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Synthesis, characterisation and cytotoxicity of platinum(II) complexes containing natural ligands with biological activity

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	ABSTRACT Cancer always is among the major causes of death worldwide and only limited progresses were carried onto in order to reduce its morbidity and mortality. Starting from its approval in 1979, cisplatin became probably the most important component of chemotherapy for treating the ovarian, testicular, lung and bladder carcinomas, as well as lymphomas, myelomas and melanoma. Although, cisplatin is a widely used anticancer drug, its clinical exploitation leads to several side effects (decline of kidney, tubular and marrow cells, auditory system). Moreover, several cancer cells are not or poorly sensitive to the cis-Pt action. As a consequence, the recognition of suitable alternatives able to reduce of side effects during the anticancer therapy, some researches were carried out for providing other cis-Pt type complexes free from these contraindications. Therefore, several hundreds of cisplatin analogues were tested during the last 20 years, and many of these species were rejected in preclinical or early clinical stages of testing. Then, the development of more selective and less toxic Pt-based drugs than cisplatin is still highly desirable. Recently, the anticancer activity of naturally occurring substances obtained from plants, such as flavonoids, curcumin and caffeine, has been tested and about 20 new drugs derived from natural products were introduced from 2001 to 2005. So, a lack of knowledge also occurs about the possibility to combine the anticancer capability of cis-Pt related complexes with the limited contraindications for the human health given by the exposure to substances obtained from plants. This study is focused on the scope to fill this gap providing information about the cytotoxic activity, of cis-Pt type complexes synthesized using ligands extracted from natural substances obtained from plants. The research was carried out according two different paths. Firstly, these new complexes were synthesized, characterized according spectroscopic investigations and then their cytotoxic activity assessed towards several cancer cell lines in terms of IC50 values. As follows, further investigations were carried out in order to provide information about the extent of interactions between DNA and the new synthesized Pt-complexes with curcumin and caffeine, that were obtaining through different characterization techniques. Our results suggest that the exploited metal complexes act as efficient DNA binders. Suitable crystals for XRD analyses were also obtained for some of the synthesized complexes.		2016	Doctor of Philosophy in Environmental and Bilding risk and Development		2016
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D.R.R.S.

POLITECNICO DI BARI

01

Doctor of Philosophy in Environmental
and Bilding risk and Development

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Coordinator: Prof. Michele Mossa

XXIX CYCLE
Curriculum: Chemistry

DICATECh

Department of Environmental, Bilding,
Engineering and Chemistry

**Synthesis, characterisation and cytotoxicity of
platinum(II) complexes containing natural ligands
with biological activity**

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SYNTHESIS, CHARACTERIZATION AND CYTOTOXICITY OF PLATINUM(II) COMPLEXES CONTAINING NATURAL LI GANDS WITH BIOLOGICAL ACTIVITY

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EXTENDED ABSTRACT (eng)

Cancer always is among the major causes of death worldwide and only limited progresses were carried onto in order to reduce its morbidity and mortality. Starting from its approval in 1979, cisplatin became probably the most important component of chemotherapy for treating the ovarian, testicular, lung and bladder carcinomas, as well as lymphomas, myelomas and melanoma. Although, cisplatin is a widely used anti-cancer drug, its clinical exploitation leads to several side effects (decline of kidney, tubular and marrow cells, auditory system). Moreover, several cancer cells are not or poorly sensitive to the cis-Pt action. As a consequence, the recognition of suitable alternatives able to reduce of side effects during the anticancer therapy, some researches were carried out for providing other cis-Pt type complexes free from these contraindications. Therefore, several hundreds of cisplatin analogues were tested during the last 20 years, and many of these species were rejected in preclinical or early clinical stages of testing. Then, the development of more selective and less toxic Pt-based drugs than cisplatin is still highly desirable. Recently, the anticancer activity of naturally occurring substances obtained from plants, such as flavonoids, curcumin and caffeine, has been tested and about 20 new drugs derived from natural products were introduced from 2001 to 2005. So, a lack of knowledge also occurs about the possibility to combine the anticancer capability of cis-Pt related complexes with the limited contraindications for the human health given by the exposure to substances obtained from plants.

This study is focused on the scope to fill this gap providing information about the cytotoxic activity of cis-Pt type complexes synthesized using ligands extracted from

natural substances obtained from plants. The research was carried out according to two different paths. Firstly, these new complexes were synthesized, characterized according to spectroscopic investigations and then their cytotoxic activity was assessed towards several cancer cell lines in terms of IC₅₀ values. As follows, further investigations were carried out in order to provide information about the extent of interactions between DNA and the new synthesized Pt-complexes with curcumin and caffeine, that were obtained through different characterization techniques. Our results suggest that the exploited metal complexes act as efficient DNA binders. Suitable crystals for XRD analyses were also obtained for some of the synthesized complexes.

key words

Platinum(II) complexes; Cytotoxicity; Flavonoids; Curcumin; Caffeine; Crystal structure, Drugs-DNA interactions.

EXTENDED ABSTRACT (ita)

Il cancro da sempre è una delle maggiori cause di morte nel mondo e vi sono stati solamente pochi progressi nella riduzione della sua morbosità e mortalità.

A partire della quando è stato approvato nel 1979, il cisplatino è divenuto probabilmente il più importante agente chemioterapico per il trattamento dei carcinomi ovarici, testicolari, del polmone e dalla vescica, come pure dei linfomi, dei mielomi e dei melanomi. Sebbene il cisplatino sia ampiamente usato come antitumorale, il suo uso clinico comporta alcuni effetti collaterali (problemi renali, alle cellule del midollo e al sistema uditivo). Inoltre, numerose cellule cancerogene sono debolmente sensibili al cisplatino. Di conseguenza si è resa necessaria la ricerca di alternative valide capaci di ridurre gli effetti collaterali del cisplatino, quindi sono state portate avanti alcune ricerche nel desiderio di trovare dei complessi analoghi del cisplatino privi delle sue controindicazioni.

Negli ultimi vent'anni sono stati testate alcune centinaia di analoghi del cisplatino e molte di queste sostanze sono state respinte durante i test preclinici o ai primi stadi dei test clinici. Quindi, ancora di grande interesse lo sviluppo di nuovi composti più selettivi nell'azione antineoplastica e meno tossici del cisplatino.

Recentemente è stata studiata l'azione antitumorale di sostanze di origine naturale quali flavonoidi, curcumina and caffeina e circa venti nuovi farmaci derivanti da prodotti naturali sono stati introdotti tra il 2001 e il 2005.

Nasce così il desiderio di combinare l'azione antitumorale di complessi analoghi del cisplatino con gli effetti benefici delle sostanze naturali.

Questo studio punta l'attenzione sul ricercare un'azione sinergica tra l'azione anti-cancerogena dei complessi analoghi del cisplatino e proprietà benefiche delle sostanze estratte dalle piante.

La ricerca si è sviluppata in più step; in primis la sintesi di nuovi complessi di Platino(II) la cui formazione è stata verificata mediante indagini spettroscopiche e sui quali sono state svolte analisi di citotossicità in vitro su selezionate linee cellulari in termini di IC_{50} .

Successivamente sono state effettuate ulteriori analisi nel desiderio di studiare il meccanismo di interazione tra il DNA e i complessi con la curcumina e la caffeina, mediante l'uso di differenti tecniche di investigazione. I risultati ottenuti suggeriscono che i complessi testati interagiscono con il DNA. I cristalli ottenuti per alcuni dei complessi selezionati sono stati utilizzati per un'ulteriore conferma della struttura del complesso sintetizzato.

key words

Complessi di Platino(II); Citotossicità; Flavonoidi; Curcumina; Caffeina;
Struttura cristallina, interazioni complesso-DNA.

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1. INTRODUCTION/INTRODUZIONE

1.1 Cancer

The cancer is the wide and unregulated proliferation of cells growing and dividing according to an uncontrolled scheme. These invade normal tissues and organs and spread throughout the body. The most important issue in cancer pathology is the distinction between benign and malignant tumours. A benign tumour is a mass of cells that lacks the ability to invade neighbouring tissue or metastasize. Thanks to the lack of such characteristics, proper of the cancerous tumour, the benign cancer is defined not cancerous. Malignant tumor is capable of both invading surrounding normal tissue and spreading throughout the body via the circulatory or lymphatic systems (Cooper, 2000); B. Alberts et al., Molecular Biology of the Cell, 4th Edition, 2002, New York, Garland). Malignant tumours are a major cause of mortality all over the world. The burden of cancer is increasing in economically developing countries (Fig. 1).

The transformation from a normal cell into a tumour cell is a multistage process, from a pre-cancerous lesion to malignant tumours. These changes are the result of the interaction between a person's genetic factor and 3 categories of external agents, including:

- Physical carcinogens, such as ultraviolet and ionizing radiation;

- Chemical carcinogens, such as asbestos, components of tobacco smoke, food contaminants and water contaminants;
- Biological carcinogens, such as infections from certain viruses, bacteria or parasites.

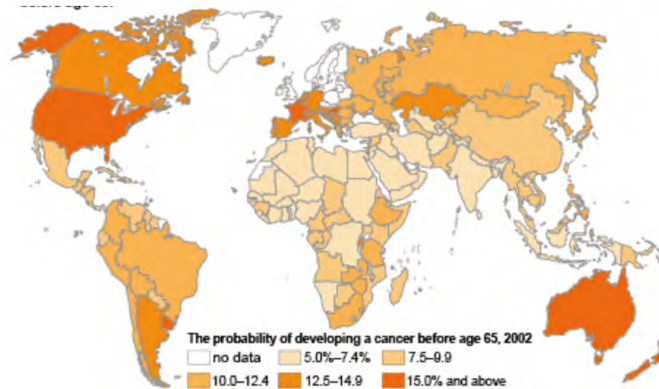


Fig. 1 - Increase cancer risk in the developed regions

Advances in detection, surgery, radiotherapy and chemotherapy are needed to improve the curability of cancer. These therapies are often coupled to particular alimentary dietary based on the assumption of natural substances from vegetables since their recognised anticancer activity.

Chemotherapy was first defined by Paul Ehrlich (Nobel Prize in medicine in 1908). It is a treatment with cytotoxic antineoplastic agents which basically interfere with cell replication of sick cells and conduct them to the apoptosis. This is related to the lack of selectivity of antineoplastic agents towards malignant cells. As a consequence, severe neuro-, oto-, hepato- and nephrotoxic effects are associated to the exposure to the chemotherapeutics. These can be distinguished according to their structures and mechanisms of action:

- Alkylating agents: damage cell function by alkylation of RNA (Ribonucleic Acid), DNA (Deoxyribonucleic acid) and proteins and therefore inhibit replication.

- Metal-based drugs: according to their different structures there are many mechanisms of action. An important example are platinum(II) complexes (cisplatin, carboplatin, oxaliplatin, that induce structural changes on DNA by covalent binding of metal centre to nucleic acids leading to the cell death.
- Topoisomerase inhibitors: they are essential enzymes that maintain the topology and integrity of DNA. Inhibition of topoisomerase interferes with all central DNA processing steps such as replication, transcription, translation and recombination.
- Antimetabolites: compete or substitute biomolecules involved DNA and RNA synthesis and therefore alter the critical pathways of nucleotide synthesis (example is 5-fluorouracil).

Coupled with the chemotherapeutic approach, the cancer treatment was carried out exploiting several natural substances extracted from plants. There is a long history of plant use in the cancer treatment so that over 60% of currently used anti-cancer agents come from natural sources.

Natural dietary agents including fruits, vegetables, and spices have drawn a great deal of attention from both the scientific community and the general public owing to their demonstrated ability to suppress cancers. Recent studies suggest that the consumption of food rich in fruits, vegetables and spices have a lower incidence of cancers (stomach, esophagus, lung, oral cavity and pharynx, endometrium, pancreas and colon).

Dietary agents consist of a wide variety of biologically active components that are responsible for the anti-cancer effects like curcumin, genistein, resveratrol, diallyl sulfide, S-allyl cysteine, allicin, lycopene, capsaicin, diosgenin, gingerol, ellagic acid, ursolic acid, silymarin, anethol, catechins, eugenol, isoeugenol, dithiolthiones, isothiocyanates, indole-3-carbinol, isoflavones, saponins, phytosterols, inositol hexaphosphate, Vitamin C, D-limonene, lutein, folic acid, beta carotene, selenium, Vitamin E and flavonoids. Many of which have been used in traditional medicines for thousands of years. These dietary agents are believed to suppress the inflammatory processes that

lead to transformation, hyperproliferation, and initiation of carcinogenesis. Their inhibitory influences may ultimately suppress the final steps of carcinogenesis i.e angiogenesis and metastasis.

1.2 Platinum(II) anticancer drugs

Cisplatin (cis-Diamminedichloroplatinum(II) or *cis*-Pt or *cis*-DDP) is a metal cytostatic owing complex accidentally discovered by Rosemberg in 60th during studies focused on the effects of electric fields on bacterial growth(Rosenberg et al., 1969).

This phenomenon appeared to be caused by the presence of small amounts of compounds, like *cis*-[PtCl₄(NH₃)₂] and *cis*-[PtCl₂(NH₃)₂] (cisplatin, Fig. 2), formed by slowly dissolving Pt electrodes in the ammonium chloride electrolyte(Chu, 1994). Rosemberg noticed that the fragmentation bacterial reproduction was inhibited in the electrolytic working space, whereas the stringy growth was about 300 times larger relative to the standard behaviour.

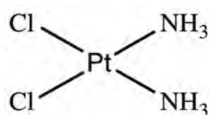


Fig. 2 - Cisplatin structure

Although there were some initial difficulties related to the popular opinion that heavy metals and related compounds could be highly toxic, Rosemberg used severe biological examinations and found that *cis*-Pt formed in the electrolytic baths, producing anticancer activity. The results obtained both *in vitro* and eventually *in vivo* systems were amazing, paving the way for a new class of anticancer compounds (Rosenberg et al., 1965). Still today, cisplatin (*cis*-Pt) is one of the most powerful chemotherapeutic agents, and it is highly active over a wide spectrum of neoplasia in the lungs, oesophagus, stomach, and cervix, as well as the urinary and reproductive systems.

The process by which *cis*-Pt occurs in cells is not fully understood, but it apparently occurs both by passive diffusion through protein membrane and a further membrane transporter that is currently not well-identified (Arnesano et al., 2013). After intravenous delivery, *cis*-Pt maintains a stable neutral status due to the large chloride

concentration in blood plasma. On the contrary, coming into the cells where low chloride concentration occurs, the Pt-complex in the cytoplasm is easily hydrolysed there where $[cis\text{-Pt}(\text{NH}_3)_2(\text{H}_2\text{O})]^{2+}$ is formed (Fig. 3), retaining the original cis-configuration of the Cl-complex (Jung and Lippard, 2007).

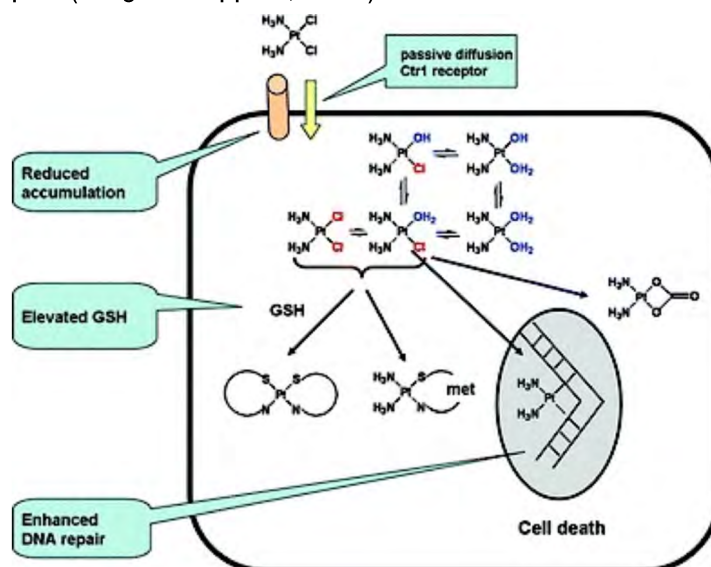


Fig. 3 - Cisplatin hydrolysis mechanism

Both the formed mono- and di-aquo cationic complexes are highly reactive and can interact with DNA, forming several adducts (Fig. 4) that induce structural modifications in the nucleic acid. If unfixed by the organism, these modifications can lead to apoptosis. The formation of the mono- and bifunctional adduct has been followed by ^{195}Pt NMR analyses that considered changes related to a chemical shift that was recorded in the NMR spectrum (Fig. 5). The active complexes enter the nucleus and become attracted by the negatively charged (Lippard, 1994).

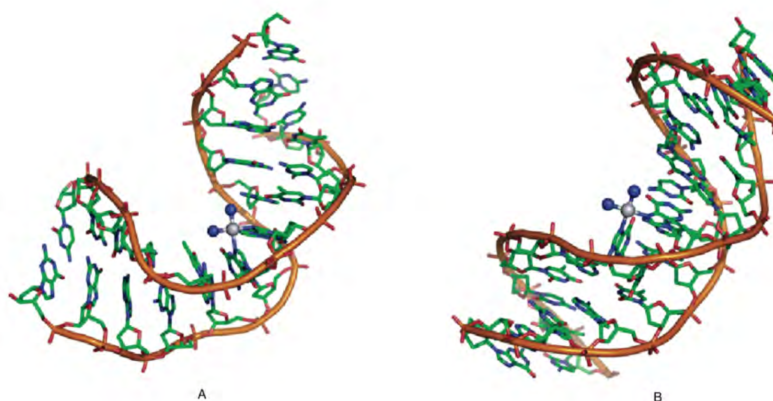


Fig. 4 - Cisplatin adducts, A) 1,2 intrastrand crosslinking, B) 1,3 intrastrand crosslinking (Lippard, 1994).

This electrostatic interaction is followed by complexation with nitrogen atoms of purine bases, normally the N-7 atoms of two vicinal guanine units that displace the two water molecules leading to intrastrand cross-linking, which deforms the DNA tertiary conformation and causes its unwinding at the complexation site (Fig. 6) (Takahara et al., 1995).

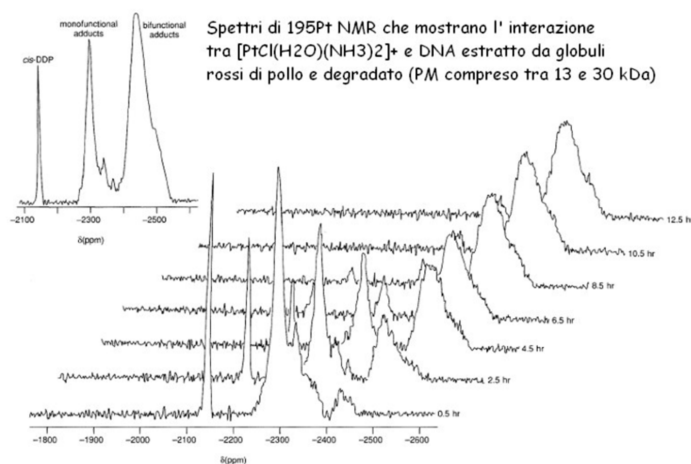


Fig. 5 - ^{195}Pt NMR spectra that show the interaction among $[\text{PtCl}(\text{H}_2\text{O})(\text{NH}_3)_2]^+$ and DNA extracted by red globules of chicken. Molecular Weight among 13 and 30 MDa (Lippard, 1994).

So, therefore high-mobility group (HMG) domain proteins become bound to DNA by intercalation of a phenylalanine unit at the unwound DNA damage site and the

protein lies along a widened minor groove, preventing DNA replication (Ohndorf et al., 1999).

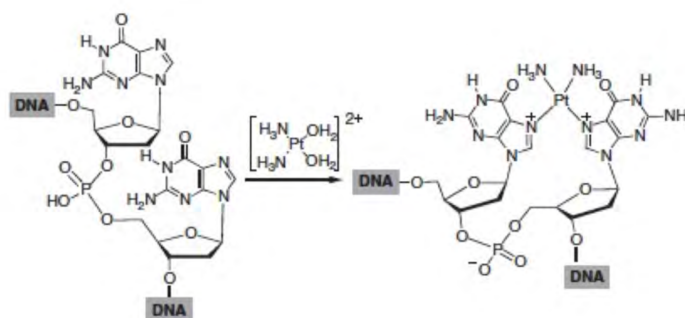


Fig. 6 - Coordination complex generated from the cisplatin active species and DNA

Although, strictly speaking, Pt coordination with DNA bases cannot be considered an alkylation reaction, cisplatin and its analogues are normally studied among the alkylating agents.

Despite this success as anticancer drug, there is still a limited range of tumors sensitive to cisplatin intervention—some cancers are inherently resistant. This behaviour is consistent with a particular membrane opposition towards the entry of this drug inside the cell or to the large glutathione contents in the cytoplasm that would favour cis-Pt removal before it can interact with the DNA. On the other hand, the possibility that the cancer cells can have a suitable restoring mechanism available that is able to remove Pt-bound DNA fragments before that apoptosis can occur cannot be ruled out (Kartalou and Essigmann, 2001). A further disadvantage is the onset of clinical acquired resistance after treatment with the platinum complex. The side effects of cisplatin treatment are dangerous and include the dose-limiting nephrotoxicity, neurotoxicity, ototoxicity, and blood-genesis.

In order to try to limit possible side effects, the coupled delivery of protective compounds (penicillamine for renal toxicity and amifostine for neurotoxicity) was carried out (Dasari and Tchounwou, 2014). Moreover, experiments were carried out in order to develop the possible structural analogues of cisplatin in order to identify less toxic drugs that are active towards the stems of resistant cell cancers (Lebwohl and Canetta, 1998). A diffuse strategy used to try solving the problem of the resistance of

some cancers to *cis*-Pt and its analogues consists of changing the nature of the coordinating ligand. The design and synthesis of more effective and less toxic platinum complexes constitutes a broad area of research with the aim of overcoming the problems associated with cisplatin in chemotherapy (Jamieson and Lippard, 1999). Toxicity can be decreased by exchanging the labile chlorine ligands for comparatively more stable bidentate leaving groups, thereby slowing down the hydrolytic activation of the drugs (Jakupec et al., 2003).

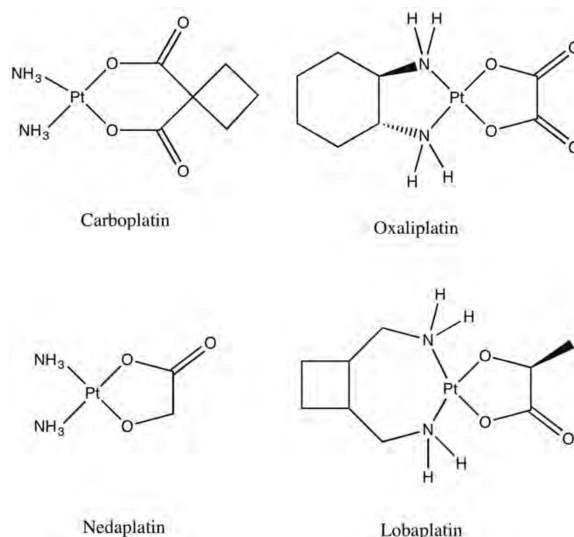


Fig. 7 - Structure of antitumor drugs in clinic, carboplatin, nedaplatin, oxaliplatin, lobaplatin.

When the chloride leaving groups are substituted for more stable ligands, such as 1,1-cyclobutanedicarboxylate, the renal effects are decreased, while the antitumor activity remains. (Christian, 1992). Three of the “second-generation” platinum drugs fulfilling the requirements mentioned above are currently used in cancer chemotherapy: carboplatin, nedaplatin and oxaliplatin, lobaplatin (Fig. 7).

Cis-diammine-1,1-cyclobutane-dicarboxylatoplatinum(II) (carboplatin), the most successful of these second-generation platinum complexes, entered the clinic in 1998, principally to satisfy the necessity to reduce the toxic side effects of the parent drug. Despite this lower toxicity, carboplatin is essentially active against the same cancer cellular lines of cisplatin. For some cancer categories, cisplatin is even therapeutically more effective than carboplatin (germ cell tumors, head and neck, and bladder)

whereas for lung cancer and ovarian cancer the result is comparable. The choice of the most appropriate analog is a function of the cancer being treated, treatment intention (palliative or curative), and other component drugs used in combination (Christian, 1992, Lokich and Anderson, 1998).

Cis-diammine(glycolato)-platinum(II) (nedaplatin) is cisplatin derivative that show less renal and gastrointestinal toxicity than cisplatin as well as reducing the nephrotoxicity and vomiting (Calvert et al., 1982, Allan and Smyth, 1986, Sasaki et al., 1991). Carboplatin has gained worldwide approval and steadily increasing acceptance as a less toxic alternative to cisplatin, while nedaplatin has received only limited regional approval (Jakupec et al., 2003).

Changing the ammines of carboplatin results in (1R,2R-diaminocyclohexane) oxalatoplatinum(II), (oxaliplatin, I.OHP), (Fig. 7). This compound was first reported in Japan;(Kidani et al., 1977, Kidani et al., 1978) and entered the clinic in France(Misset, 1998). It has been approved for secondary treatment of lung and ovarian cancers and it is first line combination therapy for colon cancer.

It shows a different range of activities compared with cisplatin(Mathé et al., 1986), and it overcomes cisplatin resistance, it is active in cisplatin-resistant cell lines(Harrap, 1995).

Cancer continues to be one of the major causes of death worldwide and only modest progress has been made in reducing the morbidity and mortality of this disease. Cancers may be caused in one of three ways, namely incorrect diet, genetic predisposition, and via the environment. As many as 95% of all cancers are caused by life style and may take as long as 20– 30 years to develop.

The prognosis for a patient with metastatic carcinoma of the lung, colon, breast, or prostate remains a concern and accounts for more than half of all cancer deaths.

Chemoprevention or chemotherapy via nontoxic agents could be one approach for decreasing the incidence of these cancers. Many naturally occurring agents have shown chemopreventive and chemotherapeutic (anticancer) potential in a variety of bioassay systems and animal models. An effective and acceptable chemopreventive or anticancer agent should have certain properties: (a) no toxic effects in healthy cells, (b)

high efficacy against numerous type of cancers, (c) known mechanism of action, (d) may be taken by mouth and (e) low cost.

The resolution of neoplasm resistance towards the *cis*-Pt can be bypassed changing the nature of ligands bound to the platinum. The following investigation of antineoplastic activity of the new synthesized complexes allows us of correlating their molecular structure to the cytotoxic activity recognising the most promising complexes. In order to attain this goal, the diethyl(pyridine-4-dimethyl)-phosphine (pmOpe), the *trans*-[PtCl₂(4-pmOpe)₂] (Fig.8) and the [Pt(O2,O3-asc)TPA₂] complexes were studied (Fig. 9).

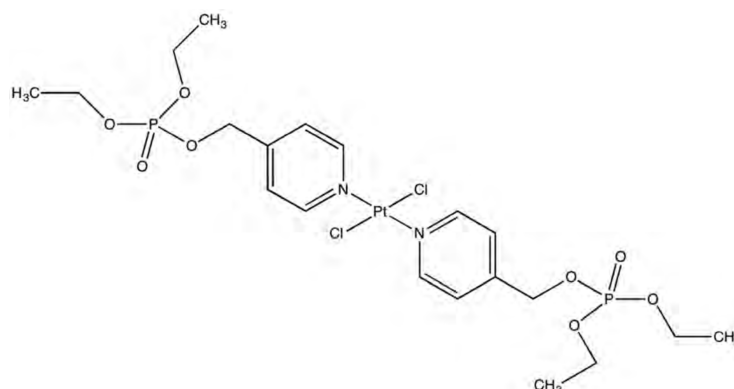


Fig. 8 - *trans*-[PtCl₂(4-pmOpe)₂] complex structure

The cytotoxic activity of the *trans*-[PtCl₂(4-pmOpe)₂] complex was assessed towards the HT 29 and A549 cell lines (colon-rectum and small cells pulmonary carcinomas, respectively) and the toxicity towards lymphoid cells of human blood was checked. The obtained results showed the significant selectivity of the above-mentioned complex whose cytotoxicity was more pronounced towards cancer cells rather than towards the unaffected human blood ones. The [Pt(O2,O3-asc)TPA₂] complex was studied for its largely solubility determined by the presence of the 1,3,5-Triaza-7-phospho-adamantane (TPA) ancillary ligand and showing a similar antiproliferative activity than *cis*-Pt if carried out on 2-5 cm wide cancer cells (T2). (Kalinowska-Lis et al., 2008).

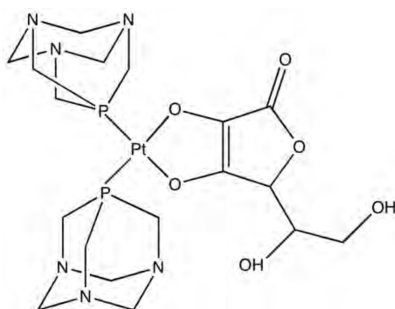


Fig. 9 - [Pt(O2,O3-asc)TPA₂] complex structure

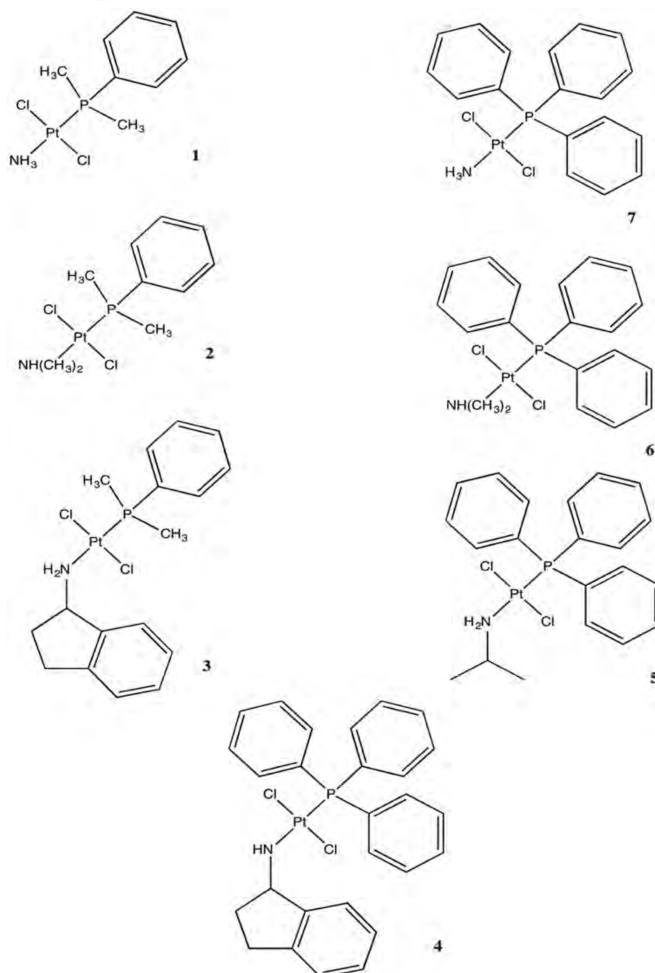


Fig.9A trans-[PtCl₂(L)(L')]-type complexes where L is an obstructed phosphine as PPh₃ or PMe₂Ph, whereas L' is NH₃, aliphatic phosphines as isopropylamine, dimethylamine or 1-aminoindane

Bergamini et al. (2008) showed a larger antiproliferative activity of several P-bearing Pt(II) complexes towards the investigated cell lines than cisplatin. Previous studies investigated the cytotoxic activity of $\text{trans-[PtCl}_2(\text{L})(\text{L}')]$ -type complexes (Fig.9A) where L is an obstructed phosphine as PPh_3 or PMe_2Ph , whereas L' is NH_3 , aliphatic phosphines as isopropylamine, dimethylamine or 1-aminoindane. These complexes show a IC_{50} values (representing the concentration of the selected species able to kill at least 50% of exposed cancer cells) and values of the drug resistance factor (RF) larger than those recognised for *cis*-Pt and other *trans*-Pt complexes towards SKOV3, CH1cisR and SR2910 cell lines (ovarian carcinoma and Ewing sarcoma, respectively).

Ramos-Lima et al.(2007) suggest that the larger activity observed during in-vitro experiments can be consistent to the lipophilic character of phosphine favouring the drug transition through the cell membrane.

Pt(II) complexes with DMSO have shown antineoplastic activity towards A2780cisR, AMD473 and A2780473R cell lines towards which *cis*-Platinum was inactive. At the same time *trans*- $[\text{PtCl}_2(\text{DMSO})(\text{tetrahydro-1,4-oxazine})]$ highlights the effectiveness of *trans*-platinum complexes as anticancer drugs.

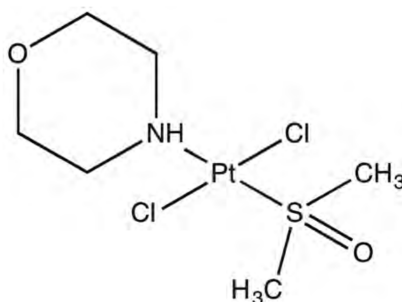


Fig. 10 - *trans* - $[\text{PtCl}_2(\text{DMSO})(\text{tetrahydro-1,4-oxazine})]$ complex structure

The first studies on *trans*-Pt complexes suggested their limited cytotoxicity if compared with *cis*-Pt. As follows, several *trans*-Pt complexes were synthesized and studied demonstrating that their cytotoxicity could be similar to the *cis*-isomers (Farrell et al., 1989).

1.3 Natural substances

Plants have a long history of use in the treatment of cancer and it is significant that over 60% of currently used anti-cancer agents are come from natural sources. In between 2001 and 2005, 23 new drugs derived from natural products were introduced for the treatment of disorders such as bacterial and fungal infections, cancer, diabetes, dyslipidemia, atopic dermatitis, Alzheimer's disease and genetic diseases.

Natural dietary agents including fruits, vegetables, and spices have drawn a great deal of attention from both the scientific community and the general public owing to their demonstrated ability to suppress cancers. Recent studies suggest that the consumption of food rich in fruits, vegetables and spices have a lower incidence of cancers (stomach, esophagus, lung, oral cavity and pharynx, endometrium, pancreas and colon).

Dietary agents consist of a wide variety of biologically active components that are responsible for the anti-cancer effects like curcumin, genistein, resveratrol, diallyl sulfide, S-allyl cysteine, allicin, lycopene, capsaicin, diosgenin, gingerol, ellagic acid, ursolic acid, silymarin, anethol, catechins, eugenol, isoeugenol, dithiolthiones, isothiocyanates, indole-3-carbinol, isoflavones, saponins, phytosterols, inositol hexaphosphate, Vitamin C, D-limonene, lutein, folic acid, beta carotene, selenium, Vitamin E and flavonoids. Many of which have been used in traditional medicines for thousands of years. These dietary agents are believed to suppress the inflammatory processes that lead to transformation, hyperproliferation, and initiation of carcinogenesis. Their inhibitory influences may ultimately suppress the final steps of carcinogenesis i.e angiogenesis and metastasis.

Flavonoids are present in a lot of plants and they belong to a group of natural substances with different phenolic structures. Flavonoids are benzo- γ -pyrone derivatives that have been classified according to their chemical structure according to presence of different substituents on the rings and the degree of benzo- γ -pyrone saturation (Fig. 11).

More than 4000 different naturally occurring flavonoids were described and their list is updating. Flavonoids have been known as plant pigments for over a century and belong to a vast group of phenolic compounds that are widely distributed in all foods

of plant origin. They have numerous beneficial properties for the human health, because they interact with numerous cellular target. Scientists are interested in the potential health effects associated with fruit and vegetable-rich diets.

They are present in the feeding of a lot of populations in different parts of world, such as in the Mediterranean coast, where the diet is rich in olive oil, citrus fruit and grapes, wine, in Netherlands where onions, apples and tea are usually consumed, in Asia where the diet is rich in vegetables, tea and fruit.

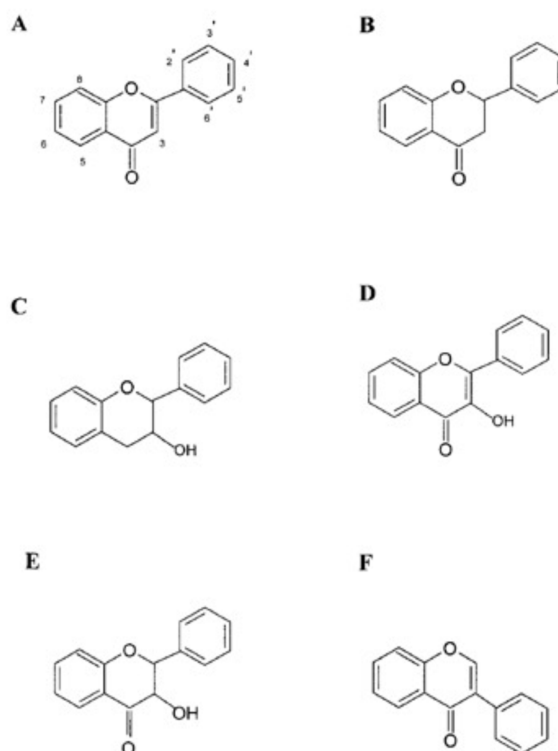


Fig. 11 - Chemical structure of some groups of flavonoids: A) flavone, B) flavanone, C) flavan-3-ol, D) flavanol, E) flavanolol, F) isoflavone

Several studies recognise the wholesome effects of flavonoids as anti-inflammatory, anti-allergic, antimicrobial, hepatoprotective, antiviral, antithrombotic, cardioprotective capillary strengthening, antidiabetic, anticarcinogenic and antineoplastic effects (Fig. 12)(Middleton et al., 2000, Kandaswami and Middleton, 1994, Castillo et al., 1989, Huang et al., 1999).

In-vivo and in-vitro experiments demonstrated that flavonoids inhibit carcinogenesis through:

- Inhibition of protein kinase activity,
- Inhibition of metastasis and angiogenesis,
- Induction of apoptosis,
- Anti-oxidative activity

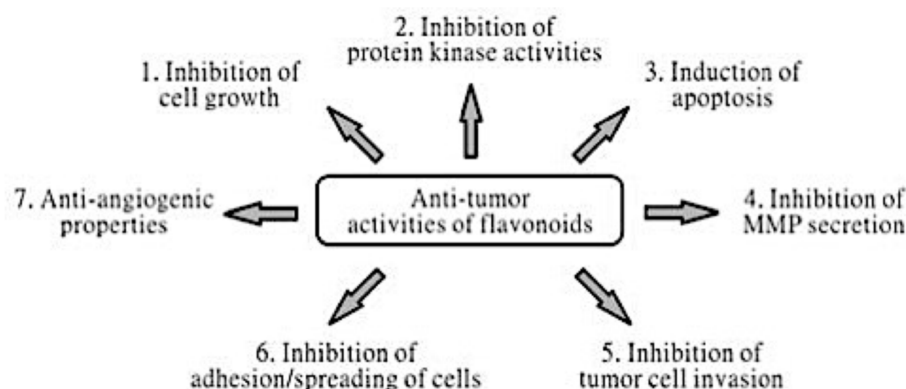


Fig. 12 - Antitumour activities of flavonoids

It is widely accepted that the inhibitory effects of flavonoids on the growth of cancer cells could be a result of their interference with the protein kinase activities taking part to the regulation of cell proliferation. On the last years several researches have evaluated the inhibitory potencies of different flavonoids on various serine/threonine and tyrosine kinases. At the cellular level, flavonoids from different chemical classes exhibit inhibitory effects on protein kinase activity.

