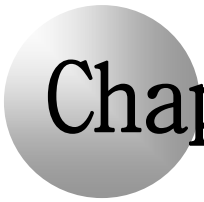
Synergy of intercalation and coordination binding to design novel DNA-targeting antineoplastic metallodrugs

Roy, Sudeshna



Introduction

1.1. Tumours and cancer

Tumor or tumour (originated from Latin 'Tumor' synonymous to swelling) is used to indicate an abnormal swelling of body part regardless of its pathogenesis (pathologic mechanism of development). This uncontrolled proliferation leads to either benign (hyperplasia) or malignant (dysplasia) tumour.[1, 2] By definition a benign tumour does not grow uncontrolled aggressively, does not invade surrounding tissues and does not metastasise. Commonly this type of growth does not possess any serious threat to health if left untreated. In some cases of serious health hazards (space-occupying and constantly growing lesions in vital organs such as the brain), or cosmetic reason (superficial skin or visible lump) the tumours are removed by surgery. A malignant tumour on the other hand is a serious and often lethal ailment. By definition a malignant tumour has the severe potential of invasion to surrounding tissues, including blood vessels and lymphatic channels.[3] In addition the tumour growth is basically uncontrolled and is often prone to metastasise in a distant organ. The major treatment consists of radiation, surgery, chemotherapy or combination of all these three therapies. In addition, some beneficial palliative treatments accompany with the main line treatments.

Cancer is generally reckoned by common people as one of the scariest diseases, but it is not a single ailment. Cancer is defined medically as a group of more than 100 life-threatening diseases which is caused by out-of-control progressive cellular growth.[4] Cancer can occur almost in every body part where cells grow and divide. In addition cancer can affect any human regardless of colour, caste and creed. If the frequency of cancer is looked up globally, a striking observation emerges. In Australia the skin cancer, in Brazil cervical cancer, in China liver cancer, in Canada leukaemia, in Japan stomach cancer, in United Kingdom lung cancer, in USA colon cancer is most prominent. This trend can be related to heredity, life style, exposure to radiation and exposure to carcinogens.

Solid malignancies form lumps and liquid tumours circulate freely in the bloodstream. Cancer can be caused or at least initiated by both external (carcinogens, tobacco and radiation) and internal (hormonal effect, inherited mutations or immune deficiency) factors. Cancer can be broadly classified into four classes as[1] (a) *Carcinoma*-originated from the cells which cover external or internal body surface as ovarian, lung, colon, breast, cervix, prostate etc. (b) *Sarcoma*-originated from the cells of supporting or connective tissues as muscle, bone, cartilage etc. (c) *Lymphoma*-originating from lymphatic

nodes and (d) *Leukaemia*-originated from immature blood cells grown in bone marrow and accumulates in blood stream.

Generally several genes are anticipated to be involved in cancer developments. Firstly, overexpression of oncogenes (damaged genes accumulated in gene sequence) plays a crucial part for cancer induction.[3, 5] When oncogenes are expressed in normal cells, they can induce cancerous growth by instructing cells to synthesise cell growth and division stimulator proteins. Oncogenes are related to healthy genes named as 'proto-oncogenes' that control normal cell-growth. Some of the controlling tools are growth factors, receptors, signalling enzymes, and transcription factors. Growth factors activate signalling enzymes inside the cell after binding to receptors on the cell surface and the activation of transcription factors inside the cell's nucleus takes place. Consequently the activated transcription factors trigger the genes required for cell growth and division. When oncogenes are in control of cellular growth, they transform the growth-signalling pathway to be constantly active and as a result cellular growth-control proteins are produced in an anarchic fashion.

A second group of genes involved in cancer are the '*tumour suppressor genes*'. Tumour suppressor genes are normal genes whose absence can lead to cancer. When a pair of tumour suppressor genes is absent in a cell or inactivated by mutation, often the induction of cancer growth happens. Individuals who are prone to have a cancer frequently inherit one defective copy of a tumour suppressor gene. Genes in an embryo are accumulated as a heritage gift from each parent; therefore a defect in any copy will not necessarily lead to cancer. In case if the normally-functional second copy accumulates mutation, the risk of being cancer-prone is higher. A specific and well-studied tumour suppressor gene activates '*p53*' that can trigger cell suicide called apoptosis.[6] In cells that have undergone DNA damage, the *p53* protein acts like a "turn-off switch" halting cell division. If the damage is irreparable, the *p53* protein automatically initiates cell suicide and prevents the genetically damaged/modified cell from growing out of control.

Another type of genes with prominent significance in cancer is called "DNA repair genes." DNA repair genes signal proteins which correct modifications in genetic sequence prior to cell division. Mutations in DNA repair genes can lead to failure in repair and consequently abnormalities in DNA are accumulated inside the cells. People with an inherited defect called *Xeroderma pigmentosum* have errors accumulated in a DNA-repair gene. This group of people often suffers from skin-cancer after prolonged and continuous

exposure to sunlight. Certain forms of hereditary colon cancer also involve defects in DNA repair.[5]

Cancer is the outcome of accumulation of mutations involving any of the tumour suppressor genes, oncogenes and DNA repair genes. This mutation initiates with single nucleotide changes or deletion (or duplication) of normal DNA sequence.[7] This defect in genetic sequence is passed down to daughter cells and subsequent generations proliferate even more rapidly and this anarchist cycle continues to death if left untreated. Cancer cells therefore acquire some special characteristics as (a) growth in absence of growth stimulatory signals, (b) growth in presence of growth inhibitory signals; (c) avoid the programmed cell death.[8, 9] In addition, cancer cells become angiogenic (formation of new blood vessels) to survive and proliferate. They attract the blood vessels inside the tumour mass to provide essential nutrients, glucose and oxygen uninterrupted and to remove metabolic wastes and CO_2.

The telomeric DNA (which resides at the end of the chromosome) controls important cellular mechanisms such as (a) frequency of cell growth and division and (b) number of cell cycle before death.[4, 6, 10] These specific moieties prevent end-to-end fusion of chromosomes. The normal cells pass through couples of cycles of growth and division, their telomeric DNA gets shorter and ultimately too short to protect the ends of chromosomal DNA. As a result the fusion of telomeres leads to chromosomal merge and the cell death is induced. To avoid this regular sequence cancer cells turn on 'telomerase' (normally expressed only early in embryologic development) and stem cells to a smaller extent. This enzyme keeps the length of telomeres longer and prevents the imminent collapse of cells.

The unexpected rapidly spreading cells and invasion and/or metastasis to different organs other than seed cause most death from human cancer (~ 90%). Invasion takes place by the direct migration and penetration by cancer cells into neighbouring tissues, whereas metastasis refers to the ability of cancer cells to penetrate into lymphatic and blood vessels, circulate through the bloodstream, and then invade normal tissues elsewhere in the body. The cancer cells modify their immediate cellular environment easily by inhibition of growth-halting receptors, overexpression of cytokines and proteases, destruction of basement membrane and matrix and ultimately the access to the blood vessels is facilitated.

The symptoms of cancer are yet-to-be substantiated though proper documentation of individual patients' data which leads to a better diagnosis. Each kind of cancer exhibits variable symptoms in spite of some common indications (a change in a wart or mole; lump

or thickening in the breast or testicles; a non-curable cough or coughing blood; a skin sore or a persistent sore throat; chronic fatigue, a change in bowel or bladder habits; constant indigestion or trouble swallowing and unusual bleeding or vaginal discharge). The main handicap to treat cancer is inability to detect it in an early stage. Therefore, regular medical check-ups especially for aged people could be the facile key to prevent and treat cancer. For the diagnosis of a cancer usually a sample of the affected tissue is tested microscopically. With the help of several advanced pathological tests, possible existence of cancer can be anticipated or confirmed. Often a next step is the biopsy, which is the surgical removal of a small piece of tissue for microscopic examination.[6, 10] In case of leukaemia the blood sample is used for confirmation. Additionally in the post-genomic era, microarrays may be used to determine specific genes which are turned on or off in the sample, or proteomic profiles may be collected for an analysis of protein activity.[3] Therefore, with the help of genomics and proteomics custom-made diagnosis protocol is possible for every patient.

Detailed and careful examination of cancer cells microscopically indicates the different traits. Generally variation in cell size and shape, a large number of irregularly shaped dividing cells, variation in nuclear size and shape, loss of normal tissue organisation, loss of specialised cell features and a poorly defined tumour boundary can be identified. After positive detection of cancer the treatment regime and dosage are determined by medical practitioners. Treatment of each individual can vary with specific type and stage of cancer, though there are certain general procedures to be followed. The main weapons to treat cancer are surgery, radiation and chemotherapy though recently a combined therapy regimen is often followed. Some newer but case-specific techniques are getting more familiar in cancer treatment regimen namely, photodynamic therapy, bone marrow and peripheral bone marrow transplantation therapy, laser treatment, angiogenesis inhibitor therapy, hyperthermia therapy, biological therapy, gene therapy, and targeted therapy.[4, 6, 10]

Chemotherapy uses drugs (organic drugs or metal-containing) to destroy cancer cells often in a non-specific way. These drugs are lethal to healthy fast-growing cells and often induce acute side-effects. Chemotherapy assists to cure, control and ease cancer symptoms. When combined with other modes of treatment chemotherapy can (a) reduce the bulk of tumour lump before surgery or radiation (neo-adjuvant), (b) kill the remaining cancer cells after surgery or radiation (adjuvant) and (c) destroy recurrent and metastatic

cancer cells. These drugs can be administered using several methods namely injection, intra-arterial (IA), intra-peritoneal (IP), intravenous (IV), topically and orally.[11-15]

1.2. Chemotherapy

1.2.1. Introduction

Chemotherapy has been in medical history from 2000 years back.[15] Arsenic and mercury concoctions were used in ancient ages as chemotherapeutics and the first book on chemotherapy appeared in the year of 1909, written by Nobel-prize winner Paul Ehrlich. The saga of chemotherapy started with usage of herbal extracts and animal organs in the prehistoric age and then the turn was of nitrogen mustard and antifolates. The modern era of chemotherapy begins with the approval of alkylating agent, cyclophosphamide in 1959. The first metallodrug, cisplatin, is introduced in medical practice in 1978. The history of chemotherapy can be time-lined in the Fig. 1.1.[12, 16]

There are several chemotherapeutic agents which can be classified as alkylating agents, proliferation inhibitors, enzyme inhibitors, DNA intercalators and antimetabolites, DNA-synthesis inhibitors and membrane permeability modifiers. Some very common drugs which are widely used in medical practice according to NIH (USA) are doxorubicin, epirubicin, bleomycin, fluorouracil, vincristine, vinblastine, etoposide, teniposide, chlorambucil, melphalan, busulfan, carmustine (BCNU), lomustine (CCNU), streptozotocin, thiotepa, dacarbazine (DTIC), methotrexate, cytarabine, azaribine, mercaptopurine, thioguanine, actinomycin D, plicamycin, mitomycin-C, asparaginase, procarbazine, hydroxyurea, topotecan, irinotecan, gemcitabine, temozolamide, capecitabine, tezacitabine, mechlorethamine, cyclophosphamide, mitoxantrone, and tegafur.[3] The gradual change in approval (by the FDA in the U.S.A) of chemotherapeutic drug invention with time is shown in Fig. 1.2.

1.2.2. Transition metals in chemotherapy

The relationship between active metals and cancer is a multifaceted issue, which combines the expertise of bioinorganic chemists, pathologists, pharmacologists and oncologists. Redox-active metals generally form reactive oxygen species (ROS) and this ROS can be used to induce DNA cleavage. The earliest report of medicinal use of metals or metal complexes dates back to the sixteenth century.[18] Several metals which are tried for efficient eradication of cancer or reduction the solid malignancy are explained briefly below.[19] The metals of interest in this thesis are platinum and ruthenium. The research and

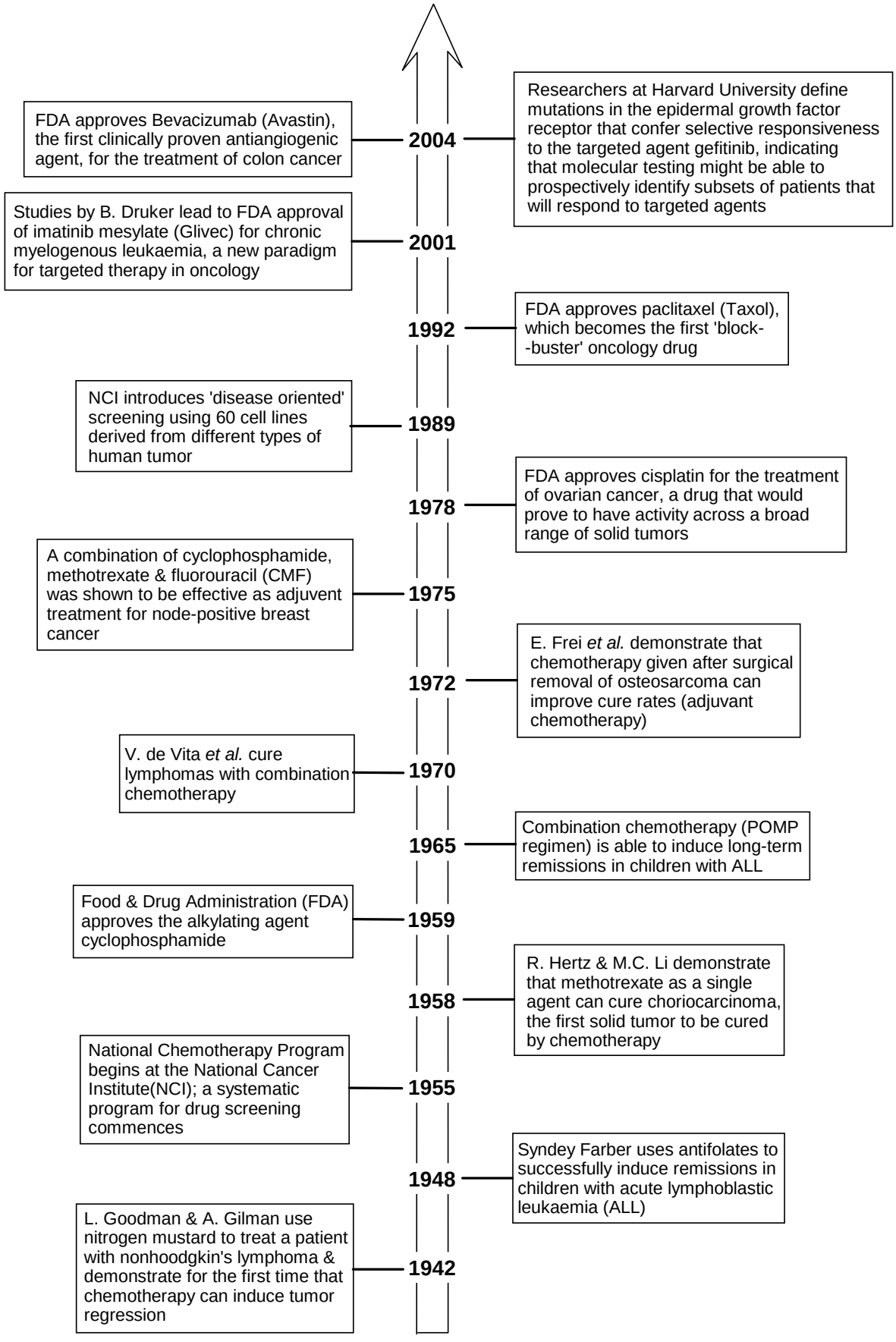


Figure 1.1. *Timeline for the history of chemotherapy according to the review article .*[17]

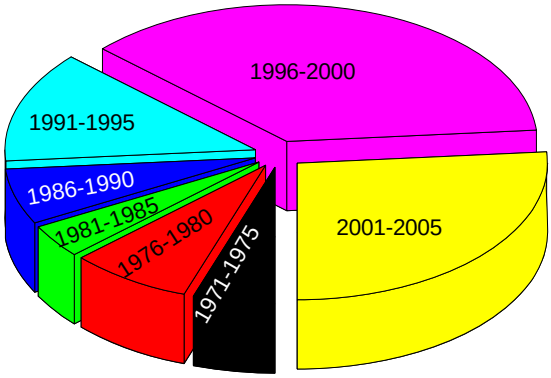


Figure 1.2. *Number of approved chemotherapeutic drugs by FDA since 1971.*[12]

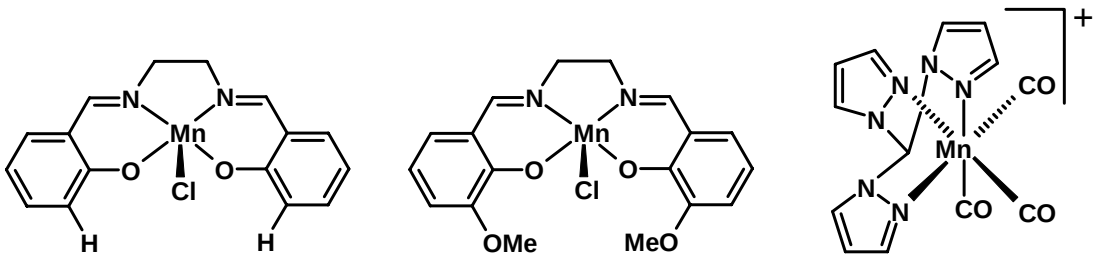
advances about platinum antitumour complexes is summarised in section 1.3, whereas section 1.4 deals with antitumour and antimetastatic ruthenium complexes.

(a) Manganese

Manganese is the central metal in some superoxide dismutases (SOD) and some cancer cells show reduced concentration of SOD2.[20, 21] SOD2 is a member of mitochondrial Fe-Mn containing superoxide dismutase family. After coding a specific protein, this gene induces the removal of detrimental side-products of oxidative phosphorylation *via* H_2O_2 and O_2. Mutation in this gene may lead to several ailments, including cancer. The malignant phenotype in melanoma is removed by transfection of plasmid cDNA SOD2 according to Church *et al.*[22] This positive effect is also proved effective for mouse fibrosarcoma and human cancer cells of the breast, lung, central nervous system, prostate and oral cavity.[20] Transfection of SOD2 induces apoptosis, a G1 delay in the cell cycle and diminishes tumour volume.[23] In human prostate cancer cells, transfection of cDNA upregulate the SOD2 by 6-fold and this elevation is sufficient for tumour reduction. This phenomenon suggests the SOD2 as a tumour suppressor gene. The transfection efficiency has been improved by using an adenoviral vector (adenovirusMnSOD) instead of plasmid. This compound in combination with BCNU [1,3-bis-(2-chloroethyl)-1-nitrosourea; glutathione peroxidase inhibitor] is used against hamster and human oral cancer cells and the cell-viability is reduced to 50% and 80%, respectively.[20]

An efficient mimic of SOD is the Mn-salen (EUK-135) compound that exhibits pharmacological efficiency in cell survival following UVB irradiation.[24, 25] Pretreatment with EUK-135 before exposure to UVB lowers the *p53* concentration is a dose-dependent

manner. In addition, it inhibits the mitogen-activated protein kinase (MAPK) pathway response to oxidative stress. Two Mn-salen type compounds have been depicted in Fig. 1.3.



(a) EUK-8 *(b) EUK-134* *(c) $[Mn(tpm)(CO)_3]^+$*

Figure 1.3. *Mimetic compounds of superoxide dismutase and catalase.*[25, 26]

A very recent improvement in photodynamic therapy using a Mn compound has been reported. The compound $[Mn(tpm)(CO)_3](PF_6)$ gets activated after irradiation with UV-light and two CO groups are released eventually in aqueous buffer. This compound [Fig. 1.3(c)] exhibits photoinduced activity in HT29 (colon cancer) cell line with a reduction in biomass comparable to 5-FU. This specific compound and several probable derivatives/modifications may be potential drugs with high specificity.[26]

(b) Arsenic

Arsenic has been a common drug in the medical world over centuries. The well-known Fowler's solution (1% $KAsO_2$ solution) was the popular and primary therapy for chronic myelogenous leukaemia (CML) until the modern radiation and advanced chemotherapy prevailed in the twentieth century. The breakthrough success came through the treatment of acute promyelocytic leukaemia (APL) with typical chromosome translocation.[27, 28]

A relatively low plasma concentration of As_2O_3 (1-2 μM) is sufficient for APL[30] treatment including all *trans*-retinoic acid (ATRA) resistant patients, or in the cases where conventional chemotherapy failed.[31] Complete remission rate in the newly diagnosed and relapsed patients suffering from APL are 85 and 93%, respectively when treated with As_2O_3. The mild side effects are responsive to either symptomatic treatment or dose reduction, while the major toxic effect with other conventional drugs, myelosuppression, is absent. The approval of this drug by the FDA as injection (Trisenox®) made it the main therapeutic for APL in adult patients who failed other chemotherapy or suffer from relapsed disease.[32] This drug is under evaluation for the treatment of chronic lymphocytic

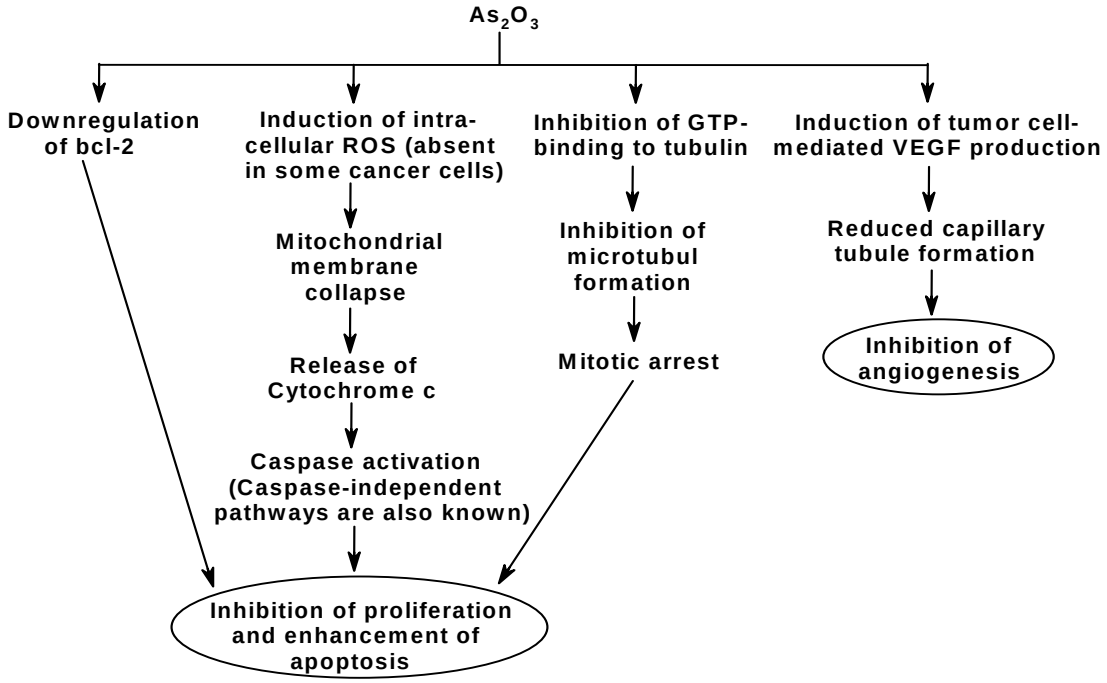


Figure 1.4. *Mode of action induced by arsenic oxide in malignancy treatment.*[29]

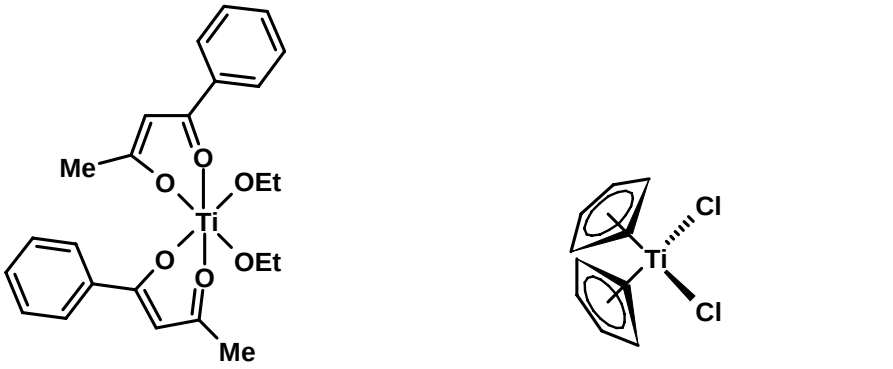
leukaemia (CLL), multiple melanoma (ML), and solid tumours such as neuroblastoma gastric or cervical tumours.[33, 34]

The cellular alterations caused by arsenic are mediated by multiple pathways as inhibition of angiogenesis, stimulation of differentiation, inhibition of proliferation and induction of apoptosis (Fig. 1.4). The anti-carcinogenic activity of arsenic is assumed [35] to be a combined effect of dosage (low *vs.* high), length of exposure (acute *vs.* chronic) and active speciation (arsenite, arsenate, monomethyl arsenic acid, dimethyl arsenic acid, *etc.*) in intracellular fluid.

