EU GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

EGE UNIVERSITY

MASTER'S THESIS

SYNTHESIS OF BIMETALLIC COMPLEXES AND INVESTIGATION OF THEIR ANTICANCER AND CATALYTIC PROPERTIES

Hatice Dilayla ALTIOK

Supervisor: Prof. Dr. Hayati TÜRKMEN

Chemistry Department

Presantation Date: 09.01.2018

Bornova- İZMİR

2018

EGE UNIVERSITY INSTITUTE OF NATURAL AND APPLIED SCIENCES

(MSc THESIS)

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Hatice Dilayla ALTIOK tarafından yüksek lisans tezi olarak sunulan "***Synthesis of bimetallic complexes and investigation of their anticancer and catalytic properties***" başlıklı bu çalışma EÜ Lisansüstü Eğitim ve Öğretim Yönetmeliği ile EÜ Fen Bilimleri Enstitüsü Eğitim ve Öğretim Yönergesi'nin ilgili hükümleri uyarınca tarafımızdan değerlendirilerek savunmaya değer bulunmuş ve 09.01.2018 tarihinde yapılan tez savunma sınavında aday oybirliği/~~oyçokluğu~~ ile başarılı bulunmuştur.

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09 / 01/ 2018

Hatice Dilayla ALTIOK

ÖZET

BİMETALİK KOMPLEKSLERİN SENTEZLENMESİ, ANTİKANSER VE KATALİTİK ÖZELLİKLERİNİN İNCELENMESİ

Hatice Dilayla ALTIOK

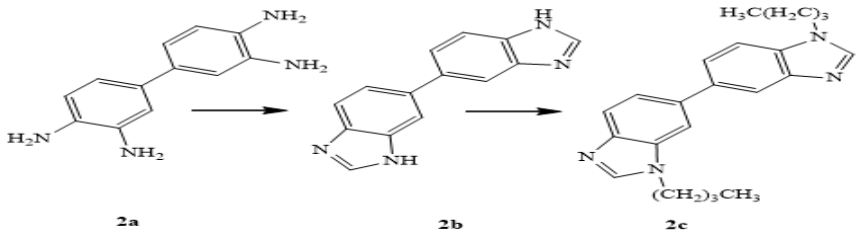
Yüksek Lisans Tezi, Kimya Anabilim Dalı
Tez Danışmanı: Prof. Dr. Hayati TÜRKMEN
Ocak 2018, 59 sayfa

Bu çalışma, 3,3′-Diaminobenzidinden türeyen ligandarın ve onların rutenyum komplekslerinin sentezlenmesini içermektedir. Literatürde bulunan monometalik kompleksler sentezlenip bu komplekslerle karşılaştırılmıştır.

Tez üç bölümden oluşmaktadır. Birinci bölümde metal komplekslerinin biyolojik aktiviteleri, bimetalik sistemlerin monometalik sistemlerle karşılaştırılması ve rutenyum komplekslerinin literatürdeki bazı örneklerine yer verilmiştir. İkinci bölümde deneysel veriler sunulmuştur. Üçüncü bölümde ise, sentezlenen komplekslerin karakterizasyonu, katalitik aktiviteleri ve antikanser özellikleri verilmiştir.

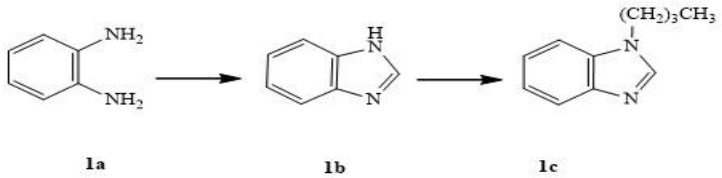
İlk olarak 3,3′-diaminobenzidin (**2a**) bileşiğinin trietilortoformat ile yüksek sıcaklıktaki reaksiyonu 3,3’-bibenzimidazol (**2b)** sentezlenmiştir. **2b** bileşiğinin bütil iyodürle etkileştirilmesi sonucunda 3,3’-dibutil-bibenzimidazol (**2c)** bileşiği sentezlenmiştir. (Şema 1). Bimetalik sistemleri monometalik olanlarla karşılaştırmak amacıyla o-fenilendiamin(**1a**), benzimidazol (**1b**), 1-bütil-1H-benzimidazol (**1c**) kullanımıştır. **1c** bileşiği **2c** ile aynı şekilde sentezlenmiştir. (Şema 2).

[Ru(*p*-simen)Cl_2]$_2$ ile ligandlar **(1a-1c, 2a-2c)** tepkimeye sokularak mono- ve bimetalik rutenyum kompleksleri elde edilmiştir. (Şema 3)



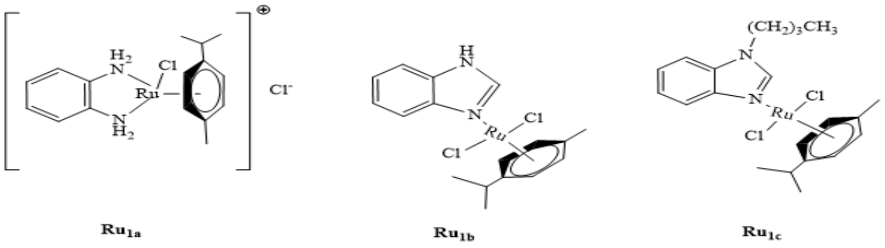
2a 2b 2c

Şema 1

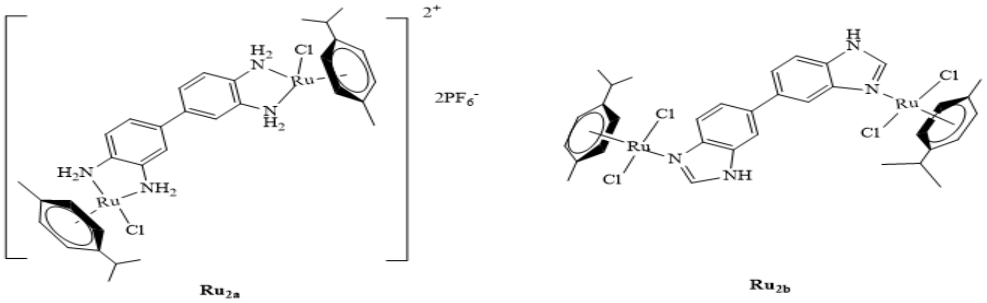


1a 1b 1c

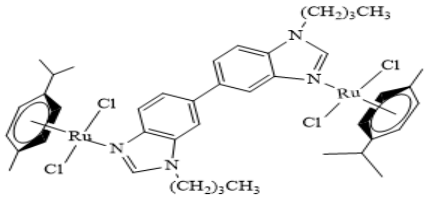
Şema 2



Ru_{1a} Ru_{1b} Ru_{1c}



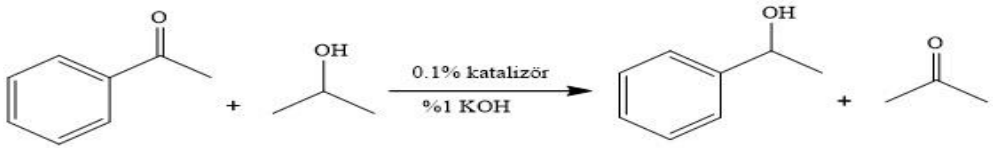
Ru_{2a} Ru_{2b}



Ru_{2c}

Şema 3

Mono- ve bimetalik komplekslerin **(Ru_{2a-2c}, Ru_{1a-1b})** asetofenonun transfer hidrojenasyonundaki katalitik aktiviteleri incelenmiştir.



(Ru_{2a-2b}, Ru_{1a}) komplekslerinin antikanser özellikleri LNCaP, PC-3, DU145, HeLa, MD-MBA, MCF-7, MDA-MB-231, HepG2 hücre hatlarında incelenmiştir.

Anahtar kelimeler: bimetalik rutenyum kompleksleri, transfer hidrojenasyon, antikanser özellikler.

ABSTRACT

SYNTHESIS OF BIMETALLIC COMPLEXES AND INVESTIGATION OF THEIR ANTICANCER AND CATALYTIC PROPERTIES

ALTIOK, Hatice Dilayla

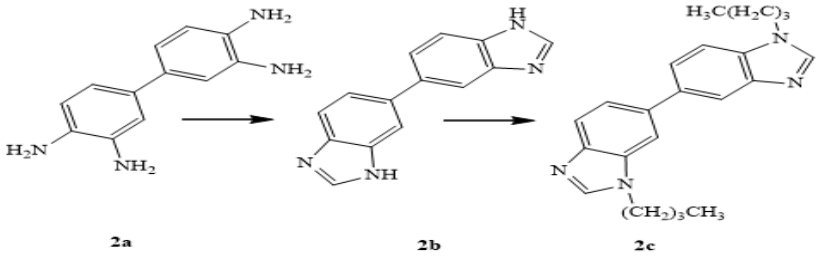
MSc in Chemistry
Supervisor: Prof. Dr. Hayati TÜRKMEN
January 2018, 59 pages

This study includes synthesis of ligands derivated from 3,3′-diaminobenzidine and their ruthenium complexes. The monometallic complexes and their ligands, reported in literature before, were prepared for comparision to bimetallic complexes.

This thesis consist of three part. The first part consist of biological activities of metal complexes, comparasion of bimetallic complexes to monometallic complexes and example of ruthenium complexes that is reported by some studies. In the second part, the experimental data were reported. In the third part, characterizaton, catalytic and anticancer properties of synthesized complexes were presented.

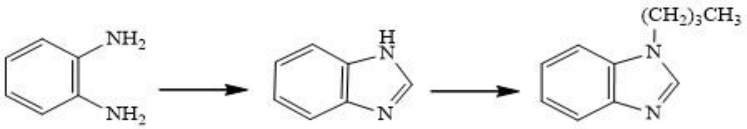
Firstly, 3,3’-bibenzimidazole **(2b)** synthesized from the reaction of 3,3′-diaminobenzidine **(2a**) with triethylorthoformate at high temperature. 3,3’-dibutyl-bibenzimidazole **(2c)** were synthesized from the reaction of **2b** compound with butyl iodide (Scheme 1). O-phenylenediamine **(1a)**, benzimidazole (**1b**), 1-butyl-1H-benzimidazole (**1c**) were used for comparision of monometallic systems to bimetallic systems. **1c** compound were prepared with **2c** same manner (Scheme 2).

Mono- and bimetallic ruthenium complexes were obtained by the reaction of Ru(*p*-cymene)$Cl_2]_2$ with ligands **(1a-1c, 2a-2c)** (Scheme 3).



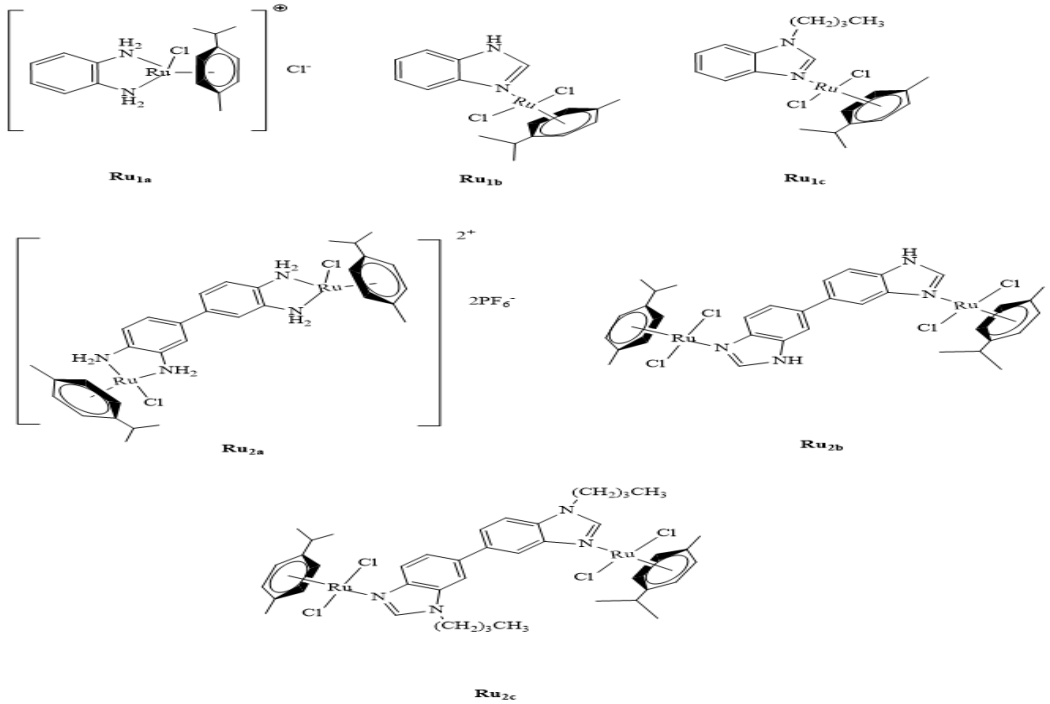
2a 2b 2c

Scheme 1



1a 1b 1c

Scheme 2



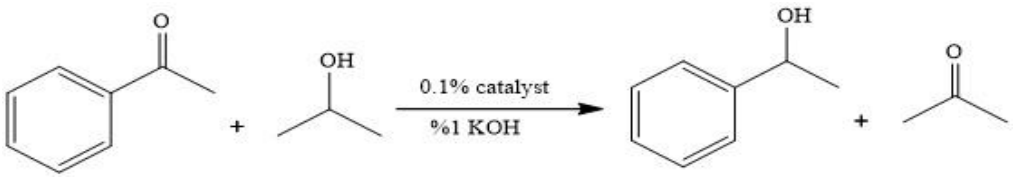
Ru_{1a} Ru_{1b} Ru_{1c}

Ru_{2a} Ru_{2b}

Ru_{2c}

Scheme 3

The catalytic activities of mono- and bimetallic complexes **(Ru_{2a-2c}, Ru_{1a-1b})** in the transfer hydrogenation of acetophenone were tested.



The anticancer activities of complexes **(Ru_{2a-2b}, Ru_{1a})** were tested in LNCaP, PC-3, DU145, HeLa, MD-MBA, MCF-7, MDA-MB-231, HepG2 cell lines.

Keywords: bimetallic ruthenium complexes, transfer hydrogenation, anticancer properties.

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ABBREVIATIONS

Abbreviations		Explanations
Ar	:	Aryl
^{13}C-NMR	:	Carbon Nuclear Magnetic Resonance
^{1}H-NMR	:	Proton Nuclear Magnetic Resonance
Bzm	:	Benzimidazole
Cat.	:	Catalyst
DAB	:	3,3′-diaminobenzidine
DCM	:	Dichloromethane
DMF	:	*N*,*N*-Dimethylformamide
DMSO	:	Dimethylsulfoxide
Hz	:	Hertz
IPA	:	Isopropyl alcohol
L	:	Ligand
NMR	:	Nuclear Magnetic Resonance
η	:	Hapticity
δ	:	Delta
s	:	Singlet
d	:	Dublet
t	:	Triplet
m	:	Multiplets

1. INTRODUCTION

1.1 Biological Activity of Metal Complexes

Metal complexes play important role as chemotherapeutic agents. The excellent properties of the metal ions and ligands provide good activity for the medicine chemistry (Pieter and Sadler 2008). They have excellent properties such as charge variation, structure and bonding, metal–ligand interaction, lewis acid properties, partially filled d shell, redox activity (Ndagi et al., 2017). Metals form positively charged ions in aqueous solution which is provide ability to bind negatively charged biological molecules (Frezza et al., 2010). The groups that are coordinated to most metals can be polarized by this metal ions easily and their hydrolysis occurs easier (Haas and Franz 2009 ; Frezza et al., 2010).

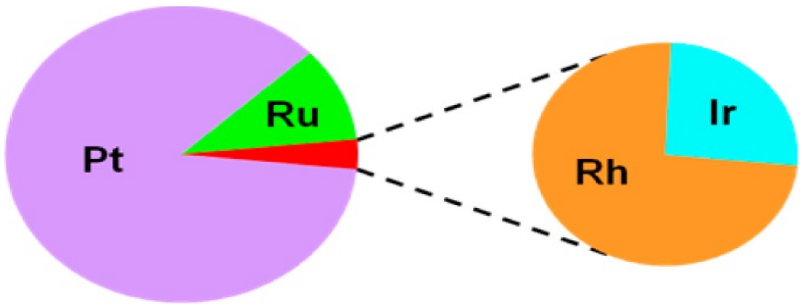
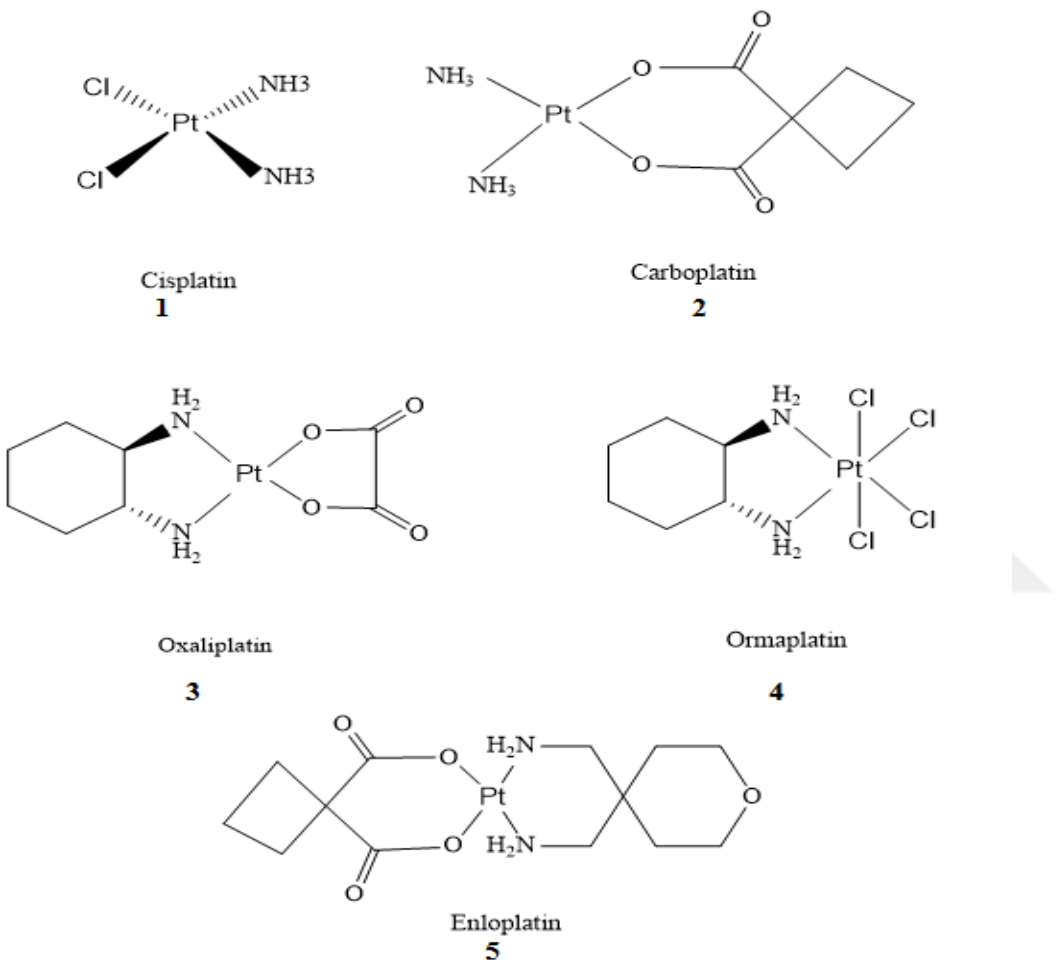


Figure 1.1 Number of publications on Pt group metals anticancer complexes (Liu and Sadler 2014)

1.2 Platinum Complexes

Cisplatin is the first known metal complex for cancer treatment. The antineoplastic properties of cisplatin was discovered by Rosenberg for demolition of Escherichia coli growth. *cis*-$[Pt(NH_3)_2Cl_2]$ was found to be active for sarcoma 180 and leukemia L1210 in mice (Johnstone et al., 2014).

