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Preface

Cancer is a complex disease that has taken a significant toll on human life, adding financial burdens and emotional distress. Chemotherapy is considered a mainstay of treatment that has been employed for 'waging a war on cancers'. Despite many success stories of chemotherapeutic drugs, pitfalls exist. Recurrence and relapse of aggressive metastatic secondary cancers pose challenges. Metastasis is the condition where cancer cells evade current treatment and spread *via* blood or to deposit elsewhere and are often difficult to treat by traditional chemotherapy regimens.

The most pertinent and important question is whether we have taken a backseat in treatment strategies or are we actively pursuing remedies for these metastatic cancers, driving the therapeutic potency to maximum curative potential, at the same time ensuring the safety profile. The fact is that, in recent years, astonishing breakthroughs have been taking place in the realm of cancer treatment involving innovative gene therapies, targeted therapy, and immunotherapies that have revolutionized the whole branches of oncology. With modern characterization techniques, and high-resolution structural data from X-ray diffraction or NMR studies, we have been able to understand and validate intricate details of interactions with targets. Nevertheless, there are failures of new drug entities, NDEs in stages of R & D, and clinical trials due to poor water solubility, systemic toxicity, and inherent drug resistance that are seen as hurdles to the success of these chemotherapeutic regimens.

We have written this book very passionately on the topic *Advances and Prospects of 3d Metal-Based Anticancer Drug Candidates* sincerely hoping to translate more inorganic metal-based chemotherapeutic drugs in the **pipeline to clinic**, keeping in mind that 3d metals are strikingly appealing for the search of efficacious chemotherapeutic anticancer drugs that will manifest better cytotoxic response over a broad spectrum of phenotypes of cancers with much lower side effects. Metal-based chemotherapeutic drug candidates exhibit interesting physicochemical properties of luminescence, redox behavior, different oxidation states, and a wide range of geometric preferences at the biological 3d space, exerting a distinct pathway of the mechanism of action (MOA) of cell death, which ensures targeting specifically at the intracellular levels *in vivo* and also prevents 'off-target' toxicity of different organs. The metallodrugs are known to possess two main druggable components (1)

tunable metal at the 'core' center and (2) the ligand 'pharmacophore' scaffold. Both these components are necessary for the integrity of the metallodrugs, often remain intact in solution, and act synergistically at the active site; however, each one has a distinct role to play in executing cell death in cancer cells.

The recent advances for the development of metal-based complexes as anticancer chemotherapeutics have appeared in many review articles, and a large number of research articles in literature include combination agent therapies, drug delivery system (DDS) based therapies, and polymeric interventions. We have meticulously presented these advances in the form of chapters in pedological order, which has indeed added value to this book. An exhaustive and elaborate section of recent coherent references added after each chapter gives a 'deep dive' to readers for further reading and comprehension of the subject.

We hope that we have done justice to the aspirations and hopes of cancer patients, clinicians, medical practitioners, and researchers, who are desperately waiting for some breakthrough or 'magic bullet' cure to treat cancers. The enlivening chapters on the role of targeting intracellular organelles such as endoplasmic reticulum, biochemical signaling mechanism, and other polymeric nanoconjugates will serve as a roadmap for the future discovery of chemotherapeutic regimens based on 3d metallodrugs anticancer drugs. A chapter on *in silico* computational techniques that include databases, quantitative structure-activity relationships, pharmacophores, homology models and other molecular modeling approaches, and data analysis tools could be utilized to predict the hypothesis and validation of binding affinity and modes of drug candidates, and to integrate experimental *in vitro* data for the optimization of novel 'lead' therapeutics molecules.

We are grateful for the help, advice, and comments from colleagues, readers, and Ph.D. scholars in our laboratory, who have contributed immensely to this field, particularly Dr Mala Chauhan, Dr Imtiyaz Yousuf, Dr Sabiha Parveen, Dr Rais Ahmad Khan, Dr Mohammed Usman, Dr Surbhi Sharma, Dr Zeenat Afsan, Dr Siffeen Zehra, Dr Salman Khursheed, Ms Robina Kouser, Mr Rijwan, Mr Salman Khan, and Ms Suffora Akhter to name a few, and not the least, special thanks are due to Dr Mohammed Fawad Ansari (UGC—Dr D.S. Kothari (PDF) fellow) who has edited all chapters and figures very meticulously. The motivation to write this book stems mainly from our 29 year research interest in 'Design and synthesis of modulated metal-based cancer chemotherapeutic agents'. The only reason the book exists is to disseminate knowledge to students interested in pursuing their careers in medicinal chemistry and developing new tailored metal-based drugs for the cure of this deadly disease.

Aligarh, Uttar Pradesh, India — Farukh Arjmand
Aligarh, Uttar Pradesh, India — Sartaj Tabassum
Aligarh, Uttar Pradesh, India — Huzaifa Yasir Khan

Contents

About the Authors

Farukh Arjmand is currently working as a Professor of Chemistry at Aligarh Muslim University, Aligarh, India. Prof. Arjmand has 29 years of research and teaching experience in the area of Inorganic Chemistry, in particular, '**Medicinal Inorganic Chemistry'**. She is working on the molecular design and synthesis of new metallodrug antitumor therapeutic candidates derived from bioactive ligand scaffolds with 3d transition and organotin(IV) metal ions. Her research group has isolated many promising, tailored metallodrugs and antitumor candidates; their structure elucidation was done by spectroscopic and single X-ray crystal diffraction methods; and their potential to act as anticancer chemotherapeutic agents was validated by DNA/RNA binding affinity, cleavage activity, and cytotoxicity profile against a panel of human cancer cell lines and molecular docking experiments. She has published **185** research papers in journals of international repute (with highest impact **24.83)**, contributed many articles to national/international conferences and symposiums, delivered many invited lectures, and has **two patents** on metallic antitumor drug entities. Dr. Arjmand has **5250 citations (Scopus)** to her credit with an h-index of **41** and an i10-index of **115**. Prof. Arjmand has successfully guided **18** Ph.D., **4** M.Phil. and **15** Master's project students and has run **six** major research projects as principal investigator (PI) on the design of metal-based drug candidates awarded by **UGC**, **CSIR**, and **DBT, Govt. of India**, and has visited many countries (China, USA, Egypt) for academic pursuits. She has joint research collaborations with national and international research institutes, IIT Kharagpur, IICT, Hyderabad, and ACTREC, Mumbai, India; USTC, China; Institut de Physique de Rennes, France; and the Ohio State University, USA. Prof. Arjmand has received many prestigious awards, viz. **Young Scientist, ICC, 2005, the Distinguished Women Scientist Award 2016, ISCB**, **2017**, and **the CRSI Bronze Medal 2019,** Chemical Research Society of India (CRSI), and **Outstanding Researchers Award 2022 in faculty of Science and Life Science, AMU**. Prof. Arjmand was awarded **prestigious FRSC** in 2023 **(Membership number: 679931)** by the Royal Society of Chemistry, UK, for her contribution to chemical science and chosen as CRSI council member in 2020 for 3 years. Prof. Arjmand is presently serving as Co-director of APJ Abdul Kalam STEM Center for Education and Research—a collaboration of the Ohio State University, USA, and AMU, Aligarh. She completed

the Academic leadership (**LeAP**) program, MHRD, Govt. of India, at AMU, India, and OSU, USA, in 2019.

Sartaj Tabassum is working as a Professor in the Department of Chemistry at Aligarh Muslim University, Aligarh. He was appointed as a lecturer in the Department of Chemistry at Aligarh Muslim University in 1993. He has published **170** papers in journals of international repute and has **4184** citations and an h-index of **37 (Scopus)**. He has presented his research work and delivered invited lectures at various conferences/symposia. He is a life member of ICC, CRSI, ISCB, DNA Society of India, and American Nano Society. He has successfully guided **19** Ph.D. and **4** M.Phil. students and many master's projects. He has successfully completed many research projects granted by TWAS, Italy, CSIR, New Delhi, and DBT, Govt. of India. As a distinguished Scientist, Prof. Tabassum was awarded the **Overseas Associateship award in 2005** by DBT, Govt of India, and **Prof. W. U. Malik Memorial Award 2022** by Indian Council of Chemists (ICC). He has signed several MoU and joint research collaborations with the University of Camerino (UNICAM), Italy, USM Malaysia, and USTC Hefei, China. He has visited many countries for academic pursuit, such as China, the USA, Italy, and King Saud University, Riyad, Saudi Arabia, as a fellow, visiting professor, and for international conferences. Prof. Tabassum is working in the area of medicinal inorganic chemistry and his main focus is the design and synthesis of heterobimetallic complexes that have the potential to act as cancer chemotherapeutics. Three patents have been granted in India (**01**) and the USA (**02**), in which he has claimed that the new drug will be very effective with minimal side effects and it will be available at a meager cost.

