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Photocytotoxic organometallic compounds

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Organometallic compounds have recently found applications in medicinal chemistry and as diagnostic tools in chemical biology. Naturally occurring biomolecules, viz., cobalamine, NiFe hydrogenase, Acetyl-CoA synthase, etc., also contain metal-carbon bonds. Among organometallic compounds having medicinal importance, (arene)ruthenium complexes, radioactive technetium complexes and ferrocene conjugates are notable ones. Applications of photoactive organometallic complexes or metal complexes conjugated with an organometallic moiety are of recent origin. Photodynamic therapy (PDT) is a promising method to treat cancer cells in presence of light. This review primarily focuses on different aspects of the chemistry of organometallic complexes showing photocytotoxic activities. Half-sandwich tungsten, iron or ruthenium complexes are known to show photonuclease and/or photo-crosslinking activity. Photoinduced organometallic CO releasing molecules also exert photocytotoxic activity. Attempts have been made in this review to highlight the photocytotoxic behavior of various metal complexes when conjugated with a photoactive organometallic moiety, viz., ferrocene.

Keywords: Medicinal chemistry, Organometallic compounds, Ferrocene, DNA photocleavage, Photocytotoxicity

Current research interest on metal based drugs stems from the discovery of cisplatin and its promising anticancer activity against various cell lines.[1-3] Established platinum-based anticancer agents used for the treatment of a variety of tumors are cisplatin, carboplatin, oxaliplatin, satraplatin, etc.[4] The chemotherapeutic action of these platinum-based drugs is associated with side effects, viz., toxicity, drug resistances, etc. This has generated interest in the search for alternative anticancer drugs with better pharmacological profile while maintaining the therapeutic efficacy. The use of organometallic compounds could be an interesting approach for the development of non-platinum anticancer drug. The current trend on using and developing water and air stable organometallic compounds in biological applications is rapidly growing. Naturally occurring bio-molecules like cobalamins (vitamin B_{12} and its derivatives), the hydrogenase enzyme family (H2ases) and intermediates during catalysis of acetyl coenzyme A synthase (ACS) and carbon monoxide dehydrogenase (CODH) provide impetus to develop the chemistry of chemotherapeutic bioorganometallics.[5-8] Organometallic technetium complex (Cardiolite®) and orthocarboxybenzoyl ferrocene are tested as myocardial radio imaging agent and for iron deficient gum diseases.[9,10] The importance of biologically active organometallic conjugates is highlighted in several recent articles.[10-15] Incorporation of an organometallic ferrocenyl moiety into breast cancer drug tamoxifen or into anti-malarial drug chloroquine showed promising results.[16,17] Half-sandwich (arene)ruthenium(II) complexes are extensively studied and some of the complexes have shown promising anticancer activity.[18-21] Organometallic compounds are also tested for photoactivated chemotherapy like photodynamic therapy (PDT) in which the drug molecules are activated by photo-irradiation of suitable wavelength of light.[22-24] Photofrin®, an oligomeric mixture of porphyrin, is one of the marketed PDT drug which suffers from side effects like skin sensitivity and hepatotoxicity.[25,26] Designing new metal-based PDT agents having lesser side effects and good therapeutic efficacy thus assumes great importance. The use of stable photoactive organometallic compounds for this purpose could be an interesting approach. Incorporation of an organometallic moiety into various organic and inorganic conjugates is likely to introduce some additional effect and could enhance their biological activity like those observed for ferrocifen and ferroquine.

Medicinal Importance of Organometallic Compounds

Organometallic compounds are generally considered as air sensitive and unstable in aqueous medium. Some organometallic compounds, however, are stable in air and water and offer interesting medicinal properties.[11] Organometallic compounds having anticancer activity include mainly the titanocene dichloride derivatives, ruthenium arene complexes, ferrocene conjugates, etc. A brief overview of the naturally occurring and medicinally important organometallic compounds is given to the following section.

Naturally Occurring Organometallic Compounds

Organometallic compounds in nature act as cofactors, active sites or intermediates in various bio-molecular transformation reactions. Cobalamins constituting a family of vitamin B_{12} are the prominent example of naturally occurring bioorganometallics.[5,27] Co-enzyme B_{12} catalyses 1,2-rearrangement and methyl group transfer. The homolytic cleavage mechanism of Co(III)-alkyl bond in cobalamin is now used to develop various prodrug candidates.[28] Other naturally occurring bioorganometallics include hydrogenase enzyme family (H_2ases), acetyl coenzyme A synthase (ACS) and carbon monoxide dehydrogenase (COdH). Ni/Fe containing organometallic hydrogenases found in archaebacteria are known to catalyze the conversion of molecular dihydrogen into protons. The Fe centre in these enzymes is coordinated to one CO and two CN ligands.[6] Acetyl coenzyme A synthase in the acetate producing bacteria acts as nature's bioorganometallic catalyst. The enzymes having Ni/Fe core form Fe-CO intermediates during acetate synthesis. The CO required for the acetate generation results from the inter-conversion of CO and CO_2 by carbon monoxide dehydrogenases(COdH's) enzymes.[7,8]

Titanocene dichloride derivatives

Metallocene dichloride compounds like titanocene dichloride (η-$C_5H_2)_2TiCl_2$, having two labile chloride in *cis*-position in a similar way as in cisplatin and undergoing hydrolysis to form DNA crosslinks, have received considerable attention as cisplatin alternatives (Fig. 1).[29] It was believed that the titanocene dichloride could bind to DNA in a similar way as cisplatin and induce apoptosis of the cancer cells. The anticancer activity of titanocene dichloride *in vitro* and *in vivo* in animal models showed promising results and the compound was taken up for clinical trial (Phase II). However, the mode of action of this compound is unclear as the formulation of the drug is not fully established in the cellular medium.[30] Due to the poor aqueous solubility and decomposition of titanocene dichloride in cellular medium, the trial has been abandoned. Benzyl substituted methoxy phenyl titanocene derivative is found to be one of the promising drug candidates towards kidney cancer LLC-PK cell line.[31a] Recently developed chelating oxalititanocene derivative is found to be more stable towards hydrolysis and is ~13 fold more cytotoxic than its dichloro analogue (Fig. 1).[31b]

(Arene)ruthenium complexes

Arene complexes of ruthenium(II) form another important class of organometallics that are studied extensively and the chemistry is reviewed by different research groups.[18-21] These complexes are generally less toxic than platinum-based drugs and are also effective in platinum resistant cancer cells. Ruthenium arene complexes can form mono-functional adduct with DNA. Sadler and coworkers[18] studied the hydrolytic behaviour of the ruthenium complex, viz. [Ru(η^6-arene)(en)Cl] (en, ethylenediamine ligand), in an aqueous medium and showed covalent adduct formation with guanine bases (Fig. 2). *Trans*-[tetrachlorobis(1H-indazole)ruthenate(III)],

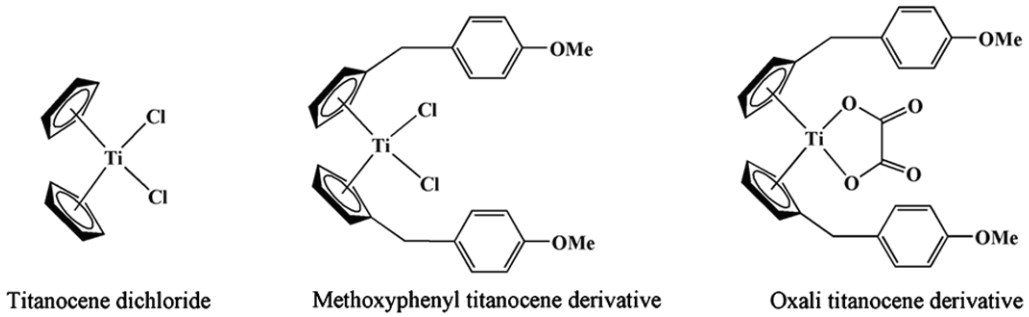

Fig. 1 – Structures of the titanocene dichloride and its derivatives.

