RSC Advances

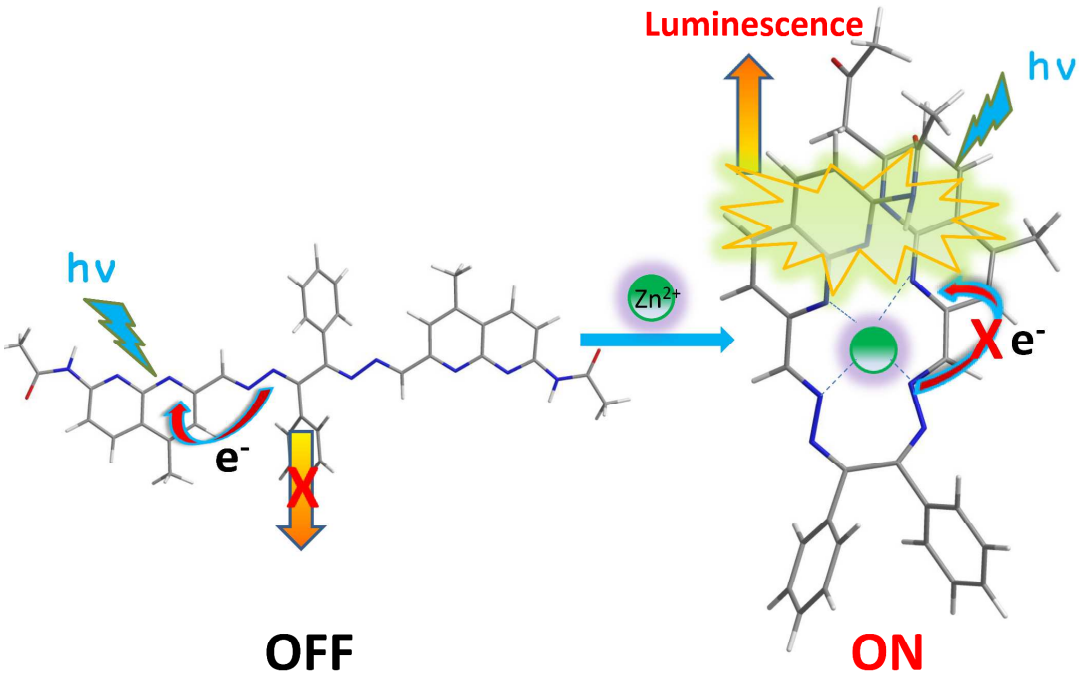
ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

TOC for

1,8-Naphthyridine-based molecular clips for off-on fluorescence sensing of Zn^{2+} in living cells

De-Hui Wang,[a]* Yuan Zhang,[b]* Zhe Gong,[a] Ran Sun,[a] De-Zhi Zhao[a] and Chang-Liang Sun*[c]



New 1,8-naphthyridine-based clip-like receptor as "off-on" fluorescent probe was designed and synthesised for selectively sensing Zn^{2+} in living cells.

RSC Advances

COMMUNICATION

1,8-Naphthyridine-based molecular clips for off-on fluorescence sensing of Zn^{2+} in living cells†

Received 00th January 20xx,
Accepted 00th January 20xx

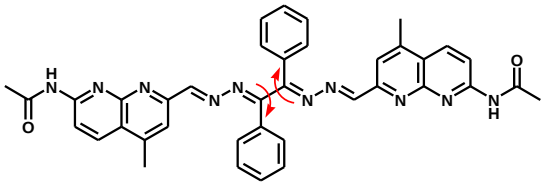
DOI: 10.1039/x0xx00000x

www.rsc.org/

De-Hui Wang, ‡[a]* Yuan Zhang, ‡[b]* Zhe Gong,[a] Ran Sun,[a] De-Zhi Zhao[a] and Chang-Liang Sun*[c]

New 1,8-naphthyridine-based clip-like receptor as "off-on" fluorescent probe was designed and synthesised for selectively sensing Zn^{2+} in aqueous media and living cells.