Huzaifa Yasir Khan received his B.Sc. (Hons.) degree in Chemistry from the Department of Chemistry at Aligarh Muslim University in 2013 and his master's degree in Chemistry from the Department of Chemistry at AMU in 2015. He was awarded the prestigious DST INSPIRE Scholarship from the Department of Science and Technology, Government of India, for 5 years during his undergraduate and master's programs. He completed his Ph.D. degree in 2021 under the supervision of Prof. Farukh Arjmand, Department of Chemistry, AMU, on the topic '**Synthesis and Characterization of tailored metal-based potential antitumor chemotherapeutic drug candidates**'. He was awarded 'INSPIRE Fellowship' (DST-JRF) and 'DST INSPIRE-Senior Research Fellowship' by the Department of Science and Technology, Government of India, during 2015–2020. He has published several good papers in reputed peer-reviewed journals, and participated in several national and international conferences. He is a life time member of CRSI, India (Chemical Research Society of India), and was a member of ACS (American Chemical Society) in the year 2019. He has been awarded **Young Scientist** for the best oral presentation by Indian Council of Chemists in the year 2022. Following his Ph.D., Dr Huzaifa Y. Khan worked as a **Postdoctoral Research Associate** at the Interdisciplinary Biotechnology unit, Aligarh Muslim University, Aligarh, funded by Indian Council of Medical Research, New Delhi, India, for 1 year. He is currently working as Assistant Professor in the Department of Chemistry at AMU, Aligarh.

Chapter 1
Introduction

1.1 Introduction

1.1.1 Overview of Metal-Based Chemotherapeutic Agents in Cancer Oncology

Medicinal inorganic chemistry is a growing field within bioinorganic chemistry that focuses on developing therapeutic and diagnostic compounds to address chronic diseases such as respiratory disorders, Alzheimer's, diabetes, HIV/AIDS, and cancer (Mjos and Orvig 2014; Thompson 2011; Farrell 1999). Among all the chronic diseases, cancer still remains a serious concern in the public healthcare domain due to the huge toll on mortality rates. The GLOBOCAN-2020 report by the International Agency for Research on Cancer (Sung et al. 2021) projected 19.3 million new cancer cases and 10 million cancer-related deaths globally in 2020, which is expected to exponentially rise (47%) to 28.4 million cases in 2040.

Cancer comprises a group of complex conditions characterized by the unregulated growth of cells and forms lumps or masses known as tumors, and sometimes these tumor cells may invade to nearby tissues or migrate to distant secondary loci resulting in more aggressive 'metastatic' or secondary cancers (Fig. 1.1) (Hanahan and Weinberg 2011; Jemal et al. 2011). There are more than 200 subgroups/phenotypes of tumors derived from different origins of tissues that arise from intratumor heterogeneity due to endless combinations of genetic/epigenetic alterations (Raatz et al. 2021). In men, lung cancer stands as the primary cause of cancer-related illnesses and fatalities, while among women it ranks third in terms of occurrence, following breast and colorectal cancers (Sung et al. 2021). Consequently, there's been a widespread effort to enhance patient survival rates through extensive research and development of rational treatment strategies/modalities for cancers.

There are many treatment strategies, for example, invasive method surgery, radiotherapy, chemotherapy, modern immunotherapy, biologically targeted therapies, etc.

F. Arjmand et al., *Advances and Prospects of 3d Metal-Based Anticancer Drug Candidates*, https://doi.org/10.1007/978-981-97-0146-9_1

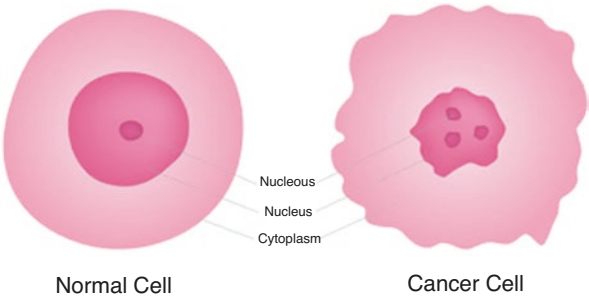


Fig. 1.1 The structure of normal cell vs cancer cell

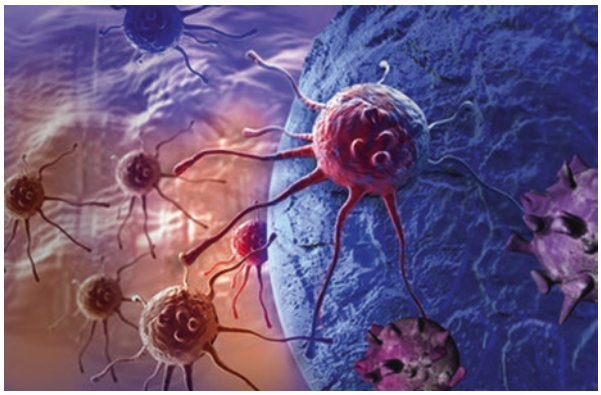


Fig. 1.2 Images of cancer cells

However, chemotherapy is widely used as a standard therapy for the treatment of solid cancers (Fig. 1.2) and is considered as the mainstay treatment option, either alone or together with radiotherapy or invasive surgical interventions (Englinger et al. 2019; Hellmann et al. 2016; van der Most et al. 2005).

In chemotherapy, cytostatic drugs are used to target the cell cycles that were identified and isolated from various sources of plants/fungi or synthetically prepared, broadly classified as alkylating agents, alkaloids, antibiotics, and antimetabolites. By 2000, approximately 57% of cancer drugs undergoing clinical trials were either natural compounds or derived from them (Cragg and Newman 2005). Some prominent noteworthy cytostatic drugs, viz., bleomycin, etoposide vinblastine, doxorubicin, taxol under trade name paclitaxel®, topotecan, irinotecan, etc. These drugs disrupt the growth of cancer cells by targeting cellular DNA or RNA (Demain and Vaishnav 2011; Dholwani et al. 2008). The US National Cancer Institute (NCI) has engaged in exploring cytostatic drugs, leading to a range of varied formulations and research strategies designed for cancer treatment. World Health Organization (WHO) has defined the criteria for the evaluation of the therapeutic potential of cytostatic drugs as follows: (1) extent of tumor response/remission; (2) the determination of reemission time; (3) survival of patients; and (4) toxicity. Chemotherapy demonstrates efficacy in managing several solid cancer types, yet in other similar

carcinomas, its curative effects are limited. However, it can extend the overall survival (OS) of patients in these cases. WHO has also classified the side effects of chemotherapeutic agents as grades 0–4 (Seeber and Schütte 1993) and most of the chemotherapeutic drugs have failed due to severe (side effects) toxicity, resistance issues, and a narrow spectrum of activity, either in preclinical or clinical trials or at R&D levels. This has led to extensive research for discovery of efficacious chemotherapeutic agents with better curative effects, exhibiting a broad spectrum of activity against most of the cancers (Schirrmacher 2019).

The accidental discovery of the archetypical inorganic compound cisplatin, *cis*-diamminedichloro platinum(II) (*cis*-$[Pt(NH_3)_2Cl_2]$) as a potent antitumor agent sparked a significant surge in medicinal inorganic chemistry research (Alderden et al. 2006; Dasari and Bernard Tchounwou 2014; Kelland 2007; Monneret 2011). It emerged as a pivotal treatment for various solid malignancies, including breast (Decatris et al. 2004), cervical, ovarian, testicular, head and neck, bladder, prostate, lung, stomach cancers, as well as sarcomas (Rosenberg and VanCamp 1970), neuroblastoma, melanoma, and multiple myeloma cancers (Rosenberg et al. 1965).

