Deanship of Graduate Studies
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Synthesis, *in vitro* and Biophysical Studies of Novel Fluorescent Platinum(IV) Prodrug Derivatives and their Delivery by Fusogenic Liposomes as Nano-carriers into Cancer Cells

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Dedication

I would like to dedicate my thesis to my husband Mohammad Ali who encouraged and supported me all time. Many thanks also to my parents, who endured this long process with me, always offering support, love and caring for me and my children.

Zeinab Breijyeh

Declaration

I certify that this thesis submitted for the degree of master, is the result of my own research, except where otherwise acknowledged, and this thesis has not been submitted for the higher degree to any other university or institution.

Signed: 

Zeinab Khalid Breijyeh

Date: 17/8/2022

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Abstract

In spite the wide spread of treatment for cancer, patients still suffer from serious side effects. Platinum(II) complexes such as Cisplatin, Oxaliplatin and Carboplatin are used to treat several types of cancer but due to general toxicity and intrinsic/acquired chemo-resistance their therapeutic application is restricted. On the other hand, Platinum(IV) prodrugs with two additional axial ligands, are more stable inert form of Pt(II) compound with less side effects and have the ability to deliver Pt(II) to cancer cell by introducing targeted or lipophilic ligand to the axial position. Few platinum(IV) complexes have advanced to clinical trials, therefore, several mechanisms such as fluorescent imaging and others have been used to understand Pt(IV) mechanism of action intracellularly by the conjugation of lipophilic fluorophore to the axial ligand of platinum compounds. In this project a series of fluorescent labeled platinum(IV) prodrugs were synthesized and characterized. Platinum(IV) complexes was conjugated to high lipophilic fluorescent long chain carboxylate ligands at the axial hydroxide ligand *via* a cleavable ester linkage to produce a fluorescent labelled platinum(IV) prodrugs. Fluorescent lipophilic amino acid derivatives and platinum(IV) prodrugs were characterized by melting point, fourier transform infrared spectroscopy, proton and carbon nuclear magnetic resonance spectroscopy, Ultra-performance liquid chromatography, electrospray ionization mass spectrometry (ESI-MS) analytical techniques. Stability study for fluorescent platinum(IV) prodrugs were investigated using Ultra-performance liquid chromatography (UPLC) at constant temperature (37°C) using 7.4 pH buffer to resemble pH of blood.

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List of Abbreviations

Abbreviation	Full name
(ε)	Extinction coefficient
(λ-em),	Emission maximum
(Φ)	Quantum yield
°C	Degree Celsius
11-DAUDA	11-((5-dimethylaminonaphthalene-1-sulfonyl)amino)undecanoic acid
^{13}C-NMR	Carbon-13 nuclear magnetic resonance
^{1}H-NMR	Proton nuclear magnetic resonance
^{31}P-NMR	Phosphorus-31 nuclear magnetic resonance
6-DAHA	6-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)hexanoic acid*
BODIPY	4,4-difluoro-4-bora-3a,4a-diaza-s-indacene
BODIPY-FL	Boron,((2-(3,5-dimethyl-1H-pyrrol-2-yl-$_{K}$N)methylene)2H-pyrrole-5-propanoate-$_{K}$N^{1})difluoro
CAN	Acetonitrile
$CDCl_3$	Deuterated chloroform
CDDP-VPA	Cisplatin-Valproate
CDI	Carbonyldiimidazole

CFDA	Carboxy fluorescein diacetate
COXis	Cyclooxtgenase inhibitors
CRM	Certified reference materials
D	Doublet
D2O	Deuterium water
DCA	Dichloro-acetate
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
Dd	Doublet of doublets
DDP	Cisplatin
DHP	Oxoplatin
DIC	Diisopropylcarbodiimde
DIPEA	N,N-Diisopropylethylamine
DiR	1,1′-dioctadecyl3,3,3′,3′-tetramethylindotricarbocyanine iodide
DMAP	Dimethylaminopyridine
DMF	Dimethylformamide
DMPC	1,2-dimyristoyl-*sn*-glycero-3-phosphocholine
DMPG	1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (sodium salt)
DNA	Deoxyribonucleic acid
DNP	Dinitrophenol
DNS-Cl	Dansyl chloride

DOEPC	1,2-dioleoyl-sn-glycero-3-ethylphosphocholine (chloride salt)
DOPE	1,2-dioleoyl-sn-glycero-3-phosphoethanolamine
DPPC	Dipalmitoylphosphatidylcholine
DPPG	1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (sodium salt)
DSC	Disuccinimidyl carbonate
DSC	Differential scanning calorimetry
ds-DNA	Double stranded- Deoxyribonucleic acid
DSPC	1,2-distearoyl-*sn*-glycero-3-phosphocholine
DSPE-MPEG2k	1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine and conjugated methoxyl poly(ethylene glycol)
DSPE-PEG2000	1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt)
DSPG	1,2-distearoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (sodium salt)
EA	Ethacrynic acid
EDANS	(5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid)

EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
$\boldsymbol{E_p}$	Reduction potential
EPR	Enhanced permeability and retention effect
ESI-MS	Electrospray ionization mass spectrometry
EtOAc	Ethyl acetate
FA	Folic acid
FDA	Food and Drug Administration
F-DDP	Fluorescein labeled Cisplatin
FF-TEM	transmission electron microscopy combined with freeze fracture
FITC	Fluorescein isothiocyanate
FLs	Fusogenic liposomes
FRET	Förster resonance energy transfer
FR-α	Folate receptor α
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
GFAAS	Graphite furnace atomic absorption spectroscopy
GFP	Green fluorescent protein
GNC	Gold fluorescence nanoclusters
GOx	Glucose oxidase
GSH	Glutathione

GST	Glutathione-S-transferase
H_2O_2	Hydrogen peroxide
HATU	O-(7-Azabenzotriazol-1-yl)-N,N,N’,N’-tetramethyluronium hexafluorophosphate
HBTU	O-(Benzotriazol-1-yl)-N,N,N’,N’-tetramethyluronium hexafluorophosphate
HCl	Hydrochloric acid
HCTU	2-(6-Chlor-1H-benzotriazol-1-yl)-1,1,3,3-tetramethylaminium-hexafluorophosphat)
HDAC	Histone deacetylase
HOBT	1-hydroxybenzotriazole
HPLC	High performance liquid chromatography
HSPC	Hydrogenated soy phosphatidylcholine
IFP	Interstitial fluid pressure
IMS	Imaging mass spectrometry
K_2PtCl_4	Potassium tetrachloroplatinate(II)
KI	Potassium iodide
KMnO4	Potassium permanganate
LA-ICP-MS	Laser ablation inductively coupled plasma mass spectrometry
LC-MS	Liquid chromatography-mass spectrometry
M	Multiplet

MALDI-MS	Matrix-assisted laser desorption-ionization mass spectrometry
MB	microbubbles
MeOH	Methanol
MLV	Multilamellar vesicles
MPEG	Methoxy-polyethylene glycol
mPEG5000-bPLA6000	Methoxy poly-(ethylene glycol)-block-poly (lactic acid
MPS	Mononuclear phagocyte system
MRI	Magnetic resonance imaging
MVV	Multilamellar vesicular vesicles
MWCNT-Fluo	Multi-walled carbon nanotubes (MWCNTs) with fluorescein
MWCNT-Rho	Multi-walled carbon nanotubes (MWCNTs) with fluorescent rhodamine-110
MWCNTs	Multi-walled carbon nanotubes
Na_2SO_4	Sodium sulfate
NADH	Nicotinamide adenine dinucleotide
nanoSIMS	Nano-secondary ion mass spectrometry
NaOH	Sodium hydroxide
NBD	N-(7-nitro-2,1,3-benzoxadiazol-4-yl)ethane-1,2-diamine

NDDP	*cis*-*bis*-neodecanoato-*trans*-R,R-1,2-diaminocyclohexane platinum(II)
NH_4OH	Ammonium hydroxide
NHS	N-hydroxysuccinimide
NIR	Near infrared
NMR	Nuclear magnetic resonance
NPs	Nanoparticles
OLV	Oligolamellar vesicles
PCPP	Polyprodrug chain
PDA	Prominence diode array
PDK	Pyruvate dehydrogenase kinase
PEG	Polyethylene glucol
$PhICl_2$	Iodobenzene dichloride
$PhSO_2NCl_2$	N,N-dichlorobenzenesulfonamide
$POCl_3$	Phosphorus oxychloride
Pt-DNA	Platinum- Deoxyribonucleic acid
Pt-VPA	Diamminedichloridobis(valproate)platinum(IV)
Q	Quartet
RES	Reticuloendothelial system
RhodG	Rhodamine Green
ROS	Reactive oxygen species
S	Singlet

SA	Succinic acid
SAXS	small angle X-ray scattering
SPC	Soy phosphatidylcholine
SWNT	Single-walled carbon nanotube
T	Triplet
TBET	Through Bond Energy Transfer
TBTU	O-(Benzotriazol-1-yl)-N,N,N’,N’-tetramethyluronium tetrafluoroborate
TEA	Trimethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TPZ	Triapazamine
UCNPs	Upconversion-luminescent nanoparticles
ULV	Unilamellar vesicles
UPLC	Ultra-performance liquid chromatography
UVVIS	Ultraviolet-visible spectroscopy
λ-max	Absorption maximum

Chapter One

Introduction

1.1 Introduction

In 1965, Rosenberg discovered that certain products of platinum were able to inhibit cell division in *Escherichia coli*, this result created much interest in the possible use of these products in cancer chemotherapy[1]. It was found that Cisplatin (*cis*-diamminedichloroplatinum(II), **1,** Figure 1.1), have a potent anti-tumor activity against sarcoma and leukemia in mice[2], while its geometric isomer Transplatin (*trans*-dichlorodiammineplatinum(II), **2,** Figure 1.1), exhibited toxicity but has no cytotoxic activity. Cisplatin **(1)**, was first synthesized by M. Peyrone in 1845 and was the first FDA approved platinum compound for treating testicular and ovarian cancers in 1978. Now it's increasingly used against head and neck, bladder, cervical, esophageal and small cell lung cancer[3].

Figure 1.1. Chemical structure of: Cisplatin **(1),** Transplatin **(2).**

Cisplatin enters tumor cell by passive diffusion or active transport and binds to DNA after replacing the chloride ligands with water molecules and forming positively charged Cisplatin species (*cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$), (Figure 1.2)[4], and in a following step it irreversibly binds to DNA. It is believed that those Pt-DNA adducts induce local conformational alterations in the ds-DNA that consequently initiates the cellular responses[5,6].

Cross-linking can occur on the same strand or on different strands of DNA. Cisplatin predominantly forms DNA intra-strand crosslinks between adjacent guanine nucleobases

(65%). This link distorts the structure of DNA and cause bending and unwinding of the double helix upon platination which promotes the release of cytochrome c and the activation of intracellular caspases. These proteases effectively induce a process of programmed cell death known as apoptosis[7].

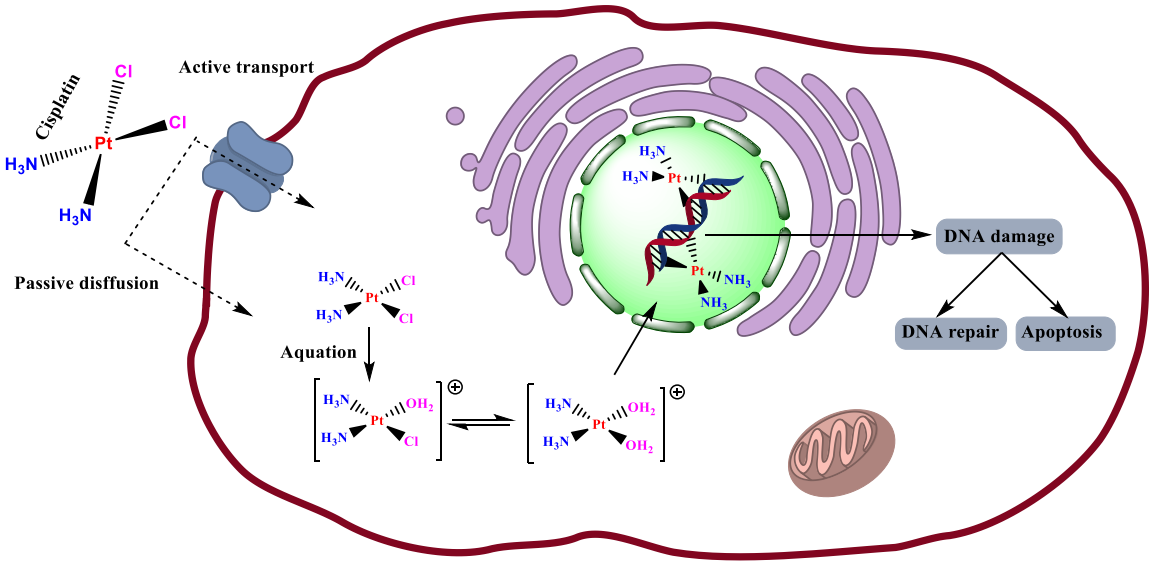


Figure 1.2. Mechanism of action of Cisplatin in tumor cells.

Cisplatin is metabolically unstable and got quickly bounded to plasma proteins, approximately 90% of the drug in the blood is bound to albumin and other plasma proteins which cause inactivation of large amount of Cisplatin and only small fraction remain active and circulate in blood stream[8].

Despite the curing effects of Cisplatin, the drug suffers serious side effects include general cell damaging effects, such as nausea and vomiting, myelosuppresion, immunosuppression, nephrotoxicity, neurotoxicity and hearing loss[9]. Cisplatin also suffers from developing acquired or intrinsic resistance explained by various mechanisms such as: reduced accumulation of platinum compounds by either enhanced efflux or

impaired influx, detoxification by GSH (and other platinophiles) conjugates and increase the level of DNA damage repair[10].

General toxicity of Cisplatin, low biological availability and acquired or intrinsic drug resistance restricted its therapeutically application and are considered serious clinical limitation of the drug[11]. In order to overcome the above problems new platinum-based compounds synthesized, developed and few of them were introduced to the clinic: Carboplatin (**3**, Figure 1.3), received regulatory approval in 1988, is a second-generation analogue which is less potent than Cisplatin but with fewer side effects. Oxaliplatin (**4,** Figure 1.3), approved in 2002, is a third generation platinum drug with a different biological profile from that of Cisplatin and carboplatin[4], Three others Pt(II) drugs have advanced to the clinic: Nedaplatin (**5,** Figure 1.3), and Miriplatin **(6,** Figure 1.3) were approved in Japan in 1995 and 2010 respectively. Nedaplatin **(5),** is a second analogue of Cisplatin with similar activity profile but low renal and gastrointestinal toxicities[12]. Miriplatin **(6),** is a lipophilic platinum complex used as trans-hepatic arterial chemotherapeutic drug for the treatment of hepatocellular carcinoma (HCC)[13]. Heptaplatin (**7,** Figure 1.3), received approval at the Korean FDA in 1999. Lobaplatin (**8,** Figure 1.3), received Chinese FDA approval in 2010[6]. These compounds have similar mechanism of action to that of cisplatin which involves DNA binding and transcription inhibition[14].

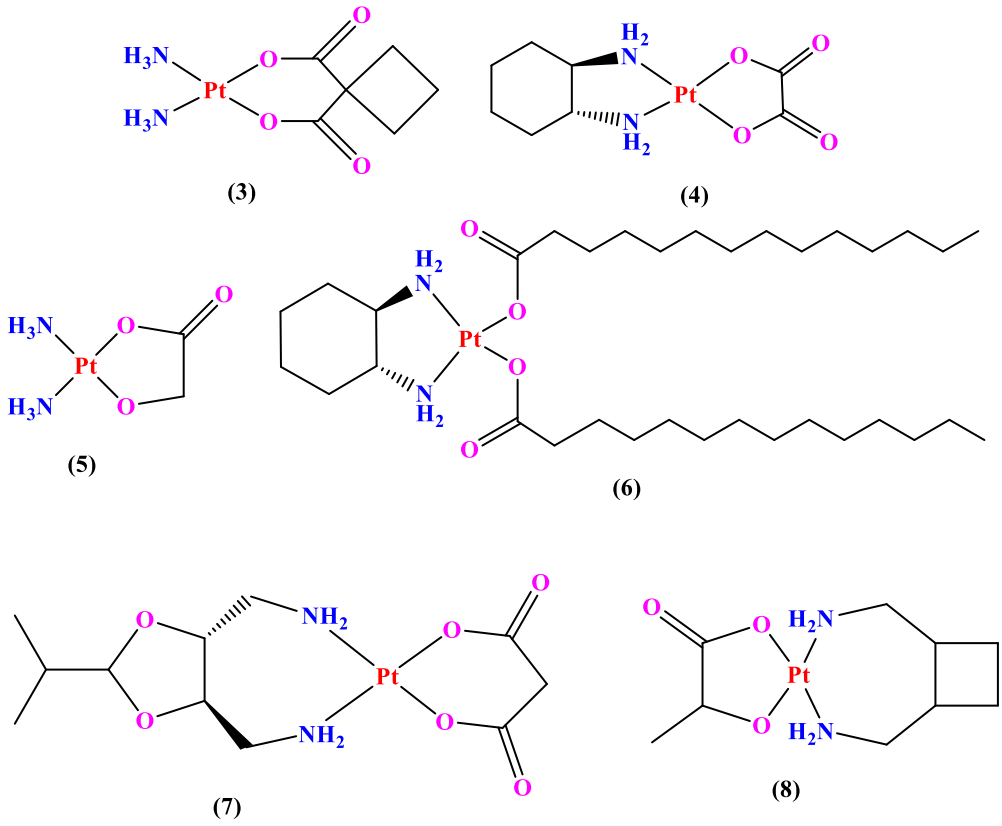


Figure 1.3. Chemical structures of carboplatin **(3),** and oxaliplatin **(4),** which approved for use worldwide, nedaplatin **(5),** Miriplatin **(6),** heptaplatin **(7)** and lobaplatin **(8),** which approved for use in Japan, Korea, and China, respectively.

Further platinum(II) drugs have advanced to clinical trials but most of them have been discontinued because of severe and unpredictable side effects that was added to lack of improved activity in phase II/III clinical trials[9]. Therefore, intense efforts have been employed to improve the efficacy of platinum complexes and reduce its side effects.

General structure for most of platinum based drugs are shown in Figure 1.4 which consists of a non-leaving group ligands, which are a central platinum-two stable ammines and leaving groups such as chloride or carboxylate to allow platinum ion to form bonds with DNA bases[15,16], and axial ligands which are present only in higher valent platinum complexes found in platinum(III) and platinum(IV)[14]. Therefore, different strategical

methods used to modify structural features for developing new non-classical platinum complexes, that are capable of forming different range of DNA adducts with anticancer activity different than that of Cisplatin[17,18], such as synthesis of: sterically hindered platinum drug[19]; multinuclear platinum drugs which contains two or more linked platinum centers that can covalently bind to DNA[20]; platinum-intercalator conjugates which act through a dual DNA binding mode[21]; and designing of chemically reductive platinum(IV) platinum complexes as prodrugs which are more kinetically inert octahedral low-spin $5d^6$ platinum(IV) complexes compared to square-planar $5d^8$ platinum(II) complexes[22].

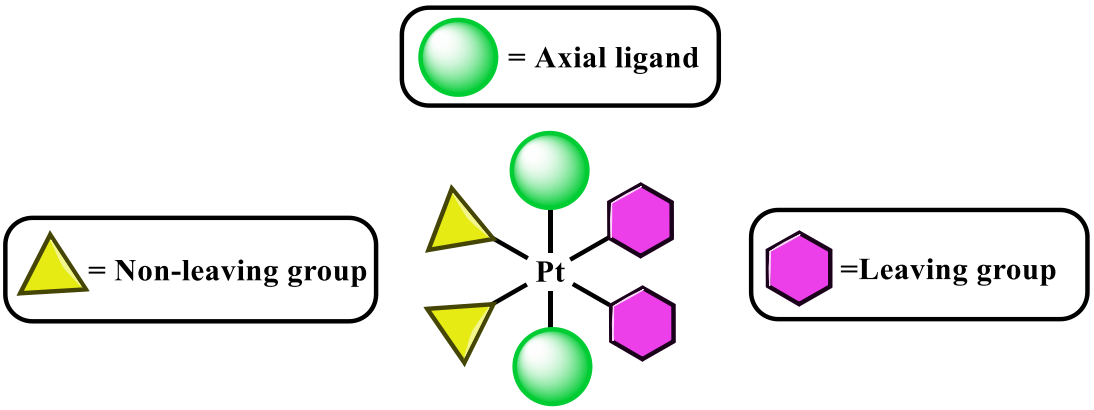


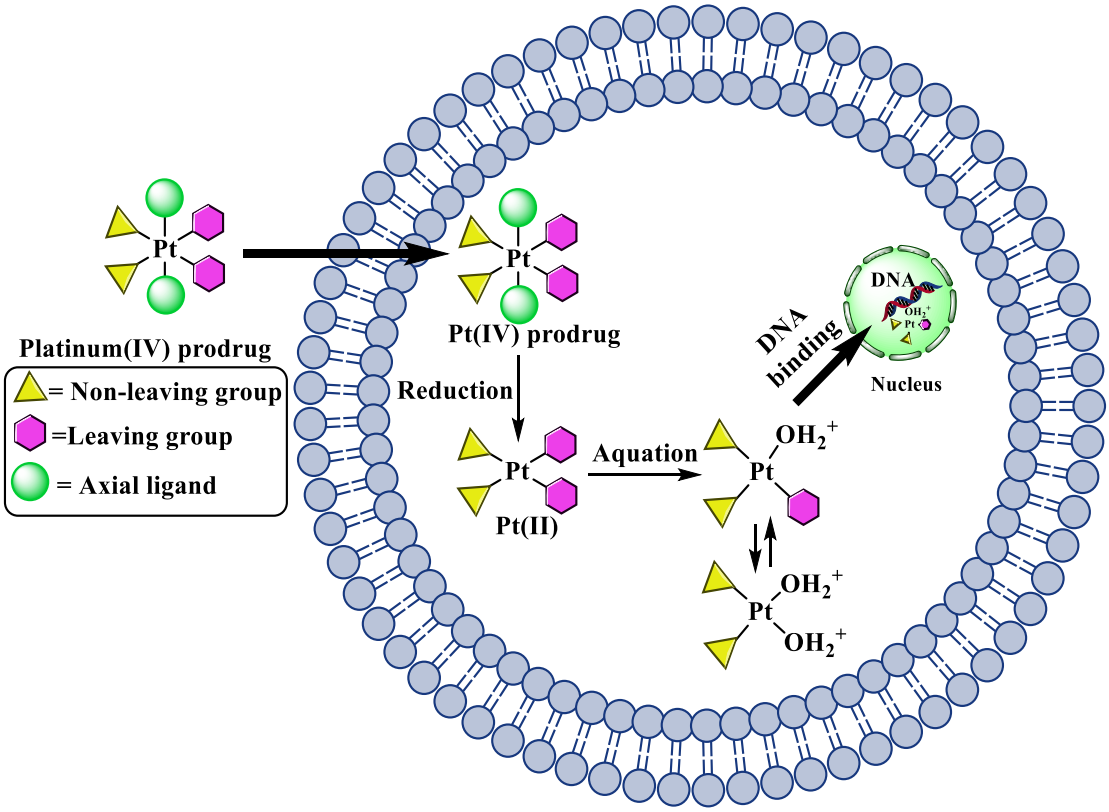
Figure 1.4. Structure of platinum complexes components; the non-leaving group, the leaving group and the axial ligands.

Platinum(IV) derivatives are likely to have better pharmacokinetics, lipophilicity, bioactivity, decreased side effects and stability under highly acidic conditions which made them suitable for oral delivery thus offer a better potential in targeting and increased efficacy compared to platinum(II) counterparts[22,23]. Nonetheless, so far FDA didn't approve any platinum(IV) complex for clinical application. Therefore, efforts are now focused on understanding the ways platinum(IV) derivatives are processed in the cell, their

molecular mechanisms of action, ways of delivering them into tumor sites with minimum side effects[24].

1.2 Platinum(IV) complexes

Due to resistance, side effects and low bioavailability of Pt(II) compounds, efforts were directed to developing new platinum drugs that can overcome these drawbacks. Pt(IV) complexes are relatively inert prodrugs with central platinum atom which upon activation gives the active Pt(II) derivatives (Scheme 1.1). The advantage of this group of compounds lies in their ability to remain in their relatively stable oxidation state [Pt(IV)] while in the blood stream. This approach is hoped to result in controlling the reactivity and the loss of the active drug which can lead to a reduction in drug toxic side effects. Pt(IV) complexes can be reduced by a plethora of agents including: Glutathione which is the primary defense against toxin and oxidants in the cell and can deactivate electrophile drugs like chemotherapeutic agents[25-27]. In addition to, ascorbate, L-Methionine, L-Cysteine and DL-homocysteine reductants which present in high abundance amount in human plasma[27-29]. Other molecules such as; serum albumin (small protein and one of the major thiols in the blood), hemoglobin, and cytochrome c may play a role in the activation of Pt(IV) under specific circumstances like the presence of NADH[29,30].

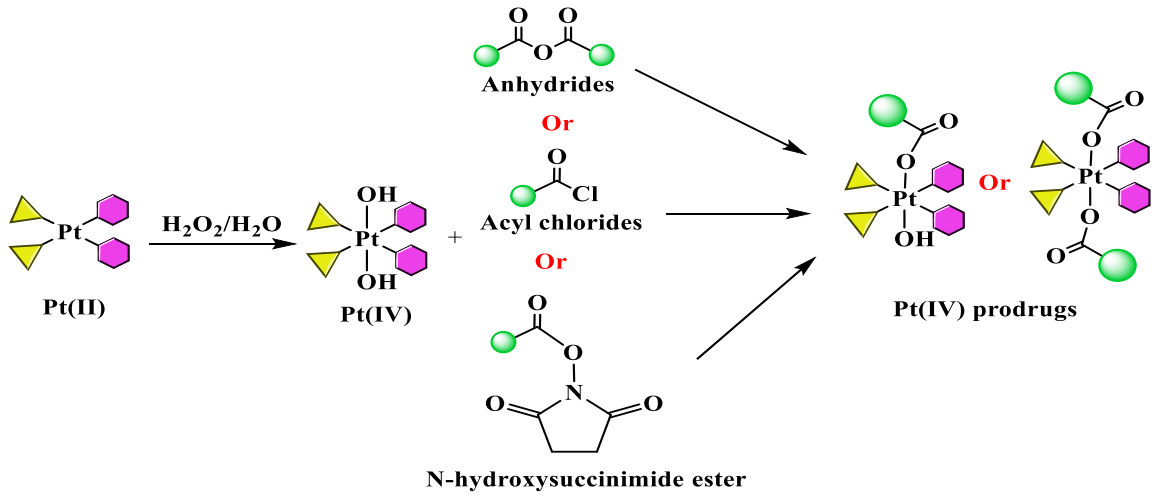


Scheme 1.1 Structure of Pt(IV) and mechanism of intracellular activation.

1.2.1 Oxidation of Platinum(II) complexes

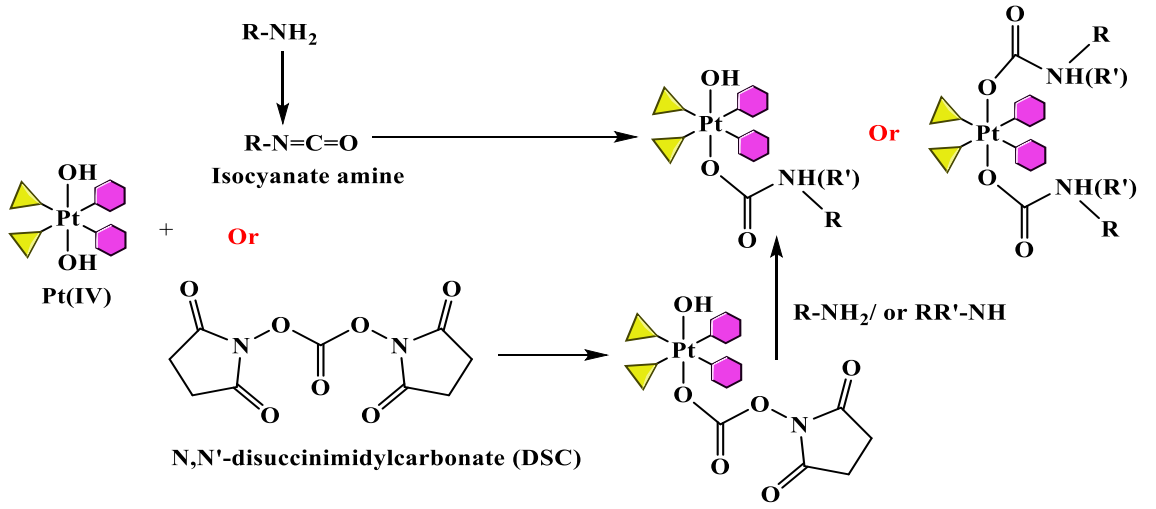
Three factors determine the final product of platinum(II) oxidation; platinum(II) precursor, oxidant and solvent. The oxidant can obtain two electrons from platinum(II) center and occupy one axial ligand in the final product and at the same time, an anion from an oxidant or solvent can become the other axial ligands[14,31]. The most widely used oxidizing agent to oxidize platinum(II) complexes is hydrogen peroxide (H_2O_2) to obtain dihydroxido platinum(IV) complexes (*cis*,*cis*,*trans*-[$PtL_2X_2(OH)_2$]), in which the two additional OH groups that occupy the axial positions came from H_2O_2, the oxidant and the other one from originate from water (H_2O), the solvent[31-33]. Different organic protic solvents (ROH) such as methanol, ethanol, and acetic acid can be used as a media which can coordinate to the platinum(IV) center with an additional ligand during the oxidation process[34,35].

Carboxylic acids could be utilized to be conjugated to the axial ligand of platinum(IV) complexes but due to its low reactivity a helping coupling reagents such as DCC, TBTU, and HBTU are used for conjugation[24,31]. Another approach is by converting the carboxylic acid to more reactive derivatives by using acyl chloride, acid anhydrides such as acyclic aliphatic or aromatic anhydrides of cyclic ones (glutaric, maleic, succinic and phthalic anhydride), or N-hydroxysuccinimide ester and then react it with the axial OH ligands (Scheme 1.2)[36-38]. Anhydrides and acyl chloride methods are widely used but they suffer from limited choice of ligands (e.g. simple aliphatic and aromatic molecules) that can be introduced to the axial position of Pt(IV) complexes[24]. Therefore, preparation of non-symmetrical bis-carboxylate complexes with the ability to introduce different bio-functional groups like nano-carriers can be achieved by two methods introduced by Hambley and Gibson *et al.*[4] where they reacted Pt(II) complexes with large excess amount of carboxylic acid and small excess of oxidizing agents, or by using of coupling reagents to activate carboxylic acids like carbodiimide (e.g. dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide (DIC), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC)), Which will finally react with dihydroxido platinum(IV) precursors[6,14,24]. Carbodiimide can also be used to prepare anhydrides by reacting free acid with carbodiimide coupling reagents and washing several times with acid and base to get the desired anhydride that can be reacted with platinum(IV) compounds[39].



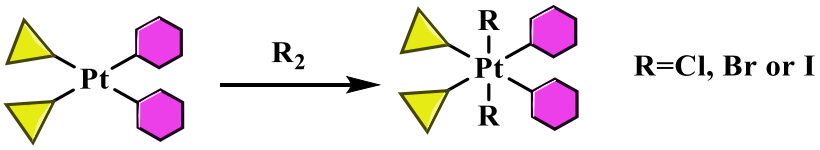
Scheme 1.2. General synthetic route for platinum(IV) prodrugs. a) Oxidation of Pt(II) by hydrogen peroxide (H_2O_2), b) Acylation of dihydroxyplatinum(IV) by using either acid anhydrides, acyl chlorides, or N-hydroxysuccininide esters.

Furthermore, the compounds that contain amine groups their conjugation can be achieved by either converting the primary amine to isocyanate (R-NH_2 to RN=C=O) or by activating the axial OH ligands by using N,N'-disuccinimidylcarbonate (DSC) then react it with primary or secondary amines[23,40-42].



Scheme 1.3. Conjugation of amine containing compound to platinum(IV) axial OH ligand by converting the primary amine to isocyanate or by activating the OH axial ligand by using N,N'-disuccinimidylcarbonate (DSC).

In addition to H_2O_2, halogens like Cl_2, Br_2, and I_2 have been also used as oxidizing agents for platinum(II) complexes where two halogens can occupy the two axial positions[31,33,43]. Despite that, the use of chlorine gas is not convenient and should follow a special request during operation in laboratory for safety reasons. Therefore, an alternative safe chlorinating agents such as iodobenzene dichloride ($PhICl_2$) or N,N-dichlorobenzenesulfonamide ($PhSO_2NCl_2$) are used to form dichlorinated platinum(IV) complexes with gradual generation of chlorine gas[31,33].



Scheme1. 4. Oxidation of platinum(II) complexes by halogens R_2 (R= Cl, Br, or I).

Other oxidative agents can be used like dichloride salt of the dithiobis(formamidinium), KMnO4 or ozone in water, and others agents all lead to the oxidation of Pt(II) complexes[14,43].

1.2.2 Advantages of platinum(IV) axial group ligands

Reduction of Pt(IV) complexes to Pt(II) is essential to elicit the anticancer active form. Thus, reduction process has a major role to play in the biological activity of Pt(IV). Table 1.1 depicts the reduction potential (E_p) of different axial ligands such as; chloro and trifluoroacetate ($OCOCF_3$) where the reduction occurs most readily, carboxylate and hydroxo with intermediate and least reduction respectively ($OCOCF_3$ > Cl > OAc > OH), therefore, the more electronegative the ligands are; the more destabilize the Pt(IV), that results in faster reduction of the higher oxidation state[27,44-46]. Another factor also affect the reduction rate is the bulkiness of the axial and equatorial ligands; for example JM-221

$Pt(NH_3)(C_6H_{11}NH_2)(OCOC_3H_7)_2Cl_2$ is reduced twice as fast as JM-216 (Satraplatin) $Pt(NH_3)(C_6H_{11}NH_2)(OCOCH3)_2Cl_2$ due to $OCOC_3H_7$ bulkier ligand[46]. Moreover, Pt(IV) complexes with ethylenediamine carrier have slower reduction rate than those with isopropylamine and cyclohexylamine carrier ligands which may refer to the fact that ethylenediamine may cause less steric hindrance and stabilize the six-coordinated state while the bulky ligands destabilize it which results in a faster reduction rate[46].

Table 1.1 Reduction potential (E_p) with various axial ligands of different platinum compounds

Compounds	**Reduction potential (E_p (mV))**
cis,*trans*,*cis*-$[PtCl_2(NH_3)_2(OH)_2]$	-880
cis,*trans*-$[PtCl_2(en)(OH)_2]$*	-664
cis,*trans*,*cis*-$[PtCl_2(NH_3)_2(OAc)_2]$	-635
cis,*trans*-$[PtCl_2(en)(OCOCH_3)_2]$	-326
cis,*trans*-$[PtCl_2(en)(OCOCH_2CH_3)_2]$	-301
cis,*trans*-$[PtCl_2(en)(OCOCH_2CH_2CH_3)_2]$	-273
cis-$[PtCl_4(NH_3)_2]$	-260
cis,*trans*-$[PtCl_4(en)]$	-4
cis,*trans*-$[PtCl_2(en)(OCOCF_3)_2]$	0

*(en): Ethylenediamine

Versatile groups can be introduced to the axial position of Pt(IV) complexes such as; lipophilic groups or bioactive ligands which can lead to an enhancement in the cellular uptake (e.g. polymers and nanoparticles)[14,47,48], enzyme inhibitors like histone deacetylase (HDAC) *inhibitors* can increase bioactivity when used[39]. Fatty acid conjugate can be used to overcome resistance [3], or improved targeting (e.g. folates)[47]. Additionally, axial ligands can be fluorescent probes (fluorescein-based probe or rhodamine-based probe)[49].

1.2.3 Pt(IV) complexes in clinical and preclinical phases

Several platinum(IV) complexes such as Tetraplatin **(9),** Iproplatin **(10)**, Satraplatin **(11)** and LA-12 **(12)** (Figure 1.5) have been developed and assessed at clinical trials[14], nonetheless up to now none was approved by the FDA. Tetraplatin (or Ormaplatin) **(9),** did not proceed to phase II due to sever neurotoxicity[5]. Iproplatin **(10)** proceeded through phase I, II to phase III, however the clinical studies were terminated due to its low effectiveness compared to cisplatin or carboplatin and it is lower susceptible to reduction and activation by biological reducing agents most probably because of the hydroxide ligands on axial position[5,50]. Satraplatin **(11),** an orally active platinum(IV) agent, exhibited improved toxicity profile with lower side effect (ototoxicity, neurotoxicity or nephrotoxicity) than Cisplatin and Carboplatin. Satraplatin entered phase I, II and III trials and due to its oral administration and low side effect in 2016 phase 1 clinical trial in pediatric patients was started. However, Satraplatin showed no overall improvement on patient survival rates and still under investigation[51]. As a result, LA-12 **(12)**, a Satraplatin analogue that contains 1-adamantylamine non-leaving ligand was developed. LA-12 showed higher cytotoxic activity, better pharmacokinetics and low side effects compared to most of platinum-based drugs including Satraplatin. Still, its exact mechanism of action is not understood and is under investigation[52-54].