(c) Titanium

A titanium compound was the first metal compound to reach the clinical trial after cisplatin. Two titanium compounds, Budotitane [*cis*-diethoxybis(1-phenylbutane-1,3-dionato)titanium(IV)][36] and titanocene dichloride (Cp_2TiCl_2) exhibit significant activity against solid tumours and reached for preclinical trials.[37-39] The chemical structures of these two active titanium compounds are shown in Fig. 1.5. Budotitane did not make it beyond the phase-I clinical trials. The *cis* labile ligands (Cl, OR) hydrolyse initially and after that also slowly the inert ligands (Cp: cyclopentadienyl, bzac: benzoylacetonate) hydrolyse and ultimately a mixture of unidentified aggregates is formed. The titanocene compound is comparatively more robust than budotitane. This compound shows moderate activity *in vitro* but is significantly promising *in vivo*.[39, 40] This compound when

administered in a phase-II trial in patients with metastatic renal cell carcinoma[41] or metastatic breast cancer[42], exhibited a too low efficacy to proceed further.



(a) Budotitane [Ti(bzac)$_2$(OEt)$_2$] ***(b) Titanocene dichloride [Cp$_2$TiCl$_2$]***

Figure 1.5. *Structures of two titanium compounds that made it to clinical trials.*[36, 39]

The mode of action of titanium compounds has been investigated by Sadler *et al.*[43, 44] The uptake of Ti(IV) from Cp_2TiCl_2 is mediated by human transferrin[45] at blood plasma pH, then release of bound Ti(IV) to ATP at cellular endosomal pH takes place. Ti(IV) then can bind to either negatively charged phosphate on the backbone of DNA or to the base nitrogen donors.[46, 47] The intracellular pH is lower than the extracellular plasma pH, therefore Ti(IV) forms stronger bonds to DNA bases. The hydrolysis of Ti compounds is quite rapid and oxido-bridged dimers are assumably the active species.

TiO_2 when finely dispersed and photo-activated was shown to significantly reduce the HeLa cells implanted in nude mice.[48] This antiproliferative activity is also retained in U937 cells after photo-activation by UV-irradiation.[49, 50] The ROS originated by photo-excited TiO_2 can potentially damage DNA and leads to cell death. The final hydrolysis product of budotitane is also TiO_2.[36]

Recent advancements in Ti-antitumour research open multiple directions to yield more specific, stable to hydrolysis and improved anti-proliferative profile. The approaches include: (a) non-metallocene, non-diketonato symmetrical titanium compound with bis-phenolato ligands,[51] (b) carbonyl substituted titanocene[52] and (c) bioorganometallic fulvene-derived titanocene.[53] Another recent advancement in titanium-anticancer drug research is Titanocene Y,[13] which is a modification of original dichloridotitanocene. This compound, having methoxyphenyl substitution on each cyclopentadienyl ring, offers greater aqueous stability, water solubility and cytotoxicity.[54-56] The structure-activity relationship is yet to be established for multiple substituted variants of the parent

compound. Some selected structures of the recently studied titanium compounds are redrawn in Fig. 1.6.

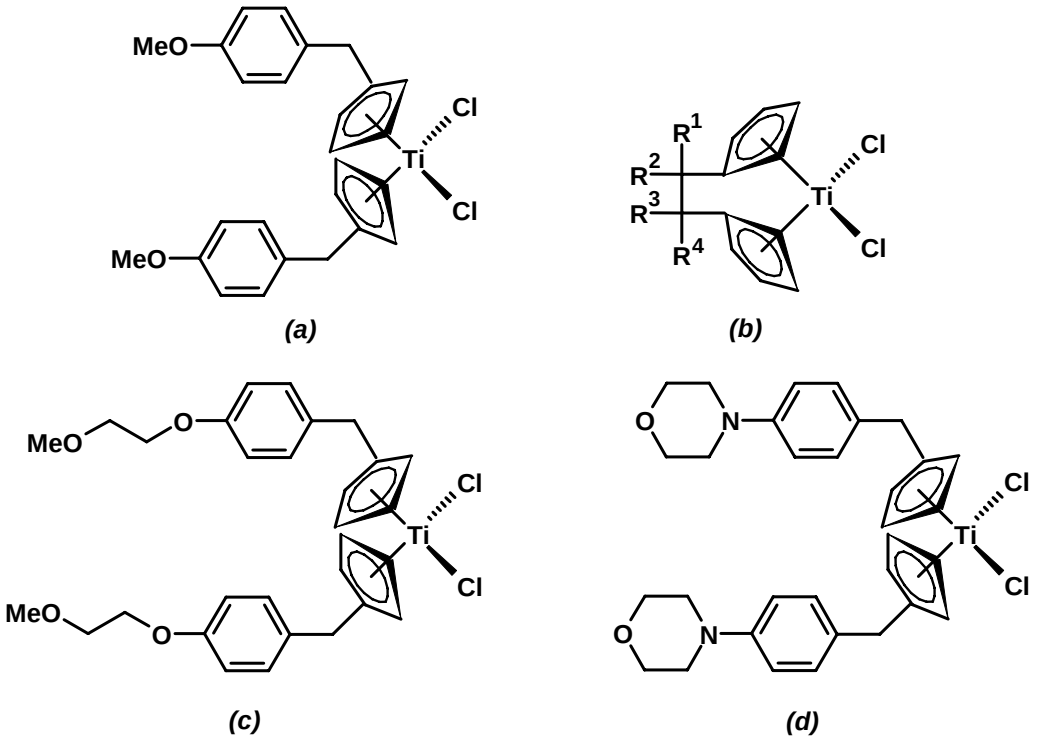


Figure 1.6. *Structures of recent titanium compounds with significant anticancer activity and (a) Titanocene Y.*[57, 58]

(d) Gold

Gold has been used in the medicinal field for centuries, starting from Egyptian, Arabian, Chinese and Indian civilizations. Several Au(III) and Au(I) compounds have more recently been studied for their medicinal potency in several diseases notably rheumatic arthritis. Au(III) has the outer shell electronic configuration as d^8, isoelectronic to Pt(II) and favours the square planar geometry similar to platinum(II) compounds. The biological and antiproliferative activity of Au(III) compounds do not arise from affinity towards DNA, which is quite distinct from typical platinum(II) compounds. The poor affinity to calf thymus DNA exerted by Au(III) compounds suggests that DNA is not the primary target.[59] In addition, the cytotoxic Au(III) compounds scarcely affect or interrupt the cell cycle.[60] Recent elaborate experimental evidences indicate a direct interference with mitochondrial functions.[61]

Mononuclear cytotoxic Au(III) compounds can be widely classified in the following category: Au(III) polyamines,[63] Au(III) polypyridines,[64, 65] Au(III) porphyrins,[66]

Au(III) dithiocarbamate[67-69] and organogold[70] compounds. Some structures of this wide group have been shown in Fig. 1.7. Most of these compounds have a strong affinity to protein targets and the thioredoxin reductase inhibition leads to mitochondrial damage. This pathway triggers mitochondrial cytochrome C release followed by apoptotic cascade.

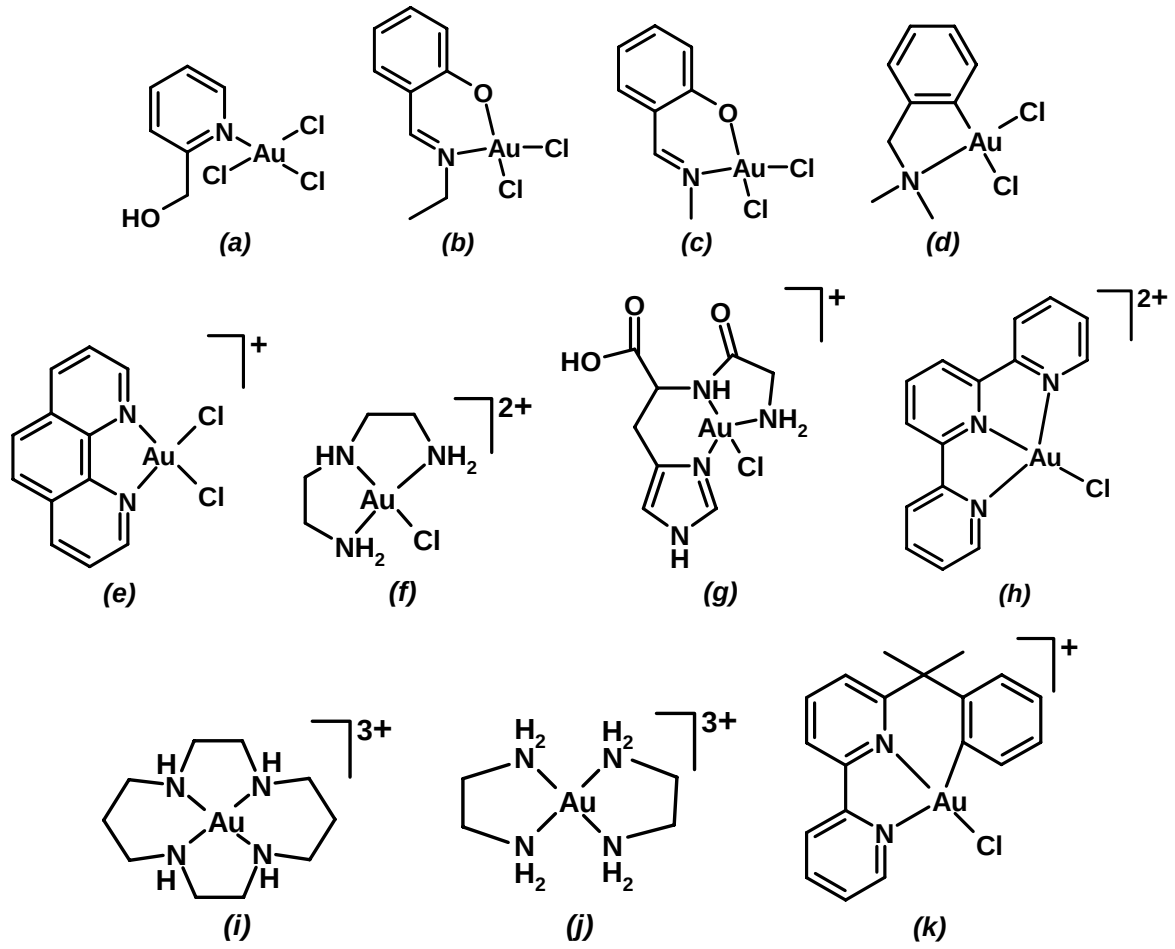
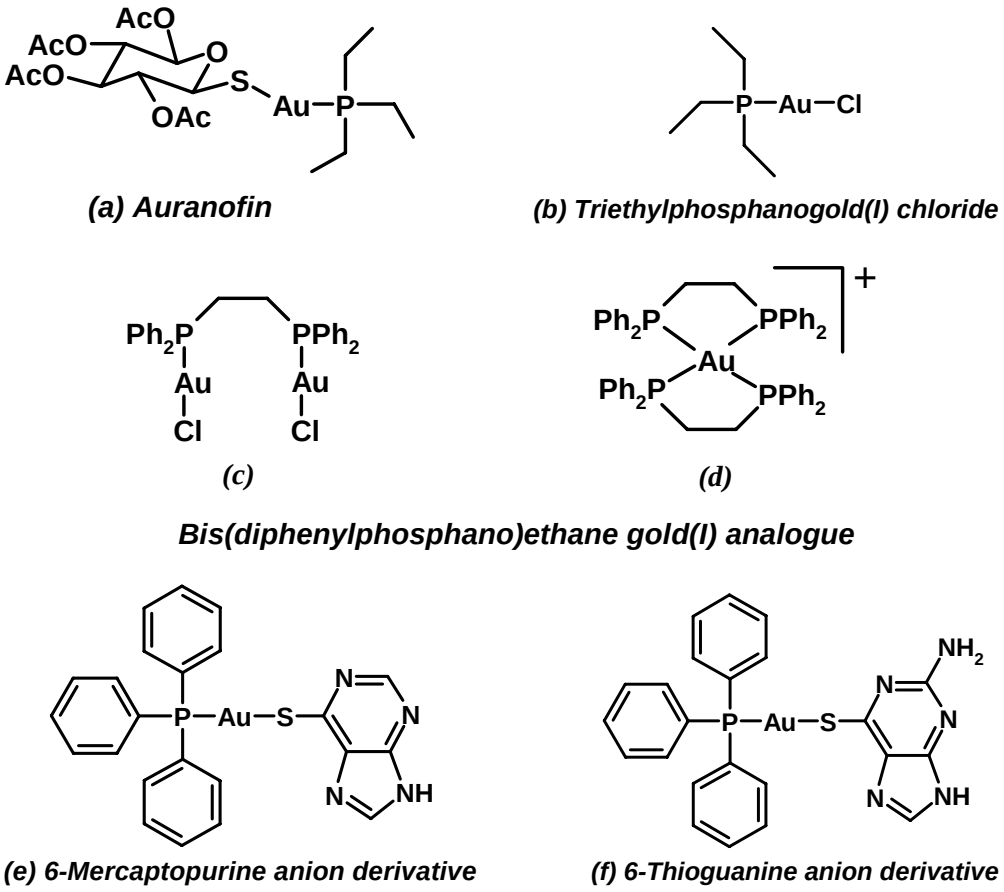


Figure 1.7. *Chemical structures of some selected Au(III) compounds with promising in vitro cytotoxicity.*[62]

Some Au(III) compounds inhibit strongly disease-specific thiol-containing cysteine protease cathepsin.[62] None of the Au(III) compounds have made it to clinical trials yet[71] despite the exciting redox properties, *in vitro* cytotoxicity and different mode of action from Pt(II) compounds.

Au(I) compounds are used as anti-inflammatory drugs in rheumatoid arthritis.[72] It has also been noticed in the medical history that patients undergoing chrysotherapy (Au-drug treatment) have reduced risks of cancer.[73] Some well-established drugs such as cyclophosphamide, 6-mercaptopurine and methotrexate exert both anticancer and anti-inflammatory properties.[74-76]

Some selected Au(I) compounds tested against cancer cells are shown in Fig. 1.8. Auranofin shows activities against Hela cell[77] and against P388 leukaemia *in vivo*.[78] A series of tertiary phosphanegold(I) compounds with a thiosugar arm are active against P388 leukaemia and B16 melanoma *in vitro* along with P388 *in vivo*.[79]



(a) Auranofin ***(b) Triethylphosphanogold(I) chloride***

(c) ***(d)***

Bis(diphenylphosphano)ethane gold(I) analogue

(e) 6-Mercaptopurine anion derivative ***(f) 6-Thioguanine anion derivative***

Figure 1.8. *Chemical structures of some selected Au(I) compounds tested against cancer cells.*[77, 80]

The coupling of organophosphanegold(I) with biologically active thiols probably exhibits dual activity of both moieties and the cytotoxicity profile improves both *in vitro* and *in vivo*.[80, 81] The bidentate phosphane ligands when coordinated to Au(I) result in a highly potent cytotoxic drug as found in both *in vitro* and *in vivo* assays. Unfortunately the clinical trials had been abandoned due to acute toxicity to lungs, liver and heart of the canine.[82-84]

(e) Gallium

The application of gallium in medicinal inorganic chemistry was initiated due to the similarity in chemical behaviour with Fe^{3+}. The resemblance extends to ionic radius, electronegativity, ionisation potential and electron affinity. Thus Ga^{3+} compounds are

expected to follow the Fe(III) route *in vivo* and to occupy the iron centres in proteins and biomolecules. Therefore a handful of gallium compounds did enter clinical trials.[85] The first two potent compounds with reported anticancer activity are $GaCl_3$ and $Ga(NO_3)_3$, which are orally administrable.

$Ga(NO_3)_3$ interferes with cellular iron metabolism[86-88] and the cellular transferrin uptakes the Ga ions.[89-91] Except the transferrin-mediated facile uptake, gallium does not follow the iron-trafficking route strictly. After $Ga(NO_3)_3$ treatment, gallium interferes with Zn metabolism[92] and exhibits an antimitotic component.[93] In addition this compound is active against the hypercalcaemia caused by malignancy, therefore the patients with advanced multiple myeloma are treated with $Ga(NO_3)_3$ for bone resorption.[86, 94] In spite of promising activity in bladder cancer and lymphoma[95] in a phase-II trial, nephrotoxicity (short infusions) and severe optical neuropathy (continuous infusions) limited the applicability. The schematic diagram of the distribution and accumulation and activation of gallium is shown in Fig. 1.9 with some uncertain pathways yet to explore in details.

Two suitable chelating ligands, 8-quinoline and 3-hydroxy-2-methyl-4H-pyran-4-one (*maltol*), after coordination to Ga^{3+} give rise to hydrolysis-stable compounds. The structures of these modified compounds are shown in Fig. 1.10.

These compounds are facilitated for intestinal absorption and membrane permeation though the reason behind this selectivity is not clear yet.[96] KP46 exhibits significant activity in an experimental model by reducing more than 50% tumour volume without any acute toxicity. These two compounds show higher bioavailability in animal species after oral administration and enhanced anti-proliferative activity compared to simple salts.[100, 101] The tolerance level is higher than expected, as a phase-I trial did not encounter any dose-limiting toxicity.[102]

(f) Vanadium

Vanadium is a trace element abundant in environment and possesses important medicinal properties.[103, 104] After oral intake, this element is rapidly distributed in tissues (spleen, lungs, kidney, and muscle) and ultimately stored in bones.[105] Vanadium is an inhibitor of terminal differentiation of murine erythroleukaemia and after incorporation in diet it reduces the chance of chemically-induced mammary carcinoma.[106] The activity arises from protein tyrosine phosphorylation, phosphoinositide breakdown, selective inhibition protein tyrosine phosphatase, activation of phosphotyrosine phosphatases. This induces changes in the invasive and metastatic potential of cancer cells after modulation of

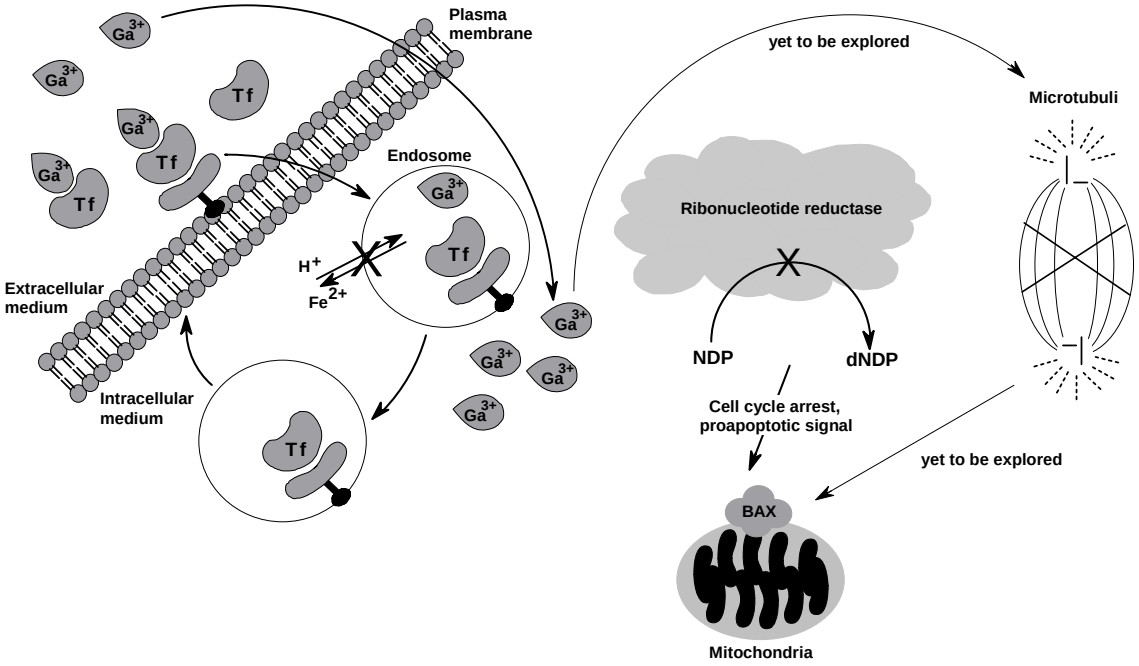
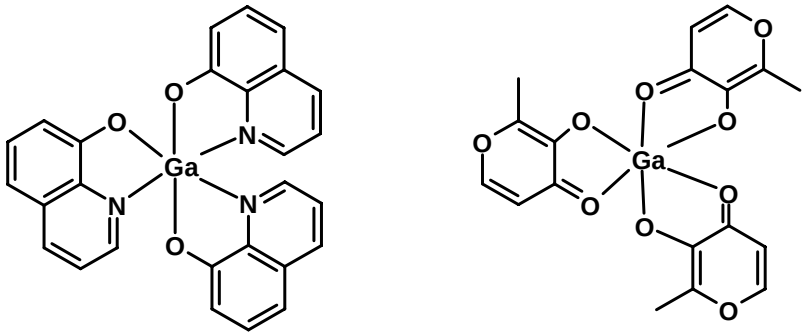


Figure 1.9. *Scheme for uptake, activation and mode of action of gallium compounds.*[96]



(a) KP46 ***(b) Gallium maltolate***

Figure 1.10. *Structures of Ga compounds studied extensively for cancer treatment.* [96-99]

cell-substrate adhesion, cell-to-cell contact and the actin cytoskeleton. As the most common side-effect of vanadium is mild gastrointestinal disturbances, it has prominent potency to be an important therapeutic.

