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Highly selective and sensitive colorimetric probes for hypochlorite anion based on azo derivatives

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ABSTRACT

Two oxime-based colorimetric probes for the hypochlorite anion (ClO^-) have been rationally designed and synthesized on basis of the mechanism of intramolecular charge transfer (ICT). Upon addition of ClO^-, the probes displayed around 20 nm redshift in the absorption maximum, accompanied with the color change from orange to pink, which were attributed to the reaction of the oxime groups with ClO^- to form aldehyde groups. The probes were highly selective for ClO^- detection without the interference of other ions and oxidants.

1. Introduction

Hypochlorite anion (ClO^-) is encountered widely in our daily life. For example, sodium hypochlorite is frequently used as a disinfectant and a bleaching agent. Typically, it is used in the concentration range of 10^{-5} to 10^{-2} M [1]. However, concentrated hypochlorite solutions are a potential health hazard to human and animals [2]. On the other hand, hypochlorite anion is one of the biologically important reactive oxygen species (ROS) [3–5], and it plays a critical role in the immune system. Endogenous ClO^- is essential to life and has important antibacterial properties. However, the abnormal production of hypochlorite can lead to tissue damage and diseases, such as cardiovascular diseases [6], neuron degeneration [7], arthritis [8], and cancer [9,10]. Therefore, it is of great interest to detect hypochlorite by sensitive and selective methods. Unfortunately, only a limited number of selective hypochlorite/hypochlorous acid fluorescent probes has been constructed [11–15], most of which respond to hypochlorite/hypochlorous acid with changes only in fluorescent intensity. However, in many cases, colorimetric-based probes are especially attractive, as it allows naked eye detection of the analyte without resort to any expensive equipment [16–27]. To our best knowledge, no colorimetric probes for hypochlorite have been reported thus far. We therefore wanted to develop a colorimetric probe which displayed a color response to hypochlorite using intramolecular charge transfer (ICT) as a signaling mechanism. An ICT system contains an electron donor and an electron acceptor. Modulation of the electronic features of the substituents could manipulate ICT to afford a large wavelength shift in the spectra of chemodosimeter before and after treatment with an analyte [11,28–34]. Therefore, we decided to employ this key feature of ICT in our colorimetric probe design.

It is noted that aldehyde groups can be protected as oximes, which are rapidly deprotected by ClO^- at room temperature [35]. In the present work, we attempted to apply this reaction to design compounds **1** and **2** as colorimetric probes for hypochlorite (Scheme 1). We hypothesized that the oxime protective group could be removed by ClO^- to give the aldehyde group [11]. Thus, oximes **1** and **2** were transformed into aldehydes **6** and **7**, respectively. As *N*,*N*-dimethylaniline is an electron-donoring group and the electron-withdrawing ability of the aldehyde group is stronger than that of the oxime group, it was expected that the maximum absorption of compounds **6** and **7** should have a redshift relative to those of compounds **1** and **2** due to stronger ICT. Therefore, "protected" **1**, **2** and "deprotected" **6** and **7** may be suitable for the development of colorimetric ClO^- probes based on regulation of the electron-withdrawing ability of the electron acceptor in the ICT system.

2. Experimental

2.1. Apparatus

NMR spectra were recorded on Mercury Plus 400NB NMR spectrometer using tetramethylsilane (TMS) as an internal standard. The elemental analyses were performed with Vario EL III Element analyzer. UV–vis absorption spectra were recorded with a UV

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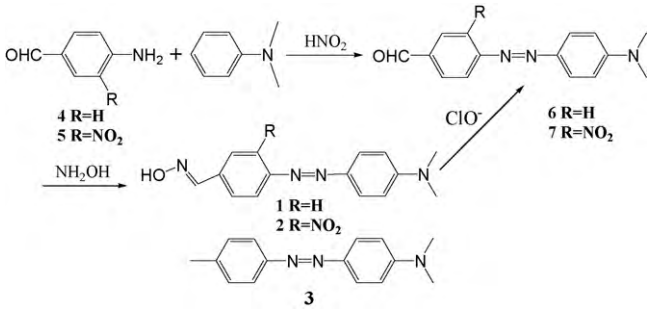

Scheme 1. Synthetic routes to compounds **1** and **2** and the structure of control compound **3**.

1102 spectrophotometer of TECHCOMP with quartz cuvette (path length = 1 cm).

