Synthesis of Ruthenium Sawhorse Complexes with Salicylic Acid, Gallic Acid and Carboxylated Thiosemicarbazone Ligands

Jiang Mingxuan, Yau Chun Huan, Tan Yong Leng Kelvin
Hwa Chong Institution
Singapore
mingxuanj@yahoo.com.sg

Abstract*—Ruthenium sawhorse complexes have great pharmaceutical potential because of their stability and non-toxicity as drug delivery agents. This project synthesizes and characterizes new sawhorse-type complexes with carboxylato bridges bonded to biologically active substituents. The thermal reactions between $Ru_3(CO)_{12}$ and carboxylic acids followed by addition of two-electron donor ligands (L), such as PPh_3, and pyridine are used to form diruthenium sawhorse complexes. Each sawhorse complex consists of an $Ru_2(CO)_4$ backbone with 2 μ_2-η^2-carboxylato bridges, with L occupying axial positions of the diruthenium unit. The characterization techniques used are infrared spectroscopy, mass spectrometry, ^1H NMR and $^{31}P\{1H\}$ NMR, elemental analysis, and single-crystal structure analysis. Unprecedented sawhorse complexes with salicylic acid with L: PPh_3 and pyridine were successfully synthesized and characterized for the first time. The characterization of the newly synthesized products with gallic acid is still undergoing. Further research is required to determine the nature of the products of the reactions involving carboxylated thiosemicarbazones whose structure eludes us.

Keywords- ruthenium, carboxylato bridges, dinuclear complexes

I. INTRODUCTION

Thiosemicarbazones (Fig.1) possess anti-cancer, anti-bacterial, anti-fungal and anti-viral properties. Carboxylated thiosemicarbazones are thiourea derivatives with an N-N-S system essential for anti-cancer properties [1]. Salicylic acid and its derivatives possess a plethora of pharmaceutical applications including anti-diabetic, anti-inflammatory, analgesic, antipyretic properties and also prevent blood clots and strokes. Salicylic acid derivatives also displayed anti-cancer properties when binded to metal complexes, such as cobalt [2]. Gallic acid and its derivatives inhibit RNA viruses and viral proteins such as that of herpes simplex virus [3]. They also display anti-oxidant and neuroprotective effects and are capable of causing cancer cell death [4]. Ruthenium sawhorse complexes (Fig.2) have huge clinical potential in the treatment of cancer, due to its release of ligands at high cancer cell concentrations [5]. Current uses of ruthenium based drugs have displayed anti-bacterial and anti-malarial properties as well. Thiosemicarbazones are highly toxic [6], but ruthenium complexes, non-toxic and stable [7], may drastically reduce its toxicity. The sawhorses are effective drug delivery agents, delivering the useful ligands safely to target sites, bind to biomolecules, before releasing the ligands to carry out their effects. The sawhorse amplifies useful properties the ligands possess, so this synergistic relationship between ruthenium sawhorses and their ligands may prove crucial to the fight against cancer and other maladies.

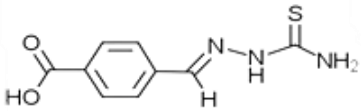


Figure 1. Carboxylated thiosemicarbazone

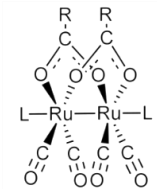


Figure 2. Ruthenium sawhorse complex

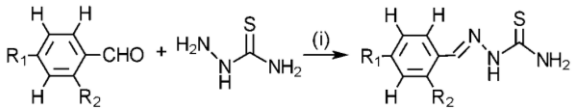
With its immense range of applications and uses of ruthenium sawhorses complexes, our research aims to synthesize, purify and characterize 6 new $Ru_2(CO)_4$ sawhorse-type complexes through the thermal reactions of $Ru_3(CO)_{12}$, with carboxylic acids-carboxylated thiosemicarbazones, salicylic acid and gallic acid, in order to unlock their potential in the pharmaceutical field.

II. EXPERIMENTAL SECTION

*A. Reactions to synthesise thiosemicarbazones **1A**, **1B***

A solution of 4-carboxybenzaldehyde (450 mg, 3 mmol) and 2-carboxybenzaldehyde (450 mg, 3 mmol) in 10 mL of 96% Ethanol respectively were reacted with thiosemicarbazide (300 mg, 3.3 mmol) at reflux for 3h. After the reaction mixture had cooled, the solids formed were filtered out, washed with ethanol and dried, to obtain yellow solids **1A** and **1B**.

