Tetrahedron 68 (2012) 7450–7455

Contents lists available at SciVerse ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

The effect of substituents on the response of 3,4-dihydro-3-(2-oxo-2-phenylethylidene)-quinoxalin-2(1*H*)-one derivatives toward binding of Cu^{2+}

Efrat Korin [a], Beny Cohen [a,*], Yue-Xia Bai [b], Cheng-Chu Zeng [b,*], James Y. Becker [a,*]

[a] *Department of Chemistry, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel*
[b] *College of Life Science & Bioengineering, Beijing University of Technology, Beijing 100124, China*

ARTICLE INFO

Article history:
Received 4 March 2012
Received in revised form 30 May 2012
Accepted 18 June 2012
Available online 26 June 2012

Keywords:
Quinoxalinone dérivatives
Cu^{2+} recognition
Fluorescence
Chelating reagent
Chemosensors

ABSTRACT

A new series of fluorogenic chelating reagents based on phenylethylidene-3,4-dihydro-1*H*-quinoxalin-2-one with different substituents (attached either to the quinoxaline-2-one (**3**) or phenyl ring (**4**)) have been investigated to examine the effect of the substituent (nature/position) on the spectral properties and response toward Cu^{2+} in the presence of other metal cations, in ethanol. It was found that all of the examined ligands exhibit a pronounced response to Cu^{2+} addition resulting in a red-shift in the UV–vis spectra and a strong quenching in the fluorescence spectra. Among the ligands examined, **3a** exhibits the highest selectivity toward various metal cations. In general it appears that the best response selectivity of these ligands toward Cu^{2+} ion is obtained by either EDG in **3** or EWG in **4**. For example, the fluorescence intensity of **3a** (with OMe substituent) increases to about three times that of the unsubstituted derivative.

1. Introduction

The design of chelating ligands capable of selective complexation with a specific metal ion has been an important goal due to their potential biological and pharmacological application. Over the years, numerous chelating reagents for different metal ions have been designed for various applications such as therapeutic reagents for the treatment of metal intoxication,[1–3] selective metal extractants,[1,4] and chemosensors.[5–8]

In recent years, considerable effort has been devoted to the development of selective chemosensors for essential trace metal such as, Zn^{2+},[9–11] Cu^{2+},[12–15] Ni^{2+},[16–18] and Co^{2+}.[18,19] All of these metal ions are important for certain biological processes but can become toxic when consumed in uncontrolled amounts. For instance, elevated levels of Zn^{2+}, Cu^{2+} or Co^{2+} are associated with neurodegenerative diseases (such as, Alzheimer and Parkinson).[20–22] In addition, elevated levels of Cu^{2+}, Co^{2+} or Ni^{2+} ions are suspected to participate in the generation of cancer.[22–24]

Among the above-mentioned metal ions, chemosensors designed for the detection and measurements of Cu^{2+} are of great interest due to its involvement in various diseases that are considered of current major importance: Alzheimer, Parkinson, Amyotrophic lateral sclerosis (ALS), Huntington, Prion, cancer, diabetes, cardiovascular diseases, and atherosclerosis.[25,26]

Previously we reported a series of organic fluorescent compounds based on *para*- and *meta*-sulfonamido-3,4-dihydro-3-(2-oxo-2-phenylethylidene)-quinoxalin-2(1*H*)-one derivatives (**1**) as potential chemosensors for cations of biological interest such as, Ca^{2+}, Mg^{2+}, Cu^{2+}, and Zn^{2+}, in both ethanol and acetonitrile.[27] In acetonitrile, derivatives **1** act as dosimeters as their oxidative decomposition by Cu^{2+} resulted in a significant color change. It should be noted that this particular behavior was exclusive for Cu^{2+} allowing its detection even in the presence of other metal cations. In ethanol, derivatives **1** show a selective binding toward Cu^{2+} as was expressed by a red-shift in the UV–vis absorption spectra and a specific fluorescence quenching behavior. In this respect, no significant response to Zn^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ca^{2+}, and Ag^{+} was observed. The changes in the UV–vis and fluorescence spectra that were observed in the presence of Cu^{2+} were attributed to the reversible formation of a metal–ligand complex.

The role of the sulfonamide moiety and the presence of two potential binding sites in **1** have not been clear in the above-mentioned results. Therefore, the present work has been focused on the synthesis and chemical modifications of **1** to simpler derivatives such as, the unsubstituted building block **2** and substituted derivatives of **3** and **4** (Fig. 1).

* Corresponding authors. Tel.: +972 8 646 1639; fax: +972-8-6472943 (B.C.); tel.: +86 10 67396211; fax: +86 10 67392001 (C.-C.Z.); tel.: +972 8 6461197; fax: +972 8 6472943 (J.Y.B.); e-mail addresses: bcohen@bgu.ac.il (B. Cohen), zengcc@bjut.edu.cn (C.-C. Zeng), becker@bgu.ac.il (J.Y. Becker).

0040-4020/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tet.2012.06.071

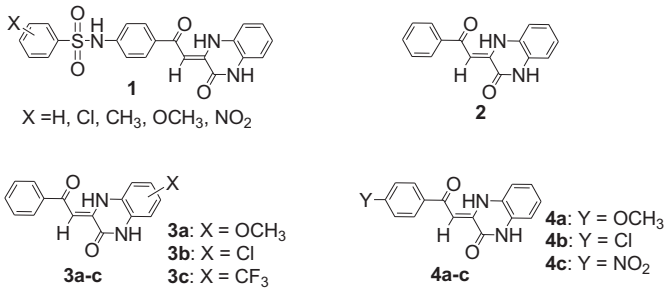


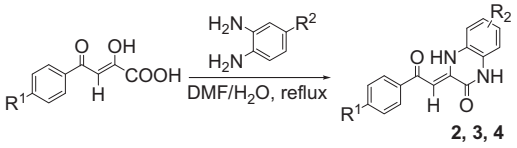
Fig. 1. Chemical structures of 3,4-dihydro-1*H*-quinoxalin-2-ones **1**–**4**.

Our main goal has been to examine the effect of different substituents on the quinoxalin-2-one moiety **3**, and at the *para*-position of the phenyl ring in **4**, on their spectral properties and response toward Cu^{2+} in ethanol, and in the presence of other metal cations.