Protein kinase C (PKC), Ca^{2+} and phospholipid-dependent, multifunctional serine- and threonine phosphorylating enzyme plays a fundamental role in an entire range of cellular activities, including tumor promotion. Specific dietary flavonoids were found to be potent inhibitor of signal transduction enzymes, PKC in vitro, protein tyrosine Kinase (PTK) and phosphoinositide 3-kinase(PIP3). Quercetin was the most efficient inhibitor of PKC, because is known that quercetin diminished the phosphorylating activity of Rous sarcoma virus (a retrovirus capables of inducing the tumor formation in vivo and in vitro) (Ferriola et al., 1989, Gschwendt et al., 1983, End et al., 1987, Hagiwara et al., 1988, Makita et al., 1996).

The capabilities of selected flavonoids to arrest solid tumour growth may be due to their inhibition of the neo-angiogenic process (Paper, 1998). Angiogenesis is the formation of new blood vessels beginning from the pre-existing ones, therefore represent a crucial trial in the development and in the progression of the tumour. When deprived of proper vascularization, the high proliferation rate in the tumour would be balanced by cell death due to the lack of diffusion of nutrients and oxygen. Angiogenesis inhibitors such as flavonoids are able to contrast with various points of angiogenesis, like basement destruction of blood vessels, proliferation and migration of endothelial cells or the lumen formation (Tosetti et al., 2002). Therefore, these compounds may have potential for the treatment of solid tumours.

In addition to the arrest of angiogenesis processes, another molecular mechanism of antiproliferation may be the inhibition of the pro-oxidant process that causes tumour promotion. Growth promoting oxidants and ROS are the major catalysts of the tumour promotion and progression stages(Chahar et al., 2011a).

Among the multitude of anticancer actions developed by flavonoids, the most relevant is to give origin to the apoptosis. Apoptosis is programmed cell death or suicide, caused by extra - or intra - cellular signals.

Dysregulation of apoptosis plays a fundamental role in oncogenesis and flavonoids have shown to induce apoptosis only in cancer cell lines and not on healthy cells. The precise molecular mechanism followed by flavonoids in inducing apoptosis is not well known. Several paths may be involved, including inhibition of DNA topoisomerase I/II activity, decrease of ROS, regulation of heat shock protein expression, modulation of signal pathways, down regulation of nuclear transcription factor kappa B (NF- κ B), activation of endonuclease, and suppression of Mcl-1 protein(Lee et al., 2002).

Among the most known properties of flavonoids there is their antioxidant activity, that derives from the presence of a large number of hydroxyl groups in their structure, and at the same time to a highly conjugated π -electron system, let flavonoids to act like free radical scavengers via hydrogen atom. Furthermore, they can arrest the formation of ROS (reactive oxygen species), such as the hydroxyl radical, through chelation of redox-active transition metal ions. This can have a positive effect on cancer

prevention by the minimization of phenomena such as oxidative damage to DNA. Research on breast cancer cell lines has shown that polyphenols exert strong inhibitory effects due to their antioxidant properties of antagonizing the oxidizing activity of H_2O_2 by scavenging ROS or inhibiting ROS production (McPhail et al., 2003, Damianaki et al., 2000, Martinez-Perez et al., 2014).

Like all flavonoids, flavonols revealed nutritional, biochemical and anticancer properties (Sakagami et al., 2000).

Flavonols as quercetin (Fig.14), 3-hydroxyflavone (Fig.13), 3',4'-dihydroxyflavone, 2',3'-dihydroxyflavone, inhibited the proliferation of normal and tumor cells, as well as *in vitro* angiogenesis, at half-maximal concentrations in the low micromolar range.

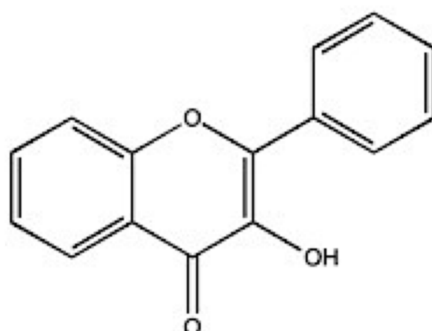


Fig. 13 - 3-Hydroxyflavone structure

It has been clearly demonstrated the antiangiogenic effects of flavonoids and precisely for this reason a series of flavonoid metabolites were tested focusing on their effect on the recombinant basic Fibroblast Growth Factor (bFGF)-stimulated proliferation of Bovine Brain Capillary Endothelial (BBCE) cells. Among all flavonols, 3-hydroxyflavone, inhibits the bFGF-induced proliferation of BBCE cells (Bovine Brain Capillary Endothelial cells) more potently than genistein. The obtained results show that the inhibitory activity of the most potent flavonoids was approximately 2 or 3 fold stronger than that of the isomeric genistein. Another group of flavonoids to which it belongs quercetin put on view inhibitory activity comparable to that of genistein, whereas several flavonoids had minimal or no effect on the proliferation of endothelial cells.

Endothelial cells were not the only target for flavonoids. Flavonoids inhibited the

proliferation of low-density cultures of various normal (HFK2 human fibroblasts and HaCaT human keratinocytes) and tumor (MCF7 human breast cancer and SHEP and WAC2 human neuroblastoma-derived) cells. 3-hydroxyflavone, as well as other flavonols, at low inhibitory concentrations was more potent than the isoflavonoid genistein.

The presence of a hydroxyl function in position 3, as occurs in flavonols, is the cornerstone of changing of the antimitotic activity in an unpredictable manner. Thus, 3-hydroxyflavone, the 3-hydroxylated flavonol derived from flavone, has antiproliferative activity much greater than other flavonols. In addition, 3-hydroxyflavone, which has no catechol functions, has antiproliferative activity comparable to the catecholic metabolites luteolin and 3',4'-dihydroxyflavone(Fotsis et al., 1997).

Quercetin (3,3',4',5,7-pentahydroxyflavone; Fig.14) belongs to an extensive class of polyphenolic flavonoid compounds almost ubiquitous in plants and plant food sources.

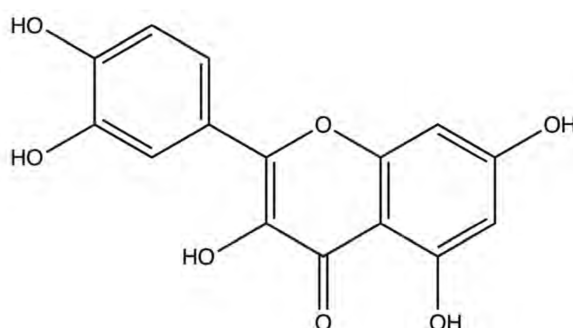


Fig. 14 - Quercetin structure

Quercetin is the major bioflavonoid in the human diet and brings many health benefits, like antioxidant, anti-inflammatory, anti-anaphylaxis, neuroprotection and so on. Quercetin mechanisms of action is still partially unclear, but one of major mechanisms of action seems to be the Tyrosine kinases inhibition.

Tyrosine kinases are a family of proteins located in or near the cell membrane involved in the transduction of growth factor signals to the nucleus. In patients with advanced cancers, intravenous administration of quercetin (dosages 60-1700 mg/m²) led to inhibition of lymphocyte tyrosine kinase at one hour in nine of eleven cases. This inhibition was seen as late as 16 hours post-administration.

In-vitro experiments have confirmed these results, both in non-malignant cells and in rat mammary tumor cells.(Levy et al., 1984, Yokoo and Kitamura, 1997) Tyrosine kinase expression is thought to be involved in oncogenesis via an ability to override normal regulatory growth control.(Boutin, 1994) Drugs targeting tyrosine kinase activity (tyrphostins) are envisioned as possible antitumor agents without the cytotoxic side-effects found with conventional chemotherapy. Quercetin was the first tyrosine kinase inhibiting compound tested in a human phase I trial(Ferry et al., 1996, Klohs et al., 1997).In addition to this, quercetin has radical scavenging potential, preventing cancer induced by oxidative stress (Chahar et al., 2011b). Another quercetin activity is the chemoprotective action through apoptosis and metastasis against tumour cell lines (Gibellini et al., 2011). Moreover, quercetin anticancer action is being done both alone and in combination with drugs. In many cases the use of quercetin increases the anti-cancer effect of conventional drugs (Du et al., 2010). In fact, during *in vitro* study using human MCF-7 cells (Michigan Cancer Foundation-7), quercetin has been reported to inhibit angiogenesis in tamoxifen-resistant cancer in breast cells (Oh et al., 2010).

Polyphenols are the antioxidant compounds most abundant in the nutrition(Scalbert et al., 2005). The term “polyphenols” describes a broad range of chemicals with this general structure. Polyphenols are biochemical compounds that have increasingly significant public health implications. Polyphenols are found in fruits and vegetables, green and black tea, red wine, coffee, nuts, chocolate, and extra virgin olive oil(Manach et al., 2004, Visioli et al., 2011). The main function of polyphenols is the prevention or the treatment at an early stage of degenerative diseases, such as cancer and cardiovascular diseases or the treatment of disease at an early stage.

Ethyl gallate is a natural compound belonging to the polyphenolic family. It is present in fruits and in a variety of plant sources including nuts, such as Indian gooseberry (*Phyllanthus emblica*) Ethyl gallate is the ester of gallic acid and it has been shown to exhibit antibiotic and relaxant functions. One of the most important action carried out by Ethyl gallate is to inhibit the lysozyme's vasodilator effect. Therefore, the use of ethyl gallate is fundamental to treat septic shock. Ethyl gallate (Fig. 15), like some polyphenols, has an anticancer action against many types of cancer

lines, but the mechanism of action is different for each type of cancer. For breast cancer MCF-7 cell lines, the results showed that ethyl gallate is an important bioactive constituent in the chemotherapy through the pathways of marked induction of cell apoptosis (Cui et al., 2015). Against human oral squamous carcinoma cell line KB, ethyl gallate acts as anticancer agent with antiproliferative effect (Mohan et al., 2015).

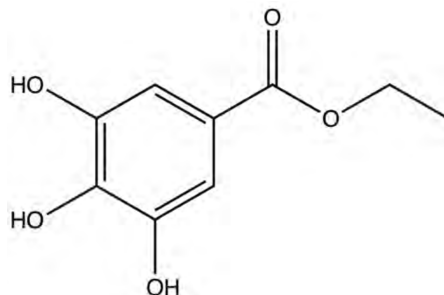


Fig. 15 - Ethylgallate structure

Curcumin (diferuloylmethane) is the principal curcuminoid derived from the *Curcuma longa* plant, commonly known as turmeric. Curcumin (Fig.16) has been used as coloring agent in foods and textiles, and as treatment for a wide variety of food. In addition to this, tumeric is present inside of traditional Indian medicine to cure biliary disorders, anorexia, diabetic wounds, hepatic disorders, rheumatism, and sinusitis and in Ayurvedic medicine because it is nontoxic and has a variety of therapeutic properties including anti-oxidant, anti-inflammatory, neuroprotective and antiseptic activity. Only recently has been demonstrated that curcumin has anti-cancer activities affecting many biological pathways involved in mutagenesis, oncogene expression, cell cycle regulation, apoptosis, tumorigenesis and metastasis (Aggarwal et al., 2003). The anti-oxidant activity of curcumin has been treated findings from Sharma demonstrating that curcumin prevent the oxidation of hemoglobine (Sharma, 1976). Studies carried out in recent years have suggested the mechanism of curcumin antioxidant activity (Sun et al., 2002). Some scientists have underlined the crucial role of the OH phenolic groups suggesting the loss of H-atom from OH (Barclay et al., 2000), others focused their attention on the central CH₂ group in the heptadienone link, arguing that the H-abstraction from

central CH₂ occurs more frequently than H-abstraction from the phenolic group that happens for only ~15% of the reactions (Jovanovic et al., 1999). Many researches show that curcumin is a highly pleiotropic molecule capable of interacting with numerous molecular targets involved in inflammation. The anti-inflammatory effect of curcumin occurs operating on the suppression of the activation of NF- κ B, an inducible transcription factor that regulates the expression of a host of genes involved in inflammation, cellular proliferation and cell survival. The suppression of this eukaryotic transcription factor inhibits anti-inflammatory process but, consequently inhibits carcinogenesis in a number of cancer types, including colorectal, pancreatic, gastric, prostate, hepatic, breast, and oral cancers, and leukemia, and at various stages of carcinogenesis (Juranka, 2009). In fact, studies have supported the concept that cancers frequently originate at the site of chronic inflammation.

Carcinogenesis is a multistep process driven by genetic instability. Continuous exposure to environmental and endogenous genotoxic agents can cause strong DNA damage. The damaged molecules can be spread during cell division giving rise to mutated clones which results in the expansion of premalignant cells. The consequence is an uncontrolled cell proliferation with invasive properties. Curcumin has been classified as a chemopreventive agent with the capability to suppress or retard the carcinogenic process induced by various chemical carcinogens.

The mechanisms by which curcumin exercises its anti-cancer effects consists of targeting many levels of regulation in the processes of cellular growth and apoptosis. In addition to the vertical effects of curcumin on various transcription factors, oncogenes and signalling proteins, its action is divided into different temporal stages of carcinogenesis, from the initial process of tumorigenesis, growth and metastasis.

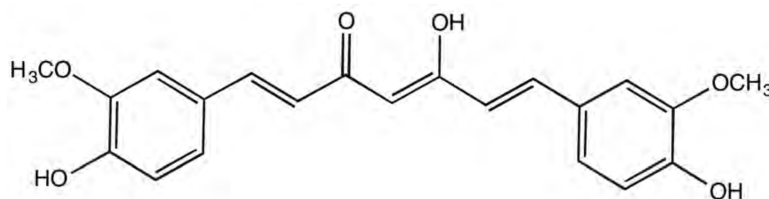


Fig. 16 - Curcumin structure

Caffeine (1,3,7-trimethylxanthine) (Fig. 17) is the most consumed psychoactive

substance in the world. Although caffeine is naturally present in many plant-based foods, the main sources in the Western diet are coffee, tea, cocoa products and cola products.

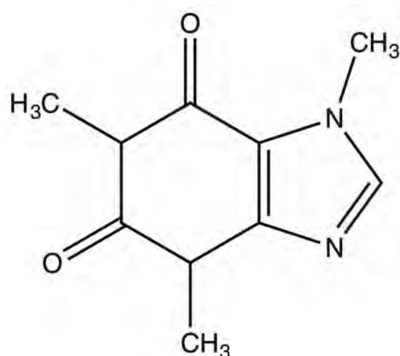


Fig. 17 - Caffeine structure

The most widely known are the disadvantages caused by the assumption of caffeine, but we should also remember the variety of benefits of caffeine. Caffeine affects brain function and researchers have been investigating whether caffeine improves memory and learning retention or hinders it (Klaassen et al., 2013). Studies suggested that assuming optimal amount of coffee protects against brain form degenerative diseases, such as Alzheimer's (Arendash et al., 2009, Cao et al., 2009). Therefore, the Mediterranean diet has lowers risk of Alzheimer's or other neurodegenerative sickness. However, cola beverage or caffeinated soft drinks do not bring any benefit because they contain a large amount of sugars, which increases the risk for hypertension or high blood pressure. In addition, coffee consumption may help to prevent chronic diseases as type 2 diabetes mellitus, Parkinson's disease and liver disease (cirrhosis and hepatocellular carcinoma).

Another study has shown that in U-87MG human glioma cells, the caffeine treatment reduces cancer cell proliferation through cell cycle arrest and apoptosis induction through caspase-3 activation and poly (ADP-ribose) polymerase cleavage. Glioblastomas, also known as glioblastoma multiform (GBM) is an aggressive malignant primary tumour found in the cerebral hemispheres of the brain (Cheng et al., 2016).

Another mechanism of anticancer action of caffeine is based on its capability of interacting with DNA base pairs intercalator.

Basically many antitumor drugs which are used in the clinical trials are DNA interacting agents with polyaromatic core able to intercalate between DNA bases. With longer exposure times (several days), caffeine potentiated the cytotoxic activity of several intercalators (Sabisz and Skladanowski, 2008).

1.4 Aim of the thesis

Both the chemotherapeutic approach and that dietary based on the assumption of natural essences demonstrated their efficiency in the anticancer activity. This finding suggested the topic of this research as the recognition of selected species derived from cis-Pt like dsp^2 square planar complexes substituted with ligands coming from different natural substances and their anticancer properties assessment. For achieving to these goals an approach based on several fronts was carried out:

- Synthesis of cis-Pt like square planar complexes substituted exploiting suitable ligands derived from several classes of natural substances and ancillary ligands like PPh_3 and DMSO.
- Recognition of their chemical-physical properties according to spectroscopic (IR, NMR, XRD) and mass spectrometric investigations coupled to theoretical model calculations.
- Recognition of drug-DNA interactions through the formation of adduct, characterising the ability of the metal-drugs to induce modification of DNA structure. Investigation of the reaction mechanisms and binding character of interactions between synthesized complexes and DNA. This goal was obtained according to chemical-physical assays using Agarose Gel electrophoresis, UV-Vis, Circular Dichroism (CD) and Fluorescence spectroscopy. These were associated to Atomic Force Microscopic (AFM) observations.
- Assessment of anti-cancer activity of newly synthesized compounds as regards of selected cell lines.

2. NOVEL Pt(II) COMPLEXES WITH NATURAL POLYPHENOLS

2.1 Materials and methods

Tap water was de-ionized by ionic exchange resins (Millipore) before use. All chemicals and solvents were purchased from commercial suppliers and used without further purification. $\text{cis-Pt(PPh}_3\text{)}_2\text{Cl}_2$ was synthesized according to literature procedures (Crudden and Alper, 1992).

Melting points were determined with a Büchi Melting Point B-540 apparatus. Elemental analyses were carried out with a Perkin Elmer 2400 CHN Elemental Analyser at the Microanalytical Laboratory of the University of Bari, Department of Pharmacy. IR spectra were recorded on a Bruker-Vector 22 spectrometer. NMR spectra were recorded on BRUKER Advance 400 spectrometer; frequencies are referenced to Me_4Si (^1H and ^{13}C), 85% H_3PO_4 (^{31}P) and H_2PtCl_6 (^{195}Pt). The signal attributions and coupling constant assessment were made on the basis of a multinuclear NMR analysis of each compound including, besides ^1D spectra, ^1H - ^{31}P HMQC, ^1H - ^{195}Pt HMQC, ^1H - ^{13}C HSQC, ^1H - ^{13}C HMBC and ^1H COSY. High resolution mass spectrometry (HR-MS) analyses were performed using a time-of-flight mass spectrometer equipped with an electrospray ion source (Bruker micrOTOF). All analyses were carried out in positive ion mode. The sample solutions were introduced by continuous infusion with the aid of a syringe pump at a flow rate of 180 $\mu\text{L}/\text{min}$. The instrument was operated at end plate offset -500 V and capillary -4500 V. Nebulizer pressure was 1.5 bar (N_2), and the drying gas (N_2) flow was 10 L/min. Capillary exit and skimmer 1 were 120 and 40 V, respectively. The drying gas temperature was set at 220°C. The software used for the simulations is Bruker Daltonics Data Analysis (version 3.3). The ESI-MS and MS/MS analyses were carried out on a triple quadrupole PE Sciex instrument (mod. API 365), equipped with a Turbospray source.

2.2 Synthesis and characterisation

2.2.1 Synthesis of *cis*-[Pt(PPh₃)₂(que)] (que = dianion of quercetin) (hereafter defined complex-1)

A solution of KOH (2.90 mmol, 162.7 mg) in ethanol (7.0 mL) kept at 40°C was added dropwise to a clear yellow solution of *cis*-[PtCl₂(PPh₃)₂] (1.45 mmol, 1.149 g) and quercetin (1.45 mmol, 0.439 g) in CH₂Cl₂ (80 mL) at room temperature under stirring. After overnight stirring at room temperature, the resulting dark orange reaction mixture was filtered (in order to remove KCl) and dried under reduced pressure. The red orange residue was dissolved in CH₂Cl₂ (10 mL) and added of n-hexane (30 mL), which caused the formation of a red-orange precipitate. This solid was filtered after overnight cooling at 4°C, washed with n-hexane (3 x 10 mL) and dried under vacuum. Yield: 1.073 g, 72.3%. Mp=212.5°C (dec). ¹H and ¹³C{¹H} NMR features are reported in tables 2 and 3, respectively, except those belonging to PPh₃ groups. PPh₃ region: ¹H NMR (400 MHz DMSO-d₆, 25 °C) δ = 7.45 ppm (m, 12 H, Hortho), 7.42 ppm (m, 6 H, Hpara), 7.29 ppm (m, 12 H, Hmeta); ¹³C{¹H} NMR (100 MHz DMSO-d₆, 25 °C) δ = 134.7 ppm (s, Cortho), 131.6 (s, Cpara), 128.8 (s, Cipso), 128.6 (s, Cmeta). ³¹P{¹H} NMR (121.5 MHz, DMSO, 25 °C): δ = 9.6 ppm (d, ¹J_{PtP} = 3565 Hz, ²J_{PP} = 23 Hz); 8.3 ppm (d, ¹J_{PtP} = 3600 Hz, ²J_{PP} = 23 Hz). ¹⁹⁵Pt NMR (85.99 MHz, DMSO, 25 °C): δ = -4062 ppm (dd). IR (KBr, cm⁻¹): 3372-3633 (b, m, O-H), 3058 (m, C-H), 1650 (s, C=O), 1617 (s, C=C), 1515 (s, C=C), 1491 (s, C=C), 1426 (s, C=C), 1354 (m, O-H), 1270 (s, C-O-C), 1162 (m, O-H), 1099 (m, C-H), 997 (m, C-O-C), 741 (m, C-H), 530 (m, Pt-O). HRMS (ESI, acetonitrile, positive ion mode) m/z: calcd. for C₅₁H₃₈O₇P₂Pt [M]⁺ 1019.1740; found 1019.1681. HRMS (ESI, acetonitrile, negative ion mode) m/z: calcd. for C₅₁H₃₇O₇P₂Pt [M-H]⁻ 1018.1672; found 1018.1678.

2.2.2 Synthesis of *cis*-[Pt(PPh₃)₂(3-Hfl)]Cl (3-Hfl = monoanion of 3-hydroxyflavone) (hereafter defined complex-2)

A solution of KOH (1.29 mmol, 72.4 mg) in ethanol (4.5 mL) kept at 40°C was added dropwise to a clear yellow solution of *cis*-[PtCl₂(PPh₃)₂] (0.64 mmol, 0.505 g) and 3-hydroxyflavone (1.29 mmol, 0.308 g) in CH₂Cl₂ (40 mL) at room temperature

under stirring. After overnight stirring at room temperature, the reaction mixture was filtered to remove KCl and the resulting orange solution was concentrated to 2 mL under reduced pressure and added of ethanol (10 mL). Addition of pentane (30 mL) caused the precipitation of an orange solid from the orange solution. The solid was washed with pentane (3 x 10 mL) and dried under vacuum. Yield: 0.403 g, 31.5 %. Anal. Calcd. for 2, C₅₁H₃₉O₃P₂PtCl: C, 61.68; H, 3.93. Found: C, 60.69; H, 4.18. Mp=219.1°C (dec). ¹H and ¹³C{¹H} NMR features are reported in tables 2 and 3, respectively, except those belonging to PPh₃ groups. PPh₃ region: ¹H NMR (400 MHz DMSO-d₆, 25 °C) δ = 7.78-7.16 ppm; ¹³C{¹H} NMR (100 MHz DMSO-d₆, 25 °C) δ = 135-128 ppm. ³¹P{¹H} NMR (121.5 MHz, DMSO, 25 °C): δ = 8.60 ppm (d, ¹J_{PtP} = 3667 Hz, ²J_{PP} = 24 Hz); 4.26 ppm (d, ¹J_{PtP} = 3991 Hz, ²J_{PP} = 24 Hz). ¹⁹⁵Pt NMR (85.99 MHz, DMSO, 25 °C): δ = -4077 ppm (dd). IR (nujol mull, cm⁻¹): 3059 (m, C-H), 1613 (s, C=O), 1568 (s, C=C), 1257 (m, C-O-C), 1093 (m, C-H), 996 (m, C-O-C), 755 (s, C-H), 515 (m, Pt-O). HRMS (ESI, acetonitrile, positive ion mode) m/z: calcd. for C₅₁H₃₉O₃P₂Pt [M]⁺ 956.2021; found 956.2021.

2.2.3 Synthesis of *cis*-[Pt(PPh₃)₂(etga)] (etga = dianion of ethyl gallate) (hereafter defined complex-3)

A solution of KOH (1.29 mmol, 72.4 mg) in ethanol (4.5 mL) kept at 40°C was added dropwise to a clear yellow solution of *cis*-[PtCl₂(PPh₃)₂] (0.64 mmol, 0.505 g) and ethyl gallate (1.29 mmol, 0.135 g) in CH₂Cl₂ (40 mL) at room temperature under stirring. After overnight stirring at room temperature, the reaction mixture was filtered to remove KCl and the resulting solution was evaporated under reduced pressure and added of CH₂Cl₂ (10 mL). Addition of pentane (30 mL) caused the precipitation of a yellow solid from the dark yellow solution. The solid was washed with pentane (3 x 10 mL) and dried under vacuum. Yield: 0.376 g, 64.6%. Anal. Calcd. for 3, C₃₉H₄₄O₅P₂Pt: C 58.97, H 4.15; found C 58.21, H 4.22. IR (nujol mull, cm⁻¹): 3496 (s, O-H), 3049 (m, C-H), 2000-1813 (w, aromatic overtone), 1688 (s, C=O), 1591 (s, C=C), 596 (s, Pt-O). ¹H NMR (400 MHz DMSO-d₆, 25 °C) δ = 7.44 ppm (m, 12 H, H_{orto}, PPh₃), 7.43 ppm (m, 6 H, H_{para}, PPh₃), 7.28 ppm (m, 12 H, H_{meta}, PPh₃), 6.56 ppm (s, 1H, 6-H),

6.41 ppm (s, 1H, 2-H), 5.60 ppm (s, 1H, 5-H), 4.10 ppm (q, 2 H, CH₂, ³J_{HH} = 7.1 Hz), 1.20 ppm (t, 3H, CH₃, ³J_{HH} = 7.1 Hz). ¹³C{¹H} NMR (100 MHz DMSO-d₆, 25 °C) δ = 167.2 ppm (s, CO), 163.1 ppm (s, C-3), 154.6 ppm (s, C-4), 144.6 (s, C-5), 134.8 ppm (s, C_{orto}, PPh₃), 131.8 ppm (s, C_{para}, PPh₃), 128.8 ppm (s, C_{meta}, PPh₃), 128.5 ppm (s, C_{ipso}, PPh₃), 116.8 ppm (s, C-1), 109.6 ppm (s, C-2), 104.3 ppm (s, C-6), 59.9 8s, CH₂), 14.8 (s, CH₃). ³¹P{¹H} NMR (121.5 MHz, DMSO, 25 °C): δ = 11.2 ppm (d, ¹J_{PtP} = 3552 Hz, ²J_{PP} = 24 Hz); 6.4 ppm (d, ¹J_{PtP} = 3660 Hz, ²J_{PP} = 24 Hz). ¹⁹⁵Pt NMR (85.99 MHz, DMSO, 25 °C): δ = -4062 ppm (pseudot). HRMS (ESI, acetonitrile) m/z: calcd. for C₄₅H₃₈O₅P₂Pt [M]⁺ 916.1841; found 916.1845; calcd. for C₄₅H₃₈O₅P₂PtNa [M+Na]⁺ 938.1739; found 938.1755; calcd. for C₄₅H₃₈O₅P₂PtK [M+K]⁺ 954.1478; found 954.1496.

2.3 Crystallographic analysis

Compounds 1 and 3 underwent batch crystallization, by slow drift of n-pentane in a solution of tetrahydrofuran containing the respective complex. Suitable crystals for diffraction analysis were obtained after 2 weeks.

The single-crystal X-ray diffraction data for crystals 1 and 3 were collected on a Kappa CCD Nonius area detector diffractometer, in φ and ω scans mode. Reflection data were measured at 293K, using graphite monochromated MoKα radiation (λ = 0.71073 Å). Data were corrected for Lorentz and polarisation effects (Duisenberg et al., 2003), and for absorption (Sheldrick, 2008).

The structures were solved by the Patterson deconvolution procedure of SIR2014 (Burla et al., 2015) and refined by the full-matrix least-squares technique based on F² with SHELXL2014/7 (Sheldrick, 2015), using collected reflections with I ≥ 2σ(I); up to 0.85 Å (crystal 1) and 0.83 Å (crystal 3) resolution; measured reflections with |(I_{obs} - I_{ca})/SigW(I)| > 10 were excluded during the refinement process.

	Compound 1	Compound 3
Chemical formula	<u>C₅₁H₃₈O₇P₂Pt</u>	<u>C₄₅H₃₈O₅P₂Pt</u>
M_r	<u>1019.84</u>	<u>915.78</u>
Crystal system	<u>Orthorhombic</u>	Triclinic
Space group	<u>Pbnc</u>	$P\bar{1}$
a, b, c (Å)	<u>15.372, 22.066, 32.445</u>	<u>11.038, 12.269, 15.242</u>
α, β, γ (°)	90.00, 90.00, 90.00	<u>72.53, 85.16, 89.25</u>
V (Å ³)	<u>11005.3</u>	<u>1961.7</u>
Z	<u>8</u>	<u>2</u>
$F(000)$	<u>4040</u>	<u>912</u>
D_x (Mg m ⁻³)	<u>1.227</u>	<u>1.550</u>
μ (mm ⁻¹)	<u>2.65</u>	<u>3.70</u>
Crystal shape	<u>Prismatic</u>	<u>Rod-shaped</u>
Colour	<u>Red</u>	<u>Pale yellow</u>
Crystal size (mm)	<u>0.25 × 0.25 × 0.10</u>	<u>0.25 × 0.13 × 0.03</u>
No. of measured reflections	<u>42439</u>	<u>20642</u>
No. of independent reflections	<u>42439, 4313</u>	<u>7631</u>
R_{int}	<u>0.110</u>	<u>0.128</u>
θ values (°)	$\theta_{max} = \underline{27.5}, \theta_{min} = \underline{5.0}$	$\theta_{max} = \underline{27.5}, \theta_{min} = \underline{5.0}$
$R[F^2 > 2\sigma(F^2)], wR(F^2), S$	<u>0.052, 0.136, 1.07</u>	<u>0.065, 0.173, 0.89</u>
No. of observed reflections [$I > 2\sigma(I)$]	<u>4010</u>	<u>5327</u>
No. of parameters	<u>373</u>	<u>480</u>
$\Delta\rho_{max}, \Delta\rho_{min}$ (e Å ⁻³)	<u>1.46, -0.58</u>	<u>1.36, -1.55</u>

Table 1- Crystal data, experimental details and refinement parameters for 1 and 3.

The non-hydrogen atoms were refined with anisotropic thermal parameters. The aromatic, methyl and hydroxyl hydrogen atoms were placed in calculated positions, refined with isotropic thermal parameters [$U(H) = 1.2U_{eq}(C)$, $U(H) = 1.5U_{eq}(Me, O)$],

and allowed to ride on their carrier atoms. Crystallographic data collection and refinement parameters are given in Table 1. Molecular graphics were generated by using MERCURY CSD 2.0 (Macrae et al., 2008) and JAV [(Burla et al., 2012). Two atoms separated by less than the sum of their Van der Waals radii have been considered in contact: for intermolecular contacts the two atoms must belong to different symmetry-equivalent molecules, for intramolecular contacts the additional requirement is that the two atoms must be separated by more than 4 bonds.

2.4 Computational details

The Density Functional Theory (DFT) calculations were used to study the hydrolysis reaction for *cis*-[Pt(PMe₃)₂(etga)], *cis*-[Pt(PMe₃)₂(3-Hfl)]⁺ and *cis*-[Pt(PMe₃)₂] using mPW1PW91 functional, which has been previously demonstrated to provide reliable structures and energies for cisplatin and its derivatives (Melchior et al., 2011).

Phenyls in PPh₃ groups have been replaced by methyl (Me) groups for computational convenience. Geometry optimizations were carried out in vacuum and with a 6-31 + G(d) basis set for all atoms except the platinum atom, which was described by the quasi-relativistic Stuttgart–Dresden pseudopotential (SDD) (Andrae et al., 1990). In order to confirm that stationary points were actually minima or transition state geometries, the analytical calculation of second derivatives of the energy and vibrational frequency analysis were carried out. Due to the key role of solvation in influencing thermodynamic and kinetic parameters in metal complex reactivity (Comuzzi et al., 2003, Del Piero et al., 2007, Melchior et al., 2013b, Di Bernardo et al., 2012, Endrizzi et al., 2015, Andrae et al., 1990), environmental effects have been introduced by representing water surrounding the reagents, transition states and the products, as a polarizable continuum (PCM) (Tomasi et al., 2005). Potential energy profiles were estimated from total electronic energies and adding zero point energy (ZPE) and thermal corrections at 298.15 °K. Total electronic energies plus thermal corrections (instead of free energies) are reported in order to uniform our data to those obtained in previous works (Melchior et al., 2011, Alberto et al., 2009, Lucas et al., 2009). DFT calculations were performed

with the Gaussian 09 program (Gaussian 09, Revision D.01, M. J. Frisch et al. Gaussian, Inc., Wallingford CT, 2016).

2.5 Cytotoxicity Assays and Combination Index (CI) Calculations

Cell Lines: U87 cells from human glioblastoma and MCF-7 cells from breast carcinoma were grown in Dulbecco's modified Eagle's medium (DMEM, Euroclone).

The complete culture medium was supplemented with 10% heat-inactivated fetal bovine serum (Euroclone), streptomycin (0.2 mg mL^{-1}), and penicillin (200 IU mL^{-1}). For U87 cells the complete medium was added with

1% of non-essential amino acids. Cells were incubated at 37°C and 5% CO_2 in a humidified incubator. The cytotoxicity assays were performed as previously described (Margiotto et al., 2014). Briefly, U87 cells from human glioblastoma and MCF-7 cells from breast carcinoma were exposed to tested compounds for a period of 72 h. All tested compounds were dissolved in dmsO prior to their dilution with complete cell culture medium to the predetermined experimental concentrations (seven concentrations ranging from 0.10 to $200 \mu\text{M}$). In all experiments the percentage of dmsO never exceeded 1%. Cytotoxicity (IC_{50}) values for the tested compounds were determined using the metabolic reduction of the soluble 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) by a mitochondrial enzyme of cultured cells into an insoluble colored formazan product. In particular, cells were seeded at a density of about 5,000 cells/well in a 96 well plate and incubated at 37°C for 72 h in the presence and absence of the tested compounds. Untreated cells were used as positive control. Then, $10 \mu\text{L}$ of 5 mg mL^{-1} MTT solution was added to each well and the plates were incubated for additional 3 h at 37°C . Next, cells were lysed by the addition of $150 \mu\text{L}$ of 50% (v/v) dmsO and 50% (v/v) ethanol solution, and the absorbance of each individual well was measured using a microplate reader at 570 nm (Wallac Victor3, 1420 Multilabel Counter, Perkin- Elmer). The reported values are the average of triplicate measurements performed in three separate experiments.

The cytotoxic effects measured for tested compounds were further analyzed according to the Chou and Talalay method on CompuSyn software (ComboSyn, Inc.,

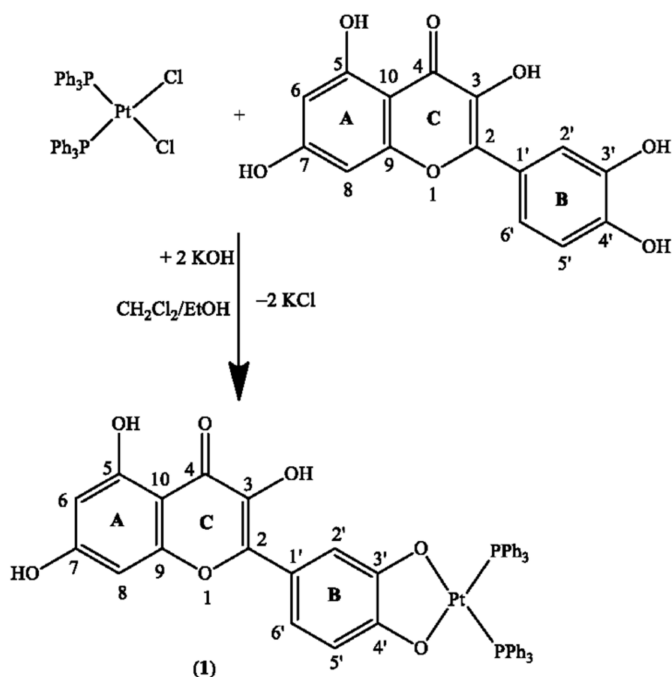
Paramus, NJ, USA). The combination index (CI) was calculated according to the equation of Chou-Talalay for two drugs: $CI = (D)1/(Dx)1 + (D)2/(Dx)2$, where (Dx)1 and (Dx)2 are the doses (concentrations) of Drug1 alone and Drug2 alone, respectively, that inhibit x% of cell viability. (D)1 and (D)2 are the doses of Drug1 and Drug2, respectively, used in combination (D)1 + (D)2, able to inhibit x% of cell viability. Complexes 2 and 3 were considered at a fixed-dose-ratio combination (1:1) of their building blocks [(D)1 = (D)2]. CI = 1 indicates an additive effect in the absence of synergism or antagonism; CI < 1 indicates synergism; CI > 1 indicates antagonism.

3. RESULTS AND DISCUSSION

3.1 Synthesis and characterisation Pt(II) complexes

The synthesis of all platinum phosphynic complexes has been carried out according to a common route (Boéré and Willis, 1985) starting from *cis*-[PtCl₂(PPh₃)₂] in presence of different ligands. Regarding the synthesis of the precursor, the problem was to synthesize a complex of Pt (II) that had an ancillary ligand to the phosphorus. The phosphorus ligand has been identified in the triphenylphosphine, a solid phosphine easily to isolate and purify. The coordination of an alcoholic oxygen atom (hard donor) to platinum (soft acid) is thermodynamically disfavored and occurs exclusively when it is supported by an additional driving force, like for example the precipitation of an insoluble side product. In fact, in all cases insoluble KCl was recovered at the end of the reaction.

