EXPERIMENTAL TECHNIQUES EMPLOYED IN THE STUDY OF METAL COMPLEXES-DNA – INTERACTIONS

TAMARA TOPALĂ[1*], ANDREEA BODOKI[1], LUMINIŢA OPREAN[1], RADU OPREAN[2]

[1] *Department of General and Inorganic Chemistry, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania*
[2] *Department of Analytical Chemistry and Instrumental Analysis, Faculty of Pharmacy, "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania*

**corresponding author: topala.liana@umfcluj.ro*

Abstract

The present paper is an overlook on metal complexes-DNA interactions, their classification and the experimental techniques employed to study them. The deoxyribonucleic acid (DNA), as the vector for genetic information in living organisms, is one of the main targets for pharmacologically active molecules, especially for anticancer agents. Understanding how drugs interact with DNA is of outmost importance as researchers struggle to design more efficient and specifically targeted therapeutics, with fewer side effects. As in the recent years bioinorganic chemistry has provided numerous examples of compounds with high affinity towards DNA, the present review focuses on transition metal complexes.

Rezumat

Acidul dezoxiribonucleic (ADN), ca vector al informaţiei genetice în toate organismele vii, este ţinta farmacologică a numeroase substanţe medicamentoase, în special a agenţilor antitumorali. Lucrarea prezintă diferite moduri de interacţiune a complecşilor metalici cu ADN-ul, precum şi metodele instrumentale utilizate frecvent în evaluarea acestor interacţiuni. Cercetările recente din domeniul chimiei bioanorganice au furnizat numeroase exemple de compuşi cu mare afinitate faţă de ADN, printre care se remarcă numeroşi complecşi ai metalelor tranziţionale.

Keywords: metal complexes-DNA interactions, UV-Vis absorption spectroscopy, fluorescence study, viscosity measurements

Introduction

Since DNA (deoxyribonucleic acid) was identified as one of the primary targets for many of the drugs that are currently in clinical use or in advanced clinical trials, including metal based anticancer drugs, the interaction mechanism of metal complexes with nucleic acids has been

intensely studied. Small molecules that bind to DNA can alter and/or inhibit its functions, disturbing gene expression, protein synthesis or interfere with replication, a major step in cell growth and division [6, 28].

Metal complexes are interesting, flexible reagents, which offer the possibility to explore the effects of the central metal ion, the contribution of the ligands and coordination geometry to the DNA binding process [19]. Transition metals are particularly suitable for this purpose, because they can adopt a wide variety of oxidation states, coordination numbers and geometries, in comparison to other main group elements [3, 25].

Several instrumental techniques are used to study these interactions. In the present paper, we discuss UV-Visible spectroscopy (metal complex titration with CT-DNA (calf thymus DNA), DNA and thermal denaturation studies), fluorescence spectroscopy, DNA viscosity measurements, as well as the type of information they provide (qualitative and/or quantitative).

Nucleic acids (deoxyribonucleic acid, DNA, and ribonucleic acid, RNA) are the macromolecules responsible for the storage and transmission of the genetic information that controls the functions of all living cells, including growth and reproduction.

The DNA structure was solved by Watson and Crick in 1953. They proposed a three-dimensional structure for nucleic acids, consisting of two antiparallel polymeric chains, with a hydrophobic core and a sugar-phosphate hydrophilic backbone.

As the two sugar-phosphate backbones twist around the central stack of base pairs, two grooves are generated along the DNA chain, a major groove, and a minor one. The grooves arise because the N-glycosidic bonds of a base pair are not diametrically opposed to each other.

The two strands of nucleotides that form the double-helical structure of DNA are held together and stabilized by hydrogen bonds formed between the complementary nitrogenous bases, electrostatic forces between the negatively charged phosphate groups and solvated cations, π-electron interactions between the bases stacked vertically and hydration [2, 28].

Types of drug-DNA interaction

Upon binding to DNA, the formed adduct can be/is stabilized through a series of interactions, such as π-stacking interactions of aromatic heterocyclic groups between base pairs, hydrogen bonding, electrostatic and van der Waals interactions [9, 19].

Small molecules can bind to the nucleic acid structure in a covalent way or in a non-covalent manner [6].

Covalent binding

Many anticancer agents currently used in therapy exert their effect by covalently binding to DNA, forming adducts via alkylation or interstrand and intrastrand crosslink. The covalent binding mode is irreversible and invariably causes the complete inhibition of DNA functions and cell death.

