ejpmr, 2018,5(2), 445-449

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SYNTHESIS OF RU-NHC COMPLEX FROM CAFFINE AND ITS ACTIVITY AGAINST MALARIAL PARASITES

Karumutchu Sitalu[1]*, B. Hari Babu[2] and A. Lakshmana Rao[3]

[1]*Department of Chemistry, Krishna University, Machilipatnam-521001.
[2]Department of Chemistry, Acharya Nagarjuna University, Guntur-522510.
[3]VV Institute of Pharmaceutical Sciences, Gudlavalleru-521356.

***Corresponding Author: Karumutchu Sitalu**

Department of Chemistry, Krishna University, Machilipatnam-521001.

Article Received on 15/12/2017 **Article Revised on 05/01/2018** **Article Accepted on 25/01/2018**

ABSTRACT

Present day organometallic science is difficult to envision without flexible N-heterocyclic carbene ligands. Because of their remarkable soundness and auxiliary differing qualities these themes are utilized in incalculable coordination buildings in current days. Specifically, transition metal complexes bearing interchangeable and promptly accessible NHCs have been built up as intense homogeneous catalysts. This field concentrates on particular applications and adjustments. Therefore, the present scientific experts can depend on complex engineered apparatus for the functionalization of carbenes, empowering access to polydentate ligand frameworks with or without hemilabile conduct. With regards to this work, different functionalized carbene ligands were utilized to accomplish or examine particular properties of ruthenium complexes. Tetracarbene ligands are generally unbending structures which empower relatively stable mixes because of their chelating coordination mode. Be that as it may, the basic differences of these themes is frequently restricted because of the low adaptability of the ligand forerunners. Using a non-cyclic, open-chain tetraimidazolium salt, we blended Ru (II) edifices whose geometry can be adjusted relying upon the response conditions. Also, these buildings demonstrated articulated movement in the TH of ketones. The present study focuses on the synthesis of Ru-NHC and its impact on malarial parasites.

KEYWORDS: Carbene compounds, methylated caffeine, XRD, malarial parasite.

INTRODUCTION

Ruthenium (III) complexes are all octahedral and low-spin with one pair electron. It can also form extensive series of halide complexes, the aqua-chloro series being probably the best characterized of all its complexes. The Ru(III)/Cl-/H_2O system has received extensive study, especially by ion exchange technique. $K_3[RuF_6]$ can be synthesized from molten salt $RuCl_3/KHF_2$ (Goldberg *et al*., 1968). The dimeric anion of bromo complexes were reported, for example, $[Ru_2Br_9]_3$- which is composed of a pair of faced-sharing octahedra. Cyano complexes of ruthenium (III) were prepared, the parent $[Ru(CN)_6]^{3-}$ was isolated as the brilliant yellow salt by aerial oxidation of dimethylsulfoxide solution of $[Ru(CN)_6]^{2+}$. Ruthenium (III) is much more amenable in coordination with N-donor ligands than is iron(III), and forms ammines with 3 to 6 NH_3 ligands (the extra ligands making up octahedral coordination are commonly H_2O or halides) as well as complexes with 2,2´-bipyridine and 1,10- phenanthroline (Dwyer *et al*., 1963).

We are interested in the synthesis of Ruthenium NHC complexes that have the potential to be used as a new class of antibiotics, DNA binders particularly for the treatment of cancer, bacterial infections, malaria, Chagas disease, Spetic shock and also as immunosuppressants. Malaria is a life threatening mosquito-borne infectious disease caused by parasites transmitted to humans through the bite of the Anopheles mosquito and affects approximately 16,00,000 people world wide (Caraballo and Hector, 2014). Infections with infected anophilous mosquitoes cause most of the morbidity and mortality in patients with Malaria (Bousema and Drakeley, 2011).

The global scope of malaria and the spread of drug-resistant *Plasmodium falciparum* make the need for improved therapy undeniable (Guerin et al, 2002). Assessment of both existing drugs and new antimalarials, alone or in combination, requires reliable methods for high-throughput testing. For decades, antimalarial drug effects have been measured in vitro by quantifying parasite uptake of radioactive substrates as a measure of growth and viability in the presence of the test drug (Desjardins et al., 1979; Elabbadi, et al., 1992). Antimalarial drugs are used for the treatment and prevention of malaria infection. Most antimalarial drugs target the erythrocytic stage of malaria infection, which is the phase of infection that causes symptomatic illness. The extent of preerythrocytic (hepatic stage) activity for

most antimalarial drugs is not well characterized (Lambros and Vanderberg, 1979). Xanthines have been used medicinally as diuretics, central nervous system stimulants and inhibitors of cyclic adenosine monophosphate (cAMP) phosphodiesterase resulting in airway smooth muscle relaxation. Caffeine is a xanthine derivative that is readily available and has low toxicity, and thus, is a good candidate for a carrier molecule for the delivery of metal cations to the lungs.