As shown in the reaction scheme, the complex 1, has been prepared by slowly adding a solution of ethanolic KOH to a solution containing an equimolar amount of *cis*-[PtCl₂(PPh₃)₂] and quercetin (scheme 1). In this reaction way, quercetin is deprotonated as anionic bidentate ligand and PPh₃ is neutral ligand.



Scheme 1 - synthesis of *cis*-[Pt(PPh₃)₂(que)] (que = dianion of quercetin) (1)

If we tried to obtain the same product by adding an ethanolic solution of the pre-formed salt of quercetin to a solution of *cis*-[PtCl₂(PPh₃)₂], the reaction was not selective at all and obtaining platinum byproduct compounds that were detected in the relevant ³¹P{¹H} NMR spectrum. Indeed, the ³¹P{¹H} NMR spectrum of the reaction mixture reported in scheme 1 showed that the singlet belonging to *cis*-[PtCl₂(PPh₃)₂] (³¹P NMR: 13.8 ppm, ¹J_{PtP} = 3677 Hz) almost disappeared after 14 hour stirring, while two major doublets (with 1:1 integration areas) with satellites mutually coupled (²J_{PP} = 23 Hz) formed. The ³¹P{¹H} NMR spectrum of the isolated product dissolved in DMSO-d₆ reported the above mentioned doublets belonging to complex 1, falling the first one at 9.6 ppm (¹J_{PtP} = 3565 Hz) and the second one at 8.3 ppm (¹J_{PtP} = 3600 Hz).

Their coupling constant $^1J_{\text{PtP}}$ values are typical of a PPh_3 group trans to an oxygen atom (Arendse et al., 1999). The $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of the complex reported a significant downfield shifting of the 3'-C and 4'-C signals compared to those of the free ligand (Table 3), due to coordination to platinum center. In fact, they changed from $\delta = 145.0$ and 147.6 ppm to $\delta = 166.6$ and 163.4 ppm, respectively, on passing from free to platinum bound quercetin, remaining nearly unchanged the chemical shifts of the carbon signals belonging to A and C rings, far away from the coordination site. Their NMR attributions were verified also by ^1H - ^{13}C HMBC and ^1H - ^{13}C HSQC two dimensional NMR experiments (Supporting Information), useful also for assessing B-ring proton signals falling under triphenylphosphane ^1H NMR region ($7.2 \div 7.5$ ppm). On the base of the latter 2D NMR analyses and ^1H COSY experiment, we assigned to the 2'-H proton the doublet at $\delta = 7.00$ ppm ($^4J_{\text{H2}'\text{H6}'} = 2.1$ Hz), to the 5'-H proton the doublet at $\delta = 6.19$ ppm ($^3J_{\text{H5}'\text{H6}'} = 8.4$ Hz) and to the 6'-H proton the chemical shift value of 7.23 ppm. The ^1H NMR spectrum in DMSO-d_6 shows (beside the above mentioned proton signals) also two doublets at $\delta = 6.12$ ppm and at $\delta = 6.32$ ppm, for 6-H and 8-H protons, respectively ($^4J_{\text{H6H8}} = 2.0$ Hz), while the sharp singlets at $\delta = 12.64$ and 8.81 ppm are assigned to 5-OH and 3-OH protons, respectively. The 7-OH proton signal is a broad peak falling at 10.63 ppm. All of the hydroxyl protons give a cross-peak with the water proton signal in the relevant NOESY-EXSY spectrum (Figure 18).

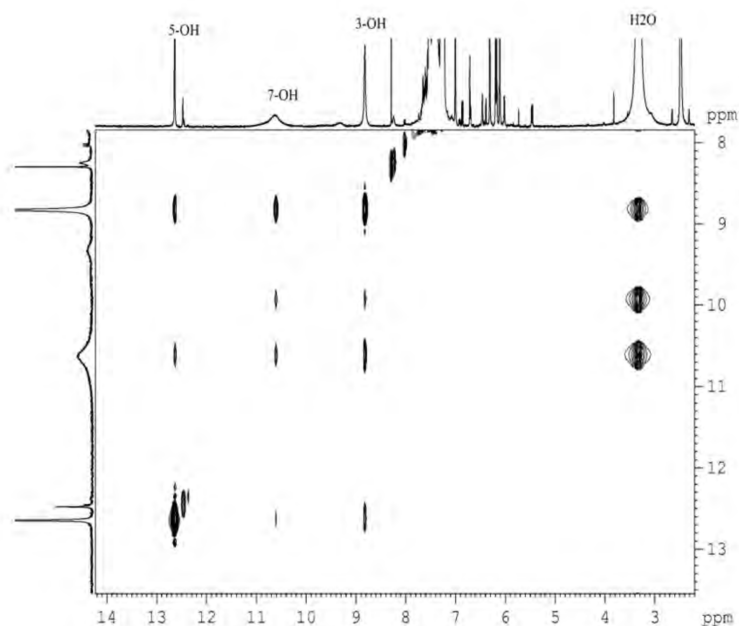


Fig. 18- ^1H NOESY-EXSY spectrum of 1 in DMSO-d_6

The chemical shifts of the proton signals belonging to A and C rings are very similar to the corresponding ones found in ^1H NMR spectrum of free quercetin (Kim et al., 1999) (Table 2). The ^{195}Pt NMR spectrum consists of a doublet of doublets at -4062 ppm, whose chemical shift is typical for Pt(II) phosphane complexes (Arendse et al., 1999). The IR spectrum shows broad signals at $3372\text{--}3633\text{ cm}^{-1}$, that can be assigned to the hydroxyl groups 7-OH, 5-OH and 3-OH, and the typical bands ascribed to the skeletal $\text{C}=\text{C}$ (1650 and 1426 cm^{-1}) and C-O-C (1270 cm^{-1}) stretching vibrations. All the bands assigned to coordinated deprotonated quercetin are shifted in comparison to those assigned to free quercetin (Nakamoto, 2008b, Nakamoto, 2008a).

	Complex 1	Quercetin	Complex 2	3-Hydroxyflavone
C ²	128.9	135.6	173.6	139.1
C ³	149.4	146.8	182.6	145.2
C ⁴	175.5	175.7	207.0	173.1
C ⁵	161	160.6	125.5	124.8
C ⁶	98.5	98.1	128.9	124.5
C ⁷	163.8	163.8	134.3	133.7
C ⁸	93.6	93.3	118.3	118.4
C ⁹	156.3	156.1	155.2	154.6
C ¹⁰	103.2	103	121.8	121.2
C ^{1'}	118.4	121.9	131.8	131.3
C ^{2'}	114.1	115.1	128.2	127.6
C ^{3'}	166.6	147.6	128.4	128.6
C ^{4'}	163.4	145.0	129.1	129.6
C ^{5'}	115.1	115.5	128.4	128.6
C ^{6'}	118.1	119.9	128.2	127.6

Table 2- ¹³C NMR spectral data of 1, 2, quercetin and 3-hydroxyflavone (dmso-d₆, 298 K). Bold typeface refers to largest changes in chemical shifts observed comparing free with metal coordinated ligand.

In particular, the C=O frequency (1661 cm⁻¹ in free quercetin) is only slightly shifted in complex 1 (1650 cm⁻¹), suggesting that the coordination of quercetin does not involve the oxygen atom of the carbonyl group. The shift is a result of ring deformation caused by platinum bonding to quercetin. The structure proposed for 1 was confirmed also by HR ESI-MS analysis. The ESI-MS spectrogram in the negative scale of a diluted acetonitrile solution of 1 showed the peak at m/z 1018.1678, whose isotope pattern is perfectly superimposable on that calculated for the anion [C₅₁H₃₇O₇P₂Pt]⁻ (corresponding to [1-H]⁻), obtained by deprotonation of a hydroxyl group under analysis conditions.

Some side products were also detected in the ³¹P{¹H} NMR spectrum of the reaction mixture as doublets or singlet in really small amount. Their formation is probably due to the fact that quercetin has different coordination sites (de Souza et al., 2003). Generally in metal(II) complexes the quercetin acts as a bidentate ligand, involving its C=O group and the 3-OH oxygen atom (Zhou et al., 2001), even in the case of Pd(II) (Malešev and Kuntić, 2007). Indeed, it has been shown that the low pK_a (7.03) of the 4' -OH moiety in the B-ring results in easy deprotonation, facilitated by charge delocalization of the anion. Also the 7-OH in the A-ring is susceptible to deprotonation at physiological pH (Lemańska et al., 2001). This implies that these two sites are also

capable of forming a metal complex. Another study on the absorption spectra of platinum complexes of quercetin suggests that the metal(II) center binds with the 3' -OH and 4' -OH sites of the B-ring of quercetin according to the structure of complex 1.

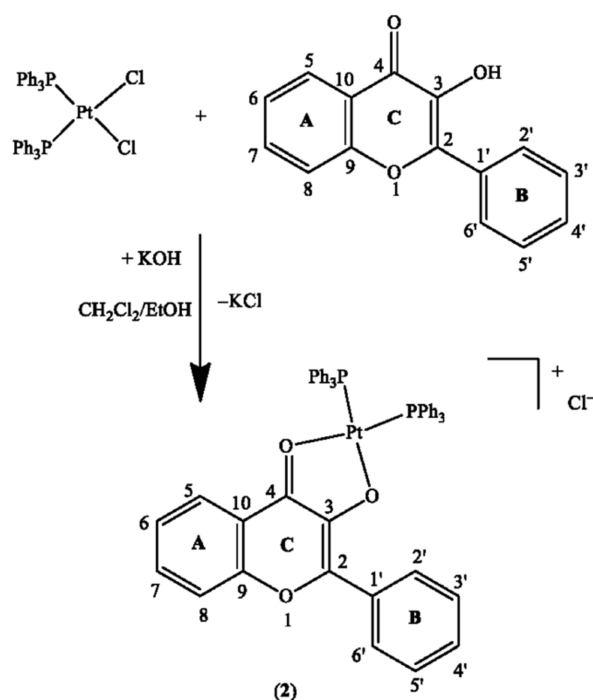
	3-H	5-H	6-H	7-H	8-H	2'-H	3'-H	4'-H	5'-H	6'-H
Complex 1	8.81	12.64	6.12 (<i>d</i> , $^4J_{H6,H8} =$ 2.1 Hz)	10.63	6.32 (<i>d</i> , $^4J_{H6,H8} =$ 2.1 Hz)	7.00 (<i>d</i> , $^4J_{H2',H6'} =$ 2.0 Hz)	-	-	6.19 (<i>d</i> , $^3J_{H5',H6'} =$ 8.4 Hz)	7.25 (<i>m</i>)
quercetin	9.60	12.42	6.18 (<i>d</i> , $^4J_{H6,H8} =$ 2.0 Hz)	10.76	6.40 (<i>d</i> , $^4J_{H6,H8} =$ 2.0 Hz)	7.67 (<i>d</i> , $^4J_{H2',H6'} =$ 2.2 Hz)	9.31	9.39	6.89 (<i>d</i> , $^3J_{H5',H6'} =$ 8.3 Hz)	7.53 (<i>dd</i> , 8.3 Hz, 2.2 Hz)
Complex 2	-	8.10 (<i>d</i> , $^3J_{H5,H6} =$ 8.4 Hz)	7.51 (<i>m</i>)	7.87 (<i>m</i>)	7.98 (<i>d</i> , $^3J_{H7,H8} =$ 7.7 Hz)	8.24 (<i>d</i> , $^3J_{H2',H3'} =$ 7.7 Hz)	7.74 (<i>m</i>)	7.55 (<i>m</i>)	7.74 (<i>m</i>)	8.24 (<i>d</i> , $^3J_{H5',H6'} =$ 7.7 Hz)
3-hydroxyflavon	9.68	8.16 (<i>d</i> , $^3J_{H5,H6} =$ 8.1 Hz)	7.50 (<i>m</i>)	7.82 (<i>m</i>)	7.78 (<i>d</i> , $^3J_{H7,H8} =$ 8.0 Hz)	8.26 (<i>d</i> , $^3J_{H2',H3'} =$ 7.9 Hz)	7.63 (<i>m</i>)	7.54 (<i>m</i>)	7.63 (<i>m</i>)	8.26 (<i>d</i> , $^3J_{H5',H6'} =$ 7.9 Hz)

Table 3- ^1H NMR spectral data of 1, 2, quercetin and 3-hydroxyflavone (dms o - d_6 , 298 K).

When 3-hydroxyflavone instead of quercetin reacted with the platinum precursor under alkaline conditions, the reacting groups were selectively 4-O and 3-O, and the cationic complex 2 formed (scheme 2), although the chloride ion is not a weakly coordinating anion.

In the $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of 2 in DMSO- d_6 , two doublets ($^2J_{\text{PP}} = 24$ Hz) with satellites at $\delta = 8.60$ and 4.26 ppm with $^1J_{\text{PtP}} = 3667$ and 3991 Hz, respectively, were observed. The ^{195}Pt NMR spectrum consists of a doublet of doublets at $\delta = -4077$ ppm, whose chemical shift is typical for Pt(II) phosphane complexes (Nakamoto, 2008b, Nakamoto, 2008a).

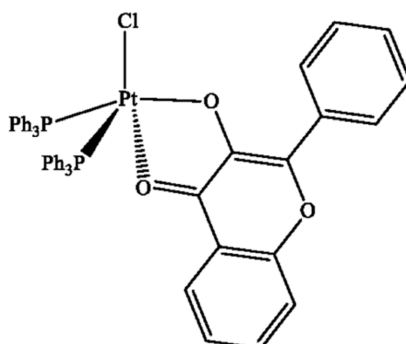
As it happened for complex 1, also the $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of complex 2 showed a significant down-shield shifting of the carbon signals close to the coordination site, on passing from free (4-C, 3-C and 2-C $\delta = 173.1$, 145.2 and 139.1 ppm, respectively) to metal bound (4-C, 3-C and 2-C $\delta = 207.2$; 173.4 and 154.0 ppm, respectively) ligand (Table 3). On the contrary, the ^{13}C chemical shift values belonging to nuclei of A- and B-rings were very similar to the ones reported for free 3-hydroxyflavone (Burns et al., 2007).



Scheme 2 - synthesis of $\text{cis-}[\text{Pt}(\text{PPh}_3)_2(3\text{-Hfl})]\text{Cl}$ (3-Hfl = monoanion of 3-hydroxyflavone) (2)

The ^1H NMR spectrum consists of a doublet at $\delta = 8.24$ ppm for the equivalent 2'-H and 6'-H protons with $^3J_{\text{H}2',\text{H}3'} = 7.7$ Hz, a multiplet at $\delta = 7.74$ ppm for the equivalent 3'-H and 5'-H protons and a triplet at $\delta = 7.55$ ppm for the 4'-H proton with $^3J_{\text{H}4',\text{H}3'} = 7.0$ Hz, besides all the expected signals belonging to the protons of the A- and triphenylphosphane rings (Table 2). The IR spectrum shows no signal in the O-H stretching region and a strong sharp peak at 1613 cm^{-1} , which can be assigned to the coordinated carbonyl group. Its 50 cm^{-1} ca. displacement with respect to the free 3-hydroxyflavone (de Souza et al., 2003, Burla et al., 2012) suggests that the metal coordination involves oxygen atoms of the carbonyl and 3-OH groups. The structure proposed for 2 was confirmed also by HR ESI-MS analysis. The ESI-MS spectrogram in the positive scale of a diluted acetonitrile solution of 2 showed the peak at m/z 956.2021, whose isotope pattern is perfectly superimposable on that calculated for the

cation $[C_{51}H_{39}O_3P_2Pt]^+$ (corresponding to *cis*- $[Pt(PPh_3)_2(3-Hfl)]^+$), obtained by loss of the chloride anion.



Scheme 3- alternative penta-coordinated structure proposed for 2.

The cationic structure proposed for 2 is very unusual, because the chlorine is not coordinated to the metal center. This ionic structure was confirmed both by XRD analysis (see below) and by NMR experiments carried out on a DMSO- d_6 solution of 2 reacted with excess $AgNO_3$. The addition of $AgNO_3$ to the yellow solution of 2 caused the precipitation of a white solid ($AgCl$), but the chemical shifts of the ^{31}P signals of 2 did not change after the displacement of the chloride for a non-coordinating anion (NO_3^-), ruling out the possibility for 2 of a neutral penta-coordinated structure (scheme 3), similar to those proposed for some dppm and dppa platinum(II) complexes bearing Cl.

To gain insights into the stability of complex 2 under physiological conditions, a 200 μM solution of 2 in cell culture media kept in the dark at 37 °C was time monitored by means of $^{31}P\{^1H\}$ (figure 19a) and 1H NMR. We found out that compound 2 completely lost its polyphenol ligand after 3 h under physiological conditions. On the basis of the all over reported findings, we could assume that 3 h might be a reasonable period of time for complex 2 to pass the cell's lipid bilayer (compound 2 bears lipophilic thiophenylphosphane ligands).

The photo degradation of 3-hydroxyflavone in polar solvents in the absence of metal ions has been extensively described in literature (Matsuura et al., 1973, Yokoe et al., 1981, Tommasini et al., 2004, Sisa et al., 2010).

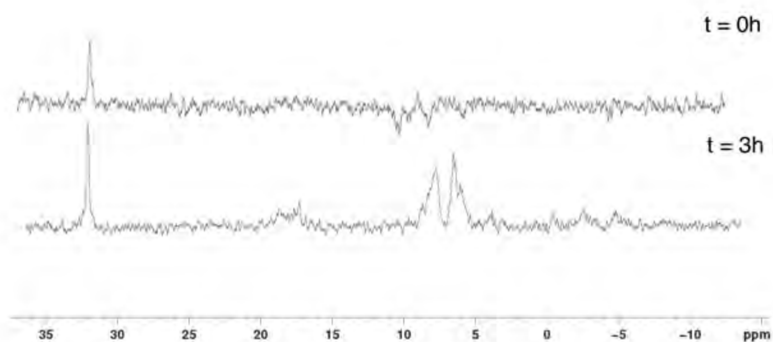
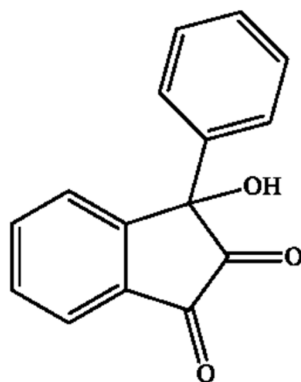


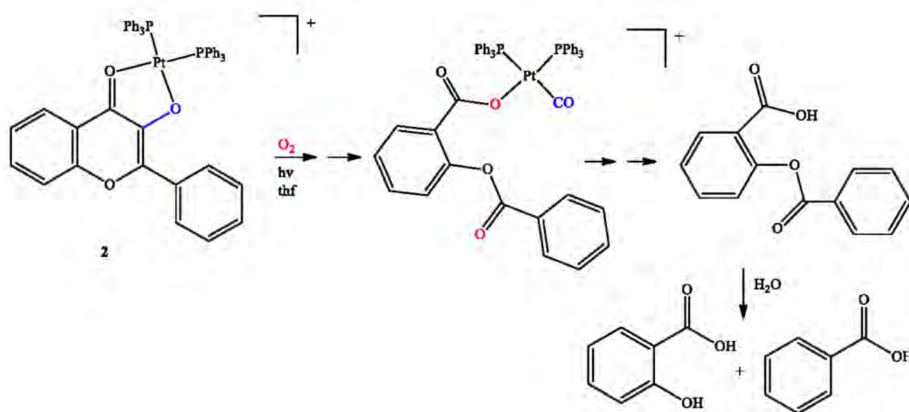
Fig. 19a- ^{31}P NMR spectra of compound **2** (DMSO- d_6) under the following conditions: (t=0) immediately after solution preparation and (t=3h) after 3 hours at 37°C under physiological conditions.

Although the nature of the photochemical processes occurring is not completely understood, the main photoproduct is reported to be 3-hydroxy-3-phenyl-1,2-indandione (Scheme 4), even under aerobic conditions. A possible explanation for the presence of salicylic acid in the elemental cell of compound **2** analysed by XRD technique can be found in ref. (Saraf et al., 2014), where it is demonstrated that the anion of 3-hydroxyflavone coordinated to Ru(II) undergoes photochemistry degradation under aerobic conditions with loss of CO.



Scheme 4- main photoproduct of the degradation of 3-hydroxyflavone in the absence of metal ions.

Also Berreau *et al.* (Grubel et al., 2013, Grubel et al., 2012) observed photoinduced oxidative degradation of some divalent metal flavonolato complexes, as the primary reaction, with formation of *O*-benzoyl salicylate. In this context, in the case of our Pt(II) complex (**2**) the salicylic acid would be the result of the aerobic degradation of the coordinated 3-idrossiflavonate, followed by hydrolysis in the presence of water, inevitably present in the thf solution, in which crystals suitable for XRD analyses grew up (scheme 5).



Scheme 5- plausible pathway for the aerobic degradation of **2**.

The observation of salicylic acid formation confirms what has been previously hypothesized: the photo-degradation of 3-hydroxyflavone in solution follows a different pathway if a metal(II) complex is present. Moreover, these findings are in agreement with the additional peaks found in the carboxylic region of the ^1H NMR spectrum of compound **2** let standing in DMSO-d_6 solution for nine days (figure 19b). In particular, the ^1H singlet at $\delta = 12.38$ ppm could belong to the carboxylic proton depside.

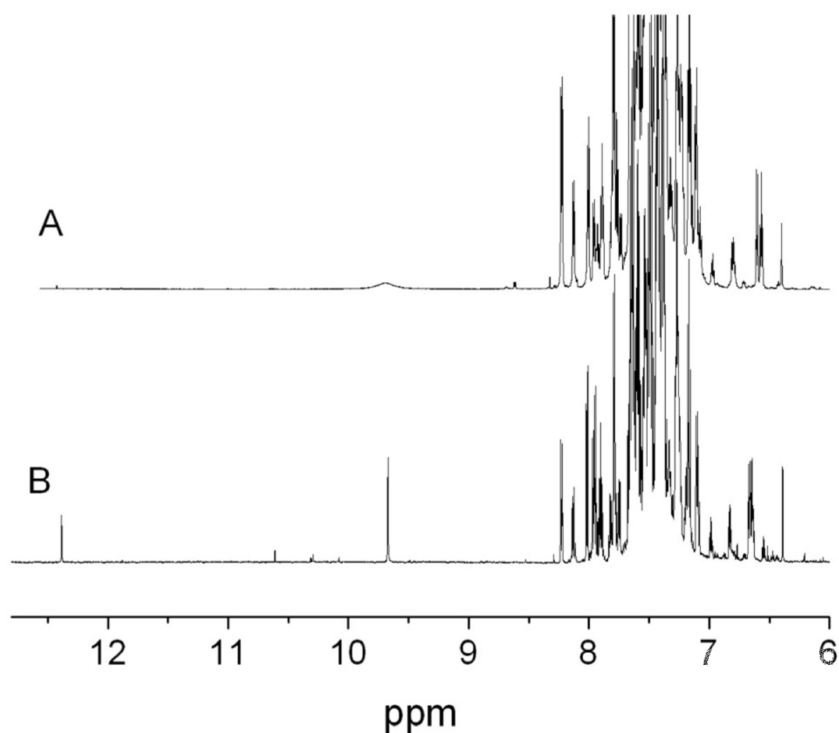
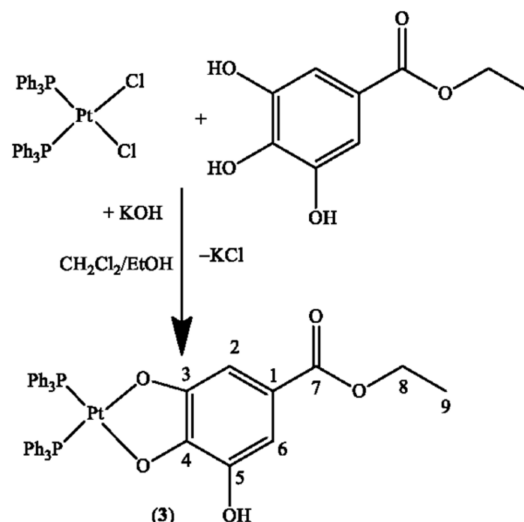


Fig. 19b- ^1H NMR spectra of compound **2** (DMSO-d_6) under the following conditions: (A) immediately after solution preparation; (B) after 9 days.

The presence of the additional fragments observed in the elemental cell of **2**, such as p-xylene and n-butane, might be due to retro Diels-Alder reactions, followed by C-C bond formation processes, whose mechanism is still deeply obscure (Lewars and March, 2007). Further studies are necessary aiming at explaining the obtained results. Overall, the photoreactivity of **2** may represent an approach toward altering the biological chemistry of this compound.

Complex 3 was synthesized by reacting ethyl gallate with the platinum precursor $\text{cis-}[\text{PtCl}_2(\text{PPh}_3)_2]$ under alkaline conditions (scheme 6).



Scheme 6- synthesis of $\text{cis-Pt(PPh}_3)_2(\text{etga})$ (etga = dianion of ethyl gallate) (3)

The $^3\text{P}\{^1\text{H}\}$ NMR spectrum of 3 in DMSO-d_6 shows two doublets with satellites at $\delta = 11.2$ and 6.4 ppm with $^1J_{\text{PtP}} = 3552$ and 3660 Hz, according to its ^{195}Pt NMR spectrum reporting a pseudo-triplet at $\delta = -4400$ ppm. The ^1H NMR spectrum consists of the triphenyl phosphane proton signals, a quartet at $\delta = 4.10$ ($^3J_{\text{H}_2',\text{H}_3'} = 7.1$ Hz) and a triplet at $\delta = 1.20$ ppm, belonging to $-\text{CH}_2$ and $-\text{CH}_3$ of the ethyl chain, and three singlets at $\delta = 6.56$ (6-H), 6.41 (2-H) and 5.60 ppm, integrated 1:1:1. The latter was assigned to the proton of the 5-OH moiety, because the ^1H - ^{13}C HSQC spectrum of 3 does not report any cross peak for this signal, but the relevant ^1H - ^{13}C HMBC spectrum shows that it couples with 5-C ($\delta = 144.6$ ppm), 6-C ($\delta = 104.3$ ppm) and 4-C ($\delta = 154.6$ ppm). The pairs of carbon nuclei 5-C and 3-C belonging to the phenyl ring, magnetically and chemically equivalent in the free ligand ($\delta = 145.6$ ppm), became very different when the complex formed and a significant downfield shift was observed

for 3-C nucleus ($\delta = 163.1$ ppm), which couples with 2-H, but it does not with the far away 6-H. The assessment of 4-C signal was given on the base of ^1H - ^{13}C HMBC cross peaks, showing that 4-C nucleus couples with both 2-H and 6-H.

The IR spectrum shows a strong sharp peak at 3496 cm^{-1} , which can be assigned to the free O-H bond stretching.

3.2 Crystallographic analysis

Compound 1: 3'-O and 4'-O form contacts with the hydrogens of the nearest phenyl rings of PPh_3 , 3-OH is in contact with 2'-C, and an H-bond is formed between 4-O and 5-O.

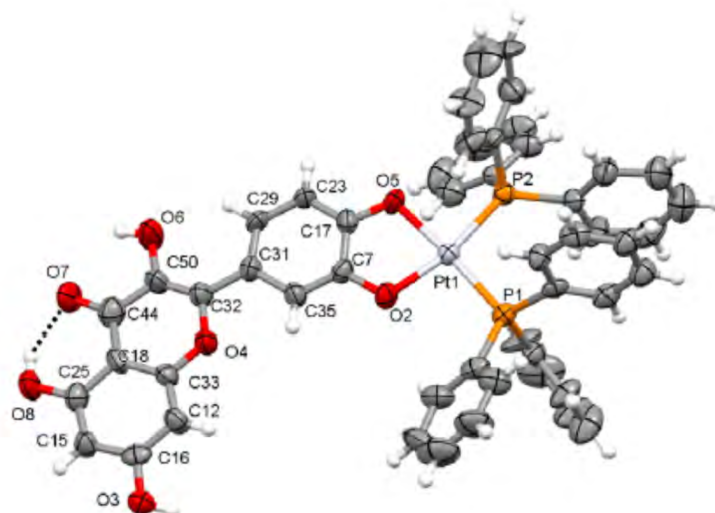


Fig. 20- Crystal structure of compound 1. Asymmetric unit with atoms shown by their thermal ellipsoids at 50% probability and short contacts highlighted in cyan (left), and crystal packing (right).

Compound **3**: 3-O always forms contacts with the hydrogen of one PPh_3 . In one of the two molecules of crystal **3a** this occurs also for 4-O, while in the other molecule two hydrogens of different PPh_3 rings are in contact with 3-O.

In complex 1 the Pt atom has a distorted square-planar coordination geometry imposed by the O,O' chelation of quercetin. In the molecular unit of 1 the Pt atom is displaced from the mean plane passing through the basal atoms P1, P2, O5 and O2 by $3.0\text{ \AA}$. The Pt-P ($2.25\text{ \AA}$) and Pt-O ($2.06\text{ \AA}$) distances are similar to those reported

for other triarylphosphane platinum(II) complexes containing chelating phenoxides (Bergamini et al., 2008). Table 4 reports selected bond lengths and angles for 1.

Bond lengths			
Pt1-P2	2.253(2)	Pt1-O2	2.059(6)
Pt1-P1	2.249(3)	C17-O5	1.338(10)
Pt1-O5	2.055(6)	C7-O2	1.386(10)
Angles			
P1-Pt1-P2	98.67(9)	P2-Pt1-O2	167.27(19)
P2-Pt1-O5	84.78(18)	P1-Pt1-O2	93.48(18)
P1-Pt1-O5	176.51(18)	Pt1-O2-C7	108.0(57)
P2-O3'-C17	110.6(6)	O5-Pt1-O2	83.0(2)

Table 4- Selected bond lengths [Å] and angles [°] for complex 1.

Compound 1 is the only one having orthorhombic symmetry, and has the larger crystal cell (cell volume 11005.3 Å³, volume per atom 22.6 Å³). The packing in the crystal cell is dominated by the interactions among the PPh₃ rings, which arrange them in planes perpendicular to the longer c axis. The dianion of quercetin are instead arranged parallel to the c axis, spacing out the stacked PPh₃ ligands (Fig.20).

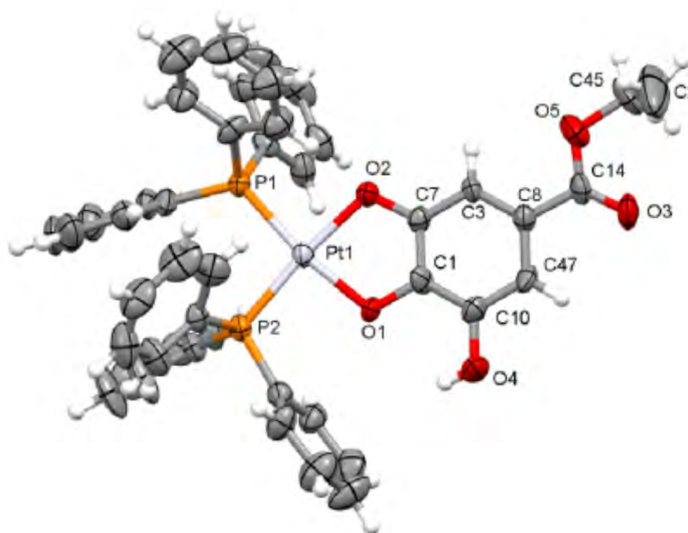


Fig. 21- Crystal structure of compound 3. Asymmetric unit with atoms shown by their thermal ellipsoids at 50% probability and short contacts highlighted in cyan (left), and crystal packing (right).

Single crystals of 2 obtained by slow diffusion of *n*-pentane into the thf reaction solution were submitted to XRD analysis, but were not of sufficient quality to warrant a complete X-ray structure determination.

Slow diffusion of *n*-pentane into a thf solution of 3 afforded crystals suitable for XRD analysis. An ORTEP(Farrugia, 2012) drawing of 3 is shown in Fig. 21, while relevant bond lengths and angles are collected in Table 5. The Pt atom adopts an almost square-planar coordination geometry imposed by the O,O' chelation of the dianion of ethyl gallate, as well as by the crystal packing on the PPh3 ligands.

Bond lengths			
Pt1-P1	2.261(3)	Pt1-O1	2.028(7)
Pt1-P2	2.260(3)	C7-O2	1.346(13)
Pt1-O2	2.053(7)	C1-O1	1.357(13)
Angles			
P1-Pt1-P2	99.17(10)	P1-Pt1-O1	169.9(2)
P1-Pt1-O2	87.6(2)	P2-Pt1-O1	90.4(2)
P2-Pt1-O2	172.3(2)	Pt1-O2-C7	109.6(6)
Pt1-O1-C1	110.3(6)	O1-Pt1-O2	83.1(3)

Table 5- Selected bond lengths [Å] and angles [°] for complex 3

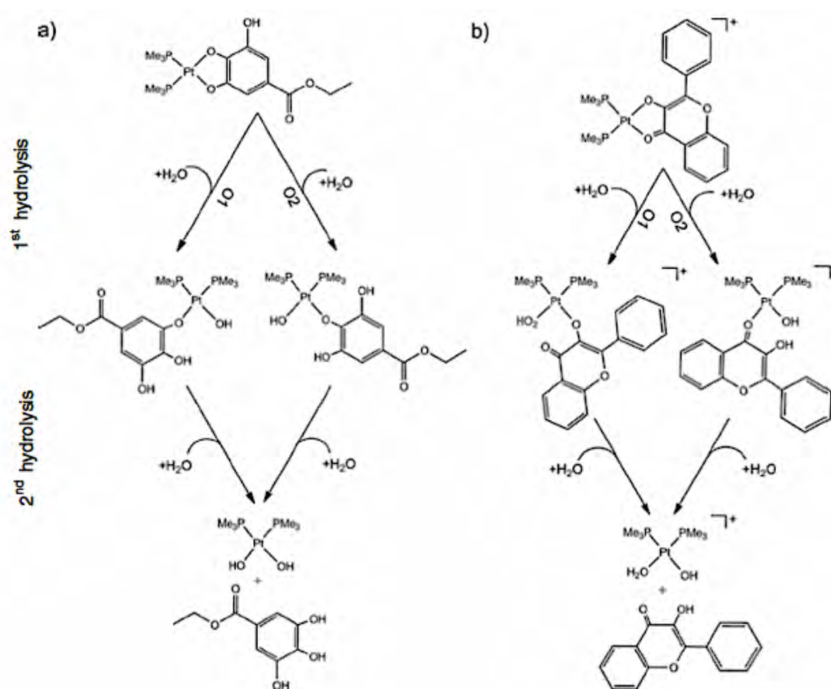
In the elementary cell, ethyl gallate moieties are all aligned in the (113) plane. Deviation from planarity of the PtP2O2 plane was observed, as measured by the improper dihedral angle value (3.2°), being the plane of the two P atoms rotated with respect to the plane of the ethyl gallate molecule.

3.3 DFT calculations

On the basis of a large number of experimental data, it is widely accepted that the hydrolysis of platinum(II) anticancer complexes is a bimolecular nucleophilic substitution (SN2) which is the ratedetermining step of the process leading to the formation of the adduct with DNA nucleobases(Jamieson and Lippard, 1999). In particular, for cis-platin the mono-aquo species is believed to be the key player, since it coordinates to the N7 atom of a guanine base in DNA producing a monofunctional adduct (Jamieson and Lippard, 1999).Once coordinated, the second chloride is released and platinum

binds a second base producing the crosslink, which distorts DNA (Jamieson and Lipard, 1999). Due to their importance, the hydrolysis reactions of platinum complexes have been studied by means of a variety of theoretical methods in the last decade (Melchior et al., 2011, Melchior et al., 2013a, Pavelka et al., 2007, Melchior et al., 2015, Lucas et al., 2009). In our case, the activation energies for the hydrolysis reactions of complexes 2 and 3 have been calculated because they could be correlated to their potential synergic cytotoxicity, since polyphenols are released in solution.

The first hydrolysis leads to the ring-opening process, with the 3-Hfl and etga derivatives remaining mono-coordinated to the metal ion centre. The second hydrolysis (second step) leads to the complete release of 3-Hfl and etga (Scheme 7). For comparison purposes, we considered also the hydrolysis of *cis*-[Pt(PMe₃)₂(Cl)₂] and that of cisplatin (Melchior et al., 2011).



Scheme 7- Hydrolysis reactions for: a) *cis*-[Pt(PMe₃)₂(etga)] and b) *cis*-Pt[(PMe₃)₂(3-Hfl)]⁺.

3.3.1 Hydrolysis reaction for *cis*-[Pt(PMe₃)₂(etga)]

The theoretical geometry of the model complex $\text{cis-[Pt(PMe}_3)_2(\text{etga})]$ is in excellent agreement with the experimental ones found for complex 3 (Table 5), thus confirming the reliability of the computational protocol used. In Figure 4 the structures of reactants (R), products (P) and transition states (TS) are shown for both reaction paths of $\text{cis-[Pt(PMe}_3)_2(\text{etga})]$ (Scheme 10a). The reaction energy (E) profiles are shown in Figure 32. The energy values are referred to the separated reactants (R) rather than reactant adducts (RA) (Figure 22), since it is wide accepted that at the beginning of the reaction, the water molecule in the second coordination shell of the metal is possible only in vacuum, but it seems an artifact in solution (Lamm and Pack, 1990). The detachment of the etga ligand in the first hydrolysis can occur in two different ways (Scheme 7a): by breaking of the bond that involves the oxygen in α position to the OH group (O1 path) or the other Pt-O bond (O2 path).

Both O1 and O2 paths (Figure 22a) showed a proton transfer from the incoming water molecule to the closer oxygen of the ligand (OL). This result has been previously reported also for the hydrolysis of other platinum drugs under neutral conditions (Pavelka et al., 2007, Lucas et al., 2009). Mono- and di-hydroxo complexes should form under physiological conditions, although it has been reported that the local pH in the vicinity of macromolecules could be different from the physiological one (Alberto et al., 2011, Despaigne et al., 2014). The energy profiles for the first step of the hydrolysis indicate that the formation of the reactant adducts (RA1-O1 and RA1-O2) is energetically favored (Figure 23a). Both transition states TS1-O1 and TS1-O2 have a penta-coordinated structure, with imaginary frequencies of 99.7 cm^{-1} for TS1-O1 and 157.8 cm^{-1} for TS1-O2 corresponding to the rupture of the platinum-ligand bond (Pt-OL) and the simultaneous formation of a new bond between the metal and a water molecule.