Cisplatin [*cis*-dichlorodiammineplatinum(II)] is maybe the most famous covalent binder used as an anticancer drug, that determines intra/interstrand crosslink through the formation of covalent bonds between Pt(II) and the nitrogen atoms in the nucleobase structures [28]. However, its effectiveness is limited by its high toxicity and the fact that some types of tumours exhibit drug-resistance [10].

Alkylating agents, such as dacarbazine, chlorambucil or nimustine, attach alkyl groups to DNA bases. In an attempt to correct the altered structure, DNA is fragmented by repair enzymes trying to replace the alkylated base [28].

Non-covalent binding

The non-covalent binding of small molecules to DNA includes intercalation between adjacent base pairs, minor and major groove binding and electrostatic interactions with the sugar-phosphate backbone. Generally, this type of interaction is considered to be less cytotoxic, reversible, and thus preferred over covalent adduct formation [28]. It also leads to structural abnormalities and interference with normal DNA functions, including replication and protein interaction.

Intercalating agents are structures containing planar heterocyclic moieties, which stack perpendicular to the DNA backbone. Intercalation stabilizes, lengthens, stiffens and unwinds the DNA double helix [14]. Typically, intercalating drugs inhibit DNA topoisomerase II and induce DNA strand breaks [17].

Minor groove binders exhibit essential features that allow them to interact with the nucleic acid, including a matching concave-shaped aromatic framework that fits in the convex DNA minor groove and having electron-donating and electron-accepting groups capable to form hydrogen bonds.

The forces that hold together such a complex are van der Waals and hydrophobic interactions, hydrogen bonding and/or charge transfer forces [14].

The first metal complexes proven to bind to DNA were synthesized by Lippard [7] and Sigman [26, 27], in the mid 1970's and 1980's: the platinum complexes, $[Pt(terpy)Cl]^+$ and $[Pt(terpy)(SCH_2CH_2OH)]^+$, and the copper complex, $([Cu(phen)_2]^{2+}$ (terpy =2,6-bis(2-pyridyl)pyridine, phen = 1,10-phenanthroline). For the platinum complexes the intercalation between

the base-pairs was confirmed with a periodicity of one platinum unit every 10 Å (every other base-pair) and a partial unwinding of the phosphate backbone. A partial intercalation and a minor-groove binding are characteristic for Sigman's bis(1,10-phenanthroline)copper(II).

Experimental techniques employed to study drug-DNA interactions

UV-Visible absorption spectroscopy studies

Metal complex titration with calf thymus DNA

Perhaps the most commonly used method to study the interaction of DNA with metal complexes is the UV-Vis absorption spectroscopy. This technique is based on monitoring the changes that occur in the UV-Vis spectrum of the metal complex or the UV-Vis spectrum of DNA upon interaction.

Usually, metal complexes show specific absorption bands in the UV region (due to π-π^* intraligand transitions [32] or ligand-to-metal charge transfer [12]), or in the visible region (*d-d* transitions [16]). Therefore, a simple way to determine whether an interaction takes place between the metal complex and DNA is to examine the modifications of the maximum of one of these bands when the nucleic acid is present in different concentrations.

Metal complexes binding to DNA through intercalation usually result in hypochromism and bathochromism or hypsochromism [12, 28, 32], while hyperchromism has been attributed to electrostatic interactions, hydrogen bonding and groove (minor or major) binding along the outside of the DNA helix [1, 13, 28]. The extent of the changes that appear in the metal complex spectrum are usually consistent with the strength of the interaction that takes place.

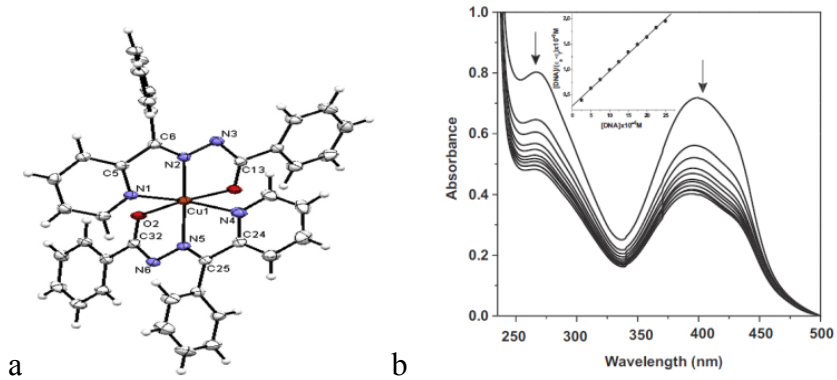
Based on the variation in absorption, the intrinsic binding constant or the association constant (K_b) of the metal complex can be calculated according to the Benesi-Hildebrand equation, modified by Wolfe et al [19]:

