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Photoactivated DNA Cleavage Of Ferrocene-Appended Copper(II) Complexes

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Abstract: Mononuclear copper(II) complexes [Cu(Fctpy)(Cl)](Cl) (**1**), [Cu(Fctpy)(bpy)]$(ClO_4)_2$ (**2**) and [Cu(Fctpy)(phen)]$(ClO_4)_2$ (**3**) (where Fctpy = 4'-(ferrocenyl)-2,2':6',2''-terpyridine) have been synthesized. Physico-chemical techniques like elemental analysis, ESI-MS, UV–Visible, Infra red and EPR spectroscopic techniques were employed to characterize the synthesized complexes. All the three complexes however successfully promoted an oxidative cleavage of plasmid DNA, producing single strand break. In addition, mechanistic SC DNA cleavage results show higher DNA cleavage activity in presence of oxidizing agent, due to the presence of hydroxyl radicals. And also the complexes showed photonuclease activity at the wavelength of 540 nm via photoredox pathway.
Keywords: Copper(II), ferrocenyl terpyridine, photoredox, SC DNA.

Introduction

The expedition for alternative drugs to the well-known cisplatin and its derivatives, which are still used in more than 50 % of the treatment regimes for patients suffering from cancer, is highly needed. More than forty years after its serendipitous discovery in 1951 [l], ferrocene still enjoys a great deal of interest from scientists in many areas of research. Due to its high stability and the well-established methods for its incorporation into more complex structures, ferrocene has become a versatile building block for the synthesis of compounds with tailor-made properties. Ferrocene can also be easily derivatized and the central iron atom is also easily oxidised from Fe(II) to Fe(III). Moreover ferrocene conjugates are stable in biological media, lipophilic and have unique redox property [2]. The molecule ferrocene itself is non-toxic but the ferrocenium ion produced is found to be toxic in various cancer cell lines [3]. These ferrocenium salts were found to inhibit the cell growth and interact with negatively charged DNA through electrostatic interaction and oxidatively damages DNA via reactive oxygen species(ROS) formation. Ferrocifen, a hormone independent chemotherapeutic agent containing ferocene moiety is found to be more effective than tamoxifen which is a hormone dependent anticancer drug. Both ferrocifen and tamoxifen are used in the treatment of breast cancer [4]. The medicinal application of ferrocenyl conjugates is currently an active area of research with many reports showing its activity *in vivo* and *in vitro* and its potential as an anti-tumour, anti-malarial and anti-fungal agent [5]. One of the most potent and effective antitumor agents was discovered in the last century [6]. Cis-Platin, the first anticancer drug, had toxic side effects such as nephrotoxicity. But on introducing ferrocenyl moiety in the transition metal complexes, the antitumour activity was found to be increased and more effective. Terpyridine ligands are more rigid than

bidentate ligands such as bipyridine and phenanthroline. Introduction of substituents in the 4' position of the terpyridine does not lead to geometrical isomers and substitution in the 4' position of the terpyridine can be easily achieved by available procedures. The photochemical and photophysical properties of the complex too can be tuned by using various substituents in the 4' position of the terpyridine. Transition metal co-ordination complexes have now been widely studied for their antimicrobial and anticancer properties [7-9]. In fact large number of ferrocenyl containing metal chelates are multinuclear molecules possessing features of both organometallic and coordination chemistry [10]. Copper complexes containing both heterocycles and ferrocenyl moiety in the same molecule have been investigated scarcely so far [11]. We have recently shown that furyl-appended copper(II) complex having a terpyridine ligand acts as multifunctional model nuclease[12]. In continuation of our efforts to explore this chemistry further, we have now synthesized mixed ligand copper(II) complexes having ferrocene appended terpyridyl ligand and bipyridine and phenanthroline bases and studied their DNA cleavage property both in the absence and presence of light. Herein, we present the synthesis, structure, and DNA cleavage activity of the ferrocene-appended terpyridyl copper(II) complexes [Cu(Fc-tpy)(Cl)]Cl (1), [Cu(Fc-tpy)(bpy)]$(ClO_4)_2$ (2) and [Cu(Fc-tpy)(phen)]$(ClO_4)_2$ (3) where Fc-tpy is 4-ferrocenyl-2,2':6,2''-terpyridine, bpy is 2,2'-bipyridine and phen is 1, 10-phenanthroline. (Figure 1).