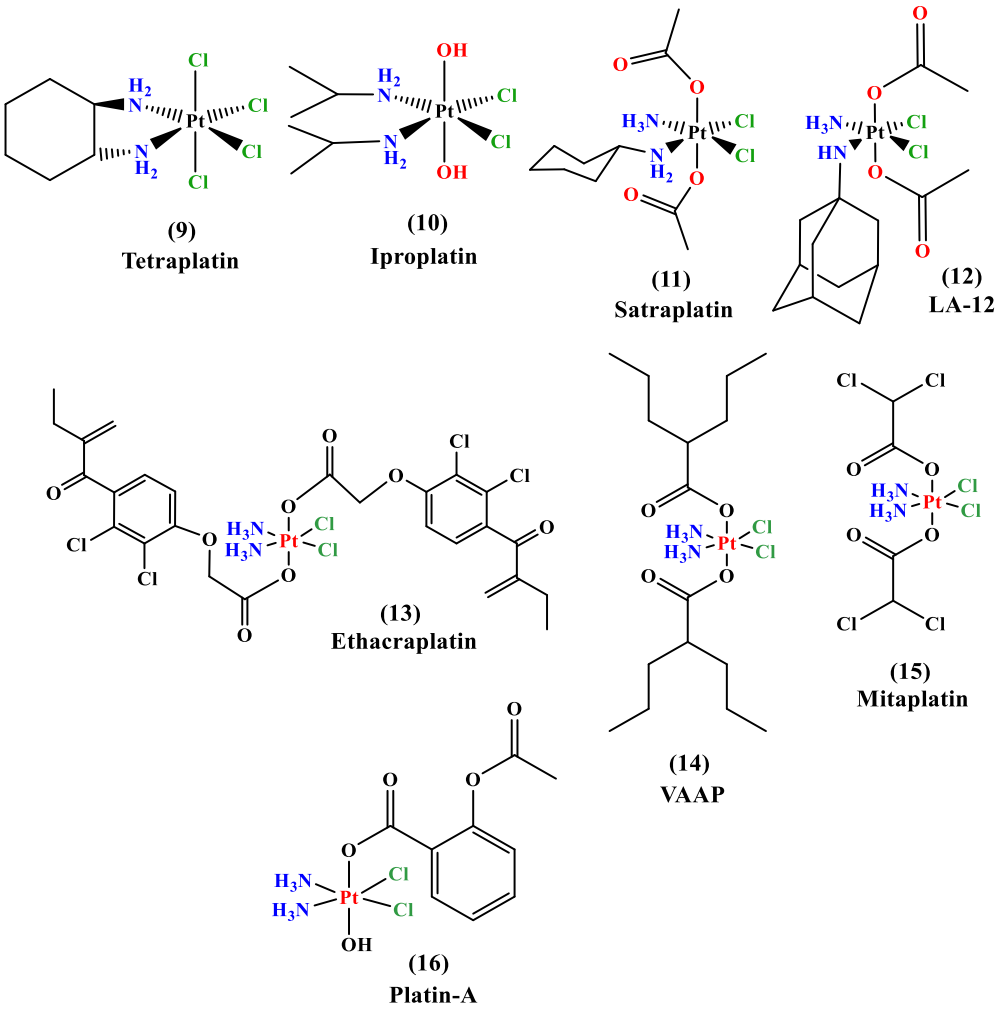


Figure 1.5. Examples of platinum(IV) anticancer agents in: 1) Clinical trials including; Tetraplatin **(9),** Iproplatin **(10),** Satraplatin **(11)** and LA-12 **(12).** 2) Preclinical trials with biologically active axial ligands such as Ethacraplatin **(13),** VAAP **(14),** Mitaplatin **(15)** and Platin-A **(16).**

Other platinum(IV) complexes were developed as dual acting agents that was attained by introducing two biologically active ligands at the axial positions. Many of them are still in preclinical stages[5]. Ethacraplatin (**13,** Figure 1.5), is a conjugated cisplatin with two ethacrynic acid (EA) ligands at the axial position. EA is a diuretic drug which has been found to be a potent inhibitor of glutathione-S-transferase (GST), (the enzyme responsible for the detoxification of platinum agents). Upon intracellular reduction of ethacraplatin, cisplatin is released with two equivalents of GST inhibitor, EA. This approach could

increase the uptake of EA and reduce the side effect and resistance to cisplatin[55,56]. Another example is the conjugation of valproic acid or phenylbutyrate (PhB), an inhibitor of histone deacetylase (HDAC) that stimulates differentiation and apoptosis in cancer cells with platinum complexes. VAAP (**14,** Figure 1.5)**,** is a bifunctional Pt(IV) divalproate complex which upon reduction give two valproic acid and cisplatin. VAAP exhibit a strong synergistic cytotoxicity profile with prolonged blood circulation times and low side effects[48,57]. Mitaplatin (**15,** Figure 1.5)**,** a platinum(IV) complex with two dichloro-acetate ligands (DCA) at the axial position. DCA is used to treat lactic acidosis and inhibits pyruvate dehydrogenase kinase (PDK) thereby restores normal mitochondrial function. Reduction of mitaplatin inside the cell releases DCA which promotes mitochondria apoptosis by releasing cytochrome c, while free cisplatin induces DNA damage and apoptotic cell death[5,58].

Another class of Pt(IV) dual action prodrugs are the conjugation with cyclooxygenase inhibitors (COXis) such as ibuprofen and indomethacin which results in increasing the potency of cisplatin and reduce side effects. Platin-A (**16,** Figure 1.5) is a Pt(IV) complex containing acetylsalicylic acid as axial ligand. The complex showed high antitumor activity than cisplatin with lower toxicity profile. Other COXis such as ketoprofen and naproxen were also studied for conjugation with platinum(IV) core by esterification reaction[59].

So far, none of the platinum(IV) complex was approved by FDA despite synthesizing and evaluating thousands of compounds. Therefore, effort must be done to know much better about platinum(IV) class of drugs and how they are processed at the cellular level and their molecular mechanisms of action to generate potent Pt(IV) complexes with improved clinical outcomes.

1.3 Fluorescence and fluorescent labeled platinum complexes

Different techniques have been used to visualize and quantify Pt-based agents and their distribution in biological tissues to understand their mechanism of action. Zoriy *et al.*[60] used laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) as an imaging tool for the detection of Pt distribution in animal tissue. Despite the high sensitivity of LA-ICP-MS it is limited to heteroatom molecules and can't distinguish between parent drugs and their metabolites[60]. Other methods used for Pt imaging are quantitative analysis using imaging mass spectrometry (IMS) and nano-secondary ion mass spectrometry (nanoSIMS), but they are impractical because they lack certified reference materials (CRM) and depend on matrix effects which make reliable quantification impossible[61,62]. Matrix-assisted laser desorption-ionization mass spectrometry (MALDI-MS) also suffer from poor ionization and signal suppression leading to false negatives[63]. X-ray based techniques also have been used to image the distribution of platinum complexes, however, they are not applicable to live cells[63]. Therefore, we need a technique that is sensitive to concentrations from nano- to micromolar with temporal resolution and sufficient spatial to analyze dynamic processes in cells, moreover, the techniques should not be invasive. Fluorescence imaging now is used frequently and is an effective technique which provide a quantitative information about the dynamic processes that occur in live cells, and has been used to study the cellular localization and uptake of platinum-fluorophore conjugates[63-66].

Fluorescence, is a term describes the process of absorption and emission of light by fluorophores[67]. The process begins when a molecule in ground state absorbing a photon

of appropriate energy that causes electron excitation to higher energy level which then relaxes and causes photon emission (fluorescence) (Figure 1.6.)[68].

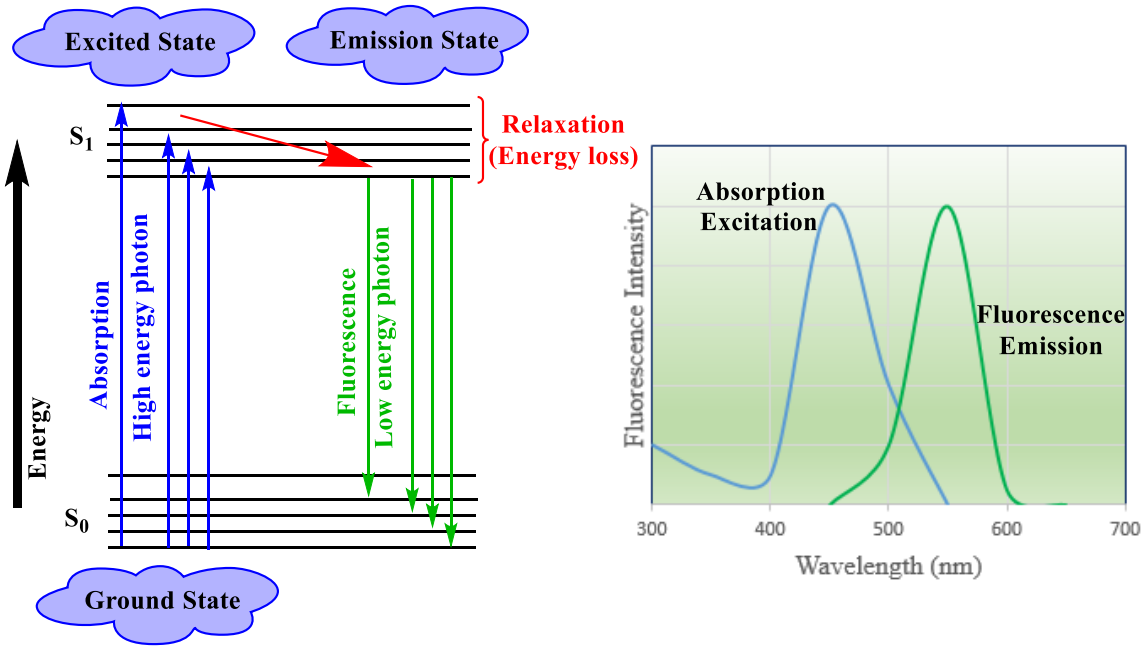


Figure 1.6. General scheme of fluorescence process. An electron is excited after absorbing a high energy photon then relaxed to a lowest energy levels releasing a photon of longer wavelength.

1.3.1 Fluorophores

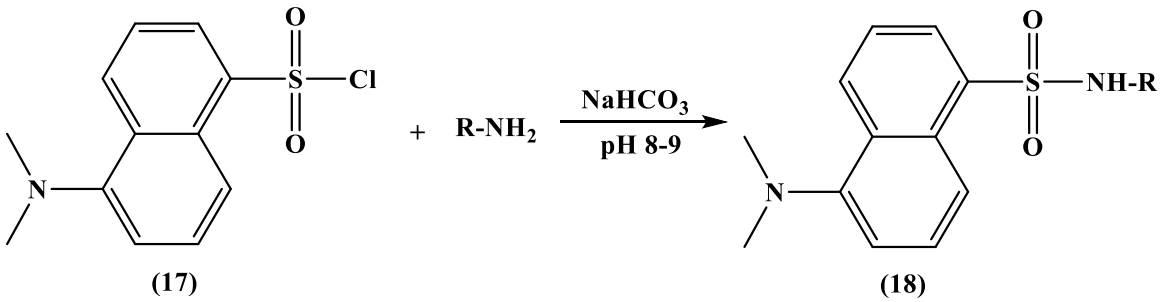
Fluorophore molecules have been used for a long time as a tool for *in vitro* imaging of biomolecules, enzyme substrates, environmental indicators, and cellular stains. In 1942 fluorescein isothiocyanate (FITC) was firstly used to label anti-pneumococcal antibodies, and over years, this method was developed to be used in living objects[69]. The usefulness of a particular fluorophore is governed by its chemical properties such as lipophilicity, reactivity, stability, and pKa, or its photo-physical properties like absorption maximum (λ-max), emission maximum (λ-em), extinction coefficient (ε) (measure of the probability of light absorption by the dye), and quantum yield (Φ) (the ratio between the number of photons emitted and the number of photons absorbed)[70].

Controlling fluorescence intensities can be achieved by several methods including: installing a blocking group onto the dye to suppress fluorescence which is restored by removing this group (e.g. Fluorescein diacetate)[71], the use of Förster resonance energy transfer (FRET) which require a transfer of energy from excited donor dye or chromophore to another acceptor dye in its ground state (e.g. Green fluorescent protein (GFP), boron dipyrromethene (BODIPY) dyes)[72,73], modifying the core structure of the dye by using chemistry (e.g. Photoconversion of (E)-o-hydroxycinnamate to coumarin)[74], or changing in the polarity of the media (e.g. nile red, 9-diethylamino-5H-benzo[alpha]phenoxazine-5-one dye)[75], or by changing the electronic structure of the dye which cause changes in fluorescent (e.g. Fluorescent $Ca2^{+}$ indicators (Fluoresceine-4))[73,76]. Fluorophores used *in vivo* application should possess several characteristic (Figure 1.7) including; 1) high brightness, 2) soluble in water, 3) does not aggregate in aqueous media and doesn't interact with biomolecules like serum proteins, 4) stay fluorescent in the biological environment, and 5) have the ability to be attached to targeting or recognition units[68].

Figure 1.7. Characteristics and advantages of fluorophores used in *in vivo* applications.

1.3.1.1 Dansyl Chloride

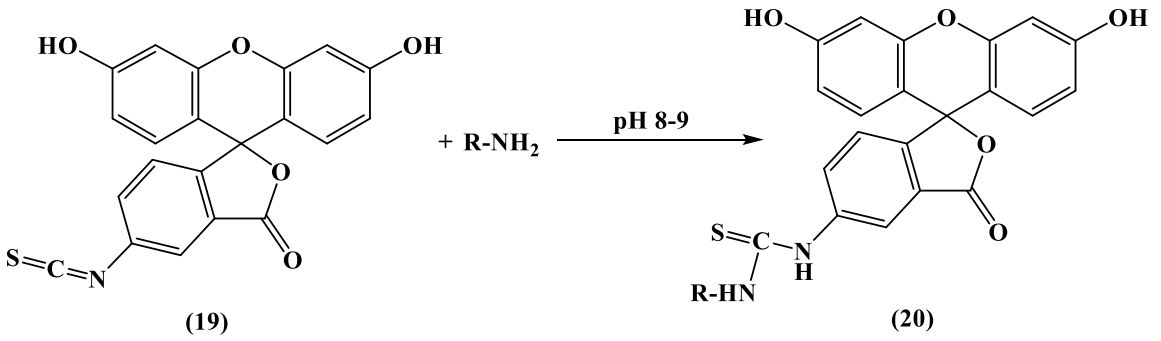
5-(Dimethylamino)naphthalene-1-Sulfonyl chloride (dansyl chloride, DNS-Cl) (**17**, Scheme 1.5) is a non-fluorescent reagent that can react with amines to give a fluorescent derivatives (**18**, Scheme 1.5) to be used in analytical chemistry for amino-acids derivatization and other detection techniques[77,78]. Dansylation can also be used for hydroxyl group (–OH) or carboxylic acid (–COOH) group. Fluorescent dansylated derivatives can be detected with ultraviolet light with great sensitivity[79].



Scheme 1.5. Reaction for labeling *N*-terminal amino acids with Dansyl chloride **(17).**

1.3.1.2 Fluorescein isothiocyanate (FITC)

Fluorescein isothiocyanate (FITC) (**19,** Scheme 1.6) is a pH sensitive dye in which its intensity diminishes when increasing solution pH[80]. FITC is used widely as fluorescence labelling reagent due to its conjugation stability and high quantum efficiency. FITC form covalent amide bond when reacting with a primary amine (**20,** Scheme 1.6) on different protein substrate which can be used as specific probes in enzyme kinetics, immunocytochemistry, flow cytometry and identification of receptors on target cells[81].



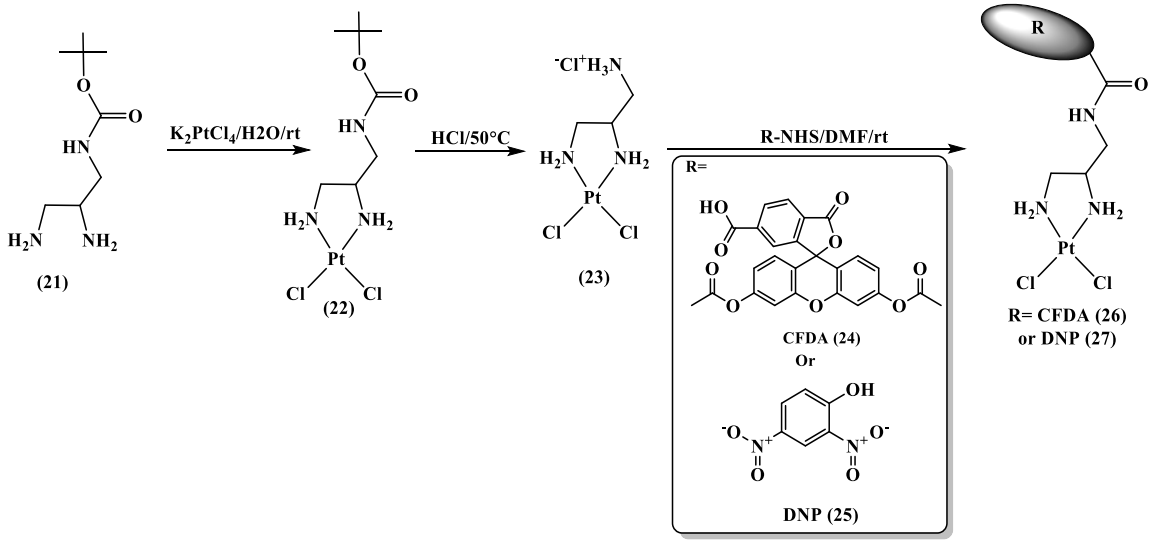
Scheme 1.6. Reaction for labeling *N*-terminal amino acids with FITC **(19).**

1.3.2 Fluorescent labelled platinum(II) complexes and their synthesis

To understand the mechanism of action in a cellular level of platinum complexes it is crucial to investigate their uptake, cellular distribution, and processing. This can feed in

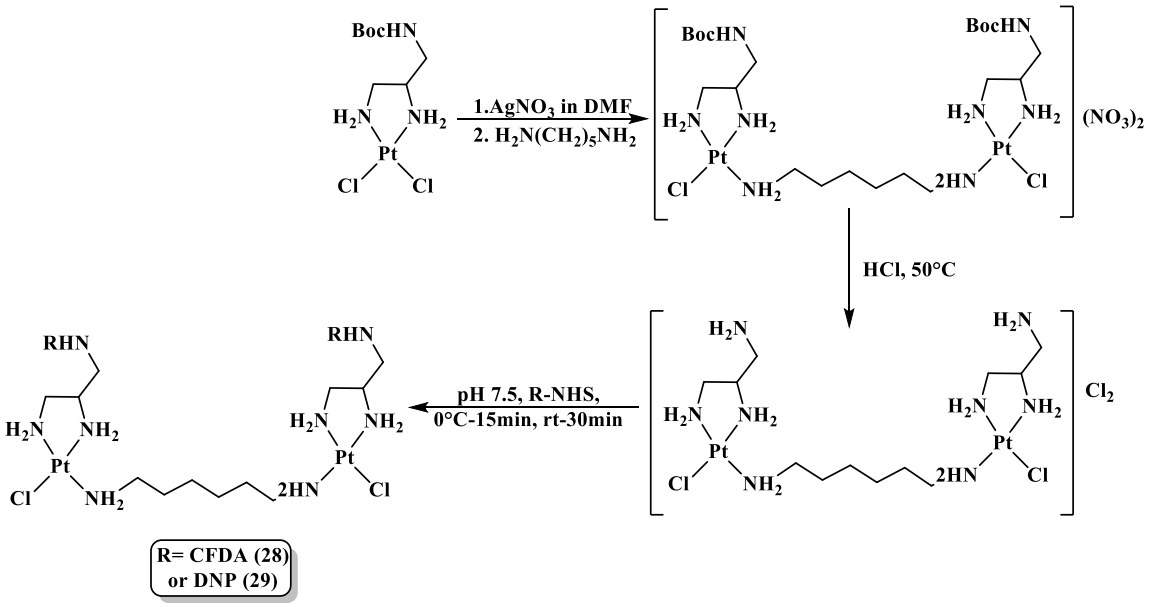
developing new tumor specific anticancer agents[82]. Unfortunately, most of platinum anticancer compounds are not intrinsically fluorescent; so, they need to be tagged to a fluorescent in order to be visualized within the cell. It has been observed that the binding of a number of transition metals to a fluorophore can increase or decrease its fluorescence properties[83,84].

Fluorophores conjugated to platinum complexes was first investigated by Molenaar *et al.* [84]whom worked on covalently linked carboxy fluorescein diacetate (CFDA) to platinum complex and showed that the complex was readily taken by cancer cells and accumulated in the nucleus by fluorescence microscopy. The fluorescent platinum compound was prepared by using N^3-(*tert*-butoxycarbonyl)-1,2,3-propanetriamine (**21,** Scheme 1.7) as starting material and reacted it with K_2PtCl_4 to produce 1-(*tert*-Butoxycarbonylaminomethyl)-1,2-ethylenediamine)dichloroplatinum(II) **(22)**. Debocylation of **(22)** at 50°C in HCl result in conversion to (1-Aminomethyl-1,2-ethylenediamine)dichloroplatinum(II) **(23)** (AMEN-Pt). Then Coupling with fluourophore was done by neutralizing the solution with NaOH to pH 7.5 and cooled to 0°C then 5-(and 6-)carboxyfluresein diacetate **(24)** in DMF or 2,4-Dinitrophenylaminohexanamidomethyl **(25)** was added to the solution to produce (1-(5-(and 6-)carboxyflureseindiacetate)aminomethyl)-1,2-ethylenediamine)dichloroplatinum(II)) (CFDA-Pt) **(26)** or (1-(2,4-Dinitrophenylaminohexanamidomethyl)-1,2-ethylenediamine)dichloroplatinum(II)) (DNP-Pt) **(27)**[84].



Scheme 1.7. Synthetic route of (1-(5-(6-)carboxyfluresein diacetate)aminomethyl)-1,2-ethylenediamine)dichloroplatinum(II)) (CFDA-Pt) **(26)** or (1-(2,4-Dinitrophenylaminohexanamidomethyl)-1,2-ethylenediamine)dichloroplatinum(II)) (DNP-Pt) **(27)**[84].

Kalayda *et al.* [82,85]also worked on the synthesis of di-nuclear platinum complexes conjugated to fluorogenic reporter such as fluorescent anthraquinone intercalators which combined the effect of intercalating agents with those of platinum complexes to give a synergistic effect and rapid accumulation in cancer cells with higher cytotoxicity. The dinuclear platinum complexes [bis((1-(5-(and 6-)-carboxyfluoresceindiacetate-aminomethyl)-1,2-ethylenediamine)chloroplatinum(II))(µ-1,6-hexanediamine)] chloride (1,1/c,c/CFDA) **(28)** and [bis((1-(2,4-dinitrophenylhexanoicaminomethylene)-1,2-ethylenediamine)chloroplatinum(II))(µ-1,6-hexanediamine)] chloride (1,1/c,c/DNP) **(29)** (Scheme 1.8), were synthesized similarly to the previously described mononuclear complexes CFDA-Pt and DNP-Pt[82,85].



Scheme 1.8. Synthetic route of [bis((1-(5-(and 6-)-carboxyfluoresceindiacetate-aminomethyl)-1,2-ethylenediamine)chloroplatinum(II))(μ-1,6-hexanediamine)] chloride (1,1/c,c/CFDA) **(28)** and [bis((1-(2,4-dinitrophenylhexanoicaminomethylene)-1,2-ethylenediamine)chloroplatinum(II))(μ-1,6-hexanediamine)] chloride (1,1/c,c/DNP) **(29)**[82,85].

Safaei and katano *et al.*[86,87] used fluorescent microscopy to identify the subcellular compartments in which Cisplatin (DDP) accumulates in human ovarian carcinoma cells by using fluorescein labeled DDP (F-DDP) (**30,** Figure 1.8), and showed that F-DDP accumulated in the Golgi, vesicles and in lysosomes. The advantage of F-DDP over CFDA is having a similar cytotoxicity and accumulation terms of Cisplatin[86,87]. F-DDP was synthesized in similar manner to the method described by Molenaar *et al.* with some modifications. Briefly N^3-*t*Boc-1,2,3-propanetriamine was reacted with K_2PtCl_4 to obtain [1-(*t*Boc-aminomethyl)-1,2-ehtylenediamine] dichloroplatinum(II). Debocylation was done at 70°C in HCl to give the platinated salt which was neutralized with NaOH to be reacted with activated 5(6)-carboxyfluorescein (activation was done by treatment with ethyldimethylaminopropyl carbodiimide and *N*-hydroxysuccinimide in dimethyl formamide).

Liang *et al.*[88] studied the trafficking and localization of Cisplatin in two type of tumor cell lines by using fluorescence Alexa Fluor (**31,** Figure 1.8) labeled Cisplatin. Co-localization experiments with a Golgi-selective stain indicate that Golgi-like vesicles involve in initial intracellular processing of Alexa Fluor-cisplatin complexes[88].

The use of fluorescein and its derivatives was found to be not ideal for intra-vital imaging due to its photo-bleaching and poor fluorescence in intracellular environments[89]. In addition, FITC and Alexa fluorophores derivatives tend to mimic the biological activity of the fluorescent rather than the platinum part itself[90]. Therefore, other green fluorescence dyes have been used in studies such as; Rhodamine Green (RhodG), and 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY; BODIPY-FL)[89].

Benedetti *et al.*[91] examined Cisplatin conjugated to N-(7-nitro-2,1,3-benzoxadiazol-4-yl)ethane-1,2-diamine (NBD) by confocal microscopy to study the mechanism of uptake and biomolecule interactions in cells for platinum drugs[91]. The advantage of using NBD is that its addition can be performed on primary amine and produce analogues very similar to the parent drugs[90]. Benedetti *et al.* conjugated NBD directly to the primary amine on both ends of TriplatinNC complex **(32,** Figure 1.8) and for Cisplatin the amine group was replaced with NBD-ethane-1,2-diamine **(33,** Figure 1.8).

Other scientists have designed fluorescent probe to detect platinum(II) complexes in intracellular environment. Shen *et al.*[92] conjugated fluorescein with dithiocarbamic acid and used it as a probe for studying cellular metabolism, localization and transformation of Pt(II) compounds, and called it FDCPt1 **(34,** Figure 1.8)[92].

Montagner *et al.*[93] designed a fluorescent turn-on probe to detect Pt(II) drugs intracellularly by binding Pt(II) complex such as cisplatin with probe Rho-DDTC **(35,** Figure 1.8) (spirolactam form of rhodamine B (RhoB)) through dithiocarbonyl bond. The probe was used also to monitor the reduction of Pt(IV) prodrugs inside cells[93]. Wee Han Ong *et al.*[49,94] developed two rhodamine-based fluorescent probe depend on Förster resonance energy transfer (FRET) and Through Bond Energy Transfer (TBET) to detect and quantify platinum(II) complexes like cisplatin. Both probes, NPR1 **(36,** Figure 1.8) and RDC1 **(37,** Figure 1.8) are coumarin-rhodamine and naphthalimide-rhodamine, respectively, conjugated to a dithiocarbamate as a recognition motif. All these probes can serve as a great tool to understand how platinum complexes are processed at cellular level[49,94].

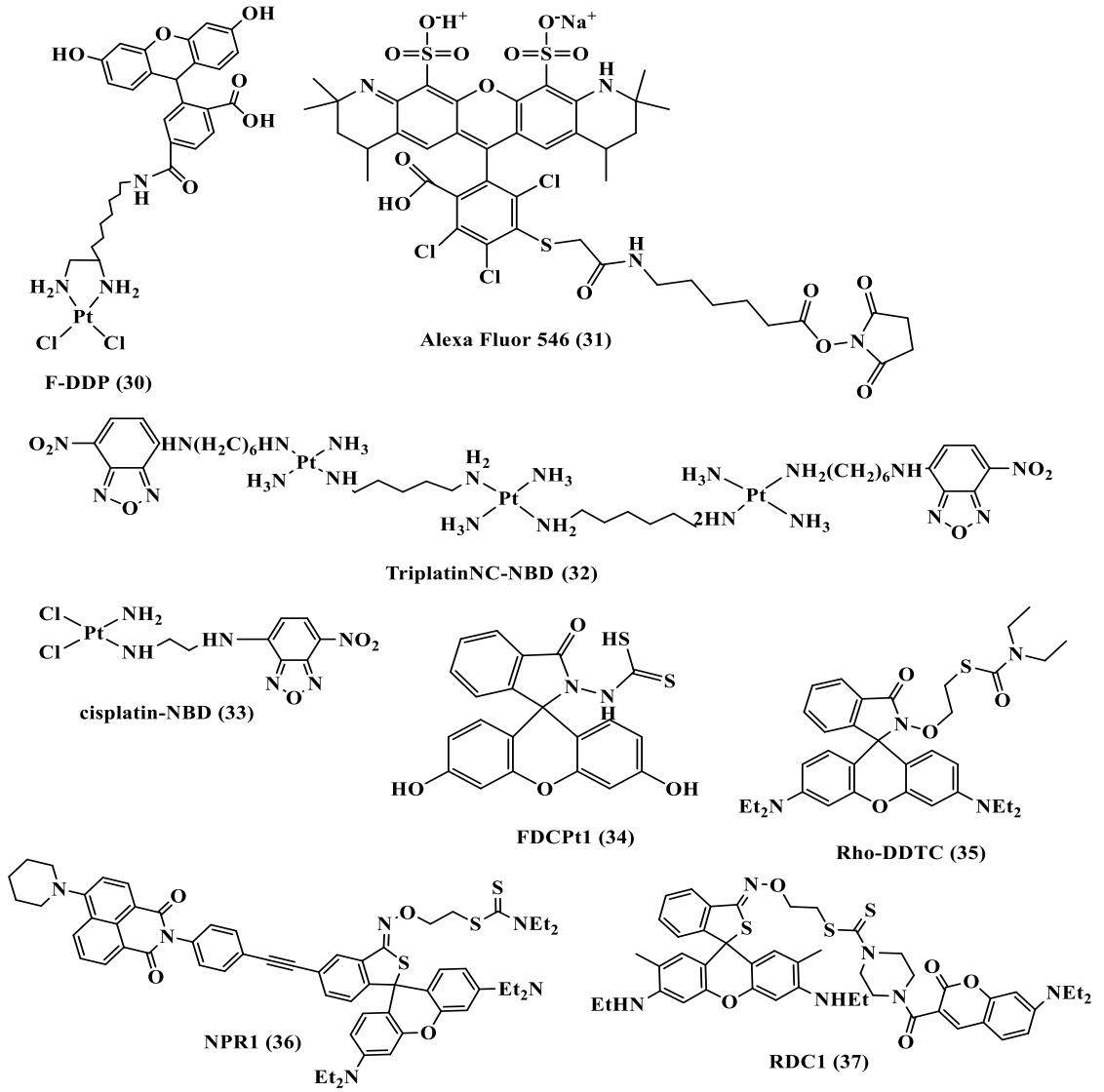
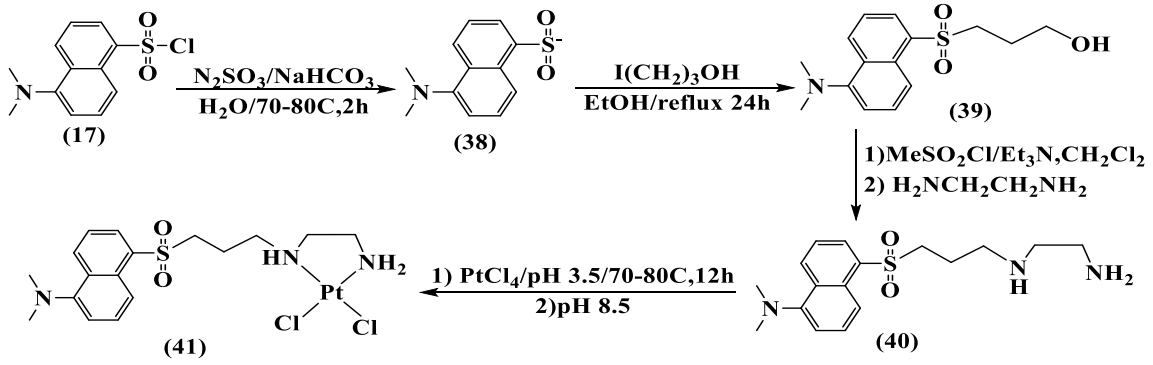


Figure 1.8. Chemical structure of F-DDP **(30),** Alexa Fluor 546 **(31),** TriplatinNC-NBD **(32),** Cisplatin-NBD **(33),** FDCPt1 **(34),** Rho-DDTC **(35),** NPR1 **(36),** RDC1 **(37).**

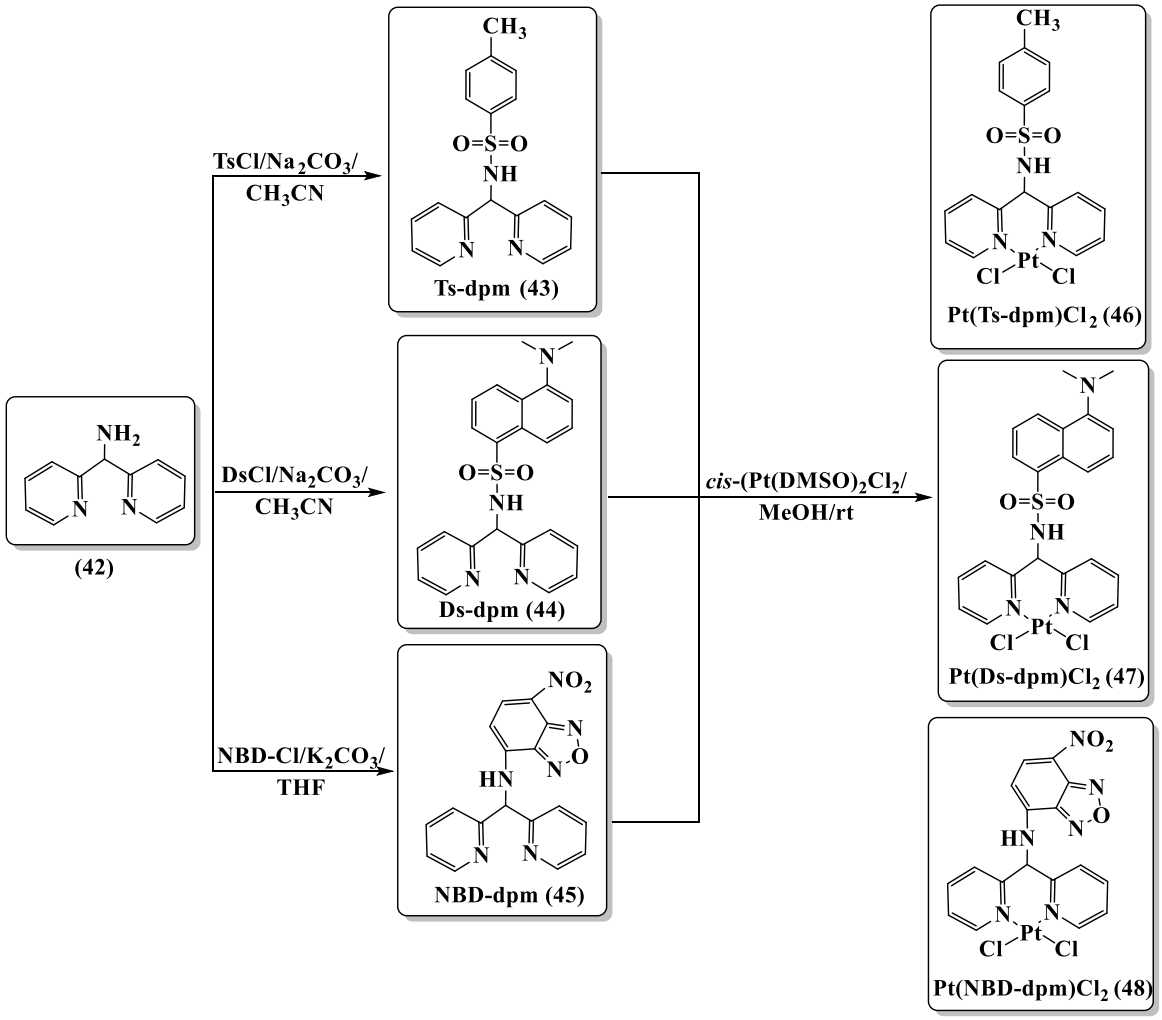
Hartwig *et al.*[95] synthesized fluorescent analogue of cisplatin by using tethered Dansyl group. The dansyl fluorescent moiety in Pt(dansen)Cl_2 **(41,** Scheme 1.9) complex provide a viable, nondestructive technique alternative to radiolabeling. In addition, the fluorescent tag made it able to observe the covalent binding to plasmid DNA in bacterial cells in this study which opened the door for the investigation of intracellular distribution, and processing by DNA repair enzymes of cisplatin analogue *in vivo*. The dansylated

ethylenediamine platinum complex (Pt(dansen)Cl_2) or dichloro((2-((3-dansylpropyl)amino)ethyl)amine)platinum(II) was synthesized by multiple steps; first Dansyl chloride **(17,** Scheme 1.9) was reacted with sodium sulfite and sodium bicarbonate to obtain sodium 5-dimethylaminonapthalene-1-sulfinate **(38,** Scheme 1.9) which was then added to a solution of 3-iodopropanol in ethanol and extracted with CH_2Cl_2 to give 5-dimethylaminonapthalene-3'-hydroxypropyl-1-sulfone **(39,** Scheme 1.9). After that the compound **(39)** was reacted with methanesulfonyl chloride in basic media and dissolved in ethylenediamine and extracted with methylene chloride to give 5-Dimethylaminonaphthalene-2''-aminoethyl-3'-aminopropyl-1-sulfone, Dansen **(40,** Scheme 1.9) which was reacted with K_2PtCl_4 in acidic media, then the final product dichloro((2-(3-dansyl)propylamino)ethylamine)platinuxn(II), (Pt(dansen)Cl_2) **(41,** Scheme 1.9) was obtained by the addition of dilute solution of sodium hydroxide to precipitate the platinum complex[95].



Scheme 1.9. Synthetic route of Pt(dansen)Cl_2 **(41),** which is started from reacting dansyl chloride **(17)** with sodium sulfite and sodium bicarbonate to obtain sodium 5-dimethylaminonapthalene-1-sulfinate **(38)** then added to a solution of 3-iodopropanol to give 5-dimethylaminonapthalene-3'-hydroxypropyl-1-sulfone **(39)** that was reacted with methanesulfonyl chloride to give 5-Dimethylaminonaphthalene-2''-aminoethyl-3'-aminopropyl-1-sulfone, Dansen **(40).** K_2PtCl_4 was then reacted with **(40)** to give dichloro((2-(3-dansyl)propylamino)ethylamine)platinuxn(II), (Pt(dansen)Cl_2) **(41)**[95].