Since the discovered of cisplatin, several thousand derivatives have been synthesized and their properties were studied. There are approximately 13 counterparts of cisplatin and they have been investigated but carboplatin was found to be most active one (Dasari and Tchounwou 2014). Scheme 1.1 shows cisplatin and its derivatives.



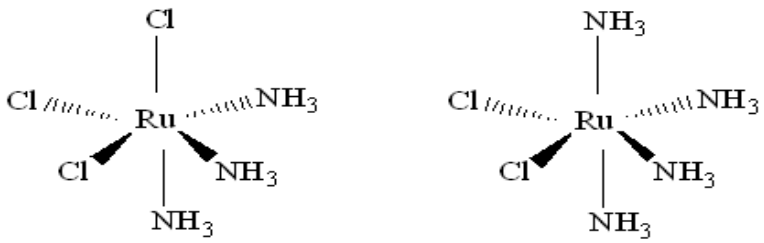
Scheme 1.1 Structures of cisplatin derivatives

For many years platin complexes have been used for cancer researches but they have some adverse effects. Firstly, toxicity is the one of the problem. Cisplatin influence both sick and healthy cells without discrimination. Tumor cells become resistant and its efficacy decreases after several chemotherapy cycles. The limitations of platinum-based drugs, dose-dependent side effects have boosted the researchers for finding other metal-based drugs (Medici et al., 2014).

1.3 Ruthenium Complexes

In the development of metal based drugs ruthenium has some advantages. Its coordination chemistry provides routes to new compounds. Ligand exchange rate of ruthenium similar to platinum complexes or can be adjusted by ligands. It can reach several oxidation states such as +2, +3 and +4 and able to tune the electron transfer rates and redox potentials. They are less toxic than platinum drug because of the able to imitate iron and binding to biomolecules (Jakupec et al., 2007).

Chloro-ammine complexes were the first ruthenium compounds that were used for cancer treatment. In 1976, ruthenium(III) complex *fac*-$Ru(NH_3)_3Cl_3$ **(6)** was used by Durig et al. to decreased filamentous growth of E. coli cells. In 1980, Clarke evaluated anticancer properties of *cis*-$Ru(NH_3)_4Cl_2$ ruthenium(II) complex **(7)**. Despite good activity of this complexes, their solubility was not enough for pharmaceutical use (Scheme 1.2)(Fink, 2009).



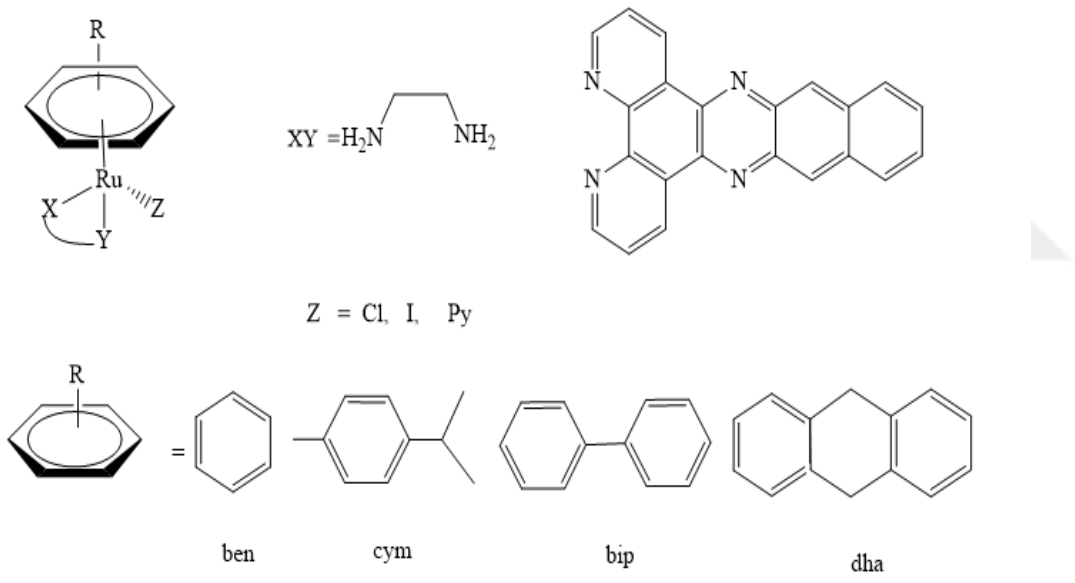
6 **7**

Scheme 1.2 Chloro ammine complexes synthesized by Durig and Clarke

Different Ru(II) and Ru(III) complexes showed anticancer activity against some of cancer type especially metastatic cancer. Clinical trial of Ru(III) complex NAMI-A **(8)** was successful on metastatic lung cancer and investigated by Sava et al, as well as KP1019 **(9)** showed activity against colorectal tumor model and several human tumors (Montel et al., 2017).

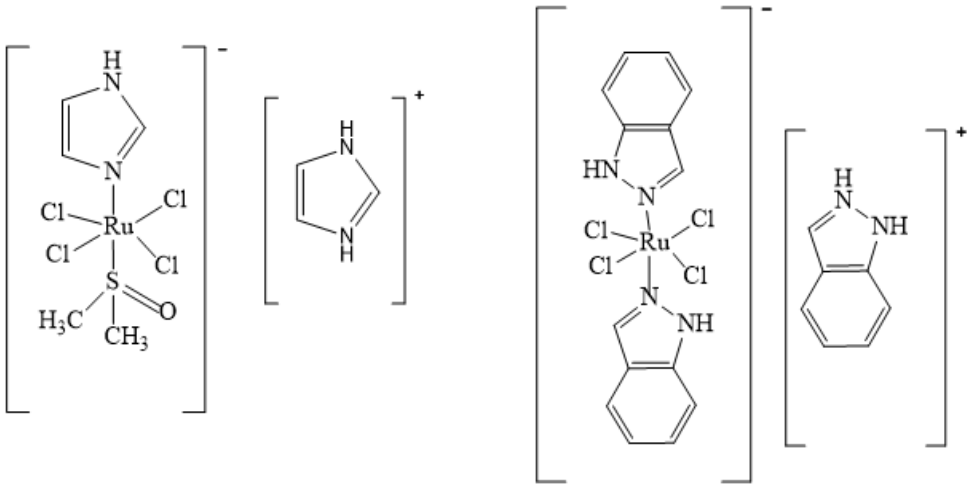
1.3.1 Ruthenium Arene Complexes

Arene ruthenium(II) complexes, also referred as piano stool complexes, are shown in scheme 1.3. The anticancer activity of these complexes good because they are soluble in water (Zeng et al., 2017).



Scheme 1.3 Ru (II) arene complexes

Dyson and Sadler lead to use of ruthenium complexes in treatment. The first use of arene ruthenium compounds as anticancer agents had been introduced by Tocher et al. in 1992. In 2001, RAPTA-C and some counterparts reported by Dyson and Sadler (Scheme 1.4 ; Scheme 1.5) (Fink 2009).



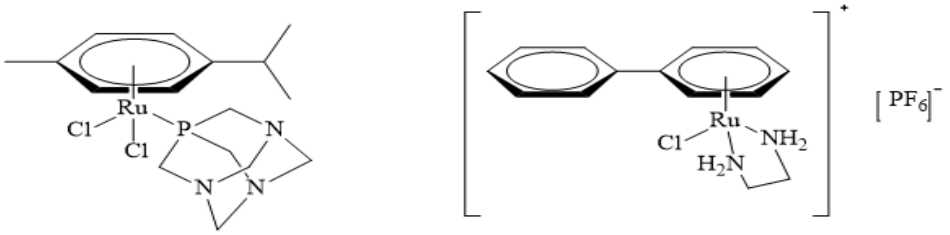
NAMI- A

8

KP1019

9

Scheme 1.4 Ruthenium (III) complexes for cancer treatment.



RAPTA-C

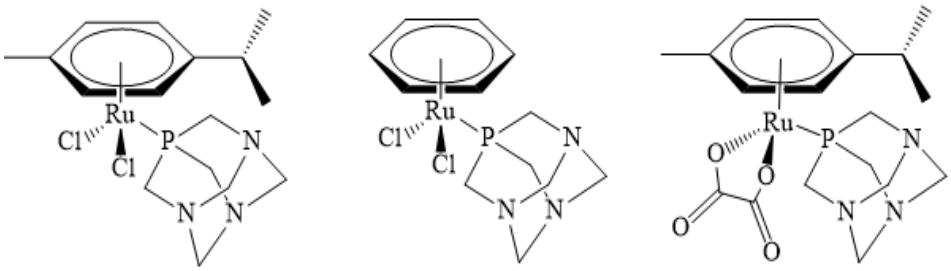
11

12

Scheme 1.5 Complexes reported by Dyson and by Sadler.

The preclinical studies showed that NAMI-A have antitumor activitiy. Smaller dosages of NAMI-A showed more significant antimetastatic activity.

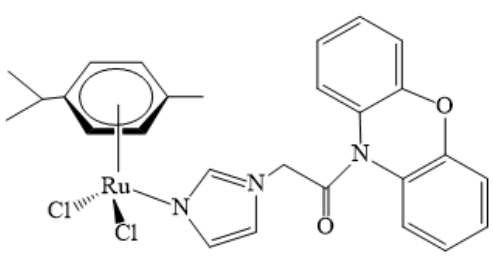
Contrary to NAMI-A, KP1019 have cytotoxic activity which include primary explanted human tumors (Antonarakis and Emadi 2010). RAPTA complexes show low toxicity in vitro, but in vivo studies of RAPTA-C **(13)** and RAPTA-B **(14)** complexes showed great activity to inhibit metastasis growth and high selectivity and extremely low general toxicity. In contrast, the chloride ligands can be substituted for a bidentate oxalate ligand with very little impact on cytotoxicity, although the rate of reaction with biomolecules is reduced (Scheme 1.6) (Renfrew et al., 2009).



13 **14** **15**

Scheme 1.6 RAPTA-C **(13)**, RAPTA-B **(14)**, and oxaliRAPTA-C **(15)**

Imidazole based Ru(II) arene complexes, synthesized by Vock et al., have been showed in scheme 1.7. Among these complexes (**16-18), 18** was the most cytotoxic and **16** was the least cytotoxic complex. **16** showed cytotoxic activity in HT29 (human colorectal adenocarcinoma) cells (Scheme 1.7) (Vock et al. 2007).



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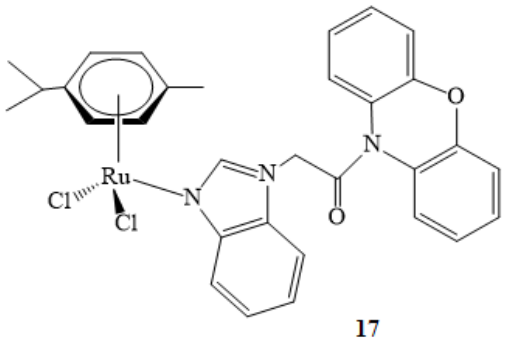

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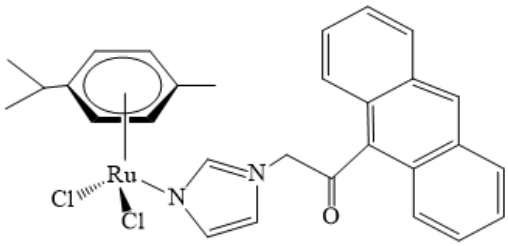

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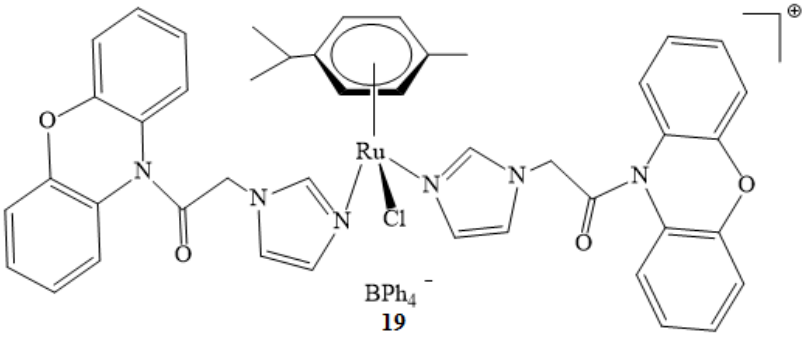

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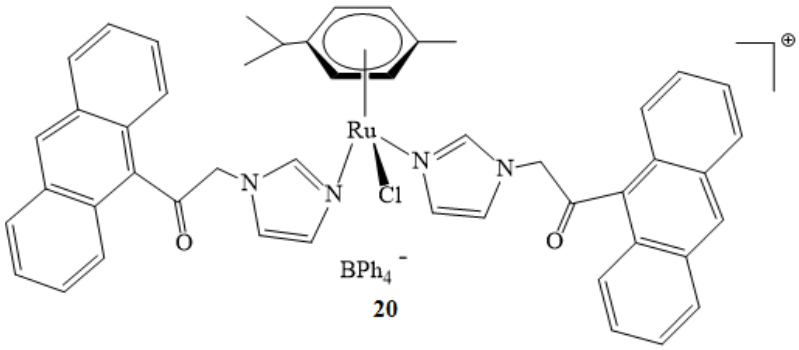

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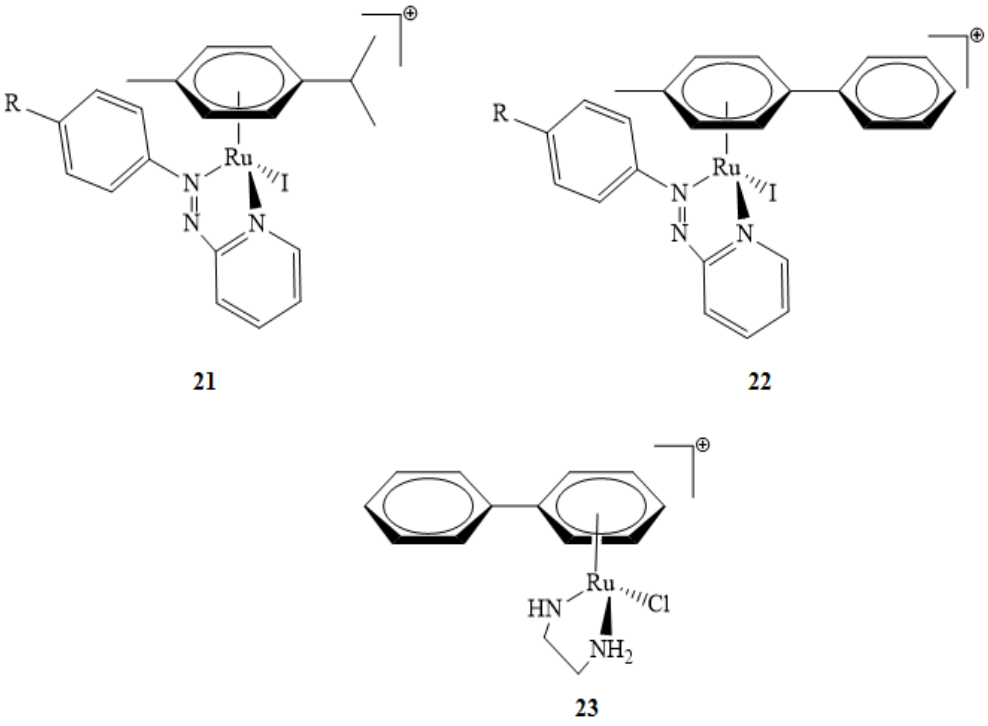
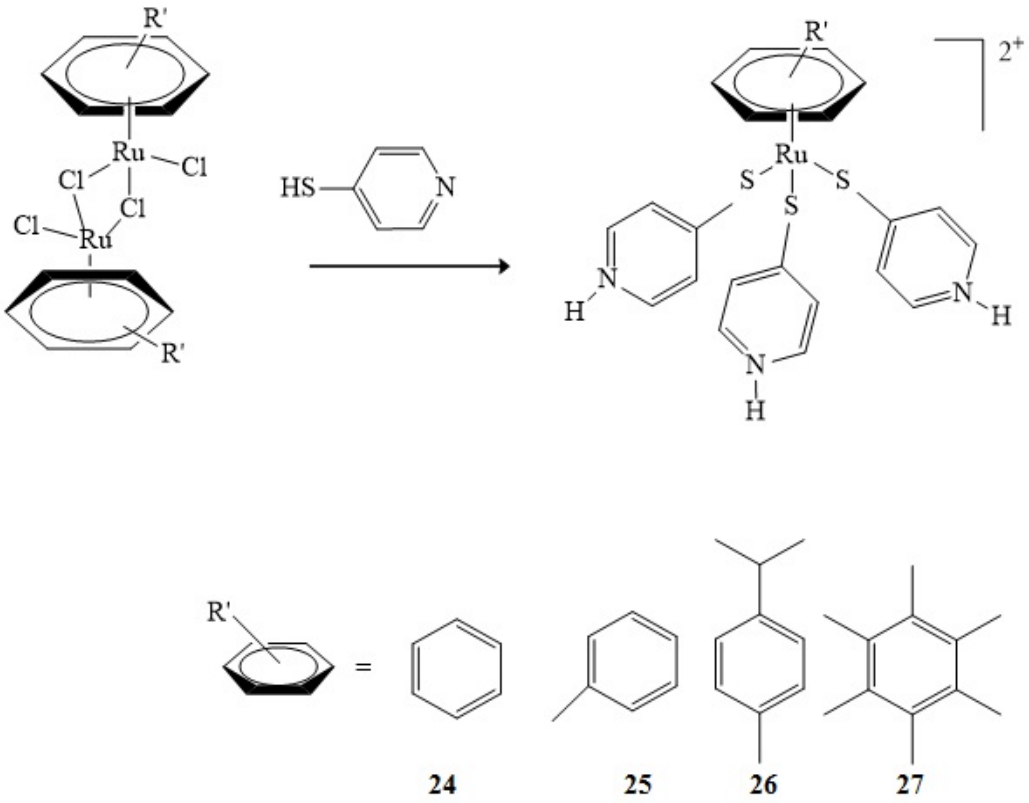
Scheme 1.7 Complexes syntesized by Vock et al.