$$[DNA]/(\varepsilon_A - \varepsilon_F) = [DNA]/(\varepsilon_B - \varepsilon_F) + 1/K_b(\varepsilon_B - \varepsilon_F)$$

where ε_A, ε_F and ε_B correspond to $A_{observed}$/[complex], the extinction coefficient for the free metal complex, and the extinction coefficient for the metal complex fully bound to DNA, respectively; K_b represents the binding constant.

In plots of $[DNA]/(\varepsilon_A - \varepsilon_F)$ versus [DNA], K_b is given by the ratio of the slope to the intercept. Values with a magnitude order of 10^5 M^{-1} for K_b are considered to be indicative of a relatively strong interaction between DNA and metal complexes [12, 13, 31, 32].

Kryshnamoorthy et al. [12] reported the synthesis of a Cu(II)-hydrazide complex, $[Cu(L)_2]$, HL = N'-(phenyl(pyridine-2-yl)methylidene)benzohydrazide, (the molecular structure of the complex is shown in Figure 1a) and the evaluation of its interaction with CT-DNA using UV-Vis absorption spectroscopy (Figure 1b), $K_b = 2.468 \times 10^5\ M^{-1}$. Based on the decrease in absorbance and the hypsochromism observed, an intercalative binding mode was assigned to this complex.



a b

Figure 1.

(a) Molecular structure of complex $[Cu(L)_2]$ and (b) the electronic absorption spectra of the complex (25 μM) in the absence and presence of increasing amounts of CT-DNA (2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5 and 25 μM). The arrows show the changes in absorbance with respect to an increase in DNA concentration. Insert: plot of [DNA] vs $[DNA]/\varepsilon_A - \varepsilon_F$ [12]

DNA thermal denaturation

Temperature has an important effect in altering the stability of the double-stranded structure of DNA, causing the formation of the single-stranded structure, the "melting" of the nucleic acid, or its thermal denaturation [2, 5]. The melting temperature (Tm) is considered to be the temperature at which 50% of DNA is denaturalized, half of the nucleic acid being found in the double-stranded state and the other half in the single-stranded state [30].

The melting temperature is directly dependant on the stability of the DNA double helix and it is altered by the presence of small molecules that may stabilize or destabilize the nucleic acid structure [17]. The interaction of such a compound with DNA induces conformational changes and usually leads to an increase of the Tm. The extent of the variation of the Tm value is influenced by the type and the strength of the interaction that takes place.

In order to determine the Tm value and study the thermal denaturation process that DNA suffers, the UV absorbance of the nucleic

acid is monitored. The UV-Vis absorption spectrum of DNA shows a broad band (200-350 nm) in the UV region with a maximum at 250-280 nm. This maximum is a consequence of the presence of chromophoric groups in purine and pyrimidine moieties responsible for the electronic transitions [5, 28].

The hydrogen bonds formed between the nucleobases limit the resonance of the aromatic rings in their structure, so the absorbance is limited as well. When the double helix separates into two single strands, the base-base interaction is reduced, increasing the UV absorbance of the DNA solution with approximately 40% in comparison with the one for the double stranded DNA at the same concentration (Figure 2) [2, 28].

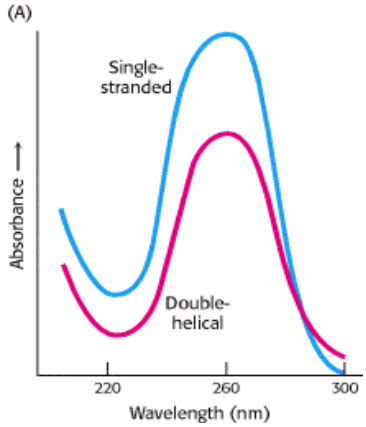


Figure 2.
Hyperchromic shift occurring during the DNA thermal denaturation process [2]

The double-stranded form is present at low temperatures, whereas the single-stranded form is found at high temperatures, corresponding to the maximum absorbance. The inflection point in the sigmoidal melting curve indicates the midpoint of the melting process, i.e. the melting temperature (Figure 3) [5].