Wilson *et al.*[96] also reported the preparation of di-2-pyridylmethane based three new ligands in which Two of them contain the fluorescent dansyl and 7-nitro-1,2,3-benzoxadiazole (NBD) units. These ligands were used to synthesis new anticancer platinum complexes with intrinsic fluorescent properties (Scheme 1.10). However, the resulting platinum(II) dichloride complexes was found to be not soluble in water, thermally and photolytically unstable with poor emission. The three ligands; N-(Di-2-pyridylmethyl)tosylamide (Ts-dpm) **(43,** Scheme 1.10), N-(Di-2-pyridylmethyl)dansylamide (Ds-dpm) **(44,** Scheme 1.10), and N-(Di-2-pyridylmethyl)-7-nitro-2,1,3-benzoxadiazole-4-amine (NBD-dpm) **(45,** Scheme 1.10), were prepared from di-2-pyridylmethanamine (**42,** Scheme 1.10) and the corresponding sulfonyl or aryl chlorides in the presence of a carbonate salt as a base. Then the ligands were treated with a suspension of *cis*-[$Pt(DMSO)_2Cl_2$] in MeOH to get the platinum complexes, Pt(Ts-dmp)Cl_2, Pt(Ds-dmp)Cl_2, Pt(NBD-dmp)Cl_2, **(46, 47, 48,** Scheme 1.10)[96].



Scheme 1.10. Synthesis of di-2-pyridylmethane (dpm) ligands; N-(Di-2-pyridylmethyl)tosylamide (Ts-dpm) **(43),** N-(Di-2-pyridylmethyl)dansylamide (Ds-dpm) **(44)** and N-(Di-2-pyridylmethyl)-7-nitro-2,1,3-benzoxadiazole-4-amine (NBD-dpm) **(45)** from reacting di-2-pyridylmethanamine **(42)** with sulfonyl or aryl chlorides and carbonate salt. Then the ligands were reacted with *cis*-[Pt(DMSO)$_2$Cl$_2$] to get platinum(II) three complexes Pt(Ts-dmp)Cl$_2$ **(46),** Pt(Ds-dmp)Cl$_2$ **(47),** Pt(NBD-dmp)Cl$_2$ **(48)[96].**

1.3.3 Fluorescent labelled platinum(IV) complexes and their synthesis

Fluorescent probes are widely used in studying Pt(IV)/Pt(II) conversion and investigating the intracellular biotransformation of platinum(IV) prodrugs[65]. New *et al*. [83]conjugated aniline containing fluorophores, coumarin 120, and coumarin 151 to the non-leaving group positions of Cisplatin analogues (*cis*-[PtCl$_2$(C120)(NH$_3$)] **49,** and *cis*-[PtCl$_2$(C151)(NH$_3$)] **50,** (Figure 1.9) and used them to monitor the loss of the aniline ligand. Pt(II)-aniline was

prepared by reacting aniline with a solution of $(NH_4)^+[PtCl_3(NH_3)]^-$ (prepared by adding ammonium chloride and tetraethylammonium chloride to cisplatin) which then treated with 30% aqueous solution of H_2O_2 to get Pt(IV)-aniline. Results showed that all the coumarin complexes have moderate cytotoxicity and concentrated in the late endosomes or lysosomes. Result showed that oxidation of the coumarin 120 complex to the Pt(IV) form (*cis,trans,cis* $[PtCl_2(OH)_2(C120)(NH_3)]$) (**51,** Figure 1.9) quenched the fluorescence signals and therefore, an increase in fluorescence indicates intracellular reduction of the Pt(IV)-coumarin complex. Despite the detection of fluorescence in A2780 cell that treated with Pt(IV)-coumarin complex the distribution pattern compared to cells treated with free coumarin ligand was similar which indicate that this method might not be ideal to monitor PT(IV) /Pt(II) redox reaction in cells[83].

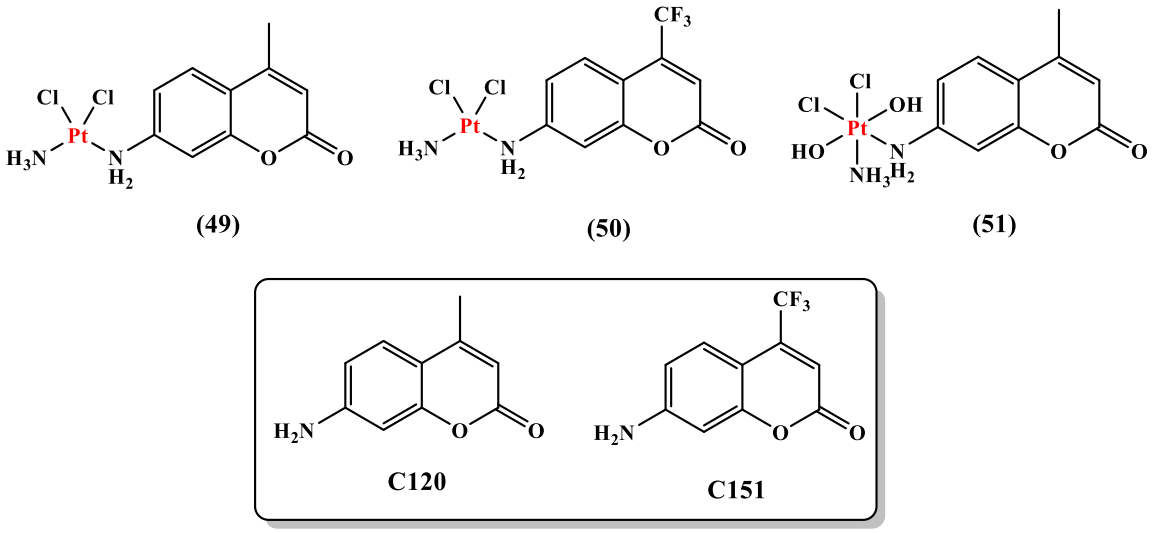
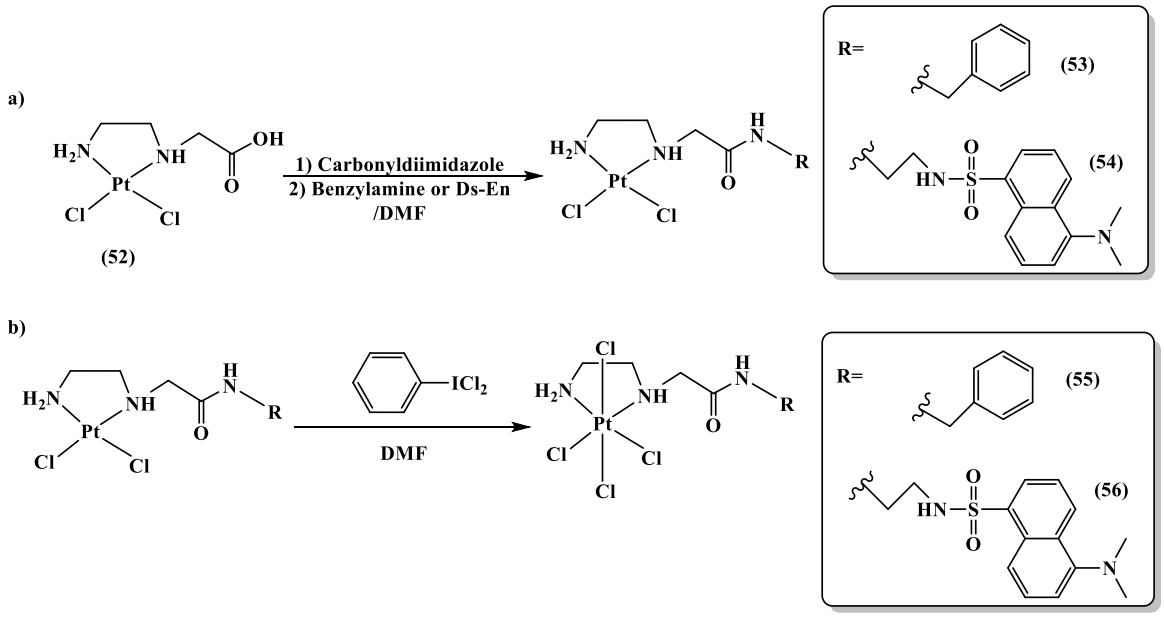


Figure 1.9. Cisplatin based platinum(II) and platinum(IV) complexes conjugated to fluorescent coumarin moiety.

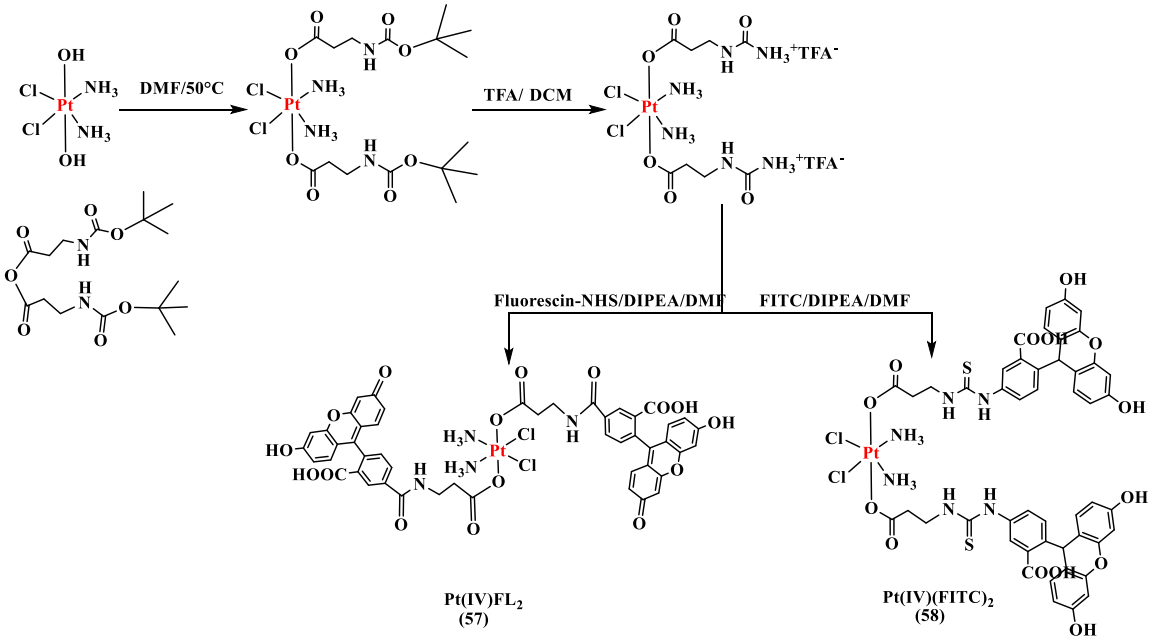
Wilson and Lippard[64] developed a platinum fluorophore conjugate to monitor platinum(IV) reduction in living systems by an emissive turn on response, platinum(II)

complex, [Pt(edma)Cl_2] **(52,** Scheme 1.11) (edma =ethylenediaminemono acetic acid), was activated by carbonyldiimidazole (CDI) and then conjugated with both benzyl amine and dansylethylenediamine to give [Pt(edBz)Cl_2] (**53,** Scheme 1.11) and [Pt(edDs)Cl_2] (**54,** Scheme 1.11) respectively. Oxidation of these platinum(II) complexes with iodobenzene dichloride yielded platinum(IV) complexes, [Pt(edBz)Cl_4] (**55,** Scheme 1.11) and [Pt(edDs)Cl_4] (**56,** Scheme 1.11). Photophysical studies of the compounds having the fluorescent Dansyl moiety showed high difference in emission efficiency which indicates that platinum(IV) [Pt(edDs)Cl_4] is more selective in fluorescence quenching, compared to the platinum(II) [Pt(edDs)Cl_2], therefore, it has the potential to be used as a probe to monitor the reduction of platinum(IV) complex in biology. Application of this complex is limited in living cell due to low yield in water and rapid rate of reduction but it provides a starting point for the design of more effective sensors[64].



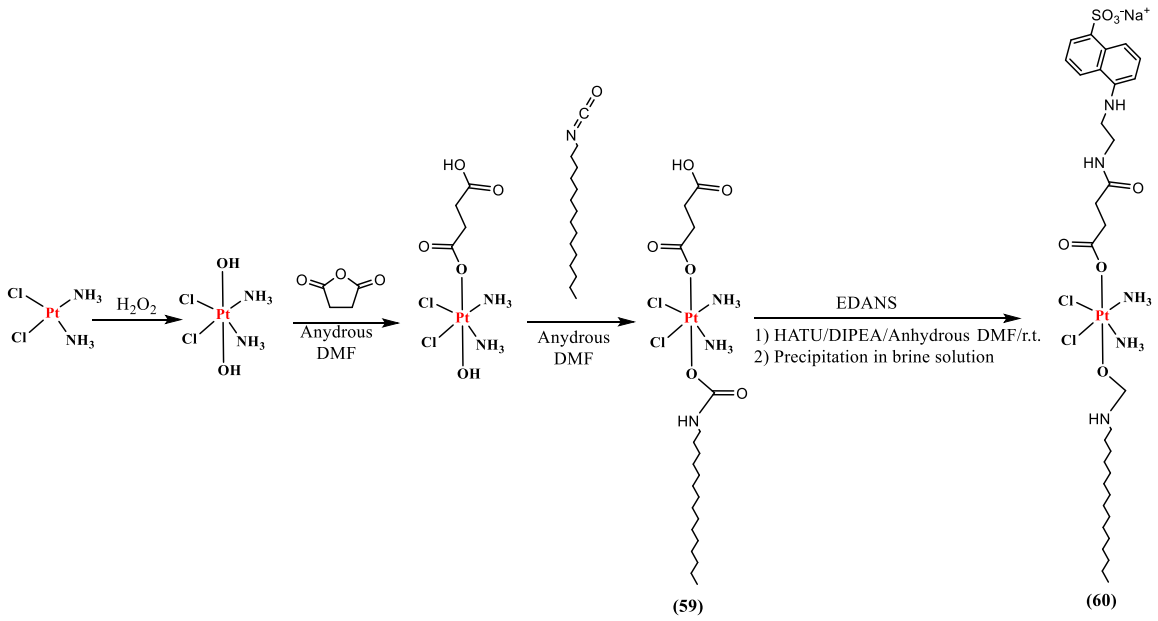
Scheme 1.11. Synthetic route of: a) [Pt(edBz)Cl_2] **(53),** and [Pt(edDs)Cl_2] **(54)** by activation of [Pt(edma)Cl_2] **(52)** with CDI and conjugation with both benzyl amine and dansylethylenediamine and b) their oxidation with iodobenzene dichloride to give [Pt(edBz)Cl_4] **(55),** and [Pt(edDs)Cl_4] **(56)**[64].

Song *et al.*[65] prepared new fluorescent Pt(IV) complexes conjugated to fluorescein NHS ester [Pt(IV)FL_2] (**57,** Scheme 1.12), and fluorescein isothiocyanate [Pt(IV)$(FITC)_2$] (**58,** Scheme 1.12) to be used in live cell imaging studies and evaluate the Pt(IV) reduction process in simulated and real biological environments. *c,c,t*-[$Pt(NH_3)_2Cl_2(OCO(CH_2)_2NH(FITC))_2$], [Pt(IV)$(FITC)_2$] was prepared by reacting *c,c,t*-[$PtCl_2(NH_3)_2(OH)_2$] with N-Boc aminopropanoic acid anhydride and trifluoroacetic acid (TFA) and then FITC and N,N-Diisopropylethylamine (DIPEA) was added to get the final compound while *c,c,t*-[$Pt(NH_3)_2Cl_2(OCO(CH_2)_2NH(FL))_2$] [Pt(IV)$FL_2$] was synthesized by using fluorescein NHS ester with DIPEA. Both Pt(IV) conjugates offered moderate cytotoxicity against human cancer cell lines[65].



Scheme 1.12. Synthesis of platinum(IV) complexes conjugated to fluorescein NHS ester **(57)** and fluorescein isothiocyanate (FITC) **(58)** by reacting platinum(IV) complex with N-Boc aminopropanoic acid anhydride and trifluoroacetic acid (TFA)[65].

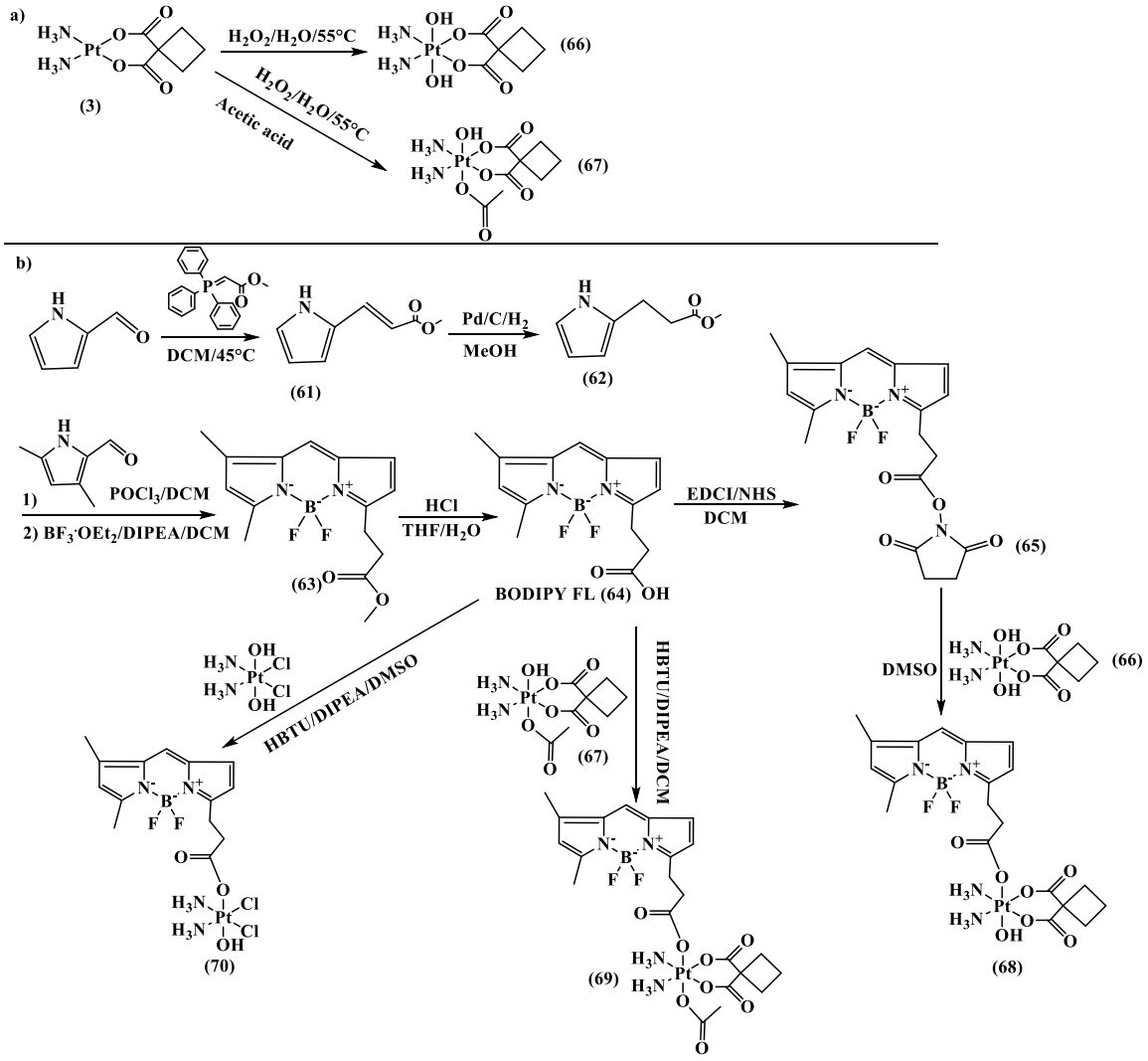
Jayawardhana *et al.*[97] synthesized a cisplatin-based Pt(IV) prodrug with a lipophilic hydrocarbon tail and anionic dansyl head group **(60,** Scheme 1.13) to study the interactions of mitochondria-damaging platinum(IV) prodrugs with cytochrome c. The prodrug was synthesized by conjugating [5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid] (EDANS) with amphiphilic platinum(IV) prodrug (C16Pt) **(59,** Scheme 1.13) which was synthesized according to the reported literature[40] by using HATU-catalysed amide bond formation reaction in DMF. Fluorescence imaging and graphite furnace atomic absorption spectroscopy (GFAAS) results showed that the lipophilic part of the prodrug facilitate its accumulation in the mitochondria of cancer cells and that the prodrug can trigger mitochondrial damage and apoptosis[97].



Scheme 1.13. Synthesis of cisplatin-based Pt(IV) prodrug **(60)** with a lipophilic hydrocarbon tail and anionic dansyl head group by conjugating [5-((2-aminoethyl)amino)naphthalene-1-sulfonic acid] (EDANS) with amphiphilic platinum(IV) prodrug (C16Pt) **(59)** by using HATU-catalysed amide bond formation reaction[40,97].

Yao *et al.* [98] developed a strategy to quantify the reduction of platinum(IV) prodrugs by using a fluorescence "turn on" sensor. In this study, boron dipyrromethene (BODIPY)

derivatives was attached to the axial position of carboplatin-based platinum(IV) prodrug[99]. The complex was synthesized by reacting methyl (triphenylphosphoranylidene)acetate with pyrrole-2-carbaldehyde to obtain compound (**61,** Scheme 1.14) which then reduced by H2 and Pd/C, to get compound (**62,** Scheme 1.14). **(62)** was coupled with 3,5- dimethylpyrrole-2-carboxaldehyde under promotion of phosphorus oxychloride ($POCl_3$) and treated with $BF_3 \cdot OEt_2$ to yield compound (**63,** Scheme 1.14). hydrolysis with ester under acidic conditions yielded the bodipy FL ligand (**64,** Scheme 1.14) which was then activated by NHS ester (**65,** Scheme 1.14) and coupled with carboplatin based platinum(IV) prodrug (**66,** Scheme 1.14) which was synthesized by oxidation of carboplatin with H_2O_2. Or by coupling it directly with compound (**67,** Scheme 1.14) which was synthesized by oxidation of carboplatin with H_2O_2 in acetic acid to yield the final complex BODI-Pt (**68, 69** Scheme 1.14), respectively[98-100]. For comparison BODIPY-FL was also coupled with cisplatin-based Pt(IV) compound **(70,** Scheme 1.14). BODI-Pt complex was found to be able to kill cancer cells by binding to DNA, inducing oncosis, and generating ROS upon irradiation[99].



Scheme 1.14. Synthesis of a) carpoplatin based platinum (IV) prodrugs. b) BODIPY and their conjugation with platinum(IV) prodrugs. Reagents: DCM, dichloromethane; Pd/C, palladium on carbon; H2, hydrogen; MeOH, methanol. POCl3, phosphorus oxychloride; BF3·OEt2, boron trifluoride−diethyl etherate; DIPEA, N,N′-diisopropylethylamine; HCl, hydrochloric acid; THF, tetrahydrofuran; HBTU, N,N,N′,N′- tetramethyl-O-(1H-benzotriazol-1-yl)uroniumhexafluorophosphate [98-101]

1.3.3.1 Nanocomposites of Pt(IV) Fluorescent-Labeled Compounds

Despite the progress in the development and tracking platinum drugs by fluorescent molecules, few compounds were assessed in clinical trials, and none have been approved by the FDA. Platinum drugs suffer from several side effect and the main challenge in cancer therapy is to deliver anticancer drugs to tumors selectively to minimize their side effects

and improve their therapeutic efficacy. Using delivery systems such as nanoplatform has great effect on improving the efficiency of cancer therapy[102,103].

Feazell *et al.*[103] prepared soluble single-walled carbon nanotube (SWNT) as a delivery system for platinum(IV) prodrug (*c,c,t*-[Pt(NH_3)$_2$$Cl_2$(OEt)($O_2CCH_2CH_2CO_2H$)]) (**71**, Figure 1.10). SWNTs was co-tethered with FITC fluorophore to investigate its mechanism in transporting Pt(IV) complex into cells and their release once inside and track their location intracellularly. Result showed that SWNTs are taken up by cells through endocytosis and a good intracellular accumulation and distribution of platinum complex which indicate that functionalized SWNTs are a good tool for transporting and delivering small molecule platinum prodrugs[103].

Zhou *et al.*[104] prepared a theranostic nanoparticles system of gold fluorescence nanoclusters (GNC) which has a highly fluorescence emission in the near infrared (NIR) region compared to organic dyes. Conjugation of GNC with Cisplatin prodrug (*cis,cis,trans*-[Pt(NH_3)$_2$$Cl_2$(OH)($O_2CCH_2CH_2CO_2H$)]) (MDDP) (**72**, Figure 1.10), and a targeting ligand folic acid (FA). FA-GNC-Pt nanoparticles was prepared by activating carboxyl group of FA-PEG5k-COOH with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBT) and reacting it with GNC-Pt in a buffer solution. FA can target breast tumors by recognizing the folate receptor α (FR-α) overexpressed on the surface of the cancer cells and selectively accumulate inside tumor and inhibit its growth. Using FA-GNC-Pt increased the fluorescence intensity and displayed much higher cytotoxicity[104].

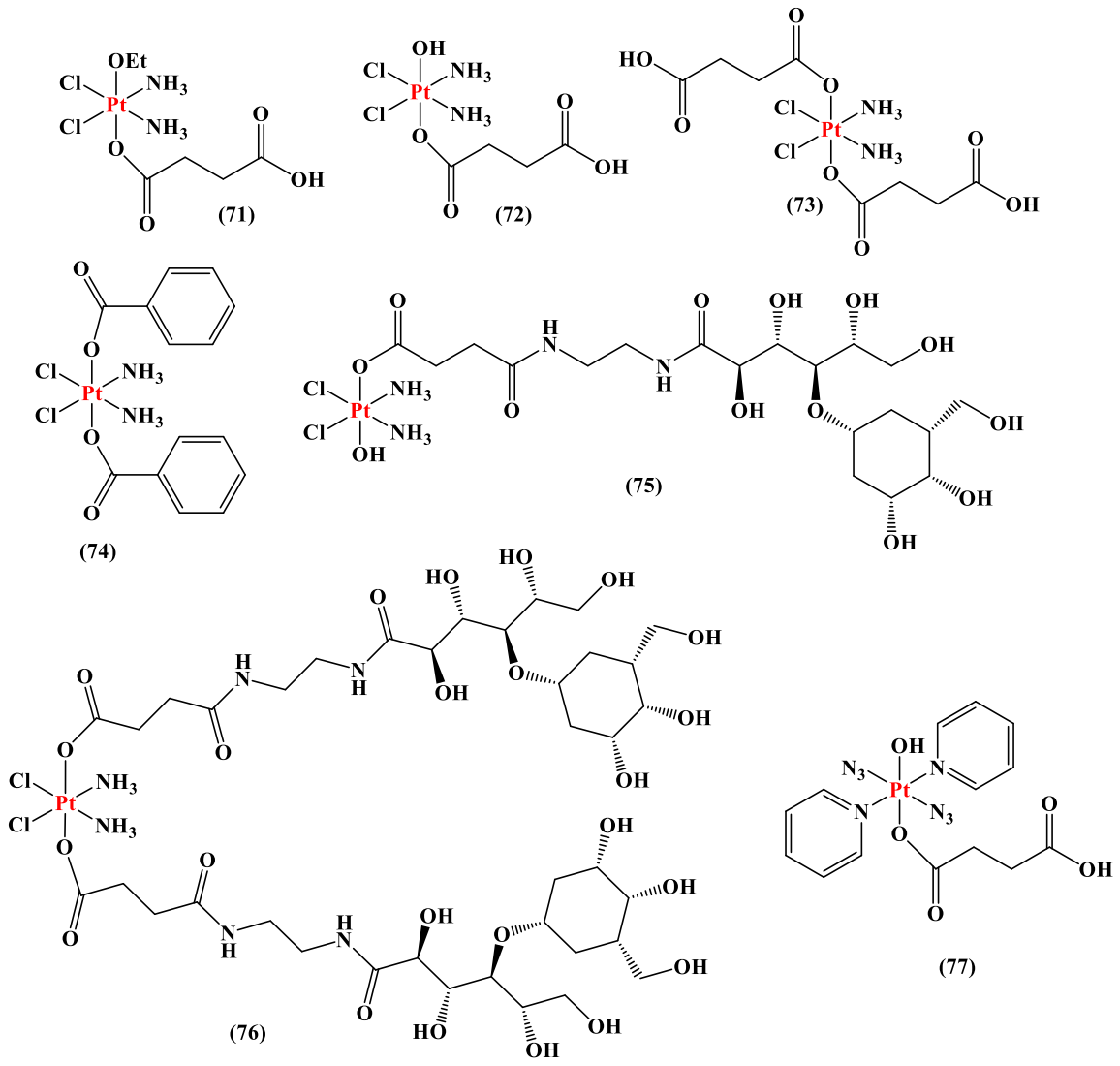


Figure 1.10. Chemical structure of *c,c,t*-[$Pt(NH_3)_2Cl_2(OEt)(O_2CCH_2CH_2CO_2H)$] **(71),** *cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OH)(O_2CCH_2CH_2CO_2H)$] (MDDP) **(72),** *cis,cis,trans*-diamminedichlorodisuccinato-platinum (IV) [Pt(IV)- $(COOH)_2$] **(73),** platinum(IV) of Cisplatin (PtBz) **(74),** photo-activatable Pt(IV) amphiphiles **(75), (76),** and *trans, trans, trans*-[$Pt(N_3)_2(OH)(O_2CCH_2CH_2CO_2H)(py)_2$] **(77).**

Cheng *et al.*[105] prepared FITC-modified gelatin for the encapsulation of oleylamine-coated Fe_3O_4 nanoparticles conjugated to anticancer platinum (IV) prodrug to be used for theranostic purpose. At first, Pt(IV) complex (cis,cis,trans-diamminedichlorodihydroxy-platinum (IV), [Pt(IV)- $(OH)_2$]) was synthesized by oxidizing Cisplatin with hydrogen peroxide and then it was reacted with succinic anhydride to produce *cis,cis,trans*-diamminedichlorodisuccinato-platinum (IV) [Pt(IV)- $(COOH)_2$] (**73,** Figure 1.10).

Thermal decomposition method was used to prepare oleyl amine-stabilized Fe_3O_4 nanoparticles. FITC modified-gelatin was prepared by reacting gelatin with FITC. second, the om-coated Fe_3O_4 in chloroform was covered with FITC-gelatin aqueous solution and transferred into water after evaporation of chloroform by ultrasonic cleaner. Finally, gelatin Fe_3O_4 solution was added to Pt(IV) prodrug that have been activated by NHS/EDC to produce the final product gelatin/Pt(IV) prodrug decorated IONPs. The nanoparticles carrying Pt(IV) prodrug showed good anticancer activities in the intracellular environment when it reduced to Pt(II), and the fluorescence of FITC on the surface of nanoparticles showed a fluorescence recovery and a complete quench after gelatin release and detachment from IONPs. Therefore, the gelatin encapsulated Fe_3O_4 nanoparticles have the potential to be used in multi-functional drug delivery system and as a fluorescence sensor for identification, tracking and monitoring the drug release[105].

Li *et al.*[102] prepared theranostic nanoplatform based on a Pt(IV) prodrug (Oxoplatin or DHP) and a near-infrared (NIR) photosensitizer (Cyanine dye or Cy). Oxoplatin and cyanine were copolymerized and incorporated into polyprodrug (PCPP) main chain which was self-assembled into nanoparticles (NPs). Result showed that PCPP NPs enabled the release of bioactive Pt(II) by reduction-triggered backbone cleavage of polyprodrugs. Moreover, PCPP NPs showed excellent tumor accumulation and antitumor efficiency in mice models after intravenous injection. Therefore, this type of nanoparticle complex can serve as a great tool for bioimaging and combined chemo–phototherapy[102].

Yoong *et al.*[106] prepared a functionalized multi-walled carbon nanotubes (MWCNTs) with fluorescent rhodamine-110 (MWCNT-Rho) that target mitochondria and a non-targeting fluorescein (MWCNT-Fluo). MWCNTs was attached to carboxylic acid groups

by oxidation to prepare MWCNT-COOH, then an amine-terminated TEG groups were added to the oxidized MWCNT-COOH in the presence of HATU as coupling agent and DIPEA to get MWCNT-TEG-NH2. MWCNT-Fluo was prepared by adding FITC to MWCNT-TEG-NH2 in the presence of DIPEA while MWCNT-Rho was prepared by adding Rho-110 chloride to MWCNT-TEG-NH2 with EDC/NHS in the presence of catalytic amount of DMAP and DIPEA. Results showed that MWCNT-Rho was associated well with mitochondria with 80% co-localization and enhanced potency (IC50 ¼ 0.34 0.07 mM) as a drug carrier for platinum(IV) of Cisplatin (PtBz) **(74,** Figure 1.10) compared to MWCNT-Fluo with poor localization of 21% and potency (IC50 2.64 mM), despite structural similarities of both complexes. These results showed that only MWCNTs- Rho-110 could act as effective drug carrier due to the importance of surface functionalization for effective drug carrier[106].

He *et al.*[107] designed a nanoparticle delivery system for two photo-activatable Pt(IV) amphiphiles **(75, 76,** Figure 1.10), photo-active Pt(IV)–azide prodrug was prepared by introducing one or two hydrophilic cancer targeting lactose ligands to the axial positions where it can be self-assembled into micelles with one lactose ligand or vesicles with two lactose ligands. This all-in-one theranostic system combine three key features; drug targeting, ligand targeting in which lactose itself is a targeting ligand and imaging agent where platinum complexes can be monitored via introducing NIR dye Cyanine to the platinum(IV) core or by CT imaging[107].

Min *et al.*[108] designed a near-infrared (NIR) light-activated nanoplatform of a photoactive platinum(IV) prodrug and an apoptosis sensing peptide that are incorporated onto the silica coated upconversion-luminescent nanoparticles (UCNPs) surface. Result showed that the

platinum(IV) prodrug complex (trans, trans, trans-[$Pt(N_3)_2(OH)(O_2CCH_2CH_2CO_2H)(py)_2$]) **(77,** Figure 1.10) is activated at the surface of the nanoparticle upon NIR light illumination and active components are selectively released which results in an efficient induced potent antitumor cytotoxcity in both cisplatin-sensitive and resistant tumor cells. Moreover, the caspases enzymes allowed the direct imaging of apoptosis in living cells by effectively cleave the NIR imaging peptide probe. Therefore, the developed platinum(IV) probe UCNPs@SiO2 conjugates can control the localized activation of the platinum prodrug at the target tumor sites, but more importantly, may also serve as personalized tumor markers to image in real time and evaluate the antitumor therapy at the cellular level[108].

1.4 Liposomes and liposomal formulation of platinum complexes

Nanoparticle drug delivery technology is an important strategy used to overcome the side effect problems *via* the enhanced permeability and retention effect (EPR) (Figure 1.11), which is a distinct feature of tumor tissue compared to normal tissue that allow large size nano-carriers to penetrate through the leaky vasculature around tumor region and accumulate due to ineffective lymphatic drainage of tumor tissues[109,110].

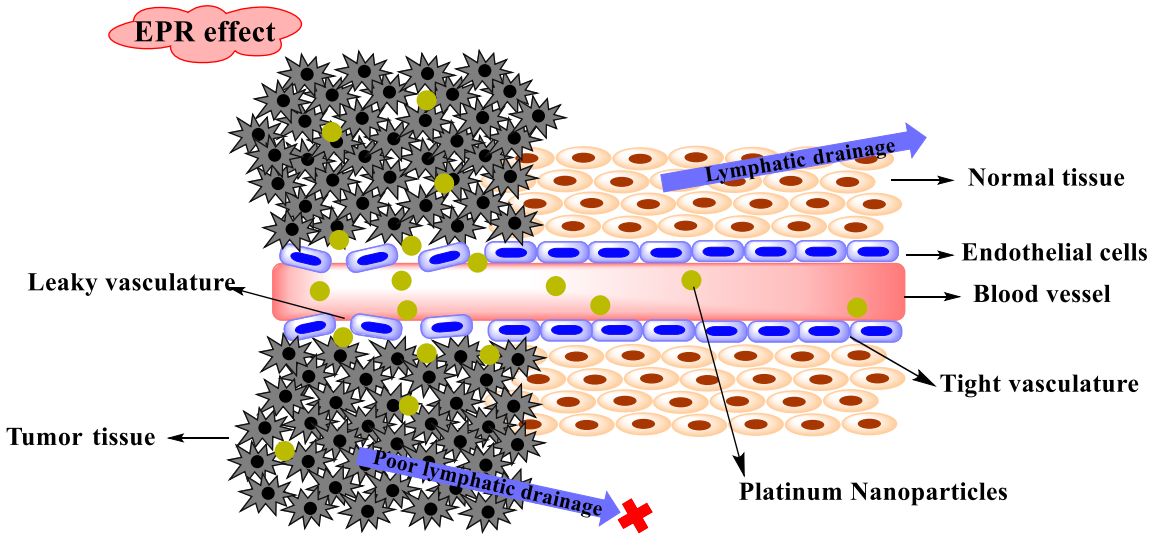


Figure 1.11. Nano particle drug delivery technology *via* the enhanced permeability and retention effect (EPR).

Platinum complexes can be attached to nanoparticles like liposomes which are a lipid bilayer vesicles consisting of an aqueous core encapsulated by natural or synthetic phospholipids that have the ability to work with both hydrophilic and hydrophobic drugs[111]. Liposomes can protect drugs from degradation before it reaches its target site, prolong their half-life in the body, increase their solubility and distribution into tumor sites, enable sustained release of drugs in the tumors, reduce their side effects, as well as, suppress development of drug resistance[5,112].