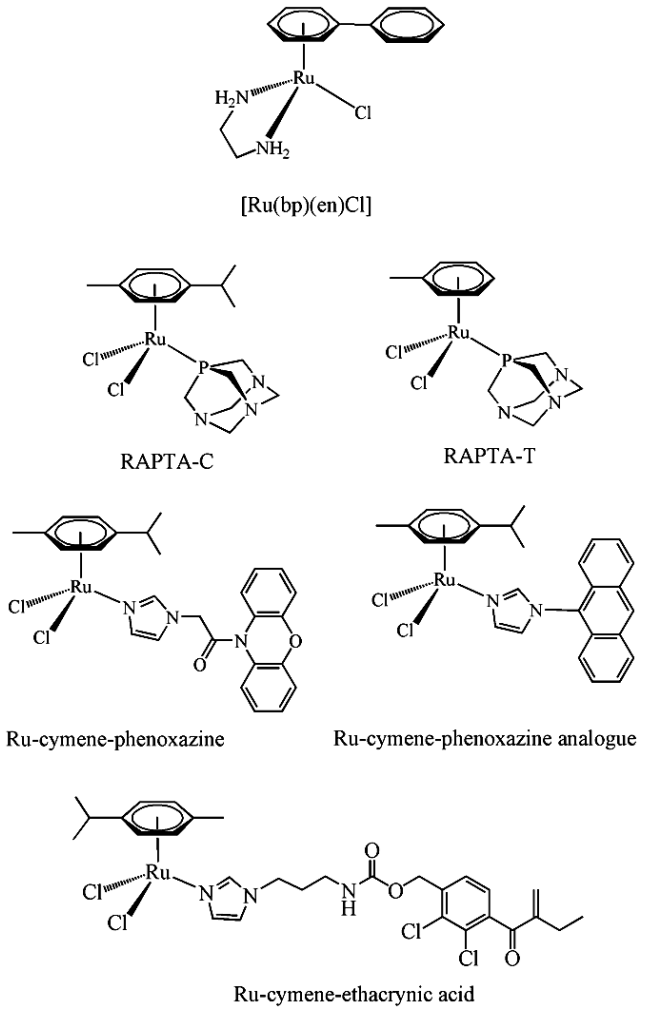

Fig. 2 – Molecular structures of the (arene)ruthenium-based anticancer drug candidates.

KP1019 and *trans*-[tetrachloro(dimethylsulphoxide)-(1H-imidazole) ruthenate(III) (NAMI-A) are two advanced level ruthenium-based drug candidates which are in clinical trial.[32]

Dyson and coworkers[33] developed a number of organometallic ruthenium arene complexes like [Ru(η^6-arene)Cl_2(pta)] (pta = 1, 3, 5-triaza-7-phosphatricyclo-[3.3.1.1]decane) that target various proteins/enzymes (Fig. 2). RAPTA complexes are less cytotoxic *in vitro* than the platinum-based drugs. In addition, *in vivo* studies of RAPTA-C and RAPTA-T indicate significant inhibition of lung metastases in CBA mice bearing the MCa mammary carcinoma. RAPTA-T complex is also a selective antimetastatic agent like NAMI-A.[34] Ruthenium arene complexes of imidazole functionalized phenoxazine are potent cytotoxic agents inhibiting the activity of P-glycoprotein (Pgp), a multidrug resistance protein.[35] Its corresponding anthracene structural analogue is also highly effective and accumulates inside the cell nucleus. Ruthenium arene complexes of ethacrynic acid functionalized imidazole are effective glutathione-S-transferase (GST) inhibitors and are significantly active cytotoxic agents compared to cisplatin or other ruthenium(II) complexes.[36]

Similar to ruthenium arene complexes, the osmium arene complexes are known to exhibit potential anticancer activity in nanomolar range towards A2780 ovarian cancer cells.[37] The arene moiety plays an important role in cellular uptake, nuclear uptake and cytotoxic activity.[38] Sadler and coworkers[39] recently developed a series of half-sandwich iridium(III) complexes having potential anticancer activity. The iridium complexes are capable of forming adduct with 9-ethyl-guanine and some complexes are shown to accumulate inside the nucleus. Apart from anticancer activity, the ruthenium-based organometallic compounds can act as structural scaffolds for kinase inhibitors with anti-metastatic activity (Fig. 2).[40] Protein kinases are enzymes that are capable of transferring phosphate groups to substrate from ATP to control various cellular processes. Megger and coworkers[41] developed various organometallic ruthenium(II)-based kinase inhibitors showing nanomolar and even picomolar ATP-competitive inhibition modeling staurosporine, a known natural kinase inhibitor.

Ferrocene conjugates

The medicinal importance of ferrocene conjugates arises from its stability in biological media, lypophilicity and unique redox activity. The biological activity of ferrocene is reviewed in the literature.[42] The one-electron oxidized product of ferrocene [(η^5-$C_5H_5)_2$Fe] (Fc), i.e. ferrocenium ion (Fc^+), exhibits interesting biological properties. Ferrocene molecule itself is nontoxic but the ferrocenium ion is toxic in various cancer cell lines.[43] The potential of the anticancer activity of the picrate and trichloro acetate salts of ferrocenium ion on Ehrlich ascites tumor (EAT) cell lines in mice was first observed in 1984.[44] Osella and coworkers[45] studied the cytotoxicity of various ferrocenium salts on Ehrlich ascites tumors in mice (Fig. 3). The Fe(III) 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. Stable decamethylferrocenium salt ($DEMFc^+BF_4^-$) exhibits cytotoxicity in MCF-7 breast cancer cells giving an IC_{50} value of 35 μ*M* (Fig. 3).[46]

$DEMFc^+$ is also able to increase the level of 8-oxoguanine (oxidized form of guanine) indicating oxidative DNA damage. Formation of reactive hydroxyl radicals was observed from EPR analysis in aqueous buffered medium. The positively charged ferrocenium ion in a nucleophilic solvent is believed to undergo decomposition to generate radical species. However, the mechanism of action of the ferrocenium derivatives is still not clear and the possible targets of the reactive species could be the nuclear DNA, cell membrane or the topoisomerase II enzyme.

Another chemotherapeutic application of the ferrocenyl moiety is in the treatment of breast cancer. Tamoxifen is a chemotherapeutic agent for (ER+) breast cancer, but the drug is ineffective in tumors that lack estrogen receptor (ER-). Jaouen and coworkers[16] substituted the beta phenyl ring of tamoxifen by a ferrocenyl moiety and the resulting compound named as "ferrocifen" is shown to be more active than tamoxifen and also effective in estrogen independent (ER-) cancer cells (Fig. 4). The mechanism of action of both ferrocifen and tamoxifen in hormone dependent cells are the same. The mechanism of action of ferrocifen in ER(-) cell lines involves a series of redox processes initiated by oxidation of the ferrocenyl moiety and finally generating quinonemethide which is susceptible to form adduct with DNA, GSH or proteins.[47] In a similar way, the anti-malarial drug chloroquine on conjugation with ferrocene becomes more effective and active against chloroquine resistant strains.[48] Apart from these two cases, various other ferrocenyl conjugates are reported to show biological activities like antibacterial and anticancer activities.[49]

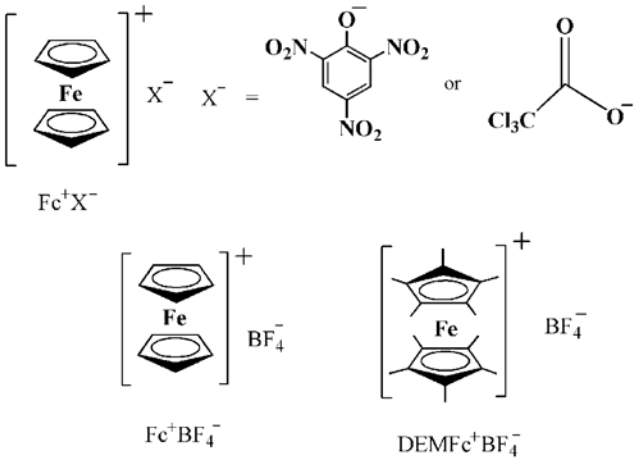

Fig. 3 – Molecular structures of the ferrocenium ions used for antiproliferative activity.

