**Self-activating DNA Cleavage by Mononuclear Copper Complexes**

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**Abstract**

Chemical nucleases have seen gradual growth in the field of chemistry and biomedical science. So far, a diverse range of small organic molecules as well as coordination complexes have been developed as chemical nucleases to cleave DNA mainly *via* three major pathways i.e., photoinduced, oxidative or hydrolytic pathways. However, most of such nucleases suffer from their practical applications in real-life samples because they require an external redox agent for functioning. In this context, small organic/inorganic compounds acting as the ‘self-activating’ chemical nucleases have emerged as viable alternatives as therapeutic agents for DNA cleavage activity with no exogeneous agent. The aim of this chapter is to highlight the self-activated DNA cleavage shown by mononuclear copper complexes with specific emphasis on their recent development and challenges.

**Keywords:** Copper Complexes, Self-activating, DNA cleavage,

1. **Introduction**

DNAses and RNAses are the naturally occurring nucleases that participate in many important biological processes such as DNA repair/recombination, DNA synthesis, DNA packaging etc.1,2 The functions of these enzymes have promoted the researchers to develop their metallonuclease mimics i.e., ‘artificial-nucleases’.3-5 The complex Cu(II)-phen (phen = 1,10-phenanthroline) is considered to be one of the first generation artificial-metallonucleases. After its successful implementation in DNA-binding and cleavage, a large number of copper-phenanthroline based derivatives have been increasingly developed over the time.3-8 Such metallonucleases exhibit mainly three types of DNA cleavage processes including hydrolytic, oxidative and photoinduced DNA cleavage. In hydrolytic cleavage process, the applied metal complex specifically induces hydrolysis of the phosphodiester bond11 while the latter (oxidative and photoinducedDNA cleavage processes)9-11 proceed either *via* nucleobase modification12 or a H atom abstraction from the sugar moiety to eventually form the reactive oxygen species (ROSs).13 The DNA cleavage may occur both *via* reactive oxygen species and a transition metal based active intermediate14 which are often produced in the presence of either light or any exogenous redox agents e.g., dithiothreitol, H2O2, mercaptopropionic acid etc.15 Due to the related toxicity of these exogenous species, the majority of the chemical nucleases suffer from their biological applications *in-vivo.* To overcome such as issue, the development of metal complexes acting as ‘self-activating’ chemical nucleases has emerged as the viable therapeutic agents. This is because ‘self-activating’ nucleases do not depend on any exogenous redox agent to exhibit DNA damage process.15,16 The focus of this chapter is to summarize different mononuclear copper complexes functioning as ‘self-activating’ metallonucleases for DNA. The sections have been divided based on the copper complexes derived from different ligand scaffolds such as phenol, phenanthroline, bipyridine and terpyridine, hydrazine and cyclen.

1. **Phenol coordinated copper complexes**

This section primarily focuses on phenol- based mononuclear Cu based ‘self-activating’ metallonucleases. Metal coordinated phenol plays a key role in the generation of ROSs which are mainly responsible for DNA cleavage activity.17 Hecht and group presented a series of alkyl-substituted resorcinols, capable of promoting Cu(II)-induced cleavage of supercoiled plasmid DNA;18 however, the cleavage mechanism was not fully elaborated. This group developed18 5-alkyl-resorcinol (**1**) and 6-alky1-1,2,4-trihydroxybenzene (**2**) (Figure 1) which exhibited Cu(II)-dependent DNA strand scission in basic medium with no requirement of exogeneous redox agent. The influence of different alkyl substituents on DNA cleavage activity of different derivatives has been scrutinized in this report. DNA cleavage process by **1** revealed the contribution of initial oxygenation of the benzene nucleus, a rapid reaction that occurs at basic pH in the presence of Cu2+ and O2. The resultant hydroxylated phenyl rings then promoted the DNA scission process. The possible mechanism involved the reduction of Cu(II) through trihydroxybenzene moiety in **2** with subsequent formation of reactive oxygen species.

