

Influence of the Structure on the Antioxidant Activity of Tetradentate Schiff Bases and their Copper(II) Complexes: Possible Mechanisms

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A influência da estrutura na atividade antioxidante de uma série de bases de Schiff e seus complexos de cobre(II) assim como possíveis mecanismos da atividade antioxidante foram investigados. As bases de Schiff são derivadas da condensação de etano-1,2-diamina ou propano-1,2-diamina com pentano-2,4-diona e/ou 1-fenilbutano-1,3-diona. Os complexos correspondentes foram sintetizados. A atividade antioxidante *in vitro*, determinada por cromatografia em camada delgada em fase reversa (RP TLC), baseada na reação com 2,2'-difênil-1-picrilhidrazil, foi expressa como capacidade antioxidante equivalente em Trolox (TEAC). A influência da estrutura foi observada com base em descritores interpretáveis e informativos calculados por métodos híbridos de teoria do funcional da densidade (DFT). As características responsáveis pela atividade antioxidante dos compostos investigados foram discutidas. Os complexos de cobre(II) apresentam atividades antioxidantes significativamente maiores que seus ligantes correspondentes. Os descritores químico-quânticos calculados forneceram uma visão do mecanismo de reação da atividade antioxidante/sequestrante das bases de Schiff tetradentadas e de seus complexos de cobre(II) e dois diferentes mecanismos de reação foram propostos.

The influence of the structure on the antioxidant activity of a series of Schiff bases and their copper(II) complexes as well as possible mechanisms of antioxidant activity were investigated. Schiff bases are derived from the condensation of ethane-1,2-diamine or propane-1,2-diamine and pentane-2,4-dione and/or 1-phenylbutane-1,3-dione. The corresponding complexes were synthesized. The antioxidant activity *in vitro*, determined using reversed-phase thin layer chromatography assay (RP TLC) based on the reaction with 2,2'-diphenyl-1-picrylhydrazyl, was expressed as Trolox equivalent antioxidant capacity (TEAC). The influence of the structure was observed on the basis of interpretable and informative descriptors calculated by hybrid density functional theory (DFT) methods. Structural features responsible for the antioxidant activity of the investigated compounds were discussed. Copper(II) complexes showed a significantly higher antioxidant activity than their corresponding ligands. Calculated quantum-chemical descriptors gave an insight to the reaction mechanism of the scavenging/antioxidant activity of tetradentate Schiff bases and their copper(II) complexes and two different reaction mechanisms were proposed.

Keywords: antioxidant activity, antioxidant mechanism, Schiff base, copper(II) complexes, DFT

Introduction

Schiff bases and their complexes have caused wide interest due to diverse spectra of biological and pharmaceutical potential, such as antitumor, antifungal, antibacterial, antimicrobial and antihelmintic uses.¹⁻¹² Schiff base complexes play an important role in designing metal complexes related to synthetic and natural oxygen carriers.¹³

The compounds of this type can be greatly modified by introducing different substituents providing very useful model compounds for investigation of different chemical processes and its effects. It is important to emphasize the structural similarity between Schiff bases possessing different donor atoms (N, O, S, etc.) and biologically active compounds found in natural biological systems.¹⁴

Nowadays, there is a great interest in new compounds with active antioxidant components, whether they are synthesized or obtained from the nature.¹⁵ Antioxidant

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activities are related to the compound capability of protecting biological systems from the potentially harmful effect of processes involving reactive oxygen species that can cause excessive oxidation. Reports on such activity investigations of Schiff bases and structurally similar compounds could be found in literature.^{16,17} The DPPH (2,2'-diphenyl-1-picrylhydrazyl) assay is one of the most important methods used for evaluation of antioxidant activity. It is technically simple and rapid and that might explain its widespread use in antioxidant screening.

Antioxidant activity can be determined spectrophotometrically or by high-performance liquid chromatography (HPLC), but also using some faster and inexpensive methods, such as thin layer chromatography (TLC).¹⁸ DPPH may be neutralized either by direct reduction via electron transfer or by radical quenching via hydrogen atom transfer.¹⁹ It is difficult to interpret reactivity patterns and mechanisms without specific information about the composition and structures of the investigated antioxidants.