While initially synthesized by M. Peyrone in 1844 (Peyrone 1844), it was B. Rosenberg in the 1960s who uncovered its anticancer properties. This led to extensive investigation, patenting (Eisenstein and Resnick 2001; Rosenberg et al. 1979), and subsequent FDA approval of cisplatin as Platinol® by Bristol-Myers Squibb in 1978.

Since then, cisplatin has been a pioneering metal-based drug globally used in cancer treatment either alone or in combination with other therapies. It remains one of the most successful and revenue-generating metallodrugs, contributing significantly to the revenue of institutions like Michigan State University through licensing royalties (Blumenstyk 1999).

Despite the success stories of cisplatin, there were some serious challenges to be addressed for its therapeutic intervention, for example, systemic toxicity issues such as neuro-nephro and/or renal-toxicity or decreased blood cell and platelet production in bone marrow (myelosuppression) (Oun et al. 2018; Farrell 1989; Miller et al. 2010; Yao et al. 2007; More et al. 2010), hearing loss in younger patients, intrinsic resistance (as observed in patients colorectal, prostate, lung or breast cancers) and extrinsic or (acquired resistance during cycles of therapy with cisplatin) (Siddik 2003; Ishida et al. 2002). In addition to patience compliances such as nausea and vomiting, loss of appetite, hair loss, etc., allergic reactions, decreased immunity to infections, and poor oral bioavailability were also observed (McWhinney et al. 2009). The resistance of cisplatin was found to be involved in various biochemical processes viz., diminished cellular absorption, increased expulsion of the drug, increased detoxification, deactivation through sulfur-containing protein binding, inhibition of apoptosis, and escalated repair of DNA damage (Sadler and Guo 1998; Timmerbosscha et al. 1992; Shen et al. 2012). To overcome the aforementioned drawbacks/challenges of cisplatin (Dhar et al. 2011; Florea and Büsselberg 2011), numerous other second-generation alternative analogues such as carboplatin, satraplatin, lobaplatin, picoplatin and oxaliplatin, and more recently the multinuclear

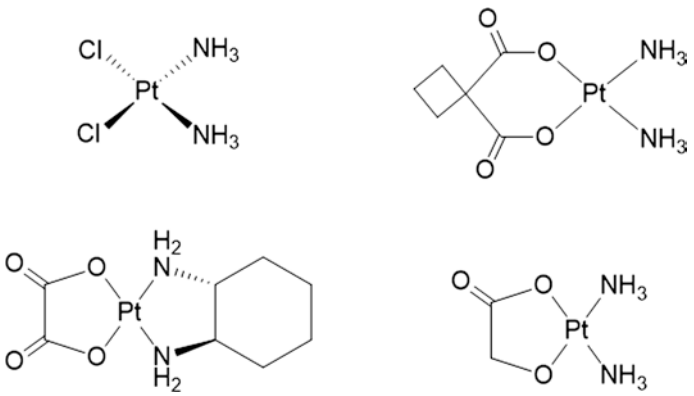


Fig. 1.3 Structure of approved platinum anticancer drugs

platinum complex BBR3464 (triplatin), etc., were developed (Desoize 2002; Rosenberg 1973, 1978) (Fig. 1.3).

Carboplatin, also known as *cis*-diammine (1,1-cyclobutanecarboxylato) platinum (II), is a chemotherapeutic agent utilized in head, neck, lung, and ovarian cancers. Its structure substitutes the two chloride ligands of cisplatin with a bidentate dicarboxylate (CBDCA) ligand. It demonstrates reduced reactivity and slower DNA binding kinetics compared to cisplatin, yet forms similar reaction products in vitro at equivalent doses. Oxaliplatin, marketed as Eloxatin® in Japan (Alcindor and Beauger 2011), nedaplatin as Aqupla® in China (Shimada et al. 2013), and lobaplatin, is another platinum-based drug containing an oxalate and diaminocyclohexane ligand (DACH). The DACH ligand significantly influences its cytotoxicity response. Oxaliplatin is licensed for combined therapy in colon cancer and nonsmall-cell lung cancer management (Chan and Coward 2013). Its superior safety profile compared to cisplatin makes it suitable for patients intolerant to cisplatin regimens. Satraplatin, referred to as bis(acetato)-amminedichloro(cyclohexylamine)platinum(IV) or JM216, possesses the unique characteristic of oral availability, administered in pill form, offering convenience for patients. Unlike cisplatin, JM216 comprises mononuclear Pt(IV), which is converted in vivo by metallo-redox proteins into the active Pt(II) complex (JM118) (Bhargava and Vaishampayan 2009; Byun et al. 2005).

BBR3464 (CT-3610), also known as triplatin tetranitrate, represents an unconventional trinuclear platinum complex with a net charge of +4 (Brabec et al. 1999). Despite entering phase II clinical trials (Fig. 1.4), its efficacy in lung cancer patients was limited, showing minimal response while inducing notable side effects like neutropenia and diarrhea. Consequently, further clinical advancement was halted (Kasparkova et al. 2002; Jodrell et al. 2004). Presently, cisplatin, carboplatin, and oxaliplatin are extensively utilized, encompassing about 50–70% of cancer treatment protocols (Dyson and Sava 2006). Despite advances in platinum-based drug design, many of these drugs have experienced reduced efficacy over time due to acquired resistance.

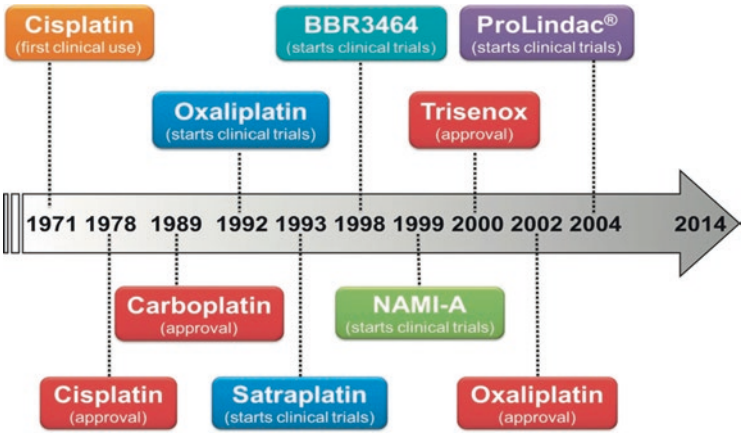


Fig. 1.4 Timeline diagram of platinum drugs for treatment of various cancers

The prevailing challenges, including systemic toxicity, drug resistance, and limited spectrum against various cancer types, have urged the exploration of alternative strategies in drug design. These include (1) employing less toxic prodrugs selectively activated within tumors, (2) using carrier groups for targeted tumor delivery, and (3) investigating nonplatinum metals.

Platinum(IV) complexes are regarded as prodrugs of platinum(II) analogs due to their slow reactivity with cellular targets (Rosenberg et al. 1969; Hall et al. 2004; Sigel and Sigel 2004; Hall and Hambley 2002). Physiologically, these Pt(IV) complexes are reduced by biomolecules such as glutathione (GSH), methionine, cysteine, metallothioneins, serum albumin, ascorbate, DNA nucleobases, nucleotides, and analogs. The reduction potential determines whether this reduction occurs in the bloodstream rather than within cells, leading to side-reactions that might induce systemic toxicity (Fig. 1.5). Administering an inert complex in its oxidation state could potentially reduce side effects by minimizing reactions with nontarget biomolecules (Rosenberg 1971). However, achieving precise molecular-level targeting of such inert complexes remains a crucial challenge. For instance, Lippard et al. incorporated estrogen-targeting groups via carboxylate linkages to the axial sites and observed increased in vitro cytotoxicity (Barnes et al. 2004). Similarly, Dyson et al. attached a glutathione-*S*-transferase targeting group and found the resulting product to be an effective GST inhibitor (Wee et al. 2005). The mechanism of action of these complexes in vivo and their retention at target sites remain areas for further exploration.