Ru(II) arene derivatives that contain iodine **(21, 22)** were found cytotoxic to A2780 (human ovarian) and A549 (human lung) cancer cell lines. These ruthenium complexes also found catalytically active for the oxidation of the tripeptide glutathione (GSH). The complex $[\eta^6$-(bip)Ru(en)Cl$]^+$ **23** (Scheme 1.8) easily reacts with GSH (Gasser et al., 2011).



Scheme 1.8 Organometallic anticancer complexes

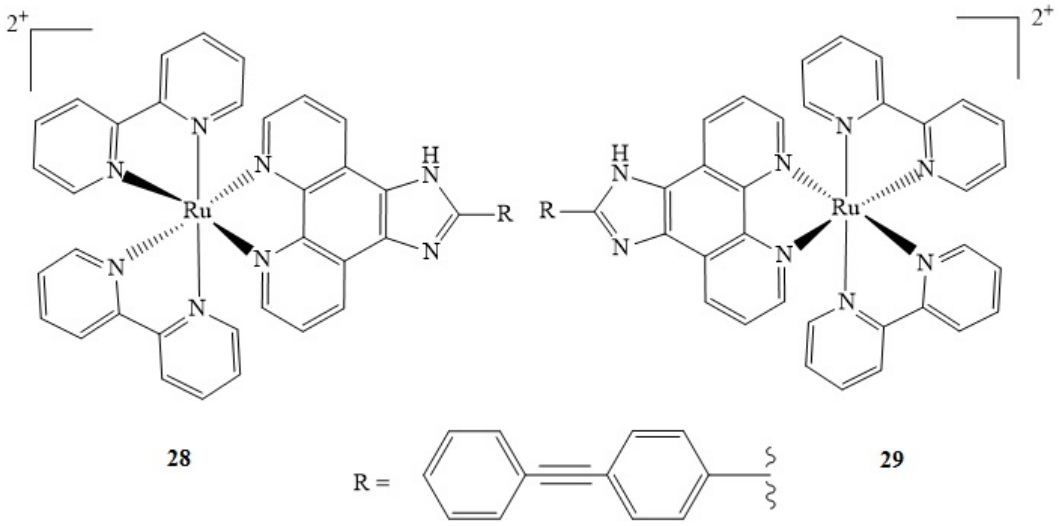
Gras et al. reported the synthesis of tris–thiolato-bridged complexes **(24-27)** (Scheme 1.9). Their cytotoxicity investigated towards A2780 ovarian cancer cells. **27** observed as the most cytotoxic one (Gras et al., 2008).



Scheme 1.9 Water soluble tris–thiolato-bridged complexes synthesized by Gras et al.

1.3.2 Polypyridyl Ru(II) Complexes

Zeng et al. syntesized chiral polypyridyl Ru(II) complexes and reported that they affect anticancer activity by intercellular localization (Zeng et al., 2017).



Scheme 1.10 Chiral polypyridyl Ru(II) complexes synthesized by Zeng et al.

1.4 Tetradentate Ligand ; 3,3' Diamino Benzidine

3,3′-diaminobenzidine is an thermodynamically and chemically stable organic compound. Between ring structures it is symmetric (Karabacak et al., 2015). 3,3′-Diaminobenzidine have toxicity and carcinogenic properties (Pozo et al., 2003). In the presence of hemoglobin, DAB is oxidized by hydrogen peroxide and gives dark brown color insoluble product which provides detection of fingerprints in blood (Sahs 1992).

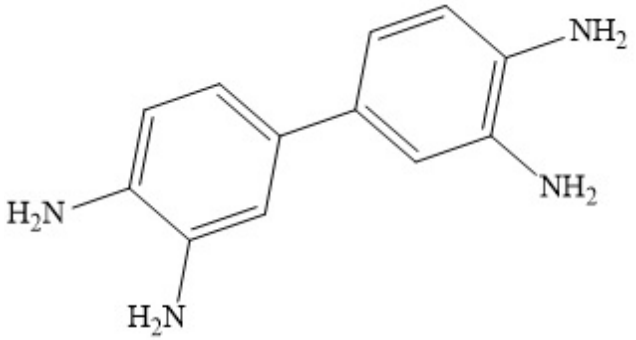


Figure 1.2 Structure of 3,3′-Diaminobenzidine

1.5 Bimetallic Complexes

The synthesis of bimetallic complexes is interest to many different areas of chemistry. This complexes allowed scientists to discover the coordination environments of various metal centers at biologically active sites, also have significant benefit to the fields of catalysis, activation of organic and inorganic molecules, solar energy harvesting and general coordination chemistry (McCready and Matthew 2015).

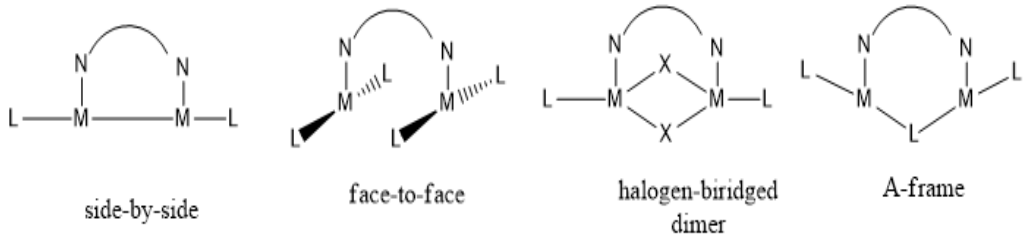
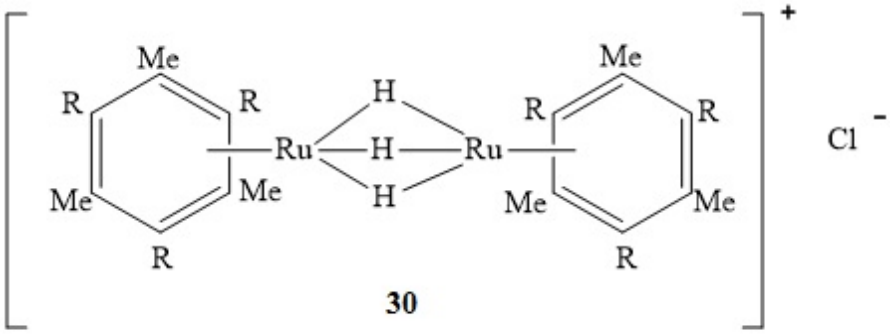


Figure 1.3 Typical structural architectures for bimetallic complexes

Bennett et al. synthesized bimetallic ruthenium(II) arene hydrido complexes and this complex was found stable and active catalyst for hydrogenation of arenes to cyclohexanes (Bennett et al., 1979).

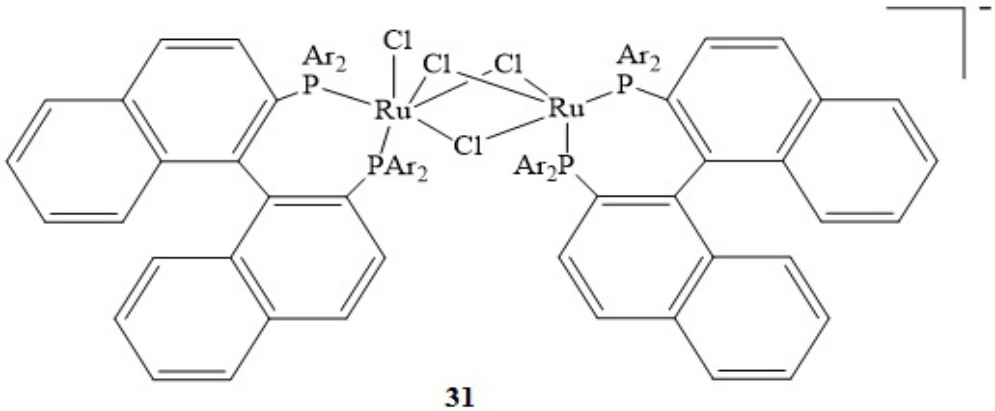


30

R = Me

Scheme 1.11 Bimetallic arene hydrido complexes of ruthenium(II)

Bimetallic BINAP ruthenium(II) complexes(Scheme 1.12) that were reported Ohta et al., were found catalytically active for the asymmetric hydrogenation of ketonic and olefinic substrates (Ohta et al., 1995).

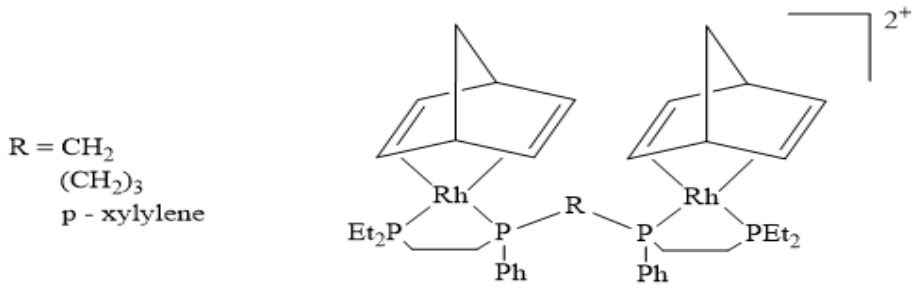


31

anion of (R) - 2 , Ar = p -MeO-C_6H_4

Scheme 1.12 Bimetallic BINAP ruthenium(II) complex

Stanley et al. used Rh(I) phospine complexes (Scheme 1.13) as catalyst for hydroformylation of alkenes. There were a difference in efficiency when two Rh(I) centers were attached via anorganic scaffold and this complex react up to 600 times faster than the closest monometallic counterparts (Timerbulatova et al., 2013).



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Scheme 1.13 Bimetallic Rh(I) phospine complexes

1.6 Catalytic Hydrogenation

Alkenes and alkynes are unsaturated hydrocarbons. A common addition reaction is hydrogenation in which hydrogen is added to each carbon in a double bond (Myers 2003).

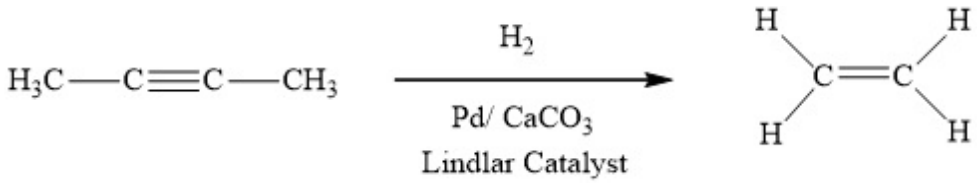
Scheme 1.14 Catalytic Hydrogenation Reaction

There are two types of catalysts according to the reaction phase that they occupy: homogeneous and heterogeneous. Homogeneous catalysts are in same phase with the reaction mixture (typically liquid or gas),while heterogeneous catalysts are in different phase. Generally, heterogeneous catalysts are solid compounds that are mixed with liquid or gas reaction mixtures (Chen 2014).

1.6.1 Heterogeneous Catalysis

Heterogeneous catalysis is a cyclic process which consists of three main steps: adsorption of the substrate on the catalyst, the catalytic reaction, and desorption of the products. After desorption, the catalyst is found in the same state as before adsorption and ready to help the next catalytic transformation (Roduner 2014).

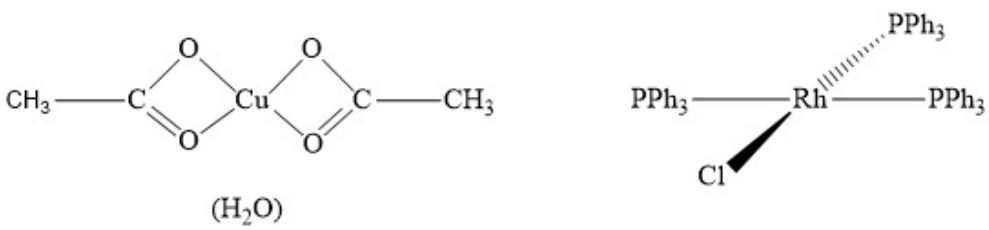
Raney nickel, palladium or platinum catalysts are the catalyst which is used for the heterogeneous catalysis (Blaser et al.; 1997). For this catalyst, the lead is used as an additive for reduction of alkynes to *cis*-alkenes (Chen 2014).



Scheme 1.15 Catalytic hydrogenation of alkynes by Lindlar Catalyst

1.6.2 Homogeneous Catalysis

The first example of homogeneous hydrogenation by metal compounds was reported by Calvin in 1938. The quinoline solutions of copper acetate reported capable of hydrogenating unsaturated substrates such as p-benzoquinone (Calvin, 1938, Calvin, 1939). Wilkinson discovered the $RhCl(PPh_3)_3$ complex (also known as Wilkinson's catalyst) which was the first very active homogeneous hydrogenation catalyst when compared with other heterogeneous catalysts (Wilkinson 1966).



Scheme 1.16 Structures of Copper Acetate and Wilkinson's Catalysis

Shrock and Osborn prepared $[Rh(dien)Ln]^+A^-$ type cationic complexes which is active catalyst homogenous hydrogenation (Shrock and Osborn 1976).

In 1977, Crabtree and colleauges developed more active catalytic system by changing solvent system, changing cordinating system with polar and noncoordinating system with CH_2Cl_2, for olefin hydrogenation. This Iridium complex known as Crabtree catalysis and most active among iridium complexes (Verendel et al., 2014).

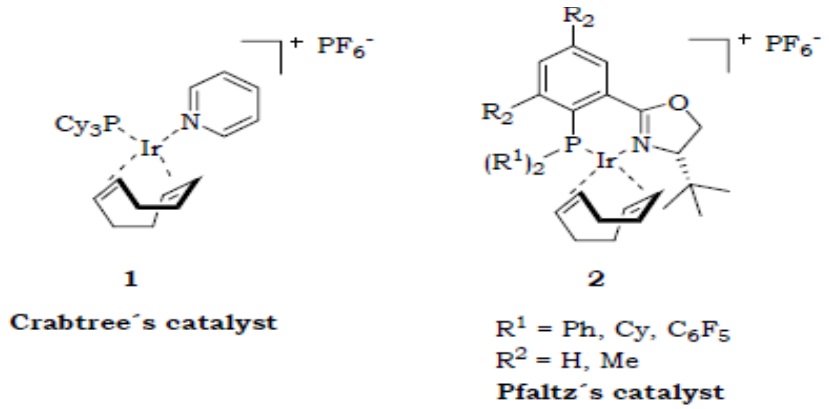
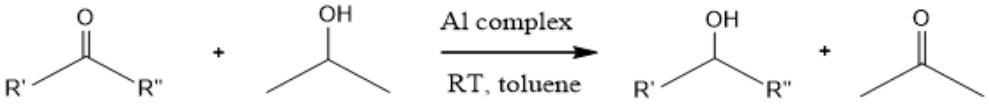


Figure 1.4 Crabtree's and Pfaltz's iridium catalysts (Church and Anderson 2008)

1.6.3 Transfer Hydrogenation

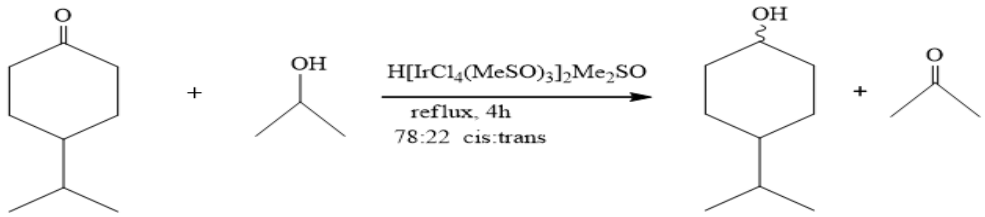
Transfer hydrogenation is obtaining reduction from unsaturated compound by addition of hydrogen to a molecule from other H_2 in the presence of catalyst and firstly demonstrated by Knoevenagel in 1903. Knoevenagel reported that dimethyl 1,4 - dihydroterephthalate disproportioned to dimethyl terephthalate and cis-hexahydroterephthalate (Knoevenagel 1903).

In the 1920s, reduction of aldehydes and ketones to alcohols by aluminium alkoxides obtained by using isopropanol (Scheme 1.17) (Meerwein and Schmidt, 1925; Verley, 1925; Ponndorf, 1926).



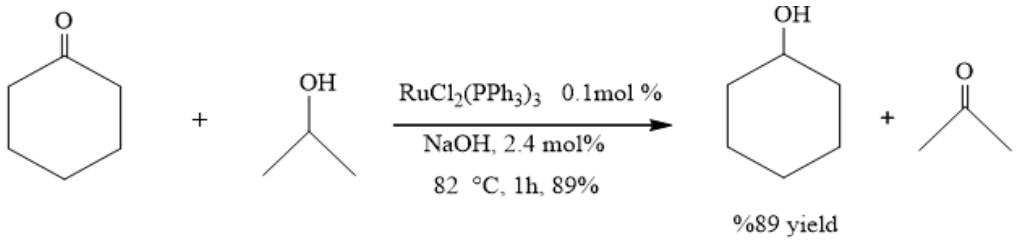
Scheme 1.17 Meerwein Ponndorf Verley Reaction

Henbest, Mitchell and co-workers reported first example of transition metal catalyst for the reduction of cyclohexanones to alcohols with isopropanol by using iridium hydride DMSO complex in 1964 (Scheme 1.18) (Haddad,et al., 1964). In the 1970s. Sasson and Blum found dichlorotris (triphenylphosphine) ruthenium(II) complex ([$RuCl_2(PPh_3)_3$]) as efficient for the transfer hydrogenation (Sasson and Blum, 1971).



Scheme 1.18 A complex for reduction of cyclohexanones

In the 1990s, Chowdhury and Backvall developed this work and they obtained increased catalytic activity for the transfer hydrogenation of aliphatic and aromatic ketones in the presence of a NaOH (Scheme 1.19)(Chowdhury and Backvall, 1991).

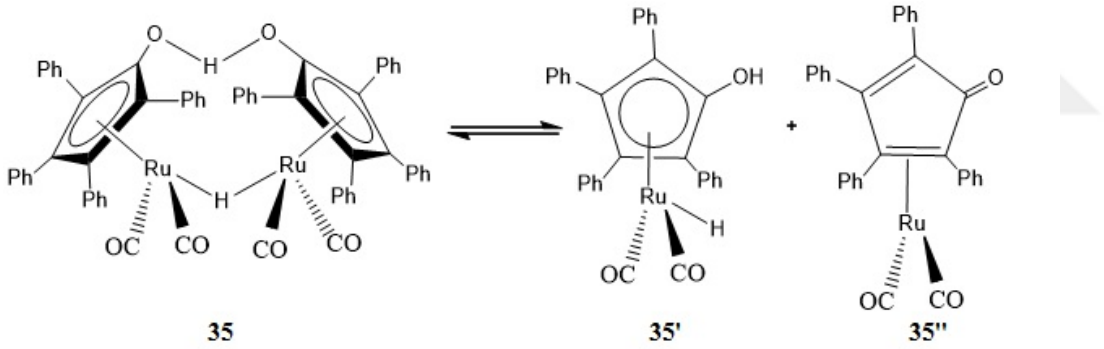


Scheme 1.19 $RuCl_2(PPh_3)_3$] complex for transfer hydrogenation reaction

1.6.3.1 Ruthenium Based Transfer Hydrogenation

Recently, ruthenium catalysts have been developed with various ligands and used in transfer hydrogenation reactions as a highly efficient, greener and economical catalytic systems in chemicals and pharmaceuticals.(Wang and Astruc 2015).