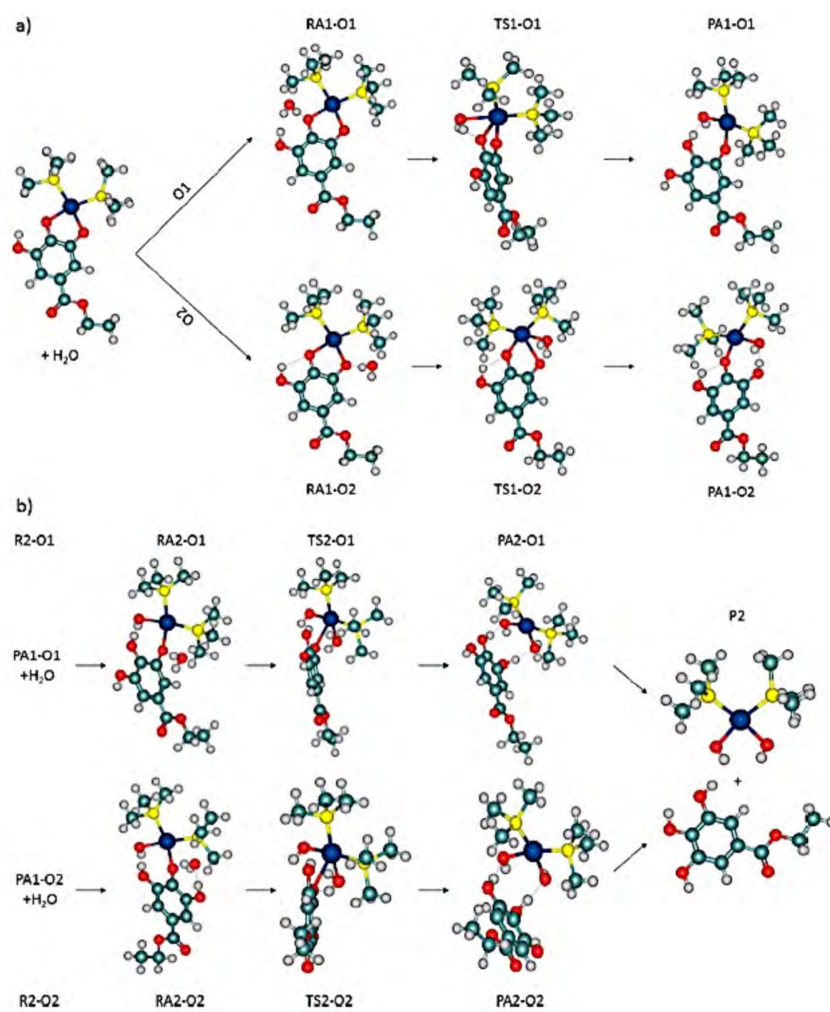


Fig. 22- Optimized structures for the hydrolysis reaction of $\text{cis-}[\text{Pt}(\text{PMe}_3)_2(\text{etga})]$. a) First step; b) second step.

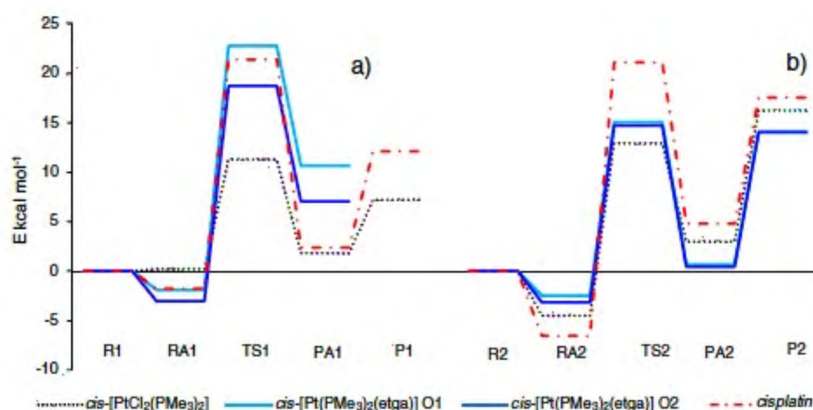


Fig. 23- Activation energy profiles of: a) the first and b) the second step of the hydrolysis reaction of *cis*-[Pt(PMe₃)₂(etga)] (both O1 and O2 paths), *cis*-[PtCl₂(PMe₃)₂] and cisplatin in PCM water. The energy values for cisplatin are taken from ref (Melchior et al., 2011)

The activation energies ($E_a = E_{\text{transition state}} - E_{\text{reactants}}$) for the two paths are rather different: for the O1 path $E_a = 22.8 \text{ kcal mol}^{-1}$, while the O2 path shows a $E_a = 18.7 \text{ kcal mol}^{-1}$. According to these results, the release of ethyl gallate by the opening of the Pt-O2 bond is kinetically preferred. Furthermore, O2 pathway leads to the most stable product [$\Delta E = E(\text{PA1-O1}) - E(\text{PA1-O2}) = 3.6 \text{ kcal mol}^{-1}$]. This difference could be due to the formation of two hydrogen bonds in the case of PA1-O2 with respect to PA1-O1 where only one hydrogen bond is possible (Figure 22a). The transition states in the second hydrolysis have a penta-coordinated structure with imaginary frequencies of 103.9 cm^{-1} for TS2-O1 and 101.7 cm^{-1} for TS2-O2 (Figure 22b) corresponding to the simultaneous rupture of the bond between platinum and the ligand (Pt-OL) and the formation of the metal-water bond. The calculated profiles (Figure 23b) show that the two reaction paths have comparable activation energy [$\Delta E_a = E_a(\text{TS2-O1}) - E_a(\text{TS2-O2}) = 0.4 \text{ kcal mol}^{-1}$]. Also the final products (P2) have almost the same energy of TS2-O1 and TS2-O2. As shown in Figure 23, the first hydrolysis step has a higher activation energy with respect to the second step, irrespective of the ring opening order, thus the first hydrolysis process is the Rate Determining Step (RDS) of the whole reaction leading to the release of ethyl gallate in solution. Regarding the first hydrolysis (Figure 23a), by comparing the energy profiles of *cis*-[Pt(PMe₃)₂(etga)] with those of the highly cytotoxic cisplatin, it appears that the E_a in O2 path is lower than the cisplatin

one ($\Delta E_a = E_a(\text{TS1-O2}) - E_a(\text{cisplatin}) = -2.7 \text{ kcal mol}^{-1}$); on the contrary the E_a in O1 path is higher than the one calculated for cisplatin ($E_a = +1.4 \text{ kcal mol}^{-1}$) (Figure 23a). For the second hydrolysis step, E_a values for both O1 and O2 paths are much lower than cisplatin one ($\Delta E_a = -6 \text{ kcal mol}^{-1}$ for O1 path and $\Delta E_a = -6.4 \text{ kcal mol}^{-1}$ for O2 path) (Figure 23b). The obtained results suggest that *cis*-[Pt(PMe₃)₂(etga)] leads to a mono-hydroxo complex with etga mono-coordinated to the metal, which promptly reacts with another water molecule giving the di-hydroxo species. The mono-hydroxo and di-hydroxo species are considered poorly reactive for DNA coordination, thus their formation can limit the efficacy of a platinum metallodrug (Wilson and Lippard, 2012). Furthermore, the low activation energy barriers of the second step and the consequent high hydrolysis reaction rate might make this complex subject to fast degradation, causing potentially poor pharmacological activity (Johnstone et al., 2016). The obtained E_a value (11.3 kcal mol⁻¹) calculated for the first hydrolysis of *cis*-[PtCl₂(PMe₃)₂] (taken as a model for the biologically inactive (vide infra) *cis*-[PtCl₂(PPh₃)₂]) is much lower than the E_a calculated for *cis*-[Pt(PMe₃)₂(etga)] and cisplatin (Figure 32). Then, the E_a calculated for the second hydrolysis of *cis*-[Pt(PMe₃)₂(etga)] is close to that of *cis*-[PtCl₂(PMe₃)₂] (Figure 23) and much lower than cisplatin. On this basis of DFT calculations, complex 3 should not be expected highly cytotoxic.

3.3.2 Hydrolysis reaction for *cis*-[Pt(PMe₃)₂(3-Hfl)]⁺

In Figures 24 the optimized structures of the complexes involved in the hydrolysis of *cis*-[Pt(PMe₃)₂(3-Hfl)]⁺ are displayed. The formation of the intermediate RA1-O2 is slightly energetically favored, while RA1-O1 formation energy is slightly positive. Also in this case, the transition states of both O1 and O2 paths have a penta-coordinated structure with imaginary frequencies of 87 cm⁻¹ for TS-O1 and 78 cm⁻¹ for TS-O2.

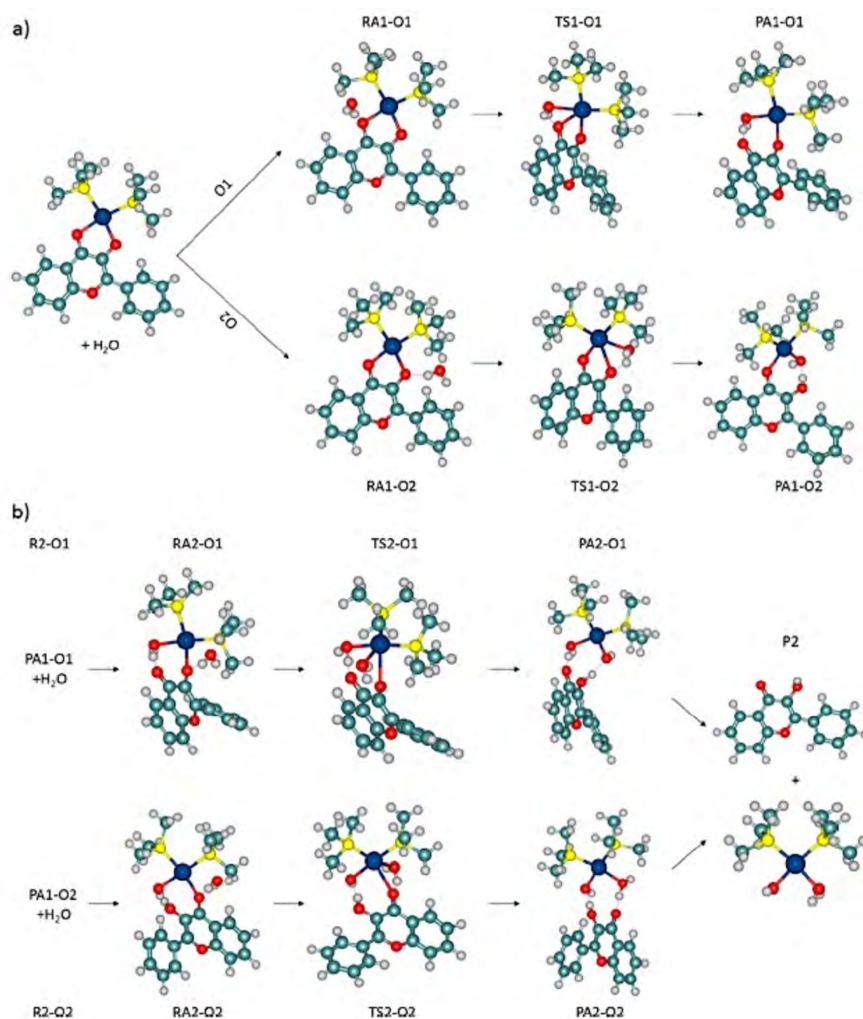


Fig. 24- Optimized structures for the hydrolysis reaction of $\text{cis-}[\text{Pt}(\text{PMe}_3)_2(3\text{-Hfl})]^+$. a) First step; b) second step.

The calculated E_a values are $14.4 \text{ kcal mol}^{-1}$ and $15.7 \text{ kcal mol}^{-1}$ for O1 and O2 path, respectively. The O1 opening produces the mono-aqua derivative, while in the O2 path a proton. In conclusion in the first hydrolysis step the O1 path, leading to a mono-aquo Pt complex, should be more favored than the O2 one (which leads to a mono-hydroxo Pt complex). In the second hydrolysis (Figure 24b), transition states with a penta-coordinated Pt geometry are obtained characterized by a single imaginary frequency (89 cm^{-1} for TS2-O1 and 83 cm^{-1} for TS2-O2). The calculated E_a values are

11.9 kcal mol⁻¹ and 9.5 kcal mol⁻¹ for O1 and O2 path, respectively. Therefore, also for *cis*-[Pt(PMe₃)₂(3-Hfl)]⁺ the first hydrolysis is the rate determining step of the whole process leading to the complete release of the 3-Hfl. Furthermore, by analyzing the obtained E_a values (Figures 24b), it emerges that the second hydrolysis step proceeds faster for *cis*-[Pt(PMe₃)₂(3-Hfl)]⁺ (both O1 and O2 paths) than for cisplatin and *cis*-[Pt(PMe₃)₂(etga)]. On the contrary, the first hydrolysis is slower for *cis*-[Pt(PMe₃)₂(3-Hfl)]⁺ with respect to *cis*-[PtCl₂(PMe₃)₂] (Figure 24a). In the O1 path the final product shows a proton transfer from the water molecule bonded to platinum to the closer oxygen of the ligand, while in the O2 path this does not occur. The final product of *cis*-[Pt(PMe₃)₂(3-Hfl)]⁺ hydrolysis is the monoaquo-monohydroxo complex (*cis*-[Pt(PMe₃)₂(H₂O)(OH)]⁺, Figure 23b). On the basis of DFT calculations, some synergic cytotoxic effect between platinum centre and 3-hydroxyflavon leaving group effect might be expected for *cis*-[Pt(PMe₃)₂(3-Hfl)]⁺, because the mono-aqua platinum species formed in both first and second hydrolysis steps, could coordinate DNA (Jamieson and Lippard, 1999). On the other hand, the theoretical hydrolysis activation energy (mainly affected by the trans influence of the carrier ligand), is significantly lower with respect to cisplatin (Figure 24) and could lead to fast deactivation of platinum compound and thus poorer biological activity (Johnstone et al., 2016).

3.4 Cytotoxicity

The antiproliferative action of the new complexes (2 and 3) was assessed in human glioblastoma (U87) and human breast cancer (MFC-7) cells, being the first cellular line sensitive to cisplatin and the latter cisplatin resistant. Complex 1 was not taken into consideration, due to the difficulty in separating it from the side-products formed in minor amounts during its synthesis. For comparison purposes, the cytotoxicities of 3-hydroxyflavone, ethyl gallate and *cis*-[PtCl₂(PPh₃)₂] were evaluated under the same experimental conditions employed for the metal complexes.

Cellular viability was determined by colorimetric MTT assay [MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]. The viability of cells in the presence of the tested compounds was compared to that observed for control cultures

and the inhibition of growth (%) was calculated. Ting concentrations (from 0.1 to 200 μM) of the different compounds for 72 h are shown in Table 6, which reports also the relevant IC₅₀ values for cisplatin taken by the literature (Despaigne et al., 2014, Wilson and Lippard, 2012).

Compound	IC ₅₀ (μM)	
	U87 ^a	MCF-7 ^a
complex 2	26.3 $\pm$ 2.1	55.2 $\pm$ 1.7
3-hydroxyflavone	27.5 $\pm$ 2.3	108.1 $\pm$ 3.5
complex 3	123.7 $\pm$ 3.8	> 200
ethyl gallate	97.7 $\pm$ 2.6	> 200
<i>cis</i> -[PtCl ₂ (PPh ₃) ₂]	> 200	> 200
cisplatin	1.76 $\pm$ 0.22 ^b	14 $\pm$ 3 ^c

[a] Cells were seeded at a density of ~5000 cells per well into 96-well microliter plates. Following overnight incubation, cells were treated with a range of drug concentrations from 0.10 to 200 μM and incubated at 37 °C under a humidified atmosphere with 5% CO₂ for a period of 72 h. Data are the mean values $\pm$ SD of three independent experiments performed in triplicate.

[b] Data taken from ref. (Despaigne et al., 2014).

[c] Data taken from ref. (Wilson and Lippard, 2012)

Table 6 - Cytotoxicity of hydroxyflavone, ethyl gallate, Pt complex 2, Pt complex 3, and *Cis*-PtCl₂(PPh₃)₂ toward U87 and MCF-7 cancer cell lines

4.0 CONCLUSIONS/CONCLUSIONI

In this chapter the synthesis and in vitro anticancer features of new cis-diphenylphosphane platinum(II) complexes bearing biologically active polyphenolate leaving group ligands has been presented. Using this synthesis route, three novel platinum(II) complexes with natural ligands have been designed and synthesised in a few steps with overall yields. The coordination modes of deprotonated quercetin, 3-hydroxyflavon and ethylgallate to platinum(II) were determined in solution by NMR spectroscopy and in the solid state by X-ray diffraction. Biological assays carried out on complexes 2 and 3 indicate that these compounds did not show higher cytotoxicity compared to cisplatin for the considered cell lines (U87 and MCF-7). Theoretical calculations confirm that the studied complexes easily release their biologically active ligand in solution, thus suggesting a possible origin of the fact that their cytotoxicity is at least similar to that of their natural leaving groups. Although complexes 2 and 3 have similar O,O' chelate structure, there is a small difference in their natural ligand coordination mode, being the leaving group a deprotonated α -hydroxy ketone in the first complex and a double deprotonated 1,2-diol in the second one. This small difference may affect the biological activity of the new compounds. In fact, a biological synergic effect between the ligand and the starting platinum compound was interestingly observed for complex 2 bearing 3-hydroxyflavonate, for which mono-aqua platinum species (able to coordinate DNA) smoothly forming both first and second hydrolysis steps, according to theoretical studies. However, the cytotoxicity of cisplatin is still higher than that of complex 2 one because of a too fast hydrolysis rate of the latter, due to the higher trans effect of the carrier ligand.

When compound 3 is considered, theoretical calculations suggest that the lack of synergic cytotoxicity effect may be due either to production of relatively inert mono- and di-hydroxo complexes under physiological conditions (determined by ethyl gallate leaving group) and to a too fast hydrolysis rate (determined by carrier ligand features).

In conclusion, this study suggests that fine tuning of chemical parameters of both leaving groups and carrier ligands is a key step in designing biologically active Pt complexes. Therefore, further studies are warranted to synthesize new platinum(II) 3-hydroxyflavonate complexes bearing carrier ligands different than triarylphosphanes.

5. NOVEL Pt(II) COMPLEXES WITH CURCUMIN AND CAFFEINE.

5.1 Pt-complexes-DNA interactions

Deoxyribonucleic acid (DNA) is one of the most important biomolecules in living organisms. DNA is a molecule that contains the instructions an organism needs to develop, live and reproduce.

DNA is an organic polymer constituted by monomeric nucleotides formed by three fundamental components: a phosphate group, a sugar pentose (deoxyribose) and a nitrogen-containing base that is linked to the deoxyribose through a N-glycosidic bond (Fig. 25).

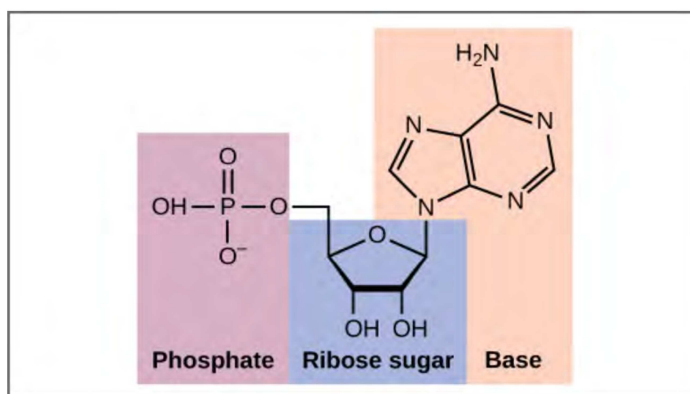


Figure 25-Example of a nucleotide, namely adenosine

There are four different nitrogenous bases, *i.e.* adenine (A), thymine (T), cytosine (C) and guanine (G) (Fig. 19), which constitute the nucleotides that are incorporated in the DNA molecule. The first two, namely adenine and guanine, are purine bases, while cytosine and thymine are pyrimidine bases.

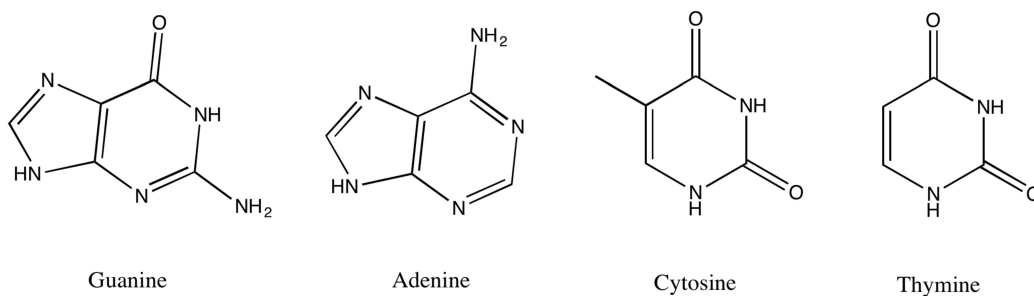


Figure 26-Nitrogenous bases

The nucleotides are covalently bonded between the phosphate and the sugar moieties of two contiguous nucleotides, to form polynucleotide chains (A.L.Lehninger; Principi di biochimica, Zanichelli (1983), 27, 765).

The structure of DNA was elucidated by Watson, Crick and Wilkins (Watson and Crick, 1953), who were awarded the Nobel Prize in Medicine in 1962. The 3D model for DNA consists of two polynucleotide chains wrapped around the same axle to form a double right-handed helix (Fig. 27).

The two strands are held together by hydrogen bonds between nitrogenous bases; hence, only certain base pairs are inserted with precision in the supramolecular structure. Actually, hydrogen bonds are established between purine and pyrimidine bases, namely between adenine and thymine through two hydrogen bonds, and between guanine and cytosine via three hydrogen bonds.

The two complementary chains run in opposite directions, the inside of the resulting double helix containing the nitrogenous bases. The nitrogenous bases are extremely close together and are perpendicular to the longitudinal axle of the double helix; the spatial arrangement of the two chains generates the major and minor grooves, which result from the non-symmetrical connection of the base pairs to the ribose-phosphate backbone.

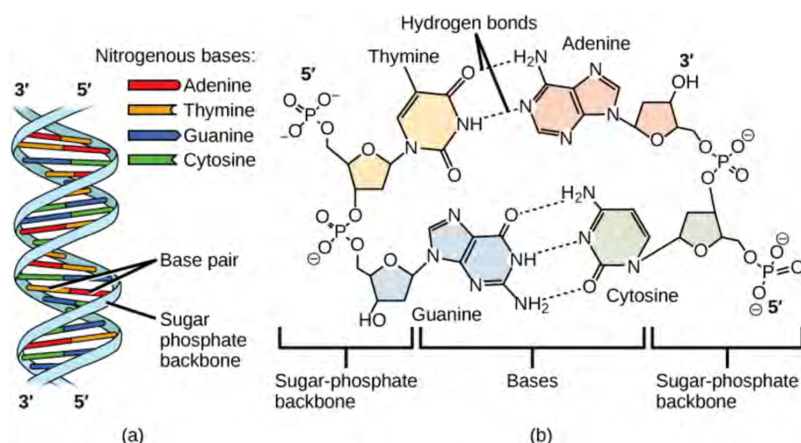


Figure 27-DNA (a) double-stranded helix, and (b) adenine-thymine and cytosine-guanine base pairs.

The DNA can have different conformations, the more common ones being the A, B and Z forms (García-Ramos et al., 2013). Initial X-ray diffraction studies, performed on concentrated DNA solutions, disclosed two different types of DNA structure *viz.* form B and form A. Form B is characterized by a double helix that makes one complete turn about its axis every about 10 base pairs, and exhibits an ample major groove and a more closed minor groove.

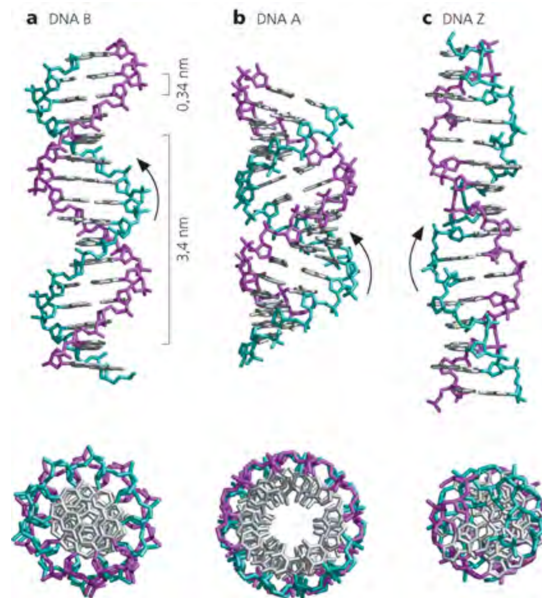


Figure 28-A, B and Z forms of DNA

A DNA is also a right-handed helix, which can be observed under non-physiological, dehydrating conditions. It is less compact since every helix turn contains 11 base pairs. This A conformation is generated when at least 4 purines (or pyrimidines) are present in a row; hence, it can be found locally, within a helix of mainly B conformation. Under physiological conditions, confirmation A characterizes RNA-DNA heteroduplexes and complexes formed by DNA-protein associations.

Z DNA is typical for sequences including chemical modifications, such as methylations or for rich d(GC) sequences. It is narrower than the A and B conformations, with a diameter of 18.4 Å and 12 base pairs per turn. Z DNA is a left-handed double helix that winds to the left in a zig-zag pattern.

The role of DNA is to encode all biological processes taking place in a living

organism. Its involvement in crucial cellular events such as DNA replication or gene expression makes it a target of choice for drugs or diagnosis agents. A large and varied range of natural and synthetic ligands that bind to DNA, hence inhibiting or altering its biological activity, have been discovered or developed as therapeutic agents. The design of new molecules with a higher specificity for DNA may lead to a greater control of gene expression and therefore to an improved anticancer efficacy.

There are many types of drug-DNA interactions: upon DNA binding, the resulting adduct can be stabilized through a series of interactions. Actually, the molecules can bind to DNA in a covalent manner or in a non-covalent mode (Sirajuddin et al., 2013).

The covalent binding mode is irreversible and invariably causes the complete inhibition of DNA functions and subsequent cell death. Many anticancer complexes, like cisplatin, exert their anticancer activity through the formation of adducts via alkylation or intra-strand and inter-strand crosslinks.

Alkylating agents belong to a class of compounds that have the ability to transfer an alkyl group to DNA. Alkylating agents preferentially proceed at the N-7 position of guanine and the N-3 position of adenine. DNA alkylation thus inhibits base pairing, which leads to DNA miscoding (errors in the transcription and translation of the genetic code).

A second mechanism by which alkylating agents may cause DNA damage is the formation of cross bridges, namely the creation of bonds between atoms in the DNA, linking two bases. This Cross linking prevents DNA from being separated to allow synthesis or transcription. The most famous anticancer alkylating agent, namely cisplatin, forms intra and inter-strand crosslinks with DNA (Fig. 4), which inhibits its replication. Cisplatin is used for the treatment of bladder, ovarian, neck, and testicular cancer (Paul and Bhattacharya, 2012).

The non-covalent binding of molecules to DNA includes various types of interactions, because the nucleic acid has a number of different binding sites, *viz.* (i) between two base pairs (intercalation), (ii) in the minor groove, and (iii) in the major groove.

The major groove of B DNA is wider than the minor groove (Fig. 29), and is therefore usually the target for large molecules, such as most DNA-binding proteins In

contrast, the minor groove, which is narrower, is the target for small molecules (Stofer and Lavery, 1994).

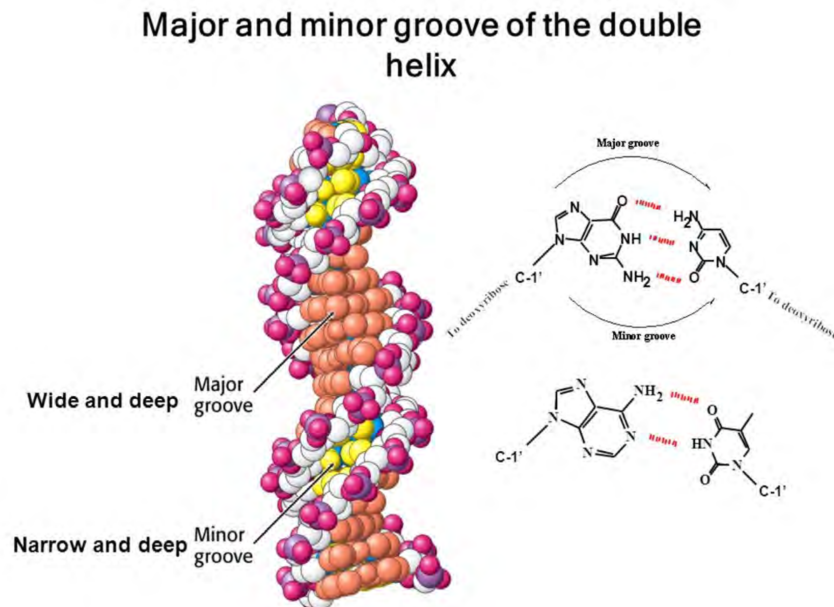


Figure 29-Major and minor groove of DNA

Some proteins can recognize a specific DNA sequence and bind to it, both grooves can be recognized but the major groove is the major binding site for such proteins. Protein–nucleic acid interactions therefore play a crucial role in central biological processes, ranging from the mechanism of replication, transcription and recombination to enzymatic events utilizing nucleic acids as substrates. For these reasons the biochemical and structural bases of the protein–DNA recognition process has been a field of intense study. X-ray crystallography, along with NMR and many other chemical and physical methods have been applied to analyse how protein and DNA interact with each other. Mainly non-covalent hydrogen-bonding interactions between DNA base pairs and the binding proteins take place; in addition, the formation of van der Waals contacts may play a role as well. Hence, the interacting protein recognises hydrogen bond donor/acceptor groups of the DNA base pairs on the major groove floor. The binding in the major groove often causes kinking or bending at the interaction site,

changing the width and depth of the DNA groove. The part of the protein that actually interacts with the DNA major groove is most commonly an α -helix moiety. An example of major groove binding molecules are synthetic zinc fingers (ZnFs), whose design originates from naturally-occurring motifs in proteins (Laity et al., 2001).

ZnFs represent a group of different binding motifs where a zinc ion coordinates and stabilizes the fold of an amino acid sequence. ZnF motifs bind to DNA, usually in a sequence-specific manner, and typically wrap around the outside of the double helix, following a right-handed helical path. ZnFs may also bind to RNA and proteins. Multiple DNA contacts are usually made, often involving more than one ZnF domains. DNA-binding ZnF proteins are involved in transcription processes.

Minor-groove binding molecules are usually small molecules built from heterocyclic or aromatic hydrocarbon rings and that possess rotational freedom. These features allow the molecule to fit into the minor groove, through displacement of water. Minor-groove binding drugs usually exhibit a crescent shape, which effectively complements the shape of the groove and facilitates their interaction *via* van der Waals contacts with the N-3 of adenine and O-2 of thymine. Most minor-groove binding drugs bind to A/T rich sequences (Abu-daya et al., 1995). This preference, in addition to the designed propensity for the electronegative pockets of AT sequences (Pullman and Pullman, 1981), is probably due to better van der Waals contacts between the ligand and groove walls in this region, since A/T regions are narrower than G/C groove regions and also because of the steric hindrance in the latter, presented by the C2 amino group of the guanine base (Geierstanger and Wemmer, 1995). However, a few synthetic polyamides like lexitropsins and imidazole-pyrrole polyamides have been designed which have specificity for G/C and C/G regions in the grooves.

The intercalation (Fig.30a) consists in the insertion of a molecule between two adjacent base pairs (Long and Barton, 1990). Intercalators typically contain planar heterocyclic moieties, which π - π stack between adjacent DNA base pairs, giving rise to a stable DNA-molecule complex. When a molecule intercalates, structural changes occur both for the DNA and the interacting agent itself; these structural alterations can be used to study the nature of the drug-DNA interactions.

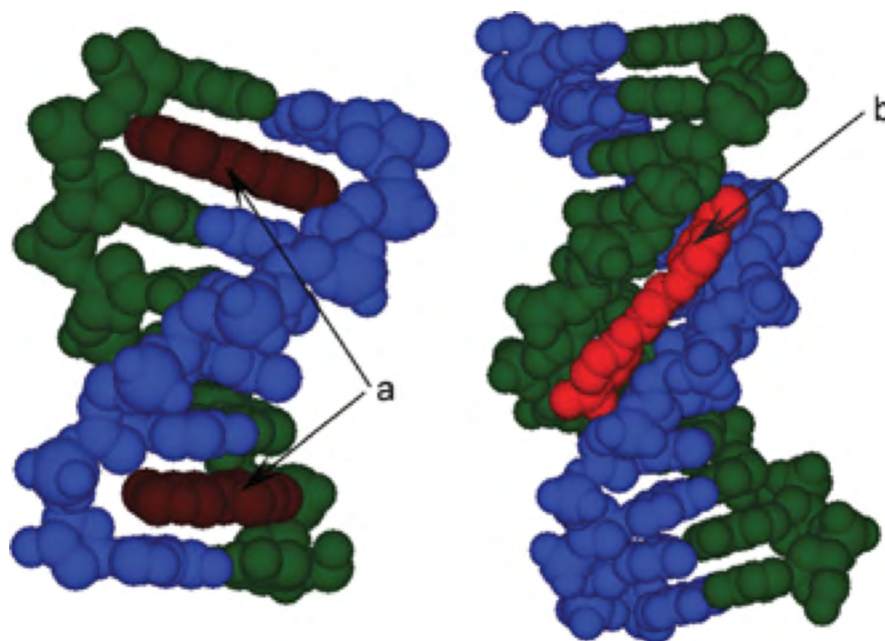


Figure 10-DNA Interaction via (a) intercalation, and (b) minor-groove binding

Thus, the intercalation planar molecules induce a geometric distortion of the structure of the double helix, lengthening it by about 3 Å per bound drug molecule, resulting in DNA unwinding, which prevents replication and transcription. The degree of unwinding depends on the structure of the intercalating molecule and the site of intercalation. A well-known example of an organic DNA-intercalating molecule is ethidium bromide (Palchaudhuri and Hergenrother, 2007).

The external DNA binding is electrostatic in nature. Some ligands are also capable of forming non-specific, outside edge stacking interactions with the DNA phosphate backbone. This binding mode usually occurs when the ligand self-associates to form higher-order aggregates, which may stack on the anionic DNA backbone in order to reduce charge–charge repulsion between ligand molecules.

5.2 Materials

5.2.1 UV-Vis spectroscopy experimental details

The absorption titrations were achieved by adding increasing amounts (0–25 μM) of calf-thymus DNA (ct-DNA) to the complexes investigated (constant complex concentration of 25 μM) in 1 mM cacodylate – 20 mM NaCl buffer at pH = 7.2. The concentration of ct-DNA was determined from its absorption intensity at 260 nm with a molar extinction coefficient of 6600 $\text{M}^{-1}\text{cm}^{-1}$. The UV-Vis experiments were performed with a Varian Cary-100 spectrophotometer.

After addition of ct-DNA to the solution of the metal complex, the resulting mixture was allowed to equilibrate at 25 °C for 5 min in the case of $[\text{PtCl}(\text{DMSO})(\text{PPh}_3)_2]\text{Cl}$, while it was incubated for 24 h at 37 °C for complexes *trans*- $[\text{PtCl}_2(\text{DMSO}(\text{caffeine}))]$ and $[\text{PtCl}(\text{DMSO})(\text{curcumin})]\text{Cl}$, after which the absorption spectra were recorded.

5.2.2 Fluorescence spectroscopy experimental details

The relative binding affinities of the complexes towards ct-DNA were investigated with EB-bound DNA (EB = ethidium bromide) in 1 mM cacodylate – 20 mM NaCl buffer at pH = 7.2, containing 2.5% DMSO (DMSO was used to dissolve the platinum(II) coordination compounds).

All samples contained 15 μM DNA and 75 μM EtBr in a fixed volume of 3 mL. In a first instance, DNA and EtBr were mixed in the buffer solution, followed by incubation for 30 minutes, to allow the intercalation of EtBr between DNA base pairs. Afterwards, increasing amounts of the studied compounds were added and the final samples were incubated for 24 h.

The fluorescence measurements were carried out with a iHR320 HORIBA JOBIN YVON spectrofluorometer. The experiments were carried out at constant concentrations of ct-DNA and EB, of respectively 150 μM_{bp} , adding increasing amounts of the complex studied (from 0 to 50 μM).

The fluorescence spectra of all complexes were recorded at room temperature applying an excitation wavelength, λ_{exc} , of 514 nm. For the experiments with the minor-groove binder, a solution of Hoechst33258 bound to ct-DNA in 1 mM cacodylate – 20 mM NaCl buffer at pH = 7.2, containing 2.5% DMSO (DMSO was required to dissolve the

platinum(II) coordination compounds) was used.

The experiments were carried out at constant concentrations of ct-DNA and Hoechst33258, of respectively 0.1 μM and 150 μM_{bp} , adding increasing amounts of the complex studied (from 0 to 50 μM).

The fluorescence spectra were recorded at room temperature applying an excitation wavelength, λ_{exc} , of 350 nm.

5.2.3 Circular Dichroism experimental details.

Circular dichroism (CD) spectra were recorded at the Serveis Científicotècnics of the Universitat de Barcelona, using a JASCO-810 spectropolarimeter, equipped with a xenon-arc lamp of 450 W, and connected to a computer to carry out the data processing. The sample compartment is cleaned before use, and dried in a current of nitrogen of 5 L/min. For the measurements, quartz cuvettes with an optical path of 0.5 cm were used. The absorption titrations were achieved by adding increasing amounts (0–25 μM) of platinum(II) complexes to ct-DNA in 1 mM cacodylate – 20 mM NaCl buffer at pH = 7.2. The concentration of ct-DNA was determined from its absorption intensity at 260 nm, with a molar extinction coefficient of 6600 $\text{M}^{-1} \text{cm}^{-1}$.

After addition of the metal complex to the solution of ct-DNA, the resulting mixture was incubated for 24 h at 37 °C, after which the absorption spectra were recorded.

5.2.4 Agarose Gel Electrophoresis experimental details

Stock solutions of the platinum(II) compounds were prepared in 1 mM cacodylate – 20 mM NaCl buffer (pH = 7.2) containing 2% DMSO. pBR322 plasmid DNA aliquots (0.2 mg mL^{-1}) in 1 mM cacodylate – 20 mM NaCl buffer were incubated with the complexes for 24 h at 37 °C.

Next, the reaction samples were quenched with 4 μL of loading buffer (5mM xylene cyanol and 4mM glycerol) and then electrophoretized on agarose gel (1% in TBE 1X buffer) for 1.20 h at 6.5 V cm^{-1} .

The electrophoresis in agarose gel was realized in a BioRad horizontal tank connected to a CONSORT EV231 electrophoresis power supply. Free DNA was used as control.