Scheme 1: Synthesis of carboxylated thiosemicarbazones **1A**, **1B** [8]



(i) EtOH, reflux for 3 hours

Product	R_1	R_2
1A	CO_2H	H
1B	H	CO_2H

B. *Reactions to synthesise ruthenium sawhorse complexes, **2A**, **2B**, **3A**, **3B**, **4A**, **4B***

Scheme 2: Synthesis of the ruthenium sawhorse complexes

$$2\ Ru_3(CO)_{12} + 6\ RCO_2H + 6\ L \rightarrow 3\ Ru_2(CO)_4(RCO_2)_2L_2 + 12\ CO + 3\ H_2$$

A solution of $Ru_3(CO)_{12}$ and RCO_2H (amount stated in Table I) in 40 mL ethanol was refluxed in a Schlenk tube for 24h. For **2A**, **3A** and **3B**, the resulting solution and PPh_3 were stirred with an additional 10mL of DCM (amount stated in Table I) at room temperature for 2h. For **4A** and **4B,** the resulting solution and 18.9 µL of pyridine were stirred at room temperature for 2h [9].

TABLE I. Various acids and ligands used and their amounts

Reaction	$Ru_3(CO)_{12}$	RCO_2H	Amount	Ligand L	Amount
2A	30 mg (0.069 mmol)	**1A**	46 mg (0.206 mmol)	PPh_3	54 mg (0.206 mmol)
2B	100 mg (0.156 mmol)	**1B**	105 mg (0.469 mmol)	PPh_3	123 mg (0.469 mmol)
3A	100 mg (0.156 mmol)	Salicylic acid	65 mg (0.469 mmol)	PPh_3	123 mg (0.469 mmol)
3B	50 mg (0.078 mmol)	Gallic acid	44 mg (0.235 mmol)	PPh_3	61 mg (0.235 mmol)
4A	50 mg (0.078 mmol)	Salicylic acid	32 mg (0.235 mmol)	Pyridine	19 mg (0.235 mmol)
4B	50 mg (0.078 mmol)	Gallic acid	44 mg (0.235 mmol)	Pyridine	19 mg (0.235 mmol)

3A and **4A**: A yellow precipitate formed, and was recrystallized to obtain yellow crystalline solids. The solvent was removed from the supernatant, with the resulting solid recrystallized in DCM to obtain brown solids.

3B and **4B**: the solvents were removed, and the solid recrystallized with DCM to obtain yellow solids and brown solids. Solids insoluble in DCM were recrystallized with ethanol to yield black solids.

TABLE II. Various yields of products from reaction **3A**, **4A**, **3B** and **4B**

Product From Reaction	Yield/ %	Mass/ mg	Amount/ mmol
3A	55	143	0.129
4A	64	57	0.070
3B	22	31	0.026
4B	24	23	0.028

2A: The solvents were removed, and recrystallization was done in ethanol to yield a yellow solid of mass 9 mg.

2B: A solution of $Ru_3(CO)_{12}$ and **1B** (mass and amount specified in Table I) in 40 mL ethanol was refluxed in a Schlenk tube overnight. The solution was filtered and the filtrate and PPh_3 (mass and amount specified in Table I) were stirred for two hours in 10mL DCM. An orange suspension formed was filtered, and the filtrate recrystallized in DCM to afford a yellow solid.

C. *Alternative Synthesis for Reaction 2A*

A solution of $Ru_3(CO)_{12}$ (100 mg, 0.156 mmol) and **1A** (0.105 g, 0.469 mmol) in 40 mL dry ethanol refluxed in a Carius tube for 24h. The solution was stirred for 4h in 10mL of DCM with 0.123 g (0.469 mmol) of PPh_3 afterwards. The solvents were removed to obtain an orange residue. The residue was purified by column chromatography on silica gel using ethyl acetate/hexane 1:1 as eluent, yielding 4 bands. Band 1: brown oil. Band 2: yellow. Band 3: Orange. Band 4: light pink. The bands 2-4 were isolated and dissolved in 10mL DCM, then recrystallized overnight, and brown solid was yielded for band 3 and 2, whereas band 4 had a reddish solid [8].

