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Spectroscopic properties of some new azo–azomethine ligands in the presence of Cu²⁺, Pb²⁺, Hg²⁺, Co²⁺, Ni²⁺, Cd²⁺ and Zn²⁺ and their antioxidant activity



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HIGHLIGHTS

- Two new azo-azomethine derivatives I and II were synthesized and characterized.
- The absorption spectra of I and II with some metal ions showed marked changes especially for I and $\rm Cu^{2+}$ ion.
- Job's plot indicated 1:1 bindingstoichiometry for receptor I with Cu²⁺ ion.
- 1:1 association constant of receptor I with Cu²⁺ was determined on the base of Benesi–Hildebrand plot.
- It was revealed both compounds show high and significant activity against DPPH method.

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ABSTRACT

Due to their potential applicability as selective receptors in optical sensors, two novel azo Schiff-base derivatives I and II are synthesized and characterized with FT-IR, ¹H NMR and elemental analysis techniques. The optical response of azo groups of I and II towards Ni²⁺, Co²⁺, Cu²⁺, Pb²⁺, Hg²⁺, Zn²⁺ and Cd²⁺ metal ions is studied in DMSO by UV-vis spectroscopy. The absorption spectra of both compounds with cations show marked changes. In solution, azo Schiff-base I produces a cation induced 95 nm blue shift for Cu²⁺ ion from 555 nm to 460 nm with remarkable color change from red to yellow. Whereas no significant color change is observed upon addition of studied metal cations to the solution of ligand II or other metal ions to the solution of ligand I. Furthermore, Job's plot indicate 1:1 binding-stoichiometry for I with Cu²⁺ ion and Benesi-Hildebrand plot is used for the determination of its association constant. Therefore receptor I is highly specific for copper ions in DMSO solution. Finally, the study of antioxidant properties of I and II with DPPH method reveals high and significant activities.

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Introduction

Azo Schiff base ligands contain both azo and azomethine groups. Azo groups possess excellent donor properties and play an important role in coordination chemistry [1–5]. Azo Schiff bases

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http://dx.doi.org/10.1016/j.saa.2014.04.026 1386-1425/© 2014 Elsevier B.V. All rights reserved. are commonly synthesized by coupling a diazonium reagent with an aromatic aldehyde to form an azo aldehyde [6]. Schiff bases are well known to have antifungal [7], antibacterial [8,9], antitumor activities [10–12]. Azo ligands containing salicyaldiminebased ligand as side chains can be used in the production of chemical sensors because they show a significant change of color of the solution and maxima of the absorption band when they interact with transition metal ions or anions [13–16]. Therefore among the wide range of materials that may be suitable for the electronic and optical applications, salicyaldimine-based ligands are of particular interest. They find applications in preparation of metallomosegens [17], various crystallographic studies [18,19] and enantioselective catalysis [20]. On the other hand, various methods like atomic absorption spectroscopy, inductively coupled atomic emission spectroscopy are widely used for the detection of metal ions at lower concentration. However, these methods require sophisticated instruments and also time consuming process. In order to overcome these difficulties a wide range of chemosensor techniques have been developed. Among them, colorimetric technique has some advantages over the others. It can be conveniently examined by naked eye, rather than by using sophisticated and expensive analytical instruments [21].

According aforementioned, two hosts **I** and **II** based on an azophenol-imine platform were prepared, characterized and studied their antioxidant activities via DPPH method. Also, optical properties of **I** and **II** were investigated in the presence some metal ions such as Cu²⁺, Pb²⁺, Hg²⁺, Co²⁺, Ni²⁺, Cd²⁺ and Zn²⁺ by UV-vis spectroscopy.

Materials and methods

General

1-(3-formyl-4-hydroxophenylazo)-4-nitrobenzene) was prepared according to literature method [6]. Starting materials were all purchased commercially and used without further purifications. Elemental analyses were performed on Elementar Vario ELIII. IR spectra were recorded on a FT-IR Spectrometer Bruker Tensor 27 in the region 4000–400 cm⁻¹ using KBr pellets. Electronic absorption spectra in the UV–vis region were recorded with T 60 UV/vis Spectrometer PG Instruments Ltd. NMR spectra were recorded on Bruker Avance 400 in CDCl₃ and DMSO with SiMe₄ as internal standard at room temperature.