In the last decade, it was shown that quantum-chemical calculations can be a valuable tool in predicting and explaining antioxidative activity of various compounds. A good correlation between some quantum-chemical descriptors (i.e., various electronic descriptors, bond dissociation enthalpy and spin density) and antioxidant activity for a series of molecules were reported.²⁰

Our group has been investigating tetradentate Schiff bases and their complexes from different aspects for a long time. Their retention was investigated on different thin layers and relationships between the structure, retention and activity/property as well as the antioxidant capacity and electrochemical behavior were discussed.²¹⁻²⁵ Experimental results motivated us to investigate the influence of the structure on the antioxidant activity of these compounds and to perform calculations by hybrid density functional theory (DFT) methods. Several works were published to describe and explain the antioxidant activity using molecular descriptors.²⁶⁻²⁸

Therefore, the aim of this work was to take into consideration the impact of structural features and substituents on the mechanism of antioxidant activity of investigated compounds. To give better insight into the mechanism of the scavenging/antioxidant activity, the quantum-chemical descriptors which are the numerical representation of the molecular structures were calculated.

Experimental

Tetradentate Schiff bases and their corresponding copper(II) complexes (doublet spin state, d^9) were prepared

according to the procedures described in our previous work.²¹ Schiff bases were obtained by condensation of diamine (ethane-1,2-diamine or propane-1,2-diamine) and corresponding β -diketones (pentane-2,4-dione and/or 1-phenylbutane-1,3-dione) in absolute ethanol by stirring under reflux. The copper(II) complexes were synthesized by addition of freshly prepared copper(II) hydroxide in hot solution of corresponding Schiff base in absolute ethanol. The mixture was stirred under reflux.

Antioxidant activity

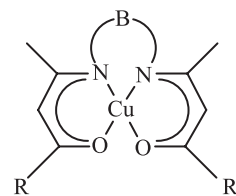
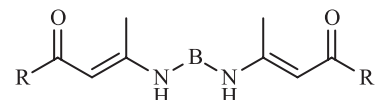
The values of antioxidant activity of the investigated compounds were extracted from our previous work.²⁵ Antioxidant activities were determined *in vitro* by TLC-DPPH assay and expressed as Trolox equivalent antioxidant capacity (TEAC, mol compound *per* mol Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The spots of 1 μ L of compound solution (0.6 mg mL⁻¹) were applied by autosampler (Linomat 5, Camag) on RP18 silica plate (Merck, Germany) as well as a series of standard solutions of Trolox (0-280 ng spot⁻¹) followed by applying 1 μ L of methanolic solution of DPPH (0.15 $\times 10^{-3}$ mol L⁻¹) at the same spots. No development was carried out. After 30 min of incubation in the dark, the plate was scanned. Camag TLC scanner with CATS evaluation software was used for scanning at 515 nm (see in the Supplementary Information (SI) section). The structures of the investigated compounds and obtained TEAC values are given in Table 1.

Calculations

Geometries of all molecules were fully optimized using hybrid DFT method, more specifically Becke three-parameterized exchange functional and Lee-Yang-Parr correlation functional (B3LYP). For the complexes, unrestricted B3LYP calculations were used with lan12dz basis set on the copper atom and 6-31G(d,p) basis set on other atoms. Inner electrons of the copper atom were described with lan12 effective core potential. Geometries of neutral ligand molecules were optimized with 6-311G(d,p) basis set. All quantum-chemical descriptors were calculated on the optimized geometries with 6-31+G(d,p) basis set on nonmetallic atoms and lan12dz with effective core potential (ECP) on the copper atom. Electronic molecular descriptors obtained after optimization were: HOMO and LUMO energies, ΔE HOMO-LUMO calculated as the difference of the energies, dipole moment, total energy, electronic energy, spin density, charge on atoms by natural bond orbital (NBO) analysis, and chelate plane angle.

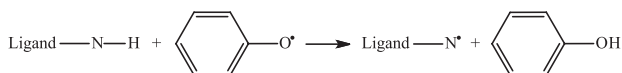
Table 1. Structures of the investigated compounds and their TEAC values

	Schiff base	R	R ₁	B	TEAC
L1	H ₂ (<i>acac</i> ₂ <i>en</i>) ^a	CH ₃	CH ₃	CH ₂ CH ₂	2.262
L2	H ₂ (<i>acac phacac en</i>) ^b	CH ₃	C ₆ H ₅	CH ₂ CH ₂	1.824
L3	H ₂ (<i>phacac</i> ₂ <i>en</i>)	C ₆ H ₅	C ₆ H ₅	CH ₂ CH ₂	1.963
L4	H ₂ (<i>acac</i> ₂ <i>pn</i>) ^c	CH ₃	CH ₃	CH(CH ₃)CH ₂	4.387
L5	H ₂ (<i>phacac</i> ₂ <i>pn</i>)	C ₆ H ₅	C ₆ H ₅	CH(CH ₃)CH ₂	5.427
	Complex	R	R ₁	B	TEAC
1	[Cu(<i>acac</i> ₂ <i>en</i>)] ^a	CH ₃	CH ₃	CH ₂ CH ₂	1.775
2	[Cu(<i>acac phacac en</i>)] ^b	CH ₃	C ₆ H ₅	CH ₂ CH ₂	1.498
3	[Cu(<i>phacac</i> ₂ <i>en</i>)]	C ₆ H ₅	C ₆ H ₅	CH ₂ CH ₂	1.442
4	[Cu(<i>acac</i> ₂ <i>pn</i>)] ^c	CH ₃	CH ₃	CH(CH ₃)CH ₂	2.809
5	[Cu(<i>phacac</i> ₂ <i>pn</i>)]	C ₆ H ₅	C ₆ H ₅	CH(CH ₃)CH ₂	3.820