1.4.1 General introduction on liposomes

Liposomes, are lipid bilayer vesicles in which the lipid molecules have head groups attracted to water molecules that arrange themselves toward the aqueous cavity while the hydrocarbon tails point to the opposite direction[111,113]. Liposomes have many advantages (Table 1.2) including ability to carry large drug payloads, biocompatibility, capacity for self-assembly, biodegradability, low toxicity and others[114,115]. On the other

hand, liposomes suffer from rapid clearance by reticuloendothelial system (RES) because they are considered a foreign object to the body and are quickly up-taken by mononuclear phagocyte system (MPS) after systemic administration and cleared from blood[114]. Liposomal formulations facilitate cancer drugs accumulation in tumor but also in some formulations they limit drug release which lead to low concentrations at the target site[116].

Table 1.2 Liposomes advantages and disadvantages

Advantages of liposomes	**Disadvantages of liposomes**
Non-toxic, non-immunogenic, biocompatible, completely biodegradable	Phospholipid may undergo oxidation and hydrolysis-like reaction.
Increase efficacy and therapeutic index of drugs	Low solubility
Increase stability of drug by encapsulation	Have a short half life
Reduce toxicity of encapsulated drug	Suffer from leakage and fusion of drug
Reduce exposure of sensitive tissue to toxic drugs	Production cost is high

1.4.1.1 Classification and characterization of liposomes

Specific factors control the behavior of liposomes in storage conditions and biological mediums like size (ranging from 25nm to several micrometers), number of bilayers (Uni-lamellar to multi-lamellar), surface charge, chemical composition and membrane permeability. Bilayer composition responsible for shelf life, interaction with specific tissues or proteins and the kinetics of the release of the entrapped drug in liposomes. The size of the liposomes affects the in vivo distribution which can determine the time the liposomes will stay in the blood stream before being removed. Whereas the surface charge of vesicles affects the physical stability because of the possible occurrence of fusion or aggregation phenomena[117,118].

Liposomes can be classified according to: 1) composition and application, 2) structural parameters, 3) preparation methods (Figure 1.12). Based on composition, liposomes are classified as conventional liposomes, fusogenic liposomes, pH-sensitive liposomes, cationic liposomes, long circulating liposomes, and immuno-liposomes[114,119]. Based on the structure, they are classified as multilamellar vesicles (MLV), Oligolamellar vesicles (OLV), multilamellar vesicular vesicles (MVV) and unilamellar vesicles (ULV)[120]. Based on the method of preparation, they are classified as thin-film hydration technique (Bangham method), solvent injection method (Ethanol and ether), reverse phase evaporation method[115,121,122].

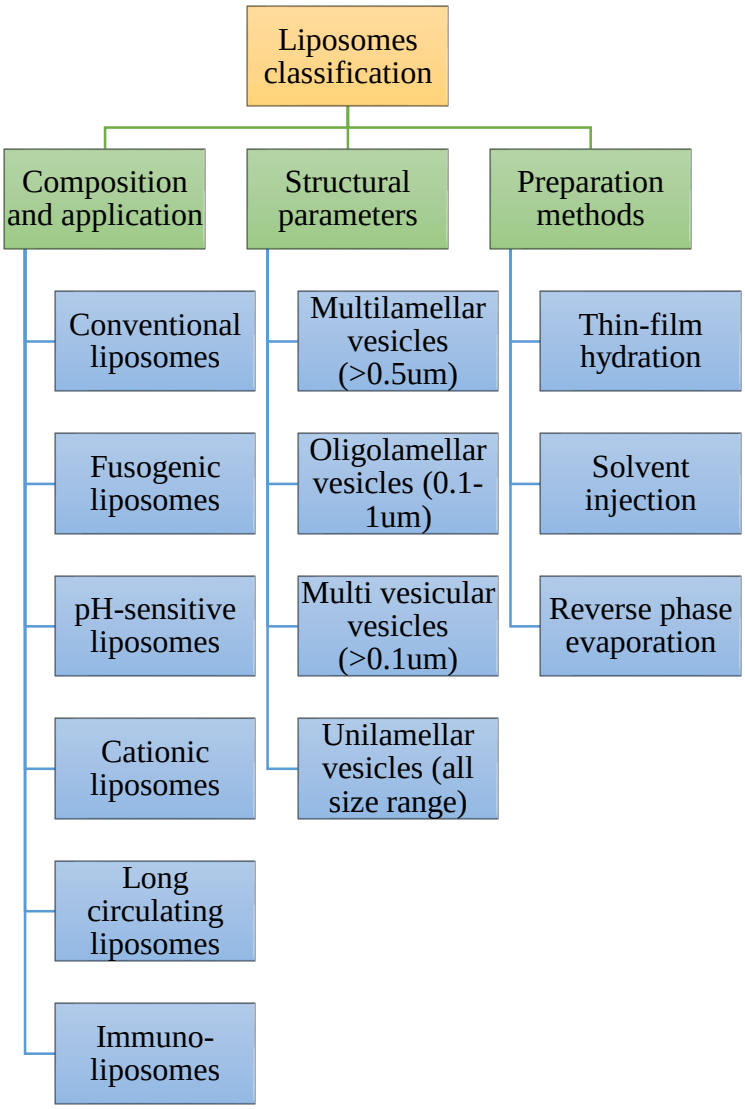


Figure 1.12. Classification of liposomes according to composition and applications, structural parameters and preparation methods.

Different methods are used to characterize liposomes physically and chemically (Table 1.3) including; quantification of phospholipids and lyso-phospholipids which is important in evaluating the efficiency of the preparation method, evaluation of lipid oxidation that reflect any changes in the bilayer integrity and presence of drug leakage. There are two pathways for liposomal degradation; the first one is the hydrolytic pathway in which the ester groups of the phospholipids can be hydrolyzed in the presence of water to produce lyso-phospholipids which leads to an increase of permeability of the lipid bilayer and

therefor destabilization of the system. The second pathway is oxidation of lipids which result in changes in the bilayer integrity and drug leakage which in turn induce aggregation and fusion phenomena.

Finally, determination of encapsulation percentage where the *in vivo* efficacy of liposomes and their physical and physicochemical properties depends on the total amount of drug encapsulated within the liposome[109,111,116]

Table 1.3. Most used methods for liposomes characterization.

Characteristics	Methodology
- Phospholipids quantification - Lyso-phospholipids quantification	- Lipid phosphorus content (Bartlett method). - Liquid chromatography combined with Bartlett method.
- Lipid oxidation	- Spectroscopy, thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas-liquid chromatography (GC).
- Encapsulation percentage	- Spectrophotometry, fluorescence spectroscopy, enzyme-based methods, electrochemical techniques and HPLC.
- Size	- Static and dynamic light scattering, microscopy techniques (light, electronic and atomic force), size-exclusion chromatography, field-flow fractionation and analytical centrifugation.
- Lamellarity	- Nuclear magnetic resonance (^{31}P-NMR), electron microscopy, small angle X-ray scattering.

1.4.1.2 Conventional vs. Fusogenic liposomes

Conventional liposomes are the first creation of liposomes which consist mainly of natural phospholipids or lipids such as 1, 2-distearoryl-sn-glycero- 3-phosphatidyl choline (DSPC), phosphatidylcholine, sphingomyelin, egg and monosialoganglioside[113]. The major drawbacks of conventional liposomes are their rapid clearance from the blood in addition to their low solubility, possibility of phospholipid oxidation and hydrolysis like reactions, leakage of drug content and high production cost[123]. To overcome these drawbacks another type of liposomes was developed with special fusogenic character called fusogenic liposomes (FLs) (Figure 1.13).

FLs was established to overcome anticancer drugs low permeability across the plasma membrane and degradation by the endocytosis pathways. FLs composed of conventional liposomes and Sendai virus which deliver encapsulated contents into the cytosol efficiently, rapidly and directly. FLs can be prepared by fusing conventional liposomes with ultraviolet inactivated Sendai virus under neutral pH and 37°C, then FL can be purified from the unreacted Sendai virus and liposomes by sucrose gradient centrifugation. These liposomes are unique because they can fuse with mammalian cell membranes and deliver their contents into the cytoplasm[124-126].

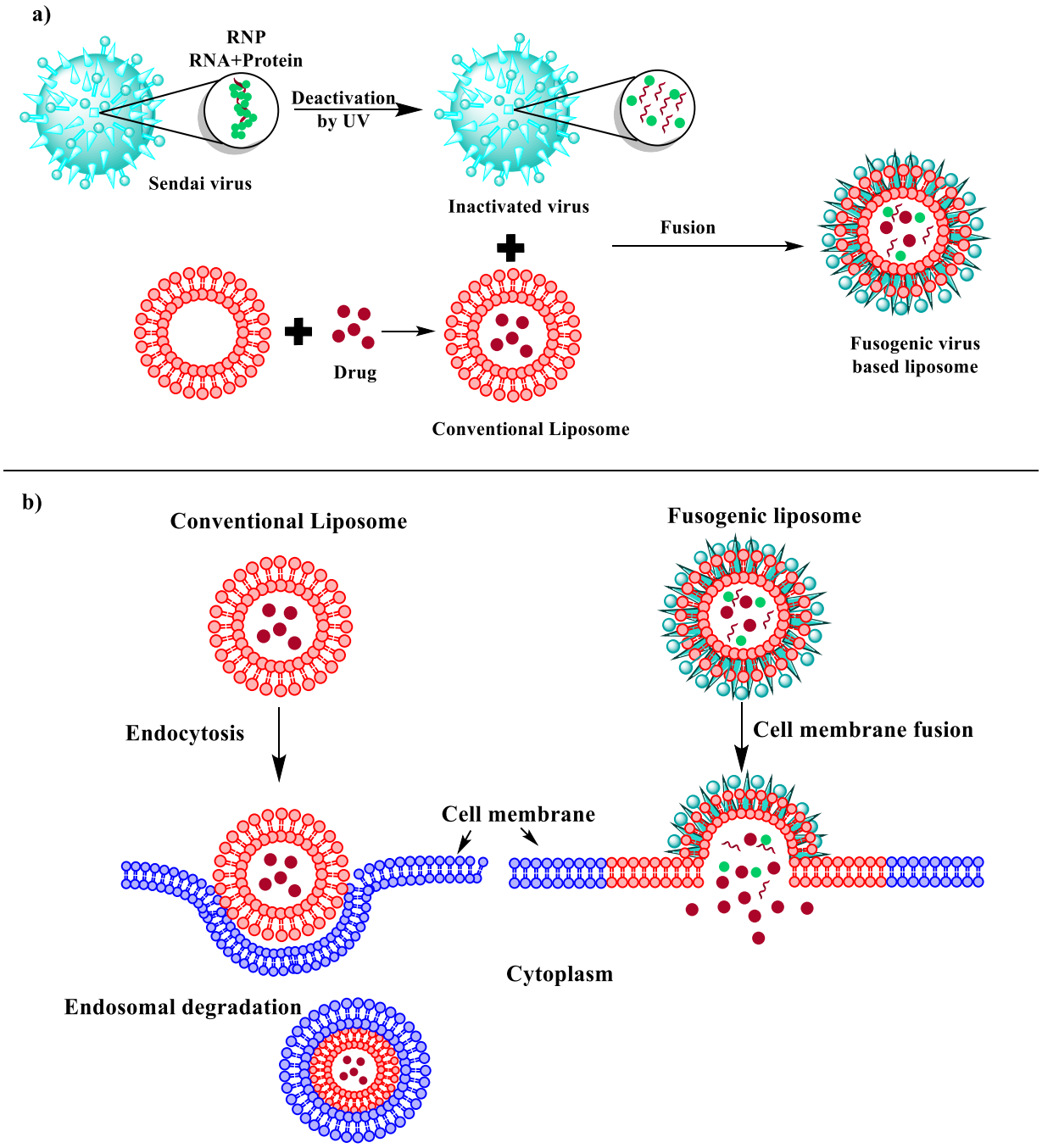


Figure 1.13. Fusogenic and conventional liposomes as nano-carriers. a) Synthesis of fusogenic liposomes from fusion of conventional liposome with inactivated sendai virus. b) Comparison between conventional and fusogenic liposomes delivery of drug content through cell membrane.

1.4.2 Platinum (II) liposomal formulations

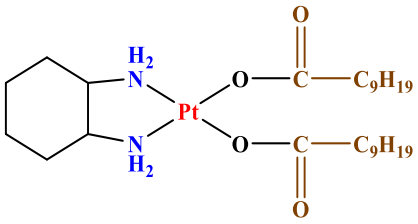
Several platinum liposomal formulations were designed to deliver platinum complexes and entered clinical trials (Table 1.4) but unfortunately, none of them was approved by the FDA.

Table 1.4. Clinically tested liposomal formulations of platinum complexes.

Formulation	L-NDDP	SPI-77	Lipoplatin	Lipoxal	LiPlaCis
Encapsulated Drug	NDDP	Cisplatin	Cisplatin	Oxaliplatin	Cisplatin
Lipid composition	DMPC/ DMPG	HSPC/ Cholesterol/ DSPE-PEG2000	HSPC/DPPG/ DSPE-PEG2000	Not Available	DSPC/ DSPG/ DSPE-PEG2000
Clinical status	Phase II	Phase II	Phase III	Phase I	Phase I

NDDP, *cis*-*bis*-neodecanoato-*trans*-*R*,*R*-1,2-diaminocyclohexane platinum(II); DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (sodium salt); HSPC, hydrogenated soy phosphatidylcholine; DSPE-PEG2000, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (ammonium salt); DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (sodium salt); DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; DSPG, 1,2-distearoyl-*sn*-glycero-3-phospho-(1′-*rac*-glycerol) (sodium salt).

The first liposomal formulation of platinum drugs that entered clinical trials is Aroplatin (L-NDDP) (**78,** Figure 1.14). The liposomal formulation is a multi-lamellar mixture of 1,2-dimyristoylphosphatidylcholine (DMPC) and 1,2-dimyristoylphosphatidylglycerol (DMPG) lipids with acidified saline solution that encapsulate NDDP a cisplatin analog (*cis*-*bis*-neodecanoato-*trans*-*R*,*R*-1,2-diaminocyclohexane platinum(II), NDDP). L-NDDP showed different bio-distribution from NDDP with higher accumulation in specific tissues and lower toxicity profile. Unfortunately, in 2005 a dose escalation phase was conducted and after reaching the maximum tolerated dose and observing an instability and degradation of the drug in liposomes the trial was terminated[109,127].



(78)
Aroplatin (L-NDDP)

Figure 1.14. Structure of liposomal formulation of platinum(II) Aroplatin.

SPI-77 is a Stealth liposomal formulation of cisplatin, which is coated with methoxy-polyethylene glycol (MPEG) that mask their recognition by MPS and removal from the blood stream[128]. SPI-77 showed a better effectiveness and toleration than free Cisplatin, but it suffered from poor release of the drug from liposomes which result in a decrease in the cytotoxic activity compared to free Cisplatin. Jaafari *et al.* tried to optimize drug release kinetics by encapsulating Cisplatin in liposomes containing different phosphatidylcholines (HSPC, DPPC, DMPC and soy phosphatidylcholine (SPC)) at various transition temperatures. Result showed that solid PEGylated liposomes with transition temperature above 37°C including DPPC and HSPC gave the highest cisplatin concentration and the best therapeutic activity compared to other formulations[109,129]. Moreover, SPI 077 formulation that contained 44% of cholesterol showed a low release of platinum drug aggregates therefore, Tesauro *et al.* [130] encapsulated cisplatin in Egg L-a-phosphatidylcholine liposomes using lower amounts of cholesterol in order to increase platinum release. Liposomal formulation showed a promising results in specific ovarian tumor cell line but further studies needs to be done.

Lipoplatin, is a cisplatin containing liposome developed by Regulon, Inc. company composed of soy phosphatidylcholine, cholesterol, dipalmitoyl phosphatidyl glycerol, and

methoxy-PEG-distearoyl phosphatidylethanolamine. Lipoplatin entered phase I, II, III clinical trials and showed that it has a lower side effects and higher accumulation in tumors with enhanced efficacy compare to free cisplatin and still under investigation. The same company also developed Lipoxal, an oxaliplatin liposomal formula with few side effects and better antitumor effect compared to free oxaliplatin[130-132] liPlaCis, is also another cisplatin liposomal formula developed by LiPlasome Pharma ApS company. The formula designed to be degraded by secretory phospholipase A2 which is abundant in tumor sites but other factors also participate in the degradation of the particles. Renal toxicity was not prevented by this formula, and that led to early stopping of this formulation in phase I stage[109]. Therefore, the same company designed a Liploxa, another oxaliplatin loaded liposomal formulation which is still under preclinical investigation[129].

1.4.3 Platinum(IV) liposomal formulations

Liposomal formulation of platinum(IV) complexes was also developed. The two extra ligands in platinum(IV) facilitate attachment to nanoparticles such as liposomes.

Browning *et al.*[133] investigated the potential of ultrasound and microbubbles (MB) (a small gas filled lipid coated bubbles) for delivering of both free and liposomal platinum(IV) prodrugs. Iproplatin (*cis,trans,cis*-dichloridodihydroxidobis(isopropylamine) platinum(IV) **(10,** Figure 1.5) was actively loaded into liposomes by using calcium acetate gradient which resulted in 3-fold improvement in drug concentration compared to passive loading methods. The resulted liposomal iproplatin (L(Pt)) was attached to MB by strain-promoted cycloaddition reaction (azide- dibenzocyclooctyne) to form an ultrasound-responsive drug delivery vehicle. Results showed that iproplatin release from the microbubble liposome was triggered by ultrasound and caused an increase in Pt

concentration in breast cancer cells. On the other hand, using L(Pt)) only or with ultrasound didn't show any appreciable platinum uptake in breast cancer cells which suggest that the microbubble-mediated ultrasonic release of Pt(IV) prodrug from liposomes has greater control over drug delivery. Iproplatin **(10)** was synthesized by adding isoprpylamine to a solution mixture of K_2PtCl_4 and KI in water. The resulted precipitated was reacted with $AgNO_3$ and then NaCl to form a yellow precipitate which was oxidized with H_2O_2 to get iproplatin. The drug was loaded into the liposomes after mixing the lipids together (DPPC, cholesterol and DSPEPEG(2000))[133].

Chen *et al.*[134] designed a hypoxia-amplifying DNA repair–inhibiting nanomedicine (HYDRI NM). The nanoparticle carries Pt(IV) in the outer hydrophobic layer and tirapazamine (TPZ) chemo drug and oxygen consumer glucose oxidase (GOx) in the hydrophilic cavity. Result showed that treating cisplatin resistant cancer cells with HYDRI NM amplified intracellular hypoxia by GOx-catalyzed oxidation of glucose and boosted the cytotoxicity and bioactivity of TPZ which in turn led to a synergistic effect with the platinum(IV) complex against drug-resistant tumors. The HYDRI NM (or GOx/TPZ@Lipo-Pt) was prepared first by using platinum(IV) prodrug as a building block which was synthesized from reacting *cis*,*trans*,*cis*-$[Pt(NH_3)_2(OH)_2Cl_2]$ with excess octane isocyanate **(79,** Figure 1.15). Second the platinum(IV) prodrug was conjugated with dipalmitoylphosphatidylcholine, cholesterol, 1,2-Distearoyl-sn-Glycero-3-Phosphoethanolamine and conjugated methoxyl poly(ethylene glycol) (DSPE-$mPEG_{2k}$) through supramolecular self-assembly method. Finally, the hypoxia-activatable dual-function chemo-drug tirapazamine (TPZ) and GOx were encapsulated into the hydrophilic cavity of the liposomes[134].

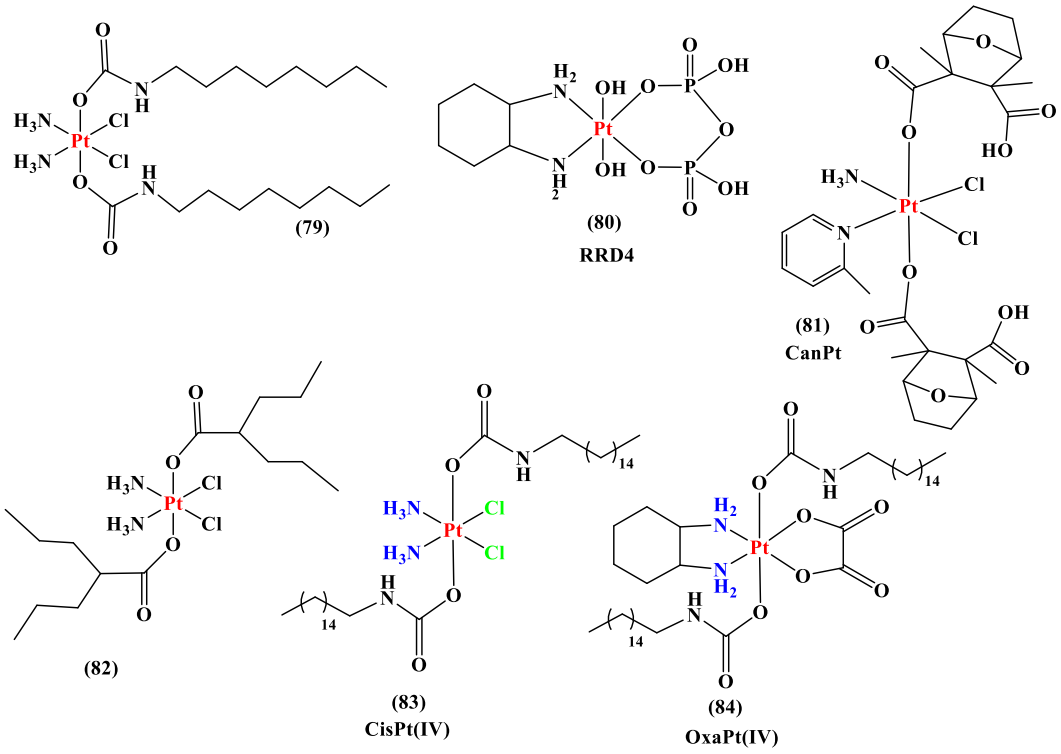


Figure 1.15. Structure of liposomal formulation of platinum(IV)Prodrugs; octane isocyanate Pt(IV) **(79),** phosphaplatins platinum(IV) (RRD4) **(80),** cantharidin platinum(IV) prodrug (CanPt) **(81),** (OC-6-33)-diamminedichloridobis(valproato) platinum(IV) **((82)**, CisPt(IV) **(83)** and OxaPt(IV) **(84).**

Belkacemi *et al.*[135] designed liposomal formulation of phosphaplatins platinum(IV) (RRD4) **(80,** Figure 1.15) and evaluated its therapeutic effects on breast tumor growth and metastasis. Result showed that free RRD4 inhibited tumor growth and metastasis while their liposomal formulation showed an enhancement in the potency of RRD4 and reduced toxicity compared to the free form of the drug. The platinum(IV) was synthesized by reacting Sodium pyrophosphate decahydrate with cis-dichloro(trans-1,2-cyclohexanediamine)platinum(II) to produce (trans-1,2-Cyclohexanediamine)(dihydrogen pyrophosphato)- platinum(II) and then was oxidized by H_2O_2 to yield (trans-1,2-Cyclohexanediamine)-trans-dihydroxo(dihydrogen pyrophosphato)platinum(IV) (RRD4). Liposomal RRD4 was then prepared by thin-film dehydration where lipids were dissolved

and mixed together (DOPC, phosphatidylcoline, Cholesterol and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(4-(p-aleimidophenyl) butyramide (MPB PE)). After that the lipid film was hydrated with RRD4 and extruded through a series of polycarbonate filter and the resulting liposomal formulation was stored at 4˚C for use[135].

Yang *et al.*[136] designed a hybrid of anti-tumor cantharidin and platinum(IV) prodrug (CanPt) **(81,** Figure 1.15). The conjugate was formulated into a liposome for drug delivery to reduce cantharidin side effects (e.g. dysphagia, hematemesis, and dysuria) which limited its use for cancer therapy. Results showed that CanPt-NPs delivered the drug to the tumor site which result in enhancement in drug accumulation, drug uptake into cancer cells and the release of platinum drug in redox-responsive manner which results in a greater apoptosis. CanPt was prepared by attaching two hydrophobic cantharidin molecules to the axial position of Pt(IV) and was loaded to the phosphate lipid bilayer of the liposomes which consist of MPEG2k-DSPE, DPPC, DSPC and cholesterol. Film hydration and extrusion by polycarbonate membrane was used to prepare the liposome[5,112,136].

Ravera *et al.*[137] examined the effect of cationic liposomes containing Cisplatin or valproate or their combination ((OC-6-33)-diamminedichloridobis(valproato) platinum(IV)) **(82,** Figure 1.15) on cisplatin sensitive human ovarian cancer cells. Valproate is a histone deacetylase (HDAC) inhibitor which plays a vital role in gene expression, cellular differentiation and cellular apoptosis, its co-administration with DNA-damaging drugs like cisplatin can give a dual action strategy in which it possesses a much higher anti-proliferative activity. Results showed that valproate has poor penetration to cells unless its transported by liposomes or conjugated to lipophilic Pt(IV). Pt-VPA **(82)** was more active than the combination of CDDP-VPA which is likely due to the lipophilic

property of Pt(IV). In addition, Pt-VPA liposomal formulation exhibited higher cellular accumulation with just two-fold increase in its anti-proliferative potency. The liposome was prepared by mixing 1,2-dioleoyl-sn-glycero-3- phosphoethanolamine (DOPE) and 1,2-dioleoyl-sn-glycero-3- ethylphosphocholine (chloride salt) (DOEPC) lipid. Then the lipid film was dispersed in an aqueous buffer-saline solution that contain Cisplatin and/or VPA. In the case of Pt(IV), LipoPt-VPA was prepared by dissolving lipophilic Pt-VPA in CHCl3 during the liposome preparation to be entangled into the membrane instead of being entrapped in the inner aqueous solution[137]

Moreover, Q. Chen *et al.*[138] developed a nanoparticle-based delivery system to deliver platinum complexes. CisPt(IV) **(83)** and OxaPt(IV) **(84)** (Figure 1.15) with two long lipid chains at the axial position were developed and encapsulated with biodegradable polymer methoxy poly-(ethylene glycol)-block-poly (lactic acid) (mPEG5000-bPLA6000). Result showed that platinum(IV) prodrug delivery system exhibited a rapid drug release from micelle under acidic and high concentration of reducing agent which conclude that this system could release platinum complexes rapidly in human cancer cells. Moreover, M(CisPt) and M(OxaPt), treated cells were arrested in S phase which resulted in apoptosis. Therefore, this system may give a new strategy for overcoming cisplatin resistance[138].

1.5 Literature Reviews

Many platinum drugs have been developed over years but their efficacy was distorted by toxicity and inability to reach target site. Many tumor sites such as; pancreatic carcinoma enhance the formation of dense desmoplastic stroma which creates a physical barrier that prevent chemotherapeutic drugs from reaching tumor cells. Moreover, increased interstitial fluid pressure (IFP) in tumor site result in low blood supply and inadequate drug delivery.

These challenges and others direct researchers to develop methods that deliver drugs to target sites such as making modifications on the surface of liposomes specifically for diagnosis, therapeutic and as a tool for molecular imaging to monitor drug delivery, response to therapy and early diagnosis[139,140]. Majority of platinum drugs have low hydrophilicity in addition to low hydrophobic affinity which result in very low drug-to-lipid ratio in liposomal formulations. Two methods have been suggested to increase the amount of platinum complexes in liposomes; either by using lipophilic platinum derivatives that can be encapsulated into large multilamellar liposomes like L-NDDP (aroplatin) or by using highly water-soluble platinum complexes like cis-diamminedinitratoplatinum(II). Several liposomal formulations entered clinical trials but none have been approved due to drawbacks like inefficient encapsulation of the drug, very low release kinetics from liposomes and poor serum stability[141,142]. To develop successful liposomal drugs, we must understand the fate of liposomes *in vivo,* this can be achieved by using reliable bioanalytical methods such as fluorescence labeling and other methods (e.g. MRI, mass spectrometry, radiolabeling and computed tomography)[143]. Despite the enormous number of imaging techniques there is still a need for highly sensitive probes that are suitable for *in vivo* imaging. Fluorescence imaging is cost-effective and non-invasive *in vivo* technique but the problem in preclinical fluorescent probes is their rapid clearance *in vivo*, which reduces their imaging sensitivity. Rapid clearance can be avoided by increasing the number of dye molecules at the target site, and encapsulation in liposomes[144].

L. Feng *et al.*[142] prepared a succinic acid (SA) cisplatin prodrug, *c,t,c* $[Pt(NH_3)_2(O_2CCH_2CH_2COOH)(OH)Cl_2]$ (Pt(IV)SA) conjugated to DSPE phospholipid

via amide bond to form a liposomal formulation with other lipids as DPPC, cholesterol, DSPE-mPEG$_{5k}$ (Figure 1.16). A lipophilic NIR dye called DiR (1,1′-dioctadecyl3,3,3′,3′-tetramethylindotricarbocyanine iodide) was used as a promising probe for *in vivo* NIR fluorescence and photoacoustic imaging. DiR-Pt(IV)-liposome was studied using UVVIS–NIR and fluorescence spectrometers which showed increasing in fluorescence intensity due to self-quenching effect of DiR when loaded into the lipid bilayer. Cells stained with a fluorescent lyso-tracker showed that the liposomal formulation entered the cell via endocytosis pathway, also the therapeutic activity of DiR-Pt(IV)-liposome was slightly higher than that of Cisplatin. Since Pt(IV)-liposome was a good carrier for hydrophobic dye DiR, it was also studied to deliver small hydrophilic molecules and bio-macromolecules into its aqueous inner cavity by using RhB and NHS-fluorescein (FITC) labeled BSA as the model molecules, results also showed that Pt(IV)-liposome-RhB and Pt(IV)-liposome-BSA-FITC entered the cell via endocytosis. This conclude that Pt(IV)-liposome have an excellent capacity for loading a different molecules and drugs[142]

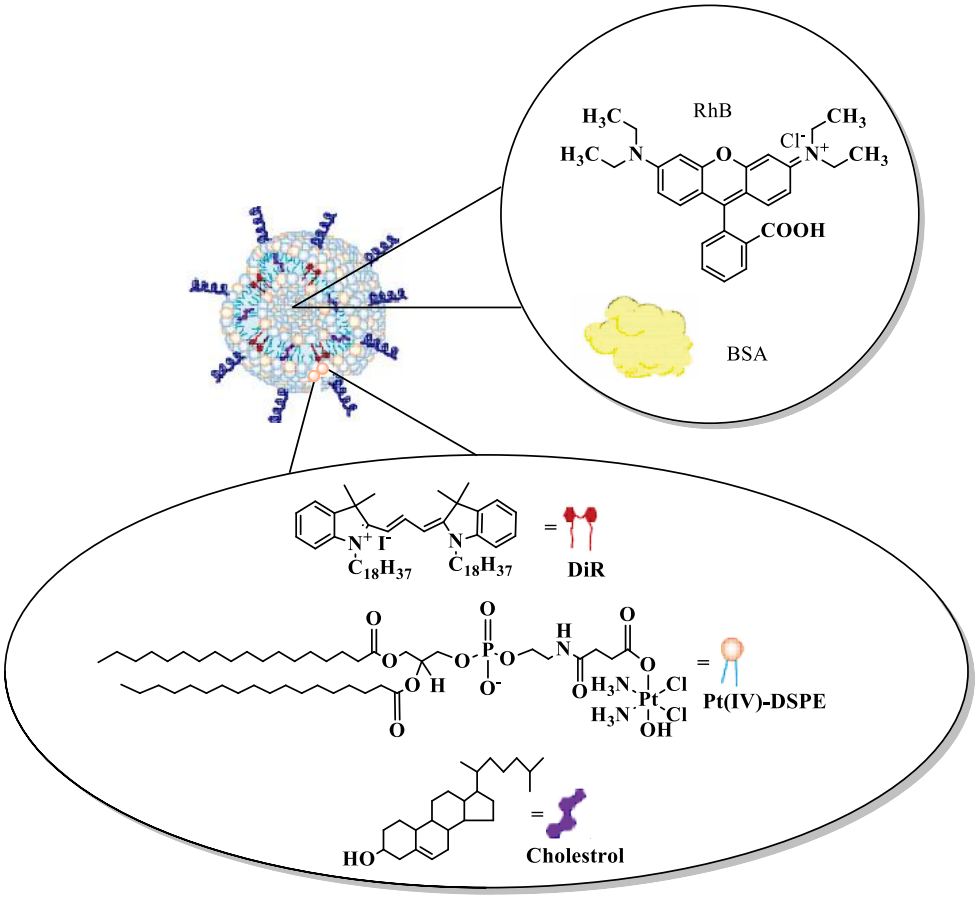


Figure 1.16. Structure of fluorescent liposome in which platinum(IV) prodrug *c,t,c* $[Pt(NH_3)_2(O_2CCH_2CH_2COOH)(OH)Cl_2]$ and the lipophilic NIR dye DiR (1,1′-dioctadecyl3,3,3′,3′-tetramethylindotricarbocyanine iodide) were loaded into the lipid layer of liposome while Pt(IV)-RhB and Pt(IV)-BSA-FITC was loaded into the aqueous inner cavity of the liposome.

1.6 Aims and Objectives of the Study

1.6.1 General objective

1- Synthesis of stable lipophilic and amine (aliphatic and aromatic) fluorescently labelled Pt(IV) prodrugs.

2- Characterization of fluorescent labelled Pt(IV) using available physical methods.

1.6.2 Specific Objectives

1- Design, synthesis and characterization of Cisplatin Pt(IV) prodrug derivatives.

2- Derivatize the Pt(IV) with lipophilic/amine containing fluorophores by conjugation to the axial hydroxido ligands.

1.6.3 Future Objectives

1- Encapsulation of the synthesized Pt(IV) derivatives in fusogenic liposomes (FLs).

2- Characterization of the FL-formulation using physical methods.

3- Characterization of encapsulated Pt(IV) using Raman microscopy, fluorescence and using it as a carrier for the delivery of the new compound into cancer cell cultures.

4- Assessing the impact of FLs on the activity of Pt(IV) derivatives using biological essays.

Chapter Two

Experimental part

2. Experimental Part

General considerations

Reactions were carried out in a fume hood in the dark. Solvents and reagents were used as received from commercial sources without further drying or purification.

2.1 Materials

Chemicals: Dansyl chloride, EDC, methanol (MeOH), dimethylformamide (DMF), N-hydroxysuccinimide (NHS), 6-Aminohexanoic acid, 11-Aminoundecanoic acid, *N*,*N*-Diisopropylethylamine, potassium iodide (KI), succinic anhydride, carbonyl diimidazole, ethylenediamine, pyridine, hydrogen peroxide (H_2O_2 30%, 35%), ammonium hydroxide (NH_4OH 25%), 2-(6-Chlor-1H-benzotriazol-1-yl)-1,1,3,3-tetramethylaminium-hexafluorophosphat) (HCTU) and acetic anhydride were purchased from ACROS Chemicals Ltd. Sodium sulfate (Na_2SO_4), 1,6-diaminohexane, dicyclohexylcarbodiimide (DCC), Sodium hydrogen carbonate, trimethylamine (TEA), hydrochloric acid (HCl), diethylether, and calcium chloride were purchased from Merck Ltd. Potassium tetrachloroplatinate(II) (K_2PtCl_4), and acetonitrile were purchased from Aldrich Chemicals Ltd. Oxalyl chloride, fluorescin isothiocyanate isomer 1 (FITC) were purchased from Alfa Aesar. Silver nitrate was purchased from Wieland. Tetrahydrofuran (THF) was purchased from J.T.Baker. Dichloromethane (DCM) waspurchased from Mallinckrodt Chemicals. Ethylacetate was purchased from MACRON fine chemicals. hydrogen peroxide (H_2O_2 3%) was purchased from Floris.

Silica gel (Silica gel 60 (0.040- 0.063 mm)) for column chromatograph and sheets for thin layer chromatography (TLC Silica gel 60 F_{254}) were all purchased from Merck Ltd.

Deuterated solvents: D_2O, $CDCl_3$ was purchased from ACROS Chemicals Ltd.

2.2 Instrumentation

Chemical hazards fuming hood, hotplates, pH meter and rotary evaporator are available at Anticancer Drugs Research Lab. Melting point, FT-IR and UPLC were done at Ibrahim Jalal's lab in the Faculty of Pharmacy, Al-Quds University. Liquid chromatography-mass spectroscopy (LC-MS) was done at Al-Quds University. Proton and carbon nuclear magnetic resonance spectroscopy (^{1}H-& ^{13}C-NMR) were done at the Hebrew University.

2.2.1 Nuclear magnetic resonance (^{1}H-, ^{13}C-, NMR)

All ^{1}H- & ^{13}C- NMR and spectra were conducted using the 500 MHz Varian NMR spectrometer. Samples were run in deuterated chloroform ($CDCl_3$). For ^{1}H-NMR, chemical shifts are reported in parts per million (ppm, δ) downfield from tetramethylsilane. Spin multiplicities are described as doublet (d), doublet of doublets (dd), singlet (s), triplet (t), quartet (q), and multiplet (m).

2.2.2 Fourier transform infrared spectroscopy (FTIR)

All infrared spectra were obtained from platinum ATR using Tensor II, Bruker, FT-IR spectrometer.

2.2.3 Electrospray ionization mass spectrometry (ESI-MS)

Was performed using a Thermos Scientific TSQ Endura Triple-stage Quadrupole. Elution was in a mixture of 40:40:20 water/acetonitrile/methanol at a flow rate of 15 μL/minute. ESI-MS were obtained delivering a diluted solution of the compound in methanol directly into the spectrometer source. The ion transfer tube temperature source and vaporizer

temperature were set to 325°C and 275° C, respectively with argon as a collision gas and nitrogen as a drying and a nebulizing gas.

2.2.4 Ultra-performance liquid chromatography (UPLC)

UPLC experiments were performed using Nexera X2 instrument Shimadzu Corporation-Japan that is equipped with Roc C18 reverse phase column (100 × 3.0 mm). The mobile phase was comprised of 0.06% TFA in water (solvent A) and acetonitrile (solvent B). A sample injection volume of 10 µL was used at a flow rate of 1 mL/min and eluted over a gradient of 0–100% for 25 min. An SPD-M20A prominence diode array detector (PDA) was used to detect the eluting peaks at 220 nm and 330nm.