Synthesis of ligands

Synthesis of 1-(3-imino-4-hydroxophenylazo-4-nitrobenzene)isonicotinohydrazide **I**

1-(3-formyl-4-hydroxophenylazo-4-nitrobenzene) (1.84 mmol, 0.500 g) in ethanol (30 ml) was added drop wise, over one hour, to a ethanol solution (15 ml) of isonicotinic acid hydrazide (1.84 mmol, 0.0252 g), immediately the color of solution changed to orange and precipitation was formed. The mixture was refluxed for 2 h (the end of reaction was controlled by T.L.C). Then the mixture was filtrated and residue solid was washed with ethanol and then recrystallized in dichloromethane-ethanol and dried (orange powder). Yield (0.600 g, 84%). IR (KBr, cm^{-1}) 3441 (OH group), 1650 (carbonyl group), 1604 (-C=N- imine), 1521 and 1458 (-N=N- cis and trans), 1360 and 1340 (NO₂ group), 1292 (C-O phenolic), 1280, 1095, 825, 668. ¹H NMR (400 MHz, CDCl₃): 12.43 (s, 1H, -CO-NH-), 11.87 (s, 1H, OH), 8.83 (s, 1H, -N=CH-), 8.38-8.43 (m,4H, ArH), 8.084 (m, 2H, ArH), 7.99 (d, 2H, pyridine ring), 7.88 (s,1H, ArH), 7.18 (d, 2H, pyridine ring). Elem. Anal. Calcd for C₁₉H₁₄O₄N₆: C, 58.46; H, 3.59; N, 21.54. Found: C, 58.21; H, 3.45; N, 22.21.

Synthesis of 1-(3-imino-4-hydroxophenylazo-4-nitrobenzene)-4methyl phenol **II**

1-(3-formyl-4-hydroxophenylazo-4-nitrobenzene) (1.84 mmol, 0.500 g) in ethanol (30 ml) was added drop wise, over one hour, to a ethanol solution (15 ml) of 4-methyl-2-aminophenol

(1.84 mmol, 0. 226 g), immediately the color of solution changed to brown and precipitation was formed. The mixture was refluxed for 3 h (the end of reaction was controlled by T.L.C). Then the mixture was filtrated and residue solid was washed with ethanol and then recrystallized in dichloromethane–ethanol and dried (redbrown powder). Yield (0.500 g, 72%). IR (KBr, cm⁻¹) 3067 (OH group), 1617 (—C=N— imine), 1518 and 1459 (—N=N— cis and trans), 1387 and 1335 (NO₂ group), 1288 (C—O phenolic), 1162, 1145, 1102, 1023, 855, 832, 686, 571. ¹H NMR (400 MHz, DMSO, d₆): 10.42 (s, 1H, OH), 9.27 (s,1H, OH), 8.42 (d, 2H, J = 6.8 Hz, ArH), 8.30 (s,1H, —HC=N—), 8.03 (m, 3H, ArH), 7.43 (s, 1H, ArH), 6.92–6.99 (m, 4H, ArH), 2.51 (s, 3H, —CH₃) Elem. Anal. Calcd for C₂₀H₁₆O₄N₄: C, 63.83; H, 4.25; N, 14.89. Found: C, 63.41; H, 3.91; N, 14.53.

Stability constant of copper complex

The stability constant K_a of 1:1 complex of **I** with Cu²⁺ transition metal ion was determined from the following Benesi–Hildebrand equation [22]:

 $1/\Delta A = 1/\Delta A_{sat} + 1/(\Delta A_{sat}K_a[guest])$

where ΔA is the absorption difference between the apparent metal complex and the free ligand and ΔA_{sat} is the absorption difference at saturation. The association constant value K_a was evaluated graphically by plotting $1/\Delta A$ against 1/[metal ion]. In this study, the concentration of the ligand was kept constant $(0.3 \times 10^{-4} \text{ M})$, and the concentration of metal ions was kept in the range ([metal ion]/[ionophore]) = 0.2–10.0 eq. Plot gave a straight line, and the K_a value was obtained from the slop and intercept of this line.

