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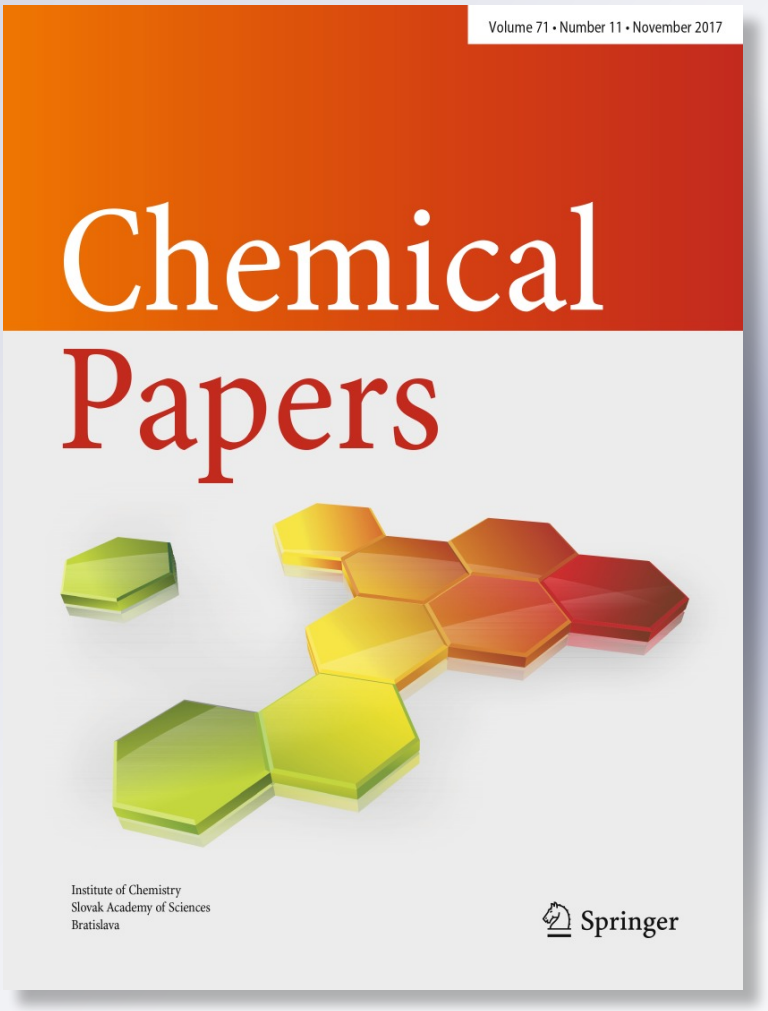

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ORIGINAL PAPER

Study of the structure, prooxidative, and cytotoxic activity of some chelate copper(II) complexes

Vladimir P. Petrović[1] · Marko N. Živanović[2] · Dušica Simijonović[1] · Jelena Đorović[1,3] · Zorica D. Petrović[1] · Snežana D. Marković[2]

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Abstract The six chelate *N*,*O*-copper(II) complexes were synthesised starting from salicylaldehyde anil Schiff bases, as ligands. Their structure is elucidated using experimental and theoretical tools. In vitro biological activities, i.e. cytotoxic and prooxidative effects against human epithelial mammary gland/breast metastatic carcinoma MDA-MB-231, epithelial colorectal carcinoma HCT-116, and foetal lung fibroblast healthy MRC-5 cell lines of investigated compounds are also determined. Complexes **Cu-1**, **Cu-6**, and especially **Cu-7** showed significant cytotoxic effects, with IC_{50} values comparable with effects of positive control CisPt. In addition, investigated complexes induced extreme oxidative and nitrosative stress in all treated cell lines. The most prominent effect is observed on HCT-116 cells, and on MRC-5 cells, while MDA-MB-231 cells showed higher resistance to the investigated cell lines, giving us direction towards the substances with more specific selectivity.