III. RESULTS AND DISCUSSION

$Ru_2(CO)_4[O_2C\text{-}C_6H_4OH]_2(PPh_3)_2$ **(3A)**

IR (DCM) $\nu_{(CO)}$: 2030 (vs), 1986 (m), 1959 (vs) cm^{-1}. **3A** solids exhibit the characteristic 3 bands around 2030 to 1950 cm^{-1} for the CO terminal ligands in the $\nu_{(CO)}$ region of the infra-red spectrum. This corresponds to literature values for the ruthenium sawhorse complexes [5]. **^{1}H NMR** (400 MHz, $CDCl_3$): δ= 9.74 (s, 1H, OH), 7.62 (m, 6H, C_6H_5, ^3J = 7.9 Hz), 7.47-7.40 (m, 9H, C_6H_5), 7.22 (t, 1H, C_6H_4, ^3J = 7.9 Hz), 6.98 (d, 1H, C_6H_4, ^3J = 8.0 Hz), 6.65 (d, 1H, C_6H_4, ^3J = 8.0 Hz), 6.49 (t, 1H, C_6H_4, ^3J = 8.0 Hz). **^{31}P{^{1}H} NMR** (162 MHz, $CDCl_3$): δ= 16.15. **^{1}H NMR** results indicate that the location and number of chemically distinct hydrogen atoms of **3A**, corresponding to that of salicylic acid and PPh_3 ligands of the desired product. **^{31}P{^{1}H} NMR** results indicate that phosphorous atoms are present, and thus suggest the successful addition of PPh_3 ligands to form the desired product. **ESI-MS m/z** = 1112 $[M]^+$ Mass spectroscopy results confirms that the desired molecular ion peak is indeed present, by coinciding with the expected mass. Anal. Calcd for $(Ru_2C_{54}H_{40}O_{10}P_2)_4.CH_2Cl_2$: C 57.38%, H 3.57%. Found: C 57.77%, H 3.70%. Hence, the new compound **3A** was indeed our desired compound, and was synthesized successfully.

Interestingly, there is a line of symmetry perpendicular to the Ru-Ru bond, as the sets of hydrogen and phosphorous peaks detected were half the anticipated number, proving that the chemical environments of the H and P atoms of one pair of salicylic acids and PPh_3 ligands are identical to the other pair.

$Ru_2(CO)_4[O_2C\text{-}C_6H_2(OH)_3]_2(PPh_3)_2$ **(3B)**

IR (DCM) $\nu_{(CO)}$: 2026 (vs), 1982 (m), 1955 (vs) cm^{-1}. **3B** solids exhibit the characteristic 3 bands around 2030 to 1950 cm^{-1} for the CO terminal ligands in the $\nu_{(CO)}$ region of the infra-red spectrum. While this corresponds to literature values for the ruthenium sawhorse complex, further characterizations such as NMR and mass spectrometry have yet to be carried out to confirm the compound's identity.

$Ru_2(CO)_4[O_2C\text{-}C_6H_4OH]_2(C_5H_5N)_2$ **(4A)**

IR (DCM) $\nu_{(CO)}$: 2031 (vs), 1981 (m), 1950 (vs) cm^{-1}. **4A** solids exhibit the characteristic 3 bands around 2030 to 1950 cm^{-1} for the CO terminal ligands in the $\nu_{(CO)}$ region of the infra-red spectrum. This corresponds to literature values for the ruthenium sawhorse complex. **^{1}H NMR** (500 MHz, $CDCl_3$): δ= 10.55 (s, 1H, OH), 8.86 (d, 2H, C_5H_5N, ^3J = 5.0 Hz), 7.97 (t, 1H, C_6H_4, ^3J = 7.5 Hz), 7.77 (d, 1H, C_6H_4, ^3J = 10 Hz), 7.59 (t, 2H, C_5H_5N, , ^3J = 7.5 Hz), 7.33 (t, 1H, C_6H_4, , ^3J = 7.5 Hz), 6.81 (m, 2H, C_6H_4, C_5H_5N) **^{1}H NMR** results of the location and number of chemically distinct hydrogen atoms of **4A** correspond to that of salicylic acid and pyridine ligands of the desired product. A similar line of symmetry is displayed in **4A**. **ESI-MS m/z** = 748 $[M + 2H]^+$ Mass spectroscopy confirms that the desired molecular ion peak is indeed present, by coinciding with the expected mass. Anal. Calcd for $(Ru_2C_{28}H_{20}O_{10}N_2)_4.CH_2Cl_2$: C 44.18%, H 2.67%. Found: C 44.16%, H 2.87%. Hence, the new compound **4A** was indeed our desired compound, and was synthesized successfully.