Research by the Sadler and Bednarski groups utilized a distinct approach involving inert platinum(IV) complexes post-irradiation with light (Bednarski et al. 2006; Mackay et al. 2006). This approach, potentially promising, aims to confine the platinum drug to the irradiated area, thereby reducing systemic toxicity. Certain platinum(IV) complexes, such as *cis, trans, cis-* and *trans, trans, trans-* $[Pt(N_3)_2(OH)_2(NH_3)_2]$, considered prodrugs of cisplatin and transplatin, form

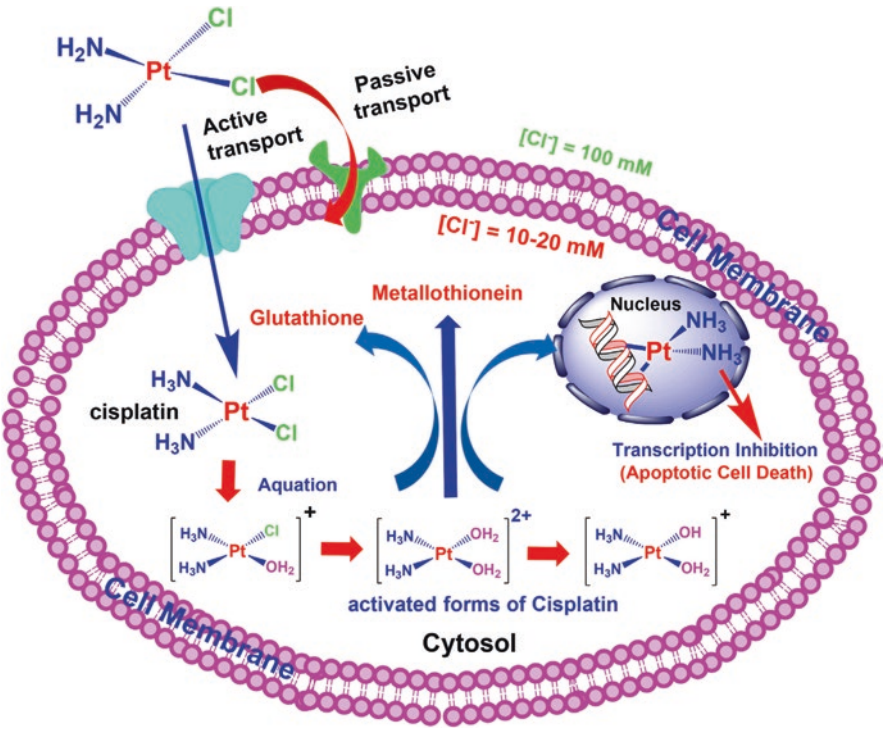


Fig. 1.5 Intracellular biochemical pathway of resistance of platinum drugs

various DNA adducts upon light exposure, showing potential as chemotherapeutic agents (Reedijk 2003; Mellor et al. 2005). This work underscores an advantage of platinum(IV) prodrugs by stabilizing a highly reactive and toxic platinum(II) species when used alone. Additionally, there's a possibility that platinum(IV) complexes selectively reduce in the hypoxic environments often present in solid tumors, making them active in such conditions (Schreiber-Brynzak et al. 2016). Hence, these complexes could potentially treat larger solid tumors effectively (Jain 1987; Hicks et al. 1997; Tunggal et al. 1999).

The failure of anticancer drugs to reach all cells at cytotoxic concentrations remains a significant concern in chemotherapy. For instance, doxorubicin can only diffuse a limited distance from blood vessels, reaching a fraction of viable cells within a solid tumor. The hypoxic regions, generally located farther from blood vessels, often contain the most aggressive and drug-resistant cancer cell phenotypes (Pallavicini et al. 1979; Graeber et al. 1996).

A large plethora of complexes from main group metals bismuth, antimony, gallium, or tin to a range of transition metals viz., titanium, vanadium, gold, iron, cobalt and copper, ruthenium or rhodium as well as cerium have been synthesized and investigated for their cytotoxic anticancer effects (Ott and Gust 2007; Clarke et al. 1999; Marzano et al. 2012). Exploration in preclinical and clinical studies has

highlighted the emergence of novel nonplatinum metal-based chemotherapeutic agents that exhibited different noncovalent modes of action as compared to classical covalent binding modes of cisplatin. Various compounds containing ruthenium (Ru), titanium (Ti), gallium (Ga), and gold (Au) have entered into clinical evaluation phases (see www.clinicaltrials.gov for current studies) (Lazarević et al. 2017; Liang et al. 2017; Chitambar 2017; Lentz et al. 2009).

Transition metal complexes are very appealing for chemotherapeutic drug design owing to their unique physico-chemical properties viz., strong Lewis acid nature, versatile coordination geometries beyond the sp, sp^2, and sp^3 hybridization of pure organic molecules, enabling a broader spectrum of stereoisomeric conformations, accommodating diverse oxidation states present in vivo as active redox pairs, capable of undergoing ligand exchange reactions to establish covalent bonds with nucleophilic donor atoms within the amenable sites of therapeutic biomolecules DNA, RNAs or proteins (Ott and Gust 2007; Ndagi et al. 2017; Jungwirth et al. 2011). Moreover, redox reactions of transition metals are known to elevate the reactive oxygen species (ROS) level, which show the capability to induce apoptosis, i.e., programmed cell death in which drug candidates specifically harm the malignant tissues, leaving the normal cells unaffected (Ma et al. 2019; Elmore 2007; Jakupec et al. 2008). These attributes enable precise adjustment of the metallodrug candidates to demonstrate potent, targeted biological interactions and a unique pharmacological reaction concerning organ distribution and penetration of tumor cell membranes.

The biological characteristics of transition metal complexes can be tuned by incorporating multifunctional bioactive ligand frameworks, crucial in reducing drug-induced toxicity and enhancing the selectivity of metallodrugs by targeting specific therapeutic areas (Storr et al. 2006). This modulation often involves 'Chelation,' the process of binding a ligand to a metal ion (derived from the word 'chela,' resembling a pincer-like claw), which fine-tunes metal properties by introducing ligands that act as active pharmaceutical agents with binding atoms (Morphy and Rankovic 2005; Gao et al. 2005; Ma et al. 2005; Boros et al. 2020). Ligands play a role in mitigating metal-ion overload effects, inhibiting specific metalloenzymes, redistributing metal ions, altering reactivity and lipophilicity, stabilizing particular oxidation states, and contributing to substitution inertness (Top et al. 2003; Heuer-Jungemann et al. 2019).

This approach enables the development of a single chemical entity capable of concurrently modulating multiple targets, potentially offering superior efficacy against complex diseases. To enhance the efficacy and safety of chemotherapeutic drugs, researchers, including our group, have modified metal complex structures by integrating suitable bioactive ligand functionalities such as chiral auxiliaries/recognition (Ma and Waxman 2008; Arjmand et al. 2023; Zhang and Lu 2021; Khursheed et al. 2022; Zehra et al. 2019, 2021).

Gold has been recognized for its medicinal potential since ancient times. In the nineteenth century, gold was considered a 'panacea' for diseases. Alchemists knew that metallic gold could dissolve in aqua regia and revert to metallic form as stable colloid solutions (Kostova 2006a; Best and Sadler 1996). Neutralized gold

solutions, known as 'aurum potabile' or drinkable gold, were widely used in therapy, yet their specific therapeutic potential for diseases remained unclear. The clinical application of Gold(I) complexes to treat rheumatoid arthritis was well-established, while Gold(I) and gold(III) complexes, being isostructural and isoelectronic with platinum(II) complexes (d^8 system), were known as anticancer agents and extensively reviewed (Ott 2009; Berners-Price and Filipovska 2011; Bertrand and Casini 2014). Gold(III) typically forms square planar complexes in solution. Since the geometry of Pt(II) cisplatin drug is square planar, an important criterion for its action as an anticancer drug, Au(III) compounds could also be used as anticancer agents based on the same rationale with decreased toxicity profile. Auranofin, a gold-based drug clinically used to treat rheumatoid arthritis, was observed to induce apoptosis in cisplatin-resistant cell lines (Fig. 1.6) (Nardon et al. 2014; Lima and Rodríguez 2011; Nobili et al. 2010). Extensive studies into the mechanism of auranofin identified several enzymatic targets for its in vivo mode of action at the molecular level (Abdalbari and Telleria 2021; Marzano et al. 2007; Milacic et al. 2008). Auranofin inhibited DNA, RNA, and protein synthesis at cytotoxic concentrations but did not directly interact with DNA, unlike other gold complexes. The cellular association, cytotoxic activity, and efflux were found to be dose-, time-, and temperature-dependent. Studies suggested that among a series of gold(I) complexes, those containing a phosphine ligand showed in vitro cytotoxicity (Mirabelli et al. 1986). Chloro(triethylphosphine)gold(I), an analog to auranofin wherein the thiosugar was replaced by chlorine, also exhibited potent cytotoxicity (Gamberi et al. 2015). Investigations into the mode of action indicated mitochondria as the target for auranofin and other gold complexes (Rush et al. 1987; Hoke et al. 1989; Huang et al. 2011; Park et al. 2014).