The synthesis of methylated caffeine with various counter ions and the formation of its biscarbene silver complexes by *in situ* deprotonation with silver and copper were already reported (Kascatan-Nebioglu, et al., 2004). The use of ruthenium chloride to deprotonate imidazolium salts is one of the most common procedures used for the synthesis of NHC ruthenium complexes.

Herein we report the synthesis and characterization of the methylated caffeine **1** and Ruthenium complexes derived from methylated caffeine and the antimalarial properties of Ru-NHC.

Methods

All manipulations were carried out in air. Caffeine and methyl iodide were purchased from Qualigens. Ruthenium Chloride was purchased from Aldrich. LB Broth, Miller and Bactor agar were purchased from Hi Media. ^{1}H and ^{13}C NMR data were recorded on a Gemini 300 MHz instrument and were referenced to residual protons and ^{13}C signals of deuterated solvents.

Synthesis of 1, 3, 7, 9- tetramethylxanthinium iodide(1)

Caffeine (9.00 g, 46.4 mmol) was refluxed with methyl iodide (15 mL) in *N, N'*-dimethyl formamide (50 mL) at 145^{o}C for 20 hours. An excess amount of acetone was added to the clear solution obtained and the precipitate filtered and washed with acetone. Crystallization from acetonitrile gave **1** as a yellowish white solid (19.4 mmol, 6.52 g, 42%). Mp: 187-190^{o}C. Anal. Calcd for $C_9H_{13}IN_4O_2.H_2O$: C, 30.52; H, 4.27; N, 15.82. Found: C, 30.27; H, 4.18; N, 15.41. ^{1}H NMR (300 MHz, *d6*-DMSO): δ 9.30 (s, ^{1}H, NCHN), 4.16 (s, ^{3}H, CH_3), 4.06 (s, ^{3}H, CH_3), 3.75 (s, ^{3}H, CH_3), 3.28 (s, ^{3}H, CH_3). ^{13}C {^{1}H} NMR (75 MHz, *d6*-DMSO): δ 153.3 (C=O), 150.2 (C=O), 139.6 (NCHN), 139.3(C=C), 107.8 (C=C), 36.9, 35.6, 31.4, 28.4 (NCH_3). ESI-MS (m/z): 209 [$C_9H_{13}N_4O_2^{+}$]. X-ray crystal structure analysis of **1**: formula $C_9H_{15}IN_4O_3$, *Mw* = 354.15, colorless crystal 0.40 x 0.40 x 0.30 mm, *a* = 7.8807(5) Å, *b* = 8.1331(6) Å, *c* = 10.8982(7) Å, α = 96.4480(10)°, *β* = 99.4090(10)°, $^{\gamma}$ = 110.5710(10)°, *V* = 634.19(7) Å3, Dcalc = 1.855 Mg.m-3, *μ* = 2.529 mm-1, *Z* = 2, triclinic, space group P-1, λ = 0.71073 Å, *T* = 100 K, ω and φ scans, 5638 reflections collected, 2944 independent (*R*int = 0.0123), 166 refined parameters, R1/*w*R2 ($I \geq 2\sigma(I)$) = 0.0148/ 0.0372 and R1/wR2 (all data) = 0.0152/0.0374, maximum (minimum) residual electron density 0.465(-0.291) e.Å-3.