In 1986, Shvo's group reported the synthesis of the ruthenium complex **35**, This complex used in transfer hydrogenation reaction later and the bimetallic complex **29** dissociates upon heating into the active reducing form **35'** and the , highly reactive species **35"** (Scheme 1.20)(Shvo et al., 1986).

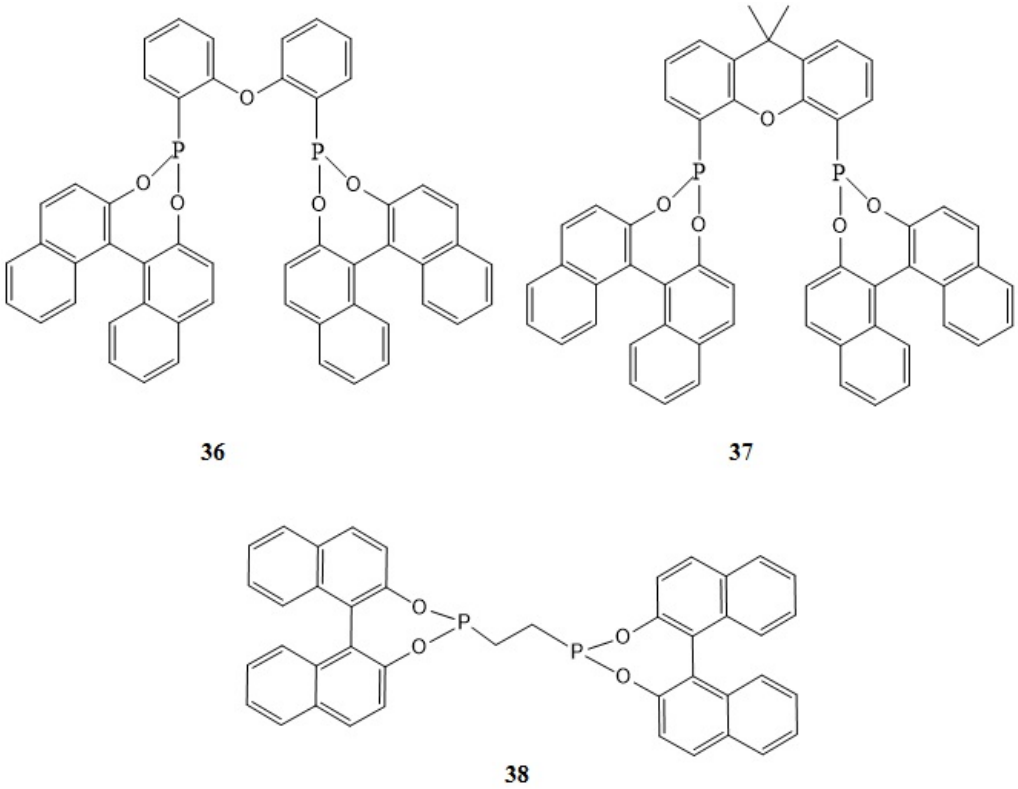


Scheme 1.20 Shvo's Ruthenium Complex

Noyori developed most successful catalyst Ru(II) catalysts based on complexes of monotosylated diamines for asymmetric transfer hydrogenation (Noyori and Hashiguchi 1997).

Reetz et al., syntesized catalyst with bidentate phosphorus ligands (Scheme 1.21). Despite their activity is better in enantioselective hydrogenation they worked better in asymmetric hydrogen transfer reaction when they bind to [Ru(*p*-

cymene)$Cl_2]_2$ (Reetz et al., 2006).



Scheme 1.21 Bidentate phosphorus donor ligands

Baratta et al., syntesized asymmetric transfer hydrogenation catalyst (Figure 1.5) by binding pyridine-derived ligand in a pincer-type to $[RuCl_2(PPh_3)_3]$ and obtained highly efficient catalyst (Baratta et al., 2010).

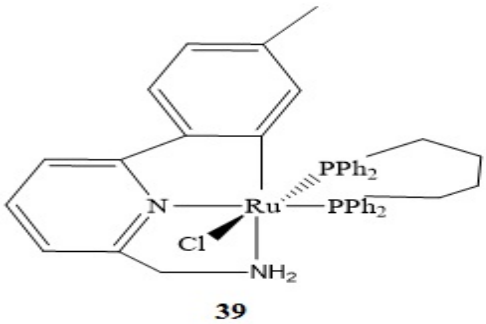
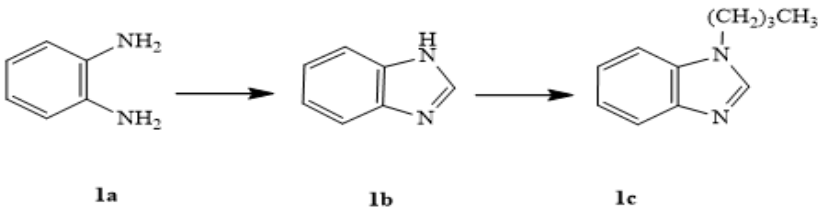


Figure 1.5 Pincer type Ruthenium complex $[RuCl(CNN)(Ph_2P(CH_2)_4PPh_2)]$

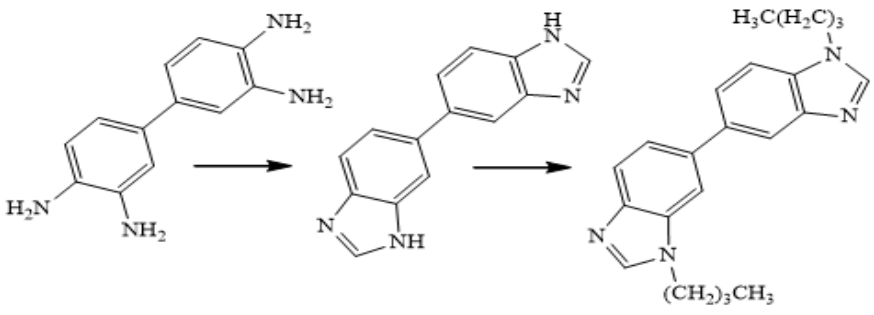
1.7 Aim of the Study

The goal of this thesis is to synthesis of bimetallic complexes and investigate their anticancer and catalytic properties. Recently, studies have shown that the electronic interactions between two metal atoms have affected the anticancer and catalytic properties. This electronic interactions between two metal atoms occur via π-conjugated bond systems; therefore they change the oxydation states of metals. In addition, ligand types affects complex stability and solubility. It is also aimed to prepare new pharmalogical compounds with monodendate or bidendate ligands that have different bonding properties and aromatic new system and to develope new systems and to specify significance of metal and ligand types in anticancer activity studies.

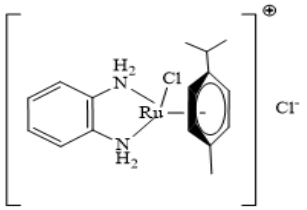
This thesis contains synthesis of mono- and bimetallic complexes. In this study ligands are 3-3' diaminobenzidine(**2a**) 3,3'-bibenzimidazole (**2b**), 3,3'-dibutyl-bibenzimidazole (**2c**), and o-phenylenediamine (**1a**), benzimidazole (**1b**) , 1-butyl-1H-benzimidazole (**1c**), with Ruthenium *p*-cymene. Ru_{2a}, Ru_{2b}, Ru_{2c} are the new compounds.



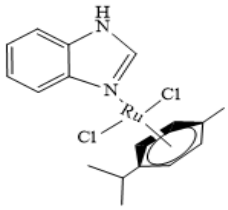
1a **1b** **1c**



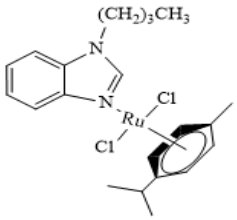
2a **2b** **2c**



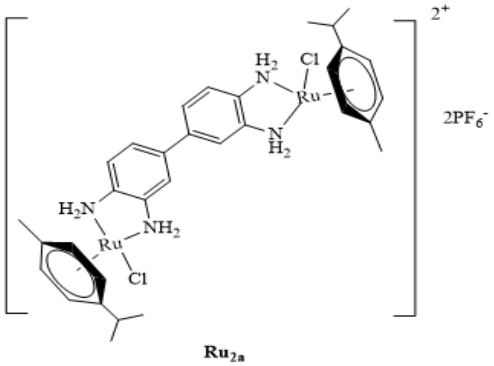
Ru_{1a}



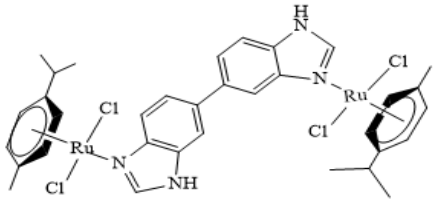
Ru_{1b}



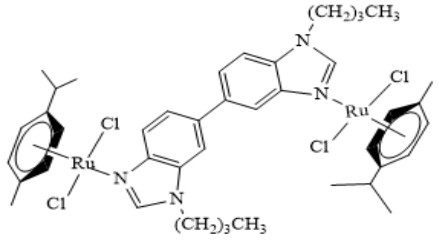
Ru_{1c}



Ru_{2a}



Ru_{2b}



Ru_{2c}

2. EXPERIMENTAL

2.1 General Methods

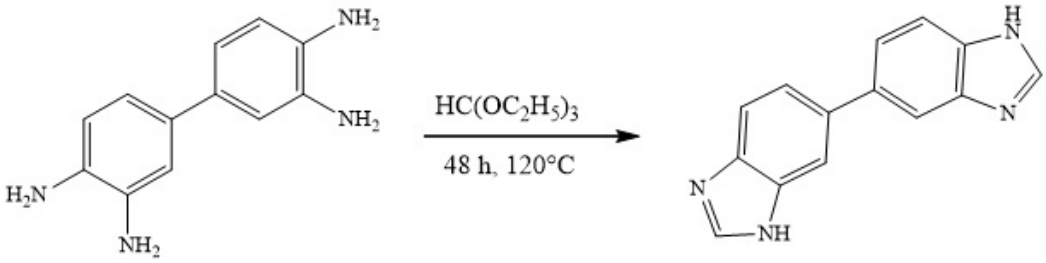
Air-sensitive reactions were carried out by using Schlenk-type flask under inert conditions and high vacuum-line techniques. The solvents were analytical grade and distilled under argon atmosphere from sodium (ethanol, methanol, toluene, diethylether,), P_2O_5 (dichloromethane).

Reagents: toluene, dichloromethane, and 3-3' diaminobenzidine (Merck), ethanol (Merck), diethylether, 2-propanol and methanol (J. T.Baker), $RuCl_3.3H_2O$ (Johnson and Mathey), a-phellandrene and 1-iodobutane (Alfa Aeser)were used as received. $[RuCl_2(p\text{-cymene})]_2$ were synthesized from procedures of [Bennett and Smith, 1974].

Instruments: ^{1}H- and ^{13}C-NMR spectra were recorded on a Varian 400 MHz spectrometer. J values were given in Hz.

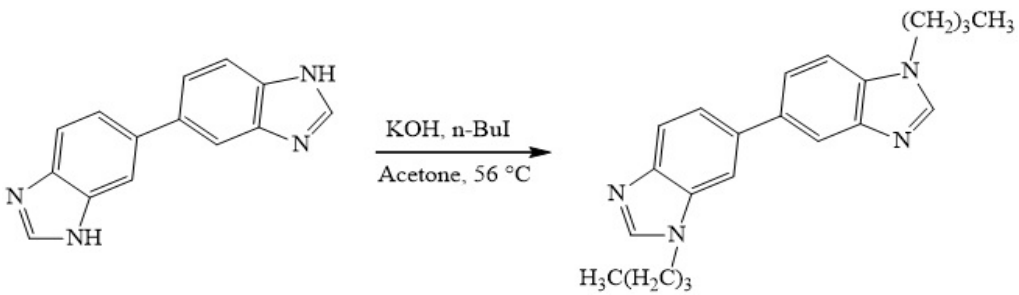
2.2 Synthesis of Ligands

2.2.1 Synthesis of 2b



1 g DAB and 20 mL triethylorthoformate were distallated under argon at 120 C. The mixture was cooled and filtrated after 48 hours. The filtrate washed with dietylether and dried under vacuum. Yield: 76%. ^{1}H NMR (400 MHz, CD_3OD): δ 8.23 (s, 2 H bzm-*H*), 7.82 (d, J = 1.1 Hz, 2 H Ar-*H*), 7.65 (d, J = 8.3 Hz, 2 H Ar-*H*), 7.51 (dd, J = 8.3, 1.1 Hz, 2 H Ar-*H*) 4,85 (s, 2 H, N-*H*). ^{13}C NMR (100 MHz, CD_3OD) δ 141.6, 137.7, 136.9, 122.5 , 115.1, 113.0

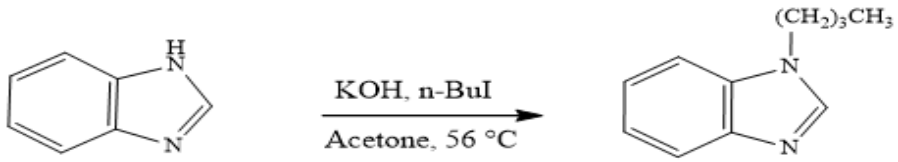
2.2.2 Synthesis of 2c



168 mg KOH (3 mmol) was added to 50 mL acetone and refluxed two hours. When the KOH dissolved and the color turned to the orange DAB (1mmol) was added and the mixture refluxed and stirred for 1 hour. 0.5 mL (2 mmol) iodobutane was added and refluxed 48 hours. The volatiles were removed by vacuum. The residue was dissolved with DCM (5 mL) and filtered . The filtrate dried under vacuum. Diethylether was added to the residue and dried under vacuum. Yield: 70%

^{1}H NMR (400 MHz, CD_3OD) δ 8.18 (d, J = 8.0 Hz, 2 H, bzm-*H*) 7.90 (m, 4 H, Ar-*H*) 7.70 (m, 4 H, Ar-*H*) 1.62 (m, 4 H, Bu-CH_2), 1.37 (m, 4 H, Bu-CH_2), 0.92 (m, 6 H, Bu-CH_3). ^{13}C NMR (101 MHz, CD_3OD) δ 143.6, 136.8, 122.7 118.6, 108.7, 31.6, 19.5, 12.5

2.2.4 Synthesis of 1c

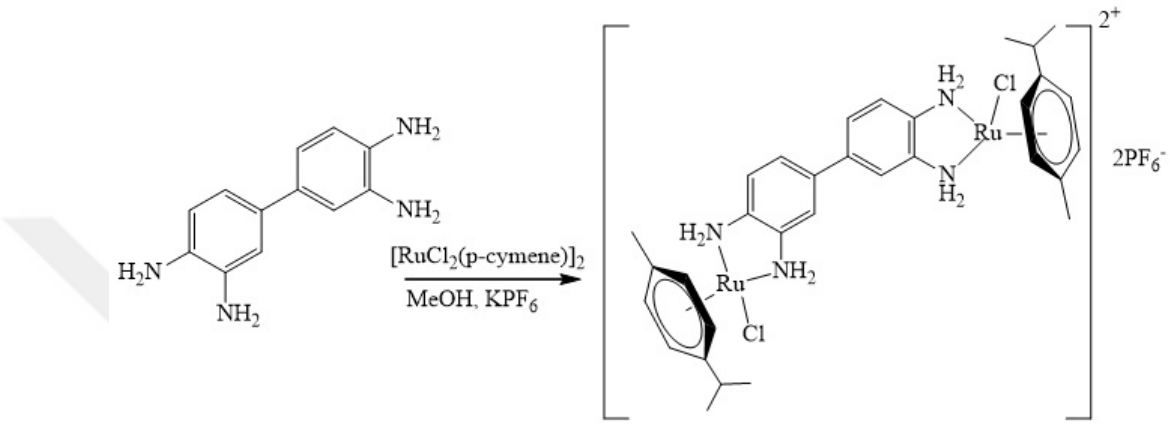


2 mmol KOH was refluxed with 50 mL acetone two hours. When the KOH dissolved and the color turned to the orange 3,3'-diaminobenzidine (1mmol) was added and refluxed two hours. 0.5 mL(1 mmol) iodobutane was added and refluxed 48 hours. The volatiles were removed under vacuum and then the residue was dissolved with DCM (5 mL) and filtered . The filtrate dried under vacuum. Diethylether was added to the residue and mixture dried by vacuum. Yield: 74%

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (s, 1H, bzm-*H*), 7.77 (ddd, *J* = 7.1, 4.0, 2.8 Hz, 2H, Ar-*H*), 7.38 (d, *J* = 1.8 Hz, 2H, Ar-*H*), 4.13 (t, *J* = 7.1 Hz, 4H, Bu-CH_2), 2.86 – 2.49 (m, 4H, Bu-CH_2), 2.42 – 0.76 (m, 6H, Bu-CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 143.8, 133.8 , 122.7, 120.3, 109.6, 29.3, 19.9 , 13.5.

