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Synopsis for the Graphical Abstract

Catalytic transfer hydrogenation and anticancer activity of arene-ruthenium compounds incorporating bi-dentate precursors

Yu-Hsiang Chang,[a] Wohn-Jenn Leu,[b] Amitabha Datta,[a] Hung-Chang Hsiao,[a] Chia-Her Lin,[c] Jih-Hwa Guh,[b,*] Jui-Hsien Huang[a,*]

A series of arene-Ru compounds were synthesized and their catalytic transfer hydrogenation and anticancer activity towards human hormone-refractory prostate cancer were investigated.

Pictogram for the Graphical Abstract

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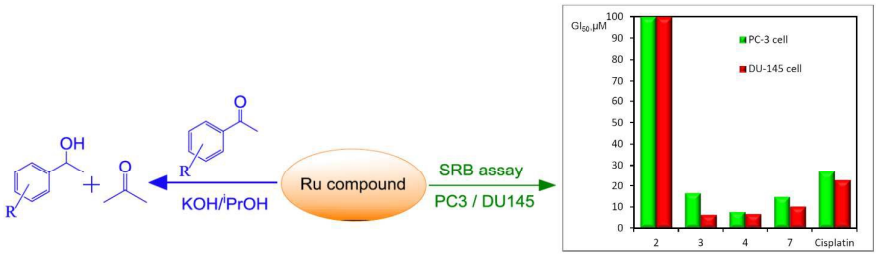

Catalytic transfer hydrogenation and anticancer activity of arene-ruthenium compounds incorporating bi-dentate precursors

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Keywords: Catalytic transfer hydrogenation; anticancer activity; ruthenium; pyrrole; keto-amine ligands

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Abstract

Ruthenium based organometallic compounds are presently an object of great attention as anticancer drugs and appear to work reasonably well on tumor cells. We develop a series of mononuclear arene-ruthenium compounds incorporating N,O and N,N bidentate ligands and their activity as anticancer drugs against human hormone-refractory metastatic prostate cancer (HRMPCs) cell lines are governed. The ruthenium compounds also indulge as effective catalysts in the transfer hydrogenation of -C=O- $\rightarrow$ -CH(OH)- system. Three types of ligands, sodium glutamate, $C_4H_3NH(2\text{-}CH_2NH^tBu)$, and $C_4H_3NH(2\text{-}CH{=}NR)$ are separately coupled with $[(\eta^6\text{-cymene})RuCl_2]_2$ (**1**) (cymene = 4-isopropyltoluene) to synthesize five Ru-derivatives, such as $[(\eta^6\text{-cymene})RuCl(\kappa^2\text{-}N,O\text{-}OOCCHNH_2CH_2CH_2COOH)]$ (**2**), $\{(\eta^6\text{-cymene})RuCl[C_4H_3N(2\text{-}CH_2NH^tBu)]\}$ (**3**), $\{(\eta^6\text{-cymene})RuCl[C_4H_3N(2\text{-}CH{=}NCH_2Ph)]\}$ (**4**), $\{(\eta^6\text{-cymene})RuCl\{C_4H_3N[2\text{-}CH{=}NCH_2(C_4H_7O)]\}\}$ (**5**) and $\{(\eta^6\text{-cymene})RuCl[C_4H_3N(2\text{-}CH^nBuNHCH_2(C_4H_7O))]\}$ (**7**). To the best of our knowledge, the aforementioned Ru compounds are not only characterized by ^1H and ^{13}C NMR spectroscopy but first time established by single crystal X-ray diffractometry. Compound **4** induces a concentration-dependent apoptosis in

PC-3 cells and initiates the conversion rate in transfer hydrogenation.

Introduction

Platinum,[1] a precious metal, is widely used in organic and medicinal chemistry. Regarding the metal-based anticancer drugs,[2] cisplatin[3] is the most well-known drug for chemotherapy; however, several concerns have raised the attention of using cisplatin due to (i) the platinum production limitation that makes the platinum price increasing dramatically,[4] (ii) drug resistance,[5] and (iii) side effects[6] including nausea, vomiting, diarrhea, and hair loss. Therefore, the development of other metal-based anticancer agents is currently an interesting approach for inorganic and bioinorganic chemists. In searching for metal-based anticancer agents with less side effects, ruthenium-based compounds have shown promising results presumably due to (i) the variable oxidation states and higher coordination number, (ii) slower ligand exchange rates, and (iii) easier reducing capacity from Ru(III) to Ru(II), i.e. less active and non-toxic Ru(III) compounds that can be activated at the site of the cancerous cells.[7] Interest in Ru anticancer drugs has been growing rapidly since NAMI-A,[8] KP1019,[9] RM175,[10] RATPA[11] and DW1[12] as shown in Scheme 1 have either entered into phase I or II clinical trial or shown highly potential for

antitumor activity. The diversity of modes of action of Ru anticancer drugs is also likely to enhance the anticancer activities and to reduce the potential to develop tumor resistance.

The catalytic hydrogenation of polar bonds and the asymmetric versions of these are key reactions in fine chemical and pharmaceutical synthesis. Ruthenium complexes display favorable reactivity and selectivity, since it has a strong tendency to perform a heterolytic activation of H_2 instead of oxidative addition to make a metal dihydride.[13] Ruthenium compounds have remarkable catalytic activity and easy accessibility in asymmetric transfer hydrogenation of ketones, a convenient route of enantiomerically pure alcohols.[14] In past years, some papers dealing with Ru(II) complexes containing N,O donor ligands have been published,[15] in order to compare their structure and reactivity with those of similar ruthenium derivatives, which show promising catalytic activity. Noyori et al.[16] before reported the half-sandwich (arene)Ru(II) complex that undergoes to catalytic transformations, such as, for example, the highly efficient asymmetric hydrogen transfer on C=O containing substrates. We also checked some articles[17] on the synthesis and reactivity of arene-ruthenium derivatives as well as N,O donor ligands.[18]

From literature survey it is clear that ruthenium complexes have

interesting anticancer properties *in vivo* and they might be a good alternative of platinum-based drugs for anticancer therapy. Besides this, we have put our attention to furnish supportive information on Ru-catalyzed asymmetric transfer hydrogenation. We prefer the Ru derivatives incorporating bidentate N,O or N,N donor ligands to put less steric hindrance around '[Ru-H]' motif that initiates the conversion rate of ketone to alcohol. As well our approach is to check the potentiality of Ru derivatives as drugs in anticancer effect against human hormone-refractory metastatic prostate cancers (HRMPCs), one of the most commonly diagnosed cancers and top leading cause of cancer-related deaths in various countries.

Results and Discussion

Synthesis and characterization of compounds 2-7. A series of ruthenium compounds mediating bidentate N,O or N,N ligands are synthesized, as shown in Scheme 2 and 3. The initial reactant, $[(\eta^6\text{-cymene})RuCl_2]_2$ (cymene = 4-isopropyltoluene),[19] (**1**), obtained from $RuCl_3.nH_2O$ and phellandrene, is refluxed with two equivs of sodium glutamate in methanol to produce the new ruthenium compound $[(\eta^6\text{-cymene})RuCl(\kappa^2\text{-}N,O\text{-}OOCCHNH_2CH_2CH_2COOH)]$ (**2**), in moderate yield (Scheme 2). Compound **2** is insoluble in organic solvents

like toluene, diethyl ether, methylene chloride, and THF but soluble in highly polar solvents like methanol and H_2O. On ^1H NMR spectroscopy, the methyl, methylene, and methine protons of **2** show resonances between δ 1.1 ~ 3.5 and the aromatic protons of cymene ring belong in the range, δ 5.5 ~6.0.

Similarly, reacting **1** with two equivs of lithium pyrrolyl amine ligand, $Li[C_4H_3N(2\text{-}CH_2NH^tBu)]$ in toluene generates compound $\{(\eta^6\text{-cymene})RuCl[C_4H_3N(2\text{-}CH_2NH^tBu)]\}$ (**3**) in satisfactory yield. Compound **3** is soluble in methylene chloride, toluene, and THF but only has partial solubility in diethyl ether. The ^1H NMR spectrum of **3** shows two doublets of doublet for the methylene protons of CH_2NH^tBu at δ 3.67 and 3.81. The amino proton of CH_2NH^tBu fragment is observed at δ 3.29 as a broad multiplet due to the coupling with adjacent methylene protons, subsequently confirmed by homo-nuclear decoupled ^1H NMR spectra. The asymmetrical environment around the Ru center can be affirmed from the four arene protons of cymene moiety showing two multiplets at δ 5.34 and 5.49.