Keywords Chelate copper(II)–Schiff bases complexes · Structure elucidation · Cytotoxic activity · Oxidative stress · Prooxidative activity

Introduction

Schiff bases are organic compounds known for almost two centuries (Da Silva et al. 2011; Schiff 1864; Vigato and Tamburini 2004; Abu-Dief and Mohamed 2015). They found utilisation in almost every part of chemistry, including organic synthesis, catalysis, analytical chemistry, food industry, as well as industry of pigments and dyes (Genin et al., 2000; Santwana, 2003; Supuran et al., 1996). The main structural fragment of these compounds is imine group, which is considered to be responsible for a broad range of biological activities (Bringmann et al. 2004; Guo et al. 2007; Souza et al. 2007; Abdel-Rahman et al. 2014, 2015). Some of them are antibacterial, antifungal, antimalarial, anti-inflammatory, antiviral, antiproliferative, and antipyretic properties (Guo et al. 2007).

In addition to this, if Schiff bases consist of some additional heteroatom to nitrogen from imine group, they can serve as chelators for numerous transition metal ions (Krishnapriya and Kandaswamy 2005; Panda and Chakravorty 2005). Various Schiff base complexes have been described, as well as their chemical and biological applications (Bian et al. 2003; Faniran et al. 1974; Mohamed et al. 2006; Abdel Rahman et al. 2016, 2017).

It is known that coordination of ligands to metal can induce increase of biological activity (Petrović et al. 2015a, b; Sharma and Khar 1998). Numerous articles studied biological activity of transition metal complexes (Čanović et al. 2016; Sun et al. 2007; Xu et al. 2002). Schiff base-derived complexes with transition metal ions,

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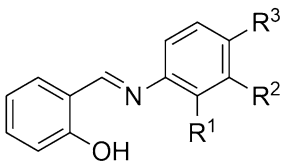
such as Cu(II), Mn(II), Co(II), Ni(II), and Pd(II), have also been reported (Ispir et al. 2008; Kavitha and Reddy 2013; Kondaiah et al. 2013; Prakash and Adhikari 2011). Among others, copper(II) complexes showed important effects such as antifungal (Creaven et al. 2010), antiviral (Wang et al. 1990), and antibacterial (Zhang 2016). For copper–Schiff base complexes it is shown that they possess significant effects against cancer cells, e.g. Joseyphus et al. pointed IC_{50} 25.8 μM and Rama et al. IC_{50} 54 μM against HCT-116, and 6.5 μM against MDA-MB-231 cancer cells (Rama and Selvameena 2015; Joseyphus et al. 2014). Similarly, we chose to examine the impact of Cu(II)–Schiff bases complexes on epithelial colorectal carcinoma HCT-116 and human epithelial mammary gland/breast metastatic carcinoma MDA-MB-231 cells, as well as on foetal lung fibroblast healthy MRC-5 cell lines.

Our previous studies were based on the influence of Schiff bases and their palladium(II) complexes on cancer cell lines. In continuation of our examinations, we wanted to synthesise complexes with a biometal/chelating agent. The choice was the life-important copper. Although this metal is essential for a lot of life processes, there are many questions concerning copper's influence on dysregulation of Cu homeostasis, which leads to diseases (Festa and Thiele 2008). In addition, copper is one of the participants in the Fenton's reaction whose radical products may cause cell damage or antioxidant/prooxidative response. Therefore, we focused our efforts towards the in vitro investigation of the prooxidative and cytotoxic effects of the Cu(II) complexes, obtained in the reactions with previously studied Schiff bases, Scheme 1 (Marković et al. 2015; Petrović et al. 2015a, b) on the cancer and healthy cell lines.

Computational methods

All calculations were performed with the Gaussian 09 software package (Frisch et al. 2009), B3LYP functional with D3 dispersion term using Becke–Johnson damping function (Grimme et al. 2010) in combination with triple split valence basis set 6-311 + G(d,p) was used for all atoms (Lee et al. 1988; Vosko et al. 1980). The structures of investigated compounds were fully optimised in the gas phase. Frequency calculations were carried out to confirm that all structures are local minima (all positive eigenvalues). The gas phase structures were used for the examination of geometrical parameters and predicting IR spectra. The computed frequencies were scaled by the factor of 0.97. The natural bond orbital analysis (Gaussian NBO version) was performed.