2. Results and discussion

2.1. Synthesis of 2–4

The unsubstituted 3,4-dihydro-2-quinoxalin-2-one derivative (**2**) and the substituted series **3** and **4** were synthesized directly by condensation of the corresponding diketo acid and *o*-phenylenediamine. However, when the reaction was performed in water, as the synthesis of **1**, a complex mixture of products, including the starting diketo acid, was obtained. Thereby, a mixture of diketo acid and *o*-phenylenediamine was refluxed in DMF/H_2O to give the desired 3,4-dihydro-2-quinoxalin-2-one derivatives **2**, **3**, and **4** in good to excellent yields (Scheme 1). High quality samples could be obtained after recrystallization from DMF.



Scheme 1. Synthesis of 3,4-dihydro-1*H*-quinoxalin-2-ones **2**, **3**, and **4**.

2.2. UV–vis and fluorescence spectra of 3,4-dihydro-1*H*-quinoxalin-2-one derivatives 2–4

2.2.1. UV–vis measurements. The absorption spectra of the building block **2** and its substituted derivatives (**3** and **4**) in ethanol solutions were investigated and their spectra are shown in Fig. 2(I) and (II), respectively. The absorption spectrum of **2** in ethanol displays a shoulder band and two intense absorption bands centered at about 393 nm (ε=2.2×10^4 M^{-1} cm^{-1}), 417 nm (ε=3.4×10^4 M^{-1} cm^{-1}), and 438 nm (ε=3.0×10^4 M^{-1} cm^{-1}),[28] respectively. These intense absorptions in the visible region can be assigned to a π–π^* transition (K band) that occurs in this conjugated system. Similar absorption spectral patterns were also observed for all other derivatives of **3** and **4**, except for **4c**, which showed only one broad absorption band around 441 nm. It can be seen from the spectral data listed in Table 1 that the absorption values are influenced by the substituent.

In the case of derivatives of **3**, it appears that the nature of the substituent affects mainly the spectral band position with no significant change in the absorbance intensity. As can be seen from Fig. 2(I) and Table 1, derivatives of **3** with electron-withdrawing groups (EWG) (e.g., **3c** with, CF_3) shift the spectrum to shorter wavelengths (~10 nm hypschromic blue-shift) relative to the unsubstituted compound **2**, whereas derivatives of **3** substituted with electron-donating groups (EDG) (e.g., **3a** with OCH_3) shift the spectrum to longer wavelengths (~19 nm red-shift) in comparison to **2**. On the other hand, examining the effect of the substituent in derivatives of **4** on their spectra reveals that all types of substituents (either EWG or EDG) caused a red-shift compared to the unsubstituted derivative (**2**), and in particular, the NO_2 substituted derivative **4c**, which caused the most significant red-shift of ~26 nm (Table 1). Furthermore, in the derivatives of **4**, the nature of the substituent also seems to have an effect on the intensity of the absorption, where the derivative substituted with Cl had the lowest absorbance intensity (ε=0.38×10^4 M^{-1} cm^{-1} at 418 nm) in comparison to **4a** and **4c** derivatives (with ε ~3×10^4 M^{-1} cm^{-1}).

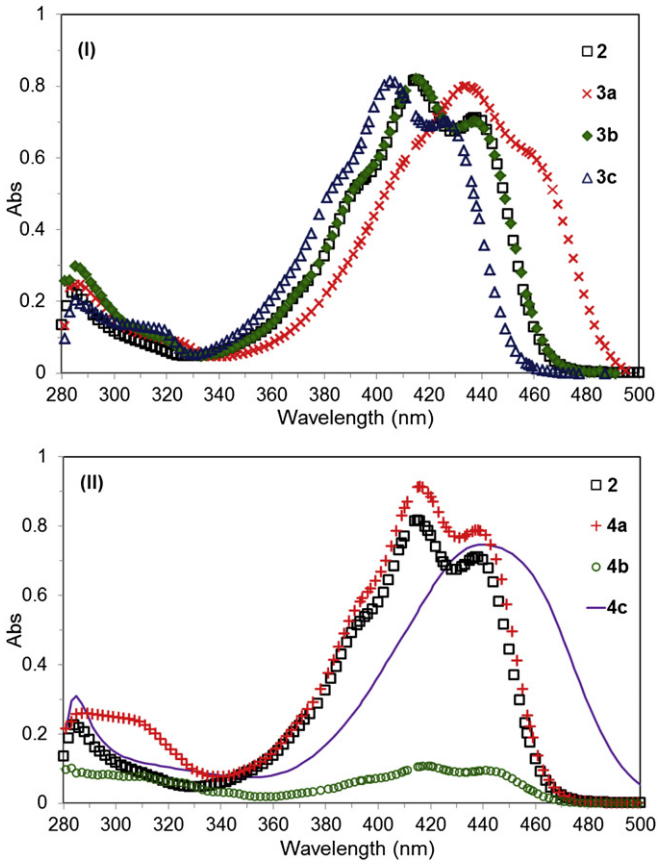


Fig. 2. Absorption spectra of 24 μM of **2** derivatives in ethanol (I) **2**, **3a**, **3b**, **3c**, (II) **2**, **4a**, **4b**, **4c**.

Table 1
Absorption and fluorescence properties of free **2** and its substituted derivatives (**3** and **4**) and the $\Delta\lambda_{max}$ of the red-shift observed in the UV–vis spectra due to addition of 100 equiv of either Cu^{2+}, Ni^{2+} or Zn^{2+}, in ethanol

'Host'	λ_{max}[a,b] (nm)	λ_{em} (nm)	F_{rel}	$\Delta\lambda_{max}$ 'host'+Cu^{2+}	$\Delta\lambda_{max}$ 'host'+Ni^{2+}	$\Delta\lambda_{max}$ 'host'+Zn^{2+}
2	~393, *415*, 438	471, 491	1.00	25	32	29
3a	~410, *434*, 458	503	3.16	17	—	4
3b	~393, *415*, 437	471, 490	1.19	29	36	31
3c	~383, *405*,426	459, 480	0.53	34	42	37
4a	~393, *417*, 438	471, 491	1.40	30	34	30
4b	393, *418*, 441	476, 491	0.27	26	—	—
4c	*441*	530	0.07	22	9	6

[a] Maximum absorption wavelength of free host (24 μM) at room temperature in ethanol; (λ_{max} ±0.5 nm).
[b] Underlined wavelengths are the peaks with the strongest absorption.