Materials And Methods

2-acetyl pyridine, copper(II) chloride dihydrate and copper(II) perchlorate hexahydrate, ferrocene-2-carboxaldehyde, agarose (molecular biology grade) and ethidium bromide were procured from Sigma Aldrich, USA and used as received. Other materials like sodium hydroxide, ammonium acetate and solvents like methanol, acetonitrile were of reagent grade. The ligand, Fctpy (ferrocenyl terpyridine) was prepared using published procedure[13]. Buffers were prepared using deionised and sonicated triple distilled water. Tris (hydroxymethyl) aminomethane–HCl (Tris–HCl) buffer (pH, 7.2) was used for DNA cleavage studies. The analyses for carbon, nitrogen and hydrogen were performed at Sophisticated Test and Instrumentation Centre (STIC), Cochin University, Kerala. UV–visible spectra of the complexes were recorded on a Perkin–Elmer Lambda 35 double beam spectrophotometer at 25^0C. Electron paramagnetic resonance spectra of the copper(II) complexes were obtained on a Varian E 112 EPR spectrometer. IR spectra were recorded as KBr pellets in the 400 - 4000 cm^{-1} region using a Shimadzu FT–IR 8000 spectrophotometer. Positive ion electron spray ionization mass spectra of the complexes were obtained by using Thermo Finnigan LCQ 6000 advantage max ion trap mass spectrometer. All the DNA gel images were taken using UVITEC gel documentation system and fragments were analyzed using UVIchem and UVI-band software.

Synthesis of [Cu(Fctpy)(Cl)]Cl (1)

The complex was prepared in good yield from the reaction of $CuCl_2 \cdot 2H_2O$ in methanol with ferrocenyl terpyridine ligand. The ligand (1.25 g, 3 mmol) and $CuCl_2 \cdot 2H_2O$ (0.5 g, 3 mmol) were dissolved in methanol individually and the solutions were warmed. To the hot solution of the ligand, copper chloride was added slowly with constant stirring when the colour changed to dark violet. The solution was cooled to room temperature and the violet precipitate of the copper–Fctpy complex separated out and was filtered and dried. Yield: 78 %. Anal. Calc. for $C_{25}H_{19}Cl_2CuFeN_3$: C, 54.42; H, 3.47; N, 7.62; Cu, 11.52; Fe, 10.12; found: C, 54.38; H, 3.44; N, 7.57; Cu, 1.46; Fe, 10. 09 %. FT-IR (KBr pellet) cm^{-1}: 3368, 3076, 1605, 1473, 788, 650, 476. ESI-MS: *m*/*z* = 515.07 $[M - L \cdot Cl]^+$.

Synthesis of [Cu (Fctpy) (bpy)] $(ClO_4)_2$ (2)

This complex was synthesized by adding a hot methanol (5 ml) solution of 2, 2'-bipyridine (156.19 mg, 1 mmol) and Fctpy (417.16 mg, 1 mmol) to a methanol solution of copper(II) perchlorate (370.53 mg, 1 mmol) and then stirring the solution at room temperature for 3 h. The resulting solution was filtered and kept aside. The product obtained was filtered and dried. Yield: 84 %. Anal. Calcd.for $C_{35}H_{27}N_5CuFeCl_2O_8$: C, 50.29; H, 3.26; N, 8.38, Cu, 7.6; Fe, 6.68; Found: C, 50.23; H, 3.23; N,8.36; Cu, 7.54; Fe, 6.62 %. FT-IR (KBr pellet) cm^{-1}: 3466, 3082, 1606, 1566, 1471, 1087, 769, 623. ESI-MS: *m*/*z* = 317.13 $[M - 2ClO_4]^{2+}$.