The most promising multi-targeted anticancer vanadium compound with apoptosis-inducing activity, among several bis(cyclopentadienyl)vanadium(IV) and oxidovanadium(IV) compounds, is bis(4,7-dimethyl-1,10-phenanthroline) sulfatooxidovanadium(IV) (metvan).[107-109] The structure of metvan [Fig.1.11(c)] along with some other analogous compounds, is shown in Fig. 1.11. At nanomolar and low micromolar concentrations, metvan induces apoptosis in human leukaemia cells, multiple myeloma cells and solid tumour cells derived from ovarian, breast cancer, testicular cancer,

glioblastoma and prostate patients. It is highly effective against cisplatin-resistant ovarian cancer and testicular cancer cell lines. Metvan is much more effective than the standard chemotherapeutic agents dexamethasone and vincristine in inducing apoptosis in primary leukaemia cells (derived from acute myeloid leukaemia, acute lymphoblastic leukaemia or chronic acute myeloid leukaemia). Metvan-induced apoptosis is associated with a loss of mitochondrial transmembrane potential, the generation of reactive oxygen species and depletion of glutathione.[110] Treatment of human malignant glioblastoma and breast cancer cells with metvan at nanomolar concentration is resulted in almost complete loss of the adhesive, migratory and invasive properties of the untreated cancer cell populations.[108, 109]

Metvan shows favourable pharmacokinetics in mice and does not cause acute or subacute toxicity at the dose levels tested (12.5–50 mg/kg). Metvan exhibits significant antitumour activity, delays tumour progression and prolongs survival time in severe combined immunodeficient mouse xenograft models of human malignant glioblastoma and breast cancer. The broad spectrum anticancer activity of metvan together with favourable pharmacodynamic features and lack of toxicity warrants further development of this oxovanadium compound as a new anticancer agent.[110, 112] Metvan could represent the first vanadium compound, as an alternative to platinum-based chemotherapy, although recently not much has been published with the updates of this compound.

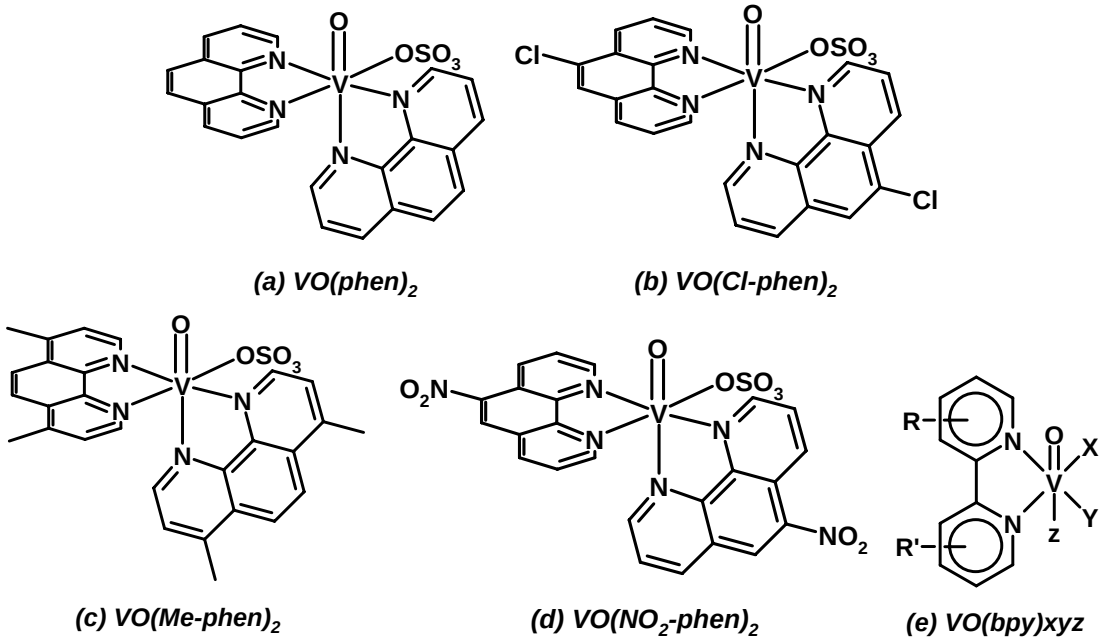


Figure 1.11. *Structures of some selected V(IV) compounds tested against cancer cells, including (c) metvan.*[107, 108, 110, 111]

(g) Iron

Iron is a biologically important metal, which takes part in essential physiological functions. Therefore the deprivation of iron supplements can be an important target for cancer growth. The thought behind the anticancer organometallic iron compound synthesis is based on three significant observations namely; (a) iron restriction by dietary supplement markedly reduces tumour growth in rodents, (b) antibodies which block transferrin binding to cell receptors inhibit cancer cell proliferation *in vitro* and *in vivo*, (c) the anticancer and DNA-cleaving agent, bleomycin, gets activated after chelation with copper or iron.

Ferricenium picrate and ferricenium trichloro acetate are the first two iron compounds exhibiting antitumour acitivity.[113] The substituted ferrocenes are active against some cancer cell lines. The mechanism of action is proposed *via* the inter-conversion between inactive ferrocene(II) and active ferrocenium(III) ions specifically happening in hypoxic cancer cells. The ferrocenium ion can interact with DNA by multiple ways such as coordinative binding to nucleophiles of nucleotides, electrostatic interaction towards negatively charged phosphate backbone, charge transfer complex formation and perhaps by intercalation with nucleotide bases.[113, 114] As an additional way the highly reactive hydroxyl radical ($OH^{\bullet}$) originated from ferricenium ion can also lead to DNA cleavage. The existence of the radical has been proved for another compound, decamethylferroceniumtetrafluorideborate ($DEMFc^{+}$). The structures of these tested iron compounds are shown in Fig. 1.12. The highly lipophilic and stable (in aqueous solution) drug, $DEMFc^{+}$ exhibits activity against human breast adenocarcinoma cells (MCF-7).[114-116]

Tamoxifen [Fig. 1.12(d), R = H] is an organic drug used for breast cancer treatment, which is mainly active against estrogen receptor positive, ERα +ve types. Substitution of one phenyl group by a ferrocenyl group leads to ferrocifen[117] [Fig. 1.12(e)], which is active against both type of human estrogen receptor namely ERα +ve and ERβ -ve.[13] The activity can be either by the ferrocenium ion,[118] or by a Fenton-like Fe^{2+}-mediated mechanism.[119] The latter pathway is the most suitable explanation of the genotoxic effect of these ferrocifens.

Another modification of the iron compound can be done by attaching a typical DNA-intercalator such as anthracene *via* an alkylamino chain [Fig. 1.12(f)].[118] Though the mechanism is yet to unfold, it is proposed that anthracene facilitates the position of the compound to the vicinity of DNA and then the ferrocenium ion mediates DNA cleavage. This compound is active against KB, HeLa, Colo-205 and Hep with IC_{50} values of 1-2 μg/mL.[118] Other apoptosis inducing cytotoxic compounds are an iron-nucleoside

compound[120] [Fig. 1.13(a)] and a pentadentate pyridyl containing compound[121] [Fig. 1.13(b)].

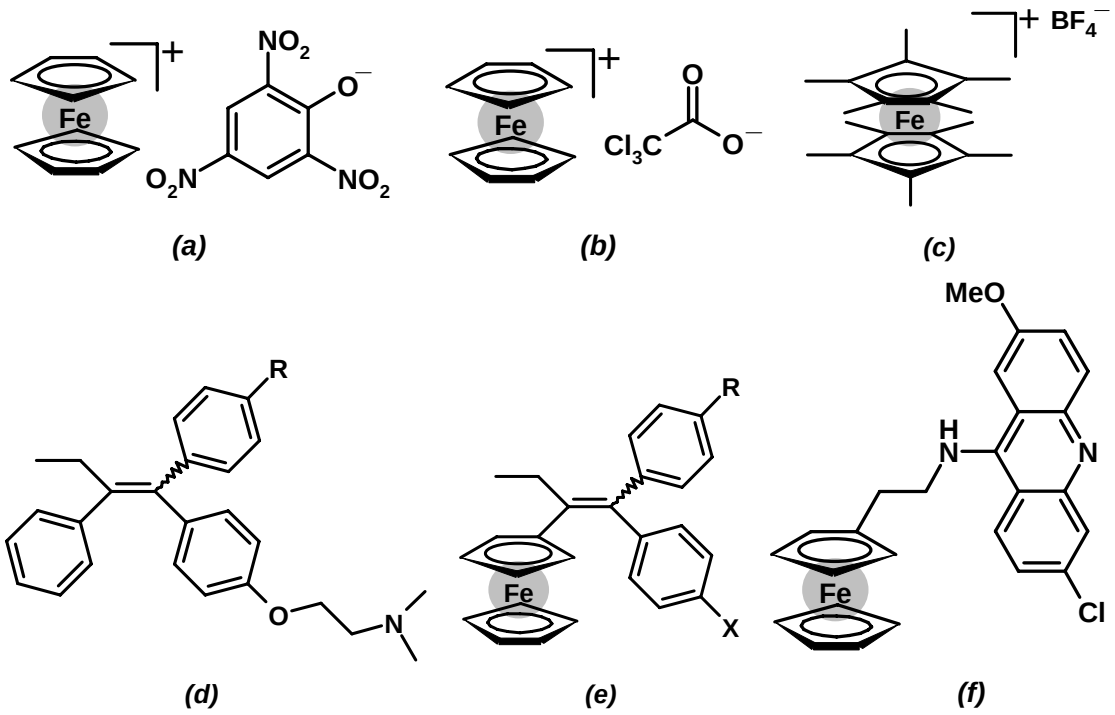


Figure 1.12. *Structures of iron compounds (a) Fc-picrate, (b) Fc-tca and (c) DEMFc, (d) tamoxifen (R = H), (e) ferrocifen, (f) ferrocenyl acridine.*[113, 117]

$$Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{\bullet -}$$
$$Fe^{2+} + O_2^{\bullet} \rightarrow Fe^{3+} + H_2O_2$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$$

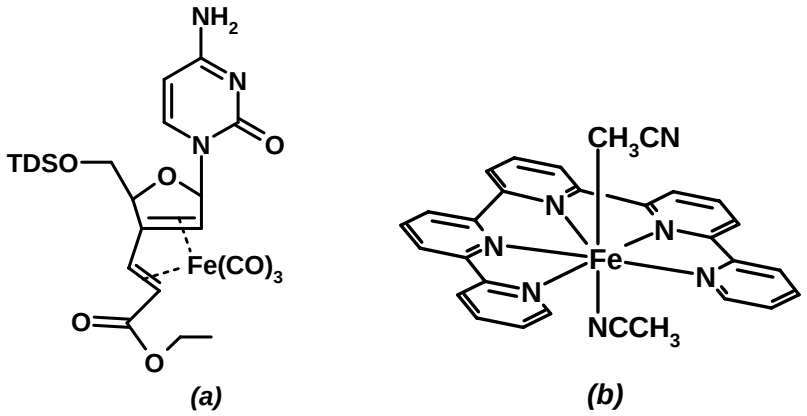


Figure 1.13. *Apoptosis inducing cytotoxic iron compounds with (a) modified nucleoside and (b) a scorpionate ligand.*[120, 121]

(h) Cobalt

Cobalt compounds are known in bioinorganic chemistry for the excellent mimic of some metalloenzymes. The cobalt compounds are widely studied for the development of antitumour agents, DNA-cleaving agents, enzyme inhibitors, hypoxic selective agents, nucleic acid probes, drug delivery devices, and positron emission tomography agents.[122-126]

The hypoxia-selective cobalt compounds have been synthesised by coupling toxic nitrogen mustard with cobalt species [Fig. 1.14(d)].[127, 130, 131] In solid tumours, some cells are far from blood vessel, it is difficult to reach them by normal chemotherapeutics as drug concentration gets lesser in the centre than the periphery. The hypoxia-selective drugs utilise one-electron bio-reduction at a transition intermediate and Co(II)/Co(III) redox

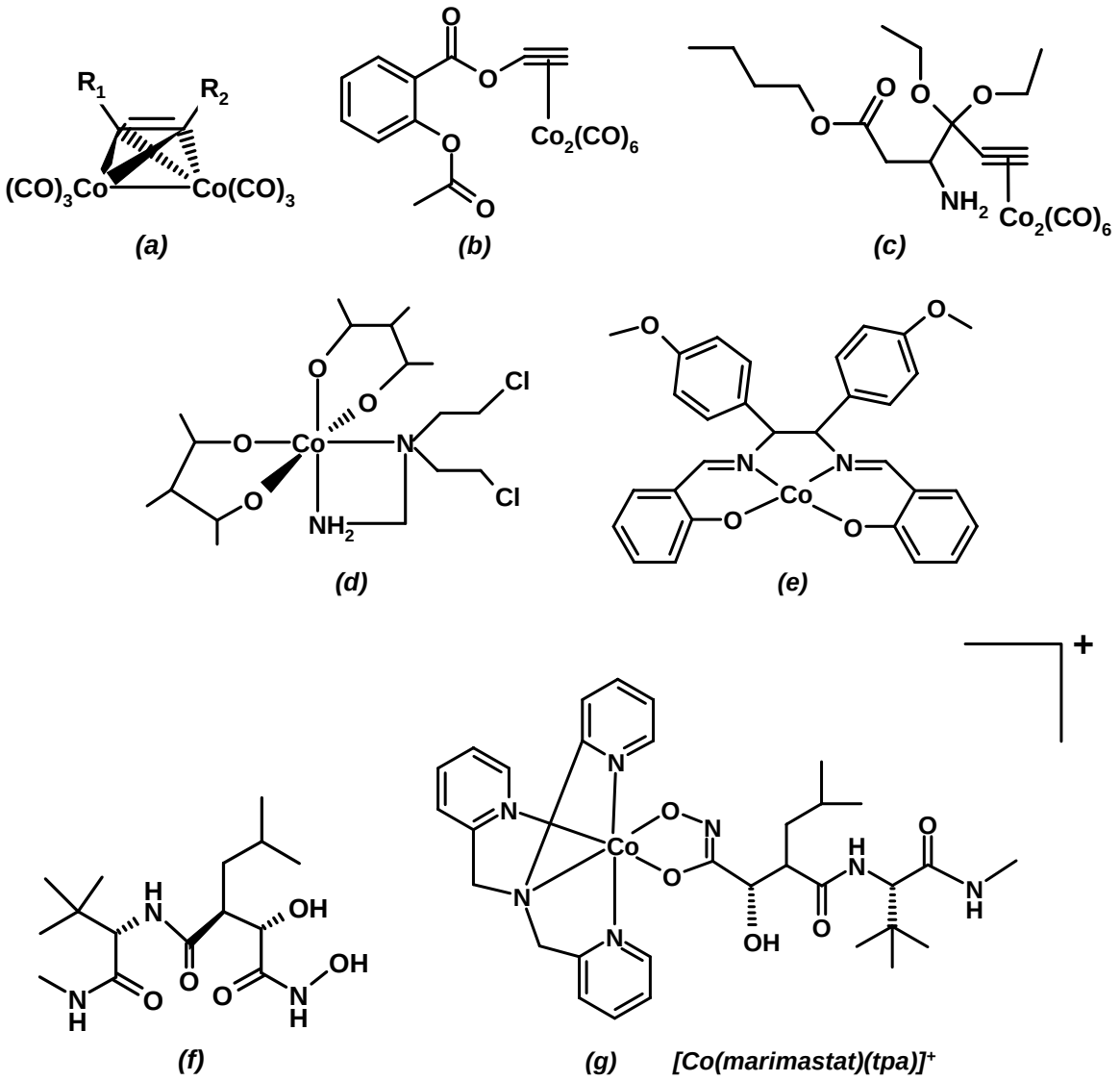


Figure 1.14. *Structures of promising antitumour Cobalt compounds*[127-131] *with (f) marimastat.*

couple can offer selectivity in this cells. These compounds show hypoxia-selective activity in Chinese hamster ovary (CHO) fibroblasts and UV4 cells *in vitro*. The ligands have significant effect on the activity of these compounds. The Schiff–base compounds, Co-salen [Fig. 1.14(e)] were tested for antitumour activity and the SAR is based on the ligands used at the ethylene diamine moiety.[129] The structures of these two types of compounds are shown in Fig. 1.14. Recent development in this area counts the Co-marimastat compound as anti-metastatic agent, which shows an even higher level of tumour growth inhibition [compared to free marimastat, Fig. 1.14(f)].[128]

Another class of cobalt compounds used for cancer treatment are dinuclear cobalt carbonyl compounds.[132] The activity firstly was exhibited by the Co-acetal [Fig. 1.14(c)] complex against murine leukaemia cell line.[133] The most active compound in this series is Co-ASS which is the cobalt-carbonyl complex with aspirin; [Fig. 1.14(b)]. This compound is active against several human cancer cell lines, but notably active against breast cancer cell lines. This compound most likely acts by inhibition of cyclooxygenases (COX1 and COX2) because the free ligand, aspirin-based, triggers the similar pharmacological effects.[134, 135]

1.3. Platinum Compounds in Chemotherapy

1.3.1. Introduction to platinum antitumour chemistry

Platinum compounds in cancer chemotherapy deserve a special attention as three metallodrugs in medicinal practice are platinum drugs. Cisplatin was first synthesised in 1844 by Peyrone in Turin and named as Peyrone's chloride.[136] The biological activity was discovered by serendipity in 1965 by physicist-turned-biophysicist Barnett Rosenberg.[137, 138] Approval of cisplatin [*cis*-diamminedichloridoplatinum(II)] by FDA for treatment of testicular and ovarian cancer was given in 1978.[139] Inspired by this unexpected success thousands of platinum (similar as parent cisplatin) compounds have been synthesised and tested for antitumour efficacy. Till to date relatively few completed the clinical trials[139, 140] and six of them are currently approved namely; cisplatin [*Platinol*®, *cis*-diamminedichloridoplatinum(II)], carboplatin [*Paraplatin*®; *cis*-diammine-1,1-cyclobutanedicarboxylatoplatinum(II)], oxaliplatin [(*R*,*R*)-diaminocyclohexane-1,2-ethanedicarboxylatoplatinum(II)], nedaplatin [*cis*-diammine-2-hydroxyacetatoplatinum(II); in Japan] and lobaplatin [*cis-1,2*-diamminocyclobutane-2-hydroxypropanoatoplatinum(II); in China] and heptaplatin [in South Korea]. The structures of these drugs are shown in Fig. 1.15.

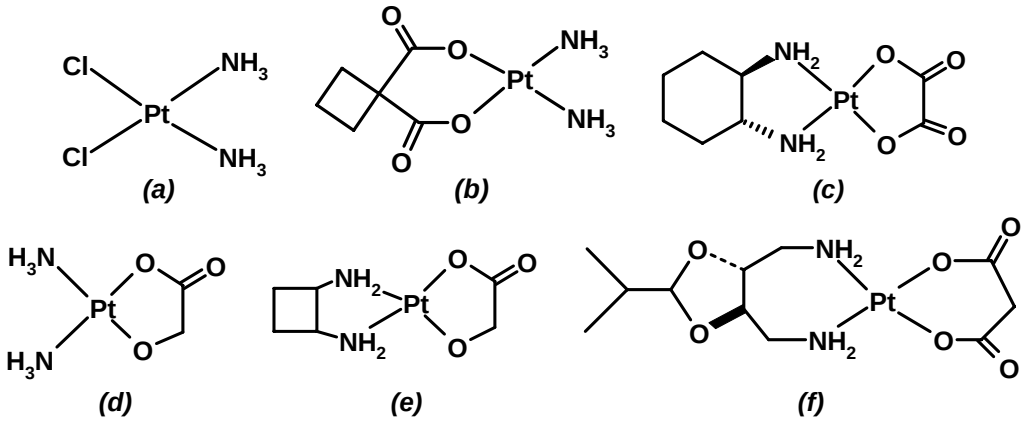


Figure 1.15. *Clinically approved platinum antitumour drugs (a) cisplatin, (b) carboplatin, (c) oxaliplatin, (d) nedaplatin (e) lobaplatin and (f) heptaplatin.*

After initial success of cisplatin, the second generation platinum drug (carboplatin) was introduced in clinic in mid-1980's. The compound is devoid of nephrotoxicity along with reduced gastrointestinal tract toxicity and neurotoxicity. The activity profile is retained when compared to cisplatin and the FDA approval was granted in 1989 for ovarian cancer treatment.