2.2.2. fluorescence measurements. The effect of substituents in the **3** and **4** series on the fluorescence spectra in ethanol was also examined. The emission spectra of unsubstituted derivative **2** (λ_{ex}=415 nm) have two bands around 471 and 491 nm (Fig. 3). As can be seen from Fig. 3 and Table 1 the fluorescence spectral properties of substituted **2** are also affected by the nature of the substituent. In both **3** and **4** series, the highest fluorescence intensity was observed for the derivative that was substituted with a strong EDG (OCH_3), while the derivative that was substituted with strong EWG (such as CF_3 and NO_2) had the lowest fluorescence intensity.

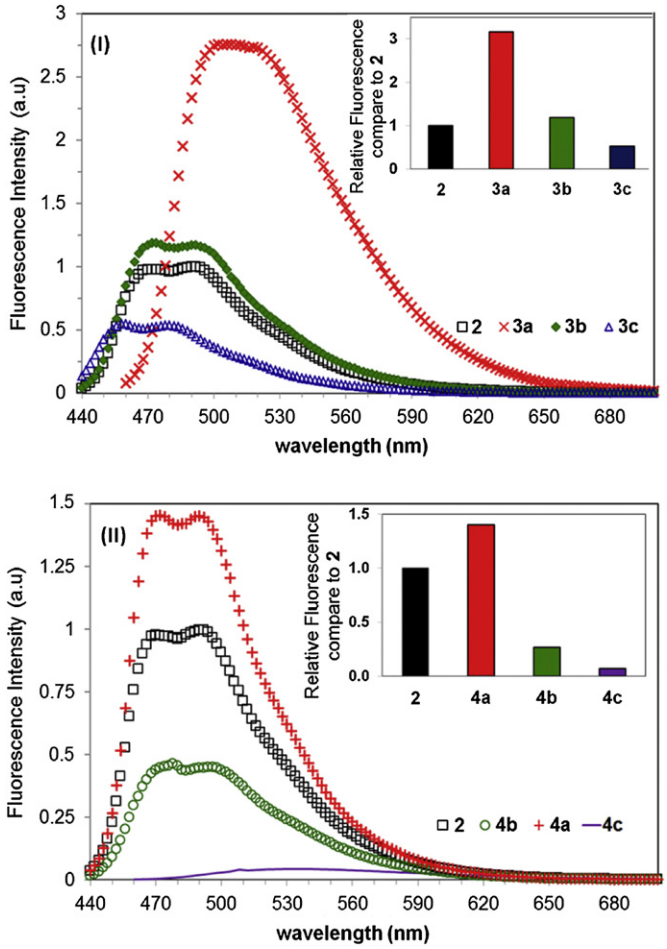


Fig. 3. (I) The emission spectra of 24 μM **2** (ex 415 nm), **3a** (ex 434 nm), **3b** (ex 415 nm), and **3c** (ex 405 nm) in ethanol. Inset: the relative fluorescence intensity of each of the investigated compounds compared to **2**. From left to right **2** (ex 415 nm), **3a** (ex 434 nm), **3b** (ex 415 nm) and **3c** (ex 405 nm) in ethanol. (II) The emission spectra of 24 μM **2** (ex 415 nm), **4a** (ex 417 nm), **4b** (ex 418 nm), and **4c** (ex 441 nm) in ethanol. Inset: the relative fluorescence intensity of each of the investigated compounds compared to **2**. From left to right **2** (ex 415 nm), **4a** (ex 417 nm), **4b** (ex 418 nm), and **4c** (ex 441 nm) in ethanol.

Examining the effect of substituent on the position of λ_{em} reveals that different trends appear for the **3** and **4** derivatives. When **3** substituted with EWG (CF_3) caused a slight blue-shift compared to **2**, while EDG caused a red-shift. On the other hand the emission spectra of all **4** derivatives exhibited red-shifts compared to **2** and the red-shift increased upon changing the substituent from EDG to EWG.

Therefore it appears that the spectral properties of **2** analogs can be tuned by modifying the nature of the substituent that is attached on either the quinoxaline-2-one or phenyl ring. This information may be used to tailor-design the spectral properties of the **2** analogs in terms of absorbance and emission maxima including fluorescence and/or absorbance intensity.

2.3. Spectral response of 3,4-dihydro-1*H*-quinoxalin-2-one derivatives 3 and 4 toward different metal ions

2.3.1. UV–vis measurements. The ability of **3** and **4** derivatives to form complexes in ethanol with different metal ions such as Zn^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ca^{2+}, Ag^{+} or Cu^{2+} was first studied by using UV–vis spectroscopy. The spectra obtained for **2** in the absence and presence of the above cations showed that addition of 100 equiv of either Cu^{2+}, Zn^{2+} or Ni^{2+} induced a spectral response. The addition of Zn^{2+} or Ni^{2+} resulted in the appearance of new peaks around 465 and 470 nm, respectively. However the addition of Cu^{2+} caused the spectra of **2** to shift to longer wavelengths (~29 nm red-shift), (see Fig. 4(I) and Table 1). It is important to note that the new band around 463 nm created by the shift after Cu^{2+} addition was 6 and 4 times more intense than the new peaks that appeared on the addition of Ni^{2+} or Zn^{2+}. Therefore among the cations examined, the most prominent response of the spectrum of **2** in ethanol was created by the addition of Cu^{2+} ions. It should be noted that spectral changes observed upon addition of Cu^{2+}, Ni^{2+} or Zn^{2+} were fully reversible, since the addition of either EDTA or $HClO_4$ (see Supplementary data, Figs. S1–S3) led to an instant return of the original absorption of the free complexing agent. Thus the UV–vis spectral response observed between **2** and Cu^{2+}/Ni^{2+}/Zn^{2+} could be assigned to the formation of a reversible complex in which the organic ligand undergoes deprotonation upon complexation.

