural information regardless of the form or state of the sample. As part of the methodology, infrared spectroscopy, elemental analysis, structural databases and DFT (Density functional theory) computational methods for structural characterization have been used. Finally, the use of methodology allowed to propose molecular models of the studied complexes.

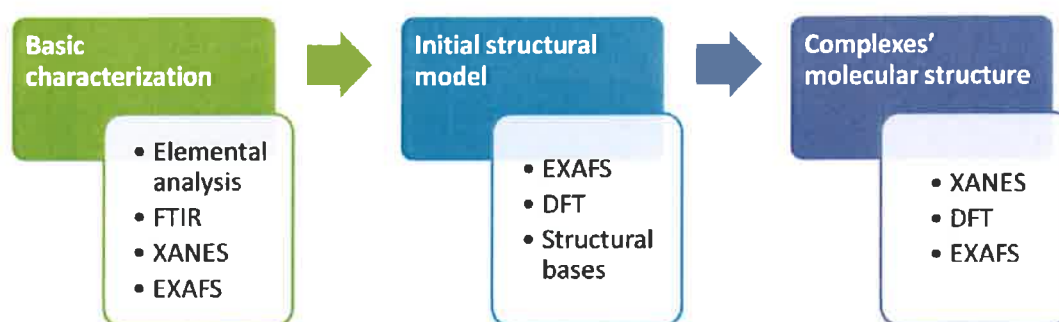


Figure 2. Scheme of methodology used in the research.

The basic structural characterization have been performed as follows. FTIR spectroscopy has been used to confirm if metal complexes were formed and to indicate ligand's functional groups active in coordination process. By comparison of XANES spectra of complexes' and reference compounds (Cu and Ag oxides or salts) the oxidation state of metal ions in studied complexes has been determined. Initial EXAFS analysis has been performed in order to get information about the nearest atomic environment around copper/silver ion. Basic characterization has been completed with elemental analysis. With the basic structural data I was able to propose two-dimensional models of studied compounds and used them for EXAFS analysis.

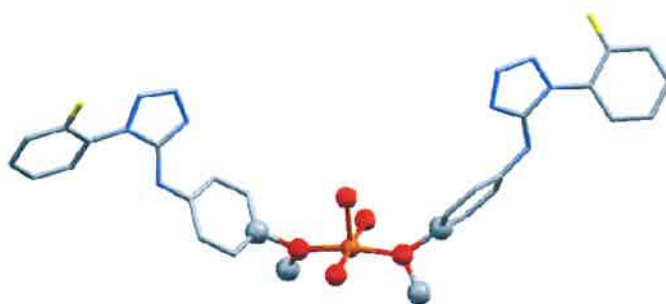
In order to get information about the nearest atomic environment in coordination site, EXAFS analysis have been performed. Information about number, type of neighboring atoms and their average distance from metallic center as well as the relative structural disorder have been determined. With this data initial structural models of each studied complex have been prepared. For verification structural databases were searched.

For model refinement DFT calculations have been performed. With this step and another EXAFS analysis the three-dimensional models have been built. In the next step XANES spectra of the best models have been calculated. The shape of XANES spectra depends on the angular distribution of atoms around the copper ions, therefore it is possible to refine the initial coordination polyhedra by comparing experimental and calculated spectra. When the agreement between theoretical and experimental spectra has been obtained, molecular models have been rechecked with EXAFS analysis for confirmation. Within the final EXAFS analysis both, single and multiple scattering paths have been included. The final models of molecular structures of investigated complexes have been proposed.

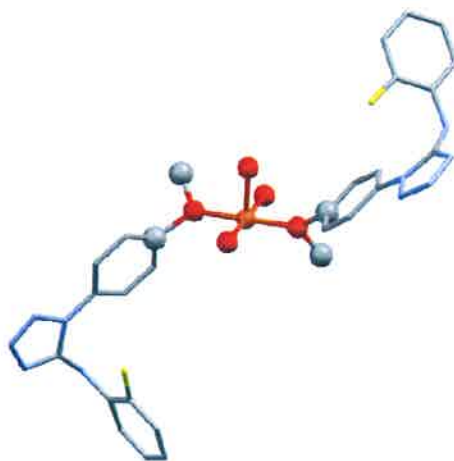
Within the presented copper complexes' models:

- the structure of all studied complexes is the single-core;

- two tetrazole ligands and three water molecules coordinate in a monodent manner to each copper ion;
- the coordination site of each copper ion is formed by five oxygen atoms at two distances, two belong to methoxy groups of tetrazole ligand, other three derived from water molecules;
- the further atomic environment is composed of four carbon atoms in two distances, derived from the methoxy and phenolic groups of the tetrazole ligands;
- the shape of a coordination polyhedra resembles the shape of a tetragonal pyramid with axial water molecule.