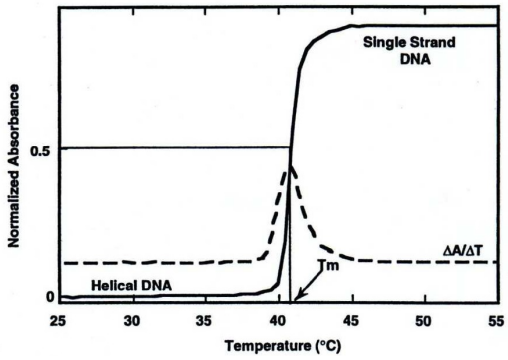


Figure 3.
The sigmoid curve in a thermal denaturation study by UV spectroscopy [5]

The DNA melting experiments are carried out by recording the absorbance spectrum of calf thymus DNA (CT-DNA) between 200 and 1000 nm, at different temperatures (usually in the range 25-90°C), in the absence and in the presence of metal complexes, and using different DNA to complex ratios. The DNA melting point can be obtained with the first derivative by applying the Savitsky-Golay algorithm.

ΔTm is defined as the difference between the Tm of free DNA (Tm_{DNA}) and the Tm obtained in the presence of the metal complex ($Tm_{DNA+complex}$) and is calculated from the experimental data as:

$$\Delta Tm = Tm_{DNA+complex} - Tm_{DNA}.$$

Thermal denaturation studies are an important tool in estimating the strength and nature of the affinity of metal complexes towards DNA and the interactions taking place. Generally, a ΔTm of a few degrees Celsius is considered to be evidence of an interaction involving groove binding and/or electrostatic binding to the phosphate groups [7, 13], while an increase of over 10°C is attributed to an intercalation binding mode, due to the stabilization of the DNA double helix [3, 20].

García-Giménez and coworkers [6] reported the synthesis of complex $[Cu(NST)_2(phen)]$ (HNST = N-(4,5-dimethyltriazol-2-yl)naphtalene-1-sulfonamide), phen = 1,10-phenanthroline) (Figure 4a, shows the molecular structure of the complex) and the DNA thermal denaturation curves recorded in the presence of different complex concentrations (Figure 4b). The value obtained for ΔTm is 12.6°C when [DNA]/[complex] = 4, which suggests a strong interaction of the copper(II) complex with the nucleic acid.

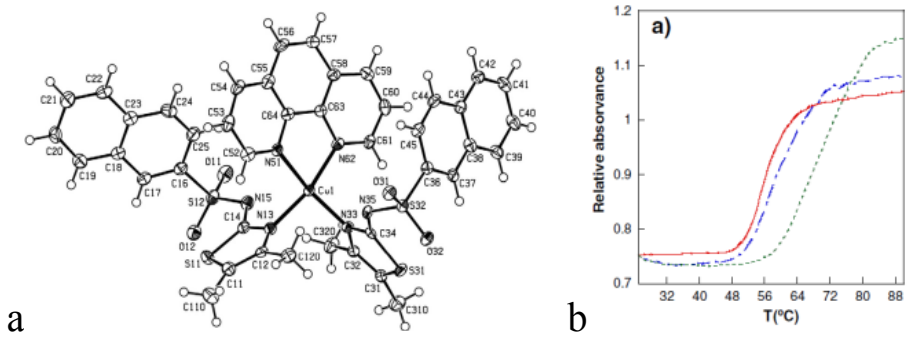


Figure 4.

(a) Molecular structure of complex $[Cu(NST)_2(phen)]$ and (b) DNA melting temperature dependence on complex concentration. 100 µM CT-DNA (red solid line) in the presence of the complex (10 µM blue dot-dashed line and 25 µM green dashed line) [6]

Competitive binding fluorescence study

Ethidium bromide (3,8-diamino-5-ethyl-6-phenyl phenanthridium bromide, EtBr) is a planar cationic dye, known as an antimicrobial agent, a carcinogen and mutagen, due to its ability of inhibiting DNA synthesis, gene transcription and translation [11].

It strongly interacts with the DNA double helix, by insertion of the phenanthridium ring between adjacent base pairs [11, 22]. The electrostatic interaction between the positively charged EtBr molecule and the anionic phosphate groups in the DNA structure is the mediator for the formation of π-stacking interactions with the nucleobases, considered to be the main binding element [21].