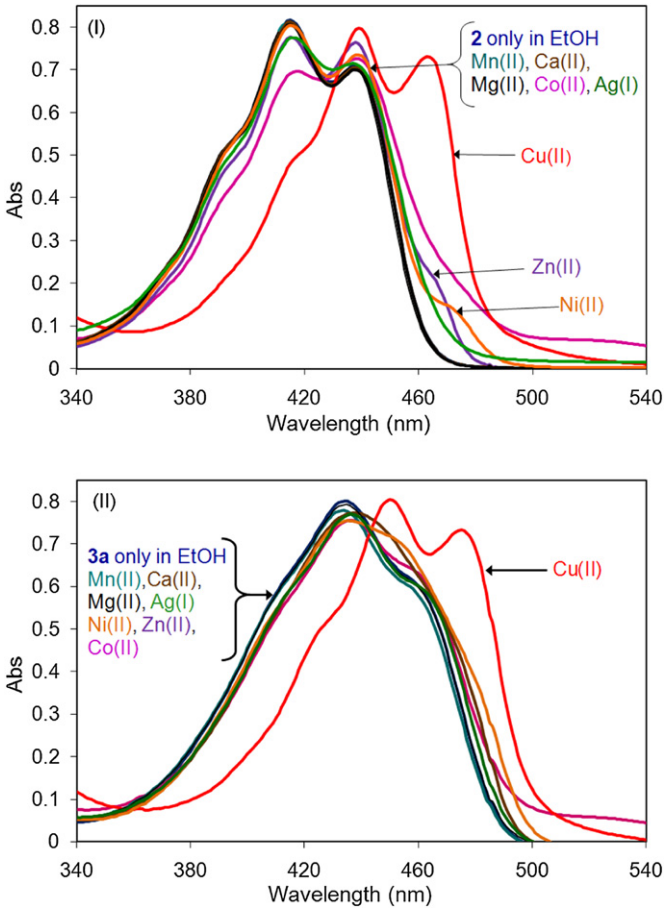


Fig. 4. UV–vis absorption spectra of the host in the absence and presence of 100 equiv of metal ions in ethanol: (I) 24 μM of **2**, (II) 24 μM of **3a**.

Furthermore, competition experiments of added Cu^{2+} to **2** solutions in the presence of one another metal ions (Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Zn^{2+} or Ag^{+}) indicated that the recognition of

Cu^{2+} by **2** was not significantly affected by either of these co-existing cations, because no change was observed in the red-shifted absorption band (except for a slight change in its intensity, see Supplementary data, Fig. S4).

A similar spectral response to that of **2** was also observed for **3b**, **3c**, **4a** absorbance spectra in the presence of 100 equiv of either: Zn^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ca^{2+} or Ag^{+}. Unlike the latter mentioned compounds, the response of **3a** toward these cations was more selective, and only the addition of 100 equiv of Cu^{2+} ions caused a significant change in the shape and position of the spectra (Fig. 4(II)). Comparing the response of the different derivatives of **3** to Cu^{2+} suggests that the nature of substituent on the quinoxaline-2-one effects the selective response toward Cu^{2+}, as switching the substituent from OCH_3 to CF_3 increases the effect of other cations (such as Zn^{2+}, Ni^{2+}, and Ag^{+}) on the spectra (in respect of intensities red-shift position and shape of the spectra).

Moreover, comparing the effect of substituent on the response toward Cu^{2+} addition reveal that for compounds from **3** series, an EWG substituent increased the red-shift while an EDG substituent decreased the red-shift observed upon Cu^{2+} addition.

Examining the response of all compounds in the **4** series to Cu^{2+} addition reveals that for all of them the spectra of the free organic compound was red-shifted upon treatment with Cu^{2+} (See Table 1). Unfortunately the absorbance spectra of **4b** and **4c** from the **4** series present some difficulty in the assessment of the effect of different metal ions on their absorbance spectra as **4b** has very low absorbance while the spectrum of **4c** overlaps with the free cation (such as, Ni^{2+} or Co^{2+}). Therefore substantial interference of free cations absorbencies is expected for these two derivatives.

In contrast to the behavior of **4b** and **4c**, the response of **4a** toward metal ions could be readily examined. The spectra of **4a** were affected by the addition of either Cu^{2+}, Ni^{2+} or Zn^{2+} in a similar manner as **2**. This shows that switching the location of OCH_3 substituent from the quinoxaline-2-one to the benzyl ring decreases the selectivity of the response.

2.3.2. Fluorescence measurements. Fluorescence spectroscopic measurements were also employed to study the cation recognition properties of **2** and its derivatives toward different metal ions. As shown in Fig. 5, the emission spectra of **2** (λ_{ex}=463 nm) in the presence of 100 equiv of metal ions such Mn^{2+}, Ca^{2+}, and Mg^{2+} did not cause any change in either position or intensity, while the addition of either Zn^{2+} or Ag^{+} results in a red-shift accompanied by significant increase in fluorescence intensity (that was doubled or quadrupled, respectively, from that of the free **2**). On the other hand, the addition of cations such as Co^{2+}, Ni^{2+}, and Cu^{2+} results in a strong fluorescence quenching. These changes in fluorescence intensity can be attributed to the nature of the cation where paramagnetic cations are known to cause a fluorescent quenching while diamagnetic transition metal ions (Zn^{2+} or Ag^{+}) can cause fluorescence enhancement.[29–31] It should be noted that the recognition of either Zn^{2+}, Ni^{2+}, Cu^{2+} or Ag^{+} was found to be reversible as the addition of $HClO_4$ reverses the fluorescence spectra back to that of free **2**. Acid addition should cause the formed complexes to dissociate back to the free ligand and the metal ion, which in turn expressed by the retrieval of the fluorescence signal of the free **2** (see Supplementary data, Fig. S5).

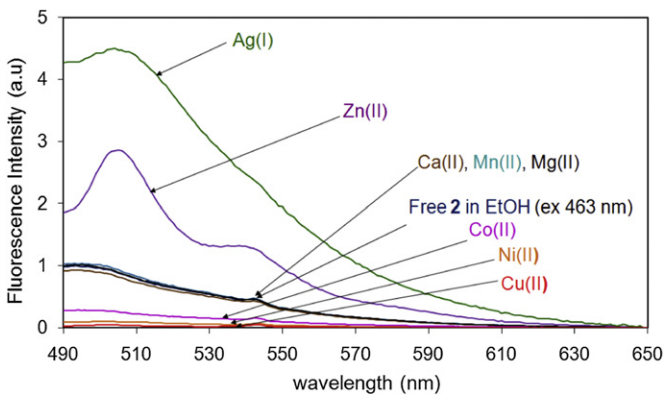


Fig. 5. Fluorescent emission spectra of **2** (24 μM) in the absence and presence of Cu^{2+}, Zn^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ca^{2+} or Ag^{+} (100 equiv) in ethanol (λ_{ex}=463 nm).