Measurement of antioxidant activity of complex by DPPH method

Antioxidant activity of compounds **I** and **II** was evaluated using DPPH. The DPPH assay was carried out using the method of Dehghan and Khoshkam [23]. Different concentrations (5–200 µg/ml) of **I** and **II** dissolved in DMSO, were added to 2 ml of DPPH solution (0.1 mM in methanol) and the reaction mixture was shaken vigorously. The reduction of DPPH absorbance was measured at 517 nm after 10 min (A_s). As a control, the absorbance of blank solution of DPPH (2 ml) was also determined at 517 nm (A_c). The percentage of radical scavenging activity (RSA%) was calculated according to the following equation:

 $RSA\% = 100(A_C - A_S)/A_C$

Results and discussion

Synthesis

In this work, to synthesis the new compounds **I** and **II**; 1-(3-formyl-4-hydroxophenylazo-4-nitrobenzene), isonicotinic hydrazic acid and 4-methyl-2-aminophenol were used as precursors. Used azophenyl derivative was synthesized according to the reported literature procedure [6]. The structures of both compounds were shown in Fig. 1. Ligands **I** and **II** were refluxed with azophenyl derivative and isonicotinic hydrazic acid or 4-methyl-2-aminophenol in ethanol, respectively. Both compounds containing imine and azo groups were characterized by a combination of FT-IR, ¹H NMR and elemental analysis and investigated by UV-vis spectroscopy. Also, optical response of receptors **I** and **II** was studied in the presence of some metal ions and their antioxidant activity.



Fig. 1. Structures of azo Schiff-base derivatives I and II.

IR spectra

The IR spectra of compounds I and II display characteristic bands of azo, hydroxo, nitro and imine groups. The absorption of the C=N stretching vibration in ligands occurs as a sharp and strong band at 1605 cm⁻¹ and 1617 cm⁻¹ for I and II, respectively. These ligands exhibit sharp and strong bands at 1335–1387 cm⁻¹ (1340 cm⁻¹ and 1360 cm⁻¹ for I and 1335 cm⁻¹ and 1387 cm⁻¹ for II), attributable to the NO₂ symmetric and asymmetric stretching vibrations. The characteristic bands of cis and trans azo groups appear at 1521 cm⁻¹ and 1458 cm⁻¹ for I and 1518 cm⁻¹ and 1459 cm⁻¹ in the spectrum of I corresponds to the carbonyl group of hydrazic acid.

¹H NMR spectra

The formation of imine group is established by the loss of signal due to the aldehyde group of azophenyl derivative at 11.43 in the ¹H NMR spectra [6] and the appearance of the signal at 8.83 and 8.30 for **I** and **II**, depending on the substituent attached to the imine nitrogen atom, respectively. The OH proton signals appear as a singlet for **I** at δ = 11.87 and **II** at 10.42 and 9.27.

The aromatic proton signals of phenyl rings of compound **I** appear two multiples and one singlet at δ = 8.38–8.43, 8.084 and 7.88, respectively. Also, the proton signals of pyridine ring of ligand **I** appear as two doublets at δ = 7.99 and 7.18. The peak appearing at δ = 12.43 corresponds to the hydrogen atom of the amide group of isonicotinic hydrazic acid derivative. Finally the aromatic proton signals of phenyl rings of compound **II** appear as two multiples, one doublet and one singlet at the range of δ = 6.99–8.42 and the protons of methyl group indicate at δ = 2.51 as one singlet.

UV-vis experiments

The absorption bands of azo compounds in UV–vis spectra are usually revealed at 300–500 nm [13,24]. These compounds show two broad absorption peaks, depending on $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions of azo groups (–N=N–). The UV–vis spectra of azo Schiff-base ligands I and II have been recorded in DMSO solutions (Figs. 2 and 3). Schiff-base ligand I shows a strong absorption band at $\lambda = 260$ nm corresponding to the $\pi \rightarrow \pi^*$ transition of aromatic rings, a broad and strong absorption band at $\lambda = 326$ attributable to the $n \rightarrow \pi^*$ transition of imine group [4] and board absorption