Further reaction of **1** with lithiated pyrrole imine system features new organo-ruthenium derivatives, as shown in Scheme 3. Reacting pyrrole-imine, $C_4H_3NH(2\text{-}CH{=}NCH_2Ph)$, with *n*-BuLi in methylene chloride generates a pyrrolyl lithium salt, $Li[C_4H_3N(2\text{-}CH{=}NCH_2Ph)]$, which afterwards precipitates

in addition of heptane. Interestingly, conjugation of two equivs of Li[C_4H_3N(2-CH=NCH_2Ph)] and **1** in toluene originates compound {(η^6-cymene)RuCl[C_4H_3N(2-CH=NCH_2Ph)]} (**4**) in 42% yield. The ^{1}H NMR spectrum of compound **4** shows four doublets for the four arene protons of cymene and two doublets for the methyl groups of CH*Me*$_2$ fragment, indicating its asymmetrical arrangement around the Ru center. The chemical shift of methylene protons of NC*H*$_2$Ph is observed at δ 5.13, overlapping with the resonance of one arene protons. Similarly, the coupling of **1** and another bidentate pyrrole-imine ligand, C_4H_3NH[2-CH=NCH_2(C_4H_7O)] is also investigated. While two equivs of C_4H_3NH[2-CH=NCH_2(C_4H_7O)] is directly added to the THF solution of **1** at room temperature, compound {(η^6-cymene)RuCl{C_4H_3N[2-CH=NCH_2(C_4H_7O)]}} (**5**) is initiated along with the elimination of two equivs of hydrogen chloride. However, the low yield (32%) could not be raised by adding strong bases like NaOH or NEt_3 to remove hydrogen chloride. Compound **5** is also obtained from the reaction of **1** with lithiated ligand, Li{C_4H_3N[2-CH=NCH_2(C_4H_7O)]}, a product of *n*-BuLi and C_4H_3NH[2-CH=NCH_2(C_4H_7O)] in methylene chloride as similar yield. After repeating re-crystallization of compound **5** from diethyl ether, the imine fragment (CH=N) shows a pair of characteristic proton and carbon resonances,

at δ 7.50 & 7.60 and at δ 160.0 & 159.7, respectively. It seems that the tetrahydrofuranyl fragment of **5** may flip away or belong towards the Ru atom (see Scheme 4), subjecting the appearance as diastereoisomers to achieve two sets of signals. They have chirality at the metal center, and an additional asymmetric carbon in the ligand.

Moreover, a lithiated compound Li[C_4H_3N(2-CH^{n}BuNHCH_2(C_4H_7O))] (**6**) is isolated while aggregating C_4H_3NH[2-CH=NCH_2(C_4H_7O)] and *n*-BuLi in heptane. The ^{1}H and ^{13}C NMR spectra of **6** supports the insertion of anionic butyl group into the C=N double bond to result a lithiated pyrrolyl amino derivative. The ^{1}H NMR spectrum of compound **6** shows the corresponding singlet of N*H* proton at δ 9.74. Treating compound **1** with **6** in toluene generates a ruthenium pyrrole compound, {(η^6-cymene)RuCl[C_4H_3N(2-CH^{n}BuNHCH_2(C_4H_7O))]} (**7**) in moderate yield. The ^{1}H and ^{13}C NMR spectra of **7** are not pure due to the overlapping of the butyl, tetrahydrofuranyl, methyl, and isopropyl moieties (see supporting information).

Molecular structure of compounds. Crystals of compounds **2**-**5** and **7**, suitable for single crystal X-ray diffraction study, are obtained from different

organic solvents. The summary of data collection and selected bond lengths & angles are listed in Table 1 and 2, respectively. Inserting monosodium glutamate in binding metals is reported;[20] however, there is no survey of crystal structures concerning glutamato-based metal derivative till date. To the best of our knowledge, compound **2** is the first Ru-based glutamate derivative, supported by X-ray diffraction analysis. The asymmetric unit consists of two independent molecules of **2**. However, these two molecules are quite similar; so one molecular unit is presented in Figure 1. Compound **2** shows a three-leg piano stool geometry with the Ru to the center of cymene at ca. 1.6 Å.[21] The glutamate moiety binds to Ru through the amine nitrogen and one of carboxylate oxygen atoms forming a five-membered chelate ring. The bond lengths of Ru to glutamate are Ru(1)-O(1) 2.080(14) and Ru(1)-N(1) 2.128(16) Å. Interestingly, it is observed that the corresponding bond lengths of the coordinate carboxylate group of the glutamate shows neither double bond nor single bond character [1.22(2) and 1.33(3) Å] since it involves in resonance while coordinating to Ru atom (Scheme 5), but the uncoordinated carboxylate unit displays a regular double bond mode [1.18(3) Å]. Compound **2** exhibits both intra- and inter-molecular hydrogen bonding network (Figure 2 and Table S2), to strengthen the molecular stability as reported earlier.[22]

The molecular geometries of compounds **3**-**5** and **7** are quite similar and depicted in Figures 3-6. The unit cells for compounds **3**-**5** all contain two molecular units where compound **3** shows an intra-molecular Cl···H hydrogen bonding with the bond distance about 2.5570(4) Å. Compound **3**-**5** and **7** show three-leg piano stool geometries with the bond lengths of Ru to the center of cymene and to the Cl atoms, ranging from 1.6751(4)~1.6843(5) Å and 2.4094(13)~2.4210(16) Å, respectively. Two types of substituted asymmetric pyrrole ligands are involved in coordination with compounds **3**, **4**, **5**, and **7**. Besides Ru-$N_{pyrrole}$ bond, a pyrrole-amine bond is incorporated in compounds **3** and **7**, whereas there exists a pyrrole-imine bond in compounds **4** and **5**. The Ru-$N_{pyrrole}$ bond lengths for compounds **3**, **4**, **5**, and **7** are very similar, ca. 2.042(4)~2.066(4) Å, despite different ligand modes. However, Ru-$N_{pyrrole\text{-}amine}$ and Ru-$N_{pyrrole\text{-}imine}$ bond lengths are slight different, Ru-$N_{pyrrole\text{-}amine}$ bond lengths (2.215(4) Å for **3** and 2.170(4) Å for **7**) are much longer than those of Ru-$N_{pyrrole\text{-}imine}$ bond lengths (2.114(5) Å for **4** and 2.119(4) Å for **5**), presumably due to the steric hindrance of the bulkier pyrrole-amine ligands.

Anticancer activity in HRMPC cells. The sulforhodamine B (SRB) assay is developed to measure drug-induced anti-proliferative activity and cytotoxicity

study on human cell-lines. The related data demonstrate that several arene-Ru derivatives display higher anti-proliferative activity than cisplatin on HRMPC cell lines, namely PC-3 and DU-145 (see SI, Table S1). The obtained data show that PC-3 cells are more susceptible to the compound-induced cytotoxicity than DU-145 cells using SRB assay. The effect of arene-ruthenium compounds on HRMPC cells while fixing with propidium iodide to analyze DNA content is represented in Figure 7. It is observed that compounds **3**, **4** and **7** induce a concentration-dependent apoptosis in PC-3 cells; among these compound **4** participates significantly as its 30 μM conc. on 48 hrs duration.

The mitochondria, a pivotal organelles in the cytoplasm, participates in potential anticancer strategy to induce cancer cell death.[23] The mitochondrial membrane potential ($\Delta\Psi_m$) is determined using flow cytometric analysis of JC-1 staining. JC-1 aggregates (red fluorescence) higher $\Delta\Psi_m$ under normal condition. After drug-induced loss of $\Delta\Psi_m$, JC-1 monomers are dominant with green fluorescence, the indicator of mitochondrial dysfunction. The data in Figure 8 demonstrate that both compounds **3** and **4** result in a significant production of JC-1 monomers (R2 green fluorescence) indicating the loss of $\Delta\Psi_m$. Thus following JC-1 assay, we observe that compound **4** is well effective for mitochondria-involved apoptotic detection.[24]

Caspases, a family of cysteine proteases, play a crucial role in apoptosis. Caspase-9 is an initiator caspase in mitochondrial apoptosis pathway in which the pro-enzyme of caspase-9 is cleaved into a 37-kDa active form.[25] Mitochondrial apoptosis pathway may induce a crosstalk activation of caspase-8, a major initiator caspase in extrinsic apoptosis pathway. Recently, the immune fluorescence microscopic examination and biochemical analysis show that pro-caspase-8 and active caspase-8 predominantly co-localize with the mitochondria mainly on outer mitochondrial membrane.[26] The corresponding data (see SI, Figure S1) demonstrate that the most potential compound **4** significantly induces the activation of caspase cascades, including the activation of caspase-9, -8, -3 and -7.