Photocytotoxic Organometallic Complexes

The molecules that show photoactivated cytotoxic activity are of importance for their selectivity in killing cancerous cells over normal cells. When compared to the coordination complexes, there are only a few reports on the organometallic complexes showing photo-induced biological activity. The organometallic complexes that are studied extensively include a variety of half-sandwich cyclopentadienyl complexes of iron and tungsten, (arene)ruthenium and related complexes, cobalt alkyl complexes, CO releasing molecules and bimetallic ferrocene conjugated transition metal complexes. Unlike coordination complexes, the organometallic complexes could induce DNA scission via different mechanistic pathways, e.g. alkyl radical pathway to abstract proton from the deoxyribose sugar or by DNA nucleobase modification. Amongst the four nucleobases, guanine is most susceptible to react with methyl radical to form methylguanine, methoxyguanine and 8-oxo-guanine. Photoactivation could result in ligand dissociation inducing cytotoxicity. Conjugation of an organometallic moiety to the photoactive metal complexes could enhance their photo-chemotherapeutic effect.

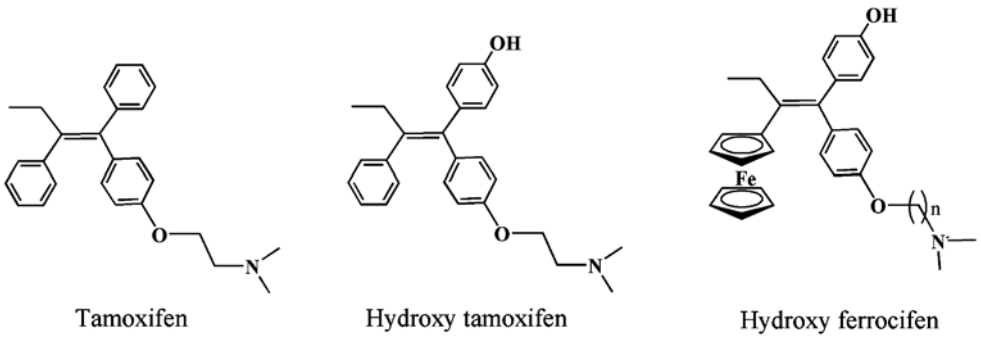

Fig. 4 – Molecular structure of tamoxifen, its active metabolite and ferrocifen (active form).

Photodynamic therapy (PDT)

Photodynamic therapy is a novel approach for selective damage of the cancerous cells by using light, leaving unexposed healthy cells unaffected (Fig. 5).[22-24,50] The advantage of phototherapy over other therapeutic methods is that the drug is not active until it is activated by photo-irradiation. Light of wavelength 620-850 nm is required in PDT to activate the drug molecules. The molecules on photoactivation get excited to higher energy singlet state (S_1) from where it can either come to the ground state (S_0) by emitting the absorbed energy or move to the excited triplet state (T_1) through intersystem crossing (ISC). With higher life time in the triplet state, the molecule in T_1 state can undergo various photochemical processes to generate radical species or it can transfer energy to molecular oxygen ($^3O_2$) to form reactive singlet oxygen species, $^1O_2$ (Fig. 6).[51] Photofrin®, a porphyrin-based FDA approved PDT drug follows singlet oxygen pathway on photoactivation with a red light of 633 nm.[22] The problems associated with photofrin is prolonged skin sensitivity and hepatotoxicity.[25,26] Photoactive organometallic complexes could be suitably designed as new PDT agents. Various organometallic complexes are known to undergo photo-induced ligand dissociation or homolysis of metal carbon bond, etc., and such photoactive organometallic compounds could be developed as PDT agents.[52-54] As observed for ferrocifen and ferroquine, conjugation of an organometallic moiety to phoactive organic or inorganic molecules could enhance the photocytotoxic activity of the compounds and could provide alternate cellular damage pathways. The chemistry of different types of photoactive organometallic complexes is discussed below.

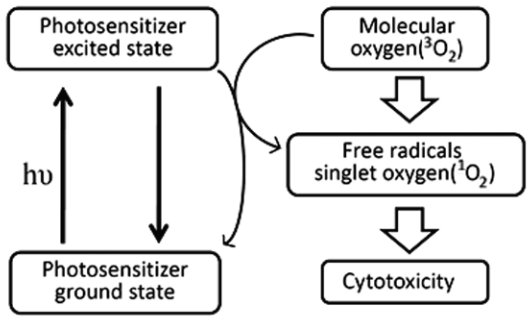

Fig. 5 – Schematic diagram of photodynamic therapy.

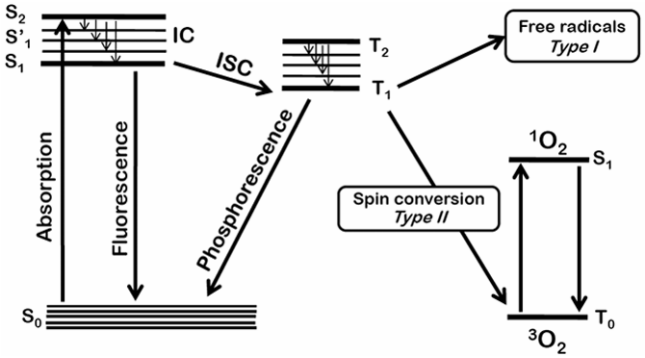

Fig. 6 – Simplified Jabłoński diagram showing various physical and chemical processes involved in PDT.

Photoactive cobalt-alkyl complexes

Organometallic compounds with a cobalt-carbon bond are biologically important, being the key intermediate in vitamin B_{12} mediated enzymatic reactions.[55] The photochemistry of the Co(III)-CH_3 bond is well known in the literature and this moiety is responsible for various biological activities. Riordan and coworkers[54] studied the plasmid DNA photocleavage activity of aqueous and air stable alkyl cobalt complexes of the formulation $[Co(Cyclam)Me(H_2O)](ClO_4)_2$ (where Cyclam is 1.4.8.11-tetraazacyclotetradecane), in ordinary light. In the presence of light, the Co(III)-CH_3 bond cleaves to generate $^{\bullet}CH_3$ radicals which is capable of cleaving the DNA (Fig. 7). The presence of TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxide) as an alkyl radical quencher reduces the DNA photocleavage activity indicating the formation of methyl radical from Co(III)-CH_3 bond homolysis. To eliminate the possibility of hydroxyl radical formation the authors used ethanol as a hydroxyl radical scavenger during DNA photocleavage reaction; the presence of ethanol did not inhibit the DNA cleavage activity. HPLC analysis of the photolyzed product of the complex in presence of sperm DNA showed the liberation of all four DNA nucleobases (A, T, G and C), indicating the nucleic acid oxidation through proton abstraction from the deoxyribose sugar moiety. The highly reactive photogenerated methyl radical could abstract the proton from the sugar as the bond dissociation energy of CH_3-H is high (104 kcal M^{-1}). Similarly, Hisaeda and coworkers[55]