2.2. Reagents

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Twice-distilled water was used throughout all experiments. *N*,*N*-dimethylaniline, 4-aminobenzaldehyde and hydroxylamine hydrochloride were purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. 4-Amino-3-nitrobenzaldehyde was prepared according to the method of Nagano [36] in the yield of 58%.

2.3. Synthesis and characterization of compounds ***1–3***

2.3.1. 4-((4-(Dimethylamino)phenyl)diazenyl)benzaldehyde (***6***)

Compound **6** was synthesized according to documented procedures [37].

^{1}H NMR (400 MHz, 400 MHz, $CDCl_3$) δ 10.06 (s, 1H), 7.90–7.99 (m, 6H), 6.76 (q, 2H), 3.12 (s, 6H).

2.3.2. 4-((4-(Dimethylamino)phenyl)diazenyl)benzaldehyde oxime (***1***)

Compound **6** (0.5 g, 2 mmol), hydroxylamine hydrochloride (0.16 g, 2.33 mmol) and Et_3N (0.236 g, 2.33 mmol) in ethanol were heated to 60 °C for 3 h. After the reaction, the solvent was removed under reduced vacuum. The resulting residue was purified by recrystallization from C_2H_5OH:H_2O (1:1) to give magenta product (0.45 g, 84%). ^{1}H NMR (400 MHz, d_6-DMSO): δ = 11.39 (s, 1H), 8.21 (s, 1H), 7.78–7.81 (m, 4H), 7.73 (d, *J* = 8.4 Hz, 2H), 6.84 (d, *J* = 9.2 Hz, 2H), 3.07 (s, 6H). ^{13}C NMR (100 MHz, d_6-DMSO): 152.7, 152.6, 147.6, 142.6, 133.9, 127.2, 124.8, 122.1, 111.5. Elemental analysis Calcd for $C_{15}H_{16}N_4O$ (268.13): C, 67.15; H, 6.01; N, 20.88. Found: C, 67.01; H, 6.24; N, 20.45.

2.3.3. 4-((4-(Dimethylamino)phenyl)diazenyl)-3-nitrobenzaldehyde (***7***)

7 was synthesized according to the previous literature [37].

^{1}H NMR (400 MHz, d_6-DMSO): 10.08 (s, 1H), 8.51 (s, 1H), 8.22 (d, *J* = 8.4 Hz, 1H), 7.88 (d, *J* = 8.4 Hz, 1H), 7.80 (d, *J* = 9.2 Hz, 2H), 6.90 (d, *J* = 9.2 Hz, 2H), 3.14 (s, 6H).

2.3.4. 4-((4-(Dimethylamino)phenyl)diazenyl)-3-nitrobenzaldehyde oxime (***2***)

Compound **7** (0.6 g, 2 mmol), hydroxylamine hydrochloride (0.16 g, 2.33 mmol) and Et_3N (0.236 mg, 2.33 mmol) in ethanol were heated to 60 °C for 3 h. After the reaction, the solvent was removed under reduced vacuum. The resulting residue was purified by recrystallization from C_2H_5OH:H_2O (1:1) to give purple red product (0.5 g, 79.8%). ^{1}H NMR (400 MHz, d_6-DMSO): δ = 11.71 (s, 1H), 8.28 (s, 1H), 8.14 (s, 1H), 7.95 (d, *J* = 8.8 Hz, 1H), 7.74–7.77 (m, 3H), 6.86 (d, *J* = 9.2 Hz, 2H), 3.11 (s, 6H). ^{13}C NMR (100 MHz, d_6-DMSO): 153.5, 147.2, 146.3, 144.3, 142.8, 134.1, 129.9, 125.8, 121.4, 118.6, 111.7. Elemental analysis Calcd for $C_{15}H_{15}N_5O_3$ (313.12): C, 57.50; H, 4.83; N, 22.35. Found: C, 57.35; H, 4.95; N, 22.03.

2.3.5. *N*,*N*-Dimethyl-4-(*p*-tolyldiazenyl)aniline (***3***)

Compound **3** was prepared similarly to **1** and **2** in 87% yield. ^{1}H NMR (400 MHz, d_6-DMSO): δ = 7.76 (d, *J* = 9.2 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.32 (d, *J* = 8.4 Hz, 2H), 6.82 (d, *J* = 9.2 Hz, 2H), 3.05 (s, 6H), 2.37 (s, 3H). Elemental analysis Calcd for $C_{15}H_{17}N_3$: C, 75.28; H, 7.16; N, 17.56. Found: C, 75.02; H, 7.38; N, 17.77.