Ochratoxin A (**3**, Figure 1) is basically a mycotoxin which is composed of a 4-chlorophenol unit amide-linked to *L*-phenylalanine moiety.19 Compound **3** itselfdoes not induce DNA cleavage process; however, the exposure of light promotes **3** to act as DNA cleaving agent. The cleavage is mediated *via* H-atom abstraction from the deoxyribose sugar moiety. Ardus *et* al. reported that compound **3** binds with Cu(II) to afford a Cu(II)-**3** species which displayed Cu(II)-dependent oxidative DNA cleavage only when Cu(II) was used in excess. The stoichiometric 1:1 Cu(II)-**3** complex inhibited the DNA cleavage process. It has also been realized that compound 3 was converted to hydroquinone when Cu(II) was added along with H2O2.

McFarland *et* al.20 demonstrated Cu(II)-dependent DNA cleavage shown by a natural product jadomycin B (**4**,Figure 1). The jadomycin B combined with Cu(II) exhibited significant DNA cleavage efficacy in absence of any external redox agent. UV-Vis spectral analysis clearly suggested no direct interaction of **4** with DNA during the cleavage process, and the stoichiometric Cu(II) species is required for efficient DNA cleavage. The DNA cleavage activity enhanced significantly upon increasing the concentration of Cu(II) which reaches maximum at 1:1 (Cu(II): **4)** stoichiometry. Excessive addition of Cu(II) species showed no further effect on DNA cleavage process. It has been observed that this Cu(II)-dependent cleavage was markedly improved upon UV light exposure suggesting the presence of a radical cation from jadomycin B and Cu(I) as probable intermediate species during the cleavage process. More importantly, the cleavage process was reduced when catalase and EDTA (scavengers for H2O2 and Cu(II)) were added in the reaction medium. The DNA cleavage mechanism involve the role of jadomycin B as e- source to reduce Cu(II). The resulting Cu(I) species then reacts with H2O2 to give ·OH radicals leading to the DNA damage.



**Figure 1** Chemical drawings of phenol-based chemical nucleases **1-4**.

Salen-based copper complexes have also gained remarkable attention as self-activating chemical nucleases.21 Vezin’s group reported22 three salen-based Cu(II) complexes (**5-7**)having -OH group at *ortho*, *meta,* or *para* position of salicylidene moiety, respectively (Figure 2). Complexes **5** and **7** were found capable of cleaving DNA without any external redox agents while **6** having -OH group at *meta* site,showed negligible DNA cleavage activity. The -OH groups produced a hydroquinone moiety to eventually indorse the generation of high-valent Cu(III) centre. Electrochemical analyses also suggested the formation of Cu(III) species in case of **5** and **7**; however, no such state could be realized for **6**. The mechanistic investigations revealed the possible interaction of hydroquinone unit with Cu(II) favouring generation of free radical and high-valent Cu(III) intermediate species for DNA cleavage.



**Figure 2** Chemical drawings of salen-based copper complexes **5-7**.

Reedijk and group23 presented another report on DNA cleavage activity shown by protected phenol group. In complex **8** based on a tridentate ligand, the phenol site was protected *via* *para*-substituted -CH3 group (Figure 3). Complex **8** was soluble in water and exhibited ‘self-activating’ nuclease activity in the absence of any external agent. Mechanistic investigations, with a diverse array of radical scavengers, suggested the key role of OH• together with ascorbic acid reductant in DNA cleavage process. Notably, Cu(I) and/or other radical species promoted no nuclease activity withoutreductant. The cleavage was further enhanced when the experiments were performed under inert atmosphere which ruled out the requirement of dioxygen (O2). It has been stated that the intercalation of **8** between the base pairs of DNA induced the “self-activating” cleavage process in which water molecules removed e- from Cu(I) to eventually oxidize DNA.