The third generation of platinum drug includes oxaliplatin, which also overcomes cisplatin resistance and is specific for common cancer (means testicular and ovarian because they comprise higher percentage of cancer cases).

Heptaplatin (*cis*-malonato-[(4R,5R)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane]platinum(II), or otherwise known as SKI-2053R is another platinum(II) drug in practice in South Korea from 1999 for treatment of gastric cancer.[141-144] This compound is approved for treatment in combination with 5-FU and showed lesser nephrotoxicity compared to cisplatin. The prominent dose-limiting toxicities comprise hepatotoxicity, nephrotoxicity and myelosuppression.[145, 146] Further research for treatment of other cancers by HTP (heptaplatin) with combination of 5-FU or paclitaxal is under progress.[147]

1.3.2. Cisplatin

At present the world's best-selling anticancer drug, cisplatin, is marketed under the names *cisplatinol*® and *platinosin*®. It is used in the standard treatment of several malignancies including testicular, ovarian, cervical, bladder, oesophageal cancer and small cell lung cancer.[148] It shows improved curing rate for testicular cancer when treated in combination with vinblastine and bleomycin and for ovarian cancer combined with

cyclophosphamide, doxorubicin, hexamethylmelamin and/or paclitaxal.[149] The testicular cancer, if detected in early stage, can reach the curing rate above 90%.

There are some major drawbacks in the use of cisplatin for cancer-chemotherapy. The poor solubility in saline, developments of resistance by the tumour cells and severe side-effects are the limitations. The resistance against drug may be intrinsic or acquired. Severe side effects include failure of the kidney and bone marrow (nephrotoxicity and haematological toxicity), nausea, intractable vomiting (emesis), peripheral neuropathy, deafness and seizures[150] and myelotoxicity. In addition cisplatin is to be administered intravenously which is inconvenient to outpatient treatment.

Some tumours such as colorectal and non-small cell lung cancers have intrinsic resistance to cisplatin whereas others, *e.g.*, ovarian or small cell lung cancers develop acquired resistance after the initial treatment.[151] Researchers identified several mechanisms contributing to resistance. This resistance is generally multi-factorial and has been shown to be due to reduced drug accumulation, inactivation by thiol-containing species (mainly glutathione and metallothionein), increased repair and/or tolerance of platinum-DNA adducts and alteration in proteins involved in apoptosis.[150, 152]

1.3.3. Interaction of cisplatin with DNA

(a) Biochemical mechanism [153, 154]

It is generally believed that binding of cisplatin to genomic DNA (gDNA) in the cell nucleus is principally responsible for the antitumour activity.[155] The damage of cisplatin-bound gDNA may interfere with normal transcription and/or replication mechanism. Consequently this disruption in DNA processing could trigger the cytotoxic processes ultimately leading to cancer-cell death. Additionally, cisplatin also forms adducts with mitochondrial DNA (mtDNA) and has been shown to form 4-6 fold higher adduct in proportion than gDNA. [148] As mitochondria are unable to carry out nucleotide excision repair (NER, a major pathway to remove cisplatin-DNA adducts),[17] mtDNA-cisplatin adduct might play an important pharmacological role in cellular processing. Prior to cisplatin binding to gDNA or mtDNA, the loss of chloride anions is essential. After injection into the bloodstream, cisplatin remains in the neutral state owing to relatively high chloride concentration in the extra-cellular fluid (~100 mM), which suppresses the hydrolysis.[156-159] It enters the cell either by passive diffusion or active transport. Inside the cell the chloride concentration is only to 2 mM-10 mM; so the hydrolysis of cisplatin yields *cis*-$[Pt(NH_3)_2(H_2O)Cl]^+$ and/or *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$. This mono- or diaqua species are

more reactive towards nucleophilic centres of biomolecules (mainly DNA) as H_2O is a better leaving group than Cl^-.[160]

(b) Binding of cisplatin to DNA[148, 157, 161, 162]

The binding of cisplatin to DNA is kinetically rather than thermodynamically controlled and the hydrolysis reaction of chloride ions is the rate-determining step for DNA binding. The N7 atoms of the imidazole rings of guanine and adenine located in the major groove of the double helix are the most accessible and reactive nucleophilic sites for platinum binding. The reaction of cisplatin with DNA may lead to various structurally different adducts. The binding sites on the nucleobases and different probable crosslinks in presence of cisplatin are shown in Fig. 1.16. Initially, monofunctional DNA adducts are formed, but most of them react further to produce interstrand or intrastrand crosslinks, which block replication and/or prevent transcription.

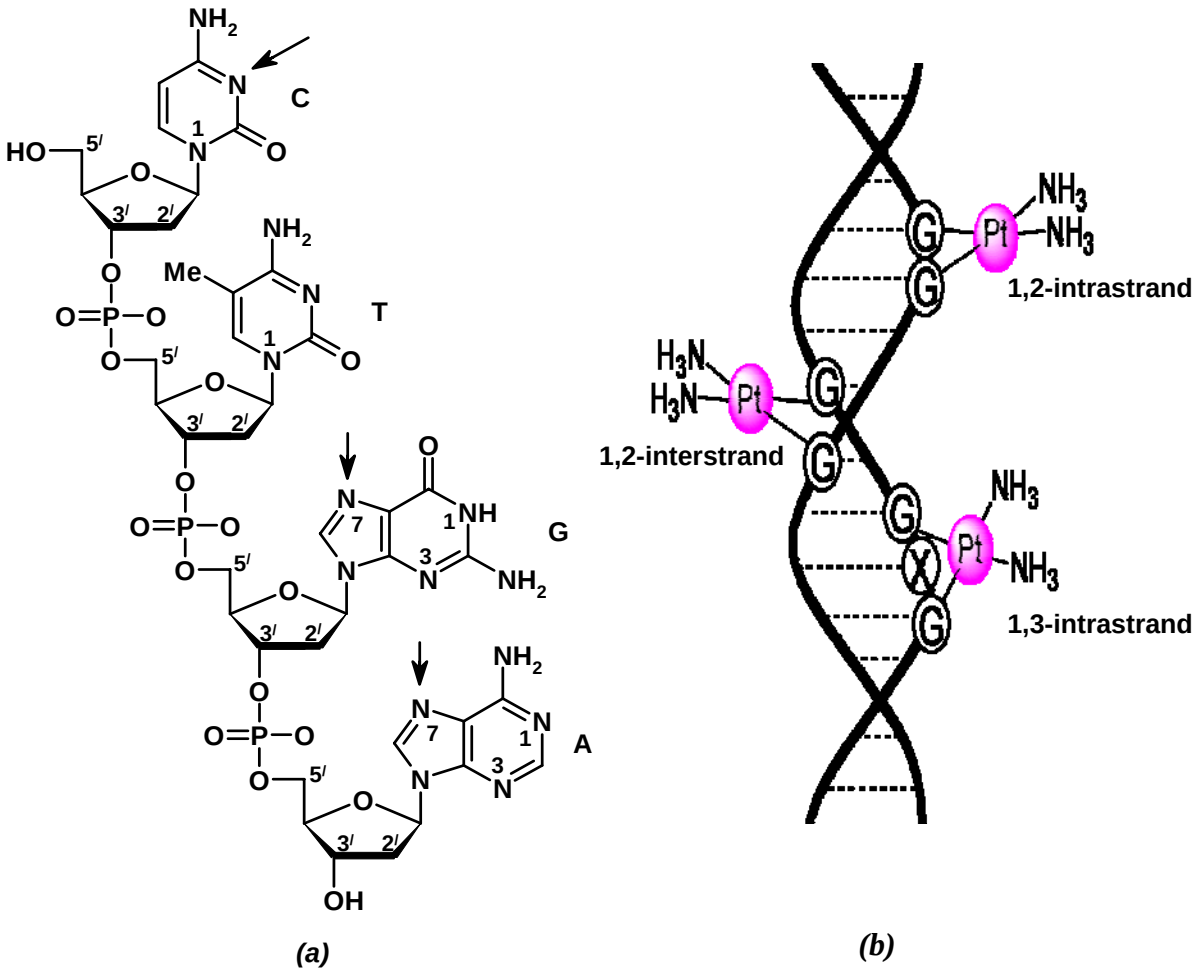


Figure 1.16. *(a) Possible platination sites on DNA nucleobases (indicated with arrows), and (b) various possible crosslinks on DNA.*

The major DNA adducts formed by cisplatin are 1,2-intrastrand crosslinks involving adjacent bases, with the 1,2-d(GpG) adduct comprising 60-65% of the adducts formed and the 1,2-d(ApG) adducts comprises up to 20-25%. The binding of cisplatin on guanine or adenine is preceded by the activation of cisplatin *via* hydrolysis. The chloride ligands get hydrolysed and mono- or bis-aqua cationic species are formed. These species first gets attracted to negatively charged phosphate backbone and the coordinative binding is followed. The first binding step shows a strong kinetic preference for the N7 atom of guanine for its strong basic property and the possibility of hydrogen bond interaction between the NH_3 protons of cisplatin with O6 of guanine. The steric hindrance for adenine and H-bond stability for guanine is schematically shown in Fig. 1.17. Minor adducts, each accounting for a few percent, include 1,3 intrastrand and interstrand crosslinks along with DNA-protein crosslinks.

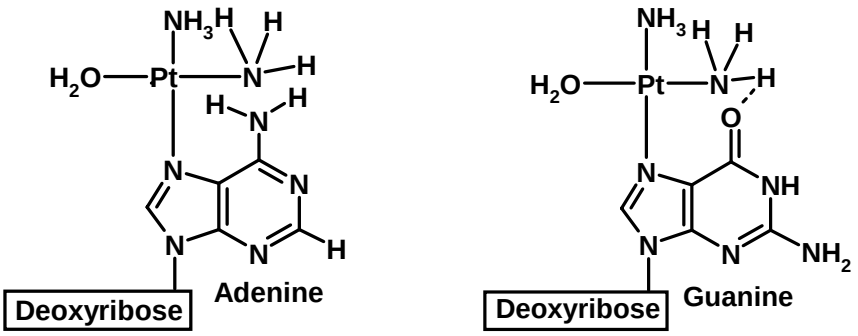


Figure 1.17. *Steric hindrance with adenine and hydrogen bonding stability with guanine exhibited by activated cisplatin.*[161]

Both the 1,2-d(GpG) and 1,2-d(ApG) intrastrand crosslinks unwind DNA by *ca.* 13°, while the 1,3-d(GpXpG) intrastrand crosslinks unwind DNA by ca. 23°. But the bending of DNA double helix is similar (32°-35°) for these three types of intrastrand adducts. The 1,2-intrastrand adducts are believed to be the major responsible for cisplatin antitumour activity.

X-ray crystallography revealed the nature of *cis*-GG crosslink on the single stranded DNA fragments, dinucleotide d(pGpG) or trinucleotide.[148, 163-166] The 3D structure with dinucleotide has shown that the two guanine rings oriented in a head-to-head configuration, with the two O6 atoms on the same side of platinum coordination plane. The dihedral angle between two guanine rings ranges from 76° to 87°, reflecting destacking of

the bases. One of the $-NH_3$ ligand is hydrogen-bonded to an oxygen atom of the 5′-phosphate group.

The three-dimensional picture of cisplatin bound to major groove (1,2-intrastrand crosslinks) is shown in Fig. 1.18.[148] The coordinative binding of cisplatin to two neighbouring guanine bases induces significant local distortion in the DNA duplex. The kink in DNA helix is towards the major groove by 40-80°. The solution (by NMR spectroscopy) and solid (X-ray diffraction) structures show variation in extent of bending depending on the used sequence of oligonucleotide.[167] The helix also opens up to the minor groove with partial unwinding. The 1,3-intrastrand crosslinks induce a bend of 27-33° towards the major groove.[168] On the contrary, the interstrand adducts are observed to induce a 80° unwinding with a kink of 20-40° towards minor groove.[169-171]

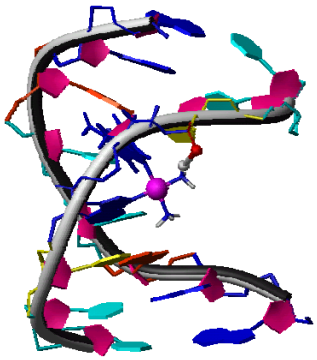


Figure 1.18. *Cisplatin binds to guanine in the major groove of DNA and produces a kink.*

1.3.4. Effects on normal gene activity

(a) Effects on DNA replication and transcription

Replication is an essential cellular process that involves unravelling of double-stranded DNA from chromatin, separation of the duplex strands and the synthesis of new DNA using the original strands as templates. DNA polymerases take an active part in the whole process. As cisplatin forms stable adducts with DNA, the replication process is inhibited, unless repair would occur.

Transcription, the cellular process where *m*RNA is produced from a DNA template, is a crucial step in protein synthesis. In these studies, cells treated with cisplatin progressed through the S-phase, the step for DNA synthesis and are arrested at the G2 phase. This G2 arrest results from the inability of the cells to transcribe genes necessary to enter mitosis. The cell cycle is schematically shown in Fig. 1.19 with different phases.

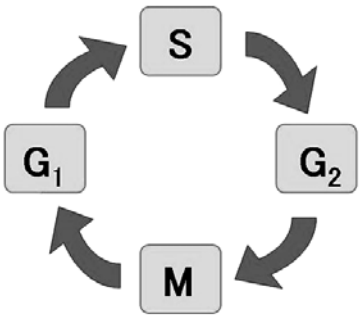


Figure 1.19. *The cell cycle: G1- the first gap phase, S- the DNA synthesis phase, G2- the second gap phase, M-the mitosis.*

The different DNA-repair pathways remove the cisplatin-DNA adducts (which blocks transcription) and the cell cycle normally continues. Other results suggest that the proteins essential for DNA-transcription are hijacked away from their usual binding sites as cisplatin occupied positions on the DNA strands to form adducts. Several proteins are known to recognise and bind to the cisplatin-DNA adduct, like HMG (high mobility group domain) proteins. These HMGs' specifically recognise the 1,2-intrastrand adducts and enhance the bending of DNA. Thus the recognition of cisplatin-DNA adduct by HMG domain proteins can modulate and influence the normal cellular processes. The crystal structure of the HMG-recognisable Pt-DNA adduct was solved by Lippard *et al.*[148, 172, 173]

(b) Telomers and telomerase

Telomers exist at the end of the eukaryotic chromosomes and consist of a tandem, G-rich repeat sequence. Their function is to protect the ends of the chromosomes from the degradation and to ensure that the genetic information is perfectly inherited at each cell division.[174-176] When they become critically shortened, cells become senescent and die. Telomerase, a ribonucleoprotein, synthesises these repeat sequences at the ends of chromosomes and is assumed to play a role in the growth of malignant tumour. Thus telomer-repeated sequences are a possible target for cisplatin since they contain many guanosine residues. Cisplatin may also bind to the RNA, or to the protein component of telomerase and effect telomerase expression.[177-179]

(c) DNA damage and cell death

In the 1980's necrosis was considered the mode of cell-death induced by DNA-damaging anticancer agents, because of the activity of the poly(ADP-ribose)polymerase

(PARP). By the 1990's it was thought that most clinically effective anticancer agents that bind to the DNA kill cancer cells by apoptosis.

It is accepted that futile attempts to repair cisplatin-induced DNA damage may finally result in the triggering of apoptosis. Apoptosis can be generally described as "programmed cell-death" or "cell-suicide".[180-182] It is a controlled pathway that requires ATP and *de novo* protein synthesis. The experimental evidences indicate that protein damage by cisplatin rather than DNA damage plays a role, triggering apoptosis. Some types of cancer cells when exposed to cisplatin, show internucleosomal DNA degradation in an approximately 180 base-pair fragment, blobbing of the cell surface and cell shrinkage. All these features match with apoptosis cell death.[183]

Besides, it is well known that in other cell lines, particularly those with resistance to cisplatin, the drug produces characteristic features of necrosis, a mode of cell death due to general cell-machinery failure.[184, 185] It has also been reported that in the same population of cisplatin treated cells, necrotic and apoptotic cell death may take place simultaneously.[186]

1.3.5. Cellular processing of platinated DNA

(a) DNA repair

It is known that even high levels of DNA platination may not always induce cell death. These are "damage recognition proteins" such as XPA, RPA, and XPC and assist in the repair of DNA lesions provoked by cisplatin. Cisplatin-DNA adducts are repaired in cells primarily through the nucleotide excision repair (NER) pathway.[148] Increased repair in cisplatin-resistant cell lines has been shown to occur both for intrastrand and interstrand adducts. NER is an ATP-dependent multi-protein compound that recognises the kink induced on DNA by 1,2-intrastrand crosslinks and subsequently excises the segment of DNA including the kink, as a 27-29 base-pair oligonucleotide. DNA polymerase then fills the gap.

(b) Cellular resistance to cisplatin

The occurrence of resistance is a serious drawback of cancer chemotherapy and cisplatin is not an exception. In addition, the patterns of cisplatin resistance vary considerably for the different tumour types. Some tumours such as colorectal cancer and non-small cell lung cancer (NSCLC) are intrinsically resistant to cisplatin chemotherapy. Other tumour types, *e.g.*, cervical, testicular, ovarian and small cell lung cancers are

predominantly sensitive to cisplatin treatment. These sensitive tumours mostly develop acquired resistance after the initial treatment.

The molecular mechanism of resistance against cisplatin can be classified into two groups: (a) mechanism that prevents cisplatin reaching DNA as its main target and (b) mechanism that blocks the induction of cell-death (by apoptosis or necrosis) after the formation of cisplatin-DNA adducts.[187] The schematic diagram of several proteins and cellular enzymes which take part in the cellular processing is given in Fig. 1.20.

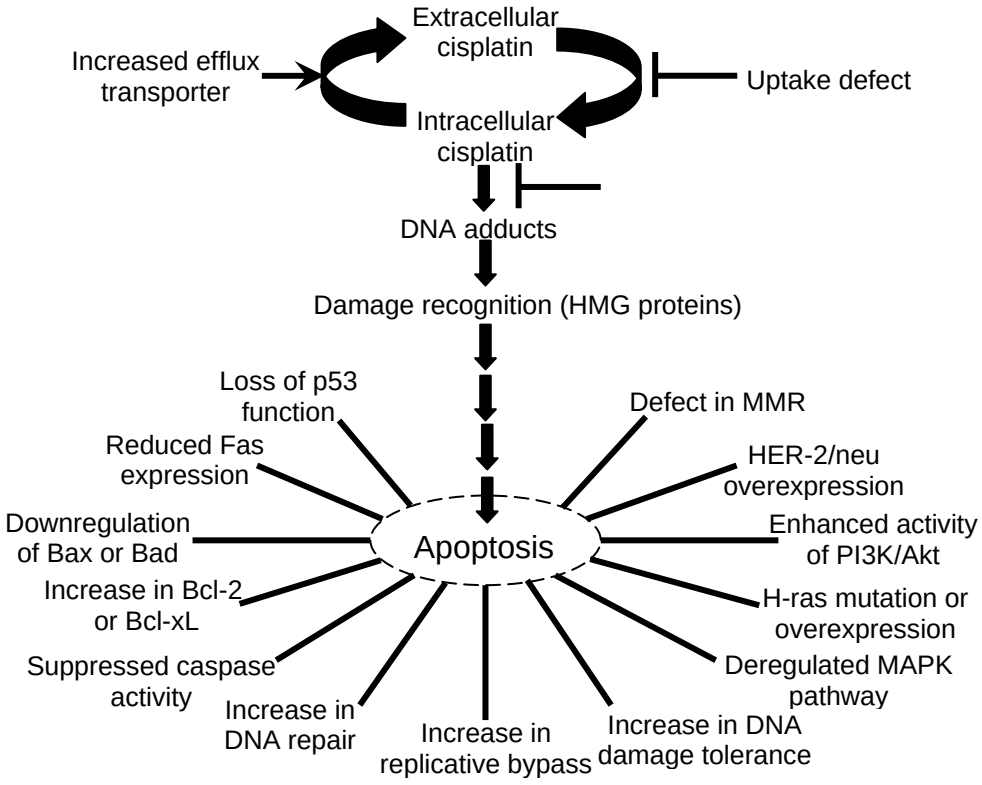


Figure 1.20. *Multiple mechanisms involved in inhibiting apoptotic signal after Siddik.*[187]

The three significant processes, which influence the resistance-mechanism, are namely: (a) reduced intracellular platinum accumulation (lead by decreased uptake of the drug and/or increased efflux of cisplatin), (b) intracellular inactivation of cisplatin by thiol-containing platinophiles (glutathione, GSH and metallothionein), and (c) failure of apoptotic pathways.

1.3.6. Second generation Pt(II) drugs

A structure-activity relationship (SAR) first summarised by Cleare and Hoeschele has been established in platinum anticancer research area.[188, 189] This basic rules help to

design new drugs which could be similar in activity compared to cisplatin and the mode of action can be also expected to be similar as cisplatin. According to the first SAR:

1. Pt(II) or Pt(IV) should have *cis* geometry with the general formula *cis*-$[PtX_2(Am)_2]$ or *cis*-$[PtX_2Y_2(Am)_2]$, where X is the leaving group and Am is the inert amine.
2. The amine should contain at least one N-H moiety.
3. The leaving group, X, should be an anion with intermediate binding strength to platinum and a weak *trans* effect to prevent release of amine in physiological milieu.

Compounds with labile leaving group, *e.g.*, ClO_4^- and NO_3^- are highly toxic, intermediate labile Cl^- or Br^- are antitumour active, whereas compounds with relatively inert leaving group, *e.g.*, I^-, N_3^- or SCN^- are inactive to tumour cells and non-toxic.