Transfer hydrogenation catalytic study. Homogeneous asymmetric hydrogenation of olefins and ketones catalyzed by chiral transition metal complexes provides a powerful means for preparing optically active organic compounds.[27] The reduction of carbonyl [C=O] groups to pure secondary alcohols is a reaction of fundamental importance, more particularly the asymmetric reduction of ketone to enantiometric secondary alcohols in modern synthetic chemistry. For asymmetric hydrogenation, Ru(II) compounds are

efficient catalysts[28] such as in the reaction of -C=O- → -CH(OH)- in alcoholic medium maintaining basic pH where H_2 or other reducing agents like N_2H_4, $LiAlH_4$, $NaBH_4$ may be used. In hydrogenation, the hydrido metal compounds ($MH(L)_n$) may participate as catalyst and then can accelerate the reduction of ketone in the catalytic cycle where reducing equivalent-H may be supplied by the reducing solvent (like isopropanol) or from other reducing agents. Presence of ancillary ligands to Ru(II) critically control the reaction efficiency and percentage of yield. Electron donating substrate may reduce the stability of hydrido-Ru(II) '[Ru-H]' motif and hence decreases the rate. Besides the presence of acidic substituent or labile-H in any part of the catalyst or substrate or solvent also scavenge '[Ru-H]' and reduces the crop yield. Possible reduction mechanism is given in Scheme 6. The results of a series of substituted acetophenone reduced to isopropyl alcohol in the presence of ruthenium compounds is shown in Table 3. Although the ruthenium compounds play as catalysts; however, the substituents on the acetophenone also play an important role in the reduction transfer hydrogenation. Compounds **4** and **5** have higher reactivity toward the transfer hydrogenation reactions (entry 3, 4, 9, 10, 15 and 16, Table 3) in comparing other substituted acetophenones like **2**, **3**, and **7**. Presumably, the pyrrole-imine ligand systems

in compounds **4** and **5** may stabilize Ru-H system due to π-donating ability of the chelated unit. Electron withdrawing groups on the aryl ring of ketones such as 4'-nitro-acetophenone dramatically reduce the conversion of ketone to alcohol (entry 19-23, Table 3). This result indicates the coordination of C=O fragments of ketones to the ruthenium atom before forming Ru-H---C-O four-membered ring (Scheme 6), a critical step for the transfer hydrogenation. The phenomenon is also verified by the low conversion of sterically bulky 2',4',6'-trimethylacetophenone and isopropyl alcohol (entry 24-28, Table 3).

Conclusion

In this article we highlight the current interest on ruthenium based metallodrugs. Besides the characterization by ^{1}H and ^{13}C NMR spectroscopy, the single crystal X-ray diffraction analysis confirms the geometry of Ru atom. The ruthenium compounds containing pyrrole-imine ligand systems show higher conversion of transfer hydrogenation from ketone-substrates to secondary alcohols. In addition, the aryl ring of acetophenone with electronic withdrawing and sterically hindered bulky groups defer the transfer hydrogenation in presence of Ru catalysts. Furthermore, the toxicity of the compounds is tested on HRMPC cell lines, namely PC-3 and DU-145.

Compound **4** is the most influential to display effective anticancer activity against HRMPCs and in apoptotic cell death through mitochondria-involved activation of caspase cascades. Further investigations with Ru-derivatives manifested by newly designed organic precursors and their catalytic approach on transfer hydrogenation as well as the potentiality on apoptosis in human cells are under way.

Experimental Section

General procedure: All reactions were performed under a nitrogen atmosphere using standard Schlenk techniques or in a glove box. Toluene and diethyl ether were dried by refluxing over sodium benzophenone ketyl. CH_2Cl_2 was dried over P_2O_5. All solvents were distilled and stored in solvent reservoirs that contained 4-Å molecular sieves and were purged with nitrogen. $[(\eta^6\text{-cymene})RuCl_2]_2$ (cymene = 4-isopropyltoluene),[19] $[LiC_4H_3N\text{-}(2\text{-}CH{=}NCH_2\text{-}C_4H_7O)]$,[29] $[LiC_4H_3N\text{-}(2\text{-}CH{=}NCH_2\text{-}C_6H_5)]$[30] and $[LiC_4H_3N\text{-}(2\text{-}CH_2NH^tBu]$[31] were prepared according to published procedures. ^1H and ^{13}C NMR spectra were recorded using a Bruker Avance 300 spectrometer. The chemical shifts for ^1H and ^{13}C spectra were recorded in ppm relative to the residual protons of $CDCl_3$ (δ =7.24, 77.0 ppm) and C_6D_6 (δ =

7.16, 128.0 ppm). Elemental analyses were performed using a Heraeus CHN-OS Rapid Elemental Analyzer at the Instrument Center of the NCHU.

Synthesis of [(η^6-cymene)RuCl(κ^2-*N,O*-OOCCHNH$_2$CH$_2$CH$_2$COOH)] (2). A 250 mL round bottom flask was charged with 1.0 g of **1** (1.6 mmol), excess of monosodium glutamate (0.52 g, 3.2 mmol), and 100 mL of methanol. The solution was then refluxed for 4 hours and filtered to avoid un-soluble solid. The filtrate was reduced to small amount and stored at -20°C to yield 0.56 g of final product (41.0%). Crystals suitable for X-ray diffraction study were obtained from a methanol solution of at -20°C. ^{1}H NMR (δ, D_2O): 1.26 (m, 6H, CH*Me*$_2$), 2.17 (m, 3H, *Me*), 2.51 (m, 2H, CH$_2$C*H*$_2$CHNH$_2$CO$_2$), 2.79 (sept, J_{HH} = 6.6Hz, 1H, C*H*Me$_2$), 3.05 (m, 1H), 5.76 (m, 4H). Anal. Calcd. for $C_{15}H_{22}NClO_4Ru$: C, 43.22; H, 5.32; N, 3.36. Found: C, 43.78; H, 5.41; N, 3.35%.

Synthesis of {(η^6-cymene)RuCl[C$_4$H$_3$N(2-CH$_2$NH^{t}Bu)]} (3). A Schlenk flask charged with 0.10 g (0.16 mmol) of **1** and 15 mL of toluene was added a 15 mL toluene solution of [LiC$_4$H$_3$N-(2-CH$_2$NH^{t}Bu] (0.052 g, 0.32 mmol) dropwise at 0°C. The mixture was stirred for 6 hours at room temperature and filtered through celite. The filtrate was concentrated to small amount of volume and

stored at -20°C to yield 0.071 g of brown-red final product (51.0%). Crystals suitable for single crystal X-ray diffraction analysis were obtained using diffusion method from a methylene chloride/heptane combined solvent. ^{1}H NMR (δ, $CDCl_3$): 0.99 (d, J_{HH} = 6.9 Hz, 3H, CH*Me*$_2$), 1.11 (d, J_{HH} = 6.9 Hz, 3H, CH*Me*$_2$), 1.46 (s, 9H, ^{t}*Bu*), 2.09 (s, 3H, *Me*), 2.52 (sept, J_{HH} = 6.9 Hz, 1H, C*H*Me$_2$), 3.29 (br, 1H, N*H*), 3.67 (dd, 1H, C*H*$_2$), 3.81 (dd, 1H, C*H*$_2$), 5.34 (m, 2H, arene C*H*), 5.49 (m, 2H, arene C*H*), 5.90 (m, 1H, pyr C*H*), 6.19 (m, 1H, pyr C*H*), 6.85 (m, 1H, pyr C*H*). ^{13}C{^{1}H} NMR (δ, $CDCl_3$): 18.2, 20.8, 23.2, 29.2, 30.7, 49.7, 57.1, 75.1, 82.1, 82.6, 86.0, 96.7, 101.1, 104.4, 109.8, 128.7, 133.7. Anal. Calcd. for $C_{19}H_{29}N_2ClRu$: C, 54.08; H, 6.93; N, 6.64. Found: C, 54.05; H, 6.92; N, 6.50%.

Synthesis of {(η^6-cymene)RuCl[C_4H_3N(2-CH=NCH_2Ph)]} (4). Similar procedure as for synthesizing compound **3** is adopted. **1** (0.20 g, 0.32 mmol) and [LiC_4H_3N-(2-CH=NCH_2-C_6H_5)] (0.13 g, 0.64 mmol) were used. The filtrate was concentrated to small amount of volume and stored at -20°C to yield 0.12 g of yellowish final product (41.7%). Crystals suitable for single crystal X-ray diffraction analysis were obtained using diffusion method from a methylene chloride/heptane combined solvent. 1H NMR (δ, $CDCl_3$): 0.83 (d, J_{HH} = 6.9 Hz, 3H, CH*Me*$_2$), 1.05 (d, J_{HH} = 6.9 Hz, 3H, CH*Me*$_2$), 2.07 (s, 3H, *Me*), 2.50 (sept,

J_{HH} = 6.9 Hz, 1H, C*H*Me$_2$), 4.81 (m, 1H, arene C*H*), 4.96 (m, 1H, arene C*H*), 5.13 (m, 3H, NC*H*$_2$ + arene C*H*), 5.43 (m, 1H, arene C*H*), 6.27 (m, 1H, pyr C*H*), 6.65 (m, 1H, pyr C*H*), 7.41 (m, 6H, phenyl + pyr C*H*), 7.50 (s, 1H, C*H*=NCH$_2$). ^{13}C{^{1}H} NMR (δ, $CDCl_3$): 18.8, 21.6, 22.7, 30.9, 80.3, 81.6, 82.6, 83.9, 99.0, 101.3, 112.9, 116.1, 138.3, 138.5, 139.2, 159.4. Anal. Calcd. for $C_{22}H_{25}N_2RuCl$: C, 58.21; H, 5.55; N, 6.17. Found: C, 57.24; H, 5.48; N, 5.88%.