EtBr is widely used as a sensitive fluorescent probe for DNA due to its high fluorescence when bound to the nucleic acid. The free EtBr molecule shows reduced emission intensity in buffer solution, as a consequence of the solvent quenching or of a photoelectron transfer mechanism [1, 25]. When bound to DNA, EtBr shows a remarkable enhancement in fluorescence, due to a steric protection that the nucleobases provide to the dye molecule [14, 29].

The presence of another species with affinity towards DNA may result in a decrease in the emission intensity of the EtBr-DNA adduct, caused by either a competition for binding sites, a change in DNA conformation or through a photoelectron transfer mechanism [1, 7, 8, 13, 31]. The affinity of metal complexes towards DNA can be measured by competitive fluorescence studies, as it is a measure of the extent of the emission intensity reduction of the EtBr-DNA adduct.

The quenching of the EtBr-DNA adduct fluorescence is studied by following the emission spectra of the species in the wavelength range of 530-680 nm with an excitation wavelength of 500 nm, upon addition of different metal complex concentrations to DNA pretreated with EtBr.

The fluorescence quenching at around 590 nm is described by the Stern-Volmer equation [45]:

$$I_0/I = 1 + K_{SV}[Q] = 1 + k_q\tau_0[Q]$$

where I_0 and I are the fluorescence intensities of the DNA-EtBr adduct in the absence and in the presence of the quencher, respectively, K_{SV} is the Stern-Volmer quenching constant, [Q] is the concentration of the quencher, k_q is the quenching constant of the biomolecule and τ_0 is the average lifetime of the molecule in the absence of a quencher. The K_{SV} value is obtained from the plot I_0/I vs. [Q]. Values with a magnitude order of 10^3 M^{-1}

for K_{SV} are considered to be indicative of a strong interaction between DNA and metal complexes [8, 12, 25].

This competitive binding technique provides information about the strength of the metal complex-DNA interaction through the apparent binding constant K_{app} as a part of the equation:

$$K_{EtBr}[EtBr] = K_{app}[Q]$$

where K_{EtBr} = 1 x 10^7 M^{-1}, [EtBr] is the EtBr concentration used in the experiment, and [Q] is the concentration of the quencher that produces a 50% decrease of the initial EtBr-DNA fluorescence. Metal complexes which show a strong interaction with DNA give K_{app} values with a magnitude order of 10^5-10^6 M^{-1} [8, 12, 13, 25].

The study of García-Giménez [8] presents the synthesis of the complex [Cu(N9-ABS)(phen)$_2$]·3.6H_2O (H_2N9-ABS=*N*-(9*H*-purin-6-yl)benzenesulfonamide) (the molecular structure of the complex is shown in Figure 5a) and the evaluation of its interaction with CT-DNA using a competitive binding fluorescence study with EtBr (Figure 5b), K_{SV} = 0.333 M^{-1}, K_{app} = 5.1 x 10^6 M^{-1}, the results of the latter study suggesting that the interaction of the complex with DNA is strong.

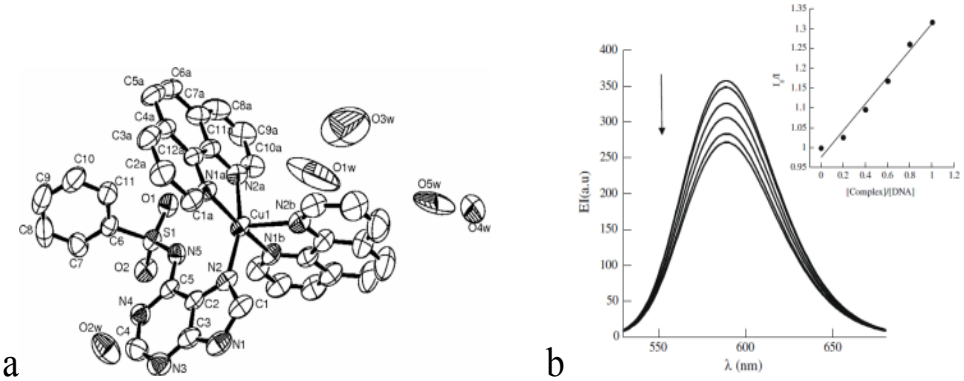


Figure 5.