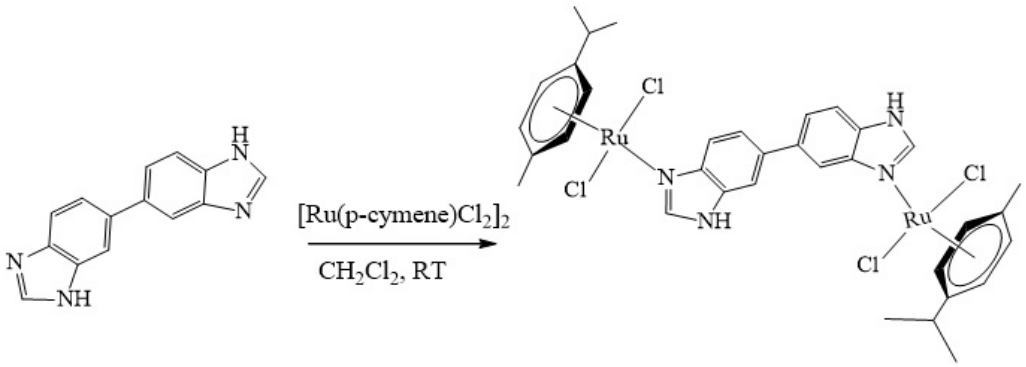
2.3 Synthesis of Ruthenium(II) Complexes

2.3.1 Synthesis of Ru_{2a}



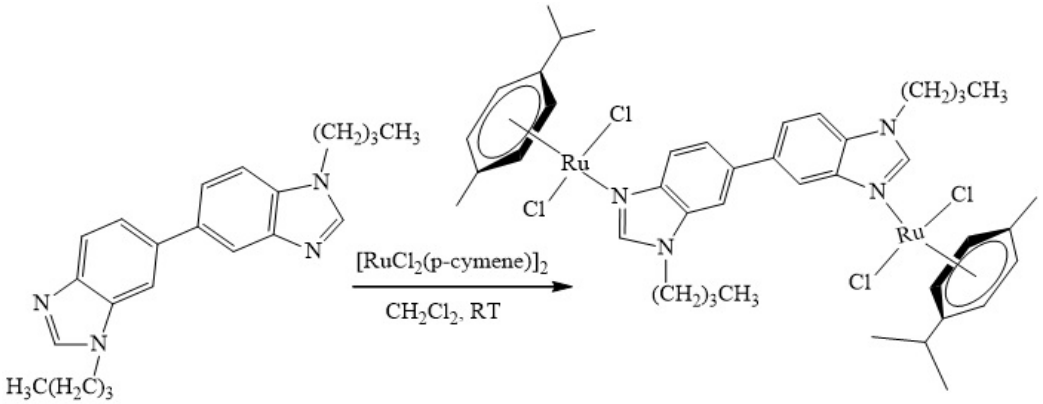
To 30 ml MeOH 0,55 mmol $[RuCl_2(p\text{-cymene})]_2$ was added and refluxed 30 minutes. 0,107 mmol DAB and 2.45 mmol KPF_6 was added to solution and refluxed 16 h. After cooled dried under vacuum. Yield: 62%. ^{1}H NMR (400 MHz, DMSO): δ 7.75 (d, *J* = 12.4 Hz, 2 H, Ar-*H*), 7.60 (d, *J* = 12.4 Hz, 2 H, Ar-*H*), 7.47 (m, 2 H, Ar-*H*), 5.92 (br, 4 H, N*H*), 5.45 (d, *J* = 6.4 Hz, 4 H, *p*-cymene-*H*), 5.71 (d, *J* = 6.4 Hz, 4 H *p*-cymene-*H*), 2.30 (s, 6 H, *p*-cymene-CH_3), 2.06 (m, 2 H, *p*-cymene-C*H*$(CH_3)_2$), 1.24 (d, *J* = 2.8 Hz, 6 H, *p*-cymene-CH(CH_3)$_2$), 1.03 (d, *J* = 2.8 Hz, 6 H, *p*-cymene-CH(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO): δ 139.7, 138.8, 126.6, 124.3, 103.8, 98.8, 82.2, 80.9, 79.1, 77.9, 30.6, 25.5, 21.6, 21.3, 18.0, 17.7.

2.3.2 Synthesis of Ru_{2b}



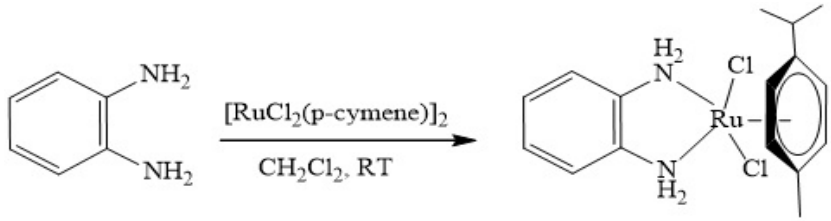
1 mmol ligand 2b was dissolved in dry DCM(15ml) under inert conditions. 1 mmol [Ru(*p*-cymene)Cl2]$_2$ was added to the solution of 2b in DCM and stirred 2 hours at room temperature. The volatiles were removed under vacuum the yellow solid was washed with diethylether. The desired product was dried under vacuum. Yield: 56% ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.52 (s, 2 H, N-*H*), 8.10 (s, H,bzm-*H*), 7.81 (s, H, bzm-*H)*, 7.57 (dd, J = 8.8, 14.40 Hz, 4 H, Ar-*H*), 7.12 (d, J = 8.0 Hz, 2 H, Ar-*H*), 6.97 (dd, J = 9.6, 9.20 Hz, 4 H, *p*-cymene-*H*), 6.85 (d, J = 8.4 Hz, 2 H, *p*-cymene-*H*), 6.72 (d, J =8.0 Hz, 2 H, *p*-cymene-*H*), 2.93 (m, 6 H, *p*-cymene-C*H*$_3$), 2.07 (m, 2 H, *p*-cymene-CH(CH_3)$_2$), 1.32 (m, 6 H, *p*-cymene-CH(CH_3)$_2$), 1.20 (m, 6 H, *p*-cymene-CH(CH_3)$_2$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.7, 138.8, 126.6, 124.3, 102.8, 30.7, 22.2, 18.4

2.3.3 Synthesis of Ru_{2c}



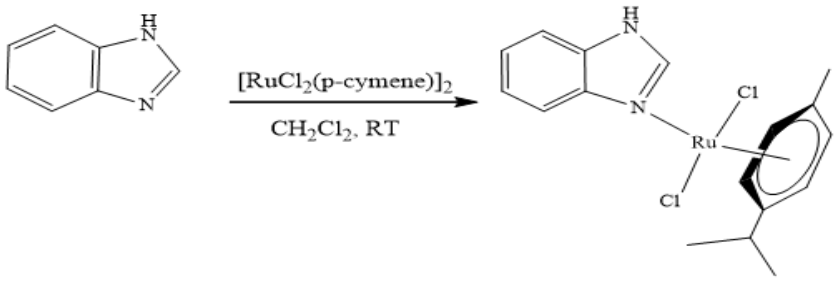
1 mmol ligand 2c was dissolved in dry DCM(15mL) under inert conditions. 1 mmol [Ru(*p*-cymene)Cl2]$_2$ was added to the solution of 2c in DCM and stirred 2 hours at room temperature. The volatiles were removed under vacuum the yellow solid was washed with diethylether. The desired product was dried under vacuum. Yield: 60% ^{1}H NMR (400 MHz, $CDCl_3$): 8.13 (s, H, bzm-*H*)), 7.92 (s, H, bzm-*H*), 7.62 (dd, J =8.0, 14.20 Hz, 4 H, Ar-*H*), 7.22 (d, J = 8.0 Hz, 2 H, Ar-*H*), 6.90 (dd, J = 9.2, 9.40 Hz, 4 H, *p*-cymene-*H*), 6.80 (d, J = 8.4 Hz, 2 H, *p*-cymene-*H*), 6.72 (d, J = 8.0 Hz, 2 H, *p*-cymene-*H*), 2.93 (m, 6 H, *p*-cymene-CH_3), 2.07 (m, 2 H, *p*-cymene-CH(CH_3)$_2$), 1.68 (m, 4 H, Bu-CH_2), 1.38 (m, 4 H, Bu-CH_2), 1.30 (m, 6 H, *p*-cymene-CH(CH_3)$_2$), 1.24 (m, 6 H, *p*-cymene-CH(CH_3)$_2$). 0.90 (m, 6 H, Bu-CH_3). ^{13}C NMR (100 Mhz, CD_3OD) δ 124.3, 101.2, 97.0, 83.5, 80.9, 78.9, 77.9, 31.2, 30.7, 27.0, 21.3, 20.91, 19.5, 17.6 , 12.6.

2.3.4 Synthesis of Ru_{1a}



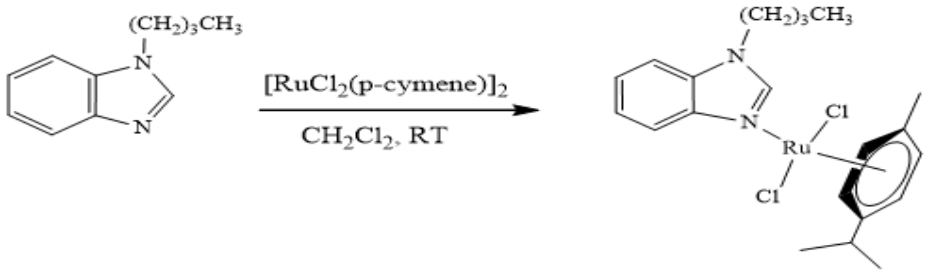
To a dichloromethane (10 ml) o-phenylene diamine (0.1 mmol) was added under inert conditions. 0.05 mmol [$RuCl_2$(*p*-cymene)]$_2$ was added to the solution. The resulting suspension was stirred for 2 h and filtered with canula. The solid was washed with diethylether and dried under vacuum. Yield 75% ^{1}H NMR (400 MHz, DMSO): δ 8.37 (d, *J* = 12.8 Hz, 1 H, Ar-*H*), 7.24 (m, 1 H, Ar-*H*), 7.14 (m, 1 H, Ar-*H*), 6.13 (d, *J* = 12.8 Hz, 1 H, Ar-*H*), 5.79 (d, *J* = 6.0 Hz, 2 H, *p*-cymene-*H*), 5.57 (d, *J* = 6.0 Hz, 2 H, *p*-cymene-*H*), 3.30 (s, 3 H, *p*-cymene-CH_3), 2.93 (m, 1 H, *p*-cymene-C*H*(CH_3)$_2$), 1.16 (d, *J* = 2.6 Hz, 6 H, *p*-cymene-CH(CH_3)$_2$). ^{13}C NMR (100 MHz, DMSO): δ 140.2, 126.9, 126.3, 103.2, 98.5, 82.3, 80.6, 30.3, 22.7, 18.4.

2.3.5 Synthesis of Ru_{1b}



To a dichloromethane (10 mL) benzimidazole (0.1 mmol) was added under inert conditions. 0.05 mmol $[RuCl_2(p\text{-cymene})]_2$ was added to the solution. The resulting suspension was stirred for 2 h and filtered with canula. The solid was washed with diethylether and dried under vacuum. Yield: 71% ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.45 (s, H, N-*H*), 8.14 (d, *J* = 1.3 Hz, H, bzm-*H*), 7.62 (d, *J* = 8.2 Hz, 4 H, Ar-*H*), 7.26 (s, 2 H, Ar-*H*), 6.96 (ddd, *J* = 8.3, 6.6, 1.7 Hz, 2 H, *p*-cymene-*H*), 6.77 – 6.56 (m, H, *p*-cymene-*H*), 5.56 (d, *J* = 5.9 Hz, H, *p*-cymene-*H*), 5.47 2.88 (dt, *J* = 13.7, 6.9 Hz, 3 H, *p*-cymene-CH_3), 2.15 (t, *J* = 18.9 Hz, H, *p*-cymene-CH(CH_3)$_2$), 2.04 (s, 3 H, *p*-cymene-CH(CH_3)$_2$), 1.42 (d, *J* = 5.9 Hz, 3 H, *p*-cymene-CH(CH_3)$_2$), 1.35 – 1.02(m, 3 H, *p*-cymene-CH(CH_3)$_2$) (Due to solubility problem ^{13}C NMR was not taken)

2.3.6 Synthesis of Ru_{1c}



1-butyl-1H-benzimidazole (0.1 mmol) was added to a dichloromethane (10 mL) under inert conditions. $[RuCl_2(p\text{-cymene})]_2$ (0.05 mmol) was added to the solution. The resulting suspension was stirred for 2 h and filtered with canula. The solid was washed with diethylether and dried under vacuum. Yield: 63% ^{1}H NMR (400 MHz, CD_3OD) δ 8.49 (s, H, bzm-*H*), 8.43 (s, H, Ar-*H*), 8.06 (s, 2 H, Ar-*H*), 7.65 (s, H, Ar-*H*), 7.84 – 7.32 (m, H, Ar-*H*), 5.95 (s, 2 H, *p*-cymene-*H*), 5.81 (t, *J* = 30.4 Hz, H, *p*-cymene-*H*), 5.71 – 5.35 (m, H, *p*-cymene-*H*), 3.07 – 2.65 (m, 3 H, *p*-cymene-CH_3), 2.18 (s, H, *p*-cymene-CH$(CH_3)_2$), 1.94 (d, *J* = 33.7 Hz, 2 H, Bu-CH_2), 1.90 – 1.70 (m, 4H, Bu-CH_2), 1.58 – 1.11 (m, 3H, *p*-cymene-CH$(CH_3)_2$), 1.05 – 0.84 (m, 3 H, *p*-cymene-CH$(CH_3)_2$), 0.82 (d, *J* = 7.2 Hz, 3H, Bu-CH_3). (Due to solubility problem ^{13}C NMR was not taken)

2.4 Catalytic Experiments

2.4.1. General method for transfer hydrogenation of synthesized complexes

1 mmol acetophenone , 0.01 mmol catalyst and propan-2-ol (2 mL) were stirred at 82 ºC for 1 hour. 56 mg KOH (1 mmol) and 2-propanol introduced to the mixture. The mixture was stirred and refluxed. At 82 ºC, aliquots were withdrawn from reaction vessel and controlled by GC.

2.5 Anticancer Studies

Antiproliferative activities of complexes were investigated by using LNCaP (androgen-sensitive human prostate adenocarcinoma), PC-3 (androgen independent prostate lymph metastasis) and DU145 (androgen independent prostate brain metastasis), HeLa(cervical adenocargicinoma), MCF-7(primary breast adenocarcinoma) MDA-MB-231 (metastatic breast adenocarcinoma), HepG2 (liver hepatocellular carcinoma) cell lines.

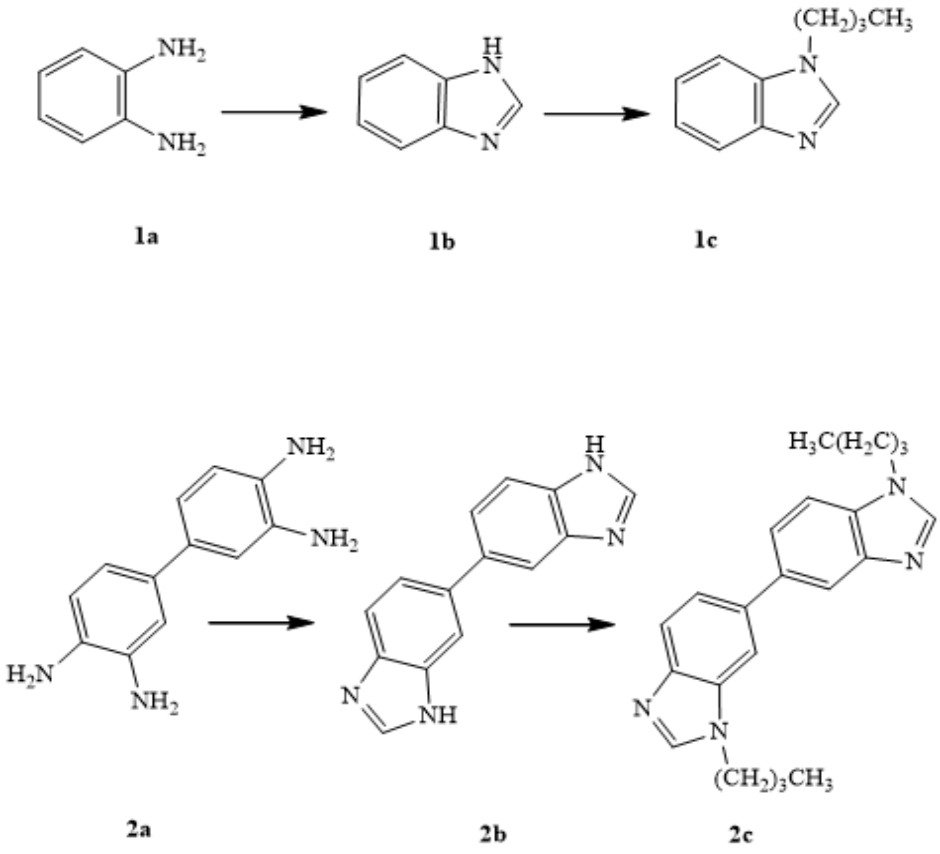
Cells were cultured in RPMI-1640 (Invitrogen, USA) and DMEM (Invitrogen, USA) nutrient media which contain %5-10 foetal bovine serum(FBS) (Sigma,USA), 100 IU/mL penicilline and streptomycin at 37 ^{0}C, %5 CO_{2}.. Cells were kept in serum that contained 10% DMSO, stored at -86 ^{0}C.

Cytotoxicity analysis was carried out by the colorimetric method MTT (3-[4,5- dimethylthiazol- 2- yl]- 2,5- diphenyl- tetrazolium bromide). This test measures the conversion of MTT dye color from yellow to purple formazan products. Cell proliferation was observed by using stock solution of MTT (M655, Sigma, USA) in PBS. Results were evaulated by Microsoft Excel (XP) and GraphPad. IC_{50} values(half maximal inhibitory concentration) of complexes were calculated. The expression levels of proteins were investigated by Western-blot analysis.

3. RESULTS and DISCUSSION

3.1 Synthesis and characterization of diaminobenzidine ligands

The aromatic ring system containing tetraamine derivative ligand and diamine derivatice ligand were synthesized related to the steps showed in Scheme 3.1. The π-conjugated system 3,3'-bibenzimidazole **2b** was synthesized by ring closing reaction of 3,3'-diaminobenzidine. **2a** with triethylformate at high temperature. **2c** were synthesized from the reaction of **2b** compound with butyl iodide. 1**b** and **1c** were prepared same manner starting from benzimidazole.