Synthesis of {(η^6-cymene)RuCl[C_4H_3N(2-CH=NCH$_2$(C_4H_7O))]} (5). A flask charged with **1** (0.20 g, 0.32 mmol) and {C_4H_3NH[2-CH=NCH$_2$(C_4H_7O)]} (0.115 g, 0.64 mmol) was added 15 mL of methanol. The solution was stirred for 3 hrs at room temperature and an excess of NaOH was added. The solution was then stirred for another 3 hrs and solvent was removed under vacuum. The residue was extracted with diethyl ether and filtered through celite. The filtrate was concentrated to small volume and stored at -20°C to yield 0.092 g of final product (32%). Crystals suitable for X-ray diffraction study were obtained from a methylene chloride solution of at -20°C. ^{1}H NMR (δ, d^8-THF): 0.88-1.05 (m, 6H, CH*Me*$_2$), 1.39-1.80 + 2.09 (m, 7H,C*H*$_2$ + C*H*$_3$), 2.51 (sept, 1H, C*H*Me$_2$), 3.57-4.30 (m, 5H, C*H*$_2$O + C*H*O + C*H*$_2$N), 5.22-5.60 (m, 4H, C_6H_4), 6.04 (m, 1H, pyr C*H*), 6.43 (t,1H, pyr C*H*), 7.32 (s, 1H, pyr C*H*), 7.50 + 7.60 (two s, 1H, C*H*=NCH$_2$). Anal. Calcd. for $C_{20}H_{27}N_2OClRu$: C, 53.62; H, 6.07; N, 6.25. Found:

C, 54.07; H, 5.62; N, 6.72%.

Synthesis of Li[C_4H_3N(2-CH^{n}BuNHCH$_2$(C_4H_7O))] (6). A flask charged with C_4H_3NH[2-CH^{n}BuNHCH$_2$(C_4H_7O)] (0.50 g, 2.80 mmol) and 10 mL of THF was slowly added a heptane solution (10 mL) of ^{n}BuLi (2.5 M, 1.12 mL, 2.80 mmol) at 0°C and stirred for 3 hrs. The suspension was filtered and the resulted solid was re-crystallized from a saturated THF solution at -20°C to yield 0.464 g of final product (68.4%). ^{1}H NMR (δ, d^8-THF): 0.86 (m, 3H, *Me*), 1.27-1.80 (m, 10H, CH_2), 2.48 (m, 2H, NCH_2), 3.55-3.76 (m, 4H, C*H*O + CH_2O + C*H*BuN), 5.83 (m, 2H, pyr), 6.60 (m, 1H, pyr), 9.74 (s, 1H, N*H*). ^{13}C NMR (δ, d^8-THF): 14.50, 23.7, 26.5, 29.6, 29.8, 37.6, 59.0, 68.2, 79.7, 106.2, 107.3, 119.3. Anal. Calcd. for $C_{14}H_{23}N_2OLi$: C, 69.40; H, 9.57; N, 11.56. Found: C, 69.80; H, 9.12; N, 11.81%.

Synthesis of {(η^6-cymene)RuCl[C_4H_3N(2-CH^{n}BuNHCH$_2$(C_4H_7O))]} (7). A flask containing **1** (0.20 g, 0.32 mmol) and 15 mL of toluene was added dropwise a toluene solution (15 mL) of **6** (0.16 g, 0.64 mmol) at 0°C. The solution was stirred for 4 hrs at room temperature and filtered through celite. The filtrate was concentrated to small volume and stored at -20°C to yield 0.33 g of final product (64.0%).Crystals suitable for X-ray diffraction study were obtained from a methylene chloride solution of at -20°C. Anal. Calcd. for

$C_{24}H_{37}N_2ClORu$: C, 56.96; H, 7.37; N, 5.53. Found: C, 57.05; H, 7.27; N, 5.58%.

X-ray structure determination. All of the crystals for compounds **2**-**5** and **7** were mounted on a glass fiber using epoxy resin and transferred to a goniostat. The data were collected on a Bruker SMART CCD diffractometer using graphite monochromated Mo-K_α radiation. The crystal data were collected at room temperature and no significant crystal decay was found. The data were corrected for absorption empirically via ψ scans. All non-hydrogen atoms were refined using anisotropic displacement parameters. For all of the structures, the hydrogen atom positions were calculated, and they were constrained to idealized geometries and treated as riding where the H atom displacement parameter was calculated from the equivalent isotropic displacement parameter of the bound atom. The structures were determined using direct-method procedures in SHELXS[32] and refined using full-matrix least-squares methods on F^2's in SHELXL.[33] All the relevant crystallographic data and structure refinement parameters are summarized in Table 1. The crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary

publications nos. CCDC-1034394 (**2**), CCDC-1034395 (**3**), CCDC-1034396 (**4**), CCDC-1034397 (**5**) and CCDC-1034398 (**7**). These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Transfer Hydrogenation. A flask was charged with Ru compound (0.005 mmol, ~0.002 g), KOH (1 mmol) and 2-propanol (5 mL). The solution was stirred at 82°C for 10 mins and cooled to room temperature. Substituted acetophenone (1 mmol, 0.116 mL) was added and the resulting solution was stirred at 82°C for 3 hrs. The volatiles then were removed under vacuum and residues were characterized by ^{1}H NMR spectroscopy.

Cell lines and cell culture. Human HRMPC cell lines PC-3 and DU-145 were provided from American Type Culture Collection (Rockville, MD). Cells were cultured in RPMI 1640 medium with 10% FBS (v/v) and penicillin (100 U/mL)/streptomycin (100 µg/mL). Cultures were maintained in a humidified incubator at 37^{o}C in 5% CO_2/95% air.

SRB assays. Cells were seeded in 96-well plates in medium with 5% FBS.

After 24 hours, cells were fixed with 10% TCA to represent cell population at the time of drug addition (T_0). After additional incubation of DMSO or the compound for 48 hours, cells were fixed with 10 % TCA and SRB at 0.4% (w/v) in 1% acetic acid was added to stain cells. Unbound SRB was washed out by 1% acetic acid and SRB bound cells were solubilized with 10 mM Trizma base. The absorbance was read at a wavelength of 515 nm. Using the following absorbance measurements, such as time zero (T_0), control growth (C), and cell growth in the presence of the compound (T_x), the percentage growth was calculated at each of the compound concentrations levels. Percentage growth inhibition was calculated as: $[1-(Tx-T_0)/(C-T_0)] \times 100\%$. Growth inhibition of 50% (IC_{50}) is determined at the compound concentration which results in 50% reduction of total protein increase in control cells during the compound incubation.

Flow cytometric assay of DNA content. After the treatment, the cells were harvested by trypsinization, fixed with 70% (v/v) alcohol at 4^{o}C for 30 min and washed with PBS. After centrifugation, cells were incubated in 0.1 mL of phosphate-citric acid buffer (0.2 M $NaHPO_4$, 0.1 M citric acid, pH7.8) for 30 min at room temperature. Then, the cells were centrifuged and re-suspended

with 0.5 mL PI solution containing Triton X-100 (0.1% v/v), RNase (100 μg/mL) and PI (80 μg/mL). DNA content was analyzed with FAC Scan and Cell Quest software (Becton Dickinson, Mountain View, CA).

Measurement of mitochondrial membrane potential ($\Delta\Psi_m$). JC-1, a mitochondrial dye staining mitochondria in living cells in a membrane potential-dependent fashion, was used to determine $\Delta\Psi_m$. Cells were treated with or without compound. Thirty minutes before the termination of incubation, the cells were incubated with JC-1 (final concentration of 2 μM) at 37°C for 30 min. The cells were finally harvested and the accumulation of JC-1 was determined using flow cytometric analysis.