1 R^1, R^2=H, R^3=OH
3 R^1, R^2=H, R^3=CH_3
4 R^1, R^2, R^3=H
5 R^1, R^2=H, R^3=F
6 R^1=H, R^2=OH, R^3=H
7 R^1=OH, R^2=H, R^3=H

Scheme 1 Schiff bases used as ligands

Experimental

Synthesis of copper(II) complexes

Copper(II) acetate (0.5 mmol) was added to a solution of corresponding Schiff base (**1**, **3**, **4**, **5**, **6**, **7**) (1 mmol) of ethanol (5 mL) and heated at reflux for 3 h. After completion of the reaction, the solvent was evaporated and remaining powder was washed with ethanol (3 × 2 mL). Obtained powder was dissolved in a mixture of dichloromethane:ethanol 1:2, recrystallized, and the resulting crystals/powder was dried under vacuum. Unfortunately, obtained crystals were not suitable for X-ray analysis. Complexes were obtained in 80–85% yield. All complexes were characterised with elemental microanalysis and IR (Fig. 1). It is worth pointing out that obtained compounds were not suitable for NMR spectroscopy due to their diamagnetic properties.

Cu-1: dark brown powder—$C_{26}H_{20}CuN_2O_4$ (FW = 487.99): C, 63.99; N, 5.74; H, 4.13%; found: C, 63.45; N, 5.81; H, 4.16%.

Cu-3: brown crystals—$C_{28}H_{24}CuN_2O_2$ (FW = 484.06): C, 69.48; N, 5.79; H, 5.00%; found: C, 69.18; N, 5.85; H, 5.04%.

Cu-4: brown crystals—$C_{26}H_{20}CuN_2O_2$ (FW = 456.00): C, 68.48; N, 6.14; H, 4.42%; found: C, 68.05; N, 6.21; H, 4.38%.

Cu-5: red-brown crystals—$C_{26}H_{18}CuF_2N_2O_2$ (FW = 491.98): C, 63.47; N, 5.69; H, 3.69%; found: C, 63.51; N, 5.76; H, 3.65%.

Cu-6: brown crystals—$C_{26}H_{20}CuN_2O_4$ (FW = 487.99): C, 63.99; N, 5.74; H, 4.13%; found: C, 63.54; N, 5.8; H, 4.16%.

Cu-7: green powder—$C_{26}H_{20}CuN_2O_4$ (FW = 487.99): C, 63.99; N, 5.74; H, 4.13%; found: C, 63.48; N, 5.83; H, 4.17%.

Cell preparation and culturing

The epithelial colorectal carcinoma HCT-116, human epithelial mammary gland/breast metastatic carcinoma