The effect of co-existing cation on the response of **2** toward copper was also investigated by measuring the emission spectra collected with Cu^{2+} in the presence 100 equiv of either of the following cations: Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Zn^{2+} or Ag^{+}. As can be seen from Fig. 6, the presence of Cu^{2+} and another metal ion had no significant interference with the quenching created by Cu^{2+} ions. Therefore the effect of Cu^{2+} ions prevails on that of other co-existing cations.

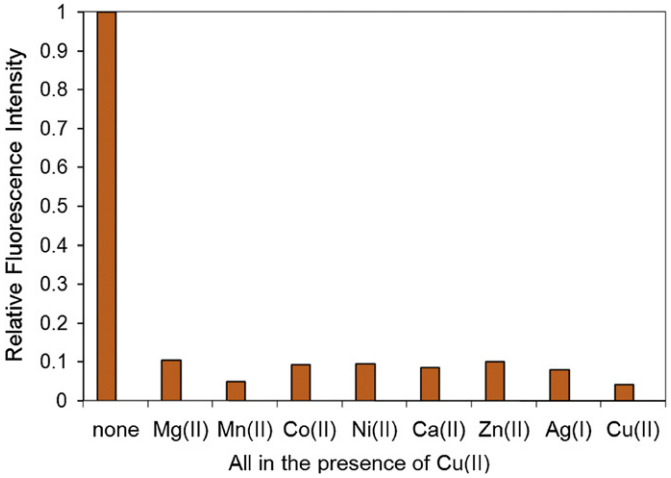


Fig. 6. Relative fluorescence intensity (F/F_0) of **2** in the presence of 100 equiv Cu^{2+} and 100 equiv of one more metal ion (either Cu^{2+}, Zn^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ca^{2+} or Ag^{+}) in ethanol.

The fluorescence emission intensity ratios (F/F_0) of different **3** and **4** derivatives in the presence and absence of 100 equiv of either Zn^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ca^{2+}, Ag^{+} or Cu^{2+} are presented in Fig. 7(I) and (II), respectively. As can be seen from Fig. 7(I) in the **3** series the two derivatives substituted with an EWG (**3b** and **3c**) behave in a similar manner toward these metal ions. For the derivative **3c**, substituted with a strong EWG (CF_3) each one of the metal ions that was added appears to affect the emission spectra (even Mg^{2+}, Mn^{2+}, Ca^{2+} induced a decrease in intensity). On the other hand **3a** with EDG, OCH_3 substituent, exhibits a more selective recognition where only the addition of 100 equiv of Co^{2+}, Ni^{2+} or Cu^{2+} induced changes on the fluorescence intensity ratio. Nevertheless it is important to note that the fluorescent enhancement observed for **3c** upon addition of 100 equiv of Zn^{2+} or Ag^{+} was the highest compared to the other complexing agents. Therefore among the **3** series, derivatives substituted with EWG groups appear to enhance the response toward the different metal ions while EDG decrease their effect resulting in a more selective compound for Cu^{2+}. Quite the opposite effect was observed for derivatives in the **4** series (Fig. 7(II)), where the fluorescence response was less selective to Cu^{2+} for compounds substituted with EDG then those with EWG.

Based on the UV–vis and more specifically the fluorescence spectra, it can be concluded that the response of **2** toward metal ions is influenced both by the nature of the substituent (EDG/EWG) and the position of substituent on the ring. This observation

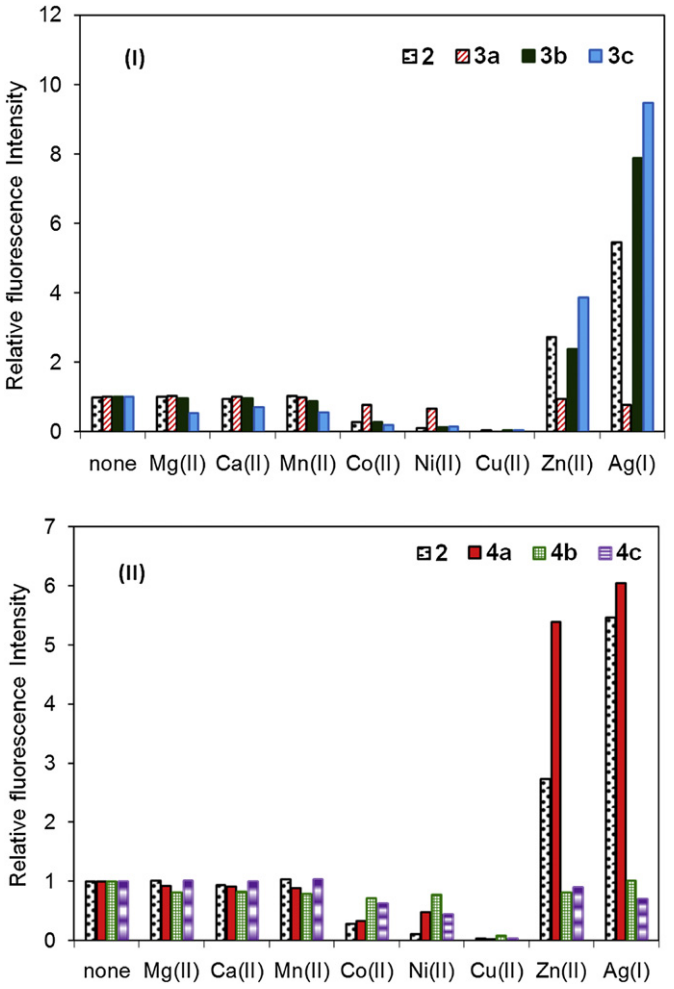


Fig. 7. Relative fluorescence intensities of **2**, **3**, and **4** derivatives (24 μM) in the absence and presence of 100 equiv Cu^{2+}, Zn^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ca^{2+} or Ag^{+} in ethanol (I) **2** (ex 463 nm), **3a** (ex 475 nm), **3b** (ex 466 nm), and **3c** (ex 460 nm), (II) **2** (ex 463 nm), **4a** (ex 468 nm), **4b** (ex 467 nm), and **4c** (ex 466 nm).

suggests that the selective response of **2** to Cu^{2+} can be optimized by simply tuning the EDG ability attached to the quinoxaline-2-one ring or the EWG ability attached to the phenyl ring.