Synthesis of (1, 3, 7, 9-tetramethylxanthine-8-ylidene) Ruthenium Chloride

Compound **1** (4.00 mmol, 1.34 g) was dissolved in methanol (100 mL) and ruthenium chloride (8.00 mmol, 1.34 g) was added. The mixture was stirred at room temperature for 40 min. The dark brown ruthenium chloride solution was filtered to give a colorless solution. The volatiles were removed *in vacuom.* Compound **Ru-NHC** (1.22 mmol, 0.5 g, 30%) was obtained as a white solid after recrystallization from ethanol. Mp: 209-212^{o} C. Anal. Calcd for C11H15Ru(N4O4)3. 2H2O: C, 32.11; H, 4.62; N, 13.62. Found: C, 31.95; H, 4.33; N, 13.18. 1H NMR (300 MHz, D2O): δ 4.19 (s, ^{3}H, CH3), 4.07 (s, ^{3}H, CH3), 3.82 (s, ^{3}H, CH3), 3.34 (s, 3H, CH3), 1.91 (s, ^{3}H, COCH3). 13C {^{1}H} NMR (75 MHz, DMSO): δ 186.2 (CAg), 176.2 (C=O), 153.9 (C=O), 151.3 (C=O), 141.2 (C=C), 109.6 (C=C), 37.8, 31.4, 29.7 (N-CH3), 23.1 (COCH3). ^{102}Ru NMR: 409.53 (broad C-Ru). ESI-MS (m/z): 523 [C18H24RuN8O4 +]3, 315 [C9H12RuN4O2 +]3, 209 [C9H13N4O2 +]. X-ray crystal structure analysis of **Ru-NHC**: formula C11N4O6H19Ru, *Mw* = 411.16, colorless crystal 0.21 x 0.18 x 0.03 mm, *a* = 8.4027(8)Å, *b* = 6.2961(6) Å, *c* = 14.1856(14) Å, α = 90°, β = 98.243(2)°, $^{\gamma}$ = 90°, *V* = 742.73(12) Å3, Dcalc = 1.839 Mg.m-3, μ = 1.393 mm-1, *Z* = 2, monoclinic, space group *P*21/m, λ= 0.71073Å, *T* = 100 K, ω and *φ* scans, 6661 reflections collected, 1940 independent (*R*int = 0.0200), 150 refined parameters, R1/*w*R2 ($I \geq 2\sigma(I)$) = 0.0244/0.0598 and R1/wR2 (all data) = 0.0256/0.0604, maximum (minimum) residual electron density 1.593 (-0.229) e.Å-3.

Cultivation of *P. falciparum* and plate setup

Prior to the experiments, the malarial parasites were cultivated as per the method described by Trager and Jensen (1976). Cultures were maintained in fresh group A-positive human erythrocytes suspended at 2% hematocrit in RPMI 1640 containing 10% human serum, 3 g of glucose per liter, 45 μg of hypoxanthine per liter, and 50 μg of gentamicin per liter. Flasks were incubated at 37° C under a gas mixture of 5% O_2, 5% CO_2, and 90% N_2. Every 3 to 4 days, infected erythrocytes were transferred into fresh complete medium with uninfected erythrocytes.

The stock culture was synchronized with 5% sorbitol, as described previously (7), and then approximately 96 h later, the level of parasitemia was determined by light microscopy by counting of a minimum of 500 erythrocytes on a Giemsa-stained thin blood smear. Parasites were noted to be late-ring and early trophozoites, with no evident schizonts. The stock culture was then diluted with complete medium and normal human erythrocytes to a starting 4% hematocrit and 0.5% parasitemia. For each test drug, plates for both assay methods were prepared in parallel with the same cells and medium. For the [3 H] ethanolamine assay, 180μl of the cell suspension was dispensed into each test well of a 96-well plate. Stock solutions of the test drugs were prepared at a concentration of 10 mM (chloroquine

in water, all other drugs in ethanol), serially diluted in complete medium, and dispensed into triplicate test wells to yield final concentrations ranging from 0 to 10^{-5} M. Final well volumes were 200 and 100 µl for the [3 H]ethanolamine and fluorescence assays, respectively. The plates were then incubated as described above.

Determination of EC_{50}

For the [3 H] ethanolamine assay, after 24 h of growth, 1 µCi (1 µl) of [3 H] ethanolamine with 19 µl of complete medium was added to each well. After an additional 24 h (a total of 48 h of growth), the contents of the plates used for the [3 H] ethanolamine assay were harvested by collecting the cells and placing them onto glass-fiber filters with a semi-automated 96-well harvester. Radiolabel uptake was quantified by scintillation counting of the filters with a Betaplate counter. The counts were plotted against the logarithm of the drug concentration and curve fitting by nonlinear regression (sigmoidal dose-response/variable slope equation) to yield the drug concentration that produced 50% of the observed decline from the maximum counts in the drug-free control wells (EC50) and then plotted and analyzed by linear regression.

RESULTS AND DISCUSSION

Direct Synthesis of Ru-NHC Complex

In situ deprotonation of methylate caffeine with Ruthenium Chloride in 1:2 ratio in methanol gave complex **Ru-NHC** (Scheme 1) (Ivanov, et al., 1989). The disappearance of the resonance for the imidazolium proton of **methylated caffeine** and the appearance of the resonance for the carbene carbon atom at 186.2 ppm together with carbonyl and methyl carbons of the acetate group at 176.2 ppm and 23.1 ppm, respectively showed the formation of the expected Ruthenium-NHC complex. Complex **Ru-NHC** has good stability in water. A water solution of **Ru-NHC** kept in the dark was observed to form crystal particles within 10 days. Complex **Ru-NHC** is composed of biologically relevant ligands and is water soluble. These properties make **Ru-NHC** a viable candidate for use as an internal antimicrobial. Furthermore, **Ru-NHC** is a relatively small molecule and may be able to diffuse into the microbes better than conventional larger antibiotics.