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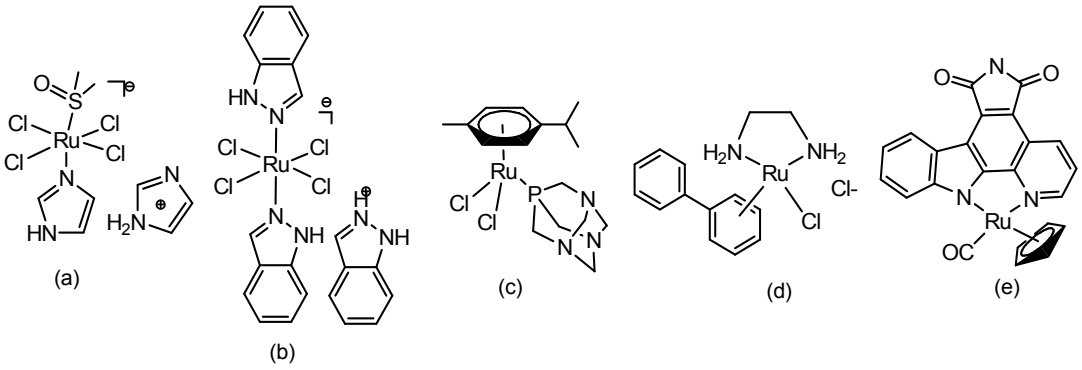
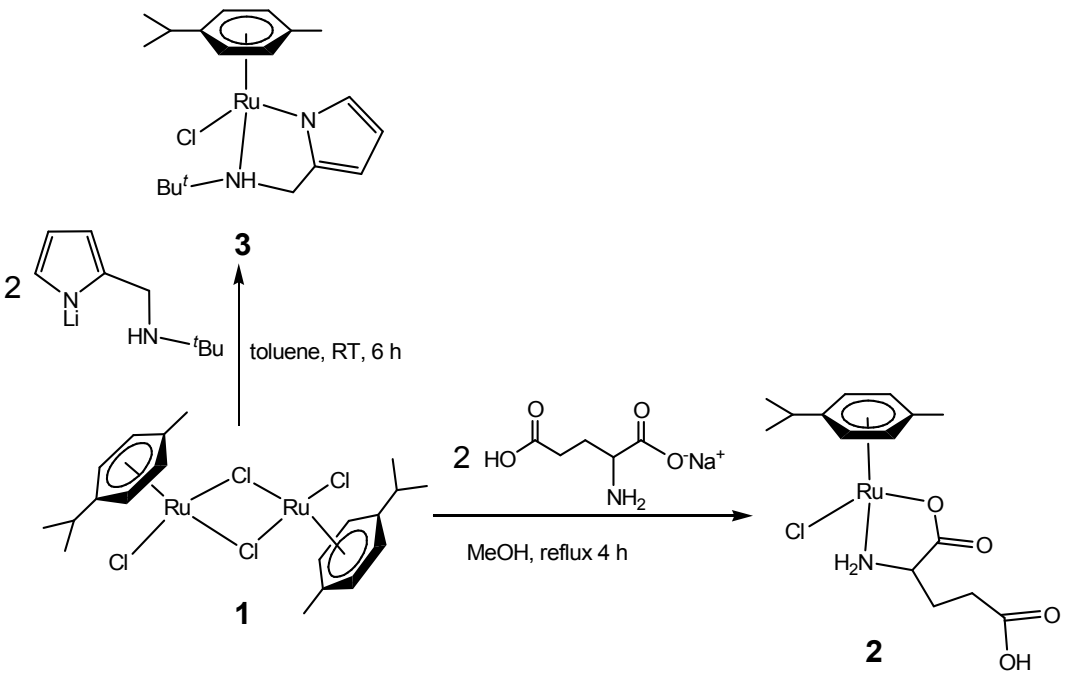
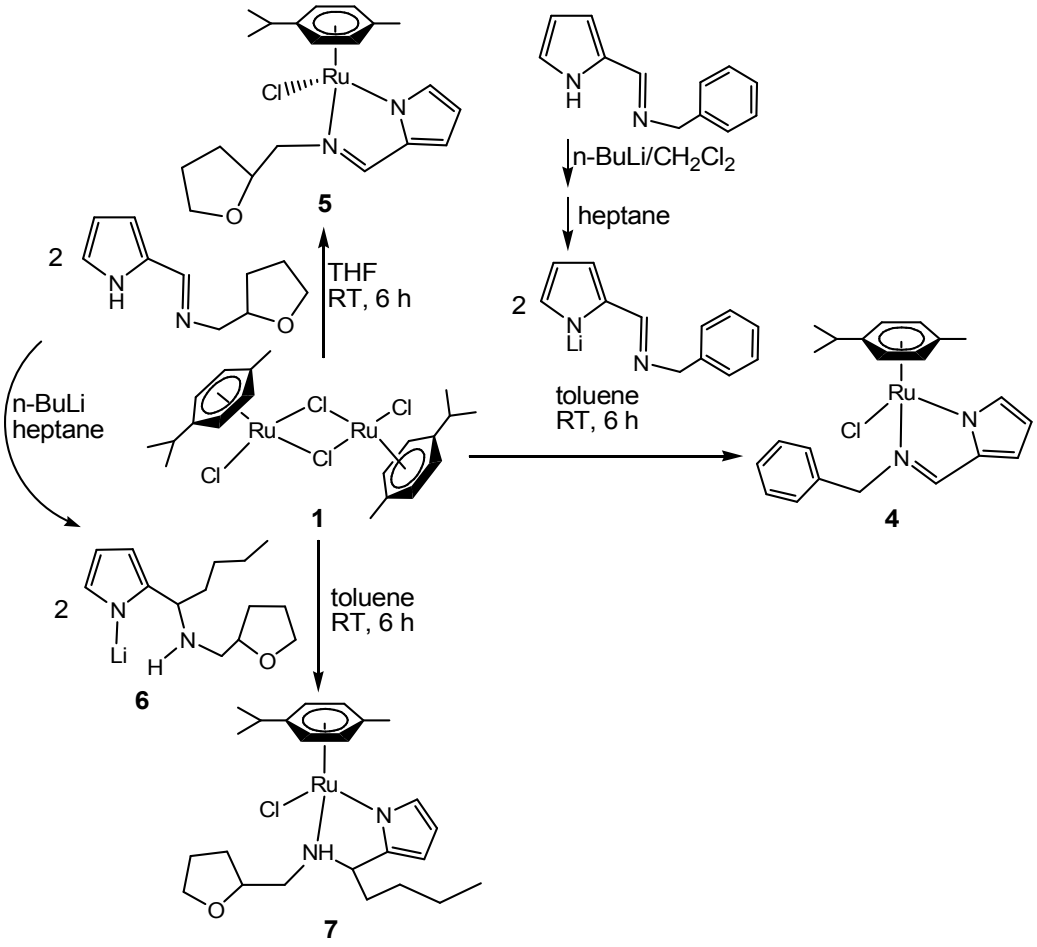
Scheme 1. Examples of Ru-based anticancer agents



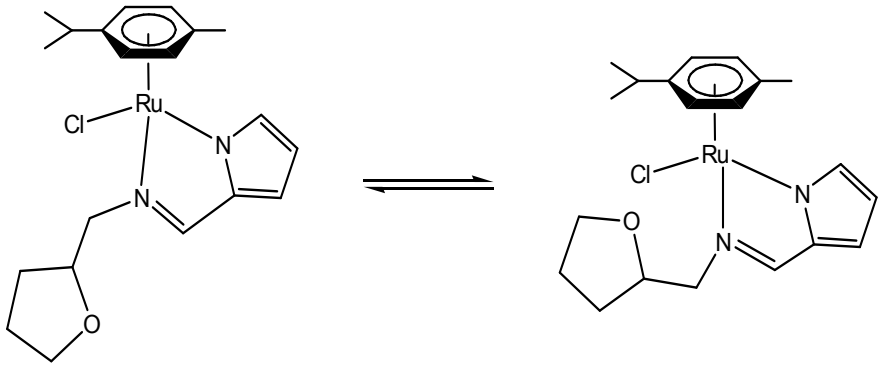
Scheme 2. Synthesis of compounds **2** and **3**



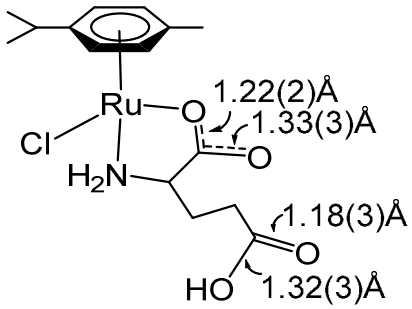
Scheme 3. Synthesis of compounds **4**-**7**