Synthesis of [Cu (Fctpy) (phen)] $(ClO_4)_2$ (3)

The compound [Cu (Fctpy) (phen)] $(ClO_4)_2$ was re-produced by following the method already reported [11]. A deep violet crystalline powder was characterized by elemental analysis, UV, ESI-MS, infrared and EPR

spectroscopy. A good match with the literature data was observed. Yield: 81 %. Anal. Calcd. for $C_{37}H_{27}N_5CuFeCl_2O_8$: C, 51.68; H, 3.16; N, 8.14, Cu, 7.39; Fe, 6.49; Found: C, 51.63; H, 3.12; N, 8.11; Cu, 7.34; Fe, 6.42 %. FT-IR (KBr pellet) cm^{-1}: 3441, 3074, 1607, 1520, 1471, 1088, 786, 623. ESI-MS: *m*/*z* = 661.00 $[M - 2ClO_4]^+$.

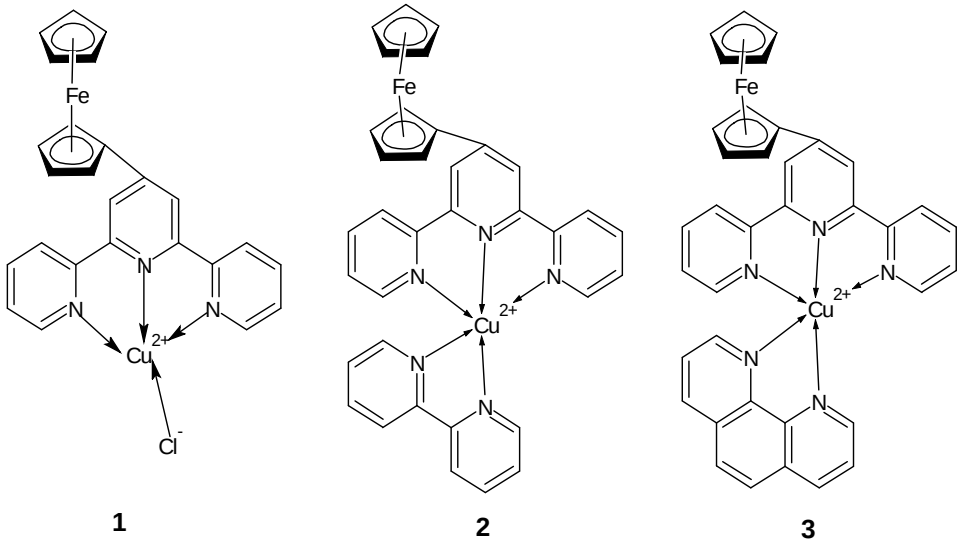

Figure 1. Mononuclear copper(II) complexes

DNA Cleavage Experiment

The experiments were carried out using SC pUC19 DNA under aerobic conditions. Samples were prepared in the dark at 25^0C by taking 3 µL of SCDNA and 6 µL of the complexes from a stock solution in DMSO followed by dilution in 10 mM Tris-HCl buffer (pH 7.2) to make the total volume 25 µL. Chemical nuclease experiments carried out under dark conditions for one hour incubation at 37^0C in the absence and presence of an activating agent H_2O_2 were monitored using agarose gel electrophoresis. Supercoiled pUC19 plasmid DNA in 5 mM Tris-HCl buffer at pH 7.2 was treated with copper(II) complex. The samples were incubated for 1 h at $37^{\mathbf{0}}$C. The reactions were quenched using loading buffer (0.25 % bromophenol blue, 40 % (w/v) sucrose and 0.5 M EDTA) and then loaded on 0.8 % agarose gel containing 0.5 mg/mL ethidium bromide. Another set of experiment was also performed using DMSO and histidine in order to find out the type of molecule involved in the cleavage mechanism. The gels were run at 50 V for 3 h in Tris-boric acid-ethylenediamine tetra acetic acid (TBE) buffer and the bands were photographed by a UVITEC gel documentation system. Photoinduced DNA cleavage experiments were also done using visible light of wavelength 540 nm.