Starting from a protected ligand, Ghosh and co-workers reported24 a square planer complex **9** generating phenoxyl radical *via* both chemical as well as electrochemical pathways (Figure 3). Upon Ce(IV) addition, complex **9** produced a phenoxyl radical which was found stable upto *ca.* 40 min under mild conditions. The stability of resultant radical species could be ascribed to *tert*-butyl group present in the ligand frame of **9**. Electrochemical analyses of **9** displayed a semi-reversible redox response around 1.0 V due to the ligand-based oxidation. The stable nature of such radical species has been verified with the help of absorption and electrochemical analyses. Nuclease activity for pBR322 DNA by complex **9** has been examined by concentration variation, time dependent and inhibition effect in presence of ethanol, DMSO, NaN3, urea, L-histidine, D2O, and catalase (Figures 3a and 3b). Mechanistic investigation confirms that complex **9** displayed an efficient DNA cleavage *via* either singlet-oxygen (1O2) or some other similar ROS. Complex **9** also acted as viable anticancer agent when tested against MCF-7 cancerous cells. Indeed, it was more effective than that of well-known anticancer drug i.e., cisplatin.

Thomas and co-workers demonstrated25 the use of mononuclear complexes **10** and **11** (Figure 3) for a moderate to high nuclease efficacy. The DNA cleavage activity of **10** and **11** was assessed with ϕX174 DNA-plasmid in the absence of any external redox agent. The mechanistic investigation suggested no participation ROSs during the DNA cleavage process. The cytotoxic effect of **10** and **11** were also examined using bladder cancer cells, resistant to cisplatin.



**Figure 3** Chemical drawings of phenol-based copper complexes **8-11**. Gel electrophoresis for pBR322 DNA (40 ng) cleavage by complex **9**. (a) Lane 1: DNA control. Lane 2: DNA + 10% acetonitrile. Lane 3: DNA + ligand (100 μM). Lanes 4−11: DNA + **9** = 5, 10, 20, 30, 40, 50, 75, and 100 μM, respectively. (b) Lane 1: DNA control. Lane 2: DNA + **9** (50 μM). Lanes 3−9: DNA + **9** (50 μM) + DMSO, urea, ethanol, NaN3, L-histidine, D2O, and catalase, respectively. Lane 10: DNA control. Lanes 11−15: DNA + **9** (50 μM) + 10, 30, 60, 90, and 120 min of incubation, respectively.

1. **Phenanthroline based copper complexes**

This section covers different phenanthroline-derived mononuclear copper complexes for their potential uses as ‘self-activating’ nucleases. In year 1979, Sigman *et* al. reported the employment of first low-molecular weight chemical nuclease i.e., complex [Cu(phen)2]2+ (phen = 1,10-phenanthroline, Figure 4). The related experiments and the resultant data played a crucial role to investigate the underlying DNA cleavage mechanism.26 However, the high dissociation constant of phenanthroline moiety and the need of an exogenous agent for functioning limited the practical applications of Cu(phen)22+ in many real-life samples. Complex Cu(phen)22+ required a reductant to generate Cu(phen)2+, an active species for DNA cleavage process. The O2-dependent oxidative DNA cleavage process involved CuII reduction which led to the production of superoxide ion by Cu(I)/O2. Further, the Cu(I) species after reacting with superoxide ion converts back to Cu(II) and H2O2. *In fine*, a reaction between Cu(I) and hydrogen peroxide results in the production of non-diffusible as well as active Cu(III)-OH/Cu(I)-OOH species. Cu(phen)2+ exhibited strong intercalation with DNA organizing the active species in very close proximity.27

Pereira-Maia and group28 presented complexes **14** and **15** for their potential applications as ‘self-activating’ chemical nucleases. These complexes consisted of two bidentate chelating units i.e., phenanthroline and doxycycline/tetracycline (Figure 4) at equatorial positions. In addition, the axial positions were occupied by one H2O molecule and one ClO4- ion in each case. Complexes **14** and **15** displayed remarkable DNA cleavage activity via binding through its major active site without any redox agent. The DNA cleavage efficiency of these complexes suppressed when a radical scavenger or inhibitor was added to the reaction medium. Furthermore, the use of DMSO as a solvent or Cu(I)-chelating agent (such as bathocuproine) resulted in the reduced DNA segmentation. These data clearly suggested the active role of ROSs in nuclease process. For a comparison, the DNA cleavage efficacy the free bidentate ligands and the parent complex CuII(phen)2 was also tested under similar conditions, and the results indicated that the complexes **14** and **15** were 100-folds more potent for DNA fragmentation process.