(a) Molecular structure of complex [Cu(N9-ABS)(phen)$_2$]·3.6H_2O and (b) the emission spectra of DNA-EtBr (50 μM) in the absence and presence of increasing concentrations of the copper(II) complex (10, 20, 30, 40 and 50 μM). The arrow indicates the changes in the emission intensity as a function of complex concentration. Insert: Stern-Volmer plot of the fluorescence titration data [8]

DNA viscosity measurements

Although spectroscopic experiments provide many important information in elucidating the type and strength of the metal complex-DNA interaction, hydrodynamic measurements are considered as the least

ambiguous and the most critical test of a DNA binding model in solution, clarifying the interaction mode of a compound with the nucleic acid [7, 8, 31].

DNA viscosity is strictly dependant on the length changes that may occur as a consequence of an interaction between DNA's double helix and a small molecule. An intercalative metal complex causes a separation of the base pairs, in order for it to be accommodated in the DNA structure, leading to a lengthening of the nucleic acid helix and an increase in its viscosity. In the case of partial and/or non-classical intercalation (binding in the grooves or in the sugar-phosphate backbone), the metal complex can bend or kink the DNA double helix, causing either a less obvious change (an increase or a decrease) or no change at all in the DNA viscosity [1, 8].

DNA viscosity experiments are carried out in especially designed viscosimeters and the temperature is strictly controlled (usually with thermostated baths, set at 25°C or 37°C [7, 8]).

Data are presented as $(\eta/\eta_0)^{1/3}$ *versus* the ratio of the compound to DNA concentration, where η is the viscosity of DNA in the presence of the metal complex and η_0 is the viscosity of the buffer alone. Viscosity values are calculated with the observed flow time of a DNA solution (t), corrected with the flow time of the buffer alone (t_0): $\eta = t - t_0$ [8].

García-Giménez and coworkers studied the influence of a series of copper(II) complexes on the relative viscosity of CT-DNA, in comparison with $[Cu(phen)_2]^{2+}$ (a well-known partial intercalator) and $CuCl_2$ (that should not influence DNA viscosity) as standards (Figure 6) [6]. The molecular structure of the complex $[Cu(NST)_2(phen)]$ is shown in Figure 4a.

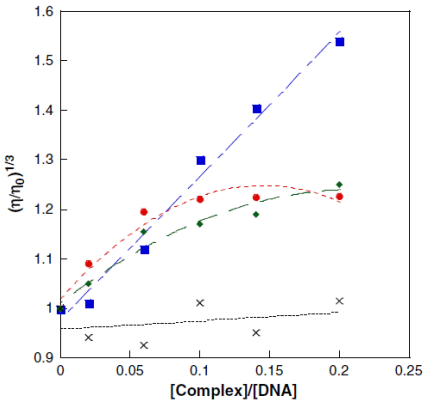


Figure 6.

Effect of increasing concentrations of $[Cu(NST)_2(phen)]$ (blue dot-dashed line), $[Cu(NST)_2(NH_3)_2]\cdot H_2O$ (red dashed line), $[Cu(phen)_2]^{2+}$ (green dashed line) and $CuCl_2$ (black dotted line) on the relative viscosity of CT-DNA [6]

Conclusions

The search for new therapeutic agents is a continuous struggle, in the context of the increasing incidence of drug resistance. Understanding the mechanism followed by drugs in exerting their effect is crucial in designing new structures, with a specific target, that would exhibit higher efficiency and fewer side effects. With this in mind, we presented the types of interactions that could occur between transition metal complexes and DNA, as a target for potential anticancer agents. The most important instrumental techniques employed to study the type and the strength of those interactions were discussed, as well as the type of information they provide (qualitative and/or quantitative): UV-Vis and fluorescence spectroscopy studies and DNA viscosity measurements.

Acknowledgements

The present work was supported financially by research Project POSDRU 107/1.5/S/78702.