3. Summary and conclusions

In conclusion, 3,4-dihydro-2-quinoxalin-2-one derivatives having EDG and EWG on either the quinoxaline-2-one (**3**) or phenyl ring (**4**) were synthesized and their effect on their spectral properties and response toward various metal ions was examined in ethanol. All of the investigated ligands derivatives posses the ability to reversibly bind to Cu^{2+} causing a red-shift in their absorption spectra and 'ON–OFF' fluorescence response. Using their fluorescence spectra it was shown that the recognition selectivity to Cu^{2+} ion can be finely tuned by the position and nature of the substituent, where either EDG in **3** or EWG in **4** enhanced the response selectivity toward Cu^{2+}. Furthermore, since the fluorescence intensity of these compounds was increased by using EDG (on either **3** or **4**) it seems that for chemosensors applications it will be better to design derivatives where the selectivity would be adjusted via EDG substituent on the quinoxaline-2-one. Here **3a** had the highest fluorescence intensity and exhibits the best selectivity to Cu^{2+}, as its fluorescence response to different metal ions was mainly affected by Cu^{2+}, Ni^{2}, and Co^{2+}. Finally, compounds **2**, **4a**, and **3b** were indifferent to the presence of Mg^{2+} and Ca^{2+} ions but appear to bind metal ions with neurological importance (Zn^{2+}, Cu^{2+}, Co^{2+}, and Ni^{2+}). Therefore, they could be good building blocks for the design of a new ligand for chelating therapy such as Clioquinol (a ligand that is able to bind the same metal ions used clinically for the treatment of Alzheimer's disease and Huntington's disease).

4. Experimental section

4.1. General

Absorption spectra were acquired with an Agilent (HP) 8453 UV–Vis Diode Array spectrophotometer (single beam) with wavelength accuracy $<\pm 0.5$ nm and photometric accuracy $<\pm 0.005$ AU. Fluorescence excitation spectra were acquired with a JASCO spectrofluorometer (Model FP-6500, Jasco Intern. Co., Japan) equipped with a 150 W xenon lamp. Both excitation and emission slit widths were 3 nm. HRMS were performed by two instruments: GCT-MS Micromass (UK) for EI-TOF, and LTQ Orbitrap XL mass spectrophotometer (Thermo Scientific, Germany and USA).

4.2. Reagents and general procedure

The solutions of the metal ions were prepared from Cu^{2+}, Zn^{2+}, Mn^{2+}, Na^{+} as perchlorate salts and Mg^{2+}, Co^{2+}, Ni^{2+}, Ca^{2+}, Ag^{+} as nitrate salts (Caution: perchlorate salts are potentially explosive). All of the reagents were purchased from commercial suppliers (Aldrich; Merck). For testing the effect of pH, 70% $HClO_4$ (Mallinckrodt chemicals) was used and base solution was made from NaOH pellets (Frutarom). The solvent ethanol was obtained from BioLab, Israel. All of the chemicals mentioned in this work were of analytical grade and used without further purification.

*4.2.1. A general procedure for the synthesis of 3,4-dihydroquinoxalin-2-ones **2**, **3**, and **4**.* A mixture of diketo acid (1 mmol), *o*-phenylenediamine (1.2 mmol), and 60 mL mixed solvent (v_{DMF}/v_{H2O}=1:1) was refluxed under stirring for 2–4 h while monitoring the reaction by TLC. After the completion of the reaction, the mixture was cooled to room temperature and a large amount of water was added. The desired products were collected by filtration, washed with water and then with ether to remove residual diamine. High quality of products was obtained after recrystallization from DMF.

*4.2.1.1. (Z)-3-(2-Oxo-2-phenylethylidene)-3,4-dihydroquinoxalin-2-one (**2**).* Yield: 75%; mp: 277–278 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=6.82 (s, 1H, C*H*=C), 7.12–7.15 (m, 3H, $Ar_{quinoxalone}$–*H*), 7.53 (s, 1H, $Ar_{quinoxalone}$–*H*), 7.50–7.60 (m, 3H, Ar–*H*), 7.97–7.99 (m, 2H, Ar–*H*), 12.04 (s, 1H, N–*H*), 13.67 (s, 1H, N–*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ=89.6, 115.8, 117.1, 124.2, 124.5, 124.6, 127.2, 127.5, 129.2, 1, 139.1, 146.1, 156.2, 188.9; IR (KBr): ν=3437, 1628, 1376, 1264 cm^{-1}; ESI-MS: *m*/*z*, 262.5 (M^{+}–1). HRMS (EI-TOF) for $C_{16}H_{12}N_2O_2$: found (M)=264.0902 (calcd 264.0899).

*4.2.1.2. (Z)-3-(2-Oxo-2-phenylethylidene)-6-methoxy-3,4-dihydroquinoxalin-2-one and (Z)-3-(2-oxo-2-phenylethylidene)-7-methoxy-3,4-dihydroquinoxalin-2-one (**3a**).* Yield: 71%; mp: 225–227 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=3.77 and 3.78 (s, 3H, OCH_3), 6.72–7.99 (m, 9H, Ar–*H*), 11.90 and 12.03 (s, 1H, N–*H*), 13.06 and 14.01 (s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ=55.9, 56.1, 88.7, 89.8, 100.4, 101.5, 110.9, 111.8, 116.7, 119.0, 120.9, 125.3, 127.2, 127.4, 128.7, 128.7, 129.1, 129.2, 132.0, 132.4, 138.9, 139.2, 146.1, 146.3, 155.6, 156.2, 156.3, 156.9, 186.2, 188.8; IR (KBr): ν=3437, 1630, 1535, 1465, 1375, 1269 cm^{-1}; ESI-MS: *m*/*z*, 292.6 (M^{+}–1). HRMS for $C_{17}H_{14}N_2O_3$: found $(M+H)^{+}$=295.10788 (calcd 295.10772); found $(M+Na)^{+}$=317.08978 (calcd 317.08966).