**Figure 4** Chemical drawings of [Cu(phen)2]2+ and phenanthroline-based complexes **14** and **15**.

Alzuet-Pina *et* al. explored29 the potential application of complexes **16** and **17** based on phenanthroline and quinoline-sulfonamide units as ‘self-activating’ chemical nucleases (Figure 5). The DNA binding ability of **16** for CT-DNA (calf-thymus DNA) was assessed using fluorescence spectroscopy, viscometry, thermal denaturation, and electrochemical studies. These analyses suggested a partial intercalation of **16** with DNA, and the binding constant value (*K*app) was depicted as 2.45 x 106 M–1. The DNA cleavage efficacy of **16** was investigated for plasmid-pUC18 in presence of light while ascorbate and H2O2 were used as inducing agents. Complex **16** was also explored as ‘self-activating’ nuclease the gel electrophoresis experiments clearly exhibited the cleavage effect (Figure 5a). The mechanistic investigations suggested the involvement of either superoxide ion or hydroxyl radical in ‘self-activating’ oxidative DNA damage.

Lehn’s group30 reported two *ortho*-quinacridine based derivatives (**18a** and **18b**)that displayed excellent DNA cleavage activity in presence of Cu(II); but with no external reagent (Figure 5). However, the DNA cleavage efficacy of **18a** and **18b** further improved upon addition of H2O2 to the reaction medium.



**Figure 5.** Chemical drawings of phenanthroline-based nucleases **16-18**.(a)Agarose gel electrophoresis of pUC18 plasmid DNA (37.5 μM) treated with **16** with 2h incubation time (37 °C); Lane1: λDNA/EcoR1 + HindIII marker, Lane 2: pUC18 control, Lane 3: 5 μM, Lane 4: 1 10 μM, Lane 5: 15 μM, Lane 6: 20 μM, Lane 7: 30 μM, Lane 8: Cu(ClO4)2 30 μM, Lane 9: Cu(ClO4)2 + *N*-(quinolin-8-yl)quinolin-8-sulfonamide (HQSQ) 30 μM.

Kulak and co-workers presented31 a series of copper complexes **19a-19c** based on hydrazone-functionalized phenanthroline ligands (Figure 6). These complexes exhibited good to excellent ‘self-activating’ nuclease activities, and the results were compared with the well-known phenanthroline complex i.e., Cu(phen)22+. In absence of any redox agent, the nuclease activity was depicted in the following order: **19b** > **19c** > **19a** > [Cu(phen)2]2+. The DNA cleavage mechanism involved the participation of different ROSs (e.g., superoxide anion and hydrogen peroxide). Complexes **19a-19c** displayed nearly same redox behaviors (*E*1/2 = -0.92 to -0.99 V) for Cu(II)/Cu(I)couple. The highest DNA cleavage efficacy of **19b** could be ascribed to the surplus groove-binding realized in **19b** as compared to other tested complexes.

Recently, Kulak’s group presented32 complexes **20a-20e** derived from the fluorinated phenanthroline derivatives (substitution using F, CF3, SF5 and SCF3). Authors report that the present series of complexes served as highly efficient chemical nucleases (Figure 6). The modulation of phenanthroline moiety in [Cu(phen)2]2+ results into reduced nuclease activity most likely due to existing steric factor after substitution. Interestingly, in this case, the incorporation of fluorine substituents led to the enhanced nuclease activity in absence of any exogenous agent. The reported order of nuclease was [Cu(phen)2]2+ > **20b** > **20c** > **20d** > **20e** > **20a**, which was further corroborated with electrochemical studies. Furthermore, complexes **20a-20e** displayed very low cytotoxic effect(IC50 less than 10 µM) after incubation with the cancer cells lines.



**Figure 6** Chemical drawings of phenanthroline-derived copper complexes **19** and **20**.

1. **Bipyridine and terpyridine derivatives**

This section mainly covers the development in the field of ‘self-activating’ metallonucleases based on 2,2’-bipyridine (bpy) and terpyridine (tpy) ligand scaffolds. In contrast to more planar phen derivatives, bpy and tpy based complexes do not directly and strongly intercalate between the base pairs of DNA. However, these complexes display strong electrostatic attraction with the phosphate backbone of DNA to exhibit cleavage activity.