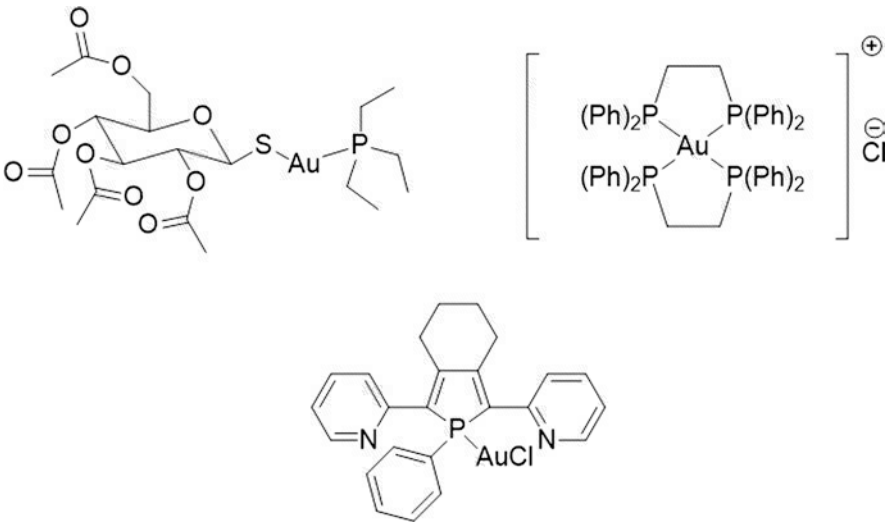


Fig. 1.6 Notable gold complexes with anticancer properties

Ruthenium holds a prominent position among metal-based chemotherapeutic anticancer agents, following platinum and gold (Clarke 2002). Ruthenium(III) complexes, namely imidazolium *trans*-[tetrachlorido(dimethyl sulfoxide) imidazoleruthenate(III)] (NAMI-A) and indazolium *trans*-[tetrachloridobis(1*H*-indazole)ruthenate(III)] (KP1019) along with its sodium analog KP1339, have undergone investigation in phase I and phase II clinical trials (Keppler and Rupp 1986; Mestroni et al. 1994). These compounds—NAMI and NAMI-A—feature a pseudo-octahedral ruthenium(III) center with distinct ligand configurations containing various axial ligands. In NAMI, one ligand is bonded in a sulfur (S)-donor mode, specifically dimethyl sulfoxide (DMSO-S), whereas in NAMI-A, an imidazole ligand occupies an axial position. The negative charge in both NAMI and NAMI-A is counterbalanced by different cations, with NAMI neutralized by a sodium ion (Na^+) and NAMI-A by an imidazolium cation. While NAMI-A exhibits exceptional stability in the solid state, it undergoes relatively rapid hydrolytic processes in aqueous solutions, with the rate strongly dependent on pH (Mestroni et al. 1994; Alessio et al. 2004; Bouma et al. 2002; Bacac et al. 2004). Intriguingly, NAMI-A-type complexes bearing azole ligands like pyrazole and thiazole were observed to be less basic than imidazole-bearing complexes and showed greater stability than NAMI-A in slightly acidic aqueous solutions, following a dissociative aquation mechanism (Bergamo et al. 2012).

Despite their structural similarity, NAMI–A & KP1019 (Fig. 1.7) exhibit notably different cytotoxic responses. While KP1339 (clinically termed IT-139) demonstrates activity against primary cancers and significantly curtails tumor growth in various in vivo models, including chemo-resistant tumors like colorectal cancers (Kostova 2006b), NAMI–A is effective against secondary tumor cells or metastatic cancers formed after migration of cancer cells from the primary site to different organs via blood or lymph (Sava et al. 2003). In vivo evaluations of NAMI-A across multiple models have shown its ability to impede the development and growth of pulmonary metastases in various solid tumors, including Lewis lung carcinoma (Sava et al. 1998), MCa mammary carcinoma (Meier-Menches et al. 2018), TS/A mammary adenocarcinoma (Alessio and Messori 2019), and human tumors in mice.

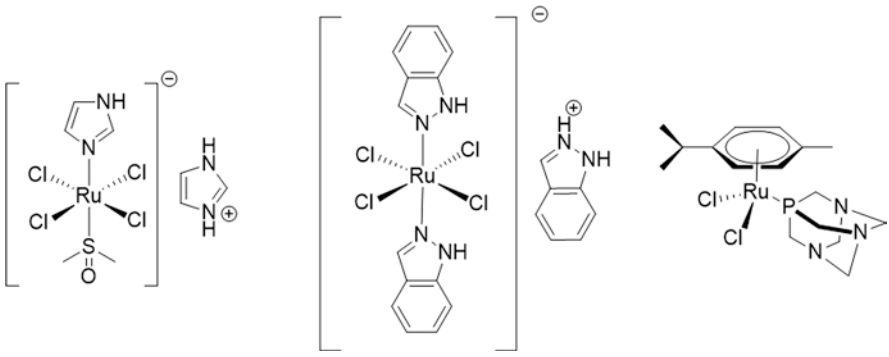


Fig. 1.7 Structure of prominent Ruthenium anticancer agents

Hence, understanding the precise mechanisms behind the anticancer activity of metallodrugs is imperative to support the clinical development of drugs and circumvent failures in later stages of research and clinical trials.

Recently, there has been an increasing interest in ruthenium(II)–arene complexes as a promising group of anticancer agents. RAPTA type complexes represented by the general formula [Ru(η^6–arene)Cl_2 (PTA)] (PTA = 1,3,5–triaza–7–phosphaadamantane), particularly, [Ru(η^6 –*p*–cymene)Cl_2(PTA)] have exhibited substantial potency in vitro, showcasing high IC_{50} values (Yan et al. 2005), (Scolaro et al. 2007; Anuja et al. 2022; Sandland et al. 2020). In vivo studies, on the other hand, demonstrated high activity towards metastatic tumors but with reduced systemic toxicity. They impede cell growth by instigating G2/M phase arrest and inducing apoptosis in cancer cells. Previous studies have also highlighted the pH-dependent selectivity of RAPTA-type complexes toward cancer cells. Tumor environments, often characterized by hypoxia and relying on glycolysis for energy, produce lactic acid, resulting in an overall reduction in cellular pH. Reports indicate tumor cell environments with pH as low as 5.5, compared to healthy cells with pH values around 7.2, supporting this hypothesis (Scolaro et al. 2005).

Titanium(IV) complexes (Fig. 1.8) have attracted significant attention due to their applications in cancer treatment, notably after two groundbreaking complexes—titanocene dichloride and budotitane—entered phase I clinical trials. Titanocene dichloride, Ti(η^5–C_5H_5)$_2Cl_2$, exhibited remarkable antitumor activity and progressed to phase I clinical trials. However, in phase II trials involving patients