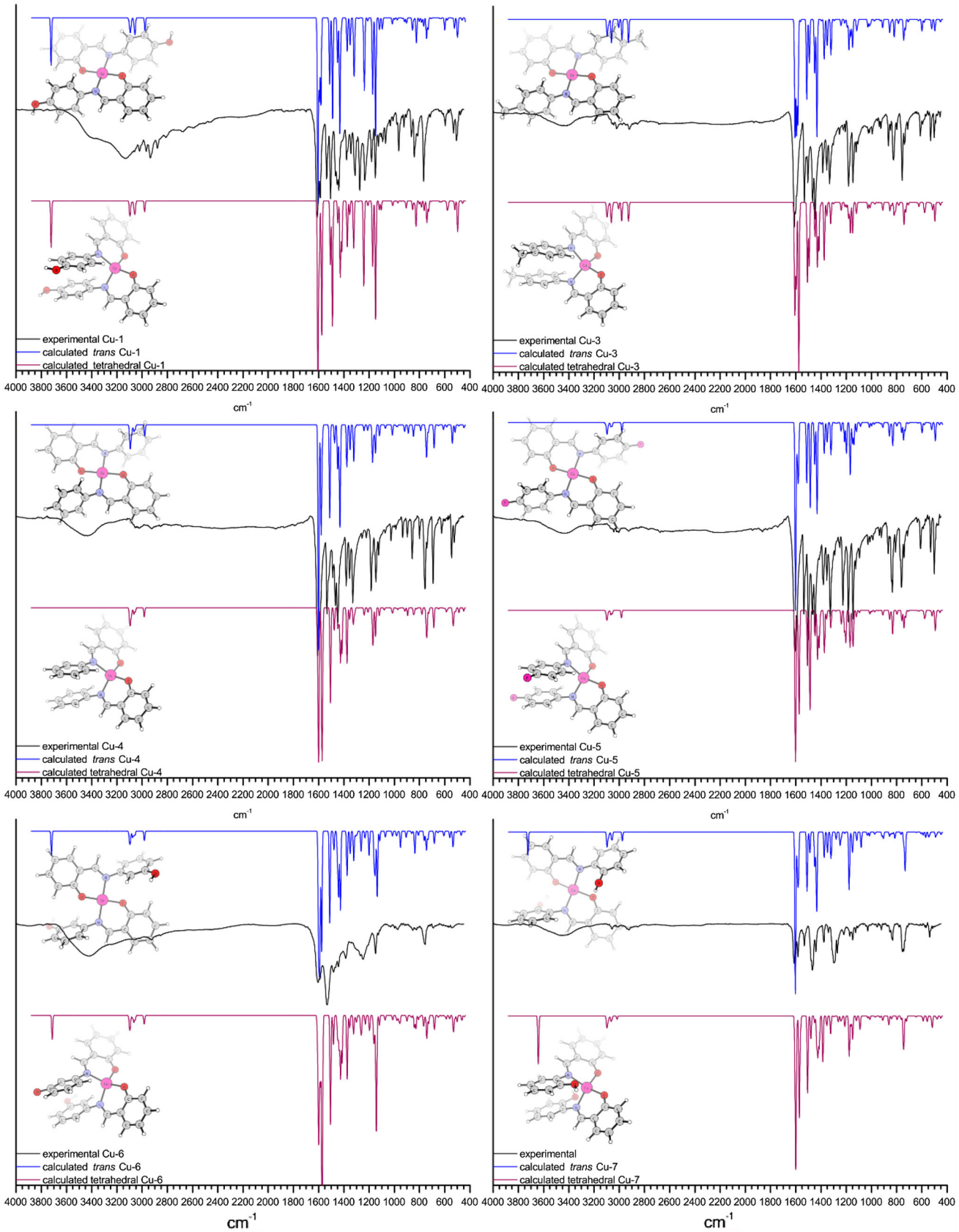


Fig. 1 Optimised structures, calculated and experimental IR spectra of *trans*-square planar and tetrahedral complexes **Cu-1**, **Cu-3**, **Cu-4**, **Cu-5**, **Cu-6**, and **Cu-7**

MDA-MB-231 cells, and foetal lung fibroblast healthy MRC-5 cell lines were purchased from the American Tissue Culture Collection (Manassas, VA, USA). The cells were propagated in controlled laboratory conditions in DMEM supplemented with 10% foetal bovine serum, 100 IU/mL penicillin, and 100 μg/mL streptomycin in humidified atmosphere with 5% CO_2 at 37 °C.

Biological in vitro assays

Determinations of cell viability, concentration of superoxide anion radical, nitrites and reduced glutathione were performed following standard procedures briefly described in our previous studies (Petrović et al. 2014, 2015a, b).

Statistics

All experiments have been performed in three individual experiments performed in triplicates for each dose and all data were expressed as mean ± standard error (SE). Statistical significance was determined using the Student's *t* test or the one-way ANOVA test for multiple comparisons. A p value <0.05 was considered as significant. The magnitude of correlation between variables was done using SPSS (Chicago, IL, USA) statistical software package (SPSS for Windows, version 17, 2008). The IC_{50} values were calculated from the dose curves by a computer program (CalcuSyn).

Chemicals

The compounds salicylaldehyde, aniline, 4-fluoroaniline, 4-nitroaniline, toluidine, 2-hydroxyaniline, 3-hydroxyaniline, 4-hydroxyaniline, copper(II) acetate, and 5,5′-dithiobis(2-nitrobenzoic acid) were obtained from Aldrich Chemical Co. Dulbecco's Modified Eagle Medium (DMEM) and PBS were obtained from GIBCO, Invitrogen, USA. Foetal bovine serum (FBS) and trypsin–EDTA were from PAA (The Cell Culture Company, Pasching, Austria). Dimethyl sulfoxide (DMSO), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), and nitro blue tetrazolium (NBT) were obtained from SERVA, Heidelberg, Germany. *N*-1-Naphthylethylenediamine dihydrochloride was purchased from Fluka chemie GMBH, Buchs, Switzerland. Sulphanilamide and sulphosalicylic acid were purchased from MP Hemija Belgrade, Serbia. All solvents and chemicals were of analytical grade.