^a*acac*: pentane-2,4-dione; *en*: ethane-1,2-diamine; ^b*phacac*: 1-phenylbutane-1,3-dione; ^c*pn*: propane-1,2-diamine.

Relative bond dissociation energies (BDE) for dissociation of N-H bond in ligand molecules in methanol solution were calculated using isodesmic reaction shown in Scheme 1.

**Scheme 1.** Isodesmic reaction for BDE calculations.

Geometries of all ligands and radicals were fully optimized using B3LYP method for closed-shell molecules and restricted open B3LYP method (ROB3LYP) for radicals with 6-311G(3df,3pd) basis set. The thermal energy, including zero-point energy, work and translational, rotational and vibrational entropy corrections were evaluated using standard statistical mechanic formulas.²⁹ Polarizable continuum model (PCM) calculations in methanol as solvent were done on optimized closed-shell molecules and radical geometries in order to introduce environment effect in BDE calculations. All DFT calculations were done in Gaussian 03 program package.³⁰

Statistical calculations were performed by NCSS 2004 software package.³¹

Results and Discussion

Antioxidant activities of the investigated Schiff bases and their copper(II) complexes were determined by TLC-DPPH assay. The relatively stable and clear zones on the RP-18 silica gel were observed, enabling the identification of radical-scavenging activity after incubation period. TLC scanner quickly and accurately determines the height and peak areas directly proportional

to the absorbance.³² The values of antioxidant activities are presented in Table 1.

From the obtained results, several interesting observations can be made. First of all, lower TEAC values (higher antioxidant activity) of copper(II) complexes (1-5, Table 1) in comparison with corresponding Schiff bases (L1-L5, Table 1) are obvious. The coordination of the Schiff bases and the presence of metal ion enhance the antioxidant potential. Furthermore, it is possible to draw a conclusion on the relationship between the antioxidant activity and the functional groups present in the complexes. It is known, from the literature, that substituents in the Schiff bases have a great influence on the activity of these compounds.³³ As can be seen in Table 1, the antioxidant activity increased by the number of phenyl groups in the complexes containing ethylenediamine as amine part (1 < 2 < 3, Table 1). The opposite dependence was observed for complexes containing propylenediamine.

Tetradentate Schiff bases

The analysis of calculated descriptors for Schiff bases and complexes revealed clear differences between compounds as a consequence of both substituents and complexation. In the attempt to explain the mechanism of the antioxidant action and the influence of the structure on the antioxidant activity of investigated compounds, Pearson's correlation matrix was carried out. The Pearson's correlation analysis tests the relationship among calculated molecular descriptors and experimentally determined DPPH scavenging activity. DPPH was chosen as free radical because of its ability to be reduced either by electron-transfer or by hydrogen atom transfer. The pair-wise method was employed (99% confidence level, $p < 0.01$). Statistically

Table 2. Pearson's coefficients between selected descriptors and DPPH scavenging activity of investigated Schiff bases

Descriptor	Dipole moment	BDE ^a	Charge O16 ^b	Charge O3 ^b	Charge H N7 ^b	Charge H N10 ^b
	-0.971	0.939	-0.828	-0.765	-0.795	-0.839

^aBond dissociation energy, ^bcharge obtained by NBO analysis.

significant correlation coefficients, between selected descriptors and DPPH scavenging activity for all investigated Schiff bases, extracted from Pearson's correlation matrixes are shown in Table 2 (Table S1 in the SI section). The descriptors (Table S3 in the SI section) used to explain the possible mechanisms of scavenging/antioxidant activity of the studied compounds were selected on the basis of the highest correlation coefficients. The Pearson's correlation matrix showed a high correlation of TEAC values of Schiff bases with dipole moment, bond dissociation energy and charge on oxygen atoms obtained by the NBO method.