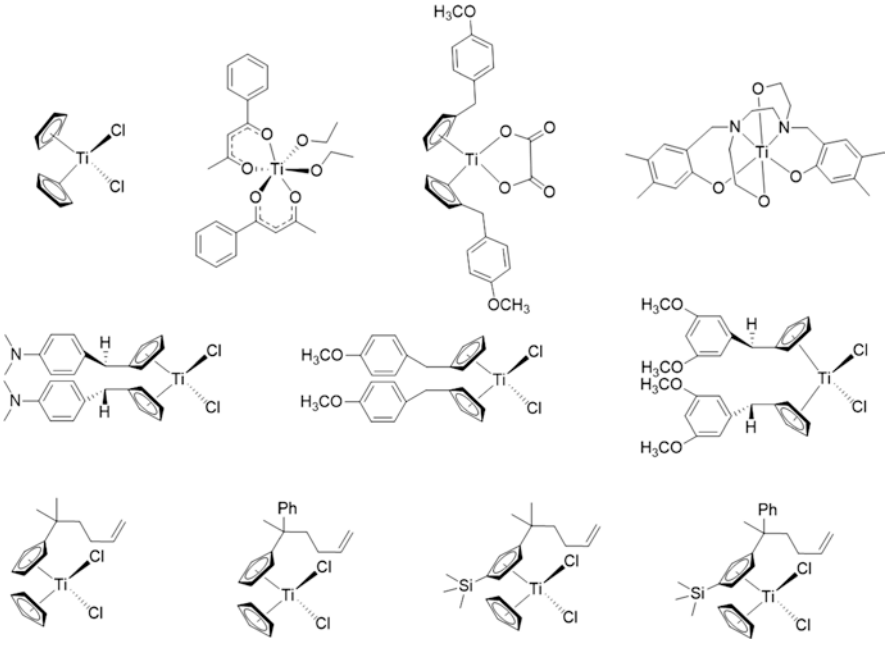
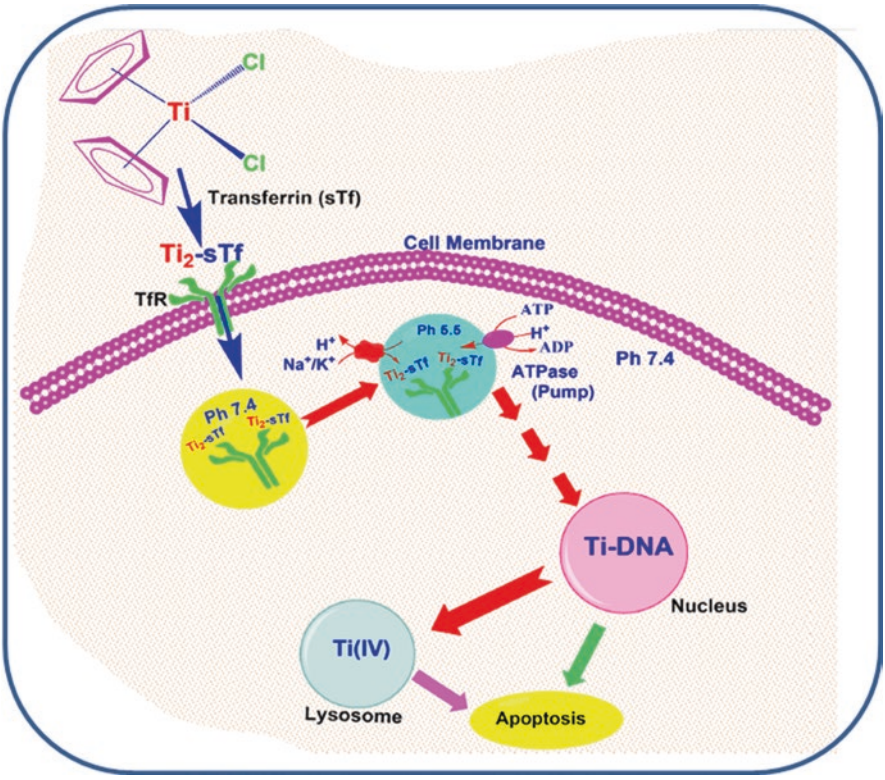


Fig. 1.8 Structures of titanium anticancer compounds

with metastatic renal-cell carcinoma or metastatic breast cancer, Cp_2TiCl_2 demonstrated inadequate efficacy (Köpf-Maier 1994; Cristododoulou et al. 1998). Titanocene dichloride was found to inhibit DNA synthesis, covalently binding to DNA and inducing apoptosis (Guo et al. 2001). Moreover, its DNA binding was mediated through the phosphate backbone rather than the nucleobases, as observed with cisplatin.

The structure of titanocene complexes showed a direct relationship with their cytotoxic effectiveness, but the mechanistic insights of cytotoxicity induced by titanocene(IV) complexes are still underway. Previous literature reports indicated that titanium ions enter cancer cells with the help of the primary iron transport protein called 'transferrin' (Köpf-Maier 1994; Cristododoulou et al. 1998; Guo et al. 2001; Tinoco et al. 2016) and move into the nucleus through active transport, facilitated by ATP. Ultimately, the binding of titanium ions to DNA triggers cell death (Köpf-Maier and Krahl 1983; Köpf-Maier 1990) (Scheme 1.1). Recent progress has unveiled the interactions between a ligand-bound Ti(IV) complex and various proteins or enzymes (Tinoco et al. 2007, 2008; Pavlaki et al. 2009), suggesting an alternative mechanism for cell death.



Scheme 1.1 Suggested mechanism of action for 'titanocene' derivatives

Copper, a coinage trace element, assumes a vital role in numerous biochemical processes, serving as a pivotal component for various metalloenzymes and proteins like cytochrome oxidase, superoxide dismutase (SOD), ascorbate oxidase, ceruloplasmin, and tyrosinase, acting either as a structural or catalytic cofactor. Positioned as the 29th element on Mendeleev's periodic table, copper is present in three oxidation states—Cu(III), Cu(II), and Cu(I)—with Cu(II) being the most favorable state due to its d^9 electronic configuration, capable of transiting to the Cu(I) state in vivo. The redox chemistry of copper holds critical significance in cell physiology, functioning as a catalytic cofactor in mitochondrial respiration, iron absorption, free radical scavenging, and elastin cross-linking processes. The accessible biological redox pairs of Cu(II)/Cu(I) bear significant implications for metallo-nuclease reactions by involving reactive oxygen species (ROS), directly produced through the interaction of copper with molecular oxygen.

Sigman et al. introduced the first copper nuclease complex, paving the way for novel research avenues exploring the interactions of copper complexes with nucleic acids (Sigman et al. 1979, 1993). Copper complexes induce DNA cleavage through hydrolysis of DNA phosphate esters and nucleobases, or oxidation of deoxyribose sugar. Additionally, they can bind to DNA/RNA through various noncovalent interactions (Fig. 1.9).

The toxicity induced by copper complexes stems from the involvement of free copper ions in generating reactive oxygen species (ROS). The proposed mechanism suggests that under certain conditions, Cu(II) can be reduced to Cu(I) in the presence of superoxide ($\cdot O_2^-$) or reducing agents like ascorbic acid or glutathione (GSH). Cu(I) can then catalyze the formation of hydroxyl radicals ($OH^\bullet$) from hydrogen peroxide (H_2O_2) via reactions like the Haber–Weiss reaction (Kehrer 2000):

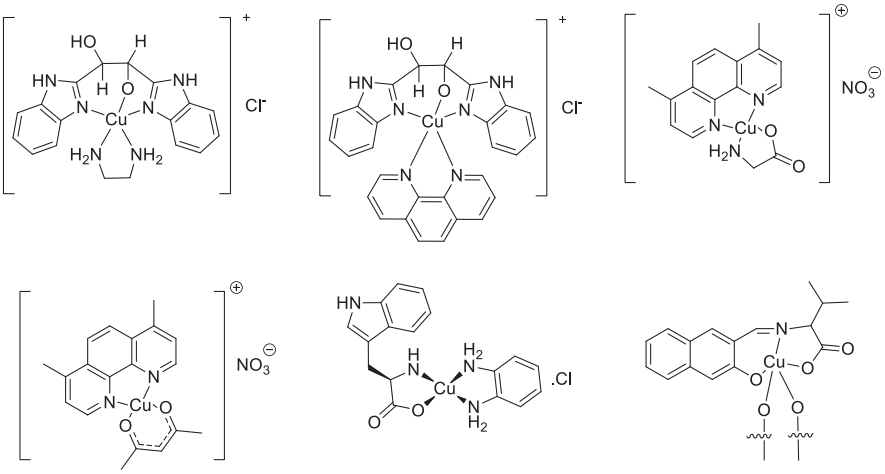


Fig. 1.9 Prominent anticancer copper-based chemotherapeutic agents

$$\begin{gathered}\mathrm{Cu(II)} + \mathrm{O_2^{\cdot -}} \rightarrow \mathrm{Cu(I)} + \mathrm{O_2} \\ \mathrm{Cu(I)} + \mathrm{H_2O_2} \rightarrow \mathrm{Cu(II)} + \mathrm{OH^{\cdot}} + \mathrm{OH^-} \\ \hline \mathrm{O_2^{\cdot -}} + \mathrm{H_2O_2} \rightarrow \mathrm{O_2} + \mathrm{OH^{\cdot}} + \mathrm{OH^-}\end{gathered}$$