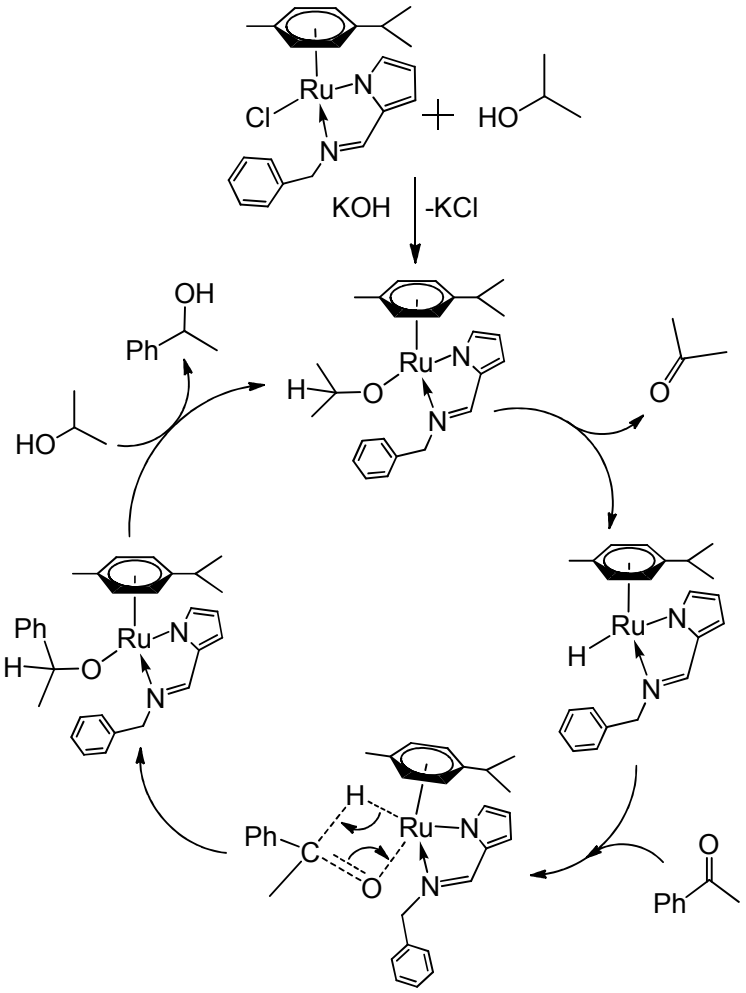
Scheme 4. The equilibrium of the tetrahydrofuranyl fragment of **5** flips away or toward the Ru atom



Scheme 5. The bond lengths of two carboxylate groups of the glutamate moiety showing a resonance form while bonding to Ru and a regular double bond mode while no metal coordinated



Scheme 6. Possible reaction mechanism of transfer hydrogenation.



Captions to Figures 1-8

Figure 1. The molecular geometry of **2**. Thermal ellipsoids were drawn at 50 % probability.

Figure 2. The molecular geometry of **2** showing the intra- (red dashed line) and inter (blue dashed line) molecular hydrogen bonding.

Figure 3. The molecular geometry of **3**. Thermal ellipsoids were drawn at 50 % probability. Hydrogen atoms except these on the amine were omitted for clarity. The intra-molecular hydrogen bond between amino proton and chloride was shown.

Figure 4. The molecular geometry of **4**. Thermal ellipsoids were drawn at 50 % probability.

Figure 5. The molecular geometry of **5**. Thermal ellipsoids were drawn at 50 % probability.

Figure 6. The molecular geometry of **7**. Thermal ellipsoids were drawn at 50 % probability. The intra-molecular hydrogen bond between amino proton and chloride was shown.

Figure 7. Effect of Ru-containing compounds on distribution of cell cycle-phases. Cells were treated in the absence or presence of the compound for the indicated times. The cells were fixed and stained with propidium iodide

to analyze DNA content by FAC Scan flow cytometer. Data are expressed as mean±SEM of three determinations. * *P*< 0.05 and *** *P*< 0.001 compared with the respective control.

Figure 8. Effect of Ru-containing compounds on mitochondrial membrane potential ($\Delta\Psi_m$). PC-3 cells were incubated in the absence or presence of the compound (30 μM, except for compound **4** of 20 μM) for 6 h. Then, the cells were incubated with JC-1 for the detection of $\Delta\Psi_m$ using flow cytometric analysis. The data are expressed as mean±SEM of three independent experiments. * *P*< 0.05 and ** *P*< 0.01 compared with the control.

.