Design of receptor for Zn^{2+} is an area of intense research activity, because they are potentially attractive for use in such areas as regulation of brain function and pathology, gene transcription, enzymes, and diseases control.[1] Recently, synthetic strategies for constructing functional Zn^{2+} receptors with various structures and novel binding properties have been well established.[2, 1b] Especially, much attention has been drawn to design different binding models for the optical imaging with fluorescent sensors for Zn^{2+} in living cells or hippocampus slices. However, only a few "off-on" fluorescent sensors for Zn^{2+} have been reported.[3, 1b] On the other hand, the background fluorescence of these sensors decrease the accuracy of Zn^{2+} detection in aqueous media or living cells. Additionally, to our best knowledge, all these reported sensors often display poor binding selectivity for Zn^{2+} over other heavy and transition-metal (HTM) ions, such as Cu^{2+}, Hg^{2+}, and Cd^{2+}.[4]



Scheme 1. The chemical structure of 1,8-naphthyridine-based molecular clips **DP1**.

Therefore, development of receptors for Zn^{2+} with visual functional fluorescent output and excellent binding selectivity has been a formidable challenge yet to be achieved. As a continuation of our research work on the di- or tripodal receptors,[5] by incorporating fluorophores onto the di- or tripodands, herein, we report the syntheses and metal binding properties of new 1,8-naphthyridine-based clip-like receptor for fluorescent discriminating of Zn^{2+} over a wide range of tested metal ions in aqueous media. The conformational switching of benzyl dihydrazone unit was selected as the receptor unit, while 1,8-naphthyridine was selected as not only the receptor unit but also the fluorophore unit (Scheme 1). The two necessary units were introduced as trigger sites to achieve efficient metal interactions and a consequently good signal response. This "OFF" nature of the ligand **DP1** can be assigned to photoinduced electron transfer **(**PET),[6] while the "ON" nature of the **DP1**+Zn^{2+} can be assigned to metal-to-ligand charge-transfer transition (MLCT).[7] Interestingly, the bright yellow (λ~480-530 nm) with high quantum yields (Φ_f)[8] and visible-light excitation of 1,8-naphthyridine groups provide an opportunity to luminescent imaging Zn^{2+} in living cells.

DPs were synthesized by the reaction of 7-acetylamino-4-methyl-1,8-naphthyridine-2-aldehyde (**NAPY**) with the respectively benzyl dihydrazone derivative and ethylenediamine in good yield. All compounds were characterized by EA, NMR and MS. The results of density functional theory calculations (DFT) for **DP1** reveal that the electronic density in the HOMO is localized mainly on the lone pair orbitals of the nitrogen atoms in the benzyl dihydrazone moiety (Fig. S6a, ESI†), suggesting a possible PET fluorescence quenching process.[9]

DP1 exhibited a 1,8-naphthyridine characteristic absorption band cenerted at 424 nm (log ε = 4.59) in CH_3CN:H_2O (1:1, v/v, containing 0.01 M HEPES, pH=7.21) solution. Indeed, as predicted by the calculations, **DP1** displayed a negligible fluorescence band with a corresponding emission maximum at 518 nm (Φ_f = 0.003) in CH_3CN:H_2O (1:1, v/v, containing 0.01 M HEPES, pH=7.21) solution when excited at 424 nm. The addition of $Zn(ClO_4)_2$ resulted in a significant fluorescence enhancement until a plateau was reached (Φ_f = 0.18). Upon adding Zn^{2+}, the fluorescence intensity of **DP1** increased by ca. 14-fold (Fig. 1), together with a significant colour change from colorlessness to bright green (Fig. 2b). At the same time, the absorbance spectra exhibited an obviously red shift take place (from 376 nm to 424 nm) upon treatment with 1

[a]*College of Chemistry, Chemical Engineering and Environmental Engineering, Liaoning Shihua University, Fushun, 113001, China. E-mail:dhuiwang@aliyun.com*
[b]*Liaoning Institute for Food Control, Shenyang, 110015, China. E-mail: yuan3660@sina.com*
[c]*Center of Physical Chemistry Test, Shenyang University of Chemical Technology, Shenyang 110142, China. E-mail: chemscl@126.com*

† Electronic Supplementary Information (ESI) available: Experimental details and additional spectroscopic data. See DOI: 10.1039/x0xx00000x

‡ D. Wang and Y. Zhang contributed equally to this work.

equivalents of $Zn(ClO_4)_2$ (Fig. S1, ESI†). The new peak at 424 nm can be safely assigned to MLCT transition ($d^{10}(Zn)$-π^*). And the color of **DP1** changes from colorlessness to light lemonade after addition of Zn^{2+}. Moreover, **DP1** featured a detection limit for Zn^{2+} of at least down to 3.92×10^{-6} M (Fig. S13, ESI†).