Results and discussion

In our previous studies we examined in detail antioxidative properties of some salicylaldehyde- and vanillin-derived Schiff bases (Marković et al. 2015; Petrović et al. 2015a, b). These compounds, as well as their respective Pd complexes, were tested in vitro on cancer cell lines (Petrović et al. 2015a, b). It was found that Pd complexes exert noticeable higher cytotoxic activity, than Schiff bases by themselves. Taking into account induced increase in cytotoxic activity by complexation with palladium, we explored the effects of complexations with copper. Here we present six copper(II) complexes, prepared from *N*-salicylidene aniline Schiff bases (Scheme 1 (Marković et al. 2015; Petrović et al. 2015a, b), Fig. 1) and copper(II) acetate (molar ratio 2:1). The obtained complexes were structurally characterised using experimental (IR) and theoretical tools (Density Functional Theory), and subjected to biological testings. For complexes (**Cu-3–6**), crystal structures are known (Bindlish et al. 1976; Burgess et al. 2001; Ren et al. 2016; Shibuya et al. 2008; Wei et al. 1964; Xu and Pei 2012; Xu et al. 2012; Yildirim et al. 2002), and they were used as starting points in theoretical optimizations. For **Cu-7**, structural characterization is given for the first time here, and to the best of our knowledge, this kind of characterization for copper complexes has not been reported elsewhere until now.

Structural characterization of the investigated complexes

The optimised geometries of investigated **Cu-1**, **Cu-3**, **Cu-4**, **Cu-5**, **Cu-6**, and **Cu-7** complexes, as well as experimental and simulated IR spectra are presented in Fig. 1. Bond lengths, angles, and dihedral angles of all complexes calculated are listed in Tables S1–S6, while corresponding atoms' labellings are depicted in Figs. S1–S6. In all investigated cases, copper is forming two six-membered chelate rings. Each of them is formed via coordination of nitrogen from azomethine group and oxygen originating from salicylaldehyde part of Schiff base, with copper(II) ion. In the case of **Cu-7** complex, chelation in five-membered fashion is also explored, Fig. S7.

Possibility of the formation of different geometric isomers of the investigated complexes is explored (Fig. 1; Figs. S1–S8; Tables S1–S7). It is assumed that the complexes exhibit square planar coordination (either *trans* or *cis*). Yet, it is found that, after the optimisations, some of the structures become distorted. In these cases, it was not obvious whether the coordination is square planar or tetrahedral. Hence, the geometry of chelate complexes is determined on the basis of the plane–plane angle defined by the two chelate rings (Shibuya et al. 2008). Angles 0°, 90°, and 180° correspond to the ideal *trans*-square planar, tetrahedral, and *cis*-square planar geometries, respectively.

On the basis of this, structures with angles of the chelate planes close to 0° (Fig. 1; Tables S1–S6) are characterised with *trans*-square planar. In all of structures delineated this

way, copper(II) exhibits nearly ideal square planar coordination, with N–Cu–O, and N–Cu–N/O–Cu–O bond angles close to 90° and 180°, respectively. The only deviation is in the case of **Cu-7**, where chelating plane angle is close to 25°. This distortion is most probably due to favourable position of OH substituent in aniline ring towards ligating oxygen atom from the other chelating ring. In that way hydrogen OH···O bond is formed. On the other hand, structures with plane–plane angles close to 125° possess geometry which is closer to tetrahedral than *cis*-square planar.

In all cases, the NBO analysis revealed that, in place of formed bonds between copper(II) and ligands, there is donation of electron density from the donor atoms to the metal centre. Oxygen lone pairs from pure p orbitals, and nitrogen lone pairs from the sp^2 orbitals delocalize to the half empty d orbital and formally empty s orbital of Cu(II). As a result, occupancies of mentioned copper orbitals are higher than before, with consequently lowered occupancies in the orbitals of donor atoms.