Scheme 3.1 Structures of synthesized ligands

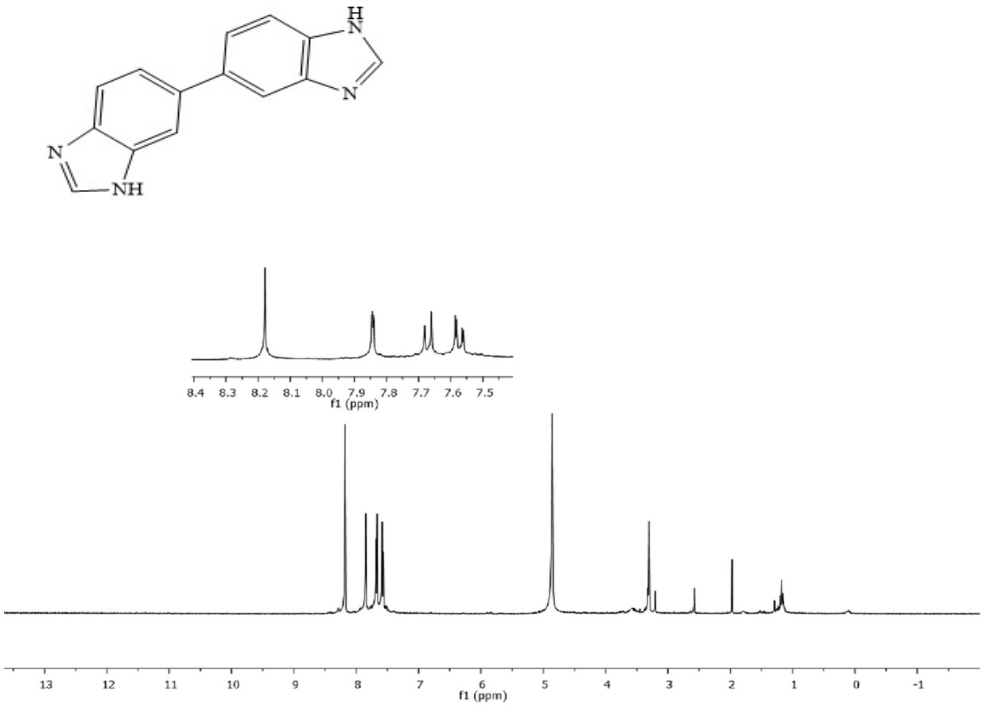


Figure 3.1 [1] H-NMR spectrum of **2b**

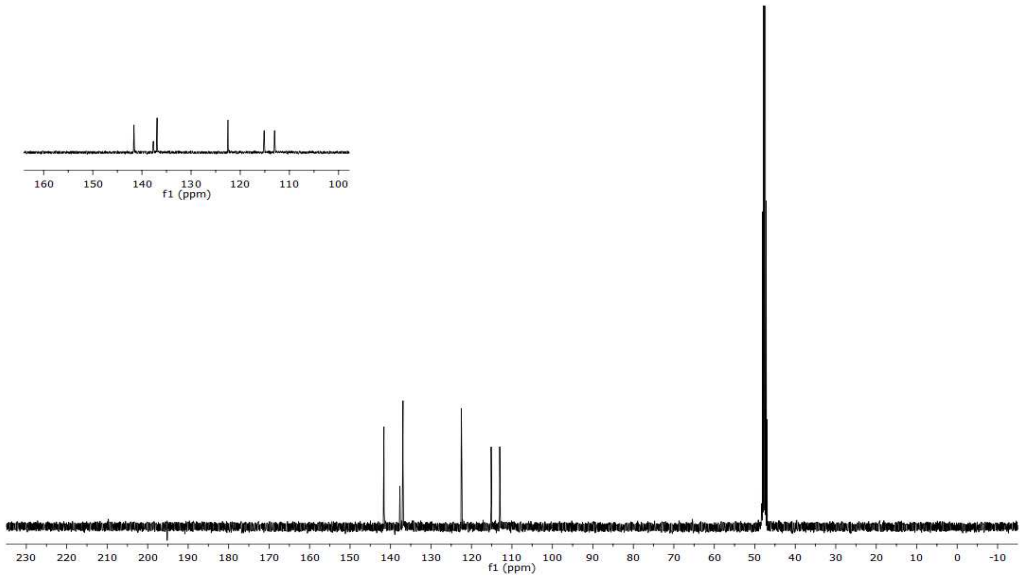


Figure 3.2 [13] C-NMR spectrum of **2b**

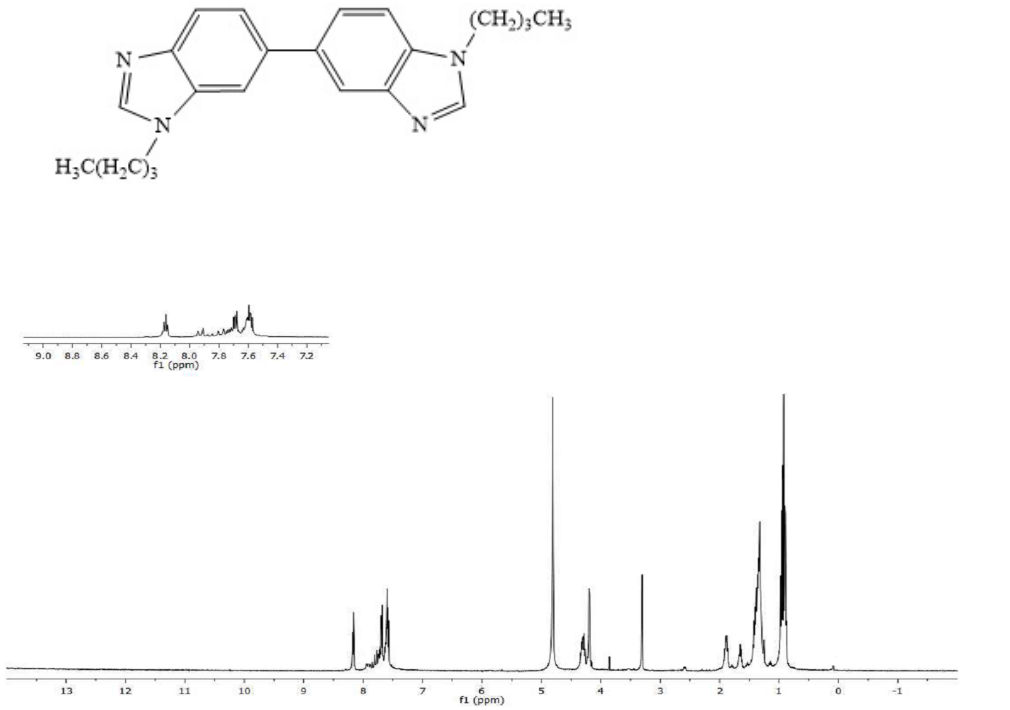


Figure 3.3 [1] H-NMR spectrum of **2c**

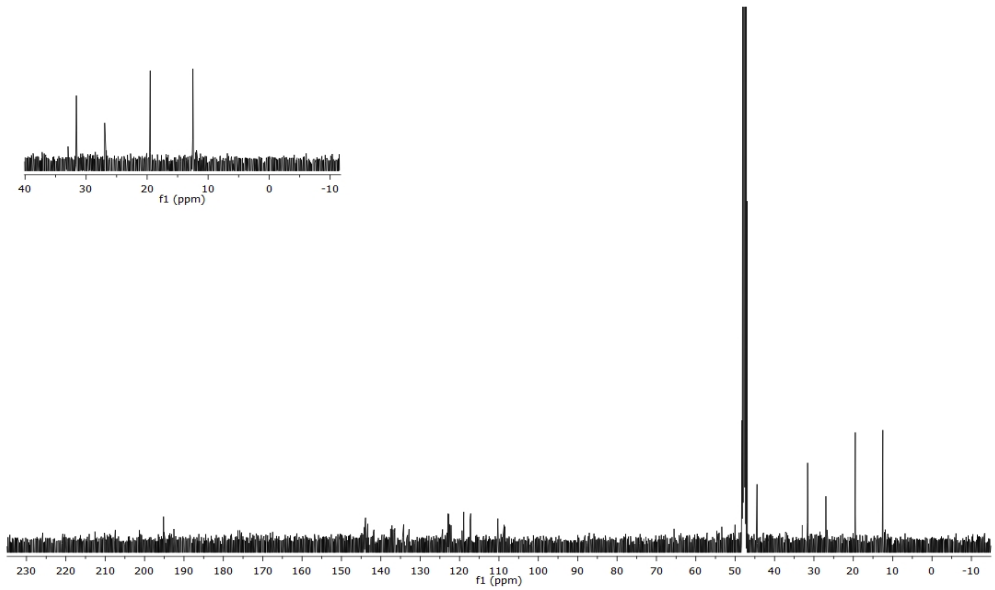


Figure 3.4 [13] C-NMR spectrum of **2c**

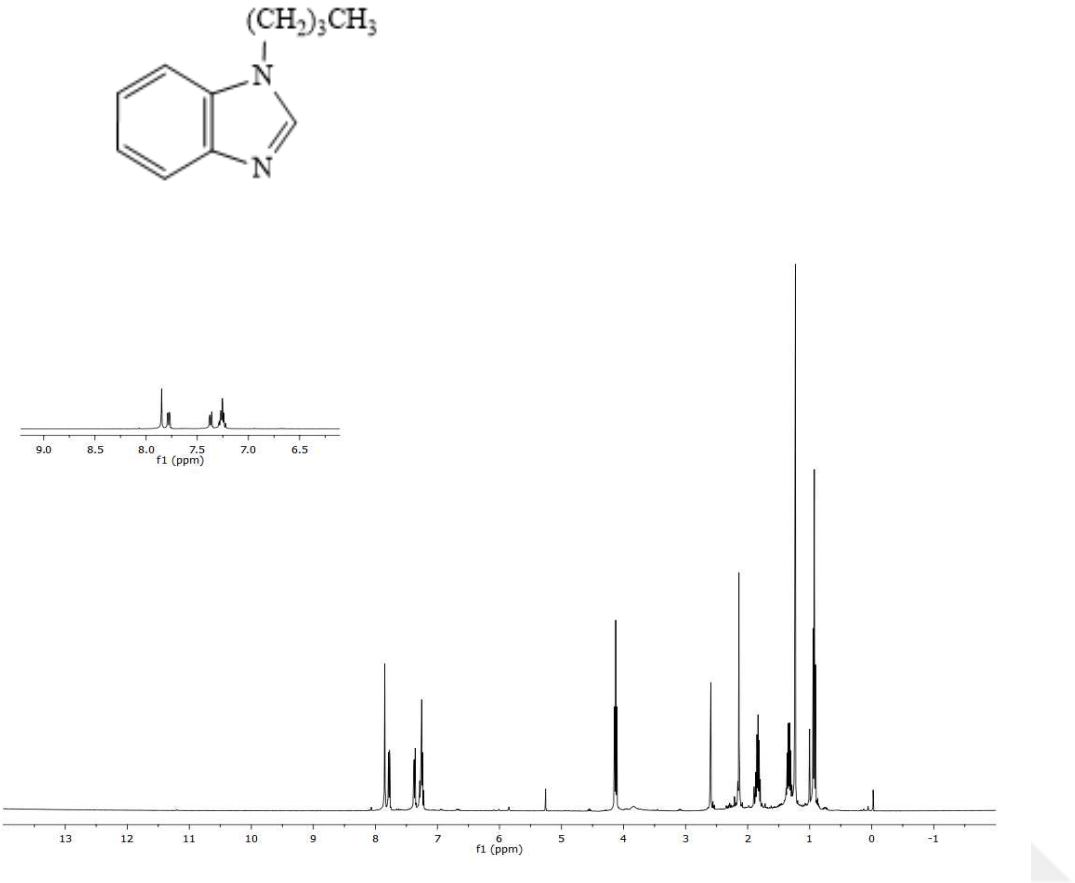


Figure 3.5 [1] H-NMR spectrum of 1**c**

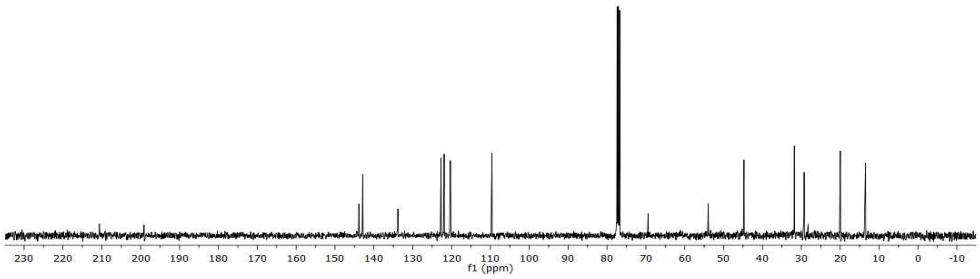
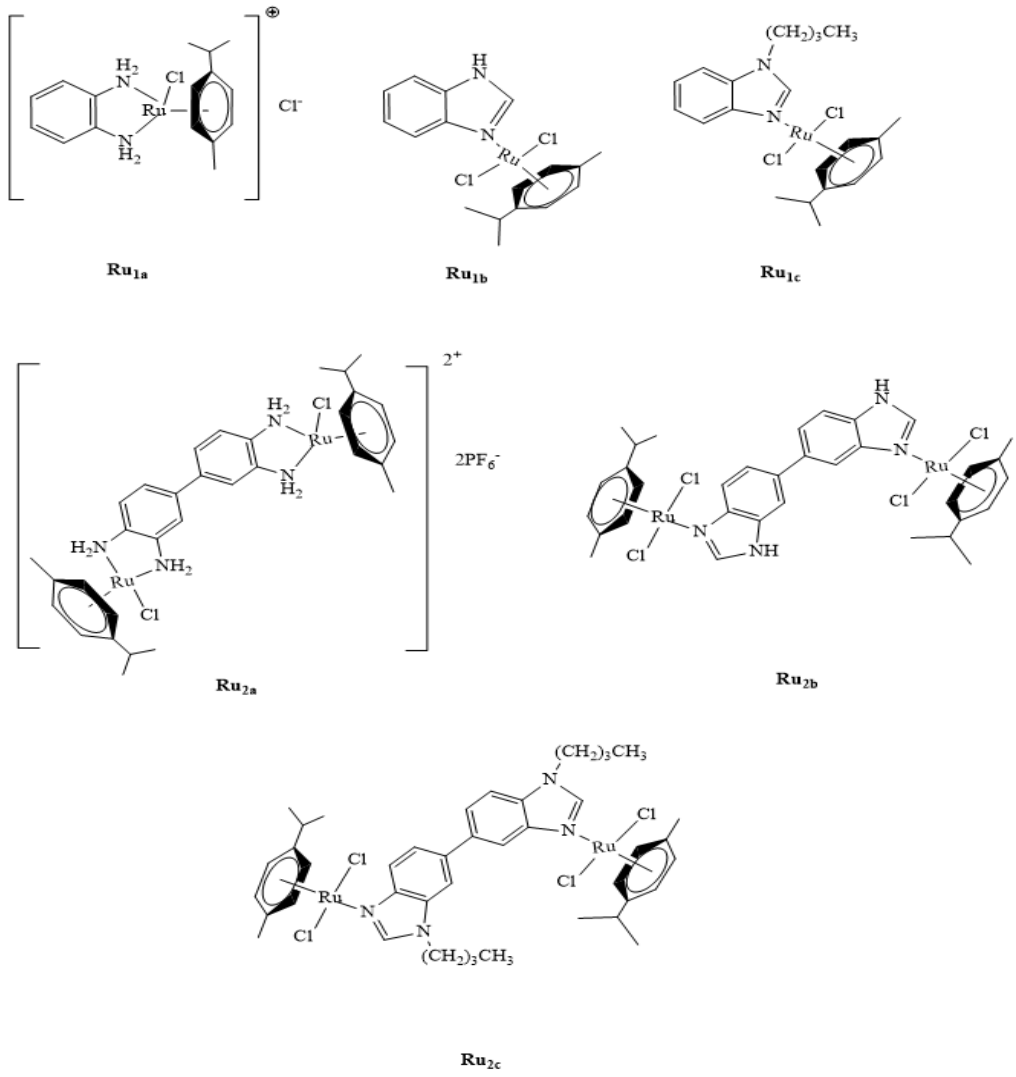


Figure 3.6 [13] C-NMR spectrum of 1**c**

3.2. Synthesis and characterization of Ru(II) complexes

Bimetallic and monometallic Ru(II) complexes were synthesized with ligand (2a-2c) and (1a-1c) (Scheme 3.2).



Scheme 3.2 Bimetallic and monometallic Ru(II) complexes

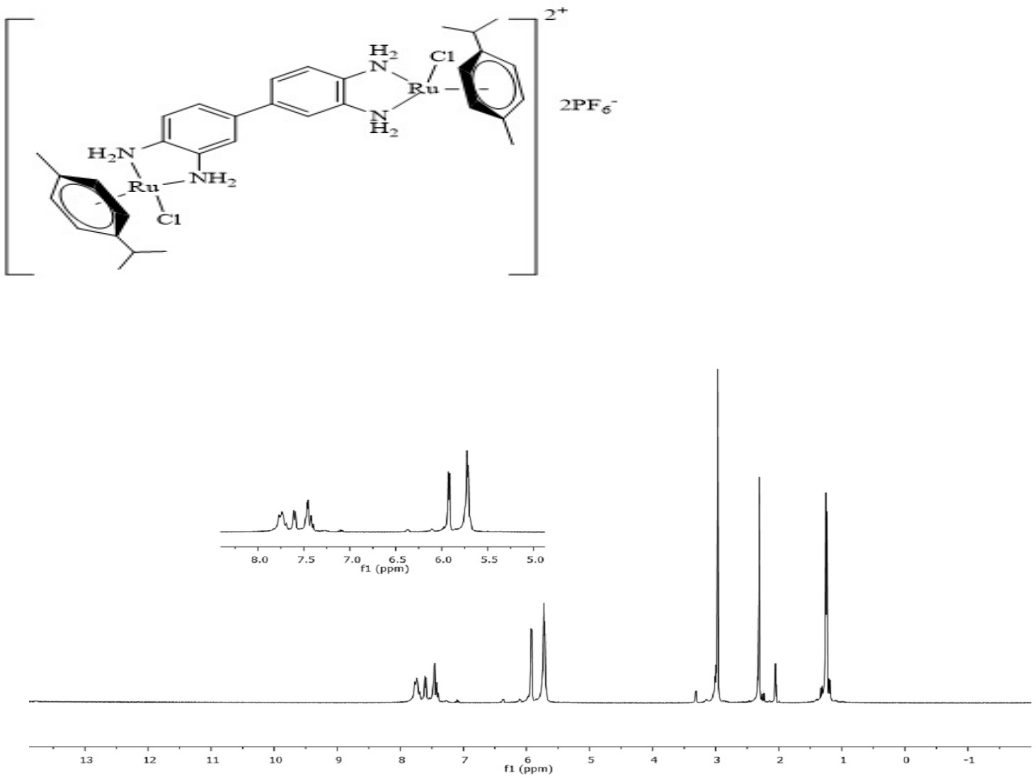


Figure 3.7 [1] H-NMR spectrumof **Ru_{2a}**

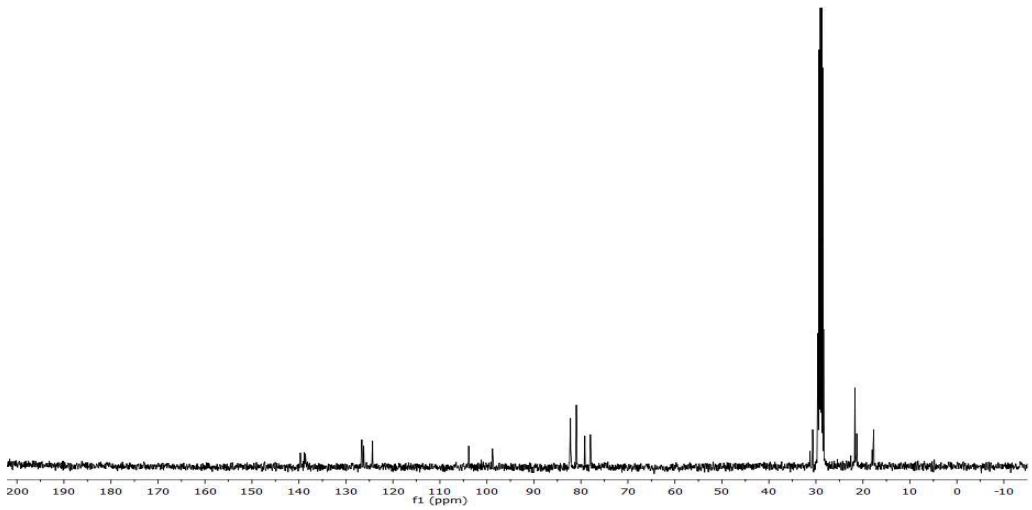
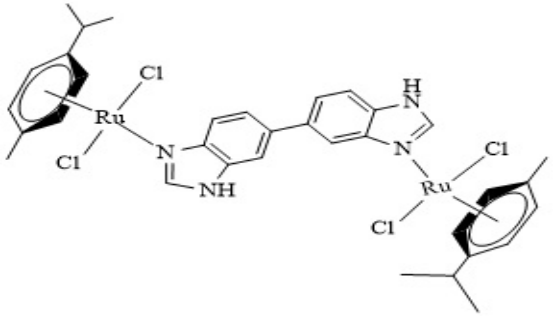