Moreover, from the NBO analysis, it was concluded that the acidity of the hydrogen atoms related to the nitrogen atoms of amine bridge is very significant for the activity. Antioxidant activity is in good correlation with charge on hydrogen atoms obtained by NBO analysis (H on N7 and H on N10, Figure 1).

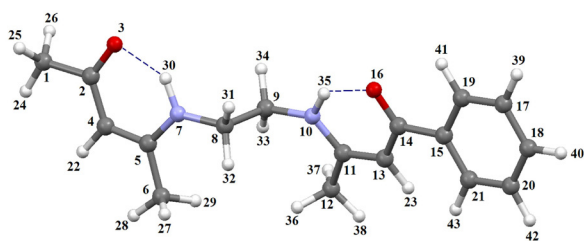


Figure 1. The B3LYP/6-31G(d,p) fully optimized geometries of the Schiff base L2 (see Table 1).

The values of correlation coefficients (Table 2) show that the higher the values of these descriptors, the lower the antioxidant activity. We believe that the mechanism by which Schiff base ligands behave as antioxidants involves the transfer of hydrogen atom from the amino group to the radical DPPH. Schiff bases are capable of forming an intramolecular H-bond (i.e., between H atom on N7 and atom O3, H atom on N10 and atom O16; dashed line, Figure 1). This weakens N-H bond and H atom is more easily released. Therefore, the donating capacity of hydrogen atom depends on dipole moments, bond dissociation energy, and charge on oxygen and hydrogen atoms. This is confirmed by the obtained results.

Low BDE values are often attributed to the high antioxidant potential.^{34,35} Bond dissociation energy was calculated for N-H bonds on N7 and N10. Difference between BDE for N7-H and N10-H is statistically

insignificant. For correlation with antioxidant activity, BDE for N7-H bond was used. The higher the value of BDE, the lower the antioxidant activity (Table 2). This is in accordance with the proposed reaction mechanism.

The global minima for Schiff bases containing propylenediamine bridge (L4, L5) (Figure 2) are close in geometry to the centrosymmetric conformation (not true centrosymmetric geometry due to the presence of chiral carbon atom). This leads to very low dipole moment values due to cancellation of the existing dipoles from two halves of the molecule. Unlike them, Schiff bases containing ethylenediamine bridge (L1, L2, L3) have different conformations of the global minima (Figure 1) and higher dipole moment values. Values calculated for the dipole moments in global minima geometry for all Schiff bases are presented in the SI section.

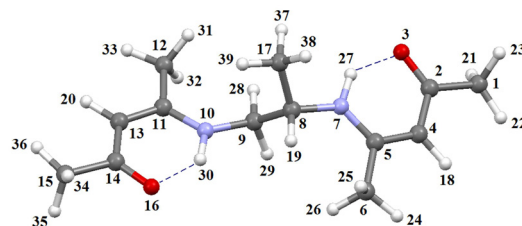


Figure 2. The B3LYP/6-31G(d,p) fully optimized geometries of the Schiff base L4 (see Table 1).

Also, the effects of substituents markedly modify intramolecular hydrogen bonding properties and thus directly affect the antioxidant activity of compounds. TEAC values (Table 1) indicate the electron-donating substituent effect on the activity. Electron-donating groups (methyl) strengthen the N-H bond so that H atom is not easily transferred to the DPPH radical (the hydrogen atom is less acidic). Based on the above mentioned descriptors and their correlation with antioxidant activity, the proposed mechanism of Schiff base action is hydrogen atom transfer.

Copper(II) complexes

Coordination of the Schiff bases and the presence of the copper(II) ion has significant effect on the increase of antioxidant activity. All copper(II) complexes possess a square-planar geometry around the metal. Angle between best planes of two chelate rings in complex 1 is only 8.99°, indicating square-planar geometry (Figure 3).

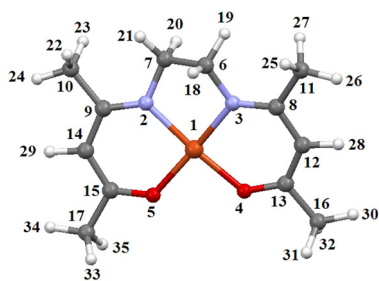


Figure 3. The B3LYP/6-31G(d,p) fully optimized geometries of copper(II) complex 1 (see Table 1).

The substitution of one or both methyl groups with phenyl groups (complexes 2 and 3) introduces new steric hindrance into complex molecule increasing the angle between chelate rings (10.86° and 11.71° , respectively). In the complexes 4 and 5, new enantiomeric center on carbon atom of methyl group in diamine bridge causes further deviation from square-planar geometry due to steric clashing between this methyl and methyl group from chelate ring (Figure 4).