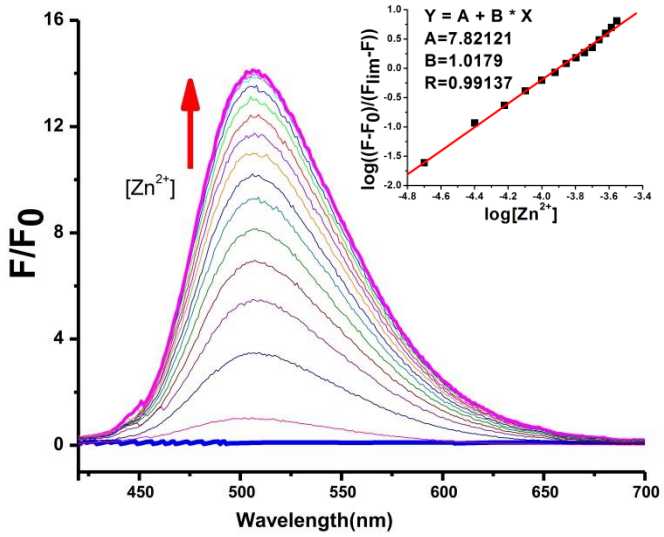


Fig. 1 Fluorescence titration of **DP1** (20 μM) with $Zn(ClO_4)_2$ in CH_3CN:H_2O(1:1, v/v, containing 0.01 M HEPES, pH=7.21). [Zn^{2+}]: 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 10.5, 11.0, 11.5, 12.0, 12.5, 13.0, 13.5, 14.0, 14.5, 15.0 equiv. λ_{ex}= 424 nm. Inset: liner of log $((F-F_0)/(F_{lim}-F)$ vs. log [Zn^{2+}]. (A present fluorescence of **DP1** at 518 nm)

Under the same conditions, no drastic fluorescence enhancement of **DP1** (20 μM) was observed in the presence of other tested metal salts of ClO_4^- (Hg^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Ag^+, Cd^{2+}, Pb^{2+}, Fe^{3+}, Fe^{2+}, Mg^{2+}, Ca^{2+}, Na^+, K^+) while Cu^{2+} caused a very small enhancement in the emission intensity. Furthermore, the competition experiments revealed that **DP1** retained the excellent Zn^{2+} specificity in the presence of a variety of other metal found in environmental and biological settings, this means that the luminescence enhancement induced by Zn^{2+} was little affected by these metal ions (Fig. 2). These results suggest that **DP1** could respond to Zn^{2+} with high selectivity by a fluorescence output manner.

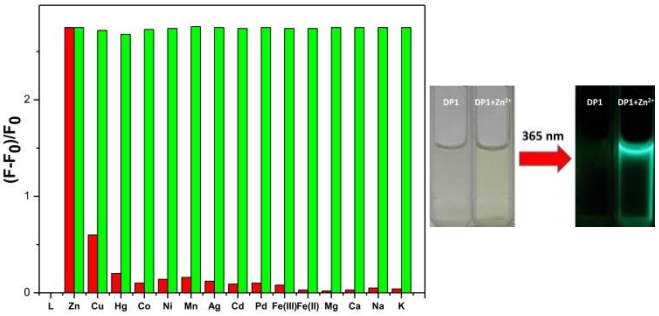


Fig. 2 (a) Fluorescence responses of **DP1** (20 μM) to various metal ions in CH_3CN:H_2O (1:1, v/v, containing 0.01 M HEPES, pH=7.21). The red bars represent the emission intensities of **DP1** in the presence of 15 eq of cations (0.3 mM) of interest, respectively. The green bars represent the emission intensities that occur upon the subsequent addition of 15 eq of Zn^{2+} (0.3 mM) to the above mentioned solutions, respectively. Excitation was provided at 424 nm, and the emission intensities were recorded at 518 nm. (b) Change in color and fluoresence of **DP1** (20 μM) upon addition of concentration of Zn^{2+}. Excitation was provided at 365 nm.