In an elegant study, Reedijk and group33 demonstarted two water-soluble complexes **21** and **22** promoting oxidative segmentation of *φ*X174 phage-DNA in absence of any external redox agent (Figure 7). The gel electrophoresis diagrams confirmed the catalytic cleavage by both **21** and **22** in double strand and single strand, respectively (Figure 7a-7d).The typical radical scavenging analysesindicated the role of OH• in the cleavage process. Relegation and cell transformation analyses further validated the oxidative cleavage over other processes.



**Figure 7**. Chemical drawings of bipyridine-based copper complexes **21** and **22**.Agarose gel electrophoresis diagrams for *φ*X17 supercoiled phage DNA (20 µg) cleavage by copper complexes **21** or **22**. Concentration variation of complex **21** (a) and complex **22** (b) in 2h incubation; Lanes 1–11: **21** or **22** (in 20 µM increments), Lane 12: DNA control. Inhibition effect for **21** (100 µM) (c) and **22** (100 µM) (d); Lane 1, no additives (control); Lane 2, 200 µM NaN3; Lane 3, 0.5 U of superoxide dismutase, Lane 4, DMSO, Lane 5, 100 µM distamycin, Lane 6, D2O; Lane 7, under argon, Lane 8, in the dark, Lane 9, 350 µM NaCl, Lane 10, 20 µM ascorbic acid.

Vilar’s group34,35 presented complex **23** having a substituted tpy ligand for its potential applications in DNA binding as well as nuclease activity (Figure 8). DNA binding capability of **23** was found dependent on the nature of substituent present on tpy ligand. This factor played an important role in determining the binding mode of 23 with DNA i.e., groove-binding or intercalation. Due to an excellent nuclease activity shown by complex **23**,cell-line studies have also been carried out to explore its anticancer property.

Usually, the complexes integrated with highly conjugated or extended aromatic systems result into remarkable DNA cleavage in combination with CuII species. For example, complex **24** bearing anthracenyl-tethered tpy derivative displayed efficient intercalation with DNA and acted as ‘self-activating’ chemical nuclease (Figure 8).36 The radical scavenging and ROS quenching studies clearly exhibited the key participation of singlet oxygen into cleavage process. Complex **24** also displayed cytotoxic effects in different cancer cell lines; however, no cytotoxicity could be realized in the normal live cells.

The substituted bpy derivatives have also been utilized as co-ligands in order to improve the ‘self-activating’ nuclease activity of different metal complexes. Gama and group demonstrated37 a series of complexes **25a-25d** derived from substituted bpy and tpy scaffolds. The bipyridine moiety has been substituted with different cyclic amines in these complexes (Figure 8). The spectral analyses suggested a good to excellent binding of **25a-25d** with duplex DNA in absence of an exogenous redox agent. An oxidative cleavage process has been established in this study. The relatively more DNA cleavage ability shown by **25b** and **25c** has been attributed to the increased electrostatic interaction between the protonated cyclic amines and phosphodiester moiety of DNA; however, in **25d**, the weaker electrostatic attraction was ascribed to charge delocalization. All the complexes **25a-25d** displayed high cytotoxic effect in A2780 cell lines. The ternary complexes exhibited an effective penetration with cell membrane and a clear localization could be seen *via* cell uptake screening. Moreover, **25a-25d** displayed remarkable cellular activity with A2780cisR cell lines along a weaker resistance factor in comparison to cisplatin.



**Figure 8.** Chemical drawings of terpyridine-based copper complexes **23-25**. Relaxation of pUC19 DNA by 1–3 after 24 h incubation. a) Lane 1: DNA only; lanes 2–5: DNA+10, 20, 50, 100 mm of 1.