Results And Discussion

Synthesis and spectral characterization

The mixed ligand complexes $[Cu(Fctpy)(B)](ClO_4)_2$, where Fctpy is the tridentate ligand 4'-(ferrocenyl)-2,2':6',2"-terpyridine and B is heterocyclic bases bipyridine (2) and phenanthroline (3) have been isolated from methanolic solution containing hexahydrated copper(II) perchlorate as the starting material. Whereas the complex [Cu(Fctpy)Cl]Cl (1) have been prepared by reacting copper(II) chloride dihydrate with 4'-(ferrocenyl)-2,2':6',2"-terpyridine in a 1:1 ratio in methanol. All the complexes were obtained in good yield and characterized by using elemental analysis, UV-Vis, ESI-MS and EPR spectral techniques. Their purity was checked by TLC on silica gel. The analytical data obtained for the new complexes agree well with the proposed molecular formula. The ESI mass spectra of [Cu(Fctpy)Cl]Cl (1), $[Cu(Fctpy)(bpy)](ClO_4)_2$ (2) and $[Cu(Fctpy)(phen)](ClO_4)_2$ (3) displayed the molecular ion peaks at m/z 515.93, 317.13 and 661.00 respectively. ESI-MS spectrum of complex **1** is depicted in figure 2. These peaks are reliable with the proposed molecular formula of the corresponding copper(II) complexes. The structures of the complexes are revealed in figure 1.

Electronic spectral analysis

The electronic absorption spectra of the copper complexes were recorded at 300 K in acetonitrile. Electronic spectrum of ferrocene itself shows two d-d bands at 324.7 and 440.0 nm and the molar extinction coefficient of ferrocene was low due to the forbidden d-d transition of the highly symmetric ferrocene[14]. Electronic specta of the present complexes are shown in figure 3. All the three complexes show two sharp bands in the area of 223-298 nm, weak band at 326-342 nm and a broad band in the region of 520-541 nm. The ligand centered π - π* and n- π* transitions due to the molecular orbitals present in the ferrocenyl terpyridine are found in the ultraviolet region. The band at ~ 289 nm could be due to terpyridine moiety. Complexes **1**-**3** show a very low intense band due to ferrocene moiety at ~340 nm. Spectrum of **1** shows a band at 298 nm and a shoulder at 341 nm, which can be attributed to intra ligand transitions of the ligand. Broad metal to ligand charge transfer (MLCT) transition has been observed at 523.5 nm. Complex **2** shows the intra ligand transitions at 269.2 nm and a shoulder at 339.6 nm. Broad MLCT band has been observed at 520.5 and 541.2 nm for complexes **2** and **3** respectively. Low intense copper centered d-d transitions are not found in the region of 650 nm for all the three complexes. This might be due to the masking of the d-d band by high intense charge transfer bands.