Binding analysis using the method of continues variations (Job's plot, Fig. S2**,** ESI†) and the linear fitting of the luminescence titration curve (coresponding the UV-Vis titration curve, Fig. S1**,** ESI†) established that a 1:1 **DP1**-Zn^{2+} complexation species was responsible for the observed fluorescence enhancement (Fig. 1), and the association constant for Zn^{2+} binding to **DP1** was calculated as $3.36\pm0.20\times10^6$ M^{-1}.[10]

The proposed mechanism was also confirmed by ^{1}H NMR and MS measurements. ^{1}H NMR titration displayed the chemical shift changes of **DP1** upon the addition of Zn^{2+}, as shown in Fig. 3. Compared to the ^{1}H NMR spectrum of the free ligand **DP1** (Fig. 3a), the Zn^{2+} binding caused the small but significant downfield shifts of almost all of the proton signals (Fig. 3b), especially for the protons in the naphthyridine ring, 0.29 ppm for H_6, 0.26 ppm for H_5 and 0.31 for H_7. And so forth, strongly suggested the participation of the naphthyridine rings in the coordination.[11,5d] Meanwhile, the singlet signal of -NH_8- proton was no obvious shift, this phenomenon indicated that O atoms of carbonyl groups were not involved in the chelation to the Zn ion, which was confirmed by our previous work.[12]

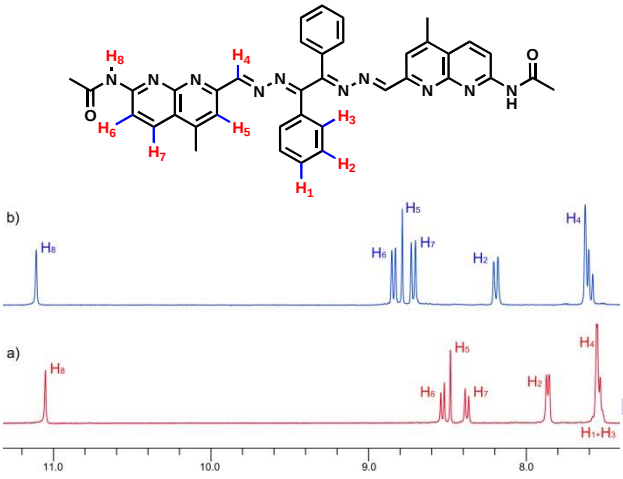


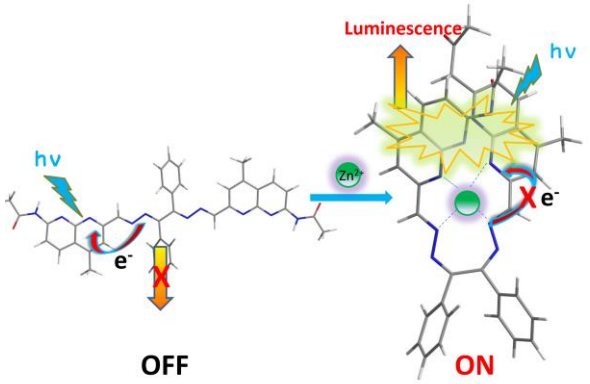
Fig. 3 Partial ^{1}H NMR spectra for pure **DP1**(a) and **DP1**+$Zn(ClO_4)_2$ (sufficit quantum) (b) in DMSO-d_6, respectively.

The binding model was further supported by the ESI-MS spectra. In the case of the solution of **DP1** in the presence of a sufficit amount of $Zn(ClO_4)_2$, an exact comparison of the most interesting experimental peak (which is observed at m/z 362.28 and 823.56) with the simulation results obtained on the basis of natural isotopic abundances reveals that this two charged species can be reasonable assigned to [**DP1**+Zn]$^{2+}$ and [**DP1**+Zn+ClO_4]$^+$, thus providing a direct evidence of a 1:1 stoichiometric host-guest complexation **(**Fig. S3**,** ESI†**)**.

To investigate the Zn^{2+} binding model of receptor **DP1**, **DP2** was designed and synthesized in multi-steps (Scheme S1, ESI†). The benzyl dihydrazone group was replaced by ethylenediamine unit which lose the character of conformational switching only. Indeed, upon addition of the Zn^{2+}, a CH_3CN:H_2O (1:1, v/v, containing 0.01 M HEPES, pH=7.21) solution of **DP2** gave rise to negligible changes in the fluorescent spectra. From this vantage point, it should be noted that the conformational switching of benzyl dihydrazone portion play an important role in binding Zn^{2+} cation by a possible multi-site coordination complexation mode, leading to the observed fluorescence

enhancement of the 1,8-naphthyridine band, like that reported by Zhou et.al.[13, 5b-d]

Based on these reasons, we proposed the following signaling mechanism for detection Zn^{2+}. **DP1** alone showed no emission at 518 nm, when Zn^{2+} ion coordinates with the nitrogen atoms of the benzyl dihydrazone and nitrogen atoms of the 1,8-naphthyridine fluorophore (Fig. S5, ESI†), the lone pair electrons of nitrogen atom no longer serve the PET process, while the MLCT leads to an enhancement of the emission (Scheme 2).