These highly reactive hydroxyl radicals could interact with biological molecules, leading to oxidative modifications in cellular components like lipids, proteins, and DNA. This process disrupts the cell's redox balance and may interfere with redox-related cellular signaling pathways (Gupte and Mumper 2009; Theophanides and Anastassopoulou 2002). Copper has been shown to induce DNA damage and base oxidation by generating ROS. GSH has been observed to inhibit free radical formation by copper ions when in the presence of hydrogen peroxide, ascorbate, and DNA. This protective effect is attributed to GSH's ability to stabilize Cu(I), preventing redox cycling and the generation of free radicals. Additionally, copper(II) also forms thiyl radicals, $\mathrm{RS^{\bullet}}$, and other ions upon interaction with molecules like cysteine and methionine, which may contribute to cellular damage (Galaris and Evangelou 2002):

$$\mathrm{RSH} + \mathrm{Cu(II)} \rightarrow \mathrm{RS^{\cdot}} + \mathrm{Cu(I)} + \mathrm{H^+}$$

Although intracellular free copper availability is limited, suggesting efficient copper chelation in cells, copper's affinity for binding to DNA exceeds that of other divalent cations, promoting DNA oxidation (Valko et al. 2005).

Copper ion binding in specific sites can alter the conformational structures of biomacromolecules like DNA/RNA or proteins, (Burkitt 1994; Kagawa et al. 1991). This binding property has been harnessed in developing various medicinal compounds with applications in antibacterial, anticancer, and anti-HIV AIDS therapies.

Copper complexes have emerged as promising anticancer agents due to their diverse potency in vitro and in vivo against a wide range of human cancer cells. Previous research suggests that cancerous tissues typically exhibit higher copper levels compared to normal cells, influencing various aspects of tumor growth, progression, and metastasis (Park 2016; Balsano et al. 2018).

Derived from a range of ligands such as Schiff bases (Kuwabara et al. 1986; Gou et al. 2017; Nunes et al. 2022; Tabassum et al. 2013; Niu et al. 2016; Kartalou and Essigmann 2001), amino acids (Zehra et al. 2019; Parveen et al. 2020; Zhang et al. 2004; Ramakrishnan et al. 2009), peptides (Arjmand et al. 2020; Fu et al. 2014, 2015), azoles (Morier-Teissier et al. 1993; Hu et al. 2018; Steiner et al. 2014; Devereux et al. 2007), terpyridines (Godlewska et al. 2013; Rajalakshmi et al. 2012; Abdi et al. 2012), polypyridyls (Kumar et al. 2011; Abosede et al. 2016; Ng et al. 2016), thiosemicarbazones (Angel et al. 2017; Palanimuthu et al. 2013; Gu et al. 2019; Balakrishnan et al. 2020), and various naturally occurring bioactive compounds like coumarins (Deng et al. 2018; Pivetta et al. 2017; Usman et al. 2017), and chromones (Das et al. 2015; Kalaiarasi et al. 2018; Yousuf et al. 2015), copper-based anticancer drugs have demonstrated significant efficacy through diverse biochemical mechanisms. These mechanisms include interfering with angiogenesis,

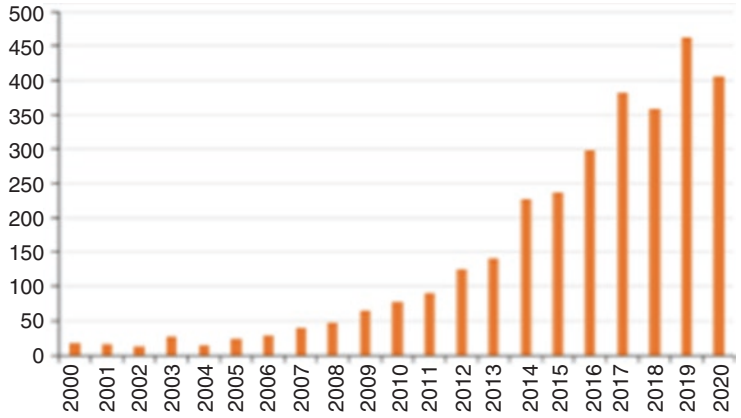


Fig. 1.10 The quantity of articles published in the Web of Science regarding 'copper and anticancer' between 2000 and 2020

proteasome activity inhibition, modulation of intracellular ROS levels, and induction of apoptosis by targeting specific cellular components (Mejía et al. 2018; Wang et al. 2010; Almond and Cohen 2002; Yang et al. 2013; Fulda 2009). As evident from the rising number of publications since 2000 (Fig. 1.10), interest in copper complexes as potential anticancer drugs has grown significantly over the past decade.

Although there has been an enormous advancement in the development of novel nonplatinum metallodrugs, there are only a handful of inorganic anticancer drugs that have crossed R&D stages, and finally reached clinical trials and were approved as drugs by the FDA. Copper-based compounds have attracted a lot of attention among metallodrugs, as they are proven to improve therapeutic potency significantly, reduce systemic toxicity, and show a broad spectrum of activity against various phenotypes of cancers including resistant cancerous cells (Santini et al. 2014; Wehbe et al. 2017). Lena Ruiz-Azuara et al. have synthesized patented mixed chelate copper(II) complexes that were registered as Casiopeínas®. Casiopeínas® represent a collection of over 100 copper-based compounds characterized by a general formula $[Cu(N–N)(O–O)]NO_3$ and $[Cu(N–N)(N–O)]NO_3$, where O–O: acetylacetonate or salicylaldehyde, N–O: α-L-aminoacidates or peptides and N–N is aromatic substituted 1,10-phenanthrolines or 2,2′-bipyridines (Ruiz-Azuara 1992, 1993, 1996, 1997) (Fig. 1.11). These compounds have undergone cytotoxicity assessments across various tumor cell lines such as colorectal (Carvallo-Chaigneau et al. 2008), neuroblastoma (Hernández-Lemus et al. 2013), medulloblastoma (Mejia and Ruiz-Azuara 2008) and breast demonstrating differential toxicity among the analogs (Ruiz-Azuara and Bravo-Gómez 2010; De Vizcaya-Ruiz et al. 2000; Gracia-Mora et al. 2001).

Casiopeína III-ia is the first copper-based anticancer chemotherapeutic agent that has entered phase I clinical trials as it exhibits significantly low cytotoxicity in comparison with phenanthroline subgroup (Alvarez et al. 2018a) Consequently, it ranks among the most extensively researched molecules within this category. Heterocyclic

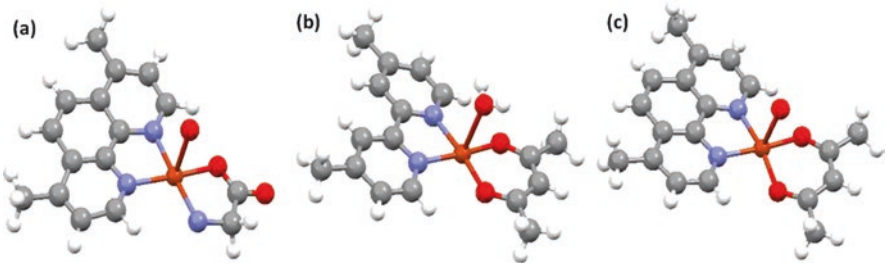


Fig. 1.11 Structure of Casiopeinas. (**a**) Cas IIgly; (**b**) CasIII –ia; (**c**) Cas IIIEa copper complexes that have entered clinical trials

aromatic ligands viz., substituted 1,10-phenanthrolines (phen) and 2,2′-bipyridines (bpy) and possessing nitrogen-donor atoms exhibited relatively high affinity for copper (Zehra et al. 2021; Alvarez et al. 2018b; Eremina et al. 2020) while the extended aromatic ring offers a high binding propensity to DNA by intercalative mode usually by synergistic approach along the metal center. The amino acid ligand acts as an ancillary secondary ligand which is biocompatible with biomacromolecules DNA, dictating the Cas III molecule to acquire distorted square planar geometry. These studies concluded that the nature, quantity, and positioning of substituents on diimine ligands, as well as modifications involving L-amino acids or O–O donors, significantly influence the selectivity or biological activity exhibited by copper(II) complexes with ternary or mixed ligands.