Gradient mobile phase was used for the prodrugs as follow:

1. Prodrug **(35)** 0min: 50%water:50%ACN.
   20min: 15%water:85%ACN.
   21min: 50%water:50%ACN.
   25min: 50%water:50%ACN.
2. Prodrug **(36)** and **(37):** 0min: 65%water:35%ACN
   20min: 50%water:50%ACN.
   21min: 15%water:85%ACN.
   25min: 65%water:35%ACN.

2.3 Synthesis of platinum compounds

2.3.1 Synthesis of *cis*-dichlorodiammineplatinum(II), [[*cis*-$[PtCl_2(NH_3)_2]$, Cisplatin](7)

Potassium tetrachloroplatinate(II) (K_2PtCl_4) **(1)** (2g, 0.0048mol) were dissolved in distilled water (40ml), and 8 equiv. (6.4g, 0.0385mol) potassium iodide (KI) were added. The mixture was stirred at room temperature for 30 minutes. 2.1 equiv. (0.68ml, 0.010mol) of 25% ammonium hydroxide solution were added drop wisely to the dark brown potassium tetraiodoplatinate(II) K_2PtI_4 **(2)** solution and stirred for 3 hours. At first one iodide was replaced by ammonia to produce a water-soluble triiodomonoamineplatinum(II) **(3)** which was subjected to further ligand exchange to give a fine yellow precipitate of *cis*-diaminodiiodoplatinum(II) (*cis*-$Pt(NH_3)_2I_2$) **(4)**. The compound was collected by filtration, washed with water and dried by vacuum filtration. *cis*-diaminodiiodoplatinum(II) (*cis*-$Pt(NH_3)_2I_2$) **(4)** was used subsequently without any further purification.

Under dark condition (2.24g, 0.0046mol) *cis*-diaminodiiodoplatinum(II) (*cis*-$Pt(NH_3)_2I_2$) **(4)** was suspended in 80 mL DDW and 1.99 equiv. $AgNO_3$ (1.569g, 0.0092mol) were added. The mixture was stirred overnight at room temperature. The precipitate of AgI was filtered off and the filtrate was collected. The filtrate containing diamminodiaquaplatinum(II)/ diamminodinitratoplatinum(II)/ *cis*-$Pt(NH_3)_2(OH_2)_2$ **(5)/** *cis*-$Pt(NH_3)_2(NO_3)_2$ **(6),** was treated with 10 equiv. (9.3ml, 0.0462mol) 5M HCl and stirred overnight at room temperature. The resulting precipitate was collected by filtration, washed with DDW and dried under vacuum. The final product ***cis*-diamminedichloroplatinum(II) (7)** was collected as a yellow powder.

Yield: 1.22 g (88%). FT-IR: 3280 and 3203 (N-H stretching), 509 (Pt-N stretching).

2.3.2 Synthesis of *cis, cis, trans*-diamminedichloridodihydroxidoplatinum(IV) [*cis,cis,trans*-$[Pt(NH_3)_2Cl_2(OH)_2]$, Oxoplatin] (8)

In aluminum foil covered RBF, Cisplatin (**7)** (1g, 0.0033mol) were suspended in distilled water (10ml) and (4ml, 0.033mol; 10-fold excess) 30% hydrogen peroxide were added. While stirred, the suspension was heated to 50°C for one hour in dark. The mixture was then cooled to room temperature and stirring continued overnight. The precipitate was collected using filtration under reduced pressure washed with distilled H_2O, and diethyl ether, and dried under vacuum. The product **Oxoplatin (8)** was obtained as a pale-yellow powder.

Yield: 876.5mg (2.62mmol, 79.4%). FTIR: 3514, 3458 (O-H stretching), 3253 (N-H stretching), 553 (Pt-O stretching). Melting point is 260. UPLC Rt 0.955 minute.

2.3.3 Synthesis of *cis, cis, trans*-ethylenediamminedichloridodihydroxidoplatinum(IV) [*cis,cis,trans*-$[PtenCl_2(OH)_2]$] (13)

$PtenCl_2(OH_2)$ was prepared in similar manner to Cisplatin and Oxoplatin synthesis.

Potassium tetrachloroplatinate(II) (K_2PtCl_4) **(1)** (1g, 0.0024mol) were dissolved in distilled water (30ml), and 8 equiv. (3.2g, 0.0192mol) potassium iodide (KI) were added. The mixture was stirred at room temperature for 30 minutes. 1.1 equiv. (176.6µl, 0.00264mol) of ethylenediamine solution were added drop wisely to the dark brown potassium tetraiodoplatinate **(2)** solution and stirred for 3 hours. A fine yellow precipitate of *cis*-ethylenediaminodiiodoplatinum(II) (*cis*-$PtenI_2$) **(9)** was formed. The compound was

collected by filtration, washed with water and dried by vacuum filtration. *cis*-ethylenediaminodiiodoplatinum(II) (*cis*-PtenI_2) **(9)** was used subsequently without any further purification.

Under dark condition (735mg, 1.43mmol) *cis*-ethylenediaminodiiodoplatinum(II) (*cis*-PtenI_2) **(9)** was suspended in 20 mL DDW and 1.99 equiv. $AgNO_3$ (484.5mg, 2.85mmol) were added. The mixture was stirred overnight at room temperature. The precipitate of AgI was filtered off and the filtrate was collected. The filtrate containing containing ethylenediamminodiaquaplatinum(II)/ ethylenediamminodinitratoplatinum(II)/ *cis*-Pten$(OH_2)_2$ **(10)/** *cis*-Pten$(NO_3)_2$ **(11),** was treated with 10 equiv. (2.86ml, 14.3mmol) 5M HCl and stirred overnight at room temperature. The resulting precipitate was collected by filtration, washed with DDW and dried under vacuum. The final product *cis*-ethylenediamminedichloroplatinum(II) **(12)** was collected as a yellow powder. *cis*-ethylenediamminedichloroplatinum(II) **(12)** (340mg, 1.04mmol) were suspended in 3% hydrogen peroxide (23.6ml, 20.8mmol; 20-fold excess). While stirred, the suspension was heated to 50°C for one hour in dark. The mixture was then cooled to room temperature and stirring continued overnight. When the precipitate was collected using filtration under reduced pressure washed with distilled H_2O, and diethyl ether, and dried under vacuum. The product **Pten$Cl_2(OH_2)$ (13)** was obtained as a pale-yellow powder.

Yield: 257mg (0.713mmol, 68.6%). Melting point is 265. UPLC Rt 0.911 minute.

2.3.4 Synthesis of Dichloro(1,2-diaminocyclohexane)dihydroxidoplatinum(IV) (Pt(DACH)$Cl_2(OH)_2$) (16)

Potassium tetrachloroplatinate(II) (K_2PtCl_4) **(1)** (1g, 0.0024mol) were dissolved in distilled water (20ml), and 1 equiv. (275mg, 0.0024mol) of DACH **(14)** was added. The mixture

was stirred at room temperature overnight. A fine yellow precipitate02+ of dichloro(1,2-diaminocyclohexane)platinum(II) **(15)** was formed. The compound was collected by filtration, washed with water and dried by vacuum filtration and used subsequently without any further purification.

Under dark condition (400mg, 1.058mmol) $Pt(DACH)Cl_2$ **(15)** was suspended in 5 mL DDW and 30% hydrogen peroxide (1.2ml, 10.58mmol, 10-fold excess) and stirred overnight. The precipitate was collected using filtration under reduced pressure washed with distilled H_2O, and diethyl ether, and dried under vacuum. The product **($Pt(DACH)Cl_2(OH)_2$) (16)** was obtained as a pale yellow powder**.**

Yield: 340.2mg (0.824 mmol, 77.8%)

2.3.5 Synthesis of (1,2-diaminocyclohexane)dihydroxidoplatinumoxalate(IV) (Oxaliplatin (IV) (20))

Dichloro(1,2-diaminocyclohexane)platinum(II) $Pt(DACH)Cl_2$ **(15)** (400mg, 1.052mmol) was dissolved in distilled water (1.6 ml), and 1.8 equiv. (321mg, 1.89mmol) of silver nitrate were added. The mixture was stirred at room temperature overnight. The obtained silver chloride was filtered off with filter paper and the filtrate containing $Pt(DACH)Cl_2(NO)_2$ **(17)** was collected. To the obtained solution 1.1 equiv. of sodium oxalate (155mg, 1.16mmol) **(18)** was added and the mixture was stirred overnight. A fine white precipitate of 1,2-diaminocyclohexane platinum oxalate (Oxaliplatin) **(19)** was formed. The compound was collected by filtration, washed with cold water and dried by vacuum filtration and used subsequently without any further purification.

Under dark condition (200mg, 0.503mmol) Oxaliplatin **(19)** was suspended in 2 mL DDW and 35% hydrogen peroxide (489 µl, 5.03mmol, 10-fold excess) and stirred overnight. The

precipitate was collected using filtration under reduced pressure washed with distilled H_2O, and diethyl ether, and dried under vacuum. The product **Oxaliplatin(IV) (20)** was obtained as a white powder**.**

Yield: 175mg, (0.405 mmol, 80.6%).

2.3.6 Synthesis of di-succinato-cisplatin (*cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCCH_2CH_2CO_2H)_2$,] (22)

Oxoplatin **(8)** (305mg; 0.913 mmol) was dissolved in 2ml dimethylformamide (DMF), and then 2 equiv. succinic anhydride **(21)** (183mg; 1.83mmol) was added to the solution. The reaction mixture was heated to 60°C in the dark for 8 hours with magnetic stirring. The solvent (DMF) was removed by rotary evaporator and the pale-yellow product was recrystallized from acetone at -20℃ and isolated via vacuum filtration to give a clear yellow color solution. This solution was further concentrated under reduced pressure. The off-white product **di-succinato-cisplatin (*cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCCH_2CH_2CO_2H)_2$] (22)** was precipitated by adding diethyl ether and was collected via vacuum filtration.

Yield: 196.4mg (40.2%), UPLC Rt=0.874.

2.4 Synthesis of Fluorescent labelled lipophilic amino acids (Dansyl and FITC derivatives) (26, 27, 31)

General procedure:

In a round bottom flask, lipophilic amino acid (5 equivalents) and sodium bicarbonate (19 equivalents) were dissolved in 50 ml DDW H_2O. While stirring, dansyl chloride/ or FITC dissolved in 14ml THF were added drop-wisely. Triethylamine (TEA, 6 equivalents) were added in portions and the mixture was stirred at room temperature

overnight with exclusion of light. Then THF was evaporated under reduced pressure, and the obtained solution was acidified with 0.5M HCl to pH 3 and extracted with ethyl acetate (3x50mL). The organic fractions were pooled and dried over anhydrous Na_2SO_4. After filtering off the solids desired product was purified using column chromatography [DCM 100%], [EtOAc 100%], or [EtOAc: DCM, 1:1, v:v] to give Dansyl/or FITC amino acid derivatives.

2.4.1 Synthesis of Dansyl amino acid derivatives (26 &27)

2.4.1.1 Synthesis of 11-((5-dimethylaminonaphthalene-1-sulfonyl)amino)undecanoic acid [$C_{23}H_{34}N_2O_4S$, 11-DAUDA, Dans-C11OOH](26)

TLC (EtOAc); Rf =4.1/4.7=0.87; Yield: 1.447g (44.9%); whitish-pink powder.

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.02(m, 12H), 1.2(m, 4H), 1.54(m, 2H), 2.2(t, 2H), 2.8(s, 6H), 4.57(t, 1H), 7.11(d, 2H), 8.17(m, 2H), 8.20(dd, 1H), 8.48(d, 1H).

FT-IR: 3251 (N-H stretching), 2912 (C-H stretching), 1739 (C=O stretching), 1578 (O-H ester stretching), 1305 (C-N stretching), 1130 (S=O stretching).

ESI-MS m/z calculated for $C_{23}H_{34}N_2O_4S$ 434.6, found 435.2 $(M+H)^+$.

Melting point: 88

UPLC Rt 9.795min

2.4.1.2 Synthesis of 6-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)hexanoic acid* [$C_{18}H_{24}N_2O_4S$, 6-DAHA, Dans-C6OOH] (27)

TLC (EtOAc); Rf =2/6.2=0.32; Yield: 1.5g (55.5%); off-white powder.

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.13(m, 2H), 1.16(m, 4H), 1.37(m, 2H), 2.11(t, 2H), 2.8(s, 6H), 4.66(t, 1H), 7.11(d, 2H), 7.45(m, 2H), 8.16(dd, 1H), 8.48(d, 1H).

^{13}C-NMR (500 MHz, $CDCl_3$) δ (ppm): 23.87($\underline{C}H_2$-CH_2-CH_2), 25.75($\underline{C}H_2$-CH_2-C=O), 29.15($\underline{C}H_2$-CH_2), 33.40($\underline{C}H_2$-C=O), 42.97(N-$\underline{C}H_2$), 45.42(N-$\underline{C}H_3$), 115.22(C=$\underline{C}$=C-), 118.66(-C=$\underline{C}$=C), 129.62(C=$\underline{C}$=C), 129.70(-C=$\underline{C}$), 129.90(C=$\underline{C}$=C), 130.46(C=$\underline{C}$=C), 134.69(C=$\underline{C}$=C), 152.05(C=$\underline{C}$-N), 179($\underline{C}$=O).

FT-IR: 3509 (O-H stretching), 3301 (N-H stretching), 2938 (C-H stretching), 1698 (C=O stretching), 1310 (C-N stretching), 1141 (S=O stretching).

ESI-MS m/z calculated for $C_{18}H_{24}N_2O_4S$ 364.46, found 365.2 $(M+H)^+$.

Melting point: 108

UPLC Rt 7.281min

2.4.2 Synthesis of FITC amino acid derivatives

2.4.2.1 Synthesis of 11-((Fluorescein-5-isothiocyanate)amino)undecanoic acid [$C_{27}H_{24}N_2O_7S$, FITC-C11OOH] (31)

TLC (EtOAc); Rf =3.5/6=0.583; Yield: 300mg (65.9%); Orange powder.

FT-IR: 3400 (O-H stretching), 3065, 3008, 2921 (N-H stretching), 1743 (C=O stretching), 1301 (C-N stretching).

Melting point: 190

UPLC Rt 10.681min

2.4.2.2 Synthesis of 6-(Fluorescein-5-isothiocyanate)amino)hexanoic acid (32)

TLC (EtOAc); Yield: 63.4mg, 47.4%, orange-brown powder

2.4.2.3 Synthesis of 4-(Fluorescein-5-isothiocyanate)amino)butyric acid (33)

TLC (EtOAc); Yield: 86mg, 67.9%, orange-brown powder

2.4.2.4 Synthesis of 4-(Fluorescein-5-isothiocyanate)amino)benzoic acid (34)

TLC (EtOAc); Yield: 85.8mg, 63.4%, orange-brown powder.

2.5 Synthesis of the platinum(IV) fluorescent-amino acid complexes (35-37)

2.5.1 Synthesis of platinum(IV) dansyl-amino acid complexes

- **General procedure (Approach-1)**

In a round bottom flask (50ml), dansyl amino acids (2.2 equivalent) were dissolved in 2 ml DMF and 2ml DCM. While stirring, (2.4 equivalent) HCTU (2-(6-Chlor-1H-benzotriazol-1-yl)-1,1,3,3-tetramethylaminium-hexafluorophosphat) and (2.4 equivalent) DIPEA (N, N-Diisopropylethylamine) were added and the mixture was stirred at room temperature for 3 hrs. To the stirred mixture (1 equivalent) *cis*, *cis*, *trans*-[$PtCl_2(NH_3)_2(OH)_2$] **(8)** was added. The reaction was stirred for 24h at 50°C with exclusion of light. Reaction progress was followed using TLC (DCM: EtOAc, 1:1, v:v). When the reaction was over two thirds of the DMF were removed under reduced pressure. To the concentrated mixture (50mL) diethyl ether was added and a sticky precipitate was formed. The liquid mixture was decantated. The addition of ether and decantation was repeated three times. The precipitate was dissolved in 3mL methanol and filtered to remove insoluble materials. To collected filtrate 50mL ether were added and the mixture was chilled overnight. The liquids were decanted and the sticky precipitate was collected and dried. The desired products were purified using column chromatography [DCM: EtOAc, 1:1, v:v] to give dansyl amino acid Pt(IV) prodrugs. The compounds were further purified by recrystallization in DCM and EtOAc.

2.5.1.1 Synthesis of di(11-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)undecanoic)*(*cis,trans,cis*-[Pt(NH_3)$_2$(Cl)$_2$)) (35)

TLC (DCM:EtOAc, 1:1, v:v); Rf = 1/6.2=0.161; Yield: 103mg (12.8%); brown powder.

^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 1.43(m, 12H), 1.44(m, 4H), 2.35(t, 2H), 2.90(s, 6H), 3.18(q, 2H), 3.71 (m, Pt-NH_3), 7.20(d, 2H), 8.11(m, 2H), 8.26(dd, 1H), 8.56(d, 1H).

UPLC Rt 17.752

2.5.1.2 Synthesis of di(6-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)hexanoic)*(*cis,trans,cis*-[Pt(NH_3)$_2$(Cl)$_2$)) (36)

TLC (DCM: EtOAc, 1:1, v:v); Rf =1/6.5=0.15; Yield: 48mg(77.9%); off-white-brown powder.

FT-IR: 3307 (N-H stretching), 2932 (C-H stretching), 1712 (C=O) stretching, 1566, 1415 (C-N stretching), 1140, 1049 (SO_2 stretching), 569, 454 (Pt-N) stretching.

Melting point: 290

UPLC Rt 10.112

- **General procedure (Approch-2)**

In a round bottom flask (50ml), dansyl fatty amino acid**s** were dissolved in 2 ml DMF. While stirring, EDC (0.5 equivalent) was added and the mixture was stirred at 50°C for 1hr. Then *cis*, *cis*, *trans*-[$PtCl_2(NH_3)_2(OfH)_2$] **(8)** was added and the reaction left stirring for 24h at 50°C with exclusion of light. Reaction progress was followed by TLC (DCM:EtAOc, 1:1, v:v). When the reaction was over DMF was concentrated under reduced pressure and diethyl ether was added to precipitate a sticky product which was then dissolved in a little amount of methanol. The desired product was precipitated by adding ether to methanol.

- **General procedure (Approach-3)**

In 50 ml round bottom flask *cis, cis, trans*-[$PtCl_2(NH_3)_2(OH)_2$] **(8)** was suspended in 2 ml pyridine. The suspension was cooled to -5 ºC in ice bath and while stirred 2.1 equivalents of acyl chloride were added drop-wise. The reaction was allowed to warm to room temperature and left stirring for 24 h. At the end of the reaction 10 ml DW were added and after stirring at room temperature for 30 min the precipitate was collected by filtration. The brownish solid was washed with 5 ml ice-cooled water then with 10 ml ether.

2.5.2 Synthesis of di(11-((Fluorescein-5-isothiocyanate)*amino)undecanoic)* (*cis,trans,cis*-[Pten$(Cl)_2$]) complexe (37)

In a round bottom flask (50ml), FITC amino acid derivative (248mg, 0.417mmol) were dissolved in 2 ml DMF. While stirring, (1.1:1.1 equivalent) NHS/EDC were added and the mixture was stirred at 50°C for 1hr. *cis, cis, trans*-[$PtenCl_2(OH)_2$] **(12)** (68.3mg, 0.189mmol) were added and the mixture was stirred for 24h at 50°C with exclusion of light. Reaction progress was followed using TLC (EtOAc 100%). When the reaction was over, DMF was removed under reduced pressure. A sticky precipitate was formed and dissolved in small amount of methanol. The solution was then added in a dropwise manner into water. The desired product **(37)** was obtained after filtration and washed with water and diethyl ether. The compound was further purified by recrystallization in methanol.

TLC (EtOAc); Rf =6/6=1=; Yield: 140mg (22.7%); Brown powder.

FT-IR: 3400 (O-H stretching), 3200 (N-H stretching), 2924, 1737 (C=O stretching), 1584, 1455 (Benzene ring stretching), 1249 (C-N stretching), 1174 (C-O ester stretching), 457 (Pt-N stretching).

Melting point: 260. UPLC Rt 1.125min

2.6 Stability studies

Sodium mono phosphate buffer (pH=7.4) was prepared by dissolving 1.7g Sodium mono basic in 250ml DDW. The pH was adjusted to 7 by adding 1M NaOH to the solution.

Stability study was done for di(11-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)undecanoic)*(*cis*,*trans*,*cis*-[$Pt(NH_3)_2(Cl)_2$]) where 500 ppm solution was prepared by dissolving 12.5mg of the prodrug in a mixture of MeOH/ACN (1:1 v/v) and the volume was completed to 25ml from prepared buffer. The solution was kept at 37 °C for 30hrs and monitored by UPLC at a wave length of 330 nm to detect hydrolytic conversion of the prodrug to its corresponding parent drug.

Chapter three

Result and Discussion

3. Result and Discussion

Cisplatin is one of the most important anticancer drugs used in clinics. However, its use has been limited by its severe side effects, and the development of resistance[4]. Using a prodrug strategy, platinum(IV)-based anticancer drugs have been developed by introducing two additional axial ligands to overcome Cisplatin limitations [145]. Platinum(IV) prodrugs are perceived as inert towards ligand exchange reactions and have to be reduced to their active drug form (Platinum(II)) to exhibit a cytotoxic effect[146]. Despite the numerous work that have been done to address the mechanism of action of platinum(II) drugs and platinum(IV) prodrugs, understanding the cellular uptake and the reduction of platinum(IV) prodrugs remains unclear[147]. Cellular imaging techniques such as fluorescence microscopy are effective and accessible for detecting and monitoring platinum in live cells by attaching fluorophore to the drug[148]. To improve the tissue localization and enhance the cellular uptake of platinum-based anticancer agents, and to diminish the undesired side effects of platinum(II) drugs fusogenic liposomes will be employed. Furthermore, to make possible studying the penetration, localization, distribution and speciation of the Pt-liposomal formulation fluorescent labelled platinum(IV) derivatives were developed. Such composite will allow employing wide range of biophysical techniques (differential scanning calorimetry (DSC), transmission electron microscopy combined with freeze fracture (FF-TEM), dynamic light scattering measurements, small angle X-ray scattering (SAXS), Fourier transform infrared spectroscopy (FTIR) and fluorescence microscopy for characterizing. The fluorescent axial ligands were designed to comprehend properties that maximize encapsulation of the Pt(IV) derivatives.

3.1 Synthetic Chemistry

Kinetically inert octahedral platinum(IV) prodrugs attracted great deal of attention as the next generation of platinum based anticancer drug candidates. Pt(IV) complexes can be functionalized with a wide range of ligands that can be either biologically inactive or active to tune their pharmacological properties to overcome the limitations of traditional platinum(II) drugs[149]. Several platinum(IV) complexes were reported with significant anticancer activity, however none so far have been approved for clinical use. Modification at the axial position of platinum(IV) may also tune their lipophilicity and solubility and therefore affect their cellular uptake[150,151].

3.1.1 Design of the axial ligand

The ligand to be attached to the axial hydroxido sites of platinum(IV) shall meet the following requirements:

1) A fluorescent tag comprised of fused heterocyclic moieties
2) Solubility modulating functional group
3) A lipophilic linker that will connect the fluorescent label platinum center.
4) An attachable functional group to make possible the conjugation to Pt(IV).

A group of ligands with the up mentioned features were designed and synthesized (**see Figure 3.1).**

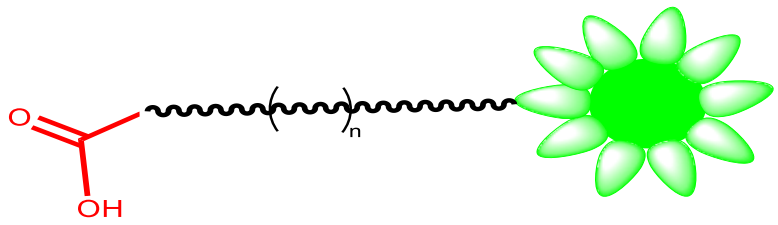


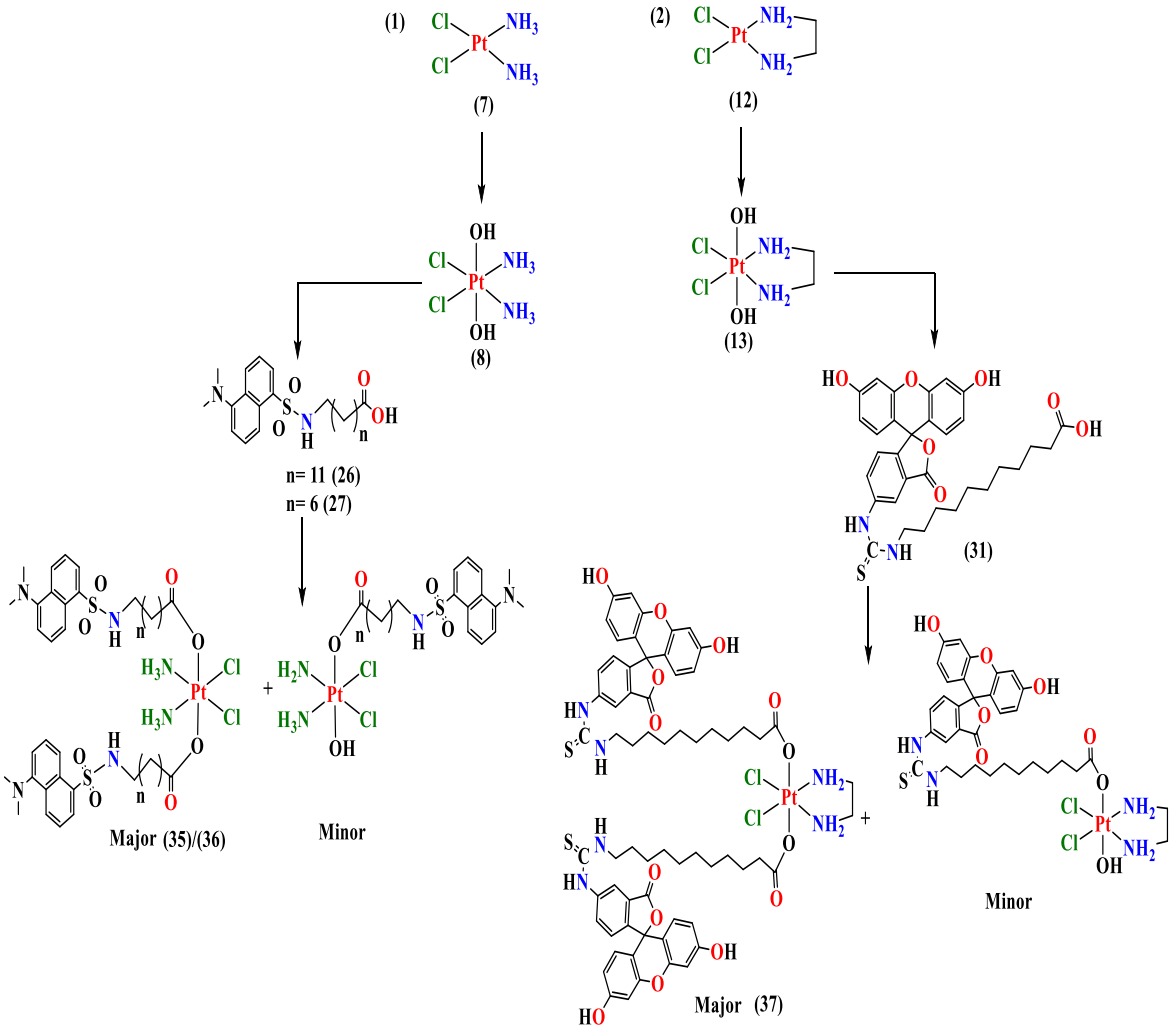
Figure 3.1. Design of fluorophore tags to be attached to the axial hydroxido ligands of platinum(IV).

Chemically, the functionalization of the axial sites of Oxoplatin was carried out by coupling of the designed ligand to the axial hydroxide sites.

3.1.2 Coupling of fluorescent labels (tags) to Pt(IV)

Several approaches have been applied to prepare fluorescent labeled Pt(IV) prodrugs. Functionalization of the axial ligands was carried out by using; Activating agents of uranium salts such as O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU); 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC); N-hydroxysuccinimide NHS/DCC (or NHS/EDC) or acyl halides.

The efficiency of carboxylation depends on the solubility of platinum(IV) di-hydroxide complexes and the reactivity of activated fluorescent fatty amino acids. Oxoplatin is poorly soluble in common solvents that's why all reactions were done in DMF as a solvent. Dichloromethane (DCM) was added to reactions for clear imaging of TLCs. Initially the reaction mixtures were pale-yellow suspensions and upon completion the mixtures turned into brownish solutions. All reactions were monitored by TLC and RP-UPLC. The compounds were divided into two classes Dansyl and FITC derivatives **(Scheme 3.1).**



Scheme 3.1. General scheme of different strategies for the synthesis of fluorescent platinum(IV) mono- and bis-carboxylate derivatives investigated in this study.

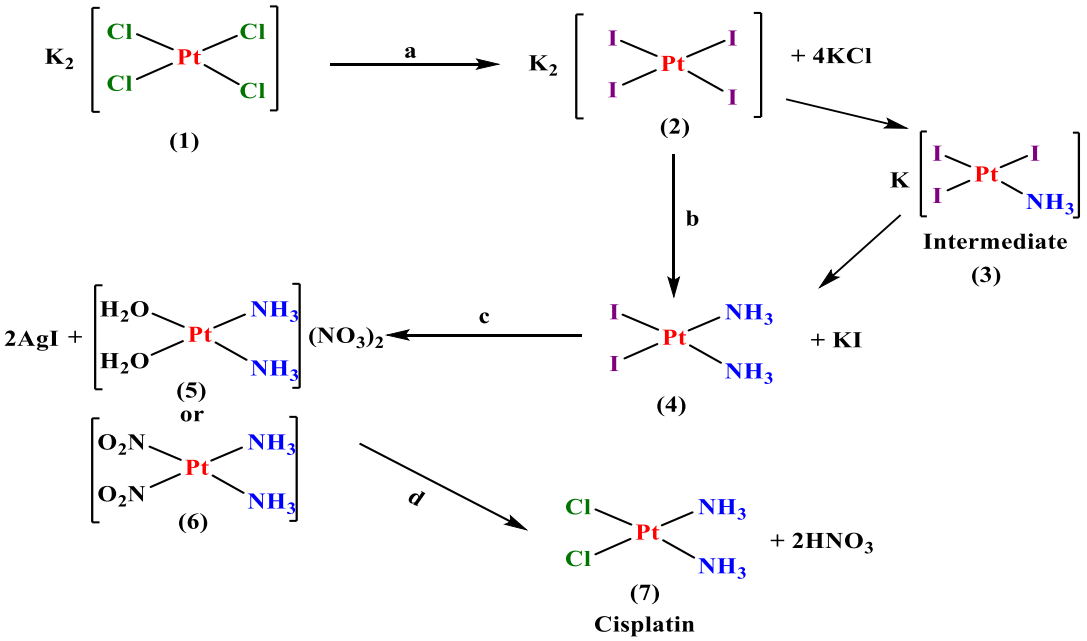
3.1.3 Synthesis of platinum(IV) complexes

The synthesis of platinum(IV) prodrugs were initiated by the synthesis of the corresponding platinum(II) analogues. In the case of Cisplatin **(7)** and $PtenCl_2$ **(12)** were prepared with 81% and 68.6% yields, respectively, in two successive steps starting from the commercially available tetrachloropaltinate(II) [K_2PtCl_4] **(1).** The oxidation of platinum(II) complexes was accomplished using hydrogen peroxide to afforded the platinum(IV) analogue as reported in previous studies[152,153].

3.1.3.1 Synthesis of *cis*-diamminedichloroplatinum(II) [Cisplatin; (*cis*-$[PtCl_2(NH_3)_2]$)] (7, Scheme 3.2)

The most used method for preparing Cisplatin is the one reported by Dhara in 1970[154]. Starting from potassium tetrachloropaltinate(II) [K_2PtCl_4] **(1)** which was subjected to ligand exchange tactic by the addition of a saturated solution of KI to form the tetraiodoplatinate(II) K_2PtI_4 **(2), (Scheme 3.2)**. Halides (Cl, Br, I) are known as labile ligands that are easily replaced by inert ligands like amines. In the first step one iodide was replaced by ammonia (NH_3) ligand to produce a water-soluble triiodomonoamineplatinum(II) **(3)** which was subjected to a further ligand exchange step ending in the water insoluble electroneutral *cis*-diaminodiiodoplatinum(II) (*cis*-$Pt(NH_3)_2I_2$) **(4).** When the intermediate water-soluble triiodomonoamineplatinum(II) **(3)** reacts with the second ammonia group there are two main options; the first is the iodo ligand displacement that is *trans* to ammonia; or the second option is the iodo ligand displacement that is *trans* to other iodo ligand. But because of the strongest *trans* effect of the iodo ligand that is relative to ammonia ensures that the trans iodide is more labile to displacement and result in the desired *cis*-diamminodiiodoplatinum(II) **(4)** isomer [4]. Earlier procedures before Dhara method didn't involve the initial step which is the converting of [K_2PtCl_4] **(1)** to K_2PtI_4 **(2)** that ensures the formation of *cis* product without contamination. On the other hand, Transplatin the other isomer is prepared by converting [K_2PtCl_4] directly to $[Pt(NH_3)_4]Cl_2$ and then treated with HCL to give Transplatin [155]. Moreover, a simple method was used to distinguish the *cis* from *trans* isomers of square planar compounds which was developed by Kurnakow in 1894 that involve the treating of the isomers with thiourea where Cisplatin produce deep yellow solution and Transplatin produces white

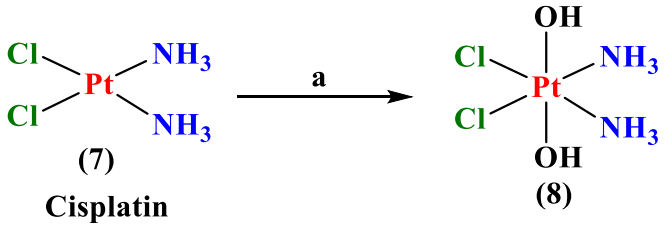
insoluble trans product [156]. In the procedure the compound *cis*-$PtI_2(NH_3)_2$ **(4)**, was collected as a yellow powder, and dried. To exchange the iodide ligand with chloride *cis*-$PtI_2(NH_3)_2$ was treated with $AgNO_3$ in aqueous conditions. This ensure the replacement of iodide with water (or nitrate), to obtain soluble diamminodiaquaplatinum(II)/ diamminodinitratoplatinum(II)/ *cis*-$Pt(NH_3)_2(OH_2)_2$ **(5)/** *cis*-$Pt(NH_3)_2(NO_3)_2$ **(6),** and the insoluble AgI which was filtered off. The filtrate containing *cis*-$Pt(NH_3)_2(OH_2)_2$ **(5)/** *cis*-$Pt(NH_3)_2(NO_3)_2$ **(6)** was treated with excess HCl to get isomerically pure yellow solid of *cis*-$PtCl_2(NH_3)_2$ **(7)** with a high yield (88%). The dried yellow powder was identified by FT-IR **(Figure 3.3)** where bands of N-H stretching (3280 and 3203), and Pt-N stretching (509) were found.



Scheme 3.2. Synthesis of Cisplatin **(7)** from potassium tetrachloroplatinate(II) (K_2PtCl_4) **(1).** Reagents and conditions: (a) potassium iodide (KI), distilled water, r.t, 30min, (b) 25% ammonium hydroxide (NH_4OH), distilled water, r.t, 24 h, 95.8%, (c) $AgNO_3$, distilled water, r.t, 24 h, (d) 5M HCl, distilled water, r.t, 24h, 88%.

3.1.3.2 Synthesis of *cis, cis, trans*-diamminedichloridodihydroxidoplatinum(IV) (Oxoplatin) (8, Scheme 3.3)

Pt(IV) counterparts (Oxoplatin **(8)**) was first synthesized by Chugaev and Khlopin in 1927[152]. Briefly, Cisplatin [*cis*-$PtCl_2(NH_3)_2$] **(7)** were suspended in H_2O_2 **(Scheme 3.3)** and double-distilled water and the solution that formed was left to stand overnight during which a yellowish crystalline precipitate formed**,** *cis, cis, trans*-diamminedichlorodihydroxyplatinum(IV) **(8)** with high yield (79.4%) compared to other studies which reported yields of 50-70%[157,158]. The platinum(IV) hydroxide ligands were originated from hydrogen peroxide and water. The dried yellow crystalline was identified using FT-IR **(Figure 3.4),** where a new sharp and intense peak appeared at 3514 (O-H stretching), and new Pt-O stretching at 553cm^1 compared with Cisplatin. UPLC **(Figure 3.5)** with melting point of 260 and retention time Rt= 0.955.

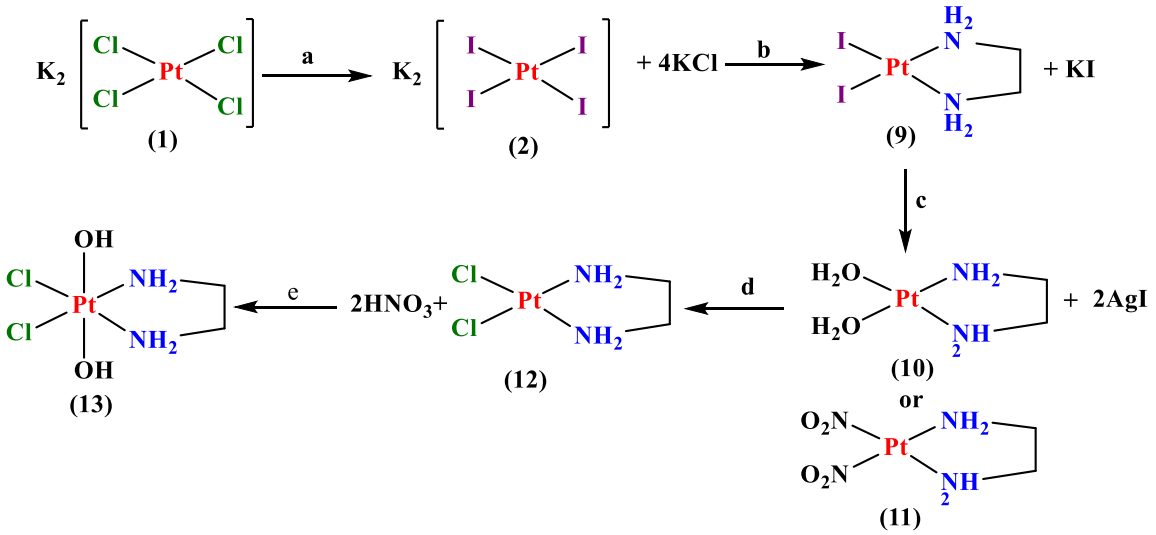


Scheme 3.3. Preparation of Oxoplatin **(8).** Reagents and conditions: (a) 30% H_2O_2, distilled water, 50°C for 1hr, r.t. for 24hr, 79.4%.