Figure 3.8 [13] C-NMR spectrum of **Ru_{2a}**



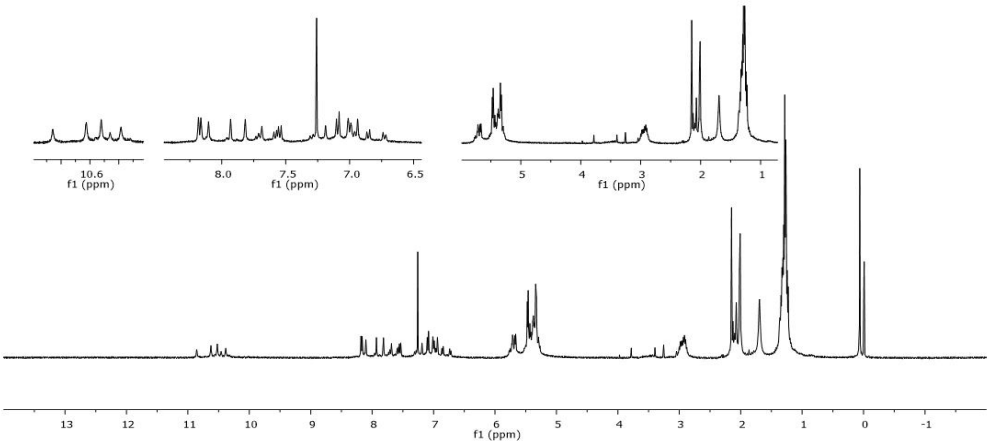


Figure 3.9 [1] H-NMR spectrum of **Ru_{2b}**

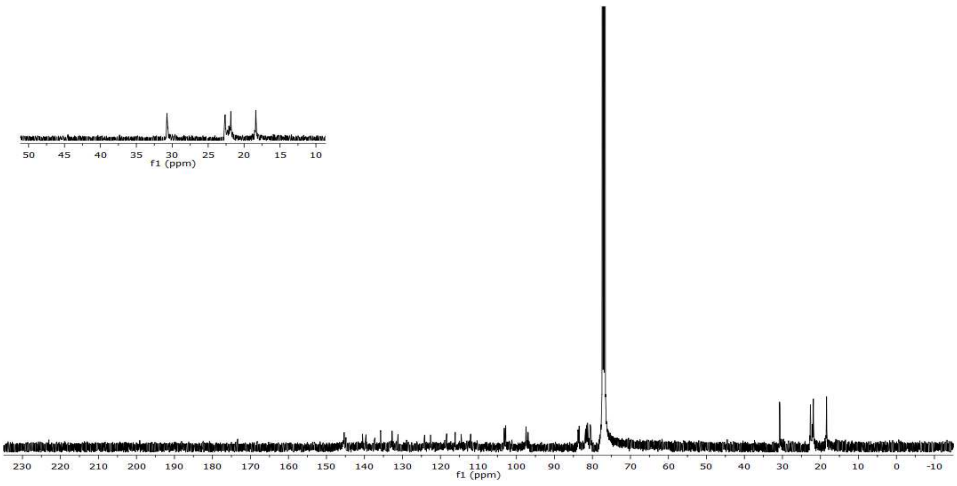


Figure 3.10 [13] C-NMR spectrum of **Ru_{2b}**

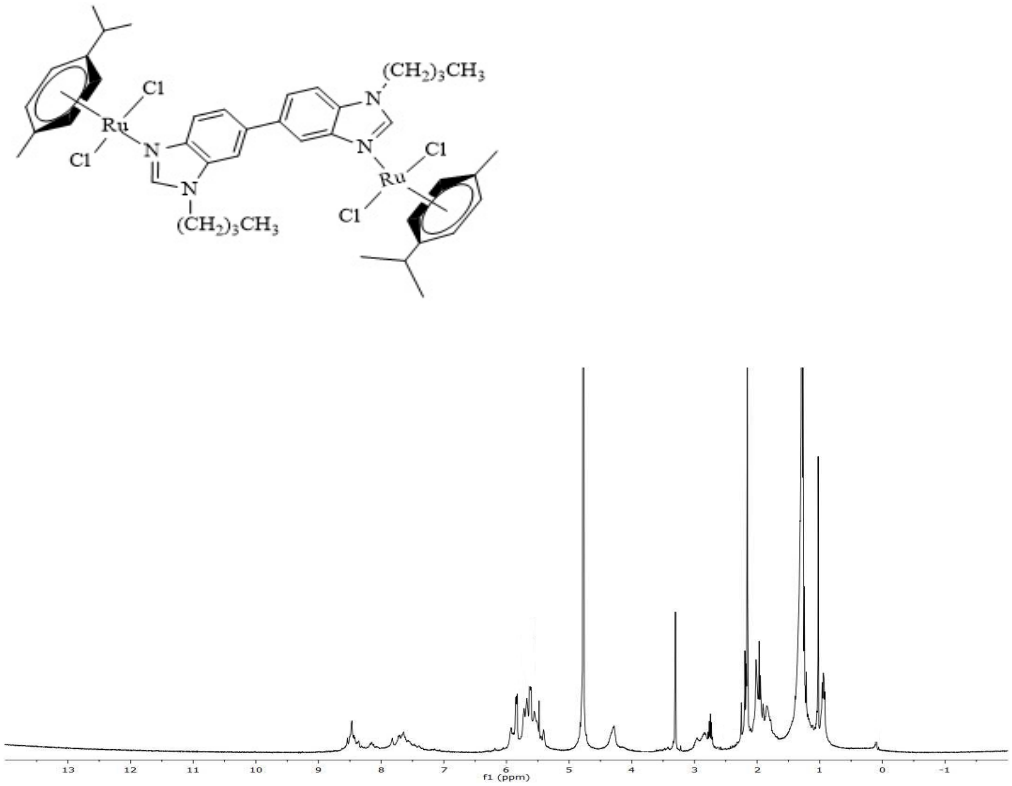


Figure 3.11 [1] H-NMR spectrum of **Ru_{2c}**

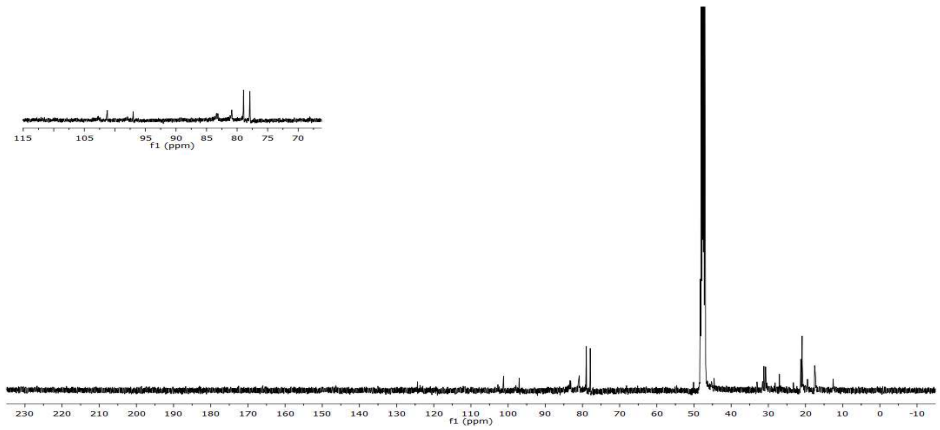
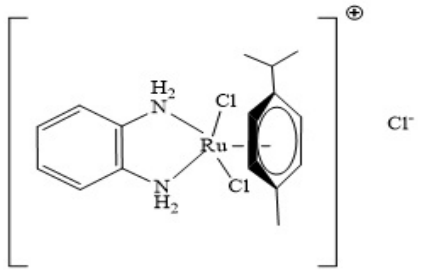


Figure 3.12 [13] C-NMR spectrum of **Ru_{2c}**



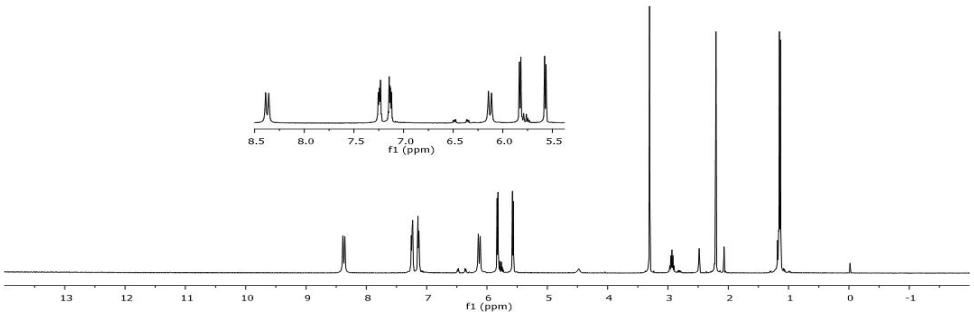


Figure 3.13 [1] H-NMR spectrum of **Ru_{1a}**

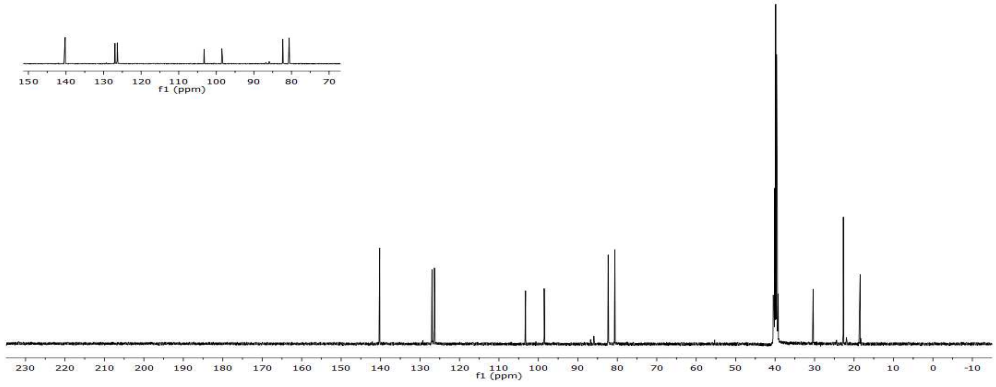


Figure 3.14 [13] C-NMR spectrum of **Ru_{1a}**

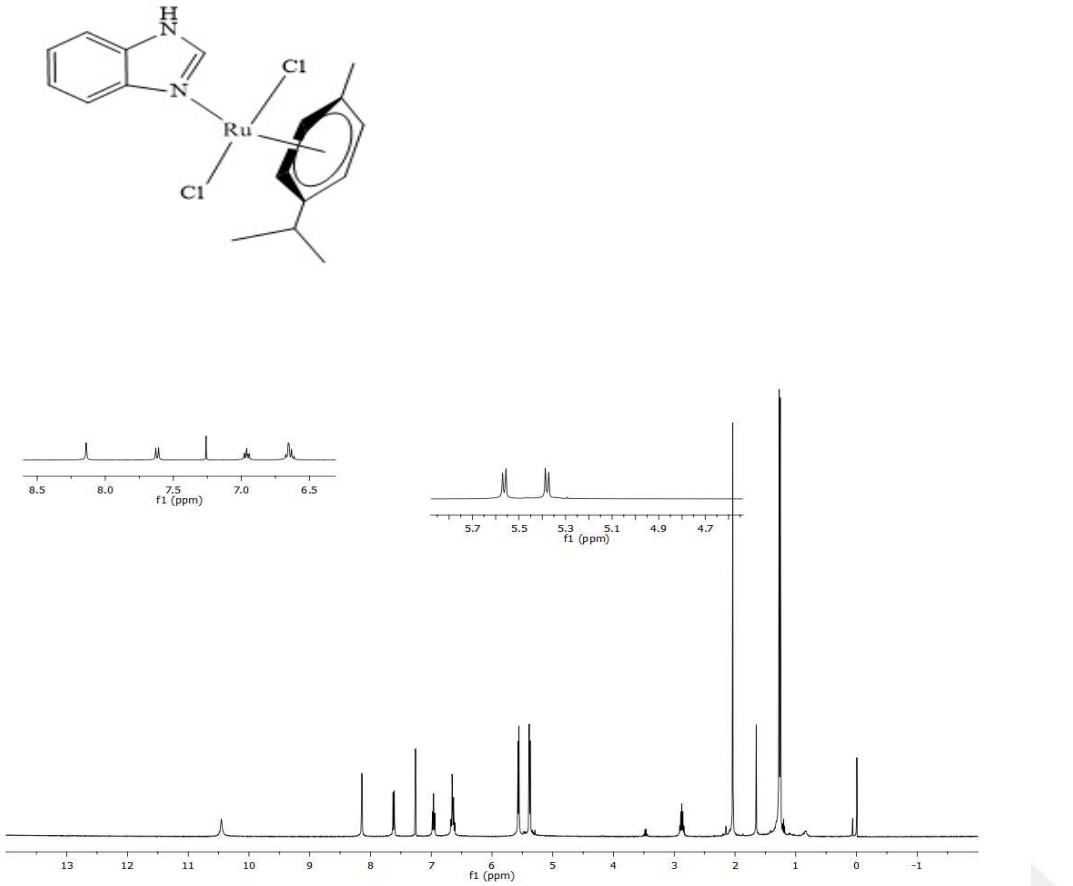


Figure 3.15 [1] H-NMR spectrum of **Ru_{1b}**

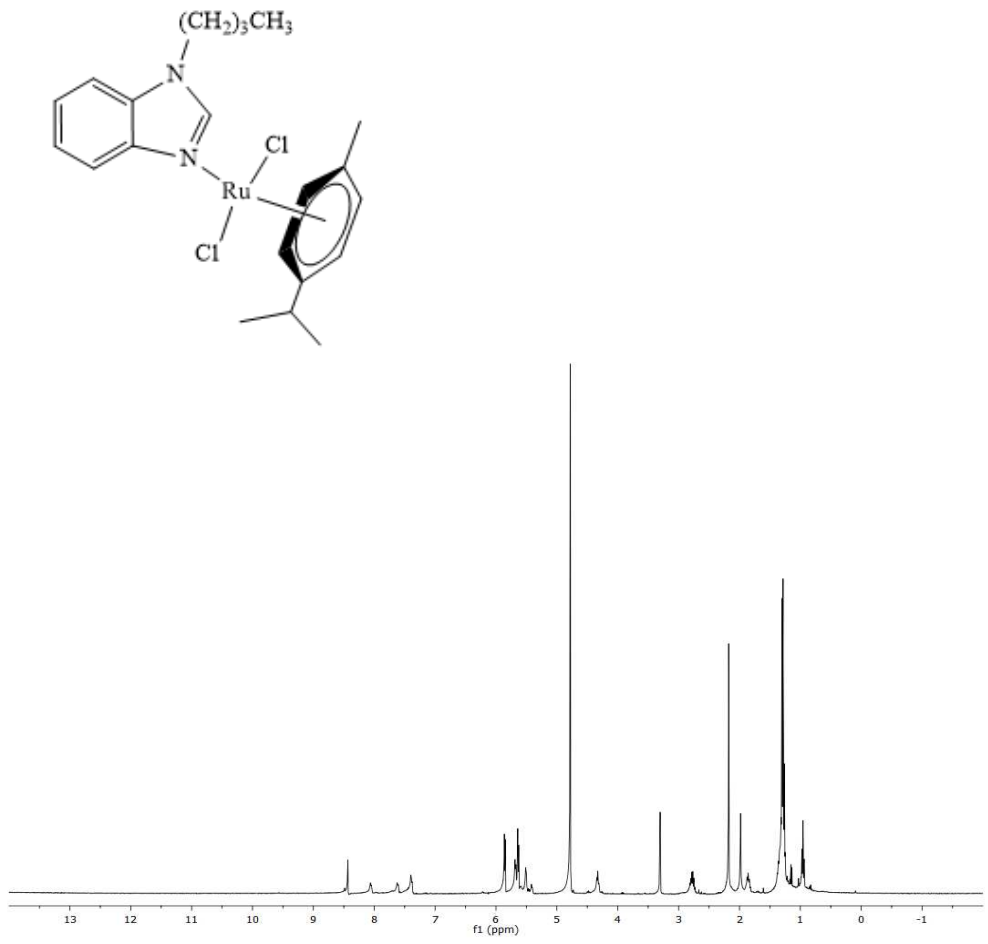
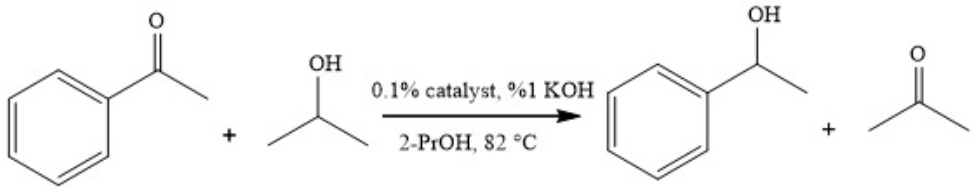


Figure 3.16 ^{1}H-NMR spectrum of **Ru$_{1c}$**

3.3 Catalytic test

3.3.1 Transfer hydrogenation of Ru(II) complexes

Catalytic activities were investigated for the complexes by transfer hydrogenation of acetophenone in the presence of KOH by 2-propanol. Bimetallic complexes(**Ru_{2a}**, **Ru_{2b}**, **Ru_{2c})** and monometallic complexes **(Ru_{1a}, R_{1b}**) were used for this test.