To confirm or to negate that calculated structures correspond to the experimentally obtained complexes, theoretical spectra obtained by means of density functional theory are compared to experimentally acquired IR spectra.

IR spectral characterization

In all investigated cases, good agreement between experimental and calculated spectra is achieved, Fig. 1. Similar to our previous work with Pd complexes (Petrović et al. 2015a, b), deviations are observed in the region above 3000 cm^{-1}. Namely, in the cases where OH group is still present, these bands are overestimated, apart from the *trans*-**Cu-7**, where OH stretches are matching experimental ones. This can be rationalised on the basis of that in this case there is intramolecular OH···O hydrogen bonding (Shibuya et al. 2008). On the other hand, this was not repeated in other calculated structures. This is most probably due to excluded possibility of intramolecular hydrogen bonding, as well as due to the intermolecular hydrogen bonding present in the solid state but not in the optimised structures. Compared to the spectra of ligands (Petrović et al. 2015a, b) (Fig. S9) bands belonging to OH stretching vibrations are changed in cases for **Cu-1**, **Cu-6**, and **Cu-7**, while in others these bands are missing, implying that Cu–O coordination occurred.

In the spectra of corresponding complexes, the C=N bands are present at lower wavenumbers 1605–1612 cm^{-1}, than in corresponding ligands, implying that N coordination occurred (Abdel-Rahman et al. 2016, 2017a, b). In addition, there are new bands in the regions of 520–550 and 450–470 cm^{-1} originating from Cu–O and Cu–N vibrations. It is worth pointing out that our IR data, both experimental and the calculated, are in accordance with the literature available (Abdel-Latif et al. 2007; Campos-Vallette et al. 1993; Kusmariya et al. 2016; Vafazadeh et al. 2010).

Based on the calculated spectra, one cannot conclude which isomer prevails. Comparison of the relative free energies among corresponding isomeric structures revealed that, except in the case of **Cu-7**, tetrahedral isomers are somewhat more stable, but one should note that differences are not pronounced (up to about 6 kJ/mol, Table S7). On the other hand, in the case of **Cu-7**, *trans*-square planar structure is by about 30 kJ/mol more stable than corresponding tetrahedral geometry. In addition, this six-membered complex is far more stable than any of equivalent five-membered chelates, Table S7. Taking into account that the crystal packing of **Cu-3** is built of both isomeric structures (Xu and Pei 2012), it is possible that in all cases, apart from **Cu-7**, both isomers are present.

Biological evaluation

Cytotoxic effects

Standardised MTT assay was used for the determination of the cytotoxicity of investigated complexes and results are expressed by IC_{50} values presented in Table 1 and Figs. S10–S12. When compared with positive control **CisPt**, the strongest effect was observed with **Cu-7**, which showed very high cytotoxicity to all three cell lines. **Cu-1** and **Cu-6** expressed significant activity on HCT-116 and MRC-5 cells. Unlike these complexes, **Cu-3**, **Cu-4**, and **Cu-5** did not express such prominent cytotoxic effect, but these substances expressed noticeable cytotoxic activity only towards HCT-116 cells. This is important since these complexes showed selectivity towards one cancer cell line versus healthy cells. On the other hand, $Cu(OAc)_2$ served as negative control and we observed no cytotoxicity on all three cell lines, Table 1. These results might suggest that investigated thermodynamically stable six-membered chelate Cu(II) complexes are stable and that they hydrolyse very slowly in cell. Our previous results showed that palladium complex analogues of **Cu-1** and **Cu-6** expressed significant cytotoxicity on these three investigated cell lines.[24] Similar to our previous findings (Kosaric et al. 2014; Petrović et al. 2014, 2015a, b), HCT-116 cells are more sensitive to investigated Cu(II) complexes than MDA-MB-231 cells. This could be explained by the nature and origin of these cells. MDA-MB-231 cells are metastatic and thus more resistant cells, while HCT-116 cells have been derived from primary tumour.