3.1.3.3 Synthesis of *cis, cis, trans*-dichloro(ethylenediamine)dihydroxyplatinum(IV) ($PtenCl_2(OH)_2$) (13, Scheme 3.4)

Ethylenediamine ligand was used because it has the ability to slower reduction rate of Pt(IV) complexes which may refer to the fact that ethylenediamine cause less steric

hindrance and stabilize the platinum complexes compared to other ligands. [PtenCl_2] was synthesized by using adaption of the Dhara method[154]. Starting from potassium tetrachloropaltinate(II) [K_2PtCl_4] **(1)** which was subjected to ligand exchange by the addition of a saturated solution of KI to form the tetraiodoplatinate(II) K_2PtI_4 **(2), (Scheme 3.4)**. Two iodide were replaced directly by ethylenediamine ligand due to its chelating characteristic to produce *cis*-ethylenediaminodiiodoplatinum(II) (*cis*-PtenI_2) **(9)** a yellow powder, which was collected and dried. To exchange the iodide ligand with chloride *cis*-PtenI_2 **(9)** was treated with $AgNO_3$ in aqueous conditions. This ensure the replacement of iodide with water (or nitrate), to obtain soluble ethylenediamminodiaquaplatinum(II)/ ethylenediamminodinitratoplatinum(II)/ *cis*-Pten$(OH_2)_2$ **(10)/** *cis*-Pten$(NO_3)_2$ **(11),** and the insoluble AgI which was filtered off. The filtrate containing *cis*-Pten$(OH_2)_2$ **(10)/** *cis*-Pten$(NO_3)_2$ **(11),** was treated with excess HCl to get isomerically pure yellow solid of *cis*-ethylenediamminedichloroplatinum(II) **(***cis*-PtenCl_2**) (12)**. Pt(IV) complex **Pten$Cl_2(OH_2)$ (13)** was synthesized by suspending *cis*-PtenCl_2 **(12)** in 3% H_2O_2 and the solution that formed was left to stand overnight during which a yellowish crystalline precipitate formed**,** *cis, cis, trans*-dichloride(ethylenediammine)dihydroxyplatinum(IV) **Pten$Cl_2(OH_2)$ (13)** with reasonable yield (68.6%) compared to other studies yield (76%)[159]. The platinum(IV) hydroxide ligands were originated from hydrogen peroxide. The dried pale yellow crystalline melting point 265 and UPLC **(Figure 3.6)** retention time Rt=0.911.

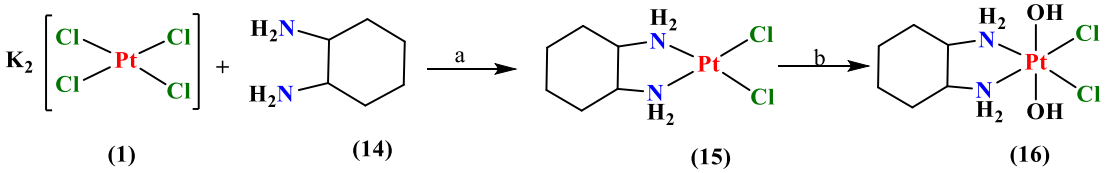


Scheme 3.4. Synthesis of *cis, cis, trans*-dichloride(ethylenediammine)dihydroxyplatinum(IV) **PtenCl$_2$(OH$_2$) (13)** from potassium tetrachloroplatinate(II) (K_2PtCl_4) **(1).** Reagents and conditions: (a) potassium iodide (KI), distilled water, r.t, 30min, (b) ethylenediamine, distilled water, r.t, 24 h, 58.3%, (c) $AgNO_3$, distilled water, r.t, 24 h, (d) 5M HCl, distilled water, r.t, 24h, 43.3%, (e) 3% H_2O_2, 50°C for 1hr, r.t. for 24hr, 68.6%.

3.1.3.4 Synthesis of Dichloro(1,2-diaminocyclohexane)dihydroxidoplatinum(IV) (Pt(DACH)Cl$_2$(OH)$_2$) (16, Scheme 3.5)

The DACH ligand was chosen because its bulkier and more hydrophobic than the ethylenediammine and diammine carrier ligands, and was found to alters the N-Pt-N bond angle. This conformation may lead to steric hindrance and significant distortion of the DNA by affecting the type, rate and lethality of adduct formed with DNA[160]. Synthesis of platinum prodrug was done in similar manner to above procedures, potassium tetrachloropaltinate(II) [K_2PtCl_4] **(1)** was subjected to ligand exchange by the addition of diaminocyclohexane (DACH) **(14) (Scheme 3.5)** to produce dichloro(1,2-diaminocyclohexane)platinum(II) **(15)** a fine yellow precipitate that was collected and dried. The oxidized form**(Pt(DACH)Cl$_2$(OH)$_2$) (16)** were synthesized by suspending Dichloro(1,2-diaminocyclohexane)platinum(II) **(15)** in 30% H_2O_2 and DDW the solution that formed was left to stand overnight during which a pale-yellowish crystalline

precipitate formed**,** Dichloro(1,2-diaminocyclohexane)dihydroxidoplatinum(IV) ($Pt(DACH)Cl_2(OH)_2$) **(16)** with good yield (77.8%). The platinum(IV) hydroxide ligands were originated from hydrogen peroxide and water.

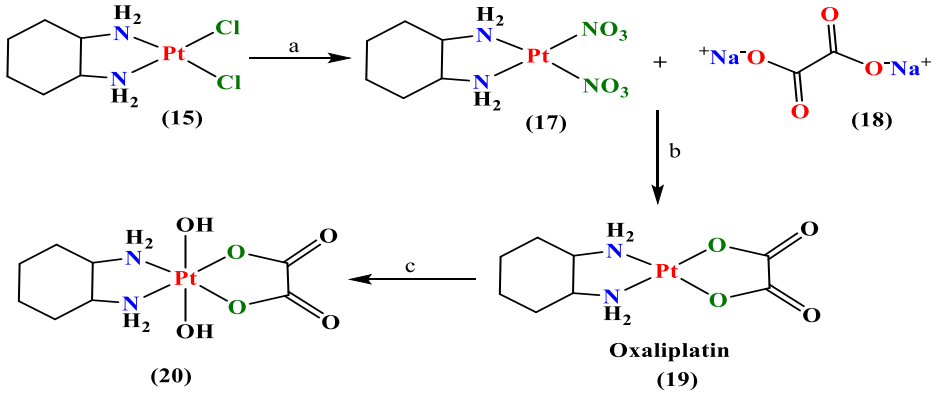


(1) (14) (15) (16)

Scheme 3.5. Synthesis of Dichloro(1,2-diaminocyclohexane)dihydroxidoplatinum(IV) ($Pt(DACH)Cl_2(OH)_2$) **(16)** from potassium tetrachloroplatinate(II) (K_2PtCl_4) **(1).** Reagents and conditions: (a) distilled water, r.t, 24hrs, 92.4% (b) 30% H_2O_2, distilled water, r.t, 24hr, 77.8%.

3.1.3.5 Synthesis of (1,2-diaminocyclohexane)dihydroxidoplatinumoxalate(IV) (Oxaliplatin(IV)) (20, Scheme 3.6)

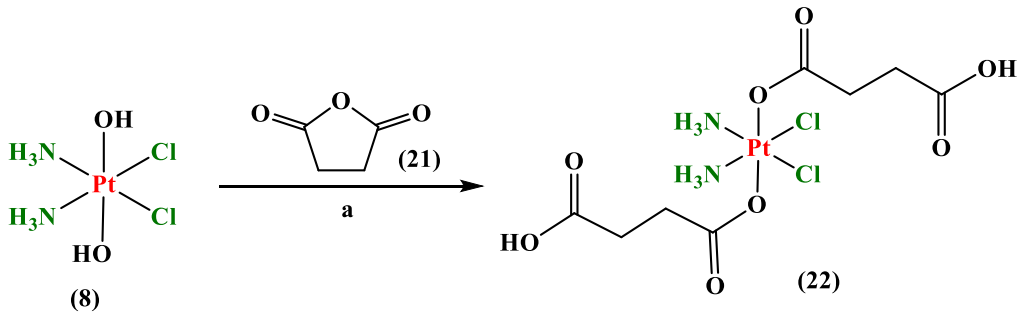
Dichloro(1,2-diaminocyclohexane)platinum(II) $Pt(DACH)Cl_2$ **(15)** was treated with silver nitrate **(Scheme 3.6).** Silver chloride a gray precipitate was formed and filtered off and the filtrate containing soluble $Pt(DACH)Cl_2(NO)_2$ **(17)** was collected. Sodium oxalate **(18)** was added to the filtrate and a fine white precipitate of 1,2-diaminocyclohexane platinum oxalate (Oxaliplatin) **(19)** was formed overnight. The compound was collected and dried to be further hydroxide with 35% hydrogen peroxide in the presence of distilled water to give **Oxaliplatin(IV) (20)** with a very good yield (80.6%).



Scheme 3.6. Synthesis of (1,2-diaminocyclohexane)dihydroxidoplatinumoxalate(IV) (Oxaliplatin(IV) **(20)** from dichloro(1,2-diaminocyclohexane)platinum(II) (Pt(DACH)Cl_2) **(15)**. Reagents and conditions: (a) silver nitrate, r.t, 24hrs, (b) r.t, 24hr, 59%, (c) 35% H_2O_2, distilled water, r.t, 24hr, 80.6%.

3.1.3.6 Synthesis of di-succinato-cisplatin (*cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCCH_2CH_2CO_2H)_2$,] (22, Scheme 3.7)

Di-succinato-cisplatin **(22)** was synthesized from Oxoplatin **(8)** and succinic anhydride **(21) (Scheme 3.7)** to produce a pale-yellow product which was recrystallized from acetone at -20°C and isolated *via* vacuum filtration to give a clear yellow color solution. The solution was further concentrated under reduced pressure to give off-white product di-succinato-cisplatin (*cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCCH_2CH_2CO_2H)_2$**)** **(22)** that was precipitated by diethyl ether and collected with a yield of 40.2%. UPLC Rt=0.874 **(Figure 3.7).**



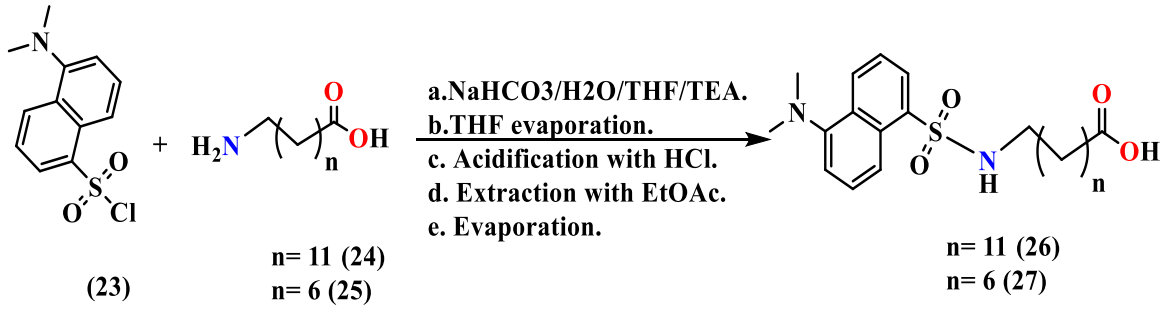
Scheme 3.7. Synthesis of di-succinato-cisplatin (*cis,cis,trans*-[$Pt(NH_3)_2Cl_2(OOCCH_2CH_2CO_2H)_2$**)** **(22)** from Oxoplatin **(8)**. Reagents and conditions: (a) DMF, 60°C, 8hrs, 40.2%.

3.1.4 Synthesis of the designed fluorescent ligands

3.1.4.1 Synthesis of Dansyl amino acid derivatives (26 &27, Scheme 3.8)

Commercially available dansyl chloride **(23)** and amino acids [11-amino undecanoic acid **(24)** or 6-amino hexanoic acid **(25)**] were all purchased and used with no further purification. 11-((5-dimethylaminonaphthalene-1sulfonyl)amino)undecanoic acid (Dans-C11-OOH) **(26)** or 6-((5-dimethylaminonaphthalene-1sulfonyl) amino)hexanoic acid (Dans-C6-OOH) **(27)** were synthesized by reacting Dansyl chloride **(23)** with amino acids in basic media **(Scheme 3.8)**. The solution was then treated with HCl and extracted with ethyl acetate to get the desired products with good yield (44.9%, 55.5% respectively). Product were characterized FTIR, ESI-MS, ^{1}HNMR, ^{13}CNMR and UPLC. FT-IR results showed new peaks at 1739cm^{1} for Dans-C11-OOH **(Figure 3.8)** and 1698cm^{1} for Dans-C6-OOH **(Figure 3.12)** which they are characteristic of carboxyl group confirming the successful synthesis of the products. To further confirm the successful synthesis ESI-MS (positive mode) was used for Dans-C11-OOH ($C_{23}H_{34}N_2O_4S$) found 435.2 $(M+H)^+$ **(Figure 3.9)** and for Dans-C6-OOH ($C_{18}H_{24}N_2O_4S$) found 365.2 $(M+H)^+$ **(Figure 3.13).** Confirming the structure for both compounds. ^{1}H-NMR **(Figure 3.10)** characterization was done for Dans-C11-OOH with sharp single peak at 2.8ppm which is a distinctive feature of Dansyl N-CH_3 protons and peaks downfield from 7.11-8.48ppm confirm the Dansyl aromatic hydrogens. In addition, aliphatic hydrogen peaks up-field from 1.02-2.29ppm reflects the hydrocarbon chain of amino undecanoic acid. Same results for Dans-C6-OOH where ^{1}H-NMR **(Figure 3.14)** showed sharp single peak at 2.8ppm for Dansyl N-CH_3 and downfield peaks from 7.1-8.48ppm that confirms the Dansyl aromatic hydrogens and up-field peaks from 1.13-2.13ppm which reflects aliphatic hydrogen of amino hexanoic acid.

^{13}C-NMR **(Figure 3.15)** was also done for Dans-C6-OOH with similar result of that of ^{1}H-NMR with aliphatic bands up-field (23.87-45.42ppm chemical shifts) and aromatic bands downfield (115.22-152.05ppm chemical shifts). Melting point for the compounds were 88 & 108 respectively. Retention time were 9.795 **(Figure 3.11)** and 7.2 respectively **(Figure 3.16).**

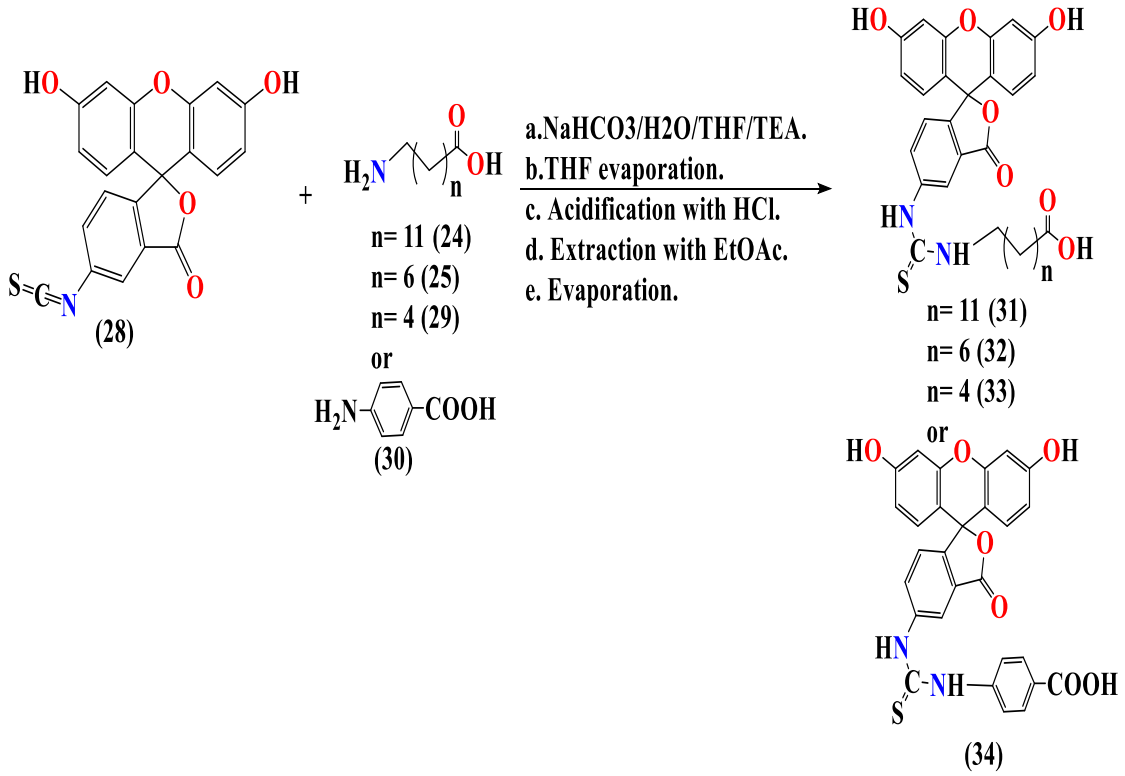


Scheme 3.8. Synthesis of 11-((5-dimethylaminonaphthalene-1sulfonyl) amino)undecanoic acid (Dans-C11-OOH) **(26)** and 6-((5-dimethylaminonaphthalene-1sulfonyl) amino)hexanoic acid (Dans-C6-OOH) **(27).** Reagents and conditions: (a) $NaHCO_3$, distilled water, THF, TEA, r.t, 24hr, (b) THF evaporation, (c) 0.5M HCl, r.t, (d) extraction 3X50ml EtOAc , (e) evaporation of organic fractions, yield 44.9% for Dans-C11-OOH **(26)** and 55.5% for Dans-C6-OOH **(27)**.

3.1.4.2 Synthesis of FITC amino acid derivatives (31-34, Scheme 3.9)

Fluorescein is an effective fluorescent reporter used in live cell imaging due to its advantages such as; having excellent photo-physical properties and can be synthesized on gram-scale. FITC amino acids were prepared in similar manner to Dansyl amino acid derivatives. Commercially available Fluorescein isothiocyanate **(28)** and amino acids [11-amino undecanoic acid **(24),** 6-amino hexanoic acid **(25),** 4-amino butyric acid **(29),** 4-amino benzoic acid **(30)**] were all purchased and used with no further purification. FITC-amino acids were synthesized by reacting FITC **(28)** with amino acids in basic media **(Scheme 3.9)**. The solution was then treated with HCl and extracted with ethyl acetate to get the desired products with good yield (47%-68%). One product was characterized and used for coupling with Pt(IV) due to the high lipophilic property of the compound which

is 11-(Fluorescein-5-isothiocyanate amino)undecanoic acid (FITC-C11-OOH) **(31).** The compound was characterized using FT-IR were the sharp peak of isothiocyanate at 2007 disappeared confirming the attachment of amino acid to FITC. Other peaks also confirmed the structure at 3400 (O-H stretching), 3065, 3008, 2921 (N-H stretching), 1743 (C=O stretching), 1301 (C-N stretching) **(Figure 3.17).** Melting point 190 and UPLC with retention time Rt= 10.681 **(Figure 3.18).**



Scheme 3.9. Synthesis of FITC amino acid derivatives; 11-(Fluorescein-5-isothiocyanate amino)undecanoic acid (FITC-C11-OOH) **(31),** 6-(Fluorescein-5-isothiocyanate amino)hexanoic acid (FITC-C6-OOH) **(32),** 4-(Fluorescein-5-isothiocyanate amino)butyric acid (FITC-C4-OOH) **(33),** 4-(Fluorescein-5-isothiocyanate amino)benzoic acid **(34).**Reagents and conditions: (a) $NaHCO_3$, distilled water, THF, TEA, r.t, 24hr, (b) THF evaporation, (c) 0.5M HCl, r.t, (d) extraction 3X50ml EtOAc , (e) evaporation of organic fractions, yield 65.9% for **(31)**, 47.4% for **(32),** 67.9% for **(33),** 63.4% for **(34).**

3.1.5 Synthesis of fluorescent labeled platinum(IV) prodrugs (35-37)

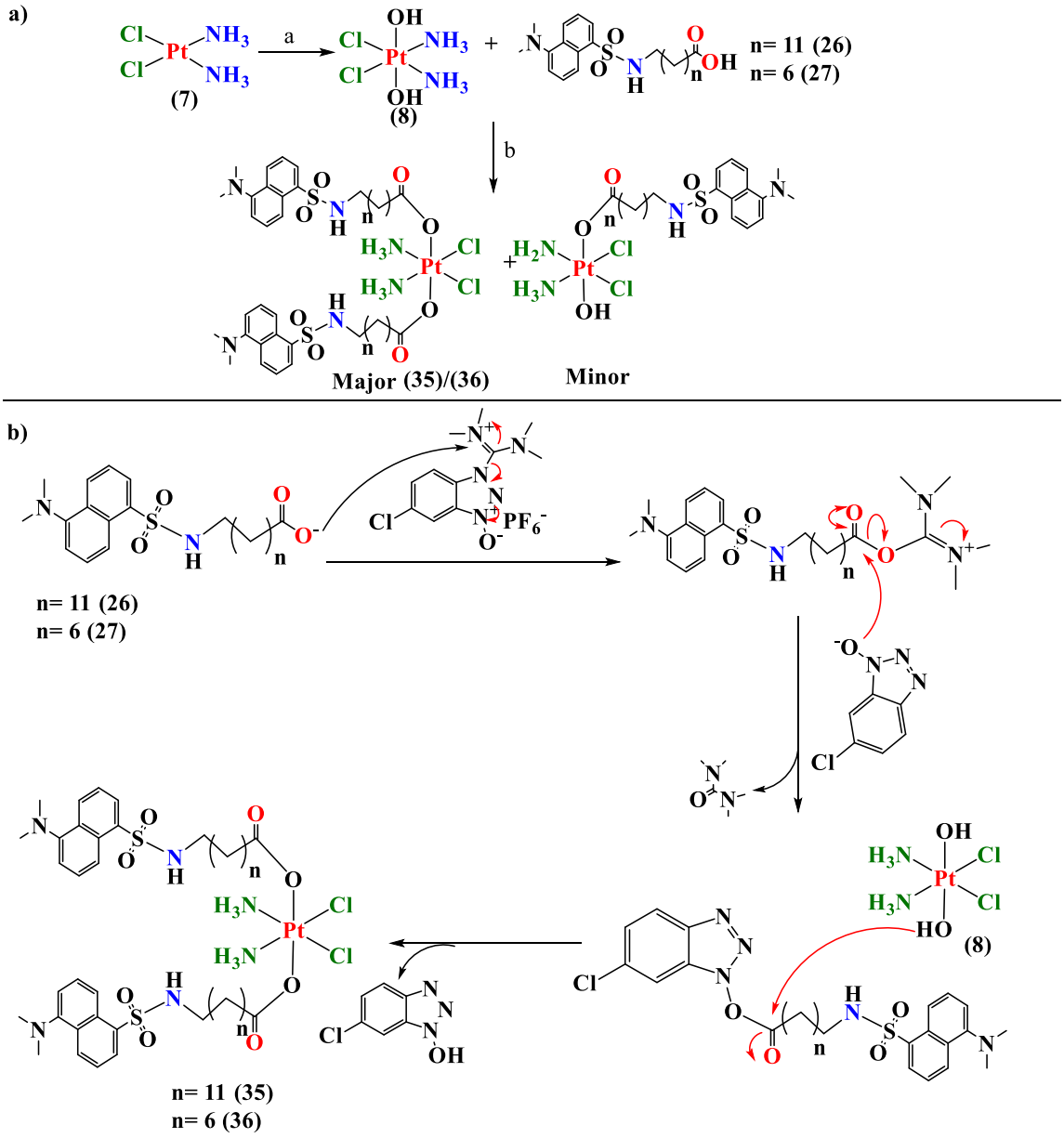
Due to the inert nature of platinum(IV) complexes, the direct ligand substitution is very slow and requires harsh conditions which made this type of reactions rarely employed to

prepare platinum(IV) prodrugs. Therefore, the key importance in such reactions is the presence of electrophiles that will react with the nucleophilic hydroxide ligand of platinum(IV) complexes. Several methods can be used; such as; anhydride and Acyl chlorides that reacts with trans dihydroxo platinum(IV) complexes to form dicarboxylates. A main difficulty in acyl chlorides reaction is the formation of hydrochloric acid (HCl) as a byproduct, which cause the removal of the hydroxy ligands by protonation. This step can be overcome by the addition of base like pyridine to sequester the HCl that is formed[14,161]. On the other hand, anhydride method results in the formation of intermediate (O-acylisourea) which is enough to couple the first hydroxide axial ligand but less to the other hydroxide ligand. In addition to that this method has low yield percentage due to low solubility of dihydroxido Pt(IV) in most organic solvents. This can be overcome by using water soluble EDC and optimization of solvent and reaction conditions to increase the yield. Other method can be used to generate dicarboxylate Pt(IV) is by using activating agents like uranium salts (e.g. HCTU) and a base which give a resonable yields with the ability to isolate Pt(IV) prodrugs from byproducts. This method bypasses the need for preparing acyl chlorides and anhydride intermediates and the need for harsh conditions[24].

3.1.5.1 Synthesis and characterization of the dansyl fatty amino acid-conjugated platinum(IV) complexes (35 & 36, Scheme 3.10) (Approach-1)

Several method was used for the synthesis of the Dansyl labeled Pt(iv) prodrug **(35, 36).** The successful method was the using of activating agents of uranium salts such as O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) to generate di-fluorescent carboxylate platinum(IV) complexes by using base and the desired dansyl amino acid derivatives **(Scheme 3.10)**. Ratio of 1.1 equivalent of activated

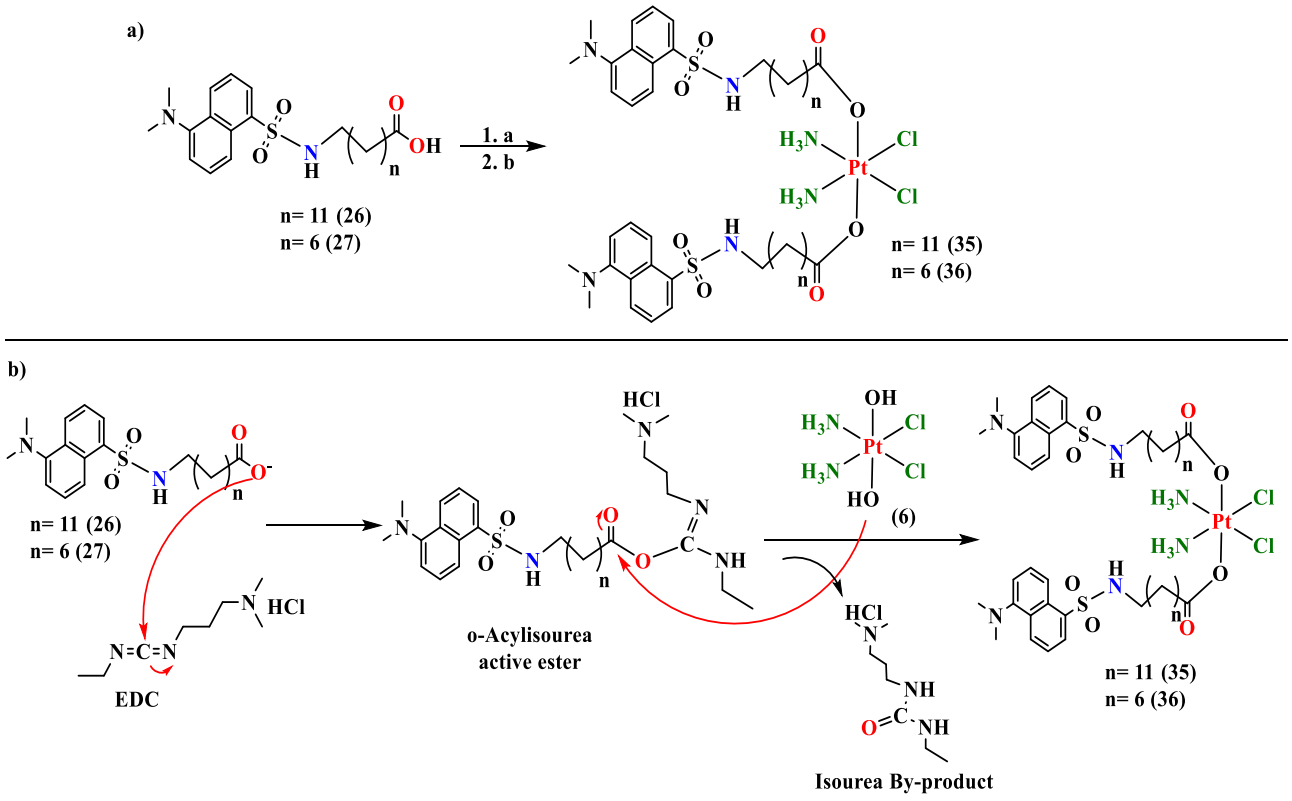
carboxylate are required to generate mono-carboxylate platinum(IV) complexes or an excess of 2.2 equivalent for the formation of di-carboxylate complexes. Uranium salts method gave rise to reasonable yields and the platinum(IV) prodrugs was isolated easily from the byproducts[24]. The synthesis occurred by conjugating 11-DAUDA **(26)** or 6-DAHA **(27)** to a Pt(iv) prodrug (Oxoplatin **8**) by the HCTU-catalyzed ester bond formation reaction. The overall yield was (12.8%), (77.9%) respectively. The Dansyl amino acid-conjugated Pt(iv) compound **(35),** was characterized by ^{1}H NMR spectroscopy **(Figure 3.19)** in which a new signal at (3.71) ppm corresponds to the amine groups of the Pt(iv) center appeared. UPLC Rt 17.752 with purity of 77% **(Figure 3.20).** Melting point of **(35)** was not done due to the sticky characteristic of the compound. Compound **(36)** was characterized by FT-IR where the band of 3509 (O-H stretching) disappeared indicating the binding of platinum(IV) to dansyl amino derivatives **(Figure 3.24).** Melting point was 290 and UPLC Rt 10.112 with purity of 63.322% **(Figure 3.25).** Low purity refers to the close RF of the starting material with the prodrug and low solubility of the prodrug **(36)** in organic solvents except methanol which made it difficult to be separated through column chromatography that's why further purifications was done to purify the product by recrystallization in DCM and ethyl acetate.



Scheme 3.10. Synthesis of platinum(IV) prodrugs approach-1; a) General scheme for the synthesis of Dansyl labeled platinum(IV) mono- and bis-carboxylate derivatives investigated in this study**.** Reagents and conditions: (a) 30% H_2O_2, distilled water, r.t, 24 h, 73.4%, (b) HCTU, DIPEA, DMF, 50 °C, 24 h, 12.8% for (33), 77% for (34), b) Mechanism of HCTU activation of carboxylic acids and its reaction with Oxoplatin**.**

3.1.5.2 Synthesis and characterization of the dansyl fatty amino acid-conjugated platinum(IV) complexes (Approach-2)

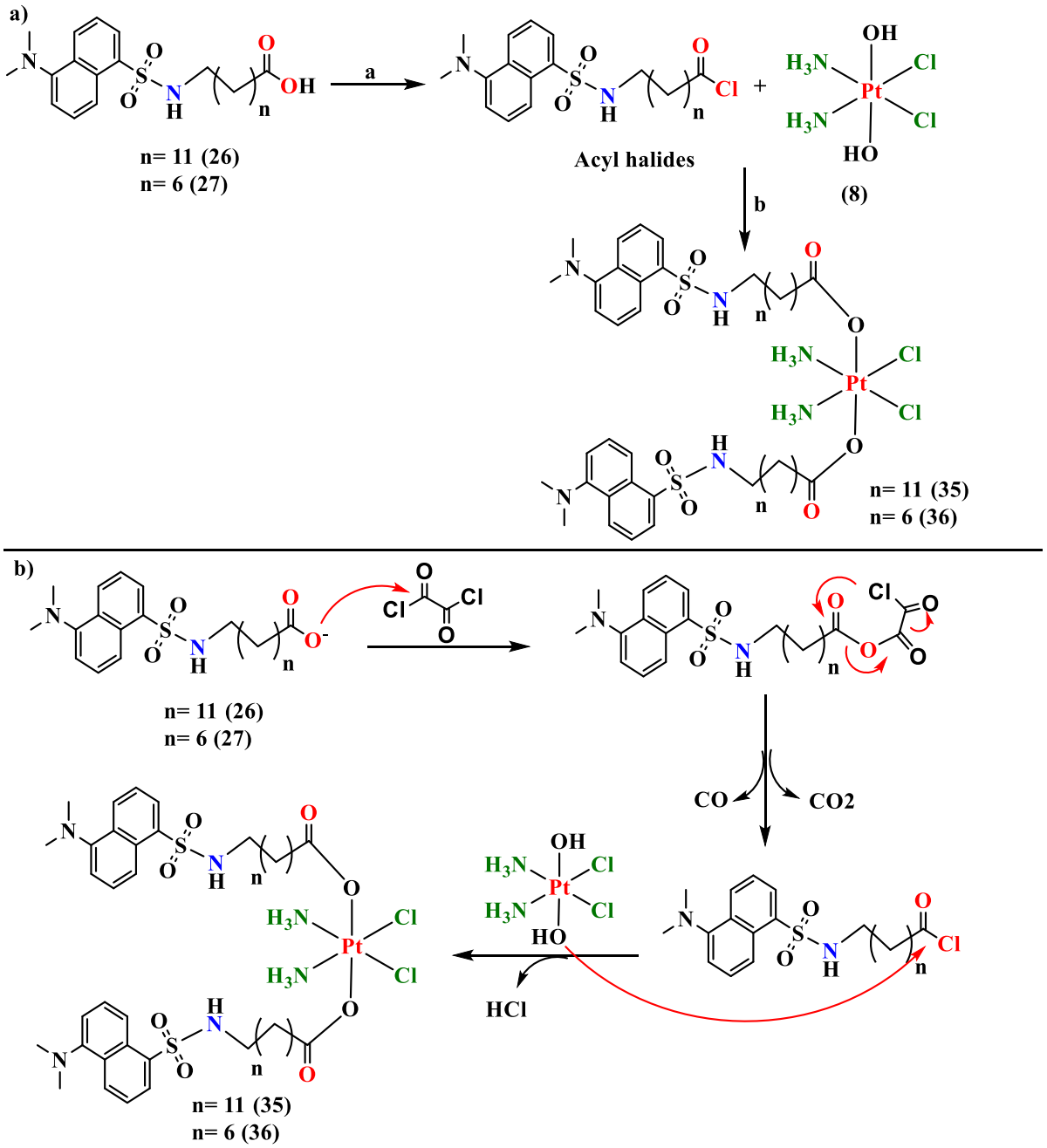
Another approach was used to prepare **(35)** & **(36)** prodrugs is by using EDC agent. Briefly, 11-DAUDA **(26)** or 6-DAHA **(27)** was conjugated to a Pt(iv) prodrug (Oxoplatin **(8))** by the EDC-mediated ester bond formation reaction **(Scheme 3.11).** The synthesis was monitored by UPLC which indicated the production of new products at Rt= 22.55 for prodrug **(35) (Figure 3.21)** and Rt= 13.23 & 22.53 for mono- and di-substituted prodrug **(36) (Figure 3.26).** The Yield for this method was very poor and the compounds were not purified due to low yield.