In order to overcome acute toxicity, resistance (acquired and inherent) and poor solubility in saline exerted by cisplatin, second generation drugs have been developed. These compounds contain coordinated less-labile carboxylate, oxalate, or glycolate. The most active drug from this series is carboplatin (it contains the chelating dicarboxylate cbdca: cyclobutanedicarboxylic acid), which exhibits improved therapeutic index and ameliorates some severe side effects.[180] This compound has a lower activity than the parent compound (cisplatin) and also lower toxicity. Therefore the drug can be administered in a higher dose (up to 2000 mg/dose)[190] and to outpatients without the problem of forced diuresis. Carboplatin is thus more patient-friendly, despite the inactivity towards cisplatin-resistant cell lines. The dose-limiting toxicity of carboplatin is myelosuppression specifically thrombocytopenia.[140] Some structural analogues of carboplatin are shown in Fig. 1.21.

Lobaplatin [Fig. 1.15(e)] exhibits similar or higher activity compared to cisplatin or carboplatin both *in vivo* and *in vitro*. This compound is devoid of cross-resistance to cisplatin *in vitro* and *in vivo* against a human embryonal cell and *P388* murine leukaemia. As a dose-limiting toxicity thrombocytopenia is observed along with constant emesis and frequent phlebitis.[191] Enloplatin was abandoned for nephrotoxicity and low activity, Zeniplatin was abandoned for myelosuppression, nephrotoxicity and relapsing renal toxicity and miboplatin was abandoned for anemia and nephrotoxicity.[192]

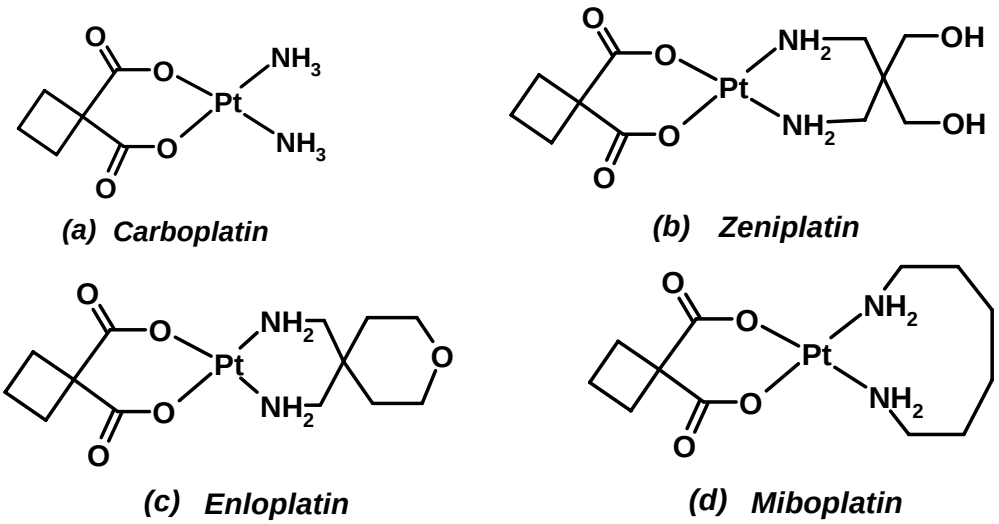


Figure 1.21. *Some second generation platinum drugs containing ammine ligands and cyclobutanedicarboxylate.*

1.3.7. Third generation Pt(II) drugs

These compounds contains different types of (chiral) amines[193] along with the carboxylate ligands. The most active drug in this series is oxaliplatin. Oxaliplatin is approved for clinical use (Eloxatin™) in Europe and China for colorectal cancer.[7] and it is also in medical practice in USA since 2005. The second interesting compound is L-NDDP [R,R-1,2-diaminocyclohexane bis-neodecanoatoplatinum(II)] and other compounds are abandoned for poor solubility, moderate activity *in vivo*, side effects, synthetic trouble and chemical instability problems. The L-NDDP is still in active research and by improvement of the pharmacological and physical properties might be a new drug. The structures of some compounds from this series are shown in Fig. 1.22. The *R,R*- isomer is the active isomer compared to *S,S*- isomer for oxaliplatin.[194-196] The recent results show that both isomer forms similar interstrand crosslinks but the helical unwinding is different. The *R,R*-isomer has cyclohexane ring which allows facilitated approach to DNA than the perpendicularly positioned dach in *S,S*-isomer. Therefore, stronger binding could be the possible reason for the high activity.[197, 198]

L-NDDP is the first liposomal formulation of a platinum compound studied in clinical trials.[199] The rationale for this modification is to solubilise the platinum compound better and to reduce its nephrotoxicity, along with surpass to cross-resistance. The phase-II clinical trial of L-NDDP against advanced colorectal carcinoma (refractory to 5-

FU/leucovorin or capecitabine and irinotecan) has been performed and the results showed possibility of advanced improved formulations.[199-202]

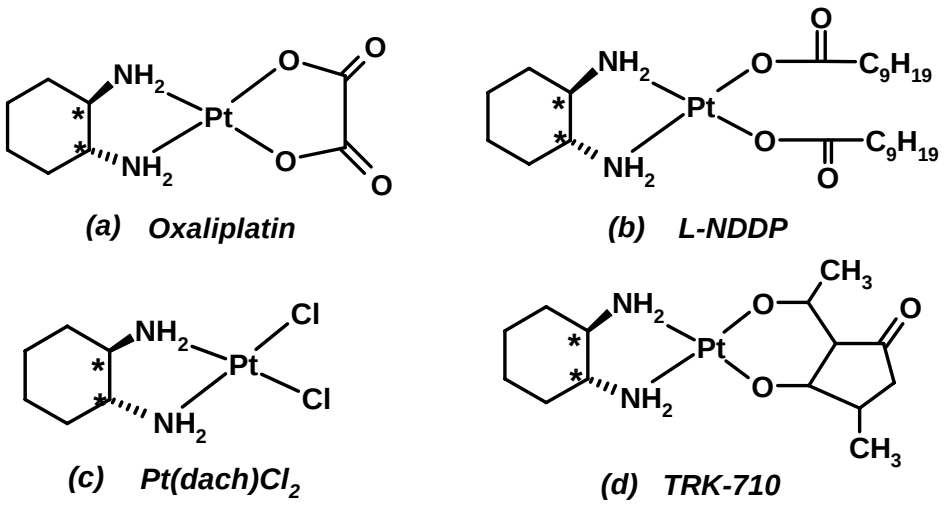


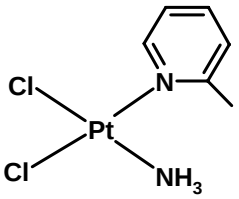
Figure 1.22. *Structures of some third-generation platinum(II) compounds (a) oxaliplatin and (b)-(d) oxaliplatin analogues (all R,R-isomers and chiral centres are marked by *).*[193]

Unfortunately, none of the successors of cisplatin could be considered superior to the prototype in terms of both lower toxicity and clinical efficacy and, thus, the pursuit for novel platinum compounds with optimised pharmacological profile remains an unsolved problem.

TRK-710 is another analogue, that has been in phase-I clinical trials in Japan. The absence of cross-resistance with cisplatin in *in vitro* and *in vivo* models particularly in the L1210/CDDP [CDDP is *cis*-diamminedichloridoplatinum(II)] model *i.e.*, cisplatin resistant leukaemia cell lines, reduced renal and bone marrow toxicity and different mode of action than cisplatin inspired the clinical development. The other dach compounds are not developed further due to severe side effects, toxicity and chemical instability.[203, 204]

A completely different approach to active drug design is to incorporate a ligand with a tunable steric crowding. This compound does not fulfil the criteria of 3rd generation drugs, however to include it in non-classical platinum drugs is not feasible. This compound follows some rules from SAR but, the steric crowding inhibits the similar mechanism of action as cisplatin. A well-known compound is picoplatin [*cis*-amminedichlorido-(2-methylpyridine)platinum(II)].[16, 17] This compound violates the SAR (structure-activity relationship) of cisplatin-like drugs but shows significant antitumour activity. It is known as AMD473 and as JM473. The steric hindrance is produced by the substituted pyridine

moiety and it is relatively less reactive towards deactivating cellular platinophiles glutathione and metallothionein. This compound (Fig. 1.23) entered clinical trials in 1997 and has been proved to be effective in the treatment of ovarian cancer cells resistant to cisplatin and carboplatin.[205-207]



Picoplatin

Figure 1.23. *Structure of sterically hindered compound, picoplatin.*[207]

Picoplatin has been evaluated by third-party investigators in more than 750 patients and has demonstrated activity in a variety of solid tumours, including lung, ovarian, colorectal and hormone-refractory prostate cancer. In addition, picoplatin has shown evidence of activity both in cisplatin-sensitive and cisplatin-resistant cell lines. In Phase-II studies, responses were seen in platinum-resistant patients with ovarian cancer, non-small cell lung cancer, small cell lung cancer and mesothelioma. Clinical studies to date also indicate that picoplatin has an acceptable safety profile and is associated with less toxicity to the kidney and peripheral nervous system than certain other currently marketed platinum chemotherapies. Evidence[208] suggests that picoplatin can be formulated for both oral and intravenous delivery.

1.3.8. Non-classical platinum compounds

The wide range of non-classical platinum compounds can be categorised as: (a) *trans* platinum(II) compounds, (b) platinum(IV) compounds (albeit these maintain some rules from SAR), (c) sterically hindered platinum compounds, (d) platinum compounds with biologically relevant carrier ligands, (e) platinum compounds with intercalator ligands, (f) mono-, bis- or tri-functional polynuclear platinum compounds. The common property of these compounds is a distinctly different mode of action towards biological targets (DNA or cellular protein) and they are therefore expected to overcome resistance in cancer cells. A few of them will be discussed briefly in the following sections.

1.3.9. Trans Pt(II) compounds[209]

Transplatin, the *trans* isomer of cisplatin shows no antitumour activity. Therefore, according to SAR the *trans* compounds would be supposed to be therapeutically inactive. But Farrell *et al*.[210] found a series of active *trans* compounds having general formula, *trans*-[$PtCl_2(L)(NH_3)$] (where L is planar heterocyclic amines). The structures of some active and inactive *trans*-compounds are shown in Fig. 1.24.

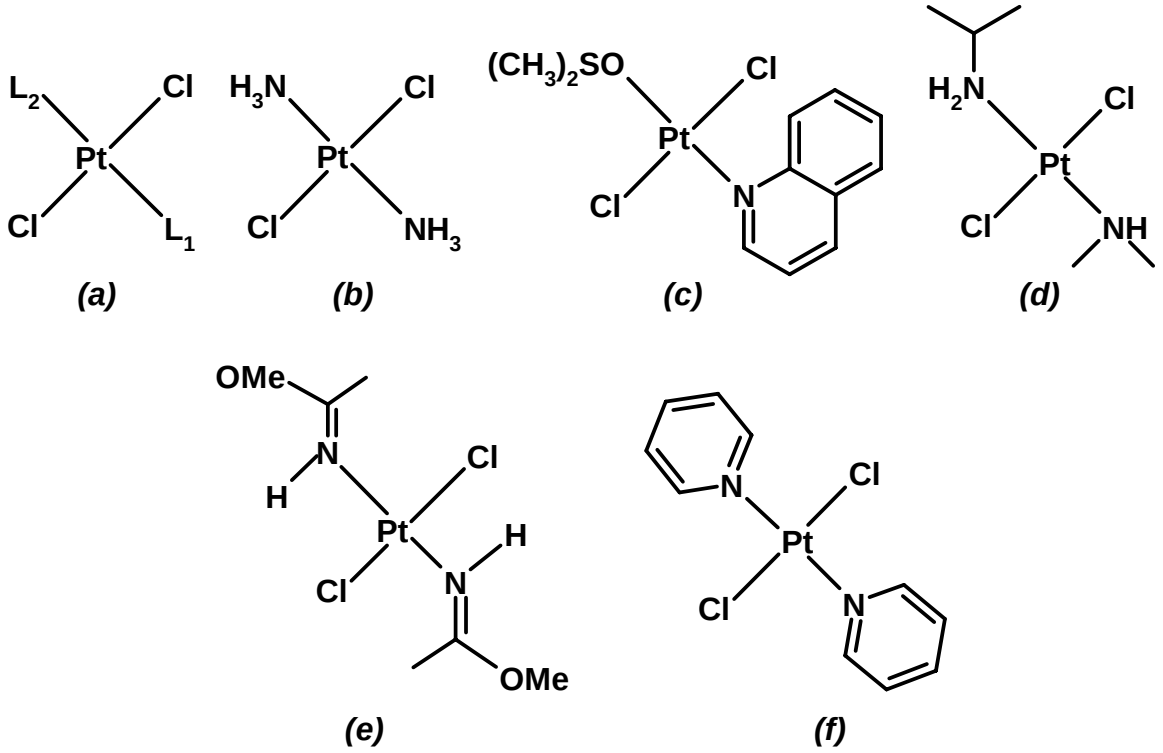


Figure 1.24. *Structure of some selected trans-platinum(II) compounds: (a) general trans compound, (b) transplatin, (c) and (d) asymmetric trans compounds, (e) trans iminoether, (f)symmetric trans with pyridinre.*[211, 212]

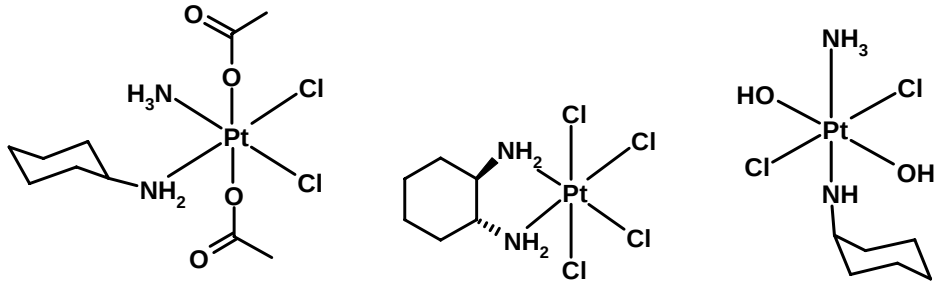
The *trans*-*E*,*E*-iminoether compound [211, 212] of platinum(II) [Fig. 1.24(e)] exhibits a marked betterment in cytotoxicity compared to inactive transplatin (not active against cancer cells) and significant antitumour activity in cisplatin resistant cancer cells. An overall classification of *trans* compounds [213] is [for Fig. 1.24(a)]:

1. L_1= L_2= pyridine, N-methylimidazole and thiol
2. L_1= quinoline and L_2= RR´SO, where R= methyl and R´= Me, Ph, $PhCH_2^-$
3. L_1= quinoline and L_2= NH_3.
4. L_1= azole and L_2= NHR_2.[214-216]

1.3.10. Pt(IV) compounds

Some platinum(IV) compounds have been investigated as orally active drugs as, having lipophilic groups at axial positions, they are suitable for intestinal absorption. Prior to reaction with DNA, they are reduced to active platinum(II) species by extracellular and intracellular reducing agents, so they can be considered to be "prodrugs".

The most successful one is satraplatin, JM216 [bis-(acetato)-amminedichlorido(cyclohexylamine)platinum(IV)]. The two other active compounds are ormaplatin [*cis*-tetrachlorido(1,2-cyclohexyldiamine)platinum(IV)] and JM335 [*trans*,*cis*,*trans*-amminedichlorido(cyclohexylamine)dihydroxidoplatinum(IV)].[217] The structures are shown in Fig. 1.25.



(a) Satraplatin ***(b) Ormaplatin*** ***(c) JM335***

Figure 1.25. *Structure of three active platinum(IV) compounds.*[13]

1.3.11. Platinum compounds with intercalator ligands

These compounds are structurally different from cisplatin having planar aromatic heterocycles as intercalators *e.g.*, bipyridine, terpyridine, phenanthroline.[218] Platinum(II) terpyridine compounds are the most studied platinum intercalators. Mononuclear compounds like $[Pt(terpy)(SC_2H_4OH)]^+$ show intercalating DNA binding, with a typical binding constant of $\sim 10^5$ M^{-1}.[219] Other platinum(II) terpyridine compounds with more labile ligands may bind to DNA, either by coordinative binding or *via* intercalation.[219] The use of a neutral fourth ligand, such as 4-picoline, yields 2+ charged species, with a good solubility in water and promoting the affinity for DNA with a binding constant range of 10^7 M^{-1}. The structures of three platinum compounds with intercalator ligand, terpyridine are redrawn in Fig.1.26. Some of these compounds have been tested against L1210 murine leukaemia cells both in culture and in mice. The $[Pt(terpy)Cl]^+$ did not show activity (IC_{50} = 450 μM) though the range of IC_{50} values for other terpy-Pt compounds have been detected to be in the range of 4-32 μM. These compounds surprisingly, are lacking activity *in vivo*, when tested in L1210 cell lines.[220]

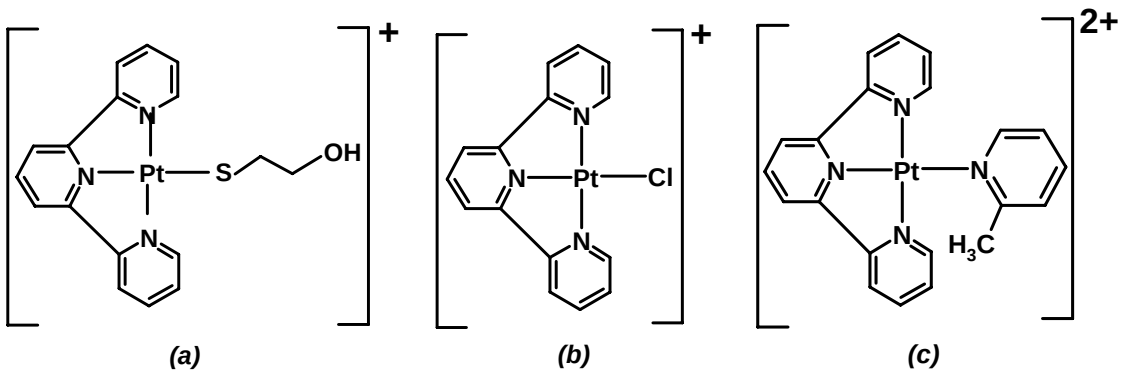


Figure 1.26. *Structures of platinum(II) compounds coordinated to the intercalator ligand, terpy.*[219]

1.3.12. Polynuclear platinum compounds[221]

The design of novel antitumour compounds is directed towards the dinuclear and trinuclear compounds[222-229] because they can bind to DNA by making long-range inter- and intra-strand crosslinks at multiple sites.[221] There is a wide scope for variation in the coordination sphere: leaving group, amine ligand and the backbone linker (to induce flexibility and to access the adjacent binding sites). The dinuclear motif was reported first in 1988 and consisted of two *cis*-$PtCl(NH_3)_2$ units linked by a flexible diamine linker.[230] This compound with the general formula, $[Cl(NH_3)_2PtH_2N(CH_2)_nNH_2PtCl(NH_3)_2]$ (n = 4, Fig. 1.27) may exist in three possible isomers: those containing both coordination units in *cis* configuration (2,2/*c*,*c*), both in *trans* (2,2/*t*,*t*) and the mixed *cis*, *trans* (2,2/*c*,*t*) species.

Within the first hours after dissolution in water the chloride ligand (one on each Pt) gets hydrolysed followed by a more rapid DNA binding than cisplatin.[231] The second binding step *i.e.*, the adduct closure is faster for the *cis* isomer. The shorter Cl-Cl distance (13.05 A° vs. 16.40 A° for *trans*-isomer) reduces flexibility and helps in the mono-/ bis-functional closing step.[231]

The high affinity of the dinuclear compounds towards DNA is the rapid electrostatic interaction due to the charge of the compounds. The structure-activity relationship between the chain-length and antitumour activity is observed for the more active *trans*-isomer. *In vivo* the activity is exhibited for n >4 and the highest is for straight-chain diamine, n = 6. When n > 8, the extended chain has reduced aqueous solubility.[232] Trifunctional dinuclear compounds bind to DNA and effectively form interstrand long-range crosslinks to the DNA. The structures of the compounds are shown in Fig. 1.28.[233]

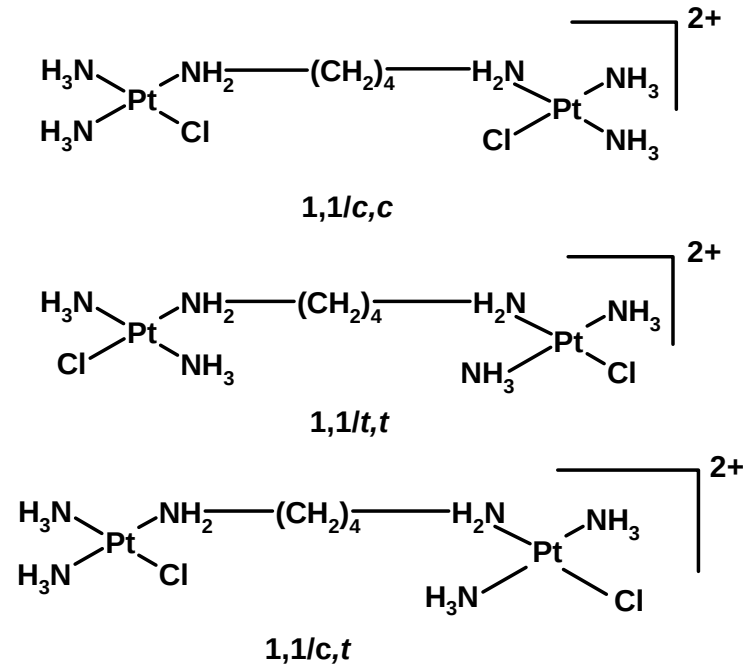


Figure 1.27. *Structures of three isomers of dinuclear platinum(II) compounds.*[231]

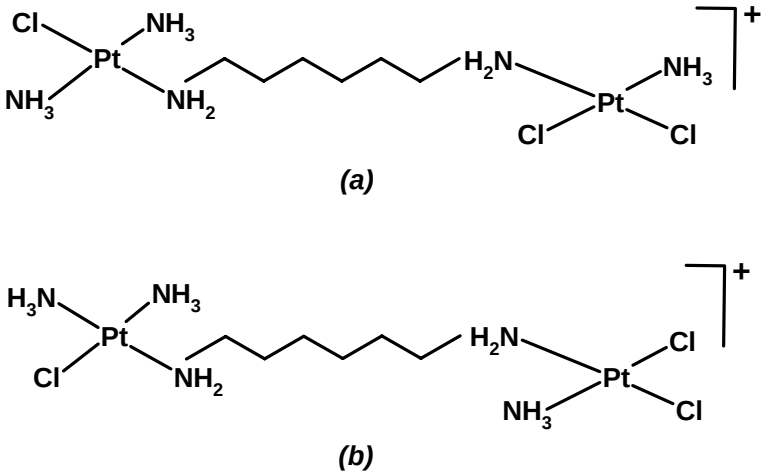


Figure 1.28. *Structures of trifunctional dinuclear platinum compounds.*[233]

Complementary physical, chemical, spectroscopic and molecular biological evidences confirm the conformational flexibility of interstrand crosslinks. The presence of only one guanine base on each Pt-centre removes the steric constraints present in two guanine-bound mononuclear *cis* Pt-centres. Thus it reduces the probability of helix bending. The cytotoxic profile of these compounds in a panel of human ovarian cancer cell lines[234] in the resistant line 41M/cisR with a resistance factor of less than one.[235] The formation of metal-mediated DNA-protein ternary compounds raises the possibility of 'suicide' lesions, which may irreversibly sequester a repair protein or transcription factor.