Cobalt stands as an essential biocompatible trace element present in all animals, vital for numerous biologically significant processes. It is predominantly found in the form of vitamin B12, cobalamin, where cobalt exists primarily in the +1 oxidation state (capable of oxidation to the +2 and +3 states) and adopts an octahedral geometry (Munteanu and Suntharalingam 2015; Renfrew et al. 2018). The various forms of cobalamin play critical roles in red blood cell formation, DNA synthesis, and regulation, as well as the maintenance of normal brain and nerve function. The first biological investigation of cobalt complexes, conducted by Dwyer et al. in 1952 (Dwyer et al. 1952, 1957), focused on assessing the toxicity of several cobalt complexes (Fig. 1.12) through intraperitoneal administration in mice. These complexes included tris-acetylacetone cobalt(III), racemic or optical isomers of tris-ethylenediamine cobalt(III) nitrate, 1;8-bis(salicylideneamino)-3;6-dithiaoctane cobalt(III) chloride, 10-bis(salicylideneamino) 7-dithiadecane cobalt(III) iodide, and tris-glycine cobalt(III) (Fig. 1.13).

The findings from the experiments showcased that the doses needed to induce lethality for the various cobalt complexes tested were notably high, ranging between 75 and 165 mg/kg, emphasizing the minimal systemic toxicity associated with cobalt. While the initial investigations with cobalt complexes didn't directly lead to the development of cobalt-based anticancer treatments, they did pave the way for researchers to delve into the biological capabilities of compounds containing cobalt.

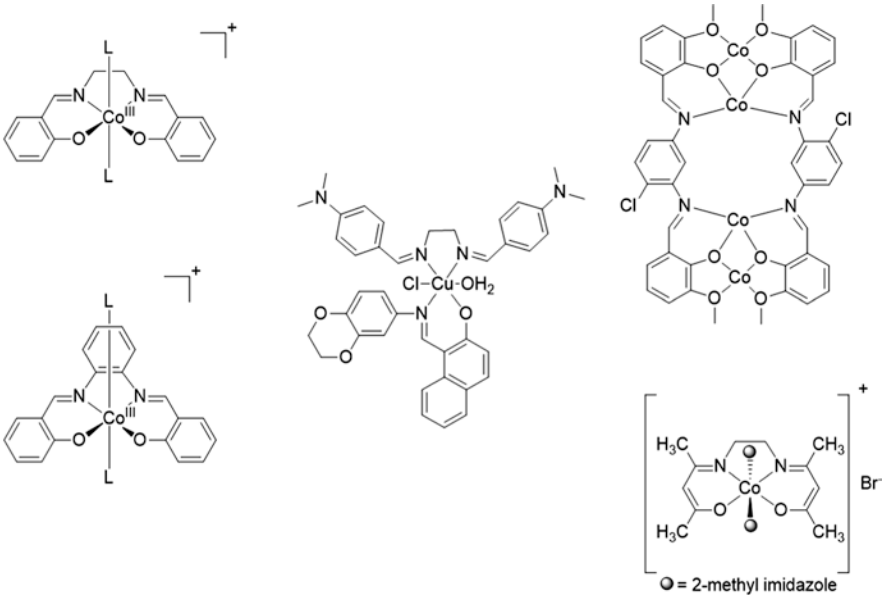


Fig. 1.12 Cobalt Schiff base complexes

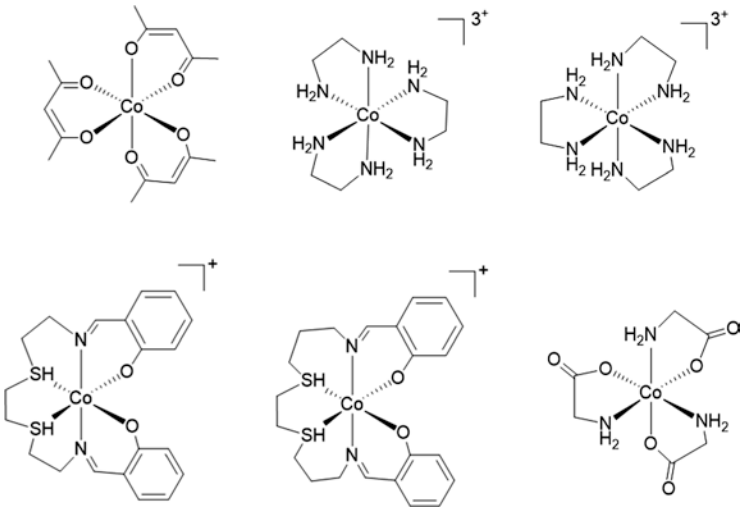


Fig. 1.13 Chemical structures of cobalt(III) complexes employed in early biological investigations

Consequently, several therapeutic cobalt compounds have been synthesized, exhibiting properties with antifungal, antiprotozoal, and antibacterial effects.

The primary breakthrough in cobalt compound medical applications is highlighted by the clinical advancement of Doxovir (CTC-96), specifically developed

for Herpes labialis or herpes simplex virus 1 (Schwartz et al. 2001). Doxovir, a cobalt(III) complex comprising bis(acetylacetone)ethylenediimine (acacen) with two axially coordinated 2-methylimidazole ligands, demonstrates potent microbicidal effects against drug-resistant strains of the herpes virus. Notably, it has recently completed phase II clinical trials. Meade et al. delineated therapeutically promising Co(III) Schiff base bioconjugates that targeted histidine residues in (zinc-finger) proteins with precision, inhibiting various transcription factors linked to cancer progression and metastasis (Harney et al. 2009, 2012; Hurtado et al. 2012).

Schiff bases, formed by the condensation of a primary amine with a carbonyl compound, notably offer flexible scaffolds in Schiff base metal complexes, suitable for structural modifications catering to diverse biological applications. Recent reports highlight simple cobalt(II) and cobalt(III) Schiff base complexes exhibiting reasonable anticancer activity. For instance, the cobalt(II) Schiff base complex, incorporating a 4-(4-aminophenyl)morpholine derivative, displayed limited activity against hepatocellular carcinoma cells (HepG2), with an IC_{50} value in the millimolar range (Dhahagani et al. 2014). Conversely, cobalt(II) complexes featuring 2,6-bis(2,6-diethylphenyliminomethyl)pyridine exhibited improved cytotoxicity against colorectal adenocarcinoma (HCT-15) and cervix adenocarcinoma (HeLa) cells, with IC_{50} values ranging from 45 to 100 μM (Martinez-Bulit et al. 2015). Another cobalt(III) complex, containing the tridentate Schiff base ligand derived from the reaction of salicylaldehyde and ethylenediamine, displayed moderate activity ($IC_{50} < 100$ μM) against human breast cancer cells (MCF-7).

Zinc, an essential element and the second most abundant trace metal in the human body, plays a pivotal role in numerous enzymatic processes, acting as a structural and catalytic component. Its involvement in vital biochemical processes such as DNA synthesis and repair, transcription factor function, and genetic message translation is crucial for cell survival, tissue protection, cellular proliferation, differentiation, apoptosis, immunity, and reproduction (Jarosz et al. 2017). Zinc's distinct chemico-physical properties differentiate it from other first-row transition metals. The dicationic Zn^{2+} element, being redox inactive with a d^{10} configuration and diamagnetic nature, exhibits strong Lewis acidity, allowing stabilization of various coordination geometries and rapid ligand exchange. Despite these advantageous properties, the lack of color in zinc complexes limits their characterization through conventional spectroscopic techniques, making them 'spectroscopically silent' in biological contexts (Penner-Hahn 2005). However, their high coordination geometry flexibility offers a range of arrangements, with the tetrahedral arrangement being prevalent in proteins.

Zinc complexes are known to exhibit a wide range of biological activities, such as anti-inflammatory, antimicrobial, anticonvulsant, antidiabetic, antioxidant, antiproliferative, antitumor, and anti-Alzheimer agents, generally exhibiting fewer side effects and much lower toxicity as compared to other transition metals or metallodrugs in medicine. The antitumor activity of Zinc complexes has been attributed to the ligand scaffold coordinated to the metal center. In this connection, a large number of Schiff bases complexes with N, O, and S donor atoms, non-Schiff base complexes such as thiosemicarbazone, amino acids, flavonoids or curcumins and