Afterwards, the DNA was stained with SYBRs safe and the gel was photographed with a BioRad Gel Doc EZ Imager

5.2.5 Atomic Force Microscopy experimental details

Stock solutions of the Pt(II) compounds were prepared in 40 mM HEPES – 10 mM MgCl_2 buffer (pH = 7.2). pBR322 plasmid DNA aliquots (0.2 mg mL^{-1}) in 40 mM HEPES – 10 mM MgCl_2 buffer were incubated with the complexes for 24 h at 37 °C.

The AFM samples were prepared by casting a 5 μL drop of test solution onto freshly cleaved muscovite green mica disks as the support. The drop was allowed to stand undisturbed for 3 min to favour the adsorbate–substrate interaction. Each DNA-laden disk was rinsed with Milli-Q water and was blown dry with clean compressed argon gas directed normal to the disk surface. The samples were stored over silica prior to AFM imaging.

5.2.6 Cell cultures and cell-viability assays

Human melanoma (A375) and lung (A549), breast (MCF-7), ovary (SKOV3) and colorectal (SW620) adenocarcinoma cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cell lines A549, A375, SKOV3 and SW620 were cultured in DMEM medium. All media were added with 10% heat-inactivated fetal bovine serum (FBS; Life Technologies, Carlsbad, CA, USA), 100 U mL^{-1} penicillin, 100 $\mu\text{g mL}^{-1}$ streptomycin, and 2 mM glutamine. The MCF-7 cell line was cultured in DMEM–F12 (HAM) media (1:1) added with 5% horse serum (Life Technologies), 100 μM sodium pyruvate (Sigma-Aldrich Chemical Co., St. Louis, MO, USA), 10 $\mu\text{g mL}^{-1}$ insulin (Sigma-Aldrich), 100 U mL^{-1} penicillin, 100 $\mu\text{g mL}^{-1}$ streptomycin, and 2 mM glutamine. Both the media and the added chemicals whenever not stated were obtained from the Biological Industries, Beit Haemek, Israel. Cells were grown at 37 °C in a 5% CO_2 atmosphere.

Cells ($1 \times 10^5 \sim 10^6 \text{ cells mL}^{-1}$) were seeded in 96-well plates and allowed to grow for 24 h. For single-point experiments, the cells reacted with the investigated drugs at two different concentrations (i.e. 10 μM and 50 μM). For the dose–response curves,

the cells were treated with different concentrations of the compounds; in the range 0.63–80 μ M for 24 h. After the treatment, a 10 μ M solution of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; Sigma-Aldrich) was added to each well and the plates were incubated at 37 °C for further 4 h. As follows, the medium was removed and the purple formazan crystals were dissolved in DMSO. The absorbance was measured at 570 nm using a multiwell plate reader (Multiskan FC, Thermo Scientific). The cell viability was calculated according to the relation:

$$\text{viability (\%)} = [(\text{absorbance of the treated wells})/(\text{absorbance of the control wells})] \times 100.$$

The IC₅₀ values (corresponding to the compound concentrations in μ M that produce 50% cell-viability reduction) were obtained from the dose–response curves using GraphPad Prism V5.0 for Windows™ (Graphpad Software, San Diego, CA, USA). All data are shown as the mean value $\pm$ S.D. of three independent experiments for single-point assays and for the dose–response curves.

5.3 Methods

5.3.1 UV-Vis spectroscopy

Absorption spectroscopy is a technique that can be used to evaluate the interaction of molecules with DNA. UV-Vis spectroscopic measurements are usually performed with a solution of the complex investigated and DNA in water or in a buffer.

The absorption wavelength and the absorbance strength of a molecule depend not only on its chemical nature but also on the molecular environment of its chromophores, e.g. alternating single and double bonds that absorb visible light. Absorption spectroscopy is therefore an excellent technique to follow ligand-binding reactions, such as complex-DNA interactions. In fact, it is perhaps the most employed instrumental technique for this purpose. The concentration of nucleic acid in solution is determined by its absorbance. The absorbance (A) is correlated to the intensity of the light passing through the reference cell (I₀) and the sample cell (I) according to the Lambert-Beer equation:

$$A = -\log \left(\frac{I}{I_0} \right) \quad (1)$$

Hence, according to this equation, the absorbance is proportional to the concentration of the substance in solution (Schmid, 2001):

$$A = \epsilon cl \quad (2)$$

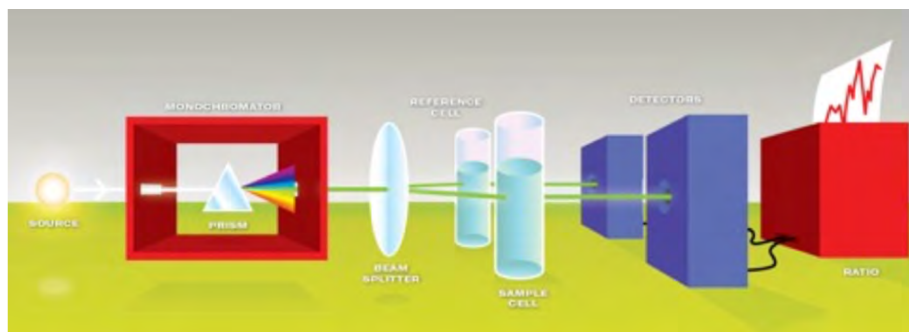


Figure 31-Schematic view of a conventional UV-Vis spectrophotometer

The UV-Vis spectrum of DNA shows a maximum of absorption at 260 nm, with a broad band between 200 and 350 nm. This absorption is due to the purine and pyrimidine bases, which contain chromophoric groups that are responsible for the electronic transitions.

Drug-DNA interactions can usually be studied by UV-Vis spectroscopy because ligands/complexes show absorption maxima in a different range of the UV region or of the visible region with respect to DNA. When a compound binds to DNA via intercalation, hypochromism associated with a red shift is observed, which is caused by a contraction of the DNA along the helix axis and also by conformational changes. These alterations have been attributed to electrostatic interactions, minor/major groove interactions or hydrogen bonding (Shi et al., 2006). Because the intercalative interactions involve stacking contacts between ligand aromatic chromophores and DNA base pairs, the extent of the hypochromism can be correlated with the strength of the intercalation. Actually, the strength of this electronic interaction is expected to decrease as the cube of the distance between the chromophore and the DNA bases. These features are consistent with a combination of the π electrons of the interacting compound and the π electrons of the DNA bases. Consequently, the energy of the $\pi-\pi^*$ transition decreases and a red shift is observed.

Conversely, hyperchromism derives from alterations of the DNA double-helix

structure. For instance, conformational or structural DNA changes upon complex-DNA formation through electrostatic interactions will be reflected by an hyperchromic effect. A hyperchromic effect may also be attributed to a partial uncoiling of the helix structure of DNA, causing a major exposition of DNA bases.

Finally, for weak interactions, hypochromic or hyperchromic effects are solely observed, without significant spectral shifts.

Based on the variation in absorption, the intrinsic binding constant or the association constant (K_b) of a metal complex interacting with DNA can be calculated applying the Benesi-Hildebrand equation:

$$\frac{[DNA]}{\varepsilon_a - \varepsilon_f} = \frac{[DNA]}{\varepsilon_0 - \varepsilon_f} + \frac{1}{K_b(\varepsilon_0 - \varepsilon_f)} \quad (3)$$

where $[DNA]$ is the concentration of DNA in base pairs, ε_a is the extinction coefficient observed at a given DNA concentration, ε_f is the extinction coefficient of the free complex in solution ($A_{obs}/[complex]$), and ε_0 is the extinction coefficient of the complex when fully bound to DNA.

A plot of $[DNA]/(\varepsilon_a - \varepsilon_f)$ vs $[DNA]$ gives a slope corresponding to $1/(\varepsilon_0 - \varepsilon_f)$ and a y-intercept equal to $1/K_b(\varepsilon_0 - \varepsilon_f)$. Thus, the intrinsic binding constant K_b is the ratio of the slope to the intercept.

K_b Values above 10^5 M^{-1} are considered to be indicative of a relatively strong interaction between DNA and the corresponding metal complex (Sirajuddin et al., 2013).

5.3.2 Fluorescence spectroscopy

A molecule can absorb a photon and thereby excite to an upper energy state, which is unstable. Relaxation of the electrons back to the ground state may lead to the emission of photons (luminescence); molecules undergoing such a process are called fluorophores.

The phenomenon of luminescence can be divided into two different types, namely fluorescence and phosphorescence. Phosphorescence takes place when a molecule does not immediately re-emit the radiation it absorbs.

In this case, the excitation of the electrons to an excited state occurs via a process called intersystem crossing, in which a singlet state passes to an excited triplet state, which then gives off a photon and relaxes to the ground state (singlet state).

Fluorescence happens when an orbital electron from a molecule (or atom) relaxes emitting a photon from an excited singlet state after having been excited.

In fluorescence, the excited state exists for a finite time, typically 10^{-10} seconds. During this time, the fluorophore undergoes conformational changes and is subject to a multitude of possible interactions with its molecular environment. For organic molecules, the ground state is usually a singlet state. According to the Franck-Condon principle, the transitions between electronic levels can be considered as instantaneous transitions when compared to molecular dynamics; therefore, they are represented by vertical lines. Thus, the Franck-Condon factor determines the probability of transitions among the initial electronic state, and the new vibrational state is called the vibronic state.

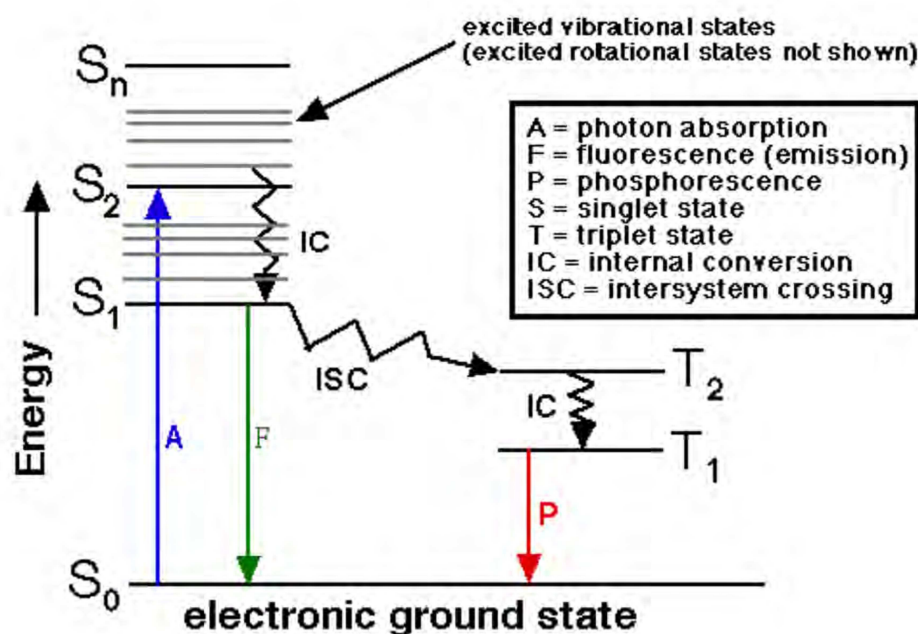


Figure 32-Jablonsky diagram

In order to investigate the possible interaction of the synthesized compounds with DNA, fluorescent-dye displacement studies have been performed with ethidium bromide (EtBr) (Fig.33) and Hoechst 33258 (Fig.35). EtBr is a well-known intercalator which binds to DNA with a weak preference for GC-rich sequences.

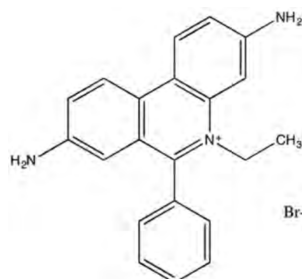


Figure 33-Representation of the chemical structure of Ethidium Bromide

Free EtBr (Fig.33) is almost non fluorescent in solution; however, upon binding to DNA through intercalation, its fluorescence increases significantly, which can be followed by spectroscopy. This increase of fluorescence is a consequence of steric protection provided by the DNA bases to the intercalating cationic agent, hence avoiding the deactivation of its excited state. This shielding effect increases the half-life of the excited state of dye, resulting in the observed increase of the fluorescent emission.

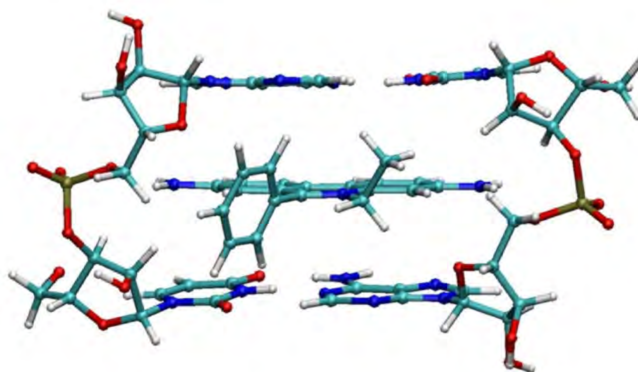


Figure 24-Ethidium bromide intercalated between two adenine-thymine base pairs.

Hoechst33258 (Fig.35) is a minor groove binder that shows high preference for AT-sequences (Reddy et al., 1999, Abu-daya et al., 1995). Its fluorescence is enhanced upon binding to DNA at stretches of at least 3 A-T base pairs, but no binding to stretches of 2 or more G-C base pairs (Loontjens et al., 1990). Hoechst 33258 requires

the binding to a [dA-dT]3-[dG-dC]1 sequence to enhance its fluorescence, with binding to the bottom of the minor groove as an essential condition.

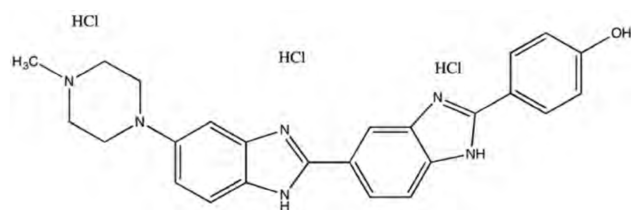


Figure 35-Structure of Hoechst 33258

The simultaneous interaction of EtBr and Hoechst33258 with double-strand DNA reveals that their respective prevailing influence is dependent on their concentration: at low concentration, the effect of EtBr is dominant, whereas an increase of the concentration of Hoechst33258 leads to its stronger effect (Palchadhuri and Hergenrother, 2007).

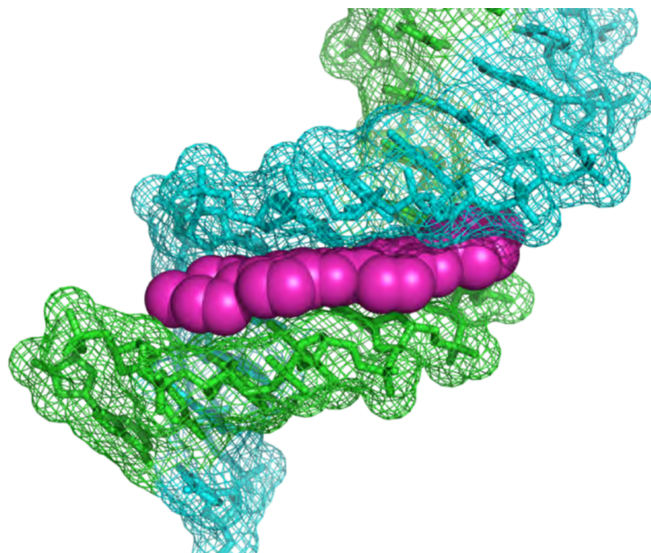


Figure 36-Hoechst 33258 interaction in the minor groove

Competitive studies are based on the addition of a second DNA-interacting molecule that will displace EtBr or Hoechst33258. Thus, a decrease of the emission intensity of the fluorescence of EtBr or Hoechst33258 reflects the degree of coordination of

the interacting compounds to DNA, as well as their binding mode. If the emission decrease is observed in the presence of EtBr, the interaction will be of intercalative mode. If the decrease occurs in the presence of Hoechst33258, then the interaction will be in the minor groove.

The experiment consists of recording the emission spectra of DNA-EtBr or DNA-Hoechst33258 adducts in the presence of increasing concentrations of the compound investigated, and to examine the corresponding variations of the maximum emission intensities.

With the objective to estimate quantitatively the affinity of different compounds studied for ct-DNA, their quenching efficiency has been determined using the Stern-Volmer equation,

$$\frac{I_0}{I} = 1 + K_{sv}[\text{complex}] \quad (4)$$

where [complex] is the quencher concentration, I_0 is the measured fluorescence intensity without quencher present, and I is the measured fluorescence intensity with complex present. After plotting (I_0 / I) against [complex], the slope gives the value of K_{sv} , i.e. the Stern-Volmer constant.

It can be noticed that the Stern-Volmer equation does not contain a variable for the fluorophore concentration and therefore suggests that the fluorescence quenching is independent of it.

Fluorescence quenching generally takes place through either a static or a dynamic mechanism. For the former type, complexation occurs in the ground state between the fluorophore and the quenching (interacting) compounds; in this case, the quenching of the excited fluorophore takes place upon irradiation. For the dynamic process, collision between the quencher and the fluorophore occurs during the excitation lifetime of the fluorophore. The efficiency of the dynamic quenching is associated with the lifetime of the excited state of the fluorophore and with the quencher concentration.

5.3.3-Circular Dichroism (CD)

Circular Dichroism spectroscopy is a valuable analytical tool useful to study the structure of biomolecules, such as proteins or DNA, in solution (Ivanov et al., 1973).

This technique is also widely used in inorganic chemistry, particularly in coordination chemistry, to investigate the interaction between a metal ion and a chiral ligand. CD has two major advantages: (i) it allows measurements with very small amounts of compound in physiological buffers and (ii) it provides one of the best methods for monitoring any structural alterations that might result from changes in environmental conditions, such as pH, temperature and ionic strength.

In the UV range, CD spectroscopy provides fundamental information concerning the possible variations that can suffer the conformation of DNA in solution. In fact, it is possible to characterize different structural forms of DNA, viz. the B-, A- and Z-forms, guanine quadruplexes, cytosine quadruplexes, triplexes and other less characterized structures.

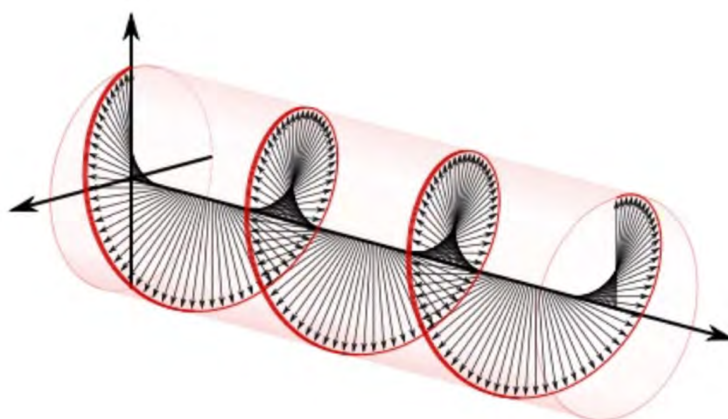


Figure 37-Schematic representation of right-handed (clockwise) polarized light

In CD, left and right circularly polarized light is produced, which passes alternately through the sample compartment (Bulheller et al., 2007). In the detector, the intensities of the two beams are measured and compared. If the sample compartment contains an optically active compound, the extinction coefficients for the left and right circularly polarized light, respectively ϵ_L and ϵ_R , may differ depending upon the wavelength (λ) of the polarized incident beam. Therefore, the difference between the two extinction coefficients, namely $\Delta\epsilon = \epsilon_L - \epsilon_R$, may not be zero ($\Delta\epsilon$ is called circular dichroism). $\Delta\epsilon$ values depend on the wavelength of the radiation used, and the representation of $\Delta\epsilon$ as a function of the wavelength is the CD spectrum of the sample.

The direct measurement gives an algebraic mean value:

$$\Delta A(\lambda) = A_L(\lambda) - A_R(\lambda) \quad (5)$$

with:

ΔA = circular dichroic absorbance,

A_L = absorbance of left circularly polarised light,

A_R = absorbance of right circularly polarised light

Using Beer-Lambert law, equation (1) can also be written as

$$\Delta A(\lambda) = A_L(\lambda) - A_R(\lambda) = [\varepsilon_L(\lambda) - \varepsilon_R(\lambda)]lc \quad (6)$$

$$\Delta A(\lambda) = [\Delta\varepsilon(\lambda)]lc \quad (7)$$

where $\Delta\varepsilon$ = molar circular dichroism or molar differential dichroic absorptivity expressed in litre mole⁻¹ cm⁻¹,

ε_L = molar absorptivity of left circularly polarised light,

ε_R = molar absorptivity of right circularly polarised light,

c = concentration of the studied solution in mole litre⁻¹,

l = optical path of the cell in centimetres.

A CD instrument determined 3 values that are: the absorbance (A), the molar circular dichroism ($\Delta\varepsilon$) and the ellipticity (θ).

$$\theta \text{ (degrees)} = 32.98 \Delta A$$

To remove the linear dependence of the pathlength and solute concentration, the molar ellipticity can be defined:

$$[\theta] = 100 \frac{\theta}{cl} = 3298 \Delta\varepsilon \quad (8)$$

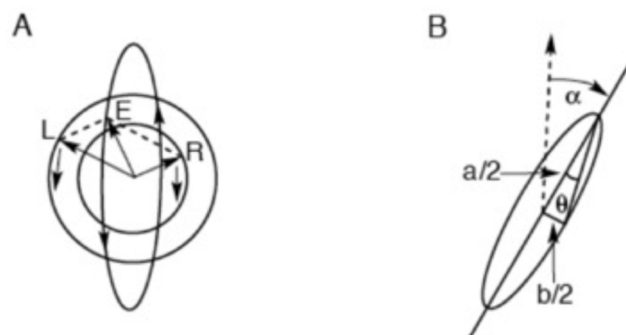


Figure 38-A) Elliptically polarized light, E, produced by the vectorial sum of right (R) and left (L) circularly polarized light. B) Ellipticity, θ , where the elliptically polarized light has been rotated through the angle α .

5.3.4- Agarose Gel Electrophoresis

Electrophoresis is a technique based on the migration of biomolecules through a matrix upon application of an electric field. The matrix may be polyacrylamide, agarose or a mixture of them. Agarose gel electrophoresis is a widely used method for the separation of DNA fragments by size, charge or conformation (Wink, 2006).

The separation is achieved thanks to the different mobility towards the anode exhibited by the negatively charged macromolecules submitted to an electric field, as consequence of their charge-to-mass ratio. For DNA, the negative charge is given by the polynucleotide chains, and is therefore approximately equal to the number of phosphate groups.

Agarose gel electrophoresis is usually carried out in an electrophoresis tank containing two indispensable elements i.e. the mobile phase and the stationary phase. The stationary phase or support is a polymer of gelatinous nature with homogeneous pore size that is submerged and shrunk in the mobile phase. The electric current provides the mobile phase, which will induce the migration of the DNA towards the positively charged anode.

For nucleic acids of great size, namely of 100bp-10kbp, agarose is the stationary phase typically used. In the present study, pBR322 DNA was used, which is a plasmid of 4.36 kbp, found as extrachromosomal genetic material in bacteria.



Figure 39-Agarose gel

This small circular, double-stranded DNA molecule exhibits two major conformations, namely the opened circular (OC) form (or relaxed form), and the circular covalently closed (CCC) form (or supercoiled form), which has a more compact structure (Figure 40).

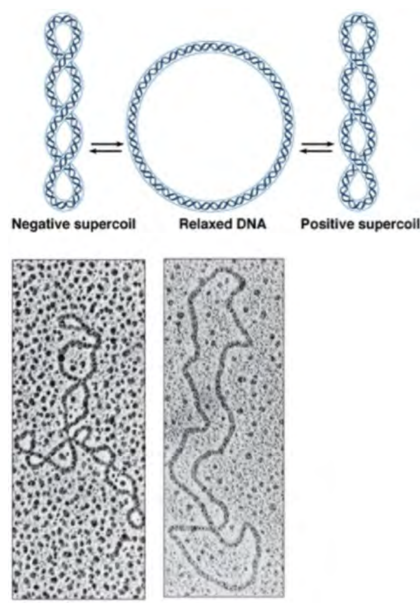


Figure 40-pBR322 DNA conformations: supercoiled and open circular forms

A third form may be observed, i.e. the linear form, which results from double-strand DNA cleavage.

Each of these conformations presents a different electrophoretic mobility, so that they will be separated by this technique, and the CCC form presents a major electrophoretic mobility than that of the OC form, due to its major degree of compaction.

Hence, the aptitude of metallic compounds to notably modify the proportion of these forms can be evaluated using this analytical procedure; actually, the changes produced by metal complexes in the tertiary structure of the DNA can be investigated (Bertini et al., 1994).

A well-known example of DNA/metal complex interaction is that of cisplatin. The interaction of this complex with plasmid DNA causes an unwinding of form CCC (resulting in lower compaction), and at the same time, promotes a certain folding of the OC form (Hintermann et al., 1981). Thus, at high cisplatin concentrations, the bands due to the two different forms coalesce to one sole band.

5.3.5- Atomic Force Microscopy (AFM)

Atomic Force Microscopy (AFM)(Cubillas and Anderson, 2014) represents an application of Scanning Probe Microscopes (SPM). The first device was introduced by Binnig and Rohrer in 1981. Early models obtained images representative of differences in electrical potential occurring between two different objects on the surface of a flat sample. They were aimed at recognizing the surface properties from the sub-microscopic to the atomic scale. In these instruments, the desired property is investigated by the changing of the z-position of a transducer (cantilever) which touches or scans a sample along the x-y plane. At the same time, a computer gathers the information from the cantilever transforming it in an image on its screen. The AFM device, developed in 1986, generated images based on the attraction and repulsion forces between the cantilever and the objects on the slide.

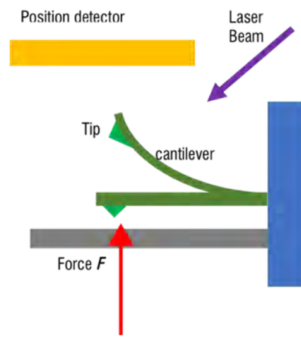


Fig. 41 -AFM apparatus

A small tip (Fig.42), made of very rigid conductive material, has fixed to the extremity of the cantilever that presses the point on the sample during the process of measure. the tip of a AFM results roundish and the terminal radius of curvature (end radius) constitutes an important parameter for the instrument resolution. The deflection degree of the lever constitutes a measure of the strength that occurs between the sample surface and the tip. The amplitude of the deflection of the lever has been verified assessing the tunnel current that is created between the microlever and a second tip posts above it. The motion of the cantilever is regulated by a piezoelectric 3D scanner, the tip flows on the sample surface moving along the three Cartesian axis.

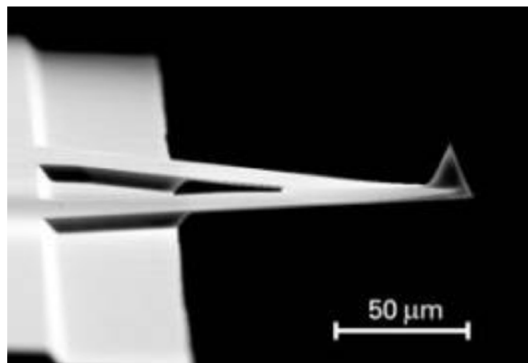


Fig. 42- AFM Tip

The AFM can works in "constant strength" mode gathering information on the strength of interaction between the sample surface and the cantilever. Otherwise, the instrument can work in "constant height" mode recording the variation of sample height. The z cantilever motions are monitored by an optic laser system recording the light

movements. In "constant strength" mode, the z-changes lead to the deflection of the laser beam. The related results consist in a 3D image of the scanning surface where, x-y changes are related to the object features, and z represents the distance between sample surface and tip. The strengths between tip and sample vary between 10^{-11} and 10^{-6} Ns. The cantilever deflection Δx is direct proportion to the strength acting on the tip, through Hook law.

$$F = -k\Delta x \quad (9)$$

where k is the strength constant of the cantilever. There are three different operation modes of Atomic Force Microscope:

- Contact mode
- No contact mode
- Tapping mode

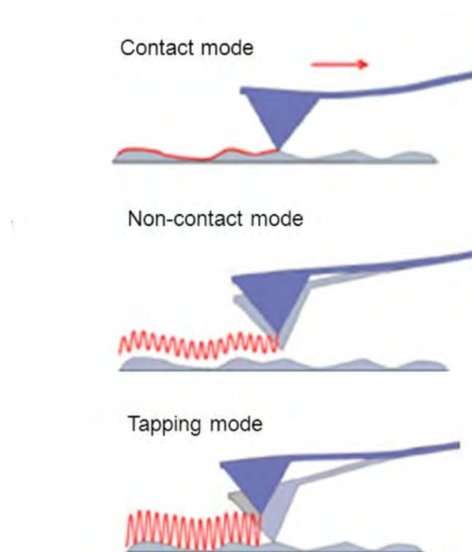


Fig. 43- Operation modes of Atomic Force Spectroscopy

In the contact mode the tip is making contact with the sample that is kept moving through micropositioning devices. The tip depicts an image of the sample surface determined by the cantilever deflection during its motion. In the contact mode the tip can scan at constant high above the surface and at force constant.

The no-contact mode requires that the cantilever position remains always the

same between sample and tip ranging close to 5-15 nm. Under this mode, the obtained image of examined surface represents the extent of Van de Waals forces occurring between tip and the sample surface.

The tapping mode (Hölscher, 2012) is a refinement of no-contact mode. This approach requires that the cantilever waves closely to the resonance frequency whose amplitude is related to the surface-to-tip distance. In any case, the high-resolution micro-topographical image of the sample surface in AFM mirrors the extent of the feedback signal resulting by the interactions between cantilever tip and scanned surface.

5.3.6 Cytotoxic Activity

This calorimetric *in-vitro* test provides the cytotoxic activity assessment of the studied complexes. This assay is based on the effect of cell proliferation leading to the yellow tetrazolium transformation MTT (3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) to in purple formazan.



Fig. 44- In vitro MTT assays

The MTT colorimetric assay is carried out in a 96-well plate. The pre-incubation times is usually ranging up to 24 h according to the properties of the cell line. These

are usually exposed to the drug from 24 to 96 h depending on the drug activity. MTT is reduced both by metabolically active cells, and by the action of dehydrogenase enzymes, forming some reducing equivalents such as NADH and NADPH. The resulting intracellular formazan can be dissolved in an organic medium and analysed spectrophotometrically. Viable cells, as opposed to no-viable cells, reduce MTT and the amount of formazan, that is formed, is proportional to the number of metabolically active cells.

The cell viability is assessed by absorbance measurements (570 nm) carried out on the crystals mother solution (Ahmadian et al., 2009). The obtained results compared to the analysis of a procedural blank allow us to assess the percentage of surviving cancer cells after the assay. This represents the extent anticancer activity of the studied drug and its IC₅₀ value.

5.4 Synthesis and characterisation

5.4.1 Synthesis of *cis*-[Pt(PPh₃)₂(curcumin)]Cl (hereafter defined complex-4)

A solution of KOH (1.4646 mmol, 0.0853 g) in ethanol (4.0 ml) at 40°C, was added dropwise to a green solution of *cis*-PtCl₂(PPh₃)₂ (0.7250 mmol, 0.5766 g) and curcumin 1.4646 mmol, 0.5408 g) (1% in excess) in CH₂Cl₂ (40 ml) at room temperature with stirring. After overnight stirring at room temperature, the reaction mixture was filtered to remove KCl and dried under vacuum. The green product was recrystallized from CH₂Cl₂ and the solid was filtered and washed with ether and dried under vacuum. Yield: (0.7950 g, 98% yield). Mp=200.1°C (dec). Anal. Calcd for C₅₉H₅₅O₆P₂Pt [Mw: 1123.41]: C, 60.99; H, 4.37. Found: C, 62.18; H, 4.77.

HRMS (ESI, acetonitrile, positive ion mode) m/z: calcd. for C₅₉H₅₅O₆P₂Pt [M]⁺ 1087.96; found. 1087.26. ¹H NMR features are reported in table 7, except those belonging to PPh₃ groups. PPh₃ region: ¹H NMR (400 MHz DMSO-d₆, 25 °C) δ = 7.60-7.36 ppm. ¹³C{¹H} NMR (100 MHz DMSO-d₆, 25 °C) δ (ppm) = 148.46 (C9,9'), 141.09(C-3,3'), 126.63 (C-5,5'), 123.71(C-6,6'), 121.4(C-4,4'), 116.3(C-7,7'), 111.79(C-10,10'), 55.70(-OCH₃). PPh₃ region: ¹³C{¹H} NMR (100 MHz DMSO-d₆, 25 °C) δ = 135-129 ppm. ³¹P{¹H} NMR (121.5 MHz, DMSO, 25 °C): δ = 8.7 ppm

(d, $^1J_{\text{PtP}} = 3873$ Hz, $^2J_{\text{PP}} = 25$ Hz). ^{195}Pt NMR (85.99 MHz, DMSO, 25 °C): $\delta = -3951$ ppm (pseudot).

IR (nujol mull, cm^{-1}): 3419 (s, O-H), 1971-1813 (w, aromatic overtone), 1620 (s, C=O), 1600 (s, C=C_{ring}), 1511 (s, C-O), 1294 (s, enol C-O), 1028 (s, C-O-C), 956 (s, benzoate *trans*-CH vibration), 744 (s, *cis*-C-H vibration of aromatic ring).

5.4.2 Synthesis of *cis*-[PtCl(curcumin)(DMSO)] (hereafter defined complex-5)
0.0686 g ($1.6389 \cdot 10^{-4}$ mol) of $\text{K}[\text{PtCl}_3(\text{DMSO})]$ (Kukushkin et al., 1968) was dissolved in 5 ml water. A solution of an equimolar quantity of curcumin (0.0710g 0.009 g (1.6043×10^{-4} mol) and KOH in ethanol (2.5 ml), followed by a 20% excess of curcumin was added dropwise to $[\text{KPtCl}_3(\text{DMSO})]$ solution. After the first drops of ethanolic solution, the colour changed from yellow to dark orange. After 36 hours under vigorous stirring at room temperature, the reaction mixture was stored in refrigerator for the night. Then, the reaction mixture was filtered off, washed with ether and dried under vacuum at 60°C for 3 hours and at room temperature overnight. Yield: (0.0786 g, 71% yield). Mp=142.4°C (dec). Anal. Calcd for $\text{C}_{57}\text{H}_{49}\text{O}_6\text{P}_2\text{ClPt}$ [Mw: 676.04]: C, 40.88; H, 3.73. Found: C, 40.50; H, 3.47. ^1H NMR features are reported in table 7, except those belonging to DMSO group. ^1H NMR (400 MHz DMSO- d_6 , 25 °C) of DMSO group: 3.90 (s, 6H). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz DMSO- d_6 , 25 °C) δ (ppm) = 161.3(C2,2'), 150.1(C8,8'), 149.4(C9,9'), 142.1 (C4,4'), 131.8, 127.8(C5,5'), 124.6 (C6,6'), 122.5 (C3,3'), 117.1 (C7,7'), 112.8 (C10,10'), 102.3 (C1), 57.2(-OCH₃). $^{13}\text{C}\{^1\text{H}\}$ NMR (100 MHz DMSO- d_6 , 25 °C) of DMSO group: δ 41.8 (-CH₃)(ppm) ^{195}Pt NMR (86.0 MHz, DMSO, 25 °C): $\delta = -3941$ ppm. IR (KBr, cm^{-1}): 3378 (s, O-H), 3049 (m, C-H), 2000-1813 (w, aromatic overtone), 1639 (s, C=O), 1599 (s, C $\equiv$ C), 1509 (s, C $\equiv$ O), 1028 (s, C-O-C), 958 (s, S-O stretching), 713 (s, *cis*-C-H vibration of aromatic ring). IR (nujol mull, cm^{-1}): 446 cm^{-1} (m, Pt-O) symmetric stretching, 379 cm^{-1} (w, Pt-O) asymmetric stretching.

5.4.3 Synthesis of *trans*-[PtCl₂(caffeine)(DMSO)] (hereafter defined complex-

6)

0.2503 g (5.97×10^{-4} mol) di [KPtCl₃(DMSO)] was dissolved in 5 ml water, and an equimolar quantity of caffeine, followed by a 10% excess, was dissolved in 9 ml water and was added dropwise to resulting solution. The first drops of caffeine changed the colour of solution from orange to peach pink, and a white precipitate formed immediately. After overnight vigorous stirring at room temperature, the reaction mixture was filtered off, washed with alcohol and ether and dried under vacuum. Yield: (0.1786 g, 87% yield). Mp=232.4°C (dec). Anal. Calcd for C₁₀H₁₆O₃SN₄Cl₂Pt [Mw: 588.22]: N, 9.52; C, 20.40; H, 9.52. Found: N, 9.34; C, 20.36; H, 2.79. ¹H NMR (700 MHz DMSO-d₆, 25 °C): δ (ppm) = 7.29 (s, 1H, 8-H), 4.39(s, 3H, 3-H), 4.12(s, 3H, 7-H), 3.52 (s, 6H, -CH₃ of DMSO group), 3.44 (s, 3H, 1-H). ¹³C{¹H} NMR (100 MHz DMSO-d₆, 25 °C) δ (ppm) = 154.3 (C6), 151.0 (C2), 145.0 (C4), 140.7 (C8), 108.4 (C5), 43.1(-CH₃ of DMSO group), 35.1 (C7), 33.4 (C3), 28.5 (C1). ¹⁹⁵Pt NMR (85.99 MHz, DMSO, 25 °C): δ = -3009 ppm. IR (nujol mull, cm⁻¹): 3419 br, 1713 (s, C=O), 1668 (s, C=O overlapped with C=C stretch), 1612 (s, C=N), 1459 (s, C=C), 1378 (s, C-N), 1236 (s, C=O ketonic group), 1141 (s, C=O ketonic group; w, C-C), 1035 (m, C=O), 844 (w, C-C).