3. Results and discussion

3.1. Spectral characteristics

The optical properties of **1**, **2**, **6**, **7** and **3** were studied in 0.1 M phosphate buffer, pH 9.2/DMF (4:1, v/v) at room temperature. In comparison to the absorption spectrum of the reference compound **3**, those of probes **1**, **2**, **6** and **7** displayed a redshift peak (Fig. 1). These bathochromic shift were apparently attributed to ICT between the *N*,*N*-dimethylaniline moiety and the oxime or aldehyde group, as designed. The presence of nitro group in **2** enhanced the intramolecular charge transfer character compared with **1**, and gave rise to 36 nm red shifted absorption spectrum compared with **1**. Additionally, the absorption spectra of **6** and **7** showed longer redshift than those of **1** and **2**, respectively. These were consistent with the fact that the aldehyde group has stronger electron-withdrawing ability than the oxime group.

As shown in Fig. 2, **1** showed maximum absorption at 453 nm with the molar extinction coefficients (ε) of 2.1×10^4 L mol^{-1} cm^{-1}, corresponding to the intramolecular charge transfer character (ICT) of the chromophore due to the push–pull effect. Upon the introduction of ClO^- (32 equiv.) into the solution of probe **1** (10 μM), the absorption maximum of **1** shifted from 453 to 475 nm.

UV–vis spectral changes (Fig. 2) of **1** in competitive ions and oxidants were undertaken in the presence of Na^+, K^+, Mg^{2+}, Zn^{2+}, Cu^{2+}, Al^{3+}, Cl^-, CH_3COO^-, SO_4^{2-}, NO_2^-, ClO_4^-, NO, ClO_3^-, CO_3^{2-}, Fe^{3+} and H_2O_2 in phosphate buffer solution, pH 9.2/DMF (4:1, v/v). The miscellaneous competitive ions and oxidants did not lead to any significant changes in the visible region. The wavelength shift of absorption resulting from the addition of the ClO^- ion was not influenced by any subsequent addition of miscellaneous ions and oxidants. All these experiments implied that the selectivity of **1**

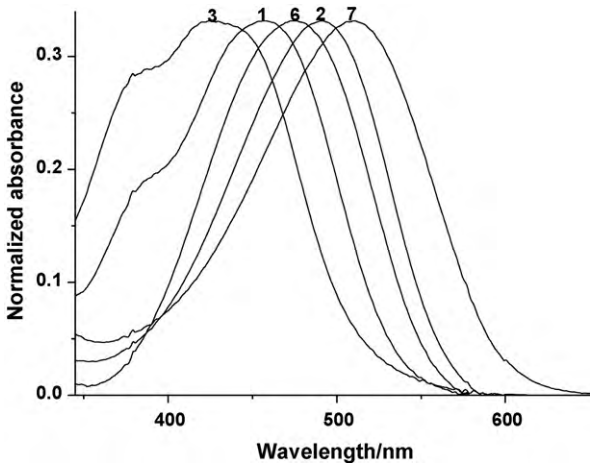

Fig. 1. Normalized absorption spectra of **1**, **2**, **6**, **7** and **3** in 0.1 M phosphate buffer, pH 9.2/DMF (4:1, v/v).

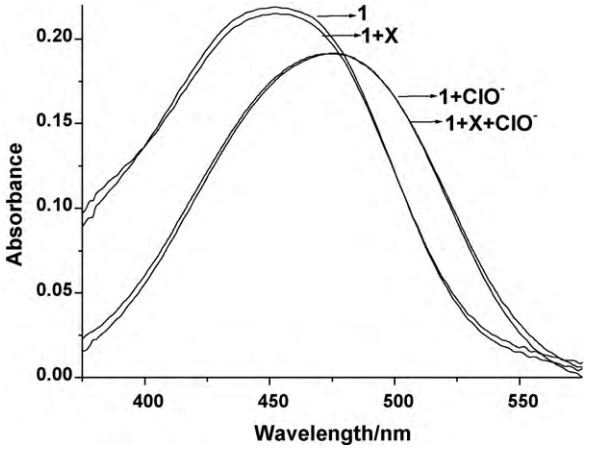

Fig. 2. UV–vis spectra of probe **1** (10 μM) in the presence of ClO^- and miscellaneous ions and oxidants including Na^+, K^+, Mg^{2+}, Zn^{2+}, Cu^{2+}, Al^{3+}, Cl^-, CH_3COO^-, SO_4^{2-}, NO_2^-, ClO_4^-, NO, ClO_3^-, CO_3^{2-}, Fe^{3+} and H_2O_2 (32 equiv.) in phosphate buffer solution, pH 9.2/DMF (4:1, v/v) at room temperature.