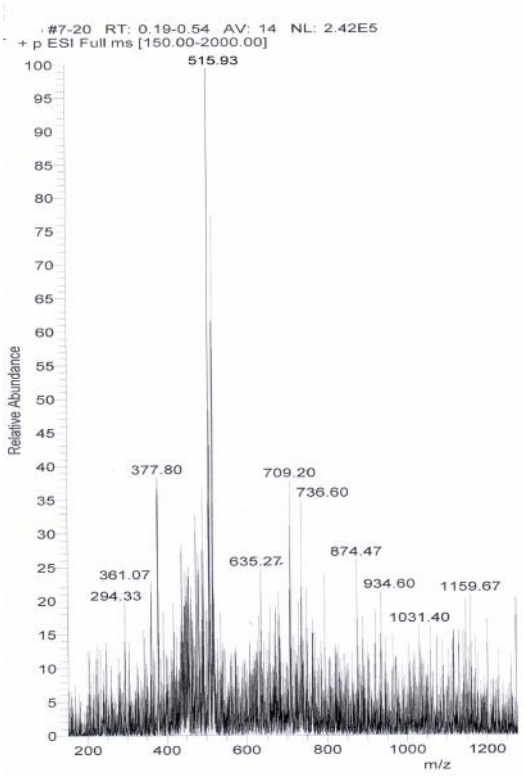

Figure 2. ESI-MS spectrum of complex 1

IR and EPR spectral analysis

Information about the nature of the binding mode and functional group attached to the metal ion can be obtained from infra-red spectrum. A strong band appeared at 1603 cm^{-1} in the ligand can be assigned to the ν(C=N) stretching vibration. In all three complexes, ν(C=N) bands are shifted by 2-4 cm^{-1} to lower wave number. This showed that the possibility of coordination of the imino nitrogen to metal ion. All these complexes exhibit a broad band in the region 3367-3466 cm^{-1} indicating the presence of lattice or coordinated water molecule [15]. The IR spectra of 1,10-phenanthroline showed peak at 739 cm^{-1} and in the complex 3 it was shifted in the range 721 cm^{-1}. This shift can be explained on the basis of the fact that the nitrogen atoms of phenanthroline donate a pair of electrons to the central metal ion, forming a coordinative covalent bond [16]. Medium intensity bands appeared at 3076, 3082 and 3074 cm^{-1} for complexes **1**, **2** & **3** respectively were attributed to C-H stretching vibration. Presence of perchlorate ion in the IR spectra of complex **2** & **3** were confirmed by the appearance of a sharp band at 1088 cm^{-1}. In all the three complexes, other medium intensity

sharp bands appear at 1471-1473 cm^{-1} have been assigned to C=C stretching vibrations. The frequency of M-N bands were appeared in the region of 476-482 cm^{-1}.

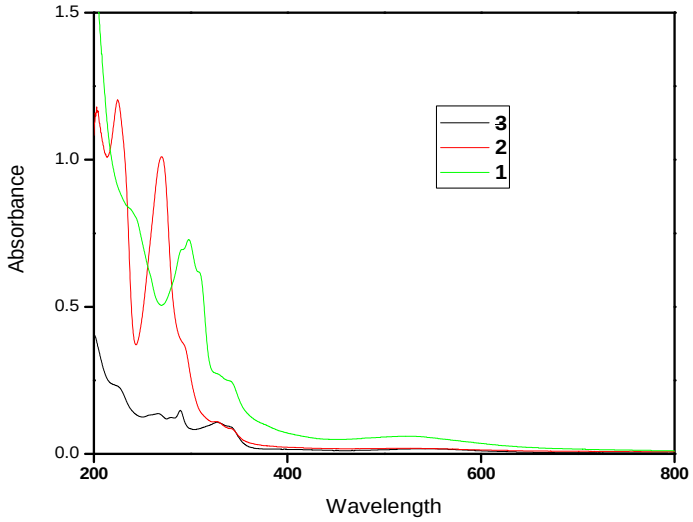

Figure 3. Electronic spectra of copper(II) complexes

Formation of the copper(II) complexes were confirmed by their broad epr spectrum and their g value. The epr spectra of complexes **1- 3** show axial signal at 300 K from a static copper (II) centre with dx^2-y^2 as the ground state. And also the spectra of all the three copper complexes at 300 K show one intense band in the high field region, which are isotropic due to tumbling motion of the molecules. The g value for complex **1**, **2** and **3** are 2.07, 2.08 and 2.09 respectively.

Chemical nuclease activity

Nuclease activity of all the three complexes were performed in the presence and absence of hydrogen peroxide as an oxidizing agent. Control experiments suggest that untreated DNA and DNA incubated with complexes **1-3**, ferrocenyl terpyridine or peroxide alone did not show any significant DNA cleavage (lanes 1–5 in figure 4). Nevertheless ferrocenyl terpyridine in the presence of hydrogen peroxide show considerable cleavage of DNA. However, in the presence of hydrogen peroxide, copper complex was found to exhibit nuclease activity. As shown in figure 4, with the increase of complex concentration, the cleavage of supercoiled DNA was found to increase apparently. At the concentration of 12 µM, only partial cleavage of DNA by the copper complexes was taken place (lanes 6-8 in figure 4). But on increasing the concentration to 24 µM, the rate of DNA cleavage also increased (lanes 9-11 in figure 4). Out of the three complexes, the cleavage efficiency of **3** was found to be more than that of the complexes **1 & 2.**

Figure. 4. Cleavage of pUC19 DNA by complexes (**1-3**) in the presence of peroxide (100 µM). DNA was incubated with complex for 60 min in Tris buffer (pH 7.2) at 37°C. Lane 1, DNA control; lane 2, DNA + peroxide (100 µM); lane 3,DNA + 1 (12 µM); lane 4, DNA + 2 (12 µM); lane 5, DNA + 3 (12 µM); lane 6, DNA + 1 (12 µM) + peroxide (100 µM),); lane 7, DNA + 2 (12 µM) + peroxide (100 µM); lane 8, DNA + 3 (12 µM) + peroxide (100 µM); lane 9, DNA + 1 (24 µM) + peroxide (100 µM); lane 10, DNA + 2 (24 µM) + peroxide (100 µM); lane 11, DNA + 3 (24 µM) + peroxide (100 µM).