Scheme 3.11. Synthesis of platinum(IV) prodrugs approach-2; a) general scheme for the synthesis of platinum(IV) prodrugs **(35)** and **(36).** Reagents and conditions: (a) DMF, EDC, 50°C, 1hr, (b) DMF, Oxoplatin **(8)** 50°C, 24hr. b) Mechanism of EDC activation of carboxylic acids and its reaction with Oxoplatin **(8).**

3.1.5.3 Synthesis and characterization of the dansyl fatty amino acid-conjugated platinum(IV) complexes (Approach-3)

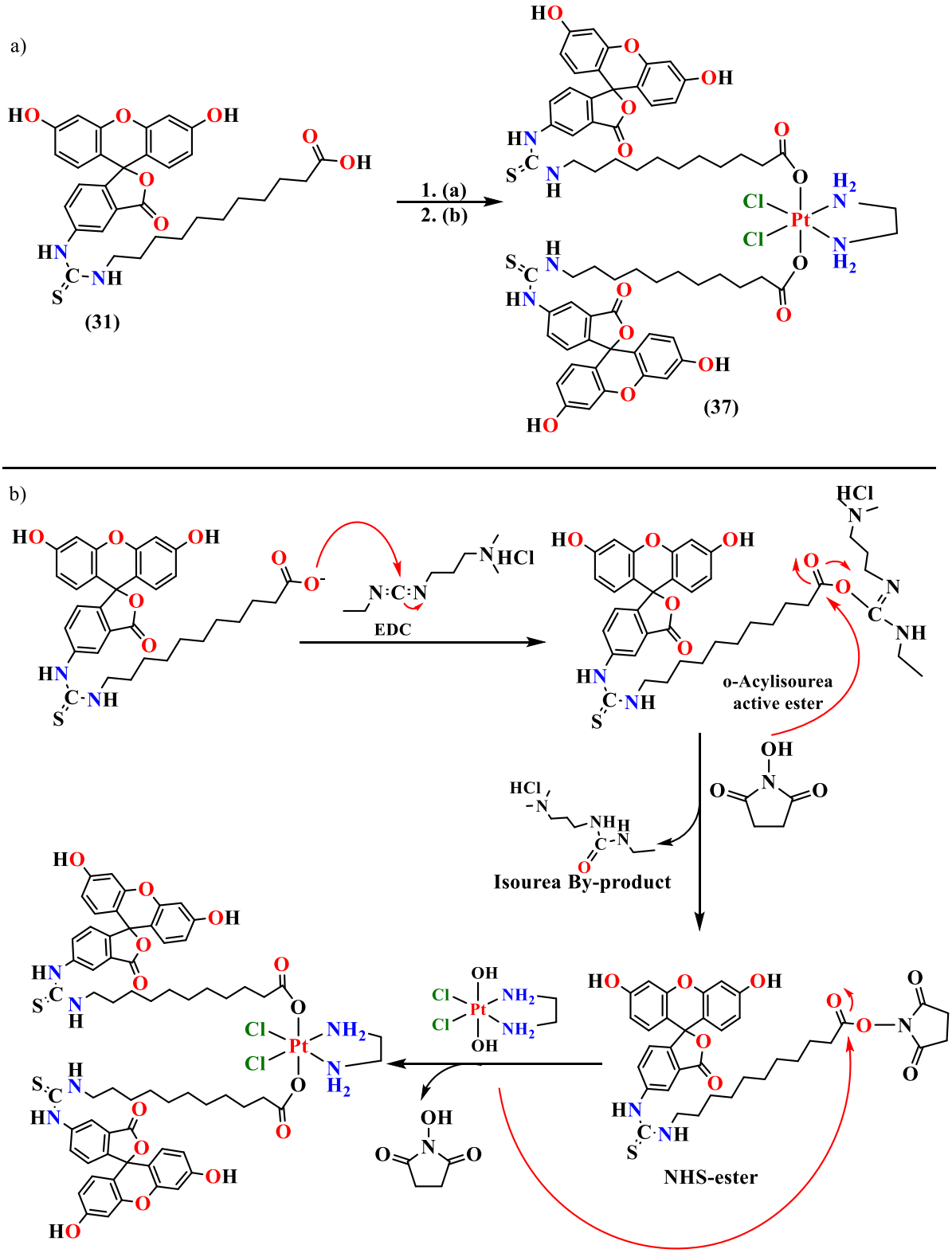
In this approach acyl halide were used to be coupled with platinum(IV) complexes. First Dansyl amino acid derivatives were converted to acyl chlorides by reacting with oxalyl chloride **(Scheme 3.12).** After that the acyl chlorides were allowed to be reacted with Oxoplatin **(8)** in basic media to neutralize HCl formed from reactions and under calcium chloride tube to prevent humidity from entering to the reactions. Despite the strict conditions while handling the reactions; the TLC & UPLC **(Figure 3.22 & 3.27)** for **(35)** and **(36),** respectively, results indicate hydrolysis of the compounds to starting materials. Very low yields and low solubility in organic solvents made it difficult to collect and continue purification of compounds. ESI-MS (positive mode) was used for Prodrug **(35)** to confirm the formation of the prodrug; calculated 1167.22 found 1167.417 $(M+H)^+$ **(Figure 3.23).**



Scheme 3.12. Synthesis of platinum(IV) prodrugs approach-3; a) General scheme for the synthesis of Dansyl labeled platinum(IV) **(35)** and **(36).** Reagents and conditions: (a) oxalyl chloride, DCM, catalytic DMF, r.t, 24 h, (b) pyridine, 24 h, r.t, b) Mechanism of oxalyl chloride activation of carboxylic acids and its reaction with Oxoplatin **(8).**

3.1.5.4 Synthesis and characterization of the FITC- fatty amino acid-conjugated platinum(IV) complexes (37, Scheme 3.13)

FITC-C11-Platinum(IV) prodrug **(37)** was synthesized via NHS/EDC method. First FITC-C11-OOH **(31)** was activated by NHS/EDC **(Scheme 3.13)** to form FITC-NHS activated ester which was reacted with *cis*, *cis*, *trans*-[$PtenCl_2(OH)_2$] **(13)** to give an overall yield of 22.7% compared to other studies with a yield of 6% [65]. The FITC amino acid-conjugated Pt(iv) compound **(37)** exhibited poor water solubility that's why concentrated solution was prepared in DMF and diluted with pH7.4 buffer immediately prior to analytical measurements. Compound **(37)** was characterized by FT-IR and UPLC. In the FT-IR peaks at 3400 (O-H stretching), 3200 (N-H stretching), 1174 (C-O ester stretching), 457 (Pt-N stretching) indicate the formation of the compound **(37) (Figure 3.28).** The compound has no solubility in water nor organic solvent except for DMF that's why for UPLC analysis the compound was dissolved in DMF and diluted with pH 7.4 buffer. UPLC of the final product indicated that the purity from the described synthetic method was 75.8% **(Figure 3.29).** Melting point of the compound was also measured with a value of 260.



Scheme 3.13. Synthesis of FITC-platinum(IV) prodrugs; a) general scheme for the synthesis of platinum(IV) prodrugs (**23**). Reagents and conditions: (a) NHS/EDC, DMF, 50°C, 1hr, (b) $PtenCl_2(OH)_2$ **(13),** DMF**,** 50°C, 24hr, yield 22.7%, b) Mechanism of NHS/EDC activation of carboxylic acids and its reaction with $PtenCl_2(OH)_2$**.**

3.2 Stability studies

Stability study was done for Dans-C11-oxoplatin **(35)** in sodium mono phosphate buffer solution at 37°C and was monitored by UPLC (Figure 3.2). Dans-C11-oxoplatin was stable under these conditions. Kinatic study was done for 30hr and the compound was stable with no degredation observed.

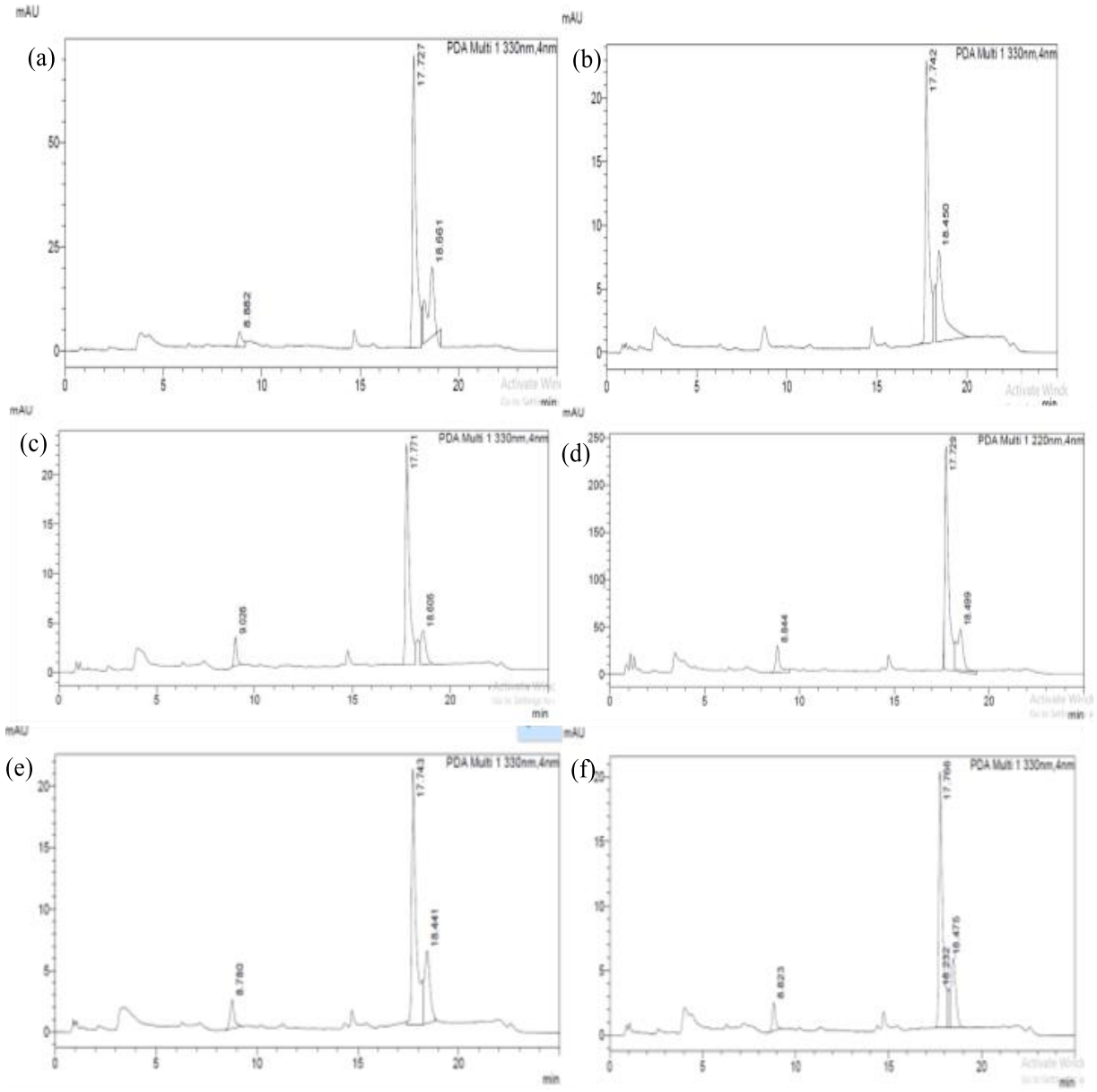


Figure 3.2. Stability study for di(11-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)undecanoic)*(*cis*,*trans*,*cis*-[$Pt(NH_3)_2(Cl)_2$]) **(21)** at zero time, 3hrs, 24 hr, 26 hr, 28 hr, and 30 hr.

3.3 Compounds characterization using different analytical techniques

3.3.1 Characterization of Cisplatin complex (7)

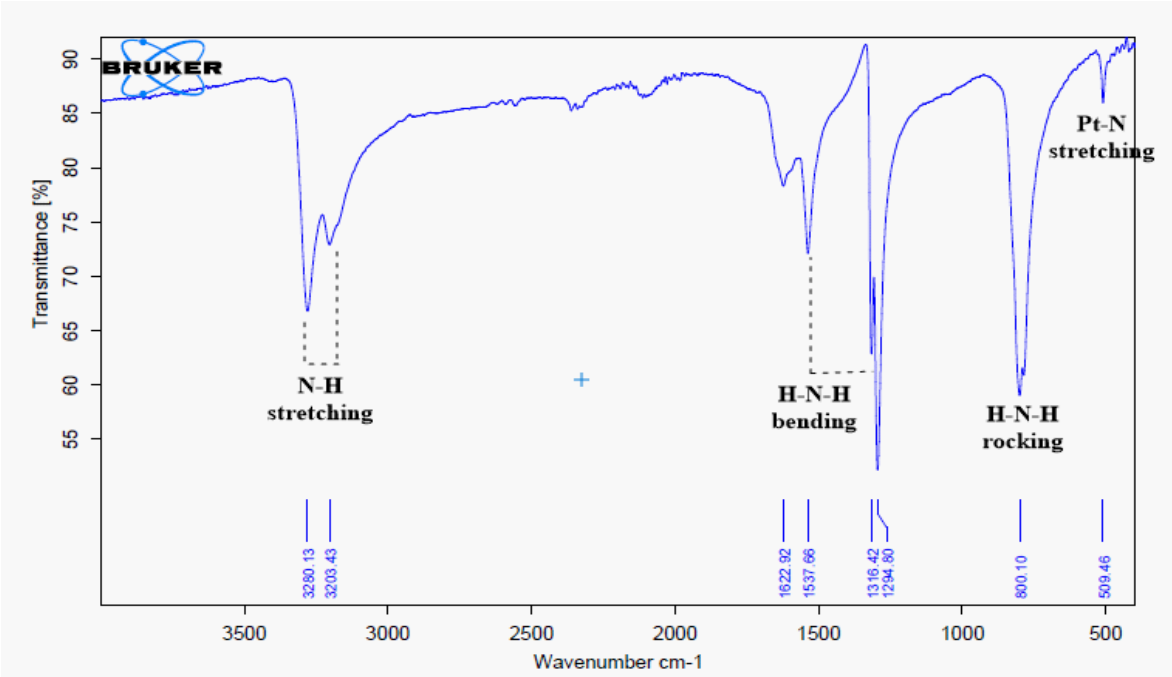


Figure 3.3. Fourier transform infrared spectra of Cisplatin **(7).**

3.3.2 Characterization of Oxoplatin complex (8)

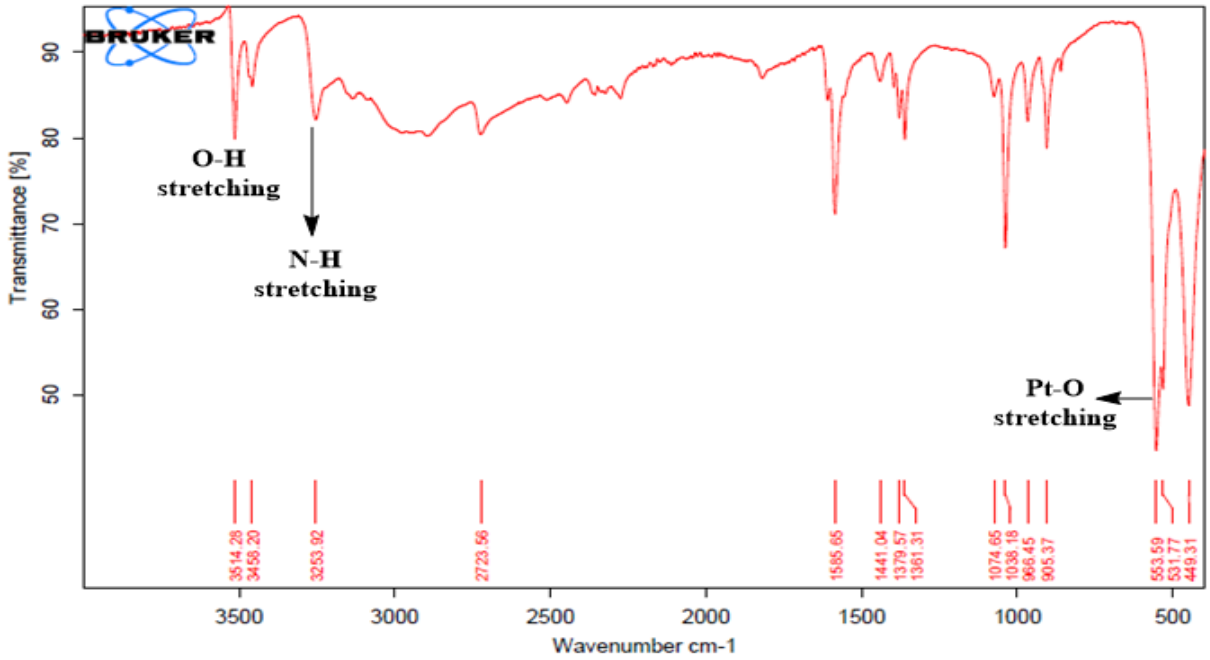


Figure 3.4. Fourier transform infrared spectra of Oxoplatin **(8).**

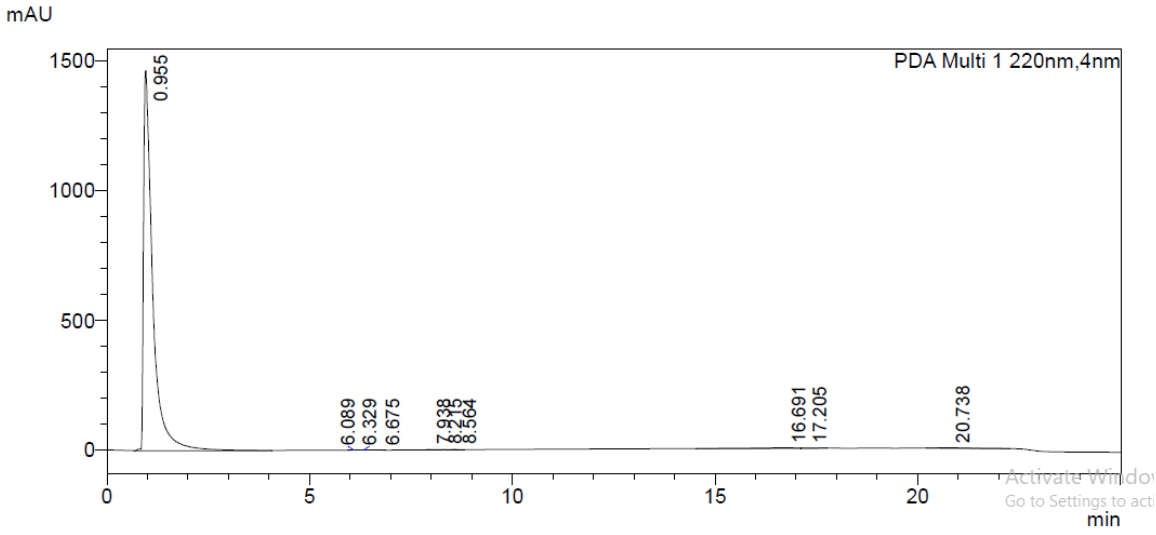


Figure 3.5. Ultra-performance liquid chromatography (UPLC) of Oxoplatin **(8).**

3.3.3 Characterization of PtenCl2(OH)2 complex (13)

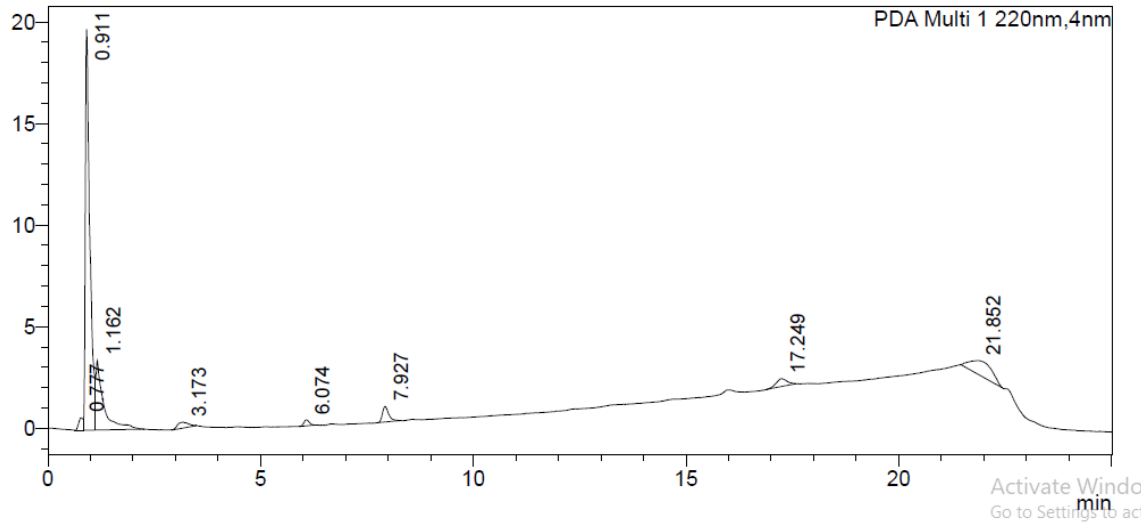


Figure 3.6. Ultra-performance liquid chromatography (UPLC) of $PtenCl_2(OH)_2$ **(13).**

3.3.4 Characterization of di-succinato-Cisplatin complex (22)

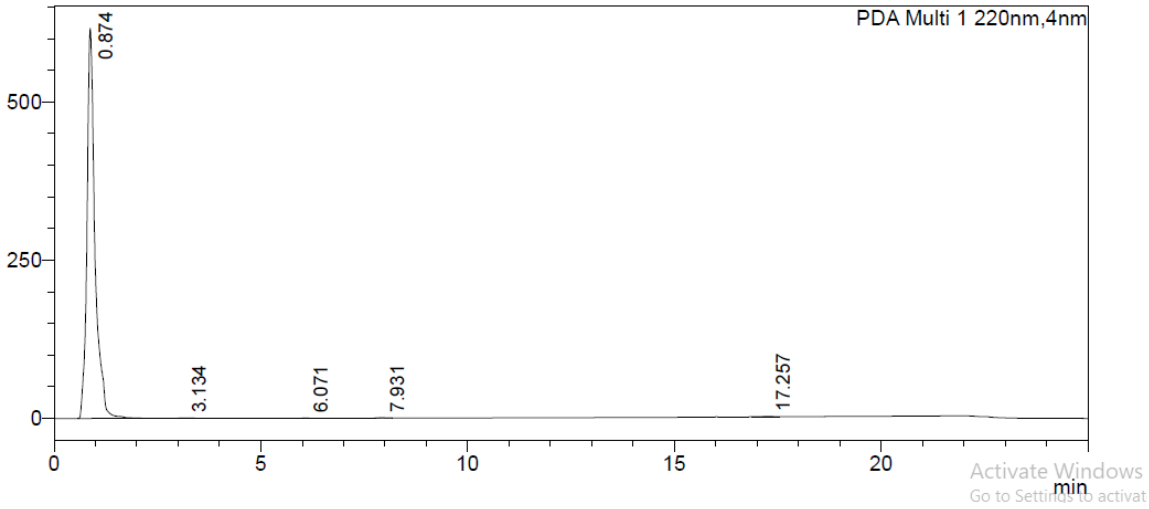


Figure 3.7. Ultra-performance liquid chromatography (UPLC) of di-succinato-Cisplatin **(22).**

3.3.5 Characterization of Dans-C11OOH complex (26)

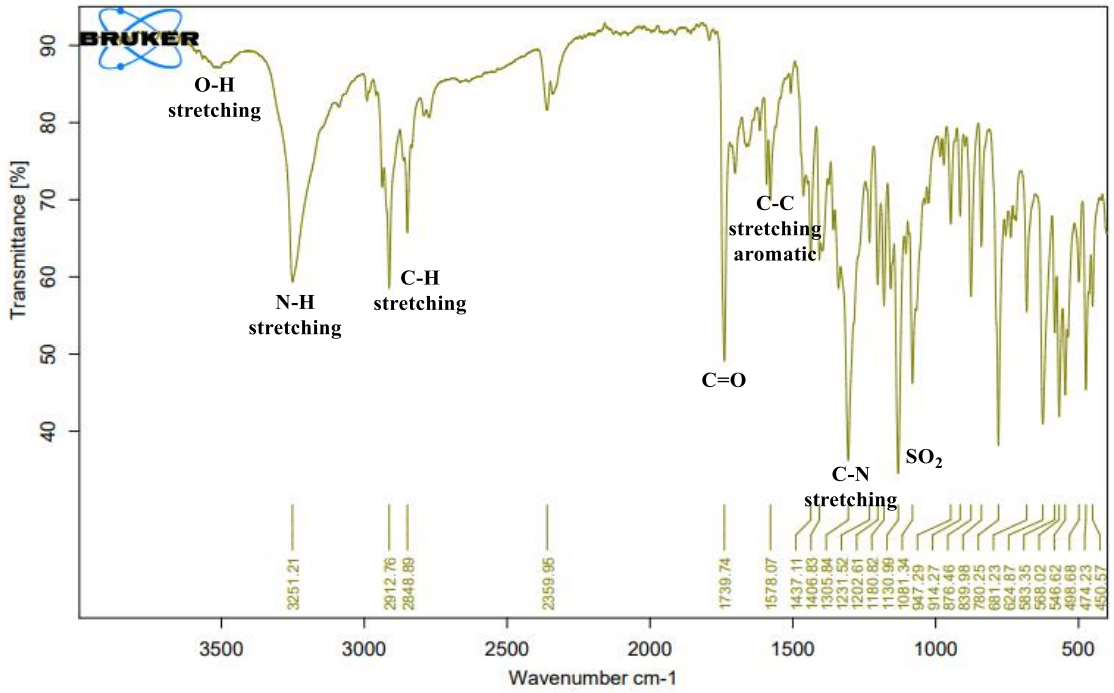


Figure 3.8. Fourier transform infrared spectra of; a) 11-((5-dimethylaminonaphthalene-1sulfonyl)amino)undecanoic acid (Dans-C11-OOH) **(26).**

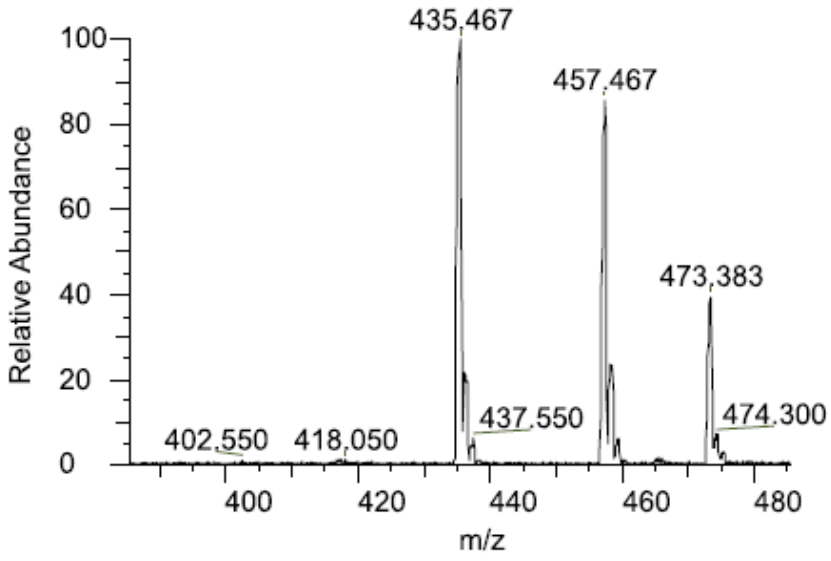


Figure 3.9. Electrospray ionization mass spectrometry (ESI-MS)) ion scan mode spectra of; 11-((5-dimethylaminonaphthalene-1sulfonyl)amino)undecanoic acid (Dans-C11-OOH) **(26)** ($C_{23}H_{34}N_2O_4S$) calculated 434.60, found 435.2$(M+H)^+$**.**

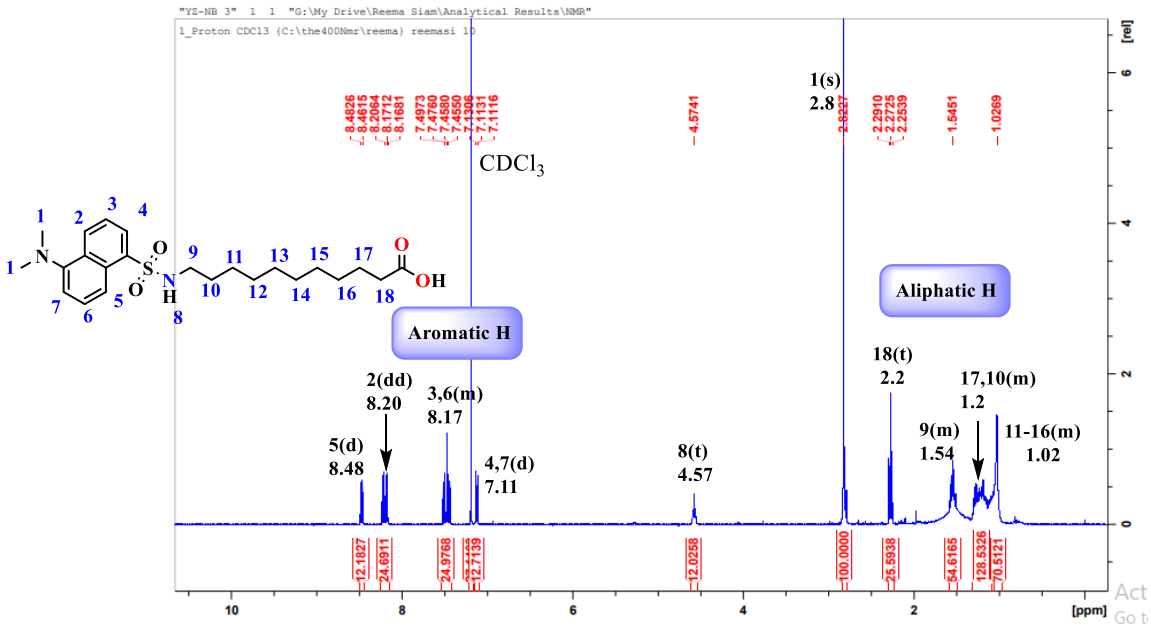


Figure 3.10 [1]HNMR spectra of 11-((5-dimethylaminonaphthalene-1sulfonyl)amino)undecanoic acid (Dans-C11-OOH) **(26)** ($C_{23}H_{34}N_2O_4S$).

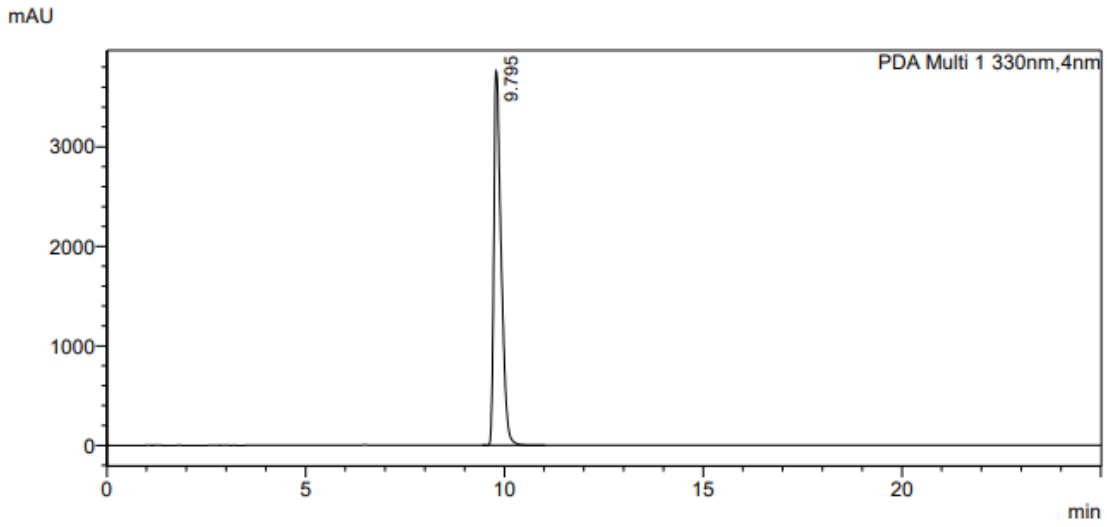


Figure 3.11. Ultra-performance liquid chromatography (UPLC) of; a) 11-((5-dimethylaminonaphthalene-1sulfonyl)amino)undecanoic acid (Dans-C11-OOH) **(26)** with retention time of 9.795.

3.3.6 Characterization of Dans-C6OOH complex (27)

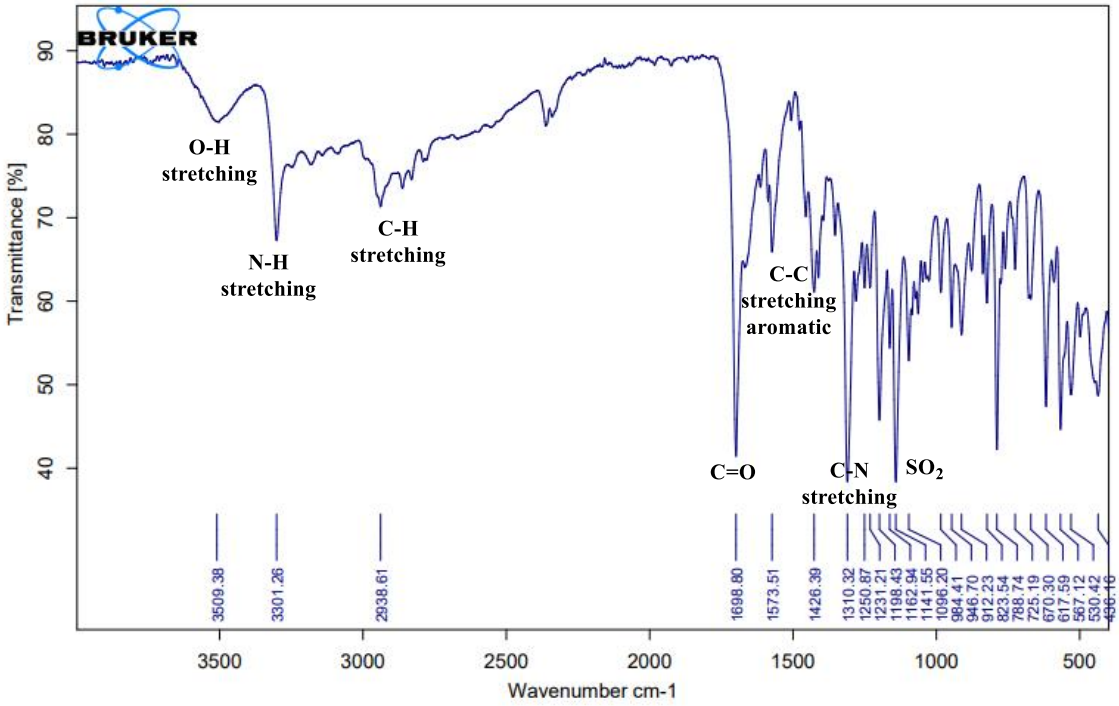


Figure 3.12. Fourier transform infrared spectra of 6-((5-dimethylaminonaphthalene-1sulfonyl) amino)hexanoic acid (Dans-C6-OOH) **(27).**

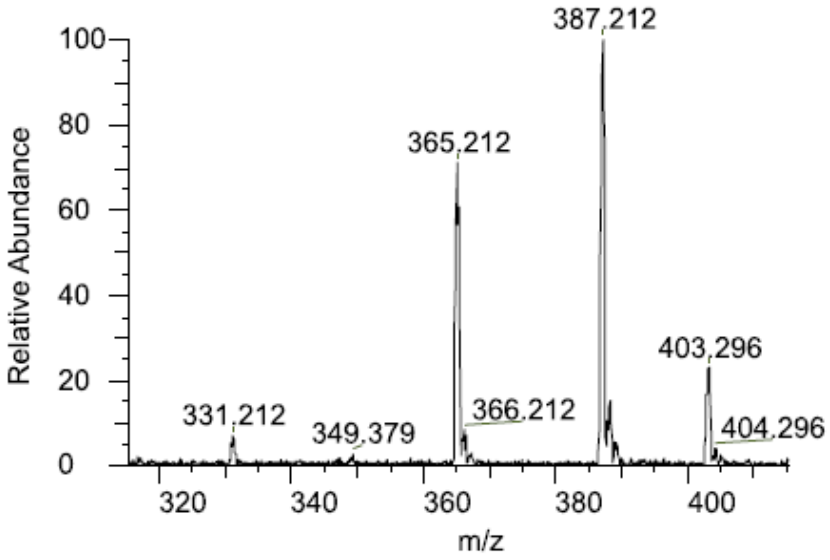


Figure 3.13. Electrospray ionization mass spectrometry (ESI-MS) ion scan mode spectra of 6-((5-dimethylaminonaphthalene-1sulfonyl) amino)hexanoic acid (Dans-C6-OOH) **(27)** ($C_{18}H_{24}N_2O_4S$) calculated 364.46, found 365.2 $(M+H)^+$**.**

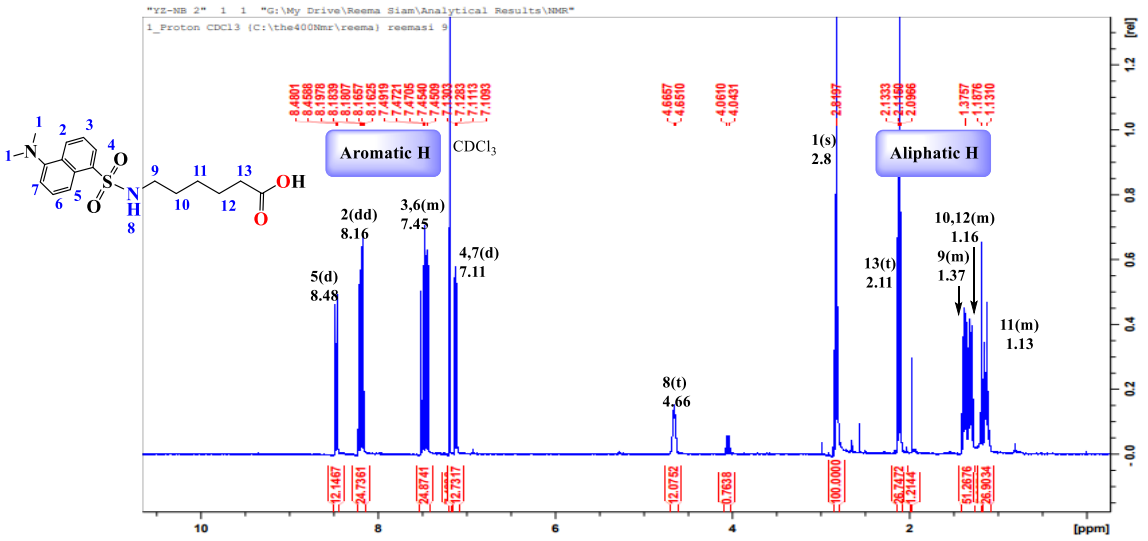


Figure 3.14. ^{1}H-NMR spectra of 6-((5-dimethylaminonaphthalene-1sulfonyl) amino)hexanoic acid (Dans-C6-OOH) **(27)** ($C_{18}H_{24}N_2O_4S$).