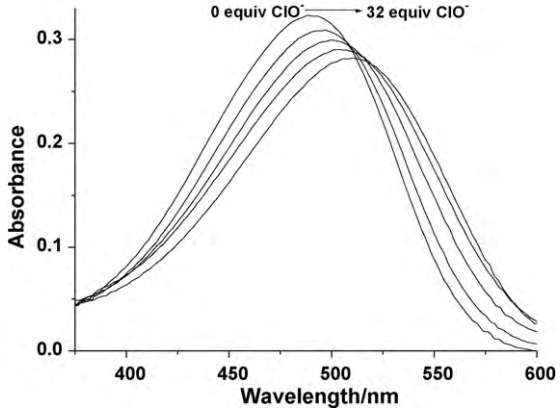

Fig. 3. UV–vis spectra of **2** (10 μM) in the presence of different concentration of ClO^- (0–32 equiv.) in phosphate buffer solution, pH 9.2/DMF (4:1, v/v) at room temperature. Each spectrum was recorded 1 min after ClO^- addition.

for the ClO^- ion over other competitive ions and oxidants was remarkably high.

The presence of nitro group in **2** enhanced the intramolecular charge transfer character compared with **1**, the absorption spectrum of **2** showed a band centered at 489 nm with an extinction coefficients of $3.2 \times 10^4\ L\,mol^{-1}\,cm^{-1}$. As shown in Fig. 3, upon addition of ClO^-, the absorption peak of **2** centered at 489 nm gradually decreased with the concomitant formation of a redshift band peaked at 510 nm, corresponding to a color change from orange to pink, which were clearly visible to the naked eyes (Fig. 4). The titration profile of **2** with ClO^- demonstrates that the detection of ClO^- is possible at the 2 μM level. The wavelength shift of absorption indicating **7** was formed by the interaction of oxime with ClO^-, increasing the electron-withdrawing ability, resulting in a stronger ICT of the chromophore. In addition, the probe was stable because no obvious change in the absorption spectrum was observed

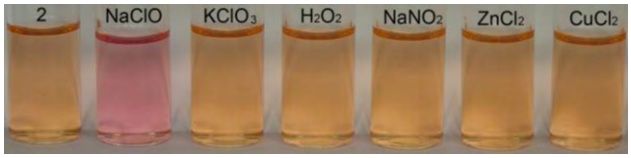

Fig. 4. Visual color changes of 2 (10 μM) in the presence of different species (32 equiv.) in phosphate buffer solution, pH 9.2/DMF (4:1, v/v).

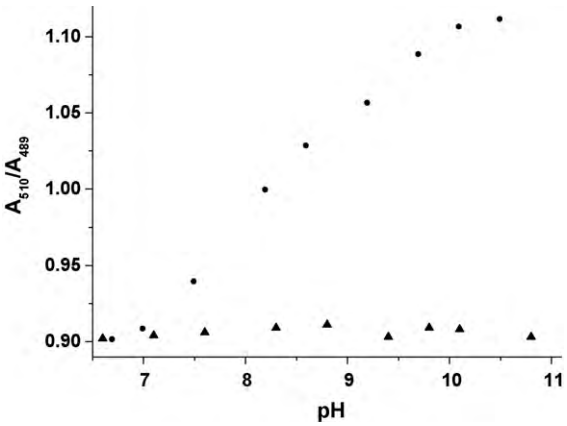

Fig. 5. Effect of pH on the reaction of 2 (10 μM) with ClO^- (32 equiv.): 2 (▲), 2 + ClO^- (●).

after the probe's solution was stored at room temperature for a week.