bands at $\lambda = 396$ and about 555 nm, corresponding to the $\pi \to \pi^*$ and $n \to \pi^*$ transitions of azo groups, respectively [13]. Azo Schiff-base derivative **II** shows one absorption peak at $\lambda = 263$ nm, apparently corresponding to the $\pi \to \pi^*$ transition of aromatic rings of ligand frame, an absorption band at $\lambda = 296$ nm, attributable to the $\pi \to \pi^*$ or $n \to \pi^*$ transition of imine group [4] and two strong and broad absorption bands at $\lambda = 386$ and 511 nm, corresponding to the $\pi \to \pi^*$ and $n \to \pi^*$ transitions of azo groups.

Complexes formed in the solution

Complexes with ligand I

The binding ability of compound I for some heavy metal cations is investigated by UV–vis absorbance method. Absorption spectra of 0.025 mM ligand I in the presence 0.025 mM of metal ions (Cu²⁺, Cd²⁺, Co²⁺, Pb²⁺, Ni²⁺, Zn²⁺ and Hg²⁺) are shown in Fig. 2. In fact, the absorbance spectra show remarkable changes after adding metal ions. In all cases, $n \rightarrow \pi^*$ transition of azo groups in ligand at λ = 555 nm increases in intensity (absorbance intensity for these metal ions is increased as follow Cd²⁺ > Co²⁺ ~ Cu²⁺ > Ni²⁺ > Pb²⁺ ~ Zn²⁺ > Hg²⁺) and the bands at λ = 396 nm and 326 nm, assigned



Fig. 2. UV-vis spectra of I (0.025 Mm) before and after adding a 0.025 Mm concentration of various metal acetates in a DMSO solution.



Fig. 3. UV-vis spectra of II (0.01 Mm) before and after adding a 0.01 Mm concentration of various metal acetates in a DMSO solution.

to the $\pi \to \pi^*$ and $n \to \pi^*$ transitions of azo and imine groups strongly decrease in intensity. Complexation of these metal ions for compound I probably affects the equilibrium of tautomeric forms and there is likely a subtle balance between metal complexation-induced release of protons from the azophenols to the quinone-hydrazone in DMSO solution [25–27]. Also, decreasing of absorption band at 326 nm and its shift to longer wavelengths can be related to the donation of the lone pairs of the nitrogen atom of imine group to the metal ion [3]. When compound I treats with Cu²⁺ ion, the largest blue shift from $n \to \pi^*$ transition at 555 nm to 460 nm (–95 nm) is obtained. It can be seen as color change of solution from red to yellow (in all case the color of solution is almost red except Cu²⁺).

Complexes with ligand II

The binding ability of compound II for some heavy metal cations is investigated by UV-vis absorbance method. Absorption spectra of 0.01 mM ligand II in the presence 0.01 mM of metal ions $(Cu^{2+}, Cd^{2+}, Co^{2+}, Pb^{2+}, Zn^{2+} and Hg^{2+})$ are shown in Fig. 3. In general. $n \rightarrow \pi^*$ transition of azo groups of ligand decreases in intensity after adding metal ions (except Co^{2+} ion). The bands at $\lambda = 386$ and 296 nm, assigned to the $\pi \to \pi^*$ and $\pi \to \pi^*$ or $n \to \pi^*$ transitions of azo and imine groups dramatically decrease in intensity (the band at 296 nm completely disappears after adding 1 equivalent of Cu²⁺ and Co²⁺ ions). As aforementioned about ligand I, it can be related to the existence a subtle balance between metal complexationinduced release of protons from the azophenols to the quinonehydrazone in DMSO solution and coordination of nitrogen atom of imine group to the metal ion center. Finally, the band at λ = 260 nm increases in intensity after adding one equivalent of Cu²⁺, Co²⁺ and Pb ²⁺ ions ($\pi \rightarrow \pi^*$ transitions are seen as sharp and strong peaks).