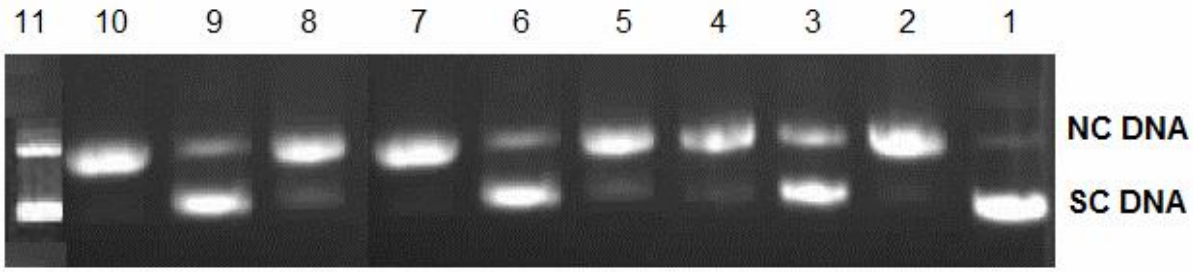

Figure. 5. Photoactivated DNA cleavage by complexes 1-3 (24 µM) in Tris buffer (pH 7.2) at 37°C in the presence of light at 540 nm. Lane 1, DNA ; lane 2, DNA + 1; lane 3, DNA + 1 + 10 mM DMSO; lane 4, DNA +1+10 mM Histidine; lane 5, DNA + 2; lane 6, DNA + 2 + 10 mM DMSO; lane 7, DNA + 2 + 10 mM Histidine; lane 8, DNA + 3; lane 9, DNA + 3 + 10 mM DMSO; lane 10, DNA + 3 + 10 mM Histidine; lane 11, DNA + Fctpy (12 µM).

Photonuclease activity of the complexes

Photoactivated DNA cleavage of the copper complexes were performed by irradiating the complexes with DNA to light for about 60 min at a wavelength of 540 nm. Since the present complexes did not show any apparent cleavage in the absence of light, the possibility of hydrolytic cleavage of DNA involving the phosphodiester bond is eliminated. On irradiation to light ferrocene terpyridine itself shows cleavage of DNA to a slight extent. All the complexes at a concentration of 24 µM show significant DNA cleavage, when they are exposed to light at a wavelength of 540 nm.

Investigation of the active oxygen species

It is known that when redox-active metal complexes interact with DNA in the presence of dioxygen or a redox reagent, reactive oxygen species are generated which causes major DNA damage [17-20]. Copper complexes can cleave DNA both through hydrolytic and oxidative processes. In the latter instance, these complexes have been shown to react with molecular oxygen or hydrogen peroxide to produce a variety of active oxidative intermediates (reactive oxygen species or ROS), including diffusible hydroxyl radicals and non-diffusible copper-oxene species [21], while in others Fenton-type chemistry which involves release of diffusible hydroxyl radicals has been suggested. In order to know the type of radical involved in the DNA cleavage reaction, we attempted to do the experiment both in dark and light in the presence of singlet oxygen quenchers and hydroxyl radical scavengers like histidine and DMSO respectively. When DMSO was added to the reaction mixture of the complex and DNA, the DNA cleavage activity of 1-3 decreases significantly (lanes 3, 6 & 9 in figure 5). Interestingly, on addition of histidine to the reaction mixture, the DNA cleavage activity was not inhibited greatly (lanes 4, 7 & 10 in figure 5). This conclusively shows the involvement of the hydroxyl radical in the observed nuclease activity of all the complexes in the presence of peroxide. The formation of hydroxyl radical could take place via a photoredox pathway involving the redox-active metal centers [22].