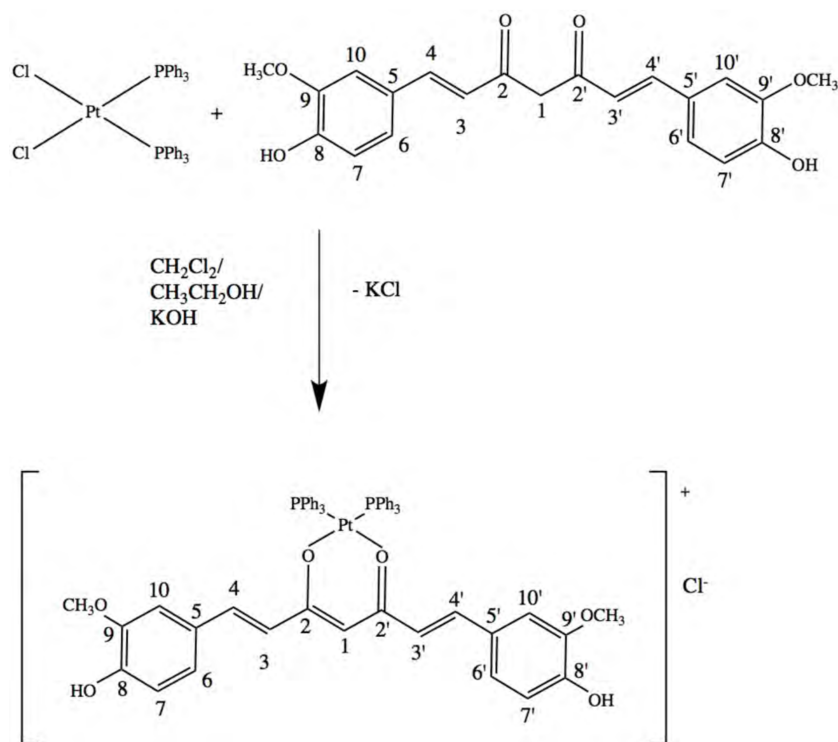
6. RESULTS AND DISCUSSION

6.1 Synthesis and characterisation of Pt(II) complexes

When curcumin reacted with the platinum precursor under alkaline conditions the cationic complex 4 formed (scheme 7), although the chloride ion is not a weakly coordinating anion. In curcumin exists a tautomeric equilibrium, with two possible enol structures through intramolecular hydrogen transfer. In some organic solvents the enol form predominates. Curcumin is a monobasic bidentate ligand and forms stable complexes with almost all the metals. The α,β -unsaturated β diketo moiety of curcumin is an excellent chelating agent (Priyadarsini, 2014).

The $^{31}\text{P}\{^1\text{H}\}$ NMR spectrum of 4 in DMSO-d_6 shows a singlet with satellite at $\delta = 8.7$ ppm with $^1J_{\text{PtP}} = 3882$ Hz, according to its ^{195}Pt NMR spectrum reporting a triplet at $\delta = -4400$ ppm. The ^1H NMR spectrum consists of the triphenyl phosphane proton signals between $\delta = 7.40$ - 7.60 ppm, the olefinic proton H4, H4' adjacent to aromatic rings appear upfield as compared to H3, H3' (doublet at $\delta = 7.56$; $^3J_{\text{H3,H4}} = 7.1$ Hz). The signals from aromatic protons H10,H10' and H6,H6' appear at downfield than H7,H7'. The latter was assigned to the proton of the 5-OH moiety, because the ^1H - ^{13}C HSQC spectrum of 4 report any relevant cross peaks: 3C,3'C signal that coupled with 3H,3'H and also with 1H, 6-6'C coupled with 6-6'H and with adjacent 7-7'H (Payton et al., 2007). The IR spectrum shows a strong sharp peak at 3419 cm^{-1} , which can be assigned to the free O-H bond stretching and an intense band at 1620 cm^{-1} assigned to carbonyl group (Kim et al., 2013).

The ESI-MS spectrogram in the positive scale of a diluted acetonitrile solution of 4 showed the peak at m/z 1087.26, whose isotope pattern is perfectly superimposable on that calculated for the cation $\text{C}_{59}\text{H}_{55}\text{O}_6\text{P}_2\text{Pt}^+$ (corresponding to $[\text{Pt}(\text{PPh}_3)_2(\text{curcumin})]^+$), obtained by loss of the chloride anion.



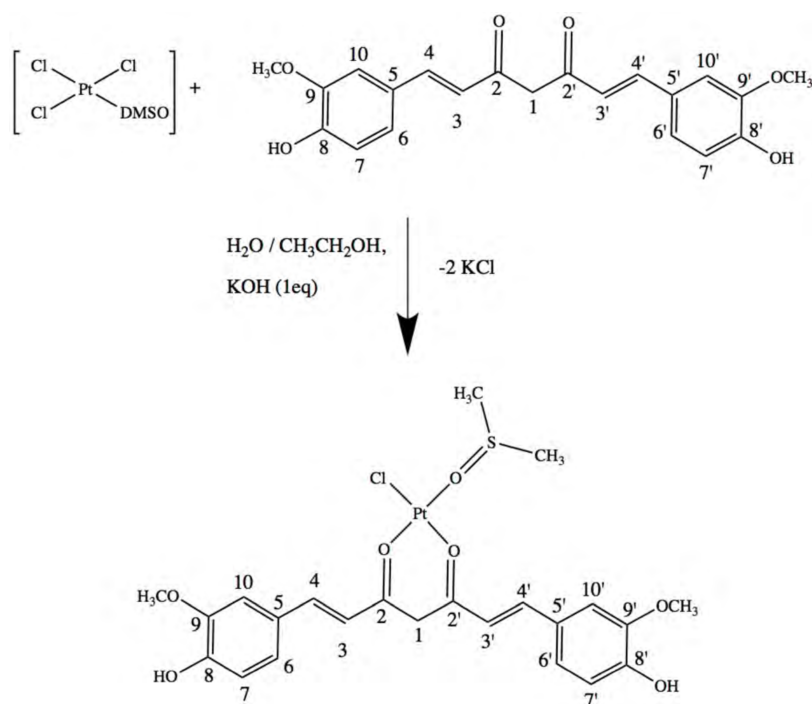
Scheme 7- synthesis of $[\text{Pt}(\text{PPh}_3)_2(\text{curcumin})]\text{Cl}$ (4)

Curcumin-metal complexes not only modify the physico-chemical properties of curcumin but they also affect the biological reactivity of the metals. For this reason, the next step of the present research was the synthesis of platinum complexes from a Pt(II) precursor with dimethyl sulphoxide as ancillary ligand. The selected precursor was $\text{K}[\text{PtCl}_3(\text{DMSO})]$ (Yu.N. Kukushkin 1968), where DMSO is the abbreviation for dimethyl sulphoxide.

The synthesis of platinum complex with curcumin and DMSO (complex 5), as ancillary ligands, has been carried out according to a new synthetic strategy shown in scheme 8.

For complex 5 spectroscopic evidences indicate that $\text{Pt-O}, \text{O'}$ -curcumin involves the 2 and 2' oxygens. This is mainly highlighted by the change of stretching frequency towards lower wavenumbers. For chelate β -diketonate complexes (De Pascali et al., 2014), the highest reported stretching frequencies for $\text{C}=\text{O}$ decrease, in agreement with the "aromatic" nature of the system resulting from delocalization of the π electrons

in the chelate ring. Depending on the identity of the metal and other ancillary ligands, DMSO can exhibit S-, O- coordination mode.



Scheme 8- synthesis of $[PtCl(curcumin)(DMSO)]$ (5)

Generally for Pt(II) and for late transition metals DMSO coordination is via the sulfur atom, but O-coordination has also been observed. The 2D NMR $^1H\{^{13}C\}$ -HMQC of 5 complex shows the ancillary DMSO ligand bonded to Pt through O atom. The O-coordination is testified by the upfield of the CH_3 protons whereas the S-coordination would provide their downfield of the chemical shift (Diao et al., 2012). The O-coordination is also confirmed by Infra-Red spectrum of complex 5, both in KBr and Nujol mull. These IR spectra of complex 5 show an intense S-O stretching band at lower frequency value than free DMSO (Fig.45) (Cotton and Francis, 1960, Nakamoto, 2008b, Nakamoto, 2008a).

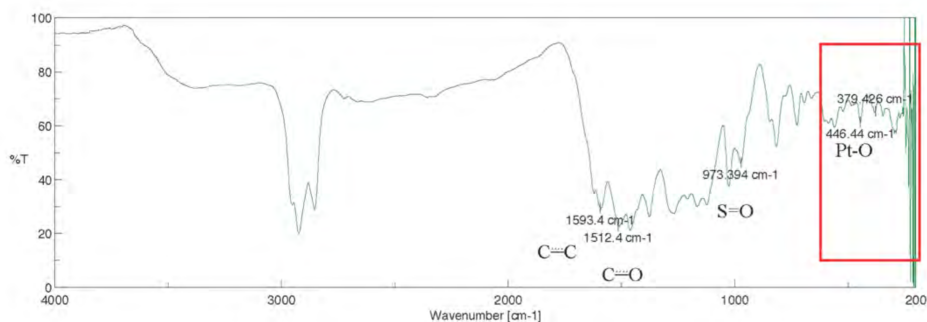


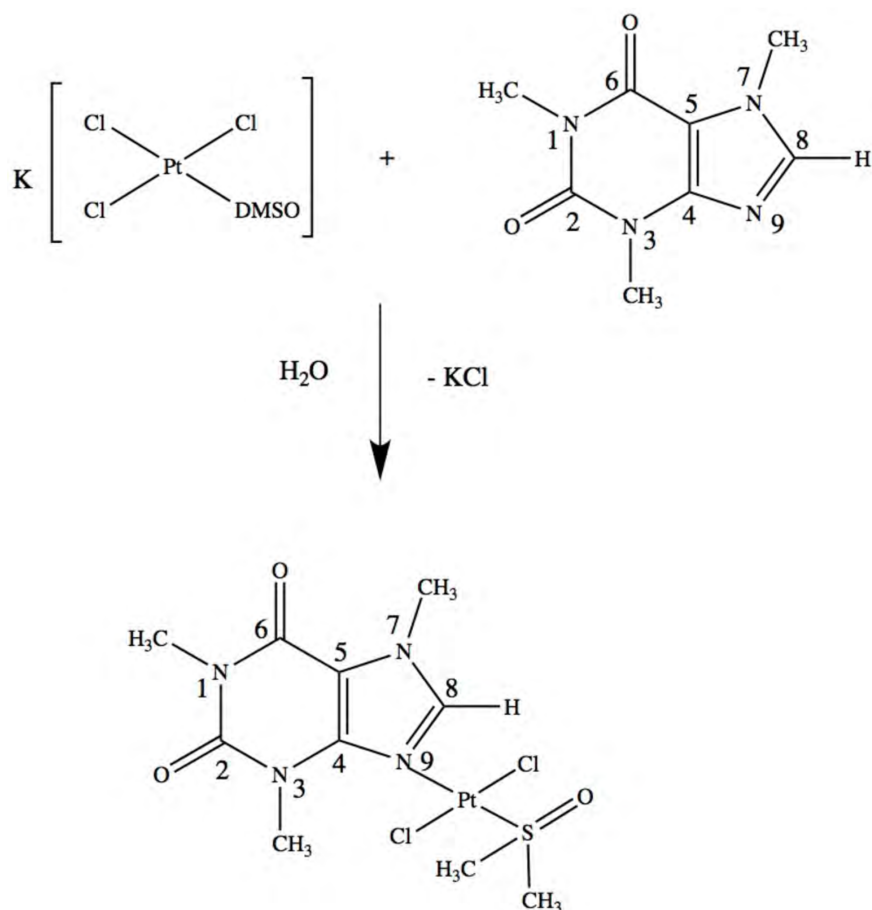
Fig. 45- Infra Red spectrum of complex 5 in Nujol mull 4000-200cm⁻¹

The ¹H NMR spectrum of complex 5 shows an exchange of the chemical shift values between 3,3'-H and 4,4'-H protons. This phenomenon is not recognised in 4 complex, where 3,3'-H and 4,4'-H protons are addressed to 3,3'-C and 4,4'-C, respectively. So, a long-range effect could be responsible of the exchange of 3,3'-H and 4,4'-H protons values recognised in complex 5 (Benassi et al., 2008).

	1-H	3,3'-H	4,4'-H	6,6'-H	7,7'-H	8,8'-H	10,10'-H	CH ₃ O-
[Pt(PPh ₃) ₂ (curcumin)]Cl	6.06 (s)	7.54 (d, ³ J _(3,4) =15.2 Hz)	6.75 (s, ³ J _(4,5) =15.2 Hz)	7.15 (dd, ³ J _(6,4) =8.0 Hz, ⁴ J _(6,5) =1.7 Hz)	6.82 (d, ³ J _(7,6) =8.1 Hz)	-	7.32 (s)	3.81 (s)
Curcumin	6.10 (s)	7.50 (d, ³ J _(3,4) =16.0 Hz)	6.70 (s)	7.20 (dd, ³ J _(6,4) =8.1 Hz, ⁴ J _(6,5) =2.0 Hz)	6.80 (d, ³ J _(7,6) =8.1 Hz)	9.60 (s)	7.30 (s)	3.90 (s)
[PtCl(curcumin)(DMSO)]	6.05 (s)	6.73 (d, ³ J _(3,4) =15.7 Hz)	7.52 (d, ³ J _(4,5) =15.7 Hz)	7.13 (dd, ³ J _(6,4) =8.1 Hz, ⁴ J _(6,5) =1.2 Hz)	6.80 (d, ³ J _(7,6) =8.1 Hz)	-	7.28 (dd)	3.90 (s)

Table 7- ¹H NMR spectral data of 4, 5 and curcumin (dmsd-d₆, 298 K).

Complex 6 was synthesized from the same precursor of complex 5 but using caffeine as natural ligand. The 2D NMR ¹H¹³C-HMBC of complex 6 shows the coupling of quaternary carbon atom and carbonyl groups. This spectrum displays that 2-C carbonyl group, at δ=151 ppm, coupled with 1-H and 3-H and the other carbonylic carbon (6-C) coupled with 1-H and 8-H. At the same time the quaternary carbon atom 4 coupled with 3-H and 8-H, and 4-C coupled with 7-H and 8-H (Sitkowski et al., 1995). A phenomenon recognised in ¹H¹³C -HMBC of complex 6 is the upfield of chemical shift of 8-C and consequently of 8-H directly bonded.



Scheme 9- synthesis of *trans*-[PtCl₂(caffeine)(DMSO)] (6)

The change in 8-H proton chemical shift is quite clear comparing ¹H-NMR of free ligand with complex 6 ¹H-NMR spectrum (Fig.47). So, a caffeine binding through 9-N atom could be responsible of the change of 8-H and 8-C values recognised in complex 6. The ¹H NMR spectrum of complex 6 shows a change of the CH₃ protons chemical shift values of DMSO. This phenomenon testifies S-coordination that implies the downfield of the CH₃ protons chemical shift(Diao et al., 2012). The S-coordination is also confirmed by Infra-Red spectrum of complex 6, because it shows an intense band at 1035 cm⁻¹ assigned to the S-O stretching (Fig. 46) (Cotton and Francis, 1960, Cotton et al., 1960).

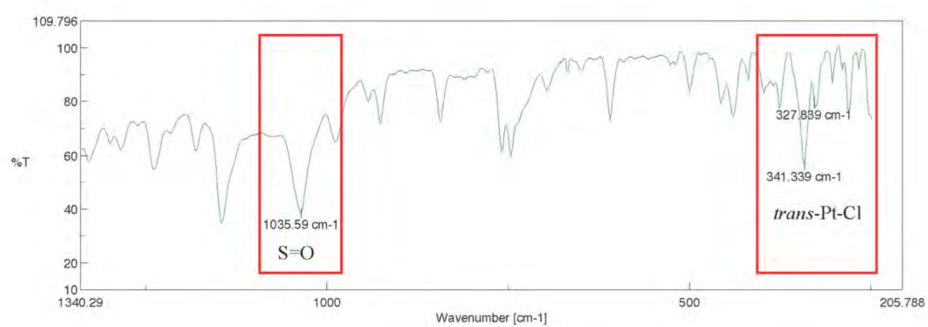


Fig. 46- Expansion of Infra-Red spectrum of complex 6 in Nujol mull between 1340-200 cm-1

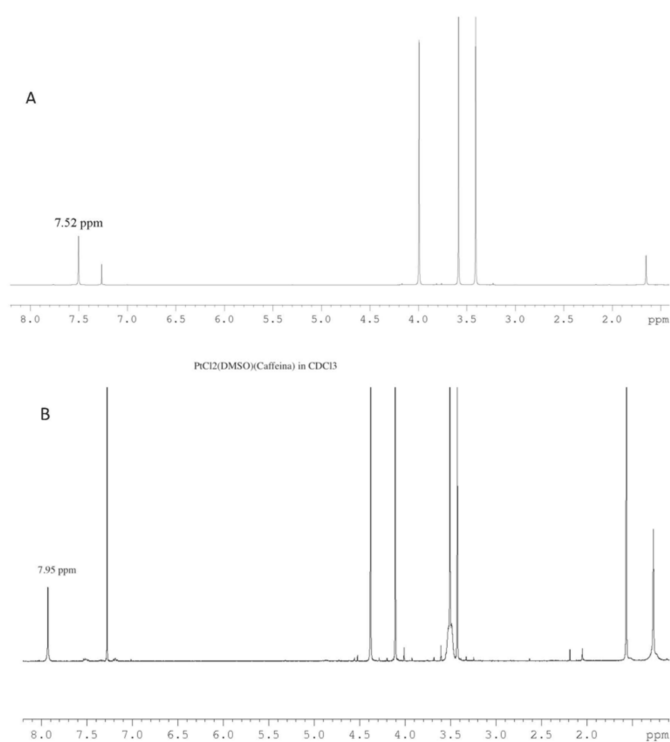


Fig. 47 ^1H NMR spectrum of free caffeine (A) in comparing with ^1H NMR spectrum of caffeine in Complex 6, both in CDCl_3

At the same time, the IR spectrum highlights the *trans*-geometry of complex 6, as testifies the Cl-Pt-Cl stretching values at 341 and 327 cm^{-1} (Fig. 46).

6.2 UV-Vis Studies

The ability of the platinum complexes to interact non-covalently with DNA was examined by UV-Vis absorption spectroscopy. Hence, absorption spectra were recorded at a constant complex concentration of 25 μM and adding increasing amounts of ct-DNA, from 0 to 100 μM . The corresponding spectra for $[\text{PtCl}(\text{DMSO})(\text{PPh}_3)_2]\text{Cl}$ are shown in Fig.35. Those for $[\text{PtCl}(\text{DMSO})(\text{curcumin})]$ and *trans*- $[\text{PtCl}_2(\text{DMSO})(\text{caffeine})]$ are depicted in Fig.49 and Fig.50, respectively.

The absorption bands in the region 200–650 nm, which are attributed to metal to-ligand charge transfer (MLCT), were used to monitor the interaction of the complexes with duplex DNA.

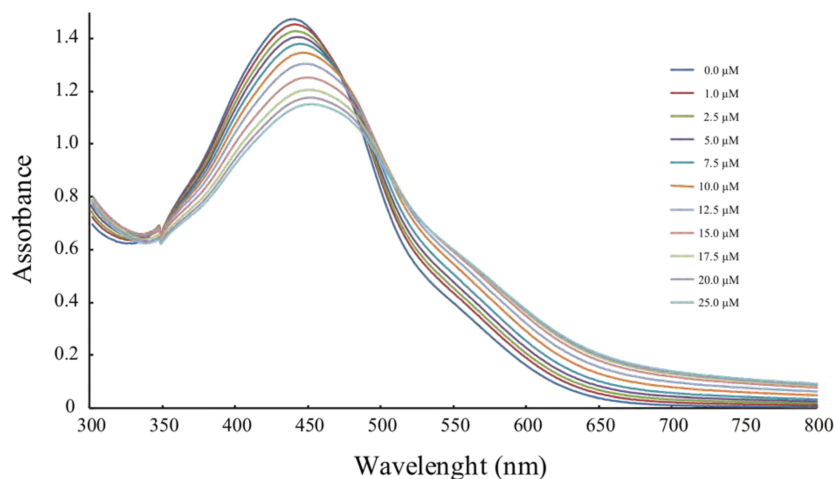


Figure 48-Absorption spectra of complex $[\text{PtCl}(\text{curcumin})(\text{PPh}_3)_2]$ in cacodylate-NaCl buffer ($\text{pH} = 7.2$) upon addition of ct-DNA. $[\text{complex}] = 25 \mu\text{M}$, $\text{ct-DNA} = 0\text{--}25 \mu\text{M}$

Hypochromism is clearly observed, which suggests an electrostatic interaction between the binding complex and the biomolecule (Navarro, 2009) (E. C. Long and J. K. Barton, *Acc. Chem. Res.*, 1990, 23, 271–273). No redshifts of the absorption bands are observed; actually, a shift towards a longer wavelength is indicative of an intercalative binding mode. (Bradley et al., 2004, Barton et al., 1984). Therefore, *trans*-

[PtCl₂(DMSO)(caffeine)] and [PtCl(DMSO)(curcumin)] do not seem to intercalate between DNA base pairs; rather it appears that *trans*-PtCl₂(DMSO)(Caffeine) and [PtCl(DMSO)(Curcumin)] tend to interact electrostatically or via groove binding with the double helix.

Indeed, addition of [PtCl(curcumin)(PPh₃)₂]Cl in small aliquots to a solution of ct-DNA resulted in a bathochromic shift of the maximum absorption associated with a significant hypochromicity. Such hypochromism is due to strong interactions between the electronic states of the intercalating chromophore and those of the DNA base pairs (Sirajuddin et al., 2013).

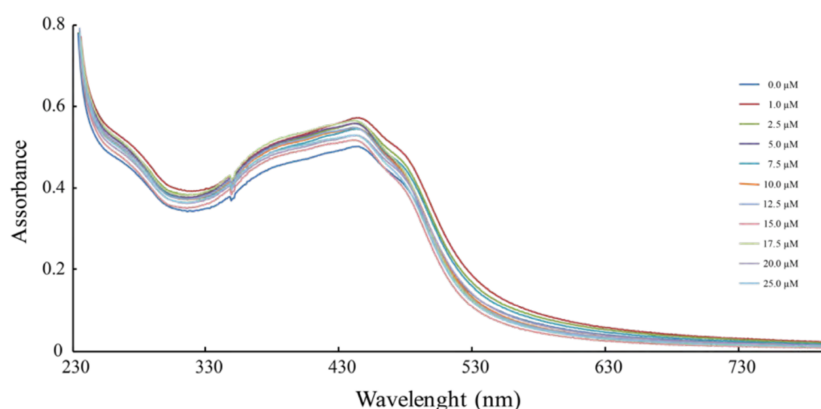


Figure 49-Absorption spectra of complex [PtCl(curcumin)(DMSO)] in cacodylate-NaCl buffer (pH = 7.2) upon addition of ct-DNA. [complex] = 25 μM, ct-[DNA] = 0-25 μM.

The value of the complex–DNA affinity constant K_b obtained for *trans*-[PtCl₂(DMSO)(caffeine)] is $3.0 \times 10^7 \text{ M}^{-1}$ and that of [PtCl(DMSO)(curcumin)] is $8.7 \times 10^5 \text{ M}^{-1}$.

For [PtCl(curcumin)(PPh₃)₂]Cl, a linear fitting of the spectroscopic data could not be obtained; therefore, only a qualitative analysis of the DNA-complex interaction could be achieved.

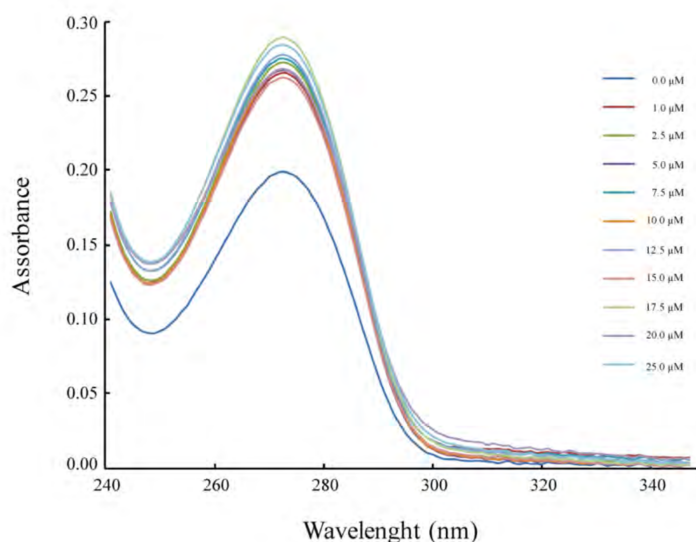


Figure 50-Absorption spectra of complex *trans*-[PtCl₂(Caffeine)(DMSO)] in cacodylate-NaCl buffer (pH = 7.2) upon addition of ct-DNA. [complex] = 25 μM, ct-[DNA] = 0-25 μM.

The affinity constant values determined for *trans*-[PtCl₂(DMSO)(caffeine)], [PtCl(DMSO)(curcumin)] are respectively $3.0 \times 10^7 \text{ M}^{-1}$ and $8.8 \times 10^5 \text{ M}^{-1}$. For [PtCl(curcumin)(PPh₃)₂]Cl, K_b could not be determined. The results obtained suggest that there are strong interactions between the metal complexes and DNA; however, other characterization studies are necessary to confirm this assumption.

Complex	Intercept ($\times 10^{-11}$)	Slope ($\times 10^{-4}$)	$K_b \text{ (M}^{-1}\text{)}$
<i>trans</i> -[PtCl ₂ (DMSO)(caffeine)]	1.0	3.0	3.0×10^7
[PtCl(DMSO)(curcumin)]	0.8	7.0	8.8×10^5

Table 8- Intrinsic binding constant K_b determined for complex *trans*-[PtCl₂(DMSO)(caffeine)], [PtCl(DMSO)(curcumin)]

6.3 Fluorescence Studies

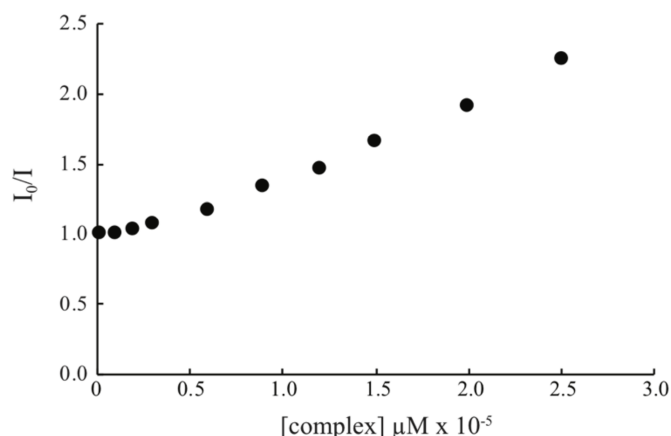


Figure 51 - Plots of I_0/I vs. [complex] for the titration of DNA–EB with $[\text{PtCl}(\text{curcumin})(\text{PPh}_3)_2]\text{Cl}$ at $\lambda_{\text{exc}} = 514 \text{ nm}$ and $\lambda_{\text{em}} = 610 \text{ nm}$: experimental data points and linear fitting of the data.

Aiming at fully understanding the behaviour of the metallic complexes in presence of DNA, fluorescence measurements have been performed with ethidium bromide (EtBr or EB), which is a planar cationic dye well-known to intercalate into the DNA double helix. A second fluorophore, namely Hoechst33258, was also used, which binds to DNA in the minor groove at low dye-to-DNA ratios.

The changes observed in the fluorescence emission spectra of a solution of EB–DNA upon addition of the Pt(II) complexes can be used to explore their ability to displace the intercalated EB. The addition of $[\text{PtCl}(\text{curcumin})(\text{PPh}_3)_2]\text{Cl}$ to EB–DNA causes a significant reduction of the fluorescence intensity, which suggests that this platinum(II) compound is capable of displacing EtBr through its interaction with ct-DNA (Fig.52A). To investigate the quenching efficiency of this process, the linear Stern–Volmer quenching constant (k_{sv}) was calculated applying the Stern–Volmer equation. K_{sv} amounts to $5.0 \times 10^4 \text{ M}^{-1}$ for $[\text{PtCl}(\text{curcumin})(\text{PPh}_3)_2]\text{Cl}$. This high value may be ascribed to the interaction of the complex between adjacent DNA base pairs (Mukherjee et al., 2016) (Fig.51)

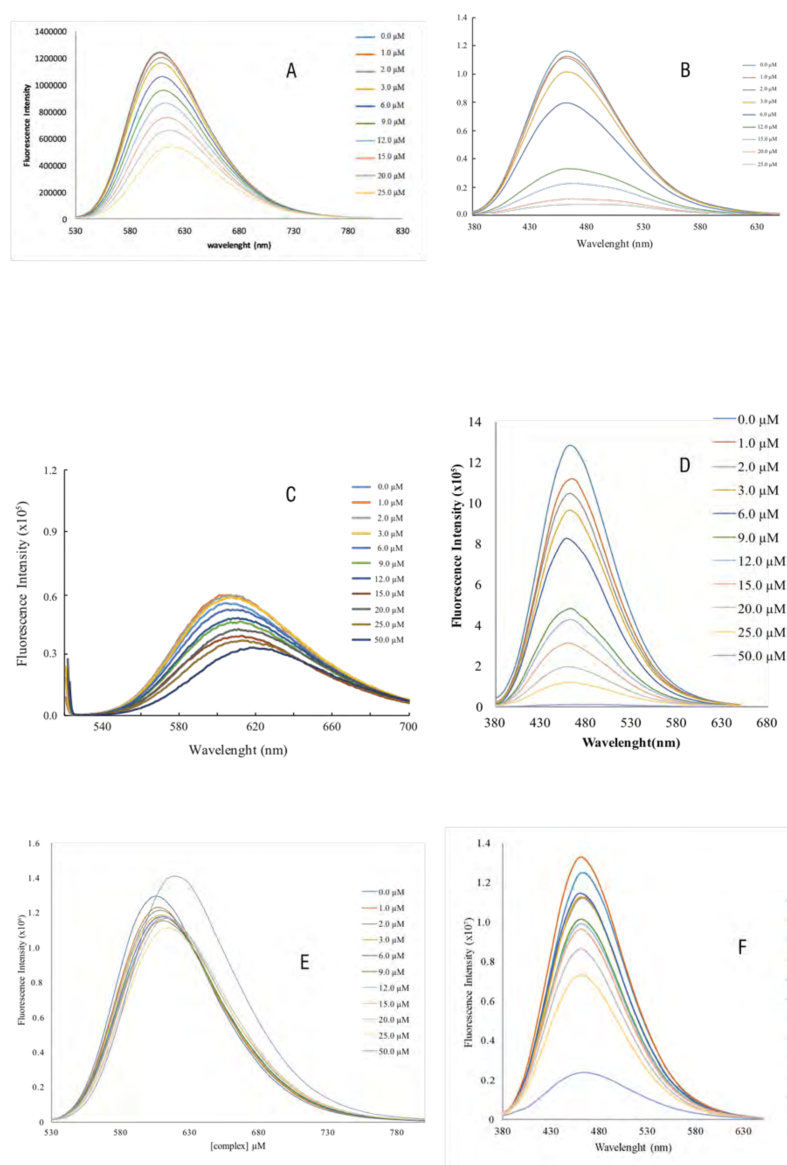


Figure 52-Emission spectra of the DNA-EB complex, $\lambda_{exc} = 514$ nm, $\lambda_{em} = 610$ nm, upon addition of increasing amounts of $[PtCl(curcumin)(PPh_3)_2]Cl$ (A), $[PtCl(DMSO)(curcumin)]$ (C) and *trans*- $[PtCl_2(DMSO)(caffeine)]$ (E). Emission spectra of the DNA-Hoechst33258 complex, $\lambda_{exc} = 350$ nm, $\lambda_{em} = 458$ nm, upon addition of increasing amounts of $[Pt(curcumin)(PPh_3)_2]Cl$ (B), $[PtCl(DMSO)(curcumin)]$ (D) and *trans*- $[PtCl_2(DMSO)(caffeine)]$ (F).

. The changes observed in the fluorescence emission spectra of a solution containing Hoechst33258-DNA upon addition of increasing amounts of this complex indicates that it most likely interacts with DNA through different ways.

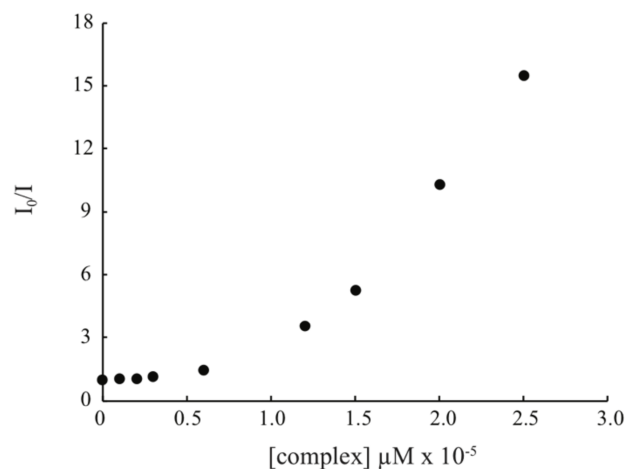


Figure 53 - Plots of I_0/I vs. [complex] for the titration of DNA–Hoechst33258 with $[\text{PtCl}(\text{curcumin})(\text{PPh}_3)_2]\text{Cl}$ at $\lambda_{\text{exc}} = 350 \text{ nm}$ and $\lambda_{\text{em}} = 458 \text{ nm}$: experimental data points and linear fitting of the data.

The study of the interaction of $[\text{PtCl}(\text{DMSO})(\text{curcumin})]$ with DNA-EtBr shows that the addition of increasing amounts of the metal complex does not produce any significant decrease of the fluorescence intensity. This result clearly suggests that $[\text{PtCl}(\text{DMSO})(\text{curcumin})]$ does not compete with ethidium bromide for DNA intercalation (Fig.52C) (Basu and Kumar, 2013).

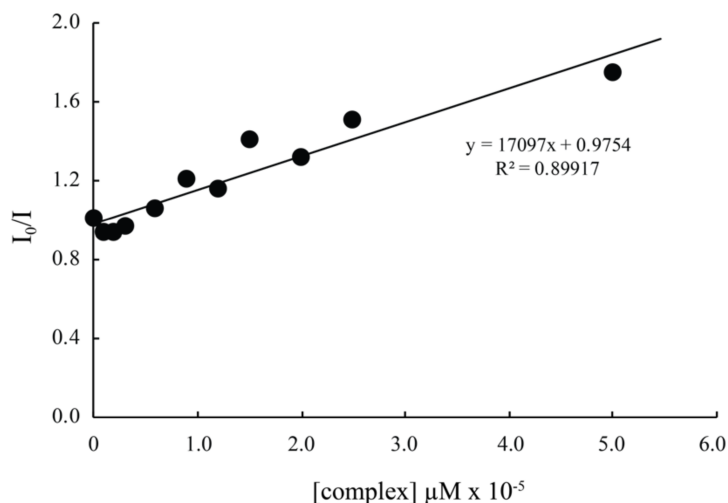


Figure 54 - Plots of I_0/I vs. [complex] for the titration of DNA-EB with [PtCl(curcumin)(DMSO)] at $\lambda_{\text{exc}} = 514 \text{ nm}$ and $\lambda_{\text{em}} = 610 \text{ nm}$: experimental data points and linear fitting of the data

Fluorescence studies of the same complex with DNA-Hoechst33258 have also been carried out.

The K_{sv} value for the interaction of [PtCl(DMSO)(curcumin)] with DNA-EtBr is $1.7 \times 10^4 \text{ M}^{-1}$ (Fig.54). For the interaction with DNA-Hoechst33258, the K_{sv} value could not be determined, because the complex induces a combined dynamic and static fluorescence quenching (Fig.55).

Fluorescence quenching can occur by different mechanisms, which are usually classified as dynamic and static quenching. Dynamic quenching refers to a process in which the fluorophore and the quencher come into contact during the transient existence of the excited state. Static quenching characterizes the formation of a fluorophore-quencher complex. In general, dynamic and static quenching can be distinguished by their differing dependence on the temperature and the excited state lifetime. Since in both cases the fluorescence intensity is related to the concentration of the quencher, the quenched fluorophore can serve as an indicator for the quenching agent.

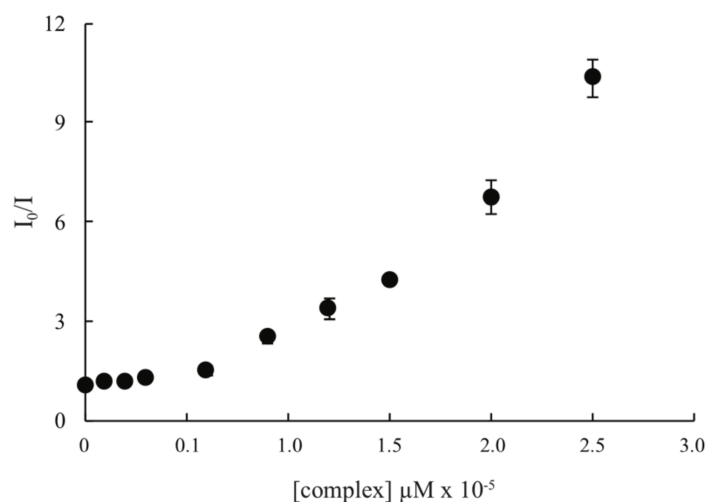


Figure 55 - Plots of I_0/I vs. $[\text{complex}]$ for the titration of DNA–Hoechst33258 with $[\text{PtCl}(\text{curcumin})(\text{DMSO})]$ at $\lambda_{\text{exc}} = 350 \text{ nm}$ and $\lambda_{\text{em}} = 458 \text{ nm}$: experimental data points and linear fitting of the data.

In the case of *trans*- $[\text{PtCl}_2(\text{DMSO})(\text{caffeine})]$, the competitive binding studies conducted in presence of DNA-EB show a weak decrease of the emission intensity when increasing amounts of metal complex are added (Fig.52E)

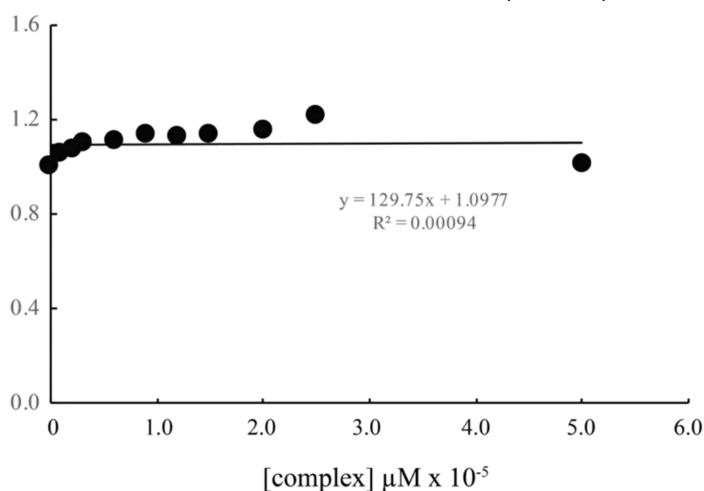


Figure 56-Plots of I_0/I vs. $[\text{complex}]$ for the titration of DNA–EB with *trans*- $[\text{PtCl}_2(\text{DMSO})(\text{caffeine})]$ at $\lambda_{\text{exc}} = 514 \text{ nm}$ and $\lambda_{\text{em}} = 610 \text{ nm}$: experimental data points and linear fitting of the data.