Colorless crystals of **Ru-NHC** were obtained from a concentrated sample in a water/ethyl acetate mixture (Figure-1). The asymmetric unit of this molecule contains the complex together with two molecules of water. The geometry around the ruthenium atom deviates significantly from linearity with a C1-Ru-O3 bond angle of $168.19(9)^0$. The Ru-carbene bond length is 2.067(3) Å (Table 1).

Methylated caffeine, 1,3,7,9-tetramethylxanthinium iodide, **1**, was synthesized by refluxing caffeine with an excess of methyl iodide in DMF using a modified literature procedure (Scheme 3.1). Compound **1** is a water-soluble solid and stable in air up to its melting point. In the ^{1}H NMR spectrum, the imidazolium proton appears at 9.30 ppm, which is consistent with the general C-H acidic proton shift of imidazolium salts (= 8-10 ppm) (Hermann, 1997; Bourissou, et al., 2000). The imidazolium carbon appears at 139.6 ppm as the most notable feature in the ^{13}C NMR spectrum. For these reasons we explored the direct synthesis of **Ru-NHC**. In situ deprotonation of **1** with ruthenium chloride in 1:2 ratio in methanol gave complex **Ru-NHC** (Scheme 3.3). Complex **Ru-NHC** has good stability in water.

The antimalarial activity of the Ru-NHC complex, **4**, was evaluated against variety of standard drugs including a panel of highly selective opportunistic agents against malarial pathogens. The drugs tested in this study are varied in their structures, mechanistic actions, and potencies; and the range of parasitemia tested for fluorescence linearity includes the entire realistic range encountered in a 48-h assay under our starting conditions.

The EC50s of the drugs tested by the radioisotopic assay methods were similar or identical (Table 2). Figure 2 shows the similarities between the dose-response curves over the range of drug concentrations tested.

In conclusion. we have synthesized a novel mixed N-heterocyclic carbene-chloride complex of ruthenium from caffeine. The Ru-NHC complex derived from methylated caffeine demonstrated antimalarial activity in comparision with standard drugs against malaria.

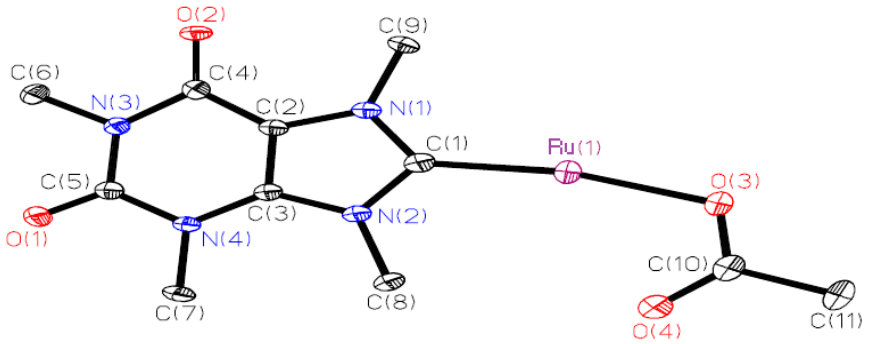
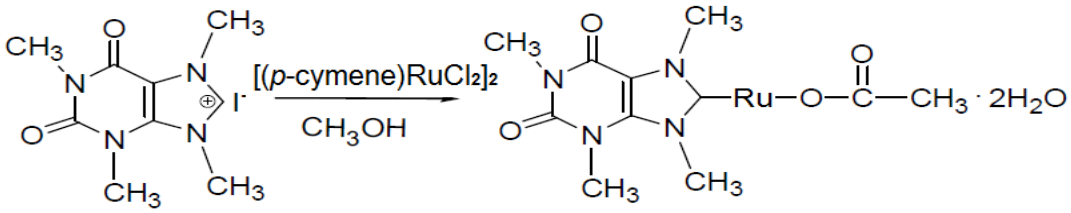


Figure 1: Molecular structure of Ru-NHC complex.



Scheme 1: Direct synthesis of Ru-NHC complex.

Table 1: Selected bond lengths and angles for Ru-NHC complex.