*4.2.1.3. (Z)-3-(2-Oxo-2-phenylethylidene)-6-chloro-3,4-dihydroquinoxalin-2-one and (Z)-3-(2-oxo-2-phenylethylidene)-7-chloro-3,4-dihydroquinoxalin-2-one (**3b**).* Yield: 69%; mp: 281–285 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=6.83 and 6.85 (s, 1H, C*H*=C), 7.12–7.99 (m, 8H, Ar–*H*), 12.07 (s, 1H, N–*H*), 13.38 and 13.53 (s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ=90.1, 90.5, 115.1, 116.7, 117.1, 118.6, 123.7, 123.8, 123.9, 125.9, 126.1, 127.5, 127.6, 127.8, 127.9, 128.4, 129.2, 132.5, 132.6, 138.9, 145.4, 145.5, 156.1, 156.2, 188.9, 189.2; IR (KBr): ν=3436, 1690, 1620, 1540, 1462, 1400, 1368 cm^{-1}; ESI-MS: *m*/*z*, 296.6 (M^+–1). HRMS for $C_{16}H_{11}ClN_2O_2$: found $(M+H)^+$=299.05801 (calcd 299.05818); found $(M+Na)^+$=321.03964 (calcd 321.04013).

*4.2.1.4. (Z)-3-(2-Oxo-2-phenylethylidene)-6-(trifluoromethyl)-3,4-dihydroquinoxalin-2-one and (Z)-3-(2-oxo-2-phenylethylidene)-7-(trifluoromethyl)-3,4-dihydroquinoxalin-2-one (**3c**).* Yield: 73%; mp: 285–287 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=6.88 and 6.90 (s, 1H, C*H*=C), 7.26–8.10 (m, 8H, Ar–*H*), 12.14 and 12.24 (s, 1H, N–*H*), 13.39 (s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ=90.7, 91.2, 112.4, 116.3, 117.6, 120.7, 127.3, 127.6, 127.7, 127.9, 129.3, 132.8, 138.9, 145.2, 156.3, 156.6, 189.3, 189.9; IR (KBr): ν=3438, 1627, 1465, 1350, 1328, 1233 cm^{-1}; ESI-MS: *m*/*z*, 330.6 (M^+–1). HRMS for $C_{17}H_{11}F_3N_2O_2$: found $(M+H)^+$=333.08453 (calcd 333.08454); found $(M+Na)^+$=355.06647 (calcd 355.06648).

*4.2.1.5. (Z)-3-[2-Oxo-2-(4-methoxyphenylethylidene)]-3,4-dihydroquinoxalin-2-one (**4a**).* Yield: 70%; mp: 238–240 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=3.85 (s, 3H, O*CH*$_3$), 6.79 (s, 1H, C*H*=C), 7.06 (d, 2H, *J*=8.8 Hz, Ar–*H*), 7.11–7.14 (m, 3H, Ar$_{quinoxalone}$–*H*), 7.46–7.48 (m, 1H, Ar$_{quinoxalone}$–*H*), 7.97 (d, 2H, *J*=8.8 Hz, Ar–*H*), 11.98 (s, 1H, N–*H*), 13.59 (s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ=55.9, 89.4, 114.5, 115.8, 116.7, 124.1, 124.7, 127.0, 129.6, 131.8, 145.5, 156.3, 162.8, 188.2; IR (KBr): ν=3437, 1626, 1416, 1368, 1260 cm^{-1}; ESI-MS: *m*/*z*, 292.6 (M^+–1). HRMS (EI-TOF) for $C_{17}H_{14}N_2O_3$: found (M)=294.1009 (calcd 294.1004).

*4.2.1.6. (Z)-3-[2-Oxo-2-(4-chlorophenylethylidene)]-3,4-dihydroquinoxalin-2-one (**4b**).* Yield: 80%; mp: 274–276 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=6.80 (s, 1H, C*H*=C), 7.16 (s, 3H, Ar$_{quinoxalone}$–*H*), 7.55–7.71 (m, 1H, Ar$_{quinoxalone}$–H), 7.58 (d, 1H, *J*=8.4 Hz, Ar–*H*), 8.01 (d, 2H, *J*=8.4 Hz, Ar–*H*), 12.09 (s, 1H, N–*H*), 13.65 (s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ=89.4, 115.9, 117.2, 124.2, 124.7, 127.3, 129.1, 129.3, 132.0, 137.2, 137.8, 146.4, 156.1, 167.4, 187.4; IR (KBr): ν=3433, 1630, 1413 cm^{-1}; ESI-MS: *m*/*z*, 297.2 (M^+–1). HRMS (EI-TOF) for $C_{16}H_{11}N_2O_2Cl$: found (M)=298.0513 (calcd 298.0509).

*4.2.1.7. (Z)-3-[2-Oxo-2-(4-nitrophenylethylidene)]-3,4-dihydroquinoxalin-2-one (**4c**).* Yield: 74%; mp: 312–313 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ=6.86 (s, 1H, C*H*=C), 7.17–7.18 (m, 3H, Ar$_{quinoxalone}$–*H*), 7.58 (d, 1H, *J*=7.6 Hz, Ar–*H*), 8.21 (d, 2H, *J*=8.4 Hz, Ar–*H*), 8.34 (d, 2H, *J*=8.4 Hz, Ar–*H*), 12.16 (s, 1H, N–*H*), 13.81 (s, 1H, N*H*); ^{13}C NMR (100 MHz, DMSO-d_6): δ=89.4, 115.9, 117.6, 124.2, 124.4, 125.2, 127.6, 128.8, 144.2, 144.4, 147.2, 149.6, 155.8, 186.1; IR (KBr): ν=3430, 1624, 1413 cm^{-1}; ESI-MS: *m*/*z*, 308.1 (M^+–1). HRMS (EI-TOF) for $C_{16}H_{11}N_3O_4$: found (M)=309.0753 (calcd 309.0750).