BBR3464, a bis-functional trinuclear compound (Fig. 1.29), has undergone phase-II clinical trial for treatment of a variety of cancers. This compound has the novel multinuclear platinum motif, where three platinum coordination units are connected through flexible polyamine linkers.[233] The high positive charge (4+) along with hydrogen-bonding capacity by the central tetraammine platinum moiety makes this compound highly susceptible for strong electrostatic attraction towards DNA. Long-range inter- and intrastrand DNA crosslinks are predominant lesions, where the sites of platination are separated by up to four base pairs. Molecular modelling and analysis of stop-sites on DNA and RNA polymerase indicate that the trinuclear compound can easily achieve the 1,6-crosslinks.[236] Notable features are the ten-fold lower maximum tolerated dose in comparison to cisplatin and structural analogues. BBR3464 maintains high antitumour activity in a subset of 6 tumours classified as mutant *p53* within 18 human tumour xenografts. After phase-II trials due to haematological cytotoxicity the development has been abandoned in 2007.[237-240]

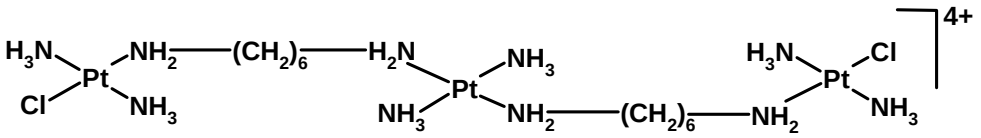


Figure 1.29. *Chemical structure of trinuclear bifunctional BBR3464 after Farrell.*[223]

1.3.13. Polyamine linked dinuclear compounds

The polyamines spermine and spermidine have polycationic character and get protonated at physiological pH. These linkers can provide non-covalent interactions with the negatively charged DNA backbone.[241, 242] The dinuclear platinum polyamine compounds[228, 243] containing spermine and spermidines as linkers (Fig. 1.30) induce the transition of B- to Z- form or to A- form.[244, 245] The major factors influencing this transition are ionic charges in solution, length of the diamine chain and covalent binding on DNA strands. Incorporation of spermidine and spermine spacers is advantageous to optimise the B- to Z- transition and this transition is assumed to be a prerequisite for antitumour activity. The structures of some spermidine- [Fig. 1.30(a)] and spermine-platinum compounds [Figs. 1.30(b) and (c)] are shown.

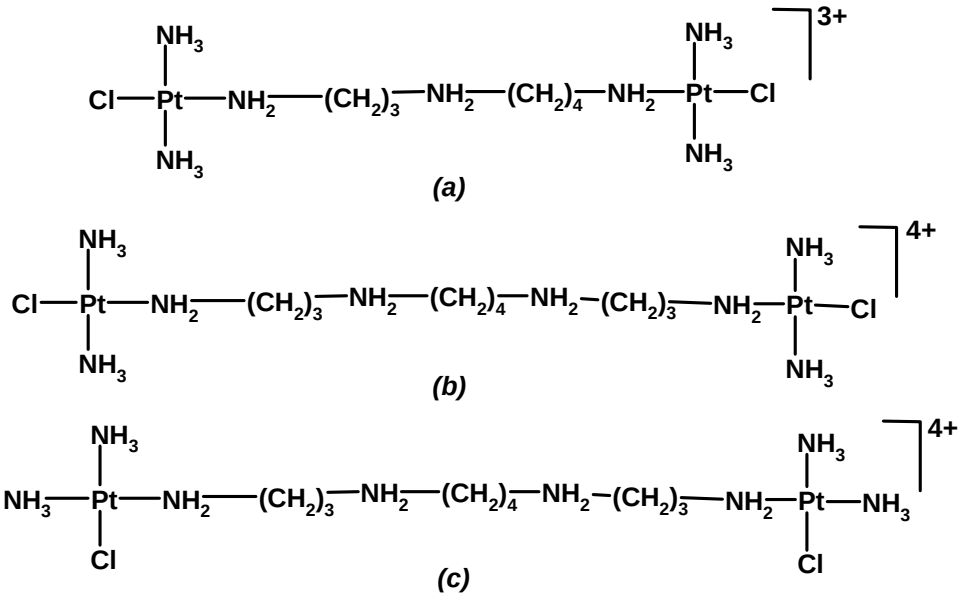


Figure 1.30. *Some dinuclear spermidine and spermine platinum compounds.*[246, 247]

The platinum compounds with spermine and spermidine show high cytotoxic activity in cisplatin-sensitive cells (L1210/0). The spermidine compound shows marked activity in the resistant cell line (L1210/CDDP). To enhance the therapeutic index of these drugs, the potential for 'prodrug' delivery has been utilised. The derivatives with less toxicity and better toleration have been synthesised and investigated for anticancer activity. Preliminary biological assays of cellular uptake and cytotoxicity confirm the utility of prodrug concept. These 'blocked polyamines' after platinum coordination are less cytotoxic than the free spermidine and therefore can be better drugs.[246, 247]

1.4. Ruthenium Compounds in Chemotherapy

1.4.1. General introduction of ruthenium chemistry

Ruthenium, found in the second row of the transition metals in group 8, directly below iron, is the lightest member of the platinum metal group. Ten radioactive isotopes of ruthenium are known, among which ^{97}Ru, ^{103}Ru, ^{106}Ru have been investigated for medical applications. Ruthenium has a vast coordination chemistry with a broad range of stable and easily accessible oxidation states ranging from –II to + VIII.

Many ruthenium compounds are in wide use for homogeneous or heterogeneous catalysis (*e.g.*, the well-known Grubb's catalyst) for olefin metathesis or polymerisation. In the relatively new supramolecular chemistry, several ruthenium compounds have been investigated extensively for their photophysical and photochemical chemistry.

Years before the discovery of cisplatin, some ruthenium compounds have been screened for their antitumour properties. In the early 1960's, Dwyer *et al.* studied the cytotoxicity of ruthenium compounds,[248] but only after the finding of the cytotoxic activity of cisplatin the entire platinum group has attained chemists' attention. In early literature, ruthenium is mentioned as a promising anticancer agent. Ru(II) compounds with 3,4,7,8-tetramethyl-1,10-phenanthroline were evaluated already in 1965 against the *Landschutz ascites* tumours in mice.[248]

In aqueous solution, the most stable oxidation states of ruthenium are II and III. The relatively high kinetic stability these compounds makes them of particular interest. Ru(III) has a marked affinity towards glycoproteins and several compounds, *e.g.*, ruthenium red, have been used for a long time as a biological stain to localise tumours. Ruthenium-ammine chemistry has been thoroughly investigated by Clarke *et al*. [96] in a search for novel anticancer drugs. Some significant properties make ruthenium compounds suitable for medical applications.

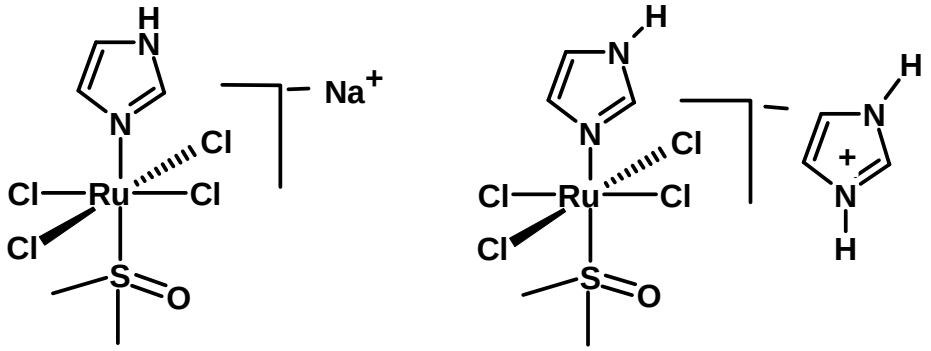
1. The rate of ligand exchange is relatively slow and comparable to Pt(II) and Pt(IV).[249]
2. The range of accessible stable oxidation states (II, III, and IV) under physiological condition and a tuneable electron-transfer rate.
3. The ability of ruthenium to mimic iron in binding to certain biological molecules.[250]
4. The octahedral geometry, compared to square-planar platinum, and the probability of a different mode of action with a varied activity profile.

Presently ruthenium medicinal chemistry has expanded to a wider spectrum of research and ruthenium compounds are being investigated as several varieties of drugs, *e.g.*, immunosuppressant, antibiotics, NO-scavengers and antitumour drugs.[251, 252] The most important groups of ruthenium compounds, *i.e.*, anticancer and antimetastatic agents,[253] will be discussed below.

1.4.2. Ru(III)-dmso compounds

Mononuclear anionic ruthenium compounds,[254] one of which reached clinical trials, are nicknamed as NAMI and NAMI-A respectively, *i.e.*, *trans*-Na[$RuCl_4$(dmSo)(Him)] (Him = imidazole) [Fig. 1.31(a)] and *trans*-(H_2Im)[$RuCl_4$(dmSo)(Him)] [Fig. 1.31(b)].[255] NAMI is the acronym for *New Antitumour Metastasis Inhibitor.* NAMI showed an increase of lifetime expectancy significantly greater than cisplatin on mice bearing Lewis lung

carcinoma, MCa mammary carcinoma or B16 melanoma. The effect of NAMI on lung metastasis of early and advanced tumours is a drastic reduction of the formation of the metastases. Instability upon storage of NAMI resulted in the development of NAMI-A, which in 1999 entered clinical trials. The first results indicated that the toxicity against the host is even lower than expected from the animal studies. It appears that NAMI and NAMI-A significantly increase the thickness of the connective tissues of the tumour capsule and around tumour blood vessels, which probably hinders metastasis formation and blood flow to the tumour.[256-259]



(a) NAMI **(b) NAMI-A**

Figure 1.31. *Chemical structures of antimetastatic Ru compounds NAMI and NAMI-A.*[255]

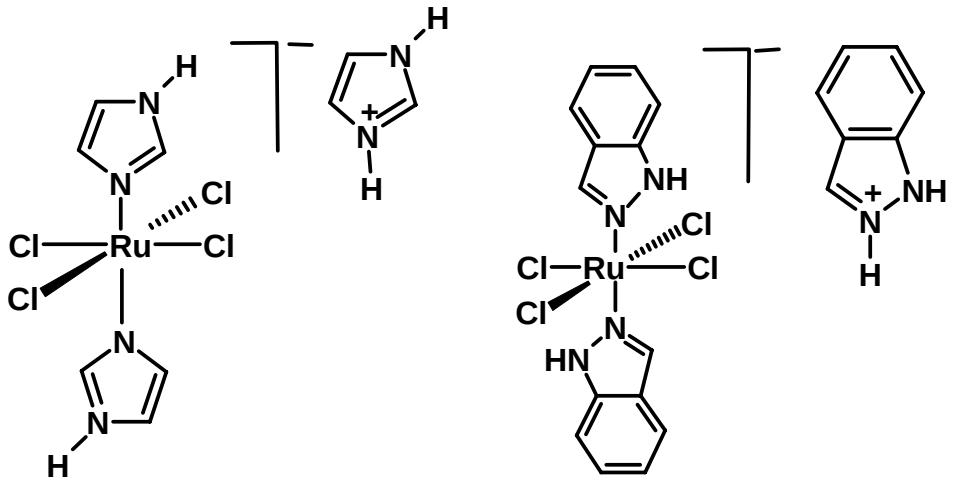
The *trans*-$[RuCl_4(dmSo)(Him)]^-$ in aqueous solution at physiological pH undergoes gradually hydrolysis of two chloride ligands [256] followed by dissociation of the dmso and Him ligands. Recent experiments strengthen the hypothesis that the preferential targets for NAMI-A are certain proteins. Probably NAMI-A exerts its antimetastatic activity by interaction with the proteins, particularly MMPs (matrix metalloproteinases) rather than the alkylation of DNA.[96]

1.4.3. Ru(III) compounds with heterocyclic monodentate ligands

In this classification, two compounds, also known as the Keppler-type compounds, showed promising anticancer properties. These are *trans*-$(H_2Im)[RuCl_4(Him)_2]$ (also called Ru-im or ICR) and *trans*-$(H_2Ind)[RuCl_4(Hind)_2]$ (also called Ru-ind or KP1019). The chemical structures of these two compounds are shown in Fig. 1.32.

Both of them exhibit marked activity against P388 leukaemia, whereas Ru-im shows better activity against Walker 256 carcinoma and Stolkholm ascitic tumour.[260, 261] In a chemically induced colorectal tumour model in rats (known to be insensitive to cisplatin) both Ru-im and Ru-ind exhibit promising response. Comparatively, Ru-ind being less toxic

can be given at higher dose and it reduces the tumour volume up to 5 %, without any mortality.[260, 261] These anionic compounds are considered as pro-drugs that hydrolyse rapidly *in vivo* to form relatively stable neutral, *trans*-[$RuCl_3(H_2O)(L)_2$] compounds.



(a) Ru-im ***(b) Ru-ind KP1019***

Figure 1.32. *Structures of the two anionic mononuclear Ru(III) compounds Ru-im and Ru-ind after Keppler.*[260, 261]

1.4.4. Ru(II)-dmso compounds

Early literature[262] shows the compound *cis*-[$RuCl_2(dmSo)_3(dmsO)$] to be an antitumour and antimetastatic agent as it reduces primary tumour growth in all the tumours tested significantly (three metastasising tumours of the mouse namely, Lewis lung carcinoma, B16 melanoma and MCa mammary carcinoma). The corresponding *trans*-compound, *trans*-[$RuCl_2(dmSo)_4$] has also promising antitumour and antimetastatic activity at lower dosages compared to the *cis* isomer. The structures of these two compounds are shown in Fig. 1.33. When the *cis*-isomer is dissolved in water, it immediately undergoes loss of the dms*O* ligand, whereas the *trans*-species yields a *cis*-diaqua compound, loosing two adjacent dm*S*o ligands. Both hydrolysed isomers then undergo slow reversible chloride dissociation, forming cationic compounds. The *trans*-compound having three reactive groups is more active in comparison to the *cis*-compound, which has three dmso ligands with considerable steric hindrance.

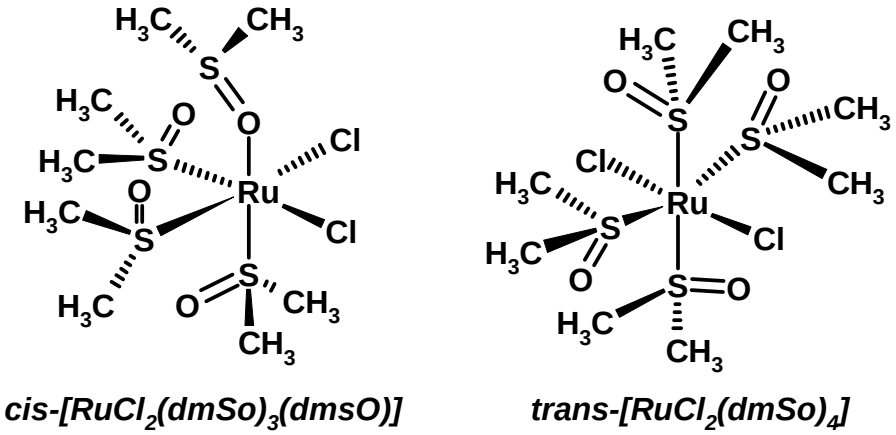


Figure 1.33. *Schematic structures of the two ruthenium(II)-dmso isomers.*[262]

1.4.5. Ru(II/III)-ammine compounds

Among the Ru-ammine compounds (Fig. 1.34), *cis*-$[RuCl_2(NH_3)_4]Cl$ and *fac*-$[RuCl_3(NH_3)_3]$ exhibit a positive response against P388 Leukaemia cell line (and in mice also).[263]

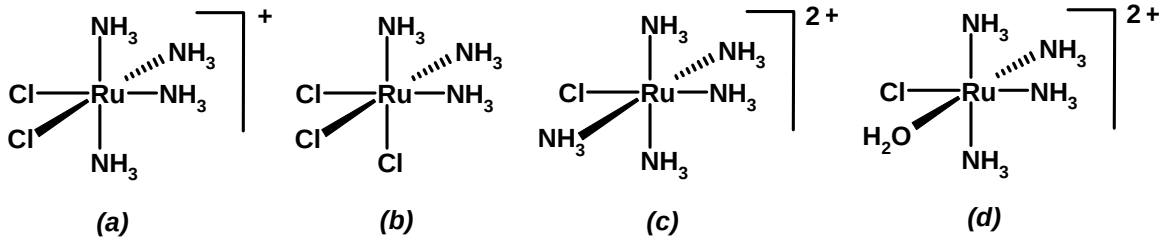


Figure 1.34. *Structures of Ru(III)-ammine compounds with different functionality.*[263]

Among the monofunctional (based on the possibility of forming monofunctional adduct with DNA purine bases as they contain single labile ligand) compounds, $[RuCl(NH_3)_5]^{1+/2+}$ and $[Ru(H_2O)(NH_3)_5]^{2+/3+}$ bind preferentially to guanine in single- and double-stranded DNA, but also with adenine and cytosine.[264] Interestingly, the pyridine analogue of this series, *cis*-$[Ru(H_2O)(py)(NH_3)_4]^{2+}$ binds exclusively to the guanine residue. Therefore it is possible by small variation in the coordination sphere to tune the DNA binding. The DNA-binding of ruthenium-ammine compounds exhibits the inhibition of DNA replication, show mutagenic activity, induce a DNA-repair system and reduce RNA synthesis. Most probably the hydrolysis of the parent compounds *in vivo* yields the active species, aquated polyammine-ruthenium complexes.[264]

These ammine compounds have been studied by Clarke *et al.* who proposed the 'activation-by-reduction' hypothesis. The inert (with respect to ligand substitution) Ru(III)

compounds are transported through the blood-stream as inactive prodrugs. In many solid tumours the existing hypoxic medium facilitates the reduction of Ru(III) to the relatively labile Ru(II) moiety, which can then react easily with target molecules like DNA bases. The current research shows that the pentaammine compounds show higher cytotoxicity at high glutathione concentration, but the lower GSH/Ru ratio alters the base where ruthenium binds (no guanine N7 binding but adenine or cytosine binding).[265] This change in usual binding site though can not be directly related to cytotoxicity.

1.4.6. Organoruthenium compounds

The organometallic compounds with the general formula, $[Ru(II)X(\eta^6\text{-arene})(en)]^+$ (X is halide, arene is benzene or substituted benzene, en is ethylenediamine), also named piano-stool compounds because of their shape, belong to this class.[266-269] These compounds show promising *in vitro* IC_{50} values in the A2780 cell line: two compounds are equipotent to carboplatin (6 μM) and the most active compound, $[Ru(\eta^6\text{-tha})(en)Cl]PF_6$ (where tha= tetrahydroanthracene) is equipotent to cisplatin (0.6 μM).[270] From a series of these compounds it has been noticed that cytotoxicity increases by increasing the hydrophobicity of the arene ligand. None of these compounds show cross-resistance in the cisplatin resistant A2780cisR cell line, but they also display cross-resistance in the multi-drug-resistant cell line 2780AD.

This 'piano-stool' series exhibits a wide spectrum of activity *in vitro* and is also active against some tumours which are resistant to cisplatin. The level of anticancer activity is dependent on the aryl unit with more extended aryls (biphenyl, tetrahydroanthracene) showing higher activity. Replacing the ethylenediamine with more bulky N-donor ligands, such as bipyridine or N,N,N',N'-tetramethylethylenediamine reduces the activity, although with 1,2-diaminobenzene the activity is retained. While the compounds can interact with a variety of different biomolecules, the biomolecular target may be DNA,[271] the chloride can be replaced by a water ligand in aqueous solution, and the compound can coordinate to the DNA bases at this position. The compounds exhibit a strong preference for G residues (binding at N7), and there is some indication that the larger aryl groups can partially insert between the DNA bases, thereby creating a bisfunctional (metal coordination and partial insertion) lesion which may account for their higher activity. However, this lesion must be distinct from that caused by cisplatin, as is not recognised by HMG proteins. The different mode of binding is consistent with the activity against cisplatin-resistant cell lines.

The water soluble compound, [Ru(η^6-p-cymene)(pta)Cl_2] (pta is 1,3,5-triaza-7-phosphatricyclo[3.3.1.1.]decane or 1,3,5-triaza-7-phosphaadamantane), exhibits pH-dependent DNA binding, which can be utilised to increase selectivity for hypoxic cells.[272] This compound is structurally similar to piano-stool compounds, but the activity profile is quite different. This Ru-organometallic (Fig. 1.35) compound shows very low toxicity toward cancer cell lines. Like NAMI-A, these agents are inactive against primary tumours, but are found to have *in vivo* activity against metastases. The RAPTA compounds, where RAPTA means ruthenium(arene)(pta), are slightly less potent antimetastatic agents than NAMI-A, but (in mice) less toxic and thus can be administered in higher doses. As NAMI-A, the indications are that proteins, rather than DNA, are the biomolecular targets for action of these compounds. Enhanced activity against lung metastases on co-administration of cisplatin has been demonstrated. Given the structural differences between the RAPTA and NAMI-A compounds, they might be expected to react differently with proteins, perhaps selecting different biomolecular targets or pathways.