Scheme 2. Proposed binding mode of **DP1** to Zn^{2+}, showing the possibility of blocking the PET process upon metal binding.

We further investigate the biological application of **DP1** in cultured cell (HeLa cells). HeLa cells incubated with **DP1** (5 μM) for 15 minutes at room temperature showed a negligible fluorescence (Fig. 6a). The cells remained viable and no apparent toxicity and side effects were observed throughout the imaging experiments, which suggested that **DP1** was cell permeable (Fig. 6c). When cells stained with compound **DP1** were further incubated with Zn^{2+} (1 mM) in phosphate-buffered saline (PBS) for 30 minutes and washed, an increase in intracellular fluorescence intensity (corresponding the fluorescence titration) was observed, suggesting the successful application in the Zn^{2+} stain experiments(Fig. 6b).

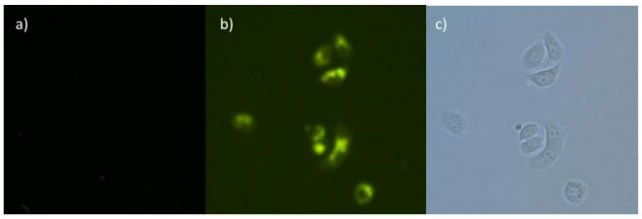


Fig. 4 Confocal fluorescence images of HeLa cells (λ_{ex} = 416 nm). (a) Cells supplemented with **DP1** for 15 min. (b) Cells supplemented with **DP1** for 15 min and then incubated with Zn^{2+} for 30 min. (c) A bright-field image of the cells in panel (b).

Conclusions

In summary, we have designed and synthesized a turn-on fluorescent chemosensor for Zn^{2+}, **DP1**, by incorporating 1,8-naphthyridine fluorophores within the benzyl dihydrazone moiety in aqueous media. Further binding model studies by ^1H NMR spectroscopy, mass spectroscopy, Job's plot, and DFT calculations demonstrated that the receptor formed a 1:1 host–guest complexation with Zn^{2+}. Due to its excellent sensitivity, high selectivity, good water solubility and favorable spectroscopic properties, **DP1** could act as a efficient sensing probe for the detection of Zn^{2+} in living cells, over biologically-relevant metal ions.

Acknowledgements

This work was supported by the Scientific Research Foundation for Doctors of Science and Technology Department of Liaoning Province (No. 20131063); Key Laboratory of Photochemical Conversion and Optoelectronic Materials，TIPC，CAS.

Notes and references

1 (a)K. Szacilowski, *Chem. Rev.*, 2008, **108**, 3481; (b) Y. Chen, Y. Bai, Z. Han, W. He and Z. Guo, *Chem. Soc. Rev.*, DOI: 10.1039/c5cs00005j; (b)G. Aragay, J. Pons and A. Merkoci, *Chem. Rev.*, 2011, **111**, 3433; (d) C. J. Frederickson, J. Y. Koh and A. I. Bush, *Nat. Rev. Neurosci.*, 2005, **6**, 449; (c)D. T. Quang and J. S. Kim, *Chem. Rev.*, 2010, **110**, 6280.

2 (a)Q. Lin, Y. Cai, Q. Li, J. Chang, H. Yao, Y. -M. Zhang and T. -B. Wei, *New. J. Chem.*, 2015, DOI: 10.1039/c5nj00023h; (b)A. Gogoi and G. Das, *Rsc. Adv.*, 2014, **99**, 55689; (c)L. Wang, W. Qin and W. Liu, *Anal. Methods.*, 2013, **4**, 1167; (d)D. Sarkar, A. K. Pramanik and T. K. Mondal, *RSC Adv.*, 2014, **48**, 25341; (e)S. H. Jung, K. -Y. Kwon and J. H. Jung, *Chem. Comm.*, 2014, **5**,952; (f)Z. Dong, X. Le, P. Zhou, C. Dong and J. Ma, *New. J. Chem.*, 2014, **4**, 1802.