Figure 1

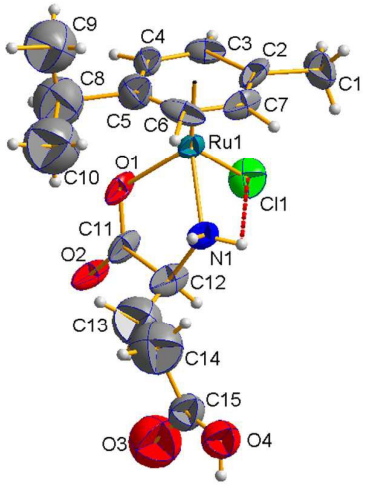


Figure 2

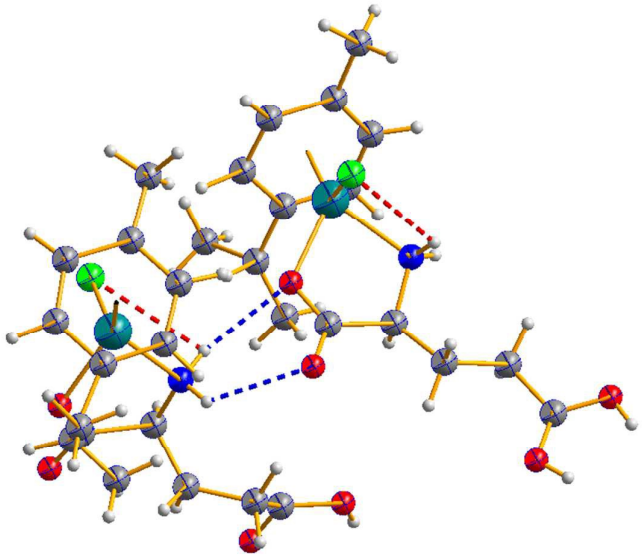


Figure 3

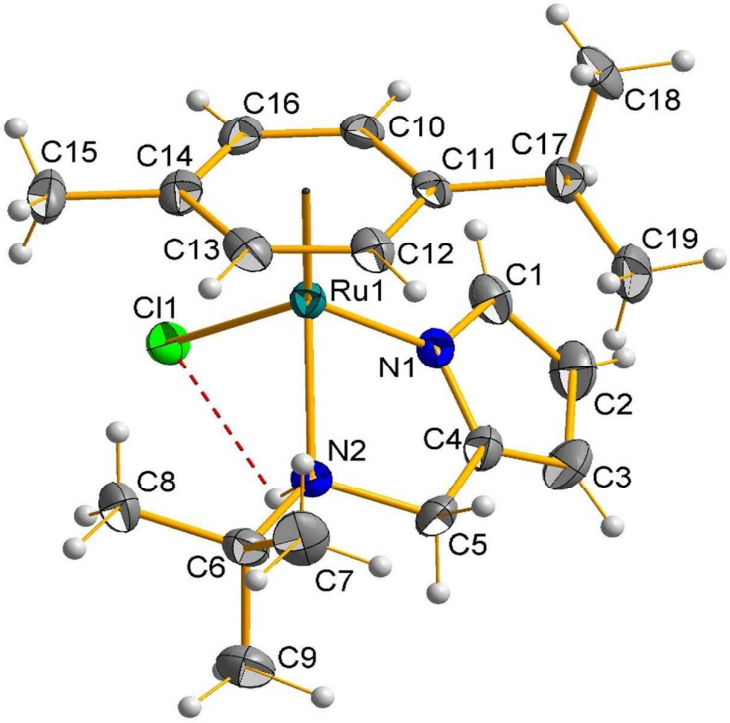


Figure 4

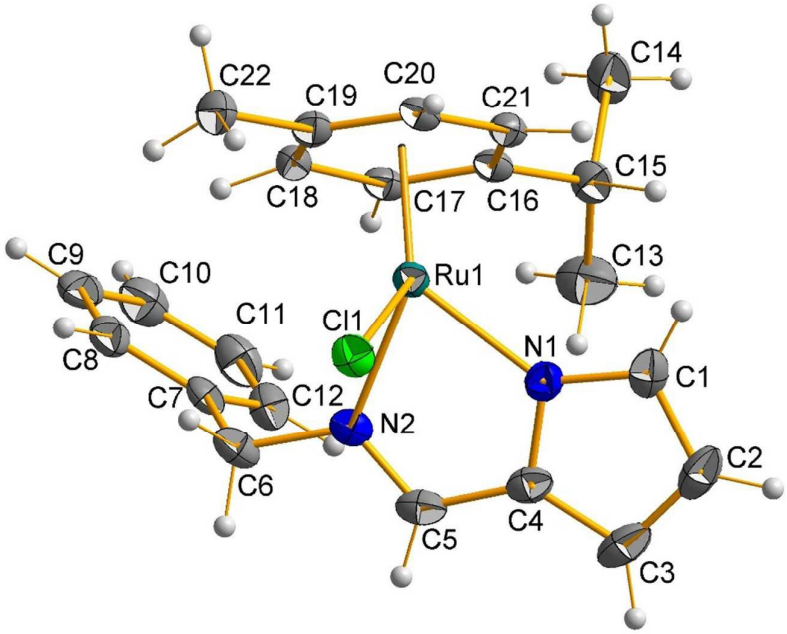


Figure 5

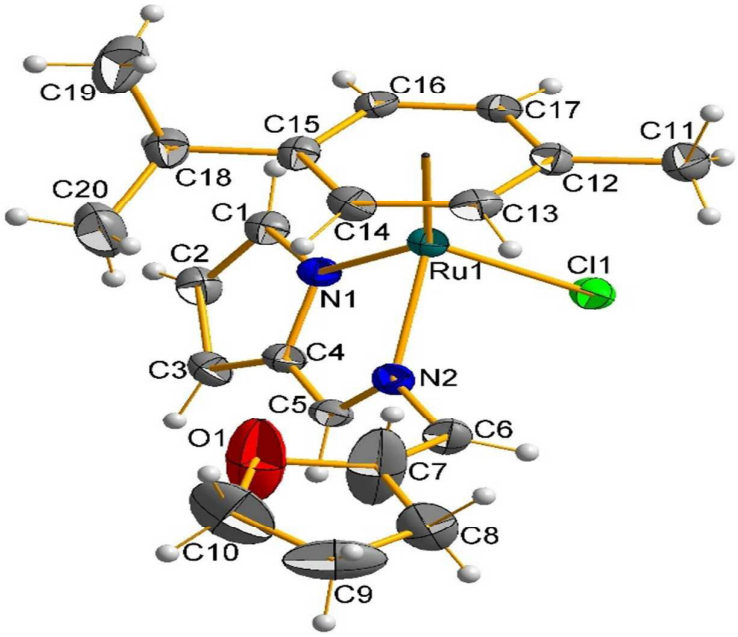


Figure 6

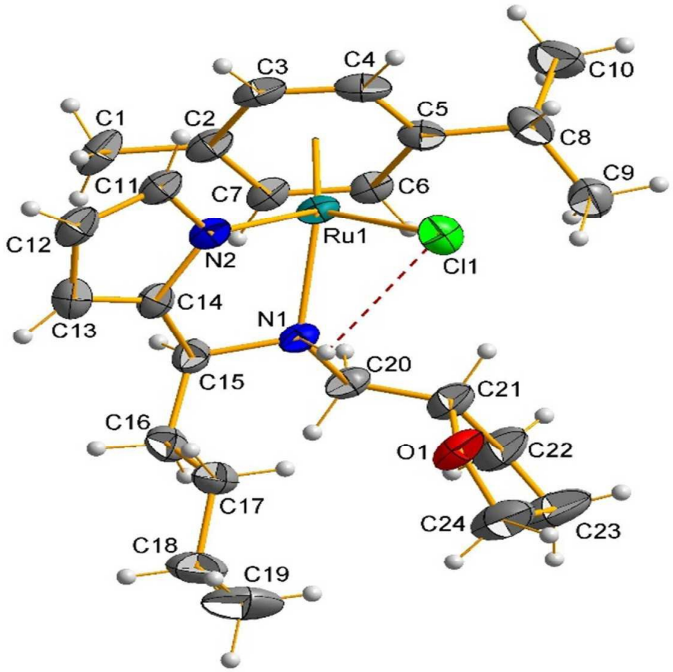


Figure 7

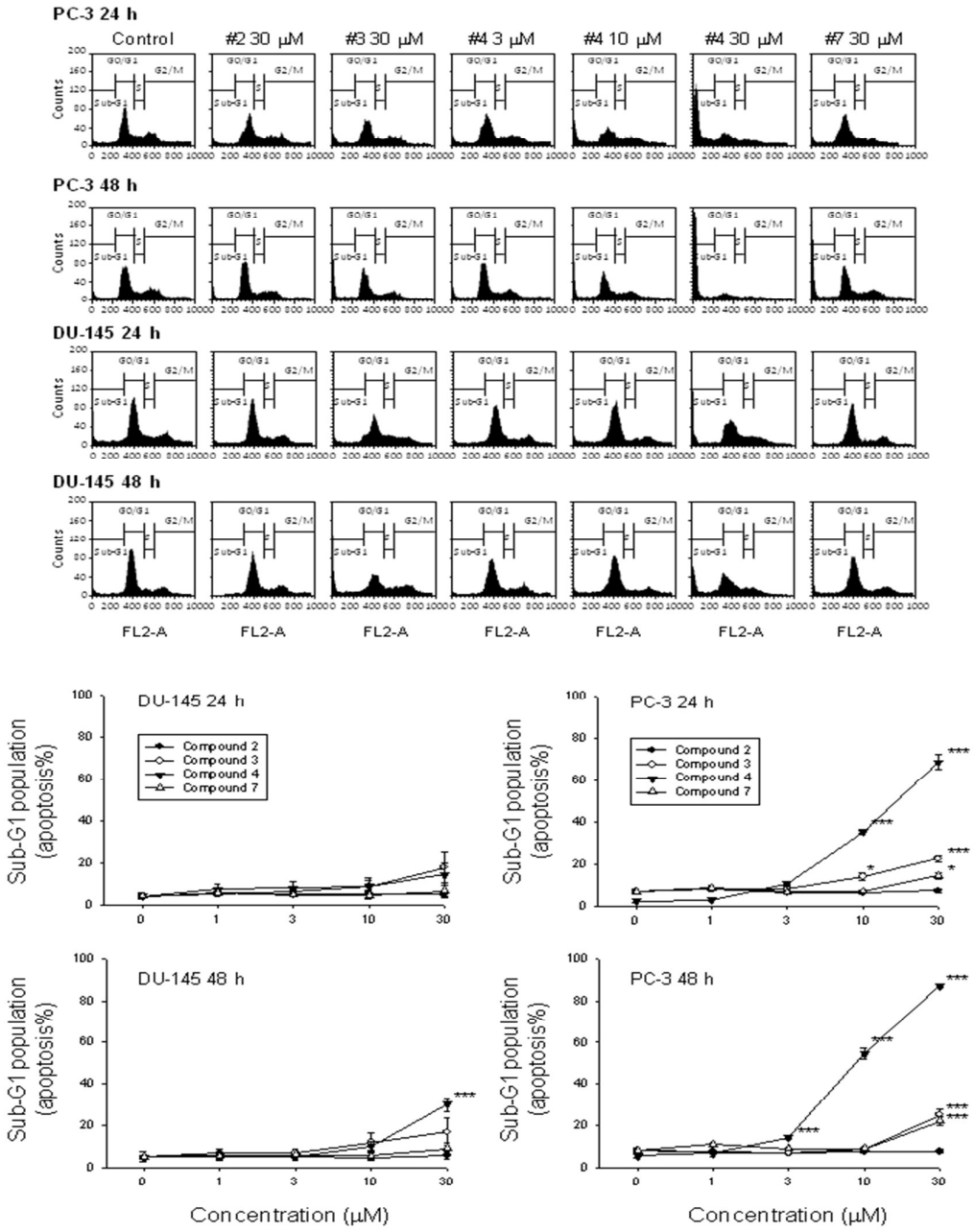


Figure 8

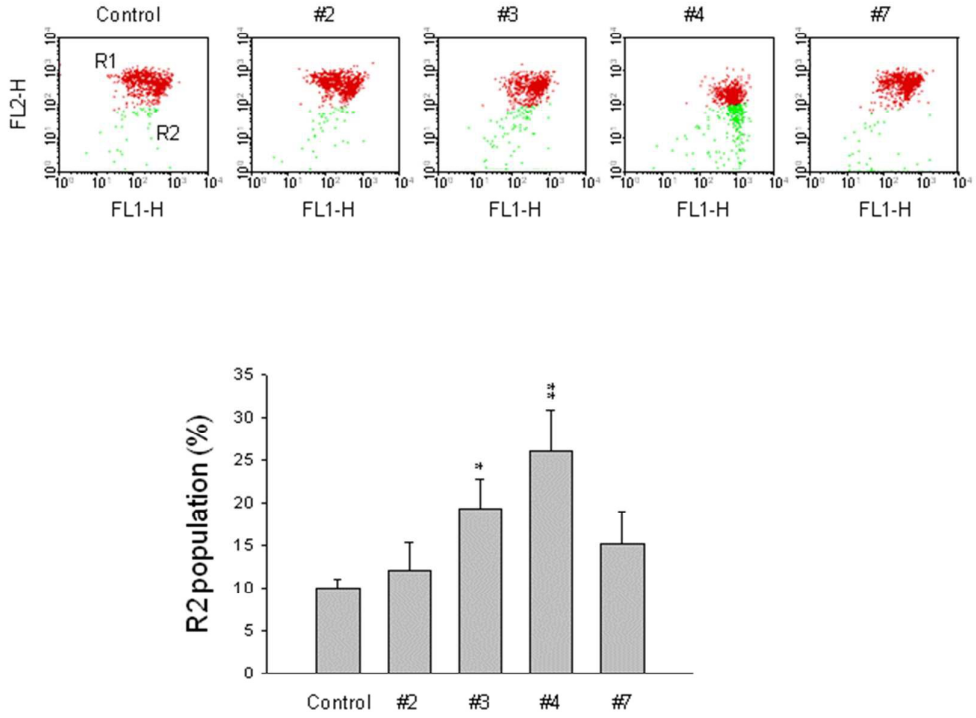


Table 1. The summary of X-ray crystal data collection of compounds **2** – **5** and **7**.