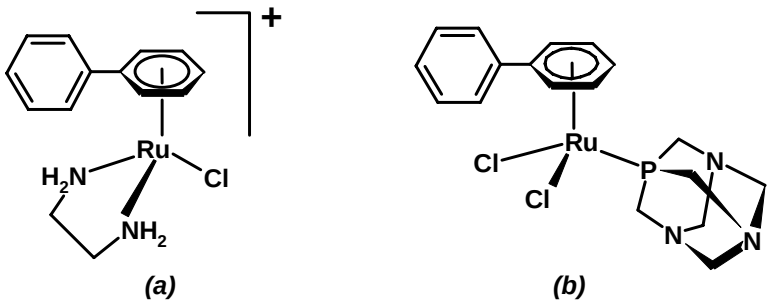


Figure 1.35. *Structures of organometallic Ru(II) compounds; (a) piano-stool and (b) RAPTA after Sadler and Dyson.*[270, 272]

1.4.7. Dinuclear ruthenium compounds

The introduction of multinuclear platinum compounds in platinum pharmacology highlights the real possibility to overcome the problem of resistance most probably due to increased interstrand DNA binding. Similarly, a series of dinuclear Ru(III) compounds, having the general formula $Na_2\{(trans\text{-}RuCl_4(dmSo))_2(\mu\text{-}L)\}$, where L is a ditopic aromatic nitrogen donor ligands, have been synthesised. These water-soluble compounds show interesting response on *in vitro* cell culture and antineoplastic activity against animal tumour models, partly related to NAMI-A activities.[255]

The dinuclear structure is maintained even after the hydrolytic process with loss of chloride ligands. *In vivo*, these species show activity comparable to that of NAMI-A in

mice bearing advanced MCa mammary carcinoma. In terms of molar concentration, dinuclear compounds are administered at a dose of 3-4 fold lower than NAMI-A. In the lung the compounds show the same concentration per mg tissue as NAMI-A but the liver and kidney show a higher accumulation.[254] Some dinuclear compounds derived from the parent NAMI-A, is shown in Fig. 1.36.

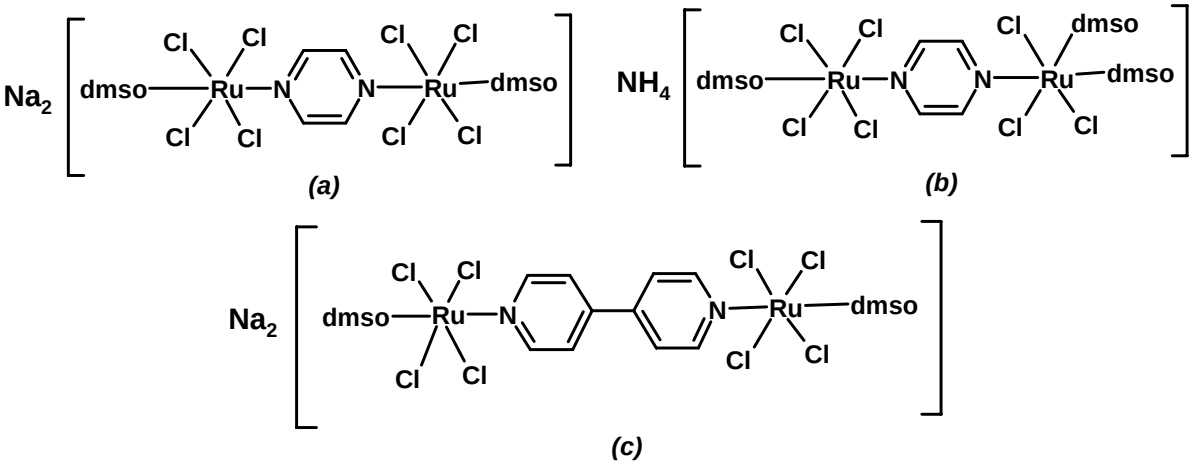


Figure 1.36. *Dimeric antitumour agents related to NAMI-A.*[255]

1.4.8. Mode of action of antitumour active ruthenium compounds[273]

The ruthenium compounds have been investigated for elucidating the mode of action against normoxic and hypoxic cell lines, binding to several cellular components, biodistribution and accumulation. Several bio-physical and spectroscopic methods have been used to follow the biological route. These compounds are expected to have different cellular mechanism than cisplatin, though some compounds are believed to target genomic DNA as the primary binding sites.

(a) Transferrin transport

The elevated requirements of tumour cells for nutrients coupled with their higher membrane permeability and angiogenesis with associated increased blood flow result in both specific and non-specific uptake of metallopharmaceuticals. Specific uptake for several metal ions appears to be mediated by transferrin (tf).[46, 274] Non-specific uptake is facilitated by the increased permeability of tumour cells. Some cationic compounds may enter cells through endocytosis following binding to anionic sites on the cell surface and the neutral ones may diffuse through the membrane.

Antitumour active ruthenium compounds are transported through blood. The generally slow ligand-exchange rate of Ru(II) and Ru(III) compounds contributes to their

inertness in the blood stream. In the blood, albumin and transferrin are abundant having numerous surface accessible histidyl imidazole nitrogens. Transferrin appears to facilitate the smooth entry of the ruthenium compounds into cells, whereas both the kinetics and equilibria of ruthenium uptake and release are highly dependent on the respective compounds.

Transferrin (tf) is an iron carrying protein present in human serum at concentrations of 2.5-3.5 mg/mL. Apotransferrin is the iron-free transferrin and can bind up to two equivalents of Fe(III). Tumour cells, specially the fast growing ones, show a much higher uptake of iron-transferrin than the surrounding normal tissues. Some ruthenium anticancer compounds are assumed to be transported to the tumour *via* transferrin. Association of the Ru-tf complex to receptor sites of cell surface is followed by endocytosis.[46, 274] The Ru(II)/Ru(III)-tf reduction potential should be biologically accessible. Such reduction facilitates the release of ruthenium from the histidine sites of transferrin, particularly at the lower pH of tumour tissue, or the transferrin endosome (pH 5.6). Transferrin uptake may lower the ruthenium toxicity, by preventing it from other binding or uptake until it has been delivered to the cells.[46]

(b) Activation by reduction

In contrast to the square-planar geometry of platinum(II) compounds, the octahedral structure of ruthenium(II) and ruthenium(III) antitumour compounds may help ruthenium compounds to y act possibly in a different manner. The platinum compounds bend DNA by crosslinking adjacent guanine residues and thereby causing a class of DNA-binding proteins to adhere to the site. The ruthenium(III) prodrugs *in vivo* are hypothesised to get activated by reduction to more active ruthenium(II) species.[264] Tumours utilise oxygen and other nutrients quite fast and the development of new blood vessels (known as neovascularisation or angiogenesis) often fails to take place with the tumour growth. Thus the O_2 content in tumour cells is often low leading to hypoxia. As a result the tumour cells are more dependent on glycolysis for energy and generate an excess of lactic acid with a lowering of pH in the cancer cells. Due to these metabolic differences, the relative electrochemical potential inside solid tumours is lower than in the surrounding healthy cells.[275, 276]Thus the different situation inside the tumour cells favours the reduction of Ru(III) to active Ru(II) species.[264]

Glutathione (GSH) and a number of redox proteins are capable of reducing Ru(III) *in vivo*. Single-electron-transfer proteins, which exist in both the mitochondrial electron-

transfer chain and in microsomal electron-transfer systems, can also perform the necessary reduction process. Oxidation of Ru(II) back to Ru(III) can occur by molecular oxygen, cytochrome C oxidase and by other oxidants, but this process is unlikely to happen in the hypoxic tumour cells. The relationship of hypoxia, DNA-binding, cytotoxicity and reactive oxygen species remains unexplored, but could lead to better understanding of redox active metallodrugs in cancer cells.

(c) DNA binding

Relatively soft transition metal ions, *e.g.*, Ru(III) and Ru(II) tend to bind preferentially to nitrogen sites on DNA bases. Both ions are known to bind preferentially at G7 sites, which are exposed in the major grooves, but they may also bind on the exocyclic adenine and cytosine residues. Ru(III) compounds with multiple chloride ligands may form monodentate or bidentate adducts with purine bases and octahedral Ru compounds might influence the global stability of DNA strands. The adjacent intrastrand G-G crosslinks are possible for the *cis*-ruthenium compounds, but these are more sterically crowded, compared to cisplatin. In spite of the fact that the original geometry of the complex may have nothing to share with the geometry of the active aqua species, the DNA-Ru compound adducts can also influence the cellular activities.

(d) Effects on nucleic acid functions

There are several possibilities in which metal-DNA adducts can interfere with DNA metabolism such as (1) blockage or lack of recognition by replicating enzymes at the metallated G, thereby halting DNA synthesis; (2) intra- or interstrand crosslinks by the metal; (3) protein-DNA crosslinks; and (4) chemical reaction of guanine residue induced by the metal ions.

As replication enzymes are sensitive to ionic environment, even simple ion pairing or hydrogen bonding interactions may cause replication errors. Local helix disruption may be caused by (a) steric effect exerted by large metal ions, (b) weakening of hydrogen bonding and π-stacking abilities of the base through polarisation of electron density toward the metal cation and (c) external hydrogen bonding. Chemical reactions of the guanine residue involve autoxidation and N-glycosidic hydrolysis.

(e) Modulation of DNA binding by glutathione

Glutathione (GSH) is present in cells at concentrations of 0.1-10 mM, but it is readily oxidised to the disulfide, GSSG. Depending on the relative concentration, experimentally it has been shown that for amminechloridoruthenium(III) compounds, GSH both facilitates and inhibits ruthenium binding to DNA. At [GSH]/[Ru(III)] ≤1,the coordination of amminechloridoruthenium(III) compounds to DNA is facilitated by GSH reduction whereas at [GSH]/[Ru(III)] ≥1, the DNA binding is inhibited by GSH. Inhibition of DNA binding by GSH is most evident at the guanine site and GSH removes all the metal ions from that site.[277] But it is less effective in removing the metal ions from adenine or cytosine sites due to lower Ru(III)/Ru(II) reduction potential.[277] Such alteration of DNA binding at physiological concentrations of GSH may have a significant effect on the mechanism of ruthenium antitumour agents by favouring the adenine or cytosine sites over guanine sites, but it is yet to be determined.

1.5. Intercalators as anticancer agents[278]

Intercalators belong to a distinct class of compounds, which have successful medical application in several ailments. Some of these compounds are already in medical practice *e.g.*, doxorubicin, actinomycin and mitoxantrone. When coupled with an active metal, these extended planar molecules are expected to enhance the binding to DNA. Their intrinsic property to slide through the base-pairs made them obvious choice for pharmaceuticals with distinct and tunable mode of actions.

1.5.1. Intercalators

DNA intercalation can be defined as the insertion or sliding of a molecule between two adjacent pairs of bases in the DNA double helix.[279] Intercalators are characterised by the possession of an extended electron-deficient planar aromatic ring system.[280] An intercalator sandwiched in DNA base pairs is shown schematically in Fig. 1.37.

Upon binding, they extend and unwind the deoxyribose–phosphate backbone and are stabilised by π-π stacking interactions with the planar aromatic bases.[281] Intercalation also leads to hydrodynamic changes in the DNA, due to the decrease in twisting between the base pair layers, the lengthening of the DNA itself, the stiffening of the helix, and the decrease in mass per unit length.[193, 282] Intercalation requires changes in the sugar-phosphate torsion angles in order to accommodate the aromatic compound. This torsion triggers other changes in the helix parameters such as unwinding and bending.[281] Many

intercalators have a preference to intercalate in the GC sequence due to lower energy needed for its unstacking.[283]

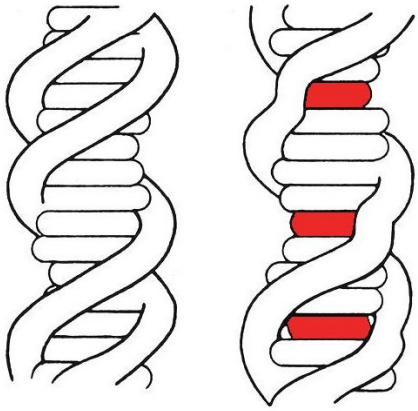


Figure 1.37. *Schematic diagram for intercalator (in red) in DNA strand.*[281]

These effects are fully reversible upon removal of the intercalator as long as the DNA duplex structure is not destroyed by the process of removal. A bound intercalator lies in a plane perpendicular to the helix axis and this position relative to the base pairs is stable.[193, 284-287]

1.5.2. Mode of action of intercalators

Intercalators are the most important group of chemicals that interact reversibly with the DNA double helix and are the most common anticancer drugs used in clinical therapy (mainly for acute leukaemia, ovarian and breast cancers).[288] They inhibit cell growth by two well-established mechanisms, (a) inhibition of replication through poisoning of topoisomerases and (b) inhibition of transcription *via* poisoning of the RNA polymerase. Either one or combined mechanisms is possible for the intercalators.

(a) Topoisomerase inhibitor

Prior to a cell division, DNA polymerases must duplicate the genetic information *via* DNA replication. Several DNA polymerases progress on the two strands of the DNA molecule, reading the sequences of nucleotides at high speed (about 80 per second). All together, they manage to duplicate the 10^8 nucleotides of a DNA molecule in a few hours to create two double-stranded DNA molecules. During this high-speed replication process, the two strands of the parent DNA must be unwound from each other to allow the progression of the DNA polymerase. Enzymes called DNA topoisomerases remove the helical twists by cutting either one or both strands (topoisomerase I and II, respectively) and reseal the cut.

Intercalators active *via* the poisoning of topoisomerases interact both with DNA and topoisomerases and stabilise the enzyme in its cleavable state.[289] The cuts cannot be resealed, and the accumulation of ternary complexes that include the drug, the enzyme and the DNA leads to DNA replication abortion.[290]

(b) RNA polymerase inhibitor

Gene expression has to transit from DNA to RNA before being translated into proteins. The synthesis of RNA is called transcription and is governed by RNA polymerases that have to read the DNA template in order to synthesise the corresponding RNA molecule. Some DNA Intercalators can impede the progression of RNA polymerases, leading to cell death. Transcription inhibitors lead to cellular death without halting the cell cycle unlike topoisomerase inhibitors.[291] A characteristic shared by the transcription inhibitors is that they bind to DNA reversibly with modest affinities, placing bulky groups in the minor groove covering 4 to 6 base pairs, and form long-lived complexes with the DNA. This ability to form long-lived DNA complexes, with half lives of hundreds of seconds, distinguishes the transcription inhibitors from the topoisomerase inhibitors, which dissociate from DNA rapidly in the millisecond to second time range.[292]

(c) Other modes

Other modes of action are observed within the anthracycline family. One occurs through metal-ion chelation of the *in situ* generated semiquinone, producing DNA-cleaving free radicals. Another one occurs *via* interaction with the cell membrane leading to alteration of membrane fluidity and ion transport, disturbing various biochemical equilibria in the cell.[293]

Intercalators can be categorised depending on their chemical group (structure of chromophores), parental origin and mode of activity (mono, bis or poly). Another way of classification could be: classical, non-classical and metallointercalators. The classical intercalators can be classified as (a) naphthalimide and related compounds, (b) pyridocarbazole family, (c) anthracyclin family, (d) echinomycin family, (e) acridine and related compounds, (f) actinomycin family and (g) benzimidazo[1,2-*c*]quinazoline family. Representative examples from each class are depicted in Figs. 1.38 and 1.39.

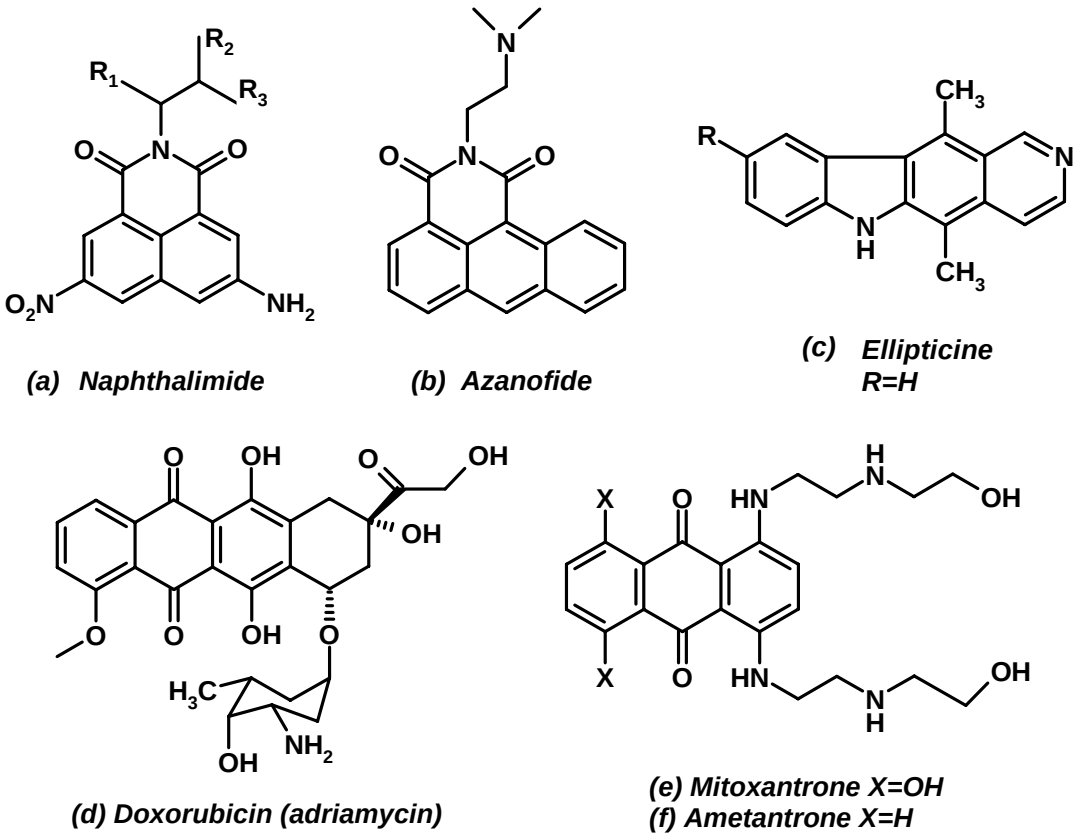


Figure 1.38. *Structures of selected representatives of different class of intercalators.*[278]

1.5.3. Metallointercalators

For a metal compound to be an intercalator, intrinsic planarity or coordinating to an extended planar ligand is required. The first series of metallointercalators was reported already in 1978[294] with the structures shown in Figs. 1.40(d)-(e). In contrast to cisplatin, intercalator ligands such as phenanthroline derivatives and their metal complexes interact with DNA by aromatic π-stacking in-between base pairs along the helix.[295] Some of these cationic intercalators are very active against leukaemia cell line (L1210) and are assumed to show activity *via* the above mentioned topoisomerase II blocking.[193, 296] For these compounds bearing dach (diaminocyclohexane) as ligand, *S,S*- isomers are more active than *R,R*-isomers.[296]

Another class of potential metallointercalators is formed by cationic porphyrins; they combine readily with metal centres in their central cavity and exhibit a strong binding affinity for DNA. Three types of binding modes are possible[297]: (1) self-induced external auto-aggregation along the DNA helix axis, (2) binding in either the major, or minor groove of DNA, and (3) intercalation between base pairs. However, intercalation only

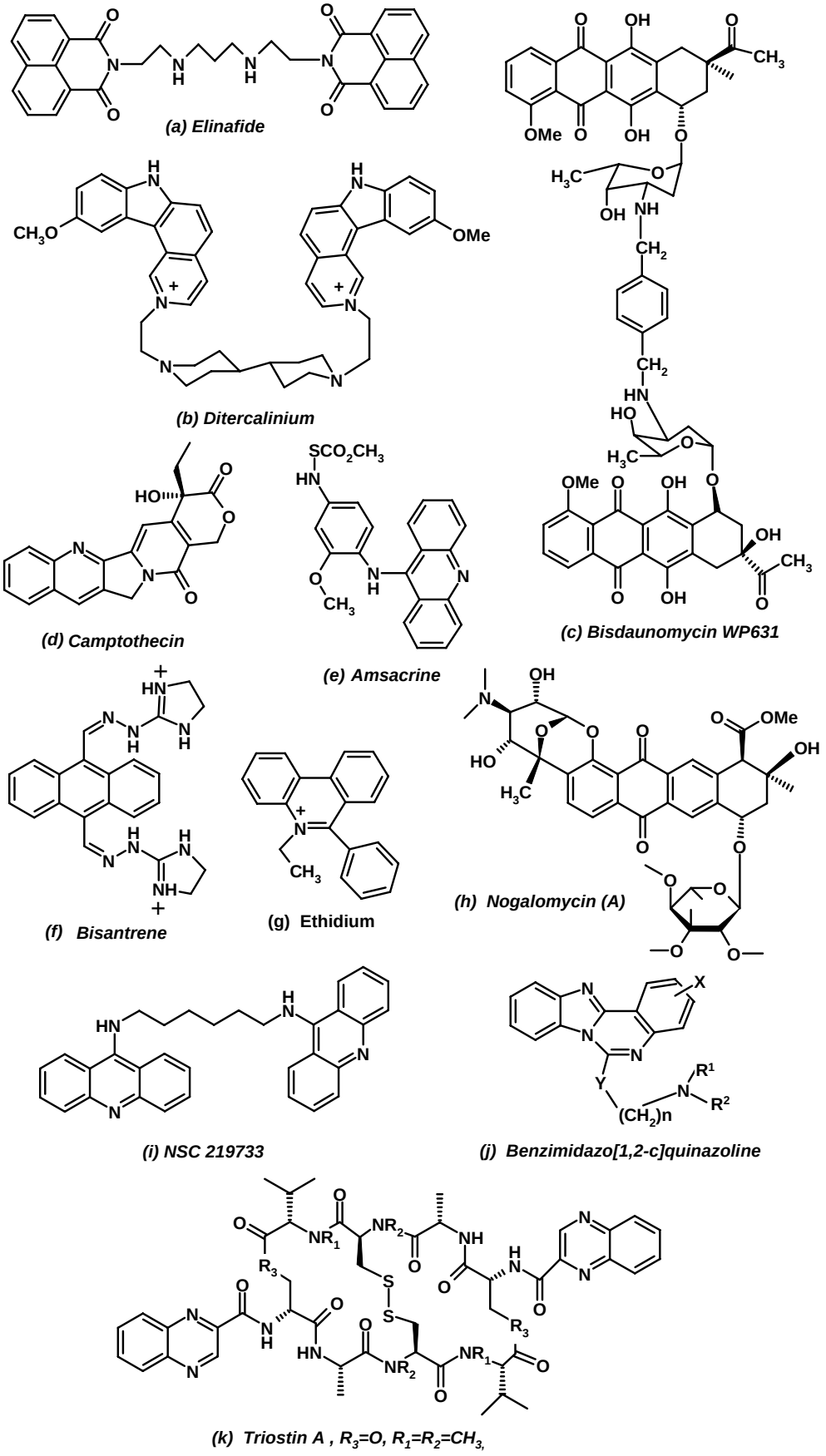


Figure 1.39. *Structures of some medically important intercalators.*[278]