3.2. Optimization of experimental conditions

Various experimental conditions, such as the concentration of the probe, reaction media, pH, temperature and time, were examined to optimize the reaction conditions. The results show that both the background and response signals increase with increasing concentration of the probe. In this study a concentration of 10 μM of the probe was used because sufficient absorption signal could be obtained at this concentration. The water solubility of the probe was limited, and the use of 20% (v/v) DMF as a co-solvent is required in this system. Time-course studies revealed that the conversion of **1** and **2** into **6** and **7** by ClO^-, respectively, was very rapid, this suggested that probes **1** and **2** can be used to monitor ClO^- in real time. Fig. 5 depicts the effect of pH on the reaction. As can be seen, **2** showed pH-dependence in the detection of hypochlorite, the absorption ratio was increased above pH 7.5, considering the pK_a of HClO is 7.6 [12,15], we concluded that **2** detects ClO^- rather than HClO. We decided to study the photophysical and sensing properties of probe **2** at pH 9.2, as the probe was much more sensitive. As a result, a reaction medium of 0.1 M phosphate buffer (pH 9.2) that contained 20% (v/v) DMF, and a reaction time of 1 min at room temperature were chosen for the present reaction.

3.3. Selectivity

For an excellent chemosensor, high selectivity is a matter of necessity. To evaluate the selectivity of **2** for ClO^-, we measured the absorption spectral changes of **2** in phosphate buffer solution, pH 9.2/DMF (4:1, v/v) upon addition of other cations, anions and oxidants. As shown in Fig. 6, no obvious absorption spectra changes were observed upon addition of Na^+, K^+, Mg^{2+}, Zn^{2+}, Cu^{2+}, Al^{3+}, Cl^-, CH_3COO^-, SO_4^{2-}, NO_2^-, ClO_4^-, NO, ClO_3^-, CO_3^{2-}, Fe^{3+} and H_2O_2 compared with the redshift of **2** with ClO^-, indicating that deoximation reaction was a key for the selective recognition of ClO^-. These phenomena suggested the high selectivity of **2** for ClO^- over other cations, anions and oxidants.

Achieving high selectivity for the analyte of interest over a complex background of potentially competing species is a challenge in sensor development. Thus, the competition experiments were conducted in the presence of 32 equiv. of different species at 10 μM of **2** mixed with ClO^- (32 equiv.), respectively. No significant variation in absorption intensity was found in comparison of that containing only ClO^- (Fig. 7). Moreover, no obvious interference in its absorption was observed when performing the titrations with ClO^- in

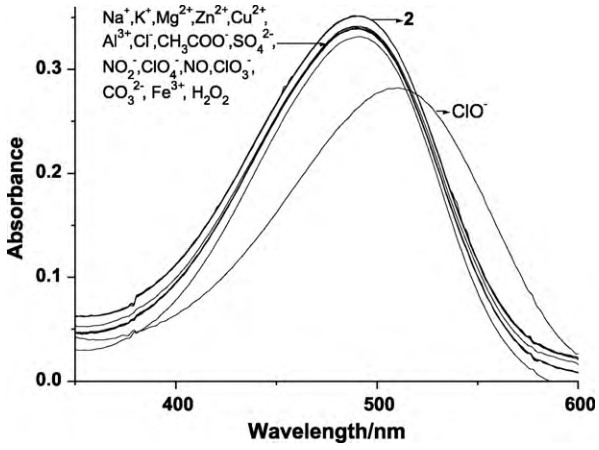

Fig. 6. UV–vis spectra of probe **2** (10 μM) in the absence and presence of 32 equiv. of various species in phosphate buffer, pH 9.2/DMF (4:1, v/v).

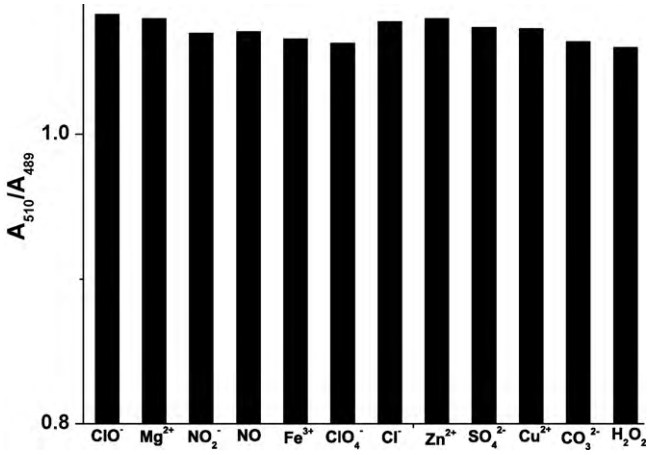

Fig. 7. A_{510}/A_{489} change profile of **2** (10 μM) to 32 equiv. of ClO^- in the presence of different competing species (32 equiv.) in phosphate buffer solution, pH 9.2/DMF (4:1, v/v).

the mixture of other cations, anions and oxidants. These facts indicated that probe **2** could be used for selective detection of ClO^- even under competition from other related species.