References

1. Arjmand F., Parveen S., Afzal M., Shahid M., Synthesis, characterization, biological studies (DNA binding, cleavage, antibacterial and topoisomerase I) and molecular docking of copper(II) benzimidazole complexes. *J. Photochem. Photobiol. B: Biology*, 2012; 114: 15-26.
2. Arsene A.L., Uivaroşi V., Mitrea N., Drăgoi C., Nicolae A., *In vitro* spectrofluorimetric analysis of the interaction of ruthenium complexes with double stranded calf thymus DNA, 35th FEBS Congress, June 26 - July 1, 2010, Göteborg, Suedia, rezumat publicat în *F.E.B.S. Journal*, 2010; 277 (suppl. 1), ISSN 1742-464X, IF 3,129.
3. Bodoki A., Hangan A., Oprean L., Alzuet G., Castiñeiras A., Borrás J., Oxidative DNA cleavage by copper ternary complexes of 1,10-phenanthroline and ethylenediamine-sulfonamide derivates. *Polyhedron*, 2009; 28: 2537-2544.
4. Bodoki A., Hangan A., Oprean l., Borrás J., Castiñeiras A., Bojiţă M., DNA-binding study and nuclease activity induced by a copper(ii), N,N'-bis[(4-methylphenyl)sulfonyl] ethylenediamine and 1,10-phenantroline ternary system. *Farmacia*, 2008; LVI(6): 607-614.
5. Fox K.R., Methods in Molecular Biology. Drug-DNA interaction protocols. Humana Press, 2007; 219-230.
6. García-Giménez J.L., Hernández-Gil J., Martínez-Ruíz A., Castiñeiras A., Liu-Gonzáles M., Pallardó F.V., Borrás J., Alzuet Piña G., DNA binding, nuclease activity, DNA photocleavage and cytotoxic properties of Cu(II) complexes of N-substituted sulfonamides. *J. Inorg. Biochem*., 2013; 121: 167-178.
7. García-Giménez J.L., Alzuet G., Gonzáles-Alvarez M., Liu-Gonzáles M., Castiñeiras A., Borrás J., Oxidative nuclease activity of ferromagnetically coupled μ-hydroxo-μ-propionato copper(II) complexes [Cu3(L)2(μ-OH)2(μ-propionato)2] (L = N-(pyrid-2-ylmethyl)R-sulfonamidato, R = benzene, toluene, naphtalene). *J. Inorg. Biochem*., 2009; 103: 243-255.
8. García-Giménez J.L., Gonzáles-Alvarez M., Liu-Gonzáles M., Macías B., Borrás J., Alzuet G., Toward the development of metal-based synthetic nucleases: DNA binding and oxidative DNA cleavage of a mixed copper(II) complex with N-(9H-purin-6-yl)benzenesulfonamide and 1,10-phenanthroline. Antitumor activity in human Caco-2 cells and Jurkat T lymphocytes. Evaluation of p53 and Bcl-2 proteins in the apoptotic mechanism. *J. Inorg. Biochem*., 2009; 103: 923-934.