$Ru_2(CO)_4[O_2C\text{-}C_6H_2(OH)_3]_2(C_5H_5N)_2$ **(4B)**

IR DCM) $\nu_{(CO)}$: 2029 (vs), 1979 (m), 1948 (vs) cm^{-1}. **4B** solids exhibit the characteristic 3 bands around 2030 to 1950 cm^{-1} for the CO terminal ligands in the $\nu_{(CO)}$ region of the infra-red spectrum. This corresponds to literature values for the ruthenium sawhorse complex. **ESI-MS m/z** = 652 $[M - 2C_6H_5N]^+$ shows that traces of our desired compound may be present. However, further characterization such as NMR is needed to confirm the compound's identity.

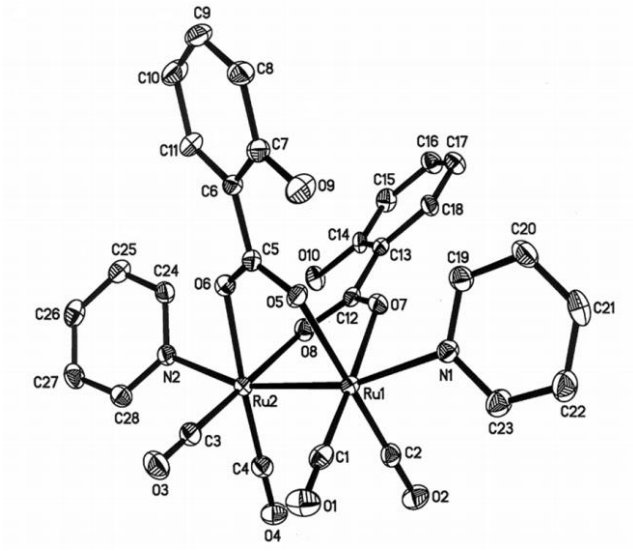


Figure 3. ORTEP drawing of **4A**, omitting hydrogen atoms for clarity

X-ray Crystallographic Studies: Crystals of diffraction quality were grown by slow diffusion of hexane in concentrated solutions of **4A** in DCM. Single-crystal structure analysis of **4A** exhibit the $Ru_2(CO)_4$ sawhorse similar to the anticipated structure, as shown in Fig. 3. The Ru-Ru distance is in range of a Ru-Ru covalent single bond. The O-C-O bond angles and Ru-O bond lengths are consistent with analogous compounds [9].

TABLE III. Selected bond lengths and angles for **4A**

Bond	Bond Length /Å
Ru(1)-Ru(2)	2.6640(4)
Ru(1)-N(1)	2.218(3)
Ru(2)-N(2)	2.222(3)
Ru(1)-O(7)	2.128(2)
Ru(1)-O(5)	2.145(2)
Ru(2)-O(6)	2.129(2)
Ru(2)-O(8)	2.143(2)

Bonds	Angle /°
O(7)-Ru(1)-O(5)	82.71(9)
O(6)-Ru(2)-O(8)	82.56(9)
O(7)-C(12)-O(7)	124.0(3)
O(6)-C(5)-O(5)	123.6(3)
C(2)-Ru(1)-C(1)	89.15(15)
C(3)-Ru(2)-C(4)	89.88 (16)

Reactions with carboxylated thiosemicarbazones: column chromatography is largely ineffective, due to decomposition of the compound on silica gel, hence only recrystallization was feasible for purification. However, the alternative synthesis of **2A** underwent column to afford a band, with a trace of **ESI-MS m/z**= 1283 [M], the Mr of our product. Unfortunately, the yield after column is very little. Recrystallization of **2A** in ethanol yielded a yellow solid of **ESI-MS m/z=** 1154 (whose mass we cannot figure out), and **IR** (KBr) $\nu_{(CO)}$: 2045 (s), 2025 (vs), 1980 (s), 1951 (vs) cm^{-1}, dissimilar to that of a sawhorse. Further research is required to determine its structure, as it does not resemble the desired compound. For **2B**, ESI-MS m/z= 1154 (whose mass we cannot figure out) for the orange solid produced. Purification via recrystallization has been carried out, which gave IR results of IR (DCM) VCO: 2156 (w), 2105 (w), 2061 (vs), 2026 (m), 1999 (vs), 1955 (m) cm^{-1}, dissimilar to that of a sawhorse. Further research is required to determine its structure, as it does not resemble the desired compound.