3.4. Mechanism

The reaction mechanism in the present system was studied. The generation of **6** and **7** as a product might be responsible for the shift of absorption spectrum of **1** and **2**, respectively. To prove this, the reaction product between **1** and ClO^- was isolated and subject to the standard characterization. In Fig. 8, the peak at 11.39 ppm, which was assigned to oxime (–C=N–OH), disappeared after reaction of ClO^- and at the same time a new peak at 10.06 ppm, which was the characteristic resonance of aldehyde (–CHO), appeared. The ^1H NMR of the isolated product was identical with that of the standard compound **6**, demonstrating that **1** was deprotected by ClO^- ions to give aldehyde **6**. The determination of ClO^- was based on this specific reaction.

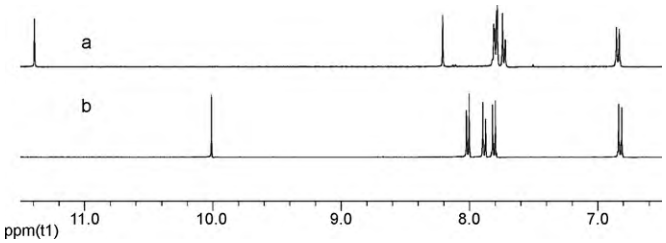

Fig. 8. Partial ^1H NMR (DMSO-d_6, 400 MHz) spectra of (a) probe **1** and (b) the separated product of probe **1** + ClO^-.

4. Conclusion

In conclusion, on the basis of a specific reaction for hypochlorite and exploiting a ICT mechanism, we have successfully developed two colorimetric probes, **1** and **2**, which are highly sensitive and specific for the detection of ClO^-. Most importantly, the recognition of ClO^- gave obvious color changes from orange to pink, which was clearly visible to the naked eyes. Moreover, the competition experiments showed the interference from other common ions and oxidants was minimal. Due to the simplicity and sensitivity of the analysis, this sensor would have many opportunities in a variety of settings requiring rapid and accurate ClO^- analysis. We anticipate that this probe will be of great benefit to biomedical researchers for studying the effects of ClO^- in biological systems.