Upon addition of *trans*-[PtCl₂(DMSO)(caffeine)] to DNA-Hoechst33258, a decrease of the fluorescence is detected, which suggests that this platinum complex apparently binds in the minor groove of the DNA double helix (Fig.52F) (Samper et al., 2014).

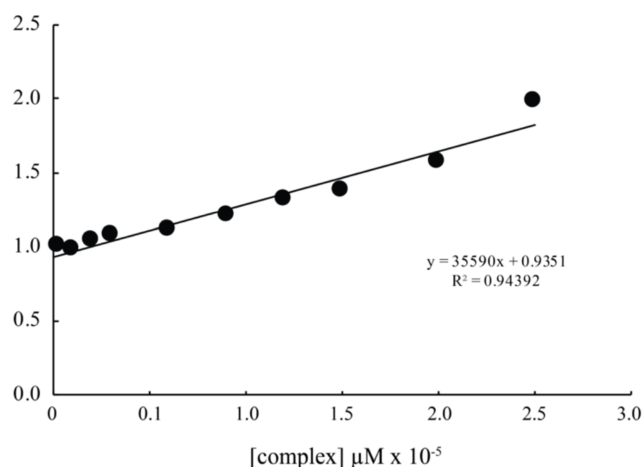


Figure 57-Plots of I_0/I vs. [complex] for the titration of DNA-Hoechst33258 with *trans*-[PtCl₂(caffeine)(DMSO)] at $\lambda_{\text{exc}} = 350 \text{ nm}$ and $\lambda_{\text{em}} = 458 \text{ nm}$: experimental data points and linear fitting of the data.

The K_{sv} value for this interaction is $3.6 \times 10^4 \text{ M}^{-1}$. For DNA-EB, a lower K_{sv} value is obtained (Fig.52), illustrating the poorer ability of *trans*-[PtCl₂(caffeine)(DMSO)] to display the DNA intercalator. This feature thus suggests that this compound may have a greater propensity to act as a groove binder, hence corroborating the UV-Vis observations (Brissos et al., 2014).

6.4 Circular Dichroism studies.

In general, the calf thymus DNA CD spectrum shows a positive band (275 nm UV: $\lambda_{\text{max}} = 260 \text{ nm}$) representing an effect of the base stacking and a negative one (245 nm) determined by the helicity characteristic of the DNA in right-handed B form.

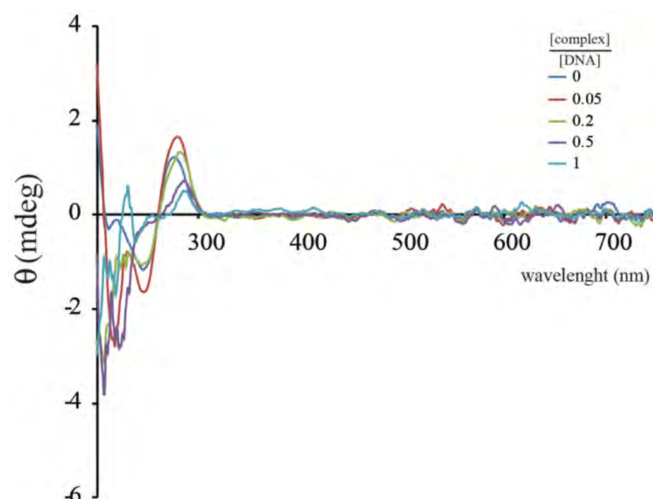


Fig. 58-Circular dichroism spectra of CT-DNA in Cacodilate (1mM) – NaCl (20mM), in the presence of increasing amounts of the *trans*-PtCl₂(DMSO)(Caffeine) complex.

Figure 58 shows the CD spectrum of the CT DNA added of *trans*-PtCl₂(DMSO)(Caffeine) complex at concentration ratio ranging from 0 to 1. The findings of Fig. 58 indicate that the CD intensity increases at low complex concentrations whereas the CD signal decreases as the *trans*-PtCl₂(DMSO)(Caffeine) concentration increases. This evidence suggests that the metal complex is partially inserted in DNA base pairs while minor groove interactions occur (Shahabadi and Moghadam, 2012). These results represent a further confirmation of evidences from the fluorescence analyses.

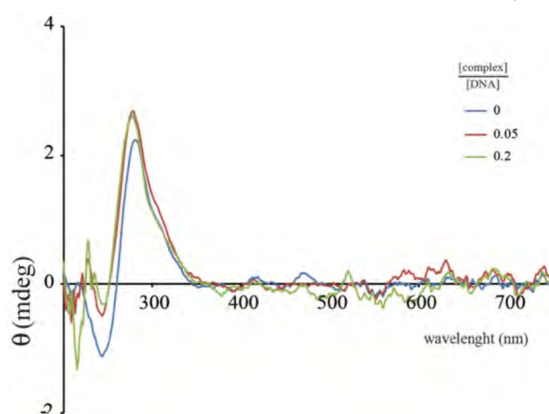


Fig. 59 - Circular dichroism spectra of ct-DNA in Cacodilate (1mM) – NaCl (20mM) buffer, in the presence of increasing amounts of the [Pt(PPh₃)₂(Curcumin)]Cl complex.

Figure 59 shows the CD spectrum of the CT DNA added of $[\text{Pt}(\text{PPh}_3)_2(\text{Curcumin})]\text{Cl}$ complex at concentration ratio spanning from 0 to 0.2. The findings of Fig. 59 indicate that the CD intensity of the positive band is increased and the negative band depressed by the metal complex addition. This behavior suggests an insertion of the $[\text{Pt}(\text{PPh}_3)_2(\text{Curcumin})]\text{Cl}$ complex in the DNA structure with some changes of the DNA conformation from B to A (Shahabadi and Moghadam, 2012, Zhu et al., 2015).

Figure 60 shows the CD spectrum of the CT DNA to $[\text{PtCl}(\text{Curumin})(\text{DMSO})]$ complex at concentration ratio spanning from 0 to 1. The spectrum highlights a decreasing extent of the positive dichroic signal and a remarkably reductions in the intensities of negative bands (Basu and Kumar, 2013). For this reason, a weak negative induced CD signal can be observed. It is suggestive of a strong complex-ct-DNA interaction. These negative-induced CD changes in complex-DNA interactions could agree with minor groove interaction modes (Nguyen et al., 2004).

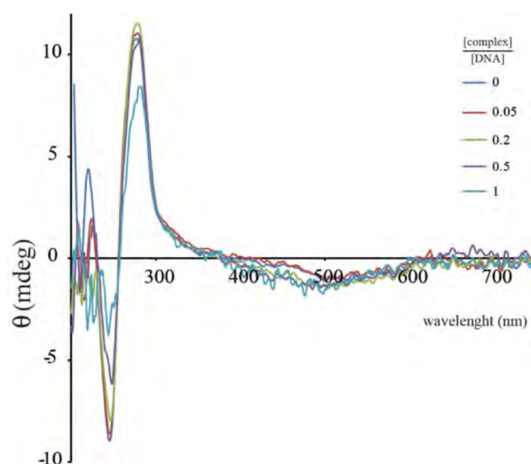


Fig. 60- Circular dichroism spectra of CT-DNA in Cacodilate (1mM) – NaCl (20mM), in the presence of increasing amounts of the $[\text{PtCl}(\text{Curumin})(\text{DMSO})]$ complex.

6.5 Agarose Gel Electrophoresis studies

The action of the complexes on the tertiary structure of DNA was established by their capability to change the electrophoretic mobility of the different forms of pBR322 plasmid DNA. In that context, agarose gel electrophoresis is a common and simple

method to visualize the different typical shapes of plasmid DNA, namely the normal supercoiled form (form I), the circular nicked form (form II), which is obtained after cutting apart only one of the two strands, and the linear form (form III), which is produced when both strands are broken. Hence, uncut plasmids (form I) will migrate more rapidly than linearized plasmid (form III).

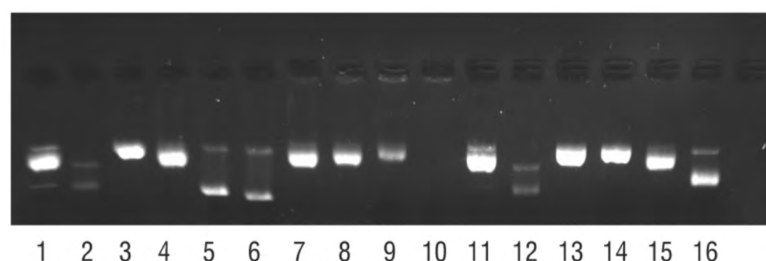


Figure 61-Agarose gel electrophoresis images of pBR322 plasmid DNA incubated for 24 h at 37 °C with increasing concentrations of complexes. [PtCl(DMSO)(curcumin)] (lanes 3 to 6), [PtCl(curcumin)(PPh₃)₂]Cl (lanes 7 to 10) and *trans*-[PtCl₂(DMSO)(caffeine)] (lanes 13 to 16).

Moreover, the nicked circles (form II), which are the bulkiest, will be the slowest migrating species in the gel (since the separation is not only by charge but also by size). Thus, increasing amounts of the complexes were incubated with pBR322 DNA for 24 h. In Figure 61, free DNA can be observed at lanes 1 and 11, cisplatin at lanes 2 and 12, [PtCl(DMSO)(curcumin)] at lanes 3 to 6, [PtCl(curcumin)(PPh₃)₂]Cl at lanes 7 to 10 and *trans*-[PtCl₂(DMSO)(caffeine)] at lanes 13 to 16. At low complex concentrations, a significant decrease of the electrophoretic mobility of form I is noticed. For all the complexes investigated, the mobility of form I decreases until its band merges with that of the nicked relaxed form (form II). This can arise from an increasing formation of an adduct between the DNA and the metal compound (Roy et al., 2009). However, at higher concentrations, the mobility of this form tends to increase for both [PtCl(DMSO)(curcumin)] and *trans*-[PtCl₂(DMSO)(caffeine)]. A bound drug-to-nucleotide ratio (rb) characterizes the coalescence point, which is the point corresponding to the amount of platinum complex required for the complete removal of all supercoiled DNA. Beyond this point, the migration rate begins to increase again as positive supercoils are produced (Keck and Lippard, 1992). This behavior is similar to that observed

for cisplatin at high concentrations. The electrophoretic behavior of pBR322 DNA incubated with the compounds $[\text{PtCl}(\text{DMSO})(\text{curcumin})]$ (Fig. 61, lane 4), $[\text{Pt}(\text{curcumin})(\text{PPh}_3)_2]\text{Cl}$ (Fig.61, lanes 7-9) and *trans*- $[\text{PtCl}_2(\text{DMSO})(\text{caffeine})]$ (Fig.61, lanes 13-15), is close to that of cisplatin at low concentrations, which seems to indicate that the interaction of this compound is not only intercalative (Grau et al., 2015, Ruiz et al., 2005).

6.6- Atomic Force Microscopy studies

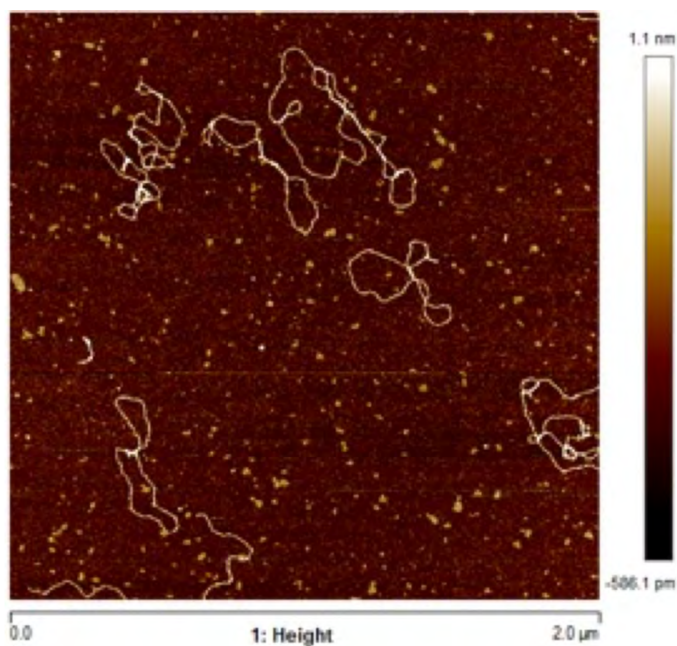


Fig. 62- AFM image of pure pBR322 plasmid DNA

AFM investigations were carried out after samples incubation with pBR322 (37 °C for 24 h in the dark). Figure 62 shows that the original open circular structures of DNA are altered after the incubation with complexes. This evidence suggests that an interaction between these complexes and DNA occur. These indications are consistent with other spectroscopic analyses.

Figure 63 shows the effects induced in DNA by the interactions with the studied complexes. Figure 63a shows the effects of the addition of the $[\text{PtCl}(\text{Curumin})(\text{DMSO})]$ complex (10 μM) to pBR322 DNA. The recognition of some crossing points suggests

the possible supercoiling inception highlighted by the appearance of some supercoiled forms.

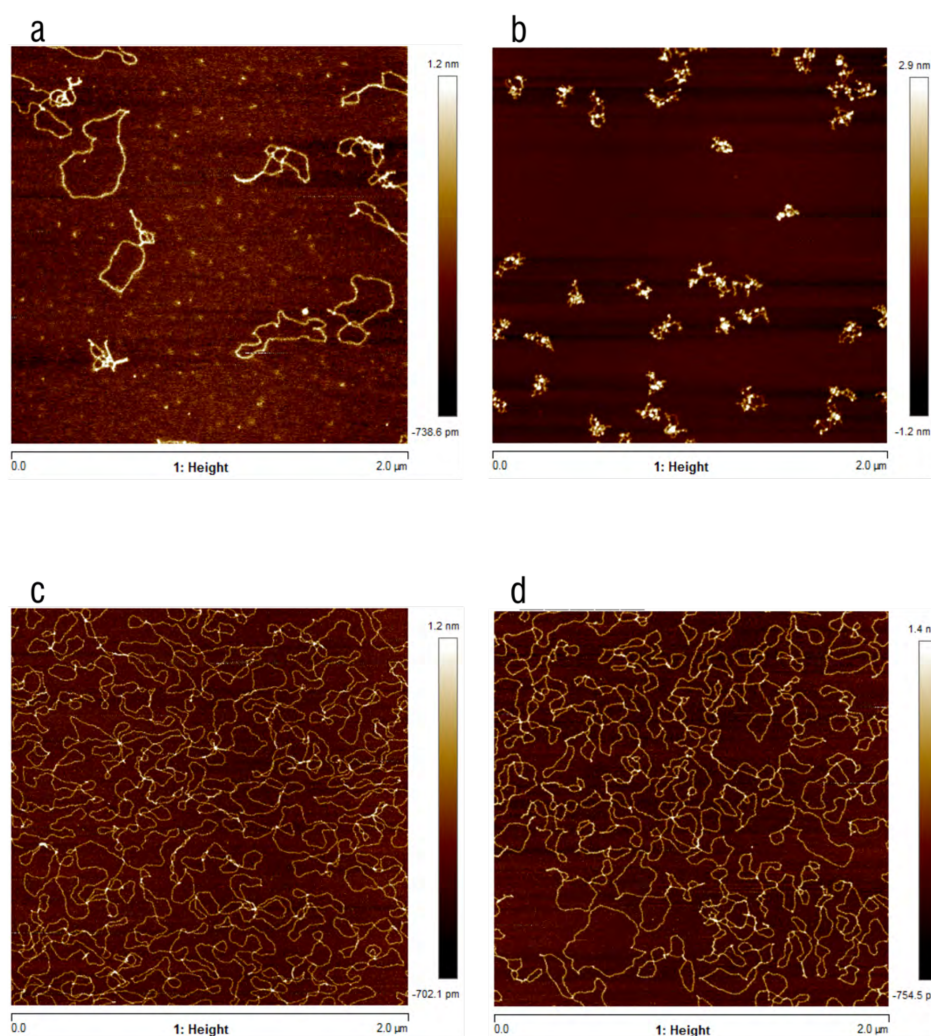


Fig. 63- Plasmid DNA in the presence of complexes $[PtCl(Curumin)(DMSO)]$ $10\mu M$ (a) and $50\mu M$ (b), $trans-PtCl_2(DMSO)(Caffeine)$ $10\mu M$ (c) and $50\mu M$ (d). $15\mu M$ bp DNA per sample.

Figure 63b shows the effects of the addition of the $[PtCl(Curumin)(DMSO)]$ complex ($50\mu M$) to pBR322 DNA. Here, the appearance of super coiled form with globular aggregates suggestive of stronger interactions between complex and DNA is shown (Liu et al., 2010, Grau et al., 2015). These evidences agree with results obtained from

electrophoresis that show the almost complete disappearance of the form II of DNA induced by the addition of the [PtCl(Curumin)(DMSO)] complex (50 μ M), as reported by the behaviour of the lane 6 (Fig. 61). Figures 61c and d show the pBR322 DNA incubated with the *trans*-PtCl₂(DMSO)(Caffeine) respectively at complex concentration of 10 and 50 μ M, respectively. Both these images show slightly modifications of the CT-DNA structure induced by interactions with the metal complex. Otherwise, these evidences can suggest some kicks more than pure pBR322 DNA and presence of supercoiled form (Gay et al., 2015). These results are consistent with the electrophoretic data (lane 16, fig.61) showing the disappearance of II bands after the addition of metal complex (50 μ M.)

6.7 Cytotoxicity studies

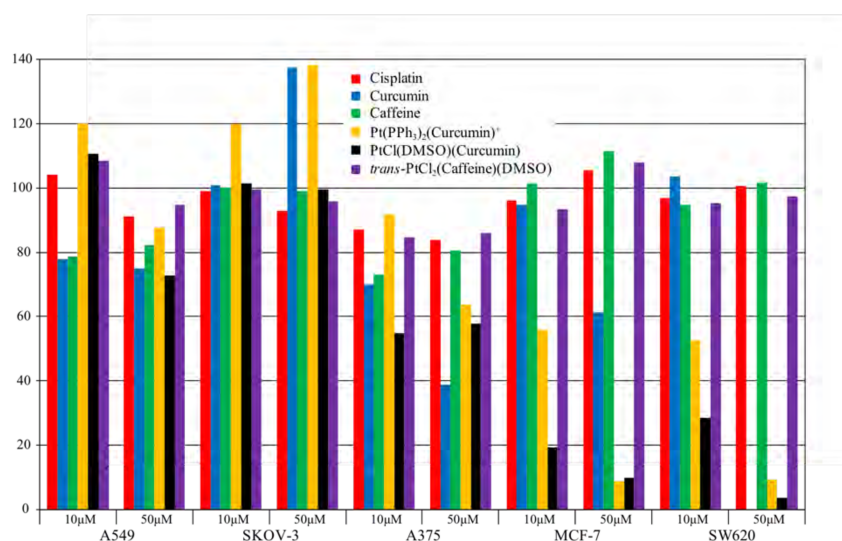


Fig. 64- Cell-viability assays (single-point screening, % of cell viability) of the free ligands Curcumin and Caffeine and [PtCl(Curumin)(DMSO)], *trans*-PtCl₂(DMSO)(Caffeine) and [Pt(PPh₃)₂(Curcumin)]Cl complexes with different cancer cell lines, namely A549 (lung adenocarcinoma), A375 (melanoma), MCF-7 (breast adenocarcinoma), SKOV3 (ovary adenocarcinoma) and SW620 (colorectal adenocarcinoma), using [compound] = 10 and 50 μ M and an incubation time of 24 h.

	A549 (lung adenocarcinoma)				SKOV-3 (ovarian carcinoma)				A375 (melanoma)				MCF-7 (breast carcinoma)				SW620 (colorectal carcinoma)			
	10µM		50µM		10µM		50µM		10µM		50µM		10µM		50µM		10µM		50µM	
	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ	X	σ
Curcumin	89,7	12,5	62,5	3,1	104,7	4,9	90,4	10,0	60,4	1,6	19,2	2,7	94,5	3,7	19,4	1,3	97,4	5,3	4,2	0,6
Caffeine	108,1	7,6	91,7	12,7	103,6	3,7	98,5	5,2	70,7	1,9	100,0	6,3	101,0	3,1	100,6	2,6	100,0	11,2	94,7	6,2
Pt(PPh ₃) ₂ (Curcumin)+	110,8	9,9	94,9	14,2	114,3	6,3	136,6	5,0	80,3	3,8	66,2	7,9	52,2	4,2	9,2	0,5	47,1	4,9	10,3	1,1
PtCl(DMSO)(Curcumin)	106,1	9,7	79,3	3,8	103,1	1,8	99,5	5,9	50,1	4,8	60,2	6,4	11,2	1,3	15,3	1,2	26,1	2,4	6,0	0,4
trans-PtCl ₂ (Caffeine)(DMSO)	106,9	6,1	93,4	6,6	103,7	4,5	97,6	4,0	85,7	12,8	89,5	10,0	100,7	1,4	106,7	2,0	102,7	6,8	97,0	10,1
Cisplatin (CDDP)	97,3	1,8	93,1	2,3	98,5	2,7	98,9	6,3	94,9	13,9	85,6	7,4	96,8	5,2	103,4	2,5	99,9	3,4	103,3	8,9

Green – higher than 75% of viability (little or no effect)

Yellow – between 50% and 75% of viability (some effect, IC₂₅ or IC₅₀ could be reached in dose-response experiments)

Red – below 50% of viability (high effect, IC₅₀ or IC₇₅ could be reached in dose-response experiments)

Table 9- Cell-viability assays (single-point screening, % of cell viability) of the free ligands Curcumin and Caffeine and [PtCl(Curcumin)(DMSO)], *trans*-PtCl₂(DMSO)(Caffeine) and [Pt(PPh₃)₂(Curcumin)]Cl complexes with different cancer cell lines, namely A549 (lung adenocarcinoma), A375 (melanoma), MCF-7 (breast adenocarcinoma), SKOV3 (ovary adenocarcinoma) and SW620 (colorectal adenocarcinoma), using [compound] = 10 and 50 µM and an incubation time of 24 h. The results are means ± SD.

Compound name	Cell line								
	MCF-7			MCF-7 10A			SW620		
	IC ₂₅	IC ₅₀	IC ₇₅	IC ₂₅	IC ₅₀	IC ₇₅	IC ₂₅	IC ₅₀	IC ₇₅
Cisplatin	>100	>100	>100	>100	>100	>100	>100	>100	>100
Curcumin	7.6±0.9	20.8±1.7	40.5±0.7	51.9±1.2	69.2±0.6	86.5±0.1	9.3±0.5	26.5±0.5	45.4±3.2
Caffeine	>100	>100	>100	>100	>100	>100	>100	>100	>100
[Pt(PPh ₃) ₂ (Curcumin)]Cl	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
[PtCl(Curcumin)(DMSO)]	6.1±0.3	12.9±1.0	35.1±3.5	39.0±0.1	59.8±0.1	94.9±1.4	5.5±0.7	15.0±1.7	31.8±1.3
trans-[PtCl ₂ (DMSO)(Caffeine)]	>100	>100	>100	>100	>100	>100	>100	>100	>100

Table 10- IC₂₅, IC₅₀ and IC₇₅ values (μM) of the free ligands Curcumin and Caffeine and [PtCl(Curcumin)(DMSO)], *trans*-PtCl₂(DMSO)(Caffeine) and [Pt(PPh₃)₂(Curcumin)]Cl complexes and cisplatin for MCF-7 (breast adenocarcinoma), MCF-7 10A (non tumorigenic epithelial cell lines) and SW620 (colorectal adenocarcinoma) after an incubation time of 24 h. The results are means ± SD of three independent experiments.

Cell-viability assays were carried out with the free ligands (curcumin and caffeine) and complexes $[\text{PtCl}(\text{Curcumin})(\text{DMSO})]$, $[\text{Pt}(\text{PPh}_3)_2(\text{Curcumin})]\text{Cl}$ and *trans*- $\text{PtCl}_2(\text{DMSO})(\text{Caffeine})$. Each metal complex was tested at 10 and 50 μM concentrations during single-point assays with the cancer cell lines A549 (lung adenocarcinoma), A375 (melanoma), MCF-7 (breast adenocarcinoma), SKOV3 (ovary adenocarcinoma) and SW620 (colorectal adenocarcinoma). Figure 64 shows the obtained results after 24 h incubation time. Results are also reported in Table 9.

As shown, *trans*- $\text{PtCl}_2(\text{DMSO})(\text{Caffeine})$ complex is inactive with all the tested cell lines. At metal complex concentration 10 μM , only $[\text{PtCl}(\text{Curcumin})(\text{DMSO})]$ complex achieves to a 11% cell viability with MCF-7 (breast adenocarcinoma) after 24-h incubation time (Fig.64). At higher metal complex concentration, the cytotoxic behaviour of $[\text{PtCl}(\text{Curcumin})(\text{DMSO})]$ complex is confirmed. Under these conditions, also $[\text{Pt}(\text{PPh}_3)_2(\text{Curcumin})]\text{Cl}$ complex shows a very good cytotoxic activity attaining to 9% cell viability. $[\text{PtCl}(\text{Curcumin})(\text{DMSO})]$ complex shows optimal cytotoxicity activity towards colon-cancer SW620 cells (26% cell at 10 μM metal complex, 24-h incubation time). Under the same conditions, the $[\text{Pt}(\text{PPh}_3)_2(\text{Curcumin})]\text{Cl}$ complex attains to 47% cell viability. Under the same experimental conditions at 50 μM , the cells viability attained by both the metal complexes is 6 % for $[\text{PtCl}(\text{Curcumin})(\text{DMSO})]$ and 10% for $[\text{Pt}(\text{PPh}_3)_2(\text{Curcumin})]\text{Cl}$. Only the Curcumin at 50 μM shows cytotoxicity activity towards breast adenocarcinoma MCF-7 and colon-cancer SW620 cells. The half maximal inhibitory concentration (IC_{50}) values for all compounds were determined for the active cell lines and for the cisplatin, chosen as reference coordination compound used clinically. The related results are reported in Tab. 10 for 24-h incubation time.

Table 10 indicates that the $[\text{PtCl}(\text{Curcumin})(\text{DMSO})]$ activity is good towards MCF7 and SW 620 cancer cells. But the most interesting evidence is represented by the selectivity of the complex action with respect to MCF-7 (cancerous) and MCF 10A (normal) cell lines.

7.CONCLUSIONS/CONCLUSIONI

In this chapter, a novel Platinum(II) complexes synthesis route has been presented which allows the easy and efficient coordination of natural ligands. It has been demonstrated that different ancillary ligands can be attached to the Pt centre via this route, in few reaction steps.

Using this synthesis route, three novel platinum(II) complexes with curcumin and caffeine have been designed and synthesised with very good yields, with respect to the starting material.

The coordination modes of curcumin and caffeine to platinum(II) were determined in solution by NMR spectroscopy and Infra-Red spectra.

Unfortunately, these complexes are insoluble in water, however their solubility in DMSO allowed DNA binding studies to be carried out.

DNA binding studies, including UV visible, fluorescence, circular dichroism with ct-DNA and atomic force microscopy, gel electrophoresis with plasmid DNA, were carried out for all complexes.

All compounds are able to interact with DNA, Complex 4 as intercalating and complex 5 and 6 as groove binders.

Almost all of them show significant cytotoxicity properties, as evidenced by the submicromolar IC₅₀ values achieved in some cases, with various cancer cell lines. Interestingly, the results obtained show that the most efficient metal complex is 5, which contains the Curcumin ligand and DMSO group and exhibits the best DNA-interacting properties. Unexpectedly, complex 6 which is capable of cleaving the DNA strands is less cytotoxic than 4 and 5 (at least in the cell lines tested). Therefore, a coordination compound displaying high DNA-cleaving abilities would not necessarily

exhibit greater cytotoxicity behaviour than a related complex which does not act as a DNA cleaver.

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REFERENCES/BIBLIOGRAFIA

- ABU-DAYA, A., BROWN, P. M. & FOX, K. R. 1995. DNA sequence preferences of several AT-selective minor groove binding ligands. *Nucleic Acids Research*, 23, 3385-3392.
- AGGARWAL, B. B., KUMAR, A. & BHARTI, A. C. 2003. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res*, 23, 363-98
- AHMADIAN, S., BARAR, J., SAEI, A. A., FAKHREE, M. A. A. & OMIDI, Y. 2009. Cellular Toxicity of Nanogenomedicine in MCF-7 Cell Line: MTT assay. *Journal of Visualized Experiments : JoVE*, 1191.
- ALBERTO, M. E., BUTERA, V. & RUSSO, N. 2011. Which one among the Pt-containing anticancer drugs more easily forms monoadducts with G and A DNA bases? A comparative study among oxaliplatin, nedaplatin, and carboplatin. *Inorg Chem*, 50, 6965-71.
- ALBERTO, M. E., LUCAS, M. F., PAVELKA, M. & RUSSO, N. 2009. The second-generation anticancer drug Nedaplatin: a theoretical investigation on the hydrolysis mechanism. *J Phys Chem B*, 113, 14473-9.
- ALLAN, S. G. & SMYTH, J. F. 1986. Small intestinal mucosal toxicity of cis-platinum--comparison of toxicity with platinum analogues and dexamethasone. *Br J Cancer*, 53, 355-60.
- ANDRAE, D., HÄUSSERMAN, U., DOLG, M., STOLL, H. & PREUS, H. 1990. Energy-adjusted ab initio pseudopotentials for the second and third row transition elements. *Theoretica Chimica Acta*, 77, 123-141.
- ARENDSE, M. J., ANDERSON, G. K. & RATH, N. P. 1999. Synthesis and Characterization of Platinum(II) Complexes of L-Ascorbic Acid. Crystal Structure of Ascorbato-C2,O5-ethylenediamineplatinum(II) Dihydrate. *Inorganic Chemistry*, 38, 5864-5869.
- ARENDASH, G. W., MORI, T., CAO, C., MAMCARZ, M., RUNFELDT, M., DICKSON, A., REZAI-ZADEH, K., TANE, J., CITRON, B. A., LIN, X., ECHEVERRIA, V. & POTTER, H. 2009. Caffeine reverses cognitive impairment and decreases brain amyloid-beta levels in aged Alzheimer's disease mice. *J Alzheimers Dis*, 17, 661-80.
- Arnesano, F. , Losacco, M. & Natile, G., 2013, An Updated View of Cisplatin Transport. *Eur. J. Inorg. Chem.*, 2013, 2701–11.

- BARCLAY, L. R., VINQVIST, M. R., MUKAI, K., GOTO, H., HASHIMOTO, Y., TOKUNAGA, A. & UNO, H. 2000. On the antioxidant mechanism of curcumin: classical methods are needed to determine antioxidant mechanism and activity. *Org Lett*, 2, 2841-3.
- BARTON, J. K., DANISHEFSKY, A. & GOLDBERG, J. 1984. Tris(phenanthroline)ruthenium(II): stereoselectivity in binding to DNA. *Journal of the American Chemical Society*, 106, 2172-2176.
- BASU, A. & KUMAR, G. S. 2013. Biophysical studies on curcumin-deoxyribonucleic acid interaction: spectroscopic and calorimetric approach. *Int J Biol Macromol*, 62, 257-64.
- BENASSI, R., FERRARI, E., LAZZARI, S., SPAGNOLO, F. & SALADINI, M. 2008. Theoretical study on Curcumin: A comparison of calculated spectroscopic properties with NMR, UV-vis and IR experimental data. *Journal of Molecular Structure*, 892, 168-176.
- BERGAMINI, P., MARCHESI, E., BERTOLASI, V., FOGAGNOLO, M., SCARPANTONIO, L., MANFREDINI, S., VERTUANI, S. & CANELLA, A. 2008. New coordination modes of L-ascorbic acid and dehydro-L-ascorbic acid as dianionic chelating ligand for platinum. *European Journal of Inorganic Chemistry*, 529-537.
- BERTINI, I., GRAY, H. B., LIPPARD, S. J. & VALENTINE, J. S. 1994. *Bioinorganic chemistry*, University Science Books.
- BOERÉ, R. T. & WILLIS, C. J. 1985. Synthetic and NMR studies on fluorinated alkoxides of platinum (II): An investigation of the trans influence of alkoxides and the nature of the Pt-O bond, using phosphine and thioether complexes. *Inorganic Chemistry*, 24, 1059-1065.
- BOUTIN, J. A. 1994. Tyrosine protein kinase inhibition and cancer. *Int J Biochem*, 26, 1203-26.
- BRADLEY, P. M., ANGELES-BOZA, A. M., DUNBAR, K. R. & TURRO, C. 2004. Direct DNA photocleavage by a new intercalating dirhodium(II/II) complex: comparison to Rh₂(μ-O₂CCH₃)₄. *Inorg Chem*, 43, 2450-2.
- BRISSOS, R. F., TORRENTS, E., DOS SANTOS MELLO, F. M., CARVALHO PIRES, W., SILVEIRA-LACERDA EDE, P., CABALLERO, A. B., CAUBET, A., MASSERA, C., ROUBEAU, O., TEAT, S. J. & GAMEZ, P. 2014. Highly cytotoxic DNA-interacting copper(II) coordination compounds. *Metallomics*, 6, 1853-68.

- BULHELLER, B. M., RODGER, A. & HIRST, J. D. 2007. Circular and linear dichroism of proteins. *Physical Chemistry Chemical Physics*, 9, 2020-2035.
- BURLA, M. C., CALIANDRO, R., CAMALLI, M., CARROZZINI, B., CASCARANO, G. L., GIACOVAZZO, C., MALLAMO, M., MAZZONE, A., POLIDORI, G. & SPAGNA, R. 2012. SIR2011: A new package for crystal structure determination and refinement. *Journal of Applied Crystallography*, 45, 357-361.
- BURLA, M. C., CALIANDRO, R., CARROZZINI, B., CASCARANO, G. L., CUOCCI, C., GIACOVAZZO, C., MALLAMO, M., MAZZONE, A. & POLIDORI, G. 2015. Crystal structure determination and refinement via SIR2014. *Journal of Applied Crystallography*, 48, 306-309.
- BURNS, D. C., ELLIS, D. A. & MARCH, R. E. 2007. A predictive tool for assessing ¹³C NMR chemical shifts of flavonoids. *Magnetic Resonance in Chemistry*, 45, 835-845.
- CALTAGIRONE, S., ROSSI, C., POGGI, A., RANELLETTI, F. O., NATALI, P. G., BRUNETTI, M., AIELLO, F. B. & PIANTELLI, M. 2000. Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential. *Int J Cancer*, 87, 595-600.
- CALVERT, A. H., HARLAND, S. J., NEWELL, D. R., SIDDIK, Z. H., JONES, A. C., MCELWAIN, T. J., RAJU, S., WILTSHAW, E., SMITH, I. E., BAKER, J. M., PECKHAM, M. J. & HARRAP, K. R. 1982. Early clinical studies with cis-diammine-1,1-cyclobutane dicarboxylate platinum II. *Cancer Chemother Pharmacol*, 9, 140-7.
- CAO, C., CIRRITO, J. R., LIN, X., WANG, L., VERGES, D. K., DICKSON, A., MAMCARZ, M., ZHANG, C., MORI, T., ARENDASH, G. W., HOLTZMAN, D. M. & POTTER, H. 2009. Caffeine suppresses amyloid-beta levels in plasma and brain of Alzheimer's disease transgenic mice. *J Alzheimers Dis*, 17, 681-97.
- CASTILLO, M. H., PERKINS, E., CAMPBELL, J. H., DOERR, R., HASSETT, J. M., KANDASWAMI, C. & MIDDLETON, E., JR. 1989. The effects of the bioflavonoid quercetin on squamous cell carcinoma of head and neck origin. *Am J Surg*, 158, 351-5.
- CHAHAR, M. K., SHARMA, N., DOBHAL, M. P. & JOSHI, Y. C. 2011a. Flavonoids: A versatile source of anticancer drugs. *Pharmacogn Rev*, 5, 1-12.

- CHAHAR, M. K., SHARMA, N., DOBHAL, M. P. & JOSHI, Y. C. 2011b. Flavonoids: A versatile source of anticancer drugs. *Pharmacognosy Reviews*, 5, 1-12.
- CHENG, Y.-C., DING, Y.-M., HUENG, D.-Y., CHEN, J.-Y. & CHEN, Y. 2016. Caffeine suppresses the progression of human glioblastoma via cathepsin B and MAPK signaling pathway. *The Journal of Nutritional Biochemistry*, 33, 63-72.
- CHRISTIAN, M. C. 1992. The current status of new platinum analogs. *Semin Oncol*, 19, 720-33.
- CHU, G. 1994. Cellular responses to cisplatin. The roles of DNA-binding proteins and DNA repair. *J Biol Chem*, 269, 787-90.
- COMUZZI, C., MELCHIOR, A., POLESE, P., PORTANOVA, R. & TOLAZZI, M. 2003. Cobalt(II) Dioxygen Carriers Based on Simple Diamino Ligands: Kinetic and ab Initio Studies. *Inorganic Chemistry*, 42, 8214-8222.
- COOPER, G. M. 2000. *The Cell - A Molecular Approach 2nd Edition*, Sunderland (MA): Sinauer Associates.
- COTTON, F. A. & FRANCIS, R. 1960. Sulfoxides as ligands. I. A preliminary survey of methyl sulfoxide complexes. *Journal of the American Chemical Society*, 82, 2986-2991.
- COTTON, F. A., FRANCIS, R. & HORROCKS JR, W. D. 1960. Sulfoxides as ligands. II. The infrared spectra of some dimethyl sulfoxide complexes. *Journal of Physical Chemistry*, 64, 1534-1536.
- CRUDDEN, C. & ALPER, H. 1992. Carbonylation. Direct Synthesis of Carbonyl Compounds. Von H. M. Colquhoun, D. J. Thompson und M. V. Twigg. Plenum Press, New York, 1991. XI, 296 S., geb. \$65.00. — ISBN 0-306-43747-3. *Angewandte Chemie*, 104, 1122-1122.
- CUBILLAS, P. & ANDERSON, M. W. 2014. Atomic Force Microscopy. *Multi Length-Scale Characterisation*. John Wiley & Sons, Ltd.
- CUI, H., YUAN, J., DU, X., WANG, M., YUE, L. & LIU, J. 2015. Ethyl gallate suppresses proliferation and invasion in human breast cancer cells via Akt-NF-kappaB signaling. *Oncol Rep*, 33, 1284-90.
- DAMIANAKI, A., BAKOGEORGOU, E., KAMPA, M., NOTAS, G., HATZOGLU, A., PANAGIOTOU, S., GEMETZI, C., KOUROUMALIS, E., MARTIN, P. M. & CASTANAS, E. 2000. Potent inhibitory action of red wine polyphenols on human breast cancer cells. *J Cell Biochem*, 78, 429-41.