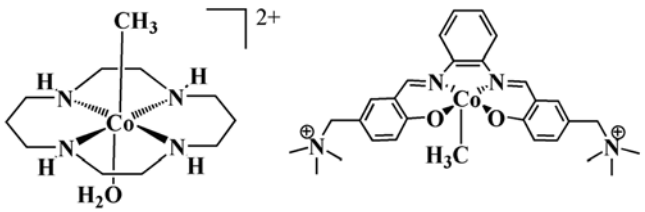

Fig. 7 – Molecular structure of alkyl Co(III) complexes showing photoinduced metal carbon bond cleavage.

developed a new series of water soluble alkyl cobalt complexes having salen type of ligand (Fig. 7). These complexes showed efficient cleavage of pBR322 DNA upon irradiation with a visible light from a 500 W tungsten lamp. Formation of methyl radical as the reactive species was evidenced from the EPR measurements using spin trapping agent DMPO (5,5'-dimethyl-1-pyrroline-*N*-oxide). Disappearance of the methyl group proton signal in the NMR upon photo-irradiation indicates cleavage of the metal-carbon bond.[56]

Shell and Lawrence[57] reported the hydroxyl radical mediated light-induced pBR322 DNA cleavage activity of hydroxocobalamin. Methylcobalamin on photolysis generates methyl radical as well as cobalt(II) species due to the hemolytic cleavage of the Co(III)-CH_3 bond. The Co(II) species thus formed readily oxidizes in the presence of oxygen to form cobalt(III) hydroxo species. The hydroxo cobalamin upon photolysis by UV-A light (>300 nm) from a pyrex filtered mercury lamp generates hydroxyl radicals converting supercoiled DNA to relaxed circular DNA (Fig. 8). The presence of hydroxyl radical scavenger essentially completely inhibits the cleavage reaction but the presence of a methyl radical quencher, viz., TEMPO, does not inhibit the cleavage activity. This clearly indicates that the initial formation of methyl radical quantity is small compared to $^{\bullet}OH$ radical generation. Although these complexes are active only in UV light, the DNA cleavage reaction provides an alternate approach to develop new types of PDT agents.

Photoactive (arene)ruthenium complexes

The cytotoxicity of many ruthenium(II) complexes related to their DNA binding affinity is reported in the literature.[58] The photoactivated ligand dissociation followed by generation of an active intermediate like an aqua complex, that is capable of binding to DNA, is important towards designing photocytotoxic ruthenium(II) complexes. Sadler and coworkers[52] developed a new class of (arene)ruthenium prodrugs showing selective photo-dissociation of the leaving group followed by coordination to a DNA base, viz., guanine. The ruthenium(II) complex has [(*p*-cym)Ru(bpm)(py)]$(PF_6)_2$ formulation, where *p*-cym is *para*-cymene, bpm is 2,2′-bipyrimidine and py is pyridine. This complex on irradiation with visible light of 400-600 nm leads to the dissociation of the Ru–py bond to form a reactive aqua complex (Ru-OH_2), as evidenced from the UV-visible and NMR spectral photolysis data, whereas the complex does not undergo any apparent hydrolysis in dark. The HR-MS and NMR spectral analysis of the aqua complex in the presence of 9-EtG (9-ethyl guanine) indicates the adduct formation at the N7 position of guanine (Fig. 9). The authors further extended the work by introducing receptor targeting peptides like dicarba analogue of octreotide and the Arg-Gly-Asp (RGD) tripeptide to the monodentate pyridyl group which can act as tumor targeting devices.[59] The complex upon blue light (420 nm) irradiation undergoes selective photo-dissociation leading to the release of the pyridyl-derivatized peptides with concomitant formation of the ruthenium aqua complex $[(p\text{-cym})Ru(bpm)(OH_2)]^{2+}$ which is capable of forming the monofunctional adduct with 9-ethyl guanine (Fig. 9). Photoreaction in the presence of DNA sequences or peptide oligonucleotide hybrid leads to the formation of a new type of two monofunctional ruthenium adduct with guanine base (GG chelate) which is caused due to the loss of *p*-cym arene moiety. This type of organometallic complexes does not form any peptide metal adduct indicating the selectivity of the complexes towards guanine bases of DNA.[59]

This strategy has been successfully used for selective delivery of the ruthenium(II) pro-drug by receptor targeting peptide followed by photoactivation. Dinuclear Ru^{II}(arene) complexes of formulation $[\{(\eta^6\text{-indane})RuCl\}_2(\mu\text{-}2,3\text{-dpp})](PF_6)_2$ (where 2,3-dpp = 2,3-bis(2-pyridyl)pyrazine) have been reported (Fig. 10).[60] The complex with a labile

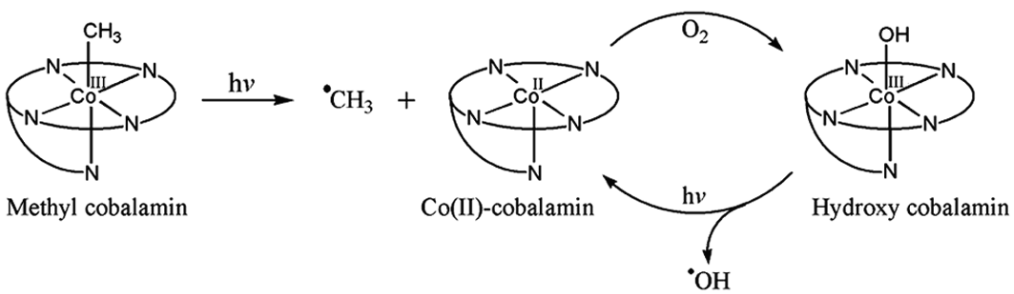

Fig. 8 – Photolysis of methyl cobalamin and hydroxyl cobalamin.