(a)



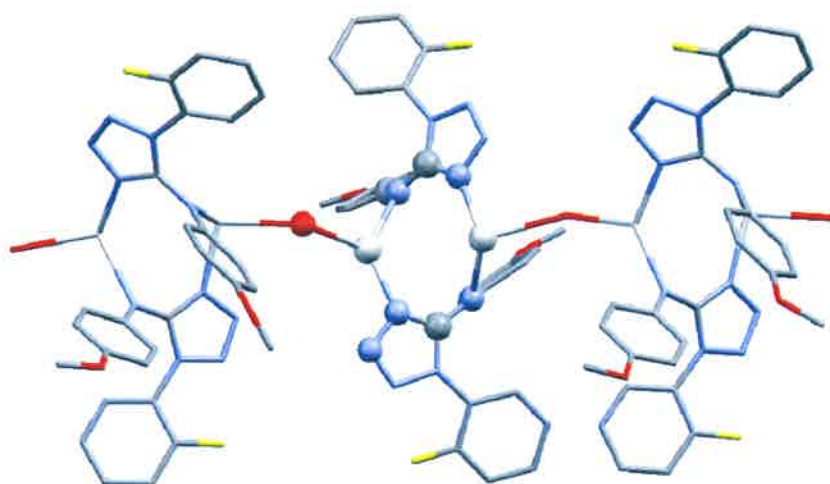
(b)

*Figure 3. Three-dimensional model of molecular structure of non-crystalline tetrazole derivative complexes with copper ions. With MD ligand – (a) and MG ligand – (b).*

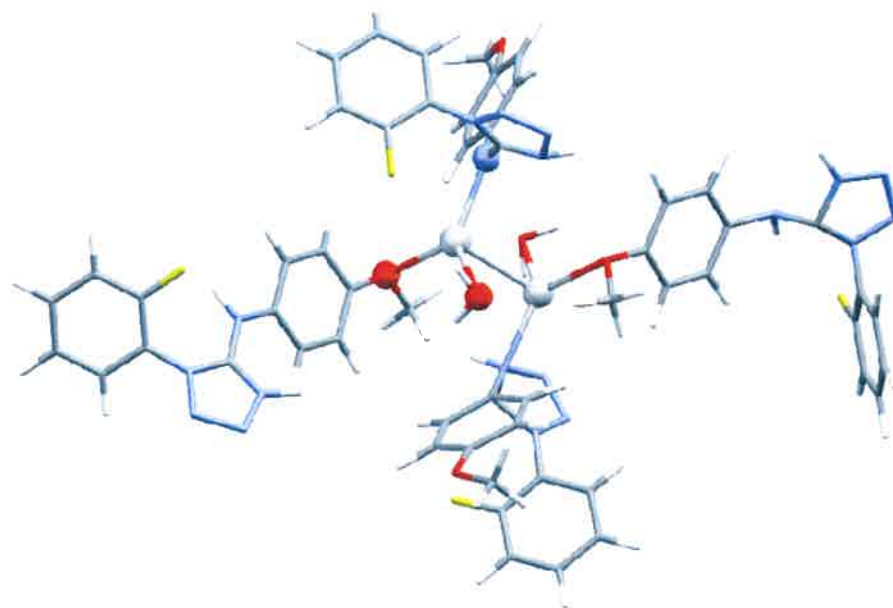
Within the presented silver complexes' models:

- the structure of all silver complexes is the dual-core;

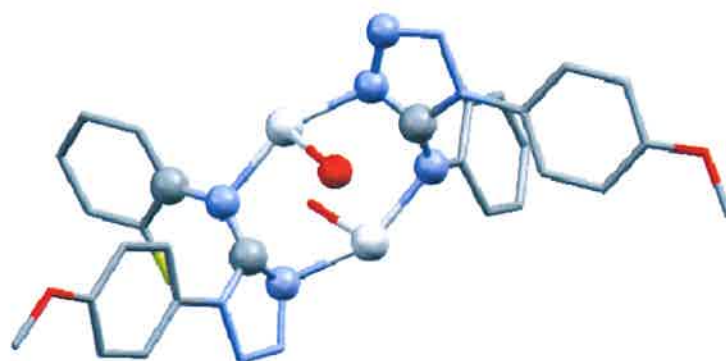
- two tetrazole ligands and two water molecules coordinate in monodentate manner to the center of each complex in powder form; wherein, the structure of a complex with MD ligand seems to be in a polymer form;
- the nearest atomic environment of silver center in the powder complexes is formed by four nitrogen atoms derived from tetrazole ligands and two oxygen atoms derived from water molecules;
  - the farther atomic environment of each silver ion in powder complexes is formed by three nitrogen atoms: one from tetrazole ring and two from the  $-NH-$  groups;
  - in case of complexes in a form of solution, to the metallic center four tetrazole ligands and two water molecules are coordinated by monodentate manner;
  - the nearest atomic environment is composed by two nitrogen atoms of  $-NH-$  groups derived from two tetrazole ligands, and two oxygen atoms derived from methoxy groups of another tetrazole ligands; the coordination site is closed with two water molecules;
  - the farther atomic environment of a single silver ion formed eight light atoms; including four carbon atoms and four nitrogen atoms;
  - the coordination polyhedra around each silver ion has the shape of a flat triangle;
- in case of complex with MG ligand, in 20mM concentration, four tetrazole ligands and four water molecules are coordinated do the metallic center in a monodentate manner;
  - the nearest atomic environment is composed of two nitrogen atom derived from  $-NH-$  groups of tetrazole ligand and two oxygen atoms derived from metoxy group; coordination site is closed with four water molecules;
  - the farther atomic environment is formed with eight light atoms; including four carbon atoms and four nitrogen atoms;
  - the coordination polyhedra around each silver ion has the shape of a deformed flat square;
- in case of complex with MG ligand, in 10mM concentration, two tetrazole ligands and four water molecules coordinate to the metallic center in a monodentate manner;
  - the nearest atomic environment is composed of six oxygen atoms, two from methoxy group of ligand and four derived from water molecules;
  - the farther atomic environment of eacg solver ion is composed of four carbon atoms.