Table 1 IC_{50} values (μM) of the investigated compounds

	HCT-116		MDA-MB-231		MRC-5	
	24 h	72 h	24 h	72 h	24 h	72 h
IC_{50}, μM						
Cu-1	25.49	18.55	>500.00	155.0	51.78	31.03
Cu-3	>500.00	38.27	>500.00	>500.00	>500.00	237.21
Cu-4	53.69	21.84	>500.00	>500.00	125.38	105.53
Cu-5	58.77	37.89	>500.00	>500.00	225.85	470.47
Cu-6	35.15	13.98	>500.00	108.2	12.17	11.42
Cu-7	9.31	2.15	67.30	27.80	12.51	11.66
$Cu(OAc)_2$	>500.00	>500.00	>500.00	>500.00	>500.00	>500.00
CisPt	219.70	19.40	322.40	38.60	188.10	22.10

Inhibitory activity was expressed as the mean of 50% inhibitory concentration of triplicate experiments

Superoxide anion radical ($O_2^{\cdot-}$) content changes

It is well known that redox equilibrium in cells is very important. Thus, we examined the influence of Cu(II) Schiff base complexes on the level of superoxide radical anion $O_2^{\cdot-}$ as an important indicator of reactive oxygen species (ROS). Results representing the level of measured $O_2^{\cdot-}$ are presented in tables S8–S10 for HCT-116, MDA-MB-231, and MRC-5 cells, respectively. These data represent the level of measured $O_2^{\cdot-}$ in whole plate well. From the obtained data, one can notice that there is significant increase of $O_2^{\cdot-}$ content for investigated Cu(II) complexes. Considering that treatment influenced significant lowering in cell number (cytotoxic effect), we recalculated all data to be related to the number of survived cells (from MTT data) (Petrović et al. 2015a, b). This way, we obtained another point of view of the level of $O_2^{\cdot-}$. Such recalculated data revealed very significant increase of $O_2^{\cdot-}$ for all complexes on all three cell lines, i.e. cells were under significant oxidative stress, Figs. S13–S15. Similar to the effects on cell viability, **Cu-1**, **Cu-6**, and especially **Cu-7** induced huge increase in $O_2^{\cdot-}$ content in dose- and time-dependent manner (Petrović et al. 2015a, b). Increased content of ROS could lead to the irreversible damage of cellular compartments and biomacromolecules (including DNA), leading to enhanced cytotoxicity. Thus, it is expected that if some substance leads to the enhanced production of ROS, it could also be cytotoxic (Deavall et al. 2012). When the results of $O_2^{\cdot-}$ and MTT are compared, significant compatibility can be found. **Cu-1** induced the highest production of $O_2^{\cdot-}$ and the highest cytotoxic effect on HCT-116 cells. **Cu-6** induced similar compatibility on MRC-5 cells. **Cu-7** showed the highest cytotoxic effect on HCT-116 cells, while the increase of $O_2^{\cdot-}$ was largest in healthy MRC-5 cells. Comparison of the structures of phenolic complexes **Cu-1**, **Cu-6**, and **Cu-7** revealed that the most active complex **Cu-7** contains free OH group in *o*-position of aniline moiety, as well as Schiff base which was used as ligand and which showed significant cytotoxic effect on HCT-116 cells (Petrović et al. 2015a, b). According to the fact that our complexes are Cu(II) based, one of the questions from where such high amount of $O_2^{\cdot-}$ comes could be explained by Fenton reaction. In mitochondria, produced H_2O_2 reacts with cytochrome iron yielding water and $O_2^{\cdot-}$. It is proved that Fenton reaction could also be catalysed by transition metals such as copper (Pham et al. 2013). It is known that many of the anti-cancer drugs are designed to induce overproduction of ROS and, therefore, prooxidative (Barrera 2012). Our results showed that increase in $O_2^{\cdot-}$ amount is larger in HCT-116 cells and in MRC-5 cells than in MDA-MB-231 cells.