Conclusions

In summary, three copper(II) mononuclear complexes **1-3** having 4'-(ferrocenyl)-2,2':6',2"-terpyridine ligand were prepared and they were characterized by various physico-chemical techniques. The complexes can all effectively promote cleavage of plasmid DNA without addition of external agents and in the presence of hydrogen peroxide at pH = 7.2 and 37^{0}C. And also photocleavage of DNA by the copper complexes was explored. The involvement of hydroxyl radical in the oxidative cleavage reactions is evidenced from the inhibition reactions in presence of DMSO.

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References

1. (a) T. J. Kealey, P. L. Pauson, Nature 1951, 168, 1039; (b) S. A. Miller, J . A. Tebboth, J. F. Tremaine, J. Chem. Soc. 1952, 632.
2. (a) D. R. Van Staveren, N. Metzler-Nolte, Chem. Rev., 104, 2004, 5931 (b) M. F. R. Fouda, M. M. Abd-Elzaher, R. A. Abdelsamaia, A. A. Labib, Appl Organometal Chem., 21, 2007, 613.
3. E. W. Neuse, J. Inorg Organomet Polym Mat, 15, 2005, 3.
4. (a) E. Hillard, A. Vessieres, L. Thouin, G. Jaouen, C. Amatore, Angew. Chem. Int. Ed., 45, 2006, 285. (b) S. Top, A. Vessieres, G. Leclercq, J. Quivy, J. Tang, J. Vaissermann, M. Huche, G. Jaouen, Chem Eur J, 9, 2003, 5223.
5. M. Patra, G. Gasser, M. Wenzel, K. Merz, J. E. Bandow, N. Metzler-Nolte, Organometallics, 29, 2010, 4312.
6. M. A. Alam, K. -e-Zahan, M.S. Hossain, F.Z. Seema, Pakistan J. Biol. Sci., 7, 2004, 891.
7. V. M. Amirkhanov, E.A. Bundya, V.A. Trush, V.A. Ovchynnikov, V.N. Zaitsev, 5th International symposium on applied bioinorganic chemistry, Corfu, Greece, 1999.
8. A. W. Beur, W.M.M. Jkirby, M. Turck, American J. Clin.Pathol., 44, 1966, 493.
9. M. H. Biswas, A.H.M. Zakaria, A. Farroque, C.M. Zakaria, M. Zakir Sultan, G. Sadik, M.S.A. Bhuiyan, Bangladesh Pharm. J., 12, 2002, 43.
10. M. Bracci, C. Ercolani, B. Floris, M. Bassetti, A. Chiesi-Vila, C. Guastini, J. Chem. Soc., Dalton Trans., 1990, 1357.
11. B. Maity, M. Roy, B. Banik, R. Majumdar, R. R. Dighe, A. R. Chakravarty, Organometallics, 29, 2010, 3632.
12. P. Dharmalingam, R. Elayaperumal, M. Kiruthika, J. Pharm. Res., 5, 2012, 4719.
13. G. W. V. Cave, C. L. Raston, J. Chem. Soc., Perkin Trans. I, 2001, 3258.
14. Y. S. Sohn, D. N. Hendrickson, H. B. Gray, J. Am. Chem. Soc. 93, 1971, 3603.
15. D. Koushik, R. Jagnyeswar, M. Mario, W. Xin-Yi, G. Song, B. Pradyot, J. Inorg. Biochem., 101, 2007, 95.
16. P. B. Pansuriya, M. N. Patel, Applied Organometallic Chemistry, 21, 2007, 739.
17. S. Thyagarajan, N. N. Murthy, A. A. Narducci-Sarjeant, K. D. Karlin, S. E. Rokita, J. Am. Chem. Soc., 128, 2006, 7003.
18. A. M. Pyle, J. K. Barton, Prog. Inorg. Chem., 38, 1990, 413.
19. J. R. Morrow, O. Iranzo, Curr. Opin. Chem. Biol., 8, 2004, 192.
20. D. M. Perrin, A. Mazumder, D. S. Sigman, In Progress in Nucleic Acid Chemistry and Molecular Biology, Cohnk W, Moldave ed.; Academic Press: New York Orlando, 52, 1996, 123.
21. M. S. S. Babu, K. H. Reddy, G. K. Pitchika, Polyhedron, 26, 2007, 572.
22. C. J. Burrows, J. G. Muller, Chem. Rev., 98, 1998, 1109.

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