	2	**3**	**4**	**5**	**7**
formula	$C_{30}H_{44}Cl_2N_2O_8Ru_2$	$C_{19}H_{29}ClN_2Ru$	$C_{22}H_{25}ClN_2Ru$	$C_{20}H_{26}ClN_2ORu$	$C_{24}H_{37}ClN_2ORu$
FW	833.71	421.96	453.96	446.95	506.08
T [K]	293(2)	296(2)	293(2)	296(2)	296(2)
crystal system	Orthorhombic	Monoclinic	Monoclinic	Monoclinic	Monoclinic
space group	$P2_12_12_1$	P 2_1/c	P 2_1/c	P 2_1/c	P 2_1/c
a [Å]	9.895(11)	9.8435(18)	11.2603(18)	9.5877(12)	17.010(7)
b [Å]	11.081(12)	18.184(4)	15.897(3)	15.183(2)	11.237(5)
c [Å]	32.12(3)	12.068(3)	13.222(3)	13.9495(17)	13.004(5)
α [º]	90	90	90	90	90
β [º]	90	116.532(16)	121.976(12)	96.417(9)	91.50(3)
γ [º]	90	90	90	90	90

V [Å^3]	3522(6)	1932.6(7)	2007.7(7)	2017.9(4)	2484.7(17)
Z	4	4	4	4	4
ρ_c [Mg m^{-3}]	1.572	1.450	1.502	1.471	1.353
μ[mm-^{1}]	1.057	0.951	0.922	0.919	0.755
F(000)	1696	872	928	916	1056
rflns collected	7489	18241	28193	20381	34152
independent rflns	4135 [R_{int}=0.0538]	4719 [R_{int}=0.1086]	5210 [R_{int}=0.1925]	5147 [R_{int}=0.0585]	6159 [R_{int}=0.0869]
data/restraints/parameters	4135/ 84 / 397	4719/ 0 / 214	5210/ 0 / 238	5147 / 0 / 229	6159/ 0 / 266
goodness-of-fit on F^2	1.104	0.967	0.960	1.029	0.994
R_1, wR_2 (I>2σ(I))	R_1 = 0.0620, wR_2 = 0.1584	R_1 = 0.0517, wR_2 = 0.1038	R_1 = 0.0569, wR_2 = 0.0942	R_1 = 0.0553, wR_2 = 0.1431	R_1 = 0.0500, wR_2 = 0.1151
R_1, wR_2 (all data)	R_1 = 0.0830,	R_1 = 0.1183,	R_1 = 0.1852,	R_1 = 0.1049,	R_1 = 0.1282,

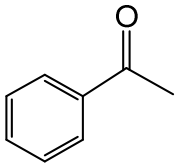
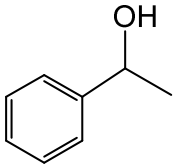
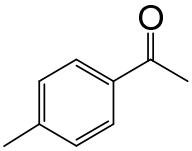
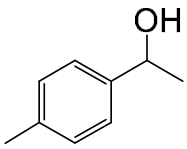
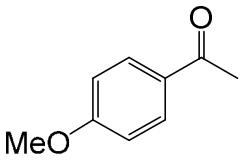
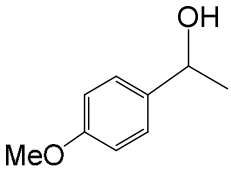
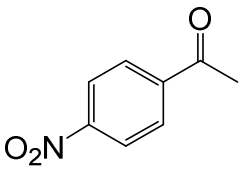
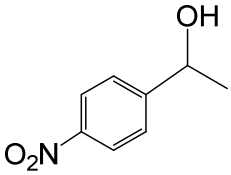
	wR_2 = 0.1735	wR_2 = 0.1293	wR_2 = 0.1309	wR_2 = 0.1644	wR_2 = 0.1474
largest diff. peak, hole [eÅ^{-3}]	1.128 and -0.737	0.507 and -0.808	0.643 and -0.669	0.724 and -0.514	0.830 and -0.613

Table 2. Selected bond lengths (Å) and angles (º) for compounds **2**-**5** and **7**.

2			
Ru(1)-O(1)	2.080(14)	Ru(1)-N(1)	2.128(16)
Ru(1)-Cl(1)	2.405(6)	Ru(2)-O(5)	2.108(12)
Ru(2)-N(2)	2.106(15)	Ru(2)-Cl(2)	2.405(6)
Ru(1)-Cymene$_{center}$(1)	1.660(4)	Ru(2)-Cymene$_{center}$(2)	1.639(4)
O(1)-Ru(1)-N(1)	77.4(7)	N(1)-Ru(1)-Cl(1)	82.7(5)
O(1)-Ru(1)-Cl(1)	85.5(4)	O(5)-Ru(2)-N(2)	77.6(6)
O(5)-Ru(2)-Cl(2)	83.5(4)	N(2)-Ru(2)-Cl(2)	82.3(4)
3			
Ru(1)-N(1)	2.042(4)	Ru(1)-N(2)	2.215(4)
Ru(1)-Cl(1)	2.4094(13)	Ru(1)-Cymene$_{center}$(1)	1.6739(5)
N(2)-Ru(1)-Cl(1)	81.55(11)	N(1)-Ru(1)-Cl(1)	86.75(11)
N(1)-Ru(1)-N(2)	75.64(17)		
4			
Ru(1)-N(1)	2.051(5)	Ru(1)-N(2)	2.114(5)
Ru(1)-Cl(1)	2.4210(16)	N(2)-C(5)	1.297(8)
Ru(1)-Cymene$_{center}$(1)	1.6840(9)		
N(2)-C(6)	1.478(7)	N(1)-Ru(1)-Cl(1)	87.59(14)

N(1)-Ru(1)-N(2)	76.5(2)	N(2)-Ru(1)-Cl(1)	84.01(14)
5			
Ru(1)-N(1)	2.048(4)	Ru(1)-N(2)	2.119(4)
Ru(1)-Cl(1)	2.4156(13)	N(2)-C(5)	1.273(6)
Ru(1)-Cymene$_{center}$(1)	1.6749(4)		
N(1)-Ru(1)-N(2)	76.41(16)	N(1)-Ru(1)-Cl(1)	87.24(12)
N(2)-Ru(1)-Cl(1)	84.09(11)		
7			
Ru(1)-N(1)	2.170(4)	Ru(1)-N(2)	2.066(4)
Ru(1)-Cl(1)	2.4119(16)	Ru(1)-Cymene$_{center}$(1)	1.6801(6)
N(2)-Ru(1)-N(1)	74.35(15)	N(1)-Ru(1)-Cl(1)	85.23(10)
N(2)-Ru(1)-Cl(1)	89.16(11)		

Table 3. Catalytic transfer hydrogenation reactions of ketones using 2-propanol and Ru(II) compounds **2-5 and 7**

Entry	Cat	Substrate	Product	Conv./%
1	**2**			89.2
2	**3**			93.9
3	**4**			96.1
4	**5**			95.7
5	**7**			92.3
6	blank			55.6
7	**2**			21.2
8	**3**			69.5
9	**4**			89.0
10	**5**			80.7
11	**7**			33.7
12	blank			17.4
13	**2**			13.2
14	**3**			19.4
15	**4**			67.8
16	**5**			59.5
17	**7**			17.4
18	blank			12.4
19	**2**			Trace
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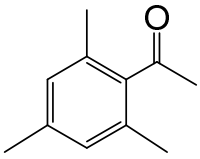
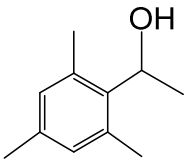
21	**4**			Trace
22	**5**			Trace
23	**7**			Trace
24	**2**			Trace
25	**3**			Trace
26	**4**			Trace
27	**5**			Trace

28	**7**	Trace