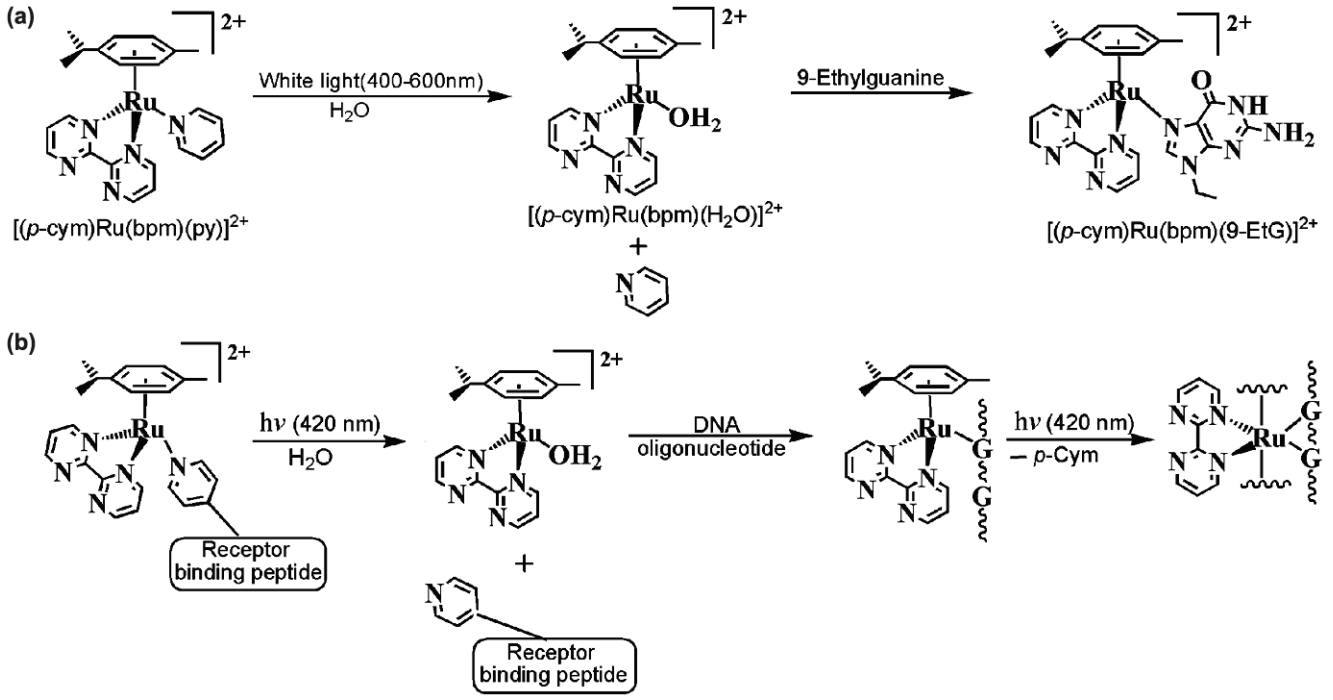

Fig. 9 – The schematic diagram showing photoinduced ligand dissociation of (arene)ruthenium(II) complexes and adduct formation with guanine base.

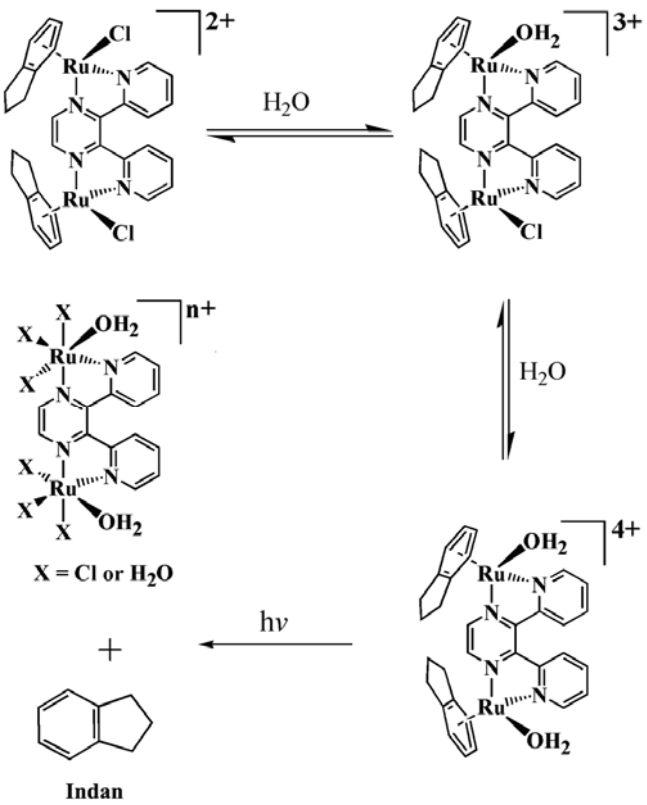

Fig. 10 – The schematic diagram showing photoinduced release of fluorescent indan from (arene)ruthenium(II) complex.

Ru-Cl bond undergoes hydrolysis in dark to give a Ru–OH_2 complex in solution and forms DNA interstrand crosslinks to a small extent. The frequency of cross linking gets significantly enhanced upon photo-irradiation with a UVA light due to the loss of an arene indane moiety. The fluorescence emission spectrum (λ_{ex} = 260 nm) of the photolyzed product is very similar to that of free indane suggesting the loss of an indane moiety. UV-visible and NMR spectral changes of the solution upon photo-irradiation also suggest the loss of the indane moiety. The binuclear (arene)ruthenium(II) complexes upon photo-irradiation form DNA crosslinking as well as produce fluorescent marker (free indane) which can be used to trace the molecule inside the cell. Photolysis of this complex in absence of oxygen also results in similar observations. These complexes are thus important for cellular applications in hypoxia selective cancer cells.

(Arene)ruthenium(II) complexes as PDT agents

Therrien and coworkers[61,62] reported a new class of (arene)ruthenium(II) conjugated tetrapyridylporphyrin derivative $[Ru_4(\eta^6\text{-arene})_4(\text{tetra-pp})Cl_8]$, having combined photodynamic as well as cytotoxic effects (Fig. 11). The complexes exhibit photocytotoxic activity towards human melanoma cells (Me300)

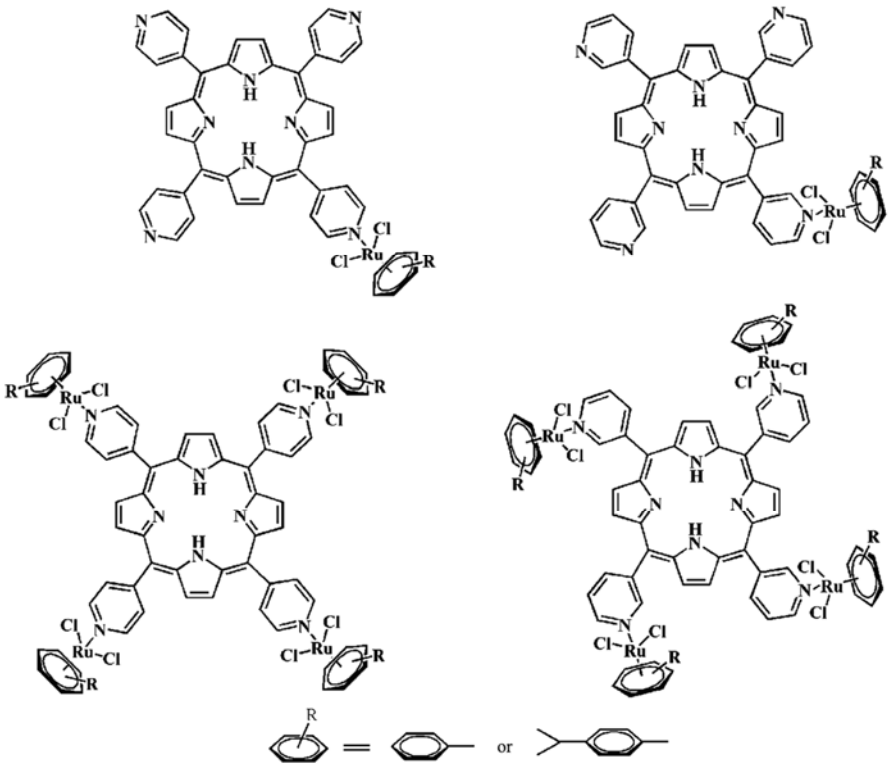

Fig. 11 – Mono and tetranuclear (arene)ruthenium-based porphyrinic PDT agents.

when irradiated with a laser light of 652 nm. The photodynamic activity of these complexes primarily depends on the nature of the pyridylporphyrin isomer (3-pyridyl or 4-pyridyl) and is also found to be dependent on the degree of substitution of the tetrapyrrole ring. 3-Pyridyl photosensitizer is reported to be more active than 4-pyridyl photosensitizer.[62] The LD_{50} values for 5 μ*M* solution of $[Ru_4(\eta^6\text{-arene})_4(\text{tetra-3-pp})Cl_8]$ is less than 0.5 J cm^{-2} whereas the same for the complex $[Ru_4(\eta^6\text{-arene})_4(\text{tetra-4-pp})Cl_8]$ is 5 J cm^{-2}. The fluorescence microscopic studies indicate that the tetranuclear complexes accumulate in the cytoplasm and intracellular organelles and not in the nucleus or lysozyme of human melanoma cells. However, the (arene)ruthenium(II) porphyrin compounds are promising organometallic photosensitizers for their potential applications in PDT.