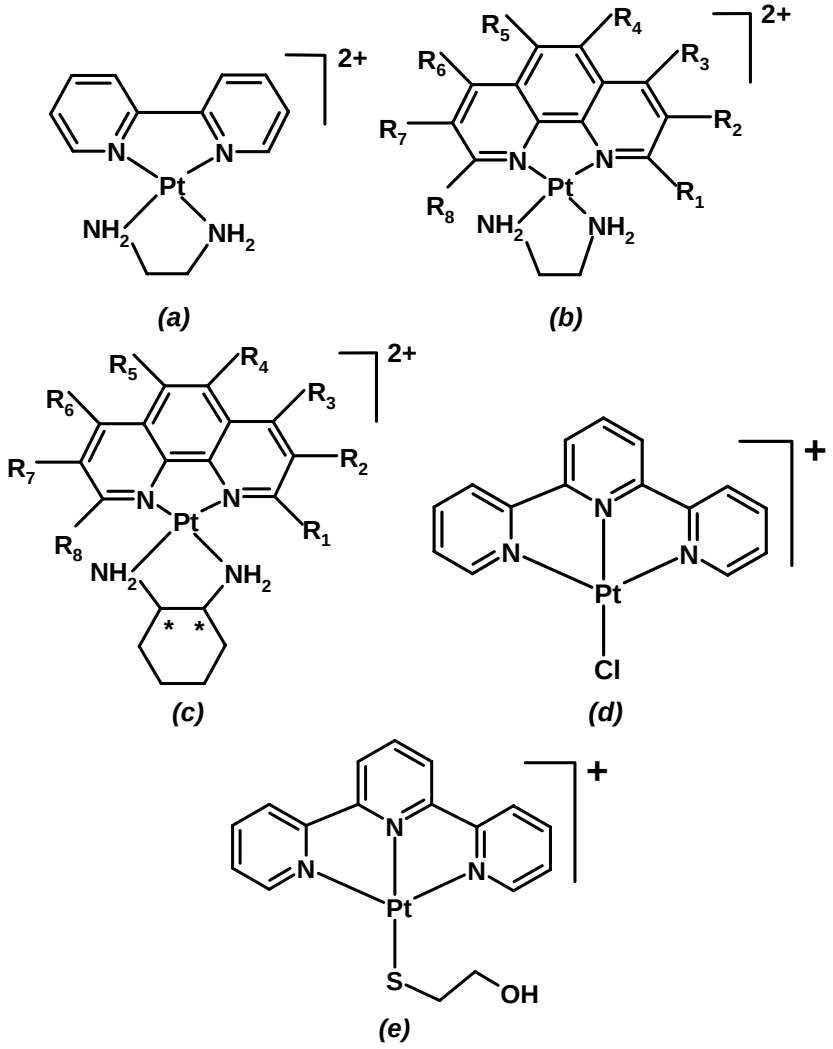


Figure 1.40. *Structures of some cationic platinum intercalators after Aldrich-Wright.*[296]

occurs if the metal lies in the same plane as the porphyrin ring and has no axial ligands. Recently dimetallo-copper-bipyridyl porphyrins[297] have been shown to bind to DNA by intercalation and external association and they cleave DNA under certain experimental conditions. These molecules are formed by combining a copper–porphyrin, which anchors the compound to DNA, with a copper–bipyridinium moiety that hydrolyses phosphodiester bonds (Fig. 1.41).

Another group of metal complexes studied extensively for intercalation is ruthenium(II) compounds with several bidentate diimines. An example of this class is dipyridophenanzine-bis(phenanthroline)ruthenium(II),[298] $[Ru(phen)_2(dppz)]^{2+}$ (Fig. 1.41). Coordination to the extended planar aromatic group, dppz, facilitates the compound to bind *via* intercalation. The binding to DNA causes an elongation of the rod-like DNA molecule consistent with classical intercalation. Ruthenium compound is octahedral therefore, whole

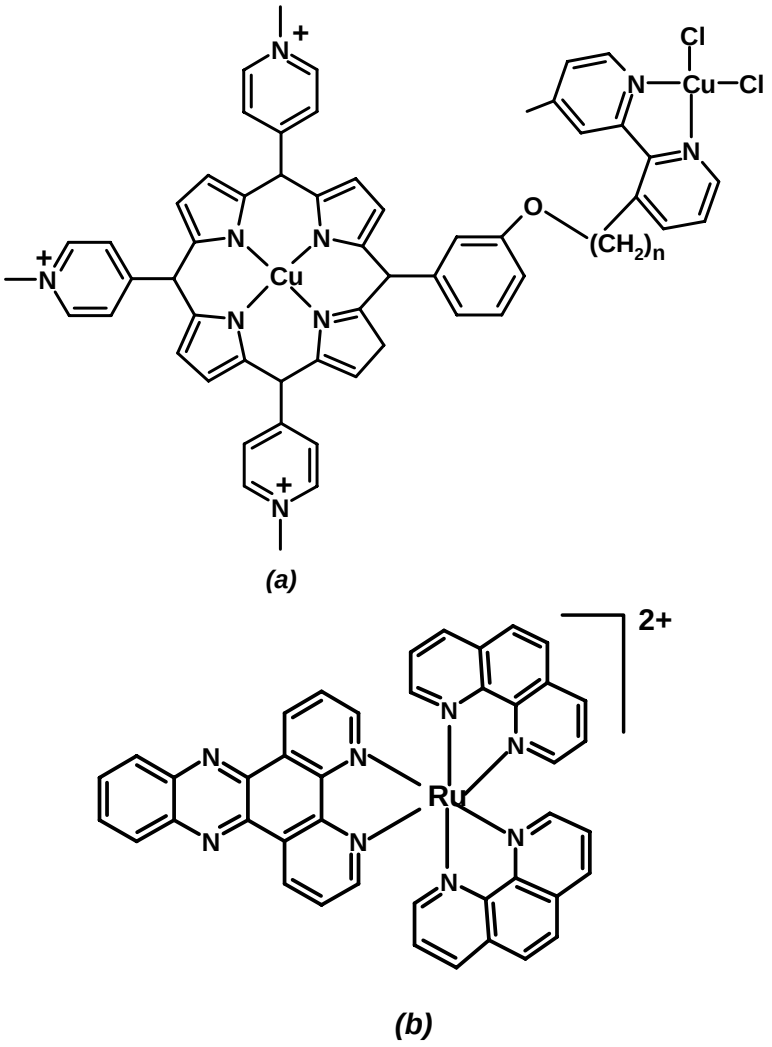


Figure 1.41. *Structures of (a) Cu-porphyrin and (b) [Ru(phen)$_2$(dppz)]$^{2+}$ intercalators.*

molecule can not slide through the bases for steric requirements. Therefore, the planar dppz portion intercalates in the base pairs with rest of the molecule sticking out.

1.6. Chemical nucleases

1.6.1. General introduction to artificial nuclease

Nucleases can be defined as enzymes capable of cleaving the phosphate diester bonds between the nucleotide subunits of DNA or RNA. On the same note, a chemical or artificial nuclease is a redox-active organic molecule or metal compound, which cleaves DNA or RNA (ir)reversibly *via* oxidative or hydrolytic mechanism.

Oxidative DNA damage, initiated by ionizing radiation,[299] photo-oxidation,[300] hydroperoxides activated by transition metals,[301, 302] hydroxyl radicals or various other oxidizing agents may lead to mutation, cancer, and cellular death.[303] The cellular response in healthy organisms to the oxidative stress is activated by three ways: (a) removal of the

damaged nucleotides and the restoration of the original DNA duplex, (b) cell-cycle arrest and (c) apoptosis. Several DNA-repair mechanisms to prevent permanent damage have been identified, namely, base excision repair, nucleotide excision repair, double-strand break repair, and cross-link repair.[237]

As the DNA is the substrate for chemical nucleases, these compounds therefore can specifically damage DNA and ultimately lead to cell death. When this phenomenon is irreversible and cancer-cell specific, a potent anticancer agent can evolve. The three major pathways to induce DNA cleavage can be named as: (a) the oxidation of the nucleobases,[304] (b) the hydrolysis of the phosphate groups,[305-307] (c) and the oxidation of the deoxyribose unit.[308] The oxidation of the nucleobases rarely leads to a direct strand scission and a second step (use of heat, a base or an enzyme treatment) is often needed to break the DNA strand.[304] The hydrolysis of the phosphate diester groups is the natural pathway to break a DNA strand. The phosphate diesters are highly stable functional groups;[309] however, some enzymes and some synthetic model compounds are known to be able to cleave DNA *via* this hydrolytic pathway.[310, 311] The oxidation of the deoxyribose unit can lead to direct DNA strand breaks. The strand scission is achieved through the initial abstraction of a hydrogen atom from the deoxyribose unit.[306, 307] Among the seven C–H bonds of the deoxyribose unit that can be oxidised, four points towards the minor groove, and three are located in the major groove (Fig. 1.42). The ease of C–H bond homolysis depends on the nature of the carbon (secondary or tertiary) attacked and the orientation of the drug with respect to the sugar C–H bonds. The tertiary C4′–H and C1′–H bonds (Fig. 1.42) are accessible from the minor groove, while the C3′–H bond is reachable only from the major groove. The secondary C–H bonds at C2′ and C5′ positions (Fig. 1.42) point towards either the minor or major grooves.[307]

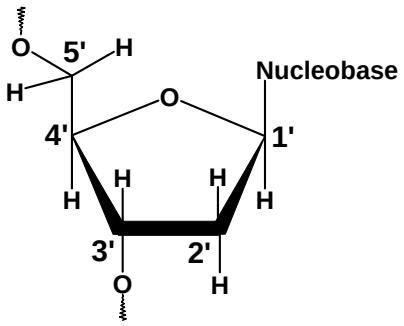
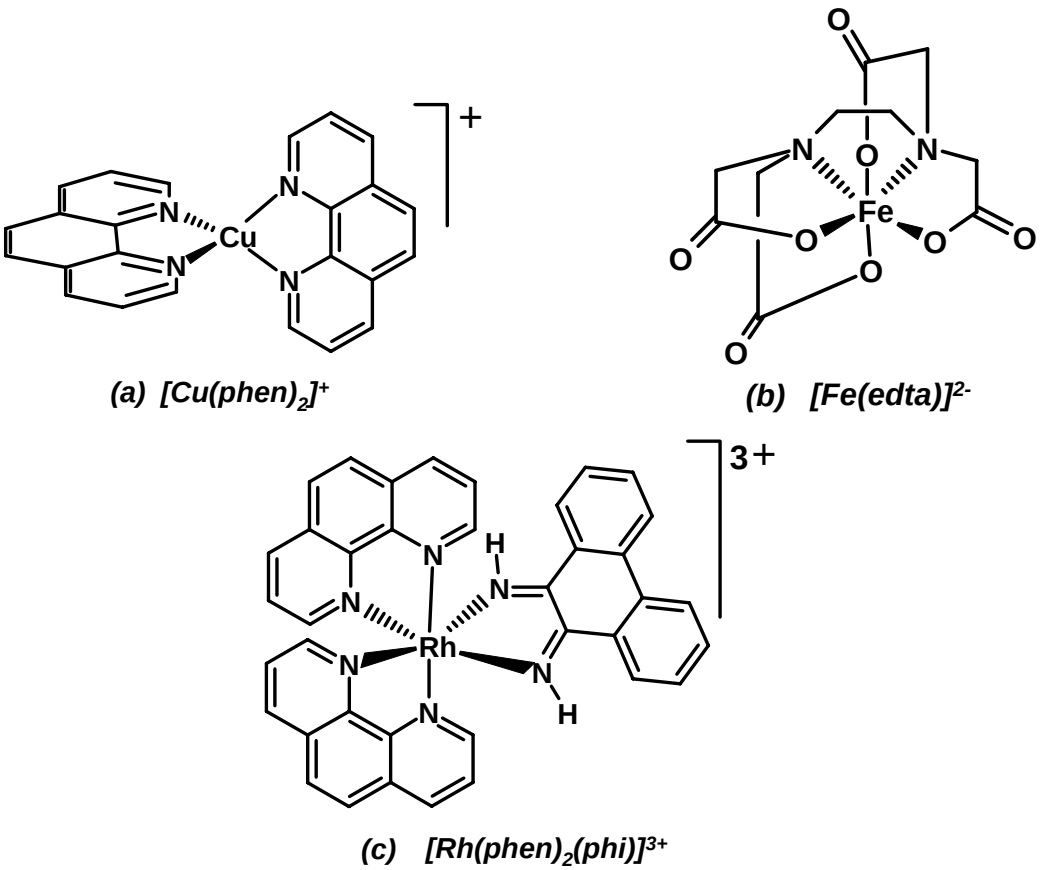


Figure 1.42. *Structure of a deoxyribose moiety with numbering scheme.*[307]

1.6.2. Oxidative cleavage

The hydrogen abstraction can be initiated either by (a) Fenton-generated hydroxyl radical ($OH^{\bullet}$), or radiation-produced hydroxyl radical ($OH^{\bullet}$). Chemical structures of some selected extensively studied synthetic nucleases are shown in Fig.1.43.

The best studied complex systems are Fe-bleomycin,[122] Fe-edta[312, 313] and $[Cu(phen)_2]$,[314, 315] Mn(III)–porphyrin and also copper, cobalt, ruthenium and rhodium compounds with phenanthroline.[302, 304, 316] These compounds are able to oxidise the deoxyribose unit of DNA in the presence of dioxygen or dihydrogen peroxide (in presence of reductant). The mechanism of action of $[Cu(phen)_2]^{2+}$ presumably follows certain steps: (a) reduction in solution to $[Cu(phen)_2]^{+}$, (b) the reversible reaction with dioxygen and formation of superoxide anion, (c) generation of dihydrogen peroxide and oxidation of $[Cu(phen)_2]$ leading to the generation of the unknown reactive species and (d) the abstraction of the protons H-1′, H-4′ and H-5′ from the deoxyribose unit. The reaction scheme has been shown below with the steps numbered. Different nuclease molecules preferentially abstract specific protons and produce different end cleavage products (Table 1.1).



(a) $[Cu(phen)_2]^{+}$ ***(b) $[Fe(edta)]^{2-}$***

(c) $[Rh(phen)_2(phi)]^{3+}$

Figure 1.43. *Structures of some synthetic nucleases with different transition metals.*

$$[Cu^{II}(phen)_2] + e^- \longrightarrow [Cu^{I}(phen)_2] \quad (1)$$

$$[Cu^{I}(phen)_2] + O_2 \rightleftharpoons [Cu^{II}(phen)_2] + O_2^{\bullet -} \quad (2)$$

$$[Cu^{I}(phen)_2] + O_2^{\bullet -} + 2H^+ \longrightarrow [Cu^{II}(phen)_2] + H_2O_2 \quad (3)$$

$$[Cu^{I}(phen)_2] + H_2O_2 \longrightarrow \text{reactive species} \quad (4)$$

Table 1.1. *Oxidative cleaving agents with preferential site of attack and the end products.*

Proton abstraction position	Position of proton in DNA strand	Examples	End products
H-C1′	Minor groove	1. $[Cu(phen)_2]^+$ 2. Neocarzinostatin	1. Oligonucleotide-3′-phosphate 2. 5-methylene-2-furanone
H-C2′	Major groove Minor groove	1. γ-radiolysis of poly(U) 2. Photolysis of oligonucleotides containing halogenated uracil	1. Oligonucleotide-3′-phosphoglycaldehyde 2. Oligonucleotide-5′-phosphoglycaldehyde
H-C3′	Major groove	Photoactive Rh(III) compounds	(a).Oligonucleotide-3′-phosphoglycaldehyde, Base propenoate (aerobic) (b).Oligonucleotide-3′-phosphate, lactone, free nucleobase (anaerobic)
H-C4′	Minor groove	1. γ-radiolysis 2. [Fe(II)-bleomycin]• 3. Neocarzinostatin	For bleomycin (a). free nucleobase, Oligonucleotide-5′-phosphate (anaerobic) (b).Oligonucleotide-5′-phosphate, Oligonucleotide-3′-phosphoglycolate, base propenal (aerobic)
H-C5′	Major groove Minor groove	1. Neocarzinostatin 2. Cationic metal porphyrins 3.Calicheamicin	1. Oligonucleotide-5′-aldehyde 2. Oligonucleotide-3′-phosphate

1.6.3. Hydrolytic cleavage

Hydrolysis of phosphate diester bond is of critical importance in most basic cellular functions, including DNA repair, excision, transcription, integration and metabolism, signal transduction.[317] The half-life of a typical phosphate diester bond in DNA in neutral

water solutions at 25 °C was estimated to be around in the order of tens to hundreds of billions of years. However, the hydrolysis of phosphate diester by natural metal-mediated enzymes is carried out within seconds. As a result, it is necessary to develop such reactive catalysts to efficiently hydrolyse DNA. The main hindrance in DNA hydrolysis is the large negative charge that inhibits attack of nucleophiles at the DNA backbone, and so charge neutralisation is one of several mechanisms used by natural nuclease molecules.

Magnesium is the primary choice of nuclease enzymes, due to high natural abundance and availability of appropriate hydration states, ligand exchange rates, redox inertness and charge density. Other multinuclear metal active sites are also well found in hydrolase molecules, with Cu^{2+}, Zn^{2+}, Fe^{2+} and Mn^{2+} to mention the most frequent cases.[306, 318]

1.7. Aim and scope of the thesis

Chemotherapeutics in the post-genomic age are supposedly designed to be specific and selective to cancer cells and spare the healthy cells. Therefore the 'smart' metal-based anticancer agent must fulfil [158] certain essential criteria: (a) activity in cisplatin-resistant cell lines, (b) saline solubility and stability, (c) facile transport in blood and *via* cellular membranes, (d) stable DNA-binding ability with slow or weak interaction to proteins and (e) selectivity and specificity towards cancer cells over healthy cells.

These challenges open a lucrative field of research for bioinorganic chemists. The goal is not limited in synthesis of new coordination compounds, the mechanism and mode of action towards the biological targets plays an important role as well. With this goal the thesis has been outlined combining (a) *synthetic* and (b) *biological activity* parts.

Phenanthroline is an extensively studied DNA footprinting agent and several of its coordination compounds are still under investigation. In Chapter 2, four different phenanthroline derivatives were studied for their anticancer activity and DNA-binding properties. In addition, two of these ligands were tested for antibacterial activity and a very selective behaviour was noticed. Their platinum compounds were investigated against a wide spectrum of human cancer cell lines and the effect of aromaticity was studied.

Hydrogen-bonds often play significant role in organic drugs or coordination compounds by providing additional stability. In Chapters 3 and 4, the combined effects of intercalation and coordination binding of platinum compounds with DNA have been summarised. A new series of water-soluble platinum compounds has emerged. Four platinum compounds were synthesised and studied in detail for their DNA and protein

binding interaction with antiproliferative properties. These compounds were modified in their carrier ligands to study the effect on activity profile.

A self-activating copper compound with an unique amino-phenol ligand (Hpyramol) is the basis of the Chapters 5 and 6.[319] The ligand[320] mediated activity was first exhibited in a Fe-compound[320] and then extended to zinc (redox inactive),[321, 322] Platinum and ruthenium (redox active and attractive candidate for anticancer agents). The distinct behaviour of the platinum, ruthenium and copper compounds towards cancer cell lines has been observed. Further biological studies including cellular uptake, conformational changes and DNA cleavage were performed to interpret the changes in activity profile upon various metal bindings.

Ruthenium compounds are suitable candidates for anticancer agents due to the different chemical properties compared to cisplatin. These compounds are more potent for metastatic malignancies, which are difficult to cure. In Chapter 7, a group of Ru(III) and Ru(II) compounds with DNA binding properties have been studied for their anticancer activity. The influence of changes in the electronic properties of the intercalator terpyridine ligands was investigated. Two Dinuclear ruthenium compounds with a flexible linker were also studied for their DNA-interaction and *in vitro* cell viability assays have been performed.

Dinuclear homo- or hetero-metallic compounds comprise a different category of potential antitumour agents. The extensive area of *'mix and match'* of suitable transition metals leads to an interesting challenge towards the synthesis of unique class of compounds. Some heteronuclear Ru-Pt compounds interact with DNA *via* intercalation and coordination.[323] The study of three dinuclear compounds with the common linker but different number of labile chloride ligands on the metal centres, *viz.*, Ru-Ru, Pt-Pt and Cu-Cu have been described in Chapter 8. These compounds have also been investigated for their mode of interaction with calf thymus DNA.

The final chapter deals with a critical evaluation of the obtained data in view of the original aim, and also presents an outlook to the future. Parts of this thesis have been published[319, 324] or will be submitted for publication.

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