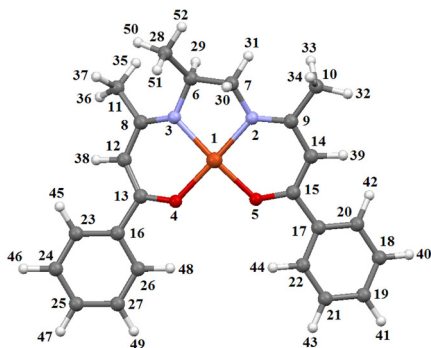


Figure 4. The B3LYP/6-31G(d,p) fully optimized geometries of copper(II) complex 5 (see Table 1).

Descriptors (see Table S4) that are highly correlated with antioxidant activity of the investigated complexes are chelate plane angle (0.906) and sum spin density (-0.887), being an indicative that the presumed mechanism of antioxidant activity is based on the electron-transfer (see Table S2).

Sum spin density is spin density on the atoms of the inner sphere of complex (N, N, Cu, O, O). This is an important factor which influences the antioxidant potency of the corresponding compound. The higher value of the sum spin density of complex results in stronger ability of the compounds to scavenge free radicals, i.e., lower TEAC values. Based on the values of these descriptors, the influence of substituents on this process can be monitored. Electron-withdrawing effects of phenyl groups affect sum spin density. Gradual substitution of methyl by phenyl groups increases the sum spin density which leads to the increase of antioxidant activity. The proposed mechanism

of antioxidant activity of studied complexes is reduction via electron-transfer. Electron localization is shown in plot of spin density (Figure 5) and it is obvious that spin density is highest at Cu atom.

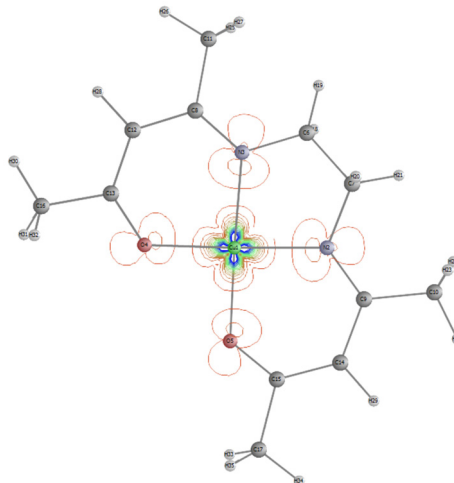


Figure 5. Plot of spin density for copper(II) complex 3.

Chelate plane angle describes deviation from square-planar structure of complexes. Higher value of this geometrical descriptor indicates a greater deviation from square-planar structure. From statistical parameters shown in Table 2, it is obvious that increase in chelate plane angle descriptor value leads to increase of the TEAC values, i.e., decrease of antioxidant activity of these complexes. Values of chelate plane angle descriptor of complexes with ethylenediamine bridge are significantly different compared with propylenediamine bridge complexes. The transfer of unpaired electron from compound to DPPH radical is more difficult if the chelate plane angle has a higher value. Therefore, complexes with propylenediamine in the amine bridge have significantly lower antioxidant activity (complexes 4 and 5) in comparison with those with ethylenediamine ligand (complexes 1, 2 and 3). The influence of chelate plane angle is in accordance with the proposed mechanism of the DPPH scavenging activity for studied complexes.

Conclusion

For better understanding the reactivity patterns and mechanisms of activity of antioxidants, the DFT-based quantum-chemical descriptors were calculated. Quantum-chemical descriptors are the numerical representation of the molecular structures. Dipole moment, bond dissociation energy, charge on oxygen atoms and charge on hydrogen atoms on N7 and N10 show high correlation with antioxidant activity of the investigated

Schiff bases. Antioxidant activity of copper(II) complexes is highly correlated with chelate plane angle and sum spin density descriptors. Selected descriptors, which have the best correlation with antioxidant activity, shed light on the reaction mechanism of scavenging/antioxidant activity of the studied compounds. DPPH may be neutralized either by direct reduction via electron-transfer or by radical quenching via hydrogen atom transfer. The H-atom transfer mechanism was defined as the most important for the antioxidant activity of the investigated Schiff bases while the proposed mechanism for the complexes was single electron transfer. The knowledge of the descriptors that affect the antioxidant activity of investigated and structurally similar compounds and possible mechanisms of antioxidant activity offer very interesting research opportunities and may be critical in the design of new ones.

Supplementary Information

Pearson's correlation matrices, descriptors, experimental details for determination of antioxidant activity are available free of charge at <http://jbcs.sbq.org.br> as PDF file.

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