Photoactive cyclopentadienyl metal complexes

Mohler's group[53,63,64] reported the DNA photocleavage activity of cyclopentadienyl tungsten and iron complexes of formulation $[CpM(CO)_3R]$ (where M = W or Fe and R = CH_3 or Ph) upon photo-irradiation using pyrex filtered mercury arc lamp (450 W). These complexes are able to produce

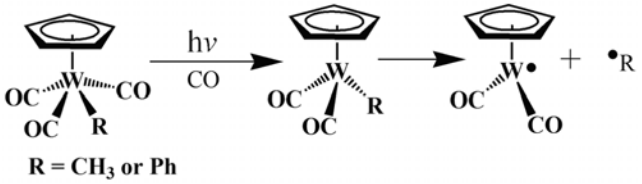

Fig. 12 – Schematic diagram showing the photolysis of cyclopentadienyl tungsten carbonyl complex and generation of alkyl radical.

reactive methyl or phenyl radicals that are capable of cleaving DNA (Fig. 12). Similar to the tungsten complex, the iron analogues of formulation $[CpFe(CO)_2R]$ (R = methyl or phenyl) also produce carbon centered radical species upon photo-irradiation. The iron complexes are found to be more efficient DNA photocleavers than the respective tungsten complexes and exhibit double stranded DNA strand scission activity.[63] The phenyl complexes are more active than the corresponding methyl complexes based on the C-H bond dissociation energy (104 kcal M^{-1} for methane and 110 kcal M^{-1} for benzene). The photogenerated carbon centred radicals abstract proton from DNA sugar moiety and induce DNA strand scission.[64] However, the cleavage activity of this type of compounds is less due to the lack of having any DNA recognition moiety.

This group further extended their work by introducing a polyamine type netropsin analogue to the cyclopentadienyl ring of the tungsten complex $[CpW(CO)_3(CH_3)]$ and to the phenyl ring of the complex $[CpW(CO)_3(Ph)]$.[65] In both the cases the complexes bind preferentially to the AT rich region of the DNA giving K_{app} values of ~10^6 M^{-1} from ethidium bromide displacement assay and the efficiency of the DNA cleavage activity is shown to increase significantly. In spite of selective DNA binding using the netropsin analogue, the Cp ring functionalized complexes do not show any sequence selective DNA strand scission indicating the generation of diffusible methyl radicals. The DNA binding (phenyl)tungsten complex, however, exhibits DNA photocleavage activity in a sequence specific manner indicating formation of non-diffusible phenyl radical as it is bounded to the netropsin analogue (Fig. 13).[65]

Diiron complexes of 1,3- and 1,4-$[Fe_2Cp_2(CO)_4(Ph)]$ are reported.[66] The complexes at higher concentration are able to linearize the plasmid DNA and show single-strand cleavage at lower concentration. Radical quenching experiments suggest the formation of carbon-centered radical formation. However, these diiron complexes are not more efficient than their respective mononuclear analogues possibly due to their inability to align correctly in the minor groove of duplex DNA. Molybdenum complexes $[MoCH_3(\eta^3\text{-allyl})(CO)_2(phen)]$ having allyl moiety are reported to show dual DNA damage pathways, viz., backbone cleavage and base modification.[67]

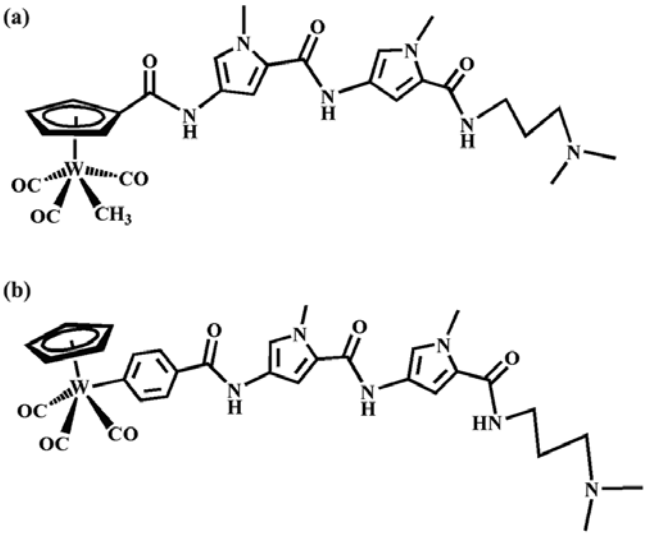

Fig. 13 – Netropsin analogue of (a) $[W(CO)_3(CH_3)]$ and (b) $[CpW(CO)_3(Ph)]$ for diffusible and non-diffusible carbon centered radicals.

The backbone cleavage is nonspecific and occurs through H1′ and/or H5′ abstraction from the deoxyribose sugar and base modification leading to G-specific cleavage, which generates methylguanine, methoxyguanine and 8-oxo-guanine.

CO releasing molecules

Carbon monoxide is an important small diatomic molecule acting as a signalling mediator in human body.[68] CO can exhibit anti-inflammatory, anti-apoptotic, and anti-proliferative effects.[69] CO being toxic, the photoinduced CO release from metal carbonyl complexes in a controlled manner could be used to monitor various activities in biological systems. Schatzschneider and coworkers[70] studied the photoinduced CO releasing property, cellular uptake and photocytotoxic activity of a tricarbonyl manganese complex $[Mn(CO)_3(tpm)](PF_6)$ (tpm, tris(pyrazolyl)methane) in HT-29 human colon cancer cells (Fig. 14). The photorelease of CO from the complex was studied from UV visible absorption spectral changes in aqueous buffered solution of horse skeletal muscle myoglobin ($MbFe^{II}$) upon 365 nm light irradiation. The appearance of two new absorption bands at 542 nm and 577 nm upon photo-irradiation indicates the formation of $MbFe^{II}CO$ complex. Using the molar extinction coefficient value of $MbFe^{II}CO$, the authors suggested the release of two CO out of three CO from $[Mn(CO)_3(tpm)](PF_6)$. Atomic absorption spectroscopy based cellular uptake assay indicates significant intracellular accumulation of manganese content and the accumulation of the complex inside the cell is reported to be concentration dependent with up to 100 μM showing no saturation indicating the passive diffusion process during the cellular uptake. Specific accumulation of the complex in the nuclear membrane and the nucleolus of the HT-29 cells evidenced from confocal Raman spectroscopic study.[71] Differential cytotoxicity study upon photoirradiation of 100 μ*M* complex is reported with crystal violet assay. A 10 min irradiation reduces

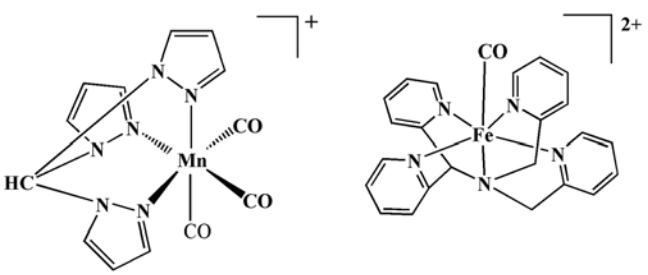

Fig. 14 – Photoinduced CO releasing Mn and Fe complexes.

the cell biomass to ~30% of the controls indicating significant photocytotoxicity against the human colon cancer cell line HT29. The photocytotoxic activity is comparable to the known cytotoxic agent, 5-fluorouracil (5-FU). The tris(2- and 4-imidazolyl) phosphane (tip) analogues of the trispyrazolylmethane complexes with various substituents also exhibit photoinduced CO releasing property.[72] Kodanko and coworkers[73] recently developed a new type of highly stable low-spin iron carbonyl complex $[Fe^{II}(CO)(N4Py)](ClO_4)_2$ which shows CO release. Myglobin assay indicates that the extent of CO release is very slow in dark and becomes very fast upon 365 nm light irradiation. The complex shows potent photocytotoxic activity against PC-3 prostate cancer cells by the generation of two active species, viz., CO and $[Fe^{II}(N4Py)]^{2+}$, in the presence of UVA light of 365 nm. To deliver CO in the tumor specific cells the authors attached short peptide chain with this iron carbonyl complex.