- DE PASCALI, S. A., MUSCELLA, A., VETRUGNO, C., MARSIGLIANTE, S. & FANIZZI, F. P. 2014. Synthesis, characterization and cytotoxicity of novel Pt(II) κ^2O,O' -acetylacetonate complexes with nitrogen ligands. *Inorganica Chimica Acta*, 412, 88-93.
- DE SOUZA, R. F. V., SUSSUCHI, E. M. & DE GIOVANI, W. F. 2003. Synthesis, Electrochemical, Spectral, and Antioxidant Properties of Complexes of Flavonoids with Metal Ions. *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry*, 33, 1125-1144.
- DEL PIERO, S., FEDELE, R., MELCHIOR, A., PORTANOVA, R., TOLAZZI, M. & ZANGRANDO, E. 2007. Solvation effects on the stability of silver(I) complexes with pyridine-containing ligands studied by thermodynamic and DFT methods. *Inorganic Chemistry*, 46, 4683-4691.
- DESCHNER, E. E., RUPERTO, J., WONG, G. & NEWMARK, H. L. 1991. Quercetin and rutin as inhibitors of azoxymethanol-induced colonic neoplasia. *Carcinogenesis*, 12, 1193-6.
- DESPAIGNE, A. A. R., DA SILVA, J. G., DA COSTA, P. R., DOS SANTOS, R. G. & BERALDO, H. 2014. ROS-mediated cytotoxic effect of copper(II) hydrazone complexes against human glioma cells. *Molecules*, 19, 17202-17220.
- DI BERNARDO, P., ZANONATO, P. L., BENETOLLO, F., MELCHIOR, A., TOLAZZI, M. & RAO, L. 2012. Energetics and structure of uranium(VI)-acetate complexes in dimethyl sulfoxide. *Inorganic Chemistry*, 51, 9045-9055.
- DIAO, T., WHITE, P., GUZEI, I. & STAHL, S. S. 2012. Characterization of DMSO coordination to palladium(II) in solution and insights into the aerobic oxidation catalyst, Pd(DMSO)₂(TFA) 2. *Inorganic Chemistry*, 51, 11898-11909.
- DU, G., LIN, H., YANG, Y., ZHANG, S., WU, X., WANG, M., JI, L., LU, L., YU, L. & HAN, G. 2010. Dietary quercetin combining intratumoral doxorubicin injection synergistically induces rejection of established breast cancer in mice. *Int Immunopharmacol*, 10, 819-26.
- DUISENBERG, A. J. M., KROON-BATENBURG, L. M. J. & SCHREURS, A. M. M. 2003. An intensity evaluation method: EVAL-14. *Journal of Applied Crystallography*, 36, 220-229.
- END, D. W., LOOK, R. A., SHAFFER, N. L., BALLES, E. A. & PERSICO, F. J. 1987. Non-selective inhibition of mammalian protein kinases by flavinoids in vitro. *Res Commun Chem Pathol Pharmacol*, 56, 75-86.

- ENDRIZZI, F., MELCHIOR, A., TOLAZZI, M. & RAO, L. 2015. Complexation of uranium(VI) with glutarimidoxime: Thermodynamic and computational studies. *Dalton Transactions*, 44, 13835-13844.
- FARRELL, N., HA, T. T., SOUCHARD, J. P., WIMMER, F. L., CROS, S. & JOHNSON, N. P. 1989. Cytostatic trans-platinum(II) complexes. *J Med Chem*, 32, 2240-1.
- FARRUGIA, L. J. 2012. WinGX and ORTEP for Windows: An update. *Journal of Applied Crystallography*, 45, 849-854.
- FERRIOLA, P. C., CODY, V. & MIDDLETON, E., JR. 1989. Protein kinase C inhibition by plant flavonoids. Kinetic mechanisms and structure-activity relationships. *Biochem Pharmacol*, 38, 1617-24.
- FERRY, D. R., SMITH, A., MALKHANDI, J., FYFE, D. W., DETAKATS, P. G., ANDERSON, D., BAKER, J. & KERR, D. J. 1996. Phase I clinical trial of the flavonoid quercetin: pharmacokinetics and evidence for in vivo tyrosine kinase inhibition. *Clin Cancer Res*, 2, 659-68.
- FOTSIS, T., PEPPER, M. S., AKTAS, E., BREIT, S., RASKU, S., ADLERCREUTZ, H., WAHALA, K., MONTESANO, R. & SCHWEIGERER, L. 1997. Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis. *Cancer Res*, 57, 2916-21.
- GARCÍA-RAMOS, J. C., GALINDO-MURILLO, R., CORTÉS-GUZMÁN, F. & RUIZ-AZUARA, L. 2013. Metal-based drug-DNA interactions. *Journal of the Mexican Chemical Society*, 57, 245-259.
- GAY, M., MONTAÑA, Á. M., BATALLA, C., MESAS, J. M. & ALEGRE, M.-T. 2015. Design, synthesis and SAR studies of novel 1,2-bis(aminomethyl)cyclohexane platinum(II) complexes with cytotoxic activity. Studies of interaction with DNA of iodinated seven-membered 1,4-diaminoplatinocycles. *Journal of Inorganic Biochemistry*, 142, 15-27.
- GEIERSTANGER, B. H. & WEMMER, D. E. 1995. Complexes of the minor groove of DNA. *Annual Review of Biophysics and Biomolecular Structure*, 24, 463-493.
- GIBELLINI, L., PINTI, M., NASI, M., MONTAGNA, J. P., DE BIASI, S., ROAT, E., BERTONCELLI, L., COOPER, E. L. & COSSARIZZA, A. 2011. Quercetin and Cancer Chemoprevention. *Evidence-Based Complementary and Alternative Medicine*, 2011.
- GRAU, J., BRISSOS, R. F., SALINAS-UBER, J., CABALLERO, A. B., CAUBET, A., ROUBEAU, O., KORRODI-GREGORIO, L., PEREZ-TOMAS, R. & GAMEZ, P.

2015. The effect of potential supramolecular-bond promoters on the DNA-interacting abilities of copper-terpyridine compounds. *Dalton Trans*, 44, 16061-72.
- GRUBEL, K., MARTS, A. R., GREER, S. M., TIERNEY, D. L., ALLPRESS, C. J., ANDERSON, S. N., LAUGHLIN, B. J., SMITH, R. C., ARIF, A. M. & BERREAU, L. M. 2012. Photoinitiated Dioxygenase-Type Reactivity of Open-Shell 3d Divalent Metal Flavonolate Complexes. *European Journal of Inorganic Chemistry*, 2012, 4750-4757.
- GRUBEL, K., SARAF, S. L., ANDERSON, S. N., LAUGHLIN, B. J., SMITH, R. C., ARIF, A. M. & BERREAU, L. M. 2013. Synthesis, characterization, and photoinduced CO-release reactivity of a Pb(II) flavonolate complex: Comparisons to Group 12 analogs. *Inorganica Chimica Acta*, 407, 91-97.
- GSCHWENDT, M., HORN, F., KITTSTEIN, W. & MARKS, F. 1983. Inhibition of the calcium- and phospholipid-dependent protein kinase activity from mouse brain cytosol by quercetin. *Biochem Biophys Res Commun*, 117, 444-7.
- HAGIWARA, M., INOUE, S., TANAKA, T., NUNOKI, K., ITO, M. & HIDAKA, H. 1988. Differential effects of flavonoids as inhibitors of tyrosine protein kinases and serine/threonine protein kinases. *Biochem Pharmacol*, 37, 2987-92.
- HARRAP, K. R. 1995. Initiatives with platinum- and quinazoline-based antitumor molecules--Fourteenth Bruce F. Cain Memorial Award Lecture. *Cancer Res*, 55, 2761-8.
- HINTERMANN, G., FISCHER, H. M., CRAMERI, R. & HÜTTER, R. 1981. Simple procedure for distinguishing CCC, OC, and L forms of plasmid DNA by agarose gel electrophoresis. *Plasmid*, 5, 371-373.
- HÖLSCHER, H. 2012. AFM, Tapping Mode. *Encyclopedia of Nanotechnology*, 99-99.
- HUANG, Y. T., HWANG, J. J., LEE, P. P., KE, F. C., HUANG, J. H., HUANG, C. J., KANDASWAMI, C., MIDDLETON, E., JR. & LEE, M. T. 1999. Effects of luteolin and quercetin, inhibitors of tyrosine kinase, on cell growth and metastasis-associated properties in A431 cells overexpressing epidermal growth factor receptor. *Br J Pharmacol*, 128, 999-1010.
- IVANOV, V. I., MINCHENKOVA, L. E., SCHYOLKINA, A. K. & POLETAYEV, A. I. 1973. Different conformations of double-stranded nucleic acid in solution as revealed by circular dichroism. *Biopolymers - Peptide Science Section*, 12, 89-110.

- JAKUPEC, M. A., GALANSKI, M. & KEPPLER, B. K. 2003. Tumour-inhibiting platinum complexes--state of the art and future perspectives. *Rev Physiol Biochem Pharmacol*, 146, 1-54.
- JAMIESON, E. R. & LIPPARD, S. J. 1999. Structure, Recognition, and Processing of Cisplatin-DNA Adducts. *Chem Rev*, 99, 2467-98.
- JEMAL, A., BRAY, F., CENTER, M. M., FERLAY, J., WARD, E. & FORMAN, D. 2011. Global cancer statistics. *CA Cancer J Clin*, 61, 69-90.
- JOHNSTONE, T. C., SUNTHARALINGAM, K. & LIPPARD, S. J. 2016. The Next Generation of Platinum Drugs: Targeted Pt(II) Agents, Nanoparticle Delivery, and Pt(IV) Prodrugs. *Chem Rev*, 116, 3436-86.
- JOVANOVIĆ, S. V., STEENKEN, S., BOONE, C. W. & SIMIĆ, M. G. 1999. H-atom transfer is a preferred antioxidant mechanism of curcumin. *Journal of the American Chemical Society*, 121, 9677-9681.
- JUNG, Y. & LIPPARD, S. J. 2007. Direct cellular responses to platinum-induced DNA damage. *Chem Rev*, 107, 1387-407.
- JURENKA, J. S. 2009. Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: a review of preclinical and clinical research. *Altern Med Rev*, 14, 141-53.
- KALINOWSKA-LIS, U., OCHOCKI, J. & MATLAWSKA-WASOWSKA, K. 2008. Trans geometry in platinum antitumor complexes. *Coordination Chemistry Reviews*, 252, 1328-1345.
- KANDASWAMI, C. & MIDDLETON, E., JR. 1994. Free radical scavenging and antioxidant activity of plant flavonoids. *Adv Exp Med Biol*, 366, 351-76.
- KARTALOU, M. & ESSIGMANN, J. M. 2001. Mechanisms of resistance to cisplatin. *Mutat Res*, 478, 23-43.
- KECK, M. V. & LIPPARD, S. J. 1992. Unwinding of supercoiled DNA by platinum-ethidium and related complexes. *Journal of the American Chemical Society*, 114, 3386-3390.
- KIDANI, Y., INAGAKI, K., SAITO, R. & TSUKAGOSHI, S. 1977. Synthesis and anti tumor activities of platinum (II) complexes of 1,2 diaminocyclohexane isomers and their related derivatives. *J.CLIN.HEMATOL.ONCOL.*, 7, 197-209.

- KIDANI, Y., NOJI, M., TSUKAGOSHI, S. & TASHIRO, T. 1978. Antitumor activity of water-soluble platinum(II) complexes of 1,2-cyclohexanediamine isomers. *Gan*, 69, 263-5.
- KIM, H. J., KIM, D. J., KARTHICK, S. N., HEMALATHA, K. V., JUSTIN RAJ, C., OK, S. & CHOE, Y. 2013. Curcumin dye extracted from *Curcuma longa* L. used as sensitizers for efficient dye-sensitized solar cells. *International Journal of Electrochemical Science*, 8, 8320-8328.
- KIM, S. Y., GAO, J. J., LEE, W.-C., RYU, K. S., LEE, K. R. & KIM, Y. C. 1999. Antioxidative flavonoids from the leaves of *Morus alba*. *Archives of Pharmacal Research*, 22, 81.
- KLAASSEN, E. B., DE GROOT, R. H. M., EVERS, E. A. T., SNEL, J., VEERMAN, E. C. I., LIGTENBERG, A. J. M., JOLLES, J. & VELTMAN, D. J. 2013. The effect of caffeine on working memory load-related brain activation in middle-aged males. *Neuropharmacology*, 64, 160-167.
- KLOHS, W. D., FRY, D. W. & KRAKER, A. J. 1997. Inhibitors of tyrosine kinase. *Curr Opin Oncol*, 9, 562-8.
- KUKUSHKIN, Y. N., VYAZ'MENSKII, Y. E. & ZORINA, L. I. 1968. The trans Influence of Dimethyl Sulphoxide in Platinum(II) compounds. *Russian Journal of Inorganic Chemistry*, 13, 1573-1575.
- LAITY, J. H., LEE, B. M. & WRIGHT, P. E. 2001. Zinc finger proteins: New insights into structural and functional diversity. *Current Opinion in Structural Biology*, 11, 39-46.
- LAMM, G. & PACK, G. R. 1990. Acidic domains around nucleic acids. *Proc Natl Acad Sci U S A*, 87, 9033-6.
- LEE, W. R., SHEN, S. C., LIN, H. Y., HOU, W. C., YANG, L. L. & CHEN, Y. C. 2002. Wogonin and fisetin induce apoptosis in human promyeloleukemic cells, accompanied by a decrease of reactive oxygen species, and activation of caspase 3 and Ca(2+)-dependent endonuclease. *Biochem Pharmacol*, 63, 225-36.
- LEMAŃSKA, K., SZYMUSIAK, H., TYRAKOWSKA, B., ZIELIŃSKI, R., SOFFERS, A. E. M. F. & RIETJENS, I. M. C. M. 2001. The influence of pH on antioxidant properties and the mechanism of antioxidant action of hydroxyflavones. *Free Radical Biology and Medicine*, 31, 869-881.

- LEWARS, E. G. & MARCH, R. E. 2007. Fragmentation of 3-hydroxyflavone; a computational and mass spectrometric study. *Rapid Communications in Mass Spectrometry*, 21, 1669-1679.
- LEVY, J., TEUERSTEIN, I., MARBACH, M., RADIAN, S. & SHARONI, Y. 1984. Tyrosine protein kinase activity in the DMBA-induced rat mammary tumor: inhibition by quercetin. *Biochem Biophys Res Commun*, 123, 1227-33.
- LIU, Z., TAN, S., ZU, Y., FU, Y., MENG, R. & XING, Z. 2010. The interactions of cisplatin and DNA studied by atomic force microscopy. *Micron*, 41, 833-839.
- LOKICH, J. & ANDERSON, N. 1998. Carboplatin versus cisplatin in solid tumors: An analysis of the literature. *Annals of Oncology*, 9, 13-21.
- LONG, E. C. & BARTON, J. K. 1990. On demonstrating DNA intercalation. *Accounts of Chemical Research*, 23, 271-273.
- LOONTJENS, F. G., REGENFUSS, P., ZECHEL, A., DUMORTIER, L. & CLEGG, R. M. 1990. Binding characteristics of hoechst 33258 with calf thymus DNA, poly[d(A-T)], and d(CCGGAATTCCGG): Multiple stoichiometries and determination of tight binding with a wide spectrum of site affinities. *Biochemistry*, 29, 9029-9039.
- LUCAS, M. F., PAVELKA, M., ALBERTO, M. E. & RUSSO, N. 2009. Neutral and acidic hydrolysis reactions of the third generation anticancer drug oxaliplatin. *J Phys Chem B*, 113, 831-8.
- MACRAE, C. F., BRUNO, I. J., CHISHOLM, J. A., EDGINGTON, P. R., MCCABE, P., PIDCOCK, E., RODRIGUEZ-MONGE, L., TAYLOR, R., VAN DE STREEK, J. & WOOD, P. A. 2008. Mercury CSD 2.0 - New features for the visualization and investigation of crystal structures. *Journal of Applied Crystallography*, 41, 466-470.
- MAKITA, H., TANAKA, T., FUJITSUKA, H., TATEMATSU, N., SATOH, K., HARA, A. & MORI, H. 1996. Chemoprevention of 4-nitroquinoline 1-oxide-induced rat oral carcinogenesis by the dietary flavonoids chalcone, 2-hydroxychalcone, and quercetin. *Cancer Res*, 56, 4904-9.
- MALEŠEV, D. & KUNTIĆ, V. 2007. Investigation of metal-flavonoid chelates and the determination of flavonoids via metal-flavonoid complexing reactions. *Journal of the Serbian Chemical Society*, 72, 921-939.
- MANACH, C., SCALBERT, A., MORAND, C., REMESY, C. & JIMENEZ, L. 2004. Polyphenols: food sources and bioavailability. *Am J Clin Nutr*, 79, 727-47.

- MARGIOTTA, N., DENORA, N., PICCINONNA, S., LAQUINTANA, V., LASORSA, F. M., FRANCO, M. & NATILE, G. 2014. Synthesis, characterization, and in vitro evaluation of new coordination complexes of platinum(ii) and rhenium(i) with a ligand targeting the translocator protein (TSPO). *Dalton Transactions*, 43, 16252-16264.
- MARTINEZ-PEREZ, C., WARD, C., COOK, G., MULLEN, P., MCPHAIL, D., HARRISON, D. J. & LANGDON, S. P. 2014. Novel flavonoids as anti-cancer agents: mechanisms of action and promise for their potential application in breast cancer. *Biochem Soc Trans*, 42, 1017-23.
- MATHÉ, G., KIDANI, Y., TRIANA, K., BRIENZA, S., RIBAUD, P., GOLDSCHMIDT, E., ECSTEIN, E., DESPAX, R., MUSSET, M. & MISSET, J. L. 1986. A phase I trial of trans-1-diaminocyclohexane oxalato-platinum (I-OHP). *Biomedicine and Pharmacotherapy*, 40, 372-376.
- MATSUURA, T., TAKEMOTO, T. & NAKASHIMA, R. 1973. Photoinduced reactions-LXXI. Photorearrangement of 3-hydroxyflavones to 3-aryl-3-hydroxy-1,2-indandiones. *Tetrahedron*, 29, 3337-3340.
- MCPHAIL, D. B., HARTLEY, R. C., GARDNER, P. T. & DUTHIE, G. G. 2003. Kinetic and stoichiometric assessment of the antioxidant activity of flavonoids by electron spin resonance spectroscopy. *J Agric Food Chem*, 51, 1684-90.
- MEDRANO, A., DENNIS, S. M., ALVAREZ-VALDES, A., PERLES, J., MCGREGOR MASON, T. & QUIROGA, A. G. 2015. Synthesis, cytotoxicity, DNA interaction and cell cycle studies of trans-diiodophosphine Pt(II) complexes. *Dalton Trans*, 44, 3557-62.
- MELCHIOR, A., MARCOS, E. S., PAPPALARDO, R. R. & MARTÍNEZ, J. M. 2011. Comparative study of the hydrolysis of a third- and a first-generation platinum anticancer complexes. *Theoretical Chemistry Accounts*, 128, 627-638.
- MELCHIOR, A., MARTINEZ, J. M., PAPPALARDO, R. R. & SANCHEZ MARCOS, E. 2013a. Hydration of Cisplatin Studied by an Effective Ab Initio Pair Potential Including Solute-Solvent Polarization. *J Chem Theory Comput*, 9, 4562-73.
- MELCHIOR, A., PERALTA, E., VALIENTE, M., TAVAGNACCO, C., ENDRIZZI, F. & TOLAZZI, M. 2013b. Interaction of d10 metal ions with thioether ligands: A thermodynamic and theoretical study. *Dalton Transactions*, 42, 6074-6082.
- MELCHIOR, A., TOLAZZI, M., MARTINEZ, J. M., PAPPALARDO, R. R. & SANCHEZ MARCOS, E. 2015. Hydration of Two Cisplatin Aqua-Derivatives Studied by

- Quantum Mechanics and Molecular Dynamics Simulations. *J Chem Theory Comput*, 11, 1735-44.
- MIDDLETON, E., JR., KANDASWAMI, C. & THEOHARIDES, T. C. 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol Rev*, 52, 673-751.
- MISSET, J. L. 1998. Oxaliplatin in practice. *Br J Cancer*, 77 Suppl 4, 4-7.
- MIYAGI, Y., OM, A. S., CHEE, K. M. & BENNINK, M. R. 2000. Inhibition of azoxymethane-induced colon cancer by orange juice. *Nutr Cancer*, 36, 224-9.
- MOHAN, S., THIAGARAJAN, K. & CHANDRASEKARAN, R. 2015. In vitro evaluation of antiproliferative effect of ethyl gallate against human oral squamous carcinoma cell line KB. *Nat Prod Res*, 29, 366-9.
- MUKHERJEE, S., MITRA, I., REDDY B, V. P., MAHATA, S., BOSE K, J. C., DASGUPTA, S., LINERT, W. & CH. MOI, S. 2016. Cytotoxic, DNA binding and drug reservoir property of Pt(II)–sulfur complexes: In-vitro kinetics, mechanism with bio-relevant molecules in aqueous medium and a theoretical approach. *Polyhedron*, 119, 84-97.
- NAKAMOTO, K. 2008a. Applications in Inorganic Chemistry. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*. John Wiley & Sons, Inc.
- NAKAMOTO, K. 2008b. Theory of Normal Vibrations. *Infrared and Raman Spectra of Inorganic and Coordination Compounds*. John Wiley & Sons, Inc.
- NGUYEN, B., HAMELBERG, D., BAILLY, C., COLSON, P., STANEK, J., BRUN, R., NEIDLE, S. & WILSON, W. D. 2004. Characterization of a Novel DNA Minor-Groove Complex. *Biophysical Journal*, 86, 1028-1041.
- OH, S. J., KIM, O., LEE, J. S., KIM, J. A., KIM, M. R., CHOI, H. S., SHIM, J. H., KANG, K. W. & KIM, Y. C. 2010. Inhibition of angiogenesis by quercetin in tamoxifen-resistant breast cancer cells. *Food Chem Toxicol*, 48, 3227-34.
- OHNDORF, U. M., ROULD, M. A., HE, Q., PABO, C. O. & LIPPARD, S. J. 1999. Basis for recognition of cisplatin-modified DNA by high-mobility-group proteins. *Nature*, 399, 708-12.
- PALCHAUDHURI, R. & HERGENROTHER, P. J. 2007. DNA as a target for anticancer compounds: methods to determine the mode of binding and the mechanism of action. *Current Opinion in Biotechnology*, 18, 497-503.
- PAPER, D. H. 1998. Natural products as angiogenesis inhibitors. *Planta Med*, 64, 686-95.

- PAUL, A. & BHATTACHARYA, S. 2012. Chemistry and biology of DNA-binding small molecules. *Current Science*, 102, 212-231.
- PAVELKA, M., LUCAS, M. F. & RUSSO, N. 2007. On the hydrolysis mechanism of the second-generation anticancer drug carboplatin. *Chemistry*, 13, 10108-16.
- PAYTON, F., SANDUSKY, P. & ALWORTH, W. L. 2007. NMR study of the solution structure of curcumin. *Journal of Natural Products*, 70, 143-146.
- PRIYADARSINI, K. I. 2014. The chemistry of curcumin: From extraction to therapeutic agent. *Molecules*, 19, 20091-20112.
- PULLMAN, A. & PULLMAN, B. 1981. Molecular electrostatic potential of the nucleic acids. *Quarterly Reviews of Biophysics*, 14, 289-380.
- RAMOS-LIMA, F. J., QUIROGA, A. G., GARCIA-SERRELDE, B., BLANCO, F., CARNERO, A. & NAVARRO-RANNINGER, C. 2007. New trans-platinum drugs with phosphines and amines as carrier ligands induce apoptosis in tumor cells resistant to cisplatin. *J Med Chem*, 50, 2194-9.
- RAVERA, M., GABANO, E., SARDI, M., MONTI, E., GARIBOLDI, M. B. & OSELLA, D. 2012. Antiproliferative activity of Pt II complexes with carboxylated phosphanes in chelated or ring-opened forms. *European Journal of Inorganic Chemistry*, 3441-3448.
- REDDY, B. S. P., SONDHI, S. M. & LOWN, J. W. 1999. Synthetic DNA minor groove-binding drugs. *Pharmacology and Therapeutics*, 84, 1-111.
- ROY, S., MAHESWARI, P. U., LUTZ, M., SPEK, A. L., DEN DULK, H., BARENDs, S., VAN WEZEL, G. P., HARTL, F. & REEDIJK, J. 2009. DNA cleavage and antitumour activity of platinum(II) and copper(II) compounds derived from 4-methyl-2-N-(2-pyridylmethyl)aminophenol: spectroscopic, electrochemical and biological investigation. *Dalton Trans*, 10846-60.
- ROSENBERG, B., VANCAMP, L. & KRIGAS, T. 1965. Inhibition of Cell Division in Escherichia Coli by Electrolysis Products from a Platinum Electrode. *Nature*, 205, 698-9.
- ROSENBERG, B., VANCAMP, L., TROSKO, J. E. & MANSOUR, V. H. 1969. Platinum compounds: a new class of potent antitumour agents. *Nature*, 222, 385-6.
- RUIZ, J., CUTILLAS, N., VICENTE, C., VILLA, M. D., LOPEZ, G., LORENZO, J., AVILES, F. X., MORENO, V. & BAUTISTA, D. 2005. New palladium(II) and platinum(II) complexes with the model nucleobase 1-methylcytosine: antitumor activity and interactions with DNA. *Inorg Chem*, 44, 7365-76.

- SABISZ, M. & SKLADANOWSKI, A. 2008. Modulation of cellular response to anticancer treatment by caffeine: inhibition of cell cycle checkpoints, DNA repair and more. *Curr Pharm Biotechnol*, 9, 325-36.
- SAKAGAMI, H., JIANG, Y., KUSAMA, K., ATSUMI, T., UEHA, T., TOGUCHI, M., IWAKURA, I., SATOH, K., FUKAI, T. & NOMURA, T. 2000. Induction of apoptosis by flavones, flavonols (3-hydroxyflavones) and isoprenoid-substituted flavonoids in human oral tumor cell lines. *Anticancer Research*, 20, 271-277.
- SAMPER, K. G., RODRÍGUEZ, V., ORTEGA-CARRASCO, E., ATRIAN, S., MARÉCHAL, J. D., CUTILLAS, N., ZAMORA, A., DE HARO, C., CAPDEVILA, M., RUIZ, J. & PALACIOS, Ò. 2014. Understanding the interaction of an antitumoral platinum(II) 7-azaindolate complex with proteins and DNA. *BioMetals*, 27, 1159-1177.
- SARAF, S. L., FISH, T. J., BENNINGHOFF, A. D., BUELT, A. A., SMITH, R. C. & BERREAU, L. M. 2014. Photochemical Reactivity of Ru(II)(η^6 -p-cymene) Flavonolato Compounds. *Organometallics*, 33, 6341-6351.
- SASAKI, Y., AMANO, T., MORITA, M., SHINKAI, T., EGUCHI, K., TAMURA, T., OHE, Y., KOJIMA, A. & SAIJO, N. 1991. Phase I study and pharmacological analysis of cis-diammine(glycolato)platinum (254-S; NSC 375101D) administered by 5-day continuous intravenous infusion. *Cancer Res*, 51, 1472-7.
- SCALBERT, A., JOHNSON, I. T. & SALTMARSH, M. 2005. Polyphenols: antioxidants and beyond. *Am J Clin Nutr*, 81, 215S-217S.
- SCHMID, F.-X. 2001. Biological Macromolecules: UV-visible Spectrophotometry. *eLS*. John Wiley & Sons, Ltd.
- SHAHABADI, N. & MOGHADAM, N. H. 2012. Determining the mode of interaction of calf thymus DNA with the drug sumatriptan using voltammetric and spectroscopic techniques. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 99, 18-22.
- SHARMA, O. P. 1976. Antioxidant activity of curcumin and related compounds. *Biochem Pharmacol*, 25, 1811-2.
- SHELDRIK, G. M. 2008. Absorption Correction Program. *In*: SADABS (ed.).
- SHELDRIK, G. M. 2015. SHELXT - Integrated space-group and crystal-structure determination. *Acta Crystallographica Section A: Foundations of Crystallography*, 71, 3-8.

- SHI, S., LIU, J., LI, J., ZHENG, K.-C., HUANG, X.-M., TAN, C.-P., CHEN, L.-M. & JI, L.-N. 2006. Synthesis, characterization and DNA-binding of novel chiral complexes Δ - and Λ -[Ru(bpy)₂L]²⁺ (L = o-mopip and p-mopip). *Journal of Inorganic Biochemistry*, 100, 385-395.
- SIRAJUDDIN, M., ALI, S. & BADSHAH, A. 2013. Drug-DNA interactions and their study by UV-Visible, fluorescence spectroscopies and cyclic voltametry. *Journal of Photochemistry and Photobiology B: Biology*, 124, 1-19.
- Crystallographica Section A: Foundations of Crystallography*, 71, 3-8.
- SISA, M., BONNET, S. L., FERREIRA, D. & VAN DER WESTHUIZEN, J. H. 2010. Photochemistry of flavonoids. *Molecules*, 15, 5196-5245.
- SITKOWSKI, J., STEFANIAK, L., NICOL, L., MARTIN, M. L., MARTIN, G. J. & WEBB, G. A. 1995. Complete assignments of the ¹H, ¹³C and ¹⁵N NMR spectra of caffeine. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 51, 839-8.
- STOFER, E. & LAVERY, R. 1994. Measuring the geometry of DNA grooves. *Biopolymers*, 34, 337-346.
- SUN, Y. M., ZHANG, H. Y., CHEN, D. Z. & LIU, C. B. 2002. Theoretical elucidation on the antioxidant mechanism of curcumin: a DFT study. *Org Lett*, 4, 2909-11.
- TAKAHARA, P. M., ROSENZWEIG, A. C., FREDERICK, C. A. & LIPPARD, S. J. 1995. Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin. *Nature*, 377, 649-52.
- TANAKA, T., MAKITA, H., OHNISHI, M., MORI, H., SATOH, K., HARA, A., SUMIDA, T., FUKUTANI, K., TANAKA, T. & OGAWA, H. 1997. Chemoprevention of 4-nitroquinoline 1-oxide-induced oral carcinogenesis in rats by flavonoids diosmin and hesperidin, each alone and in combination. *Cancer Res*, 57, 246-52.
- TOMASI, J., MENNUCCI, B. & CAMMI, R. 2005. Quantum mechanical continuum solvation models. *Chem Rev*, 105, 2999-3093.
- TOMMASINI, S., CALABRÒ, M. L., DONATO, P., RANERI, D., GUGLIELMO, G., FICARRA, P. & FICARRA, R. 2004. Comparative photodegradation studies on 3-hydroxyflavone: influence of different media, pH and light sources. *Journal of Pharmaceutical and Biomedical Analysis*, 35, 389-397.
- TOSETTI, F., FERRARI, N., DE FLORA, S. & ALBINI, A. 2002. Angioprevention': angiogenesis is a common and key target for cancer chemopreventive agents. *FASEB J*, 16, 2-14.

- VISIOLI, F., DE LA LASTRA, C. A., ANDRES-LACUEVA, C., AVIRAM, M., CALHAU, C., CASSANO, A., D'ARCHIVIO, M., FARIA, A., FAVE, G., FOGLIANO, V., LLORACH, R., VITAGLIONE, P., ZORATTI, M. & EDEAS, M. 2011. Polyphenols and human health: a prospectus. *Crit Rev Food Sci Nutr*, 51, 524-46.
- YANG, M., TANAKA, T., HIROSE, Y., DEGUCHI, T., MORI, H. & KAWADA, Y. 1997. Chemopreventive effects of diosmin and hesperidin on N-butyl-N-(4-hydroxybutyl)nitrosamine-induced urinary-bladder carcinogenesis in male ICR mice. *Int J Cancer*, 73, 719-24.
- YOKOE, I., HIGUCHI, K., SHIRATAKI, Y. & KOMATSU, M. 1981. Photochemistry of flavonoids. III. Photorearrangement of flavonols. *Chemical and Pharmaceutical Bulletin*, 29, 894-898.
- YOKOO, T. & KITAMURA, M. 1997. Unexpected protection of glomerular mesangial cells from oxidant-triggered apoptosis by bioflavonoid quercetin. *Am J Physiol*, 273, F206-12.
- YU.N. KUKUSHKIN , Y., E. VYAZ'MENSKII 1968. the trans influence of dimethyl sulphoxide in platinum (II) compounds. *russian journal of inorganic chemistry*, 13, 4.
- WATSON, J. D. & CRICK, F. H. C. 1953. Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid. *Nature*, 171, 737-738.
- WILSON, J. J. & LIPPARD, S. J. 2012. In vitro anticancer activity of cis-diammineplatinum(II) complexes with beta-diketonate leaving group ligands. *J Med Chem*, 55, 5326-36.
- WINK, M. 2006. *An introduction to molecular biotechnology*, Wiley Online Library.
15. Crystal structure determination and refinement via SIR2014. *Journal of Applied Crystallography*, 48, 306-309.
- ZHOU, J., WANG, L., WANG, J. & TANG, N. 2001. Antioxidative and anti-tumour activities of solid quercetin metal(II) complexes. *Transition Metal Chemistry*, 26, 57-63

CURRICULUM



EDUCATION

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|-----------------------|--|
| 2014 (Jan)-2016 (Dec) | PhD in Inorganic Chemistry and Spectroscopy in the Environmental, Regional and Construction Risk and Development School (Polytechnic University of Bari). |
| 2013 (July) | Master (Inorganic Chemistry) at the University of Pisa (Italy).
Thesis: Synthesis, characterization, in vitro antitumor activity of some complexes of Pt (II) and their interaction with phospholipids model of the cell membrane (110 pp). |
| 2010 | Bachelor (Physical Chemistry) at the University of Palermo (Italy) Thesis: Physical-chemical characterization of biocompatible copolymers (26 pp). |

2004	Maturità Classica (high school) at the Liceo G. Garibaldi in Palermo (Italy)
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EXPERIENCE

2016 Feb-Dec	Research period at the Lab of Bio-Inorganic Chemistry (Prof. P. Gamez) in University of Barcelona in the setting of PhD studies.
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2016 Sept 25-30	School on Analytical Chemistry for PhD's students on environmental chemistry, chemometrics, LC-MS, piezo-electric, optical, electronical and electro-chemical bio-sensors, doping, speciation of trace elements, sampling and calibration of gaseous mixtures, industrial process management.
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2013 Sept-Dec	Four-months molecular spectroscopy (NMR and IR) stage on food matrices for metabolomics analyses and geographical determination of oil and wine origin (Bruker Corporation, Milan, Italy). During this period the following courses were attended:
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November, 25-29	Theoretical and practical course on NMR spectroscopy focused on structural molecular analysis (Bruker Corporation, Milan, Italy).
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November, 18-21	Theoretical and practical course on NMR spectroscopy focused on molecular detection (Bruker Corporation, Milan, Italy).
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October, 24-25	Basic course on OPUS/IR software for molecular spectroscopy determination (Bruker Corporation, Milan, Italy).
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October, 2-3	Theoretical and practical course on NMR spectroscopy focused on food and clinical applications (Bruker Corporation, Milan, Italy).
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2011 – 2012	Internship during the master thesis activity at the Lab of Inorganic Chemistry, University of Pisa (tutor: Dr. S. Samaritani). Synthesis and characterization of square-Pt(II) complexes.
(2011-2012):	Internship during the master thesis activity at the Lab of NMR spectroscopy at ICCOM-CNR (Pisa, Italy) (tutor: Dr. C. Forte Petrini). Study of interactions between square-Pt(II) complexes and a DNA model-membrane.
2010	Internship Lab of Physical Chemistry, University of Palermo for studying the specific viscosity of organic compounds, in presence of suitable solvents (tutor: Prof. M. L. Turco Liveri).
2008	Basic course on IR, Raman and NMR spectroscopy at the Bruker Corporation (Milan, Italy).
2006 – 2008	Training researcher during oceanographic cruises organised by the Italian National Research Council, Istituto per l'Ambiente Marino Costiero (IAMC-CNR) UOS Mazara del Vallo. (supervisor: Dr. Angela Cuttitta). • July 2006: Seawater sampling with CTD-rosette device, sample treatment and storage for trace element analyses. • August 2007: Seawater sampling for microbiological investigation (Bongo device). • August 2008: Seawater sampling and in-situ pH, TDS and alkalinity determination. Sediment collection with box corer device for paleo-environmental analyses.

PAPERS

2017 -Parisi M.G., Cammarata I., Cammarata M., Censi V., Rare Earth, zirconium and hafnium distribution in coastal areas: the example of Sabella spallanzanii. Chemosphere (submitted).

- 2017 - Censi P., Inguaggiato C., Chiavetta S., Schembri C., Sposito F., Censi V., Zuddas P., 2017. Zirconium, hafnium and Rare Earth Elements behaviour during halite crystallization. *Chem. Geol.* (in press).
- 2016 -Censi P., Sposito F., Inguaggiato C., Venturi M., Censi V., 2016. Weathering of evaporites. Natural versus anthropogenic signature on the composition of river waters. *Rend. Fis. Acc. Lincei* 27, 29-37.
- 2016 -Dell'Anna M. M., Censi V., Denora N., Franco M., Carrozzini B., Caliandro R., Mastrolilli, P., 2016. Triphenylphosphane Pt(II) complexes containing biologically active natural polyphenols: Synthesis, crystal structure, molecular modeling and cytotoxic studies, *Jour.of Inorg. Biochem.* 163, 346-361.
- 2014 – Nierzwicki L., Wieczor M., Censi V., Baginski M., Calucci L., Samaritani S., Czub J. Forte C., 2014. Interaction of cisplatin and two potential antitumoral platinum(II) complexes with a model lipid membrane: a combined NMR and MD study. *Phys. Chem. Chem. Phys.*, 17, 1458-1468.

EXPERTISE

- Electronic spectroscopy: (UV-vis, fluorescence, Circular dichroism), X-Ray spectrometry (single crystal and powder samples), ICP-Optical Emission Spectroscopy, NMR spectroscopy in liquid phase (1H, 19F, 31P, 13C, 195Pt) and in solid phase (2H, 31P, 13C).
- Optical spectroscopy: AFM (atomic force spectroscopy)
- Vibrational spectroscopy: IR both in liquid and solid phases,
- Environmental sample collection (solids and liquids), their handling for trace element analyses (enrichments onto ion-exchange resins, filtration and storage), lab measurements (alkalinity and pH analyses).
- Biological technique: Gel electrophoresis
- Sediment, soil and natural water collection, environmental determination of inorganic and organic compounds.

COMPUTER SKILLS

- Software packages: MatLab, Mestre Nova, Office, ChemDraw, Origin, TopSpin, CrystalMaker.
- Operating System: (Excellent) Mac OsX, (Good) Windows

LANGUAGES

- Italian (mother tongue),
- English (B2a)
- French (A2)
- Spanish (B1)