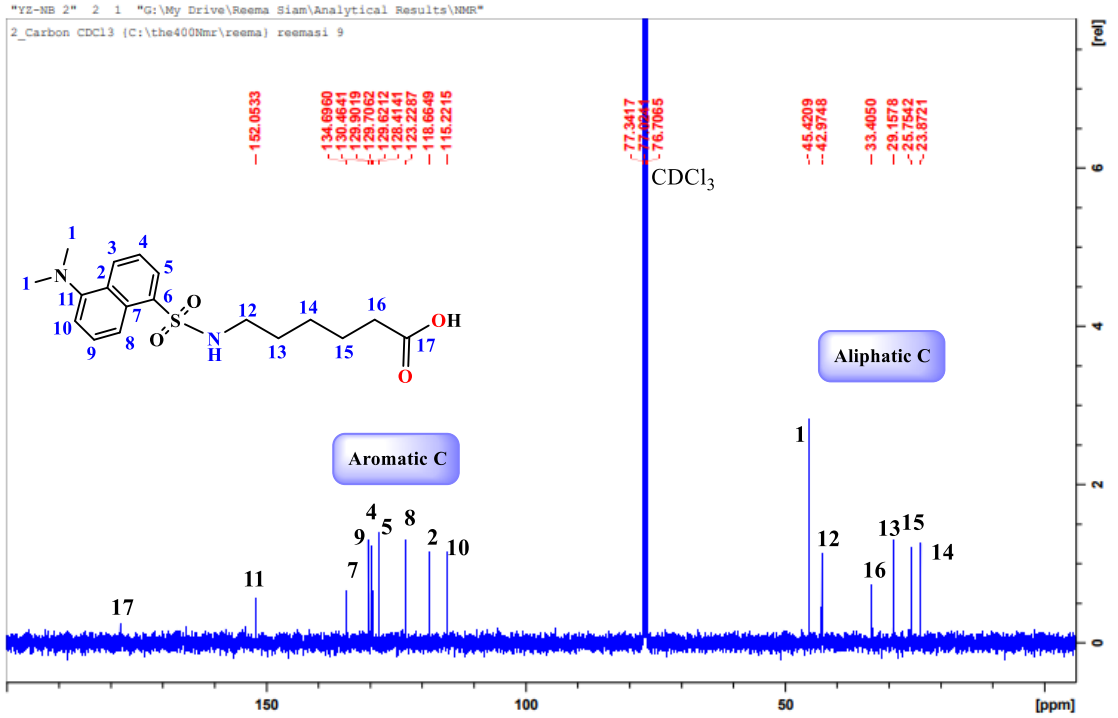


Figure 3.15. ^{13}C-NMR spectra of 6-((5-dimethylaminonaphthalene-1sulfonyl) amino)hexanoic acid (Dans-C6-OOH) **(27)** ($C_{18}H_{24}N_2O_4S$).

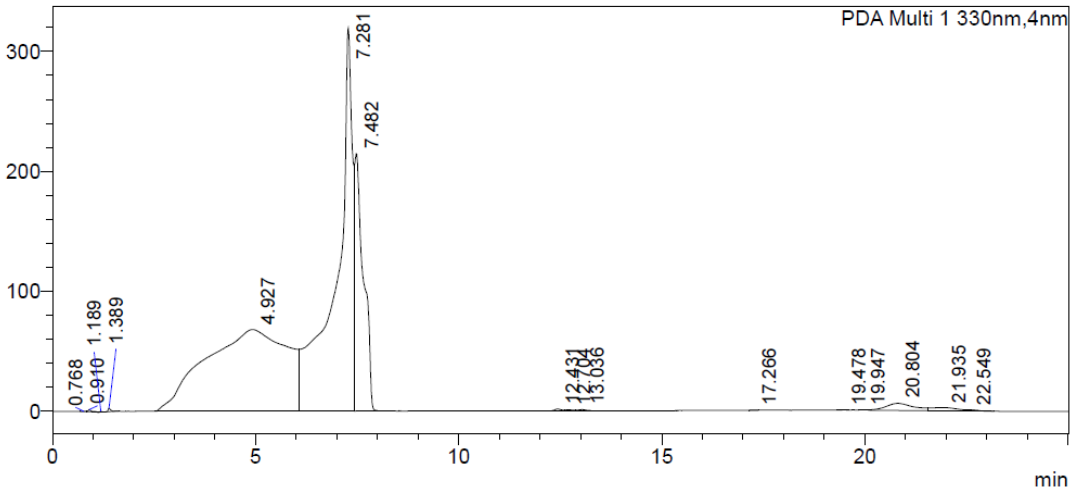


Figure 3.16. Ultra-performance liquid chromatography (UPLC) of 6-((5-dimethylaminonaphthalene-1sulfonyl) amino)hexanoic acid (Dans-C6-OOH) **(27)** with retention time of 7.28.

3.3.7 Characterization of FITC-C11OOH complex (31)

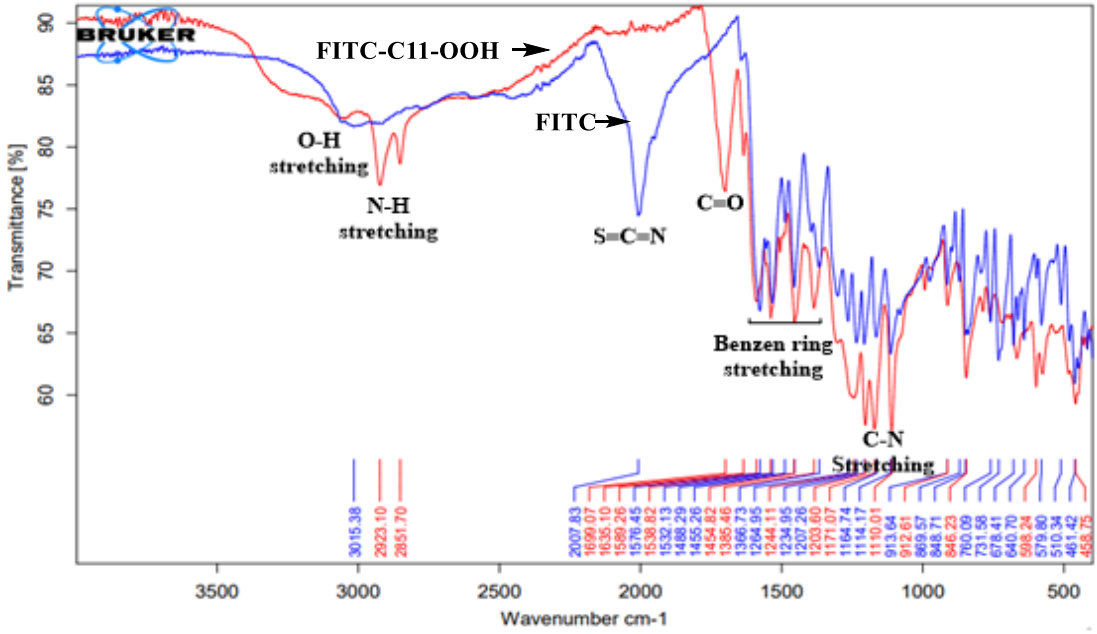


Figure 3.17. Fourier transform infrared spectra of 11-((Fluorescein-5-isothiocyanate)amino)undecanoic acid (FITC-C11-OOH) **(31)** compared to FITC **(28).**

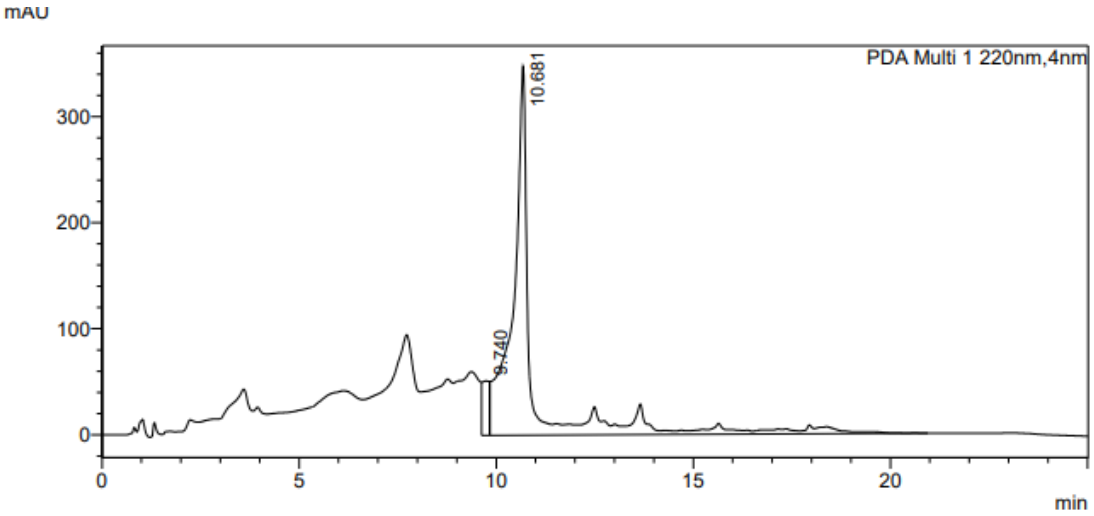


Figure 3.18. Ultra-performance liquid chromatography (UPLC) of 11-((Fluorescein-5-isothiocyanate)amino)undecanoic acid (FITC-C11-OOH) **(31)** with retention time of 10.681.

3.3.8 Characterization of di(11-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)undecanoic)*(*cis,trans,cis*-[Pt(NH$_3$)$_2$(Cl)$_2$)) (35)

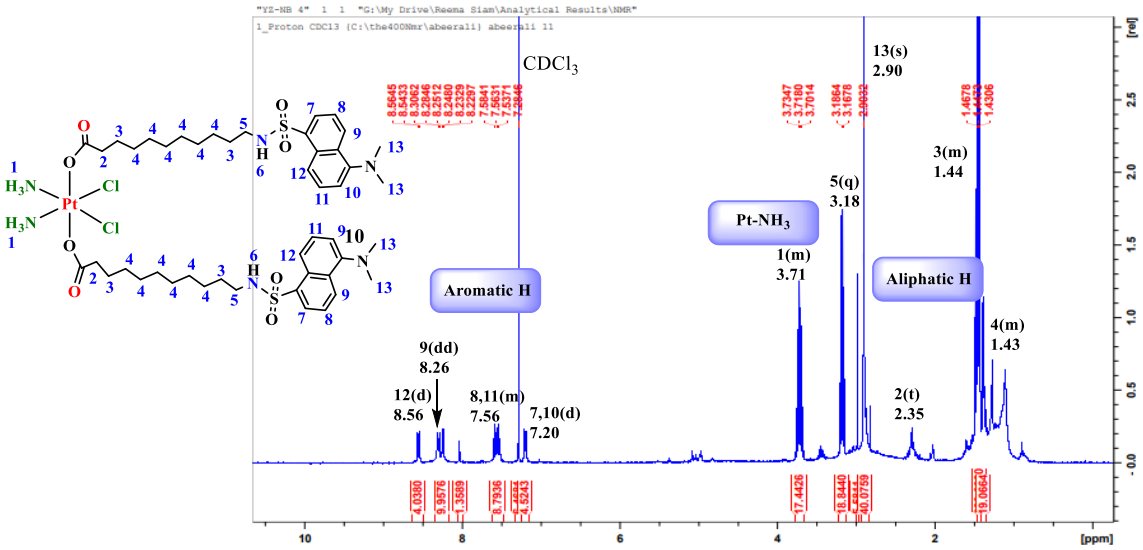


Figure 3.19. 1HNMR spectra of di(11-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)undecanoic)* (*cis,trans,cis*-[Pt(NH$_3$)$_2$(Cl)$_2$])) **(35)**

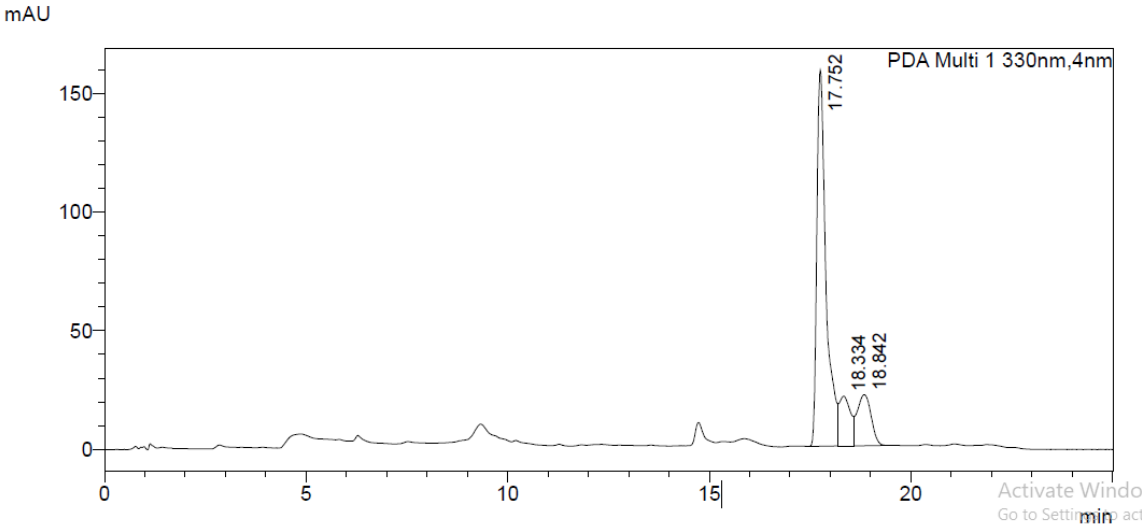


Figure 3.20. Ultra-performance liquid chromatography (UPLC) of di(11-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)undecanoic)*(*cis*,*trans*,*cis*-[$Pt(NH_3)_2(Cl)_2$])) **(35)** with retention time of 17.752. (Gradient of 50%:50%, water: ACN)

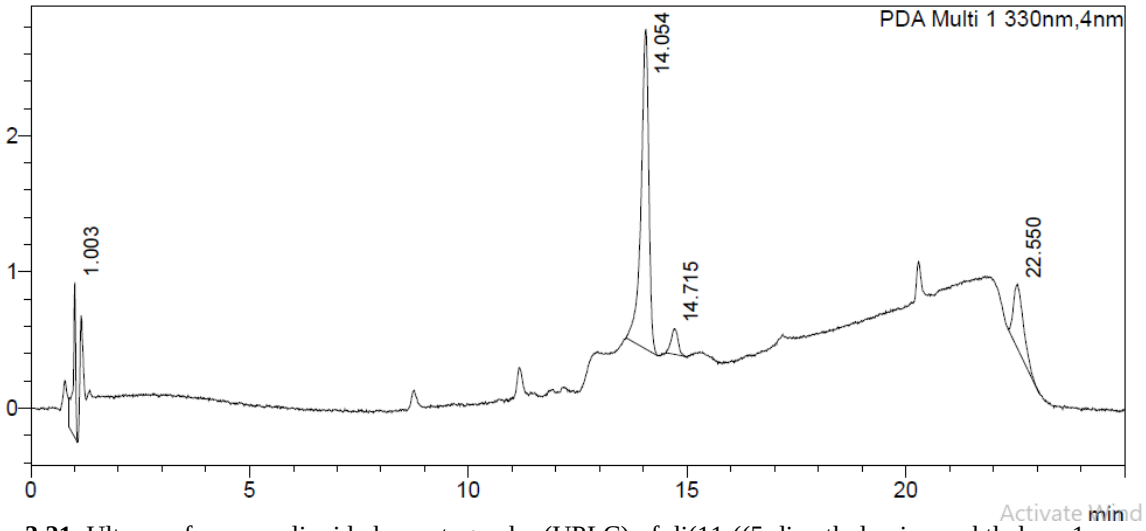


Figure 3.21. Ultra-performance liquid chromatography (UPLC) of di(11-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)undecanoic)*(*cis*,*trans*,*cis*-[$Pt(NH_3)_2(Cl)_2$])) **(35)** From appraoch-2 EDC mediated with retention time of 22.5. (Gradient of 65%:35%, water: ACN)

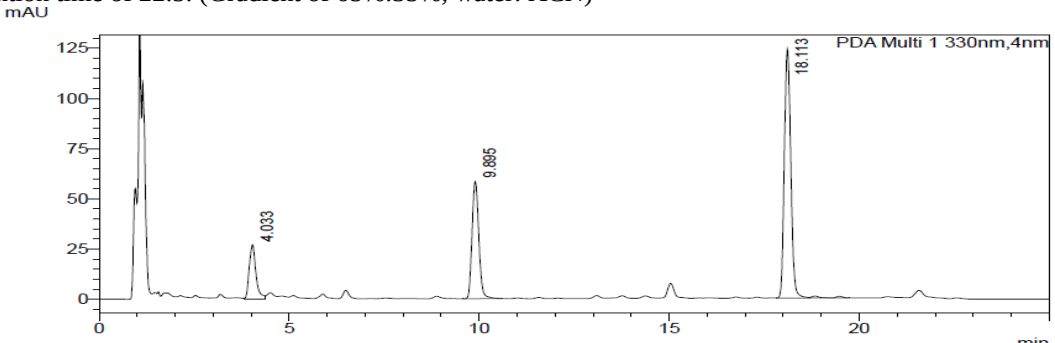


Figure 3.22. Ultra-performance liquid chromatography (UPLC) of di(11-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)undecanoic)*(*cis*,*trans*,*cis*-[$Pt(NH_3)_2(Cl)_2$])) **(35)** From appraoch-3 acyl halide mediated with retention time of 18.113. (Gradient of 50%:50%, water: ACN)

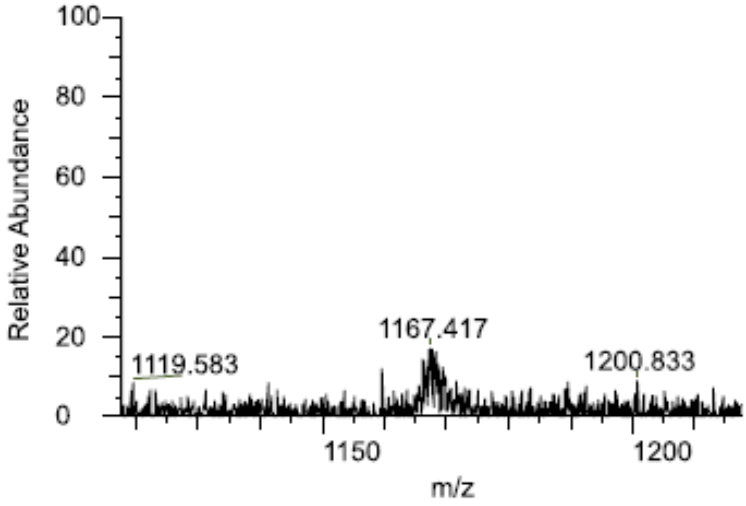


Figure 3.23. Electrospray ionization mass spectrometry (ESI-MS) ion scan mode spectra of di(11-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)undecanoic)*(*cis*,*trans*,*cis*-[$Pt(NH_3)_2(Cl)_2$])) **(35)** From appraoch-3 acyl halide mediated calculated 1167.22 , found 1167.417 $(M+H)^+$**.**

3.3.9 Characterization of di(6-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)hexanoic)*(*cis*,*trans*,*cis*-[$Pt(NH_3)_2(Cl)_2$]) (36)

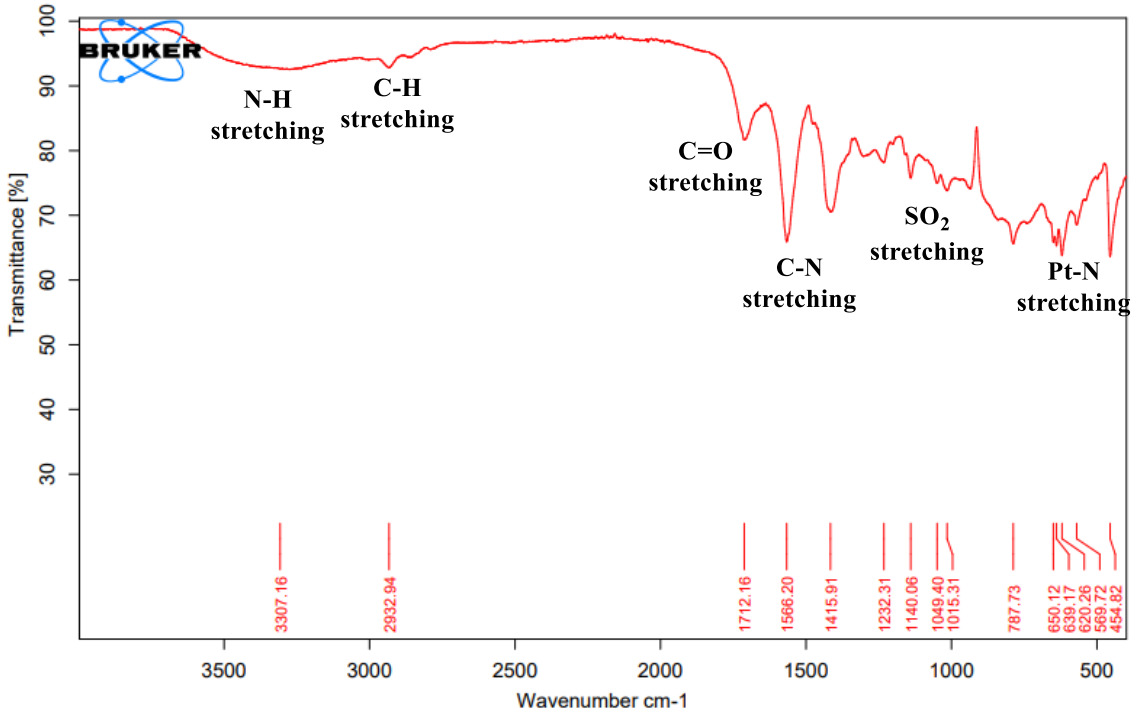


Figure 3.24. Fourier transform infrared spectra of di(6-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)hexanoic)*(*cis*,*trans*,*cis*-[$Pt(NH_3)_2(Cl)_2$]) **(36).**

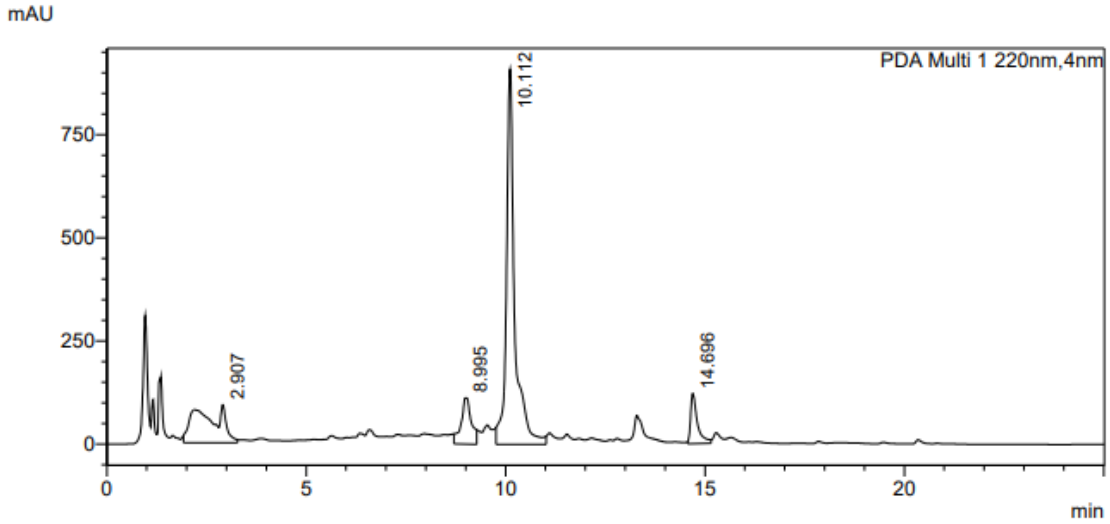


Figure 3.25. Ultra-performance liquid chromatography (UPLC) of di(6-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)hexanoic)*(*cis*,*trans*,*cis*-[Pt(NH_3)$_2$(Cl)$_2$])) **(36)** from approach-1 with retention time of 10.112.

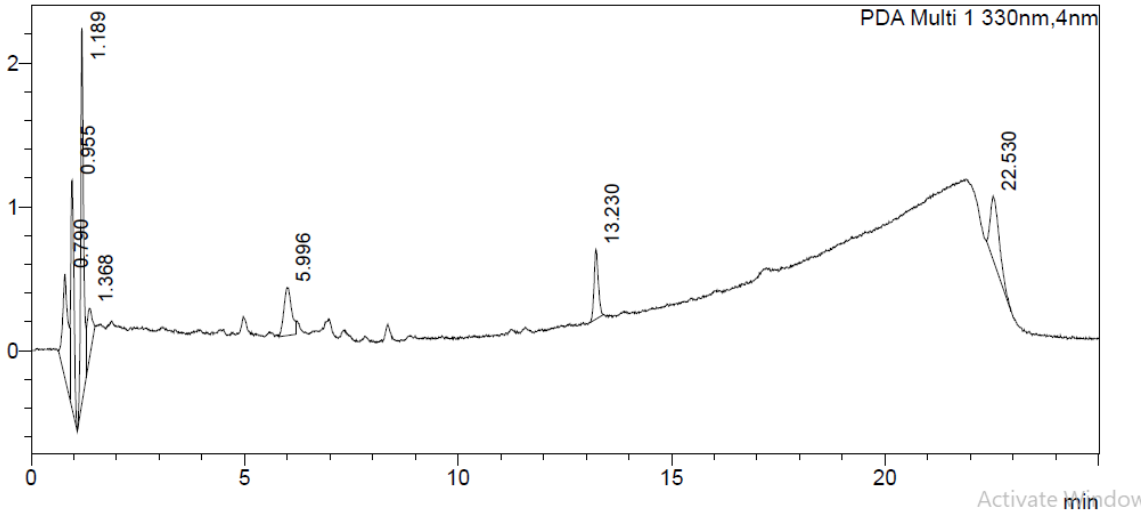


Figure 3.26. Ultra-performance liquid chromatography (UPLC) of di(6-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)hexanoic)*(*cis*,*trans*,*cis*-[Pt(NH_3)$_2$(Cl)$_2$])) **(36)** from approach-2 EDC mediated**.**

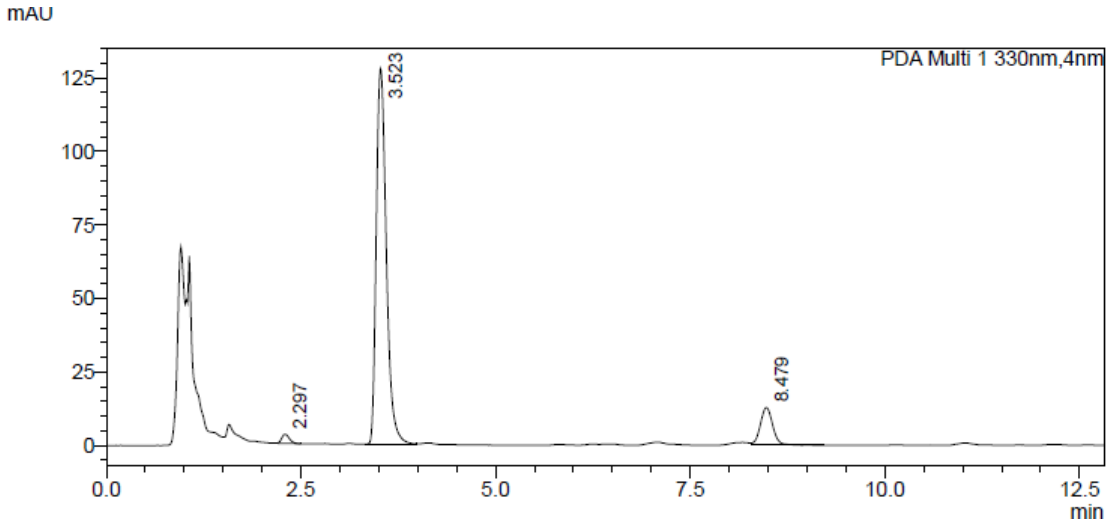


Figure 3.27. Ultra-performance liquid chromatography (UPLC) of di(6-((5-dimethylaminonaphthalene-1-sulfonyl)*amino)hexanoic)*(*cis*,*trans*,*cis*-[$Pt(NH_3)_2(Cl)_2$])) **(36)** from approach-3 acyl halide mediated**.**

3.3.10 Characterization of di(11-((Fluorescein-5-isothiocyanate)*amino)undecanoic)*(*cis*,*trans*,*cis*-[$Pten(Cl)_2$])) (37)

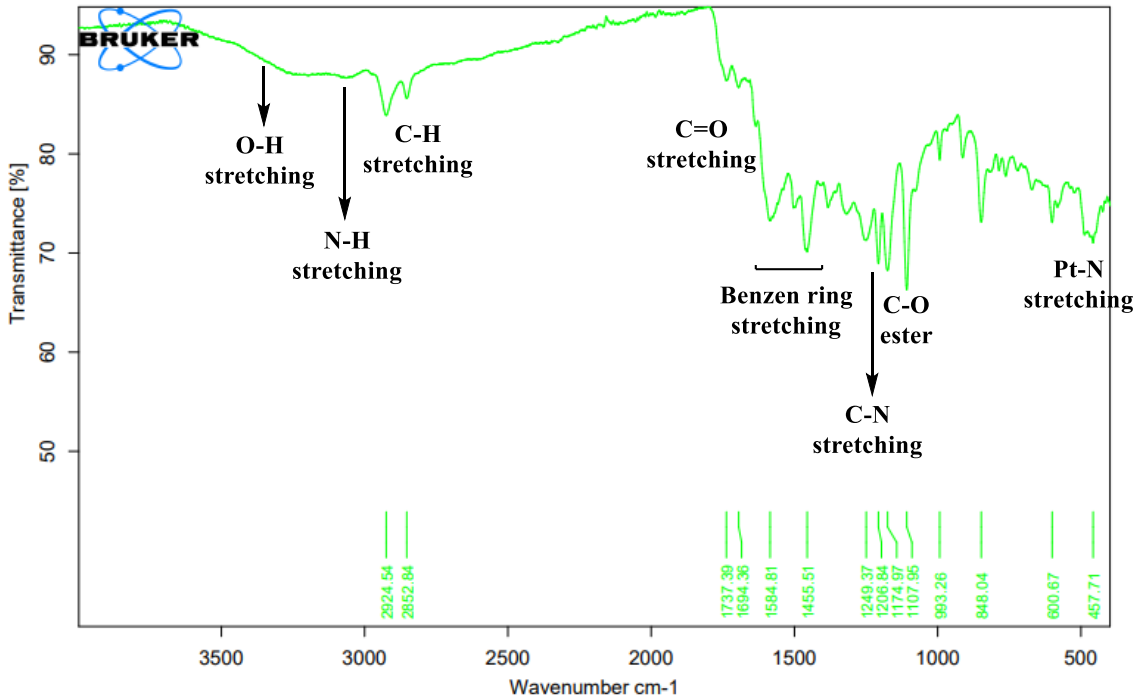


Figure 3.28. Fourier transform infrared spectra of di(11-((Fluorescein-5-isothiocyanate)*amino)undecanoic)*(*cis*,*trans*,*cis*-[$Pten(Cl)_2$]) **(37)**

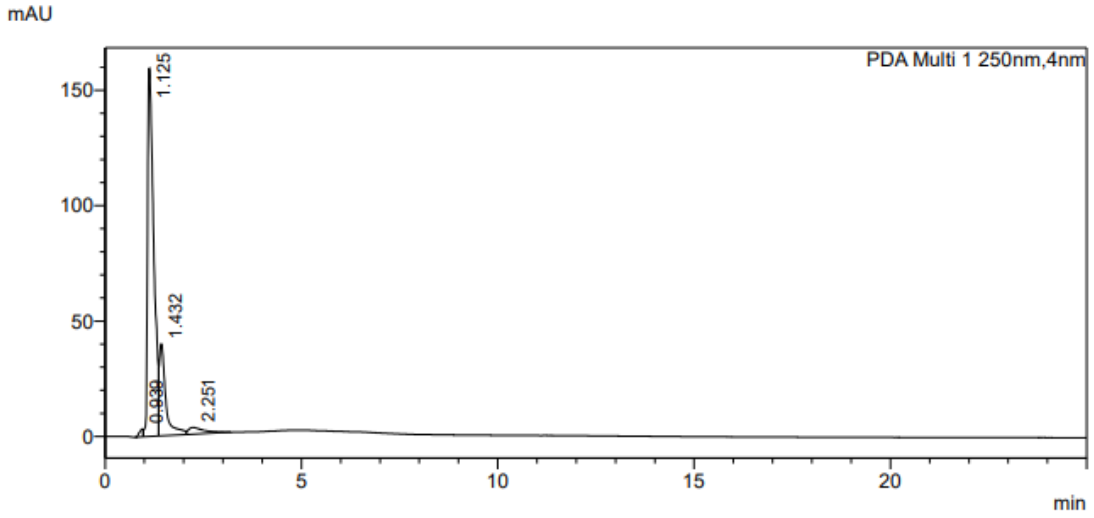


Figure 3.29. Ultra-performance liquid chromatography (UPLC) of di(11-((Fluorescein-5-isothiocyanate)*amino)undecanoic)*(*cis*,*trans*,*cis*-[Pten(Cl)$_2$)) **(37)** with retention time of 1.125.

Chapter Four

Conclusion and Future plans

4. Conclusion and future plans

4.1 Conclusion

This research project focuses on synthesizing fluorescent labeled platinum(IV) prodrugs to understand how Pt(IV) are processed at the cellular level and their molecular mechanisms of action. The compounds prepared in the current study were gone through two steps; first synthesizing of lipophilic fluorescent amino acids tags, and second step is coupling of these tags to Pt(IV) complexes. Several methods have been used to prepare the prodrugs, the most useful one was the use of the uranium salt activating agent such HCTU which gave a reasonable yield compared to other methods like EDC and acyl halides. The synthesized compounds were purified using chromatography techniques, and characterized by (1H-NMR, 13C-NMR, FT-IR, UPLC spectroscopy and ESI-MS spectrometry). Stability study was done for prodrug **(35)** and showed stability in 7.4 sodium monophosphate buffer at 37°C over 30 hrs.

4.2 Future plans

To improve the tissue localization and enhance the cellular uptake of platinum-based anticancer agents, and to diminish the undesired side effects of platinum(II) drugs fusogenic liposomes will be employed. Furthermore, to make possible studying the penetration, localization, distribution and speciation of the Pt-liposomal formulation fluorescent labelled platinum(IV) derivatives will be developed. The Pt(IV) synthesized prodrugs will be encapsulated into fusogenic liposomes (FLs). The FL-formulation will be characterized using physical methods and the encapsulated Pt(IV) will be characterized using Raman microscopy, fluorescence microscopy. In addition, the impact of FLs on the activity of Pt(IV) derivatives will be assessed using biological essays and *in vitro*

assessment of the biological activity of Pt(IV) loaded fusogenic liposoemes against cancer cells will be studied. A wide range of biophysical techniques will be employed such as (differential scanning calorimetry (DSC), transmission electron microscopy combined with freeze fracture (FF-TEM), dynamic light scattering measurements, small angle X-ray scattering (SAXS), Fourier transform infrared spectroscopy (FTIR) and fluorescence microscopy for characterizing. The fluorescent axial ligands were designed to comprehend properties that maximize encapsulation of the Pt(IV) derivative.

Chapter Five

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5. References

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تصنيع، دراسة الخصائص البيوفيزيائية لطلائع البلاتين (IV) الفلوروسنتية كسوابق الادوية وتحميلها بواسطة جسيمات شحمية كناقلات نانونية الى الخلايا السرطانية

اعداد الطالبة: زينب بريجية

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الملخص

على الرغم من الانتشار الواسع لعلاج السرطان، لا يزال المرضى يعانون من آثار جانبية خطيرة. يتم استخدام مركبات الجيل الثاني من البلاتين مثل سيسبلاتين وأوكساليبلاتين وكاربوبلاتين لعلاج عدة أنواع من السرطان ولكن بسبب السمية العامة والمقاومة الكيميائية الجوهرية/المكتسبة، يتم تقييد تطبيقها العلاجي. من ناحية أخرى، فإن الجيل الرابع من مركبات البلاتين ذات الروابط المحورية الإضافية، هي عبارة عن شكل خامل أكثر استقرارًا من مركبات الجيل الثاني مع آثار جانبية أقل ولديها القدرة على تسليم الشكل الفعال وهو الجيل الثاني إلى الخلايا السرطانية عن طريق ربط مركبات دهنية او مركبات تستهدف الخلايا السرطانية إلى الروابط المحورية. تقدم عدد قليل من المركبات البلاتينية الجيل الرابع إلى التجارب السريرية، لذلك، يجب استخدام العديد من الآليات مثل التصوير الفلوري وغيرها لفهم آلية عملها داخل الخلية وذلك عن طريق اقتران مركبات مضيئة دهنية إلى الروابط المحورية الجديدة. في هذا المشروع، تم تصنيع سلسلة من طلائع المركبات البلاتينية المضيئة حيث تم ربط المركبات البلاتينية باحماض أمينية دهنية مضيئة طويلة.

تم تشخيص طلائع الأحماض الأمينية الدهنية المضيئة عن طريق، نقطة الانصهار، التحليل الطيفي للأشعة تحت الحمراء، والرنين المغناطيسي النووي، وكروماتوجرافيا سائلة فائقة الأداء، وتقنيات قياس الطيف الكتلي للتأين بالرش الكهربائي للتأكد من المركبات المصنعة. وتم دراسة هذه الطلائع داخل المختبر باستخدام كروماتوجرافيا سائلة فائقة الأداء على درجة حرارة ثابتة (37 درجة مئوية) ودرجة حموضة 7.4 مشابهة لدرجة حموضة الدم.

عمادة الدراسات العليا

جامعة القدس

تصنيع، دراسة الخصائص البيوفيزيائية لطلائع البلاتين (IV) الفلوروسنتية كسوابق الادوية وتحميلها بواسطة جسيمات شحمية كناقلات نانونية الى الخلايا السرطانية

زينب بريجية

رسالة ماجستير

القدس، فلسطين

١٤٤٤/٢٠٢٢

تصنيع، دراسة الخصائص البيوفيزيائية لطلائع البلاتين (IV) الفلوروسنتية كسوابق الادوية وتحميلها بواسطة جسيمات شحمية كناقلات نانونية الى الخلايا السرطانية

اعداد

زينب بريجية

بكالوريوس صيدلة- جامعة القدس، فلسطين

اشراف الدكتور

يوسف نجاجرة

قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في العلوم الصيدلانية من كلية الدراسات العليا جامعة القدس- فلسطين.

١٤٤٤/٢٠٢٢