Photocytotoxic ferrocene conjugates

Ferrocene is an important organometallic constituent and used in various biological applications. The medicinal importance of ferrocenyl moiety is evidenced from its incorporation in tamoxifen as well as chloroquine.[16,17] The sodium salt of *o*-carboxylbenzoyl ferrocene which is known as "Ferrocerone" is already in clinical use for the treatment of iron deficiency anemia and gum diseases.[10] Apart from biological applications, ferrocene conjugates show very good photochemistry which involves photoinduced charge transfer, electron transfer and oxidative processes. Ferrocene can be used for the development of new ferrocene-based photo-chemotherapeutic agents.[74-76] The ferrocenium ion being a cytotoxic agent, designing new photoactive ferrocene conjugates is a promising area of research. Literature reports on photobiological activity of ferrocene conjugates are rare. Ferrocene conjugated imidazophenanthroline derivative reported from our group is an active DNA photocleaving agent in visible light of wavelengths 476 nm, 532 nm and 647 nm whereas its phenyl analogue is essentially inactive (Fig. 15).[77] The ferrocenyl conjugate cleaves DNA through hydroxyl radical generation. The UV photolysis experiment in aqueous DMF primarily suggests that the ferrocenyl moiety could undergo photooxidation. The imidazophenanthroline derivative of ferrocene shows significant photocytotoxic activity in HeLa cells giving IC_{50} value of 13 μ*M* in visible light of wavelength 400-700 nm, while it is less toxic in dark with an IC_{50} value of 33 μ*M*. The compound induces cell death via apoptotic pathway which is evidenced from Hoechst staining of the HeLa cells.

Photoactive bimetallic ferrocenyl conjugates

Various bio-essential transition metal complexes having low energy visible absorption bands are known to exhibit photo-induced DNA cleavage and cytotoxic activity.[78-80] Ferrocene molecule with the metal having $3d^6$ electronic configuration shows interesting excited state spectral properties. The conjugation of photoactive ferrocenyl moiety could change the excited state behavior of the metal complexes and may lead to homolytic bond cleavage, metal centre reduction, ferrocene oxidation, radical generation, etc. and could generate active species which are very important for therapeutic purposes. The lipophilic ferrocenyl moiety could also make the metal complexes pass through the cell membrane. Hence, designing new bimetallic ferrocene-conjugated transition metal complexes is an interesting approach to develop metal based photosensitizers for their potential applications in PDT. Ferrocene appended copper(II) complexes with various tridentate ligands are reported to show photoinduced DNA cleavage activity and cytotoxicity (Fig. 16).[81] Ferrocene-conjugated terpyridyl (Fc-tpy) copper(II) complexes $[Cu(Fc\text{-}tpy)(B)](ClO_4)_2$ (where B = dipyridoquinoxaline and dipyridophenazine) show an intense charge transfer absorption band at ~540 nm which is essential to observe DNA photocleavage and photocytotoxic activity in visible light. The complexes exhibit significant red light induced DNA scission activity via photo-redox pathway forming reactive $^{\bullet}OH$ radicals, whereas their phenyl analogue lacking the ferrocenyl moiety are less active indicating positive role of the ferrocenyl moiety in the DNA photocleavage reactions. The photolysis study using UV-A light of 365 nm indicates formation of an oxidized ferrocenyl

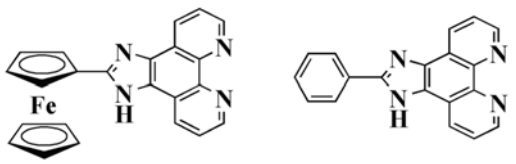

Fig. 15 – Photocytotoxic ferrocenyl imidazophenanthroline derivative and its phenyl analogue.

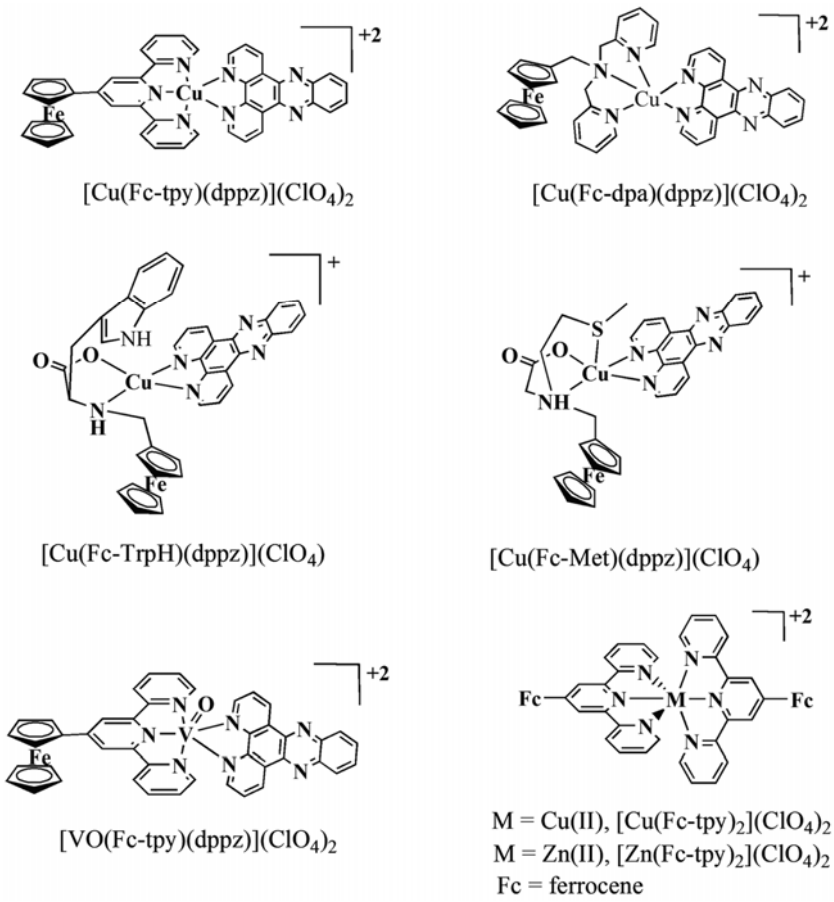

Fig. 16 – Ferrocene-conjugated transition metal complexes showing DNA photocleavage and photocytotoxic activity.