4.3. Preparation of sample solutions for the evaluation of the photophysical properties of the ligands and their response to metallic cations

Stock solutions of ligands **2**, **3a**, **3b**, **3c**, **4a**, **4b**, and **4c** (2.4×10^{-5} M) in ethanol were prepared. Their UV–vis and fluorescence spectra were collected. To evaluate selectivity to specific metallic cation stock solutions of 1.2×10^{-1} M of Cu^{2+}, Zn^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ca^{2+} or Ag^+ were prepared in ethanol. Test samples were prepared by adding 80 μL of one of the mentioned metal stock to 4 mL of a host stock solution. For the competition experiment test solutions were prepared by adding 80 μL of the competing cations and 80 μL of Cu^{2+} to 4 mL of the **2** stock solution.

4.4. Evaluation of the effect of EDTA/acid ratio on the reversibility of the spectral response of 2 and its complexes

Stock solutions of 2.4×10^{-5} M of **2** in ethanol and 1.2×10^{-1} M of EDTA in tri-distilled water were prepared. To test the effect of EDTA on copper/nickel/zinc complexes with **2**, 80 μL of EDTA was added to 4 mL of **2** and 80 μL of one of these metallic cations (1.2×10^{-1} M). To test the acidity effect on these complexes 80 μL of 70% $HClO_4$ was added to a solution that contain 4 mL of **2** and 80 μL of one of these metallic cation (1.2×10^{-1} M) (Caution: $HClO_4$ is a strong oxidant).

Acknowledgements

C.-C.Z. thanks the National Basic Research Program of China (No. 2009CB930200), Beijing Natural Science Foundation (No. 7112008) and Beijing City Education Committee (KM201010005009). The authors are thankful to Mrs. E. Solomon for technical assistance. J.Y.B. is grateful to Mrs. Irene Evens for supporting this research.

Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2012.06.071. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Hancock, R. D.; Martell, A. E. *Chem. Rev.* **1989**, *89*, 1875–1914.
2. Flora, S. J. S.; Pachauri, V. *Int. J. Environ. Res. Public Health* **2010**, *7*, 2745–2788.
3. Andersen, O. *Chem. Rev.* **1999**, *99*, 2683–2710.
4. Ngeontae, W.; Aeungmaitrepirom, W.; Tuntulani, T.; Imyim, A. *Talanta* **2009**, *78*, 1004–1010.
5. Domaille, D. W.; Que, E. L.; Chang, C. J. *Nat. Chem. Biol.* **2008**, *4*, 168–175.
6. Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3–40.
7. Prodi, L.; Bolletta, F.; Montalti, M.; Zaccheroni, N. *Coord. Chem. Rev.* **2000**, *205*, 59–83.
8. Keefe, M. H.; Benkstein, K. D.; Hupp, J. T. *Chem. Rev.* **2000**, *205*, 201–228.
9. Xu, Z.; Yoon, J.; Spring, D. R. *Chem. Soc. Rev.* **2010**, *39*, 1996–2006.
10. Liu, Z. C.; Wang, B.-D.; Yang, Z.-Y.; Li, T.-R.; Li, Y. *Inorg. Chem. Commun.* **2010**, *13*, 606–608.
11. Li, H.-Y.; Gao, S.; Xi, Z. *Inorg. Chem. Commun.* **2009**, *12*, 300–303.
12. Wang, M. X.; Meng, X. M.; Zhu, M. Z.; Guo, Q. X. *Chin. Chem. Lett.* **2007**, *18*, 1403–1406.
13. Helal, A.; Rashid, M. H.; Choi, C.-H.; Kim, H.-S. *Tetrahedron* **2011**, *67*, 2794–2802.
14. Zhang, J.; Yu, C.; Qian, S.; Lu, G.; Chen, J. *Dyes Pigments* **2012**, *92*, 1370–1375.
15. Cátia, I. C.; Esteves, M.; Raposo, M. M.; Susana, P. G. *Tetrahedron* **2010**, *66*, 7479–7486.
16. Kumar, M.; Bhalla, V.; Dhir, A.; Babu, J. N. A. *Dalton Trans.* **2010**, *39*, 10116–10121.
17. Dodani, S. C.; He, Q.; Chang, C. J. *J. Am. Chem. Soc.* **2009**, *131*, 18020–18021.
18. Abebe, F. A.; Eribal, C. S.; Ramakrishna, G.; Sinn, E. *Tetrahedron Lett.* **2011**, *52*, 5554–5558.
19. Maity, D.; Govindaraju, T. *Inorg. Chem.* **2011**, *50*, 11282–11284.
20. Budimir, A. *Acta Pharm.* **2011**, *61*, 1–14.
21. Budimir, A.; Humbert, N.; Elhabiri, M.; Osinska, I.; Biruš, M.; Albrecht-Gary, A. M. *J. Inorg. Biochem.* **2011**, *105*, 490–496.
22. Matés, J. M.; Segura, J. A.; Alonso, F. J.; Márquez, J. *Free Radical Biol. Med.* **2010**, *49*, 1328–1341.
23. Valko, M.; Rhodes, C. J.; Moncola, J.; Izakovic, M.; Mazur, M. *Chem.-Biol. Interact.* **2006**, *160*, 1–40.
24. Williams, D. R. *Chem. Rev.* **1972**, *72*, 203–213.
25. Jomovaa, K.; Valkob, M. *Toxicology* **2011**, *283*, 65–87.
26. Desai, V.; Kaler, S. G. *Am. J. Clin. Nutr.* **2008**, *88*, 855S–858S.
27. Korin, E.; Cohen, B.; Xu, Y.-S.; Zeng, C.-C.; Becker, J. Y. *Tetrahedron* **2011**, *67*, 6252–6258.
28. Iwanami, Y.; Seki, T.; Inagaki, T. *Bull. Chem. Soc. Jpn.* **1971**, *44*, 1316–1321.
29. Rurack, K. *Spectrochim. Acta, Part A* **2001**, *57*, 2161–2195.
30. Descalzo, A. B.; Zhu, S.; Fischer, T.; Rurack, K. *Advanced Fluorescence Reporters In Chemistry And Biology II: Molecular Constructions, Polymers And Nanoparticles*; Springer: New York, 2010.
31. Valeur, B. *Molecular Fluorescence, Principles and Applications*; Wiley-VCH: Weinheim, 2001.