3 (a)P. Wang, J. Wu, P. Zhou, W. Liu and Y. Tang, *J. Mater. Chem. B.*, 2015, DOI: 10.1039/c5tb00142k; (b)S. Goswami, A. Manna, S. Paul, A. K. Maity, P. Saha, C. K. Quahc and H. -K. Fun, *RSC. Adv.*, 2014, **4**, 34572; (c)G. J. Park, Y. J. Na, H. Y. Jo, S. A. Lee, A. R. Kim, I. Noh and C. Kim, *New. J.Chem.*, 2014, **38**, 2587; (d)N. Khairnar, K. Tayade, S. K. Sahoo, B. Bondhopadhyay, A. Basu, J. Singh, N. Singh, V. Gite and A. Kuwar, *Dalton. Trans.*, 2015, **44**, 2097; (e)K. Tayade, B. Bondhopadhyay, H. Sharma, A. Basu, V. Gite, S. Attarde, N. Singh and A. Kuwar, *Photochem. Photobiol. Sci.*, 2014, **13**, 1052; (f)S. Chall, S. S. Mati, S. Konar, D. Singharoy and S. C. Bhattacharya, *Org. Biomol. Chem.*, 2014, **12**, 6447; (g)B. K. Datta, D. Thiyagarajan, S. Samanta, A. Ramesh and G. Das, *Org. Biomol. Chem.*, 2014, **12**, 4975; (h)P. Saluja, V. K. Bhardwaj, T. Pandiyan, S. Kaur, N. Kaur and N. Singh, *RSC Adv.*, 2014, **4**, 9784; (i)Z. Xu, K. -H. Baek, H. N. Kim, J. Cui, X. Qian, D. R. Spring, I. Shin and J. Yoon, *J. Am. Chem. Soc.*, 2010, **132**, 601.

4 (a)J. -C. Qin, L. Fan, B. -D. Wang, Z. -Y. Yang and T. -R. Li, *Anal. Methods.*, 2015, **7**, 716; (b)A. Ganguly, B. K. Paul, S. Ghosh, S. Kar and N. Guchhait, *Analyst.*, 2013, **138**, 6532; (c)P. -S. Yao, Z. Liu, J. -Z. Ge, Y. Chen and Q. -Y. Cao, *Dalton. Trans.*, 2015, **44**, 7470; (d)Z. Kowser, H. Tomiyasu, X. Jiang, U. Rayhan, C. Redshawb and T. Yamato, *New. J. Chem.*, 2015, DOI: 10.1039/c4nj02363c; (e)N. Sharma, S. I. Reja, V. Bhalla and M. Kumar, *Dalton Trans.*, 2015, **44**, 6062; (f)P. Li, X. Zhou, R. Huang, L. Yang, X. Tang, W. Dou, Q. Zhao and W. Liu, *Dalton. Trans.*, 2014, **43**, 706; (g)A. Jana, P. K. Sukul, S. K. Mandal, S. Konar, S. Ray, K. Das, J. A. Golen, A. L. Rheingold, S. Mondal, T. K. Mondal, A. R. Khuda-Bukhshc and S. K. Kar, *Analyst.*, 2014, **139**, 495; (h)S. Xia, G. Liu and S. Pu, *J. Mater. Chem. C.*, 2015, **3**, 4023; (i)Z. -P. Zheng, Q. Wei, W. -X. Yin, L. -T. Wan, X. Huang, Y. Yu and Y. -P. Cai, *RSC Adv.*, 2015, **5**, 27682; (j)M. Yang, Y. Zhang, W. Zhu, H. Wang, J. Huang, L. Cheng, H. Zhou, J. Wu and Y. Tian, *J. Mater. Chem. C.*, 2015, **3**, 1994; (k)A. Rai, N. Kumari, R. Nair, K. Singh and L. Mishra, *RSC Adv.*, 2015, **5**, 14382; (l)Y. S. Kim, G. J. Park, J. J. Lee, S. Y. Lee, S. Y. Lee and C. Kim, *RSC Adv.*, 2015, **5**, 11229; (m)D. Sarkar, A. K. Pramanik and T. K. Mondal, *RSC Adv.*, 2015, **5**, 7647; (n)S. Goswami, S. Paul and A. Manna, *RSC Adv.*, 2013, **3**, 25079; (o)W. -H. Ding, W. Cao, X. -J. Zheng, W. -J. Ding, J. -P. Qiao and L. -P. Jin, *Dalton. Trans.*, 2014, **43**, 6429; (p)K. B. Kim, H. Kim, E. J. Song, S. Kim, I. Noh and C. Kim, *Dalton. Trans.*, 2013, **42**, 16569; (q)M. C. Aragoni, M. Arca, A. Bencini, C. Caltagirone, A. Garau, F. Isaia, M. E. Light, V. Lippolis, C. Lodeiro, M. Mameli, R. Montis, M. C. Mostallino, A. Pintus and S. Puccioni, *Dalton. Trans.*, 2013, **42**, 14516.