moiety followed by its degradation in the presence of oxygen. Covalent conjugation of the ferrocenyl moiety and the copper centre could be responsible for the observation of an intense charge transfer absorption band and this could make the molecule more photoactive than its control. To rationalize the effect of conjugation, similar copper(II) complexes of a ferrocenyl-dipicolylmethaneamine ligand were reported.[82] These complexes show only two visible absorption bands at 430 and 560 nm due to the presence of ferrocene and copper centered *d-d* band. However, the DNA photocleavage activity of these complexes is less compared to the ferrocenyl-terpyridine copper(II) complexes. The results indicate the importance of conjugation within the bimetallic core on the DNA photocleavage activity. The DNA photocleavage activity of ferrocene-appended L-methionine reduced Schiff base copper(II) complexes of phenanthroline bases is reported and the complexes show significant DNA cleavage activity in red light whereas in absence of the ferrocenyl moiety only L-met copper(II) complex shows lower cleavage activity.[83] Mechanistic study using radical trapping method indicates formation of reactive hydroxyl radicals via photo-redox pathway in presence of light, while the L-met copper(II) complex without any ferrocenyl moiety cleaves DNA via type II pathway forming singlet oxygen species.[84] The results indicate the involvement of the ferrocenyl moiety in the DNA photocleavage reactions. Similarly, the ferrocene-conjugated L-tryptophan copper(II) complexes of phenanthroline bases also show red light-induced DNA cleavage activity via $^{\bullet}OH$ radical pathway.[85] The ferrocenyl ligands alone do not show any DNA photocleavage activity but become active upon binding to the copper(II) centers.

The cytotoxicity of some of the ferrocene-conjugated copper(II) complexes are reported in HeLa or MCF-7 cells in the presence of visible light of wavelength 400-700 nm. The dipyridophenazine copper(II) complex of the ferrocenyl-terpyridine

ligand shows cytotoxicity (IC_{50} = 10.5 μ*M*) against HeLa cells in dark which gets significantly enhanced in presence of visible light giving IC_{50} value of 3.7 μ*M*.[81] Similarly, the cytotoxicity of the ferrocenyl L-tryptophan/L-methionine copper(II) complexes of dipyridophenazine ligand increase significantly upon photoirradiation. The IC_{50} values in dark are: 8.95 μ*M*, 6.10 μ*M* in HeLa cells and 2.99 μ*M*, 4.13 μ*M* in MCF-7 cells for L-trp and L-met copper(II) complexes, respectively. The IC_{50} values in light (400-700 nm) are: 1.29 μ*M*, 4.27 μ*M* in HeLa cells and 0.65 μ*M*, 2.08 μ*M* in MCF-7 cells for the L-trp and L-met copper(II) complexes, respectively. The complexes induce caspase-independent apoptosis in the HeLa cells in visible light. The corresponding zinc(II) complex of the ferrocenyl L-tryptophan reduced Schiff base ligand does not show any photocytotoxicity (IC_{50}, >80 μ*M*) indicating significant dark toxicity of the complexes arising due to the presence of the copper(II) center.[85] Nuclear chromatin cleavage is reported in acridine orange/ethidium bromide (AO/EB) dual staining for the ferrocenyl complexes. Copper(II) complexes exhibiting significant dark toxicity could be due to the formation of reactive copper(I) species by cellular reducing agents like glutathione. The oxidovanadium(IV) complex of ferrocenyl terpyridine ligand is reported to show less dark toxicity.[86] The VO^{2+} complex exhibits significant DNA photocleavage activity in red light of wavelengths 633, 647 and 785 nm. The oxidovanadium(IV) complex is less toxic in dark giving an IC_{50} value of 35.5 μ*M* and significant photocytotoxicity (IC_{50} = 13.4 μ*M*) in visible light.

Binary ferrocenyl terpyridyl complexes of Fe(II), Co(II), Cu(II) and Zn(II) are reported to explore the effect of the 3*d* metal ion on the DNA photocleavage activity and photocytotoxicity.[87] The UV visible absorption pattern of these complexes is similar and the complexes show charge transfer band(s) within 530-590 nm including the Zn(II) complex. The complexes show DNA cleavage activity in visible light via the formation of reactive hydroxyl radicals. The photolysis experiments suggest that the ferrocenyl moiety is susceptible to oxidation in an aqueous medium generating hydroxyl radicals. However, the cellular behavior of the complexes is different in cancer cells. The copper(II) and zinc(II) complexes are cytotoxic, while other complexes showed inactivity. The cytotoxicity of the copper(II) and zinc(II) complexes in HeLa cells gets enhanced upon visible light (400-700 nm) irradiation giving IC_{50} values of 3.1 and 7.5 μ*M*, respectively. The binary copper(II) complex shows significant dark toxicity (IC_{50} = 6.7 μ*M*) whereas the zinc(II) complex exhibits less dark toxicity (IC_{50} = 49.1 μ*M*). The phenyl analogue of the Zn(II) complex is shown to be inactive under similar experimental conditions. The results indicate that the presence of a ferrocenyl moiety is a requirement to achieve significant photocytotoxicity of the zinc(II) complex which shows apoptotic cell death as evidenced from the flow cytometric analysis.

Other Photoactive Organometallic Compounds

There are reports on iridium and rhenium complexes of dipyridophenazine base showing photoinduced DNA cleavage activity. Sheldrick and coworkers[88] studied the DNA binding and DNA photocleavage activity of a series of S-coordinated methionine derivative of iridium complexes having dipyridophenazine ligand. The complexes show double-stranded DNA photocleavage activity due to the close proximity of (η^5-C_5Me_5)Ir^{III} intercalator to DNA in the presence of light from a high pressure Hg lamp (Fig. 17).[88] Similarly, the tricarbonyl rhenium complexes of formulation [Re(dppz or dppn)$(CO)_3$(py)](CF_3SO_3) (where dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine and dppn = benzo[*i*]dipyrido[3,2-*a*:2′,3′-*c*]phenazine) are reported to interact with duplex DNA intercalatively and show oxidative DNA cleavage activity[89] at λ >350 nm. The dppz complex is reported to involve direct oxidation of pBR322 DNA, whereas the dppn complex acts as an oxygen sensitizer producing superoxide and hydroxyl radicals to cleave DNA.

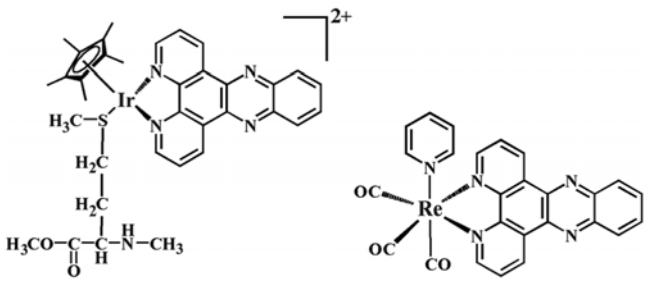

Fig. 17 – Organometallic iridium and rhenium complexes showing photoinduced DNA cleavage activity.

Conclusions and Outlook

This review mainly describes the recent developments of various stable and photoactive organometallic compounds showing various biological activities like photoinduced DNA cleavage activity, cytotoxicity or photo-crosslinking properties for their potential PDT applications. Photoactivation of therapeutic agents offers various advantages like control over their biological activity, selectivity, etc. Organometallic compounds are shown to undergo various photochemical pathways to generate reactive species. Attachment of organometallic moieties to various transition metal complexes are shown to change their mechanism of action. Although the exact role of some organometallic moieties is not fully understood, these moieties are shown to offer various mechanistic pathways that are different from the purely organic or inorganic drug candidates. Organometallic compounds generally show cellular activity in UV light or high energy visible light which are not desirable for any PDT application. The ferrocenyl-appended metal complexes are found to show DNA cleavage activity and photocytotoxicity in red light. The chemistry presented in this review is expected to initiate further studies in design and synthesis of organometallic complexes for their photochemotherapeutic applications within the PDT spectral window.

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