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References

[1] T. Aoki, M. Munemori, Anal. Chem. 55 (1983) 209–212.
[2] L.C. Adam, G. Gordon, Anal. Chem. 67 (1995) 535–540.
[3] S. Khatib, R. Musaa, J. Vaya, Bioorg. Med. Chem. 15 (2007) 3661–3666.
[4] A. Gomes, E. Fernandes, J.L.F.C. Lima, J. Biochem. Biophys. Methods 65 (2005) 45–80.
[5] X.H. Li, G.X. Zhang, H.M. Ma, D.Q. Zhang, J. Li, D.B. Zhu, J. Am. Chem. Soc. 126 (2004) 11543–11548.
[6] S. Sugiyama, K. Kugiyama, M. Aikawa, S. Nakamura, H. Ogawa, P. Libby, Arterioscler. Thromb. Vasc. Biol. 24 (2004) 1309–1314.
[7] Y.W. Yap, M. Whiteman, N.S. Cheung, Cell Signal. 19 (2007) 219–228.
[8] M.J. Steinbeck, L.J. Nesti, P.F. Sharkey, J. Parvizi, J. Orthop. Res. 25 (2007) 1128–1135.
[9] E.A. Podrez, H.M. Abu-Soud, S.L. Hazen, Free Radic. Biol. Med. 28 (2000) 1717–1725.
[10] D.I. Pattison, M.J. Davies, Biochemistry 45 (2006) 8152–8162.
[11] W.Y. Lin, L.L. Long, B.B. Chen, W. Tan, Chem. Eur. J. 15 (2009) 2305–2309.
[12] X.Q. Chen, X.C. Wang, S.J. Wang, W. Shi, K. Wang, H.M. Ma, Chem. Eur. J. 14 (2008) 4719–4724.
[13] Z.N. Sun, F.Q. Liu, Y. Chen, P.K.H. Tam, D. Yang, Org. Lett. 10 (2008) 2171–2174.
[14] Y.K. Yang, H.J. Cho, J. Lee, I. Shin, J. Tae, Org. Lett. 11 (2009) 859–861.
[15] S. Kenmoku, Y. Urano, H. Kojima, T. Nagano, J. Am. Chem. Soc. 129 (2007) 7313–7318.
[16] A. Mitra, B. Ramanujam, C. Rao, Tetrahedron Lett. 50 (2009) 776–780.
[17] P. Montes-Navajas, L.A. Baumes, A. Corma, H. Garcia, Tetrahedron Lett. 50 (2009) 2301–2304.
[18] D. Saravanakumar, S. Devaraj, S. Iyyampillai, K. Mohandoss, M. Kandaswamy, Tetrahedron Lett. 49 (2008) 127–132.
[19] J.W. Li, H. Lin, Z.S. Cai, H.K. Lin, Spectrochim. Acta A 72 (2009) 1062–1065.
[20] X.M. Huang, Z.Q. Guo, W.H. Zhu, Y.S. Xie, H. Tian, Chem. Commun. (2008) 5143–5145.
[21] H.T. Niu, D.D. Su, X.L. Jiang, W.Z. Yang, Z.M. Yin, J.Q. He, J.P. Cheng, Org. Biomol. Chem. 6 (2008) 3038–3040.
[22] R. Shunmugam, G.J. Gabriel, C.E. Smith, K.A. Aamer, G.N. Tew, Chem. Eur. J. 14 (2008) 3904–3907.
[23] H. Zheng, G.Q. Shang, S.Y. Yang, X. Gao, J.G. Xu, Org. Lett. 10 (2008) 2357–2360.
[24] R.L. Sheng, P.F. Wang, Y.H. Gao, Y. Wu, W.M. Liu, J.J. Ma, H.P. Li, S.K. Wu, Org. Lett. 10 (2008) 5015–5018.
[25] F.H. Zelder, Inorg. Chem. 47 (2008) 1264–1266.
[26] J.H. Huang, Y.F. Xu, X.H. Qian, Org. Biomol. Chem. 7 (2009) 1299–1303.
[27] Q.H. Liu, X.L. Yan, J.C. Guo, D.H. Wang, L. Li, F.Y. Yan, L.G. Chen, Spectrochim. Acta A 73 (2009) 789–793.
[28] F. Qian, C.L. Zhang, Y.M. Zhang, W.J. He, X. Gao, P. Hu, Z.J. Guo, J. Am. Chem. Soc. 131 (2009) 1460–1468.
[29] D. Srikun, E.W. Miller, D.W. Domaille, C.J. Chang, J. Am. Chem. Soc. 130 (2008) 4596–4597.
[30] Y. Zeng, G.X. Zhang, D.Q. Zhang, D.B. Zhu, Tetrahedron Lett. 49 (2008) 7391–7394.
[31] S.Z. Hu, Y. Guo, J. Xu, S.J. Shao, Org. Biomol. Chem. 6 (2008) 2071–2075.

[32] W.Y. Lin, L.L. Long, L. Yuan, Z.M. Cao, B.B. Chen, W. Tan, Org. Lett. 10 (2008) 5577–5580.
[33] X.J. Zhang, X.S. Ren, Q.H. Xu, K.P. Loh, Z.K. Chen, Org. Lett. 11 (2009) 1257–1260.
[34] X.J. Peng, J.J. Du, J.L. Fan, J.Y. Wang, Y.K. Wu, J.Z. Zhao, S.G. Sun, T. Xu, J. Am. Chem. Soc. 129 (2007) 1500–1501.
[35] J.M. Khurana, A. Ray, P.K. Sahoo, Bull. Chem. Soc. Jpn. 67 (1994) 1091–1093.
[36] Y. Gabe, Y. Urano, K. Kikuchi, H. Kojima, T. Nagano, J. Am. Chem. Soc. 126 (2004) 3357–3367.
[37] D.Q. Zhang, M. Zhang, Z.Q. Liu, M.X. Yu, F.Y. Li, T. Yi, C.H. Huang, Tetrahedron Lett. 47 (2006) 7093–7096.