1. **Thiazole/imidazole based chemical nucleases**

Chemical nucleases based on thiazole derivatives have also gained huge attention of researchers in this field.38 Reedijk and group reported39 complexes **26** and **27** consisting of thiazole derivatives that displayed DNA cleavage (*φ*X174 phage DNA) with and without redox agent (Figure 9). The solvated (DMF coordinated) complex **27** was easily synthesized by preparing a solution of **26** in DMF (*N,N*’-dimethylformamide). DNA cleavage analyses together with some radical scavenging studies exhibited the absence of diffusible radicals in reaction medium, and thus, DNA fragmentation could be attributed to the non-hydrolytic process. Most likely, the untrapped ROS participated in the clevage process. The lability of bromide ions in **26** offered some additional sites present for interaction with biological species and molecular oxygen. The redox characteristics of **26** led to the dioxygen activation *in-situ* that indicated a CuIII-radical inducing the oxidative cleavage process. The free ligand alone displayed high cytotoxicity for selected L1210 and A2780 cancer cell lines, comparable to **26** as well as cisplatin; however, the ligand alone did not promote any DNA cleavage activity.

Chao’s group40 demonstrated a series of Cu(II) complexes **28a-28c** acting as ‘self-activating’ nucleases under dark conditions. These complexes interacted with DNA *via* groove binding (Figure 9). The nuclease activity was remarkably enhanced (*ca.* 10-fold) when an external redox agent was used. The mechanistic investigations clearly revealed the *in situ* reduction of dioxygen (O2) to H2O2 which was initiated by Cu(II) in **28a-28c**. Comet assay further validated the key participation of Cu(II) in DNA segmentation process. Complexes **28a-28c** displayed good antitumor activities when incubated with different cancer cell lines such as HeLa, BEL-7402 and HepG2 cells.



**Figure 9.** Chemical drawings of copper complexes **26-28**.

1. **Hydrazones based chemical nucleases**

Earlier investigations clearly suggest that the metal complexes derived from hydrazone-based ligands can strongly interact with DNA to eventually exhibit remarkable DNA cleavage activities.41 Our group, for the first time, reported42,43 a series of complexes **29-31** hiving different hydrazone derivatives (Figure 10). Complexes **29-31** showed ‘self-activating’ nuclease response; however, the activity was improved with the addition of redox agent. Complex **29** involved the generation of a new species with calf-thymus DNA (ct-DNA) as evident by UV-Vis analyses. The concentration variations as well as the time-dependent cleavage of supercoiled pBR322 DNA by complex **29** is shown in Figure 10(a). The involvement of either singlet oxygen or hydroxyl radical has been reported as the mode of the cleavage process shown by complexes (Figure 10b).



**Figure 10.** Chemical drawings of hydrazone-based copper complexes **29** -**31**. Gel electrophoresis diagrams for the cleavage of supercoiled pBR322 DNA (100 ng) by complex **29** with 1.5h incubation at37 °C. (a) Concentration variation: Lane 1, DNA control; lane 2, DNA+10% DMF; lanes 3–9, DNA + **29** =10, 25 40, 50, 60, 80, 100 μM respectively, lanes 10–14, DNA + 29 + incubation time 5, 15, 30, 60 and 90 min respectively. (b) Nuclease by **29** (50 μM) in presence of radical scavengers (20 mM): lane 1, DNA; lane 2, DNA + Cu2+ salt (100 μM); lane 3, DNA+**29** (50 μM); lane 4–10, DNA+ **29** + DMSO, ethanol, urea, catalase (1 U), D2O, L-histidine, NaN3, respectively.

1. **Cyclen derivatives as self-activating chemical nucleases**

Cyclen (i.e., 1,4,7,10-tetraazacyclododecane) and related derivatives show strong binding with different metals, and they have been widely utilized in the binding and cleavage of DNA.44 In a recent study, Kulak and group45 investigated the Copper-dependent oxidative cleavage of DNA using cyclen derivatives. The ability order for DNA cleavage was depicted as oxacyclen > sulfacyclen > cyclen.

Lin and group46 presented another cyclen-based complex **32** functionalized with Merrifield Resin (MR) to for potential nuclease activity (Figure 11).Complex **32** served as a highly efficient ‘self-activating’ metallonuclease, and it could successfully be applied upto four cycles with no loss in its cleavage efficiency. The mechanistic examination exhibited that the DNA fragmentation occurred *via* oxidative route involving either singlet oxygen or similar ROSs.