UV-vis titrations

UV–vis spectra, obtained upon the addition of copper ions to the solution of azo Schiff-base derivative I have been presented in Fig. 4. It can be seen that the absorbance band at λ = 555 nm shifts to the shorter wavelengths, so that after adding one equivalent of copper ion, the largest shift is obtained (–95 nm). This is observed as color change from red to yellow with the addition of concentration of cation. The absorption band at λ = 460 nm increases in intensity with the addition of concentration of cation while the bands at λ = 396 and 326 nm, assigned to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition of azo and imine groups of ligand frame decrease in



Fig. 4. Changes in the UV-vis spectra of I (0.03 Mm) upon titration by Cu(CH₃COO)₂ in a DMSO solution, where the concentration of Cu(CH₃COO)₂ varies from 0.006 to 0.3 mM.

intensity. It can be attributed to the formation of complexes with copper ions. The band at 460 nm gradually increases in intensity vice versa the bands at 396 and 326 nm strongly and continually decrease with the extra addition of copper ions to the ligand solution in DMSO. The spectra features in Fig. 4 are consistent with a 1:1 ratio between azo derivative I and Cu^{2+} ion. Further support of the 1:1 binding ratio comes from Job's plot experiment, where the absorptions of the complex at 460 nm is plotted against molar fractions of ligand under the conditions of an invariant total concentration. As a result, the concentration of I. Cu^{2+} complex approaches a maximum when the molar fraction of [I]/ ([I] + [Cu^{2+}]) is about 0.5 (Fig. 5).

Among studied cations, especial remarkable changes are only observed in the absorbance spectra and colors of ligand solutions with different equivalents of Cu^{2+} ion (the largest blue shift ($\Delta \lambda = -95$ nm)). Therefore, 1:1 association constant of I with Cu^{2+} is determined on the base of Benesi–Hildebrand plot at $\lambda = 460$ nm. It is revealed that the stability constant azo Schiff-base derivative I with copper is $K_a = 2.033 \times 10^4$ M⁻¹.

DPPH radical scavenging activity of ligands I and II

The nitrogen centered stable free radical 1,1-diphenyl-2-pic-rylhydrazyl (DPPH) has often been used to characterize



Fig. 5. Jop plot for I and Cu^{2+} , where the absorption at 460 nm was plotted against the mole fraction of I at an invariant total concentration of 0.3×10^{-4} M in DMSO.

antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at $\lambda = 517$ nm, which is purple in color. This property makes it suitable for spectrophotometer studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts it into 1,1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties. In this study, antioxidant activities of azo-Schiff base derivatives I and II were determined by using DPPH method. DPPH radical scavenging values of compounds I and II at different concentrations are recorded. Both compounds show good antioxidant activity against DPPH method especially II (over than 72% for 40 µg/ml) (Suppl: Fig.3).

There are two postulated mechanisms for the reaction of compound **II** as an antioxidant [28]. The first mechanism depends on the benzyl hydrogen atom, where this atom is under the influence of two effects, namely resonance and inductive. The resonance effect of benzyl hydrogen makes the release of hydrogen as a free radical easy while the inductive effect on benzene ring pushes the electrons towards a carbon free radical, resulting in the molecule becoming stable. The second postulated mechanism fellows the route of the keto–enol form. For compound **I**, the two suggested mechanisms depend on the keto–enol forms (Suppl:S2-S4).

Conclusion

Two new azo Schiff-base derivatives I and II have been synthesized and characterized with several techniques. The study of UVvis spectra of compounds I and II with Ni²⁺, Co²⁺, Cu²⁺, Pb²⁺, Hg²⁺, Zn²⁺ and Cd²⁺ metal ions show remarkable changes in the intensity of the $\pi \to \pi^*$ and $n \to \pi^*$ transitions, assigned to the azo and imine units. Especial changes in the UV-vis spectra are observed with the addition of copper ion to the DMSO solution of receptor I. The largest blue shift is obtained. Therefore ligand I can be used as visible chemosensor owing to the noticeable color change in the presence of Cu²⁺ ions. Upon the appearance of new absorption band and increasing of this band in intensity with the addition of concentration of Cu²⁺, 1:1 binding-stoichiometry is determined from Job plot. Association constant I with Cu²⁺ obtained on the base of Benesi-Hildebrand plot. Significantly, the selectivity of receptor I for Cu²⁺ over other tested metal ions is extremely high. Finally, it is revealed I and II have high antioxidant activity against DPPH method.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2014.04.026.

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