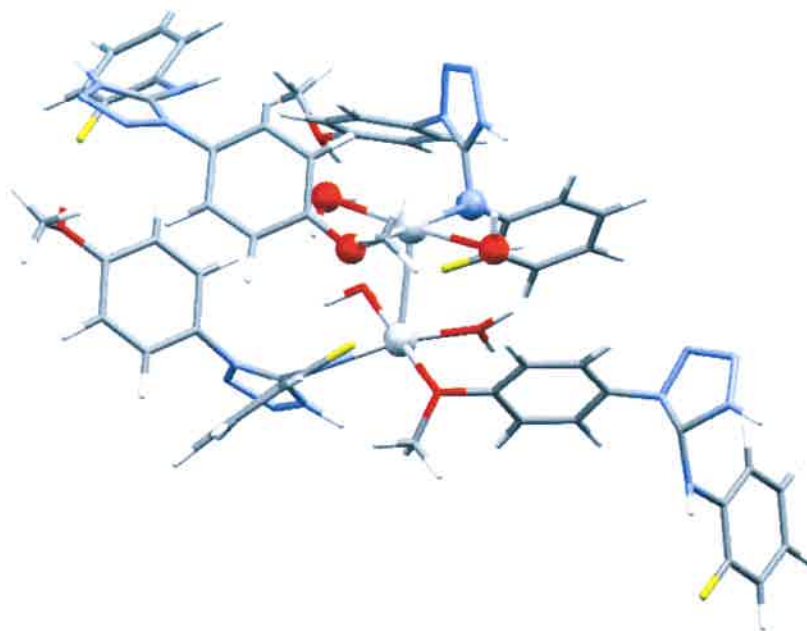
(a)



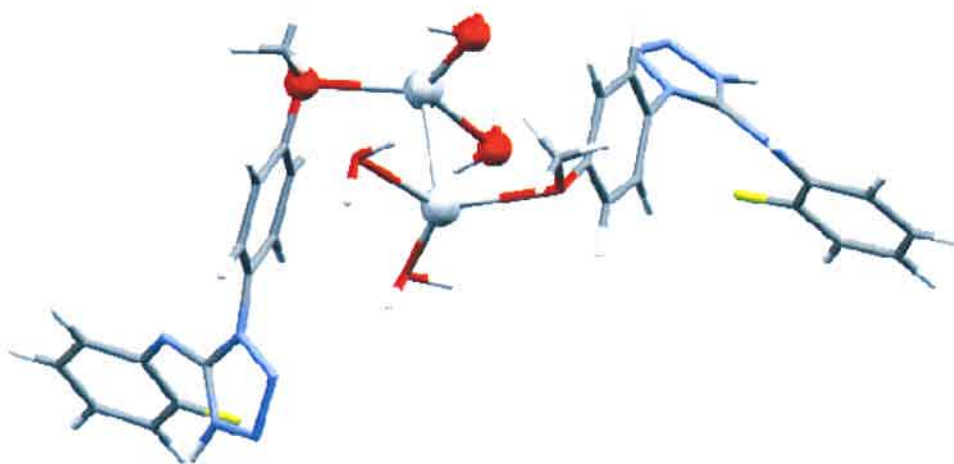
(b)



(c)



(d)



(e)

*Figure 4. Three-dimensional models of molecular structure of non-crystalline tetrazole derivative complexes with silver ions. With MD ligand in powder form – (a), with MD ligand in solvate form – (b), with MG ligand in powder form – (c), with MG ligand in concentration 20mM – (d), with MG ligand in concentration 10mM – (e).*

## Literature

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