This group further reported47 another series of cyclen-based complexes (i.e., **33a**, **33b** and **34**) tethered with different substituents such as methyl, benzyl and anthracenyl (Figure 11). These complexes showed strong DNA binding affinity and concomitant ‘self-activating’ oxidative DNA cleavage activity. The cleavage activity was superior in the case of complex **34** having planar anthracene unit in the side chain. DNA cleavage experiments carried out using radical scavengers clearly indicated the role of either singlet oxygen or similar reactive species (Figure 11b).



**Figure 11.** Chemical drawings of cyclen-based copper complexes **32-34**. Agarose gel electrophoresis diagram for pUC 19 DNA (7 µg/mL) cleavage by **34**; (a) Time dependent: Lane 1: DNA control, Lanes 2–6: DNA + **34** + 1,2, 4, 8 and 12 h, respectively. (b) Inhibition studies: Lane 1: DNA control; Lane 2: DNA + **34**; Lanes 3–6: DNA + **34** + 2.5 mM of NaN3, SOD, *tert*-butyl alcohol, DMSO respectively.

Another Cu(II)-cyclen based “self-activating” nucleases **35a-35c** were demonstrated by Li *et* al. (Figure 12).48 The incorporation of ferrocene unit in cyclen resulted in strong DNA interaction and high cleavage efficiency. The cleavage occurred without using any exogenous redox agent. Complex **35c** having hydroxymethyl group (R= -CH2OH) group in spacer group showed the highest DNA cleavage activity among others. The involvement of singlet oxygen was found mainly responsible for cleavage process, as evident by mechanistic investigation. The same group presented49 ferrocene-bridged complexes **36a** and **36b** exhibiting efficient nuclease activity *via* self-activation (Figure 12). Similar to **35c**, complex **36b** integrating -CH2OH with spacer unit exhibited superior DNA cleavage in comparison to complex **36a**. The existing electrostatic attraction between the phosphate backbone of DNA and **36a** have been corroborated by electrochemical studies. The cleavage experiments performed using several radical scavengers indicated that either singlet oxygen or the hydroxyl radical, or a combination of both may be responsible for the obtained nuclease activity.

The incorporation of acridine groups (1 or 2 units) in such cyclen derivative possessed a strong DNA binding via intercalative mode.50 Complexes **37** and **38** based on acridine-cyclen ligand scaffolds served as excellent “self-activating” nucleases under physiological conditions (Figure 12). The gel electrophoresis diagrams for pUC19 DNA cleavage shown by **37** and **38** in presence of radical scavengers, time-dependent cleavage and concentration variation are shown in Figures 12a-12c. The mechanistic investigations clearly suggested the existence of oxidative DNA cleavage via singlet oxygen species.



**Figure 12.** Chemical drawings of cyclen-based copper complexes **35-38**. Agarose gel electrophoresis diagrams for pUC19 DNA cleavage by copper complexes **37** and **38**: (a) Mechanistic investigation on cleavage of pUC 19 DNA (7 mg/mL) by **37** (143 µM) for

12 h. lane 1, DNA control; Lane 2, DNA + **37**; Lanes 3-5, DNA + **37** + presence of tert-butyl alcohol, DMSO and NaN3 (0.1 M) respectively. (b) Time dependant cleavage in presence of **38** (72 µM): lanes 1, DNA control; lanes 2-5, DNA + **38** + reaction times of 3, 6, 9 and 12 h, respectively. (c) Concentration effect by **38** (72 µM) in 12 h: lane 1, DNA control; lanes 2-4, DNA concentrations of 36, 72 and 107 mM, respectively.

1. **Summary and Conclusions**

The present chapter covers a variety of mononuclear copper complexes serving as ‘self-activating’ chemical nucleases. The subsequent sections have been categorised based on their ligand systems. The DNA cleavage ability of these ‘self-activating’ chemical nucleases typically occurs by the production of ROS such as superoxide ion (O2-·), hydroxyl radical (·OH), singlet oxygen (1O2)/singlet oxygen like species and H2O2. In some cases, transient metal bound species was also responsible for DNA cleavage. These ‘self-activating’ chemical nucleases based on copper complexes presented in this chapter will be useful in the development of future metal complexes based chemical nucleases.

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