Nitrite (NO_2^-) content changes

Besides ROS-induced oxidative stress, the cell redox equilibria could also be altered by reactive nitrogen species (RNS). Nitrites could be considered as an indicator of NO which is one of the most important RNS factors. Results representing nitrite levels are presented in Tables S11–S13 and nitrite levels related to the number of viable cells in Figs. S19–21 for HCT-116, MDA-MB-231 and MRC-5 cells, respectively. It was observed that investigated complexes induced increase in nitrite level. The most significant effect was in cases of **Cu-1**, **Cu-6**, and **Cu-7** in the dose- and time-dependent manner. **Cu-4** increased nitrites only in the highest concentration. Similarly, as in NBT assay, when recalculated to the number of survived cells, the NO_2^- increase is significant and related to the cell viability. Thus, our results again suggest that cells are under high nitrosative stress. The origin of nitrites is quite complicated for explanation because it could be derived from many sources, e.g. from catabolism of investigated substances (our substances contain nitrogen atoms), and/or from the induction of enzymes that could be involved in

NO production such as iNOS and many others. NO (and thus nitrite) level is greatly influenced by $O^{2\cdot-}$ production. According to Ferrer-Sueta and Radi (2009), $O^{2\cdot-}$ possess great affinity towards NO, forming peroxynitrites, which exert great oxidative potential. $NO^{\cdot}$ and $O_2^{\cdot-}$ react rapidly in vivo, and the formed $ONOO^-$ also quickly reacts with thiol groups of GSH and many proteins (Lancaster 2006) inducing non-controlled and non-desired posttranslational modifications. The largest production of NO_2^- is observed in healthy MRC-5 cells.

Reduced glutathione (GSH) content changes

Glutathione is a tripeptide, γ-L-glutamyl-L-cysteinyl-glycine, responsible for preventing the influence of ROS and RNS that could cause damage to the cell structures and affect cellular processes and cell viability. Tables S14–S16 and Figs. S16–S18 represent results obtained for GSH measurements on HCT-116, MDA-MB-231 and MRC-5 cells, respectively. Considering the level of GSH related to the number of survived cells, these results suggest significant increase in GSH level induced by investigated substances, especially after 72 h from treatment. The highest production is observed in healthy MRC-5 cells. On HCT-116 cells all investigated substances induced increase of GSH in similar extent. On MDA-MB-231 cells, **Cu-7** induced the most significant increase, while in MRC-5 cells **Cu-1**, **Cu-6**, and **Cu-7** induced the largest increase. Such a denominated increase in GSH level could be related to the increased oxidative and nitrosative stress as a positive feedback of cell. This GSH production could be related to previous intensive production of ROS/RNS. Interaction of GSH with redox changed active moieties of proteins includes oxidation to GSSG with subsequent NADPH-dependent reduction to GSH by glutathione reductase (Nordberg and Arnér 2001). In addition, this might be achieved in interaction of GSH with tested substances, or with some reactive species originating from phenolic complexes **Cu-1**, **Cu-6**, and **Cu-7** (Radu et al. 2010).

Conclusions

The results presented in this paper include the synthesis of the six chelate *N*,*O*-copper(II) complexes, investigation of their structures using experimental and theoretical tools, as well as in vitro biological evaluation on cancer and healthy cell lines. On the basis of obtained experimental and theoretical data, it is found that the complexes **Cu-1**-**6** exhibit either *trans*-square planar or tetrahedral geometry, while **Cu-7** exclusively adopts *trans*-square planar geometry. In vitro study of biological activity of synthesised Cu(II) complexes revealed that, similar to our earlier findings with Pd(II) Schiff complexes, **Cu-1**, **Cu-6**, and **Cu-7** induced significant oxidative/nitrosative stress followed by enhanced cytotoxicity of healthy and cancer cells. Compared to **CisPt**, investigated **Cu-7** showed higher cytotoxic effect against treated cells with no selectivity. Such cytotoxic effect is a consequence of increased production of superoxide radical anions and nitrites as a result of prooxidative action of these complexes. It is important to emphasise the effect of **Cu-3**, **Cu-4**, and **Cu-5**. They exhibited higher cytotoxicity against HCT-116 cells than against healthy MRC-5 cells, with no effect against MDA-MB-231 cells. Compared to the results of analogue Pd(II) complexes, Cu(II) complexes exerted higher selectivity to healthy MRC-5 cells.

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