IV. Conclusion and Future Work

We have found that the reaction of $Ru_3(CO)_{12}$ with salicyclic acids afforded the desired ruthenium sawhorse complexes **3A**, **4A**, and further characterization using single crystal X-ray structural analysis is being carried out to fully characterize the compound. Further characterization using NMR and mass spectrometry is being carried out for **3B**, **4B**, before any conclusions regarding their identity can be made. Although the structures of products **2A** and **2B** have eluded us, we plan to purify and characterize them further to uncover their identity. In addition, we can explore alternative synthetic routes to synthesize our desired products **2A** and **2B.**

In the long run, we hope to determine the biological activities of the various sawhorses which we have synthesized and characterized, against diseases such as cancer, and discover their applications in the pharmaceutical field.

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V. APPENDIX

Characterization of selected compounds

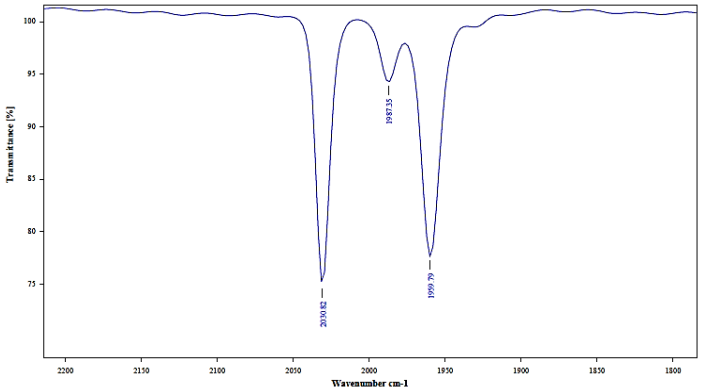

Figure 4. Infra-red spectrum **of 3A**

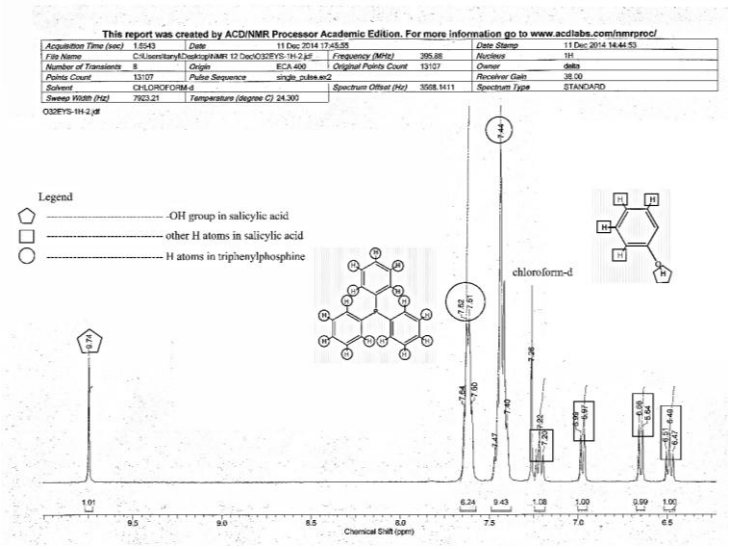

Figure 5. **^{1}H NMR** spectrum **of 3A**

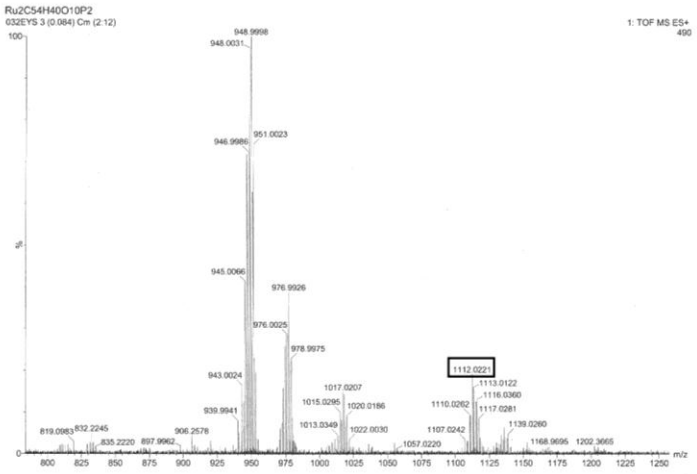

Figure 6. Mass spectrum of **3A**