Bond Lengths and Angles	Ru-NHC Complex
N1-C1	1.337(3) Å
N2-C1	1.359(3) Å
N1-C1-N2	106.2(2)o
C1-RuII	1.998(3) Å
C1-RuII-O3	171.68(9) o

Table 2: EC50 of Ru-NHC complex in comparision with standard drugs with [^{3}H] Ethanolamine.

Drug/Complex	EC_{50} value
Chloroquine	19 ± 2.0
Quinine	41 ± 2.6
Mefloquine	51 ± 4.0
Ru-NHC complex	46 ± 3.2

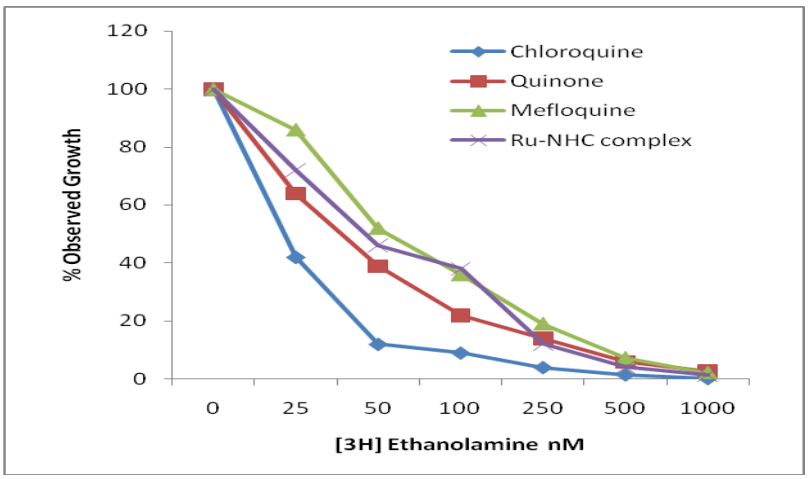


Figure 2: Dose response curves for Ru-NHC along with standard drugs.

REFERENCES

1. Bourissou, D.; Guerret, O.; Gabbaï, F. P.; Bertrand, G.; Chem. Rev., 2000; 100: 39.
2. Bousema T, Drakeley C. Epidemiology and Infectivity of Plasmodium falciparum and Plasmodium vivax Gametocytes in Relation to

Malaria Control and Elimination. Clinical Microbiology Reviews, 2011; 24(2): 377-410. doi:10.1128/CMR.00051-10.
3. Caraballo, Hector (May 2014). "Emergency Department Management Of Mosquito-Borne Illness: Malaria, Dengue, And West Nile Virus". Emergency Medicine Practice. 16(5).
4. Desjardins, R., G. Canfield, J. Haynes, and J. Chulay. 1979. Quantitative assessment of antimicrobial activity in vitro by a semiautomated microdilution technique. Antimicrob. Agents Chemother, 16: 710–718.
5. Dwyer, F.P., H.A. Goodwin and E.C. Gyarfas. 1963. Mono- and Bis-(2,2´- bipyridine) and (1,10-phenanthroline) Chelates of Ruthenium and Osmium. I. Monochelates of Bivalent, Tervalent and Quadrivalent Ruthenium. Aust. J. Chem., 16(1): 42-50.
6. Elabbadi, N., M. Ancelin, and H. Vial. 1992. Use of radioactive ethanolamine incorporation into phospholipids to assess in vitro antimalarial activity by the semiautomated microdilution technique. Antimicrob. Agents Chemother., 36: 50–55.
7. Goldberg, R.N. and L.G. Hepler. 1968. Thermochemistry and oxidation potentials of platinum group metals and their compounds. Chem. Rev., 68(2): 229-252.
8. Guerin, P., P. Olliaro, P. Druilhe, R. Laxminarayan, F. Binka, W. Kilama, N. Ford, and N. White. 2002. Malaria: current status of control, diagnosis, treatment, and a proposed agenda for research and development. Lancet Infect. Dis., 2: 564–573.
9. Herrmann, W. A.; Angew. Chem., Int. Ed. Engl., 1997; 36: 2162.
10. Ivanov, E.I.; Kalayanov, G.D.; Yaroshchenko, I.M.; Stepanov, D.E. Khimiya Geterotsiklicheskikh Soedinenii, 1989; 11: 1570.
11. Kascatan-Nebioglu, A.; Panzner, M. J.; Garrison, J. C.; Tessier, C. A.; Youngs, W. J. Organometallics, 2004; 23: 1928.
12. Lambros, C. and J. Vanderberg. 1979. Synchronization of Plasmodium falciparum erythrocytic stages in culture. J. Parasitol., 65: 418–420.
13. Trager, W., and J. Jensen. 1976. Human malaria parasites in continuous culture. Science, 193: 673–675.