9. Khan G.S., Shah A., Zia-ur-Rehman, Baker D., Chemistry of DNA minor groove binding agents. *J. Photochem. Photobiol. B: Biology*, 2012; 115: 105-118.
10. Jaividhya P., Dhivya R., Akbarsha M.A., Palaniandavar M., Efficient DNA cleavage mediated by mononuclear mixed ligand copper(II) phenolate complexes: The role of co-ligand planarity on DNA binding and cleavage and anticancer activity. *J. Inorg. Biochem*., 2012; 114: 94-105.
11. Karacan P., Okay O., Ehtidium bromide binding to DNA cryogels. *Reactive & Functional Polymers*, 2013; 73: 442-450.
12. Krishnamoorthy P., Sathyadevi P., Cowley A.H., Butorac R.R., Dharmaraj, Evaluation of DNA binding, DNA cleavage, protein binding and *in vitro* cytotoxic activities of bivalent transition metal hydrazone complexes. *Eur. J. Med. Chem*., 2011; 46: 3376-3387.
13. Kumar P., Gorai I., Santra M.K., Mondal B., Manna D., DNA binding, nuclease activity and cytotoxicity studies of Cu(II) complexes of tridentate ligands. *Dalton Trans*., 2012; 41: 7573-7581.
14. Lepecq J.B., Paoletti C., A fluorescent complex between ethidium bromide and nucleic acids: physical-chemical characterization. *J. Mol. Biol*., 1967; 27(1): 87-106.
15. Lippard S.J., Bond P.J., Wu K.C., Bauer W.R., Stereochemical requirements for intercalation of platinum complexes into double-stranded DNA's. *Science*, 1976; 194(4266): 726-728.
16. Lippard S.J., Berg J.M., Principles of BioInorg Chem. University Science Books, Mill Valley, 1994; 61-66.
17. Macías B., Villa M.V., Lapresa R., Alzuet G., Hernández-Gil J., Sanz F., Mn(II) complexes with sulfonamides as ligands. DNA interaction studies and nuclease activity. J. Inorg. Biochem., 2012; 115: 64-71.
18. Manikandamathavan V.M., Parameswari R.P., Weyhermüller T., Vasanthi H.V., Nair B.U., Cytotoxic copper(II) mixed ligand complexes: Crystal structure and DNA cleavage activity. Eur. J. Med. Chem., 2011; 46: 4537-4547.
19. Mudasir, Yoshioka N., Inoue H., DNA binding of iron(II) mixed-ligand complexes containing 1,10-phenanthroline and 4,7-diphenyl-1,10-phenanthroline. *J. Inorg. Biochem*., 1999; 77: 239-247.
20. Mudasir, Wahyuni E.T., Tjahjono D.H., Yoshioka N., Inoue H., Spectroscopic studies on the thermodynamic and thermal denaturation of the CT-DNA binding of methylene blue. *Spectrochim. Acta A. Mol. Biol. Spectrosc*., 2010; 77: 528-534.
21. Nafisi S., Saboury A.A., Keramat N., Neault J.F., Tajmir-Riahi H.A., Stability and structural features of DNA intercalation with ethidium bromide, acridine orange and methylene blue. *Journal of Molecular Structure*, 2007; 827: 35-43.
22. Drăgoi C.M., Mitrea N., Arsene A.L., Ilie M., Nicolae A.C., Jurkat E 6.1 cell line studies regarding the effects of some bio-indols on the membrane fluidity. Farmacia, 60(1): 13-20.
23. Nelson S.M., Ferguson L.R., Denny W., A non-covalent ligand/DNA interactions: Minor groove binding agents. *Mutat. Res*., 2007; 623: 24-40.
24. Samari F., Hemmateenejad B., Shamsipur M., Rashdi M., Samouei H., Affinity of two novel five-coordinated anticancer Pt(II) complexes to human and bovine serum albumins: A spectroscopic approach. *Inorg. Chem*., 2012; 51: 3454-3464.
25. Sathyadevi P., Krishnamoorthy P., Jayanthi E., Butorac R.R., Cowley A.H., Dharmaraj N., Studies on the effect of metal ions of hydrazone complexes on the interaction with nucleic acids, bovine serum albumin and antioxidant properties. *Inorganica Chimica Acta*, 2012; 384: 83-96.
26. Sigman D.S., Graham D.R., D'Aurora V., Stern A.M., Oxygen-dependent cleavage of DNA by the 1,10-phenanthroline cuprous complex. Inhibition of Escherichia coli DNA polymerase I. *J. Biol. Chem*., 1979; 254(24): 12269-12272.
27. Sigman D.S., Landgraf R., Perrin D.M., Perason L., Nucleic acid chemistry of the cuprous complexes of 1,10-phenanthroline and derivatives. *Met. Ions Biol. Syst*., 1996; 33: 485-513.
28. Sirajuddin M., Ali S., Badshah A., Drug-DNA interactions and their study by UV-Visible, fluorescence and cyclic voltametry. *J. Photochem. Photobiol. B: Biology*, 2013; 124: 1-19.

29. Strothkamp K.G, Strothkamp R.E., Fluorescence measurements of ethidium binding to DNA. *J. Chem. Educ*., 1994; 71(1): 77-79.
30. Tselepi E., Katsaros N., The interaction of [Ru(NH3)5Cl]2+ and [Ru(NH3)6]3+ ions with DNA. *J. Inorg. Biochem*., 1989; 37(4): 271-282.
31. Tsiliou S., Kefala L.A., Perdih F., Turel I., Kessissoglou D.P., Psomas G., Cbalt(II) complexes with non-steroidal anti-inflamatory drug tolfenamic acid: Structure and biological evaluation. *Eur. J. Med. Chem*., 2012; 48: 132-142.
32. Xue F., Xie C.Z., Zhang Y.W., Qiao Z., Xu J.Y., Yan S.P., Two new dicopper(II) complexes with oxamido-bridged ligand: Synthesis, crystal structures, DNA binding/cleavage and BSA binding activity. J. Inorg. Biochem., 2012; 115: 78-86.
33. Zhang R., Wu X., Yalowich J.C., Hasinoff B.B., Design, synthesis and biological evaluation of a novel series of bisintercalating DNA-binding piperazine-linked bisanthrapyrazole compounds as anticancer agents. *Bioorg. Med. Chem*., 2011; 19: 7023-7032.

Manuscript received: June 2012