5 (a)D. Wang, X. Zhang, C. He and C. Duan, *Org. Biomol. Chem.*, 2010, **8**, 2923; (b)G. He, X. Zhao, X. Zhang, H. Fan, S. Wu, H. Li, C. He and ...

Duan, *New. J. Chem.*, 2010, **34**, 1055; (c)Z. -H. Lin, L. -X. Xie, Y. -G. Zhao, C. -Y. Duan and J. -P. Qu, *Org. Biomol. Chem.*, 2007, **5**, 3535; (d)G. He, Y. Zhao, C. He, Y. Liu and C. Duan, *Inorg. Chem.*, 2008, **47**, 5169.

6 A. R. Sarkar, D. E. Kang, H. M. Kim and B. R. Cho, *Inorg. Chem.*, 2014, **53**, 1794.

7 (a)S. Goswami, S. Das, K. Aich, D. Sarkar, T. K. Mondal, C. K. Quah and H.-K. Fun, *Dalton Trans.*, 2013, **42**, 15113; (b)S. Goswami, K. Aich, S. Das, C. Das Mukhopadhyay, D. Sarkarc and T. K. Mondalc, *Dalton Trans.*, 2015, **44**, 5763; (c) N. C. Lim, J. V. Schuster, M. C. Porto, M. A. Tanudra, L. Yao, H. C. Freake and C. Bruckner, *Inorg. Chem.*, 2005, **44**, 2018.

8 (a)J. -L. Zuo, W. -F. Fu, C -M. Che and K. -K. Cheung, *Eur. J. Inorg. Chem.*, 2003, **2**, 255; (b) C. -M. Che, C. -W. Wan, K. -Y. Ho and Z. -Y. Zhou, *New J. Chem.*, 2001, **25**, 63.

9 H. S. Jung, P. S. Kwon, J. W. Lee, J. I. Kim, C. S. Hong, J. W. Kim, S. Yan, J. Y. Lee, J. H. Lee, T. Joo and J. S. Kim, *J. Am. Chem. Soc.*, 2009, **131**, 2008.

10 K. A. Connors, *Binding Constants.*, John Wiley, New York,1987.

11 (a)J. Wang and X. Qian, *Chem. Commun.*, 2006, 109; (b)K. Wu, Y. Gao, Z. Yu, F. Yu, J. Jiang, J. Guo and Y. Han, *Anal. Methods.*, 2014, **6**, 3560; (c)Y. Shiraishi, H. Maehara, K. Ishizumi and T. Hirai, *Org. Lett.*, 2007, **9**, 3125; (d)U. N. Yadav, P. Pant, S. K. Sahoob and G. S. Shankarling, *RSC Adv.*, 2014, **4**, 42647.

12 S. -M Chi, Y. -F. Wang, X. Gan, D. -H. Wang, W. -F. Fu, *Cent. Eur. J. Chem.*, 2009, **7**, 923.

13 (a)G. K. Wang, Q. L. Mi, L. Y. Zhao, J. J. Hu, L. Guo, X. J. Zou, B. Liu, X. G. Xie, J. F. Zhang, Q. H. Zhao and Y. Zhou, *Chem. Asian J.*, 2014, **9**, 744; (b)C. -M. Che and J. -S. Huang, *Coord. Chem. Rev.*, 2003, **242**, 97. (d)M. H. Mei and S. K. Wu, *New. J. Chem.*, 2001, **25**, 471; (e)Y. -P. Yen and K. -W. Ho, *Tetrahedron Lett.*, 2006, **47**, 1193.