3.3.2 Activity of Bimetallic Ru (II) Complexes

Entry	Complex	Conv. (%)	Time (h)
1	**Ru_{2a}**	83	
2	**Ru_{2b}**	20	1
3	**Ru_{2c}**	27	
4	**Ru_{2a}**	90	
5	**Ru_{2b}**	27	2
6	**Ru_{2c}**	36	

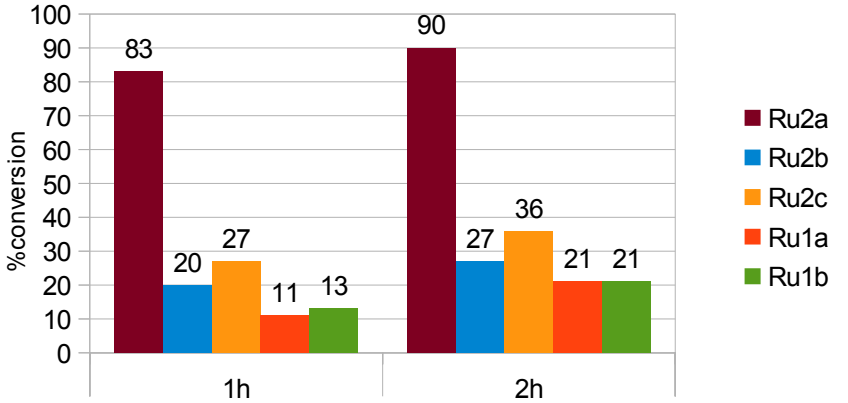
Table 3.1 Catalytic Activities of Bimetallic Complexes

3.3.3 Activity of Monometallic Ru (II) Complexes

Entry	Complex	Conv. (%)	Time (h)
1	**Ru_{1a}**	11	
2	**Ru_{1b}**	13	1
3	**Ru_{1a}**	21	
4	**Ru_{1b}**	21	2

Table 3.2 Catalytic Activities of Monometallic Complexes

3.3.4 Activity Comparision of mono- and bi-metallic Ru(II) Complexes



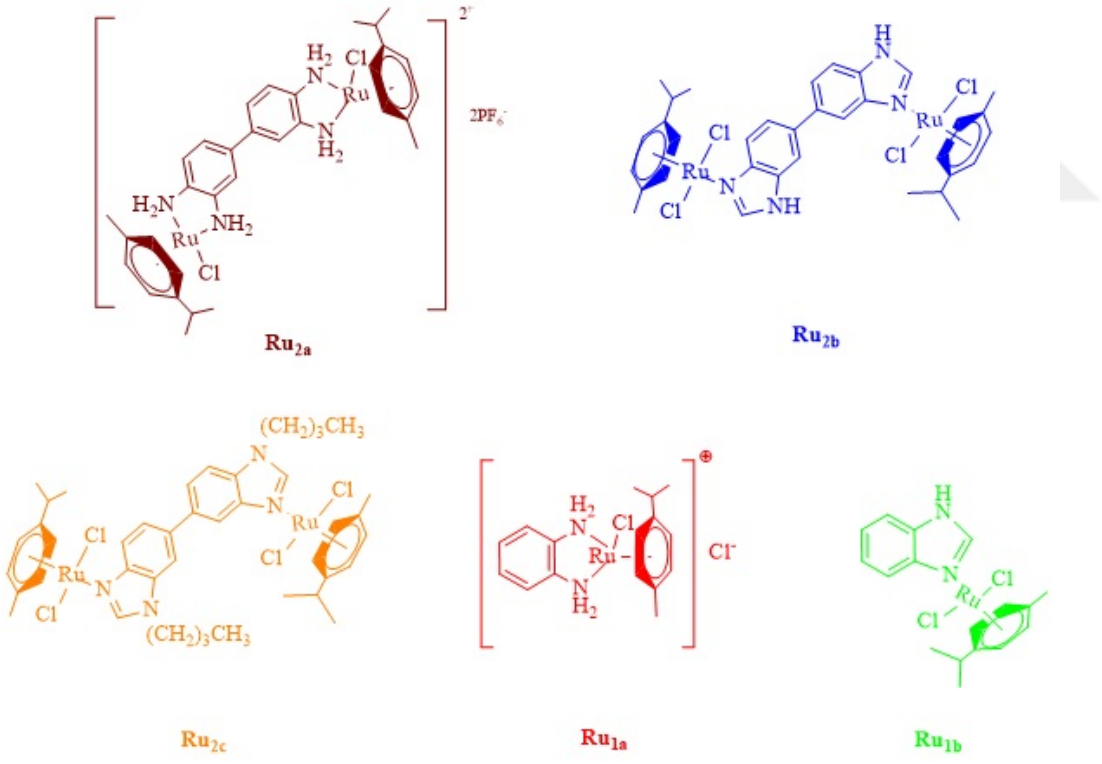


Figure 3.17 Transfer hydrogenation of acetophenone by the synthesized complexes

3.4 Anticancer Activity of Ru(II) Complexes

3.4.1 Cell Culture Studies

Antiproliferative activities of Ru_{2a} and Ru_{1a} complexes were investigated in LNCaP (androgen-sensitive human prostate adenocarcinoma), PC-3 (androgen independent prostate lymph metastasis) and DU145 (androgen independent prostate brain metastasis) cell lines.

3.4.2 Cytotoxicity Analysis (MTT):

Cytotoxicity analysis was carried out by the colorimetric method with MTT (3[4,5- dimethylthiazol-2-yl]-2,5- diphenyl-tetrazolium bromide). This analysis were carried out for determine most active compound at low concentration. IC_{50} values (half maximal inhibitory concentration) of complexes were calculated.

Figure 3.18 Time and concentration dependent cytotoxicity of Ru_{2a} and Ru_{1a} complexes in DU145 cell line

Figure 3.19 Time and concentration dependent cytotoxicity of Ru_{2a} and Ru_{1a} complexes in PC-3 cell lines

Ru_{2a} exhibited cytotoxic activity at high concentrations. Ru_{1a} complex showed cytotoxic activity even at low concentrations in PC-3 cell lines.

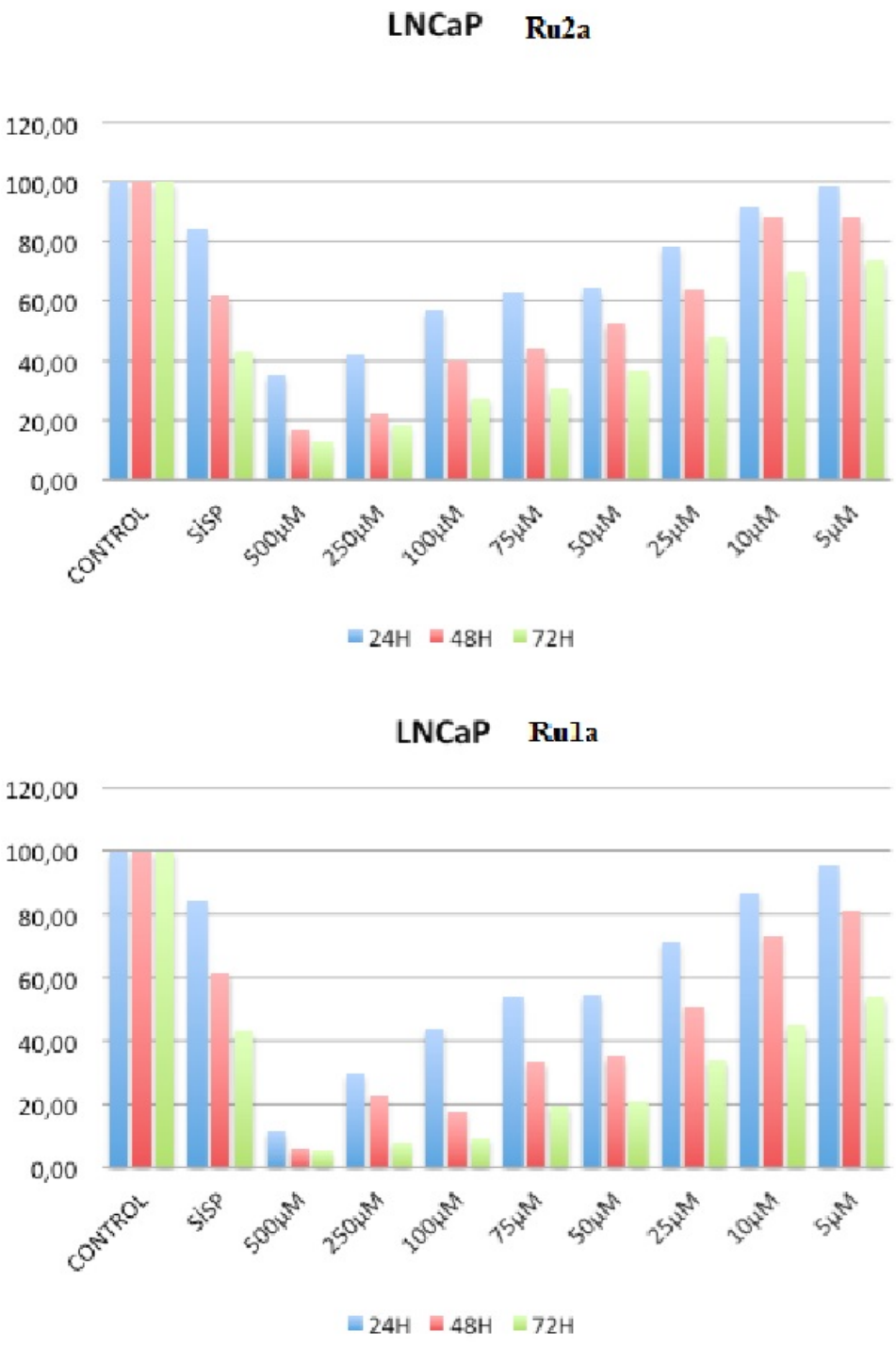


Figure 3.20 Time and concentration dependent cytotoxicity of Ru_{2a} and Ru_{1a} complexes in LNCaP cell lines

IC_{50} (uM)	DU145			PC-3			LNCaP		
	24sa	48sa	72sa	24sa	48sa	72sa	24sa	48sa	72sa
Ru2a	182	153	149	259	190	161	120	60	30
Ru1a	111	87	62	43	34	26	80	25	8

Figure 3.21 IC_{50} values of Ru_{2a} and Ru_{1a} complexes

IC_{50} values of Ru_{1a} complex were found 43, 34, 26 µM for 24, 48 ve 72 hours respectively in PC-3 cell line, in DU145 cell line 111, 87, 62 µM, in LNCaP cell line 80, 25, 8 µM. Ru_{1a} was found to be most active one and used for western-blot tests. Investigation of apoptosis related protein level was carried out by using western-blot analysis.

$Ru_{2a,}$ Ru_{1a} and Ru_{2b} were investigated by diluting concentrations, including 1:2, 1:4, 1:8, 1:16 and 1:32. Cytotoxic analysis of extracts were performed by MTT.

In HeLa (cervical adenocarcinoma), cell lines, Ru_{2a} was found more active than others. All complex were toxic in MD-MBA cell lines and MCF-7 (breast cancer cells), MDA-MB-231(metastatic breast cancer cell), HepG2 (liver hepatocellular cell) cell lines cytotoxicity decreased by dilution. In MCF-7 and HepG2 cell lines, Ru_{2b} was more active.

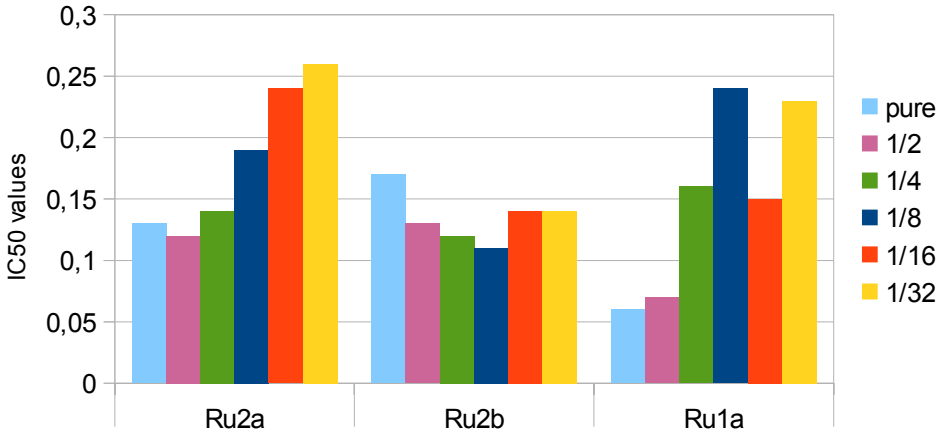


Figure 3.22 Concentration dependent graphic in HeLa cell lines

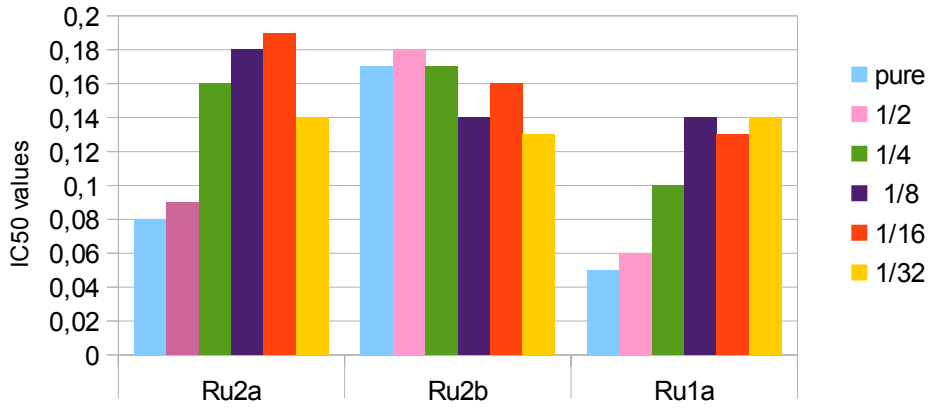


Figure 3.23 Concentration dependent graphic MCF-7 cell lines

3.4.3 Western-Blot Analysis

The purpose of the Western-blot method is to determine expression levels of proteins. Ru_{1a} complex was investigated by Western-blot analysis because this complex found to be most active one in MTT analysis.

Ru_{1a} complex was apply to LNCaP, PC-3 and DU145 cell lines in 50-100-150 μM concentration range for 24 hours. Apoptosis related proteins (PARP polymerase, active caspaze 3-9, p53) were isolated and investigated by Western-blot method.

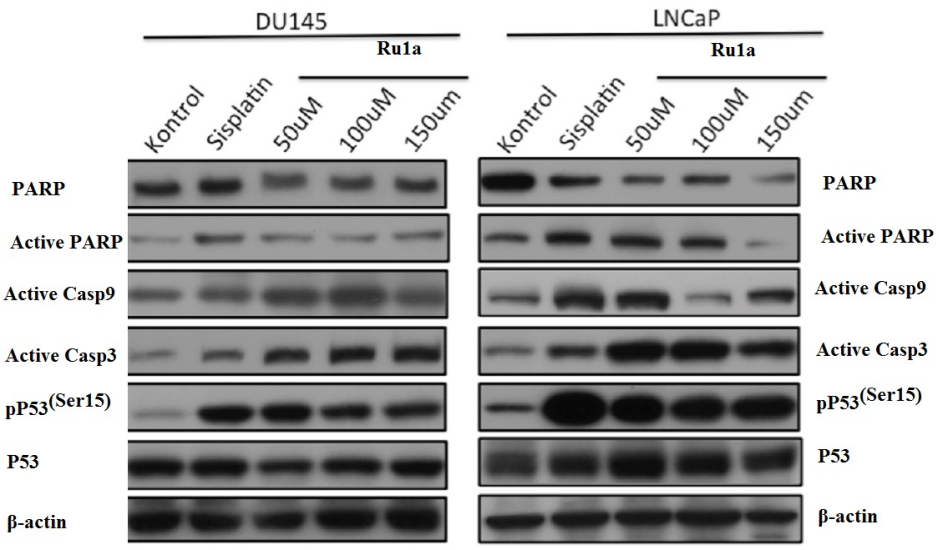


Figure 3.24 Changes in the apoptosis related protein level in DU145 and LNCaP cell lines

Investigation of changes in the apoptosis related protein level showed that Ru_{1a} complex increase the apoptotic effect in the DU145 and LNCaP cell lines. Phosphorylation of P53 with Ser15 increased the expression in two cell lines. The complex caused same level stimulation with cisplatin in DU145 cell lines while caused less stimulation than cisplatin in LNCaP cell lines. PARP level

decreased because of polymerase and active PARP level increased. Lastly, investigation of active caspase 3 and 9 level showed that activation of caspase 3 increased dose dependently and higher than protein level of cisplatin.

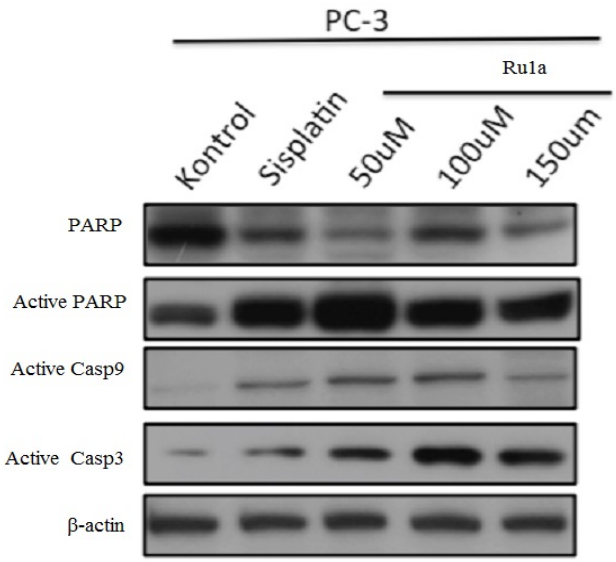


Figure 3.24 Changes in the apoptosis related protein level in PC-3 cell lines

In PC-3 cell line, dose dependently increase were obtained in all apoptosis related proteins. Especially, PARP activation was found high and apoptosis induction was found to be higher than cisplatin. Cytotoxic activity of Ru_{1a} complex was obtain in PC-3 cell line at low concentrations. Results were found compatible with MTT analysis results.

3.5 Conclusions

In this thesis, we reported preparation and characterization of amine ligands and their monometallic and bimetallic ruthenium complexes. Their catalytic activities in transfer hydrogenation of acetophenone and anticancer activities in LNCaP, PC-3, DU145, HeLa, MD-MBA, MCF-7, MDA-MB-231, HepG2 cell lines were investigated.

The structures of the new bimetallic complexes (**Ru_{2a}**, **Ru_{2b}**, **Ru_{2c}**,), and monometallic complexes (**Ru_{1a}**, **Ru_{1b}**, **Ru_{1c}**) have been characterized by spectroscopic techniques.

Transfer hydrogenation reduction of acetophenone performed by the **Ru_{2a}**, **Ru_{2b}**, **Ru_{2c}**, **Ru_{1a}**, **Ru_{1b}** complexes. Bimetallic complex **Ru_{2a}** exhibit the best activity in the transfer hydrogenation of acetophenone.

A comparision of anticancer activity by MTT analysis showed that cytotoxic activities of complexes varied depending on the cell line. In LNCaP, PC-3 and DU145 cell lines monometallic **Ru_{1a}** showed the best cytotoxicity. The **Ru_{1a}** complex showed cytotoxic activity even at low concentrations in PC-3 cell lines and choosen for Western-blot test to investigate of changes in the protein levels. Bimetallic complexes showed the better cytotoxicity than monometallic ones in HeLa, MCF-7, MDA-MB-231 cell lines.

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